



2020 Quantitative BioImaging Conference

January 6 – 9, 2020

University of Oxford, Mathematical Institute

Andrew Wiles Building, Woodstock Rd.

Oxford OX2 6GG, UK

Phone: +44 (0) 1865 615130

Special Thanks to:

The CLF OCTOPUS Imaging Facility, UKRI/STFC Rutherford Appleton Laboratory

The Scientific Machine Learning Group, UKRI/STFC Rutherford Appleton Laboratory

The Company of Biologists Ltd

Cancer Research UK – Oxford Centre

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Sponsors

- CLF OCTOPUS Imaging Facility, UKRI/STFC Rutherford Appelton Laboratory
- Scientific Machine Learning Group, UKRI/STFC Rutherford Appelton Laboratory



The Company of Biologists Ltd



Cancer Research UK – Oxford Centre



ZEISS House

Marketing Presenter

Abbelight Marketing Session

Wednesday (8 January) 16:00 – 16:15 in Lecture Room 1

Conference Exhibitors

Innopsys

Abbelight

Oxxius SA

ZEISS House

Cairn Research

NKT Photonics Ltd

Andor Technology Ltd

Teledyne Photometrics

Nikon Optical U.K. Limited

Oxford Nanoimaging Ltd (ONI)

Andrew Wiles Building Floor Plan



Session Room 1 (L1)

Session Room 2 (L2)

Foyer : Exhibits, Posters, Coffee/Lunch Breaks, Receptions

Travel Information

Travel by Air

For delegates arriving from Heathrow or Gatwick Airports there are frequent and reliable coach services run by the Oxford Bus Company, terminating at Gloucester Green coach station. Average journey time from Heathrow is 1hr 10mins, and average journey time from Gatwick is 2 hours 10 minutes. Be aware, these times can go up if there are traffic problems. For more information about coach service from Heathrow and Gatwick airports, visit: <http://www.oxfordbus.co.uk>

For delegates arriving from London Stansted or Luton Airports, there are less frequent coach and rail connections with Oxford. See the National Express Airports web site for more information: <http://www.nationalexpress.com/destinations/airports.cfm>

Travel by Coach

London to Oxford: There are frequent non-stop motorway express services which provide a substantially cheaper alternative to rail. Tickets can be purchased on the coach itself or through their app. Services run from London (Marble Arch, Victoria Coach Station and Grosvenor Gardens) to Gloucester Green. The journey is approximately 1hour and 40 minutes.

X90 <http://www.oxfordbus.co.uk>

Oxford Tube <http://www.oxfordtube.com/>

Travel by Car

The Mathematics Institute does not offer any car parking facilities and does not recommend travel by car. However information on use of the nearest public car parks (below) can be found on the Oxford City Council website (https://www.oxford.gov.uk/directory/8/car_parks_in_oxford)

[St. Giles' East](#) (3LW, St. Giles', Oxford, UK)

[Gloucester Green](#) (Gloucester St., Oxford OX1 2BN, UK)

[Oxford Parking](#) (52 Broad St., Oxford OX1 3BS, UK)

Travel within Oxford

Taxi ranks are located at the Oxford Train Station and Gloucester Green. Minimum fares are usually £5.

Radio Taxis (Oxford): +44 (0) 1865 242424

Oxford City Taxis: +44 (0) 1865 959595

Royal Cars: +44 (0) 1865 777333

Train Travel

The Oxford [OXF] railway station lies to the west of the city centre and is a 20 minute walk or taxi ride to the venue. The new Oxford Parkway [OXP] railway station is located to the North of Oxford, and is a short bus or taxi ride to the venue.

For train times visit <http://www.nationalrail.co.uk>

For more information on travel from London Heathrow and Gatwick airports to Oxford as well as other travel information for Oxford and the surrounding areas, please visit the University of Oxford's visitor information page (<https://www.ox.ac.uk/visitors/visiting-oxford/how-get-oxford?wssl=1>)

Conference Registration and Badge Pickup

A copy of your Registration Confirmation email will help in processing your registration on-site at the Registration Desk of the conference.

Monday (6 January) 08:00 to 16:00

Overlap with Opening Reception (19:00 to 22:00)

Registration Desk in
Andrew Wiles Building – Main Foyer
Mathematical Institute, University of Oxford
Radcliffe Observatory, Woodstock Road
Oxford OX2 6GG, UK
(<https://tinyurl.com/yyf9k5zl>)

Tuesday (7 January) 08:00 to 18:00

Registration Desk in
Andrew Wiles Building – Main Foyer
Mathematical Institute, University of Oxford
Radcliffe Observatory, Woodstock Road
Oxford OX2 6GG, UK

Wednesday (8 January) 08:00 to 18:00

Registration Desk in
Andrew Wiles Building – Main Foyer
Mathematical Institute, University of Oxford
Radcliffe Observatory, Woodstock Road
Oxford OX2 6GG, UK

Thursday (9 January) 08:00 to 14:00

Registration Desk in
Andrew Wiles Building – Main Foyer
Mathematical Institute, University of Oxford
Radcliffe Observatory, Woodstock Road
Oxford OX2 6GG, UK

Scientific Organizing Committee QBI 2020

Chairs

Martin BOOTH (University of Oxford)

Jens RITTSCHER (University of Oxford)

Raimund OBER (Texas A&M University / University of Southampton)

Members

Edward COHEN (London)

Maria HARKIOLAKI (Harwell)

Iain STYLES (Birmingham)

Daniel ROLFE (Harwell)

Ian DOBBIE (Oxford)

Karen HAMPSON (Oxford)

Dominic WAITHE (Oxford)

Carlas SMITH (Delft)

Program Committee

- **Sean Andersson**, Boston
- **Martin Booth**, Oxford
- **Chris Calderon**, Boulder
- **Alessandra Cambi**, Nijmegen
- **Ed Cohen**, London
- **Susan Cox**, London
- **Joerg Enderlein**, Goettingen
- **David Gruenwald**, Worcester
- **Florian Jug**, Dresden
- **Charles Kervrann**, Rennes
- **Sandrine Leveque-Fort**, Paris
- **Diane Lidke**, Albuquerque
- **Keith Lidke**, Albuquerque
- **Raimund Ober**, College Station/Southampton
- **Crysanthe Preza**, Memphis
- **Sripad Ram**, San Diego
- **Jens Rittscher**, Oxford
- **Yoav Shechtman**, Haifa
- **Sally Ward**, College Station/Southampton
- **Winfried Wiegraebe**, Seattle
- **Daniel Wuestner**, Odense
- **Christophe Zimmer**, Paris

Quantitative BioImaging Society

Meeting to discuss future plans and events for the QBI Society

Wednesday (8 January) at 20:30

Room

Andrew Wiles Building – Main Foyer

Mathematical Institute, University of Oxford

Radcliffe Observatory, Woodstock Road

Oxford OX2 6GG, UK

Session Chair: Raimund Ober

Meals and Refreshments

The meals and refreshments are provided in the Andrew Wiles Building Main Foyer free of charge to conference attendees.

Monday (6 January)

11:00 to 11:15 – Coffee Break

12:15 to 13:00 – Lunch

14:45 to 15:00 – Coffee Break

17:00 to 17:15 – Coffee Break

19:00 to 22:00 – Opening Reception and Poster Viewing

Tuesday (7 January)

09:50 to 10:20 – Coffee Break

12:05 to 13:30 – Lunch

15:00 to 16:30 – Coffee Break (sponsored by Chroma Technologies) and Poster Session

Wednesday (8 January)

10:10 to 10:40 – Coffee Break

12:00 to 13:30 – Lunch

15:00 to 16:30 – Coffee Break and Poster Session

Thursday (9 January)

09:40 to 10:10 – Coffee Break

12:10 to 13:30 – Lunch

15:30 to 16:00 – Coffee Break

18:30 to 21:00 – Closing Reception

Conference Technical Information

Internet Wi-Fi Access

There are two methods to access WiFi :

Eduroam – for those who have academic affiliation, log in with your home academic institution credentials

Icloud – a free commercial provider that can be accessed with the following instructions :

1. Switch on your smartphone, tables or laptop and check that WiFi is enabled
2. Select ‘_The Cloud’ from the available network list
3. Open your internet browser – the venue landing page will appear. If it does not, type in bbc.co.uk to prompt the browser to load the landing page
4. If it is your first time using The Cloud WiFi network, follow the simple one-time registration process by sharing some simple details
5. Once registered you can access the internet via The Cloud

Posters

Posters should be in vertical format, and the maximal dimensions should be 841 mm in width x 1189 mm in height (A0 Portrait).

Please note the very specific poster format. The poster will be slipped into the board mount and other formats cannot be accommodated. Board numbers will be assigned at the registration desk. Posters with odd numbered poster board assignments will be presented in the Tuesday (7 Jan) Poster Session (15:00-16:30). Posters with even numbered poster board assignments will be presented in the Wednesday (8 Jan) Poster Session (15:00-16:30).

Set up

Monday (6 January) starting at 15:00 and no later than Monday at 18:45 (for the Monday 19:00 Poster Viewing)

Take down

Friday (11 January) beginning at 17:00 and no later than Friday at 18:00

Keynote Speakers

Jennifer Lippincott-Schwartz

Senior Group Leader, Janelia Research Campus, Howard Hughes Medical Institute, Ashburn, USA



“Lippincott-Schwartz stayed on at the NIH, as a primary investigator and chief of the Section on Organelle Biology in the Cell Biology and Metabolism Branch. Hoping to expand her research into live-cell imaging, she began searching for a way to tag proteins in live cells. Eventually her group created a photoactivatable form of green fluorescent protein (GFP), which allowed them to switch a tagged protein’s green glow on and off using flashes of light.

Her discovery led to a fruitful collaboration with physicists Eric Betzig and Harald Hess (now group leaders at Janelia), who proposed a new function for the photoactivatable protein. The scientists used the protein to generate photoactivatable fluorophores, or dyes, which enabled them to illuminate different sets of molecules sequentially, creating a microscope image far more detailed than previously possible. The method, called super-resolution microscopy, garnered Betzig the 2014 Nobel Prize in Chemistry. Using super-resolution microscopy and other fluorescence imaging techniques, Lippincott-Schwartz’s NIH lab group probed subcellular processes, such as lipid droplet formation and organelle dynamics, for nearly three decades.

In 2016, Lippincott-Schwartz moved her lab to Janelia, where she continues to investigate cell biology, but in the context of the brain. Her group studies neurobiology on a cellular level, looking into processes such as organelle trafficking and metabolism, to better understand how nerve cells communicate and behave in normal and diseased brain function.” (sources: photo – [Wikipedia](#); text – [HHMI Janelia](#))

Dr. Lippincott-Schwartz’s presentation, ‘Eukaryotic organelles: deciphering their interdependency, structure and dynamics with new imaging technologies’, is scheduled for 08:30 on Tuesday, 7 January.

Wolfgang Baumeister

Director of Molecular Structural Biology, Max Planck Institute of Biochemistry, Planegg, Germany



“Cryoelectron microscopy offers the key to investigate the structure of large, complex and flexible protein assemblies in its natural environment in intact cells and in isolated form to the quasi-atomic level. Wolfgang Baumeister and his team of the research department “Molecular Structural Biology” have studied the 3D structure of the 26S proteasome (image) by an integrative approach of single particle electron microscopy, combining the classification of reconstructed 26S complexes in different conformational states, by structure modelling and the integration of atomic models of single protein components obtained by x-ray crystallography. The 26S proteasome consists of 66 protein subunits and

recognizes, unfolds and degrades other protein molecules in a controlled manner. Large protein assemblies have important roles in the living cell, but they are often unstable, may exist only temporarily and can thus hardly be investigated in biochemical preparations in purified form. The department of Wolfgang Baumeister pioneered and developed the method of cryo-electron tomography which enables the reserches to visualize macromolecular structures in a functional and close-to-native state in frozen-hydrated cells. By recording micrographs from different viewing angles and integrating them to a 3D image (tomogram) the researchers study the structure of macromolecular assemblies in prokaryotic cells and of eukaryotic cellular components such as the nuclear pore complex, the actin filament network, and the pre- and postsynaptic structures of neurons *in situ*.” (source: [Max Planck Institute](#))

Dr. Baumeister’s presentation, ‘Structural biology in situ: the promise and challenges of cryo-electros tomography’, is scheduled for 08:30 on Thursday, 9 January.

Invited Speakers

- Jennifer Lippincott-Schwartz, HHMI Janelia Research Campus
- Wolfgang Baumeister, Max Planck Institute of Biochemistry
- Marino Zerial, Max Planck Institute of Molecular Cell Biology and Genetics
- Dylan Owen, University College London
- Melike Lakadamyali, University of Pennsylvania
- Steve Presse, Arizona State
- Kristin Grussmayer, EPFL
- Aleksandra Radenovic, EPFL
- Christian Soeller, University of Exeter
- Alexander Jesacher, Innsbruck Medical University
- Dirk-Peter Herten, University of Heidelberg
- Michael Dustin, University of Oxford
- Gerhard Schütz, Vienna University of Technology
- Frederick Maxfield, Cornell University
- Daniel Wuestner, University of Southern Denmark
- Christoph Wuelfing, University of Bristol
- Thibault Lagache, Columbia University
- Thierry Pecot, Hollings Cancer Center at the Medical University of South Carolina
- Ilaria Testa, KTH Royal Institute of Technology
- Gail McConnell, University of Strathclyde - Glasgow
- Charles Kervrann, INRIA Rennes
- Edward Cohen, Imperial College - London
- Anish Abraham, College Station
- Jean-Baptiste Sibarita, University of Bordeaux
- Thomas Walter, Institute Curie
- Luke Lavis, HHMI Janelia Research Campus
- Lei Tian, Boston University
- Andrew York, San Francisco
- Bernd Bodenmiller, University of Zurich
- Josephine Bunch, National Physical Laboratory
- Jan-Otto Hooghoudt, Aalborg University
- Rasmus Waagepetersen, Aalborg University
- Lucy Collinson, The Francis Crick Institute
- Roarke Horstmeyer, Duke University
- Badri Roysam, University of Houston
- Sandrine Leveque-Fort, University of Paris - Sud
- Frederick Klauschen, Institute of Pathology, Charite - University Medicine Berlin
- Shanon Seger, Hoffman La-Roche, Switzerland
- Daniel Sage, EPFL
- Juliette Griffié, EPFL
- Fang Huang, Purdue University
- David Holcman, Institut de Biologie de l'Ecole Normale Supérieure
- Justin W. Taraska, Bethesda
- N. Liv, UMC Utrecht
- Robert West, Stanford University
- Marta Trub, University of Basel

Workshops

Spatial Statistics

6 January at 08:30 to 11:00 in Lecture Room 1

This workshop, chaired by Dr. Edward Cohen, will present some of the key theory of spatial point patterns and the methodology used for exploratory data analysis. Topics covered will include Poisson processes, complete spatial randomness, clustering and regularity, the definitions and estimators of key summary functions (including the nearest neighbor distribution, pair correlation function and Ripley's K-function), testing for complete spatial randomness, cluster model fitting and multichannel data. Implementation of these statistical tools will center around worked examples using the SpatStat package in R.

Approaching Quantitative Microscopy – counting molecules and cluster analysis

6 January at 11:15 to 12:15 and 13:10 to 14:45 in Lecture Room 1

The workshop, chaired by Dr. Dirk-Peter Herten will introduce the theme of quantitative microscopy in counting molecules and cluster analysis:

- 11:15 Introduction by Dr. Dirk-Peter Herten
- 11:25 'Quantitative super-resolution imaging in biology' presented by Dr. Melike Lakadamyali
- 11:50 'Quantitative super-resolution imaging with DNA-PAINT' presented by Dr. Christian Soeller
- 12:15 Lunch
- 13:00 'Cluster analysis of SMLM data' presented by Dr. Dylan M. Owen
- 13:25 'Quantitative imaging using super-resolution optical fluctuation imaging' presented by Kristin S. Grussmayer
- 13:50 'Counting by photon statistics' presented by Johan Hummert
- 14:15 'Models of single molecule dynamics from single photon detections: a Bayesian nonparametric approach' presented by Dr. Steve Pressé
- 14:40 Final discussion

Challenge Session

Challenges in Subcellular Trafficking

6 January at 15:00 to 17:00 and 17:15 to 19:00 in Lecture Room 1

Subcellular trafficking in cells is a topic that is much less understood than processes that occur, for example, on the plasma membrane. The reason is that standard imaging approaches are often not well suited to addressing these problems. Even if data can be acquired, the analysis often poses problems that are difficult to surmount.

- presentations and discussion presented by Luke Lavis, Jean-Christophe Olivo-Marin, Frederick Maxfield, Chyrsanthe Preza, Jean Salamero, Justin Taraska, E. Sally Ward, Daniel Wuestner, Jennifer Lippincott-Schwartz, Marino Zerial, and Raimund Ober

Tutorial

From time to time, we present from overviews and tutorial lectures on topical research areas to introduce the attendees to a field of interest. At this year's QBI conference, we have the following tutorial presentation:

Quantitative Phase Imaging

9 January at 09:10 to 09:40 in Lecture Room 1

- 'Quantitative phase Imaging' presented by Dr. Alexander Jesacher

Minisymposia

Imaging in Immunology

7 January at 13:30 to 15:00 in Lecture Room 1

- 'Supramolecular attack particles: a new cytotoxic biomaterial' presented by Dr. Michael L. Dustin
- 'Superresolution microscopy images: What they tell us about protein clusters – and what they don't' presented by Dr. Gerhard J. Schütz
- 'Applications of single-molecule localization microscopy to studying negative regulators of T cell activation' presented by Dr. Dylan M. Owen
- 'Quantitative analyses of T cell signaling' presented by Dr. Christoph Wuelfing

Volumetric Imaging

7 January at 10:20-11:20 in Lecture Room 1

- '3D parallelized RESOLFT optical nanoscopy' presented by Dr. Liaria Testa
- 'A bolt-on single-objective light-sheet design with uncompromised numerical aperture' presented by Dr. Andrew York
- 'Mesoscale imaging with the Mesolens' presented by Dr. Gail McConnell

Spatial Statistics in Microscopy

8 January at 13:30 to 15:00 in Lecture Room 1

- 'Inferring correlation in inhomogeneous and multitype point patterns – a review and new contributions' presented by Dr. Rasmus P. Waagepetersen
- 'Bayesian inference of the spatial distribution of proteins from three-cube Föster resonance energy transfer data' presented by Dr. Jan-Otto Hooghoudt
- 'Quantitative analysis of molecular coupling with SODA' presented by Dr. Thibault Lagache
- 'QuantEv: A quantitative approach for analyzing the spatio-temporal distribution of 3D events in fluorescence microscopy' presented by Dr. Thierry Pecot

Bioimage Informatics

7 January at 16:30 to 17:30 in Lecture Room 1

- 'Machine learning for computational phenotyping: how to overcome the need for massive image annotation' presented by Dr. Thomas E. Walter
- 'Statistical analysis and modeling of single particle trajectories to reveal the flow and dynamics of the ER' presented by Dr. David Holcman
- 'Quantitative multicolor super-resolution microscopy' presented by Dr. Jean-Baptiste Sibarita

Correlative Microscopy

9 January at 10:10 to 11:10 in Lecture Room 1

- 'Towards quantitative correlative microscopy' presented by Dr. Lucy M. Collinson
- 'Imaging the nanoscale structure of endocytosis with correlative super-resolution light and electron microscopy' presented by Dr. Justin W. Taraska
- 'Live-cell correlative microscopy of single organelles' presented by Dr. Nalan Liv

Modern Machine Learning Approaches to Image Analysis Uncertainty Quantifications in Microscopy

8 January at 10:40 to 11:40 in Lecture Room 1

- 'Deep learning based phase microscopy with uncertainty quantification' presented by Dr. Lei Tian
- 'Using machine learning to design intelligent computational microscopes' presented by Dr. Roarke Horstmeyer
- 'Comprehensive in-situ profiling of brain cellular alterations in the injured brain for accelerating drug discovery' presented by Dr. Badri Roysam

Tissue Multiplexing Techniques and Applications in Oncology

9 January at 13:30 to 15:30 and 16:00 to 16:55 in Lecture Room 1

- 'Introduction to the minisymposium on tissue multiplexing – is more the merrier or messier?' presented by Dr. Sripad Ram
- 'Multiplexing immunohistochemistry and transcriptomic data' presented by Dr. Robert West
- 'Implementation of multiplexing and image analysis to support drug discovery and development' presented by Dr. Shanon Seger
- 'Deep learning approaches for reliable quantification of multi-omics cell imaging datasets to interrogate RNA and protein spatial and temporal subcellular interactions' presented by Dr. Emmanuel Bouilhol
- 'Understanding and modeling inflammation induced immune-suppression in tumors using multi-plex immunofluorescence and live cell microscopy' presented by Dr. Stephen Lockett
- 'Highly multiplexed imaging in health and disease by imaging mass cytometry' presented by Dr. Bernd Bodenmiller
- 'Multiplex immune cell profiling to gain insights into progression in early cancer' presented by Dr. Natalia Garcia Martin
- 'Towards computational multiplex fluorescent microscopy: Machine learning-based integrated prediction of morphological and molecular tumor profiles' presented by Dr. Frederick Klauschen
- 'Multiplex imaging of lung tumors with CODEX technology, presented by Dr. Marta Trüb

New Frontiers in Single Molecule Microscopy

8 January at 08:30 to 08:50 in Lecture Room 1

- 'Single molecule localization microscopy with modulated excitation' presented by Dr. Sandrine Leveque-Fort

Past Conference Chair Lecture

This lecture recognizes the contributions of the prior conference chair. Dr. Charles Kervrann of Inria, Rennes was conference chair for the 2019 Quantitative BioImaging conference in Rennes, France.

7 January at 09:10 to 09:40 in Lecture Room 1

- 'Statistical and computational methods for intracellular trajectory analysis in fluorescence microscopy' presented by Dr. Charles Kervrann

QBI Student/Post-doc Chapter

We are excited to introduce the second annual meeting of the student-postdoc chapter of the Quantitative Bioimaging conference, with the goal of increasing the engagement and active participation of current and future young investigators in this field. We believe this chapter will provide a platform for students and postdocs to learn and present their work, while also engaging in chapter activities to enhance their professional skills and network with the leaders in the field. This is a great opportunity to get involved and make an impact as budding young investigators.

They will be organizing a ***Career Opportunities in Academia and Industry*** discussion on 7 January at 20:15 to 21:15 in Lecture Room 1 and having a ***Meet the Expert*** session on 8 January at 12:00 to 13:30 in the Andrew Wiles foyer food area as well as holding a ***Social Meeting*** for students and postdocs on 7 January at 21:15 to 22:00 (location will be announced closer to the meeting).

Participation in these events is open to all interested student and postdoc QBI registrants.

Given that this chapter is in its infancy, any suggestions for this and future meetings are welcome.

Please contact studentpostdocchapter@quantitativebioimaging.com if you have any suggestions.

Best Poster Award

Students presenting a poster are eligible for the **Best Student Poster Presentation Award**. An independent jury will assess the poster presentations and determine the awardee. The winner will be given the opportunity to present his/her work in a plenary lecture at the next QBI conference.

Award Lecture – 2019 Winner:

Elias E. Nehme

‘DeepSTORM3D: Deep learning for dense 3D single molecule localization microscopy’

7 January at 09:40 to 09:50 in Lecture Room 1

2020 Best Poster Announcement:


9 January at 18:00 to 18:15 in Lecture Room 1

Advanced Imaging Techniques

QBI 2020 Conference Sessions

6 Jan - 9 Jan













Monday January 6

Registration: Workshops	08:00	16:00	Registration Desk/Area
Workshop: Introduction to Spatial Statistics Session Chair: Edward Cohen	08:30	11:00	Room 1
Spatial Statistics Workshop (# 535)  Edward Cohen Imperial College London, London, GB	08:30	11:00	
Coffee Break	11:00	11:15	Foyer
Workshop: Approaching Quantitative Microscopy - counting molecules and cluster analysis I Session Chair: Dirk-Peter Herten	11:15	12:15	Room 1
Participants: Kristin S. Grussmayer, Dylan M Owen, Christian Soeller, Melike Lakadamyali, Johan Hummert, Steve Pressé			
Lunch (provided)	12:15	13:00	Foyer
Workshop: Approaching Quantitative Microscopy - counting molecules and cluster analysis II Session Chair: Dirk-Peter Herten	13:10	14:45	Room 1
Coffee Break	14:45	15:00	Foyer
Challenge Session: Challenges in Subcellular Trafficking I Participants: Luke Lavis, Jean-Christophe Olivo-Marin, Frederick Maxfield, Chyrsanthe Preza, Jean Salamero, Justin Taraska, E. Sally Ward, Daniel Wuestner, Jennifer Lippincott-Schwartz, Marino Zerial, Raimund Ober	15:00	17:00	Room 1
Coffee Break	17:00	17:15	Foyer
Challenge Session: Challenges in Subcellular Trafficking II	17:15	19:00	Room 1
Registration Desk Opens: Main Conference	18:00	21:00	Registration Desk/Area
Opening Reception and Poster Viewing	19:00	22:00	Foyer and Poster Areas
Plenary Discussion: Uses and Misuses of Machine Learning	20:00	21:00	Room 1










QBI 2020 Conference Sessions

6 Jan - 9 Jan















Tuesday January 7
















Registration Desk Opens	08:00	18:00	Registration Desk/Area
Opening Remarks	08:15	08:30	Room 1
Key Note Lecture 1: Jennifer Lippincott-Schwartz	08:30	09:10	Room 1
<u>Eukaryotic organelles: deciphering their interdependency, structure and dynamics with new imaging technologies (# 595)</u> 	08:30	09:10	
<u>Jennifer A. Lippincott-Schwartz</u> HHMI Janelia Research Campus,			
Past Chair Lecture	09:10	09:40	Room 1
<u>STATISTICAL AND COMPUTATIONAL METHODS FOR INTRACELLULAR TRAJECTORY ANALYSIS IN FLUORESCENCE MICROSCOPY (# 538)</u> 	09:10	09:40	
<u>Charles Kervrann</u> Inria, Rennes, FR			
Poster Award Lecture	09:40	09:50	Room 1
<u>DeepSTORM3D: Deep learning for dense 3D single molecule localization microscopy (# 360)</u> 	09:40	09:50	
<u>Elias E Nehme</u> , Daniel Freedman, Racheli Gordon, Boris Ferdman, Lucien Weiss, Onit Alalouf, Reut Orange, Tomer Michaeli, Yoav Shechtman Technion - Israel Institute of Technology, Haifa, IL			
Coffee Break	09:50	10:20	Foyer
Minisymposium: Advanced Imaging Techniques	10:20	11:20	Room 1
Session Chair: Martin Booth			
<u>3D parallelized RESOLFT optical nanoscopy (# 572)</u> 	10:20	10:40	
<u>Ilaria Testa</u> KTH, SciLifeLab,			
<u>A BOLT-ON SINGLE-OBJECTIVE LIGHT-SHEET DESIGN WITH UNCOMPROMISED NUMERICAL APERTURE (# 576)</u> 	10:40	11:00	
Alfred Millett-Sikking, <u>Andrew York</u> Calico Labs,			
<u>Mesoscale imaging with the Mesolens (# 579)</u> 	11:00	11:20	
<u>Gail McConnell</u> University of Strathclyde,			
Session: Volumetric imaging	11:20	12:00	Room 1
Session Chair: Martin Booth			
<u>Quantitating light sheet microscopy (# 460)</u> 	11:20	11:40	
<u>James Manton</u> , Nick Barry MRC Laboratory of Molecular Biology,			
<u>Label-free live-cell imaging at 150 nm and 100 Hz by ROCS-Microscopy (# 487)</u> 	11:40	12:00	
<u>Alexander Rohrbach</u> University of Freiburg,			
Session: Applications in Cell Biology	10:20	12:05	Room 2
Session Chair: Daniel Wüstner			
<u>Multiplex Single Molecule Pull-down (SiMPull) measurements of EGFR site-specific phoshporylation (# 494)</u> 	10:20	10:35	
<u>Diane Lidke</u> University of New Mexico, Albuquerque, US			
<u>Quantitative analysis of EGF receptor dynamics, confinement and interaction with the cytoskeleton using nanoparticle labelling, single-molecule tracking and hydrodynamic force application (# 492)</u> 	10:35	10:50	
Chao Yu, Maximilian Richly, Cedric Bouzigues, <u>Antigoni Alexandrou</u> Ecole polytechnique,			
<u>Condensation of endoplasmic reticulum membrane domains (# 325)</u> 	10:50	11:05	
<u>Konstantin S.A. Speckner</u> , Lorenz Stadler, Matthias Weiss University of Bayreuth, , DE			
<u>Super-resolution Microscopy Elucidates Curvature Generation By Endocytic Clathrin Coats In Live Cells and Tissues (# 402)</u> 	11:05	11:20	
<u>Comert Kural</u> Ohio State University,			

















<u>Nanoscale cellular dynamics with super-resolution spectroscopy_(# 444)</u>  <u>Erdinc Sezgin</u> ,Christian Eggeling <i>University of Oxford, , GB</i>	11:20	11:35	
<u>Quantitative imaging of intracellular receptors in cardiac myocytes using DNA-PAINT_(# 466)</u>  <u>Anna Meletiou</u> ,Christian Soeller <i>University of Exeter,</i>	11:35	11:50	
<u>Novel intensity-based approach to characterize astrocytic calcium signals_(# 510)</u>  <u>Andre Zeug</u> ,Franziska Müller, Evgeni Ponimaskin, Gebhard Stopper <i>Hannover Medical School,</i>	11:50	12:05	
Lunch (provided)	12:05	13:30	Food Area
Minisymposium: Imaging in Immunology Session Chair: Sally Ward	13:30	15:00	Room 1
<u>Supramolecular attack particles: a new cytotoxic biomaterial_(# 599)</u>  Stefan Balint, Maria Harkiolaki, Roman Fischer, Benedikt Kessler, <u>Michael Loran Dustin</u> <i>University of Oxford,</i>	13:30	14:00	
<u>Superresolution Microscopy Images: What they tell us about protein clusters – and what they don't_(# 423)</u>  <u>Gerhard J. Schütz</u> <i>Vienna University of Technology, , Austria</i>	14:00	14:20	
<u>Applications of single-molecule localisation microscopy to studying negative regulators of T cell activation_(# 413)</u>  <u>Dylan M Owen</u> ,Sabrina Simoncelli <i>University of Birmingham,</i>	14:20	14:40	
<u>Quantitative analyses of T cell signaling_(# 520)</u>  <u>Christoph Wuelfing</u> , <i>University of Bristol,</i>	14:40	15:00	
Session: Advances in Optical Approaches Session Chair: Martin Booth	13:30	15:00	Room 2
<u>Towards quantum enhanced quantitative superresolution microscopy_(# 296)</u>  <u>Dan Oron</u> ,Ron Tenne, Uri Rossman, Gur Lubin <i>Weizmann Institute of science,</i>	13:30	13:45	
<u>Metal-Induced Energy Transfer_(# 309)</u>  <u>Jörg Enderlein</u> <i>Georg August University, Göttingen, DE</i>	13:45	14:00	
<u>Homogeneous multifocal excitation for high-throughput super-resolution imaging_(# 297)</u>  <u>Dora Mahecic</u> ,Davide Gambarotto, Kyle Douglass, Denis Fortun, Maeva Le Guennec, Virginie Hamel, Paul Guichard, Suliana Manley <i>Ecole Polytechnique Federale de Lausanne,</i>	14:00	14:15	
<u>Multiconjugate Adaptive Optics for Microscopy_(# 414)</u>  <u>Karen M Hampson</u> ,Jiahe Cui, Matthew Wincott, Syed Asad Hussain, Kaustubh Banerjee, Pouya Rajaeipour, Hans Zappe, Caglar Ataman, Martin Booth <i>University of Oxford,</i>	14:15	14:30	
<u>Random Illumination Microscopy_(RIM) : nanoscopy in living tissues with neither calibration nor adaptive optics_(# 349)</u>  <u>Thomas Mangeat</u> ,Simon Labouesse, awoke negash, Emmanuel Martin, Renaud Poincloux, Xiabo Wang, Magali Suzanne, Mathieu Pinot, Roland LE BORGNE, nicolas Sandeau, Kamal Belkebir, Hugues giovannini, Marc Allain, Jérôme Idier, Anne Sentenac <i>CBI, Toulouse, FR</i>	14:30	14:45	
<u>Thermophoretic trap for single amyloid fibril and protein aggregation studies_(# 411)</u>  <u>Tobias Thalheim</u> <i>Peter Debye Institute for Soft Matter Physics, Leipzig University,</i>	14:45	15:00	
Poster Session	15:00	16:30	Poster Area
Chroma Technologies sponsored Coffee Break	15:00	16:30	Foyer
Minisymposium: Bioimage Informatics Session Chair: Charles Kervrann	16:30	17:30	Room 1
<u>Machine Learning for Computational Phenotyping: how to overcome the need for massive image annotation_(# 530)</u>  <u>Thomas Edgar Walter</u> <i>Mines ParisTech, Paris, FR</i>	16:30	16:50	
<u>Statistical analysis and modeling of single particle trajectories to reveal the flow and dynamics of the ER_(# 555)</u>  <u>David Holcman</u> <i>ENS,</i>	16:50	17:10	



Quantitative Multicolor Super-Resolution Microscopy (# 585) 	17:10	17:30	
Jean-Baptiste Sibarita <i>Interdisciplinary Institute for NeuroScience, , France</i>			
Session: Bioimage informatics Session Chair: Charles Kervrann	17:30	18:00	Room 1
Quantifying the Robustness of Topological Defects in Orientation Fields from Bio-Images (# 278) 	17:30	17:45	
Karl B. Hoffmann , Ivo Sbalzarini <i>Max Planck Institute of Molecular Cell Biology and Genetics,</i>			
KDML: a Knowledge-Driven Machine Learning for Analysing High Throughput Imaging Data (# 366) 	17:45	18:00	
Heba Sailem , Jens Rittscher, Lucas Pelkmans <i>University of Oxford, Oxford, GB</i>			
Session: Advanced Microscopy Techniques	16:30	17:45	Room 2
Measuring diffusion dynamics at high photon-count rates with FCS and STED-FCS (# 450) 	16:30	16:45	
Falk Schneider , M. Julia Roberti, Erdinc Sezgin, Christian Eggeling, Iztok Urbančič <i>University of Oxford, Oxford, GB</i>			
Automated, User-independent Correction of Artifacts in Fluorescence Correlation Spectroscopy Measurements using Convolutional Neural Networks (# 346) 	16:45	17:00	
Alexander Seltmann , Christian Eggeling, Dominic Waithe <i>Friedrich-Schiller-University Jena,</i>			
Optimization of experimental and acquisition parameters for improved SOFI super-resolution microscopy imaging (# 446) 	17:00	17:15	
Dario Cevoli <i>Université de Lille,</i>			
An extended quantitative model for super-resolution optical fluctuation imaging (SOFI) (# 350) 	17:15	17:30	
Peter Dedecker, Wim Vandenberg , Sam Duwé, Marcel Leutenegger <i>KU Leuven, Leuven, BE</i>			
Fluorescence Speckle Correlation Spectroscopy (# 307) 	17:30	17:45	
Anirban Sarkar, Irène Wang, Jörg Enderlein, Jacques Derouard, Antoine Delon <i>Université Grenoble Alpes, , FR</i>			
Dinner Break (on own)	18:00	18:30	(on own)
Workshop: Single molecule localization microscopy flight simulator Session Chair: Daniel Sage	18:30	20:15	Room 1
SMLM flight simulator (WORKSHOP) (# 536) 	18:30	20:15	
Juliette Griffie , Daniel Sage <i>EPFL, Lausanne, CH</i>			
Career opportunities in academia and industry (organized by QBI student and post-doc chapter)	20:15	21:15	Room 1
QBI Student/Post-doc Chapter Social Meeting	21:15	22:00	Off site

6 Jan - 9 Jan

Registration Desk Opens	08:00	18:00	Registration Desk/Area
Minisymposium: New Frontiers in Single Molecule Microscopy	08:30	08:50	Room 1
Single Molecule localization microscopy with modulated excitation (# 602)  Pierre Jouchet, Clement Cabriel, Nicolas Bourg, Abigail Illand, Marion Bardou, Christian Poüs, Emmanuel Fort, Sandrine Leveque-Fort ISMO-CNRS, ORSAY, FR	08:30	08:50	
Session: New Frontiers in Single Molecule Microscopy	08:50	10:10	Room 1
Isotropic 3D molecular resolution in cells with MINFLUX nanoscopy (# 272)  Francisco Balzarotti , Jasmin Pape, Klaus Gwosch, Stefan W. Hell Research Institute for Molecular Pathology, Goettingen, DE	08:50	09:10	
Bayesian Grouping of Localizations: Sub-Nanometer Precision, Counting and Resolution Doubling (# 470)  Keith A Lidke University of New Mexico, Albuquerque, US	09:10	09:30	
Uniting Structured Illumination and Localization Microscopy (SIMFLUX) (# 418)  Carlas Smith TU-Delft,	09:30	09:50	
Quantifying Localization Accuracy in Single-Molecule Super-Resolution Microscopy (# 282)  Hesam Mazidi, Tianben Ding, Arye Nehorai, Matthew D Lew Washington University in St. Louis,	09:50	10:10	
Session: Imaging in Immunology	08:30	10:00	Room 2
Leukocyte mech(n)anobiology: super-resolution microscopy reveals actin nanoscale architecture at cell protrusions (# 387)  Alessandra Cambi , Koen van den Dries Radboud UMC, RIMLS, Nijmegen, NL	08:30	08:45	
Thermoregulation of immune cell dynamics (# 368)  Stefan Wieser , Jaime Ortega Arroyo, Bernard Ciraulo, Ivan Company Garrido, Romain Quidant, Verena Ruprecht ICFO - The Institute of Photonic Sciences, Castelldefels, ES	08:45	09:00	
Lipid Membrane Sensing and Remodeling during the immune synapse (# 391)  Jorge Bernardino de la Serna , Esther A Garcia Gonzalez Imperial College London,	09:00	09:15	
Quantitative analysis of macropinocytosis in Dictyostelium captured with light-sheet microscopy. (# 419)  Josiah Lutton , Daniel Moore, Sharon Collier, Piotr Baniukiewicz, Till Bretschneider University of Warwick,	09:15	09:30	
Single-molecule approach to study mechanical control of B cell antigen recognition (# 398)  Anna Teresa Bajur , Maria Iliopoulou, Kaltelyn Spillane King's College London,	09:30	09:45	
Quantifying binding affinities and kinetics of biomolecular complexes with mass photometry (# 463)  Fabian Soltermann , Weston Struwe, Philipp Kukura Oxford University,	09:45	10:00	
Coffee Break	10:10	10:40	Foyer
Minisymposium: Modern Machine Learning Approaches to Image Analysis Uncertainty Quantification in Microscopy Session Chair: Yoav Shechtman	10:40	11:40	Room 1
Deep learning based Phase Microscopy with Uncertainty Quantification (# 517)  Lei Tian , Yujia Xue, Shiyi Cheng, Yunzhe Li Boston University,	10:40	11:00	
Using machine learning to design intelligent computational microscopes (# 518)  Roarke Horstmeyer , Amey Chaware, Colin Cooke, Kanghyun Kim, Pavan Konda Duke University,	11:00	11:20	
Comprehensive In-situ Profiling of Brain Cellular Alterations in the Injured Brain for Accelerating Drug Discovery (# 412)  Badri Roysam University of Houston,	11:20	11:40	

Session: Modern machine learning approaches to image analysis uncertainty quantification in microscopy Session Chair: Christopher P Calderon	11:40	11:55	Room 1
Leveraging Self-Supervised Denoising for Image Segmentation (# 475)  Mangal Prakash , Tim-Oliver Buchholz, Manan Lalit, Pavel Tomancak, Florian Jug, Alexander Krull MPI-CBG/CSBD,	11:40	11:55	
Session: Software	10:40	12:10	Room 2
3Dscript: animating 3D/4D microscopy data using a natural language- based syntax. (# 522)  Ralph Palmisano Friedrich-Alexander University Erlangen-Nürnberg,	10:40	10:55	
PhenoPlot-Web for visualising microscopy data (# 482)  Andrea Chatrian, Jens Rittscher, Heba Sailem University of Oxford, Oxford, GB	10:55	11:10	
CLIJ: GPU-accelerated real-time image analysis is the key to smart microscopy. (# 439)  Robert Haase , Daniela Vorkel, Akanksha Jain, Nicola Maghelli, Pavel Tomancak, Eugene W. Myers Max Planck Institute for Molecular Cell Biology and Genetics,	11:10	11:25	
Virtual Reality for Quantification of Multidimensional Single-Molecule Data (# 422)  Thomas Blanc , Bassam HAJJ, Mohamed El Beheiry, Jean-Baptiste Masson Institut Curie,	11:25	11:40	
Developing microscope software with Python (# 348)  Mick A Phillips, David Miguel Susano Pinto , Tiago Susano Pinto, Richard M Parton, Chris Weisiger, Andrew Jefferson, John W Sedat, Ilan Davis, Ian M Dobbie University of Oxford,	11:40	11:55	
Metadata and Performance Tracking for Fluorescent Microscopes (# 506)  Mathias Hammer, Alex Rigano, Farzin Farzam, Andrew Shanaj, Colton Horman, Carlas Sierd Smith, Russell Ulbrich, Caterina Strambio de Castilla, David Grunwald, Maximiliaan Huisman UMass Medical School, WORCESTER, US	11:55	12:10	
Lunch (provided)	12:00	13:30	Food Area
QBI Postdoc/Student Chapter: Meet the Expert	12:00	13:30	Food Area
Minisymposium: Spatial Statistics in Microscopy Session Chair: Edward Cohen	13:30	15:00	Room 1
Inferring correlation in inhomogeneous and multitype point patterns – a review and new contributions (# 527)  Rasmus Plenge Waagepetersen Aalborg University,	13:30	14:00	
Bayesian Inference of the Spatial Distribution of Proteins from Three-Cube Förster Resonance Energy Transfer Data (# 586)  Jan-Otto Hooghoudt , Rasmus Plenge Waagepetersen Aalborg University,	14:00	14:20	
Quantitative analysis of molecular coupling with SODA (# 625)  Thibault Lagache , Lydia A Danglot, Jean-Christophe Olivo-Marin Institut Pasteur,	14:20	14:40	
QuantEv: A Quantitative Approach for Analyzing the Spatio-Temporal Distribution of 3D Events in Fluorescence Microscopy (# 526)  Thierry Pecot , Zengzhen Liu, Jerome Boulanger, Jean SALAMERO, Charles Kervrann Medical University of South Carolina,	14:40	15:00	
Session: Applications in BioPharma Session Chair: Sripad Ram	13:30	15:00	Room 2
Machine learning and airy-light-sheet microscopy reveals morphometric indicators in organoids for informed anti-cancer drug discovery. (# 495)  Craig Terence Russell EBI_EMBL,	13:30	13:45	
Multiplexed imaging of lung tissue using a macroscopic light sheet fluorescence microscope with uniform axial resolution (# 376)  Arianna Gentile Polese, Gregory Seedorf, Leonardo Saunders, Dominik Stich, Douglas Parker Shepherd Arizona State University,	13:45	14:00	
Quantification of bone tissue heterogeneity and cell distribution patterns from digital histology: application to osteosarcoma (# 445)  Anthony Mancini , Anne Gomez-Brouchet, Michel quintard, Sylvie Lorthois, Pascal Swider, Pauline Assemat IMFT,	14:00	14:15	
Leveraging Automated Blood Cell Morphology for Myelodysplastic Syndrome Diagnosis and Prognosis Prediction (# 485)  José Guilherme de Almeida , George Vassiliou, Moritz Gerstung European Bioinformatics Institute,	14:15	14:30	















<u>Image-based nanoparticle characterization to aid drug design (# 367)</u> 	14:30	14:45	
<u>Shoh Michael Asano</u> , Katherine J. Hales <i>Pfizer,</i>			
<u>Automating FLImP – macromolecular structure fingerprints for personalised cancer therapy (# 339)</u> 	14:45	15:00	
<u>Daniel James Rolfe</u> , <i>Rutherford Appleton Laboratory,</i>			
Coffee Break	15:00	16:30	Foyer
Poster Session	15:00	16:30	Poster Area
Marketing Presentation: AbbeLight	16:00	16:15	Room 1
Session: Spatial Statistics and Clustering	16:30	18:30	Room 1
<u>Functional summary statistics for Poisson processes on bounded convex three dimensional shapes (# 516)</u> 	16:30	16:45	
<u>Scott Ward</u> , Edward Cohen, Niall Adams <i>Imperial College London, London, GB</i>			
<u>Generalized Statistical Object Distance Analysis (GSODA) For Object Based Colocalization In Quantitative Microscopy (# 486)</u> 	16:45	17:00	
<u>Suvadip Mukherjee</u> , Thibault Lagache, Daniel Gonzalez-Obando, Vannary Meas-Yedid, Lydia A Danglot, Jean-Christophe Olivo-Marin <i>Institut Pasteur,</i>			
<u>Cluster Analysis of Localization Microscopy Data with Machine Learning (# 461)</u> 	17:00	17:15	
<u>David Williamson</u> , Garth Burn, Sabrina Simoncelli, Ruby Peters, Juliette Griffie, Daniel Davis, Dylan Owen <i>King's College London,</i>			
<u>FOCAL3D: A 3D clustering algorithm for single-molecule localization microscopy (# 371)</u> 	17:15	17:30	
<u>Daniel Felipe Nino</u> , Josh Milstein <i>University of Toronto, Mississauga, CA</i>			
<u>Toward Absolute Molecular Numbers in DNA-PAINT (# 313)</u> 	17:30	17:45	
<u>Johannes Stein</u> , Florian Stehr, Patrick Schueler, Philipp Blumhardt, Florian Schueder, Jonas Muecksch, Ralf Jungmann, Petra Schwille <i>Max-Planck-Institute of Biochemistry,</i>			
<u>Molecular counting with calibrated fluorescent labelling and photobleaching step analysis (# 493)</u> 	17:45	18:00	
<u>Klaus Yserentant</u> , Johan Hummert, Felix Braun, Wioleta Chmielewicz, Dirk-Peter Herten <i>Heidelberg University, Heidelberg, DE</i>			
<u>Stoichiometric quantification of spatially dense assemblies with qPAINT (# 317)</u> 	18:00	18:15	
<u>Daniel J Nieves</u> , <i>University of Birmingham,</i>			
<u>Statistical modeling of spatial interactions in biological patterns, with application to plant nuclear organization (# 489)</u> 	18:15	18:30	
Javier Arpon, Kaori Sakai, Valérie Gaudin, <u>Philippe Andrey</u> , <i>INRA, Versailles, FR</i>			
Session: 3D methods	16:30	18:30	Room 2
<u>3D model-based restoration methods for 3D-SIM (# 320)</u> 	16:30	16:45	
Cong Tuan Son Van, <u>Chrysanthe Preza</u> , Hasti Shabani <i>The University of Memphis,</i>			
<u>4Pi-SMLM and the 3D architecture of the Nuclear Pore Complex (# 384)</u> 	16:45	17:00	
<u>Mark Bates</u> , <i>Max Planck Institute for Biophysical Chemistry, Goettingen, DE</i>			
<u>Off-focus imaging exploits supercritical-angle fluorescence emission for precise SMLM (# 397)</u> 	17:00	17:15	
<u>Alexander Jesacher</u> , Philipp Zelger, Lisa Bodner, Lukas Velas, Gerhard Schütz <i>Medical University of Innsbruck,</i>			
<u>Analysis of relative positions in 3D PALM and dSTORM provides high-resolution information on ordered biological complexes (# 441)</u> 	17:15	17:30	
<u>Alistair Curd</u> , Ruth Hughes, Michelle Peckham <i>University of Leeds,</i>			
<u>Selective volumetric microcopy for single molecule imaging in living cells (# 421)</u> 	17:30	17:45	
Tommaso Galgani, Remi Galland, Virgile Viasnoff, Jean-Baptiste Sibarita, <u>Bassam HAJJ</u> , <i>Institut Curie - CNRS, Paris, FR</i>			
<u>3D Multicolor Nanoscopy at 10,000 Cells a Day (# 299)</u> 	17:45	18:00	
<u>Andrew E S Barentine</u> , Yu Lin, Miao Liu, Phylicia Kidd, Leonhard Balduf, Michael Grace, Siyuan Wang, Joerg Bewersdorf, David Baddeley <i>Yale University,</i>			

















<u>High-throughput multicolor 3D localization in live cells by depth-encoding imaging flow cytometry.</u> (# 335) 	18:00	18:15	
<u>Lucien Weiss</u> , Yael Shalev Ezra, Sarah Goldberg, Boris Ferdman, Onit Alalouf, Yoav Shechtman <i>Technion, Israel Institute of Technology, Haifa, IL</i>			
<u>3D-SRRF: Real-Time Live-Cell-Compatible Volumetric Super-Resolution Microscopy</u> (# 426) 	18:15	18:30	
<u>Romain F Laine</u> , Tommaso Galgani, Bassam HAJJ, Ricardo Henriques <i>UCL,</i>			
Dinner Break (on own)	18:30	20:30	(on own)
Workshop: Introduction to programming for microscopy image analysis Session Chair: Anish V. Abraham	18:30	21:30	Room 1
QBI Society Meeting	20:30	22:00	Foyer
















QBI 2020 Conference Sessions

6 Jan - 9 Jan

Thursday January 9

Registration Desk Opens	08:00	14:00	Registration Desk/Area
Keynote Lecture: Wolfgang Baumeister Session Chair: Alessandra Cambi	08:30	09:10	Room 1
Structural biology in situ: The promise and challenges of cryo-electron tomography_(# 617)  Wolfgang Baumeister Max Planck Institute of Biochemistry, , DE	08:30	09:10	
Tutorial: Quantitative Phase Imaging Session Chair: Jörg Enderlein	09:10	09:40	Room 1
Quantitative Phase Imaging_(# 396)  Alexander Jesacher Medical University of Innsbruck,	09:10	09:40	
Coffee Break	09:40	10:10	Foyer
Minisymposium: Correlative Microscopy	10:10	11:10	Room 1
Imaging the nanoscale structure of endocytosis with correlative super-resolution light and electron microscopy_(# 577)  Justin W Taraska ,Kem Sochacki National Institutes of Health,	10:10	10:30	
Live-cell correlative microscopy of single organelles_(# 590)  Nalan Liv University Medical Center Utrecht,,	10:30	10:50	
Towards quantitative correlative microscopy_(# 528)  Lucy M Collinson Francis Crick Institute,	10:30	10:50	
Session: Correlative/multimodal microscopy Session Chair: Alessandra Cambi	11:10	12:10	Room 1
Multimodal and multicolour microscopy to identify biomolecules in large-scale EM_(# 310)  Pascal de Boer ,Aditi Srinivasa Raja, Jacob Hoogenboom, Ben Giepmans University Medical Center Groningen,	11:10	11:25	
Quantitative 3D super-resolution imaging of chromatin 'blobs'_(# 420)  Lothar Schermelleh University of Oxford,	11:25	11:40	
Cryo-CARE Facilitates Sub-Tomogram Averaging in Cryo Transmission Electron Microscopy_(# 453)  Tim-Oliver Buchholz ,Mareike Jordan, Gaia Pigino, Florian Jug MPI-CBG/CSBD,	11:40	11:55	
Towards correlative multimodal nanospectroscopy_(# 329)  Iztok Urbancic ,Falk Schneider, Erdinc Sezgin, Silvia Galiani, Christian Eggeling Jozef Stefan Institute,	11:55	12:10	
Session: Tracking Session Chair: Sean Andersson	10:10	12:10	Room 2
Novel particle tracking approach with CNN-based candidate selection and two-step linking by Bayesian network_(# 503)  Mariia Dmitrieva ,Jens Rittscher University of Oxford,	10:10	10:25	
Precise mapping of intracellular diffusion and drift from SPT data analysis_(# 409)  Antoine Salomon ,Charles Kervrann Inria Rennes, Rennes, FR	10:25	10:40	
Probabilistic overall reconstruction of membrane-associated molecular dynamics from partial observations in rod-shaped bacteria_(# 390)  Yunjiao LU ,Charles Kervrann, Alain Trubuil, Pierre Hodara INRA, Jouy-en-Josas, FR	10:40	10:55	
A 2-step algorithm for the estimation of time-varying Single Particle Tracking models_(# 381)  Boris I Godoy ,Ye Lin, Sean Andersson Boston University, Boston, US	10:55	11:10	
Single-Particle Diffusion Characterization by Deep Learning_(# 359)  Yoav Shechtman ,Naor Granik, Yael Roichman, Lucien Weiss, Elias E Nehme, Eran Perlson Technion, Israel Institute of Technology, Haifa, IL	11:10	11:25	

<u>Faster FLImP – Towards High throughput image acquisition and track selection (# 341)</u>  <u>Ben Davis</u> , Michail Vrettas, Michael Hirsch, Sarah Needham, Marisa Martin-Fernandez, Daniel James Rolfe STFC,	11:25	11:40	
<u>Quantitative Comparison of Single Particle Tracking Algorithms Across Different Signal and Noise Levels (# 275)</u>  Ye Lin, <u>Sean B. Andersson</u> Boston University,	11:40	11:55	
<u>Effect of pixelation on the parameter estimation of single molecule trajectories (# 569)</u>  Milad Rafiee Vahid, Bernard Hanzon, <u>Raimund J Ober</u> Texas A&M University, College Station, US	11:55	12:10	
Lunch (provided)	12:10	13:30	Food Area
Minisymposium: Tissue Multiplexing Techniques and Applications in Oncology I Session Chair: Jens Rittscher	13:30	15:30	Room 1
<u>Introduction to the minisymposium on tissue multiplexing – is more the merrier or messier? (# 591)</u>  <u>Sripad Ram</u> Pfizer, Inc., , United States	13:30	13:40	
<u>Multiplex immunohistochemistry and transcriptomic data (# 593)</u>  <u>Robert West</u> Stanford University Medical Center,	13:40	14:00	
<u>Implementation of multiplexing and image analysis to support drug discovery and development (# 571)</u>  <u>Shanon Seger</u> Roche,	14:00	14:20	
<u>Deep Learning approaches for reliable quantification of multi-omics cell imaging datasets to interrogate RNA and protein spatial and temporal subcellular interactions (# 291)</u>  <u>Emmanuel Bouilhol</u> , Macha Nikolski, Benjamin Dartigues, Hayssam Soueidan, Robyn Brackin, Anca savulescu, Musa Mhlanga CNRS, Merignac, FR	14:20	14:35	
<u>Understanding and Modeling Inflammation Induced Immuno-Suppression in Tumors Using Multiplex Immunofluorescence and Live Cell Microscopy (# 336)</u>  <u>Stephen Lockett</u> , Anne Gilmore, William Heinz, David Scheiblin, Sarah Flaherty, Noemi Kedei, Veena Somasundaram, Lisa Ridnour, Adelaide Wink, Caleb Kim, Valentin Magidson, Erina Kamiya, Jinqiu Chen, David Wink National Cancer Institute,	14:35	14:55	
<u>Highly multiplexed imaging in health and disease by imaging mass cytometry (# 607)</u>  <u>Bernd Bodenmiller</u> University of Zurich,	14:55	15:15	
<u>Multimodal mass spectrometry imaging of tumours (# 550)</u>  <u>Josephine Bunch</u> National Physical Laboratory,	15:15	15:30	
Session: Methods for Single Molecule Analysis Session Chair: Keith A Lidke	13:30	15:30	Room 2
<u>Single Molecule Localization Microscopy using time modulated illumination (# 481)</u>  <u>Pierre Jouchet</u> , Clement Gabriel, Nicolas Bourg, Marion Bardou, Christian Poüs, Emmanuel Fort, Sandrine Leveque-Fort CNRS, Orsay Cedex, FR	13:30	13:45	
<u>Identifying analysis algorithm induced image artefacts in localisation microscopy (# 469)</u>  <u>Richard John Marsh</u> , Ishan Costello, Susan Cox King's College London, London, GB	13:45	14:00	
	14:00	14:15	
<u>SIMPLE: Structured illumination based point localization estimator with enhanced precision (# 386)</u>  <u>Loïc Reymond</u> , Stefan Wieser, Verena Ruprecht ICFO - The Institute of Photonic Sciences,	14:15	14:30	
<u>Spatiotemporal Resolution as an Information Theoretical Property of Stochastic Optical Localization Nanoscopy (# 369)</u>  <u>Yi Sun</u> The City College of City University of New York, New York, US	14:30	14:45	
<u>Haste makes waste – Slow imaging increases resolution and labeling efficiencies in SMLM (# 322)</u>  <u>Robin Diekmann</u> , Maurice Kahnwald, Jonas Ries EMBL,	14:45	15:00	
<u>Towards Bayesian based smart microscopy (# 283)</u>  <u>Juliette Griffie</u> , Paul Rolland, Daniel Sage, Robin Lang, Volkan Cevher, Suliana Manley EPFL, Lausanne, CH	15:00	15:15	

<u>Single molecule localisation lightfield microscopy_(# 290)</u>  <u>Leila A Muresan</u> <i>University of Cambridge, Cambridge, GB</i>	15:15	15:30	
Coffee Break	15:30	16:00	Foyer
Minisymposium: Tissue Multiplexing Techniques and Applications in Oncology II Session Chair: Sripad Ram	16:00	16:55	Room 1
<u>Multiplex Immune Cell Profiling to Gain Insights into Progression in Early Cancer (# 484)</u>  Jens Rittscher, <u>Natalia Garcia Martin</u> <i>University of Oxford,</i>	16:00	16:15	
<u>Towards computational multiplex fluorescence microscopy: Machine learning-based integrated prediction of morphological and molecular tumor profiles (# 627)</u>  <u>Frederick Klauschen</u> <i>Charité University Hospital Berlin,</i>	16:15	16:35	
<u>Multiplex imaging of lung tumours with CODEX technology_(# 584)</u>  <u>Marta Trüb</u> ,Petra Herzig, Nicole Kirchhammer, Franziska Werner, Kirsten Mertz, Viktor Kölzer, Abhishek Kashyap, Alfred Zippelius <i>University of Basel / University Hospital Basel,</i>	16:35	16:55	
Session: Probe Development Session Chair: Daniel Wuestner	17:00	17:45	Room 1
<u>Mechanical investigations of mEos4b blinking: longer single particle tracking with less track interruptions_(# 311)</u>  Siewert Hugelier,Elke De Zitter, Daniel Thédié, Viola Monkemöller, Joel Beaudouin, Virgile Adam, Martin Byrdin, Luc Vanmeervelt, Dominique Bourgeois, Peter Dedecker <i>KU Leuven, Heverlee, BE</i>	17:00	17:15	
<u>Separation of cell signalling responses based on a new model for photoswitching in FRET biosensors (# 380)</u>  <u>Thijs Roebroek</u> <i>KULeuven,</i>	17:15	17:30	
<u>Innovative buffer for long-lived fluorescence imaging for 2D and 3D dSTORM (# 357)</u>  Angelina Provost, corentin Rousset, Camille Fourneaux, Philippe Bouvet, Jean-Jacques Diaz, Catherine Ladavière, marie-therese charreyre, Arnaud Favier, Christophe Place, <u>Karine MONIER</u> <i>CRCL,</i>	17:30	17:45	
Session: Point spread function analysis Session Chair: Yoav Shechtman	16:00	17:15	Room 2
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<u>Simultaneous multicolor single-molecule imaging using discrete PSF engineering_(# 379)</u>  <u>Wim Vandenberg</u> ,Marcel Leutenegger, Fabian Hertel, Bartosz Krajnik, Arno Bouwens, Robin Van den Eynde, Ezra Bruggeman, Thomas Schlichthärle, Alexander Auer, Ralf Jungmann, Peter Dedecker <i>KU Leuven, Leuven, BE</i>	16:15	16:30	
<u>Measurement of the depth-dependence of point spread functions near the glass-water interface in high-numerical aperture microscopy_(# 373)</u>  <u>Petar N Petrov</u> ,William E. Moerner <i>Stanford University, Stanford, US</i>	16:30	16:45	
<u>A General Framework for Fitting an Arbitrary Model to SMLM Data (# 312)</u>  Yu-Le Wu, Markus Mund, Philipp Hoess, Aline Tschanz, <u>Jonas Ries</u> <i>EMBL, Heidelberg, DE</i>	16:45	17:00	
<u>ZOLA-3D: a software for optimal 3D SMLM with arbitrary PSF (# 524)</u>  <u>Benoit Lelandais</u> ,MICKAEL LELEK, Jonas Ries, Christophe Zimmer <i>Institut Pasteur, paris, FR</i>	17:00	17:15	
Session: Machine Learning Session Chair: Christopher P Calderon	17:15	18:00	Room 2
<u>DeepFocus: a Near One-Shot Micro-Manager Auto-focus Plugin using Convolutional Neural Networks (# 388)</u>  <u>Adrian Shajkofci</u> ,Michael Liebling <i>Idiap Research Institute, Martigny, CH</i>	17:15	17:30	
<u>Probabilistic Noise2Void: Unsupervised Content-Aware Denoising_(# 473)</u>  <u>Alexander Krull</u> ,Tomas Vicar, Mangal Prakash, Manan Lalit, Florian Jug, Pavel Tomancak <i>MPI-CBG/CSBD,</i>	17:30	17:45	
<u>Bootstrapping Unsupervised Image Denoising with Parametric Noise Models (# 476)</u>  <u>Mangal Prakash</u> ,Manan Lalit, Pavel Tomancak, Alexander Krull, Florian Jug <i>MPI-CBG/CSBD,</i>	17:45	18:00	
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QBI 2020 Conference

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324	An image-based machine learning approach as screening tool of pathological calcium oxalate crystallization inhibitors Anna Kletzmayer , Jean-Christophe Leroux, Mattias E. Ivarsson	27	Tue, Jan 07, 15:00
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331	Organization and dynamics of Escherichia coli outer membrane proteins Patrick George Inns , Nicholas Housden, Joanna Szczepaniak, Renata Kaminska, Colin Kleanthous	51	Tue, Jan 07, 15:00
337	Automated lens-free, fluorescent microscopy to study cell populations Dorothee Kraemer , Cédric Allier, Lionel Hervé, Kiran Padmanabhan, Adlen Foudi, Sophie Morales	5	Tue, Jan 07, 15:00
342	Multi-parameter Single Molecule Spectroscopy Imaging Reveals Intra- and Inter- Molecular Conformations of HIV-1 Envelope on Native Viruses Irene Carlon-Andres , Sergi Padilla-Parra	33	Tue, Jan 07, 15:00
343	Towards multi-dimensional FLImP Michail Vrettas, Daniel James Rolfe, Ben Davis	57	Tue, Jan 07, 15:00
344	Breaking New Ground in Live and Label-free Volumetric Imaging Niall Hanrahan, Simon Lane, Raquel Munoz, Sumeet Mahajan		Sat, Feb 29, 15:10
345	User-friendly Building of Reconstruction Algorithms with GlobalBiolm Laurène Donati , Emmanuel Soubies, Michael Unser	20	Wed, Jan 08, 15:00
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355	High density 3D localization of fluorescent molecules Valentin Debarnot , Thomas Mangeat, Pierre Weiss	52	Wed, Jan 08, 15:00
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363	High-Throughput Structured Illumination Microscopy (HiT-SIM) Robin Van den Eynde	56	Wed, Jan 08, 15:00
364	Quantitative comparison of camera technologies for cost-effective super-resolution optical fluctuation imaging (SOFI) Robin Van den Eynde	2	Wed, Jan 08, 15:00
365	Quantitative image-analysis of organoids with high-throughput digital holography microscopy Thanh-an Pham , Nathalie Brandenberg, Sylke Hoehnel, Benjamin Rappaz, Michael Unser, Matthias Lütolf, Daniel Sage	69	Tue, Jan 07, 15:00

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372	An LPV approach to the estimation of time-varying Single Particle Tracking models Boris I Godoy , Nicholas Vickers, Sean Andersson	72	Wed, Jan 08, 15:00
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378	Axial stimulated emission depletion spectroscopy to study lipid dynamics Aurélien Barbotin , Jztok Urbancic, Martin Booth, Christian Eggeling, Erdinc Sezgin	47	Tue, Jan 07, 15:00
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400	Edge correction in density estimation from repeated point patterns in biological imaging Jasmine Burguet , Philippe Andrey	24	Wed, Jan 08, 15:00
404	Quantitative Analysis of Spatial-Temporal Imaging of Auxin to Cytokinin Ratio During Nodule Development in Soybean (Glycine max) by Two-Photon Induced Fluorescence Ni Putu Dewi Nurmalasari , Paul Gaillard, Senthil Subramanian, Steve Smith	45	Tue, Jan 07, 15:00
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415	4Pi aberration correction in single molecule switching microscopy Jingyu Wang , Martin Booth, Ian M Dobbie, Jacopo Antonello, Mick A Phillips, Richard M Parton, Chao He	7	Tue, Jan 07, 15:00
416	A universal framework for sensorless adaptive optics Qi Hu , Jingyu Wang, Matthew Wincott, Jacopo Antonello, Martin Booth	8	Wed, Jan 08, 15:00
417	Implementation of Confocal Adaptive Optical Microscopy for Clinical Tissue Analysis Jiahe Cui , Raphael Turcotte, Karen M Hampson, Martin Booth	.	Tue, Jan 07, 15:00
424	Deciphering genuine transient pauses in super-localized trajectories of single-molecule tracers Jaladhar Mahato , Arindam Chowdhury	26	Wed, Jan 08, 15:00
427	Calibration of a Remote Focusing Microscope Using Deflectometry Syed Asad Hussain , Karen M Hampson, Matthew Wincott, Jacopo Antonello, Martin Booth	62	Wed, Jan 08, 15:00
430	Quantification of DNA by Mass Photometry Yiwen Li , Katharina Haeussermann, Weston Struwe, Philipp Kukura	39	Tue, Jan 07, 15:00
431	Site-selective visualization and tracking of DNA methylation in fixed cells Krstian Ubych , Paco Fernandez-Trillo, Clare Davies, Robert Neely	41	Tue, Jan 07, 15:00
433	Ultrashort dual colour laser at 1.55 and 1.88 μm for the detection of tumour through Raman imaging Syed Asad Hussain	64	Wed, Jan 08, 15:00
435	ConCoR: A software pipeline for high-throughput image analysis of time-dependent colocalization data Greg Bass , Isabelle Glauser, Jenny Chia, Anne Verhagen, Steve Dower, Milica Ng	9	Tue, Jan 07, 15:00
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455	3D Model Building Pipeline for Light-Sheet Imaging of Hippocampal Neurons for Simulation and Analysis of Calcium Dynamics <u>James Barnard Wilsenach</u> , Charlotte M. Deane, Gesine Reinert	63	Tue, Jan 07, 15:00
457	Extending molecular counting by photon statistics <u>Johan Hummert</u> , Klaus Yserentant, Wioleta Chmielewicz, Dirk-Peter Herten	34	Wed, Jan 08, 15:00
458	Cell segmentation and tracking for mathematical modelling of cell motility <u>Rituparna Sarkar</u> , Pascal Bochet, Maria Manich, Aleix Boquet-Pujadas, Nancy Gullien, Elisabeth Labruyere, Jean-Christophe Olivo-Marin	65	Tue, Jan 07, 15:00
462	Investigating membrane proteins interactions and membrane mimetic systems using mass photometry <u>Anna Olerinyova</u> , Philipp Kukura, Weston Struwe, Adar Sonn Segev	43	Tue, Jan 07, 15:00
465	Adaptive optics in bioimaging for improved quantitative exploitation of microscopy images <u>Audrius Jasaitis</u> , Cynthia Veilly, Fabrice Harms, Xavier Levecq	32	Wed, Jan 08, 15:00
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480	Single-molecule and super-resolution investigation of chemokine receptor behaviour within the T cell immunological synapse <u>James Felce</u> , Michael Loran Dustin	13	Tue, Jan 07, 15:00
483	ICYTOMINE: A User-Friendly Tool for Reproducible Analysis of Gigapixel Images Daniel Felipe González Obando, Diana Mandache, Jean-Christophe Olivo-Marin, <u>Vannary Meas-Yedid</u>	36	Wed, Jan 08, 15:00
488	Full field interferometry for characterizing and counting biotic nanoparticles <u>Samer Alhaddad</u> , Yasmina Fedala, Ignacio Izeddin, Claude Boccara, Martine Boccara	28	Wed, Jan 08, 15:00
490	Quantitative mapping of fluorescently tagged ribosomes throughout the cell cycle of S. cerevisiae using FCS-calibrated imaging <u>Madlen Luckner</u> , Gabriele Schreiber, Edda Klipp, Salvatore Chiantia	55	Tue, Jan 07, 15:00
491	Versatile uniform illumination method toward quantitative single molecule localization microscopy <u>Adrien - Mau</u> , Nicolas Bourg, Sandrine Leveque-Fort	10	Wed, Jan 08, 15:00
499	Computational analysis of filament polymerization dynamics in cytoskeletal networks <u>Paulo Caldas</u> , Christoph Sommer, Philipp Radler, Martin Loose	14	Wed, Jan 08, 15:00
507	Metadata and Performance Tracking for Fluorescent Microscopes III - MetaMax <u>Maximiliaan Huisman</u> , Carlos Sierd Smith, Mathias Hammer, Russell Ulbrich, David Grunwald	42	Wed, Jan 08, 15:00
508	Metadata and Performance Tracking for Fluorescent Microscopes I - Metadata <u>David Grunwald</u> , Mathias Hammer, Alex Rigano, Maximiliaan Huisman, Farzin Farzam	44	Wed, Jan 08, 15:00
509	Metadata and Performance Tracking for Fluorescent Microscopes II - Optics <u>David Grunwald</u> , Mathias Hammer, Maximiliaan Huisman, Farzin Farzam	40	Wed, Jan 08, 15:00
512	Towards Quantitative 3D Super-Resolution Correlative Light-Electron Microscopy <u>Jonas Euchner</u> , Klaus Yserentant, Dirk-Peter Herten	46	Wed, Jan 08, 15:00
515	Pixelwise H-score: a novel digital image analysis-based metric to quantify membrane biomarker expression from IHC images <u>Sripad Ram</u>	29	Tue, Jan 07, 15:00

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533	Predicting Patient Outcome from Immunohistochemistry Images by Quantifying Spatial Distribution of Immune Cells Morgan Oneka , Jenny Lazarus, Souptik Barua, Arvind Rao, Timothy Frankel	67	Tue, Jan 07, 15:00
606	Persistent Homology for the Spatial Characterisation of Tumour Blood Vessel Networks Bernadette Jana Stolz	74	Wed, Jan 08, 15:00
608	Quantifying the heterogeneity of macromolecular machines by mass photometry Adar Sonn Segev , Katarina Belacic, Tatyana Bodrug, Gavin T Young, Nicholas G. Brown, David Haselbach, Philipp Kukura	75	Tue, Jan 07, 15:00
609	Dissecting the Single-Cell Dynamics of Xist RNA in X Chromosome Inactivation by 3D Structured Illumination Microscopy Lisa Selina Rodermund	77	Tue, Jan 07, 15:00
611	Leveraging Molecular and Cellular Fiducial Markers for Correlative Bio-Imaging Chidinma Adanna Okolo , Ilias Kounatidis, Thomas M Fish, Perrine Paul-Gilloteaux, Ian M Dobbie, Maria Harkiolaki	78	Wed, Jan 08, 15:00
614	Cryo 3D Correlation imaging for studies of host-pathogen interactions; the case of Staphylococcus aureus clearance by primary macrophages Ilias Kounatidis , Maria Harkiolaki, Petros Ligoxygakis	79	Tue, Jan 07, 15:00
615	Combining spatial statistics to describe localisation of tumour associated macrophages in digital pathology Joshua A Bull	80	Wed, Jan 08, 15:00
616	Morphological analysis of images of dried droplets of saliva for determination the degree of endogenous intoxication Peter (Petr) V. Lebedev-Stepanov , Marina Buzoverya, Konstantin Vlasov, Yulia Potekhina	81	Tue, Jan 07, 15:00

Glycine receptor copy numbers and packing density at spinal cord synapses revealed by quantitative dual-colour super-CLEM

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The strength of synaptic transmission depends on the number and distribution of neurotransmitter receptors at synapses [1]. At inhibitory synapses in the spinal cord, direct interactions between glycine receptors (GlyRs) and the synaptic scaffold protein gephyrin regulate receptor diffusion and trapping [2]. To quantitatively characterise the sub-synaptic distribution of GlyRs in the spinal cord, we implemented single molecule counting in knock-in animals expressing endogenous mEos4-tagged GlyRs. Dual-colour super-resolution images of GlyRs and gephyrin are correlated with electron microscopic images (super-CLEM) to relate copy numbers to structural features of identified synapses. Serial sectioning further reveals the 3D orientation of the synapse as well as the exact size of the postsynaptic domain, from which we can derive absolute receptor densities at glycinergic synapses in different neuronal populations [3]. Our data suggest that the receptors are clustered at a density of about 1000 GlyR complexes/ μm^2 in the dorsal horn and 1500/ μm^2 in the ventral horn, pointing to striking differences in the occupancy of receptor binding sites. Ongoing analyses address the open question whether GlyRs are heterogeneously distributed within the synaptic domain [4].

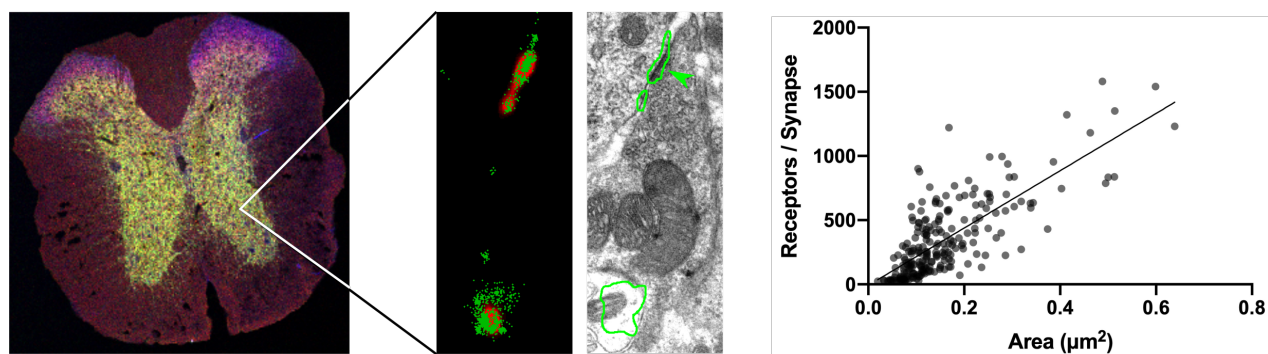


Fig. Left: endogenous mEos4-tagged GlyRs (green) in spinal cord tissue. Middle: Dual-colour super-resolution imaging of synaptic GlyRs (green, arrowhead) and gephyrin (red) overlaid with an electron micrograph. Image size: 1 x 2.5 μm . Right: Absolute GlyR densities at synapses.

- [1] Nusser et al. (1997). Differences in synaptic GABA(A) receptor number underlie variation in GABA mini amplitude. *Neuron* 19:697-709.
- [2] Choquet & Triller (2013). The dynamic synapse. *Neuron* 80:691-703.
- [3] unpublished data
- [4] Yang & Specht (2019). Subsynaptic domains in super-resolution microscopy: The treachery of images. *Front Mol Neurosci* 12:161. doi: 10.3389/fnmol.2019.00161

Isotropic 3D molecular resolution in cells with MINFLUX nanoscopy

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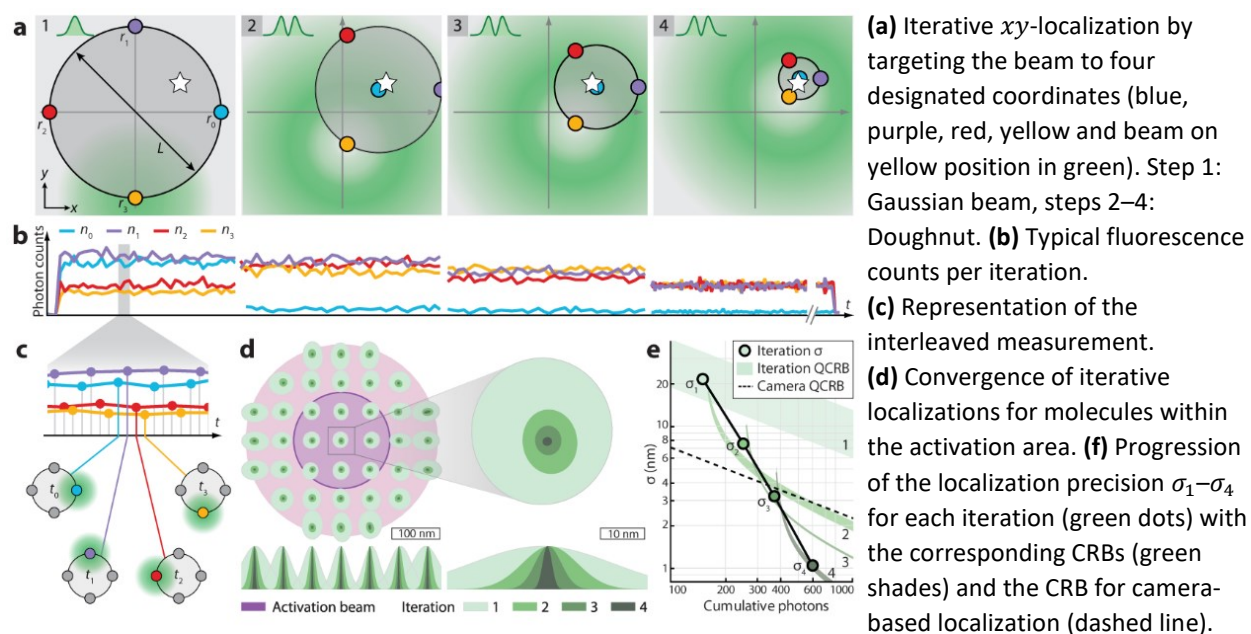
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Superresolution microscopy methods such as STED and PALM/STORM have revolutionized far-field optical fluorescence microscopy by manipulating state transitions of the emitters, offering potentially unlimited resolution. In practice, however, the resolution of an image is limited by the finite photon budget of fluorescent probes, while their finite emission rate imposes a spatial-temporal trade-off in tracking applications. By synergistically combining the strengths of both superresolution families, the recent MINFLUX concept [1,2] tackles these limitations by rendering each emitted photon more informative.

Here, we present a MINFLUX strategy with high photon efficiency in arbitrarily large regions that allows imaging in fixed and living cells [3]. This is accomplished by iteratively approaching each photo-activated emitter with a set of MINFLUX localizations, while gradually shrinking the probed region size. This allows isotropic localization precision and surpasses the typical $\propto N^{-1/2}$ dependence, as photons are made increasingly informative as they are acquired. Additionally, this MINFLUX embodiment operates in three dimensions and for multi-color recordings.



- [1] F. Balzarotti *et al.*, “Nanometer resolution imaging and tracking of fluorescent molecules with minimal photon fluxes,” *Science*, vol. 355, no. 6325, pp. 606–612, 2017.
- [2] Eilers, H. Ta *et al.*, “MINFLUX monitors rapid molecular jumps with superior spatiotemporal resolution.,” *Proc. Natl. Acad. Sci. U.S.A.*, p. 201801672, 2018
- [3] K. C. Gwosch, J. K. Pape, F. Balzarotti *et al.*, “MINFLUX nanoscopy delivers multicolor nanometer 3D-resolution in (living) cells,” *bioRxiv*, p. 734251, 2019.

Quantitative Comparison of Single Particle Tracking Algorithms Across Different Signal and Noise Levels

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KEYWORDS: single particle tracking, localization, parameter estimation

Single Particle Tracking (SPT) is a powerful class of tools for acquiring information about the dynamics of biological macromolecules moving inside living cells. In this work, we are interested in comparing algorithms with respect to the performance of localization and motion

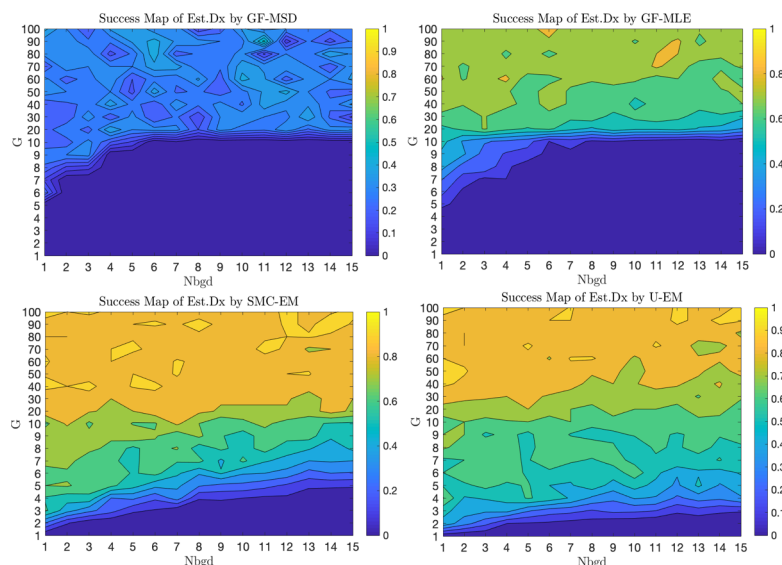


Fig. 1: Success map of estimated diffusion coefficient by GF-MSD, GF-MLE, SMC-EM and U-EM, respectively.

model estimation at very low signal levels.

A standard approach for estimation is to use Gaussian Fitting (GF) to determine the position of an emitter in an image, followed by linking to produce a trajectory. These trajectories are then analyzed by using Mean Square Displacement (MSD) or Maximum Likelihood (ML) approach to estimate motion parameters, such as diffusion coefficients. However, both MSD and ML assume a linear measurement model with simple additive Gaussian

noise, which begins to fail as image intensity decreases. We have introduced two nonlinear methods that more accurately model camera-based measurements: Sequential Monte Carlo combined with Expectation Maximization (SMC-EM) [1], and Unscented Kalman Filter combined with Expectation Maximization (U-EM) [2]. To compare the performance of SMC-EM and U-EM with the standard approach in localization and parameter estimation at low signal levels, we simulated SPT experiments of a single particle diffusing in two dimensions. Images were generated from a basic camera model together with shot noise. The results of estimated diffusion coefficient in x direction using these four schemes are shown in Fig.1. In these figures, *successive* is defined as an estimate within 25% of its true value [3].

The results indicate that across different signal and noise level settings (especially with low signal levels), both SMC-EM and U-EM outperform GF-MSD and GF-MLE in localization and parameter estimation. It is important to note that U-EM is significantly cheaper in computation than SMC-EM. In future work, we would like to take into consideration time-varying motion model and other observation models with camera-specific noise.

[1] T. T. Ashley and S. B. Andersson. "Method for simultaneous localization and parameter estimation in particle tracking experiments." *Physical review. E, Statistical, nonlinear, and soft matter physics* 92 5 (2015): 052707.

[2] Y. Lin and S. B. Andersson. "Simultaneous localization and parameter estimation for single particle tracking via Sigma Points based EM." *IEEE 58th Conference on Decision and Control (CDC)* (2019).

[3] X. Michalet. "Mean square displacement analysis of single-particle trajectories with localization error: Brownian motion in an isotropic medium." *Physical Review E* 82.4 (2010): 041914.

Quantifying the Robustness of Topological Defects in Orientation Fields from Bio-Images

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Topological defects, also known as singular points, are distinguished points in orientation fields that attract increasing interest in the analysis of bio-medical images and processes. The orientations of elongated objects, such as filaments or elongated cells, are crucial for structure and function on various length scales. The topological defects present in such orientation fields have been linked to physical properties like shape curvature [1], cell extrusion and death [2], and the level of active stresses generated by motor proteins [3].

However, due to their discrete nature, topological defects *cannot* depend continuously on the orientations in a numerical representation of an image. Therefore, small fluctuations in an imaged orientation field, e.g. due to imaging noise or imperfect orientation estimation, can alter the location of topological defects or cause a pair of defects to be annihilated or to be created. It is thus important to know the robustness of defects detected in an image against

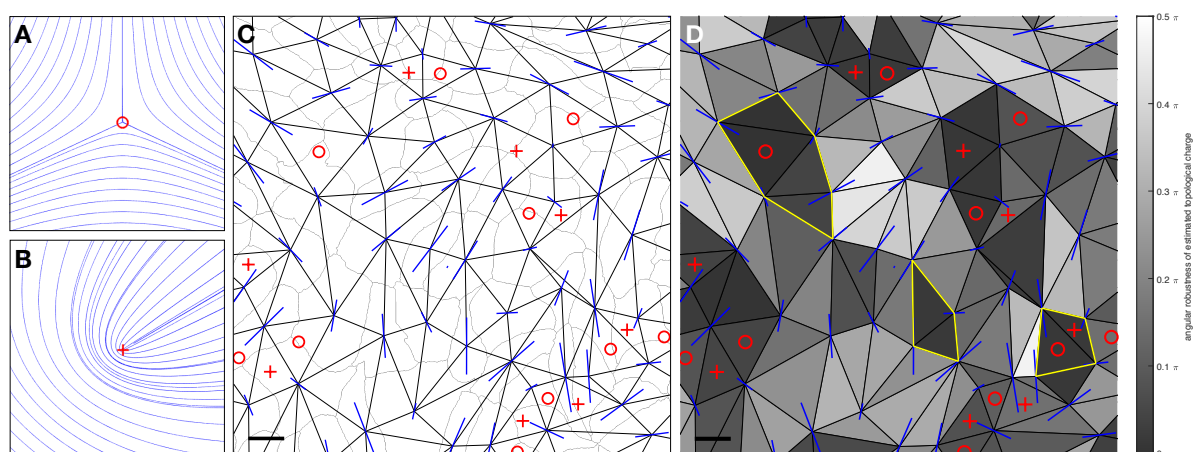


Figure 1. **A,B.** Prototypical defect patterns of topological charge $-1/2$ (**A**, "o") and $+1/2$ (**B**, "+"). **C.** Topological defects identified in the irregular orientation field formed by elongations of MDCK cells in a tissue (raw image: Chen-Ho Wang, MPI-CBG Dresden). Scale bar is $20\mu\text{m}$. Gray lines show segmented cell outlines, blue bars indicate cell elongation, and black lines provide a Delaunay triangulation of cell centers. **D.** Triangulation colored by defect robustness (dark: low robustness, bright: high robustness). Low robustness areas enclosed by yellow borders predict high chance for defect pair annihilation (right area), generation (middle area), or relocation (left area).

Here, we provide such a robustness measure. It quantifies the amount of orientation change that is maximally tolerable for a given defect to persist in its kind and position. Our robustness measure is easily calculated alongside defect identification on the finest scale of resolution. It can also be computed at non-defect points, where it indicates the chance of defect pair generation, and in arbitrary areas indicating the robustness of the total topological charge within that area (see Figure 1).

This complements defect identification with rigorous uncertainty quantification, allows anticipating defect dynamics from a single static image, and adds prior knowledge to subsequent analysis steps like defect tracking, making topological defects an even more valuable observable for biophysical tissue dynamics.

References

- [1] Ellis, Pearce, Chang, *et. al.* Nature Physics (2018): Curvature-induced defect unbinding and dynamics in active nematic toroids
- [2] Saw, Doostmohammadi, Nier, *et. al.* Nature Letter (2017): Topological defects in epithelia govern cell death and extrusion
- [3] Guillamat, Ignés-Mullol, and Sagués. Proc Natl Acad Sci (2016): Control of active liquid crystals with a magnetic field

Filament Sensor – A tool for near real time analysis of stress fiber formation in stem cells

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Human mesenchymal stem cells (hMSC) can be differentiated into various lineages and are thus interesting for biomedical research. However, differentiation can not only be achieved by chemical means, but mechanical cues alone are sufficient. Biocompatible matrices of different elasticities E can induce reversible differentiation. Differentiation is usually checked by changes in lineage specific protein expression, which occur over a period of days to weeks, but significantly different structures of stress fibres are observable within the first 24 hours of planting [1]. Therefore, stress fibre structure, quantified by an order parameter S , can be used as an early morphological marker. It also dictates the morphology of the nucleus and hence could directly affect gene transcription.

We use a massively parallel live-cell imaging set-up to record cells under physiological conditions (37 °C, 5 % CO₂) over a period of 24-48 hours. This way we obtain a large, statistically sufficient data set. To minimize the impact of the fluorescent marker, we use an optimized lifeact-TagRFP transfection of hMSCs to visualize the structure and formation of actin-myosin stress fibres. We aim for a full representation of filament processes over time and space, allowing for statistical analysis. The current understanding and classification of stress fibres (dorsal, ventral, arcs) is based on their location in the cell and function during migration. In contrast, we concentrate on an unbiased classification due to their temporal and spatial persistence that should also correlate with function. This might for example been represented by significantly different persistence in space and time and crosstalk with other cytoskeletal components. For this task we developed the ‘Filament Sensor’ [2, 3], a freely available tool for near real-time analysis of stress fibres. We present experimental data where we can distinguish the cytoskeletal structures of hMSCs on 1 kPa, 10 kPa and 30 kPa elastic substrates with 99 % confidence. We are working on single filament tracking, a sophisticated analysis of the structure in terms of orientation fields, 3D filament tracing and tracking, and correlation of focal adhesions and stress fibers.

[1] A. Zemel, et al., Nat. Phys., 2010.

[2] www.filament-sensor.de.

[3] B. Eltzner, et al., PLoS One, 2015.

Quantifying Localization Accuracy in Single-Molecule Super-Resolution Microscopy

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Single-molecule localization microscopy (SMLM) uses images of blinking fluorescent molecules to reconstruct high-resolution images of a target structure. SMLM reconstruction algorithms seek to estimate the most likely set of fluorophore positions and brightnesses (i.e., a super-resolution image) that is consistent with the observed noisy images of blinking molecules. A key question left unresolved by existing SMLM methodologies is: How well do the SMLM data support an algorithm's statistical estimates that comprise an SMLM image, i.e., what is our statistical confidence in each measurement?

Given a mathematical model of the imaging system and the noise statistics within each image, we have developed a method, called Wasserstein-induced flux (WIF),¹ to reliably quantify the confidence of *individual* localizations within an SMLM reconstruction. These confidences yield a consistent measure of localization accuracy under various imaging conditions such as changing molecular density and optical aberrations, without knowing the ground-truth positions. Our WIF confidence map outperforms other image-based methods in detecting artifacts in high-density SMLM while revealing detailed and accurate features of the target structure (Fig. 1). WIF also reveals the experimental mismatches present in computational imaging models and sample heterogeneity due to hidden parameters (e.g., molecular orientation) in both simulated and experimental SMLM datasets.

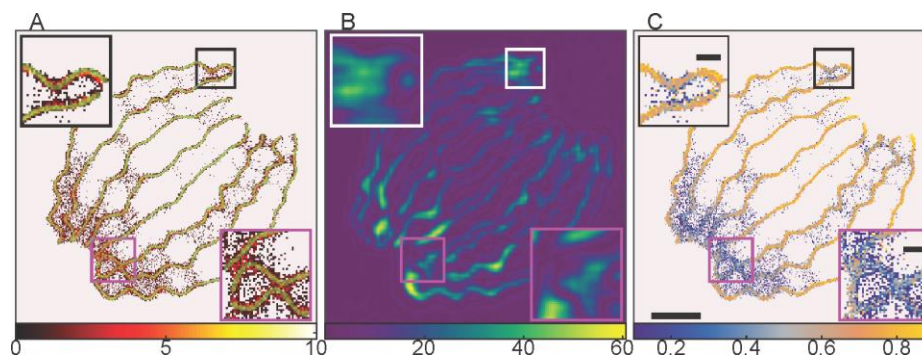


Fig. 1. Confidence quantification for high-density SMLM data. (A) Recovered structure (red) using FALCON overlaid with the ground truth (green). (B) Error map recovered by SQUIRREL (brighter colors correspond to larger errors). (C) WIF confidence map (brighter colors indicate higher confidence) obtained by averaging localization confidences in each pixel. Colorbars: (A) number of localizations, (B) error, and (C) WIF confidence per 20×20 nm². Scalebars: (C) 1 µm, insets: 200 nm.

¹ H Mazidi, T Ding, A Nehorai, MD Lew. bioRxiv 721837; doi: <https://doi.org/10.1101/721837>

Towards Bayesian based smart microscopy

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Fluorescence microscopy is an incredible toolbox for the investigation of the cellular machinery over multiple length scales. It presents unique advantages such as protein specificity, multi-color, the access to 2D, 3D and whole cell data, from both fixed or live sample, to name a few. This, however, comes at a cost: complex microscopy set ups, with many user definable parameters to set for the image acquisition, and no clear guidelines to do so. This lack of robust framework for the optimization of acquisition parameters often impacts negatively on the output data: image quality deterioration and limited reproducibility are common issues faced by the field. In the face of these limitations, how much can we trust the statistics extracted from these images, independently of the analysis tool used? We propose here a Bayesian based framework for the optimization of acquisition parameters allowing for user-free microscopy acquisition, while insuring the reproducibility of the generated images. It is a first step towards sample specific automated microscopes or “smart microscopes”. We will focus on the application of this framework to SMLM relying on a novel real time SMLM simulator that we have developed for its validation.

Untying the Gordian Knot: Spatiotemporal Single Particle Tracking using Point Cloud Reduction

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Keywords: 3D Single Particle Tracking, Cellular Imaging, Point Cloud Reduction, Trajectory overlap

Single particle tracking algorithms have two well-known issues: photoblinking and trajectory overlap. Conventional methods compensate by using *a priori* knowledge of the particle, introducing bias into trajectory calculations [1]. Data driven approaches avoid systemic bias by relying on internal consistencies to fill knowledge gaps. Increasing the number of compatible points present in a sample strengthens the conclusions one can make about the underlying structure. As such, interpreting single particle localization data as a weighted point cloud improves the accuracy of the derived model.

We present an application of point cloud clustering to 3D single particle tracking. Our novel approach uses 3D particle localizations provided by the Alternating Direction Method of Multipliers [2] to build a point cloud for each frame. Particles are initialized via agglomerative clustering of points representing fluorescent signals. Clusters are linked between frames by using a hybridization of weighted moving least squares regression and sample consensus to develop a local second-order parametric model of the particle motion. The second-order model predicts the position of the particle in future frames [3] bridging together points across intersection and photoblinking events. Fusion and fission events are resolvable by sharing points between particles, which allow particles to overlap, follow each other, and separate freely.

The effectiveness of our point cloud method is evaluated with real protein motion on surfaces and vesicle trafficking in a cell-like environment. Future applications include tracking intracellular phenomena that exhibit high degrees of photoblinking and trajectory overlap.

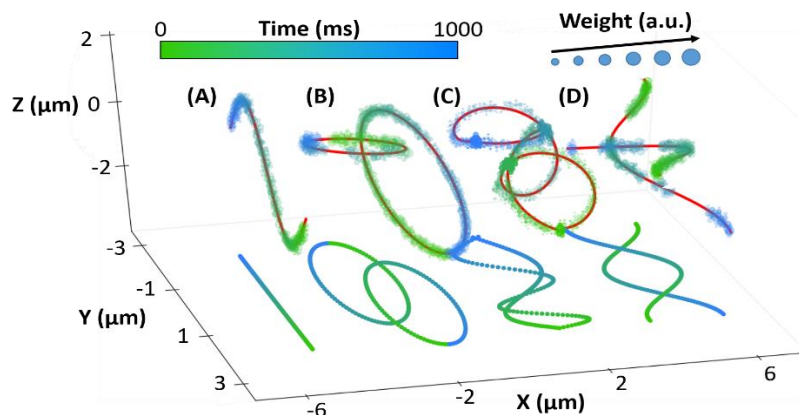


Figure 1: Four simulated test cases resolved using our algorithm using 3D elementary parametric motions. The point cloud is represented in 3D with the recovered analytical model shown in red. The ground truth trajectories are projected onto the xy plane. Tests conducted: (A) linear and nonlinear motion, (B) overlapping motion, cyclic motion, chained trajectories, (C) intersecting motion, fusion and fission events, accelerating motion (D) photoblinking events.

- [1] Hansen, A. S., et al. (2018). "Robust model-based analysis of single-particle tracking experiments with Spot-On." *Elife* 7.
- [2] Shuang, B., et al. (2016). "Generalized recovery algorithm for 3D super-resolution microscopy using rotating point spread functions." *Scientific Reports* 6: 30826.
- [3] Veenman, C. J., et al. (2001). "Resolving motion correspondence for densely moving points." *Ieee Transactions on Pattern Analysis and Machine Intelligence* 23(1): 54-72.

PSF-broadening due to fluorescence emission: principle and implications on imaging

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Keywords: fluorescence, point-spread-function, full-width-half-maximum, weighted averaging

Fluorescence imaging plays an important role in biological research [1]. It provides a valuable tool in terms of specificity and yields a high imaging contrast. However, the use of fluorescence structures also comes with some drawbacks [2]. Beside the need for labeling also the asymmetric emission spectrum of most dyes or fluorescent proteins limits the imaging quality: it results in a broadening of the detection point-spread-function (PSF).

Given the emission spectrum of any fluorescent structure, the measurable PSF needs to be expressed as an incoherent summation of individual 'single wavelength' PSFs. The full-width-half-maximum (FWHM) of the PSF scales inversely with the wavelength and most emission spectra exhibit a fluorescence tail at longer wavelengths. Yielding in some lateral broadening compared to the PSF obtained from the peak emission only.

We present an analytical explanation of this effect and its dependency on the numerical aperture, the maximum emission wavelength and the shape of the emission spectrum. Our theoretical investigation is verified by numerical simulations, which shows a broadening on the order of 10 nm. Additionally we propose a way to overcome this broadening by splitting the emission spectrum in multiple wavelength-segments. Those are subsequently recombined using weighted averaging in Fourier space [3]. Our simulation shows a reduction of the 'broadened' FWHM by 8 nm, without compromising detection efficiency or increasing the noise level by image processing.

References:

[1] Vonesch, Cédric, et al. "The colored revolution of bioimaging." *IEEE signal processing magazine* 23.ARTICLE (2006): 20-31.

[2] Ettinger, Andreas, and Torsten Wittmann. "Fluorescence live cell imaging." *Methods in cell biology* vol. 123 (2014): 77-94. doi:10.1016/B978-0-12-420138-5.00005-7

[3] Becker, Jan, Ronny Förster, and Rainer Heintzmann. "Better than a lens-A novel concept to break the SNR-limit, given by Fermat's principle." *arXiv preprint arXiv:1811.08267* (2018).

Single molecule localisation lightfield microscopy

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Ever since the seminal work of Levoy (1), lightfield microscopy is gaining more relevance in biological applications. The technique allows imaging large volumes in one shot, capturing lightfield by inserting a microlens array into the optical system. Although diffraction limits the spatial and angular resolution, resolution can be enhanced using computationally demanding deconvolution algorithms (2). In this work we present how lightfield techniques bring a novel facet to single molecule microscopy, due to a huge increase of the depth of field of the former but with the typical outstanding resolution of the latter technique.

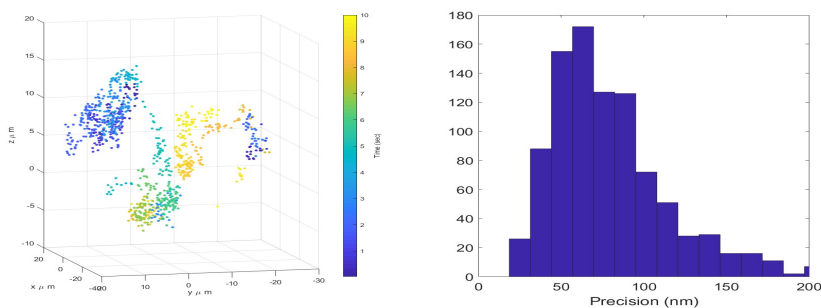


Fig. Localisations of diffusing beads

We briefly present the systems developed to achieve imaging of single molecules via lightfield microscopy, with a few tenths of nanometers resolution over a depth of field over 10 micrometers. We will focus on the computational aspects such as calibration based on bead images, proof of concept for multi-emitter detection and localisation algorithms as well as precision analysis.

Finally, the performance of the reconstruction is demonstrated on several biological samples.

(1) M.Levoy, R.Ng, A.Adams, M.Footer, and M.~Horowitz, Light field microscopy, ACM Transactions on Graphics (25), 924--934 (2006).

(2) M.Broxton, L.Grosenick, S.Yang, N.Cohen, A.Andalman, K.Deisseroth, and M.Levoy, Wave optics theory and 3-d deconvolution for the light field microscope, Optics Express, 21, 25418--25439 (2013).

Deep Learning approaches for reliable quantification of multi-omics cell imaging datasets to interrogate RNA and protein spatial and temporal subcellular interactions

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Abstract BodyQuantitative analysis of single-cell biomolecular processes has recently expanded its scope by encompassing cellular imaging data thanks to advances in both image acquisition techniques and the emergence of computer vision algorithms using machine learning. Moreover, as more and more bioimaging data become available, it is now possible to apply Deep Learning algorithms to single cell images taking advantage of these large-scale datasets particularly suited for training such algorithms in order to quantify the mRNA or protein counts within cellular images.

As cells can vary not only in quantitative molecular information but also in the way these molecules are organized in space and time, we propose DypFISH, a trans-omics approach to quantitatively investigate the spatial and temporal subcellular localizations of mRNA and proteins, using fluorescence microscopy data at high resolution.

We have both developed a complete a computing platform using Deep Learning for mRNA and protein quantification and created training datasets that make it possible for our models to be robust. Our framework enables automatic single cell segmentation as well as the as localization of relevant cellular elements such as nucleus, MTOC, mRNA spots and proteins. Cell and nucleus segmentation is based on the well-known U-net architecture, whereas mRNA spots and MTOC detection is based on unsupervised GAN-type algorithms.

We then introduce a range of analytical techniques for quantitatively interrogating the previously acquired single cell imaging spatiotemporal data, using MTOC orientation to reveal spatial clustering and interactions at multi-omics level.

Multivariate spectral analysis for identification of spectral features of peripheral nerves using Raman microspectroscopy

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Identification of peripheral nerves during surgery is desired to avoid neural disturbance following surgery as an aspect of improving the quality of life of patients. In the previous study, we have provided a proof of principle demonstration of peripheral nerve detection of Wistar rats and human periprosthetic tissues by using Raman spectroscopy [1,2]. The Raman spectroscopy identified the peripheral nerves with the molecular information appeared in Raman spectra. To realize more reliable prediction of peripheral nerves in terms of Raman spectroscopy, the identification of predominant Raman spectral features for the discrimination of peripheral nerves is required for the optimization of Raman spectroscopic measurement and spectral analysis. In the present study, we investigated predominant spectral features of peripheral nerves appeared in Raman spectra for more detail by performing multivariate spectral analysis.

Figure 1a shows representative Raman spectra of peripheral nerves and its adjacent tissues. To identify the predominant spectral features of peripheral nerves, we performed principal component analysis (PCA) to the Raman spectra and predicted each tissue by regression analysis using principal components (PCs) obtained by the PCA (Fig. 1b). Furthermore, the dimensional reduction of PCs in the regression analysis was also performed. As a result, we found the notable decrease of the sensitivities for peripheral nerves with the dimensional reduction of PC3, which indicated that the PC3 included spectral features for the prediction of peripheral nerves against the other tissues. We also applied to the Raman imaging of peripheral nerves, which enabled automatic identification of peripheral nerves based on Raman spectra. These results indicated that the dimensional reduction method is effective to identify the spectral features for the identification of peripheral nerves, and our proposed approach may provide a unique and powerful tool for peripheral nerve detection for nerve-sparing surgery in the future.

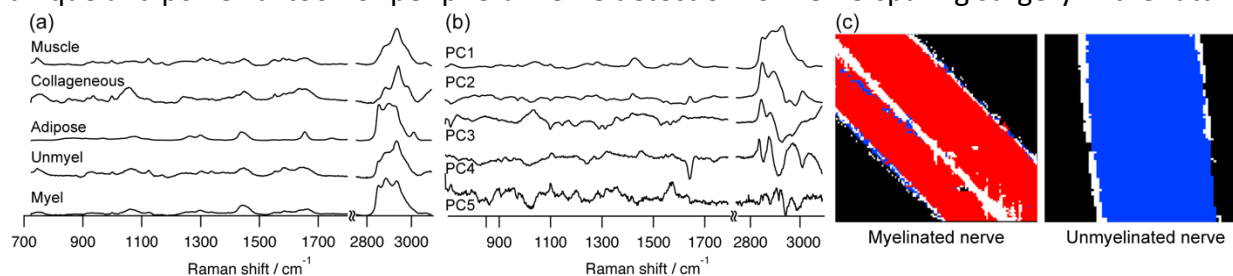


Fig. 1 Label-free Raman spectroscopic identification of peripheral nerves and its adjacent tissues. (a) Representative Raman spectra of these tissues. (b) Principle component spectra obtained by principal component analysis. (c) Raman identification of peripheral nerves.

[1] T. Minamikawa, Y. Harada, N. Koizumi, K. Okihara, K. Kamoi, A. Yanagisawa, and T. Takamatsu, *Histochem. Cell Biol.* 139 (1), 181-193 (2013).

[2] T. Minamikawa, Y. Harada, and T. Takamatsu, *Sci. Rep.* 5, 17165 (2015).

Joint denoising deconvolution

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Deconvolution of fluorescence microscopy images is known to be difficult due to the band-limited nature of the optical transfer function but also due to the intrinsic noisy nature of the acquired images. While the usual approach to stabilize the recovery of the fluorophore density consists in regularizing the solution by adding an a priori on the smoothness of the recovered image, we propose here to a scheme for jointly reduce the noise and deconvolve the image under settings commonly observed in wide-field or lightsheet microscopy. In this context, the algorithms recover not only the deconvolved image but also a denoised image. We rely on the Chambolle-Pock minimization algorithm to efficiently minimize the cost function composed of coupling terms and regularization terms for the respective images. Simulation shows that when exploring the hyperparameter space this approach outperforms deconvolving directly the image or deconvolving after a denoising step. We also compare this approach to other popular deconvolution methods on benchmark images. Finally, we demonstrate this approach on real images.

Towards quantum enhanced quantitative superresolution microscopy

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Far-field optical microscopy beyond the Abbe diffraction limit, making use of nonlinear excitation (e.g. STED), or temporal fluctuations in fluorescence (PALM, STORM, SOFI) is already a reality. In contrast, overcoming the diffraction limit using non-classical properties of light is very difficult to achieve due to the difficulty in generating quantum states of light and their inherent fragility. Here, we experimentally demonstrate practical superresolution microscopy based on quantum properties of light naturally emitted by fluorophores used as markers in fluorescence microscopy. Our approach is based on photon antibunching, the tendency of fluorophores to emit photons one by one rather than in bursts.

Since the non-classical intensity correlations carry higher spatial frequency information, they can be utilized to enhance image resolution. We demonstrate how antibunching can improve the resolution capabilities of image-scanning confocal microscopy in all three dimensions¹ (a 2D example is shown in Figure 1). These features allow us to enhance the spatial resolution with which multiple emitters can be imaged and localized compared with techniques that rely on temporally integrated brightness using CCD cameras, as well as to apply various algorithmic resolution augmentation methods².

Notably, the use of photon antibunching readily lends itself to quantitative imaging modalities since the degree of antibunching is intimately related to the emitter density. Potential approaches to such quantitative superresolution microscopy using a simple confocal microscope setup will be discussed.

References

- [1] R. Tenne et al., "Super-resolution enhancement by quantum image scanning microscopy", *Nature Photonics* 13, 116 (2019).
- [2] U. Rossman et al., "Rapid quantum image scanning microscopy by joint sparse reconstruction", to appear in *Optica*

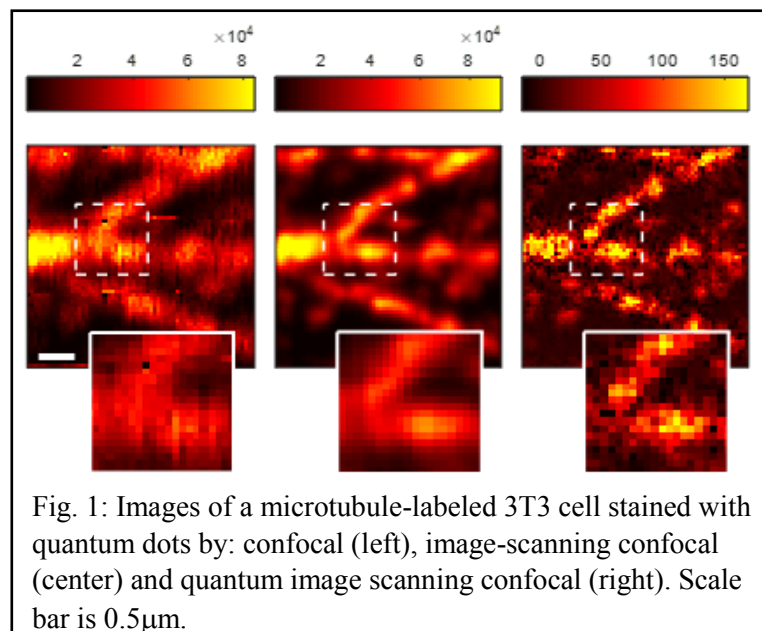


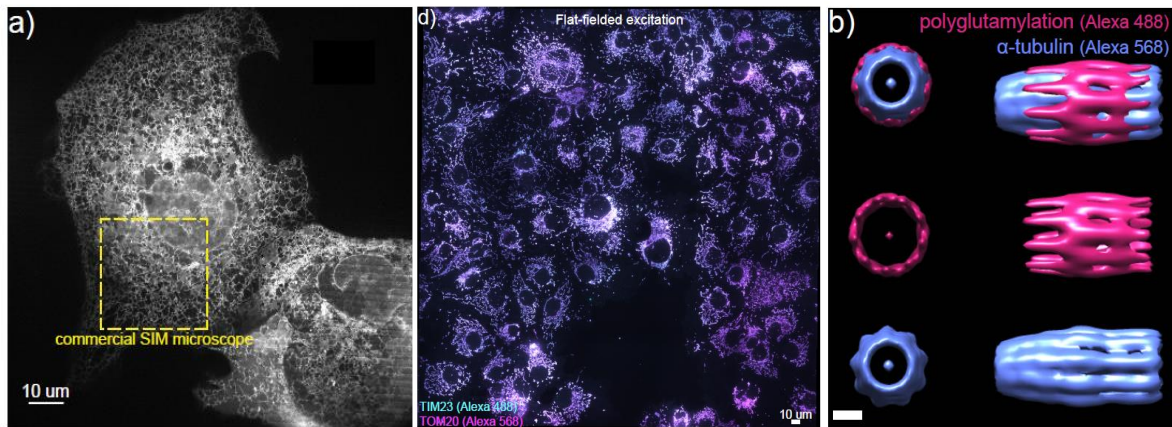
Fig. 1: Images of a microtubule-labeled 3T3 cell stained with quantum dots by: confocal (left), image-scanning confocal (center) and quantum image scanning confocal (right). Scale bar is 0.5 μ m.

Homogeneous multifocal excitation for high-throughput super-resolution imaging

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Super-resolution microscopies have become an established tool allowing biologists to resolve features below the diffraction limit of light. However, speed and field of view (FOV) still present major bottlenecks in their application to quantitative biology, which requires access to large datasets to overcome the inherent variability and noise of biological processes. Here, by extending existing Koehler integrator technology, we develop a novel method to generate homogeneous multi-focal excitation and implement it into an instant structured illumination microscope (iSIM). The iSIM is capable of imaging speeds up to 100 fps, which combined with our homogeneous excitation and sCMOS detection enables fast, multi-color, volumetric imaging over >100 μm FOVs at doubled resolution (Figure, left). We further extend the effective FOV by trading off the speed of iSIM imaging to cover multiple FOVs and stitch them back into a larger image (Figure, center). Furthermore, by iterating this procedure at high speed, we can perform fast live-cell super-resolution imaging of dozens of cells spanning multiple FOVs. Finally, we combine our flat-fielded iSIM setup with recently developed ultrastructure expansion microscopy (U-ExM) to collect thousands of 3D images of expanded *Chlamydomonas* centrioles at an effective resolution of ~35 nm, within an hour – a task which would have otherwise taken weeks or months. From the collected library of particles, we employ particle averaging and reconstruction techniques to create a high-resolution 3D model of different tubulin PTMs within the centriole (Figure, right). This novel high-throughput approach lends possibility to combining super-resolution with more advanced kinds of analysis, such as high-content screening, structural modelling and machine learning applications.

3D Multicolor Nanoscopy at 10,000 Cells a Day

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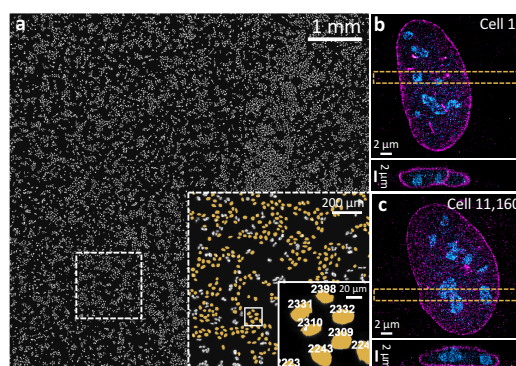
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Single-molecule switching (SMS) nanoscopy techniques like STORM/(F)PALM circumvent the diffraction limit by sacrificing temporal resolution. SMS typically requires several minutes to reconstruct a single super-resolution image, severely limiting its throughput. High-speed SMS has been achieved using high laser power and sCMOS cameras, however, conventional analysis pipelines cannot handle the resulting 70 TB/day data volume. We have overcome this by developing a platform for high-throughput SMS, enabling us to perform automated super-resolution imaging of ~10,000 3D fields of view a day.

Our integrated system comprises a biplanar-astigmatism microscope specifically designed to produce high volumes of 3D and two-color SMS data as well as a computer cluster for real-time analysis. It can record SMS images of whole nuclei (25 x 25 x 4 μm) in 8 s, and can image autonomously for at least 30 hrs. The computer cluster runs PYthon Microscopy Environment (PYME) software, which we have developed to include a microscopy-specific compression algorithm and distributed storage framework to receive the full 800 MB/s bandwidth of sCMOS cameras in real-time. We additionally use the computer cluster to run our now fully GPU-accelerated sCMOS-specific localization algorithms to localize 39,000 emitters/s, which is real-time at our 800 Hz imaging for up to 49 emitters/frame. The distributed analysis backend in PYME additionally supports batch-processing post-localization analysis recipes over thousands of super-resolution images or point-clouds.

This platform converts SMS imaging from a qualitative or small-scale quantitative tool into a valid imaging technique for large-scale quantitative hypothesis testing. After preliminary high-throughput demonstrations characterizing nuclear phenotypes, we are now leveraging our advances to study the organization of the interphase nucleus, specifically studying the size and positioning of lamin-associated domains with the nuclear envelope.



11,160 lamin- and nucleophosmin-stained U2-OS cells imaged in ~30 hr.

Simple, compact and robust phase and fluorescence microscope for statistical analysis of cell cycle

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We designed a surprisingly simple, compact and robust microscope for phase and fluorescent imaging. The phase-contrast image is reconstructed from a single, approximately 100 μm out-of-focus image while fluorescence image is recorded in-focus. No mechanical movement of neither sample nor objective or any other part of the system is needed to change between the phase-contrast and fluorescence modality. The change of focus is achieved with the chromatic aberration of the system. Our microscope is sufficiently compact to fit into a standard biological incubator, which together with simplicity and robustness greatly facilitate the long-time observation of cell cultures.

We can observe a thousand cells in parallel in a single field of view (1.2 mm^2) with resolution down to 2 μm . We show FUCCI marked HeLa cell culture observed over several days directly in the incubator. FUCCI (fluorescence ubiquitination cell-cycle indicator), is a genetically encoded, two-colour (red and green), indicator of the progression through the cell cycle: the cells in G1 phase show red fluorescence nuclei while the cells in S, G2 and M phase display green fluorescence within the nuclei.

We use phase images for segmentation and tracking of the individual cells which allows us to determine the level of fluorescence in each cell in the green and red fluorescence channel. We compare the obtained statistics to the flow cytometer data and show that we can produce a statistically relevant time-resolved measurement of a cell population while keeping access to the individual cells.

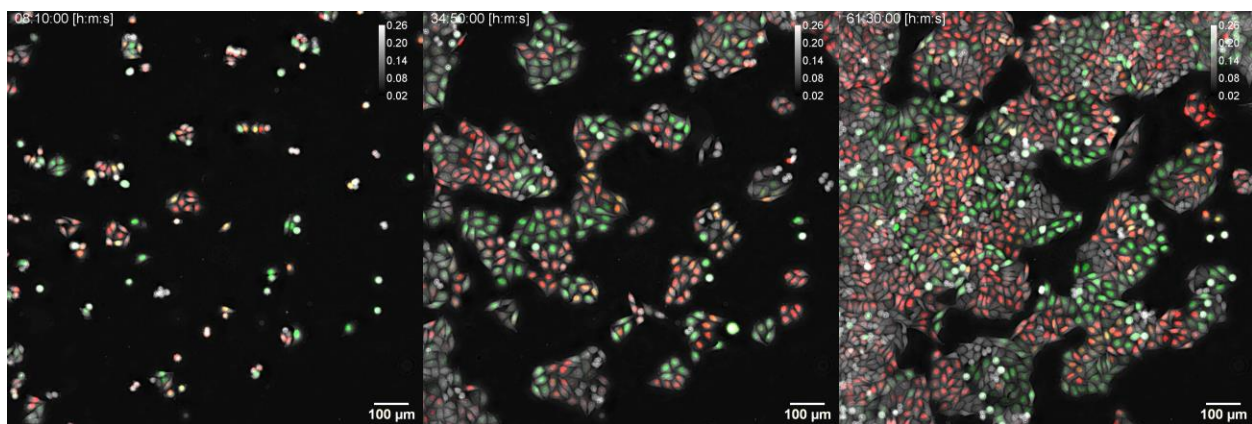


Figure 1: FUCCI marked HeLa cells in phase and two colour fluorescence observed over 60 hours directly in the incubator.

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Keywords: localization microscopy, phase retrieval, point spread function engineering.

Phase retrieval techniques have been extensively used in applications such as computer generated hologram design and aberration correction in imaging systems[1]. The latter can be achieved by estimating the Optical Transfer Function (OTF) of the system from recorded\generated data (e.g. a z-stack of a fiducial marker). In fluorescence microscopy, specifically super resolution localization techniques, accounting for aberrations necessitates an algorithm which approximates the given images with high model precision, both for 2D and 3D applications (where a Point Spread Function (PSF) altering component is introduced in the optical path).

Previous work in this field and in adaptive optics usually incorporated scalar diffraction theory to model the imaging setup and reconstruct the (OTF) with a set of Zernike polynomials, which is computationally efficient and works reasonably well in most cases[2]. However, this approach isn't sufficient for applications (mainly with high NA oil immersion objectives) where polarization effects have to be accounted for. Examples include imaging samples with fixed dipoles, introducing surface plasmon imaging setups[3] or when Supercritical Angle Fluorescence (SAF) is present in the system (imaging close to the coverslip).

Here, we develop and demonstrate a GPU-accelerated software that can deal with any of the above optical configurations, and give a fast (up to 2 minutes) OTF reconstruction in a pixel-wise manner; this is specifically relevant when Zernike polynomials are not a suitable basis, e.g. for a double helix PSF. The method is based on an analytical derivation of the vectorial diffraction model and, unlike typical approaches, the technique is highly robust to initialization of the OTF, to the point that no prior knowledge of the mask is required. We show experimentally that this method improves localization results by creating a reliable PSF generator from a z-stack that matches with acquired 3D data under the constraints of having SAF, a linear polarizer (due to using a liquid crystal spatial light modulator to introduce the designed aberrations) and refractive index mismatch between the immersion oil and sample medium.

- [1] M. J. Booth, "Adaptive optics in microscopy," *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences*. 2007.
- [2] P. N. Petrov, Y. Shechtman, and W. E. Moerner, "Measurement-based estimation of global pupil functions in 3D localization microscopy," *Opt. Express*, vol. 25, no. 7, p. 7945, Apr. 2017.
- [3] N. Karedla *et al.*, "Three-dimensional single-molecule localization with nanometer accuracy using Metal-Induced Energy Transfer (MIET) imaging," *J. Chem. Phys.*, 2018.

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New image acquisition systems generate large number of images and large volume images. Such data sets are hard to store, to process and to analyze for one user in a workstation. Many solutions exist for data management (Omero, OpenImadis...), image analysis (Fiji, Icy, CellProfiler, Python libraries...) and statistics (R...). Each of them has its specificities and several bridges have been developed between pieces of software. Nevertheless, in many use-cases, we need to perform analysis using tools that are available in different pieces of software and different languages. It is then tedious to create a workflow that brings the data from one tool to another. It needs programming skills and most of the time, a dedicated script using a dedicated file system for processed data management is developed.

The aim of the BioImage-IT project is to create a “bandmaster” application that allows any scientist to annotate data, process data and analyze data using only one single high level application. This BioImage-IT application is based on 3 components:

- an image annotation method based on a *json* file system,
- an image processing and analysis tools integration method based on Docker and XML commands description,
- an application with a graphical interface to easily annotate data, run processing tools, and visualize data and results.

This software architecture has three main goals. First, data are annotated using a file system. This means that data are not dependent on any software like a SQL database, and each experiment can then be stored in a different directory and moved from one server to another or to any drive with a simple copy pasting operation. Second, the processing tools are used as binary packages managed by Docker. Docker makes it easy to deal with the dependencies and allows to use several versions of the same tool. Any existing tool can then be integrated as it is without re-inventing the wheel. Third, using a single “bandmaster” application allows to automatically generate metadata for any processed data, making available the traceability and repeatability of any result.

BioImage-IT is developed in the context of the France-BioImaging research infrastructure in coordination with the IPDM-FBI (Image Processing and Data Management) node in order to provide a standardized image processing tool set and data management for the imaging facilities.

3D tracking of endocytic and exocytic events using lattice light sheet microscopy

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The study of the whole cell dynamics of endocytic/exocytic-recycling events has proven difficult until recently because of lack of sensitivity, limited speed, photobleaching and phototoxicity associated with conventional imaging modalities. The Lattice Light Sheet Microscope (LLSM) [1] allows overcoming these difficulties, yet reaching high spatial resolution. This allows 3D images to be captured over long time at a high acquisition frequency, and enables the study of signalling, transport, and stochastic self-assembly in complex environments.

In addition, this imaging technique and 3D-tracking will allow to look at molecular machinery throughout the full sequence of events that lead to exocytic fusion event or endocytic carrier formation, from initial membrane recruitment and budding and intracellular trafficking throughout the entire endocytic membrane system. Using 3D segmentation maps will permit to quantify different intracellular distribution pathways from the plasma membrane to a particular cellular destination and *vice et versa*.

We present preliminary results of the coordination of vesicle recycling from the endosomal recycling compartment up to the plasma membrane using LLSM imaging and 3D tracking. In addition, we introduce a quantitative analysis of endocytosis dynamics of AP2 adaptor complex, Galectin-3 (Figure 1) and Transferrin using single particle tracking analysis of 3D+time data. These examples demonstrate the advantage of lattice light sheet microscopy for imaging endocytic/exocytic events in single cells.

[1] Chen, B.C. et al. Science. 346 (6208): 1257998–1257998. (2014)

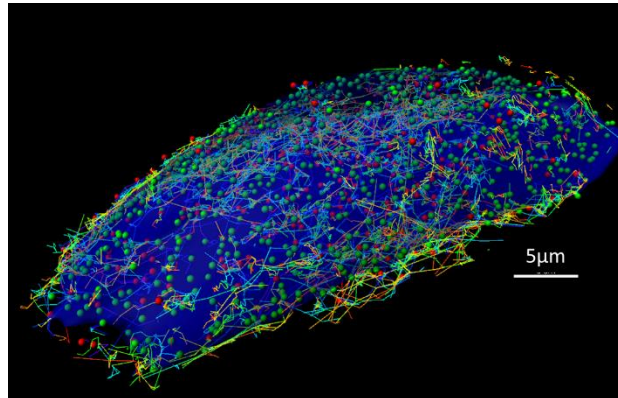


Figure 1. 3D tracking of Gal3-Atto647n (red) vs AP2-eGFP (green) adaptor protein in SUM159 cell.

Fluorescence Speckle Correlation Spectroscopy

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Keywords: Fluorescence correlation spectroscopy, light scattering, speckle, diffusion

Fluorescence correlation spectroscopy is used extensively for quantitative characterization of biomolecules at very low concentration. However, light aberration and scattering from the tissues are two major factors that affect the results strongly. Although adaptive optics arrangement can correct the aberrations of light to some extent, it fails completely to eliminate the light scattering effect. Recently, exploiting the fact that autocorrelation of a speckle pattern is a sharply peaked point spread function and the optical memory effect, noninvasive imaging of fluorescent sample has been possible [1]. In this study, we employ a modality based on speckle correlation enabled via optical memory effect to study 2D diffusion of fluorescent sample hidden behind a scattering film.

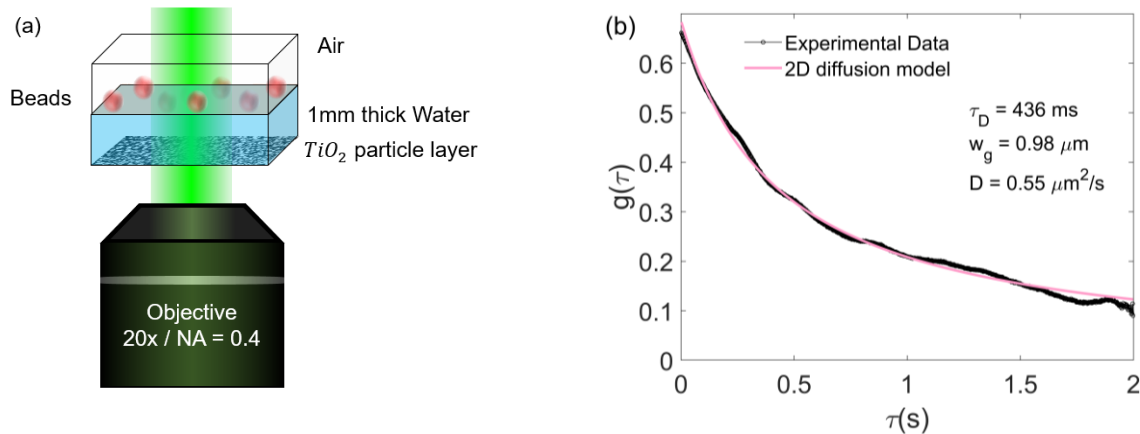


Fig. 1: (a) Schematic of the experiment (shown in part). (b) Temporal image cross-correlation acquired from a single bead fluorescence speckle is plotted with delay.

We realized a 2D diffusing model system by confining fluorescent polystyrene beads of 1 μ m diameter at the water/air interface behind a TiO₂ diffuser. Beads were excited by speckle illumination and the emission speckle was detected in an epi-fluorescence configuration. 2D diffusion constant obtained by temporal image cross-correlations of a single bead emission speckle is compared with that obtained by single particle tracking without the scattering layer. Quantitative agreement between these results establishes the potential application of this technique in correlation spectroscopy. Superimposed multiple beads speckle patterns were also studied and the results will be presented at the meeting.

[1] M. Hofer *et al*, Opt. Express **26**(8), 9866-9876 (2018).

Metal-Induced Energy Transfer

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Metal-Induced Energy Transfer (MIET) Imaging is a recently developed method [1] that allows for nanometer resolution along the optical axis. It is based on the fact that, when placing a fluorescent molecule close to a metal, its fluorescence properties change dramatically, due to electromagnetic coupling of its excited state to surface plasmons in the metal. This is very similar to Förster Resonance Energy Transfer (FRET) where the fluorescence properties of a donor are changed by the proximity of an acceptor that can resonantly absorb energy emitted by the donor. In particular, one observes a strongly modified lifetime of its excited state. This coupling between an excited emitter and a metal film is strongly dependent on the emitter's distance from the metal. We have used this effect for mapping the basal membrane of live cells with an axial accuracy of ~3 nm. The method is easy to implement and does not require any change to a conventional fluorescence lifetime microscope; it can be applied to any biological system of interest, and is compatible with most other super-resolution microscopy techniques that enhance the lateral resolution of imaging [2-4]. Moreover, it is even applicable to localizing individual molecules [5-6], thus offering the prospect of three-dimensional single-molecule localization microscopy with nanometer isotropic resolution for structural biology. I will also present latest developments of MIET where we use a single layer of graphene instead of a metal film that allows for increasing the spatial resolution down to few Ångströms [7].

References

1. Chizhik, A. I., Rother, J., Gregor, I., Janshoff, A., & Enderlein, J. (2014). *Nature Photonics* **8**:124-127.
2. Baronsky, T., Ruhlandt, D., Brückner, B. R., Schäfer, J., Karedla, N., Isbaner, S., ... & Chizhik, A. I. (2017). *Nano Lett.* **17**:3320-3326.
3. Chizhik, A. M., Ruhlandt, D., Pfaff, J., Karedla, N., Chizhik, A. I., Gregor, I., ... & Enderlein, J. (2017). *ACS Nano* **11**:11839-11846.
4. Chizhik, A. M., Wollnik, C., Ruhlandt, D., Karedla, N., Chizhik, A. I., Hauke, L., ... & Rehfeldt, F. (2018). *Mol. Biol. Cell* **29**:846-851.
5. Karedla, N., Chizhik, A. I., Gregor, I., Chizhik, A. M., Schulz, O., & Enderlein, J. (2014). *ChemPhysChem* **15**:705-711.
6. Isbaner, S., Karedla, N., Kaminska, I., Ruhlandt, D., Raab, M., Bohlen, J., ... & Tsukanov, R. (2018). *Nano Lett.* **18**:2616-2622.
7. Ghosh, A., Sharma, A., Chizhik, A. I., Isbaner, S., Ruhlandt, D., Tsukanov, R., Gregor, I., Karedla, N., & Enderlein, J. (2019). *Nature Photonics*, doi: 10.1038/s41566-019-0510-7.

Multimodal and multicolour microscopy to identify biomolecules in large-scale EM

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Electron microscopy (EM) is a powerful tool for imaging biomolecules at near-molecular resolution in cells and tissue, which is essential to understand the regulation of life and disease. However, restricted fields of view (FOVs) at high resolution hamper the quantitative power of EM. We routinely perform large-scale 2D EM on complete cells and tissue sections to maintain both large FOVs and high resolution, called nanotomography for nano-anatomy [1]. However, identifying specific molecules in these large grey-scaled datasets is troublesome. With correlated light and electron microscopy (CLEM) multimodal fluorescent and EM labels show specific biomolecules in a high resolution context. Though, targeting and spectral variety are often limited. Therefore we optimized and routinely apply energy dispersive X-ray analysis (EDX) for nanometer resolution elemental fingerprinting on bio-samples [2,3]. To identify proteins of interest, we now develop super-resolution fluorescence techniques for integrated CLEM: fluorescence signals are recorded during scanning of the focused electron beam, local modifications of the signal by the electron beam allow localization of fluorescence molecules with improved resolution. Fluorescent proteins are optimized for such techniques via mutagenesis screens for high fluorescence yield in vacuum and either stability or lability under electron irradiation. The first recent analyses revealed mutants with increased brightness and an increase in vacuum quantum yield. Thus, the quantitative power of EM is improved by nanotomography in combination with analytical EM methods and optimized CLEM methods.

[1] www.nanotomography.org

[2] Scotuzzi et al. Multi-color EM by element-guided identification of cells, organelles and molecules, 2016, *Scientific Reports*

[3] Pirozzi et al. ColorEM: analytical electron microscopy for element-guided identification and imaging of the building blocks of life, 2018, *Histochem Cell Biol*

Mechanical investigations of mEos4b blinking: longer single particle tracking with less track interruptions.

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Blinking, repeatedly entering a dark state, in fluorescent proteins (FPs) has proven to be a researcher's best friend. With a large contrast between the bright and dark state, reversibly photoswitchable FPs (RSFPs) have proven to be invaluable tools for super-resolution techniques¹. Just a simple change from a standard FP towards an RSFP can give a researcher diffraction unlimited information about his/her research question. Blinking in FPs has however also proven to be a researcher's worst enemy. The prolonged dark states cause interruptions in single particle tracking (SPT) experiments, which limits the visualization of the dynamics to only a fraction of it, and, causes several other severe complications². Therefore, the fidelity of the SPT results is reduced because of this. Green-to-red photoconvertible FPs (PCFPs), such as mEos4b³, in which the red state can be irreversibly activated are well-known labels for SPT-PALM (photo activated localisation microscopy). A spectroscopic and mechanistical investigation of the long-lived dark state in photoconverted mEos4b has shown that the blinked state absorbs cyan light efficiently. This highly resembles a photoswitched off-state, and, by addition of weak 488-nm light, this dark state swiftly reverts to the fluorescent state. These insights can be exploited for the fast recovery of the bright state by additional cyan light illumination. It largely eliminates slow blinking and enables recording much longer single particle tracks, with a minimum effort, with a higher quality imaging as a consequence. Using this strategy, heterogenous Microtubule-Associated Protein 4 (MAP4) diffusion behavior was studied, and its diffusion state changes could be sampled with a far better precision.

^[1]X. Zhou & M. Lin. Photoswitchable fluorescent proteins: ten years of colorful and exciting applications. *Curr. Opin. Chem. Biol.* 17(4): 682–690 (2013).

^[2]E. De Zitter, et al. Mechanistic investigation of mEos4b reveals a strategy to reduce track interruptions in sptPALM. *Nat. Methods.* 16: 707–710 (2019).

^[3]M. Paez-Segala et al. Fixation-resistant photoactivatable fluorescent proteins for CLEM. *Nat. Methods.* 12: 215–218 (2015).

A General Framework for Fitting an Arbitrary Model to SMLM Data

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Quantitative analysis of superresolution microscopy data is currently a major bottleneck, specifically for single molecule localisation microscopy (SMLM) where the data consists of lists of localisation coordinates. Structures of interest are usually analysed by simple geometric analysis or by averaging. In many cases, we can postulate an underlying basic geometry for the structures and are interested in the values of the parameters describing the model. However, an approach for fitting a parametrized model at the level of localisations is still missing.

Here, we present a new general framework for fitting an arbitrary model to SMLM data at the level of localisations. This framework is inspired by maximum likelihood estimation, which is commonly used to fit point clouds (Figure): the model is a probability density function and localisations are assumed to be observations drawn from the density function (distribution). We implemented a standardized framework to easily incorporate own continuous or discrete models for both 2D and 3D data and included visualization routines to assess the quality of the fit.

We demonstrate the power, versatility and flexibility of the framework by fitting models to various biological structures including clathrin-mediated endocytosis (Figure), nuclear pore complexes, and microtubules. Our results show that the framework enables a precise quantification of structural features in SMLM images.

This framework is implemented as an open-source Matlab tool and will be available along with instructions and model templates.

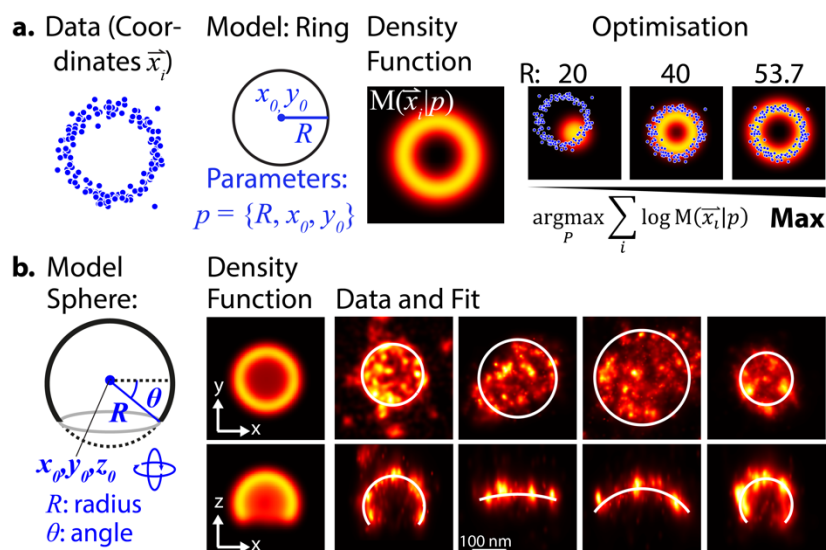


Figure: a. Schematic of the approach: point-clouds are fitted to a parametrized model using a maximum-likelihood inspired optimization.

b. Example on clathrin-coated pits: The model consists of a partially covered sphere that is fitted to the localizations by varying radius R , coverage angle, position x_0 , y_0 , z_0 and rotation in 3D.

Self-calibrating molecular counting for DNA-PAINT

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Keywords: DNA-PAINT, Super-resolution microscopy, Single Molecule Localization Microscopy (SMLM), molecular counting, Fluorescence Correlation Spectroscopy (FCS)

Single molecule localization microscopy (SMLM) has enabled the quantification of cellular structures down to the level of individual molecules. However, the counting of molecules relies on preliminary knowledge of the blinking behavior of individual targets or on a calibration to a reference. For successful biological applications, great care has to be taken since a plethora of factors influence the quality and applicability of calibration-dependent approaches to count targets in localization clusters particularly in SMLM data obtained from heterogeneous samples. Here, we present localization-based Fluorescence Correlation Spectroscopy (lbFCS) as the first self-calibrating molecular counting approach for DNA-PAINT microscopy and, to our knowledge, for SMLM in general. We demonstrate that lbFCS overcomes the limitation of previous DNA-PAINT counting approaches and allows the quantification of target molecules independent of the localization cluster density. In accordance with the promising results of our systematic investigation on DNA origami structures, lbFCS could potentially provide quantitative access to biological targets featuring heterogeneous cluster sizes in the future.

Stoichiometric quantification of spatially dense assemblies with qPAINT

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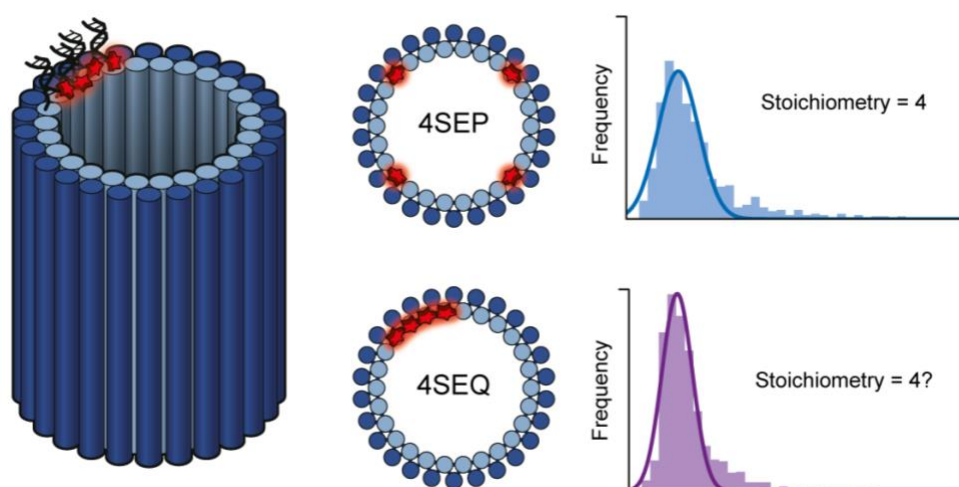
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DNA-PAINT imaging is emerging as a popular choice for single molecule localisation microscopy (SMLM), due to the specificity, programmability, and ability to multiplex, afforded by using DNA molecules as the imaging probe/docking strand^[1]. Recently, DNA-PAINT SMLM has been extended to allow quantitative measurements of DNA docking strand number(s), termed quantitative PAINT (qPAINT)^[2]. qPAINT is a useful method for counting well-separated molecules within nanoscale assemblies^[2, 3]. However, whether cross-reactivity of DNA docking strands, in densely packed arrangements, perturbs measurements is unknown. Here, we establish that qPAINT measurements are robust even when target molecules are separated by as little as 3 nm, sufficiently close that single-stranded DNA binding sites can interact^[4].



References

1. Nieves, D. J. *et al.*, (2018). *Genes (Basel)*. **9**, (10.3390/genes9120621)
2. Jungmann, R. *et al.*, (2016). *Nat Methods*. **13**, 439-442. (10.1038/nmeth.3804)
3. Jayasinghe, I. *et al.*, (2018). *Cell Rep*. **22**, 557-567. (10.1016/j.celrep.2017.12.045)
4. Baker, M. A. B., Nieves, D. J. *et al.*, (2019). *Nanoscale*. **11**, 12460-12464. (10.1039/c9nr00472f)

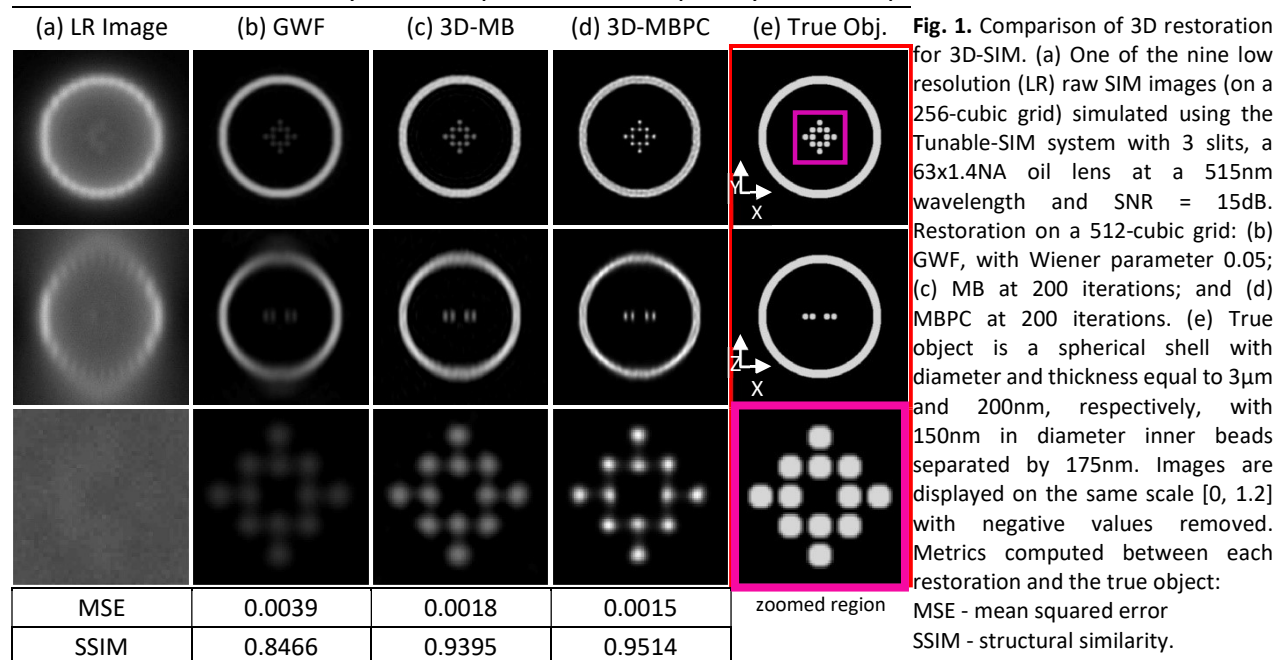
3D model-based restoration methods for 3D-SIM

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We extend our previous 3D model-based method in [1] by introducing a positivity constraint (3D-MBPC) and apply it to noisy simulated structured illumination microscopy (SIM) data from a tunable 3D-SIM system with desirable features [2] over traditional SIM [3]. In this study we compare numerical results from the 3D-MBPC with two other methods: the Generalized Wiener Filter (GWF) [3] and the 3D model-based (3D-MB) [1] (Fig. 1). Additionally, in the model-based framework we take into account the information redundancy in the raw data and compute the restoration using a reduced number of the forward raw images as previously suggested in [4,5]. The performance of both the 3D-MB and 3D-MBPC using reduced data is investigated in this study and compared to the result obtained with the traditional GWF without data reduction. The tradeoff between accuracy and computational complexity is also explored.



REFERENCES

- [1] H. Shabani et al., "Three-dimensional deconvolution based on axial-scanning model for structured illumination microscopy," 2019 IEEE 16th International Symposium on Biomedical Imaging (ISBI 2019), Venice, Italy, 2019, pp. 552-555.
- [2] A. Doblas et al., "Tunable-frequency three-dimensional structured illumination microscopy with reduced data-acquisition," *Opt. Express* 26(23), 30476–30491 (2018).
- [3] M. G. L. Gustafsson et al., "Three-dimensional resolution doubling in wide-field fluorescence microscopy by structured illumination," *Biophys. J.* 94(12), 4957–4970 (2008).
- [4] F. Orieux, et al., "Fast myopic 2D-SIM Super Resolution Microscopy with Joint Modulation Pattern Estimation", *Inverse Problems* 33(12), 1–22, 2017.
- [5] H. Shabani, "Three-dimensional (3D) image formation and reconstruction for structured illumination microscopy using a 3D tunable pattern", PhD Dissertation, The University of Memphis, 2019.

Acknowledgements: This work was funded by the National Science Foundation (NSF) (DBI award 1353904, PI: C. Preza).

Haste makes waste – Slow imaging increases resolution and labeling efficiencies in SMLM

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Keywords: SMLM, dSTORM, DNA-PAINT, PALM, intensities, labeling efficiency, molecular counting

Single-molecule localization microscopy (SMLM) is a slow super-resolution technique as it requires acquisition of thousands of frames per image. To increase throughput, microscopists like to use high excitation intensities [1] which were believed to not strongly impair the image quality [2].

We set this believe on trial and found that both the effective labeling efficiency as well as the number of photons per localization strongly depend on the excitation intensity. Unconventionally low intensities of less than 1 kW/cm² result in more than 20,000 photons per localization for Alexa 647, and, hence, localization precisions on the order of one nanometer. Consequently, we could easily resolve individual molecules in the nuclear pore complex labeled by Alexa 647 dyes [3] which are only 11 nm apart. This takes dSTORM to the regime of DNA-PAINT [4]. While DNA-PAINT suffers from high backgrounds which restrict achievable precisions and probes which are not readily commercially available, both downsides are overcome by our approach.

Our findings also have a consequence for fast imaging. Using low intensities instead of high intensities [5] for the initial off-switching, increased the labeling efficiencies by a factor of two for fast imaging with high laser powers during data acquisition.

Applying the same pipeline to the photoconvertible fluorescent protein mMaple (PALM approach), we found a similar trend of decreasing photon counts with higher intensities. However, mMaple could still be localized at precisions better than 10 nm when imaging at 300 frames per second, giving rise to live-cell PALM.

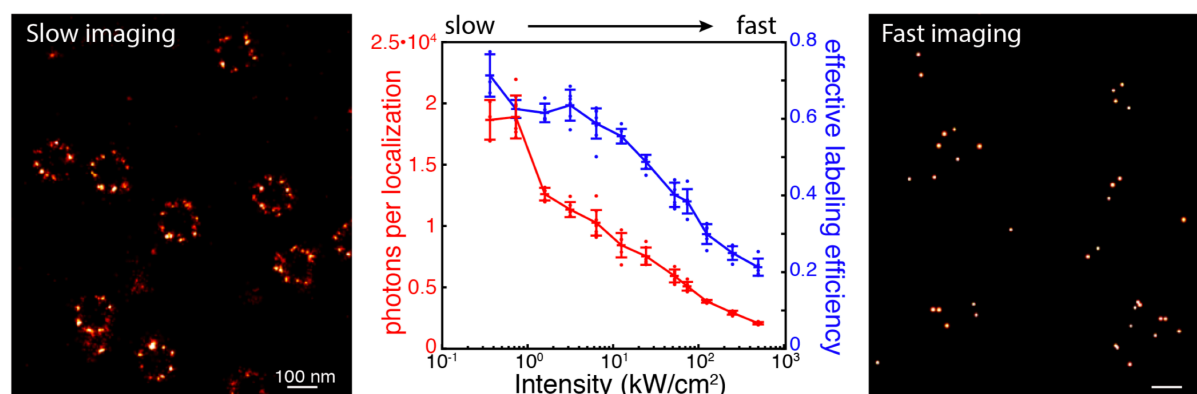


Figure 1. Imaging speeds affect image quality in SMLM. Nup96-SNAP labeled with BG-AF647 in GLOX/BME buffer for exposure times between 500 ms (left) and 0.4 ms (right).

[1] Barentine, Lin et al. Biorxiv 2019, doi 10.1101/606954

[2] Lin et al. Plos One 2015, doi 10.1371/journal.pone.0128135

[3] Thevathasan, Kahnwald et al. Biorxiv 2019, doi 10.1101/582668

[4] Schlichtharle et al. Angewandte Chemie 2019, doi 10.1002/ange.201905685

[5] van de Linde et al. Nature Protocols 2011, doi 10.1038/nprot.2011.336t

An image-based machine learning approach as screening tool of pathological calcium oxalate crystallization inhibitors

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Keywords: image based drug screening, crystallization disorders, kidney disease

Calcium oxalate (CaOx) crystal-induced nephropathies comprise a broad range of kidney disorders, which leave the patient at risk for developing serious complications, such as chronic kidney and end-stage renal diseases¹. CaOx crystallization inhibitors are being investigated as novel treatment modalities, however rapid and inexpensive screening methods that allow for a comparison across studies are lacking so far.

Herein, we describe a strategy for the prompt assessment of an inhibitor's impact on CaOx crystallization dynamics. Crystallization in human urine was induced by spiking with 1 mM sodium oxalate, representing a simplified pathophysiological setting. Light microscopy provided a simple and quick readout to follow the crystallization process. A semi-supervised machine learning approach was implemented to quantify the changes in the crystallization pattern with respect to the size and proportion of different CaOx crystal hydrates formed under different conditions. In brief, single crystals were segmented using classic computer vision techniques, and shape, intensity and texture features for each crystal were extracted. A training dataset was created by manually adjusting single features to distinguish the different crystal hydrate forms on single images. A support vector machine classifier was trained and subsequently used to classify crystal polymorphs on testing images.

The assay was then employed to assess the influence of a small library of *myo*-inositol hexakisphosphate (IP6) analogues on CaOx crystallization. We found a charge-dependent inhibition of CaOx monohydrate (COM) crystals, while CaOx dihydrate (COD) crystallization remained relatively unaffected. By designing new, higher charged IP6 analogues, we achieved a step-wise inhibition of first COM, then COD, and finally complete CaOx crystallization.

To conclude, the presented screening assay might serve as a useful framework to compare novel CaOx inhibitors in a rapid and inexpensive manner, allowing for a more efficient screening process and a better means of comparison between studies.

¹ Mulay, S. R. & Anders, H.-J. Crystal nephropathies: mechanisms of crystal-induced kidney injury. *Nat. Rev. Nephrol.* **13**, 226–240 (2017).

Condensation of endoplasmic reticulum membrane domains

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The endoplasmic reticulum (ER) is a highly dynamic and interconnected membrane system that extends throughout mammalian cells and interacts with almost every other organelle. Overall the ER is a continuous membrane system in which structurally different compartments provide distinct chemical environments for specific cellular tasks. The network-like smooth ER, for example, fuels protein traffic along the secretory pathway from specialized and long-lived membrane domains, called ER exit sites (ERES).

In mammalian cells, ERES self-assemble as droplet-like domains, arranged as a dispersed punctuate pattern of some hundred diffraction-limited points with a quasi-crystalline ordering [1]. Although ERES were seen to freely diffuse along ER segments on short timescales, they appear stationary on longer periods [2]. Notably, their dynamics is different from the microtubule-dependent, shivering motion of ER tubules that host the ERES [3].

Here, we quantitatively characterize the self-organization of ERES by fluorescence live-cell imaging when the topology and geometry of the ER network is altered. If ER junction stabilizing proteins were selectively knocked down by RNA interference, the ER morphology became more sheet-like and fewer ERES were observed. At the same time their size and intensity increased and the pattern of ERES lost its purely point-like character.

These findings strongly indicate that self-organization of ERES on the ER membrane system is caused by a diffusion-driven condensation phenomenon, similar to a liquid-liquid phase separation. When topological constraints are lifted, e.g. by knocking down ER-shaping proteins, diffusion of ERES constituents across ER tubules is facilitated and the previously hampered coalescence of ERES into coarse-grained droplet-like structures is rendered possible.

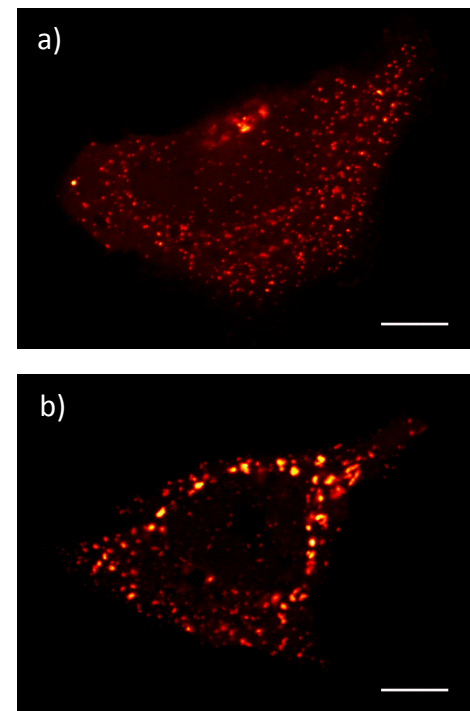


Figure 1: Endoplasmic reticulum exit sites in HeLa cells. a) wildtype b) after knockdown (scalebar is 10 μm).

[1] S. Heinzer, S. Wörz, C. Kalla, K. Rohr & M. Weiss, *J. Cell Sci.* 121(1), 55 (2008).

[2] L. Stadler, K. Speckner & M. Weiss, *Biophys. J.* 115(8), 1552 (2018).

[3] K. Speckner, L. Stadler & M. Weiss, *Phys. Rev. E* 98, 012406 (2018).

Simulation and implementation of digital micromirror devices (DMDs) for cost-effective structured illumination microscopy

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Structured illumination microscopy (SIM) provides gentle, high-speed super-resolution imaging. The fastest implementations today rely on *spatial light modulators* (SLMs) to generate the light interference patterns required for SIM. While liquid-crystal-based SLMs are well-established for SIM, those based on *digital micromirrors* are seldomly used and not well documented. The substructure inherent to these devices, in combination with the *coherent illumination* used for

SIM, yields so-called *blaze grating* effects that must be correctly modelled to employ them.

Here, we present both our *simulation approach* for DMDs in a structured illumination microscope, and the cost-effective *implementation* of such a system, along with calibration and demonstration measurements of fixed and live-cell samples.

We use numerical simulations to predict the (highly wavelength-dependent) ideal alignment of the DMD device (fig. 1), and can extract manufacturing variations by comparing simulations and reference measurements.

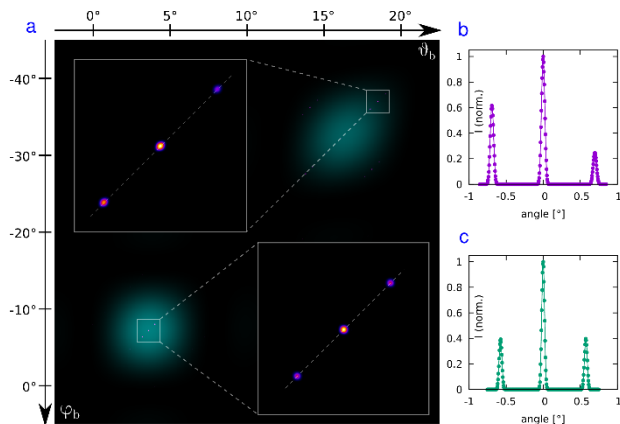


Figure 1: Numerical simulation of SIM pattern generated by a DMD. Sub-optimal alignment (a, top row) generates mismatched strength in the interference orders (b), while optimal alignment (a, bottom row) generates well-matched interference (c).

We demonstrate the use of a DMD in a cost-effective structured illumination microscope (fig. 2), where we reach 140nm resolution and sub-second image acquisition speed, all on a system with an over-all cost below \$20.000.

Both the simulation software and blueprints for the cost-effective SIM system are freely and openly available as part of the fairSIM.org project.

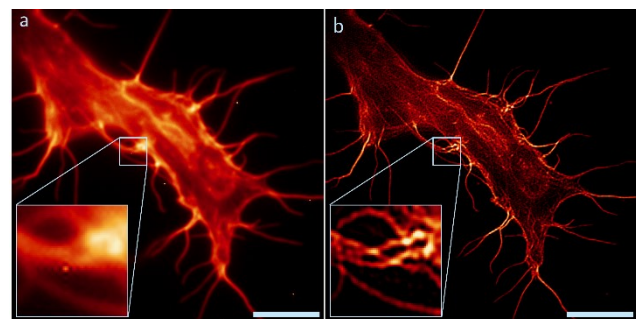


Figure 2 Wide-field (a) and SIM (b) imaging of HEK293T cells, transfected with mScarlet labelling actin. Imaged on a DMD-based, low-cost SIM microscope. Scale bar 5μm, inset (2.1μm)²

Towards correlative multimodal nanospectroscopy

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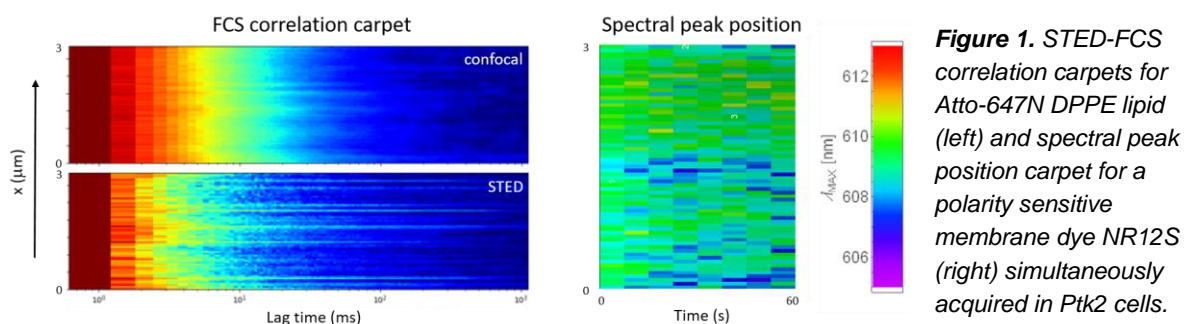
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Keywords: membrane dynamics, fluorescent probes, STED microscopy

Nanoscale organisation and dynamics of cellular structures is key to the function of living cells. Their plasma membrane, in particular, is a vital structure as it mediates all communication and trafficking, and is known to be dynamically compartmentalised to facilitate these processes. However, the exact properties and detailed functions of these heterogeneities are still largely unknown, primarily due to the small size and transient nature of the supramolecular complexes.

Amongst the most established methods to characterise the membrane environment, especially in terms of diffusion properties and lipid order, are fluorescence correlation spectroscopy and spectral imaging with polarity-sensitive probes, respectively. Recently, both have benefited from the increased resolution of STED nanoscopy [1,2] – STED-FCS in particular, as the differences in the apparent diffusion kinetics at different length scales provides additional information on underlying molecular interactions, underlying free or hindered diffusion. However, as all fluorescence methods rely on the use of extrinsic probes, the behaviour of these reporter molecules needs to be well characterised and understood. Ideally, combining several readouts into a correlative approach can offer richer description, less prone to artefacts and misinterpretations.

To this end, we combined line-interleaved scanning STED-FCS and spectral STED imaging to validate the connection between local diffusion properties and lipid packing for various types of fluorescent membrane probes in both model and cellular membranes (Figure 1). Experiments as well as simulations indicated that dye exclusion from a subdiffraction-sized zone, e.g. due to its partitioning preference for disordered membrane environment, can result in an apparent site of hindered diffusion.



- [1] E. Sezgin, F. Schneider, S. Galiani, I. Urbancic, D. Waithe, B. C. Lagerholm, C. Eggeling, *Measuring nanoscale diffusion dynamics in cellular membranes with super-resolution STED-FCS*, Nature Protocols, vol. 14, no. 4, pp. 1054–1083, 2019.
- [2] E. Sezgin, F. Schneider, V. Zilles, I. Urbancic, E. Garcia, D. Waithe, A. S. Klymchenko, C. Eggeling, *Polarity-sensitive probes for superresolution stimulated emission depletion microscopy*, Biophysical journal, vol. 113, no. 6, str. 1321-1330, 2017.

Conquering the tumour microenvironment: Using image analysis and spatial statistics to map the phenotypic heterogeneity of human cancers

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Tumours are highly heterogeneous populations of cells and intratumoral heterogeneity correlates with worse prognosis in many cancers¹. A growing body of evidence is highlighting that the tumour microenvironment contributes to both tumour growth and viability.

However, these studies have not focused on interrogating the spatial statistics of sub-clones defined by activated signalling pathways. This is mainly due to the limitations of currently available technologies to define complex immunophenotypes *in situ*. Here we circumvent this limitation by undertaking imaging mass cytometry which allows for simultaneous measurement of up to 40 markers whilst retaining the spatial relationships within the sample.

Using imaging mass cytometry we constructed an antibody panel that combines markers for tissue architecture as well as tumour and immune cell phenotyping that was then applied to a tissue microarray containing a number of different tumour types.

Analysis of imaging mass cytometry data is complex due to the low resolution and the highly multiplexed nature of the data. Here we demonstrate the generation and use of a fully automated algorithm to detect biologically meaningful cell populations in their spatial context (Tissue Phenomics, Definiens AG, Munich, Germany). On the lower hierarchical level, a heuristic segmentation algorithm first identifies the center of each nucleus using a threshold-based method on the denoised nuclear marker channels. Based on the nuclei center a knowledge-based cell model segments cellular objects which are classified to the predefined cell classes using a maximum likelihood evaluation. The cell class definition maps marker expression to cell type (Fig 1a). On the upper hierarchical level, e-Cadherin positive and α -Mentherin positive regions are detected (Fig 1b) enabling the numerical quantification of cell populations within the regions, and the spatial analysis in terms of Ripley's K function.

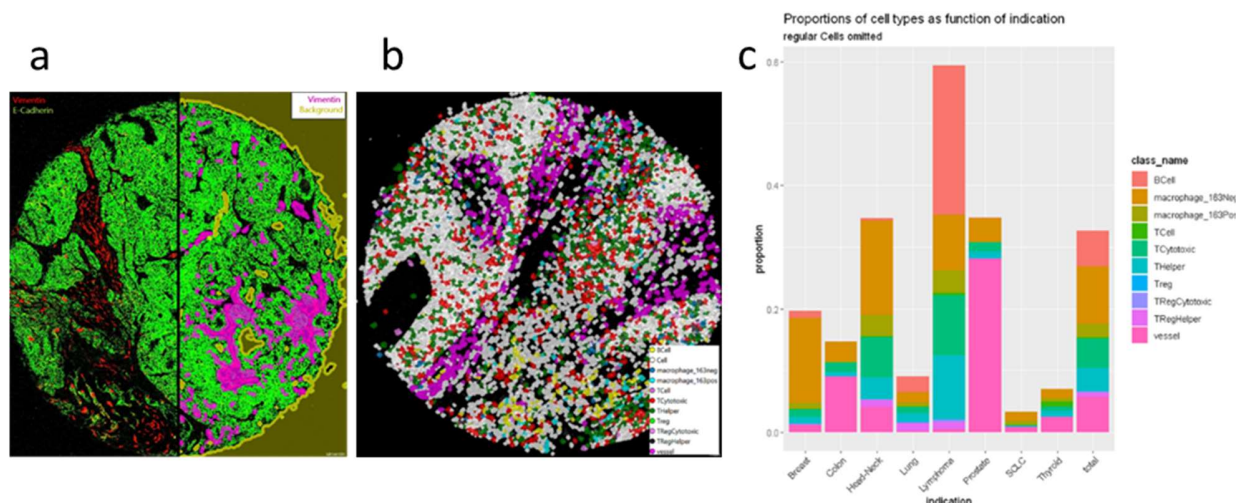


Figure 1. Examples for detected and classified cells (a) and α -Mentherin positive regions (b). (c) Shows the distribution of prevalences of cell populations across various cancer indications. We observe cancer-specific signatures of immune cell prevalences indicating biologies of the tumor microenvironment. Moreover, by analyzing the spatial distributions of immune cells we detect specific patterns which again may lead to different strategies for treatment.

¹ Yuan Y, Cold Spring Harb Perspect Med. 2016 Aug; 6(8)a026583.

Organization and dynamics of *Escherichia coli* outer membrane proteins

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Despite their diminutive size, bacterial cells—such as *E. coli*—can display subcellular protein organization. Many examples have been demonstrated for proteins in the cytoplasm and inner membrane. However, much less is known about such organization in the outer membrane of *E. coli*. Proteins in the outer membrane can be divided into two groups: beta barrel outer membrane proteins (OMPs) and lipoylated proteins that are anchored to the inner leaflet of the outer membrane. In the present study, we sought to determine the dynamics and organization of these two protein classes in the outer membrane of the model organism, *E. coli*. For an OMP, we focused on OmpF, which allows the passive diffusion of small nutrients and metabolites across the outer membrane. For a lipoprotein, we focused on Pal, which associates with the cell wall to stabilize the outer membrane. Both serve important functions in bacteria and both are present at similarly high copy numbers (~100,000/cell). Imaging these proteins required distinct approaches: OmpF was visualized using exogenous, high-affinity colicin-N based fluorescent labels, which we confirmed bind exclusively to OmpF *in vivo*. Pal, by contrast, was imaged using a chromosomally encoded Pal-PAmCherry fusion. Using high throughput widefield fluorescence microscopy analysis, OmpF distribution was shown to fall into six broad categories, all of which are distinct to the punctate distribution observed previously for low copy number OMPs such as BtuB (Rassam et al (2015) Nature 523, 333). Fluorescence recovery after photobleaching (FRAP) and single particle tracking (SPT) indicated that OmpF lacks long range diffusion, instead showing confined diffusion restricted to small areas on the cell surface. Pal distribution differs significantly to that of OmpF. Photoactivated localization microscopy based single particle tracking (PALM-SPT) indicated uniform Pal distribution in the outer membrane of non-dividing cells that accumulated in the septum of dividing cells. SPT data also indicated highly restricted, confined diffusion in the OM, but, unlike OmpF, Pal diffusion characteristics are attributed to non-covalent binding to the cell wall.

High-throughput multicolor 3D localization in live cells by depth-encoding imaging flow cytometry

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Keywords: 3D localization, high throughput, multicolour imaging

Traditionally, 3D microscopy requires z-scanning of either the sample (for optical sectioning) or fiducials (for calibration). In microfluidic imaging systems, where high flow rates rapidly move objects through the imaging volume, scanning over a single object is typically not practical. In this work we demonstrate high-throughput 3D co-localization of fluorescent emitters by combining imaging flow cytometry with point-spread function (PSF) engineering. We encode the depth of fluorescent emitters into the PSF of the flow-imaging system, and then leverage the statistical properties of laminar flow to calibrate each measurement [1]. This is accomplished by adding a calibration standard, i.e. beads, to the imaging buffer, then analyzing the distribution of shapes present in collected images (Figure 1). This calibration is then applied to sample images, thus attaining 3D localizations. Notably, our method can be implemented into existing commercial apparatus with only a minor hardware addition.

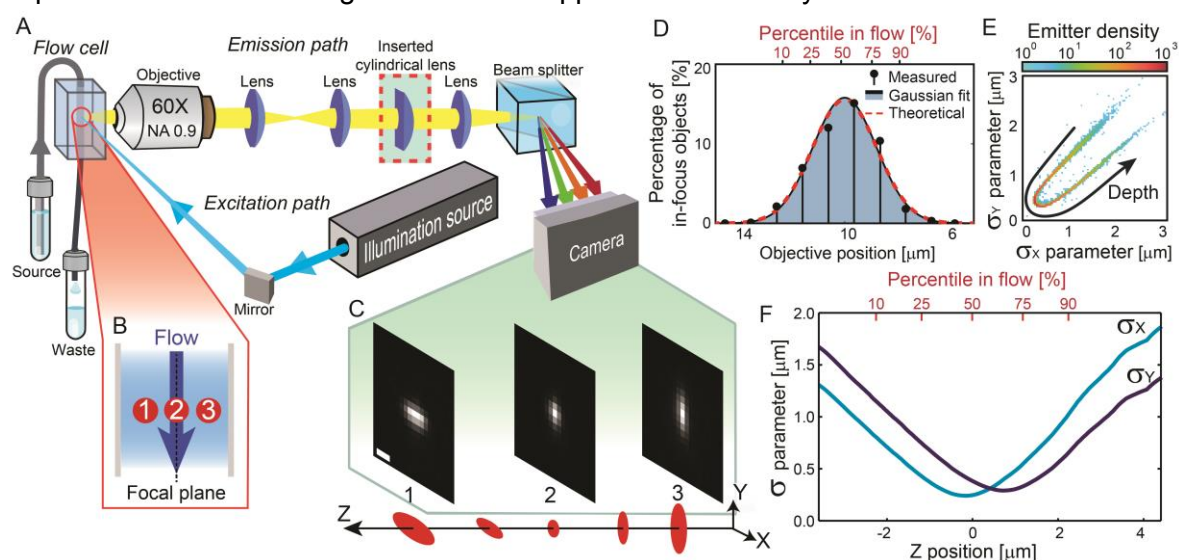


Figure 1. 3D calibration by depth-encoded imaging flow cytometry. A) The optical system. B) objects imaged by the device have their depth encoded in the C) image shape. D) The flow profile in the device is used to calibrate the E) the image shape distributions to F) extract the 3D PSF calibration.

We validate our approach by co-localizing fluorescent beads in six color channels. To demonstrate the method in live-cell imaging, we characterized chromatin compaction states in live yeast cells at unprecedented throughput: 1000s of cells per minute. Finally, we show that our technique is generally applicable to other engineered PSFs and can be used to improve the depth range of the device by more than 4X.

[1] Weiss, L.E., Ezra, Y.S., Goldberg, S.E., Ferdman, B., & Shechtman, Y. (2019). High-throughput multicolor 3D localization in live cells by depth-encoding imaging flow cytometry. BioRxiv.

Understanding and Modeling Inflammation Induced Immuno-Suppression in Tumors Using Multi-plex Immunofluorescence and Live Cell Microscopy

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Key words: inflammation, multi-plex, live-cell

Tumorigenesis is promoted by inflammation through immune-suppression and is mediated by intercellular mechanisms between immune cells, host stroma, vasculature, extra-cellular matrix and tumor cells. Thus, we are integrating sample preparation (in vitro and ex vivo), image acquisition and analysis to investigate these phenomena at cell to tissue scales. Microscopic imaging of multiplex immunofluorescence (MIF) labeled thin ($\approx 10 \mu\text{m}$) tissue sections is the choice technique for elucidating these mechanisms, because 20+ labels can be cyclically applied to the same section while preserving the spatial organization of cells. However, since samples are fixed, MIF only reports one time point. To overcome this, we model tumor-immune cell interactions in vitro by imaging live co-cultured cells in a restricted exchange environment chamber (REEC). Cells in the chamber consume oxygen and nutrients diffusing from a single source, generating radial gradients of these molecules, which in turn generate distinct cell phenotypes depending on radial position.

We applied these technologies to further our understanding of proinflammatory signaling as it pertains to immune response to treatment and the identification of novel therapeutic targets associated with inducible nitric oxide synthase (Nos2) and cyclooxygenase (Cox2) in the 4T1 syngeneic mouse model of human triple-negative breast cancer. High expression of both proteins has been reported to strongly predict poor survival in patients with ER- cancer. Treatment with indomethacin, a Cox2 inhibitor, or irradiation or both significantly increased CD8 positive T cell infiltration into 4T1 tumors, with a notable increase in the CD8 to CD4 cell count ratio with indomethacin treatment alone. This suggests a successful inhibition of immune-suppression by the drug. We discovered that Nos2 and Cox2 expression is spatially distinct in 4T1 mouse tissue, in spheroids grown from 4T1 cells and amongst 4T1 cell cultured in the REEC. This suggests that the feed-forward loop, which drives expression of Nos2 and Cox2, is an intercellular mechanism. Moreover, in the latter two environments Nos2 cells were localized in hypoxic regions, as anticipated since hypoxia and nutrient deprivation drive Nos2 expression.

Ongoing studies are: (1) discovering a transient epithelial to mesenchymal transition during migration of 4T1 cells in a low oxygen and low glucose (hypoxic) environment in the REEC, and (2) developing 3D imaging methods for thicker ($> 50 \mu\text{m}$) sections, including tissue clearing and expansion, so that individual cell analysis is more accurate.

We gratefully acknowledge contributions of the Pathology / Histotechnology Laboratory at NCI – Frederick and support from Dr. David Goldstein, Office of Science and Technology Resources, CCR. Funded by NCI Contract No. HHSN261200800001E.

Automated lens-free, fluorescent microscopy to study cell populations

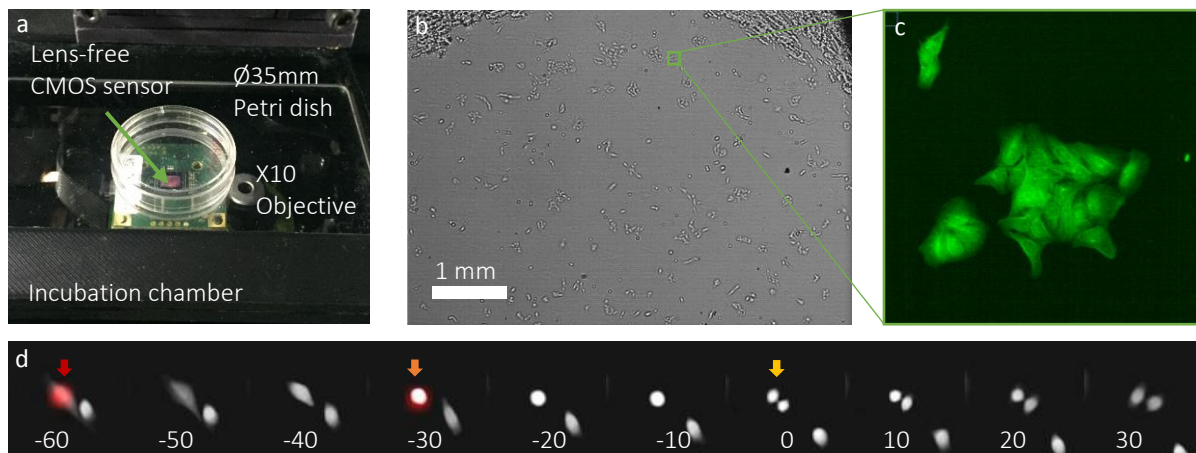
Kraemer, D. C., Allier, C., Hervé, L., Padmanabhan, K., Foudi, A., Morales, S.

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Cellular systems are inherently heterogeneous and cellular heterogeneity fundamentally shapes population behavior. The description of populations often falls short of portraying single cell dynamics. Equally, rare events can appear negligible in the ensemble of population statistics. Vice versa, a focus on individual cells may lack the holistic description of the entire cell population. To combine population statistics with a more in depth cellular view, we are developing a microscope that combines lens-free wide-field quantitative phase imaging (30mm²) with fluorescence microscopy. The new microscope performs live-cell imaging that alternates between the cell population and a small field of view, thereby enabling to reconcile the two domains. The microscope is fully automated and performs decisions to point the fluorescence image acquisition to the next meaningful event, a concept we term “smart microscopy”.

To date we focused on mitosis observation, an important cellular event, crucial in determining cell fate and development. Using a machine learning based detection approach on the reconstructed lens-free images resulted in highly reliable mitosis monitoring. Importantly, it allows to reveal in advance cells that are due to enter mitosis. Fluorescence imaging can thereupon be guided to acquire time-lapse movies of cells entering division up to the cytokinesis. With this setup we expect to gather thousands of time-lapse sequences in a single experiment.



a: The imaging setup, with the Petri dish placed in an incubation chamber, b: The lens-free image after reconstruction, c: Zoomed in view of a cluster of cells, d: Time-lapse acquisition of a cell undergoing mitosis as detected by the algorithm, time between images is 10 minutes. Red arrow indicates detection, one hour before cell division; orange arrow indicates detection of cell rounding; yellow arrow shows the splitting event.

Automating FLImP – macromolecular structure fingerprints for personalised cancer therapy

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FLImP (Fluorescence Localisation Imaging with Photobleaching) is a single-molecule fluorescence microscopy-based method which reveals fingerprints of macromolecular structures in cell membrane receptors with ~5nm resolution. It was developed in STFC to investigate interactions of the Epidermal Growth Factor Receptor (EGFR) family, whose interactions are critical to the development and treatment of many cancers. The FLImP fingerprints of these receptors, essentially high-resolution distributions of receptor-receptor separations in cells, reveal structures and interactions in unprecedented detail in-situ in cells where their effects are evident and have life-changing implications.

Exploited in tandem with single-molecule tracking, molecular simulations and prior structural knowledge FLImP provides a powerful tool for biomedical research into cancer. Translating this partially-automated but still labour-intensive method to a fast, automated assay accessible in the clinic will provide a tool with unprecedented precision for biomedical research and for personalised diagnosis and treatment of cancer. We are now working on this project, using machine learning methods to achieve full automation of our pipeline from data acquisition to the final FLImP fingerprint and its clinical exploitation, and improving many aspects of FLImP to increase precision and efficiency.

I will present our work in the development and application of FLImP to understand EGFR signalling in cancer, outline our recent progress in improvement and automation of FLImP and our future plans.

- [1] Needham, S. R. et al. Measuring EGFR separations on cells with similar to 10 nm resolution via fluorophore localization imaging with photobleaching. PLoS ONE 8, e62331 (2013).
- [2] Needham, S. R. et al. EGFR oligomerization organizes kinase-active dimers into competent signalling platforms. Nat. Commun. 7, 13307 (2016).
- [3] Zanetti-Domingues, L. C., et al., The architecture of EGFR's basal complexes reveals autoinhibition mechanisms in dimers and oligomers, Nat. Commun. 9, 4325 (2018).

Faster FLImP - Towards High throughput image acquisition and track selection

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Fluorophore **L**ocalization **I**maging with **P**hotobleaching (FLImP) is a single molecule localization technique for determining receptor oligomer structural fingerprints with $\sim 5\text{nm}$ resolution¹. To date, the FLImP image acquisition and analysis process has been a manually intensive, requiring operators to select an appropriate region of interest for time-series imaging, facilitate detection of subsets suitable for drift correction and selection of tracks appropriate for subsequent analysis.

This work outlines recent progress towards automation of the FLImP image acquisition and track selection process, including the use of semantic segmentation based deep learning for automatic region of interest detection and methods to automate the process of FLImP track selection while extending the technology to assess multiple separations from the same track. Together this work dramatically increases the rate of data acquisition with the ultimate goal of increasing the accessibility and utility of single molecule imaging technologies and their application in meeting emerging biological and clinical challenges.

¹ Sarah R. Needham, et. al. (2013): "Measuring EGFR separations on cells with similar to 10 nm resolution via fluorophore localization imaging with photobleaching", PLoS ONE 8(5): e62331. doi:10.1371/journal.pone.0062331.

Multi-parameter Single Molecule Spectroscopy Imaging Reveals Intra- and Inter- Molecular Conformations of HIV-1 Envelope on Native Viruses

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Keywords: HIV-1, smFRET, FLIM, bNAbs

HIV-1 Envelope (Env) engaged with CD4 and co-receptors (either CXCR4 or CCR5) mediates viral membrane fusion between HIV-1 viruses and the host. Even if structure-based reverse vaccinology represents a big hope in the field to develop a new vaccine, it can be argued that new approaches are needed to better define the plasticity of Env epitopes in the presence and absence of broadly neutralizing antibodies (bNAbs). Indeed, the structural flexibility of Env can prevent access to the epitope while still establishing functional interactions with the cellular receptor and co-receptor.

Here, we present a novel approach based on coupling single molecule FRET with multiphoton Fluorescence Lifetime Imaging to measure HIV-1 Env conformational dynamics in single HIV-1 viruses labelled in V4 and V1 Env domains, in the presence and absence of bNAbs (10E8, b12, PGT145). Analyses of the apparent FRET efficiency and lifetime fluctuations showed the inter- and intra-molecular dynamics of HIV-1 Env proteins in single viruses and, more importantly, when engaged with T Cells during the HIV-1 pre-fusion reaction.

The combination of single molecule FRET, lifetime imaging and real-time single virus tracking using multiphoton excitation allowed us to define previously unknown intra- and inter-molecular steps during the pre-fusion reaction in live cells. These new insights will help to better characterize Env rearrangements and epitope exposure during the pre-fusion reaction and the mechanism/s by which bNAbs disrupt HIV-1 fusion in live T cells.

Towards multi-dimensional FLImP

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Fluorophore **L**ocalization **I**maging with **P**hotobleaching (FLImP) is a single molecule localization technique for determining receptor oligomer structural fingerprints with ~ 5 nm resolution¹. While the current FLImP technology allows determination of molecule separations within an oligomer complex, higher-order information, such as that regarding the relative positions of these objects is not presently considered. This work outlines recent progress towards extending the FLImP methodology to resolve this problem, in conjunction with techniques to improve the accuracy of FLImP localizations, particularly when working with crowded fields. In addition, with the push towards automation and higher-throughput single molecule microscopy, this work forms part of a larger project that aims to fully automate FLImP data acquisition and analysis processes with the ultimate goal of increasing the accessibility and utility of single molecule imaging technologies and their application in meeting emerging biological and clinical challenges.

1 Sarah R. Needham, et. al. (2013): "Measuring EGFR separations on cells with similar to 10 nm resolution via fluorophore localization imaging with photobleaching", PLoS ONE 8(5): e62331. doi:10.1371/journal.pone.0062331.

Breaking New Ground in Live and Label-free Volumetric Imaging

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Keywords: light sheet, label-free, multiphoton, non-linear, volumetric

In recent years light sheet fluorescence microscopy has been used to great effect in rapid 3D imaging of biological systems. However, this requires labelling by exogenous dyes or endogenously expressed genetically engineered fluorescent proteins that can be perturbative to natural processes and cause issues such as bleaching and phototoxicity. This prevents long-term live imaging without photodamage. Label-free imaging methods combined with light sheet microscopy have great potential to reduce potentially damaging sample preparation steps, and enhance technological capabilities for imaging samples live in their native state over prolonged periods.

In this work we show long-term imaging of mammalian cells (single cells and 3D cultures) using non-linear label-free techniques such as two-photon excited autofluorescence (2PEF) on a light sheet microscope. 2PEF is excited using near-infrared laser wavelengths, which are ideal for deep penetration into biological samples and cause reduced phototoxicity. We use a type of Airy beam [1] to obtain a field-of-view that is 10x larger than with corresponding Gaussian illumination for non-linear imaging. We experimentally establish the trade-off between the field-of-view and signal-to-noise for damage-free imaging by non-linear techniques in order to enable long term monitoring. We have been able to establish a light sheet microscopy system such that 3D imaging of highly sensitive oocytes (mouse) is possible and their normal cell cycle can be imaged without photodamage. We can perform multi-colour live imaging and monitor metabolic activity by imaging FAD (flavin adeno-dinucleotide) and NADH (nicotin-amide dehydrogenase), and in full 3D where required. Our work shows that the ability to image sensitive biological samples in 3D, while maintaining good image contrast without exogenous fluorescent labels and ensuring minimal photodamage to image changes and sub-cellular dynamics, can be a powerful tool for furthering research and applications in life sciences, and especially, in developmental biology.

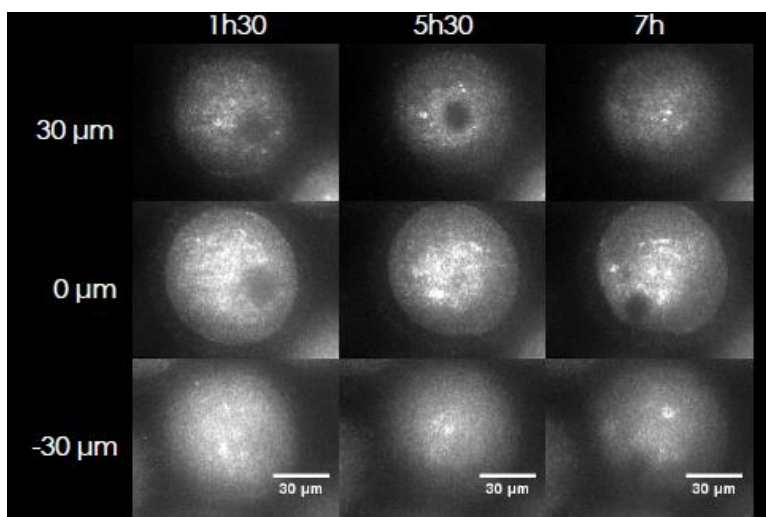


Figure 1. Two-photon label-free images of a mouse oocyte in 3 different planes acquired on an Airy beam light sheet microscope. Autofluorescence from NADH provides good contrast without bleaching and photodamage and images functional dynamics. Exc: 740 nm (120 fs, 80 MHz laser), Em: 450 nm

[1] T. Vettenburg, H. C. Dalgarno, J. Nylk, C. Coll-Lladó, D. E. K. Ferrier, T. Čižmár, F. J. Gunn-Moore & K. Dholakia, *Nature Methods*, 11, 541–544, 2014.

User-friendly Building of Reconstruction Algorithms with GlobalBioIm

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Abstract

The current shift towards computational imaging has made reconstruction procedures an integral part of many advanced imaging systems. A consequence is that imaging scientists now commonly require efficient and reliable computational tools for solving their inverse problems.

To this end, we recently developed *GlobalBioIm*, an open-source Matlab library that standardizes the resolution of a wide range of imaging problems [1]. This toolbox gives access to cutting-edge reconstruction algorithms, and can be extended to new modalities and methods by combining elementary modules. The versatility and efficiency of *GlobalBioIm* have been highlighted in a series of recent high-impact works [2-4].

Driven by these encouraging applications, we have devoted our efforts towards improving the usability of *GlobalBioIm* by those with limited expertise in inverse problems and optimization theory. The outcome is a new user-friendly Matlab interface (Figure 1) that allows non-experts to intuitively build tailored reconstruction algorithms with minimal effort.

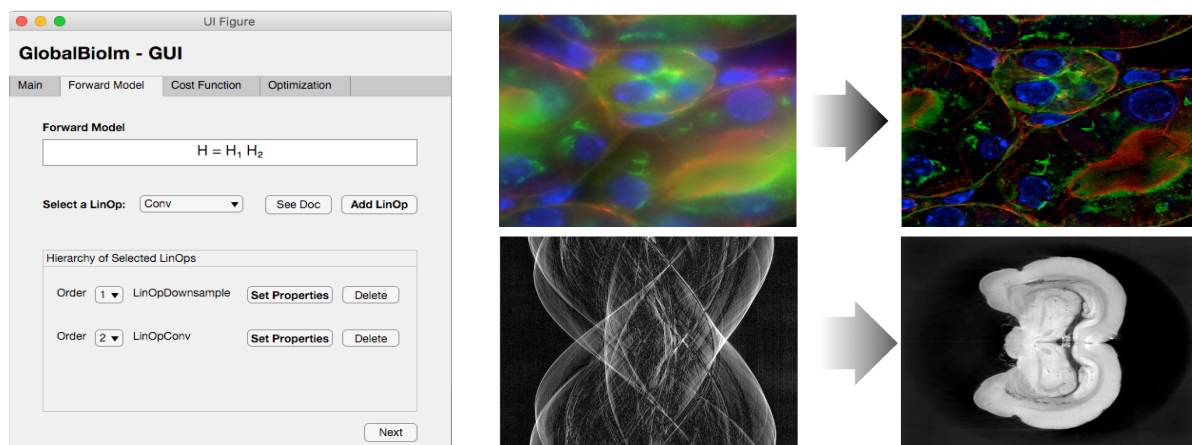


Figure 1: The new *GlobalBioIm* GUI (left) can be used to solve various inverse problems (right).

This work was supported by the grant ERC-692726-GlobalBioIm.

- [1] E. Soubies et al, *Pocket guide to solve inverse problems with GlobalBioIm*, Inverse Problems, 2018
- [2] D. Gambarotto et al, *Imaging cellular ultrastructures using expansion microscopy*, Nature Methods, 2018
- [3] C. Chen et al, *Imaging neural activity in the ventral nerve cord of [...] Drosophila*, Nature Communications, 2018
- [4] E. Soubies et al, *Nanometric axial resolution of fibronectin [...] with multi-angle-TIRF*, Nature Sci. Reports, 2019

Automated, User-independent Correction of Artifacts in Fluorescence Correlation Spectroscopy Measurements using Convolutional Neural Networks

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Fluorescence correlation spectroscopy (FCS) is a well-established tool for studying molecular dynamics. Unfortunately, the single-molecule sensitivity and high dynamic resolution of FCS comes with the cost of a variety of hardware- and sample-related artifacts, such as photobleaching, contamination from additional slow moving particles, or sudden drops in intensity because of detector anomalies¹. These artifacts distort the measured fluorescence traces, as well as the analysis by auto-correlation, and render them useless. In practice, these corrupted traces are often filtered by hand. Here, we show that a variety of commonly seen artifacts can be corrected by automatic cropping of fluorescence traces through using convolutional neural networks (CNNs). The models were trained on data generated using an established FCS trace simulation algorithm based on 2-dimensional stochastic Brownian motion². Next, the models were validated on experimental data. Finally, we performed a statistical comparison between the auto-correlation results of the traces corrected by our system and the auto-correlation results of a set of non-corrupted traces. Our approach offers a reproducible, user-independent solution on how to handle FCS measurements distorted by different artifacts without having to discard the whole trace.

1. Sezgin E, Schneider F, Galiani S, Urbančič I, Waithe D, Lagerholm BC, Eggeling C., Apellaniz B, Sicard T, Julien JP, Eggeling C, Nieva JL. Measuring nanoscale diffusion dynamics in cellular membranes with super-resolution STED-FCS. *Nature Protocols*, 14(4), 1054-1083, (2019)
2. Waithe D, Schneider F, Chojnacki J, Clausen MP, Shrestha D, de la Serna JB, Eggeling C. Optimized processing and analysis of conventional confocal microscopy generated scanning FCS data. *Methods*, 140-141, 62-73, (2018)

SpineJ: A software tool for quantitative analysis of nanoscale spine morphology

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Keywords: Wavelet-based filtering, gradient field, Delaunay triangulation

Dendritic spines feature nanoscale structural details, such as spine necks, which are functionally very important but cannot be properly resolved by conventional optical techniques. By breaking the diffraction limit of light, super-resolution fluorescence microscopy provides access to the intricate morphology of neurons in living brain tissue, resolving their finest structural details, which are critical for neuronal function. However, as existing image analysis software tools have been developed for diffraction-limited images, they are generally not well suited for quantifying nanoscale structures like dendritic spines. The working standard is therefore manual analysis, a time-consuming solution inevitably introducing variability and bias.

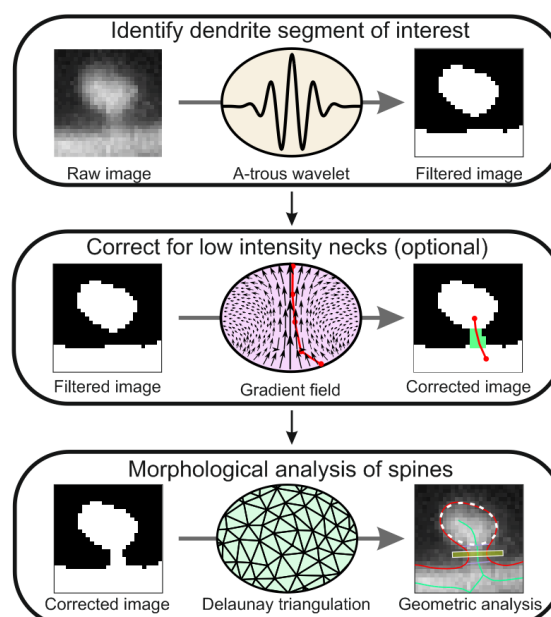


Figure 1: SpineJ workflow.

We introduce SpineJ, a new ImageJ plugin to extract geometric information about nanoscale details of dendritic spines. Its workflow is composed of three main steps: (i) Interactive wavelet-based filtering to binarize the dendritic structures of interest from the background. (ii) Semi-automatic reconnection of spines that may have been erroneously separated from the dendritic shaft, which can happen with very thin and weakly fluorescent necks. (iii) Skeletonization-based segmentation allowing quantitative morphological analysis of spine neck width, length and head surface.

SpineJ only requires basic ImageJ skills and comes with an intuitive user-friendly GUI that allows swift collection of spine geometry data. Its strength lies in its ability to analyze spine neck geometry in a quick and reproducible manner, necessitating much less time than manual analyses while exhibiting diminished variabilities of the measurements.

Developing microscope software with Python

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Keywords: control, hardware, python, microscope, software

We have created Microscope and Cockpit, two Python packages to dramatically reduce effort required for bespoke microscope development. Scientists that would rather be building microscopes are instead spending their time in low-level software development and GUI toolkits to control the microscopes. This software ends up being tied to the specifics of the devices, impossible to maintain, or incur ongoing licensing costs. Effort is wasted as scientists in different labs, and even in the same lab, keep independently implementing solutions to the same problems.

Microscope is a Python package that provides remote control of microscope devices, abstract base classes of different type of devices, and concrete classes for the devices we had access to. Remote control enables us to control arbitrarily complex microscopes by distributing devices across multiple machines, while the common interface enables us to swap devices from different vendors without changes to the underlying code.

On top of Microscope we have created Cockpit, a graphical interface for microscope control and running both simple and complex experiments. The Cockpit interface adjusts to the available devices, providing device independent access to a wide range of hardware. Experiments are written in Python and provide generic experiment types such as time lapse or Z-stacks, as well as much more complex experiments, independent of the actual device details.

We have successfully deployed Cockpit on a number of bespoke microscope systems, and used Microscope in student projects and for the development of microscope tools.

Random Illumination Microscopy (RIM) : nanoscopy in living tissues with neither calibration nor adaptive optics

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We propose a new method, Random Illumination Microscopy (RIM) to combine the best features of Structured Illumination Microscopy (SIM) and optical fluctuation microscopy, while minimizing their theoretical and practical flaws. Our approach is inspired by SIM in which the use of inhomogeneous excitation patterns gives access to object information that are inaccessible in brightfield microscopy [1]. To overcome the major issue, both from the experimental and algorithmic point of view, of the control and knowledge of the excitation patterns, we replace the periodic illumination of classical SIM by unknown speckles [2-4]. We develop a robust reconstruction scheme which only necessitates the knowledge of the statistical behavior of the random speckles. Our inversion approach avoids the widely used sparsity constraint [4] to better respect the dynamic range of the fluorescence density (especially in the case of high surface densities of fluorochromes). We demonstrate the versatility of this approach by imaging

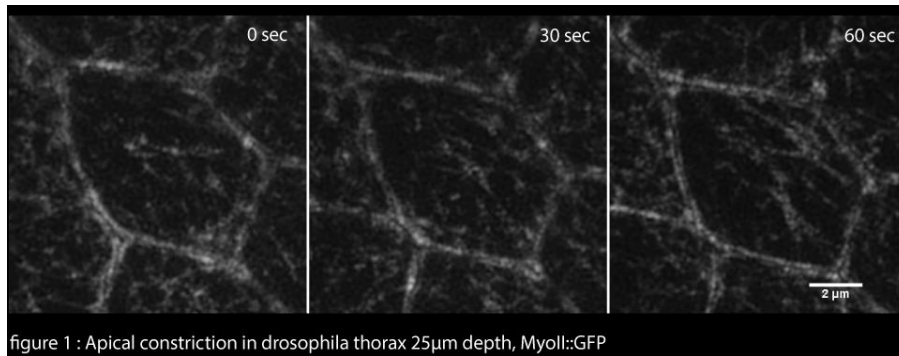


figure 1 : Apical constriction in drosophila thorax 25µm depth, MyoII::GFP

various types of cells and thick model organisms in a simple widefield configuration. The low toxicity and high temporal resolution of Random Illumination Microscopy enables the imaging of critical

biological function like mitosis, cell migration or subcellular force production during a morphogenetic drosophila pupa until 50µm depth.

[1] Gustafsson, M.G.*et al.* Three-dimensional resolution doubling in wide-field fluorescence microscopy by structured illumination. *Biophysical Journal* **94**, 4957–4970 (2008).

[2]. Emeric, M. *et al.* Structured illumination microscopy using unknown speckle patterns. *Nature Photonics*. **6**, 312-315 (2012).

[3]. Negash, A. *et al.* Improving the axial and lateral resolution of three-dimensional fluorescence microscopy using random speckle illuminations. *J. Opt. Soc. Am. A* **33**(6), 1089-1094 (2016).

[4]. Labouesse, S. *et al.* *IEEE Transactions on Image Processing*, **26**(5), 2480-2493 (2017).

An extended quantitative model for super-resolution optical fluctuation imaging (SOFI)

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Keywords: Super Resolution, SOFI, fluorescent microscopy

Super-resolution optical fluctuation imaging (SOFI) provides super-resolution (SR) fluorescence imaging by analyzing fluctuations in the fluorophore emission. The technique has been used both to acquire quantitative SR images and to provide SR biosensing by monitoring changes in fluorophore blinking dynamics. Proper analysis of such data relies on a fully quantitative model of the imaging. However, previous SOFI imaging models made several assumptions that can not be realized in practice. In this work[1] we address these limitations by developing and verifying a fully quantitative model that better approximates real-world imaging conditions. Our model shows that (i) SOFI images are free of bias, or can be made so, if the signal is stationary and fluorophores blink independently, (ii) allows a fully quantitative description of the link between SOFI imaging and probe dynamics, and (iii) paves the way for more advanced SOFI image reconstruction by offering a computationally fast way to calculate SOFI images for arbitrary probe, sample and instrumental properties.

[1] Vandenberg et al., "An extended quantitative model for super-resolution optical fluctuation imaging (SOFI)," Opt. Express 27, 25749-25766 (2019)

High density 3D localization of fluorescent molecules

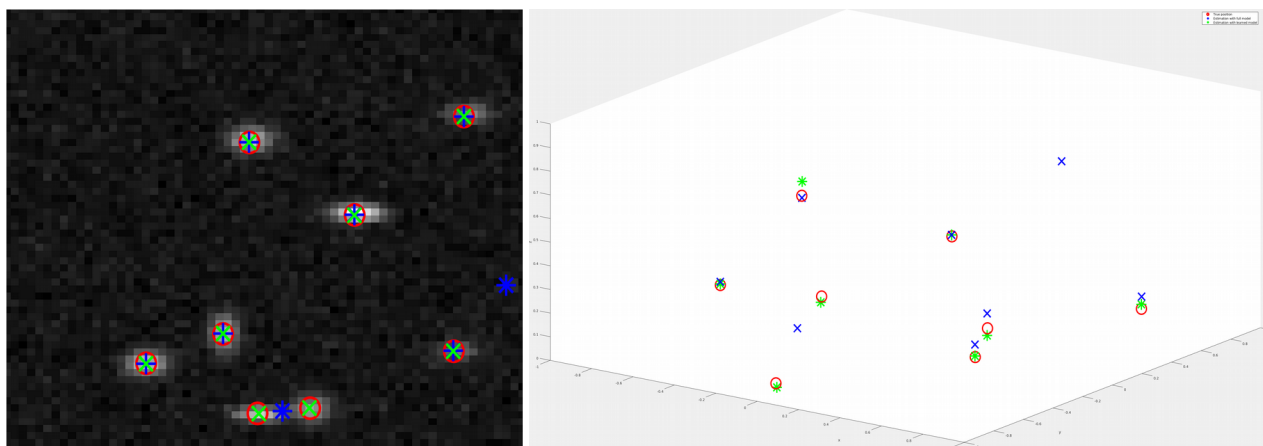
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Single molecule localization microscopy allows to break the diffraction limit and provides significantly increased resolutions. The efficiency of the computational approaches however strongly depends on the density of excited molecules. In low density, simple correlation based methods allow to achieve results on par with the state-of-the-art. This regime is time consuming which makes it incompatible with high content screening microscopy. On the other hand, the high-density localization estimation is much more complex but speeds up the acquisition process, increasing the range of potential biological applications. Methods based on off-the-grid total variation minimization were shown to be very promising in terms of accuracy in the recent 2D localization challenge [1] and enjoy many desirable theoretical properties [2]. These methods however suffer from a serious computational bottleneck, making them irrelevant in 3D localization microscopy for now.

We propose to use a different mathematical formulation of the problem that allows recovering the complexity of the 2D problem while still preserving the good properties of the original model. Instead of modeling the full 3D forward model, we propose to decompose the PSFs on a low dimensional basis learnt from a single 3D PSF model (astigmatism, double-helix,...). This leads to a 2D vectorial total variation minimization problem which can be tackled efficiently using recent advances in semi-infinite programming [3]. The approach considerably reduces the numerical complexity of the method. These ideas can be incorporated in deep learning architectures [4], which may come with additional advantages in the long run.



Red circle: ground truth position. Green star: estimated location with low dimensional basis. Blue cross: estimated location with full 3D PSF model. Left: 3D localization plot. Right: lateral localization (x and y axis).

[1] Sage, D., Pham, T. A., Babcock, H., Lukes, T., Pengo, T., Velmurugan, R., ... & Archetti, A. (2018). Super-resolution fight club: A broad assessment of 2D & 3D single-molecule localization microscopy software. *BioRxiv*, 362517.

[2] Denoyelle, Q., Duval, V., & Peyré, G. (2017). Support recovery for sparse super-resolution of positive measures. *Journal of Fourier Analysis and Applications*, 23(5), 1153-1194.

[3] Flinth, A., de Gournay, F., & Weiss, P. (2019). On the linear convergence rates of exchange and continuous methods for total variation minimization. *arXiv preprint arXiv:1906.09919*.

[4] Speiser, A., Turaga, S. C., & Macke, J. H. (2019). Teaching deep neural networks to localize sources in super-resolution microscopy by combining simulation-based learning and unsupervised learning. *arXiv preprint arXiv:1907.00770*.

Innovative buffer for long-lived fluorescence imaging for 2D and 3D dSTORM

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Keywords: dSTORM, centrosome, Eternity buffer

Direct stochastic optical reconstruction microscopy (dSTORM), developed in the last decade, has revolutionised optical microscopy by enabling scientists to visualise objects beyond the resolution provided by conventional microscopy (200 nm). dSTORM is based on the localisation of individual fluorophores that stochastically oscillate between an ON (bright) and OFF (dark) state, obtained in a special buffer containing a thiol reducer and a low oxygen concentration. This latter condition is typically obtained through the use of oxygen scavenging enzymes, such as the enzyme pair glucose oxidase/catalase in the presence of glucose. Although efficient, this enzymatically deoxygenated *buffer is short-lived and must be replaced every 2-3 h, which is both time- and buffer-consuming.*

Our current study aimed at developing a simple strategy to maintain an efficient long-term blinking phenomenon within an aqueous buffer to keep high compatibility with most dSTORM imaging modalities. The alternative buffer obtained (named Eternity [1]), resulted in the photostability of our 2D dSTORM imaging for up to several weeks. Furthermore, the in-house designed 1 µm in diameter fluorescently-labelled (Alexa 647) LipoParticle, enabled us to evaluate this novel buffer independently of biological variability, demonstrating (i) the stability of the LipoParticle/Eternity buffer combination at pH 8 and 5, (ii) the quality of our 3D dSTORM image reconstruction. Finally, the Eternity buffer revealed 2D dSTORM images of centrosomes *in cellulo* over a period of 17 days enabling the detection of individual appendages and demonstrated a localisation precision within the 10 nm range for *in cellulo* 2D imaging of centrosomes, as well as their reliable reconstruction in 3D dSTORM.

- [1] A. Provost, C. Rousset, L. Bourdon, S. Mezhoud, E. Reungoat, C. Fourneaux, T. Bresson, M. Pauly, N. Béard, L. Possi-Tchouanlon, B. Grigorov, P. Bouvet, J-J Diaz, C. Chamot, E-I Pécheur, C. Ladavière, M-T Charreyre, A. Favier, C. Place, K. Monier, *Innovative particle standards and long-lived imaging for 2D and 3D dSTORM*, Scientific Reports, Final revision, 2019.

Single-Particle Diffusion Characterization by Deep Learning

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Keywords: single particle tracking, neural networks, diffusion

Diffusion is a vital mechanism in cellular biology, playing a crucial part in numerous processes such as protein organization, transport mechanisms, membrane translocation etc. Improvements in single molecule tracking techniques have led to substantial evidence that many cellular systems do not display classical Brownian motion, but rather exhibit the more interesting case of anomalous diffusion. Anomalous diffusion differs from Brownian motion in that the mean squared displacement (MSD), does not grow linearly in time, but instead behaves in a general form of a power-law with some exponent $0 < \alpha < 2$. Where $\alpha < 1$ indicates sub-diffusion, and $1 < \alpha < 2$ indicates super-diffusion. Characterization of the underlying physical process manifested in anomalous diffusion remains a challenging problem. Even extracting the diffusion power-law α with the MSD in its various forms is hindered by experimental conditions e.g., photobleaching, that prevent the acquisition of long trajectories of tracer proteins.

Here, we present a novel method for identification and characterization of anomalous diffusion processes, in which we leverage Deep Learning to overcome several key limiting factors of existing methods [1]: namely, we classify single particle trajectories to different anomalous diffusion models and estimate relevant parameters, requiring a fraction of the data needed by current common approaches and achieving higher accuracy. We first show classification of single-particle trajectories by diffusion type: Brownian motion, fractional Brownian motion and continuous time random walk. Next, we demonstrate the applicability of our network architecture for estimating the Hurst exponent for fractional Brownian motion and the diffusion coefficient for Brownian motion on both simulated and experimental data. These networks achieve greater accuracy than time-averaged MSD analysis on simulated trajectories while only requiring as few as 25 steps. When tested on experimental data, both net and ensemble MSD analysis converge to similar values; however, the net needs only half the number of trajectories required for ensemble MSD to achieve the same confidence interval. Finally, we extract diffusion parameters from multiple extremely short trajectories (10 steps) using our approach.

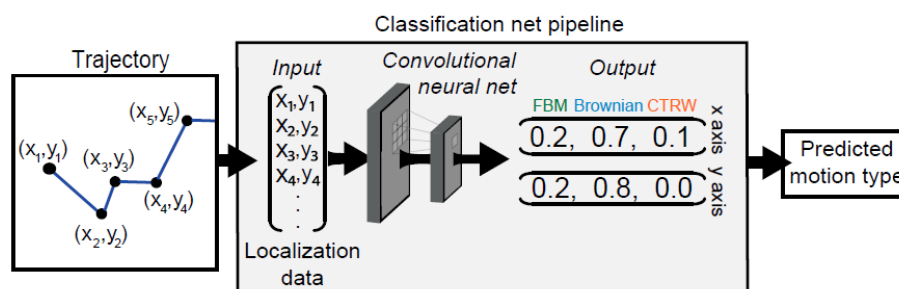


Figure 1. A schematic representation of the classification neural network is shown. The net input is the position coordinates of a single trajectory and outputs are either the model classification, as depicted here, or the relevant diffusion-process parameters (adapted from[1]).

- [1] Granik, Naor, Lucien E. Weiss, Elias Nehme, Maayan Levin, Michael Chein, Eran Perlson, Yael Roichman, and Yoav Shechtman. "Single particle diffusion characterization by deep learning." *Biophysical Journal* 117,2 (2019).

DeepSTORM3D: Deep learning for dense 3D single molecule localization microscopy

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KEYWORDS: 3D single molecule localization microscopy, psf engineering, deep learning.

Localization microscopy is an imaging technique in which the positions of individual nanoscale point emitters (e.g. fluorescent molecules) are determined at high precision from their images. This is the key ingredient in single/multiple-particle-tracking and several super-resolution microscopy approaches like (f)PALM and STORM. Localization in three-dimensions (3D) can be performed by modifying the image that a point-source creates on the camera, namely, the point-spread function (PSF), using additional optical elements (e.g. a DOE/SLM). However, localizing multiple adjacent emitters in 3D poses a significant algorithmic challenge, due to the lateral overlap of their PSFs.

Here we present two fundamental contributions to tackle the problem of high-density overlapping PSFs in 3D localization [1]. First, we employ a convolutional neural network (CNN) for 3D localization from dense fields of overlapping emitters with engineered PSFs, and demonstrate it using the Tetrapod PSF. Second, we design an optimal PSF for high-density 3D microscopic particle localization for a large axial range of 4 μm . This is done by incorporating a physical-simulation layer in the CNN with an adjustable phase modulation, thus jointly learning the reconstruction (decoding) and the optimal PSF (encoding).

Our approach is highly flexible and can be easily adapted to any 3D localization data set. We demonstrate our approach numerically as well as experimentally in two different scenarios: 3D STORM imaging of mitochondria over a large axial range of 4 microns, and single snapshot imaging of dozens of fluorescently labeled telomeres occupying a mammalian nucleus.

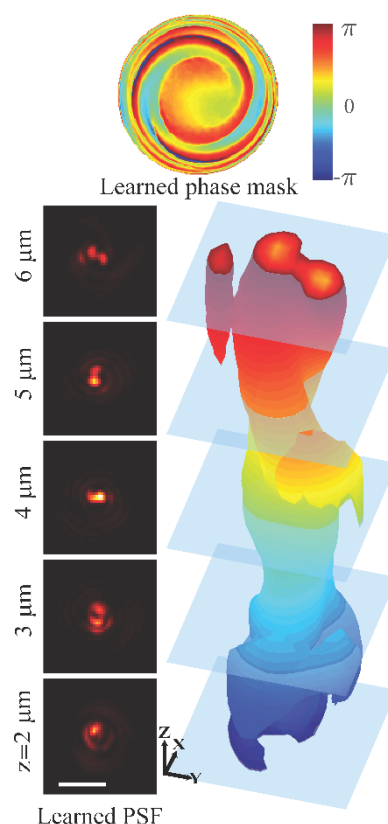


Figure 1. PSF Learning. Learned phase mask (top), with a simulation of the learned PSF as function of the emitter axial position (bottom).

[1] Nehme, E., Freedman, D., Gordon, R., Ferdman, B., Michaeli, T., & Shechtman, Y. (2019). Dense three-dimensional localization microscopy by deep learning. *arXiv preprint arXiv:1906.09957*.

Creating interactive, explorable simulations of cell migration at tissue scale using CPMjs

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Over the past decade, visualization of immune cells via time-lapse microscopy has revealed the incredible diversity of migratory behavior in the immune system. However, the short duration of many imaging experiments makes it hard to assess how cell movement affects immune cell function on longer timescales. Computational modelling can be used to simulate the long-term effects of several “hypotheses” *in silico*, and comparing these models to short-term *in vivo* observations helps immunologists uncover how immune cell movement translates to function.

The Cellular Potts Model (CPM) is a well-established framework for modelling cell migration. It is complex enough to reproduce several key features of immune cell migration (such as realistic movement, cell-cell interactions, and chemotaxis; see Figure 1) – yet efficient enough to allow the simulation of thousands of interacting cells at the same time. While mature software suites for developing and running CPMs already exist (CompuCell3D, Morpheus), rapid technological advances have only recently made it feasible to run simulations directly in web browsers. This provides a unique opportunity to make CPMs available to a broader audience: sharing simulations with other users (collaborators or students) via a web page is simple and requires no additional software. Within HTML-based user interfaces, it is easy to build fully “explorable” simulations where users can interactively change simulation setup and parameters and observe the results.

Here we present CPMjs, the first JavaScript framework allowing users to build highly customizable 2D and 3D CPM simulations directly in the web browser. Novice users can rapidly set up and customize simulations by starting from one of the several included predefined models, whereas advanced users can build their own simulations from scratch and extend the CPM with custom energy terms. CPMjs performs comparably to other frameworks written in C++ or Java. We demonstrate how CPMjs can help immunologists interpret immune cell migration data, thus unraveling the crucial role of migration in the immune system.

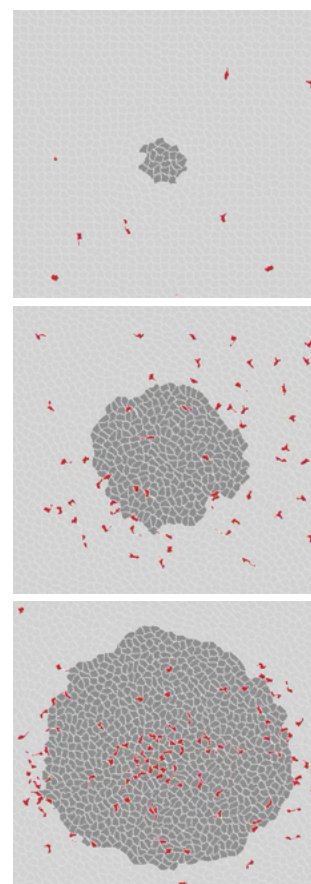


Figure 1. A CPM simulation of T cells (red) infiltrating a growing tumor (dark gray).

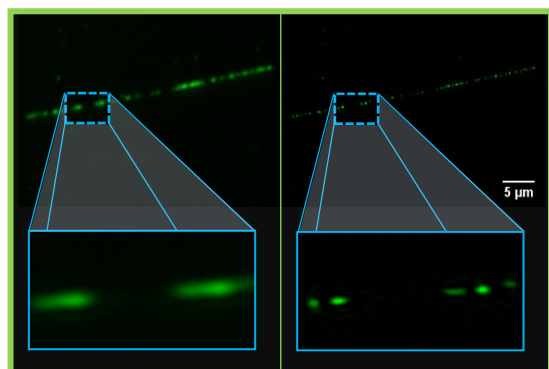
High-Throughput Structured Illumination Microscopy (HiT-SIM)

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A key advantage of structured illumination microscopy (SIM) is its very high acquisition speed, which is important for imaging dynamics, but also for rapidly visualizing a large sample. In this contribution, we present our work on High-Throughput Structured illumination microscopy (HiT-SIM), an implementation of SIM where fast and robust imaging of entire sample dishes or slides is a key deliverable. Our system is based on a spatial light modulator to generate a structured illumination pattern, allowing for a full SIM image acquisition within 20 ms. Additionally, our system is equipped with a water objective, for fast scanning purposes, and an integrated Z-drift correction module, for keeping focus over time and while scanning over an uneven surface. Combined, these components allow for our microscope to quickly image large sample areas (e.g. an entire cover slide could be imaged well under 1 hour with a 60X objective and under 20 minutes with a 20X objective, only requiring the user to engage the initialization of the imaging sequence). SIM reconstruction is handled via the fairSIM software.



We will present an application of the system to rapid species identification through barcoding of genomic DNA, and the high-throughput imaging of complex biological samples.

Figure 1: Comparing widefield (left panel) to the SIM reconstructed image (right panel). A 60x Water objective was used.

Quantitative comparison of camera technologies for cost-effective super-resolution optical fluctuation imaging (SOFI)

R. Van den Eynde¹, Alice Sandmeyer², Wim Vandenberg¹, Sam Duwé¹, Wolfgang Hübner², Thomas Huser², Peter Dedecker¹ and Marcel Müller^{1,2}

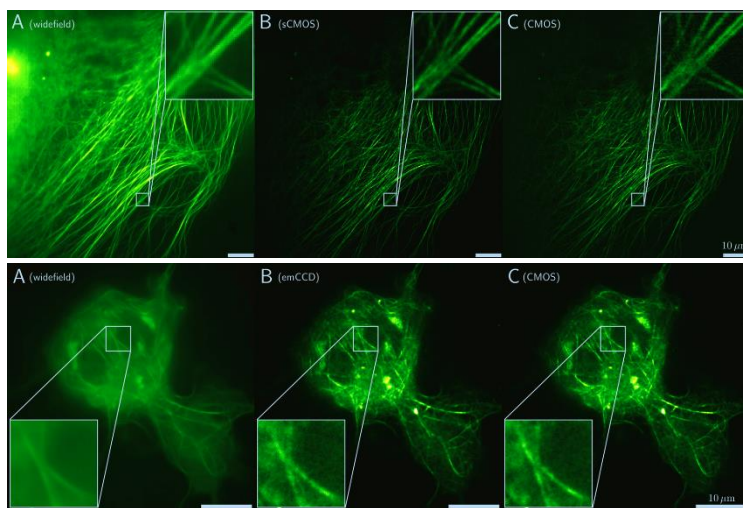
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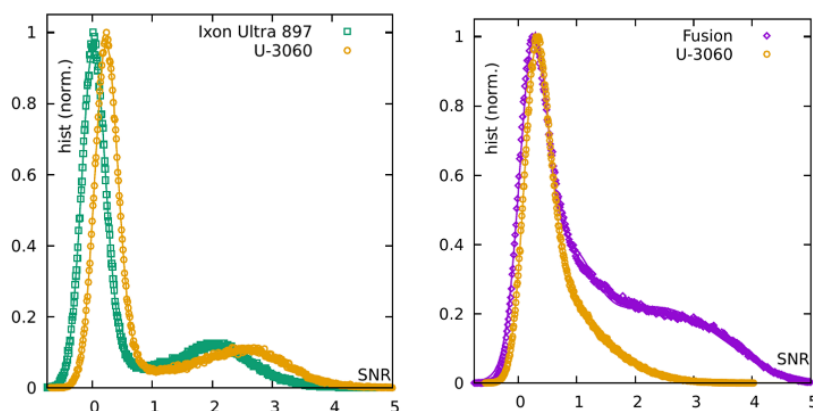
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Super-resolution (SR) fluorescence microscopy is typically carried out on research microscopes equipped with high-NA TIRF objectives and powerful laser light sources. Super-resolution optical fluctuation imaging (SOFI) is a fast SR technique capable of live-cell imaging, that is compatible with many wide-field microscope systems. However, especially when employing fluorescent proteins, a key part of the imaging system is a very sensitive and well calibrated camera sensor. The substantial costs of such systems preclude many research groups from employing SR imaging techniques. Here, we examine to what extent SOFI can be performed using a range of imaging hardware comprising different technologies and costs.

Figure 1: Comparing SOFI imaging (250 frames) of Cos-7 cells stained with ffdronpa. A) Widefield image (average). B) SOFI image (sCMOS/emCCD). C) SOFI image (CMOS).



In particular, we quantitatively compare the performance of an industry-grade CMOS camera to both state-of-the-art emCCD and sCMOS detectors, with SOFI specific metrics. We show that SOFI data can be obtained using a cost-efficient industry-grade sensor, both on commercial and home-



built microscope systems, though our analysis also readily exposes the merits of the per-pixel corrections performed in scientific cameras.

Figure 2: Comparing SNR between cameras. Left panel) emCCD (green) VS CMOS (orange). Right panel) sCMOS (purple) VS CMOS (orange).

Quantitative image-analysis of organoids with high-throughput digital holography microscopy

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KEYWORDS: Quantitative imaging, DHM, organoids, high-throughput, image analysis

Organoids imitate organ physiology better than any previously described *in vitro* tissue model, which makes them ideal candidates to capture our increasingly complex diseases. Their biological relevance raised high expectations in drug development and in regenerative medicine.

However, the existing readouts are simplistic and provide limited information. In this study, we show that more phenotypically accurate measures can be extracted from intestinal organoids using digital holographic microscopy (DHM). This is done in U-shape microwell that we recently designed. DHM provides quantitative phase information in a non-invasive manner, thus enabling time-lapse analysis of organoids and providing quantitative information on their morphology and intracellular content.

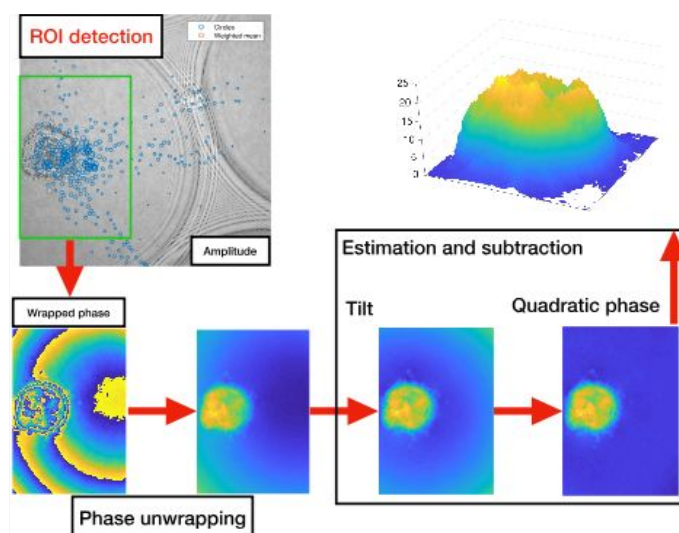
The holographic images (phase + amplitude) present inhomogeneity in the amplitude, a phase delay coming from the image formation model, a deformation due the microwell, and misalignment coming from the robotized system.

To tackle these problems, we developed an automated pipeline (Fig. 1) which notably features:

- Detection of organoids from partial view of microwell based on a circular Hough Transform;
- Phase unwrapping that relies on a robust non-iterative algorithm [1];
- Flattening the background to correct the phase induced by the meniscus.

Our automated image-analysis pipeline enables the extraction of the quantitative phase information from a large number of organoid cultures. As results, we succeeded to quantify the time evolution of the growth of the organoids over long period of time.

[1] H. Takajo and T. Takahashi, "Noniterative method for obtaining the exact solution for the normal equation in least-squares phase estimation from the phase difference," J. Opt. Soc. Am. A 5, 1818-1827 (1988).



KDML: a Knowledge-Driven Machine Learning for Analysing High Throughput Imaging Data

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Keywords: high throughput imaging, colon cancer, nuclear morphology

Characterisation of gene functions in a context-dependent manner is crucial for identifying of gene roles in health and disease. High Throughput Imaging allows the determination of gene function by monitoring phenotypic changes following genetic perturbations. However, a framework for a comprehensive analysis of high throughput imaging datasets is still lacking due to the challenges in data analysis and complexity of gene functions. To address these challenges,

we developed a Knowledge-Driven Machine-Learning framework (KDML) to automatically annotate imaging data of genetically modified cells with various biological functions. KDML utilises existing biological databases to train an ensemble of support vector machine classifiers that can identify any associated phenotypes. We apply KDML to an image-based genetic screen in a colorectal cancer cell line where cells stained with DAPI and Viral Protein 6. The dataset is composed of ~100,00 images capturing the phenotype of 500 million cells. We extensively profile cell morphology and microenvironment resulting in 1,710 features for each gene perturbation image (Figure 1). We show that KDML can identify genes associated with many biologically relevant phenotypes such as protrusions and multicellular organisation. Experiments are underway to extend KDML using deep convolutional neural networks. In summary, KDML is a flexible and systematic framework for analysing high throughput imaging datasets and identifying context and tissue-dependent gene functions.

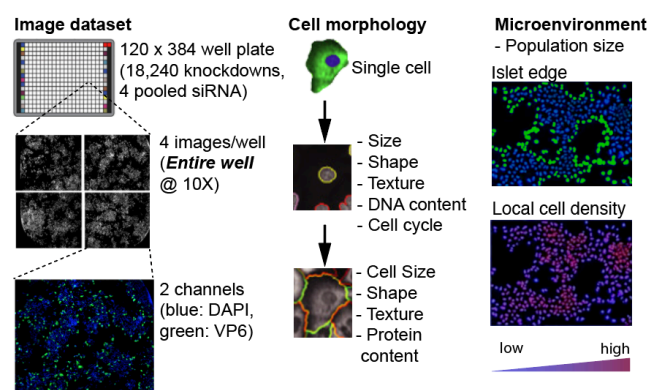


Figure 1. Colon cancer Imaging dataset and extracted features

[1] Sailem H., Rittscher J., Pelkmans L., (2019) KDML: a machine-learning framework for inference of multi-scale gene functions from genetic perturbation screens, bioRxiv 761106 [preprint]. September, 2019, doi: <https://doi.org/10.1101/761106>.

Image-based nanoparticle characterization to aid drug design

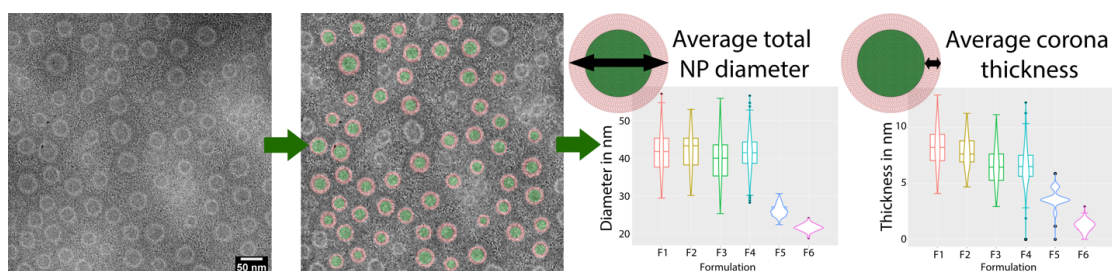
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Nanoparticles (NPs) are emerging as a novel drug delivery platform, whereby small molecule therapeutics are encapsulated within their core and/or conjugated to the exterior of these metallic or polymeric NPs. These NPs have the potential to target abnormal, disease-specific tissues with reduced off-target effects. Integral to the NP formulation stage and the subsequent targeting assessment is the precise morphological characterization of the NPs, including its polymeric corona with or without bound therapeutic payload. Several methods, such as nanoparticle tracking analysis and dynamic light scattering, are well suited for bulk measurements of NPs, but usually lack the individual size information and assessments of their morphological variability. To this end, the most accurate NP depiction to date is via cryogenic transmission electron microscopy, where NPs are flash frozen in their native state and subsequently imaged under cryogenic conditions under high magnification. However, the analysis of these images is challenging due to signal noise and low contrast materials, and measurements are often subjected to manual procedures to establish their characterization properties. This often leads to subsampling and underrepresentation of NP parameters and can be prone to end-user biases.



This study presents a semi-automatic analysis workflow, which was developed to process the acquired images and characterize each NP core and its polymer corona. The image segmentation performed in MATLAB utilizes template-matching, as well as by exploiting the predominantly circular symmetry of the NPs, allowing for robust and precise sizing measurements even under noisy imaging conditions. The resulting data is then combined, analyzed and visualized in an R program. Using this approach, we were able to characterize individual NPs of different formulations with high confidence, as well as provide sufficient NP sample size for morphological evaluations aiding the drug design and NP formulation process.

Thermoregulation of immune cell dynamics

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Keywords: fever, immune cell migration, neutrophils, holographic temperature microscope

Fever is an evolutionary conserved response able to stimulate and boost both innate and adaptive immunity. Temperature variations in general modify the homeostatic setpoint such as blood flow in warm blooded animals and can change gene-expression patterns on the single cell level [1]. Neutrophils and dendritic cells (DCs) patrol tissues under various temperature levels and their effective encounter and response to 'danger signals' is key for optimal immune defense [2]. Still, the thermo-regulation of immune cell dynamics remains largely elusive. Here we show that the local temperature controls immune cell dynamics, such as shape changes, cell migration speed and persistence in 2D/2.5D/3D biomimetic micro-environments.

To unravel the impact of thermal force onto the dynamics of single immune cells in details we developed an all-optical platform based on a digital holographic microscope that allows for: (1) shaping the local heat source (2) measuring the actual temperature profile, and (3) following the dynamics of single cells in three dimensions by fluorescent microscopy. The heat transfer into the sample using plasmonic structures can be quantitatively determined in a label-free manner by measuring the phase difference caused by the thermally-induced refractive index variations of the aqueous medium. We achieved to measure differences in temperature from a diffraction-limited heat source as low as 0.3 K at 0.004ms temporal resolution. This allowed us to analyze the response dynamics of single immune cells under well-defined temperature profiles in space and time.

We show that the temperature response towards heat and cold is symmetric and occurs faster than one second. These findings support the idea that fever's ability to boost immunity could be partially driven by a non-genetically physical response, working independently but in synergy with complex pathophysiological networks. Furthermore the dynamic response is maintained over time leading to temperature defined setpoints of cell migration speed and persistence. Finally we tested actin polymerization, myosin contractility and calcium signaling to get a mechanistic insight into the temperature sensitivity of neutrophils.

Altogether I will discuss how temperature variation strongly shapes single cell migration patterns for effective immune responses.

1. Evans SS, Repasky EA, Fisher DT: *Fever and the thermal regulation of immunity: the immune system feels the heat*. **Nat Rev Immunol** 2015, 15(6):335-349.
2. Maiuri P, Rupprecht JF, Wieser S, Ruprecht V, Benichou O, Carpi N, Coppey M, De Beco S, Gov N, Heisenberg CP *et al*: *Actin flows mediate a universal coupling between cell speed and cell persistence*. **Cell** 2015, 161(2):374-386.

Spatiotemporal Resolution as an Information Theoretical Property of Stochastic Optical Localization Nanoscopy

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Abstract

We propose and analyze a novel concept – spatiotemporal resolution for stochastic optical localization nanoscopy (SOLON)¹. Consider that a SOLON system acquires a data movie of N frames with the frame time of Δ_t seconds. The temporal resolution is equal to $\Delta_T = N\Delta_t$. An information-achieving unbiased Gaussian (IAUG) estimator localizes the locations of emitters and achieves the Fisher information² of the data movie. The area-wise spatial resolution for the m th emitter $\theta_m = (x_m, y_m)$, denoted by $\Delta(\theta_m)$, is defined as the area of the ellipse where the IAUG estimator for θ_m is located with a pre-specified probability. The spatiotemporal resolution is defined as the product of the spatial resolution and the temporal resolution in the large data limit as N tends to infinity

$$R(\theta_m) = \lim_{N \rightarrow \infty} \Delta_T \Delta(\theta_m)$$

in second nm². For 3D imaging, θ_m is a vector of any two coordinates for the m th emitter.

By analysis, it is obtained that if all the data frames are jointly utilized in localization of emitters, the spatiotemporal resolution is a finite constant depending only on the system parameters and the distribution of emitters. It implies that the spatial resolution and the temporal resolution can be traded off with each other, i.e. one increasing with the other decreasing, and vice versa. Therefore, as the number of acquired data frames N increases, the spatial resolution $\Delta(\theta_m)$ can be arbitrarily reduced.

On the other hand, if each single data frame is independently utilized in emitter localization, as done in most localization algorithms in the literature^{3,4}, the spatiotemporal resolution is infinity. This implies that the spatial resolution and the temporal resolution cannot be traded off with each other. Therefore, as the number of acquired data frames N increases, the spatial resolution cannot be arbitrarily reduced.

These results reveal that the spatiotemporal resolution is an important information-theoretical property of stochastic optical localization nanoscopy. It points out the importance to jointly utilize the entire data movie in localization of emitters.

References

1. Rust, M. J., Bates, M. & Zhuang, X. (2006). *Nat. Methods*, Oct., 3(10), pp. 793-796.
2. Sun, Y. (2013). *J. Biomed. Optics*, Oct., 18(11), pp. 111418-14.
3. Sage, D. et al. (2015). *Nat. Methods*, Aug., 12(8), pp. 717-724.
4. Sage, D. et al. (2019). *Nat. Methods*, May, 16(5), pp. 387-395.

Localization microscopy shows the 3D organization of LPS in the *Escherichia coli* outer-membrane

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European Research Council

Established by the European Commission

Keywords: Lipopolysaccharides (LPS), *E. coli* outer-membrane (OM), SMLM, clustering analysis, 4Pi microscope

Antibiotic resistance in bacteria is on the rise for all classes of antibiotics. In the case of pathogenic Gram-negative bacteria, many are intrinsically resistant to some classes of antibiotics due to the outer-membrane (OM) acting as a diffusion barrier. The OM is asymmetric, where the outer leaflet is formed of lipopolysaccharides (LPS) as the main lipid while the inner leaflet constitutes of phospholipids. LPS plays a major role in the barrier function of the OM. While a great deal is known about the biogenesis of the OM and how LPS is deposited on the surface of Gram-negative bacteria, surprisingly little is known about how these molecules are organized in a live bacterium. Here we study the organization of the million or so LPS molecules in *E. coli*.

Using click-based chemistry to fluorescently label LPS in *E. coli* [1, 2] and 3D localization microscopy using a 4Pi microscope (W-4PiSMSN) [3], combined with new algorithms developed for elliptical bacteria [4], we have obtained the highest resolution 3D organization to-date of LPS in a live bacterium (Figure 1). Current work is focused on determining how this distribution relates to that of outer-membrane proteins in *E. coli*.

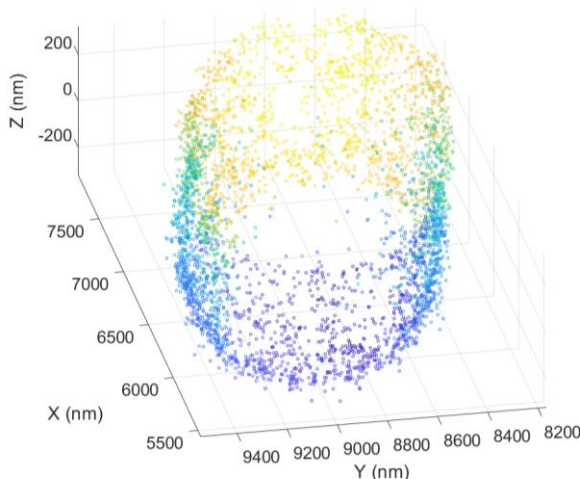


Figure 1. 3D SMLM shows the localisation of LPS-AF647 in an *E. coli* MG1655 cell. A 640 nm z slice of the cell is shown. Color varies with x position.

1. Dumont, A., et al., *Click-mediated labeling of bacterial membranes through metabolic modification of the lipopolysaccharide inner core*. *Angew Chem Int Ed Engl*, 2012. **51**(13): p. 3143-6.
2. Fugier, E., et al., *Rapid and Specific Enrichment of Culturable Gram Negative Bacteria Using Non-Lethal Copper-Free Click Chemistry Coupled with Magnetic Beads Separation*. *PLOS ONE*, 2015. **10**(6): p. e0127700.
3. Huang, F., et al., *Ultra-High Resolution 3D Imaging of Whole Cells*. *Cell*, 2016. **166**(4): p. 1028-40.
4. Ward, S., E.A.K. Cohen, and N. Adams. *Fusing multimodal microscopy data for improved cell boundary estimation and fluorophore localization of Pseudomonas aeruginosa*. in *2018 52nd Asilomar Conference on Signals, Systems, and Computers*. 2018.

FOCAL3D: A 3D clustering algorithm for single-molecule localization microscopy

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The advent of single-molecule localization microscopy (SMLM) has paved the way for the novel field of quantitative nanoscopy. A major aspect of quantitative nanoscopy involves the analysis of molecular aggregates (i.e. clusters) which are commonly formed by a host of different processes in both eukaryotes and prokaryotes. As more advances are made in 3-dimensional SMLM, the problem of accurately identifying clusters from a typical 3D SMLM data set has become increasingly important. While several methods to address this problem already exist, perhaps most notably the popular clustering algorithm DBSCAN, many of the existing techniques suffer from long computation times and/or a highly subjective choice of parameters. To address some of these issues, we've developed a 3-dimensional generalization of FOCAL, a clustering algorithm previously developed in our lab (1), which we've dubbed FOCAL3D (2). The algorithm improves on the speed of DBSCAN by discretizing space into a grid. Clusters are then differentiated from noise based on a size and density threshold whose values are coupled to the chosen grid size. Unlike DBSCAN, the clustering parameters in FOCAL3D can be objectively optimized. Finally, we illustrate the performance of our algorithm by analyzing a 3D SMLM data set of nuclear pore complexes in wild type U-2 human osteosarcoma cells (3).

1. Mazouchi, A. and Milstein, J. N. (2016). Fast optimized cluster algorithm for localizations (FOCAL): a spatial cluster analysis for super-resolved microscopy. *Bioinformatics*, 32(5), 747–54.
2. Nino, D.F. , Djayakarsana, D., and Milstein, J.N. (in progress). FOCAL3D: A 3-dimensional clustering package for single-molecule localization microscopy
3. Sage, Daniel, Thanh-An Pham, Hazen Babcock, Tomas Lukes, Thomas Pengo, Jerry Chao, Ramraj Velmurugan, et al. "Super-Resolution Fight Club: Assessment of 2D and 3D Single-Molecule Localization Microscopy Software." *Nature Methods*, April 8, 2019.

An LPV approach to the estimation of time-varying Single Particle Tracking models

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KEYWORDS: single particle tracking; time-varying models, maximum likelihood

Single Particle Tracking (SPT) is a class of experimental techniques and mathematical algorithms for following the motion of nanometer-scale particles moving inside living cells, including viruses, proteins, and strands of RNA. There are several models in the current literature to describe this movement including the simple diffusion model and more general linear Markov systems. These models are completely specified by a set of parameters such as the diffusion coefficients. These parameters can be estimated by analyzing SPT data such as wide-field fluorescence images.

Previous works by the authors considered the case where parameters change during a single SPT trajectory. One way to account for this time-varying nature in the system is by using a jump Markov model. The disadvantage of this approach is that it does not allow for continuously varying parameters and requires the definition of transition probabilities which may have no physical meaning. Another way to deal with time-varying parameters is to consider a local estimation approach, where the parameters are considered time-invariant in a window of nominated length. The window then moves along by one time point, and a new estimation is carried out, tracing in this way time-varying parameters. The latter has shown to be successful in the time-varying SPT context, however, it is not clear how to choose the best window lengths and the performance can vary greatly as a function of this choice.

Linear parameter varying (LPV) systems are used to model systems that have a piecewise linear time-invariant (LTI) nature, allowing the model to switch between the different LTIs, according to a *a-priori* known scheduling signal p .

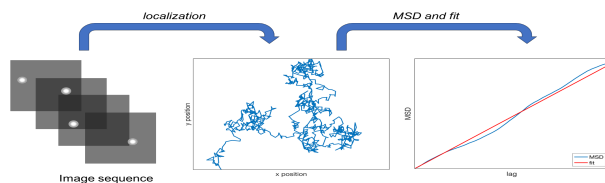


Figure 1. Illustration of a generic SPT scheme. (left) Data in the form of CCD camera images are processed to produce (center) trajectories, which are analysed to produce (right) data to fit a mathematical model

parameters arising in motion models. Here, we use ML estimation to find the associated LTI models that are scheduled by the scheduling variable p . A key requirement of the LPV approach is that the variable p needs to be known in advance. This variable, in our SPT application, indicates when we have a change in the parameter of interest. Hence, one way to estimate p is by simply applying change detection (CD) techniques to the particle trajectories. CD techniques can be applied to detect both linear and nonlinear changes in the systems. Since our parameter of interest appears non-additively in our model (through the variances), we apply the CUSUM algorithm, which is based on the Likelihood ratio test. The CUSUM algorithm is a sufficient statistic to detect nonadditive changes.

Here, we develop an LPV approach to track time-varying parameters in SPT using Maximum Likelihood estimation. In particular, these parameters refer to the noise variances of a random walk model with noisy observations. In the context of SPT, ML has been utilized previously to estimate key

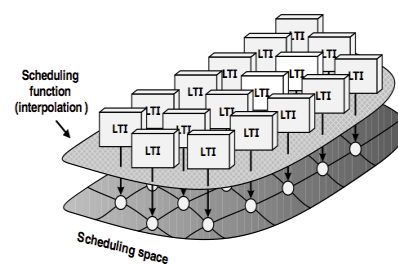


Figure 2. Different LTI systems associated with the value of a 2-D scheduling parameter

[1] Qian, H., Sheetz, Michael P., and Elson, Elliot L. Single particle tracking. Analysis of flow and diffusion in two-dimensional system. Biophys. J, vol. 60, pp.910-921, October 1991.

[2] M. Basseville, I. Nikiforov. Detection of abrupt changes: Theory and Application, Prent. Hall 1993.

Measurement of the depth-dependence of point spread functions near the glass-water interface in high-numerical aperture microscopy

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In order to maximize collected photons and reduce the size of the diffraction-limited point spread function (PSF), single-molecule fluorescence imaging experiments commonly rely on high numerical aperture (NA) microscope objectives with immersion media of refractive index exceeding that of the sample medium. This sample geometry (Fig. 1) results in a refractive index boundary at the glass coverslip. Refraction at this interface induces focal shift between the actual focal plane (AFP) and nominal focal plane (NFP), enables the collection of supercritical fluorescence, and yields a depth-dependent PSF shape. These effects have been studied both theoretically and in experiment.¹⁻⁵

However, there are discrepancies between the results of experiments and theoretical calculations, as well as between experiments. These discrepancies are attributed to the lack of a systematic experimental characterization of the effects of the interface on multiple length scales relevant to single-molecule imaging experiments. This lack is due to the difficulty in obtaining precise, accurate knowledge of emitter depth within an aqueous medium.

Here, we present an experimental study of the glass-water interface in a high-NA microscope in a typical geometry used in single-molecule imaging. Using a polymer with a refractive index matched to that of water, we position stationary emitters a distance z above the glass surface (Fig. 1). Characterization of the polymer layer thickness via atomic force microscopy and surface profilometry enables precise and *accurate* knowledge of the emitter positions, providing ground truth measurements independent of the optical readout. By varying the polymer thickness, we demonstrate depth-dependent measurements of the focal shift and show how refractive index mismatch affects the double-helix PSF. We compare our experimental results to theoretical calculations.

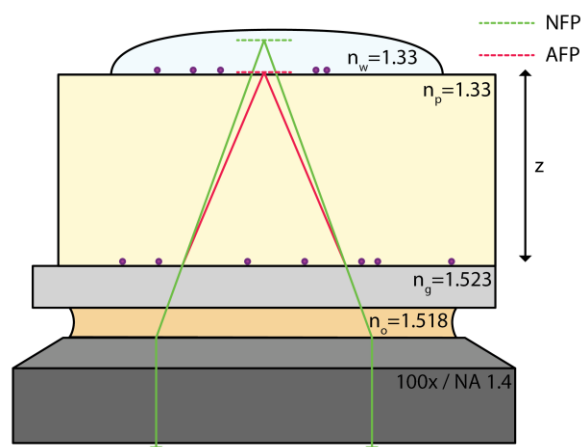


Figure 1. Sample geometry (not to scale). A polymer of thickness z , with refractive index matched to water, is sandwiched between a glass coverslip and a layer of water. Emitters on either polymer surface enable measurement of the focal shift and depth-dependent PSF features. The polymer thickness can be measured separately.

1. S.H. Wiersma *et al.*, *J. Opt. Soc. Amer. A* **14**(7), 1997
2. A. Egner & S.W. Hell, *Handbook of Biological Confocal Microscopy*, 2006
3. B. Huang *et al.*, *Nat. Methods* **5**(12), 2008
4. R. McGorty *et al.*, *Opt. Lett.* **39**(2), 2014
5. B.P. Bratton & J.W. Shaevitz, *PLoS ONE* **10**(8), 2015

The dual approach to non-negative super-resolution

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Keywords: super-resolution, non-smooth optimization, duality

In this work, we consider the problem of sparse deconvolution, which is relevant to super-resolution microscopy. The problem concerns recovering locations and weights of point sources from noisy measurements of their convolution with a known blurring kernel. Mathematically, we represent the input signal in 1D as a sum of Dirac delta functions:

$$x = \sum_{i=1}^k a_i \delta_{t_i},$$

for all $i = 1, \dots, k$ with non-negative weights $a_i > 0$ and source locations $t_i \in [0,1]$, $i = 1, \dots, k$, and the measurements are given by noisy samples at locations $s_j \in [0,1]$ for $j = 1, \dots, m$ of the convolution of x with a known kernel ϕ (for example a Gaussian), which represents the point spread function of the imaging device:

$$y_j = \int_{[0,1]} \phi(t - s_j) x(dt) + \eta_j = \sum_{i=1}^k a_i \phi(t_i - s_j) + \eta_j$$

for all $j = 1, \dots, m$, where η_j with $\sum_{j=1}^m \eta_j^2 \leq \delta^2$ represents the additive noise with magnitude δ .

A standard way of solving this problem is by considering the total variation (TV) norm minimization problem:

$$\min_{x \geq 0} \|x\|_{TV} \text{ subject to } y_j = \int_{[0,1]} \phi(t - s_j) x(dt), \quad \forall j = 1, \dots, m,$$

or the dual problem:

$$\max_{\lambda \in \mathbb{R}^m} y^T \lambda \text{ subject to } \sum_{j=1}^m \lambda_j \phi(t - s_j) \leq 1, \quad \forall t \in [0,1].$$

Our proposed approach is to consider the exact penalty formulation of the dual problem above:

$$\min_{\lambda \in \mathbb{R}^m} -y^T \lambda + \Pi \cdot \max \left\{ \sup_s \sum_{j=1}^m \lambda_j \phi(s - s_j) - 1, 0 \right\},$$

for a large enough value of the parameter $\Pi > 0$. We solve this problem using a non-smooth optimization algorithm, specifically the level method as given in [1].

As far as we are aware, this is a new approach to solving the sparse deconvolution problem. We provide a novel analysis of the algorithm in terms of perturbation bounds of the primal and dual variables as the measurements are perturbed by additive noise, and we show the power of the method by applying it to data from super-resolution microscopy.

Model-based Imaging Biomarkers for Non-rectangular Regions of Interest

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Keywords: multi-resolution model, image analysis, random effects

Quantitative imaging biomarkers are extracted from medical images for a variety of purposes including noninvasive disease detection, cancer monitoring, and precision medicine. However, the existing methods for extraction of such biomarkers tend to be ad-hoc and not reproducible. In this study, a general and flexible statistical approach is proposed for handling one-, two- and three-dimensional medical images in an objective and principled way. It can directly handle non-rectangular regions of interest with varying shapes among patients. Specifically, a model-based decomposition of spatial processes is developed, where individual patients share common component functions but each patient has unique random weights on these components.

Existing techniques for extracting potential imaging biomarkers were developed largely for rectangular images. By contrast, our proposed model decomposes image intensities into several multi-resolution thin-plate spline functions[2], which can describe rectangular or non-rectangular images in the same way. Model fitting and selection are based on maximum likelihood, while biomarker generation is via optimal prediction of the underlying true signals[1]. We apply our method to a cancer image data set and extract biomarkers in association with a clinical endpoint. The results show that the proposed biomarkers have a similar model goodness-of-fit, compared to existing features. Our novel proposal also has advantages including approximate orthogonality, no need for ad-hoc quantization of intensities, and automatic tuning parameter selection.

[1] Cressie, Noel, and Gardar Johannesson. *Fixed rank kriging for very large spatial data sets*. Journal of the Royal Statistical Society: Series B (Statistical Methodology) 70.1 (2008): 209-226.

[2] Tzeng, ShengLi, and Hsin-Cheng Huang. *Resolution adaptive fixed rank kriging*. Technometrics 60.2 (2018): 198-208.

Multiplexed imaging of lung tissue using a macroscopic light sheet fluorescence microscope with uniform axial resolution

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Keywords: volumetric imaging, tissue quantification, multiplexed imaging

A key compromise in light sheet fluorescence microscopy (LSFM) is the tradeoff between high axial resolution and large field of view (FOV) [1]. Recent advancements have demonstrated that uniform axial resolution during high numerical aperture (NA) imaging can be maintained over a large FOV by optical engineering of the light sheet [2, 3] or by scanning the thinnest portion of a Gaussian focus across the FOV [4]. For large samples, we previously demonstrated that using an electrotunable lens (ETL) in both the excitation and detection pathways of a LSFM enables the correction of common optical aberrations and uniform axial resolution for low to moderate NA imaging of cleared tissue samples [5].

Motivated by the need for rapid and accurate imaging of multiple cubic centimeter cleared tissue samples at cellular resolution, we created a simple to construct, low-cost, portable macroscopic LSFM with uniform axial resolution. To increase the accessibility, affordability, and portability of this macro LSFM we utilized a low-cost, high efficiency industrial CMOS camera. Using an ETL conjugate to the back focal plane of a cylindrical lens, we were able to remotely change the focusing position of the resulting Gaussian light sheet across the FOV of the detection optics. At each light sheet focal position, we captured a full camera frame. Post-acquisition, we fused all images using a complex wavelet transform to generate a final uniform axial resolution image. We verified that this complex wavelet transform algorithm provided a near-equivalent axial resolution to axially scanned LSFM [4] by replacing the industrial CMOS with a programmable shutter scientific CMOS.

Using this macro LSFM, we investigated how three distinct approaches to optical tissue clearing, solvent based [6], aqueous based [7], and hydrogel embedding based [8] each preserved alveolar structure in the distal rat lung. Using quantum dot labeled primary antibodies, we measured, quantified, and built 3D computational models of multiple proteins throughout entire rat lung lobes. Using these computational models, we extracted the surface area and volume of an average alveoli in each lung. We found that only the hydrogel embedding approach correctly preserved the distribution of surface area and volume when compared to previous serial sectioned histological imaging experiments. These results have important implications for those groups seeking to use volumetric imaging and tissue clearing to build quantitative spatial maps of cells and structures.

1. Power RM, Huisken J (2017) A guide to light-sheet fluorescence microscopy for multiscale imaging. *Nature Methods*, 14(4):360–373.
2. Fahrbach FO et al (2013) Light-sheet microscopy in thick media using scanned Bessel beams and two-photon fluorescence excitation. *Optics Express*, 21(11):13824–13839.
3. Chang B-J et al (2019) Universal light-sheet generation with field synthesis. *Nature Methods*, 16(3):235–238.
4. Dean KM et al (2015) Deconvolution-free Subcellular Imaging with Axially Swept Light Sheet Microscopy. *Biophysical Journal*, 108(12):2807–2815.
5. Ryan DP et al (2017) Automatic and adaptive heterogeneous refractive index compensation for light-sheet microscopy. *Nature Communications*, 8(1):612.
6. Klingberg A et al (2017) Fully Automated Evaluation of Total Glomerular Number and Capillary Tuft Size in Nephritic Kidneys Using Lightsheet Microscopy. *Journal of the American Society of Nephrology*, 28(2):452–459.
7. Li W, Germain RN, Gerner MY (2019) High-dimensional cell-level analysis of tissues with Ce3D multiplex volume imaging. *Nature Protocols*, 14:1708–1733
8. Park Y-G et al (2018) Protection of tissue physicochemical properties using polyfunctional crosslinkers. *Nature Biotechnology*, 37:73–83

Feature representations for the identification of mRNA localization patterns from smFISH data

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Messenger RNAs do not always distribute randomly inside cells: in some cases, RNA molecules localize in specific regions of the cytoplasm, i.e. they distribute according to a specific localization pattern. Subcellular localization of mRNAs is thought of as playing an important role for the spatial control of gene expression. However, the function and mechanism of RNA localization are not yet well understood. In addition, it is still unclear which localization patterns exist and which mRNAs distribute according to which localization pattern. These questions can be addressed by large-scale image-based assays, where individual mRNA molecules are visualized by single molecule FISH (smFISH).

Here, we present extensions of our recently published simulation and analysis framework [1], [2], where we first segment cells and nuclei, detect individual RNA molecules, describe the spatial distribution of RNA molecules inside the cell by features and apply supervised and unsupervised machine learning in order to infer localization patterns. One of the key questions in this approach is to find representations that capture the essential characteristics of the point clouds, but are robust with respect to the main covariates, namely cell morphology and expression levels. In this work we quantitatively compare different sets of features: hand-crafted features from spatial statistics and deep features from Convolutional Neural Networks (CNN) trained on simulated data. Finally, we explore domain adaptation techniques to bridge the gap between simulated and real data.

1. A. Samacoits, R. Chouaib, A. Safieddine, A. M. Traboulsi, W. Ouyang, C. Zimmer, M. Peter, E. Bertrand, T. Walter, and F. Mueller. A computational framework to study sub-cellular RNA localization. *Nature Communications*, 9(1), 2018.
2. R. Dubois, A. Imbert, A. Samacoits, M. Peter, E. Bertrand, F. Müller and T. Walter. A deep learning approach to identify mRNA localization patterns. In IEEE 16th International Symposium on Biomedical Imaging (ISBI 2019); 2019; pp 1386–1390.

Axial stimulated emission depletion spectroscopy to study lipid dynamics

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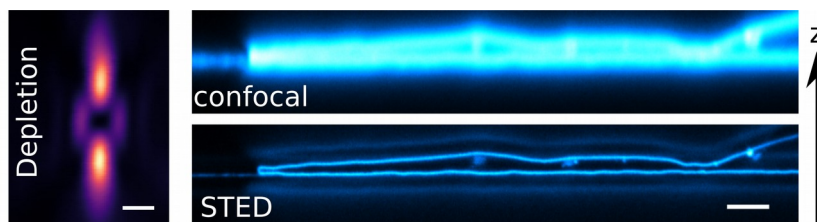
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Stimulated emission depletion (STED) super-resolution microscopy is a versatile tool for cellular imaging, for example due to its flexibility in tuning performance. STED microscopy increases the resolution of conventional microscopes by means of a depletion laser that exhibits a central intensity minimum in its center and that inhibits the fluorescence in the peripheral focal areas of high intensity. In its most common implementation, STED microscopy makes use of a ring-shaped depletion beam to increase lateral resolution (so called 2D STED). In addition to pure imaging, STED microscopy is also employed with various spectroscopy techniques. Particularly, combining STED and fluorescence correlation spectroscopy (FCS) enables lengthscale-dependent measurements of diffusion speeds, providing useful information about the molecular environment[1].

2D STED improves only the lateral resolution and leaves the axial resolution unchanged, which remains limited by the diffraction to about 650 nm. This is problematic in crowded environments, or when structures are in close proximity e.g. cellular bottom and top membrane. Here, we explore the use of a bottle-shaped depletion pattern that increases mostly the axial resolution (z-STED) and that can be used together with available spectroscopic tools to investigate lipid dynamics in living cells (Figure 1).

Figure 1: Figure 1: z-STED microscopy. A bottle-shaped depletion pattern (left, scalebar: 500 nm) is used to increase the axial resolution of a STED microscope. This can be used to resolve closely separated membranes in Ptk2 cells (right, scalebar: 2 μ m)



- [1] E. Sezgin *et al.*, "Measuring nanoscale diffusion dynamics in cellular membranes with super-resolution STED-FCS," *Nat Protoc*, vol. 14, no. 4, pp. 1054–1083, Apr. 2019.

Simultaneous multicolor single-molecule imaging using discrete PSF engineering

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Keywords: Localization microscopy, DNA-PAINT, PSF engineering

Simultaneous multicolor SMLM or single-molecule imaging typically requires the use of multiple cameras, or splitting the detector field of view into distinct regions for each emission band. The result is a setup that is either more expensive and complex, especially in terms of alignment and robustness, or one that sacrifices field of view and hence allows less data to be collected.

In this presentation I will introduce the Circulator, an optical device that allows simultaneous multiplexing of SMLM imaging by encoding the emission color into the PSF, without requiring more than one camera or sacrificing field of view. In addition, the Circulator can be readily introduced into existing imaging systems. The spectral encoding capacity of this instrument will be shown by 3-color PAINT imaging. This combination is Especially fruitful since the highly specific and reversible interactions between DNA strands that PAINT relies on inherently bring multiplexing a (nearly) unlimited number of targets in the same sample into range.

Beyond super-resolution microscopy, the system can be used to acquire more data in multicolor single-molecule experiments, such as FRET or multicolor single-particle tracking, or to perform single-molecule spectroscopy.

Separation of cell signalling responses based on a new model for photoswitching in FRET biosensors

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Keywords: FRET biosensor, cell signalling, multiplex imaging, photoswitching

The possibility to visualize multiple cell signalling pathways simultaneously is a highly desired asset to the cell biologist toolkit. However, the limited spectral range over which fluorescence occurs restrains the amount of biosensors that can be distinguished based on colour.

In this ongoing work we implement the dynamics of photoswitchable fluorescent proteins to separate cell signalling activities of two spectrally identical biosensors. We have derived a new quantitative model that describes FRET biosensors with a photoswitchable donor in ON and OFF switched states. Subsequently, we extended this photoswitching model to two spectrally identical FRET biosensors and show that we can unmix the signals from both biosensors using the information from the dynamics of donor photoswitching.

We have first developed a photoswitchable variant of the cyan-yellow PKA biosensor AKARev (psAKAR). Next, we performed time-lapse imaging of psAKAR in combination with the non-photoswitchable Erk kinase biosensor EKARev. Using the photoswitching model, we demonstrate that we can separate the responses of both biosensors after sequential stimulation of Erk and PKA signalling pathways in living cells.

To further maximize use of the available spectral space, we then explored the possibility to multiplex a third orthogonal biosensor. In HEK293T cells expressing psAKAR, EKARev and an additional calcium sensor, we were able to successfully monitor the changes in PKA, Erk and Ca^{2+} activity during stimulation of cell receptors. These revealed an intricate link between the receptors and their various downstream signalling pathways, emphasizing the demand for multiparametric imaging in biosciences.

In summary, we have successfully developed a new quantitative method for orthogonal FRET biosensor imaging based on donor photoswitching. The additional layer of orthogonality expands the number of choices for multiplex bio-imaging, with the added value of freeing up the spectral space which can now be used to extract additional information from any imaging experiment.

A two-step algorithm to the estimation of time-varying Single Particle Tracking models

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KEYWORDS: single particle tracking; time-varying models, change detection

Single Particle Tracking is a powerful class of methods for studying the dynamics of biomolecules inside living cells. These techniques reveal both trajectories of individual particles, with a resolution well below the diffraction of light, as well as parameters that define the motion.

There are several models in the literature to describe this movement of biomolecular macromolecules inside their native environments. One of the most common is the simple diffusion model. It is a common assumption in the SPT community that motion parameters do not change in a given trajectory. This can lead to biased estimates if the parameter has actually a time-varying nature. The authors have considered this element in previous works. For example, one way to account for the time-varying nature in such system is by using a jump Markov model. Unfortunately, this approach does not allow one to track continuous changes in the time-varying

parameters and requires the need to include transition probabilities which may lack physical meaning. Another way to deal with time-varying parameters is to apply a local estimation approach, where the parameters are considered time-invariant in a window of nominated length. The window then moves along by one sample, and a new estimation is carried out, tracing in this way time-varying parameters. This general scheme has been shown to be successful in the

time-varying SPT context, however, it is not clear how to choose the best window lengths. Smaller windows are more sensitive and react more quickly to changes but also produce noisier estimates. Longer windows, by contrast, are more robust but are slower to respond.

Here, we use the window approach in a slightly different way, focusing on simple diffusion and described here for a single change in the parameter value. To be precise, we first apply a standard windowed scheme to estimate the values of the diffusion coefficient before and after a change has occurred. We then use these values to perform offline change detection. Since our parameter of interest appears non-additively in our model (the variances), we apply the CUSUM algorithm, which is based on the Likelihood ratio test. To avoid a forward bias in the change point (as shown in Fig. 2), the CUSUM algorithm is applied in both the forward and backward directions on the data and the two results averaged. The model parameters are then re-estimated using windows of maximum (and possibly non-equal) size by splitting the data into two segments: from the initial point to the time of change, and then from that time until end of the data (or the time of the next change in the more general setting). We demonstrate that by implementing this technique, the bias seen in Fig. 2 can be eliminated, given very precise estimates for the diffusion.

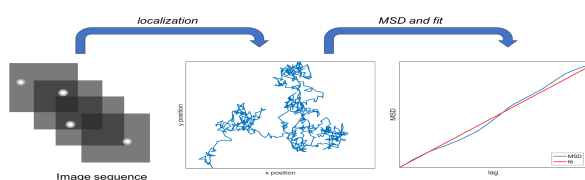


Figure 1. Illustration of a generic SPT scheme. (left) Data in the form of CCD camera images are processed to produce (center) trajectories, which are analysed to produce (right) data to fit a mathematical model

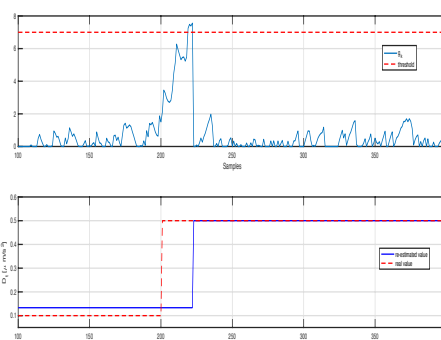


Figure 2. Change detection performance (upper plot); 2nd step in the estimation (lower plot)

- [1] Qian, H., Sheetz, Michael P., and Elson, Elliot L. Single particle tracking. Analysis of flow and diffusion in two-dimensional system. Biophys. J, vol. 60, pp.910-921, October 1991.
- [2] M. Basseville, I. Nikiforov. Detection of abrupt changes: Theory and Application, Prent. Hall 1993.
- [3] J. Fan, I. Gijbels. Local polynomial modeling and its applications, CRC Press, 1996.

Method of Synthetic Motion for Testing Single Particle Tracking Microscopes

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KEYWORDS: Experimental Method Development, Single Particle Tracking, Fluorescence Microscopy

disease on a cellular level. To understand cellular function and disease, it is necessary to understand how these molecules transport, bind, undergo conformational changes, and are consumed within cells and tissues. The study of cells at these length scales necessitates the development of methods that can localize and track single particles at the nanometer scale, collectively known as single particle tracking (SPT). However successful these techniques are, it is difficult to compare them directly [1], especially as one is most interested in their performance on a specific experimental setup.

In this work, we demonstrate the use of synthetic motion as a basis for testing, calibrating, optimizing, and comparing SPT microscope techniques. Synthetic motion, first described by Michael Saxton [2], is a method of moving a fluorophore, fixed to a microscope slide, along a trajectory, which is a realization of a stochastic motion model. This paradigm allows a single particle to move with a known ground truth in the field of view of an SPT microscope, allowing for comparison to the estimated positions, trajectory, and motion model parameters. Having the ground truth then allows for detailed and precise experimental analysis and comparison of specific SPT microscopes with very different detection and signal processing. However, when implementing synthetic motion, one must take into account the physical limitations of the actuation modality used to achieve motion on the microscope.

Piezo-actuated microscope stages, a common tool in biophysics labs, provide large actuation range, precise positioning, and high speeds and thus make a great platform for realizing synthetic motion. However, achieving accurate and precise positioning requires overcoming several challenges that arise due to the nature of the piezo-actuators and the quantization, noise, and delay of digital controllers used in the implementation. Previously we analyzed one such limitation where the stage exhibits a bandwidth [3]. We now analyze the effects of controller quantization, actuator slew rate, and amplifier input slew rate limitations. Additionally, we show how a feedforward-feedback control system can increase the bandwidth of the stage while maintaining the precise and accurate motion needed for the fast acquisition rates of modern SPT microscopes or when intraframe motion blur is required. With these challenges overcome, synthetic motion becomes a powerful tool for the experimental analysis of SPT algorithms, hardware, and microscope instrument designs.

- [1] Chenouard, Nicolas, et al. "Objective comparison of particle tracking methods." *Nature methods* 11.3 (2014): 281.
- [2] Saxton, Michael J. "Wanted: a positive control for anomalous subdiffusion." *Biophysical journal* 103.12 (2012): 2411-2422.
- [3] NA. Vickers, SB Andersson, "Monte Carlo Simulation of Brownian Motion Using a Piezo-Actuated Microscope Stage", in Proceedings of American Control Conference 2019, Philadelphia, PA, USA, July 10-12, 2019

Quantitative analysis of the structural organization and diffusion properties of a bacterial biofilm matrix

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Keywords: Diffusion, Fluorescence Correlation Spectroscopy, Single Particle Tracking, Bacterial biofilms, Bacteriophages, Polymer physics

Bacterial biofilms are surface-adherent communities of bacteria surrounded by extracellular polymeric substances (EPS) consisting of secreted polysaccharides and other macromolecules. In healthcare settings bacterial biofilms represent a severe threat, causing chronic infections and contamination of medical devices. To remove biofilms, multiple strategies have been developed, e.g. treatment with antibiotics or bacteriophages, which are advantageous to specifically target bacterial species. In this context, it remains unclear to which extent the EPS matrix imposes a physical barrier to the transport of bacteriophages through the biofilm.

To address this question, we have reconstituted the EPS matrix of the bacterium *Pantoea stewartii*, responsible for a severe disease of corn plants, and investigated the diffusion properties of fluorescent particles using fluorescence correlation spectroscopy and single particle tracking. This approach allows to study the EPS spatial organization under various physico-chemical conditions. We show that small probes diffuse freely in the EPS with diffusion coefficients similar to those measured in water. In contrast, large probes are drastically slowed down, showing anomalous subdiffusion. The diffusion hindrance increases with EPS concentration, revealing two distinct scaling regimes. At physiological concentrations, the EPS shows typical characteristics of an entangled polymer network, with an average mesh size of ca. 50-100 nm, estimated from the diffusion hindrance measured for differently sized particles. Tracking single fluorescently labeled bacteriophages in this regime, we observe a population of strongly confined particles, characterized by distinct subdiffusive dynamics with anti-correlation of successive steps.

To overcome the physical barrier imposed by the EPS, bacteriophages are equipped with EPS depolymerizing enzymes. By treating the EPS with bacteriophage enzymes, we show that upon EPS degradation strongly confined diffusion rapidly turns to free diffusion. Thus, our approach allows the investigation of dynamic changes of the biofilm matrix organization and demonstrates that the entangled EPS matrix imposes a probe size dependent diffusion barrier under physiological conditions. Our data suggests that the ability to degrade EPS provides a key for bacteriophages to evade trapping in the biofilm.

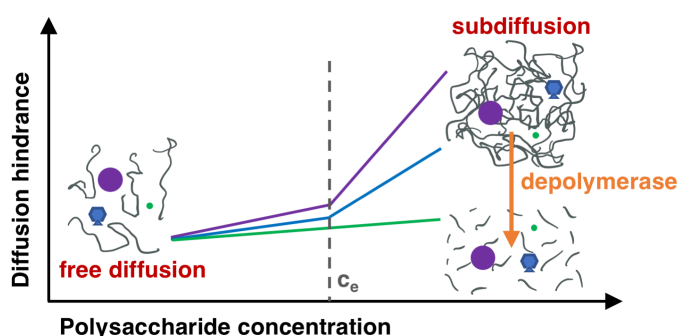


Figure 1. Schematic of the concentration dependent diffusion properties of the biofilm EPS matrix. Diffusion measurements indicate a transition to an entangled polymer network at the polysaccharide concentration c_e . Depolymerization of the matrix recovers free unhindered diffusion.

4Pi-SMLM and the 3D architecture of the Nuclear Pore Complex

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Super-resolution localization microscopy techniques such as PALM and STORM enable the visualization and quantification of sub-cellular structures at the nanometer scale [1, 2]. Interferometric fluorescence detection, in particular, yields the highest possible localization precision along the Z dimension, allowing for fluorescence microscopy datasets with isotropic 3D image resolution of 10 nm or better [3]. At this level of detail, it becomes possible to visualize the sub-structure of protein complexes, and we have applied this approach to study Nuclear Pore Complex (NPC) organization. The

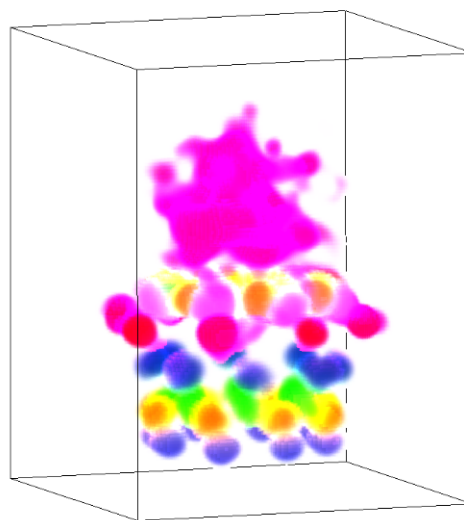


Figure 1: Multicolor 3D rendering of six different NPC components, imaged by 4PI-STORM.

combination of genetically engineered cell lines, small fluorescent tags, super-resolution 3D imaging, and particle alignment methods adjusted for localization microscopy data, allowed us to obtain multicolor reconstructions of NPCs which may be directly compared with cryo-electron microscopy structures. Our approach allows for the alignment and visualization of an arbitrary number of distinct structural elements in the NPC, and these results represent the first example of multicolor 3D light microscopy of a single protein complex at 10 nm resolution (Figure 1). Furthermore, as the number of aligned particles increases, individual fluorescent tags within the NPC structure may be localized with arbitrarily high precision. Finally, the specificity and high contrast of the fluorescence data allows new insights into NPC biology which are not accessible by other methods, and our work represents a further step in the development of light microscopy as a tool for structural biology studies.

[1] M. Bates, B. Huang, and X. Zhuang, *Curr. Opin. Chem. Biol.*, **12**, 505-514 (2008)

[2] A. Szymborska *et al.*, *Science*, **341**, 655-658 (2013)

[3] G. Shtengel *et al.*, *PNAS*, **106**, 3125-3130 (2009)

Single-Cell / Single-Molecule microscopy on endogenous pulsed-labeled p53 dissects the regulation layers involved in its activation.

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In response to specific stimuli inducible transcription factors (iTFs) activate the transcription of the target genes. The activation process has often multiple regulation steps that enable a fine modulation of the response. These steps involve the gathering of iTF in the nucleus and in many cases oligomerization and accumulation of post-transcriptional modifications. An instance of multi-layer regulation of iTF activity is provided by the tumor suppressor p53, which decodes different stress stimuli into different transcriptional programs ranging from cell cycle arrest, DNA damage repair to apoptosis.

To dissect if these processes are independently regulated in living cells, we combine different fluorescence microscopy approaches. With Single Molecule Tracking (SMT) we monitor the DNA binding kinetic with single-molecule sensitivity, while with Number and Brightness (NB) we probe homo-oligomerization at the single-cell level. We show that the modulation of p53 oligomerization and binding to chromatin does not merely descend from the increase in p53 levels and that the formation of p53 tetramers is necessary but insufficient to induce the stabilization of p53 binding and its activation as a transcription factor. Moreover, thanks to a newly developed approach to NB experiments and data analysis, for the first time, we are able to measure oligomerization dynamics at single cell level. The approach developed here will be useful to identify key regulatory events in the inducible activation of transcriptional networks and in their re-wiring in disease.

SIMPLE: Structured illumination based point localization estimator with enhanced precision

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Keywords: Particle localization, structured illumination, microscopy

Modern challenges in biology push the existing microscopy techniques to their limits, often leading to restricting compromises such as image resolution, dimensionality, sample type and fixation. To study dynamical processes in living cells sets particularly strict requirements to spatio-temporal resolution, non-invasiveness and low photo-toxicity. In order to cope with those restrictions, we introduce a novel microscopy technique at the nexus of structured illumination microscopy (SIM) and single molecule localization microscopy (SMLM) termed Structured Illumination Microscopy based Point Localization Estimator (SIMPLE).

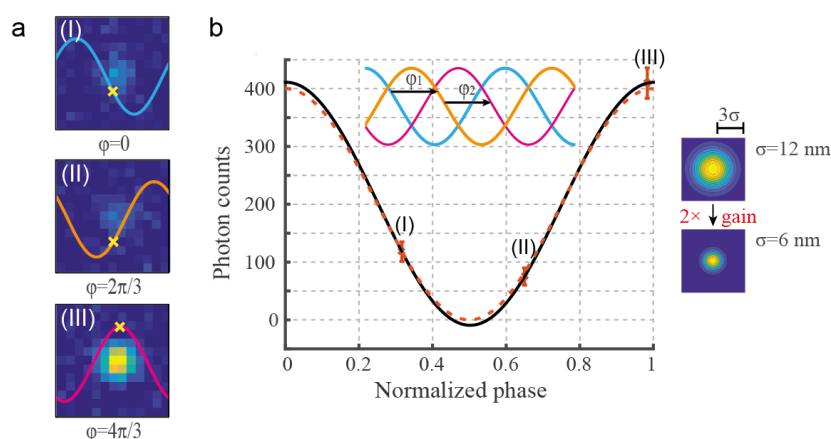


Figure 1. Schematic of the SIMPLE principle. (a) Different camera images of a single emitter and its associated position on the sinusoidal illumination pattern for equidistant phase-shifts. (b) Localization achieved through fitting the modulated photon intensity.

SIMPLE - a highly parallelized extension of MINIFLUX [1] - features a homogeneous 2 to 6 fold increase in localization precision compared to conventional centroid estimation methods [2]. By using fast and precise phase-shifted sub-diffraction sinusoidal illumination patterns as nanometric ruler, we propose field of view wide particle localization through induced photon count modulation over a 20 μm field of view. The technique has been validated both *in silico* and experimentally on a custom TIRF-SIM setup using a digital micro-mirror device (DMD) as a spatial light modulator.

1. Balzarotti, F. *et al.* Nanometer resolution imaging and tracking of fluorescent molecules with minimal photon fluxes. *Science* (80-.). **355**, 606–612 (2017).
2. Reymond, L. *et al.* SIMPLE: Structured illumination based point localization estimator with enhanced precision. *Opt. Express* **27**, 24578 (2019).

Leukocyte mech(n)anobiology: super-resolution microscopy reveals actin nanoscale architecture at cell protrusions

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Keywords: imaging in immunology, cell migration, super-resolution microscopy

Basement membrane transmigration during embryonal development, tissue homeostasis and tumor invasion relies on invadosomes, a collective term for invadopodia and podosomes.

Leukocytes such as macrophages and dendritic cells form hundreds of podosomes and use them for adhesion, migration and possibly for antigen-uptake. Recently we demonstrated the involvement of podosomes also in protrusion-based mechanosensing, but an adequate structural framework for this process is still missing.

Here, by using multi-color super-resolution microscopy in fixed and living human primary dendritic cells, we reveal the modular actin nano-architecture that enables podosome protrusion and mechanosensing. The podosome protrusive core contains a central branched actin module encased by a linear actin module, each harboring specific actin interactors and actin isoforms. From the core, two actin modules radiate: ventral filaments bound by vinculin and connected to the plasma membrane and dorsal interpodosomal filaments crosslinked by myosin IIA. On stiff substrates, the actin modules mediate long-range substrate exploration, associated with degradative behavior. On compliant substrates, the vinculin-bound ventral actin filaments shorten, resulting in short-range connectivity and a focally protrusive, non-degradative state.

Our findings redefine podosome nanoscale architecture and reveal a paradigm for how actin modularity drives invadosome mechanosensing in cells that breach tissue boundaries [1].

[1] van den Dries et al <https://www.biorxiv.org/content/10.1101/583492v1>.

DeepFocus: a Near One-Shot Micro-Manager Auto-focus Plugin using Convolutional Neural Networks

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Keywords: auto-focus, deep learning, micro-manager

Modern microscopy techniques rely on tens of different components, such as lenses, mirrors, light sources, and moving stages, which can be controlled remotely. In life sciences, the autofocus system is a control loop used extensively for acquisition of time lapses, especially in development biology or cellular biology. Indeed, over time, imaged specimens tend to drift from the focal plane (where the image is the sharpest), because of specimen growth, movement or medium flow caused by temperature changes. Autofocus (AF) systems seek to analyze from the image or other data sources, what would be the optimal shift of the imaged sample in terms of axial position to maximize image sharpness. AF algorithms are either using iterative minimization of a one-dimensional objective function, the focus score, to move the object to its optimum. In order to converge in a non-local optimum, these algorithms need to acquire tens to hundreds of images at different axial positions. However, such a high number of image acquisitions can be damaging for the sample, especially in fluorescence microscopy. Furthermore, depending on the software implementation and the imaging modality (eg. confocal imaging), the acquisition of hundreds of images can take up to several minutes.

More recently, the development of single shot AF techniques (needing only one or a few shots) has boomed, thanks to Convolutional Neural Networks (CNN) which are able to draw direct correlations between the image and the optimal shift. The drawback of these direct methods is that a very long and computation-intensive training phase must be repeated whenever the point spread function (PSF) of the optical system changes. Furthermore, available AF algorithms using open-source control software, such as Micro-Manager do not currently supports the benefits, and do not provide solutions for other forms of quantitative assays, such as capturing a timelapse acquisition through a thick sample, or 3D tracking.

We developed an autofocus algorithm that is able to find the right local focus from a couple of images only, thanks to a CNN focus scoring function, derived from [1] and [2], that is invariant to changes in sample, modality, and noise, and infer more information content about the object axial position compared to other scoring functions. This method is developed for the acquisition of both thin samples (such as tissue slides or cell colonies on petri dishes), and thicker samples that need stack acquisition (such as developing embryos). We are releasing the software as an open-source Micro-Manager plugin.

Funded by Swiss Science National Foundation Grant Nr. 200020_179217 "Computational biomicroscopy: advanced image processing methods to quantify live biological systems (2018-2022)"

[1] Shajkofci A. and Liebling M., "Semi-Blind Spatially-Variant Deconvolution in Optical Microscopy with Local Point Spread Function Estimation by Use of Convolutional Neural Networks", IEEE ICIP 2018.

[2] Shajkofci A. and Liebling M., "Spatially-Variant CNN-based Point Spread Function Estimation for Blind Deconvolution and Depth Estimation in Optical Microscopy", submitted.

Title: A comprehensive system calibration protocol for widefield fluorescence microscopy

Widefield fluorescence microscopy has long been an invaluable tool in biomedical research. More recently, application of this technique has further increased with the introduction of new fluorophores and significant advances in optical instrumentation. More specifically, this technique has been used in a broad range of biological studies involving, for example, colocalization analysis, ratiometric imaging, 3D single molecule tracking and multicolor super-resolution imaging. Advanced widefield microscopy setups are generally implemented with many optical components, including mirrors, dichroic filters, excitation/emission filters, beam splitters, objective lenses, and optical cameras. The complexity of such microscopy configurations imposes an inherent risk of optical aberrations and systematic errors which can affect the quality and analysis of the acquired image data. Many methods have been introduced over the years to characterize specific aspects of fluorescence microscopy such as the system's point spread function and field illumination. However, methods for the assessment of the various optical components, such as the objective lens, optical filter, and other key components required for microscope imaging, are lacking, and in general, there is a shortage of software tools for this analysis. Therefore, we present here a comprehensive system calibration protocol for improving the entire experimental pipeline, starting with image acquisition and ending with automated data analysis and workflow documentation. The protocol details the characterization of optical components, the assessment of data quality and validity, and correction of aberrations to allow for the attainment of the performance limits of the imaging system. This protocol allows researchers ranging from novice microscopists to imaging professionals to implement an optimal widefield imaging system.

Probabilistic overall reconstruction of membrane-associated molecular dynamics from partial observations in rod-shaped bacteria

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Keyword: Total Internal Reflection (TIRF) Microscopy, Time-lapse microscopy, Multi object tracking, Image reconstruction, probabilistic modelling, stochastic processes, parametric estimation

Understanding the mechanisms that maintain the structure of rod-shaped bacteria is a challenging problem in cell biological research. We try to gain insight into this problem by observing the dynamics of the cell wall construction workers, the membrane-associated molecular machines (MMs) thanks to the recent development in bioimaging, especially with total internal reflection fluorescence microscopy (TIRFM). Due to the cylindrical form of the bacteria and the selective visualization of the TIRFM, only around one third of the perimeter can be observed. From the partial observed bacteria surface images, using single-particle tracking (SPT), earlier studies showed that a fraction of the MMs performs directed motion, across the image field quasi-orthogonally to the cylinder axis. No re-entry event considered, these motion segments were studied in an independent way. Here we address the problem of the connection of motion segments on a cylindrical surface, assuming that one MM may re-enters into the observed region (OR), a certain time after having left it. The directed MM motions are assumed as Brownian motion with drift, the birth and death events of the MMs are supposed to happen independently and uniformly on the surface. Given a set of observed segments entering and exiting the OR, we propose a probabilistic framework to calculate the probabilities of the events of birth, death and re-entry, based on speed and diffusion of the motion and the time of exit and entry. Even though two third of the surface is hidden as shown in Figure 1, this framework allows us to derive a computational procedure aiming at connecting segments belonging to the same trajectory, and then recovering directed MMs dynamics on the whole surface. The performance of the method is demonstrated on simulation data mimicking MMs dynamics observed in TIRFM. Further work will be focused on the application of the framework to real MMs motion segments, obtained by pre-processing TIRFM acquisitions using SPT.

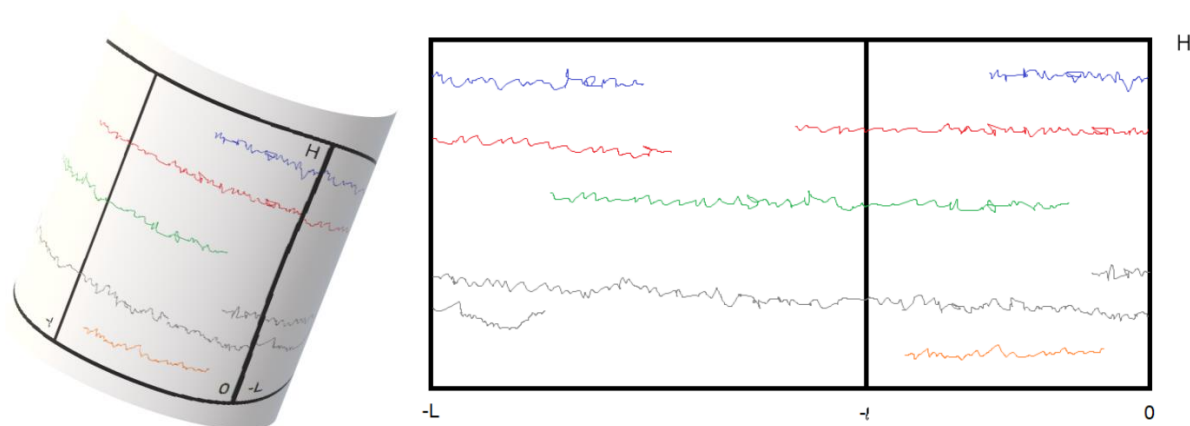


Figure1: Trajectories on the cylinder and its 2D representation. The unobserved region is $(-L, -l] \times [0, H]$ and the observed region is $(-l, 0] \times [0, H]$.

Lipid Membrane Sensing and Remodelling during the immune synapse

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Keywords: T cell Immune Synapse, Cell Membrane Biophysics, Membrane Lipids, RICS

Lymphocyte T cells are responsible for cell-mediated adaptive immune responses, involving transient interactions of the T-cell receptor (TCR) with peptides presented by MHC proteins. A productive interaction triggers the T-cell signaling forming the immunological synapse (IS). Initially, Lck, a membrane-anchored tyrosine kinase, phosphorylates the TCR, ultimately producing basal plasma membrane microclusters and calcium release. For this purpose, the preferred imaging method to unravel nanoclustering at surface-membrane close contact zones during in-vitro T cell activation is TIRF-based super-resolution imaging. Classically, clustering characterisation of the resting (non-activated) state relied on observing T cell onto surfaces coated with Poly-L-lysine;

whereas T cells, onto functionalised surfaces coated with anti-CD3, and -CD28 antibodies, resembled the T cell activation during the IS. In the last years, some super-resolution studies suggested protein nanoclustering already at resting state, which contradicts the consensus picture of protein aggregation upon activation. Recently, results from T cells in controlled suspension [1,2](immersed in a hydrogel gradient) and later on following its activation highlighted that unnatural cell membrane interactions might hinder our understanding of early T cell activation and the subsequent IS. Whereas these studies focused in the protein clustering and its relation to cortical actin dynamics, they overlooked at the role of the lipids membranes in the early activation and during the IS. Typically, protein clustering is assumed to be coordinated with a higher lateral lipid packing at the membranes. We aim at gaining some knowledge on how lipids would behave in these highly dense localised protein aggregation conditions. For this purpose, to better understand how T cell membranes sense and remodel during the immune synapse, we employ fluorescence imaging correlation spectroscopy based methods and reveal the membrane lipid spatiotemporal localisation, diffusion, and collective assembly at the plasma membrane.

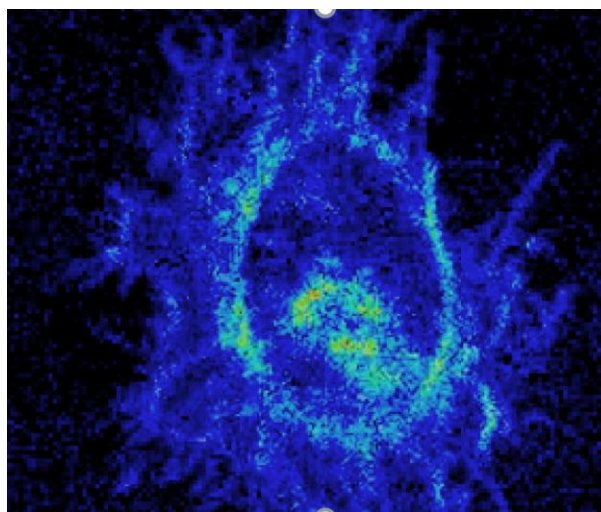


Figure 1. T cell during the Immune Synapse. Jurkat T-cell labelled with Di-4-ANEPPQ. Heat map, normalised fluorescence.

[1] J Bernardino de laSerna et al, *T-Cells in Suspension Do Not Show Pre-Clustered LCK*, Biophysical Journal, 2016.

[2] AM Santos et al, *Capturing resting T cells: the perils of PLL*, Nature immunology, 2018.

Analysis of Nieman Pick C2 Protein in Regulation of Intracellular Membrane Transport by Combined and Correlative Fluorescent and Soft X-ray Microscopy

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Keywords: Correlative fluorescent/cryo-SXT microscopy, applied image analysis, cholesterol trafficking

Cellular cholesterol homeostasis depends on sterol efflux from late endosomes and lysosomes (LE/LYS). Using fluorescent microscopy combined and correlated with Cryo-Soft X-ray Tomography (cryo-SXT), we have studied Niemann Pick C2 (NPC2) protein mediated cholesterol export from LE/LYS.

Cryo-SXT is an optical imaging technique which uses X-rays instead of visible light to visualize cellular ultrastructure in 3D tomograms. Cryo-SXT operates within the X-ray absorption of the *K* edges of carbon and oxygen. Within this *window*, hydrated biological samples do not need to have any additional labeling, since a natural absorption contrast will occur from the carbon-rich biological structures [1]. By cryo freezing, mammalian cells can be imaged in a near-native-state while being moderately protected from radiation damage, enabling tilt series image collection. The tomograms are subsequently aligned and reconstructed, providing the additional advantage of not having to section the sample in order to obtain 3D information [2].

We have used skin fibroblasts from NPC2 lacking patients as a model system to study efflux of plasma membrane derived sterol from LE/LYS. By quantitative fluorescence microscopy we showed that dehydroergosterol [3], trapped in LE/LYSs can slowly efflux from NPC2 lacking fibroblasts, however this process was heavily accelerated upon internalization of bovine NPC2 protein [4, 5]. From cryo-SXT we visualized membrane contact sites between endosomes and other organelles resembling potential routes of intracellular cholesterol transfer [6]. With correlative fluorescent and cryo-SXT we imaged extracellular vesicles containing TopFluor cholesterol and fluorescent NPC2 protein, being potential cholesterol efflux routes.

1. Harkiolaki, M., et al., *Cryo-soft X-ray tomography: using soft X-rays to explore the ultrastructure of whole cells*. Emerging Topics in Life Sciences, 2018.
2. Carzaniga, R., et al., *Cryo-soft X-ray tomography: a journey into the world of the native-state cell*. Protoplasma, 2014. **251**(2): p. 449-58.
3. Solanko, K.A., et al., *Fluorescent Sterols and Cholesteryl Esters as Probes for Intracellular Cholesterol Transport*. Lipid Insights, 2015. **8**(Suppl 1): p. 95-114.
4. Berzina, Z., et al., *Niemann-Pick C2 protein regulates sterol transport between plasma membrane and late endosomes in human fibroblasts*. Chem Phys Lipids, 2018. **213**: p. 48-61.
5. Lund, F.W., et al., *SpatTrack: an imaging toolbox for analysis of vesicle motility and distribution in living cells*. Traffic, 2014. **15**(12): p. 1406-29.
6. Dupont, A., et al., *Analysis of cholesterol export from endo-lysosomes by Niemann Pick C2 protein using combined fluorescence and X-ray microscopy*. Submitted for publication, 2018

Quantitative Phase Imaging

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Keywords: Invited Tutorial: Quantitative Phase Imaging

The basic motivation for optical imaging is that light carries information about objects that it interacts with. This information can be contained in several properties: Intensity, wavelength, polarization or phase.

The light's phase information is somewhat special: It can usually not be directly accessed but requires specific approaches that turn its information into detectable intensity variations. Several techniques have been developed to this end, each adapted to specific measurement conditions.

This tutorial will focus on methods that facilitate quantitative phase imaging, such as holographic microscopy, wavefront sensing and “transport-of-intensity (TIE)”-based techniques.

Amongst other things, it will be explained why Zernike phase contrast cannot be considered quantitative and why phase imaging is not restricted to coherent light.

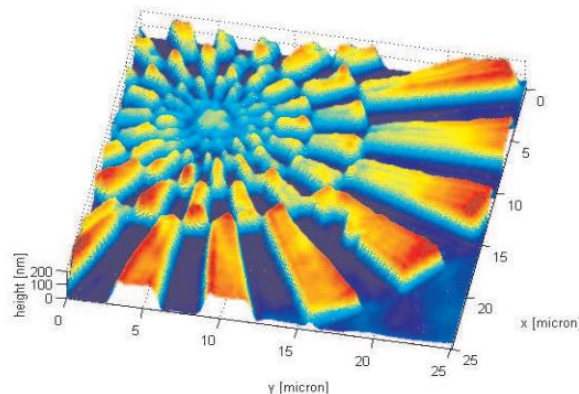


Figure 1 Quantitative height map of a lithographically made microstructure, measured using phase-stepping interferometry

Off-focus imaging exploits supercritical-angle fluorescence emission for precise SMLM

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Keywords: SMLM, SAF, localization microscopy

Fluorescent emitters very close to the coverslip are known to couple “forbidden light”, also denoted as “supercritical angle fluorescence”, at high propagation angles into the glass.

Using a specific 2-channel imaging setup, this light has been shown to improve the axial localization precision in SMLM [1, 2].

We show that off-focus imaging using a standard microscope can provide comparable localization performance in x-y and even better results along z for coverslip distances below 200 nm.

We support our findings by calculations of Cramér-Rao bounds and experimental results from dSTORM imaging of stained microtubule networks.

[1] J. Deschamps, M. Mund, and J. Ries, *3D superresolution microscopy by supercritical angle detection*. Optics Express, 2014. **22**(23): p. 29081-29091, 10.1364/OE.22.029081.

[2] Bourg, Nicolas, et al. "Direct optical nanoscopy with axially localized detection." *Nature Photonics* 9.9 (2015): 587.

Single-molecule approach to study mechanical control of B cell antigen recognition

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B cells have the remarkable capability to mount sensitive and specific antibody responses to antigen, which is an essential mechanism of defence against infection and the basis for many successful vaccines¹. However, many pathogens are highly mutable and conventional vaccination approaches fail to constrain them². The rational design of new vaccines will be driven a mechanistic understanding of how B cells recognise and discriminate antigens to trigger antigen-specific antibody responses. A critical role of B cell receptor (BCR) mechanotransduction during B cell antigen recognition and activation has been recently demonstrated³⁻⁹, but there remains a huge gap in our understanding of the events leading from the initial BCR-antigen binding event to the initiation of intracellular signalling. A challenging aspect of this problem is that B cells integrate a number of chemical, mechanical, and spatial cues at the molecular level when deciding whether or not to respond to antigen. Biophysical approaches capable of quantitative and reproducible control of B cell activation will be key to discriminating the different mechanisms that have been proposed.

Here, we combine single-molecule fluorescence microscopy, calcium imaging, and DNA-based molecular force sensors¹⁰ to determine how BCR binding to membrane-tethered antigen initiates intracellular signalling, and how chemical and physical cues such as antigen affinity and mechanical tension regulate this process. With this multifaceted approach we can resolve the formation of individual BCR-antigen bonds, quantify bond lifetimes and bond numbers, track the growth of individual BCR clusters, and observe the onset and strength of intracellular signalling, all while controlling the mechanical signals transmitted to the BCR¹¹. With these experiments we can capture the transition from the B cell resting state to an activated state, giving new insight into the mechanisms of antigen-triggered B cell activation.

1. Janeway, C. *Immunobiology* (2001).
2. Elhanati, Y. *et al. Philos. Trans.* (2015).
3. Pierobon, P. & Lennon-Duménil, A. M. *J. Cell Biol.* (2017).
4. Spillane, K. M. & Tolar, P. *Mol. Immunol.* (2018).
5. Kwak, K. *et al. Sci Immunol.* (2018).
6. Nowosad, C. R., Spillane, K. M. & Tolar, P. *Nat. Immunol.* (2016).
7. Tolar, P. & Spillane, K. M. *Advances in Immunology* (2014).
8. Spillane, K. M. & Tolar, P. *J. Cell Biol.* (2017).
9. Wan, Z. *et al. J. Immunol.* (2013).
10. Spillane, K. M. & Tolar, P. *Methods in molecular biology* (2018).
11. O'Donoghue *et al.*, *eLife* (2013).

Edge correction in density estimation from repeated point patterns in biological imaging

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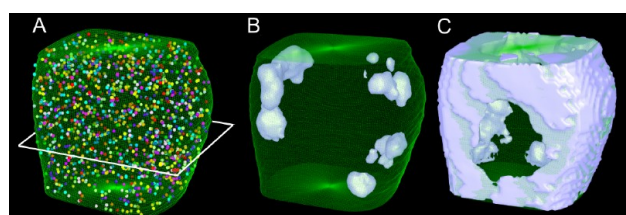
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Elucidating how biological structures organize in space is crucial for the understanding of the way they function. Microscopy is a means of choice by which the 3D organization of biological systems can be revealed. In this context, the consideration of structures composed of small elements that are confined within finite, 3D domains (endosomes in a cell, specific neurons in the brain, etc.) is not trivial. Such data are commonly assimilated to 3D point patterns. Because experiments are typically repeated, datasets are thus samples of point patterns. To analyze such replicated discrete data, appropriate statistical methods are mandatory.

A typical approach for the analysis of point patterns is to estimate point density of the underlying process, i.e., the expected number of points per unit volume at each position in space. Formally, we consider here that point pattern samples are generated by an unknown, stochastic mechanism called a point process, i.e., a process that generates sets of points. Then, we try to assess this process by estimating point density over space, so that we build a spatial cartography of densities. Such an explicit and integrative representation is very informative for the analysis of the spatial organization of a point process. However, at domain boundary, edge effect arises that induces a systematic negative bias in density estimation, which could possibly impair the reading and interpretation of such a map.

To correct this bias, we introduce an edge correction method for a maximum likelihood density estimator based on distance statistics. We propose corrections relying on locally planar or spherical shape approximations of the boundary. Based on corrected estimators, we propose a strategy for the statistical mapping of a 3D bounded point process that adapts to the shape of the domain boundary. The robustness of the correction method is quantitatively demonstrated using point patterns simulated over domains with various shapes. Eventually, we illustrate the practical usefulness of the method by analyzing the 3D spatial organization of compartments revealed by fluorescent microscopy within plant cells. The method should be useful for the statistical analysis of biological structures at different scales.



A: superimposed compartments of 21 cells, within the envelope of a mean cell. BC: iso-density surfaces enclosing the highest estimates density values and above a common threshold, computed with uncorrected (B) or corrected estimators (C).

Super-resolution Microscopy Elucidates Curvature Generation By Endocytic Clathrin Coats In Live Cells and Tissues

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Clathrin-mediated endocytosis is the most extensively studied internalization mechanism of membrane lipids and proteins from the cell surface. Over the past decades, a multitude of biophysical and biochemical methodologies have been employed to elucidate structural and dynamic properties of endocytic clathrin coats. However, fundamental aspects of clathrin-mediated endocytosis remain controversial due to the lack of experimental approaches that allow correlation of ultra-structural and dynamic properties of clathrin-coated structures. Using electron micrographs, it was originally proposed that clathrin initially grows into a flat array (i.e., clathrin plaques) on the plasma membrane prior to transitioning into a curved coat. Flat-to-curved transition of clathrin coats during endocytic vesicle formation was rejected by others as it requires a substantial structural rearrangement, which is energetically unfavorable. As an alternative, it was suggested that curved clathrin-coated structures form gradually without a major structural rearrangement. In this study, we used structured illumination microscopy in the total internal reflection mode to monitor curvature formation by clathrin coats during assembly of individual endocytic complexes within cultured cells and tissues of developing metazoan organisms. Our analyses very clearly demonstrate that endocytic clathrin coats acquire curvature without a major flat-to-curved transition that requires an extensive reorganization of the clathrin lattice. Altogether, our results signify the importance of employing methodologies comprising high resolution in both spatial and temporal dimensions for constructing dynamic models.

Quantitative Analysis of Spatial-Temporal Imaging of Auxin to Cytokinin Ratio During Nodule Development in Soybean (*Glycine max*) by Two-Photon Induced Fluorescence

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Keywords: Two-Photon, Nodule, Auxin

Legumes have a symbiotic relationship with rhizobia, which supply nitrogen to the legume while the legume provides a carbon source for the rhizobia. The legume forms a specialized root organ called a nodule, which is infected by the symbiotic rhizobia. Two phytohormones, auxin and cytokinin are key regulators of nodulation in soybean. The binary vector DR5::GFP-NLS (auxin) and TCSn::TdT-NLS (cytokinin) was transfected into the plants, expressing green (GFP) and red (TdT) fluorescent proteins in proportion to the local auxin and cytokinin concentration in each cell. A nuclear localization signal (NLS) was added to the construct to concentrate the fluorophores in the nucleus. We use multi-photon induced fluorescence microscopy to volumetrically map the spatial variation of these key regulatory phytohormones, and computational methods to quantify the absolute and ratiometric variations in auxin/cytokinin and their spatial and temporal variations during nodule development. Auxin to cytokinin ratio is high in the early stages and decreases in the later stages of development of the primordium. Importantly, specific auxin to cytokinin ratios correlate to specific tissue or cell types.

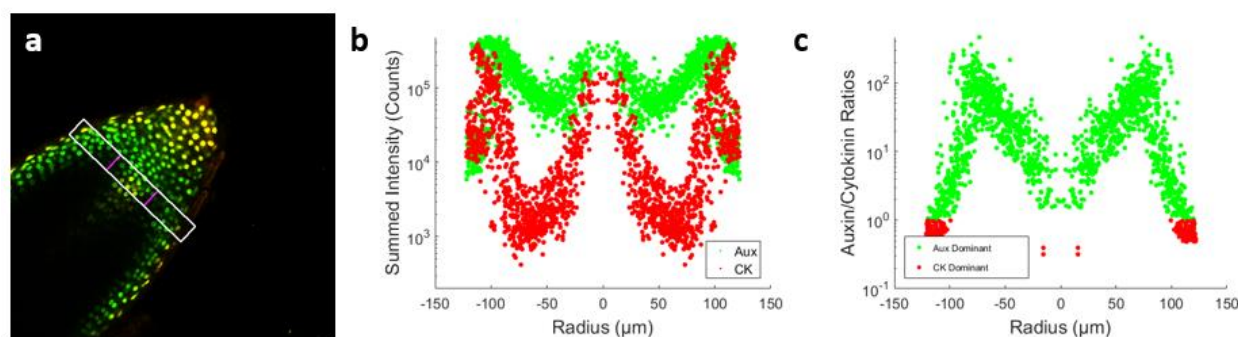


Figure 1 (a) Multi-photon induced fluorescence images of GFP and TdT fluorescent proteins showing spatial-temporal variation of auxin and cytokinin in primary root tip in soybean (*Glycine Max*/W82) (b) quantification of Auxin and Cytokinin output and (c) Auxin to Cytokinin ratio.

[1] J. Fisher, P. Gaillard, C. Fellbaum, S. Subramanian, S. Smith, *Plant Cell Environ.* 41, 2080–2092, 2018.

[2] J. Fisher, P. Gaillard, N. Nurmallasari, C. Fellbaum, S. Subramanian, S. Smith, *in Progress in Biomedical Optics and Imaging - Proceedings of SPIE* 10498, 2018.

Investigating spatial relationships of host-associated microbes using correlative light and electron microscopy

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Keywords: FISH-CLEM, ePhageFISH, Spatial Microbial Ecology

The majority of symbiotic microorganisms thriving in hosts still remain uncultivated in the lab and therefore are assigned to the “microbial dark matter”. In recent years, advances in culture independent sequencing-based methods have accelerated insights into taxonomy, function and activity of whole host associated microbial communities. However, these methods rely on homogenised bulk tissue and thus represent averaged signals of populations detached from their cellular and spatial origin. Therefore, efforts bridging sequencing data back to their spatial context at a cellular level are urgently needed to progress from a community to a cell centred understanding of symbiont physiology, cell-cell interactions, and the distribution of functional niches. Here, we present the application of correlative light and electron microscopy in symbiosis research with the aim of closing the gap between ultrastructure, taxonomic identity and function. In a case study with the uncultured sponge symbiont Poribacteria we present a protocol for fluorescence *in situ* hybridization-correlative light and electron microscopy (FISH-CLEM) that enabled the identification of poribacterial cells in sponge tissue at electron microscopy resolution. Cellular 3D reconstructions revealed bipolar, spherical structures of low electron density, which are likely carbon reserves. Highly expressed proteins related to cellular compartmentation, as detected by metatranscriptomics, were localized in poribacterial cells by combining, to our knowledge, for the first time FISH-CLEM with immunohistochemistry (FISH-IHC-CLEM). Based on our findings, we hypothesize that Poribacteria carry out propanediol degradation, atypically localized along the cytoplasmic membrane. In the second part, we establish a protocol for enhanced PhageFISH, that maps the distribution of selected viruses at single virus resolution both integrated into the host genome and as free viral particles. Overall, the established toolkit allows us to effectively combine -omics approaches with quantitative spatial approaches and should thus be applicable in the wider context of microbiology.

Precise mapping of intracellular diffusion and drift from SPT data analysis

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Keywords: Diffusion, drift, mapping, classification, single-particle tracking.

It is of primary interest for biologists to be able to visualize diffusion and drift inside a cell. These two notions are used to characterize particle motion in the Langevin equation:

$$dX = b(X)dt + \sigma(X)dw$$

where \mathbf{X} designates the (2D or 3D) spatial coordinates of the particle, \mathbf{b} the drift vector, σ the diffusion coefficient, and \mathbf{w} standard gaussian white noise.

We propose a new mapping method inspired from the method of N. Hozé [1], providing results in the form of bitmap images for the diffusion and vector field matrices for the drift by scanning SPT data by spatial blocks, and from the work of V. Briane [2-3], who developed two methods to classify SPT data by particle motion types and detect switches in-between them.

To obtain satisfying sharpness - i.e. to avoid the blurring effect due to calculation of both diffusion and drift in cell coordinates where no data is available - we replace the scanning movement of an averaging window by a trajectory-following movement of a product of spatial and temporal gaussian weights. Each diffusion point and each drift vector are calculated at coordinates corresponding exactly to the coordinates given by the preliminary particle tracking, which gives us more detailed results in the form of point clouds and vector fields.

Classification from V. Briane enables us to label trajectories and even their sub-parts automatically by motion type: confined motion (the particle is bound to a specific point), Brownian motion (the particle moves randomly), and directed motion (the particle moves in a determined direction). We then use this information to calculate diffusion coefficient and drift maps separately on each motion class with the most suitable formula.

[1] Hozé et al., Heterogeneity of AMPA receptor trafficking and molecular interactions revealed by superresolution analysis of live cell imaging, Proceedings of the National Academy of Sciences Oct 2012, 109 (42) 17052-17057; DOI: 10.1073/pnas.1204589109

[2] Briane et al., A statistical analysis of particle trajectories in living cells, Physical Review E, 2018

[3] Briane et al., A Sequential Algorithm to Detect Diffusion Switching along Intracellular Particle Trajectories, Bioinformatics, 2019.

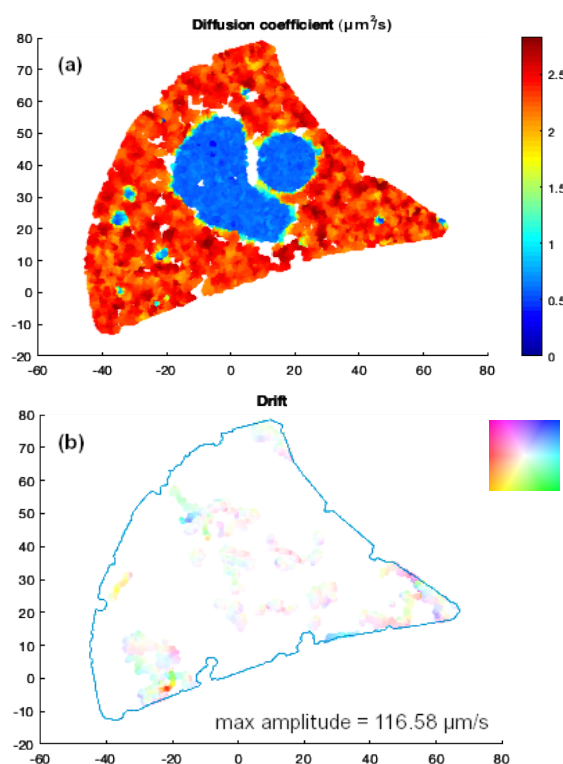


Figure 1. (a) Intracellular diffusion and (b) drift maps processed from SPT data generated with FluoSIM cell simulator. In (b), the hue represents the orientation of the vectors while the saturation represents their amplitude.

3D TIRF nanoscopy with sub-10 nm resolution

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Keywords: TIRF, axial, DNA-PAINT

Imaging the three-dimensional organization of biological structures down to the size of their structural proteins, ~ 4 to 10 nm, can open exciting opportunities in the life sciences. Although this level of resolution has been reached in the (x,y) plane by DNA-PAINT and MINIFLUX techniques [1-2], the issue is not yet solved for the axial counterpart. Axial resolution of fluorescence nanoscopy using a single objective lens lies in the range of 35 to 120 nm and while the 4Pi configuration allows axial resolutions well below 35 nm this comes at the cost of increased technical complexity [3].

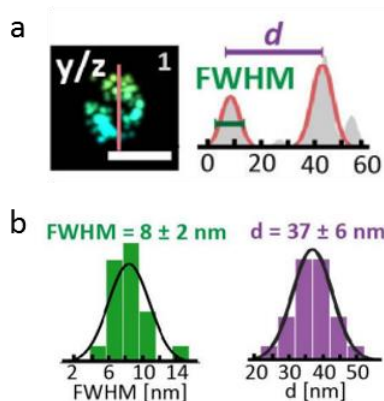


Figure 1. (a) Axial reconstruction & z-profile of immunolabelled microtubules in COS cells by SIMPLE.

(b) Histograms of the FWHM and peak to peak distances calculated from z-profiles of single microtubules ($n \sim 20$). Scale bar represents 25 nm.

Here, we present Supercritical Illumination Microscopy by Photometric z-Localization Encoding (SIMPLE), an easy-to-implement photometric method to determine the axial position of molecules near a dielectric interface under total internal reflection (TIR) excitation. SIMPLE consists of calibrating the detected fluorescence signal considering both the exponential decay of the excitation field (with a small non-evanescent component) and the z-dependent emission of single molecules that are close to the interface, in order to retrieve the axial position of single molecules from a direct measurement of their detected fluorescence signal. In combination with DNA-PAINT, SIMPLE delivers sub-10 nm resolution in all three dimensions, enabling the direct recognition of protein assemblies at the molecular level (Figure 1).

- [1] R. Jungmann, C. Steinhauer, M. Scheible, A. Kuzyk, P. Tinnefeld and F. C. Simmel, *Single-Molecule Kinetics and Super-Resolution Microscopy by Fluorescence Imaging of Transient Binding on DNA Origami*. Nano Lett., vol. 10, p.4756–4761, 2010.
- [2] F. Balzarotti, Y. Eilers, K. C. Gwosch, A. H. Gynnå, V. Westphal, F. D. Stefani, J. Elf and S. W. Hell, *Nanometer Resolution Imaging and Tracking of Fluorescent Molecules with Minimal Photon Fluxes*. Science, vol. 355, p.606–612, 2017.
- [3] D. Aquino, A. Schönle, C. Geisler, C. v Middendorff, C. A. Wurm, Y. Okamura, T. Lang, S. W. Hell and A. Egner, *Two-Color Nanoscopy of Three-Dimensional Volumes by 4Pi Detection of Stochastically Switched Fluorophores*. Nat. Methods, vol. 8, p.353–359, 2011.

Thermophoretic trap for single amyloid fibril and protein aggregation studies

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Keywords: Amyloid Fibrils, Trapping, Tracking, Fluorescence Microscopy, Image Analysis

Several neurodegenerative diseases are related to the formation of aggregates of peptides. Single soluble monomers or oligomers attach into highly ordered, insoluble amyloid fibrils via different transient structures. These important intermediate steps of the fibril growth are commonly hidden in the ensemble average of experimental methods and crucial details such as secondary nucleation processes or fibril fragmentation cannot be captured directly.

We recently presented a method [1] which is able to trap single amyloid fibrils freely diffusing in solution. This so-called thermophoretic trap generates a static temperature field by converting optical energy from a focused laser into heat with a nano-plasmonic metal structure fabricated with the help of laser lithography (Figure 1). Properties of an individual fibril, like its Soret coefficient, which characterizes the motion in a temperature gradient, as well as the translational and rotational diffusion coefficient, can be extracted over a time period of several 10 minutes up to several hours.

I will present in my talk studies on the length dependence of the translational and rotational diffusion coefficient and the Soret coefficient for single amyloid fibrils matching theoretical predictions. The growth of individual trapped fibrils with a resolution of a few 10 nm will be discussed. These growth investigations utilize the high sensitivity of the rotational diffusion coefficient to length changes of the fibril ($D_r \sim L^{-3}$). Furthermore, fragmentation as a secondary growth process for amyloid fibrils could be monitored which has not been seen directly in other studies.

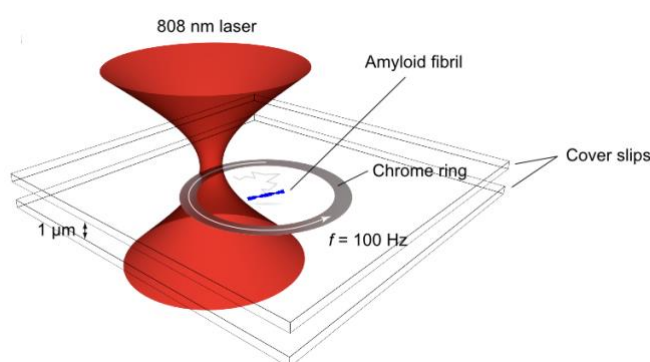


Figure 1. Sketch of the thermophoretic trap. Figure taken from [1].

- [1] M. Fränzl, T. Thalheim, J. Adler, D. Huster, J. Posseckardt, M. Mertig and F. Cichos, *Thermophoretic trap for single amyloid fibril and protein aggregation studies*, Nature Methods 16, 611-614 (2019)

Comprehensive *In-situ* Profiling of Brain Cellular Alterations in the Injured Brain for Accelerating Drug Discovery

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Keywords: Brain Histocytometry, CNN, Concussion, Cellular Alterations

The altered brain needs our urgent attention. While international brain mapping initiatives remain focused on the structure and working of the healthy brain, the need to map the unhealthy brain is compelling and urgent. Pathological conditions like traumatic brain injury (concussion), ischemia, binge alcohol, tumor growth, and experimental drug treatments inflict complex and multi-scale brain cellular alterations that deserve to be mapped in a comprehensive manner.

At the cellular level, traumatic brain injury (TBI) initiates a complex web of pathological alterations in all the types of brain cells, ranging from individual cells to multi-cellular functional units at multiple scales ranging from niches to the layered brain cytoarchitecture. These alterations represent a mixture of changes associated with the primary injury, secondary injuries, regenerative processes, inflammation, tissue remodeling, drug treatments, and drug side effects. Many of these alterations can be subtle and/or latent, only discernible by sensing changes in cell morphology and/or the expression and/or intra-cellular distribution of specific molecular markers, and can be spread across brain regions that are distant from the injury/damage site.

Unfortunately, current immunohistochemistry (IHC) methods reveal only a fraction of these alterations at a time, miss the many other alterations and side effects that are occurring concurrently, and do not provide quantitative readouts. The potential consequence of unobserved and untreated cellular alterations is high, as they may contribute to confounding, co-morbid, or persistent conditions (e.g., depression, headaches, stress-related health problems). Importantly, the current state of drug development for brain pathologies leaves much to be desired, with a recent review concluding that “most of the pharmacologic and non-pharmacologic treatments have failed to demonstrate significant efficacy on both the clinical symptoms as well as the pathophysiologic cascade responsible for the permanent brain injury”.

In this talk, I will describe a practical approach to pathological brain tissue mapping with a focus on rational therapeutics development. Our approach is based on replacing the many low information content assays with a single comprehensive assay based on imaging and analyzing highly multiplexed whole brain sections using 10 – 50 molecular markers, sufficient to analyze all the major brain cell types and their functional states over extended regions. Analyzing these images is challenging due to their complexity, variability, and sheer size. To overcome these challenges, we describe a combination of signal reconstruction, deep Cell detection and segmentation, and high-dimensional data analysis approaches to generate quantitative readouts of cellular alterations at multiple scales ranging from individual cells to multi-cellular units, large cellular ensembles (e.g., cortical layers), and atlas-mapped brain regions for comparative analysis. These data can be used for testing hypotheses, screening individual drugs and combination therapies, and initiating system-level studies.

Applications of single-molecule localisation microscopy to studying negative regulators of T cell activation

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Keywords: Invited Minisymposium speaker: Imaging in Immunology, super-resolution, T cells, cluster analysis, PTPN22, DNA-PAINT

It is now known that many key T cell proteins are not randomly distributed on the cell surface, but instead display a range of distributions from mild heterogeneity to highly clustered. Such distributions can be well studied using super-resolution imaging based on single-molecule localization microscopy (SMLM), however, the analysis of the resulting data requires new statistical approaches. Here, we will first introduce a number of statistical software tools to extract biologically meaningful, quantitative information from SMLM data, applicable to 2D or 3D imaging, fixed and live-cell conditions, multi-colour data and data acquired by PALM, dSTORM and DNA-PAINT. I will then present a biological application of these methods – the use of multi-colour quantitative DNA-qPAINT and quantitative cluster analysis to study the clustering and co-localisation of Csk, TRAF3 and PAG at the T cell immunological synapse, with important implications for autoimmune disease.

Multiconjugate Adaptive Optics for Microscopy

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Keywords: Adaptive Optics, Microscopy

Adaptive optics systems typically use a single corrective device, which is usually a deformable mirror. A single device cannot correct for field-dependent aberrations and so provides correction over a limited field of view. In order to increase the size of the corrected field, additional deformable mirrors can be added. However, this requires many changes to the optical set-up. A solution is to add a transmissive adaptive optics element. We have developed a multiconjugate adaptive optics system for field dependent aberration correction. The sensor consists of an EMCCD and a lenslet array with a 500 μm pitch and 47 mm focal length. A 140-element electrostatic membrane deformable mirror is used in a conjugate pupil plane, with a custom 37-element electrostatic fluidic transmissive adaptive element placed conjugate to the sample. The correction scheme is demonstrated in a multi-purpose microscope used for two-photon and confocal fluorescence imaging as shown in Figure 1.

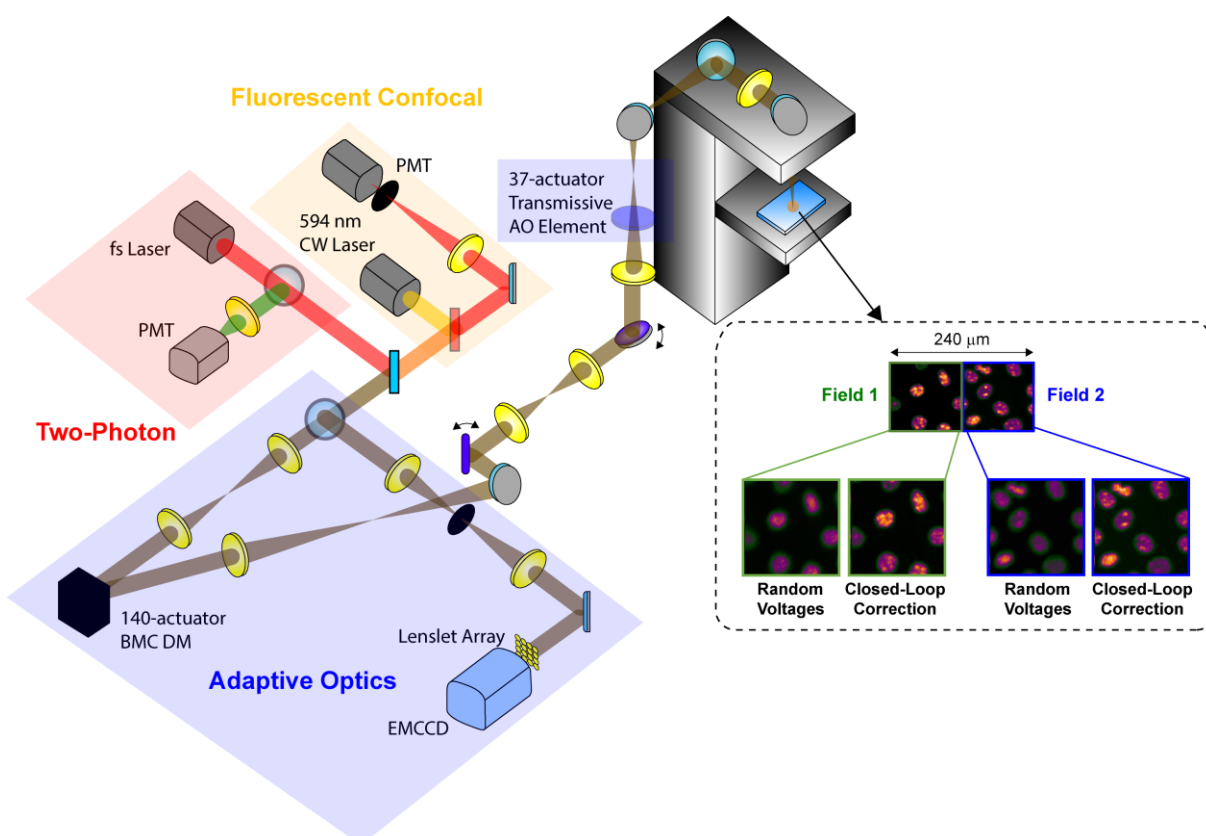


Figure 1. Multi-purpose microscope. Inset shows an image of HeLa cells. Random voltages were applied to both devices and corrected in closed loop for each field in the image.

4Pi aberration correction in single molecule switching microscopy

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Keywords: 4Pi Microscope, Single molecule switching, Aberration modes, adaptive optics, localization

Single molecule switching (SMS) microscopy can provide nanometre spatial resolution for biological studies in cells; however, the axial resolution of standard SMS microscopes is inferior to the transverse resolution. By using two opposing objective lenses for coherent detection, a SMS microscope in 4Pi configuration enables ultra-high axial resolution with an improved signal collection efficiency. Due to the nature of 4Pi imaging, even a moderate sample thickness will inevitably introduce aberrations that affect the focusing performance of the system. More importantly, the aberrations experienced by the two imaging paths of the 4Pi configuration are different and will vary differently as the imaging position moves axially. For these reasons, the axial resolution and imaging efficiency deteriorate significantly in thick samples. It limits the axial imaging range and thus makes imaging large cells with uncompromised resolution impossible without compensating the depth dependent aberrations[1].

The nature of aberrations in a 4Pi cavity has been described and the effect on the system performance was studied[2]. Based on the knowledge, we experimentally demonstrate aberration correction methods using a novel sensorless Adaptive Optics (AO) approach. Two deformable mirrors (DM) are employed in the microscope, one for each objective but working together as a single device to control a set of 4Pi aberration modes that is synthesized from aberration modes across the two pupils in a 'covariant' and 'contra-variant' style. We estimate the aberrations based on various imaging metrics and compare the performance of this method with correction of separate paths[1] for cell imaging on a 4Pi SMS microscope. This method produces consistent spatial resolutions in a large axial range for volumetric imaging.

[1] Huang, F., Sirinakis, G., et al., 2016. "Ultra-high resolution 3D imaging of whole cells". *Cell*, 166(4), pp.1028-1040 (2016).

[2] Xiang Hao, Jacopo Antonello, Edward S. Allgeyer, Joerg Bewersdorf, and Martin J. Booth, "Aberrations in 4Pi Microscopy," *Opt. Express* 25, 14049-14058 (2017)

A universal framework for sensorless adaptive optics

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Keywords: Methods development – Optical methods development

Sensorless adaptive optics (AO) methods are phase aberration correction methods improving image quality in microscopy [1]. The range of sensorless AO schemes that exist have different physical implementations, but can all be fitted into a general mathematical framework that permits comparison between the schemes. One of the major differences among these sensorless methods is that they employ different aberration representations, and hence different modes to modulate and correct phase aberrations. These range from piston correction over many pupil zones [2] [3], through pupil segmentation approaches correcting piston, tip and tilt over zones [4] [5], to full pupil modal correction [6]. In order to compare the effectiveness of these methods, we simulated a two-photon microscope with random system aberration to image a volume object as a demonstration; we used the range of sensorless AO methods to correct the same aberration and compare the output image quality after correction. We expect the general trends revealed through studying the two-photon microscope will also be observed in other imaging systems. Results showed that the modal method performs better in general, while small zone methods offer better correction when the initial aberration has extremely complex shapes. In the case when noise becomes significant in the imaging system, modal methods are much more robust than small zone methods. Our experimental results also support our conclusions drawn from simulation results. These conclusions provide a guideline when selecting a more suitable sensorless AO method for a particular imaging system; they provide us a deeper understanding on all sensorless methods and also help to develop new sensorless AO schemes.

- [1] M. J. Booth and B. R. Patton, "Chapter 2 - adaptive optics for fluorescence microscopy," in *Fluorescence Microscopy*, Boston, Academic Press, 2014, pp. 15-33.
- [2] J. Tang, R. N. Germain and M. Cui, "Superpenetration optical microscopy by iterative multiphoton adaptive compensation technique," *Proceedings of the National Academy of Sciences*, vol. 109, pp. 8434-8439, 2012.
- [3] L. Kong and M. Cui, "In vivo fluorescence microscopy via iterative multi-photon adaptive compensation technique," *Opt. Express*, pp. 23786-23794, 2014.
- [4] N. Ji, D. E. Milkie and E. Betzig, "Adaptive optics via pupil segmentation for high-resolution imaging in biological tissues," *Nature methods*, vol. 7, pp. 141-147, 2009.
- [5] D. E. Milkie, E. Betzig and N. Ji, "Pupil-segmentation-based adaptive optical microscopy with full-pupil illumination," *Opt. Lett.*, vol. 36, pp. 4206-4208, 2011.
- [6] M. J. Booth, M. A. A. Neil and T. Wilson, "Aberration Correction for Confocal Imaging in Refractive Index Mismatched Media," *J Microsc*, vol. 192, pp. 90-98, 1998.

Implementation of Confocal Adaptive Optical Microscopy for Clinical Tissue Analysis

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Keywords: Adaptive Optics, Confocal, Clinical tissue analysis

The main objective of tumour surgery is to attempt complete resection of tumorous tissue with preservation of normal functional tissue. An incomplete resection with remaining infiltrative growing cells increases the risk of recurrence, leading to a shorter life expectancy. Successful total resection relies on accurate intraoperative definition of tumour borders. However, due to intrinsic heterogeneous properties of biological tissues, the resolution, contrast, and imaging depth is compromised such that diagnostic quality imaging is limited to the superficial layers of cells.

Adaptive optics is a technique that can compensate for specimen-induced aberrations by applying an equal but opposite phase pattern, usually to a conjugate pupil plane, using an appropriate dynamic device. The incorporation of aberration correction in confocal surgical microscopes would permit better in situ identification of tumour boundaries at a cellular level.

We have developed a compact confocal microscope with the implementation of adaptive optics using off-the-shelf components. It takes advantage of back-scattered light in the near-infrared (NIR) region, eliminating the need for fluorescent markers and thus is label-free. Two imaging modes are achieved: one with a narrow central field of view (FOV) and cellular level resolution; and the other with a compromised larger FOV that facilitates the process of finding the region of interest. A low NA objective lens is identified, allowing for an effective working distance of 2 mm and an ALPAO DM69 deformable mirror is used for aberration correction.

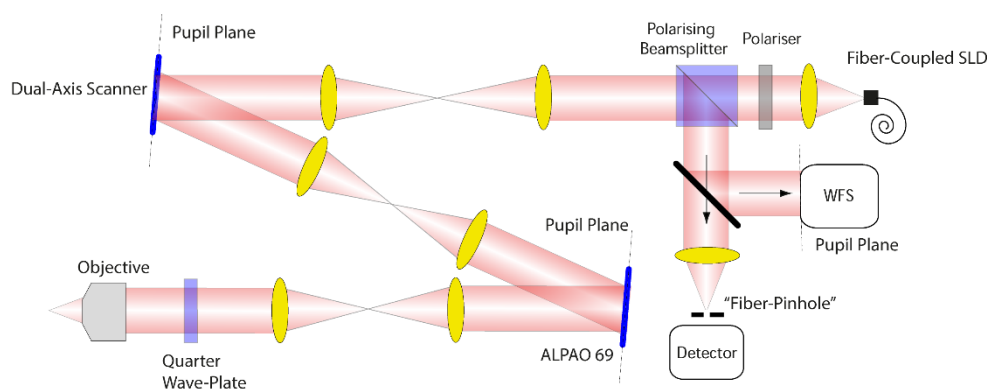


Figure 1. Schematic of compact clinical confocal microscope using adaptive optics.

Uniting Structured Illumination and Localization Microscopy (SIMFLUX)

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Keywords: super-resolution microscopy, SIM, localization microscopy

MINFLUX offers a breakthrough in single molecule localization precision, but suffers from a tiny field-of-view and a lack of practical parallelism. SIMFLUX makes use of extended illumination patterns similar to those used in Structured Illumination Microscopy (SIM). Here, we combine centroid estimation and illumination pattern induced photon count variations in a conventional widefield imaging setup to extract position information over a typical micron sized field-of-view. We show a near twofold improvement in precision over standard localization microscopy with the same photon count on DNA-origami nanostructures and Tubulin, imaged with PAINT and dSTORM.

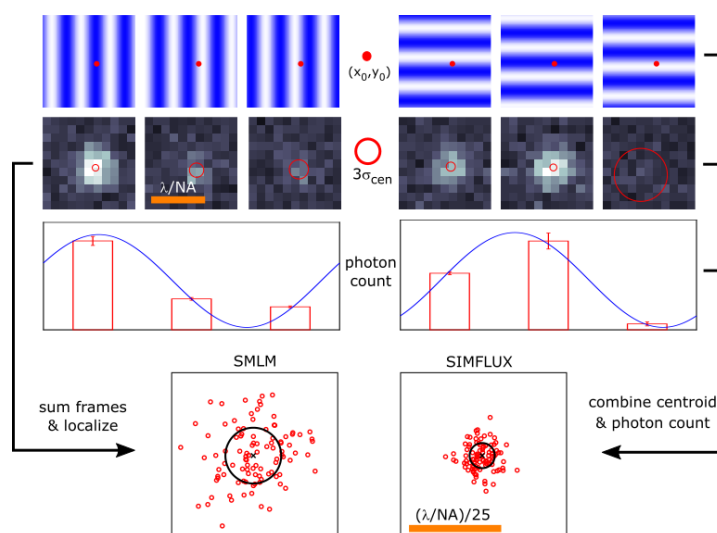


Figure 1. Principle of SIMFLUX. A total of 6 images are recorded with 3 shifted patterns per orthogonal orientation of the line pattern. Combining the centroid estimates of the 6 frames with the photon count in relation to the pattern shift improves the localization precision with a factor of around two compared to the standard centroid estimate on the sum of the 6 frames.

- [1] Jelmer Cnossen, Taylor Hinsdale, Ramsus Thorsen, Florian Schueder, Ralf Jungmann, Carlas S Smith, Bernd Rieger, Sjoerd Stallinga, *Localization microscopy at doubled precision with patterned illumination*, bioRxiv 554337; doi: <https://doi.org/10.1101/554337>

Quantitative analysis of macropinocytosis in *Dictyostelium* captured with light-sheet microscopy

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Keywords: light sheet microscopy, macropinocytosis, segmentation, cell surface analysis

Macropinocytosis is the uptake of extracellular fluid, employed in important biological processes, including antigen processing and cancer cell feeding [1]. This process involves the formation of cups on the cell surface membrane, which draw in fluid and form vesicles. In this work we aim to analyse cell surface activity during macropinocytosis in *Dictyostelium* cells, to better understand the mechanisms involved.

We used lattice light sheet microscopy (LLSM) to record cell activity, which yielded high spatial ($\sim 0.1 \mu\text{m}/\text{pixel}$) and temporal resolution ($\sim 1 \text{ s}/\text{frame}$). Cells imaged expressed markers for PIP3 and actin, both of which are present during macropinocytosis in *Dictyostelium* cells [2]. In order to segment the cell surfaces, we developed a novel random walker-based segmentation method with a curvature-based enhancement term, with the aim of capturing the detailed structure of macropinocytotic cups and filopodia, both of which induce complex geometries on the cell surface. We show that our method performs better than the standard random walker and power watershed in real cell images and synthetic data, and that our method performs better than the random forest pixel classifier in real cell images.

Surface models were generated using the above segmentation method, which enabled correspondence mappings between surfaces in adjacent time frames [3]. By mapping fluorescence values onto these surface models, it is possible to analyse the dynamics of actin and PIP3 at the cell surface, with the aim to investigate these factors and the cell surface geometry during cup formation and closure, and during vesicle formation.

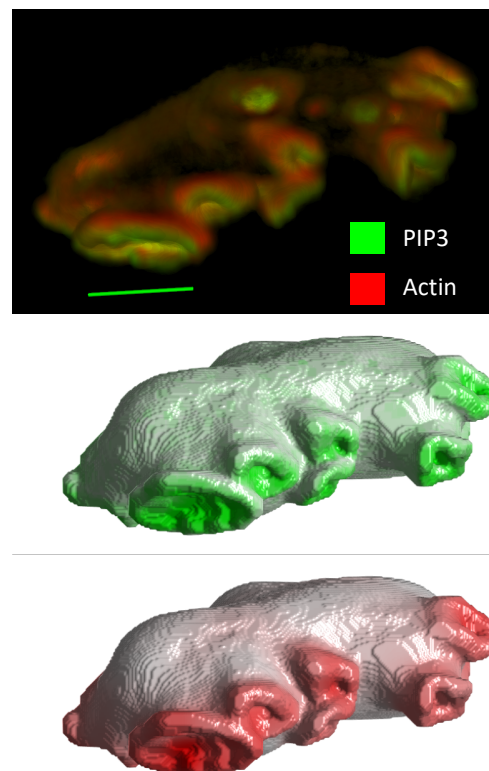


Figure 1. Top: LLSM-imaged cell expressing markers for PIP3 (green), and Actin (red) activity (scale bar $5 \mu\text{m}$). Middle: projection of PIP3 fluorescence onto segmented cell surface. Bottom: projection of actin fluorescence onto segmented cell surface.

[1] J. P. Lim and P. A. Gleeson, "Macropinocytosis: an endocytic pathway for internalising large gulps," *Immunology and Cell Biology*, vol. 89, no. 8, pp. 836–843, 2011.

[2] Kay, Robert R., Thomas D. Williams, and Peggy Paschke. "Amplification of PIP3 signalling by macropinocytic cups." *Biochemical Journal* 475.3 (2018): 643-648.

[3] Lombaert, Herve, et al. "FOCUSR: feature oriented correspondence using spectral regularization--a method for precise surface matching." *IEEE transactions on pattern analysis and machine intelligence* 35.9 (2012): 2143-2160.

Quantitative 3D super-resolution imaging of chromatin ‘blobs’

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Keywords: 3D structured illumination microscopy, high-content imaging, topologically associated domains

Three-dimensional (3D) chromatin organisation plays a key role in regulating genome function in higher eukaryotes. Despite recognition that the genome partitions into ~1Mb-sized topological associated domains (TADs) based on ensemble Hi-C measurements, many features of the physical organisation at the single cell level remain underexplored. Using in vivo and in situ 3D super-resolution microscopy, supported by 3D scanning electron microscopy of cryo-preserved samples, we reveal a sequential curvilinear arrangement of irregular shaped ~200-300 nm diameter nucleosomal assemblies with viscoelastic properties (‘blobs’) juxtaposed to an RNA-populated chromatin-depleted interchromatin network. Quantitative mapping of genome function markers uncovers a zonal distribution, with strong confinement of structural proteins and transcriptionally active/permissive marks to a narrow region at blob surfaces, and enrichment of repressive marks towards the interior. This correlation between nanoscale topology and genome function is relaxed in postreplicative chromatin, and accentuated upon ATP depletion and hyperosmolarity induced chromatin condensation and, remarkably, after inactivation of cohesin [1]. Our findings establish TAD-sized nanodomains (‘blobs’) as physical modules of mesoscale genome organization with functional chromatin states being defined by radial position and exposure to a phase-separated interchromatin space.

- [1] Miron E, Oldenkamp R, Pino DMS, Brown JM, Faria AR, Shaban HA, Rhodes JDP, Innocent C, de Ornellas S, Buckle V, Schermelleh L. Chromatin arranges in filaments of blobs with nanoscale functional zonation. *bioRxiv*, 566638.

Selective volumetric microcopy for single molecule imaging in living cells

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In recent years, light-sheet fluorescent microscopy (LSFM), or selective-plane illumination microscopy (SPIM), has been shown to be the leading standard in 2- and 3-dimensional high-resolution imaging of live samples over a prolonged duration due to its high sectioning capability, high contrast, reduced photobleaching and reduced phototoxicity. Cells are three-dimensional objects and intracellular events are typically not constrained to one focal plane, thus, the conventional single plane microscopy technology is inadequate for detailed studies of fast intracellular and/or single molecule dynamics in three dimensions. In addition, the focal plane may frequently be at the wrong place at the wrong time, thereby missing important aspects of dynamic events.

To overcome these limitations, several recent technologies have been developed (for review check [1]). However, these technologies are not ideal for volumetric, 4D

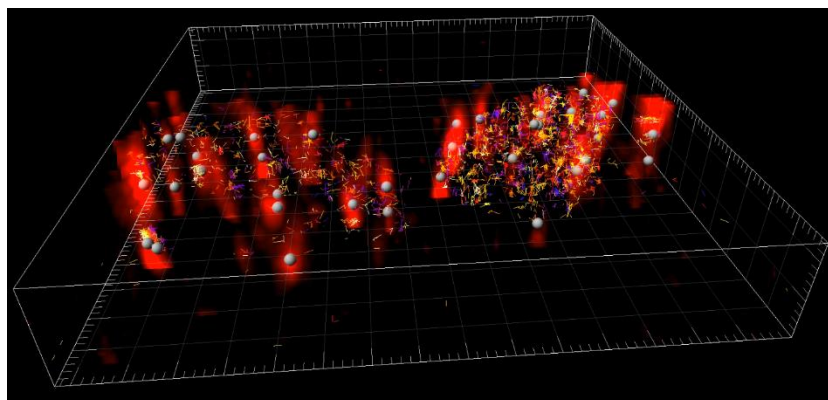


Figure 1: Reconstructed volume of MFM acquisition of H2B single molecules in live cells. The excitation was confined to the observed volume only.

imaging at the single molecule level in living cells. They either have limited resolution at the level of signal detection (spatial resolution) and thus cannot be used for single-molecule imaging, or they require scanning of individual planes on the z-axis thus limiting the volumetric and temporal resolution that could be mostly problematic in live cells.

We have developed a technique that, for the first time, combines the use of LSFM with a multifocus microscope (MFM) which can simultaneously image different focal planes [2], thus enabling the volumetric 4D imaging of samples at the single-molecule resolution (Figure 1). We opted for single objective SPIM (soSPIM) configuration in which a single high numerical aperture objective is used to excite and collect the emission of the sample [3]. We tuned the sheet thickness to reach an optimum excitation volume matching a desired imaging volume of MFM. The principle will be illustrated with dense single particle imaging and live cell imaging of nuclear structures.

References:

- [1] Gustavsson et al., Optics express 2018
- [2] Hajj et al. PNAS 2014
- [3] Galland et al. Nature Methods 2015

Virtual Reality for Quantification of Multidimensional Single-Molecule Data

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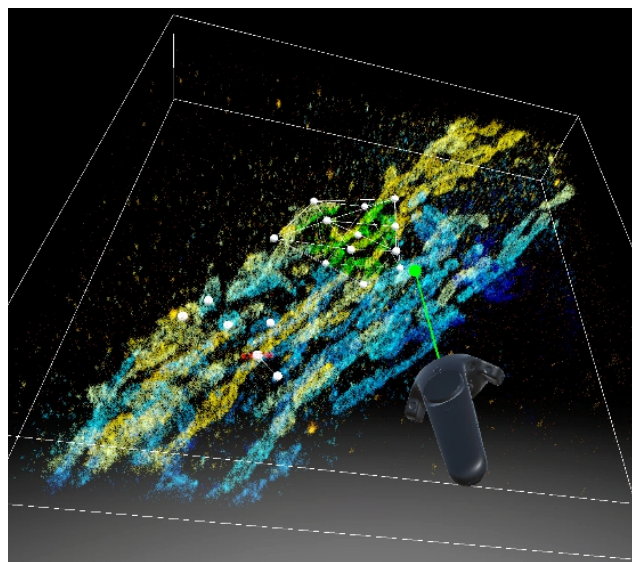
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Recent advances in single-molecule (SM) imaging modalities are uncovering the complexity and the organization of molecular entities involved in a variety of cellular processes. New microscopy techniques are able to reveal not only the 3D distribution of single molecules over entire cellular depths but also their orientation and 3D dynamics [1,2,3]. Exploiting geometric information stored in SM localizations is a real challenge. It is the consequence of the complexity in analyzing and visualizing point clouds. Tools for visualizing and interacting with multidimensional SM data are lacking (e.g. $n > 4$). In fact, currently available tools tend to present SM data on a 2D screen. Selecting a 3D region of interest on a 2D screen for further processing is tedious and challenging.

Virtual reality (VR) has re-emerged recently as an interesting technology in a variety of applications [4]. VR presents a natural way to visualize and interact with 3D data thanks to stereoscopic visualization and motion tracking [5]. It effectively provides an intuitive sense of object geometry and position in space.

We present here a new platform for visualizing and interacting with SM multi-dimensional data using VR environments.

Our immersive platform, entitled GeNuage, (i) allows an efficient presentation and rendering of n -dimensional SM data (millions of points) in a very robust and smooth way, (ii) provides tools for measuring, quantification and selection within the VR immersive environment and (iii) offers an easy interfacing with other platforms such as MATLAB and Python for downstream analysis (Figure 1).



The strengths of our platform will be illustrated with some examples on real SM and synthetic multidimensional data.

References:

- [1] Hajj et al. PNAS 2014
- [2] Aristov et al. Nature Communications 2018
- [3] Augusto Valades Cruz et al. PNAS 2016
- [4] El Beheiry et al. Submitted
- [5] El Beheiry et al. Journal of Molecular Biology (2019)

Superresolution Microscopy Images: What they tell us about protein clusters – and what they don't

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Keywords: Invited Minisymposium speaker: Imaging in Immunology, Single molecule localization microscopy, T cell, immunological synapse, T cell receptor

The organization and dynamics of proteins and lipids in the plasma membrane, and their role in membrane functionality, have been subject of a long-lasting debate. Developments in superresolution microscopy have facilitated for the first time the direct imaging of cellular structures at length scales far below the optical diffraction limit. Indeed, when applied to the plasma membrane the presence of a variety of protein nanoclusters was revealed, which lead to speculations whether nanoclustering was a general feature of plasma membrane proteins. Particularly in T lymphocytes, clustering of signaling proteins has been proposed to represent a fundamental mechanism for cell activation. Recently, however, doubts were raised whether imaging artifacts inherent to PALM/STORM might have influenced or even caused the observation of some of those protein clusters. To approach these concerns, we developed a method to robustly discriminate clustered from random distributions of molecules detected with single molecule localization microscopy-based techniques like PALM and STORM [1]. In my talk I will present the application of superresolution techniques to different proteins expressed at the T cell plasma membrane. Particularly, I will show that the T cell receptor complex is randomly distributed at the plasma membrane of non-activated T cells [2].

1. Baumgart, F., et al., *Varying label density allows artifact-free analysis of membrane-protein nanoclusters*. Nat Meth, 2016. **13**(8): p. 661-664.
2. Rosso, B., et al., *TCRs are randomly distributed on the plasma membrane of resting antigen-experienced T cells*. Nature Immunology, 2018. **19**(8): p. 821-827.

Deciphering genuine transient pauses in super-localized trajectories of single-molecule tracers

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Keywords: single molecule tracking, navigation within localization error, transient pause

Diffusion dynamics of single-molecule (SM) tracers have allowed for understanding of the local physical properties of inhomogeneous systems such as cellular media, membranes, and gel/polymer networks (Figure 1a). Further, super-resolved tracking of individual molecular tracers has revealed diverse mechanisms and transient events which have changed prior perceptions on chemical and bio-molecular interaction mechanisms. Intriguingly, diffusion of SMs in crowded heterogeneous environments is most often found to be anomalous (sub-diffusive) and it has been shown that non-Brownian transport is a consequence of the navigation mechanism, such as those involving momentary pauses (continuous time random walk, CTRW). Typically, positions of mobile SM tracers can be spatially localized down to few tens of nanometers (referred to as the localization error, Figure 1b-c), which is at least an order of magnitude bigger than molecular dimensions of the probes. Thus, super-localized single-particle tracking (SPT) is unable to provide unambiguous dynamical information of probe molecules within the localization error (LE ~20-40 nm). However, the waiting-time of each tracer within the LE is usually correlated to transient pauses (or momentary binding) during tracer navigation in heterogeneous environments.

With the premise that translational motion of tracers in a 3D network is associated with relatively fast (>1 KHz) dipolar reorientation, we conceptualize that true transient pauses within the localization-error can be revealed upon investigation of the SM dipolar reorientational propensity (Figure 1c). In essence, when the reorientation of a single dipole is hindered (or very slow), it is highly improbable that the tracer can undergo translational motion unless confined in a channel of molecular dimensions. To realize this concept, we have developed a polarization-resolved SM tracking (PR-SMT) method, which allows for simultaneous investigation of both translational and rotational dynamics of single-dipoles. As a case study, we investigated the navigational dynamics of single Rhodamine 6G (Rh6G) molecules in a hydrated (plasticized) poly(vinylpyrrolidone) (PVP) thin-film network. While the SMs exhibit a wide range of transport characteristics, the majority of tracers are found to undergo subdiffusive motion.¹ Here, we demonstrate how PR-SMT can be used to identify transient yet genuine pauses *within* the LE for individual trajectories. Our PR-SMT measurements and analysis on 100 SM tracers reveal the dynamics of SM probes within the LE can be quite heterogeneous and provides insights on the local nanoscale environments as well as navigation mechanisms of molecular tracers in a viscous heterogeneous media.

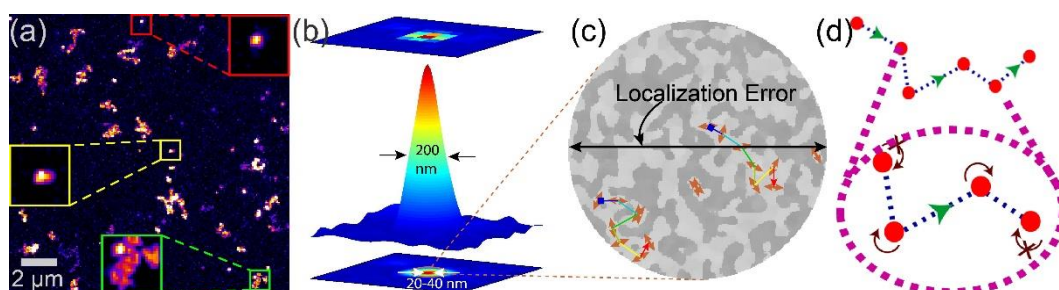


Figure 1. (a) Navigation of individual SMs in PVP. (b): super-localization of tracer (c) movement of tracers within LE (d) reorientational dynamics of tracers while navigation within LE

1. S. Bhattacharya, D.K. Sharma, S. Saurabh, S. De, A. Sain, A. Nandi and A. Chowdhury, *J. Phys. Chem. B*, vol. 117, no.25, pp. 7771-7782

3D-SRRF: Real-Time Live-Cell-Compatible Volumetric Super-Resolution Microscopy

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Keywords: SRRF, MFM, 3D, live-cell imaging, super-resolution microscopy

The direct and real-time observation of cellular mechanisms in live biological systems at high resolution in 3D remains a challenge for the microscopy community. This is mainly because conventional super-resolution microscopy (SRM) techniques require intense illumination or long acquisition times, limiting their compatibility with live-cell imaging. Recently, some of us pioneered the SRRF approach [1,2], which only requires low illumination and few frames to enable SRM. SRRF exploits the spatial (radial pattern) and temporal (intensity fluctuations) features of fluorescent emitters in the sample to extract spatial resolution beyond the diffraction-limit. The original method, however, can only perform 2D (lateral) resolution improvement. To be able to extend this approach in the axial dimension, it is necessary to acquire multiple axial planes simultaneously, similarly to the 2D data.

Multifocus microscopy (MFM), developed by some of us, offers a unique true simultaneous volumetric imaging [3,4], where different focal planes are acquired in parallel on the same camera. Hence, the emitters' 3D spatial patterns can be acquired in a single camera frame, allowing for the capture of its volumetric fluctuations. Here, we present an approach to extend SRRF to true 3D-SRM where the resolution is improved throughout the volume, including in the axial direction, thanks to MFM. This requires engineering the reconstruction algorithm to perform a precise spatial calibration of the 3D data and to handle the difference in spatial sampling between the lateral and axial dimensions.

We demonstrate the approach on simulated data as well as on mitochondrial dynamics in living cells. This method is currently able to provide true 3D SRM in living cells every ~2s, within an imaging volume of 20 x 20 x 3.6 μm^3 . This constitutes an invaluable tool for the dynamic study of numerous biological mechanisms.

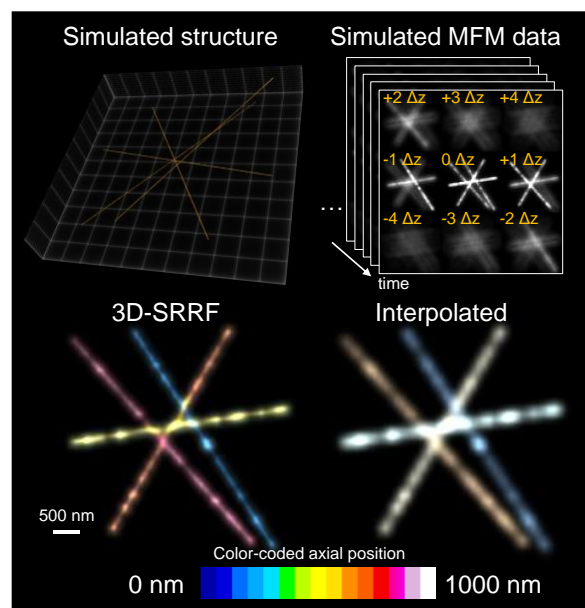


Figure 1. Simulated and reconstructed SRRF-MFM data. The simulated structure consists of horizontal lines with axial spacing.

- [1] N. Gustafsson, S. Culley, G. Ashdown, D. M. Owen, P. M. Pereira, and R. Henriques, "Fast live-cell conventional fluorophore nanoscopy with ImageJ through super-resolution radial fluctuations," *Nat. Commun.*, vol. 7, no. 12471, p. 12471, Aug. 2016.
- [2] S. Culley, K. L. Tosheva, P. Matos Pereira, and R. Henriques, "SRRF: Universal live-cell super-resolution microscopy," *Int. J. Biochem. Cell Biol.*, vol. 101, pp. 74–79, Aug. 2018.
- [3] S. Abrahamsson, J. Chen, B. Hajj, S. Stallinga, A. Y. Katsov, J. Wisniewski, G. Mizuguchi, P. Soule, F. Mueller, C. D. Darzacq, X. Darzacq, C. Wu, C. I. Bargmann, D. A. Agard, M. Dahan, and M. G. L. Gustafsson, "Fast multicolor 3D imaging using aberration-corrected multifocus microscopy," *Nat. Methods*, vol. 10, no. 1, pp. 60–63, Dec. 2012.
- [4] B. Hajj, J. Wisniewski, M. El Beheiry, J. Chen, A. Revyakin, C. Wu, and M. Dahan, "Whole-cell, multicolor superresolution imaging using volumetric multifocus microscopy," *Proc. Natl. Acad. Sci.*, vol. 111, no. 49, pp. 17480–17485, Dec. 2014.

Calibration of a Remote Focusing Microscope Using Deflectometry

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Keywords: Remote Focusing Microscope, Deflectometry, Deformable Mirror

Remote focussing (RF) is an important technique in the field of microscopy as it provides focussing without movement of the microscope objective or sample stage to get stacks of images over large depths. With appropriate calibration, RF can be implemented using a deformable mirror. To determine the mirror's surface profile in terms of Zernike Polynomials (ZP), researchers have used optical interferometry [1]. However, the technique has limited dynamic range, and is complicated and expensive [2]. An alternative approach is to use deflectometry, which measures the ZP by using the distorted image of an illumination pattern to obtain the gradient of the phase in the x and y directions i.e. $\partial\Phi/\partial x$ and $\partial\Phi/\partial y$, where Φ is the phase [3]. To implement this for any surface we need a surface under test (Deformable Mirror, Mirao 52-e), a display, a camera (Andor Zyla) and a computer for processing of the captured images (Fig.1). This method could also be used in closed loop and to measure intermediate fast dynamic shapes of surfaces under test. These qualities make this method very advantageous when comparing it with interferometry for this application.

In the presented report we have used the above-mentioned qualities of deflectometry to train a Deformable Mirror (DM) that was used in closed loop for calibration of a Remote Focusing (RF) microscope. Combining high dynamic range and inexpensive deflectometry with a DM inside a RF microscope makes the image capturing procedure faster than conventional scanning methods. Moreover, it also decreases errors while trying to image large distances inside the biological sample [4]. Since our method also incorporates a DM, we could also correct optical aberrations to get high quality volumetric images at relatively high speed compared to conventional focus methods.

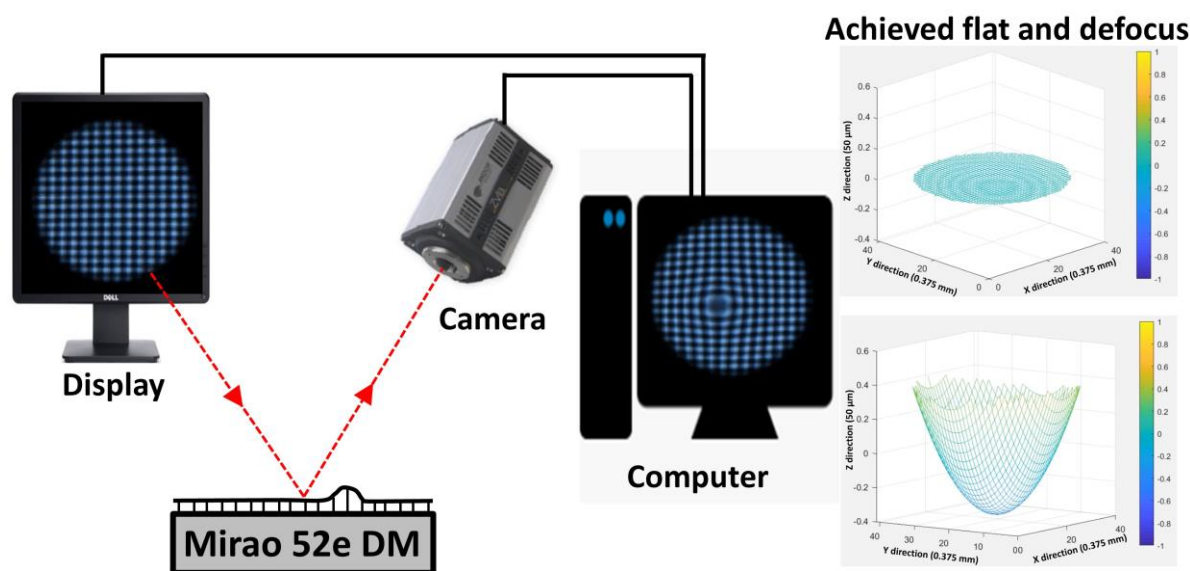


Fig.1 Experimental concept used in the investigation. The experimental set-up could be deployed inside the remote focussing microscope to enable online calibration and monitoring.

- [1] M. Booth, T. Wilson, H.-B. Sun, T. Ota, and S. Kawata, *Appl. Opt.* **44**, 5131 (2005).
- [2] C. Faber, E. Olesch, R. Krobot, and G. Häusler, in edited by J. Schmit, K. Creath, C. E. Towers, and J. Burke (2012), p. 84930R.
- [3] L. Huang, M. Idir, C. Zuo, and A. Asundi, *Opt. Lasers Eng.* **107**, 247 (2018).
- [4] M. Žurauskas, O. Barnstedt, M. Frade-Rodriguez, S. Waddell, and M. J. Booth, *Biomed. Opt. Express* **8**, 4369 (2017).

Quantification of DNA by Mass Photometry

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Keywords: label-free detection, mass photometry, DNA quantification, DNA-protein interactions

A primary advantage of label-free detection methods over fluorescent measurements is its quantitative detection capability, since an absolute measure of adsorbed material facilitates kinetic characterization of biomolecular interactions. Mass photometry based on interferometric scattering microscopy (iSCAT) is a novel, label-free imaging and quantification approach, having demonstrated accurate mass measurement of single biomolecules in solution [1]. Here, we show that mass photometry is equally applicable to nucleic acid detection and mass quantification. Individual dsDNA molecules binding to a cover glass surface

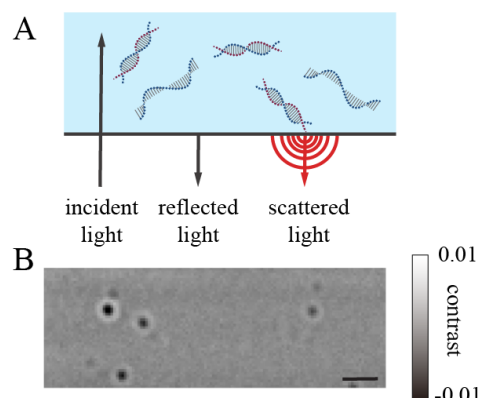


Figure 1. Concept of mass photometry. (A) Scheme of the experimental approach. (B) Differential interferometric image of dsDNA ladder. Scale bar, 1 μm

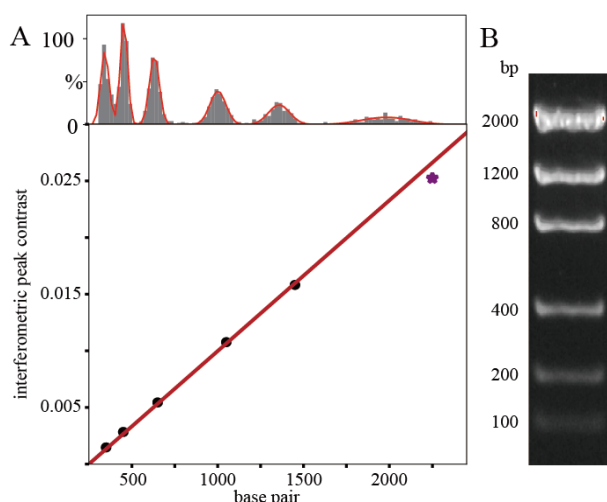


Figure 2. (A) Histogram of single-molecule binding events and their scattering contrasts for the dsDNA ladder along with the Gaussian fit to it (upper panel). Contrast versus base pair for the dsDNA ladder (lower panel). **(B)** Gel image of the dsDNA ladder.

(Fig. 1A) produce strong interferometric contrast, with a detection limit on the order of 60 base pairs (Fig. 1B). Characterisation of standard DNA ladders yields mass distributions with well-resolved peaks, exhibiting a clear correlation between interferometric contrast and the number of base pairs.

Our results, in combination with similar capabilities for ss-DNA and polypeptides, illustrate the potential of mass photometry to provide quantitative insights on nucleic acids, and their interactions with proteins.

[1] Young, Hundt and et al., Science 360, 423–427 (2018)

Site-selective visualization and tracking of DNA methylation in fixed cells

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Keywords: DNA methylation, chromatin structure

DNA methylation plays a crucial role in epigenetic gene regulation and protein function modulation. Abnormal levels of CG methylation are found in cancer cells. For example, hypermethylation is found in regions of the genome rich in cytosine and guanine, so-called CpG islands; yet, across the entire genome hypomethylation is the dominant trait.¹ Even though the methylated cytosine residues can be detected with single nucleotide resolution using sequencing methods, little is known about the impact of DNA methylation in chromatin organization in nucleus.

Herein, we report a novel approach for the 3D visualization of DNA methylation architecture in mammalian cells. We employ methyltransferase-directed labelling of the epigenome using a synthetic cofactor analogue for these enzymes bearing a clickable moiety for fluorescent labelling of DNA (Fig. 1).² We combine this with expansion microscopy and inverted selective plane illumination microscopy (iSPIM) to reveal the organized structures of unmethylated regions of chromatin in healthy and cancerous cells. Using this method we were also able to track the changes in the level of methylation between cancerous and non-cancerous cells as well as cell with depleted methylation.

The application of methyltransferase enzymes enables the site-selective labelling of the unmethylated DNA. Using the state-of-the-art microscopy techniques we were able to take images of its three dimensional architecture in cells.

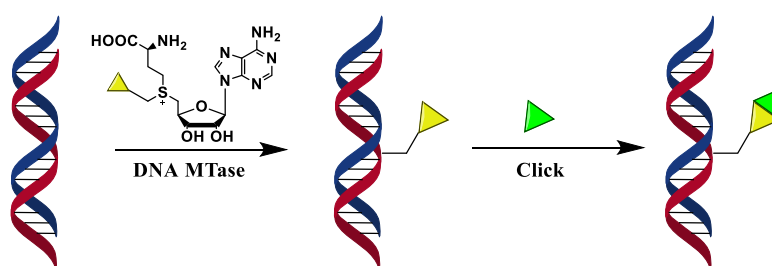


Figure 1. Fluorescent labelling of DNA using methyltransferase enzymes and synthetic cofactors

Acknowledgments: This work was supported by the EPSRC (EP/N020901/1).

[1] Ehrlich M., *Oncogene* 2002, 21, 5400-5413. [2] Deen J., et al. *Nucleic Acids Research*, 2018, 46, e64

Ultrashort dual colour laser at 1.55 and 1.88 μm for the detection of tumour through Raman imaging

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Keywords: Carbon nanotube saturable absorber, mode-locked fibre laser, laser at 1.55 and 1.88 μm lasers

Ultrashort laser pulses have been used for various applications. To obtain these pulses different solutions have been adopted. One of these methods is to use passive techniques by using a saturable absorber (SA). In the family of SAs, Graphene (Gr) and Carbon Nanotube (CNT) are considered to be the broadest SA that could provide mode-locking at various wavelengths [1]. This means that they can mode-locked laser wavelengths whose difference in spectrums is large.

Stain-free histopathology with coherent Raman imaging is an interesting topic as it provides doctors a tool to distinguish between tumours and healthy tissue in-vivo during the surgery [2,3]. This saves time and effort as in this case we do not need to perform any kind of biopsy and the patient do not need to go through the trauma of being operated on. To achieve this task, we need two lasers that could provide pulses at two different wavelengths that are synchronised in time. In the past, two lasers were used that were synchronised by using timing controls. However, this made the system complicated and expensive. In this study, I have presented a laser that is able to provide two lasers that are approximately ~ 250 nm apart [4]. Since a common CNT was used, the pulses that came out were synchronised in time. Moreover, since a fibre laser is presented in the study, the laser could be made compact and is robust.

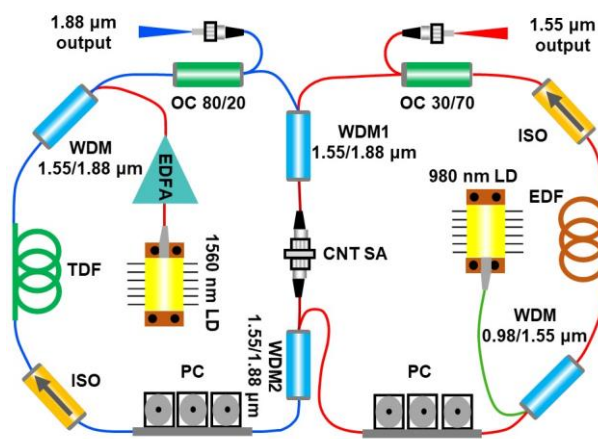


Fig. 1. Dual wavelength passively mode-locked Erbium and Thulium lasers by using CNT SA. WDM: Wavelength division multiplexer; LD: Laser diode; PC: Polarization controller; EDFA: Erbium doped fibre amplifier; TDF: Thulium doped fibre; EDF: Erbium doped fibre; OC: Optical coupler; and ISO: Isolator.

- [1] U. Keller, *Nature* **424**, 831 (2003).
- [2] C. W. Freudiger, R. Pfannl, D. A. Orringer, B. G. Saar, M. Ji, Q. Zeng, L. Ottoboni, W. Ying, C. Waeber, J. R. Sims, P. L. De Jager, O. Sagher, M. A. Philbert, X. Xu, S. Kesari, X. S. Xie, and G. S. Young, *Lab. Investig.* **92**, 1492 (2012).
- [3] M. Ji, S. Lewis, S. Camelo-Piragua, S. H. Ramkissoon, M. Snuderl, S. Venneti, A. Fisher-Hubbard, M. Garrard, D. Fu, A. C. Wang, J. A. Heth, C. O. Maher, N. Sanai, T. D. Johnson, C. W. Freudiger, O. Sagher, X. S. Xie, and D. A. Orringer, *Sci. Transl. Med.* **7**, 309ra163 (2015).
- [4] S. A. Hussain, *IEEE Photonics Technol. Lett.* **31**, 990 (2019).

ConCoR: A software pipeline for high-throughput image analysis of time-dependent colocalization data

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Keywords: applications in biopharma, cell biology, image analysis, colocalization, automation

Colocalization assays are widely used to determine how frequently two or more fluorescent labels spatially correspond. Several statistical measures are available to describe these correlations, but interpreting them is not straightforward for a dynamic system, where both the number of objects and their fluorescence intensity may be in flux. The central aim of our study was to develop a high-throughput, open-source software pipeline specifically for time-dependent colocalization analysis.

We developed ConCoR (Contextualized Colocalization in R), an image analysis pipeline which combines a macro in ImageJ with a set of functions in the statistical programming language R. The ImageJ macro takes a grayscale image stack as an input, applies a threshold (for which several options are implemented), counts the number of nuclei per image, quantitates pixel overlap statistics using the OBCOL plugin, and saves all data to a spreadsheet; the macro iterates this process over all images in the dataset. The R program is then used to assign labels, collate the data, determine mean and 95% confidence interval for each time-point, and display the outputs as a report (Figure 1). We validated our pipeline using confocal image data from six independent time-course experiments in which recombinant human serum albumin (rHSA) is rescued via the FcRn recycling pathway.

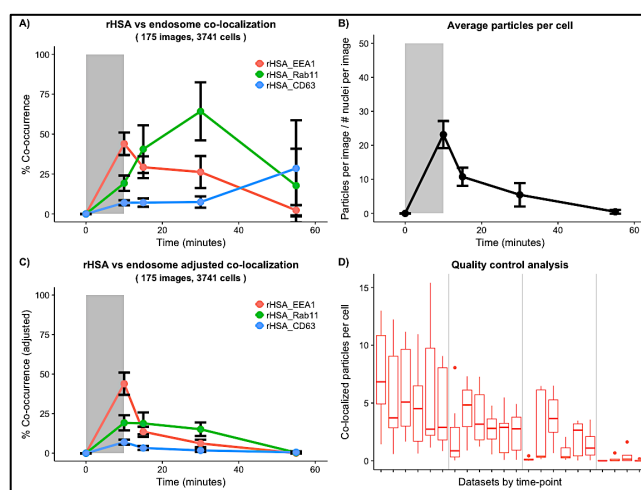


Figure 1. To resolve the apparent discrepancy between our observations and the statistical quantitation (A), our pipeline contextualizes percent colocalization (C) by normalizing to the number of detected objects at each time-point (B). Variance within and between datasets can be visualized using box plots (D).

We found that the conventional method of simple percent colocalization produces undesirably large confidence intervals and some unintuitive results, especially at later time-points (Figure 1A). To resolve the apparent discrepancy between our observations and the quantitation, our pipeline contextualizes percent colocalization by normalizing to the number of rHSA objects (Figure 1B & 1C). This method provides a more intuitive measure of how the distribution of rHSA to endocytic vesicles changes over time and more closely matches our observations from the raw images.

ConCoR enables high-throughput quantification of fluorescence imaging data. It automatically generates reports that are standardizable, reproducible, high-resolution, and readily shareable. It produces quality-control box plots that help visualize variance in the data for each time-point (Figure 1D). It enables the side-by-side comparison of simple colocalization and contextualized colocalization metrics. While HSA-FcRn data was used in its development, our open-source pipeline is generalized and may be useful in contextualizing and evaluating a wide range of colocalization experiments.

Models of single molecule dynamics from single photon detections: a Bayesian nonparametric approach

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Keywords: single molecule, single photon, Bayesian inference, stochastic processes

Fluorescence time traces report on dynamical properties of biomolecules. The basic unit of information contained within these traces is the individual photon. Such single photons carry instantaneous information from the biomolecule, from which they are emitted, to the detector on rapid, sub-microsecond, timescales. Thus, from confocal microscopy it is theoretically possible to monitor biomolecular dynamics at such timescales. In practice, however, signals are stochastic and in order to deduce dynamical information through traditional means, such as fluorescence correlation spectroscopy (FCS) and related techniques, fluorescence signals are collected and temporally auto-correlated over several minutes. So far, it has been impossible to analyze dynamical properties of biomolecules on timescales approaching data acquisition as this demands that we estimate the instantaneous number of biomolecules emitting photons and their positions within the confocal volume. The mathematical structure of this problem demands that we abandon the normal ("parametric") Bayesian paradigm. Here, we exploit novel mathematical tools, namely Bayesian nonparametrics, that allow us to deduce in a principled fashion the same information normally deduced from FCS but from the direct analysis of significantly smaller datasets starting from individual photon arrivals. We discuss the implications of this method in helping dramatically reduce phototoxic damage on the sample and the ability to monitor out-of-equilibrium processes [1,2].

[1] M. Tavakoli, S. Jazani, I. Sgouralis, O.M. Shafraz, S. Sivasankar, B. Donaphon, M. Levitus, S. Pressé "Pitching single-focus confocal data analysis one photon at a time with Bayesian nonparametrics", in review (2019).

[2] S. Jazani, I. Sgouralis, O.M. Shafraz, M. Levitus, S. Sivasankar, S. Pressé "An Alternative Framework for Fluorescence Correlation Spectroscopy", Nat. Comm., 10, 3662 (2019).

FCS and anomalous diffusion model as tools to monitor molecular interactions in living cells

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Keywords: biophotonics, subdiffusion, anomaly coefficient, interactome, FCS, molecular dynamics, P-TEFb, HEXIM1,

Diffusion of transcription factor (TF) plays a key role in the control of their target search. Dynamic proteins in the nucleus classically display a subdiffusive behavior which can be modelled with anomalous diffusion [1], yet the underlying physical mechanisms are still debated. We addressed this question by monitoring the dynamics of HEXIM1, a multifunctional hub involved in transcription regulation. By studying photon fluctuations of fluorescently labelled HEXIM1-mEGFP with FCS (fluorescence correlation spectroscopy), we explored the contribution of TF interactome to the generation of anomalous diffusion. We show that HEXIM1 interactions with 7SK RNA and with positive transcription elongation factor b (P-TEFb) determine HEXIM1 anomalous diffusion. Moreover, numerical simulations allowed us to establish that, in a regime where interactions change very slowly relative to the residence time, the proportions of distinct oligomeric HEXIM1 subpopulations define the apparent anomaly parameter of the whole population. Altogether our data open new prospects in which the alpha coefficient in anomalous diffusion becomes a rule to track and report modifications in molecular interactome.

[1] Wachsmuth M, Waldeck W, Langowski J. Anomalous diffusion of fluorescent probes inside living cell nuclei investigated by spatially-resolved fluorescence correlation spectroscopy. *J Mol Biol.* 2000 May 12;298(4):677-89.

CLIJ: GPU-accelerated real-time image analysis is the key to smart microscopy

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GPU-acceleration, real-time, bio-image analysis, smart microscopy

Long-term light sheet microscopy is the method of choice for studying tissue morphogenesis in early embryo development of organisms such as *Tribolium castaneum*. This gentle imaging technique allows acquiring huge amounts of data in high spatio-temporal resolution. On the one hand, the amount of data is a challenge and reducing it by concentrating imaging on relevant time ranges is worthwhile. On the other hand, sparing light intensity by reducing temporal resolution would also be beneficial for sample health having photo toxicity in mind. A smart microscope could detect when processes of interest are happening and react to these events by modulating temporal resolution for example.

We propose online image processing for quantitative analysis during acquisition to detect biological events such as begin of gastrulation. Therefore we use our recently developed general-purpose GPU-accelerated image processing toolkit CLIJ [1]. To demonstrate its potential, we analysed images from nuclei-GFP expressing transgenic *Tribolium* embryos. After background subtraction and cylinder maximum projection we count visible spots to be able to estimate the developmental stage of the embryo. We show that this computationally heavy task can be solved using CLIJ in an NVidia P6000 GPU in less than a second. As this is far below the frame duration we use for imaging, we claim achieving real-time performance.

Real-time image analysis has high potential in smart autonomous microscope control and GPU-acceleration is a key technology for that. CLIJ paves the path for life-scientists interested in online real-time image analysis during acquisition.

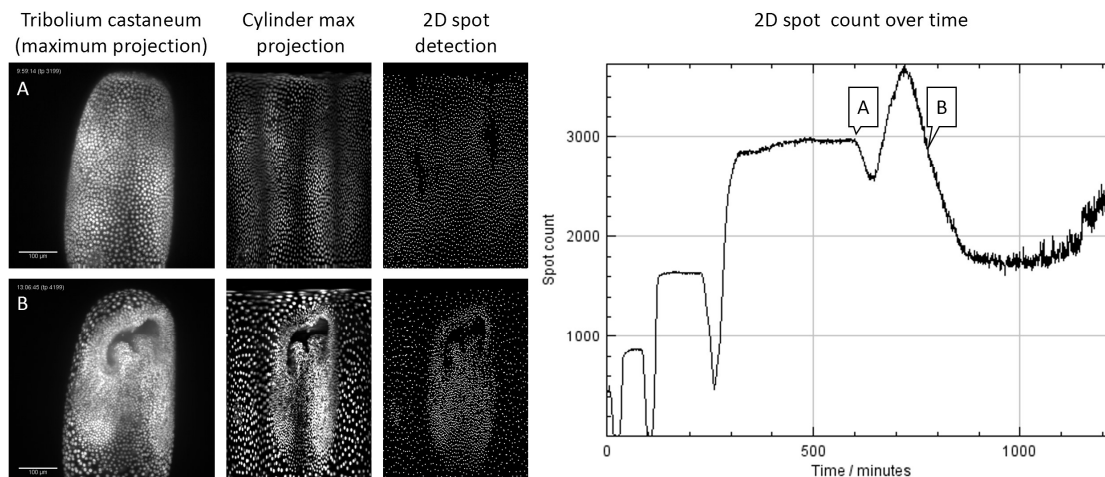


Figure 1: *Tribolium castaneum* shown as maximum and cylinder maximum projection before (A) and after (B) onset of gastrulation. The number of spots detected in the 2D cylinder maximum projection image suggests that gastrulation can be predicted from spot counts.

[1] Haase, R. et Al. CLIJ: GPU-accelerated image processing for everyone. BioRxiv preprint. <https://doi.org/10.1101/660704>

Toward SMLM acquisitions assisted by real-time quality control

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Since super-resolution microscopy (SR) aims at providing improved image resolution below the diffraction limit, quantification at the nanoscale requires single-molecule localization microscopy (SMLM) as it provides insights at the molecular level.

Through a robust theoretical study [1], we demonstrated that there is a strong and constant link between the local density (D), the signal-to-noise-ratio (SNR) and the precision of localization (Fig. 1). We showed the impact of inter-particle distances, intensity of each particle, local background on the results of detection/estimation algorithms and defines regimes where one-gaussian (Fig.1, green area), multiple-gaussian (Fig.1, yellow to orange area) fitting algorithms are required, or when the enumeration of particles is not anymore possible due to too high density of particles/frame (Fig.1, red area). Thus, we have designed UNLOC [4], an ImageJ plugin dedicated to high-density SMLM acquisitions.

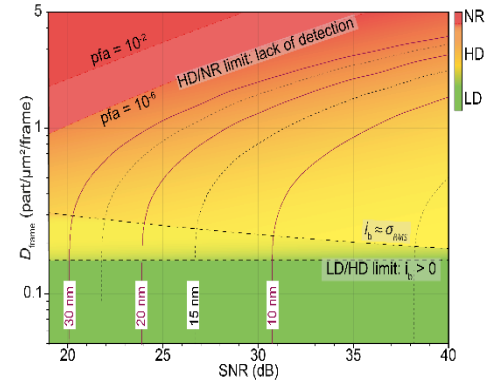


Figure 1. Theoretical density/SNR and localization precision diagram

Still, there is a need to evaluate in real time the density and SNR value in order to control the quality of SMLM acquisitions and assist the user to the record of the data.

If such tools already exist [2-3], they are not easy to use by experimentalists who do not have strong expertise in image analysis. Moreover, these tools are not fully compatible with a real-time quality control during the acquisition.

Here, we propose a fast and quantitative tool to visualize the quality of SMLM raw data by calculating spatially and temporally the maps of local density D and SNR. This provides a direct and unambiguous way (1) to evaluate quantitatively the quality of the raw data in real time and (2) to choose the algorithm strategy to analyze these data (single or multiple emitter fitting, etc.).

We show the relevance of this new fast and parameter-free tool on simulated and real data showing the potential interest for experimentalist who would like to evaluate the relevance of their acquisitions.

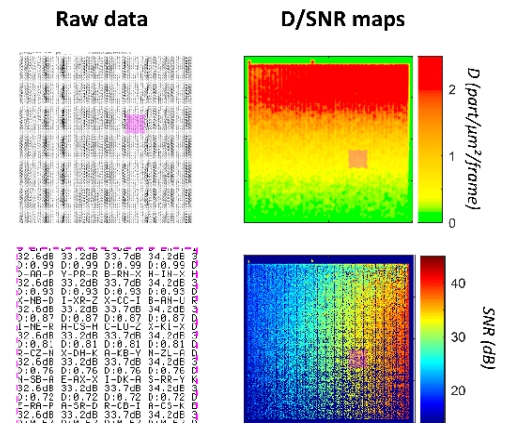


Figure 2. D and SNR maps

Next improvements are oriented to real-time computation and the feedback control of the acquisition parameters.

[1] Mailfert *et al.*, *Biophys J*, 2018.

[2] Banterle *et al.*, *Journal of Structural Biology*, 2013.

[3] Culley *et al.*, *Nat Meth*, 2018.

[4] UNLOC (UNsupervised particle LOCalization) ImageJ plugin: <http://ciml-e12.univ-mrs.fr/App.Net/mtt/>

Analysis of relative positions in 3D PALM and dSTORM provides high-resolution information on ordered biological complexes

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Keywords: 3D localisation microscopy, macromolecular complex, symmetry

'Super-resolution' microscopy techniques now provide the ability to precisely locate specific molecules within cells and cellular structures, to within ~10 nm. However, the internal organisation of macromolecular complexes at such length scales is still very difficult to access. Single-particle averaging techniques adopted from electron microscopy show some promise, but are of limited use in many fluorescently-labelled samples. As well as limits on precision for the location of each detected molecule, low labelling density of a sample, and heterogeneity in orientation of macromolecular complexes, pose significant problems. We find that we can address these and allow the investigation of such structures with an alternative method of analysing distance histograms obtained from 3D relative positions of localisations, and their fits to those of model macromolecular complex geometries.

We apply this technique to fluorescence localisation data on nuclear pore (Nup107, 3D D-STORM) and DNA-origami (3D DNA-PAINT) complexes, extracting accurate organisational information on 3D shape, symmetry and distances. We also extract the known ~20-nm repeat of α -actinin-2 (ACTN2) organisation in the cardiomyocyte Z-disk lattice, despite high background and low labelling efficiency in 3D dSTORM, using an Affimer [1], and in PALM, using mEos2. Finally, we extract the diameter of the arrangement of a centriolar protein (Cep152), and are able to use our technique for refining particle selection in single-particle image averaging.

We expect this analysis to be applied to study regularity and symmetry in many other complexes, including advancing the understanding of the Z-disk in striated muscle.

[1] Tiede, C. *et al.*, Affimer proteins are versatile and renewable affinity reagents, *eLife*, vol. 6, e24903, 2017.

Acknowledgements: The authors gratefully acknowledge funding from the UK Medical Research Council under grant MR/K015613/1; the Wellcome Trust (Institutional Strategic Support Fund at University of Leeds and travel award to MP). The dSTORM system was funded by University of Leeds alumnus M. Beverley. Isolated cardiomyocytes were a kind gift from the Steele Group, University of Leeds. We thank Jonas Ries for sample preparation and imaging of the Nup107 cells; Thomas Schichlthaerle and Ralf Jungmann for sample preparation and imaging of the DNA-origami complexes; Christian Sieben and Suliana Manley for assistance with centriolar dSTORM simulations; and Hari Shroff, Michelle Baird and Yasuharu Takagi for contributions to sample preparation and imaging of cardiomyocytes.

Dynamic contrast for micro- and macro-scale multiplexed fluorescence imaging against autofluorescence and ambient light

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We introduced Out-of-Phase Imaging after Optical Modulation (OPIOM), which exploits reversible photoswitchable fluorophores (RSFs) as labels and combines optimized periodic illumination with phase-sensitive detection to specifically retrieve the label signal.¹⁻⁶ OPIOM can extract the fluorescence emission from a targeted label in the presence of spectrally interfering fluorophores and autofluorescence^{1,3,4,6} and image biological processes in real time.³ First implemented in fluorescence microscopy,^{1,3} OPIOM discriminated in cells up to four fluorescent proteins exhibiting a same green fluorescence either sequentially or in parallel.³

More recently we demonstrated that OPIOM is not limited to microscopy but is relevant as well for other modalities of fluorescence imaging. We first built a fluorescence macroscope for RSF imaging against detrimental autofluorescence and ambient light.⁴ OPIOM strongly enhanced sensitivity and signal-to-noise ratio for fluorescence detection in blot assays over direct fluorescence observation under constant illumination. It successfully overcame the strong autofluorescence of growth media currently used in microbiology and achieved multiplexed fluorescence observation of colonies of spectrally similar fluorescent bacteria. Finally it easily discriminated RSFs from the autofluorescent and reflective background in labeled leaves, even under interference of incident light at sunlight intensities.

We also transposed OPIOM to wide-field fluorescence microendoscopy with one-photon excitation.⁶ It provided intrinsic optical sectioning, which facilitated the observation of RSFs at targeted positions within the sample. It was efficient to eliminate the interference of autofluorescence arising from both the fiber bundle and the specimen when observing targeted RSFs in several biological samples. Finally, it enabled multiplexed observations of two spectrally similar RSFs, which differed by their photoswitching dynamics.

Compatible with multiple imaging modalities, and simple and cheap instrumentation, OPIOM is expected to find numerous applications in fluorescence micro- and macro-imaging ranging from biological assays, bioprocessing, clinical diagnostics, to outdoor observations of plants and surfaces.

References:

1. *Angew. Chem. Int. Ed.*, **2015**, 54, 2633;
2. *Chem. Sci.*, **2015**, 6, 2968;
3. *Nat. Commun.*, **2017**, 8, 969;
4. *Light Sci. Appl.*, **2018**, 7, 97;
5. *Phys. Chem. Chem. Phys.*, **2018**, 20, 23998;
6. *Optica*, **2019**, 6, 972

Statins – not just about cholesterol reduction

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Keywords: Statin, Membrane Organization, Fluorescence Correlation Spectroscopy (FCS)

Statins are amongst the best-selling drugs and is appreciated mainly for its cholesterol-lowering feature. They have proven to be hugely beneficial in the prevention of cardiovascular diseases, such as heart attack and stroke. The drug inhibits the formation of a metabolite mevalonate that is essential for cholesterol synthesis. However, mevalonate is also essential for other biological functions related to cholesterol-independent secondary isoprenoid pathway. Given the rising concerns of side-effects of statin, it becomes necessary to demonstrate the cause of such incidences. Using fluorescence-based various microscopic techniques, we provide evidence that the isoprenoid pathway is equally important and is potentially related to the underlying benefits (or adverse effects) of statin. Our study suggests that statin has global effects in cell: increasing the membrane fluidity and affecting the membrane order and topological organization of proteins. We also analyzed the mobility of a very important immunologically relevant membrane receptor using fluorescence correlation spectroscopy (FCS). There were significant changes in the mobility of the receptor. We are currently investigating the biological implications of these changes. Using fluorescence resonance energy transfer (FRET), we had also shown earlier that statin would change the features of interactions of molecules in the plasma membrane. Overall, the current study together with our previous findings support the idea that statin and its related benefits are not just about cholesterol-reduction.

Nanoscale cellular dynamics with super-resolution spectroscopy

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Keywords: Cellular biophysics, cell membrane, super-resolution STED

Cellular homeostasis is usually maintained via complex signalling mechanisms. Although these signalling events involve a multitude of signal transduction molecules, they are usually triggered by interactions between a ligand and a receptor on the cell membrane. The investigation of spatiotemporal interactions between signalling components in the membrane has been hampered by the complex structure of the cell membrane: signalling in the plasma membrane involve not only the core protein components, but also membrane lipids, the actin cytoskeleton and the glycocalyx. Moreover, early phases of signalling occur at very fast temporal (milliseconds) and small spatial (nanometre) scales, which further hampers thorough elucidation of these processes. Therefore, studies aiming at a complete understanding of signalling require both advanced imaging techniques with high spatiotemporal resolution and well-defined reconstituted systems that can pinpoint the role of each functional component. Here, I will explain how we utilize super-resolution STED microscopy combined with fluorescence correlation spectroscopy (STED-FCS) to access the diffusion characteristics of fluorescently labelled lipids and proteins in the live cell plasma membrane. Our data showed that nanoscale mobility of lipids and proteins in the plasma membrane is highly heterogeneous and this heterogeneity gives invaluable information on the molecular bioactivity. We also developed simplified biomimetic of cell membrane to clarify the principles underlying cell signalling at the molecular level and to dissect the essential drivers of these processes.

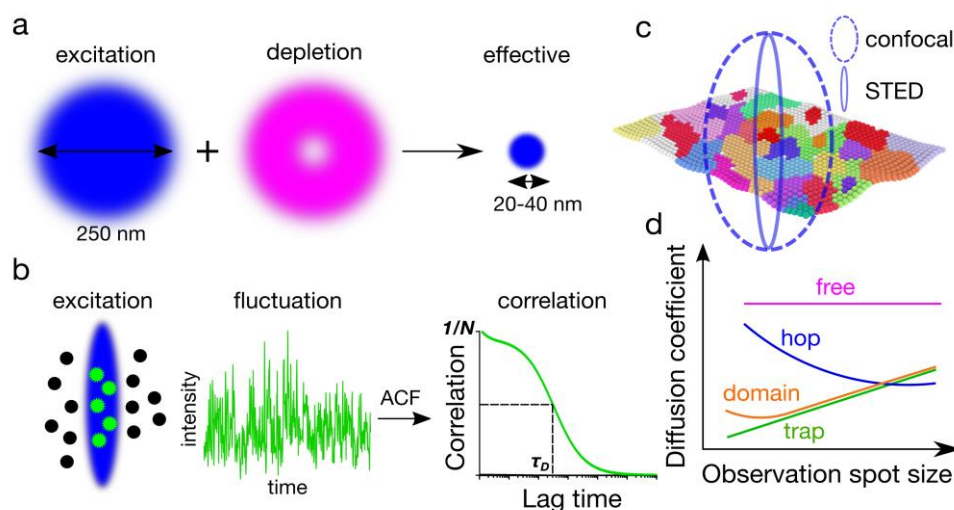


Figure 1: Principles of STED-FCS. a) STED microscopy relies on depletion of spontaneous fluorescence emission from excited molecules (blue) via stimulated emission induced by an additional laser featuring a local intensity zero in the centre (purple) b) FCS measures the average transit time of diffusing fluorescent molecules (green) through the observation volume of a microscope (blue) from recordings of the temporal fluorescence intensity fluctuations and subsequent calculation of the temporal autocorrelation function (ACF) of the fluctuations in the detected fluorescence signal. c) Sketch of nanoscale plasma membrane heterogeneity which can be detected with STED-FCS but not with a large confocal observation volume. d) STED-FCS results in a series of diffusion coefficient measurements from FCS recordings that are obtained at various differently-sized effective observation spots. Please see Sezgin et al, Nature Protocols, 2019 for details.

Quantification of bone tissue heterogeneity and cell distribution patterns from digital histology: application to osteosarcoma

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Keywords: *porous media, segmentation, k-nearest neighbors, immunohistology, effective properties*

Like most sarcomas with complex genomics [1], or more generally bone tissues, osteosarcoma is a type of tumors exhibiting a strong spatial heterogeneity of the micro-environment. This heterogeneity makes the diagnostic complex and can induce strong spatial variability in the response to treatments [2]. New research strategies are consequently needed to understand the impact of spatial heterogeneity on the diagnostic accuracy and on the treatment efficiency, and more generally to understand the links between tissue scale bone matrix structures and underlying biology occurring at the cell scale.

The aim of this interdisciplinary work is to obtain the quantification of correlations between clinical data, heterogeneity of bone tissues and mechanobiological parameters.

To this purpose, original numerical developments [3] were initiated in our group to study the intratumoral and healthy bone tissue heterogeneity from histological and immunohistological sections [4]. The code aimed at obtaining quantitative metrics of the cell population distribution (fig 1d), of the bone matrix micro-architecture (porosity, fig 1e) and of the transport properties (such as effective diffusivity). Because tissues exhibit naturally a complex spatial scales cascade, it can be modeled, at the tissue scale, as a three phases porous medium (fluid, solid, cell populations). Using methodologies related to porous media analysis [5], characteristic lengths were extracted and correlations of phenomena occurring cell and tissue scale examined. Further developments permitted the calculation of effective mechanical properties. The methodology used successive algorithms of machine learning for the histological image segmentation and a combination of iterative algorithms and filters for the correlation calculations. Results put forward the strength of this approach for the identification of new markers in the study of pathological bone tissues.

Acknowledgements: This research was supported by the CGSO (Emergence grant) and the NIH NCI (IMAT program Award Number R21CA21429).

References:

- [1] Gambera et al, Nat Com, 9:3994, 2017.
- [2] Crenn et al, Am J Cancer Res, 7(11): 2333, 2017.
- [3] Assemat et al, ORS 2018. New-Orleans USA.
- [4] Gomez-Brouchet et al, Oncoimmunology, 6(9), e1331193, 2017.
- [5] Cherblanc et al, Transp Porous Med, 30(5):1127,2001.

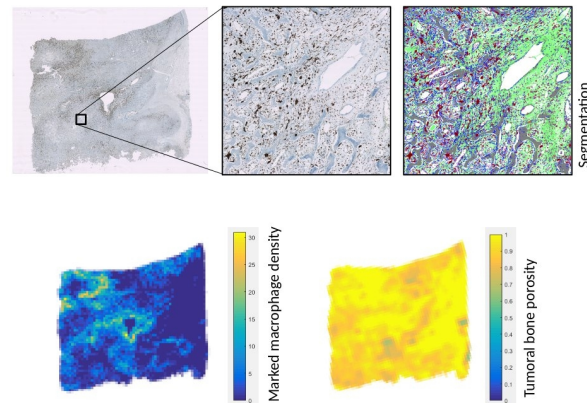


Figure 1 : a) Immunohistological section of a resection sample stained with CD68, b) zoom of the section, c) segmentation, d) marked cell density on macro-pixel with characteristic size evaluated with a semi-variogram and e) quantification of the 2D tissue porosity.

Optimization of experimental and acquisition parameters for improved SOFI super-resolution microscopy imaging

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Super-resolution optical fluctuation imaging (SOFI) is a stochastic super-resolution fluorescence imaging technique. It relies on the spatio-temporal evaluation of the fluctuations of the fluorophores' emissions between sufficiently distinguishable optical states (Dertinger, 2009). Among the advantages of SOFI there are the availability of a full theoretical model, its flexible range of operative conditions and its quantitative nature (Vandenberg, 2019). The acquisition of a suitable dataset is critical for the successful application of the super-resolution algorithm. Many parameters can influence the characteristics of the dataset: probe properties (e.g. brightness or blinking kinetics), characteristics of the sample (such as emitter sparsity and background presence) and choices of the operator during data acquisition (e.g. laser power or number of frames accumulated). Despite the solid theoretical model describing SOFI, deriving analytically the effect of each of these experimental parameters into the SOFI signal is not necessarily straightforward. For this reason, the experimental conditions are usually determined through a trial and error process, relying on the expertise of the operator. This work proposes the derivation of an empirical model based on simulations to rationalize the weight and influence of a selection of experimental parameters on the global signal-to-noise ratio of the SOFI image. SNR has been already discussed as a reliable evaluator of the quality of a SOFI image (Vandenberg, 2016). A collection of fluorescence microscopy measurements were simulated (Girsault, 2016), mimicking the behavior of DNA-paint dyes (Schnitzbauer, 2017). A selection of experimental parameters (blinking behaviour, brightness, background and measurement time) were examined. A D-optimal design of experiment was used to find the optimal parameter combination yielding the highest SNR for SOFI orders 2 and 3. The validity of this combination was tested using real data measurements. This strategy can be exploited as an optimization tool, aimed at guiding the operator during data acquisition.

Dertinger, T., Colyer, R., Iyer, G., Weiss, S., & Enderlein, J. (2009). Fast, background-free, 3D super-resolution optical fluctuation imaging (SOFI). *Proceedings of the National Academy of Sciences*, 106(52), 22287-22292.

Girsault, A., Lukes, T., Sharipov, A., Geissbuehler, S., Leutenegger, M., Vandenberg, W., & Lasser, T. (2016). SOFI simulation tool: a software package for simulating and testing super-resolution optical fluctuation imaging. *PLoS One*, 11(9), e0161602.

Schnitzbauer, J., Strauss, M. T., Schlichthaerle, T., Schueder, F., & Jungmann, R. (2017). Super-resolution microscopy with DNA-PAINT. *Nature protocols*, 12(6), 1198.

Vandenberg, W., Duwé, S., Leutenegger, M., Moeyaert, B., Krajnik, B., Lasser, T., & Dedecker, P. (2016). Model-free uncertainty estimation in stochastic optical fluctuation imaging (SOFI) leads to a doubled temporal resolution. *Biomedical optics express*, 7(2), 467-480.

Vandenberg, W., Leutenegger, M., Duwé, S., & Dedecker, P. (2019). An extended quantitative model for super-resolution optical fluctuation imaging (SOFI). *Optics Express*, 27(18), 25749-25766.

Measuring diffusion dynamics at high photon-count rates with FCS and STED-FCS

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Keywords: STED-FCS, diffusion, high count rates

Probing diffusion dynamics has become a routine measurement all across the life sciences, chemistry and physics. It provides valuable insights into reaction dynamics, oligomerisation, molecular (re-)organisation or cellular (membrane) heterogeneities [1]. Fluorescence correlation spectroscopy (FCS) is a popular tool to determine diffusion dynamics in cellular or artificial membranes (2D diffusion) and solution or the cytoplasm (3D diffusion). Recording intensity fluctuations over time which result from molecules traversing through the observation volume is the basis for FCS. Temporal autocorrelation of the signal allows for the calculation of the autocorrelation curve which provides insights into the underlying dynamics [2]. Thus far the concentration regime for reliable measurements has been limited by the detection electronics which could not efficiently and accurately time-tag photons at high photon-count rates. This restricted the range of measurable fluorophore concentrations and data quality of the FCS recordings, especially in combination with super-resolution stimulated emission depletion (STED)-FCS.

Here, we investigate the applicability and reliability of FCS at high photon-count rates (average intensities of more than 1 MHz and concentrations higher than 1 μ M) using novel detection equipment such as hybrid detectors and real-time gigahertz sampling of the photon stream on a commercially available microscope. By measuring the diffusion of fluorophores in solution and cytoplasm of live cells, as well as in model and cellular membranes, we show that accurate diffusion and concentration measurements are possible in these previously inaccessible high photon count regimes. This may reduce the bias when performing live cell measurements where varying expression levels occur routinely and increases the experimental flexibility. In STED-FCS data quality suffers from the depletion and can be greatly improved by using higher confocal count rates. The presented data show a path towards robust FCS and STED-FCS measurements in living cells.

References

- [1] E. Sezgin *et al.*, "Measuring nanoscale diffusion dynamics in cellular membranes with super-resolution STED-FCS," *Nat. Protoc.*, vol. 14, no. 4, pp. 1054–1083, Apr. 2019.
- [2] J. Lackowicz, *Principles of Fluorescence Spectroscopy*, Third. Boston, MA: Springer US, 2006.

ALFRED: user-friendly approach to obtain length and curvature and its application to microtubule networks in nerve cells

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Keywords: Applications in cell biology, Methods development - Image analysis, Methods development - Software development

Axons are the long, cable-like processes of neurons which form the nerves that wire our bodies. These delicate structures usually need to survive for an organism's lifetime (up to a century in humans) whilst being key lesion sites in injury (e.g. spinal cord injury), neurodegeneration (e.g. motorneuron disease) or age-related nervous system decay. We are particularly interested in analyses of continuous parallel bundles of filamentous polymers called microtubules (MTs), which run all along the axonal core providing the structural backbones. In ageing and neurodegeneration, axons can form swellings with disorganised MTs that have lost their bundled conformation, causing detrimental accumulations of organelles and vesicles.

Understanding the causes for such MT disorganisation requires their qualitative and quantitative description, i.e. the definition of informative parameters and development of suitable strategies to measure them reliably and efficiently.

The combined algorithms for image processing and the consequent measurement of parameters such as length, straightness or curvature were implemented computationally in a user-friendly MATLAB application ALFRED (Advanced Labelling, Fitting, Recognition and Enhancement of Data), which can import several image formats, skeletonize and virtualise these images and apply our analysis, from graph theory, to Hough transforms and smoothing spline fitting. Furthermore, our evaluations using well-defined patterns show that ALFRED measures their parameters speedily, with high accuracy, and low dependence on image resolution.

Given that it also encompasses a user-friendly interface (Figure 1), ALFRED will have a broad spectrum of applications going far beyond our own scientific interests, as the algorithms implemented can be applied to any line or contoured objects in the images.

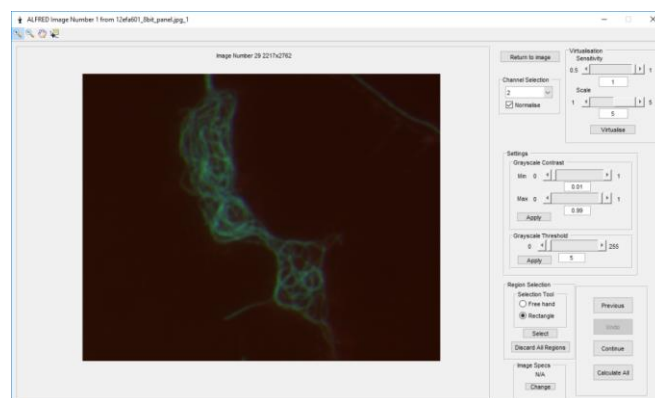


Figure 1. Printscreen of ALFRED User Interface, with a *Drosophila neuronal microtubules fluorescence image*.

Cryo-CARE Facilitates Sub-Tomogram Averaging in Cryo Transmission Electron Microscopy

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Keywords: sub-tomogram averaging, deep learning, cryo-TEM

Cryo transmission electron microscopy (cryo-TEM) suffers from low contrast and low signal-to-noise ratio (SNR), due to beam induced electron damage [1]. To obtain well contrasted high resolution molecular maps, sub-tomogram averaging is necessary. During this rather tedious workflow, dozens, hundreds, or even thousands of noisy particles get selected (picked), are aligned to each other, and the aligned volumes averaged into one less noisy, higher resolution volume. With cryo-CARE [2] we showed how state-of-the-art image restoration techniques can be applied to cryo-TEM data. By splitting direct detector movie acquisitions into halves, we are able to train convolutional neural networks via Noise2Noise [3] training. Restored data show increased contrast at lower noise levels, leading to improved interpretability as well as improved automated downstream processing.

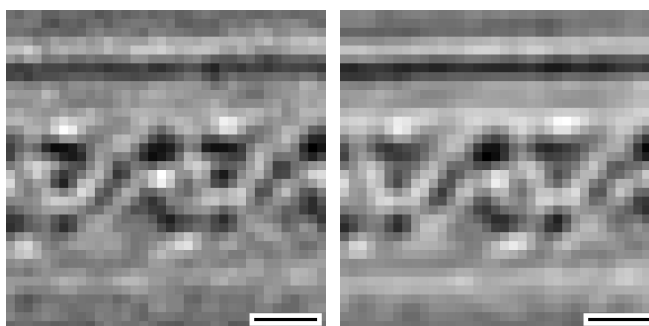


Figure 1. Sub-tomogram averages generated from **(left)** 146 raw particles and **(right)** 70 cryo-CARE restored particles. Scalebars are 10nm.

Here, we investigate the possibility of generating sub-tomogram averages from cryo-CARE restored data. Given a tomogram, we compare sub-tomogram averages generated from raw data and cryo-CARE restored data. This provides the answers to the following two questions: (i) How does cryo-CARE affect the resolution of a tomogram? (ii) How many cryo-CARE particles are needed to obtain an average of comparable quality to a given average created from raw data?

We show that sub-tomogram averages generated from cryo-CARE restored data result in high quality averages, while requiring significantly fewer particles. In Figure 1, a sub-tomogram average generated from 146 raw particles is compared to an average generated from 70 cryo-CARE restored particles. Our results suggest that cryo-CARE can help to improve cryo-TEM investigations when only a limited number of particles are available in the data.

- [1] E Knappek and J Dubochet, "Beam damage to organic material is considerably reduced in cryo-electron microscopy," *Journal of molecular biology*, vol. 141, no. 2, pp. 147–161, 1980.
- [2] Buchholz, Tim-Oliver, et al. "Cryo-CARE: content-aware image restoration for cryo-transmission electron microscopy data." *2019 IEEE 16th International Symposium on Biomedical Imaging (ISBI 2019)*. IEEE, 2019.
- [3] Lehtinen, Jaakko, et al. "Noise2noise: Learning image restoration without clean data." *arXiv preprint arXiv:1803.04189* (2018).

Cluster analysis of SMLM data

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Keywords: SMLM, cluster analysis, Invited Workshop: Molecular Counting: Organizer: Dirk-Peter Herten

Single-molecule localization microscopy (SMLM) produces outputs which are unlike those of conventional systems; consisting of point patterns rather than pixelated images. Here, we will present a toolbox of analysis methodologies for quantifying such point patterns and extracting quantitative, biologically relevant information. These methods include 2D, 3D and live-cell cluster analysis via a model-based Bayesian approach, cluster analysis by machine learning using neural networks and fibrous network analysis. We will show that these methods can be used on a wide range of SMLM implementations including PALM, dSTORM and quantitative DNA-PAINT. In DNA-qPAINT, we demonstrate the use of cluster analysis to multi-colour colocalisation and co-clustering. Experimental data is acquired by imaging signaling molecules, and the actin cytoskeleton at the T cell immunological synapse, where the spatio-temporal arrangement of proteins has been shown to have important signaling outcomes.

3D Model Building Pipeline for Light-Sheet Imaging of Hippocampal Neurons for Simulation and Analysis of Ca^{2+} Dynamics

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Keywords: light-sheet microscopy, simulation, pipeline

Individual neurons of the central nervous system have a complex branching tree morphology. This morphology and the non-random distribution of synapses along each branch has important implications for how each neuron stores information and integrates electrochemical signals from other neurons. We seek to understand the spatio-temporal patterns of synaptic activation and intracellular integration that lead to action potential generation (neuronal firing). Using 3D microscopy images, obtained via a novel light-sheet microscopy method (from collaborators in the Emptage Lab, Oxford Department of Pharmacology) [1], we have combined deconvolution (proprietary: M2 Lasers) and thresholding procedures with state of the art image segmentation and neuron tracing methods to automatically extract 3D volumetric models (traces) of pyramidal neurons in the rat hippocampus [2].

Using these 3D structural models in conjunction with the light sheet image stacks we then applied current intensity-based region growing and clustering procedures to automatically select out dendritic spines (markers of excitatory synapses) from neighboring background pixels in each stack. The results are detailed maps of single neuronal tree structure, including 3D volume estimates for both dendrites and spines (Figure 1.). These morphological models are being used to simulate intracellular electrical activity and calcium ion concentration within the NEURON simulation environment [3]. Hypotheses from the simulations will be tested against data from 3D dynamical (3Dt) imaging of the intracellular calcium ion concentration which will be produced in the next phase of the light-sheet experiment.

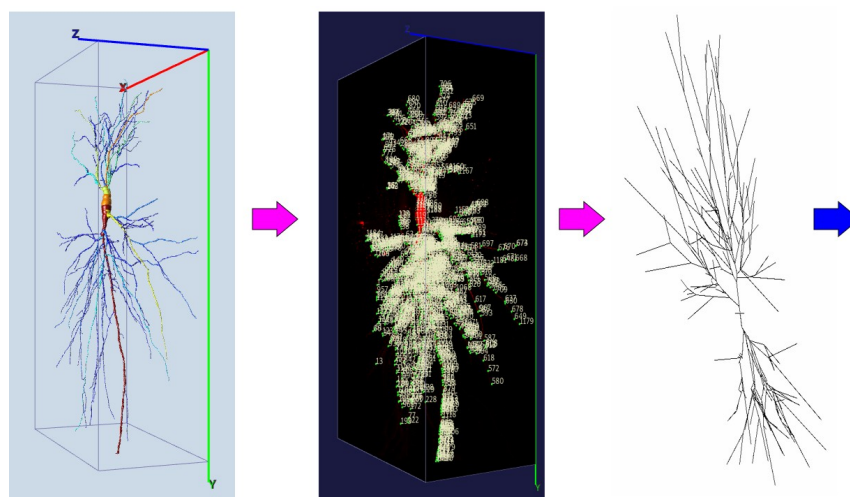


Figure 1. Diagram showing current and future steps of the neuronal model building pipeline. Current steps (purple arrows), following initial de-convolution and thresholding, are the extraction of the neuronal tree (left) which is then used in the spine detection step (middle) to define the search space of neighbouring pixels. These steps were performed using plug-ins available in the current version of the vaa3D image processing package [2]. Finally a biophysical model is set up in the NEURON simulation environment (right) to be used in simulations (blue arrow).

- [1] P.Haslehurst, Z. Yang, K. Dholakia and N. Emptage, Biomedical Optics Express, vol. 9, no. 5, 2018.
- [2] H. Peng, A. Bria, Z. Zhou, G. Iannello and F. Long, Extensible visualization and analysis for multidimensional images using Vaa3D, Nature Protocols, vol. 9, no. 1, 2014.
- [3] N.T. Carnevale and M.L. Hines, The NEURON Book, Cambridge University Press, 2006

Extending molecular counting by photon statistics

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Keywords: Methods Development – Image Analysis, Optical Methods Development

Molecular counting by fluorescence microscopy offers unique possibilities to investigate complex intracellular protein assemblies regarding their composition and function. One method which offers key advantages is Counting by Photon Statistics [1]. It is calibration-free, can cope with variations in fluorophore brightness, and is fast in comparison with single molecule localization or photo bleach step analysis based method. However, the technique is so far limited to the confocal resolution and therefore quantification of isolated, diffraction-limited objects.

Here we present the extension of the CoPS concept to detection layouts with non-uniform detection probabilities for the individual detectors. In the original four detector layout this offers a higher dynamic range and a more robust emitter number estimation due to the possibility to compensate imperfections of the optical setup. Additionally it will enable us to extend CoPS to be used in combination with novel array detectors, which were recently demonstrated to increase confocal resolution without loss of detected photons [2]. With the wealth of spatial and temporal information collected by these detectors both the spatial and temporal resolution of the emitter number measurement could potentially be improved. We explore this possibility by extensively benchmarking the novel analysis with synthetic data generated by Monte-Carlo simulations.

[1] Großmayer, K. S. & Herten, D.-P. Time-resolved molecule counting by photon statistics across the visible spectrum. *Phys. Chem. Chem. Phys.* **19**, 8962–8969 (2017)

[2] Castello, M. *et al.* A robust and versatile platform for image scanning microscopy enabling super-resolution FLIM. *Nature Methods* **1** (2019)

Cell segmentation and tracking for mathematical modelling of cell motility

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Keywords: Image analysis, cell motility, cell segmentation

The elucidation of the mechanisms underlying cell deformation and motility is a topic of major interest in cell biology because they are heavily involved in fecondation, embryonic development, immune responses, cancer metastasis and infectious diseases. The project focuses on the implementation of robust, fully automated image analysis tools, which are able to model and extract quantitative measures of cell architecture, shape, motility and intra-cellular biophysical quantities from multi-dimensional (2D/3D) time-lapse microscopy data. Our first objective is to employ image analysis and machine learning techniques to analyse the shape dynamics and motility of migrating cells, aiming to establish the relationship between morphology, kinematics and the surrounding micro-environment. Second, we aim to use image analysis based computational models to measure intracellular quantities such as, pressure and force, in order to decipher the mechanisms by which cells regulate their cytoskeleton to initiate migration [1]. The computational models developed for the study of cell morphology and motility, at both cellular and sub-cellular levels are based on *Entamoeba histolytica*, a highly motile protozoan. Identifying individual cells from a population - computing the accurate cell boundary and position over time are crucial for the accurate implementation of the afore-mentioned computational models [2]. For the past few years, deformable models and active contours have been used extensively for image segmentation [3,4]. However, the accuracy of these segmentation methods is sensitive to initial cell detection and image feature selection. To overcome these drawbacks of the classical segmentation methods, we aim to employ machine learning tools in combination with traditional active contour models to provide a cell segmentation method. The developed approach is capable of detecting individual cell boundaries in a population and follow them over time to ensure robust analysis of cell morpho-dynamics.

- [1] A. Boquet-Pujadas *et al.*, *Bioflow: a non-invasive, image-based method to measure speed, pressure and forces inside living cells*, Scientific reports, vol. 7, no. 1, p. 9178, 2017
- [2] A. C. Dufour *et al.*, *Signal processing challenges in quantitative 3-d cell morphology: More than meets the eye*, IEEE Signal Processing Magazine, vol. 32, no. 1, pp. 30–40, 2014
- [3] R. Sarkar, S. Mukherjee, and S. T. Acton. *Dictionary learning level set*. IEEE Signal Processing Letters 22.11 (2015): 2034-2038.
- [4] C. Zimmer, *et al.* *Segmentation and tracking of migrating cells in videomicroscopy with parametric active contours: A tool for cell-based drug testing*, IEEE transactions on medical imaging 21.10 (2002): 1212-1221.

Quantitating light sheet microscopy

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Keywords: light sheet microscopy, image formation, volumetric imaging

Light sheet microscopy reduces photodamage and increases optical sectioning from the levels of wide-field microscopy by illuminating the sample with a thin sheet of light constrained to the vicinity of the focal plane. While initially demonstrated with Gaussian illumination profiles, a number of alternative profiles have now been suggested and demonstrated [1–5]. We have recently constructed a flexible light sheet microscope capable of generating different illumination profiles via field synthesis [6], which has motivated us to study the fundamental properties of these profiles.

First, we show that light sheet microscopy, irrespective of the exact light sheet profile used, is non-quantitative. Furthermore, we demonstrate that the simple correction suitable for 2D images necessarily fails when applied to 3D data and justify this through our model of image formation in a light sheet microscope.

Next, we present our unified approach for studying light sheet illumination profiles, detailing the underlying principles governing their behaviour and performance. Using these principles, high-numerical-aperture vectorial diffraction theory and experimental results, we investigate the main light sheet profiles in use and show why simplified metrics such as full width at half maximum are unsuitable for fair and accurate comparisons.

Our results demonstrate that conventional Gaussian light sheets are fundamentally longer than often considered and are in fact suitable for sub-cellular imaging. Indeed, theoretical considerations and experimental results indicate that dithered lattice light sheets are in practice no better than Gaussian light sheets, contrary to popular belief. In contrast, we show that Bessel light sheets do provide an opportunity for improved axial resolution, albeit at the cost of lower contrast.

Finally, we introduce two new approaches for improved axial resolution in light sheet microscopy. In the first, we introduce a new class of illumination profile, Lorentzian light sheets, suitable for high-resolution imaging of mesoscopic samples. These sheets maintain widths less than those of comparably long Gaussian sheets, while also allowing for the extraction of much higher resolution data through deconvolution. In the second, we show that the experimental apparatus required for field synthesis can be exploited to produce images with higher contrast and effective resolution while maintaining the same illumination profile and photodose.

- [1] J. Huisken, et al., “Optical Sectioning Deep Inside Live Embryos by Selective Plane Illumination Microscopy”, *Science* **305**, 1007–1009 (2004).
- [2] F. O. Fahrbach, et al., “Microscopy with self-reconstructing beams”, *Nature Photonics* **4**, 780–785 (2010).
- [3] T. A. Planchon, et al., “Rapid three-dimensional isotropic imaging of living cells using Bessel beam plane illumination”, *Nature Methods* **8**, 417–423 (2011).
- [4] E. S. Welf, et al., “Quantitative Multiscale Cell Imaging in Controlled 3D Microenvironments”, *Developmental Cell* **36**, 462–475 (2016).
- [5] B.-C. Chen, et al., “Lattice light-sheet microscopy: Imaging molecules to embryos at high spatiotemporal resolution”, *Science* **346**, 1257998 (2014).
- [6] B.-J. Chang, et al., “Universal light-sheet generation with field synthesis”, *Nature Methods* **16**, 235–238 (2019).

Cluster Analysis of Localization Microscopy Data with Machine Learning

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Keywords: localization microscopy, cluster analysis

Quantifying the clustering of points within single-molecule localization microscopy data is useful to understanding the spatial relationships of the molecules in the underlying sample. The conversion of point pattern data into a meaningful description of clustering is difficult, especially for biologically derived data, as the definitions of clustering are often subjective or simplistic. Many existing computational approaches are also limited in their ability to process large-scale data-sets or to deal effectively with inhomogeneities in clustering. Here we have developed a supervised machine-learning approach to cluster analysis which is fast and accurate. Trained on a variety of simulated clustered data, the network can then classify all points from a typical localization microscopy data-set (several million points from the entire field of view) as being either clustered or not-clustered, with the potential to include additional classifiers to describe different types of clusters. Clustered points can then be further refined into like-clusters for the measurement of cluster area, shape, and point-density. We demonstrate the performance on simulated data and experimental data of the kinase Csk and the adaptor PAG in both naive and pre-stimulated primary human T cell synapses.

Investigating membrane proteins interactions and membrane mimetic systems using mass photometry

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Keywords: membrane proteins, mass measurement, single molecule

Membrane proteins constitute 30% of eukaryotic proteins in the cell and are integral to a majority of cell signalling pathways, making them major targets in pharmaceutical development. Studying their structure, interactions and dynamics, however, has proven difficult due to inherent challenges associated with membrane proteins, such as structure preservation and solubilisation. Even though there has been significant progress in our understanding of membrane protein function and structure, characterising their interactions as well as kinetic properties remains a challenge. Here, we report a single-molecule based method to investigate membrane protein complexes in a range of mimetic systems using mass photometry, thus enabling the study of oligomerisation, protein-protein interactions as well as small molecule effects. Our approach is based on mass photometry which uses interferometric scattering microscopy, capable of quantifying biological macromolecules and their interactions in solution.¹ We show that mass photometry can characterise a variety of mimetic systems, including detergents, lipid nanodiscs, amphipols and native nanodiscs, producing a molecular weight distribution of the MMS particles in solution that is comparable to literature. These results then form the basis of detailed characterisation of membrane structure in terms of both oligomeric state and lipid environment, presenting a general route to studying membrane proteins in vitro.

¹ Young, G. *et al.* Quantitative mass imaging of single biological macromolecules. *Science* (80-.). 360, 423–427 (2018).

Quantifying binding affinities and kinetics of biomolecular complexes with mass photometry

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Mass photometry, biomolecular interactions, binding affinities, kinetics

The binding affinity, kinetics and stoichiometry of biomolecular complexes are key parameters for understanding cellular processes as well as for the development of new biotherapeutics. Despite the maturity of many existing biosensor techniques, they are often hampered by the requirement for surface immobilisation, introduction of tags, the inability to differentiate between co-existing species (e.g. oligomers) and quantifying the relative concentration of each component. Here, we show that mass photometry based on interferometric scattering microscopy (iSCAT)^{1,2}, provides a direct method to determine binding affinities and kinetics of biomolecular complexes in a label-free fashion at the single molecule level. We illustrate the capabilities by quantifying antibody-antigen and antibody-receptor interactions ranging in strength over 4 orders of magnitude (0.1 – 1000 nM). The ease and speed of use (< 1 min), low sample consumption (< picomole protein), minimal sample or assay preparation in combination with clear avenues to high-throughput and data analysis makes mass photometry a powerful new method for characterising biomolecular interactions and dynamics *in vitro*.

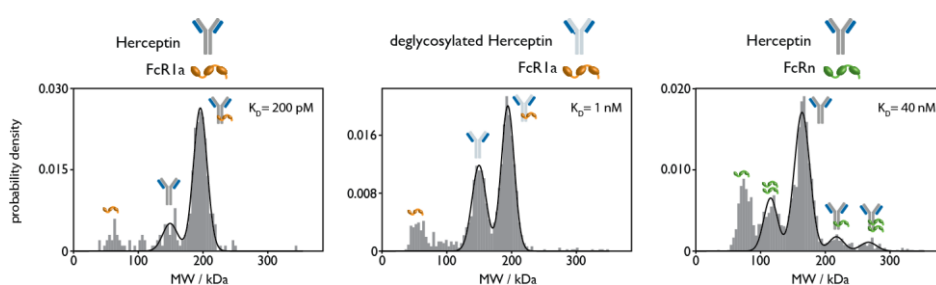


Figure 1 A selection of biomolecular interactions and binding affinities quantified with mass photometry. Stoichiometry and binding affinities were obtained directly by molecular counting.

[1]: Cole, D., et al. (2017). "Label-Free Single-Molecule Imaging with Numerical-Aperture-Shaped Interferometric Scattering Microscopy." ACS Photonics 4(2): 211-216.

[2]: Young, G., et al. (2018). "Quantitative mass imaging of single biological macromolecules." Science 360(6387): 423-427.

Adaptive optics in bioimaging for improved quantitative exploitation of microscopy images

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Keywords: adaptive optics, PSF engineering, SMLM, Light-Sheet microscopy

Adaptive optics (AO), when applied to microscopy, has proved to provide significant improvement of images in various microscopy modalities such as Single Molecule Localization Microscopy (SMLM), confocal, 2-photon or Light-Sheet setups [1-3]. The capability of AO to manipulate the wavefront enables both correction of aberrations induced either by the optical setup or the sample, and a precise engineering of the Point Spread Function for advanced quantitative exploitation of images, in particular in the case of SMLM.

Here we report AO components (wavefront sensor, deformable mirrors) as well as implementation of AO that are optimized for microscopy use in several modalities:

- In SMLM, we report an AO module providing both correction of aberrations of both the microscope setup and of the sample itself, following an image based approach in order to minimize photobleaching. PSF engineering is also applied in order to provide 3D localization. The AO module was recently optimized in order to provide long-term unequaled temporal stability down to 5nm rms over time. Such a module provides a significant increase of counts as well of the localization precision.
- In optical sectioning microscopy setups, such as Light-Sheet or 2-photon microscopy, we report a new AO approach that enables direct wavefront sensing (WFS) and aberration correction without the need for a guide star, which significantly reduces the constraints linked to AO implementation in particular regarding sample preparation. Direct WFS provides minimal photobleaching when compared to image-based AO approach, enabling compatibility with *in vivo* experiments. We report first images based on the approach, demonstrating significant SNR increase of images of neuronal networks in the drosophila brain, targeting improved quantitative recording of neuronal activity.

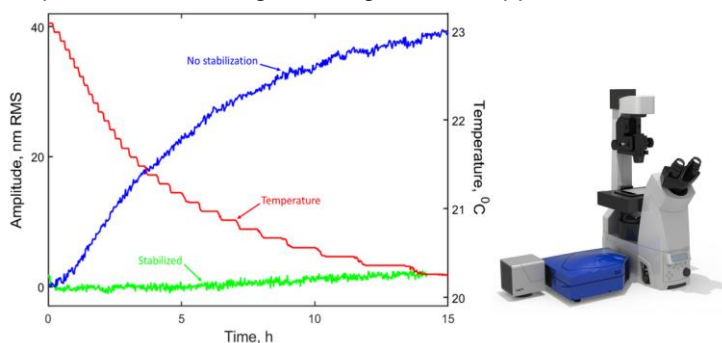


Figure 1. The PSF stability measurement (left) at the changing ambient temperature in plug & play AO device (right).

1. M. J. Booth. "Adaptive optics in microscopy", *Phil. Trans. R. Soc. A* (2007) **365**, 2829.
2. I. Izeddin *et al.* "PSF shaping using adaptive optics for three-dimensional single-molecule super-resolution imaging and tracking", *Opt. Express* (2012) **20**, 4957.
3. K. M. Dean *et al.* "Imaging subcellular dynamics with fast and light-efficient volumetrically parallelized microscopy", *Optics* (2017) **4**, 263.

Quantitative imaging of intracellular receptors in cardiac myocytes using DNA-PAINT

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Keywords: DNA-PAINT, cardiac receptors, super-resolution

Super resolution microscopy by single molecule localisation has the potential of revolutionising our understanding of how cells work at the molecular level. Particularly, DNA-PAINT (1) (Point Accumulation Imaging in Nanoscale Topography) achieves ~10nm resolution by using freely diffusing dyes that bind transiently to the target molecules. By combining DNA PAINT's unique capabilities with Quencher Exchange PAINT (2) to achieve multi-colour imaging, we have established a protocol for imaging two calcium channels located in cardiac muscle tissue, pivotal to release calcium into heart muscle cells, the key process that governs heart contraction.

In the heart, voltage-gated calcium channels located in invaginations of the cell membrane trigger calcium release from an ion channel in the sarcoplasmic reticulum, the ryanodine receptor (RyR) (3). In addition, the inositol 1,4,5 triphosphate receptor (IP3R), also located within the sarcoplasmic reticulum can release calcium in response to IP3 release, and potentially activate adjacent RyRs if these are sufficiently close. Super resolution microscopy allowed us to study the spatial distributions of these proteins at the nanoscale, and with the possibility of dual colour imaging it is leading the way to accurately determine their colocalisation.

Super-resolution RyR-IP3R images of left ventricular pig tissue were processed and analysed using the open source Python Microscopy environment (PyME) software (<http://python-microscopy.org/>). Images were taken from both sham treated samples and samples of hearts after myocardial infarction (MI). Quantitative analysis shows an increase in both individual cluster sizes and the overlap between RyR and IP3R distribution in the MI samples. In the sham cases 9% of the RyR clusters exhibited overlap with IP3R in contrast to 20.9% overlap in the MI cases, leading to a ~2.3-fold increase in colocalisation. Out of 10 RyR receptors in sham data, 4.2 overlapped with IP3 receptors, in comparison to 7.2 IP3Rs overlapping in MI. RyR cluster sizes were increased by 1.7%, and IP3R sizes by 1.2%. These data provide evidence to support a connection between myocardial infarction and the spatial relationship of the two intracellular calcium receptors compatible with increased functional cross-talk after MI.

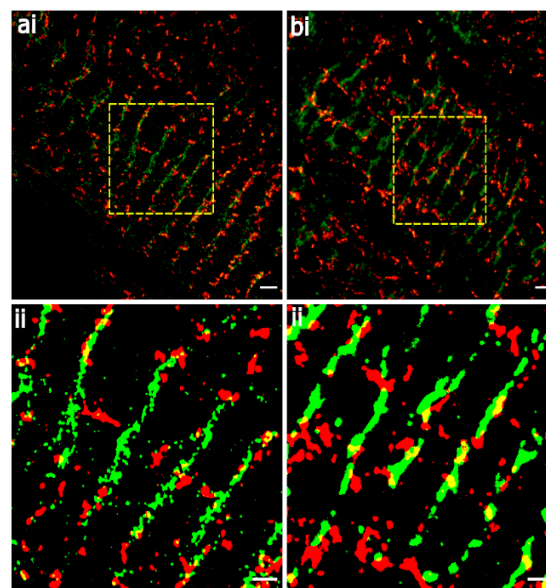


Figure 1. i) typical SHAM image versus a MI one. ii) Magnified regions of masks comparing the Z lines of the sarcolemma in a SHAM Pig sample with a MI Pig sample. RyR-red, IP3R-green, overlap: yellow, scale bars: i) 1 μm, ii) 0.5 μm

1. Jungmann R, Avendaño MS, Woehrstein JB, Dai M, Shih WM, Yin P. Multiplexed 3D cellular super-resolution imaging with DNA-PAINT and Exchange-PAINT. *Nat Methods*. 2014 Mar;11(3):313–8.
2. Lutz T, Clowsley AH, Lin R, Pagliara S, Di Michele L, Soeller C. Versatile multiplexed super-resolution imaging of nanostructures by Quencher-Exchange-PAINT. *Nano Res*. 2018 Dec 1;11(12):6141–54.
3. Baddeley D, Jayasinghe ID, Lam L, Rossberger S, Cannell MB, Soeller C. Optical single-channel resolution imaging of the ryanodine receptor distribution in rat cardiac myocytes. *Proc Natl Acad Sci USA*. 2009 Dec 29;106(52):22275–80.

Characterization of Flat-Fielding Systems for Quantitative Microscopy

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Keywords: flat-field, correction, flat-top

The illumination sources used at the heart of many microscopy techniques have Gaussian profiles — with the beam intensity peaking at the center and decaying radially. In quantitative microscopy, this fact leads to a variable, field-dependent image quality and compromised data reliability. Several flat-fielding mechanisms exist to tackle this issue, including field-mapping and beam-integration methods. Such techniques transform an input Gaussian profile into a uniform one, reducing the variations in image quality. In this master's project, beam shaping systems of both kinds are characterized experimentally to find out which method is more suitable for wide-field illumination and multipoint-scanning illumination. The systems studied are two commercial field-mapping systems called PiShaper and TopShape, and a third based on beam-integration, the Köhler integrator, which is built during the project. The analysis includes quantitative measures for things like the uniformity, steepness of the transition at the intensity edges, the transmission efficiency, as well as qualitatively studying things like the ease of alignment and use. The impartial benchmarking of these systems, which have grown in popularity as beam homogenizers in recent years, is crucial so that scientists can make an informed choice of system when doing flat-field correction.

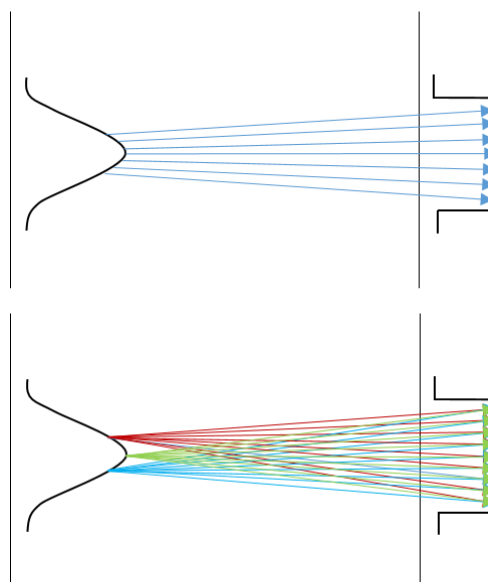


Figure 1. Mechanisms for shaping a Gaussian beam into a flat-top beam. Top: field mapping. Bottom: beam integration.

Quantitative imaging of sparse targets with DNA-PAINT.

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Super-resolution, DNA-PAINT, background and non-specific labelling reduction

Single molecule localisation microscopy is offering unprecedented super-resolution using ordinary light microscopes. The progression from the need for special chemical switching buffers and particular photoswitchable dyes to instead photostable fluorophores on programmable oligonucleotides is opening the field to a variety of users. DNA-PAINT relies on the transient interaction of dye modified oligonucleotides (an imager) to stationary complementary sequences affixed in some fashion to the target molecule of choice¹. It is the immobilisation of these imager strands to docking sites that are visualised as single molecule events and their fluorescence duration results in the ability to resolve structures at the low nanometre scale. However, how can one be sure that one is observing *real* structures, especially in biological samples? We have found that in completely unlabelled tissue samples the relatively short ~10 base pair sequences chosen to image can result in varying amounts of non-specific localisations depending on their general composition, Figure 1. Our experiments suggest that nonspecific binding increases with increasing GC content of imagers. Here we explore strategies to help minimise both the non-specific imager binding and the potentially significant build-up of *false-positive* clusters by choice of imager sequence and reduction of imager concentration.

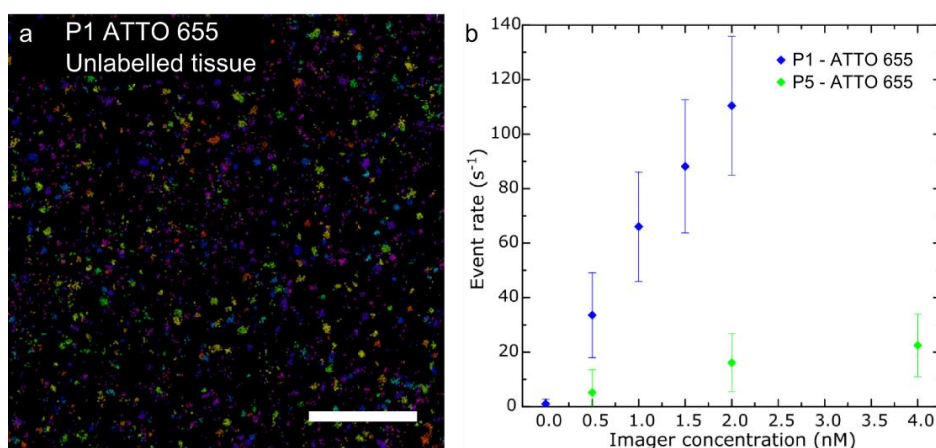


Figure 1. Increased non-specific imager binding in biological samples. **(a)** An example of localisation data acquired when using a P1 imager sequence, modified with an ATTO 655 dye, to probe a fixed unlabelled cardiac tissue section shows a large number of non-specific clusters. **(b)** The binding rate of P1 and P5 ATTO 655 imagers to non-specific sites increased proportionally to imager concentration with a greater number of non-specific bindings observed for the P1 imager sequence. **Scale bar:** 2 μ m.

1. Jungmann R, Avendaño MS, Woehrstein JB, Dai M, Shih WM, Yin P. Multiplexed 3D cellular super-resolution imaging with DNA-PAINT and Exchange-PAINT. *Nat Methods*. 2014;11(3):313-318. doi:10.1038/nmeth.2835.

Identifying analysis algorithm induced image artefacts in localisation microscopy.

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Keywords: Super-resolution, Artefacts, Image-quality

Localisation microscopy can produce very high resolution images with high authenticity when the experimental conditions are right. Outside of these optimum conditions the reconstructed image can contain substantial artefacts. When the emitter density is high enough that individual molecule point spread functions overlap the localisation algorithm becomes biased – producing artefacts. High emitter density is a necessary condition for the fast acquisition times needed for live cell imaging, or from the poor 'blinking' associated with multi colour imaging. Specialist algorithms have been developed but all suffer similar biases toward areas of high structural density. Additionally they often report these localisations with higher precision/confidence making identification of these artefacts very difficult.

Our recently introduced analysis method HAWK [1] does not display this same bias as the emitter density increases, instead a loss of precision is observed. Here we exploit this difference to identify sub diffraction scale regions of the sample where the emitter density exceeds the limits of a particular algorithm, producing artefacts. Reconstructions produced with and without prior HAWK processing or analysed (flattened, binarised & skeletonised) and compared. Where differences occur the reconstruction has become unreliable. Quantification of these differences is then projected onto the original reconstruction producing a map of the identified likely artefacts (see Figure 1) The analysis is repeated at different (sub diffraction) length scales to ascertain the actual reliable resolution obtained in different regions of the test image

Our method is used to compare the ability of different algorithms to accurately reproduce known sub diffraction scale structures from simulated and experimental high density data. It does not require a reference image or to be able to resolve structure in the HAWK reconstruction.

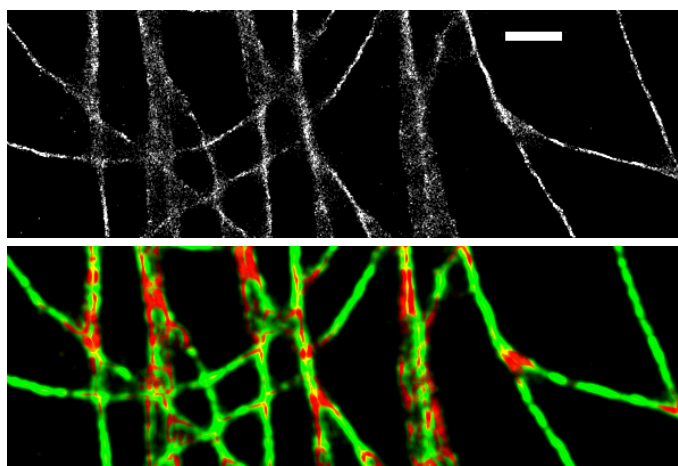


Figure1 : Assessment of local reconstruction accuracy for experimental microtubule data from the Single Molecule Localisation Microscopy Challenge [2]. The reconstruction produced from single emitter fitting (top) is compared with the result produced from HAWK. Analysing the differences between the images gives a measure of the accuracy of the reconstruction (bottom). Areas of high confidence are displayed in green and likely artefacts are red. Scale bar 1 μm

[1] R Marsh *et.al.*, *Nature Methods*, **15**,689-692 (2018)

[2] Daniel Sage *et.al.*, *Nature Methods* **12**, 717-724 (2015)

Bayesian Grouping of Localizations: Sub-Nanometer Precision, Counting and Resolution Doubling

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KEYWORDS: Super-resolution, Bayesian Inference

Single-molecule-localization-based super-resolution methods such as DNA-PAINT and (d)STORM result in multiple observed localizations from each dye or binding site that are not *a priori* assigned to the specific dyes or binding sites. We describe a method for Bayesian Grouping of Localizations (BaGoL) that combines localizations from multiple blinking/binding events [1]. BaGoL can improve localization precision to better than 1 nm, can be used for inference on the number of fluorophores in small clusters, and can be applied to any SMLM data set for improved resolution.

The known statistical distribution of the number of binding/blinking events per dye/docking strand along with the localization precision of each localization event are used together to estimate the number and location of emitters in closely spaced clusters. The method uses Reversible Jump Markov Chain Monte Carlo to find the uncertainty of both the number of dyes/docking strands and their locations. The results can be returned from the most likely model or a posterior distribution that is a weighted average over all models.

We describe the requirements and sensitivity of BaGoL to prior knowledge on the blinking distribution and show the application to many structures imaged with dSTORM and DNA-PAINT.

1. Fazel, M., M.J. Wester, B. Rieger, R. Jungmann, and K.A. Lidke, *Sub-Nanometer Precision using Bayesian Grouping of Localizations*. bioRxiv, 2019: p. 752287.

Towards a molecular mechanism of dynamin polymerization with mass photometry

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Keywords: Mass photometry, dynamin, membrane fission, protein-protein interactions,

Dynamin, the prototypical member of dynamin superfamily, is a large multi-domain GTPase complex and essential for membrane fission in clathrin-mediated-endocytosis. Dynamin shows extensive tendency to polymerize (in-solution and on the membrane), a characteristic that is essential to carry out membrane fission. Despite extensive studies, it has been difficult to understand the underlying mechanism of dynamin polymerization due to difficulties associated with differentiating different oligomeric states on membranes *in vitro* and *in vivo*, and their evolution and dependence on conditions. Here, we use mass photometry, based on interferometric scattering microscopy, to characterise the details of dynamin self-association in-solution at the single molecule level. We find that dynamin exists in equilibrium between monomers, dimers and tetramers and that this equilibrium is essential for the process of producing larger polymers. By decreasing the ionic strength of the buffer, we can stimulate dynamin polymerization in-solution and observe that oligomerization is regulated by tetramer abundance and is initiated by the addition of dimers to the tetramer. Subsequent dimer addition extends the polymer length. These results provide the critical insights into the mechanism of dynamin oligomerization and show that unlike previously proposed, dynamin oligomerization does not occur by tetramer-tetramer interactions. They further provide a general blueprint for studying nucleation processes based on single molecule mass measurement in solution.

Probabilistic Noise2Void: Unsupervised Content-Aware Denoising

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Keywords: Denoising, CARE, Unsupervised Learning

Today, Convolutional Neural Networks (CNNs) are the leading method for image denoising. They are traditionally trained on pairs of images [1,2], which are often hard to obtain for practical applications. This motivates self-supervised training methods such as Noise2Void (N2V) [3] that operate on single noisy images. Self-supervised methods are, unfortunately, not competitive with models trained on image pairs.

Here, we present *Probabilistic Noise2Void* (PN2V), a novel method to train CNNs to predict per-pixel intensity distributions using single noisy images together with a suitable description of the camera's noise characteristic. This combination allows us to obtain a complete probabilistic model for the noisy observations and true signal in every pixel (see Figure 1).

We evaluate PN2V on publicly available microscopy datasets, under a broad range of noise regimes, and achieve competitive results with respect to supervised state-of-the-art methods.

PN2V is widely applicable since: (i) the required camera noise model is sample-independent and has to be measured only once using a simple sequence of calibration images, (ii) the training of the network does not require any form of annotation. We believe that this will allow PN2V to bring high quality denoising to many new applications, which do not allow for the acquisition of paired training data.

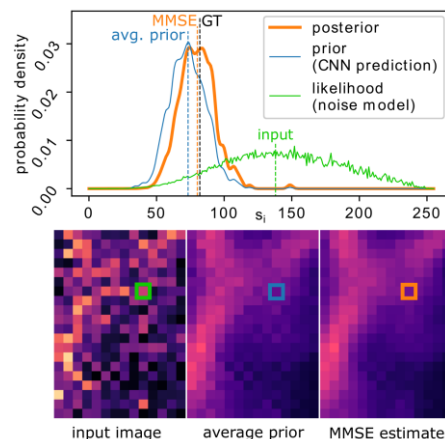


Figure 1. Image denoising with PN2V: The final MMSE estimate (orange dashed line) for the true signal s_i of a pixel (position marked in the image insets on the bottom) corresponds to the center of mass of the posterior distribution (orange curve). Given an observed noisy input value x_i (dashed green line), the posterior is proportional to the product of the prior (blue curve) and the observation likelihood (green curve). PN2V describes the prior by a set of samples predicted by our CNN. The likelihood is provided by an arbitrary noise model. Black dashed line is the true signal of the pixel (GT).

- [1] Weigert, M., et al.: Content-aware image restoration: Pushing the limits of fluorescence microscopy. In: *Nature Methods* (2018).
- [2] Lehtinen, J., Munkberg, J., Hasselgren, J., Laine, S., Karras, T., Aittala, M., Aila, T.: Noise2Noise: Learning image restoration without clean data. In: *ICML* (2018)
- [3] Krull, A., Buchholz, T.O., Jug, F.: Noise2void - learning denoising from single noisy images. In: *CVPR* (2019)

Leveraging Self-Supervised Denoising for Image Segmentation

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Keywords: Segmentation, Denoising, Deep Learning

Deep learning has arguably emerged as the method of choice for the detection and segmentation of biological structures in microscopy images [1,2,3]. However, deep learning based techniques are usually data hungry and need copious amounts of annotated training data, a condition which can be prohibitively expensive for most real life biomedical/microscopy datasets. Moreover, quantitative analysis tasks such as cell/nuclei detection, segmentation, and/or tracking becomes even harder in the presence of noise. Consequently, potent denoising methods can help improve the quality of (semi-)automated image analysis pipelines.

Here, we present two ideas on how state-of-the-art self-supervised image restoration networks can improve cell/nuclei segmentation in microscopy image data. Given a body of data, we first use Noise2Void [4] to train state-of-the-art denoising networks. Then we investigate the following two questions: (i) Do segmentation networks perform better on denoised data? (ii) Is it possible to gain additional performance by fine-tuning a pre-trained denoising network so that it directly performs the desired segmentation task (as opposed to training the segmentation task in a vanilla network without any prior training)?

We show that our proposed ideas are particularly interesting in cases where only limited training data for the final segmentation task (segmentation labels) are available - a situation that is typically true in the context of bioimaging data. Our results demonstrate that both proposed scenarios can greatly improve segmentation results.

- [1] Falk, Thorsten, et al. "U-Net: deep learning for cell counting, detection, and morphometry." *Nature methods* 16, no. 1 (2019): 67.
- [2] Litjens, Geert, et al. "A survey on deep learning in medical image analysis." *Medical image analysis* 42 (2017): 60-88.
- [3] Schmidt, Uwe, Martin Weigert, Coleman Broaddus, and Gene Myers. "Cell Detection with Star-convex Polygons." In *International Conference on Medical Image Computing and Computer-Assisted Intervention*, pp. 265-273. Springer, Cham, 2018.
- [4] Krull, Alexander, Tim-Oliver Buchholz, and Florian Jug. "Noise2void-learning denoising from single noisy images." In *Proceedings of the IEEE Conference on Computer Vision and Pattern Recognition*, pp. 2129-2137. 2019.

Bootstrapping Unsupervised Image Denoising with Parametric Noise Models

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Keywords: Denoising, Deep Learning, Noise Model, Gaussian Mixture Model

With the advent of Convolutional Neural Networks (CNNs), the field of biomedical image denoising has taken rapid strides in recent years. After traditional supervised works such as CARE [1], more recently it was shown that denoising networks can be trained self-supervised from noisy data alone [2,3]. Such methods mask individual pixels in the input image and try to predict their values from surrounding areas. However, this strategy can lead to inferior results, when compared to traditionally trained models that have access to clean (ground-truth) data.

Probabilistic Noise2Void (PN2V) [4] was recently proposed to improve the quality of reconstructions by additionally considering a camera specific noise model, measured from a sequence of calibration images. A noise model characterizes the distribution of noisy pixel values, expected for all possible true signals. The original formulation of PN2V uses a set of histograms to achieve this.

In our work, we first show limitations of histogram based noise models, such as their hunger for data and their inability to deal with missing image intensities during calibration/recording of said noise models. To counter such limitations, we propose two improvements of PN2V, namely (i) the use of parametric, Gaussian Mixture Model (GMM) based noise models, and (ii) a bootstrapping mechanism that allows approximating a suitable noise model if no noise model is available. We show that this new formulation allows learning of a wide variety of continuous noise distributions from limited calibration data, and ultimately leads to improved image reconstructions on all tested example datasets.

- [1] Weigert, M. et al. "Content-aware image restoration: pushing the limits of fluorescence microscopy." *Nature methods* 15, no. 12 (2018): 1090.
- [2] Krull, A., Buchholz, T.-O., and Jug, F.. "Noise2void-learning denoising from single noisy images." In *Proceedings of the IEEE Conference on Computer Vision and Pattern Recognition*, pp. 2129-2137. 2019.
- [3] Batson, J., and Royer, L.. "Noise2self: Blind denoising by self-supervision." In *Proceedings of the Thirty-sixth International Conference on Machine Learning*. 2019.
- [4] Krull, A., Vicar, T., and Jug, F.. "Probabilistic Noise2Void: Unsupervised Content-Aware Denoising." *arXiv preprint arXiv:1906.00651* (2019).

Quantitative Imaging Using Super-Resolution Optical Fluctuation Imaging

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Keywords: quantitative imaging, counting, molecular parameter estimation, Super-resolution, SOFI

Invited Workshop: Molecular Counting: Organizer: Dirk-Peter Herten

Although super-resolution methods developed in the past decade are starting to provide near molecular-scale resolution, it is not yet reached in routine experiments. Therefore, dedicated alternative approaches are necessary for quantification of label (or emitter) numbers from fluorescence microscopy experiments.

Super-resolution optical fluctuation imaging (SOFI) provides a resolution beyond the diffraction limit by computing higher-order statistics of a time series of independent, stochastically blinking fluorophores. Using n^{th} order spatio-temporal cross-cumulants the spatial resolution as well as the sampling can be increased up to n -fold in all three spatial dimensions. In the super-resolution community, SOFI is generally known as imaging/analysis modality for high density blinking data. However, cumulant analysis offers much more than improved resolution under challenging conditions. Using a combination of three cumulant orders, balanced (b)SOFI [2] can provide access to molecular parameter estimates. Emitter density, brightness and on-time ratio of fluorophores are directly calculated from the image data without the need for calibration measurements for e.g. over-counting corrections. I will provide an introduction into SOFI and then focus on different aspects of quantitative data analysis that we and others introduced. bSOFI enabled e.g. the determination of the paxilin density in focal adhesions of mouse embryonic fibroblasts [3] and was recently used to study the nanoscale distribution and clustering of CD4 glycoprotein mutants in the plasma membrane of T cells [4].

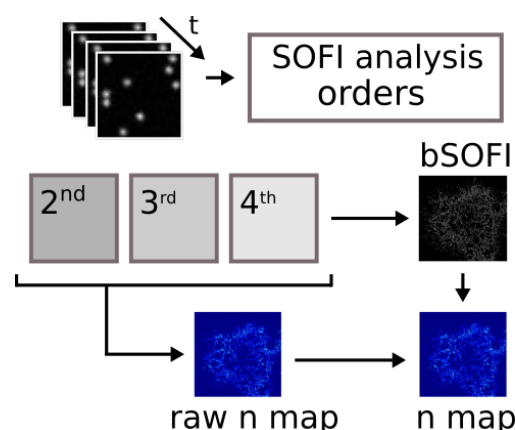


Figure 1: Processing workflow for quantitative SOFI analysis to estimate molecular densities. Modified from [1].

- [1] Grüssmayer, K. S., Yserentant K. (equal contribution), and Herten, D.-P.. Photons in – numbers out: Perspectives in quantitative fluorescence microscopy for in situ protein counting, *Methods and Applications in Fluorescence* **7**, 012003 (2019)
- [2] Geissbuehler, S., Bocchio, N. L., Dellagiacoma, C., Berclaz, C., Leutenegger, M. & Lasser, T. Mapping Molecular Statistics with Balanced Super-Resolution Optical Fluctuation Imaging. *Opt Nanoscopy* **1**, 1–7 (2012).
- [3] Deschout H, ..., Lasser T and Radenovic A, Complementarity of PALM and SOFI for super-resolution live-cell imaging of focal adhesions *Nature Communications* **7** 13693 (2016)
- [4] Lukeš, T., ..., Theo Lasser & Marek Cebecauer. Quantifying protein densities on cell membranes using super- resolution optical fluctuation imaging, *Nature Communications*, 1731 (2017).

Biolapse Toolbox

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Keywords: Convolutional LSTM, Tracking, Multi-class Classification.

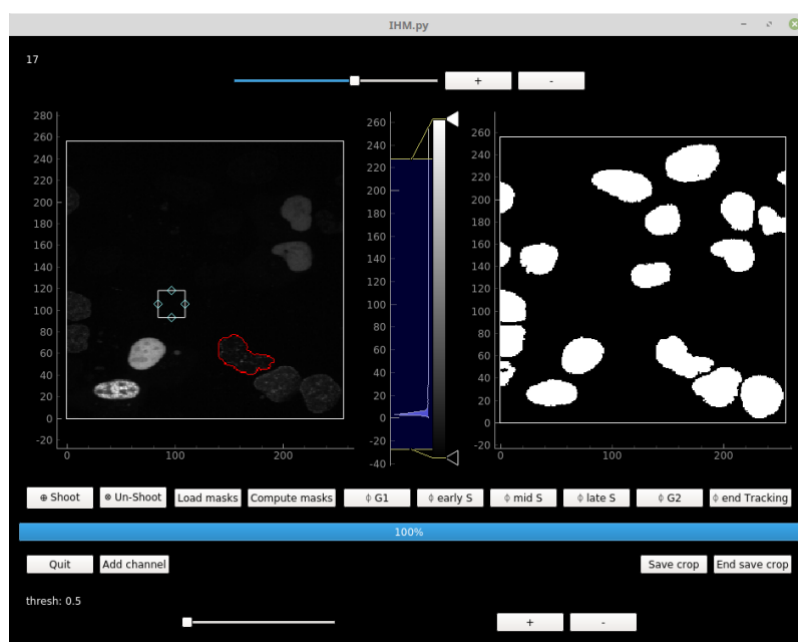


Figure 1. Screenshot of the Biolapse toolbox.

We provide a turnkey toolbox dedicated to the segmentation, tracking and analysis of biological events. The segmentation task is performed by a state-of-the-art convolutional neural network which integrates temporal information: C-LSTM [1]. We provide codes to retrain the neural network on any dataset. Tracking can be executed via a user friendly graphical interface. From this interface one can analyze a biological object across time, that is: extract masks or features, estimate the duration of an event. The feature extraction is carried out by an encoder [2], we also provide codes for a classifier that takes into account the ordinal nature of the data and the seasonality of potential biological phases. This toolbox stems from a collaboration between biologists and image processing experts. We pay particular attention to make our code reusable for non-computer-science specialists. A concrete application of this work is the mitosis analysis for PCNA marked cells, where the toolbox is used to compute statistics on the duration of mitosis phases. The code is open-source and freely available at: <https://github.com/lebrat/Biolapse>.

[1] A. Assaf and T. Riklin Raviv, «2019 IEEE 16th International Symposium on Biomedical Imaging (ISBI 2019).», Venice, 2019.

[2] F. J. Huang, B. Y-Lan and Y. LeCun, «Unsupervised learning of invariant feature hierarchies with applications to object recognition.,» at 2007 IEEE conference on computer vision and pattern recognition, 2007.

Single-molecule and super-resolution investigation of chemokine receptor behaviour within the T cell immunological synapse

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The immunological synapse (IS) serves as a highly dynamic yet tightly organised platform for the transfer of information between T cells and a number of different antigen-presenting cell (APC) types. It is required for the correct activation of T cells in response to infection, as well as the prevention of autoimmunity, and licensing of APCs for subsequent anti-microbial processes. Chemokine receptors are G-protein coupled receptors that recognise inflammatory and homeostatic chemokines, mediating leukocyte migration towards sites of infection or within specialised tissues of the immune system. In recent years it has become apparent that a number of chemokine receptors, particularly CXCR4, also contribute to the stability and longevity of the IS, and do so through signalling processes distinct from those involved in cell migration. Nonetheless, the spatiotemporal organisation of this behaviour within the complex architecture of the IS is unstudied, and hence its integration with the known signalling events of T cell activation is not understood. Here we use total-internal reflection fluorescence (TIRF) microscopy to examine CXCR4 and other chemokine receptors within primary human CD4⁺ T cells during the process of IS formation on supported lipid bilayers. Single-molecule tracking demonstrated substantial changes in receptor dynamics over the lifetime of the synapse, both in terms of spatial location and nature of diffusion. Combining TIRF with super-resolution structured illumination microscopy (SIM) also revealed the segregation of some chemokine receptors from other signalling domains, most significantly those of the T cell receptor. These observations shed light on the mechanisms by which T cell activation is influenced by chemokine receptors, and hence how such processes are regulated during a coordinated immune response.

Single Molecule Localization Microscopy using time modulated illumination

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Keywords: Single Molecule Localization Microscopy, 3D Imaging, depth imaging

In Single Molecule Localization Microscopy (SMLM), the localization of a fluorescent emitter is performed by a Gaussian fitting of its image. Thereby, the precision of the measurement depends on the shape of the Point Spread Function (PSF) and thus is rapidly degraded with the optical aberrations. Other strategies had been developed for single particle tracking based on structured illumination in order to encode the position information in the fluorescent signal [1-4]. In this case, the precision does not depend on the spatial properties of the emission but relies on the spatial properties of the excitation.

We propose a new method, called ModLoc, based on the temporal modulation of the fluorescent signal using a shifting fringe pattern and adapted to single molecule blinking constraints. The fluorescence emission is proportional to the illumination received by the emitter and its temporal response is related to its position in the structured pattern. The extraction of 4 temporal samples of the fluorescence emission is needed to retrieve the response of the emitter and get its position along the modulation axis. Thus, ModLoc can achieve an enhancement of the localization precision of a factor of 2 compare to classical Gaussian fitting method. For SMLM, the random temporal properties of the emission and the cameras low frame rate hampers frame by frame demodulation for all active emitters. We developed a smart fast demodulation system inserted in front of the camera, synchronized with the pattern displacement, making possible the signal demodulation in a single acquired frame.

We will discuss the ModLoc performances with experimental results compare to theoretical calculations, simulations and the application of ModLoc along the z axis for 3D imaging in depth in order to obtain an almost isotropic 3D resolution.

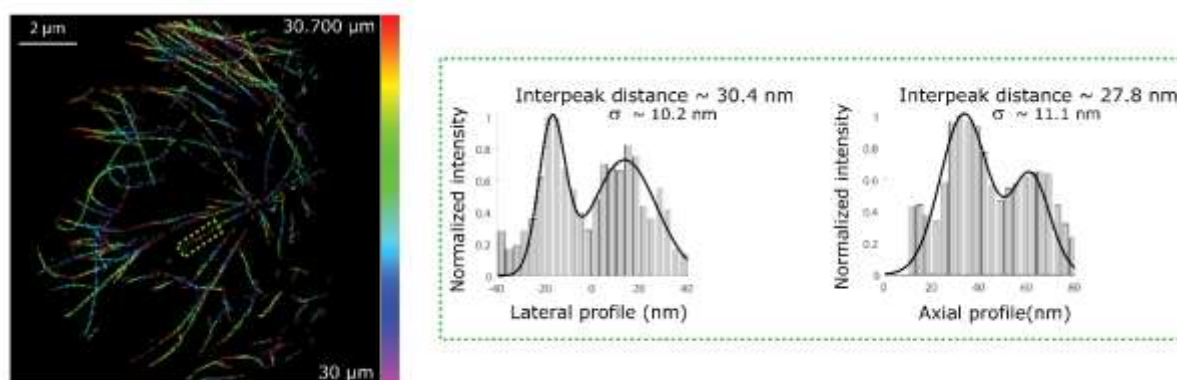


Figure 1: Tubulin of cos 7 cell grown in collagen-I and labeled with primary and secondary antibody conjugated with AF 647. Observation at 30 μm in dSTORM condition imaging. Both lateral and axial profiles are plotted to show performances of ModLoc for 3D imaging in depth.

- [1] Virgilio Failla et al., Applied Optics, 2002
- [2] Busoni et al., Journal Of Applied Physics, 2005
- [3] Chasles et al., Optics Letters, 2006
- [4] Balzarotti et al., Science, 2017

PhenoPlot-Web for visualising microscopy data

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Keywords: Microscopy, data visualisation, phenotypic data

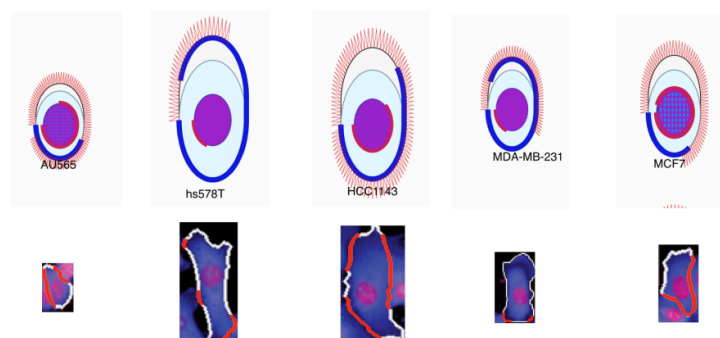


Figure 1: Cell glyphs (top row) representing morphological features of cells (bottom row).

Recent advances in computer vision, and particularly in deep neural networks (DNNs), have empowered researchers to segment diverse biological structures in microscopy from single cells in fluorescence microscopy to complex tissue structures. Accurate segmentation of tissue structures translate into more universally representative measurements of shape, texture and structural features, and opens up new possibilities to compare such complex biological specimens in a more quantitative and objective manner. When studying the responses of cells to changes in the external environment, or examining differences in tissue organization across patients affected by various conditions, image measurements can be linked causally with changes in condition or analysed in their distribution, in order to provide insight into the underlying biology.

Despite these opportunities, interpretation of handcrafted and DNN-extracted numerical features remains difficult: this is due to the limited ability of current data visualizations to display a high number of features in a way that makes comparison intuitive for humans.

Typical visualizations, such as bar and line charts or heatmaps, can display a limited number of features at once. Furthermore, the methods above fail to represent phenotypes intuitively and are difficult to relate to cell and tissue images. To address these challenges, we have implemented PhenoPlot [1] as a web-based application to facilitate visualisation of cell and tissue imaging data. Figure 1 shows an example of visualization for a dataset of breast cancer cells. Different visual elements map into numerical features so that a quantitative comparison can be made by eye.

[1] Sailem HZ, Sero JE & Bakal C (2015) Visualizing cellular imaging data using PhenoPlot. *Nat. Commun.* **6**: 1–6 Available at: <http://dx.doi.org/10.1038/ncomms6825>

ICYTOMINE:**A User-Friendly Tool for Reproducible Analysis of Gigapixel Images**

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Keywords: big data management, digital pathology, deep learning, open-source

Whole slide imaging (WSI) achieves high throughput and high resolution images and results in large images, called virtual slides offering many outlooks for clinical and research studies. However, as the analysis of virtual slides largely remains the work of human experts, the clinical analysis does not exploit fully this high potential content. The manual assessment of slides is indeed known to have high variability and limited reproducibility. In addition, automatic processing of these large images raises many computational challenges like memory constraints due to gigapixel images or high variability of histology images (See **Fig. 1**). To fully exploit virtual slides that would improve high-quality reproducibility, we present Icytomine, a user-friendly framework for processing large images from slide scanners. Icytomine integrates in one unique framework the tools and algorithms that were developed independently on Icy^[1] and Cytomine^[2] platforms to store, visualize and computationally quantify and analyze digital pathology images.

Icytomine also supports the generation of big datasets to feed deep learning algorithms. We illustrate this feature by designing a dedicated program that uses state of the art Convolutional Neural Networks to detect and classify glomeruli in kidney biopsies coming from a multi-centric clinical study. Our dataset contains 100 slides stained by Masson's trichrome from 7 research centers and digitized with scanners produced by 4 different manufacturers at 20X. We show that by streamlining the analytical capabilities of Icy with the functionalities provided by Cytomine, we achieve highly promising results.

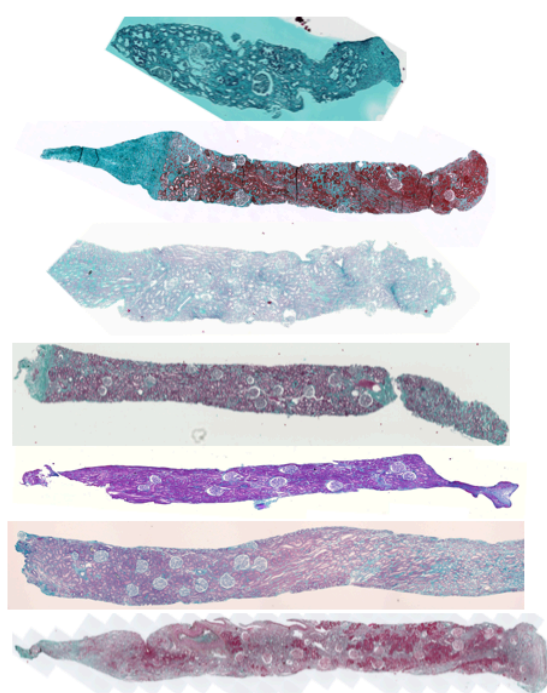


Fig. 1 Color variation in Masson's trichrome staining, due to the multi-centric staining: i) tissue preparation protocol is not exactly the same across pathology services, ii) image acquisition system, iii) age of the slide.

[1] F de Chaumont et al., "Icy: an open bioimage informatics platform for extended reproducible research.," *Nature Methods*, vol. 9, no. 7, pp. 690–6, 2012.

[2] R. Marée et al., "Collaborative analysis of multi-gigapixel imaging data using Cytomine," *Bioinformatics*, vol. 32, no. 9, pp. 1395–1401, 2016.

Multiplex Immune Cell Profiling to Gain Insights into Progression in Early Cancer

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Keywords: Theme: Tissue multiplexing techniques and applications in oncology

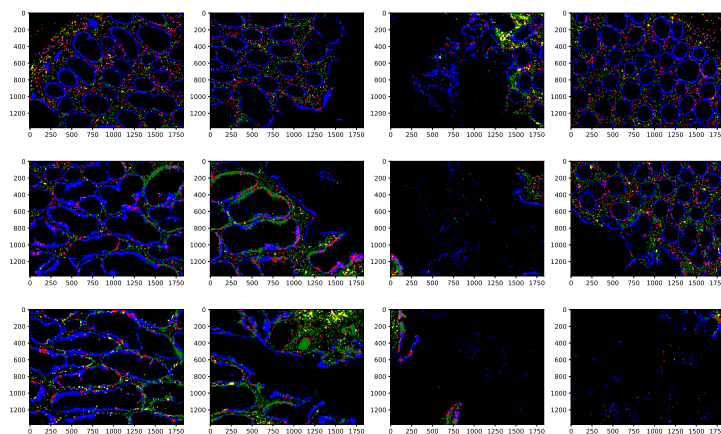


Figure 1: Cell phenotyping to identify clusters showing clusters of B-cells and Helper T-cells while cytotoxic and regulatory T-cells are spread more uniformly in the epithelium.

The precise and robust measurement of a broad range of biomolecules in the tissue architecture context opens up new opportunities for improving our understanding of disease, monitoring therapeutic response, and the development of high-dimensional clinical tests. In this study we apply multiplex IF staining platform (Perkin-Elmer Vectra) on a diverse set of cancer cases. These include samples of different tissue types from Genomics England and a set of colorectal polyps from the S:CORT (Stratification in Colorectal Cancer) cohort. Aim of our study is to map the inflammatory environment in a range of tumour types and associate with whole genome sequencing and clinical data, making a comparison of early to later stage cancers.

Spatially resolved digital image analysis has been carried out to determine the precise location of each immune cell population in the tumor microenvironment in relation to neoplastic and pre-neoplastic cell populations. Here, advanced image analysis techniques are used to classify cells into different phenotypes and to analyse their spatial statistics. The effect of using more traditional as well as deep learning-based segmentation techniques is analysed.

Identified regions were then projected into the channels of interest (CD4, CD8, CD20, FOXP3 and cytokeratin (CK)) in order to record the protein expression intensities of each marker at each of the cells. Cells with a single marker expressed were allocated the corresponding phenotype: CD4 for helper T-cells, CD8 for cytotoxic T-cells, CD20 for B-cells, FOXP3 for regulatory T-cells and CK for epithelial cells. Methods of statistical change detection are used to analyse effects of disease progression.

Leveraging Automated Blood Cell Morphology for Myelodysplastic Syndrome Diagnosis and Prognosis Prediction

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Keywords: machine-learning, haemato-oncology, clinical informatics

Blood, as a diagnostic tool, is incredibly powerful. In the analysis of a whole blood slide (WBS), blood is stained and visualized under a light microscope, granting haematologists and cytomorphologists subcellular resolution into its components, which are mainly white blood cells (WBC), red blood cells (RBC) and platelets [1]. However, while guidelines for WBS analysis exist, the reasons for a specific diagnostic are prone to being quite subjective [2] and to depend on the skills of the person conducting the analysis and on the complexity of the case [3]. As such, if WBS are to be leveraged in a machine-learning setting, it is imperative to perform this analysis in a fully automated, scalable and objective way.

This work draws on that necessity – to shift WBS analysis from a qualitative to a purely quantitative framework. To do so, we developed a protocol that is summarised in Figure 1 and consists on 1) tiling the WBS into smaller tiles, 2) a quality control (QC) to filter tiles, 3) segment WBC (using U-Net, a deep-learning framework for cell segmentation) and RBC (using a simple, parametric protocol) and 4) characterise cell morphology. We applied this protocol to a cohort of individuals suffering from myelodysplastic syndrome (MDS) or anaemia (a disease and also a symptom of MDS) to determine its relevance for diagnosis and prognosis. MDS is a cancer that can further develop to acute myeloid leukaemia and its diagnosis requires WBS, bone marrow slides and cytogenetics, while its prognosis requires further genotyping. Using our protocol, we morphologically characterise between thousands and millions of cells *per* individual. Then, we aggregate the first few moments of each feature for all individuals and use this for diagnosis and prognosis prediction. We show that, with our protocol, we are able to use a WBS to predict that an individual is afflicted by a disease (AUC = 0.99), discriminate between anaemia and MDS (AUC = 0.81), and predict which individuals are SF3B1 mutants (AUC = 0.89), which indicates generally a better prognosis.

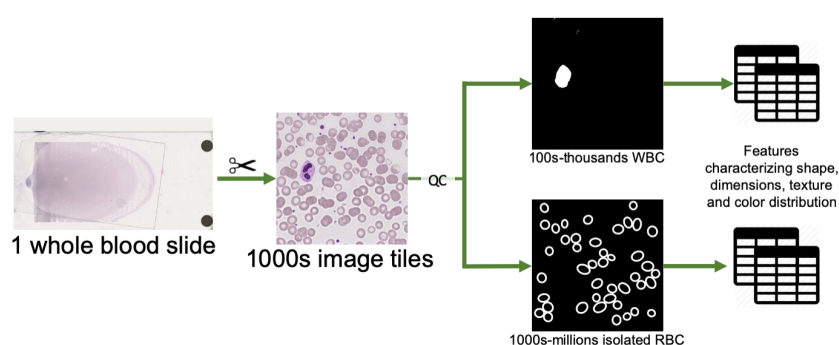


Figure 1 - Pipeline to detect and characterise blood cells in whole blood slides.

- [1] B. J. Bain, "Diagnosis from the Blood Smear," *N. Engl. J. Med.*, 2005.
- [2] L. de Swart *et al.*, "Cytomorphology review of 100 newly diagnosed lower-risk MDS patients in the European LeukemiaNet MDS (EUMDS) registry reveals a high inter-observer concordance," *Ann. Hematol.*, vol. 96, no. 7, pp. 1105–1112, 2017.
- [3] M. Brereton, B. De La Salle, J. Arden, K. Hyde, and J. Burthem, "Do We Know Why We Make Errors in Morphological Diagnosis? An Analysis of Approach and Decision-Making in Haematological Morphology," *EBioMedicine*, 2015.

Generalized Statistical Object Distance Analysis (GSODA) For Object Based Colocalization In Quantitative Microscopy

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The analysis of the spatial distribution of molecules and organelles in bioimaging remains a gold-standard for understanding cellular processes at the molecular level. Recent developments in molecule labeling, optics (e.g. full-field OCT for digital pathology, single molecule localization microscopy in fluorescence microscopy, etc.) and automated image analysis of biological objects such as molecular complexes, organelles or cells etc. call for the development of advanced statistical tools to analyze the objects' spatial distribution. In that context, we have recently introduced SODA [1] to study spatial relations between two (or more) spatial point processes (see Fig. 1(a1)). However, in many applications as illustrated in Fig. 1(a2)-(a3), the reduction of analyzed objects to the position of their center-of-mass affects the robustness of analysis. To generalize SODA (G-SODA) and extend its applicability to more generic region based colocalization studies, we leverage the flexibility of level sets [2] to implicitly embed closed regions, and propose a mathematical model to analytically estimate the model parameters to describe the colocalization properties of the underlying random process. GSODA preserves the statistical characteristics of SODA, but extends its use to a wider gamut of colocalization studies. Furthermore, by restricting the level set function to the region of interest by using suitable boundary conditions, GSODA eliminates the need to explicitly correct for edge-artifacts, which could be challenging to compute for complex shapes. Finally, due to the recent advances in real-time polygon based digital morphology, GSODA allows computationally elegant solution for processing big data in bio-image informatics applications. This makes the proposed technique both robust and computationally efficient, and provides a generic approach to colocalization analysis for a variety of bioimaging studies in fluorescent microscopy, super resolution imaging, and histopathology.

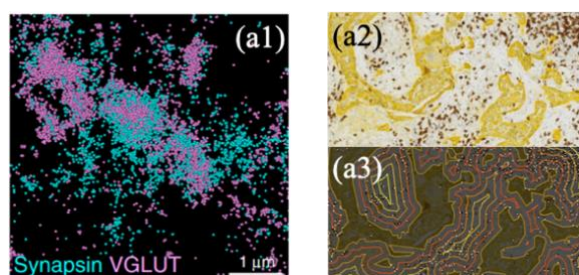


Fig. 1: Two potential applications of object based colocalization is presented here. The first example (a1) involves estimating protein colocalization in super-resolution microscopy. In (a2), an example is taken from histopathology where the objective is to study the spatial colocalization of the immune cells (points) to the tumor regions (a3).

References

- [1] T. Lagache et al., "Mapping molecular assemblies with fluorescence microscopy and object-based spatial statistics," *Nature communications*, vol. 9.1, p. 698, 2018.
- [2] J. A. Sethian, "Level set methods and fast marching methods: evolving interfaces in computational geometry, fluid mechanics, computer vision, and materials science", *Cambridge University Press*, 1999.

Label-free live-cell imaging at 150 nm and 100 Hz by ROCS-Microscopy

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KEY WORDS: Label-free, super-resolution imaging, 100Hz, dark-field, total internal reflection, actin cytoskeleton.

Thermal and active cellular forces drive biological structures. These movements become the faster the smaller the biological structures because of less friction and steric hindrance. This is an important aspect for super-resolution microscopy, since unfortunately the resolution of smaller structures requires more photons and time. Therefore, novel concepts enabling smart trade-offs between temporal and spatial resolution have to be developed.

Our variant of an oblique illumination super-resolution microscopy [1, 2] is based on rotating coherent scattering (ROCS), which generates thousands of high contrast images without post-processing at frame rates of more than 100 Hertz and without labeling. The sample is scanned over all azimuthal illumination angles within a single camera exposure time and allows for variant illumination and detection modes such as bright-field, dark-field or total internal reflection (TIR). Thus, structures as small as 150 nm become separable through local destructive interferences. Within one rotation, the speckles do not disappear, but change to the object itself - thereby improving contrast and resolution [3]. ROCS is applied to different cells revealing unexpected dynamic biological processes [4].

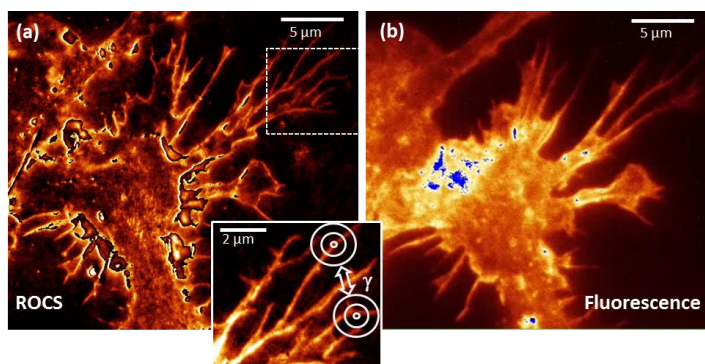


Figure 1: (a) ROCS image (DF mode) of a living mouse-macrophage recorded with a 405 nm laser within 9 ms. (b) The corresponding LifeAct GFP-actin labeled fluorescence image was recorded within 200 ms. Both images were recorded in TIR mode. Inset: The correlation between two spherical waves is quantified by the degree of coherence γ .

- [1] Jünger F, Olshausen P, Rohrbach A., Fast, label-free super-resolution live-cell imaging using rotating coherent scattering (ROCS) microscopy (2016) *Sci Rep*, 6, 30393
- [2] Olshausen P, Rohrbach A., Coherent total internal reflection dark-field microscopy: label-free imaging beyond the diffraction limit (2013) *Opt Lett*, 38, 20, 4066 – 4069
- [3] D. Ruh, J. Mutschler, M. Michelbach, and A. Rohrbach, "Superior contrast and resolution by image formation in rotating coherent scattering (ROCS) microscopy," *Optica* 5, 1371-1381 (2018).
- [4] F. Jünger and A. Rohrbach, "Strong cytoskeleton activity on millisecond time scales during particle binding and uptake revealed by ROCS microscopy," *Cytoskeleton* 75, 410–424 (2018).

Full field interferometry for characterizing and counting biotic nanoparticles

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Keywords: interferometer label free, tracking, nanoparticles, diffusion

The development of label-free optical methods to characterize nanosized particles is key for studying a wide range of processes ranging from biomedical to environmental studies. Marine environments are rich in nanoparticles such as viruses of various origins and composition with a vital role in the balance of oceans microbiome; membrane vesicles are another type of nanoparticles that are found in marine environment as well as in the intestine microbiota; it has been hypothesized that dysregulations of bacterial populations by viruses constitute a favorable ground for inflammatory diseases of the intestine; several studies have suggested that the presence of small viruses in the intestine and in aquatic environments has its origins in anthropogenic pollution. It is thus of primary importance to enumerate and distinguish these different types of biotic nanoparticles in marine and in other ecosystems.

At the *Institut Langevin*, we have studied the diffusion properties of those and other type of nanoparticles in aquatic environment introducing a novel, highly sensitive, and non-destructive approach using optical interferometry. This technique allows to detect and track label-free nanoparticles with a resolution in the order of tens of nanometers [1]. Our approach is based on detecting the interference signal that takes place near the focus of the imaging system between a strong illumination beam and the light scattered by the nanoparticle (figure 1). The measurement is robust since both beams follow the same path (common path interferometer), and the amplitude of the recorded signal is related to the size and nature of the nanoparticle. Moreover, our measurements are complemented with single-particle tracking of their motion; the analysis of the trajectories of each individual nanoparticle gives us access to a better understanding of its mobility and the surrounding environment.

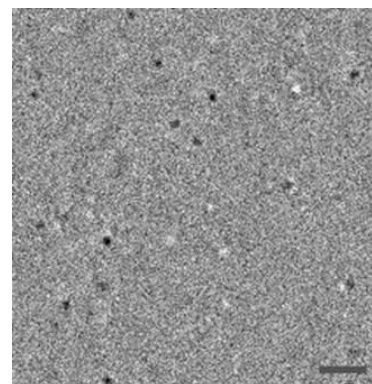


Figure 1: SiO₂ nanoparticles of diameter 100 nm image immersed in water (scale bar 140 μm)

So far, different types of viruses have been the focus of our main studies. It has been noticed that the trajectories of some viruses characterized by the existence of a rigid tail deviated from pure Brownian diffusion. These observations are particularly relevant as diffusion and spatial exploration by the viruses are critical to understand how microbial interactions shape the biogeochemical cycles in the ocean. We will present and discuss these results obtained with our method.

[1]. Full-field interferometry for counting and differentiating aquatic biotic nanoparticles: from laboratory to Tara Oceans, Boccara M, Fedala Y, Venien Bryan C, Bailly-Bechet M, Bowler C, and Boccara AC, **Biomed. Opt. Express** 7, 3736-3746 (2016)

Statistical modeling of spatial interactions in biological patterns, with application to plant nuclear organization

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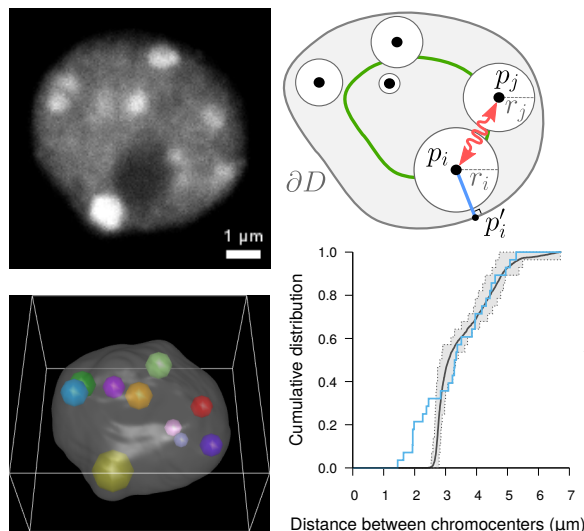
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Keywords: 3D Biological imaging; Object patterns; Spatial statistics; Nuclear organization; Heterochromatin

The spatial organization in the cell nucleus is tightly linked to genome functions such as gene regulation. Similarly, specific spatial arrangements of biological components such as macromolecular complexes, organelles and cells are involved in many biological functions. Deciphering the principles underlying these organizations is thus crucial to characterize and understand biological systems. Spatial interactions among elementary components, such as attraction or repulsion, are key determinants of spatial patterns. Spatial statistics and the statistical analysis of point patterns are the methodology of choice to analyze these interactions. However, spatial statistical methods have been essentially developed for application contexts such ecology or forestry, where data are typically single observations of 2D points distributed within a sampling window. These methods are not appropriate for the analysis of spatial data gathered through imaging experiments, which are generally repeated observations of 3D patterns of real-sized objects distributed within domains of arbitrary shapes. The lack of appropriate statistical methods and models to process biological spatial data currently limit our ability to finely dissect spatial interactions.

Here, we developed a computational framework to overcome these limitations. The framework combines unbiased statistical tests, novel spatial descriptors and models more elaborate than the classical random model. We used constitutive heterochromatin, a dynamic, structural and functional nuclear compartment, as a model system to demonstrate the potential of our framework. Our results provide an unbiased demonstration of a peripheral location of chromocenters but challenge the common view on chromocenter organization by showing that peripheral positioning alone is not sufficient to account for their spatial arrangement. We reveal that chromocenters are organized along both radial and lateral directions in the nuclear space. Furthermore, we highlight a multiscale organization of chromocenters with a close to maximal repulsion at large scale. Our results reveal complex principles underlying heterochromatic patterns with possible scale-dependent antagonistic effects. The proposed generic framework will be useful to identify determinants of spatial organizations and to question their interplay with biological functions.



Statistical spatial modeling of 3D biological organizations: application to constitutive heterochromatin in Arabidopsis thaliana. Nuclei and chromocenters (constitutive heterochromatin) were segmented in 3D confocal images of isolated leaf nuclei. Observed patterns were compared to the predictions of several spatial interaction models. A maximum repulsion model was consistent with the long-range organization between chromocenters.

Quantitative mapping of fluorescently tagged ribosomes throughout the cell cycle of *S. cerevisiae* using FCS-calibrated imaging.

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Keywords: FCS-calibrated imaging, cell cycle, ribosomes

FCS-calibrated imaging integrates the strength of imaging-based time-lapse microscopy to analyze spatial protein dynamics, and fluorescence correlation spectroscopy (FCS), which provides quantitative parameters such as concentrations, oligomerization, or diffusion coefficients [1].

We use this method to quantify the abundance of ribosomes of the model organism *S. cerevisiae* throughout its cell cycle on a single cell level. To date, contradictory information on the total ribosome number ranging, on average, from 50,000 to 200,000 ribosomes per cell are reported from ensemble measurements. However, the overall protein expression pattern of a cell highly depends on the number of ribosomes, and the distribution of stalk proteins among the ribosome population that determines differences in the translation rate of certain mRNAs (so-called ribosome-filter hypothesis). Thus, detailed information on the number and type of ribosomes on the single cell level will not only promote the understanding of protein expression patterns, but also support mathematical modeling in systems biology.

To address this issue, we first perform FCS measurements on yeast cells expressing EGFP monomers to obtain a calibration curve relating the arbitrary fluorescence intensities of image voxels to protein concentrations. Next, we determine the fluorescence probability of EGFP by comparing the molecular brightness of EGFP homo-dimers to the respective monomer [2]. This finally allows us to precisely determine the abundance of EGFP-tagged ribosomal proteins on the single cell level throughout the cell cycle of *S. cerevisiae*. In summary, we observe a broad distribution of the total ribosome number, but steady ribosome density that stays constant throughout the cell cycle of unstressed yeast cells.

In future experiments we will additionally analyze the distribution of stalk proteins among the ribosome population to investigate whether or not differences in protein expression patterns are related to the type of ribosomes present in certain cell cycle phases.

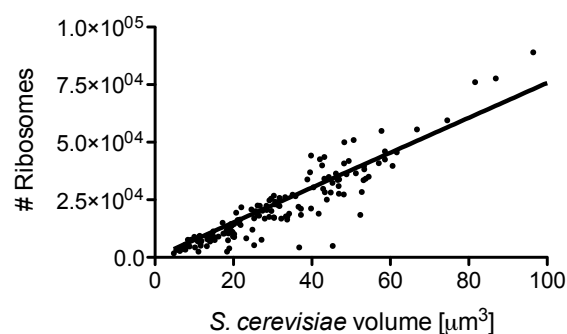


Figure 1: Correlation between volume and total ribosome number of single yeast cells. The density but not total number of ribosomes is approximately constant on the single cell level. $N=2$ with 156 analyzed cells; Slope= 758 ribosomes/ μm^3 , $R^2=0.84$.

[1] Politi, AZ. *et al.*, 2018. Quantitative mapping of fluorescently tagged cellular proteins using FCS-calibrated four-dimensional imaging. *Nature Protocols*, 13:1445.

[2] Dunsing, V., Luckner, M., *et al.*, 2018. Optimal fluorescent protein tags for quantifying protein oligomerization in living cells. *Scientific Reports*, 8(1):10634.

Versatile uniform illumination method toward quantitative single molecule localization microscopy

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Gaussian-shaped illumination in Single Molecule Localization Microscopy (SMLM) and fluorescence microscopy induces non-uniform excitation at the sample plane and makes poor use of the available laser power, restricting the resulting field of view (FOV). As a result, large field quantitative imaging is only achievable in SMLM with great difficulty.

We will present an illumination scheme which is based on a classical 300mW, 647nm laser and benefits from the flexibility of a scanning-mirror system to achieve uniform illumination over the maximum $(200\mu\text{m})^2$ FOV of a sCMOS camera. Optimal imaging is achieved even with low laser power by adapting the integration time and the scanning speed to the blinking properties. In SMLM, quantification with nanorulers [Fig.1] shows that our setup can provide uniform photon count, on-time and resolution. To our knowledge, this is the only uniform illumination scheme that provides adaptable uniform optical sectioning, from epifluorescence to TIRF [Fig.2] and thus is adapted for both wide-field, (d)STORM [Fig.3] and PAINT quantitative experiments.

Fig.1

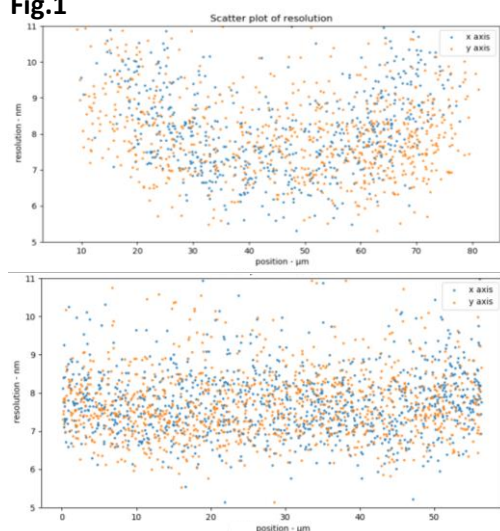


Fig.2

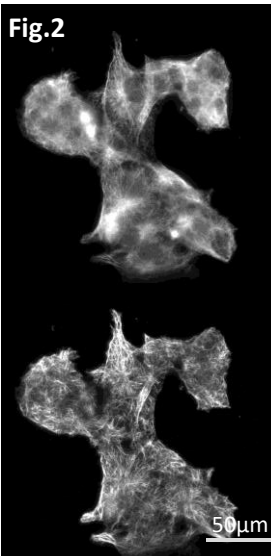


Fig.3

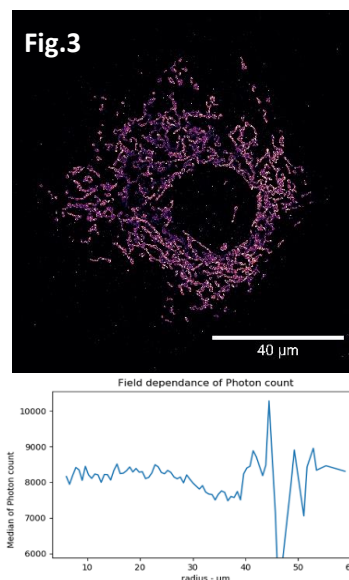


Figure 1 : Resolution quantification along axis for 40nm-spaced nanorulers, with gaussian (up) and uniform (down) illumination.

Figure 2: EPI (up) and TIRF (down) illumination on $200*200\mu\text{m}^2$ field of view.

Figure 3: Large field uniform SMLM image of mitochondria, showing uniform photon count repartition

Quantitative analysis of EGF receptor dynamics, confinement and interaction with the cytoskeleton using nanoparticle labelling, single-molecule tracking and hydrodynamic force application

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Keywords: membrane nanodomains, Bayesian inference, cytoskeleton

We compared the confinement behavior and interaction with the actin cytoskeleton of 3 raft-confined receptors (EGFR and two toxin receptors) and a non-raft receptor, the transferrin receptor in MDCK cells. Using labelling with Eu-doped YVO₄ nanoparticles, we could acquire long-term trajectories and extract the confinement energy landscape felt by the receptors with Bayesian inference (Türkcan et al., Biophys. J. 2012). Using information criteria (Türkcan and Masson, PloS ONE 2013), and clustering-based analysis we showed that the raft-confined receptors can be well described with a second-order polynomial energy landscape, whereas the transferrin non-raft receptors are better described by a fourth-order polynomial energy landscape. Moreover, the stiffness of the energy landscape for the raft-confined receptors is inversely proportional to the square root of the nanodomain radius, as expected for a receptor at thermal equilibrium moving inside a parabolic energy landscape. This means that the so-called nanodomain radius is not the physical size of the nanodomain but takes on a value determined by the thermal energy of the receptor moving inside the parabolic energy landscape.

By growing the cells in a microchannel, we could apply a hydrodynamic force which is amplified by the nanoparticle radius and is thus able to displace the receptors inside the membrane while exerting negligible force on the cells (Türkcan et al., Biophys. J. 2013). EGFR and the two raft-confined toxin receptors are displaced over several microns, together with their confining nanodomain, and return close to their initial position after the flow force stops. In stark contrast, the transferrin receptors are also displaced but remain close to the final position reached under force when the flow stops (see Fig. 1). This implies that EGFR and the two toxin receptors are attached to the cytoskeleton, EGFR probably via its actin-binding domain, and the two toxin receptors, which do not interact directly with actin, via the nanodomain binding to the cytoskeleton.

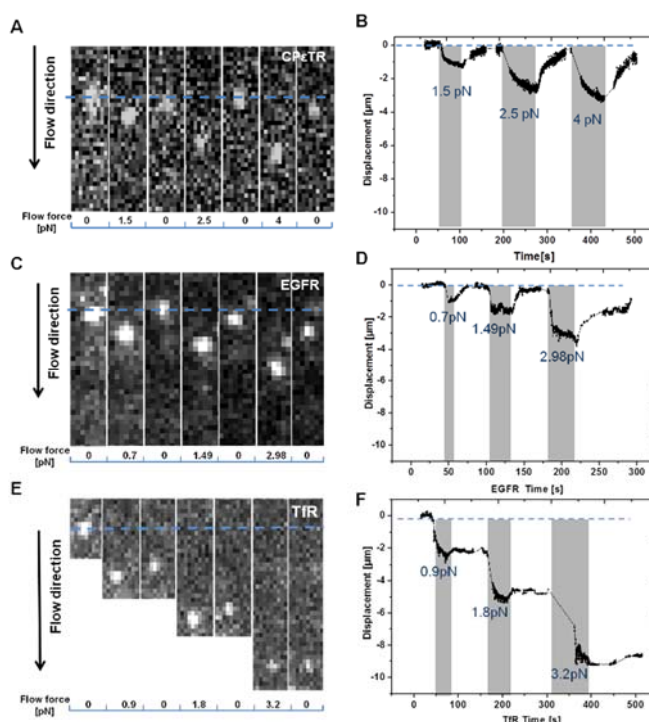


Figure 1. Receptor displacements over time for a series of different flow forces. Top: *Clostridium Perfringens* ϵ -toxin. Middle: EGFR. Bottom: Transferrin receptor.

Although this work is centered on EGFR, the comparison with other receptors leads us to classify the studied receptors in three categories: i) receptors confined in raft nanodomains and binding to actin (like EGFR), ii) receptors confined in raft nanodomains and indirectly bound to actin via the nanodomain constituents, and iii) non-raft receptors which are not bound to actin but are only hindered sterically in their motion by the actin filaments.

Molecular counting with calibrated fluorescent labelling and photobleaching step analysis

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Keywords: Protein counting, Degree of labelling, emitter counting

At the molecular level, biological processes are driven by protein assemblies which are tightly regulated with respect to their composition, localization and activity over time. Fluorescence microscopy allows investigating such assemblies at the single particle level and within their native context, but requires labelling with fluorescent markers. If the fraction of labelled target proteins is known, the number of protein copies in an assembly can be determined in situ. Different methods for fluorescence-based counting of molecules within protein assemblies or other structures have been developed in the past [1]. A straightforward approach for molecular counting is to determine the number of fluorophores by observing discrete photobleaching steps under continuous illumination.

Here, we present a new algorithm for automated photobleaching step analysis (PBSA) based on previously published work [2,3]. We show that the proposed algorithm is able to robustly recover the number of fluorophore bleaching steps with minimal runtime even for complex traces. The bias and precision of the algorithm was evaluated using semi-synthetic data and experimental data obtained from DNA origami with a defined number of emitters per assembly. To relate the number of deposited fluorophores to the number of protein copies within a structure, we developed a calibration probe which allows measuring the absolute degree of labelling (DOL) for the two self-labelling protein tags HaloTag and Snap-tag. We furthermore validated the established approaches using the nuclear pore complex as structure with defined and known stoichiometry.

The combination of calibrated labelling schemes and emitter counting methods such as PBSA now enables molecular counting in situ. In the future, advanced emitter counting approaches such as Counting by Photon Statistics may enable time-resolved observation of protein complex stoichiometries in live specimen [4].

[1] Großmayer, K. S., Yserentant, K., and Herten, D. P. (2018). Photons in-numbers out: Perspectives in quantitative fluorescence microscopy for in situ protein counting. *Methods and Applications in Fluorescence*.

[2] Kalafut, B. and Visscher, K. (2008). An objective, model-independent method for detection of non-uniform steps in noisy signals. *Computer Physics Communications*, 179(10), 716-723.

[3] Tsekouras, K., Custer, T. C., Jashnsaz, H., Walter, N. G., and Pressé, S. (2016). A novel method to accurately locate and count large numbers of steps by photobleaching. *Molecular Biology of the Cell*, 27(22), 3601-3615.

[4] Großmayer, K. S., & Herten, D. P. (2017). Time-resolved molecule counting by photon statistics across the visible spectrum. *Physical Chemistry Chemical Physics*, 19(13), 8962-8969.

Multiplex Single Molecule Pull-down (SiMPull) measurements of EGFR site-specific phosphorylation

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Keywords: single molecule imaging, SiMPull, computational modelling, Epidermal Growth Factor Receptor, cell signalling

The Epidermal Growth Factor Receptor (EGFR/ErbB1/HER1) plays an important role in both physiological and cancer-related processes. EGFR has 20 cytoplasmic tyrosines, at least six of which are known to recruit signaling molecules and differential phosphorylation of these tyrosines is thought to couple receptor activation to distinct signaling pathways. However, limitations of existing techniques, such as western blotting and those of quantitative mass spectrometry-based proteomics, preclude the detection or accurate quantification of phosphorylation events in intact, individual signaling proteins. To address this capability gap, we modified the previously described single molecule pull-down (SiMPull) technique to allow for robust

quantification of cell-surface receptor phosphorylation. Briefly, cells are lysed and the protein of interest is then captured by antibodies bound to the glass coverslip. If the proteins are fluorescently tagged, either by fluorescent proteins or subsequent antibody labeling, their presence will be quantified by single molecule imaging (Fig. 1). We made a number of critical improvements that allowed us to directly detect and rigorously quantify the phosphorylation state of thousands of individual membrane receptors. These improvements enabled the first direct detection of activation-dependent multisite phosphorylation on full-length EGFR. We found that, while multisite phosphorylation occurs, the extent of phosphorylation at individual tyrosines varies and only a fraction of receptors are phosphorylated at saturating EGF levels. This heterogeneity cannot be detected with other methods. Our results show that there are distinct populations of receptors leading to early diversification of function. To gain insight into the mechanisms that regulate EGFR phosphorylation patterns, we coupled SiMPull measurements with rule-based modeling of EGFR signaling. Simulation and experimental results demonstrated that site-specific variations are dependent on the relative abundance of signaling partners that limit access by tyrosine phosphatases. Evaluation of the impact of low- and high-affinity ligands, along with oncogenic EGFR mutants, showed that single site and multisite phosphorylation levels are dependent on dimer lifetimes. The new level of detail provided by multiplex SiMPull measurements has revealed new insights into how ligand-regulated receptor dimerization dynamics and adaptor protein concentrations play critical roles in EGFR signaling.

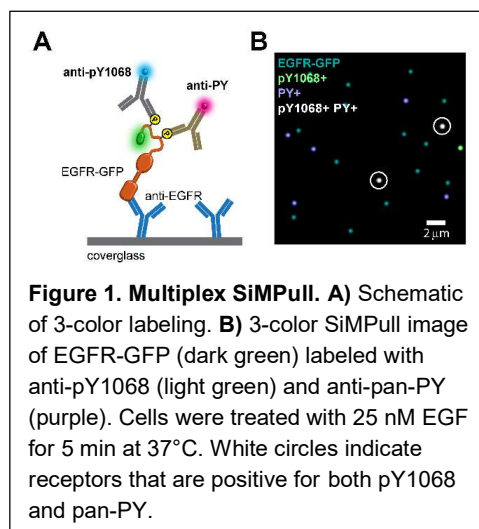


Figure 1. Multiplex SiMPull. **A)** Schematic of 3-color labeling. **B)** 3-color SiMPull image of EGFR-GFP (dark green) labeled with anti-pY1068 (light green) and anti-pan-PY (purple). Cells were treated with 25 nM EGF for 5 min at 37°C. White circles indicate receptors that are positive for both pY1068 and pan-PY.

Machine learning and light-sheet fluorescence microscopy reveals morphometric indicators in organoids for informed anti-cancer drug discovery

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Keywords: light-sheet microscopy, airy-beam, machine learning, big data, organoids, cancer, data mining, image analysis

Recent advances in organoid culture and fast-volumetric imaging technology have facilitated previously inaccessible phenotypic characterisation of disease aetiology. Here, we present a combined methodological and analytical pipeline for detecting and quantitating the efficacy of chemotherapeutic-drug-induced morphometric differences in human-derived colorectal malignant tumour organoids¹. Patient-derived organoids, which more closely mimic the complexity of the *in vivo* environment than traditional cell cultures, have been shown to be a good predictor of therapeutic response² and also offer potential for personalised medicine. By using a customised commercial airy-light-sheet microscope³, we were able to rapidly record ($\sim 0.2\text{Hz}$) the spatial fluorescence distribution of large specimen volumes ($\sim 1\text{mm}^3$), enabling us to screen chemotherapeutic compounds in relatively large sample sizes ($n > 200$ organoids). We show how applying data-mining techniques to these rich volumetric imaging datasets can provide a series of measures of therapeutic efficacy⁴ and compare our results to traditional screening assays. Furthermore, we propose a machine-learning derived titration-invariant drug similarity measure, which can be used to correlate the effects of different drug treatments with potential application in large-scale drug discovery programs.

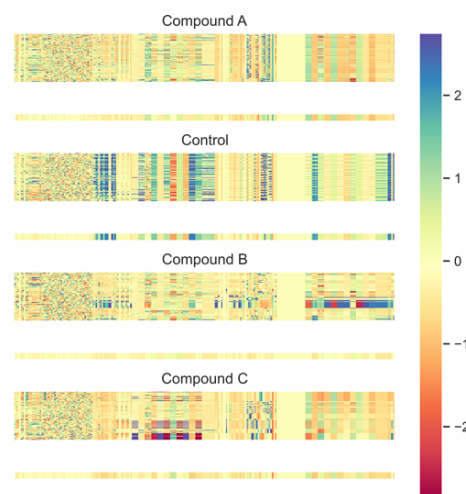


Figure 1. After segmentation each cell nucleus is represented by 400 shape and texture features. Each row represents a different nucleus, the colour bar shows the amplitude of each feature, each column a different feature. The single row below each compound is the median feature profile for each drug.

1. Badger, L. M. *et al.* 3D imaging of colorectal cancer organoids identifies responses to Tankyrase inhibitors. *bioRxiv* 705277 (2019). doi:10.1101/705277
2. Vlachogiannis, G. *et al.* Patient-derived organoids model treatment response of metastatic gastrointestinal cancers. *Science* **359**, 920–926 (2018).
3. Vettenburg, T. *et al.* Light-sheet microscopy using an Airy beam. *Nat. Methods* **11**, 541–544 (2014).
4. Caicedo, J. C. *et al.* Data-analysis strategies for image-based cell profiling. *Nat. Methods* **14**, 849–863 (2017).

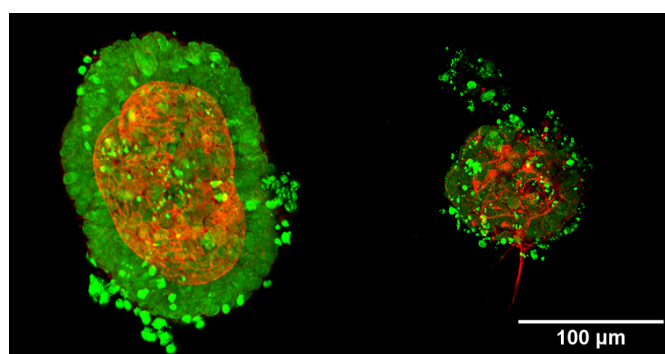


Figure 2. Representative light sheet fluorescence microscopy images of colorectal cancer organoids, stained for DNA (DAPI, green) and F-actin (phalloidin, red). Left panel shows an untreated organoid and right image shows the same organoid type treated with an anticancer therapeutic.

Computational analysis of filament polymerization dynamics in cytoskeletal networks

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Keywords: cytoskeleton, protein dynamics, fluorescence confocal microscopy, image analysis, tracking

The polymerization–depolymerization dynamics of certain proteins play essential roles in the self-organization of cytoskeletal structures, in eukaryotic as well as prokaryotic cells. Cytoskeletal filaments show unique properties, distinct from equilibrium polymers and essential for various complex processes in the living cell, from chromosome segregation to cell motility. While advances in fluorescence microscopy and *in vitro* reconstitution experiments have helped to study the dynamic properties of these complex systems, methods to collect and analyze rates of polymerization as well as depolymerization events in a non-biased, high-throughput manner, are still missing.

To address these limitations, we developed a novel image analysis protocol to study polymerization dynamics of active filaments in an unbiased, highly automated manner. This approach relies on standard time-lapse imaging protocols and open-source software tools (ImageJ and python scripts), which makes it readily available to study various dynamic processes *in vivo* and *in vitro*. The workflow comprises three steps: (i) generate moving fluorescent spots corresponding to growing and shrinking filament ends by image subtraction, a technique commonly used for motion detection and background subtraction; (ii) build treadmilling trajectories by detecting and tracking the fluorescent spots using TrackMate; (iii) analyze the trajectories to quantify velocity and directionality of filaments. As an example, we use treadmilling filaments of the bacterial tubulin FtsZ to demonstrate that this method is able to specifically detect, track and analyze growth and shrinkage of polymers, even in dense networks of filaments.

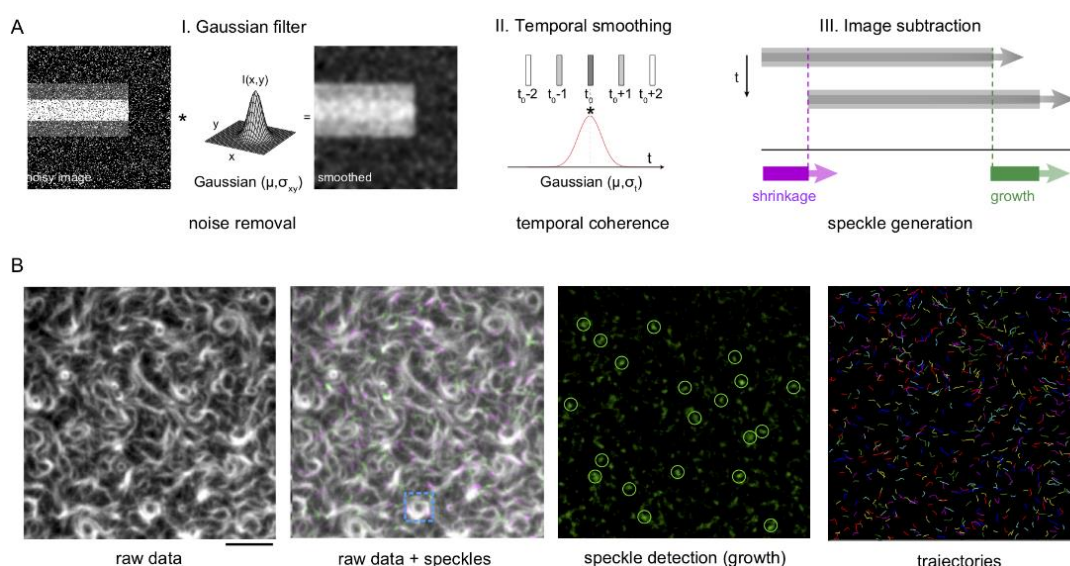


Fig. 1: Protocol to generate fluorescent speckles (A) and the outcome when analyzing fluorescently-labelled FtsZ filaments on a supported lipid bilayer (B).

We believe that this automated method can facilitate the analysis of a large variety of dynamic cytoskeletal systems, using standard time-lapse movies obtained from experiments *in vitro* as well as in the living cell.

Novel particle tracking approach with CNN-based candidate selection and two-step linking by Bayesian network.

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Particle tracking is a subject of intense development and a wide range of tracking approaches have been proposed, many with a high accuracy [1]. However, the experimental set-up and imaging conditions have a major influence on the tracking performance, and often tracking methods are tailored for a specific experimental set-up. The proposed tracking approach exploits the recent advancements in machine learning techniques and combines it with classical image analysis approaches thereby allowing more comprehensive and flexible framework suitable for both, interpretable settings and result analysis.

As prerequisite for tracking technique, the proposed method requires target detection and frame-to-frame object association. The detection solution has a few consecutive steps. Firstly, the particle visibility is improved in the frame sequence using classical background subtraction approach. Secondly, the particle candidates are detected with the multi-scale spot enhancing filter (MSSEF). And finally, the candidate selection process employs a light-weighted Convolutional Neural Network (CNN) classifier to eliminate spurious detections. The data association part is executed as a two-step linking process. A set of short tracks (tracklets) are first formed based on the Euclidean distance between the detected particles. Only detections with a reliable small distance in temporal and spatial domain are linked. Then, the tracklets are linked to assemble final tracks. This linking is based on the connectivity score defined by an inference over a Bayesian network which provides the probability of a tracklet pair to be connected.

The presented tracking approach is illustrated for the confocal fluorescence microscopy imaging of protein trafficking in epithelial cells of the *Drosophila* egg chamber. The labelled protein can be detected throughout its passage in the secretory system, in the Golgi, in vesicles and at the plasma membrane. This confounded the ability to detect protein in the vesicles. The proposed CNN-based candidate pruning reduces false detections, while two-step linking provides an efficient association of the detections. The presented method achieves a root mean square error (RMSE) of 1.39 for the vesicle localisation and 0.7 for the degree of track matching (α).

[1] N Chenouard, I Smal, F de Chaumont, M Maska, *et al*, "Objective comparison of particle tracking methods," Nature Methods, vol. 11, 2015.

Measuring differential diffusion rates of distinct protein states by sptPALM imaging in live microbial cells

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Keywords: SMLM, single particle tracking, super-resolution microscopy

Correct spatiotemporal organization is essential for many cellular processes and the formation of protein gradients plays a significant role in establishing specific intracellular localization patterns. With the help of sptPALM imaging, we can measure the spatial organization and dynamics of single molecules in live microbial cells at high specificity, sensitivity and spatiotemporal resolution. In particular, it enables us to measure the individual molecules in the heterogeneous cellular environment where we can observe their behavior in presence of the other components of the cellular machinery. This distinguishes sptPALM experiments from in vitro single-molecule assays and their very controlled environment in test tubes.

By using sptPALM imaging, we thus can follow the localization dynamics and differential diffusion rates of distinct conformational protein states which drive and regulate intracellular pattern formation. In this talk, I will introduce some of our recently developed experimental and analytical sptPALM tools alongside with our specific microbiological questions.

Metadata and Performance Tracking for Fluorescent Microscopes.

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KEYWORDS: fluorescence microscopy, quantitative image analysis, single molecule, nanoscopy, image comparability, imaging QC

Abstract: Microscopy images need to be accompanied by a description of the sample, its preparation and experimental layout as well as technical parameters under which images were taken. The term "metadata" is used to refer to such accompanying information, but the exact meaning of "metadata" frequently varies with context. A major challenge with metadata for technical parameters is the large variability of what is recorded by different microscopes. Metadata can be as simple as the pixel size or as complex as the results of an entire internal instrument calibration routine and everything in between. To enable full quantitative analysis to extract the maximal information content of images and to make images from different microscopes comparable, we propose 1) an OME based, extended metadata model to capture complete hardware and settings used for image acquisition, 2) an extension of metadata to contain optical calibration- and performance documentation, 3) a tier system for metadata requirements that scales the amount of metadata to be reported with the complexity of the imaging data and 4) an interactive, easy-to-use GUI-based software tool to ease the often tedious and time consuming metadata collection process and lower the experimental record-keeping burden. However, there are certain crucial pieces of information that simply are not captured in even the most rigorous and precise routines for record-keeping and calibration, as they simply cannot be measured without the aid of (often costly, cumbersome and complicated) external devices. Here, we present an inexpensive, easy-to-use calibration device combining a power sensor with a light source that allows the user to measure excitation power and perform basic detector calibration routines. This provides crucial meta-data on experimental conditions and allows current and future model-based data processing tools to get as much quantitative information as possible out of the images. The device also provides an elegant, possibly automated way to track microscope performance over time.

Metadata and Performance Tracking for Fluorescent Microscopes III- MetaMax

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KEYWORDS: fluorescence microscopy, quantitative image analysis, single molecule, nanoscopy, image comparability, imaging QC

Microscopy images need to be accompanied by a description of the sample, its preparation and experimental layout as well as technical parameters under which images were taken. The term “metadata” is used to refer to such accompanying information, but the exact meaning of “metadata” frequently varies with context. A major challenge with metadata for technical parameters is the large variability of what is recorded by different microscopes. Metadata can be as simple as the pixel size or as complex as the results of an entire internal instrument calibration routine – and everything in between.

To enable full quantitative analysis – to extract the maximal information content of images – and to make images from different microscopes comparable, we propose 1) an OME based, extended metadata model to capture complete hardware and settings used for image acquisition, 2) an extension of metadata to contain optical calibration- and performance documentation and 3) a tier system for metadata requirements that scales the amount of metadata to be reported with the complexity of the imaging data.

However, there are certain crucial pieces of information that simply are not captured in even the most rigorous and precise routines for record-keeping and calibration, as they simply cannot be measured without the aid of (often costly, cumbersome and complicated) external devices. Here, we present an inexpensive, easy-to-use calibration device that, among other things, allows the user to measure excitation power and perform basic detector calibration routines. In doing so, the “M_e_t_a_M_a_x_”_tool provides crucial meta-data to evaluate potential photo-toxicity and allows current and future model-based data processing tools to get as much quantitative information as possible out of the images.

MetaMax also provides an elegant, possibly automated way to track microscope performance over time. This work is accompanied by a second contribution on a metadata model and a third optical performance calibration.

[1] Smith et al., “Probability-Based Particle Detection That Enables Threshold-Free and Robust in Vivo Single-Molecule Tracking”, *MBoC* (2015), 22:4057–62; doi:10.1091/mbc.E15-06-0448.

[2] Smith et al., “An automated Bayesian pipeline for rapid analysis of single-molecule binding data”, *Nature Comm.* (2019), 10:272; doi.org/10.1038/s41467-018-08045-5

Metadata and Performance Tracking for Fluorescent Microscopes I- Metadata

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KEYWORDS: fluorescence microscopy, quantitative image analysis, single molecule, nanoscopy, image comparability, imaging QC

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Here we present the proposed metadata model and tier system [1] and a tool [2] to collect metadata in a tier dependent manner through a GUI that enables non-experts to be thorough and complete in their metadata documentation of hardware and setting used. This work is accompanied by a second contribution on optical calibration and a third contribution on performance calibration.

[1] <https://github.com/WU-BIMAC/MicroscopyMetadata4DNGuidelines>

[2] <https://github.com/WU-BIMAC/4DNMicroscopyMetadataTool>

Metadata and Performance Tracking for Fluorescent Microscopes II- Optics

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KEYWORDS: fluorescence microscopy, quantitative image analysis, single molecule, nanoscopy, image comparability, imaging QC

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Here we present cooperative data on the use of diffraction limited multicolor beads [1] and a number of imaging and resolution standards for optical calibration of a microscope [2-6].

This work is accompanied by a second contribution on a metadata model and a third contribution on performance calibration.

[1] Theer, Mongis and Knop, “PSFj: know your fluorescence microscope”, Nature Methods (2014), 11:981; doi.org/10.1038/nmeth.3102

[2] <https://github.com/cmongis/psfj/>

[3] Geller MRS4.2, http://www.gellermicro.com/mag_standards/MRS-4.2_190114.pdf

[4] Argolight, <http://argolight.com/products/argo-sim/>

[5] Siemensstar, <https://www.edmundoptics.com/p/high-resolution-microscopy-star-target/38664/>

[6] PSFcheck, <https://doi.org/10.1364/OE.26.021887>, psfcheck.com

Novel intensity-based approach to characterize astrocytic calcium signals

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Keywords: Ca^{2+} -imaging, GCaMP, quantification, astrocytic culture

Astrocytes, an abundant type of glial cells in the mammalian brain and spinal cord, play an important role in regulation of neuronal network functions. Because of their unique morphology, astrocytes can modulate the functional properties of thousands of synapses over defined anatomical regions. It has become evident that astrocytes can also be directly involved in modulation of synaptic signalling and synaptic plasticity, and furthermore that these functions are related to their intracellular Ca^{2+} dynamics. The Ca^{2+} events in astrocytes can occur spontaneously and mainly rely on Ca^{2+} release from intracellular stores.

Continuous development of methods for quantitative detection of $[\text{Ca}^{2+}]_i$ opens up new horizons for scientists to study physiological activities of astrocytes at a subcellular levels. The unique characteristic of astrocytic Ca^{2+} activity, however, still require the development of event detection algorithms, which correctly describe its versatile characteristics. Here we describe a novel approach for the analysis and quantification of astrocytic Ca^{2+} activity, obtained by intensity based genetically encoded Ca^{2+} indicators (GCaMPs).

Since the Ca^{2+} indicator signal not only scales with $[\text{Ca}^{2+}]_i$, but also with indicator concentration, we show that it is mandatory for quantification to correctly estimate fluorescence intensity at basal $[\text{Ca}^{2+}]_i$ (F_0). For that we developed a robust pixel based algorithm to estimate F_0 on the basis of fluorescence fluctuation analysis, which runs in a fully automatic manner. Furthermore, on the basis of $\Delta F/F_0$ we developed a novel Ca^{2+} event detection algorithm, which handles multiple threshold levels and enables to identify and characterize dynamic and overlapping patterns of activity. This allows to obtain activity parameters such as spatial extent, duration, maximum Ca^{2+} release, release and uptake velocity (Fig.1). Due to the multi-threshold approach our strategy is much more flexible while versatile and not as biased as other single threshold techniques commonly applied. This empowers to better characterize the complexity of astrocyte Ca^{2+} signalling.

Our strategy is robust and suitable for many kinds of imaging, including its usage in two-photon excitation, confocal and epi-fluorescent microscopy.

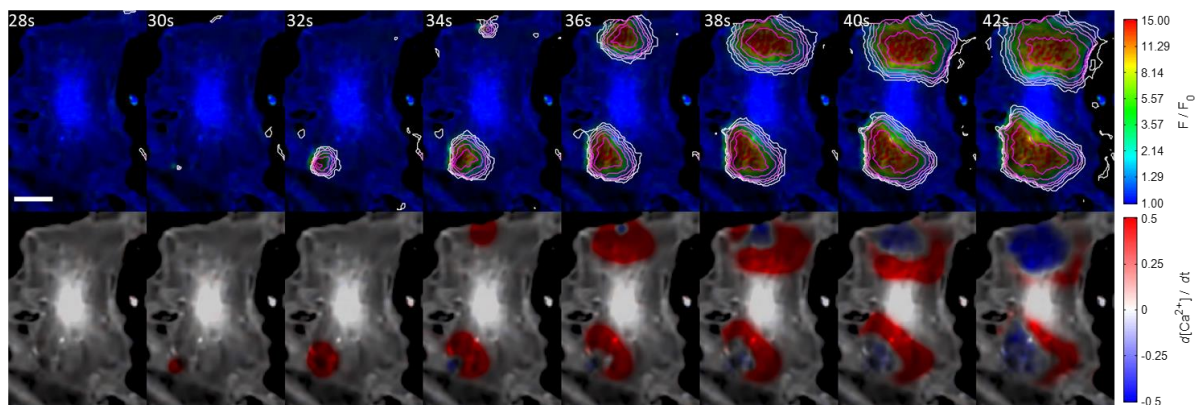


Fig. 1: Characterization of endogenous Ca^{2+} activity patterns of mouse hippocampal astrocytes.

Towards Quantitative 3D Super-Resolution Correlative Light-Electron Microscopy

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Keywords: correlative microscopy, super resolution microscopy, volumetric imaging

Determining the subcellular distribution of a protein can be a challenging task. Correlating topological information obtained by electron microscopy with protein-specific super-resolution fluorescence data can be used to gain the necessary insight [1]. However, commonly used sample preparation protocols for correlated light and electron microscopy (CLEM) are typically complex and time-consuming [2]. The preservation of fluorophores while at the same time optimizing samples for electron microscopy still represents a major challenge. Resin embedding, which is required for serial sectioning of samples, is known to strongly reduce the detectable fluorescence signal of both, fluorescent proteins and organic fluorophores. A comprehensive evaluation of different embedding conditions revealed that the degree of fluorescence preservation is strongly dependent on the resin composition and polymerization condition.

We present optimized resin embedding protocols for Alexa Fluor 647 and other commonly used dSTORM fluorophores. This allows for super-resolution fluorescence microscopy with pre-embedding labeling and minimal fluorescence loss followed by scanning electron microscopy (SEM). Serial thin sectioning is not only a necessary step for 3D SEM imaging but is also beneficial for dSTORM imaging due to the absence of out-of-focus signal and out-of-focus bleaching.

Combining the presented workflow with well-defined fluorescent labelling using protein tags will enable quantitative super-resolution fluorescence microscopy in a CLEM context and thereby make full use of information obtainable from 3D CLEM.

- [1] Müller, A., *et al.* (2017). A Global Approach for Quantitative Super Resolution and Electron Microscopy on Cryo and Epoxy Sections Using Self-labeling Protein Tags. *Scientific Reports* 7, 23.
- [2] Franke, C., *et al.* (2019). Correlative single-molecule localization microscopy and electron tomography reveals endosome nanoscale domains. *Traffic* 20, 601-617.

Pixelwise H-score: a novel digital image analysis-based metric to quantify membrane biomarker expression from IHC images

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Keywords: Digital pathology, Biomarker analysis

Background: Immunohistochemistry (IHC) assays play a central role in evaluating biomarker expression in tissue sections for diagnostic and research applications. Manual scoring of IHC images, which is the current standard of practice, is based on qualitative criteria and are known to have several shortcomings in terms of reproducibility and scalability to large scale studies. While digital image analysis (DIA) based approaches hold significant promise to overcome these limitations, current DIA methods pose several challenges that have limited their widespread use in analyzing clinical samples.

Methods: We introduce a novel DIA metric, the pixelwise H-score (pix H-score), that quantifies biomarker expression from whole-slide scanned IHC images. Pix H-score is unique in that it does not rely on the detection of individual cells or the delineation of subcellular compartments (e.g. nucleus and cell membrane) which are necessary for traditional scoring algorithms such as the H-score. All DIA metrics are calculated using either commercially available (HALO, Visiopharm) or open-source (QuPath) digital pathology software packages.

Results: We compute the pix H-score, the ATM score [1] and the traditional H-score [2] from IHC images for PD-L1 and P-cadherin. Our results show that the pix H-score exhibit tight concordance to multiple orthogonal measurements such as mRNA level and pathologist score, and provide consistently better performance over other DIA metrics.

Discussion: We anticipate that the new metric introduced here will be broadly applicable to quantify biomarker expression from a wide variety of IHC images. Although not shown here, the new metric can also be applied to immunofluorescence images. Moreover, these results underscore the benefit of digital image analysis-based approaches which offer an objective, reproducible and highly scalable strategy to quantitatively analyze IHC images.

1. K. R. Choudhury, K. J. Yagle, P. E. Swanson, K. A. Krohn and J. G. Rajendran, "A Robust Automated Measure of Average Antibody Staining in Immunohistochemistry Images", *Journal of Histochemistry and Cytochemistry*, 2010, 58: 96-107.

2. Y. Hatanaka, K. Hashizume, K. Nitta, T. Kato, I. Itoh, and Y. Tani, "Cytometrical image analysis for immunohistochemical hormone receptor status in breast carcinomas", *Pathology International*, 2003, 53: 693-699.

Functional summary statistics for Poisson processes on bounded convex three dimensional shapes

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Keywords: Poisson process, functional summary statistics, convex shapes

Current methodologies in spatial point pattern analysis have been developed for planar and spatial data. Although this theory is applicable to a wide variety of practical applications, for example tree patterns in ecology, many point patterns are not observed in Euclidean space. For example in microbiology, researchers are interested in patterns arising on the cellular membranes of bacteria. In this scenario point patterns are restricted to specific shapes such as spheres and ellipsoids. Research has only recently extended the theory to point pattern analysis on a sphere [1,2,3] with many other shapes left unexplored, mainly due to the lack of symmetries existing for general bounded subspaces of Euclidean space. Our work discusses extensions of typical functional summary statistics, such as Ripley's K-function and the empty space function, for Poisson processes defined on convex shapes in three dimensions. By using the invariance of Poisson processes under transformations between metric spaces (known as the Mapping Theorem) we can transform a Poisson process from any convex shape to a Poisson process on the unit sphere and take advantage of its rotational symmetries to construct functional summary statistics. This allows us to determine whether patterns exhibit complete spatial randomness or determine if there exists spatial preference on the original convex space.

- [1] S. M. Robeson, A. Li, and C. Huang, Point-pattern analysis on the sphere, *Spatial Statistics*, 10, (2014), pp 76-86.
- [2] T. Lawrence, A. Baddeley, R. K. Milne, and G. Nair, Point pattern analysis on a region of a sphere, *Stat*, 5, (2016), pp 144-157.
- [3] J. Møller, and E. Rubak, Functional summary statistics for point processes on the sphere with an application to determinantal point processes, *Spatial Statistics*, 18, (2016), pp 4-23.

Deep learning based Phase Microscopy with Uncertainty Quantification

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Keywords: Invited Minisymposium speaker: Modern Machine Learning Approaches, uncertainty quantification, deep learning, computational microscopy, quantitative phase imaging

Emerging deep learning based computational microscopy techniques promise novel imaging capabilities beyond traditional techniques. In this talk, I will discuss our recent efforts in building such techniques that provide improved scalability and reliability. I will demonstrate a physics guided deep learning imaging approach that enables designing highly efficient multiplexed data acquisition schemes and fully leverages the powerful deep learning-based inverse problem framework (Fig. 1a). We apply this approach to large space-bandwidth product phase microscopy using intensity-only measurements, implemented on a simple LED-array based computational microscopy platform [1] (Fig. 1b). The trained network is shown to be robust to sample variations and various experimental imperfections (Fig. 1c). I will discuss an uncertainty quantification framework to assess the reliability of the deep learning predictions. Quantifying the uncertainty provides per-pixel evaluation of the prediction's confidence level as well as the quality of the model and dataset. This uncertainty learning framework is widely applicable to build reliable deep learning-based biomedical imaging techniques.

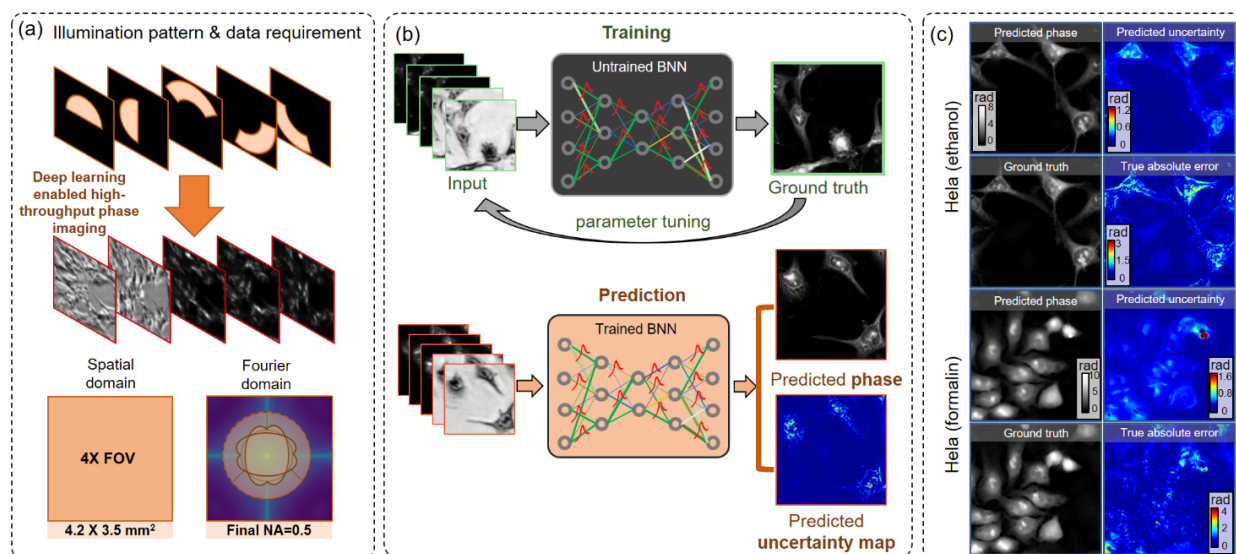


Fig. 1. Overview of the deep learning based phase microscopy technique. (a) We take only 5 intensity measurement under asymmetric illumination (2 brightfield and 3 darkfield) to encode large space-bandwidth product phase information. (b) A Bayesian convolution neural network (BNN) is developed to perform phase prediction and pixel-wise uncertainty level estimation. (c) High-resolution phase images predicted by the BNN along with quantitative uncertainty maps. Compared to ground truth phase images, we verify that the predicted uncertainty maps can be used as surrogate of true prediction errors, which is widely applicable to various deep learning driven imaging techniques.

[1] Yujia Xue, Shiyi Cheng, Yunzhe Li, and Lei Tian, "Reliable deep-learning-based phase imaging with uncertainty quantification," *Optica* 6, 618-629 (2019).

Using machine learning to design intelligent computational microscopes

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Keywords: Computational microscopy, Machine learning, Invited Minisymposium speaker: Modern Machine Learning Approaches

Deep learning algorithms offer a powerful means to automatically analyze the content of biomedical images. However, many biological samples of interest are difficult to resolve with a standard optical microscope. Either they are too large to fit within the microscope's field-of-view, or too thick, or are quickly moving around. In this talk, I will discuss our recent work in addressing these challenges by using deep learning algorithms to design new experimental strategies for microscopic imaging. Specifically, we use deep neural networks to jointly optimize the physical parameters of our computational microscopes - their illumination settings, lens layouts and data transfer pipelines, for example - for specific tasks. Examples include learning specific illumination patterns that can improve classification of the malaria parasite by up to 15%, and establishing fast methods to automatically track moving specimens across gigapixel-sized images.

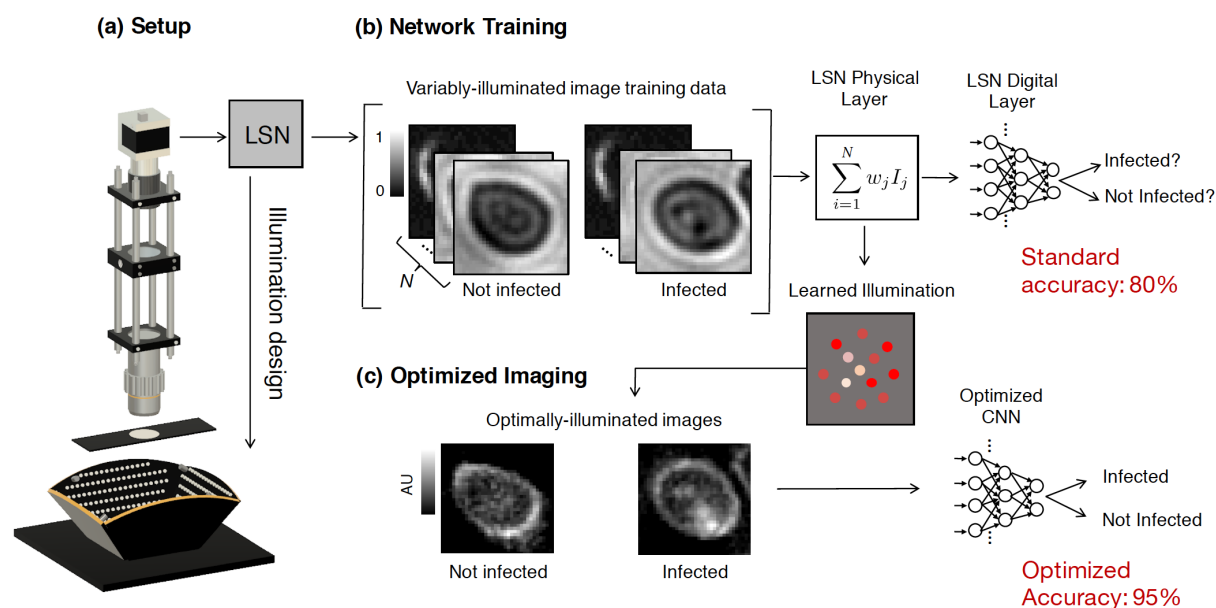


Figure 1: Example deep "learned sensing" pipeline. This system jointly optimizes a deep neural network for image classification, along with a microscope's illumination unit, for the task of malaria parasite identification.

Quantitative analyses of T cell signaling

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Keywords: large-scale imaging, computational image analysis, T cell activation, immunological synapse

T cell activation is shaped by dynamic subcellular protein distributions. In T cell signaling the association of signaling intermediates with supramolecular structures, such as F-actin sheets or supramolecular protein complexes, controls the efficiency of T cell activation. In the killing of tumor target cells by cytotoxic T cells cytoskeletal components have to polarize toward the target cell for effective cytolysis. In both cases dozen of proteins cooperate in the regulation of T cell activation. Therefore, any investigation of the regulation of T cell activation through these spatiotemporal protein networks requires the acquisition of live cell imaging data sets containing thousands of activating T cells. Unbiased analysis of image data sets of that size is best accomplished using computational methods. The Murphy laboratory using imaging data from our group has developed a computational image analysis pipeline that automatically segments T cells, aligns them with each other, and transforms their coordinates onto a standardized cell shape model. Thus, thousands of cells can be analyzed in a voxel-by-voxel aligned fashion. We have validated and applied this approach to understand the regulation of actin dynamics by the T cell costimulatory receptor CD28, polarization defects in tumor infiltrating lymphocytes and for causality inference in signaling networks.

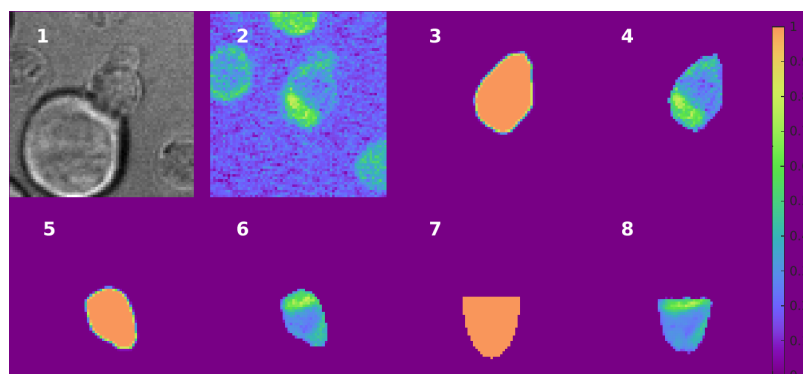


Figure 1. Computational image analysis pipeline with raw 3D data (1,2), T cell segmentation (3,4), T cell alignment (5,6) and cell shape transformation (7,8).

3Dscript: animating 3D/4D microscopy data using a natural-language-based syntax.

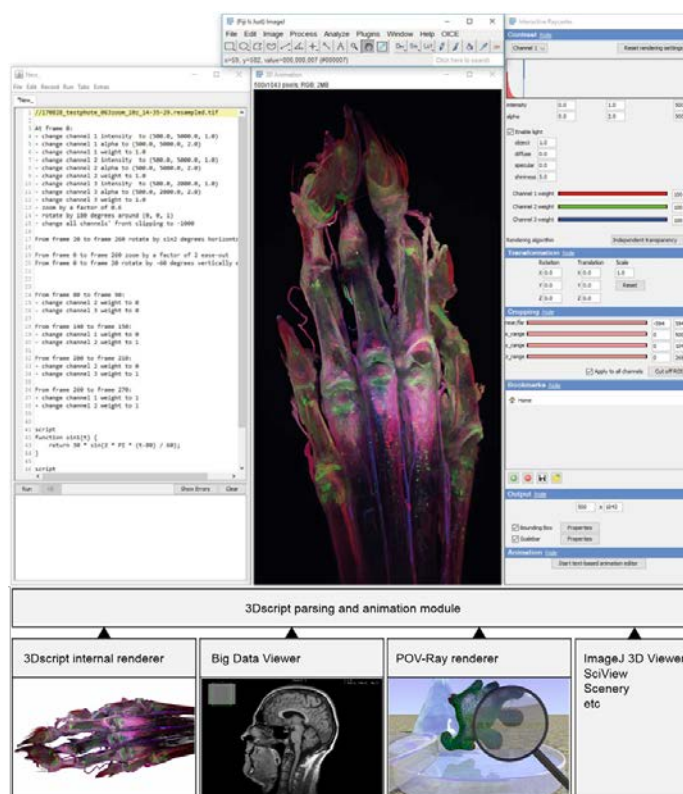
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Keywords: 3D Animation, Natural Language Syntax, 3Dscript, Fiji/ImageJ Plug-In

We introduce a software framework that assembles 3D animations of imaging data using a textual description with a syntax based on natural English language. In contrast to the established key frame based approach, smooth and complex motion sequences are intuitively implemented by concatenating multiple instructions. [1] The syntax is extensible and we demonstrate its integration into existing 3D visualization software. The user typically stores different rendering settings and scene transformations in a number of key frames along a timeline, and the rendering engine creates a smooth animation by interpolating between them. Key frames save the spatial transformation of an object as a state, e.g. three angles for a rotation, and not as a transition. This at least 4 key frames are required to define a 360-degree rotation with constant speed unambiguously. Even more are required if the motion is non-linear, which is essential for achieving smooth and pleasant motions. Software packages therefore typically provide a shortcut to insert key frames for rotations around common axes. More complex motions such as combined rotations around multiple axes, however, are difficult to achieve.



The syntax based on natural English language maximizes experience and provides easy access for users of distinct fields. Each instruction is an English sentence. Complex motion sequences are implemented by combining multiple instructions which are applied in the order they appear in the text. The syntax comprises phrases for standard non-linear accelerations, resulting in smooth and natural animations. Self-defined macro functions can be used to define motion and other parameters as functions over time. We provide 3Dscript as a Fiji/ImageJ plugin including an hardware-accelerated 3D renderer and a dedicated editor featuring auto-completion and recording. Although tailored to the included rendering engine, both the syntax and our animation framework can be easily adapted by other rendering software.

The source code is available at <https://github.com/bene51/3Dscript>, binary releases for Windows, Linux and Mac OS X are available from our Fiji update site, <https://romulus.oice.unierlangen.de/updatesite/>.

User documentation are available at <https://bene51.github.io/3Dscript>.

[1] B. Schmid et al., Nat Methods. 2019 Apr;16(4):278-280. doi: 10.1038/s41592-019-0359-1

ZOLA-3D: a software for optimal 3D SMLM with arbitrary PSF

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Keywords: 3D SMLM, Phase retrieval, Engineered PSF, Double-helix, Dual-Objective

Super-resolution microscopy based on single molecule localization (SMLM) typically achieves lateral resolutions of 20 nm in 2D images. Point spread function (PSF) engineering is widely used as a means to enable 3D SMLM reconstructions from 2D image sequences obtained without scanning. The most common engineered PSF is astigmatism [1], which enables axial resolutions of about 50 nm over an axial range of $\sim 1 \mu\text{m}$. Larger axial ranges can be achieved with more sophisticated PSFs, such as double helix [2], saddle-point or tetrapod PSFs [3]. PSF engineering that improves the axial range necessarily entails a reduction of lateral and axial resolutions. This degradation of resolution can be compensated by combining two opposing objectives that collect photons from both sides of the sample [4,5]. However, imaging deep inside the sample can lead to spherical aberrations, particularly in dual-objective systems. To reconstruct 3D super-resolution images with optimal resolutions with such complex and aberration-prone microscopes, a generic localization method based on a flexible and accurate modeling of the PSF is needed.

We recently developed ZOLA-3D, an easy-to-use Fiji plugin that enables 3D SMLM image reconstruction using a realistic modelling of the PSF, and which accounts for depth-dependent spherical aberrations [6]. ZOLA was initially designed for PSFs obtained with continuous phase masks such as astigmatism, saddle-point or tetrapod PSFs. Here, we present an extension of ZOLA that makes it compatible with discontinuous phase masks, used e.g. for the double-helix PSF, and with dual objective systems. We will show and compare ZOLA reconstructions of 3D images obtained with astigmatism, saddle-point, tetrapod or double-helix PSFs, as well as a dual-objective system (**Figure 1**). The Figure shows a reconstruction of nuclear pore complex images obtained with our dual-objective setup combined with astigmatism. Lateral and axial resolutions are approximately 20 and 40 nm respectively for an axial range of $1 \mu\text{m}$, allowing us to resolve individual nucleoporins.

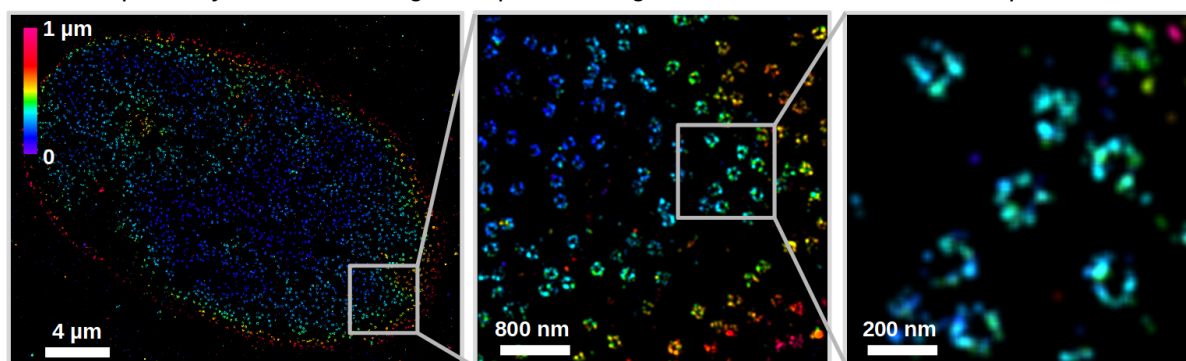


Figure 1: Super-resolution 3D image of nuclear pores in a U2OS cell reconstructed with ZOLA-3D. Imaging was performed with a dual-objective setup combined with astigmatism. Lateral and axial localization resolutions are approximately 20 and 40 nm, respectively, allowing us to resolve individual Nup96 nucleoporins labeled with SNAP-tag and Alexa647.

[1] B. Huang et al. Science, vol 319, 2008.

[2] S.R.P. Pavani et al. Proceedings of the National Academy of Science, vol 106, 2009.

[3] P.N. Petrov et al. Opt. Express, vol 25, 2017.

[4] S. Ram et al. Opt. Express, vol 8, 2009.

[5] Xu et al. Nature Methods, vol 9, 2012.

[6] A. Aristov et al. Nature Communications, vol. 9, 2018.

QUANTEV: A QUANTITATIVE APPROACH FOR ANALYZING THE SPATIO-TEMPORAL DISTRIBUTION OF 3D EVENTS IN FLUORESCENCE MICROSCOPY

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KEYWORDS: spatio-temporal distribution, fluorescence microscopy, quantitative analysis. Invited Minisymposium speaker: Spatial Statistics in Microscopy. Organizer: Ed Cohen.

We present QuantEv (see Fig. 1), a generic and non-parametric framework to quantitatively analyze and visualize the spatio-temporal distribution of observed events from different conditions in fluorescence microscopy. For each dimension of the 2D/3D chosen coordinate system, weighted densities of spatial and/or temporal features are automatically estimated. A statistical analysis taking into account the variability over replicated experiments is then applied.

In a first application, we apply QuantEv to visualize and quantify the cell shape influence on Rab6 trafficking and to localize regions in the cell associated with particular Rab6 trafficking stages. We also apply QuantEv on Rab6 trajectories to understand how these membrane associated proteins move from and towards the Golgi located at the cell center in apparent close proportions [1], while they traffic in majority from the Golgi to the cell periphery [2-3].

In a second application, we confirm by using QuantEv that the Rab11 transport intermediates are uniformly distributed around the Endosomal Recycling Compartment (ERC) at the plasma membrane plane. We also investigate the progressive effect of actin disruption on the ERC localization with respect to time. We finally apply QuantEv to analyze the joined influence of actin disruption and cell shape on the radial distribution of Rab11 vesicles trafficking.

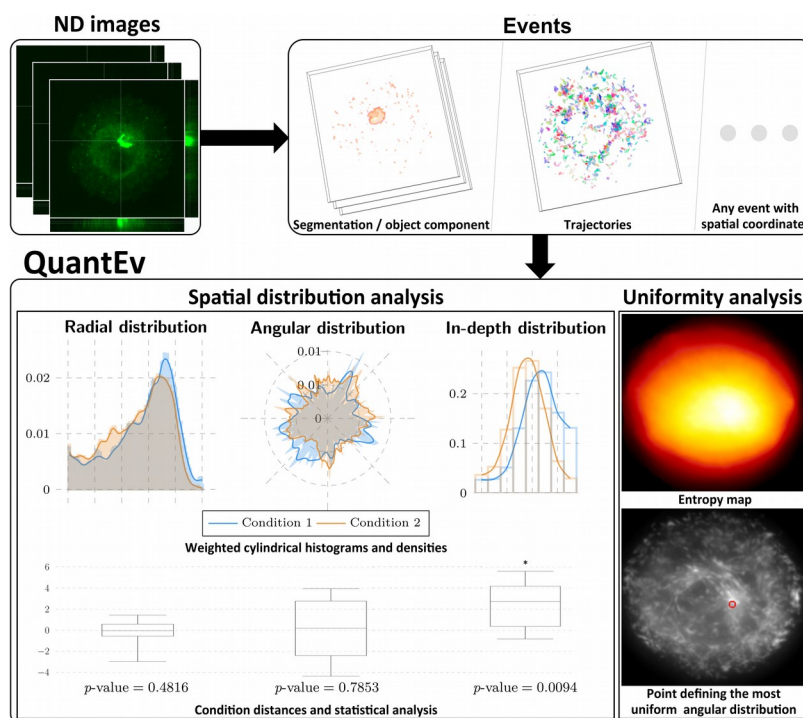


Figure 1. QuantEv workflow.

In a third application, we use QuantEv to investigate the relationship between the cell cycle phase of mouse intestinal epithelium cells and their localization with respect to the basal membrane.

[1] I. Grigoriev, D. Splinter, N. Keijzer, P.S. Wulf, J. Demmers, T. Ohtsuka, M. Modesti, I.V. Maly, F. Grosveld, C.C. Hoogenraad and A. Akhmanova. *Rab6 regulates transport and targeting of exocytotic carriers*. Dev Cell. 2007; 13(2):305–314.

[2] J. White, L. Johannes, F. Mallar, A. Girod, G. Stephan, S. Reinsh, P. Keller, B. Tzschaschel, A. Echard, B. Goud and E. Stelzer. *Rab6 Coordinates a Novel Golgi to ER Retrograde Transport Pathway in Live Cells*. J Cell Biol. 1999 Nov;147(4):743–760.

Inferring correlation in inhomogeneous and multitype point patterns – a review and new contributions

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Keywords: inhomogeneous point pattern, K-function, pair correlation function, bandwidth selection

The K-function and the closely related pair correlation function are the most important non-parametric summary statistics for characterizing correlation in spatial point patterns. The pair correlation function provides more detailed information about the correlation patterns of a point process than the K-function. This is because the K-function is a cumulative quantity obtained by integrating the pair correlation function. On the other hand, estimation of the K-function from spatial point pattern data is more straightforward than estimation of the pair correlation function which involves selection of certain smoothing parameters. The K-function and the pair correlation function can also be applied for inhomogeneous point patterns by appropriately adjusting for variations in the inhomogeneous intensity function.

In this talk we will review estimation of the K-function and pair correlation function for inhomogeneous point patterns as well as cross K and cross pair correlation functions for multi-type point patterns. We will for instance consider bandwidth selection for kernel estimation of the pair correlation function. We will also discuss difficulties that arise when an inhomogeneous intensity function is estimated using kernel smoothing and we will introduce some new K-function and pair correlation function estimates that may resolve these difficulties.

- [1] Baddeley, A. J., Møller, J. and Waagepetersen, R. (2000) Non- and semi-parametric estimation of interaction in inhomogeneous point patterns, *Statistica Neerlandica*, **54**, 329-350.
- [2] Jalilian, A. and Waagepetersen, R. (2018) Fast band width selection for estimation of the pair correlation function, *Journal of Statistical Computation and Simulation*, **88**, 2001-2011.

Towards quantitative correlative microscopy

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Keywords: Correlative light and electron microscopy, nanoSIMS, cell biology, big data, citizen science, machine learning

Correlative light and electron microscopy (CLEM) combines the benefits of fluorescence and electron imaging, allowing researchers to track rare biological events in the context of cell structure. Depending on the biological question, correlative workflows can be tailored to incorporate almost any light and electron imaging modality. For example, in collaboration with Max Gutierrez at the Crick and Haibo Jiang at the University of Western Australia, we combined live confocal microscopy with Serial Block Face Scanning Electron Microscopy and nanoSIMS to image the intracellular fate of antibiotics during *Mycobacterium tuberculosis* infection (Greenwood et al., 2019).

However, correlative microscopy tends to be 'n=1', largely due to the complexity of the experimental workflows and the lack of automated image analysis algorithms. To overcome this bottleneck, our technology development work has focused on improving the speed, accuracy and accessibility of CLEM. In this talk, I will discuss the benefits of preserving fluorescent proteins in cells and tissues prepared for electron microscopy, which facilitates 'smart tracking' correlative workflows, and our collaboration with astrophysicists, citizen scientists (Fig.1) and machine learning specialists to automate segmentation and visualisation of cell organelles at the nanoscale for large-scale downstream quantitative analysis of cells in health and disease.

1] D. Greenwood et al. *Science*. 364:1279-1282, 2019.

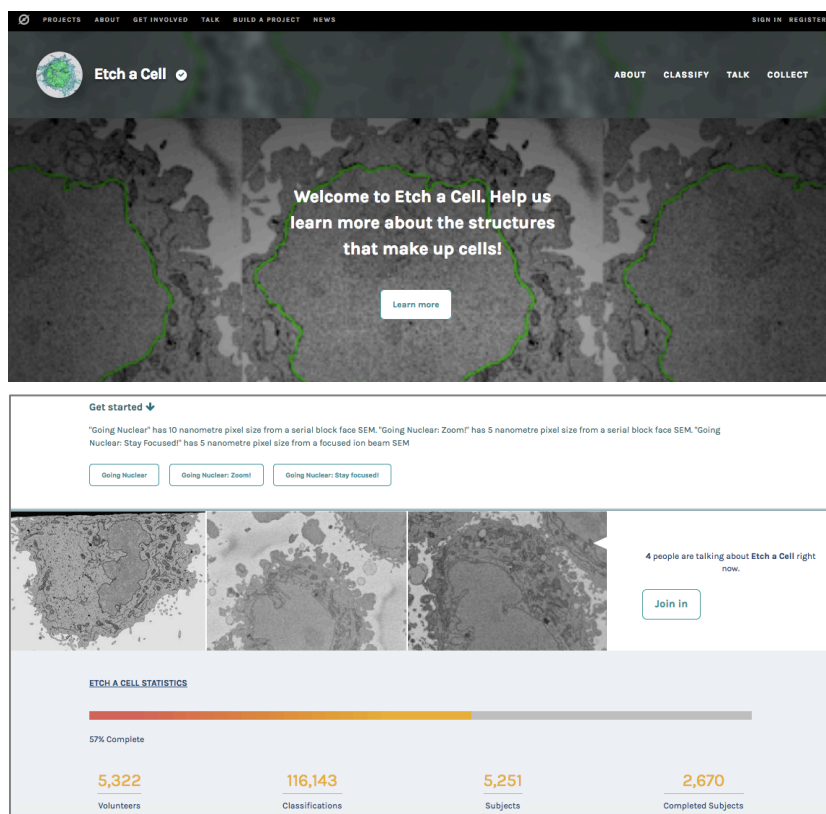


Figure 1. The Etch a Cell interface on the Zooniverse platform, which currently hosts three projects whose aim is to segment the nuclear envelope from HeLa cells imaged using SBF SEM and FIB SEM.

Quantitative super-resolution imaging with DNA-PAINT.

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DNA-PAINT is a single-molecule localisation based super-resolution approach which employs the transient interaction of dye attached oligonucleotides in solution (imager strands) with immobilised complementary sequences (docking strands) attached to target molecules by a suitable marker technology¹. A particular advantage of DNA-PAINT is its suitability for quantitative imaging based on the analysis of the kinetics of binding between imager and docking strands in a procedure termed qPAINT². qPAINT exploits that the average time between imager events binding

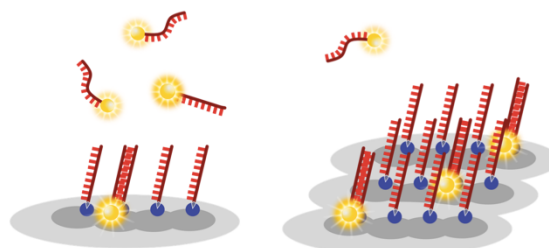


Figure 1. qPAINT analysis of DNA-PAINT. Key to the qPAINT analysis is the observation that regions containing higher numbers of docking strands will experience binding of imager strands more often. The average time between binding events is a sensitive measure that is inversely proportional to the number of docking strands.

to docking strands is inversely proportional to the number of docking strands, see also Fig. 1 (at a fixed concentration of imager strands in solution). In this presentation I will describe the principles underlying qPAINT, give examples of its application to biological samples^{3,4} and illustrate the workflow underlying successful implementation of qPAINT. This includes the use of test samples to validate a qPAINT analysis pipeline⁵ as well as strategies to calibrate qPAINT which can be challenging in biological samples. Additional practical aspects of the implementation of qPAINT include the detection (and ideally elimination) of non-specific imager binding and the blinking of imager dyes while bound to the docking strand which have the potential to distort qPAINT data. Finally, it is important to keep in mind that even fully calibrated and corrected qPAINT will only count those biomolecules in the sample that are labelled. Accordingly, the labelling efficiency is another variable that may be critical for the biological interpretation of qPAINT data.

1. Schnitzbauer, J., Strauss, M. T., Schlichthaerle, T., Schueder, F. & Jungmann, R. Super-resolution microscopy with DNA-PAINT. *Nat. Protoc.* **12**, 1198–1228 (2017).
2. Jungmann, R. *et al.* Quantitative super-resolution imaging with qPAINT. *Nat. Methods* **13**, 439–442 (2016).
3. Jayasinghe, I. *et al.* True Molecular Scale Visualization of Variable Clustering Properties of Ryanodine Receptors. *Cell Rep.* **22**, 557–567 (2018).
4. Böger, C. *et al.* Super-resolution imaging and estimation of protein copy numbers at single synapses with DNA-point accumulation for imaging in nanoscale topography. *Neurophotonics* **6**, 035008 (2019).
5. Lin, R., Clowsley, A. H., Lutz, T., Baddeley, D. & Soeller, C. 3D super-resolution microscopy performance and quantitative analysis assessment using DNA-PAINT and DNA origami test samples. *Methods* (2019). doi:10.1016/j.ymeth.2019.05.018.

Machine Learning for Computational Phenotyping: how to overcome the need for massive image annotation

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1

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Keywords: High Content Screening, RNA localization, Digital pathology, Machine Learning, Deep Learning

The phenotype of a biological system, such as a cell or an organism, is the set of its observable properties. Computational phenotyping refers to automatically inferring a quantitative description of a phenotype from images. Thanks to its inherent capacity of combining many visual aspects in a computational workflow, computer vision is the method of choice for computational phenotyping.

The most powerful computer vision method for image classification today is Deep Learning. However, one of the pre-requirements of Deep Learning is the existence of a large set of annotated samples. While such data sets can often be generated in medical imaging, where standardized images and the corresponding diagnosis are result of routine examinations, massive annotations are often unrealistic in bioimaging projects. In this talk, I will present different ways of overcoming the need for massive image annotation by the following three strategies:

- **Weak supervision:** here, we only coarsely annotate a bag of samples. For instance, we could label an entire tissue or an entire experiment, instead of labeling each individual cell or tissue tile. We can use these bag labels nevertheless to train classifiers on the individual samples.
- **Learning from simulations:** we can design simulators of microscopy images, that can generate images with known ground truth. Classifiers can be learned from the simulated images and applied to real data. This often implies the use of domain adaptation in order to bridge the gap between simulations and reality.
- **Experimental annotation:** we can design experimental workflows that provide annotations for large image sets without manual intervention. For instance, we can use specific fluorescent markers that annotate cellular phenotypes, and build a classifier able to predict these labels from bright field images.

I will show applications for each of these strategies in the fields of digital pathology, drug screening and RNA localization screening.

[1] Dubois, R., Imbert, A., Samacoits, A., Peter, M., et al. (2019). "A deep learning approach to identify mRNA localization patterns". IEEE 16th International Symposium on Biomedical Imaging (ISBI 2019), pp. 1386–1390. doi: [10.1109/ISBI.2019.8759235](https://doi.org/10.1109/ISBI.2019.8759235).

[2] Samacoits, A., Chouaib, R., Safieddine, A., Traboulsi, A.-M., et al. (2018). "A computational framework to study sub-cellular RNA localization". Nature Communications 9.1, p. 4584. doi: [10.1038/s41467-018-06868-w](https://doi.org/10.1038/s41467-018-06868-w).

[3] Naylor, P., Boyd, J., Laé, M., Rey, F., et al. (2019). "Predicting Residual Cancer Burden In A Triple Negative Breast Cancer Cohort". 2019 IEEE 16th International Symposium on Biomedical Imaging (ISBI 2019), pp. 933–937. doi: [10.1109/ISBI.2019.8759205](https://doi.org/10.1109/ISBI.2019.8759205).

[4] Naylor, P., La, M., Rey, F., and Walter, T. (2018). "Segmentation of Nuclei in Histopathology Images by deep regression of the distance map". IEEE Transactions on Medical Imaging, pp. 1–12. doi: [10.1109/TMI.2018.2865709](https://doi.org/10.1109/TMI.2018.2865709).

Label-free intracellular dynamics studies using quantitative phase imaging with a high definition wavefront sensor

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Keywords: quantitative phase imaging, vesicle tracking

The interaction between cells and their environment or between intracellular compartments are essential for the maintenance of cellular functions. These are based on complex vesicular transport processes which are crucial to understand intracellular, extracellular and intercellular communication.

Progress about intracellular trafficking is currently essentially made by constant innovation in fluorescence-based technique. They now reach a single-molecule resolution in living cells. It is possible to follow molecules all along their travel inside the cell. In this case, the main limitation is that fluorescent probes could bias the vesicle behavior and alter their transport inside the cell. Also, very fast vesicular transport of tiny vesicles from one side of the cell to the other part is usually very hard to follow because of the vesicle velocity: weak fluorescence level implies exposure times longer than the transfer time. To compensate for the weak fluorescence yield, high level of fluorescence excitation is requested but induces the so-called photobleaching effect, which inhibits fluorescence after a given number of photons are emitted. Phototoxic effects can also occur. [1]

In this paper we propose to use a label-free quantitative phase imaging modality in order to study in details the behavior of vesicles at the intracellular scale and their interaction with the cytoskeleton. Quantitative phase imaging techniques are conventionally used in microscopy, enhancing the contrast for imaging semi-transparent samples with a non-invasive (i.e. label-free) and fast approach. For instance, phase correlation imaging introduced by [2] showed that global statistics on the whole cell or a population can give an insight of motions and dynamics. The resolution and the high sensitivity brought by the High-Definition wavefront sensor, developed by Phasics and based on quadriwave lateral shearing interferometry [3,4], enables to detect a large number of vesicles while imaging whole cell at high magnification. We can use any wavelength with this imaging technique, like far red or near infrared, which is less absorbed by cells and less energetic than blue or green excitation of fluorescence. With our new detection and localization algorithm, we can thus get large content of vesicle information for all individual vesicles for all consecutive frames. Besides, the tracking algorithm provides information about the interaction between vesicles and the surrounding environment, as well as the motion type (Brownian or active transport along the cytoskeleton). We will show how we can extract information about intracellular transport from quantitative phase images series.

- [1] P. Laissue, R. Alghamdi, P. Tomancak, E. Reynaud, H. Shroff, "Assessing phototoxicity in live fluorescence imaging," *Nature Methods* 14, 657-661 (2017)
- [2] L. Ma et al., "Phase correlation imaging of unlabeled cell dynamics," *Scientific Reports* 6, 32702 (2016)
- [3] P. Bon, G. Maucort, B. Wattellier, S. Monneret, "Quadriwave lateral shearing interferometry for quantitative phase microscopy of living cells," *Optics Express* 17 (15), 13080-13094 (2009).
- [4] P. Bon, J. Savatier, M. Merlin, B. Wattellier, S. Monneret, "Optical detection and measurement of living cell morphometric features with single-shot quantitative phase microscopy," *J. Biomed. Opt.* 17 (7), 076004 (2012).

Predicting Patient Outcome from Immunohistochemistry Images by Quantifying Spatial Distribution of Immune Cells

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Keywords: spatial statistics, immunohistochemistry, cancer immunology

The spatial arrangement of different cell types is vital to determining the function of a tissue. In cancer, cytotoxic T-cells (CTLs) target and kill cancerous cells; thus, high infiltration of CTLs into cancerous cells can be indicative of patient survival. The G-function is a spatial distribution metric which , calculates the probability of finding at least one cell of type j within a radius length r of cell type i . It is expressed mathematically as $G_{x,y}(r) = Prob(\rho(x_i, X_j) \leq r)$, where $\rho = \min\{|x_i - x_j| : x_j \in X_j\}$ is the distance between a cell of type i and the nearest cell of type j . When paired with a Kaplan-Meier correction to adjust for edge effects, the G-function provides a concrete method to quantify relationships between two cell types. Using cell location data obtained from immunohistochemistry imaging, we have used the G-function to quantify the infiltration of CTLs into tumor cells, and the metric calculated from the G-function has predicted patient outcome for several different cancers[1,2,3]. While traditional G-function analysis characterizes the relationship between two cell types, the G-function can be expanded to quantify relationships among three or more cell types, allowing for a more comprehensive analysis of the tumor microenvironment.

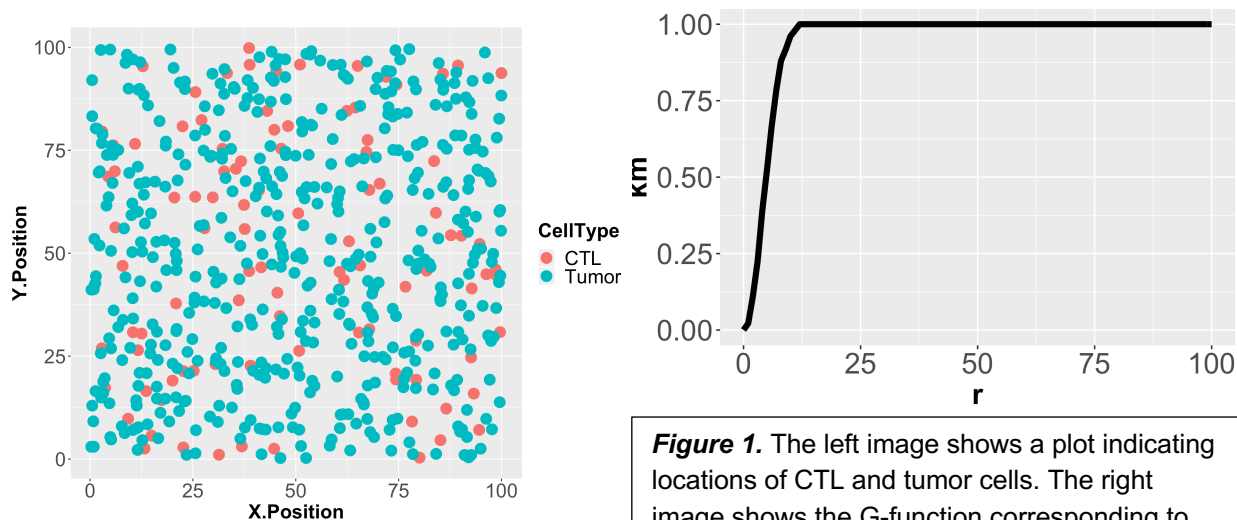


Figure 1. The left image shows a plot indicating locations of CTL and tumor cells. The right image shows the G-function corresponding to the given spatial arrangement.

- [1] Lazarus, J., Oneka, M. D., Barua, S., Maj, T., Lanfranca, M. P., Delrosario, L., ... Magliano, D. (2019). Mathematical Modeling of the Metastatic Colorectal Cancer Microenvironment Defines the Importance of Cytotoxic Lymphocyte Infiltration and Presence of PD-L1 on Antigen Presenting Cells. *Annals of Surgical Oncology*, 26(9), 2821–2830.
- [2] Barua, S., Fang, P., Sharma, A., Fujimoto, J., Wistuba, I., Rao, A. U. K., & Lin, S. H. (2018). Spatial interaction of tumor cells and regulatory T cells correlates with survival in non-small cell lung cancer. *Lung Cancer*, 117(December 2017), 73–79.
- [3] Carstens, J. L., Sampaio, P. C. De, Yang, D., Barua, S., Wang, H., Rao, A., ... Kalluri, R. (2017). Spatial computation of intratumoral T cells correlates with survival of patients with pancreatic cancer. *Nature Communications*, 8, 1–13.

Spatial Statistics Workshop

QBI 2020

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Spatial data is classed as any data that represents observations over a spatial domain. Typically, this spatial domain will be 2-dimensional but is also often 3-dimensional. Spatial data can be broadly categorised into two types: (i) data sampled over a continuous domain, (ii) event data. Examples of (i) include the air temperature recorded at a set of weather stations and air pollution levels recorded at a set of pollution sensors. Event data, on the other hand, are the locations of specific events or objects. For example, the locations of a particular species of plant in a rain forest, or the location of robberies that occurred in a particular city during some specified time window. It is the statistical analysis of event data that this workshop focuses on as these are the type of data most commonly seen in bioimaging, be it the location of single molecules or the location of clatharin coated pits.

When a set of object/event locations are treated as random it is known as a *spatial point pattern*, and rigorous statistical theory and methodology is required for correct inference. This workshop will present some of the key theory of spatial point patterns and the methodology used for exploratory data analysis. Topics covered will include: Poisson processes, complete spatial randomness, clustering and regularity, the definitions and estimators of key summary functions (including the nearest neighbour distribution, pair correlation function and Ripley's K-function), testing for complete spatial randomness, cluster model fitting and multichannel data.

Implementation of these statistical tools will centre around worked examples using the SpatStat package in R.

SMLM flight simulator workshop

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Abstract

Super resolution has enabled the study of the cellular architecture under the diffraction limit of light for the first time, while retaining the advantages of conventional fluorescence microscopy (e.g. staining specificity). Although, single molecule localisation microscopy (SMLM) leads to the biggest gain in spatial resolution (typically 10-30nm), it remains paradoxically rarely used as a routine tool in biology laboratories, and overall, suffers from the lack of reproducibility of the produced images. Both the complexity of the image acquisition with many user-defined parameters to set (e.g. intensity of the excitation/activation laser, frame rate), and the lack of guide lines to do so, contribute to these limitations. We will present during this workshop a novel easy-to-use simulation platform, or **SMLM flight simulator**, that aims at tackling both issues. This simulator runs in real-time allowing effective interactivity. It generates hundreds of images from a 3D structure defined by hundreds of thousands of points. Our flight simulator consists of a virtual microscope set up that recreates the environment of SMLM acquisition setup. The platform is designed for instance for the training of new users in producing reliable SMLM data sets. It also allows for the validation of new quantitative methods, as well as enables experienced users to fully characterised the feasibility of future experiments.

We will provide in this workshop a quick overview of the SMLM flight simulator platform, focusing on applications to a range of cellular structures. We practice simulations on given structure or on structure provided by the community.

Take away:

- For experienced SMLM users: You will be able to generate synthetic data sets with the simulator, that can be used for further **validation of your quantitative methods**.
- For new comers to SMLM: You will have experienced a **synthetic imaging session** and learn what to look for when doing an SMLM acquisition to get reliable output images.

Program of the workshop:

1: Introduction of the workflow of parameters tuning / simulation / localization / assessment

2: Discussion on well characterised biological structures that can be used for simulations (e.g. microtubules). We will try to simulate 3D structure selected by the participant.

3: Practical work:

- Installation of the SMLM flight simulator package
- Tutorial on the user interface
- Generation of SMLM synthetic data sets from a structure chosen by the participant

4: Discussion and analysis of the produced results

Requirements

No a priori knowledge is required for participation to this workshop. Basic knowledge of SMLM.

A computer with Fiji installed is required.

STATISTICAL AND COMPUTATIONAL METHODS FOR INTRACELLULAR TRAJECTORY ANALYSIS IN FLUORESCENCE MICROSCOPY

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Keywords: diffusion, classification, estimation, trajectory, MSD, tracking, statistical tests

The characterization of molecule dynamics in living cells is essential to decipher biological mechanisms and processes. This topic is usually addressed in fluorescent video-microscopy from particle trajectories computed by object tracking algorithms. However, classifying individual trajectories into predefined diffusion classes (e.g. sub-diffusion, free diffusion (or Brownian motion), super-diffusion), estimating diffusion model parameters, or detecting diffusion regime changes, is a difficult task in most cases. To address this challenging issue, we propose a computational framework based on statistical tests (with the Brownian motion as the null hypothesis) to analyze short and long trajectories, and derive spatial diffusion maps. The methodological approach is well-grounded in statistics and is more robust than previous techniques, including the Mean Square Displacement (MSD) method and variants.

In this talk, I will present the concepts and methods and focus on dynamics of biomolecules involved in exocytosis and endocytosis mechanisms, observed in total internal reflection fluorescence (TIRF) and lattice light sheet microscopy. The algorithms, dedicated to short or long trajectories, are flexible in most cases, with a minimal number of control parameters to be tuned (p-values). They can be applied to a large range of problems in cell imaging and can be integrated in generic image-based workflows, including for high content screening applications.

References:

1. V. Briane, C. Kervrann, M. Vimond. *Statistical analysis of particle trajectories in living cells*, Phys. Rev. E 97, 062121
2. V. Briane, M. Vimond, C. Valades Cruz, A. Salomon, C. Wunder, C. Kervrann. *A sequential algorithm to detect diffusion switching along intracellular particle trajectories*, Bioinformatics, btz489, 2019.
3. V. Briane, M. Vimond, C. Kervrann. *An overview of diffusion models for intracellular dynamics analysis*, Briefings in Bioinformatics, bbz052, 2019
4. V. Briane, M. Vimond, A. Salomon, C. Kervrann. *A computational approach for detecting micro-domains and confinement domains in cells: a simulation study*, 2019. (in revision)

Multimodal Mass Spectrometry Imaging of Tumours

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Introduction: Tumours are well known to be highly heterogeneous, but how heterogeneity impacts tumour progression and drug resistance remains unclear. To address this, a deeper and spatially resolved understanding of underlying metabolism is required. Mass spectrometry (MS) is one of the most powerful techniques for chemical analysis and when combined with an imaging modality allows molecules to be visualised in a spatially resolved manner, from the nano- to the macroscale. By pursuing a multiscale and multi-omics approach with a range of mass spectrometry imaging (MSI) techniques, we aim to deepen our understanding of the metabolic deregulation in cancer development and growth. Here we present early results from the Cancer Research UK Grand Challenge, pilot studies of colorectal cancer models.

Methods: Colon samples from several genetically engineered mouse models (GEMMs) as well as patient derived organoids and xenografts, were produced and processed at various sites. Samples were sectioned onto glass slides and stored at -80 °C until use. MALDI-MSI was carried out on a rapifleX ToF-ToF and a Synapt G2-Si. DESI-MSI was carried out on a Synapt G2-Si or Xevo G2-XS or an Orbitrap Q-Exactive. SIMS analysis was carried out on a ToF-SIMS V hybrid orbitrap. Data was analysed in MATLAB (2017a) via the SpectralAnalysis software package, using a range of existing and novel computational methods including non-negative matrix factorisation (NMF), t-distributed stochastic neighbour embedding (t-SNE) as well as k-means and two-phase graph cuts clustering methods.

Results: The multisite, multimodal datasets collected, enable objective assessment of preferential molecular detection of metabolites and lipids between sites, techniques, polarities and mass analysers. This provides insights into the relative sensitivity of different techniques to particular molecular classes and enables studies to be targeted towards specific protocols or instruments that provide optimal coverage of metabolic pathways of interest. A summary of the multimodal method combinations and pathways implicated in various models will be presented.

Statistical analysis and modeling of single particle trajectories to reveal the flow and dynamics of the ER

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Keywords: SPTs, Modeling, stochastic processes, Endoplasmic reticulum

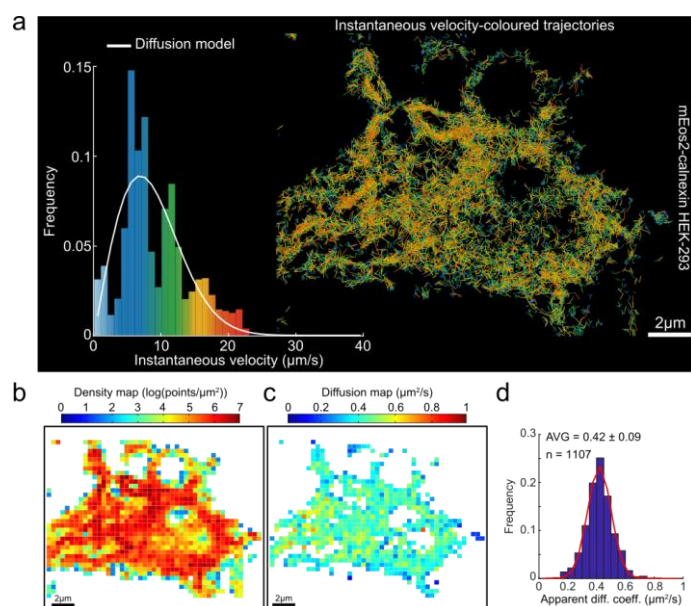


Figure 1.: reconstruct of the ER from single particle trajectories (SPTs). a: color map according to the amplitude of velocity. b density map, c diffusion map, d apparent diffusion coefficient.

Modeling single particle trajectories can be used to reveal the organization of subcellular compartments at a nanoscale resolution. We recently showed that diffusion and active motion contribute to protein delivery in the endoplasmic reticulum (ER) network, composed of membranous sheets and tubules, regulating the delivery of proteins. In this talk, I will present modeling and analysis of network properties based on a unidirectional transport mechanism, extracted from large ensemble of trajectory data. The modeling is based on stochastic processes and the concept of active graph. [1,2,3]

[1] D Holcman, P Parutto, JE Chambers, M Fantham, LJ Young, SJ Marciniak, ... [Single particle trajectories reveal active endoplasmic reticulum luminal flow](#), Nature cell biology 20 (10), 1118

[2] P Parutto, J Heck, M Heine, D Holcman, [Reconstructing wells from high density regions extracted from super-resolution single particle trajectories](#) bioRxiv, 642744

[3] M Dora, D Holcman [Active unidirectional network flow generates a packet molecular transport in cells](#) arXiv preprint arXiv:1810.07272

Effect of pixelation on the parameter estimation of single molecule trajectories

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KEY WORDS: single molecule tracking, pixelated detectors, maximum likelihood estimation, Fisher information matrix, Cramér-Rao lower bound.

The advent of single molecule microscopy enables the tracking of single molecules in cellular environments. Estimation of the parameters of the model that describes the molecule trajectory in such environments is a critical task in the analysis of single molecule tracking data. In [1], we studied the parameter estimation of single molecule trajectories for the fundamental data model, in which we had an ideal unpixelated detector and the measurements were the time points and locations of the detected photons. In practice, pixelated detectors, e.g., charge-coupled device (CCD) and electron multiplying CCD (EMCCD) cameras are used in fluorescence microscopy, and therefore, the time points and exact locations of detection of the photons are not available. In this case, the only available information is the pixel areas in which the photons impact the detector. Hence, the parameter estimation of single molecule trajectories from pixelated images are not a trivial problem. The majority of available methods model the effect of pixelation by using an additive noise in the fundamental data model. However, in general, this approximation does not describe the underlying stochastic model precisely.

Here, we investigate the effect of pixelation on the parameter estimation of single molecule trajectories. In particular, we develop a stochastic framework to calculate the maximum likelihood estimates of the parameters of the general motion of the molecule in living cells. We also calculate the Cramér-Rao lower bound (CRLB), given by the inverse of the Fisher information matrix, on the variance of the parameter estimates. We apply the developed methodology to simulated single molecule trajectory data and show that, even in cases that we have a small number of photons detected, the parameters of the molecule trajectory are estimated accurately.

[16] M. R. Vahid, B. Hanzon, and R. J. Ober, "Fisher information matrix for single molecules with stochastic trajectories," *SIAM Journal on Imaging Science* (under review), arxiv.org/abs/1808.02195.

Implementation of multiplexing and image analysis to support drug discovery and development

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Key words: Fluorescence, Image Analysis, TSA, Multiplex

In histology development of more stable fluorescent dyes, signal amplification and new labelling methods supports the application of multiplexing to drug development.

New methods require new validation steps and a case by case selection of the most appropriate technology. In our lab we evaluated different multiplexing technologies (classical fluorescence, TSA, DNA barcode,...) and selected fit for purpose assays for different projects.

Two projects where multiplexing has been combined with image analysis for research and early development will be presented as case examples. The validation steps for the staining establishment and quality checks to move forward with image analysis will be described.

In the early development phase, fluorescent image analysis is widely used on rodent tissue to explore the mechanism of action of test compounds. In our example we used a four color fluorescent panel to assess in the postmortem brain of PS2/APP transgenic mice interaction of microglia with A β plaques.

In immunology the subtyping of immune cells is a key information and often use as an efficacy biomarker or a toxicology readout. In this second example, we will describe 2 B cell phenotyping panels established on human tissue for further use in cynomolgus monkey.

3D parallelized RESOLFT optical nanoscopy

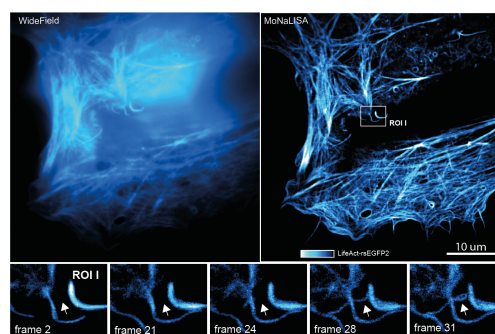
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Keywords: 3D super-resolution fluorescence microscopy, switchable proteins, live super resolution, Invited Minisymposium speaker: Volumetric Imaging. Organizer: Martin Booth.

In recent years, the development of parallelized STED/RESOLFT super resolution microscopy techniques have shown impressive results combining diffraction unlimited lateral spatial resolution (< 70 nm) with increasingly fast acquisition speeds (~ 1 second) and optical sectioning. However, the diffraction-unlimited resolution improvement in these systems are always limited to the lateral dimension. Here we present a novel design of illumination patterns that allows for 3-dimensional isotropic diffraction unlimited resolution while maintaining the rapid recording speed of a parallelized imaging system. Much like in the original RESOLFT-MoNaLISA set-up [1] the system is based on controlling the spatial distribution of ON-OFF states in a sample labelled with reversibly switchable fluorescent proteins (RSFPs) [2]. Using RSFPs as labels allows for high resolution imaging at minimal light doses and is thus very suitable for live cell imaging. Designing illumination patterns that are modulated in all three spatial dimensions and combining these with the complete OFF-switching of RSFP allow to confine the emission spots in all the three spatial dimensions to sub-diffraction sized. The extended frequency content in the emission pattern manifests itself as isotropic diffraction unlimited resolution in the final images.



The spatial modulation in all three dimensions is made possible by the incoherent superposition of several independent illumination patterns, each pattern highly modulated in one spatial direction. Proper co-alignment of these patterns results in sharply confined zero intensity volumes that, together with saturation of the fluorophore OFF-state, can create sub-diffraction sized emission volumes. Quantification and reassignment of the emitted photons from these volumes allows for reconstruction of the final isotropic super resolution images. We recorded 3D stacks across time of moving organelles and cytoskeleton in living human cells with spatial details < 100 nm in all the three dimensions.

[1] Masullo L A, Boden A, Pennacchietti F, Coceano G, Ratz M and Testa I 2018 Enhanced photon collection enables four dimensional fluorescence nanoscopy of living systems *Nature communications* **9**

[2] Pennacchietti F, Serebrovskaya E O, Faro A R, Shemyakina, II, Bozhanova N G, Kotlobay A A, Gurskaya N G, Boden A, Dreier J, Chudakov D M, Lukyanov K A, Verkhusha V V, Mishin A S and Testa I 2018 Fast reversibly photoswitching red fluorescent proteins for live-cell RESOLFT nanoscopy *Nature methods* **15** 601-4

A BOLT-ON SINGLE-OBJECTIVE LIGHT-SHEET DESIGN WITH UNCOMPROMISED NUMERICAL APERTURE

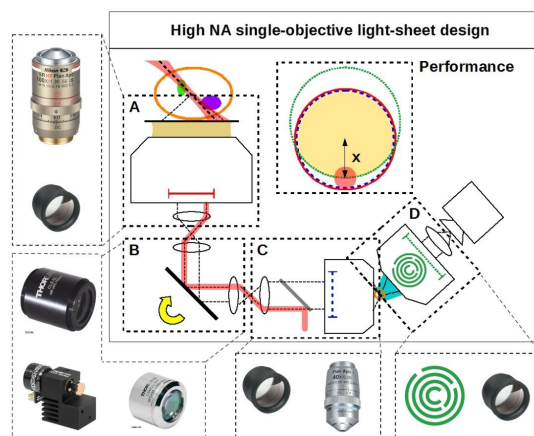
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Keywords: Invited Minisymposium speaker: Volumetric Imaging. Organizer: Martin Booth.

Spinning disk confocal modules are “core facility friendly”; they insert conveniently between a commercial microscope base and camera, improve image quality and add no significant drawbacks. In contrast, high numerical aperture (NA) light sheet microscopy often requires radical sample modification, substantial user re-training and fully customized hardware. We present a “core-facility friendly” light-sheet: a “black box” that inserts between a commercial microscope base and camera, greatly reducing photo-toxicity without degrading image quality or breaking compatibility with existing sample preparation.



In 2008, based on the ingenious ‘Remote-Refocus’ of E.J. Botcherby and T. Wilson [Botcherby 2007], C. Dunsby [Dunsby 2008] invented a brilliant single-objective light sheet technique called ‘Oblique Plane Microscopy’ (OPM) that bypassed many typical light sheet drawbacks. However his theoretical NA of 0.74 for a water immersion objective was significantly lower than the 1.33 limit for an aqueous sample. In 2018 B. Yang and B. Huang [Yang 2018] made a clever modification to OPM to achieve an NA of 1.06 and incorporated the elegant galvo-based volumetric scanning method invented by M.B. Bouchard and E.M.C. Hillman [Bouchard 2015].

Building on this work, we present a simple, robust and modular light sheet design with an NA in the 1.2-1.3 range, and discuss the key concepts and considerations for high NA single objective light sheet.

[Botcherby 2007] An optical technique for remote focusing in microscopy; E.J. Botcherby, R. Juškaitis, M.J. Booth and T. Wilson; Optics Communications, vol 281(4), p880-887, (2007) <https://doi.org/10.1016/j.optcom.2007.10.007>

[Dunsby 2008] Optically sectioned imaging by oblique plane microscopy; C. Dunsby; Optics Express, vol 16(25), p20306-20316, (2008) <https://doi.org/10.1364/OE.16.020306>

[Bouchard 2015] Swept confocally-aligned planar excitation (SCAPE) microscopy for high-speed volumetric imaging of behaving organisms; M.B. Bouchard, V. Voleti, C.S. Mendes, C. Lacefield, W. B. Grueber, R.S. Mann, R.M. Bruno and E.M.C. Hillman; Nature Photonics, vol 9, p113-119, (2015) <https://doi.org/10.1038/nphoton.2014.323>

[Yang 2018] High Numerical Aperture Epi-illumination Selective Plane Illumination Microscopy; B. Yang, Y. Wang, S. Feng, V. Pessino, N. Stuurman and B. Huang; bioRxiv, (2018) <https://doi.org/10.1101/273359>

Imaging the nanoscale structure of endocytosis with correlative super-resolution light and electron microscopy.

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Keywords: Invited Minisymposium speaker: correlative microscopy, super-resolution imaging, electron microscopy, endocytosis.

The plasma membrane separates the cell's interior from the outside world. The exchange of signals and materials across this barrier is regulated by a multitude of channels, transporters, receptors, and trafficking organelles. Mapping the molecular structure and dynamics of the plasma membrane is key to understanding how human cells function in health and malfunction in disease. Electron microscopy can produce high resolution images of the membrane. Historically, it has been challenging to locate and identify proteins within these images. Recently-developed super-resolution localization microscopy, however, can image fluorescently-labeled molecules with better than 20 nm precision. We developed a correlative super-resolution light and EM method (CLEM) that couples these complementary methods to produce images where identified proteins are mapped within the dense native structure of the cell at the molecular scale. This correlative method is uniquely suited to determine the nanometer-scale organization of the plasma membrane and associated organelles.

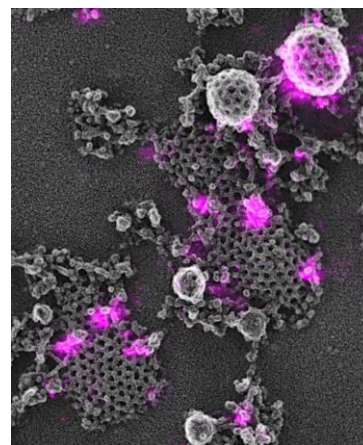


Figure 1. Correlative super-resolution light and platinum Replica EM image of dynamin at single clathrin coated pits in a HeLa cell.

Clathrin-mediated endocytosis is the primary mechanism human cells use to internalize receptors, nutrients, hormones, and other cargo at the plasma membrane. It is fundamental to the function of cells and tissues. Using our CLEM method, we studied 19 key proteins involved in clathrin-mediated endocytosis. Our data provides a comprehensive molecular architecture of endocytic vesicles in human cells (Figure 1). We discovered that key regulatory and cargo proteins distribute into distinct nanoscale spatial zones; inside, outside, and at the edge of the clathrin coat in human cells. The presence and amount of many factors within these zones change during vesicle growth. From these data we present a model that the formation and curvature of single endocytic vesicles is driven by the recruitment, re-organization, and loss of proteins within these distinct nanoscale zones.

Currently, gaps exist between understanding protein structures and their dynamic cellular contexts. Our studies aim to fill these gaps by developing and using new ultra-high resolution imaging tools to determine the nanoscale structures, organizations, and dynamics of molecules that are important for membrane traffic in neurons, endocrine, and immune cells. As we piece together structures in their specific cellular contexts, a new holistic understanding of how biological processes work will be gained. These studies will help to map the fundamental architecture of cellular machines to better understand how these complex assemblies function in healthy cells and malfunction in disease.

Mesoscale imaging with the Mesolens

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Keywords: Mesoscopy, fluorescence, confocal, widefield, 3D

For more than a century, the design of low-magnification microscope objectives has been guided by the angular acuity of the human eye (approximately 1 second of arc). At x4 magnification, this requires a numerical aperture no greater than 0.1 or 0.2, which can be achieved cheaply and easily by simple optical designs. With the advent of confocal and multiphoton microscopy, however, it became apparent that the poor axial resolution of more than 30 microns with low magnification objectives was intolerable for these 3D methods.

To overcome this, we have developed a new and complex objective with a magnification of 4x and an NA of just less than 0.5 which we call the Mesolens. We specified this lens for mammalian embryology, and have shown that it can image every cell of a 6mm-long embryo 3mm thick with sub-cellular resolution if the tissue is cleared appropriately. A by-product of the high NA is that the optical throughput is approximately 20x greater than a conventional 4x objective. The pupil size of the lens is so great that it cannot be used with a conventional microscope frame, so we have built the imaging system around the lens, and use either wide-field camera or point-scanning fluorescence detection to create images.

I will present an overview of the Mesolens technology, and I will give some new and emerging examples of the application of this instrument in biomedical research.

Multiplex imaging of the tumour microenvironment with CODEX technology

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Keywords: CODEX, tumour imaging, neighbourhood analysis. Minisymposium speaker: Tissue multiplexing session.

CODEX (for CO-Detection by indEXing) is a novel multiplex imaging method, which allows for simultaneous detection and quantifications of up to 55 epitopes from a single tissue section. Marker detection is achieved by staining the tissues with cocktail of antibodies and subsequent visualisation of 3 markers with fluorescent reporters in each cycle until all data is collected. CODEX enables acquisition of a large number of markers while retaining the spatial context of the staining from the single tissue specimen. This is followed by a computational analysis, which consist of image processing and cytometric analysis. Image processing is performed by Codex Analysis Manager software and includes such steps as best focus definition, drift compensation, image cropping and stitching, flat field correction, deconvolution, segmentation and cytometric signal quantification. Cytometric analysis is achieved with Multiplex Analysis Viewer, which is a plug-in for Fiji developed by CODEX programmers. It consists of unsupervised clustering, cell annotation, phenotype mapping and neighbourhood analysis. The strength of CODEX technology in tumour imaging comes from possibility to look at complexity of all immune cell subsets *in situ* alongside tumour cells and the opportunity for unsupervised analysis and quantification of cellular interactome. We are investigating mechanism of anti-tumour immunity in both mouse tumour models and cancer patients.

We would like to open a discussion on several technical aspects and analysis challenges of this multiplex technology, such as statistical quantification, comparable and consistent marker quantification between tissues from different patients/animals and possible algorithms for neighbourhood analysis.

Quantitative Multicolor Super-Resolution Microscopy

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Keywords: Single Molecule Localization Microscopy, Colocalization, Tracking, Spectral Imaging, STED Microscopy.

Single Molecule Localization Microscopy techniques such as (spt)PALM, (u/DNA)PAINT, (d)STORM and quantum dot tracking have given unprecedented insight into molecular organization and dynamics in fixed and living cells. They allow monitoring the nano-organization and molecular interaction of individual proteins at millisecond temporal resolution and high spatial resolution (<30 nm) by fitting the point spread function (PSF) of individual emitters and tracking their position over time. By comparison, STED microscopy is very powerful at revealing the nanoscale cellular morphology, using fluorophores diffusing in the cytosol of extra-cellular space.

Here, I will present our recent analytical and microscopy developments allowing pushing forward the limits of quantitative multicolor super-resolution microscopy, and more particularly: i) a single molecule tracking technique that allows for simultaneous 3D single particle tracking of multiple distinct species without compromising spatio-temporal resolution, including advanced data analysis that can fully exploit the 5-dimensional data (x,y,z,t,λ) [1]; ii) A correlative super-resolution imaging platform allowing monitoring protein organization and dynamics within their cellular context [2].

[1] Levet, F., Julien, G., Galland, R., Butler, C., Beghin, A., Chazeau, A., Hoess, P., Ries, J., Giannone, G., Sibarita, J.B., A tessellation-based colocalization analysis approach for single-molecule localization microscopy, *Nature communications*, 10 (2019) 2379.10.1038/s41467-019-10007-4

[2] Inavalli, V., Lenz, M.O., Butler, C., Angibaud, J., Compans, B., Levet, F., Tonnesen, J., Rossier, O., Giannone, G., Thoumine, O., Hosy, E., Choquet, D., Sibarita, J.B., Nagerl, U.V., A super-resolution platform for correlative live single-molecule imaging and STED microscopy, *Nat Methods*, DOI 10.1038/s41592-019-0611-8(2019).10.1038/s41592-019-0611-8

Bayesian Inference of the Spatial Distribution of Proteins from Three-Cube Förster Resonance Energy Transfer Data

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Keywords: Invited Minisymposium speaker: Spatial Statistics in Microscopy. Organizer: Ed Cohen.

Förster resonance energy transfer (FRET) is a quantum-physical phenomenon where energy may be transferred from one molecule to a neighbour molecule if the molecules are close enough. Using fluorophore molecule marking of proteins in a cell it is possible to measure in microscopic images to what extent FRET takes place between the fluorophores [1]. This provides indirect information of the spatial distribution of the proteins. Questions of particular interest are whether (and if so to which extent) proteins of possibly different types interact or whether they appear independently of each other.

In this presentation we propose a new likelihood-based approach to statistical inference for FRET microscopic data. The likelihood function is obtained from a detailed modeling of the FRET data generating mechanism conditional on a protein configuration. We then follow a Bayesian approach and introduce a spatial point process prior model for the protein configurations depending on hyper parameters quantifying the intensity of the point process. Posterior distributions are evaluated using Markov chain Monte Carlo. We propose to infer microscope related parameters in an initial step from reference data without interaction between the proteins. The new methodology is applied to simulated and real data sets [2].

[1] Lakowicz, J. R., Principles of fluorescence spectroscopy. Springer, 2009.

[2] Hooghoudt, J.O, Barroso, M, Waagepetersen, R., The *Annals of Applied Statistics*, Towards Bayesian Inference of the Spatial Distribution of Proteins from Three-Cube Förster Resonance, vol. 11, no. 3, 2017.

Quantitative super-resolution imaging in biology

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Keywords: super-resolution microscopy, DNA origami, protein stoichiometry

Protein stoichiometry is highly important for protein function. Changes from monomeric to oligomeric stoichiometry can often be the difference between healthy and diseased function. However, quantifying protein copy number with high spatial resolution is often challenging. Super-resolution methods hold promise for overcoming this hurdle, since the single molecule information obtained during the imaging can be leveraged to extract quantitative information. However, the quantification is confounded by the photophysics of the fluorophores used as well as the unknown labeling stoichiometry (in the case of primary and secondary antibody labeling). My lab has been developing nanotemplates and calibration standards that can be used to extract quantitative information on protein stoichiometry at the nanoscale. I will describe these nanotemplate based approaches and highlight biological applications.

- [1] N. Durisic, L.L. Cuervo, A.S. Alvarez, J. Borberly and M. Lakadamyali, *Nature Methods*, 11, 156-162 (2014)
- [2] F. C. Zanicchi, C. Manzo, A. S. Alvarez, N. D. Derr and M. Lakadamyali, *Nature Methods*, 14, 789-792 (2017)
- [3] F. C. Zanicchi, C. Manzo, R. Magrassi, N. D. Derr and M. Lakadamyali, *Biophysical Journal*, 116, 2195-2203 (2019)

Live-cell correlative microscopy of single organelles

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Keywords: Correlative Microscopy, Live-cell Imaging, Volume Electron Microscopy

The function of intracellular organelles is intrinsically determined by their structure. Understanding these structure-function relationships requires an integrated approach in which the spatio-temporal localization and activation of molecules is directly linked to sub-organellar membrane organization. In the last century new techniques in light microscopy (LM) and electron microscopy (EM) have led to a choice of imaging techniques that target specific molecular, functional or ultrastructural parameters. However, no microscope can yet provide a complete read-out of all parameters simultaneously. Our current understanding of the fundamental processes of life is therefore largely based on averages of dynamic, functional data from LM studies and averages of ultrastructural data from EM studies. The links between these imaging modalities are mostly indirect, which causes a major gap in our knowledge on the functional organization of individual organelles.

Correlative live-cell imaging - electron microscopy (live cell-CLEM) approaches provide elegant means to directly link molecular composition and dynamic behavior of organelles to their morphology at nanometer resolution. A major challenge however, is to retrace single, especially small, organelles in 3 dimensions (3D). Mastering this challenge, we have recently presented an approach where we have

used live-cell volume CLEM to link the dynamics and ultrastructural context of individual, highly dynamic organelles [1]. We show that live-cell imaged organelles can be reliably retraced in 3D EM data, and fusion, fission and trafficking dynamics of single organelles can be linked to their ultrastructure.

We extend the approach to simultaneously image multiple functional probes in live cells and convert these data to EM. The method integrates subcellular dynamics, functional character, and ultrastructure onto a single organelle, is promising to address also many biological questions related to membrane trafficking, organelle transport, and biogenesis.

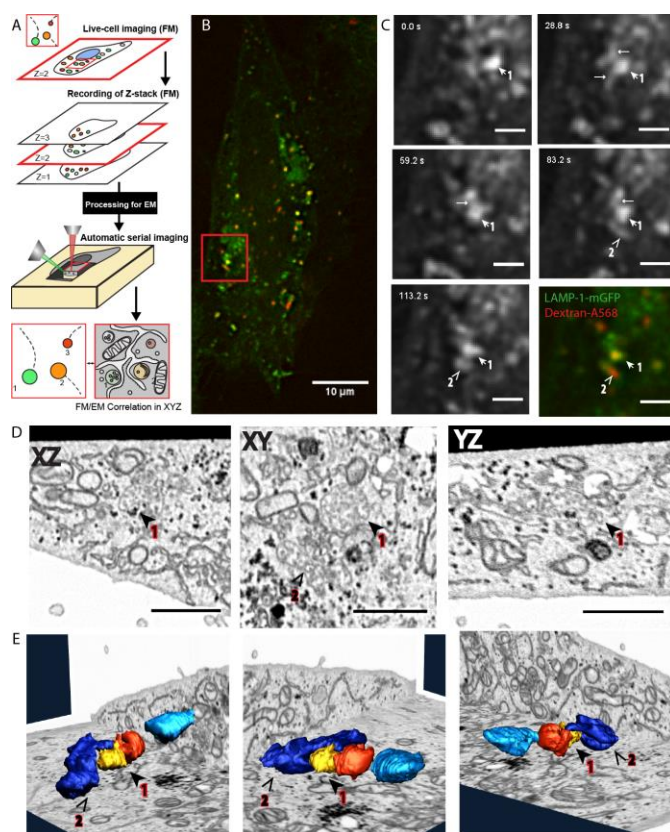


Figure 1. Volume-CLEM providing a direct link between live cell dynamics and 3D ultrastructure on the single organelle level [2].

[1] J. Fermie *et al.*, "Single organelle dynamics linked to 3D structure by correlative live-cell 3D electron microscopy," *Traffic*, Feb. 2018.

[2] T. Ando *et al.*, "The 2018 correlative microscopy techniques roadmap," *J. Phys. D. Appl. Phys.*, vol. 51, no. 44, p. 443001, Nov. 2018.

Introduction to the minisymposium on tissue multiplexing – is more the merrier or messier?

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Tissue multiplexing techniques represent a relatively new technical advancement that enables the labeling of several biomarkers within a single tissue section. The recent past has witnessed an explosive growth of multiplexing techniques that leverage different sample labeling schemes and readout formats. At present, multiplexing techniques can detect anywhere from 4 to 60 biomarkers within a single tissue section, generating an unprecedented level of data. Common to all multiplexing techniques is the image analysis workflow which converts the high dimensional raw data into useful information (i.e., number, location and phenotypes of different cells).

The goal of the minisymposium is to showcase the recent developments in tissue multiplexing technologies and the underlying image analysis problems. In this introductory talk, a brief overview of the different multiplexing technologies will be presented along with some of the challenges in the analysis of multiplex images.

Counting by Photon Statistics

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In recent years, interest in the quantification of protein copy numbers in cellular protein complexes steadily increased [1]. Since the first successful emitter number quantification by photon antibunching [2] it has become clear that single photon coincidence measurements can be a powerful tool for molecular counting as they provide an absolute number estimation. In the meantime, these coincidence measurements have been extended to higher order coincidences [3] and mapping of emitter numbers in confocal and STED microscopy [4]. Additionally, a complementary approach recently showed that coincidence information can also be used for resolution enhancement beyond the diffraction limit [5]. Together, these developments demonstrate that photon coincidence information can enhance fluorescence microscopy and aid in developing it towards a fully quantitative method without need of calibration.

As part of the workshop on quantitative microscopy, we will give a detailed introduction into photon statistics based molecular counting with the idea of giving potential users an overview of the state of the art. The sample requirements, especially regarding labeling, will be discussed with the goal to highlight the potential of the technique as well as its current limitations. To do so we present extensive benchmarks using synthetic data and experimental results from standard samples with a known stoichiometry.

[1] Grußmayer et al., *Meth. Appl. Fluoresc.*, **7**, 012003 (2019)

[2] Weston et al., *Anal. Chem.*, **74**, 5342-5349 (2001)

[3] Ta et al., *Laser Phys.* **20** (2010)

[4] Ta, et al., *Nature Communications* **6**, (2015)

[5] Tenne et al., *Nature Photonics* **13**, (2019)

Multiplex immunohistochemistry and transcriptomic data

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Keywords: transcriptome, RNA-seq, multiplex immunohistochemistry, MIBI, Cancer

Invited Minisymposium speaker: Tissue multiplexing session. Organizer: Sripad Ram/Jens Rittscher.

Understanding the interactions between tumor and the tumor microenvironment is an important area of cancer research, especially given the increasing use of immunotherapies. There are numerous approaches to identifying cell types in the context of the tumor microenvironment. These include examining regions of interest with *in situ* techniques and identifying individual cell types using antibody directed approaches. These approaches generate information on what cells are present in the tumor microenvironment. In some instances, spatial relationships can be examined. These approaches generally treat the cell types as static entities. The assumption being that the presence of a cell type indicates a specific biologic response. It is generally ignored that different cell types may have different cell states or transcriptomic responses to different environments.

Combining multiplex immunohistochemistry data with transcriptomic data can address some of these issues. Spatially oriented multi-layered data can generate both new findings and provide confirmation. Our group explores these data through the Breast PreCancer Atlas. We examine a large cohort of ductal carcinoma in situ samples with clinical context. We combine the multiplex ion beam imaging (MIBI) with SMART-3seq, a laser capture microdissection-directed transcriptional profiling method. Using established bioinformatic pipelines, we can use the transcriptomic data to assess cell type composition of tumor microenvironment and tumor samples and compare these findings to the multiplex immunohistochemistry data. We can also predict changes in cell types gene expression patterns. These combination assay approaches create datasets that can generate novel observations and invite the creation of new analytic tools.

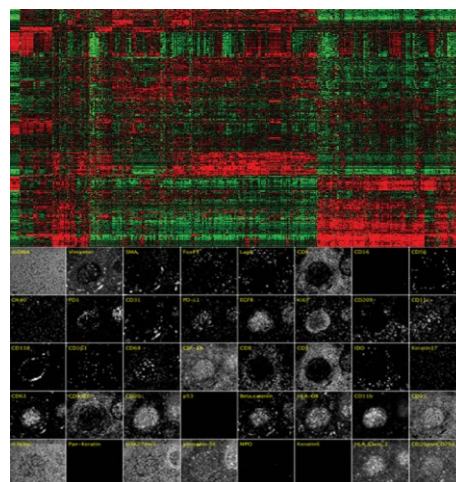


Figure 1. Gene expression profiling matched with multiplex immunohistochemistry on a cohort of 400 samples of cancer and tumor microenvironment.

Eukaryotic organelles: deciphering their interdependency, structure and dynamics with new imaging technologies

Jennifer Lippincott-Schwartz, Janelia Research Campus, HHMI, Ashburn, VA

Powerful new ways to image the internal structures and complex dynamics of cells are revolutionizing cell biology and bio-medical research. In this talk, I will focus on how emerging fluorescent technologies are increasing spatio-temporal resolution dramatically, permitting simultaneous multispectral imaging of multiple cellular components. In addition, results will be discussed from whole cell milling using Focused Ion Beam Electron Microscopy (FIB-SEM), which reconstructs the entire cell volume at 4 voxel resolution. Using these tools, it is now possible to begin constructing an “organelle interactome”, describing the interrelationships of different cellular organelles as they carry out critical functions. The same tools are also revealing new properties of organelles and their trafficking pathways, and how disruptions of their normal functions due to genetic mutations may contribute to important diseases.

Supramolecular attack particles: a new cytotoxic biomaterial

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Keywords: cytotoxicity, lymphocytes, immunological synapse

T cells communicate through direct cell-cell contact across gaps of only 13 nm (immunological synapse) (1) through secreted cytokines or cytolytic proteins (2). I will present data from my lab on the role of CD2 expression in immunological synapse structure and on an intermediate mode of communication based on T cell export of vesicles and non-vesicular particles to antigen presenting cells across immunological synapses. I will present data that CD2-CD58 interactions amplify TCR signals and the low CD2 expression has a similar impact to engaging PD1 on signaling parameters (<https://www.biorxiv.org/content/10.1101/589440v1>). Vesicles that are generated in the immunological synapse by budding from the plasma membrane into the extracellular space are referred to as synaptic ectosomes (SYNECT) (3-5). SYNECTs combine both antigen recognition and effector function and thus may maintain specificity even if they escape the initial synaptic interaction. In contrast, cytotoxic CD8⁺ T cells release non-lipid bilayer enveloped cytotoxic microparticles into the synaptic cleft (Balint, Fisher, Kessler, Harkiolaki and Dustin, in preparation). SMAPs are held together by multiple non-covalent protein-protein and protein-carbohydrate interactions and are referred to as supramolecular attack particles (SMAPs). SMAPs lack TCR, but appear to incorporate innate recognition molecules. The composition, structure, function and potential applications of SYNECTs and SMAPs will be discussed.

Five Selected Publications

1. Grakoui A, Bromley SK, Sumen C, Davis MM, Shaw AS, Allen PM, Dustin ML. The immunological synapse: a molecular machine controlling T cell activation. *Science*. 1999;285(5425):221-7. PubMed PMID: 10398592.
2. Somersalo K, Anikeeva N, Sims TN, Thomas VK, Strong RK, Spies T, Lebedeva T, Sykulev Y, Dustin ML. Cytotoxic T lymphocytes form an antigen-independent ring junction. *J Clin Invest*. 2004;113(1):49-57. PubMed PMID: 14702108.
3. Choudhuri K, Llodra J, Roth EW, Tsai J, Gordo S, Wucherpfennig KW, Kam LC, Stokes DL, Dustin ML. Polarized release of T-cell-receptor-enriched microvesicles at the immunological synapse. *Nature*. 2014;507(7490):118-23. Epub 2014/02/04. doi: 10.1038/nature12951. PubMed PMID: 24487619
4. Papa I, Saliba D, Ponzoni M, Bustamante S, Canete PF, Gonzalez-Figueroa P, McNamara HA, Valvo S, Grimbaldston M, Sweet RA, Vohra H, Cockburn IA, Meyer-Hermann M, Dustin ML, Doglioni C, Vinuesa CG. TFH-derived dopamine accelerates productive synapses in germinal centres. *Nature*. 2017;547(7663):318-23. doi: 10.1038/nature23013. PubMed PMID: 28700579
5. Saliba DG, Cespedes-Donoso PF, Balint S, Compeer EB, Korobchevskaya K, Valvo S, Mayya V, Kvalvaag A, Peng Y, Dong T, Tognoli ML, O'Neill E, Bonham S, Fischer R, Kessler BM, Dustin ML. Composition and structure of synaptic ectosomes exporting antigen receptor linked to functional CD40 ligand from helper T-cells. *eLife*. 2019;8:600551. Epub 2019/08/31. doi: 10.7554/eLife.47528. PubMed PMID: 31469364.

Persistent Homology for the Spatial Characterisation of Tumour Blood Vessel Networks

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Keywords: Tumour vasculature, persistent homology, spatial networks

The formation of blood vessels in tumours, tumour-induced angiogenesis, has long been recognised as one of the hallmarks of cancer [1]. Tumours not only rely on vasculature for the delivery of nutrients, cells, and oxygen, but blood vessels also provide physical support, serve as a starting point for metastasis, form a niche for tumour stem cells and even ensure immune suppression around the tumour. Structurally, tumour blood vessels form a chaotic network of highly inefficient blood vessels characterised by many loops and twists. Even though these features are obvious to the human eye, quantifying them has so far been very difficult. Methods from topological data analysis can characterise the “shape” of (high-dimensional) data by studying properties such as connectedness and holes. In recent years these methods have become more accessible with many available software packages [2]. Here, we show how persistent homology (PH), a method from topological data analysis which considers data at different scales, can be used to characterise structural features of tumour blood vessels. Our approach [3] can spatially capture loops in tumour blood vessel networks and their changes over time in response to treatment with radiotherapy and vascular targeting agents. We further find features connected to tortuosity of blood vessels which follow trends similar to the loops for different treatments.

- [1] Douglas Hanahan and Robert A. Weinberg, *Hallmarks of cancer: The next generation*, Cell **144**, 2011: 646 - 674.
- [2] Nina Otter, Mason A. Porter, Ulrike Tillmann, Peter Grindrod and Heather A. Harrington, *A roadmap for the computation of persistent homology*, European Physical Journal - Data Science **6**(17), 2017: 1 - 38.
- [3] Helen M. Byrne, Heather A. Harrington, Ruth Muschel, Gesine Reinert, Bernadette J. Stolz and Ulrike Tillmann, *Topology characterises tumour vasculature*, Mathematics Today **55**(5), 2019: 206 - 210.



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Abstract, Prof. Bernd Bodenmiller

“Highly multiplexed imaging in health and disease by imaging mass cytometry”

Cancer is a tissue disease. Heterogeneous cancer cells and normal stromal and immune cells form a dynamic ecosystem that evolves to support tumor expansion and ultimately tumor spread. The complexity of this dynamic system is the main obstacle in our attempts to treat and heal the disease. The study of the tumor ecosystem and its cell-to-cell communications is thus essential to enable an understanding of tumor biology, to define new biomarkers to improve patient care, and ultimately to identify new therapeutic routes and targets.

To study and understand the workings of the tumor ecosystem, highly multiplexed image information of tumor tissues is essential. Such multiplexed images will reveal which cell types are present in a tumor, their functional state, and which cell-cell interactions are present. To enable multiplexed tissue imaging, we developed imaging mass cytometry (IMC). IMC is a novel imaging modality that uses metal isotopes of defined mass as reporters and currently allows to visualize over 50 antibodies and DNA probes simultaneously on tissues with subcellular resolution. In the near future, we expect that over 100 markers can be visualized. We applied IMC for the analysis of breast cancer samples in a quantitative manner. To extract biological meaningful data and potential biomarkers from this dataset, we developed a novel computational pipeline called histoCAT geared for the interactive and automated analysis of large scale, highly multiplexed tissues image datasets. Our analysis reveals a surprising level of inter and intra-tumor heterogeneity and identify new diversity within known human breast cancer subtypes as well as a variety of stromal cell types that interact with them. In summary, our results show that IMC provides targeted, high-dimensional analysis of cell type, cell state and cell-to-cell interactions within the TME at subcellular resolution. Spatial relationships of complex cell states of cellular assemblies can be inferred and potentially used as biomarkers. We envision that IMC will enable a systems biology approach to understand and diagnose disease and to guide treatment.

Quantifying the heterogeneity of macromolecular machines by mass photometry

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Sample purity is central to *in vitro* studies of protein function and regulation, as well as to the efficiency and success of structural studies requiring crystallization or computational alignment of individual molecules. Much effort has therefore been aimed at developing and optimizing techniques capable of accurately reporting on sample heterogeneity including SDS-PAGE, size exclusion chromatography (SEC) and negative staining EM (nsEM), with the latter being the most detailed tool today to evaluate sample quality for protein complexes. High throughput screening, however, remains impractical with nsEM, due to the associated workflows.

Mass photometry (MP) is a novel approach that can quantify the mass of protein complexes under native-like conditions at the single-molecule level. It is based on the detection of scattering signals from individual proteins, and is therefore a fast and simple method for measuring mass distributions in solution. Here, we show that MP can accurately report on sample heterogeneity using minimal volumes with molecular resolution within minutes, providing a rapid alternative to nsEM. We benchmark our approach by comparison to nsEM, and include workflows involving chemical crosslinking and multi-step purification of a multi-subunit ubiquitin ligase. When applied to proteasome stability, we detect and quantify assemblies invisible to nsEM. In cases where the sample exhibits heterogeneity in the structure as well as mass, nsEM fails to give quantitative analysis while MP can provide detailed information on the complex heterogeneity. Our results illustrate the unique advantages of MP for rapid sample characterization, prioritization and optimization.

Dissecting the Single-Cell Dynamics of Xist RNA in X Chromosome Inactivation by 3D Structured Illumination Microscopy

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Keywords: Methods development – Image analysis, Techniques – Structured illumination, Applications in cell biology, Techniques – Super resolution microscopy

Dosage compensation in XX female mammals is achieved by X chromosome inactivation (XCI). The key player of this process is the long noncoding RNA Xist, which associates in cis with the inactive X chromosome elect (Xi) to mediate gene silencing and heterochromatization. Xist spreads across the entire Xi, but little is known about the dynamics of this process, particularly in the context of single cells. We aim to comprehensively assess the single-cell dynamics, spreading and maintenance of Xist in mouse ES cells (mESC) using HaloTag technology in combination with 3D Structured Illumination Microscopy (3D-SIM) to achieve high spatial and temporal resolution.

We have generated XY and XX mESCs expressing inducible transgenic or endogenous bgl-stem-loop tagged Xist in tandem with BglG-Halo fusion protein. Initial experiments have demonstrated that we can visualize distinct functional Xist RNP particles and analyse their spreading behaviour across the Xi chromosome territory during the first hours of XCI. Novel dual-colour fluorescence pulse chase experiments, employing sequential use of two different Halo ligands, allowed us to gain exciting insights into nascent Xist dynamics, turnover on chromatin, localisation and density during the different stages of XCI. We suggest that newly synthesised Xist spreads across the Xi in a 3D spatial manner by localising to sites previously occupied by Xist, and that its turnover depends both on the stage of XCI and chromosomal context. Comparison of transgenic with endogenous Xist has identified major differences in Xist dynamics and spreading which likely explain the highly efficient silencing of Xist in its endogenous chromosomal context.

Photo-Activated Thermal Imaging at Sub-Diffraction Resolution

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Keywords: optical methods development, super-resolution, infrared photo-thermal imaging

Conventional non-contact thermal imaging provides temperature maps based on the intensity of infrared radiation emitted by the sample and detected by a microbolometer-based thermal camera under the assumption of grey body radiance. The typically low numerical aperture of collecting lenses sets a diffraction-limited spatial resolution of $\sim 100\text{-}500\text{ }\mu\text{m}$. However, the limit is effectively worsened to $\sim 1\text{ mm}$ by heat diffusion in the sample, so that high sensitivity ($\sim 0.1^\circ\text{C}$) temperature mapping with tens-of-microns resolution across extended (mm-/cm-sized) fields of view is not routinely achieved.

Sub-diffraction thermal imaging is demonstrated here by the development and validation of a super-resolution approach¹, that combines the photo-thermal effect induced by the sample absorption of modulated laser light with the a-posteriori localization of the resulting temperature variations. By the non-linear fit of the isolated temperature peaks in the thermal camera frames, light-absorbing and heat-releasing centers get localized and rendered in the final super-resolution image. While best-fit amplitudes code for local temperature values, peak coordinates provide morphological information on the absorbing sample with a resolution assigned by the $\sim 10\text{-}\mu\text{m}$ excitation laser-spot size.

We initially validate the approach on synthetic ink samples. By comparing our results with transmitted-light images of the same structures, we confirm accurate imaging capability at $60\text{-}\mu\text{m}$ resolution. We prove therefore a resolution gain of a factor of 6 and 20 with respect to the diffraction-limited prediction and the effective $1200\text{-}\mu\text{m}$ resolution of our thermal camera in conventional operation. We further perform proof-of-principle experiments on complex biological samples. We image explanted murine skin biopsies treated with Prussian blue 30-nm nanocubes, and provide temperature-based super-resolution maps of the distribution of the absorbing nanostructures across mm-sized tissue sections. Our results suggest potential applications and impact of photo-activated super-resolved thermal imaging in the characterization of the morphology and functional state of both biological tissues and synthetic materials.

¹ M. Bouzin et al., Photo-activated raster scanning thermal imaging at sub-diffraction resolution, Accepted, Nat. Commun., 2019

Leveraging Molecular and Cellular Fiducial Markers for Correlative Bio-Imaging

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Beamline B24 is the biological correlative cryo-imaging beamline at the UK synchrotron Diamond Light Source, delivering 3D soft X-ray imaging and structured illumination fluorescence imaging of cryopreserved biological materials such as cells and tissues to nanometer resolution. Our unique, semi-automated and user-friendly correlative microscopy workflow offers unambiguous multidimensional localisation of biomolecules within the cellular suprastructure and is available to the scientific community via a peer-reviewed application process.

Cryo Soft X-ray Tomography (cryo-SXT) 3D data allows the identification and quantification of cellular ultrastructure and subcellular organelles. It is used to study the structural effects of biological, chemical and mechanical cues on cell populations and provides wide field cellular context for data from other imaging modalities such as fluorescence microscopy. At B24, fluorescence localisation data is provided by the structured illumination super resolution fluorescence microscopy method using a bespoke cryo-structured illumination microscope (cryo-SIM). The particular attraction of this correlative imaging system is that samples that are due to be used for X-ray imaging can be first imaged on the cryoSIM to gather 3D fluorescence information at high resolution and identify areas of interest before being taken for cryo-SXT, thereby allowing for the accumulation of easily correlatable and unambiguous localisation data. Effectively, the same cryogenically preserved near-native cell population can now be imaged at nanometer resolution through a variety of imaging methods within and beyond the diffraction limit of available light sources. The resulting data is not compromised by chemical fixation, mechanical milling or slicing, additional staining or sample-to-sample variations and therefore allows the unambiguous interpretation of imaging data across modalities to nanometer resolution.

Recently, as part of a project to ensure accurate information capture and precise correlation across state-of-the-art imaging modalities, we have carried out a comparative study using different commercial nanoparticles and bio-trackers for correlation of X-ray and fluorescence images. We have analysed size, distribution and contrasting properties of a range of potential biomarkers and their relative contribution to correlative analysis of real imaging data. We present here a novel biological fiducialisation system, which uses a combination of nanoparticles and biomarkers to ensure minimal disruption of physiological cell structures while delivering precise correlation of data through an intuitive and user-friendly *in silico* workflow.

Cryo 3D Correlation imaging for studies of host-pathogen interactions: the case of *Staphylococcus aureus* clearance by primary macrophages.

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Keywords: *Staphylococcus aureus*, macrophages, soft-X ray, correlative imaging, structural immunology

Summary

Usually, bacteria coexist with us without harm, however, in certain circumstances they can develop into opportunistic pathogens, for example after surgery. In such cases, the first defense is the macrophage. These cells engage with pathogens to clear infection and signal adaptive responses. Despite its importance for host survival, we lack detailed knowledge of the cell biology of this interaction inside primary mammalian macrophages. Here, we will use *Drosophila* macrophages, which are functionally equivalent to those of mammals, to decipher the functional significance of various subcellular compartments during interaction [1]. We used a correlative approach to “track” the interactions of microbe and host by assembling series of “snapshots” at different times during the process for the first time under near-physiological conditions.

Introduction

Following *Staphylococcus* systemic infection, the first layer of immune defence that is activated are the blood circulating phagocytes which protect the human body by ingesting harmful foreign particles like dead cells, bacteria and fungi, including *Staphylococcus* cells in order to prevent bacteraemia. Thus, phagocytes are crucial in fighting infections as well in maintaining healthy tissues. Here we propose to use for the first time a correlative approach that combines the use of a super resolution cryo-fluorescence (cryo-SIM) with cryo-soft X-ray tomography (cryo-SXT) with the aim of understanding host morphological changes during *Staphylococcus* infection without the need for staining, chemical modification or sectioning. Specifically, we investigate the mitochondrial and the lysosomal alterations within macrophages after *Staphylococcus* infection following the B24 workflow.. We studied cells from a leading immunity model organism, *Drosophila melanogaster*, historically an invaluable resource in the study of innate immunity pathways and host-pathogen interactions. In specific, we used circulating haemocytes (equivalent to human blood cells) of *Drosophila* larvae that have a primary phagocytosis role as the host model to study *Staphylococcus* infections [2].

Methods

The workflow that was followed encompasses sample preparation, correlative microscopy, data acquisition and data processing and analysis.

Results

We will show for the first time an established protocol in order to a) extract, b) cryo-freeze, c) use Cryo-soft X-ray tomography on *Drosophila* primary phagocytes. We will present a unique correlative microscopy platform that allows ultrastructural immunology studies under near- physiological condition studying an host-pathogen interaction model, the one between *Staphylococcus* and primary *Drosophila* macrophages. The application of the B24 correlative microscopy workflow helped us to visualise and accordingly (a) detect, (b) localise and (c) quantify the ultrastructural changes leading to a better understanding of the above interaction.

Conclusion

Our study will allow us to visualise the potential impact of *Staphylococcus* infections on the ultrastructure of the cell under near-physiological condition to nanometer resolution and can conceivably assist drug development at a preclinical level.

References

1. Kounatidis I, Ligoxygakis P. *Drosophila* as a model system to unravel the layers of innate immunity to infection. *Open Biol.* 2012;2(5):120075.
2. Wang L, Kounatidis I, Ligoxygakis P. *Drosophila* as a model to study the role of blood cells in inflammation, innate immunity and cancer. *Front Cell Infect Microbiol.* 2014;3:113. Published 2014 Jan 9. doi:10.3389/fcimb.2013.00113

Combining spatial statistics to describe localisation of tumour associated macrophages in digital pathology

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Keywords: Spatial statistics, digital pathology

The increasing digitization of immunohistochemistry slides provides opportunities for pathologists to automate routine tasks and improve current workflows. Techniques for identifying immune cells in biopsies or surgically resected tumours are now widespread, with several open-source or commercial image analysis platforms providing cell identification tools [1]. While these tools can be used to calculate statistics such as the density of immune cells in a given tumour region, a property which correlates with patient prognosis, spatial statistics are not generally considered.

We apply several spatial statistics to macrophage distributions in human IHC images and in simulated datasets. We show that key features of the spherical contact distribution, the pair correlation function, and the J -function [2,3] vary predictably as the degree of immune cell infiltration from stromal to tumour regions increases in simulated data.

We develop a method based on maximum likelihood estimation that combines features of these three spatial statistics to define a new infiltration index which can automatically classify the extent of macrophage infiltration into tumour nests based on only the observed spatial (x and y) coordinates of the immune cells. We validate our approach by applying it to macrophage distributions from clinical datasets, obtaining infiltration indices which match the qualitative assessments of experienced pathologists. We then show how our approach could be used to automatically distinguish between slides which exhibit low immune infiltration and those which have artificially low infiltration due to imaging artefacts or lack of tissue, and which should be flagged for review and annotation by a pathologist.

[1] Bankhead, P., Loughrey, M. B., Fernández, J. A., Dombrowski, Y., McArt, D. G., Dunne, P. D., McQuaid, S., Gray, R. T., Murray, L. J., Coleman, H. G., James J. A., Salto-Tellez, M., Hamilton, P. W. (2017). QuPath: Open source software for digital pathology image analysis. *Scientific Reports*, 7(1), 16878. <https://doi.org/10.1038/s41598-017-17204-5>

[2] Stoyan, D., & Stoyan, H. (1994). *Fractals, Random Shapes and Point Fields: Methods of Geometrical Statistics*. Chichester: Wiley.

[3] van Lieshout, M. N. M., & Baddeley, A. (1996). A non-parametric measure of spatial interaction in point patterns. *Statistica Neerlandica*, 50(3), 344–361. <https://doi.org/10.1111/j.1467-9574.1996.tb01501.x>

Morphological analysis of images of dried droplets of saliva for determination the degree of endogenous intoxication

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Keywords: bioimage informatics, screening microscopy, correlative microscopy, spatial statistics in microscopy, application in medicine

Endogenous intoxication (EI) is an integral pathology which gives the general information about state of health of the patient. There is a correlation of biochemical data (costly, require long-term invasive studies) and morphological indicators based on image analysis of the visual image of a droplet of saliva dried in standard conditions (a simple and cheap non-invasive procedure). It is important to develop cheap, fast, non-invasive, computerized morphological methods for a medical diagnostics of EI based on the unique clinical experience obtained in the past decade to apply for mass rapid screening and monitoring of patients during the clinical examination, and also for individual checkup the health of any patient (figure 1).

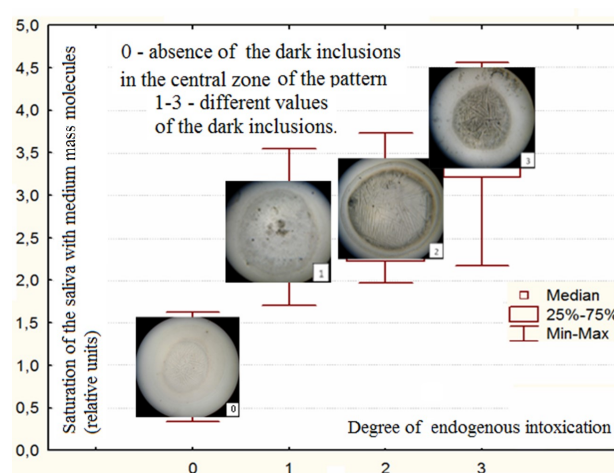


Figure 1. Correlation between morphology of the dried saliva drop pattern and biochemical parameters.

The method is proposed and laboratory setup is elaborated for determination of the EI by determination of morphological properties of dried pattern of evaporated saliva droplet. The set of microphotographs of the salivary dried patterns is formed taking into account the degree of EI [1]. Criteria for expert evaluation of the EI degree from images of saliva dried pattern image are proposed. For the first time, computer software was created and tested to determine the EI degree. This makes it possible to process a large number of digital images of saliva patterns, which allows evaluating the monitoring of the patient health. Proposed method is a combination of bioinformatics and biochemistry approaches for obtaining diagnostic information from a morphological analysis of standardized dried patterns of saliva.

- [1] Lebedev-Stepanov P.V., Buzoverya M.E., Vlasov K.O. and Potekhina Yu.P. 'Morphological analysis of images of dried droplets of saliva for determination the degree of endogenous intoxication' *J. of Bioinformatics and Genomics* **2018**, 4(9), 1.

Structural Biology *in situ*: The Promise and Challenges of Cryo-Electron Tomography

Prof. Dr. Wolfgang Baumeister
Max Planck Institute of Biochemistry

Traditionally, structural biologists have approached cellular complexity in a reductionist manner by characterizing isolated and purified molecular components. This 'divide and conquer' approach has been highly successful. However, awareness has grown in recent years that only rarely can biological functions be attributed to individual macromolecules. Most cellular functions arise from their acting in concert. Hence there is a need for methods developments enabling studies performed *in situ*, i.e. in unperturbed cellular environments. *Sensu stricto* the term 'structural biology *in situ*' should apply only to a scenario in which the cellular environment is preserved in its entirety. Cryo electron tomography has unique potential to study the supramolecular architecture or 'molecular sociology' of cells. It combines the power of three-dimensional imaging with the best structural preservation that is physically possible.

Quantitative analysis of molecular coupling with SODA

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Keywords: Spatial statistics, colocalization (coupling), super-resolution microscopy

To understand protein functions and molecular network organisation, it is important to determine the localisation of proteins at subcellular level. The in cellulo probing of molecular assemblies is commonly performed with microscopy techniques which visualise the localisations of molecules. The analysis of the spatial proximity between different molecules of interest is traditionally achieved by labelling proteins with different fluorophores (in general red and green fluorophores), and quantifying their spatial co-localisation. Traditional co-localisation index are based on signal correlation (e.g. Pearson Correlation Coefficient) and overlap (e.g. Manders Overlap Coefficient) and thus depend on the size of the point spread function (PSF) of the microscope. In addition, these methods cannot be used for single-molecule localisation microscopy where the nanometre scale localisation of molecules is directly estimated from the sequential activation of blinking molecules. Thus, distance-based methods that evaluate the spatial association (coupling) between objects (spots or localisations) based on their relative positions rather than their fluorescence correlation or overlap have been introduced. Yet, while these distance-based methods have demonstrated their statistical power in detecting coupling between objects, they do not clearly measure the number of objects' couples. Moreover, the topographical organisation of molecules within the cell frequently bias the interpretation of co-localisation coefficients. Indeed, confined molecules can strongly overlap or be very close (localisations) even if randomly distributed.

We will present a novel method and software named Statistical Object Distance Analysis (SODA) that characterises the relative spatial positioning of several distributions of molecules, in a quantitative and automatic manner. Contrary to previous co-localisation methods, SODA allows to map statistically coupled objects within the cell, i.e., to compute a coupling probability for each single pair of objects. As SODA uses a marked-point process framework, it does not depend on the PSF characteristics and is robust to noise. In addition, by analysing the morphology (size, intensity and shape) and the distance separating coupled molecules, it provides, at a population level, a detailed and exhaustive description of the molecular assemblies. We will exemplify the capabilities of SODA to quantitatively assess the geometry of molecular assemblies with two examples: 1- The characterisation of the spatial organisation of thousands of synapses using three-colour structured-illumination microscopy (SIM) images of hippocampal neurons, and 2- the statistical computation of the stoichiometry and the distance between single localisations of two synaptic vesicle proteins imaged with 3D-STORM.

Towards computational multiplex fluorescence microscopy: Machine learning-based integrated prediction of morphological and molecular tumor profiles

Alexander Binder, Michael Bockmayr, Miriam Hägele, Stephan Wienert, Daniel Heim, Katharina Hellweg, Albrecht Stenzinger, Laura Parlow, Jan Budczies, Benjamin Goeppert, Denise Treue, Manato Kotani, Masaru Ishii, Manfred Dietel, Andreas Hocke, Carsten Denkert, Klaus-Robert Müller, Frederick Klauschen

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Recent advances in cancer research largely rely on new developments in microscopic or molecular profiling techniques offering high level of detail with respect to either spatial or molecular features, but usually not both. Here, we present a novel machine learning-based computational approach that allows for the identification of morphological tissue features and the prediction of molecular properties from breast cancer imaging data. This integration of microanatomic information of tumors with complex molecular profiling data, including protein or gene expression, copy number variation, gene methylation and somatic mutations, provides a novel means to computationally score molecular markers with respect to their relevance to cancer and their spatial associations within the tumor microenvironment.

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Shining Light on Topology in Early T Cell Triggering – A Novel Bilayer System

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Keywords: T cell, fluorescence microscopy, TIRF, cell membrane

The very first cell-cell interactions of a T cell with an antigen presenting cell (APC) are key to understand antigen discrimination in adaptive immunity. The use of supported lipid bilayers (SLBs) to mimic the APC simplifies the imaging approach and allows to define its protein composition. In studies of the immune synapse these are commonly functionalized with peptide bound MHC (pMHC) and ICAM-1, in this case both an adhesion molecule and a steric barrier to engage pMHC. By replacing it with CD58, a small adhesion molecule, and the extracellular part of CD45, as glycocalyx, we aimed to produce a more realistic model system for further insights into T cell triggering.

On this surface, adhesion characteristics of T cells could be fine-tuned by varying the CD45 to CD58 ratio: low densities of CD45 allow cell attachment and spreading; higher densities prevent contact formation. At intermediate glycoprotein densities cells only attached and triggered if pMHC was present. We utilized the size-based exclusion of CD45 in the SLB together with cell membrane labelling to image the close contact formation via live TIRF microscopy. Initial small, diffraction limited contacts grew and were highly dynamic, dominated by centripetal movements at later stages. The small, very first contacts represented the footprint of microvilli, a dominant feature of T cell topology. T cells treated with agents that reduce microvilli on their cell membrane were unable to detect pMHC molecules in our model SLB.

The presented SLB system allowed specific imaging of the dynamics of close contact zones, which are distinct from simple membrane proximity. T cell microvilli provided a structure to both penetrate the glycocalyx barrier and detect antigens.



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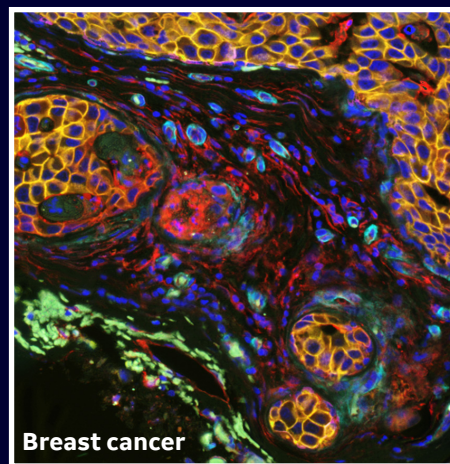
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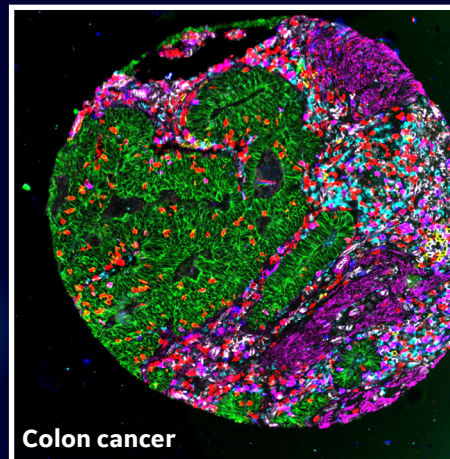
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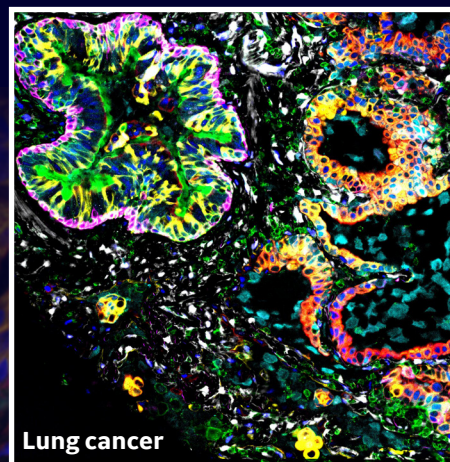
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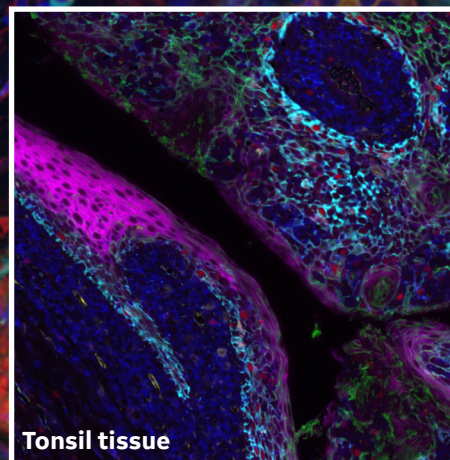
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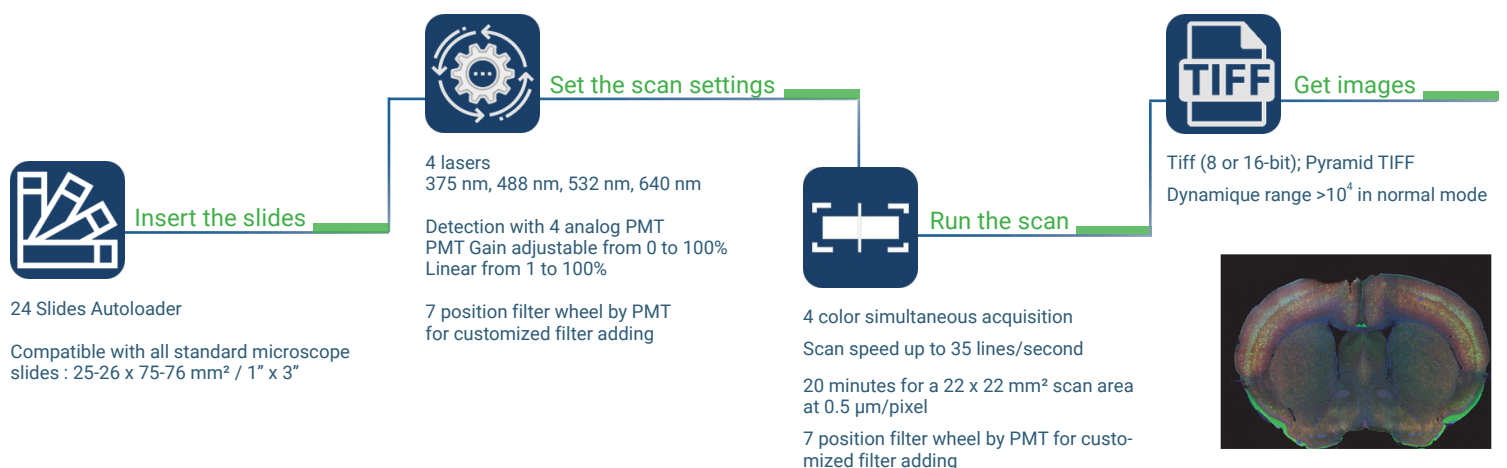
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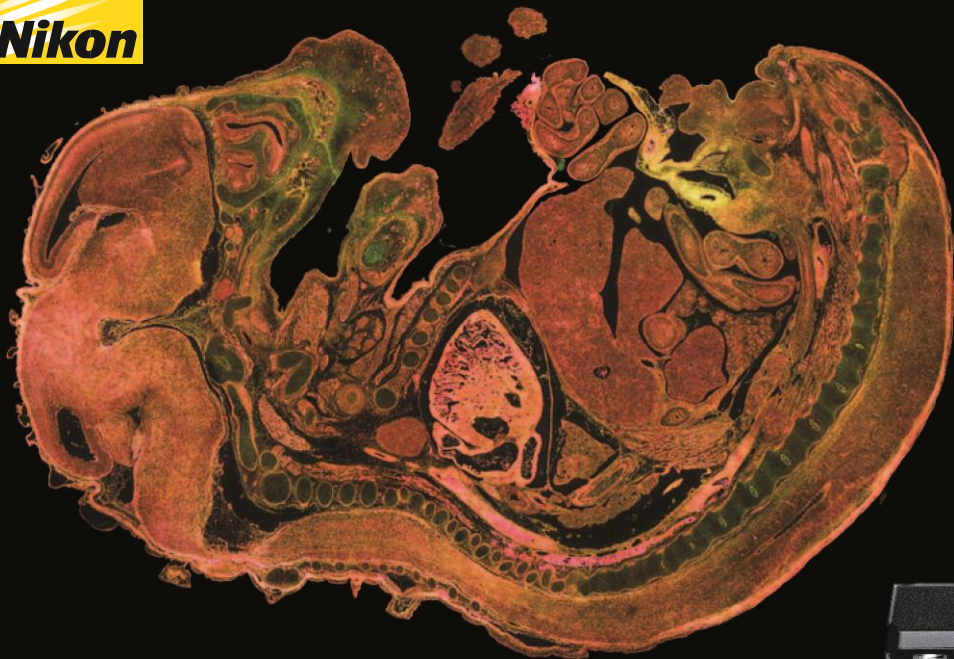
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and between different scans



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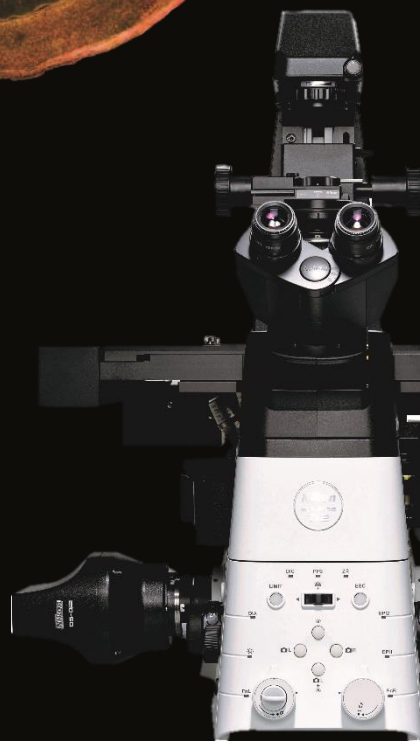


ECLIPSE Ti2

The Eclipse Ti2-E is easy to use, and is ideal for fast imaging without compromising on image quality.

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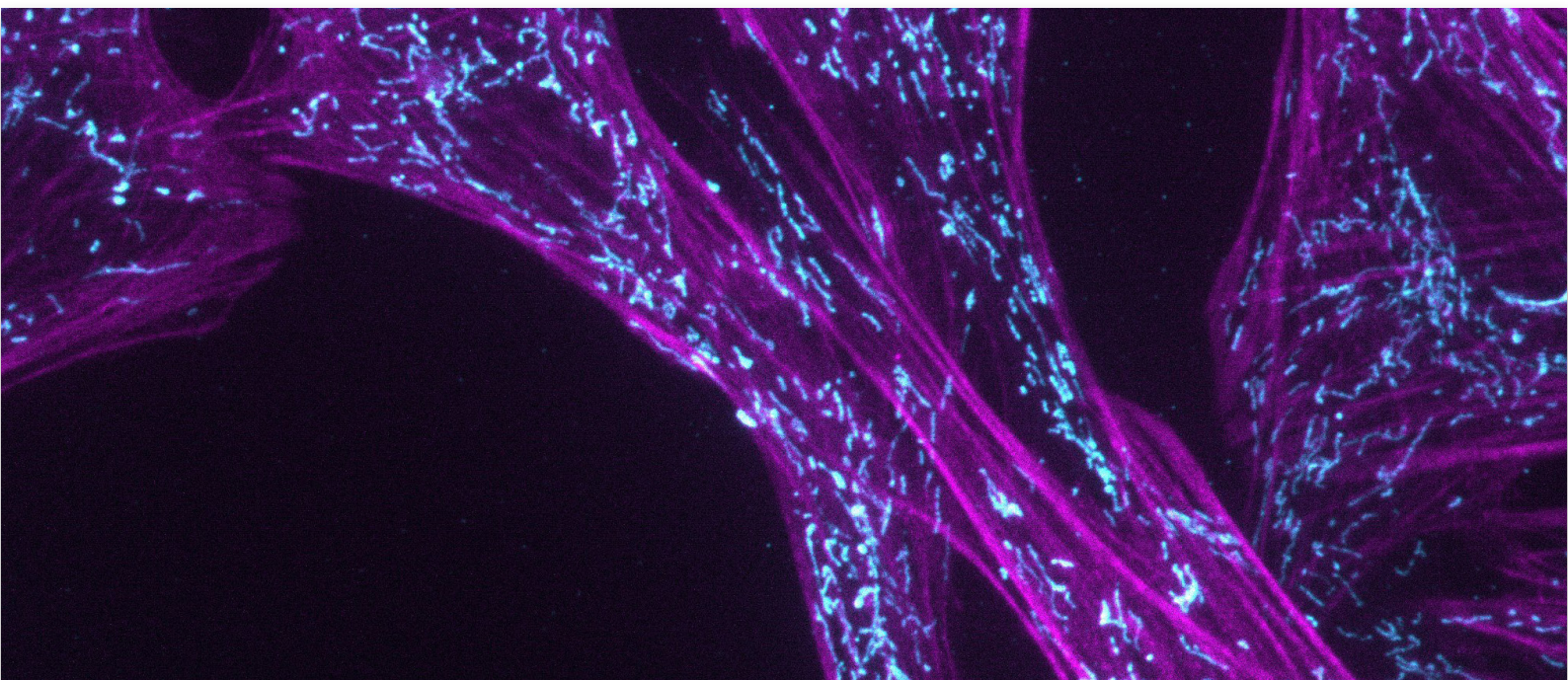


Image credit: Sam Lord, UCSF. Taken with Photometrics 6.5 μm sCMOS Camera

KINETIX

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10 Megapixel Back-Illuminated sCMOS

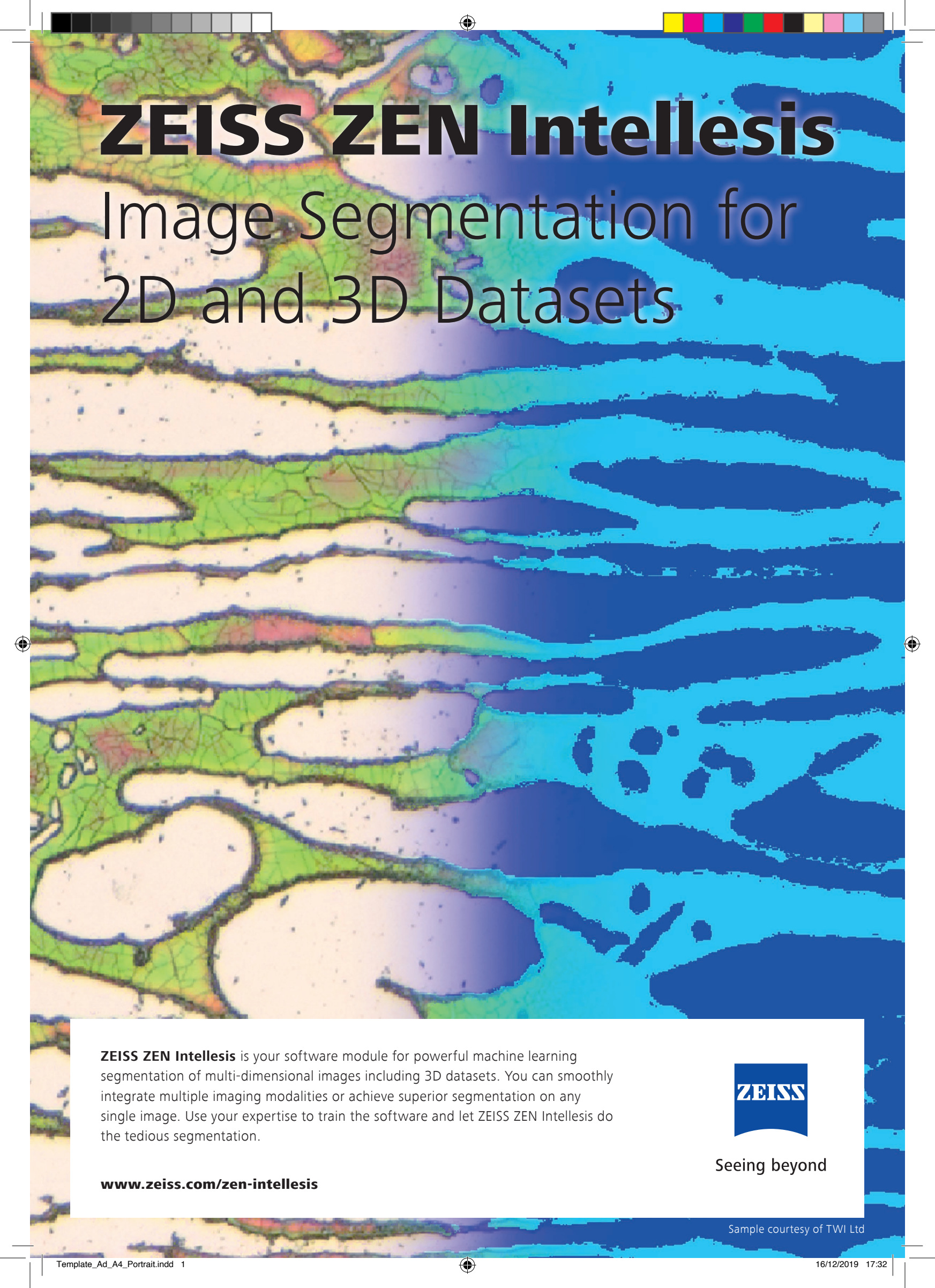
- » 400 Frames Per Second
- » 29mm Field Of View
- » 6.5 μm x 6.5 μm Pixels
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Seeing beyond

Sample courtesy of TWI Ltd