

# 2018 Quantitative BioImaging Conference

Georg August University Göttingen, Faculty of Physics, Göttingen, Germany

### January 4 – 6, 2018

Friedrich-Hund-Platz 1 37077 Göttingen

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# **Sponsors**







# **Exhibitors**

















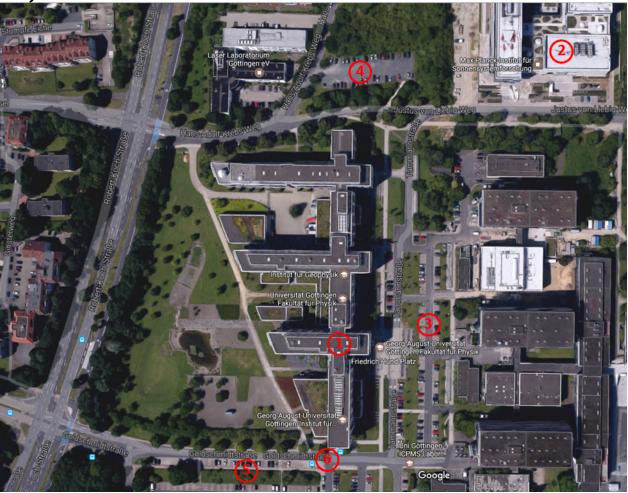






# **Maps and Parking**

### Major locations of interest for conference attendees



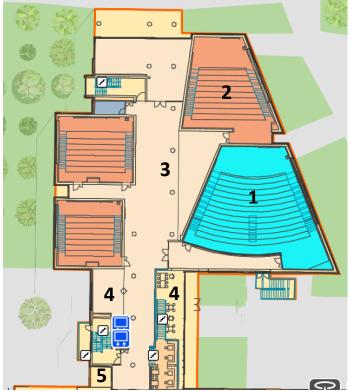
- 1 Main entrance, Faculty of Physics, Georg-August University
- 2 Max Planck Institute for Solar System Research, the conference dinner will be organized in foyer
- 3, 4, 5 Parking places
- 6 Bus stops, the name of the bus stop is "Tammannstraße"

### Major locations of interest for conference attendees

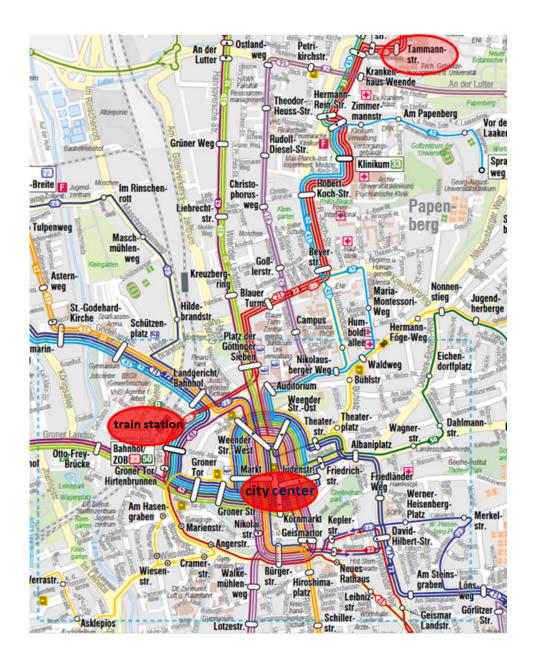


The conference location is section **A** of Faculty of Physics, Georg-August University, ground floor.

### **Conference floor layout**



- 1 Hörsaal 1, Lecture hall 1
- 2 Hörsaal 2, Lecture hall 2
- 3 Exhibition/Poster session area
- 4 Lunches/Coffee breaks areas
- 5 Registration desk



From the main train station one can take bus number 21 and 23 till "Tammanstraße".

From the city center (bus stops "Jüdenstraße" or Weender Straße-Ost) one can take bus N 22 till "Tammanstraße". If you want to go by bus to the city center from Faculty of Physics you may take bus N 22 which will bring you directly to the city center (bus stops "Weender Straße-West" or "Markt"), or you may take bus N 21 or 23 and leave at the bus stop "Platz der Göttinger Sieben". From the bus stop "Platz der Göttinger Sieben" is app. 5 min to walk to the city center.

If you want to go by bus to the Main train station (bus stop "Haupt Bahnhof"), take bus N 21 or N 23. Bus ticket in one direction costs  $2,30 \in$  and can be bought from the bus driver.

High resolution bus plan: https://www.goevb.de/fileadmin/goevb/fahrplan/download-dateien/LNP\_2015.pdf Buses time table: https://www.goevb.de/fahrplan/fahrplaninformation/

### **Conference Registration and Badge Pickup**

### Wednesday, Jan. 3, 2018

#### 17:00 to 19:00

Georg August University Göttingen Faculty of Physics Foyer, section A of the building, ground floor Friedrich-Hund-Platz 1 37077 Göttingen Germany

### Thursday, Jan. 4, 2018 through Saturday, Jan. 6, 2018

#### 8:00 to 18:00

Georg August University Göttingen Faculty of Physics Foyer, section A of the building, ground floor Friedrich-Hund-Platz 1 37077 Göttingen



### **Scientific Organizing Committee QBI 2018**

#### Prof. Dr. Stefan Hell

Max-Planck-Institute of Biophysical Chemistry, Göttingen <u>http://www.mpibpc.mpg.de/hell</u>

**Prof. Dr. Holger Stark** Max-Planck-Institute of Biophysical Chemistry, Göttingen <u>http://www.mpibpc.mpg.de/stark</u>

Prof. Dr. Thomas Jovin Prof. Dr. Donna Arndt-Jovin Max Planck Institute for Biophysical Chemistry, Göttingen http://www3.mpibpc.mpg.de/groups/jovin/index.php/ResearchGroups/HomePage

#### Dr. Mark Bates

Max Planck Institute for Biophysical Chemistry, Göttingen <u>https://www.researchgate.net/profile/Mark\_Bates4</u>

**Prof. Dr. Alexander Egner** Laser-Laboratorium Göttingen e.V., Göttingen <u>http://www.llg-ev.de/en/optical-nanoscopy/index.html</u>

#### Prof. Dr. Stefan Jakobs

University Medicine Göttingen, Georg August University, Göttingen <u>https://jakobs.mpibpc.mpg.de</u>

#### **Prof. Dr. Fred Wouters**

University Medicine Göttingen, Georg August University, Göttingen <u>https://www.uni-goettingen.de/en/58060.html</u>

#### Dr. Gertrude Bunt

University Medicine Göttingen, Georg August University, Göttingen http://www.germanbioimaging.org/wiki/index.php/MOLCI Goettingen

#### Prof. Dr. Tim Salditt

Institute for X-ray physics, Dept. Physics, Georg August University, Göttingen <a href="http://www.roentgen.physik.uni-goettingen.de/members/?id=1">http://www.roentgen.physik.uni-goettingen.de/members/?id=1</a>

#### Prof. Dr. Christoph Schmidt,

3rd Institute of Physics - Biophysics, Dept. Physics, Georg August University, Göttingen <u>https://www.uni-goettingen.de/en/58028.html</u>

#### Prof. Dr. Axel Munk,

Institute for Mathematical Stochastics, Georg August University, Göttingen http://www.stochastik.math.uni-goettingen.de/index.php?language=en&id=munk

# **Quantitative BioImaging Society**

### Meeting to discuss future plans and events for the QBI Society

Georg August University Göttingen, Faculty of Physics (conference site)

03.01, Wednesday, 19:00, Hörsaal 2

Session Chair: Raimund Ober

### **Meals and Refreshments**

The following meals and refreshments are provided free of charge to attendees.

### Thursday, January 4, 2018

10:40 to 11:10 Coffee Break Georg August University Göttingen, Faculty of Physics (conference site) Coffee and snacks

12:10 to 13:40 Lunch Georg August University Göttingen, Faculty of Physics (conference site) Full meal

15:00 to 16:30 Coffee Break and Poster Session Coffee break sponsored by Chroma



Georg August University Göttingen, Faculty of Physics (conference site) Coffee and snacks

18:30 to 22:00Poster SessionGeorg August University Göttingen, Faculty of Physics (conference site)Beer and pretzels

### Friday, January 5, 2018

10:10 to 11:10 Coffee Break Coffee break sponsored by the Allen Institute for Cell Science Georg August University Göttingen, Faculty of Physics (conference site) Coffee and snacks

12:40 to 13:40 Lunch Georg August University Göttingen, Faculty of Physics (conference site) Full meal 15:00 to 16:20 Coffee Break and Poster Session Georg August University Göttingen, Faculty of Physics (conference site) Coffee and snacks



18:30 to 22:00
Conference Dinner
Max Planck Institute for Solar System Research, the conference dinner will be organized in foyer
Justus-von-Liebig-Weg 3
37077 Göttingen
Full meal

### Saturday, January 6, 2018

10:40 to 11:10 Coffee Break Georg August University Göttingen, Faculty of Physics (conference site) Coffee and snacks

12:40 to 13:40 Lunch Georg August University Göttingen, Faculty of Physics (conference site) Full meal

15:20 to 16:50 Coffee Break and Poster Session Georg August University Göttingen, Faculty of Physics (conference site) Coffee and snacks

# **Internet Access**

#### **Wi-Fi-Access**

Together with your confirmation of attendance you received a username and a password for "GuestOnCampus". To connect to the wireless guest network follow these steps:

1. Connect to the "GuestOnCampus" WiFi.

2. Visit a website via http e. g. <u>wlan.gwdg.de</u>.

3. Enter the login information from your confirmation of attendance, e.g.

Username: 2018QBIXXX@guestnet

Password: XXXXXXXX

Once you are authenticated, you may access the Internet.

Note:

- This account is valid from 02/01/2018 until 07/01/2018.
- Guest network access is intended for scientific use only.
- Usage will be monitored and may be terminated at any time.

• The connection is unencrypted. Do not send any passwords or personal information over an unencrypted protocol. If possible use only HTTPS websites.

The Eduroam network is also available at the Faculty of Physics, Georg-August University for those who have access provided by their participating institutions.

## **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.

Best Poster Prize QBI 2018 Announcement: 18:10, Hörsaal 1, 06.01.2018

# **Minisymposium**

#### **05.01.18**, Software design for quantitative microscopy image analysis

Jens Rittscher Oxford University, UK "Microscopy software support on websites"

Mark A. Tsuchida Open Imaging "Design approaches to Micro-Manager"

Winfried Wiegraebe Allen Institute for Cell Science "Quantitative microscopy pipeline for building a model of the human cell"

Jerry Chao Texas A&M University

"A software framework for advanced microscopy data analysis with application to single molecule microscopy"

#### 06.01.18, Digital microscopy and image informatics

Yinyin Yuan Institute for Cancer Research, UK "Deciphering the tumor ecosystem with histology image analysis"

Nasir Rajpoot University of Warwick, UK "Mining for Histology Footprints of Cancer Subtypes"

Martin Kristensson and Lars Pedersen Visiopharm "Advanced virtual multiplexing"

**Sripad Ram** Pfizer, Inc, San Diego, US

"Advanced Image Analytics For Characterizing The Type And Distribution Of Immune Cells In The Tumor Microenvironment"

**France Rose** 

Institut de Biologie de l'École Normale Superieure (IBENS), France

"Quantifying the spatial heterogeneity of cell responses to cancer drugs"

# **Plenary Discussion**

04.01.18, 19:30-20:30 Hörsaal 1, Organizer: David Grunwald

### Calibration of microscopy instruments

- 19:30 19:35 Opening remarks: David Grunwald
- 19:35 19:50 Olaf Selchow (Microscopy & BioImaging Consulting)
- 19:50 20:05 Ute Resch (BAM)
- 20:05 20:20 Mark Browne (Andor)
- 20:20 20:30 Max Huisman (UMMS)

# **Keynote Speakers**

Stefan Hell, Max Planck Institute for Biophysical Chemistry, Göttingen, Germany A MINimum for Maximum Resolution [ABSTRACT # A]
9:00, 04.01, Thursday, Hörsaal 1

Philip Tinnefeld, Ludwig-Maximilians-Universität München, München, Germany Reference Structures for Quantitative Microscopy [ABSTRACT # E]
9:00, 05.01, Friday, Hörsaal 1

Theo Lasser, École Polytechnique Fédérale de Lausanne, Lausanne, Switzerland VOIR fait SAVOIR [ABSTRACT # K] 9:00, 06.01, Saturday, Hörsaal 1

# **Invited Speakers**

Peter Dedecker, University of Leuven, Leuven, Belgium

Alberto Diaspro, Italian Institute of Technology, Genoa, Italy

Ana Diaz, Paul Scherrer Institute, Villigen, Switzerland

Yuval Ebenstein, Tel Aviv University, Tel Aviv, Israel

Yuval Garini, Bar-Ilan University, Ramat Gan, Israel

*Martin Hof*, J. Heyrovský Institute of Physical Chemistry, Czech Academy of Sciences, Prague, Czech Republic

Ralf Jungmann, Max Planck Institute of Biochemistry, Martinsried, Germany.

Philipp Kukura, University of Oxford, Oxford, UK

Alexander Rohrbach, University of Freiburg, Freiburg, Germany

*Hari Shroff*, National Institute of Biomedical Imaging and Bioengineering, Bethesda, Maryland, USA

Matthias Weiss, University of Bayreuth, Bayreuth, Germany

### Program

### Wednesday, 03.01.2018

17:00 Registration desk open – Georg August University Göttingen, Faculty of Physics, conference site

19:00 **QBI Society Meeting**, Hörsaal 2 Session Chair: Raimund Ober

### Thursday, 04.01.2018

Conference Opening (8:45 - 9:00), Hörsaal 1

8:45 Welcome address by Jörg Enderlein & Raimund Ober

Super-resolution (9:00 - 10:40), Hörsaal 1 Session Chair: Jörg Enderlein

- 9:00 **Stefan Hell (Keynote Lecture), Max Planck Institute for Biophysical Chemistry, Göttingen, Germany** A MINimum for Maximum Resolution [ABSTRACT # A]
- 9:50 Alberto Diaspro (Invited Speaker), Italian Institute of Technology, Genoa, Italy Liquid Tunable Microscopy [ABSTRACT # B]
- 10:20 Kevin Vynck, CNRS IOGS Univ. Bordeaux, Talence, France Propagation Of Polarized Light In Turbid Media: Challenges And New Perspectives [ABSTRACT # 105]

Coffee Break (10:40 - 11:10)

#### DNA and Super-resolution (11:10-12:10), Hörsaal 1

Session Chair: Alberto Diaspro

- 11:10 **Yuval Ebenstein (Invited Speaker)**, Tel Aviv University, Tel Aviv, Israel Super-Resolution Tracking of Molecular Motors and DNA in Nanochannel Arrays [ABSTRACT # C]
- 11:40 Ralf Jungmann (Invited Speaker), LMU Munich and Max Planck Institute of Biochemistry, Martinsried near Munich, Germany

Super-Resolution Imaging with DNA Molecules [ABSTRACT # D]

Lunch (12:10 - 13:40)

#### **Parallel Sessions**

#### Analytical Techniques for Single Molecules I (13:40 - 15:00), Hörsaal 1

Session Chair: Sebastian Kruss

- 13:40 Stefan Niekamp, UCSF/HHMI, San Francisco, United States
   Multi-color high-resolution localization microscopy methods enable nanometer distance measurements [ABSTRACT # 42]
- 14:00 Jonas Ries, EMBL, Heidelberg, Germany Fast, robust and precise 3D localization for arbitrary point spread functions [ABSTRACT # 13]
- 14:20 **Petar Petrov**, Stanford University, Stanford, United States Modeling engineered point spread functions for 3D single-molecule localization microscopy [ABSTRACT # 20]
- 14:40 Amin Zehtabian, Freie Universität Berlin, Berlin, Germany Adaptive enhancement of microtubule filaments in SMLM images using nonlinear partial differential equations and genetic algorithms [ABSTRACT # 87]

Fluorescence Correlation Spectroscopy and Diffusion Techniques (13:40 - 15:00), Hörsaal 2
Session Chair: Ingo Gregor

- 13:40Falk Schneider, University of Oxford, Oxford, United KingdomStatistical analysis of scanning FCS data differentiates free from hindered diffusion [ABSTRACT # 64]
- 14:00 Antoine Delon, Université Grenoble Alpes, Grenoble, France Fluorescence correlation spectroscopy through micro-beads: a minimal model to study the impact of a cellular layer [ABSTRACT # 103]
- 14:20 Mariano Gonzalez Pisfil, PicoQuant GmbH Humboldt-Universität zu Berlin, Berlin, Germany Multi-species diffusion in membrane utilizing scanning FCS and super-resolution microscopy [ABSTRACT # 79]

14:40 **Quan Wang, Princeton University, Princeton, United States** Single-molecule diffusometry in a feedback trap [ABSTRACT # 9]

Coffee Break and Poster Session (15:00 - 16:30)

Coffee break sponsored by Chroma

16:10-16:25 *Marketing Presentation, Hörsaal 1:* Marcelle Koenig, PicoQuant GmbH, Berlin, Germany Quantitative Ultrafast FLIM [ABSTRACT # 6]

**Parallel Sessions** 

Single Molecule Clustering and Colocalization (16:30 - 18:10), Hörsaal 1 Session Chair: Peter Dedecker				
16:30	Adela Staszowska, King's College London, London, United Kingdom The Renyi divergence allows precise and accurate cluster radius measurement for localization microscopy [ABSTRACT # 22]			
16:50	<b>Iain Styles</b> , University of Birmingham, Birmingham, United Kingdom Persistent homology as a tool to probe structure in single molecule microscopy datasets [ABSTRACT # 46]			
17:10	Andreas Arnold, TU Wien, Wien, Austria Temporal accumulation analysis allows detection of small protein oligomers in the plasma membrane [ABSTRACT # 76]			
17:30	<b>Charles Kervrann</b> , Inria, Rennes, France GcoPS: a geo-copositionning system for live cell imaging and superresolution microscopy [ABSTRACT # 50]			
17:50	Florian Levet, INSERM, Bordeaux, France Polygon-based colocalizatin analysis for multicolor single-molecule localization microscopy data [ABSTRACT # 61]			
	mental Advances for Cell Imaging (16:30 - 18:10), Hörsaal 2 a Chair: Thomas Jovin			
16:30	<b>Ed Cohen</b> , Imperial College London, London, United Kingdom Spatial Statistics and Resolution [ABSTRACT # 111]			
16:50	Ingo Gregor, Georg-August-University, Göttingen, Germany Rapid non-linear image scanning microscopy [ABSTRACT # 60]			
17:10	Elias M. Puchner, University of Minnesota, Twin Cities, Minneapolis, United States Quantitative and motion-corrected super-resolution imaging of intracellular organelles in living cells [ABSTRACT # 107]			
17:30	Verena Richter, Aalen University, Aalen, Germany Axial tomography in single cell fluorescence microscopy [ABSTRACT # 12]			
17:50	<b>Bassam Hajj, Institut Curie - CNRS, Paris, France</b> Volumetric dual color microscopy for 3D imaging of densely labeled cellular structures [ABSTRACT # 24]			
Poster Session with Beer & Pretzel (18:30 - 22:00)				
19:30-20:30 <b>Plenary Discussion, Hörsaal 1</b> : Calibration of microscopy instruments Organizer: David Grunwald				

### Friday, 05.01.2018

#### Advanced Imaging and Super-resolution (9:00 - 10:40), Hörsaal 1

Session Chair: Raimund Ober

- 9:00 *Philip Tinnefeld* (Keynote Lecture), Ludwig-Maximilians-Universität München, München, Germany Reference Structures for Quantitative Microscopy [ABSTRACT # E]
- 9:50 Matthias Weiss (Invited Speaker), University of Bayreuth, Bayreuth, Germany Monitoring Self-Organization Events in the Early Embryogenesis of *Caenorhabditis elegans* with Light Sheet Microscopy [ABSTRACT # G]
- 10:20 **Hidreza Heydarian (2017 Poster Award Winner), Delft University of Technology, Delft, The Netherlands** Template-free 2D-particle fusion of localization microscopy images produces λ/150 resolution [ABSTRACT # 19]

Coffee Break (10:40 - 11:10)

Coffee break sponsored by the Allen Institute for Cell Science

#### Quantitation in Cell Biology and Membranes (11:10 - 12:40), Hörsaal 1

Session Chair: Yuval Ebenstein

- 11:10 **Yuval Garini (Invited Speaker)**, Bar Ilan University, Ramat Gan, Israel Studying Chromatin Dynamics by Advanced Live Cell Imaging Methods [ABSTRACT # H]
- 11:40 **Martin Hof (Invited Speaker)**, Czech Academy of Sciences, Prague, Czech Republic Lipid Driven Nano-Domains are Fluid [ABSTRACT # I]
- 12:10 Alexander Rohrbach (Invited Speaker), University of Freiburg, Freiburg, Germany Label-Free Imaging of Cellular Dynamics at 100 Hz and 140 nm Resolution [ABSTRACT # J]

Lunch (12:40 - 13:40)

#### Parallel Sessions

**Minisymposium: Software Design for Quantitative Microscopy Image Analysis** (13:40 - 15:00), **Hörsaal 1** Session Chair: Raimund Ober

- 13:40 Winfried Wiegraebe, Allen Institute for Cell Science, Seattle, United States Quantitative microscopy pipeline for building a model of the human cell [ABSTRACT # 118]
- 14:00 Raimund Ober, Texas A&M University, College Station, United States A software framework for advanced microscopy data analysis with application to single molecule microscopy [ABSTRACT # 112]

- 14:20 Jens Rittscher, Oxford University, Oxford, United Kingdom Microscopy software support on websites, [ABSTRACT # 108]
- 14:40 **Mark Tsuchida**, Open Imaging, Inc., San Francisco, United States Design approaches to micromanager [ABSTRACT # 109]

**Single Molecule Application** (13:40 - 15:00), **Hörsaal 2** Session Chair: Yuval Garini

- 13:40 **Steffen J. Sahl**, MPI for Biophysical Chemistry, Göttingen, Germany Fluorescence nanoscopy of aggregation-prone mutant Huntingtin proteins: recent advances [ABSTRACT # 85]
- 14:00 Andreas Gahlmann, University of Virginia, Charlottesville, United States
   3D single-molecule tracking of confined diffusers: resolving cytosolic complex formation in living bacterial cells
   [ABSTRACT # 7]
- 14:20 Daniel Thédié, Université de Grenoble Alpes, CNRS, CEA, Grenoble, France
   Photoswitching of green MEOS2 by intense 561nm light perturbs efficient green-to-red photoconversion in quantitative
   localization microscopy [ABSTRACT # 104]
- 14:40Stephan Bergmann, Bielefeld University, Bielefeld, GermanyPhotoactivation localization microscopy of cardiomyopathy associated plakophilin-2 mutants [ABSTRACT # 17]

Coffee Break and Poster Session (15:00 - 16:20)

16:00 – 16:15 *Marketing Presentation, Hörsaal 1:* Kirti Prakash, Oxford Nanolmaging, Oxford, United Kingdom Meet the nanoimager: the next generation of single-molecule and superresolution imaging

**Parallel Sessions** 

Analysis of Microscopy and Cell Biological Data Using Machine Learning and Other Techniques (16:20 - 18:20), Hörsaal 1 Session Chair: Ed Cohen

16:20 **Christopher Calderon**, Ursa Analytics, Denver, United States Using deep convolutional neural networks to circumvent morphological feature specification when classifying subvisible protein aggregates from micro-flow images [ABSTRACT # 54]

16:40 **Matthias Häring**, Max-Planck-Institute for Dynamics and Self-Organization, Göttingen, Germany Segmentation of low-quality biomedical images using deep convolution networks [ABSTRACT # 88]

#### 17:00 Benedict Diederich, IPHT Leibniz Institute of Photonic Technology's, Jena, Germany

Machine learning to reconstruct 3D scattering data from partially coherent imaging data [ABSTRACT # 93] Felipe Delestro, ENS, Paris, France 17:20 High density tracking of soma activity in 3D confocal images in vivo [ABSTRACT # 36] 17:40 Daniel Wüstner, University of Southern Denmark, Odense, Denmark Computational analysis of fluorescence loss in photobleaching (FLIP) experiments [ABSTRACT # 8] 18:00 Zoltan Cseresnyes, HKI, Jena, Germany Quantitative image analysis of label-free cells [ABSTRACT # 56] Imaging of Cell Biological Phenomena Using Different Imaging Techniques (16:20 - 18:20), Hörsaal 2 Session Chair: Ralph Jungmann Chiara Gramaccioni, Univ. of Cosenza, Arcavata di Rende, Italy 16:20 Nanotomography and X-ray fluorescence microscopy for quantitative Iron concentration map in inflamed cells [ABSTRACT # 11] 16:40 Maximilian Gorelashvili, University Hospital Würzburg, Würzburg, Germany Light sheet fluorescence microscopy (LSFM) based quantitative structural analysis of megakaryocytes in intact murine bone [ABSTRACT # 86] 17:00 Sebastian Kruss, Göttingen University, Göttingen, Germany Near infrared chemical imaging of small molecules [ABSTRACT # 25] 17:20 Michael Müller, Universitätsmedizin Göttingen, Göttingen, Germany Quantitative imaging of subcellular redox-dynamics in complex preparations [ABSTRACT # 71] 17:40 Eva Kreysing, FZ Juelich, Juelich, Germany Quantitative measurement of action-potential-induced dynamics at the cell-substrate interface using Surface Plasmon Resonance microscopy [ABSTRACT # 51] 18:00 Dirk-Peter Herten, Universität Heidelberg, Heidelberg, Germany From Super-Resolution to Quantitative Microscopy [ABSTRACT # 95] Conference dinner (18:30-22:00) Max Planck Institute for Solar System Research (fover)

Justus-von-Liebig-Weg 3 37077 Göttingen

### Saturday, 06.01.2018

#### X-Ray and Advanced Microscopy (9:00 - 10:40), Hörsaal 1

Session Chair: Martin Hof

- 9:00 **Theo Lasser (Keynote Lecture)**, École Polytechnique Fédérale de Lausanne, Lausanne, Switzerland VOIR fait SAVOIR [ABSTRACT # K]
- 9:50 Ana Diaz (Invited Speaker), Paul Scherrer Institut, Villigen, Switzerland Three-Dimensional Absolute Density Mapping of Biological Matter on the Nanoscale with Coherent X-rays [ABSTRACT # L]
- 10:20 Thomas Jovin, Max Planck Institute for Biophysical Chemistry, Göttingen, Germany Adjustable Enhanced ("super") Resolution With A Multiaperture, Rapid Optical-sectioning Fluorescence Microscopy Module ("iPAM") [ABSTRACT # 10]

Coffee Break (10:40 - 11:10)

**High Speed Imaging, Single Molecule Imaging and Super-resolution** (11:10 - 12:40), **Hörsaal 1** Session Chair: Theo Lasser

11:10 *Hari Shroff* (Invited Speaker), National Institute of Biomedical Imaging and Bioengineering, Bethesda, Maryland, United States

High Speed Imaging At And Beyond The Diffraction Limit [ABSTRACT # M]

- 11:40 **Philipp Kukura (Invited Speaker)**, University of Oxford, Oxford, United Kingdom Single Molecule Imaging Mass Spectrometry in Solution [ABSTRACT # N]
- 12:10 **Peter Dedecker (Invited Speaker)**, University of Leuven, Leuven, Belgium Sub-diffraction imaging of cellular biosensors [ABSTRACT # 0]

Lunch (12:40 - 13:40)

Parallel Sessions

#### Minisymposium: Digital Microscopy and Image Informatics (13:40 - 15:20), Hörsaal 1

Session Chair: Sripad Ram

- 13:40 Sripad Ram, Pfizer, Inc, San Diego, United States
   Advanced Image Analytics For Characterizing The Type And Distribution Of Immune Cells In The Tumor
   Microenvironment [ABSTRACT # 102]
- 14:00 **Yinyin Yuan**, Institute for Cancer Research, United Kingdom Deciphering the tumor ecosystem with histology image analysis [ABSTRACT # 99]

- 14:20 Nasir Rajpoot, University of Warwick, Coventry, United Kingdom Mining for histology footprints of cancer subtypes [ABSTRACT # 97]
- 14:40 Lars Pedersen, Visiopharm, Denmark Advanced virtual multiplexing [ABSTRACT # 98]
- 15:00 **France Rose**, Institut de Biologie de l'École Normale Superieure (IBENS), Paris, France Quantifying the spatial heterogeneity of cell responses to cancer drugs [ABSTRACT # 72]

Experimental Techniques for Single Molecule Microscopy (13:40 - 15:20), Hörsaal 2

Session Chair: Philipp Kukura

- 13:40 **Kristýna Holanová**, Institute of Photonics and Electronics of the AS CR, v. v. i, Prague, Czech Republic High-fidelity fast tracking of protein motion [ABSTRACT # 33]
- 14:00 **Kyle Douglass**, EPFL, Lausanne, Switzerland Autonomous and adaptive illumination for the real-time control of fluorescence photodynamics [ABSTRACT # 32]
- 14:20 Christiaan Hulleman, Delft University of Technology, Delft, The Netherlands
   Generating linearly polarized light in epifluorescence microscopes for cryogenic super-resolution [ABSTRACT # 37]
- 14:40 Keith Lidke, University of New Mexico, Albuquerque, United States
   Multi-structure super-resolution imaging using sequential imaging and DNA strand displacement [ABSTRACT # 55]
- 15:00 **Roman Tsukanov**, Universität Göttingen, Göttingen, Germany Nanometer axial colocalization of single emitters using metal-induced energy transfer [ABSTRACT # 49]

Coffee Break and Poster Session (15:20 - 16:50)

Parallel Sessions

**Single Molecule Microscopy in Applications and Super-resolution** (16:50 - 18:10), **Hörsaal 1** Session Chair: Jörg Enderlein

16:50 **Eyal Nir**, Beer Sheva University, Beer Sheva, Israel Imaging a computer controlled fast and processive DNA bipedal walker [ABSTRACT # 48]

17:10 **Sebastian Isbaner**, Georg-August-University, Göttingen, Germany Superresolution upgrade for confocal spinning disk systems [ABSTRACT # 14]

17:30 **Diane Lidke**, University of New Mexico, Albuquerque, United States Optimized single molecule pull-down (SiMPull) reveals heterogeneity in EGFR phosphorylation [ABSTRACT # 43] 17:50 **Yoav Shechtman**, Technion, Israel Institute of Technology, Haifa, Israel Three-dimensional tracking of DNA loci in living cells using a large-depth range Tetrapod point-spread-function [ABSTRACT # 119]

Analytical Techniques for Single Molecule Microscopy II (16:50 - 18:10), Hörsaal 2 Session Chair: Hari Shroff

Session Chair: Hari Shroff

- 16:50 **Daniel Nino**, University of Toronto, Toronto, Canada Molecular counting from fluorophore blinking statistics [ABSTRACT # 5]
- 17:10 **Zach Marin**, University of Auckland, Auckland, New Zealand Simulating (F)PALM/(d)STORM data based on measured photokinetic properties [ABSTRACT # 40]
- 17:30 Johannes Hohlbein, Wageningen University and Research, Wageningen, The Netherlands Phasor based single-molecule localization microscopy in 3D (PSMLM-3D): an algorithm for MHz localization rates using standard CPUS [ABSTRACT # 89]
- 17:50 **Rasmus Thorsen**, TU Delft, Delft, The Netherlands Photons count underestimation in single molecule imaging [ABSTRACT # 58]

Conference Closing (18:10 – 18:30), Hörsaal 1

18:10 **Conference Closing** by **Raimund Ober & Jörg Enderlein** Best Poster Prize QBI 2018 Announcement

Social gatherings in restaurants (18:30 - ... )

# **Poster schedule**

**Topic**:Single molecule - General theory, Super-resolution-Experimental techniques,Fluorescence correlation spectroscopy**Thursday 04.01, 15:00-16:30** 

Abstract ID	Titel	Authors	Presenter
1	Quality of biological images, reconstructed using localization microscopy data	Blazej Ruszczycki, Tytus Bernas	Blazej Ruszczycki
15	An integrated platform for rapid semi-confocal imaging and spatially resolved fluctuation microscopy	Adal Sabri, Andreas Veres, Matthias Weiss	Adal Sabri
77	Model based adaptive optics for single molecule localization microscopy	Carlas Smith	Carlas Smith
78	Performance of dual-focus fluorescence correlation spectroscopy (2fFCS) in highly scattering and aberrating media	Aditya Katti, Joerg Enderlein, Antoine Delon	Aditya Katti
84	Brownian Dynamics Simulation of Semiflexible Peptides	Steffen Mühle	Steffen Mühle
100	Leaflet-dependent diffusion in lipid bilayers using Metal-Induced Energy Transfer Fluorescence Lifetime Correlation Spectroscopy (MIET-FLCS)	Akshita Sharma, Narain Karedla, Sebastian Isbaner, Arindam Ghosh, Ingo Gregor, Jörg Enderlein	Akshita Sharma
110	A hidden Markov model approach to characterising the photo-switching behaviour of fluorophores	Ed Cohen, Lekha Patel	Ed Cohen
121	The utility of quantitative bio-imaging analysis to develop a Direct Immunofluorecence miscroscopy tools for cutanuous Leishmaniasis diagnosis	Nasreddine Saïdi, Meriem Benabid, Thouraya Boussofara, Ines Ben Sghaier, Aïda Bouratbine, Yousr Galaï	Nasreddine Saïdi
129	Direct Measurement Of Protein-protein Interactions And Dynamics At Cell-cell Adhesion Sites Via Fluorescence Fluctuation Spectroscopy	Valentin Dunsing, Magnus Mayer, Filip Liebsch, Gerhard Multhaup, Salvatore Chiantia	Valentin Dunsing
130	Multimodal Investigation Of The Spindle Alignment Machinery: From Fast Dynamics To Super-resolution Structure	Allen Leary, Eric Yen, Kristy Shulist, Jackie Vogel	Allen Leary
134	Direct analysis of oligomerization and nuclear shuttling dynamics of viral proteins studied by molecular brightness analysis and FCS	Madlen Luckner, Valentin Dunsing, Salvatore Chiantia, Andreas Herrmann	Madlen Luckner
132	Fluorescence Lifetime Correlation Spectroscopy (FLCS) of microsecond rotational isomerization in a fluorescent protein	Arindam Ghosh, Sebastian Isbaner, Manoel Veiga, Ingo Gregor, Jörg Enderlein, Narain Karedla	Narain Karedla
134	Direct analysis of oligomerization and nucle ar shuttling dynamics of viral proteins studied by molecular brightness analysis and FCS	Madlen Luckner, Valentin Dunsing, Salvatore Chiantia, Andreas Herrmann	Madlen Luckner

# **Topic**: Single molecular tracking, Single molecule clustering, Cell Biology: Experimental techniques, Instrumental advances for Cell imaging **Thursday 04.01, 18:30-22:00**

Abstract ID	Titel	Authors	Presenter
47	UNIVERSAL MODULAR SUBSYSTEM FOR 3D LOCALIZATION BASED IMAGING AND TRACKING USING ENGINEERED POINT SPREAD FUNCTIONS	Anurag Agrawal, Scott Gaumer, Katie Heiser, Rafael Piestun, Leslie Kimerling	Anurag Agrawal
52	Quantitative measurement of action-potential- induced dynamics at the cell-substrate interface using Surface Plasmon Resonance Microscopy	Eva Kreysing	Eva Kreysing
57	Reflected Light Scanning Focused Refractive Index Microscopy of Cultured Cells	Hossein Hassani, Eva Kreysing, Andreas Offenhäusser	Hossein Hassani
59	Single particle tracking inside biological fibers	Jacqueline He, Sean Andersson, Matthew Jacobsen, Gwen Hoffmann, Michael Smith	Sean Andersson
67	Combining density-based artifact-free and Bayesian cluster analysis tools for studying the characteristics of membrane-protein organization	Saskia Kutz, Helge Ewers	Saskia Kutz
68	Analysis of the lipid distribution of the neuronal membrane using Time-of-flight secondary ion mass spectrometry (ToF-SIMS) imaging	Nhu Phan, Angle de Castro, Silvio Rizzoli	Nhu Phan
70	DNA origami as a nanoscale platform for studying T- cell activation	Joschka Hellmeier, Eva Sevcsik	Joschka Hellmeier
90	Measuring the Absolute Degree of Labeling for Self- Labeling Protein Tags	Klaus Yserentant, Felix Braun, Siegfried Hänselmann, Wioleta Chmielewicz, Dirk-Peter Herten	Klaus Yserentant
131	Characterization of calcium signals in astrocytes from intensity based fluorescence indicators	Volodymyr Cherkas, Gebhard Stopper, Franziska Müller, Andre Zeug	Andre Zeug
133	Kinetic Imaging of Cholesterol Trafficking between Pla sma Membrane and Late Endosomes	Alice Dupont, Frederik W. Lund, Maria Louise V. Jensen, Lukasz M. Solanko, Maria Szomek, Katarzyna A. Solanko, Maciej Modzel, Ahmed S. Mehadi, Gitte K. Nielsen, Christian W. Heegaard and Daniel Wüstner	Alice Dupont
135	AUTOMATED DETECTION, TRACKING AND MOTION ANALYSIS OF CYTOSKELETAL KERATIN FILAMENTS IN MICROSCOPIC TIME -LAPSE FLUORESCENCE RECORDINGS	Dmytro Kotsur, Roman Yakobenchuk, Julian Mattes, Rudolf E. Leube, Reinhard Windoffer	Reinhard Windoffer

**Topic**: Single molecule theory: 3D, Applications of single molecular imaging, Software, Cell Biology: Applications, Analysis of cell biological data, Other advanced Imaging techniques **Friday 05.01, 15:00-16:20** 

Abstract ID	Titel	Authors	Presenter
18	Tracking the Dynamics of the Endoplasmic Reticulum	Konstantin Speckner, Lorenz Stadler, Matthias Weiss	Konstantin Speckner
26	Extraction of biophysical parameter sets from label- free quantitative phase microscopy images for cell culture quality assessment	Lena Kastl, Michael Isbach, Dieter Dirksen, Jürgen Schnekenburger, Björn Kemper	Björn Kemper
27	Kinetic Requirements for Spatiotemporal Chemical Imaging with Fluorescent Nanosensors	Daniel Meyer, Annika Hagemann, Sebastian Kruss	Daniel Meyer
28	Nanosensor bioconjugates and their application in imaging	Florian Mann, Niklas Herrmann, Elena Polo, Daniel Meyer, Sebastian Kruss	Florian Mann
30	NANOSCOPY ON-A-CHIP FOR WIDE FIELD-OF-VIEW CELL OBSERVATIONS	Anders Engdahl, Robin Diekmann, Thomas Huser, Mark Schüttpelz	Anders Engdahl
35	Imaging of chromatin and membrane reorganization during NET formation	Elsa Neubert, Daniel Meyer, Michael P. Schön, Luise Erpenbeck, Sebastian Kruss	Elsa Neubert
44	Activity-dependent nanoscale distribution of GABAARs and GlyRs at mixed synapses using quantitative SMLM.	Xiaojuan Yang, Felipe Delestro, Auguste Genovesio, Antoine Triller, Christian Specht	Xiaojuan Yang
45	Ultra High throughput, single molecule mapping of replicating DNA	Nikita Menezes Braganca, Francesco De Carli, Wahiba Berrabah, Valérie Barbe, Auguste Genovesio, Olivier Hyrien	Nikita Menezes Braganca
53	Development of a distance-based scoring method for fungal soil pathogen dispersion in digital micrographs	Arni Gambe-Gilbuena	Arni Gambe-Gilbuena
65	In vivo quantification of arthritis-induced alterations of murine bones based on PET/CT image data	Bianca Hoffmann, Carl- Magnus Svensson, Maria Straßburger, Hans Peter Saluz, Marc Thilo Figge	Bianca Hoffmann
66	Polymer brushes in motion-measuring flow profiles with nanometre resolution	Jan Christoph Thiele	Jan Christoph Thiele
69	Segmentation of dark field images from scanning X-ray micro-diffraction	Chiara Cassini, Andrew Wittmeier, Sarah Köster	Chiara Cassini
80	Data-standards for fluorescence microscopy: increasing data quality and fidelity for biological measurements	Maximiliaan Huisman, Carlas Smith, Mathias Hammer, David Grunwald	Maximiliaan Huisman
81	Identification of Drebrin as an I-band sarcomere component	Christina Jayachandran, Irina Majoul, Elena Ciirdaeva, Malte Tiburcy, Dieter Klopfenstein, Wolfram-	Eugenia Butkevich

		Hubertus Zimmermann, Christoph Schmidt, Eugenia Butkevich	
113	A software framework for meta-optimization of data analysis techniques with application to single molecule data analysis	Anish Abraham, Jerry Chao, David Kim, Sally Ward, Raimund Ober	Raimund Ober
125	Concept Of Image-based Biomarkers For A Prospective Personalized Radiation Therapy	Bettina Heise, Tanja Etzelstorfer, Hans Geinitz	Bettina Heise
126	Fairsim – A Modular Open-source Solution For 2d And 3d Sim Reconstruction	Marcel Müller, Mario Lachetta, Andreas Markwirth, Viola Mönkemöller, Wolfgang Hübner, Peter Dedecker, Mick Phillips, Ian Dobbie, Lothar Schermelleh, Thomas Huser	Marcel Müller

**Topic**: Single molecular experimental techniques, Machine learning, Camera characterization, Single molecular applications

Saturday 06.01,	15:20-16:50
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Abstract ID	Titel	Authors	Presenter
16	Assessing the Spatial Heterogeneity of Crowding via Imaging and FRET	Claudia Donth, Matthias Weiss	Claudia Donth
38	Photon yield enhancement of red fluorophores at cryogenic temperatures	Christiaan Hulleman, Weixing Li, Ingo Gregor, Bernd Rieger, Jörg Enderlein	Christiaan Hulleman
62	Machine learning supported image analysis of microfluidic droplets: Using Random Forest classifiers and Bayesian inference for identification of experimental conditions	Carl-Magnus Svensson, Oksana Shvydkiv, Lisa Mahler, Stefanie Dietrich, Thomas Weber, Mahipal Choudhary, Miguel Tovar, Martin Roth, Marc Thilo Figge	Carl-Magnus Svensson
73	Multi-plane 3-color Super Resolution Optical Fluctuation Imaging (SOFI)	Soheil Mojiri, Ingo Gregor, Joerg Enderlein	Soheil Mojiri
75	Dynamics using Metal Induced Energy Transfer (DynaMIET): Probing Nanoscale Biomolecular Dynamics at Single-Molecule Level	Arindam Ghosh, Roman Tsukanov, Narain Karedla, Alexey Chizhik, Ingo Gregor, Joerg Enderlein	Arindam Ghosh
82	Competition and cooperation between Drebrin-like protein and tropomyosin within the sarcomere	Eugenia Butkevich	Eugenia Butkevich
83	Investigating complex formation in the alternative NLRP3 inflammasome pathway by Raster image correlation spectroscopy	Ivo M. Glück, Thomas S. Ebert, Moritz M. Gaidt, Veit Hornung, Don C. Lamb	Ivo M. Glück
91	DNA Bipedal Motor Walking Dynamics: Experimental and Theoretical Study of the Dependency on Step Size	Dinesh Chandra Khara, John S. Schreck, Tommy E. Tomov, Yaron Berger, Thomas E. Ouldridge, Jonathan P. K. Doye, Eyal Nir	Dinesh Chandra Khara
93	Machine Learning to Reconstruct 3D Scattering Data from Partially Coherent Imaging Data	Benedict Diederich	Benedict Diederich
94	Activity-induced ionotropic glutamate receptor dynamics at super-resolution in vivo	DIVYA SACHIDANANDAN, SINA WÄLDCHEN, NADINE EHMANN, MARKUS SAUER, ROBERT KITTEL	DIVYA SACHIDANANDAN
106	One Man's Noise is Another's Signal Quantifying DiffusionDynamics of GPCRs using Fluorescence Correlation Spectroscopy	Ashwin Balakrishnan, Katrin Heinze	Ashwin Balakrishnan
114	Validation of remote focusing multifocal plane microscopy for high accuracy 3D tracking of single molecule with cellular context	Sungyong You, Sreevidhya Ramakrishan, Jerry Chao, David Kim, Anish Abraham, Dongyoung Kim, Sally Ward, Raimund Ober	Sungyong You
116	LEAF MICRO-MORPHOLOGY OF THE WEST AFRICAN SPECIES OF TRAGIA PLUM. EX L. (EUPHORBIACEAE)	Akeem Kadiri, Dele Olowokudejo	Akeem Kadiri

	AND ITS SYSTEMATIC RELEVANCE		
120	Deciphering the Receptome by dSTORM	Ralph Götz, Patrick Eiring, Alexander Kuhlemann, Sören Doose, Markus Sauer	Ralph Götz
122	Better than a lens	Jan Becker, Polina Feldmann, Christian Karras, Walter Müller, Ronny Förster, Rainer Heintzmann	Jan Becker
127	Holostorm: A New Approach Of High Spatial Resolution Imaging For The Characterization Of Intracellular Infections	Alexander Jügler, Patrick Then, Rainer Heintzmann	Alexander Jügler
128	Enhancing And Validating The Performance And Applicability Of Sofi Using New Measurement And Analysis Strategies	Wim Vandenberg, Peter Dedecker	Wim Vandenberg

### **Abstracts**

#### Abstract #A

### **A MINimum for Maximum Resolution**

#### Stefan W. Hell

Max Planck Institute for Biophysical Chemistry, Göttingen, GER

Max Planck Institute for Medical Research, Heidelberg, GER

A fresh look at the foundations [1] of fluorescence nanoscopy, also known as super-resolution microscopy, shows that an in-depth description of the basic principles spawns new powerful concepts such as MINFIELD [2], MINFLUX [3] and DyMIN [4]. Although they differ in some aspects, these concepts harness a local intensity minimum (of a doughnut or a standing wave) for determining the coordinate of the fluorophore(s) to be registered. Most strikingly, by using an intensity minimum of the excitation light to establish the fluorophore position, MINFLUX nanoscopy has obtained the ultimate (super)resolution: the size of a molecule [3].

- [1] Hell, S.W. Far-Field Optical Nanoscopy. Science 316, 1153-1158 (2007).
- [2] Göttfert, F., Pleiner, T., Heine, J., Westphal, V., Görlich, D., Sahl, S.J., Hell, S.W.

Strong signal increase in STED fluorescence microscopy by imaging regions of subdiffraction extent. **PNAS** 114, 2125-2130 (2017).

- [3] Balzarotti, F., Eilers, Y., Gwosch, K. C., Gynnå, A. H., Westphal, V., Stefani, F. D., Elf, J., Hell, S.W. Nanometer resolution imaging and tracking of fluorescent molecules with minimal photon fluxes. Science 355, 606-612 (2017).
- [4] Heine, J., Reuss, M., Harke, B., D'Este, E., Sahl, S.J., Hell, S.W.

Adaptive-illumination STED nanoscopy. PNAS (advance online, 2017).

#### Liquid Tunable Microscopy.

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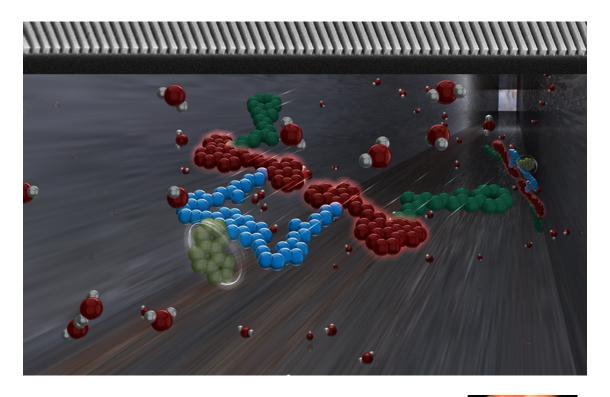
Taking inspiration from the philosophical and sociological speculation by Zygmunt Bauman (Bauman Z., Liquid modernity, Polity Press, Cambridge, 2000), a new paradigm for optical microscopy is proposed in terms of design, implementation and applications. Current advances in optical microscopy (Diaspro A., van Zandvoort M.A.M.J. (eds) Super-resolution Imaging in Biomedicine, CRC press, 2016), related to the accessibility of data at the nanoscale in living systems or in matter physics studies, made super resolved microscopy, label free approaches, time and space encoding and decoding strategies, single molecule imaging and tracking approaches impregnated with a liquidity capable of condensing in itself the most significant aspects of the status of the art; a new paradigm for microscopy (Diaspro A., Circumventing the diffraction limit, Il Nuovo Saggiatore, 2014). The Liquid Tunable Microscopy perspective is related to the integration of gated STED, light sheet microscopy, image scanning microscopy, expansion microscopy and label free "new" approaches in a unique architecture. Three different directions will be also discussed, namely: i) intrinsic fluorescence of biological macromolecules; II) converging technologies for fluorescence unlimited super resolved microscopy; iii) label free microscopy based on Mueller matrix signature coming from angular scattering processes and exploiting differential polarisation interactions and refractive index mismatches in the VIS-IR regions (Diaspro A. et al, Polarized Light Scattering of Nucleosomes and Polynucleosomes: in Situ and in Vitro Studies, IEEE Trans. Biomedical Engineering, 1991; Mazumder N., Diaspro A. et al, Mueller matrix signature in advanced fluorescence microscopy imaging, J. Opt, 2017). Such technologies will converge to the liquid tunable microscope. It is liquid because it overlaps in an efficient and optimised way different mechanisms of contrast and it is tunable because it offers a real time scalability in terms of spatial and temporal resolution like a radio tuned on the preferred radio station. It is smart because is able to adapt its architecture to the current scientific question and is open to additional light-matter interaction modules. The liquid tunable microscope will find its application in deciphering how macromolecular complexes dynamically change in structure and transiently interact each other to perform the vital functions of a cell towards the understanding human diseases.

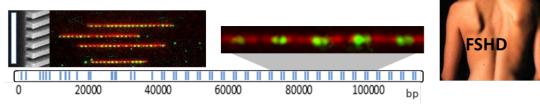
#### Super-resolution tracking of molecular motors and DNA in nanochannel arrays

#### Yuval Ebenstein

Department of Physical Chemistry, Tel-Aviv University, Tel-Aviv, Israel

Silicon nano channel arrays with channel cross section on the order of 50-100nm are now routinely fabricated and have recently been commercialized. We utilize single molecule imaging in nanochannels in order to track individual, light activated, molecular machines, and assess whether sufficient amount of work could be exerted by the motor to propel the nano machine and overcome the dominant viscosity forces in this extremely low Reynolds number regime. We report a two-fold enhancement in diffusion constant upon motor activation. In addition, nanochannels allow stretching individual DNA molecules by applying an electric field. By attaching fluorescent molecules to genetic and epigenetic marks on the DNA molecule, the stretched DNA is visualized as a pattern of fluorescent spots along the DNA molecules. We show how physical extension of long DNA molecules in nanofluidic channels reveals this information in the form of a linear, optical "barcode". Recent results from our lab demonstrate our ability to detect genetic structural variations (SVs), DNA repeats, and various forms of DNA epigenetic modifications. Applying super-resolution imaging techniques and accounting for DNA fluctuation dynamics allowed us to improve mapping precision 15 fold over commonly used techniques.





### Super-resolution imaging with DNA molecules

Ralf Jungmann

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Super-resolution fluorescence microscopy is a powerful tool for biological research, but obtaining multiplexed images for a large number of distinct target species in whole cells and beyond remains challenging. Here we use the transient binding of short fluorescently labeled oligonucleotides (DNA-PAINT, a variation of point accumulation for imaging in nanoscale topography) for simple and easy-to-implement multiplexed super-resolution imaging that achieves sub-5-nm spatial resolution in vitro on synthetic DNA structures.

We report a multiplexing approach (Exchange-PAINT) that allows sequential imaging of multiple targets using only a single dye and a single laser source. We experimentally demonstrate ten-color super-resolution imaging in vitro on synthetic DNA structures as well as four-color two-dimensional imaging and three-color 3D imaging of proteins in fixed cells.

Finally, we demonstrate whole cell imaging using DNA- and Exchange-PAINT and optical sectioning, now allowing DNA-based super-resolution imaging deep inside cells, away from the glass coverslip.

In combination with molecular barcoding based on engineered blinking kinetics, we strive to bring systems biology approaches to super-resolution microscopy by eventually detecting hundreds of molecular species simultaneously in single cells or tissues.

#### Reference Structures for Quantitative Microscopy

#### **Philip Tinnefeld**

TU Braunschweig – BRICS, Institut für Physikalische und Theoretische Chemie, BRICS -Braunschweig Institute for Systems Biology, LENA - Laboratory for Emerging Nanometrology Rebenring 56, 38106 Braunschweig

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In recent years, DNA nanotechnology has matured to enable robust production of complex nanostructures and hybrid materials. We have combined DNA nanotechnology with sensitive optical detection to create functional single-molecule devices that enable new applications for quantitative microscopy. After the development of nanorulers for superresolution microscopy (1), we present a new approach for sample stabilization and for fluorescence intensity and stoichiometry reference structures. Finally, we present DNA origami force spectroscopy that enables the application of forces to biomolecules without physical connection to the macroscopic world (2).

1. Schmied, J.J. et al. DNA origami-1. based standards for quantitative fluorescence microscopy. Nature protocols 9, 1367-1391 (2014).

2. Nickels, P.C. et al. Molecular force spectroscopy with a DNA origami-based nanoscopic force clamp. Science 354, 305-307 (2016).

### Monitoring self-organization events in the early embryogenesis of *Caenorhabditis elegans* with light sheet microscopy

Matthias Weiss

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Embryogenesis is a remarkably robust, but still poorly understood self-organization phenomenon. Due to its simplicity, high reproducibility, optical transparency, and well-characterized genetics, the small nematode *Caenorhabditis elegans* is a superb model organism to study self-organization events during early development. Using single plane illumination microscopy (SPIM), we have explored how physical cues determine the cell arrangement in early embryogenesis. In particular, we have shown that the coupling of cellular volumes and cell cycle times in combination with a mechanically guided arrangement process is key for a fail-safe embryogenesis of *C. elegans* [1,2]. Going beyond mere imaging, we have used pixel-wise fluorescence correlation spectroscopy (SPIM-FCS) to spatiotemporally quantify the diffusion of proteins in individual cells of the embryo in cytoplasm and on membranes [3]. As a result of our efforts, we were able to monitor the emergence of an intracellular diffusion gradient concomitant to the formation of a chemical polarity gradient that determines the first symmetry breaking in the embryo, namely the formation of the anterior-posterior body axis.

- [1] R. Fickentscher, P. Struntz & M. Weiss, Biophys. J. 105, 1805 (2013)
- [2] R. Fickentscher, P. Struntz & M. Weiss, Phys. Rev. Lett. 117, 188101 (2016)
- [3] P. Struntz & M. Weiss, J. Phys. D 49, 044002 (2016).

# Studying chromatin dynamics by advanced live cell imaging methods

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The DNA in a human cell which is ~3 meters long is packed in a tiny nucleus of ~10  $\mu$ m radius. Although it is dynamic, it is well organized. By using advanced microscopy methods for live cell imaging, we study the mechanisms that organizes the chromatin in the nucleus.

These methods allowed us to identify that a protein, lamin A, forms chromatin loops thereby restricting the chromatin dynamics significantly in the whole nucleus volume. The methods we use are based on measuring the signal and fluctuations of fluorescent molecules and the use of biophysical models based on Smoluchowski equation and modified diffusion equations. It allowed us to identify not only the type of the protein, but also its bound and free fractions, concentration and diffusion properties.

The methods includes single particle tracking, a new method that we have developed named time resolved intensity photobleaching (TRIP) as well as fluorescence correlation spectroscopy (FCS) and more. We also use single-molecule methods for verifying the DNA-protein interaction, including tethered particle motion (TPM) and atomic force microscopy (AFM). The methods, their use, the biophysical modeling and the biological insight will be described.

#### LIPID DRIVEN NANODOMAINS ARE FLUID

# R. Šachl<sup>1</sup>, M, Amaro<sup>1</sup>, A. Koukalová<sup>1</sup>, L.Veľas<sup>1</sup>, G. Gröbner<sup>2</sup>, P.T.F. Williamson<sup>3</sup>, and <u>M. Hof<sup>1</sup></u>

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KEYWORDS: FLIM-FRET, Monte Carlo, FCS, NMR, Ganglioside GM1, sphingomyelin

It is a fundamental question whether sphingomyelin (SM)- and cholesterol (Chol)- driven nanodomains exist in cells and in model membranes. Studies on model membranes revealed SM and Chol driven micrometer-sized liquid-ordered domains. Although the existence of such microdomains has not been proven for the plasma membrane, such lipid mixtures have been used as a model system for 'rafts'. On the other hand, super resolution results indicate that the plasma membrane might organize into nanocompartments. However, due to the limited resolution of those techniques their unambiguous characterization is still missing. In this lecture, a combination of Förster resonance energy transfer and Monte Carlo simulations (MC-FRET) [1] identifies directly 10 nm large nanodomains in liquid-disordered model membranes composed of lipid mixtures containing SM and Chol [2]. Our MC-FRET approach can determine the sizes and concentrations of nanodomains down to 2 nm and enables studying the nanodomain inter-leaflet coupling. Combining MC-FRET with solid-state wide-line and high resolution magic angle spinning NMR as well as with fluorescence correlation spectroscopy [3] we demonstrate that these nanodomains containing hundreds of lipid molecules are fluid [2]. Addition of GM1 ganglioside, a molecule which forms already fluid 6 nm sized clusters in fluid phosphatidylcholine bilayers [4,5], leads to growth of those nanodomains while preserving the fluidity [5]. Similarly, addition of oxidized phospholipids increases the size of the nanodomains. Our MC-FRET approach indicates that the latter nanodomains are registered in between the leaflets

[1] R. Šachl et al. Biophys. J., 101, L60-L62, 2011

[2] A. Koukalová et al. Scientific Rep. vol. 7 p. 5460, 2017.

<sup>[3]</sup> R. Šachl et al. J. Phys. D 49 189601, 2016

<sup>[4]</sup> R. Šachl et al. BBA- Mol. Cell Res. 1853, 850-857, 2015.

<sup>[5]</sup> M. Amaro et al. Angew. Chem. vol. 55 p. 9411-9415, 2016.

Label-free imaging of cellular dynamics at 100Hz and 140nm resolution

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Many new, exciting imaging techniques have emerged during the last decade, providing significantly improved spatial resolution and contrast. However, this extra information comes at the cost of more photons required to illuminate the cell, which requires more time and energy and often damages biological structures. The smaller the structures to be investigated, the faster they usually move inside living cells, because of both Brownian motion and coordinated work of molecular motors. Therefore, alternative imaging approaches are necessary.

In this talk I will present a novel technique called rotating coherent scattering (ROCS) microscopy. This imaging method is characterized by label-free, coherent imaging through scattering of a rotating laser beam, which reveals a variety of unexpectedly fast processes inside/of living cells. The technique operates at up to 100 Hz with a spatial resolution of currently 140nm, it can acquire thousands of images without loss in image quality and does not require postprocessing, such that the cells can be observed online.

#### **VOIR fait SAVOIR**

#### Theo Lasser

#### École Polytechnique Fédérale de Lausanne, Lausanne, Switzerland

Super-resolution optical fluctuation imaging (SOFI) provides an elegant concept for 3D super-resolution imaging. We intend to expand the scope of this imaging technique based on new applications in life sciences and medicine.

As a first example we exploit the higher order cumulant statistics of SOFI, which allows to assess quantitatively the receptor distribution and clustering on T-cells. In a further extension we combine SOFI with a novel label-free white light quantitative phase tomography to provide high-speed 3D imaging (>100 Hz) and spatial super-resolution. Finally we would like to report on our recent progress concerning the gut-Alzheimer Disease link. This project demands a realm of optical techniques ranging from functional brain imaging to a novel way for a fast read-out of the microbiome. These selected examples based on new optical concepts demonstrate the growing potential of optical imaging for medicine and lifesciences.

Three-dimensional absolute density mapping of biological matter on the nanoscale with coherent X-rays

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Owing to their penetration length, hard X-rays with energies of some keV or, equivalently, with wavelengths between about 1 and 2 Ångstrom, are an interesting probe to image opaque samples of several tens of micrometers thickness with a few tens of nanometers resolution without having to physically cut them. Typical challenges in hard X-ray microscopy include the fabrication of good efficient lenses and the inherently low absorption contrast of materials. At high brilliance synchrotron sources we can select the coherent fraction of the beam and use a microscopy technique called X-ray ptychography in order to reconstruct the complex-valued transmissivity of the sample, providing both absorption and phase contrast, without the need of a lens [1]. Tomographic reconstruction of images acquired at different rotation angles finally yield three-dimensional (3D) density maps with accurate density values [2,3], which can reach isotropic spatial resolutions better than 15 nm in 3D for high contrast specimens [4].

At the Swiss Light Source we explore the application of X-ray ptychographic tomography for nondestructive microscopy of thick biological matter. Despite featuring very low contrast at hard X-rays, we have measured the 3D mass density distribution of frozen hydrated cell solutions, giving accurate mass densities for different organelles within the cells [5,6]. This method has been further used as one of the experimental proofs of the synthesis of starch in yeast [7]. More recently, we have imaged neuronal structures in a frozen hydrated mouse brain specimen where axons are clearly resolved in 3D [8].

In this presentation I will give an introduction to X-ray ptychographic tomography and present some recent results on biological specimens, with a special focus on the capability to map 3D mass density distributions on the nanoscale. Finally I will discuss the potential of the method in combination with other X-ray and electron microscopy techniques such as elemental mapping with X-ray fluorescence and transmission electron microscopy.

- [1] J. M. Rodenburg et al., Phys. Rev. Lett. 98, 034801 (2007)
- [2] M. Dierolf et al., Nature 467, 436 (2010)
- [3] A. Diaz et al., Phys. Rev. B 85, 020104(R) (2012)
- [4] M. Holler et al., Nature 543, 402 (2017)
- [5] A. Diaz et al., J. Struct. Biol. 192, 461 (2015)
- [6] A. Diaz et al., J. Struct. Biol. 193, 83 (2016)
- [7] B. Pfister *et al.*, eLife **5**, e15552 (2016)
- [8] S. Shahmoradian et al., just accepted in Sci. Rep. (2017)

#### High speed imaging at and beyond the diffraction limit

Hari Shroff

#### National Institute of Biomedical Imaging and Bioengineering National Institutes of Health Bethesda, MD USA

#### *E-mail: hari.shroff@nih.gov*

Keywords: Structured illumination microscopy, light sheet microscopy, adaptive optics, neurodevelopment, developmental biology

I will discuss our efforts to improve structured illumination microscopy (SIM) and light-sheet microscopy.

SIM doubles the spatial resolution of light microscopy, requiring lower light intensities and acquisition times than other super-resolution techniques. I will present SIM implementations that enable resolution doubling in live samples > 10-20x thicker than possible with conventional SIM, as well as hardware modifications that enable effectively 'instant' SIM imaging at rates 10-100x faster than other SIM. New applications of instant SIM, including combination with total internal reflection (TIRF) and with adaptive optics will also be discussed.

The second half of the talk will focus on the development of inverted selective plane illumination microscopy (iSPIM), and subsequent application to the noninvasive study of neurodevelopment in nematodes. I will discuss progress that quadruples the axial resolution of iSPIM by using a second specimen view, enabling imaging with isotropic spatial resolution (dual-view iSPIM, or diSPIM). Newer multiview microscopes with more objectives and more views, further improving spatial resolution, will also be described. Applications of these technologies will be presented, including computational methods for untwisting worm embryos and calcium imaging in freely moving embryos. Finally, I will describe efforts to quantitatively map the 'behaviorome' of freely moving embryos using light sheet microscopy.

Single molecule imaging mass spectrometry in solution

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Mesoscopic structure and dynamics are central to a variety of key processes in nature, such as crystallisation, force generation or metastasis. An impressive number of techniques have been developed over the past decades aimed at understanding the underlying mechanisms, leading to remarkable levels of knowledge with respect to both molecular and in some cases atomic structure as well as the overall kinetics of (dis)assembly of the relevant macromolecular complexes and structures. A distinct challenge, however, remains regarding our ability to directly visualise and thereby quantify these interactions and dynamics at the fundamental, single molecule level. I will show how ultrasensitive optical microscopy based on light scattering alone, in the form of interferometric scattering microscopy (iSCAT), can be used to study mesoscopic dynamics with single molecule sensitivity, specificity and resolution. I will illustrate these capabilities with studies of phase separation, interfacial dynamics and biological filaments and present recent results that demonstrate the capability of iSCAT to operate as a single molecule mass spectrometer in solution, which could be transformative for our ability to study protein-protein and protein-drug interactions.

#### Sub-diffraction imaging of cellular biosensors

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It is generally accepted that living cells make extensive use of spatial compartmentalization and structuring to limit the interactions that can occur, giving rise to e.g. signaling specificity. In this presentation I will discuss our recent work on trying to visualize biosensor responses in live cells with a spatial resolution below the diffraction limit. Our efforts thus far have centered on the use of stochastic optical fluctuation imaging (SOFI) [1] combined with engineered sensor variants that possess appropriate single-molecule fluorescence dynamics. After introducing the fundamentals of the imaging and the fluorophores, I will discuss two recent results. In the first study, we developed an interaction sensor based on bimolecular fluorescence complementation (BiFC), by creating variants of a photochromic fluorescent protein. Using this label we could visualize protein interactions at a spatial resolution of about 100 nm. In the second study, we developed a variant of AKAR (PKA activity reporter) that encoded the activity of PKA into changes in fluorescence dynamics. Using SOFI, we could read out this kinase activity with about 100 nm spatial resolution and a temporal resolution of a few second. In this way we observed dynamic PKA activity microdomains or 'hot spots', whose existence was corroborated with STORM-based experiments using antibodies selective for PKA phosphorylation sites.