



## 2017 Quantitative Bioimaging Conference

Texas A&M University, College Station, TX, USA

January 5 – 7, 2017



**Bethancourt Ballroom**  
**Memorial Student Center**

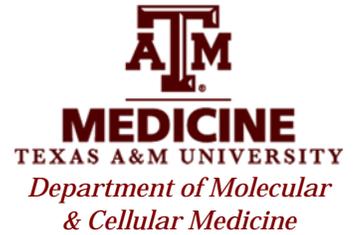
275 Joe Routt Blvd #2240  
College Station, TX 77843  
Phone: (979) 845-8901



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# Sponsors



## Exhibitors

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The Company of Biologists is a not-for-profit publishing organisation dedicated to supporting and inspiring the biological community. We are run by distinguished practicing scientists. We exist to profit science, not shareholders. We inspire new thinking and support the worldwide community of biologists.

We do this by publishing leading peer-reviewed journals, facilitating scientific meetings and communities, providing travel grants for young researchers and by supporting societies and gatherings.

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#### Disease Models & Mechanisms

[dmm.biologists.org](http://dmm.biologists.org)

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Journal of  
Cell Science

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Biology Open



## Introducing the SPINDLE™ 3D nano-scale imaging made accessible & affordable

### At a glance

Double Helix's innovative integrated image capture and image processing system gives researchers the ability to easily capture and analyze 3D images of cellular structures down to the single molecule level. Using Double Helix's patented DH-PSF phase mask technology as its foundation, 3D super-resolution imaging is now affordable for cutting-edge applications, including molecular level imaging and tracking.

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*Super-resolution Microscopy Cubes* set the new standard for laser based microscopes. These cubes are optimized for mounting flat super-resolution 1mm thick dichroics. Maximize SNR and minimize artifacts in TIRF, Confocal, PALM, STORM, SIM, and other super-resolution techniques.

Learn more and order at [semrock.com](http://semrock.com)

# Getting to and around College Station

## Traveling to College Station by plane

### Through Houston, Texas

**Route 1 (for all travelers):** Many major airlines offer flights to Houston's George Bush Intercontinental Airport (IAH). From IAH, United Airlines provides direct flights (approximately 1 hour) to College Station's Easterwood Airport (CLL), which is just 4 miles away from the conference venue (TAMU's Memorial Student Center). Alternatively, you can get to CLL by taking a shuttle from IAH. The shuttle service is provided by **Ground Shuttle**. Another option is to drive a rental car to College Station. The distance between IAH and CLL is approximately 100 miles.

**Route 2 (primarily for US domestic travelers):** Fly to Houston's William P. Hobby Airport (HOU), which provides primarily US domestic service, but offers a few international options. From HOU, you can get to College Station's Easterwood Airport (CLL) by Ground Shuttle (see Route 1). Alternatively, you can drive a rental car to College Station. The distance between HOU and CLL is approximately 110 miles.

### Through Dallas, Texas

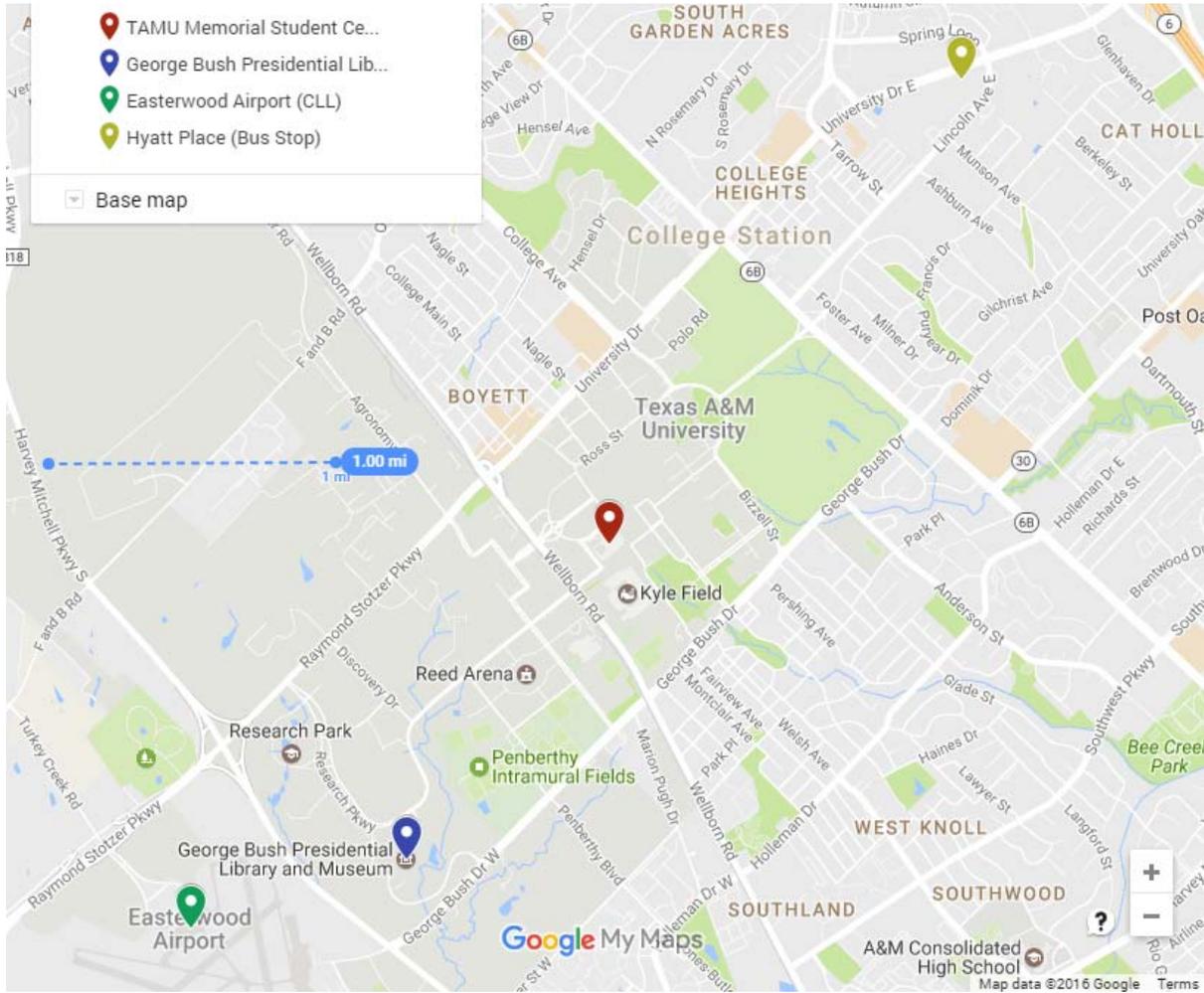
**Route 3 (for all travelers):** Many major airlines offer flights to Dallas/Fort Worth International Airport (DFW). From DFW, American Airlines provides direct flights (approximately 1 hour) to College Station's Easterwood Airport (CLL). Alternatively, you can drive a rental car to College Station. The distance between DFW and CLL is approximately 200 miles.

## Getting around Bryan/College Station

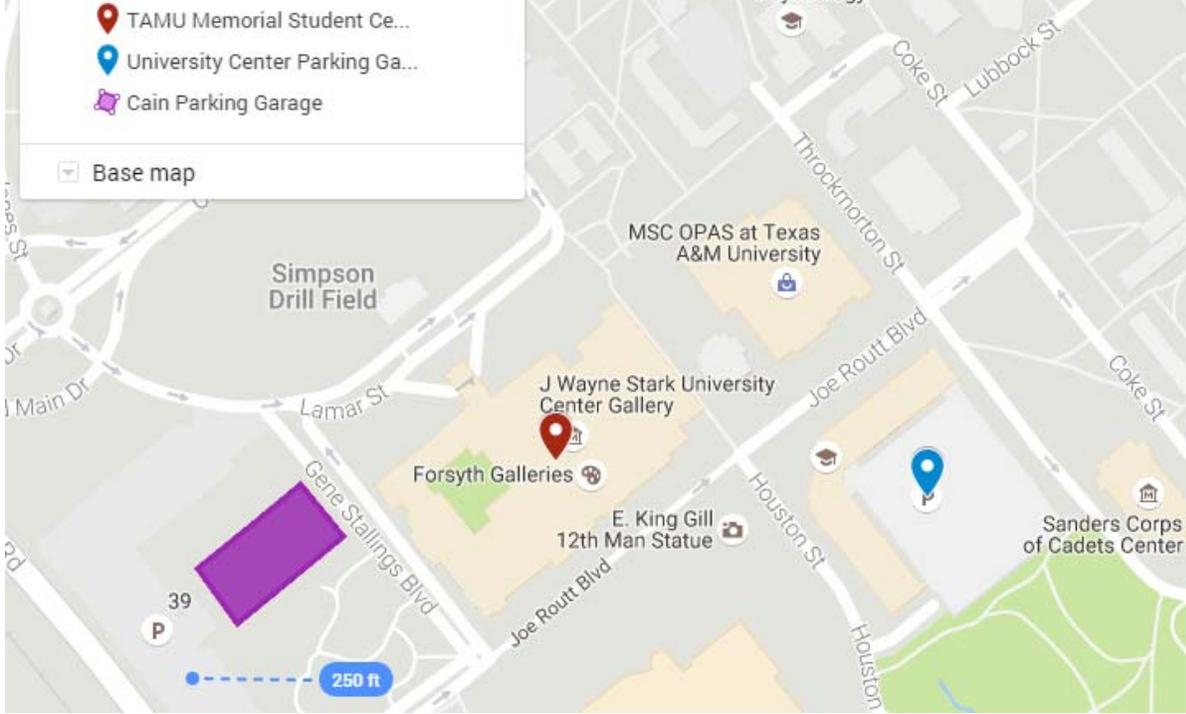
Many hotels offer free shuttles to and from College Station's Easterwood Airport (CLL). Uber and local taxi services are also available.

# Maps and Parking

## Major locations of interest for conference attendees



# TAMU Memorial Student Center, the Cain visitor parking garage, and the University Center visitor parking garage



Visitor parking rates (for reference only; actual rates may differ slightly) for the **University Center Parking Garage\*** and the **Cain Parking Garage^**:

Duration	6 am to 4:59 pm	5 pm to 5:59 am
Entry – 1 hour	\$2	\$1
1 hour – 2 hours	\$4	\$2
2 hours – 3 hours	\$6	\$3
Each additional hour	\$1	\$0.50

\*Maximum daily rate of \$15.

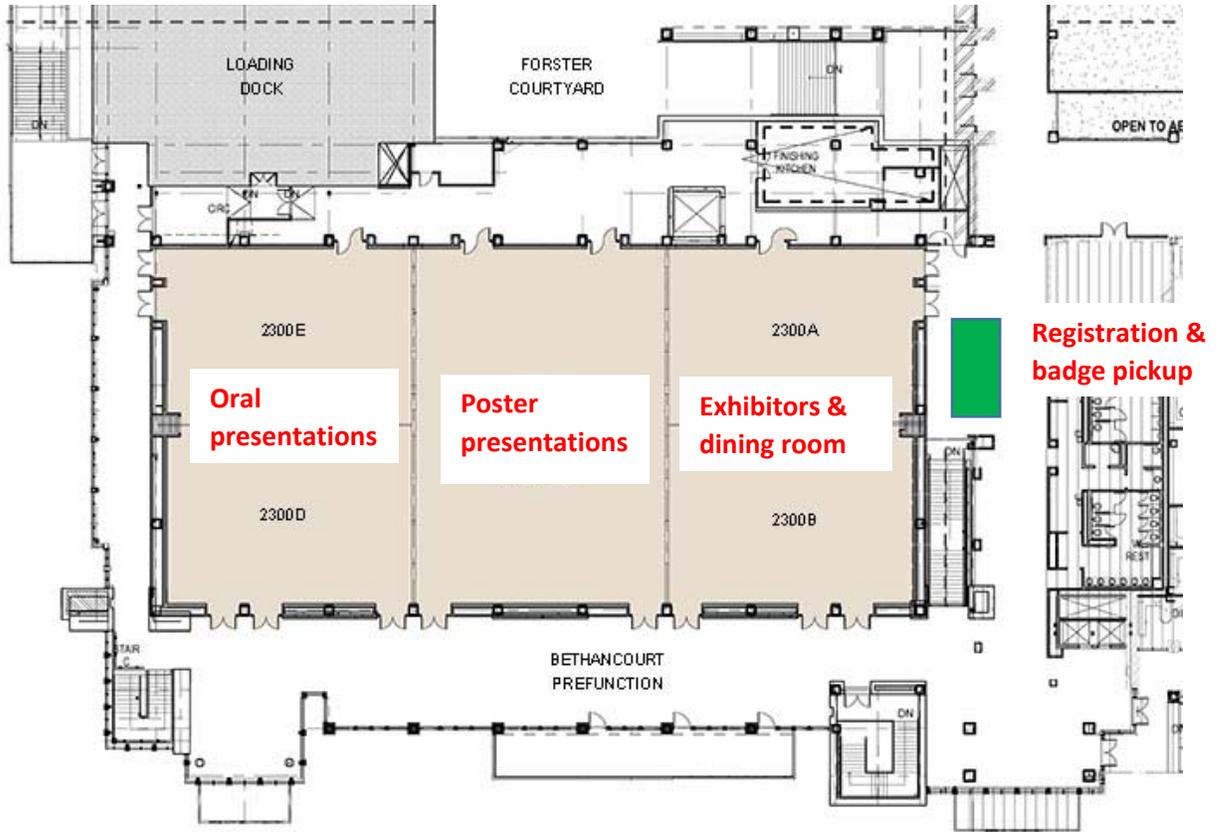
^No maximum daily rate.

Lost ticket fee: \$15.

Citation for inability to pay: \$40.

Payment: cash, American Express, MasterCard, Visa, and Discover accepted. No checks.

# Conference floor layout



# Conference Registration and Badge Pickup

## **Wednesday, Jan. 4, 2017**

5:00 pm to 7:00 pm

Hyatt Place, 1100 University Drive East, College Station, TX 77840

Refreshments provided

## **Thursday, Jan. 5, 2017 through Saturday, Jan. 7, 2017**

8:00 am to 6:00 pm

TAMU Memorial Student Center (conference site)

# Quantitative BioImaging Society

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

Hyatt Place, 1100 University Drive East, College Station, TX 77840

**8:30 pm, Friday, Jan. 6, 2017**

**All conference attendees are welcome to attend.**

Refreshments provided

# Conference Bus Schedule

## Thursday, January 5, 2017

7:30 am to 8:15 am

Hyatt Place to TAMU Memorial Student Center

The first and last buses are scheduled to leave Hyatt Place at approximately 7:30 am and 8:15 am, respectively. Buses in between will leave Hyatt Place approximately 15 minutes apart.

9:30 pm to 10:15 pm

TAMU Memorial Student Center to Hyatt Place

The first and last buses are scheduled to leave the Memorial Student Center at approximately 9:30 pm and 10:15 pm, respectively.

## Friday, January 6, 2017

7:45 am to 8:30 am

Hyatt Place to TAMU Memorial Student Center

The first and last buses are scheduled to leave Hyatt Place at approximately 7:45 am and 8:30 am, respectively. Buses in between will leave Hyatt Place approximately 15 minutes apart.

7:00 pm to 7:45 pm

TAMU Memorial Student Center to Hyatt Place

The first and last buses are scheduled to leave the Memorial Student Center at approximately 7:00 pm and 7:45 pm, respectively.

## Saturday, January 7, 2017

7:45 am to 8:30 am

Hyatt Place to TAMU Memorial Student Center

The first and last buses are scheduled to Hyatt Place at approximately 7:45 am and 8:30 am, respectively. Buses in between will leave Hyatt Place approximately 15 minutes apart.

**6:30 pm to 7:15 pm**

**TAMU Memorial Student Center to George Bush Library**

**The first and last buses are scheduled to leave the Memorial Student Center at approximately 6:30 pm and 7:15 pm, respectively.**

**9:30 pm to 10:15 pm**

**George Bush Library to TAMU Memorial Student Center to Hyatt Place**

**The first and last buses are scheduled to leave George Bush Library at approximately 9:30 pm and 10:15 pm, respectively.**

# Social Events

*All social events are free of charge to conference attendees. If you would like to bring a guest to the Conference Closing Reception and Dinner at the George Bush Library, please let us know by email to [inquiries@quantitativebioimaging.com](mailto:inquiries@quantitativebioimaging.com).*

## Registration and Social Gathering

Hyatt Place, 1100 University Drive East, College Station, TX 77840

**5:00 pm to 7:00 pm, Wednesday, Jan. 4, 2017**

**Refreshments provided**

## Conference Opening Reception and Dinner

TAMU Memorial Student Center (conference site)

**6:50 pm, Thursday, Jan. 5, 2017**

## Conference Closing Reception and Dinner

**(Tour of the George Bush Presidential Library and Museum)**

George Bush Presidential Library and Museum, 1002 George Bush Drive West, College Station, TX 77845

**6:25 pm to 10:00 pm, Saturday, Jan. 7, 2017**

# Meals and Refreshments

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

## Wednesday, January 4, 2017

5:00 pm to 7:00 pm

**Registration and Social Gathering**

Hyatt Place, 1100 University Drive East, College Station, TX 77840

**Refreshments**

## Thursday, January 5, 2017

10:30 am to 11:00 am

**Coffee Break**

TAMU Memorial Student Center (conference site)

**Coffee and snacks**

12:50 pm to 2:10 pm

**Lunch**

TAMU Memorial Student Center (conference site)

**Full meal**

3:40 pm to 5:10 pm

**Coffee Break and Poster Session**

TAMU Memorial Student Center (conference site)

**Coffee and snacks**

6:50 pm to 8:00 pm

**Conference Opening Reception and Dinner**

TAMU Memorial Student Center (conference site)

**Full meal**

## **Friday, January 6, 2017**

**10:30 am to 11:00 am**

**Coffee Break**

TAMU Memorial Student Center (conference site)

**Coffee and snacks**

**12:40 pm to 2:00 pm**

**Lunch**

TAMU Memorial Student Center (conference site)

**Full meal**

**3:30 pm to 5:00 pm**

**Coffee Break and Poster Session**

TAMU Memorial Student Center (conference site)

**Coffee and snacks**

**8:30 pm**

**QBI Society Meeting**

Hyatt Place, 1100 University Drive East, College Station, TX 77840

**Refreshments**

## **Saturday, January 7, 2017**

**10:20 am to 10:50 am**

**Coffee Break**

TAMU Memorial Student Center (conference site)

**Coffee and snacks**

**12:40 pm to 2:00 pm**

**Lunch**

TAMU Memorial Student Center (conference site)

**Full meal**

**3:20 pm to 4:40 pm**

**Coffee Break and Poster Session**

TAMU Memorial Student Center (conference site)

**Coffee and snacks**

**6:40 pm to 10:00 pm**

**Conference Closing Reception and Dinner**

George Bush Presidential Library and Museum,

1002 George Bush Drive West, College Station, TX 77845

**Dinner reception**

# Internet Access

The **TAMU\_Visitor** network is available to all guests at the TAMU Memorial Student Center (conference site). Take the following steps to connect:

1. Choose the TAMU\_Visitor network from the list of available networks on your device.
2. Enter an email address and a 24-hour password.
3. Enter your full name, mobile telephone number, and email address in the fields provided. Click the box next to “I accept the terms of use” and then click “Register”.
4. You will receive both an email and a text message with your TAMU\_Visitor username and password.
5. Enter the provided username and password. After a successful connection, you will be taken to the Texas A&M University web site.
6. You may close this connection browser window and use your device normally.

The **Eduroam** network is also available at the TAMU Memorial Student Center for those who have access provided by their participating institutions.

# Bulletin Boards

Bulletin boards for announcements, job advertisements, etc. will be available at the conference site.

# Keynote Speakers

## Ernst Stelzer

**Buchmann Institute for Molecular Life Sciences, Frankfurt, Germany**

Optical sectioning with light sheets forms the basis for highly corrected and fully automated microscopes [ABSTRACT # 97]

**9:00 am, Thursday, Jan. 5, 2017**

## Marino Zerial

**Max Planck Institute, Dresden, Germany**

Multi-scale analysis of cell and tissue organization

**9:00 am, Friday, Jan. 6, 2017**

## Robert Singer

**Albert Einstein College of Medicine, New York, USA**

Quantitation of the movements of single mRNA molecules in living cells

**9:00 am, Saturday, Jan. 7, 2017**

# Tutorials

## Dylan Owen

**King's College London, UK**

Statistical methods for the quantification of molecular coordinate distributions from single-molecule localisation microscopy data

**11:00 am, Thursday, Jan. 5, 2017**

## Karl Rohr

**University of Heidelberg, Germany**

Tracking of cells and particles

**2:10 pm, Thursday, Jan. 5, 2017**

## Carol Cogswell

**University of Colorado at Boulder, USA**

PSF engineering as a tool for three-dimensional super-resolution microscopy

**2:00 pm, Friday, Jan. 6, 2017**

# Software Workshop

**Thursday, Jan. 5, 2017, 8:00 pm**

**Jean-Christophe Olivo-Marin**

Institut Pasteur, Paris, France

**Keith Lidke**

University of New Mexico, Albuquerque, NM, USA

**Susan Cox**

King's College, London, UK

**Nico Stuurman**

University of California San Francisco, USA

**Winfried Wiegraebe**

Allen Institute for Cell Science, Seattle, USA

# Minisymposium

## Digital microscopy and image informatics

**Friday, Jan. 6, 2017**

### **Sripad Ram**

**Pfizer Inc.**

Introduction to digital microscopy and image informatics

**12:10 pm**

### **James Mansfield**

**Andor Technology Ltd.**

Imaging sensors for digital pathology, microscopy and intraoperative imaging

**5:20 pm**

### **Gustavo Rohde**

**University of Virginia, Charlottesville, USA**

Modeling information in images of cells and tissues: towards universal encoders

**5:50 pm**

### **Jens Rittscher**

**Oxford University, UK**

Towards screening more complex 3D cultures

**6:20 pm**

# Minisymposium

## Single molecule imaging approaches to nuclear pore complexes

**Saturday, Jan. 7, 2017**

### Siegfried Musser

**Texas A&M University Health Science Center, College Station, USA**

Introduction to workshop on single molecule approaches to nuclear pore complex biophysics

**9:50 am**

### Edward Lemke

**EMBL, Heidelberg, Germany**

Decoding molecular plasticity in the dark proteome of the nuclear pore complex

**10:50 am**

### Anton Zilman

**University of Toronto, Canada**

Simple biophysics of the collective conformational transitions of the intrinsically disordered proteins of the Nuclear Pore Complex [ABSTRACT # 95]

**11:10 am**

### Jan Liphardt

**Stanford University, USA**

Is the nuclear pore complex an active molecular pump with a cyclical operating cycle?

**11:30 am**

## David Grunwald

University of Massachusetts Medical School, Worcester, USA

Observing mRNA nuclear export in cells one RNA at a time

**11:50 am**

# Program

## Thursday

### Conference Opening (8:45 AM - 9:00 AM)

Session Chair: Raimund J. Ober

8:45 AM **Michael K. Young** *President, Texas A&M University.*  
Welcome Address

### Light Sheet and Structured Illumination Microscopy (9:00 AM - 10:30 AM)

Session Chair: Jörg Enderlein

9:00 AM **Ernst Stelzer** (*Keynote*) *Goethe University, Frankfurt, Germany.*  
Optical sectioning with light sheets forms the basis for highly corrected and fully automated microscopes [Abstract #97]

9:50 AM **Reto Fiolka** *UT Southwestern Medical Center, Dallas, USA.*  
Rapid and light efficient 3D microscopy [ABSTRACT # 15]

10:10 AM **Sjoerd Stallinga** *Delft University of Technology, The Netherlands.*  
Noise propagation in Structured Illumination Microscopy

### Coffee Break (10:30 AM - 11:00 AM)

### Single Molecule Techniques and Clustering (11:00 AM - 12:50 PM)

Session Chair: Keith Lidke

11:00 AM **Dylan Owen** (*Tutorial*) *King's College London, UK.*  
*Statistical methods for the quantification of molecular coordinate distributions from single-molecule localisation microscopy data*

11:30 AM **Bernd Rieger** *Delft University of Technology, The Netherlands.*  
Pushing resolution in super-resolution microscopy towards 1 nm

11:50 AM **Edward Cohen** *Imperial College London, UK.*  
New results in spatial statistics for single molecule microscopy

12:10 PM **Wenxiao Wang** *Rice University, Houston, USA.*  
Super temporal-resolved microscopy (STReM) [ABSTRACT # 66]

12:30 PM **Peter Kner** *University of Georgia, Athens, USA.*  
Wavefront Correction for Superresolution Microscopy

### Lunch (12:50 PM - 2:10 PM)

### Tracking (2:10 PM - 3:40 PM)

Session Chair: Jean-Christophe Olivo-Marin

- 2:10 PM **Karl Rohr** (Tutorial) *University of Heidelberg, Germany*  
Tracking of Cells and Particles
- 2:40 PM **Nancy Thompson** *University of North Carolina at Chapel Hill, USA.*  
DC-sign mediated dengue virus entry into cells [ABSTRACT # 34]
- 3:00 PM **Charles Kervrann** *Inria, Rennes, France*  
Classification of diffusion dynamics from particle trajectories [ABSTRACT # 33]
- 3:20 PM **Chris Calderon** *Ursa Analytics, Denver, USA.*  
Leveraging Time Series Analysis and Machine Learning to Quantify Intra and Inter Trajectory Heterogeneity in Particle Tracking Experiments

### Coffee Break and Poster Session (3:40 PM - 5:10 PM)

#### Parallel Sessions

### Microscopy Techniques Applied to Biological Problems (5:10 PM - 6:50 PM)

Session Chair: E. Sally Ward

- 5:10 PM **Emily Mace** *Baylor College of Medicine and Texas Children's Hospital, Houston, USA.*  
Non-diffraction limited microscopy demonstrates the role of local actin dynamism in NK cell effector function [ABSTRACT # 58]
- 5:30 PM **Alexey Zheltikov** *Texas A&M University, College Station, USA.*  
Fluorescent-imaging quantitative characterization of single-cell laser thermogenetic neurostimulation [ABSTRACT # 55]
- 5:50 PM **Beiyan Nan** *Texas A&M University, College Station, USA.*  
The actin homologue MreB and gliding motors show interdependent movements in the bacterium *Myxococcus xanthus* [ABSTRACT # 9]
- 6:10 PM **Munenori Ishibashi** *UT Health Science Center at Tyler, USA.*  
Fast cycling of integrin-clutch formation as revealed by single-molecule imaging [ABSTRACT # 45]
- 6:30 PM **Winfried Wiegraebe** *Allen Institute for Cell Science, Seattle, USA.*  
The Allen Institute for Cell Science: Building a microscope pipeline to map the human cell [ABSTRACT # 96]

### Light Sheet Microscopy (5:10 PM - 6:50 PM)

Session Chair: Sjoerd Stallinga

- 
- 5:10 PM **Hugh Sparks** *Imperial College London, UK.*  
Light sheet microscopy for high content 3-D imaging of 3-D tissue cultures in a 96-well plate format [ABSTRACT # 73]
- 
- 5:30 PM **Ryan McGorty** *University of San Diego, USA.*  
Selective-plane illumination differential dynamic microscopy [ABSTRACT # 5]
- 
- 5:50 PM **Alvin Yeh** *Texas A&M University, College Station, USA.*  
Multicolor selective plane illumination microscopy to image the dynamics and interactions of genetically defined lineages [ABSTRACT # 88]
- 
- 6:10 PM **Farzin Farzam** *University of New Mexico, Albuquerque, USA.*  
Automated multiple target superresolution imaging [ABSTRACT # 61]
- 
- 6:30 PM **Keith Lidke** *University of New Mexico, Albuquerque, USA.*  
Single objective light-sheet microscopy for high-speed whole-cell 3D super-resolution [ABSTRACT # 53]

**Dinner (6:50 PM - 8:00 PM)**

**Software Workshop (8:00 PM)**

*Session Chair: Raimund J. Ober*

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**Jean-Christophe Olivo-Marin** *Institut Pasteur, Paris, France.*

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**Keith Lidke** *University of New Mexico, Albuquerque, NM, USA.*

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**Susan Cox** *King's College, London, UK.*

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**Nico Stuurman** *University of California San Francisco, USA.*

Development and maintenance of Open Source software tools for the microscopy community [ABSTRACT # 21]

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**Winfried Wiegand** *Allen Institute for Cell Science, Seattle, USA.*

Building a microscope pipeline to map the human cell [ABSTRACT # 96]

# Friday

## Quantitation in Cell Biology (9:00 AM - 10:30 AM)

Session Chair: E. Sally Ward

9:00 **Marino Zerial (Keynote)** *Max Planck Institute, Dresden, Germany.*

AM Multi-scale analysis of cell and tissue organization

9:50 **Yannis Kalaidzidis** *Max Planck Institute, Dresden, Germany.*

AM Quantitative analysis of object colocalization in fluorescent microscopy images

10:10 **Mark Kittisopikul** *UT Southwestern Medical Center, Dallas, USA.*

AM Detecting junctions using spectral methods in orientation-scale space [ABSTRACT # 14]

## Coffee Break (10:30 AM - 11:00 AM)

## Quantitation in Cell Biology (Cont.) (11:00 AM - 12:10 PM)

Session Chair: Diane Lidke

11:00 **John Hancock** *University of Texas Health Science Center, Houston, USA.*

AM The K-Ras plasma membrane anchor encodes lipid-binding specificity that regulates signal output

11:30 **E. Sally Ward** *Texas A&M University Health Science Center, College Station, TX, USA.*

AM Cellular Antibody Trafficking

11:50 **Raimund J. Ober** *Texas A&M University, College Station, TX, USA.*

AM Multifocal Plane Microscopy for the Analysis of Subcellular Trafficking Pathways [ABSTRACT # 89]

## Digital Microscopy and Image Informatics (12:10 PM - 12:40 PM)

Session Chair: Raimund J. Ober

12:10 **Sripad Ram** *Pfizer Inc.*

PM Introduction to Digital Microscopy and Image Informatics

## Lunch (12:40 PM - 2:00 PM)

## PSF Engineering (2:00 PM - 3:30 PM)

Session Chair: Jörg Enderlein

2:00 **Carol Cogswell (Tutorial)** *University of Colorado at Boulder, USA.*

PM PSF engineering as a tool for three-dimensional super-resolution microscopy

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2:30 **Rafael Piestun** *University of Colorado at Boulder, USA.*

PM To Be Announced

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2:50 **Yoav Shechtman** *Technion-Israel Institute of Technology, Haifa, Israel.*

PM Optimal and multicolor 3D localization microscopy by point spread function engineering

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3:10 **Chrysanthe Preza** *University of Memphis, Memphis, USA.*

PM Implementation of PSF engineering using a fabricated submicron phase mask to reduce the effect of spherical aberration in 3D wide field fluorescence imaging [ABSTRACT # 52]

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### Coffee Break and Poster Session (3:30 PM - 5:00 PM)

### Marketing Presentation (5:00 PM - 5:20 PM)

Session Chair: Prashant Prabhat

5:00 **Marketing Presentation** *Oxford Nanoimaging*

PM

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### Parallel Sessions

### Single Molecule Techniques (5:20 PM - 7:00 PM)

Session Chair: Bernd Rieger

5:20 **Mohamedreza Fazel** *University of New Mexico, Albuquerque, USA.*

PM Multiple emitter fitting using reversible jump Markov Chain Monte Carlo [ABSTRACT # 60]

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5:40 **Lekha Patel** *Imperial College London, UK.*

PM Modelling and estimating the behaviour of photo-switching fluorophores [ABSTRACT # 51]

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6:00 **Hamidreza Heydari** *Delft University of Technology, The Netherlands.*

PM Lambda/100 resolution by template-free 2D particle fusion in localization microscopy [ABSTRACT # 3]

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6:20 **Christian Franke** *Universität Würzburg, Germany.*

PM Three-dimensional localization microscopy based on accurate intensity estimation [ABSTRACT # 10]

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### Digital Microscopy and Image Informatics (Cont.) (5:20 PM - 6:50 PM)

Session Chair: Sripad Ram

5:20 **James Mansfield** *Andor Technology Ltd.*

PM Multiplexing in Digital Pathology: The Whys and Hows

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5:50 **Gustavo Rohde** *University of Virginia, Charlottesville, USA*

PM Modeling information in images of cells and tissues: towards universal encoders

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6:20 **Jens Rittscher** *Oxford University, UK.*

PM Towards screening more complex 3D cultures

---

**Dinner in Town (Individually) (7:00 PM - 8:30 PM)**

**Hyatt Place Conference Room: Quantitative BioImaging Society Meeting (8:30 PM - 10:00 PM)**

# Saturday

## Single Molecule Applications in Biology (9:00 AM - 9:50 AM)

Session Chair: David Grunwald

9:00 AM **Robert Singer** (Keynote) *Albert Einstein College of Medicine, New York, USA.*  
Quantitation of the movements of single mRNA molecules in living cells

## Nuclear Pore Complex (9:50 AM - 10:20 AM)

Session Chair: David Grunwald

9:50 AM **Siegfried Musser** *Texas A&M University Health Science Center, College Station, USA.*  
Introduction to Workshop on Single Molecule Approaches to Nuclear Pore Complex Biophysics

## Coffee Break (10:20 AM - 10:50 AM)

## Nuclear Pore Complex (Cont.) (10:50 AM - 12:10 PM)

Session Chair: Siegfried Musser

10:50 AM **Edward Lemke** *EMBL, Heidelberg, Germany.*  
Decoding molecular plasticity in the dark proteome of the nuclear pore complex

11:10 AM **Anton Zilman** *University of Toronto, Canada.*  
Simple biophysics of the collective conformational transitions of the intrinsically disordered proteins of the Nuclear Pore Complex [ABSTRACT # 95]

11:30 AM **Jan Liphardt** *Stanford University, USA.*  
Is the nuclear pore complex an active molecular pump with a cyclical operating cycle?

11:50 AM **David Grunwald** *University of Massachusetts Medical School, Worcester, USA.*  
Observing mRNA nuclear export in cells one RNA at a time

## Poster Award Winner (12:10 PM - 12:30 PM)

12:10 PM **Max Huisman (2016 Poster Award Winner)** *University of Massachusetts Medical School, Worcester, USA.*  
Reconstructing Discrete Features Of Nucleocytoplasmic Transport Using Projected Density Maps

## Lunch (12:30 PM - 2:00 PM)

## Optics (2:00 PM - 2:40 PM)

Session Chair: Keith Lidke

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2:00 PM **Jörg Enderlein** *Georg-August-Universität, Göttingen, Germany.*  
Multiphoton image scanning microscopy [ABSTRACT # 2]

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2:20 PM **Tim Yeh** *University of Texas at Austin, USA.*  
Deep and high-resolution three-dimensional tracking of single particles using nonlinear and multiplexed illumination [ABSTRACT # 82]

**Single Molecule Techniques (2:40 PM - 3:20 PM)**

*Session Chair: Keith Lidke*

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2:40 PM **Aleksandra Radenovic** *EPFL, Lausanne, Switzerland.*  
The power of correlative super-resolution imaging

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3:00 PM **Suliana Manley** *EPFL, Lausanne, Switzerland.*  
Expanding Horizons with Large FOV, Automated Localization Microscopy

**Coffee Break and Poster Session (3:20 PM - 4:50 PM)**

Parallel Sessions

**Cameras, Detectors, and Single Molecule Microscopy (4:50 PM - 6:15 PM)**

*Session Chair: Raimund J. Ober*

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4:50 PM **Andrew Barentine** *Yale University, New Haven, USA.*  
Towards real-time localization of SCMOS single-molecule-switching data [ABSTRACT # 32]

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5:10 PM **Michael Hirsch** *Science and Technology Facilities Council, Didcot, UK.*  
Bayesian fluorophore separation measurement in unresolved PSF using realistic EMCCD noise model [ABSTRACT # 36]

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5:30 PM **Keith Bennett** *Hamamatsu Photonics K.K., Hamamatsu, Japan.*  
Quantitative evaluation of accuracy and variance of individual pixels in a Scientific CMOS (SCMOS) camera for computational imaging [ABSTRACT # 30]

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5:50 PM **Narain Karedla** *Georg-August-Universität, Göttingen, Germany.*  
Single-molecule metal induced energy transfer (smMIET): resolving nanometer distances at single molecule level [ABSTRACT # 1]

**Various Microscopy Techniques (4:50 PM - 6:10 PM)**

*Session Chair: Jörg Enderlein*

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4:50 PM **Andreea Trache** *Texas A&M Health Science Center, College Station, USA.*  
Regulation of integrin function studied by integrated optical and atomic force microscopy [ABSTRACT # 75]

5:10 PM	<b>Michal Pawlowski</b> <i>Rice University, Houston, USA.</i> Selected applications of image mapping spectrometry [ABSTRACT # 77]
5:30 PM	<b>YongKeun Park</b> <i>KAIST, Daejeon, South Korea.</i> 3D quantitative phase imaging techniques for non-invasive label-free imaging of cells and tissues [ABSTRACT # 35]
5:50 PM	<b>Nicholas Smith</b> <i>Osaka University, Suita, Japan.</i> Label-free raman and phase imaging for multidimensional data-based quantitative analysis of immune cells [ABSTRACT # 70]

**Conference Closing** (6:10 PM - 10:00 PM)

6:10 PM      **Raimund J. Ober & Jörg Enderlein**  
Closing Remarks

6:30 PM      **Bus Ride to Reception**

6:45 PM      **Conference Dinner Reception / Tour of George H.W. Bush Presidential Library and Museum**

# Poster Schedule

Listed below are the posters to be presented during the conference. All posters will be displayed for all three days. The listing of the posters indicate the day on which the poster presenter should be available to discuss the poster during the poster session.

We will be able to accommodate standard poster dimensions of 3 ft by 4 ft (36 in by 48 in) on all of our poster boards. If you have or plan to have a poster with dimensions larger than the standard dimensions, please let us know by email ([inquiries@quantitativebioimaging.com](mailto:inquiries@quantitativebioimaging.com)) as soon as possible so that we can try to make arrangements to accommodate it.

All speakers in oral sessions are very much encouraged to also present a poster. The poster presentations are organized in such a way that in most cases the poster presentation follows after the corresponding oral session so that the poster presentation can be used to supplement the oral presentation.

The poster boards are numbered. These numbers coincide with the abstract numbers for the presentation.

Date	Talk/Poster Only	Abstract No.	Topic	Title	Authors	Presenter
1/05 Thu.	Poster + Talk	1	Single Molecule Techniques	Single-molecule metal induced energy transfer (smMIET): Resolving nanometer distances at single molecule level	Narain Karedla, Isbaner Sebastian, Roman Tsukanov, Alexey I. Chizhik, Ingo Gregor, Anna M. Chizhik, Jörg Enderlein	Narain Karedla [Georg-August-Universität, Germany]
1/05 Thu.	Poster + Talk	5	Light Sheet Microscopy	Selective-plane illumination differential dynamic microscopy	Devynn Wulstein, Kathryn Regan, Rae Robertson-Anderson, Ryan McGorty	Ryan McGorty [University of San Diego, USA]
1/05 Thu.	Poster	11	Single Molecule Techniques	Determining receptor interaction kinetics through single molecule imaging and computational modeling	Luciana de Oliveira, Robel Yirdaw, Khuloud Jaqaman	Luciana de Oliveira [University of Texas Southwestern Medical Center, United States]
1/05 Thu.	Poster	13	Quantitation in Cell Biology	Quantitative three-dimensional imaging of cell-matrix interactions and cytoskeletal dynamics with light-sheet microscopy	Kevin Dean, Erik Welf, Philippe Roudot, Meghan Driscoll, Reto Fiolka, Gaudenz Danuser	Kevin Dean [UT Southwestern Medical Center, U.S.A.]
1/05 Thu.	Poster + Talk	15	Light Sheet Microscopy	Rapid and light efficient 3d microscopy	Reto Fiolka, Kevin Dean, Erik Welf, Phillippe Roudot, Meghan Driscoll, Gaudenz Danuser	Reto Fiolka [UT Southwestern, United States]
1/05 Thu.	Poster	27	Microscopy Techniques Applied to Biological Problems	Calcium imaging with GCaMP6s reveals differential responses to nicotine in sex-segregated hippocampal cultures	Katherine Rees, Amanda Mahnke, Shelby Weyand, Alexandra Sharp, Ursula Winzer-Serhan	Katherine Rees [Texas A&M Health Science Center, ]
1/05 Thu.	Poster	31	Tracking	Localization and parameter estimation in particle tracking using sequential Monte Carlo with expectation maximization	Sean Andersson	Sean Andersson [Boston University, USA]

Date	Talk/Poster Only	Abstract No.	Topic	Title	Authors	Presenter
1/05 Thu.	Poster + Talk	33	Tracking	Classification of diffusion dynamics from particle trajectories	Vincent Briane, Myriam Vimond, Charles Kervrann	Charles Kervrann [Inria, France]
1/05 Thu.	Poster + Talk	34	Tracking	DC-SIGN mediated dengue virus entry into cells	Nancy Thompson	Nancy Thompson [University of North Carolina at Chapel Hill, USA]
1/05 Thu.	Poster	37	Microscopy Techniques Applied to Biological Problems	Decision-making of bacteriophage P1	Kailun Zhang, Jimmy Trinh, Qiuyan Shao, Jingwen Guan, Lanying Zeng	Kailun Zhang [Texas A&M University]
1/05 Thu.	Poster	40	Microscopy Techniques Applied to Biological Problems	Internalization and cellular targeting of rhGAA in WT and GAA KO mice	Greg Ulinski	Greg Ulinski [Sanofi-Genzyme, USA]
1/05 Thu.	Poster	41	Microscopy Techniques Applied to Biological Problems	Visualize phage DNA degradation by CRISPR system at the single-cell level	Jingwen Guan, Xu Shi, Qiuyan Shao, Jimmy Trinh, Roberto Burgos, Lanying Zeng	Jingwen Guan [Texas A&M University, USA]
1/05 Thu.	Poster	42	Microscopy Techniques Applied to Biological Problems	Increased gene expression noise mediated by DNA replication promotes cell-fate bifurcation	Qiuyan Shao, Michael Cortes, Gabor Balazsi, Lanying Zeng	Qiuyan Shao [Texas A&M University, US]
1/05 Thu.	Poster	46	Fluorescent Labels	Delivery of non-genetically encodable imaging probes inside live cells	Jean-Philippe Pellois	Jean-Philippe Pellois [Texas A&M University, USA]
1/05 Thu.	Poster	48	Microscopy Techniques Applied to Biological Problems	Identification and characterization of focal and global calcium signaling events in smooth muscle cells of the microcirculation	Ray Mitchell, Emily Holzman, Pooneh Bagher	Pooneh Bagher [Texas A&M University Health Science Center, USA]
1/05 Thu.	Talk	50	Single Molecule Techniques and Clustering	New results in spatial statistics for single molecule microscopy	Edward Cohen, Raimund Ober	Edward Cohen [Imperial College London, UK]
1/05 Thu.	Poster + Talk	53	Light Sheet Microscopy	Single objective light-sheet microscopy for high-speed whole-cell 3D super-resolution	Marjolein Meddens, Sheng Liu, Patrick Finnegan, Thayne Edwards, Conrad James, Keith Lidke	Keith Lidke [University of New Mexico, United States]
1/05 Thu.	Poster	62	Tracking	Evaluation of organic dyes for high-speed single particle tracking	Hanieh Mazloom-Farsibaf, Keith Lidke	Hanieh Mazloom-Farsibaf

Date	Talk/Poster Only	Abstract No.	Topic	Title	Authors	Presenter
						[University of New Mexico, United States]
1/05 Thu.	Poster	65	Single Molecule Techniques	Characterization and fitting of complex fluorescence point spread functions	Rashad Baiyasi, Stephan Link, Christy Landes	Rashad Baiyasi [Rice University, USA]
1/05 Thu.	Poster + Talk	66	Single Molecule Techniques	Super temporal-resolved microscopy (STReM)	Wenxiao Wang, Hao Shen, Bo Shuang, Benjamin Hoener, Lawrence Tauzin, Nicholas Moringo, Kevin Kelly, Christy Landes	Wenxiao Wang [Rice University, United States]
1/05 Thu.	Poster + Talk	73	Light Sheet Microscopy	Light sheet microscopy for high content 3-D imaging of 3-D tissue cultures in a 96-well plate format	Hugh Sparks, Chris Dunsby	Hugh Sparks [UK, Imperial College London]
1/05 Thu.	Poster	80	Fluorescent Labels	Surface-Dependent Diffusion Dynamics of Intracellular Quantum Dots	Mohammad Zahid, Liang Ma, Sung Jun Lim, Andrew Smith	Mohammad Zahid [University of Illinois at Urbana-Champaign, United States of America]
1/05 Thu.	Poster	83	Microscopy Instrumentation	Light-sheet microscopy using attenuation-compensating Airy beam	Cong Liu, Yen-liang Liu, Tim Yeh	Cong Liu [University of Texas at Austin, USA]
1/05 Thu.	Poster	86	Microscopy Instrumentation	Maximizing the performance of fluorescence microscopes by optical filters	Prashant Prabhat, Jordan Myers	Prashant Prabhat [Semrock Inc., United States]
1/05 Thu.	Poster + Talk	88	Light Sheet Microscopy	Multicolor selective plane illumination microscopy to image the dynamics and interactions of genetically defined lineages	Alvin Yeh, Nathan Hart, Holly Gibbs, Arne Lekven	Alvin Yeh [Texas A&M, USA]
1/05 Thu.	Poster + Talk	89	Tracking	3D single molecule tracking with cellular context: trafficking of prostate-specific membrane antigen-specific antibody in prostate cancer cells	Dongyoung Kim, Sungyong You, Sally Ward, Raimund Ober	Sungyong You [Texas A&M, USA]
1/05 Thu.	Poster + Talk	97	Light Sheet and Structured Illumination Microscopy	Optical sectioning with light sheets forms the basis for highly corrected and fully automated microscopes	Ernst Stelzer	Ernst Stelzer [Goethe Universität, Germany]

Date	Talk/Poster Only	Abstract No.	Topic	Title	Authors	Presenter
1/06 Fri.	Poster	12	Microscopy Techniques Applied to Biological Problems	Live cell monitoring of glycine betaine by FRET-based genetically encoded nanosensor	Mohammad Ahmad	Mohammad Ahmad [Jamia Hamdard (Hamdard University), India]
1/06 Fri.	Poster + Talk	14	Quantitation in Cell Biology	Detecting junctions using spectral methods in orientation-scale space	Mark Kittisopikul, Takeshi Shimi, Robert D. Goldman, Khuloud Jaqaman	Mark Kittisopikul [UT Southwestern, USA]
1/06 Fri.	Poster	17	Microscopy Techniques Applied to Biological Problems	Plasmonic nanocarrier for NIR light-triggered protein release	Naomi J. Halas, Oara Neumann	Oara Neumann [Rice University, United States]
1/06 Fri.	Poster	20	Software	ThunderSTORM: a comprehensive ImageJ plugin for PALM and STORM data analysis and super-resolution imaging	Josef Borkovec, Martin Ovesný, Pavel Křížek, Zdeněk Švindrych	Guy Hagen [University of Colorado at Colorado Springs, USA]
1/06 Fri.	Poster	21	Software	Development and maintenance of Open Source software tools for the microscopy community.	Nico Stuurman, Mark Tsuchida, Ron Vale	Nico Stuurman [UCSF/HHMI, USA]
1/06 Fri.	Poster	22	Microscopy Techniques Applied to Biological Problems	A confocal super-resolution approach to study the dynamics of golgi apparatus in lung cancer metastasis	Priyam Banerjee, Xiaochao Tan, Jonathan M Kurie	Priyam Banerjee [UT MD Anderson Cancer Center, USA]
1/06 Fri.	Poster	24	Quantitation in Cell Biology	Quantification of keratin intermediate filament dynamics in non-motile and motile cells	Georg Dreissen, Nadieh Kuijpers, Anne Pora, Bernd Hoffmann, Rudolf Merkel, Rudolf Leube, Reinhard Windoffer	Reinhard Windoffer [RWTH University Aachen, Germany]
1/06 Fri.	Poster	25	Microscopy Techniques Applied to Biological Problems	Cooperation, competition, and confusion mediated subcellular decision-making revealed via 4-color live-cell imaging	Jimmy Trinh, Tamás Székely, Qiuyan Shao, Gábor Balázs, Lanying Zeng	Jimmy Trinh [Texas A&M University, United States]
1/06 Fri.	Poster	26	Quantitation in Cell Biology	Computer aided feature extraction for biological images	Ana Chang-Gonzalez, Tyler Lovelace, Wonmuk Hwang	Ana Chang-Gonzalez [Texas A&M University, USA]
1/06 Fri.	Poster	28	PSF Engineering / Adaptive Optics	An N-interface point spread function model suitable for wide-field fluorescent imaging of thick specimen with variable refractive index	Sreya Ghosh, Chrysanthe Preza	Chrysanthe Preza [University of Memphis, United States]
1/06 Fri.	Poster	29	PSF Engineering / Adaptive Optics	Imaging thick specimens using optical-sectioning microscopy coupled with block-based 3D image restoration	Sreya Ghosh, Chrysanthe Preza	Chrysanthe Preza [The University of Memphis, USA]

Date	Talk/Poster Only	Abstract No.	Topic	Title	Authors	Presenter
1/06 Fri.	Poster + Talk	30	Cameras and Detectors	Quantitative evaluation of accuracy and variance of individual pixels in a Scientific CMOS (SCMOS) camera for computational imaging	Shigeo Watanabe, Teruo Takahashi, Keith Bennett	Keith Bennett [Hamamatsu Photonics K.K., Japan]
1/06 Fri.	Poster	43	Quantitation in Cell Biology	Analyzing waves of tumor cell migration	Ohad M. Doron, Yossi Levi, Yael Ramm, Dganit Shkedy, Ilan Tsarfaty	Ilan Tsarfaty [Tel Aviv University, Israel]
1/06 Fri.	Poster	49	Microscopy Techniques Applied to Biological Problems	Visualizing scaffold protein functions in living cells	Nicholaus Trenton, Satya Bellamkonda, David Tsao, Tyler Mclaughlin, Volker Schweikhard, Michael Diehl	Nicholaus Trenton [Rice University, USA]
1/06 Fri.	Poster + Talk	52	PSF Engineering / Adaptive Optics	Implementation of PSF engineering using a fabricated spheric phase mask to reduce the effect of spherical aberration in 3D wide field fluorescence imaging	Nurmohammed Patwary, Hasti Shabani, Ana Doblaz, Genaro Saavedra, Chrysanthe Preza	Chrysanthe Preza [University of Memphis, USA]
1/06 Fri.	Poster + Talk	55	Microscopy Techniques Applied to Biological Problems	Fluorescent-imaging quantitative characterization of single-cell laser thermogenetic neurostimulation	V. V. Belousov, Sean Blakley, Alexei Zheltikov	Alexei Zheltikov [Texas A&M University]
1/06 Fri.	Poster	56	Electron Microscopy	An accurate forward imaging model for electron tomography	Sreya Ghosh, Tsvi Katchalski, Xiaohua Wan, Christopher Churas, Mason Mackey, Ranjan Ramachandra, Albert Lawrence, Mark Ellisman	Sreya Ghosh [University of California, San Diego, United States]
1/06 Fri.	Poster	57	Quantitation in Cell Biology	Large-scale analysis of spatiotemporal organellar network evolution	Allyson Loy, Abigail Courtney, Barbara Reaves, Frederick Quinn, Chakra Chennubhotla, Shannon Quinn	Allyson Loy [University of Georgia, United States]
1/06 Fri.	Poster + Talk	58	Microscopy Techniques Applied to Biological Problems	Non-diffraction limited microscopy demonstrates the role of local actin dynamism in nk cell effector function	Emily Mace, Alexandre Carisey, Jordan Orange	Emily Mace [Baylor College of Medicine and Texas Children's Hospital]
1/06 Fri.	Poster	59	Quantitation in Cell Biology	plusTipTracker3D: quantification, visualization and analysis of microtubule dynamics with lattice light-sheet microscopy	Philippe Roudot, Wesley Legant, Ana David, Daniel Gerlich, Eric Betzig, Gaudenz Danuser	Philippe Roudot [UT Southwestern Medical Center, U.S.A.]
1/06 Fri.	Poster	63	Quantitation in Cell Biology	Observing the effect of mechanobiology by lung cancer cell during metastasis	Richard Han, Charles Wanna, Shail Mehta, Don Gibbons, Jane Grande-allen	Richard Han [Rice University, USA]

Date	Talk/Poster Only	Abstract No.	Topic	Title	Authors	Presenter
1/06 Fri.	Poster	64	Electron Microscopy	Asymmetric cryo-EM structure of the canonical Allovivivirus Q $\beta$ reveals a single maturation protein and the genomic ssRNA in situ	Karl Gorzelnik, Zhicheng Cui, Catrina Reed, Joanita Jakana, Ry Young, Junjie Zhang	Karl Gorzelnik [Texas A&M University, USA]
1/06 Fri.	Poster	68	PSF Engineering / Adaptive Optics	Generalized recovery algorithm for 3D super-resolution microscopy using rotating point spread functions	Bo Shuang, Wenxiao Wang, Hao Shen, Lawrence J. Tauzin, Charlotte Flatebo, Jianbo Chen, Nicholas A. Moringo, Logan D. C. Bishop, Kevin F. Kelly, Christy F. Landes	Bo Shuang [Rice University, United States]
1/06 Fri.	Poster	69	Quantitation in Cell Biology	Quantitative analysis of endoplasmic reticulum dynamics in plant cells.	Lawrence Griffing	Lawrence Griffing [Texas A&M University, USA]
1/06 Fri.	Poster	72	Electron Microscopy	Electron microscopy study of the influence of rose phenol glycoside on the amylin fibrils	Narek Hovnanyan, Svetlana Sharoyan, Alvard Antonyan, Sona Mardanyan	Karlen Hovnanyan [Institute Molecular biology of NAS RA, Armenia]
1/06 Fri.	Poster	76	PSF Engineering / Adaptive Optics	Three-dimensional superresolution and tracking of overlapping emitters using the double-helix SPINDLETM module	Anthony Barisc, Anurag Agrawal, Ajoke Williams, Saumya Jain, Joshua Wheeler, Robert Walters, Roy Parker, Rafael Piestun	Anthony Barisc [Double Helix LLC, USA]
1/06 Fri.	Poster	81	PSF Engineering / Adaptive Optics	Resolving fluorescent objects beyond the diffraction limit by reducing the illuminated region and applying numerical optimization	Jiun-Yann Yu, Simeng Chen, Jian Xing, Stephen Becker, Carol Cogswell	Jiun-Yann Yu [University of Colorado Boulder, ]
1/06 Fri.	Poster	84	Microscopy Techniques Applied to Biological Problems	Development of biophysical markers that quantify metastatic potentials of prostate cancer cells using TSUNAMI microscope	Yen-Liang Liu, Aaron Horning, Evan Perillo, Cong Liu, Mirae Kim, Rohan Vasisht, Hannah Horng, Andrew Dunn, Chun-liang Chen, Tim Yeh	Yen-Liang Liu [The University of Texas at Austin, United States]
1/06 Fri.	Poster	85	PSF Engineering / Adaptive Optics	A new way of optimizing point spread functions for 3D fluorescence super-resolution microscopy	Simeng Chen, Jiun-yann Yu, Jian Xing, Stephen Becker, Carol Cogswell	Simeng Chen [University of Colorado Boulder, United States]
1/06 Fri.	Poster	87	Microscopy Techniques Applied to Biological Problems	Using advanced microscopy techniques to image antibody-dependent macrophage-cancer cell interactions	Ramraj Velmurugan, Dilip Challa, Sripad Ram, Raimund Ober, E. Sally Ward	Ramraj Velmurugan [Texas A&M University Health Sciences Center, USA]

Date	Talk/Poster Only	Abstract No.	Topic	Title	Authors	Presenter
1/06 Fri.	Poster	92	Software	Software framework for single molecule data analysis	Anish Abraham, David Kim, Jerry Chao, Sally Ward, Raimund Ober	Anish Abraham [Texas A&M University, USA]
1/06 Fri.	Poster	96	Software	The Allen Institute for Cell Science: Building a microscope pipeline to map the human cell	Winfried Wiegreaebe	Winfried Wiegreaebe [Allen Institute for Cell Science, USA]
1/07 Sat.	Poster + Talk	2	Various Microscopy Techniques	Multiphoton Image Scanning Microscopy	Ingo Gregor, Robert Ros, Jörg Enderlein	Jörg Enderlein [Georg-August-University, Germany]
1/07 Sat.	Poster + Talk	3	Single Molecule Techniques	$\lambda/100$ resolution by template-free 2d particle fusion in localization microscopy	Hamidreza Heydarian, Emile Broeders, Maximilian Strauss, Florian Schueder, Ralf Jungmann, Sjoerd Stallinga, Bernd Rieger	Hamidreza Heydarian [Delft University of Technology, The Netherlands]
1/07 Sat.	Poster	8	Various Microscopy Techniques	Temporal Area Maps	George Mcnamara	George Mcnamara [GeoMcNamara, United States]
1/07 Sat.	Poster + Talk	9	Microscopy Techniques Applied to Biological Problems	The actin homologue MreB and gliding motors show interdependent movements in the bacterium Myxococcus xanthus	Beiyan Nan, David Zusman	Beiyan Nan [Texas A&M, USA]
1/07 Sat.	Poster	10	Single Molecule Techniques	Three-dimensional localization microscopy based on accurate intensity estimation	Christian Franke, Markus Sauer, Sebastian Van De Linde	Christian Franke [University of Strathclyde, United Kingdom]
1/07 Sat.	Poster	16	Various Microscopy Techniques	MRI-Active Au Nanomatryoskhas	Luke Henderson	Luke Henderson [Rice University, USA]
1/07 Sat.	Poster	18	Electron Microscopy	Variable-pressure scanning electron microscopy images of sorghum predict resistance to storage insect pests	Michael Pendleton, Bonnie Pendleton, Gary Peterson	Michael Pendleton [Texas A&M University, USA]
1/07 Sat.	Poster	19	Microscopy Techniques Applied to Biological Problems	Augmentation of interactions between dimers stabilizes higher order oligomers of membrane-bound K-Ras: a single molecule perspective	Suparna Sarkar-Banerjee, Abdallah Sayyed-ahmad, Priyanka Prakash, Kwang-jin Cho, M. Neal Waxham, John F. Hancock, Alemayehu A. Gorfe	Suparna Sarkar-Banerjee [McGovern Medical School at UTHealth, USA]
1/07 Sat.	Poster	23	Structured Illumination	Blur reduction in TIRF and HILO microscopy	Reto Fiolka	Reto Fiolka [UT Southwestern, USA]

Date	Talk/Poster Only	Abstract No.	Topic	Title	Authors	Presenter
1/07 Sat.	Poster + Talk	32	Cameras and Detectors	Towards real-time localization of SCMOS single-molecule-switching data	Andrew Barentine, Yu Lin, Michael Grace, Leo Balduf, Kenny Chung, Joerg Bewersdorf, David Baddeley	Andrew Barentine [ <i>Yale University, United States</i> ]
1/07 Sat.	Poster + Talk	35	Various Microscopy Techniques	3D quantitative phase imaging techniques for non-invasive label-free imaging of cells and tissues.	Seungwoo Shin, Kyoohyun Kim, Yongkeun Park	Yongkeun Park [ <i>KAIST, </i> ]
1/07 Sat.	Poster + Talk	36	Cameras and Detectors	Bayesian fluorophore separation measurement in unresolved PSF using realistic EMCCD noise model	Chris Duncan, Michael Hirsch, Dan Rolfe, Sarah Needham, Marisa Martin-Fernandez	Michael Hirsch [ <i>Science and Technology Facilities Council, UK</i> ]
1/07 Sat.	Poster	38	Microscopy Techniques Applied to Biological Problems	Application of different imaging approaches for characterization of interactions of ErbB2 receptor and tetraspanin-enriched microdomains	Emma Lonnen, Robert Neely, Ela Claridge, Natalie Poulter, Elena Odintsova	Elena Odintsova [ <i>University of Birmingham, United Kingdom</i> ]
1/07 Sat.	Poster	44	Single Molecule Techniques	Inducing sparsity AT LN2 temperatures: towards polarization-selective single-molecule excitation and emission depletion	Robert Moerland, Christiaan Hulleman, Sjoerd Stallinga, Bernd Rieger	Robert Moerland [ <i>Delft University of Technology, The Netherlands</i> ]
1/07 Sat.	Poster + Talk	45	Microscopy Techniques Applied to Biological Problems	Fast cycling of integrin-clutch formation as revealed by single-molecule imaging	Munenori Ishibashi, Yukihiro Miyanaga, Satomi Matsuoka, Jun Kozuka, Yuichi Togashi, Tatsuo Kinashi, Masahiro Ueda	Munenori Ishibashi [ <i>Osaka University, Japan</i> ]
1/07 Sat.	Poster	47	Single Molecule Techniques	Super-resolution imaging of biological samples via spectral separation of quantum dots	Kemal Keseroglu, Andrei Sapelkin	Kemal Keseroglu [ <i>Queen Mary University of London, United Kingdom</i> ]
1/07 Sat.	Poster + Talk	51	Single Molecule Techniques	Modelling and estimating the behaviour of photo-switching fluorophores	Lekha Patel, Nils Gustafsson, Raimund Ober, Ricardo Henriques, Edward Cohen	Lekha Patel [ <i>Imperial College London, United Kingdom</i> ]
1/07 Sat.	Poster + Talk	60	Single Molecule Techniques	Multiple emitter fitting using reversible jump Markov Chain Monte Carlo	Mohamadreza Fazel, Keith A. Lidke	Mohamadreza Fazel Keith A. Lidke [ <i>Univesity of New Mexico, US</i> ]
1/07 Sat.	Poster + Talk	61	Single Molecule Techniques	Automated multiple target superresolution imaging	Farzin Farzam, Keith A Lidke	Farzin Farzam [ <i>University of NewMexico, USA</i> ]

Date	Talk/Poster Only	Abstract No.	Topic	Title	Authors	Presenter
1/07 Sat.	Poster	67	Single Molecule Techniques	Probing in situ dynamics of Nanoparticle-Protein Interactions	Sishan Chen, Stephan Link, Sudeshna Chatterjee, Franziska Hollenhorst, Rashad Baiyasi, Wenxiao Wang, Logan Bishop, Wei-Shun Chang, Christy Landes	Sishan Chen [Rice University, USA]
1/07 Sat.	Poster + Talk	70	Various Microscopy Techniques	Label-free raman and phase imaging for multidimensional data-based quantitative analysis of immune cells	Nicholas Smith, Alison Hobro, Nicolas Pavillon	Nicholas Smith [Japan, Osaka University]
1/07 Sat.	Poster	74	Microscopy Techniques Applied to Biological Problems	Disruption of oncogenic Ras-driven dependencies using membrane targeted dietary bioactives (MTDBs)	Natividad Fuentes, Rola Barhoumi, Robert S. Chapkin	Natividad Fuentes [Texas A&M University, United States]
1/07 Sat.	Poster + Talk	75	Various Microscopy Techniques	Regulation of integrin function studied by integrated optical and atomic force microscopy	Harini Sreenivasappa, Rola Barhoumi, Jerome Trzeciakowski, Andreea Trache	Andreea Trache [Texas A&M University Health Science Center, USA]
1/07 Sat.	Poster + Talk	77	Various Microscopy Techniques	Selected applications of Image Mapping Spectrometry	Michal Pawlowski, Jason Dwight, Tomasz Tkaczyk	Michal Pawlowski [USA, Rice University]
1/07 Sat.	Poster	79	Microscopy Techniques Applied to Biological Problems	Studying T7 phage particle transport in artificial mucus by TSUNAMI 3D tracking microscopy	Yuan-I Chen, Jasmim Leal, Yen-Liang Liu, Debadyuti Ghosh, Tim Yeh	Yuan-I Chen [The University of Texas at Austin, USA]
1/07 Sat.	Poster + Talk	82	Various Microscopy Techniques	Deep and high-resolution three-dimensional tracking of single particles using nonlinear and multiplexed illumination	Evan Perillo, Yen-Liang Liu, Cong Liu, Andrew Dunn, Tim Yeh	Tim Yeh [University of Texas at Austin, USA]
1/07 Sat.	Poster	90	Single Molecule Techniques	Localization of single molecules from fluorescence microscopy images using a state space-based method	Milad Rafiee Vahid, Jerry Chao, E. Sally Ward, Raimund Ober	Milad Rafiee Vahid [Texas A&M University, United States]
1/07 Sat.	Poster	93	Structured Illumination	Investigating the effect of different 3D illumination patterns on structured illumination microscopy performance	Hasti Shabani, Nurmohammed Patwary, Ana Doblas, Genaro Saavedra, Chrysanthe Preza	Hasti Shabani [University of Memphis, USA]
1/07 Sat.	Poster + Talk	95	Nuclear Pore Complex	Simple biophysics of the collective conformational transitions of the intrinsically disordered proteins of the Nuclear Pore Complex	Anton Zilman	Anton Zilman [University of Toronto, Canada]

Date	Talk/Poster Only	Abstract No.	Topic	Title	Authors	Presenter
1/07 Sat.	Poster	99	Various Microscopy Techniques	Quantitative hyperspectral imaging of plasmon-labeled single molecule isoforms in live cells	Kyuwan Lee	Kyuwan Lee <i>[Albert Einstein College of Medicine, United States]</i>

## 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.

# Abstracts by Topic

## Cameras and Detectors

30, 32, 36

## Electron Microscopy

18, 56, 64, 72

## Fluorescent Labels

46, 80

## Light Sheet Microscopy

5, 15, 53, 73, 88, 97

## Microscopy Instrumentation

83, 86

## Microscopy Techniques Applied to Biological Problems

9, 12, 17, 19, 22, 25, 27, 37, 38, 40, 41, 42, 45, 48, 49, 55, 58, 74, 79, 84, 87

## Nuclear Pore Complex

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## PSF Engineering / Adaptive Optics

28, 29, 52, 68, 76, 81, 85

## Quantitation in Cell Biology

13, 14, 24, 26, 43, 57, 59, 63, 69

## Single Molecule Techniques

1, 3, 10, 11, 44, 47, 51, 60, 61, 65, 66, 67, 90

## Single Molecule Techniques and Clustering

50

## **Software**

**20, 21, 92, 96**

## **Structured Illumination**

**23, 93, 97**

## **Tracking**

**31, 33, 34, 62, 89**

## **Various Microscopy Techniques**

**2, 8, 16, 35, 70, 75, 77, 82, 99**

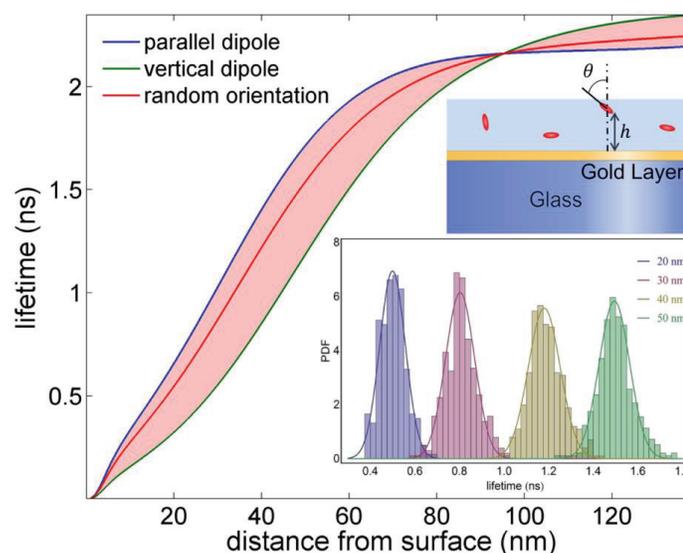
**SINGLE-MOLECULE METAL INDUCED ENERGY TRANSFER (smMIET):  
RESOLVING NANOMETER DISTANCES AT SINGLE MOLECULE LEVEL**

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**KEYWORDS:** Axial resolution, Förster resonance energy transfer, dipole emitter, super-resolution

We present a new concept for measuring distance values of single molecules from a surface with nanometer accuracy using the energy transfer from the excited molecule to surface plasmons of a metal film [1]. We measure the fluorescence lifetime of individual dye molecules deposited on a dielectric spacer as a function of a spacer thickness. By using our theoretical model [2], we convert the lifetime values into the axial distance of individual molecules. Similar to Förster resonance energy transfer (FRET), this allows emitters to be localized with nanometer accuracy, but in contrast to FRET the distance range at which efficient energy transfer takes place is an order of magnitude larger. Together with orientation measurements [3], one can potentially use smMIET to localize single emitters with a nanometer precision isotropically, which will facilitate intra- and intermolecular distance measurements in biomolecules and complexes, circumventing the requirement of the knowledge of mutual orientations between two dipole emitters which severely limits the quantification of such distances from a conventional single-pair FRET (spFRET) experiment.



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## MULTIPHOTON IMAGE SCANNING MICROSCOPY

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KEYWORDS: Multiphoton excitation fluorescence, second harmonic excitation, structured illumination, image scanning microscopy

The past 20 years have seen a revolution in optical microscopy, with the invention of a multitude of new superresolution methods and techniques, such as STED, PALM, STORM, SOFI, SIM etc. However, not many of them are easily adapted to multiphoton excitation microscopy. However, for many applications in life sciences, multiphoton excitation microscopy offers two important advantages: deep penetration depth of infrared excitation light into tissue, which is important when interested in imaging deep into highly scattering and opaque tissue such as the brain; and the ability to use, besides fluorescence, coherent processes such as second or third harmonic generation (SHG or THG) for image generation. Recently, we have experimentally realized a new modality for enhanced resolution imaging which is physically very similar to the more familiar Structured Illumination Microscopy (SIM): Image Scanning Microscopy or ISM [1,2]. This technique is based on a conventional Confocal Laser Scanning Microscope (CLSM), but replaces the typical pinhole and point detector of a CLSM by an imaging wide-field camera. Because ISM uses the same excitation modality as a CLSM, one can use multiphoton excitation in ISM with the same ease as in a normal CLSM. This makes ISM ideally suited for non-linear optical microscopy including coherent microscopy such as SHG, THG, or even CARS.

We present theory and realization of ISM when using two-photon excitation for fluorescence as well as SHG. Our experimental system provides excellent sensitivity and high frame-rate in combination with doubled lateral resolution if compared to a classical two-photon laser-scanning microscope. We demonstrate the performance of multi-photon ISM by imaging fixed and living specimens. The modular design of our multi-photon ISM allows its straightforward implementation into any existing multi-photon CLSM.

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## λ/100 RESOLUTION BY TEMPLATE-FREE 2D PARTICLE FUSION IN LOCALIZATION MICROSCOPY

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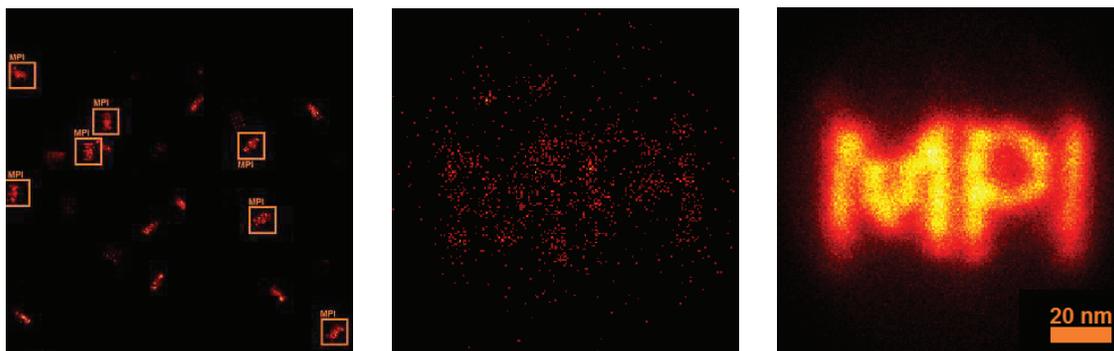
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**KEY WORDS:** localization microscopy, particle averaging, super-resolution reconstruction

Low density labeling and limited photon count of emitters are among the factors that limit the achievable resolution in localization microscopy. This problem can be mitigated if instances of the same structure are fused (combined) properly. This technique is similar to Single Particle Analysis from cryo-EM. Typically, these particles have arbitrary pose, are degraded by low photon count, false localizations and missing labels. If the structure to be imaged is known a priori, one approach is to register all particles on a template. However, it introduces the so-called template bias which can occur if too strong prior knowledge is imposed on the data. To address this issue, a novel template-free data fusion method is proposed which assumes no prior knowledge about the sample.

Pairs of particles are registered in a tree structure from leaves to root. For each pair, a cost function which uses the uncertainties is optimized for rigid transform parameters. To speed up the optimization, a Gaussian mixture registration method is used. The test samples consist of logos shaped by DNA-origami and imaged with DNA-PAINT. The average number of localizations per particle is  $\sim 1500$  and the mean lateral uncertainty is 1.4 nm. With 1024 particles, a logo of  $\sim 1.5 \cdot 10^6$  localizations is reconstructed. We evaluate the resolution by Fourier Ring Correlation. While the initial particles have FRC values in the range of 12-26 nm, the top reconstructed particle is  $\sim 5.2$  nm which is close to the best possible resolution one can get from this data as the distance of the binding sites is  $\sim 5$  nm.



Superresolution image of MPI logo. **Left:** Regions of interest containing raw MPI logos. **Middle:** A zoom-in on one of the ROIs. **Right:** The fusion of 1024 identical MPI logos.

## SELECTIVE-PLANE ILLUMINATION DIFFERENTIAL DYNAMIC MICROSCOPY

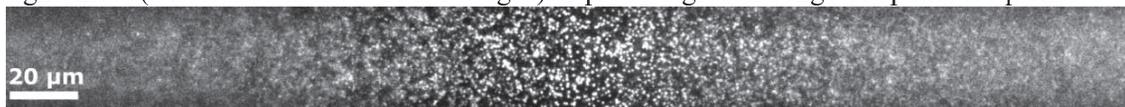
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**KEY WORDS:** selective-plane illumination microscopy, light-sheet microscopy, digital Fourier microscopy, diffusion

Using differential dynamic microscopy (DDM) we are able to extract quantitative information about the diffusion of objects imaged with selective-plane illumination microscopy (SPIM). DDM was first described in 2008 by Cerbino and Trappe [1]. They described how the dynamics of diffusing colloidal particles could be captured by analyzing images acquired with transmitted light microscopy. In brief, the method works by measuring the temporal decay of density fluctuations for each accessible spatial frequency in the acquired images. Such a technique bears much in common with other image correlation or spectroscopy methods. Later work has demonstrated DDM's applicability to fluorescence wide-field [2], dark-field [3] and confocal [4] microscopies. Notably, this technique allows for studying the dynamics of particles or molecules whether or not they can be individually resolved.

Recently, we demonstrated that DDM can be applied to SPIM [5]. While this should not come as a surprise, we found that DDM can extract information about the dynamics on portions of the images *outside* the region where the light-sheet is most focused. For Gaussian light-sheets only the focused and thinnest portion of the light-sheet is typically used. However, with DDM we measure the dynamics even in the regions of the image where the higher background (due to the thicker sheet) makes resolving individual particles or molecules impossible. Furthermore, we find that the spatial frequencies accessible to DDM depend on the light-sheet thickness. Where the sheet is thinnest, we extract higher spatial frequency dynamics. Where the sheet thickens, we extract the lower spatial frequency dynamics. Therefore, using DDM on SPIM-acquired images not only extends the field-of-view from which we can extract usable information it also exploits the Gaussian nature of the light-sheet (a nuisance in most SPIM usages) to probe a greater range of spatial frequencies.



**Figure 1.** Image of 200-nm fluorescent beads taken with SPIM using a 20 $\times$  detection objective. Even though particles can only be individually localized in the central region of this image we can use DDM to extract the dynamics over the entire 330  $\mu\text{m}$  length.

We have demonstrated our technique, SPIDDM, using colloidal particles (Fig. 1) and DNA molecules. Currently, we are measuring the diffusion of DNA in various crowded environments with SPIDDM and are investigating how the accessible dynamics vary with light-sheet thickness.

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McNamara – Temporal Area Maps 2017 The Chase

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I present my most recent update of "The Chase" digital movie. The movie is available online at <https://vimeo.com/175151196>

Panoramic version of a classic biology movie by David Rogers of a neutrophil (polymorphonuclear leukocyte) chasing 3 bacteria. Please see [works.bepress.com/gmcmnamara/73/](https://works.bepress.com/gmcmnamara/73/)

for my four 2010 MetaMorph MetaMatters newsletter articles on "The Chase" (four PDFs in the download). The original Rogers movie is reprinted in the bottom left quadrant of the Panorama video (I added this in 2012).

This movie (created and uploaded 20160717) has two lines of additional text at bottom, including the bepress "73" link. Here is my previous text description, from [works.bepress.com/gmcmnamara/18/](https://works.bepress.com/gmcmnamara/18/)

update of my panoramic movie of "The Chase".

Bottom left: original movie (jumpy as cells move to new fields of view).

Top has four segments: 1. Panorama version of David Rogers 1950's timelapse movie. 2. Addition of white cell outlines for the neutrophil. 3. Topographic surface map (TSM) with outlines. Made using MetaMorph Imaging System version 7.7. TSM command was present in version 1.0 of MetaMorph, released in 12/1992. 4. Temporal area map (TAM) with outlines. bottom right shows the same TAM data in monochrome (with blue for non-covered areas) plus TAM histogram. Made using MetaMorph Imaging System version 7.7. TAM was made using the "Stack Arithmetic: Sum" command, which was a MetaMorph very early build, circa summer 1992, pre-version 1.0, which was released in 12/1992.

Temporal area maps were invented by George McNamara and Prof. Robert P. Futrelle, circa 1984, when we were at the University of Illinois Urbana-Champaign. Later in 1990 George Used Image-1/AT image acquisition/processing/analysis software in the laboratory of Prof. Neal First, University of Wisconsin-Madison to make digital TAM's (Dr. McNamara recieved information from the Image-1/AT manufacturer, Universal Imaging Corp., to program the Matrox MVP-AT digitizer board to do the image summing). Dr. McNamara joined UIC in summr 1992 as the MetaMorph applications scientist.

A key feature of TAM's for scientific imaging is that the data histograms of the TAM data (1's for every time point pixel or voxel covered) for multiple cells can be added together as histogram data, and different experimental conditions compared by a non-parametric statistical test, such as the "K-S" statistic.

I note here that Fiji ImageJ [imagej.net/Fiji/Downloads](http://imagej.net/Fiji/Downloads) can open AVI files, enabling anyone (ex. high school and college students) to work with the image data.

**The actin homologue MreB and gliding motors show  
interdependent movements in the bacterium *Myxococcus xanthus***

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MreB is a bacterial actin homologue involved in a wide range of functions including cell wall biosynthesis and chromosome segregation. MreB also plays crucial roles in *Myxococcus xanthus* gliding motility, which is impaired when MreB polymerization is inhibited. However, it remains unknown how MreB facilitates and adapts to the fast motion of the gliding machinery. Here, we tracked the dynamics of single MreB molecules in *M. xanthus* using single particle tracking photo-activated localization microscopy (sptPALM). We found that a subpopulation of MreB moved actively in helical trajectories, similar to the movement of the gliding machinery. MreB interacts directly with motor protein AglS, a MotB homologue, and MreB movements were lost in the mutants that carried truncated motors, indicating that MreB movement is driven by the gliding complexes. The speed of MreB movement was two orders of magnitude higher than that in *Bacillus subtilis* and *E. coli*. Unlike these organisms, the fast motion of MreB was not blocked by inhibitors of cell wall biosynthesis in *M. xanthus*. Our results imply that in *M. xanthus*, the function of MreB is adapted to provide a scaffold for the gliding motors and that the gliding machinery drives the movement of MreB filaments.

**Three-dimensional localization microscopy based on accurate intensity estimation****Christian Franke<sup>1</sup>, Markus Sauer<sup>1</sup> and Sebastian van de Linde<sup>1,2</sup>****<sup>1</sup>Department of Biotechnology and Biophysics, University of Würzburg, Biocenter, Am Hubland, 97074 Würzburg, Germany****<sup>2</sup> Department of Physics, University of Strathclyde, John Anderson Building, 107 Rottenrow, Glasgow, G4 0NG, UK****Email: cfranke@physik.uni-wuerzburg.de, s.vandelinde@strath.ac.uk****KEY WORDS:** 3D localization microscopy, photometry, dSTORM, super - resolution

Localization microscopy provides super-resolution images with impressive lateral resolution achieved by fitting a two-dimensional Gaussian to the emission pattern of individual fluorophores. We show that state-of-the-art single-molecule localization software systematically underestimates the true fluorescence intensity. Accurate determination of single-molecule fluorescence intensities is of particular importance not only for the localization precision, but also for the characterization of novel dyes and chemical buffers. We therefore propose a new photometric method, termed temporal, radial-aperture based intensity-estimation (TRABI) to determine accurate photon numbers within different regions of the emission pattern of single fluorescent molecules, thus generating a  $z$ -dependent photometric parameter. Utilizing a biplane imaging scheme we demonstrate 3D imaging at a localization precision down to 12 nm within an axial range over 800 nm. Furthermore, we show that TRABI can be used to extract 3D information of existing 2D localization microscopy data without additional hardware requirements. [1]

[1] C. Franke, M. Sauer, S. van de Linde (2016) *under revision*.

## DETERMINING RECEPTOR INTERACTION KINETICS THROUGH SINGLE MOLECULE IMAGING AND COMPUTATIONAL MODELING

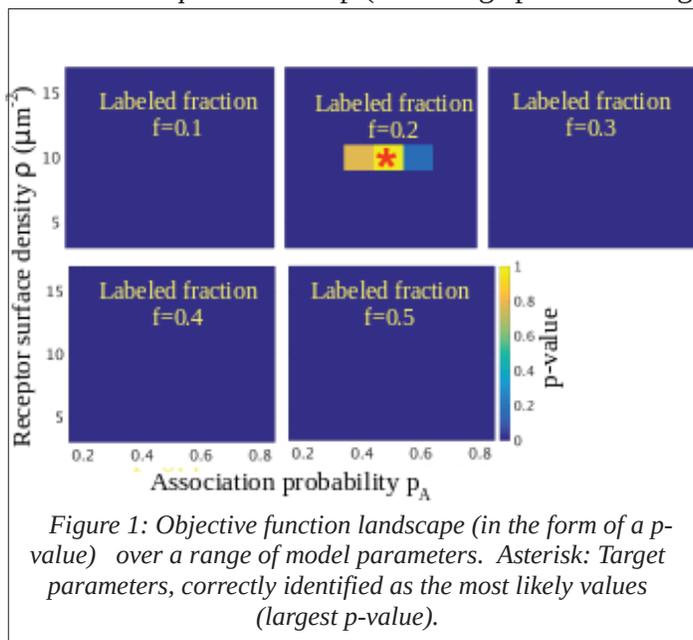
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**KEY WORDS:** Monte Carlo simulations, receptor interaction kinetics, single molecule imaging

The spatiotemporal organization of receptors in the plasma membrane is critical for transmembrane signal transduction. Using single molecule imaging it is possible to monitor inter-receptor interactions with high spatiotemporal resolution in their native cellular context. However, all single-molecule approaches reveal interactions only between the labeled receptors, which are a small subset of the full population. As a result, it has remained a challenge to calculate receptor interaction kinetics, especially association rates, from single-molecule data. To tackle this challenge we are developing a stochastic mathematical modeling approach to derive receptor association and dissociation rates from single-molecule image data. The approach consists of (1) building a mathematical model of receptor dynamics and interactions, mimicking the biological system and data acquisition setup (including sparse labeling) and (2) estimating the unknown model parameters by fitting the model to experimental single-molecule data using the method of indirect inference for stochastic model calibration [1,2]. To test this approach, we have been simulating trajectories of diffusing receptors that interact with each other, considering a wide range of model parameters and investigating whether our approach is able to accurately recover the unknown model parameters. Our tests demonstrate that the approach has the sensitivity to determine association and dissociation rates as well receptor density and labeled fraction (see figure 1 for an example). Next we will test the method under various experimental conditions including data with errors.



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## LIVE CELL MONITORING OF GLYCINE BETAINE BY FRET-BASED GENETICALLY ENCODED NANOSENSOR

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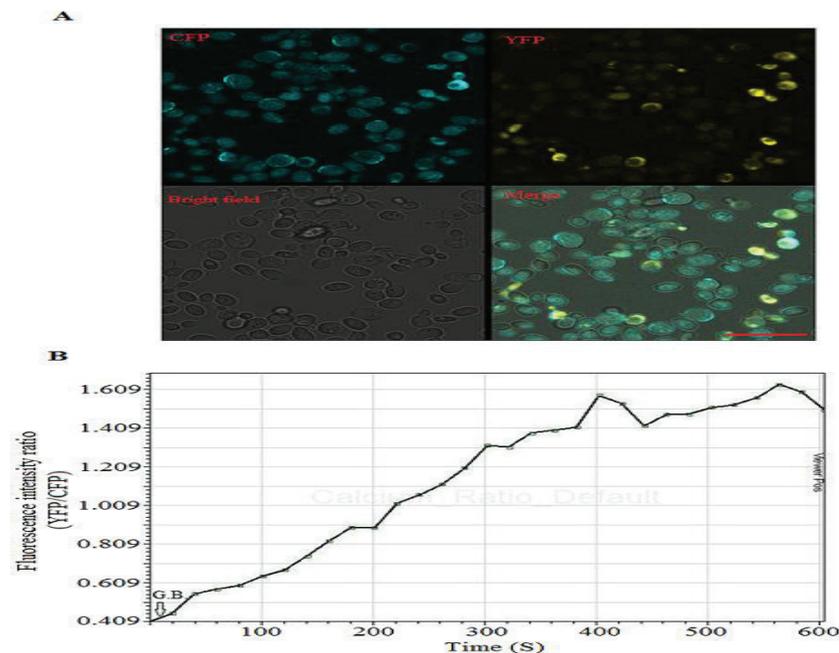
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**Key Words:** Glycine betaine, salinity, FRET, nanosensor, fluorescent proteins

### Abstract:

Glycine betaine (GB) accumulates in the cell at exceedingly high level under the conditions of high salinity and plays a crucial role in the maintenance of osmolarity of the cell without affecting the physiological processes. Since, analysis of stress-induced physiological conditions in living cells requires real-time monitoring of cellular GB level. A genetically encoded FRET-based nanosensor has been developed in this study that allows the real-time monitoring of GB levels inside living cells. GB binding protein (ProX) was sandwiched between the cyan fluorescent protein (CFP) and yellow fluorescent protein (YFP), which are known as Förster resonance energy transfer (FRET) pair. This trimeric sensor protein was named as Glycine Betaine Optical Sensor (GBOS). Conformational change in ProX, which was used as sensory domain, reported the change in the level of this compatible solute under *in vitro* and *in vivo* conditions. Binding of the GB to the sensory domain fetches close to both the fluorescent moieties that result in the form of increased FRET ratio. So, any change in the concentration of GB is correlated with change in FRET ratio. This nanosensor also reported the GB cellular dynamics in real-time in *Escherichia coli* cells after the addition of its precursor, choline. GBOS was also expressed in yeast and mammalian cells and live cell imaging was performed to monitor the intracellular GB level, quantitatively. Therefore, the GBOS represents a unique FRET-based nanosensor which allows the non-invasive ratiometric analysis of the GB in living cells.



## Quantitative Three-Dimensional Imaging of Cell-Matrix Interactions and Cytoskeletal Dynamics with Light-Sheet Microscopy

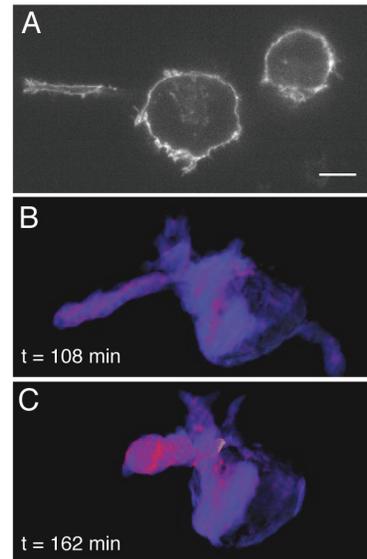
**Kevin M. Dean, Erik S. Welf, Philippe Roudot, Meghan K. Driscoll, Reto Fiolka, and Gaudenz Danuser.**

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**KEY WORDS:** light-sheet microscopy, cell biology, tissue microenvironment

The formation and maintenance of anteroposterior cell polarity is a fundamental biological process that critically influences cell motility, embryogenesis, tissue formation, and disease processes, including metastasis. To establish polarity, cells must integrate extracellular cues, selectively activate localized molecular circuits, and reorganize their cellular architecture. However, due to limitations in fluorescence microscopy, spontaneous symmetry breaking and polarity maintenance is primarily studied on stiff and infinitely flat optical substrates that bear little resemblance to 3D microenvironments encountered *in vivo*. To address this challenge, we developed a battery of light-sheet microscope platforms that enable sensitive, multicolor, isotropic high-resolution (sub-400 nm) imaging of cells in reconstituted 3D microenvironments [1-4]. Here, I present ongoing work that combines three-dimensional light-sheet microscopy with computer vision in an effort to provide insight into the dynamics of cell-collagen adhesions, Rho GTPase activity, and cytoskeletal adaptation as cells initiate and maintain polarization.



RhoA GTPase dynamics during protrusion contraction in a 3D collagen microenvironment. (A) Raw image of RaichuRhoA-GR. Scale bar 10 microns. (B) Surface rendering of FRET ratio at 108 min and (C) 162 min, where an increase in RhoA activity is associated with actomyosin contraction of the cellular protrusion..

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## Detecting Junctions Using Spectral Methods in Orientation-Scale Space

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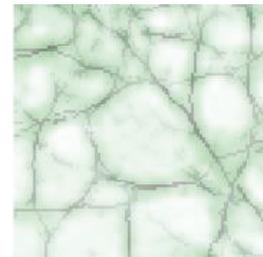
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**Introduction:** Lamin intermediate filaments form an intricate meshwork subjacent to the nuclear envelope to provide mechanical and functional support to the nucleus of a cell [1]. Defects in these meshworks are clinically correlated with the human disease Emery-Dreifuss muscular dystrophy. A quantitative understanding of these defects may thus yield biomedical insights into the nature of this disease. We have previously used steerable filters designed with Canny-like criteria to analyze these meshwork structures by determining the optimal orientation of the filter that best matches the neighborhood around each pixel [1, 4]. The orientation filter response can be described as a continuous periodic function along an orientation dimension in addition to the spatial dimensions, thus forming an orientation space [2]. However, the multiscale junctions inherent to lamin meshworks pose a challenge for these filters. To address this, we created an orientation-scale space and examined local maxima within that space.

**Methods: 1)** We first add a scale dimension to orientation space. The scalability of a filter would allow the scale response to be accurately interpolated based on a discrete number of filter responses. To achieve this we employ the filter developed by van Ginkel et al. used to construct an orientation space and note it is bandlimited in terms of scale-frequency and is thus scalable [2]. Since the scale response is defined on bounded intervals, Chebyshev polynomials are used to interpolate scale responses. **2)** Within the continuous dimensions of orientation and scale, we find local maxima that represent predominant orientations and scales. For this we use procedures to find the extrema of Fourier series and Chebyshev polynomials, as implemented in the MATLAB spectral methods toolbox, *chebfun* [3]. **3)** We then develop a Non-Local Maxima Suppression (NLMS) technique to only preserve the orientation-scale space responses at local maxima in orientation-scale space while zeroing other responses, thus detecting line centers, including at junctions.



NLMS of Lamin A immunofluorescence

**Results:** We apply the above methods to analyze both synthetic and real structured illumination microscopy images of lamin intermediate filaments. Maximum response projections of orientation-scale space after NLMS (Figure) can detect pixels missed by our previous approach [1, 4], thus producing a complete segmentation of junctions. This is because our method uses multiple local maxima per pixel in orientation-scale space rather a single optimal orientation as in the Canny line detector [4]. In conclusion we have developed a framework to extract and analyze multiple orientations at optimal scales, allowing for accurate junction and line detection across many scales in dense lamin intermediate filament meshworks.

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## Rapid and Light Efficient 3D Microscopy

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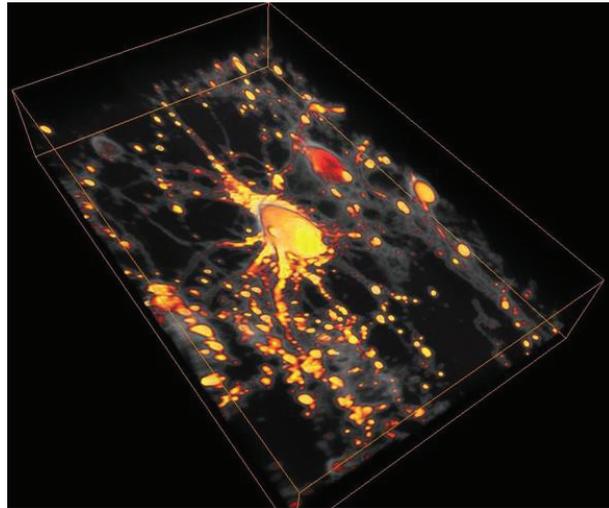
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**KEY WORDS:** light-sheet microscopy, live cell imaging, 3D microscopy, time-lapse imaging

Three-dimensional (3D) live cell imaging with Nyquist sampling in both space and time requires microscopes that are light efficient, minimally phototoxic, and capable of fast volumetric image acquisition rates ( $\sim 1$ -14 Hz) at high spatial resolution. So far, imaging of very rapid dynamics or faint objects has been restricted to two-dimensional imaging modalities such as Total Internal Reflection Fluorescence microscopy. However, such imaging assays impose artificial culturing conditions of cells on hard glass coverslips and ignore one spatial dimension entirely.

Here we report on the development of light-sheet technologies with high spatiotemporal resolution that are capable of monitoring intracellular dynamics and cell morphology in 3D. This has enabled us to quantitatively measure membrane dynamics and PI-3 Kinase signaling in melanoma cancer cells in physiologically relevant microenvironments at volumetric imaging rates of 1 Hz [1].

Further, we have imaged and tracked rapid intracellular transport of clathrin coated vesicles [2] and Rab5a vesicles in 3D with volumetric acquisition rates of up to 7 Hz. The rapid image acquisition has enabled us to track motor proteins that achieved peak velocities of up to 10 microns/s. Finally, we present time-lapse imaging of calcium signaling of cultured Neurons at a volumetric imaging rate of 14 Hz, which enabled us to follow action potentials in 3D over time. Owing to this progress in microscope technology, we believe that biological processes that hitherto have been only accessible to 2D imaging can now be performed in 3D, thereby greatly expanding the number of biological questions that can be addressed with light microscopy.



Calcium signaling cortical neurons labeled with GcaMP-6f and imaged at a volumetric acquisition rate of 14.4Hz (2800 images per second).

1. Erik S. Welf, Meghan K. Driscoll, Kevin M. Dean, C. Schäfer, J. Chu, Michael W. Davidson, Michael Z. Lin, G. Danuser, and R. Fiolka, *Developmental Cell* **36**, 462-475 (2016).
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## MRI-Active Au Nanomatryoskhas

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Multifunctional plasmonic nanoparticles have shown extraordinary potential for near-IR photothermal and triggered-therapeutic release treatments of solid tumors. However, the critical ability to efficiently track these particles *in vivo* remains difficult. To address this challenge, we have created MRI-active Au nanomatryoskhas. These are Au core-silica layer-Au shell nanoparticles with Gd(III) ions encapsulated within the silica layer between the inner core and outer Au layer of the nanoparticle (Gd-NM). This multifunctional nanoparticle retains its strong near-IR Fano-resonant optical absorption properties essential for photothermal or other near-IR light-triggered cancer therapy, while simultaneously providing increased T<sub>1</sub> contrast in magnetic resonance imaging by concentrating Gd(III) within the nanoparticle. By design, this system secures chelated Gd(III) within the NM, reducing toxicity but still enabling interaction with neighboring water molecules for MR enhancement. Measurements of Gd-NM revealed a substantially enhanced T<sub>1</sub> relaxivity ( $r_1 \sim 17 \text{ mM}^{-1} \text{ s}^{-1}$ ) even at 4.7 T as compared to conventional Gd(III)-DOTA chelating agents ( $r_1 \sim 4 \text{ mM}^{-1} \text{ s}^{-1}$ ) currently in clinical use. The observed relaxivities are consistent with Solomon-Bloembergen-Morgan (SBM) theory, describing the longer-range interactions between the Gd(III) and protons outside the nanoparticle. This novel system has the potential to enable tracking of therapeutic nanoparticles *in vivo*, an essential step for optimizing light-induced, nanoparticle based therapies. Furthermore, our system has shown great promise in loading a variety of metal ions to enhance MRI contrast and decrease toxicity.

# Plasmonic Nanocarrier for NIR Light-Triggered Protein Release

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## ABSTRACT

Protein therapeutics are becoming important in the pharmaceutical industry with many of them currently undergoing clinical trials. Some of these molecules present high systemic toxicity and, transporting them to the exact site of action without being released in the blood stream is crucial for their safety and efficacy. We present a near-infrared light induced delivery system able to release a therapeutic protein inside cells in a controlled manner. The plasmonic protein delivery system consists of aptamer-functionalized nanoshells, which bind tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ) through non-covalent interactions. Upon illumination of the nanocarrier at its plasmon resonance with a continuous wave laser, aptamer-protein interactions are thermally disrupted and active TNF- $\alpha$  is released. Cell studies show that the nanocarrier is internalized by RAW 264.7 macrophages and MDA-MB-231-eGFP breast cancer cells, accumulating inside the cells and are not cytotoxic under the experimental conditions of this study; however, the released TNF- $\alpha$  is active and induces cell death. The light induced delivery system shown in this study can be extended to the treatment of different types of tumors by choosing the appropriate aptamer-protein system.

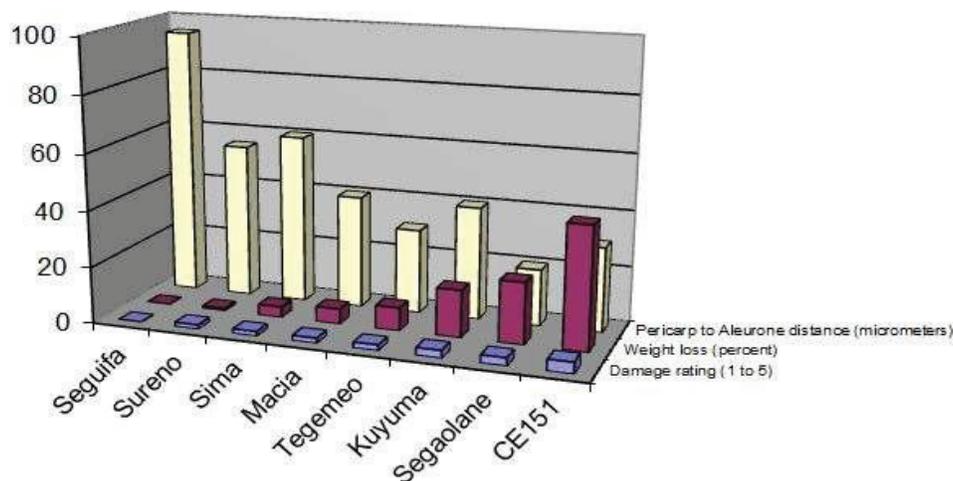
## VARIABLE-PRESSURE SCANNING ELECTRON MICROSCOPY IMAGES OF SORGHUM PREDICT RESISTANCE TO STORAGE INSECT PESTS

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**KEY WORDS:** maize weevil, stored grain, sorghum resistance, pericarp, aleurone

The most damaging insect pest of stored grain worldwide is the maize weevil, *Sitophilus zeamais* Motschulsky. A female maize weevil chews into a kernel of sorghum, *Sorghum bicolor* (L.) Moench, deposits an egg, and seals the hole. The egg hatches into a larva that feeds inside the kernel, pupates, and emerges as an adult. Six generations of weevils can be produced each year. Each female weevil can lay as many as 400 eggs in a lifetime [1]. To determine resistance of grain of eight genotypes of sorghum, 5 g of grain of each genotype were put with three female and two male newly emerged maize weevils into ventilated vials. Damage was scored (1 to 5, with 5 being most) and the kernels were weighed every three weeks for 105 days. A Tescan Vega 3 XMU Variable-Pressure Scanning Electron Microscope (VPSEM) was used to determine if the morphology of sorghum genotypes was related to resistance. To prepare sorghum for VPSEM, a representative kernel of each sorghum genotype was cut in half, coated with gold using a Hummer sputter coater, and placed in the VPSEM for observation. The degree of resistance to maize weevil was positively correlated to the distance from the pericarp to the aleurone layer of kernels of the eight sorghum genotypes. Seguifa sorghum was most resistant and had the greatest distance from the pericarp to aleurone layer. Funding provided in part by USAID and SMIL under Cooperative Agreement No. AID-OAA-A-13-00047.



Graph demonstrating the correlation between damage and distance from pericarp to aleurone.

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## Augmentation of interactions between dimers stabilizes higher order oligomers of membrane-bound K-Ras: a single molecule perspective

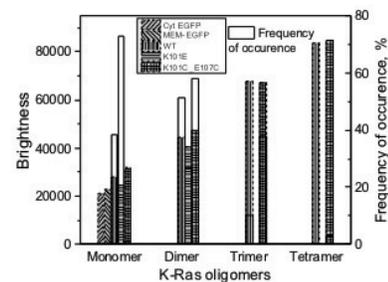
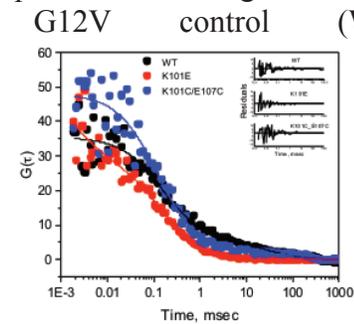
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**KEY WORDS:** K-Ras, dimers, oligomers, single molecule, FRAP, FCS, RICS

Ras proteins are small GTPases that control cell growth, differentiation and proliferation (1). They primarily reside on the inner leaflet of the plasma membrane and are crucial anticancer drug targets (2). The structural organization of Ras proteins on the plasma membrane has attracted major interest in recent years. Computational work by our group predicted three interfaces that are variously involved in the oligomerization of K-Ras, the most frequently mutated isoform of Ras proteins (3, 4). In the current study, we examined a charge reversal K-Ras mutant K101E and a double cysteine mutant K101C/E107C that represent constructs with predicted weak and strong potential for oligomerization, respectively relative to the oncogenic G12V control (WT). We used fluorescence recovery after photobleaching (FRAP), fluorescence correlation spectroscopy (FCS) and image correlation spectroscopy (ICS) to monitor the distribution of dimers and higher-order oligomers of these proteins. Our data show that the distribution of monomers, dimers and higher-order oligomers is such that the K101E mutant is mostly monomeric, K-Ras G12V exists as both dimer and higher-order oligomer whereas the percentage of dimers and larger oligomers is much greater in K101C/E107C (Figures), consistent with our prediction and data from signaling assays. These results establish that K101 and E107 play a key role in K-Ras oligomerization and establish the co-existence of diverse K-Ras oligomeric states.



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**ThunderSTORM: a comprehensive ImageJ plugin for PALM and STORM data analysis and super-resolution imaging**

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ThunderSTORM [1] is an open-source, interactive, and modular plug-in for ImageJ designed for automated processing, analysis, and visualization of data acquired by single molecule localization microscopy methods such as PALM and STORM. ThunderSTORM offers an extensive collection of processing and post-processing methods so that users can easily adapt the process of analysis to their data. ThunderSTORM also offers a set of tools for creation of simulated data and for quantitative performance evaluation of localization algorithms using Monte-Carlo simulations. ThunderSTORM and the online documentation are both freely accessible at <http://zitmen.github.io/thunderstorm/>. Help using the software can be found at <https://github.com/zitmen/thunderstorm/wiki>. Further information about ThunderSTORM can be found at [https://www.researchgate.net/profile/Guy\\_Hagen](https://www.researchgate.net/profile/Guy_Hagen).

1. M. Ovesný, P. Křížek, J. Borkovec, Z. Švindrych, and G. M. Hagen, "ThunderSTORM: a comprehensive ImageJ plug-in for PALM and STORM data analysis and super-resolution imaging," *Bioinformatics* **30**, 2389–2390 (2014). PMID: [24771516](https://pubmed.ncbi.nlm.nih.gov/24771516/) PMCID: [PMC4207427](https://pubmed.ncbi.nlm.nih.gov/PMC4207427/) DOI: [10.1093/bioinformatics/btu202](https://doi.org/10.1093/bioinformatics/btu202)

## Development and maintenance of Open Source software tools for the microscopy community.

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Computer software is essential to orchestrate movement of the motorized microscope components, grab data from the sensor(s) at user-specified intervals and order, and provide visual feedback. Research groups working on new microscopy techniques use extensive resources to develop specialized software for their specific microscopes, whereas groups that merely use light microscopes in their research usually rely on less-flexible commercial software packages. We sought to reduce the gap between these groups by creating an Open Source platform for microscope control that microscope developers can use to write software code needed to run new microscopes, which can be deployed on any possible microscope hardware, and that is easy to use.



This “μManager” software is freely available (<http://micro-manager.org>), works with most microscope hardware, has a large number of code contributors (from industry and academia) and involved users, is actively supported by a significant fraction of companies creating microscope hardware, and is used on several thousand microscope systems world-wide. A critical aspect of the success of this tool is the community-maintained documentation (<http://micro-manager.org>). Recent additions include a plugin that operates the ASI diSPIM microscope (1), a plugin for exploration of 3D space (2) in the microscope (Micro-Magellan), and a SAIM (scanning angle interference microscopy) plugin (3), that can measure z-heights at nm precision using a standard TIRF microscope.

Because funding structures are not conducive to infrastructural contributions such as μManager and also to provide better user support, the project transitioned from an academic environment to a small startup company, called Open Imaging (<http://open-imaging.com>). Open Imaging maintains the open source code base, continues to make downloads freely available, and provides direct end-user support. The soon to be released version 2.0 of the software (mainly developed by Open Imaging) provides major improvements in the user and programmer's interfaces, yet maintains extensive and robust hardware support. Future maintenance and development (and therefore availability) of this valuable resource depends on support of Open Imaging by the research community.

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Disclosure: NS, MAT, and RDV are co-founders of Open Imaging, Inc.

1. <http://dx.doi.org/10.1038/nprot.2014.172>
2. <http://dx.doi.org/10.1101/038117>
3. <http://dx.doi.org/10.1101/050468>

## A CONFOCAL SUPER-RESOLUTION APPROACH TO STUDY THE DYNAMICS OF GOLGI APPARATUS IN LUNG CANCER METASTASIS

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**KEY WORDS:** Golgi apparatus, Lung cancer metastasis, Confocal microscopy, Deconvolution, HyVolution

The Golgi apparatus is a critical effector of tumor cell polarization and directional motility during epithelial-mesenchymal transition (EMT), but the way in which organization and functions of the Golgi are regulated during EMT remains unclear. The structure-function relationship of the Golgi is a much debated topic, owing to the difficulties associated with biochemical purification of the Golgi and lack of high throughput optical techniques to resolve the Golgi elements, many of which are below the Abbe's diffraction limit. While electron microscopy approaches have a very high spatial resolution, such approaches have limited quantitative output and temporal resolution. Conventional widefield fluorescence and confocal microscopy approaches are diffraction limited whereas the super-resolution microscopy approaches are limited by throughput, difficulties associated with reproducible sample preparation across panels cell types, live cell compatibility and fluorophore combinations.

Here, we are presenting a quantitative assay to measure the structure-function dynamics of the Golgi apparatus below the diffraction limit, using HyVolution - a combination of high sensitivity hybrid (GaAsP) detector equipped confocal platforms to acquire diffraction limited high-resolution images using defined sets of acquisition parameters and subsequent computational super-resolution by image deconvolution. HyVolution has been reported to achieve a lateral resolution of 140 nm, which is ~1.6 times higher than the resolution achievable with conventional confocal microscopy. This assay is amenable to fixed and live cell and tissue imaging formats, using commercially available fluorophores and enabled screening of the Golgi morphology and function in panels of cell lines.

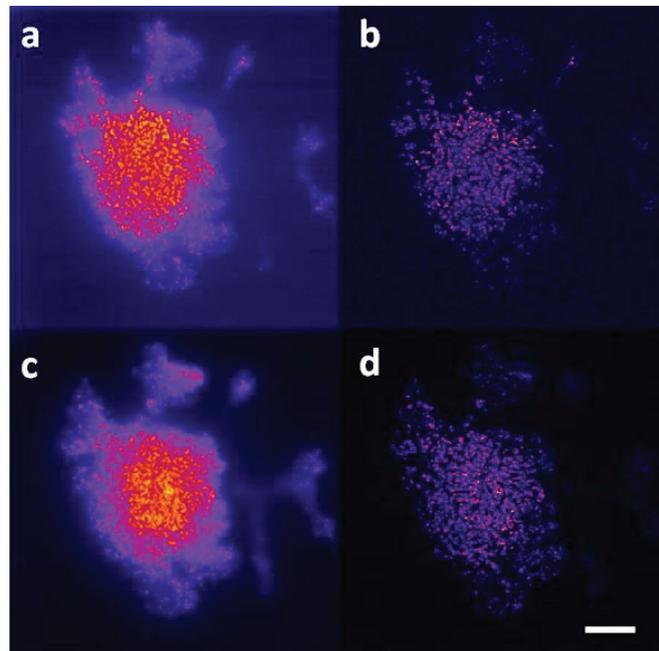
Using this approach, we found that the EMT- activating transcription factor ZEB1 leads to the formation of a polarized and compact Golgi structure with improved ribbon linking, enhanced Golgi matrix connectivity, and increased anterograde vesicle trafficking. Progesterin and adiponectin q receptor 11 (PAQR11), a ZEB1 derepressed Golgi-resident protein is required for ZEB1 to induce the structure-function changes in the Golgi, anterograde trafficking, cell migration and metastasis. Prevalent optical microscopy based assays of Golgi structure and function allows diffraction-limited quantitative assessment of Golgi compaction but does not allow precise measurement of Golgi element size and number, ribbon linking or compares compactness across several cell types. Our HyVolution based technique allowed comparison of panels of murine and human lung cancer cells based on their Golgi compaction, element size, number, ribbon linking and correlate the same with cell migration, invasion and metastasis.

**Blur reduction in TIRF and HILO microscopy****Reto Fiolka****Department of Cell Biology****UT Southwestern Medical Center, Dallas, TX, USA.****Email: [reto.fiolka@utsouthwestern.edu](mailto:reto.fiolka@utsouthwestern.edu)****KEY WORDS:** Fluorescence microscopy, live cell imaging, time-lapse imaging, Total Internal Reflection Fluorescence microscopy

Total internal reflection fluorescence (TIRF) microscopy [1] has become an indispensable tool in the live sciences, as it allows very sensitive imaging within the evanescent field used for illumination.

In practice, TIRF imaging can be plagued by imperfections of the evanescent field, which can produce image blur that compromises image analysis [2]. Furthermore, TIRF is limited to a thin layer of the basal membrane of a cell. Highly inclined and laminated optical sheet (HILO [3] ) microscopy was proposed to extend the reach of TIRF microscopy by illuminating the sample just below the critical angle for total internal reflection. However, oblique illumination schemes inevitably introduce out-of-focus blur, which lowers the contrast of the image.

Here, I show that by combining structured illumination with TIRF and HILO microscopy, out-of-focus blur can be rejected robustly, thereby drastically increasing the image contrast in both techniques. For densely labeled samples, this results in a drastic increase in image clarity, which is demonstrated by imaging a translocation biosensor. Further, structured TIRF and HILO imaging of the actin cytoskeleton and the dynamics of clathrin coated vesicles with improved image contrast is presented. Finally, a robust reconstruction algorithm is introduced that does not require deconvolution and avoids common artifacts encountered in structured illumination microscopy (SIM).



Comparison of SKMEL2 skin cells labeled with CLC-RFP as imaged by (a) TIRF, (b) structured TIRF, (c) HILO and (d) sHILO. Scale bar: 10 microns.

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QUANTIFICATION OF KERATIN INTERMEDIATE FILAMENT DYNAMICS IN  
NON-MOTILE AND MOTILE CELLS

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Rudolf E. Leube<sup>2</sup>,  
and Reinhard Windoffer<sup>2</sup>

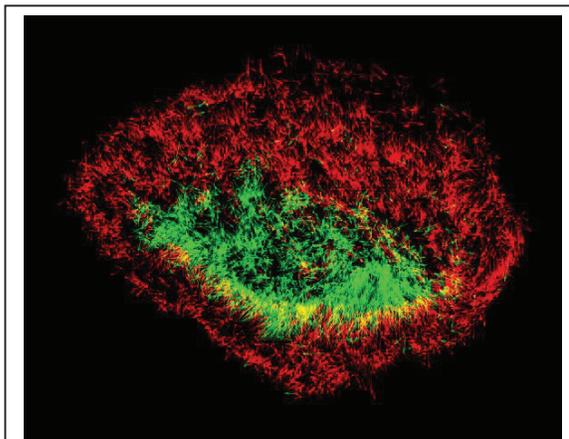
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KEY WORDS: intermediate filament, network dynamics, filament assembly, filament disassembly, actin

The cytoskeletal keratin intermediate filaments play a dominant role in maintaining epithelial tissue homeostasis. They establish a tissue-specific and mechanically resilient transcellular network by tight association with desmosomal cell-cell contacts. The cellular environment impacts keratin-dependent cell mechanics through various signaling cascades. The more than 60 disorders of keratin-related diseases and observations from knock out mice reveal additional roles of keratins that go far beyond pure mechanical functions [1]. The understanding of keratin dynamics is essential to mechanistically unravel aspects of the multiple keratin functions in living cells and tissues. A central aspect of keratin dynamics in living cells is the constant assembly and disassembly of the keratin network [2].



Flow fields of keratin (green) and actin (red) of a migrating keratinocyte. Arrows indicate direction of flow. Note the separation of keratin and actin domains in the cell.

To quantify this keratin turnover we developed tools to calculate intracellular movements of fluorescently labelled keratin in living cells via cross correlation. Furthermore, the intracellular localization of assembly and disassembly sites was determined. To overcome cell shape variations and to make results comparable, the derived heat maps were adapted to a circular normalized cell shape in non-migrating cells [3]. For migrating cells, shape was normalized to a near semi-circle reflecting the typical shape of migrating keratinocytes. Cells double labelled for keratin and actin were also analysed for intracellular movement to identify relationships between these two cytoskeletal components. Using these tools

we could identify biochemical and mechanical modulators of keratin dynamics.

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## COOPERATION, COMPETITION, AND CONFUSION MEDIATED SUBCELLULAR DECISION-MAKING REVEALED VIA 4-COLOR LIVE-CELL IMAGING

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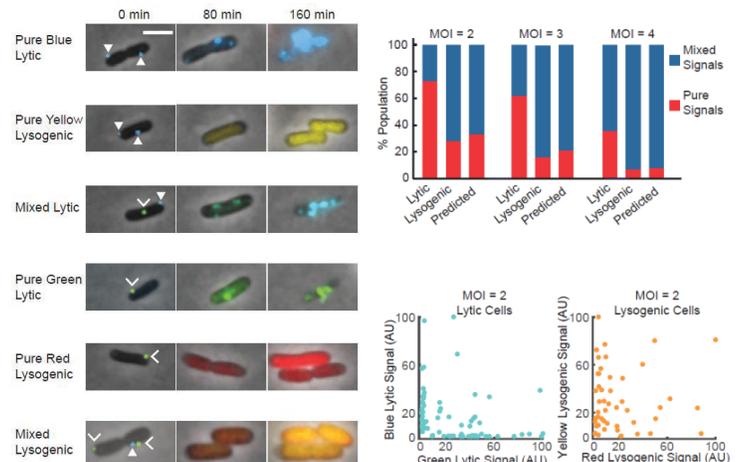
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**Keywords:** Fluorescence microscopy, decision-making, bacteriophages, computational modeling

The fates of single cells as well as whole organisms and populations depend on the decisions they make<sup>1</sup>. As decision-making is complex and ubiquitous, we use a simple paradigm for cell-fate decision-making<sup>2</sup>, the system of bacteriophage lambda development after infecting its host bacterium *Escherichia coli*, to deconstruct its fundamentals. Phage propagation bifurcates into either lysis, where virions are cloned and the host cell bursts, or lysogeny, where the phage DNA integrates with the host genome to replicate passively. Despite decades of research resulting in the characterization of the major pathways and key genes comprising the decision, it is still unclear how the phages integrate all of the factors to arrive at the ultimate cell fate, and this may be due to the insufficient resolution of past studies.

By taking advantage of higher resolution techniques, researchers have refined this model to better account for pre-existing host variations and the presence of independent cell fate commitments, or “voting,” by different co-infecting phages for decisions<sup>3</sup>. We seek to delve further into the idea of viral individuality by observing decision-making at the single-virus level using our 4-color fluorescence reporter system, complemented by computational modeling and quantitative analysis.



We find that individual phage DNAs have interactions which vary with the cell fate. Phages compete to dominate each other to maximize their own propagation during lysis. Phages cooperate by sharing proteins during lysogeny, commonly resulting in mutual propagation. In addition, both lytic and lysogenic pathways can be pursued in the same cell resulting in final cell lysis or lysogeny depending on the environmental conditions. With simple computational models, we predict that the cellular machinery involved in DNA replication and the timing of phage infections are key factors that give rise to phage competition, which are confirmed experimentally. Our study shows an example of how increased resolution combined with quantitation and computation can extract new details from an established system to enhance our understanding of complex processes.

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- 3 Zeng, L. *et al.* Decision making at a subcellular level determines the outcome of bacteriophage infection. *Cell* **141**, 682-691, doi:10.1016/j.cell.2010.03.034 (2010).

**Computer Aided Feature Extraction for Biological Images**  
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**KEY WORDS:** bioimage informatics, biofilament networks, brain morphology, image-based modeling

With the advances in bioimaging technology that are increasing in spatial and temporal resolution, novel computational tools that can rapidly process large image datasets and extract quantitative information are much needed. Current computational tools require intensive manual input and cannot efficiently perform automated feature extraction on large image stacks. We introduce the Computer Aided Feature Extraction (CAFE)<sup>1</sup> program, which is a command-driven and programmable environment to process images and build the corresponding structural models.

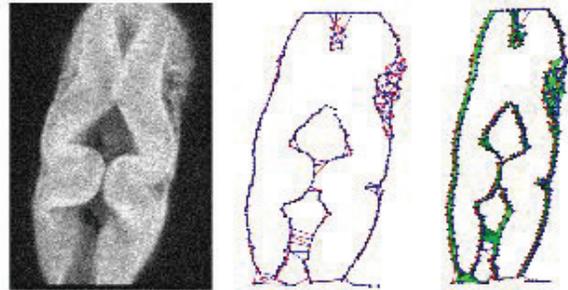


Figure 1. Zebrafish embryo brain from raw autofluorescence scan to CAFE reconstruction. (A) Image courtesy of A. T. Yeh, H. Gibbs, and A. Lekven. (B) Bead-filament and (C) surface models generated by CAFE. (C) is viewed at an angle.

Unlike pixel-based filtering approaches, CAFE analyzes features in biological images (Figure 1A) and represents them using simple geometric elements such as beads, filaments (Figure 1B), surfaces (Figure 1C), or networks (Figure 2). Images are analyzed based on global and local pixel intensity values. Depending on the application, the user can specify what form of reconstruction is required by modifying a CAFE input script.

These various representations allow for image analysis based on key features as well as enabling measurement and structural modeling in 2- or 3-dimensions. This methodology allows for versatility in analysis, fast processing speed, and customizable reconstruction forms. Application of CAFE to a variety of images, including triaxial collagen networks, linear microtubule filaments, and surface reconstruction of embryonic zebrafish images, is demonstrated. CAFE is expected to be a transformative tool for utilizing vast amount of bioimages to gain critical information and enable image-based simulation.



Figure 2. Structural model of a mitotic spindle constructed from CAFE from a stack of cryo-sectioning fluorescence microscopy images.

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**CALCIUM IMAGING WITH GCaMP6s REVEALS DIFFERENTIAL RESPONSES TO NICOTINE IN SEX-SEGREGATED HIPPOCAMPAL CULTURES**

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Sex differences have been shown to occur at the cellular level, even before puberty and the onset of high rates of sex hormone production. We have previously shown that chronic neonatal nicotine treatment can have long term effects on hippocampal excitability in a sex-specific manner. Since calcium is an important regulator of cellular function, we studied intracellular calcium dynamics in hippocampal cells in response to acute and chronic nicotine using the genetically encoded calcium indicator, GCaMP6s. Sex-segregated primary hippocampal cultures were derived from postnatal day 1 mouse pups, and transduced with an AAV5-viral vector encoding GCaMP6s. After 14-15 days *in vitro*, cells were imaged using a confocal microscope. GCaMP6s fluorescent signal dynamics, indicative of changes in intracellular calcium levels, could be visualized in cell bodies, and into proximal and distal neuronal dendrites and spines, and glial processes and micro-domains. Male and female hippocampal cultures had similar neuron to glia ratios, gross neuronal morphology and equivalent AAV5 transduction rates of neurons and glia. However, male and female neurons showed different responses to acute nicotine administration. In the presence of bicuculline (10  $\mu$ M), male and female neuronal somas showed differential responses to increasing doses of bath-applied nicotine (0.01  $\mu$ M to 10  $\mu$ M). Notably, at 0.1  $\mu$ M nicotine, female neurons reached their peak calcium response ( $p = 0.005$  compared to 0.01  $\mu$ M nicotine) and exhibited more robust calcium signal amplitude compared to male neurons ( $p = 0.042$ ) which reach their peak response at 10  $\mu$ M nicotine ( $p = 0.011$  compared to 0.01  $\mu$ M nicotine). Furthermore, we examined the effects of acute, bath-applied nicotine in cultures chronically exposed to 0.1  $\mu$ M nicotine for 5 days. Data suggest that chronic nicotine treatment increased the overall calcium response amplitude to acute nicotine in neurons. Glia cells from male and female cultures changed firing patterns in response to acute nicotine, and chronic nicotine exposure increased their calcium dynamics to bath-applied acute nicotine. These data reveal novel sex differences in the response to acute and chronic nicotine administration in hippocampal cultures. The results demonstrate sex differences that occur at the cellular level and in the absence of sex hormones, and therefore, may be due to either differences in intrinsic sex programming or patterning *in utero* in response to hormone surges. The observed differential responses may explain sex-specific long-term changes in hippocampal excitability that are seen after chronic neonatal nicotine administration.

**AN  $N$ -INTERFACE POINT SPREAD FUNCTION MODEL SUITABLE FOR WIDE-FIELD FLUORESCENT IMAGING OF THICK SPECIMEN WITH VARIABLE REFRACTIVE INDEX**

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**KEY WORDS:** Point spread function, spherical aberrations, refractive index, computational optical sectioning microscopy.

Wide-field fluorescence imaging of biological samples, like lung tissue, which are thick ( $>200\ \mu\text{m}$ ) and have variable refractive index (RI) introduces the challenge of space-variant (SV) imaging. The mismatch between the design and experimental conditions introduces spherical aberrations that cause every point in the specimen to be associated with its own unique 3D point spread function (PSF). Development of a method [1] that restores SV images requires the accomplishment of 4 integrated goals: 1) a methodology to derive the sample RI distribution; 2) the  $N$ -interface PSF model [2]; 3) the computationally tractable PCA based block-based (BB) forward imaging model that formulates space-variant imaging [3]; and 4) a conjugate-gradient based restoration approach [1]. The two widely [4,5] used models for PSF generation, assume that the specimen can be represented by a uniform average RI, which is a generalization, and is addressed by our  $N$ -interface PSF model [2]. In this study, we focus on the second goal, that is development of the  $N$ -interface PSF model, that combines microscope and specimen derived parameters in a rigorous vectorial formulation. Experimental evaluation and verification of the PSF model is accomplished using images from 175-nm fluorescent beads in a test sample. Simulation results highlight significant changes, including the direction of intensity attenuation, due to the change in the spatial location within the specimen and the associated RI. Fluorescent point sources (Fig. 1) imaged through rat lung tissue show a 42% improvement in accuracy of the  $N$ -interface PSF model over the existing PSF model prediction [1].

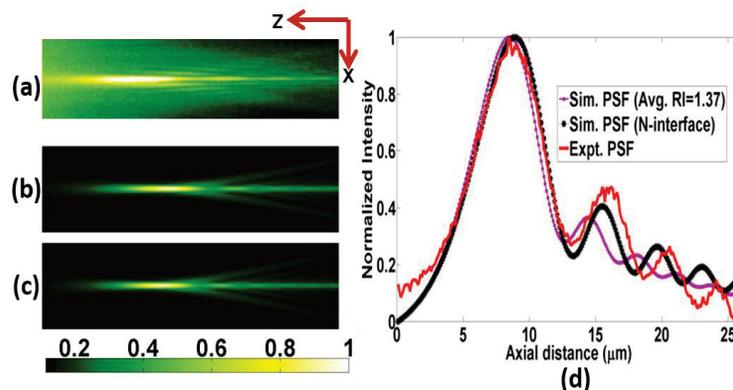


Fig1: Evaluation of the  $N$ -interface PSF model from wide-field image of a rat lung slice with bright fluorescing beads located below it.: (a) Experimentally acquired PSF. PSF predicted by the (b)  $N$ -interface model and (c) existing model [1]. (d) Axial intensity profile through the PSFs (a-c). Lens parameters: 40x/1.3 NA oil immersion (RI = 1.512); Wavelength: 509 nm; 3D grid size: 677x765x300; Axial and lateral spacing: 0.16 x 0.16  $\mu\text{m}$ .

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# IMAGING THICK SPECIMENS USING OPTICAL-SECTIONING MICROSCOPY COUPLED WITH BLOCK-BASED 3D IMAGE RESTORATION

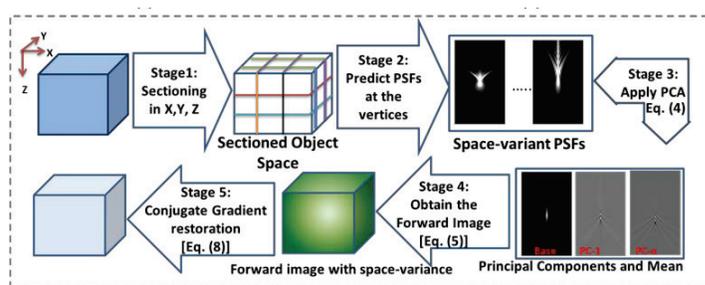
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**KEY WORDS:** Block-based 3D image restoration, 3D image formation theory, space-variant point-spread function, computational optical sectioning microscopy.

Three-dimensional (3D) imaging of optically thick samples with refractive index (RI) variance is prone to spatially variant (SV) spherical aberration (SA), which causes the system's point-spread function (PSF) to vary at every point within the imaging volume and consequently resulting in restoration artifacts when commercial deconvolution is used for data processing. A block-based restoration (BBR) method that overcomes this problem is presented here. The BBR method (Fig. 1), based on a block-based forward imaging model [1] that approximates SV imaging using multiple SV-PSFs [2], solves a maximum-likelihood optimization problem using a fast converging conjugate-gradient iteration scheme [3]. Simulations using objects comprised of similar structures suspended in media with variable RI were used to show the effect of SV and to establish the advantage of BBR over current restoration methods. Experimental and synthetic images of a controlled test sample, comprised of 15  $\mu\text{m}$  FluoSphere beads distributed in an air to mountant interface (max RI difference = 0.47), were restored successfully using the BBR method while consistency between experiment and simulation is demonstrated. In the BBR method information from DIC and fluorescence images is combined to identify regions with changing RI within the imaging volume. The effect of the block size on the restoration was investigated with simulations using a numerical lung phantom relevant to biological studies. Results show that the BBR method is capable of restoring object intensity using only 5-18% of information needed to otherwise account for SV imaging. The BBR method is the first step towards accurate and efficient reconstruction of thick biological samples with varying RI.



**Figure 1:** Schematic diagram of the BBR method stages: 1) Object space sectioned along the X, Y and Z axes; 2) Corresponding SV PSFs computed at block vertices; 3) Principle components and corresponding coefficients computed from principal components analysis (PCA) [1]; 4) Forward image computed using interpolated weighting coefficients; 5) Object intensity computed through iterative restoration. Eqs. 4, 5 and 8 mentioned here are defined in Ref [3].

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**QUANTITATIVE EVALUTATION OF ACCURACY AND VARIANCE OF  
INDIVIDUAL PIXELS IN A SCIENTIFIC CMOS (sCMOS) CAMERA FOR  
COMPUTATIONAL IMAGING**

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**KEY WORDS:** scientific camera, sCMOS, quantitative imaging, read noise distribution, fixed pattern noise, photoresponse non-uniformity (PRNU), dark signal non-uniformity (DSNU), linearity, quantum efficiency map, individual pixel variance, individual pixel mean

“Scientific” CMOS (sCMOS) camera architecture fundamentally differs from CCD and EMCCD cameras. In scientific digital CCD and EMCCD cameras conversion from charge to the digital output is through a single electronic chain and the read noise and the conversion factor from photoelectrons to digital outputs are highly uniform for all pixels, although quantum efficiency may spatially vary.

In CMOS cameras, the charge to voltage conversion is separate for each pixel and each column has independent amplifiers and analog to digital converters, and pixel-to-pixel variation in quantum efficiency is possible. The “raw” output from the CMOS image sensor, includes pixel-to-pixel variability in the read noise, gain, offset and dark current. Scientific camera manufacturers digitally compensate the raw signal from the CMOS image sensors.

Unless corrected, inaccuracies and noise in image can introduce artifacts in computational imaging, such as lightsheet microscopy or localization microscopy, especially for maximum likelihood estimation methods. We measured the *distributions* and *spatial maps* of individual pixel offset, dark current, read noise, linearity, photoresponse non-uniformity (PRNU)(Fig.1) and variance distributions of individual camera pixels for a Hamamatsu ORCA Flash 4.0 V3 sCMOS camera. Measurements are taken with highly uniform and controlled illumination over low light conditions from dark conditions and at multiple low light levels between  $\sim 20$  to  $\sim 1,000$  photons / pixel per frame.

We further show that using individual pixel variance to “correct” pixel gain for photo response non-uniformity (PRNU) correction is fundamentally incorrect and instead increases PRNU in cameras with accurate factory calibration.

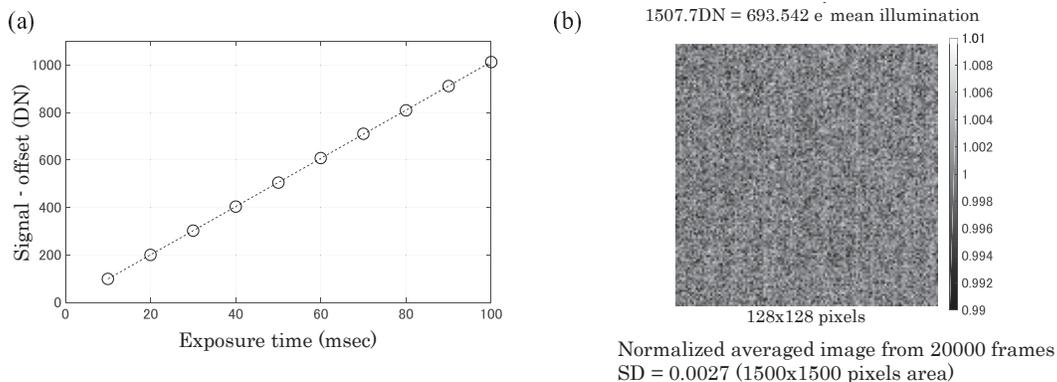


Fig. 1. (a) Low-light linearity. Plot offset subtracted signal (DN) against camera exposure time (msec). Signal ranges from 100 DN to 1000 DN (50 to 500  $e^-$  mean signal). (b) Normalized photo-response non-uniformity (PRNU) of 128x128 area at 694  $e^-$  mean signal from 20000 frames. Statistical analysis shows SD = 0.0027 (1500 x 1500 pixels area).

## LOCALIZATION AND PARAMETER ESTIMATION IN PARTICLE TRACKING USING SEQUENTIAL MONTE CARLO WITH EXPECTATION MAXIMIZATION

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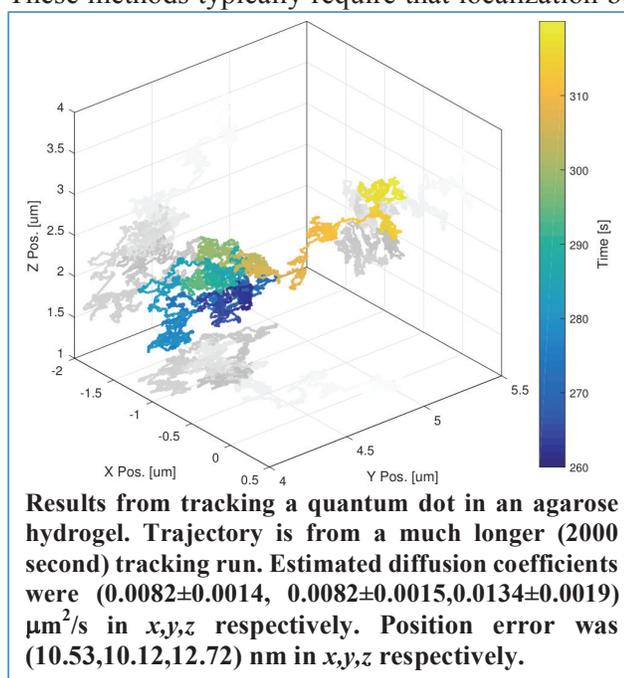
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**KEY WORDS:** parameter estimation, system identification, particle filter, particle tracking

While there have been several breakthroughs in recent years in the optimal estimation of motion parameters from single- and multiple-particle tracking data, the most common method for extracting such parameters remains the use of the mean square displacement [1]. Recent improvements include an optimal framework for diffusive motion that accounts for both motion blur and Gaussian white noise [2]. This has been extended to allow for Ornstein-Uhlenbeck motion, modeling confined diffusion [3]. These methods typically require that localization be performed prior to the estimation of motion parameters rather than in conjunction and are limited to Gaussian-distributed position error. While these assumptions hold well when analyzing motion in two-dimensions, they begin to break down when considering three dimensional motion over a large distance along the optical axis.

We describe an approach that uses Sequential Monte Carlo combined with Expectation Maximization for joint localization and motion parameter estimation from raw data such as image sequences [4]. The method can handle almost arbitrary observation models, including engineered point spread functions and camera-specific noise models, as well as a broad class of motion models. Its effectiveness is

demonstrated through both simulation and experiment. The experiments involve the use of our extremum seeker based algorithm for tracking single particles based on a confocal measurement modality [5], illustrating both the utility of that approach to tracking as well as the ability of the estimation procedure to handle such different measurement modalities.



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**Towards Real-Time Localization of sCMOS Single-Molecule-Switching Data**

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**KEY WORDS:** maximum likelihood estimation, GPU localization, distributed analysis, distributed storage

Single-Molecule-Switching Nanoscopy (SMSN) methods such as PALM or STORM can retrieve spatial information from within a cell at 20-80 nm resolution, about 10-fold better than diffraction-limited conventional microscopy. However, the low temporal resolution typical of SMSN imaging limits its usefulness for samples of more than tens to hundreds of cells.

The use of sCMOS cameras [1] and high-power lasers have recently enabled acquisition at speeds an order of magnitude faster than with EMCCD cameras [2]. Despite significant advances in analysis speed for EMCCD based SMSN [3], the higher acquisition speeds enabled by sCMOS cameras again create a data analysis bottleneck. This bottleneck is further compounded if we pursue high-quality fits, as sCMOS cameras have both non-negligible read-noise and shot-noise, necessitating a fit that employs a more complicated noise model than that required for EMCCD cameras [1].

By optimizing our existing GPU-accelerated, sCMOS-specific Gaussian fit, and by shifting point detection and ROI extraction from the CPU to the GPU using PyCUDA [4], we have reduced the run-time of our sCMOS-specific molecule localization pipeline by more than an order of magnitude. Our optimized fit can process 20,000 ROIs per second with 15x15 pixel ROIs, and our accelerated candidate detection and ROI extraction is capable of processing 5,000 frames per second with 200x200 pixel frames. This allows us to perform astigmatic fitting at over 200 frames per second for 256x256 pixel images on a single computer. We have included this new sCMOS fitting routine as an optional module in our PYTHON-Microscopy Environment (PYME) software suite. We have additionally expanded the distributed analysis and distributed storage capabilities in PYME, allowing us to multiplex our analysis across a computer cluster, which will be able to scale to an analysis rate of more than 1000 raw frames per second.

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## Classification of diffusion dynamics from particle trajectories

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**Keywords:** *diffusion, Brownian motion, classification, trajectory, MSD, tracking, statistical tests*

Measuring confined diffusion, Brownian motion and active transport is central in cell biology since they represent the main modes of mobility of molecules in living cells. In quantitative microscopy, there are multiple techniques that allow molecule mobility analysis. Single Particle Tracking (SPT) is attractive since it can reveal individual dynamics, close to molecular resolution. However, robust statistical procedures to classify individual trajectories are lacking. Consequently, we have especially developed TOTH to process individual trajectories provided by particle tracking algorithms and 2D+t and 3D+t images acquired with standard microscopy methods such as wide-field or confocal microscopy or with super-resolution microscopy such as spt-PALM.

In our framework, we assume that the motions of particles follow a certain class of random process: the diffusion processes. We have proposed a statistical method able to classify the motion of the observed trajectories into three groups: “confined”, “directed” and “free diffusion” (namely Brownian motion). THOTH is an alternative to the commonly used Mean Square Displacement (MSD) analysis. We assessed our procedure on both simulations and real cases; an example of confined diffusion is the Ornstein-Uhlenbeck process while an example of directed diffusion is the Brownian motion with constant drift. The method is currently applied to investigate membrane trafficking (Rab11/Langerin (see Figure 1) and Rab11/TfR protein sequences) using the following procedure:

1. Tracking of particles with any competitive algorithm.
2. Statistical test /classification applied on tracks longer than ten time points.
3. Estimation of diffusion parameters (e.g. drift, diffusion, ...).

Each trajectory is labeled with the most likely process and the parameters of the underlying process are estimated. Future work will concern the detection of change of motion dynamic over time.

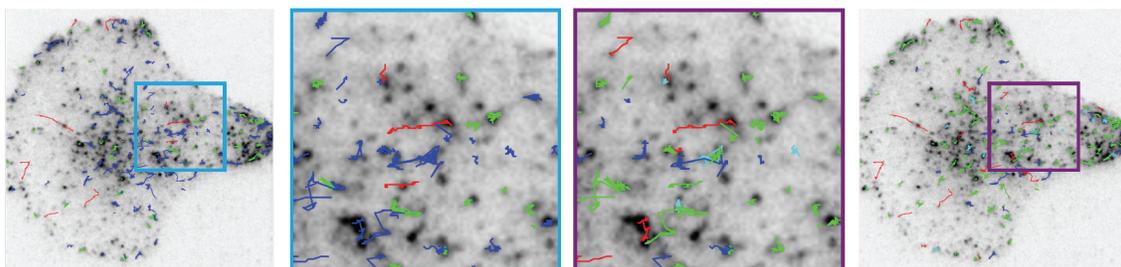


Figure 1. Labeling of the dynamics of trajectories on the Langerin protein sequence (Courtesy of UMR 144 CNRS-Institut Curie and PICT IBIISA). We display only the trajectories appearing on the first 100 frames. The color code is red for directed Brownian, green for confined diffusion, blue for Brownian, light blue for motionless. Left panel is labeled with TOTH, right panel with the MSD method.

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## DC-SIGN mediated dengue virus entry into cells

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DC-SIGN (a single-pass C-type lectin) is a receptor for dengue virus (DENV) and a variety of other pathogens. This receptor exists in small clusters on the plasma membranes of dendritic and other cells, and facilitates viral entry and infection. DENV binding, surface transport, endocytosis, intracellular trafficking and infection have been investigated for NIH3T3 cells that express wild type DC-SIGN or various mutants, as well as for primary dendritic cells. These measurements have employed confocal microscopy, super-resolution imaging, dual-color co-localization microscopy, large-scale automated single particle tracking, and single molecule and particle counting. A variety of results will be discussed.

### 3D QUANTITATIVE PHASE IMAGING TECHNIQUES FOR NON-INVASIVE LABEL-FREE IMAGING OF CELLS AND TISSUES

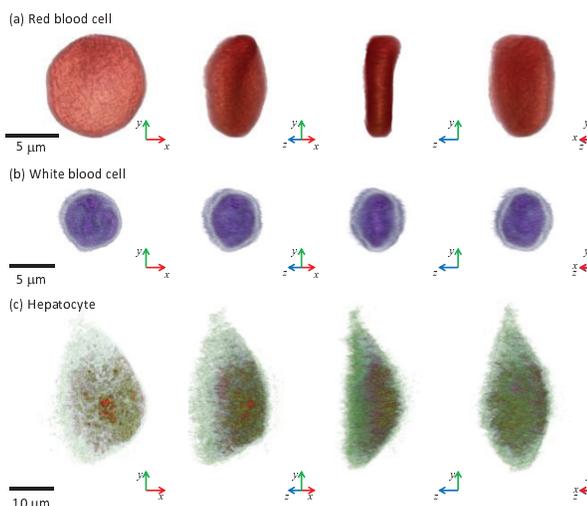
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**KEY WORDS:** quantitative phase imaging, optical diffraction tomography, holotomography, label-free imaging

Quantitative phase imaging (QPI) has emerged as an invaluable tool for imaging small transparent objects, such as biological cells and tissues. QPI employs various interferometric microscopy techniques to quantitatively measure the optical phase delay of samples. In particular, the measured optical phase delay provides information about the morphological and biochemical properties of biological samples at the single-cell level. Recently, QPI techniques have been widely applied to study the pathophysiology of various biological cells and tissues, including red blood cells (RBCs), white blood cells, bacteria, neurons, and cancer cells.

In this talk, we will present the recently developed 3-D holotomography setup using a dynamic mirror device, which is an optical analogous to X-ray computed tomography. In particular, we will discuss the visualization of 3D refractive index distributions of biological cells and tissues measured with the 3-D holotomography using the transfer function method, which has been widely used in the visualization field [1-4]. The outcome demonstrates outstanding visualization of 3D refractive index maps of live cells, which will be potentially used in various applications in biology and medicine.

we will also discuss about the commercialization of the technique.



**Figure 1** 3-D Images of representative biological cells.

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# Bayesian fluorophore separation measurement in unresolved PSF using realistic EMCCD noise model

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Sara Needham      Marisa Martin-Fernandez

September 15, 2016

## Abstract

Electron multiplied charge coupled devices (EMCCD) are frequently used in a wide range of scientific applications to take images under low-light conditions and photon-counting experiments. However, the combination of statistical processes that relate the recorded number of electrons from the incident photons in such a device result in pixel noise which has a complicated form, and as a result the analysis of EMCCD images often assumes simplified forms of the noise model to facilitate ease of interpretation.

We present the application of the realistic Poisson-Gamma-Normal EMCCD noise model of Hirsch et al 2012 to typical super-resolution analysis of fluorescent molecules, such as that which is done in FLImP analyses (Needham et al 2014, 2016), which measures the separation of two fluorophores in an optically unresolved PSF. The application of this complicated pixel noise model requires the removal of certain simplifying assumptions in the current method, such as the analytic determination of maximum-likelihood model parameters, and is facilitated by the application of a fully Bayesian treatment with posterior sampling methods. We discuss the improvements which result from this formalism, as well as the limitations in the use of a realistic noise model in comparison to current methods through application to simulated data sets.

These improvements in the analysis will enable relaxing assumptions of the model to allow exploitation of a greater proportion of FLImP data. This will improve statistics of FLImP studies, increasing their potential to provide key insights into receptor-protein interactions on mammalian cells, beyond dimerisation to investigations on oligomerisation.

## DECISION-MAKING OF BACTERIOPHAGE P1

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**KEY WORDS:** cellular decision-making, bacteriophage P1, single cell, fluorescent phage

Upon infection by bacteriophage P1, a bacterium cell can make the decision for either entering the lytic pathway, where new viruses are produced inside the cell and released to the environment after cell lysis, or entering the lysogenic pathway, so that the virus DNA remains in a dormant state as a unit copy plasmid, without harming the host cells [1].

Consistent with the literature [2], our bulk experiments show that the lysogenization frequency of P1 against the average phage input (i.e. ratio of phage over cell concentration) follows a Poisson distribution of  $N \geq 1$ , and the lysogenization frequency can reach 30% under optimal conditions. In contrast, a cell-fate paradigmatic system, bacteriophage lambda, follows a Poisson distribution of  $N \geq 2$  indicating the cell requires 2 or more phages to lysogenize a cell [3]. Recently, studies at higher resolution have yielded new insights about the lysis-lysogeny decision-making, such as individual phage voting and concurrent development of lytic and lysogenic pathways within the same cell [4-6]. The gene regulatory network for lysis-lysogeny decision of P1 is fundamentally different from lambda such as three immunity regions for P1 versus one for lambda, and the repressor gene *cI* of P1 is early expressed while *cI* of lambda is late. Here, we ask how the different regulatory networks contribute to the different cell-fate decision pattern, and how stochasticity plays a role in the decision-making process.

In order to study P1 at the single-cell/single-phage level, we report the lytic and lysogenic pathways fluorescently, i.e. genome modifications of *gene 23* encoding the major capsid protein and *cI* for major repressor. From this reporter system, the fluorescently fused capsid protein gp23 allows us to track the infecting phages on the host cell during the infection. The lytic pathway is characterized by the production of many new fluorescent phages, followed by cell lysis. The lysogeny can be detected by the expression of C1 during cell growth and division. Our aim is to create a spatiotemporal map linking the microscopic infection parameters—number and position of infecting phages, cell age and growth rate, etc.—with the eventual decision made.

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## Application of different imaging approaches for characterization of interactions of ErbB2 receptor and tetraspanin-enriched microdomains.

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ErbB receptor tyrosine kinases play a significant role in normal cellular processes in adult organisms and in embryogenesis. Genetic modifications in these molecules and epigenetic changes in their expression result in cardiac dysfunction, and in cancer progression. We have identified a tetraspanin CD82 as a modulator of EGFR signalling and intracellular trafficking (Odintsova et al., 2006, 2013). Our recent unpublished work indicated that CD82 also has an effect on the dynamics of ErbB2 receptor at the plasma membrane in breast cancer cells.

Tetraspanins are four transmembrane domain proteins known to organise associated proteins into specific functional platforms – tetraspanin-enriched microdomains (TERMs) – which could have either activating or attenuating roles. Interactions within TERMS are dependent on palmitoylation of the proteins and lipid content of the membrane. We investigated how changes in expression levels of CD82 affected biophysical properties of ErbB2 receptor and the nanoscale organization of TERMS. Using TIRFM and single particle tracking (SPT) we observed changes in receptor's characteristics, such as diffusion coefficient, dwell time and size of confinement zone in cells overexpressing CD82 or its palmitoylation-deficient mutant. We have investigated various methods to image real time interactions of ErbB2 with membrane microdomains, including dual colour single molecule tracking, and live cell SOFI - the results of which indicate a possible effect on ErbB2 diffusion by interaction with CD82-enriched microdomains.

We calculated cluster diameters and molecular density within clusters of CD82 and ErbB2 in cells with different expression levels of ErbB2 and CD82 by using Ripley's K-function cluster analysis of STORM data (Owen et al., 2010) acquired by imaging of fixed cells. We found that although the cluster diameter of both proteins is similar in the cell types we investigated, CD82 cluster density was increased in cells overexpressing CD82. ErbB2 cluster density was also affected by CD82 expression, decrease/increase being correlated with the expression level of the receptor. Interestingly, CD82-palmitoylation-deficient mutant was found in more dense clusters than the wild type protein. This finding indicates that although palmitoylation is essential for the interactions of individual tetraspanins with other proteins it is not important for the formation of clusters of one type of molecules. We found that CD82 overexpression has an implication on the basal level of phosphorylation of ErbB2 receptors and downstream signalling molecules. We are evaluating a multiscan DBSCAN cluster analysis and optimising imaging conditions for dual colour STORM imaging in order to compare characteristics of co-localised clusters.

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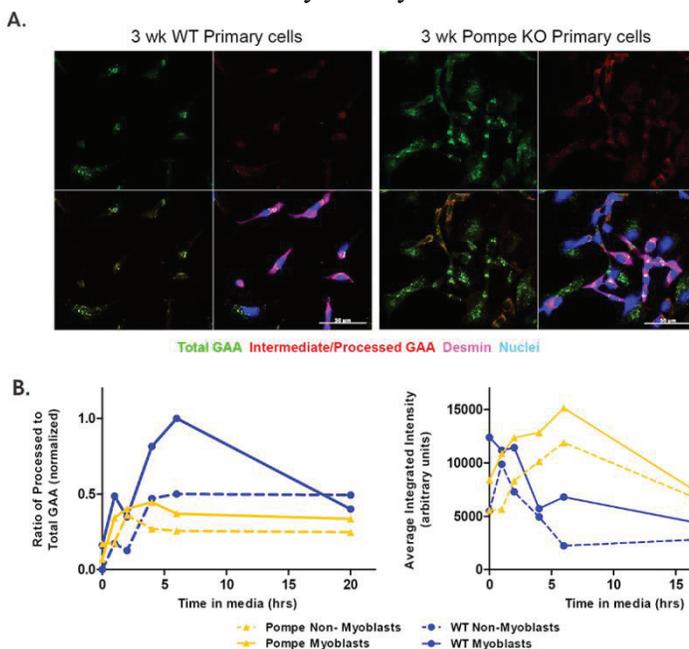
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## INTERNALIZATION AND CELLULAR TARGETING OF rhGAA IN WT AND GAA KO MICE

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Pompe disease (glycogen storage disease type II) is a rare and debilitating genetic disease characterized by deficiency of lysosomal acid alpha-glucosidase (GAA). Enzyme replacement therapy (ERT) utilizing recombinant human GAA (rhGAA, Myozyme) currently represents the most effective therapeutic approach [1]. However, the precise cellular and sub-cellular localization of administered enzyme and its processing status in various target cell types remains poorly characterized. To address this need, we have developed novel analyses involving immunofluorescence staining with two different anti-GAA antibodies which recognize either unprocessed or processed enzyme along with antibodies which permit identification of specific cell types or subcellular organelles. Laser scanning confocal microscopy and quantitative image analysis were used to measure rhGAA internalization, subcellular targeting, and processing kinetics in primary cell cultures from WT and GAA KO mice. A unique advantage of the microscopy-based approach employed is that it permits identification and analysis of specific cell types in mixed cell populations, which is essential when dealing with primary cell cultures. Data derived from studies of these primary cultures suggests that myoblasts process internalized enzyme somewhat more slowly than non-myoblast cells and that both myoblast and non-myoblast cells from GAA KO mice internalize GAA more slowly than cells derived from WT mice. In parallel with these efforts, immunofluorescence and confocal microscopy were used to better understand the tissue and cell type targeting of rhGAA *in vivo*. Our results indicate that a large amount of the administered rhGAA is internalized by macrophages and endothelial cells of the liver and spleen, and that these cells represent a non-productive ‘sink’ for administered enzyme. In comparison, administered rhGAA appears to be relatively inefficiently targeted to skeletal muscle tissue where it is observed in endothelial cells, macrophages, and myocyte-associated cells which may be satellite cells. Potential internalization by satellite cells suggests a novel targeting mechanism involving internalization of enzyme by satellite cells

followed by fusion of enzyme-laden satellite cells with diseased myocytes. While enzyme was easily observed in muscle tissue, it was seldom observed in myocytes themselves. We believe that this observation may be due to the inability of our current tissue preparation methods to preserve accumulated glycogen in tissue, the substrate of GAA and a molecule with which administered rhGAA may be associated. It is hoped that the data generated from these and related future studies will provide additional insight into the pathophysiology of Pompe myocytes and help to inform efforts to improve current generation rhGAA through more efficient targeting to affected tissues and cell types.



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# VISUALIZE PHAGE DNA DEGRADATION BY CRISPR SYSTEM AT THE SINGLE-CELL LEVEL

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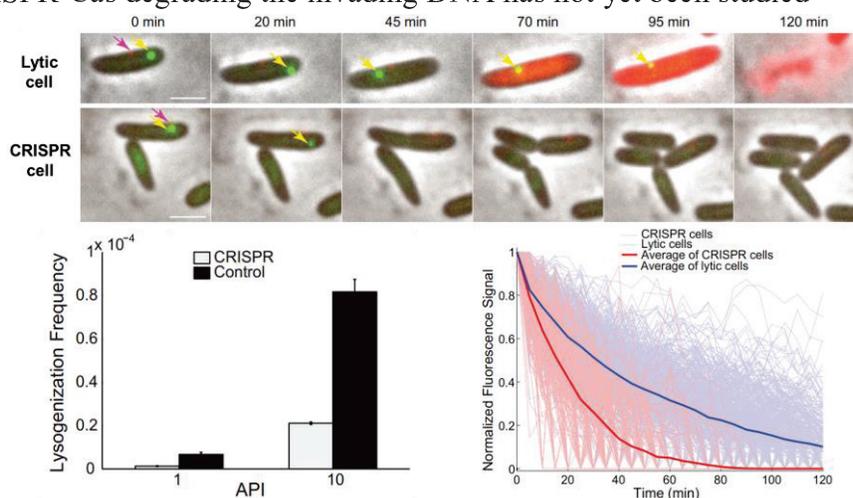
**KEY WORDS:** CRISPR, degradation, single cell, bacteriophage, fluorescence microscopy

The CRISPR-Cas system is a widespread defense system evolved in bacteria in order to protect themselves from invasive nucleic acids, such as plasmids or bacteriophage DNA [1]. A great number of studies have focused on the components and mechanism of this system, however, the direct visualization of CRISPR-Cas degrading the invading DNA has not yet been studied at the single-cell level. Here,

we take advantage of the bacteriophage (or simply phage) lambda system to visualize CRISPR action *in vivo*. Phage lambda is prepared to be fluorescently labeled in order to be visualized when infecting the cell, and with fully methylated DNA packaged in its head, which can be then visualized as a

fluorescent spot through the binding of SeqA-YFP protein once the DNA is ejected into the *E. coli* reporter host cell [2]. The CRISPR system is introduced by artificial CRISPR-Cas plasmids transformed into the host *E. coli* cells. Upon phage infection, the ejected DNA degradation can be tracked over time.

With this CRISPR in our phage DNA reporter system, the lysogenization frequency decreases significantly when comparing to a non-CRISPR control system, consistent with that reported in the literature [3]. At the single-cell level, when comparing cells where CRISPR is active against those lytic cells where CRISPR does not function, the phage DNA spot intensity in CRISPR cells decreases much faster than that in lytic cells. The CRISPR degradation typically finishes around  $37 \pm 17$  minutes, whereas, phage DNA spots in lytic cells persist until the cells lyse or beyond the end of the movies (120 minutes). In addition, we characterize some parameters accounting for the effect of cell-to-cell variability on the CRISPR function, such as initial spot intensity and cell size.



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## Increased Gene Expression Noise Mediated by DNA Replication Promotes Cell-Fate Bifurcation

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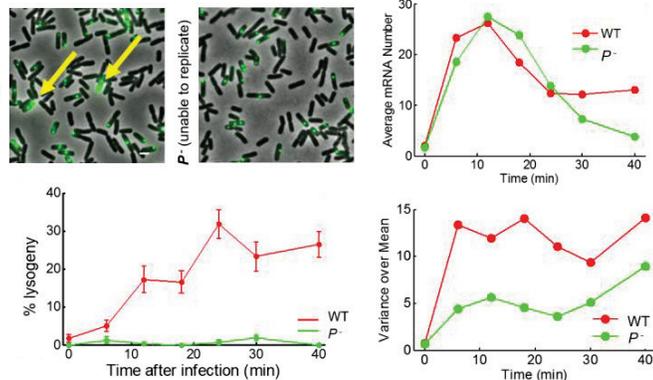
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Cellular decision-making is a common process among all life forms, and a key step that organisms take to integrate the environmental signals to choose an optimal response to improve their overall fitness [1]. The genetic circuits selected to carry out this task determine the cell fate in a seemingly probabilistic way, and this raises the question of how the components of each circuit together contribute to the cell-fate bifurcation.

To investigate this problem, we utilize a well-established system for cellular decision-making, the paradigm of bacteriophage lambda infection, which leads to two distinct outcomes – lysis or lysogeny [2]. The genetic components involved in this lysis/lysogeny decision have been well characterized, and recent single cell/phage level studies have revealed some previously hidden deterministic factors that contribute to the cell-fate decision [3]. However, those factors cannot explain away all the randomness in the decision-making process. Whether this noise comes from the intrinsic properties of the genetic circuit itself remains to be addressed. To answer this question, we quantify the spatiotemporal gene expression of key cell-fate determining genes in their native circuit with wild type phage infection using single-molecule fluorescence *in situ* hybridization (smFISH) and compare them to multiple phage mutants with defects in the cell-fate bifurcation. Simultaneous monitoring of the early mRNA transcription and the resulting cell-fate distributions showed that the weak negative gene

regulator Cro which is required for lytic development, decreases the overall mRNA transcription level. Interestingly, we also observed that early phage DNA replication, whose role in the decision-making process has been overlooked, increases the noisiness of the early mRNA transcription and is essential for the bifurcation. Phages defective in DNA replication fail to establish both the lysogenic and lytic state. We seek to



understand the source of noisiness and to investigate how DNA replication mediates increased noisiness using quantitative imaging (smFISH and immunofluorescence) combined with mathematical modelling. We further hypothesize that this noisiness at the mRNA level which might be amplified at the protein level, is critical for the bifurcation by allowing some cells to enter the lysogenic state while the others default to the lytic. Insights gained from this study will promote better understanding the properties of selected genetic circuits and how they allow cell-fate bifurcation.

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### Analyzing Waves of Tumor Cell Migration

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The ability of cells to migrate is crucial for embryonic development, wound healing, tumorigenesis and metastasis. Tracking large numbers of cells and analyze their morphodynamic behavior is pivotal for understanding the molecular, metabolic and physical mechanisms of cell motility leading to metastasis phenotype. We describe here an analytical framework designed for an automated high-throughput quantification of single cell migration and morphodynamics. The analysis combines and associates vast amount of spatiotemporal data across multiple experiments into quantitative measures. We demonstrate the power of the software by quantifying variations in cell population migration rates while manage to explicitly detect and quantify single cell morphodynamic signatures during collective cell migration, single cell EMT and Amoeboid migration in vitro and in vivo.

Using a tumor progression model of SKOV-3 cells, we have isolated MGTC (multi-generation tumor cells) clones that are highly metastatic cells compared to their SKOV-3 predecessor. MGTC.1-3 demonstrated increased Met basal phosphorylation and enhanced p-Met/Met ratios. Using this analytical framework we further characterized the emergence of the coordinated migration wave propagating backward from the wound front, which reflects the formation of clusters of coordinately migrating cells that are generated further away from the wound edge and disintegrate close to the advancing front. This wave was evident in differentiate tumor cells but not in poorly differentiated cells. Morphodynamic analyses demonstrate that while SKOV-3 showed low Velocity, Displacement<sup>2</sup> and high Collectivity, Directionality, Area and Sphericity, whereas MGTC.1-3 exhibited higher velocity with poor directionality, no collectivity and relatively lower area and sphericity. In both the SKOV-3 and MGTC.1-3, Met activation by HGF/SF induced increased Velocity and the overall morpho-dynamic unique signature. Assessments of the relations between the waves revealed that amplified coordinated migration is associated with the emergence of directional migration.

We also examined the amoeboid cell motility induced by Met signaling and its interaction and phosphorylation of the adaptor proteins. Met expression induced small rounded cell structures with slow dynamics, whereas Met and Grb2 expression increased directional motility and cell size. Met and Gab1 expression dramatically increased velocity, however cells did not move with directionality.

Taken together the analytical framework enable new insight and suggest a basic cellular mechanism for long-term cell guidance and intercellular communication during single and collective cell migration induced by modified downstream Met signaling leading to motility plasticity and enabling metastasis

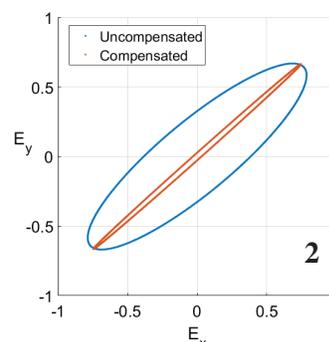
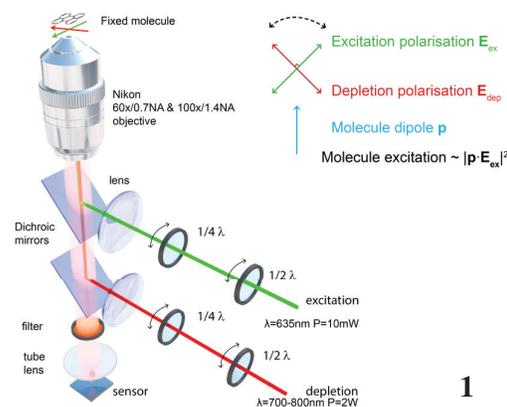
## INDUCING SPARSITY AT LN<sub>2</sub> TEMPERATURES: TOWARDS POLARIZATION-SELECTIVE SINGLE-MOLECULE EXCITATION AND EMISSION DEPLETION

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**KEY WORDS:** fluorescence, dipole emitter, STED, cryogenic temperature

The accuracy with which a molecule can be localized is ultimately limited by the amount of emitted photons that can be collected before photobleaching. At cryogenic temperatures, photobleaching is strongly suppressed, and a localization precision of better than 1 nm has been reached [1]. However, room-temperature techniques that induce sparsity in a fluorescently labeled sample, such as photoswitching, may not work or only very inefficiently. In order to maintain typical labeling densities, alternative routes are required to introduce a sparse activation of fluorophores at low temperatures.

Here, we show our latest results on promoting sparsity with polarization-selective single-molecule excitation and emission depletion. We use the fact that, below saturation, the excitation of a dye molecule with a fixed transition dipole moment is proportional to  $|\mathbf{p} \cdot \mathbf{E}|^2$ , where  $\mathbf{p}$  is the dipole moment of the molecule and  $\mathbf{E}$  is the electric field vector. Hence, rotating the polarization results in a  $\cos^2 \alpha$  dependency of the emission, leading to a slight increase in sparsity in the sample. Moreover, we will show our progress towards implementing a non-linear polarization-dependent emission depletion scheme [2, 3], to obtain a  $\cos^n \alpha$  dependency with  $n$  substantially larger than 2 (Fig. 1). We aim at a linear polarization state with a better than 1:100 polarization extinction ratio, regardless of the chosen angle or the additional optics required between excitation and the sample. Therefore, we fully calibrate our optical system, and compensate for spurious polarization changes due to, e.g., dichroic mirrors and non-perfect waveplates. Careful calibration of both excitation and emission depletion light paths allows us to drastically improve on a naive implementation of polarization selectivity. For the excitation path alone, we have improved on the linearity of the polarization by up to a factor of 12, which in intensities means a suppression of the unfavorable polarization of over two orders of magnitude.



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## FAST CYCLING OF INTEGRIN-CLUTCH FORMATION AS REVEALED BY SINGLE-MOLECULE IMAGING

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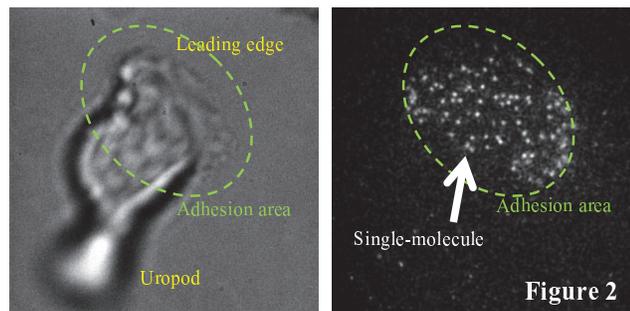
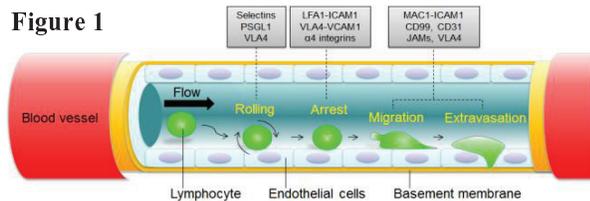
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**KEY WORDS:** Single-molecule; Integrin; Cell adhesion; Immune cell; Chemokine

Integrins are transmembrane receptors that are important for cell adhesion and migration [1]. Integrin LFA-1 regulates immune cell adhesion and trafficking by binding to its ligand ICAM-1 upon chemokine stimulation (Fig.1). Integrin-mediated mechanical linkage between extracellular ICAM-1 and the intracellular actin cytoskeleton, i.e. molecular clutch formation, is important for cell adhesion [2]. Several experiments combined with  $Mn^{2+}$  treatment have suggested stable clutch formation accompanied by high-affinity ligand binding for cell adhesion [3]. Yet the relationship between cell adhesion and the stability of clutch formation is controversial, because no significant increase in ligand binding affinity has been observed even when cell adhesion was promoted [4-7]. We analyzed the molecular dynamics of integrin in living cells (Fig.2), clarifying that transient, unstable clutch formation regulates the adhesion of lymphocytes undergoing dynamic migration after being stimulated with chemokines. We show that integrin/ligand binding for a few seconds with low mobility by anchoring to the actin cytoskeleton successfully promotes chemokine-stimulated cell adhesion. Stable integrin/ligand binding was observed for  $Mn^{2+}$ -induced high-affinity LFA-1, although the binding was not required for physiological adhesion. Based on our findings, we propose that fast cycling of the clutch formation enables dynamic cell adhesion and migration [8].

It should also be noted that previous studies on integrin dynamics have focused on only integrin's lateral mobility on the membrane, leaving the binding kinetics to the ligand unclear [e.g., 9]. We used single-molecule imaging techniques to monitor not only the integrin LFA-1, but also its ligand, ICAM-1. This ability is a technical accomplishment that should appeal to people interested in such quantitative live-cell imaging.



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## Delivery of non-genetically encodable imaging probes inside live cells

Jean-Philippe Pellois, Texas A&M University, College Station, Texas, USA

I will present a novel approach for the delivery of non-genetically encodable macromolecules and imaging agents into human cells. This approach relies on a simple co-incubation protocol with the reagent dfTAT. [1, 2] Macromolecules are delivered in two dfTAT-mediated steps: endocytosis followed by extremely efficient endosomal escape. I will discuss how dfTAT promotes cytosolic delivery on a molecular level and describe how this reagent can successfully deliver various cargos. Overall, this approach should allow new opportunities for the quantitative probing of cellular processes and, as such, be of value to the bioimaging community.

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## SUPER-RESOLUTION IMAGING OF BIOLOGICAL SAMPLES VIA SPECTRAL SEPARATION OF QUANTUM DOTS

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**KEY WORDS:** Super Resolution Biological Imaging, Spectral Separation, Quantum Dots, DNA Origami, Synaptic Proteins

The aim of this study is to develop a methodology for super-resolution biological imaging on the basis of spectroscopic signal separation using semiconductor quantum dots (QDs). This approach allows to shift super-resolution method from temporal to spectral domain and thus promises much faster image acquisition than currently possible. Previously, we showed that resolution down to at least 40 nm can be achieved by labeling alpha-tubulin structure in HEK293T cells with off-the-shelf QDs and using a standard confocal microscope having a spectroscopic capability (e.g. Leica TCS SP2). In this study, we tested our method against a standard in order to verify the resolution. Triangular DNA origami was used as a reference standard with the side length of 120 nm. Each side was labeled with QDs in a middle giving 60 nm distance between QDs, well below the optical resolution limit. Attachment of QDs to the DNA origami triangles was verified by AFM. The super-resolution images we obtained using a standard confocal microscope (Leica TCS SP2) were found to be consistent with AFM results, thus verifying our methodology.

Once verified, we used our method to observe the distribution of the receptor clusters of NMDAR and AMPAR, which are receptor proteins in postsynaptic region of the nerve cells. Furthermore, we also applied our method to observe distribution of the presynaptic scaffolding proteins (Bassoon and Piccolo) and postsynaptic scaffolding proteins (Homer1 and PSD95) located around the synaptic cleft.

Identification and characterization of focal and global calcium signaling events in smooth muscle cells of the microcirculation

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Global increases in intracellular calcium in vascular smooth muscle cells (SMCs) are associated with the formation of calcium-dependent cross-bridges between actin and myosin leading to SMC contraction and vasoconstriction of small resistance vessels. In contrast, focal increases in SMC calcium initiates a vasodilatory pathway due to hyperpolarization of the SMCs downstream of activation of large conductance calcium-activated potassium channels (BK<sub>Ca</sub>). Subtle changes in diameter (vasoconstriction and vasodilation) in resistance vessels, such as arteries and arterioles, function to regulate pressure and perfusion of blood to active tissues. As a result, it is crucial to identify, characterize, and differentiate between global and focal calcium signals in SMCs of the microcirculation. Isolated mouse mesenteric arteries from C57BL/6J mice were cannulated and then pressurized, Fluo-4 loaded SMCs were imaged using an Olympus FV3000 laser scanning confocal microscope equipped with high sensitivity GaAsP detectors. Global calcium signaling events were observed as propagating waves that would encompass the entire length of the cell, while focal events radiated a few microns from a single observable point. Calcium waves and focal events were observed spontaneously, and were modulated in the presence of a range of pharmacological agents. Calcium activity was measured by placing regions of interest (ROI) around the entire cell border, and by placing smaller ROIs within the cell borders at sites of activity. Propagation velocities were determined by measuring calcium levels at two distinct sites, a known distance apart, in images acquired at high speeds (>100 frames/sec). Our data demonstrate the presence of two classifications of calcium signaling events, focal calcium events and global calcium waves, which are discernable based on the properties of the fluorescence response. These data have important implications for future application in models of human diseases, such as hypertension, where calcium signaling is thought to be altered.

**VISUALIZING SCAFFOLD PROTEIN FUNCTIONS IN LIVING CELLS**

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**KEY WORDS:** Scaffold proteins, IQGAP1, live cell imaging, total internal reflection microscopy, TIRF, structured illumination microscopy, SIM

Multi-domain, cytoskeletal scaffolding protein IQGAP1 is involved in regulating a wide array of cellular functions, including coordinating signaling cascades, controlling actin growth, and maintaining spatial-temporal interactions within the cell. Although numerous bulk analyses have been performed on living cells which show the effect of IQGAP1 on diverse functions such as cell morphology, movement, and vesicular trafficking, there is a lack of understanding of the mechanism of action of IQGAP1 on its scaffolding partners at the micro- and nano- scale. Here we show that through the use of high contrast imaging modalities and live-cell imaging analyses we can measure the dynamics of a novel use of IQGAP1, which regulates an actin shell surrounding a novel membranous compartment that localizes selectively to the basal cortex of polarized epithelial cells (MCF-10A). These highly dynamic structures are identified as multivesicular membrane processing stations which stabilize an actin coating and constrain its growth. Through the use of novel live-cell, high contrast imaging techniques, we have developed a robust process for analyzing compartment dynamics in living cells. Our findings suggest that IQGAP1 interacts with formins in the cell to modulate the actin structure through a negative feedback loop, and provide a framework for future analyses of similar types of cellular scaffolds through microscopy techniques.

# The effect of limited resolution on the analysis of spatial point patterns

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Resolution is a key property of an optical system, yet it remains difficult to define and apply. The classical notions of resolution such as Abbe's and Rayleigh's criteria had a major influence on the development of optical methodologies. However, they were developed in the context of observations with the human eye and have severe limitations in the usage of modern optical imaging methods. For example, modern imaging systems in many cases cannot be separated from the sophisticated image processing algorithms that are used to analyze the acquired data. An important example is localization based super-resolution microscopy (PALM,(d-)STORM) where the final result of an experiment depends not only on the optical system but also, crucially to the quality of the final image, on the data analysis step. Data analysis in this case is non-trivial as it involves a series of steps such as segmentation of the data, followed by the fitting of a point spread function model.

Spatial point processes have been extensively used in a range scientific disciplines to model spatial data. For example, the pattern of cellular membrane receptors has been investigated using spatial statistics approaches such as Ripley's  $K$ -function and the pair correlation function. We show that insufficient resolution can have a significant impact in the analysis of spatial point patterns. We quantify this effect and in doing so we define a new measure of resolution based on spatial statistical theory. We show this approach to defining resolution can be used to analyze the resolution of the complete optical system, from acquisition to data analysis, but can also be used to investigate the analysis algorithm by itself.

## Modelling and estimating the behaviour of photo-switching fluorophores

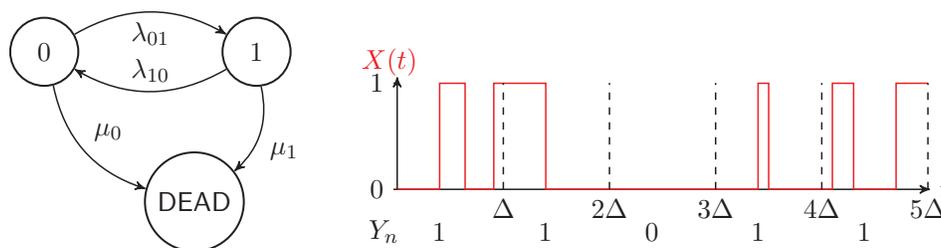
Lekha Patel<sup>\*1</sup>, Nils Gustafsson<sup>\*2</sup>, Raimund Ober<sup>3</sup>, Ricardo Henriques<sup>2</sup>, and Edward Cohen<sup>1</sup>

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Several super-resolution techniques take advantage of the inherent stochasticity of a photoswitchable fluorophore's photon emission state and a careful choice of fluorophore must be made for the intended application. Furthermore, knowledge of the photo-switching characteristics has been employed in advanced analytical methods developed to analyze data sets in which fluorophores partially overlap, for example, 3B analysis [1] and DeconSTORM [2]. For this reason, characterizing photoswitching behaviour has received much attention, e.g. [3].



The contribution of this work is two fold. We begin by presenting a model to characterise the photo-switching behaviour of a fluorophore. We first model an individual fluorophore's true photon emission state as a continuous-time homogeneous Markov process. From this, we derive the discrete time imaged process that indicates if a fluorophore is detected in a given frame. In doing so, we highlight a key difference between what is observed and the underlying photo-switching process. We then construct a novel procedure for estimating the underlying transition rates by formulating a hidden Markov model to link the discrete time observed process with its continuous time signal. We implement a multivariate extension of this method to allow for the more common scenario of multiple emitters. We demonstrate with a simulation study the effectiveness of our model and power of our estimation method.

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## IMPLEMENTATION OF PSF ENGINEERING USING A FABRICATED SQUBIC PHASE MASK TO REDUCE THE EFFECT OF SPHERICAL ABERRATION IN 3D WIDE FIELD FLUORESCENCE IMAGING

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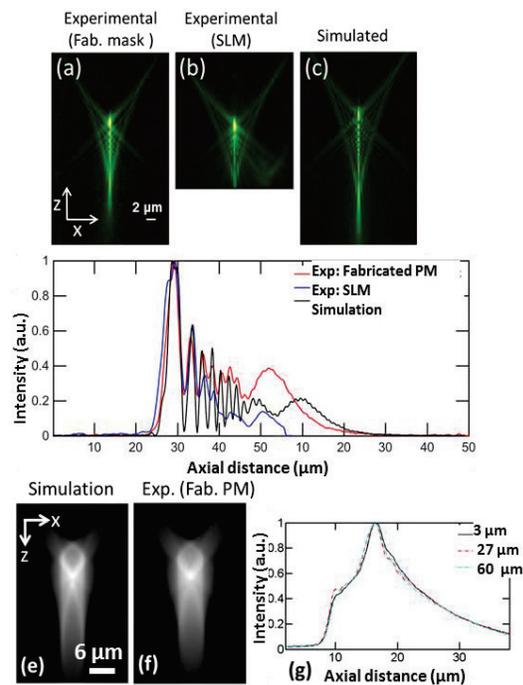
We study a squared cubic (SQUBIC) phase mask (PM) fabricated on Zeonex E48R™ glass plate to implement the wavefront encoded (WFE) imaging in a commercial upright wide field microscope (Zeiss Axio Imager.Z1). In earlier studies we have demonstrated the experimental implementation of the SQUBIC PM WFE using a liquid crystal spatial light modulator (LC-SLM) [1]. Due to the limitation of the finite pixels size of the SLM [2], the implementation of the SQUBIC phase mask was limited to the PM with lower spatial frequency (SF) although it is predicted by theory that SQUBIC PM with higher SF renders stable PSFs over a wide range of depths [1]. Below is the equation of the SQUBIC phase mask [3] where  $A$  is the design parameter (i.e. strength parameter), and with the increase of the value of  $A$  stability of the PSF with depth increases (higher  $A$  produces PM with high SF) [1]:

$$\varphi(\rho, \alpha) = 2\pi A \left[ \frac{\sqrt{1 - \rho^2 \sin^2(\alpha)} - 1}{1 - \cos(\alpha)} + \frac{1}{2} \right]^3$$

where  $\alpha = \sin^{-1}(NA/n)$  with  $n$  the refractive index of the lens' immersion medium and  $NA$  is the numerical aperture of the objective lens. The comparison between the PSFs (Figs. a-d) show that better agreement between the axial intensity profiles of the simulation and experiment is obtained when the fabricated PM is used for the WFE implementation compared to that of the SLM implementation (Fig. d). Image of a 6  $\mu\text{m}$  in diameter spherical shell having shell thickness equal to one (a test object) shows that the intensity distribution of the experimental forward image (SQUBIC WFE implemented using the fabricated PM) has a structure similarity coefficient [4] of 0.99 when compared with that of the simulated image (Figs. e-f). It is also observed that over the 60  $\mu\text{m}$  depth, the SQUBIC WFE system intermediate images show a variability of only 3% (Fig g).

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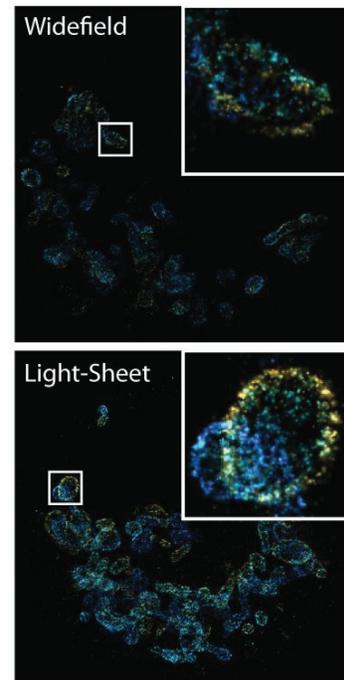
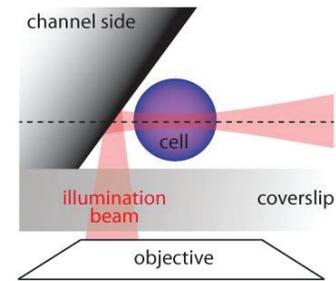
## SINGLE OBJECTIVE LIGHT-SHEET MICROSCOPY FOR HIGH-SPEED WHOLE-CELL 3D SUPER-RESOLUTION

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SM-SR in 3D throughout a whole cell is difficult because wide-field illumination cannot be targeted to the in-focus image plane. This results in high background fluorescence that severely degrades detection and localization precision. We have developed a technique, termed Single Objective Light-Sheet Microscopy (SO-LSM) that uses a common, single objective, inverted epi-fluorescence microscope along with a reflective surface to generate illumination only in the image focal plane [1]. We use a reflective, planar surface which is placed at an angle of  $45^\circ$  on a cover slip to reflect the beam (see Figure). This surface forms the side wall of a microfluidic channel incorporated into a microfluidic device. A light-sheet is generated through the objective and reflected by the surface such that it illuminates only the in-focus plane of the cell. The light-sheet is scanned through the cell for whole-cell imaging and an astigmatic lens in the emission light path enables the 3D localization of individual emitters, creating a whole-cell 3D super-resolution image. SO-LSM provides greatly improved localization accuracy due to background reduction. An additional benefit of SO-LSM comes from reduced photobleaching of un-imaged, out of focus fluorophores. Moreover, the light-sheet confines the laser light in one dimension, thereby increasing the excitation intensity, which makes the probes photo-switch faster thereby significantly reducing the acquisition time. The microfluidics device provides a closed environment that allows for fast and automated buffer exchange, which is utilized to maintain a reduced oxygen environment needed for many SM-SR probes. SO-LSM is a system that is easily implemented on a regular wide-field fluorescence microscope. In conclusion, SO-LSM increases image quality due to better localization of more emitters, acquired in less time (see Figure) than widefield illumination. The development of this microscope and its imaging and analysis routines will therefore be of great value to investigators seeking a relatively straightforward, inexpensive method for whole-cell 3D super-resolution microscopy and high-speed live cell imaging.



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## FLUORESCENT-IMAGING QUANTITATIVE CHARACTERIZATION OF SINGLE-CELL LASER THERMOGENETIC NEUROSTIMULATION

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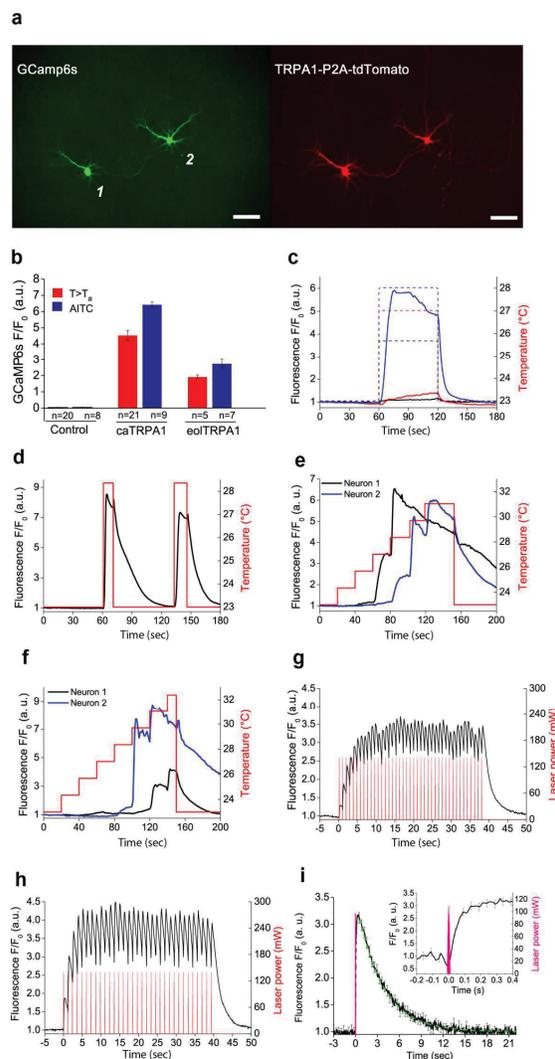
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Fluorescence imaging using fluorescent  $\text{Ca}^{2+}$ -ion sensors is shown to provide a powerful tool for a quantitative characterization of single-cell laser thermogenetic neurostimulation. In our experiments, thermogenetic activation is performed on individual cultured mouse neurons expressing TRPA1 channels from *Crotalus atrox* western diamondback rattlesnakes, providing thermogenetic neurostimulation above the channel activation threshold  $T_a \approx 28^\circ\text{C}$ . The neurons are co-transfected with tdTomato and GCaMP6s sensors for fluorescence visualization of TRPA1 channel expression (through a red fluorescence signal from tdTomato, Fig. 1a, right), and the buildup of the inward  $\text{Ca}^{2+}$  current across the neuronal membrane (through green fluorescence from GCaMP6s, Fig. 1a, left). When combined with *in situ* quantum thermometry using NV centers in diamond integrated into an optical fiber,



this approach enables a robust and reliable detection of thermogenetic stimulation of single neurons above  $T_a$  (Figs. 1b-1d), helps discriminate between activated and silent neurons (Figs 1e, 1f), and provides a unique tool to measure complex patterns of neural activity in TRPA1-channel-expressing neurons stimulated by tailored trains of laser pulses (Figs 1g, 1h).

Fig. 1. Fluorescent-imaging quantitative characterization of single-cell thermogenetic neurostimulation. (a) Fluorescent images of neurons co-expressing GCaMP6s and caTRPA1-P2A-tdTomato. The scale bar is 60  $\mu\text{m}$ . (b) Stimulation of the TRPA1-expressing neurons using IR irradiation or chemical agonist AITC gives rise to  $\text{Ca}^{2+}$  transients of comparable amplitudes;  $n$  is the number of cells in the group. (c) IR stimulation of neuron 1 heated to 25.5, 27, and 28 $^\circ\text{C}$  (black, red, and blue dotted lines, respectively). (d) Multiple cycles of neuron 1 activation by mild above-threshold heating. (e, f) Determination of the neuronal activation threshold with the power of IR laser radiation, focused on neuron 1 (e) and neuron 2 (f), increased in a stepwise fashion. Localized heating activates only one of two neighboring cells. (g)-(i) Activation of caTRPA1-expressing neurons by trains of femtosecond IR laser pulses with a central wavelength of 1440 nm. The OPO is operated in the pulsed mode to deliver pulse trains (red line) with a duration  $\tau \approx 8$  ms (g) and 11 ms (h) and repetition rate  $f_i \approx 1.18$  Hz (g) and 0.87 Hz (h). (i)  $\text{Ca}^{2+}$  dynamics in caTRPA1-expressing neurons subjected to a single 15-ms train (pink shading) of femtosecond laser pulses with a central wavelength of 1440 nm. Dynamics of GCaMP6s fluorescence is shown in the inset.

## AN ACCURATE FORWARD IMAGING MODEL FOR ELECTRON TOMOGRAPHY

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**KEY WORDS:** Forward imaging model, point spread function, transmission electron microscopes, depth variant imaging.

Electron tomography (ET) offers tools to observe cellular ultrastructure and understand electro-chemical processes that help to further research in genomics and molecular biology [1]. Imaging biological samples pose a challenge due to their complexity, low contrast, and signal to noise, which can be resolved by improved restoration algorithms. Understanding image formation is key for these methods which should account for aberrations (spherical, astigmatism) introduced due electron optics, errors in alignment, curvilinear trajectories, sample warping, noise, and the stochastic nature of the problem due to missing cone of frequencies. Advances in imaging ensure there are multiple tools, namely IMOD [2], EMAN [3], TxBR [4], etc., that offer restoration algorithms but in the absence of a universal evaluation system it is hard to identify their specific applicability and advantages. Our recent research focussed on developing an ET simulator (ET-SPEC) [7], to allow the simulation of a tomographic image. ET-SPEC together with a biologically derived 3D phantom acquired from advanced block face tomography offers forward image that can be used to compare and contrast restoration methods. In this poster, we focus on the effect of defocus dependent point spread functions (PSFs) [1,5,6] on image formation. PSFs which are dependent on a variety of factors like lens characteristics, specimen thickness and composition, wavelength of illumination and size of source, introduce loss of resolution with a redistribution of intensities. Our observations regarding the PSFs and their frequency domain counterpart contrast transfer functions, are folded into the ET-SPEC, offering an accurate understanding of blurring and loss of resolution as well as the ability of restoration algorithms to resolve them.

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## LARGE-SCALE ANALYSIS OF SPATIOTEMPORAL ORGANELLAR NETWORK EVOLUTION

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We are designing and implementing a high-throughput, large-scale computational pipeline to detect changes in organellar morphology of cells. The framework will quantify changes in organellar shape, quantity, and spatial distribution over large sequences of Z-stack microscope images and digital videos, improving our understanding of cellular mechanisms as they respond to their environments. Any tagged subcellular component can be tracked. Our framework takes the novel approach of examining subcellular components as nodes in a social network. Characterizing ensembles of cellular machinery, such as labeled mitochondria or other organelles of interest, as networks allows our framework to study organellar evolution as a function of interconnectedness of cellular components. In addition to quantifying global information such as quantity and appearance, our framework's approach can also provide more detailed local feedback regarding how subsets of the organellar ensembles evolve. This framework will be scalable, employing "big data" paradigms to analyze large quantities of image data at very high resolutions. It will enable rapid quantitative analysis of organellar temporal evolution for extremely large data and provide detailed results at high statistical resolutions.

This current methodology will accurately characterize the spatio-temporal evolution of the organellar social networks in a large collection of time-lapse videos of human cells infected with pathogens, treated to toxins and chemicals or exposed to various environmental stimuli [1]. Our initial image processing is examining the temporal changes in distribution of spatial frequencies of mitochondria in cells infected with the intracellular bacterial pathogen, *Listeria monocytogenes* versus uninfected healthy cells. Mitochondria are vital organelles and changes in number or shape can be a clear indicator of cell health. As with successful intracellular pathogens, *L. monocytogenes* can modify or eliminate mitochondria as the bacillus ultimately takes control of the fate of the host cell. We are assessing *L. monocytogenes* strains containing genetic mutations in key virulence genes in order to identify the genes responsible for multiple stages of this phenotype [2]. These statistics provided, in some sense, a quantitative representation of cellular morphology, and in turn captured the changes in that morphology as a response to the applied stimulus.

This can have a positive impact on efforts spanning multiple fields. The most immediate consequence will be genotype-phenotype associations and the efficient screening of large genomic libraries. By designing a scalable, open-source framework, extremely large assays can be collected and analyzed. The collaborators have access to a significant amount of established computing and imaging resources between their respective universities, ensuring a rapid influx of raw data that can be readily tested and deployed in a production setting. Besides contributing to our improved understanding of host cell interactions with various stimuli, practical applications could involve replacement of expensive animal studies and more rapid development of countermeasures.

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## NON-DIFFRACTION LIMITED MICROSCOPY DEMONSTRATES THE ROLE OF LOCAL ACTIN DYNAMISM IN NK CELL EFFECTOR FUNCTION

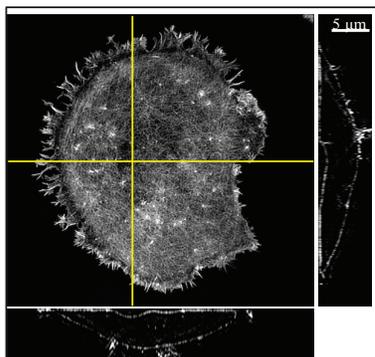
Emily M. Mace<sup>1\*</sup>, Alexandre F. Carisey<sup>1\*</sup> and Jordan. S. Orange<sup>1</sup>

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\*equal contribution

**KEY WORDS:** NK cell, F-actin, immunological synapse, non-diffraction limited microscopy, stimulated emission depletion microscopy (STED), structured illumination microscopy (SIM).

As essential players of the innate immune system, Natural Killer (NK) cells are responsible for the targeted lysis of virally infected or transformed cells. Upon activation, NK cells establish a specialized



*NK cell activated for 10 min on aCD18+aNKp30 and imaged by 3D-SIM to illustrate 3D F-actin architecture at the immunological synapse.*

cell-cell interface with the potential target termed the immunological synapse (IS). Killing of the target cell is controlled by a tightly regulated series of checkpoints that ultimately lead to the directed secretion of specialized secretory lysosomes (lytic granules) by NK cells at the IS.

Previous work has shown the requirement for intact actin cytoskeleton in both cell adhesion and lytic granule exocytosis. In addition, we have previously shown that F-actin remodeling is required for lytic granule access to the IS and that granule secretion occurs in minimally sized, granule-permissive F-actin hypodensities that form as part of a filamentous mesh at the IS. Despite the known critical role of F-actin nucleators and modulators in immune function, the nature and regulation of the local dynamic F-actin architecture at the IS is poorly understood.

The most recent developments in microscopy allow the visualization of this structure with unprecedented spatial and temporal resolution. We have combined these technologies with image feature reconstruction and mobility measurements to define the dynamic F-actin architecture at the NK cell lytic synapse. We find that the F-actin mesh undergoes high dynamism of F-actin fibers that is independent from local granule movement. Myosin IIA is required for F-actin contraction to form the mesh, whereas inhibition of Arp2/3 complex function profoundly reduces F-actin dynamism. Finally, we have identified additional actin architectures in addition to the actin mesh that are formed at the IS. These include a large lamellipodium that is responsible for the spreading onto the target cell. Further, velocity analysis has distinguished a novel feature of the lytic synapse; immobile bright actin loci within the actin mesh.

These detailed and quantitative measurements describe dynamic yet highly regulated F-actin at the NK cell IS. Further, they establish a timeline of the different actin architectures observed and suggest the differential modulation of specialized structures required for adhesion and granule exocytosis.

## plusTipTracker3D: Quantification, Visualization and Analysis of Microtubule Dynamics with Lattice Light-Sheet Microscopy

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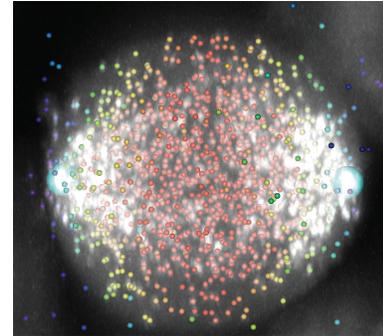
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**KEY WORDS:** Multiple particle tracking, light sheet microscopy, 3D visualization

Lattice light-sheet microscopy [1] enables the evaluation of complex and dynamic subcellular events unaltered by spatial integration of the signal or arbitrary slicing of the cellular volume. Scaling to this higher statistical power, quantification methods must harness the limitations that can arise from fluorophores performance (either lower signal-to-noise or spatiotemporal sampling) and the challenges to validate and interpret 3D data analysis. Mitosis provides an excellent case study of these challenges as it presents a variety of highly dynamic behaviors that are potentially occluded by their spatial density. In this work, we propose a new version of the plusTipTracker software [2] that allows automated +TIPs tracking and estimation of microtubule growth speed and growth phase duration in 3D. The software also delivers inferences of the frequency and duration of pauses and shrinkage events. We introduce a novel formulation for +TIP detection, which uses a highly sensitive local statistical thresholding [3]. Furthermore, to facilitate the analysis of tracking results, tracks are represented in a 3D dynamical frame of reference based on the spindle poles. This non-supervised, multi-scale approach, combined with tracking detects the poles throughout the whole image sequence. +TIP aggregates are then mapped into the spherical coordinate system centered on their nucleation poles. To represent the repartition of growth rates in the spindle, we then generate 2D stereographic projections of the microtubule growth events localized at fixed radii. In addition to conventional projections in the Cartesian system, we established a minimally supervised communication channel with the Amira 3D rendering software (FEI) to enable further analysis at the single microtubule level. Our results are validated with the analysis of +TIP dynamics after chemical inhibition of microtubule polymerization and depolymerization and compared with recent work performed on spindle dynamics analysis in 3D [4]. We will also present recent work on the quantification of heterogeneous motion in 3D using our new piecewise-stationary motion modeling smoother applied to 3D data.



Maximum intensity projection of an HeLa cell in anaphase expressing GFP-labeled EB3 imaged with Lattice Light-Sheet Microscopy.

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## MULTIPLE EMITTER FITTING USING REVERSIBLE JUMP MARKOV CHAIN MONTE CARLO

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**KEY WORDS:** super-resolution, multiple particle fitting, Bayesian inference, MCMC

In single molecule based super-resolution imaging, high labeling density or the desire for greater data collection speed can lead to clusters of overlapping PSFs in the raw super-resolution image data. Multi-emitter fitting algorithms can identify and localize the particles in those dense regions of the data. In this work, we seek to improve the state-of-the art in multi-emitter fitting by employing Reversible Jump Markov Chain Monte Carlo (RJMC) [1]. Markov Chain Monte Carlo (MCMC) can be used to find the posterior distribution of a set of parameters, which in the multi-emitter fitting problem consists of the particle positions and intensities and a local background. RJMC takes this concept further and allows jumps between parameter spaces, in this case allowing the addition or subtraction of emitters in the model. We allow four mechanisms to make jumps between spaces of various models, called birth, death, merge and split. Birth (death) allows the addition (deletion) of an emitter anywhere in the sample. Split allows the possibility that an existing particle can be actually two or more neighboring particles. Merge allows the chance of two adjacent particles join into a single particle.

Here we describe the mathematical formalism for the RJMC model and compare the results under a range of conditions to several other multi-emitter fitting approaches [2,3]. Figure 1 shows the result of the RJMC analysis for a small region of simulated data.

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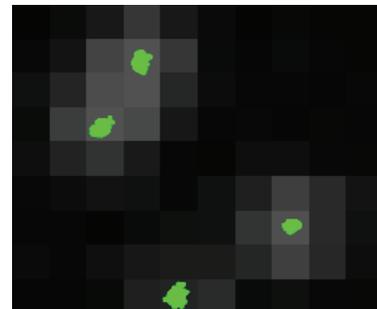


Fig. 1: Simulated data with the RJMC results. Green shows the posterior position density generated by the Markov Chain.

**AUTOMATED MULTIPLE TARGET SUPERRESOLUTION IMAGING**

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**KEY WORDS:** sequential imaging, automation, multiple targets

Single molecule superresolution of multiple targets is challenging due to limited availability of dyes with the required photophysical properties. An additional difficulty that naturally arises when using multiple dyes is the need for different optical paths and thus multiple cameras (or different regions on one camera) and differential aberrations, which become important at the <100 nanometer scale. In order to overcome these problems, we recently showed in our lab that it is possible to image multiple targets using a single optical path with a single dye and a buffer that is optimized for that dye using a sequential imaging approach [1]. To facilitate sequential imaging we have automated several steps in the data collection and image processing pipeline. This automation improves reliability and reduces the user time required at the microscope. Here we describe in detail the automation procedures and algorithms. We show the results of four ‘color’ super-resolution imaging using this approach.

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**EVALUATION OF ORGANIC DYES FOR HIGH-SPEED SINGLE PARTICLE TRACKING****Hanieh Mazloom-Farsibaf and Keith A. Lidke****Physics and Astronomy Department, University of New Mexico, US****Email: hmfarsibaf@unm.edu****KEY WORDS:** single particle tracking, organic dyes, photostability

Single particle tracking can be used to study the dynamics of proteins and lipids in the membrane of living cells. In some cases, such as the study of short-lived interactions or the observation of proteins and lipids confined to the membrane compartments [1,2], high-speed tracking is required. When using fluorescence imaging, quantum dots and organic dyes can both be used as bright tags. Quantum dots (QDs) are more photostable in comparison to the organic dyes but they have ultimately limited brightness from the ~10 times longer fluorescence lifetime and suffer from blinking. In some cases, the physical size of QDs prevents them from moving under the cell in tight coverslip-cell junctions, precluding TIRF imaging. Therefore, in some applications the use of organic dye labels are preferred. The disadvantage of organic dyes is the relatively poor photostability and smaller absorption cross section.

We evaluate and compare four far-red dyes for use in high-speed single particle tracking: LD650 (Prepared by Lumidyne Technologies Cat No. is 07), CF640R (Prepared by Sigma-Aldrich, Cat No. is SCJ4600044), Atto 655 (Prepared by Sigma-Aldrich, Cat No. is 76245), DyLight™ 650 (Prepared by ThermoFisher Scientific, Cat No. is 62265). Since the photophysics of dyes can be altered by proximity to or immobilization to a dielectric surface, i.e. the coverslip, we evaluate dyes by tracking dye-labeled IgE bound to the FcεRI receptors at frame rate up to 1000 Hz. We evaluate and rank the dyes by photon emission rate, emission stability (e.g. lack of blinking) and photostability.

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## OBSERVING THE EFFECT OF MECHANOBIOLOGY BY LUNG CANCER CELL DURING METASTASIS

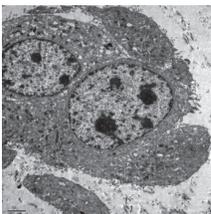
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A mouse model of human lung adenocarcinoma driven by mutations in *K-ras* and *p-53* genes was adapted to investigate roles of mechanics and cellular epithelial to mesenchymal transition (EMT) during metastasis. Tumor cells were grown in collagen gels under static tension, so that the mechanobiological properties and the cell-matrix interactions, in particular the collagen structural changes could be elucidated.

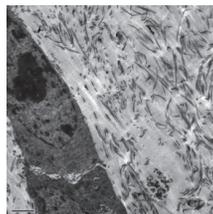
Metastatic (344SQ) and non-metastatic (393P) tumor cells were encapsulated in rat tail collagen-I, and studied under static tension (anchored) for 7 days. Collagen gels were processed for light microscopy (picosirius red for collagen), confocal microscopy (ZO-1, DAPI and collagen), two photon microscopy, cell macerated SEM and TEM. The elastic modulus were determined via mechanical testing and the alignment of collagen fibrils were analyzed with MATLAB.

Under static tension, the 344SQ contracted 15.3% more and were 14.7kPa stiffer than 393P (at collagen=2mg/ml). Ultra-structurally, the 393P were rounded and clustered together, whereas the 344SQ were elongated and closely associated with collagen. The tight junction protein (zonula occludens-1 ZO-1) were overt and widely expressed in 393P, whereas ZO-1 in 344SQ were scarcely and limited to edges. Under two-photon microscope, the 344SQ cells were closely associated with collagen and clustered to form a ribbons-like structures. The collagen fibril alignment in 344SQ was much more aligned and was significantly different to 393P ( $p<0.001$ ).

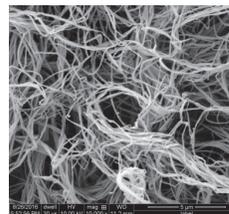
The metastatic cells were closely associated with collagen and exerted more contractile force than the non-metastatic hence the stiffer gels. EMT during metastasis enhanced the cell-matrix interactions; the re-modulated metastatic cell distribution in collagen gel contained a higher tolerance to tension compares to clustered non-metastatic cells. Further study is required to understand the interaction of mechanical, cytokine and ECM signaling.



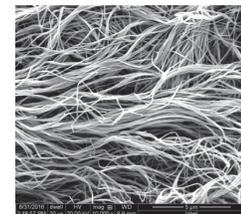
Transmission electron micrograph of 393P.



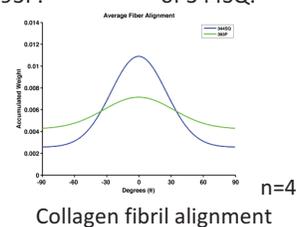
Transmission electron micrograph of 344SQ.



Scanning electron micrograph of 393P.



Scanning electron micrograph of 344SQ.



Karl Gorzelnik

9/7/16

Texas A&M University, College Station, Texas, USA

Asymmetric cryo-EM structure of the canonical Allovivivirus Q $\beta$  reveals a single maturation protein and the genomic ssRNA in situ

Single-stranded (ss) RNA viruses infect all domains of life. To date, for most ssRNA virions, only the structures of the capsids and their associated protein components have been resolved to high resolution. Q $\beta$ , an ssRNA phage specific for the conjugative F-pilus, has a T=3 icosahedral lattice of coat proteins assembled around its 4,217 nucleotides of genomic RNA (gRNA). In the mature virion, the maturation protein, A2, binds to the gRNA and is required for adsorption to the F-pilus. Here, we report the cryo-electron microscopy (cryo-EM) structures of Q $\beta$  with and without symmetry applied. The icosahedral structure, at 3.7Å resolution, resolves missing loops of the coat protein while the asymmetric structure, at 7Å resolution, reveals A 2 and the gRNA. A 2 contains a bundle of  $\alpha$ -helices and replaces one dimer of coat proteins at a two-fold axis. The helix-bundle binds gRNA, causing denser packing of RNA in its proximity, which asymmetrically expands the surrounding coat protein shell to potentially facilitate RNA release during infection. We observe a fixed pattern of gRNA organization among all viral particles, with the major and minor grooves of RNA helices clearly visible. A single layer of RNA directly contacts every copy of the coat protein, with one third of the interactions occurring at operator-like RNA hairpins. These RNA-coat interactions stabilize the tertiary structure of gRNA within the virion, which could further provide a roadmap for capsid assembly.

## Characterization and fitting of complex fluorescence point spread functions

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Super-resolution fluorescence microscopy is a robust method of sub-diffraction limited optical microscopy. However, it has been demonstrated that fluorophores close to metallic nanowires generate non-standard point spread functions that cannot be fit with traditional models like a Gaussian function. Complex point spread function shape is based on location and orientation relative to nanowires, but there has not yet been a method proposed to accurately localize these fluorophores. I have generated point spread functions for fluorophores near silver nanowires using finite-difference time-domain simulations, and developed a fitting model based on weighted linear combinations of Hermite-Gaussian beam modes.

The method of steepest descent algorithm for the Hermite-Gaussian model with LASSO (least absolute shrinkage and selection operator) regularization recovers sparse weight functions for test images. Decomposition of finite-difference time-domain PSFs for fluorophore rotation about the optical axis also recovers sparse weight functions, and displays variation based on fluorophore orientation. In the future this method can be further developed to determine the location and position of fluorophores close to metallic nanowires, providing useful insights into the minute surface dynamics taking place.

## Super Temporal-Resolved Microscopy (STReM)

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Super-resolution fluorescence microscopy breaks the optical diffraction limit in spatial domain and is widely applied to biological imaging and particle tracking. However the temporal resolution remains low compared to the timescale of many scientifically relevant processes. Typically, dynamic events faster than the detector frame rate cannot be resolved temporally in super-resolution microscopy. The temporal resolution thus limits the application of super-resolution microscopy to processes occurring within the detector frame time. We report Super Temporal-Resolved Microscopy, STReM, to improve the temporal resolution by as much as 20 times faster than the detector frame rate. STReM encodes the information of high temporal resolution by physically rotating a double helix phase mask that is commonly used for 3D super-resolution imaging. We also demonstrate both in simulation and experiments that STReM is capable of detecting events with high temporal resolution. Finally we show that a compressive sensing algorithm can be used to recover fast 2D transport.

**PROBING IN SITU DYNAMICS OF NANOPARTICLE–PROTEIN INTERACTIONS**

Sishan Chen,<sup>1</sup> Sudeshna Chatterjee<sup>1</sup>, Franziska Hollenhorst<sup>1</sup>, Rashad Baiyasi<sup>2</sup>, Wenxiao Wang<sup>2</sup>, Logan D.C. Bishop<sup>1</sup>, Wei-Shun Chang<sup>1,4</sup>, Christy Landes<sup>1,2,3,4</sup> and Stephan Link,<sup>1,2,3,4</sup>

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**KEY WORDS:** protein corona, protein binding dynamics, correlation spectroscopy, super-localization microscopy, 3D particle tracking.

We are interested in nanoparticle-protein dynamics in physiologically relevant environments. Our goals are to characterize in situ interactions of nanoparticles and proteins, to determine protein binding dynamics and conformation, and finally to identify the relationship between protein coronas and physiological outcome. These goals are pursued using a combination of techniques including fluctuation correlation spectroscopy, super-localization microscopy, and 3D particle tracking.

**GENERALIZED RECOVERY ALGORITHM FOR 3D SUPER-RESOLUTION  
MICROSCOPY USING ROTATING POINT SPREAD FUNCTIONS**

**Bo Shuang, Wenxiao Wang, Hao Shen, Lawrence J. Tauzin, Charlotte Flatebo, Jianbo Chen, Nicholas A. Moringo, Logan D. C. Bishop, Kevin F. Kelly, and Christy F. Landes**  
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**KEY WORDS:** ADMM, phase mask, parallel computation

Super-resolution microscopy with phase masks is a promising technique for 3D imaging and tracking. Due to the complexity of the resultant point spread functions, generalized recovery algorithms are still missing. We introduce a 3D super-resolution recovery algorithm that works for a variety of phase masks generating 3D point spread functions. A fast deconvolution process generates initial guesses, which are further refined by least squares fitting. Overfitting is suppressed using a machine learning determined threshold. Preliminary results on experimental data show that our algorithm can be used to super-localize 3D adsorption events within a porous polymer film and is useful for evaluating potential phase masks. Finally, we demonstrate that parallel computation on graphics processing units can reduce the processing time required for 3D recovery. Simulations reveal that, through desktop parallelization, the ultimate limit of real-time processing is possible. Our program is the first open source recovery program for generalized 3D recovery using rotating point spread functions.

## Quantitative analysis of endoplasmic reticulum dynamics in plant cells

Lawrence Griffing, Texas A&M University, College Station, Texas, USA

The endoplasmic reticulum (ER) of plants shows two kinds of movement: movement of tubules (living nanotubules, 50-60 nm in diameter) and cisternae that results in rapid remodeling of the 3D ER network, and movement within the tubules of luminal and membrane proteins. High rates of ER motion 1-4 micrometers per second, dependent on internal molecular motors and the cytoskeleton, are analyzed following acquisition on line-scanning and spinning disk confocal microscopy, total internal reflection fluorescence, and lattice light sheet imaging. Various computational approaches are used to quantify the network remodeling including optic flow algorithms, persistency mapping, polygonal mesh counts, and morphometric algorithms that parse tubular vs. cisternal regions for quantitation. These approaches are complementary in that they provide measures of advective streaming of the ER (optic flow) and measures and locations of the interaction of the ER with the plasma membrane via membrane contact sites (persistency mapping). Advective and diffusive movement within tubules and cisternae can be quantified using photoactivation, fluorescence recovery after photobleaching (FRAP), fluorescence correlation spectroscopy using laser line-scanning (RICS), and particle tracking of induced protein aggregates in the ER. The interrelationships between the two kinds of movement, movement of the network and movement within the network will be discussed.

**LABEL-FREE RAMAN AND PHASE IMAGING FOR MULTIDIMENSIONAL  
DATA-BASED QUANTITATIVE ANALYSIS OF IMMUNE CELLS**

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**KEY WORDS:** spontaneous Raman scattering, digital holographic phase, label-free imaging, macrophage, lymphocyte, principal component analysis, spectroscopy

Label-free microscopy for bioimaging has a number of unique advantages over more common techniques. Raman imaging provides a spatial map of molecular signals in living cells to evaluate the cellular composition and how it changes over time. Using no labels, the light-sample interaction is also not affected by staining protocols. However, in the case of label-free Raman imaging, the data is very powerful in terms of information content yet poor in terms of signal-to-noise in the collected data. It therefore useful to consider what other types of information could be measured at the same time as the Raman data. In our lab, after surveying possible complementary imaging modes compatible with the Raman imaging process [1], we implemented simultaneous digital holographic microscopy with an excitation wavelength outside the Raman spectral region of interest, providing us with quantitative phase maps from the sample at the same time as the Raman data acquisition [2]. This then gives one phase data set that is accurate in spatial location and well-resolved in time but poor in terms of chemical information. The complementary Raman mode provides the ability to isolate and distinguish chemical components in sub-cellular locations, but with a lower degree of spatio-temporal resolution. In particular, components in the cell that move during the Raman acquisition require such a dual-mode imaging approach so that blurred artifacts in the Raman images can be recognized from the time-resolved phase imaging mode. It also allows us to measure additional data which can be used to discriminate cell types and behavior, since the molecular contributions to the phase mode are not exactly the same as those that contribute most to Raman contrast [3]. The use of both sets of information also allows high-throughput analysis [4]. These types of measurements are highly useful for analysis of pathogen triggers of immune response such as bacteria or malarial byproducts [5,6].

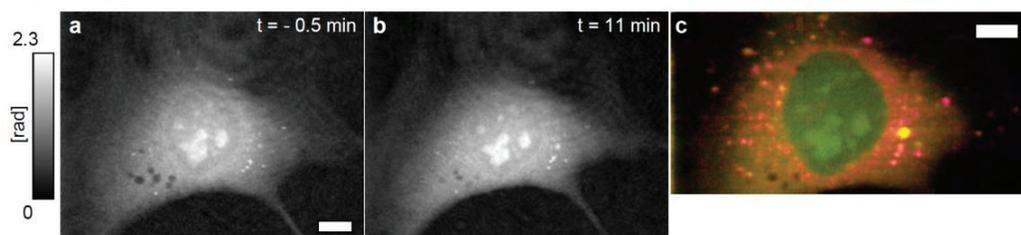


Fig. 1. Simultaneous digital holographic (quantitative phase) and Raman scattering image of a live mouse embryonic fibroblast cell. From Pavillon et al, see Reference [2] for details.

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## ELECTRON MICROSCOPY STUDY OF THE INFLUENCE OF ROSE PHENOL GLYCOSIDE ON THE AMYLIN FIBRILS

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**KEY WORDS:** electron microscopy, amylin, fibrils, phenol glycoside from rose petals

Electron microscopy analysis permits to visualize the microstructures of amyloid fibrils [1,2]. The fibrillization of pancreas peptide hormone amylin and the disaggregating effects of several plant preparations have been studied earlier [3]. At present, despite existing of many publications, the questions concerning the mechanism of aggregation and disaggregation of amyloid fibrils and of action of plant preparations on these processes are not clear. In the present work we studied the effect of phenol glycoside fraction from rose petals (PhG) on the preformed fibrils of amylin using transmission (TEM) and scanning (SEM) electron microscopy methods (JEOL-1400 and JSM-5410, respectively). TEM analysis allowed evaluating the diameter of preformed amylin fibrils as equal of 5-10 nm (Figure1a). SEM study of the action of 0.02% (w/v) PhG revealed the increasing of the fibril sizes (Figure1b). The disaggregation and despiralization of fibrils in the presence of this plant preparation was observed (Figure 1c). The formation of narrow tubes with a central cavity of the diameters from 0.4 to 1.1 $\mu$ m can be seen. The formation of such nanotubes with a central water-filled cavity shown in this picture can be considered as a way of disaggregation of preformed amylin fibrils by PhG. This phenomenon might be considered as one of the probable mechanisms of disaggregation and prevention of the formaton of amyloid fibrils by plant preparations in general.

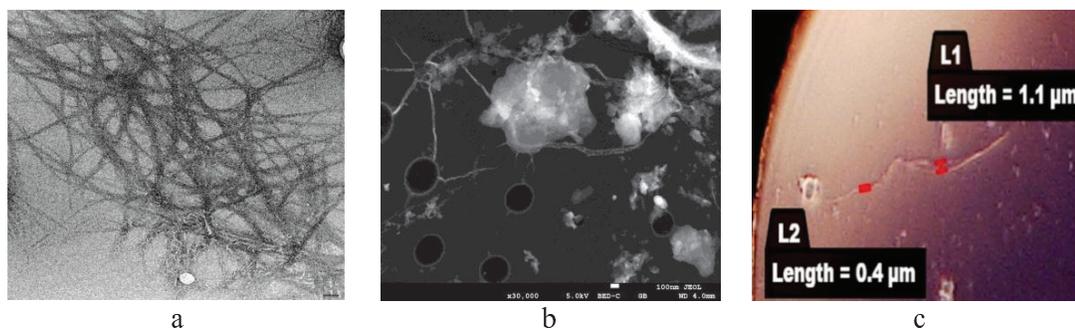


Fig.1a. TEM. Fibrils of amylin in HEPES buffer, pH 7.2; negative contrasting; scale bar: 100 nm.

Fig.1b SEM. The dimention of amylin fibrils increased under action of 0.02% of PhG; scale bar: 100 nm.

Fig.1c. SEM. Disaggregation and despiralization of amylin fibrils under action of PhG. Diameters of fibrils is between 0.4 and 1.1 $\mu$ m

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## Light sheet microscopy for high content 3-D imaging of 3-D tissue cultures in a 96-well plate format

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**KEY WORDS:** Oblique plane microscopy, light sheet microscopy, quantitative analysis, high content analysis

Light sheet fluorescence microscopy is a high-speed 3-D imaging technique providing low out-of-plane photobleaching and phototoxicity. However, conventional light sheet fluorescence microscopy uses two microscope objective lenses orientated at an angle of 90° relative to one another in order to image a sample and so is not compatible with imaging conventional 96-well plates. Oblique plane microscopy (OPM)<sup>1</sup> is an alternative approach to light sheet microscopy that uses a single high numerical aperture microscope objective to provide both fluorescence excitation and detection. OPM can be implemented on a commercially available fluorescence microscope frame to rapidly image volumes with subcellular resolution<sup>2,3</sup>.

We present the development of a stage scanning OPM (ssOPM) approach for light sheet fluorescence imaging in 96-well plates. The plate is translated at a constant velocity through the tilted light sheet of the OPM system and image acquisition is electronically synchronised to the motion of the stage. This approach enables rapid 3-D imaging across each well of a 96-well plate with sub-cellular resolution.

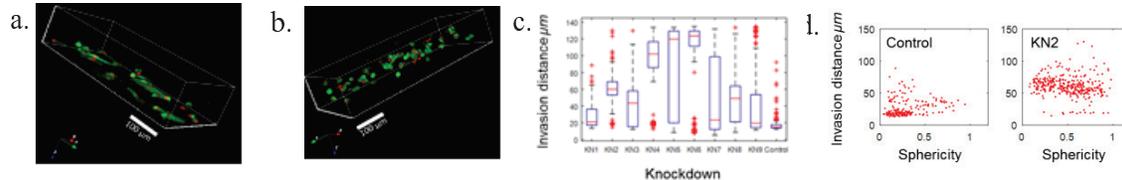


Figure 1 (a) control & (b) knockdown (KN2) sub-volumes rendered in 3-D (c) variation in invasion distributions, measured as intensity weighted centroid distance from the coverslip, across knockdowns (d) invasion vs. sphericity scatter plots for control and KN2 conditions.

WM-266-4 melanoma cells with GFP labelled membranes were seeded onto the bottom of collagen I gel in a 96-well plate. The plate was subsequently incubated for 24 hours allowing cells to invade up into the gel prior to fixation and nuclear staining with DRAQ5<sup>TM</sup>. Image volumes of  $6 \times 0.32 \times 0.14 \text{ mm}^3$  were acquired in 60 seconds for each well of a plate for a range of siRNA knockdowns, see example data shown in fig. 1. The total volume of raw image data for the plate was 0.3 TB. We demonstrate the use of automatic 3-D morphological quantification of the image data to quantify morphological phenotypes for each knockdown.

In conclusion this work will present the development of a stage-scanning OPM plate-reader system for automatic 3-D fluorescence imaging with sub-cellular resolution in 96-well plates.

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## Disruption of oncogenic Ras-driven dependencies using membrane targeted dietary bioactives (MTDBs)

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Approximately 30 to 50% of colorectal cancers contain KRas mutations, which confer resistance to standard therapy and have therefore been termed “undruggable.” Since no curative treatments for KRas driven colon cancer are available, there is a critical need to develop toxicologically innocuous KRas therapeutic approaches that are free of safety problems intrinsic to drugs administered over long periods of time. Uptake of extracellular lipids and proteins by the process of macropinocytosis is a hallmark of Ras-driven tumors. The internalization of substrates provide fuel for the metabolic needs that underlie tumor phenotypes. Recently, it has become apparent that macropinocytosis is influenced by the hierarchical organization of the plasma membrane.

We have hypothesized that membrane targeted dietary bioactives (MTDBs), at physiologically relevant levels, modulate plasma membrane organization and thereby suppress KRas-dependent signaling. Using colonic epithelial cell models non-transformed young adult mouse colonocyte (YAMC) cells containing wild type Ras, as well as oncogenic KRas expressing SW48 human colon adenocarcinoma cells, we investigated the ability of select MTDBs, e.g., docosahexaenoic acid (DHA, 22:6 $\Delta$ <sup>4,7,10,13,16,19</sup>), eicosapentaenoic acid (EPA, 20:5 $\Delta$ <sup>5,8,11,14,17</sup>) and curcumin, to modulate KRas activation and macropinocytosis following cell activation.

Using the membrane order sensitive dye, Di-4-ANEPPDHQ, we initially characterized the rigidity of the plasma membrane in colonocyte cell lines expressing oncogenic HRasG12V and KRasG12D. Expression of oncogenic HRasG12V ( $p < 0.0001$ ) and KRasG12D ( $p < 0.0001$ ) increased plasma membrane rigidity relative to parental cells expressing wild type Ras. In contrast, EPA and DHA reduced plasma membrane rigidity in YAMC cells. In complementary experiments, Ras activation was assessed using fluorescence resonance energy transfer (FRET) biosensors targeted to HRas or KRas domains. Interestingly, only DHA and not EPA or curcumin suppressed the temporal activation of H- and KRas following stimulation with epidermal growth factor (EGF). In contrast, all MTDBs tested (DHA, EPA and curcumin) reduced EGF stimulated macropinocytosis by 50% ( $p < 0.0001$ ), 48% ( $p < 0.0001$ ) and 57% ( $p < 0.01\%$ ), respectively.

Establishing a role of MTDBs in colon cancer prevention would have a major translational impact because these dietary bioactives are safe, well tolerated, relatively inexpensive, and provide additional health benefits, such as reduction in mortality.

## REGULATION OF INTEGRIN FUNCTION STUDIED BY INTEGRATED OPTICAL AND ATOMIC FORCE MICROSCOPY

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KEY WORDS: integrins, extracellular matrix, TIRF microscopy, atomic force microscopy

Vascular smooth muscle (VSM) cells sense and respond to mechanical cues by involving integrin receptors that mediate their adaptive remodeling to the microenvironment. Integrins are transmembrane proteins which have important roles in VSM cell physiology by regulating cell adhesion and contractile function. Our study investigates the relationship between integrin  $\alpha_5\beta_1$  and  $\alpha_v\beta_3$  spatial distribution and function with respect to extracellular matrix. To determine the role of extracellular matrix in selective integrin activation at focal adhesions, cells co-expressing green fluorescent protein fusions for wild-type (wt) integrin  $\alpha_5$ -mApple and  $\beta_3$ -EGFP were plated on fibronectin (FN) or vitronectin (VN). Total internal reflection fluorescence (TIRF) microscopy was used to perform measurements of integrin area relative to cell area. Our results showed that spatial distribution of integrin  $\alpha_5\beta_1$  and  $\alpha_v\beta_3$  is matrix dependent. In control or VN substrates integrin  $\alpha_v\beta_3$  was mainly present at cell edges and formed streak-like adhesions, while  $\alpha_5\beta_1$  was mainly found in adhesions located towards the center of the cell. However, cells plated on FN significantly decreased integrin  $\alpha_v\beta_3$  localization at cell edges, and enhanced overall recruitment of  $\alpha_5\beta_1$  at focal adhesions, especially at cell periphery. Moreover, downregulation of integrin  $\alpha_v\beta_3$ , by expressing a dominant negative (DN) mutant further enhanced the recruitment of integrin  $\alpha_5\beta_1$  at cell periphery for cells plated on FN and induced formation of larger focal adhesions, as also measured by vinculin recruitment. Fluorescence recovery after photobleaching measurements performed at peripheral adhesions showed a slower recovery rate for  $\alpha_5$  integrin in  $\beta_3$ -DN vs.  $\beta_3$ -wt expressing cells. In addition, atomic force microscopy (AFM) was used to test if  $\beta_3$  downregulation affects integrin  $\alpha_5\beta_1$  functional binding to the matrix. Fibronectin functionalized AFM probes were used to measure integrin-matrix adhesion forces at single molecule level. Integrin  $\alpha_5\beta_1$  binding strength to FN was not affected in  $\beta_3$ -DN expressing cells in respect to control, however, the availability of free integrin  $\alpha_5\beta_1$  on cell surface was reduced. Taken together, these results show that downregulation of integrin  $\alpha_v\beta_3$  triggers a compensatory mechanism in which integrin  $\alpha_5\beta_1$  replaces  $\alpha_v\beta_3$  at peripheral adhesions, further enhancing matrix-dependent focal adhesion remodeling. Thus, TIRF imaging performed in combination with AFM represents a novel approach that enables complex functional studies of integrin mechanoreceptors, and is widely applicable to other cell types.

## THREE-DIMENSIONAL SUPERRESOLUTION AND TRACKING OF OVERLAPPING EMITTERS USING THE DOUBLE-HELIX SPINDLE™ MODULE

Anthony Barisc<sup>1,2</sup>, Anurag Agrawal<sup>1</sup>, Ajoke Williams<sup>1</sup>, Saumya Jain<sup>3</sup>, Joshua R. Wheeler<sup>3</sup>, Robert Walters<sup>3</sup>, Roy Parker<sup>3,4</sup>, and Rafael Piestun<sup>1,2</sup>

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**KEY WORDS:** point spread function, 3D single molecule imaging, Double-Helix

The Double-Helix Point Spread Function technique engineers the response of a microscope such that the image of each point emitter is in the form of two lobes. The center of the pair of lobes determines the lateral position of the emitter and the angle between the lobes determines the axial position<sup>1</sup>. We present an implementation of the Double-Helix phase mask technology integrated into an optical module - SPINDLE™, in conjunction with matched algorithms that extends the imaging capabilities of a microscope to nanometer scale 3D localization for imaging<sup>2</sup> and tracking<sup>3</sup>.

Previous localization algorithms required sparse, well-separated images of the DHPSF. More recently, we described an algorithm<sup>4</sup> capable of resolving multiple emitters within a single diffraction-limited 3D volume. Here, we show the results of the algorithm for 3D tracking of fluorescent beads in glycerol and for 3D super-resolution imaging of stress granules<sup>5</sup>. In Fig. 1 a), the two molecules are well separated, and the reconstructed image in b) displays the two distinct images of the DHPSF in red and green. The experimental image in c) exhibits substantial overlap of the images of the DHPSF, and yet the reconstruction in d) demonstrates the capability to distinguish fluorophores even when the separation is small. This algorithm in conjunction with the module showed, for the first time, detailed 3D structure of stress granules and the distribution of its cores at sub-diffraction resolution clarifying the formation of core structures within stress granules<sup>5</sup> (Fig. 2)

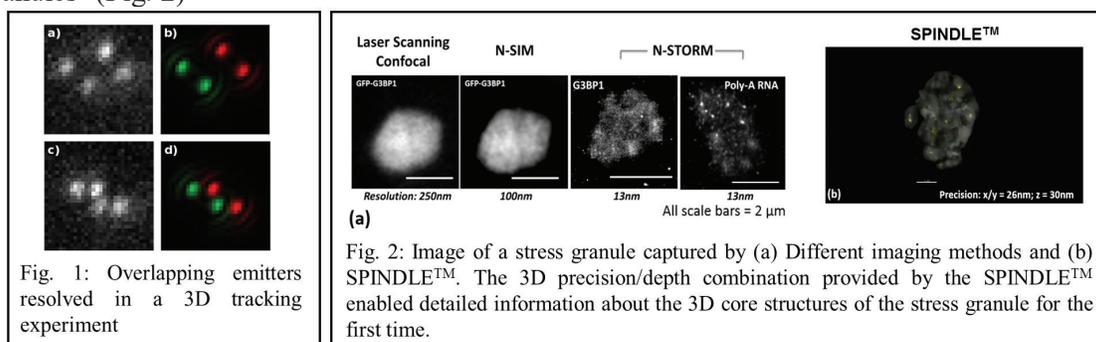


Fig. 1: Overlapping emitters resolved in a 3D tracking experiment

Fig. 2: Image of a stress granule captured by (a) Different imaging methods and (b) SPINDLE™. The 3D precision/depth combination provided by the SPINDLE™ enabled detailed information about the 3D core structures of the stress granule for the first time.

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## Selected applications of Image Mapping Spectrometry

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**KEY WORDS:** snapshot spectrometry, imaging, multispectral imaging, medical imaging, fluorescence, microscopy

The hyperspectral imaging modality allows acquisition of three dimensional data cubes that include spatial and spectral intensity signatures. In our system, a custom designed mapping mirror is combined with imaging optics tailored to it that directly maps the sample image into a 3D datacube of  $(x,y,\lambda)$  components[1,2]. We have combined the IMS with a microscope[3], and with a microscope-compatible structural illumination system to acquire a 4D data stack  $(x,y,z,\lambda)$ [4], which allowed us to analyse the spectral characteristic of samples at controllable depths ( $z$  dimension). We have used the hyperspectral imaging modality to simultaneously observe multidimensional cellular signalling events [5-7], specifically  $\text{Ca}^{2+}$  and  $\text{Zn}^{2+}$  dynamics in  $\alpha$ - and  $\beta$ -cells. The operating spectrum of the IMS system overlaps with the characteristic absorption curves of hemoglobin and thus, allows direct measurement of relative oxy-/deoxy-hemoglobin concentrations in superficial layers of the skin [2]. When combined with a retinal camera, the IMS allows for snapshot, motion artifact-free analysis of oxygenation of the retina[8,9] and assessment of the condition of the visual organs in case of degenerative changes related to e.g.: exudative AMD, glaucoma, retinitis pigmentosa, and iridocyclitis. By virtue of the simultaneous acquisition of spatio-spectral data, we have the ability to uniquely identify spectrally the mutually exclusive fluorophores that share a spatial location[7]. Using linear unmixing algorithms, we are able to monitor dynamic changes in the fluorescence rate of multiple collocated fluorophores, without the need for time consuming spectral and spatial scanning. Recently, we have developed a new IMS build around low read noise ( $<1.4e$  rms) using a scientific sCMOSS detector that is capable of video rate data acquisition (30 fps). Our system operates in a spectral range of 515 – 842 nm and acquires a datacube size of  $210 \times 279 \times 54$   $(x,y,\lambda)$  samples. Our instrument in the bright field imaging mode enables the acquisition of absorption/reflection spectral signatures dependent on the illumination mode selected (dia-/epi-). When combined with excitation and emission filter(s), our system allows for the analysis of samples stained with multiple fluorophores allowing simultaneous acquisition of the spatio-spectral footprint of the investigated object that is free from motion and spectral scanning related artifacts.

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## Studying T7 phage particle transport in artificial mucus by TSUNAMI 3D tracking microscopy

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**KEY WORDS:** T7 phage, 3D single-particle tracking, mucus penetration

Whereas cystic fibrosis (CF) is the most common autosomal recessive genetic disorder in the Caucasian population, there is no established curative treatment for the disease at this moment. Patients with CF have thickened mucus, which not only traps pathogens but also becomes a barrier for drug penetration. Here we want to study the transport properties of various mucus-penetrating formulations using an advanced 3D single-particle tracking instrument termed TSUNAMI (Tracking of Single particles Using Nonlinear And Multiplexed Illumination)[1], which is a custom-built, feedback-control microscope that relies on a two-photon spatiotemporally multiplexed excitation scheme and temporally demultiplexed detection method to discern the 3D position of a single particle. Our model system is the engineered T7 phage particles that express specific peptides on their capsid surfaces[2]. By tracking single T7 phage particles in artificial mucus, we can evaluate the viscoelastic properties of mucus and understand the roles of specific peptides in promoting the T7 mucus penetration.

In our preliminary experiments, one kind of T7 phage was stained with SYBR-gold[3]. Using an 850 nm femtosecond laser for two-photon excitation, the brightness of single T7 particle was about 300 kHz, leading to a signal-to-noise ratio of 15. The average track duration was  $4.24 \pm 2.45$  seconds. The diffusivity ( $D_0$ ) extracted from the mean-squared-displacement (MSD) curves (based on the first 5 points) indicated the existence of a slow moving species ( $D_0 = 0.043 \pm 0.013 \mu\text{m}^2/\text{s}$ ) and a relatively fast moving species ( $D_0 = 0.106 \pm 0.025 \mu\text{m}^2/\text{s}$ ). We are collecting more 3D diffusion trajectories from various T7 phage particles in mucus and evaluate their MSD curves. Our goal is to correlate the transport properties with the peptide sequences, elucidate the fundamental mucus penetration mechanism and establish a model for particles penetration in mucus.

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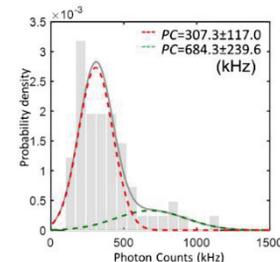


Figure 1. Photon count rate of SYBR-Gold stained T7 phages.

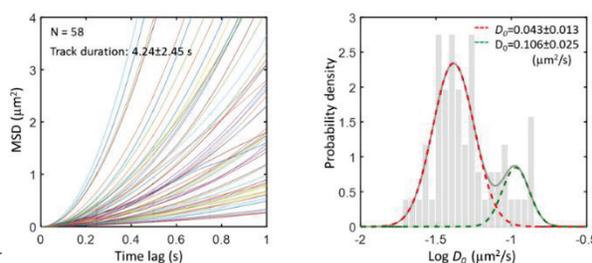


Figure 2. Diffusivity of T7 phage particles in artificial mucus.

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## Surface-Dependent Diffusion Dynamics of Intracellular Quantum Dots

Mohammad U. Zahid, Liang Ma, Sung Jun Lim, and Andrew M. Smith

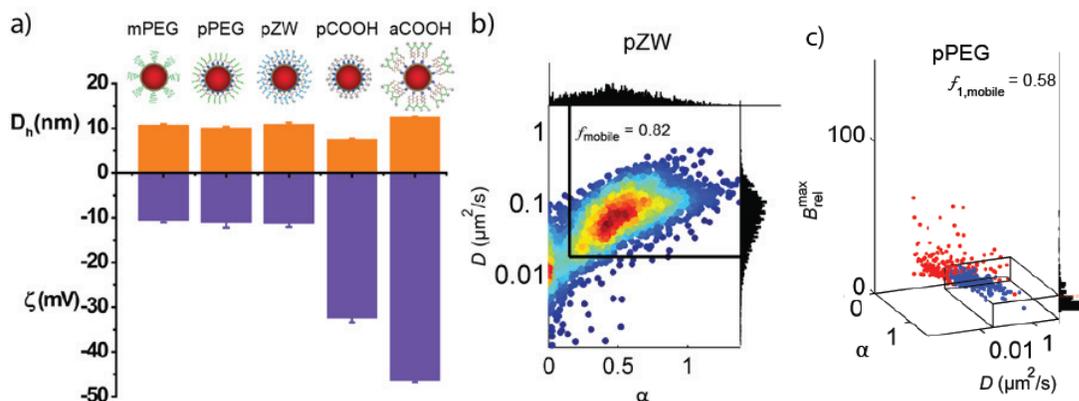
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**KEY WORDS:** Quantum Dots (QDs), Intracellular Delivery, Single-Molecule Imaging, Single-Particle Tracking

Quantum dots (QDs) are fluorescent nanocrystals that exhibit exceptional brightness and stability, characteristics that have made them particularly valuable for single-particle and single-molecule tracking. However, their use in the cellular cytoplasm has been challenging due to nonspecific binding. Recently we demonstrated the generation of highly compact and stable QDs coated with multidentate polymers (1). The hydrodynamic diameters are in the range of 7–12 nm and the particles exhibit exceptionally low nonspecific interactions compared with commercial QDs. We have now studied how surface chemistry impacts intracellular diffusion of QDs delivered to cells using osmotic pinosome lysis (OPL) (2). We independently analyzed the impact of coating stability and electrostatic charge (i.e. zeta potential,  $\zeta$ ), while largely keeping the hydrodynamic diameter ( $D_h$  by dynamic light scattering) fixed, as shown in Fig. 1a. We imaged intracellular QDs using HILO microscopy and tracked them using the u-Track software package (3). We calculated the fraction of single QDs and the total number of QDs delivered to each cell by quantitative analysis of single-molecule brightness. We correlated these values with intracellular mobility by quantitative analysis of their mean squared displacement (MSD) using an anomalous diffusion model with diffusion coefficient ( $D$ ) and confinement parameter  $\alpha$  as  $MSD = 4Dt^\alpha$  (4). We found that both the number of QDs delivered to the cytosol and the fraction that are single and mobile are highly sensitive to the physicochemical nature of the QD surface. Specifically, the stable, zwitterionic polydentate ligand pZW yielded intracellular mobility with  $D$  near  $0.1 \mu\text{m}^2/\text{s}$  (Fig. 1b) and  $>90\%$  single QDs (Fig. 1c). In contrast, less stable and more anionic coatings resulted in higher levels of QD clustering and substantially reduced diffusion. In conclusion, unstable or charged surfaces increase cell uptake, but intracellular mobility requires stability and electrostatic neutrality.



**Figure 1.** (a) Size and zeta potential characterization of different QDs. (b) Density plot of  $D$  vs.  $\alpha$  (anomalous diffusion model). For QDs coated with pZW, 82% are mobile after OPL delivery to CHO cells. (c) 3D plot of  $D$ ,  $\alpha$ , and relative particle brightness of intracellular pPEG QDs: 90% are single; 58% are single and mobile.

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**RESOLVING FLUORESCENT OBJECTS BEYOND THE DIFFRACTION LIMIT BY REDUCING THE ILLUMINATED REGION AND APPLYING NUMERICAL OPTIMIZATION**

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**KEY WORDS:** numerical optimization, diffraction limit, optical transfer function

We present a new fluorescence imaging method that resolves structural details much finer than the conventional diffraction-limited resolution. In our method, we step a focused illumination spot across the region of interest, and acquire one image at each step. We then feed the acquired images to a numerical optimization algorithm to determine the intensity distribution of the fluorescent photo-emitters. These simple procedures together yield output images with resolution significantly higher than conventional diffraction-limited imaging systems.

Such results are controversial because they show higher resolution can be achieved without increasing optical transfer function bandwidth, which is not compatible with the standard resolution theory of band-limited imaging systems. In this talk we present both spatial and frequency-domain perspectives that together explain the resolution-enhancing mechanism underlying our imaging method.

## Deep and high-resolution three-dimensional tracking of single particles using nonlinear and multiplexed illumination

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Molecular trafficking within engineered three-dimensional multicellular models is critical to the understanding of the development and treatment of various diseases including cancer. However, current tracking methods are either confined to two dimensions or limited to an interrogation depth of 15  $\mu\text{m}$ . Here we present a new 3D tracking method capable of quantifying rapid molecular transport dynamics in highly scattering environments at depths up to 200  $\mu\text{m}$ . The system has a response time of 1 ms with a temporal resolution down to 50  $\mu\text{s}$  in high signal-to-noise conditions, and a spatial localization precision as good as 35 nm. Built upon spatiotemporally multiplexed two-photon excitation, this approach requires only one detector for 3D particle tracking and allows for two-photon, multi-color imaging. 3D tracking of epidermal growth factor receptor (EGFR) complexes at a depth of 100  $\mu\text{m}$  in tumor spheroids is demonstrated. Our 3D tracking microscope is built upon spatiotemporally multiplexed two-photon excitation and uses time-gated analysis *via* a photon counting histogram to discern the molecular 3D position. Feedback control then steers the excitation to lock-on to the single molecule as it travels at a high speed. The molecular trajectories are reconstructed from the recorded actuator positions from the feedback control loop operating at 1-5 ms. Dynamics down to 50  $\mu\text{s}$  can be inferred from analysis of the photon counting histogram. In our method, the first PMT channel is used for particle tracking while the second and the third PMT channels can be used for two-photon scanning microscopy, colocalization analysis, and energy transfer studies. We have coined this technique **TSUNAMI** (Tracking Single particles Using Nonlinear And Multiplexed Illumination).

## LIGHT-SHEET MICROSCOPY USING ATTENUATION-COMPENSATING AIRY BEAM

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**KEY WORDS:** light-sheet microscopy, Airy beam, digital micromirror device, spatial light modulation

Diffraction-free light fields such as Airy beam have attracted significant attention due to their ability to retain their transverse intensity profile during propagation. This ability is highly desirable for light-sheet microscopy whose field of view (FOV) is usually limited by the Rayleigh range of the Gaussian beam used to create the light sheet [1]. However, the extended FOV of Airy beam light-sheet microscopy can be comprised by the non-uniformity of illumination, especially in thick, highly scattering and absorbing tissue. To provide a more uniform illumination, light-sheet excitation from both sides of the sample is adopted [2], which results in higher system complexity and optical alignment difficulties. Here we introduce the concept of digital micromirror device (DMD)-based Airy beam light-sheet microscope (DMD-ALS) that can compensate the intensity loss caused by scattering and absorption in biological tissues. In our modelling, it can provide thin and uniform illumination over a large range, thus eliminating the necessity of double-sided illumination. The attenuation-compensating Airy beam is generated by phase-modulating a Gaussian laser beam with a DMD displaying the Lee hologram [3]. The fluorescence intensity profile along the optical axis is measured and used for the calculation of DMD spatial pattern in real time. This feedback adaptive procedure ensures optimal attenuation compensation for a variety of biological tissues with different optical properties, without human intervention. Taking advantage of the fast frame-rate (~32kHz) of DMD, we expect the adaptive control algorithm converges within 50ms.

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## DEVELOPMENT OF BIOPHYSICAL MARKERS THAT QUANTIFY METASTATIC POTENTIALS OF PROSTATE CANCER CELLS USING TSUNAMI MICROSCOPE

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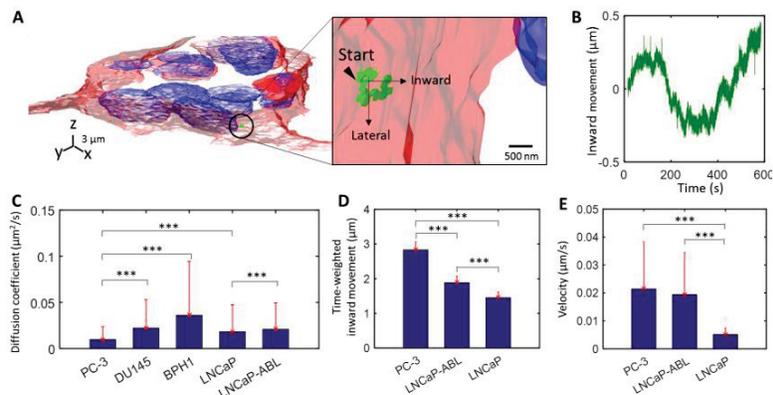
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**KEY WORDS:** single particle tracking, metastasis, epidermal growth factor receptor

Dysregulated trafficking of receptor tyrosine kinases has been linked to oncogenesis [1]. Here we study the trafficking patterns and dynamics of epidermal growth factor receptor (EGFR) trafficking of benign (BPH1), non-invasive malignant (LNCaP), and highly invasive malignant (PC-3) prostate cells using an advanced 3D single-particle tracking technique termed TSUNAMI (Tracking of Single particles Using Nonlinear And Multiplexed Illumination) [2]. As a feedback-control tracking system, TSUNAMI is capable of tracking fluorescent nanoparticle-tagged EGFR for up to 10 minutes and in the z-direction for up to  $\pm 50$  microns. To analyze the long 3D trajectories generated by the TSUNAMI microscope, a trajectory analysis algorithm is developed to classify trajectories and extract the dynamic parameters [3], such as diffusivity, inward movement, and internalization duration. These parameters can be used to quantify the metastatic potentials of prostate cancer cell lines. For instance, the diffusivities of EGFRs on the highly invasive malignant PC-3 cells ( $0.010 \pm 0.014 \mu\text{m}^2/\text{s}$ ) are around one-quarter of those estimated from the benign BPH1 cells ( $0.036 \pm 0.058 \mu\text{m}^2/\text{s}$ ), possibly due to the abnormally high expression of EGFRs on the PC-3 cells. The highly invasive PC-3 cells also exhibit longer ( $2.83 \pm 0.23 \mu\text{m}$  vs.  $1.45 \pm 0.16 \mu\text{m}$ ) and faster ( $0.021 \pm 0.016 \mu\text{m}/\text{s}$  vs.  $0.005 \pm 0.002 \mu\text{m}/\text{s}$ ) inward movement as compared with the non-invasive LNCaP prostate cancer cells, which could be due to the high endocytotic activity associated with the invasive PC-3 cells. In addition, the dynamics parameters extracted from the EGFR trajectories are correlated with the expression levels of a number of epidermal-mesenchymal-transition (EMT)-related genes. The high EGFR expression is related to the decrease of EGFR diffusivity, and the increase of dynamins coheres with more active inward movement. After EMT induction, the non-invasive LNCaP prostate cancer cells also exhibit gene expression profiles and EGFR trafficking patterns similar to those shown in invasive PC-3 cells. Our work demonstrates that EGFR trajectory-derived dynamics parameters are linked to metastatic potentials. A new class of biophysical markers can be established based on the analysis of membrane protein trajectories.



**Figure 1.** Biophysical features revealed by 3D single-particle tracking. (A) A representative trajectory demonstrates the internalization of an EGFR complex. (B) Time trace of EGFR inward movement. (C) Diffusivities of EGFRs on the plasma membrane of various prostate cell lines. EGFR internalization characterized by the trajectory-derived parameters: (D) inward movement and (E) velocity. The error bars represent standard deviations from 15-63 measurements and the \*\*\* indicates that the associated p value of two-sample t-test is less than 0.001.

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## A NEW WAY OF OPTIMIZING POINT SPREAD FUNCTIONS FOR 3D FLUORESCENCE SUPER-RESOLUTION MICROSCOPY

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**KEY WORDS:** PSF engineering, numerical optimization, 3D super-resolution microscopy, fluorescence imaging

We present a way of optimizing point spread functions (PSFs) for a new type of super-resolution microscopy. Common methods for optimizing PSFs for 3D super-resolution microscopy use the signal to noise ratio and the Cramer-Rao lower bound (CRLB) as the optimization criteria [1-2]. However, these criteria are only reliable in the context of localization-based microscopy, which assumes that each acquired image has point spread functions far apart from one another. In this talk, we describe a new type of super-resolution microscopy technique that can be used to image three-dimensional (non sparse) structures, and introduce the method we have developed to optimize PSFs for this technique.

In our new super-resolution microscope, a series of images are acquired as a focused excitation spot scans across a sample, and a least-squares algorithm is then used to estimate the spatial and intensity distribution of the fluorescent photo-emitters. Therefore, the resolution in our method depends on the estimation accuracy of closely packed photo-emitters. To find the optimal PSF, we first numerically model a ground-truth object, simulate its resultant images, and analyze them with the previously mentioned algorithm. We then use an iterative method to search for the optimal PSF by minimizing the difference between the analyzed results and the modeled ground-truth object. Using this new approach, we have been able to demonstrate super-resolution of extended fluorescent objects with an accuracy of around 100nm in all three dimensions.

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## Maximizing the Performance of Fluorescence Microscopes by Optical Filters

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**Semrock, a unit of IDEX Health & Science**

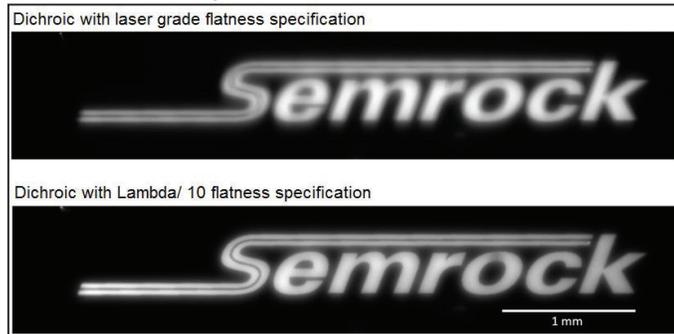
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**KEY WORDS:** Optical Filters, TIRF, Super resolution microscopy, Multi-photon microscopy

Years ago, microscopy approaches such as total internal reflection fluorescence (TIRF), as well as super-resolution microscopy techniques such as stimulated emission depletion (STED) and localization microscopy, were only available to engineers, physicists, and cross-disciplinary researchers. Due to a larger number of commercially available systems, access to these techniques has greatly expanded, allowing progress in scientific fields to propagate. As microscopy needs in biological applications vary to a great degree, users may find themselves tailoring advanced microscopy techniques such as TIRF, super-resolution, or multi-photon to solve a specific problem. One way to achieve success and maximize performance is through the use of application-specific optical filters.

Though elements such as a dichoric beamsplitter are ever present in even the most elementary of epifluorescence microscopes, they can have a profound effect if specifications such as flatness are not tailored to the techniques of interest. These can include interference fringing and increased background noise [1]. Any astigmatism or other aberration will reduce effective resolution, and alter spot size and/or shape from the ideal diffraction-limited PSF provided by a perfectly flat dichroic, which could render some techniques useless [2].

We discuss the fundamental filter requirements for advanced imaging techniques such as multiphoton, TIRF, SIM, PALM, STORM, patterned illumination, laser-based confocal microscopy, and even LED or broadband light source [3] based super-resolution techniques. These application-specific optical filter solutions should allow users to maximize their system performance.



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## Using advanced microscopy techniques to image antibody-dependent macrophage-cancer cell interactions

Understanding the complex behavior of effector cells such as monocytes or macrophages in regulating cancerous growth is of central importance for cancer immunotherapy, especially with the ability of macrophages to bind antibodies through Fc receptors. Earlier studies using CD20-specific antibodies have demonstrated that immune cells can mediate the Fc $\gamma$  receptor (Fc $\gamma$ R)-mediated transfer of the targeted receptors from tumor cells through a process called trogocytosis, while antibody-dependent phagocytosis of the entire target cell is another established macrophage-mediated action on tumor cells.

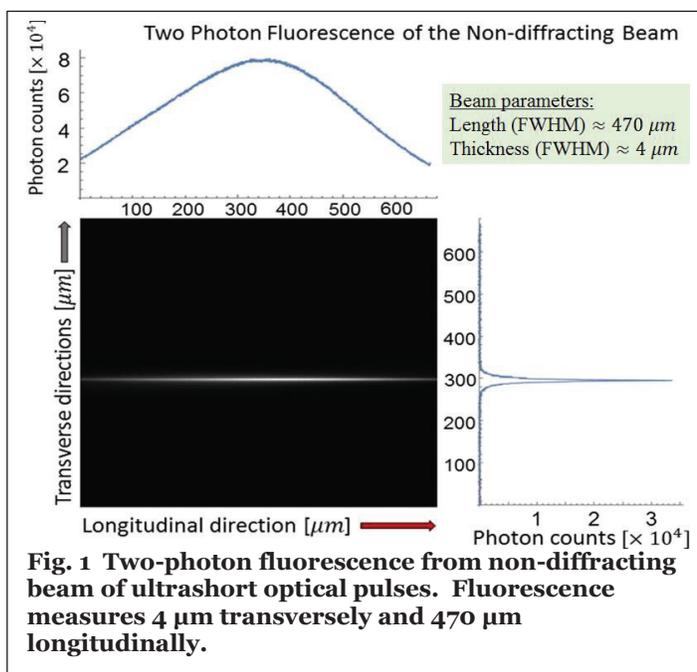
We use advanced microscopy methods and quantitative flow cytometric assays to study the mechanisms and effects of these antibody-mediated processes on cancer cells. Multifocal plane Microscopy (MUM) has allowed us to image the antibody-mediated macrophage-cancer cell synapse in high spatio-temporal resolution. Our results indicate that trogocytosis occurs through the extrusion of tubule-like structures from the target cell surface, thus enabling the selective internalization of the target membrane without affecting its integrity. MUM combined with high accuracy 3D single-particle tracking algorithms also allows us to track the fate of the material after internalization by trogocytosis. Together with our long-term imaging modalities and quantitative flow-cytometric assays, we have identified that though trogocytosis does not cause immediate cell death, persistent trogocytosis of HER2 positive breast cancer cells can lead to the death of target cells in an antibody-dependent manner. Together, our results add to our understanding of the numerous interactions macrophages can have with cancer cells, and how therapeutic antibodies modulate their effects.

MULTICOLOR SELECTIVE PLANE ILLUMINATION MICROSCOPY TO IMAGE THE DYNAMICS AND INTERACTIONS OF GENETICALLY DEFINED LINEAGES

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KEY WORDS: ultrashort optical pulses, selective plane illumination microscopy, two-photon excited fluorescence, multiplexed microscopy

The vertebrate central nervous system is formed gradually by partitioning proliferating primordial cell populations into compartments and refining these populations into distinct functional subunits. The development of these functional units is guided by signals emitted from secondary organizers usually located at lineage restricted boundaries. The midbrain-hindbrain boundary (MHB) organizer (or isthmus organizer) is an evolutionarily conserved compartment boundary that instructs the formation of the tectum and cerebellum from the mesencephalon and anterior hindbrain, respectively. The formation of this organizer is essential for brain development, however, little is understood about the dynamics and interactions underlying MHB formation and its function. In the zebrafish, the MHB is positioned early in development during gastrulation (specification of germ layers) when the embryo is undergoing rapid cell movements and divisions that envelope the yolk. At this early stage, the MHB is not morphologically apparent, but is positioned at the interface of two gene expression domains (*otx/gbx*) which sharpens over the course of two or three hours. To better understand the dynamics and interactions underlying MHB formation, we have developed multispectral, two-photon, selective plane illumination microscopy (2P-SPIM) based on broadband, ultrashort optical pulses that can excite and segment multiple genetically encoded fluorophores with emission maxima that span the visible spectrum [1]. Spectral 2P-SPIM will be used to image transgenic zebrafish lines with fluorescent reporters of the two target genes. To extend the depth of field for imaging the zebrafish embryo (yolk diameter  $\sim 700 \mu\text{m}$ ), a non-diffracting Bessel beam is produced in the sample by illuminating the back aperture of the focusing objective with an annular beam shaped by an all-reflective axicon. With this experimental configuration, two-photon excited fluorescence may be generated at the focus with a depth of field and radial thickness of  $470 \mu\text{m}$  and  $4 \mu\text{m}$ , FWHM, respectively, as shown in Fig. 1. We will present our progress in the visualization of initial formation and positioning of the MHB in the developing zebrafish embryo.



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### 3D single molecule tracking with cellular context: trafficking of prostate-specific membrane antigen-specific antibody in prostate cancer cells

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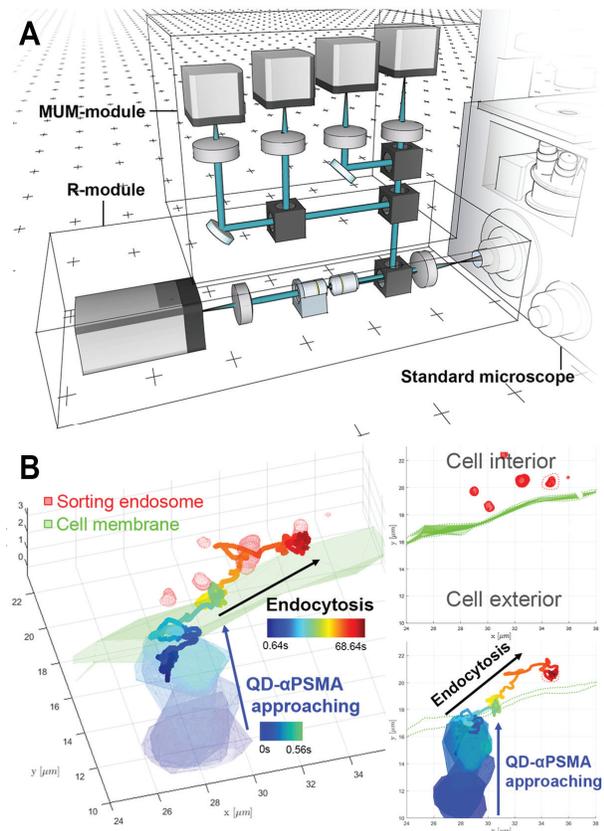
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Single molecule microscopy combined with 3D imaging enables the study of dynamic processes in living cells at the level of individual molecules. Multifocal plane microscopy (MUM) exemplifies such a modality, and has been shown to be capable of capturing the rapid subcellular trafficking of single molecules in thick samples by simultaneously imaging distinct focal planes within the sample. Regardless of the specific modality, however, the obtained 3D trajectories of single molecules often do not fully reveal the biological significance of the observed dynamics. This is because the missing cellular context is often also needed in order to properly understand the events observed at the molecular level. We introduce the remote focusing-MUM (rMUM) modality (Figure A), which allows 3D single molecule imaging with the simultaneous z-stack imaging of the surrounding cellular structures. Using rMUM, we demonstrate the 3D tracking of prostate-specific membrane antigen (PSMA) with a PSMA-specific antibody in a prostate cancer cell.

By making possible the observation of single molecule dynamics within the relevant cellular context, rMUM allows, in our current application, the identification and analysis of different stages of the PSMA-specific antibody trafficking pathway (Figure B). These stages can be summarized as follows. First, quantum dot-labeled PSMA-specific antibody is observed in the cell exterior to very rapidly diffuse towards the plasma membrane. Following a period of both unconstrained and constrained diffusion on the lateral plasma membrane, the antibody undergoes endocytosis and is immediately transported towards a sorting endosome by highly directed motion. Subsequently, the antibody is observed to enter the sorting endosome and diffuse along the spherical surface of the endosome's inner leaflet of the endosomal membrane. The results obtained thus provide a significantly more complete understanding of PSMA dynamics at the single molecule level and within the cellular environment.



## Localization of single molecules from fluorescence microscopy images using a state space-based method

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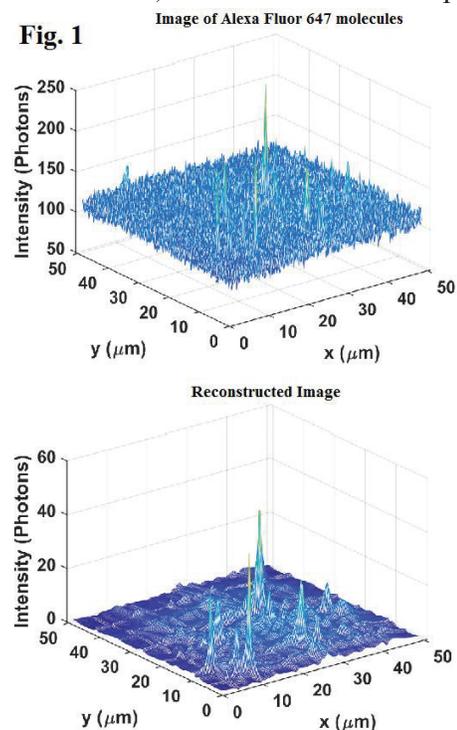
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**KEY WORDS:** image reconstruction, maximum likelihood estimation, multi-order system, singular value decomposition, state space, superresolution.

In single molecule superresolution microscopy, images of subsets of stochastically photoactivated fluorophores are acquired over a sequence of frames. In order to construct a high-resolution image of the structures labeled by these fluorophores, the locations of the fluorophores are accurately estimated in each frame. The majority of available fluorophore localization methods consist of two separate steps: a detection step in which regions of the image that potentially contain emitting fluorophores are identified, and an estimation step which carries out fluorophore localization inside these regions. Most estimation methods are fitting-based, i.e., they estimate the locations of fluorophores by fitting a point spread function model to the image data and finding the location coordinates that minimize the difference between the model and data according to some criterion.

Here, to solve the localization problem for single molecule images, we develop a non-fitting state space algorithm which combines the detection and estimation steps. In single molecule fluorescence images, the locations of peaks of intensity correspond to the locations of molecules. Our proposed algorithm models such an image by the frequency response of a multi-order system obtained using the state space realization algorithm used in [1] for the noise reduction of fluorescence microscopy images based on the singular value decomposition of a Hankel matrix. The locations of the poles of such a system determine the peak locations in the frequency domain, and therefore correspond to the locations of molecules.

The locations obtained in this manner can be used as initial conditions in an estimation routine, e.g., the maximum likelihood estimator, to improve the accuracy of the estimates. For a given image, the algorithm also produces an analytical expression for a reconstruction of the image in the form of a multi-order system (see Fig. 1). The performance of the algorithm is evaluated with both simulated and experimental data.



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## Software framework for single molecule data analysis

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Single molecule analysis is a complex task that single molecule practitioners with varying levels of expertise engage in. Current analysis software tools limit the type of analyses novice practitioners can perform and provide limited features for expert practitioners to advance the field. These limitations cannot be addressed by continually developing new software or updating existing tools because single molecule analysis requirements are far too complex and varying. Software to address the complex and varying nature of single molecule analysis is better developed using a software framework designed for single molecule analysis. Such a software framework would provide the following: a library of software building blocks from which new analysis procedures and tools can be developed quickly, uniform communication patterns between software blocks to facilitate developing analysis procedures for novel situations, and established development patterns serving as templates using which new features can easily be incorporated.

The Microscopy Image Analysis Tool (MIATool) <sup>[1]</sup> software, previously used for routine microscopy image analysis, has been adapted and improved upon as one such software framework for single molecule image data analysis. The new version of the MIATool framework consists of three subsystems. The processing subsystem provides software elements to develop flexible analysis procedures of arbitrary complexity. This allows expert users to efficiently develop analysis procedures for novel scenarios. The data subsystem provides a uniform method to access raw data efficiently in all analysis scenarios and allows analysis results to be associated with raw data meaningfully. This is accomplished using multidimensional matrix-like data structures containing references to images and regions of interest <sup>[2]</sup>. Given the centrality of molecule localization to the analysis of single molecule data, and given the variety of available localization approaches with varying numerical complexities, a subsystem dedicated to this aspect of the analysis and, more generally, parameter estimation, is provided in the framework. Designed in hierarchical layers, this subsystem provides a variety of options for fitting point spread function models to image data <sup>[3]</sup>.

Elements from the MIATool framework can be quickly coupled together and packaged with easy-to-use graphical interfaces to form analysis tools for a variety of routine and novel analysis situations for novice users. The software elements, communication interfaces, and design patterns of the framework provide advanced users with building blocks to design complex analysis procedures for novel scenarios without writing a significant amount of customized software.

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## INVESTIGATING THE EFFECT OF DIFFERENT 3D ILLUMINATION PATTERNS ON STRUCTURED ILLUMINATION MICROSCOPY PERFORMANCE

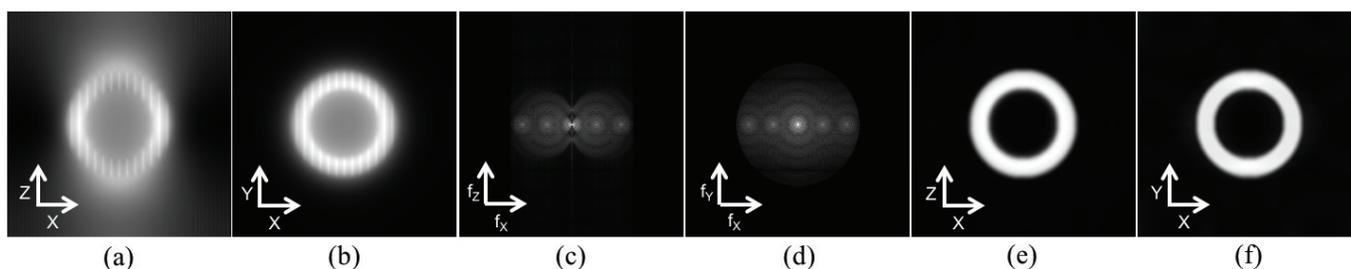
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**KEY WORDS:** structured illumination microscopy, axial modulation, reconstruction, tunable modulation frequency

Structured illumination in optical microscopy has led to several super-resolution techniques. Among different patterns, the lateral sinusoidal excitation was used for the first demonstration of structured illumination microscopy (SIM), which provides the fastest SIM acquisition system compared to the multi-spot illumination approach. Moreover, the 3D patterns that include lateral and axial variations in the illumination attract more attention recently as they address resolution enhancement in three dimensions. A three-wave interference technique in SIM has been developed to provide super-resolution and optical sectioning based on the coherent illumination [1]. In this study, we investigate the novel tunable technique that creates a 3D pattern from a set of multiple incoherently illuminated parallel slits that acts as light sources for a Fresnel biprism [2]. This novel setup is able to modulate the illumination pattern in the object space both axially and laterally with adjustable modulation frequencies [2,3]. The lateral modulation folds the whole information of the object (modulated and unmodulated) into the conventional 2D optical transfer function (OTF). Thus, the lateral support of the OTF does not change with lateral modulation. However, the axial modulation changes the OTF support axially. The 3D forward model for the new system is developed to consider the effect of the axial modulation within the 3D patterned illumination. The performance of 3D-SIM based on the three-wave interference and the multi-slit biprism setup are investigated in simulation and compared based on two different criteria. First, restored images obtained for both 3D-SIM systems using the generalized Wiener filter [1] are compared to determine the effect of the illumination pattern on the reconstruction. Second, the effective OTF of both systems is studied to determine the axial and lateral resolution enhancement that is obtained in each case.



Forward and restored images of spherical shell for three-wave interference technique in SIM. (a)-(b): axial and lateral views of forward image in space domain, (c)-(d) axial and lateral views of forward image in frequency domain, and (e)-(f) axial and lateral views of restored image.

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## Simple biophysics of the collective conformational transitions of the intrinsically disordered proteins of the Nuclear Pore Complex

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Nuclear Pore Complex (NPC) is a key cellular transporter that controls nucleocytoplasmic transport in eukaryotic cells, and is involved in large number of regulatory processes. It is a remarkable device that combines high selectivity with robustness and speed. Its unique transport mechanism is still not fully understood. Recently, the Nuclear Pore Complex transport mechanism inspired creation of artificial selective nano-channels that mimic its structure and function for nano-technology applications.

The centerpiece of NPC transport is the assembly of intrinsically disordered polypeptides, known as FG nucleoporins, lining its passageway, which serve as a template for binding of the cargo-carrying transport proteins. Their conformations and collective dynamics during transport are difficult to assess *in vivo*. *In vitro* investigations provide partially conflicting results, lending support to different models of transport, which invoke various conformational transitions of the FG nucleoporins induced by the cargo-carrying transport proteins.

I will present a theoretical and computational framework that provides rigorous biophysical underpinnings for the Nuclear Pore Complex spatial architecture and transport mechanism. It shows that the spatial organization of FG nucleoporin assemblies with the transport proteins can be understood within a first principles biophysical model with a minimal number of key physical variables, such as the average protein interaction strengths and spatial densities. These results reconcile some of the outstanding controversies and suggest how molecularly divergent NPCs in different species can perform essentially the same function.

# The Allen Institute for Cell Science: Building a microscope pipeline to map the human cell.

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The textbook sketch of the human cell we are familiar with is based more on imagination than on solid data. We believe that understanding the organizational structure of a healthy cell and its evolution over time is an essential starting point to understand changes caused by disease.

In late 2015, the Allen Institute for Cell Science (AICS) launched, developing a light microscopy pipeline to create large data sets that will help scientists in academia and industry to put their models into context and to generate new hypothesis. We use gene-edited hiPS cells, since they are diploid and relatively homogeneous. In the first iteration, we are identifying the major cellular machines and signaling pathways using genome-edited fluorescently tagged proteins. To orient ourselves within the cell, we will also visualize one or two referential markers. Using these live cells, we will acquire large numbers of 3D images and time series.

We use robotics to seed cells on Matrigel in glass bottom plates, feed them and grow them to colonies. We detect the colonies in an overview scan and image them in high resolution with multiple spinning disk systems. We plan to complement these measurements with data from Airy scan super-resolution or other light microscopy modalities. The pipeline will be able to acquire at least 50.000 3D time-lapse movies per year that will yield up to 1PB of data. To manage and process the data we plan using OMERO and CellProfiler.

We are committed to open science. We will not only share all of our data, tools, and other results with the scientific community but also will have an ongoing dialog on tools and questions to address. We depend on your input and feedback to build a pipeline that best complements the achievements and needs of the scientific community.

## Optical sectioning with light sheets forms the basis for highly corrected and fully automated microscopes

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In light sheet-based fluorescence microscopy (LSFM), optical sectioning in the excitation process minimizes fluorophore bleaching and photo-toxic effects<sup>1</sup>. Since biological specimens survive long-term three-dimensional imaging at high spatio-temporal resolution, light sheet-based microscopes (LSM) have become an indispensable tool in developmental<sup>2</sup>, three-dimensional cell<sup>3</sup> and plant biology<sup>4,5</sup>. LSFM is based on two main optical paths<sup>6</sup>. The detection path consists of a microscope objective, a spectral filter, a tube lens and a camera. The excitation path is perpendicular to the detection path and directs a light sheet into the side of the specimen<sup>7,8</sup>. The thin light sheet and the focal plane of the detection objective overlap. LSFM provides at least three important degrees of freedom, which are usually not available in an epifluorescence microscope: 1) the axial and 2) lateral locations of the light sheet and 3) the axial location of the focal plane. Further probably less important degrees of freedom are the tilt and the incline of the light sheet. LSFM takes full advantage of modern cameras, massively parallelizing the data acquisition process<sup>9</sup> and recording ten to one hundred images per second with a high dynamic range. LSFM does not rely on traditional features required for ergonomic reasons. A powerful multiple-sensors-based image processing pipeline is, therefore, an inherent feature. Traditional fluorescence microscopy enforces specimen preparation schemes that rely on hard and flat surfaces. LSFM places the specimen in the center and the optics in its perimeter. Completely new schemes for specimen preparation become available. Three-dimensional integrity is maintained and specimens can be used in experiments hitherto regarded impossible. Fluorescence microscopy has several basic limitations. Firstly, excitation light is absorbed by fluorophores and by endogenous organic compounds. The latter are degraded much like fluorophores and thus lost for vital metabolic processes. Secondly, the number of fluorophores in any volume element at any given time is finite, and fluorophores degrade statistically upon excitation. In consequence, the number of retrievable photons from a fluorophore-labeled specimen is limited. Finally, life on Earth is adapted to the solar flux, which is less than 1.4 kW/m<sup>2</sup>. This might not be a hard limit, but it indicates that irradiance should not exceed a few nW/μm<sup>2</sup> or a few hundred mW/cm<sup>2</sup> when dynamic biological processes are observed. These challenges cannot be ignored with living biological samples. LSFM is the best technology we have so far, which makes a sincere and honest effort to address these challenges: 1) it provides optical sectioning, 2) a true axial resolution, 3) reduces fluorophore bleaching and 4) photo toxicity at almost any scale, 5) allows one to record millions of pixels in parallel and 6) dramatically improves the viability of the specimen.

[www.researcherid.com/rid/A-7648-2011](http://www.researcherid.com/rid/A-7648-2011) or [scholar.google.com/citations?user=EV5RvqkAAAAJ](http://scholar.google.com/citations?user=EV5RvqkAAAAJ)

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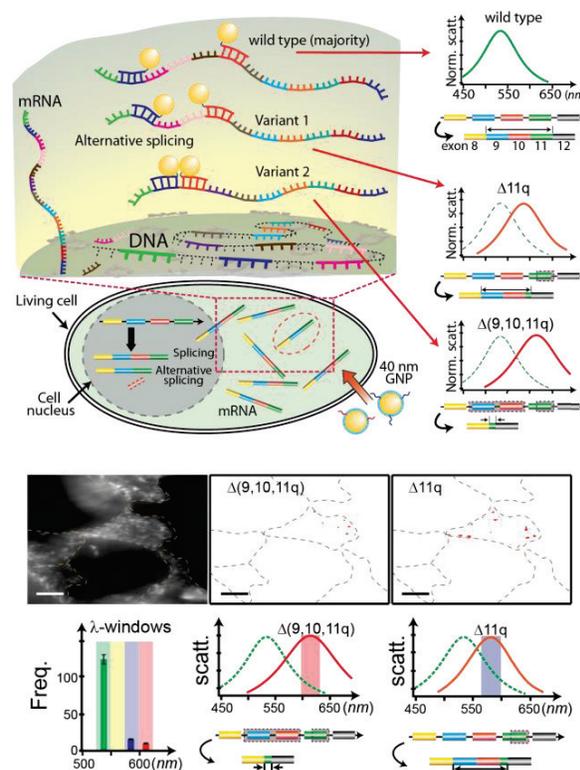
## QUANTITATIVE HYPERSPECTRAL IMAGING OF PLASMON-LABELED SINGLE MOLECULE ISOFORMS IN LIVE CELLS

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**KEY WORDS:** surface plasmon coupling, hyperspectral imaging, single molecule detection

A single molecule is a fundamental unit of the molecular processes in biology, and structural isoforms of molecules are playing crucial roles in a variety of diseases. However, there is currently a lack of scientific methods for imaging structural isoforms of single molecules in live cells. In this poster, I will present that the hyperspectral imaging of plasmonic coupling probes can be used to detect and quantify single mRNA isoforms in different conditions including live cells. The probes are made from plasmonic nanoantennae functionalized with molecular probes targeting specific structural characteristics of a single mRNA, forming nanoantenna couplings that exhibit distinct spectral peaks and shifts due to the plasmonic interaction. With this approach, we demonstrated a spectral imaging technique that the spatial and temporal distribution of multiplexing isoforms of mRNA is monitored at single-copy resolution. This study provides insights into single molecules and its isoforms as well as its transport in living cells, which could improve our understanding of more advanced molecular complexes in pharmacogenomics, molecular diagnosis, and therapies[1].



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