Frontiers in Live Cell Imaging

April 19-21, 2006
Natcher Conference Center, NIH
Bethesda, MD

Sponsors

Cell Migration Consortium
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National Institute of General Medical Sciences
www.nigms.nih.gov

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Frontiers in Live Cell Imaging

April 19-21, 2006
Natcher Conference Center, NIH
Bethesda, Maryland

Organizers
Yu-li Wang (University of Massachusetts)
Rick Horwitz (University of Virginia)

Planning Committee
Yu-li Wang (University of Massachusetts)
Rick Horwitz (University of Virginia)
Jim Deatherage (National Institute of General Medical Sciences)
Cathy Lewis (National Institute of General Medical Sciences)
Richard Rodewald (National Institute of General Medical Sciences)

Arrangements
Nikki Watson (University of Virginia)
Melinda Gray (Capital Consulting Corporation)
Eileen Young (University of Virginia)
Hal Noakes (University of Virginia)

NIH Events Coordinator
Kathy Bass (Office of the Director, NIH)

Contractor
Melinda Gray
Capital Consulting Corporation
11821 Parklawn Drive, Suite 100
Rockville, MD 20852

Cover Picture
C.L. Rieder (Wadsworth Center)

Contents

The following companies have provided generous sponsorship and are displaying their products during the conference:

Meeting Room C1
Olympus America, Inc.
Evident Technologies
Hamamatsu Photonic Systems

Meeting Room C2
Carl Zeiss MicroImaging Inc.
Andor Technology
Semrock, Inc.

Meeting Room G1
Leica Microsystems, Inc.
Omega Optical
Compix Inc.

Meeting Room G2
Nikon Instruments, Inc.
Invitrogen Corporation
Prior Scientific Inc.

Welcome to Conference Participants

Welcome to Conference Participants

Over the past three decades, advances in the microscopy of living cells have dramatically transformed the biological sciences. Critical developments include fluorescence, confocal and two-photon optics, green fluorescent protein, photoelectronic detectors, and image deconvolution. The future promises exciting new capabilities, such as visualizing single molecules, monitoring and manipulating molecular interactions, mining information in images, and seeing into complex tissues.

Progress in live cell imaging has been driven largely by a convergence of cell manipulation, probe chemistry, solid-state physics, electronics, optics, and image analysis. These advances often took place independently, at least initially, without the benefit of direct interaction and cross-stimulation among investigators in different fields. While the achievement has been impressive, the pace of development may be accelerated by promoting cross-disciplinary interactions and collaborations. To achieve this goal, we have brought together leading scientists from diverse fields relevant to live cell imaging, with the goals of reviewing recent progress, charting new directions, and promoting interactions. To emphasize the forward-looking nature of this meeting, we have balanced presentations on bona fide live cell imaging with those likely to have an impact on it. In addition, we encourage all participants to address future development in their presentations and discussions. Since imaging represents one of the major thrusts in the NIH Roadmap, the meeting will end with a discussion of recent and forthcoming NIH initiatives that seek to promote imaging technology development and interdisciplinary research.

We welcome the participation of 12 companies and the display of their most recent products. Their generous donations have facilitated the participation of trainees and young investigators. We thank Jim Deatherage, Cathy Lewis, and Richard Rodewald of the National Institute of General Medical Sciences for their help in organizing this conference. Finally, we thank Dr. Nikki Watson and Ms. Melinda Gray, who have managed all logistical issues through their highly efficient and tireless efforts.

Yu-li Wang (University of Massachusetts) and Rick Horwitz (University of Virginia), Chairs

General Information

General Information

Airport Information

Baltimore/Washington International Thurgood Marshall Airport: 410-859-7111 or www.bwiairport.com
Washington Dulles International Airport: 703-572-6240 or www.metwashairports.com/dulles/
Reagan National Airport: 703-417-3500 or www.metwashairports.com/national/

Ground Transportation

Local Taxi Companies:
- Barwood Cab: 301-984-1900
- Regency Cab: 301-990-9000
- Montgomery Taxi Cab: 301-762-2001

Metrorail (see "Maps," p. 5)
NIH Shuttle

Conference and Visitor Badges

At all times, visitors to the NIH Campus are required to wear a Visitor Badge issued by NIH security personnel. Registrants are also requested to wear their conference badges to all conference events.

Internet Access and Business Center

The Natcher Conference Center does not have an official business center. Wireless Internet access is not available to non-NIH personnel due to security reasons. If you have fax or copy needs, please ask for assistance at the conference registration desk located in the lower level of the atrium.

Conference Meals

A continental breakfast, box lunch, and an evening buffet dinner will be provided at the meeting. Please refer to the Program Schedule (p. 7) for dates and times. Please be aware that food and beverages are not permitted in Natcher Auditorium and meeting rooms.

A conference dinner will be held on the evening of April 20 at the Hyatt Regency Bethesda. This is a separate ticketed event. Onsite registration for this event is not available.

The Natcher Conference Center cafeteria is located on the main level of the building and is open to the public. A variety of refreshments are available for purchase from 7:00 a.m. to 2:00 p.m.

Luggage Storage

Unfortunately, the Natcher Conference Center does not have storage facilities for luggage and personal belongings; however, our conference group does have access to a small storage room. You are welcome to use this room for your luggage if needed. Please keep in mind that this room will not be continuously staffed. The Natcher Conference Center is a very safe and secure location, but we cannot be responsible for any items left in this storage room. Please check with staff at registration for use of this storage room.

Telephones and Restrooms

Telephones are available at each end of the Natcher Conference Center on the lower level. Restrooms are located on each end of the Natcher Conference Center on both the upper and lower levels.

Poster Sessions

Poster sessions will be held on Wednesday, April 19, and Thursday, April 20, in the atrium on the main level of the Natcher Conference Center. Sessions are scheduled to take place from 1:30 p.m. to 3:00 p.m. Presenters will be attending their posters at these times to discuss their research and answer any questions.

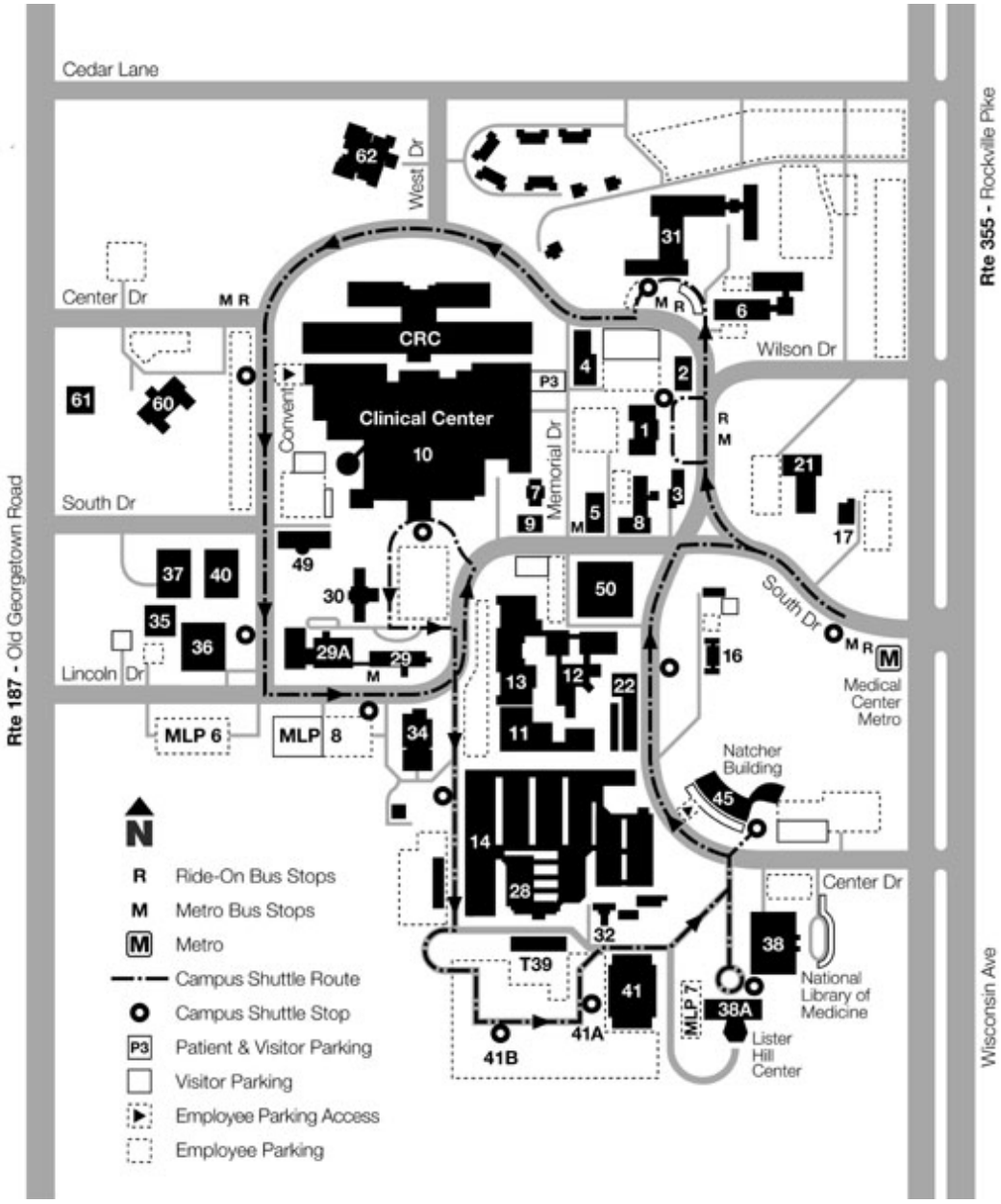
Posters have been organized by the topics listed below. A complete author index with topic and poster board assignment appears on pp. 79-81 in this program book.

I. Probes
II. Optics and Devices
III. Image Processing and Informatics
IV. Imaging Single Molecules
V. Imaging Cells
VI. Imaging Tissues

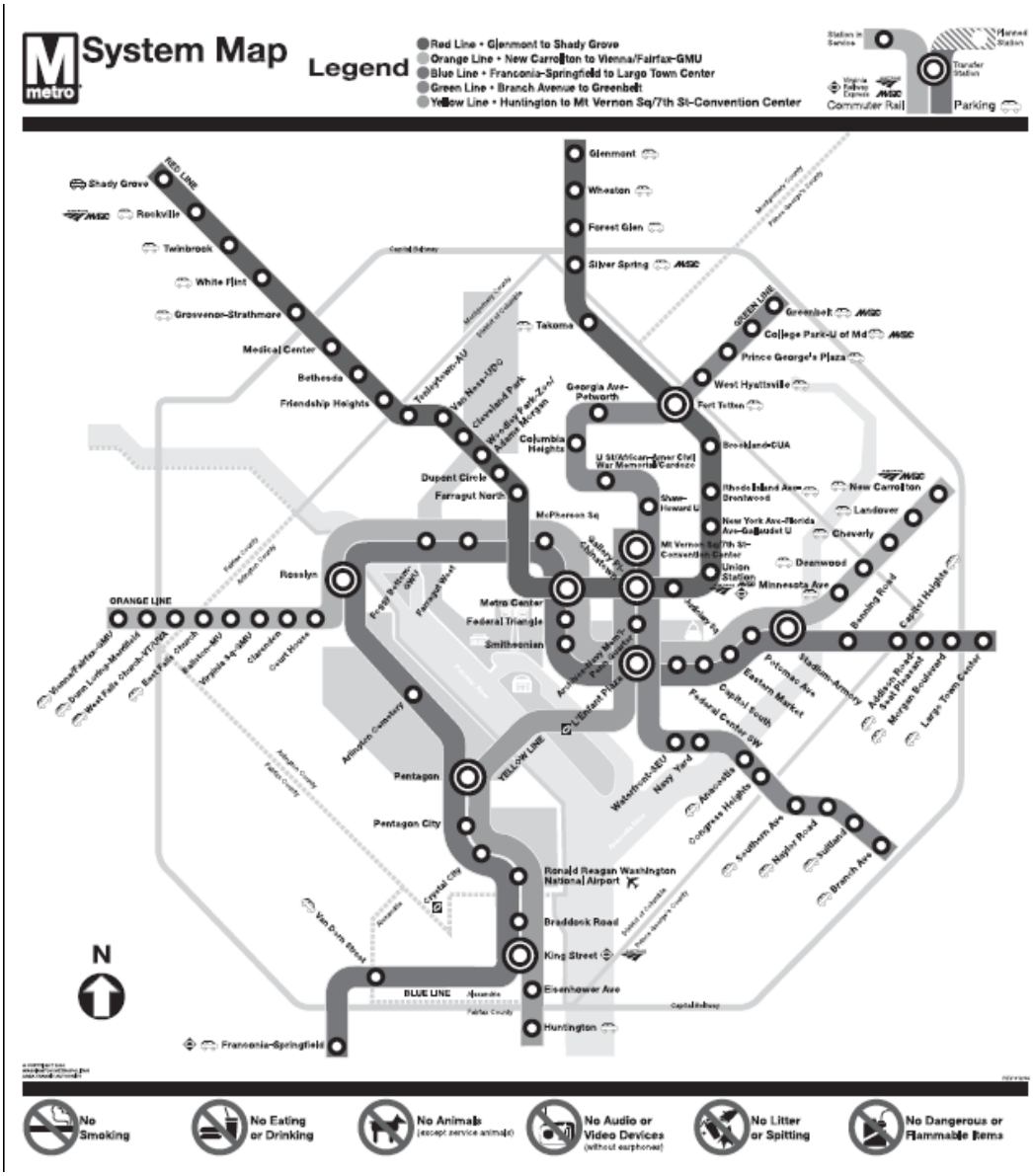
Maps

Maps

NIH Campus



Metro


System Map
Legend
Red Line • Glenmont to Shady Grove
Orange Line • New Carrollton to Vienna/Fairfax-GMU
Blue Line • Franconia-Springfield to Largo Town Center
Green Line • Branch Avenue to Greenbelt
Yellow Line • Huntington to Mt Vernon Sq/7th St-Convention Center
Commuter Rail
Parking
RED LINE
Shady Grove
Rockville
Twinbrook
White Flint
Grosvenor-Strathmore
Medical Center
Bethesda
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Cleveland Park
Woodley Park-Zoo/Adams Morgan
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McPherson Sq
Metro Center
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Wheaton
Forest Glen
Silver Spring
Takoma
GREEN LINE
Greenbelt
College Park-U of Md
Prince George's Plaza
West Hyattsville
Georgia Ave-Petworth
Fort Totten
Columbia Heights
U St/African-Amer Civil War Memorial/Cardozo
Shaw-Howard U
Mt Vernon Sq/7th St-Convention Center
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Archives-Navy Mem'l-Penn Quarter
Union Station
BrookLand-CUA
Rhode Island Ave-Brentwood
New York Ave-Florida Ave-Gallaudet U
NEW CARROLLTON
New Carrollton
Landover
Cheverly
Deanwood
Minnesota Ave
Benning Road
Capitol Heights
Addison Road-Seat Pleasant
Morgan Boulevard
Largo Town Center
Stadium-Armory
Potomac Ave
Eastern Market
Capitol South
Federal Center SW
L'Enfant Plaza
Rosslyn
Foggy Bottom-GWU
Farragut West
ORANGE LINE
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Dunn Loring-Merrifield
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East Falls Church
Ballston-MU
Virginia Sq-GMU
Clarendon
Court House
YELLOW LINE
Arlington Cemetery
Pentagon
Pentagon City
Waterfront-SEU
Navy Yard
Anacostia
Congress Heights
Southern Ave
Naylor Road
Suitland
Branch Ave
Ronald Reagan Washington National Airport
Crystal City
Braddock Road
King Street
Eisenhower Ave
Huntington
BLUE LINE
Van Dorn Street
Franconia-Springfield
N
No Smoking
No Eating or Drinking
No Animals
No Audio or Video Devices
(without earphones)
No Litter or Spitting
No Dangerous or Flammable Items

Program Schedule

Program Schedule

Wednesday, April 19, 2006, Natcher Conference Center

7:00 a.m. - 8:30 a.m. **Registration and Continental Breakfast**

8:30 a.m. - 11:30 a.m. **Session 1. Imaging Single Molecules I**
Session Chair: W.E. Moerner

Tackjip Ha
Next Generation Single Molecule Fluorescence Technologies

Petra Schwille
Proteomics on a Single Molecule Scale – Facts and Visions

Nancy L. Thompson
Submicroscopic Cluster Sizes by Quantum Dot Blinking & Membrane Dynamics by Total Internal Reflection with Fluorescence Correlation Spectroscopy

Coffee Break

Session 2. Imaging Single Molecules II
Session Chair: W.E. Moerner

Xiaowei Zhuang
Investigating Viral Entry Mechanisms by Live Cell Imaging and Single-Virus Tracking

Paul R. Selvin
Single Molecule Studies of Molecular Motors, in Vivo

W.E. Moerner
Single-Molecule Emitters as Reporters of Dynamics and Function in Living Cells

12 noon - 1:30 p.m. **Box Lunch Provided in the Atrium (Upper and Lower Level)**

1:30 p.m. - 3:00 p.m. **Poster Session**

3:00 p.m. - 6:00 p.m. **Session 3. Molecular Dynamics in Single Cells I**
Session Chair: Jennifer Lippincott-Schwartz

Rebecca Heald
Analysis of Ran-GTP Regulated Mitotic Gradients in Xenopus Egg Extracts and Somatic Cells

Edward D. Salmon
Imaging Protein Number and Dynamics at the Kinetochore-Microtubule Interface

Claire M. Waterman-Storer
Analyzing the Integration of Actin Dynamics with Adhesion in Cell Migration Using Correlational qFSM

Coffee Break

Session 4. Molecular Dynamics in Single Cells II
Session Chair: Jennifer Lippincott-Schwartz

Sanford M. Simon
The Dynamics of Molecules during Exo and Endocytosis

Akihiro Kusumi
Single-Molecule Imaging of Signal Transduction of GPI-Anchored Receptor

Jennifer Lippincott-Schwartz
Deciphering Protein Turnover, Topology, and Transport in Living Cells

6:00 p.m. - 7:30 p.m. **Buffet Dinner provided in the Atrium (Upper and Lower Levels)**

7:30 p.m. - 9:00 p.m. **Session 5. Extracting Information from Images**
Session Chair: Robert F. Murphy

Gaudenz Danuser
Computer Vision of Cytoskeleton Regulation

Roland Eils
Automated Large-Scale Classification of Cellular Phenotypes in High-Content 4D-Microscopy Screens

Robert F. Murphy
Location Proteomics: Automated Image Understanding for Systems Biology

Thursday, April 20, 2006, Natcher Conference Center

7:30 a.m. - 8:30 a.m. **Continental Breakfast**

8:30 a.m. - 11:00 a.m. **Session 6. Probes and Sensors I**
Session Chair: Klaus M. Hahn

Robert M. Dickson
Novel Raman-Active and Highly Fluorescent Noble Metal Quantum Dots

Alice Y. Ting
New Methodologies for Optical Imaging of Living Cells

Atsushi Miyawaki
Visualization of the Spatial and Temporal Dynamics of Intracellular Signaling

Coffee Break

Session 7. Probes and Sensors II
Session Chair: Klaus M. Hahn

Gerald Marriott
Molecular Optical Switches: Design, Characterization, and Applications in Biophysics and Cell Biology

Klaus M. Hahn
New Windows on Living Cells – Facile Generation of Biosensors to Visualize Endogenous Protein Conformations

11:10 a.m. - 11:50 a.m. **Keynote Lecture 1**
Roger Y. Tsien
The Future of Live Cell Imaging

12 noon - 1:30 p.m. **Box Lunch Provided in the Atrium (Upper and Lower Level)**

1:30 p.m. - 3:00 p.m. **Poster Session**

3:00 p.m. - 5:30 p.m. **Session 8. Structural and Cellular Dynamics in Tissues I**
Session Chair: Scott E. Fraser

Ernst H.K. Stelzer
SPIMaging Technology: Leaving Flatland Behind

Peter Friedl
Dynamic Imaging of Tumor Cell Invasion and Remodeling of Extracellular Matrix

John S. Condeelis
How Imaging Revealed the Molecular Mechanism of Tumor Cell Chemotaxis During Metastasis

Coffee Break

Session 9. Structural and Cellular Dynamics in Tissues II
Session Chair: Scott E. Fraser

R. Clay Reid
Micro-Architecture of Visual Cortex: Two-Photon Imaging of Functional Maps with Single-Cell Precision

Scott E. Fraser
To be announced

7:00 p.m. - 9:30 p.m. **Conference Dinner and Keynote Lecture 2**
Hyatt Regency Bethesda - One Bethesda Metro Center (corner of Old Georgetown and Wisconsin)

Dinner Speaker: Roberto Ragazzoni
Astronomical Optics and Light Microscopy: Common Challenges and Diverse Opportunities

Friday, April 21, 2006, Natcher Conference Center

7:30 a.m. - 8:30 a.m. **Continental Breakfast**

8:30 a.m. - 11:30 a.m. **Session 10. New Directions in Optical Imaging I**
Session Chair: John W. Sedat

Enrico Gratton
To be announced

X. Sunney Xie
New Advances in Optical Imaging of Living Organisms

Tony Wilson
Active and Adaptive Optics in Microscopy

Coffee Break

Session 11. New Directions in Optical Imaging II
Session Chair: John W. Sedat

Eric Betzig
New Approaches to Cellular Imaging with High Spatial and Temporal Resolution

Carolyn Larabell
X-ray Tomography: 3-D Imaging of Whole Cells at Better Than 50 nm Resolution

John W. Sedat
OMX, a Microscope Platform for the Future?

12 noon - 1:00 p.m. **Lunch Break (Cafeteria)**

1:00 p.m. - 3:30 p.m. **NIH Funding Opportunities**
NIH Staff

Discussion of NIH Funding Opportunities. NIH staff members from CSR, NCI, NCRR, NIBIB, NIDDK, and NIGMS will discuss and answer questions from participants about agency programs, grant mechanisms, new initiatives, NIH Roadmap imaging initiatives, and advanced application strategies for imaging research and technology development. Following staff presentations, there will be a staff poster session.

Conference Speaker Biographies and Presentation Synopses

Conference Speaker Biographies and Presentation Synopses

Name Ha, Taekjip tjha@uiuc.edu

Position Associate Professor of Physics, University of Illinois at Urbana-Champaign (UICU), and Investigator, Howard Hughes Medical Institute

Presentation Title Next Generation Single Molecule Fluorescence Technologies

Presentation Summary

A nearly universal feature of single-molecule fluorescence studies of biological molecules is the revelation of conformational fluctuations. Almost as universal is our inability to decipher what these fluctuations mean, physically and biologically. We aim to remedy this situation by probing the functional effects of mechanical constraints that select only a subset of these conformations. Two different approaches will be discussed:

1. Combined single-molecule FRET and optical trap

   Why is such a hybrid instrument needed? Single-molecule fluorescence measurements can step-by-step piece together the motion of biomolecules. Yet, biomolecules in a cell perform their functions under a variety of environmental conditions, such as force and load for molecular motors. To understand their behavior, we must emulate these environmental variables. Techniques such as optical trapping apply these forces and are now well established. Yet, by themselves, they do not have the selectivity to isolate which parts of the biomolecule are undergoing shape changes responsible for the molecule's behavior. By combining fluorescence with manipulation, we make this connection. The latest results on mapping the conformational energy landscape of the Holliday junction through simultaneous fluorescence and force measurements will be presented.

2. Nucleic acids-based control of biomolecular conformations

   The goal is to use a biological motif to control another biological motif in an unnatural way. A new nanomechanical device called a single-molecule nanometronome will be discussed with an outlook toward controlling protein conformations using nucleic acids motifs.

Home Page http://bio.physics.uiuc.edu/

Education

1996, Ph.D., Physics, University of California at Berkeley
1991, M.A., Physics, University of California at Berkeley
1990, B.S., Physics, Seoul National University, Korea

Selected Activities & Honors

2002 - Fluorescence Young Investigator Award
2003 - Alfred P. Sloan Fellowship
2003 - Beckman Fellow at Center for Advanced Studies, UIUC
2003 - Xerox Faculty Research Award, UIUC
2003 - Cottrell Scholars Award
2005 - Howard Hughes Medical Institute investigator
2005 - Fellow, American Physical Society

Research Interests

Physical concepts and experimental techniques to study fundamental questions in molecular biology
Single-molecule fluorescence spectroscopy and microscopy
Nanomechanical tools such as magnetic and optical tweezers

Biological systems under study include:

1. DNA recombination intermediate called Holliday junction and its associated enzymes
2. Helicases that unzip DNA
3. Chromatin remodeling complexes
4. Folding and catalysis of ribozymes
5. DNA replication machinery
6. Chromatin remodeling complexes
7. Membrane-binding proteins

Key Publications Relevant to Imaging

S. Myong, I. Rasnik, C. Joo, T.M. Lohman and T. Ha, "Repetitive shuttling of a motor protein on DNA," *Nature* 437, 1321-1325 (2005)

J. Liu, A.C. Déclais, S.A. McKinney, T. Ha, D.G. Norman and D.M.J. Lilley, "Stereospecific effects determine the structure of a four-way DNA junction," *Chemistry & Biology* 12, 217-288 (2005)

S. Hohng and T. Ha, "Single-molecule quantum dot FRET," *Chem Phys Chem* 6, 956-960 (2005)

J.Y. Lee, B. Okumus, D.S. Kim and T. Ha, "Extreme conformational diversity in human telomeric DNA," *Proc Natl Acad Sci USA* 102, 18938-18943 (2005)

S.A. McKinney, A. Freeman, D.M.J. Lilley and T. Ha, "Observing spontaneous branch migration of Holliday junctions one step at a time," *Proc Natl Acad Sci* USA 102, 5715-5720 (2005)

M.K. Nahas, T.J. Wilson, S. Hohng, K.Jarvie, D.M.J. Lilley and T. Ha, "Observation of internal cleavage and ligation reactions of a ribozyme," *Nature Structural & Molecular Biology* 11, 1107-1113 (2004)

Name Schwille, Petra schwille@biotec.tu-dresden.de

Position Professor, Biophysics/BIOTEC, Technische Universität (TU) Dresden

Presentation Title Proteomics on a Single Molecule Scale – Facts and Visions

Presentation Summary

After years of exciting development of novel spectroscopic and microscopic techniques to investigate individual biomolecules, the first successful attempts have been made to enter the field of cell biology and study these functional elements in their native environment. Of particular importance are methods such as single-molecule imaging that allow the tracking of large-scale translocations and confocal spectroscopy such as fluorescence correlation spectroscopy (FCS) that illuminates only a diffraction-limited confined spot and focuses on fast time-scale fluctuations for analysis of molecular dynamics. In conjunction with two-photon excitation and spectrally resolved detection, FCS is a particularly powerful means for the study of molecular association or enzymatic turnovers. It is fair to state that this technique raises strong hopes for the possibility of quantitative in situ proteomics. In past years, we applied FCS to a variety of cell-associated phenomena, among them protein-protein binding, enzymatic reactions, endocytosis, and gene delivery. To study processes on cell membranes and to elucidate the delicate interplay between membrane proteins and the surrounding lipids, we devised cell-like model membrane systems mimicking the formation of membrane domains whose cellular counterparts are potentially active as recruitment platforms for signaling proteins.

Home Page http://www.biotec.tu-dresden.de/schwille/group/homeindex.html

Education

- 1987-1993, study of physics and philosophy, Universities of Stuttgart and Göttingen, Germany
- 1993-1996, doctoral thesis, Max-Planck-Institute for Biophysical Chemistry, Göttingen
- 1996, Ph.D. in Physics, MPI Göttingen and TU Braunschweig
- 1996-1997, Postdoctoral Research, MPI for Biophysical Chemistry, Göttingen
- 1997-1999, Postdoctoral Research, Cornell University, Ithaca, NY, USA
- 1999-2002, Junior Group Leader, MPI for Biophysical Chemistry, Göttingen

Selected Activities & Honors

1997-1999 - Feodor-Lynen-Fellowship of the Alexander von Humboldt Foundation for Postdoctoral Research, Cornell University, Ithica, NY, USA
August 1998 - "Biofuture" Prize German Ministry of Education and Science
August 2001 - Lecturer award by the German Chemical Industry Fund
January 2003 - Young Investigator Award for Biotechnology of the Peter und Traudl Engelhorn Foundation
January 2004 - Philip Morris Research Prize
July 2005 - Elected Max Planck Fellow

Research Interests

Single-molecule biophysics, cellular biophysics, membrane organization and dynamics, artificial cell systems, microfluidics

Key Publications Relevant to Imaging

Kim S.A., Heinze K.G., Bacia K., Waxham M.N., Schwille P. (2005) Two-photon cross-correlation analysis of intracellular reactions with variable stoichiometry, *Biophys J* 2005 88(6), 4319-4336

Bacia K., Schwille P., and Kurzchalia T. (2005) Sterol structure determines the separation of phases and the curvature of the liquid-ordered phase in model membranes, *Proc Natl Acad Sci USA* 102(9):3272-3277

Haustein E. and Schwille P. (2004) Single-molecule spectroscopic methods, *Curr Op Struct Biol* 14, 531-540

Bacia K., Schütte C.G., Kahya N., Jahn R., and Schwille P. (2004) SNAREs prefer liquid-disordered over "raft"-(liquid-ordered) domains when reconstituted into Giant Unilamellar Vesicles, *J Biol Chem* 279(36):37951-37955

Kim S.A., Heinze K.G., Waxham M.N., and Schwille P. (2004) Intracellular calmodulin availability accessed with two-photon cross-correlation, *Proc Natl Acad Sci USA* 101:105-110

Name Thompson, Nancy L. nlt@unc.edu

Position Professor of Chemistry, University of North Carolina at Chapel Hill

Presentation Title Submicroscopic Cluster Sizes by Quantum Dot Blinking & Membrane Dynamics by Total Internal Reflection with Fluorescence Correlation Spectroscopy

Presentation Summary The use of quantum dot blinking to measure submicroscopic distances will be introduced. The development of total internal reflection with fluorescence correlation spectroscopy to characterize membrane dynamics, including measurement of protein diffusion very close to supported membranes and measurement of ligand-receptor interaction kinetics, will also be described.

Home Page http://www.chem.unc.edu/people/faculty/thompsonnl/nltindex.html

Education B.S., Physics and Mathematics, Guilford College, 1977
Ph.D., Physics, University of Michigan at Ann Arbor, 1982
Damon Runyon-Walter Winchell Postdoctoral Fellow, Chemistry, Stanford University, 1985

Selected Activities & Honors Honors: Fellow of the American Physical Society, 2006; Francis Stuart Chapin Professor, 1993-1996; Hettleman Prize, University of North Carolina, 1991; NSF Faculty Award for Women in Science, 1991; Alumni Excellence Award, Guilford College, 1991; Dreyfus Teacher-Scholar Award, 1990; Margaret Oakley Dayhoff Award, Biophysical Society, 1989; NSF Presidential Young Investigator Award 1986

Editorial Boards: *Biophysical Journal*, 4/92-10/98; *Journal of Fluorescence*, 5/92-5/98, 8/03-present; *Comments on Molecular and Cellular Biophysics*, 7/90-3/94

Grant Review Panels: NSF Molecular Biophysics Panel, 5/97-10/02; NIH Biomedical Research Training Study Section, 4/96-3/00; NIH Biomedical Research Technology Study Section, 7/87-6/91

Research Interests Fluorescence microscopy and spectroscopy, membrane dynamics

Key Publications Relevant to Imaging

Lagerholm BC, Weinreb GE, Jacobson K, Thompson NL (2006) Spatial mapping of quantum dots in submicroscopic clusters, submitted

Pero JK, Haas EM, Thompson NL (2006) Size dependence of protein diffusion very close to membrane surfaces: Measurement by total internal reflection with fluorescence correlation spectroscopy, submitted

Thompson NL, Pero JK (2006) Total internal reflection – fluorescence correlation spectroscopy, in Reviews in Fluorescence (CD Geddes and JR Lakowicz, editors), Kluwer Academic/Plenum Publishers, in press

Lagerholm BC, Weinreb GE, Jacobson K, Thompson NL (2005) Detecting microdomains in intact cell membranes. *Annual Review of Physical Chemistry* 56: 309-336

Lieto AM, Thompson NL (2004) Total internal reflection with fluorescence correlation spectroscopy: Nonfluorescent competitors. *Biophysical Journal* 87: 1268-1278

Lieto AM, Cush RR, Thompson NL (2003) Ligand-receptor kinetics measured by total internal reflection with fluorescence correlation spectroscopy. *Biophysical Journal* 85: 3294-3302

Starr TE, Thompson NL (2002) Local diffusion and concentration of IgG near planar membranes: Measurement by total internal reflection with fluorescence correlation spectroscopy, *Journal of Physical Chemistry B* 106, 2365-2371

Starr TE, Thompson NL (2001) Total internal reflection with fluorescence correlation spectroscopy: Combined surface reaction and solution diffusion. *Biophysical Journal* 80: 1575-1584

Starr TE, Thompson NL (2000) Formation and characterization of planar phospholipid bilayers supported on TiO_2 and $SrTiO_3$ single crystals. *Langmuir* 16: 10301-1030

Name Zhuang, Xiaowei zhuang@chemistry.harvard.edu

Position Professor of Chemistry and Chemical Biology, Professor of Physics, Harvard University

Investigator, Howard Hughes Medical Institute

Presentation Title Investigating Viral Entry Mechanisms by Live Cell Imaging and Single-Virus Tracking

Presentation Summary Viruses must deliver their genome into cells to initiate infection. As a critical step in the infection process, viral entry is a subject of fundamental importance in cell biology, as well as a therapeutic target for viral disease treatment. However, understanding the molecular mechanisms of viral entry is challenging due to the existence of multiple entry pathways and multiple steps on each pathway, as well as the complication that a large fraction of entry events are futile. Real-time, live-cell imaging offers us the opportunity to track the behavior of individual virus particles and to determine the entry pathway(s) leading to infection. By imaging the interactions between virus particles and cellular structures in real time, we can produce viral entry "movies" that allow the entry pathways to be dissected into microscopic steps and the molecular mechanism of each step to be determined. Combining this approach with conventional virology assays, we have elucidated the entry mechanism of several different types of animal viruses. Two or three examples will be presented in this talk.

Home Page http://zhuang.harvard.edu

Education
1987-1991, B.S., Physics, University of Science and Technology of China
1991-1996, Ph.D., Physics, University of California, Berkeley
1997-2001, Chodorow Postdoctoral Fellow, Biophysics, Stanford University

Selected Activities & Honors
2006 - Vice Chair, Gordon Research Conference on "Single Molecule Approaches to Biology"
2005 - Camille Dreyfus Teacher-Scholar Award
2004 - Alfred P. Sloan Research Fellowship
2004 - MIT Technology Review Worlds' Top 100 Young Innovators Award
2003 - MacArthur Fellowship
2003 - Packard Fellowship for Science and Engineering
2003 - CAREER Award, National Science Foundation
2003 - Searle Scholarship
2003 - Beckman Young Investigator Award
2002 - Young Investigator Award, Office of Naval Research
2003-2006 - Ad Hoc NIH study section member

Research Interests Single-molecule biophysics, live cell imaging, nucleic acid-protein interactions, virus infection, cellular trafficking

Key Publications Relevant to Imaging

M. Lakadamyali, M.J. Rust, X. Zhuang, "Ligands for clathrin-mediated endocytosis are differentially sorted into distinct populations of early endosomes," *Cell* 124, March 10 (2006)

M. Bates, T.R. Blosser, X. Zhuang, "Short-range spectroscopic ruler based on a single-molecule optical switch," *Phys. Rev. Lett.* 94, 101801 (2005)

M.J. Rust, M. Lakadamyali, F. Zhang, X. Zhuang, "Assembly of endocytic machinery around individual influenza viruses during viral entry," *Nature Struct. Mol. Biol.* 11, 567-573 (2004)

H.P. Babcock, C. Chen, X. Zhuang, "Using single-particle tracking to study nuclear trafficking of viral genes," *Biophys. J.* 87, 2749-2758 (2004)

D. Rueda, G. Bokinsky, M.M. Rhodes, M.J. Rust, X. Zhuang, N.G. Walter, "Single-molecule enzymology of RNA: Essential functional groups impact catalysis from a distance," *Proc. Natl. Acad. Sci. USA* 101, 10066-10071 (2004)

M. Lakadamyali, M.J. Rust, H.P. Babcock, X. Zhuang, "Visualizing infection of individual influenza viruses," *Proc. Natl. Acad. Sci. USA* 100, 9280-9285 (2003)

G. Bokinsky, D. Rueda, V.K. Misra, A. Gordus, M.M. Rhodes, H.P. Babcock, N.G. Walter, X. Zhuang, "Single-molecule transition-state analysis of RNA folding," *Proc. Natl. Acad. Sci. USA* 100, 9302-9307 (2003)

X. Zhuang, H. Kim, M. Pereira, H. Babcock, N. Walter, S. Chu, "Correlating structural dynamics and function in single ribozyme molecules," *Science* 296, 1473-1476 (2002)

X. Zhuang, L. Bartley, H. Babcock, R. Russell, T. Ha, D. Herschlag, S. Chu, "A single-molecule study of RNA catalysis and folding," *Science* 288, 2048-2051 (2000)

Name Selvin, Paul R. selvin@uiuc.e

Position Professor of Physics and Biophysics, University of Illinois at Urbana-Champaign

Presentation Title Single Molecule Studies of Molecular Motors, in Vivo

Presentation Summary We have been able to measure 1.5 nm in 1-500 msec, using fluorescence imaging. We have seen single molecular motors moving both in purified systems and inside living cells. In living (*Drosophilia*) cells, we have seen individual cargoes being moved by individual "conventional" kinesin and dynein. We find that both kinesin and dynein move cargo 8 nm per ATP. Amazingly, these two molecular motors do not engage in a tug-of-war but appear to be cooperative, giving the particle extra speed. We also show that myosin VI, which moves in opposite direction to all other myosins, actually walks in a hand-over-hand fashion, despite recent controversy. Finally, we have also achieved the inverse of this technique on melanophores in frog eggs. These cells are nonfluorescent but contain melanin, an opaque material. Here we use bright-field microscopy and achieve < 2 nm and < 2 msec resolution. We show that a heterotrimeric kinesin, and kinesin II, displays 8 nm steps, and myosin V moves 36 nm, all in vivo. Furthermore, they move farther total distances when intermediate filaments are removed. And for the first time, we have been able to see a cargo being passed between myosin V and kinesin/dynein.

Home Page http://www.physics.uiuc.edu/People/Faculty/Selvin/

Education 1983, B.S., Physics, University of Michigan
1990, Ph.D., Physics, University of California, Berkeley
1990-1993, Postdoctoral Research in Chemistry, University of California, Berkeley

Selected Activities & Honors 2004, Fellow, American Physical Society
2004-2007, John Bardeen Faculty Scholar
2004, Michael & Kate Bárány Award for Young Investigators, Biophysical Society
2000, Cottrell Scholar
2000, Xerox Scholar, UIUC

Research Interests Molecular motors, ion channels, DNA haplotyping

Key Publications Relevant to Imaging

Hyokeun Park, Bugs Ramamurthy, Dan Safer, Mirko Travaglia, Li-Qiong Chen, Clara Franzini-Armstrong, Paul R. Selvin & H. Lee Sweeney. Full-length myosin VI dimerizes and moves processively along actin filaments upon monomer clustering, *Molecular Cell*, 21, 331–336 (2006).

Comert Kural, Hwajin Kim, Gohta Goshima, Vladimir I. Gelfand, Paul R. Selvin. Kinesin & Dynein Move a Peroxisome In Vivo: A Tug-of-War or Coordinated Movement? *Science*, 308, 1469-1472 (2005).

Name Moerner, W.E. wmoerner@stanford.edu

Position

Harry S. Mosher Professor of Chemistry and Professor, by courtesy, of Applied Physics

Director, Exploratory Center on Single-Molecule Fluorophores for Cellular Imaging

Presentation Title

Single-Molecule Emitters as Reporters of Dynamics and Function in Living Cells

Presentation Summary

This talk will describe studies where both single autofluorescent proteins and single fluorescent dyes provide sufficient signal-to-noise for imaging in living cells. A focus is on new classes of single-molecule fluorophores with alternate modes of reporter function. In addition, following the behavior of single molecules inside the cell allows unprecedented detection of selected cellular functions.

Home Page

http://www.stanford.edu/group/moerner/research.html

Education

1975, B.S., Physics, Washington University, St. Louis
1975, A.B., Mathematics, Washington University, St. Louis
1975, B.S., Electric Engineering, Washington University, St. Louis
1978, M.S., Physics, Cornell University
1982, Ph.D., Physics, Cornell University

Selected Activities & Honors

- Fellow, Optical Society of America, May 28, 1992
- Fellow, American Physical Society, November 16, 1992
- IBM Outstanding Technical Achievement Award for Single-Molecule Detection and Spectroscopy, November 22, 1992
- Samuel L. McElvain Lecturer, Department of Chemistry, University of Wisconsin, 1993
- Ehrenfest Colloquium Lecturer, University of Leiden, The Netherlands, March 1994
- Arthur D. Little Lecturer, Department of Chemistry, Massachusetts Institute of Technology, 1995
- William Draper Harkins Lecturer, Department of Chemistry, University of Chicago, 2001
- Earle K. Plyler Prize in Molecular Spectroscopy, American Physical Society, 2001
- Fellow, American Academy of Arts and Sciences, October 13, 2001
- Geoffrey Frew Fellow, Australian Academy of Sciences, 2003
- Fellow, American Association for the Advancement of Science, 2004

Research Interests

Probing local environments using single-molecule spectroscopy and microscopy, biophysics and imaging of single molecules in vitro and in vivo, trapping nanoparticles in solution: the anti-brownian electrokinetic (ABEL) trap, single-molecule nanophotonics (using metallic nanoantennas to modify how a single quantum-mechanical object interacts with light), single-molecule spectroscopy, imaging and auantum optics

Key Publications Relevant to Imaging

S. Y. Nishimura, S. J. Lord, L. O. Klein, K. A. Willets, M. He, Z. Lu, R. J. Twieg, and W. E. Moerner, "Diffusion of Lipid-Like Single-Molecule Fluorophores in the Cell Membrane," *J. Phys. Chem. B* (in press, 2006).

P. J. Schuck, K. A. Willets, D. P. Fromm, R. J. Twieg, and W. E. Moerner, "A Novel Fluorophore for Single-Molecule Two-Photon-Excited Fluorescence," *Chem. Phys.* 318, 7-11 (2005).

S. Y. Kim, A. N. Semyonov, R. J. Twieg, A. L. Horwich, J. Frydman, and W. E. Moerner, "Probing the Sequence of Conformational Changes in the Molecular Chaperonin GroEL with Fluorescence Spectroscopy," *J. Phys. Chem. B* 109, 24517-24525 (2005).

M. Vrljic, S. Y. Nishimura, W. E. Moerner, and H. M. McConnell, "Cholesterol depletion suppresses the translational diffusion of class II MHC proteins in the plasma membrane," *Biophys. J.* 88, 334-347 (2005).

J. Deich, E. M. Judd, H. H. McAdams, and W. E. Moerner, "Visualization of the Movement of Single Histidine Kinase Molecules in Live Caulobacter cells," *Proc. Nat. Acad. Sci. USA* 101, 15921-15926 (2004).

E. J. G. Peterman, H. Sosa, and W. E. Moerner, "Single-Molecule Fluorescence Spectroscopy and Microscopy of Biomolecular Motors," invited review, *Ann. Rev. Phys. Chem.* 55, 79-96 (2004).

K. A. Willets, O. Ostroverkhova, S. Hess, M. He, R. J. Twieg, and W. E. Moerner, "Novel Fluorophores for Single-Molecule Imaging," *Proc. SPIE* 5222, 150-157 (2003).

W. E. Moerner, "Optical Measurements of Single Molecules in Cells," *Trends Analyt. Chem.* 22, 544-548 (2003).

Name Heald, Rebecca heald@socrates.berkeley.edu

Position Associate Professor, Department of Molecular and Celll Biology, University of California Berkeley

Presentation Title Analysis of Ran-GTP Regulated Mitotic Gradients in Xenopus Egg Extracts and Somatic Cells

Presentation Summary The RanGTPase cycle provides directionality to nucleocytoplasmic-transport-regulating interactions between cargoes and nuclear transport receptors of the importin-beta family. The Ran-importin-beta system also functions in mitotic spindle assembly and nuclear pore and nuclear envelope formation. The common principle underlying these diverse functions throughout the cell cycle is thought to be anisotropy of the distribution of RanGTP (the RanGTP gradient), driven by the chromatin-associated guanine nucleotide exchange factor RCC1. However, the existence and function of a RanGTP gradient during mitosis in cells are unclear. We have examined the Ran-importin-beta system in cells by conventional and fluorescence lifetime microscopy using a biosensor, termed "Rango," that increases its fluorescence resonance energy transfer signal when released from importin-beta by RanGTP. Rango is predominantly free in mitotic cells but is further liberated around mitotic chromatin. In vitro experiments and modeling show that this localized increase of free cargoes corresponds to changes in RanGTP concentration sufficient to stabilize microtubules in extracts. In cells, the Ran-importin-beta cargo gradient kinetically promotes spindle formation but is largely dispensable once the spindle has been established. Consistent with previous reports, we observe that the Ran system also affects spindle pole formation and chromosome congression in vivo. Our results demonstrate that conserved Ran-regulated pathways are involved in multiple, parallel processes required for spindle function but that their relative contribution differs in chromatin- vs. centrosome/kinetochore-driven spindle assembly systems.

Home Page http://mcb.berkeley.edu/labs/heald/

Education 1985, B.S., Chemistry, Hamilton College
1993, Ph.D. Cell Physiology, Harvard Medical School
1993-1997, Postdoctoral Research in Cell Biology, European Molecular Biology Lab

Selected Activities & Honors

American Cancer Society Postdoctoral Fellowship (1993-1996)
Pew Scholar Award in Biomedical Sciences (1999-2003)
Hellman Faculty Fund Award (2000)
Monitoring Editor, *Journal of Cell Biology* (2001-present)
Member, Cancer Research Coordinating Committee (2003-present)
Physiology Course Faculty, MBL Woods Hole, 2004, 2005
Member, NIH study section NDT (2004-present)
Editorial Board, *Molecular Biology of the Cell* (2005-present)
Alumni Scientist Medal in Biochemistry, Hamilton College (2005)
American Society for Cell Biology, Women in Cell Biology Junior Career Recognition Award (2005)
Editorial Board, *ACS Chemical Biology* (2006-present)

Research Interests

Cell division, mitotic spindle assembly, chromosome segregation, chromosome architecture, microtubule dynamics

Key Publications Relevant to Imaging

Kalab, P., Weis, K. and R. Heald (2002) Visualization of a RanGTP gradient in interphase and mitotic *Xenopus* egg extracts. *Science* 295, 2452-56.

Kalab, P., Pralle, A., Isacoff, E.Y., Heald, R., and K. Weis (2006) Analysis of a RanGTP-regulated gradient essential for mitosis in somatic cells. *Nature*, in press.

Name Salmon, Edward D. tsalmon@email.unc.edu

Position Ballou UNC Distinguished Professor, Biology, University of North Carolina at Chapel Hill

Presentation Title Imaging Protein Number and Dynamics at the Kinetochore-Microtubule Interface

Presentation Summary

Kinetochores are protein assemblies that play several key functions required for achieving accurate chromosome segregation. They provide attachment sites to link centromeric DNA to the plus ends of spindle microtubules. We have counted the number of major protein subunit complexes that produce this attachment site linkage in budding yeast where there is only one attachment site per kinetochore. Counts are obtained from the ratio of fluorescence from GFP-protein fusions to the fluorescence of two GFP-Cse4 proteins within the single nucleosome wrapped by the 150 bp centromeric DNA. At the microtubule interface, there are 8 Ndc80 complexes that link the kinetochore to 16 Dam1/DASH subunits that are known to form a ring around the plus-end of the microtubule in vitro. Measurements of fluorescence redistribution after photobleaching (FRAP) show that these and the other core linker protein complexes are stable components of the kinetochore. Kinetochores in vertebrate cells have multiple attachment sites for spindle microtubule plus ends (about 25 in humans). By using two-color fluorescence imaging and labeling the ends of the elongated Ndc80 complex red and green, we have found that one end is located near the microtubule plus end while the other is oriented toward the inner kinetochore. We have measured microtubule assembly within attachment sites by the dynamics of red fluorescence tubulin speckles within the microtubule lattice relative to green fluorescent kinetochore protein. Microtubules slide through the attachment site at velocities of 55 dimers per second coupled to the polymerization and depolymerization phases of plus-end dynamics instability. Pulling force is produced during depolymerization while a resistive force is generated during polymerization. Because of their multiple attachment sites, mammalian kinetochores must correct errors in attachment such as merotelic attachments where kinetochores become attached to microtubules from opposite poles rather than to just one pole as normal. We have used measurements of fluorescence dissipation after photoactivation (FDAP) to obtain the half-life of microtubule plus-end attachment in PtK1 cells expressing PA-GFP-tubulin. Half-lives vary from 2 minutes in early prometaphase to greater than 30 minutes in anaphase. Turnover is controlled by Aurora B kinase, which concentrates within the inner centromere behind the kinetochore. Partial inhibition of Aurora B activity produces substantial inhibition of kinetochore microtubule turnover, and a sixfold increase in chromosome mis-segregation in anaphase due to merotelic kinetochores. Finally, kinetochores are the site of the spindle checkpoint that produces a Mad1/Mad2 wait-anaphase signal when attachment sites are not filled and tension not achieved by metaphase chromosome alignment. We have used both FRAP and FLIP methods to obtain the kinetic constants for spindle checkpoint dynamics at kinetochores in order to understand how a single unattached kinetochore can prevent the whole cell from entering anaphase.

Home Page	http://www.bio.unc.edu/faculty/salmon/lab/
Education	B.S., Engineering Science, Brown University, 1967 Ph.D., Biomedical Electronic Engineering, University of Pennsylvania, 1973
Selected Activities & Honors	Council of the American Society for Cell Biology, 1998-2001 Ballou UNC Distinguished Professor, 2002 Keith R. Porter Lecture for 2004, American Society for Cell Biology Meetings NIH MERIT Award
Research Interests	Mechanisms of mitotic spindle assembly and chromosome movement, microtubules and microtubule motors, developing new live cell imaging methods for assays of protein function in cells
Key Publications Relevant to Imaging	See PubMed

Name Waterman-Storer, Clare M. waterman@scripps.edu

Position Associate Professor, Department of Cell Biology, The Scripps Research Institute

Presentation Title Analyzing the Integration of Actin Dynamics with Adhesion in Cell Migration Using Correlational qFSM

Presentation Summary Cell migration requires transmission of force generated in the actin filament (f-actin) cytoskeleton through molecularly complex focal adhesions (FAs) to the extracellular environment. We discovered a remarkable dynamic complexity for different classes of structural and regulatory molecules within single FAs in migrating epithelial cells. We developed correlational fluorescent speckle microscopy to measure correlated motions between molecules to analyze coupling of FA proteins to the dynamic actin cytoskeleton. Different classes of FA molecules exhibit varying degrees of correlated motions with f-actin, indicating differential transmission actomyosin force through FA. Our results suggest that dynamic interactions between FA proteins and f-actin constitute a friction clutch between f-actin and the extracellular environment that is regulated during morphodynamic transitions of cell migration.

Home Page http://speckle.scripps.edu/waterman_research.htm

Education
B.A., Biochemistry, Mount Holyoke College (1989)
M.S., Exercise Science, University of Massachusetts (1991)
Ph.D., Cell Biology, University of Pennsylvania (1995)
Postdoctoral Research, Cell Biology, University of North Carolina (1999)

Selected Activities & Honors
2005 Director's Pioneer Award, Office of the Director, National Institutes of Health
2005 R.R. Bensley Award in Cell Biology, American Association of Anatomists
2004 Nikon Instruments Partner in Research
2003-2006 Keith Porter Fellow, Keith R. Porter Endowment for Cell Biology
2003 Finalist, W.M. Keck Foundation Distinguished Young Scholars in Medical Research.
2002 Women in Cell Biology Junior Career Award, American Society for Cell Biology
2001 Award for Achievement in Optical Microscopy, Optical Imaging Association (OPIA), Microscopy Society of American (MSA)
2000 Carl Zeiss Lecture, Micro 2000, Meeting of the Royal Microsocpical Society, London

Research Interests Microscopes and motility: systems integration in directed cell migration, microtubule and actin interactions in cell migration, quantitative fluorescent speckle microscopy, systems biology approach to integration of subcellular machines

Key Publications Relevant to Imaging

Ponti A., A. Matov, M.C. Adams, S.L.Gupton, C.M. Waterman-Storer and G. Danuser G. (2005) Periodic patterns of actin turnover in lamellipodia and lamellae of migrating epithelial cells analyzed by quantitative fluorescent speckle microscopy. *Biophys J* 89:3456-3469.

Adams, M.C., A. Matov, D. Yarar, S.L. Gupton, G. Danuser and C.M. Waterman-Storer. (2004) Signal analysis of total internal reflection fluorescent speckle microscopy (TIR-FSM) and widefield epifluorescence FSM of the actin cytoskeleton and focal adhesions in living cells. *J Microscop* 216:138-152.

Ponti, A., M. Machachek, S.L. Gupton, C.M. Waterman-Storer and G. Danuser. (2004) Two uncoupled actin networks drive the protrusion of migrating cells. *Science* 305:1782-1786.

Vallotton P., C.M. Waterman-Storer and G. Danuser. (2004) Computational fluorescent speckle microscopy: A new window on actin cytoskeleton dynamics in motile cells. *Proc Natl Acad Sci USA.* 101:9660-9665.

Vallotton P., A. Ponti, C.M. Waterman-Storer E.D. Salmon, and G. Danuser. (2003) Recovery, visualization, and analysis of actin and tubulin polymer flow in live cells: a fluorescent speckle microscopy study. *Biophys J* 85:1289-1306.

Ponti, A., P. Vallotton, W.C. Salmon, C.M. Waterman-Storer, and G. Danuser, (2003) Computational analysis of f-actin turnover in cortical actin meshworks using fluorescent speckle microscopy. *Biophys J* 84:3336-3352.

Adams, M.C., W.C. Salmon, S.L. Gupton, C.S. Cohan, T. Wittmann, N. Prigozhina and C.M. Waterman-Storer. (2003) A high-speed multi-spectral spinning disc confocal microscope system for fluorescent speckle microscopy of living cells. *Methods* 29:29-41.

Name Simon, Sanford M. simon@mail.rockefeller.edu

Position Professor, The Rockefeller University

Presentation Title The Dynamics of Molecules during Exo and Endocytosis

Presentation Summary The dynamics of the molecules that have been implicated in endocytosis and exocytosis were quantified at the plasma membrane to put constraints on the models to describe these processes.

Home Page http://www.rockefeller.edu/labheads/simon/simon-lab.php

Education

B.S, Princeton University, Neuroscience, 1977
Ph.D., NYU Medical Center, Calcium and Transmitter Release in the Squid Synapse, 1984
Postdoctoral Research, Gunter Blobel, Rockefeller University

Selected Activities & Honors

2004-present, NIH Microscopy Imaging Study Section
2004, Bard College Medal of Science
1999-2004, NSF Biophotonics Study Section

Research Interests Exocytosis, endocytosis, protein translocation across membranes, transport through nuclear pores, tumorigenesis and metastasis, apoptosis

Key Publications Relevant to Imaging

J.Z. Rappoport, A. Benmerah, and S.M. Simon. Analysis of the AP-2 adaptor complex and cargo during clathrin-mediated endocytosis. *Traffic.* 6 (7):539-547, 2005.

E.B. Voura, J.K. Jaiswal, H. Mattoussi, and S.M. Simon. Tracking metastatic tumor cell extravasation with quantum dot nanocrystals and fluorescence emission-scanning microscopy. *Nat. Med.* 10 (9):993-998, 2004.

J.K. Jaiswal, E.R. Goldman, H. Mattoussi, and S.M. Simon. Use of quantum dots for live cell imaging. *Nat. Meth.* 1 (1):73-78, 2004.

J.K. Jaiswal and S.M. Simon. Potentials and pitfalls of fluorescent quantum dots for biological imaging. *Trends Cell Biol.* 14 (9):497-504, 2004.

J.K. Jaiswal, S. Chakrabarti, N.W. Andrews, and S.M. Simon. Synaptotagmin VII Restricts Fusion Pore Expansion during Lysosomal Exocytosis. *PLoS. Biol.* 2 (8):E233, 2004.

M. Fix, T.J. Melia, J.K. Jaiswal, J.Z. Rappoport, D. You, T.H. Sollner, J.E. Rothman, and S.M. Simon (2004). Imaging single membrane fusion events mediated by SNARE proteins. *Proc Natl Acad Sci USA* 101 (19):7311-7316.

J.K. Jaiswal, H. Mattoussi, J.M. Mauro, and S.M. Simon (2003). Long-term multiple color imaging of live cells using quantum dot bioconjugates. *Nat. Biotechnol.* 21 (1):47-51.

J.Z. Rappoport, B.W. Taha, and S.M. Simon. Movement of plasma-membrane-associated clathrin spots along the microtubule cytoskeleton. *Traffic*. 4 (7):460-467, 2003.

J.Z. Rappoport, B.W. Taha, S. Lemeer, A. Benmerah, and S.M. Simon. (2003) The AP-2 complex is excluded from the dynamic population of plasma membrane-associated clathrin. *JBC* 278 (48):47357-47360.

G. Kreitzer, J. Schmoranzer, S.H. Low, X. Li, Y. Gan, T. Weimbs, S.M. Simon, and E. Rodriguez-Boulan. Three-dimensional analysis of post-Golgi carrier exocytosis in epithelial cells. *Nat. Cell Biol*. 5 (2):126-136, 2003.

J. K. Jaiswal, N. W. Andrews, and S. M. Simon. (2002) Membrane proximal lysosomes are the major vesicles responsible for calcium-dependent exocytosis in nonsecretory cells. *JCB* 159 (4):625-635.

Y. Chen, A. C. Pant, and S. M. Simon. (2001) P-glycoprotein does not reduce substrate concentration from the extracellular leaflet of the plasma membrane in living cells. *Cancer Res*. 61 (21):7763-7769.

M. A. Lampson, J. Schmoranzer, A. Zeigerer, S. M. Simon, and T. E. McGraw. (2001) Insulin-regulated release from the endosomal recycling compartment is regulated by budding of specialized vesicles. *Mol. Biol. Cell* 12 (11):3489-3501.

M. Goulian, and S. M. Simon (2000). Tracking single proteins within cells. *Biophys. J*. 79:2188-2198.

Y. Chen, and Simon, S.M. (2000). In situ biochemical demonstration that P-glycoprotein is a drug efflux pump with broad specificity. *J. Cell Biol*. 148:863-870.

J. Schmoranzer, M. Goulian, D. Axelrod, S.M. Simon (2000). Imaging constitutive exocytosis with total internal reflection fluorescence microscopy. *J. Cell Biol*. 149:23-31.

Name Kusumi, Akihiro akusumi@frontier.kyoto-u.ac.jp

Position Professor, Institute for Frontier Medical Sciences, Kyoto University, Kyoto, Japan

Presentation Title Single-Molecule Imaging of Signal Transduction of GPI-Anchored Receptor

Presentation Summary How the GPI-anchored receptor, which lacks the intracellular domain, induces intracellular signaling has been a long-standing enigma. Here, we found the recruitment of signaling molecules beneath the induced receptor clusters. More importantly, single-molecule tracking revealed that such recruitment takes place transiently.

Home Page http://www.nanobio.frontier.kyoto-u.ac.jp/lab/e.html

Education B.S., Biophysics, Kyoto University, Japan (1975)
Ph.D., Biophysics, Kyoto University, Japan (1980)

Selected Activities & Honors Sefoh Award, Japanese Society for Microscopy
Editorial Board, *Cell Struct. Func.*
Editorial Board, *Bioimages*
Editorial Board, *Biophys. J.*
Member of Faculty 1000

Research Interests Microdomains in biological membranes, single molecular imaging of biological membranes

Key Publications Relevant to Imaging

Kusumi, A., C. Nakada, K. Ritchie, K. Murase, K. Suzuki, H. Murakoshi, R. S. Kasai, J. Kondo, and T. Fujiwara. Paradigm shift of the plasma membrane concept from the two-dimensional continuum fluid to the partitioned fluid: high-speed single-molecule tracking of membrane molecules. Annu. Rev. Biophys. Biomol. Struct. 34, 351-378 (2005).

Kusumi, A., H. Ike, C. Nakada, K. Murase and T. Fujiwara. Single-molecule tracking of membrane molecules: plasma membrane compartmentalization and dynamic assembly of raft-philic signaling molecules. Sem. Immunol. 17, 3-21 (2005).

Kusumi, A., H. Murakoshi, K. Murase, and T. Fujiwara. Single-molecule imaging of diffusion, recruitment, and activation of signaling molecules in living cells. In "Biophysical aspects of transmembrane signaling," Chapter 5, S. Damjanovich, Ed. Springer-Heidelberg pp. 123-152. (2005).

Suzuki, K., K. Ritchie, E. Kajikawa, T. Fujiwara, and A. Kusumi. Rapid hop diffusion of a G-protein-coupled receptor in the plasma membrane as revealed by single-molecule techniques. Biophys. J. 88, 3659-80 (2005).

Koyama-Honda, K. Ritchie, T. Fujiwara, R. Iino, H. Murakoshi, R. S. Kasai, and A. Kusumi. Fluorescence imaging for monitoring the colocalization of two single molecules in living cells. Biophys. J. 88, 2126-2136 (2005).

Ritchie, K., X.-Y. Shan, J. Kondo, K. Iwasawa, T. Fujiwara, and A. Kusumi. Detection of non-Brownian diffusion in the cell membrane in single molecule tracking. Biophys. J. 88, 2266-2277 (2005).

Murase, K., T. Fujiwara, Y. Umemura, K. Suzuki, R. Iino, H. Yamashita, M. Saito, H. Murakoshi, K. Ritchie, and A. Kusumi. Ultrafine membrane compartments for molecular diffusion as revealed by single molecule techniques. Biophys. J. 86(6):4075-4093 (2004).

Murakoshi, H., R. Iino, T. Kobayashi, T. Fujiwara, C. Ohshima, A. Yoshimura, and A. Kusumi. Single-molecule imaging analysis of Ras activation in living cells. Proc. Natl. Acad. Sci. USA 101, 7317-7322 (2004).

Nakada, C., K. Ritchie, Y. Oba, M. Nakamura, Y. Hotta, R. Iino, R.S.Kasai, K. Yamaguchi, T. Fujiwara, and A. Kusumi. Accumulation of anchored proteins forms membrane diffusion barriers as neurons develop polarization. Nat. Cell Biol. 5(7): 626-632 (2003).

Name Lippincott-Schwartz, Jennifer lippincj@mail.nih.gov

Position Chief, Section on Organelle Biology, Cell Biology and Metabolism Branch, National Institute of Child Health and Human Development, National Institutes of Health

Presentation Title Deciphering Protein Turnover, Topology, and Transport in Living Cells

Presentation Summary

The development of fluorescent proteins as molecular tags over the past decade has spurred a revolution by allowing complex biochemical processes to be correlated with the functioning of proteins in living cells. Fluorescent proteins such as green fluorescent protein (GFP) from the jellyfish *Aequorea victoria* and its variants can be fused to virtually any protein of interest to analyze protein geography, movement, and chemistry in living cells. As such, they have provided an important new tool for understanding protein function, filling an urgent need now that the genome sequence of many organisms is complete. Here, experimental protocols using photoactivatable GFP that allow in cellula pulse-chase analysis of proteins undergoing degradation or selective delivery to different organelles will be presented. In addition, a new fluorescent-based protease protection assay for determining the topology of transmembrane and lumenal proteins associated with a wide variety of cellular compartments (including the ER, Golgi apparatus, autophagosomes and peroxisomes) and protein complexes will be described.

Home Page http://lippincottschwartzlab.nichd.nih.gov/

Education M.S., Biology, Stanford University
Ph.D., Biochemistry, Johns Hopkins University

Selected Activities & Honors J. Cell Science, Associate Editor; Molecular Biology of the Cell, Associate Editor; Current Protocols Cell Biology, Associate Editor; Council member, Biophysics Society. Feulgin Prize, Society of Histochemistry

Research Interests Intracellular protein trafficking and sorting, organelle disassembly/reassembly and inheritance strategies, membrane dynamics and the cytoskeleton, green fluorescent protein technologies

Key Publications Relevant to Imaging

Patterson, G. and J. Lippincott-Schwartz (2002) A photoactivatable GFP for selective photolabeling of proteins and cells. *Science* 297, 1873-1877.

Lippincott-Schwartz, J., Altan-Bonnet, N., and G. Patterson (2003) Photobleaching and photoactivation: following protein dynamics in living cells. *Nature Reviews/Molecular Cell Biology*. Suppl: S7-14.

Presley, J.P., Ward, T, Miller, C., Siggia E, Phair, R.D., and J. Lippincott-Schwartz (2002) Dissection of COPI and Arf1 dynamics *in vivo* and role in Golgi membrane transport. *Nature* 417, 187-193.

Lippincott-Schwartz, J., Snapp, E., and Kenworthy, A. (2001) Studying protein dynamics in living cells. *Nature Cell Biology Reviews* 2, 444-456.

Cole, N.B., Smith, C., Sciaky, N., Terasaki, M., Edidin, M. and Lippincott-Schwartz, J. (1996) Diffusional mobility of Golgi proteins in membranes of living cells. *Science* 273, 797-801.

Lorenz, H., Hailey, D.W., and J. Lippincott-Schwartz, A fluorescent protease protection assay using GFP chimeras to determine protein topology, localization and assembly. *Nature Methods*, in press.

Name Danuser, Gaudenz gdanuser@scripps.edu

Position Associate Professor, Laboratory for Computational Cell Biology, Department of Cell Biology, The Scripps Research Institute

Presentation Title Computer Vision of Cytoskeleton Regulation

Presentation Summary In this talk I will report applications of computer vision methods to extract mechanistic information about the regulation of actin cytoskeleton dynamics in cell migration and microtubule dynamics in mitosis. Based on these examples, I will illustrate how we build integrated computational image analysis pipelines that model various aspects of an imaging experiment, from the photophysics of a fluorophore, to the optics and camera electronics of the microscope, to the mechanics and kinetics of the probed macromolecular structures, in order to quantitate the dynamics of cellular processes. I will also present recent work in the lab where we have expanded the scope of these analyses to multivariate and multiplexed spatiotemporal correlation to infer the causality and timing of molecular activities involved in the regulation of cell migration and mitosis.

Home Page http://lccb.scripps.edu

Education M.Sc. Measurement Sciences, ETH Zurich, Switzerland (1993)
Ph.D. Electrical Engineering, ETH Zurich, Switzerland (1997)
Postdoctoral Bioimaging, The Marine Biological Lab, Woods Hole, MA (1997-1999)

Selected Activities & Honors

- Editorial Board Member, Biophysical Journal
- Associate Editor, IEEE Transactions on Image Processing
- Human Frontiers in Sciences Program Young Investigator Award (jointly with Claire Waterman-Storer and Inke Naethke)

Research Interests Mechanochemical regulation of cytoskeleton dynamics and integration of deterministic and stochastic models thereof with computational image analysis, multispectral high-resolution live cell light microscopy, computer vision and image understanding of molecular processes in living cells; pattern recognition in cell dynamic behavior; inference of regulatory networks from multispectral/multimodal live cell light microscopy

Key Publications Relevant to Imaging

Cameron L., Yang G., Cimini D., Canman J.C., Kisurina-Evgenieva O., Khodjakov A., Danuser G., and Salmon E.D.S. A pulling-in mechanism produces the majority of kinetochore microtubule poleward flux in PtK1 cells. *J. Cell Biol.* In Press. 2006.

Machacek M. and Danuser G. Two morpho–dynamic states reveal the molecular mechanism of transversal protrusion waves in migrating epithelial cells. *Biophys. J.* 90:1439-1452. 2006.

Dorn J.F., Jaqaman K., Rines D.R., Jelson G.S., Sorger P.K., Danuser G. Yeast kinetochore microtubule dynamics analyzed by high-resolution three-dimensional microscopy**.** *Biophys. J*. 89:2834-2854. 2005.

Ponti A., Machacek M., Gupton S., Waterman-Storer C.M.*, and Danuser G.* Two uncoupled actin networks drive cell protrusion. *Science*. 305:1782-1786. 2004. *equal contribution

Vallotton P., Gupton S., Waterman-Storer C., and Danuser G. Simultaneous mapping of F-actin flow and turnover in migrating cells by Quantitative Fluorescent Speckle Microscopy. *Proc. Natl. Acad. Sci. USA*. 101:9660-9665. 2004.

Berney C. and Danuser G. FRET or no FRET? A comparative study. *Biophys. J.* 84:3992-4010. 2003.

Ponti A., Vallotton P., Salmon W., Waterman-Storer C. and Danuser G. Computational analysis of f-actin turnover in cortical actin meshworks using fluorescent speckle microscopy. *Biophys. J.* 84:3336-3352. 2003.

Danuser G. and Oldenbourg, R. Probing f-actin flow by tracking shape fluctuations of radial bundles in lamellipodia of motile cells. *Biophys. J.*, 79:191-201. 2000.

Name Eils, Roland r.eils@dkfz-heidelberg.de

Position Department Head and Professor, German Cancer Research Centre; Division of Theoretical Bioinformatics, Department of Bioinformatics and Functional Genomics, University of Heidelberg

Presentation Title Automated Large-Scale Classification of Cellular Phenotypes in High-Content 4D-Microscopy Screens

Presentation Summary High-content cellular screens of protein function as performed on genome-wide cell arrays combined with gene knockdown assays provide rapidly increasing amounts of data. In typical applications, many 10 TBytes of multi-dimensional (spatiotemporal) image data are produced every week. The analysis of such image data presently constitutes a substantial bottleneck in the functional analysis pipeline and calls for the development of automated image analysis and pattern recognition tools for automatically processing large-scale image data. We developed a versatile automated image analysis pipeline that allows us to automatically detect, track over time, and classify cells into different cellular phenotypes. This platform will be suited for any large-scale, high-content cellular screen.

Home Page http://www.dkfz.de/tbi

Education

- 1990, M.Sc. Mathematics, RWTH Aachen
- Oct. 1992-Sept. 1995, Ph.D. study at University of Heidelberg (Ruprecht-Karl-Universität Heidelberg); Specialization: *Mathematics and Scientific Computing* (Ph.D. in Mathematics, first class)
- Oct. 1995-Sept. 1996, Postdoctoral student at the IWR
- Jan. 1996-Dec. 1996, Guest researcher at the Institut Albert Bonniot, Université Grenoble, France (Host: Prof. M. Robert-Nicoud, Faculty of Medicine). Research area: Development of 3-dimensional image analysis methods

Selected Activities & Honors June 1999, BioFuture prize from the German Ministery for Education and Research (approx. 1.2 million)
2005, Microsoft Research award "Computational tools for advancing science"
2005, Award for new innovative research by Helmholtz Society: "Systems Biology of Complex Diseases"

Research Interests Multidimensional image analysis and classification, functional nuclear and cellular architecture, modeling and simulation of cellular processes

Key Publications Relevant to Imaging

W. Tvaruskó, M. Bentele, T. Misteli, R. Rudolf, C. Kaether, D.L. Spector, H.H. Gerdes, R. Eils (1999) Time-resolved analysis and visualization of dynamic processes in living cells, *Proc. Natl. Acad. Sci USA* 96:7950-7955.

D. Gerlich, J. Beaudouin, M. Gebhard, J. Ellenberg, R. Eils (2001) 4-D imaging and quantitative reconstruction to analyse complex spatiotemporal processes in live cells. *Nature Cell Biology* 3:852-855.

Gerlich, D., B. Kalbfuss, J. Beaudouin, N. Daigle, R. Eils*, J. Ellenberg (2003) Inheritance of chromosome topology throughout mitosis. *Cell* 112:751-764. *corresponding author

Lenart P., C.P. Bacher, N. Daigle, A. Hand, R. Eils, M. Terasaki, J. Ellenberg (2005) A contractile nuclear actin network drives chromosome congression in oocytes. *Nature* 436(7052):812-8.

Bacher C.P., M. Guggiari, B. Brors, S. Augui, P. Clerc, P. Avner, R. Eils*, E. Heard* (2006) Transient colocalization of X-inactivation centres accompanies the initiation of X inactivation. *Nat. Cell Biol.* 2006 Jan 24; [Epub ahead of print] **corresp. author and equal contribution.*

Name Murphy, Robert F. murphy@andrew.cmu.edu

Position Professor of Biological Sciences and Biomedical Engineering; Director, Center for Bioimage Informatics; Carnegie Mellon University

Presentation Title Location Proteomics: Automated Image Understanding for Systems Biology

Presentation Summary Systems biology requires comprehensive, systematic data on all aspects and levels of biological organization and function. The creation of accurate, predictive models of cell behavior will require detailed information on the distributions of all macromolecules within cells and the ways in which those distributions change over the cell cycle and in response to mutations or external stimuli. Current information on subcellular location in protein databases is limited to sets of terms assigned by human curators. These entries do not permit basic operations that are common to other biological databases, such as measurement of similarity between the distributions of two proteins, and they are not able to fully capture the complexity of protein patterns. The field of location proteomics seeks to provide automated, objective, high-resolution descriptions of protein location patterns within cells. Automated classifiers have been shown to recognize all major subcellular patterns in fluorescence microscope images with very high accuracy (over 98% on single 3-D images) and to discriminate patterns that visual examination cannot. Consensus clustering methods can be used to identify location families, in a manner analogous to clusters found for other domains, such as protein sequence families. The next step is to express each unique pattern as a generative model that can be incorporated into comprehensive models of cell behavior. The combination of these methods with large scale imaging approaches will allow realistic cell modeling that reflects detailed knowledge of the subcellular location of all proteins.

Home Page http://murphylab.web.cmu.edu

Education A.B., Biochemistry, Columbia College, 1974
Ph.D., Biochemistry, California Institute of Technology, 1980
Damon Runyon Postdoctoral Fellow, Biophysics, Columbia University, 1979-1983

Selected Activities & Honors Presidential Young Investigator Award, NSF*;* Guest Editor (with Gaudenz Danuser and Erik Meijering), Special Issue on Molecular and Cellular Bioimaging, *IEEE Transactions on Image Processing*; Guest Editor (with Jelena Kovacevic), Special Issue on Molecular and Cellular Bioimaging, *IEEE Signal Processing Magazine*; Member, Editorial Advisory Board, *Journal of Proteome Research;* Councilor, International Society for Analytical Cytology; Chair, NIH BDMA Study Section; Chair, Cytometry Development Workshop, Finance Chair, ISBI 2006

Research Interests Bioimage informatics, fluorescence microscopy, machine learning, ipattern analysis, subcellular location, model-based image interpretation

Key Publications Relevant to Imaging

M.V. Boland, M.K. Markey, and R.F. Murphy (1998). Automated Recognition of Patterns Characteristic of Subcellular Structures in Fluorescence Microscopy Images. *Cytometry 33*:366-375.

M.V. Boland and R.F. Murphy (2001). A Neural Network Classifier Capable of Recognizing the Patterns of all Major Subcellular Structures in Fluorescence Microscope Images of HeLa Cells. *Bioinformatics 17:*1213-1223.

R.F. Murphy, M. Velliste, and G. Porreca (2003). Robust Numerical Features for Description and Classification of Subcellular Location Patterns in Fluorescence Microscope Images. *J. VLSI Sig. Proc. 35:* 311-321.

K. Huang and R.F. Murphy (2004). From Quantitative Microscopy to Automated Image Understanding. *J. Biomed. Optics 9:*893-912.

K. Huang and R.F. Murphy (2004). Boosting Accuracy of Automated Classification of Fluorescence Microscope Images for Location Proteomics. *BMC Bioinformatics 5:*78.

X. Chen and R.F. Murphy (2005). Objective Clustering of Proteins Based on Subcellular Location Patterns. *J. Biomed. Biotech. 2005(2):*87-95.

T. Zhao, M. Velliste, M.V. Boland, and R.F. Murphy (2005). Object Type Recognition for Automated Analysis of Protein Subcellular Location. *IEEE Trans. Image Proc. 14:*1351-1359.

S.-C. Chen, and R.F. Murphy (2006). A Graphical Model Approach to Automated Classification of Protein Subcellular Location Patterns in Multi-Cell Images. *BMC Bioinformatics* 7*:*90.

Name Dickson, Robert M. dickson@chemistry.gatech.edu

Position Associate Professor, Department of Chemistry and Biochemistry, Georgia Institute of Technology

Presentation Title Novel Raman-Active and Highly Fluorescent Noble Metal Quantum Dots

Presentation Summary Highly fluorescent and Raman-active, water-soluble, several-atom gold and silver quantum dots have been created in dendritic and peptide matrices and are readily observed on the single-molecule level. These quantum dots behave as multi-electron artificial atoms with size-tunable, discrete electronic transitions between states of well-defined angular momenta. Correlation of Au nanocluster size with transition energy is well-fit by the simple relation, Efermi/N1/3, indicating protoplasmonic fluorescence arising from intraband transitions of free electrons (the jellium model). These conduction electron transitions are the low number limit of the plasmon—the collective dipole oscillations occurring when a continuous density of states is reached. Photon antibunching experiments further indicate that single-electron transitions instead of collective oscillations are responsible for the size-dependent emission. Providing the missing link between atomic and nanoparticle behavior in noble metals, these highly emissive, water-soluble noble metal quantum dots offer complementary transition energy-size scalings at smaller dimensions than do semiconductor quantum dots. The unique, discrete excitation and emission coupled with facile creation in aqueous solution open new opportunities for noble metal quantum dots as biological labels, energy transfer pairs, and other light-emitting sources in nanoscale optoelectronics.

Home Page http://www.chemistry.gatech.edu/faculty/Dickson/ or http://web.chemistry.gatech.edu/~dickson/group/index.htm

Education B.S., Chemistry, Haverford College (1991)
Ph.D., Physical Chemistry, University of Chicago (1996)
Postdoctoral Fellow, Chemistry, UC-San Diego (1996-1998)

Selected Activities & Honors Outstanding Achievement in Research Program Development Award, April 2006; Visiting Lecturer, National Science Council, Taiwan, December 2003; Camille Dreyfus Teacher-Scholar Award, 2002; Blanchard Assistant Professor, 2001; Alfred P. Sloan Foundation Fellow, March, 2001; National Science Foundation, Faculty Early CAREER Award, February 2000; Research Corporation, Research Innovation Award, September 1999; Co-Organizer, 14th Annual Bud Suddath Symposium in Biosciences, Atlanta, GA, April 2006; External Oversight Committee Member, South Carolina INBRE Program 2005; Ad Hoc Reviewer and Participant, NIH Nanotechnology Study Section, October 2004 and July 2005; NHLBI Study Section, June 2004; BECM Study Section, June 2002 and 2003; BBCB Study Section, February 2003; Reviewer and Participant, NSF Review Panel, May 2003; Organizer and Presenter, “High Resolution Light Microscopy” at the American Physical Society, Indianapolis, March 2002 (Division of Molecular, Optical and Atomic Physics)

Research Interests

Single-molecule spectroscopy, fluorescence and Raman microscopy, laser spectroscopy and dynamics, nanoparticle synthesis and photophysics, optical data storage, single-molecule electroluminescence, nanoscale/molecular electronics, biolabeling

Key Publications Relevant to Imaging

L.A. Peyser-Capadona, J. Zheng, J.I. Gonzalez, T.-H. Lee, S.A. Patel, and R.M. Dickson, "Nanoparticle-Free Single Molecule Anti-Stokes Raman Spectroscopy," *Phys. Rev. Lett.* 94, 058301 (2005).

J. Zheng, C. Zhang, and R.M. Dickson, "Highly Fluorescent, Water-Soluble, Size-Tunable Gold Quantum Dots," *Phys. Rev. Lett.,* 93, 077402 (2004).

J.T. Petty, J. Zheng, N.V. Hud, and R.M. Dickson, "DNA Templated Ag Nanocluster Formation," *J. Amer. Chem. Soc.*, 126, 5207-5212 (2004).

J. Zheng, J.T. Petty, and R.M. Dickson, "High Quantum Yield Blue Emission from Water-Soluble Au_8 Nanodots," *J. Amer. Chem. Soc.,* 125, 7780-7781 (2003).

J. Zheng and R.M. Dickson, "Individual Water-Soluble Dendrimer-Encapsulated Silver Nanodot Fluorescence," *J. Amer. Chem. Soc.,* 124, 13982-13983 (2002).

A.P. Bartko and R.M. Dickson, "Imaging Three-Dimensional Orientations of Single Molecules," *J. Phys. Chem. B*, 103, 11237-11241 (1999).

Name Ting, Alice Y. ating@mit.edu

Position Assistant Professor, Department of Chemistry, Massachusetts Institute of Technology

Presentation Title New Methodologies for Optical Imaging of Living Cells

Presentation Summary New reporters and methodologies for optical imaging of protein trafficking and function in living cells will be described. Topics include site-specific protein labeling by biotin ligase, imaging of single AMPA receptor molecules using quantum dots, protein-protein interaction detection methodology using biotin ligase, and FRET reporters of enzyme activity.

Home Page http://web.mit.edu/chemistry/Ting_Lab/

Education Harvard University Cambridge, M.A., A.B., Chemistry (with E.J. Corey), 6/1996
University of California, Berkeley, Ph.D., Chemistry (with Peter Schultz), 12/2000
University of California, San Diego, Postdoctoral Research, Biochemistry (with Roger Tsien), 6/2002

Selected Activities & Honors McKnight Technological Innovations in Neuroscience Award, Alfred P. Sloan Foundation Research Fellowship, EJLB Foundation Scholar Research Program Award, National Institutes of Health Career Development Award, Office of Naval Research Young Investigator Award, Camille and Henry Dreyfus New Faculty Award, Pfizer-Laubach Career Development Chair

Research Interests Fluorescence imaging, reporter development, organic synthesis, neuroscience, protein engineering, RNA engineering, in vitro evolution, photophysics, electron microscopy, nanotechnology, protein trafficking

Key Publications Relevant to Imaging

A monovalent streptavidin with a single femtomolar biotin binding site. M. Howarth, D. J.-F. Chinnapen, K. Gerrow, P. C. Dorrestein, M. R. Grandy, N. L. Kelleher, A. El-Husseini, A. Y. Ting. *Nature Methods*, in press (2006).

Targeting quantum dots to surface proteins in living cells with biotin ligase. M. Howarth, K. Takao, Y. Hayashi, A. Y. Ting. *Proc. Natl. Acad. Sci.*, **102**, 7583-7588 (2005).

Site-specific labeling of cell surface proteins with biophysical probes using biotin ligase. I. Chen, M. Howarth, W. Lin, and A. Y. Ting. *Nature Methods*, **2**, 99-104 (2005).

A genetically-encoded fluorescent reporter of histone phosphorylation in living cells. C.-W. Lin and A. Y. Ting. *Angew. Chem. Int. Ed.*, **43**, 2940-2943 (2004).

Genetically-encoded fluorescent reporters of histone methylation in living cells. C.-W. Lin, C. Y. Jao, and A. Y. Ting. *J. Am. Chem. Soc.*, **126**, 5982-5983 (2004).

Name Miyawaki, Atsushi matsushi@brain.riken.jp

Position Group Director, Laboratory Head RIKEN, Brain Science Institute
Advanced Technology Development Group, Laboratory for Cell Function Dynamics

Presentation Title Visualization of the Spatial and Temporal Dynamics of Intracellular Signaling

Presentation Summary Since the isolation of green fluorescent protein (GFP) from the bioluminescent jellyfish in 1992 and later with its relatives, researchers have been awaiting the development of a tool to enable the direct visualization of biological functions. This has been increasingly enhanced by the marriage of GFP with fluorescence resonance energy transfer (FRET) and fluorescence cross-correlation spectroscopy (FCCS) and is further expanded upon by the need for "post-genomic analyses." I propose that it is time to evaluate the true asset of "bio-imaging" for its potential and limitations in order to utilize and truly benefit from this novel technique.

Home Page http://www.brain.riken.go.jp/bsi/a_miyawaki.html

Education M.D., Medicine, Keio University School of Medicine (1987)
Ph.D., Signal Transduction, Osaka University School of Medicine (1991)

Selected Activities & Honors The 4th (2004) Yamazaki-Teiichi Prize Winner (Biological Science & Technology)

Research Interests Fluorescence imaging, signal transduction, photophysics

Key Publications Relevant to Imaging

Miyawaki, A. (2005) Innovations in the imaging of brain functions using fluorescent proteins. Neuron 48, 189-199.

Tsutsui, H. et al. (2005) Semi-rational engineering of a coral fluorescent protein into an efficient highlighter. EMBO Rep. 6, 233-238.

Miyawaki, A. (2004) Fluorescent proteins in a new light. Nat. Biotechnol. 22, 1374-1376.

Ando, R. et al. (2004) Regulated fast nucleocytoplasmic shuttling observed by reversible protein highlighting. Science 306, 1370-1373.

Nagai, T. et al. (2004) Expanded dynamic range of fluorescent indicators for calcium by circularly permuted yellow fluorescent proteins. Proc. Natl. Acad. Sci. USA 101, 10554-10559.

Shimozono, S. et al. (2004) Slow calcium dynamics in pharyngeal muscles in Caenorhabditis elegans during fast pumping. EMBO Rep. 5, 521-526.

Karasawa, S. et al. (2004) Cyan-emitting and orange-emitting fluorescent proteins as a donor/acceptor pair for fluorescence resonance energy transfer. Biochem. J. 381, 307-312.

Hama, H. et al. (2004) PKC signaling mediates global enhancement of excitatory synaptogenesis in neurons triggered by local contact with astrocytes. Neuron 41, 405-415.

Mizuno, H. et al. (2003) Photo-induced peptide cleavage in the green-to-red conversion of a fluorescent protein. Mol. Cell 12, 1051-1058.

Miyawaki, A. et al. (2003) Lighting up cells: labeling proteins with fluorophores. Nat. Cell Biol. S1-7.

Miyawaki, A. (2003) Visualization of the spatial and temporal dynamics of intracellular signaling. Dev. Cell 4, 295-305.

Ando, R. et al. (2002) An optical marker based on the UV-induced green-to-red photoconversion of a fluorescent protein. Proc. Natl. Acad. Sci. USA 99, 12651-12656.

Sawano, A. et al. (2002) Lateral propagation of EGF signaling after local stimulation is dependent on receptor density. Dev. Cell 3, 245-257.

Nagai, T. et al. (2002) A variant of yellow fluorescent protein with fast and efficient maturation for cell-biological applications. Nat. Biotechnol. 20, 87-90.

Name Marriott, Gerard gm@physiology.wisc.edu

Position Professor, Department of Physiology, University of Wisconsin, Madison

Presentation Title Molecular Optical Switches: Design, Characterization, and Applications in Biophysics and Cell Biology

Presentation Summary My talk will focus on the design, synthesis, and spectroscopic properties of new families of optical switches. Molecular optical switches exist in two structurally and spectroscopically distinct states; rapid and reversible transitions between these states are achieved via excited state reactions without the release of photoproducts and with high quantum efficiency. I describe different classes of optical switch that are used to prepare specific protein conjugates or to chelate divalent metal ions. Selected applications of optical switches will be presented, including optical modulation of specific protein fluorescence in cells and high-fidelity, reversible manipulation of specific dipolar interactions within protein conjugates. Finally, I will show how combinatorial chemistry is being used to generate a library of optical switches whose members may find novel applications in cell biology, bioengineering, and bioimaging.

Home Page http://www.physiology.wisc.edu/faculty/marriott.html

Education
B.Sc., Biochemistry, Birmingham University, England (1980)
Ph.D., Biochemistry, University of Illinois (1987)
Postdoctoral Research, Biophysical Chemistry, Max Planck Institute for Biophysical Chemistry (1987-1989)

Selected Activities & Honors
1988 Alexander von Humboldt Fellowship Award
1990 Japan Society for the Promotion of Science Fellowship Award
1998 Young Fluorescence Investigator Award, American Biophysics Society
1998 Editor, Caged Compounds, Methods in Enzymology, Volume 291
2003 Editor, Biophotonics, Methods in Enzymology, Volumes 360 and 361
2003 Organizer, Workshop on Biophotonics, Manoa Valley, O'ahu, HI
2003 Organizer, 2nd International Symposium on Biophotonics, San Antonio, TX

Research Interests Molecular basis of cell motility, molecular motors, imaging and optical probes (optical switching of biomolecular activity)

Key Publications Relevant to Imaging

Sakata, T., Yan, Y. and Marriott, G. Optical switching of dipolar interactions on proteins. *Proc. Natl. Acad. Sci. USA*.102, 4759-2764 (2005).

Sakata, T. Yan Y. and Marriott**,** G. A family of site selective optical switches. *J. Org Chem*. 70, 2009-2013 (2005).

Yan,Y and Marriott, G. Analysis of Protein Interactions using Fluorescence Technologies. *Curr. Opin. Chem. Biol.* 7, 1-6. (2003).

Wegner, H.J. Lee, *G. Marriott*, and R.M.Corn. Fabrication of histidine-tagged fusion protein arrays for surface plasmon resonance imaging studies of protein-protein and protein-DNA interactions. *Anal. Chem.* 75, 4740-4746 (2003).

Yan, Y. and Marriott, G. Determining proximity relationships on single protein complexes using fluorescence resonance energy transfer imaging microscopy and fluorescence polarization imaging microscopy. *Meth. Enzymol.* 36, 560-582 (2003).

Roy, P., Rajfur, Z., Jones, D. Marriott, G. and Jacobson, K. Local photorelease of caged thymosin b4 in locomoting keratocytes causes cell turning. *J. Cell Biology.* 153, 1035-1048 (2001).

G. Marriott and J.W. Walker. Caged proteins and caged peptides. *Trends in Plant Science.* 4, 330-34 (1999).

Choidas, A., Jungbluth, A., Sechi, A., Ullrich, A. and Marriott, G. The suitability and application of a GFP-actin fusion protein for long-term imaging of the organization and dynamics of the cytoskeleton in mammalian cells. *Eur. J. Cell Biol.* 77, 81-90 (1998).

Marriott, G. and Ottl, J. Photocleavable crosslinking reagents. *Meth. Enzymol.* 291, 155. (1998).

Name Hahn, Klaus M. khahn@med.unc.edu

Position Thurman Professor of Pharmacology, University of North Carolina at Chapel Hill

Presentation Title New Windows on Living Cells – Facile Generation of Biosensors to Visualize Endogenous Protein Conformations

Presentation Summary We are developing new methods for studying spatiotemporal dynamics of signaling with minimal perturbation. By conjugating novel bright dyes to affinity reagents, activities of endogenous proteins can be studied with high sensitivity. This approach opens the door to high-throughput generation of biosensors via phage display and other screening for biosensor affinity elements.

Home Page http://www.med.unc.edu/wrkunits/2depts/pharm/faculty/labpages/hahnlab/index.html

Education University of Pennsylvania, B.S., Biochemistry and Philosophy, 1981
University of Virginia, Ph.D., Chemistry, 1986
Postdoctoral Research, Center for Fluorescence Imaging in the Biomedical Sciences, Carnegie Mellon University (with D. Lansing Taylor and Alan Waggoner)

Selected Activities & Honors National Institutes of Health James A. Shannon Directors Award
2002-present, Whitaker Institute of Biomedical Engineering, University of California, San Diego
2004-present, Ronald Thurman Professor of Pharmacology, University of North Carolina Medical School, Chapel Hill
2004-present, Lineberger Comprehensive Cancer Center, University of North Carolina
2005-present, UNC Biomedical Imaging Research Center

Research Interests Novel methods to quantify the spatiotemporal dynamics of signaling in individual living cells, robotic microscopy, automated visualization of subcellular signaling dynamics and genome wide screens of modulatory pathways, novel chemistries for manipulation of protein activity in vivo, spatiotemporal control of MAP kinase signaling networks, dynamics of adhesion signaling in cell polarization and motility

Key Publications Relevant to Imaging

Kraynov, V.S., C.E. Chamberlain, G.M. Bokoch, M.A. Schwartz, S. Slabaugh and K.M. Hahn. Localized Rac Activation Dynamics Visualized in Living Cells. *Science*, **290**:333-337, 2000.

Toutchkine, A., V. Kraynov, and K.M. Hahn. Solvent-Sensitive Dyes to Report Protein Conformational Changes in Living Cells, *J. Amer. Chem. Soc.*, **125**:4132-4145, 2003.

Nalbant, P., L. Hodgson, V. Kraynov, A. Toutchkine, K.M. Hahn. Activation of Endogenous Cdc42 Visualized in Living Cells. *Science*, **305**:1615-1619, 2004.

Shen, F., L. Hodgson, A. Rabinovich, O. Pertz, K.M. Hahn, and J.H. Price. Functional Proteomics for Cell Migration. *Cytometry,* in press.

Pertz, O., Hodgson, L., Klemke, R., and Klaus M. Hahn. Spatio-Temporal Dynamics of RhoA Activity in Migrating Cells. *Nature*, in press.

Name Tsien, Roger Y. rtsien@ucsd.edu

Position Professor, Departments of Pharmacology and Chemistry and Biochemistry, University of California, San Diego; Investigator, Howard Hughes Medical Institute

Presentation Title The Future of Live Cell Imaging

Presentation Summary I will review some recent successes and tribulations in the development of new ways to image cells, then speculate on further advances that ought to be possible.

Home Page http://www-chem.ucsd.edu/Faculty/bios/tsien.html

Education B.S., Chemistry and Physics, Harvard College, 1972
Ph.D., Physiology, University of Cambridge, 1977
Postdoctoral Research (with T.J. Rink), Physiological Laboratory, Cambridge, U.K., 1978-1981

Selected Activities & Honors Searle Scholar, 1986: Lamport Prize, New York Academy of Sciences, 1989: Javits Neuroscience Investigator Award, 1991: Passano Foundation Young Scientist Award; W. Alden Spencer Award in Neurobiology, College of Physicians and Surgeons, Columbia University 1992; Bowditch Lectureship, American Physiological Society, 1994; President's Lectureship, American Thoracic Society, 1995; Doctorate *honoris causa,* Katholieke Universiteit Leuven; Artois-Baillet-Latour Health Prize, Belgium; Gairdner Foundation International Award, Canada; Basic Science Prize, American Heart Association; elected to Institute of Medicine, 1997; elected to American Academy of Arts and Sciences and National Academy of Sciences; EG&G Wallac Award for Innovation in High Throughput Screening, Society for Biomolecular Screening, 2000; Herbert Sober Lectureship, American Society for Biochemistry and Molecular Biology; Pearse Prize, Royal Microscopical Society, 2002; Award for Creative Invention, American Chemical Society; Anfinsen Award, Protein Society; Heineken Prize for Biochemistry and Biophysics, Royal Netherlands Academy of Sciences; Max Delbrück Medal, Max-Delbrück-Centrum für Molekulare Medizin, Berlin, 2003; Hugh Davson Distinguished Lecturer, American Physiological Society Cell and Molecular Physiology Section; Konrad Bloch Lectureship, Harvard University; Todd Visiting Professor of Chemistry, University of Cambridge, England; Keith Porter Lectureship, American Society for Cell Biology, 2004; Wolf Prize in Medicine, Wolf Foundation, Israel (shared with Prof. R. Weinberg); Grass Foundation Lectureship, Society for Neuroscience; Keio Medical Science Prize, Keio University, Tokyo, 2005; Perl Prize in Neuroscience, University of North Carolina; J. Allyn Taylor International Prize in Medicine, Robarts Institute, London, Ontario; Distinguished Scientist Award, San Diego Chapter of American Chemical Society, 2006; Award, Association of Biomolecular Resource Facilities; Lewis S. Rosenstiel Award for Distinguished Work in the Basic Medical Sciences, Brandeis University

Research Interests

Fluorescent and photolytic probes, signal transduction

Key Publications Relevant to Imaging

Kunkel M.T., Ni Q., Tsien R.Y., Zhang J., Newton A.C., 2005. Spatiotemporal dynamics of protein kinase B/Akt signaling revealed by a genetically-encoded fluorescent reporter. *J. Biol. Chem.* **280**: 5581-5587.

Tsien R.Y., 2005. Building and breeding molecules to spy on cells and tumors. *FEBS Letters* **579**: 927-932.

Hoffmann C., Gaietta G., Bünemann M., Adams S.R., Oberdorff-Maass S., Behr B., Vilardaga J.P., Tsien R.Y., Ellisman M.H., and Lohse M.J., 2005. A FlAsH-based FRET aproach to determine G-protein-coupled receptor activation in living cells. *Nature Methods* **2**: 171-176.

Wang Y., Botvinick E.L., Zhao Y., Berns M.W., Usami S., Tsien R.Y., Chien S., 2005. Visualizing the mechanical activation of Src. *Nature* **434**: 1040-1045.

Martin B.R., Giepmans B.N.G., Adams S.R., Tsien R.Y., 2005. Mammalian cell-based optimization of the biarsenical-binding tetracysteine motif for improved fluorescence and affinity. *Nat. Biotech*. **23**: 1308-1314.

Shaner N.C., Tsien R.Y., 2005. A guide to choosing fluorescent proteins. *Nat. Methods* **2**: 905-909.

Name Stelzer, Ernst H.K. stelzer@embl.de

Position Scientific Group Leader, European Molecular Biology Laboratory (EMBL), Heidelberg, Germany

Presentation Title SPIMaging Technology: Leaving Flatland Behind

Presentation Summary

Confocal theta microscopy was invented about 10 years ago to provide biologists with a new tool for the investigation of large specimens with high, isotropic three-dimensional resolution. By purely instrumental means, the axial resolution is easily improved by factors of three and more when compared to confocal microscopy. The fundamental principle of theta microscopy is the detection of fluorescence light at an angle of about 90° to the illumination axis. Our new implementation of the theta principle uses light sheets to illuminate a single plane in an object and a regular fluorescence microscope arranged at an angle of 90° to record the fluorescence light emitted only from this single plane. In contrast to a theta microscope, our new device illuminates and observes an entire plane and allows us to take full advantage of modern camera systems. The new instrument features optical sectioning by the excitation of fluorescence in a well-defined and very narrow volume. A major advantage over conventional and confocal microscopy is that only the plane that is observed is illuminated. This reduces the effects of phototoxicity (e.g., photobleaching) by orders of magnitude. Three-dimensional images are created by scanning the specimen through the stationary volume of illumination. Such stacks can be treated very much like stacks recorded with other microscopic three-dimensional imaging techniques. However, an important extra feature is the rotation of the sample, which changes the orientation of the excitation and detection axes with respect to the sample. This enables us to illuminate and image parts of the sample that would otherwise be hidden or obscured. Data stacks recorded at different angles are combinable in a postprocessing step and yield a high-resolution image of the complete sample. Our implementation of light-sheet based microscopy (single-plane illumination microscopy [SPIM]) is very well suited for the investigation of millimeter-size samples but also works extremely well with yeast cells and even microtubule asters. We have observed many different species with our instruments and have results from many experiments that demonstrate the properties and performance of the microscope. We are absolutely sure that this technology is of interest for all scientists working in the life sciences.

Home Page http://www.embl-heidelberg.de/ExternalInfo/stelzer/frames.html?themes

Education Diploma Thesis, Physics, MPI Biophysik and University of Frankfurt (1982)
Ph.D. Thesis, University of Heidelberg (1987)

Selected Activities & Honors
Ernst Abbe Lecture, 1999
Organizer EMBO Courses in Heidelberg, Gent, Lisboa, and Singapore (~10)
Organizer and Co-Organizer of Focus on Microscopy
Associate Editor of various journals

Research Interests

Optical physics and optical instrumentation, three-dimensional cell biology, mathematical treatment of noisy systems, mathematical treatment of biological systems

Key Publications Relevant to Imaging

Keller, P.J., F. Pampaloni, and E.H.K. Stelzer. 2006. Life sciences require the third dimension. Current Opinion in Cell Biology 18:117.

Christoph J. Engelbrecht, Ernst H.K. Stelzer. 2006. Measured & calculated PSFs in light sheet based microscopy (SPIM). Optics Letters, in press.

C. Taxis, P.J. Keller, Z. Kavagiou, L.J. Jensen, J. Colombelli, P. Bork, E.H.K. Stelzer, and M. Knop: Spore number control and breeding in Saccharomyces cerevisiae: a key role for a self-organizing system. Journal of Cell Biology 171:627-640 (2005).

Colombelli J, Grill SW, Stelzer EHK. 2004. UV diffraction limited nanosurgery of live biological tissues. Rev of Sci Instr 75(2):472-478.

Huisken J, Swoger J, Del Bene F, Wittbrodt J, Stelzer EHK (2004) Optical sectioning deep inside live embryos by selective plane illumination microscopy. Science 305:1007-1009.

Rohrbach A, Tischer C, Neumayer D, Florin EL, Stelzer EHK (2004) Trapping and tracking a local probe with a Photonic Force Microscope. Rev of Sci Instr 75(6):2197-2210.

Name Friedl, Peter peter.friedl@gmx.de

Position Professor, Rudolf Virchow Center for Experimental Biomedicine and Department of Dermatology, University of Würzburg

Presentation Title Dynamic Imaging of Tumor Cell Invasion and Remodeling of Extracellular Matrix

Presentation Summary Confocal reflection and multiphoton second-harmonic-generation imaging represent powerful approaches to visualize native extracellular tissue scaffolds in live cell settings. Using 3-D collagen lattices and in vivo models of cancer invasion, we have resonstructed at high resolution the subcellular location of pericellular proteolysis during the migration process, resulting in extracellular matrix (ECM) realignment and migration track formation. The findings show how cell invasion and proteolytic ECM remodeling form a functional unit to generate multicellular invasion processes and large-scale remodeling of tissue structures and further reveal novel compensation strategies that rescue cell migration after protease inhibitor treatment.

Home Page http://www.rudolf-virchow-zentrum.de/forschung/friedl.html

Education M.D., University of Bochum, Germany (1992)
Ph.D., Cell Biology and Immunology, McGill University, Montreal, Canada (1996)
Specialist accreditation: Dermatologist (2002), Clinical Allergologist (2003)

Selected Activities & Honors Editorial Board Member, *Histochem. Cell Biol., Clin. Exp. Metast.*
Speaker, Graduate Program, "Target Proteins" (2004)
Felix-Wankel Animal Protection Award (1994)
German Skin Cancer Award (2004)
Oscar-Gans Research Award of the German Dermatological Society (2005)

Research Interests Mechanics and molecular dynamics during cell migration in 3-D ECM, cellular and molecular diversity and plasticity of cell migration, optical reconstruction of native tissue scaffolds (reflection, SHG, FLIM), role of cell migration in immune cell-cell signaling

Key Publications Relevant to Imaging

Friedl P, Maaser K, Klein CE, Niggemann B, Krohne G, Zänker KS (1997) Migration of highly aggressive MV3 melanoma cells in 3-D collagen lattices results in local matrix reorganization and shedding of alpha2 and ß1 integrins and CD44. Cancer Res. **57**, 2061-2070.

Gunzer M, Schäfer A, Borgmann S, Grabbe S, Zänker KS, Bröcker EB, Kämpgen E, Friedl P (2000) Antigen presentation in three-dimensional extracellular matrix: interactions of T cells with dendritic cells are dynamic, short lived, and sequential. Immunity **13**, 323-332.

Friedl P, Gunzer M (2001) Dynamics of antigen presentation: The serial encounter model. Trends Immunol. **22**, 187-191.

Hegerfeldt Y, Tusch M, Bröcker EB, Friedl P (2002) Collective cell movement from primary melanoma explants: plasticity of cell-cell-interaction, ß1 integrin function and migration strategies. Cancer Res. **62**, 2125-2130.

Wolf K, Mazo I, Leung H, Engelke K, von Andrian UH, Deryugina EI, Strongin AY, Bröcker EB, Friedl P (2003) Compensation mechanism in tumor cell migration: Mesenchymal-amoeboid transition after blocking of pericellular proteolysis. J. Cell Biol. **160**, 267-277.

Friedl P, Wolf K (2003) Tumour cell invasion and migration: Diversity and escape mechanisms. Nat. Rev. Cancer **3**, 362-374.

Wolf K, Muller R, Borgmann S, Bröcker EB, Friedl P (2003) Amoeboid shape change and contact guidance: T cell crawling through fibrillar collagen is independent of matrix remodeling by MMPs and other proteases. Blood **160**, 267-277.

Friedl P (2004) Prespecification and plasticity: shifting mechanisms of cell migration. Curr. Opin. Cell Biol. **16**, 14-23.

Friedl P, den Boer AT, Gunzer M (2005) Tuning immune responses: diversity and adaptation of the immunological synapse. Nat. Rev. Immunol. **5**, 532-545.

Friedl P, Wolf K, von Andrian UH, Harms G (2006) Biological second and third harmonic generation microscopy. Curr. Prot. Cell. Biol. (accepted)

Name Condeelis, John S. condeeli@aecom.yu.edu

Position Professor and Co-Chair, Department of Anatomy and Structural Biology, Albert Einstein College of Medicine

Presentation Title How Imaging Revealed the Molecular Mechanism of Tumor Cell Chemotaxis During Metastasis

Presentation Summary The recent convergence of technologies for expression profiling and intravital imaging has revealed the identities of the genes involved in the survival, adjuvant-resistance, and chemotaxis of invasive cancer cells inside living tumors. These genes fall into well-defined pathways and are coordinately regulated in metastatic tumor cells. This pattern is called the invasion signature. Of particular relevance to the chemotactic behavior of metastatic cancer cells is the finding that the genes coding for pathways leading to the minimum motility machine (i.e., the cofilin, capping protein, and N-WASP-Arp2/3 pathways) that regulate actin polymerization during invasion, and the directionality of cell protrusion during chemotaxis to EGF, are coordinately upregulated. This latter result is particularly relevant to the contribution of the tumor microenvironment to metastasis because chemotaxis to blood vessels is involved in the escape of cancer cells from primary mammary tumors. The cofilin and N-WASP pathways have been studied for their ability to alter metastatic outcome, and the results confirm the importance of the invasion signature in predicting metastasis in mammary tumors. This confirmation has stimulated the development of caged-cofilin and N-WASP biosensors which have been used to directly image the consequences of activation of these pathways in chemotaxis and invasion of tumor cells and have revealed the molecular mechanism of chemotaxis.

Home Page http://www.aecom.yu.edu/aif/intravital_imaging/introduction.htm

Education B.S., Physics, Rensselaer Polytechnic Institute, 1971
Ph.D., Biology, State University of New York, Albany, 1975
Postdoctoral Research, Biophysics, Harvard University, 1977

Selected Activities & Honors Rita Allen Foundation Scholar, 1979-84; Irma T. Hirschl Career Scientist Award, 1984-89; Editorial Board, Cell Motility and the Cytoskeleton, 1986-94, J. Cell Biol. 1995-2001; Cell Biology Study Section, NIH (member, 1989-93, chair, 1991-1993); Gordon Conferences on Motile and Contractile Systems, Co-Chair (1992) and Chair (1994) Board of Scientific Councilors, NHLBI, NIH, 1997, 2001; President, New York Society of Experimental Microscopists, 1997-1998; Consultant, NCI Tissue Microenvironment Initiative, 2001-2004 ; Council, the American Society for Cell Biology, 2006-2009

Research Interests Cell migration and chemotaxis, intravital confocal imaging of tumor invasion

Key Publications Relevant to Imaging

Wyckoff, Wang, Lin, Wang, Pixley, Graf, Segall, Pollard, and Condeelis (2004) A paracrine loop between tumor cells and macrophages is required for tumor cell migration in mammary tumors. *Cancer Res* 64:7022-7029.

Goswami, Wang, Wyckoff, and Condeelis (2004) Breast cancer cells isolated by chemotaxis from primary tumors show increased survival and resistance to chemotherapy. *Cancer Res* 64:7664-7667.

Ghosh, Song, Mouneimne, Sidani, Lawrence and Condeelis (2004) Cofilin promotes actin polymerization and defines the direction of cell motility. *Science* 304:743-746.

Lorenz, Yamaguchi, Wang, Singer, and Condeelis (2004) Imaging sites of N-WASP activity in lamellipodia and invadopodia of carcinoma cells. *Curr Biol* 14:697-703.

Mouneimne, Soon, DesMarais, Sidani, Song, Yip, Ghosh, Eddy, Backer and Condeelis (2004) Phospholipase C and cofilin are required for carcinoma cell directionality in response to EGF stimulation. *J Cell Biol* 166:697-708.

Yamaguchi, Lorenz, Kempiak, Sarmiento, Coniglio, Symons, Segall, Eddy, Miki, Takenawa, and Condeelis. (2005) Molecular mechanism of invadopodium formation: the role of the N-WASP/Arp2/3 complex pathway and cofilin. *J Cell Biol* 168:441-452.

Condeelis, Singer, and Segall (2005) The great escape: When cancer cells hijack the genes for chemotaxis and motility. *Annu Rev Cell Devel Biol* 21:695-718.

Condeelis and Pollard (2006) Macrophages: obligate partners for tumor cell migration, invasion and metastasis. *Cell* 124:263-266.

Name Reid, R. Clay clay_reid@hms.harvard.edu

Position Professor, Department of Neurobiology, Harvard Medical School

Presentation Title Micro-Architecture of Visual Cortex: Two-Photon Imaging of Functional Maps with Single-Cell Precision

Presentation Summary In the visual cortex, neurons within a column have similar functional properties, such as selectivity for stimulus orientation. Columns with distinct properties, such as different preferred orientations, tile the cortical surface in orderly patterns. This functional architecture was discovered with the relatively sparse sampling of microelectrode recordings. Optical imaging of membrane voltage or metabolic activity subsequently showed the overall geometry of functional maps, but averaged over many cells (resolution >100 mm). In recent experiments, we have labeled thousands of visual cortical neurons with a calcium-sensitive indicator in vivo. We then imaged the activity of neuronal populations at single-cell resolution with two-photon microscopy up to a depth of 400 mm. In cat visual cortex, functional maps were organized at a fine scale. Neurons with different preferences for stimulus direction or orientation were segregated with extraordinary spatial precision in three dimensions, with columnar borders one to two cells wide. These results indicate that functional maps in the cerebral cortex can be built with single-cell precision.

Home Page http://neuro.med.harvard.edu/site/reidweb/index.html

Education

1982, B.S., Yale University, Physics and Philosophy, Mathematics
1988, Ph.D., Rockefeller University, Biophysics
1991, M.D., Cornell University Medical College

Postdoctoral Training:
1988-90 Center for Neural Science, New York University (with Robert Shapley)

1991-93, Laboratory of Neurobiology (T. Wiesel), The Rockefeller University (with Dan Ts'o)

Selected Activities & Honors

Klingenstein Fellowship in the Neurosciences
2001, Young Investigator Award, Society for Neuroscience

Research Interests Two-photon calcium imaging of cortical activity, physiology and functional anatomy of the mammalian visual system, electrophysiology/multi-electrode recording

Key Publications Relevant to Imaging

Reid RC and Shapley RM (1992) The spatial structure of L, M, and S cone inputs to receptive fields in primate lateral geniculate nucleus. *Nature* 356:716-718.

Reid RC and Alonso JM (1995) Specificity of monosynaptic connections from thalamus to visual cortex. *Nature* 378:281-284.

Dan Y, Atick JJ and Reid RC (1996) Efficient coding of natural scenes in the lateral geniculate nucleus: Experimental test of a computational theory. ***J. Neurosci.*** 16:3351-3362.

Alonso JM, Usrey WM and Reid RC (1996) Precisely correlated firing of cells in the lateral geniculate nucleus. *Nature* 383:815-819.

Usrey WM, Reppas JB and Reid RC (1998) Paired-spike interactions and synaptic efficacy of retinal inputs to thalamus. *Nature* 395:384-387.

Dan Y, Alonso JM, Usrey WM and Reid RC (1998) Coding of visual information by precisely correlated spikes in the LGN. *Nature Neurosci.* 1:501-507.

Tavazoie ST and Reid RC (2000) Diverse receptive fields in the lateral geniculate nucleus during thalamocortical development. *Nature Neurosci.* 3:608-616.

Reinagel P and Reid RC (2000) Temporal coding of visual information in the thalamus. ***J. Neurosci.*** 20:5392-5400.

Kara P, Reinagel P and Reid RC (2000) Low response variability in simultaneously recorded retinal, thalamic, and cortical neurons. *Neuron* 27: 635-646.

Reid, RC and Shapley, RM (2002) Space and time maps of cone photoreceptor signals in macaque lateral geniculate nucleus. ***J. Neurosci,*** 22:6158-6175.

Ohki, K, Chung, S, Ch'ng, YH, Kara, P, Reid, RC. (2005) Functional imaging with cellular resolution reveals precise microarchitecture in visual cortex. *Nature* 433:597-603.

Name Fraser, Scott E. sefraser@caltech.edu

Position Anna L. Rosen Professor of Biology
Director, Biological Imaging Center
California Institute of Technology

Presentation Title To be announced

Home Page http://biology.caltech.edu/Members/Fraser

Education
B.S., Physics, Harvey Mudd College, 1976
Ph.D., Johns Hopkins University, Biophysics, 1979
Postdoctoral Research, MBL, Woods Hole; Johns Hopkins University, 1980

Selected Activities & Honors
2002, NASA/JPL Space Account Award
2002, R&D 100 Award
1999, Forbes Lecture, MBL
1997, Chipperfield Lecture, MIT
1995, Marcus Singer Medal
1990, Fellow, AAAS
1984-1987, McKnight Scholar Award
1983, Kaiser-Permanente Award

Research Interests Pattern formation during embryogenesis, in vivo imaging

Key Publications Relevant to Imaging

Liebling, M., Forouhar, A.S., Gharib, M., Fraser, S.E., and Dickinson, M.E. (2005) Four-dimensional cardiac imaging in living embryos via postacquisition synchronization of nongated slice sequences. J. Biomed. Opt. 10:054001.

Kulusa, P.M., Lu, C.C., and Fraser, S.E. (2005) Time-lapse analysis reveals a series of events by which cranial neural crest cells reroute around physical barriers. Brain Behav. Evol. 66:255-265.

Tyszka, J.M., Ewald, A.J., Wallingford, J.B., and Fraser, S.E. (2005) New tools for visualization and analysis of morphogenesis in spherical embryos. Dev Dyn. 234:974-983.

Rieger, S., Kulkarni, R.P., Darcy, D., Fraser, S.E., and Koster, R.W. (2005) Quantum dots are powerful vital labeling agents in zebrafish embryos. Dev Dyn. 234:670-681.

Kulkarni, R.P., Wu, D.D., Davis, M.E., and Fraser, S.E. (2005) Quantitating intracellular transport of polyplexes by spatial-temporal image correlation spectroscopy. Proc. Natl. Acad. Sci., 102:7523-7528.

Tyszka, J.M., Fraser, S.M., and Jacobs, R.E. (2005) Magnetic resonance microscopy: recent advance and applications. Curr. Opin. Biotechnol. 16:93-99.

Koster, R.W., and Fraser, S.E. (2004) Time-lapse microscopy of brain development. Methods Cell Biol. 76:207-235.

Jones, F.A., Baron, M.H., Fraser, S.E., and Dickinson, M.E. (2005) Dynamic in vivo imaging of mammalian hematovascular development using whole embryo culture. Methods Mol. Med. 105:381-394.

Megason, S.G., and Fraser, S.E. (2003) Digitizing life at the level of the cell: high-performance laser-scanning microscopy and image analysis for in toto imaging of development. Mech. Dev. 120:1407-1420.

Fraser, S.E. (2003) Crystal gazing in optical microscopy. Nature Biotechnol. 21:1272-1273.

Hadjantonakis, A.K., Dikinson, M.E., Fraser, M.E., and Papaioannou, V.E. (2003) Technicolour transgenics: imaging tools for functional genomics in the mouse. Nat. Rev. Genet. 4:613-625.

Name Gratton, Enrico egratton22@yahoo.com

Position Professor, Department of Biomedical Engineering, University of California at Irvine

Presentation Title To be announced

Home Page http://lfd.uiuc.edu/staff/gratton/index.html

Education

Ph.D., Physics, University of Rome, (1969)
Postdoctoral research, Biochemistry, University of Illinois at Urbana-Champaign

Selected Activities & Honors

American Physical Society Fellow (1991)
Gregorio Weber Award for Excellence in Fluorescence Theory and Application (2005)
Associate Editor, *Biophysical Journal*, *Journal of Fluorescence*
Organizer, International Weber Symposium on Innovative Fluorescence Methodologies in Biochemistry and Medicine (1986-present)
Scientific Advisory Board, Max Planck Institute for Biophysical Chemistry (2004-present)

Research Interests

Design of new fluorescence instruments, protein dynamics and hydration of proteins, IR spectroscopy of biological substances, Nucleic acids—fluorescent probe interactions

Key Publications Relevant to Imaging

Ragan, T., H. Huang, P. So and E. Gratton. 3D particle tracking on a two-photon microscope. *J. Fluorescence* (2006).

Gratton, E., S. Breusegem, N. Barry, Q. Ruan, and J. Eid. Fluctuation correlation spectroscopy in cells: Determination of molecular aggregation. Chapter 1 in: *Biophotonics-Optical Science and Engineering for the 21st Century*, Kluwer Academic/Plenum Publishers. Shen, X. Van Wijk, R. (Eds.) XVIII, pp. 1-14. ISBN: 0-387-24995-8 (2005).

Levi, V., Q. Ruan, M. Plutz, A. S. Belmont, and E. Gratton. Chromatin dynamics in interphase cells revealed by tracking in a two-photon excitation microscope. *Biophys. J.* 89, 4275-4285 (2005).

Digman, M. A., C. M. Brown, P. Sengupta, P. W. Wiseman, A. R. Horwitz and E. Gratton. Measuring Fast Dynamics in Solutions and Cells with a Laser Scanning Microscope. *Biophys. J*. 89, 1317-1327 (2005).

Digman, M. A., P. Sengupta, P. W. Wiseman, C. M. Brown, A. R. Horwitz, and E. Gratton. Fluctuation Correlation Spectroscopy with a Laser-Scanning Microscope: Exploiting the Hidden Time Structure. *Biophys. J. Biophys. Lett.* 88: L33-36L (2005).

Sanchez, S. A. and E. Gratton. Lipid-protein interactions revealed by two-photon microscopy and fluorescence correlation spectroscopy. *Acc. Chem. Res.* 38, 469-477 (2005).

Levi, V., Q. Ruan, and E. Gratton. 3-D Particle Tracking in a Two-Photon Microscope: Application to the Study of Molecular Dynamics in Cells. *Biophys. J.* 88, 2919-2928 (2005).

Barcellona, M. L., S. Gammon, T. Hazlett, M. Digman and E. Gratton. Polarized fluorescence correlation spectroscopy of DNA-DAPI complexes. *Microscopy Res. Tech.* 65(4-5), 205-217 (2004).

Ruan, Q., M. A. Cheng, M. Levi, E. Gratton and W. W. Mantulin. Spatial-temporal studies of membrane dynamics: scanning fluorescence correlation spectroscopy (SFCS). *Biophys. J.* 87, 1260-1267 (2004).

Kis-Petekova, K. and E. Gratton. Distance Measurement by Circular Scanning of the Excitation Beam in the Two-Photon Microscope. *Microscopy Res. Tech.* 63 (1), 34-49 (2004).

Gaus, K., E. Gratton, E. P. W. Kable, A. S. Jones, I. Gelissen, L. Kritharides and W. Jessup. Visualizing lipid structure and raft domains in living cells with two-photon microscopy. *Proc. Nat. Acad. Sci. USA* 100, 15554-15559 (2003).

Muller, J. D. and E. Gratton. High-pressure fluorescence correlation spectroscopy. *Biophys. J*. 85, 2711-2719 (2003).

Name Xie, X. Sunney xie@chemistry.harvard.edu

Position Professor of Chemistry and Chemical Biology, Harvard University

Presentation Title New Advances in Optical Imaging of Living Organisms

Presentation Summary The presentation will start with a brief summary of our work on live cell fluorescence imaging of gene expression, one protein molecule at a time, and then focus on recent developments in coherent antiStokes Raman scattering (CARS) microscopy. CARS microscopy allows noninvasive chemical imaging based on vibrational spectroscopy without the use of fluorophores. The applications to tracking cellular metabolism and in vivo imaging at video rate will be discussed.

Home Page http://bernstein.harvard.edu/

Education
B.S., Chemistry, Peking University, 1984
Ph.D., Chemistry, University of California at San Diego, 1990
Postdoctoral Fellow, University of Chicago, 1990-1992

Selected Activities & Honors
Senior Research Scientist to Chief Scientist, Environmental Molecular Sciences Laboratory, Pacific Northwest National Laboratory, 1992-1999
Professor of Chemistry and Chemical Biology, Harvard University, 1999-present
Coblentz Award, 1996
Raymond and Beverly Sackler Prize in the Physical Sciences, 2003
NIH Director's Pioneer Award, 2004
Fellow of the American Association for the Advancement of Science, 2006
Fellow of Biophysical Society, 2006

Research Interests Single-molecule spectroscopy and single-molecule enzymology; molecular interactions, conformational dynamics, and underlying mechanisms of macromolecule machines; quantification of gene expression in living cells; coherent antiStokes Raman scattering microscopy

Key Publications Relevant to Imaging

Yu, Ji; Xiao, Jie; Ren, Xiaojia; Lao, Kaiqin; Xie, X. Sunney, "Probing Gene Expression in Live Cells, One Protein Molecule at a Time," *Science*, Mar. 17 issue, (2006).

English, Brian P.; Min, Wei; van Oijen, Antoine M.; Lee, Kang Taek; Luo, Guobin; Sun, Hongye; Cherayil, Binny J.; Kou, S.C.; Xie, X. Sunney, "Ever-fluctuating Single Enzyme Molecules: Michaelis-Menten Equation Revisited," *Nat. Chem. Bio.*, 2, 87 (2006).

Evans, Conor L.; Potma, Eric O.; Puoris'haag, Mehron; Côté, Daniel; Lin, Charles P.; Xie, X. Sunney, "Chemical Imaging of Tissue *in vivo* with Video-rate Coherent anti-Stokes Raman Scattering Microscopy," *Proc. Natl. Acad. Sci. USA* 102, 16807 (2005).

Min, Wei; Luo, Guobin; Cherayil, Binny J.; Kou, S. C.; and Xie, X. Sunney, "Observation of a Power-Law Memory Kernel for Fluctuations within a Single Protein Molecule," *Phys. Rev. Lett.* 94, 198302 (2005).

Yang, Haw; Luo, Guobin; Karnchanaphanurach, Pallop; Louie, Tai-Man; Rech, Ivan; Cova, Sergio; Xun, Luying; and Xie, X. Sunney, "Protein Conformational Dynamics Probed by Single-molecule Electron Transfer," *Science* 302, 262 (2003).

van Oijen, Antoine M.; Blainey, Paul C.; Crampton, Donald J.; Richardson, Charles C.; Ellenberger, Tom and Xie, X. Sunney, "Single-Molecule Kinetics of Λ Exonuclease Reveal Base Dependence and Dynamic Disorder," *Science* 301, 1235 (2003).

Zumbusch, Andreas; Holtom, Gary R.; Xie, X. Sunney, "Vibrational Microscopy Using Coherent Anti-Stokes Raman Scattering," *Phys. Rev. Lett*. 82, 4142 (1999).

Sánchez, Erik J.; Novotny, Lukas; Xie, X. Sunney, "Near-field Fluorescence Microscopy Based on Two-photon Excitation with Metal Tips," *Phys. Rev. Lett.* 82, 4014 (1999).

Lu, H. Peter; Xun, Luying; Xie, X. Sunney, "Single-Molecule Enzymatic Dynamics," *Science* 282, 1877 (1998).

Xie, X. Sunney; Dunn, Robert C. "Probing Single Molecule Dynamics," *Science* 265, 361 (1994).

Name Wilson, Tony tony.wilson@engineering-science.oxford.ac.uk

Position Professor of Engineering Science, University of Oxford

Presentation Title Active and Adaptive Optics in Microscopy

Presentation Summary Among the key elements of a confocal microscope are the light source, the detector, and the nature of the focused spot of light that is used to probe the specimen. I will describe various ways in which the imaging performance of confocal microscopes may be enhanced by appropriate choice of source, detector, and imaging point-spread function.

Home Page http://acara.eng.ox.ac.uk/som/

Education B.A. (1976), M.A., D. Phil. (1979), University of Oxford
C. Eng, C. Phys, FIEE, FInstP

Selected Activities & Honors
Honorary Fellow, Royal Miroscopical Society
Prince of Wales Award for Industrial Innovation and Production
British Technology Group's Academic Enterprise Competition
National Physical Laboratory Metrology Award, 1984 and 1994
IEE Metrology Prize Award
National Physical Laboratory Award, Basic Science Category
General Editor, *Journal of Microscopy*
Honorary Executive Secretary, Royal Microscopical Society

Research Interests Applied optics, optical imaging, confocal microscopy

Key Publications Relevant to Imaging

Author of three books

Editor of 17 conference proceedings

Author or co-author of around 300 peer-reviewed papers

Booth MJ, Wilson T (2004) Low-cost, frequency-domain, fluorescence lifetime confocal microscopy. *J. Microsc.* 214:36-42.

Watson TF, Juskaitis R, Wilson T (2002) New imaging modes for lenslet-array tandem scanning microscopes. *J. Microsc.* 205:209-212.

Neil MA, Juskaitis R, Booth MJ, Wilson T, Tanaka T, Kawata S (2002) Adaptive aberration correction in a two-photon microscope. *J. Microsc.* 200:105-108.

Booth MJ, Neil MA, Juskaitis R, Wilson T (2002) Adaptive aberration correction in a confocal microscope. *Proc. Natl. Acad. Sci. USA* 99:5788-5792.

Karadaglic D, Juskaitis R, Wilson T (2002) Confocal endoscopy via structured illumination. *Scanning* 24:301-304.

Booth MJ, Neil MA, Wilson T (2002) New modal wave-front sensor: application to adaptive confocal fluorescence microscopy and two-photon excitation fluorescence microscopy. *J. Opt. Soc. Am. A Opt. Image. Sci. Vis.* 19:2112-2120.

Cole MJ, Siegel J, Webb SE, Jones R, Dowling K, Dayel MJ, Parsons-Karavassilis D, French PM, Lever MJ, Neil MA, Juskaitis R, Wilson T (2001) Time-domain whole-field fluorescence lifetime imaging with optical sectioning. *J. Microsc.* 203:246-257.

Booth MJ, Wilson T (2001) Refractive-index-mismatch induced aberrations in single-photon and two-photon microscopy and the use of aberration correction. *J. Biomed. Opt.* 6:266-272.

Name Betzig, Eric betzige@hhmi.org

Position Group Leader, Janelia Farm Research Campus, Howard Hughes Medical Institute

Presentation Title New Approaches to Cellular Imaging with High Spatial and Temporal Resolution

Presentation Summary Two new microscope concepts will be described: a proposed optical lattice microscope with predicted improvements in speed, resolution, photobleaching propensity, and molecular sensitivity and a newly demonstrated fluorescence microscope capable of near-molecular resolution.

Home Page

Education B.S., Physics, California Institute of Technology, 1983
Ph.D., Applied and Engineering Physics, Cornell University, 1988

Selected Activities & Honors 1992, William L. McMillan Award
1993, National Academy of Sciences Award for Initiatives in Research

Research Interests Super-resolution microscopy, live cell imaging, adaptive optics, high-speed image acquisition and processing

Key Publications Relevant to Imaging

E. Betzig, J.K. Trautman, "Near-field optics: microscopy, spectroscopy, and surface modification beyond the diffraction limit," *Science* **257**, 189 (1992)

E. Betzig, R.J. Chichester, "Single molecules observed by near-field scanning optical microscopy," *Science* **262**, 1422 (1993)

E. Betzig, R.J. Chichester, F. Lanni, and D.L. Taylor, "Near-field fluorescence imaging of cytoskeletal actin," *Bioimaging* **1**, 129 (1993)

J.K. Trautman, J.J. Macklin, L.E. Brus, and E. Betzig, "Near-field spectroscopy of single molecules at room temperature," *Nature* **369**, 40 (1994)

E. Betzig, "Proposed method for molecular optical imaging," *Opt. Lett.* **20**, 237 (1995)

J. Hwang, *et al.*, "Nanoscale complexity of phospholipids monolayers investigated by near-field scanning optical microscopy," *Science* **270**, 610 (1995)

E. Betzig, "Excitation strategies for optical lattice microscopy," *Opt. Express* **13**, 3021 (2005)

Name Larabell, Carolyn larabel@itsa.ucsf.edu

Position Professor, Department of Anatomy, University of California at San Francisco; Faculty Scientist, Physical Biosciences Division, Lawrence Berkeley National Laboratory

Presentation Title X-ray Tomography: 3-D Imaging of Whole Cells at Better Than 50 nm Resolution

Presentation Summary X-ray tomography generates 3-D images of rapidly frozen whole cells at better than 50 nm resolution. At the energy of x-rays used, carbon and nitrogen of cellular structures absorb an order of magnitude more than the surrounding cellular water, producing high-contrast images. Using x-ray absorbing markers, molecules can be localized to cellular structures. Collection of the tomographic data set requires about 3 minutes, making it possible to examine large numbers of cells.

The National Center for X-ray Tomography is funded by the National Center for Research Resources, National Institutes of Health, and the Office of Biological and Environmental Research, U.S. Department of Education, to continue development of this technology and to make it available to the scientific community.

Home Page http://ncxt.lbl.gov

Education B.S., Cell Biology, Arizona State University, 1976-1981
Ph.D., Cell Biology, Arizona State University, 1983-1988
Postdoctoral Fellow, Neurobiology, Stanford University, 1988-1989
Postdoctoral Fellow, Developmental, University of California, Davis, 1989-1990

Selected Activities & Honors Keith R. Porter Fellowship
Member, NIH Microscopic Imaging Study Section
Member, NIH Special Emphasis Study Sections, National Academy of Sciences Study on Revealing Chemistry Through Advanced Imaging Technologies

Research Interests Cell and developmental biology, cytoskeletal dynamics, development of imaging techniques

Key Publications Relevant to Imaging

X-ray Imaging

Le Gros, M.A., McDermott, G. and Larabell, C.A. (2005) X-ray tomography of whole cells. Curr Opin Struct Biol 15, 593-600.

Larabell, C.A., and Le Gros, M.A. (2004). X-ray tomography generates 3-D reconstructions of the yeast, Saccharomyces cerevisiae, at 60-nm resolution. Mol. Biol. Cell 15, 957-962.

Miao, J. Hodgson, K.O., Ishikawa, T., Larabell, C.A., LeGros, M.A., and Nishino, Y. (2003). Imaging whole *Escherichia coli* bacteria using single particle x-ray diffraction. PNAS USA. 100, 110-112.

Meyer-Ilse, W., Hamamoto, D., Nair, A., Lelievre, S.A., Denbeaux, G., Johnson, L., Pearson, A.L., Yager, D., LeGros, M.A., and Larabell, C.A. (2001). High resolution protein localization using soft x-ray microscopy. J. Micros. 201, 395-403.

Live Cell Light Imaging

Alivisatos, A. P., Gu, W. W. and Larabell**,** C. (2005) Quantum dots as cellular probes. Annu Rev Biomed Eng 7, 55-76.

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Position Professor, Biochemistry and Biophysics, University of California, San Francisco

Presentation Title OMX, a Microscope Platform for the Future?

Presentation Summary

In order to begin using the advanced microscopes developed over the years in our laboratories for real biology, it was necessary to go beyond the optical breadboard used to prototype the technologies. Structured illumination (SI) provides the widest possible application and the most practical integration with conventional microscopes. Toward this end, we designed an entirely new microscope from the ground up. Called OMX (for experimental), it was designed to do very rapid 3-D multiwavelength imaging on live samples and to be the first practical implementation of SI. OMX can record conventional widefield images simultaneously in four wavelengths, allowing four different labels to be visualized at once within a living cell at about 30-100 frames/second and optimized for every last photon. OMX can also do simultaneous two-color 3-D SI, which provides a remarkable 100nm XY and 350nm Z resolution. Because it was designed with novel, very rapid XYZ positioning, it can record two new modes, synthetic projection and synthetic stereo, at 30 and 20 3-D volumes per second. These newly created methods allow ~10µm-thick Z volumes to be imaged in-focus as either a single projection or a stereo pair providing very rapid, low-light-level imaging of cellular behavior in vivo. New biology gleaned from this technology will be presented. A new direction, using adaptive optics, will be discussed.

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Education

Pasadena College, Pasadena, CA, Chemistry, B.A., 1963
University of California, San Francisco, Medicine, 1963-1964
California Institute of Technology, Biology/Biophysics, Ph.D, 1970
Medical Research Council, Cambridge, England, Molecular Biology, Postdoctoral Research, 1970-74

Selected Activities & Honors

Helen Hay Whitney Foundation Fellow
Sigma XI

Research Interests Chromosomal structure and dynamics, image processing and restoration

Key Publications Relevant to Imaging

Moss, W.C., Haase, S., Lyle, J.M., Agard, D.A., and Sedat, J.W. (2005) A novel 3D wavelet-based filter for visualizing features in noisy biological data. J. Microsc. 219:43-39.

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Poster Topics

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Topic I: Probes

A Novel Green Fluorescent Protein Mutant as Improved Ratiometric Excitation or Emission pH Biosensor

Ranieri Bizzarri, Caterina Arcangeli, Daniele Arosio, Francesco Cardarelli, Paolo Faraci, Fernanda Ricci, and Fabio Beltram

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Intracellular pH is an important modulator of cell function since the activity of most proteins is affected by small changes of proton concentration. In spite of the fast diffusivity of H+ ions, the cellular distribution of pH is not uniform and varies in distinct cell microdomains. This makes protein-based fluorescent pH biosensors more advantageous than organic indicators since they can be selectively targeted to subcellular compartments by genetic engineering. Among fluorescent proteins the class of green fluorescent proteins (GFPs) stands as a naturally optimized structure for fluorescent probing of environmental pH. However, the development of ratiometric probes, which display multiple excitation or emission maxima with opposite dependence on pH, appears the only way to address the problem of variable cellular expression. Although some GFP-based ratiometric pH biosensors have been reported, most are not optimized for the physiological pH range of the cellular processes or rely upon FRET couples whose control is difficult to achieve.

Homology-Guided Directed Evolution of Red and Far-Red Fluorescent Proteins

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Live cell imaging has been revolutionized by the advent of fluorescent proteins such as GFP, DsRed, and their respective variants. When genetically fused to a protein of interest, these fluorescent proteins allow the researcher to qualitatively and quantitatively monitor protein dynamics in living cells. An essential property of a fluorescent protein, particularly for quantitative experiments, is that the fluorescent protein does not interact with itself or other cellular components and that it does not interfere with any cellular process. In the past several years, many new fluorescent proteins have become available, either through the cloning of novel genes from various marine organisms or through the directed evolution of previously characterized fluorescent proteins. Unfortunately, however, many of these new fluorescent proteins can perturb the very cellular processes that they are supposed to monitor. These perturbations are particularly evident when the protein of interest is present at high local concentrations or in regions of restricted diffusion, such as membranes. To circumvent this problem, we are using a directed evolution approach that is guided by the natural variation among fluorescent proteins. The result is new variants of DsRed that show reduced interference with cellular components and processes. We take advantage of the fact that approximately 50 homologous fluorescent proteins have been cloned. By comparing the DsRed sequence to homologous sequences and biasing the weight of close homologs, we are able to broadly and

efficiently sample sequence variations that are naturally present in fluorescent proteins. We have created new red (ex. 554, em. 588) and far-red (ex. 597, em. 627) monomeric DsRed variants that are engineered to be inert in cells. These new proteins are bright and photostable, and they do not perturb cellular function in several test assays.

Structural Dynamics of the Calcium Transport Complex From Skeletal Muscle Using GFP Derivatives and Bis-Arsenical Fluorophores

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Fluorescence resonance energy transfer (FRET) was used to investigate structural interactions between sarcolipin (SLN) and Ca-ATPase (SERCA), two membrane proteins that compose the calcium transport complex of skeletal muscle sarcoplasmic reticulum. SLN, a phosphorylated regulatory subunit, was labeled at the N-terminus with cyan (CFP) and yellow (YFP) derivatives of green fluorescent protein from jellyfish. SERCA, the large catalytic subunit, was labeled at the N-terminus with CFP and red fluorescent protein (coral DsRed), with retention of activity only in the cyan fusion. SERCA was also tagged with tetra-cysteine motifs (CCXXCC) in cytoplasmic domains for labeling with bis-arsenical fluorescein (FLASH). Native SERCA contains a three-cysteine cluster (670CRRACC) that labels poorly with FLASH. The Arg671-to-Cys substitution created a tetracysteine site (670CCRACC) that binds threefold to fourfold more FLASH than the wild-type enzyme. Other tetracysteine mutants of SERCA showed similar enhancement of FLASH labeling. Fluorescence microscopy demonstrated that SERCA and SLN are localized to the endoplasmic reticulum (ER) when expressed in Sf21 insect cells using the baculovirus system. Steady-state FRET experiments were used to monitor SLN-SLN and SERCA-SLN interactions. Selective photobleaching of YFP-SLN resulted in a large increase in CFP-SLN fluorescence emission, providing novel FRET evidence for self-association of SLN. In vivo photobleaching experiments demonstrated a maximum FRET efficiency of 40-60%, a linear relationship between acceptor bleach and donor enhancement, and a lag time of 30-60 seconds for Forster Transfer recovery, indicating that SLN exists as a stable bimolecular complex in cell ER. SERCA-SLN interactions were monitored using FRET measurements between CFP-SERCA/YFP-SLN in live Sf21 cells and CFP-SLN/670FLASH-SERCA in Sf21 cell homogenates. Both approaches gave a maximum FRET efficiency of 20-30%, providing novel constraints on oligomerization of SERCA-SLN subunits. Thus, genetically encoded FRET probes clearly demonstrate SLN-SLN and SERCA-SLN interactions and provide key data on structural dynamics of the calcium transport complex.

Simultaneous Imaging of RhoA and Cdc42 Activation in Living Cells

Louis Hodgson and Klaus M. Hahn

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The Rho family GTPases Cdc42 and RhoA are crucial regulators of motility, proliferation and apoptosis. The effects of Cdc42 and RhoA in cells are thought to be strongly determined by transient, localized changes in nucleotide state (GTP binding induces interaction with downstream effectors). The activation of Cdc42 and RhoA must be precisely coordinated in time and space during motility and other cell behaviors. We report the use of two fluorescent biosensors with complementary wavelengths, enabling study of Cdc42 and RhoA activation in the same living cell. The Cdc42 activation biosensor (MeroCBD: Merocyanine-labeled Cdc42 Binding Domain) is based on novel solvatochromic dyes designed for live cell imaging, whose fluorescence properties are strongly sensitive to changes in solvent or protein environment. The sensor design enables sensitive study of endogenous, unlabeled Cdc42. The single-chain, genetically encoded RhoA biosensor is based on FRET between CFP and YFP within a single chain containing also RhoA and a binding domain from Rhotekin. The single-chain RhoA biosensor is constructed to preserve normal interactions with GDI by maintaining an intact and free RhoA C-terminus. Data showing the coordination of Cdc42 and RhoA activation dynamics in vivo will be shown.

FRET Reporters of Histone Modifications and Transglutaminase-Mediated Site-Specific Protein Labeling in Living Cells

Chiu-Wang Lin and Alice Y. Ting

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We report new methodologies for monitoring posttranslational protein modification in living cells and site-specific incorporation of biophysical probes onto cell surface proteins. Genetically encoded, fluorescence resonance energy transfer (FRET)-based reporters were constructed for sensing cellular histone phosphorylation and methylation events. The reporters are four-part chimeric proteins with a peptide sequence from the N-terminus of histone H3, a phosphoserine (14-3-3 tau) or methyllysine (HP1 or Pc chromodomain) recognition domain, and CFP and YFP fluorescent proteins on the ends. Phosphorylation of the H3-serine28 phosphorylation reporter with Msk1 kinase in vitro resulted in a 20% YFP/CFP emission ratio increase. When tested in living HeLa cells, the reporter exhibited a rapid increase of the emission ratio 5-15 min prior to the nuclear membrane breakdown and peaked during cell division. Two methylation reporters were constructed for sensing H3-lysine9 and H3-lysine27 methylation. The reporters gave 60% and 28% emission ratio changes in vitro after vSET-catalyzed methylation and also showed significant FRET level differences in fibroblast cell lines either expressing or lacking the methyltransferase Suv39h1/2.

Transglutaminases have previously been used to conjugate small-molecule probes bearing primary amines to glutamine-containing proteins in vitro. We show that transglutaminase can also be used to mediate ligation of amine probes to cell surface proteins expressing a 6- or 7-amino acid consensus sequence (Q-tag). We demonstrate ligation of biotin, fluorescein, and

benzophenone probes to Q-tag-CFP and EGF receptor-Q-tag constructs expressed on the surface of live HeLa and HEK cells. The NFκB p50 transcription factor fused to a Q-tag was labeled with a benzophenone photo-affinity probe in vitro. Upon UV irradiation, we observed increased levels of p50 homodimerization in the presence of DNA or the binding protein Myotrophin.

Fluorophores to Sense Activation of Endogenous, Untagged Proteins in Living Cells

Dmitriy Gremyachinskiy, Louis Hodgson, Chris Johnston, David Siderovski, and Klaus M. Hahn

Fluorescent biosensors are very valuable for studying the dynamics of protein activation in vivo. However, they have been limited by the photophysical characteristics of available fluorophores and frequently the need to derivatize the targeted proteins. We describe here novel fluorophores for biosensor designs that report activation of endogenous, untagged proteins. A series of merocyanines was synthesized through combination of donor/acceptor heterocycles with a range of electron affinities. The photophysical properties of 30 dyes were compared (photostability, solvent-dependent shifts in excitation and emission maxima, quantum yields, and extinction coefficients). Water-soluble, functionalized dyes were synthesized via a new route for facile incorporation of an amino-sulphonate chain. Bright dyes (e > 200,000, QY > 0.5) with large fluorescence changes were used in biosensors. Dyes were validated in vitro and in vivo by substantially improving a published sensor of endogenous Cdc42 activity and by producing a novel sensor of G-alpha-i activity.

Real-Time Quantitative Monitoring of a Lipid Second Messenger, Diacylglycerol, in Cell Membranes Using a Protein Sensor

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Department of Chemistry, University of Illinois at Chicago, Chicago, IL, USA

Many dynamically regulated lipid molecules, including diacylglycerol (DAG) and phosphoinositides, serve as second messengers that orchestrate the spatiotemporal dynamics of cell signaling and membrane trafficking. Therefore, real-time quantification of these lipids will provide the critical information about the regulation of cell signaling and membrane trafficking. We have developed a new methodology to quantify the cellular concentration of DAG that activates C1-domain-containing proteins, including protein kinases C, using an engineered C1 domain that is chemically labeled with an environmentally sensitive fluorophore. This new sensor, when delivered into various mammalian cells by means of lipid carriers, allows a sensitive and robust quantification of DAG concentration without significant inhibitory effects on downstream signaling pathways. Thus, unlike fluorescence protein-tagged C1 domains previously used for visualizing DAG, this sensor serves as a genuine quantitative DAG probe that can be used to investigate how spatiotemporal dynamics of cellular DAG regulates multiple and divergent signaling pathways.

Unnatural Substrates of Biotin Ligase for Site-Specific Labeling of Cellular Proteins

Irwin Chen, Hemanta Baruah, Yoon-aa Choi, Mark Howarth, and Alice Y. Ting

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We report a highly specific, robust, and rapid new method for labeling cell surface proteins with biophysical probes. The method employs the *E. coli* enzyme biotin ligase (BirA), which sequence-specifically ligates biotin to a 15-amino acid acceptor peptide (AP). We found that BirA also accepts a ketone isostere of biotin as a cofactor, ligating this probe to the AP with similar kinetics and retaining the high substrate specificity of the native reaction. Because ketones are absent from cell surfaces, recombinant AP-fused cell surface proteins can be tagged with the ketone probe and then specifically conjugated to hydrazide- or hydroxylamine-functionalized molecules. We demonstrate this two-stage protein labeling methodology on purified protein, in the context of mammalian cell lysate, and on epidermal growth factor receptor expressed on the surface of live HeLa cells. Both fluorescein and benzophenone photoaffinity probes are incorporated, with total labeling times as short as 20 minutes.

To expand the scope of the methodology to intracellular labeling, we are reengineering biotin ligase to accept analogs bearing an azide functionality. Through this abiotic functional handle, diverse probes can be specifically conjugated via Huisgen or Staudinger ligations. We will describe our efforts to evolve a BirA mutant through in vitro compartmentalization that can utilize an azido analog of biotin.

We also seek to reengineer the peptide specificity of biotin ligase so that two or more proteins can be labeled simultaneously in the same cell. To this end, we have identified biotin ligases that have severely impaired or no reactivity toward the AP. Efforts to isolate an exclusive peptide substrate for these orthogonal biotin ligases through panning against phage-displayed peptide libraries will be described.

Single-Particle Tracking in Living Cells With Biotin Ligase and Re-Engineered Streptavidin

Mark Howarth[1], John Zimmer[1], Daniel Chinnapen[1], Pieter Dorrestein[2], Kimberley Gerrow[3], Melanie Grandy[1], Keizo Takao[4], Neil Kelleher[2], Alaa El-Husseini[3], Yasunori Hayashi[4], Moungi Bawendi[1], and Alice Ting[1]

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Fusions to fluorescent proteins have made an enormous contribution to our understanding of cell biology. However, fluorescent proteins have many limitations for single-particle tracking. Our objective is to develop methods for the labeling of proteins in living cells with biophysical probes, using the enzyme biotin ligase. Biotin ligase site-specifically biotinylates a lysine within a 15-amino acid acceptor peptide (AP) sequence. Mammalian cell surface proteins tagged with AP

can be biotinylated by biotin ligase added to the medium, while endogenous proteins remain unmodified. The biotin group then serves as a handle for targeting streptavidin-conjugated quantum dots (QDs). This helps address two deficiencies of antibody-based labeling: the size of the QD-conjugate after antibody attachment and the instability of many antibody-antigen interactions. Labeling with biotin ligase requires only 2 minutes and is specific for the AP-tagged protein. We used biotin ligase labeling to perform time-lapse imaging of single QDs bound to AP-tagged AMPA receptors in neurons. One problem with the use of streptavidin-conjugated QDs is that each streptavidin can bind 4 biotinylated surface proteins and so cause cross-linking. To combat this, we have engineered a chimeric streptavidin tetramer with a single functional biotin binding site. This "monovalent" streptavidin has the same affinity and off-rate for biotin as wild-type streptavidin. Labeling of biotinylated cell surface proteins with dye-conjugated monovalent streptavidin produced stable binding but did not cause clustering. Conjugation of monovalent streptavidin to QDs should produce QDs that are small, do not cross-link, and show binding that is stable over hours. Development of streptavidin tetramers with defined numbers of biotin binding sites may also find many applications in the construction of new sensors and nanostructured materials.

Towards Cellular Reporters for Detection of Endogenous Protein-Protein Interactions

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Networks of protein-protein interactions mediate cellular responses to the environment and direct the execution of cellular pathways. Existing technologies for detecting protein-protein interactions require either cell lysis (thus sacrificing spatial and/or temporal resolution) or fusion of reporter proteins to the protein partners of interest, thus perturbing their expression, function, and ability to interact with other biomolecules. Ideally, a method for reporting protein-protein interactions should detect endogenous proteins in a noninvasive manner, with high temporal and spatial resolution, and with sufficient sensitivity to allow single-cell imaging. We herein describe a methodology that should allow detection of unaltered, endogenous protein-protein interactions in single living cells. The overall strategy of our method is analogous to existing enzyme complementation assays, where an enzyme is dissected into two fragments, which are fused to the protein partners of interest. Interaction of the two proteins brings the reporter fragments into close proximity where they can fold into their active 3-D structure. In our method, however, the enzyme is a ribozyme (RNA enzyme), and protein fusion is circumvented by using RNA aptamers. The RNA aptamers, fused to the ribozyme halves, act as scaffolds that recognize the endogenous proteins of interest. Protein interaction results in ribozyme reconstitution and hence generation of a fluorescence signal that can be monitored in real time. We report preliminary results obtained when employing this methodology to the study of the homodimerization of the protein p50, a member of the family of NF-κB transcription factors. Ultimately, due to the modular nature of the system, the method should be generally applicable to nearly the entire proteome by simply changing the aptamer pair.

Topic II: Optics and Devices

Amplitude, Phase, and Polarization Microscopy

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Optical microscopy is one of the most widely used methods of investigation in medicine and biology. Many biological samples, including live cells and tissues, are transparent and behave, essentially, as phase objects. They produce little or no information in the bright field image so techniques such as phase contrast and Nomarski microscopy (differential interference contrast [DIC]) are employed for "optical staining." These techniques generate rather qualitative object information. Quantitative phase information is desired in many areas of cell imaging: live cell dynamics, evaluation of cellular mass distribution, cell motility and morphology, and image-based cytometry.

I present an imaging device, the sampling field sensor (SFS), capable of providing quantitative estimates of the phase and polarization (including birefringence) information from phase-only (transparent) samples. It is based on phase shift interferometry, a highly accurate phase measurement technique. However, unlike other interferometers, the SFS is virtually insensitive to vibrations, thus making possible for the first time the use of accurate quantitative phase measurement techniques in the typical biomicroscopy lab.

When coupled to a microscope using coherent illumination, the SFS will provide quantitative images of transparent cells or tissue architectures without fluorescent labeling or staining. Here I show the working principle of the SFS and experimental data demonstrating its phase and polarization measurement capability and its unprecedented vibration insensitivity. In addition, I present numerical simulations of imaging transparent cellular structures in the presence of detection noise due to embedding transparent clutter or instrumental error. Phase measurement is demonstrated by imaging micron-size, human-made, phase-only structures similar to those encountered in cell imaging, which also allow estimating the phase measurement accuracy of the instrument. Vibration insensitivity is demonstrated by lambda/100 accurate measurements obtained when strong shocks were applied to the optical breadboard (the optical path length of the instrument is 2 m).

Optics' Limitations on Spatial Resolution in Fluorescence Optical Mapping

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Optical mapping of excitable tissues has exposed the rich, complex dynamics of propagation behavior. Key to interpretation of macroscopic fluorescent recordings is the ability to localize the source of the signal, or spatial resolution. Sensor sensitivity restricts the ability to resolve differences in fluorescence intensity (i.e., intensity resolution) and thus couples spatial resolution to the gradient of the fluorescence signal, or contrast, which can vary across both space and time. As contrast is strongly affected by the performance of optics (e.g., the modulation transfer

function), we examined the relative role lenses play in determining spatial resolution and challenged assumptions borrowed from light microscopy where diffraction errors predominate. Simple geometric models and empirical testing revealed that, in low-magnification, widefield macroscopic fluorescence imaging, lenses can profoundly limit effective spatial resolution in the focal plane. Light sources outside the focal plane appear blurred, reducing contrast (and hence resolution) even further with the extent of degradation dependent on the distribution of fluorescence in three dimensions. In situations where high spatial frequency is expected – such as areas of slow propagation or fine anatomical detail – the true effective resolution of the imaging system must be considered to avoid incorrectly interpreting low spatial frequency content of macroscopic images.

As a part of a long-standing interest in the biophysics of GFP mutants, we developed a ratiometric pH biosensor, which shows an optimized working range around physiological pH. This mutant allowed the selection of excitation lines (in the range 400-500 nm) and emission intervals (in the range 480-600 nm) to obtain a ratiometric signal dependent only on the pH value with a tunable dynamic range. The presence of anions that bind reversibly to this protein variant was demonstrated not to affect the pH-dependent absolute calibration curve. The GFP biosensor was used to monitor the intracellular pH in living cells under different physiological conditions and was targeted to the nucleolar or nuclear regions of the cell.

Novel Simultaneous Multi-Focal Plane Imaging Technique for the Study of the Cellular Trafficking of Immunoglobulin G and its Receptor FcRn in Three Dimensions

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The study of protein trafficking pathways in three dimensions is important for understanding cellular processes. Widefield fluorescence microscopy is extensively used to study intracellular trafficking events. On the other hand, total internal reflection fluorescence (TIRF) microscopy can be used to image events, such as exocytosis, that occur on the cellular membrane. However, the imaging of three-dimensional trafficking pathways from the cell interior to the cellular membrane requires that the focus of the objective be changed since the events occur at different focal planes. However, the typical devices used to change the focal plane are relatively slow. Thus, for example, when the cell interior is being imaged, important events on the cell membrane can be missed.

To overcome these limitations, we have developed a novel microscopy technique that enables the simultaneous imaging of multiple focal planes within a cell (1). This technique uses multiple detectors, each of which simultaneously images a distinct plane within the sample. By labeling the cellular organelles with different fluorophores and illuminating the sample in both TIRF and widefield mode with multiple laser lines, this technique can be used to study protein trafficking events in the cell interior as well as simultaneously on the cellular membrane.

We have made use of this technique to study the trafficking pathways of immunoglobulin G (IgG) and its salvage receptor, FcRn, in human endothelial cells. Such trafficking pathways from inside the cell to the cellular membrane would be difficult to study using conventional imaging

techniques. The use of quantum dot-IgG complexes has allowed us to track events for long periods of time in three dimensions.

[1]P. Prabhat, S. Ram, E.S. Ward and R.J. Ober, IEEE Transaction on Nanobioscience, 3(4), 237-242, (2004).

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Translational Diffusion of Fluorescent Proteins and Aggregates by Molecular Fourier Imaging Correlation Spectroscopy

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The ability to noninvasively observe the translational diffusion of proteins and protein complexes is important to many biophysical problems related to dynamic processes in living cells. We report high signal-to-noise (S/N $\geq$ 250) measurements of the translational diffusion in viscous solution of a fluorescent biomolecular species: DsRed, a mutant of the green fluorescent protein that forms oligomeric complexes. Our experiments are carried out using a new technique: molecular Fourier imaging correlation spectroscopy (M-FICS). M-FICS is an interferometric method that detects a collective Fourier component of the fluctuating density of a small population of fluorescent molecules and provides information about the distribution of the molecular species present. In addition to the self-diffusion of the DsRed tetramer, we observe the self-diffusion of multiple aggregate species. A theoretical analysis is presented that expresses the detected signal fluctuations in terms of the relevant time correlation functions for molecular translational diffusion. Polarization-resolved variations of M-FICS will provide simultaneous information about protein translational motions, intramolecular fluctuations, and protein-protein interactions.

Dynamic Imaging: RICS Reveals Fast Dynamics in the Cell Interior

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Dynamic imaging in which fast events in the cell interior are visualized is becoming an important new imaging modality. Interactions among molecules can be transient and last for a few milliseconds, or a stable complex can form. New methodologies are needed to extract this information in live cells. We have developed a novel method of analysis from images obtained with a conventional laser scanning microscope that exploits the time sequence of raster-scanned images. This approach, termed “raster-scan image correlation spectroscopy” (RICS), exploits the intrinsic time structure of a confocal raster-scan images to determine spatiotemporal correlations at several time scales simultaneously, corresponding to the pixel sequence, the line sequence, and the frame sequence. With this method, maps of protein diffusion inside living cells, binding processes, and aggregation maps from a photon-counting histogram (PCH) analysis are generated

for the entire image. In this study we present the dynamics of the EGFP-tagged adaptor protein, paxillin, at focal adhesions. We discuss the unique features of the RICS approach to extract spatiotemporal correlation in a very wide time scale. With the advent of fast camera systems, we have also been able to obtain dynamic information with total internal reflection fluorescence (TIRF) microscopy. Here we also present the dynamic interaction between CFP-actinin and YFP-actin during cellular migration. For this analysis the emissions from the CFP and YFP are simultaneously recorded, and cross-correlation methods are used to study the interaction among these two proteins.

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Towards Non-Invasive, High-Resolution Hyperspectral Imaging of Cells and Tissues

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Coherent anti-Stokes Raman scattering (CARS) is a nonlinear scattering method, using three photons (a pump, Stokes, and probe photon) to generate an anti-Stokes photon, the energy of which bears relationship to vibrational levels of molecules within the sample. Microscopy based on CARS (μCARS) can be used for chemical imaging cells and tissues without extrinsic tagging. We have developed an adaptation of μCARS microscopy that uses a single Ti:Sapphire laser, but gives > 2500 cm-1 bandwidth, sufficient for simultaneous identification of many distinct species in chemically complex samples, including discrimination between cells with subtly different differentiation states. Using power levels that are not toxic to rapidly dividing cells, we are able to acquire a single hyperspectral pixel in milliseconds, so that the technique is amenable to 3-D imaging of live, developing cells and tissue. We present progress toward reducing the negative effects of a nonresonant background that is intrinsic to this method and typically obscures vibrational signatures of cell components, such as amide bands from proteins.

Automated Detection and Analysis of T-Cell Intracellular Calcium Using a Nanophysiometer

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Cell function is mediated by complex, stochastic cascades of intracellular and extracellular signaling molecules, which leads to specific cellular responses. Understanding the connectivity and causality between signaling molecules requires the ability to independently observe isolated, individual cells and groups of cells in statistically relevant quantities at sufficiently high bandwidth and under controllable and repeatable conditions throughout the course of the cellular

events being observed. Currently available techniques for studying cellular signaling pathways are limited in one or more of these respects.

We have developed a fully automated, timed acquisition system incorporating a novel microfluidic nanophysiometer cell platform. The nanophysiometer, constructed using PDMS and soft-lithography techniques, consists of many spatially separated cell traps. The cellular microenvironment is controllable and easily manipulated by administration of biologically active agents. An automated microscope was programmed for fluorescence acquisition from hundreds of isolated cells or cell clusters at bandwidths up to 10 Hz and up to five colors.

We loaded 1,000 primary T cells into the nanophysiometer and recorded bright field images and calcium-indicator fluorescence during and after exposure to the calcium ionophore ionomycin. Data were collected at precise time intervals, and all images were processed by custom software that detects the presence of cells and their fluorescence at each time point. 85% of the traps were occupied with at least one cell, with an average of 2.6 cells per occupied trap (range 1-6). Analysis revealed two subsets (subset I, apoptotic; subset II, nonapoptotic) of calcium activity characterized by differences in maximum fluorescence increase above baseline of 31% for subset I versus 10% for subset II ($p<0.05$). We have shown that this nanophysiometer/microscopy system is ideal for studying controlled activation of large numbers of T cells by individual dendritic or artificial antigen-presenting cells and that the software allows detection of differences in the temporal behavior of cells to better understand complex cell signaling activities.

Two-Photon Fluorescent Microlithography for Live-Cell Imaging

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Fluorescent dyes added to UV-cure resins allow the rapid fabrication of fluorescent micropatterns on standard glass coverslips by two-photon optical lithography. We use this lithographic method to tailor fiduciary markers, focal references, and calibration tools for fluorescence and laser scanning microscopy (LSM). Fluorescent microlithography provides spatial landmarks to quantify molecular transport, cell growth, and migration and to compensate for focal drift during time-lapse imaging. We show that the fluorescent-patterned microstructures are biocompatible with cultures of mammalian cell lines and hippocampal neurons. Furthermore, the high-relief topology of the lithographed substrates is utilized as a mold for poly(dimethylsiloxane) (PDMS) stamps to create protein patterns by microcontact printing, representing an alternative to current etching techniques. We present two different applications of such protein patterns for localizing cell adhesion and guidance of neurite outgrowth.

Ultrasensitive Magnetic Resonance by Mechanical Detection

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Magnetic resonance is a ubiquitous technique for the interrogation of chemical and biological systems. Despite this prominence, the intrinsic low sensitivity of inductive detection has prohibited the application of magnetic resonance to single cells and molecules. Magnetic resonance force microscopy (MRFM) has been proposed as a route to magnetic resonance imaging with single-nucleus sensitivity. MRFM brings the possibility of subsurface, nondestructive, chemically specific imaging to the length scale of a single molecule.

In MRFM a magnet-tipped microcantilever is brought close to the sample. Due to the high field gradient from the magnetic tip, only electron or nuclear spins in a thin slice of the sample satisfy the resonance condition. These spins can be manipulated using a nearby radio frequency coil and registered as a force, or a force-gradient, on the microcantilever's magnetic tip. The resulting forces are only a few attonewtons, (1 aN = 10-18 N), making them some of the smallest ever measured. We have successfully fabricated custom microcantilevers capable of detecting such small forces and have demonstrated a sensitivity of ~105 proton spins at 4 Kelvin (1 Hertz bandwidth).

We will improve the sensitivity of our measurements by attacking two remaining technical challenges. First, we have explored the dominant source of noise in our measurement: noncontact friction between the magnetic tip and the sample. We have demonstrated, at room temperature, that this noncontact friction can result from dielectric fluctuations within the sample. These findings open a new route to the detection of dielectric fluctuations at the nanoscale and suggest that appropriate sample preparation may minimize noise in MRFM measurements. Second, we are exploiting advanced electron beam lithography to reduce the size of our magnetic tips from 10 micrometers to 50 nanometers. Using these new tips, we project a sensitivity of 1,000 protons.

Topic III: Image Processing and Informatics

Visual-Serving Optical Microscopy (VSOM) for Synergistic, Bidirectional Coupling of Living Cells With Remote Computer Algorithms

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We have converted an automated digital imaging fluorescence microscope into a visual-serving optical microscopy (VSOM) instrument capable of performing a new class of live cell assays. Visual-serving, a term from the fields of computer vision and robotics, refers to the automated, real-time control of mechanical devices (e.g., syringe pumps) based on the ongoing analysis of digital image content (e.g., individual cell responses). In VSOM live cell assays, cells are one part of a dynamic data-driven application system (DDDAS). As stated on the NSF website, "DDDAS is a paradigm whereby applications/simulations and measurements become a symbiotic feedback control system. DDDAS entails the ability to dynamically incorporate additional data into an executing application, and in reverse, the ability of an application to dynamically steer the measurement process." We will present a VSOM DDDAS software architecture and VSOM experimental results that demonstrate synergistic, bidirectional coupling of complex, live cell systems with remote computer algorithms. The architecture consists of (i) a local instrument control application (ICA) that controls the microscope and peripherals, (ii) an orchestration component that coordinates experiments at a high level (BPEL Maestro™, Parasoft Corporation, Monrovia, CA), and (iii) a remote analytics component that analyzes experimental data and proposes actions in real time. The instrument, the orchestration component, and the analytics component are physically located in different cities. The orchestration component expresses the high-level control logic of the experiment in business process execution language (BPEL) and indirectly controls the experiment via web service interactions with the ICA and remote computer algorithms provided by collaborators. Repetitive, remote computer-generated cell perturbations and the resultant physiological responses provided by live cells thus represent a bidirectional communication that couples living cells with remote computer resources. Such resources can repeatedly form and test hypotheses that may be based on models, simulations, or other on-line biological knowledge.

Robotic Microscope for Signaling Pathway Analysis

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Responses to a stimulus vary widely from cell to cell within a cell population—bulk biochemical assays average out subtle differences in activation kinetics and location, obscuring precise mensuration of signaling. To understand spatiotemporal control of signaling, many cells need to be examined individually so that behavioral variations across a cell population can be taken into account. We are working toward building a robotic microscope that can automatically image hundreds of cells per experiment and utilize image processing tools that can extract and display

data from across the population. Expensive commercial imagers use only air objectives that have low light-gathering capability and are not suitable for imaging many live-cell biosensors. A software-based fast autofocus routine was implemented to overcome focal drift during multi-field, time-lapse experiments. Modular subroutines were written in both LabView and C++ languages to enable implementation by users on PCs with readily available laboratory imaging systems. The versatile modules can be integrated to construct "robotic imagers" based on off-the-shelf microscope components. The robustness of the autofocus was tested with oil-immersion objective lenses, which present challenges relative to air immersion lenses. An autofocus standard deviation of 5.30 nm (n = 50) was achieved. The instrument was used to scan fields of cells expressing a genetically encoded RhoA GTPase biosensor based on CFP-YFP FRET. Upon nocodazole treatment (30 μM), serum-starved mouse embryo fibroblast (MEF) cells showed an increase of RhoA activity in the cell periphery. With automated data acquisition and analysis, and new means to represent results spanning cell populations, this robotic microscope will hasten our understanding of spatiotemporal dynamics in signaling, and enable genome-wide screening of proteins regulating signaling.

Intelligent Interfaces for Interactive Analysis of High-Content Cellular Images

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RNA interference (RNAi) is a revolutionary approach for silencing gene expression and has become an essential method for studying genes through RNAi-induced cellular phenotype analyses. High-content screening (HCS) is a powerful technology for generating cellular images that are rich in phenotypic information. The convergence of RNAi and HCS (RNAi-HCS) has led to high-throughput, image-based studies of cellular phenotypes under various stimulations and systematic RNAi. However, great challenges arise when combining the technologies. The cellular images generated by the RNAi-HCS technology are relatively new to image analysis and computer vision research. Existing HCS image analysis tools are inadequate to capture the profound aspects of cellular phenotypes regarding cellular morphology and therefore limit the scope of RNAi-HCS screening to analyses on simple marker readouts. Domain knowledge is yet to be accumulated for developing image processing techniques and algorithms to analyze RNAi-HCS images encoding complex cellular morphology. This requires teamwork between experimental and computational biologists. To this end, we have developed a prototype system for interactive mining of cellular phenotypes—imCellPhen. The imCellPhen system allows human experts to visually explore large-scale cellular image databases in effective ways and retrieve images similar to ones of their interests from a large-scale HCS image database. In addition, imCellPhen enables biologists to provide feedbacks to the system, which are automatically used by imCellPhen to train computational models to recognize the important parameters in the images. Such an interactive procedure for image exploration and processing not only satisfies the information needs of biologists but also allows domain knowledge to be implicitly accumulated by the intelligent interfaces of imCellPhen. We demonstrate imCellPhen in the context of genome-wide, RNAi-base morphological screens of *Drosophila* primary neural cells. The scheme and techniques developed in this research can eventually be applied to study other types of cells using the RNAi-HCS technology, as well as image-based drug screening.

Arclength Parameterization and Optical Flow Calculation of Membrane Velocity Reveals Role of Actin in the Phases of Cell Spreading

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We present an image analysis scheme to quantify membrane velocity for time-lapse sequences of cell spreading, polarization, and movement. Segmentation of each image in the time-lapse sequence is performed by fitting a Gaussian mixture model to the distribution of pixel intensities using the Expectation Maximization algorithm, determining a threshold value used to separate the foreground and background classes. We then parameterize the cell contour by arclength, accommodating cells of complex morphologies, and calculate membrane velocity using an optical flow technique. The high temporal and spatial resolution of this technique combined with its low computational cost enables us to accurately measure motility parameters of mouse fibroblasts spreading on a fibronectin matrix. We show the dependence of these motility parameters on the concentration of cytochalasin D (CD), a classical modulator of actin-based motility. While CD decreases the overall rate of area growth during cell spreading, it has different effects depending on the phase of spreading. In particular, we find that the contractile phase of spreading is very sensitive to CD while the rapid spreading and basil phases are relatively insensitive to CD. These results support our working hypothesis that different protein complexes and mechanisms are involved in the different phases.

Wavelet-Based Analysis of Cellular Signaling Domains

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We present a new strategy for the identification of the functional signaling domains based on wavelet analysis of fluorescence resonance energy transfer (FRET) microscopy data. A FRET-based, plasma membrane-tagged, protein kinase C (PKC) reporter (Violin J.D. et al, J. Cell. Biol., 2003) was expressed in COS1 cells, and this system was utilized as a model for developing a wavelet-based approach for ascertaining signaling domains. Continuous wavelet transforms (CWTs) were applied to FRET signals, resulting from activation of PKC with phorbol-12,13 dibutyrate, acetylcholine, or calcium influx through periodically stimulated voltage-gated calcium channels. The differences between wavelet coefficient matrices revealed several heterogeneous functional domains reflecting the dynamic balance between PKC and phosphatase activity. The transient activity of several domains was not represented by the average activity over the entire membrane. Two-dimensional signals in the defined plasma membrane region were converted to one dimension via a linearization procedure for the 1-D CWT analysis. To overcome the limitations imposed by this procedure, we have extended our methodology to incorporate a 2-D CWT. Signaling microdomains identified by the 1-D and 2-D CWT methods were comparable for fluorescence- and computer-generated images. Our results demonstrate that 1-D and 2-D CWT is

an effective new approach to study cellular signaling and may provide a framework for future 3-D applications.

Robust Analysis for Live Cell Synaptic Vesicle Recycling Assays

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The use of live cell time-lapse subcellular microscopy to assay the exo-endocytic recycling of synaptic vesicles provides an important tool for understanding presynaptic function. However, the analysis for these experiments is challenging because of the large number of small, fluorescently labeled nerve terminals with a weak and unstable signal. The large number of synapses makes manual analysis impractical. The weak signal further makes the analysis result unreliable. These impose a critical limitation on assay outcomes.

We have developed image analysis methods for fully automated identification of FM dye-labeled presynaptic terminals. We have also developed robust methods for time-lapse signal enhancement, feature measurement, and model parameter fitting. In this study, we compare the fully automated results with previously published results obtained manually. In addition to providing faster and unbiased analysis, the results show that robust analysis methods can also improve assay quality as measured by the Z score and S:N metric.

Two and Three Dimensional Segmentation of Whole Cells and Cell Nuclei in Tissue

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To understand the molecular basis of cell-cell interactions that drive tissue development, function, and tumorigenesis, it is necessary to quantitatively analyze specific molecules in adjacent individual cells or cell nuclei in intact tissue. However, the widespread use of such approaches is prevented by the lack of an efficient method that ensures the correct segmentation of each whole cell from 3-D microscope images. Therefore, we developed computer programs, based on dynamic programming, for identifying near-optimal surfaces of cells or cell nuclei labeled with fluorescent cell surface or nuclear markers.

The 2-D algorithm defines the optimal border as having an average intensity per unit length greater that any other possible border around the same cell or nucleus. Correct segmentation of each cell/nuclei is guaranteed by the algorithm, requiring modest user interaction that consists of manually indicating two points per cell, one inside and the other on the border. Thereafter, segmentation is automatic. Virtually 100% of cells are correctly detected, because determination

of the optimal path is not significantly affected by noise, intermittent labeling of the cell's border, or a diffuse border. Additionally, the algorithm allows subsequent interactive correction of any segmentation errors.

Extension to 3-D segmentation begins with 2-D segmentation in an x-y plane approximately through the center of the cell, followed by automatic finding of the two surfaces in the planes above and below the initial x-y plane. The algorithm does not find the true optimal surface but closely approximates it by successively finding optimal partial surfaces. Following segmentation, the user may interactively correct any perceived errors by adding points that are required to be on the surface. The algorithm has been tested on a wide variety of biological tissue samples and will segment moderately irregularly shaped cells with concavities.

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Topic IV: Imaging Single Molecules

A New Resolution Measure With Application to Single Molecule Studies—How Far Can We Go Beyond Rayleigh's Limit?

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In single-molecule microscopy, quantitative analysis of the acquired data plays a crucial role in the interpretation of results. In the recent past, several groups have demonstrated nanometer-level accuracy in estimating single-molecule location, and some groups have even reported measurement of single-molecule distances well below Rayleigh's resolution limit. In general, to carry out such studies, it is important to have a methodology available to be able to assess the fundamental limits of a microscope setup for a given experimental configuration. Here, by adopting an information-theoretic stochastic framework, we present results that quantify the capabilities of an optical microscope for single-molecule applications.

We consider two problems that are of current interest in single-molecule studies (i.e., the resolution problem and the localization accuracy problem). For the resolution problem, we derive a novel resolution measure that predicts the accuracy with which the distance between two single molecules can be determined for a given imaging condition. Our new resolution measure shows that the resolution of an optical microscope is not limited but that the resolvability of single molecules depends on the collected photon count. We also investigate the effect of deteriorating experimental factors such as pixelation of the detector and additive noise sources on the resolution measure. By imaging closely spaced single molecules with different distances of separation, we experimentally verify that these distances can be determined with accuracy as predicted by the resolution measure. For the localization accuracy problem, we present analytical formulae for the fundamental limit to the 2-D/3-D localization accuracy of a single molecule. We also show how the fundamental limit is affected by deteriorating experimental factors such as pixelation of the detector and additive noise sources.

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Single Molecule Microscopy to Dissect the Spatiotemporal Heterogeneity of Toll-Like Receptor Activation Clusters

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Live cell single-molecule imaging is a powerful approach to monitor the spatiotemporal dynamics of biological reactions. Especially in the case of membrane located signal transduction processes, the mobility and cluster sizes of proteins in membranes are believed to play an important role.

Our experimental setup consists of an inverted microscope, laser excitation between 458 and 647 nm, and two-channel detection on an ultra-sensitive CCD-camera. Most importantly, the setup allows for the detection of individual YFP molecules, which enables us to take full advantage of this protein-labeling technique (i.e., fixed label stoichiometry and biocompatibility). We achieve a spatiotemporal resolution of 35 nm and 5 ms under carefully controlled physiological conditions.

We applied this technique to the investigation of Toll-like receptors, the primary sensors of the innate immune system, which recognizes and defends microbial invasions. An HEK293 cell line stably expressing hTLR2::YFP was established and shown to be functional in interleukin 8 responses on stimulation with bacterial lipoprotein and cell extracts from *Mycobacterium marinum*.

Individual TLR2s were continuously detected over 45 minutes in the plasma membrane of a single cell. At about 5-15 minutes after stimulation with bacterial lipoproteins, we observed a transient formation of TLR2 clusters. While the fraction of clusters with respect to monomers was found to be dose dependent, kinetics of the cluster formation was not.

Our next aim is to detail the characterization of the TLR activation cluster by simultaneously monitoring fluorescent-labeled receptors, coreceptors, ligands, and established lipid raft markers.

One at a Time: Tracking NGF Signaling Endosomes in Live Neurons

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Nerve growth factor (NGF) is a signaling molecule that is critical for neuronal survival, differentiation, and maintenance. NGF signaling begins at the nerve terminal, where it binds and activates the receptor tyrosine kinase TrkA; the NGF signal is then transmitted retrogradely through the axon to the cell body. The mechanisms by which the NGF signal is propagated from the axon terminal to the cell body have been the topic of great interest. Here we report the first direct observation of individual NGF-containing endosomes being actively transported within the axons of dorsal root ganglia neurons toward their cell bodies. We used quantum dots as fluorescence probes and in vivo single-molecule imaging techniques to track the transport of NGF in living neurons. Live imaging of NGF axonal transport revealed rapid, directional movement interrupted by frequent pauses. The net movement was exclusively retrograde, but short-distance antegrade movements were observed occasionally. Furthermore, quantitative analysis revealed that the majority of endosomes contained a single NGF dimer, in contrast to the general belief that multiple NGF molecules reside in each signaling endosome. Our approach uncovered unprecedented details of NGF retrograde transport and serves as a powerful tool to examine further the formation, trafficking, and signaling properties of NGF signaling.

Tracking of Quantum Dot-Labeled CFTR Reveals Dramatic Immobilization in the Plasma Membrane Produced by PDZ Interactions

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The cystic fibrosis transmembrane conductance regulator (CFTR) protein is a cAMP-regulated chloride channel, which when mutated, can cause cystic fibrosis. Here we report very low plasma membrane mobility of CFTR, which was increased greatly by disrupting PDZ domains coupling to actin via the C-terminal PDZ-binding motif. Single-molecule tracking of quantum dot-labeled CFTR was done in transfected cells expressing low, native-like levels of human wild-type CFTR containing an extracellular epitope (3-HA) tag. In multiple cell lines, CFTR diffused little in the plasma membrane, generally not moving beyond a 100-200 nm radius. However, most CFTR became mobile over >1000 nm with D ~2–3 × 10-10 cm2/s after (a) C-terminal truncation (Δ26 or Δ70) or blocking (C-terminal GFP addition) the PDZ-binding motif; (b) expression of a dominant-negative EBP50/NHERF1 mutant lacking its C-terminal ezrin binding domain; or (c) skeletal disruption (latrunculin). CFTR also became mobile when its expression was increased by > tenfold over native levels, suggesting that excess CFTR is not properly coupled to its C-terminus binding partners. Our data demonstrate remarkable and previously unrecognized immobilization of CFTR in cell plasma membranes and provide direct evidence that C-terminal coupling to the actin skeleton via EBP50/ezrin is responsible for its immobility.

Single-Molecule Fluorescence Studies of the Sliding Clamp and Clamp Loader From *Methanosarcina Acetivorans*

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DNA polymerases generally become more processive with the loading of a circular clamp (proliferating cell nuclear antigen, PCNA) behind the polymerase. The clamp has been shown to increase the length of the replicated DNA by tethering the polymerase to the DNA. A protein called replication factor C (RFC) that is conserved in many organisms and is necessary for life loads the PCNA to the DNA. In this study, we visualize the loading of PCNA by RFC from the archaeon *Methanosarcina acetivorans* by single-molecule FRET. Recent studies by Chen and colleagues[1] show that MacRFC contains two distinct small subunits, the first RFC to be studied with this feature. Only indirectly had Mac RFC been observed to load PCNA using polymerase activity studies; however, we are now able to directly observe the interaction between the PCNA and DNA using fluorescent labels at both bulk solution and single-molecule levels. We find that RFC is able to load the PCNA to several different DNA constructs and that PCNA is able to be trapped on the DNA by use of bound proteins and four-way junction DNA.

[1]Chen et al. (2005) JBC 280, 41852-41863.

Single-Molecule FRET Studies of DNA Mismatch Repair Initiation Dynamics

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We use single-molecule FRET to measure DNA conformational changes in the presence of mismatch repair initiation gene MutS. MutS is the cofactor that recognizes a single DNA mismatch and facilitates a cascade of events that leads to repair of the mismatch. Prior results have shown that when MutS is bound at a mismatch, two DNA conformations ensue: bent and unbent. We are using single-molecule FRET to measure the conformational dynamics of mismatch DNA from bent to unbent and relate these relative conformations to total repair efficiency.

We have measured conformation fluctuations of two DNA mismatches when Thermus aquaticus (Taq) MutS is bound: an unpaired thymine (T-bulge) and a single G:T mismatch. Results show that conformational dynamics vary from mismatch to mismatch, with GT mismatch DNA displaying faster conformational changes as well as forming high-energy, high-FRET conformations that do not occur for T-bulge DNA. We have also isolated binding-unbinding events of MutS to a G:T mismatch and are able to combine DNA conformational dynamics with MutS binding kinetics. These dynamics bring us closer to understanding DNA mismatch repair initiation by MutS and how DNA conformations affect the capability of the DNA to be repaired.

Combination of FRET and AFM for the Study of Single Molecules

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We are developing a combination of fluorescence resonance energy transfer (FRET) and atomic force microscopy (AFM). This novel imaging approach will be of great interest for the study of biological systems at the single-molecule level. For example, the technique will enable us to identify specific proteins within multiprotein complexes. The protein of interest is fluorescently labeled with the acceptor dye of a FRET pair, and the corresponding FRET donor is immobilized on the AFM tip. In our experiments, we are using quantum dots as FRET donors. Quantum dots have narrow Gaussian emission profiles with high emission intensities and high photostability. Their surface structure allows for relatively easy attachment to the AFM tips. Such properties render them ideal FRET donors in our experiments. FRET occurs when acceptor and donor come close together. The strong distance dependency of FRET is thus exploited to preserve high spatial resolution. The FRET signal is measured at each sample position scanned by the tip and produces a photon count image that can be registered with the AFM topography image. Superposition of the fluorescence and height features allows identification of individual protein components and the ability to visualize small protein molecules within or next to large proteins that would otherwise be invisible to AFM.

Topic V: Imaging Cells

Following the Steps of Nuclear Envelope Fusion and Fission in Budding Yeast

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Nuclear envelope fusion is the last step in diploid formation during yeast mating, whereas nuclear envelope fission is one of the last steps of yeast mitosis, coincident with cytokinesis. We are using time-lapse microscopy of live yeast cells with different fluorescent-tagged compartments to follow nuclear fusion during mating and nuclear envelope fission during mitosis. We have followed the spindle pole body marker, Spc42-RFP, and the nuclear envelope/ER luminal marker, GFP-HDEL, during nuclear fusion. We see close juxtaposition of two spindle pole body dots just prior to nuclear envelope fusion. To distinguish between outer and inner nuclear envelope fusion, we have followed the GFP-HDEL marker and an RFP-tagged nucleoplasm marker. Since we can detect nuclear envelope luminal mixing before nucleoplasm mixing 25% of the time with 1-minute time points and 50% of the time with 30-second time points, we conclude that outer and inner membrane fusions occur in distinct steps. These markers are being examined in several mutants defective in either the initiation or completion of nuclear fusion.

To determine the requirements for nuclear envelope fission, we have examined mutants conditionally defective for bud emergence and/or nuclear migration. These mutants have in common that they complete the nuclear division cycle within the mother cell, but nuclear envelope fission does not occur. Upon shifting a conditional mutant to permissive temperature to initiate bud emergence, the nucleus spanning the neck region undergoes fission, whereas mitotic nuclei wholly within either the mother or the bud do not undergo fission. These data suggest that the neck region is important for the initiation of nuclear envelope fission. However, the contractile ring is not necessary for fission, since septin mutants failing to undergo cytokinesis still undergo fission 75% of the time. These data suggest that fission is controlled by signaling from the neck rather than by mechanical contraction.

Coordinate Regulation of Sphingosine Kinase 1 Localization and Activity and Actin Cytoskeletal Dynamics in Macrophages

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Sphingosine kinase (SK) plays a key role in diverse cellular processes such as apoptosis, proliferation, stress responses, regulation of calcium fluxes, and phagocytosis. The product of SK activity, sphingosine-1-phosphate (S-1-P), is a major intracellular signaling molecule for macrophage antimicrobial defense, due to its induction of the rise in cytosolic Ca2+, which is required for phagosome maturation. The translocation of SK1 from the cytosol to the phagosome

membrane contributes to the spatial restriction of Ca2+-signaling. Here, we sought to further characterize the determinants of SK1 localization to specific compartments: the phagosome membrane and the plasma membrane. Resting RAW 264.7 macrophages exhibited constitutive association of wild-type (WT)-SK1 with cortical actin filaments. Stimulation with PMA increased the colocalization of SK1 with actin filaments, especially in plasma membrane ruffles. Cells transfected with catalytically inactive (CI)-SK1G82D or with phosphorylation-defective (PD)-SK1S225A (a mutant that cannot undergo ERK1/2-mediated, phosphorylation-dependent activation) exhibited significantly less membrane translocation and colocalization with actin filaments in response to PMA. In contrast, WT-, CI-, and PD-SK1 each colocalized with actin filaments at the membrane of nascent phagosomes containing complement- or IgG-opsonized latex beads. Biochemical analysis of intact macrophages and a cell-free assay confirmed the constitutive- and stimulus-enhanced association of SK1 with the F-actin-containing, detergent-insoluble fraction. Inhibition of actin cytoskeletal dynamics with latrunculin B or jasplakinolide disrupted the localization of SK1 and modulated its enzymatic activity. Conversely, inhibition of SK resulted in dysregulation of actin filaments. These studies indicate that the subcellular localization and activity of SK1 are tightly coupled to actin cytoskeletal dynamics.

Recruitment of CD63 to *Cryptococcus neoformans* Phagosomes Requires Acidification

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The immune system utilizes highly specific cells to provide a comprehensive defense against microbial pathogens. One cell type that initiates the immune response is the dendritic cell. These cells reside in peripheral tissue and are responsible for phagocytosis of pathogens. These cells then travel to regional lymph nodes where they are capable of activating naive T cells. The transit of pathogen through the endocytic pathway and intersection of the antigen processing and presentation pathway are incompletely understood. One molecule that has been found in this pathway is CD63, a member of the tetraspanin family. The subcellular localization of CD63, and its interaction with the Class II MHC antigen presentation pathway, were examined in the context of phagocytosis by live cell imaging, using mRFP1-tagged mouse CD63 expressed in primary bone marrow-derived cell cultures established from Class II MHC-eGFP mice.

Upon phagocytosis of the human fungal pathogen *Cryptococcus neoformans* and polystyrene beads, CD63 was recruited selectively to *C. neoformans*-containing phagosomes in a MyD88-independent, acidification-dependent manner. Bead-containing phagosomes, within a *C. neoformans*-containing cell, acidified to a lesser extent and failed to recruit CD63 to a level detectable by microscopy. CD63 recruitment to yeast phagosomes occurred independently of Class II MHC and LAMP-1. These observations indicate that the composition of distinct phagosomal compartments within one and the same cell is determined by phagosomal cargo and may thus affect the outcome of antigen processing and presentation.

POLKADOTS Are Signaling Foci for the T-Cell Receptor-Mediated Activation of NF-κB

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Stimulation of the T-cell receptor (TCR) leads to the activation of several transcription factors, including NF-κB, that are crucial for the proliferation and gain of effector functions. TCR activation leads to a dynamic spatial redistribution of several signaling proteins; however, the significance of these redistribution events is largely unknown. We have previously described TCR-induced subcellular structures called POLKADOTS that are enriched in the NF-κB signaling intermediate, Bcl10.

We now show that POLKADOTS form only under conditions resulting in efficient NF-κB activation. Furthermore, we find that POLKADOTS formation is dependent upon the functional interaction of the NF-κB signaling proteins Bcl10 and MALT1. Utilizing a photoactivatable GFP, we demonstrate that POLKADOTS contain both a highly stable and a rapidly equilibrating protein component. FRET analyses show that POLKADOTS are enriched in interactions between Bcl10 and partner signaling proteins. This research strongly suggests that POLKADOTS are signaling foci for cytosolic intermediates in the TCR-mediated activation of NF-κB.

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Oskar mRNA Reveals Independence of Staufen

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The intracellular transport of mRNAs from sites of transcription in the nucleus to specific destinations in the cytoplasm is a highly conserved mechanism found throughout eukaryotic life. Increasingly, mRNA transport proves to be dependent on the interplay of nuclear and cytoplasmic proteins where specific nuclear events dictate the cytoplasmic destinations of various mRNAs.

The subtle changes that occur at distinct steps in the transport of the RNA-protein complex have been shown to be impervious to biochemical analyses. To investigate these fine changes in the composition of the transport complex, we have build on techniques that we have previously developed, which enable us to detect the rapidly changing physical interactions between a single protein, Staufen, and the oskar mRNP during Drosophila oogenesis. We have used molecular beacons to track individual particles of oskar mRNPs from their birth in the nucleus of a nurse cell to their eventual localization within the oocyte. Tracking mRNPs with high spatial and temporal resolution, we successfully dissected the transport of oskar mRNPs into components that require energy-dependent motion and those that occur by diffusion. We concluded that

during mRNP transport, most energy-dependent events occur in the oocyte and not in the nurse cells. To relate the biophysical descriptions of oskar mRNP transport to a molecular context, we sought to relate Staufen to oskar mRNPs, with emphasis on when it is required in the transport process. Contrary to previously described models, we observed Staufen to be in particles distinct from oskar mRNPs until the very last stage in the transport process, near the posterior of the oocyte, despite frequent collision events at the ring canals—the cellular bridges between the oocyte and the neighboring nurse cells. We believe that this study of the biophysical processes that oskar mRNP and Staufen undergo refine the model of the transport mechanism that facilitates oskar mRNA localization in the developing oocyte. At the same time, we hope that our approach will help explore other systems in which the fleeting moments of interaction between nucleic acids and proteins have not yet been resolved.

Mechanisms of Taxol's Function on Living Cells

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The poles of the mitotic spindle are assembled by the pair of centrosomes, which recruit structural proteins, such as NuMA, to the minus ends of microtubules (MTs). Dynamic MTs are necessary for proper formation of mitotic spindle poles; NuMA is transported to the spindle pole in a dynein-dependent fashion, which itself requires dynamic MT plus-ends for "search-capture." To investigate the mechanisms of spindle pole assembly, BSC-1 cells constitutively expressing either GFP-α tubulin or GFP-NuMA were continuously treated with μM concentrations of Taxol to completely suppress plus-end microtubule dynamics and then imaged by time-lapse fluorescence video microscopy as they entered mitosis. At the onset of mitosis, the microtubule network in Taxol-treated cells dissociates from the centrosome, and microtubules are recruited to the nuclear envelope and the cell cortex. The duplicated centrosomes often fail to separate, do not remain attached to the nucleus, and fail to assemble prominent microtubule asters. A significant number of centrosomes do not have any microtubules associated with them. In addition, suppressing microtubule dynamics leads to significant delays in nuclear envelope breakdown, resulting in NuMA "leaking" out of the nucleus and moving predominantly to the cortex, rather than to the centrosomes. Unexpectedly, the cortically arrayed NuMA and microtubules bud off into multiple cytasters that migrate, cluster, and fuse together around the chromosomes but fail to assemble a proper bipolar microtubule array. This ultimately leads to failure of cytokinesis and the generation of a polyploid cell. Our data are consistent with the model that plus-end microtubule dynamics are required for search-capture during prophase to properly localize NuMA, thereby ensuring that the spindle poles assemble only at the centrosomes. Our live cell imaging has also directly revealed the cellular mechanism for the anti-mitotic activity of Taxol.

Mechanism Controlling Perpendicular Alignment of the Spindle to the Axis of Cell Division in Fission Yeast

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In animal cells, the mitotic spindle is aligned perpendicular to the axis of cell division. This ensures that sister chromatids are separated to opposite sides of the cytokinetic actomyosin ring (CAR). We show that, in fission yeast, spindle rotation is dependent on the interaction of astral microtubules with the cortical actin cytoskeleton. Interaction initially occurs with a region surrounding the nucleus, which we term the "astral microtubule interaction zone" (AMIZ). Simultaneous contact of astral microtubules from both poles with the AMIZ directs spindle rotation and requires both actin and two type V myosins, Myo51 and Myo52. Finally, we show that disruption of the actin cytoskeleton is monitored by a checkpoint that regulates the timing of sister chromatid separation. We find that whereas sister kinetochore pairs normally congress to the spindle midzone before anaphase onset, this congression is disrupted when the actin cytoskeleton is disturbed. By analyzing the timing of kinetochore separation, we find that this anaphase delay requires the Bub3, Mad3, and Bub1 but not the Mad1 or Mad2 spindle assembly checkpoint proteins. In agreement with this, we find that Bub1 remains associated with kinetochores when actin is disrupted. These data indicate that, in fission yeast, the integrity of the actin cytoskeleton is monitored by a subset of spindle assembly checkpoint proteins.

Grasping the Molecular Clutch With High-Resolution Velocity Mapping

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Cell migration is regulated in part by the connection between adhesion components and the actin cytoskeleton. The very large number of proteins involved in cell protrusion and adhesion, along with their complex interactions, make it difficult to assess their role in cell migration. We apply spatiotemporal image correlation spectroscopy (STICS) to determine protein flow velocities throughout the lamella and use these measurements to explore how F-actin-based motility is regulated by interactions with adhesion components that constitute the hypothetical molecular clutch. STICS is a new technique that can be applied to virtually any fluorescence image time series in order to extract protein velocity magnitudes and directions. The technique reveals hidden protein dynamics even in situations where there are high or low densities of fluorescent protein and also works in the presence of static macromolecular complexes. There is no requirement for sophisticated sample preparation or special labeling. The technique has been successfully applied to laser scanning confocal images and total internal reflection fluorescence (TIRF) CCD camera images, and the analysis is done in a matter of minutes. Using STICS we construct detailed cellular velocity maps for actin and the adhesion-related proteins α-actinin, α 5-integrin, talin, paxillin, and FAK. Our results provide evidence that there are two slippage points between actin and the extra cellular matrix, one at the integrin level and the other at the α-actinin level. The method proves to be a useful new tool for quantifying directed protein transport from image time series of fluorescently tagged proteins.

Differential Spatio-Temporal Localization and Regulation of cAMP Generation and PKA and RhoA Activity in Normal and Cancer Breast Cells in Invasion

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The identification of the tumor-specific signal transduction modules involved in promoting metastasis is of particular importance. Recent studies have underlined the importance of PKA as a gating element in a number of different processes, including cancer invasion, and there has been a paradigm change in the understanding of PKA-dependent regulation in which specificity is secured by tight localization of signaling components. By using cAMP/PKA fluorescence resonance energy transfer (FRET)-based sensors and fluorescence lifetime imaging microscopy (FLIM) analysis, it is possible to both visualize the location and measure the amount of microcompartmentalized cAMP production and PKA activity. We first compare the localization of cAMP generation and PKA activity in basal and serum-deprived conditions in human breast normal, MCF-10, and cancer MDA-MB-435 cells. The results are consistant with an even distribution and activation of cAMP and PKA between the cell body and pseudopodia in the normal cells, whereas in the cancer cells there are higher levels of cAMP and PKA in the pseudopodia. Importantly, the mobilization of cAMP and subsequent activation of PKA by serum deprivation occurs preferentially along the cell membrane of the pseudopodia that protrude in the direction of movement. This polarized expression and activation of the PKA signal system are particularly strong at the pseudopodial tips of the cancer cell and result in a phosphorylation of RhoA. In line with these data, we further observe that serum deprivation-dependent activation of PKA preferentially inhibits RhoA located at the cancer but not normal cell pseudopodial tip. Transfection of a phosphorilation-deficient RhoA mutant (RhoAS188A) inhibits a pseudopodial p160ROCK/p38 signaling cascade, which activates NHE1 and increases invasion only in the cancer cells. These data further support the idea that invasion-specific signal transduction modules in cancer cells are organized in invasion-specific cell structures that finely orchestrate invasive activity.

The Palmitoylated Trafficking Signal in GAD65 Mediates Sorting Into Spatially Distinct Trans-Golgi Membrane Microdomains of Decreased Mobility

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GAD65, the smaller isoform of the GABA-synthesizing enzyme glutamic acid decarboxylase, is anchored posttranslationally to the cytosolic phase of Golgi membranes. Palmitoylation of GAD65 is not required for membrane anchoring but is critical for the post-Golgi trafficking of the

protein to presynaptic clusters via axon-specific endosomes and a Rab5a-dependent pathway. Here we show that palmitoylation results in translocation of GAD65 to geometrically distinct parts of Golgi membranes. Prior to palmitoylation, the protein forms a ribbon-like staining pattern juxtaposed to the trans-Golgi marker TGN38 and does not colocalize with the cation-independent mannose-6-phosphate receptor (CI-M6PR) in Golgi ministacks formed by nocodazole treatment. In contrast, palmitoylated GAD65 is interspersed with TGN38 and colocalizes with CI-M6PR in Golgi ministacks following nocodazole treatment. Fluorescence recovery after photobleaching (FRAP) analyses reveal a lower rate of recovery of wild-type (wt) than palmitoylation-deficient GAD65 in Golgi membranes, and a fraction of the wt protein does not recover from photobleaching. Live cell imaging in the presence of Brefeldin A (BFA) reveals a redistribution of palmitoylation-deficient GAD65 to the endoplasmic reticulum in a pattern and at a rate similar to the Golgi resident protein galactosyltransferase (GalT) but at a threefold lower rate than the redistribution of wt ARF1 to the cytosol following BFA treatment. In contrast, a significant fraction of palmitoylated GAD65 redistributes into perinuclear punctate structures that harbor CI-M6PR. High-resolution confocal microscopy and live cell imaging reveal that GAD65-GFP-containing vesicles are observed only for the wt protein, while the palmitoylation-deficient mutant remains in the Golgi compartment and in a diffuse pattern throughout the cell. The results suggest that palmitoylation provides a signal for sorting of GAD65 into trans-Golgi membrane microdomains of decreased fluidity that are critical waystations for its subsequent targeting to post-Golgi vesicles and presynaptic clusters.

Visualizing and Probing the Order and Molecular Dynamics of Lipid Rafts in Mast Cells

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Cholesterol-rich microdomains (or “lipid rafts”) within the plasma membrane are thought to exist in a liquid-ordered phase and have been hypothesized to play functionally important roles in cell signaling and membrane trafficking. To visualize raft domains and relate raft structure and formation dynamics to their biological function—specifically, mast cell signaling—we use fluorescence lifetime imaging (FLIM), fluorescence anisotropy imaging, and single-point lifetime and anisotropy decay measurements with high temporal resolution. When the high-affinity receptor for IgE receptor (FcΣRI) is crosslinked with antigen or a secondary antibody for long periods in the cold, molecules associated with cholesterol-rich microdomains (e.g., saturated lipids [the lipid analog diI-C18 or glycosphingolipids] and lipid-anchored proteins [glycosylphosphatidylinositol-anchored proteins or acylated proteins]) co-redistribute with the crosslinked FcΣRI. We find that increases in steady-state environmental rigidity and structural order (lifetime and anisotropy imaging), as well as the molecular dynamics, occur in regions of diI-C18 that colocalize with patches of IgE-FcΣRI. To model the complexity observed in live cells, these methods are applied to supported lipid bilayers to demonstrate the difference in fluorescence lifetime and anisotropy, as reported by the incorporated fluorescent probe, upon varying degrees of lipid order (e.g., gel phase versus fluid phase). Our results suggest that lifetime and, particularly, anisotropy are effective reporters of local membrane nanostructure. Our current efforts are directed toward steady-state and dynamic studies on adherent mast cells stimulated at

25 °C and 37 °C to better understand the critical functional relationships occurring between proteins and lipids under physiological conditions.

Mdv1 Interacts With Assembled Dnm1 to Promote Mitochondrial Division

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The dynamin-related GTPase, Dnm1, self-assembles into punctate structures that are targeted to the outer mitochondrial membrane where they mediate mitochondrial division. Posttargeting, Dnm1-dependent division is controlled by the actions of the WD repeat protein, Mdv1, and the mitochondrial TPR-like outer membrane protein, Fis1. Our previous studies suggest a model where at this step Mdv1 functions as an adaptor linking Fis1 with Dnm1. To gain insight into the exact role of the Fis1/Mdv1/Dnm1 complex in mitochondrial division, we performed a structure-function analysis to study the function of the Mdv1 adaptor using fluorescent microscopy, electron microscopy, and two-hybrid assays. Additionally, fluorescent time-lapse analysis of Mdv1 behavior in the cell indicates that Dnm1 and Mdv1 have a specific order of assembly onto the mitochondrial membrane and both complexes are present at division. Our analysis suggests that dynamic interactions between Mdv1 and Dnm1 play a key role in division by regulating Dnm1 self-assembly.

Spatial Anatomy of a *Bacillus subtilis* Biofilm

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Many microbial cells differentiate from free-living planktonic cells to surface-associated sessile communities known as biofilms. These multicellular structures of bacteria are encased in a self-produced extracellular matrix composed of polysaccharides and proteins. The remarkable architectures of many different laboratory-grown biofilms suggest a high degree of cellular specialization within these communities. We hypothesize that the interplay between cell-cell signaling and the microenvironments within the biofilm produces spatial and temporal patterns of gene expression that lead to localized cellular differentiation. *Bacillus subtilis*, a gram-positive, motile, rod-shaped bacterium, is best known for its ability to develop natural genetic competence and sporulate in response to starvation and high population density. The regulatory processes controlling *B. subtilis* sporulation and competence have been extensively characterized in planktonic cultures. Because several developmental events are well defined in *B. subtilis*, it is an ideal model system for the study of cellular differentiation within a multicellular community. To form a biofilm, motile *B. subtilis* cells differentiate into nonmotile chains of cells that form parallel bundles held together by extracellular matrix material. These bundles eventually produce aerial structures that serve as preferential sites for sporulation. In addition to sporulation, we determine the spatial organization of different developmental events, including cessation of

motility, induction of genetic competence, and production of extracellular matrix. We utilize cell-type-specific promoters and fluorescent reporter molecules coupled with fluorescence microscopy to map out the spatial anatomy of a *B. subtilis* biofilm.

Exploring the Intracellular Path of Nonviral Nucleic Acid Delivery

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One of the most challenging aspects in the application of genetic-based therapies is the physical delivery of nucleic acid to specific regions within target cells. In the past, viral delivery vehicles have been used to cross the cell membrane and intercellular medium en route to the nucleus. Although they are efficient gene carriers, viral vectors have displayed some disadvantages, such as insertional mutagenesis into the host genome, severe immune response to viral proteins, and limited DNA capacity. For these reasons, the development of nonviral vectors is under intense research. Current nonviral vectors, while circumventing the disadvantages of viral vectors, have yet to meet the delivery efficiency of viruses. This investigation focuses on cationic polymers that can complex nucleic acids into particles, called polyplexes, and are capable of cellular internalization via endocytosis. After internalization, the polyplexes must escape endocytic vesicles to avoid degradation of the valuable nucleic acid in lysosomal compartments. This escape and subsequent intracellular path constitute a crucial research focus needed to aid the further tailoring and development of nonviral vectors to increase their transfection efficiency. In this investigation, mammalian cells are transfected with fluorescently labeled vectors. After transfection for 4 hours, the cells are fixed, mounted, and visualized via fluorescence microscopy. Both DNA and the polymeric vector can be visualized in discrete intracellular compartments. Additionally, fluorescent DNA can be found in the nucleus of these cells. Future work involves live-cell imaging and incorporates measurement of the intracellular pH of the transfected DNA and colocalization of vectors with specific intracellular targets labeled via immunocytochemistry.

Topic VI: Imaging Tissues

Imaging Linker Cell Migration in *C. Elegans*

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C. elegans is an excellent model system for imaging individual cells in vivo because its small size and optical transparency allow virtually every cell to be visible at high magnification throughout its development. We are studying the cell biology of an individual migratory cell, the linker cell (LC), in the context of a developing worm by imaging live worms that express GFP-tagged proteins to examine protein localization in the LC. The LC is a relatively large cell that undergoes a stereotypic migration over the course of three larval stages, thereby leading the developing gonad from the mid-body region, through a series of turns, to the posterior of the organism. We currently have as markers a cytoplasmic YFP, mig-2::MIG-2::GFP (a Rac homolog), and nmy-2::NMY-2::GFP (non-muscle myosin). Using confocal microscopy, we have observed polarized localization of MIG-2 and NMY-2 within the LC that varies over the course of its migration. We are using worms that express cytoplasmic YFP in the LC to do confocal time-lapse microscopy of the migration.

Non-Invasive, Quantitative Analysis of Circulation in the Zebrafish *Danio Rerio* Using Widefield and Laser Scanning Confocal Microscopy and Digital Image Processing

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Embryos of the vertebrate zebrafish *Danio rerio* are transparent and form a functional circulatory system within 26 hours postfertilization. These attributes make them ideal organisms in which to study genetic and pharmacological modifiers of the cardiovascular system. However, traditional techniques to trace the route of blood flow and measure erythrocyte dynamics require specialized equipment (and considerable practice). Here we present two complementary methods for characterizing the development and function of zebrafish circulation using both widefield and laser scanning confocal microscopy. Through a technique we refer to as "in silico microangiography," time-lapse DIC images are digitally processed to extract the trajectory of circulating erythrocytes, resulting in a composite image set that depicts the functional vasculature of the embryo. Unlike traditional dye-based microangiography, in silico microangiography does not require pericardial injections and provides information about the density and distribution of erythrocytes as a function of time. Additionally, we have developed a high-speed imaging technique to visualize the flow of individual cells through the vasculature of zebrafish embryos. Confocal linescans acquired from regions perpendicular to the vessels of interest provide quantitative measures of erythrocyte flux, velocity, vessel diameter, and heart rate. Applications of these techniques to the analysis of pharmacological and genetic modifiers of cardiovascular

function are presented. Together, these noninvasive, whole organism microscopy methods will facilitate the analysis of vertebrate circulation to address questions relevant to drug development and molecular genetics.

Four Color, 4D Time-Lapse Confocal Imaging of Chick Embryos

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Insight into the molecular mechanisms that direct cell motility has benefited from innovations in targeted cell labeling and the ability to resolve intracellular dynamics with live cell imaging. Advances in culture and imaging techniques now permit questions about cellular and molecular events to be posed within the intact embryo, but typically use a single color. Here, we report the delivery of multiple (up to 4), multicolor fluorescent protein (FP) constructs and 4-D, multispectral time-lapse confocal imaging of cell movements in living chick embryos. Cell cytoskeletal components are fluorescently tagged after microinjection and electroporation of a cocktail of FP constructs into specific regions of chick embryos. We present the results of testing various two-, three-, and four-color combinations using multispectral imaging and linear unmixing and develop a set of advantageous optical parameters for 4-D imaging. We show that the number of multicolor-labeled cells in a typical chick embryo decreases as the number of FPs per cell increases. We present an example of monitoring multiple cytoskeletal dynamics in vivo in a highly migratory population of cells called the neural crest. We suggest that this technique offers a powerful tool to simultaneously analyze cellular and molecular events during chick embryogenesis.

Monitor Dynamic Cell-Cell Communication in 3D by Two Photon Uncaging and Imaging

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Gap junction (GJ) channels allow adjacent cells to communicate via the transfer of small molecules and ions and play important roles in cell homeostasis and synchronization. Using a new class of photo-activatable fluorophores[1], we recently developed a new fluorescence imaging technique, LAMP, for studying dynamic junctional communication in live cells. By comparison with techniques previously available, LAMP assay shows a number of advantages, including noninvasiveness, applicability to fully intact cell populations, high spatiotemporal resolution, quantification of dye transfer rates, and capability of tracking changes in coupling strength[2,3].

To extend LAMP method to study GJ communication in three dimensions (3-D), we resorted to two-photon (2-P) uncaging and imaging. The fluorescence 2-P action cross-sections of the parent fluorophores were measured between 710 nm and 880 nm, displaying a maximum of 30 GM (10-50 cm4 s) at 790 nm. Moreover, through the mechanism of substrate-assisted photolysis, the 2-P uncaging cross-section (Δu) of the caged fluorophores is also fairly high, reaching 0.6 GM at 740 nm. Importantly, Δu falls off rapidly above 770 nm, dropping to a negligible level at 790 nm

or longer wavelengths. This allows us to activate the caged form at 740 nm and monitor the movement of the dye at 790 nm. By employing "orthogonal" infrared light to activate and monitor dye movements (infrared-LAMP), we fully exploit the high spatial selectivity of 2-P excitation.

To map cell-cell communication networks with high spatial resolution in 3-D, we prepared mouse pancreatic acini and imaged GJ coupling between acinar cells by infrared-LAMP. We observed very strong coupling between acinar cells in intact acini, whereas dye transfer between doublet or triplet acinar cells was rarely observed. Thus, in physiological preparations containing intact cell populations that maintain normal cell-cell communication channels, infrared-LAMP should facilitate studying how the junctional coupling is involved in regulating different biological processes.

The unique photochemical and fluorescent properties of this new class of photo-activatable fluorophores prompted us to prepare reactive derivatives for bioconjugation. Initial tests of these reagents showed promising results of applying them for future studies to track movements of biomolecules in cells.

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Deciphering Electric Fish Communication Signals Using In Vivo Two-Photon Calcium Imaging

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Temporal coding, the encoding of the temporal characteristics of sensory pathways by the precise timing of neural spikes, plays a significant role in the processing of information in many sensory systems. Mormyrid electric fish (*Brienomyrus brachyistius*) communicate using electric organ discharge waveforms (EODs), generated by an electric organ in the tail, and have the ability to discriminate the sex and species of an individual based on their EODs. These electric signals are encoded with microsecond precision by Knollenorgan electroreceptors (KOs), which translate the signal variance into a spike-timing neural code. The current model proposes that stimulus-evoked spikes, originating in two different regions of the skin, will arrive at small cells in the anterior exterolateral nucleus (ELa) of the mormyrid midbrain via both an excitatory input that is delayed and an inhibitory input with a shorter delay. We are currently investigating this delay-line anti-coincidence model by stimulating KOs with transverse electric field geometry designed to stimulate receptors sequentially and uniform geometry designed to stimulate them synchronously while optically recording small-cell activity using in vivo two-photon calcium imaging. The

fluorescent calcium indicator dyes are either retrogradely loaded into the small cells from ELp or AM directly loaded into ELa. The exposed position of ELa allows us to employ functional optical imaging techniques both to record activity-dependent fluorescence in vivo from single small cells to observe the effects of stimulus timing on small-cell activation and to study many small cells simultaneously to test whether a spatial map of small-cell activity is generated as a function of stimulus duration and stimulus geometry. Thus, in vivo two-photon calcium imaging offers a better understanding of functional changes on the level of single neurons and networks and provides a window to deciphering neuronal interactions in networks within the intact brain.

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