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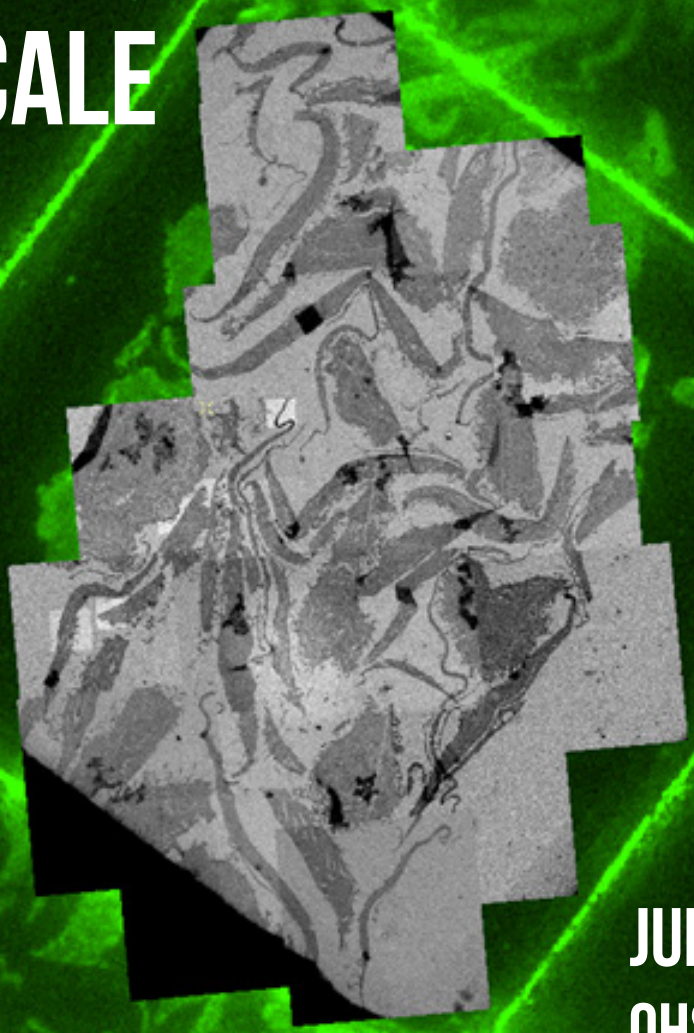

JUNE 4 - 6, 2014
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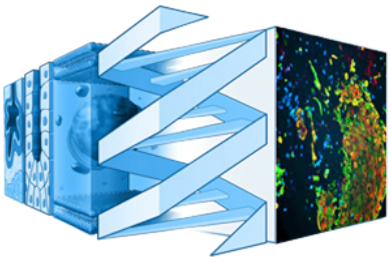

OHSU CENTER FOR SPATIAL
SYSTEMS BIOMEDICINE

FEI™

SCHEDULE

JUNE 4

9:00 AM Introduction to FEI and the Workshop Agenda
Auditorium

Session A: Helios SDB	Session a: MAPS and Auto Slice and View
Session B: Tecnai with iCorr	Session b: Correlative LM/TEM and Sample Prep
Session C: Titan Krios	Session c: CryoTEM
Session D: CorrSight LM	Session d: MAPS and Sample Prep

9:30 AM Session I
Multiple Locations

11:00 AM Session II
Multiple Locations

12:30 PM Lunch for registered attendees
Old Library Lobby

2:00 PM Session III
Multiple Locations

3:30 PM Session IV
Multiple Locations

5:00 PM Questions and Agenda for Days 2 and 3
Auditorium

JUNE 5

8:00 AM Registration Opens

8:30 AM OCSSB Symposium Welcome
Joe W. Gray, OCSSB | OHSU

8:40 AM **Session I: Molecular Electron Microscopy**
James Chen, OHSU

8:45 AM Lessons from the HIV-1 envelope trimer: CryoEM strategies for studying challenging glycoproteins at high resolution
Andrew Ward, Scripps

9:10 AM Adeno-associated virus (AAV) Cell Attachment – Structural Studies
Michael Chapman, OHSU

SCHEDULE

JUNE 5

9:35 AM — Life sciences computing and visualization with Intel xeon phi coprocessors
James Jeffers, Intel Inc.

10:00 AM — Coffee Break

10:20 AM — Architecture, symmetry and mechanism of glutamate receptors
Eric Gouaux, Vollum Institute | OHSU | HHMI

11:15 AM — Mechanism of substrate degradation by the 26S Proteasome
Gabe Lander, Scripps

11:40 AM — The structural basis of ESCRT-III mediated membrane remodeling and fission
Adam Frost, University of Utah

12:05 PM — Lunch and Poster Session
Poster session sponsored by Applied Scientific Instrumentation (ASI)

2:00 PM — **Session II: Correlative Light & Electron Microscopy**
Summer Gibbs, OHSU

2:05 PM — **Keynote: Zooming in on cells and molecular structures with correlative light and electron microscopy**
Abraham Koster, Leiden University Medical Center

2:55 PM — Correlation of the same fields imaged in the TEM, Confocal, LM and MicroCT by image registration: From specimen preparation to displaying a final composite image
Doug Keene, Shriners Hospital for Children

3:20 PM — Making correlative experiments easier
Gregor Heiss, FEI Munich GmbH

3:45 PM — Coffee Break

4:00 PM — Harnessing the power of EM to define defects in cellular differentiation
Nick Smith on behalf of Melissa Wong, OHSU

4:25 PM — A light in the dark: Correlative light and electron microscopy of breast cancer cells
Danielle Jorgens, OHSU

4:50 PM — Her2 Localization in Breast Cancer
Anke Mulder, OHSU

SCHEDULE

JUNE 6

8:30 AM Registration Opens

9:00 AM **Session III: Super-Resolution Light Microscopy**
Xiaolin Nan, OHSU

9:05 AM Advanced light microscopies at OHSU
Stefanie Kaech Petrie, OHSU

9:30 AM Using single molecule dynamics to understand cellular function
James and Catherine Galbraith, OHSU

10:00 AM Poster Talk: Photoactivated Localization Microscopy with Bimolecular Fluorescence Complementation (BiFC-PALM): Nanoscale Imaging of Protein-Protein Interactions in a Cell
Andrew Nickerson, OHSU

10:20 AM Coffee break

10:45 AM Expanding the fluorescence toolkit for super-resolution microscopy
Joshua Vaughan, University of Washington

11:30 AM High-contrast visualization of endogenous proteins in live tissue
Haining Zhong, OHSU

12:00 PM Lunch and Poster session
Poster Session sponsored by Applied Scientific Instrumentation (ASI)

2:00 PM **Session IV: Novel Tools & Probes for Cellular Imaging**
Kimberly Beatty, OHSU

2:05 PM 3D microscopy of whole mouse lungs (and human airway) using optical clearing and fluorescent casting
Gregory Scott, OHSU

2:30 PM **Keynote: Design and evolution of genetically encoded probes to illuminate cellular activities**
Robert Campbell, University of Alberta | Sponsored by Life Technologies

3:35 PM Poster Talk: Combining quantitative phase microscopy with fluorescent reporters: quantifying the alteration of nuclear structure following radiation damage
Kevin Phillips, OHSU

3:50 PM World-class electron microscopy capabilities at OHSU
Claudia López, OHSU

SCHEDULE

JUNE 6

4:15 PM Closing remarks and ajournment
Joe Gray, OHSU | OCSSB

POSTERS

Poster 1: Live Imaging of HER2 Receptor Trafficking and Downstream Spatial Signaling Distribution of AKT in Breast Cancer Using Quantum Dots

Lam, Wai Yan1; Ramunno-Johnson, Damien1,2; Bowcock, Alec1; Chin, Koei1,2; Gray, Joe1,2; Bruchez, Marcel3,4; Vu, Tania1,2

1Department of Biomedical Engineering, Oregon Health and Science University, Portland, OR 97239
2OHSU Center for Spatial Systems Bioscience, Oregon Health and Science University, Portland, OR 97239
3Department of Chemistry, Carnegie Mellon University, Pittsburgh, PA 15213
4Department of Biological Sciences, Carnegie Mellon University, Pittsburgh, PA 15213

Signal transduction of extracellular cues to the intracellular environment is a highly dynamic process that must occur in a spatially and temporally precise manner. This spatiotemporal coordination of upstream signaling molecules and downstream targets is critical to ensuring specificity of interactions between proteins during normal signaling, and understanding how such pathways become dysregulated in disease models can yield insight into how signaling is propagated. However, technological means to probe the location of single-protein complexes quantitatively, dynamically, and with context to location of subcellular structures remain an unaddressed challenge. HER2 receptors and Akt are two PI3K pathway effectors that are dysregulated and major therapeutic targets in breast cancer. For HER2, a receptor tyrosine kinase (RTK) that initiates signaling via receptor heterodimerization, it is not fully understood how receptors traffic upon activation to regulate receptor availability upstream and receptor interactions downstream during signaling. Previous studies suggest HER2 undergo recycling, and other studies looking at endosomal signaling in RTKs raise the question of whether spatiotemporal location of HER2 in relation to downstream effectors is key in propagating signaling. But inability to study such receptor events in live cells with high spatiotemporal resolution using current methods limits our understanding of signaling. Downstream of HER2 is Akt, a serine-threonine kinase thought to translocate from cytoplasmic locations to the plasma membrane, nucleus, and possibly Golgi to regulate aspects of tumor progression. Little is known about how specific upstream receptor behaviors regulate downstream effects such as localization of activated PI3K effectors like Akt. Furthermore, current immunofluorescence and biochemical methods to locate Akt using diffuse dyes and fractionation methods overlook key spatial information about the organization of individual Akt complexes in relation to important cellular structures.

Poster 2: Focused Ion Beam Characterization of Bicomponent Polymer Fibers for Biological Applications
K. C. Wong1,4,*, C.M. Haslauer2,3, and D.P. Griffis1,4

1Analytical Instrumentation Facility, North Carolina State University
2 North Carolina State University and UNC-Chapel Hill, Joint Department of Biomedical Engineering
3Nonwovens Cooperative Research Center, The Nonwovens Institute, North Carolina State University
4Department of Materials Science and Engineering, North Carolina State University

Previous work has shown that focused ion beam (FIB) micromachining can be effectively utilized for the cross sectional analysis of polymers such as core-shell solid micro-spheres and hollow latex nano-spheres. While these studies have clearly demonstrated the precise location selection and micromachining control provided by the FIB technique, the samples studied consisted of only a single polymer. In this work, FIB is used to investigate bicomponent polymeric fiber systems by

taking advantage of the polymer components' differing physical properties. An in situ FIB approach for cross sectioning and visualizing the cross sectional morphology of the polymeric components in a bicomponent polymeric fiber with the island-in-the-sea (I/S) structure is presented. I/S fibers investigated were fabricated through the melt spinning process and were composed of bicomponent combinations of polylactic acid (PLA) and EastOne™, a proprietary water dispersible polymer. An FEI Quanta 200 3D was used to reliably and repeatedly provide the distribution of the EastOneTM polymer within the PLA fiber using only the Ga+ FIB beam. Moreover, when the EastOneTM polymer dissolved away from the PLA fiber by a water treatment process leaving pores and channels where the EastOneTM polymer once was, the FIB beam was used to cross section as well as to examine a wide range of pore sizes (nanometers to micrometers) and porosity. Such channels and pores were shown to support the viability and differentiation of human adipose-derived adult stem cells down the osteogenic lineage. FIB techniques are advanced to characterize fine features pertaining to polymeric material system. Topographical contrast as a result of differential sputtering and high surface specificity combined with high signal-to-noise using FIB induced secondary electron (iSE) imaging is shown to provide a useful approach for the rapid characterization of the cross sectional morphology of the bicomponent polymeric materials examined in this study. The ability to visualize these features plays a significant role in the design of scaffolds for tissue engineering applications.

Poster 3: Stx18 plays a key role in surface stabilization of MR1 and Mtb antigen presentation

Melanie J. Harriff1,2, Wilmon F. Grant2, Elizabeth T. Canfield2, Ansen Burr1, Luis F. Moita3*, David M. Lewinsohn1,2,4*

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4Molecular Microbiology and Immunology Oregon Health & Science University
*Co-last authors

Mucosa-Associated Invariant T (MAIT) cells may play an important role in immune response to pathogens, including Mycobacterium tuberculosis (Mtb). MAIT cells are restricted by the MHC-related protein (MR1), a highly conserved non-classical Class I molecule. Recently, Vitamin B metabolites were identified as ligands that bind and stabilize MR1, and subsequently activate MAIT cells. Currently, little is known about the intracellular trafficking pathways for MR1 or the mechanisms for processing and presenting these unusual ligands. To determine the mechanisms by which mycobacterial antigen is processed and presented to MAIT cells on MR1, we screened a lentiviral shRNA library specific for 150 genes involved in vesicular trafficking. Knockdown of Sec22b and Stx18 in antigen presenting cells was associated with a decrease in IFN- release by MAIT cell clones, but not HLA-E or HLA-B45 restricted T cell clones. To analyze the movement of MR1 in cells, we expressed an MR1-GFP fusion protein and analyzed MR1 trafficking at basal conditions or following incubation with ligand. We find that at basal conditions, MR1 localizes to vesicles that have features of late endosomes. In the presence of 6-formyl pterin (6-FP) or 7-hydroxy-6-methyl-8-D-ribitylumazine (RL-6-Me-7-OH), two vitamin B metabolites, MR1 is stabilized on the cell surface. We subsequently showed that Stx18 knockdown resulted in a decrease in 6-FP dependent surface stabilization of MR1 and corresponding increase in the number and size of intracellular vesicles, suggesting that Stx18 is required for trafficking of loaded MR1 from intracellular vesicles to the cell surface. Interestingly, while Sec22b altered the size and number MR1 containing vesicles, it did not impact the surface stabilization of MR1 in the presence of 6FP. From these data we hypothesize that the MR1 containing vesicle is a source of MR1 that becomes loaded with mycobacterial ligands, and that Stx18 is required for proper loading and trafficking of MR1 to the cell surface.

POSTERS

Poster 4: Fluorogenic Probes for Mycobacterial Esterase Profiling

Katie Tallman and Kimberly E. Beatty

Department of Physiology and Pharmacology and OHSU Center for Spatial Systems Biomedicine
Oregon Health and Science University, Portland, OR, 97239 USA
*Correspondence to: Katie Tallman (tallman@ohsu.edu)

Fluorogenic probes go from a dark to a bright state following enzyme-mediated hydrolysis and provide a rapid readout of enzyme activity. We have developed three novel fluorogenic esterase probes: two derived from the far-red fluorophore 7-hydroxy-9H-(1,3-dichloro-9,9-dimethylacridin-2-one) (DDAO), and one from the chlorinated fluorescein 2',7'-dichlorofluorescein (DCF). Notably, DDAO excites above 600 nm but does not require near-infrared detection capabilities. We spectrally characterized and validated our probes with a panel of commercially available esterases and lipases. All three probes were efficiently hydrolyzed in the presence of enzyme, but resisted spontaneous cleavage in aqueous solution. We used these probes to identify differences in enzyme activity patterns produced by a variety of mycobacterial species, including members of the Mycobacterium tuberculosis (M. tb.) complex. We anticipate that these probes will enable us to annotate the M. tb. genome and to identify new diagnostic targets.

Poster 5: Design and Development of BODIPY-based photoswitchable fluorophores for Multi-Spectral Super Resolution Microscopy (MSSRM)

Amy M. Bittel, Andrew K. Nickerson, Li-Jung Lin, Xiaolin Nan, Summer L. Gibbs

Department of Biomedical Engineering, Center for Spatial Systems Biomedicine, Knight Cancer Institute, Oregon Health and Science University, Portland, OR, USA
*Correspondence to: Summer L. Gibbs, gibbss@ohsu.edu

Super resolution microscopy (SRM) has overcome the historic spatial resolution limit of light microscopy, enabling fluorescence visualization of cellular structures and multi-protein complexes at the nanometer scale. Using single-molecule localization microscopy, the precise location of a stochastically activated population of photoswitchable fluorophores is determined during the collection of many images to form a single image with resolution of ~10-20 nm, an order of magnitude improvement over conventional microscopy. However, the spectral resolution of current SRM techniques are limited by existing fluorophores with only up to four colors imaged simultaneously, limiting the number of intracellular components that can be studied in a single sample. In the current work, a library of novel BODIPY-based fluorophores was synthesized using a solid phase synthetic platform with the goal of creating a set of photoswitchable fluorophores that can be excited by 5 distinct laser lines but emit throughout the spectral range (450-850 nm) enabling multispectral super resolution microscopy (MSSRM). The key photoswitching properties of all new fluorophores were quantified using custom Matlab analysis. To ensure the accuracy of our photoswitching measurements, our methodology to detect and quantitate the photoswitching properties of individual fluorophore molecules was validated by comparing measured photoswitching properties of three commercial dyes to published results. We also identified two efficient methods to positionally isolate fluorophores on coverglass for screening of the BODIPY-based library.

POSTERS

Poster 6: Characterization of Fluorophore Behavior during Electron Microscopy Sample Preparation for Improved Contrast in Correlative Light and Electron Microscopy

Haiyan Li, Danielle Jorgens, Anke Mulder, Christopher Arthur, Summer L. Gibbs*

Department of Biomedical Engineering, Center for Spatial Systems Biomedicine, Knight Cancer Institute, Oregon Health and Science University, Portland, OR, USA
*Correspondence to: Summer L. Gibbs, gibbss@ohsu.edu

Correlative light and electron microscopy (CLEM) provides a comprehensive imaging tool to examine both biological structures and their function(s) by detecting fluorescently labeled cellular structures at sub-micrometer level, while visualizing their absolute structure at Å resolution. However, it remains difficult to preserve fluorescence signal from labeled proteins during sample preparation for electron microscopy (EM). In the study herein we examined the decrease in fluorescence intensity caused by the variety of reagents required for EM sample preparation. Four classes of fluorophore scaffolds, specifically including BODIPY FL, Atto 488, Cy3B, and Alexa Fluor 647, were characterized for their fluorescence intensity in solution, bound to bovine serum albumin (BSA), and following intracellular immunofluorescence. We demonstrated that heavy metal stains used for EM sample preparation including, osmium tetroxide and uranyl acetate, significantly decreased fluorescence intensity. However, interestingly, some fluorophore scaffolds were more susceptible to heavy metal induced fluorescence decrease than others. Liquid chromatography-mass spectrometry (LCMS) analysis was performed on the heavy metal treated fluorophores and suggested that the observed fluorescence intensity decrease may result from heavy metal complex-induced structural degradation of the fluorophore scaffolds. When the fluorophore scaffolds were conjugated to BSA or antibodies for intracellular immunofluorescence, their intensity was less affected by uranyl acetate staining then by osmium tetroxide staining as compared to the bare fluorophores in solution. These preliminary data indicate that judicious selection of fluorophore families for CLEM studies as well as optimization of heavy metal staining may improve fluorescence imaging contrast for future CLEM studies.

Poster 7: Nuclear Lamins

Bingbing Li

Department of Physiology and Pharmacology, Oregon Health and Science University, Portland, OR, 97239 USA
*Correspondence to: Bingbing Li (lib@ohsu.edu)

Nuclear lamins, including lamin A/C, B1, and B2 in humans, form nuclear lamina inside the nucleus to maintain the structure of nucleus. In addition to this traditional view of lamins as nucleoskeleton proteins, they also participate in a variety of nuclear metabolism functions including DNA repair, DNA replication and RNA transcription. Thus, misregulation of lamins has been observed in a variety of diseases ranging from cancer to premature aging (progeria). However, the exact mechanisms that underling these functions are poorly understood. Complicating our understanding of the molecular mechanisms of lamins is the existence of a separate nuclearplasmic pool of lamins, which makes classical genetic strategies inefficient in teasing out such mechanisms. Small molecule regulators of lamins would provide a unique and powerful tool to dissect the molecular mechanisms of lamins' functions. However, there are currently no small molecules known to bind lamins to regulate their functions. In this presentation, we will discuss our discovery of lamin-binding ligands (LBL) from a phenotypic screening of a uniquely synthesized compound library followed by chemical proteomics identification of their binding targets.

POSTERS

Poster 8: Differences in heterocycle basicity distinguish homocysteine from cysteine using aldehyde-bearing fluorophores

Aabha Barve, Mark Lowry, Jorge O. Escobedo, Robert M. Strongin.

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We report the detection of homocysteine over cysteine based upon characteristic differences between 5- and 6-membered heterocyclic amines formed upon reaction with aldehyde-bearing compounds. Homocysteine-derived thiazinane-4-carboxylic acids are more basic than cysteine-derived thiazolidines-4-carboxylic acids. Fluorescence enhancement in response to homocysteine is achieved by tuning pH and excitation wavelength.

Poster 9: Thyroid Hormone Action in X-Linked Adrenoleukodystrophy

Hartley, Meredith D.; Galipeau, Danielle; Potter, Gregory P.; Poicus, Edvinas; Marracci, Gail; Chaudhary, Priya; Petryniak, Magdalena A.; Bourdette, Dennis N.; Scanlan, Thomas S.

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X-linked adrenoleukodystrophy (X-ALD) is a rare genetic disease that affects the adrenal cortex and central nervous system. In the most severe clinical phenotype, which presents in 35-40% of patients, the disease manifests as inflammatory cerebral demyelination at age 5-8 and patients survive for only 1-2 years. X-ALD is caused by mutation of ABCD1, which leads to an accumulation of very long chain fatty acids (VLCFAs) in patients. Thyroid hormone has several tantalizing connections to X-ALD that indicate a thyroid hormone-based strategy may represent a successful approach for the treatment of X-ALD. Thyroid hormone is an important signal during neurodevelopment. It induces the differentiation of oligodendrocyte precursor cells (OPCs) into mature myelinating oligodendrocytes. In addition, thyroid hormone induces transcription of ABCD2, which encodes a close homolog of the mutated ABCD1. Overexpression of ABCD2 has been shown to compensate for defective ABCD1 in disease models. Both of these mechanisms of action would provide routes for thyroid hormone action impact the disease progression of X-ALD patients. Unfortunately, thyroid hormone is not a viable treatment due to the toxic effects associated with hyperthyroidism. However, synthetic thyroid hormone analogs represent a safe alternative. Sobetirome, a potent thyromimetic developed in the Scanlan Laboratory and currently in clinical trials, mimics thyroid hormone action while avoiding thyrotoxic effects through tissue and receptor-selective action. In this study, we have evaluated thyroid hormone and sobetirome action in a variety of models including cultured oligodendrocytes, fibroblasts derived from X-ALD, and mouse models of demyelination. We have observed that sobetirome can effectively mimic thyroid hormone action to induce maturation of oligodendrocytes in vitro and to upregulate ABCD2 in both cellular and in vivo mouse models. Furthermore, thyroid hormone and sobetirome treatment effectively hastened remyelination in the lysolecithin model of demyelination in mice, which was demonstrated by analysis of tissue samples with both fluorescence and electron microscopy. These results represent the first efforts towards validating the thyromimetic sobetirome as an efficacious treatment strategy for X-ALD.

POSTERS

Poster 10: Depth dependent point spread function measurement for an Oil immersion objective of confocal microscope

Ahmad Raza Khan1, 2, Anda Cornea1, Sune Nørhøj Jespersen 3, and Christopher D Kroenke1, 2

1Division of Neuroscience, Oregon National Primate Research Center, Oregon Health & Science University, Beaverton, Oregon, United States.
2Advanced Imaging Research Center, Oregon Health & Science University, Portland, Oregon, United States.
3Center of Functionally Integrative Neuroscience, Aarhus University Hospital, Aarhus, Denmark.

Point spread function (PSF) describes the response of an imaging system to a point source/ object. The image is constituted by an operation know as convolution of object and PSF of imaging system. PSF is greater in through plane direction (z-direction) than in plane (xy-plane). This inhomogeneity in PSF is a confounding factor for local intensity variation in 3D microscopic images obtained on confocal microscope. Local intensity variation of an image provide a great deal of information regarding edge and corner detection, texture analysis, image registration and structure tensor (ST) analysis in this case (Weickert et al 1999). In order to measure and correct this inhomogeneity of PSF in thick specimen, a tri-layered tissue sandwiched with micro beads was utilized to inquire about the depth dependent variation in PSF in thick tissue (Figure 1).In addition to that the study also demonstrated depth dependent correction of 3D image stack in order to perform 3D ST analysis. Microscopy based ST analysis validate d-MRI data with homologues parameters at 1µm resolution which cannot attain by strongest MRI.

Poster 11: High Performance Antifade Mounting Media for High Resolution Fluorescence Microscopy

Scott Sweeney, Daniel Cash, Kamran Jamil, Eric Welch,

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While often overlooked, choosing the correct mounting medium is an important consideration when trying to obtain the best possible fluorescent images from fixed cell and tissue samples and is an integral part of the imaging workflow. The choice of mounting medium is largely dependent upon sample preparation, the type of imaging involved and the specific fluorophores used. More specifically, the mounting medium should have a refractive index that is compatible with the microscope and objectives to be used and it should be compatible with the dyes and fluorescent proteins with which the sample will be visualized. Antifade mounting media protect fluorescent dyes from oxidative photobleaching during fluorescence imaging. A number of commercial products exist that provide antifade protection, however these products are often limited by (i) the fluorophores that they protect (i.e. certain classes of dyes), (ii) lack of protection for fluorescent proteins, (iii) low signal to noise (typically in the violet and blue channels) and (iv) refractive index mismatch with oil-immersion objectives. Here, we compare the performance of best-in-class commercial antifade mounting media, including VECTASHIELD®, ProLong® Gold, SlowFade® Gold, ProLong® Diamond and SlowFade® Diamond for photobleach, signal to background and real world imaging performance. We show that ProLong® and SlowFade® Diamond provide the best performance for the protection of all classes of dyes (e.g. rhodamine, AlexaFluor and Cy dyes) and fluorescent proteins while providing very good signal to background performance and that these mounting media yield high quality fluorescent images.

POSTERS

Poster 12: Combining quantitative phase microscopy with fluorescent reporters: quantifying the alteration of nuclear structure following radiation damage

Sophia Bornstein1, Casey Kernan2, Charles Thomas1, Melissa Wong2, Owen J.T. McCarty2,3, Kevin G. Phillips3

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2Department of Cell & Developmental Biology, Oregon Health & Science University, Portland, OR, USA
3Department of Biomedical Engineering, Oregon Health & Science University, Portland, OR, USA
Correspondence to: Kevin G. Phillips (phillkev@ohsu.edu)

The combination of label-free quantitative phase microscopy with fluorescent labeling of molecular constitutes of the cell will enable new insights into cellular structure and function. However, the successful union of label-free and label-based approaches requires a quantitative understanding of cellular perturbations arising from cell membrane permeabilization needed for intracellular immunolabeling. In this study we characterize the role of cell membrane permeabilization in UM-SCC-22A cells with the detergent Triton X-100 in altering dry mass density organization and cell volume in the presence and absence of DNA and H2AX fluorescent labeling. We find that cell dry mass density is reduced by 28% following cell membrane permeabilization, independent of staining, and that visualization of nuclear architecture, regularly obscured by cytoplasmic constituents, is greatly enhanced under differential interference contrast. Further, we establish that cell volume is not altered by Triton X-100 membrane permeabilization. With this quantitative understanding of cell alterations induced by Triton X-100, we quantify nuclear and cytoplasmic subcellular density alterations resulting from 8 Gy x-ray irradiation and correlate these signatures with DNA and H2AX expression patterns. This synergistic imaging approach elucidates both biophysical and biochemical alterations in cells following radiation damage and can be widely used in similar investigations exploring therapeutic cellular perturbations.

Poster 13: Quantitative co-imaging of mRNAs and protein in Her2-positive breast cancer single cells treated with Lapatinib, a HER-family tyrosine kinase inhibitor

Sunjong Kwon1, Michel Nederlof2, Patrina Pellett3, Aurelie Snyder4, Koei Chin1, Joe W. Gray1

1Department of Biomedical Engineering, Oregon Health & Science University, Portland, OR 97239. 2Quantitative Imaging Systems, Inc. 941 Penn Ave, Ste 902, Pittsburgh, PA 15222. 3Cell Technologies, GE Healthcare, 1040 12th Ave NW, Issaquah, WA 98027. 4Advanced Light Microscopy Core, Oregon Health & Science University, Portland, OR 97239.

Both intrinsic and extrinsic micro-environmental factors influence pathway function in individual cancer cells, which results in establishing tumor heterogeneity. We need tools to explore the heterogeneity in histological contexts; therefore, we have been developing imaging methods to allow simultaneous quantification of RNAs and proteins at the single cell level. Simultaneous visualization of the activation of these pathways and subsequent gene expression in single cells is needed to understand sensitivities and responses to drug treatment in cancer cells with cell-to-cell variability. To quantify individual transcripts in breast cancer single cells, we first established single molecule fluorescent in situ hybridization (smFISH) technology using multiple short probes (20-nucleotide length) labeled with single fluorophores. Her2 mRNA particle counts are closely related to DNA copy number data in a variety of breast cancer cells. Unexpectedly, Her2 mRNA particles are enriched in the nuclei of both Her2+ and non-Her2+ cells, representing around 30 % of their total particles, and suggesting their potential subcellular RNA localization and consequent translational regulation. The nuclear Her2 mRNA aggregates present in diffraction-limited resolution imaging are resolved using super-resolution structural illumination microscopy (SR-SIM). Next, we established "immune-smFISH," combining immunocytochemistry and smFISH for the simultaneous co-imaging of protein and mRNA, and applied it to time-lapse analyses of Her2 mRNA expression and phosphoAkt protein levels in Her2-positive breast cancer single cells treated with the HER-family tyrosine kinase inhibitor Lapatinib. Akt signaling activities down-regulated by Lapatinib treatments are recovered after 48 hrs, as previously reported by Western blot analysis. However, phosphoAkts are not recovered in SKBR3 cells grown on glass coverglasses, as opposed to plastic, possibly due to extrinsic micro-environmental factors. Nuclear morphometries are

also analyzed by measuring the size, intensity, aspect ratio, perimeter, roundness, and circularity of DAPI-stained nuclei, whose differences might cause expression level changes. Our imaging methods provide information about the association between transcription level, cellular localization, and protein expression in individual cells, and would be applied to pinpoint target cancer cells of aberrant signaling and subsequent end-point gene expression in human tumor biopsy samples and xenograft tissues.

Poster 14: Photoactivated Localization Microscopy with Bimolecular Fluorescence Complementation (BiFC-PALM): Nanoscale Imaging of Protein-Protein Interactions in a Cell

Andrew Nickerson, Li-Jung Lin, Tao Huang, and Xiaolin Nan

Department of Biomedical Engineering, Knight Cancer Institute, and Oregon Center for Spatial Systems Biology (OCSSB), Oregon Health and Science University, Portland, OR 97239

Among existing methods for detecting PPIs, bimolecular fluorescence complementation (BiFC) has been widely used because interactions can be visually detected and spatially mapped in cells as fluorescence. Like all conventional light microscopy, however, the spatial resolution in BiFC has been diffraction limited at ~250 nm, providing little information of PPIs at the molecular scale. To visualize PPIs at nanometer spatial resolution, we combine BiFC with photoactivated localization microscopy (PALM). PALM uses photoactivatable fluorescent proteins (PA-FPs) and operates by stochastic activation and sub-diffractive localization of individual PA-FP molecules. A photoactivatable mCherry (i.e., PAmCherry1, a commonly used PALM probe) is split into two non-fluorescent fragments, each of which is genetically fused to a target protein. When the two target proteins interact, the two fragments are brought into proximity to re-generate a PAmCherry1 molecule. We found that re-generated PAmCherry1 molecules retain photoactivation, single-molecule brightness, and other properties of the original PAmCherry1, allowing for PALM imaging at ~18 nm resolution. With BiFC-PALM, we obtained super-resolution maps of interactions between the small GTPase Ras and its downstream protein kinase Raf in mammalian cells. The images clearly showed that Ras/Raf complexes form nanoscale clusters on the cell membrane, a previously hypothetical attribute of Ras/Raf interaction. Our study demonstrated the feasibility and power of BiFC-PALM for studying PPIs at the nanometer scale. It can be readily extended to other PA-FPs and biological systems, such as HER2/HER3 heterodimerization in breast cancer cells.

Poster 15: Structural Analysis of the ClpAP-ClpS Protease Complex by Single-Particle Electron Microscopy

Alice England and James Chen

Department of Biochemistry and Molecular Biology, OHSU

Substrate recognition by the E. Coli protease complex ClpAP is regulated by the adaptor protein ClpS. ClpS binds N-end-rule substrates and delivers them to the axial pore of the ClpA unfoldase for degradation in the ClpP proteolytic chamber, where the specific interactions between the substrate, ClpS and the ClpA D1 domain mediate the substrate recognition, delivery, unfolding, and degradation. The ClpAP-ClpS complex with a GFP substrate was imaged and reconstructed by single-particle electron microscopy. The structure provides further insight into the role of ClpS in ClpAP protein degradation processes as well as a better understanding of the ClpA-ClpP interactions.

POSTERS

Poster 16: Spiro guanidine-rhodamines as fluorogenic probes for total LPA analysis

Wang, L.; Sibrian-Vazquez, M.; Escobedo, J. O.; Strongin, R. M.

Department of Chemistry, Portland State University, 1719 SW 10th Ave.SB2, Room 262, Portland, OR, 97201

Ovarian cancer is one of the most lethal cancers affecting women. This is due to the inability to detect it while still localized to the ovaries. Early detection would result in a 90 % survival rate. LPA, a putative biomarker for early stage ovarian cancer, is the subject of our research. Two fluorogenic probes (GRBI and GRBII) for total LPA analysis in biological samples have been developed in our lab.

Poster 17: The Visual Detection of Homocysteine in Natural Media Via Simple

Lovemore Hakuna, Jorge O. Escobedo and Robert M. Strongin*

Department of Chemistry, Portland State University, 1719 SW 10th Ave.SB2, Room 262, Portland, OR, 97201

Elevated levels of Hcy are linked with cardiovascular disease (CVD), dementia, osteoporosis and Alzheimer's disease. According to the World Health Organization, global CVD-related deaths are projected to reach an annual rate of 23.6 million by 2030. A wide variety of innovative indicators and probes for aminothiols detection have been reported. Many are commercially available. However, most lack the selectivity needed to distinguish among the structurally-related biothiols. Current commercial detection methods use separations, fragile and expensive enzymatic or immunogenic materials and complex instrumentation. Available kits are also generally non-selective for specific thiols, and use multi-step procedures and fragile materials that can require storage at temperatures below -20 ºC, thereby limiting their use, for instance, in emerging nations with limited access to refrigeration or electricity. Our main research goal is to apply fundamental chemical principles towards creating sensing reagents that will enable researchers and clinicians to readily determine specific bioactive thiols, such as Hcy, in order to better understand their unique roles in disease progression and diagnostics. A relatively simple and inexpensive photochemical method for the selective detection of Hcy directly in human blood plasma will be presented. The method uses a commercially available probe (benzyl viologen). The fundamental chemical mechanism that enables detection of Hcy over GSH and other thiols is the reduction of the probe by the α-amino carbon centered radical of Hcy that is generated selectively due to a favorable intramolecular hydrogen atom transfer (HAT) of the Hcy thiyl radical. This method has potential practical application in home test kits or point-of-care diagnostics because it involves the use of a non-toxic chromogen, an inexpensive commercial visible light source and simple sample processing, involving only reduction and filtration prior to photolysis. It enables quantification of Hcy directly in a patient's plasma sample.

Poster 18: Lysophosphatidic Acid Biomakers in Ovarian Cancer

Jialu Wang

Department of Chemistry, Portland State University

Lysophosphatidic acids (LPA) have been reported to be biomarkers for many diseases including ovarian cancer. Patients with ovarian cancer have a 90% survival rate if diagnosed in early stages. Rapid and simple methods for the detection of lysophosphatidic acids are being developed. A HPLC-post column fluorescence detection method can separate and detect individual LPA subspecies. Molecular imprinted polymers are being synthesized to extract LPA from plasma.

POSTERS

Poster 19: Role of Fluorophores in Biochemistry and Medical Diagnostics

Shelly Chu

Fluorophores play an important role in biochemistry and medical diagnostics. Fluorescent probes are widely used for the detection of specific biomolecules in cells or to study specific cellular events. The aim of the research described herein is to design and synthesize fluorescent probes and their corresponding functional conjugates to investigate the trafficking mechanisms of gentamicin and cisplatin involved in their cellular uptake. The goal is to aid our understanding of the mechanisms whereby these widely used drugs induce serious side effects such as kidney failure and deafness.

Poster 20: Dimerization-dependent RAS signaling studied with super resolution microscopy

Tao Huang, Li-Jung Lin, Andrew Nickerson, Frank McCormick, Joe Gray, Steven Chu, and Xiaolin Nan

Membrane-bound Ras small GTPases regulate cell proliferation, survival, and differentiation through PI3K-Akt-mTOR and RAF-MEK-Erk (aka MAPK) pathways, and are frequently activated in in human cancer. However, it remains unclear how RAS operates on the membrane to activate its effectors, leaving it an intractable drug target. We have used photoactivated localization microscopy (PALM) to examine the nanoscale spatial organization of individual RAS proteins on the cell membrane; PALM is a recent super-resolution microscopy technique that enables cellular imaging at ~10 nm spatial and single-molecule stoichiometric resolutions. With PALM, we have previously discovered that RAS-mediated activation of Raf/MAPK is dependent on dimerization of Ras-GTP. We now show that RAS dimerization also regulates PI3K-Akt activation. Specifically, at endogenous expression levels mutant RAS forms dimers and activates PI3K/Akt, but at much lower expression mutant RAS is monomeric and only activates PI3K-Akt when artificially dimerized. These results are analogous to our previous observations on RAS-dimer mediated MAPK activation. Consistently, single molecule tracking in living cells revealed that RAS proteins exist in multiple diffusion states – mobile and immobile fractions – and the immobile fraction increases with expression level. These observations establish dimerization as a key component in RAS-mediated oncogenic signaling, and provide the molecular basis for targeted therapy against mutant RAS in human cancer.

Poster 21: Probe Signaling Nano-structures with Correlative Superresolution and Electron Microscopies

Ying Zhang, Tao Huang, Andrew Nickerson, Li-Jung Lin, Claudia Lopez, Danielle Jorgens, and Xiaolin Nan

Department of Biomedical Engineering, Knight Cancer Institute, and OHSU Center for Spatial Systems Biomedicine (OCSSB)

Cellular processes are often regulated by both temporal and spatial mechanisms. While temporal regulation has been extensively studied, spatial mechanisms and particularly those involve nanoscale cellular structures are poorly understood. For example, membrane nanodomains such as lipid rafts and Ras nanoclusters, are enriched in certain lipids and proteins and implicated in many cell signaling processes. The complex composition and small length scale of these structures pose challenges to existing visualization tools – both light and electron microscopies. We combined superresolution microscopy (SRM) with electron microscopy (EM) to study membrane nanodomains. In this approach, SRM resolves individual proteins tagged with photoactivable fluorescent proteins at ~10 nm nanometer spatial resolution, and EM provides the detailed cellular context in which these proteins reside. We demonstrate this approach with preliminary studies using cells expressing tubulin tagged with mEos4, a photoactivatable fluorescent protein commonly used in SRM with photoactivated localization microscopy (PALM). We acquired images of microtubules with SRM images of microtubules and Scanning EM images of the same cells; the two sets of images were then registered for correlative visualization of microtubules and the whole cell. The next step is to label nanodomain proteins (e.g. Ras, CD59, or PLAP) for correlative observations of Ras nanoclusters and lipid rafts. We anticipate that this approach will greatly facilitate our understanding of the structure – function relationship of these signaling membrane nanodomains.

THANK YOU!

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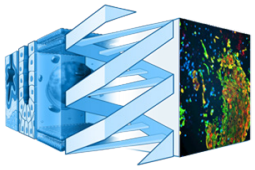

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