Optical Microscopy and Super-Resolution Imaging I

1607-Pos Board B558
An Automated Protocol for Performance Benchmarking a Widefield Fluorescence Microscope
Michael Halter, Elianna Bier, Paul C. DeRose, Gregory A. Cooksey, Steven J. Choquette, Anne L. Plant, John T. Elliott.
Biosystems and Biomaterials Division, National Institute of Standards and Technology, Gaithersburg, MD, USA.
Widefield fluorescence microscopy is a highly used tool for visually assessing biological samples and for quantifying cell responses. Despite its widespread use, few published methods exist for evaluating and benchmarking the analytical performance of a microscope. Easy-to-use benchmarking methods would facilitate the use of fluorescence imaging as a quantitative analytical tool in research applications. We describe and evaluate an automated method to characterize a fluorescence imaging system's performance by benchmarking the detection threshold, saturation and linear dynamic range to a reference material. The benchmarking procedure is demonstrated using two different materials as the reference material, uranyl-ion-doped glass and Schott 475 GG filter glass. Both are suitable candidate reference materials that are homogeneously fluorescent and highly photostable, and the Schott 475 GG filter glass is currently commercially available. In addition to benchmarking the analytical performance, we also demonstrate that the reference materials provide for accurate day to day intensity calibration. A script written in MicroManager, an open-source microscopy control software, has been developed to automate the procedure and return the benchmarked parameters. The MicroManager script is now publicly available. Ideally, this work will lead to common operating procedures that will help to assure that fluorescence microscopy results are meaningful, traceable, and comparable from day to day and between laboratories.

1608-Pos Board B559
Respiration Rate Measurements of Single Bacterial Cells
Michael C. Konopka.
Chemistry, The University of Akron, Akron, OH, USA.
One of the limitations of a population-based analysis method is that it averages over a large number of cells. While this helps average out fluctuations in the measured signal, it potentially can cover up subpopulations that could be functionally important. Using a single-cell approach, one can individually measure cells and look at the variation within the isogenic cell population.
Respiration can be an indicator of physiological state and therefore is an excellent target for analysis of heterogeneity within the sample. Single-cells are isolated in microwells containing Pt-porphyrin embedded microspheres on a glass chip. These microwells are diffusionally sealed with a lid actuator which can be raised at the end of the measurement to reoxygenate the sample. Since the phosphorescence lifetime of the Pt-porphyrin depends on the oxygen concentration, the Pt-porphyrin embedded microspheres can be used as an oxygen sensor. Monitoring the consumption of oxygen in the sealed microwells over time allows a respiration rate to be calculated for the individual cells. This method is compatible with other optical imaging techniques.

1609-Pos Board B560
Development of Physiologically Based Pharmacokinetic Model (PBPK) of Cancer Treatment in Mice
Jeremy C. Bonor.
Bio, University of Delaware, Newark, DE, USA.
In hormone-dependent cancers, tumor growth is driven by the binding of the hormone to its receptor. Vitamin D3 can have both preventive and therapeutic effects by regulating cell growth, cell cycle, apoptosis, and differentiation - a role much greater than earlier views that focused primarily on bone health and maintenance of calcium homeostasis. Epidemiological studies have found a significant association between low serum levels and low dietary intake of Vitamin D3 and the incidence, degree of malignancy, metastases, and mortality of cancers of the breast, prostate, colon, and ovaries. We used Semiconductor Nanoparticles (Quantum Dots, (QDs)) in conjunction with a binding procedure to develop a Calcitriol-QD conjugate. Based on this research a Physiologically-Based Pharmacokinetic (PBPK) model has been developed to determine steady-state distribution of Calcitriol-QD in mice. The multi-compartmental PBPK model represents relevant organ/tissues with physiological accuracy. Four processes characterized the change in the concentration of the protein in every compartment: blood flow in, blood flow out, protein turnover and receptor binding in the organ. The unique aspects of the model are the determination of elimination using receptor kinetics and generation using protein turnover. The model also predicts steady state concentrations of Calcitriol-QD in tissues in mice and may be used for possible scale-up of dosage regimens in humans. Due to the QDs fluorescent characteristics, our Calcitriol conjugates can be applied successfully to image the distribution and uptake of Calcitriol into cells. Our data show that we successfully generated a Calcitriol-QD conjugate that is biologically active and stable for at least 48h at RT. Additionally we were able to image the uptake and the distribution of Calcitriol in real time and determine its interaction with the cell membrane and accumulation in the cell nucleus.

1610-Pos Board B561
Raising the Speed Limit on 3D-3Way FRET Microscopy
Brandon L. Scott, Adam D. Hoppe.
Chemistry and Biochemistry, South Dakota State University, Brookings, SD, USA.
The spatial organization of protein-protein interactions inside of cells is an important component of cellular signaling. These interactions can be imaged using fluorescent resonance energy transfer (FRET) microscopy methods. We have described a generalized mathematical solution and method for any number of interacting FRET pairs (N-Way FRET). Here, we combine N-Way FRET with our three-dimensional FRET stoichiometry reconstruction (3D-FSR) to allow 3D-3Way-FRET image deconvolution. By developing a multi-camera instrument, we are able to acquire the 6 images needed per z-plane with sub-second time resolution, allowing observation of live cell dynamics. Furthermore, we implemented improved fast Fourier transforms and parallelized calculations to achieve a 10-fold decrease in the computational time required to reconstruct 3D-3Way-FRET data. This reduction has made it feasible to analyze dynamic 2Way and 3Way FRET data with 3D reconstructions, increase the number of reconstruction iterations, as well as explore stopping criteria for the algorithm.

1611-Pos Board B562
Stretched, Oriented DNA Arrays (SODA) for Fluorescence Based Single-Molecule Experiments in Complex Environment
Eugeniu Ostrofet, Seungkyu Ha, Richard Janissen, Theo van Laar, Nynke Dekker.
Bionanoscience, Kavli Institut of Nanoscience, TU Delft, Delft, Netherlands.
All biochemical and biophysical processes that support cellular activity take place in complex, crowded environments. Up to 30% of the weight of a cell consists of proteins, DNA and other large biological macromolecules. Consequently, 1-dimensional protein motions along DNA while replication or transcription have to be studied and understood in the context of a DNA molecule that is not naked, but instead bound by a wide variety of obstacles - roadblocks. Inspired by previous, pioneering work on DNA curtains, we used the intrinsic propriety of some macromolecules and polymers to create self-assembled, organized structures, adapted for visualization using TIRF microscopy of interactions between genome processing enzymes and roadblocks in crowded environment.

1612-Pos Board B563
Dual-Switching FRET (dsFRET) Imaging Based on Photoswitchable Donor-Acceptor Pair
Yingqi Wang, Ji Tang, Xiaodong Liu.
Department of Biomedical Engineering, School of Medicine, Tsinghua University, Beijing, China.
Fluorescence resonance energy transfer (FRET), especially with fluorescent proteins of donor and acceptor, has been widely used to measure biomolecular interactions. To overcome limitations of existing approaches for quantitative FRET, we here put forward a novel platform of dual-switching FRET (dsFRET), with a photoswitchable donor as well as a photoswitchable acceptor. With the photoswitchable capability from the FRET pair we constructed, neither donor-only nor acceptor-only samples would be required as control reference for calculation. Experiments of dsFRET and traditional 3^3-FRET were conducted and compared in both dimer and two-hybrid forms. Our data demonstrate that dsFRET has higher accuracy and stability than 3^3 -FRET, mainly benefited from *in-situ* references. Further development of dsFRET has been pursued, to enhance the performance of this new methodology, and also to extend its applications, such as in subcellular FRET and *in-vivo* FRET.

1613-Pos Board B564
Dynamic Turnover of FtsZ-Ring in Live Cell
Xinxing Yang, Christopher Herrick Bohrer, Jie Xiao.
Biophysics and Biophysical Chemistry, The Johns Hopkins University, Baltimore, MD, USA.
In bacterial, cell division is initiated at the midcell by the formation of a ring-like structure called Z-ring. The Z-ring is assembled by the polymerization of a tubulin homolog, FtsZ, and serves as a scaffold to recruit more than twenty other division proteins. Like tubulin, FtsZ is a GTPase. Thus, a model has been proposed wherein nucleotide-dependent changes in the conformation of