Platform: Imaging & Optical Microscopy - Applications

1029-Plat
Probing Molecular Number Variability in Intracellular Structures using Photoswitchable Fluorescent Proteins
Elias M. Puchner, Jessica M. Walter, Robert Kasper, Bo Huang, Wendell A. Lim.
UCSF, San Francisco, CA, USA.
The development of advanced fluorescence microscopy has opened new opportunities to investigate biological systems beyond the optical diffraction-barrier. Single molecule localization microscopy techniques such as STORM and PALM offer additional information about the local number and stoichiometry of subcellular biological molecules and complexes. However, the photophysical properties of compatible fluorescent probes vary with different cell types and experimental conditions, complicating quantitative image reconstruction. These photophysical properties can lead to both over-counting and under-counting artifacts as a consequence of dye blinking and undetected fluorophores. We propose a single molecule calibration system in the budding yeast S. cerevisiae to account for both effects: we first determine the relevant photophysical parameter of the photoactivatable fluorescent proteins (PAFPs) in cells to correct for blinking and then measure the number of observed PAFPs in protein complexes with known stoichiometry. We apply this concept to measure variability and correlations of PI3P binding sites on early endocytic vesicles.

1030-Plat
Tracking the Mechanical Dynamics of Stem Cell Chromatin
Elizabeth Hinde[1], Francesco Cardarelli[2], Aaron Chen[3], Michelle Khine[3], Enrico Gratton[1].
[1]Laboratory for Fluorescence Dynamics, University fo California, Irvine, CA, USA, [2]Center for Nanotechnology Innovation @NEST, Istituto Italiano di Tecnologia, Pisa, Italy, [3]University fo California, Irvine, CA, USA.
A plastic chromatin structure has emerged as fundamental to the self-renewal and pluripotent capacity of embryonic stem (ES) cells. Direct measurement of chromatin dynamics in vivo is however challenging as high spatiotemporal resolution is required. Here we present a new tracking based method which can detect chromatin fiber movement and quantify the mechanical dynamics of chromatin in live cells. We use this method to study how the mechanical properties of chromatin movement in hESCs are modulated spatiotemporally during differentiation into cardiomyocytes (CM). Notably, we find that pluripotency is associated with a highly discrete, energy-dependent frequency of chromatin movement, that we refer to as a ''breathing'' state. We find that this ''breathing'' state is strictly dependent on the metabolic state of the cell and is progressively silenced during differentiation, thus representing a hallmark of pluripotency maintenance. This is a result that could not have been observed without the nanometer resolution provided by this novel tracking method.

1031-Plat
Snapshot Hyperspectral Imaging for Dual-FRET in Live Cells
Amicia D. Elliott[1], Noah Bedard[2], Alessandro Ustione[1], David Sprinzen[1], Michelle A. Baird[3], Michael W. Davidson[3], Tomasz S. Tkaczyk[2], David W. Piston[1].
[1]Vanderbilt University, Nasvhille, TN, USA, [2]Rice University, Houston, TX, USA, [3]The Florida State University, Tallahassee, FL, USA.
Fluorescent biosensors based on Forster resonance energy transfer (FRET) are commonly used for studying the dynamics of molecules in living cells. While numerous fluorescent biosensors are available, most of them are built around cyan/yellow fluorescent protein (FP) pairs. This severely limits our ability to perform simultaneous measurements of multiple biosensors. A few biosensors have been based on green/red FP pairs, but simultaneous imaging with these and a traditional cyan/yellow FP pair becomes challenging due to the highly overlapping emission spectra of all these FPs. One way to overcome these challenges is to use a novel snapshot spectral imaging device, the Image Mapping Spectrometer (IMS), which we have previously used for imaging concurrently Ca^{2+} and cAMP in pancreatic β-cells [Elliott, et al, JCS, 2012]. Here, we show the simultaneous imaging of two FRET pairs, a caspase3-cleavable green/red FRET sensor, and a cAMP-sensitive cyan/yellow FRET sensor. These signals are used to determine the relationship between cAMP signaling and apoptosis in pancreatic β-cells, towards a better understanding the pathology of type 1 and type 2 diabetes.
Spectral imaging data analysis can be difficult under the best conditions, and this becomes even more difficult when imaging multiple FRET pairs. Linear unmixing is the most commonly used method for extracting multiple spectra from an image. However, linear unmixing requires high quality reference spectra, which may change dynamically because of cellular autofluorescence background. The recently described spectral phasor analysis method offers a potentially more robust approach since it does not require reference spectra. To establish the validity of this new analysis, we provide a quantitative comparative analysis of linear unmixing and spectral phasor methods to analyze the data.

1032-Plat
Intracellular Measurement of Macromolecular Crowding by a Genetically-Encoded Indicator
Takamitsu Morikawa[1], Katsumi Imada[2], Keiko Yoshizawa[3], Toshio Yanagida[1,3], Takeharu Nagai[4], Tomonobu M. Watanabe[1,3].
[1]Graduate School of Frontier Biosciences, Osaka University, Suita, Osaka, Japan, [2]Department of Macromolecular Science, Graduate School of Science, Osaka University, Toyonaka, Osaka, Japan, [3]RIKEN Quantitative Biology Center (QBiC), Suita, Osaka, Japan, [4]The Institute of Scientific and Industrial Research, Osaka University, Suita, Osaka, Japan.
Intracellular environment is overcrowded with enormous number of various macromolecules such as proteins. For example, the total concentration of macromolecules in Escherichia coli (E. coli) cells is known to be about 350 mg/ml. This molecular crowding reduces the substantial space for molecular diffusion which must affect the biological phenomena including enzymatic reaction, protein-protein interaction, protein-DNA interaction, and protein folding. Since the crowding conditions are a ubiquitous feature in not only prokaryotic cells but also eukaryotic cells, biophysical and biochemical measurements in vitro using cell extracts maintaining natural cellular contents are one of essential approaches. However, this type of experiments inevitably lost a spatiotemporal information of molecular crowding in living cells. To investigate the crowding effect on biomolecular functions in living cells, we have been trying to develop a genetically-encoded fluorescent indicator for molecular crowding. During the course of engineering in a yellow variant of green fluorescent protein from Aequorea Victoria, we found that a variant inserted glycine residue just before Thr145 (YFP1G) changed in its fluorescence intensity according to the protein concentration while cyan variant of fluorescent protein (CFP) did not. Then, we fused CFP with YFP1G (CFP-YFP1G) to make a ratiometric indicator. The emission ratio (YFP/CFP) of CFP-YFP1G was decreased by increasing in protein concentration.When expressed in E. coli, fluorescence signal of CFP-YFP1G was dramatically changed in synchronization with the cell cycle, i.e. protein concentration increased with cell growth but decreased after cell division. In addition, we obtained a similar result in mammalian cells. This CFP-YFP1G will become an indispensable tool to figure out relationship between biomolecular functions and macromolecular crowding.

1033-Plat
Imaging of Temperature Distribution in a Living Cell
Kohki Okabe[1,2], Seiichi Uchiyama[1], Noriko Inada[3], Yoshie Harada[4], Takashi Funatsu[1].
[1]University of Tokyo, Tokyo, Japan, [2]JST, PRESTO, Kawaguchi, Japan, [3]Nara Institute of Science and Technology, Ikoma, Japan, [4]Kyoto University, Kyoto, Japan.
Temperature is a fundamental physical quantity that governs every biological reaction within living cells, and temperature distribution reflects cellular thermodynamics and function. In medical studies, the cellular pathogenesis of diseases (e.g., cancer) is characterized by extraordinary heat production. Therefore, intracellular temperature imaging of living cells should promote better understanding of cellular events and the establishment of novel diagnoses and therapies. However, imaging of temperature distributions in living cells has never been achieved. Here we demonstrate the first intracellular temperature imaging based on a fluorescent polymeric thermometer and fluorescence lifetime imaging microscopy (FLIM). The spatial and temperature resolutions of our thermometry were at the diffraction limited level (200 nm) and 0.2 °C, respectively. The intracellular temperature distribution we observed indicated that the nucleus and centrosome of a COS7 cell both showed a significantly higher temperature than the cytoplasm and that the temperature gap between the nucleus and the cytoplasm differed depending on the cell cycle. The heat production from mitochondria was also observed as a proximal local temperature increase. These findings demonstrate an intrinsic connection between temperature and organelle function. Thus, our intracellular temperature imaging has a significant impact on the comprehension of cell function and will provide insights into the regulatory mechanisms of intracellular signaling.