Monday, March 4
9:30 am – 11:00 am
Room 303
Bruker Corporation

ADVANCES IN DYE DEVELOPMENT AND MICROSCOPY FOR LIVE CELL SUPER RESOLUTION MICROSCOPY WITH THE VUTARA 352

Expanding the frontier of super-resolution imaging requires advances in both microscopy hardware and fluorescent labels. Here we describe a cooperative effort to improve both technological fronts with the ultimate goal of live-cell super-resolution microscopy. Bruker's Vutara 352 super-resolution microscope has been designed for live-cell super-resolution microscopy with both high spatial and temporal resolution capabilities. The patented biplane module allows simultaneous two-color imaging in 3D while the sCMOS detector enables fast imaging of biological phenomena. Although this microscope system is capable of live-cell super-resolution imaging, it has been stymied by limitations in the current generation of live-cell-compatible fluorophores. Extant live-cell probes are either fluorescent proteins with low photon counts—and therefore low localization precision—or organic dyes, which require high laser power resulting in phototoxicity in living samples. To remedy this problem, we developed spontaneously blinking (SB) versions of the Janelia Fluor and Alexa Fluor dyes, which blink under physiological conditions at low laser power while still providing high photon counts. In particular, the spontaneously blinking Janelia Fluor 549 (SB-JF549) and red-shifted SB-JF646 are cell-permeable and are easily conjugated to HaloTag or SNAP-tag ligands, making them ready to use in live cell multi-color super-resolution experiments. The SB dyes, in combination with the Vutara 352, provide a powerful methodology for simultaneous imaging, localization and visualization of live-cell single-molecule localization data, while offering numerous statistical tools to quantify the data into publishable results.

Speaker
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