Rapid Subcellular Imaging with Light-Sheet Fluorescence Microscopy

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By illuminating only the parts of a sample that are in focus, Light-Sheet Fluorescence Microscopy (LSFM) reduces photo-toxicity and provides intrinsic optical sectioning. The high spatial duty cycle of LSFM, *i.e.* the entire focal plane is illuminated at once, also allows for very rapid volumetric imaging. However, further improvements in imaging speed are necessary to follow rapid intracellular events, such as motor driven transport or secondary ion signaling waves, with sufficient spatiotemporal sampling over whole-cell dimensions.

Here, we present two approaches that can overcome technological and conceptual limitations of 3D acquisition rates in subcellular LSFM. For adherent cells on a coverslip, typically placed at 45 degrees relative to the detection and illumination axes, we realized that more than one focal plane can be imaged instantaneously in a lossless manner. In a first implementation of this concept, we used three staggered and independently detected light-sheets, resulting in crosstalk-free, highly light-efficient, 3D parallelized image acquisition. This allowed imaging of subcellular dynamics throughout whole cells at up to 14Hz (2700 frames per second) and over 1000s of time points.

FEP films, which have a refractive index close to water, can be aligned parallel to the focal plane of the detection objective in LSFM without causing significant aberrations to the illumination beam. This greatly speeds up imaging of adherent cells as much fewer focal planes are needed to form a z-stack compared to cells on coverslips at a 45 degree angle. However, light sheets of extraordinary high aspect ratio (beam waist to propagation length) are needed to provide a sufficiently large field of view and subcellular axial resolution. Here we use a two-photon, high-aspect ratio Bessel beam that allows us to synthesize light-sheets that span a field of view of 100x100microns at 350nm axial resolution. We image cells cultured on FEP films that are aligned parallel to the propagation axis of the Bessel beam to realize rapid whole cell 3D imaging. We believe that both approaches hold the potential to significantly speed up volumetric acquisition, which in turn allows 3D imaging of intracellular dynamics that were hitherto limited to observation with 2D imaging modalities.

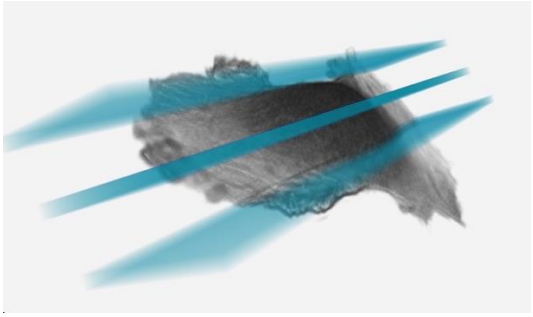

Figure 1: Rendering of parallelized volumetric imaging with three staggered light-sheets.