OBLIQUE PLANE MICROSCOPY WITH 200 NM-SCALE RESOLUTION.

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Light-sheet fluorescence microscopy has generated significant interest in biological research as a result of its ability to volumetrically image biological specimens with high optical sectioning, high spatiotemporal resolution, and low photobleaching and phototoxicity. However, its adoption has been modest in part because light-sheet microscopes require expert maintenance, alignment and operation. Furthermore, the orthogonal imaging geometry sterically occludes many commonly used sample mounting methods (e.g., microfluidics and multi-well plates), and because the sample is in direct contact with water-dipping objectives, it remains challenging to maintain the sterile conditions necessary for longitudinal imaging.

Oblique plane microscopy, because it can image biological samples in a traditional inverted microscopy format and is commpatible with all coverslip-based sample mounting techniques, overcomes many of these challenges [1]. In oblique plane microscopy, a highly inclined light-sheet is launched from a single high-NA objective, and the resulting fluorescence is relayed in an aberration-free remote focusing format to an obliquely oriented imaging system [2]. However, until recently, oblique plane microscopy has failed to achieve the necessary resolution for sub-cellular imaging. Here, we build upon previous work [3], and describe the design of an improved oblique plane microscope that incorporates a bespoke glass-tipped objective [4] and delivers ~200 nm-scale resolution after deconvolution and camera framerate-limited volumetric imaging. We demonstrate its optical performance and image clathrin-mediated endocytosis, cell migration, natural killer cell-mediated cell apoptosis, migration through confined microfluidic chambers, and optogenetic activation of Rac1 and PI3Kinase.

Figure 1: Maximum intensity projection of retinal pigment epithelial cell expressing vimentin-GFP.

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