High-Numerical Aperture Oblique Plane Microscopy

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Owing to its selective plane illumination and massively parallelized and photon-efficient camera-based detection, light-sheet fluorescence microscopy enables longitudinal imaging of living specimens with greatly reduced photobleaching and phototoxicity. In most cases, this is achieved by illuminating the sample with an orthogonal illumination and detection geometry, or by introducing reflective surfaces in the immediate vicinity of the specimen. Unfortunately, for high-numerical aperture systems, the former approach places the sample in direct contact with non-sterile optical surfaces, the latter approach has a limited volumetric imaging capacity, resolution, and field of view, and both are incompatible with many biological experiments (e.g., imaging cells migrating through a confined microfluidic channel). In contrast, an oblique plane microscope illuminates the specimen with a light-sheet that is obliquely launched from an objective, collects fluorescence with the same objective, and then uses aberration-free relay optics to refocus the fluorescence onto a traditional two-dimensional scientific camera [1]. However, for years, imperfect relay optics limited the resolution and performance of such imaging systems. Here, we describe an oblique plane microscope that implements custom remote refocusing optics and enables imaging at the highest numerical aperture and thus resolution [2, 3]. Owing to its careful design, it achieves 220 nm scale resolution (after deconvolution) throughout a 180x180-micron field of view and camera framerate-limited imaging (~800 fps) in a conventional and user-friendly microscope geometry. Performance of the system is evaluated with wavefront measurements and a combination of resolution targets analyzed in both spatial and frequency domains. Biological imaging is demonstrated on diverse specimens spanning single cells, developing embryos, and large-scale tissue slices (see also Figure 1 for one example).

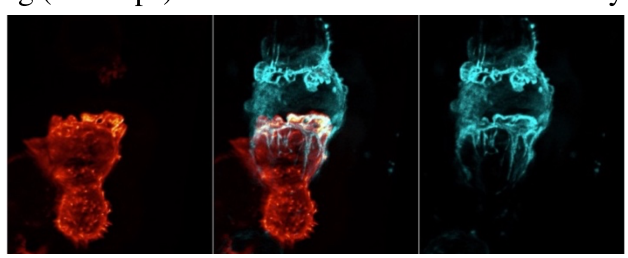

Figure 1 – Formation of an immunological

[1] C. Dunsby, Optics Express **16** (2008).
[2] E. Sapoznik, B.-J. Chang, J. Huh, R. J. Ju, E. V. Azarova, T. Pohlkamp, E. S. Welf, D. Broadbent, A. F. Carisey, S. J. Stehbens, K.-M. Lee, A. Marín, A. B. Hanker, J. C. Schmidt, C. L. Arteaga, B. Yang, Y. Kobayashi, P. R. Tata, R. Kruithoff, K. Doubrovinski, D. P. Shepherd, A. Millett-Sikking, A. G. York, K. M. Dean, and R. P. Fiolka, eLife **9** (2020).
[3] A. Millett-Sikking, K. M. Dean, R. Fiolka, A. Fardad, L. Whitehead, and A. G. York, Zenodo (2019).