LIGHT-SHEET MICROSCOPY OPTIMIZED FOR DEPTH PENETRATION TO STUDY EMBRYOGENESIS

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Fluorescence light sheet microscopy (FLSM) has gained widespread recognition in recent years, due to its distinct advantages for the 3-dimensional (3D) imaging of living biological samples (for reviews see [1,2]). FLSM uses a planar sheet of light to illuminate a sample, generating fluorescence over an optical section of the sample that is collected by a wide-field microscope camera oriented orthogonal to the light sheet. The orthogonal geometry between the illumination and detection pathways enables massive parallelization in both illumination and detection; furthermore, it permits optical and physical access to samples (3D cell cultures or whole embryos) in ways that are impossible in the collinear geometry of standard microscopes. Because of these features, FLSM significantly outperforms standard laser-scanning confocal microscopy in imaging speed, phototoxicity, and signal to noise in many imaging applications. An important aspect of any 3D imaging technique is its imaging depth limit (how deep into a sample useful information can be collected). In this respect, standard FLSM fares only slightly better than confocal microscopy. To overcome this hurdle, we have optimized FLSM for imaging of live thick samples by minimizing the degradation of the light sheet due to scattering, while preserving acceptable axial resolution. Using this approach we have imaged whole, live fruit fly embryos and zebrafish embryos. We achieve higher depth penetration than standard FLSM, while maintaining sub-cellular resolution, at imaging speed of about ten times faster than standard confocal microscopy.

The figure below demonstrates the improvement in depth penetration that we achieve with our approach, showing an axial slice through a live H2A-GFP transgenic fruit fly at stage 15, imaged by the standard light sheet technique (left) and by our technique (right). Each bright dot in the images denote a cell nucleus. The images have size of 250 microns x 100 microns. The y-axis of the images represents the axial axis as seen by the detection optics, while the light sheet propagation direction is orthogonal to the image plane shown.