

## **Selective Plane Illumination Microscopy, A New Imaging Modality Available at the Indiana Center for Biological Microscopy**

**Seth Winfree, Nathaniel Smith, Ken Dunn, Malgorzata Kamocka and Bruce Molitoris**

**Indiana Center for Biological Microscopy, Division of Nephrology, Department of Medicine, Indiana University School of Medicine, Indianapolis, IN**

Microscopy is a primary tool for studying 3D tissue models. Microscopy provides the only means of distinguishing the behaviors of individual cells in a heterogeneous context that obscures biochemical assays. SPIM (Selective Plane Illumination Microscopy) is a new approach that is ideally suited to the unique problems involved in high-resolution imaging of 3D tissue models. In the simplest form of SPIM, a cylindrical lens is used to generate a thin lightsheet (1-10 microns) that illuminates a sample. An imaging objective lens, placed orthogonal to this lightsheet is used to collect an image of fluorescence that is selectively excited in this single illuminated plane. The sample is then rotated, and the process is repeated until a multiview dataset of the entire sample is collected. These cross-section images are then assembled to give a complete 3D image of the sample. This approach offers several advantages over conventional methods of imaging thick tissues.

First, SPIM provides superior axial resolution for large field-of-view images, deconvolved SPIM volumes have isotropic 3D resolution. Second, SPIM is a “gentle” imaging approach and is better suited to imaging living tissues than either confocal or multiphoton microscopy, supporting studies of cell migration, development, signaling and physiology. Third, imaging speeds can be 30 to 200 fold faster than scanning confocal or multiphoton systems, enabling resolution of dynamic events, and rapid collection of large image datasets.

We describe the assembly and customization of an OpenSPIM based lightsheet microscope (IU OpenSPIM) as a platform for developing new imaging technologies. To this end we have implemented software and hardware for multi-channel laser control and temperature and perfusion control. We present examples of high-resolution, live and high speed imaging, demonstrating these capabilities. The IU OpenSPIM is a centerpiece in the development of new software for 3D tissue cytometry and a novel screening platform.