Dual Inverted Selective Plane Illumination Microscopy (diSPIM)

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- The diSPIM system from ASI can be mounted on to an existing inverted microscope, or configured as a complete system with ASI's RAMM platform. The system offers a number of advantages over conventional microscopy systems:
  - Use conventional mounting / glass cover slips
  - Generate 3D volumes with isotropic resolution (330 nm in all directions)
  - Axial resolution is ~2x better than confocal- or spinning disk systems
  - Achieve a ~7-10 fold reduction in photobleaching
  - Acquisition rates up to 200 images per second or 2-5 volumes per second (roughly 10x faster than spinning-disk microscopes)
  - Compared to Bessel beam plane illumination approaches, diSPIM offers equivalent (or better) axial resolution (~330 nm), 10-100x faster volumetric imaging rates (0.5-1 s instead of 10-100 s), and the ability to image over ~10x more time points (~1000 instead of ~100), due to the significantly lower illumination dose employed and the lack of extraneous illumination outside the focal plane.
  - Microscope has multicolor capability, and has been tested successfully on cells cultured on cover slips, cells embedded in collagen gels, and nematode and zebrafish embryos.

Other Features

In addition to the dual inverted Selective Plane Illumination Microscopy (diSPIM) systems which is a double-side system, light sheet excitation and emission on each side, ASI also has a number of other configurations including:

1) Fixed sheet systems. (single sided) The light sheet is stationary, with only mechanical adjustment for the sheet position. The two objectives are manually adjusted to correctly focus on the sheet. The specimen is scanned through the sheet using the X and Z stages to generate volume images. Advantages of this system is that it is the least expensive - not requiring either galvo scanners or a piezo objective positioner. Disadvantage is the difficulty in correctly overlapping the objective focal planes with fixed positioners and the relatively slower stage scanning at odd angles.

2) Standard single-sided system. Light sheet from one side, emission objective on the other. The light sheet can be scanned using galvos to sweep across the sample volume. There is an emission objective piezo so the viewing objective can be positioned to follow the light sheet as it is scanned through the sample.

Advantages: Rapid scanning, straight-forward set-up.
Disadvantages: Better XY resolution than Z resolution
Fig. 1. Dual-View iSPIM Setup
0.8 NA water-immersion objectives (A/B) are mounted orthogonally onto a Z translation stage that is bolted directly onto the illumination pillar of an inverted microscope. In conjunction with other optics (Supplementary Fig. 1), both objectives produce a light sheet at the sample. Excitation A(B) occurs via objective A(B), and the resulting fluorescence is collected through perpendicular objective B(A), and imaged onto Camera B(A) via dichroic mirrors, emission filters, and lenses. Excitation (blue) and detection (red) are shown occurring simultaneously along both light paths in the lower schematic, but in reality volumetric imaging occurs sequentially as shown in the upper right inset. During acquisition, sample and objective A(B) are held stationary, the light sheet is scanned through the sample using galvanometric mirrors (not shown), and a piezoelectric objective stage moves objective B(A) in sync with the light sheet, ensuring that excitation/detection planes are coincident. The sample is mounted onto a rectangular coverslip that is placed onto a 3D translation stage, ensuring correct placement relative to objectives. The sample may also be viewed through objective C (see upper left inset), dichroic mirror, emission filter, lens and Camera C placed in the conventional light path of the inverted microscope. This objective is particularly useful in finding or screening samples.