Dual-View Inverted Selective Plane Illumination Microscopy (diSPIM)

Introduction

Light sheet microscopy overcomes the challenges of imaging physiological processes. Light exposure can result in phototoxic effects and photodamage on biological samples, which can disrupt cellular functions. Light sheet microscopy uses two perpendicular objectives, one for illumination and the other for detection, and a z-stack of images is collected by sweeping the light sheet through the sample. Widefield microscopy illuminates samples throughout the axial direction, which excites out-of-focus fluorescence and decreases the contrast of the in-focus signal. Minimizing out-of-focus light reduces the background signal and images with high contrast are comparable to confocal microscopy. Less light exposure decreases the photodamage on living samples, which allows imaging of large and sensitive samples in 3D over a long period of time.

Single-view inverted selective plane illumination microscopy (iSPIM) is a light sheet microscopy technique that uses planar light sheets and widefield detection. It was created as a collaboration between Hari Shroff at NIH and Applied Scientific Instrumentation (ASI). It is currently manufactured and distributed by Applied Scientific Instrumentation (ASI).

iSPIM

In iSPIM, a laser beam is focused in one direction by a cylindrical lens and objective to form a thin planar light sheet a few micrometers thick. The planar light sheet has a hyperbolic profile with a beam waist that has the most tightly focused mid-point of the illumination profile. The beam waist can be calculated using the equation:

$$\omega_0 = \frac{1.4f\lambda}{2D_{\text{lens}}}$$

Where \(\omega_0\) is the beam waist thickness, \(f\) is the focal length of the illumination optics, \(\lambda\) is the illumination wavelength, and \(D_{\text{lens}}\) is the diaphragm of the illumination optics.

The sample should fit within the beam waist to ensure even illumination because light expands away from the waist. The confocal parameter defines the distance of near-homogenous planar illumination using the following equation:

$$b_n = 2n\chi_R = nb$$

Where \(n\) is the refractive index of the medium, \(b\) is the confocal parameter, and \(\chi_R\) is the Raleigh range.

The iSPIM “head” can be mounted on an inverted microscope with two objectives that dip into a reservoir filled with media to image samples. Live samples can be imaged over long periods of time with easy access for media changes. Compared to most other LSFM techniques that require samples to be embedded in a gel and suspended between objectives, preparation for iSPIM is simple and the sample remains stationary.
Adherent cells can be cultures on coverslips and whole organisms or tissues can be mounted to coverslips. In addition, transparent tissues can be imaged in the aggressive solvents used in clearing solutions with special cleared tissue objectives.

**diSPIM**

iSPIM is similar to most microscopy systems where the axial resolution is two-to-three-fold worse than the lateral resolution. The dual-view inverted selective plane illumination microscopy (diSPIM) system creates 3D images by collecting volumes from perpendicular directions and computationally combining data from the lateral views to create a volume with isotropic resolution (330 nm) in x, y, and z.

The poor axial resolution from one view is overcome by information from the other view. The “head” contains z piezos to ensure the detection objective moves as the light sheet sweeps in the plane of focus. The initial alignment of the diSPIM may seem challenging, but realignment takes approximately 10 minutes. The diSPIM is ideal for 3D imaging of live samples because volumes are quickly acquired and consistently in focus.

The system can be bought as a standalone microscope which means that it isn’t necessary to have an existing microscope to use it. Neither does the system require any special camera modes or extra features, making the system very simple to implement.

**diSPIM Camera Choice**

Any CMOS camera is recommended to be used with iSPIM and diSPIM but we believe that the best performance can be achieved with the 95% quantum efficient, back-illuminated Scientific CMOS camera, the Photometrics Prime 95B™.

The Prime 95B offers the sensitivity necessary for 3D imaging of live samples. A 95% quantum efficiency with large 11 µm pixels gives the Prime 95B the ability to detect faint fluorescent signals. This increase in sensitivity allows researchers to decrease exposure times to have faster acquisition times and minimize light dose. Phototoxicity using diSPIM and the Prime 95B has a >10x reduction in photobleaching compared to a confocal microscope. A significant decrease in phototoxic effects and minimal photobleaching allow data collection of live cells with high resolution over a long period of time.
The diSPIM system provides high spatiotemporal resolution imaging in all three dimensions. The observed plane is illuminated and images can be acquired at 200 fps, this speed is >30x faster than confocal laser scanning microscopy. The Prime 95B can acquire full frame images at 82 fps, but image acquisition for diSPIM requires a global shutter to image fast moving objects. When using Global Shutter readout all pixels are exposed at the same instant in time. Maximum frame rate speed is lower because there are two phases, an active image accumulation phase and a subsequent readout phase. However, a C. elegans embryo z-stack takes only 2.5 seconds [Figure 2]. This is fast enough to image most biological processes without significant motion blur, such as, late stage worm embryos, small objects that transverse the cell. Fine processes or projections of live cells are clearly resolved with diSPIM and the Prime 95B. The signal to noise ratio using diSPIM is comparable to confocal spinning disk. To further increase signal to noise, the Prime 95B is equipped with PrimeEnhance™, an active denoising process that reduces the effects of photon shot noise at low signal levels to provide a real-time quantitative increase in signal to noise ratio by 3x to 5x. Increasing laser power to resolve dim signals on live samples can be avoided by using PrimeEnhance.

The diSPIM system with Prime 95B cameras is an ideal solution for acquiring 3D images of live samples with isotropic resolution. The sensitivity and speed of this system allows minimal light dose with clear resolution of fast moving objects. Minimal photobleaching and phototoxic effects allows live samples to be imaged over a long period of time.

Figure 2: iSPIM image of C. elegans embryos expressing elt-2::mCherry::H2B to label gut cell nuclei. A single volume of 100 slices were acquired by the Prime 95B in 2.5 seconds. Huygens Professional (SVI, Hilversum, The Netherlands) to perform two processes. The Image Stabilizer was used to compensate for sample jitter. Followed by deconvolution with 40 iterations using the CMLE algorithm with the channels parameters were set to 'Light Sheet'. Images were exported as 16-bit TIF-files with linked scaling. C. elegans were a gift from the authors, from: Choi et al., Dev Bio; 427 (2017) 49-60.

References


http://dispim.org/