

Unlocking the Cell's Secrets with 3D Imaging



Characterizing cells and cellular organization is required for many areas of biology as well as pathology. Traditional pathology examines one slice of tissue at a time under a microscope. Other times living cells and tissue are imaged using fluorescence microscopy. Advances in microscopic imaging now allow samples to be imaged in three dimensions much faster and with fewer imaging artifacts than was previously possible.

Selective Plane Illumination Microscopy (SPIM) is a revolutionary technique that is replacing conventional confocal microscopy as well as slide scanning for many applications. SPIM — also referred to as **Light Sheet Fluorescence Microscopy (LSFM)** or simply “**light sheet**” — is a fast and gentle imaging method that combines the speed of widefield imaging with optical sectioning and low photobleaching. The defining feature of SPIM is selective illumination of the detection focal plane. Because only a thin section of the sample is illuminated at any given time, photodamage is minimized and optical sectioning improves SNR compared with widefield fluorescence. Light sheet imaging is much faster than point-scanned confocal, and has a much lower light dosage than spinning-disk confocal.

ASI Light Sheet Microscopy Solution		
Dual Inverted Selective Plane Illumination Microscopy (diSPIM)	AMS-AGY Objective	Fiber-Coupled Laser Scanner
ASI's original light sheet microscopy configuration is an excellent general-purpose light sheet system. It uses two dipping objective lenses mounted over an inverted microscope, and the sample is mounted in an open dish. One objective is used to create the light sheet and the other to image. For dual-view imaging, 3D datasets are acquired from both objectives and computationally merged to yield a single 3D dataset with isotropic resolution.	The Calico-invented objective makes it easy to implement high-resolution single-objective light-sheet (SOLS) microscopy using a variety of primary objective lenses. The SOLS configuration permits a single objective to be used near the sample, like conventional microscopes, but still reap the benefits of light sheet microscopy. The disadvantage is a more complicated optics system away from the sample.	A compact and versatile light sheet generator creates a light sheet by scanning a beam quickly within the camera exposure time using a MEMS mirror. An optional anti-stripping mirror varies the beam's angle of incidence on the sample to mitigate shadowing effects. Another option uses a cylindrical lens to create a static light sheet. The scanner interfaces with the microscope using a C-Mount port.

What to look for using SPIM

SPIM Advantages:

- Minimizes photodamage because excitation light is confined near the focal plane
- Good optical sectioning
- Faster acquisition

SPIM Disadvantages:

- Requires extra optics
- Each instrument more specialized to a particular sample type