SOLUTIONS FOR
LIGHT SHEET
MICROSCOPY

ASI

APPLIED SCIENTIFIC
INSTRUMENTATION

Light Sheet: What and Why

Selective Plane Illumination Microscopy (SPIM) is a fast and gentle imaging technique that combines the speed of widefield imaging with optical sectioning and low photo-bleaching. It has become an important fluorescence imaging modality, especially for volumetric imaging. SPIM is also referred to as Light Sheet Fluorescence Microscopy (LSFM) or simply "light sheet." (Although some reserve the term SPIM for static light sheets, we use the terms LSFM, light sheet, and SPIM interchangeably.)

The defining feature of light sheet imaging is illumination of the focal plane from the side. Only a thin section of the sample is illuminated at any given time, which simultaneously improves photon efficiency (minimizing bleaching and phototoxicity) and provides optical sectioning (improving SNR). Light sheet imaging is much faster than point-scanned confocal microscopes because detection is done on a 2D image sensor.

Light sheet microscopy has rapidly gained popularity for volumetric imaging because of the combination of three key features:
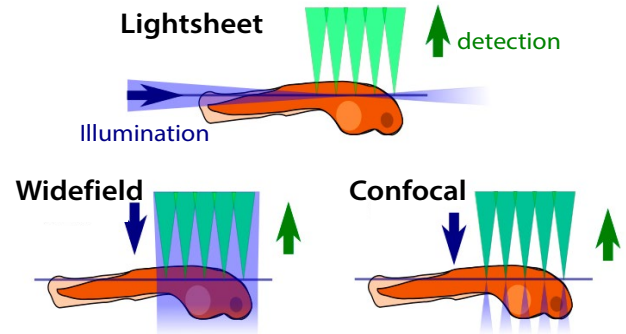
JKrieger / CC BY-SA 3.0. "LSFM: Lightsheet Fluorescence Microscopy, WF: widefield microscopy, CF: confocal microscopy." Wikimedia Commons, June 7th 2013, 20:10, https://commons.wikimedia.org/wiki/File:Lsfm_lightsheetinsample.svg. Retrieved and modified May 1st 2019
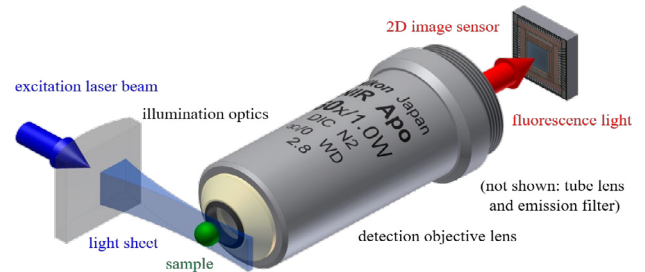
Jan Krieger / CC BY-SA 3.0. "The principal setup of a light sheet fluorescence microscope." Wikimedia Commons, October 24th 2012, 14:08, https://commons.wikimedia.org/wiki/File:Spim_prinziple_en.svg. Retrieved and modified May 1st 2019

1. Photodamage is minimized because excitation is confined near the focal plane, e.g. living samples stay alive for much longer.

2. Good optical sectioning is obtained, often comparable to confocal microscopy.

3. Acquisition is orders of magnitude faster than a traditional confocal microscope because of wide-field detection.

The main disadvantage of SPIM is that extra optics are required to generate the light sheet, unlike most confocal and widefield microscopes which have a single optical path. Thus, the advantages of SPIM come at the cost of more narrow applicability of any particular instrument. This has led to the explosion of different light sheet microscope configurations (and acronyms!), each with their own advantages and disadvantages for particular applications.

ASI offers several standard light sheet microscope configurations including the diSPIM, ct-dSPIM, and oSPIM. ASI's modular components make it easy to implement many other light sheet configurations.

How ASI Can Help

ASI has been taking a modular approach to constructing microscopes for over a decade. Our modular elements for SPIM include:

• Motorized and piezo stages

• Light sheet generators, for both static and scanned light sheets

• Tunable lenses

• All associated control electronics, including synchronizing elements including cameras and lasers with sub-millisecond precision

• Optomechanics, e.g. filter holders, kinematic mirrors, tube lenses

• Objective lenses for designed for light sheet

ASI's modular components can be easily combined to make an array of light sheet microscope configurations. Objective lenses, lasers, filters, and cameras are required to complete the system; users can procure these items themselves, use the services of various system integrators reselling ASI hardware, or purchase them via ASI.

Dual Inverted Selective Plane Illumination Microscopy (diSPIM)

The diSPIM is a flexible and easy-to-use implementation of Selective Plane Illumination Microscopy (SPIM) that allows for dual views ("d") of the sample while mounted on an inverted ("i") microscope. The SPIM objective lenses are placed at right angles above a sample mounted horizontally in an open dish, each objective 45° from vertical. The SPIM head is mounted on top of an inverted microscope, either a conventional inverted microscope or one made from ASI's RAMM/MIM components.

In a dual-view use case, the SPIM objectives are used alternatingly for illumination and detection, creating two datasets of the same sample viewed from orthogonal directions. The datasets can be computationally merged to yield a single 3D dataset with isotropic resolution. The dual-view diSPIM thus has two (usually symmetric) optical paths including two scanners (light sheet generators) and two cameras, in addition to the inverted microscope.

Sample

Objectives

A B

C

Piezoelectric objective stage
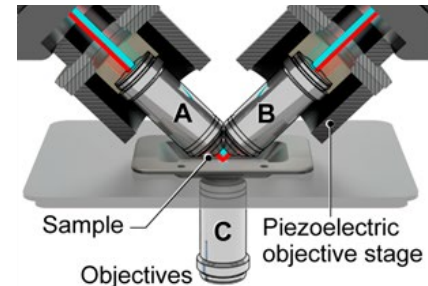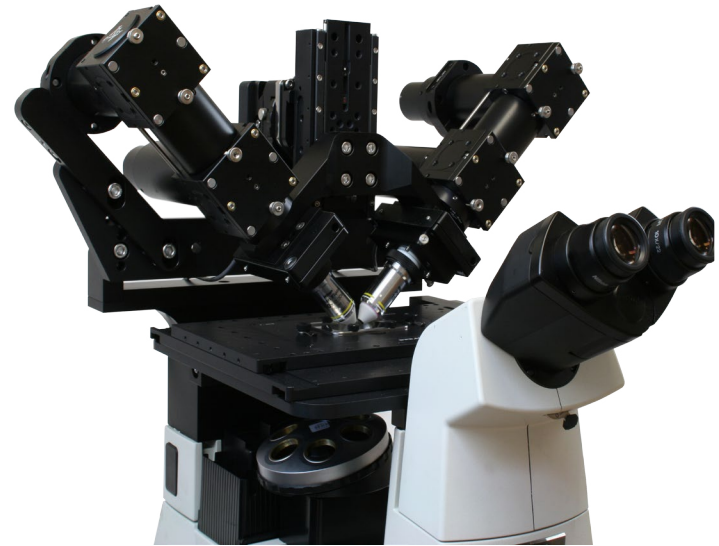
iSPIM is a subset of diSPIM with a single camera and scanner but otherwise identical in concept. The iSPIM often uses a single high-NA detection objective paired with a low-NA illumination objective, allowing it to attain better lateral resolution than the diSPIM at the expense of axial resolution.

The iSPIM and diSPIM have been used to image c. elegans and zebrafish embryos, cells and spheroids embedded in collagen gels, cells cultured on cover slips, and many other samples.

Features:

- Low phototoxicity: >10x reduction vs. confocal/spinning disk
- Rapid 3D imaging with isotropic resolution
- ~2x better axial resolution than confocal/spinning disk
- Acquisition rates up to 200 planes per second
- Sample mounting on coverslip or open dish
- Modular and flexible

diSPIM Specifications

Field of View*	>400 μm diagonal
Resolution*	380 nm @ 500 nm wavelength in XYZ
Sample Size*	Large flat samples up to 200 mm thick, or up to 3.5 mm radius hemisphere

* For Nikon 40x/0.8 WD objective and sCMOS camera.

Mounting	Cover slip or open dish
Imaging Depth	Limited by scattering, usually 50 -200 μm depending on sample
Software	Various free/open-source and proprietary
Photomanipulation	Available
Incubation	25-40 °C with CO_2 and humidity control (others possible)
Compatible Cameras	Any sCMOS with external trigger
Compatible Lasers	Any with TTL control (dual fiber output beneficial)
Acquisition Modes	Synchronized slice/piezo Stage scan Fixed sheet
Multi-D Acquisition	Any combination of: Time Points Multi-position Multi-color (up to 4)

Dual Selective Plane Illumination Microscopy for Cleared Tissue (ct–dSPIM)

The ct-SPIM and ct-dSPIM configurations are optimized for imaging cleared tissue ("ct"). They use an SPIM objective geometry identical to iSPIM/diSPIM respectively but without an inverted microscope. The sample is typically mounted on a motorized XYZ stage. Because of the size of the sample, stage scanning is the only viable acquisition mode. Acquisition speed is generally limited by the camera.

The ct-dSPIM has been successfully used to image various samples including whole mouse brains, cleared human brains, and slices of other types of cleared tissue.
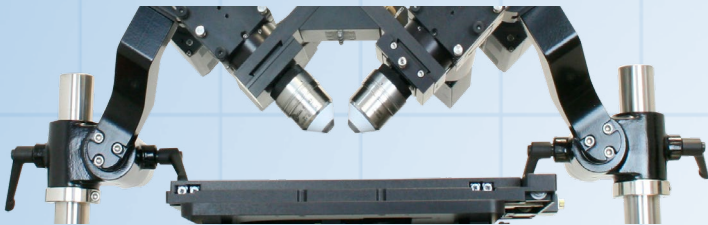
ct-dSPIM Specifications

Field of View*	>1.1 mm diagonal
Resolution*	<800 nm @ 500 nm wavelength in XYZ
Sample Size*	5 mm thick up to 200 mm in XY, or up to 12 mm radius sphere

* For 54-10-12 objective and sCMOS camera

Mounting	Open dish with objectives immersed in media
Imaging Depth	>5 mm into flat samples (aberrations often limit)
Software	Various free/open-source and proprietary
Compatible Cameras	Any sCMOS with external trigger
Compatible Lasers	Any with TTL control (dual fiber output beneficial)
Acquisition Modes	Stage scan recommended for large samples
Multi-D Acquisition	Any combination of: Time Points, Multi-position Multi-color (up to 4)
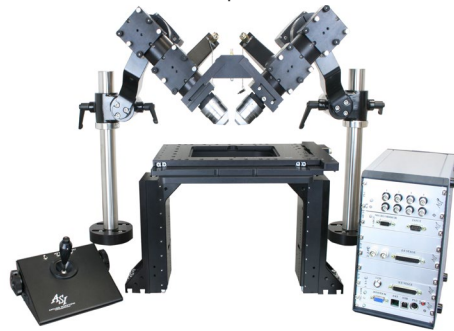
Multi-Immersion Objectives

ASI and Special Optics have developed two dipping objective lenses designed for light sheet microscopy of cleared tissue samples, including ASI's ct-dSPIM. These objectives work in any refractive index media without a correction collar because of a unique curved first surface. They are robust to immersion in harsh media including DBE and BABB.

The original objective lens (54-10-12) has nominal NA of 0.4, WD of 12 mm, and allows imaging over 5 mm deep into a flat sample. The second one (54-12-8) has an increased NA of 0.7 with a WD of 10 mm. Both NA and magnification vary slightly with the refractive index of the immersion medium, but WD is constant.

Like other ASI components, these objective lenses are available for sale individually or as part of complete systems.

More objective lenses for light sheet microscopy are planned; inquire for details.

	54-10-12	54-12-8
Numerical Aperture	0.4 @ RI 1.45	0.7 @ RI 1.45
Immersion Media RI	1.33 – 1.56	1.33 – 1.56
Effective Focal Length	12 mm @ RI 1.45	8.4 mm @ RI 1.45
Working Distance	12 mm (5 mm deep @ 45°)	10 mm (2 mm deep @ 45°)
Field of View	1.2 mm Ø	1.0 mm Ø

# Oblique Single Plane Illumination Microscope (oSPIM)

The oSPIM is an excellent platform for imaging live cells or other samples on a coverslip using fast and gentle light sheet microscopy. The oSPIM is a single-view light sheet system where the light sheet is generated at an oblique angle using an oil immersion objective below the sample dish. Fluorescent emission is observed using a high NA water dipping objective from the top, perpendicular to the illumination sheet (the objective is tilted 60° from horizontal). The arrangement with high NA objectives both above and below the sample dish allows for high-resolution imaging in a geometry convenient for cell culture work.

The oSPIM is two microscopes in one. The lower inverted microscope can be used for imaging the same sample using conventional modalities, such as widefield fluorescence, confocal, or TIRF. It is also utilized for to generate the light sheet. The tilted top microscope is dedicated to light sheet imaging.

Features:

- Low phototoxicity: >10x reduction vs. confocal/spinning disk
- Water dipping emission objective yields 280 nm lateral resolution (NA 1.1)
- Rapid 3D imaging at oblique angle to coverslip, up to 200 planes per second
- Sample mounting in cell culture dishes
- Fully functional "conventional" fluorescent microscope in addition to the light sheet modality
- Modular and flexible

90.0°

OBJECTIVE
VERTICAL
CLEARANCE
UP FROM
COVERSLIP
SURFACE
.006in
[0.15mm]
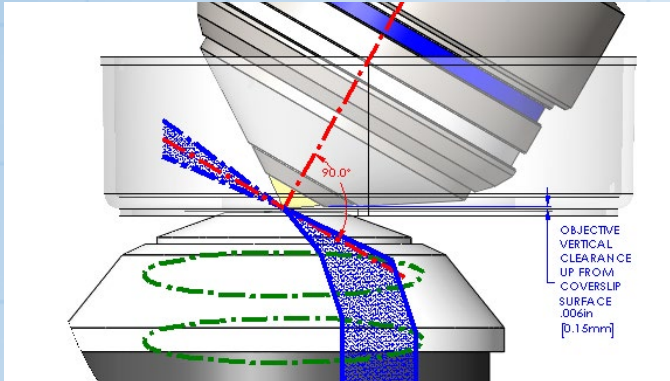
oSPIM Specifications

Field of View*	>250 µm diagonal
Resolution*	280 nm XY, ~670 nm Z @ 500 nm wavelength
Sample Size*	Best for thin transparent samples such as cells or tissue cultures mounted on coverslips

* For 60x NA 1.1 imaging objective and sCMOS camera.

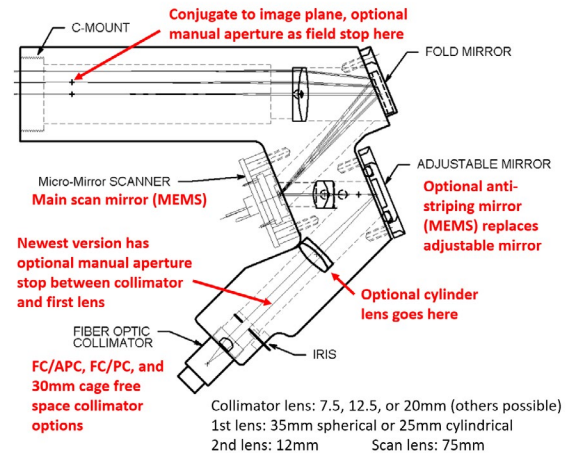|	
Mounting	35 mm Ø or larger glass-bottom dish, coverslip
Imaging Depth	Limited by scattering, usually 30 -150 µm depending on sample
Software	Various free/open-source and proprietary
Photomanipulation	Available
Incubation	25-40 °C with CO2 and humidity control (others possible)
Compatible Cameras	Any sCMOS with external trigger
Compatible Lasers	Any with TTL control
Acquisition Modes	Synchronized slice/piezo Stage scan Fixed sheet
Multi-D Acquisition	Any combination of: Time Points Multi-position Multi-color (up to 4)

SPIM Components

Fiber-coupled laser scanner: ASI developed a compact light sheet generator or "scanner" which is integral to our light sheet microscopes and useful for other applications. Scanners utilize a 2D MEMS mirror to steer the beam, enabling them to be compact and vibration-free. The standard version creates a "digital" or scanned light sheet, but versions exist for creating static sheets, for filling the BFP in other applications (e.g. FRAP), and with an anti-striping option.

Tunable lens: One application is to translate the beam waist synchronously with the camera's rolling shutter to implement ASLM.

Stages: ASI's servo-driven stages have industry-leading performance. An important feature for SPIM applications is the ability to move the sample slowly at a very uniform speed. Stages can be combined in various ways including 3D/4D stackups.

Your component here: We often work with researchers to develop new components. If you need something we don't yet offer, talk with us!

C-MOUNT

Conjugate to image plane, optional manual aperture as field stop here

FOLD MIRROR

ADJUSTABLE MIRROR

Micro-Mirror SCANNER
Main scan mirror (MEMS)

Optional anti-striping mirror (MEMS) replaces adjustable mirror

Newest version has optional manual aperture stop between collimator and first lens

Optional cylinder lens goes here

FIBER OPTIC COLLIMATOR

IRIS

FC/APC, FC/PC, and 30mm cage free space collimator options

Collimator lens: 7.5, 12.5, or 20mm (others possible)
1st lens: 35mm spherical or 25mm cylindrical
2nd lens: 12mm   Scan lens: 75mm

Custom SPIM Configurations

ASI's modular components are available for individual sale, but their true power comes from the ease of combining them in arbitrary ways to create the microscope you need.

The most difficult aspect of implementing a light sheet microscope is synchronizing the various components to each other including sample movement, cameras, lasers, and sheet-generating elements. ASI's controller coordinates these elements with sub-millisecond precision.

There is a free and open-source Micro-Manager plugin for controlling SPIM microscopes with ASI components. The plugin makes it easy to align the system, find the sample, and run acquisitions. ASI provides Micro-Manager device adapters for all our components, which are accessible in Python, Matlab, and other programming environments.

Whether you are trying to create a new configuration or replicate an existing configuration with robust and scalable hardware and software, ASI has the expertise and capability to help you.

Micro-Manager Control Plugin
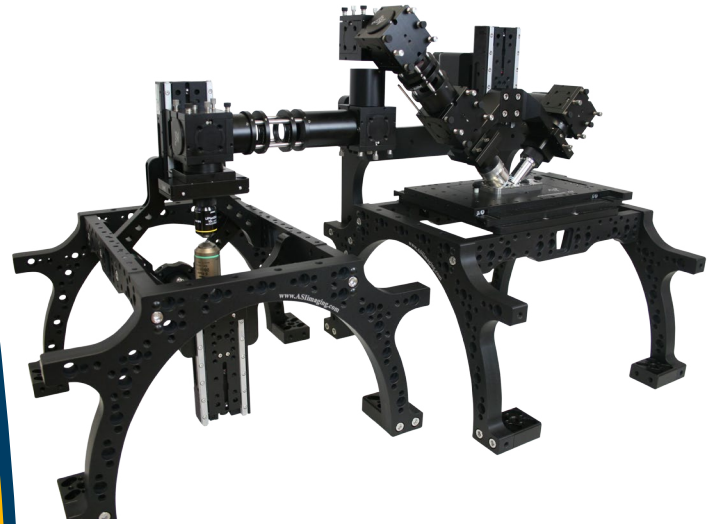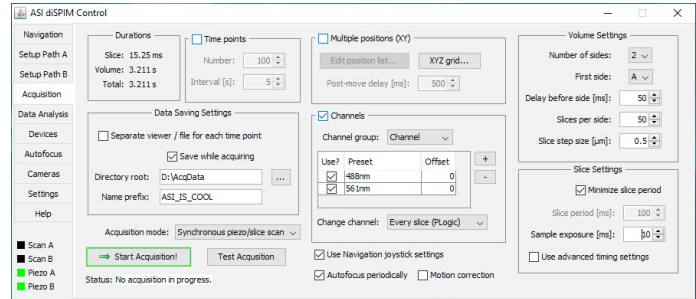
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we can help you!

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