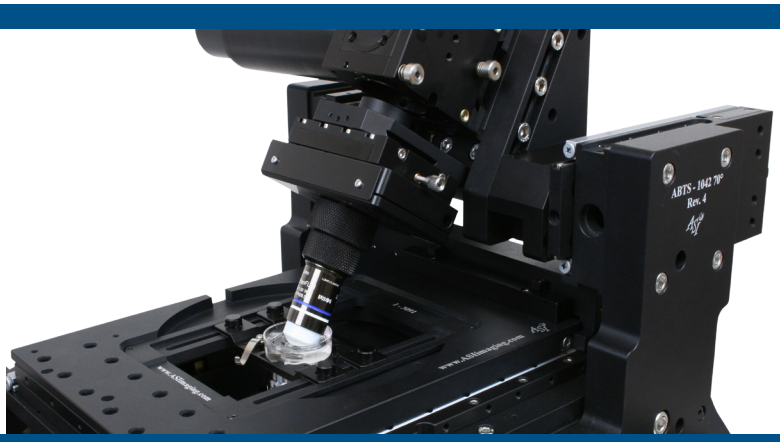
APPLIED SCIENTIFIC INSTRUMENTATION

Oblique Single Plane Illumination Microscope



ASI's 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, with the objective tilted 60 degrees, perpendicular to the illumination sheet. The arrangement with high NA objectives both above and below the sample dish allow for high-resolution imaging in a convenient geometry for cell culture work.

The oSPIM is two microscopes in one. The lower microscope can be used for fluorescent imaging using conventional modalities, such as wide field fluorescence, confocal, or TIRF. It is also utilized as the light sheet excitation objective. The tilted top microscope is dedicated to light sheet images.

Features

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

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

** Depends on objective and camera; these are for 60x NA 1.1 and sCMOS camera.*

Mounting	35 mm Ø or larger glass-bottom dish, cover slip
Imaging Depth	Limited by scattering, usually 30 -150 µm depending on sample
Software	Various free/open-source and proprietary
Photomanipulation	Available using inverted microscope objective
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)

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Basic System Configurations

The oSPIM is configured as an option for the ASI RAMM frame modular microscope system. Special hardware is used to mount the top-side microscope to the RAMM frame at a 60 degree angle. The bottom-side microscope includes a standard epi-fluorescence pathway as well as the scanner that generates the light sheet, but can easily combine modalities such as TIRF or confocal.

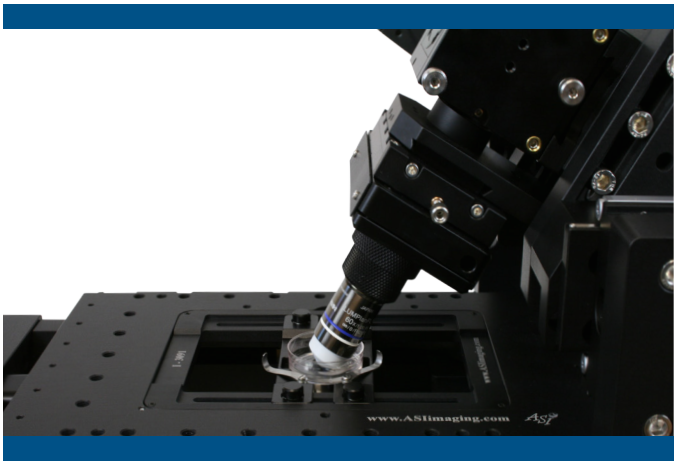
ASI manufactures the optomechanical elements, including the motorized stages, 2D galvos for creating and moving the light sheet, and the piezo objective movers. Objectives, lasers, and cameras are required to complete the system; users can procure these other items themselves, use the services of various system integrators selling the oSPIM, or purchase them via ASI.

Because the light sheet data is from only one objective the associated post-processing is minimal and completely conventional. This stands in contrast to the diSPIM, where data analysis to obtain isotropic views is often the practical bottleneck. The trade-off is that the resolution is not isotropic. Compared with confocal or other forms of epi-fluorescence, the light sheet dataset is tilted 30 degrees w.r.t. the coverslip which allows a different view of the sample that can be advantageous.

A Micro-Manager plugin for acquisition is open-source and freely available.

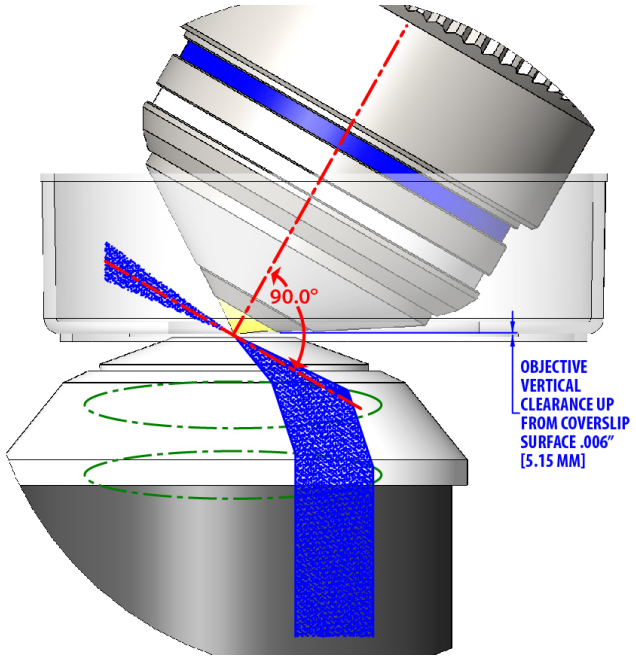
ASI also offers a dual-view oblique SPIM system (doSPIM) in which the bottom objective is also tilted. This configuration does not offer the ability to have a conventional inverted microscope. It requires special sample holders and two sets of cameras and scanners. The advantage is that it offers improved resolution by combining the two near-orthogonal views into a single high-resolution view much as the diSPIM does.

Hardware is available to mount the upper imaging objective to most commercial inverted microscopes (customers need to couple the ASI scanner for light sheet generation appropriately).



Example Variations:

- Photomanipulation using inverted microscope
- TIRF illumination on bottom microscope
- Cylindrical lens light sheet scanner instead of Gaussian beam scanner
- Dual-view oblique SPIM



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