# Dual Inverted Selective Plane Illumination Microscopy
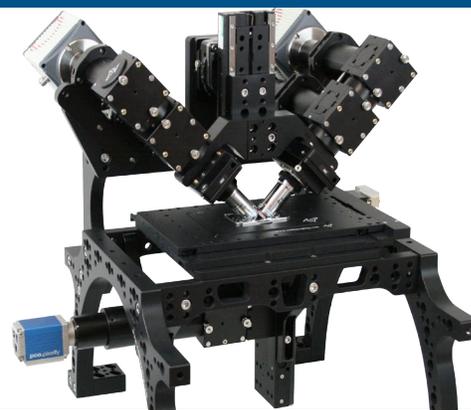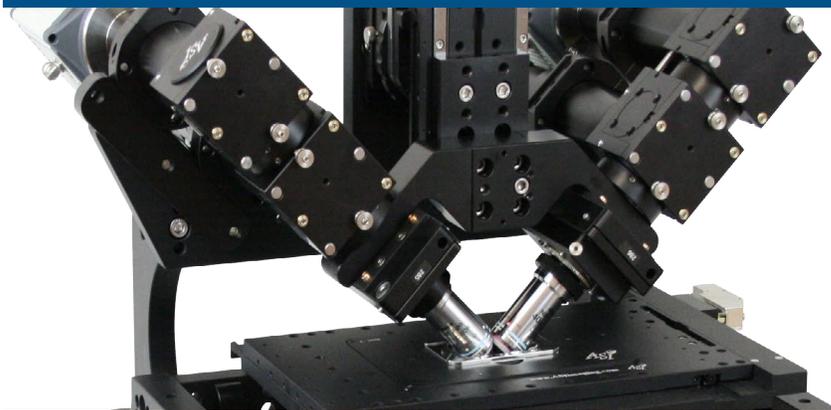
ASI offers all of the necessary hardware to implement the diSPIM, which is a flexible and easy-to-use implementation of Selective Plane Illumination Microscopy (SPIM) that allows for dual views (d) of the sample while mounted on an inverted (i) microscope. The diSPIM "head" can be mounted on various inverted microscopes including ASI's RAMM frame.

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 diSPIM, or purchase them via ASI.

The diSPIM has been tested successfully on cells cultured on cover slips, cells embedded on collegen gels, c. elegans and zebrafish embryos, and many other samples.

## Features

- Low photobleaching: >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
- Conventional sample mounting on coverslip or open dish
- Modular and flexible

## More Information

You may find more detailed information at dispim.org or asiimaging.com.

## Specifications

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|---|---|
| **Field of View*** | >400 µm diagonal |
| **Resolution*** | 380 nm @ 500 nm wavelength in XYZ |
| **Sample Size*** | Unlimited for flat samples, up to 3.5 mm radius hemisphere |

*\* Depends on objective, these are for Nikon 40x/0.8 WD.*

| | |
|---|---|
| **Mounting** | Cover slip or open dish |
| **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 $CO_2$ 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) |

# Basic System Configurations

**1) Single-Sided System (iSPIM):** Light sheet created from one objective and imaged using the other objective. The light sheet is moved through the sample, most often by moving the light sheet using the scanner (galvo) which is synchronized with a piezo stage moving the imaging objective.

**Advantages:** Fastest acquisition, least expensive, straight-forward set-up.
**Disadvantages:** Better XY resolution than Z resolution.

**2) Double-Sided System (diSPIM):** Both sides have a light sheet scanner, piezo objective positioner, and camera. During an experiment a stack of images is collected from both views, and the two datasets can be merged computationally to yield a 3D dataset with isotropic resolution (the usual problem of poor axial resolution is overcome by information from the other view). Can operate in single-sided mode if desired.

**Advantages:** XY & Z resolutions are all very good – yielding a combination of speed and resolution that is unsurpassed for live cell imaging.
**Disadvantages:** More hardware to buy. Data post-processing required for isotropic resolution.
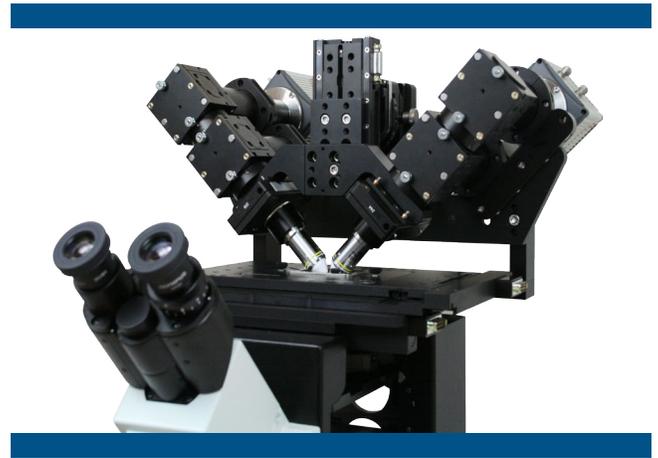


**Example Variations:**
- Photomanipulation using inverted microscope
- Filter wheels on imaging paths
- Asymmetric single-sided system, e.g. using same objectives as Lattice Light Sheet
- Non-gaussian beam
- 2-photon microscopy

# diSPIM Concept

Two immersion objectives (A/B) are placed at right angles above a sample mounted horizontally in an open dish, each objective 45° from vertical. A light sheet is created from objective A and imaged using objective B onto camera B. By moving the light sheet through the sample a stack of images is acquired, most often by moving the light sheet with galvo mirrors (not shown) synchronously with the imaging objective via a piezo stage. For some applications, the 3D information from a single view or stack is sufficient (iSPIM). For dual-view systems (diSPIM), the role of the two objectives is reversed to collect another stack from a perpendicular direction; although excitation and detection are shown schematically simultaneously, they are actually sequential. The two datasets can be computationally merged to yield a 3D dataset with isotropic resolution, and the usual problem of poor axial resolution is overcome by information from the other view. The sample may also be viewed through objective C, which belongs to an inverted microscope (either from ASI or from another microscope vendor). Objective C can be also used for photomanipulation, even during the light sheet acquisition.