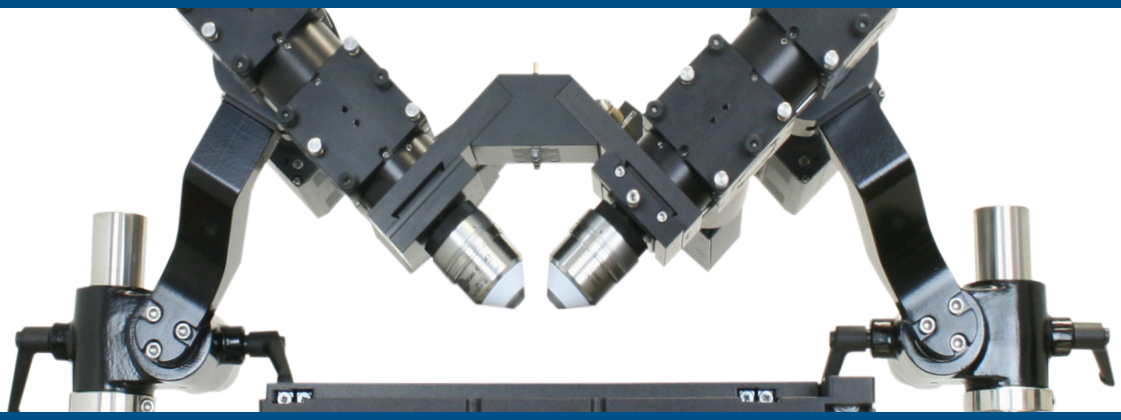
APPLIED SCIENTIFIC INSTRUMENTATION

Dual Selective Plane Illumination Microscopy for Cleared Tissue



ASI's ct-dSPIM is a flexible and easy-to-use implementation of Selective Plane Illumination Microscopy (SPIM) that allows for dual views (d) of large samples such as cleared tissue (ct).

The ct-dSPIM is one of many light sheet microscope configurations possible using ASI's modular components. We manufacture the optomechanical elements, including the motorized stages and 2D galvos for creating and moving the light sheet. ASI partnered with Special Optics to develop an objective optimized for light sheet imaging of cleared tissue. Lasers and sCMOS cameras are required to complete the system; users can procure these themselves, use the services of various system integrators selling ASI SPIM systems, or purchase them via ASI.

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

Features

- Image acquisition >10^8 voxels/sec
- Sub-micron resolution in XYZ (sample-permitting)
- Sample mounting in open dish
- Image >5 mm deep into flat samples or up to 12 mm radius sphere
- Media RI range from 1.33 to 1.56, aqueous or organic media
- Modular and flexible setup

More Information

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

Specifications

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

* *Depends on objective, these are for ASI/Special Optics 16.7x/0.4 multi-immersion.*

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 such as Micro-Manager and 3i SlideBook
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: Multi-position Multi-color Time Points

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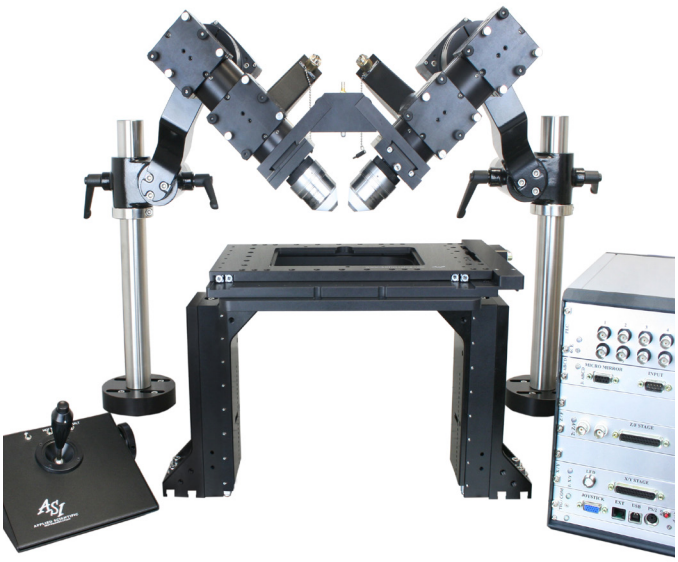
Basic System Configurations

1) Single-Sided System (iSPIM): Light sheet created from one objective and imaged using the other objective.

Advantages: Fastest acquisition, least expensive.
Disadvantages: Better XY resolution than Z resolution.

2) Dual-Sided System (diSPIM): Both objectives have a light sheet scanner and camera. Data is collected from both views sequentially, and the two datasets can be merged computationally to yield a 3D dataset with isotropic resolution. Can operate in single-sided mode if desired.

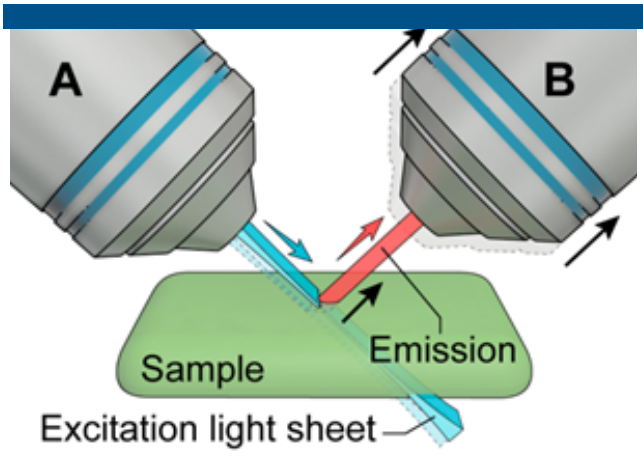
Advantages: XY and Z resolutions are all very good.
Disadvantages: More hardware to buy. Isotropic resolution requires double the acquisition time plus data post-processing.

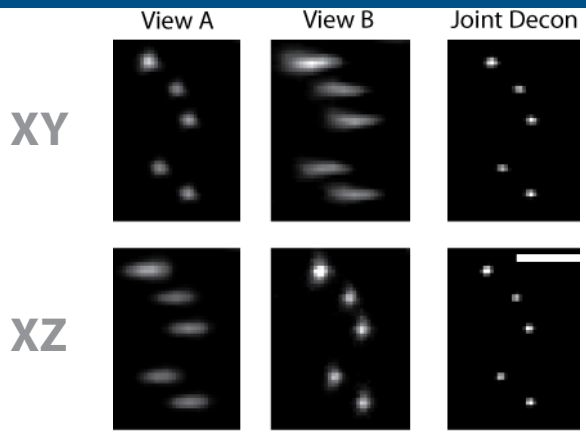


Example Variations:

- Filter wheels on imaging paths
- Asymmetric single-sided system, e.g. using other manufacturer's high-NA clearing objective lens
- Non-gaussian beam
- 2-photon microscopy

Dual-view SPIM Concept





Joint Decon: A. York and Y. Wu
Wu et al. Nat. Biotechnol. 31, 1032-138 (2013), Kumar et al. Nature Protocols 9, 2555-2573 (2014), Ingaramo et al.

Two objectives 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 one objective and imaged using the other objective. A stack of images is collected by moving the light sheet through the sample; in the case of ct-dSPIM, the sample is normally moved through a stationary light sheet using the XY stage. For some applications, the 3D information from a single view or stack is sufficient. For dual-view systems, the role of the two objectives is reversed to collect another stack from a perpendicular direction. The two datasets can be computationally merged to yield a 3D dataset with isotropic resolution; the usual problem of poor axial resolution is overcome by information from the other view.

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