D*i*SPIM – A Flexible Dual-View Light Sheet Microscope Platform

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ASI's D*i*SPIM Team

John Zemek	President, ASI
Jon Daniels	D*i*SPIM Lead Engineer
Gary Rondeau	Technical Director, ASI

NIH Research Collaborators and Inventors

Hari Shroff	NIH/NIBIB Chief Scientist
Yicong Wu	NIBIB Staff Scientist
Abhishek Kumar	Postdoctoral Fellow

Micromanager Development

Nico Stuurman	UCSF Vale Lab

1st LightSheet Fluorescence Microscopy International Conference
& 6th LSFM International workshop BARCELONA 25-26 SEPT. 2014

History:

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*i*SPIM and D*i*SPIM – Hari Shroff & Yicong Wu

Yicong Wu, et.al.,**Inverted selective plane illumination microscopy (*i*SPIM) enables coupled cell identity lineaging and neurodevelopmental imaging in *Caenorhabditis elegans*** PNAS 2011 108 (43) 17708-17713

Yicong Wu, et.al., **Spatially isotropic four-dimensional imaging with dual-view plane illumination microscopy**. Nature Biotechnology 31, 1032–1038 (2013)

ASI contributions:

*i*SPIM/D*i*SPIM Mount

RAMM Frame Platform

Micro-mirror light sheet scanners

MicroManger integrated control

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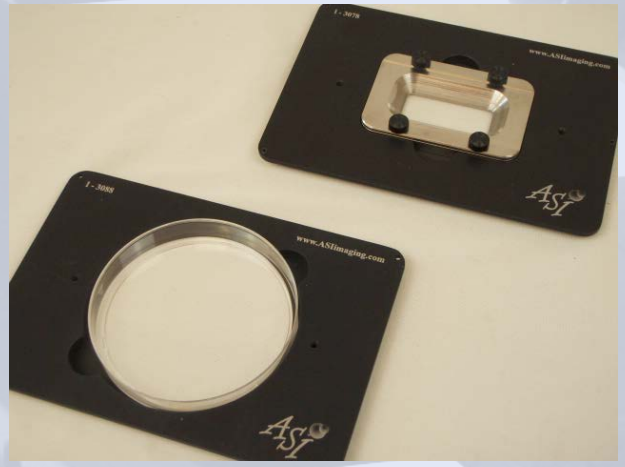
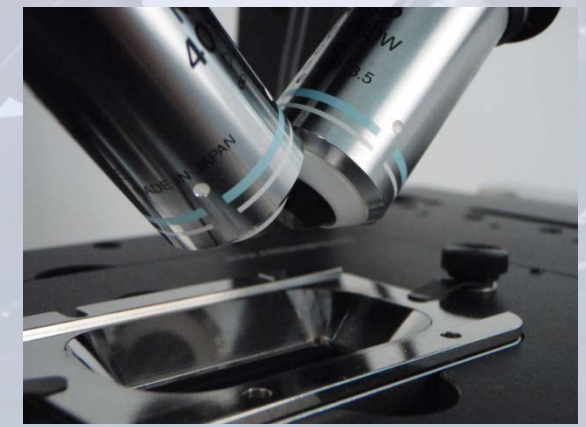
Light Sheet Microscopy

- use your existing inverted stand
- or the ASI RAMM Frame
- with conventional sample mounting





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D*i*SPIM on Inverted Microscope

SPIM Mounts for:

- Leica DMI-6000
- Nikon TE-300, Ti
- Olympus IX-71/81, IX-73/83
- Zeiss Axio-Observer

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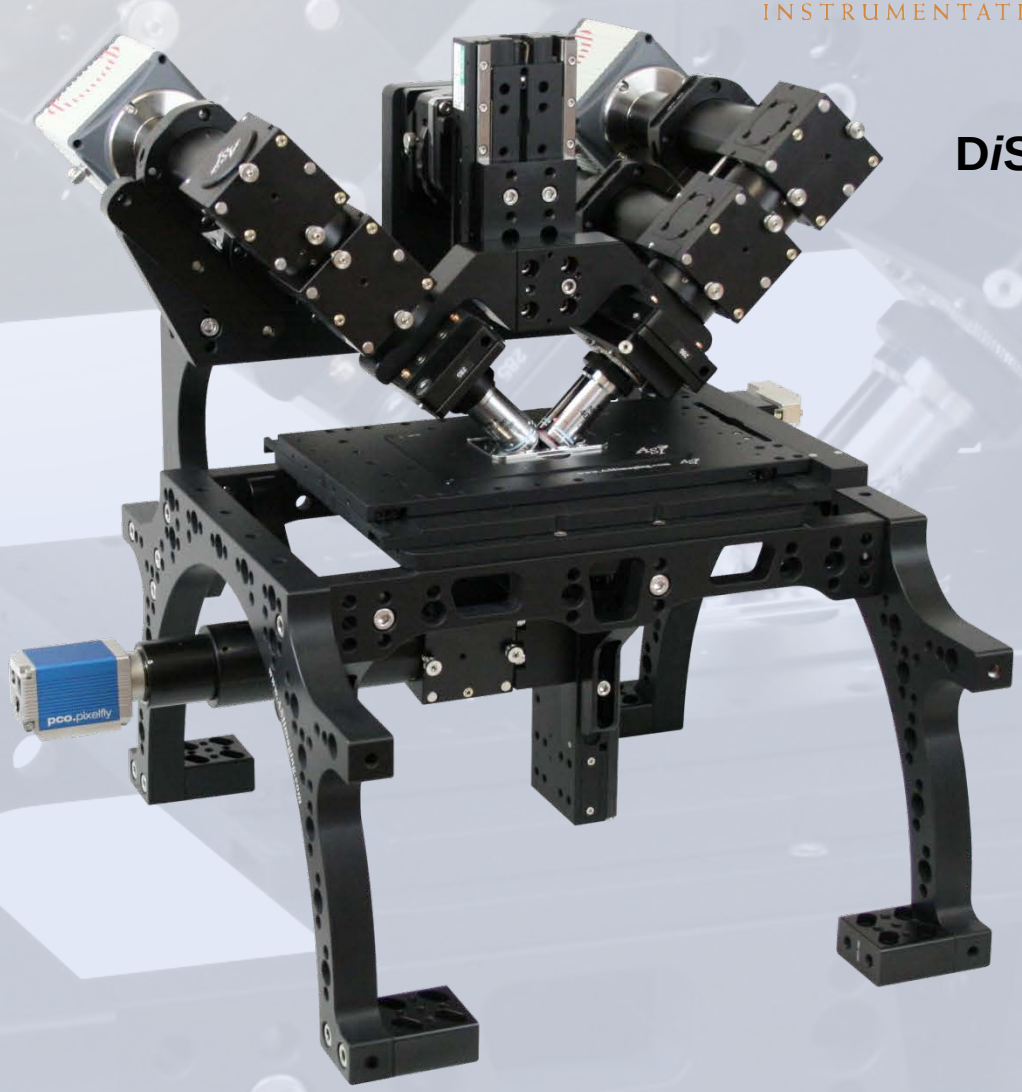
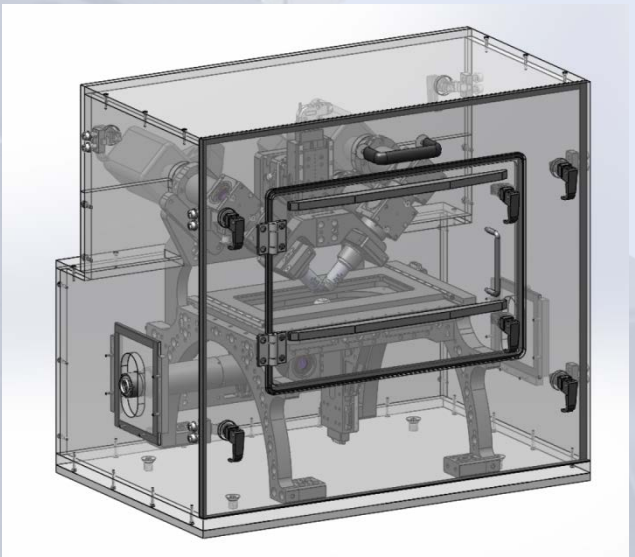


D*i*SPIM on ASI RAMM System

- Flexible Modular Inverted Microscope
- Compact footprint
- Complete environmental chamber option



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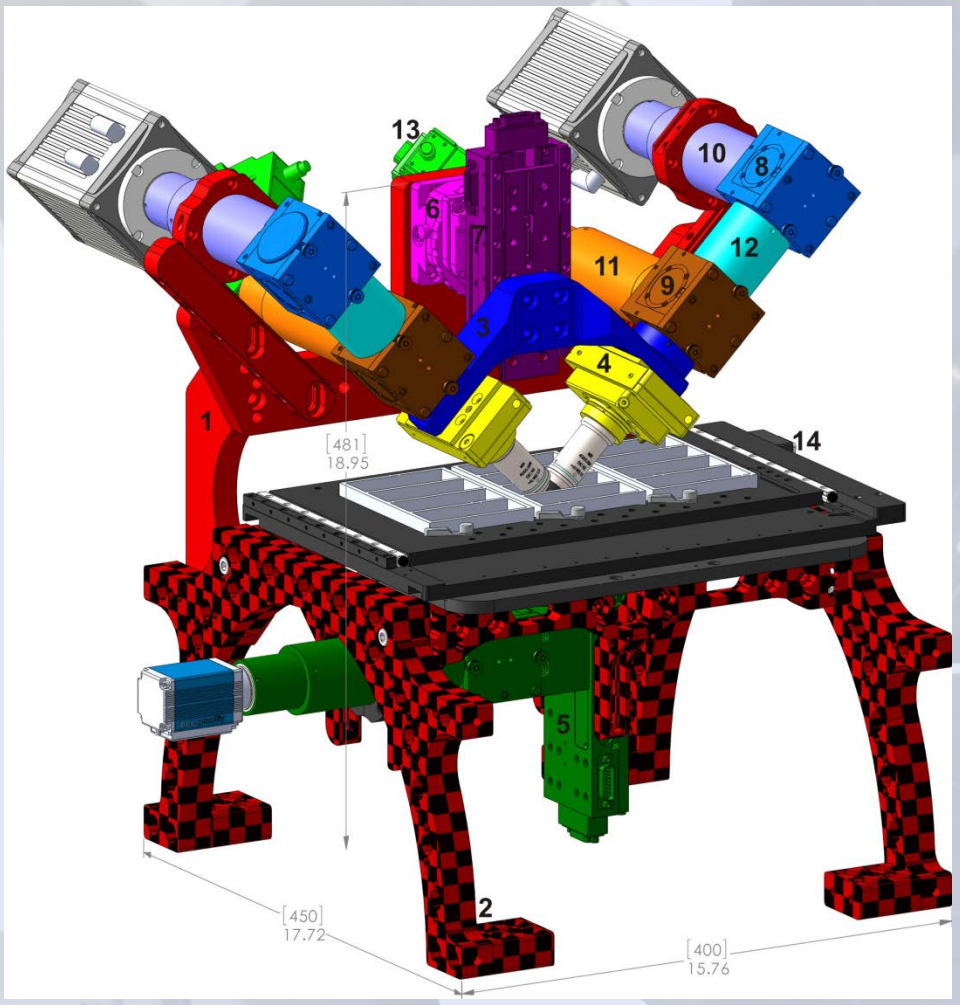

D*i*SPIM Parts and Function

1. SPIM mount
2. RAMM frame
3. Objective mount
4. Objective piezo
5. Bottom-side microscope
6. CDZ centering stage
7. SPIM LS-50 Z-drive
8. Camera mirror cubes
9. Excitation filter cubes
10. Camera tube lens
11. Scanner tube lens
12. Spacer
13. Light sheet scanners
14. MS2500 large stage

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Performance:

- 3D volumes w/ isotropic resolution (330 nm using Nikon 40X NA 0.8 objectives)
- Acquisition rates up to 200 images per second or 2-5 volumes per second (roughly 10x faster than spinning-disk microscopes)
- Achieve a ~7-10 fold reduction in photo-bleaching compared to con-focal methods.

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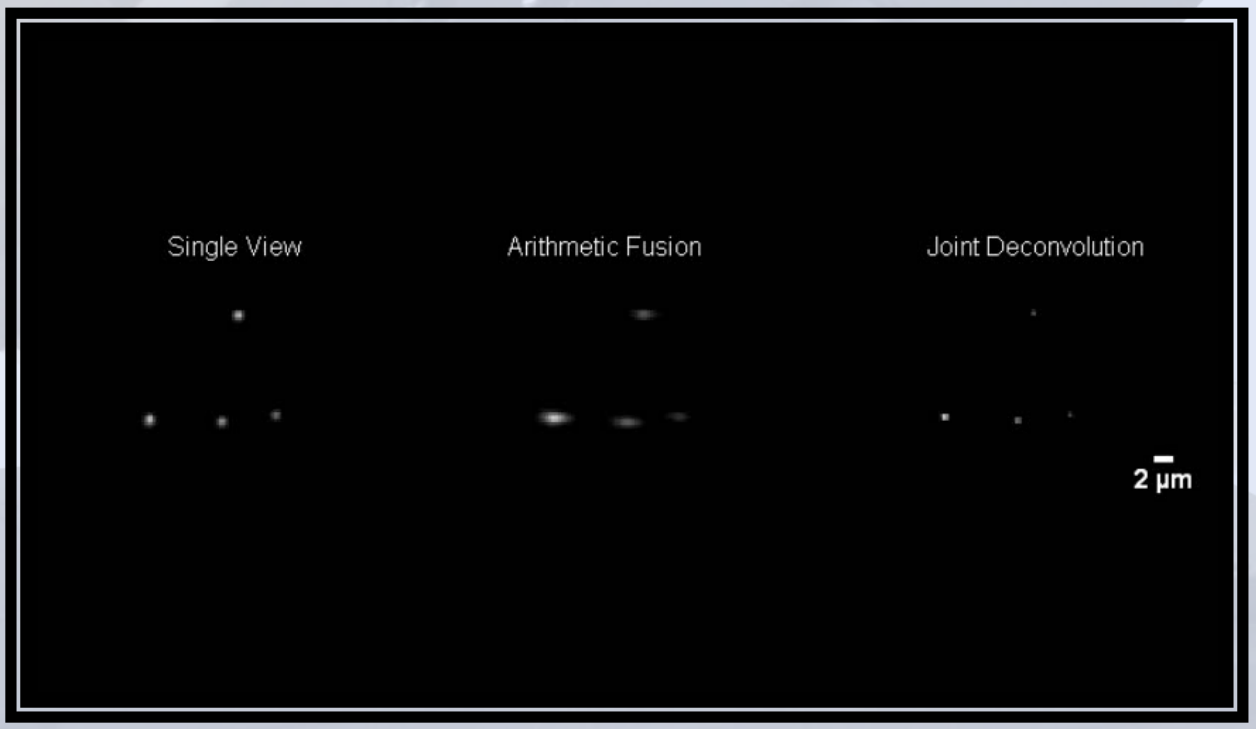

Comparison of Subdiffractive Beads with Different Fusion Schemes

- Left: Single-view.
- Middle: Arithmetic fusion.
- Right: Joint deconvolution.

The 3D projections are rotated with respect to the Y axis.

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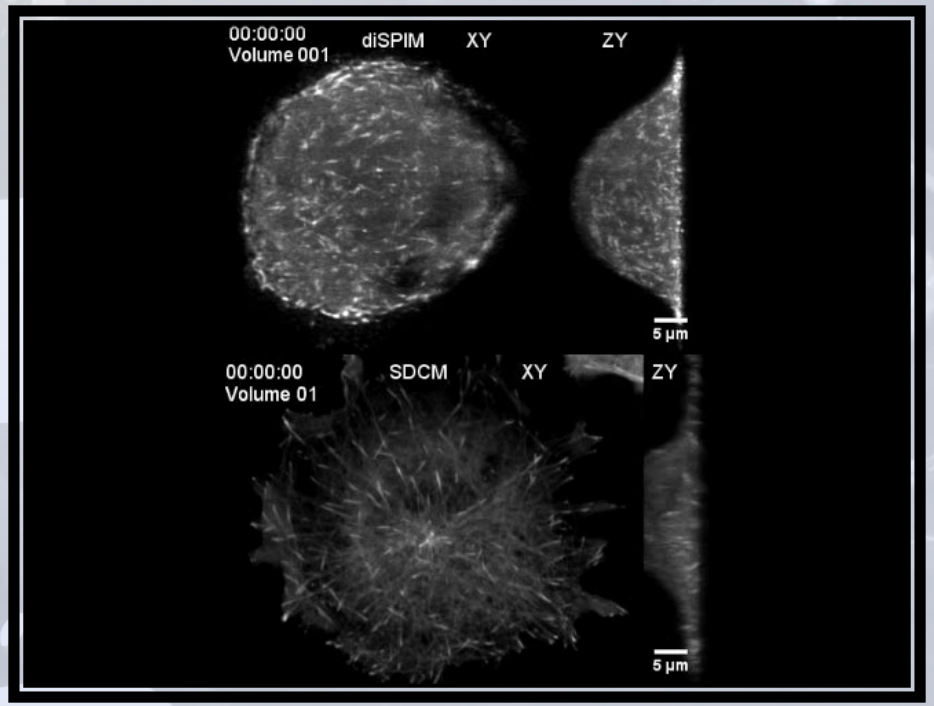

Comparison Between SDCM & D*i*SPIM on GFP-EB3 Microtubules in Live Human Umbilical Vein Endothelial Cells

- Top Row: DiSPIM
- Bottom Row: Spinnig Disk Confocal

SDCM and DiSPIM images have similar initial SNR and were taken at equivalent illumination doses, but diSPIM enables collection of 3x more volumes, 3.2x more planes per volume and 7.6 –fold less photobleaching.
Note that in SDCM, the cell is significantly bleached after 1 minute.

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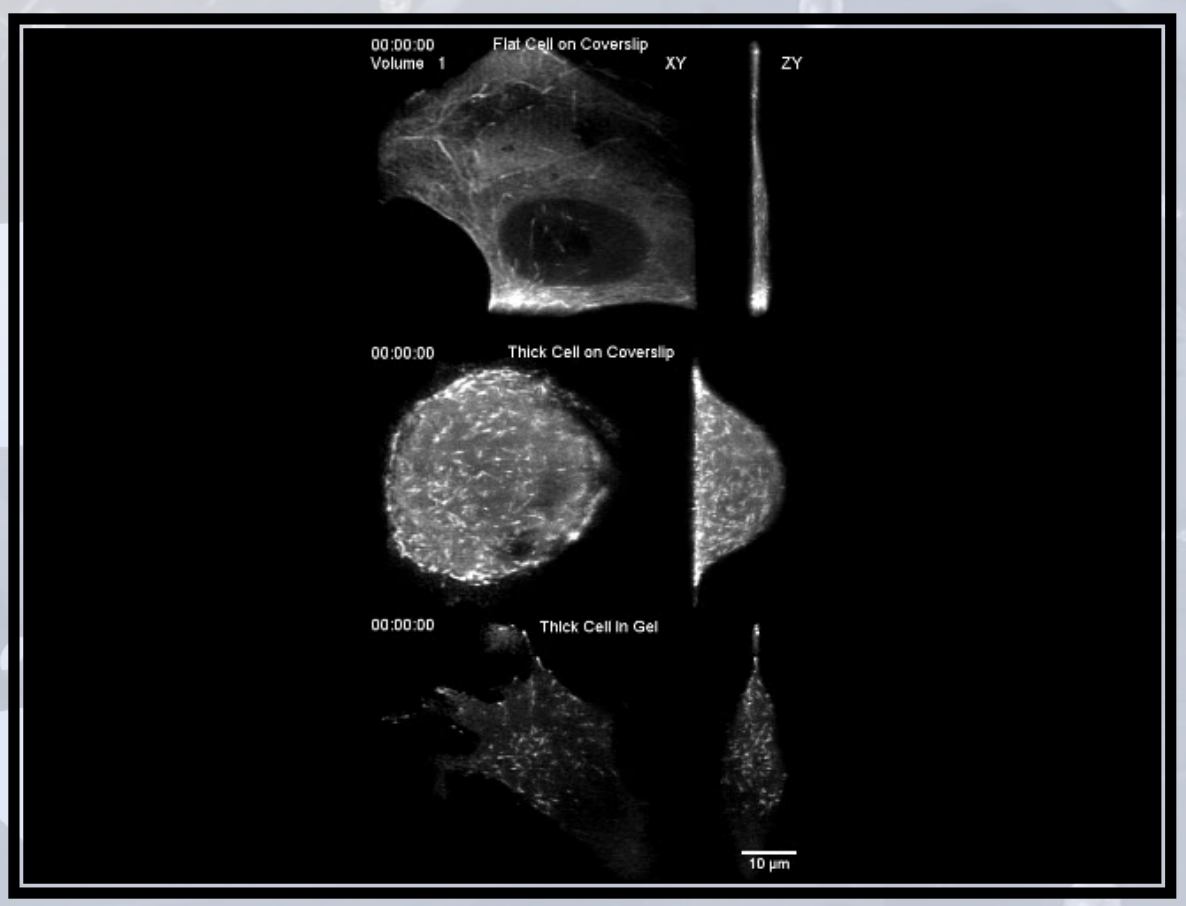

Comparison of 3D GFP-EB3 Microtubule Dynamics in Human Umbilical Vein, Endothelial Cells of Different Thickness and in Different Cellular Environments with diSPIM

All cells were sampled at 15 volumes per minute over the entire 5 minute imaging duration. Maximum intensity XY and ZY views are shown. The clarity of MT tips and the stability of the fluorescence signal enable reliable microtubule tracking in 4D.

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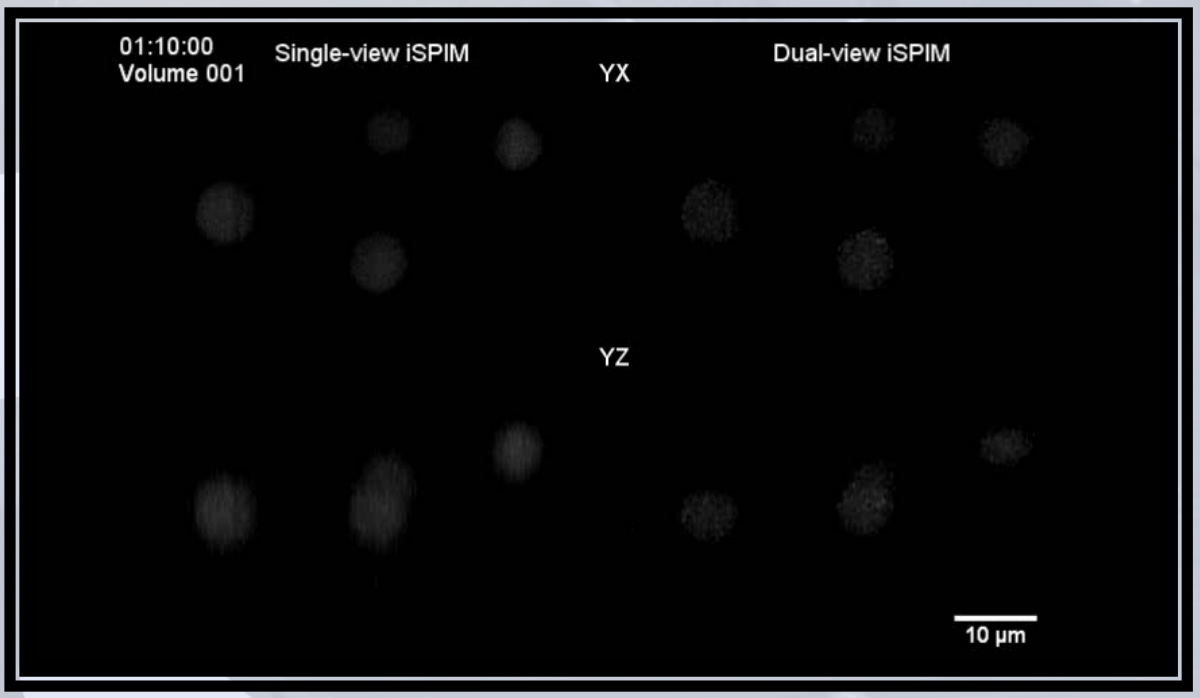

Comparative iSPIM and diSPIM Volumetric Time Series of GFP Histones in a Live BV24 Nematode Embryo from the 4 Cell Stage up to Hatching

Volumes were sampled every minute at 50 planes per volume with 1 µm inter-plane spacing. Embryos develop normally under these conditions and diSPIM offers significantly increased resolution (especially axially) compared to iSPIM (where nuclei appear distorted in the axial view). Maximum intensity XY and ZY views are shown.

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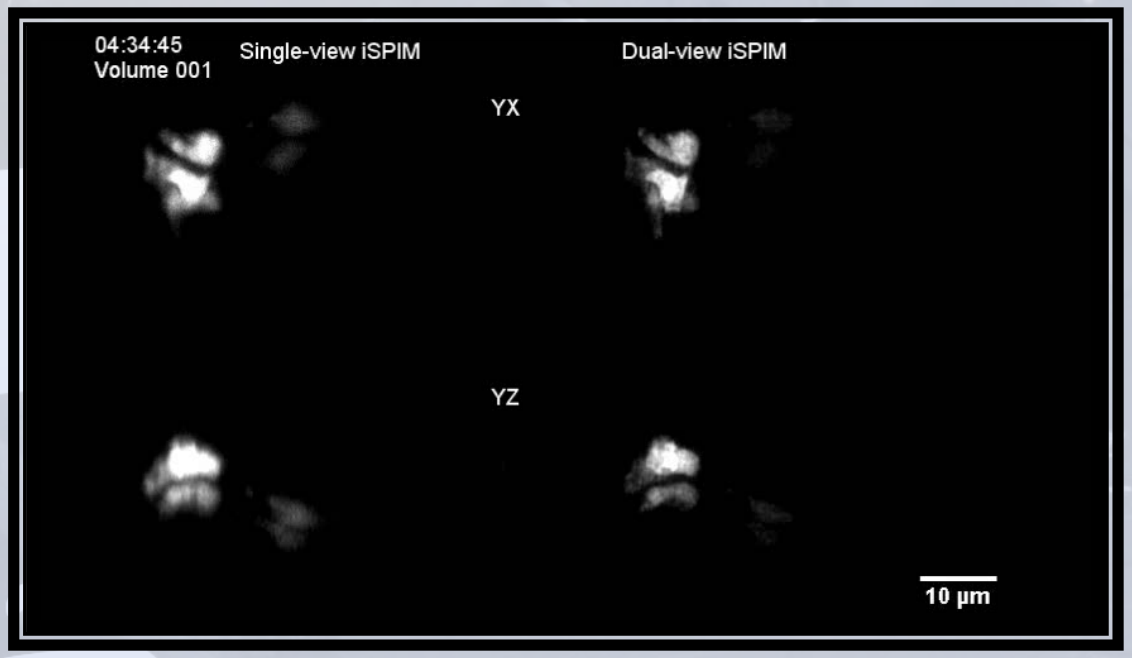
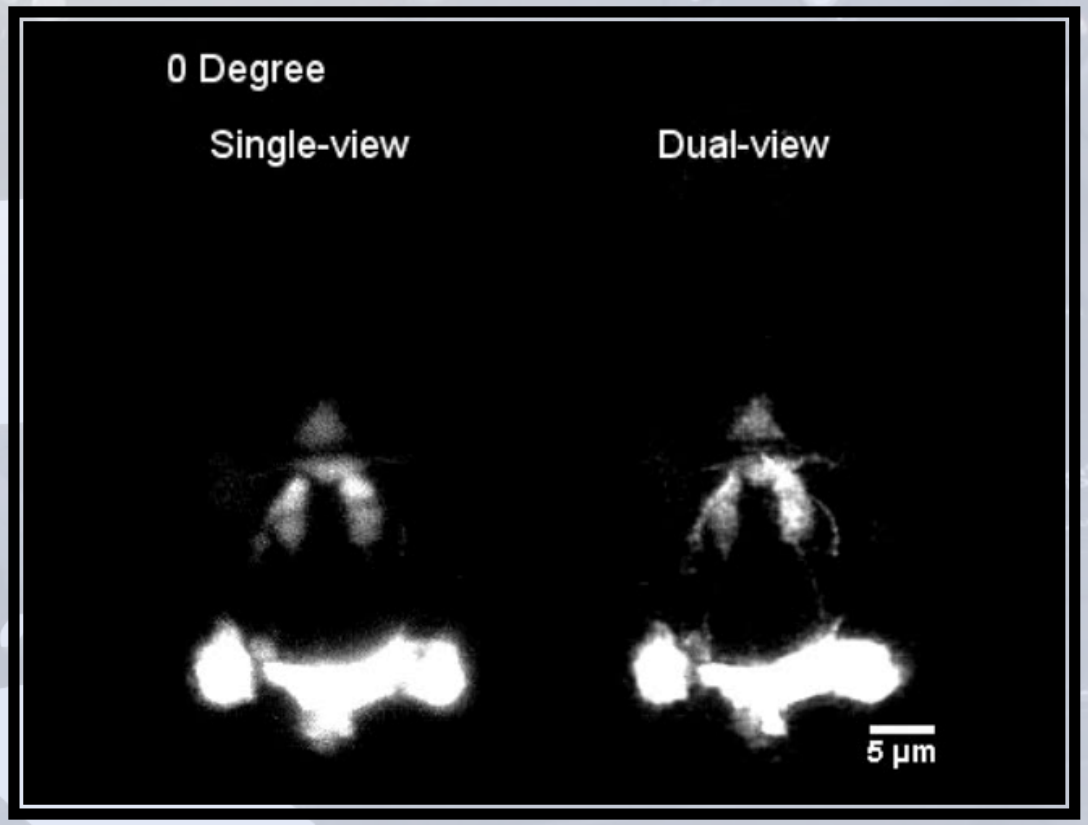


Comparison Between iSPIM and diSPIM when Visualizing Neuronal Processes in Developing Embryo.

Volumes were sampled every 15 seconds at 100 planes per volume per view with 0.5 µm inter-plane spacing. Maximum intensity XY and ZY views are shown.



Comparison Between iSPIM and diSPIM, Highlighting Differences in a Single Volume with GFP-Labeled AIY Neurons

The 3D projections.

ASI's D*i*SPIM Implementation

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- Built with modular hardware for evolving applications
  - Cameras fixed to frame
  - Scanners move vertically with objectives
- Uses modular control electronics for evolving automation needs
  - DISPIM state machine resides on Micro-Mirror card
  - Flexible TTL communication on Tiger back plane
- MicroManager support for ASI hardware
  - ASI support with complete imaging package

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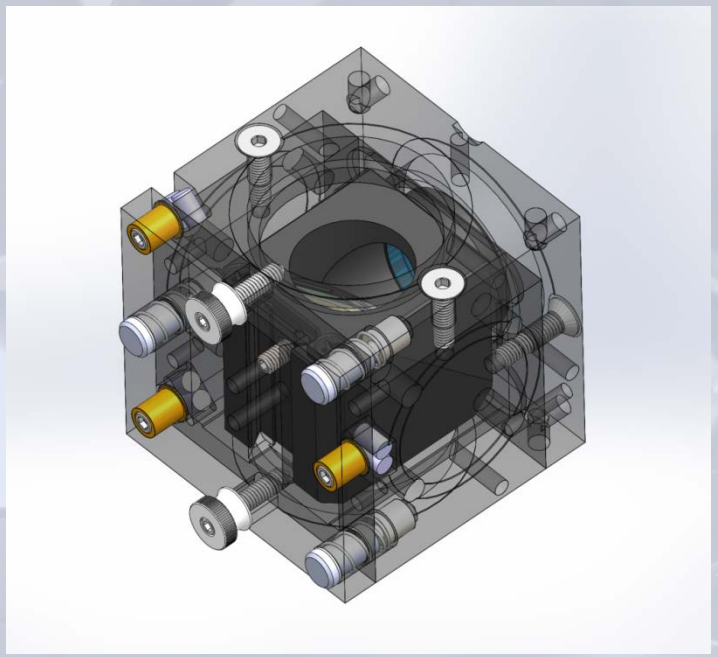
ASI's Modular Microscope Hardware

- Adjustable Cubes
  - Mirrors
  - Beam Splitters
- Tube lens
- Camera mounts

Building complex hardware is straightforward



Adjustable Beam-Splitter Cube

Four cameras

Transmitted light for bottom scope

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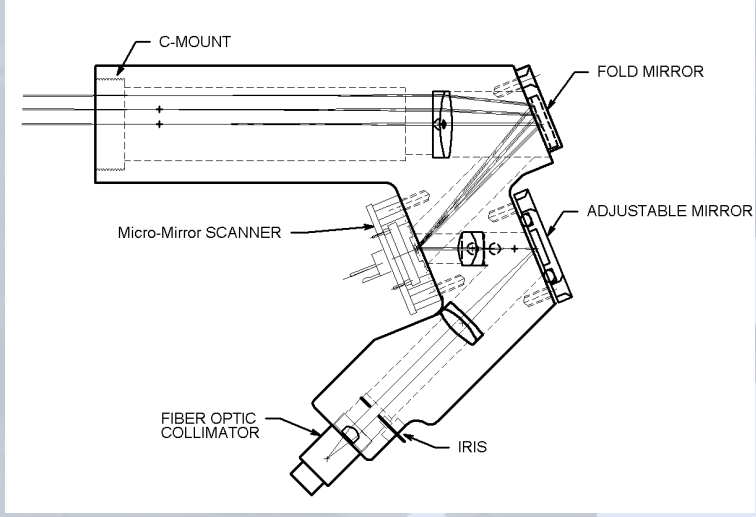
Fiber-Coupled Light Sheet Scanners

- Two axes of deflection
- C-mount device – 19mm FN
- Single mode fiber coupled – FC/PC or FC/APC
- 1kHz Bandwidth



- Four-axis Tiger control card
- Compact for D*i*SPIM integration

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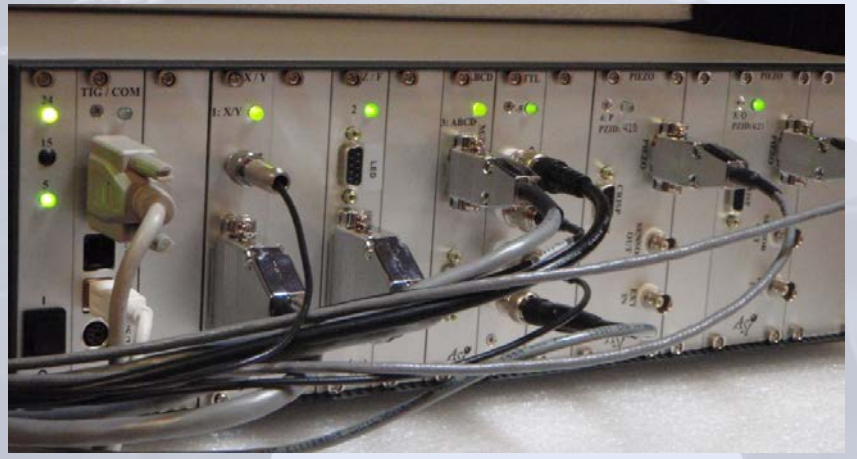
D*i*SPIM Hardware and Automation

Ten automated motion controlled axes

- XY stage
- SPIM Z
- Lower Z
- Two objective piezos
- Two 2-axis micro-mirror scanners

All controlled with ASI's *Tiger* multi-axis controller

Piezos and scanners controlled with either internal DACs or external voltage control



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Control of the D*i*SPIM Hardware

External voltage control of scanners and piezos

- Labview – available from Shroff group
- Third party developers

ASI's D*i*SPIM *Tiger* control firmware

- Micro-Manger D*i*SPIM plug-in
- Third party developers

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Micro-Manager DiSPIM plug-in Supports

Cameras:
- Andor Zyla
- PCO Edge
- Hammamatsu Flash 4

Lasers: with two-port switchers
- Toptica MCLE
- Applied Spectral ILE

- Passively split lasers

Modes:
- Single sided (iSPIM)
- Double side (DiSPIM)

- Synchronized scanner and piezo
- Slice-scan only

Planned:
- Fixed Sheets – stage scan

Colors:
- Single color or MM_Core multi-color

Planned:
- Full four-color hardware control

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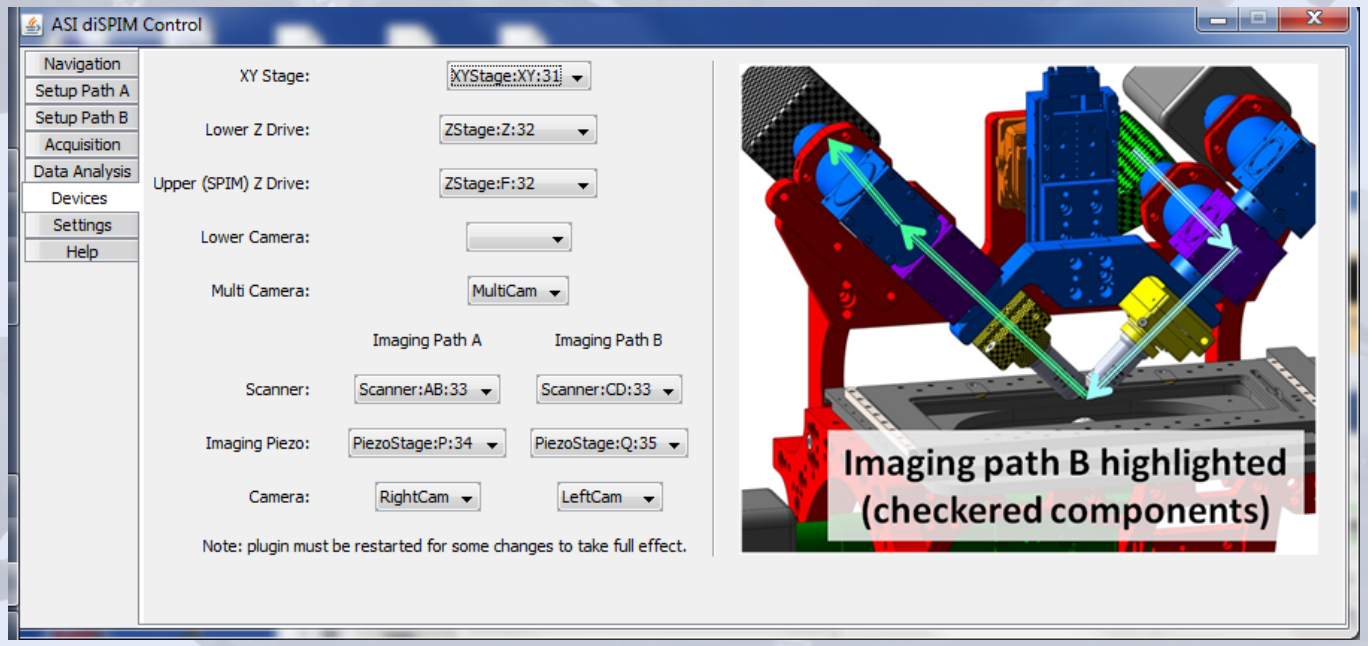
Micro-Manager DiSPIM plug-in

Select devices; stages, scanners, piezo, cameras known to Micro_Manager



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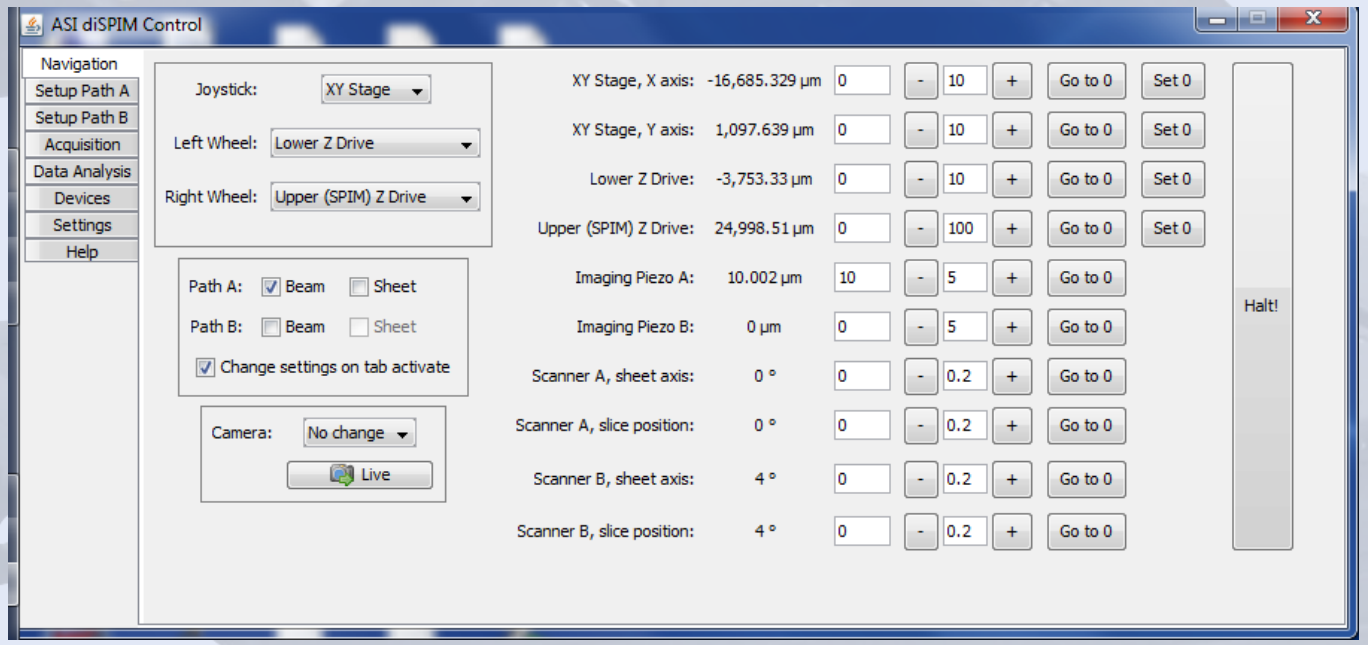
Micro-Manager DiSPIM plug-in

Navigate all 10 Axes

Flexible Manual control of just about anything with either Joystick or Control Knobs



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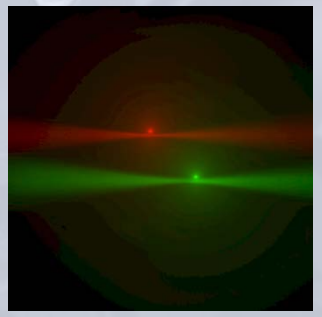
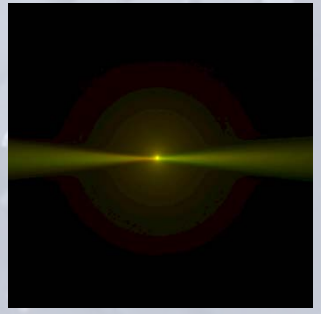
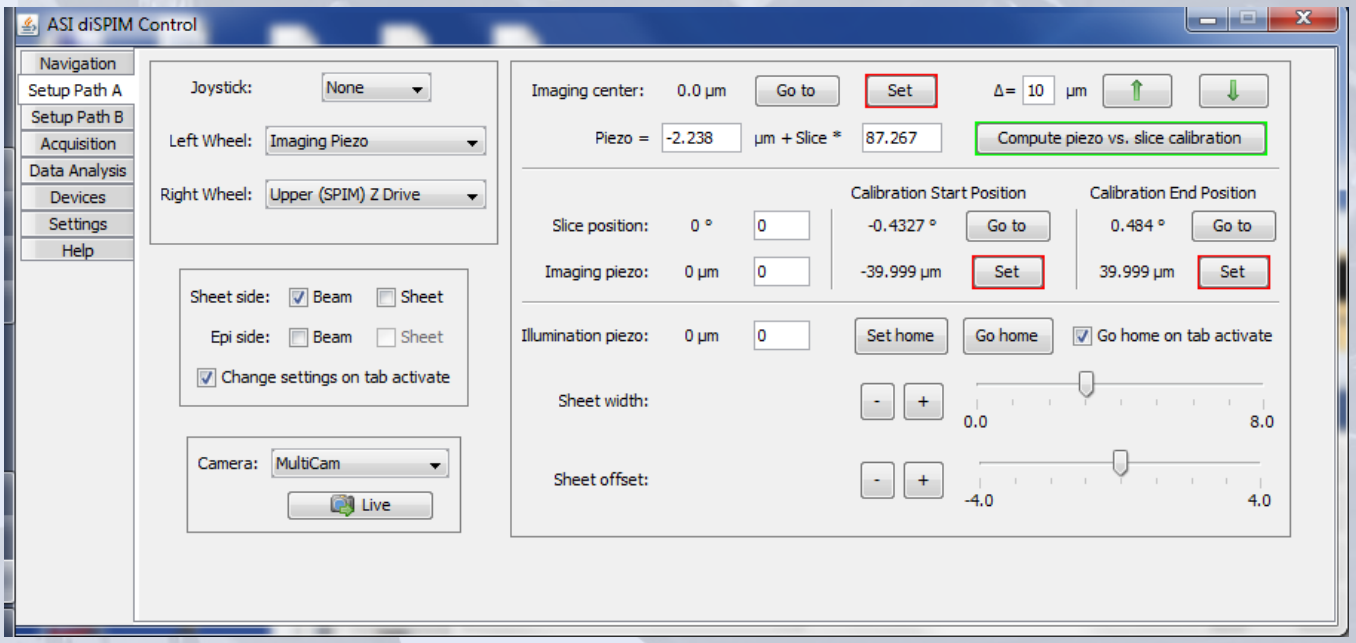
Micro-Manager DiSPIM plug-in





Control cameras for alignment – MultiCam.

Align the objectives and cross calibrate scanners and piezos.



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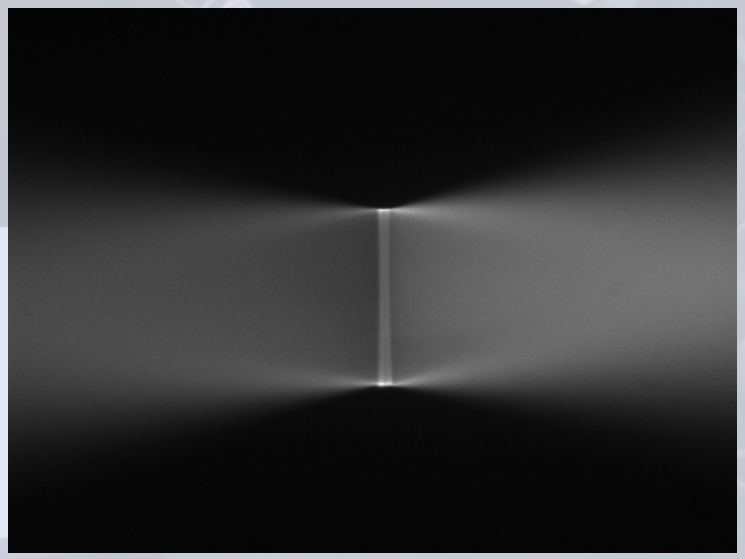
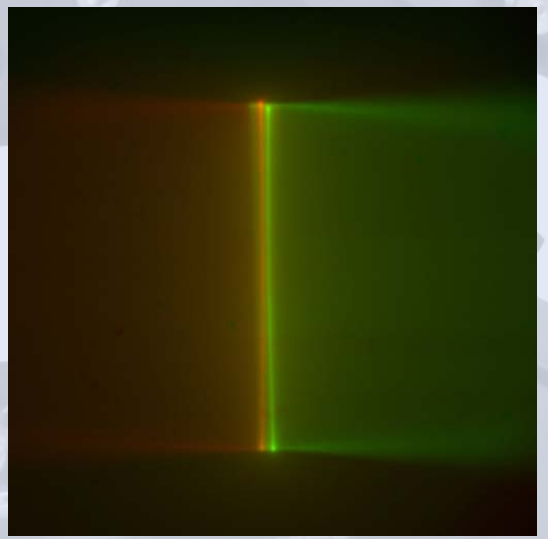
Micro-Manager DiSPIM plug-in

Control the lasers, scanners and cameras

Aligning Light Sheets hitting cover-slip in dye solution



Bottom Camera



MultiCam

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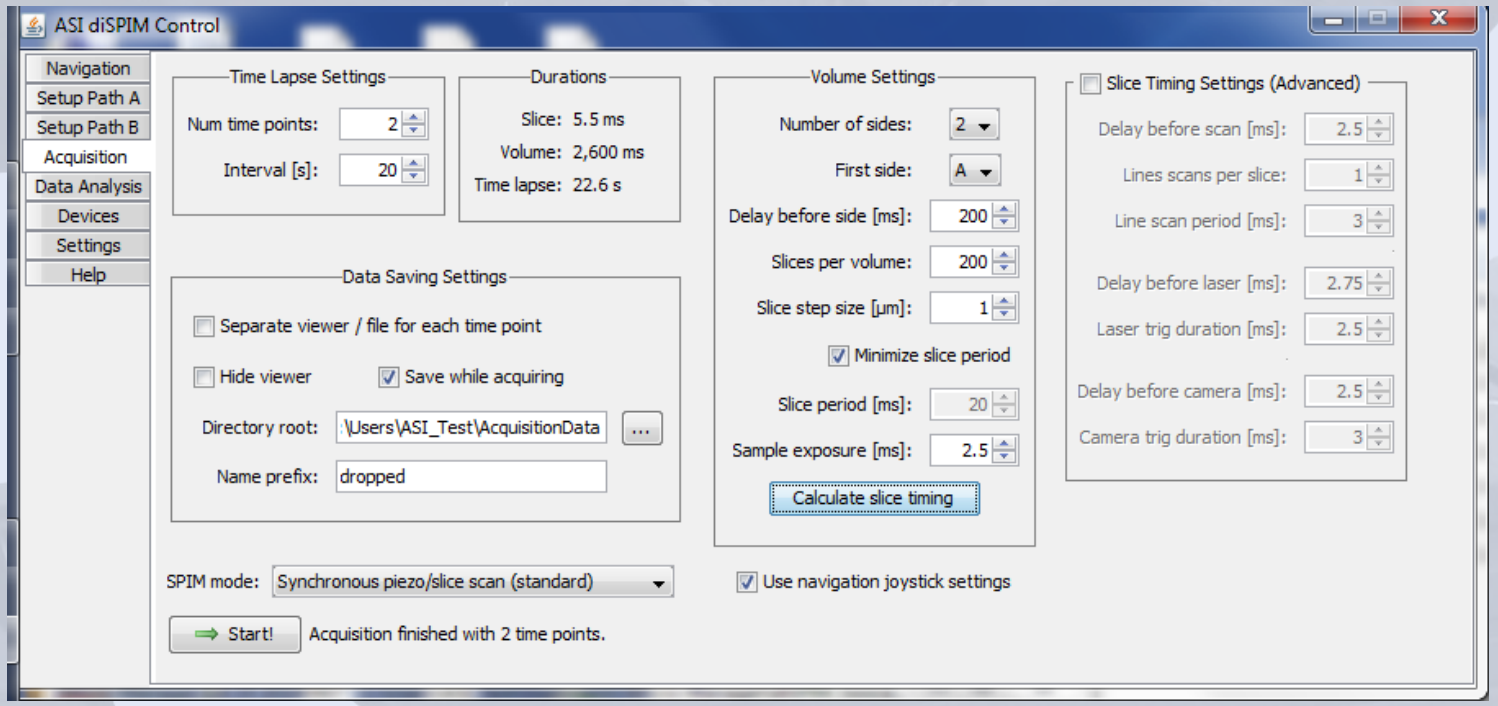
Micro-Manager DiSPIM plug-in

Set up acquisitions and Aquire!



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Micro-Manager DiSPIM plug-in

Analysis!

- MIPAV
- FIJI