

# DiSPIM – A Flexible Dual-View Light Sheet Microscope Platform



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## ASI's DiSPIM Team

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## NIH Research Collaborators and Inventors

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Postdoctoral Fellow

## Micromanager Development

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**1<sup>st</sup> LightSheet Fluorescence Microscopy International Conference**  
& 6<sup>th</sup> LSFM International workshop **BARCELONA 25-26 SEPT. 2014**



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## History:

### *i*SPIM and *Di*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/*Di*SPIM Mount

RAMM Frame Platform

Micro-mirror light sheet scanners

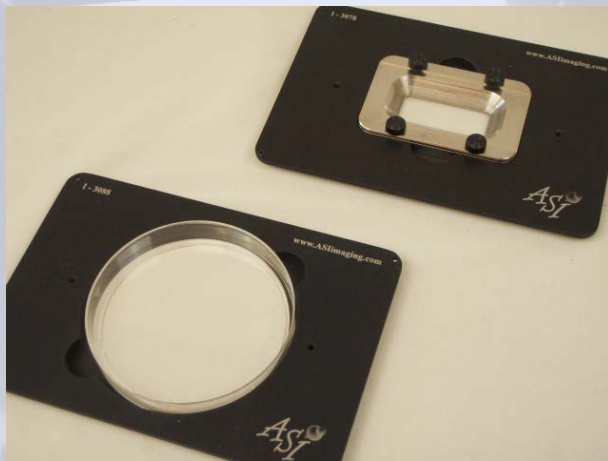
MicroManger integrated control



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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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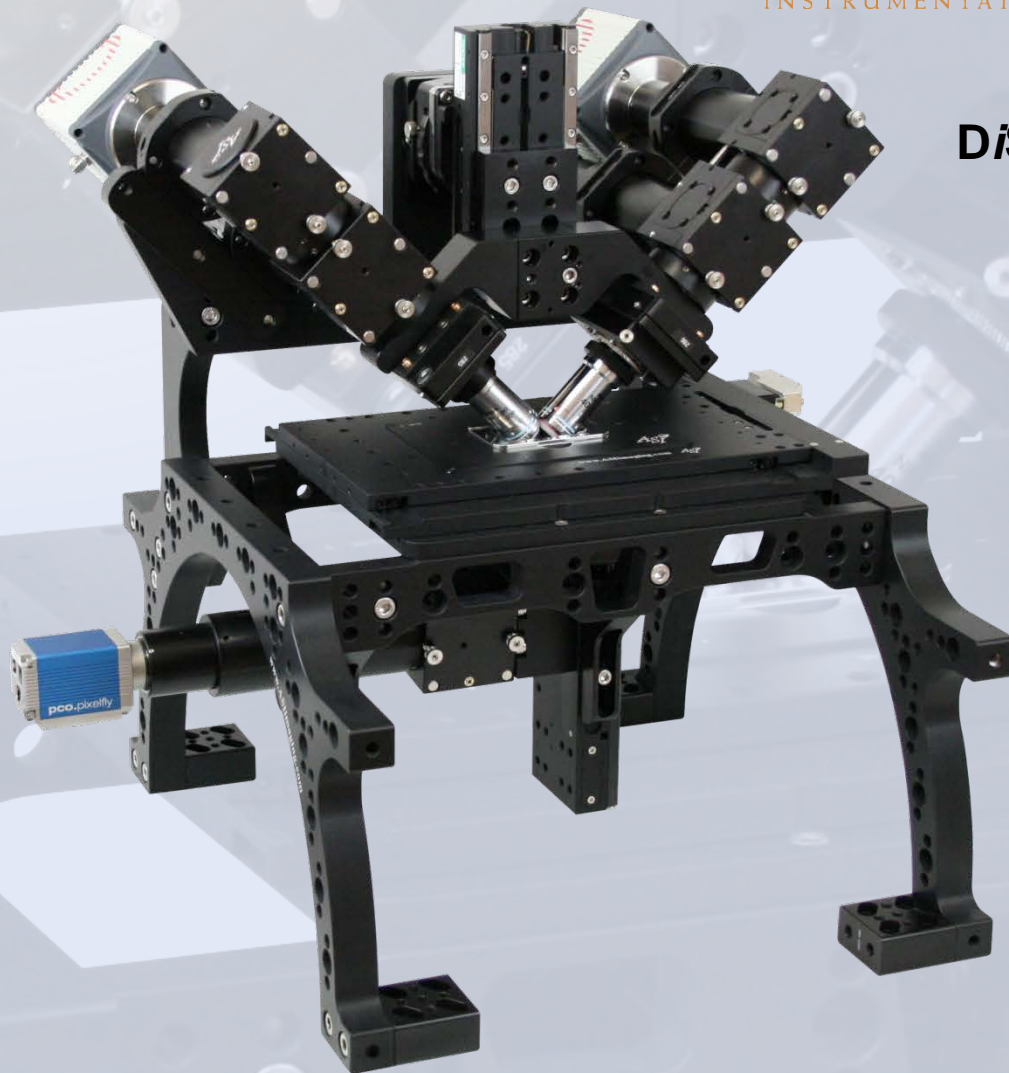
## **DiSPIM on Inverted Microscope**

### **SPIM Mounts for:**

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

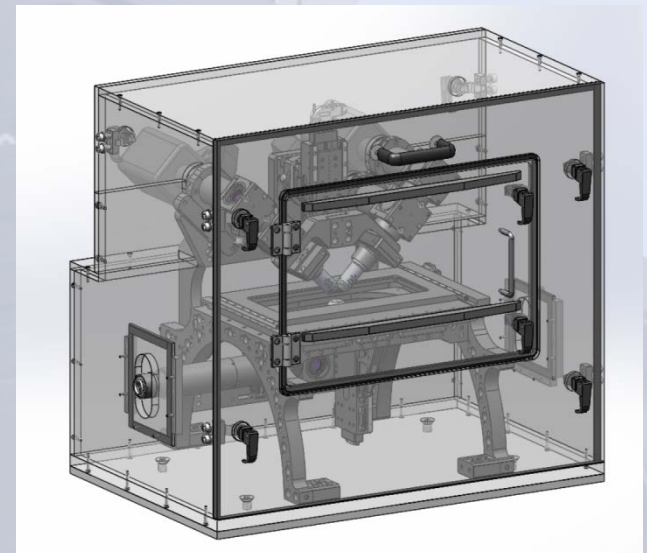


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## DiSPIM on ASI RAMM System

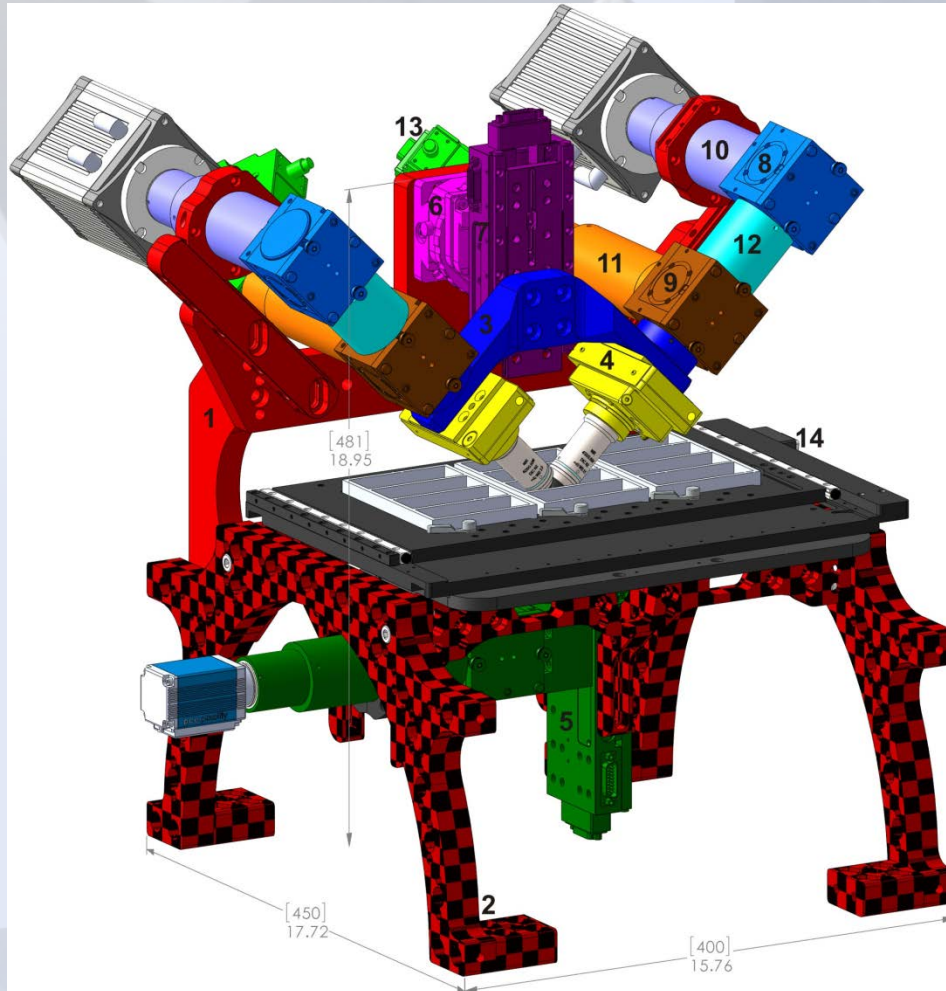
- Flexible Modular Inverted Microscope
- Compact footprint
- Complete environmental chamber option







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## DiSPIM 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

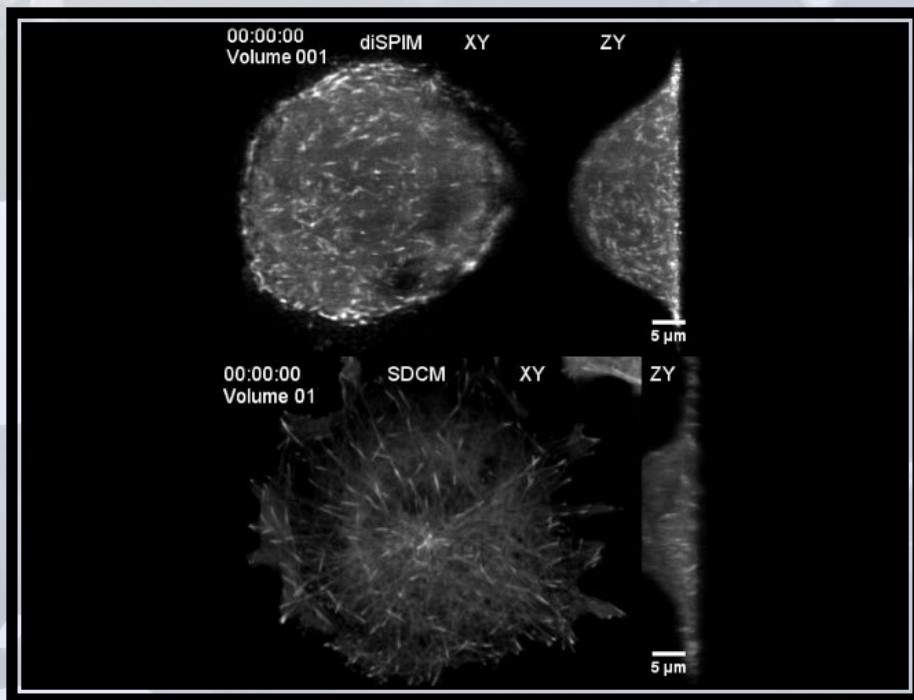
- Left: Single-view.
- Middle: Arithmetic fusion.
- Right: Joint deconvolution.

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





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## Comparison Between SDCM & DiSPIM 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.

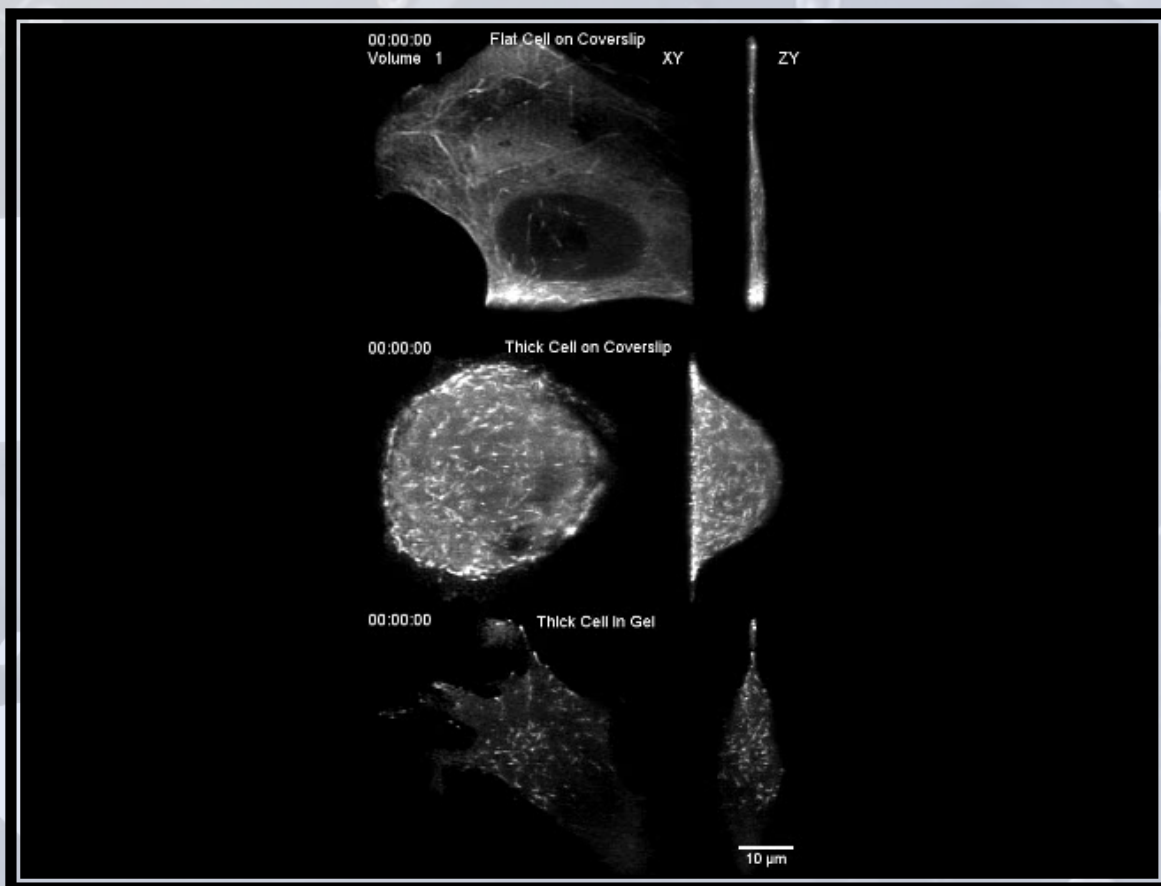
Note that in SDCM, the cell is significantly bleached after 1 minute.



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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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01:10:00  
Volume 001

Single-view iSPIM

YX

Dual-view iSPIM

YZ

10  $\mu$ m

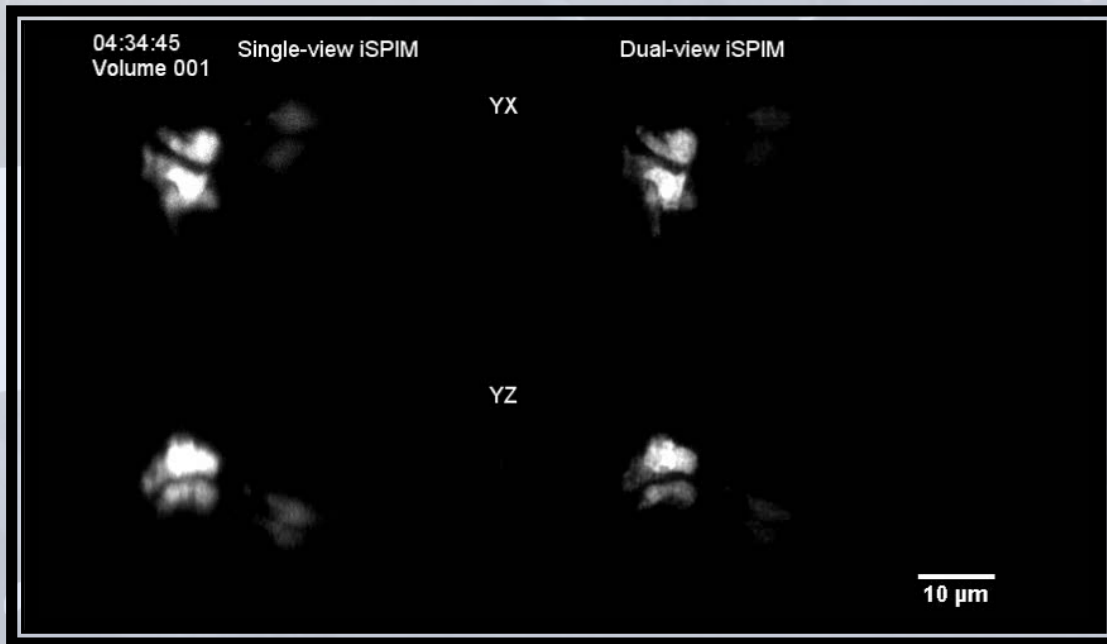
## Comparative iSPIM and diSPIM Volumetric Time Series of GFP Histones in a Live BV24 Nematode Embryo from the 4 Cell Stage up to Hatching

Volumes were sampled every minute at 50 planes per volume with 1  $\mu$ m 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  $\mu\text{m}$  inter-plane spacing. Maximum intensity XY and ZY views are shown.



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0 Degree

Single-view

Dual-view



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

The 3D projections.



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## ASI's DiSPIM Implementation

- 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

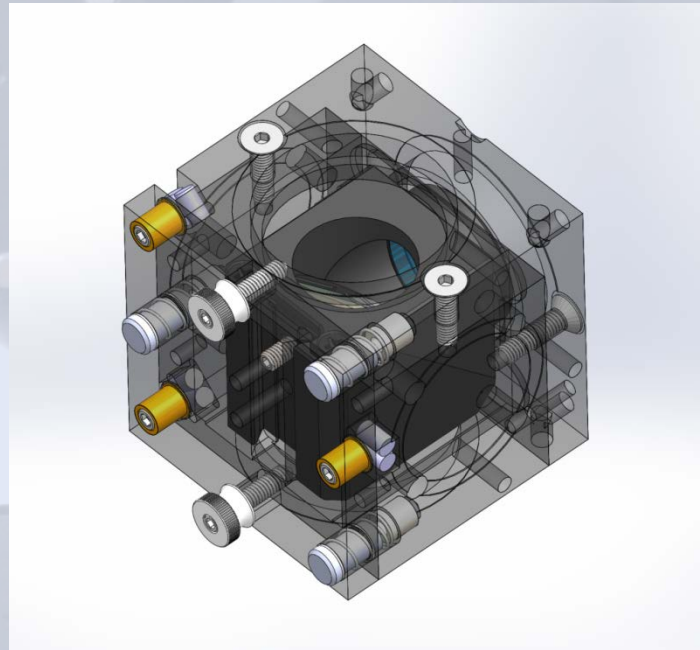
- Adjustable Cubes

- Mirrors
- Beam Splitters

- Tube lens

- Camera mounts

Building complex  
hardware is  
straightforward



Adjustable Beam-Splitter Cube



Four cameras



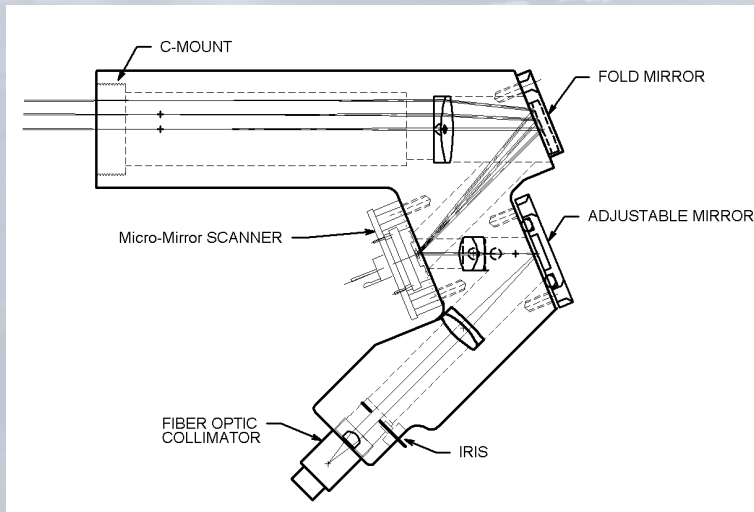
Transmitted  
light for  
bottom scope



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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 DiSPIM integration



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## DiSPIM 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 DiSPIM Hardware

### External voltage control of scanners and piezos

- Labview – available from Shroff group
- Third party developers

### ASI's DiSPIM *Tiger* control firmware

- **Micro-Manger DiSPIM plug-in**
- Third party developers



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# Micro-Manager DiSPIM plug-in Supports

## Cameras:

- Andor Zyla
- PCO Edge
- Hamamatsu 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

The screenshot shows the 'ASI diSPIM Control' software window. On the left is a navigation menu with options: Navigation, Setup Path A, Setup Path B, Acquisition, Data Analysis, Devices, Settings, and Help. The main area contains several configuration sections:

- XY Stage:** XYStage:XY:31
- Lower Z Drive:** ZStage:Z:32
- Upper (SPIM) Z Drive:** ZStage:F:32
- Lower Camera:** (empty dropdown)
- Multi Camera:** MultiCam
- Imaging Path A:** Scanner: Scanner:AB:33, Imaging Piezo: PiezoStage:P:34, Camera: RightCam
- Imaging Path B:** Scanner: Scanner:CD:33, Imaging Piezo: PiezoStage:Q:35, Camera: LeftCam

At the bottom, a note states: "Note: plugin must be restarted for some changes to take full effect."

On the right side of the window is a 3D CAD model of the microscope's internal components. A text box at the bottom of the model reads: "Imaging path B highlighted (checkered components)". The model shows various mechanical parts in red, blue, and purple, with green arrows indicating light paths.





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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



ASI diSPIM Control

Navigation  
Setup Path A  
Setup Path B  
Acquisition  
Data Analysis  
Devices  
Settings  
Help

Joystick: XY Stage

Left Wheel: Lower Z Drive

Right Wheel: Upper (SPIM) Z Drive

Path A:  Beam  Sheet  
Path B:  Beam  Sheet  
 Change settings on tab activate

Camera: No change

XY Stage, X axis:	-16,685.329 $\mu\text{m}$	0	-	10	+	Go to 0	Set 0
XY Stage, Y axis:	1,097.639 $\mu\text{m}$	0	-	10	+	Go to 0	Set 0
Lower Z Drive:	-3,753.33 $\mu\text{m}$	0	-	10	+	Go to 0	Set 0
Upper (SPIM) Z Drive:	24,998.51 $\mu\text{m}$	0	-	100	+	Go to 0	Set 0
Imaging Piezo A:	10.002 $\mu\text{m}$	10	-	5	+	Go to 0	
Imaging Piezo B:	0 $\mu\text{m}$	0	-	5	+	Go to 0	
Scanner A, sheet axis:	0 $^{\circ}$	0	-	0.2	+	Go to 0	
Scanner A, slice position:	0 $^{\circ}$	0	-	0.2	+	Go to 0	
Scanner B, sheet axis:	4 $^{\circ}$	0	-	0.2	+	Go to 0	
Scanner B, slice position:	4 $^{\circ}$	0	-	0.2	+	Go to 0	

Halt!

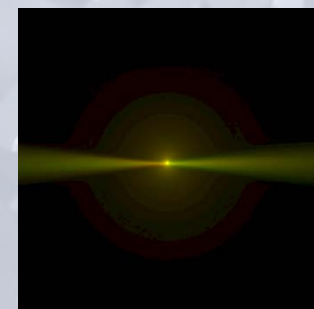
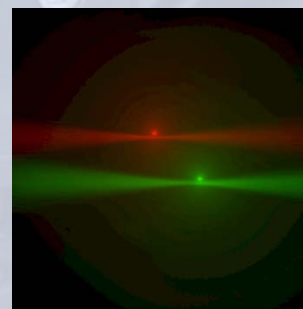


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# Micro-Manager DiSPIM plug-in

Control cameras for alignment – MultiCam.

Align the objectives and cross calibrate scanners and piezos.



ASI diSPIM Control

Navigation

Setup Path A

Setup Path B

Acquisition

Data Analysis

Devices

Settings

Help

Joystick: None

Left Wheel: Imaging Piezo

Right Wheel: Upper (SPIM) Z Drive

Sheet side:  Beam  Sheet

Epi side:  Beam  Sheet

Change settings on tab activate

Camera: MultiCam

Live

Imaging center: 0.0  $\mu\text{m}$  Go to Set  $\Delta = 10 \mu\text{m}$   $\uparrow$   $\downarrow$

Piezo = -2.238  $\mu\text{m}$  + Slice \* 87.267 Compute piezo vs. slice calibration

	Calibration Start Position	Calibration End Position
Slice position: 0 °	0 -0.4327 ° Go to	0.484 ° Go to
Imaging piezo: 0 $\mu\text{m}$	0 -39.999 $\mu\text{m}$ Set	39.999 $\mu\text{m}$ Set

Illumination piezo: 0  $\mu\text{m}$  0 Set home Go home  Go home on tab activate

Sheet width: - + 0.0 8.0

Sheet offset: - + -4.0 4.0

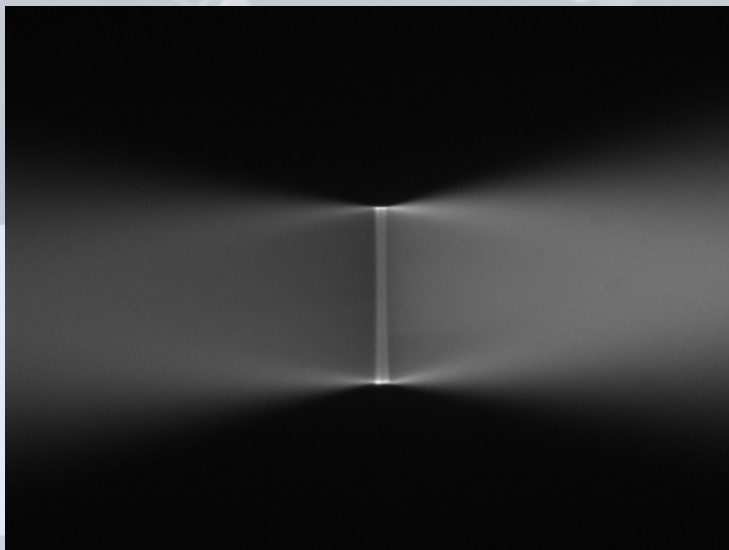


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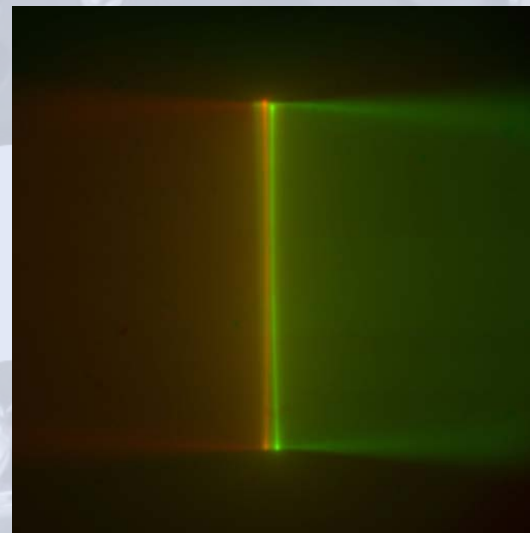
## Micro-Manager DiSPIM plug-in

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!

The screenshot shows the ASI diSPIM Control software interface. The window title is "ASI diSPIM Control". On the left is a navigation pane with the following items: Navigation, Setup Path A, Setup Path B, Acquisition, Data Analysis, Devices, Settings, and Help. The main area is divided into several sections:

- Time Lapse Settings:** Num time points: 2, Interval [s]: 20.
- Durations:** Slice: 5.5 ms, Volume: 2,600 ms, Time lapse: 22.6 s.
- Volume Settings:** Number of sides: 2, First side: A, Delay before side [ms]: 200, Slices per volume: 200, Slice step size [µm]: 1, Minimize slice period (checked), Slice period [ms]: 20, Sample exposure [ms]: 2.5. A "Calculate slice timing" button is present.
- Data Saving Settings:** Separate viewer / file for each time point (unchecked), Hide viewer (unchecked), Save while acquiring (checked), Directory root: :\\Users\\ASI\_Test\\AcquisitionData, Name prefix: dropped.
- Slice Timing Settings (Advanced):** Delay before scan [ms]: 2.5, Lines scans per slice: 1, Line scan period [ms]: 3, Delay before laser [ms]: 2.75, Laser trig duration [ms]: 2.5, Delay before camera [ms]: 2.5, Camera trig duration [ms]: 3.

At the bottom, the SPIM mode is set to "Synchronous piezo/slice scan (standard)" and "Use navigation joystick settings" is checked. A "Start!" button is visible, and a status message reads "Acquisition finished with 2 time points."



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# Micro-Manager DiSPIM plug-in

Analysis!

- MIPAV
- FIJI

