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

ASI's D*i*SPIM Team:

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

NIH Collaborators and Inventors:

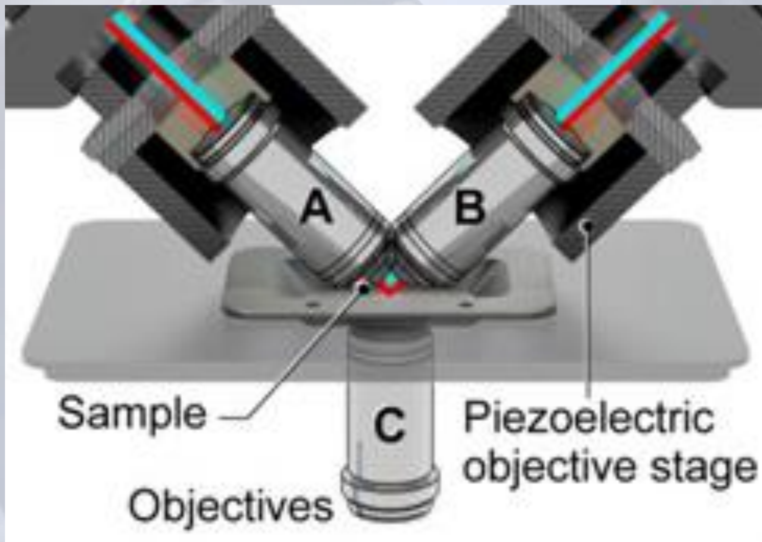
Hari Shroff	NIH/NIBIB Chief Scientist
Yicong Wu	NIBIB Staff Scientist
Abhishek Kumar	Postdoctoral Fellow

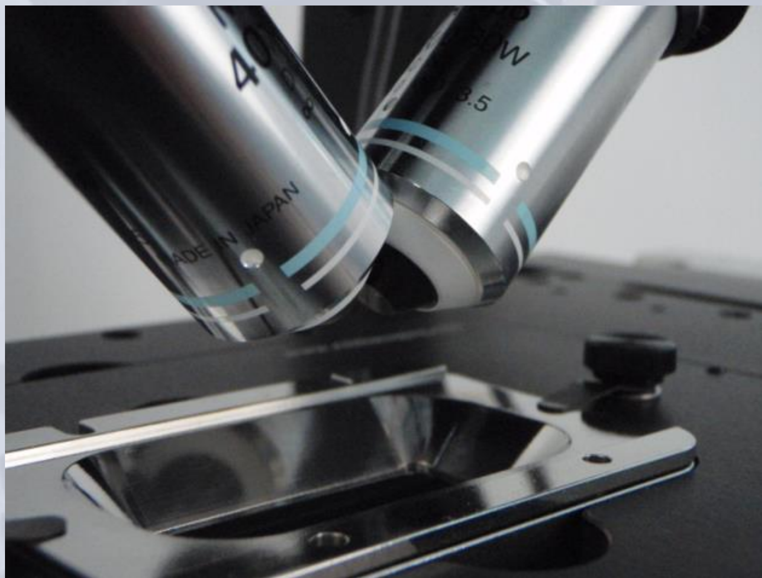
Micro-Manager Development:

Nico Stuurman	UCSF Vale Lab
Jon Daniels	ASI

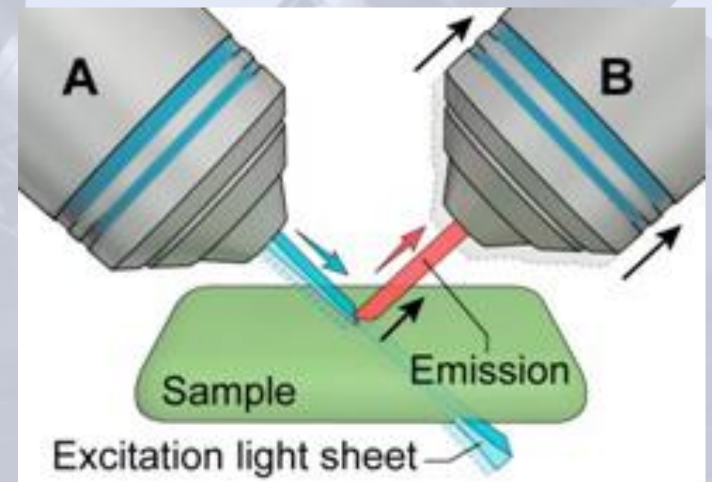
2nd LightSheet Fluorescence Microscopy International Conference
& 7th LSFM International workshop GENOA JULY 5 - 8 2015

DiSPIM = dual-view SPIM on inverted microscope





- Light sheet on inverted microscope
- Two (fixed) views → isotropic resolution
- Conventional sample mounting



Add-on for Conventional Inverted Microscope

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- Existing mounts for:
  - Leica DMI-6000
  - Nikon TE-300, Ti
  - Olympus IX-71/81
  - Olympus IX-73/83
  - Zeiss Axio-Observer
  - Others can be designed

On ASI RAMM Frame

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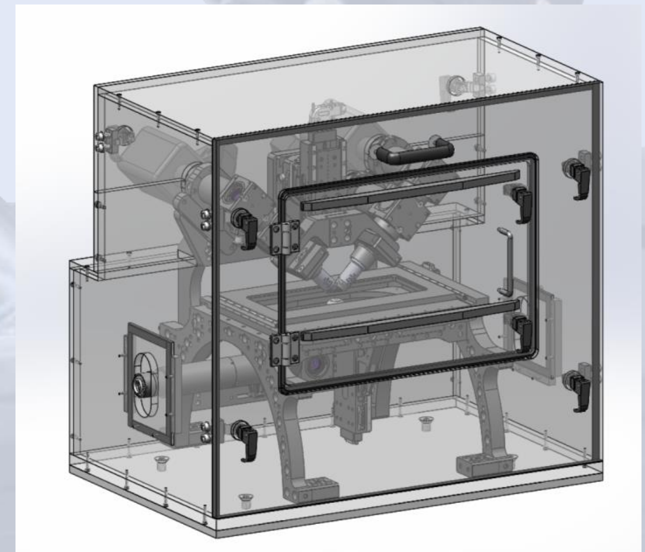
- Flexible Modular Inverted Microscope
- Environmental chamber option



Outline

- Introduction
- History
- Images
- Implementation details
- Micro-manager plugin
- Conclusion

History

- Hari Shroff & Yicong Wu (NIH)
  - Original iSPIM paper: Wu et.al., PNAS 108 (43) 17708-17713 (2011)
  - Original diSPIM paper: Wu et.al., Nature Biotechnology 31, 1032–1038 (2013)
- ASI contributions:
  - Modular microscope, including DiSPIM-specific parts
  - Fiber-coupled scanners
  - Controller-based synchronization
  - Micro-manager plugin

iSPIM Concept

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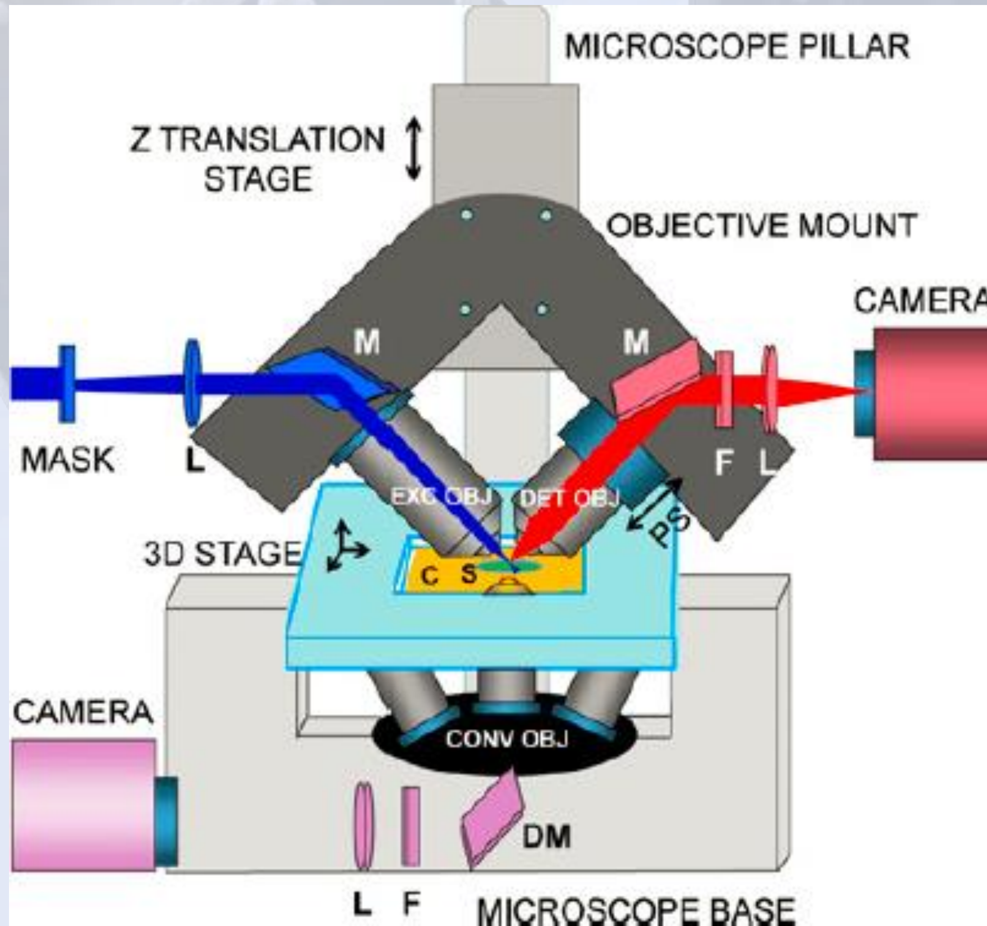

Wu et. al, PNAS 108, 17708-17713 (2011)

- SPIM on inverted microscope
- Sample mounted on standard glass coverslip
- 30x faster than spinning disk for same SNR

DiSPIM Concept

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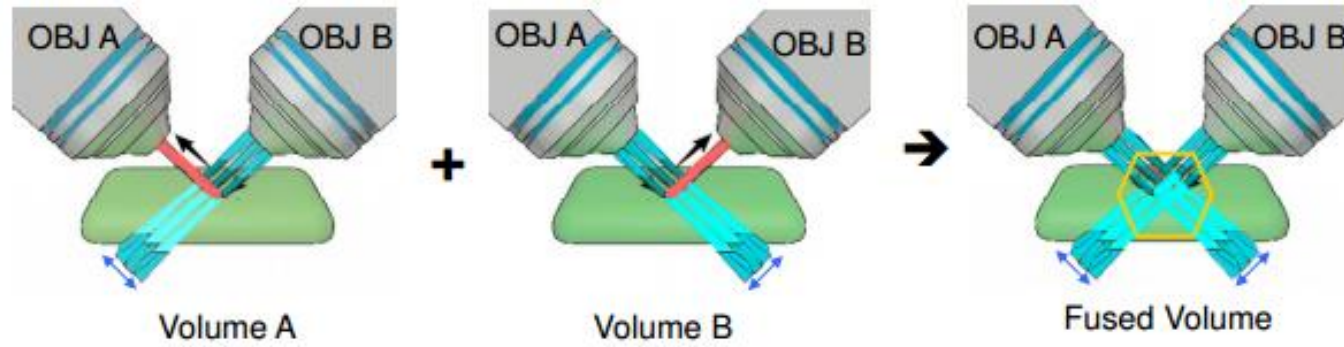
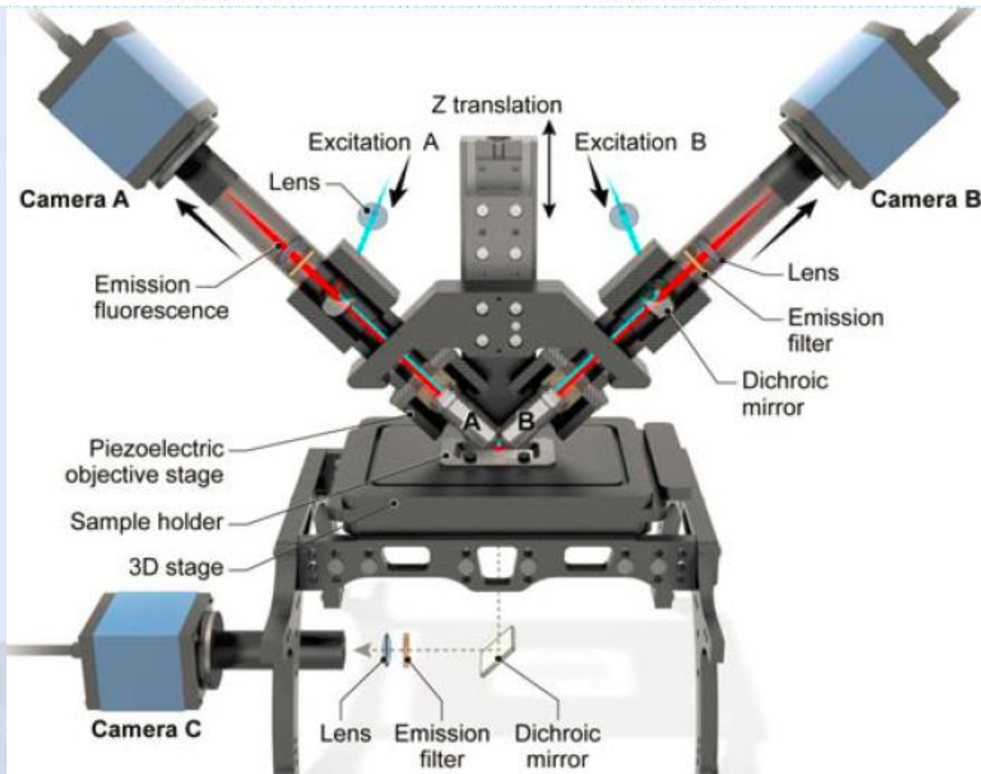


Wu et. al, Nat. Biotech. 31, 1032-1038 (2013)



- iSPIM but use both objectives for imaging => dual orthogonal views => isotropic resolution after computationally combining

Isotropic Resolution

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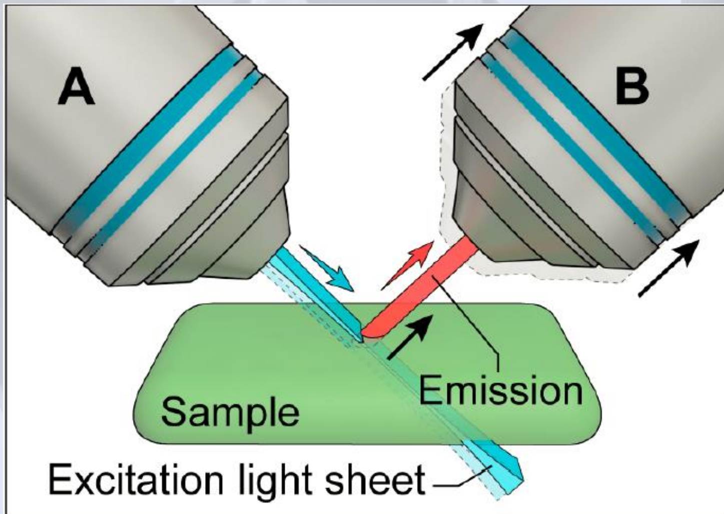

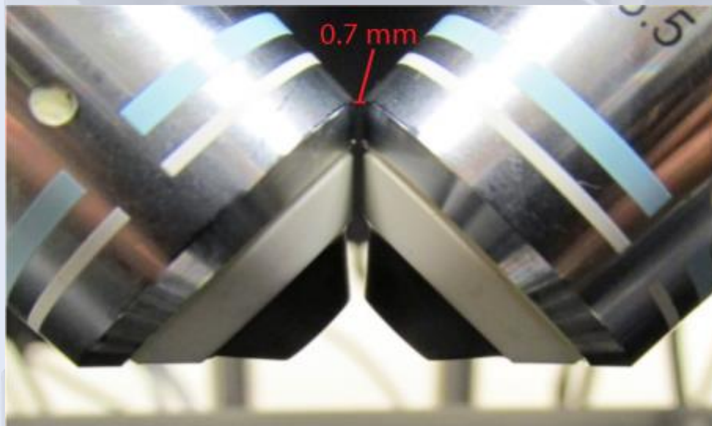

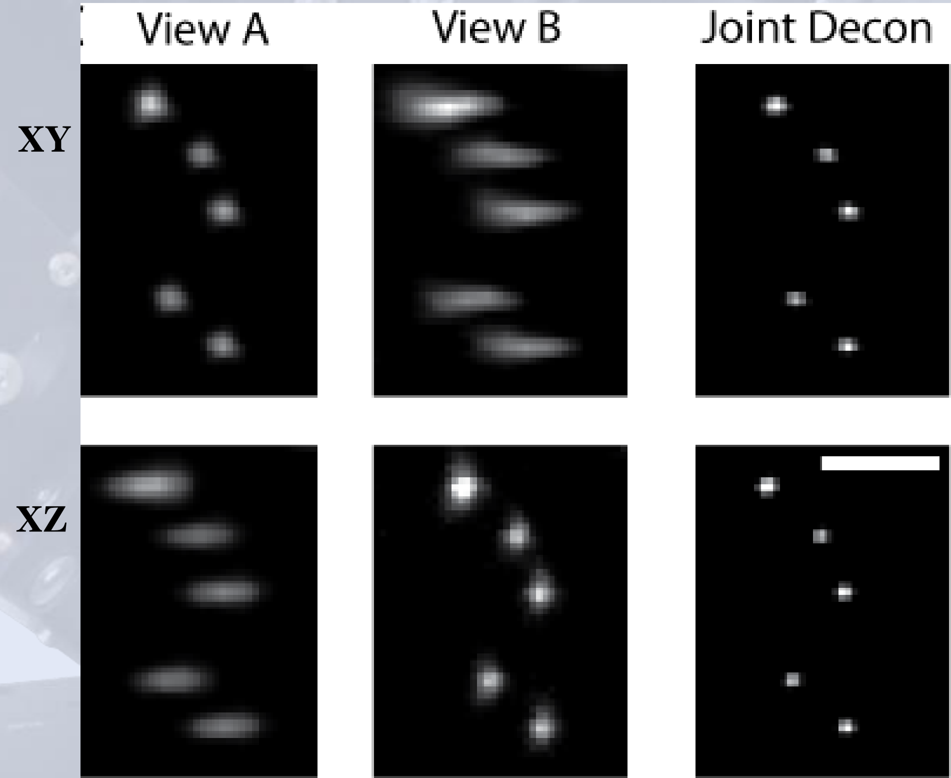

Joint Decon: A. York and Y. Wu

Wu et al. *Nat. Biotechnol.* 31, 1032-138 (2013), Kumar et al. *Nature Protocols* 9, 2555-2573 (2014), Ingaramo et al. *Chem Phys Chem* 15, 794-800 (2014)

Early diSPIM

Modern DiSPIM

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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. XY stage (large MS2500)

Modern diSPIM

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- Fits 90 x 75 cm air table
- Environmental control system
- Microscope alone costs <$100k, additional $75-150k for cameras, laser launch, epi light source, environmental control, computer, etc.

diSPIM Performance

- 3D volumes w/ isotropic resolution
  - Isotropic resolution requires registration/fusion
  - 330 nm resolution with Nikon 40X NA 0.8
- Acquisition rates up to 200 images per second (2-5 volumes per second)
- Achieve a >10x reduction in photo-bleaching compared to con-focal methods.

Why DiSPIM?

- Sample mounting on coverslip/dish
- Isotropic resolution without rotating sample
  - Observe fast processes or moving objects
- Commercial but open and customizable
- Can be integrated with your favorite techniques or equipment

iSPIM vs. DiSPIM

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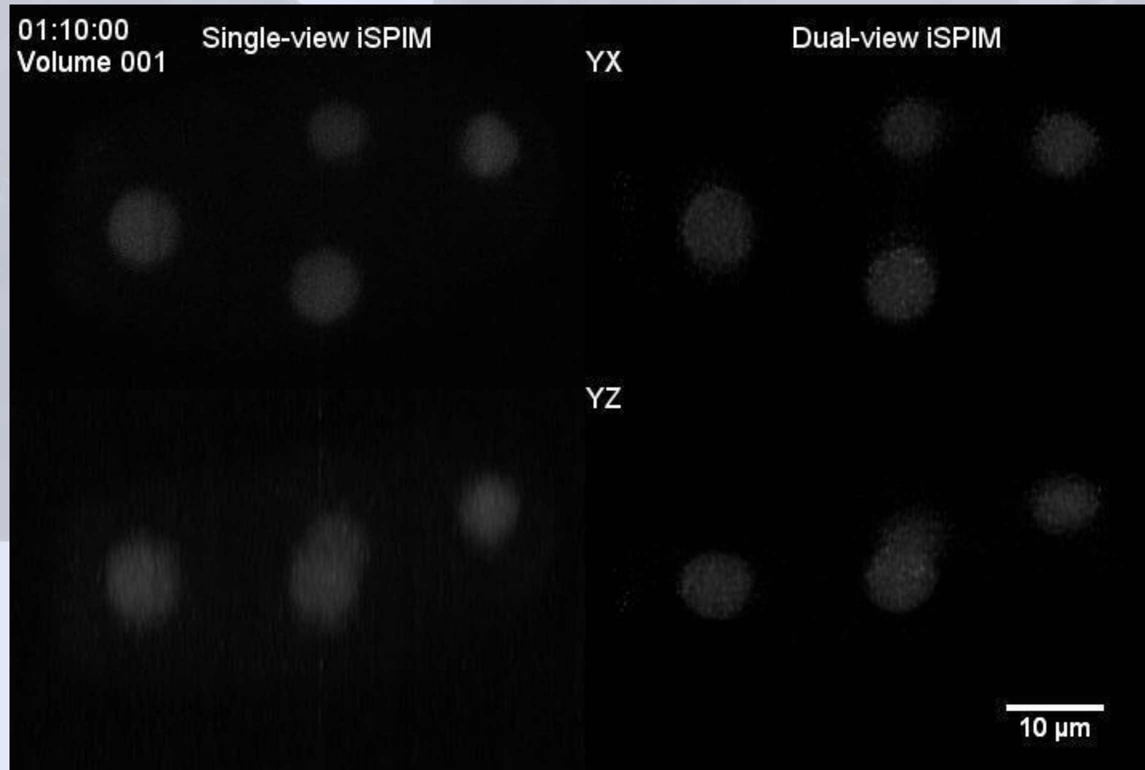

Wu *et. al, Nat. Biotechnol.* 31, 1032-1038 (2013)

GFPhistones in a live BV24 *C. elegans* embryo from the 4 cell stage up to hatching. Sampled every minute at 50 planes per volume with 1 µm inter-plane spacing at 200 Hz.

DiSPIM vs. SDCM

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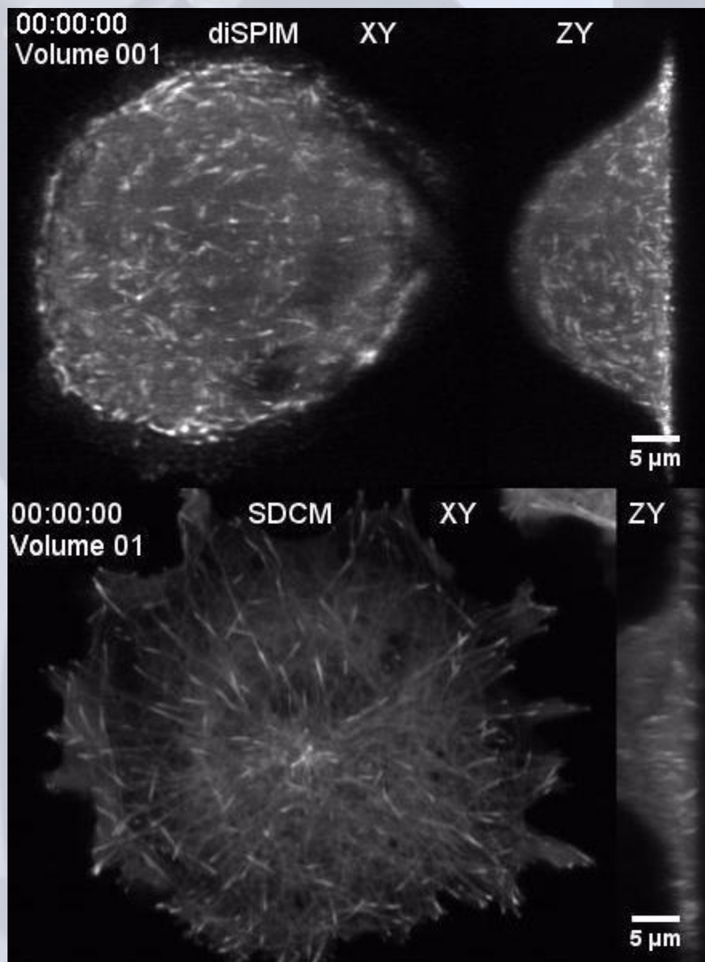
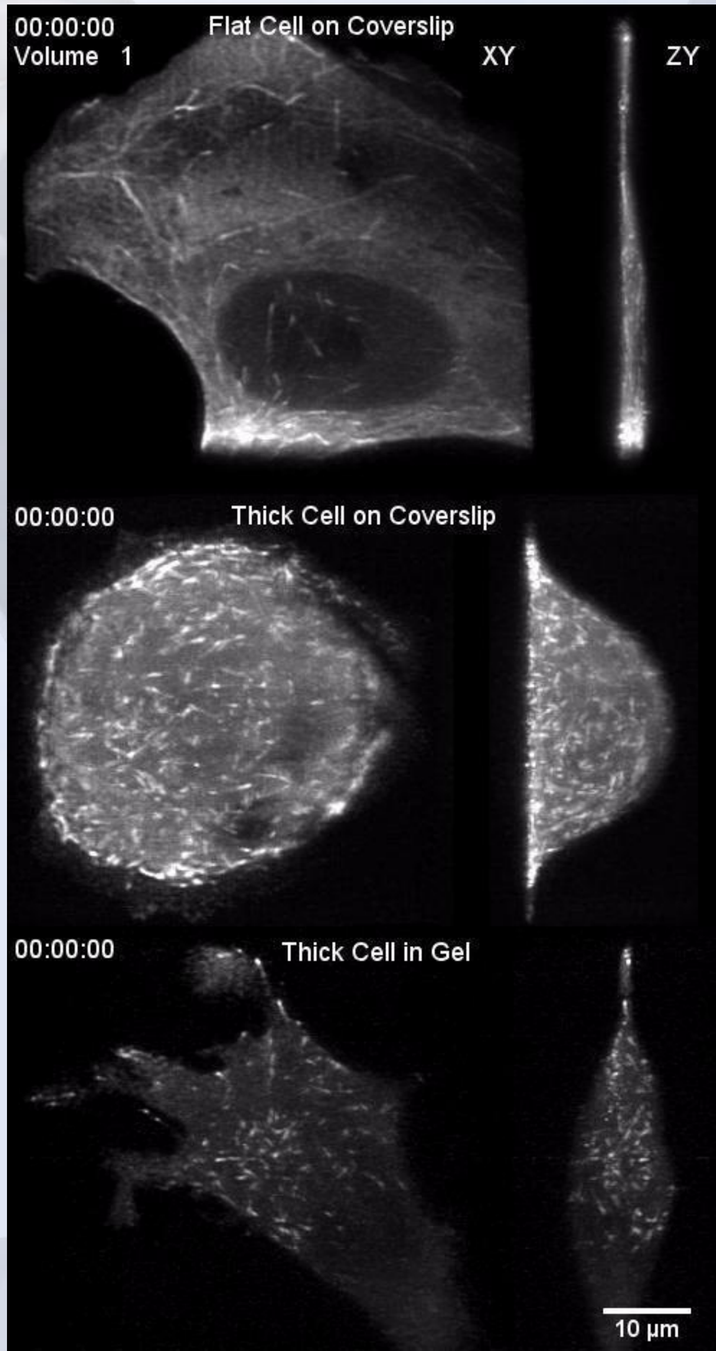


- GFP-EB3 Microtubules in Live Human Umbilical Vein Endothelial Cells
- At same SNR and illumination, diSPIM enables collection of 3x more volumes, 3.2x more planes per volume and 7.6-fold less photobleaching

Wu *et. al, Nat. Biotechnol.* 31, 1032-1038 (2013)



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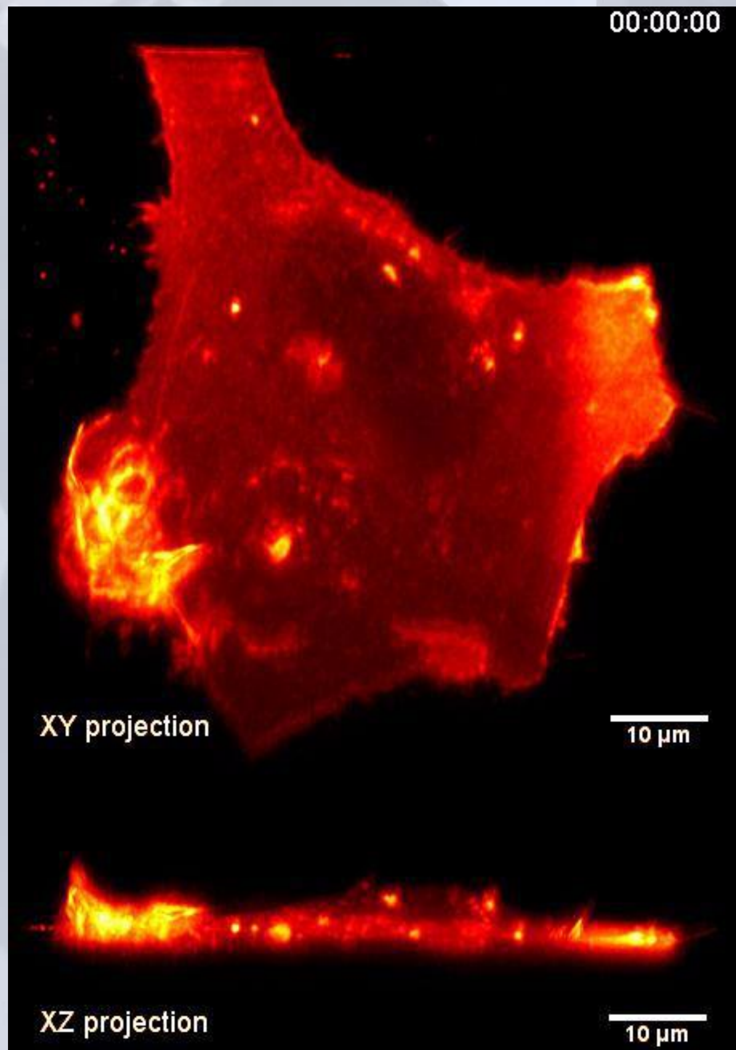
- C3D GFP-EB3 microtubule dynamics in human umbilical vein, endothelial cells
- Different thickness and cellular environments

Wu *et. al, Nat. Biotechnol.* 31, 1032-1038 (2013)

APPLIED SCIENTIFIC INSTRUMENTATION



- Cultured human lung fibroblast cell, expressing GFP-tagged H-Ras, and demonstrating dynamic cellular movements

Kumar *et. al, Nat. Protocols* 9, 2555–2573 (2014)

Cancer Spheroids in Culture

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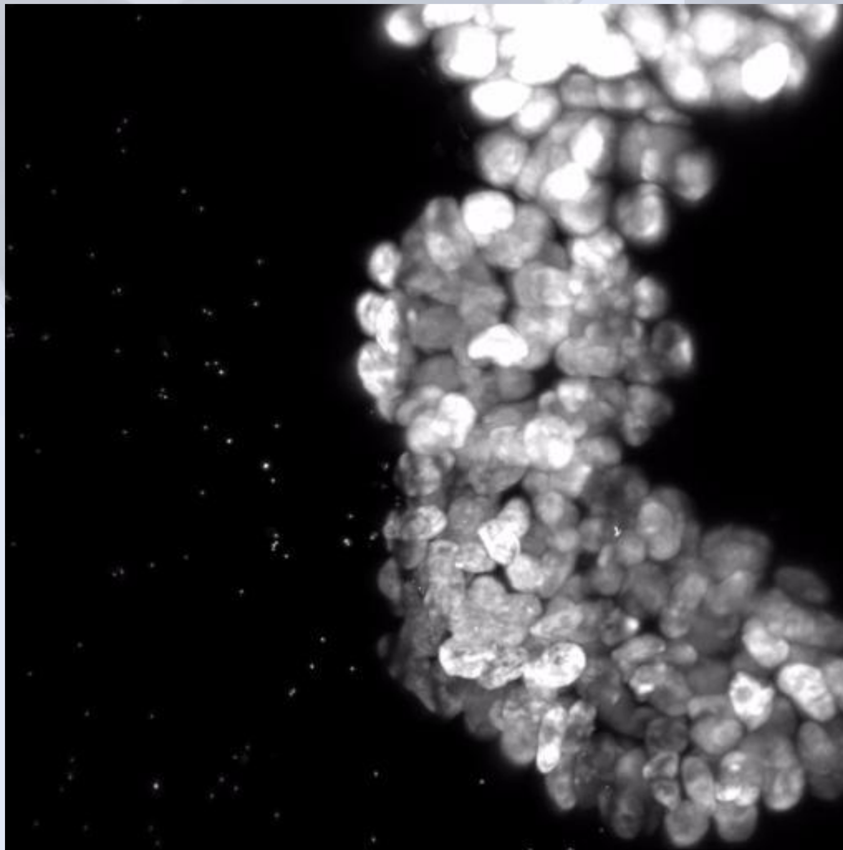
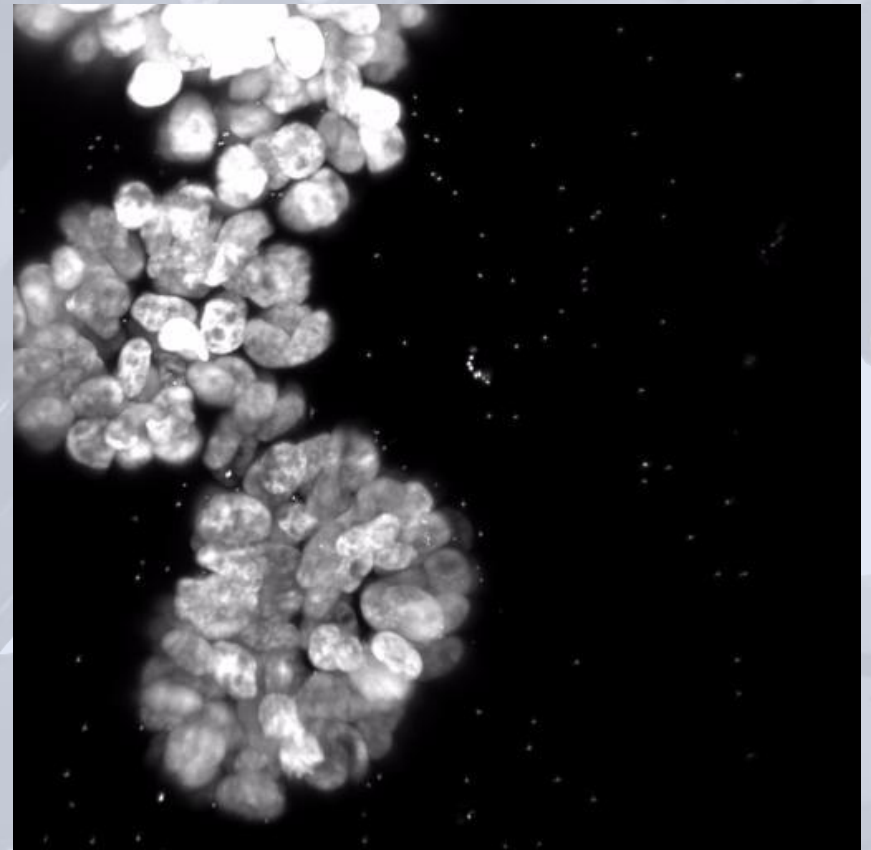
View A

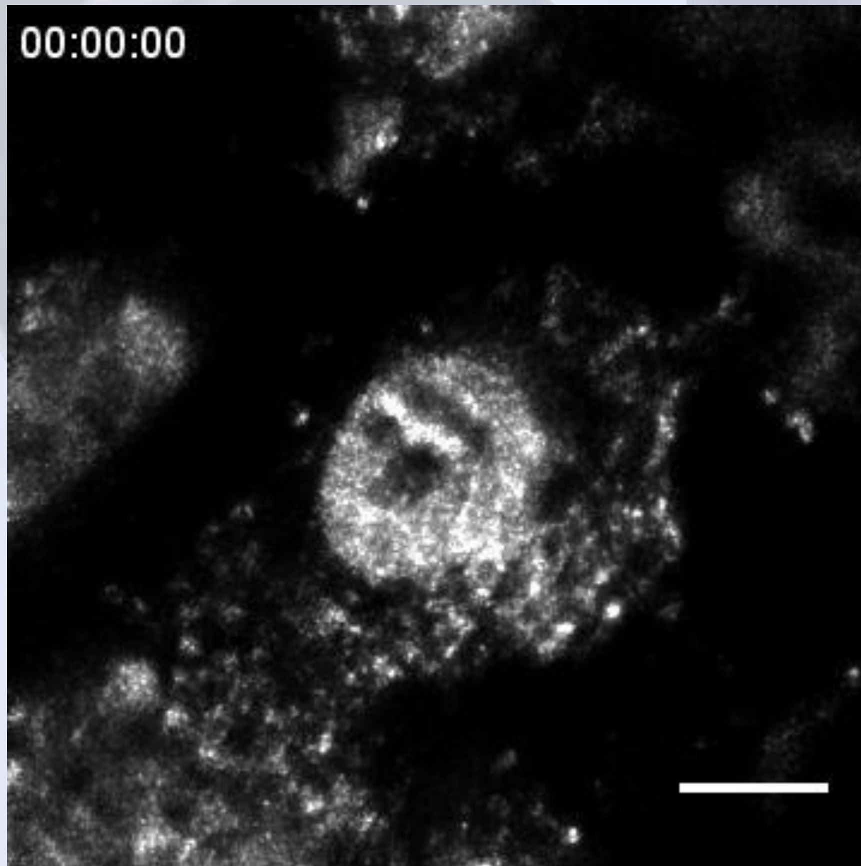


View B



Maximum projection vs. time, 2 views (not fused)
Courtesy Christian Conrad, Univ. of Heidelberg

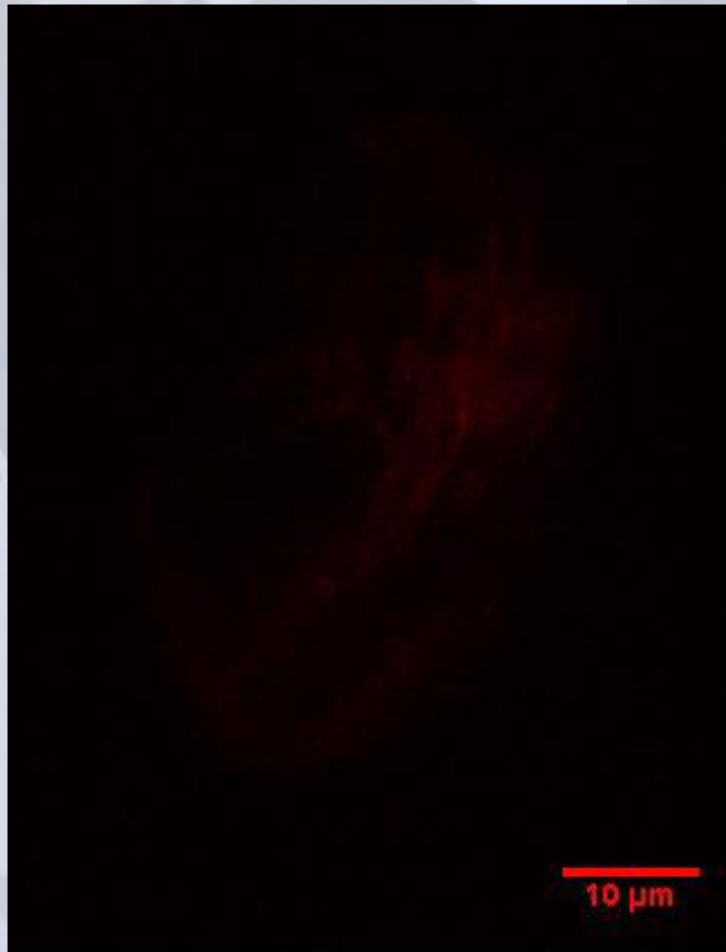
APPLIED SCIENTIFIC INSTRUMENTATION



- A549 cells infected for 16 hours with WSN PA-GFP and then imaged for 30 min with an entire cell volume captured every 2 seconds. Scale bar is 10 µm.

Lakdawala et al., PLoS Pathogen 10(3): e1003971 (2014).

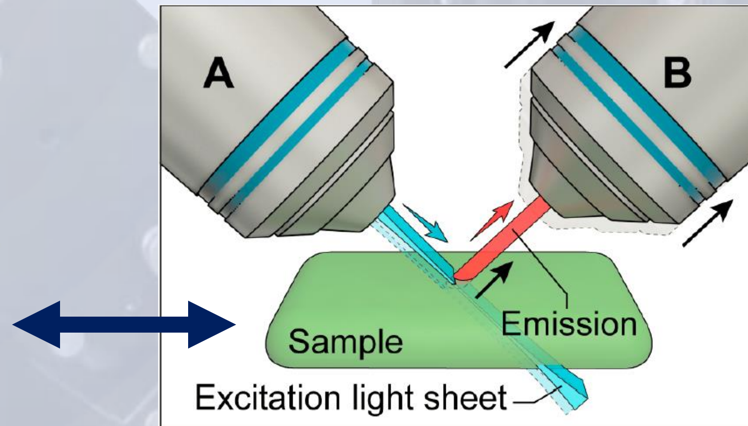
Dual-color Imaging



- C. elegans embryo

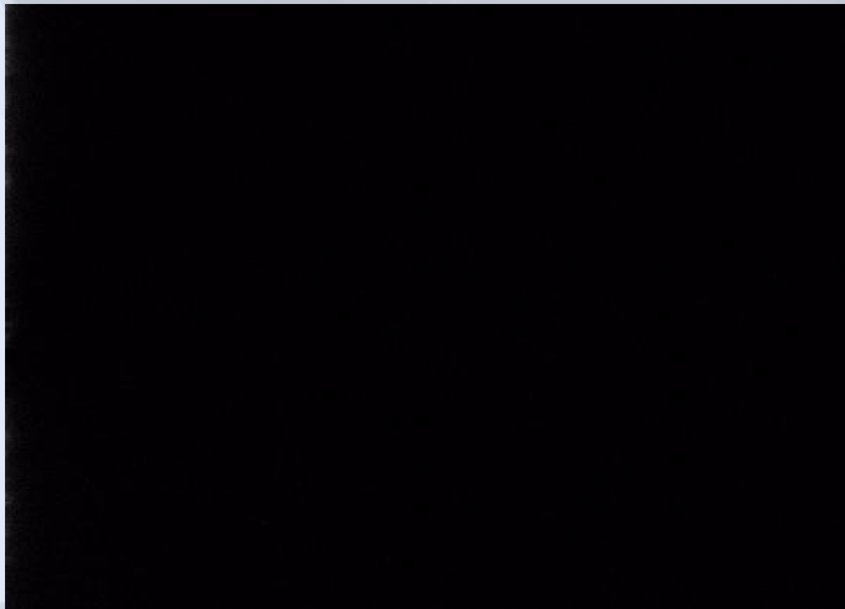
Courtesy Abhishek Kumar and Ryan Christiensen

Stage Scanning



APPLIED SCIENTIFIC INSTRUMENTATION

Courtesy Abhishek Kumar



Scanned sheet

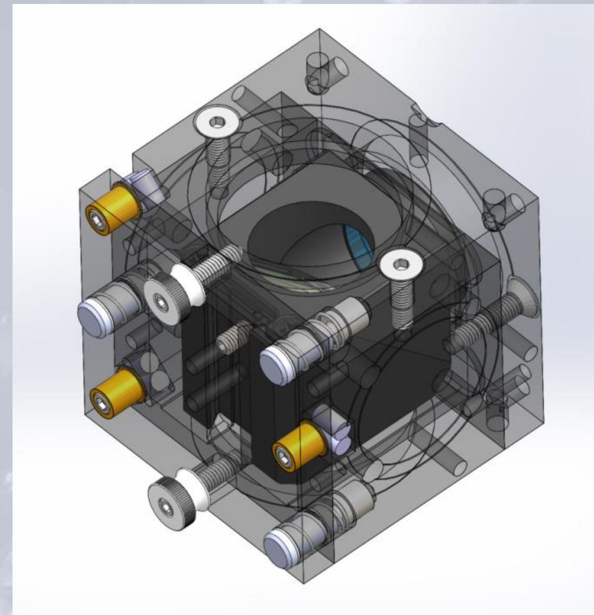
Stage scanning

ASI DiSPIM Implementation

- Built with modular hardware for evolving applications and configurations
- Modular control electronics for evolving needs
- Micro-Manager support w/ASI support
- Systems available from ASI directly or via multiple system integrators
- Many additional features/variations being developed by labs worldwide

ASI's Modular Microscope Hardware

- Adjustable cubes
- Tube lenses
- Camera mounts
- Illumination adapters
- Linear stages
- Filter wheels
- And many more LEGO-like components



Adjustable Beam-Splitter Cube

Four cameras

Transmitted light for bottom scope

Easy to build complex or custom microscope

Light Sheet Scanners

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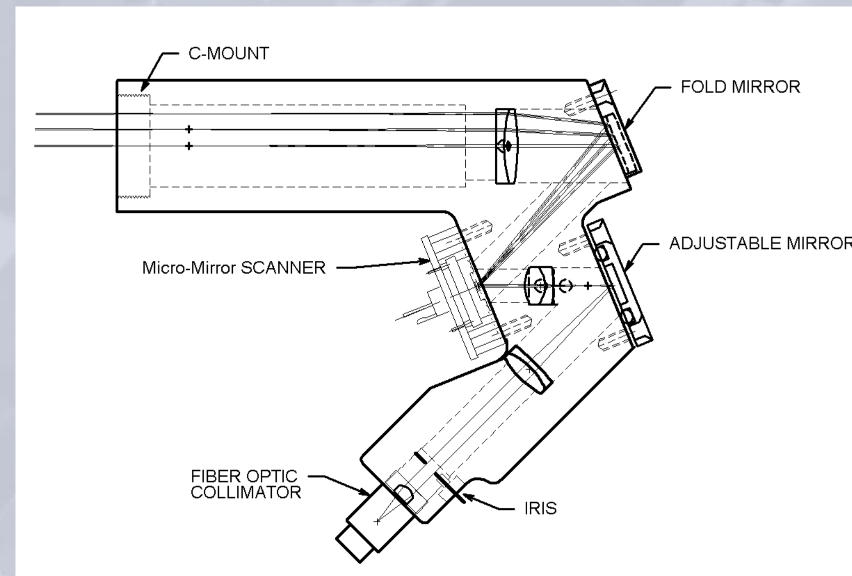
- Compact 2-axis scanner with fiber input
  - One axis makes sheet, other selects slice position
- C-mount device (19mm FN)
- 1kHz scanner bandwidth
- Anti-striping option
- Tiger control card
- Many other applications



Tiger Controller

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- Basic diSPIM has 10 controlled axes
  - XY stage
  - SPIM height
  - Lower Z
  - Two objective piezos
  - Two 2-axis scanners
  - Plus TTL triggers
- All controlled with ASI's Tiger modular controller
- Piezos and scanners controlled with either internal DACs or external voltage control

Hardware Synchronization

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- Light sheet, piezos, cameras, and lasers must be tightly synchronized
- 2 approaches:
  - Generate synchronized control voltages
    - Labview code available from Shroff group
    - Third party software or write your own
  - Use synchronization within Tiger controller
    - Micro-Manger DiSPIM plug-in
    - Third party software or write your own

Micro-manager plugin features

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- Acquisition Modes:
  - Single or double-sided
  - Synchronized slice and piezo
  - Fixed sheet
  - Stage scan
- Multi-Dimensional Acq.
  - Time points
  - Multi-position
  - Multi-color
- Supported cameras:
  - Andor Zyla
  - PCO Edge
  - Hamamatsu Flash 4
  - Others possible
- Supported lasers:
  - Lasers with dual port switch or passively split
  - Lasers on/off via TTL
  - Up to 4 colors

Navigation Tab

- Move all 10 axes
- Flexible manual control with joystick and wheels

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
Live

Scan A
Scan B
Piezo A
Piezo B

Axis	Position	Target		Step			
XY Stage, X axis:	-2,819.6 µm	0	-	10	+	Go to 0	Set 0
XY Stage, Y axis:	690.52 µm	0	-	10	+	Go to 0	Set 0
Lower Z Drive:	5,792.94 ...	0	-	100	+	Go to 0	Set 0
Upper (SPIM) Z Drive:	20,000.0...	0	-	100	+	Go to 0	Set 0
Imaging Piezo A:	0 µm	0	-	5	+	Go to 0	
Imaging Piezo B:	0 µm	0	-	5	+	Go to 0	
Scanner A, sheet (X):	4 °	0	-	0.2	+	Go to 0	
Scanner A, slice (Y):	4 °	0	-	0.2	+	Go to 0	
Scanner B, sheet (X):	4 °	0	-	0.2	+	Go to 0	
Scanner B, slice (Y):	4 °	0	-	0.2	+	Go to 0	

Halt!

Setup Tabs

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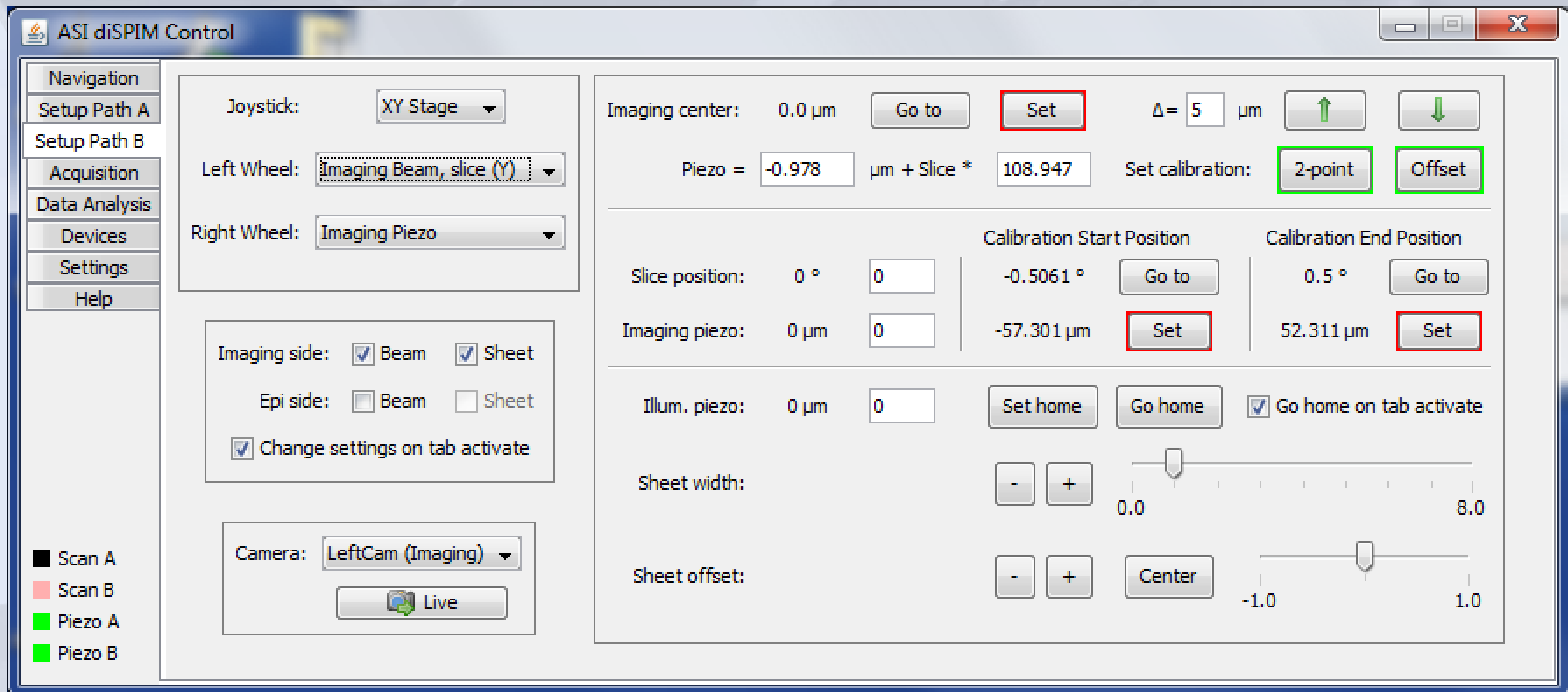
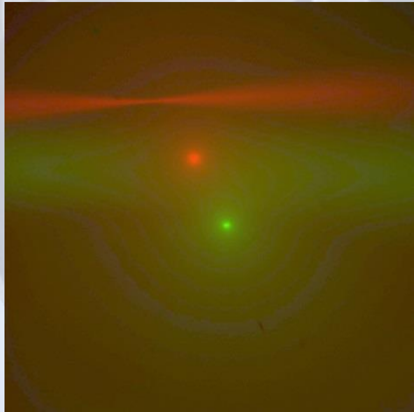
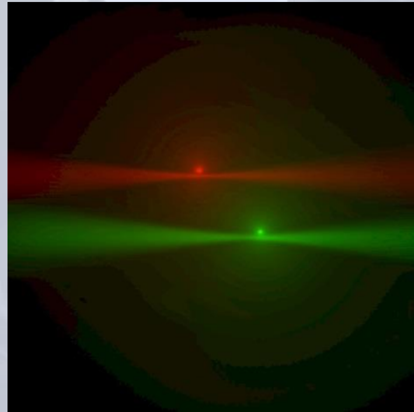
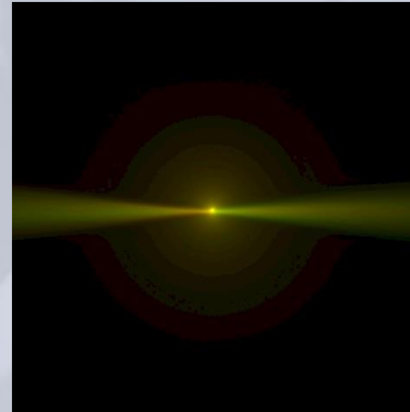
- Useful for mechanical alignment
- Piezo/scanner cross-calibration



Microscope Alignment

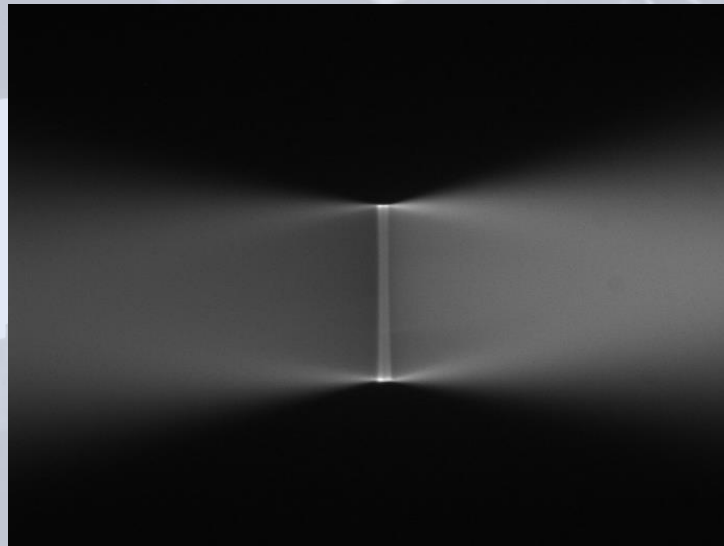




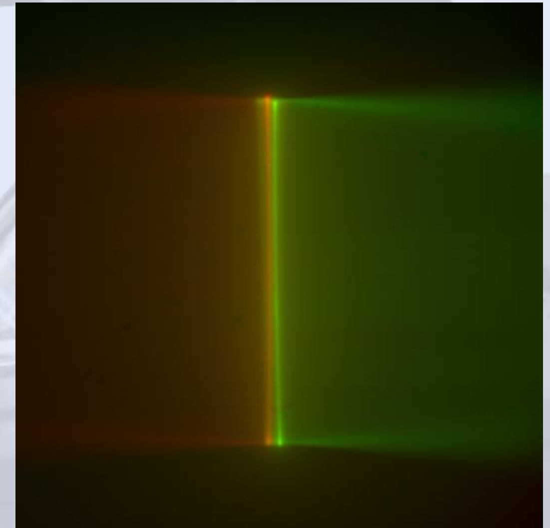


Co-aligning objectives and centering cameras

Aligning light sheets hitting cover-slip in dye solution



Bottom Camera



MultiCam

Acquisition Tab

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- Set parameters and acquire

ASI diSPIM Control

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

Durations
Slice: 30.75 ms
Volume: 1,738 ms
Total: 1.738 s

Time points
Number: 3
Interval [s]: 10

Multiple positions (XY)
Edit position list...
Post-move delay [ms]: 1

Volume Settings
Number of sides: 2
First side: A
Delay before side [ms]: 500
Slices per volume: 12
Slice step size [µm]: 1
Minimize slice period
Slice period [ms]: 20
Sample exposure [ms]: 20.5
Calculate slice timing
Use advanced timing settings

Data Saving Settings
Separate viewer / file for each time point
Hide viewer
Save while acquiring
Directory root: \ASI_Test\AcquisitionData
Name prefix: test

Channels
Channel group: Excitation Source

Use?	Preset
✓	488nm
✓	561nm

Change channel: Every volume

SPIM mode: Synchronous piezo/slice scan (standard)

Start! No acquisition in progress.

Use Navigation joystick settings

Scan A
Scan B
Piezo A
Piezo B

Data Analysis Tab

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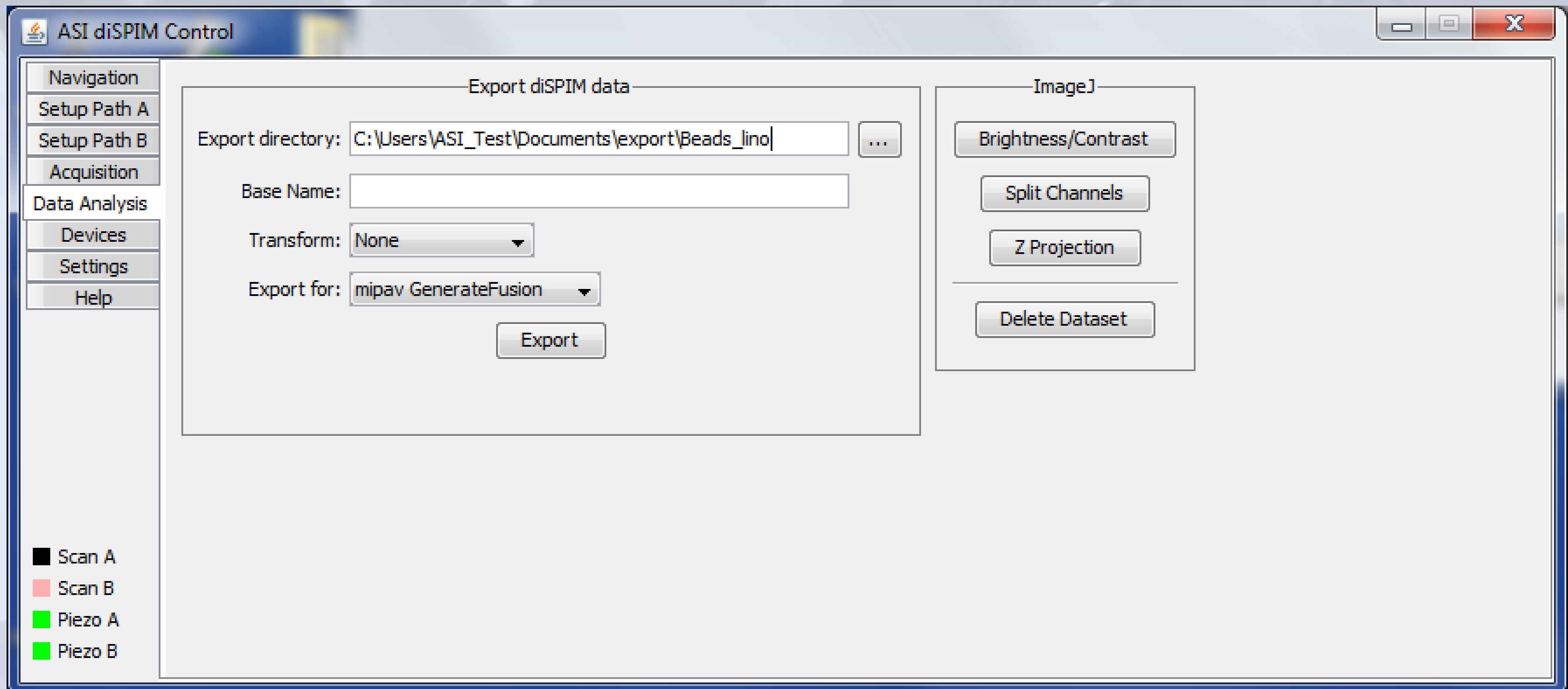
- Export to MIPAV
- Open directly in Fiji Multi-View Reconstruction



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Lots of options

- Many system configurations possible
- Multiple system integrators offering diSPIM
  - 3i is most advanced/proficient of integrators
  - Can also buy direct from ASI
- Multiple acquisition softwares
  - Including open source Micro-manager
  - 3i's Slidebook, others to come
- Multiple data analysis softwares
  - Including open source

Extensions

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- Single-sided system with high-NA objective
- Photomanipulation from bottom
  - Optogenetics
  - Wounding
- 2-photon
- Imaging from bottom simultaneously
- Gated laser to avoid certain regions of sample

dispim.org wiki

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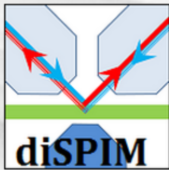
dispim.org/start

Login



navigation

- docs
  - manual
  - comparison_of_spim_
  - comparison_with_cor
  - system_integrators
  - user_list
- hardware
  - add-ons
  - asi_manual
  - environmental_contro
  - troubleshooting
- media
  - publications
  - video_tutorials
- software
  - fiji_mvr
  - labview
  - micro-manager
  - mipav_generatefusior

registered users

search

Go Search

toolbox

- What links here
- Recent changes
- Media Manager
- Site index
- Printable version
- Permanent link
- Cite this article

article | discussion | show pagesource | old revisions

Table of Contents

- The Idea
- The Implementation
- The Benefits
- This Wiki

The Idea

The *diSPIM* 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 was co-developed by the NIH-based lab of Hari Shroff and Applied Scientific Instrumentation (ASI). SPIM is also referred to as light sheet fluorescence microscopy or LSFM because it uses a sheet or plane of light to illuminate the sample perpendicular to the imaging direction.

The Implementation

The diSPIM has two (usually symmetric) optical paths for light sheet imaging. Two objectives are placed at right angles above a sample mounted horizontally. A light sheet is created from one objective and then imaged using the other objective. A stack of images is collected by moving the light sheet through the sample. For a few users, the 3D information from a single view or stack is sufficient (iSPIM). For dual-view systems, the role of the two objectives is reversed to collect another stack from a perpendicular direction, and then 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).

The diSPIM "head" can be mounted on various inverted microscopes including ASI's RAMM frame (shown here). diSPIM systems can be obtained from various system integrators. Various open-source and proprietary software packages are available for both data acquisition and data visualization. Most of the underlying microscope hardware is identical regardless of the system integrator and software used.

The choice of diSPIM objectives is limited because they must be co-focused without bumping into each other. The most commonly-used objectives for diSPIM are 40x water-dipping objectives with a NA of 0.8 (Nikon CFI Apo 40XW NIR).

Most often sCMOS cameras are used for SPIM imaging. There are working diSPIM systems with Hamamatsu Flash4, Andor Zyla, and PCO Edge cameras.

ASI makes a compact fiber-coupled 2D galvo or "scanner" which is an integral part of the system. It creates the light sheet by fast scanning in one axis and moves the sheet through the sample using the other axis. The output of the excitation laser (or laser launch) simply is fed into the scanner; it is helpful to have a 2×1 optical switch or dual-output laser launch so the excitation can all be steered to the scanner in the active light path.

For applications where environmental control is important, the diSPIM can easily be fitted with an incubator enclosure and appropriate equipment to keep samples alive and happy.

The bottom objective (the inverted microscope) most often has a lower-magnification objective and less expensive camera for locating the sample. Epi-illumination can easily be added.

The Benefits

Like other SPIM techniques, diSPIM illuminates only the focus plane and is thus ideal for imaging living cells and organisms because it minimizes photobleaching and phototoxicity effects.

Compared with confocal or spinning disk systems, there is ~2x improvement in axial resolution, >10x reduction in photobleaching, and speed comparable to spinning disk. See a more detailed comparison with confocal.

A major advantage compared with most other SPIM implementations is that sample mounting is extremely simple, similar to an inverted microscope. Most commonly, specimens are placed on a 24x50mm coverslip which is held in a special chamber that holds the dipping media. See a more detailed comparison with other SPIM techniques.

Thank you! Any questions?

Commercial SPIMs

SPIM Type	# views	Mounting	Software	Comments
DiSPIM	2 fixed	Coverslip/dish with media	Open & various proprietary	Flexible configuration
Zeiss Z-1	Unlimited (rotation)	Capillary with agarose	1 proprietary	OpenSPIM is open alternative
Leica TCS SP8 DLS	1 fixed	Dish with media	1 proprietary	Combined with confocal
LaVision BioTec Ultramicroscope	1 fixed	??	1 proprietary	Mainly for large fixed samples