Innovations in ASI Light Sheet Microscopes: The Oblique SPIM

ASI's SPIM Team

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American Society for Cell Biology Meeting
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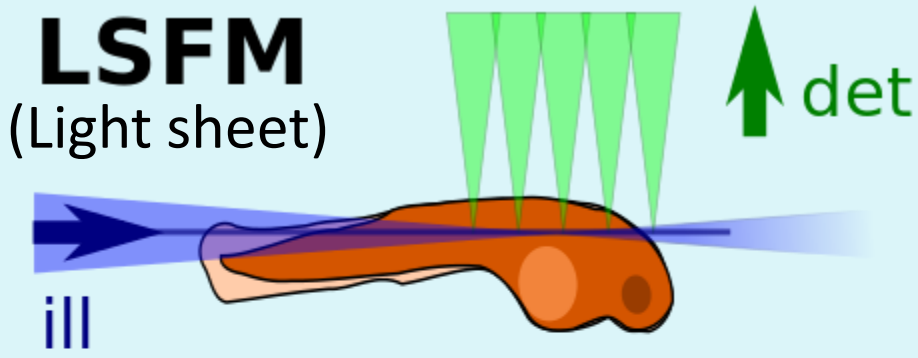
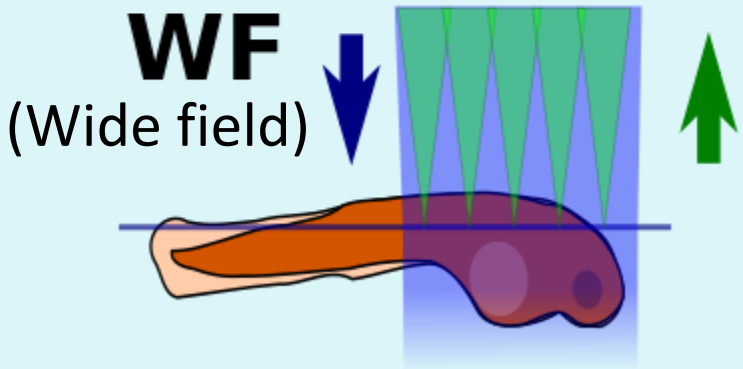
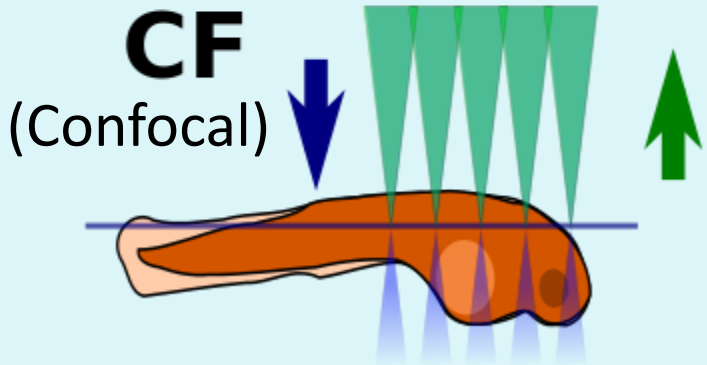
What is light sheet microscopy?



LSFM ~= SPIM = Selective Plane Illumination Microscopy





https://commons.wikimedia.org/wiki/File%3ALsfm_lightsheetinsample.svg (CC BY-SA 3.0)

Why light sheet microscopy?

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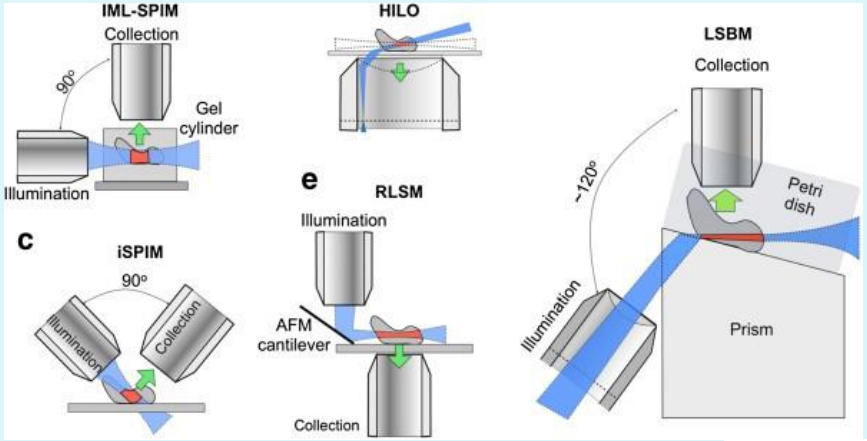
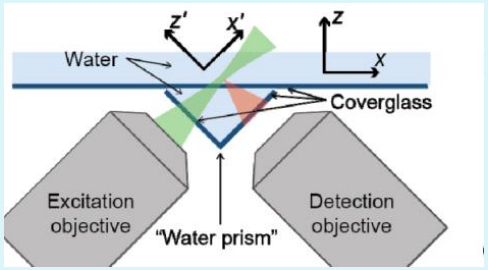
- Minimize photodamage/bleaching
  - Better utilize “photon budget”
  - Keep living things living
- Rapid acquisition
  - 2D parallel imaging
- Main cost is optics for generating light sheet

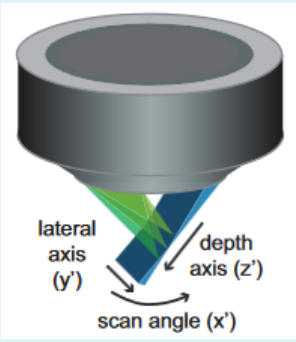
Light sheet configurations



Optical Nanoscopy 2013 2:7.

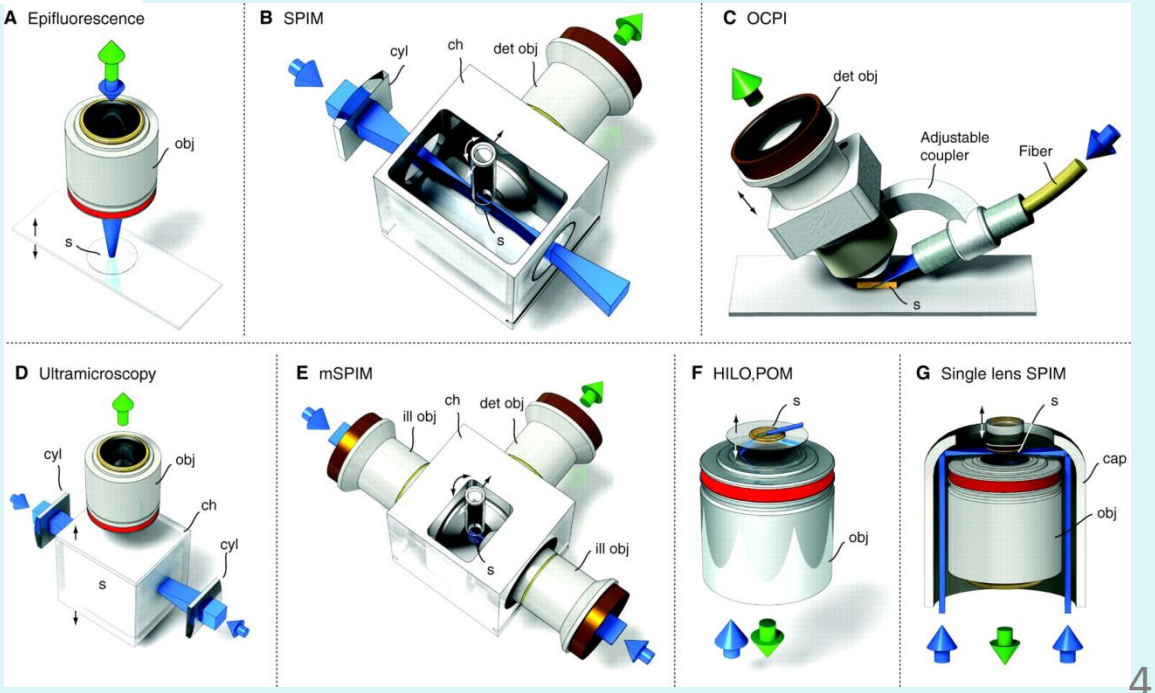


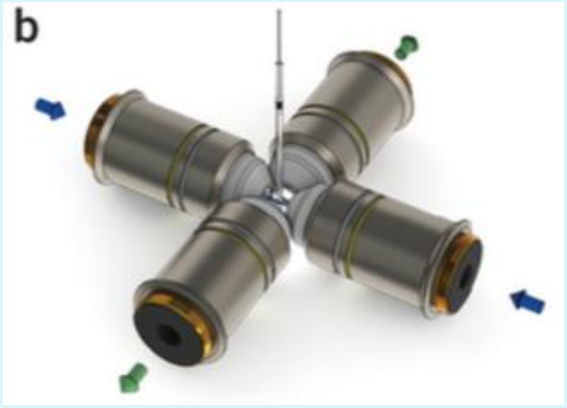
Optics Express 2015; 23: 16142-16153



Nature Phot. 2015 9:113.

Development 2009 136:1963.

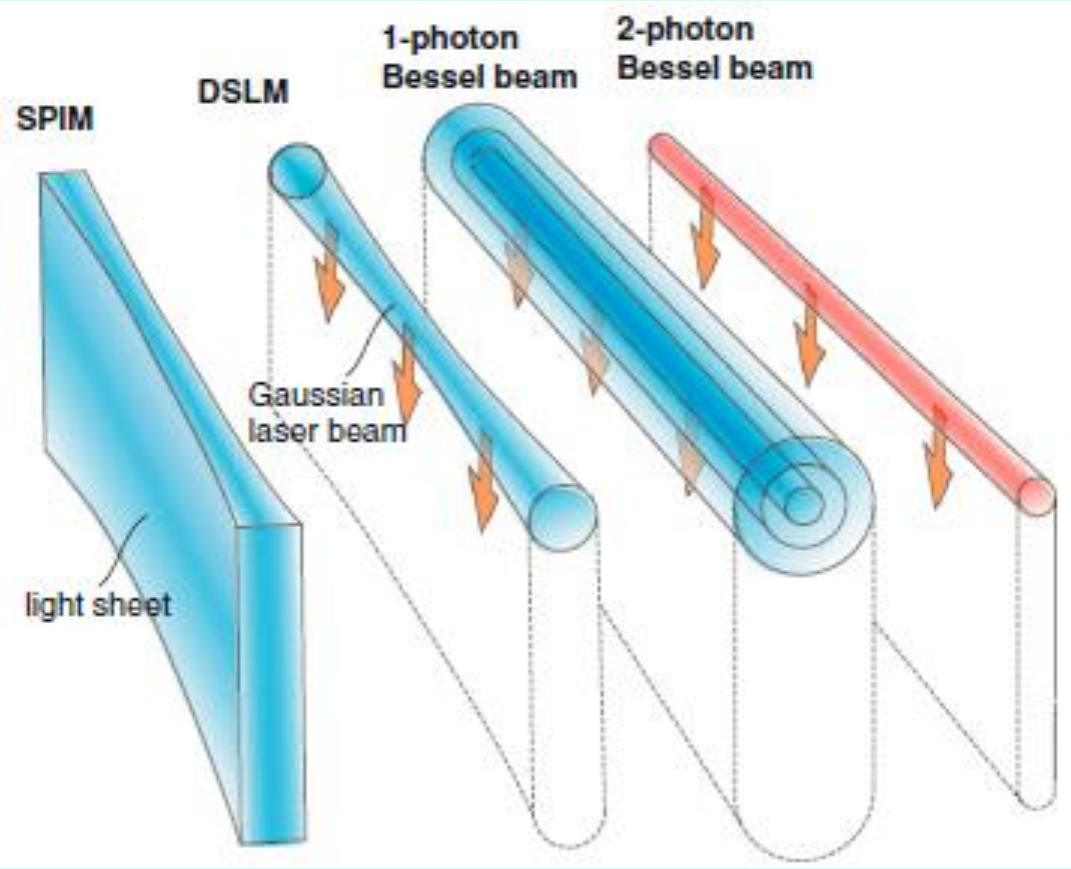




Nature Meth 2015 12:30.

Light sheet generation

- Sheet thickness trades off with width of thin region (FOV)
- Increasingly complex optics can give increasingly better thinness and/or FOV



Weber et al., Cur. Opinion in Genetics and Development 21, 566-572 (2011)

Resolution is anisotropic

Lateral resolution ~ 0.61*λ/NA

Axial resolution ~ 1.22*λ/NA^2

NA	Lateral Res @ GFP [nm]	Axial Res @ GFP [nm]	Ratio (all λ)
0.4	778	3889	5.0
0.6	519	1728	3.3
0.8	389	972	2.5
1	311	622	2.0
1.2	259	432	1.7

Improving (axial) resolution

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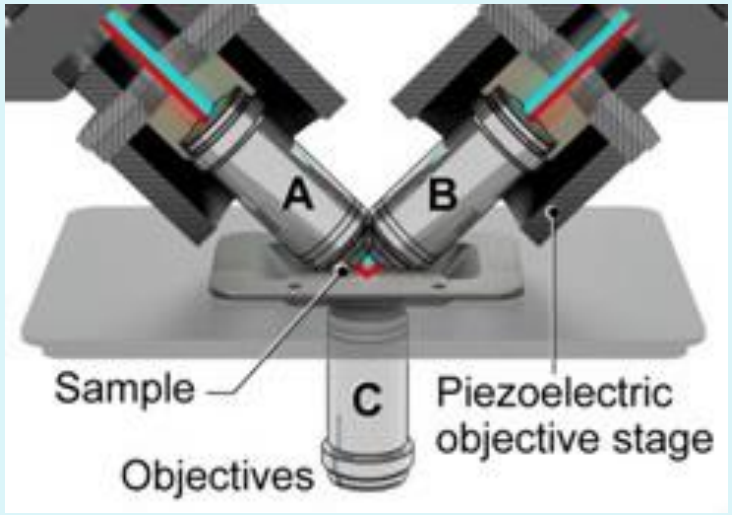
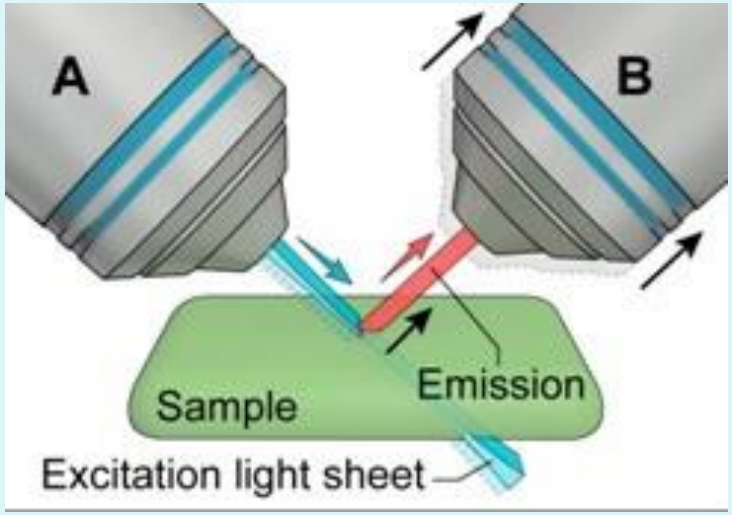
- Improve axial resolution of imaging objective
  - i.e. higher NA **(oSPIM/doSPIM, lattice light sheet)**
- Create light sheet thinner than objective's axial resolution **(lattice light sheet)**
- Combine datasets from different angles
  - Axial direction becomes lateral **(diSPIM/doSPIM)**
- Physically section sample
  - Not practical for most samples

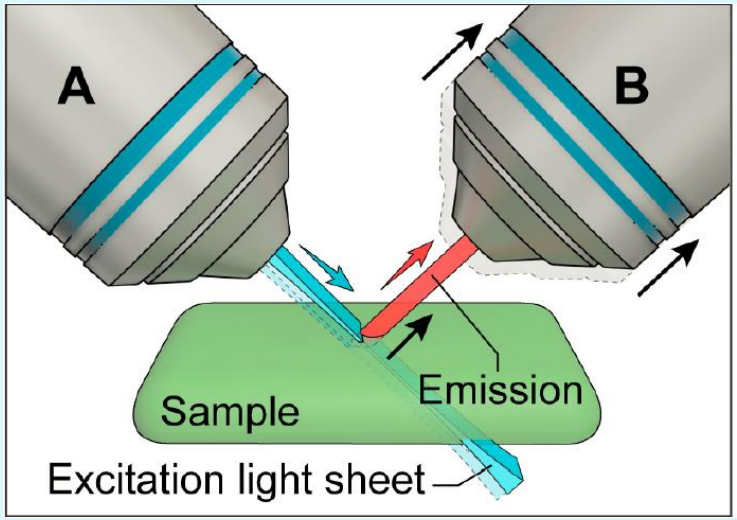
diSPIM = dual-view SPIM on inverted microscope

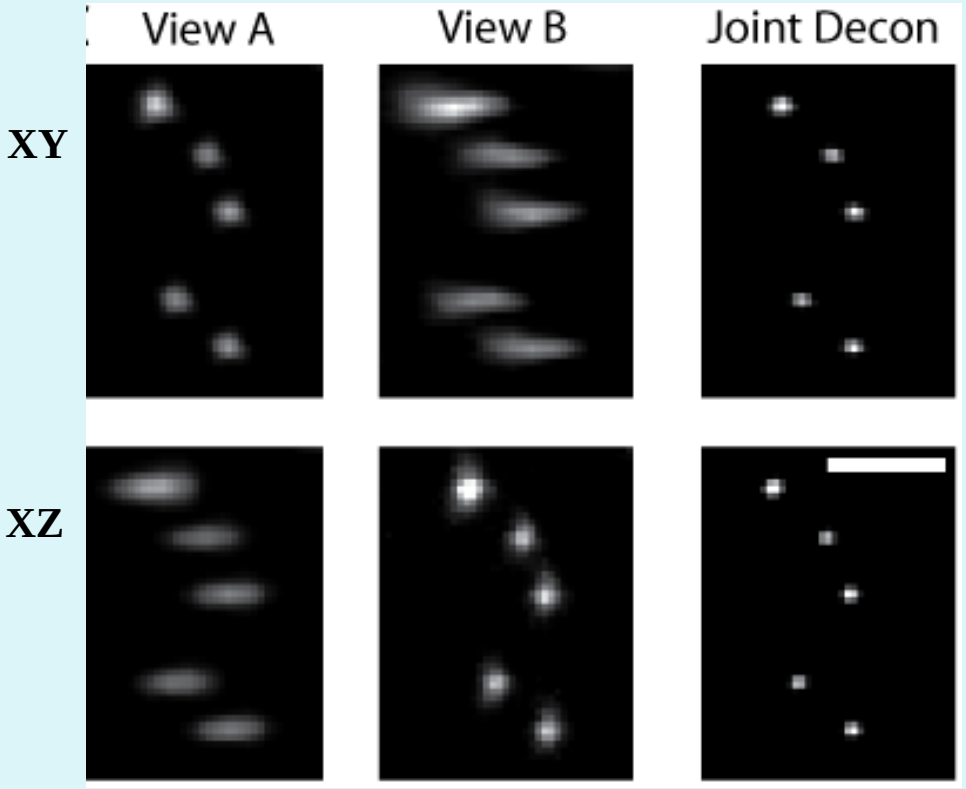




- Light sheet on inverted microscope
- Two (fixed) views → isotropic resolution
- Open-dish sample mounting
- Stacks by moving objective/light sheet or by moving stage

Isotropic resolution by fusion

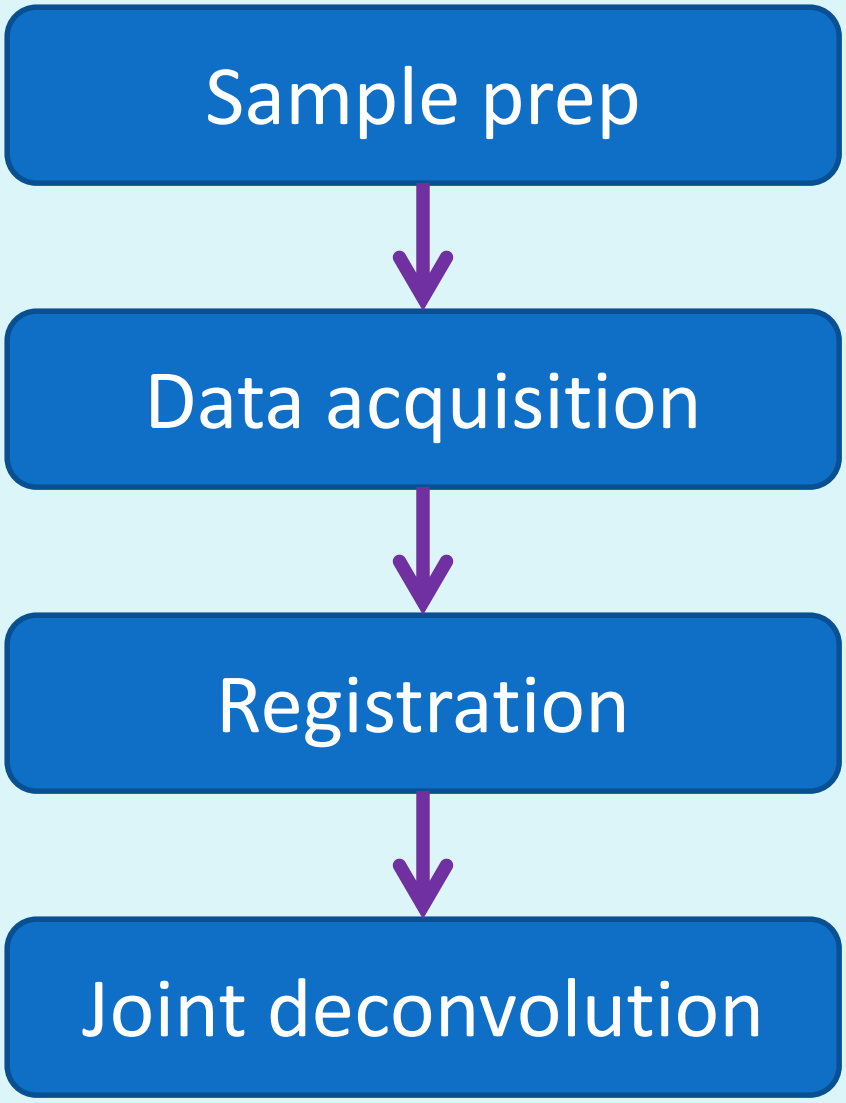




Joint Decon: A. York and Y. Wu

Wu et al. *Nat. Biotechnol.* 31, 1032-138 (2013),
Kumar et al. *Nature Protocols* 9, 2555-2573 (2014)

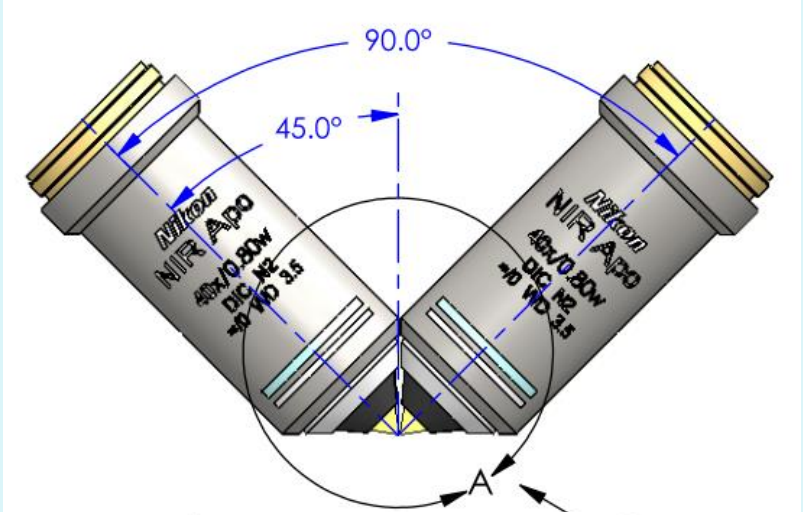
diSPIM workflow

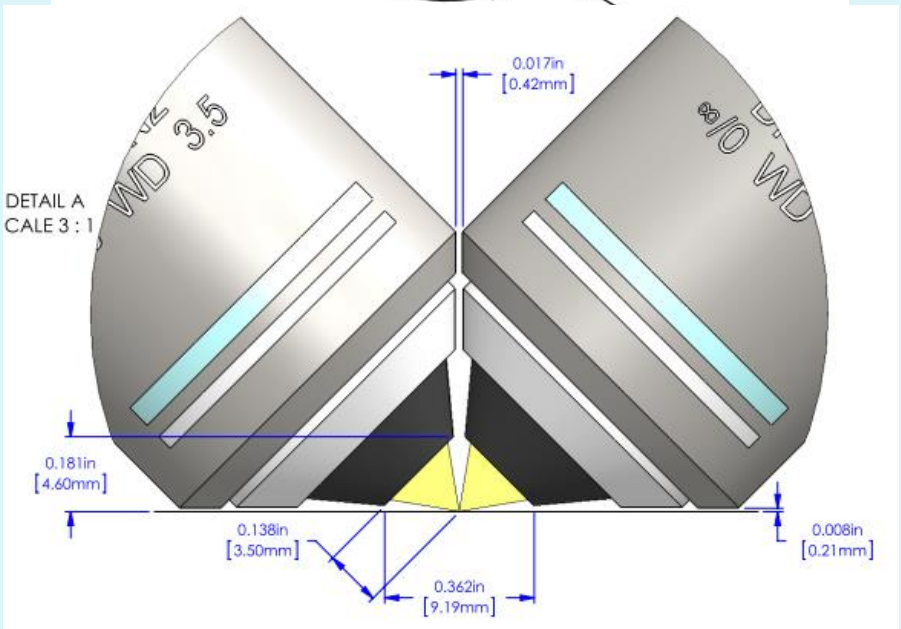


Shroff group LabView
Micro-Manager diSPIM plugin
3i's Slidebook
Others in future?

MIPAV GenerateFusion
Fiji Multiview Reconstruction
3i's Slidebook
Others in future?

diSPIM objective geometry

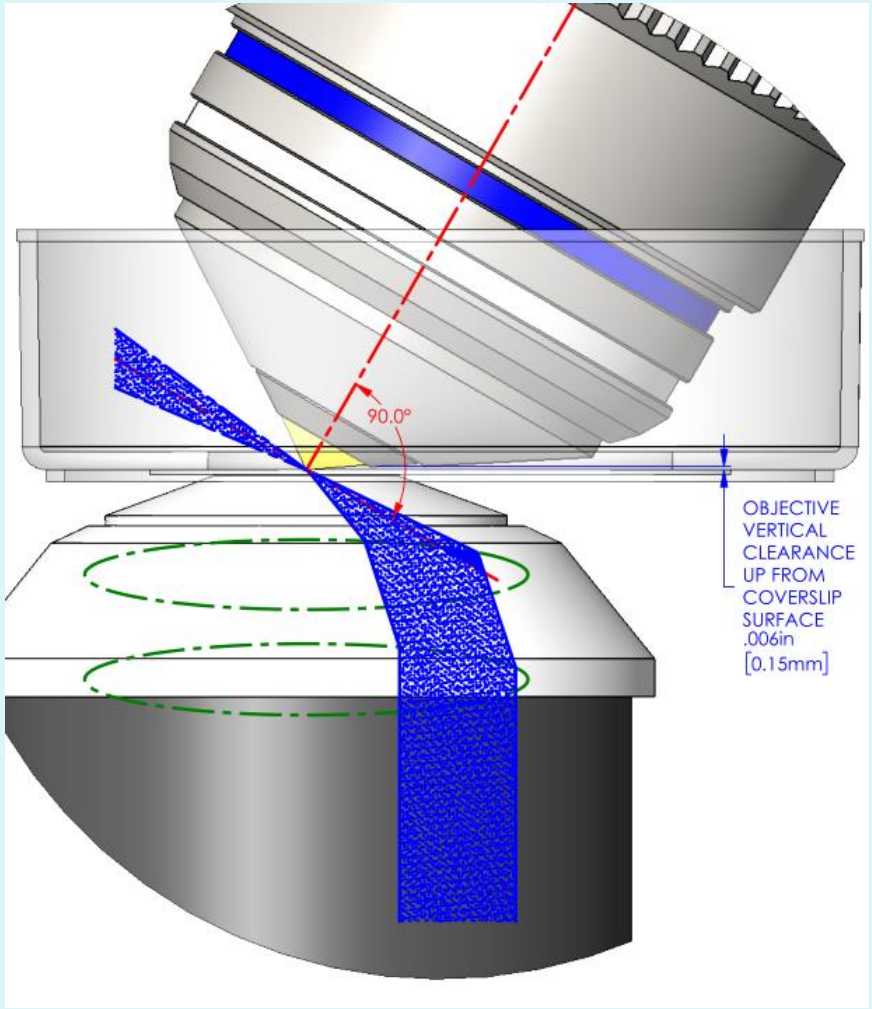




- Have to co-focus without physically bumping => limited NA
- NA 0.8 (Nikon 40x) is close to maximum possible NA for water objectives at 90°

oSPIM objective geometry

- Create light sheet sideways from objective by illuminating off-center in BFP (partway to TIRF)
- → >90° objective angle
- → higher NA objectives
- → better resolution



Oblique SPIM resolution

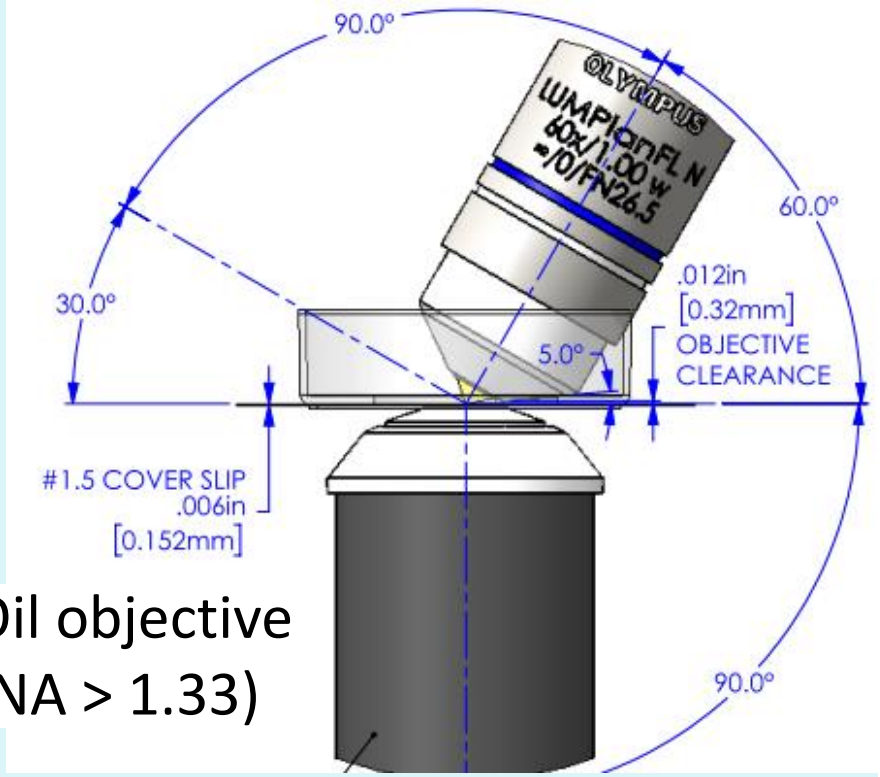
NA	Lateral @ GFP [nm]	Axial @ GFP [nm]
0.4	778	3889
0.6	519	1728
0.8	389	972
1	311	622
1.2	259	432

iSPIM/diSPIM, isotropic “lateral” resolution with post-processing

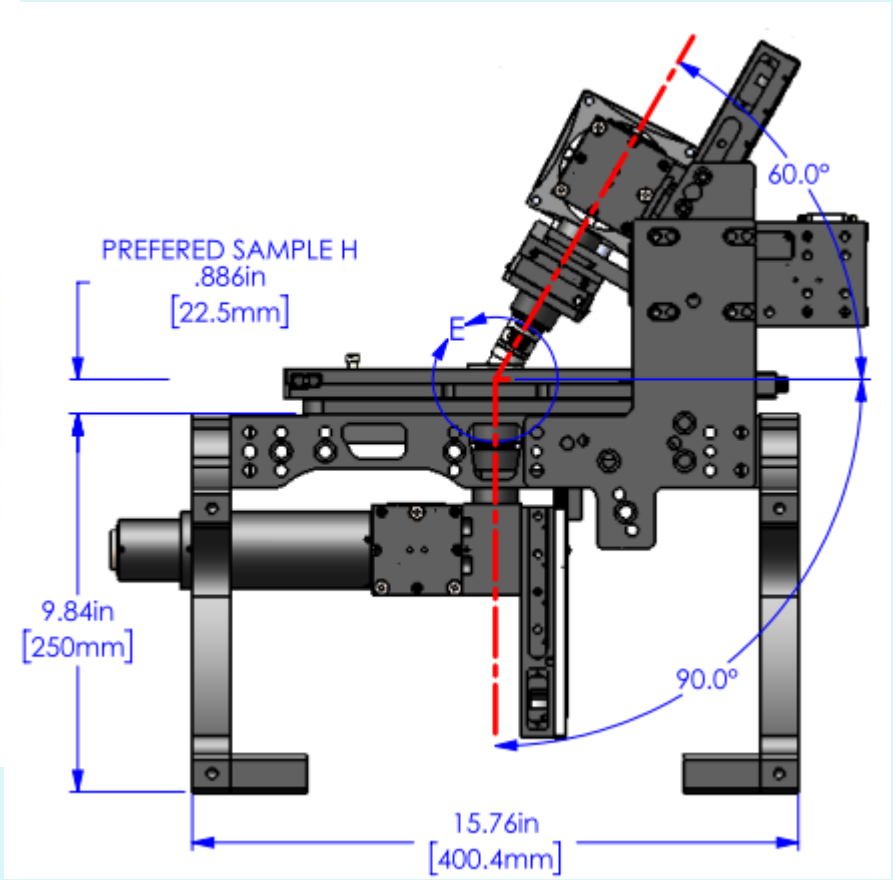
oSPIM @ NA 1.0 vs. (d)iSPIM: lateral resolution 20% better axial resolution 36% better vs. iSPIM, 60% worse vs. diSPIM

NB: oSPIM/doSPIM design should work up to NA 1.1, but has been used only with NA 1.0 objectives so far

oSPIM implementation (single)



Oil objective
(NA > 1.33)



Bottom objective creates tilted light sheet for imaging with top objective

doSPIM implementation (dual)

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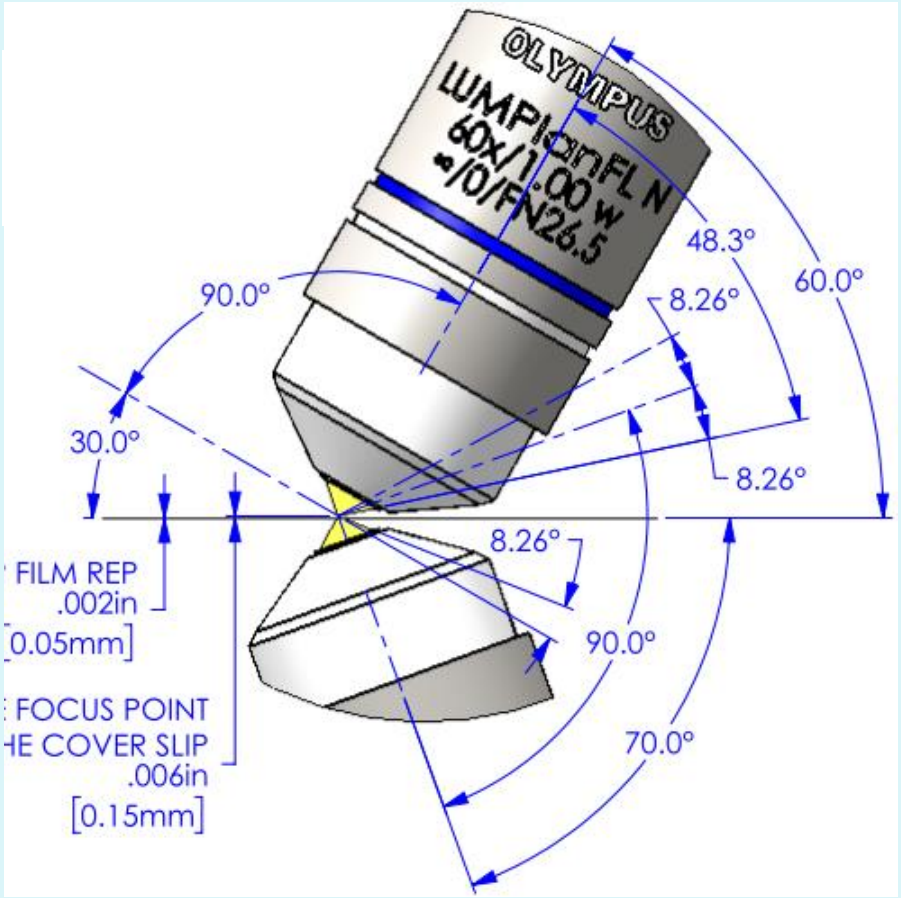

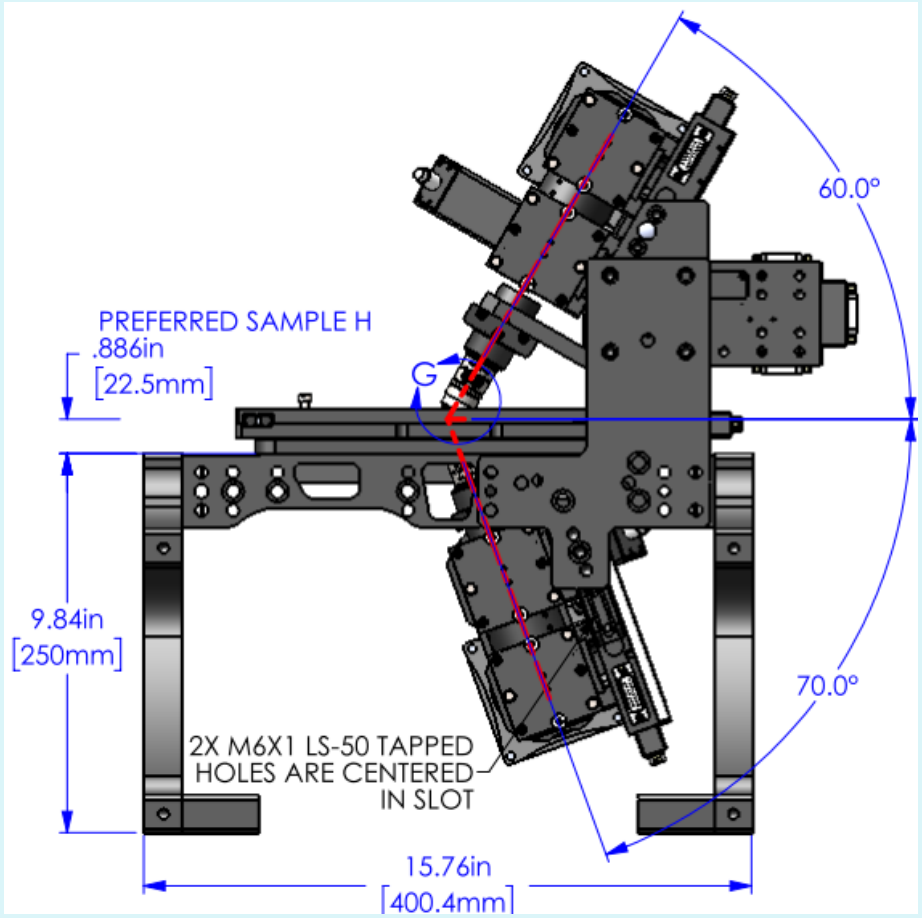

Dual-view system, objectives sequentially generate light sheet and image like diSPIM

Comparison of oSPIM vs. doSPIM

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oSPIM

- Single-view
  - Anisotropic resolution; no need for registration/fusion
- Glass coverslip bottoms OK
- Alignment easier
- One camera/scanner
- Conventional inverted microscope
- Easily combined with spinning disk, TIRF, etc.

doSPIM

- Dual-view
  - Resolution benefit after registration/fusion
- Requires FEP-bottom dishes
- Alignment more difficult
- Two cameras/scanners
- No “normal” bottom view
- Not easily combined with other techniques

oSPIM/doSPIM status

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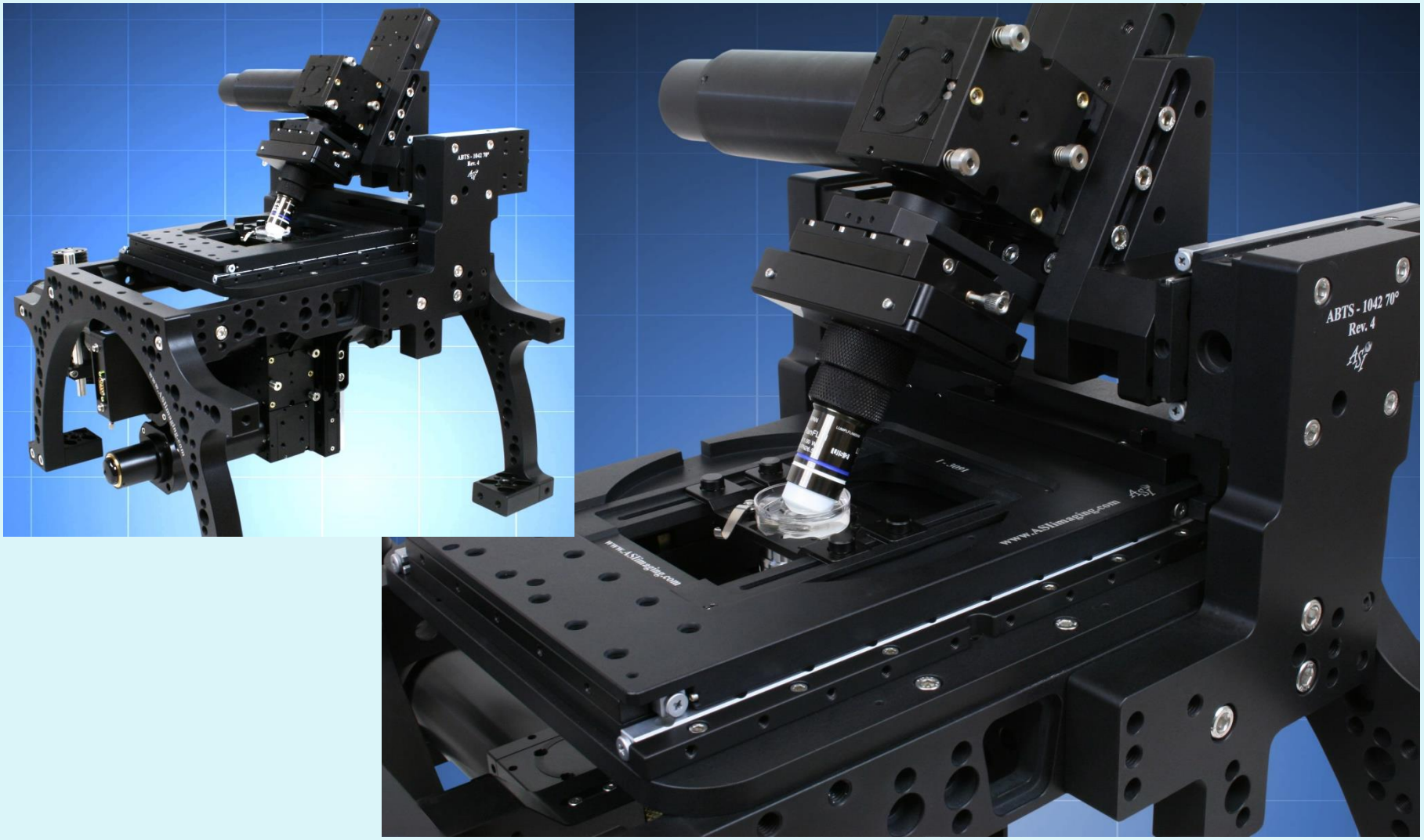
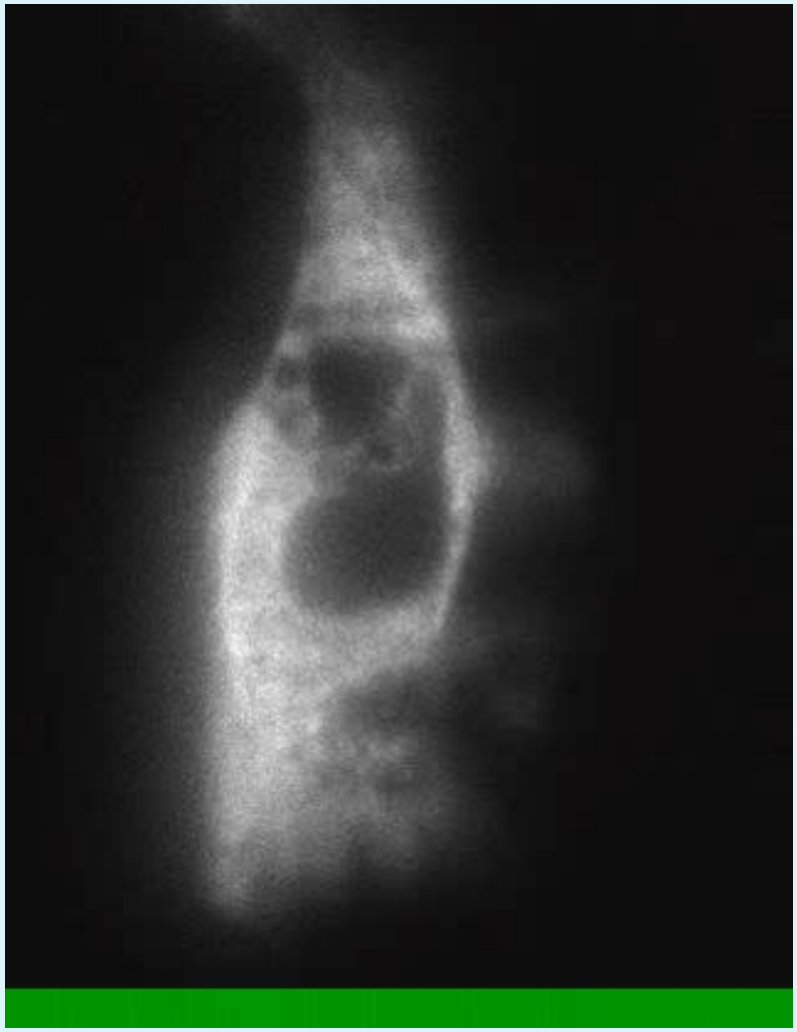
- 2015: Patent application, initial development
- 1H'16: various successful demos
- Late 1H'16: initial sales of system
- Sep 2016: publication of similar setup to oSPIM independently conceived
  - "πSPIM: high NA high resolution isotropic light-sheet imaging in cell culture dishes." Sci. Rep. 6:32880 (2016)
    - Resolution benefit oversold by having tiny FOV

oSPIM in real life



oSPIM raw data
fixed sheet – S2 cells



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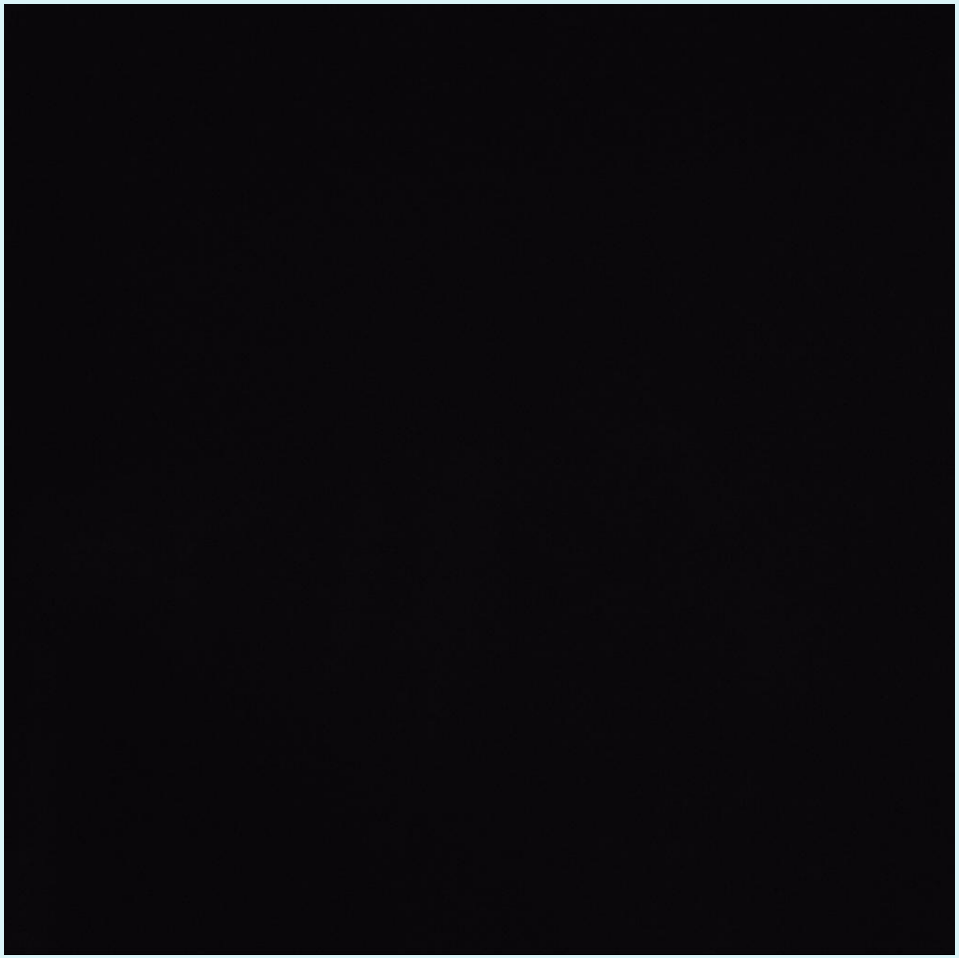
oSPIM raw data
piezo stack – S2 Cells



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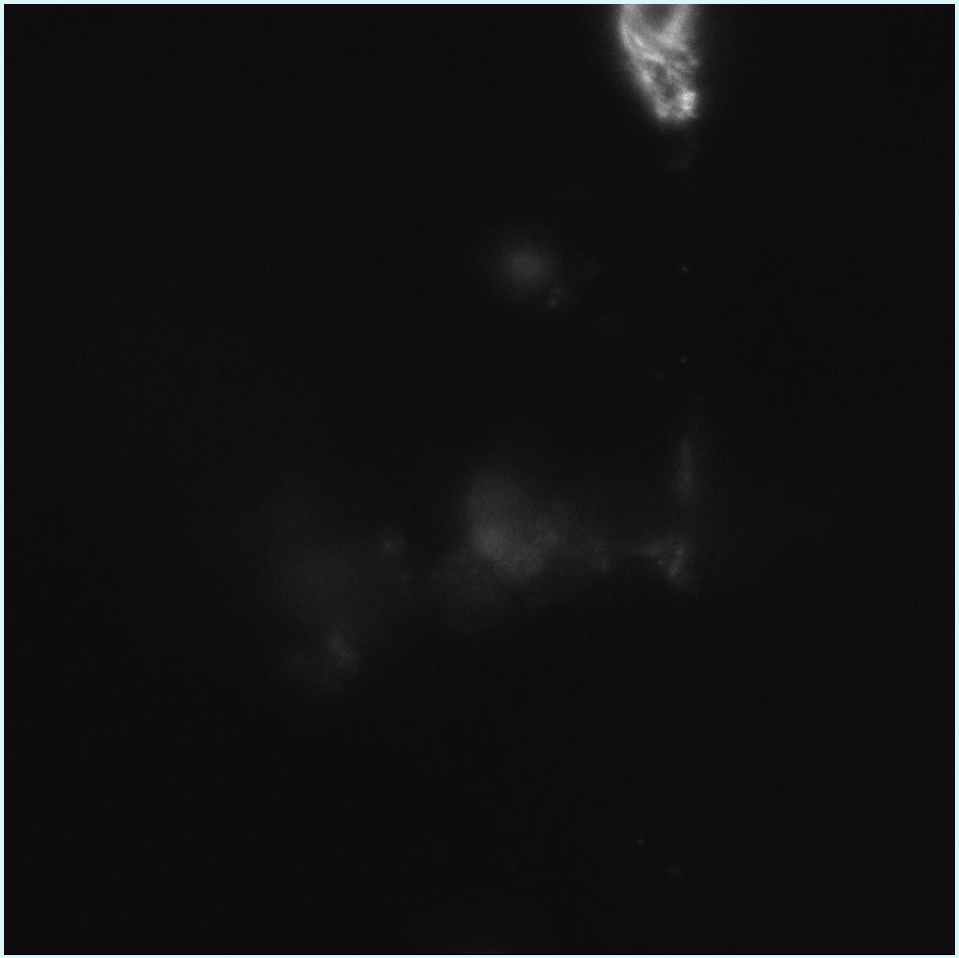
oSPIM raw data
stage scan – S2 Cells



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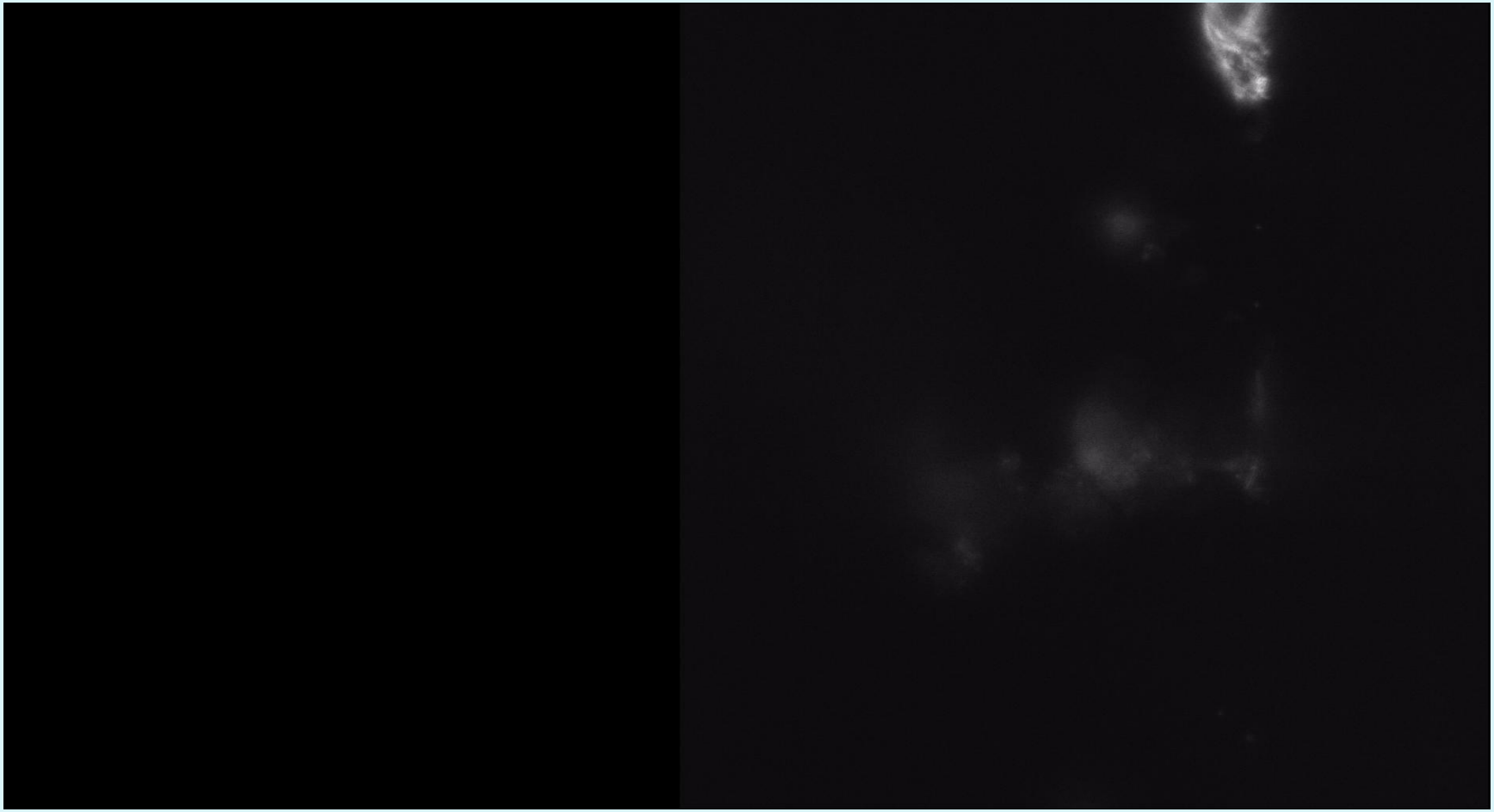
oSPIM deskewed stage scan – S2 Cells



Comparison of ASI SPIM systems

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	iSPIM	diSPIM	oSPIM	doSPIM
Cost	$$	$$$	$$	$$$
Requires registration and fusion	No	Yes	No	Yes
Lateral resolution	+++ or ++++	+++	++++	++++
Axial resolution	+ or ++	+++	++	++++
Minimal mounting	24x50 coverslip	24x50 coverslip	Glass bottom 35mm dish	FEP bottom 35mm dish
Inverted microscope	Mount on any	Mount on any	ASI RAMM included	Not possible
Sheet excitation direction	Above sample	Above sample	Below sample	Below sample

oSPIM is particularly attractive for cell biology: 35mm dishes, simple use, and easy to combine with other modalities

ASI SPIM ongoing developments

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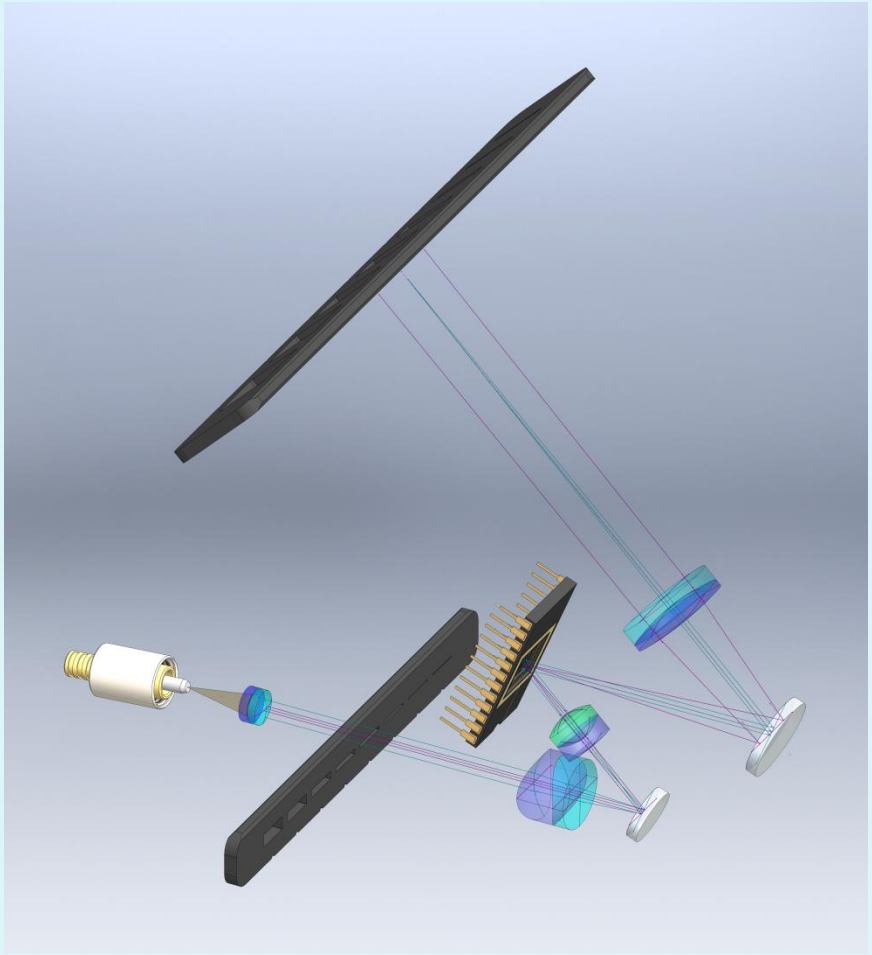
- Sheet generation using cylindrical lens
- Structured illumination for better-than-Gaussian light sheet profile
- “Virtual slit” using camera rolling shutter
- Objectives for cleared tissue samples

Cylindrical lens scanner



- Faster b/c don't need to scan to make sheet
- Separate apertures for field stop and sheet thickness vs. length
- Retains desirable features of original ASI scanner:
  - Fiber coupled
  - Same compact form factor
  - Easy control in Micro-manager or other SW

Beating Gaussian Illumination

- Create interference pattern in sample plane to get thinner sheet for same sheet length (FOV)
- Easy to implement and results in beam profile between Gaussian and dithered Bessel/Lattice
- Similar to concept in “Multicolor 4D Fluorescence Microscopy using Ultrathin Bessel Light Sheets.” Sci. Rep. 6:26159 (2016)

Virtual slit confocal

- Synchronize camera rolling shutter with beam to reject out of focus and scattered light
- Allows near-simultaneous two-sided acquisition
- Already implemented for diSPIM in LabView and for other light sheet systems
  - "Using Stage- and Slit-Scanning to Improve Contrast and Optical Sectioning in Dual-View Inverted Light Sheet Microscopy." Biol. Bull. 231:26-39 (Aug 2016)

LWD multi-immersion objective for cleared tissue

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SPECIAL OPTICS
A NAVITAR COMPANY

Specification	Value	Comments
Numerical Aperture	0.4 @ RI 1.45	0.37 – 0.43 over RI range
Immersion Media RI	1.33 – 1.56	Includes all major clearing solutions
Effective Focal Length	12 mm @ RI 1.45	15.3x – 17.9x over RI range w/ 200 mm TL
Working Distance	12 mm (for all RI)	5.1 mm imaging depth with flat sample, 12 mm diameter sphere
Field of View	1.2 mm diameter	
Spherical Correction	480 – 1000 nm	Diffraction-limited for most media and λ
Chromatic Correction	480 – 720 nm	Performance depends on media, optimized for CLARITY and TDE
Correction Collar	None	Designed for immersion w/o coverslip
Form Factor	Nikon style	62.7 mm parfocal distance, M25 threads
Price	$15k	Available early 2017

Thank you for coming!

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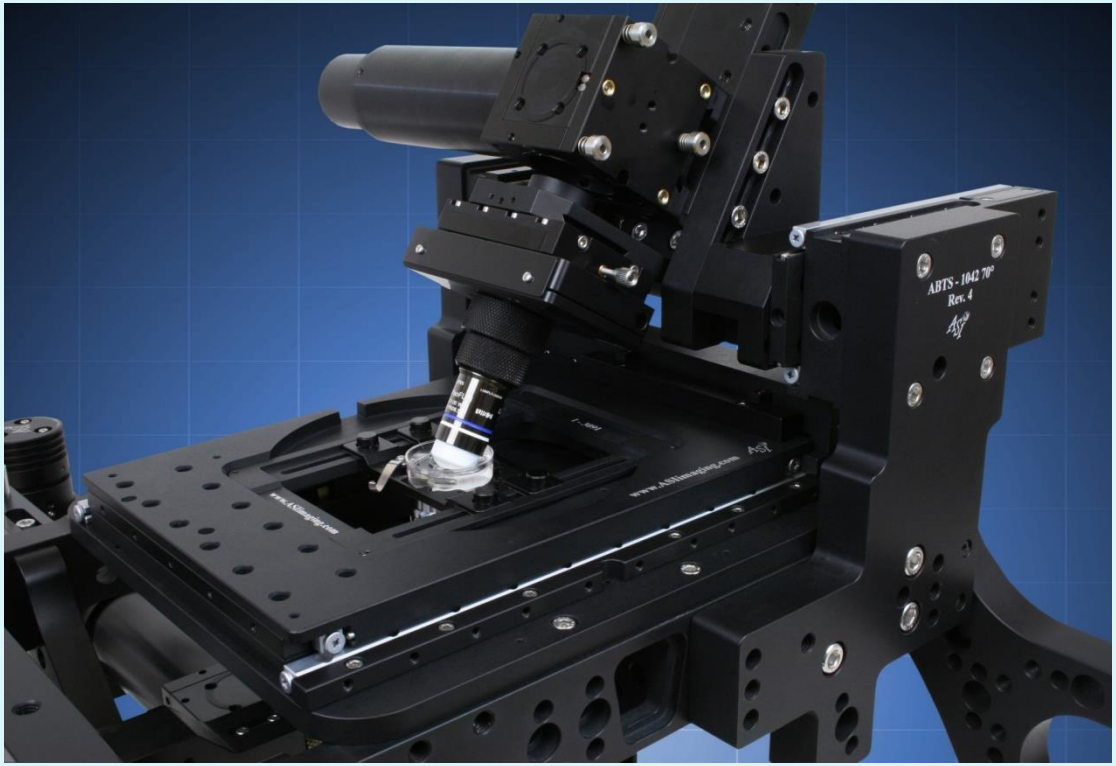

Come see a working oSPIM in Booth #829