Simplifying DIY Light Sheet Microscopes

ASI's SPIM Team

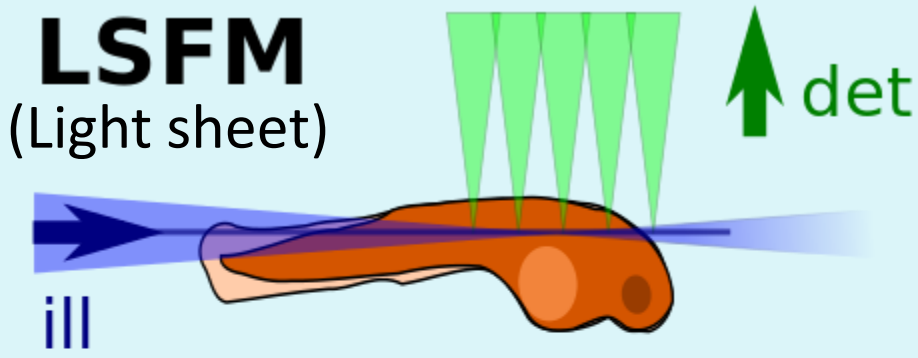
John Zemek	President
Gary Rondeau	Technical Director
Jon Daniels	SPIM Lead Engineer

EMBO I EMBL symposium "Seeing is Believing"
04 October 2017, Heidelberg Germany

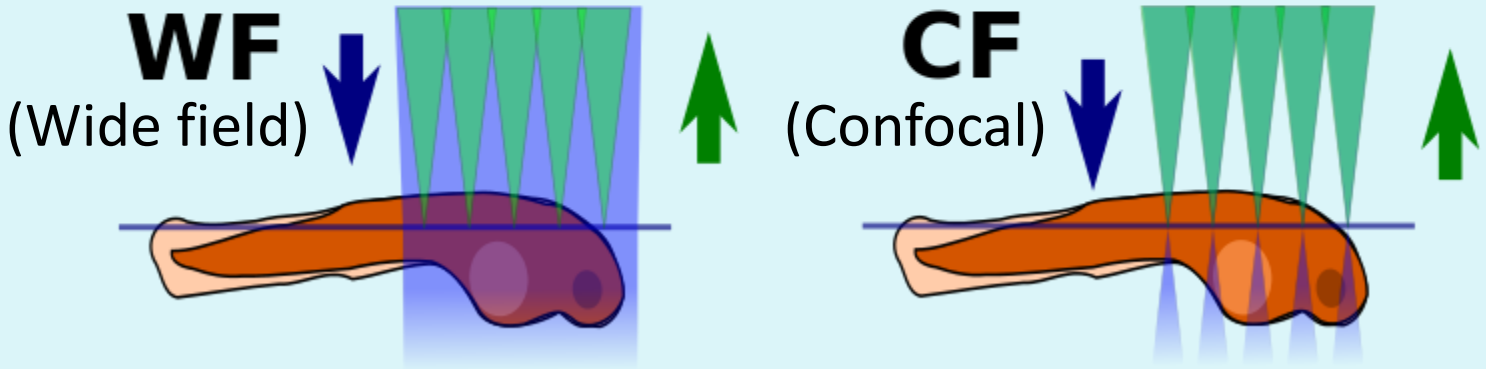
Outline

- Why light sheet microscopy?
- How can ASI help?
- Examples:
  - iSPIM/diSPIM
  - oSPIM or πSPIM
  - dSPIM for cleared tissue
  - SPIM for functional imaging in zebrafish
- Synchronization and software

What is light sheet microscopy?

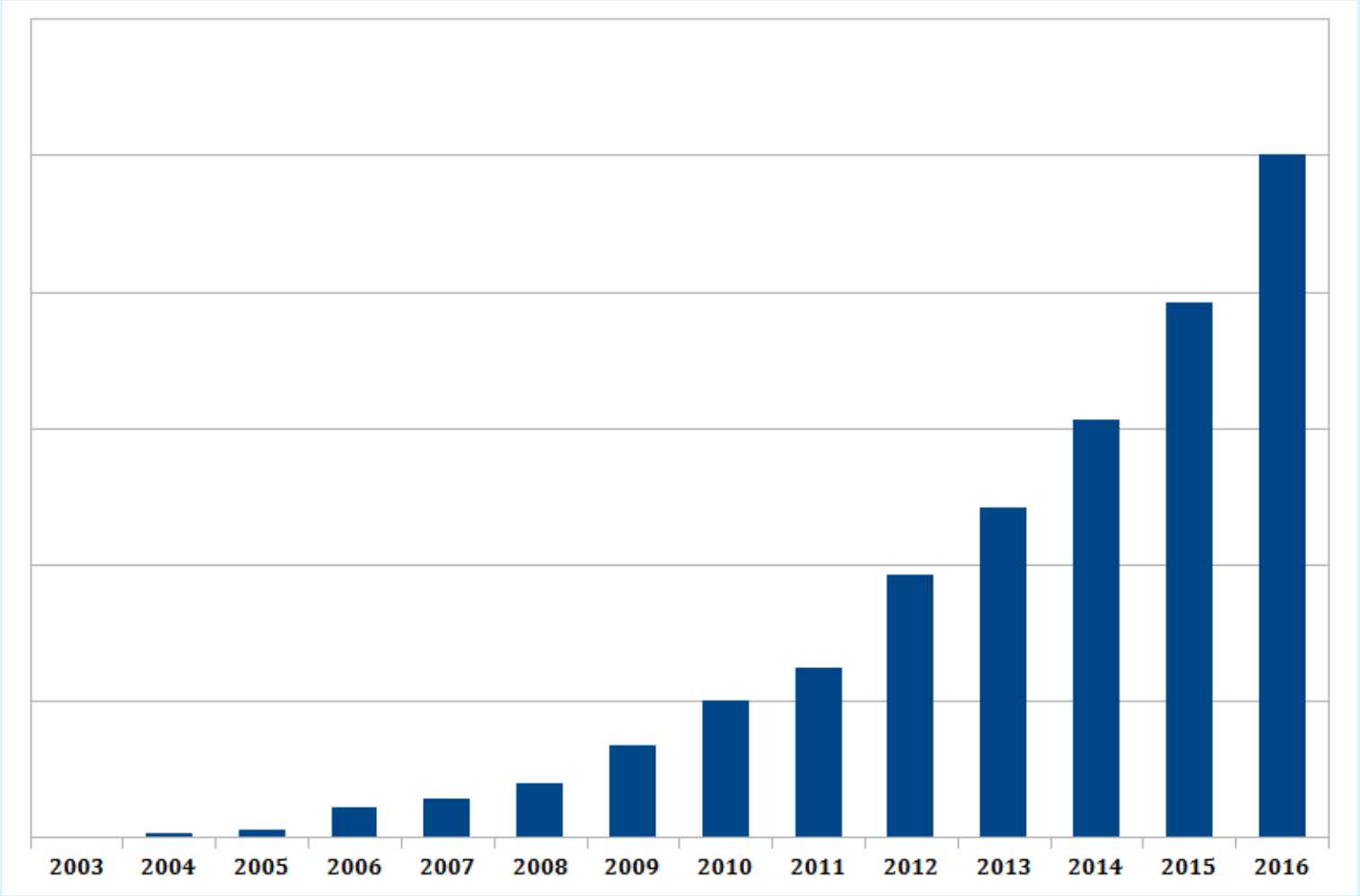


LSFM ~= SPIM = Selective Plane Illumination Microscopy



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

Publications using light sheet

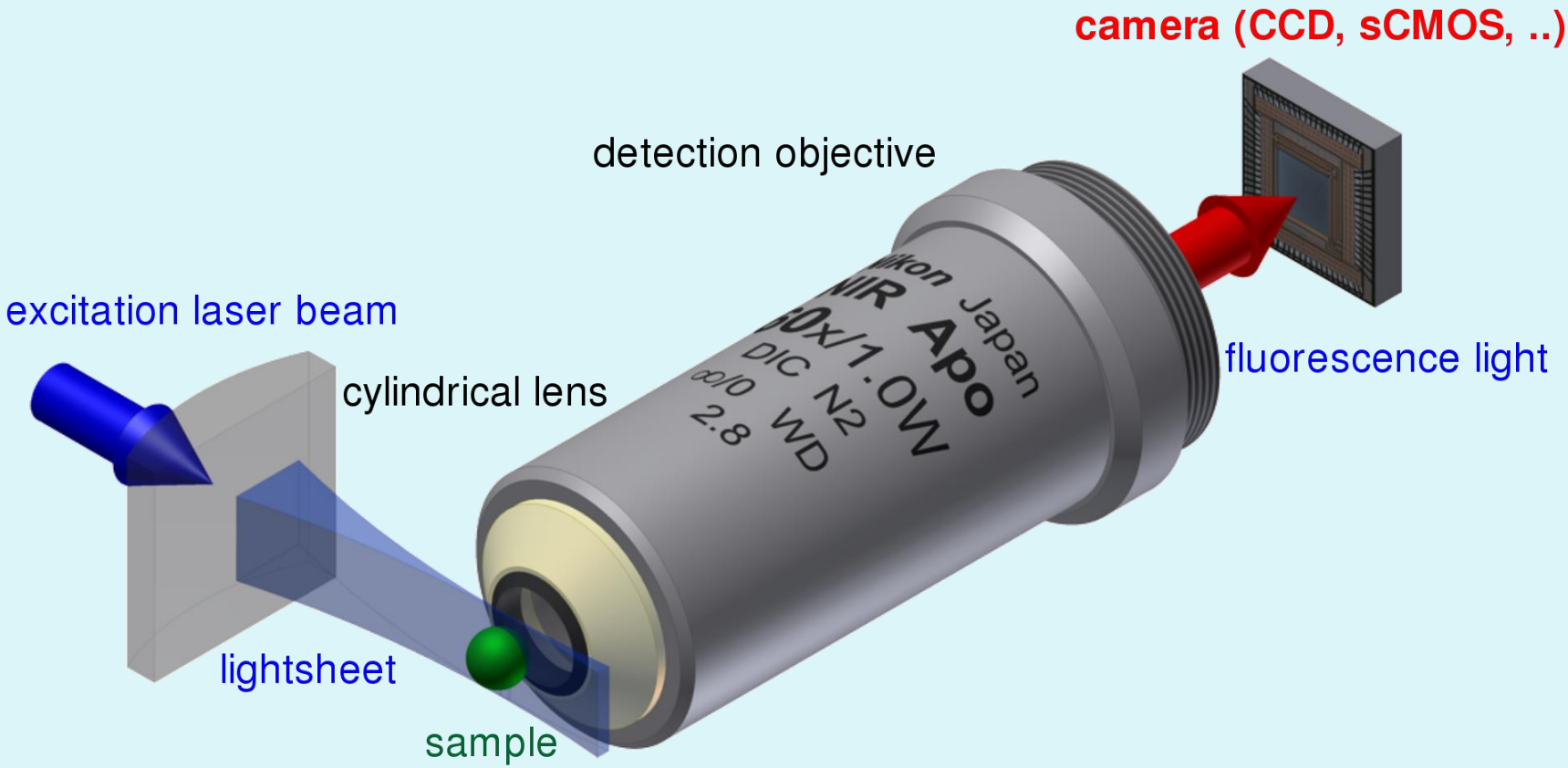


Why light sheet microscopy?

APPLIED SCIENTIFIC INSTRUMENTATION

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

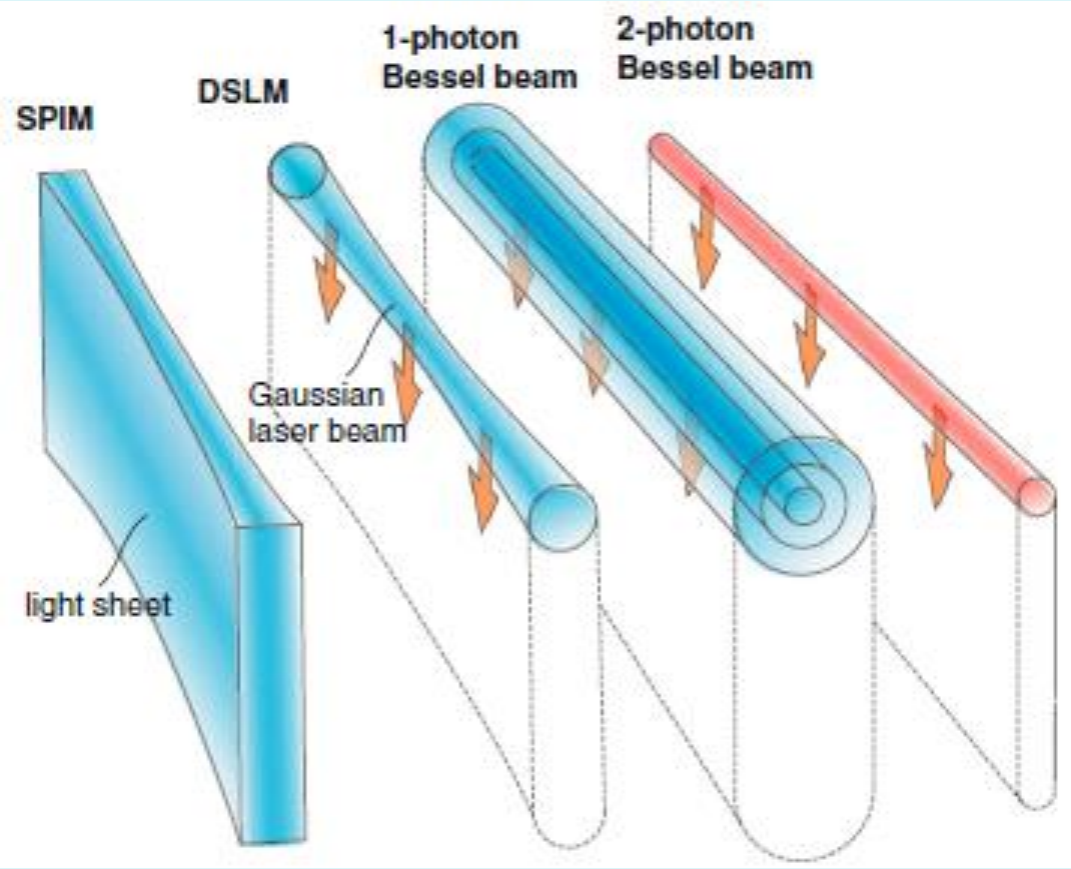
Simple light sheet microscope



By Jan Krieger, CC BY-SA 3.0,
https://commons.wikimedia.org/w/index.php?curid=22333698

Generating the sheet

- 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)

An aside: terminology

APPLIED SCIENTIFIC INSTRUMENTATION

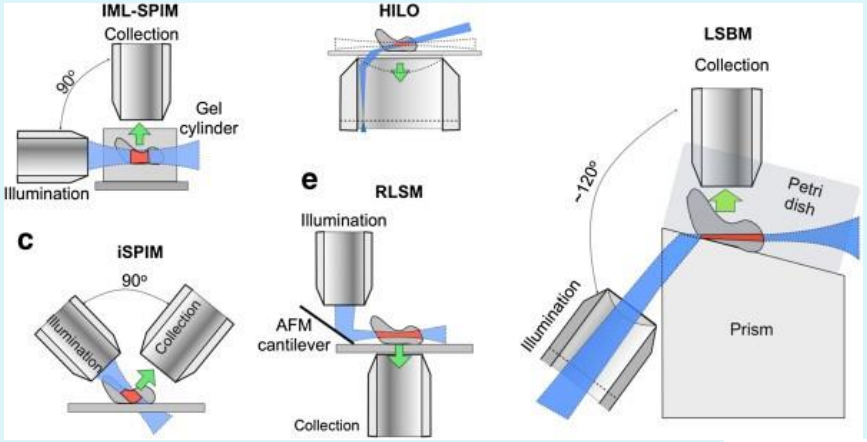
LSFM = light sheet fluorescence microscopy

SPIM = selective plane illumination microscopy

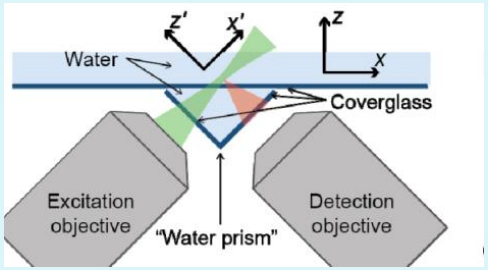
DLSM = digital light sheet microscopy

- Some reserve “SPIM” for static light sheet; we use “SPIM” = LSFM for scanned or static sheet
  - Important thing is planar illumination
  - ASI systems have option of light sheet generator for static sheet or scanned sheet so we name by the geometry instead of the light sheet type

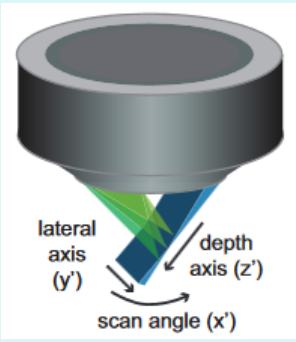
Sub-sampling of configurations



Optical Nanoscopy 2013 2:7.

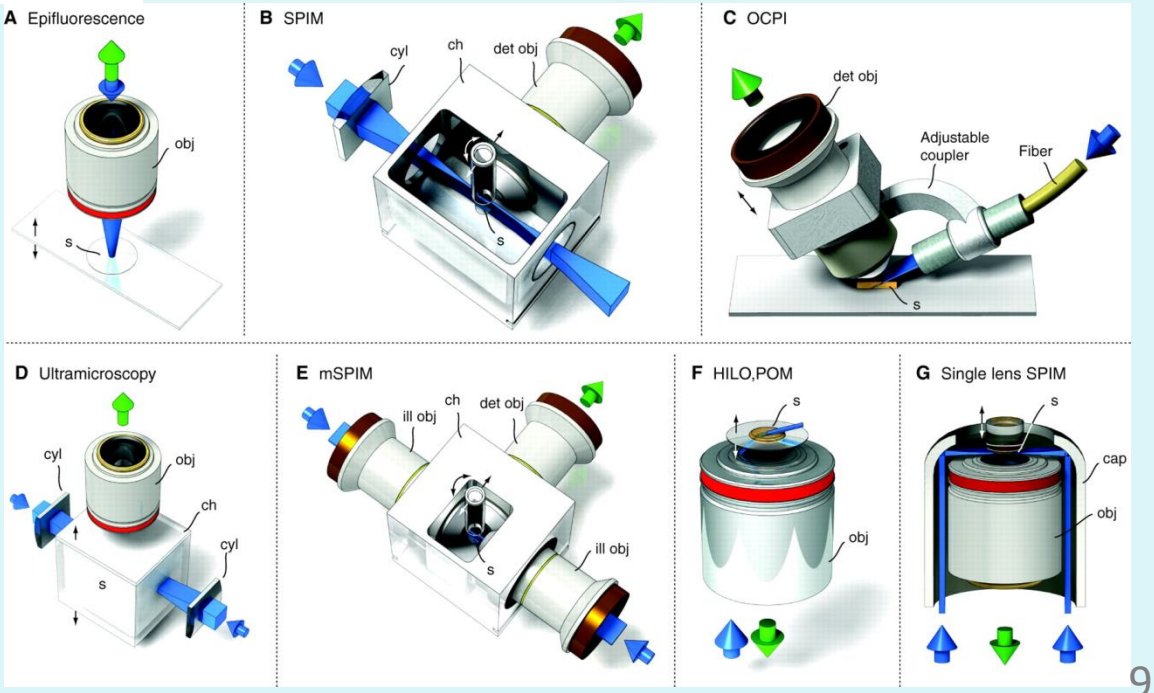


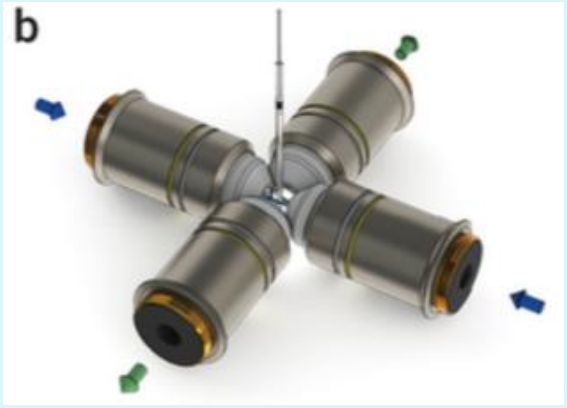
Optics Express 2015; 23: 16142-16153



Nature Phot. 2015 9:113.

Development 2009 136:1963.





Nature Meth 2015 12:30.

Why so many configurations?

APPLIED SCIENTIFIC INSTRUMENTATION

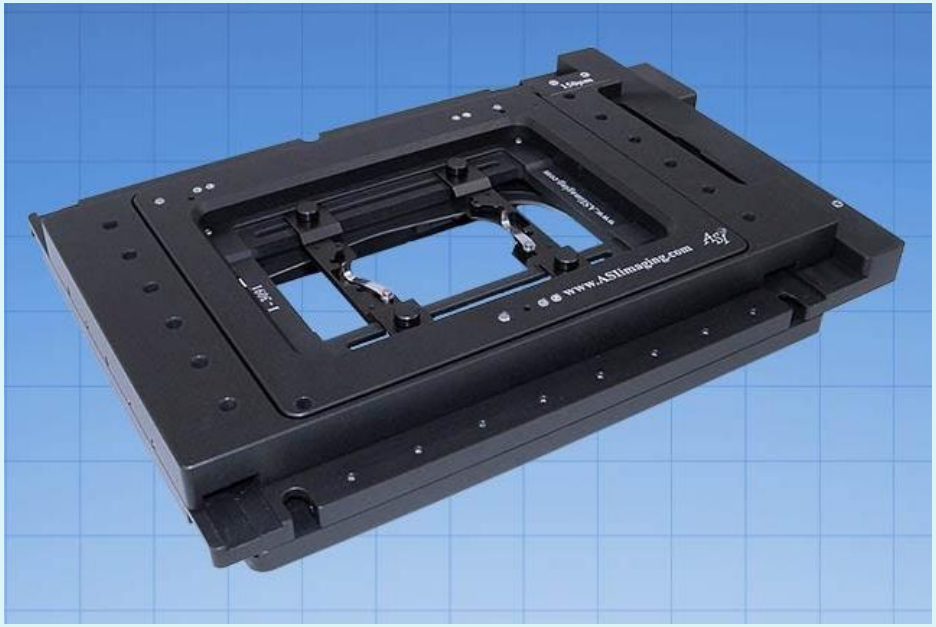
- Different samples ⇔ different microscopes
  - (Everybody wants their own paper)
  - Steric constraints
  - Mounting requirements
  - Imaging requirements → different motion control
- Paradigm shift: single costly microscope for all samples → multiple inexpensive microscopes each customized for sample/application

How can ASI help?

- ASI's core competencies
  - Motion control
  - Modular microscopes
- How we work
  - Customer-driven
  - Collaborate with leading researchers
  - Everything happens under one roof

Motion control: ASI's heritage

- 1D and 2D motorized stages
- Piezo stages
  - Stage top-plates
  - Objective movers



Control electronics

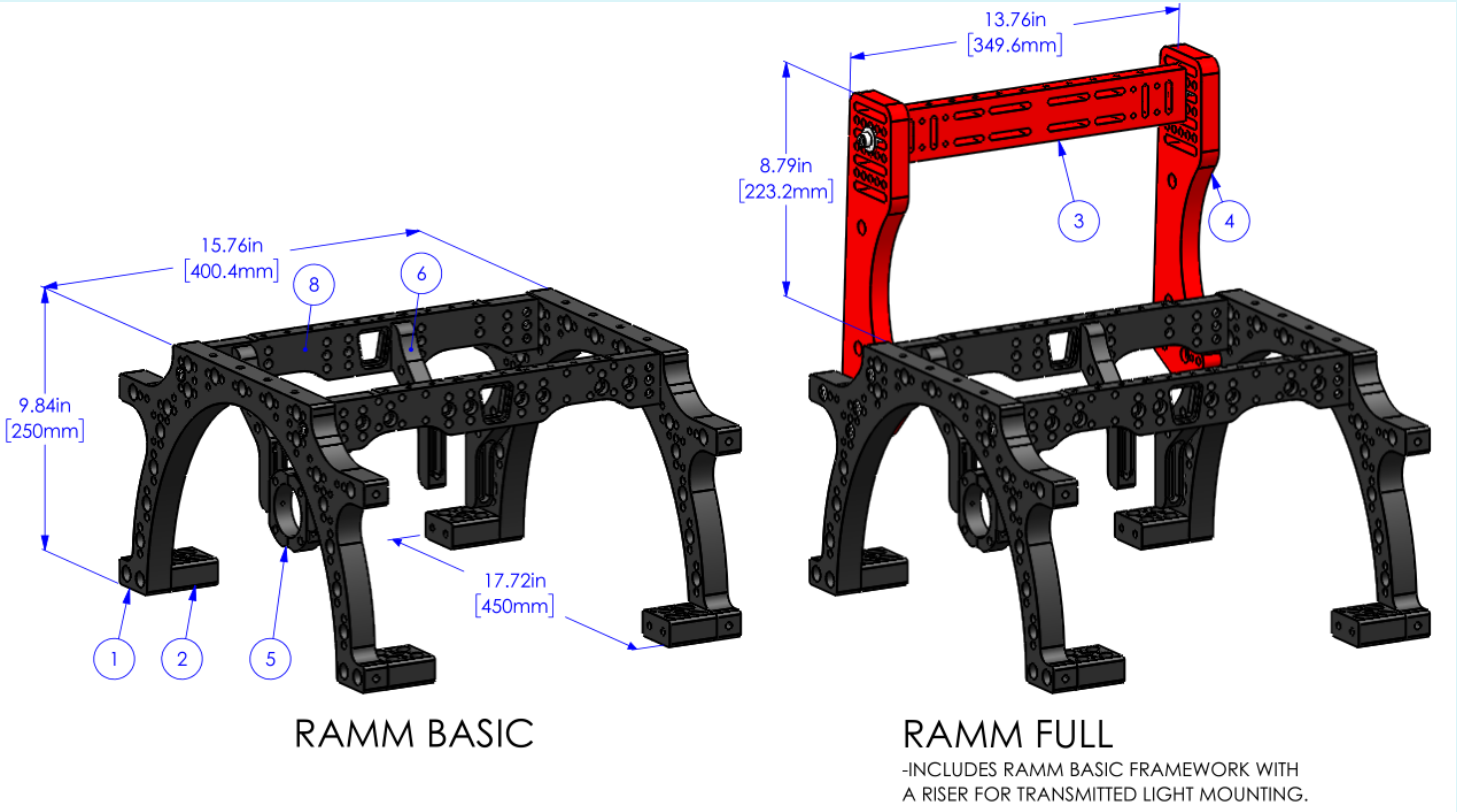
MS2000 4-axis controller best for simple microscopes up to one piezo axis.

TG-1000 Modular controller for motorized and piezo stages, filter wheels, laser scanners, PMTs, LEDs, tunable lenses, laser triggering, etc.

Modular microscopes

- Microscope as simple or complex as required
- User-accessible light path in compact form-factor without free-space optics
- Easily upgraded and modified in field
- Many modules available; more are designed every year

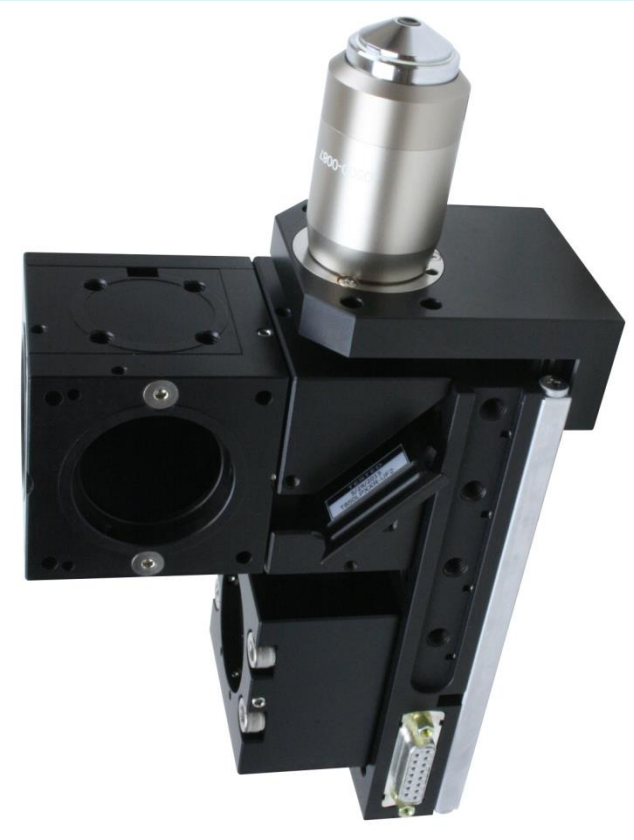
RAMM frame



- Supports the microscope assembly and the stage in a manner that minimizes drift and vibration
- Many mounting holes and support points for easy adaptation

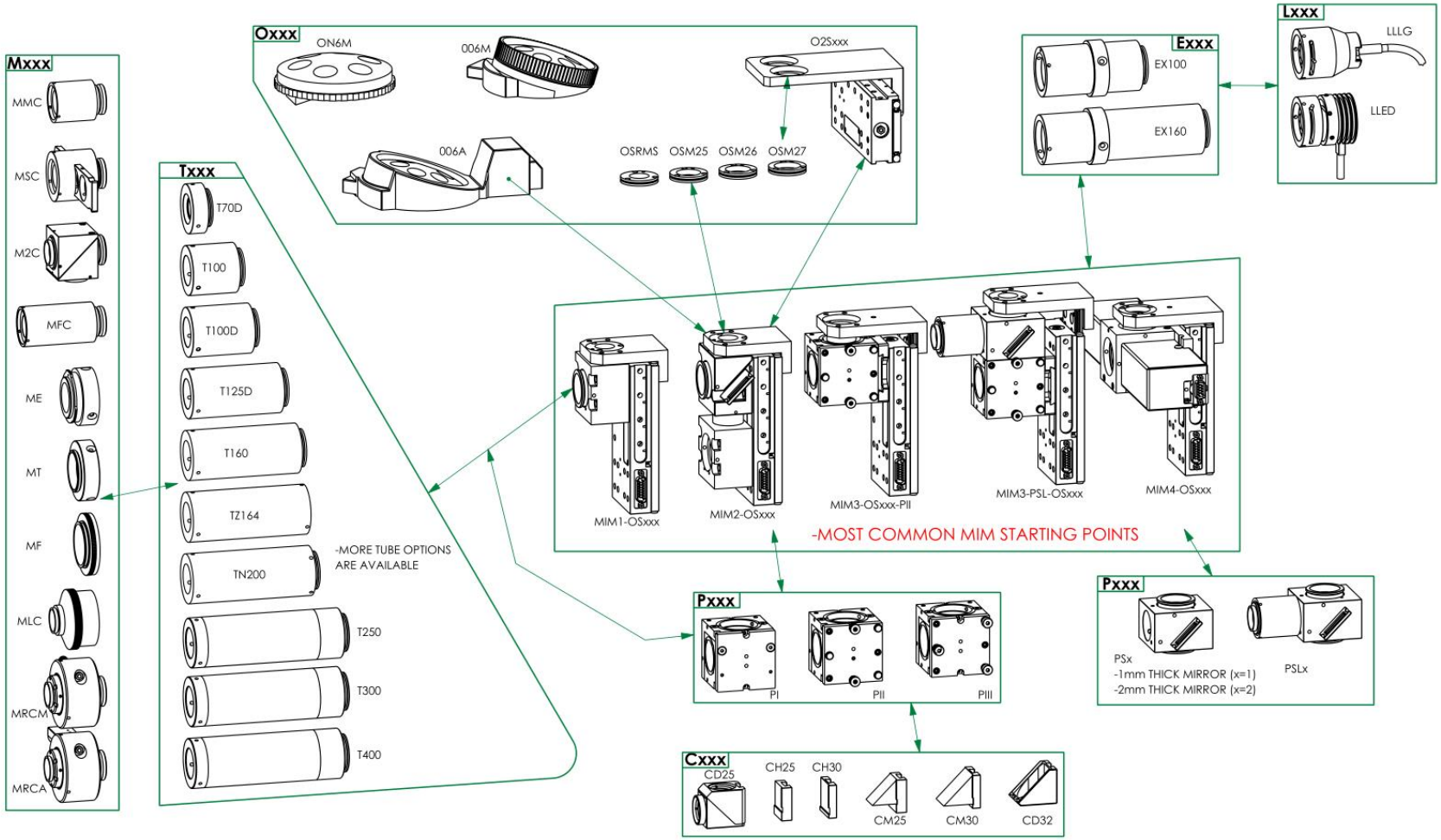
Modular infinity microscopes

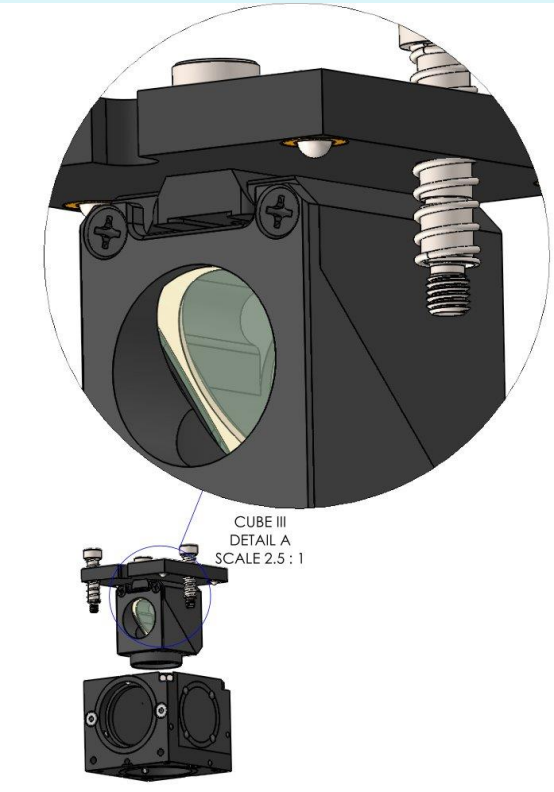
- Include LS-50 Focus Drive “backbone”
- Beam-splitter and Mirror attached to LS-50
- Wide selection of imaging and illumination optical paths can be attached to CUBEs
- A single objective or manual & automated nosepieces are supported



MIM2-OSM25-PII

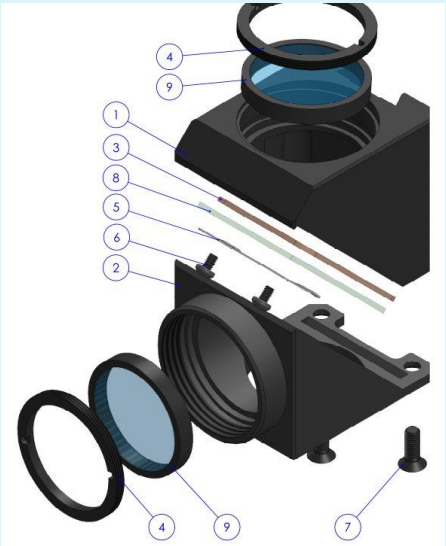
MIM system map

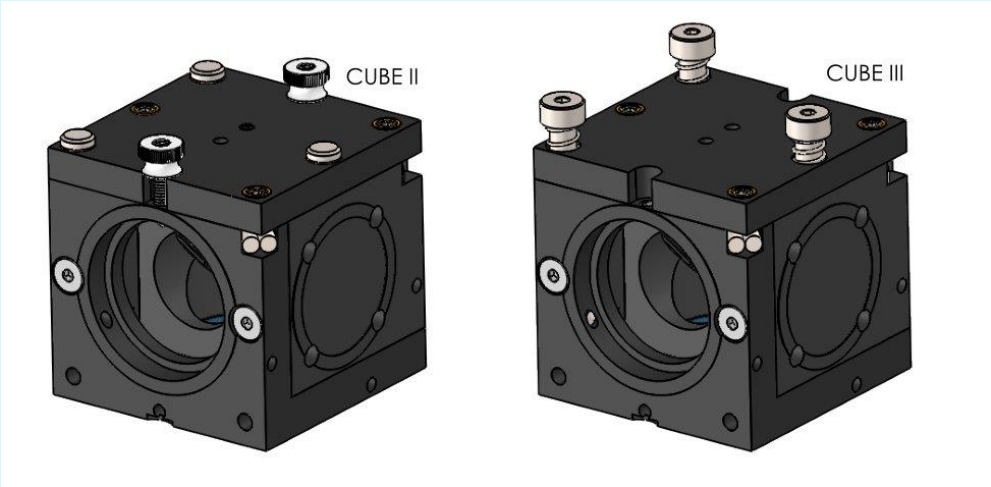




Cubes

- 60mm CUBES function to define optical combinations and paths
- Internal filter cube (C60-D_CUBE) holds standard 25mm filters and 25mm x 36mm dichroics or mirrors
- CUBE-II and CUBE-III have adjustable mirror tilt
- CUBE-II has quick-change latches



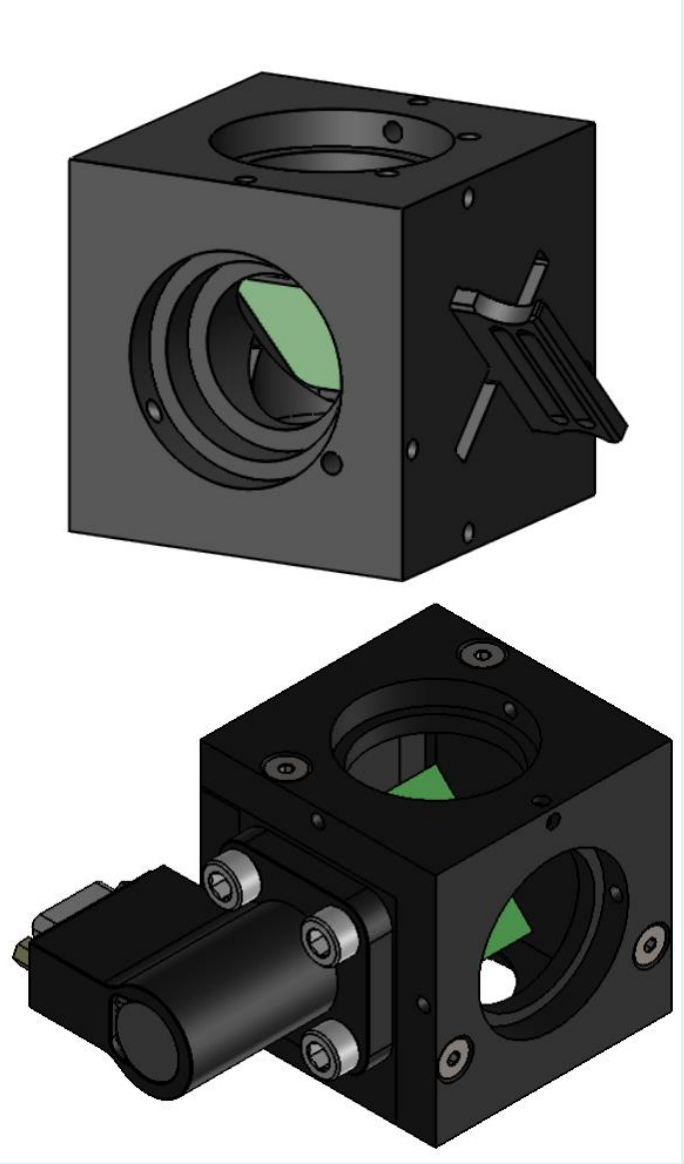


Port switches

Port switches to select illumination path or camera.

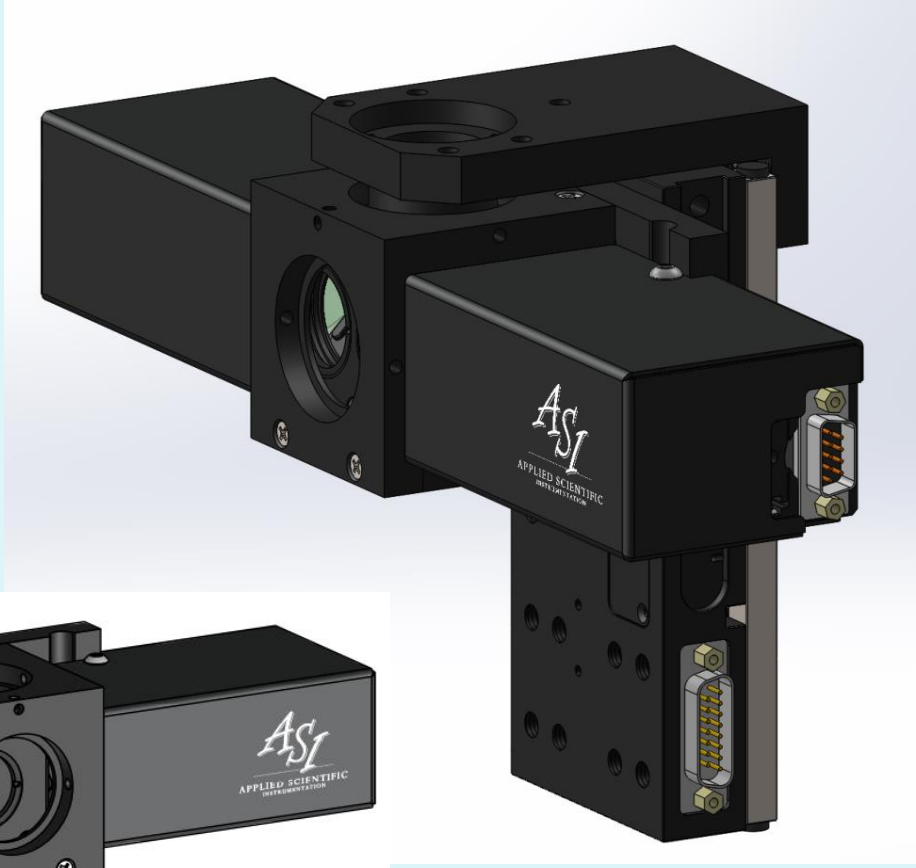
C60-3WMS Three-way Manual
Selects between two side ports or straight-through port depending upon position (or presence) of the mirror slide.

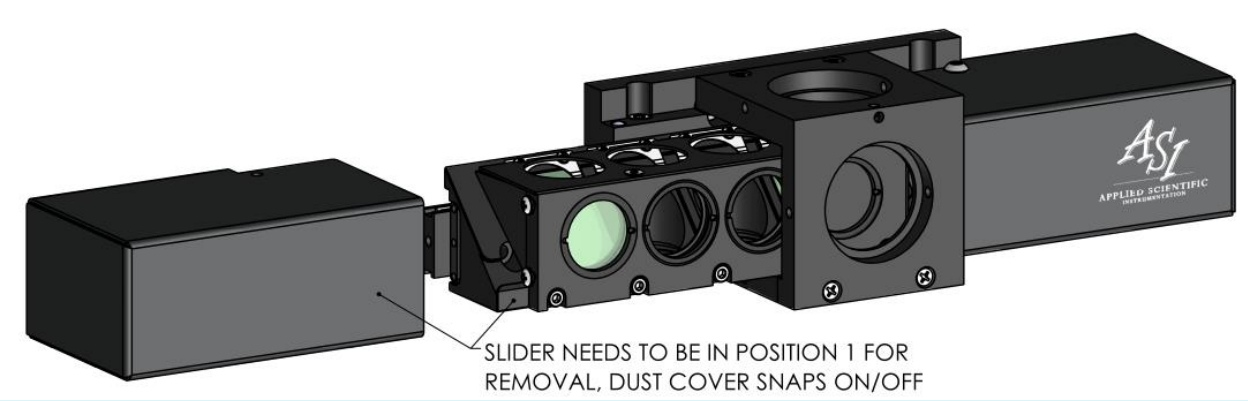
C60-PORT_SWITCH Motorized
Automated for switching the common port between the two side ports.



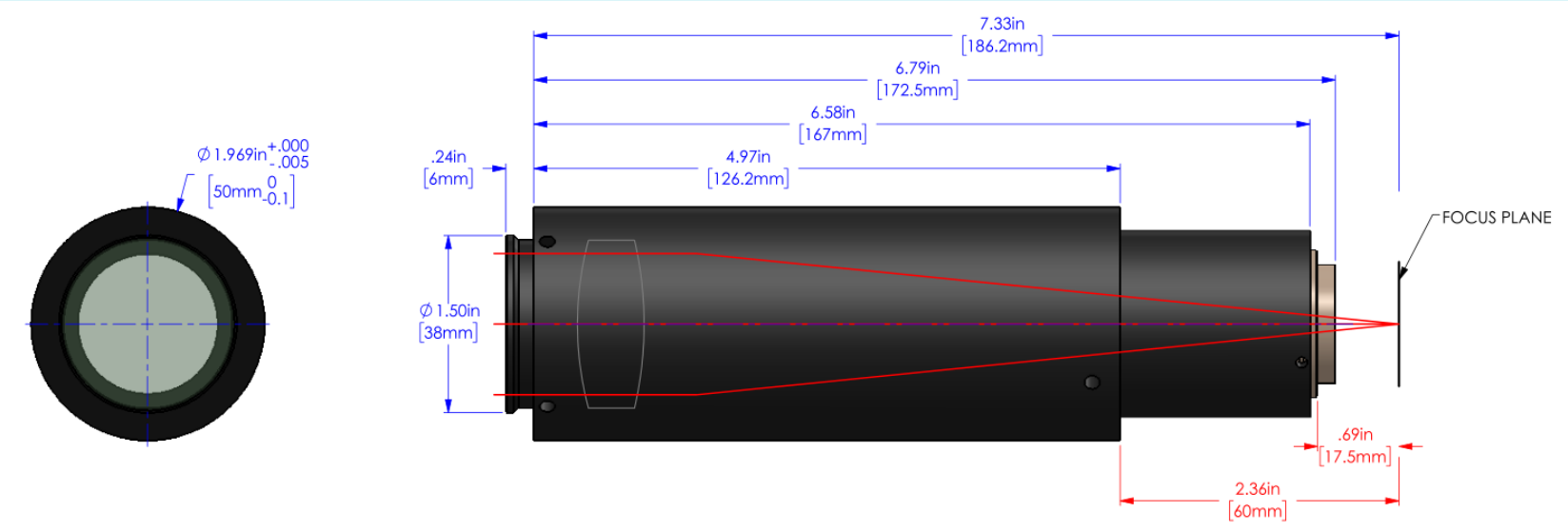
Filter slider

- Automated or Manual
- Same form-factor as standard C60-CUBEs
- Removable filter cartridge for filter loading



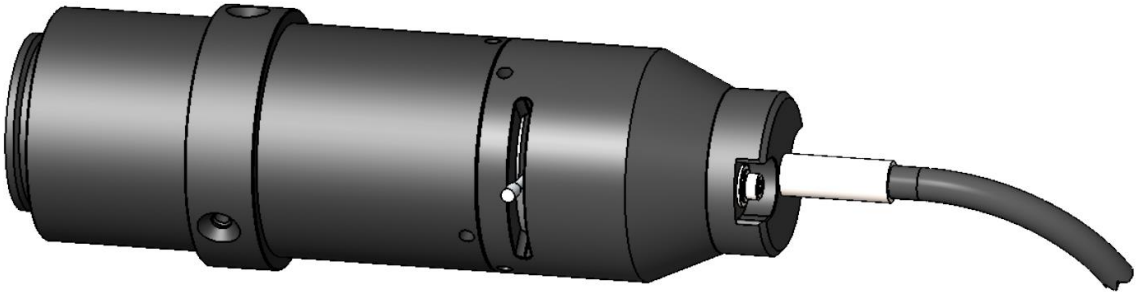


Tube lenses

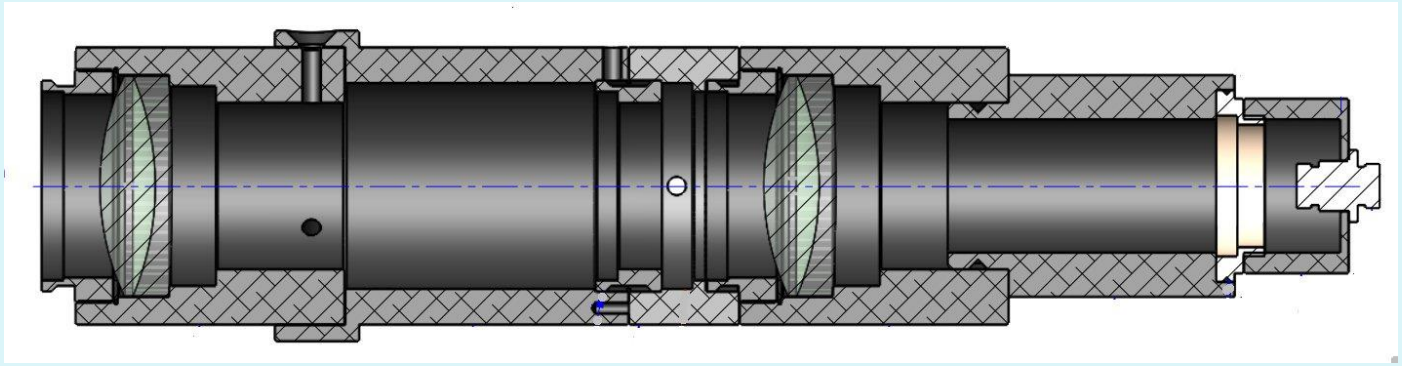


- All Tube Lenses use same format – lengths vary
- “Collimated space” fitting is our 38mm C60-RING
- Focal plane is 60mm from end of lens tube
- “Focus space” fitting is “Zeiss-like” 30mm dovetail
- Many choices from 70mm to 500mm focal length

Epi-illumination



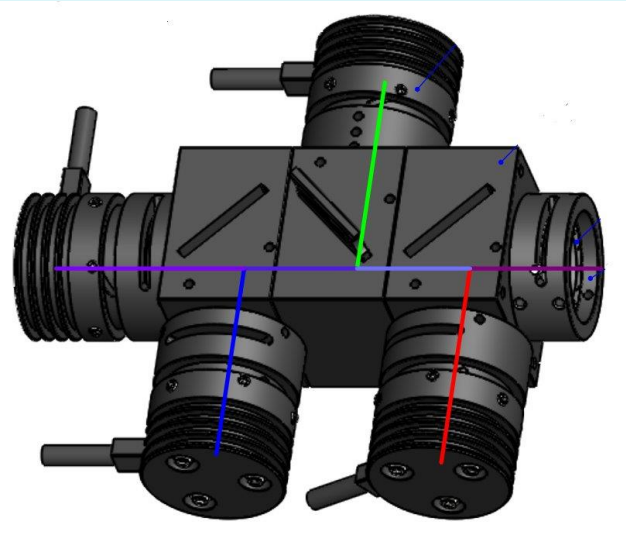
LLG-E100 Condenser and Liquid Light Guide Adapter



LFP-E100 Fiber-Coupled Laser Illuminator Assembly

Illumination assemblies made with modular lens components, easy to tailor for particular application

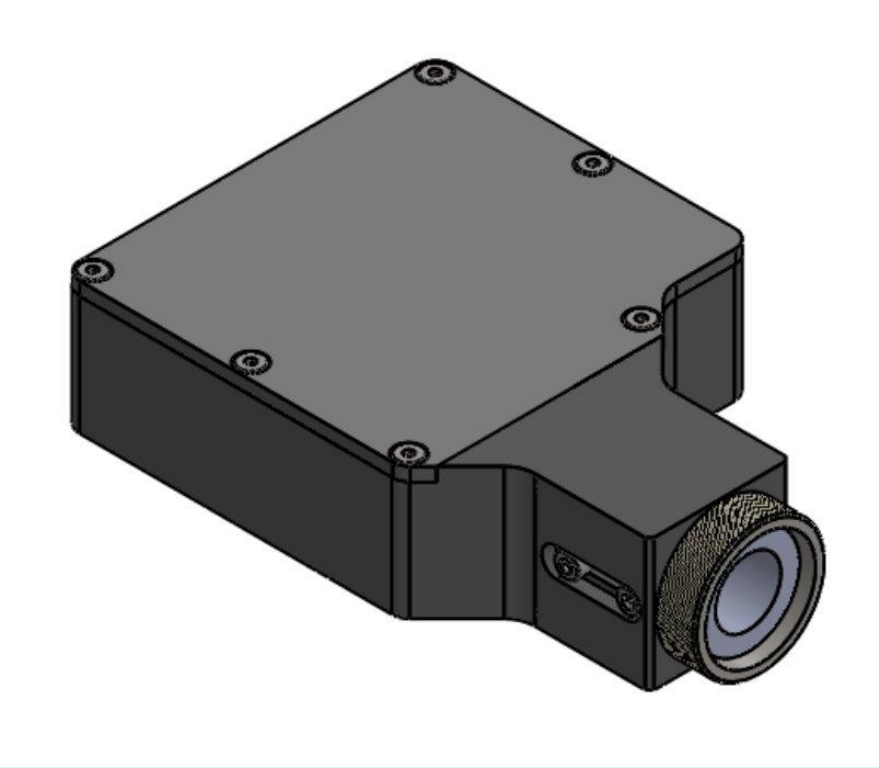
Multi-LED illuminators



Individual LEDs easily combined into an illuminator assembly controlled by single TGLED electronics card

CRISP focus stabilization

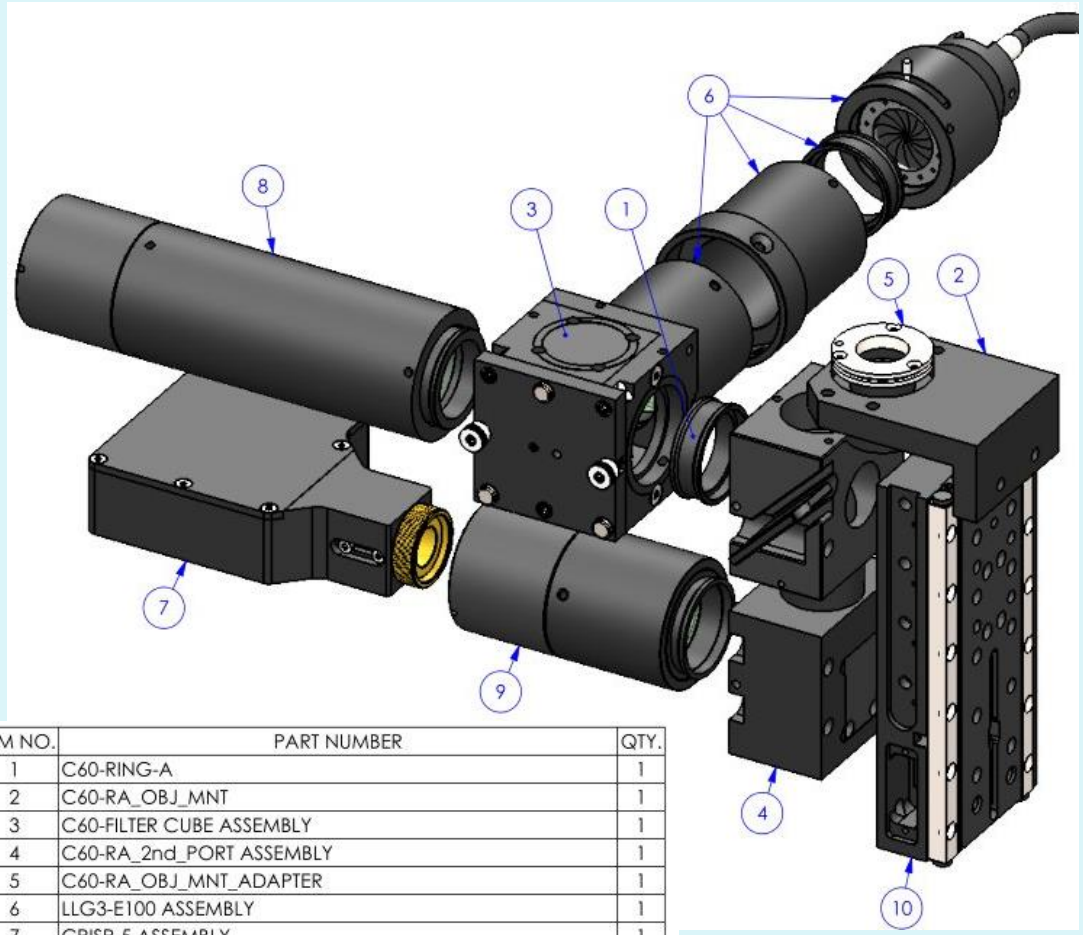
- CRISP system holds focus using a reference surface with a refractive index mismatch such as glass/air or glass/water slide interface.
- Uses IR LED projected onto sample



- Continuous hardware focus correction by integrating with with Z drive (motorized or piezo)

Example: putting it together

Exploded diagram of a MIM2 microscope system for a single objective with camera port, liquid light guide epi-illumination source, and CRISP autofocus.



ITEM NO.	PART NUMBER	QTY.
1	C60-RING-A	1
2	C60-RA_OBJ_MNT	1
3	C60-FILTER CUBE ASSEMBLY	1
4	C60-RA_2nd_PORT ASSEMBLY	1
5	C60-RA_OBJ_MNT_ADAPTER	1
6	LLG3-E100 ASSEMBLY	1
7	CRISP-5 ASSEMBLY	1
8	TN200 ASSEMBLY with C60-5060 C-MOUNT	1
9	C60-TUBE-100 ASSEMBLY with C60-5060 C-MOUNT	1
10	LS-50 LE	1

Transmitted light options

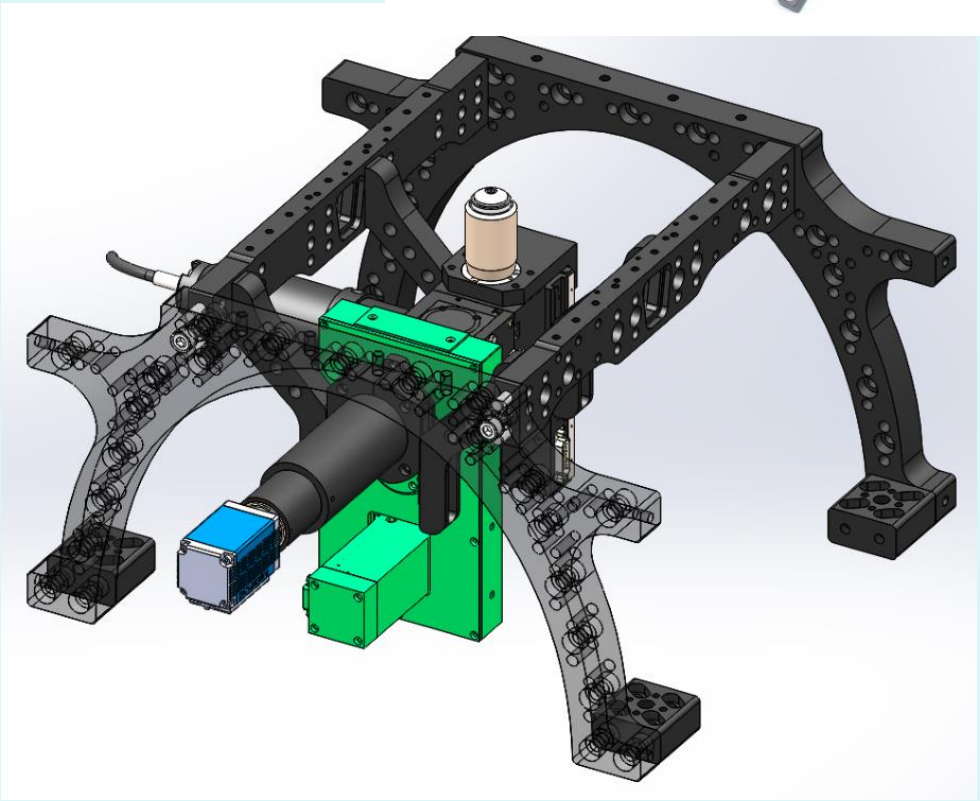
- Olympus IX2-LWUCD condenser
- ASI White LED Lamp
- ASI adjustable condenser carrier
- Olympus nose piece for DIC or Phase contrast brightfield imaging modes

TIRF on the RAMM

- TIRF fiber-coupled illuminator includes either manual or motorized micrometer for setting the injection point and TRIF angle.
- Simple cage section for focusing laser spot exactly at the objective back focal plane.

Filter wheels

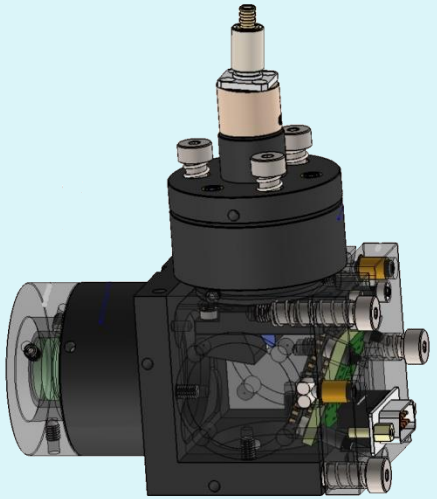
- Engineered for very low vibration
- Can be installed in the C-mount fitting or in collimated space.
- Wheels for eight 25mm filters or six 32mm filters available.
- TGFW control card handles two wheels.

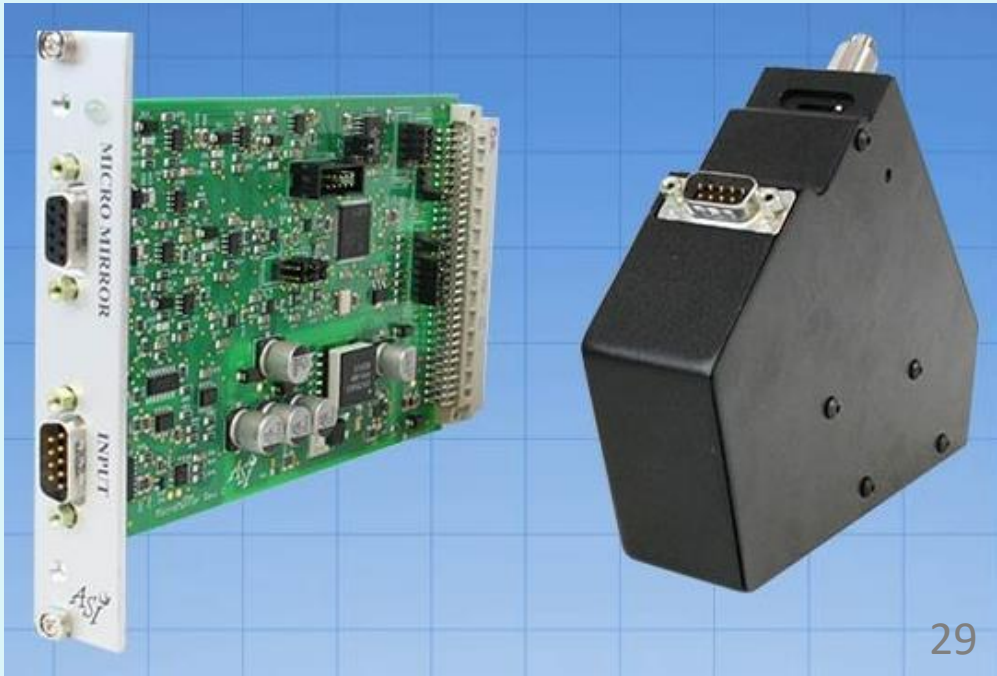


Fiber-coupled scanners

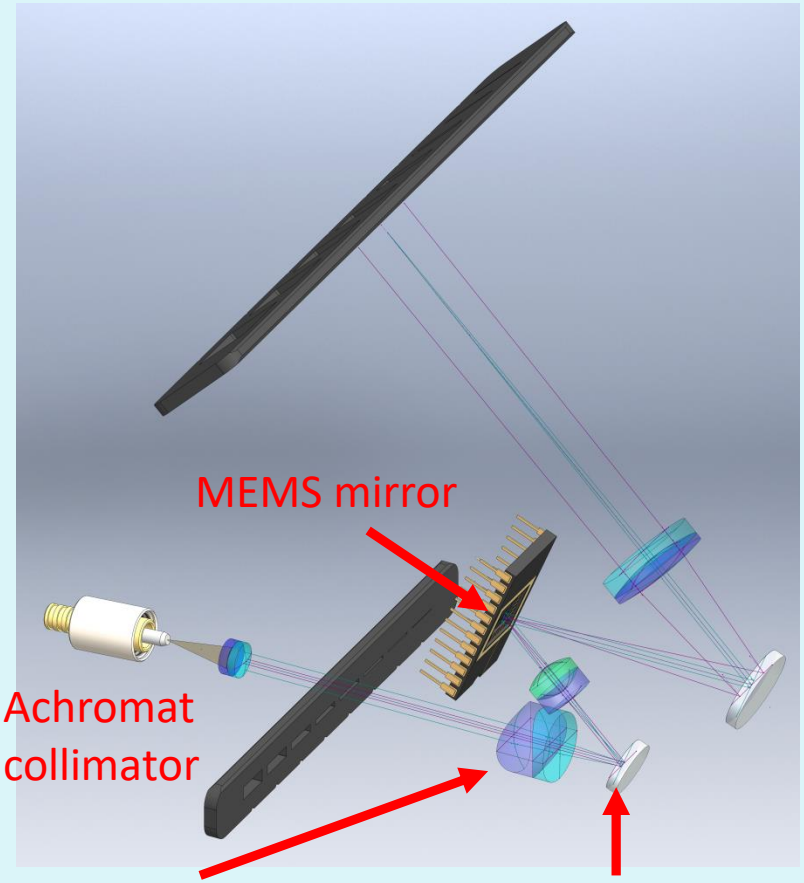
APPLIED SCIENTIFIC INSTRUMENTATION

- 2-axis scanners with MEMS mirrors are compact, light weight, and zero vibration
- Fiber in, focused scanned beam at output C-mount image plane
- Applications include:
  - Light sheet
  - FRAP
  - Photo-stimulation



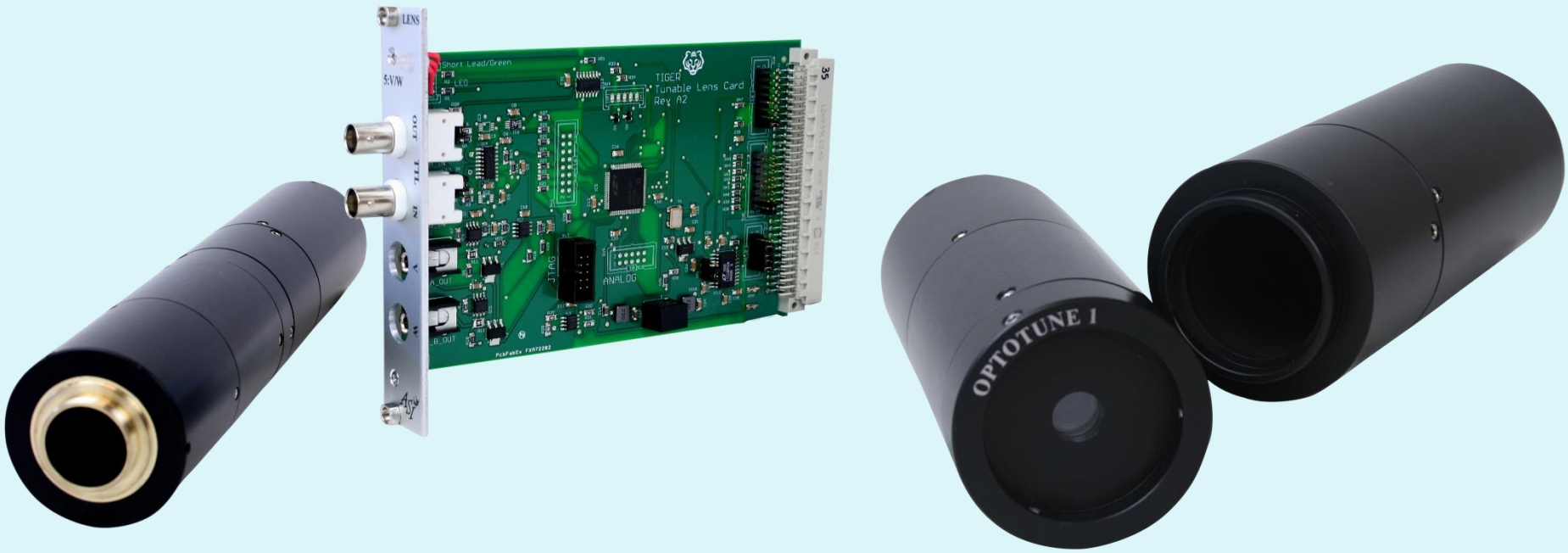


Cylindrical lens scanner



- Static sheet vs. scanned
  - Faster frame rates b/c no scanning and no need to blank laser during camera readout
  - Less expensive
  - Intensity varies across sheet
  - Can't use virtual slit mode
  - No "stop motion" effect
- Cylindrical lens and Gaussian beam only differ by single lens
  - use either one with any system

Tunable lens



- Optotune electronically tunable lens integrated into ASI system including synchronizable electronics
- For imaging path applications, have relay lens system with C-mount interfaces on both ends

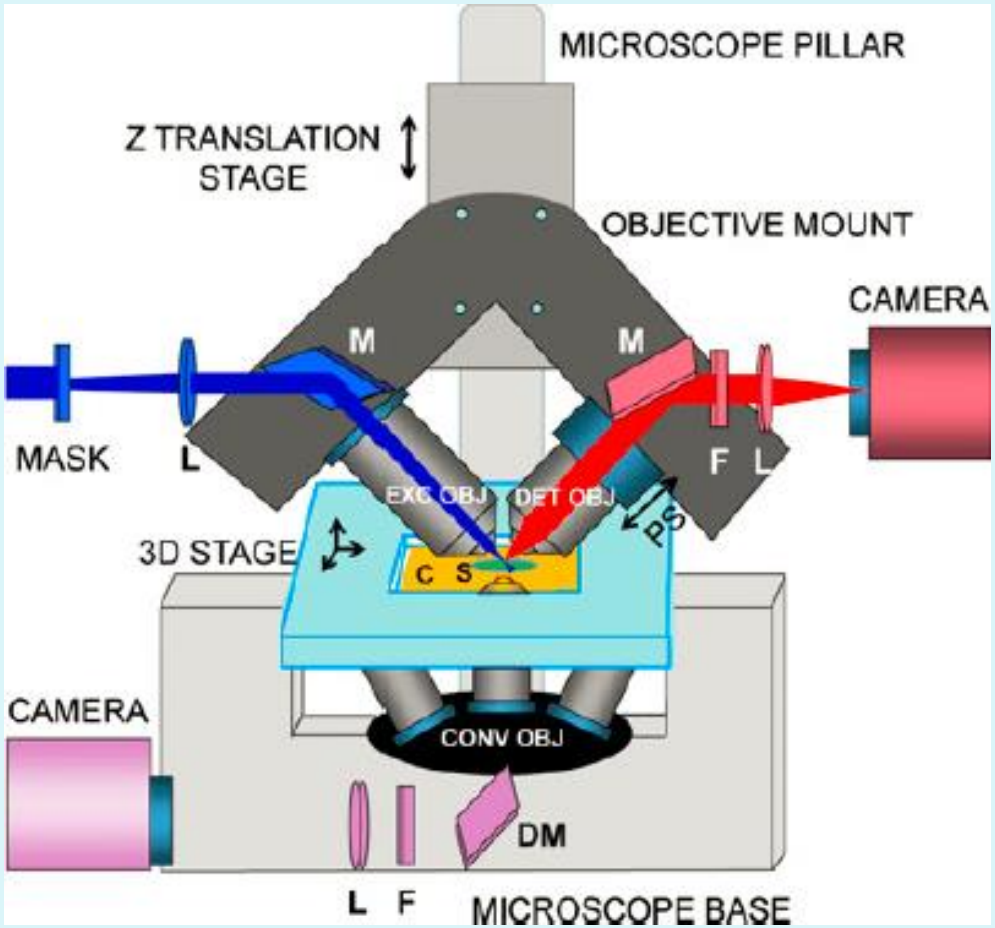
Software support

- MS2000 controller (up to 4 axes) supported in almost all microscopy softwares
- TG1000 controller (modular) supported in Micro-manager and some others
  - ASI actively maintains Micro-Manager device adapters for all our hardware devices
- LabView drivers available from ASI
- Everything happens via serial commands

Outline

- Why light sheet microscopy?
- How can ASI help?
- Examples:
  - iSPIM/diSPIM
  - oSPIM or πSPIM
  - dSPIM for cleared tissue
  - SPIM for functional imaging in zebrafish
- Synchronization and software

Original iSPIM Concept



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

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

Resolution is anisotropic

Lateral resolution ~ $0.61*\lambda/NA$

Axial resolution ~ $1.22*\lambda/NA^2$

(Other equations exist depending on how you define thresholds)

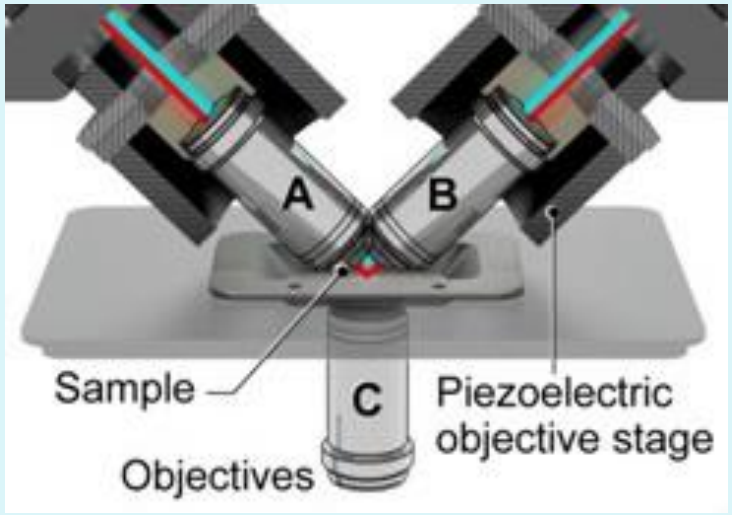
NA	Lateral Res @ GFP [nm]	Axial Res @ GFP [nm]	Ratio (all λ)
0.4	778	3889	5.0
0.6	519	1728	3.3
0.8	389	972	2.5
1	311	622	2.0
1.2	259	432	1.7

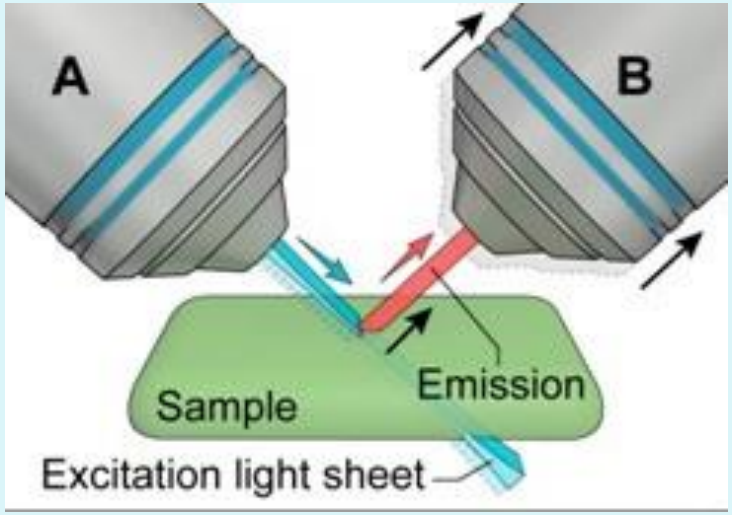
Improving (axial) resolution

APPLIED SCIENTIFIC INSTRUMENTATION

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

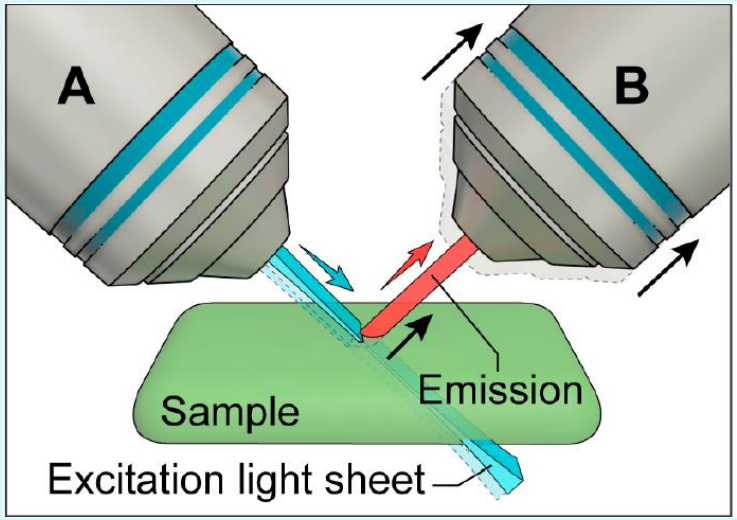
diSPIM = dual-view SPIM on inverted microscope

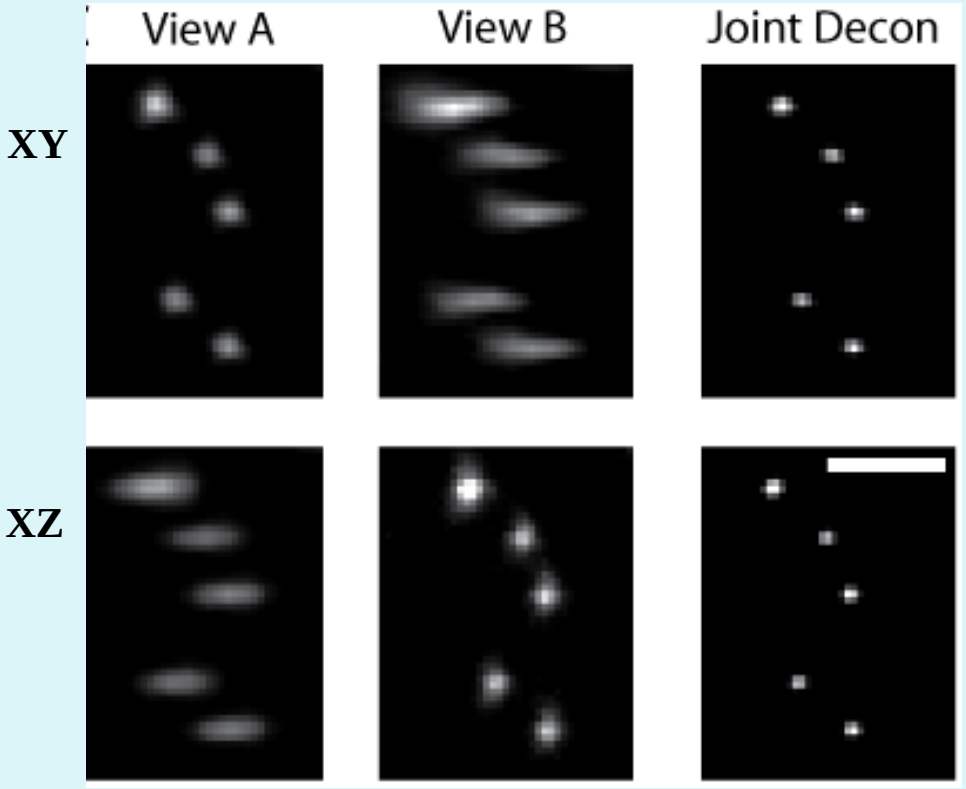




- Two (fixed) views → isotropic resolution
- Open-dish sample mounting
- Stacks by moving objective/light sheet or by moving stage

Isotropic resolution by fusion



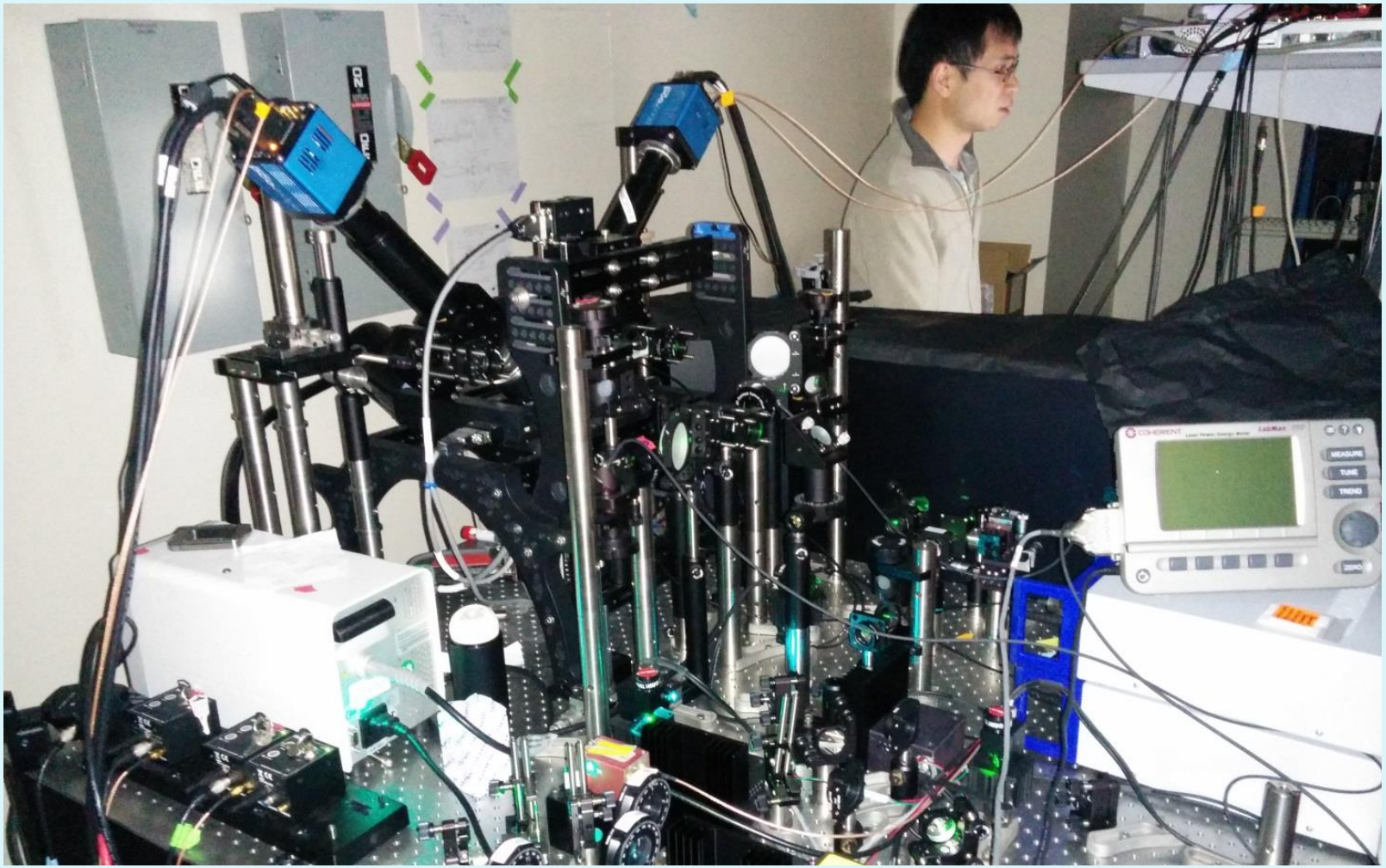


Joint Decon: A. York and Y. Wu

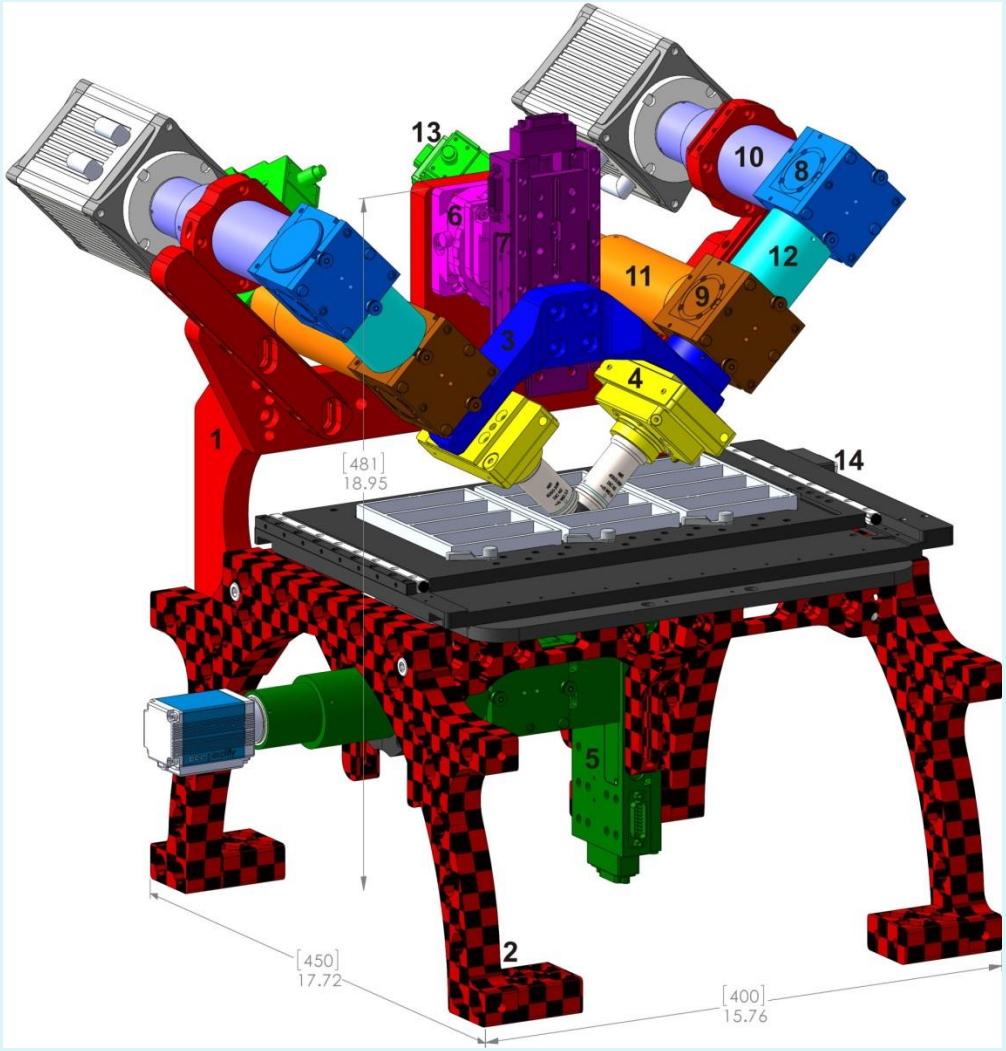
Wu et al. *Nat. Biotechnol.* 31, 1032-138 (2013),
Kumar et al. *Nature Protocols* 9, 2555-2573 (2014)

Early diSPIM (2011?)

APPLIED SCIENTIFIC INSTRUMENTATION

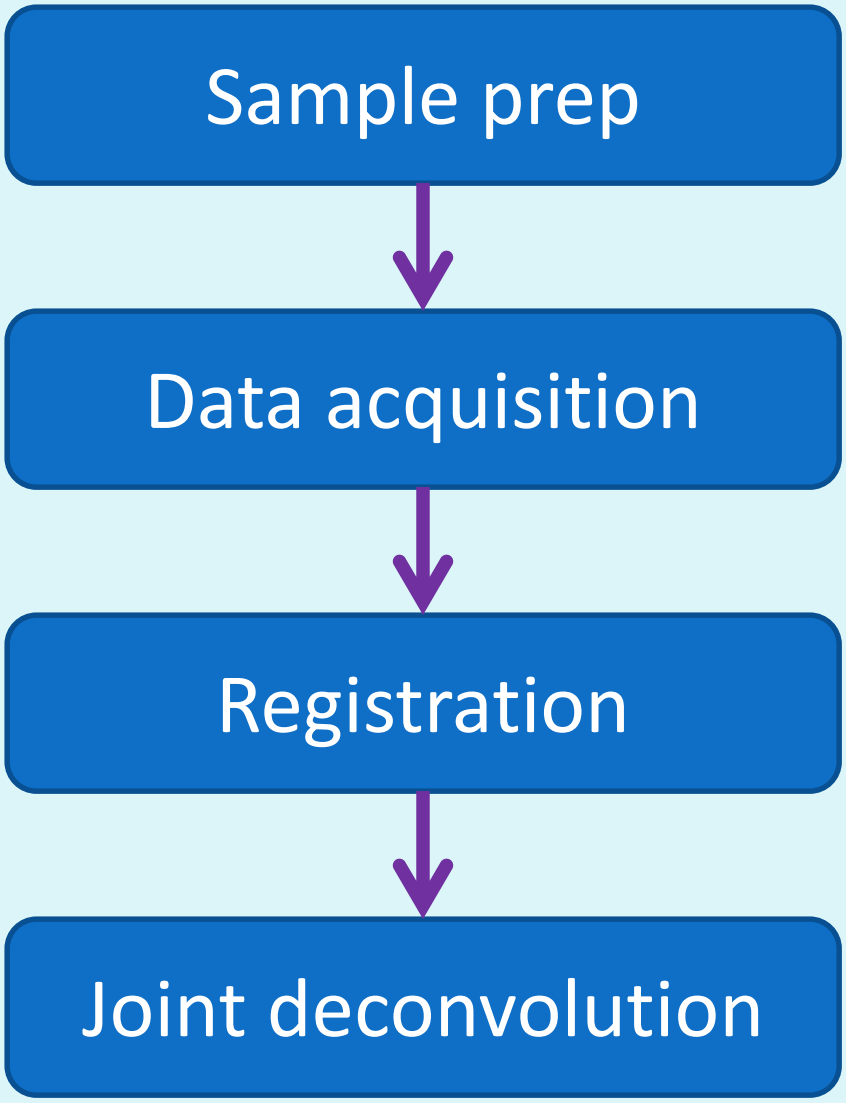


Modern diSPIM



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)

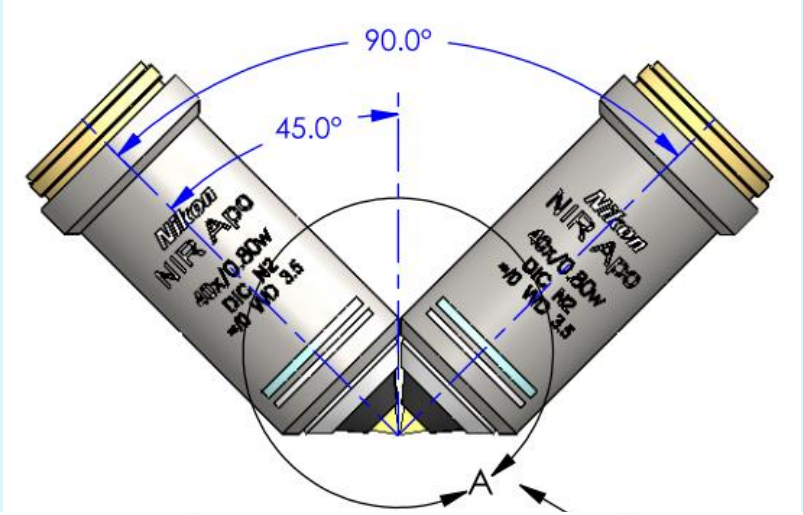
diSPIM workflow

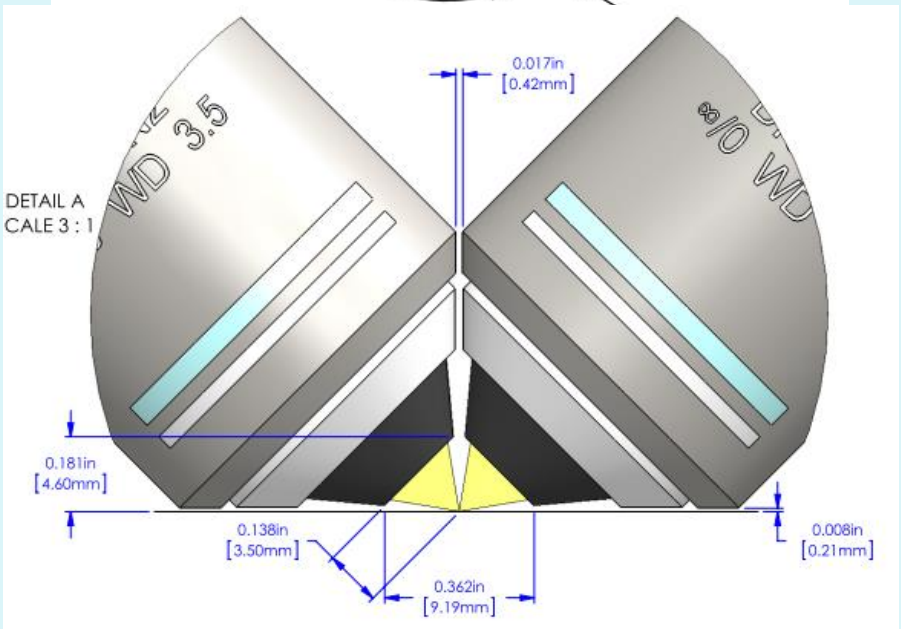


Shroff group LabView
Micro-Manager diSPIM plugin
3i's Slidebook
Others in future?

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 symmetric water objectives at 90°

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

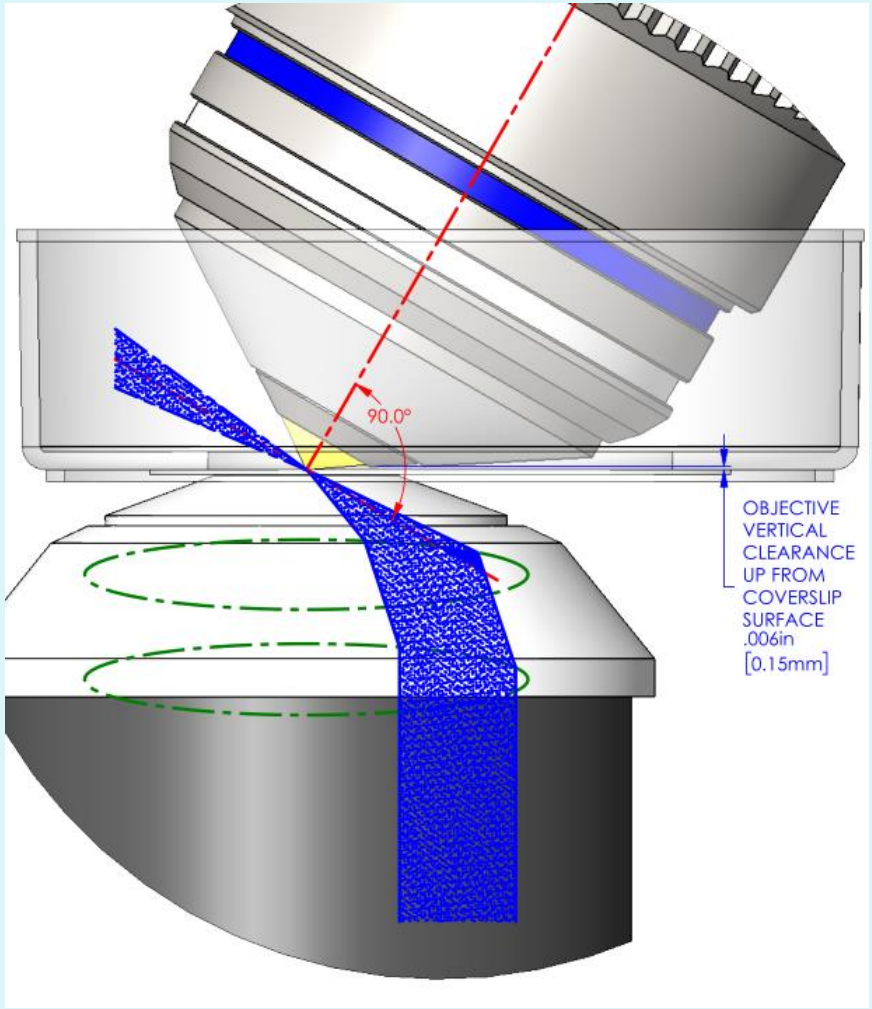
iSPIM/diSPIM, isotropic “lateral” resolution with post-processing

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

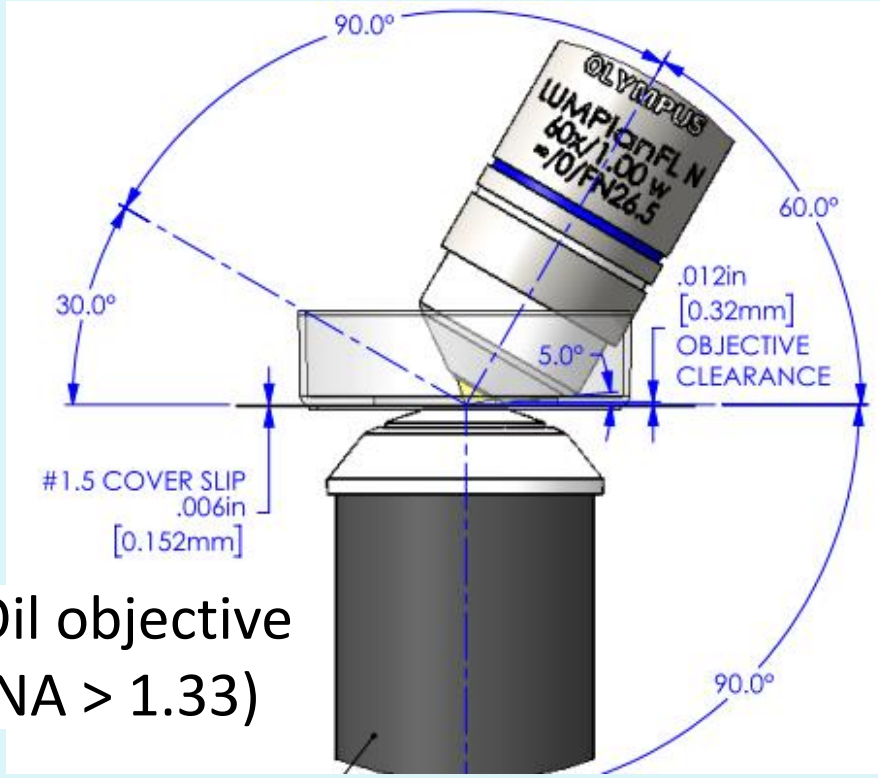
NB: oSPIM/doSPIM design works up to NA 1.1

oSPIM objective geometry

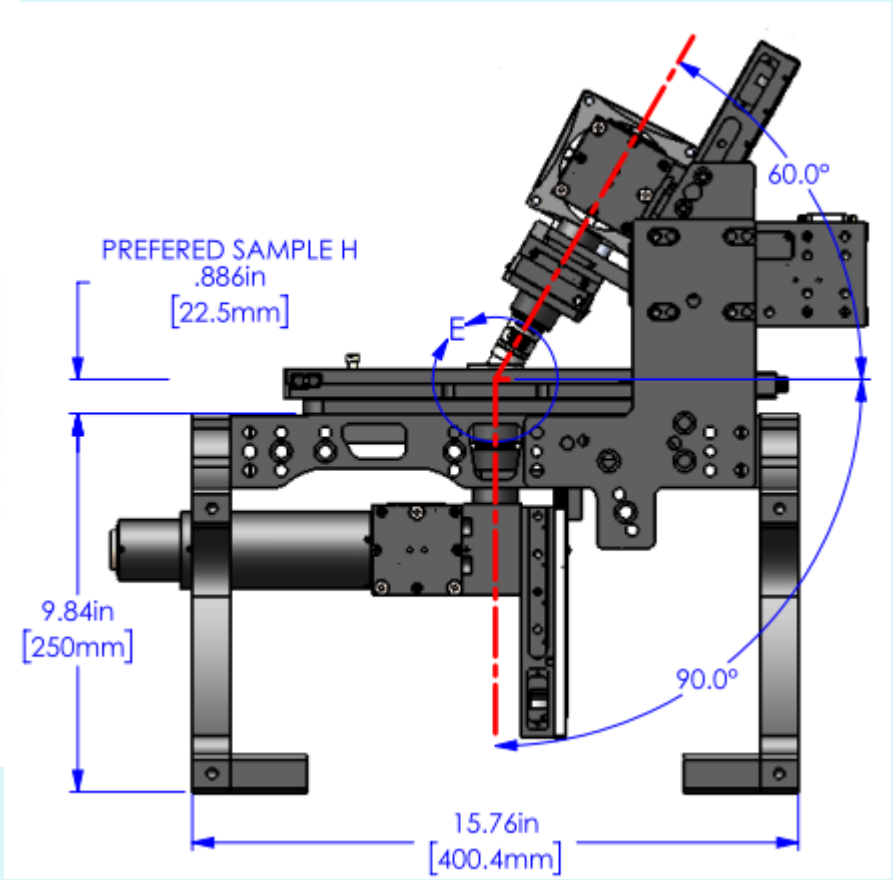
- Create light sheet sideways from objective by illuminating off-center in BFP (partway to TIRF)
  - → >90° objective angle
  - → higher NA objectives
  - → improved resolution
- Independently invented as “πSPIM” Sci. Rep. 6:32880 (2016)



oSPIM implementation (single)



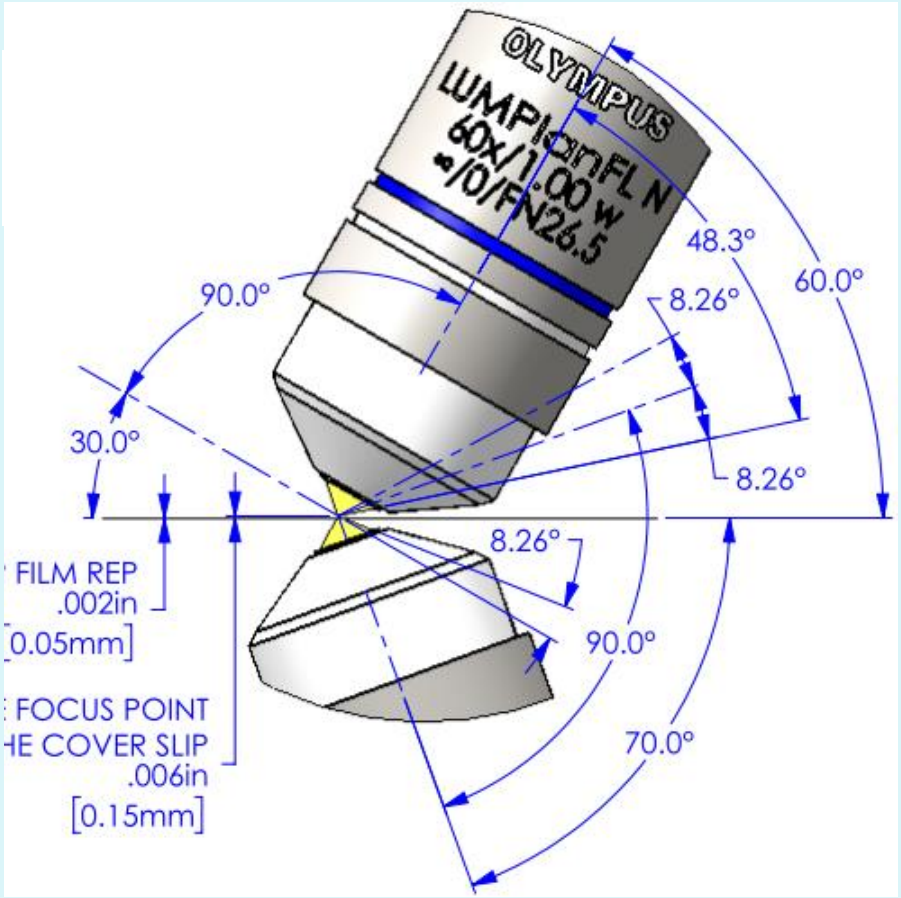
Oil objective
(NA > 1.33)

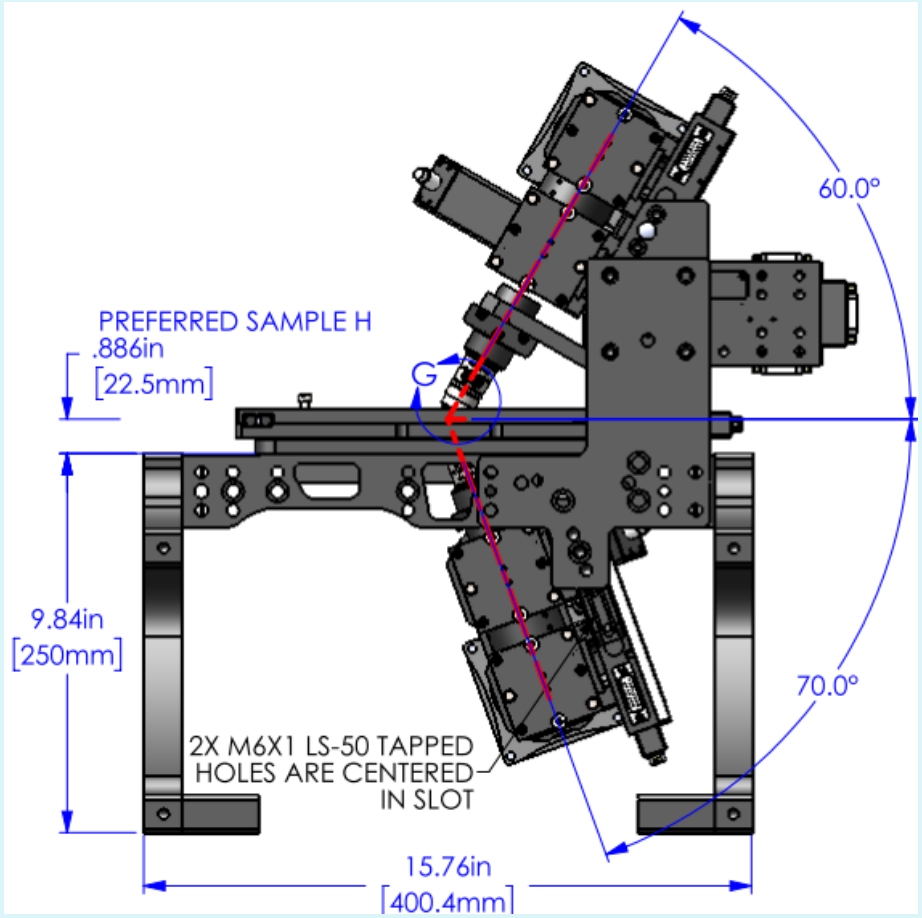


Bottom objective creates tilted light sheet for imaging with top objective

doSPIM implementation (dual)

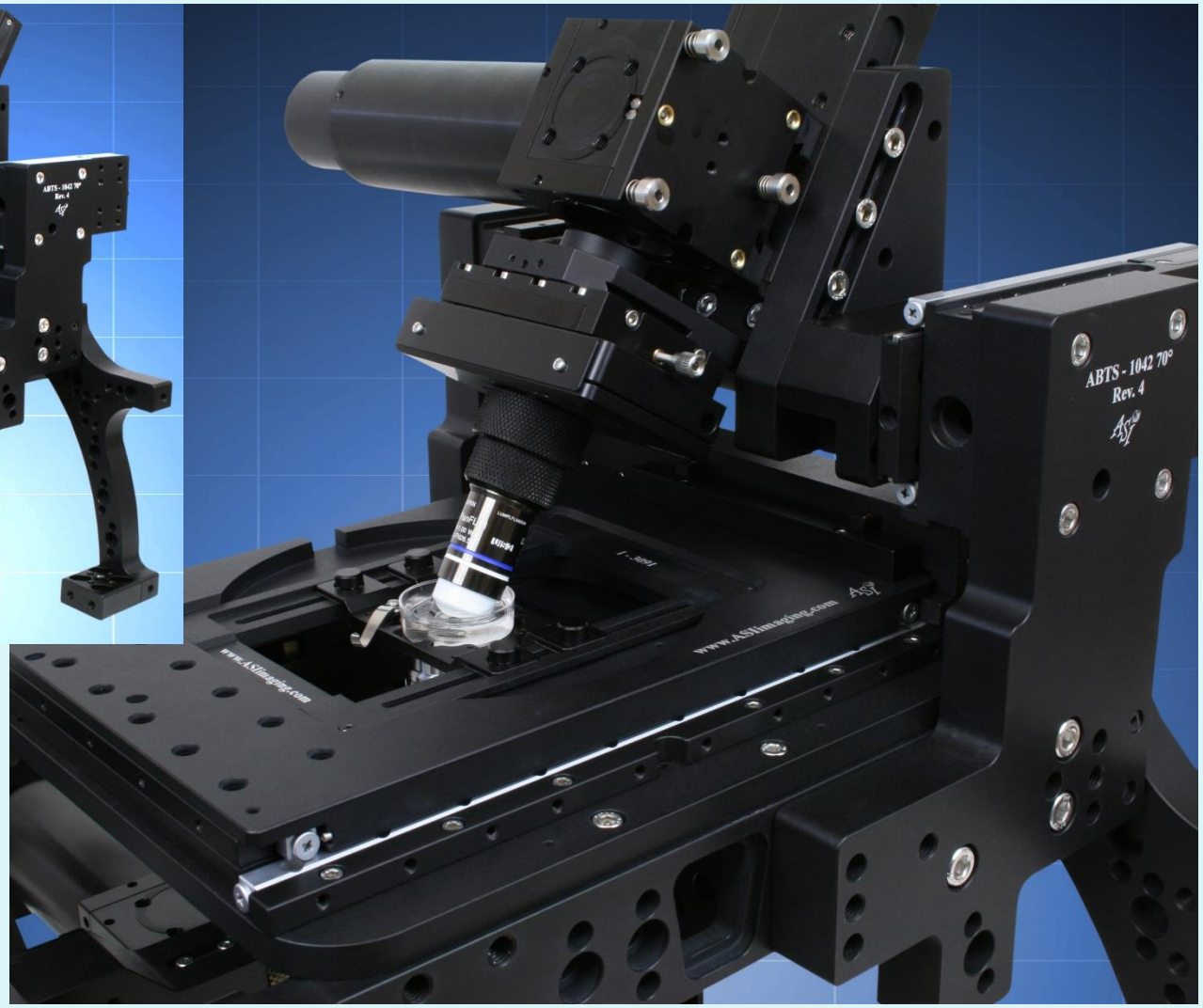
APPLIED SCIENTIFIC INSTRUMENTATION





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

oSPIM in real life



Just 3 new parts for
a new configuration!
(all mounting blocks)

Cleared tissue objective

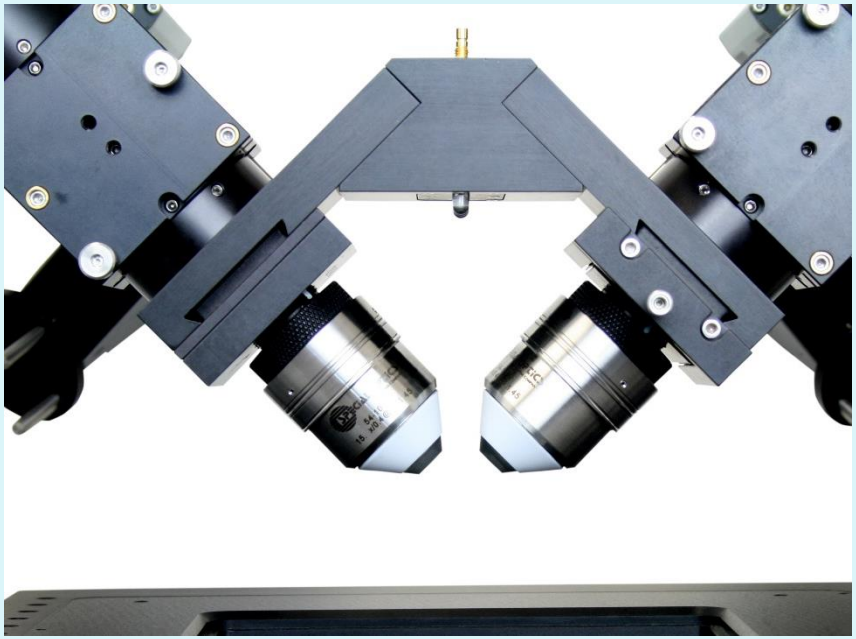
APPLIED SCIENTIFIC INSTRUMENTATION

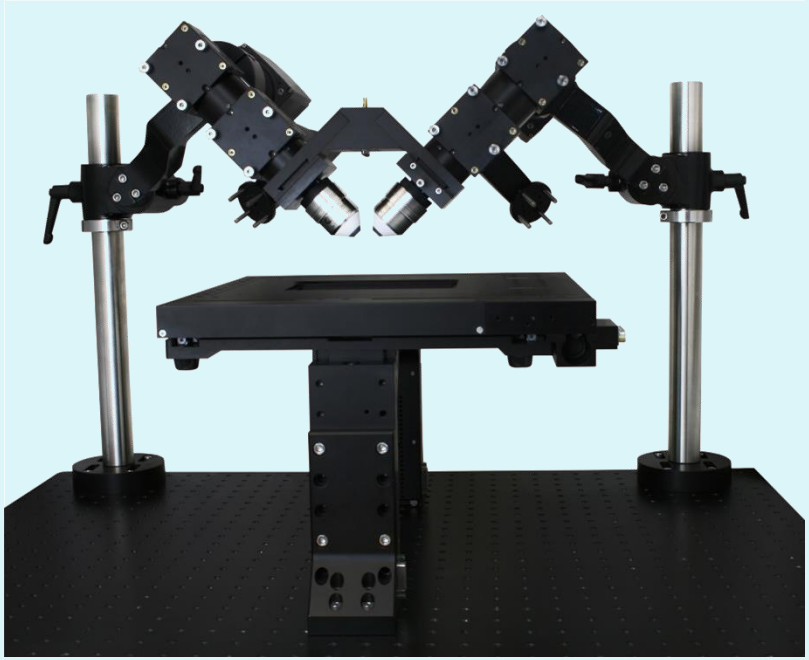
SPECIAL OPTICS A NAVITAR COMPANY

Spec	Value	Comments
Numerical Aperture	0.4 @ RI 1.45	0.37 – 0.43 over RI range
Dipping 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 for flat sample @ 45°
Field of View	1.2 mm Ø	
Correction Collar	None	For immersion w/o coverslip
Price	$15k	Available Oct 2017

dSPIM

- Sample on XYZ stage and SPIM head is fixed
  - Better for large samples like cleared tissue



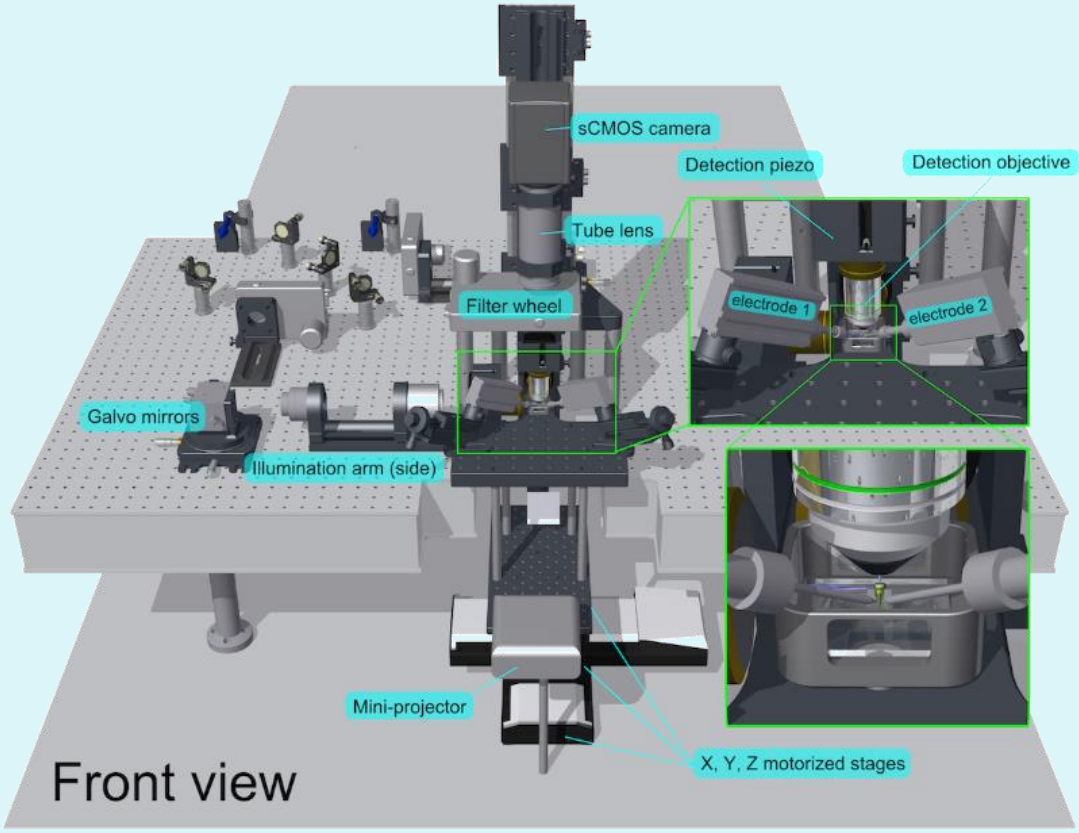


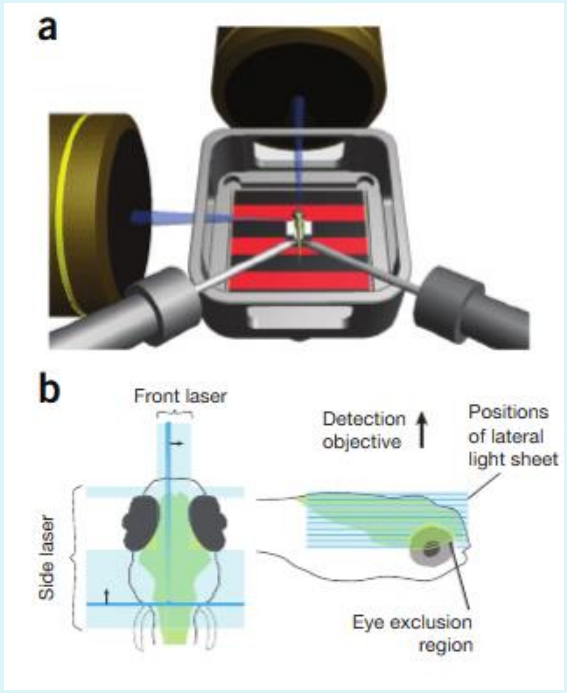
- No inverted microscope
- No objective piezos (stage scanning)

dSPIM features

- Image >5 mm deep into cleared samples with XY extent limited only by stage (200x200 mm)
- Sub-micron stage repeatability → easy stitching
- Redesigned SPIM head
  - reduce collimated space
  - wider apertures
  - more modular

Functional zebrafish imaging





Vladimirov et al. *Nature Methods 11*, 883-884 (2014)

- ASI offers all the required parts already, just have to connect them in this configuration

Synchronization

- Light sheet, piezos and/or XY stage, cameras, and lasers must be tightly synchronized → need hardware synchronization
- 2 approaches:
  - Generate synchronized control voltages yourself
  - **Use synchronization within Tiger controller**
    - High-level software specifies timing parameters
    - Saves lots of implementation effort to let ASI controller coordinate sub-millisecond timing of components

Don’t forget about software

- Developing control software is major task…
  - User interface
  - Hardware control
  - Save images with metadata
  - Live view for alignment
- … but it’s already been done for you!
- **Spend time working on science, not developing microscope control software!**

Micro-manager plugin features

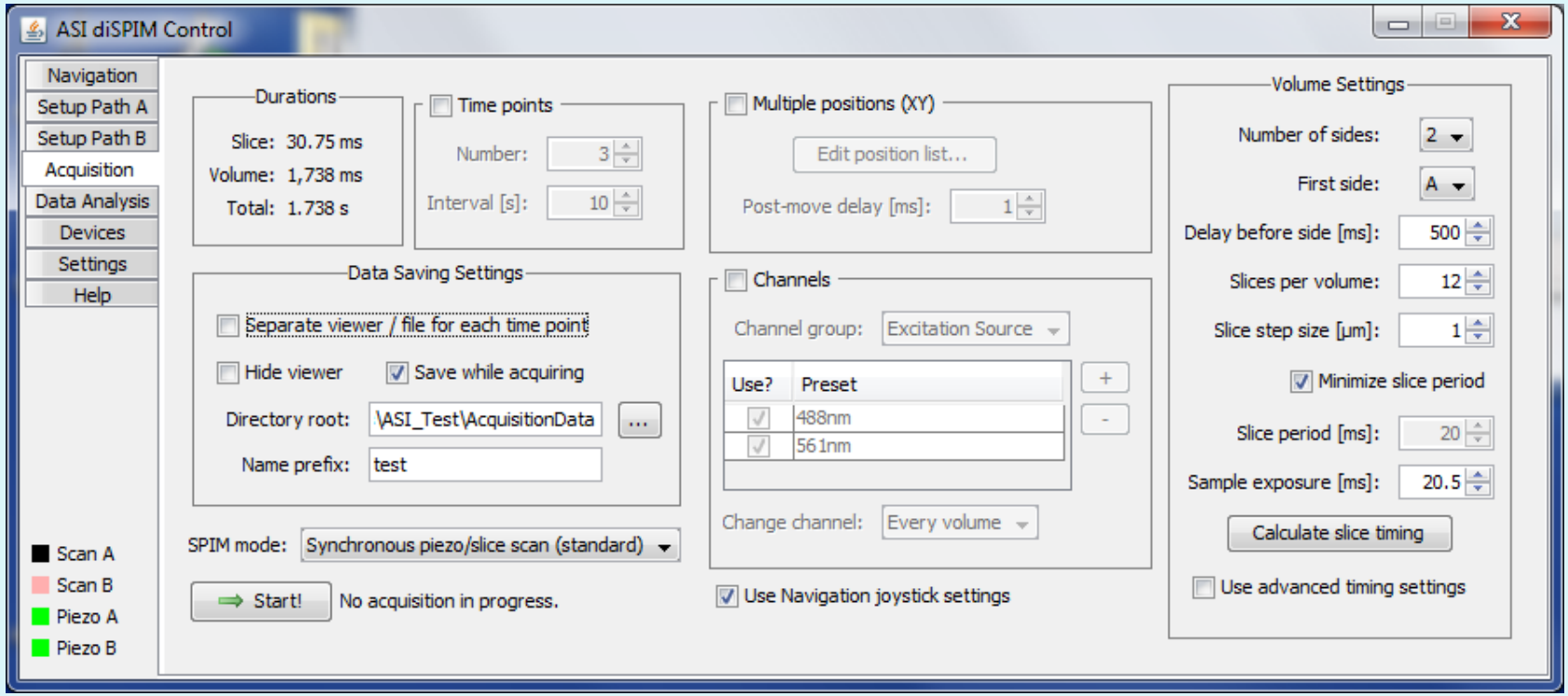
APPLIED SCIENTIFIC INSTRUMENTATION

- Acquisition Modes:
  - Synch. slice/piezo
  - Fixed sheet
  - Stage scan
  - Virtual slit confocal
- Multi-Dimensional Acq.
  - Time points
  - Multi-position
  - Multi-channel
- Supported cameras:
  - Andor Zyla
  - PCO Edge
  - Hamamatsu Flash 4
  - Photometrics 95B
- Supported lasers:
  - Lasers with dual port switch or passively split
  - 4 channels on/off via TTL

Works for iSPIM, diSPIM, oSPIM, dSPIM, and more

Micro-manager plugin

- Built in to MM: Plugins->Devices->ASI diSPIM
- Fully open source including ASI contributions



Advantages of Micro-Manager

APPLIED SCIENTIFIC INSTRUMENTATION

- Free and open-source
  - Zero cost, download anywhere anytime
  - Fully modifiable and liberally licensed
  - Documentation and community support make it easy to augment code if you want/need
- Facilitates reproducibility
  - Easy to change hardware e.g. different camera
  - Easy to distribute your to other labs

ASI SPIM ongoing developments

- Structured illumination for better-than-Gaussian light sheet profile
- Tunable lens for remote focusing
- Tunable lens for adjusting beam waist
- 2-photon light sheet
- Combining XYZ tracking of moving samples with light sheet

Conclusion

- ASI makes it easy to build custom light sheet microscopes
  - Modular hardware components
  - Synchronization done in controller
  - Functional yet user-extensible software
- ASI loves working with leading scientists to create the next thing; **how can we help you?**