# Simplifying DIY Light Sheet Microscopes


ASI's SPIM Team

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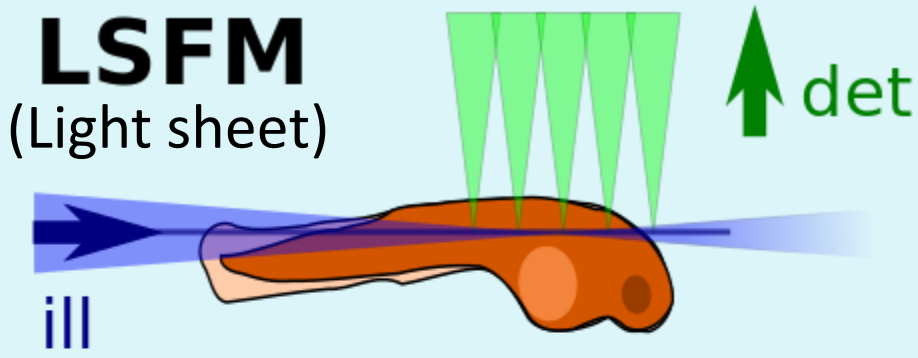
*EMBO I EMBL symposium "Seeing is Believing"*
*04 October 2017, Heidelberg Germany*

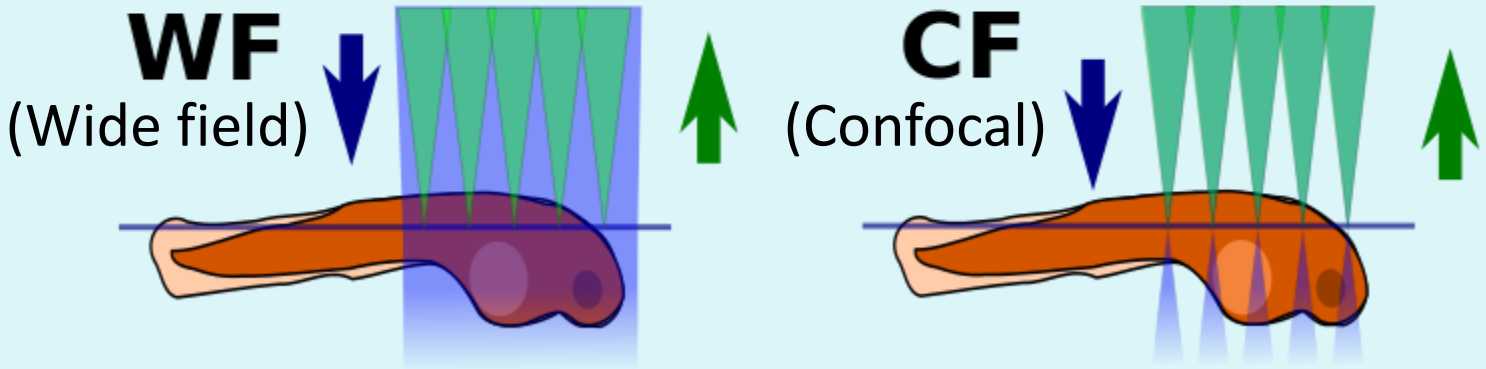
# Outline

- 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?

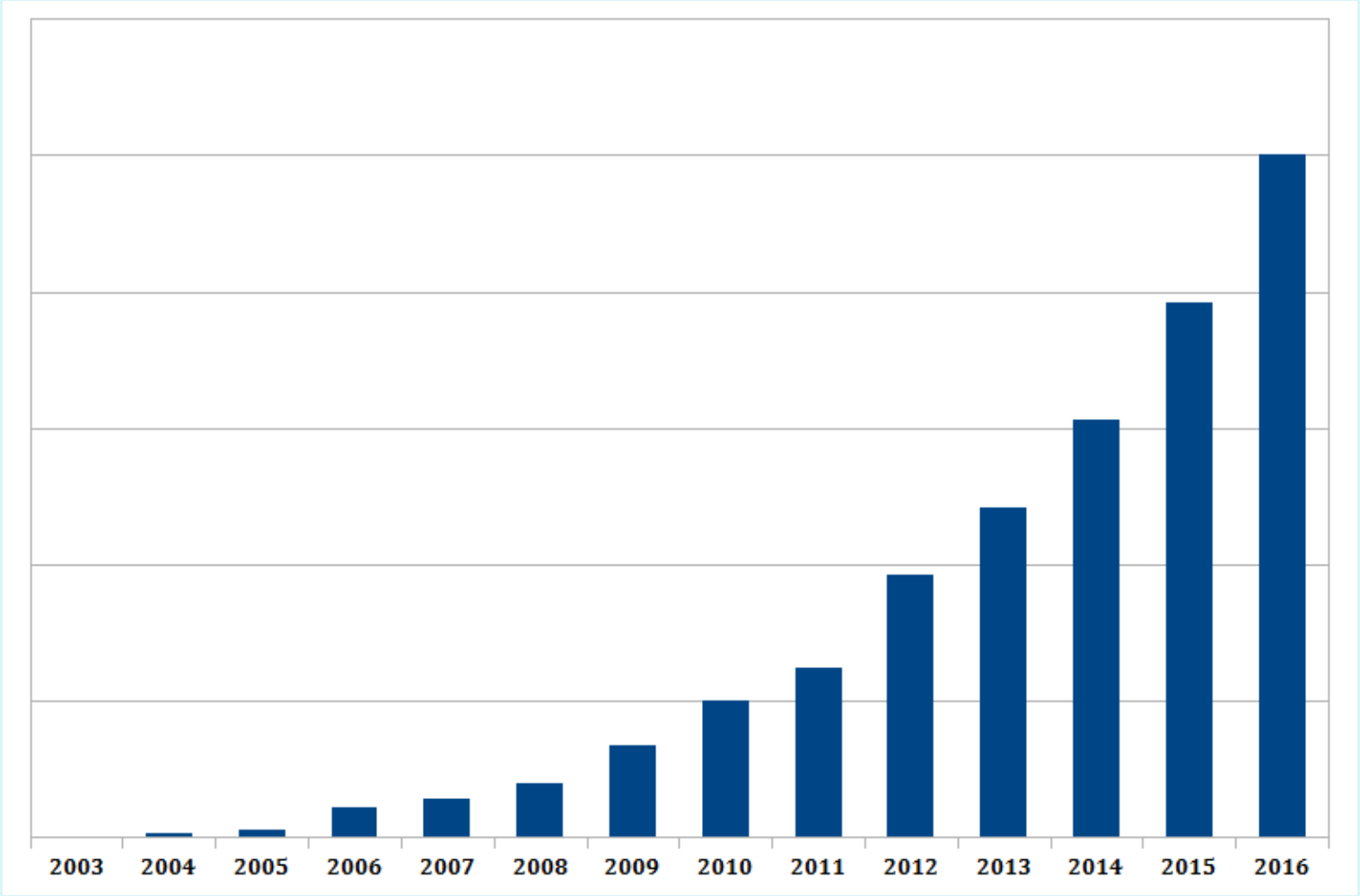



LSFM ~= SPIM = Selective Plane Illumination Microscopy




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

# Publications using light sheet



# Why light sheet microscopy?

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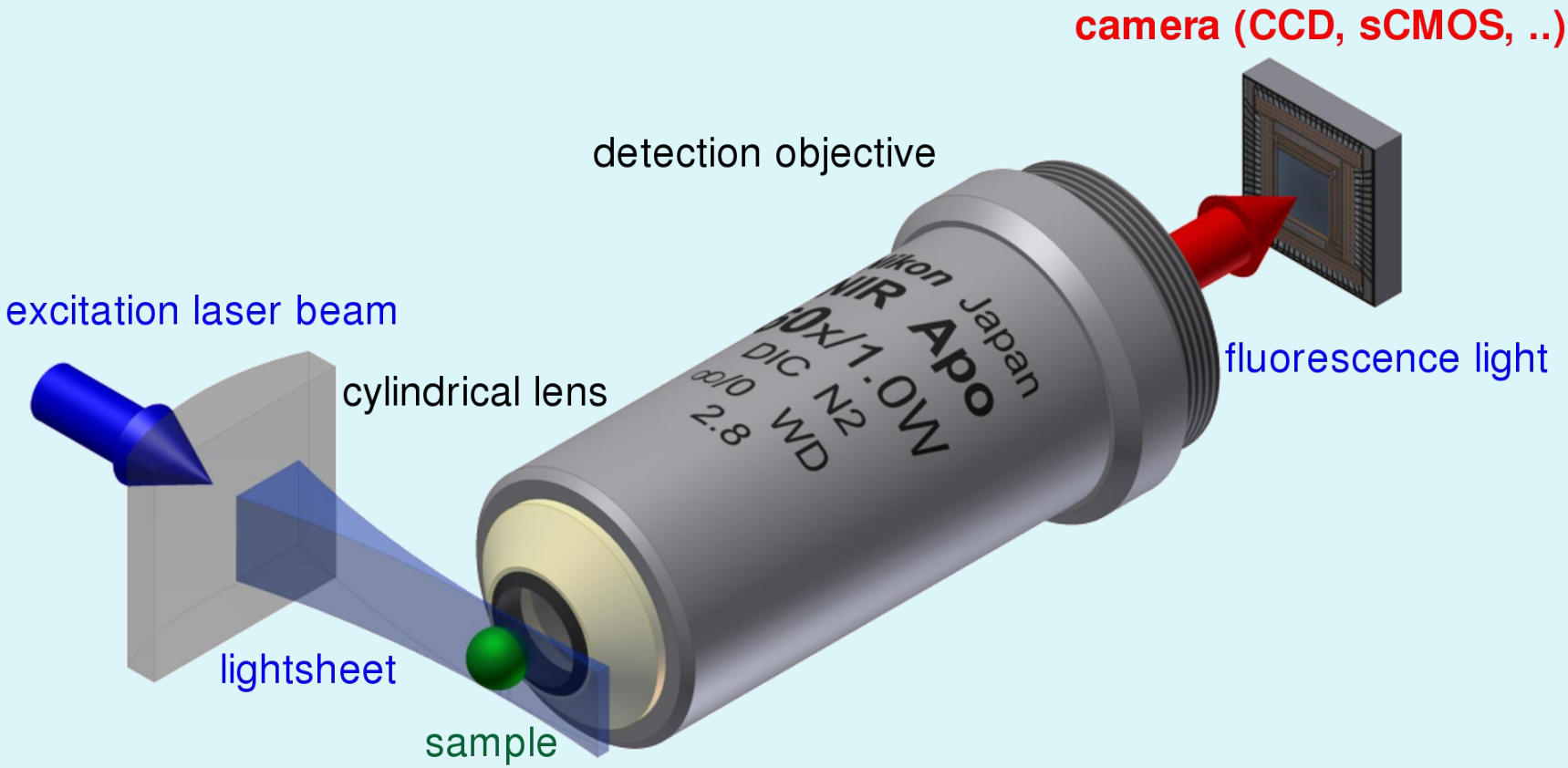
- Minimize photodamage/bleaching
  - Better utilize “photon budget”
  - Keep living things living
- Rapid acquisition
  - 2D parallel imaging
- Main cost is optics for generating light sheet

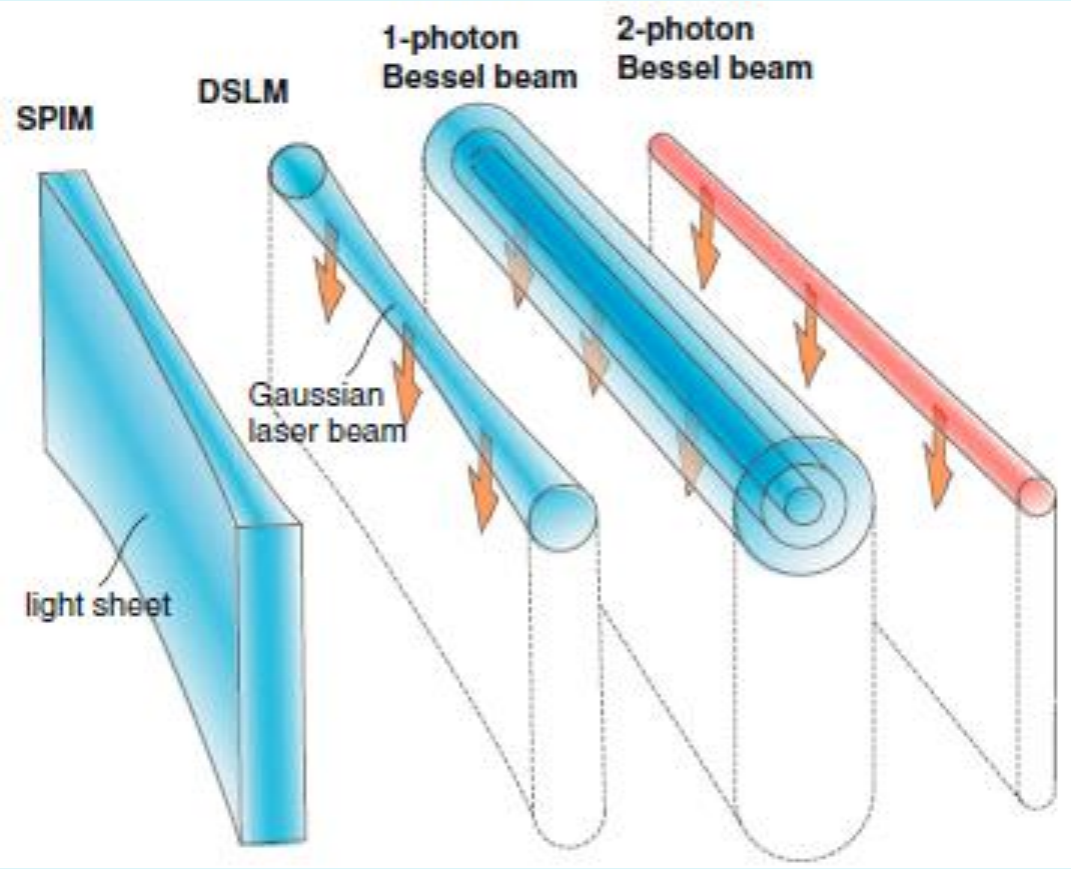
# Simple light sheet microscope




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

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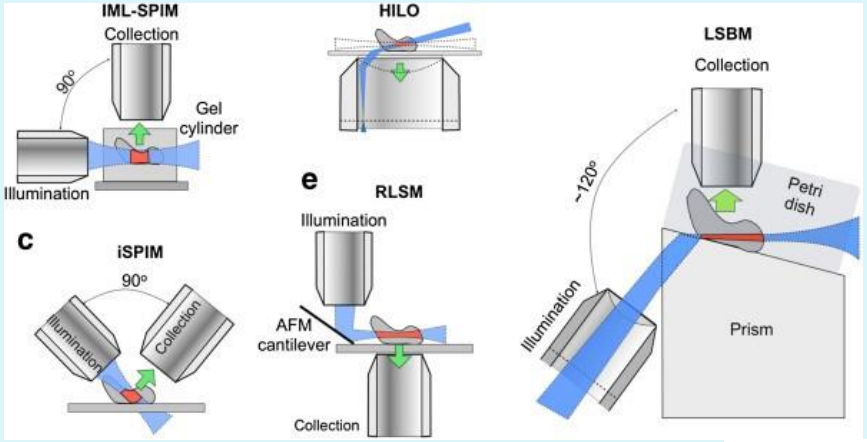
LSFM = light sheet fluorescence microscopy

SPIM = selective plane illumination microscopy

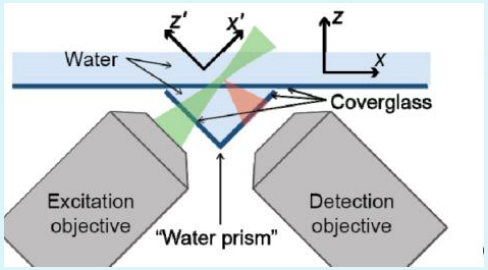
DLSM = digital light sheet microscopy

- 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

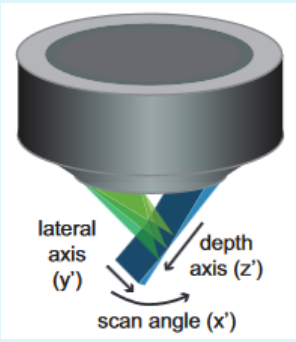
# Sub-sampling of configurations




Optical Nanoscopy 2013 2:7.

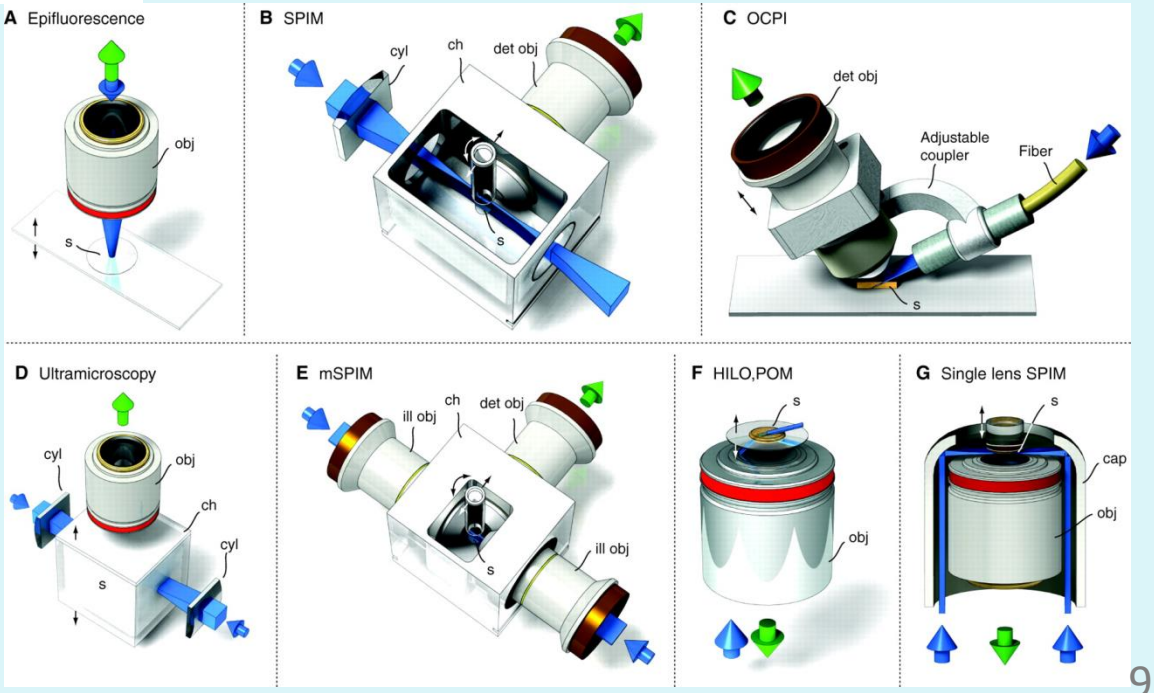


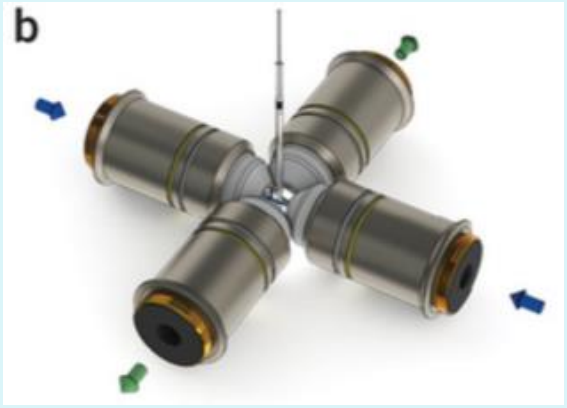

Optics Express 2015; 23: 16142-16153




Nature Phot. 2015 9:113.

Development 2009 136:1963.




Nature Meth 2015 12:30.

# Why so many configurations?

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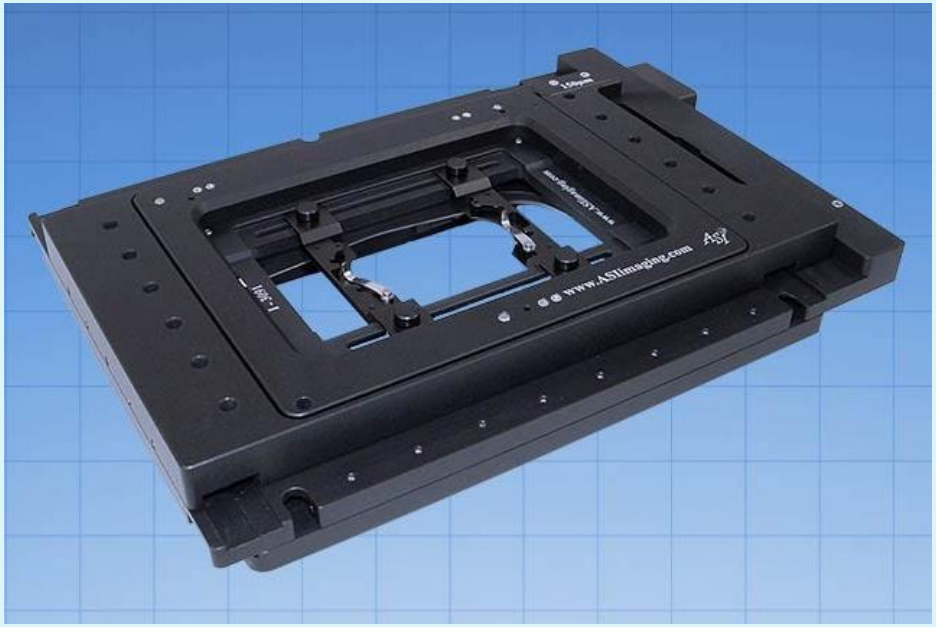
- Different samples ⇔ different microscopes
  - (Everybody wants their own paper)
  - Steric constraints
  - Mounting requirements
  - Imaging requirements → different motion control
- Paradigm shift: single costly microscope for all samples → multiple inexpensive microscopes each customized for sample/application

# 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

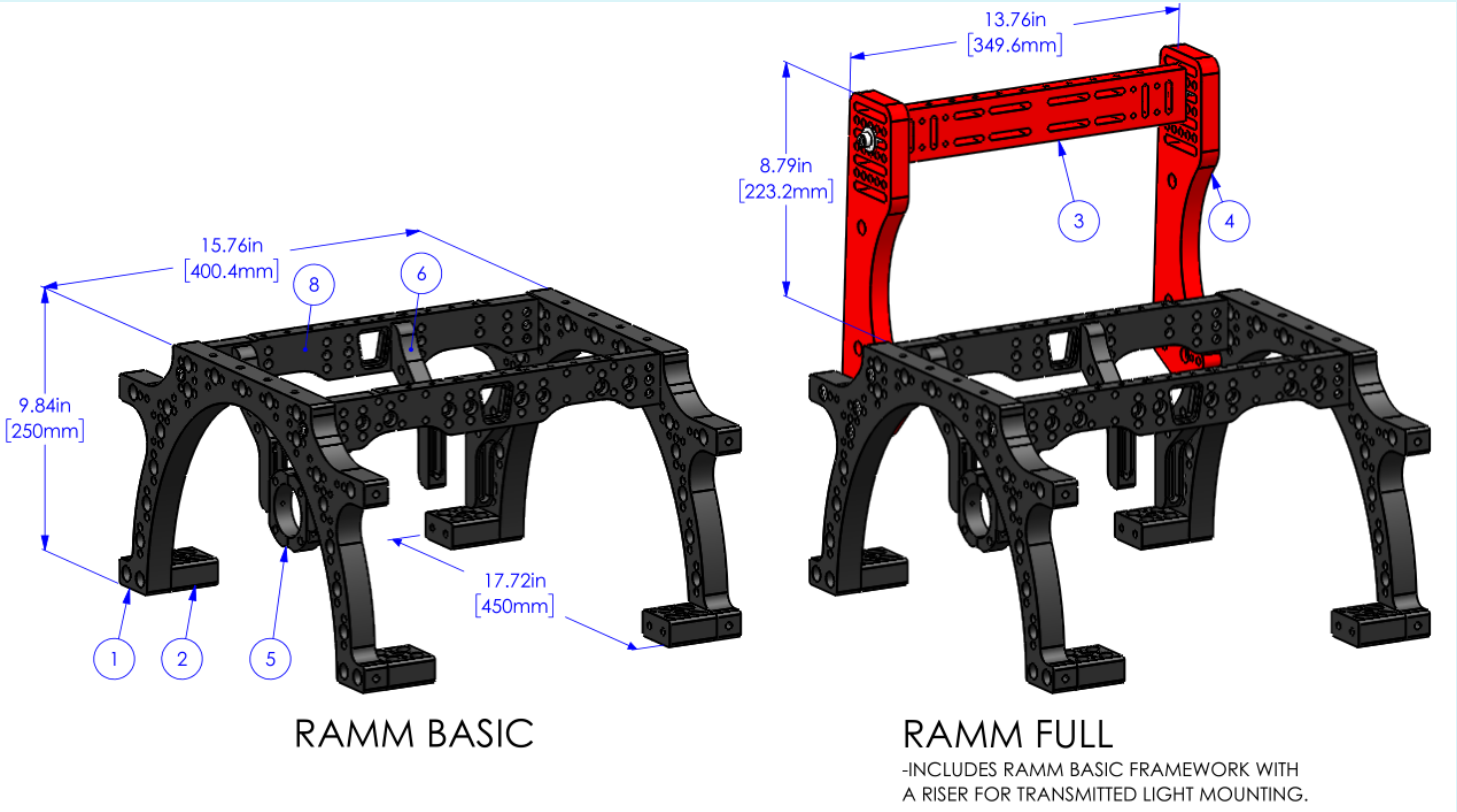
**MS2000** 4-axis controller best for simple microscopes up to one piezo axis.


**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

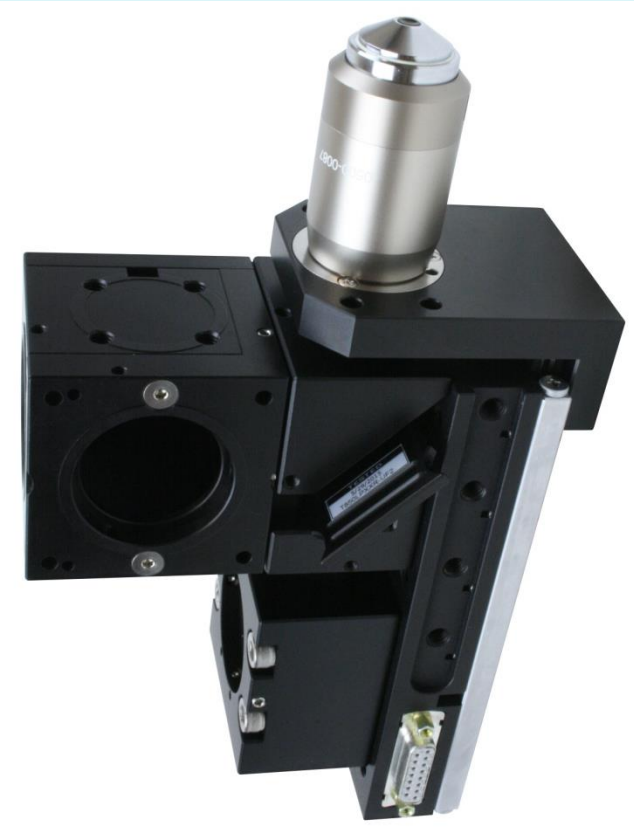
# RAMM frame




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

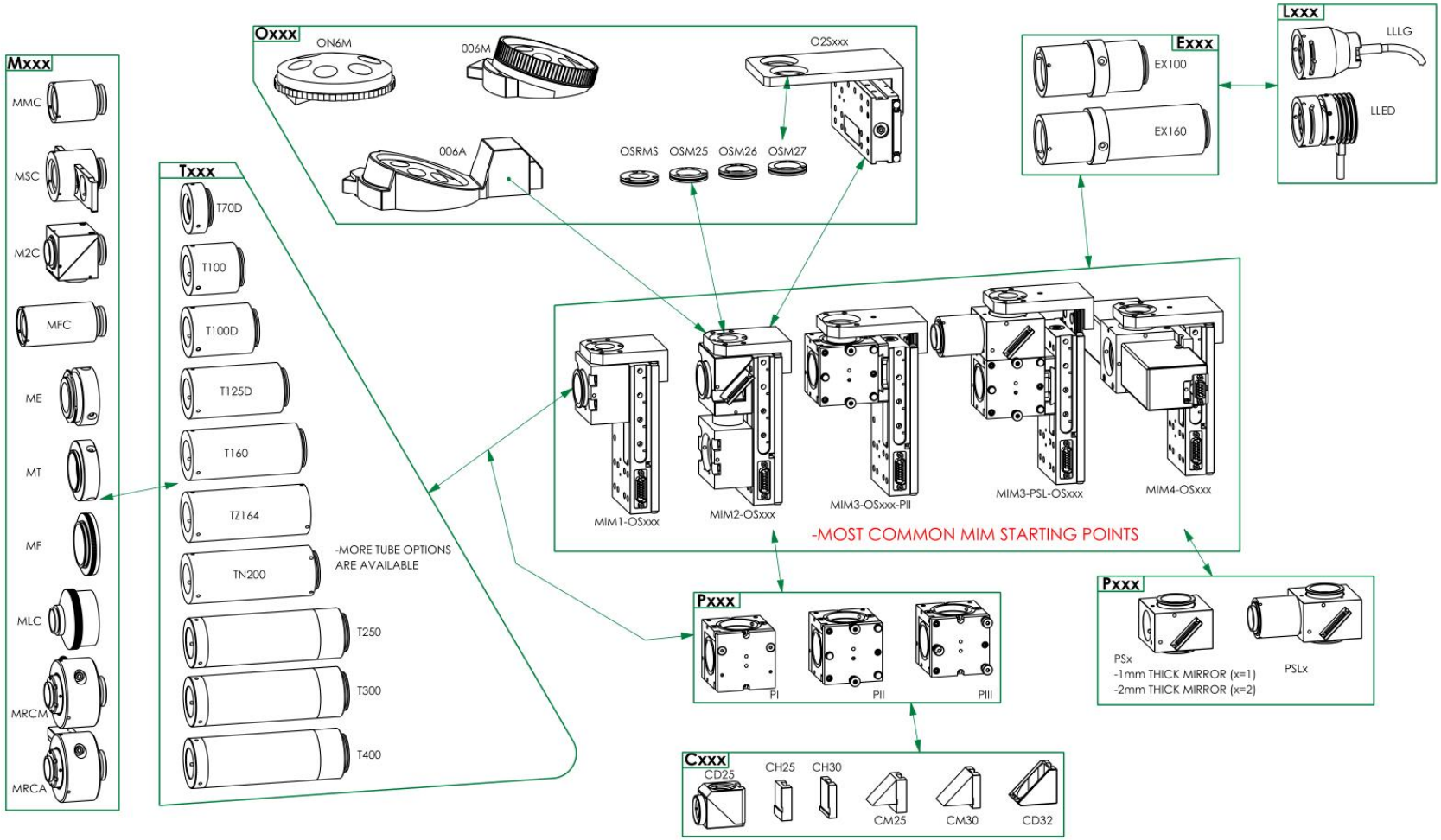
# Modular infinity microscopes

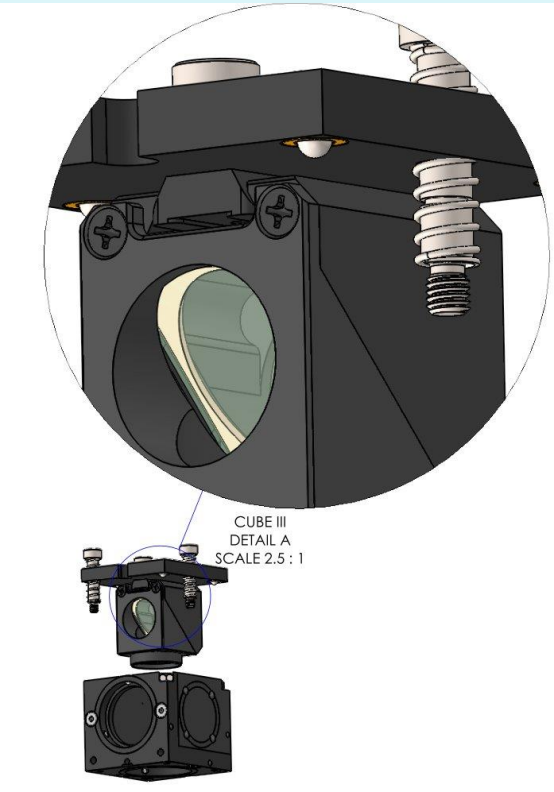
- Include LS-50 Focus Drive “backbone”
- Beam-splitter and Mirror attached to LS-50
- Wide selection of imaging and illumination optical paths can be attached to CUBEs
- A single objective or manual & automated nosepieces are supported



MIM2-OSM25-PII

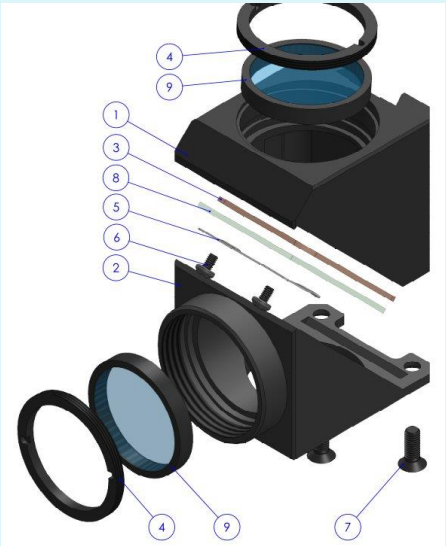
# MIM system map

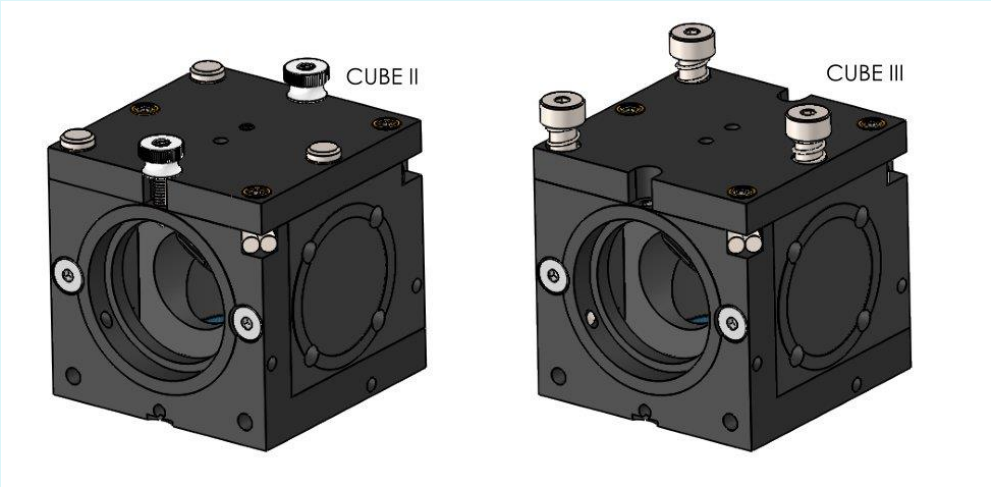





# Cubes

- 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



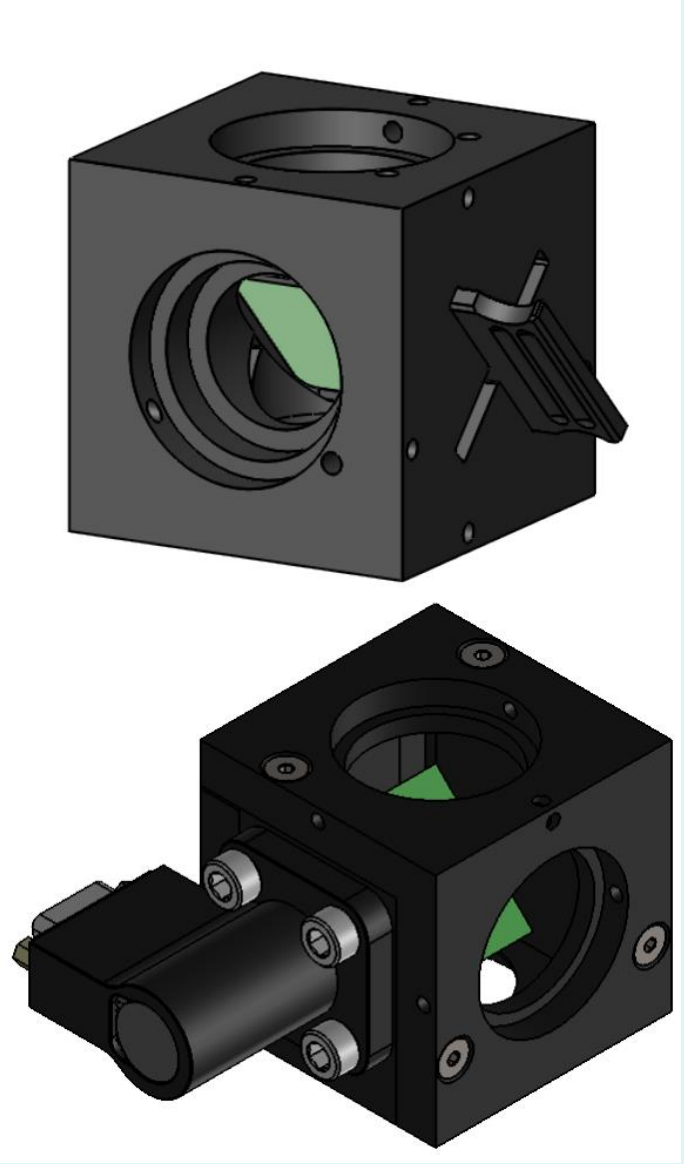



# Port switches

Port switches to select illumination path or camera.

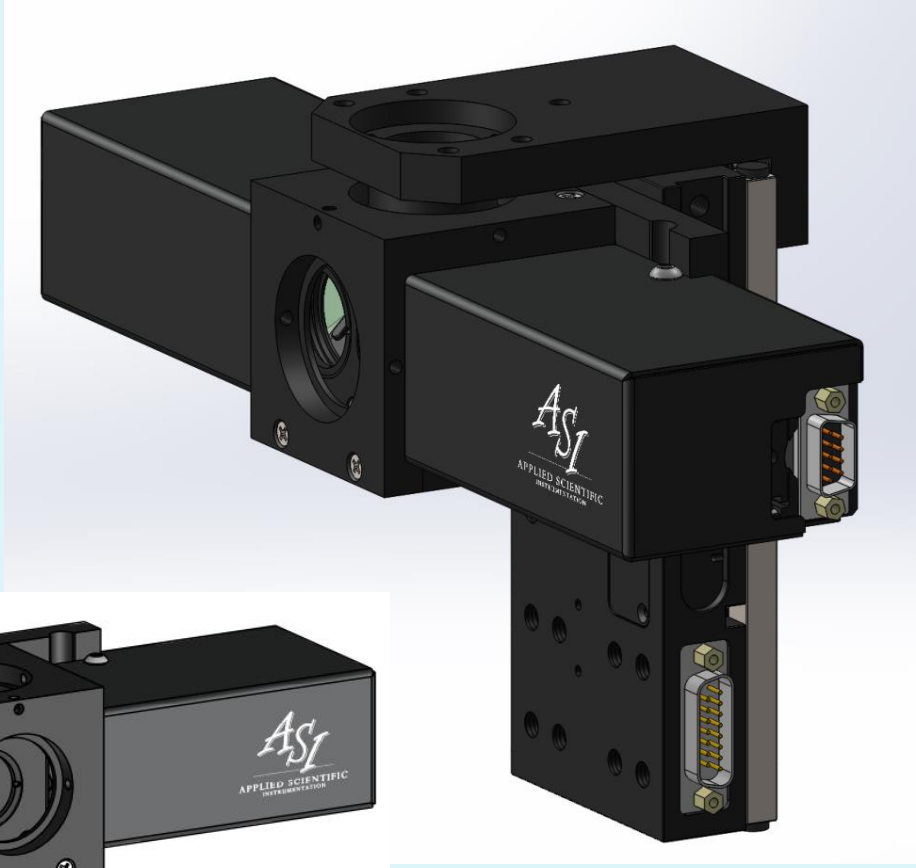
**C60-3WMS  Three-way Manual**
Selects between two side ports or straight-through port depending upon position (or presence) of the mirror slide.

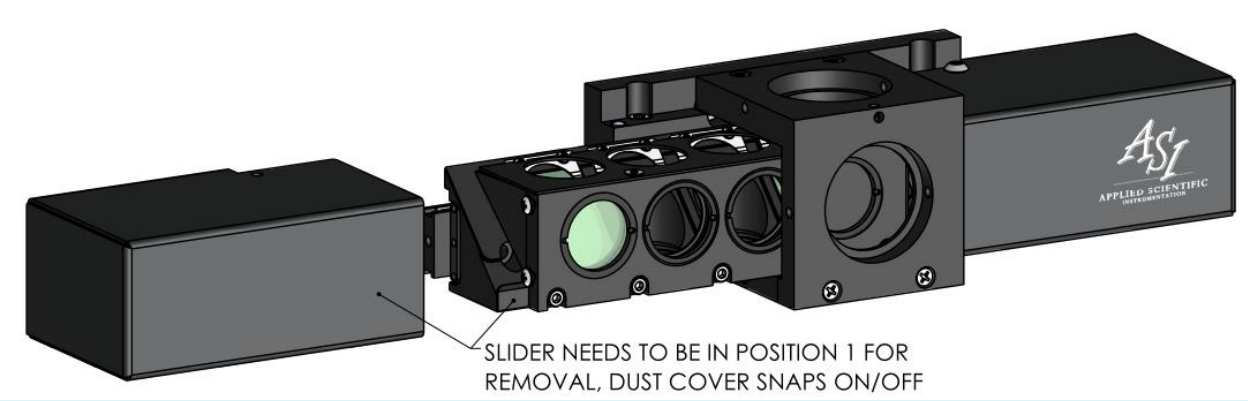
**C60-PORT_SWITCH  Motorized**
Automated for switching the common port between the two side ports.



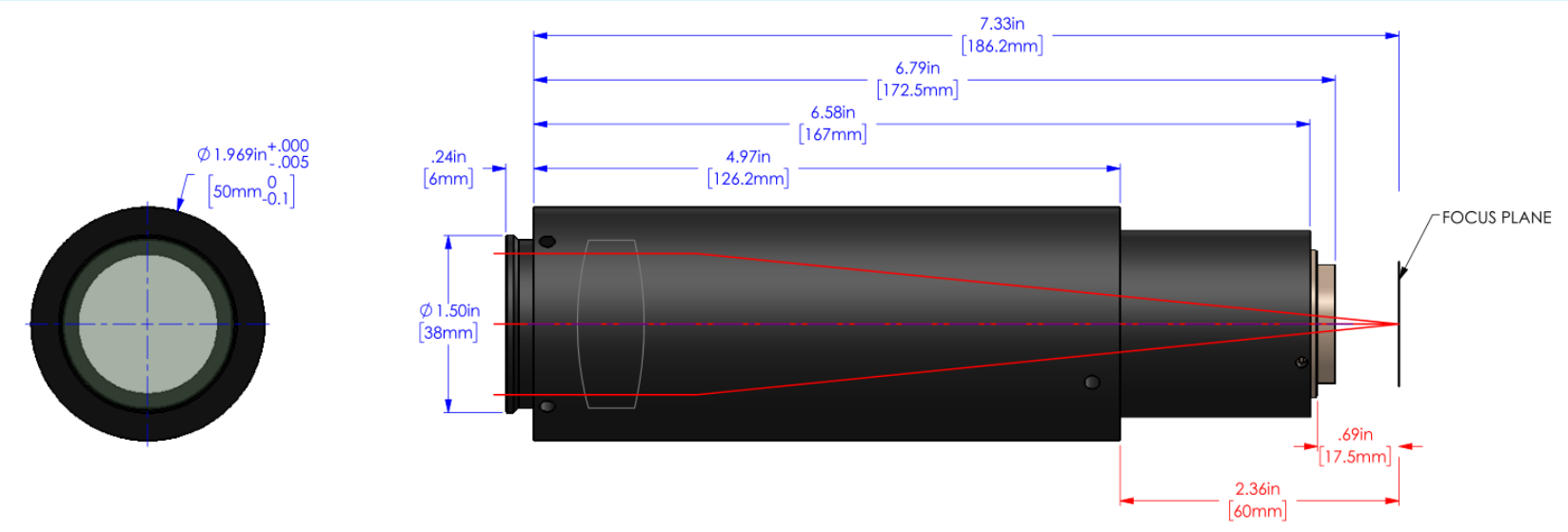
# Filter slider

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



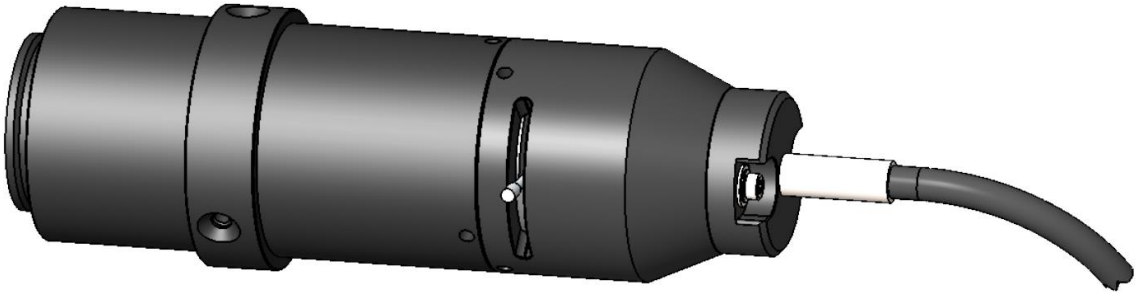


# Tube lenses

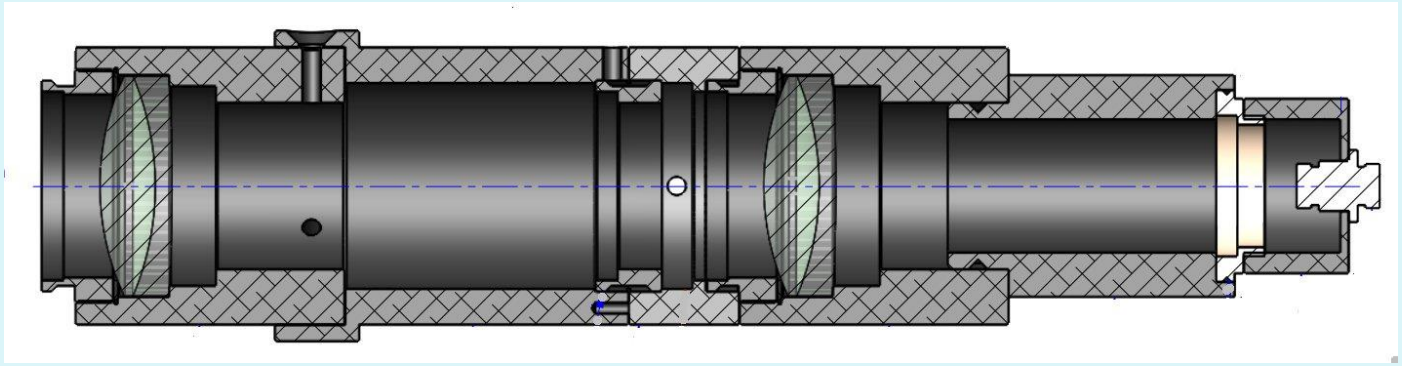



- All Tube Lenses use same format – lengths vary
- “Collimated space” fitting is our 38mm C60-RING
- Focal plane is 60mm from end of lens tube
- “Focus space” fitting is “Zeiss-like” 30mm dovetail
- Many choices from 70mm to 500mm focal length

# Epi-illumination



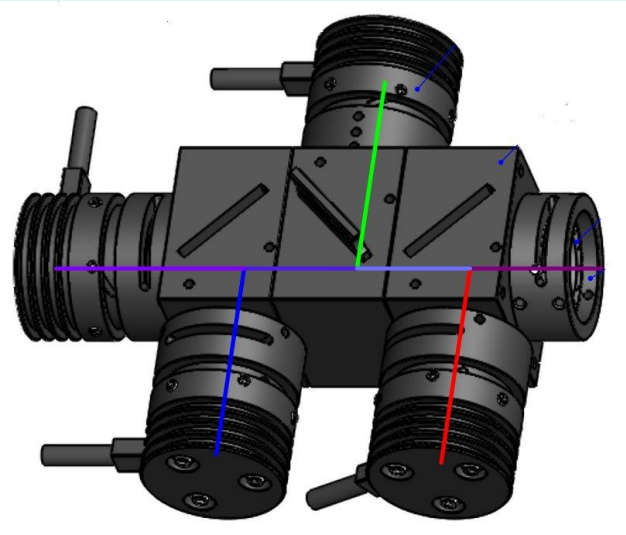
**LLG-E100** Condenser and Liquid Light Guide Adapter



**LFP-E100** Fiber-Coupled Laser Illuminator Assembly

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

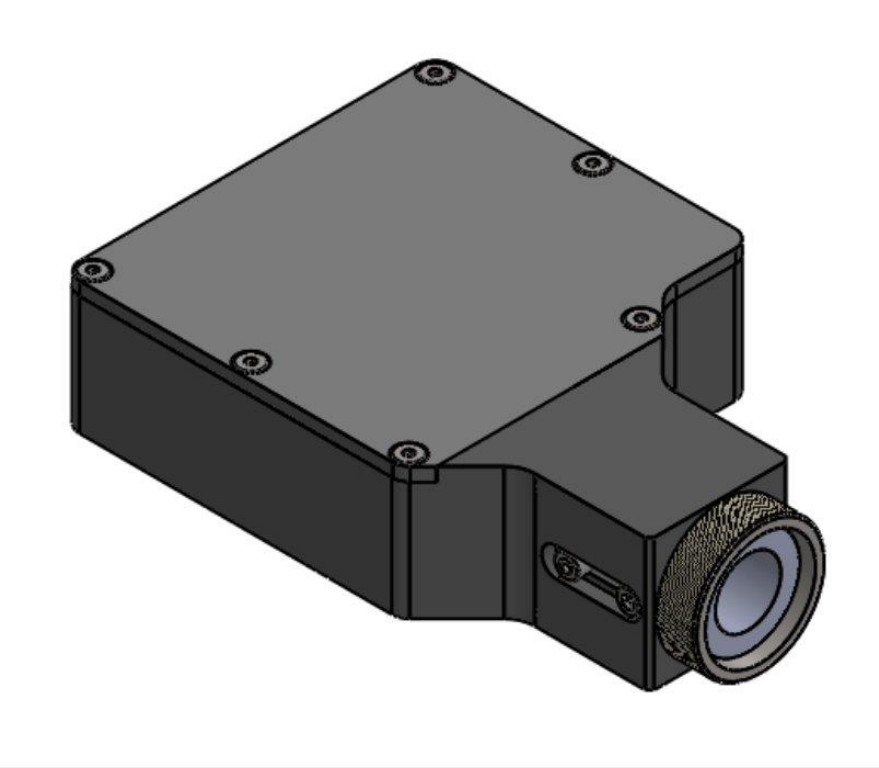
# Multi-LED illuminators


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

# CRISP focus stabilization

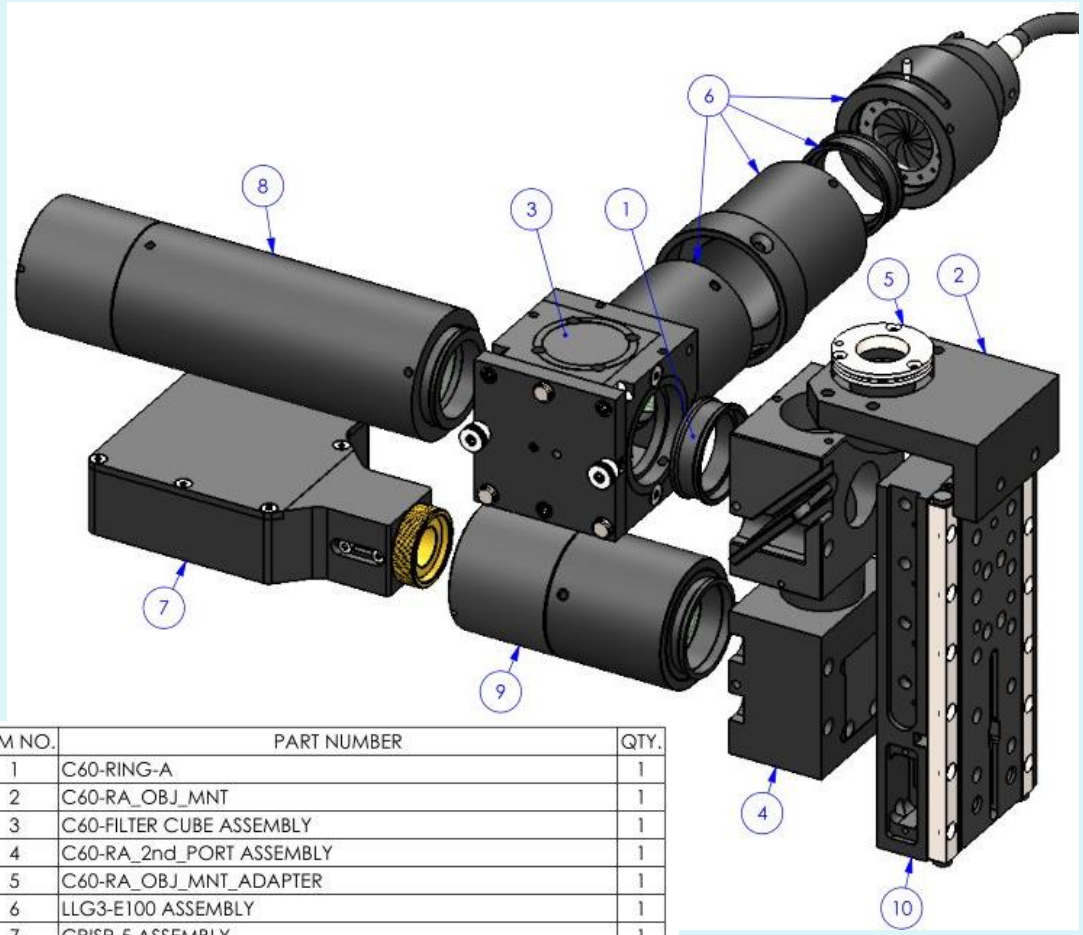
- CRISP system holds focus using a reference surface with a refractive index mismatch such as glass/air or glass/water slide interface.
- Uses IR LED projected onto sample



- 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

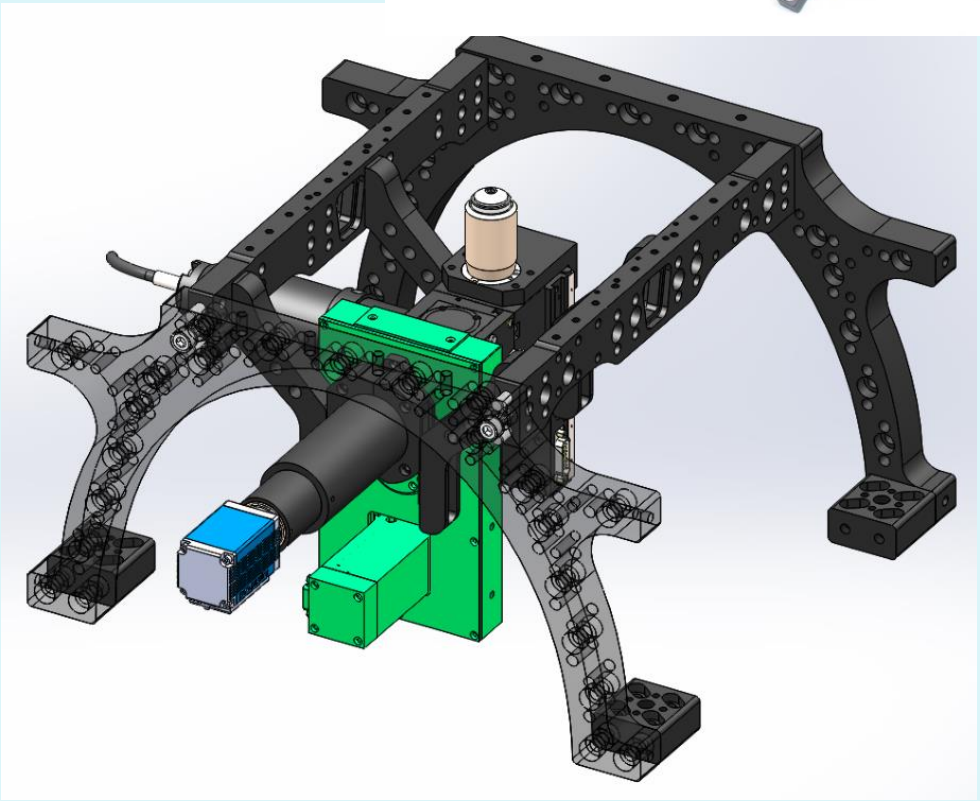
- Olympus IX2-LWUCD condenser
- ASI White LED Lamp
- ASI adjustable condenser carrier
- Olympus nose piece for DIC or Phase contrast brightfield imaging modes

# 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

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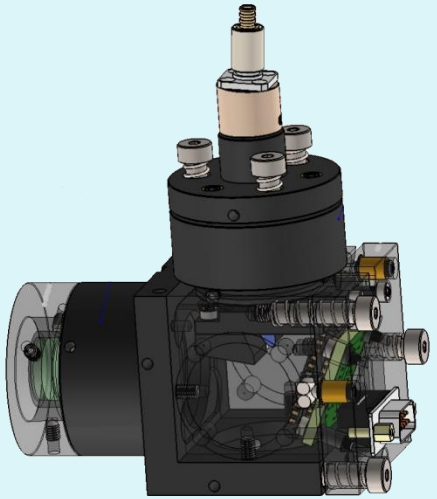
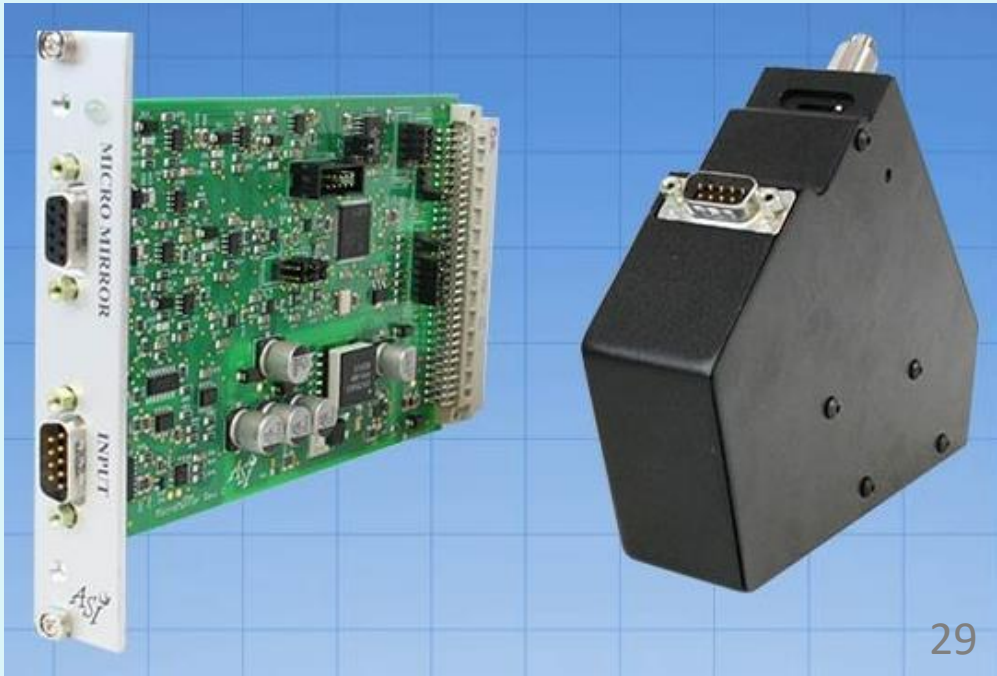
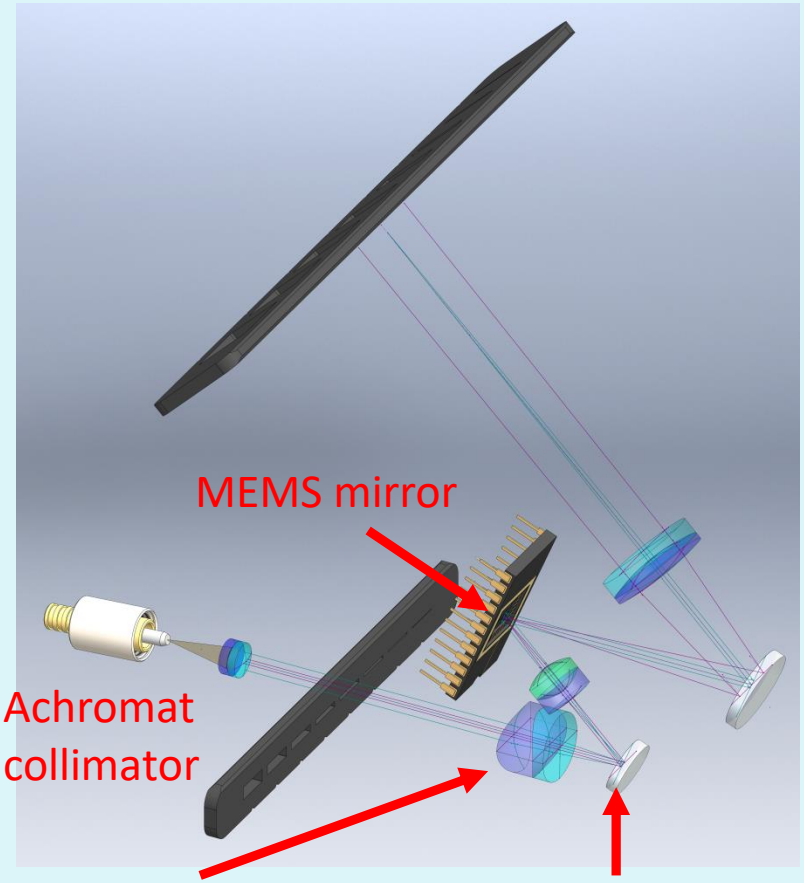
- 2-axis scanners with MEMS mirrors are compact, light weight, and zero vibration
- Fiber in, focused scanned beam at output C-mount image plane
- Applications include:
  - Light sheet
  - FRAP
  - Photo-stimulation



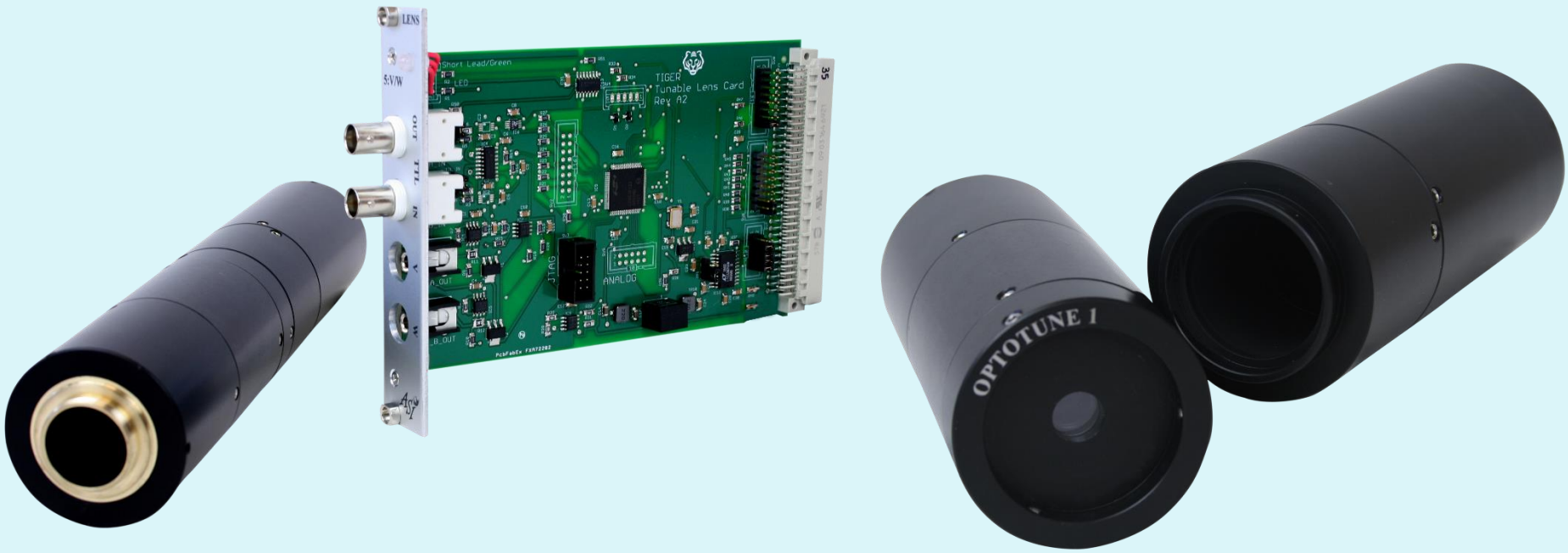


# Cylindrical lens scanner




- 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

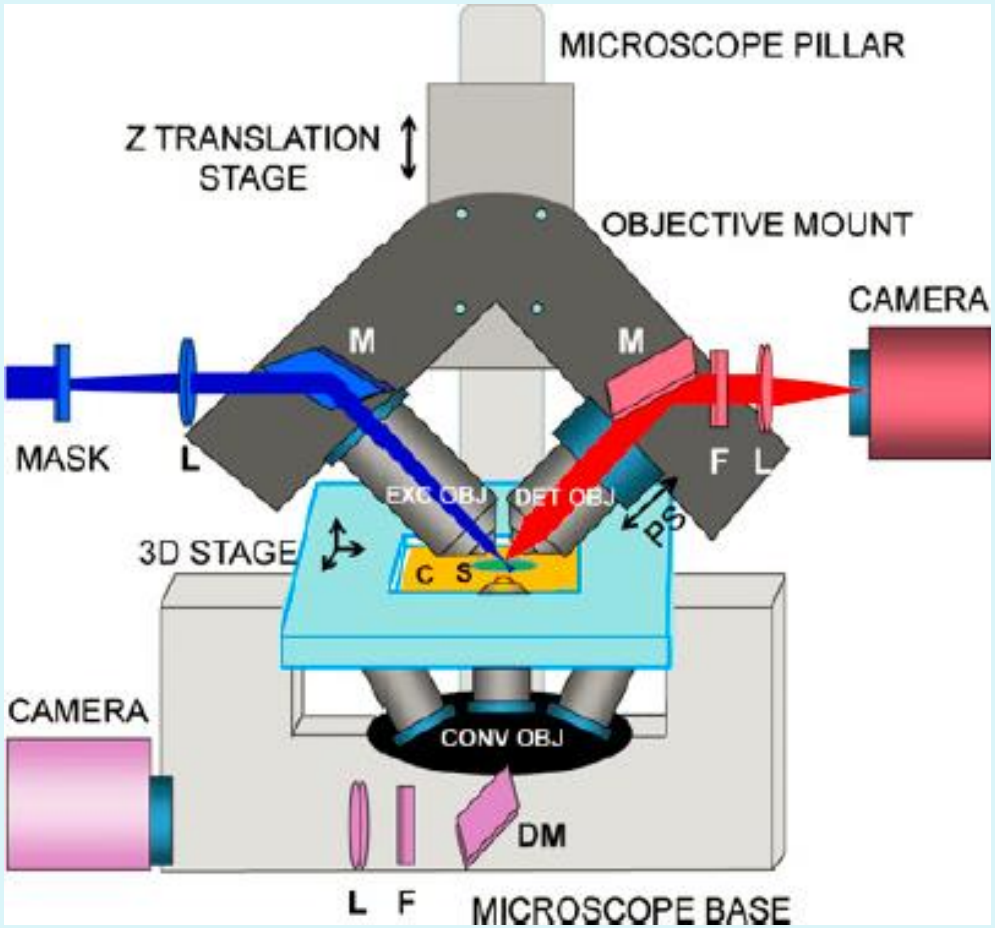
# Software support

- 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

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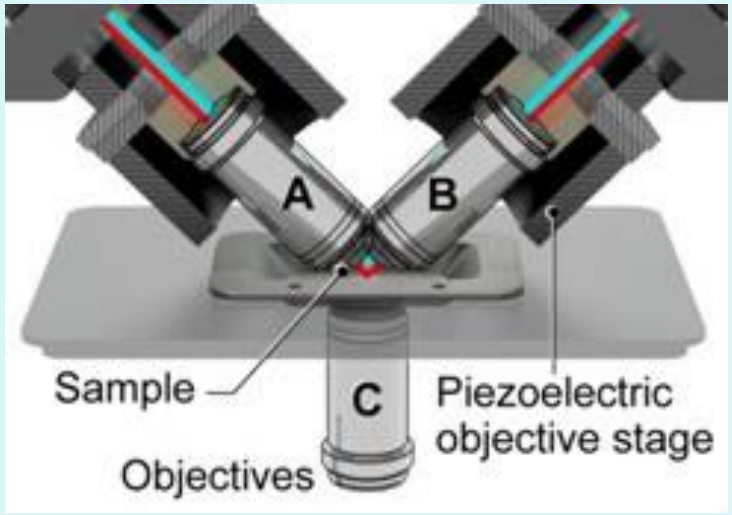
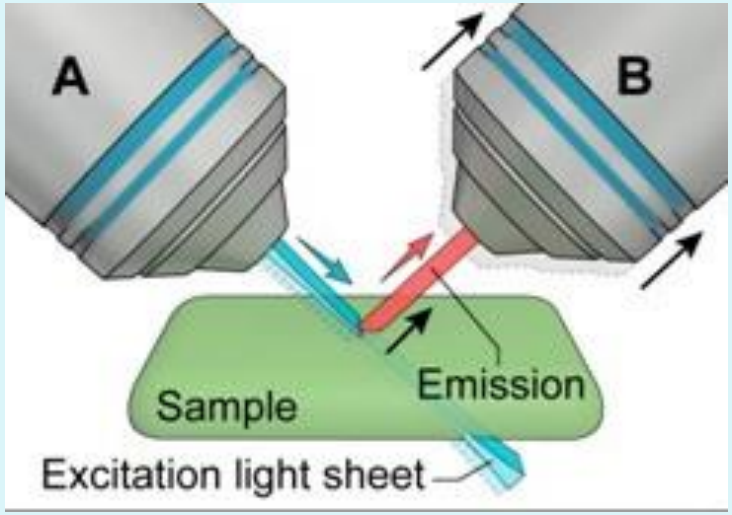
- Improve axial resolution of imaging objective
  - i.e. higher NA **(any single-view SPIM e.g. oSPIM)**
- Create light sheet thinner than objective's axial resolution **(lattice light sheet)**
- Combine datasets from different angles
  - Axial direction becomes lateral **(diSPIM, OpenSPIM)**
- Physically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

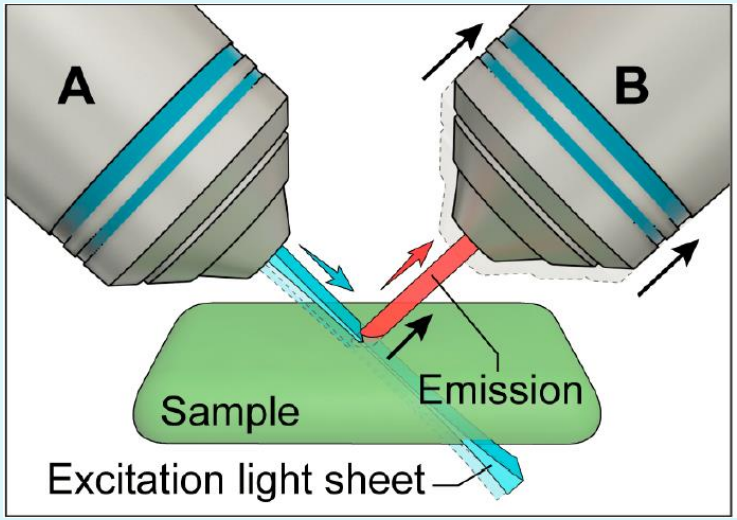
# diSPIM = dual-view SPIM on inverted microscope

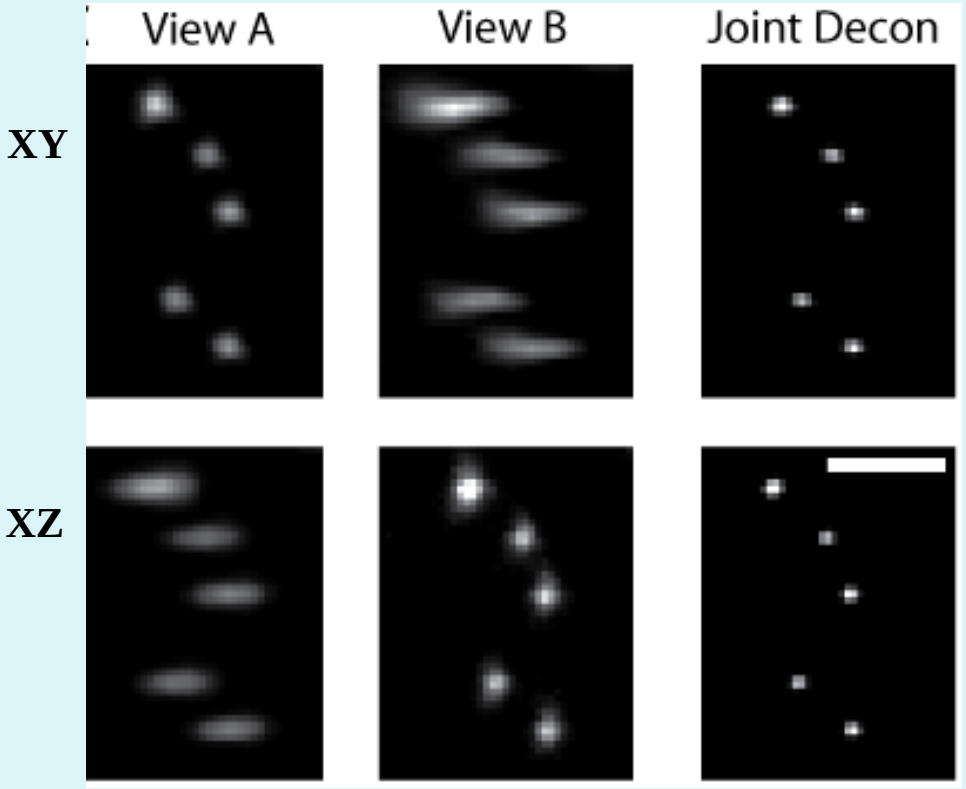



- Two (fixed) views → isotropic resolution
- Open-dish sample mounting
- Stacks by moving objective/light sheet or by moving stage

# Isotropic resolution by fusion



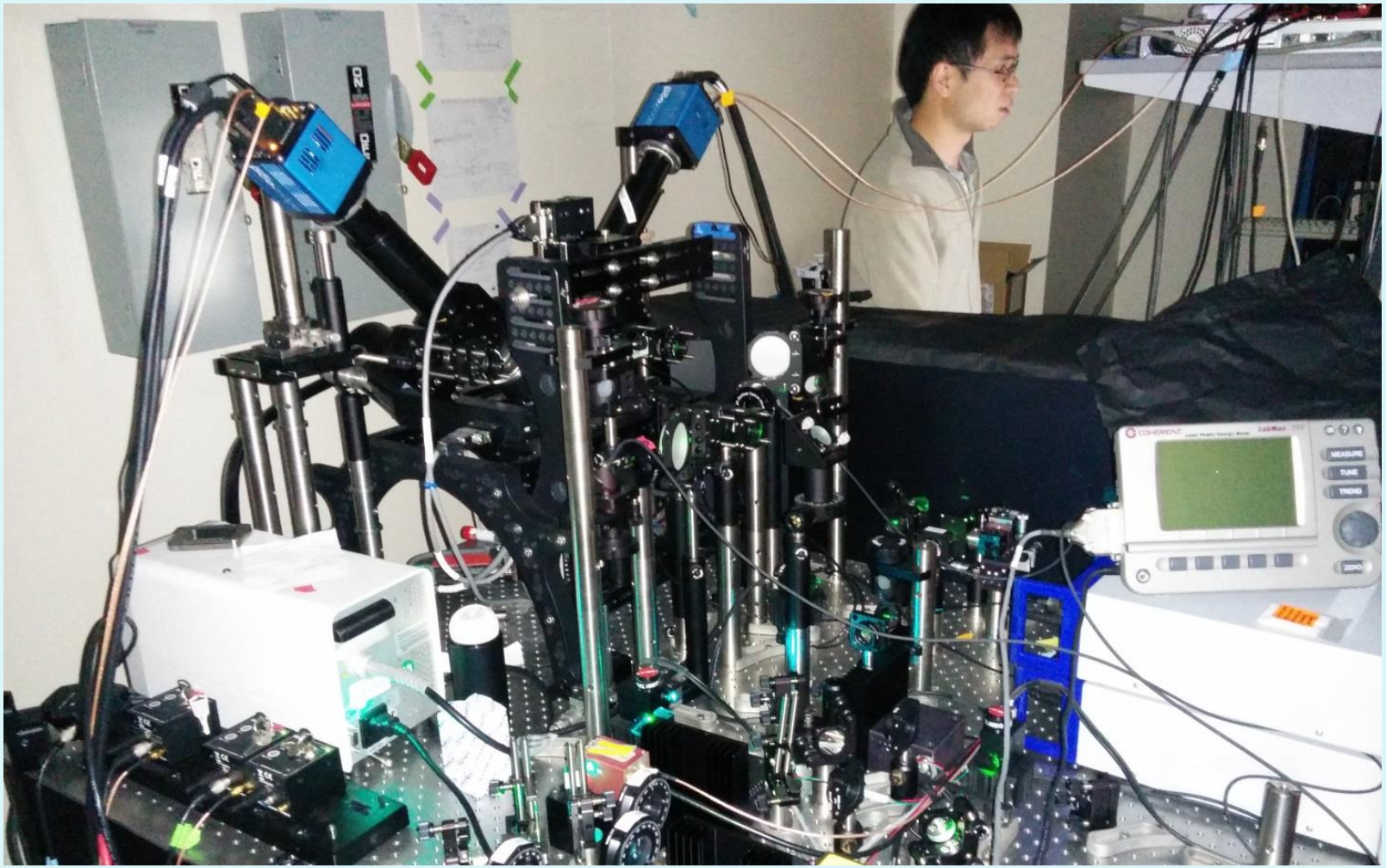




Joint Decon: A. York and Y. Wu

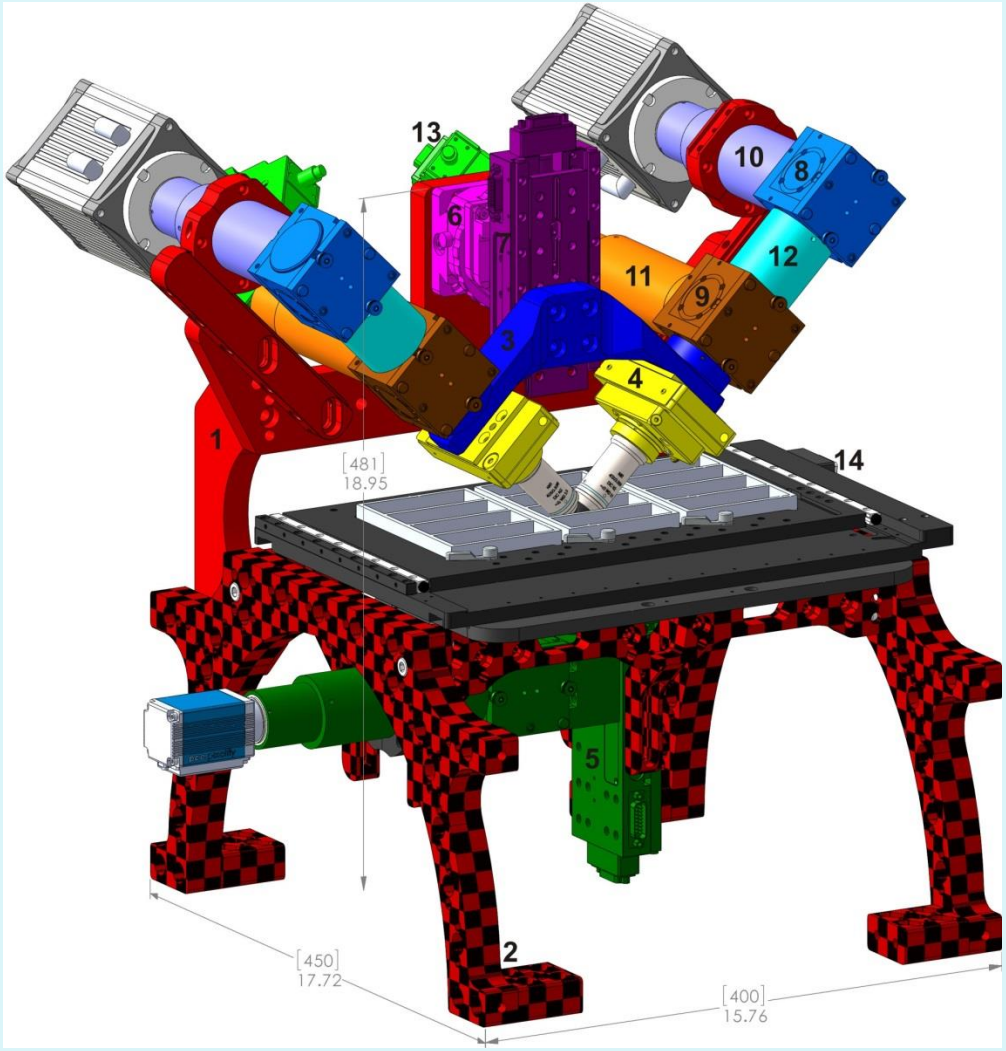
Wu et al. *Nat. Biotechnol.* 31, 1032-138 (2013),
Kumar et al. *Nature Protocols* 9, 2555-2573 (2014)

# Early diSPIM (2011?)

APPLIED SCIENTIFIC INSTRUMENTATION

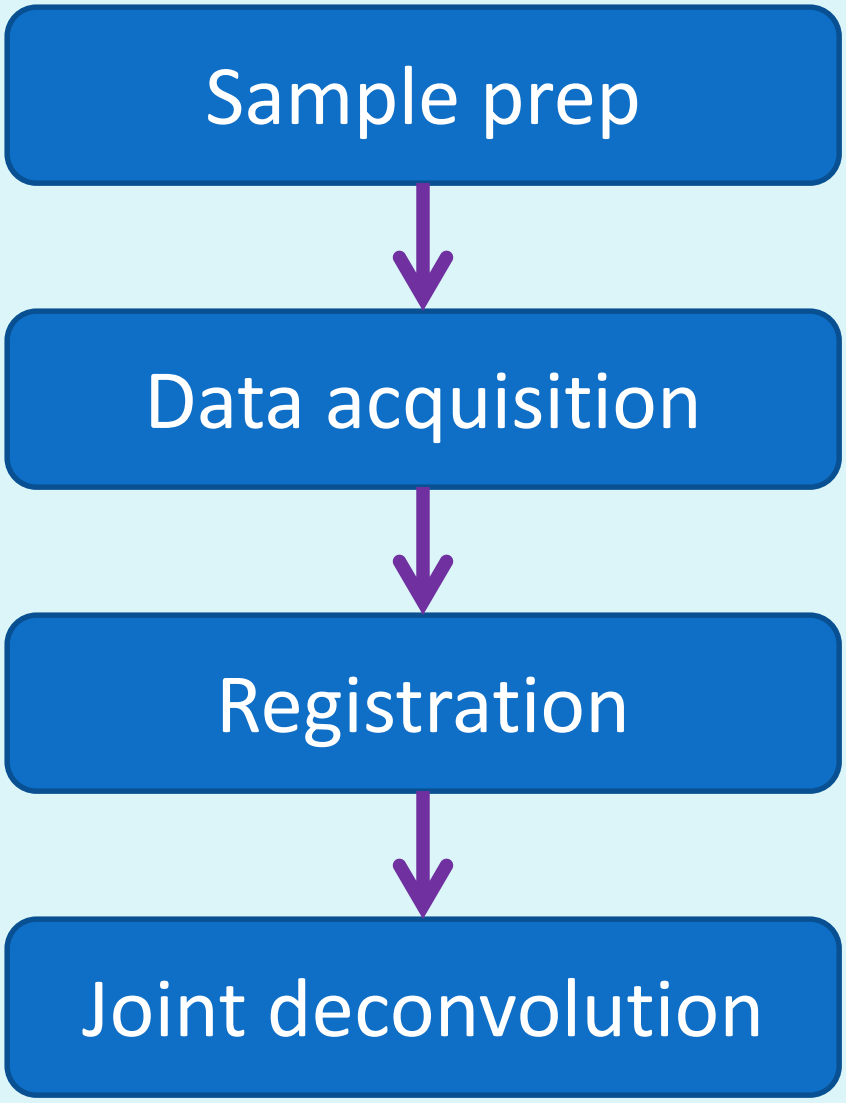


# Modern diSPIM




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)

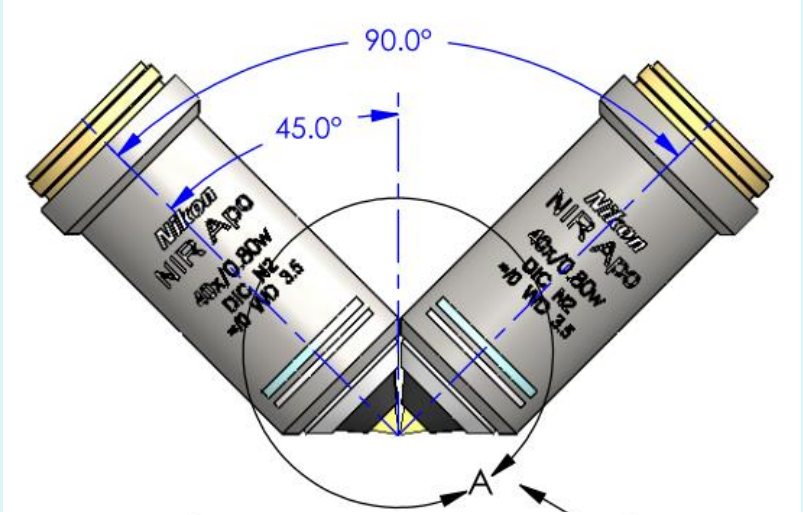
# diSPIM workflow

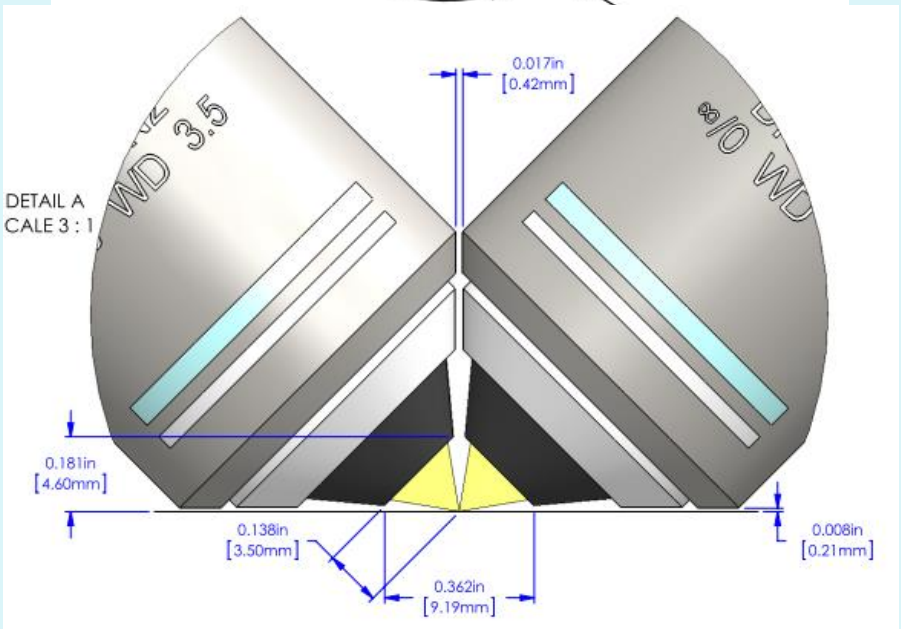



Shroff group LabView
Micro-Manager diSPIM plugin
3i's Slidebook
Others in future?

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 |

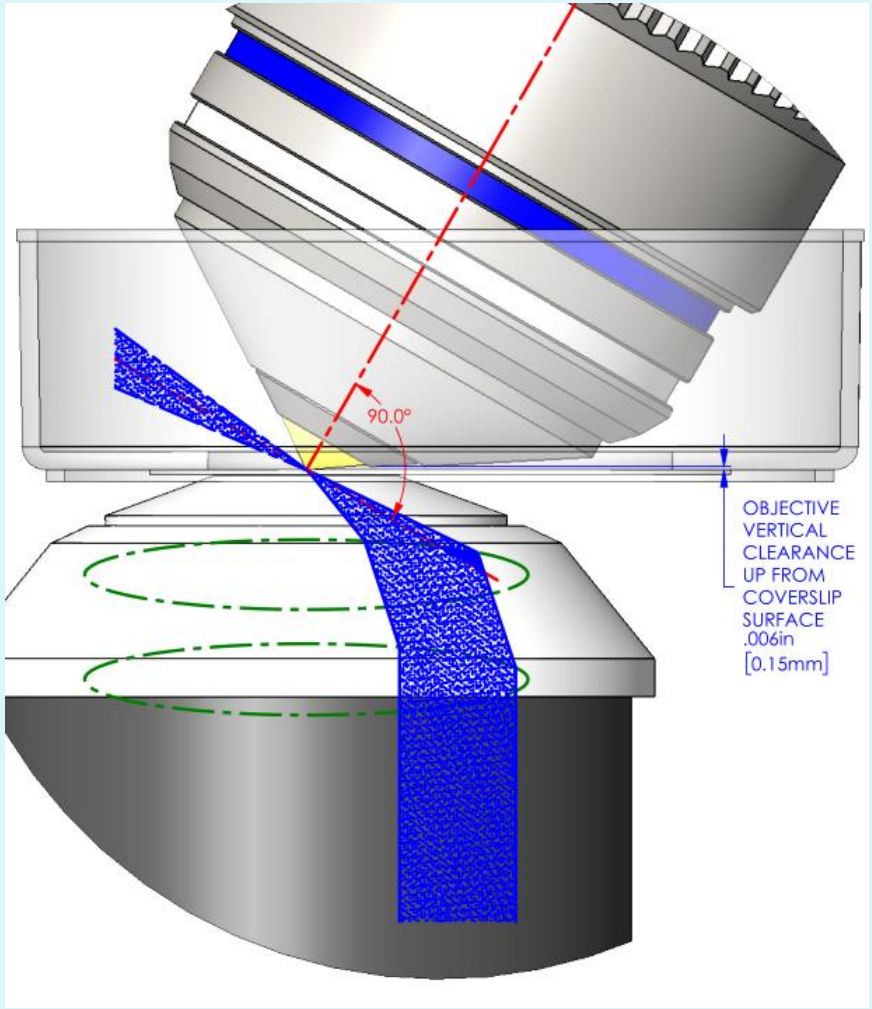
iSPIM/diSPIM, isotropic “lateral” resolution with post-processing

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

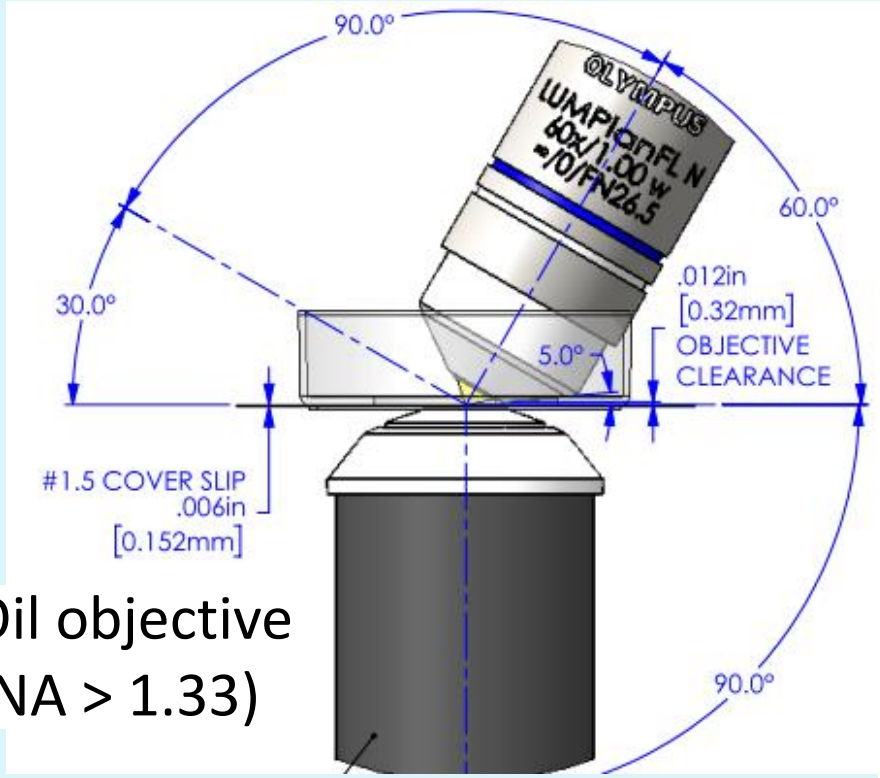
**NB: oSPIM/doSPIM design works up to NA 1.1**

# oSPIM objective geometry

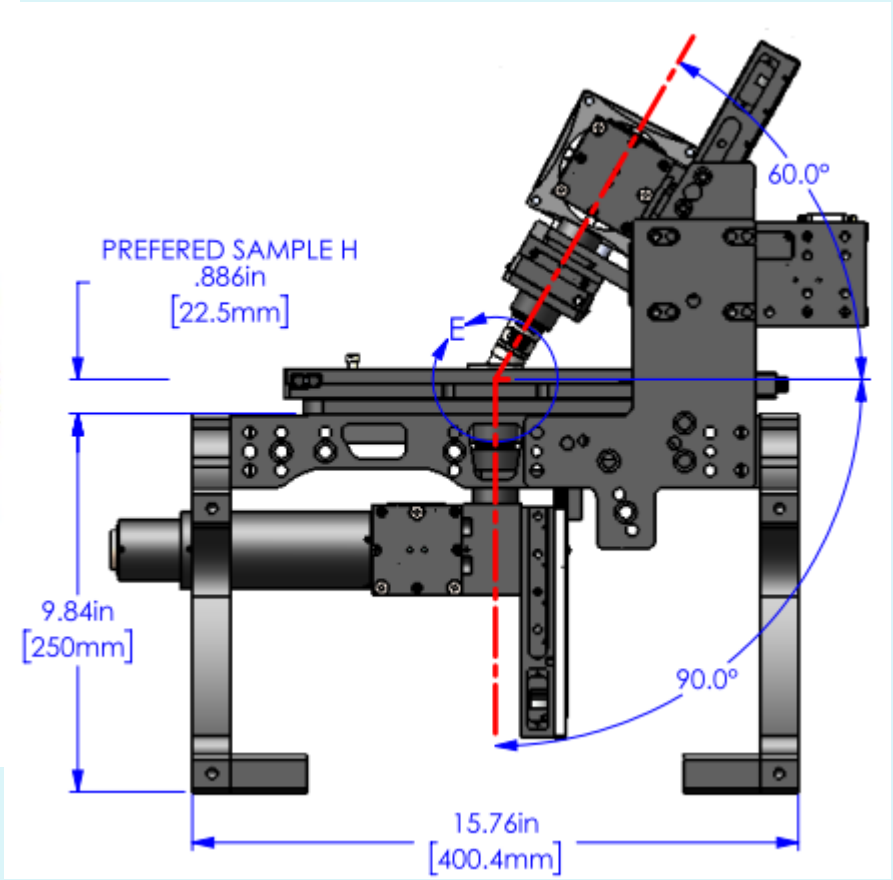
- Create light sheet sideways from objective by illuminating off-center in BFP (partway to TIRF)
  - → >90° objective angle
  - → higher NA objectives
  - → improved resolution
- Independently invented as “πSPIM” Sci. Rep. 6:32880 (2016)



# oSPIM implementation (single)




Oil objective
(NA > 1.33)




**Bottom objective creates tilted light sheet for imaging with top objective**

# doSPIM implementation (dual)

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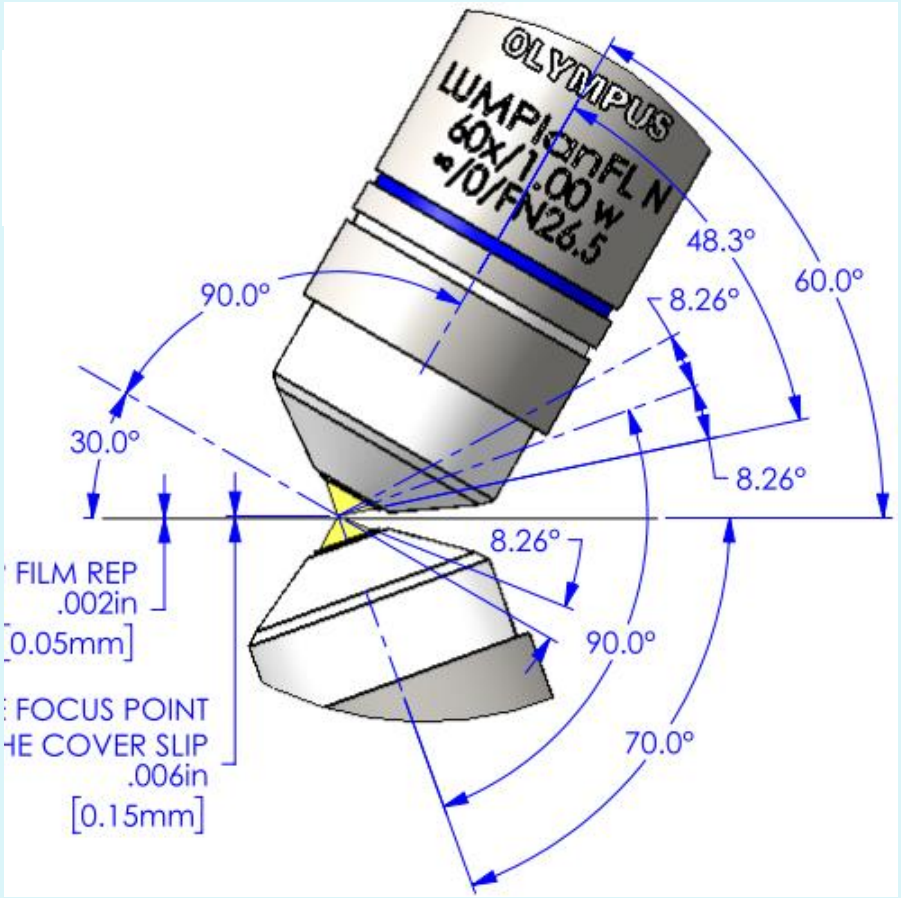
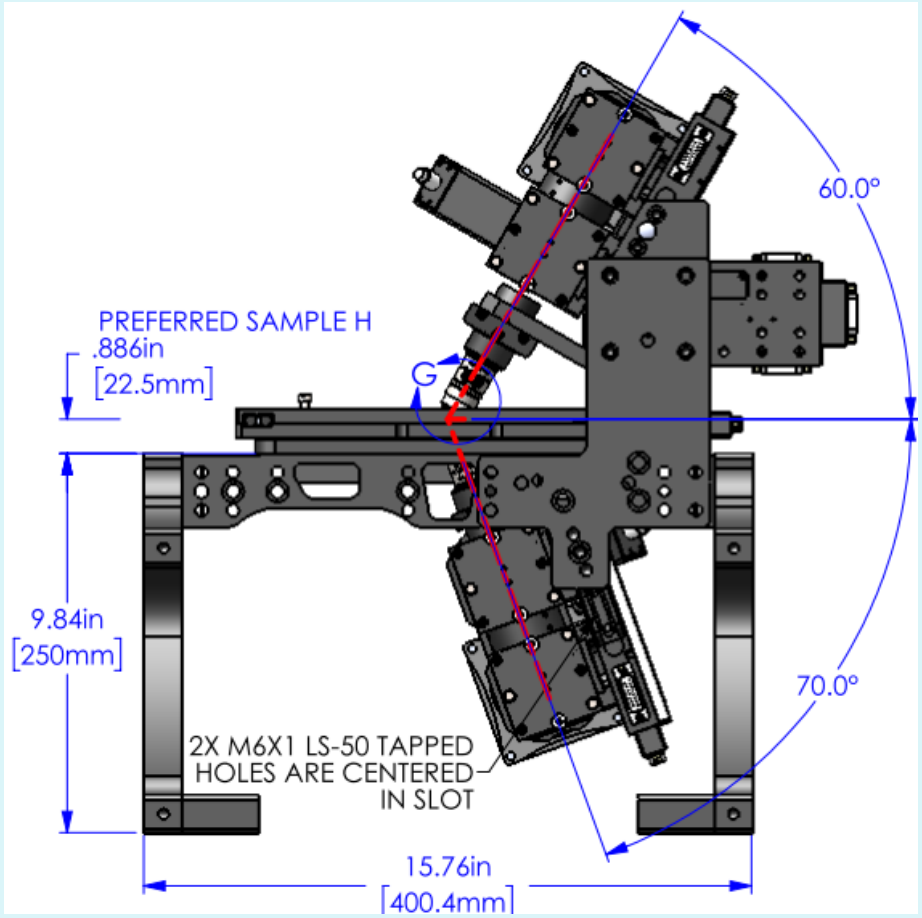
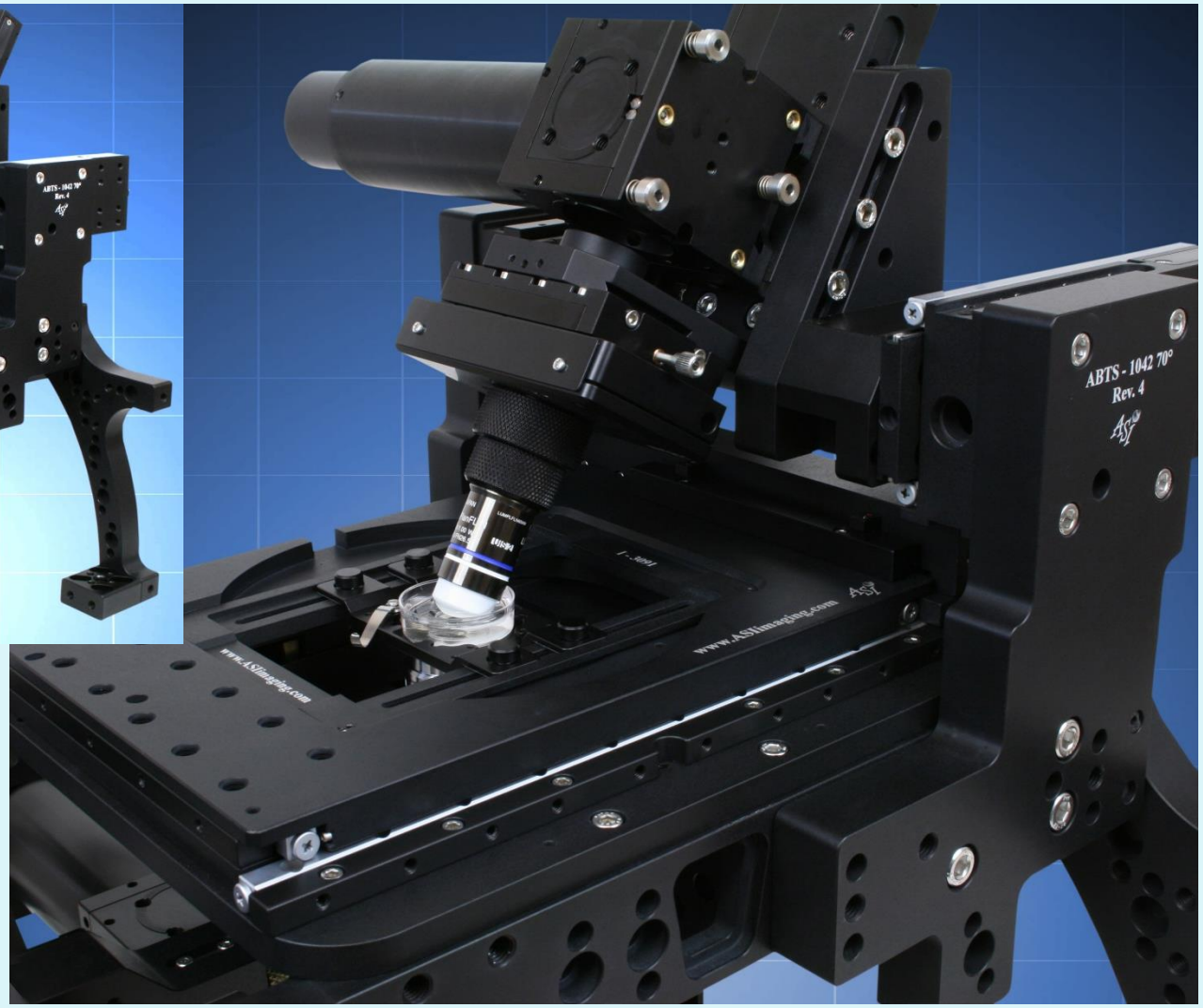



**Dual-view system, objectives sequentially generate light sheet and image like diSPIM**

# oSPIM in real life




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

# Cleared tissue objective

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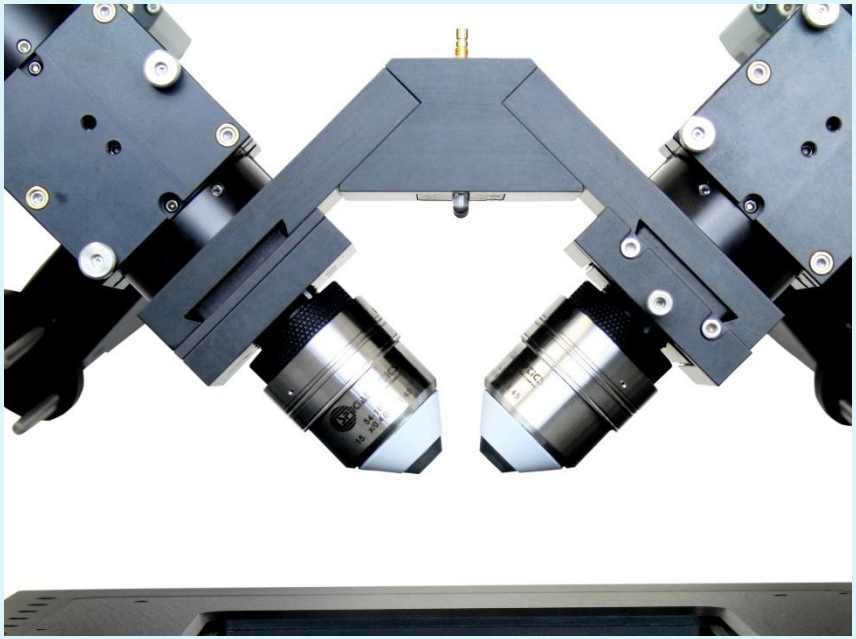
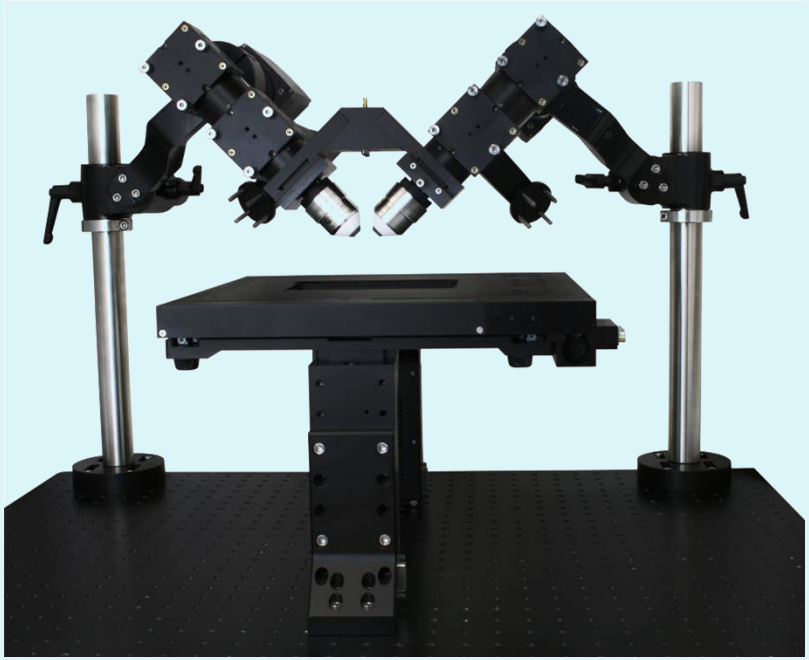
SPECIAL OPTICS A NAVITAR COMPANY

| 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



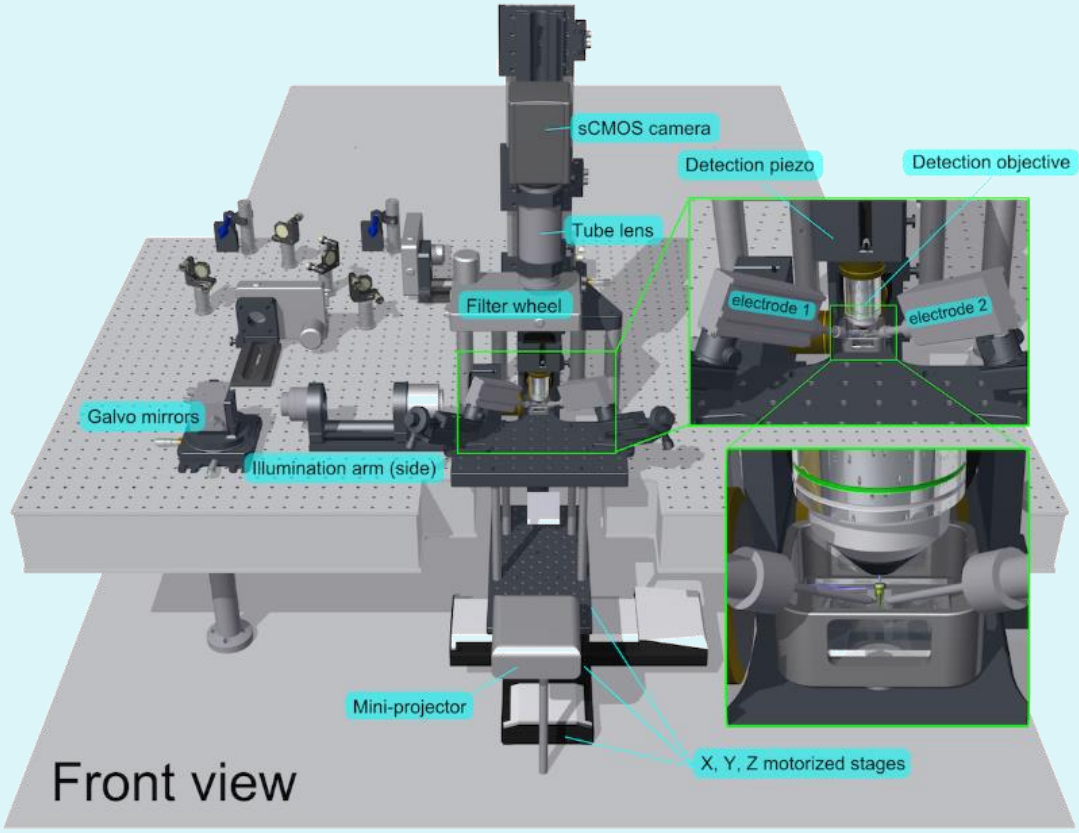


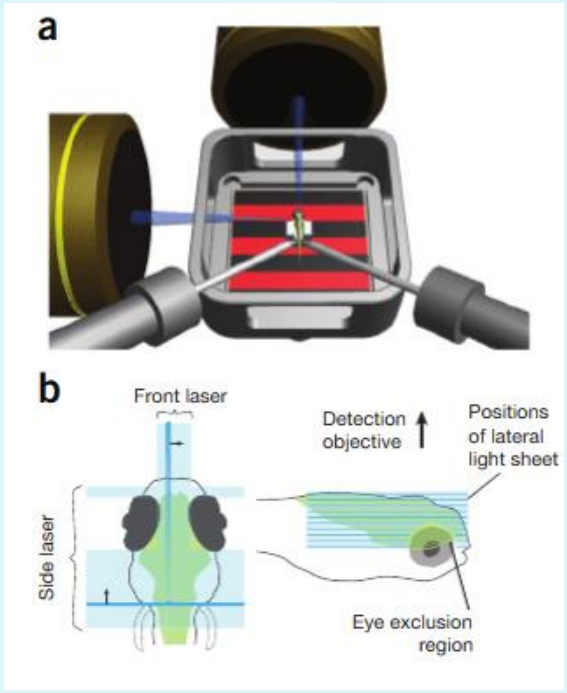
- No inverted microscope
- No objective piezos (stage scanning)

# 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!

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- Developing control software is necessary task that you don’t get much credit for…
  - User interface
  - Hardware control
  - Save images with metadata
  - Live view for alignment
- … but it’s already been done for you!
  - Spend your time on science, not on software

# Micro-manager plugin features

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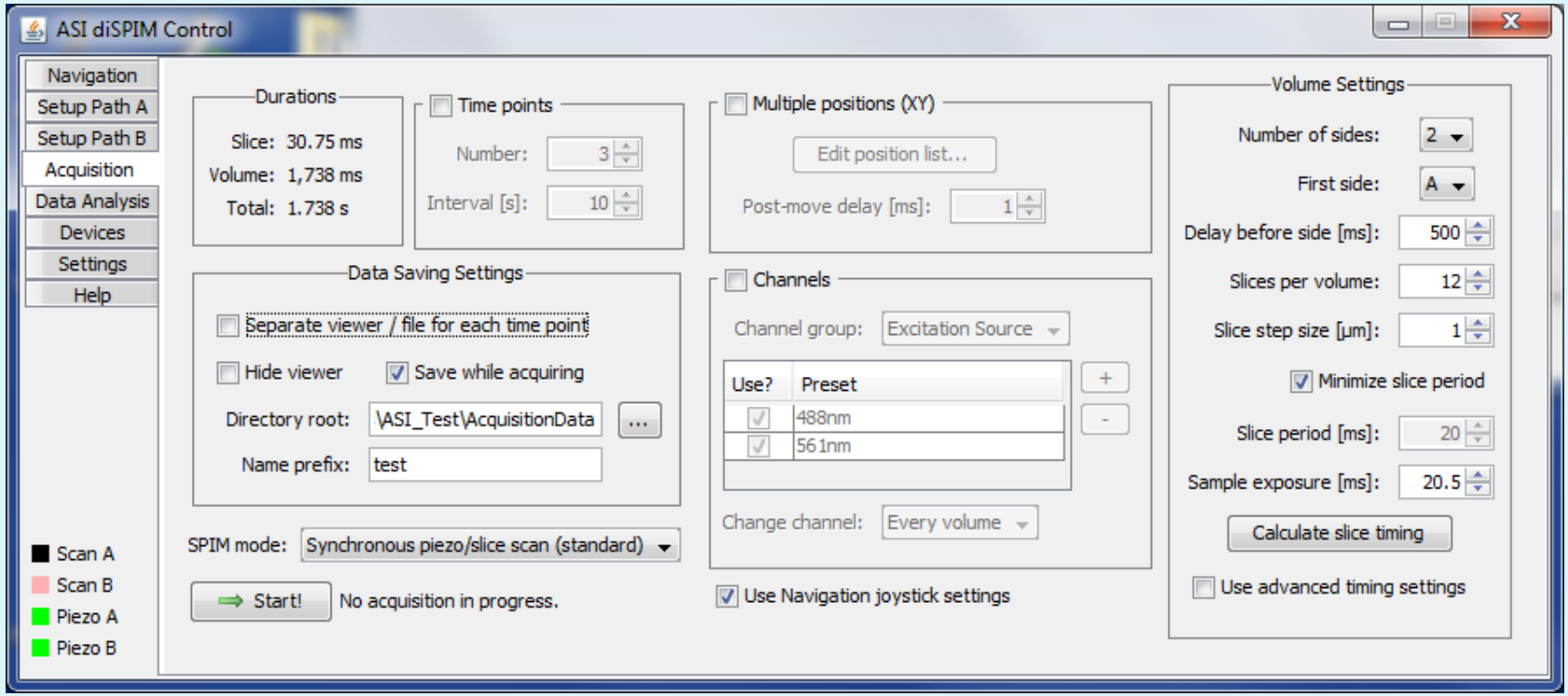
- Acquisition Modes:
  - Synch. slice/piezo
  - Fixed sheet
  - Stage scan
  - Virtual slit confocal
- Multi-Dimensional Acq.
  - Time points
  - Multi-position
  - Multi-channel
- Supported cameras:
  - Andor Zyla
  - PCO Edge
  - Hamamatsu Flash 4
  - Photometrics 95B
- Supported lasers:
  - Lasers 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

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- 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 for other labs to use same software

# 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?**