Light Sheet Microscopy: Basic principles, iSPIM/diSPIM, oSPIM/doSPIM

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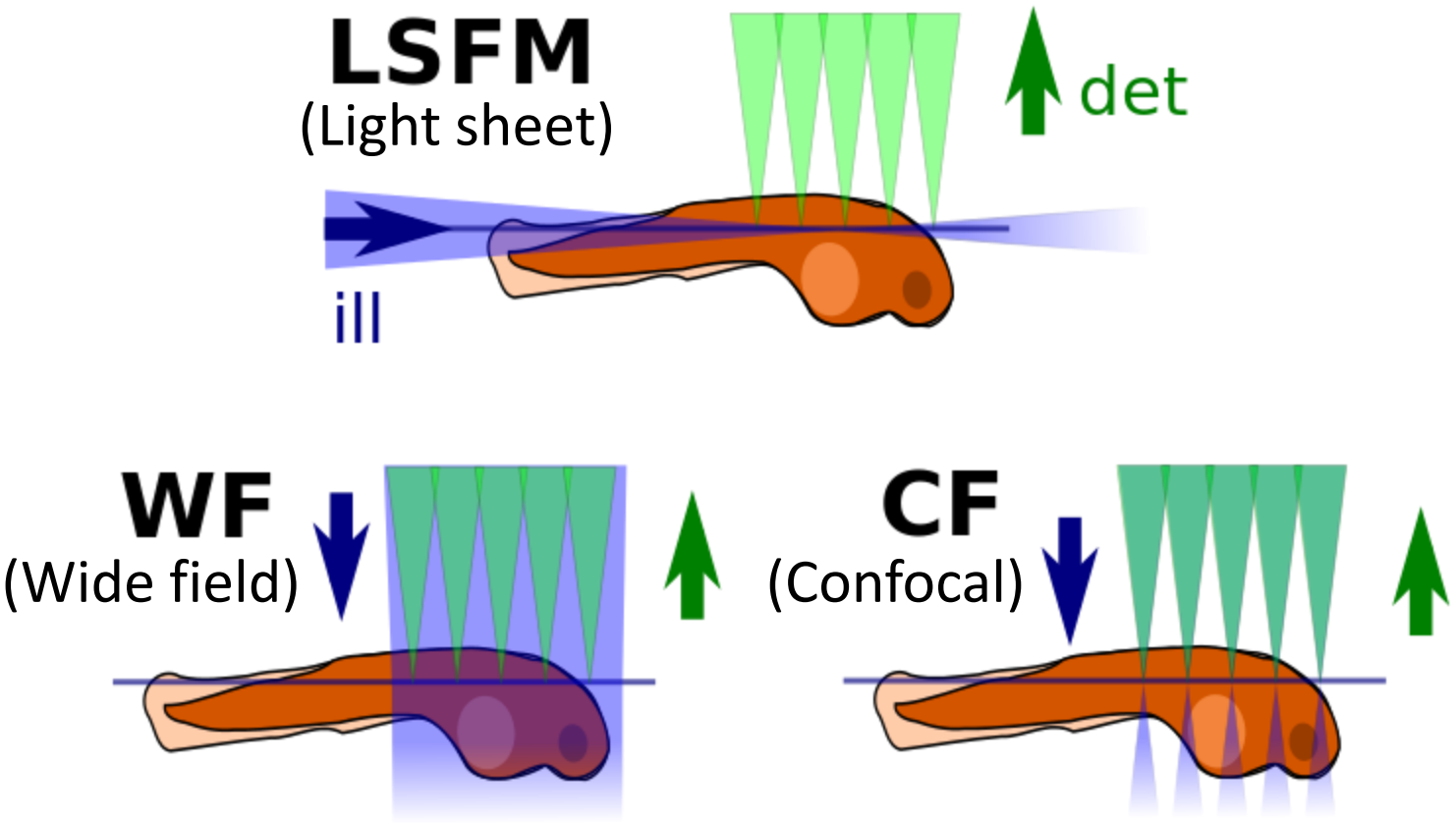
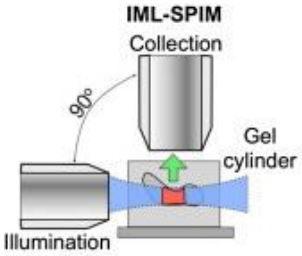
What is Light Sheet Microscopy?

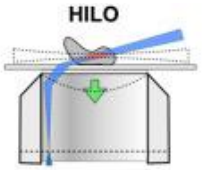


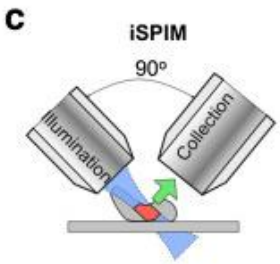
Why Light Sheet Microscopy?

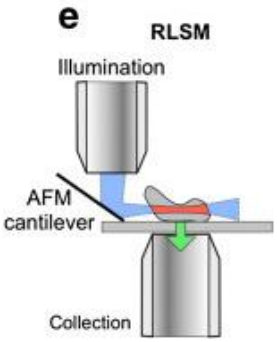
- Minimize photodamage/bleaching
  - "photon budget" (+ nonlinear effects)
  - Keeps living things alive longer
- Rapid acquisition
  - 2D parallel imaging
- What does it cost? Optics for light sheet illumination

Some Light Sheet Configurations

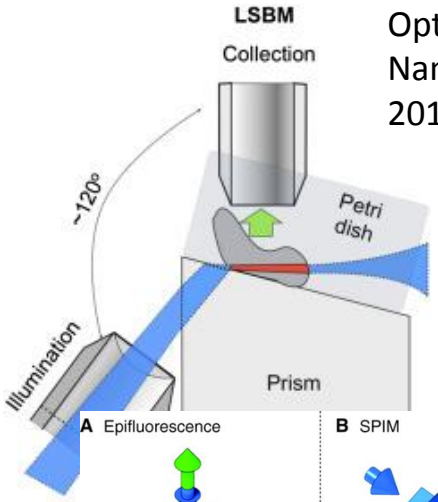




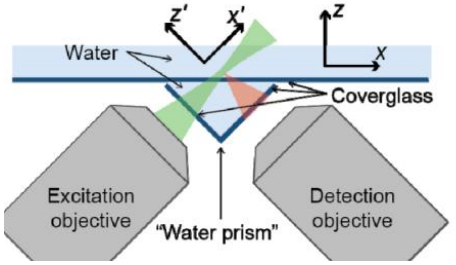




Nature Meth 2015 12: 30–34

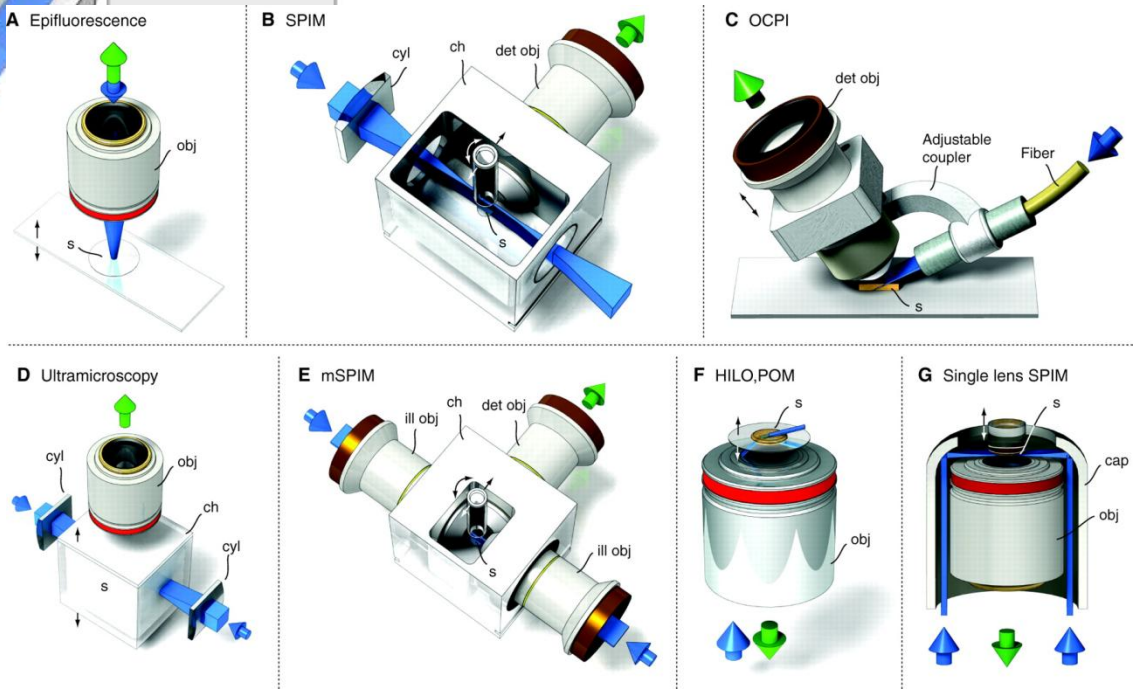


Optical Nanoscopy 2013 2:7



Optics Express 2015; 23: 16142-16153

Development 2009; 136:1963-1975



Commercial Light Sheets

Type	# views	Mounting	Software	Comments
iSPIM/ diSPIM	1/2 fixed (isotropic)	Coverslip or dish with media	Free/open + various proprietary	Modular/flexible configuration, allows simultaneous photomanipulation
Zeiss Z.1	Unlimited (isotropic)	Capillary with agarose	Single proprietary	Rotation allows imaging scattering samples from both sides
Leica TCS SP8 DLS	1 fixed	Dish with media	Single proprietary	Add-on to existing Leica confocal
LaVision BioTec	1 fixed	Dish with media	Single proprietary	Optimized for large fixed samples (low mag, low res)

In early commercialization: oSPIM/doSPIM (ASI), Lattice Light Sheet (3i), MuVi-SPIM (Luxendo)

Light Sheet Resolution is Anisotropic

- Lateral = 0.61*λ/NA Axial = 2*λ/NA^2

  (confocal usually provides small improvement but still anisotropic)

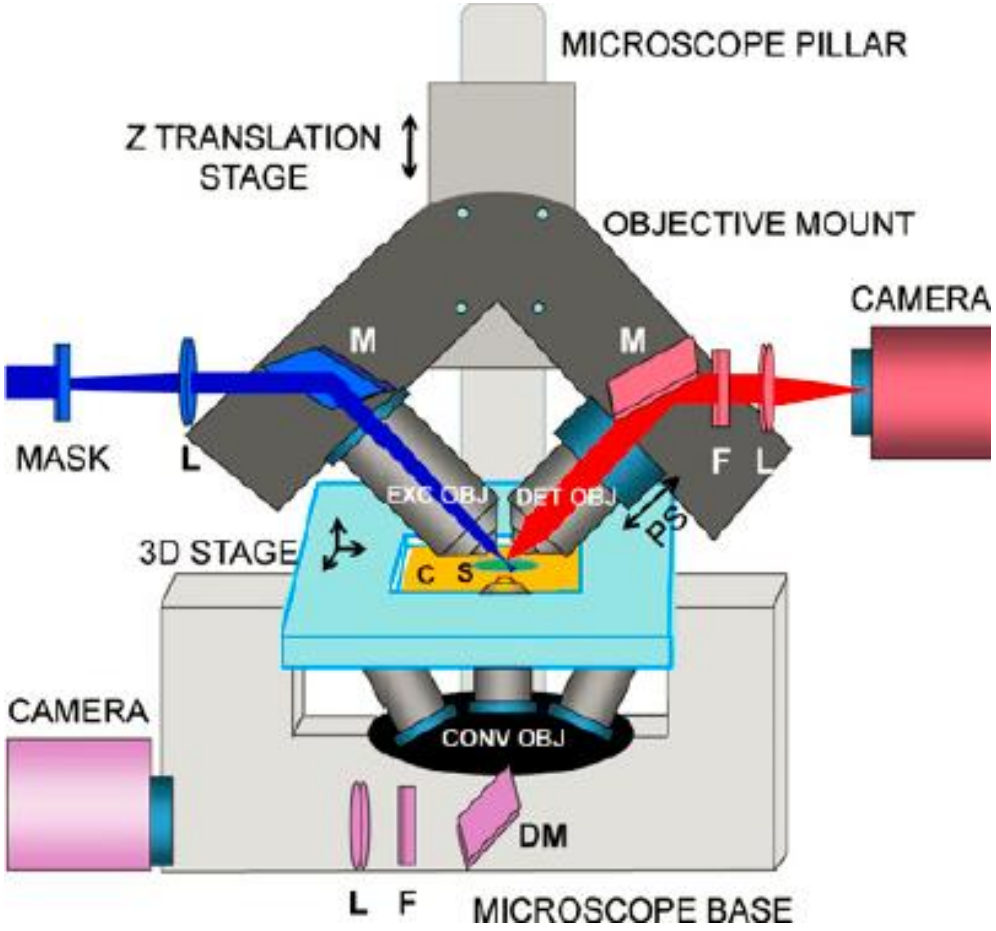
NA	Lateral @ GFP [nm]	Axial @ GFP [nm]
0.3	1037	11333
0.6	519	2833
0.8	389	1594
1.0	311	1020
1.2	259	708
1.4	222	520

NA	Lateral [AU]	Axial [AU]
0.3	2.00	21.8
0.6	1.00	5.44
0.8	0.75	3.06
1.0	0.60	1.96
1.2	0.50	1.36
1.4	0.43	1.00

Ways of Improving (Axial) Resolution

- 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

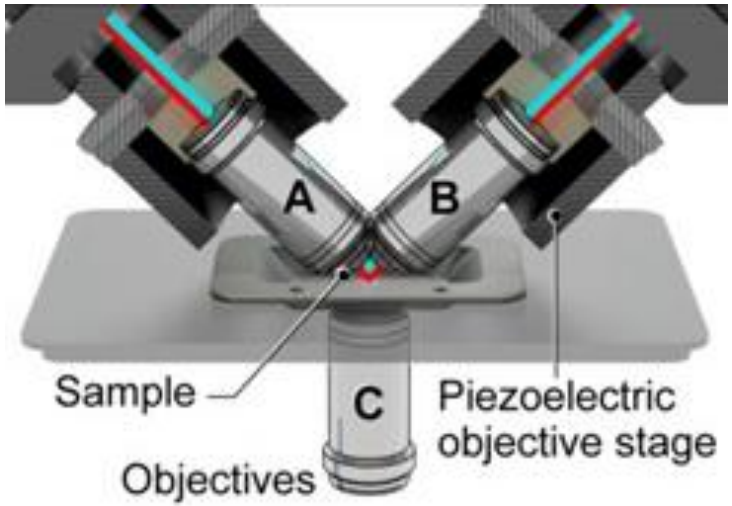
Original iSPIM Concept



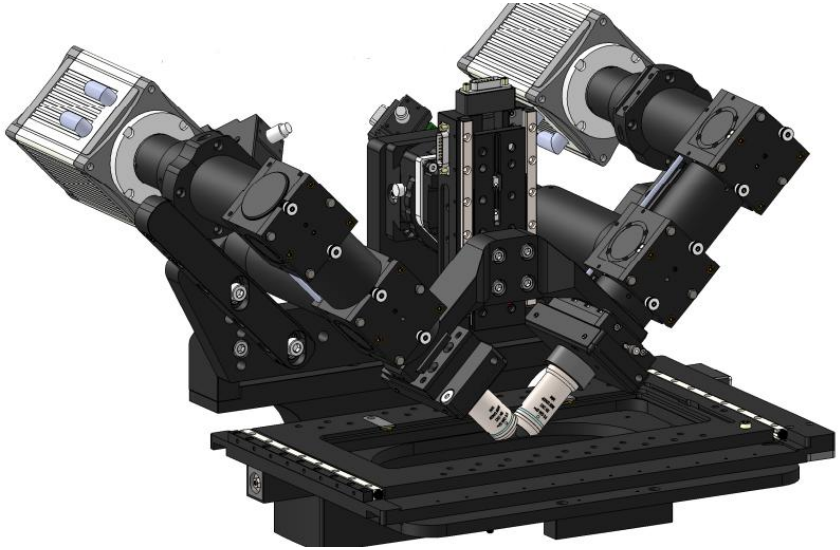
- SPIM on inverted microscope
- Sample mounted on standard glass coverslip
- 30x faster than spinning disk for same SNR

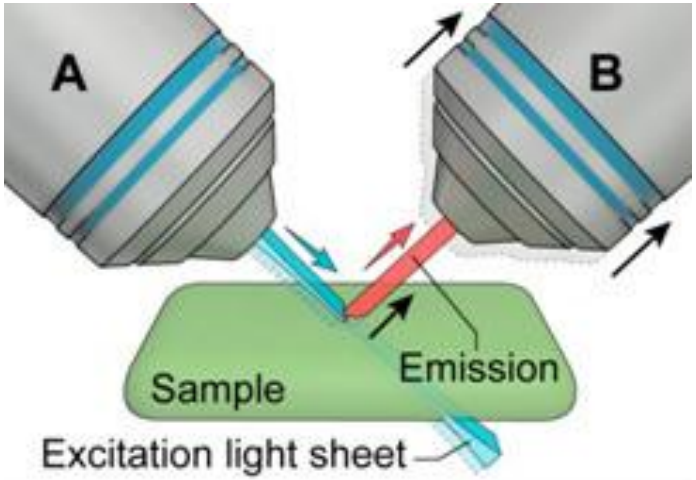
Wu et. al, PNAS 108, 17708-17713 (2011)

diSPIM = dual-view SPIM on inverted microscope

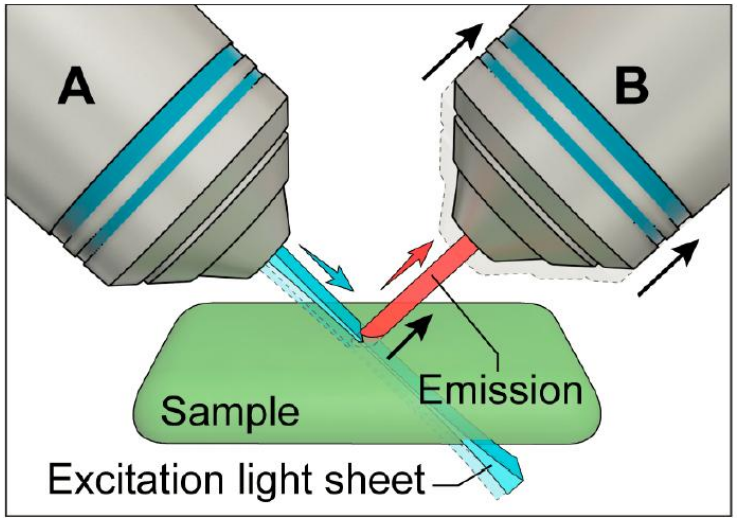


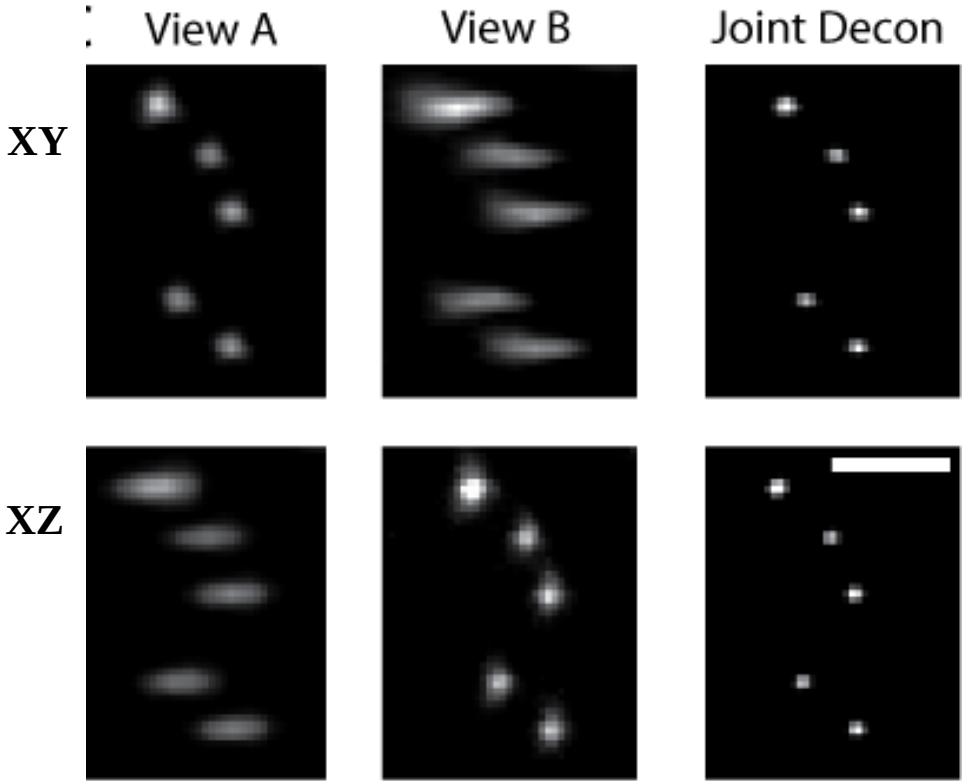
- Light sheet on inverted microscope
- Two (fixed) views → isotropic resolution
- Open-dish sample mounting





Isotropic Resolution

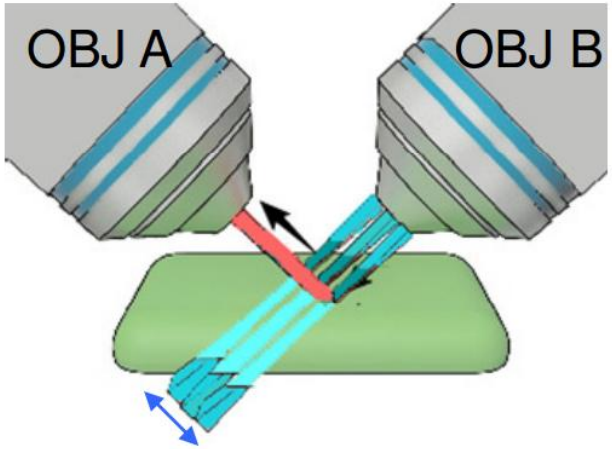


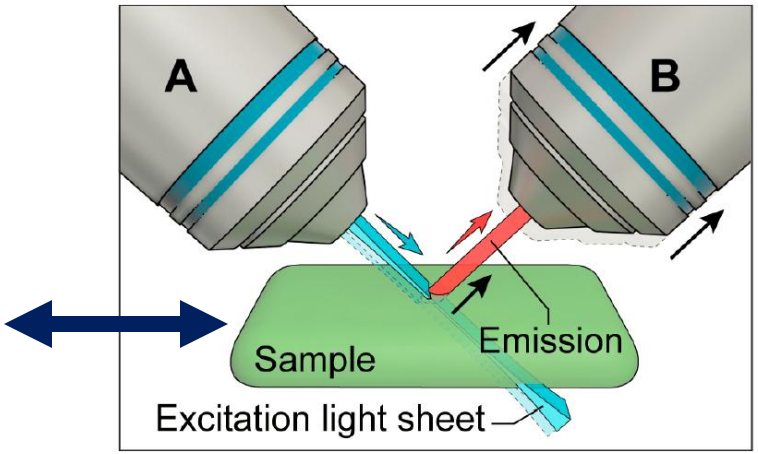


Joint Decon: A. York and Y. Wu

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

Two ways of creating stacks



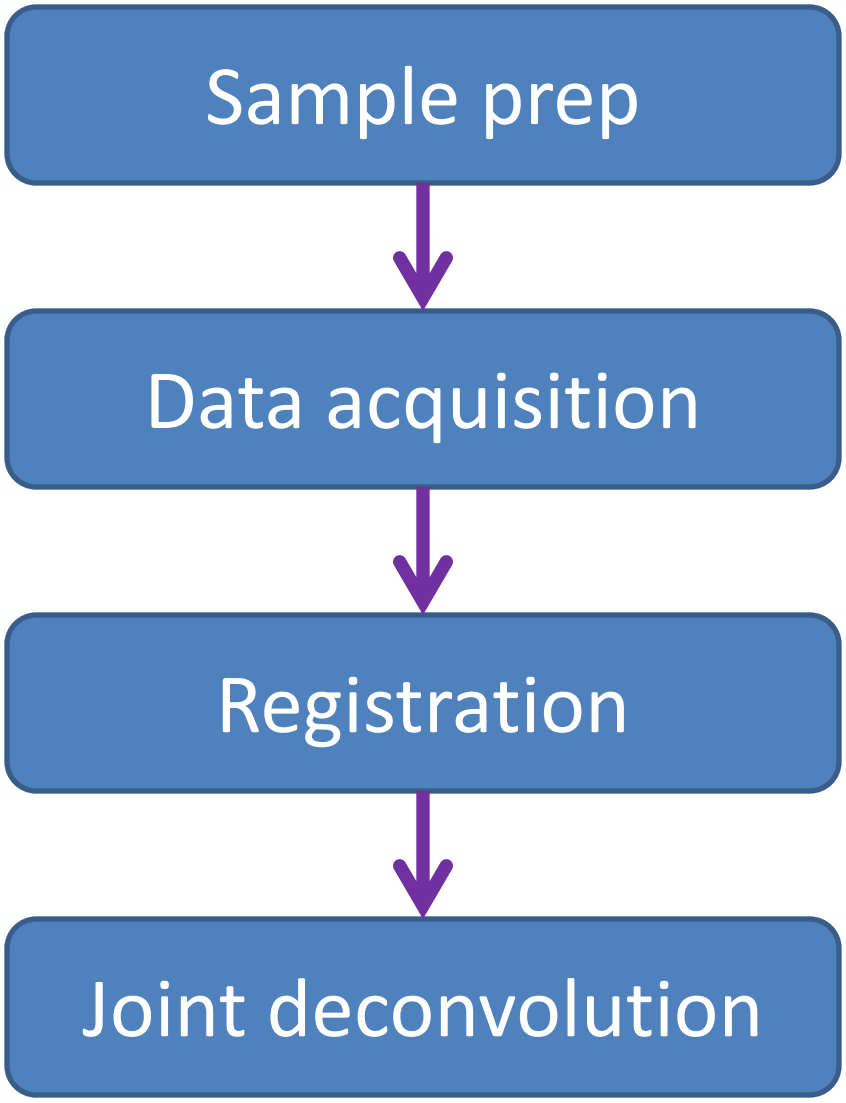


Move light sheet and imaging objective together through sample

Move sample through fixed light sheet using stage

All ASI SPIM systems support both imaging modes

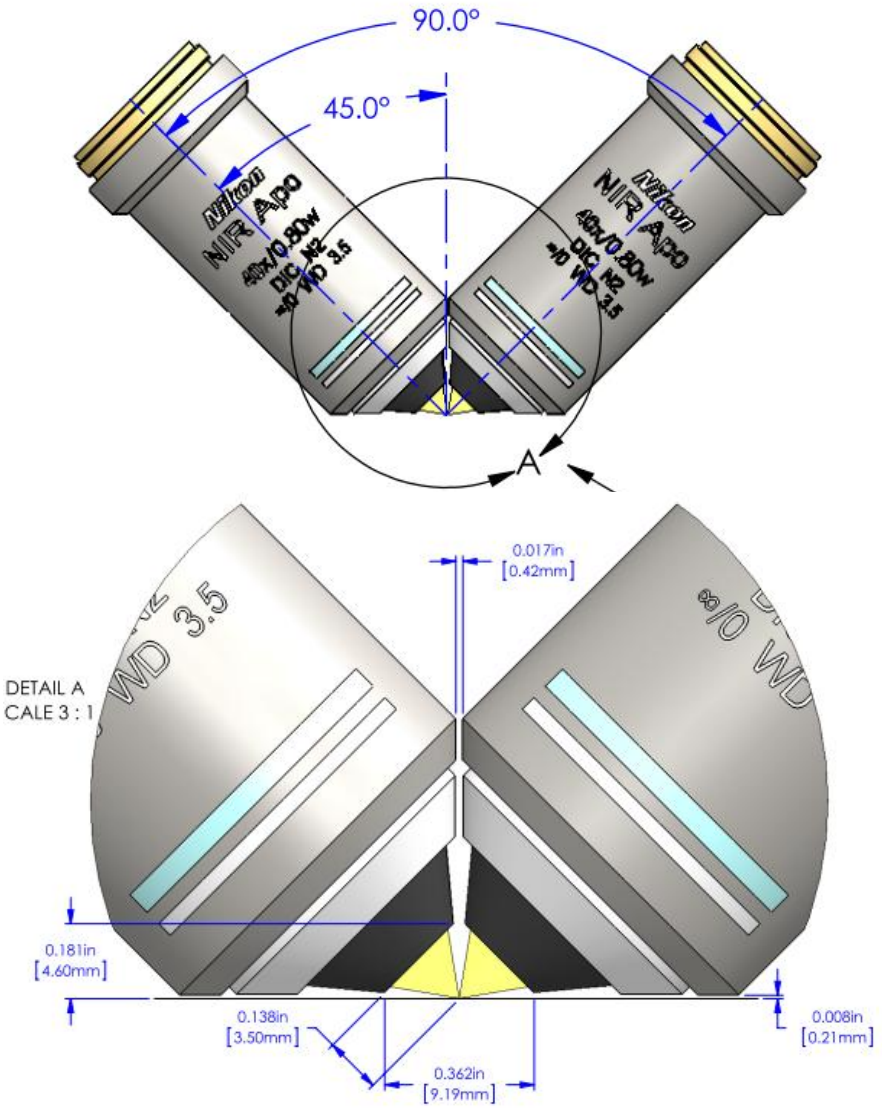
diSPIM Workflow



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

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

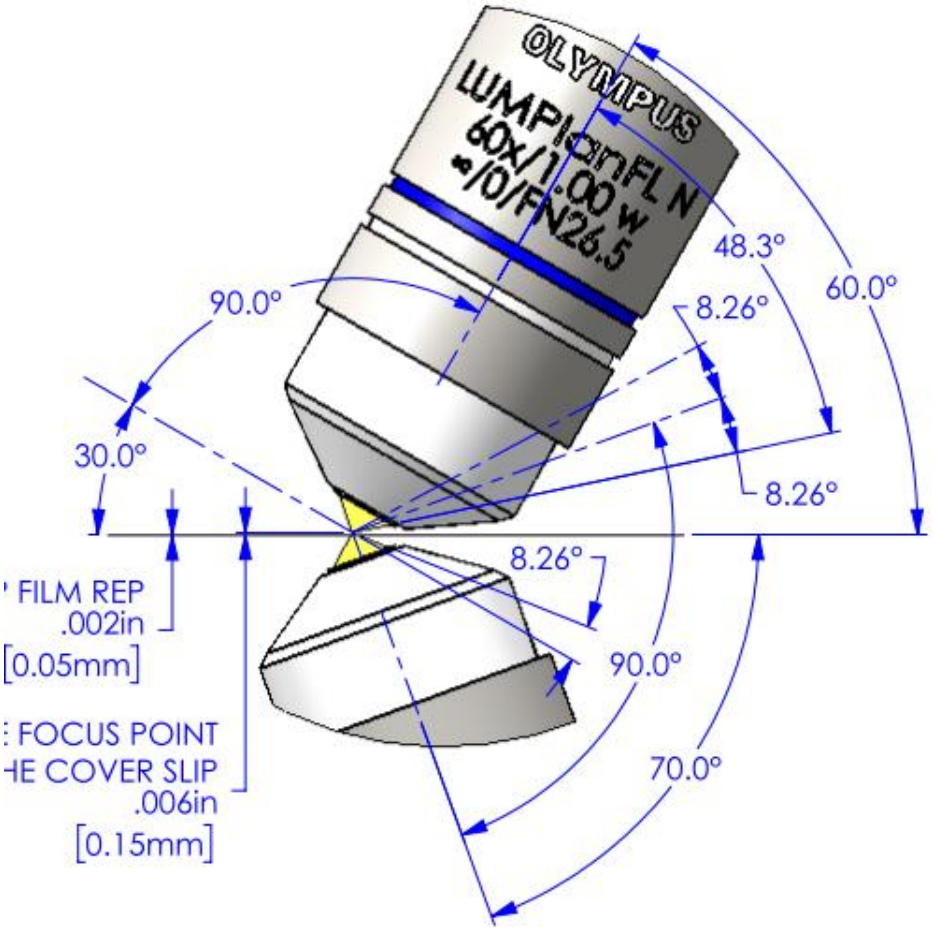
diSPIM Objectives



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

“(d)oSPIM” = (dual) oblique SPIM

- Create light sheet out sideways from objective (coincident with imaging plane) by illuminating off-center in BFP (partway to TIRF)
- => >90° objective angle
- => higher NA possible



Θ = 48° for NA 1.0 in water

Θ = 56° for NA 1.1 in water

oSPIM/doSPIM Resolution

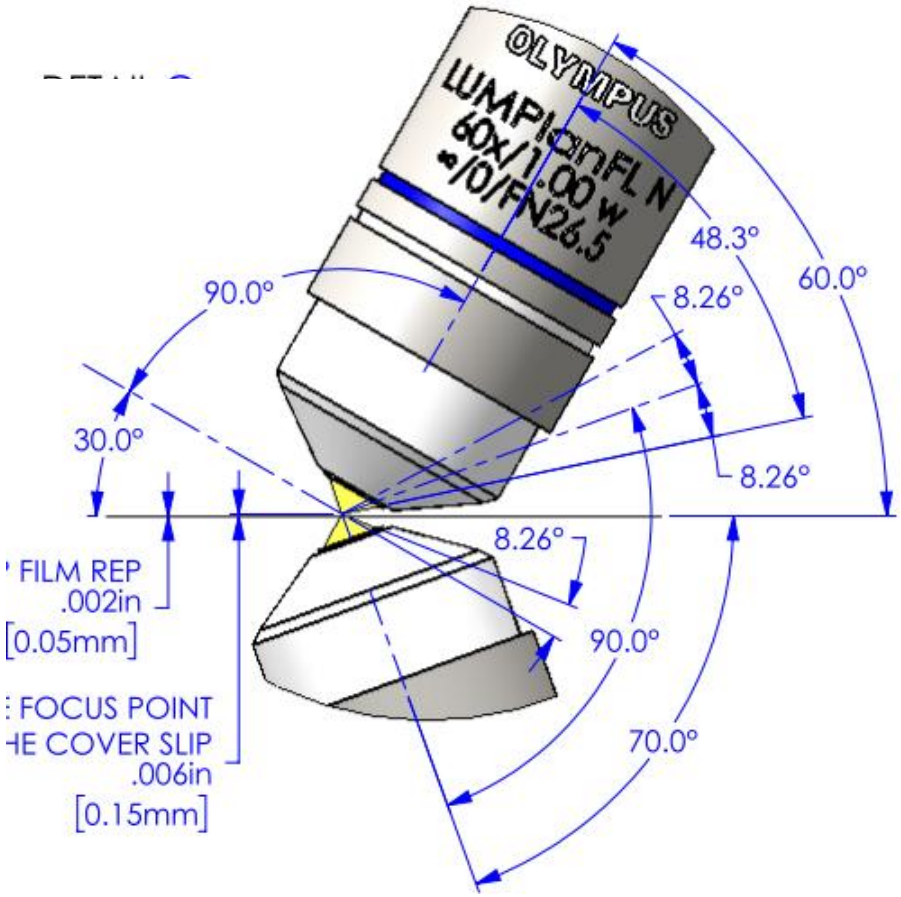
NA	Lateral @ GFP [nm]	Axial @ GFP [nm]
0.3	1037	11333
0.6	519	2833
0.8	389	1594
1.0	311	1020
1.2	259	708
1.4	222	520

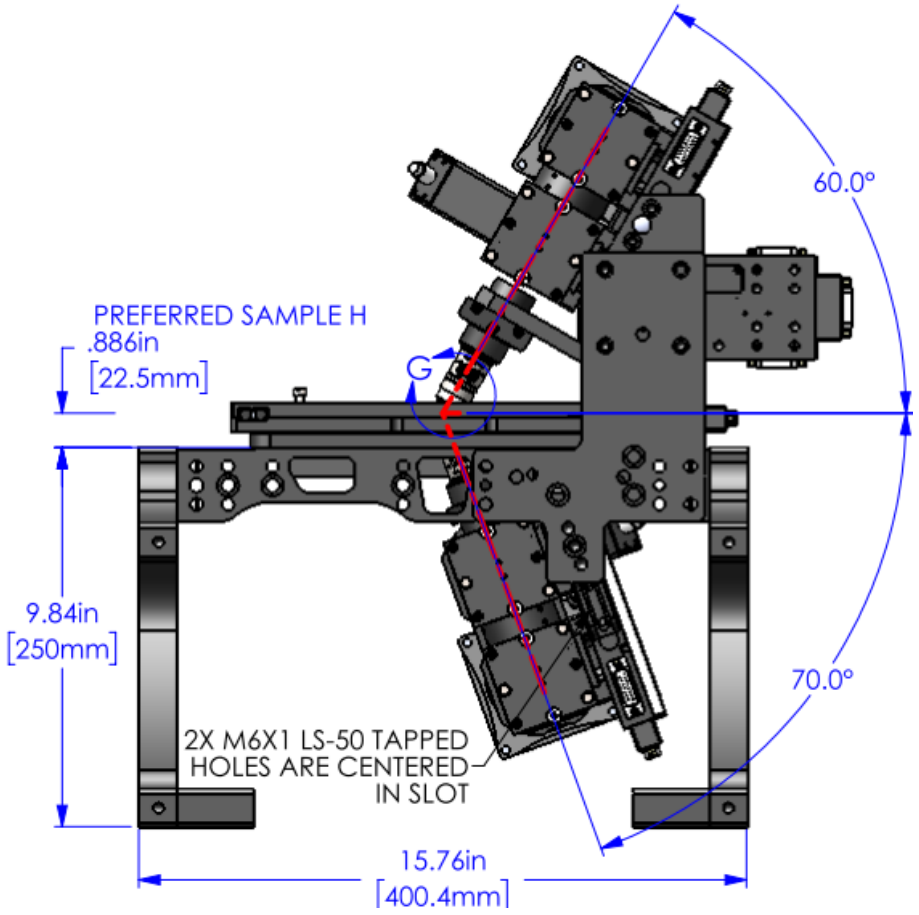
iSPIM/diSPIM, isotropic “lateral” resolution with post-processing

oSPIM/doSPIM, @ NA 1.0 has 20% better lateral resolution than diSPIM and (single-view) axial resolution ~1um

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

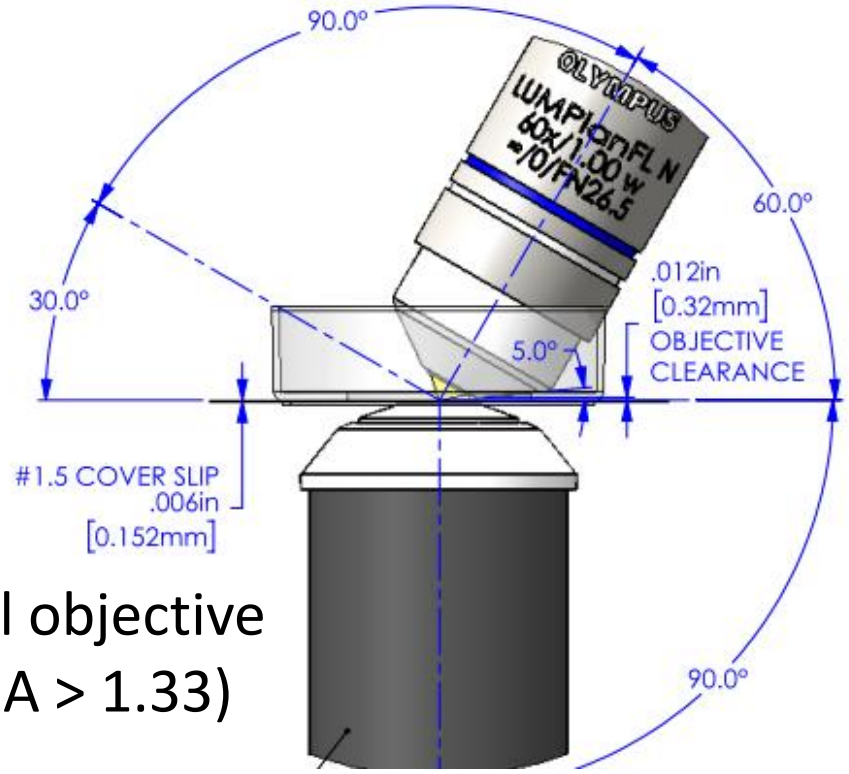
doSPIM implementation



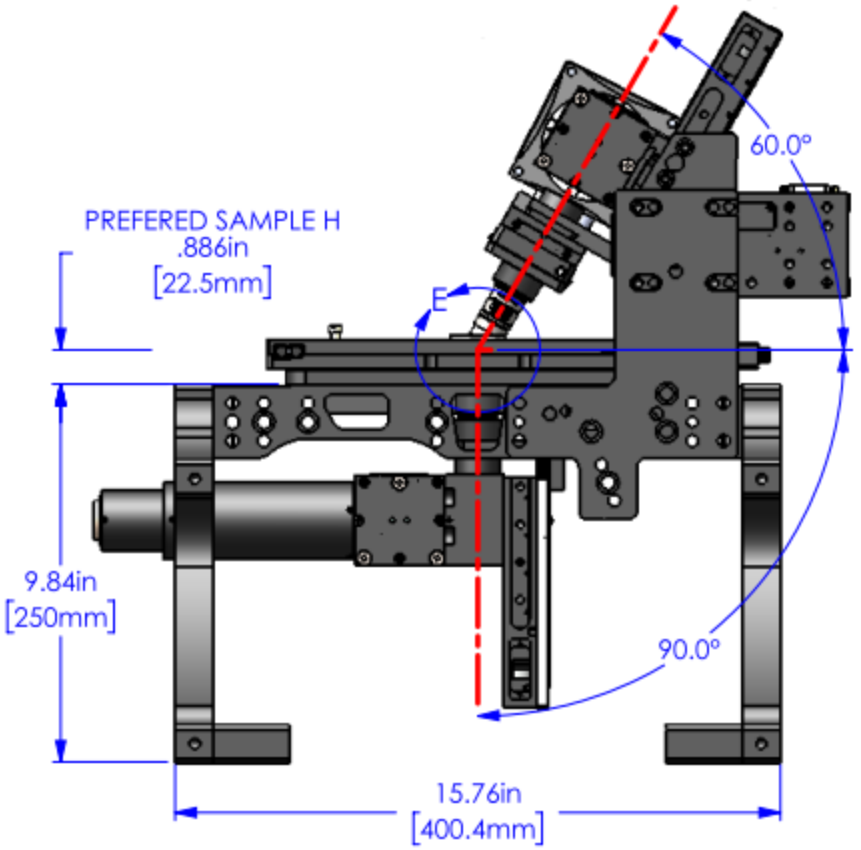


Dual-view system, objectives sequentially generate light sheet and image

oSPIM Implementation



Oil objective
(NA > 1.33)



Bottom objective creates tilted light sheet for imaging with top objective

Comparison of oSPIM vs. doSPIM

oSPIM

- Single-view (no registration/ fusion but anisotropic resolution)
- Glass coverslip bottoms OK
- Alignment easier
- One camera/scanner
- Still have conventional inverted microscope but requires RAMM frame
- Can couple to spinning disk, TIRF, or other techniques

doSPIM

- Dual-view (requires registration/fusion for resolution benefit)
- Requires FEP-bottom dishes
- Alignment more difficult
- Two cameras/scanners
- No “normal” bottom view

oSPIM/doSPIM status

- 2015: Patent application, initial development
- 1H’16: various successful demos
- Late 1H’16: initial sales of system
- Most of iSPIM/diSPIM hardware and software “infrastructure” can be re-used for oSPIM/doSPIM
  - Micro-Manager plugin has oSPIM option already

ASI SPIM Comparison

iSPIM/diSPIM

- 24x50mm coverslips or larger dishes
- Most mature system
- Can use non-RAMM inverted stand
- Light sheet comes from above sample (through buffer)

oSPIM

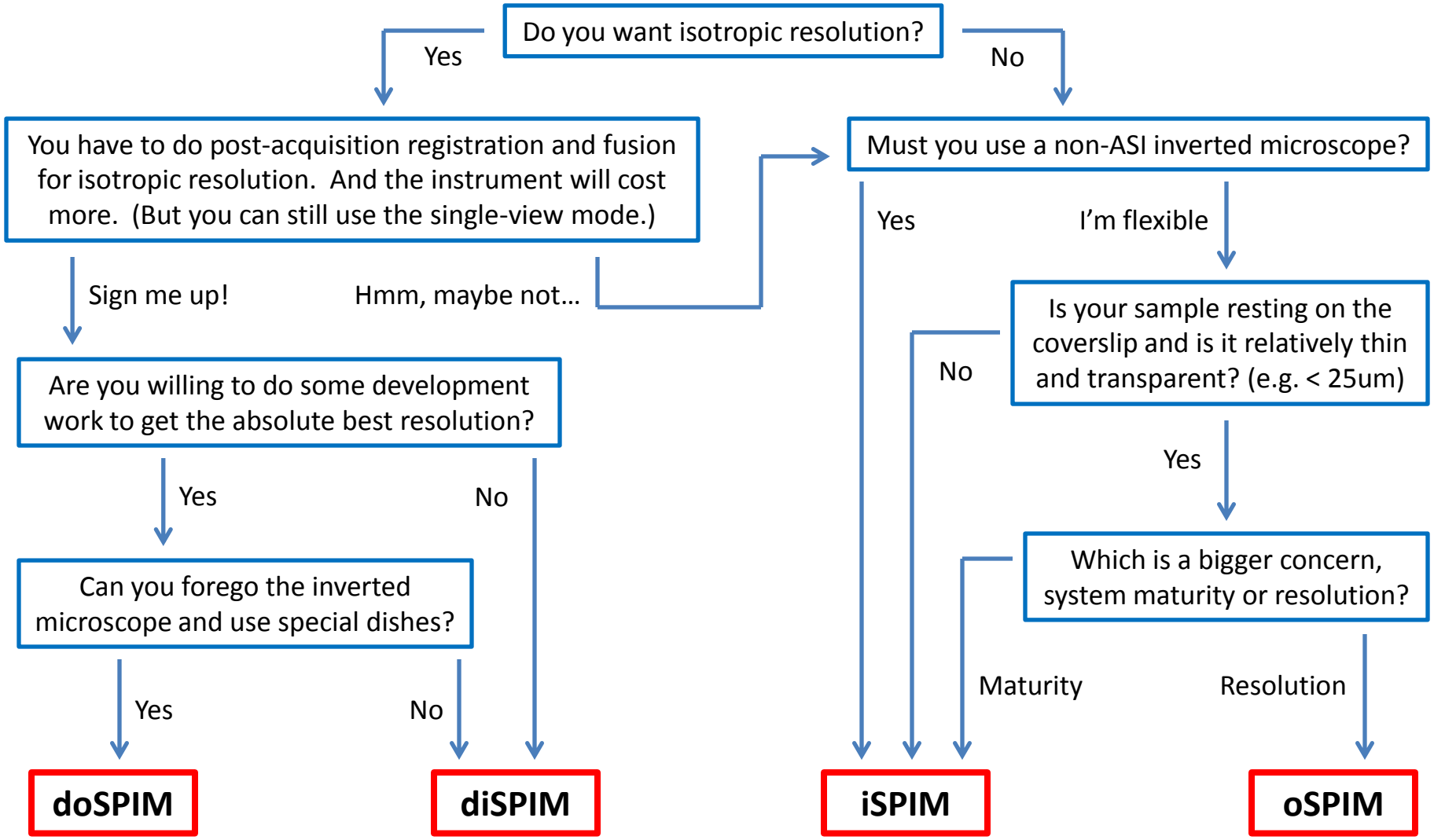
- 35mm glass-bottom dishes or larger
- Better-than-diSPIM lateral resolution and better-than-iSPIM axial resolution (not isotropic)
- Light sheet from below (through coverslip)

doSPIM

- Only FEP-bottom dishes
- Best resolution
- First adopters must write custom registration/fusion code
- No inverted scope
- Light sheet comes from both above and below

Single-view (iSPIM/oSPIM) is less expensive than dual-view (diSPIM/doSPIM) because only one camera and scanner required and no laser switcher. Furthermore single-view avoids the (sometimes problematic) step of registration/fusion but also does not lead to isotropic resolution.

Decision Tree



Future directions for ASI SPIM
(some in various stages of development)

- Rapidly scan beam axially for thinner sheet
- Sheet generation using cylindrical lens
- “Virtual slit” using camera rolling shutter
  - Near-simultaneous two-sided acquisition
- Multi-photon excitation
- Use lattice light sheet objectives
- + photo-track
- Cleared tissue samples
- + structured illumination (SIM)