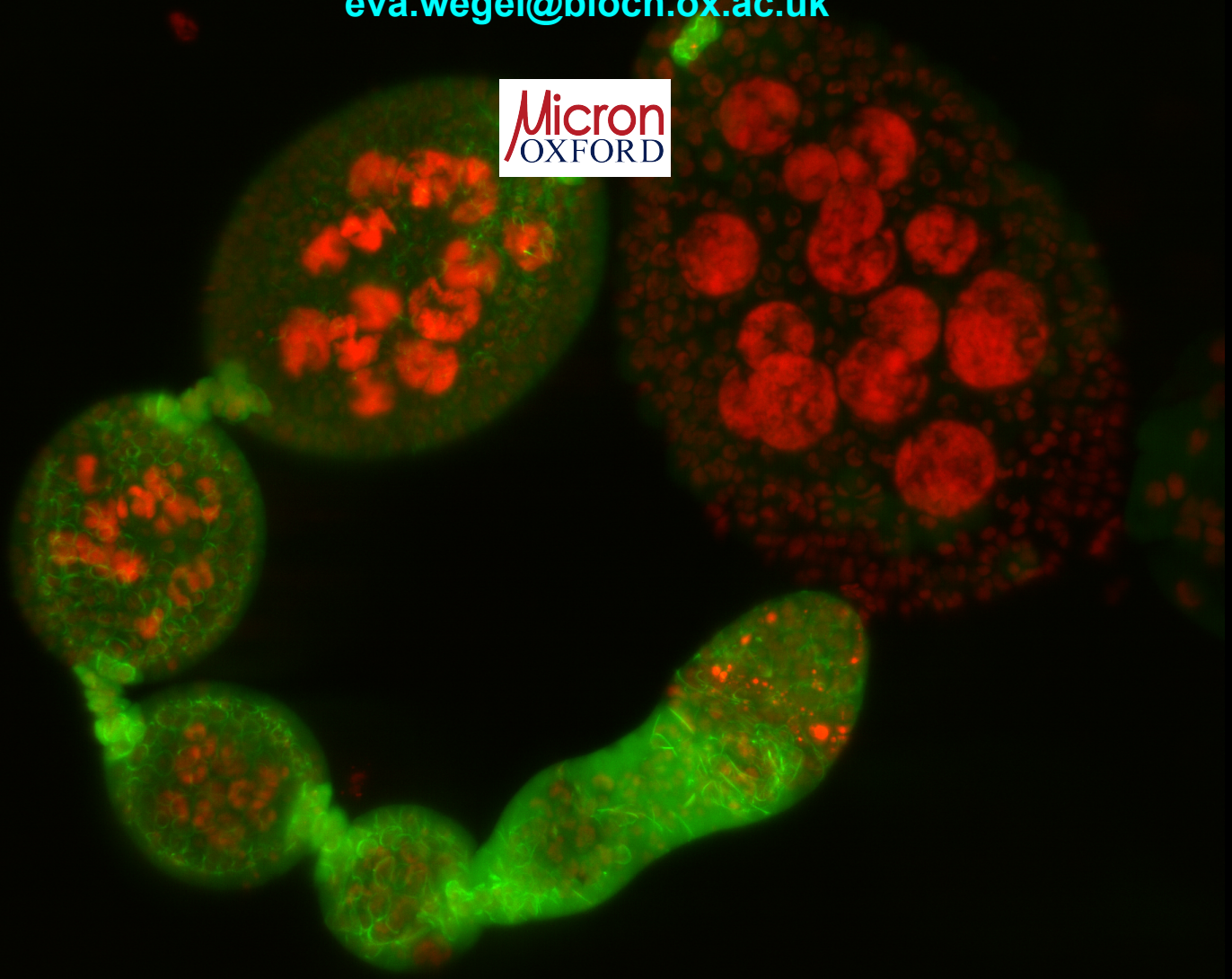
Light-sheet microscopy

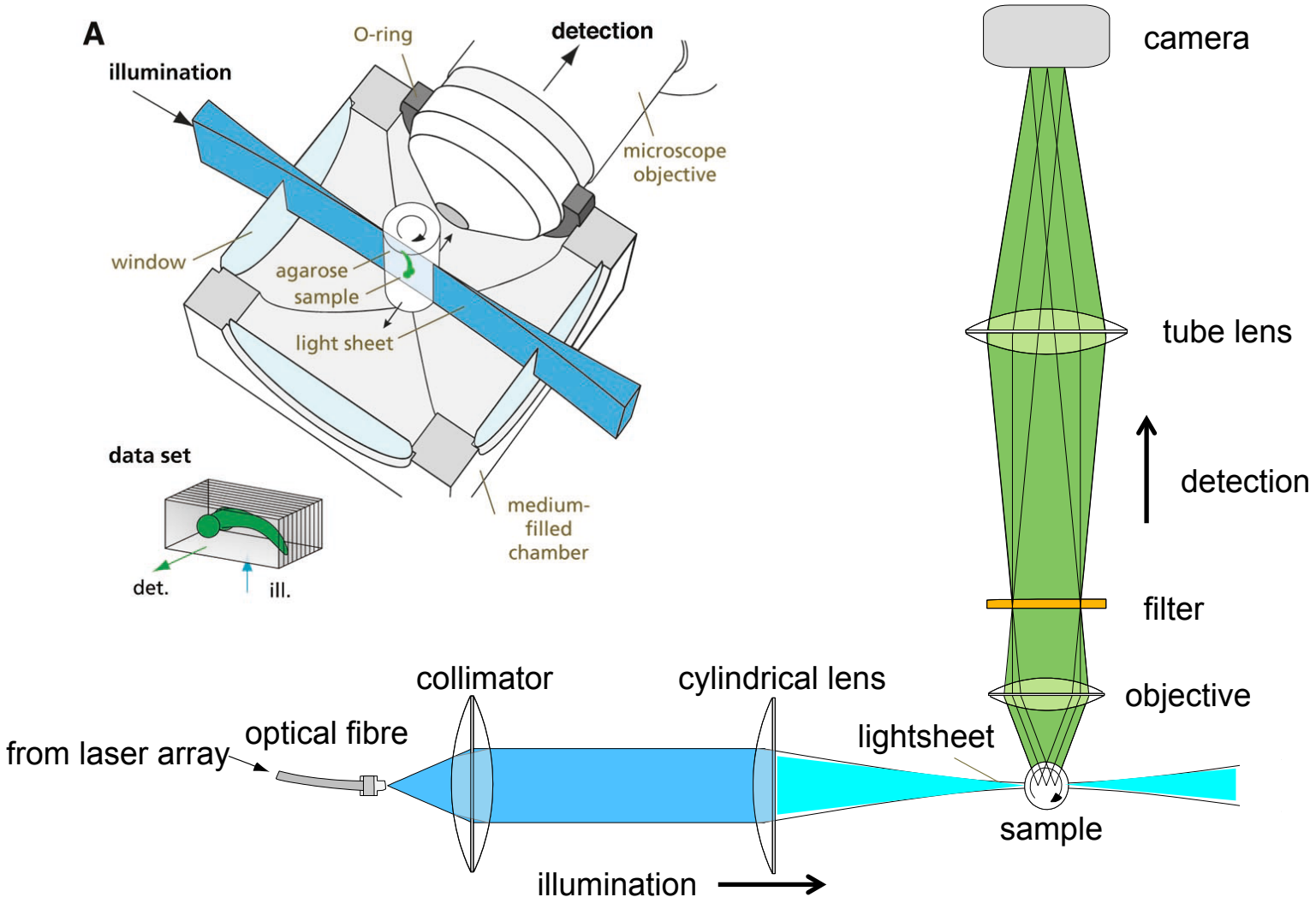
Eva Wegel
eva.wegel@bioch.ox.ac.uk



The principle of lightsheet microscopy

Jan Huisken et al., Science 305, 1007 (2004), Selective Plane Illumination Microscope (SPIM)

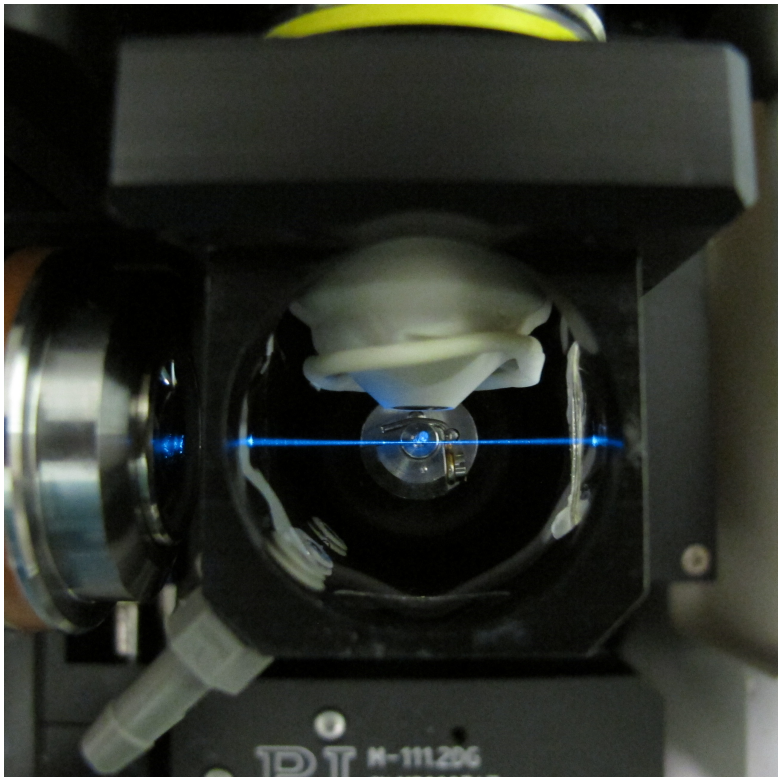
Excitation light is focussed by a cylindrical lens to a sheet of light that illuminates only the focal plane of the detection optics.

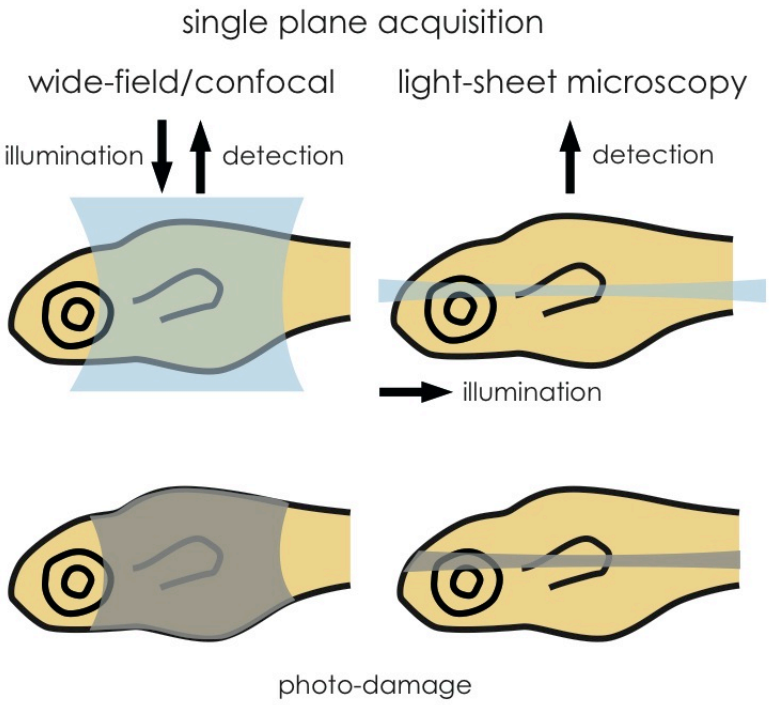


The principle of light-sheet microscopy

A thin sheet of light (thickness ca. 2–6 µm) illuminates the sample from the side and not from above or below:

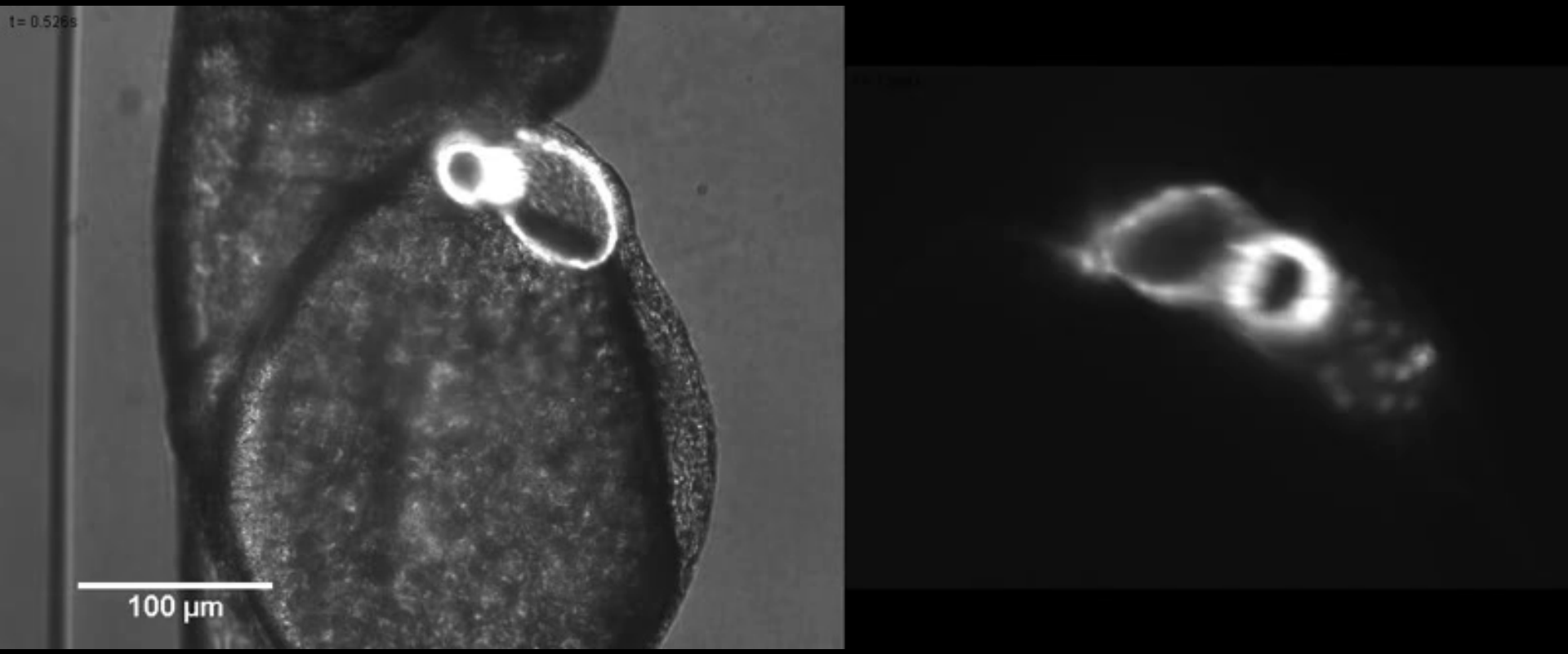
- Reduced photodamage/bleaching and increased longevity of live samples
- Reduced out-of-focus blur through optical sectioning





Compared with confocal and two-photon fluorescence microscopy, light-sheet exposes the embryo to at least three orders of magnitude less light energy, but still provides up to 50 times faster imaging speeds and a 10–100-fold higher signal-to-noise ratio.

Keller et al, Cold Spring Harb Protoc 2011; doi: 10.1101/pdb.prot065839

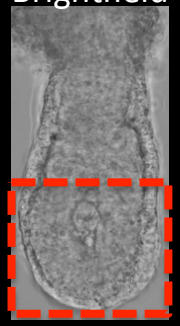


Beating heart of a 48 hpf Tg(cmlc2:EGFP) zebrafish. Left: overlay of transmitted light and fluorescence signal. Right: fluorescence signal.

openspim.org

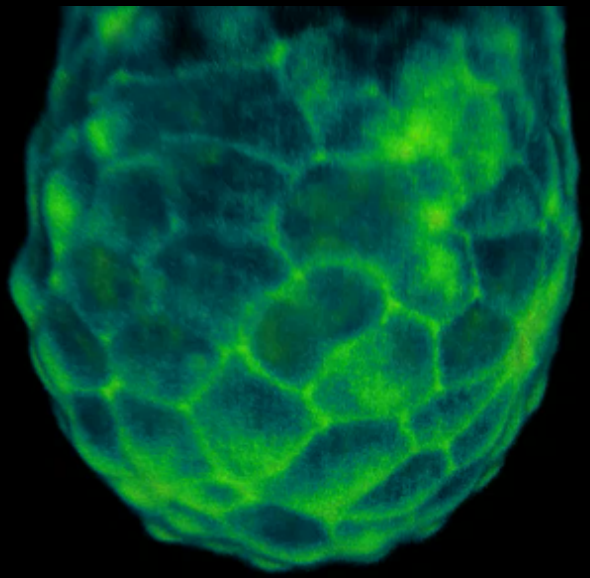
LifeAct Z1 Timelapse

Brightfield

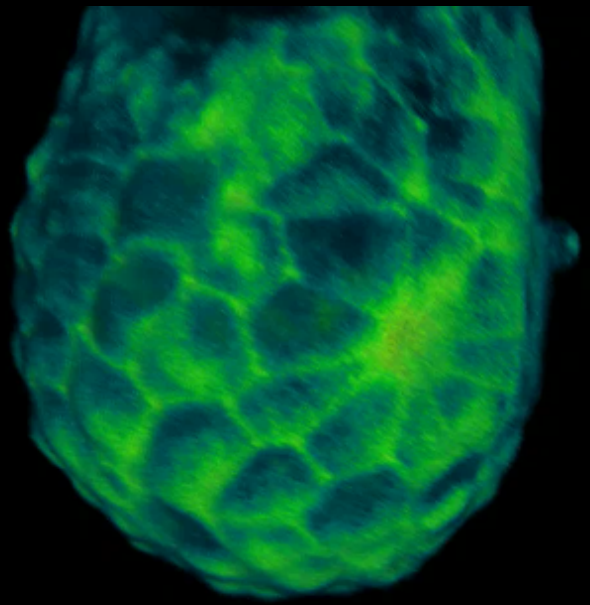


LifeAct mouse E5.5
z-stack every 2 min
s 1% 488 laser 39 ms exposure
Single lightsheet
Duration 70 mins
3D Opacity rendering - Volocty

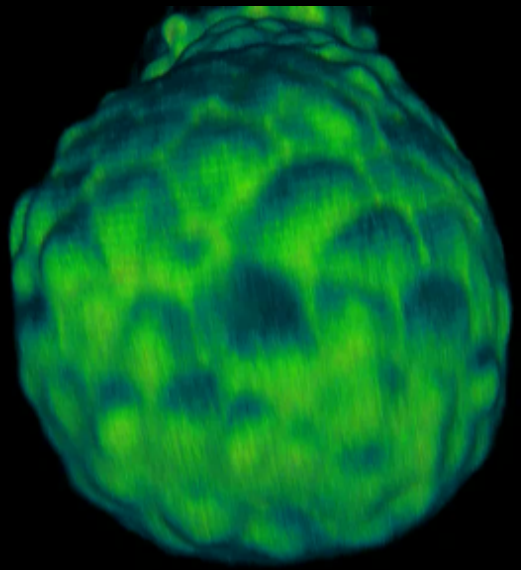
Front View



Right-Side

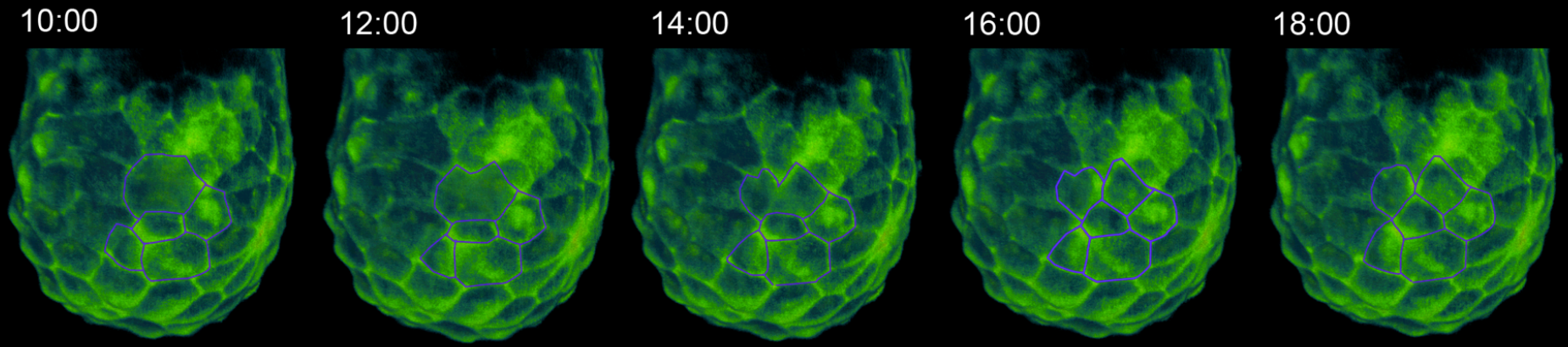


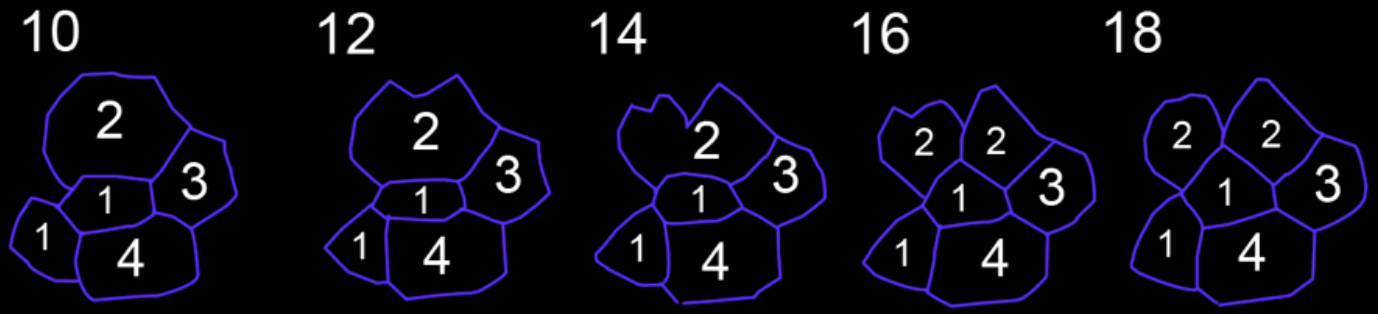
Distal View



Matthew Stower, Srinivas lab

LifeAct Z1 Timelapse

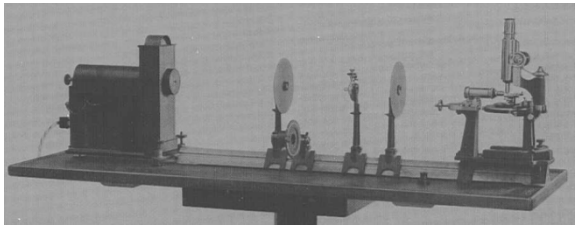


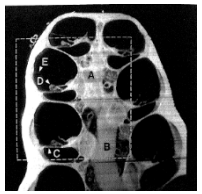


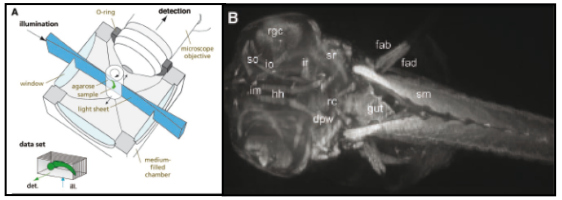
Matthew Stower, Srinivas lab

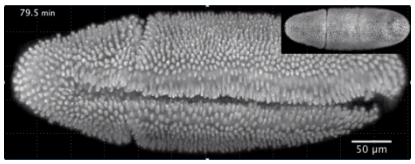
A short history of light-sheet microscopy

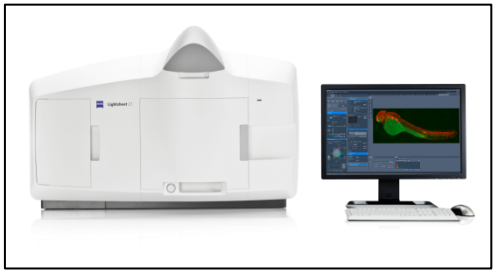
- 1903: First Lightsheet by Siedentopf and Zsigmondy (Ultramicroscopy, Colloid Chemistry)
- 1964-1993: Various light-sheet microscopy implementations, mostly for surface visualization, fluid dynamics, etc.
- 1993: First application of light-sheet illumination to a biological specimen (fluorescence imaging of cleared guinea pig cochlea) by Voie et al.
- 2004: Ground-breaking renaissance of the illumination technique by Huisken et al. (“SPIM”, MultiView, live imaging in developmental biol.)
- 2005 – today: a large number of innovative implementations of light-sheet fluorescence microscopy for a wide range of applications
- 2012: The first commercial light-sheet fluorescence microscope







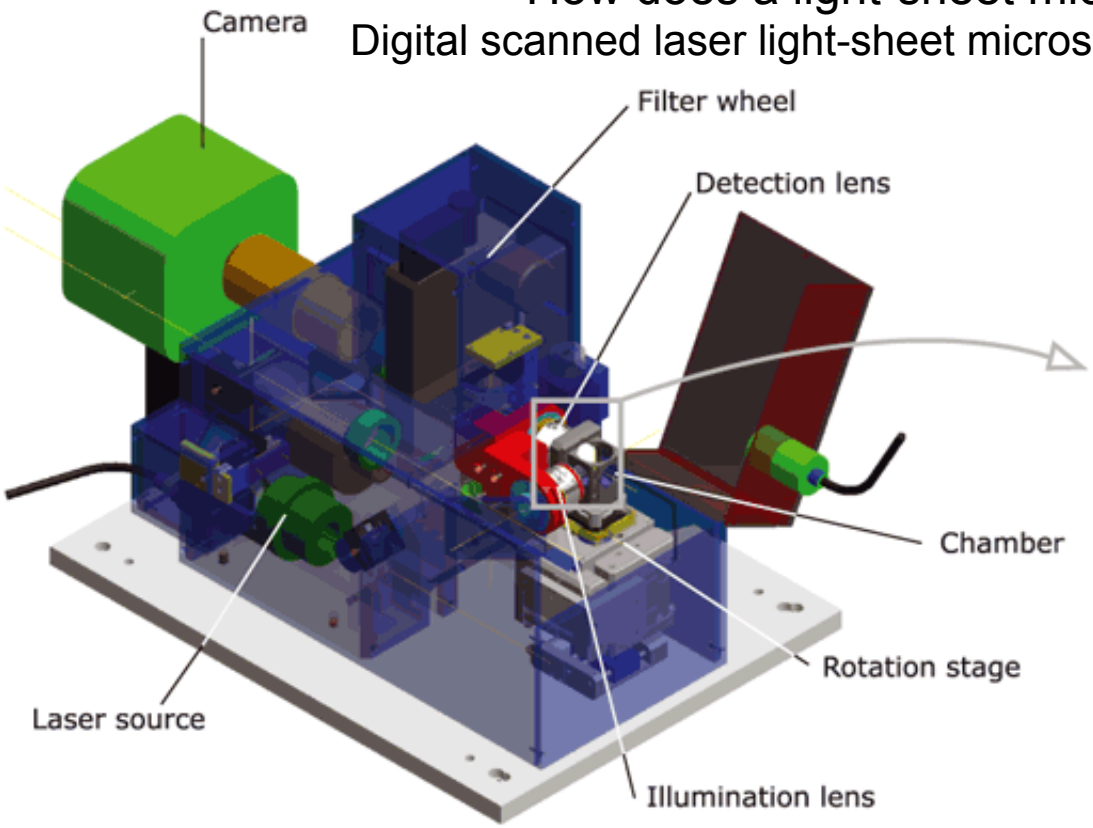


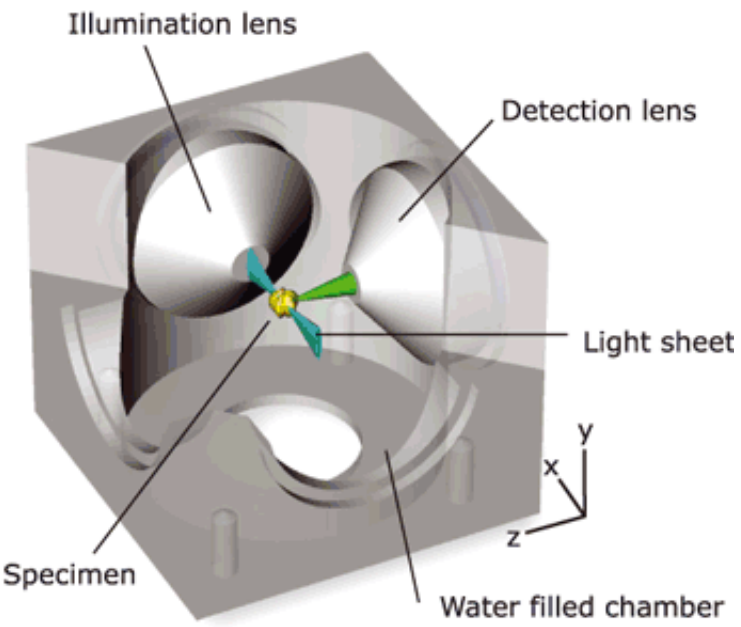


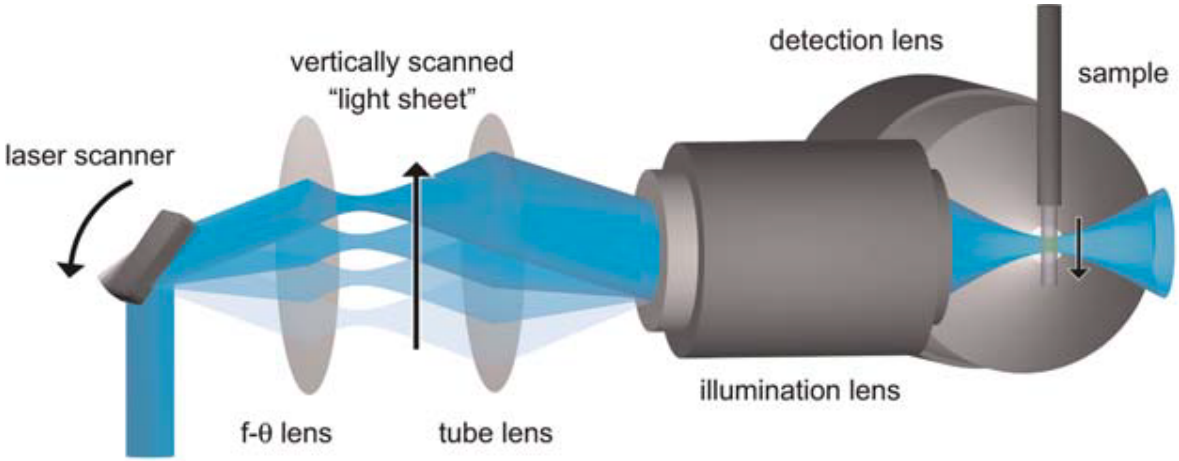
For a more detail review see also: Keller and Dodt Curr Opin Neurobiol. 2012

How does a light-sheet microscope work?

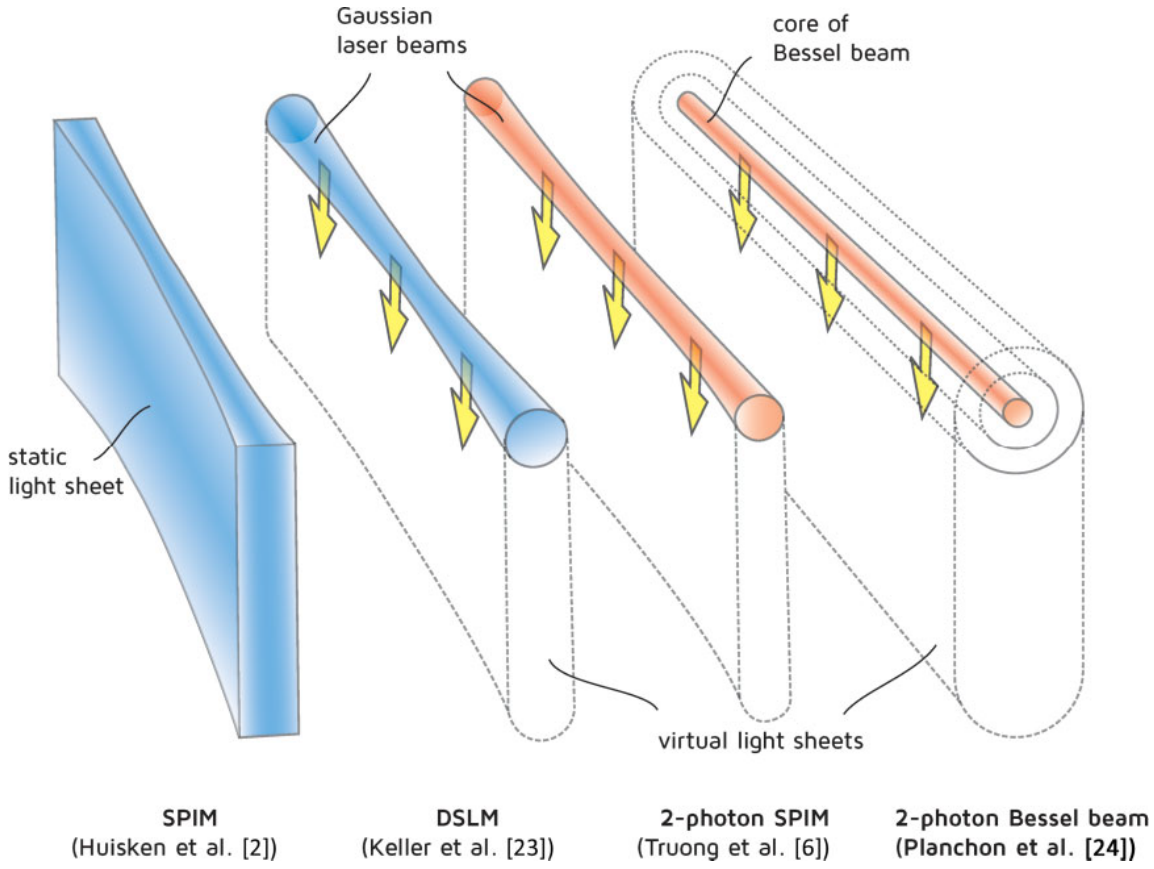
Digital scanned laser light-sheet microscope (DSLM, Keller, 2006)



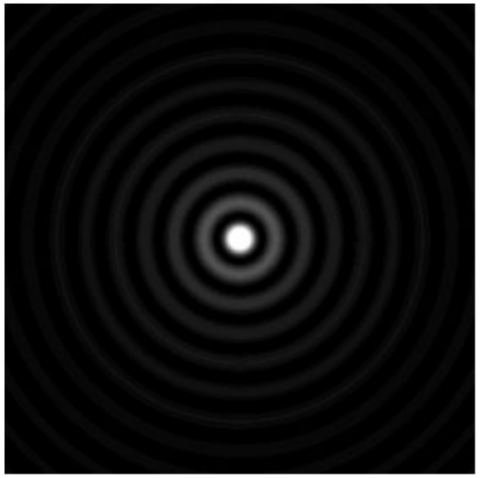




Ways of producing a light-sheet



Better depth penetration



Core of a Bessel beam with light rings of equal energy surrounding it

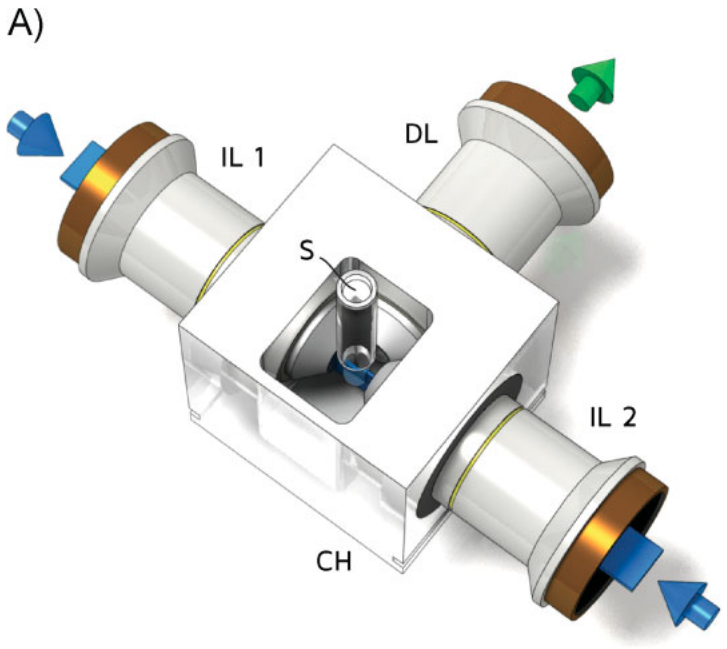
Jan Huisken
Bioessays 34: 406–411 (2012)

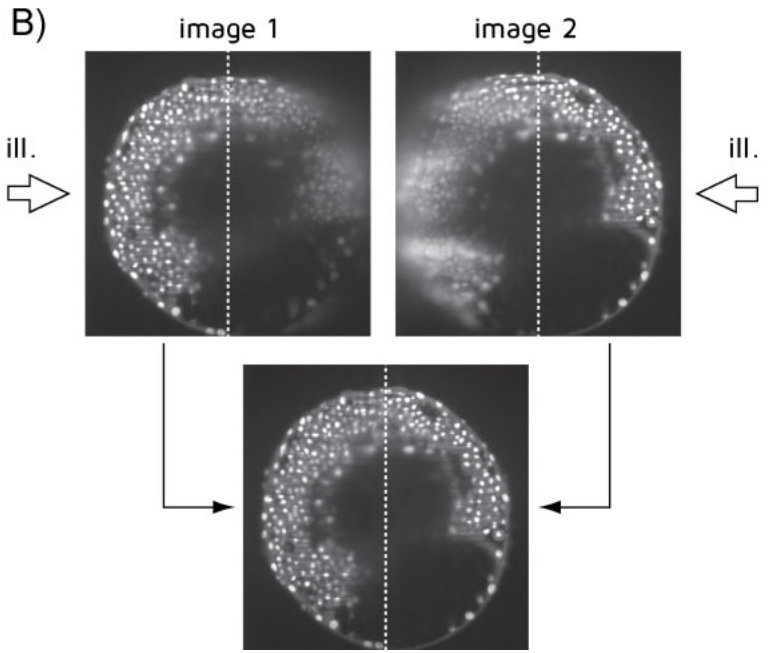
Dual-sided illumination in multi-directional SPIM

Implemented on the Zeiss Z1 light-sheet microscope in Micron

The detection lens sits between two illumination lenses. The sample is illuminated first from one side and then the other.

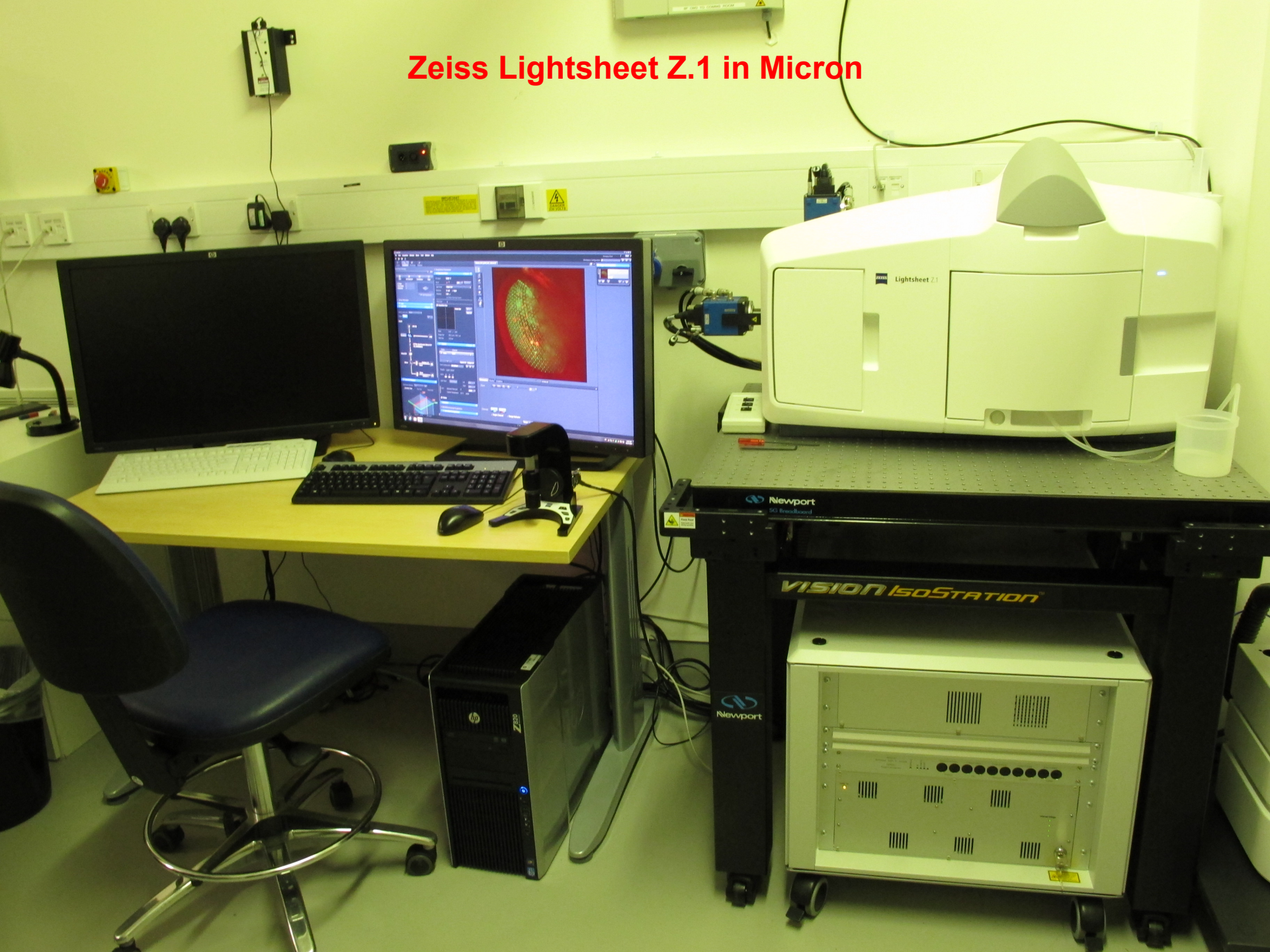
- Advantage: Only half the number of rotation angles are necessary
- Disadvantage: Aligning both illumination lenses is difficult



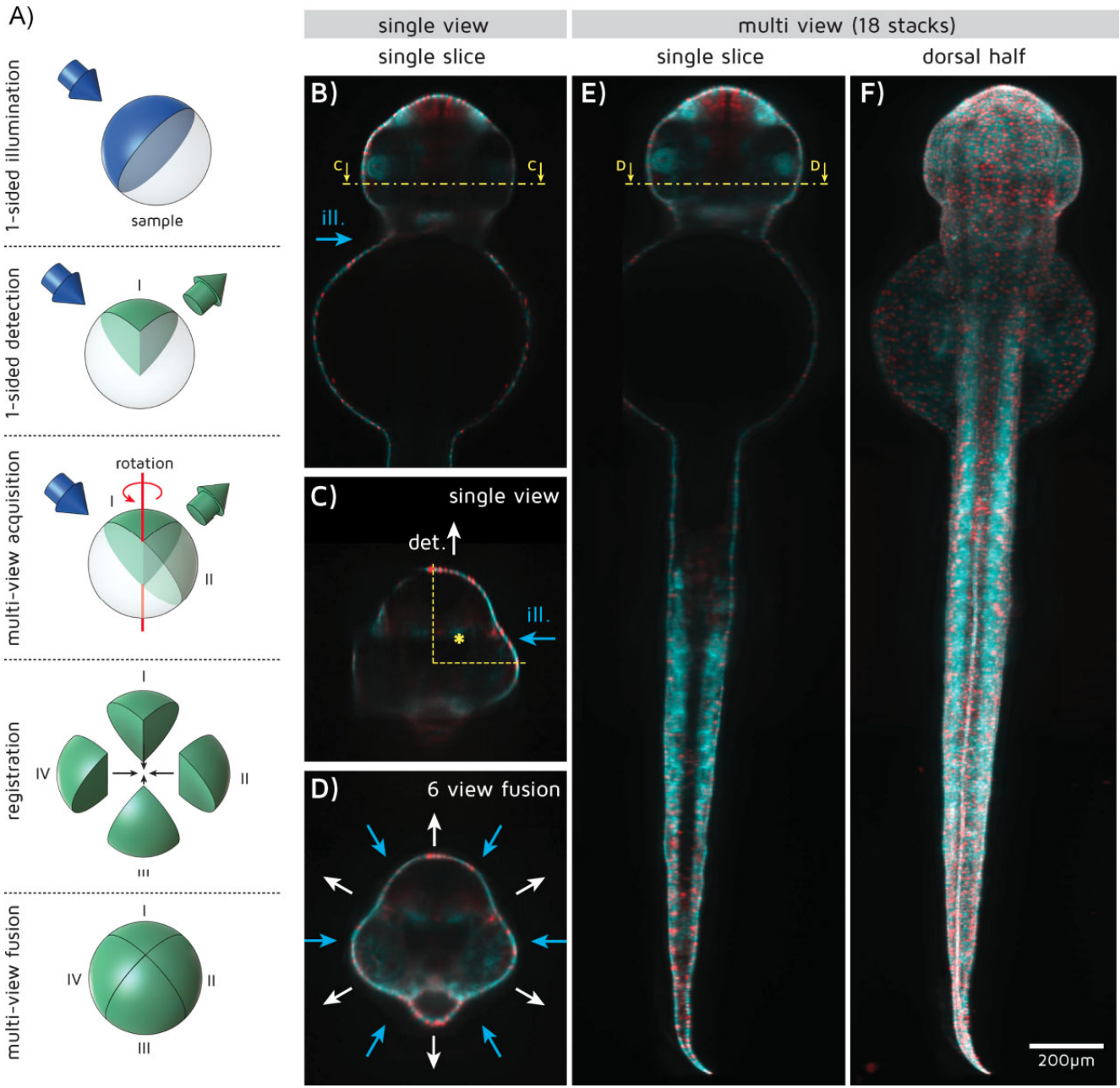


Jan Huisken
Bioessays 34: 406–411 (2012)

Zeiss Lightsheet Z.1 in Micron



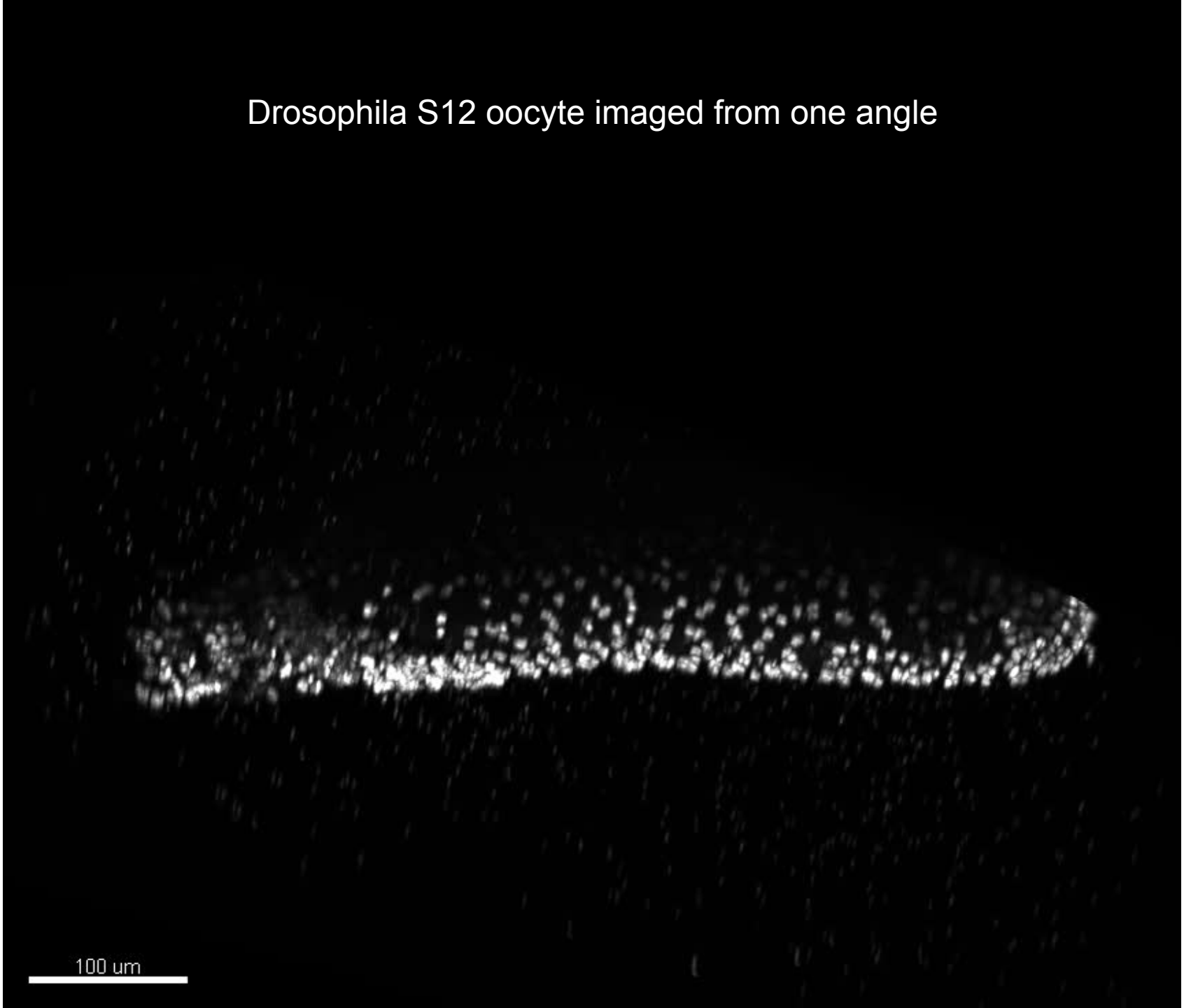
Multi-View Fusion

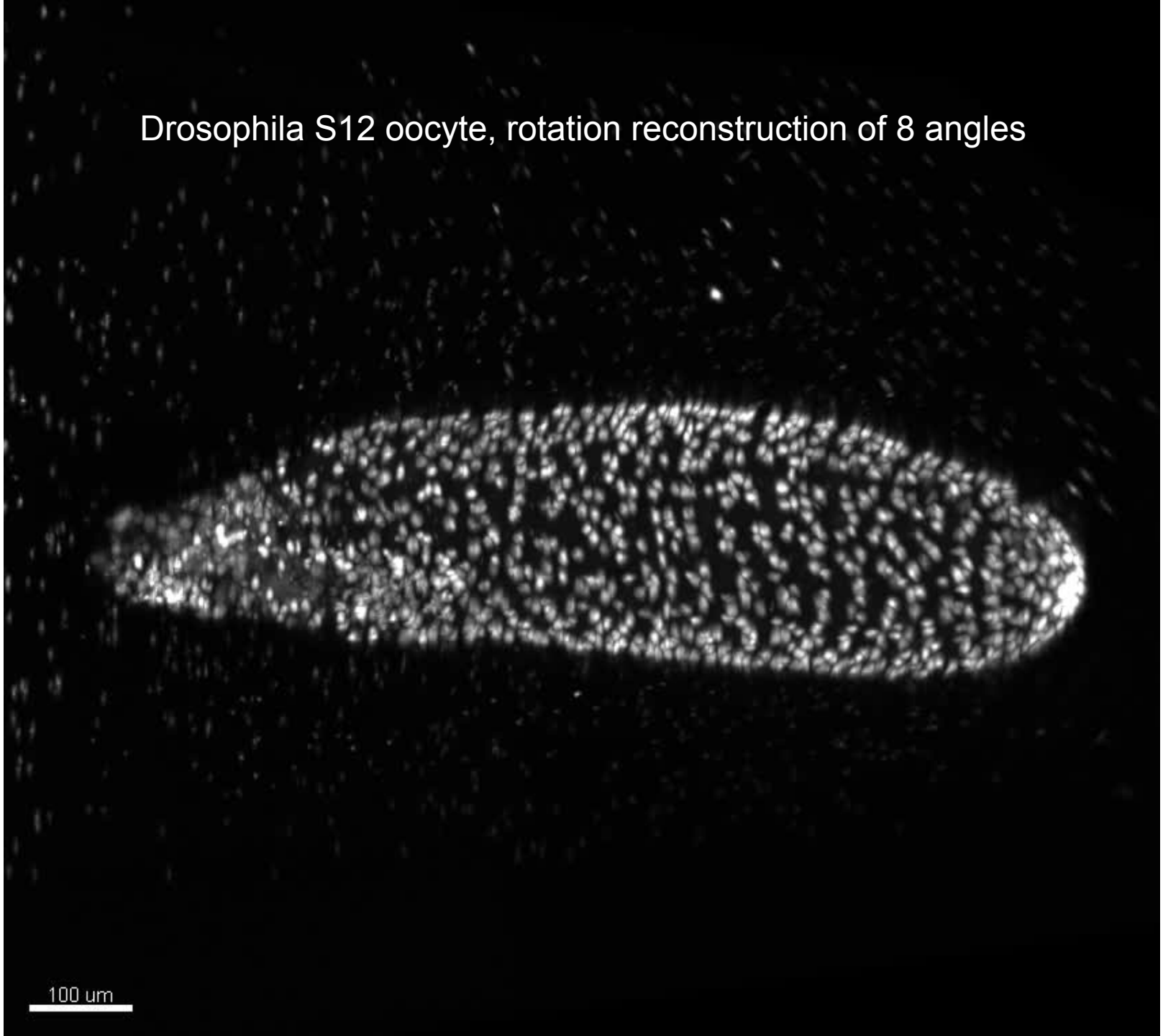


Nuclei in red
Membranes in cyan

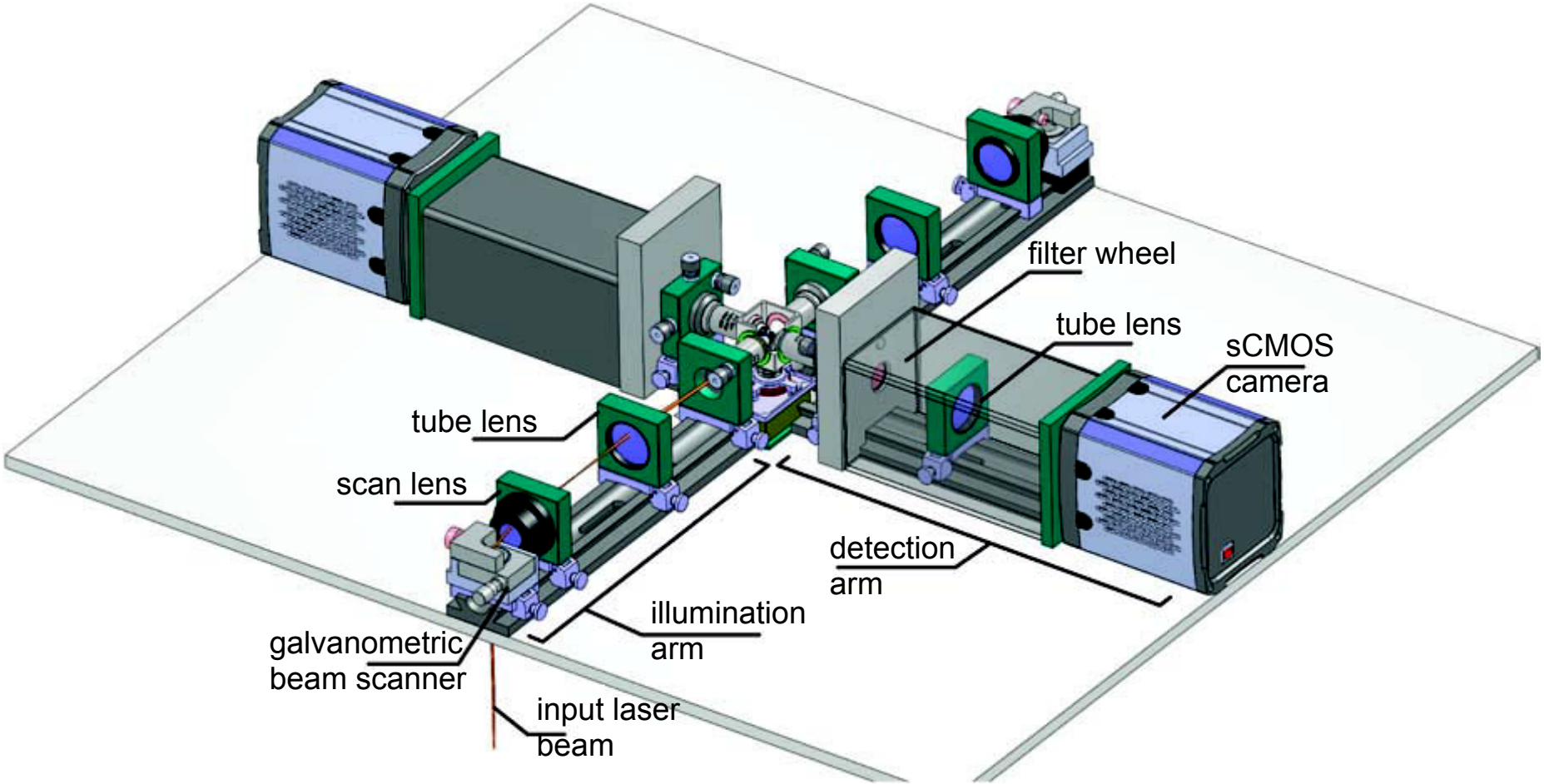
Jan Huisken
Bioessays 34: 406–411
(2012)

Drosophila S12 oocyte imaged from one angle




Drosophila S12 oocyte, rotation reconstruction of 8 angles
100 um

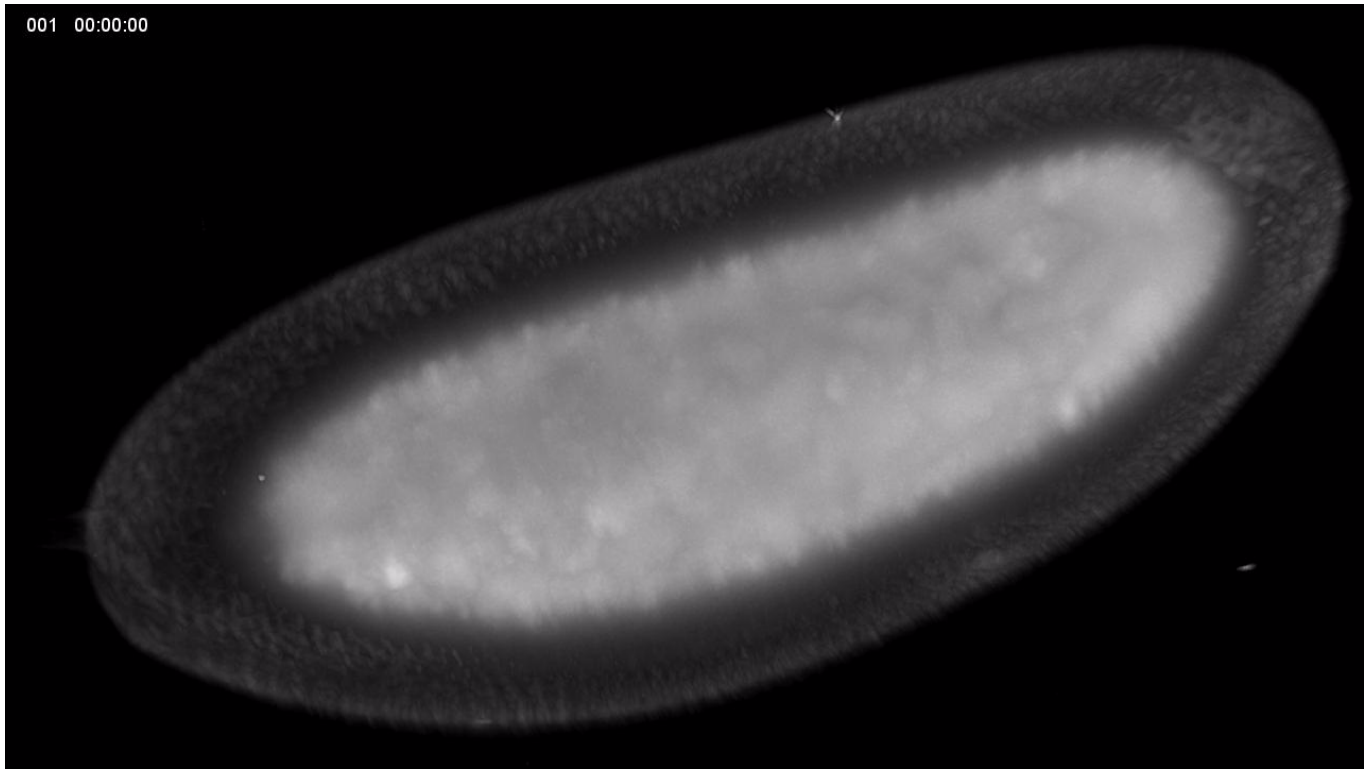
Multiview selective-plane illumination microscope



The two cameras simultaneously acquire stacks through the sample first with illumination by one light-sheet and then by the other thus eliminating the need for sample rotation and increasing acquisition speed.

Uros Krzic et al., Nature Methods 9 (7), 730-733 (2012)

Drosophila Embryo Development
In toto imaging of expression pattern

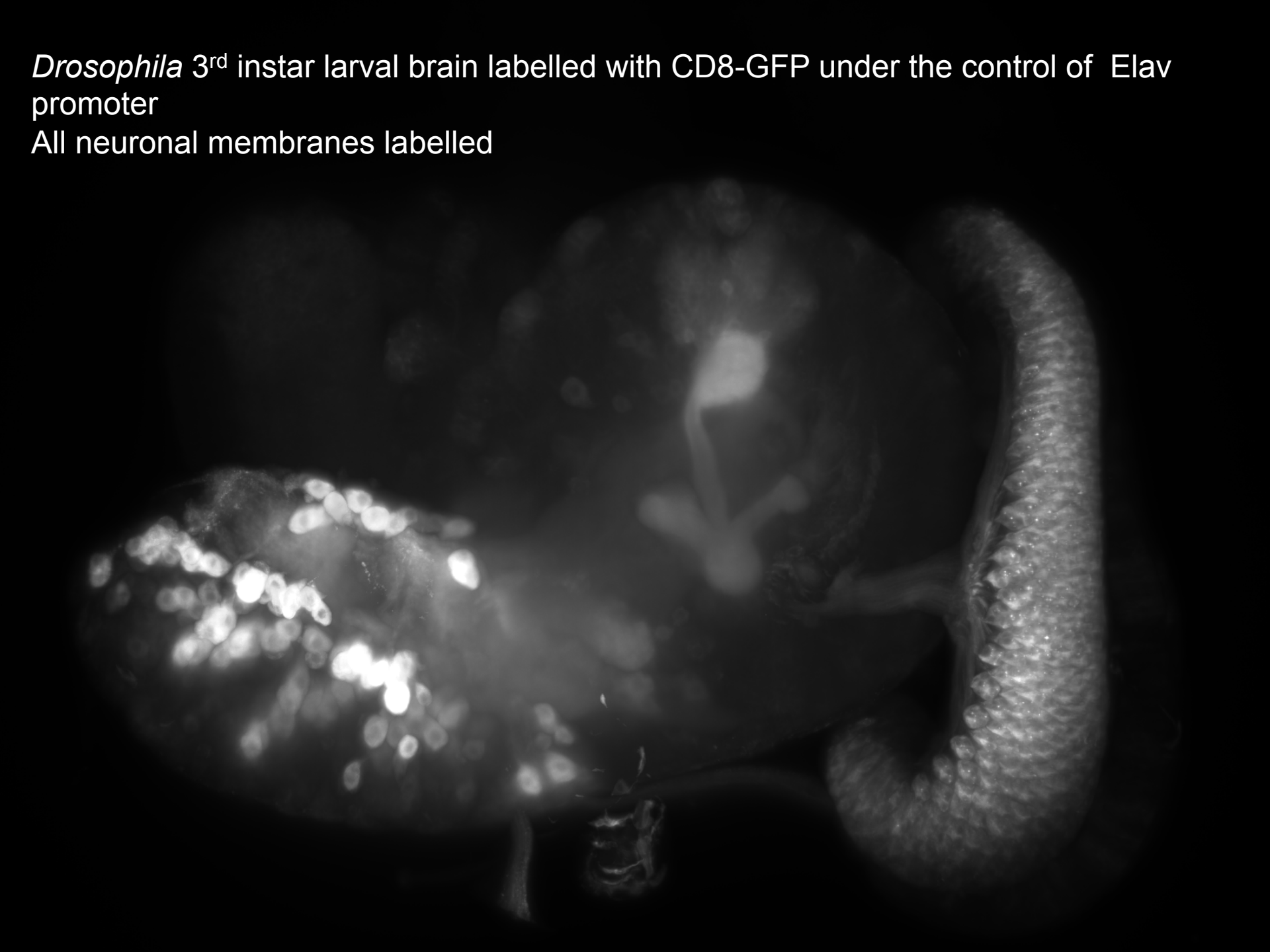


3D rendering of reconstructed multi-view recordings of a Drosphila embryo expressing a GFP tagged BAC clone. The BACs are from Hugo Bellen.
The gene is csp (cystein string protein), a general marker of the central nervous system.

Imaged with a Zeiss LSFM prototype from 6 angles every 10 minutes. Fluorescent beads (fiducal markers for data registration) are visible around the embryo, so is yolk.

Movie by M. Weber and P. Tomancak from MPI-CBG Dresden, Germany with permission from the authors

Drosophila 3rd instar larval brain labelled with CD8-GFP under the control of Elav promoter
All neuronal membranes labelled



Drosophila 3rd instar larval brain labelled with CD8-GFP under the control of Elav promoter
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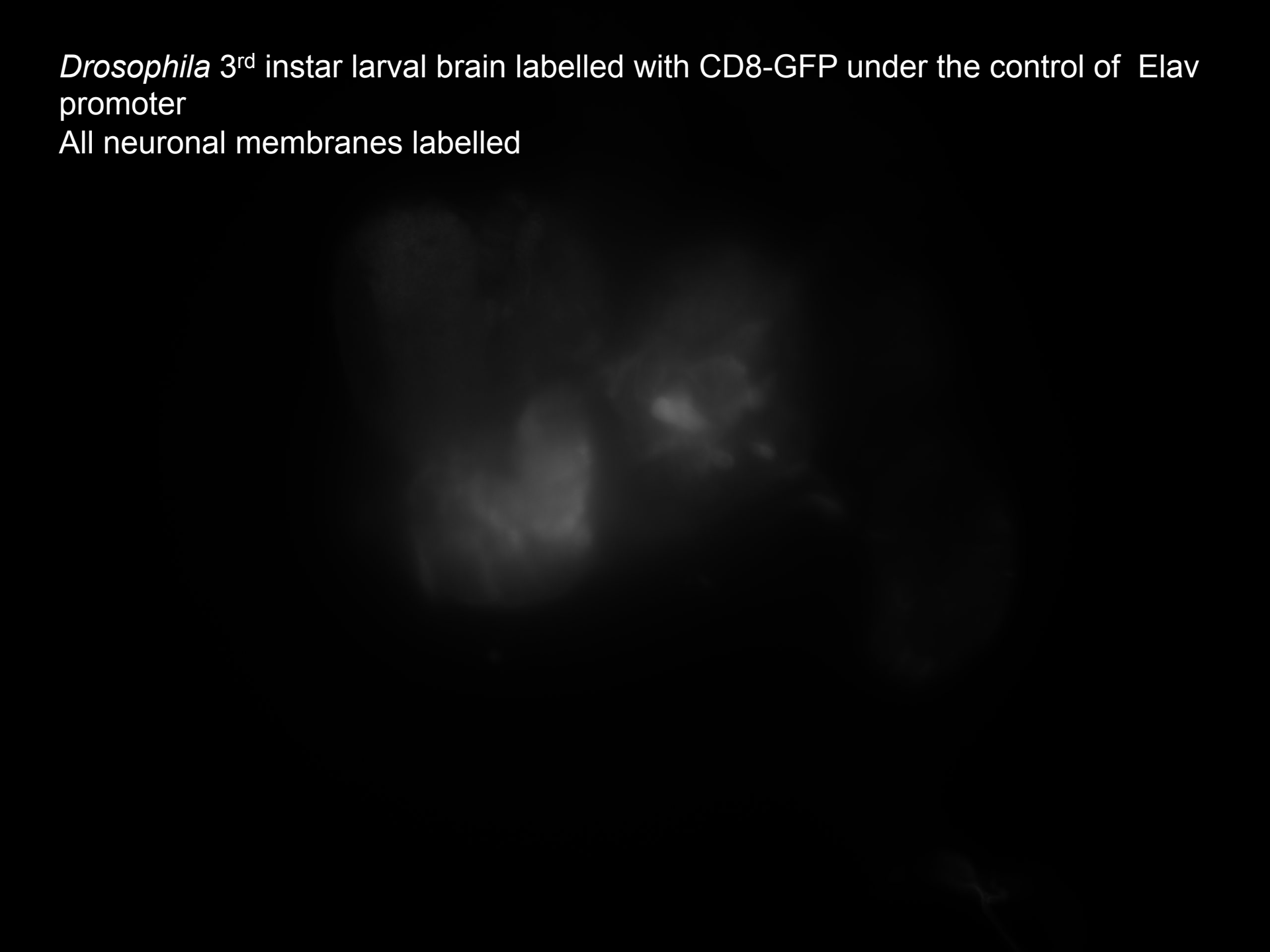
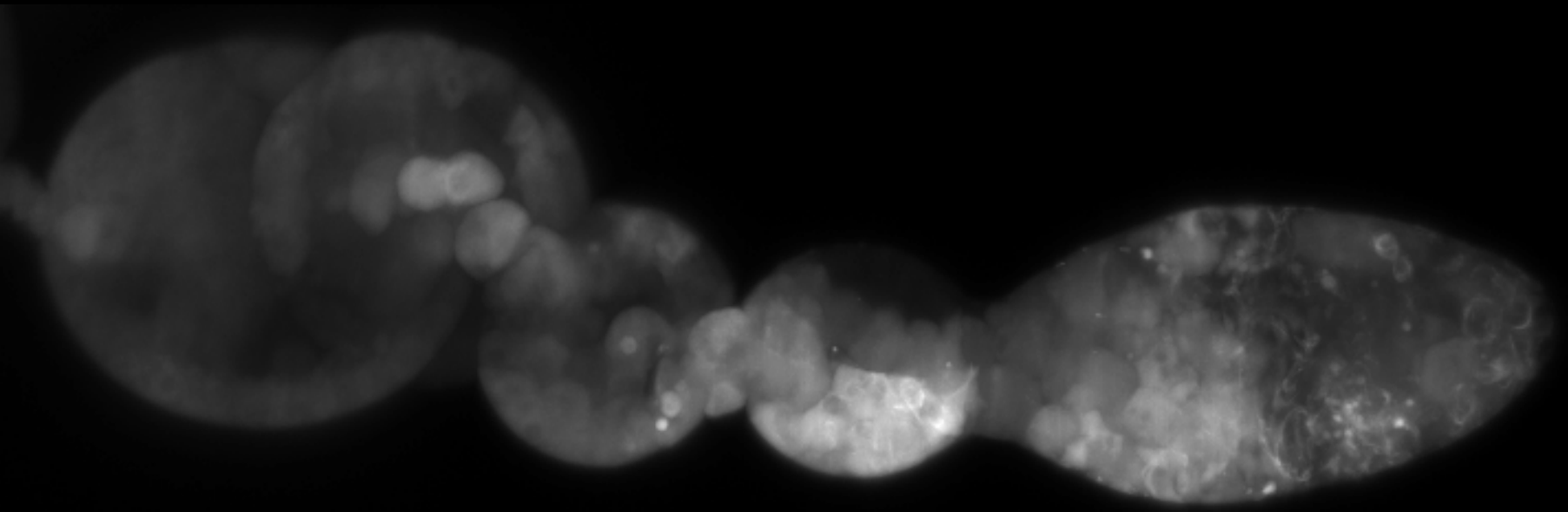
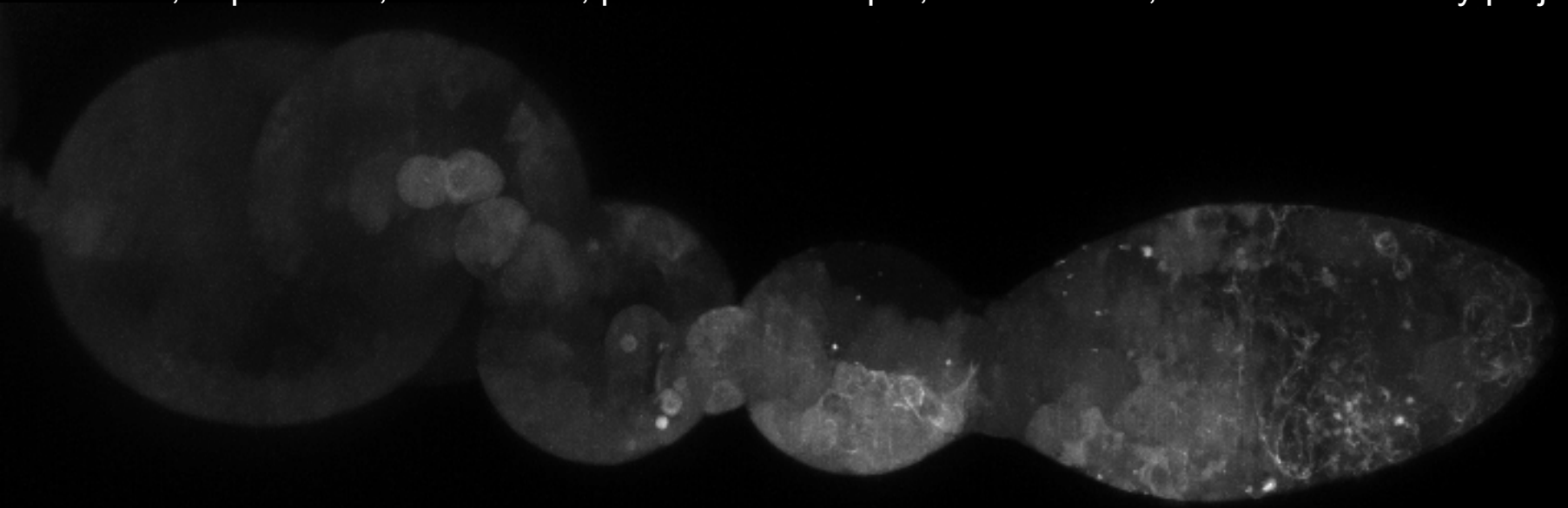


Contrast enhancement strategies: Deconvolution

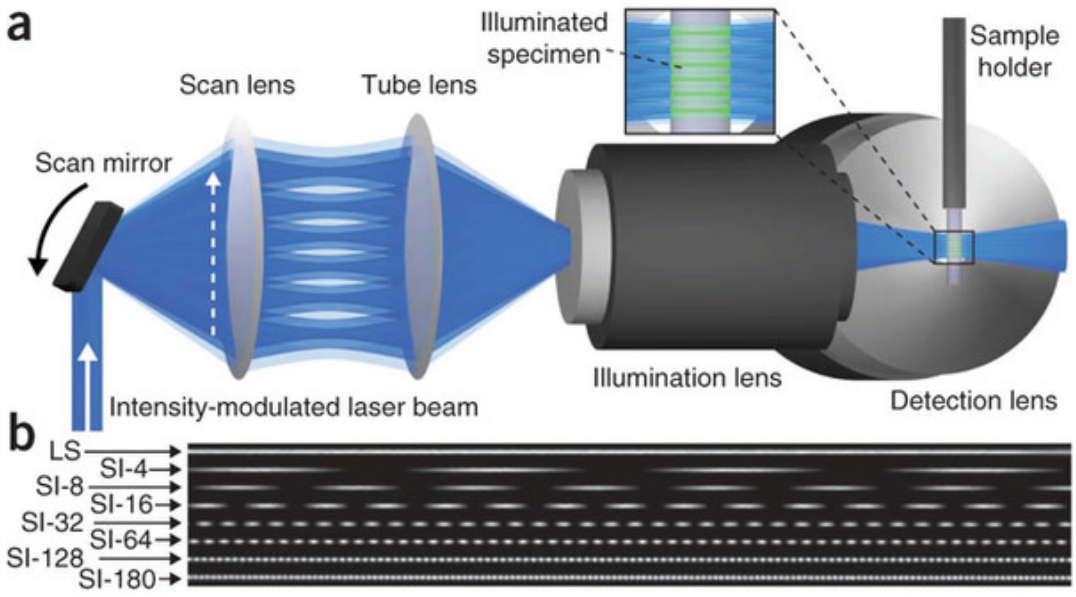
Ovariole, Jupiter-GFP, 20x NA 1.0, pixel size 0.291 µm, raw data, maximum intensity projection

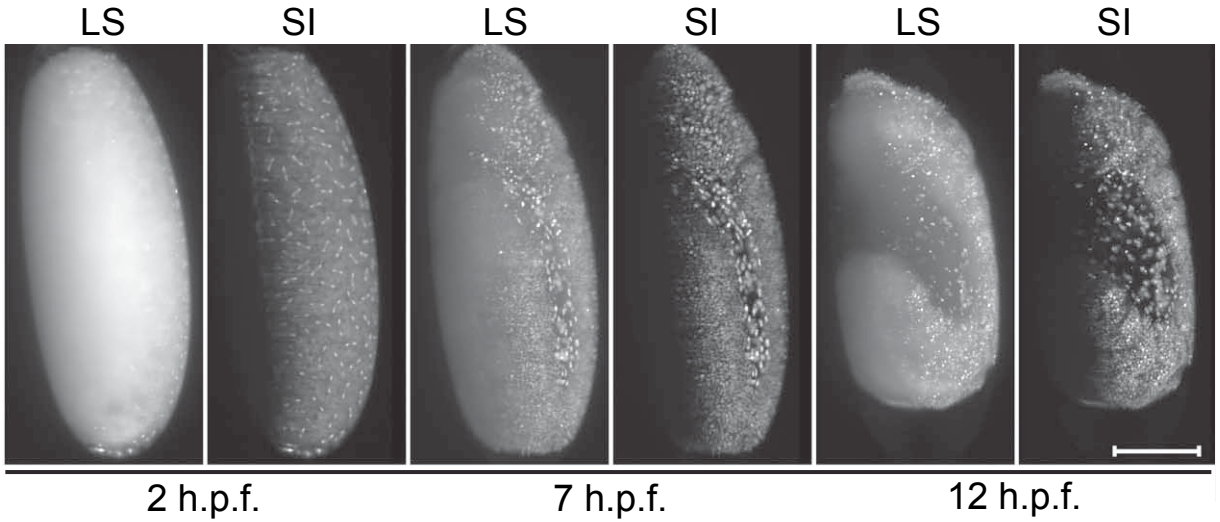


Ovariole, Jupter-GFP, 20x NA 1.0, pixel size 0.291 µm, deconvolved, maximum intensity projection



Contrast enhancement strategies: Incoherent structured illumination





Keller, P.J. et al., Nature Methods 7 (8), 637-642, 2010

Key applications of light-sheet microscopy

- Imaging of **morphogenesis** and **spatio-temporal patterns** of cells during **embryogenesis of Drosophila, Zebrafish and other model organisms** ("*In toto*" imaging)
- **Fast imaging** of morphogenesis and cellular dynamics **in embryos and small organisms** (cardiac development, blood flow, angiogenesis and vascular development, neuro-development by calcium imaging)
- Imaging of **3D cell culture**, spheroids and cysts, tissue culture, organotypic cultures
- Fluorescence imaging of **marine organisms** (Ciona, squid, plankton, flat worms, etc.)
- Structural imaging of **larger (mm) organisms, also fixed and cleared** (early mouse embryos, Zebrafish & Medaka Fish)

When should I use a lightsheet microscope?

The lightsheet overlaps with the scanning confocal and the 2-photon
These are guidelines, there are no hard and fast rules! If in doubt try all three microscopes.

- The sample is >= 100 µm thick (also try 2-photon)
- I need a view from all sides
- I want long-term live imaging >= 20 µm into the sample
- My sample is not happy on a slide
- The scanning confocal takes too long to image my thick sample
- I don’t need maximum resolution

Table 2. Lateral and Axial Extents and Focal Volumes of Simulated PSFs in a SPIM (ligthsheet microscope) Compared with Conventional, Confocal, and Two-Photon Microscopes[a]

Lens	Technique	$FWHM_{lat}$ j..m	$FWHM_{ax}$ j..m	Volume al
10 X /0.3 W	FM	1.00	15.17	7943.0
	CFM	0.68	10.38	2513.1
	2hv-FM	1.22	18.57	14472.0
	SPIM	1.00	5.73	3000.2
40 X /0.8 W	FM	0.37	1.94	139.1
	CFM	0.25	1.33	43.5
	2hv-FM	0.45	2.38	252.3
	SPIM	0.37	1.65	118.3
100 X /1.0 W	FM	0.29	1.15	50.6
	CFM	0.20	0.79	16.5
	2hv-FM	0.36	1.40	95.0
	SPIM	0.29	0.99	43.6
100 X /1.2 W	FM	0.24	0.69	20.8
	CFM	0.16	0.47	6.3
	2hv-FM	0.29	0.84	37.0
	SPIM	0.24	0.65	19.6

[a]All calculations do not take pixilation or bead sizes into account. The parameters are X_{ill}=0.488 j..m; NA_{ill}=0.034, 0.068, 0.108, and 0.108 for the 10X /0.3 W, 40X /0.8 W, 100X /1.0 W, and 100X /1.2 W lenses in the SPIM; camera pixel pitch 6.45 j..m; number of pixels=1024X 1344; X_{det}=0.520 j..m; n=1.33; X_{ill} =0.900 j..m for two-photon excitation. These parameters vary from those used in Table 1.

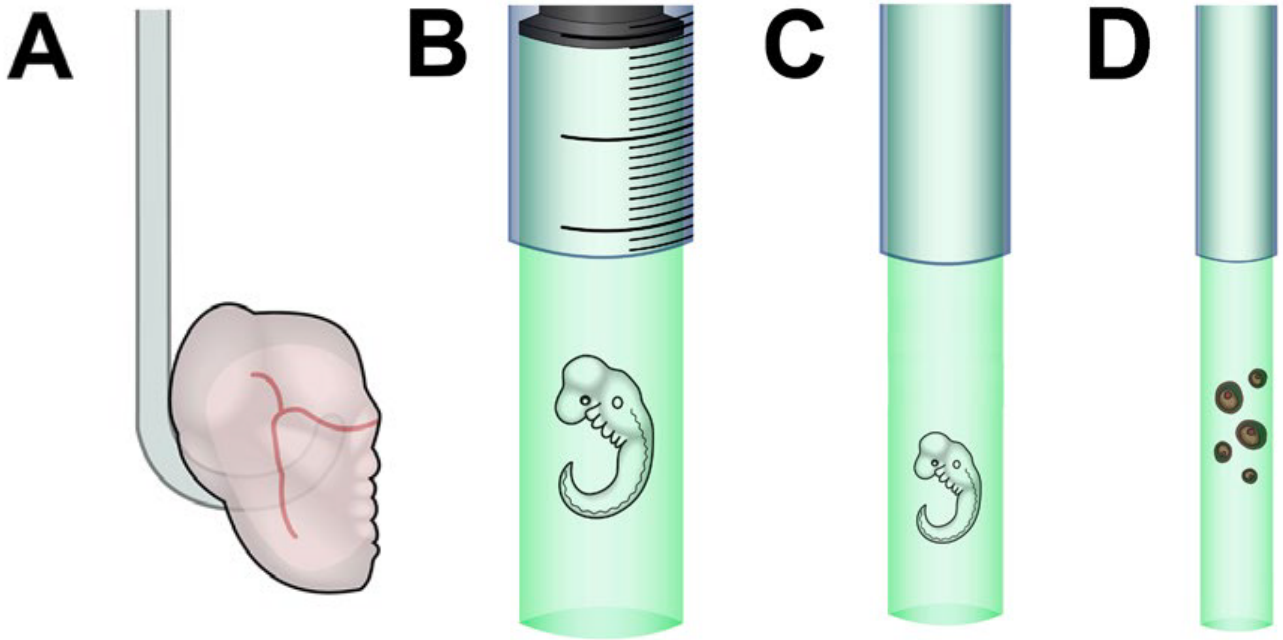
The focal volumes are those of an ellipsoid **V** =4/3FWHMlat/22FWHMax/2 and specified in attoliters 1 al=10−18 l=10−21 m3.

The resolution of a lightsheet microscope exceeds that of a 2-photon microscope.
The lateral resolution of a scanning confocal exceeds that of a lightsheet microscope.
Only when using a 10x objective is the axial resolution of a lightsheet microscope superior to that of a confocal.

Engelbrecht CJ, Stelzer EHK, Optics Letters 31 (10), 2006

Sample Preparation

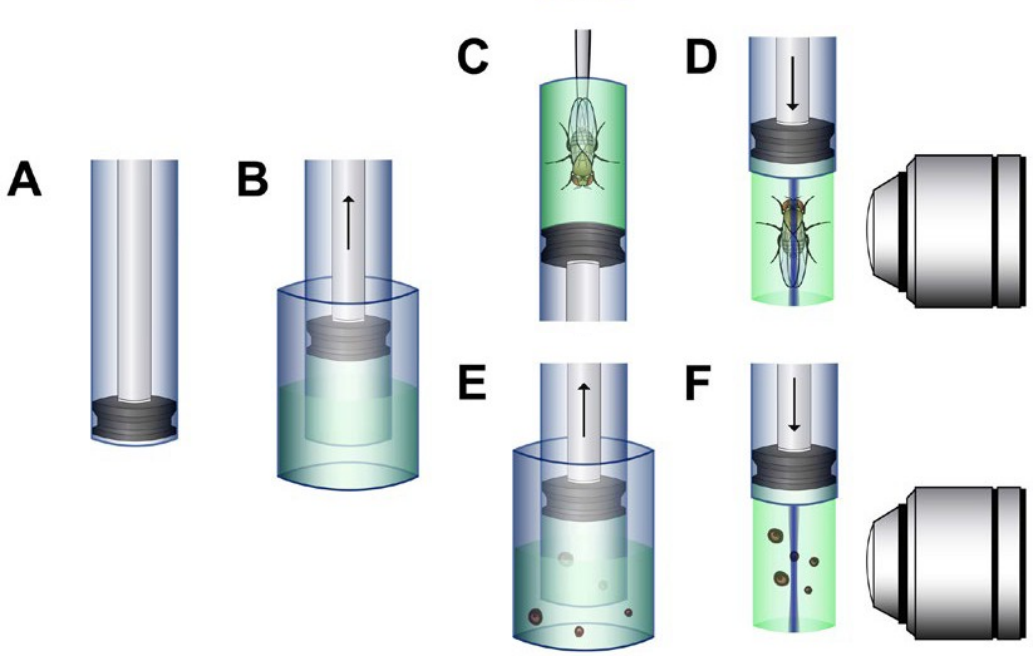
Embedding for in vivo imaging:
- Want to be as close as possible to RI of water (1.33)
- Low melting point agarose cylinder made with 0.8 – 1.0% agarose only suitable for samples that do not change size or shape during the experiment (*Drosophila* embryos), can be used with fiducial markers for multi-view registration
- Sample should be 1/3 to 2/3 the diameter of the capillary or syringe
- Embedding medium and chamber liquid should be made with the same buffer



Sample on a hook and in agarose cylinders of varying diameters

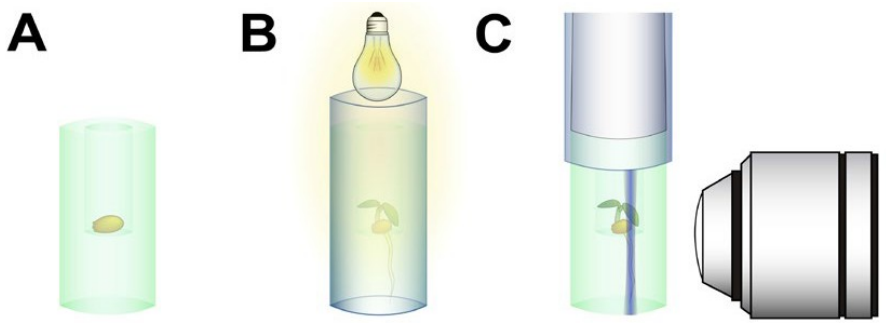
Flood P.M., Kelly R., Gutiérrez-Heredia L. and E.G. Reynaud, Zeiss Lightsheet Z.1 sample preparation

Sample Preparation



A-D
Draw melted agarose into a capillary
Add sample, allow to set, extrude
E-F
Draw sample in agarose into a capillary
Allow to set and extrude

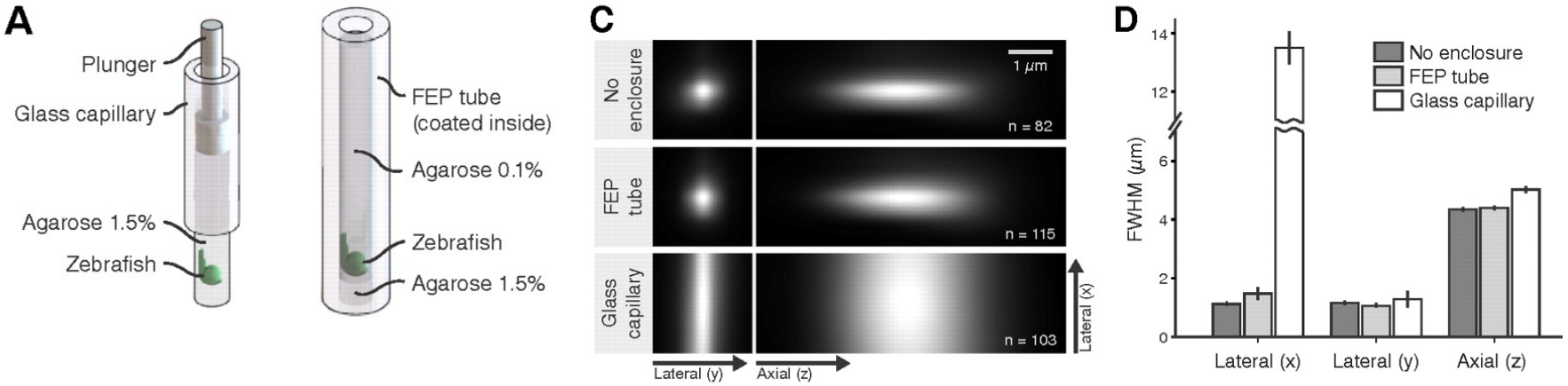
A seed is allowed to germinate in a hollow agarose cylinder. The root grows into the agarose and can then be imaged.



Flood P.M., Kelly R., Gutiérrez-Heredia L. and E.G. Reynaud, Zeiss Lightsheet Z.1 sample preparation

Sample Preparation

Embedding samples that will not develop in 1% agarose:
- fluorinated ethylene propylene (FEP) tubes filled with 0.1% agarose or other viscous medium to restrict movement of the sample and plugged with 1.5% agarose



Kaufmann, A et al, Development 139, 3242-3247 (2010)

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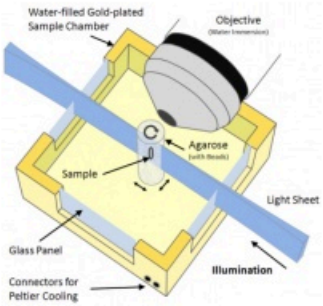
Welcome to the OpenSPIM Wiki

The Idea

OpenSPIM is an *Open Access* platform for applying and enhancing **Selective Plane Illumination Microscopy** (*SPIM*).

SPIM principle

The SPIM technology offers fast, optically-sectioning, minimally-invasive 3D acquisition of fluorescing specimen over time. It achieves that by focusing a thin laser light-sheet into the specimen, taking two-dimensional images of the illuminated slice with a perpendicularly positioned detector (CCD camera). Three-dimensional stacks are obtained by moving the specimen orthogonal to the light-sheet between consecutive images. By mounting the sample in a rigid medium, e.g. agarose, and hanging it into the sample chamber in front of the detection lens, it is possible to rotate the sample and collect 3d stacks from multiple angles (views).



OpenSPIM

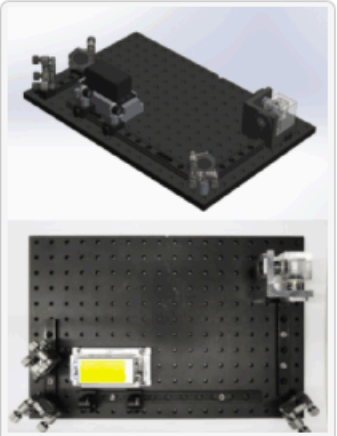
OpenSPIM is a platform to build, adapt and enhance SPIM technology. It is designed to be as accessible as possible:

- detailed, easy-to-follow build instructions
- off-the-self components and 3D-printed parts
- Modular and extensible design
- completely open blueprints
- completely Open Source

The build instructions are intended to allow scientists without prior knowledge in building optical systems to make their own OpenSPIM set-up. If a 3D printer is not readily available, the parts are designed to be easily machined by any competent work shop. The set-up is small enough to fit inside a suitcase. The software is built on top of the Open Source projects µManager and Fiji.

OpenSPIM is designed to be maximally cost-effective allowing anyone to build an entry level system and further tweak it for the specific imaging needs. Parallel set-ups (SPIM farms) can be realized to enable medium throughput, long-term, time-lapse imaging.

OpenSPIM aims to creates a powerful synergy between Open Software and Open Hardware that can serve as a nucleus for further development of the SPIM technology.



OpenSPIM buildup

SPIM application

The End