Light-sheet microscopy

Imaging developmental processes in vivo

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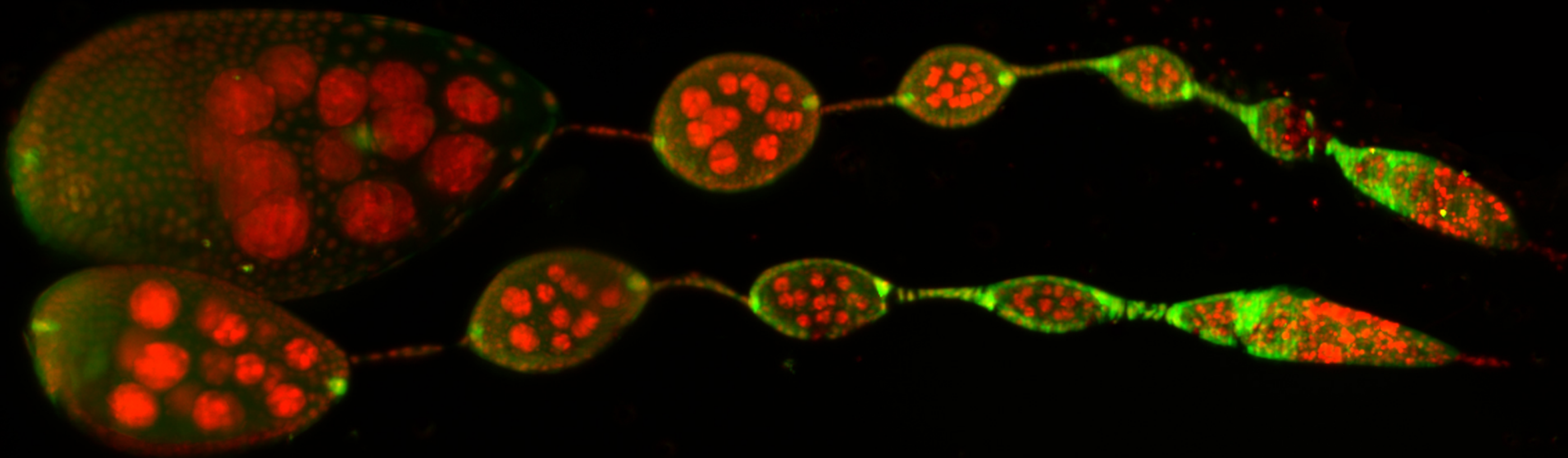
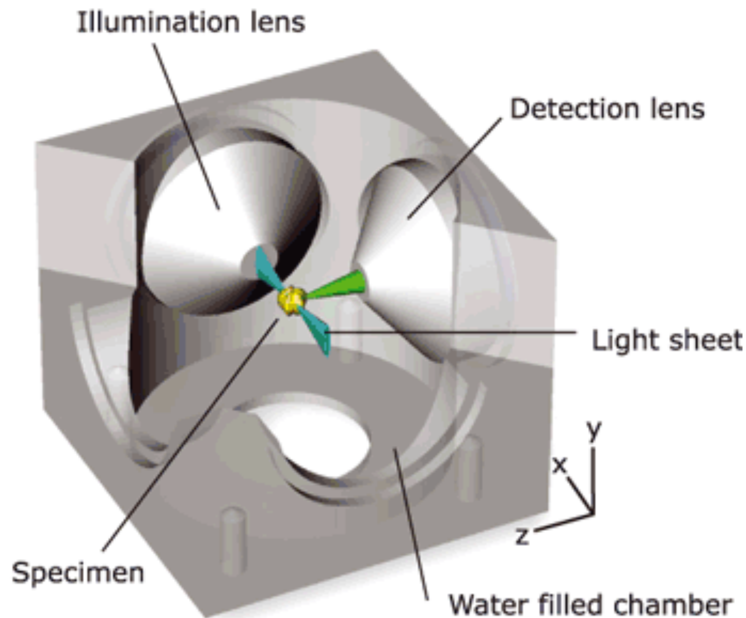


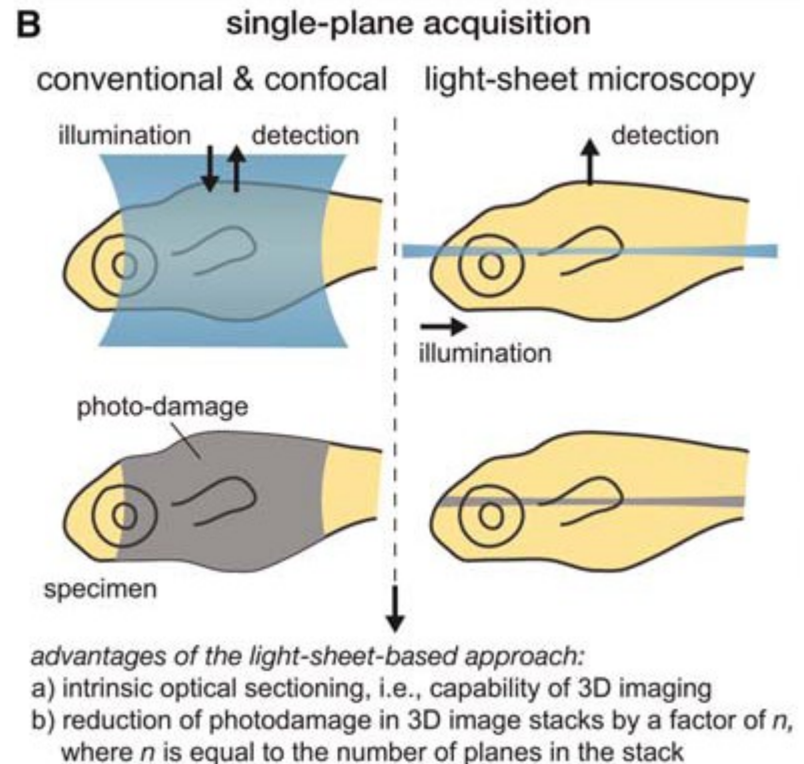
Micron OXFORD

How does a light-sheet microscope work?

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





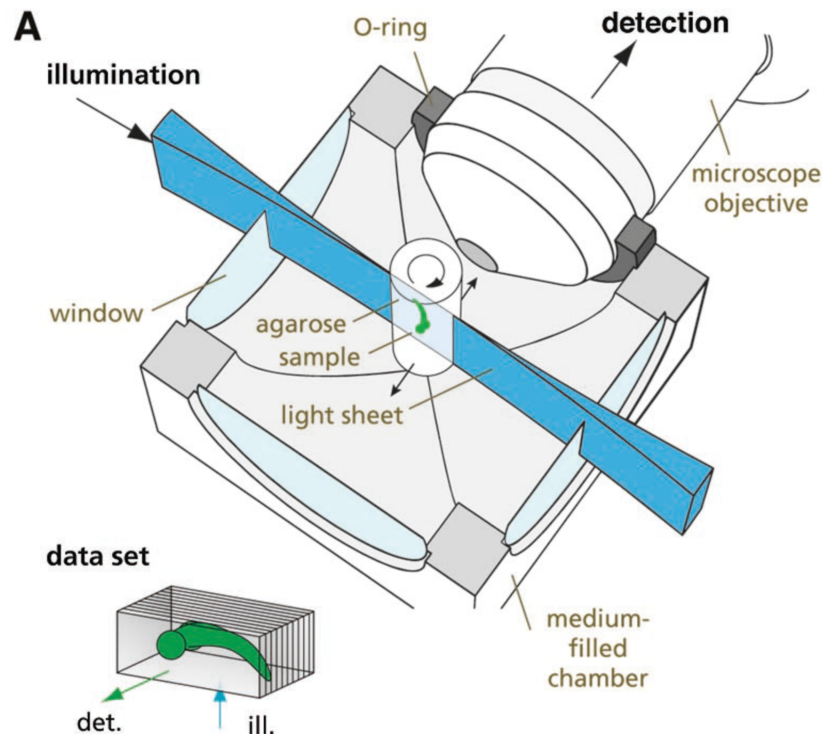
Compared with confocal and two-photon fluorescence microscopy,DSLM exposes the embryo to at least three orders of magnitude less light energy, but still provides up to 50 times faster imaging speeds and a 10–100-fold higher signal-to-noise ratio.

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

How does a light-sheet microscope work?
Selective Plane Illumination Microscope (SPIM)

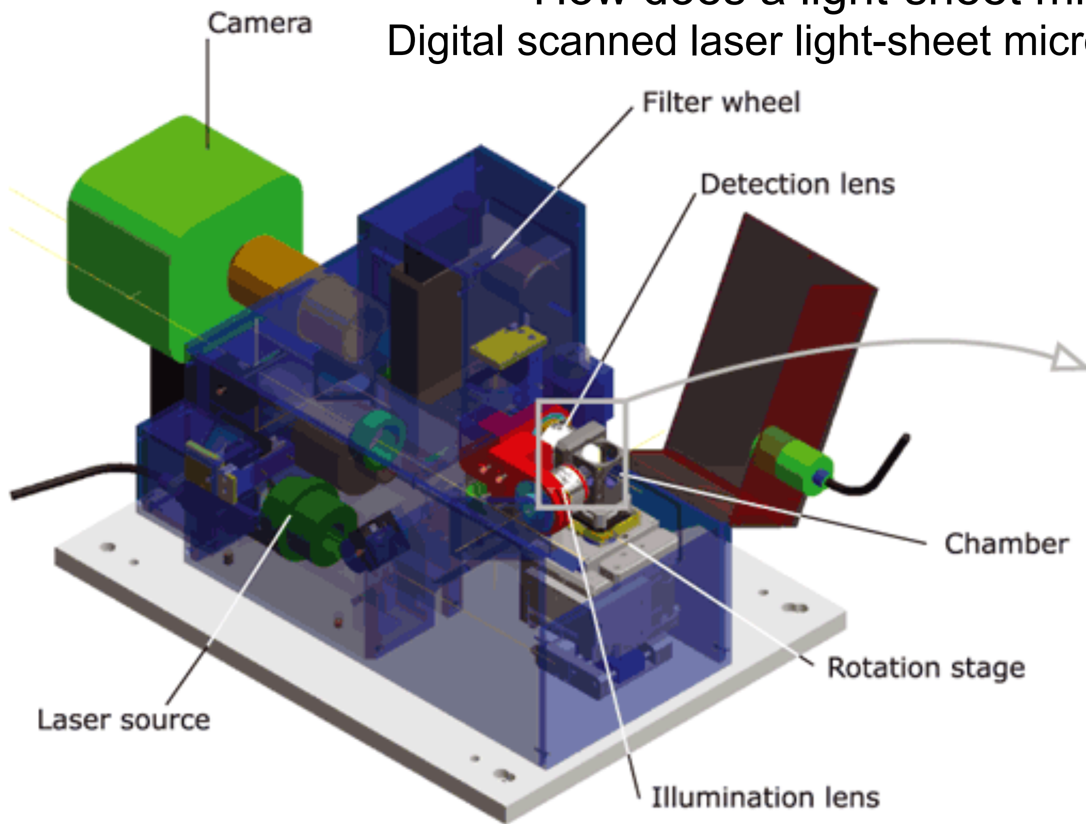
Jan Huisken et al., Science 305, 1007 (2004)

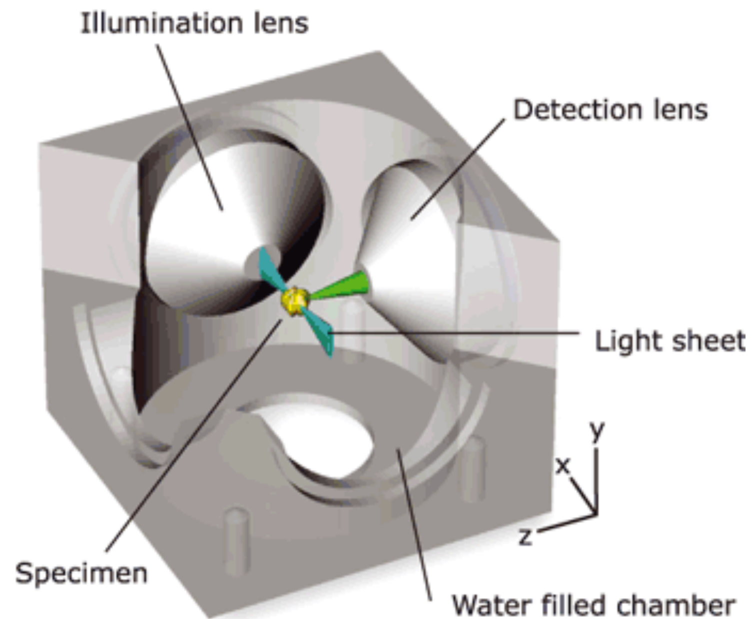
Excitation light is focussed by a cylindrical lens to a sheet of light that illuminates only the focal plane of the detection optics

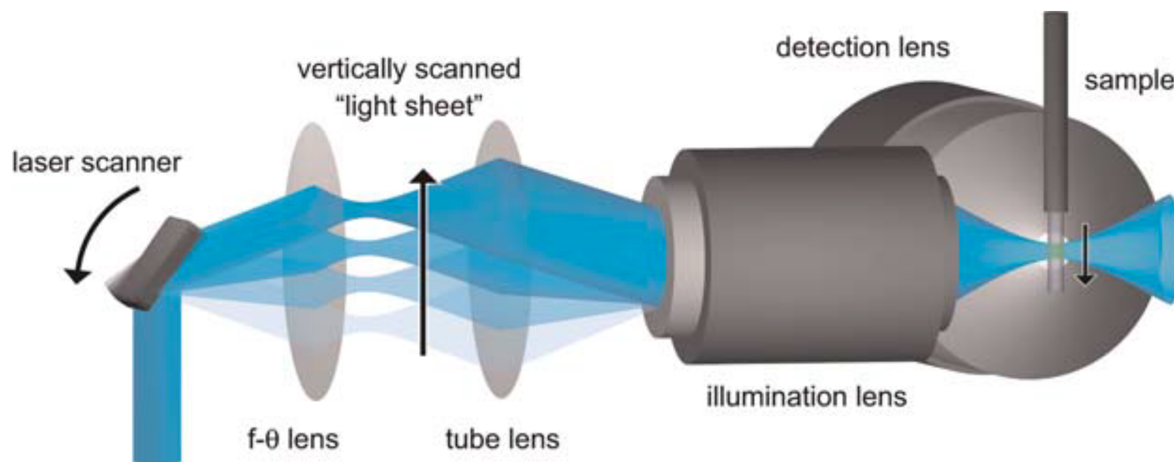


How does a light-sheet microscope work?

Digital scanned laser light-sheet microscope (DSLM) in Micron







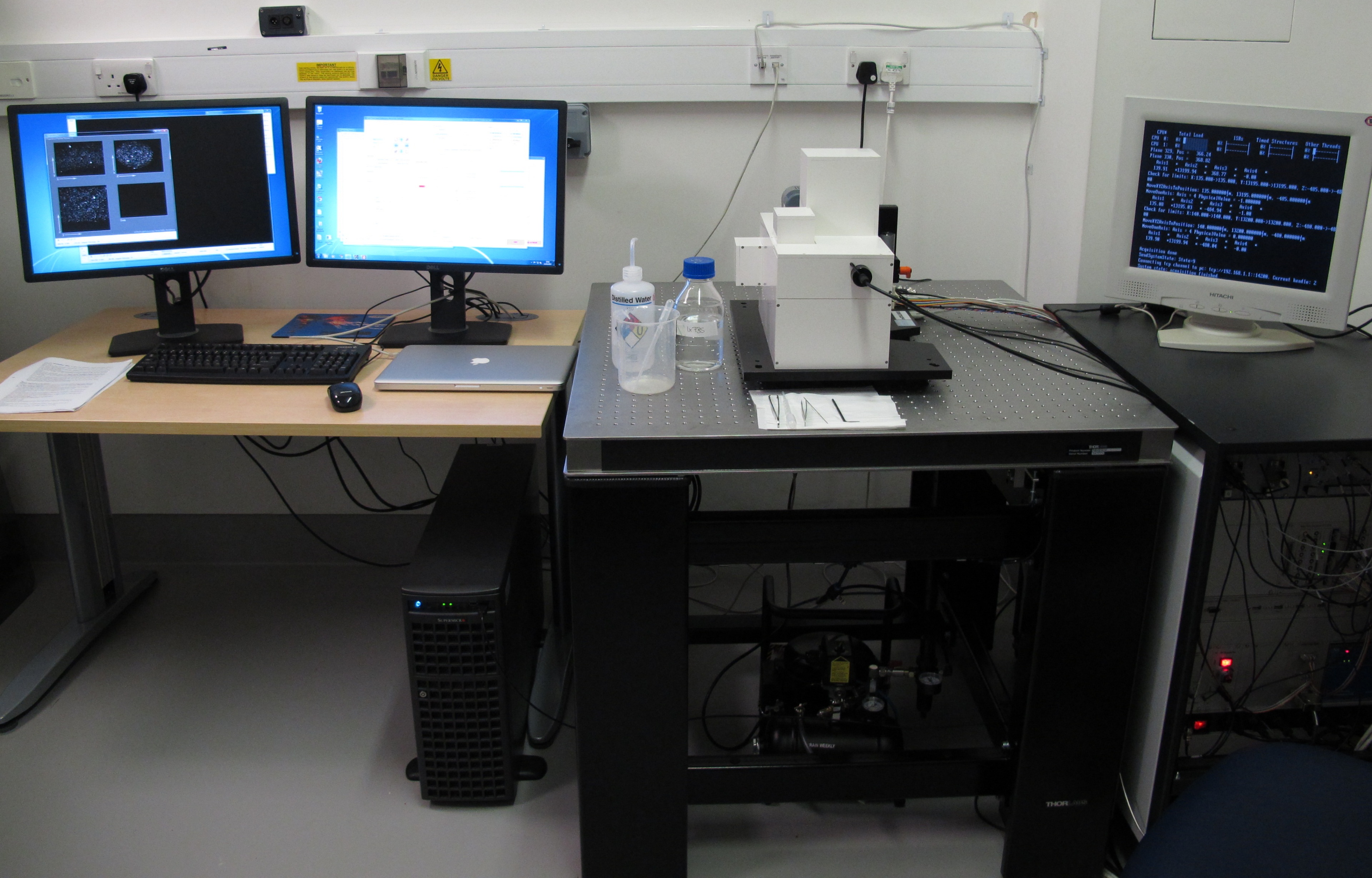
Available laser lines:

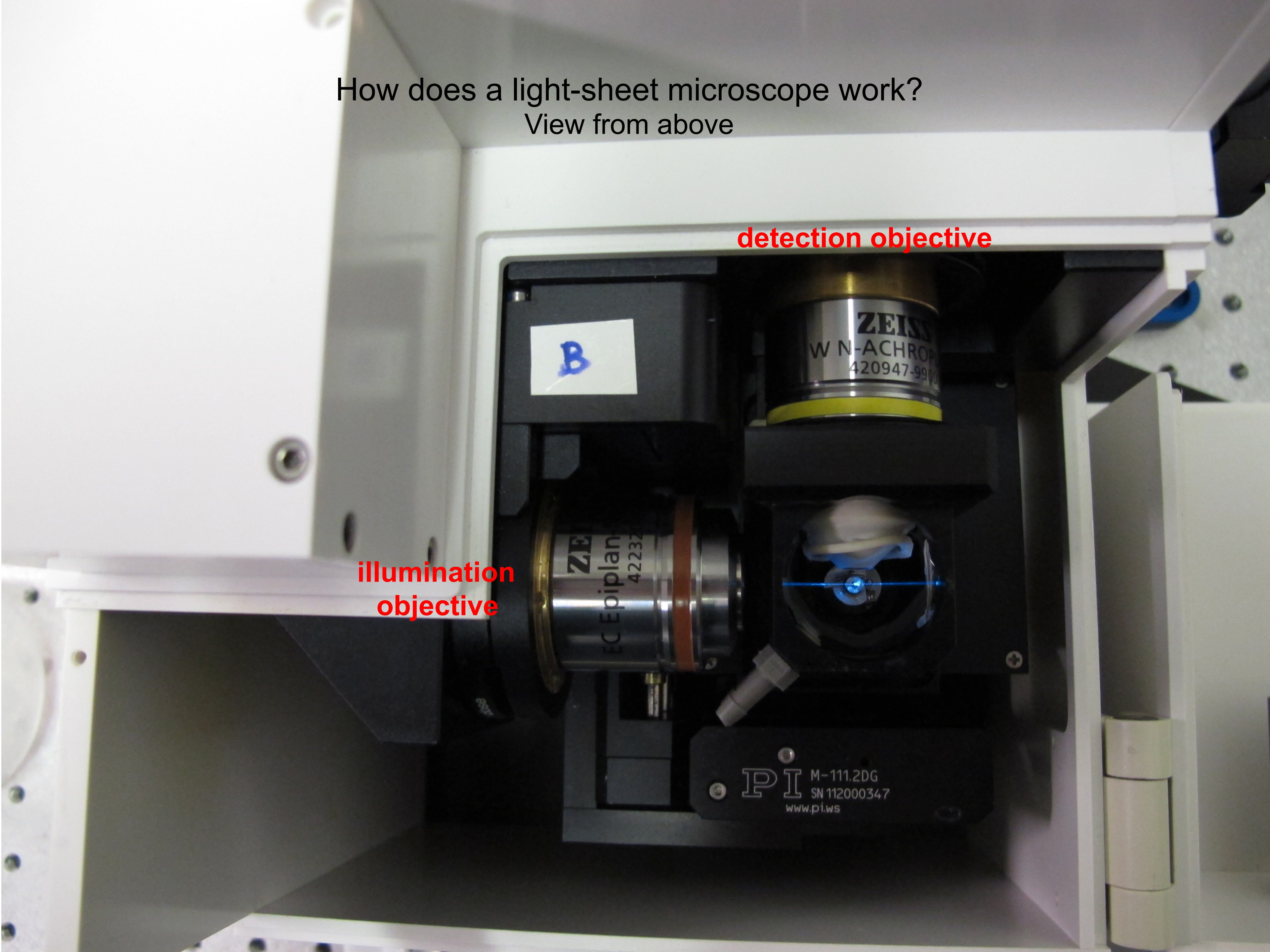
488 nm

561 nm

638 nm

The light-sheet microscope in Micron




How does a light-sheet microscope work?
View from above
detection objective
illumination
objective
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EC Epiplan
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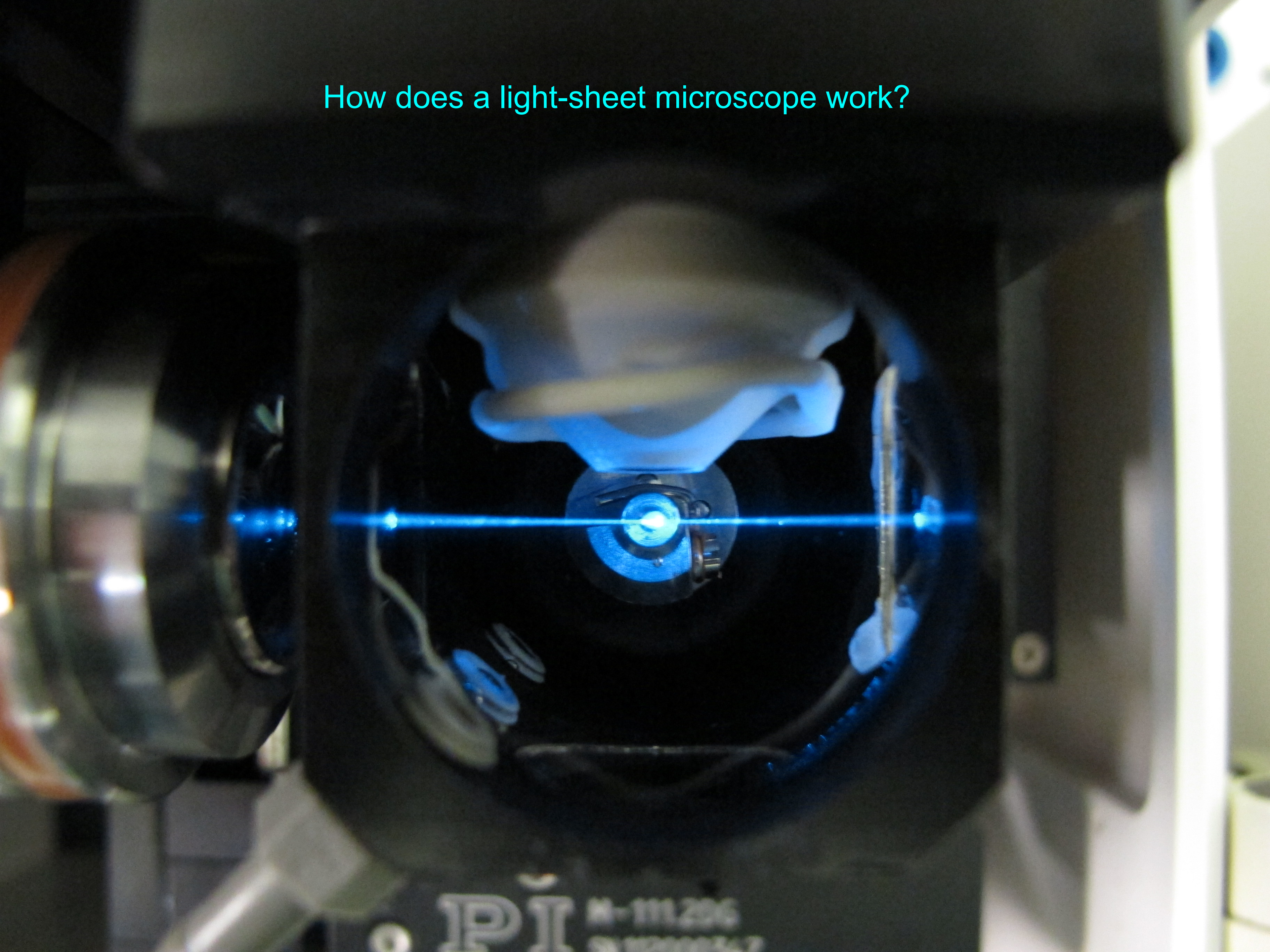
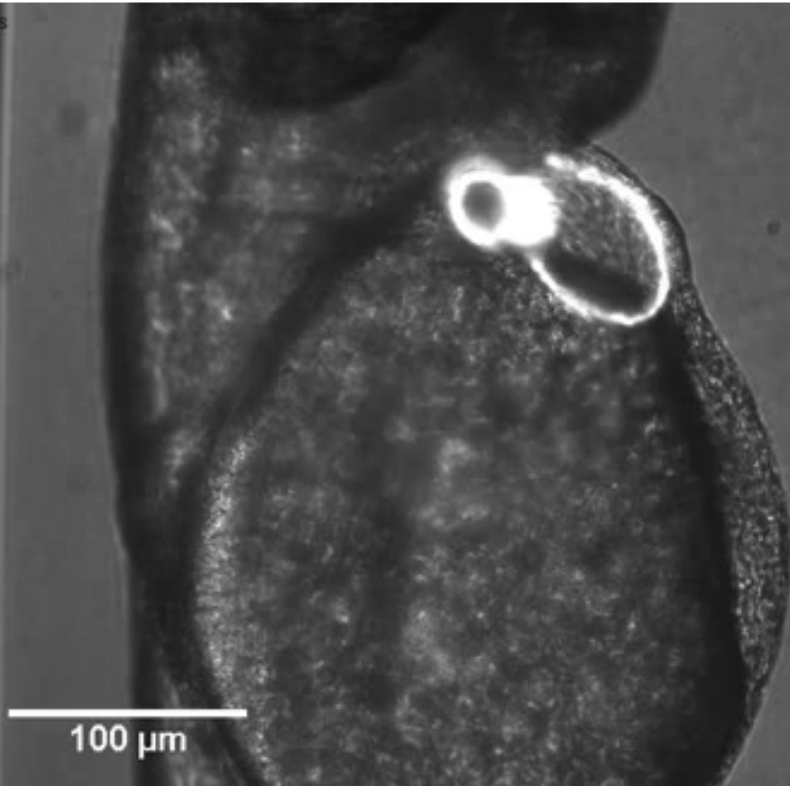
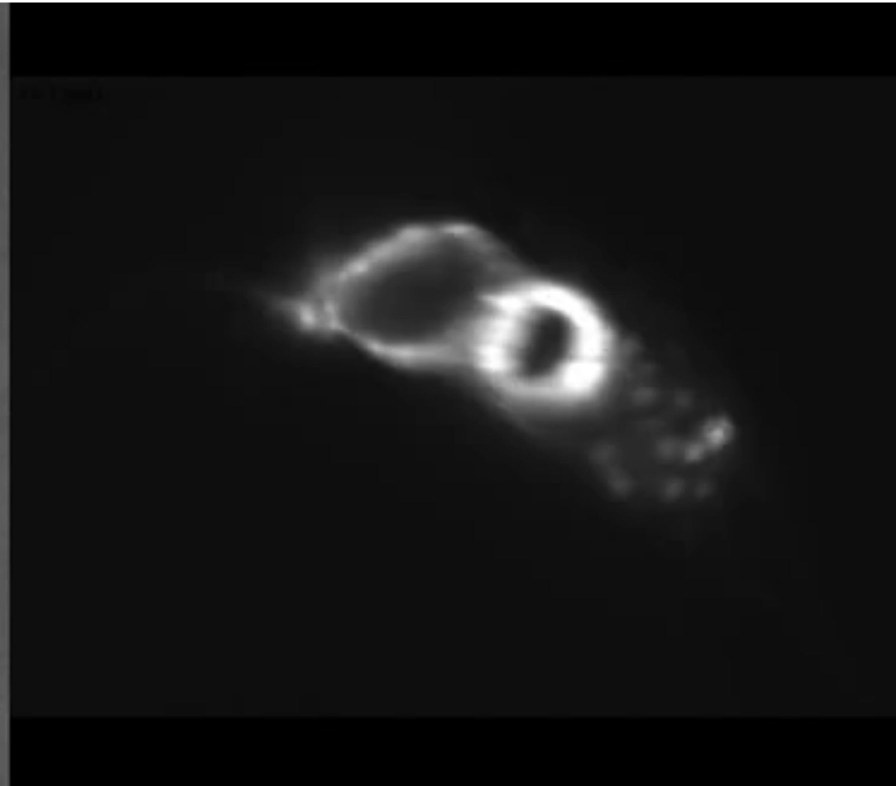
www.pi.ws


How does a light-sheet microscope work?
PI

How does a light-sheet microscope work?



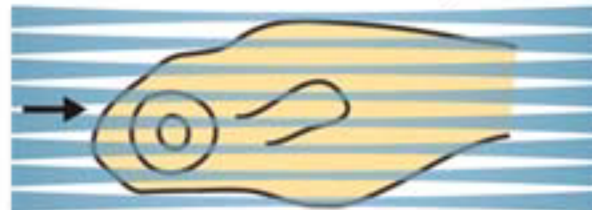


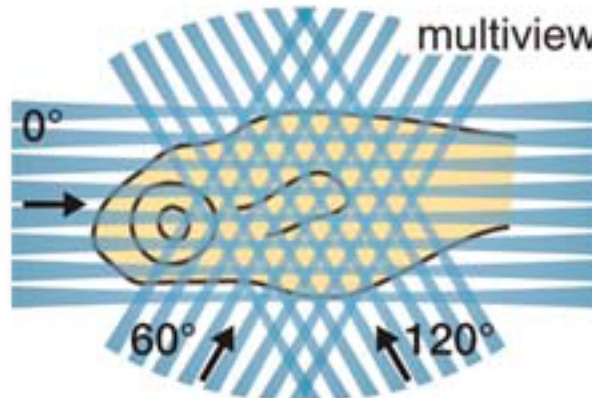
Beating heart of a 48 hpf Tg(cmlc2:EGFP) zebrafish. Left: overlay of transmitted light and fluorescence signal. Right: fluorescence signal.
openspim.org

3D data acquisition

light-sheet microscopy

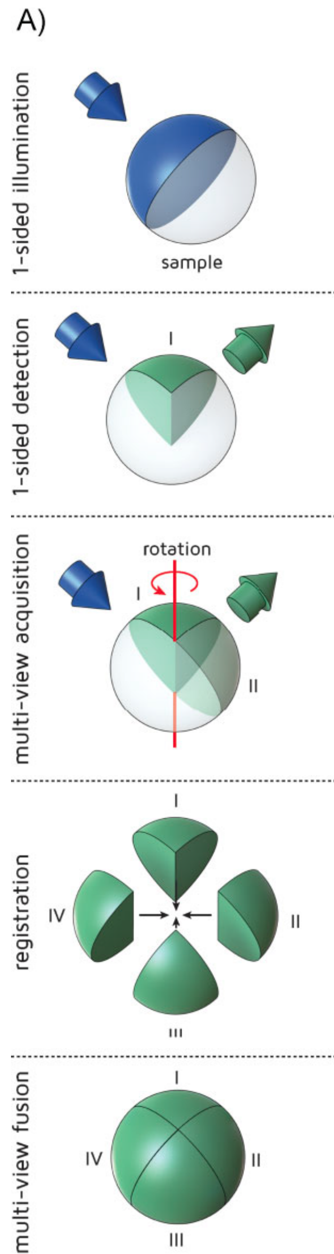
single view





improved axial resolution
reconstruction of large specimen

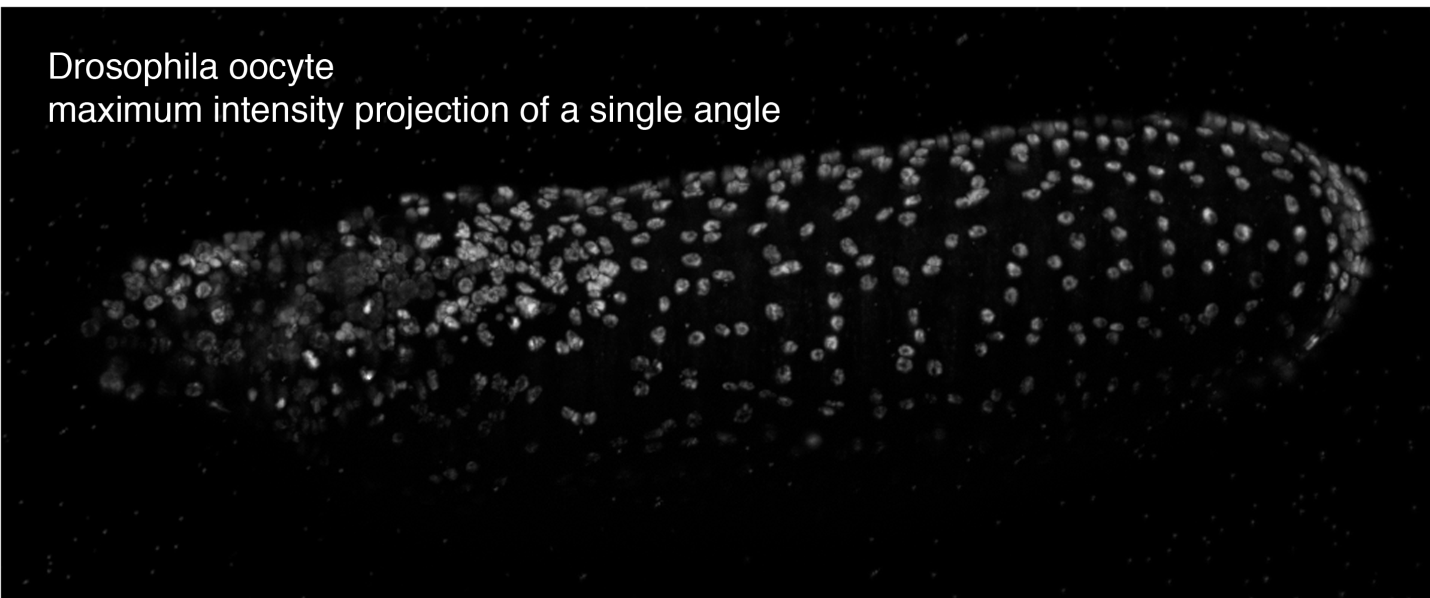
Multi-View Fusion

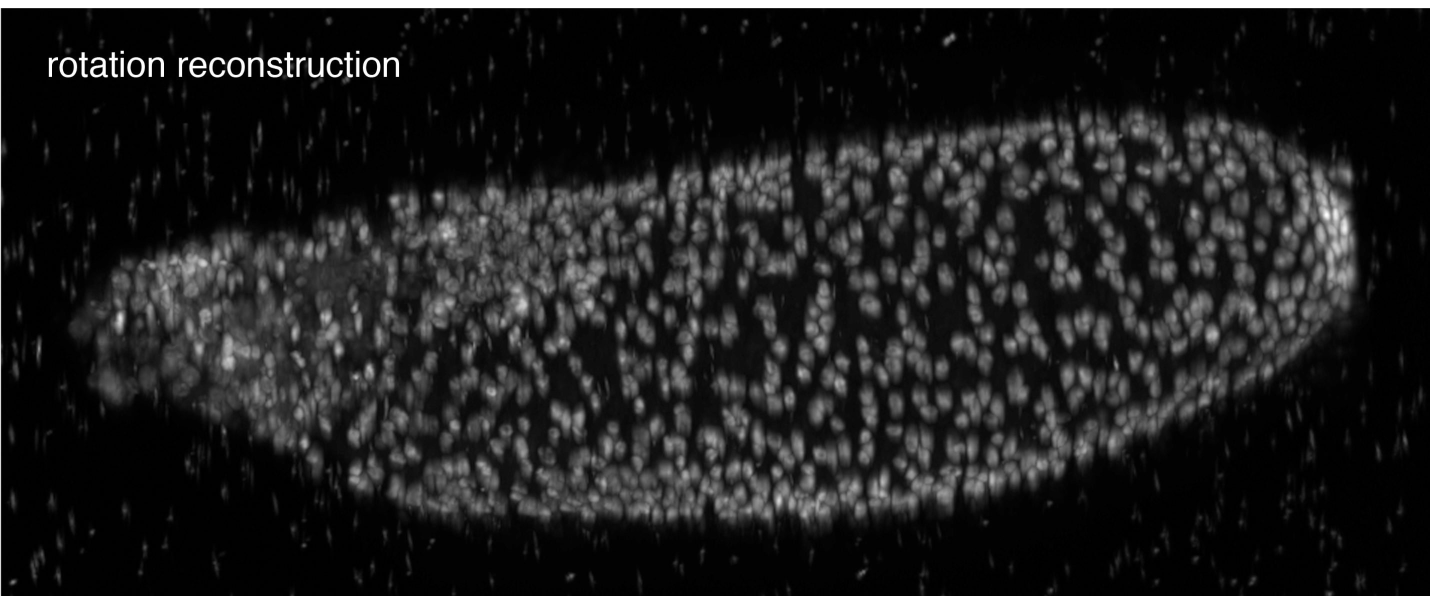


Nuclei in red
Membranes in cyan

Jan Huisken
Bioessays 34: 406–411

Multi-View Fusion

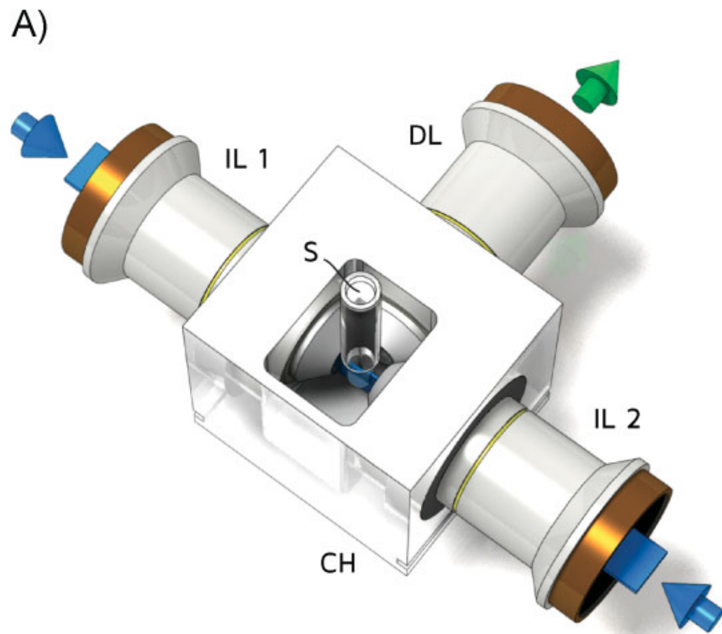


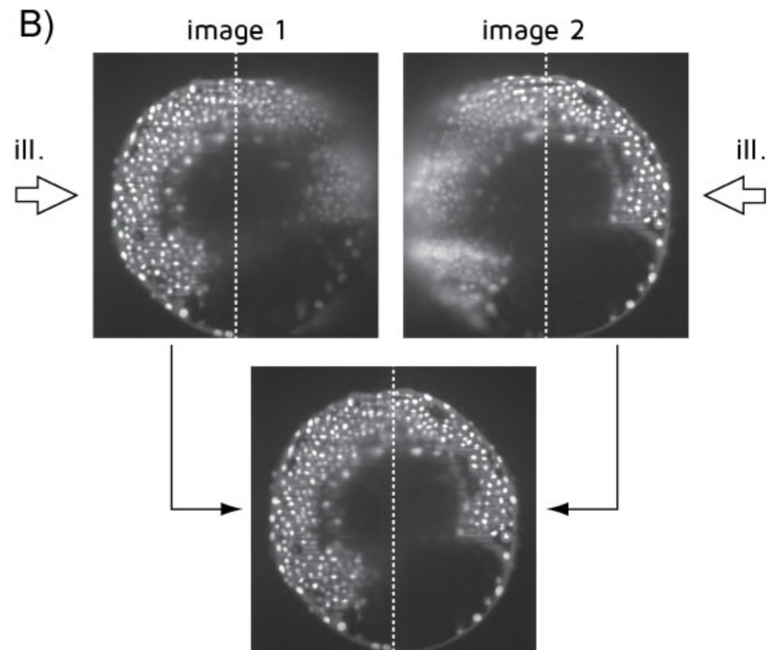


Dual-sided illumination in multi-directional SPIM

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

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





Jan Huisken
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Resolution of the light-sheet microscope

Theoretical lateral resolution of a light sheet microscope like any wide-field microscope: approximately half the wavelength, ca. 250 nm

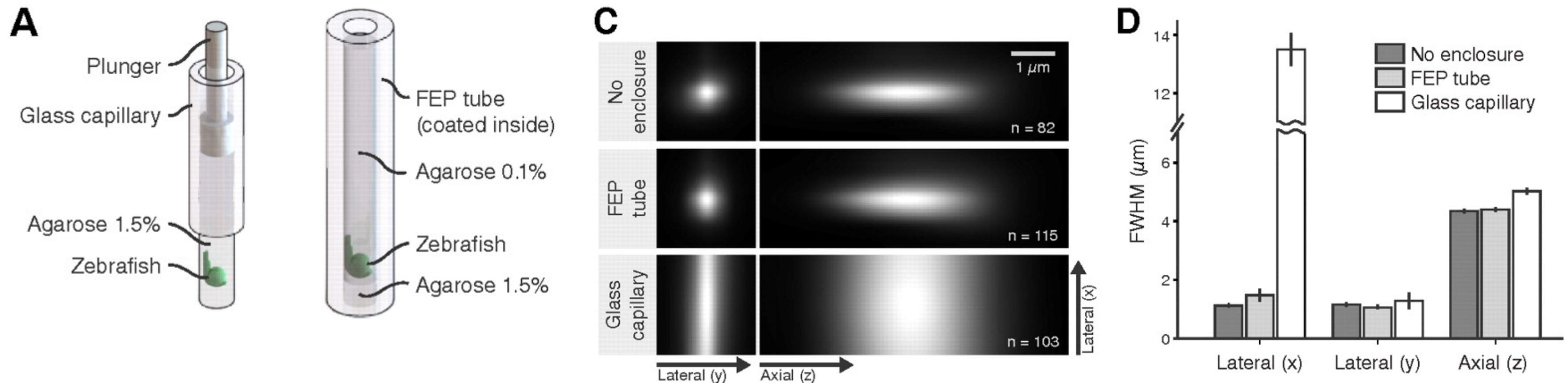
Achievable lateral resolution limited by the resolution of the camera and the optics:
1.48 µm for 10x detection objective NA 0.3
0.74 µm for 20x detection objective NA 0.5
0.41 µm for 40x detection objective NA 0.75

Axial resolution is given by the light sheet thickness (ca. 2–6 µm, depending on the field of view) in the case of the commonly used long working distance objectives with large depth of focus.

With a 10x NA 0.3 objective the axial resolution of a light-sheet microscope is better than that of a laser scanning confocal.

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 1 – 1.5% 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
- 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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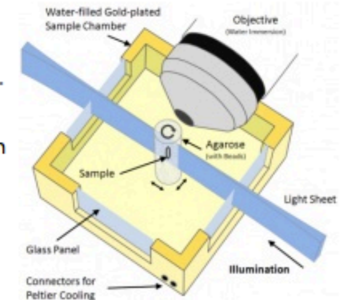
Welcome to the OpenSPIM Wiki

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).

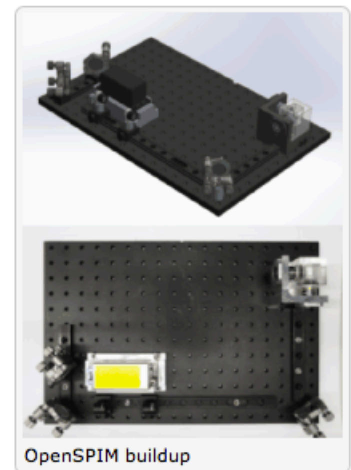


OpenSPIM

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

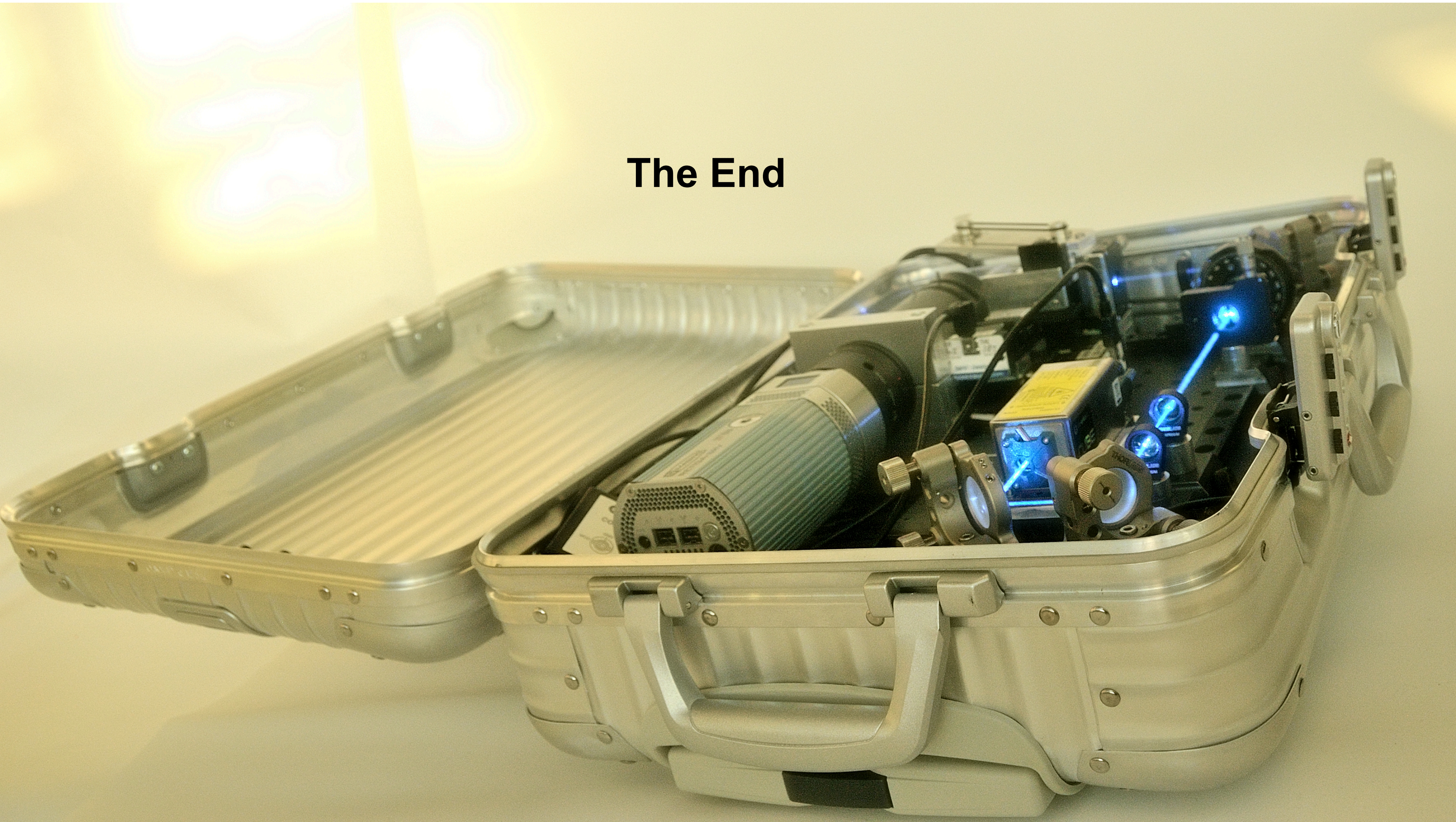
- detailed, easy-to-follow build instructions
- off-the-self components and 3D-printed parts
- Modular and extensible design
- completely open blueprints
- completely Open Source

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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