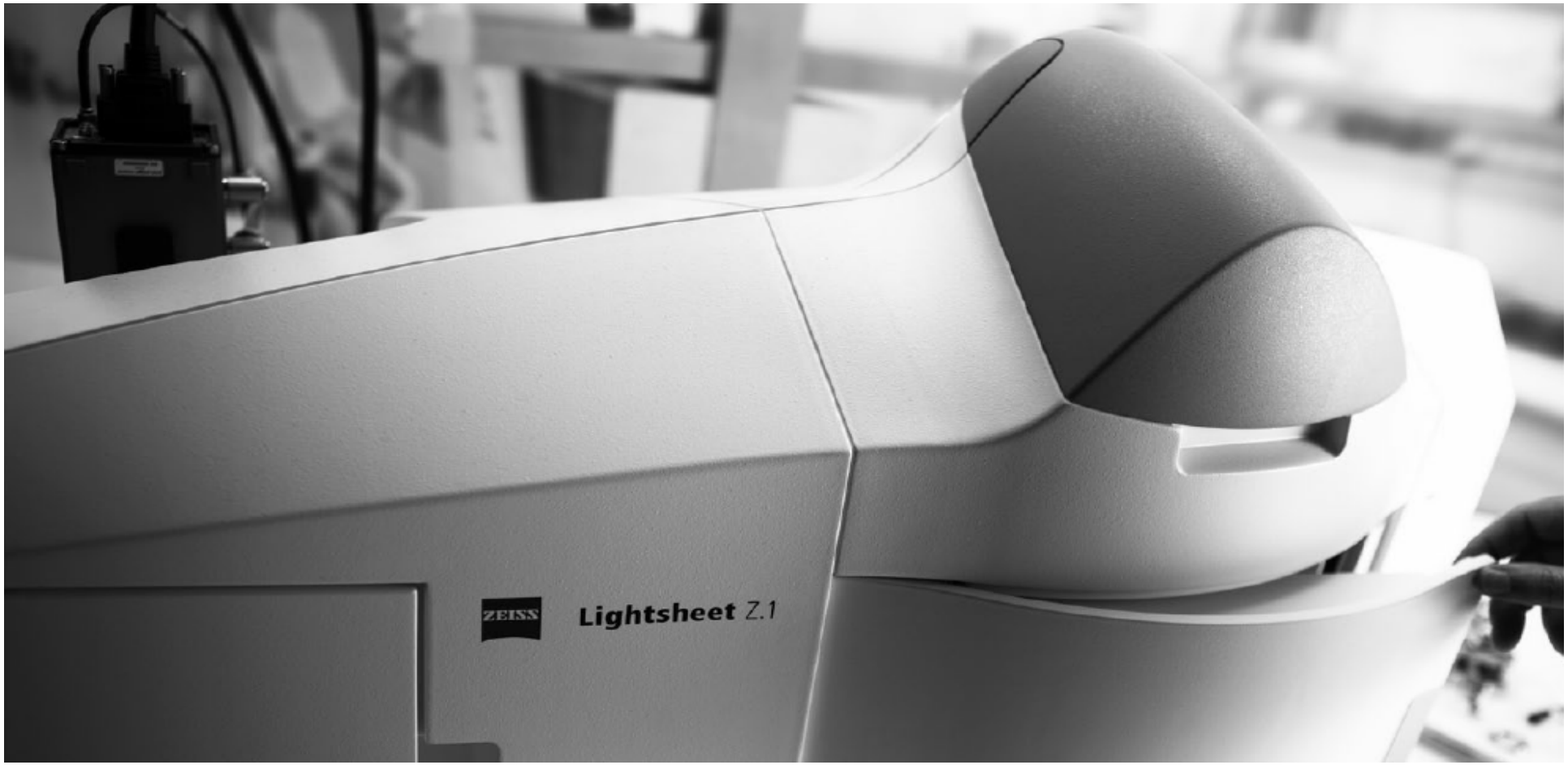
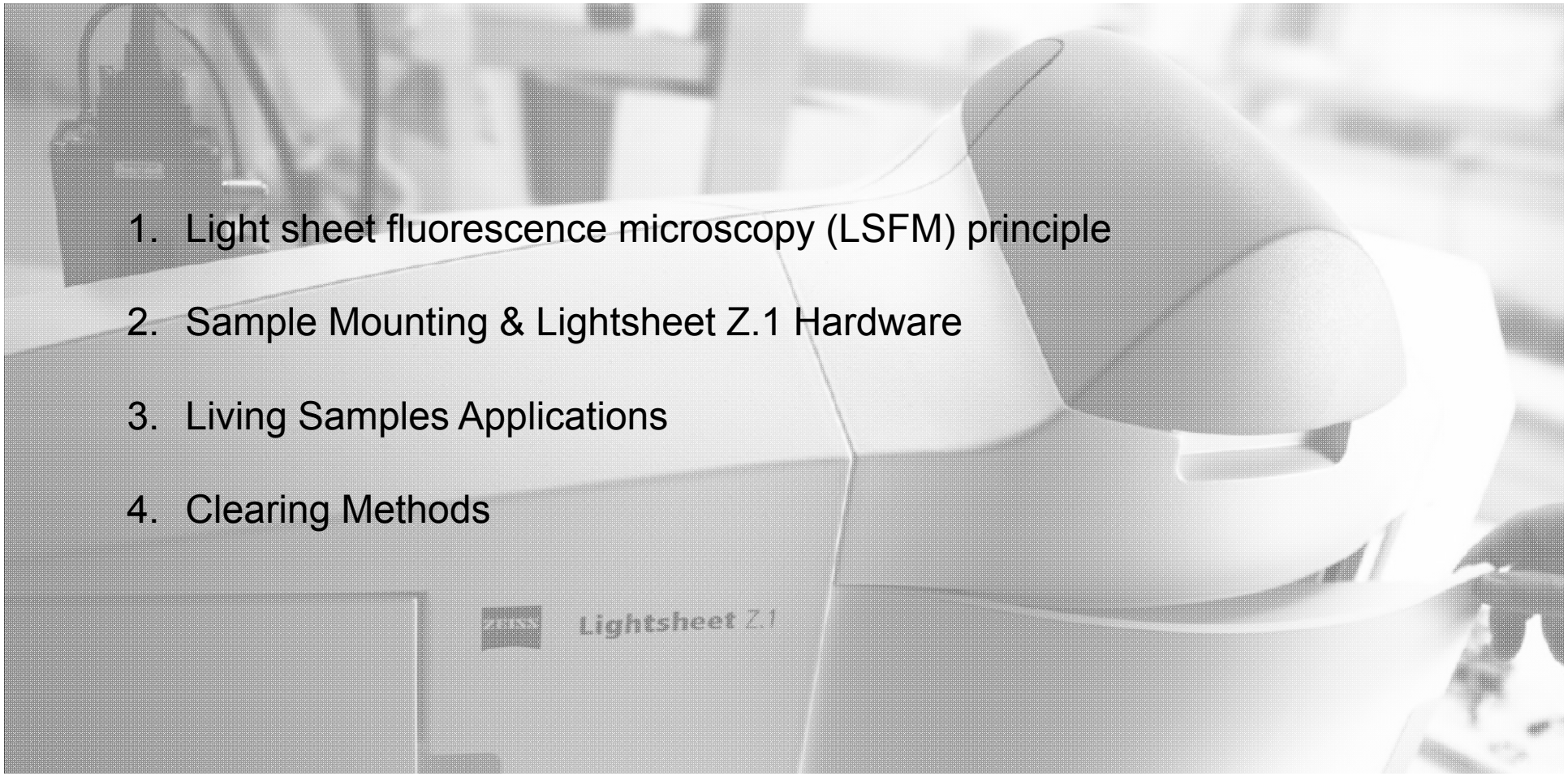
Light Sheet Fluorescence Microscopy
A system built around your samples



Emmanuel ELIAS

Advanced Imaging Microscopy Specialist

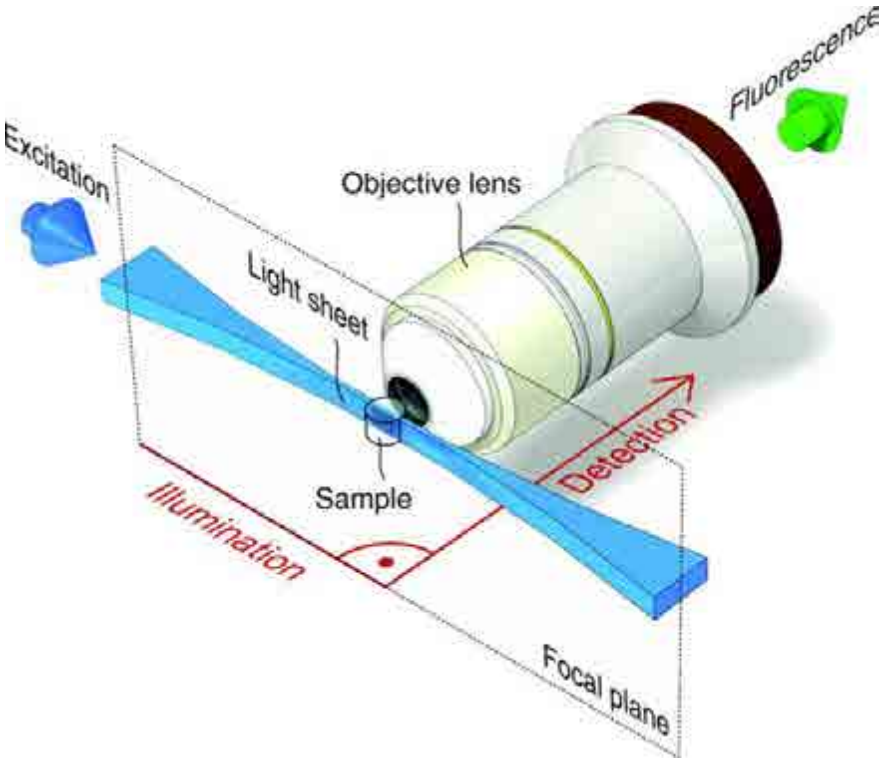
Agenda



1. Light sheet fluorescence microscopy (LSFM) principle
2. Sample Mounting & Lightsheet Z.1 Hardware
3. Living Samples Applications
4. Clearing Methods

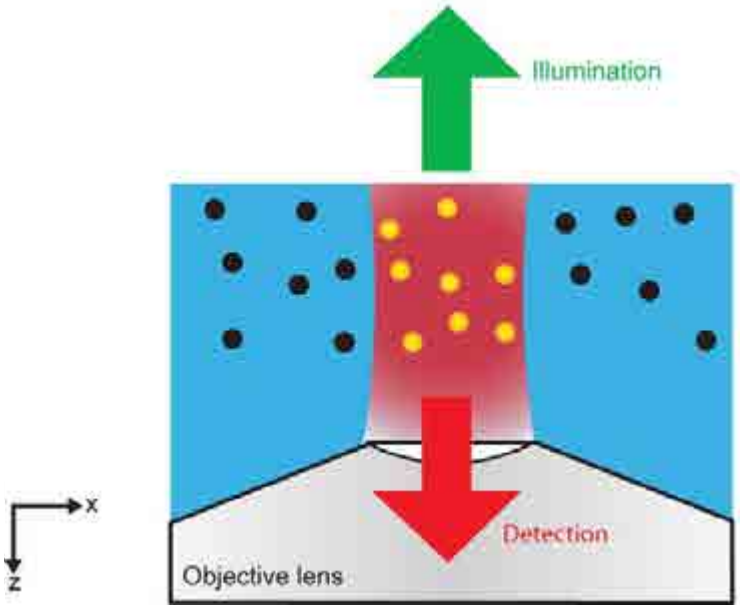
Light sheet fluorescence microscopy (LSFM)
The principle

- Orthogonal light paths for Illumination and detection in a horizontal microscope
- Illumination source is a laser
- In SPIM, light sheet is shaped by a cylindrical lens
- In DSLM, LightScanning mirrors move the beam along the focal plane (y-direction) to create the light sheet

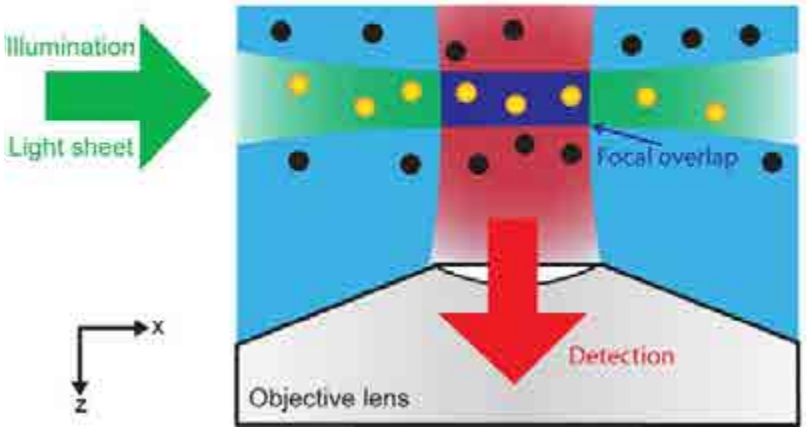


Light Sheet Fluorescence Microscopy
Epi-illumination vs. Light sheet illumination

Epi-Illumination



Light sheet illumination



- Inherent optical sectioning capability of the illumination method
- No excitation of out-of-focus fluorescence

Figure from the PhD thesis of Jörg Ritter (2011), University of Bonn, Germany

Built around your sample
Any View: Rotation

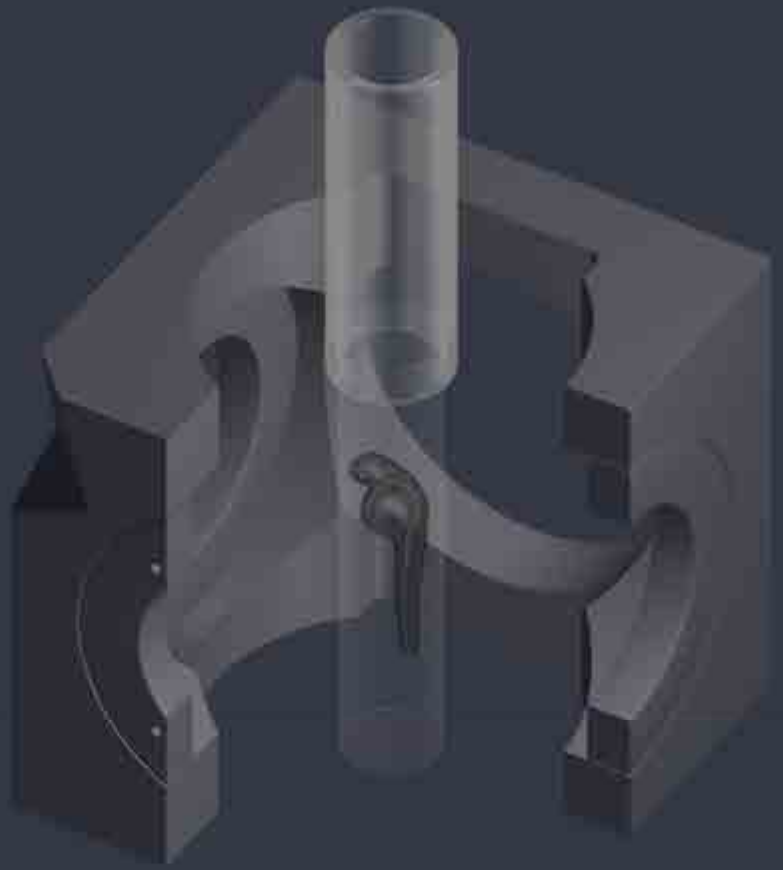
Sample mounted vertically in a hydrogel

- Ideal for larger, living specimens
- Easy to prepare & store using common laboratory materials
- Translates & rotates : easy positioning, moves for generating Z-stacks, allows multiple viewing perspectives
- Suspended in a medium/buffer

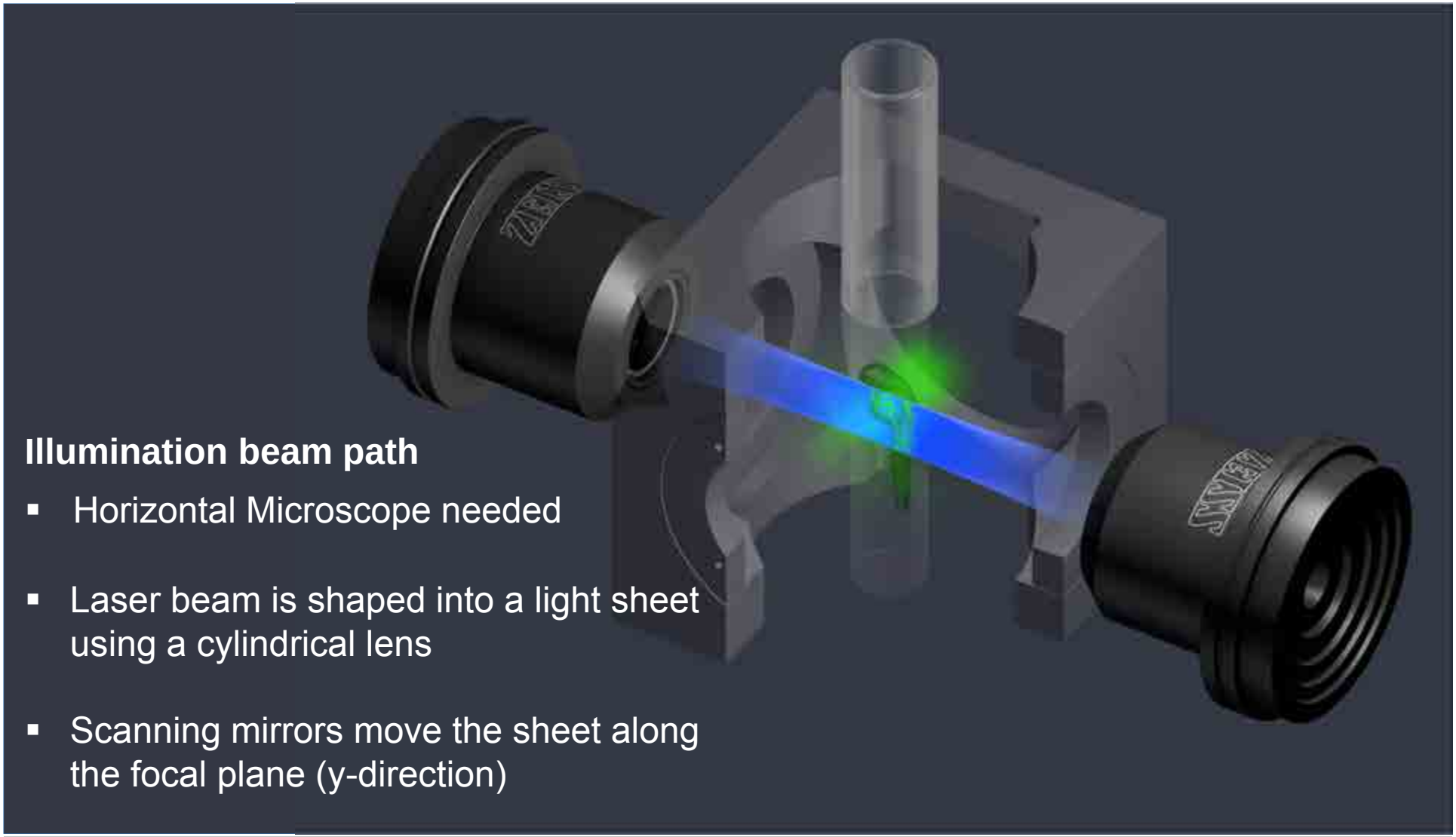
Built around your sample
A stable environment

Chamber for aqueous sample environment

- Physiological conditions maintained
- Aqueous medium and minimized aberrations
- Compact & stable temperature controlled incubation (hot & cold) with CO_2



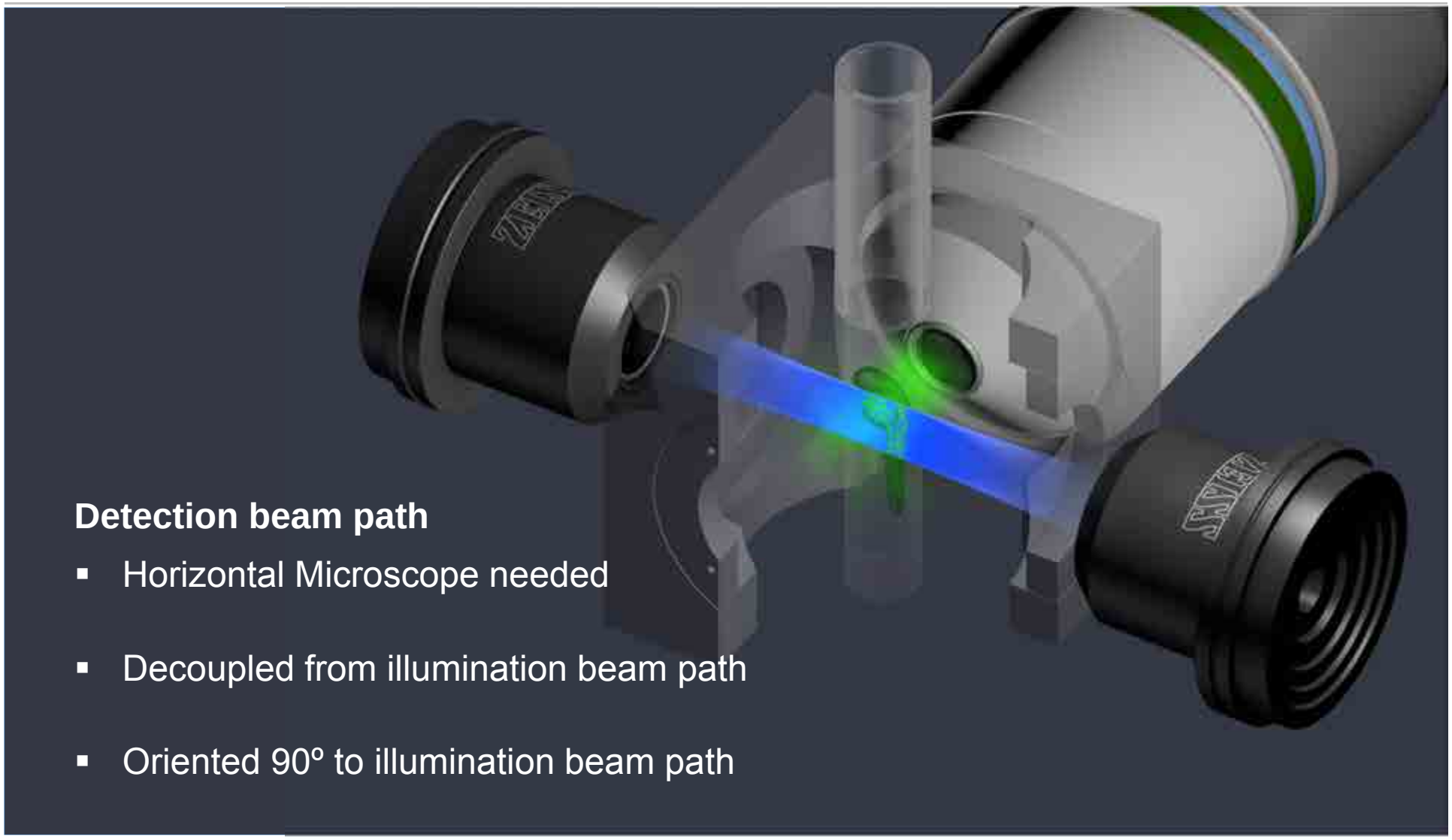
Built around your sample
Illumination in a horizontal microscope



Illumination beam path

- Horizontal Microscope needed
- Laser beam is shaped into a light sheet using a cylindrical lens
- Scanning mirrors move the sheet along the focal plane (y-direction)

Built around your sample
Detection in a horizontal microscope

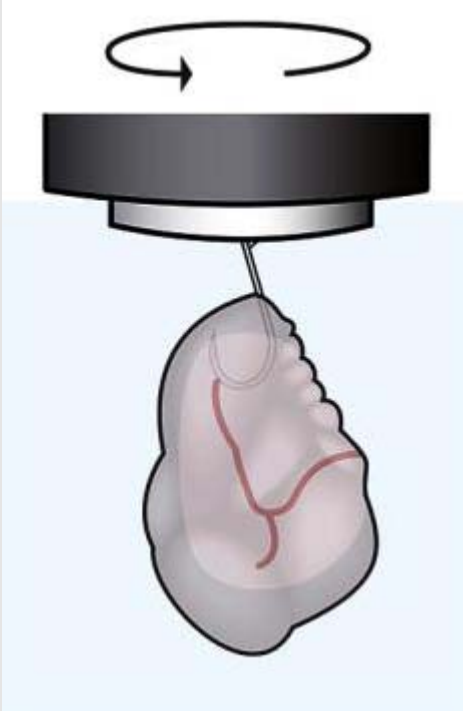


Detection beam path

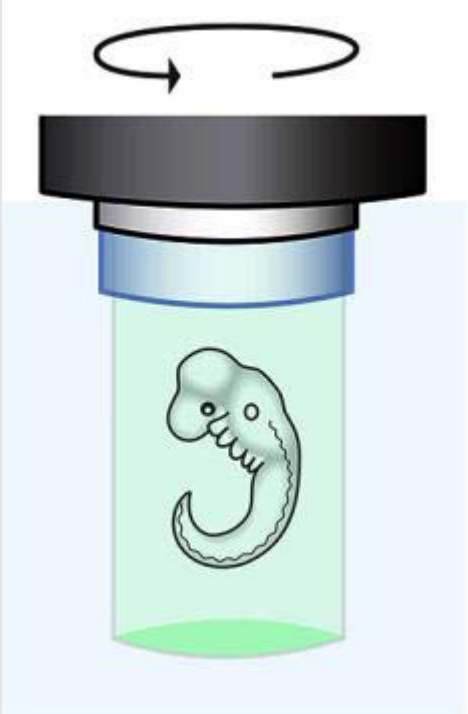
- Horizontal Microscope needed
- Decoupled from illumination beam path
- Oriented 90º to illumination beam path

Lightsheet Z.1
Sample Mounting Options

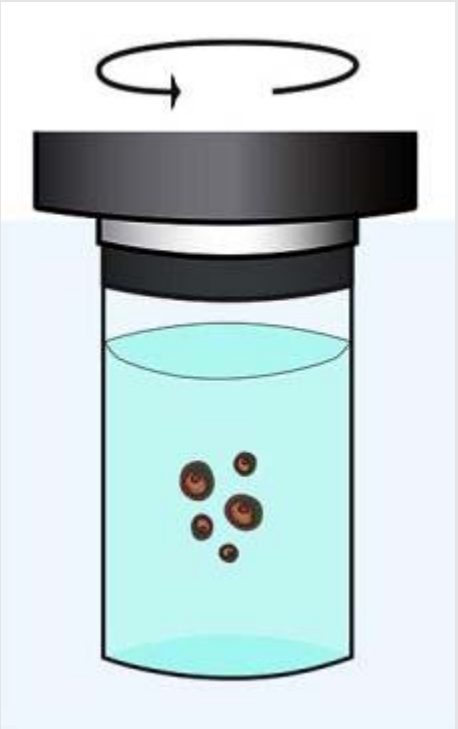
Hanging Samples



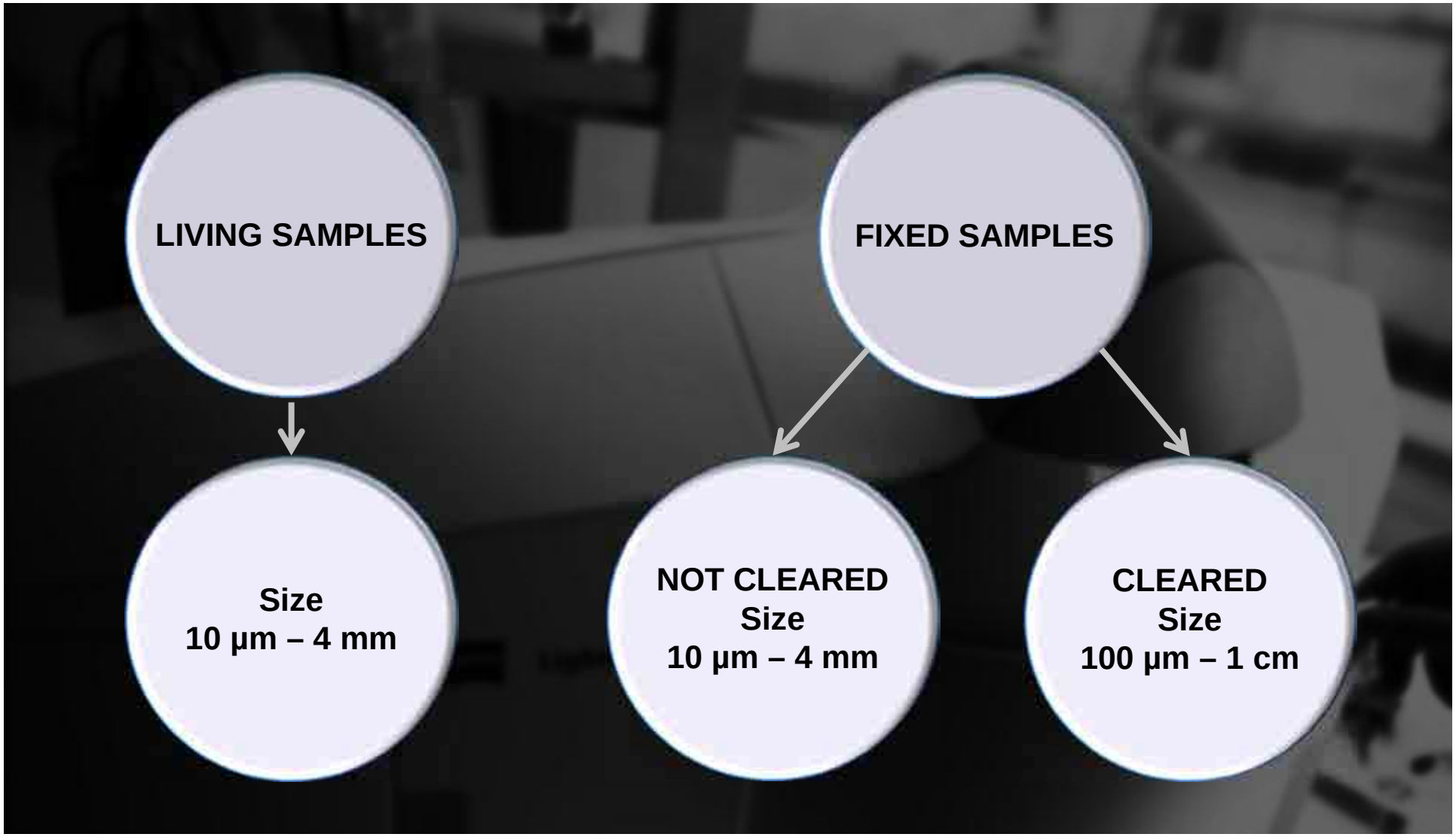
Embedded Samples



Enclosed Samples



Light sheet fluorescence microscopy (LSFM)
For which samples & applications



How do we generate a lightsheet?

The Laser Scanning Approach

- Small light sheet is shaped by a cylindrical lens
- This small light sheet covers ~ 30% of field of view (in vertical (y) direction)
- Scanning mirrors move the light sheet along the focal plane (y-direction), with appropriate amplitude to cover FoV
- Light sheet thickness (in detection (z) direction in the image center): 2 µm – approx. 14 µm (@ 488 nm) depending on FoV, Zoom settings and sample properties)
- Available wavelengths: 405 nm, 445 nm, 488 nm, 515 nm, 561 nm, 638 nm (with different power options)

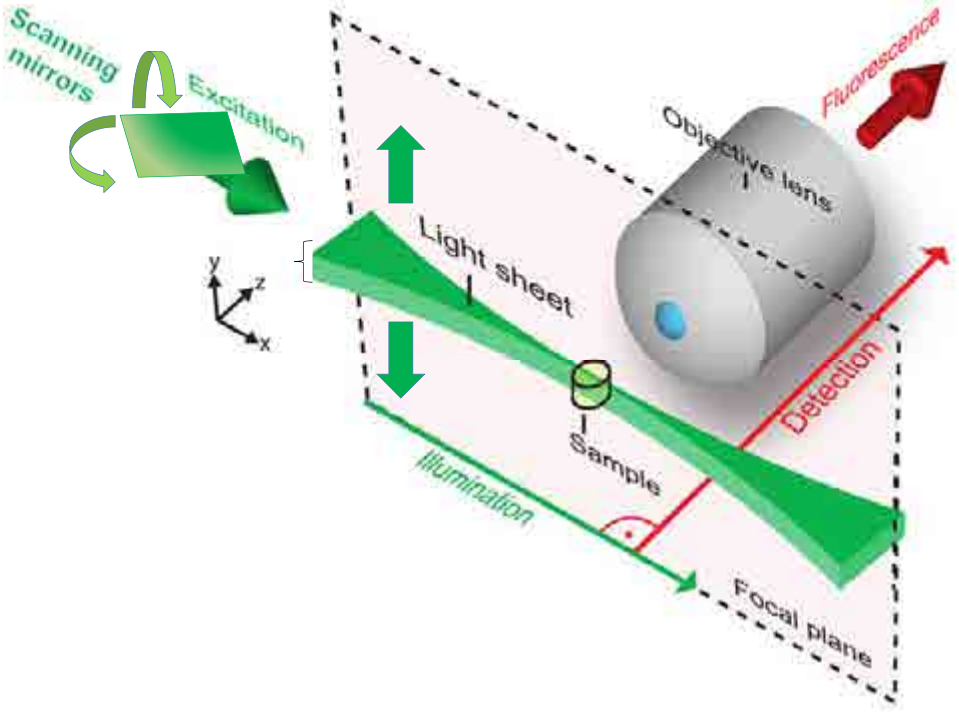


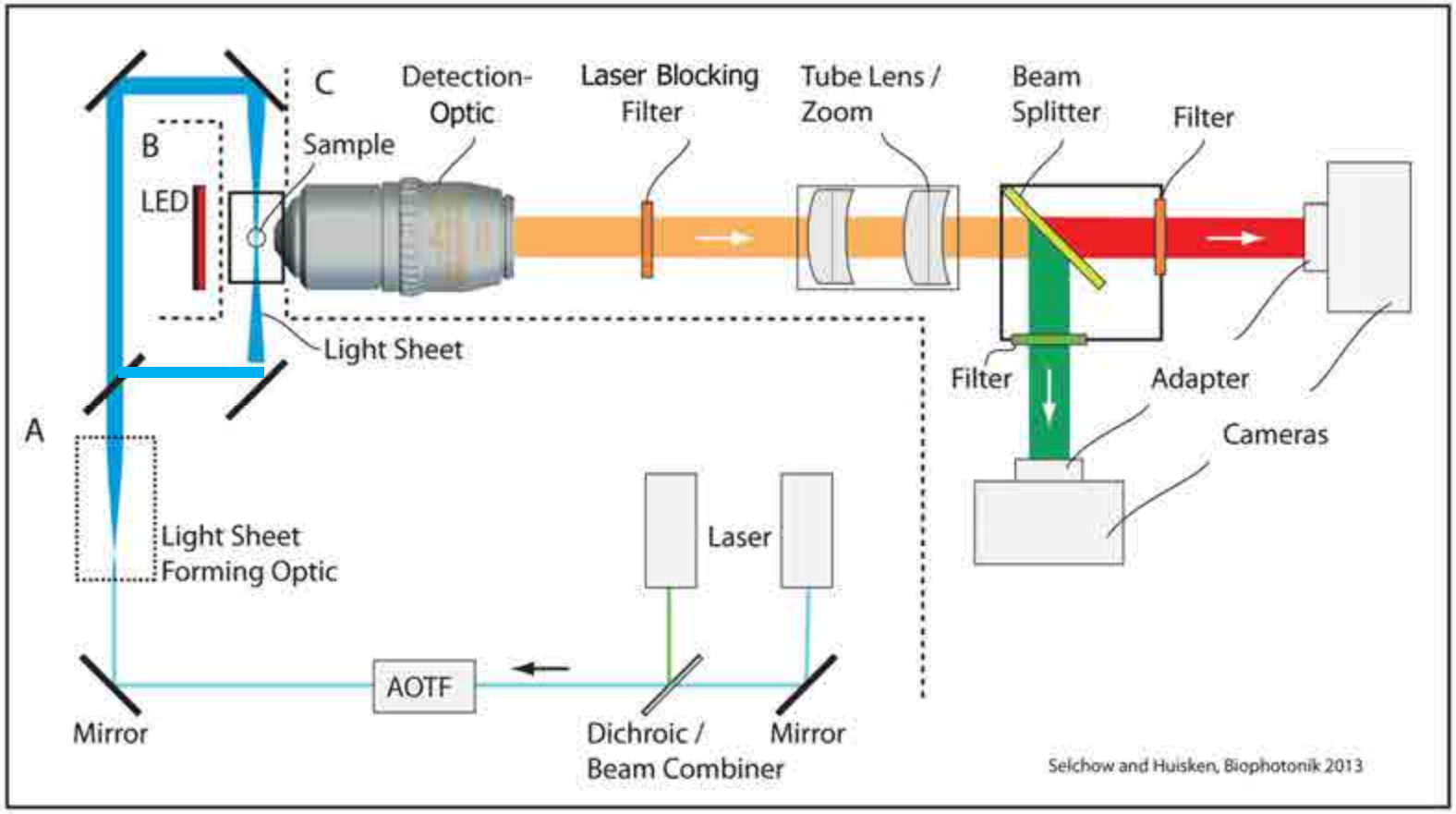
Figure from the PhD thesis of Jörg Ritter (2011), University of Bonn, Germany)

Lightsheet Z.1
Sample observation by „webcam“ and LED illumination

Your sample

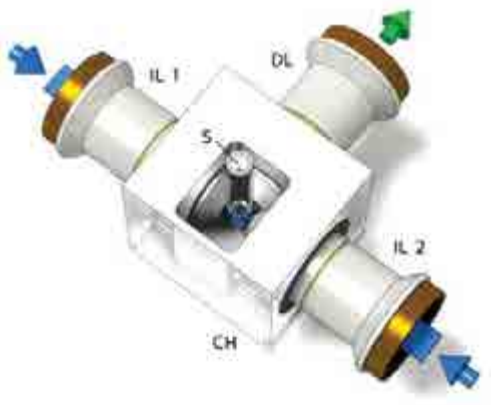
... gently embedded in a physiological environment!

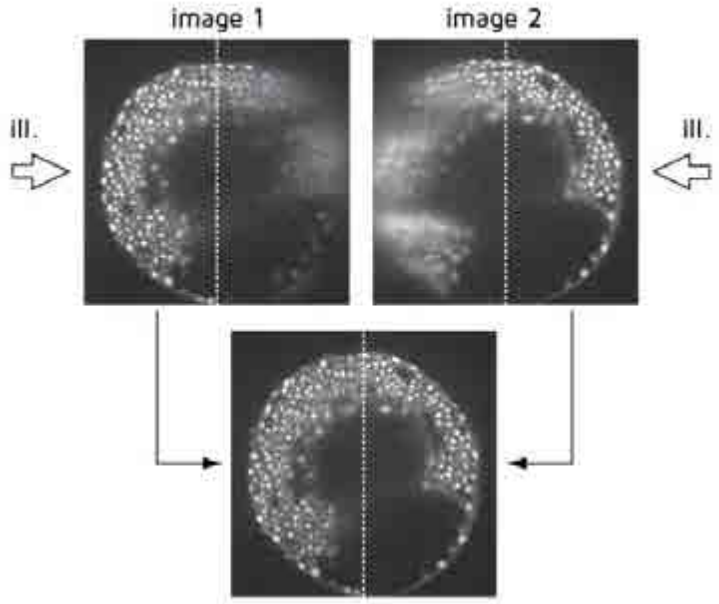
Built around your Sample
The horizontal microscope beam path for Lightsheet Z.1



Dual side illumination
Putting your sample in its true light

- Lightsheet Z.1 is equipped with two opposite illumination optics: Light Sheets can be generated from two sides
- Light is absorbed, blocked and scattered along the axis of the light sheet.
- Illumination from both sides enhances the quality of information
- Dual side illumination is done sequentially

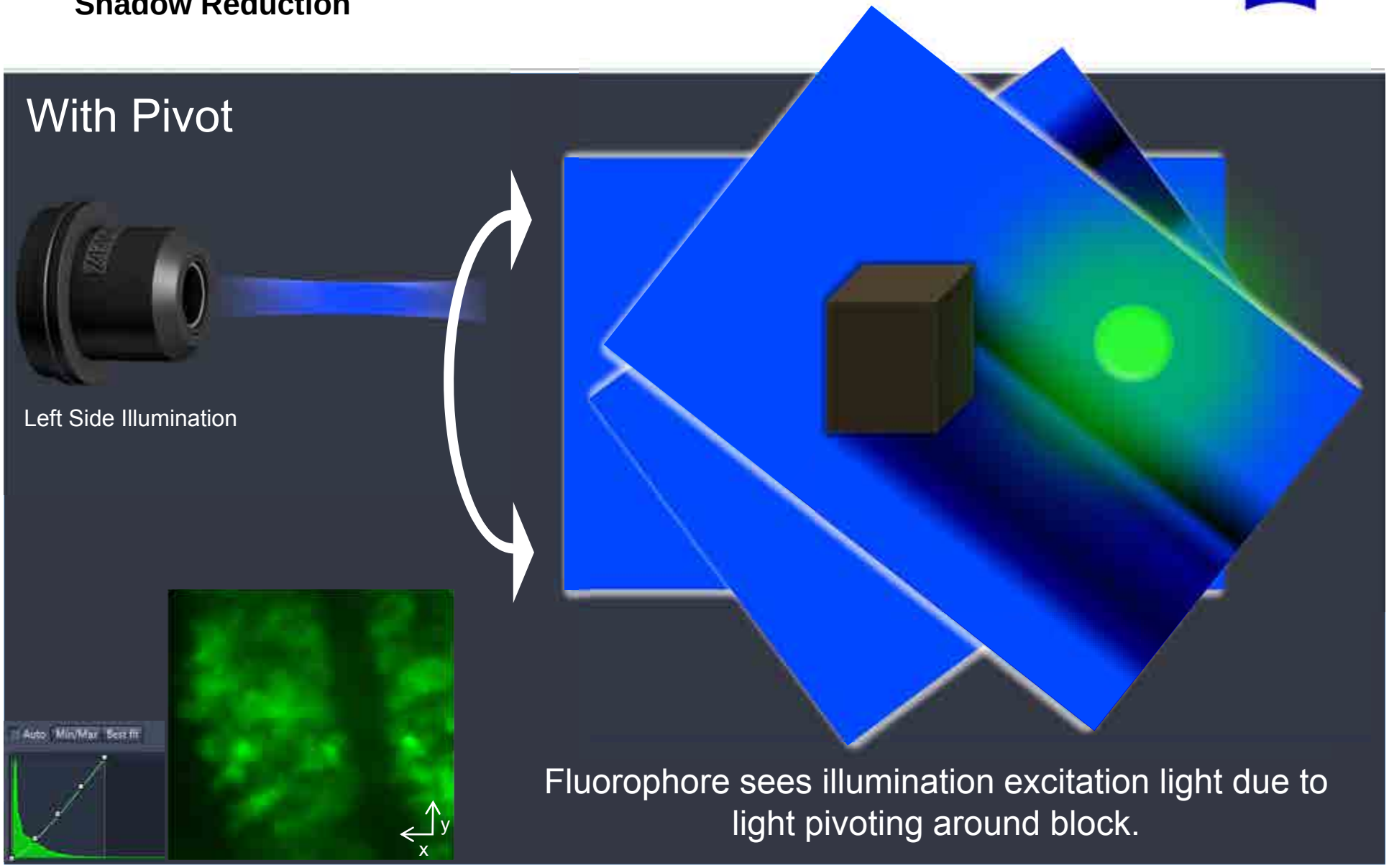




Adapted from Huisken, Bioessays, 2012

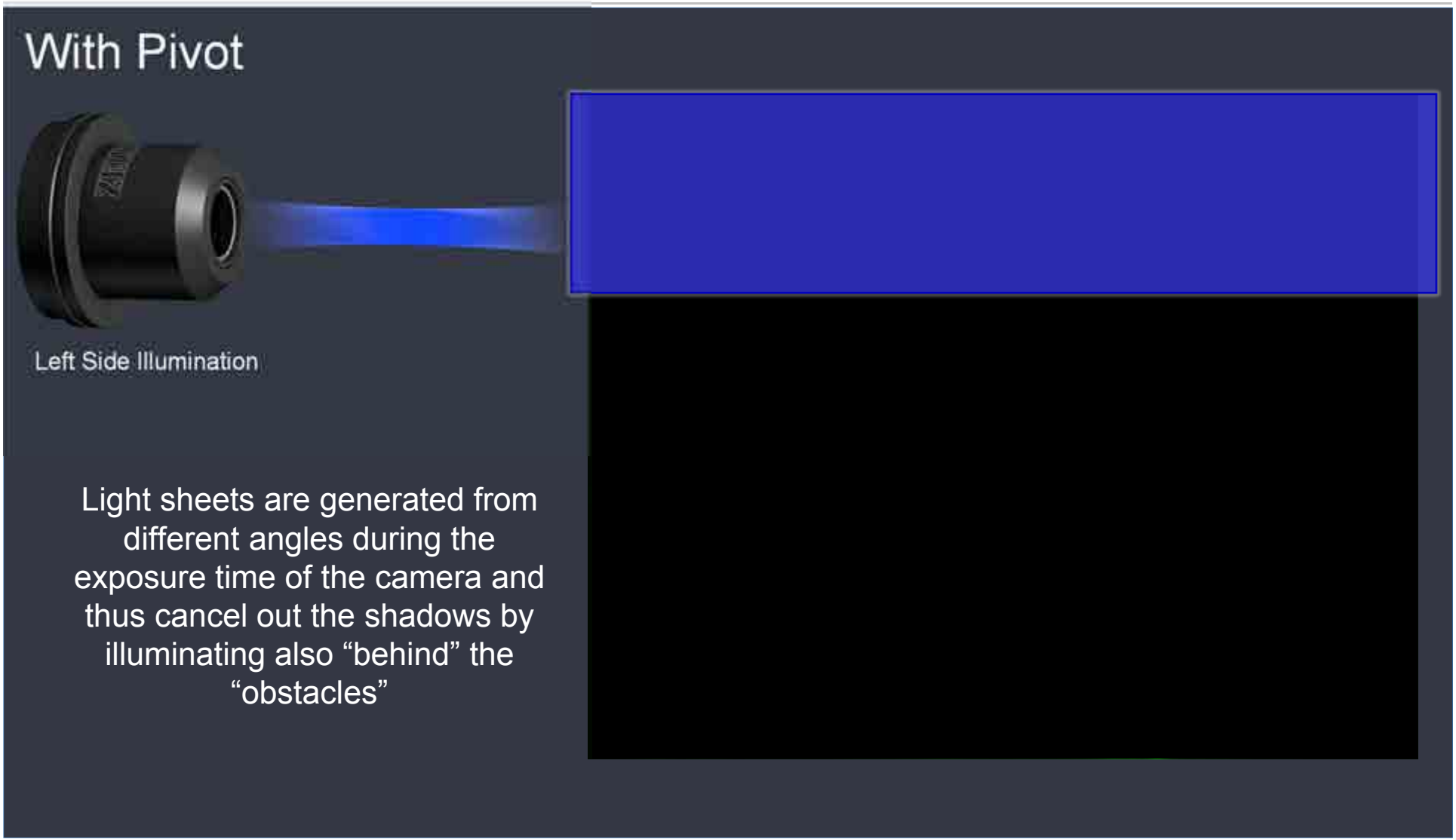
Pivot Scan

Shadow Reduction



Pivot Scan

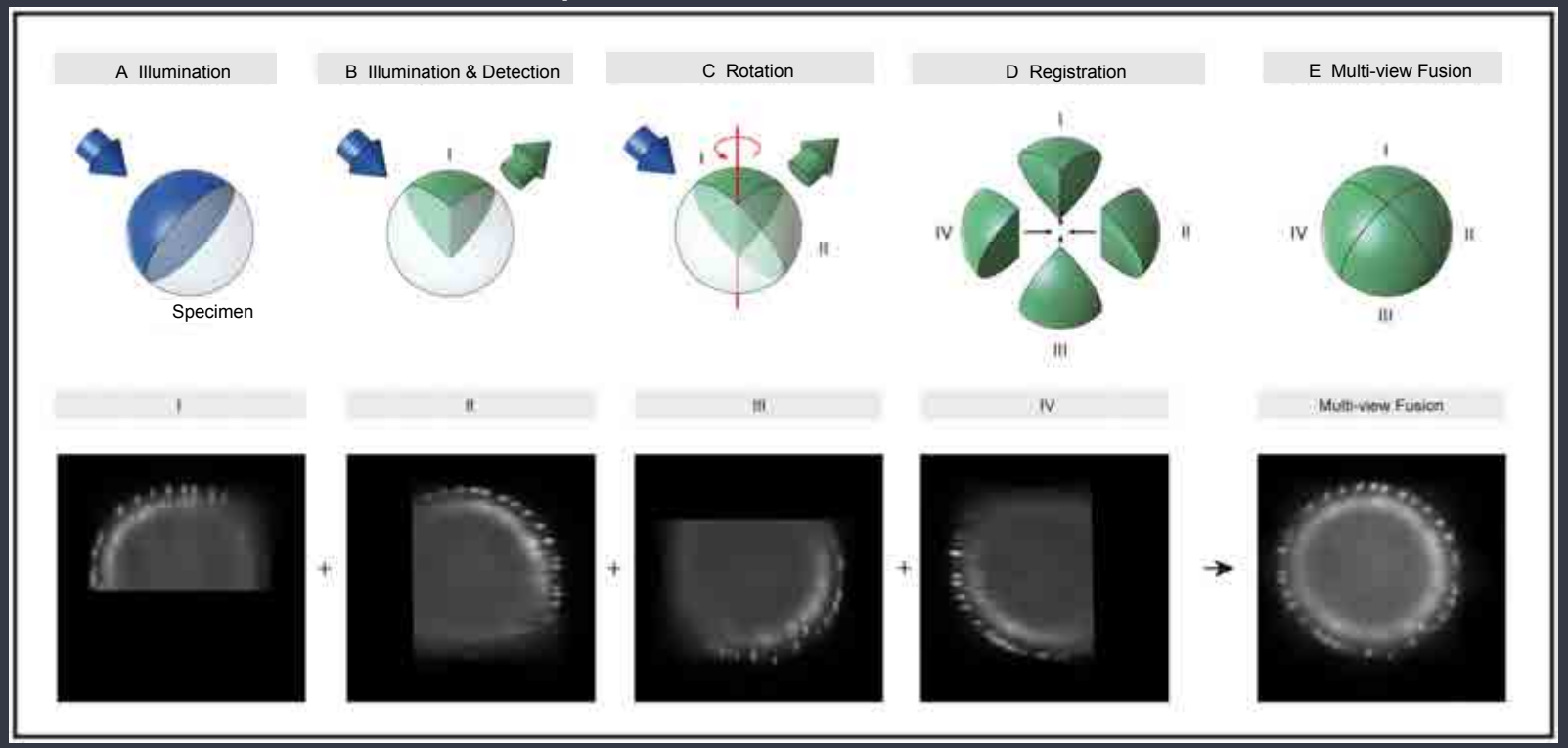
Shadow Reduction



Light sheets are generated from different angles during the exposure time of the camera and thus cancel out the shadows by illuminating also “behind” the “obstacles”

An Additional Degree of Freedom
Multiview: Imaging from different viewing perspectives

Multiview Imaging: Sequential acquisition of multiple z-stacks from different directions via sample rotation.

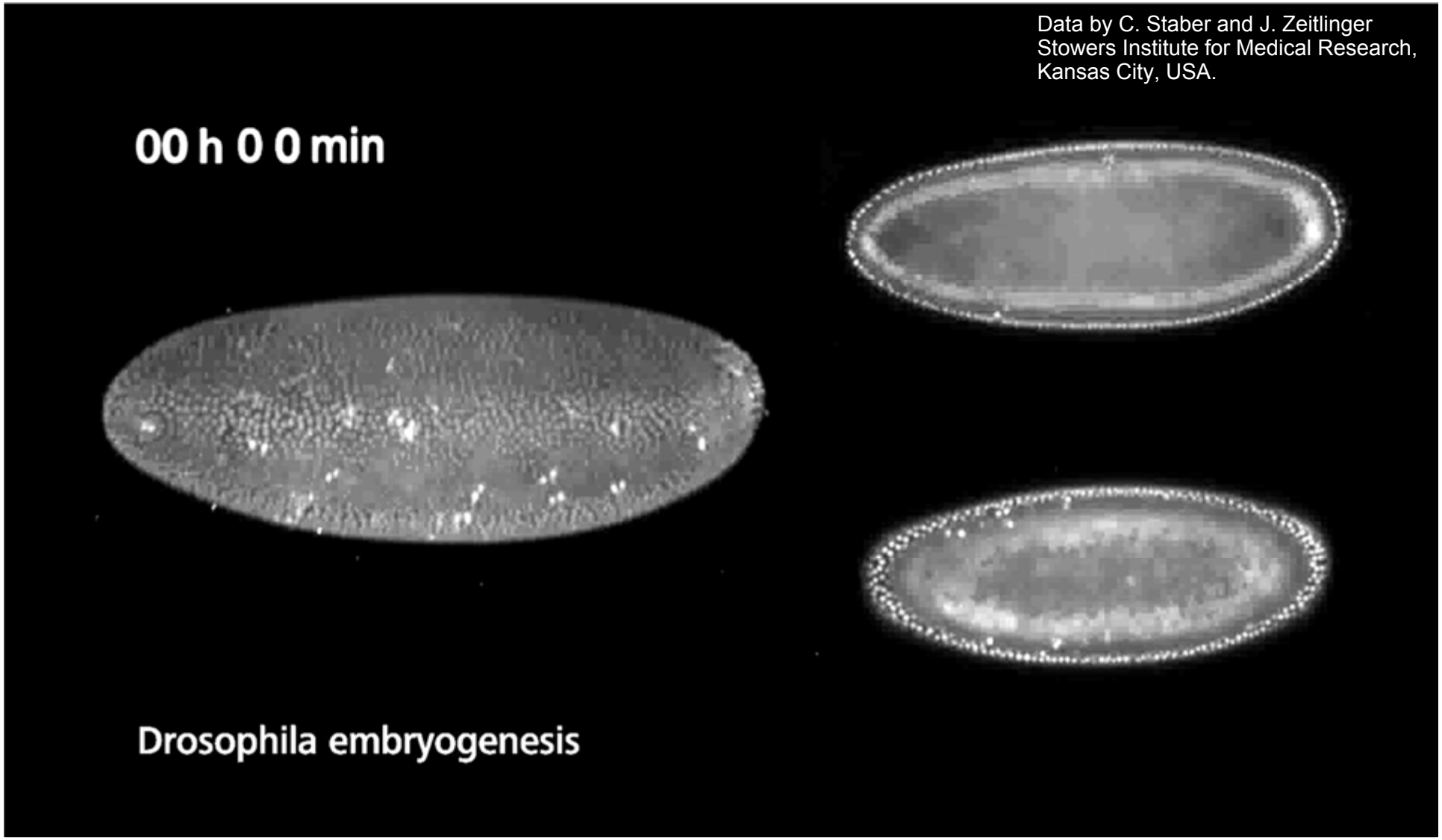


Selchow & Huisken Biophotonik, 2013

Benefits:

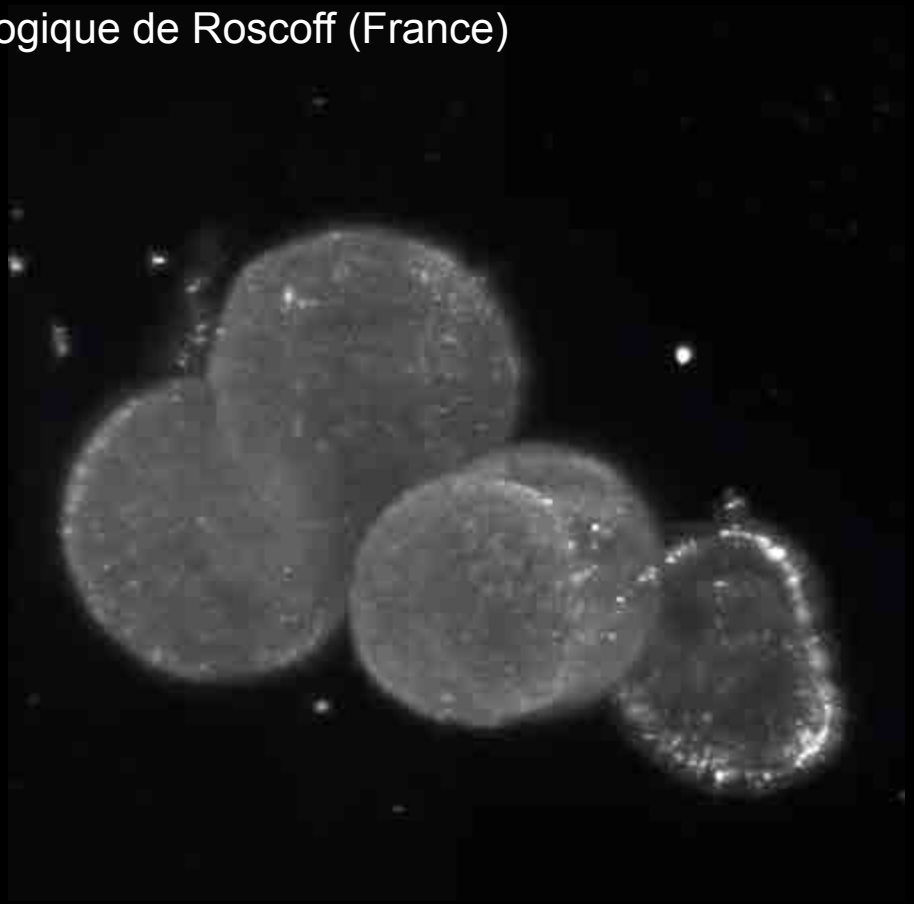
- Complementary information from different viewing angles.
- Potentially improved resolution (depends on specimen)

“Life is all about dynamic processes of complex multicellular organisms in a three-dimensional world.”

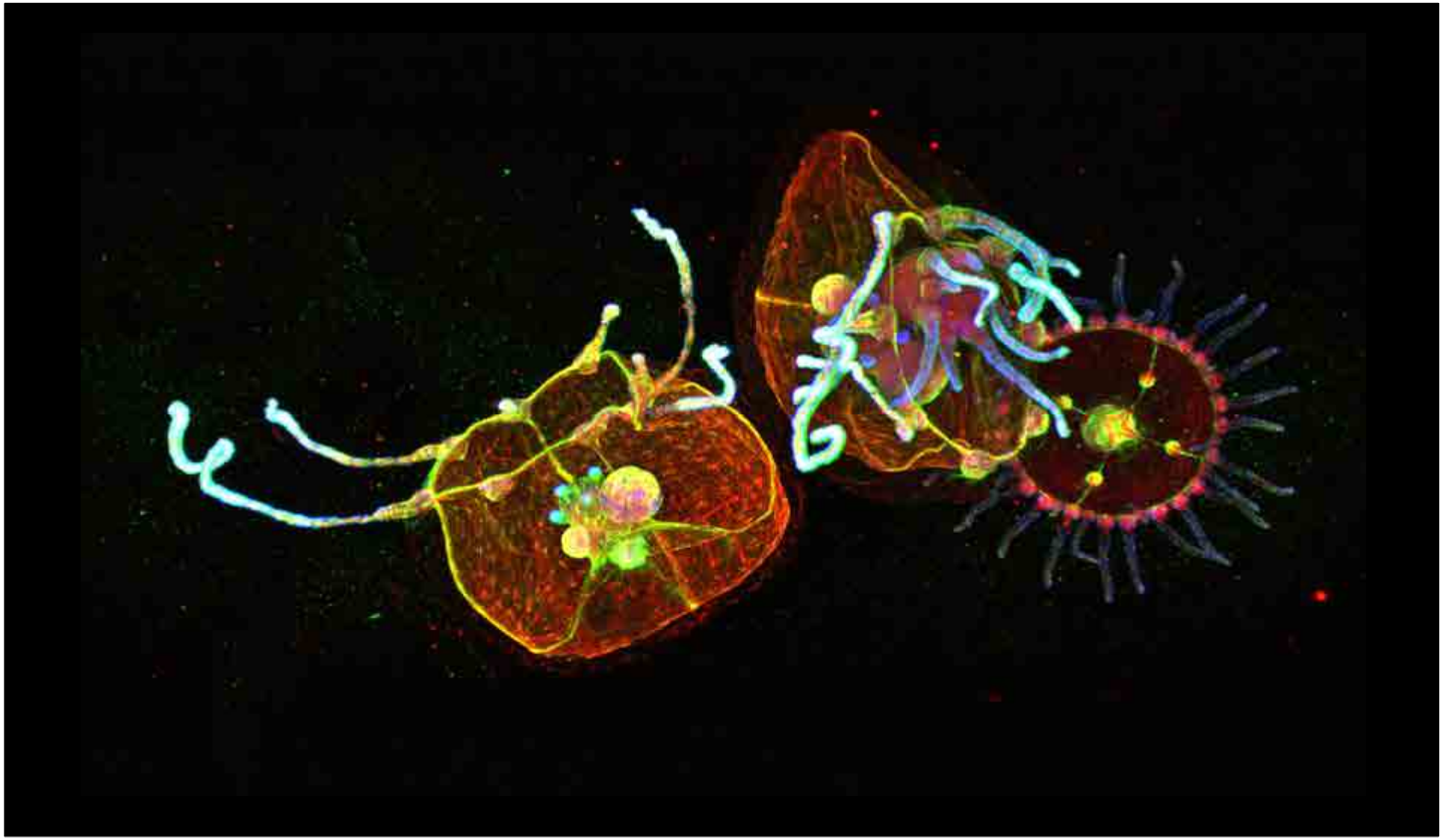


Sea Urchin Embryo
Membrane staining by FM4-64

Bertrand Cosson
UPMC-Station Biologique de Roscoff (France)



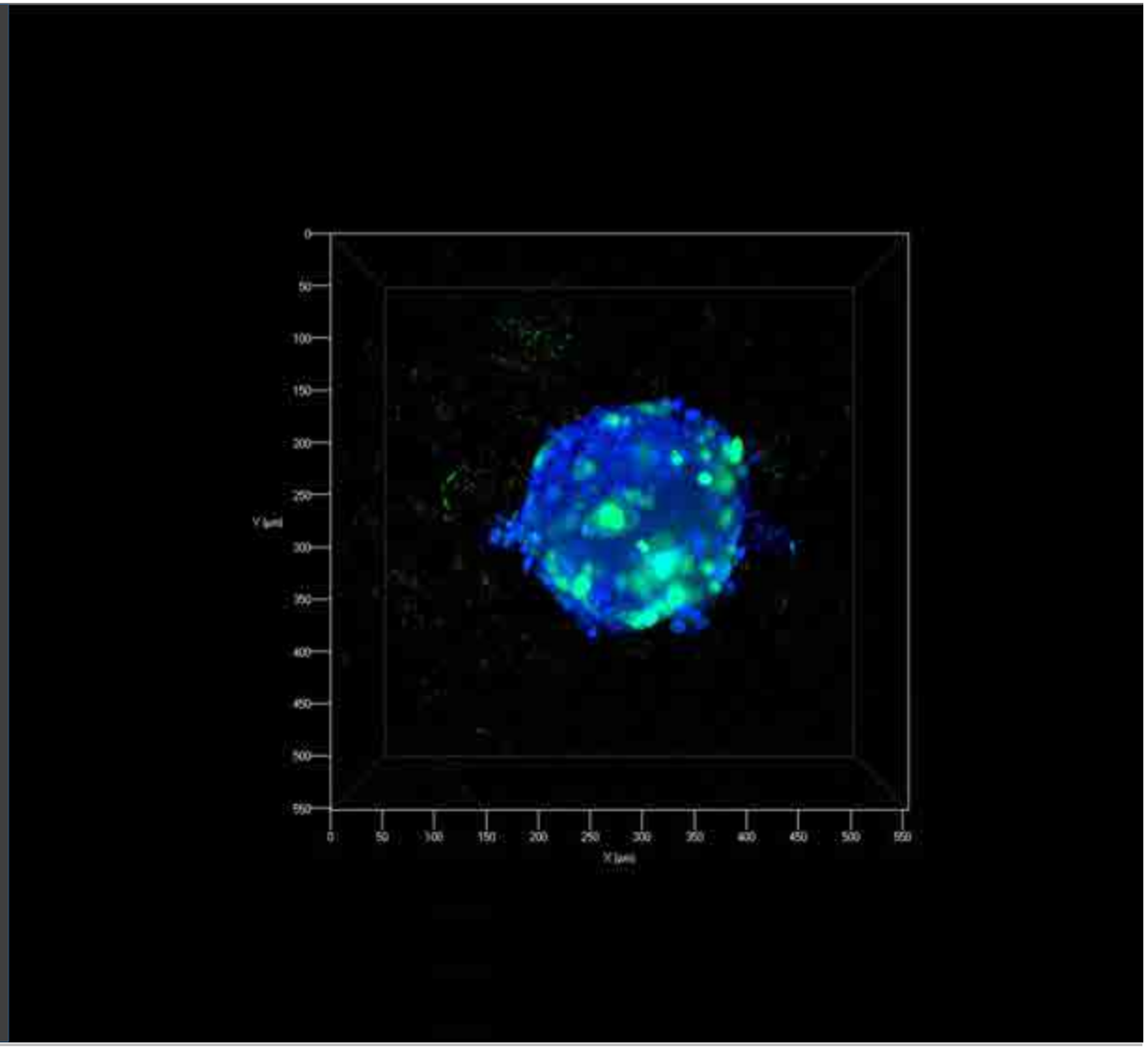
Cnidarian Larvae
EMBO Course in Fiskebäckskil (Sweden)



Spheroids Application
Fast imaging of cellular spheroids

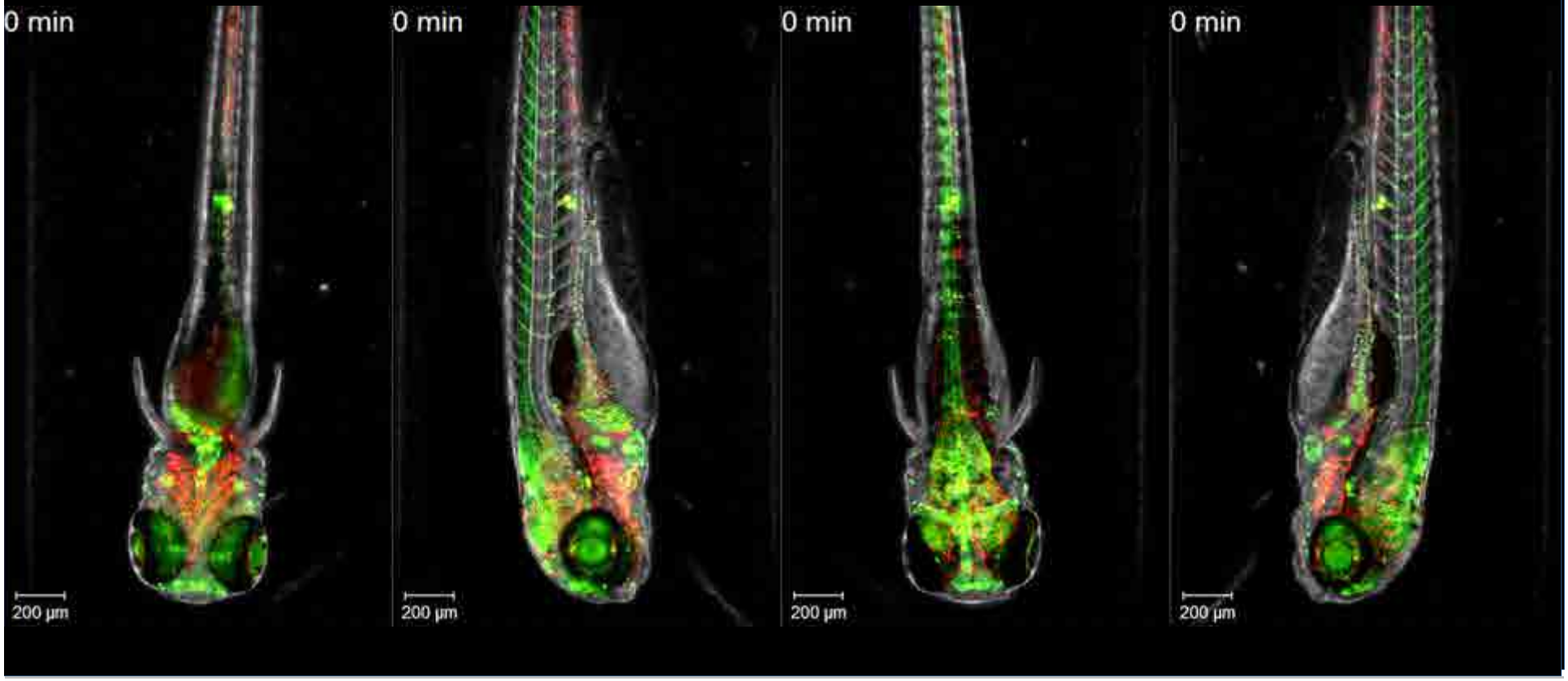
- HBE (Human Bronchial Epithelial) Cells infected with transgenic human adenovirus C2
- Dual side illumination
- Multiview Acquisition
- Hoechst stained of DNA, Transgenic human adenovirus C2, eGFP
- 3D Reconstruction

- *Data by Prof. Dr. Urs Greber (Institute of molecular life sciences, University of Zurich)*



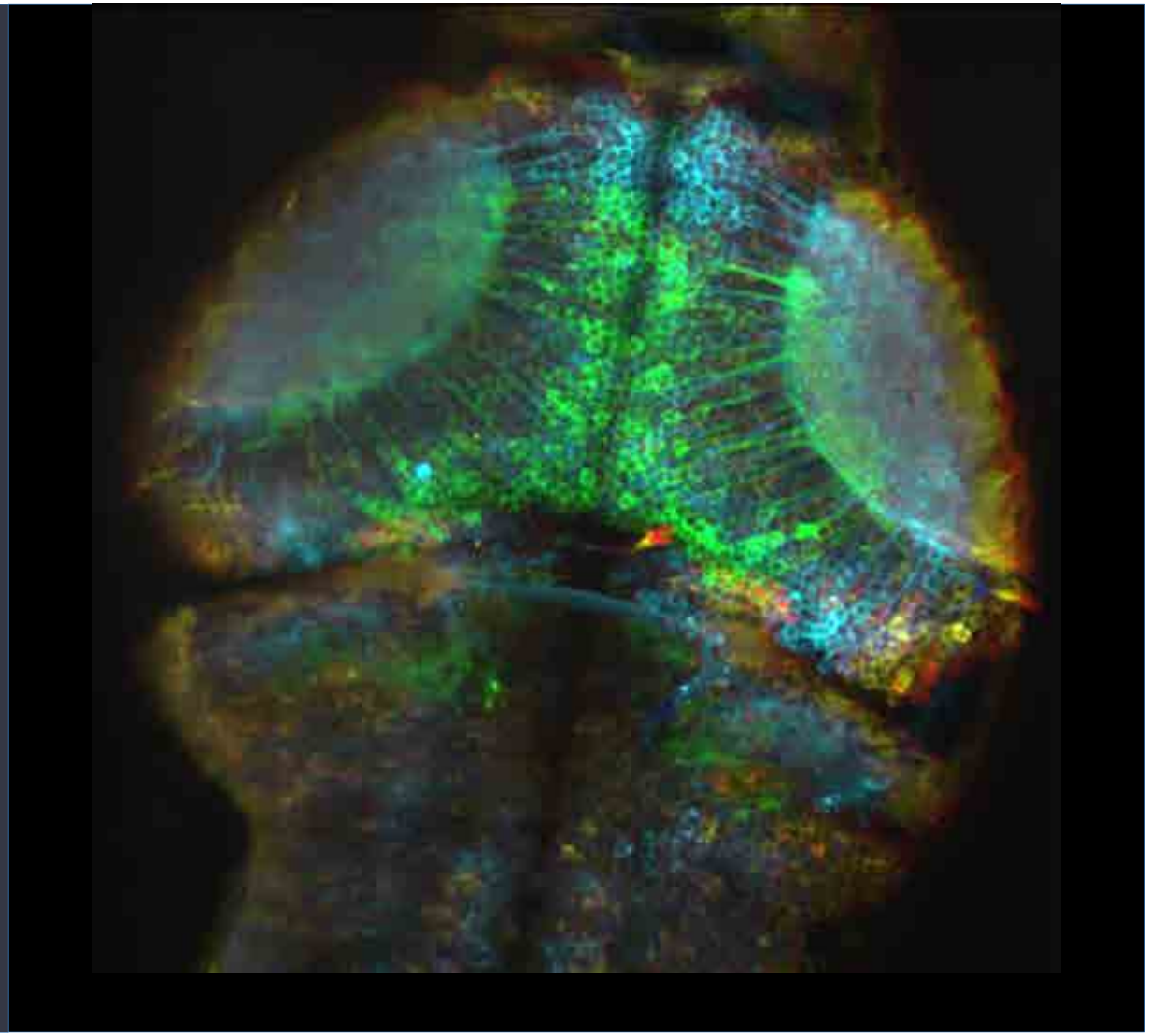
Zebrafish Development
Structural imaging of larger organisms

- Zebrafish, 2 days old embryo
- Views: 4
- Multiview registration and fusion



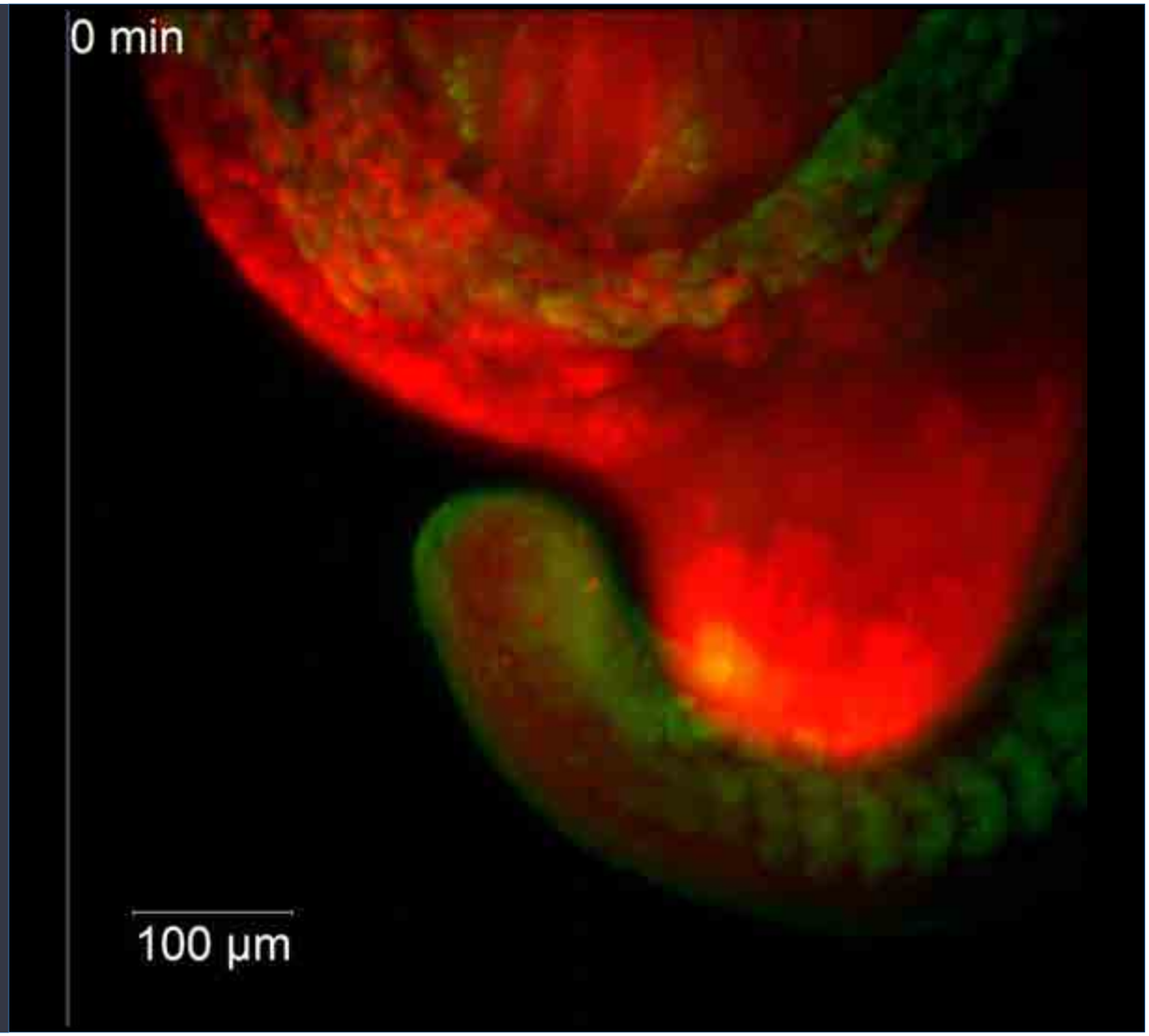
Zebrafish Development
Calcium Imaging

- Zebrafish
- Calcium Imaging
- Dual Side Illumination
- Maximum Intensity Projection
- Color coded projection
- Z Stack, 60 sections
- 60 fps, 10 minutes acquisition
- Curie Institute, BDD



Zebrafish Development
Muscle Development

- Zebrafish
- Dual Side Illumination
- Time laps, 1 View
- Objective: W Plan Apochromat 20x/1.0
- Dr. Yosuke Ono, Philip Ingham Lab, IMCB, A*STAR, Singapore

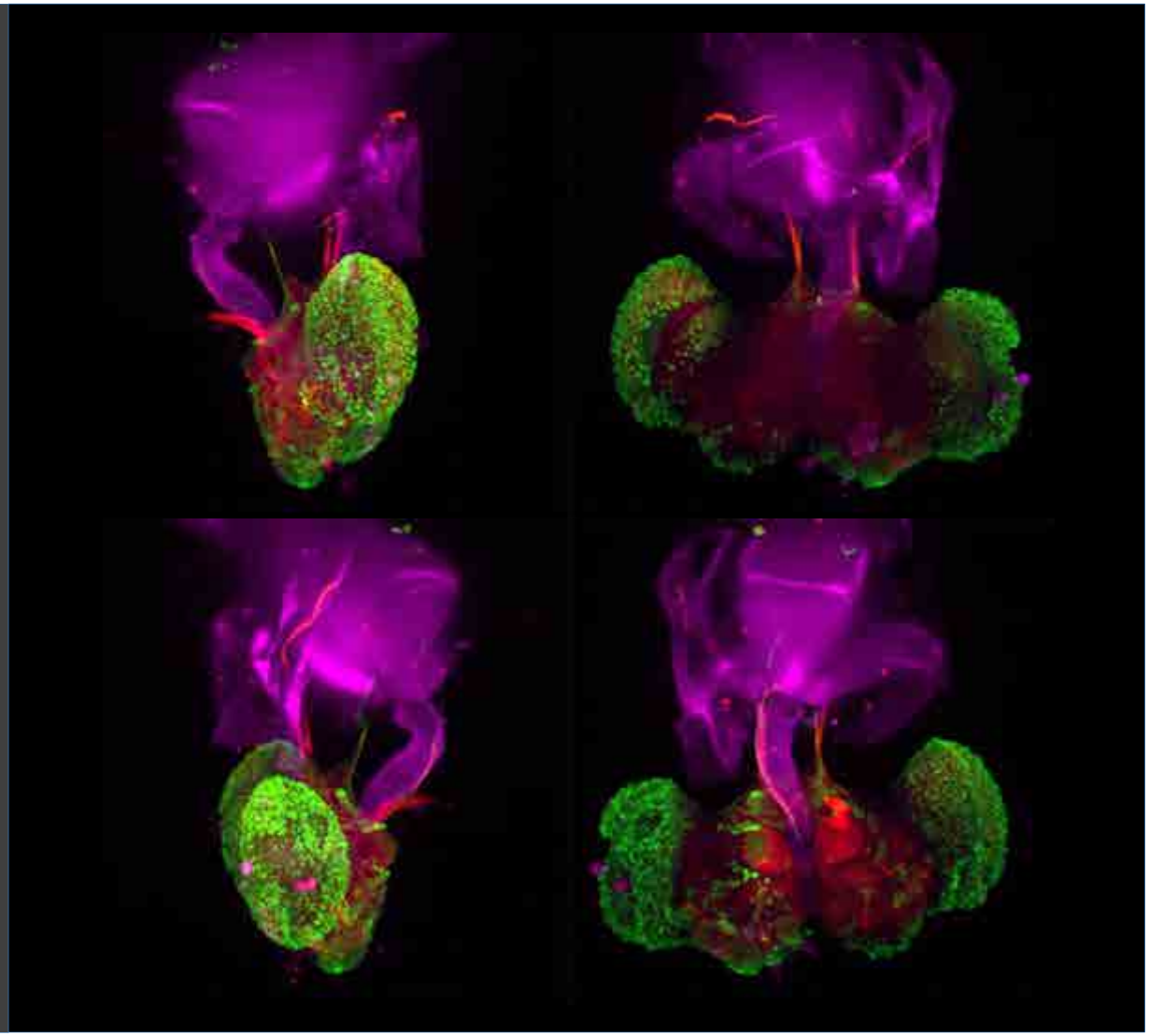


Zebrafish Heart Development
Fast fluorescence imaging

- Zebrafish heart of 2 day embryo
- Red label: blood vessels, endocardium
- Green label: myocardium
- Acquisition rate: 80 fps
- Movie: **20 fps**
- Light sheet fluorescence microscopy allows imaging the beating heart with maximal frame rates (80 to 100 fps) for extended periods of time with minimal light exposure to the specimen.
- *Data by* Michaela Mickoleit, Michael Weber (Huisken Lab, MPI-CBG)

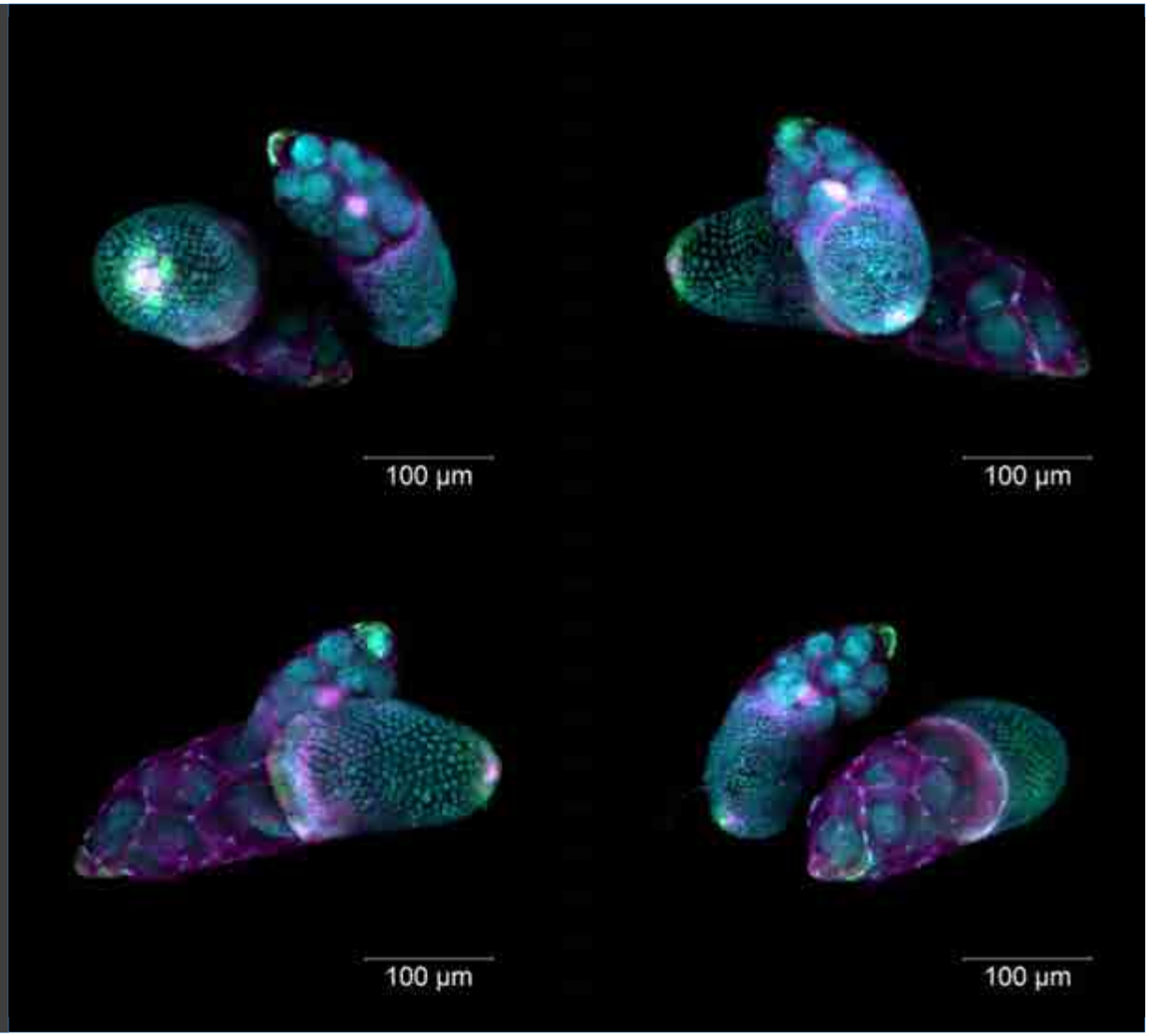
Drosophila melanogaster
Adult Brain

- *Drosophila melanogaster,* Adult Brain
- Magenta: Proboscis Muscles, Rhodamine phalloidin
  Red: Neuroglian, Alexa 647
  Green: GFP-Neurons (Alexa 488)
- Views: 4 (90 degree)
- Dual side illumination
- Size: about 700 µm in length
- Maximum intensity projection
- *Data by Ali Asgar Bohra, K. Vijay Raghavan, NCBS Bangalore India*



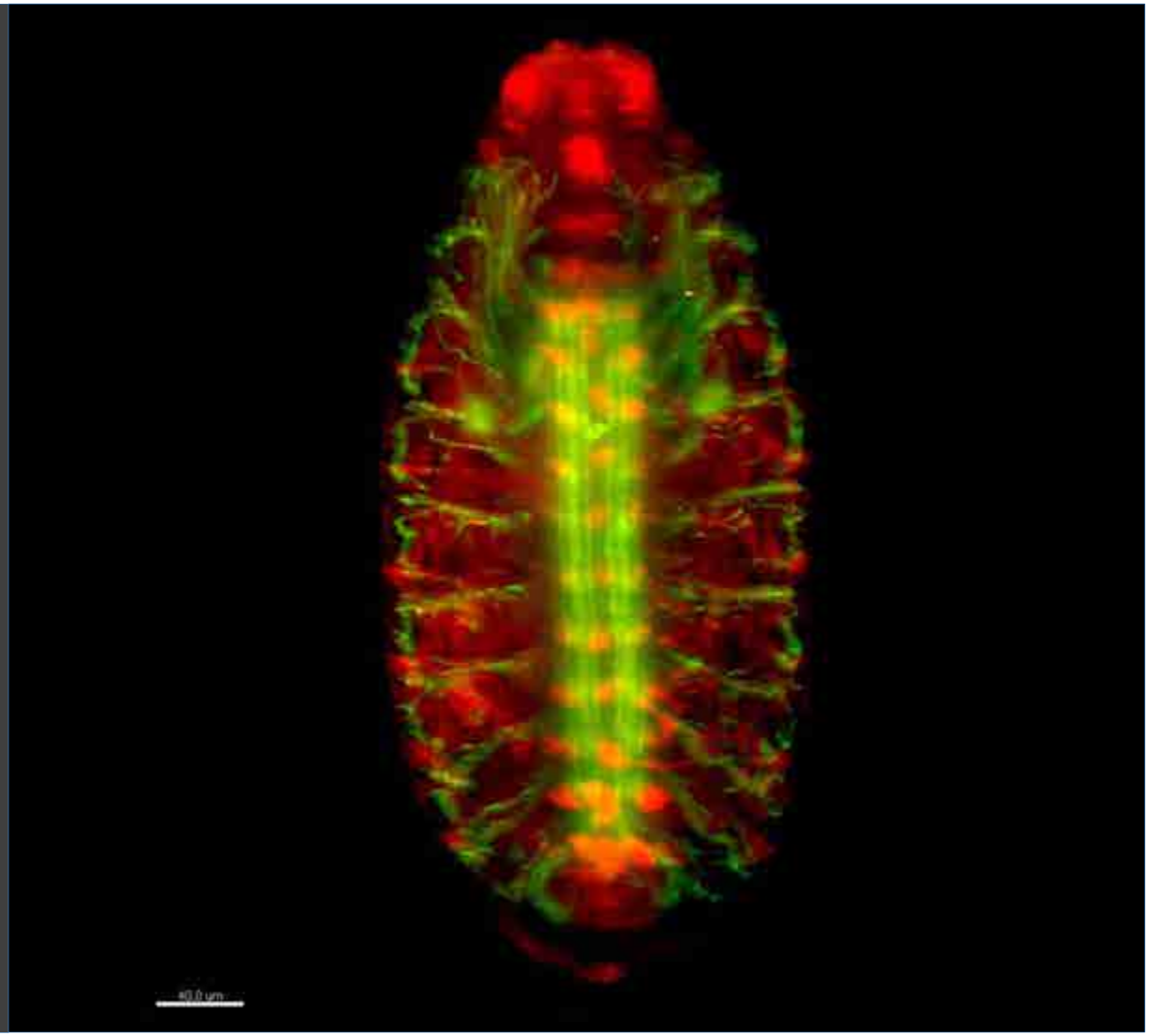
Drosophila melanogaster
Ovaries

- *Drosophila melanogaster,* Ovaries
- *Green: GFP*
- *Magenta: Membrane*
- *Blue: DAPI*
- Views: 4 (90 degree)
- Dual side illumination
- Objective: W Plan Apochromat 20x/1.0
- Maximum intensity projection
- Dr. Mohit Prasad, Department of Biological Sciences, IISER, Kolkata

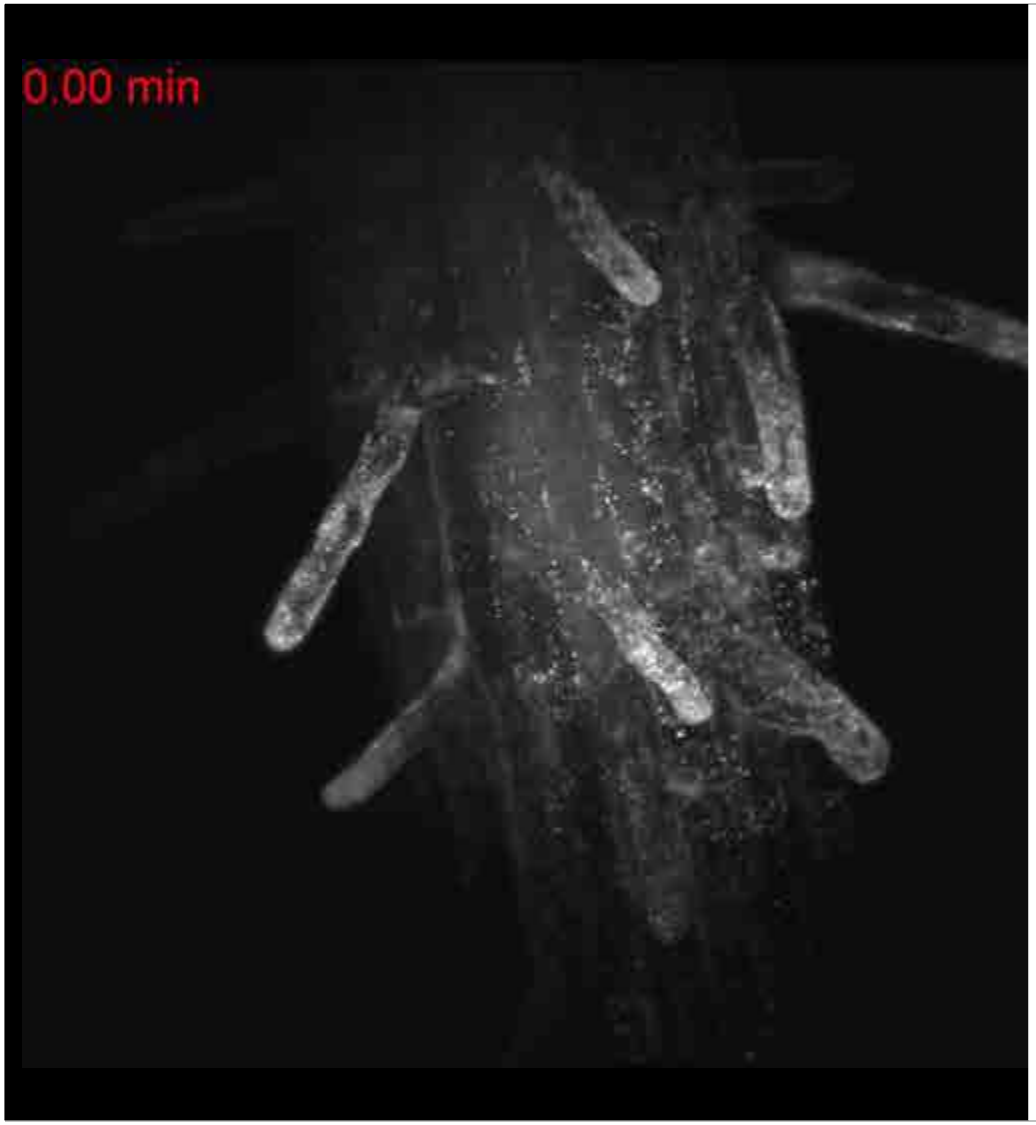


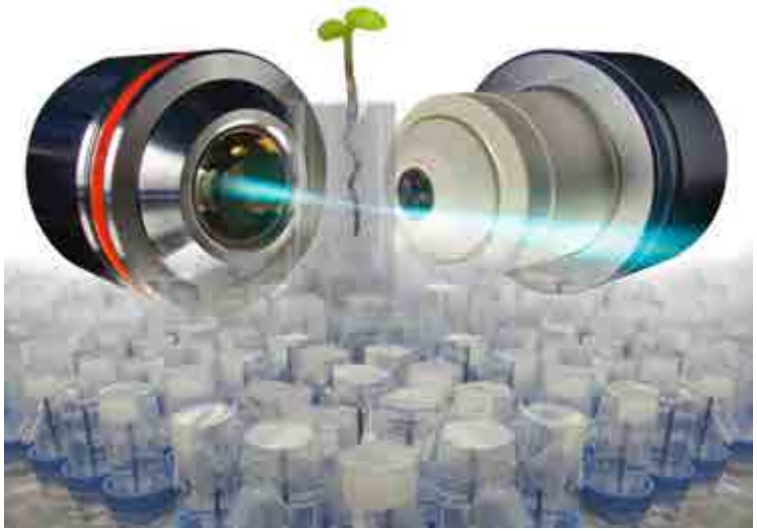
Drosophila melanogaster
Embryo

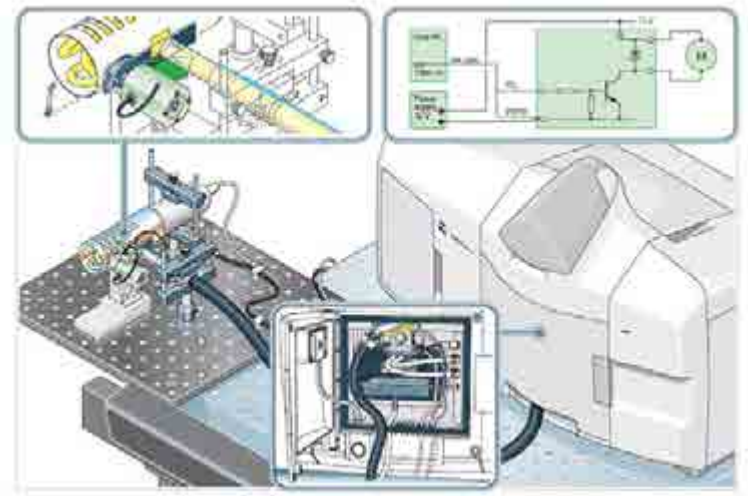
- *Drosophila melanogaster,* Embyro wild type, labelled nervous system
- Views: 4 (90 degree)
- Dual side illumination
- 3D Rendering
- Cecilia S. Lu, Okinawa Institute of Science and Technology, Okinawa, Japan



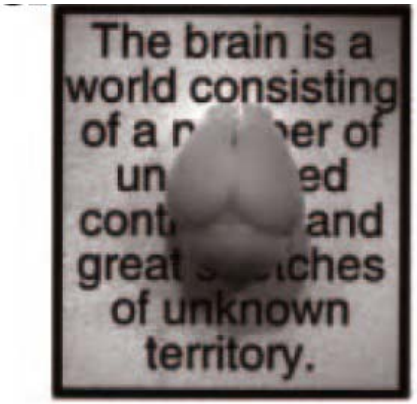
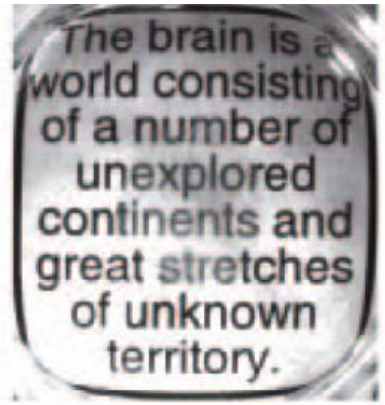
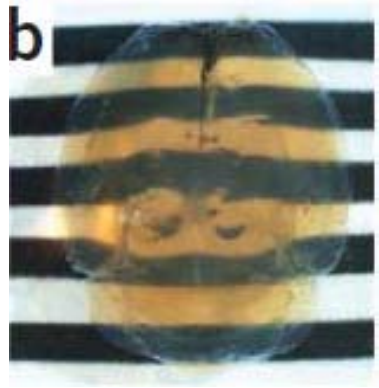
Arabidopsis Root
RAB F2A:YFP





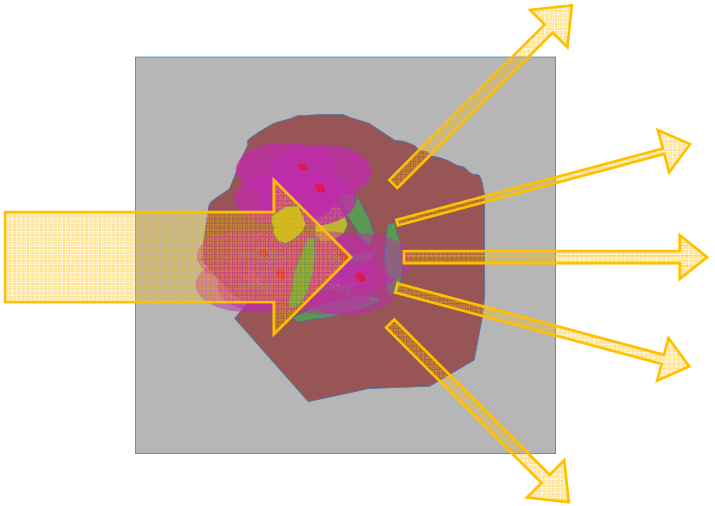


Optical Clearing
Reducing scattering to achieve large depth penetration

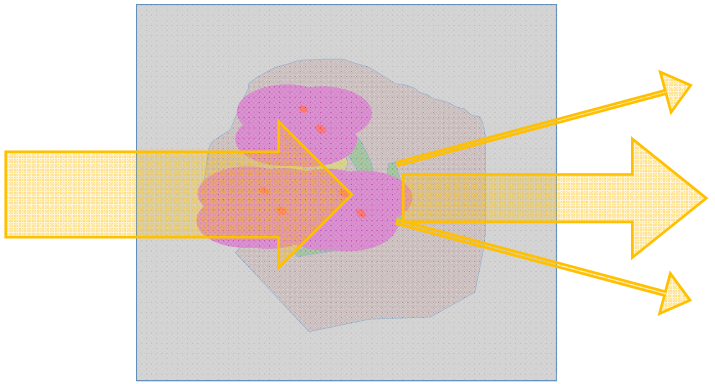
	Sca/e	Clarity	3DISCO
Before			
After			
	(Hama et al, Nat Neuroscience 2011)	(Chung et al , Nature 2013)	(Ertürk et al, Nature Methods, 2012)

How does optical clearing work?
… in a nutshell

- Clearing methods match the refractive index of the tissue while maintaining the three-dimensional structure of the sample and fluorescence of the labels
- Exchange of light scattering structures (lipid membranes, tissue water) within the sample by:
  - **Organic chemicals** with high R.I. (BABB, Spalteholz)
  - **Aqueous solutions** with high concentrations of sugar (SeeDB), urea (Sca/e),…
  - **Electrophoretic** tissue clearing (Clarity)



Absorption and scattering in opaque tissue



Transparent tissue after optical clearing

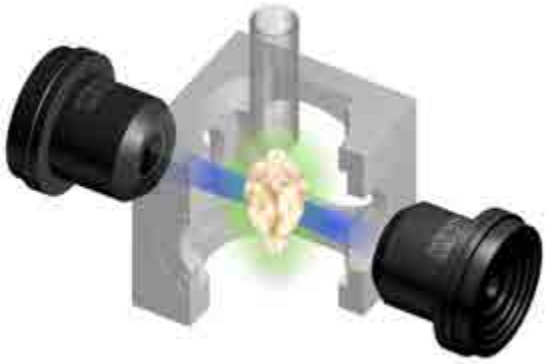
Lightsheet Z1 & clearing method
High quality optics for depth penetration

Switch from live sample imaging to clearing applications in minutes

5.6 mm free working distance

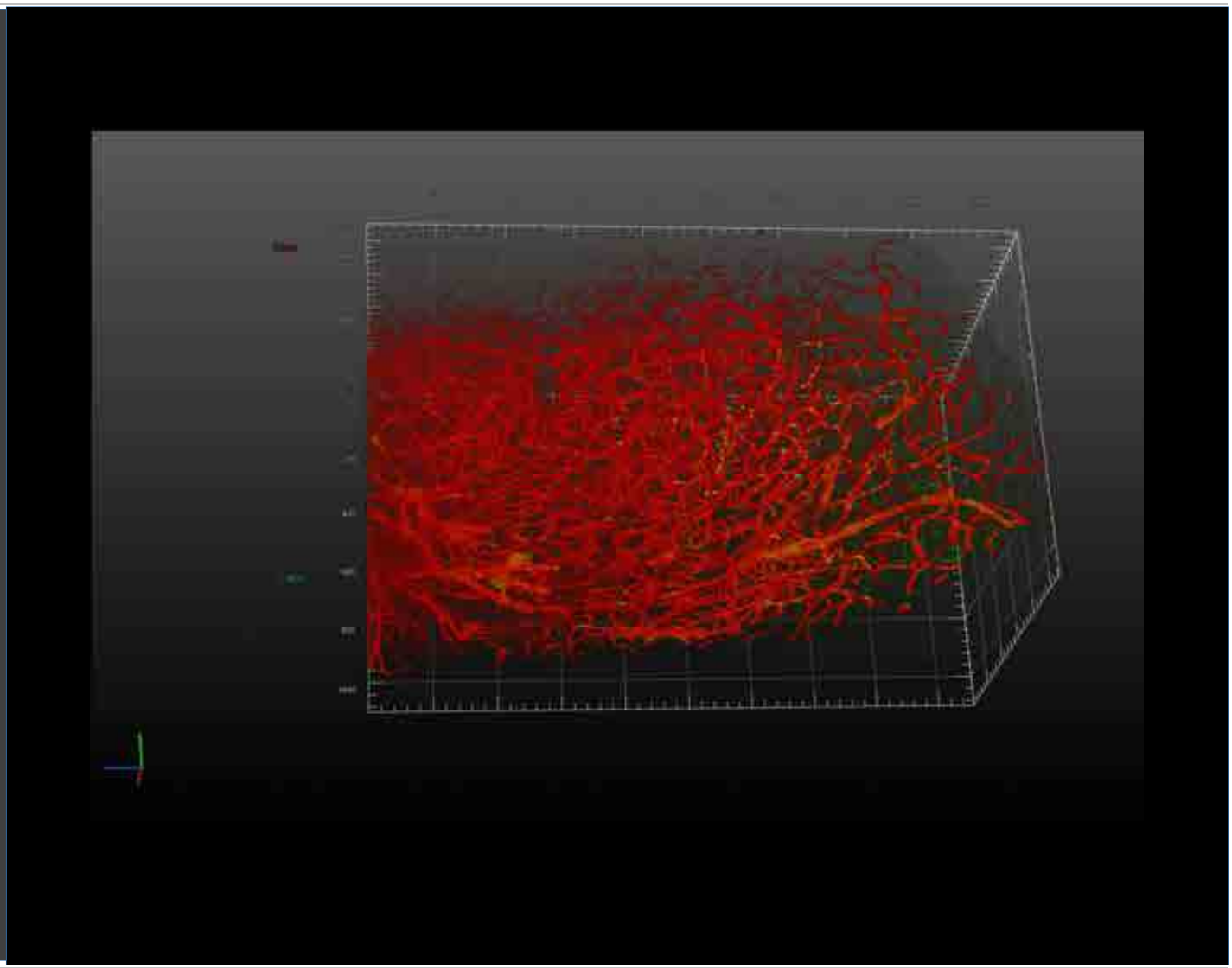
Sample size : up to 1 x 1 x 2 cm

- 20x N.A. 1,0 for n = 1,38 (± 0,03)
- 20x N.A. 1,0 for n = 1,45 (± 0,03)
- 5x N.A. 0,16 for n = 1,45



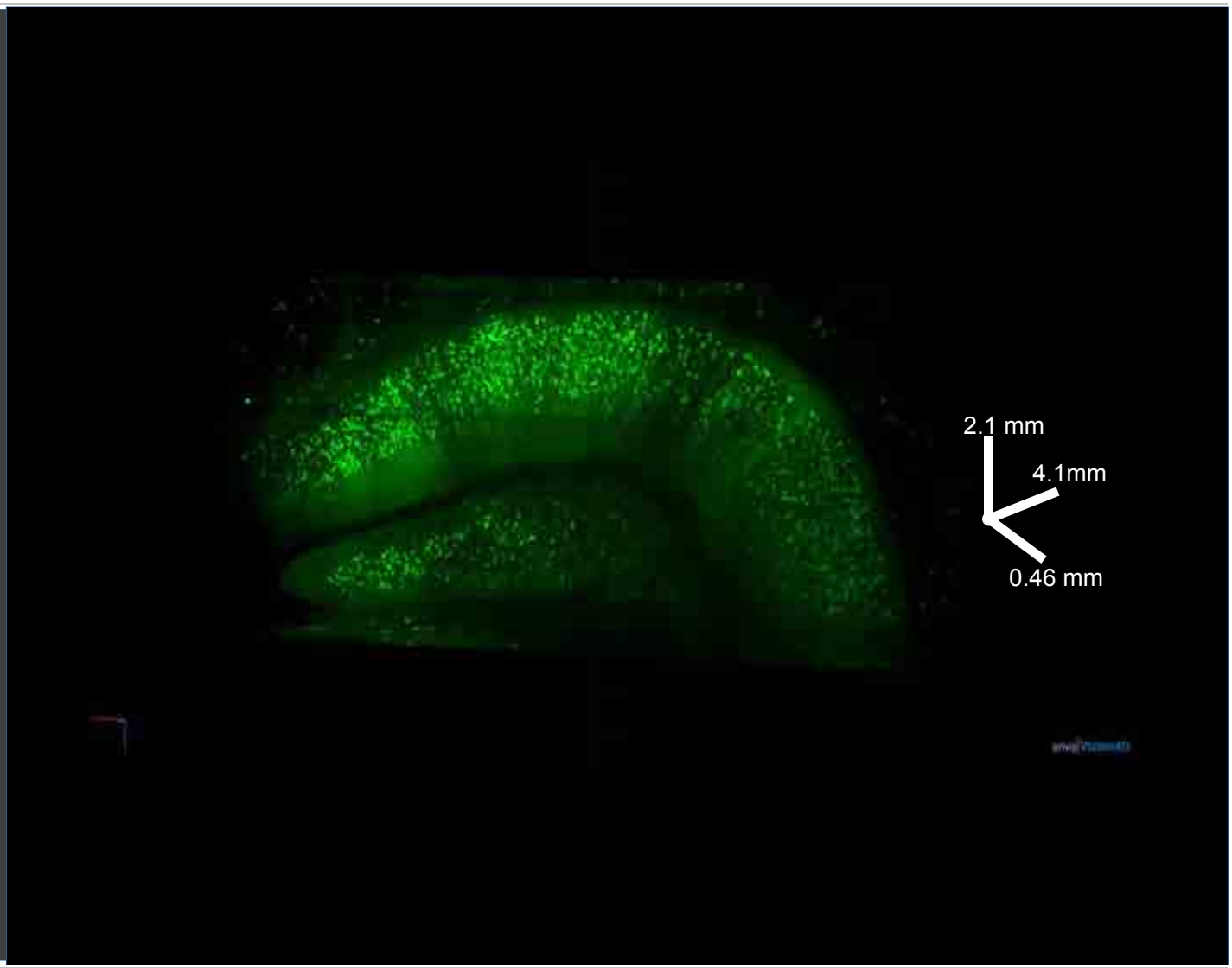
Mouse brain cleared with CLARITY/RIMS
Imaged with Lightsheet Z.1

- Blood vessels in CLARITY cleared mouse brain
- Volume size: 1,1 x 1,1 x 1,8 mm
- *Data by: Jean-Marie Vanderwinden,Université Libre de Bruxelles, ULB Neuroscience Institute - Neurophysiology lab*



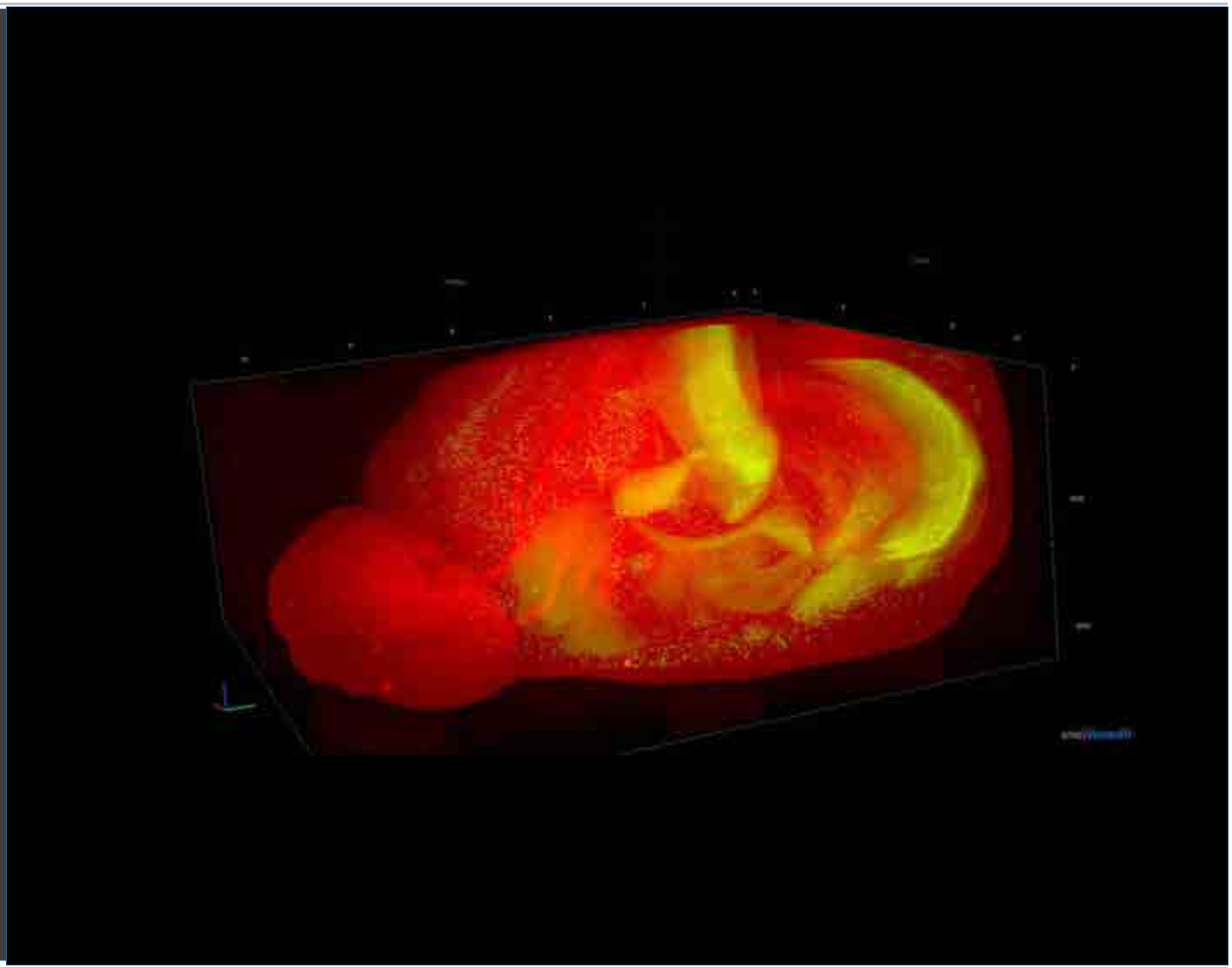
Mouse hippocampus cleared with LUMOS
Imaged with Lightsheet Z.1

- Thy1-EGFP M-line mouse hippocampus, optically cleared in LUMOS clearing agent.
- Volume Size: 4.12 x 0.466 x 2.11 mm (14755 x 1636 pixel and 8882 z-sections)
- *Data by O.Efimova National Research Center "Kurchatov Institute", Moscow, Russia*

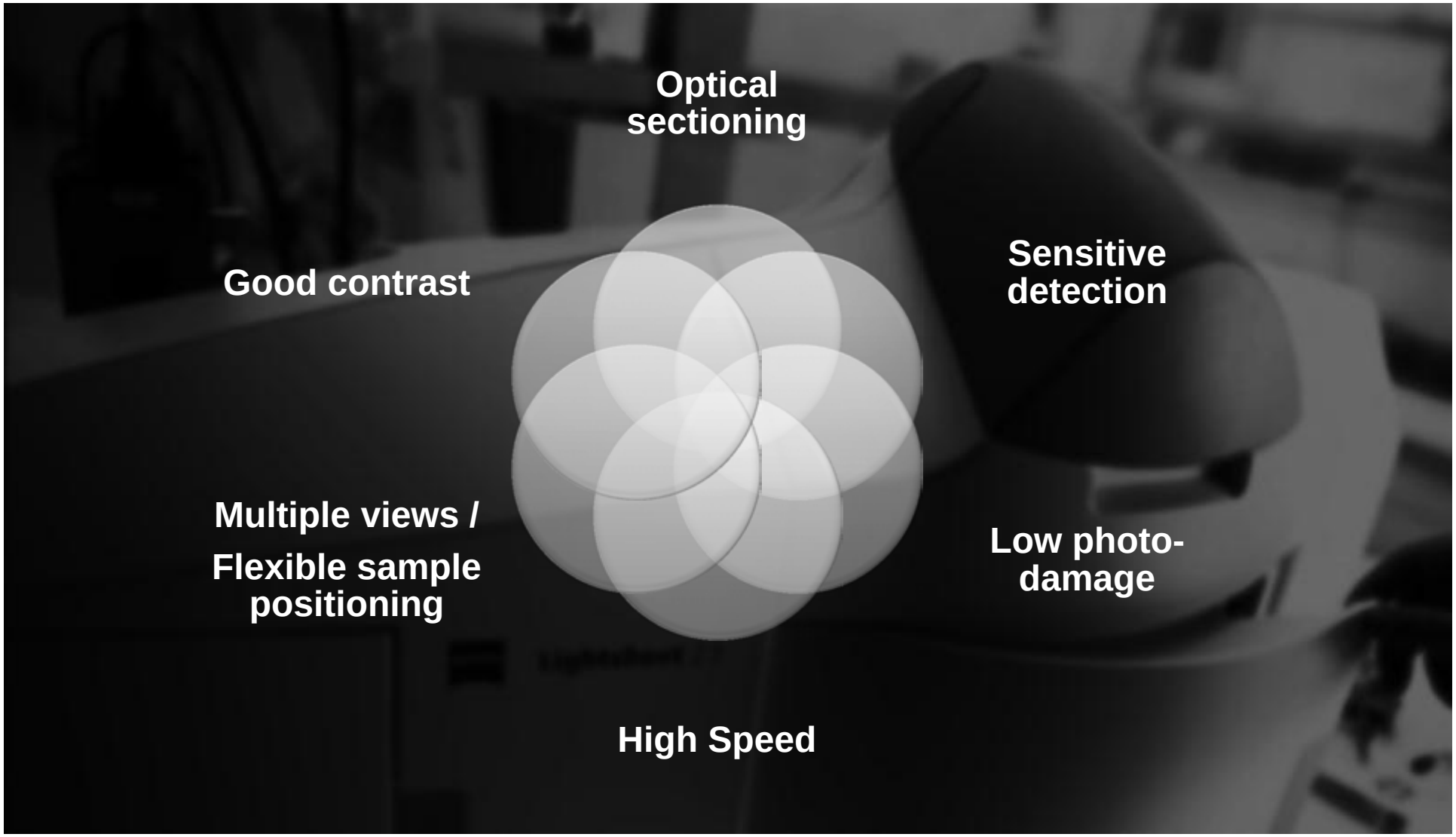


Mouse whole brain cleared with LUMOS
Imaged with Lightsheet Z.1

- Thy1-EGFP M-line mouse whole mount, optically cleared in LUMOS clearing agent, co-stained with Propidium Iodide
- Volume Size: 11.1 x 11 x 4.5 mm (8600 x 8500 pixel and 2588 z-sections)
- *Data by O.Efimova National Research Center "Kurchatov Institute", Moscow, Russia*



Good to remember
about the turn-key system Lightsheet Z.1



Lightsheet Z.1
Light Sheet Fluorescence Microscopy by Carl Zeiss

We make it visible.