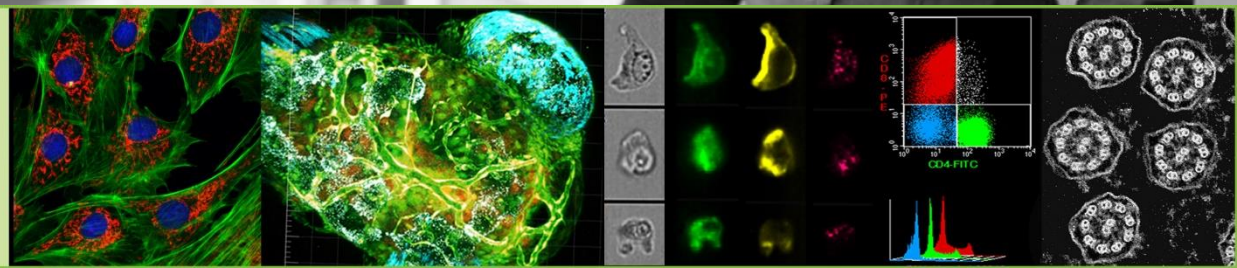
Light Sheet Fluorescence Microscopy

by ZEISS

Adapted by Dirk Pacholsky, BioVis Facility, Uppsala, Sweden

For Methods for Cell Analysis, 2014

UPPSALA UNIVERSITET SciLifeLab BioVis Facility

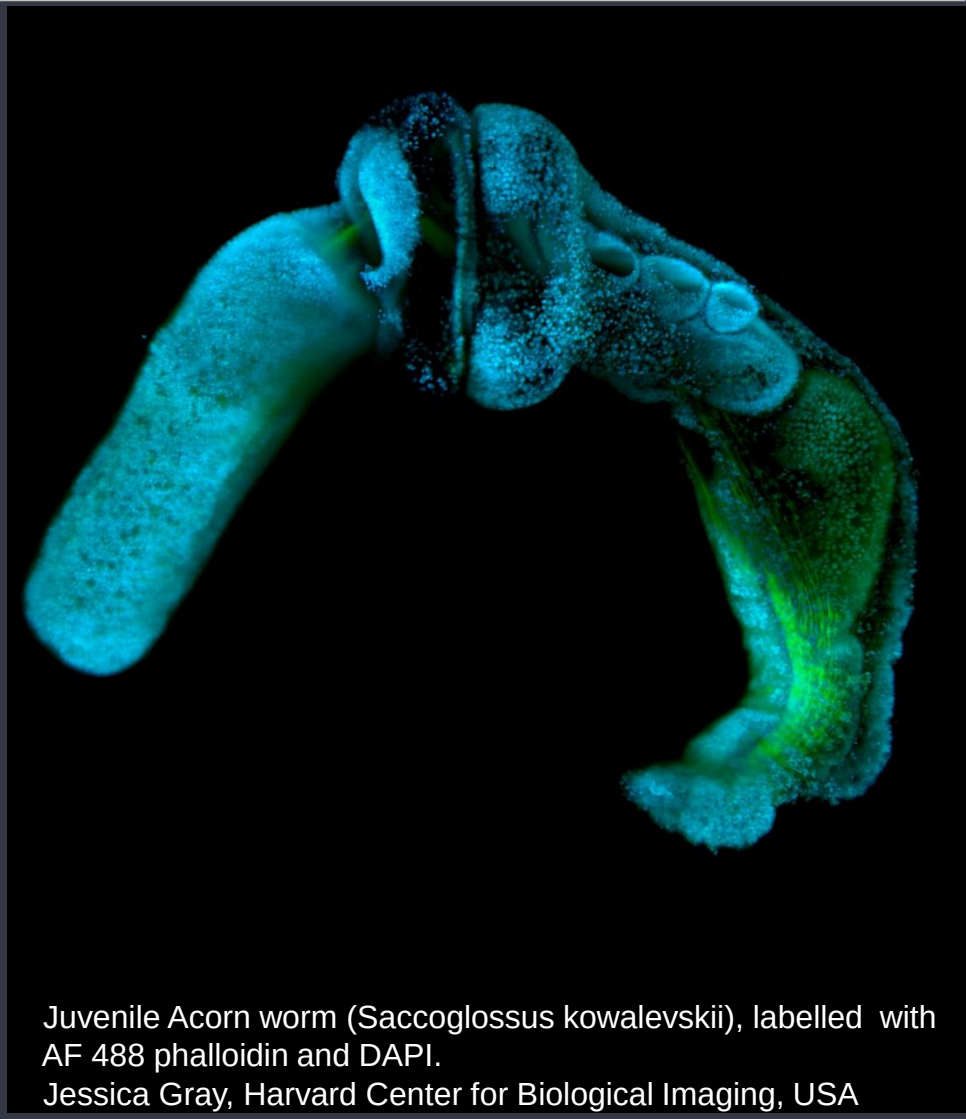


Imaging of living, multi-dimensional specimens
What are the challenges?

Challenges:

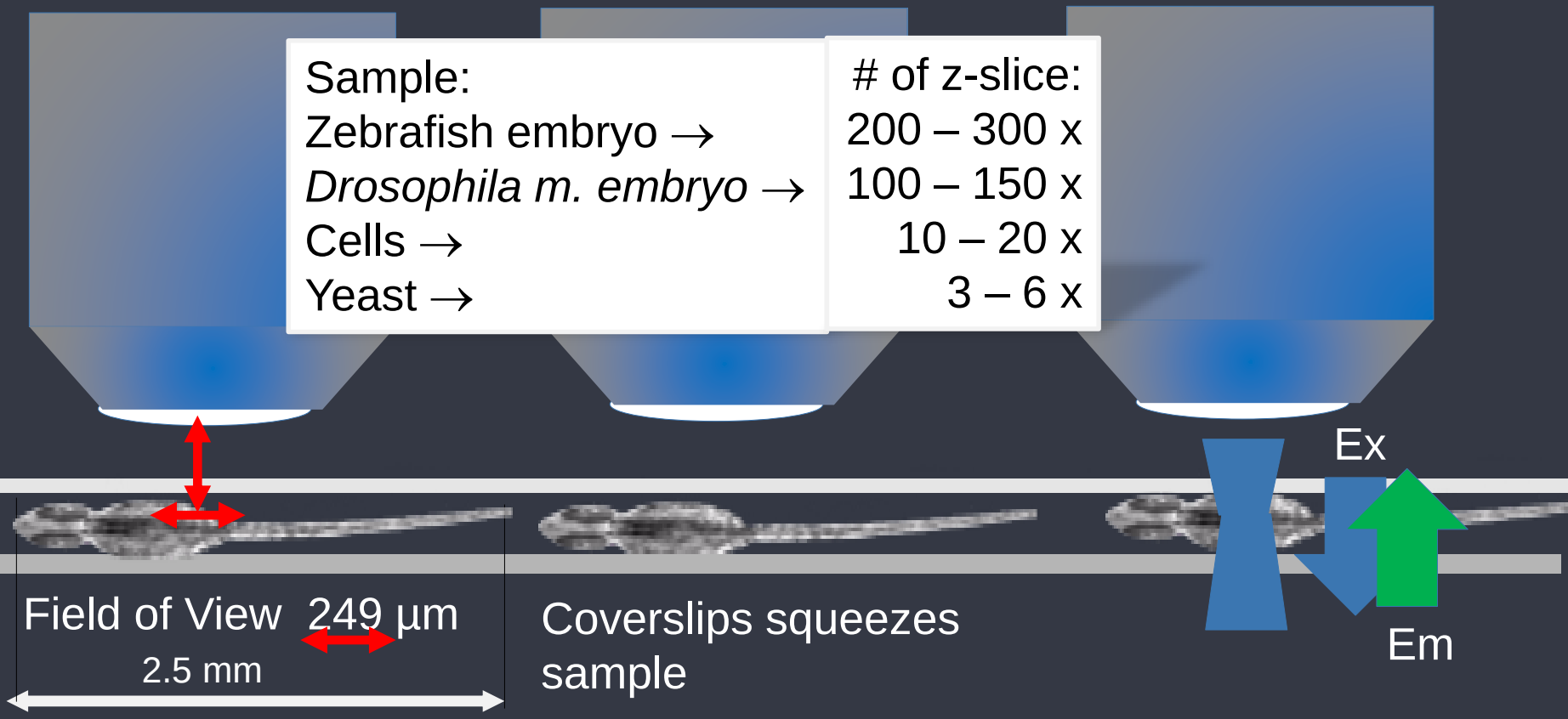
1. Bleaching and photo-damage
2. Capturing highly dynamic processes in 3D
3. Sample size
4. Sample viewing position
5. Out of focus fluorescence for optical sectioning

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



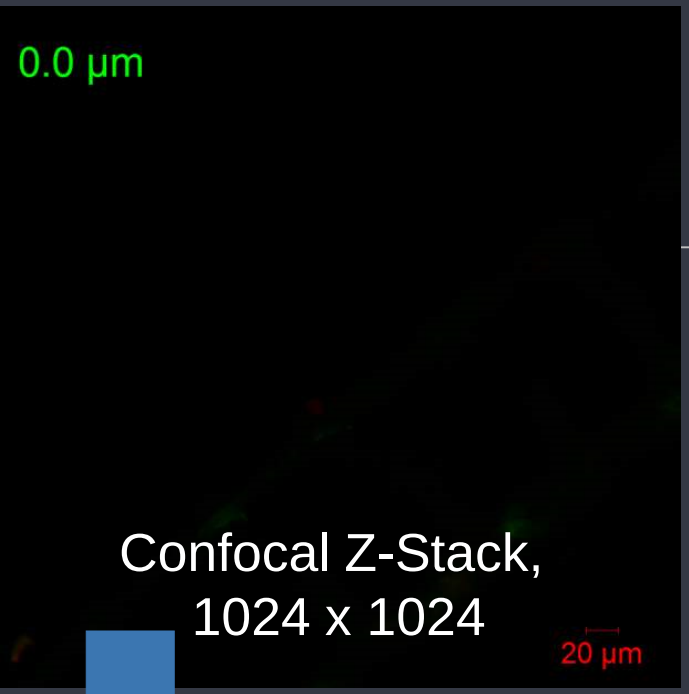
Juvenile Acorn worm (Saccoglossus kowalevskii), labelled with AF 488 phalloidin and DAPI.
Jessica Gray, Harvard Center for Biological Imaging, USA

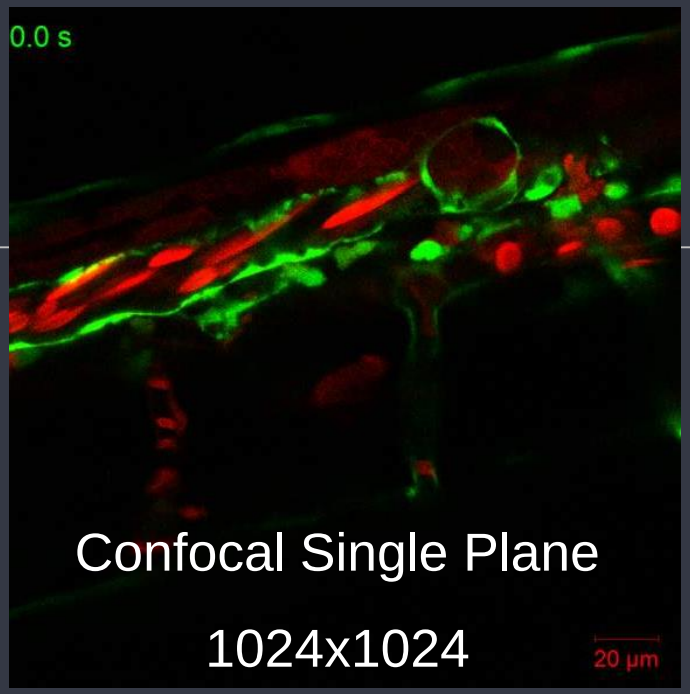
Challenges
environment, dimensions, imaging

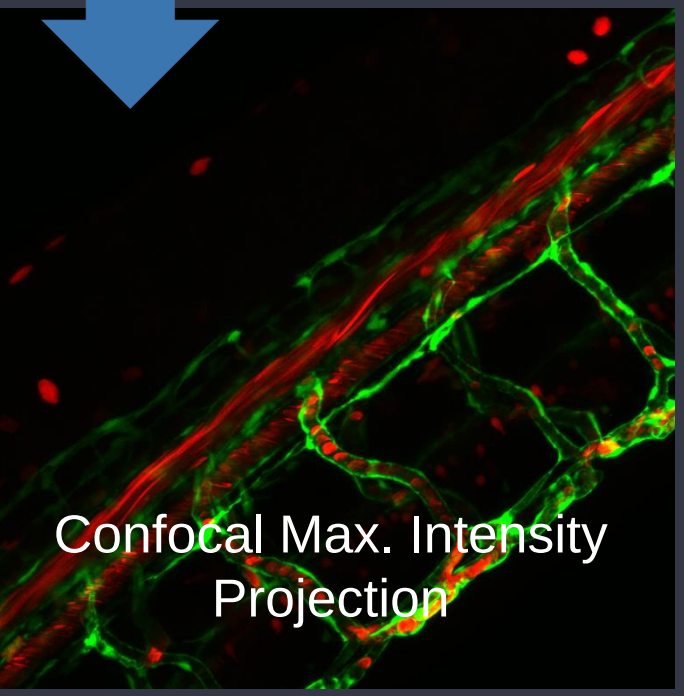


Widefield vs Optical sectioning

Point scanning vs time & light damage







Challenge
Life is dynamic!

Slow scan → moving objects are blurred

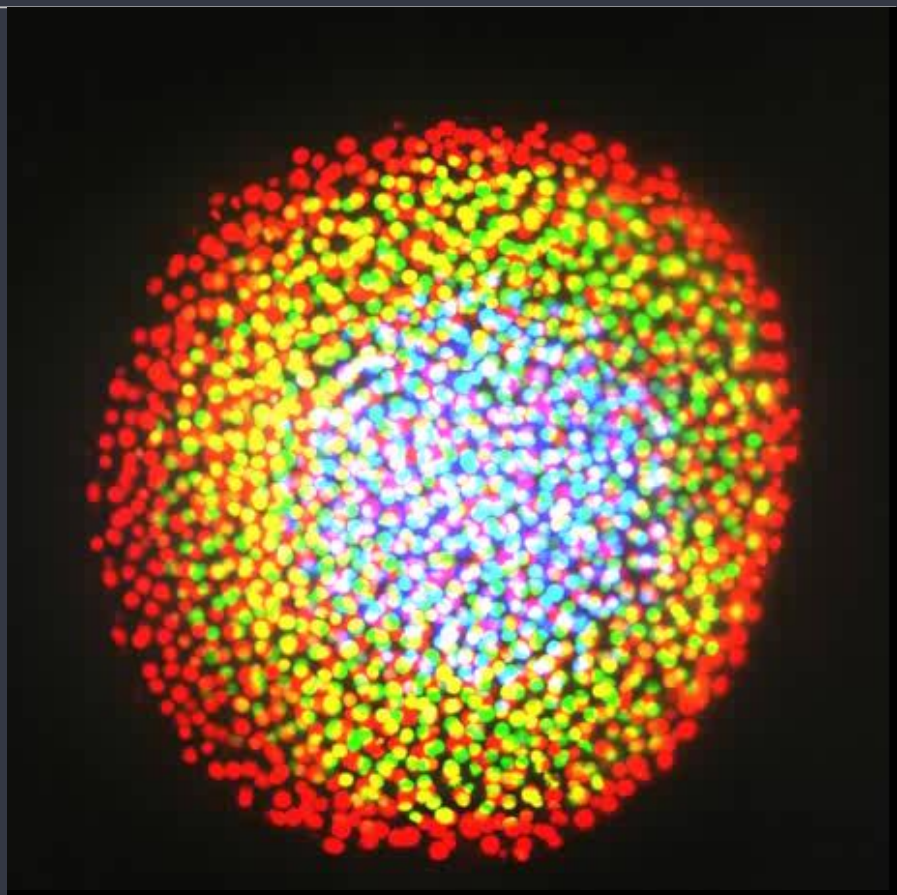
Faster scan → compromise in resolution

erythrocytes in blood **vessel**

Imaging of living, multi-dimensional specimens
What are the motivations?

Motivations:

1. Minimize bleaching and reduce photo-damage
2. Sufficient spatio-temporal resolution for observing dynamic 3D processes.
3. Image large, living 3D specimens without squishing or needing to microtome
4. Sample freely positionable in 3D including rotation, not limited to 2D
5. Avoid out-of-focus fluorescence for clean, optical sectioning



Zebrafish embryo expressing histone H2B fused to Dendra2 which labels DNA
Katherine Rogers, Department of Molecular and Cellular Biology, Harvard University, USA

Live Cell Imaging of multi-dimensional specimens

What kind of microscope would you need?

Optical sectioning

(2PM, LSM, SD vs Widefield)

Perfect environment

(CO2,O2, T)

Sensitive detection

(Camera vs PMT)

Multiple views /

Flexible sample positioning

(2Pi...?)

Low photo-damage

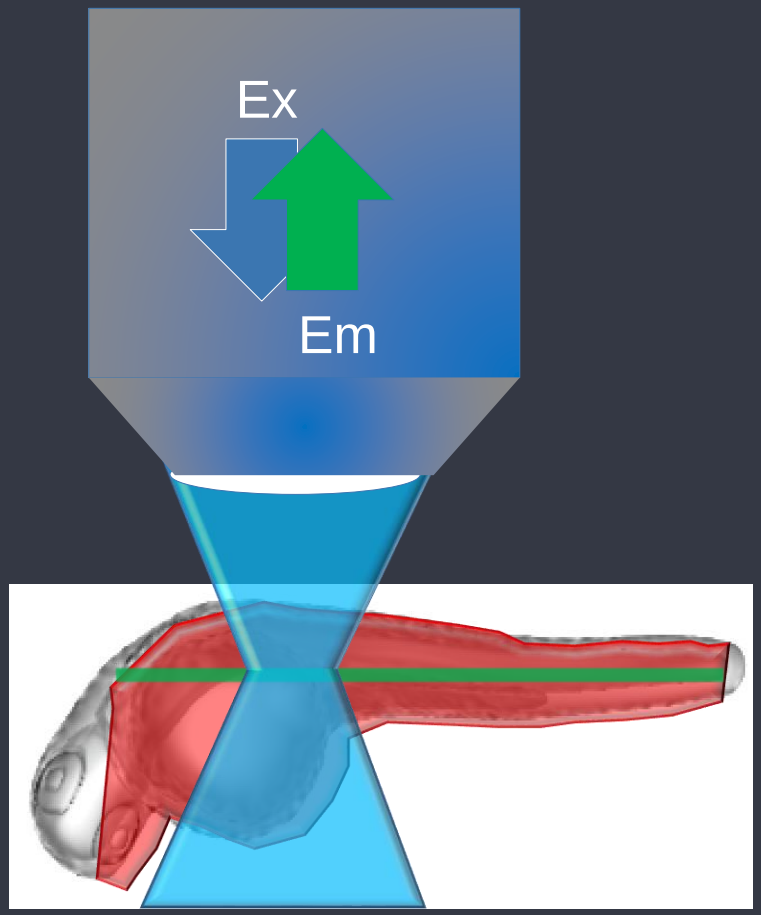
(2PM, SD vs ...)

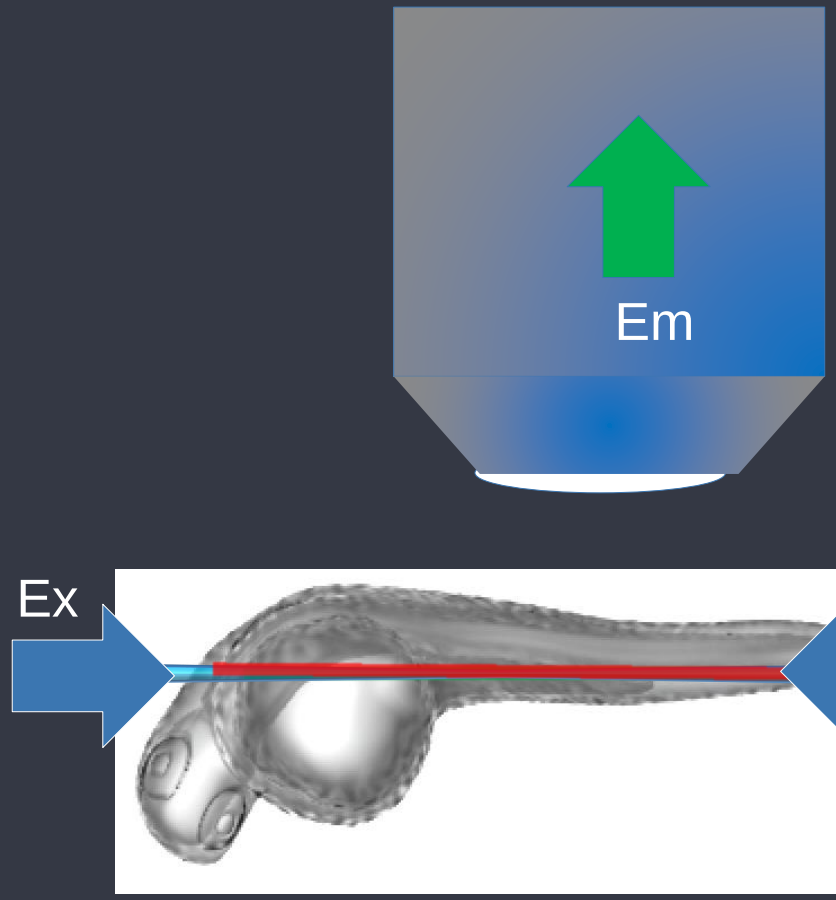
High Speed

(Widefield or Spinning Disc/camera vs pointscan)

Epi-illumination (e.g. LSM) versus Light Sheet illumination

Widefield/Pointscan versus light sheet of defined thickness





What illumination to use?
Epi-illumination vs. Light Sheet illumination

→ Orthogonal light paths for Illumination and Detection in a horizontal microscope.

→ Inherent optical sectioning capability of the illumination Method

→ No excitation of out-of-focus fluorescence
→ Whole field of view il´luminated
→ Camera based light collection

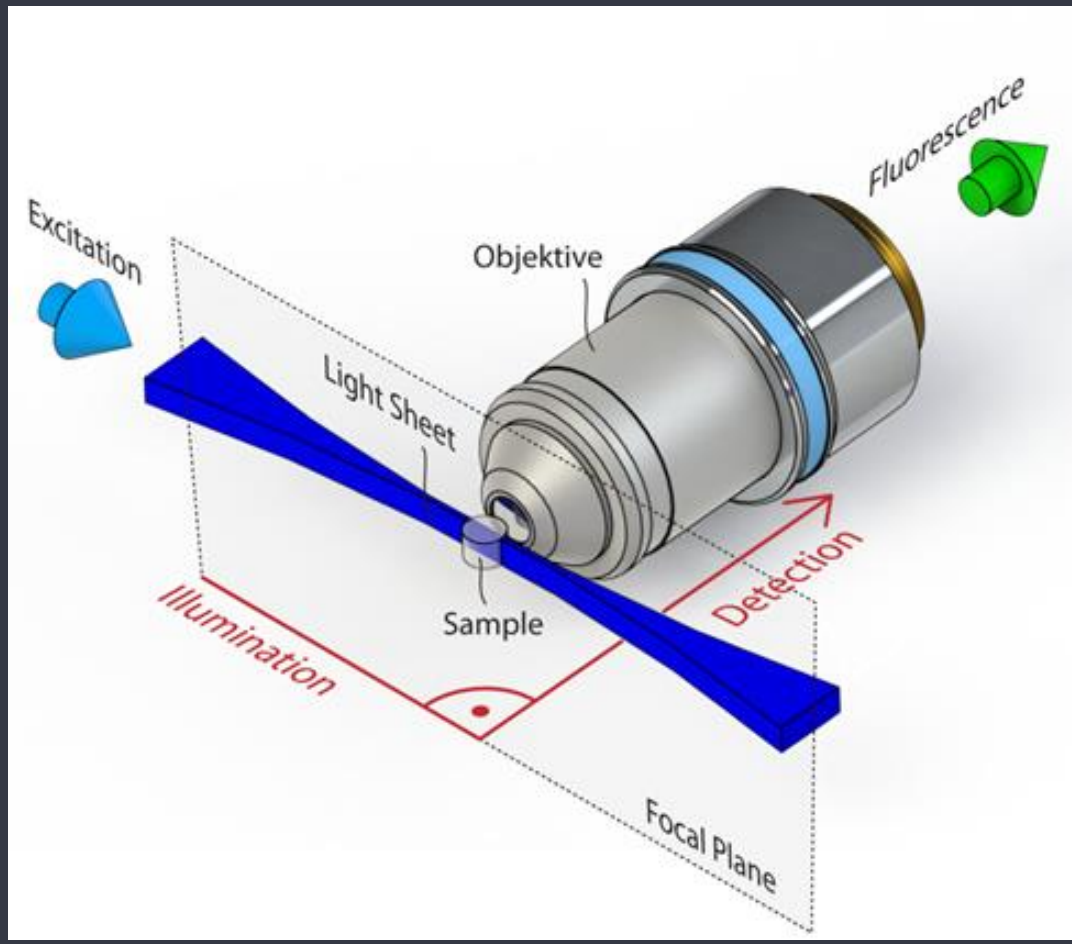
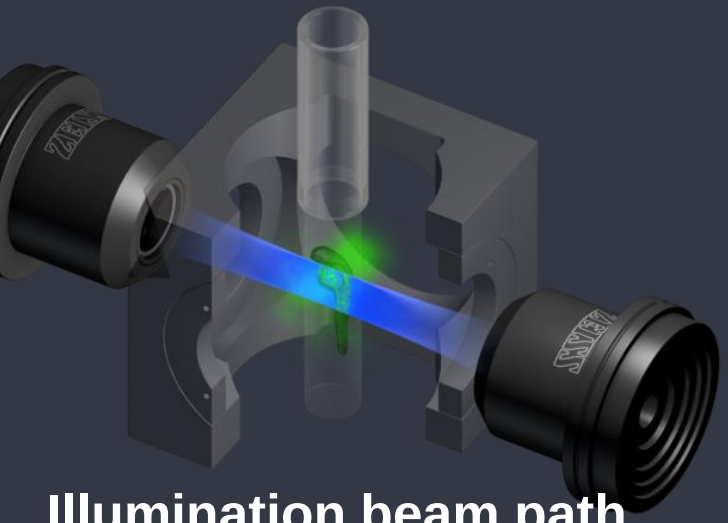


Figure from the PhD thesis of Jörg Ritter (2011), University of Bonn, Germany

Light sheet Light
Illumination in a horizontal microscope

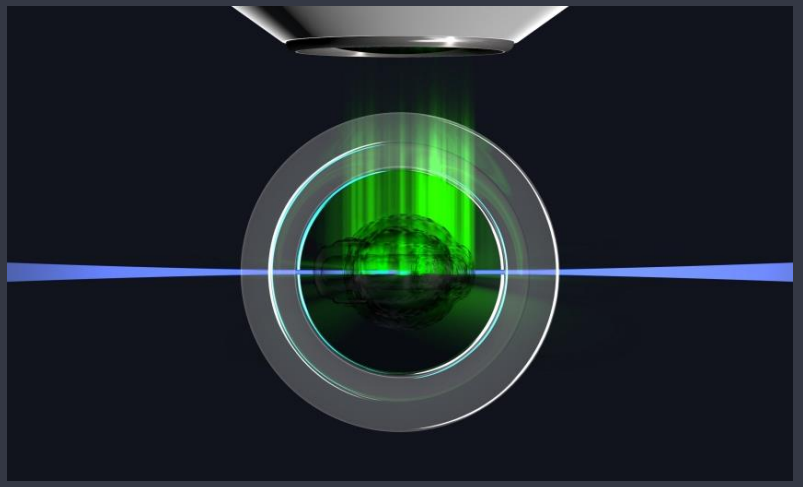


Illumination beam path

Horizontal Microscope needed

Laser beam is shaped into a Light Sheet

Scanning mirrors move the sheet along the focal plane (z-direction)



Detection beam path

Horizontal Microscope needed

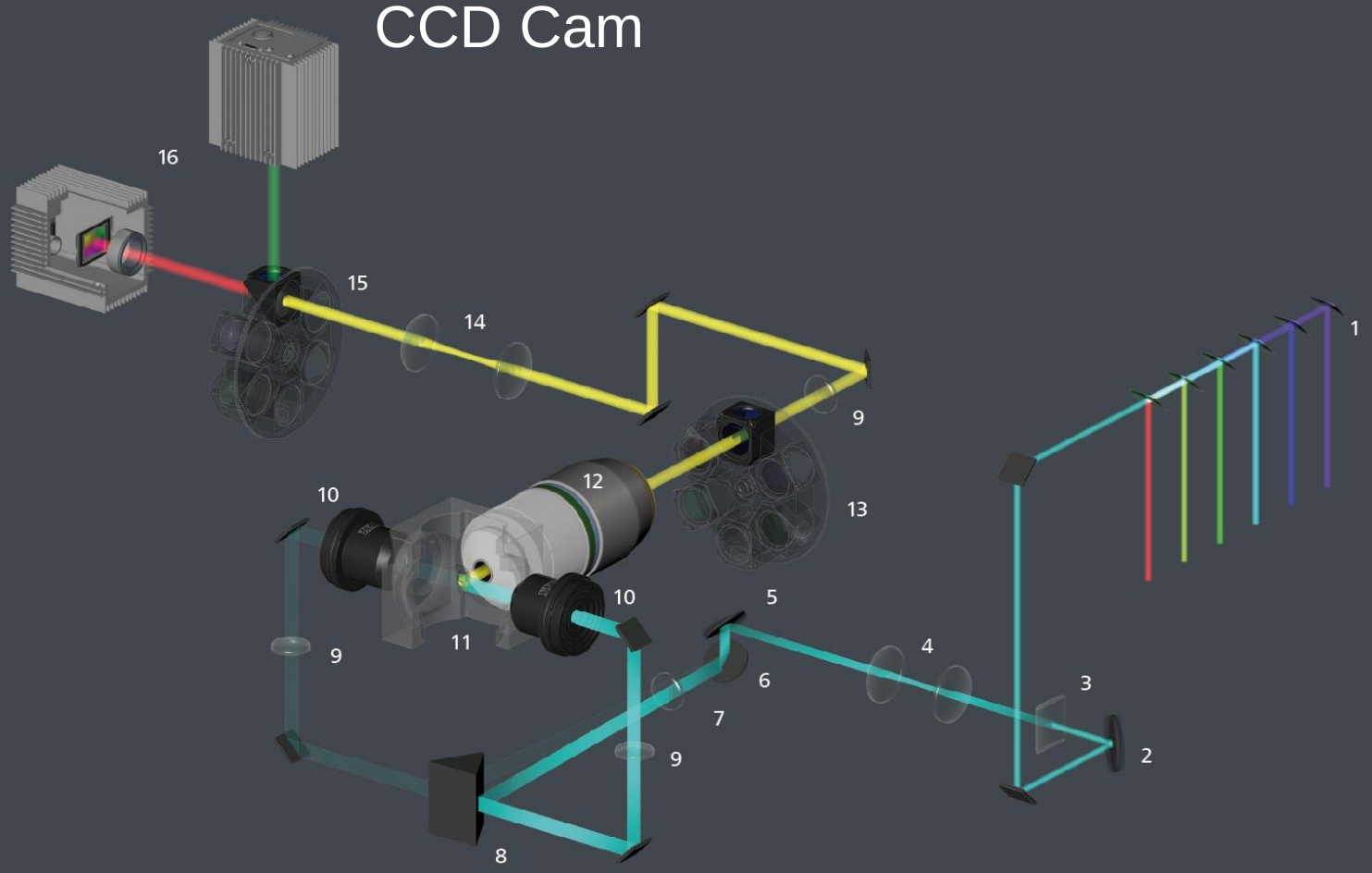
Decoupled from illumination beam path

Oriented 90º to illumination beam path

The horizontal microscope beam path for Lightsheet Z.1

Lightsheet Z.1 Beam Path

1. Lasers
2. Pivot Scanner
3. Cylindrical Lens
4. Illumination Zoom
5. Light Sheet Scanner
6. Switching Mirror (right/left)
7. Scan Objective
8. Mirrored Prism
9. Tube Lens
10. Illumination Optics
11. Sample Chamber with Sample
12. Detection Optics
13. Laser Blocking Filter
14. Detection Zoom
15. Emission Filter Module
16. Detection Modules



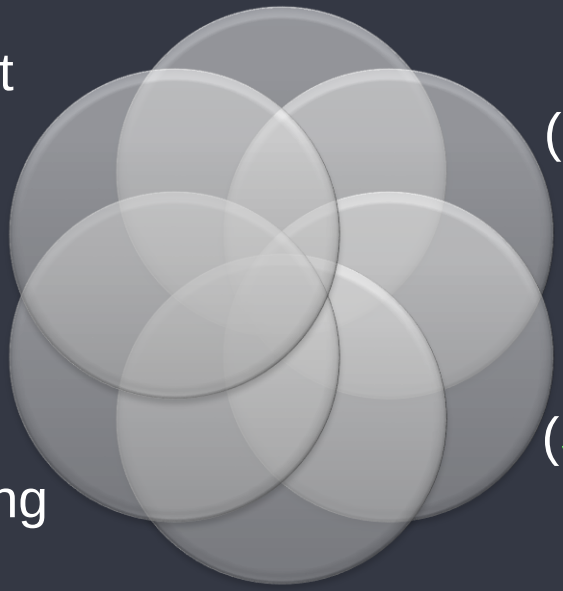
Live Cell Imaging of multi-dimensional specimens

What kind of microscope would you need?

Optical sectioning
(SPIM – light sheet)

Perfect environment
(CO2,O2, T)

Sensitive detection
(SPIM-CCD CMOS)



Multiple views /
Flexible sample positioning
(2Pi...?)

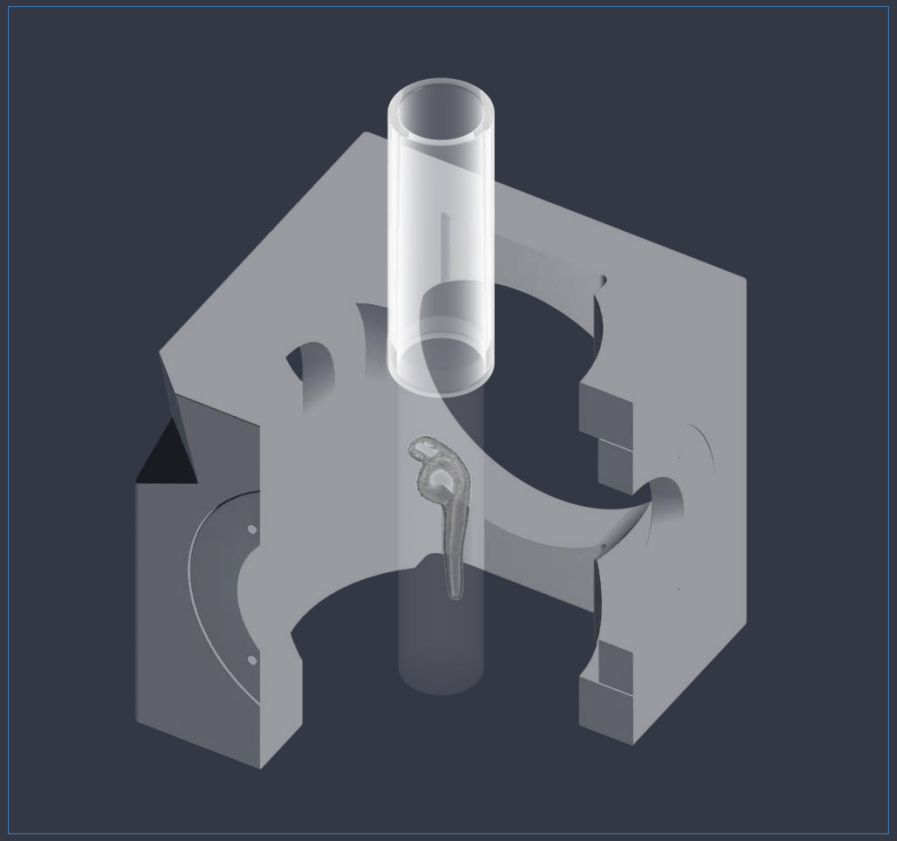
Low photo-damage
(SPIM-Lightsheet, fast acquisition)

High Speed
(SPIM, fast acquisition)

Sample mount : ”cultivation” chamber (CO_2, O_2, Temp)

Chamber for aqueous sample environment

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



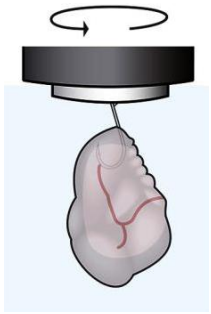
Sample mount for any view: Rotation

Sample mounted vertically in a Hydrogel

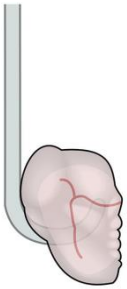
- Ideal for larger, living specimens
- Easy to prepare & store using common laboratory materials
- Translates & rotates:
  - easy positioning
  - moves for generating Z-stacks
  - allows multiple viewing perspectives (Multiview)
- Suspended in a medium/buffer

Sample mount for any type: Types of Sample Mountings

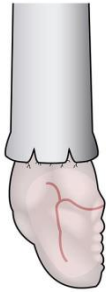
HANGING



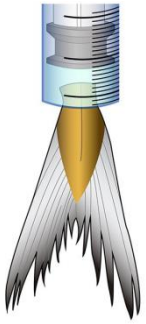
Hooked



Glued

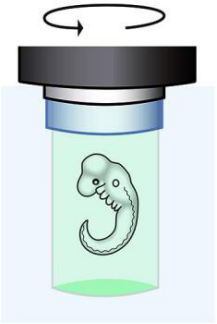


Clamped/ Submerged



Suspended

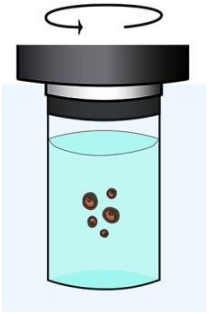
EMBEDDED



Syringe

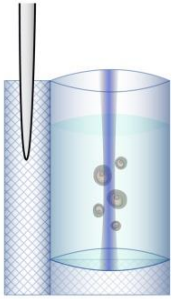
Glass Capillaries

ENCLOSED

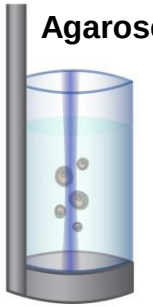


FEP Tubing

Polymer Foil



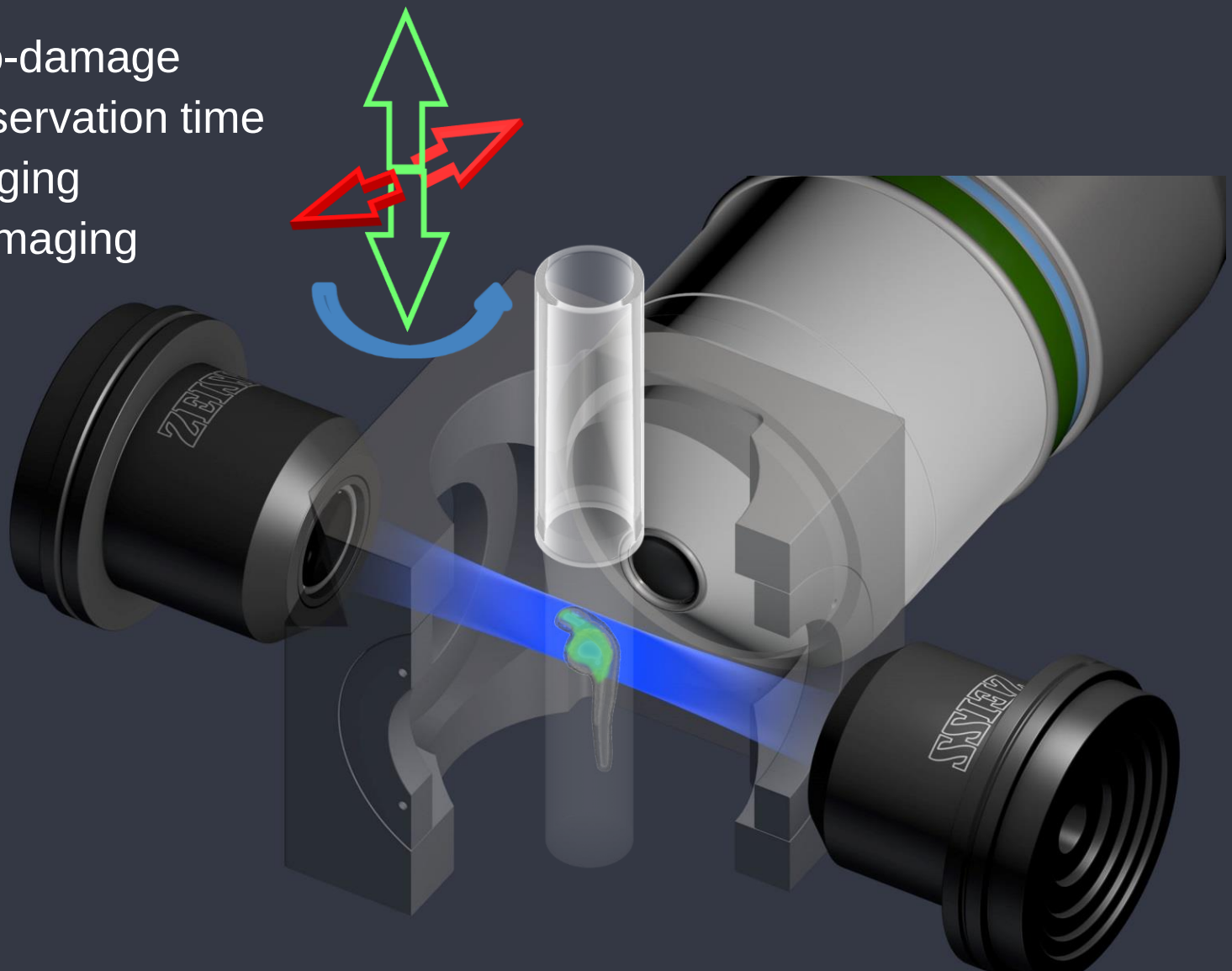
Agarose Beaker



Light Sheet Fluorescence Microscopy (LSFM /SPIM)

Fast Z-Stack Movie Z- Stack with continuous drive

Less photo-damage
Longer observation time
Faster imaging
Multiview imaging



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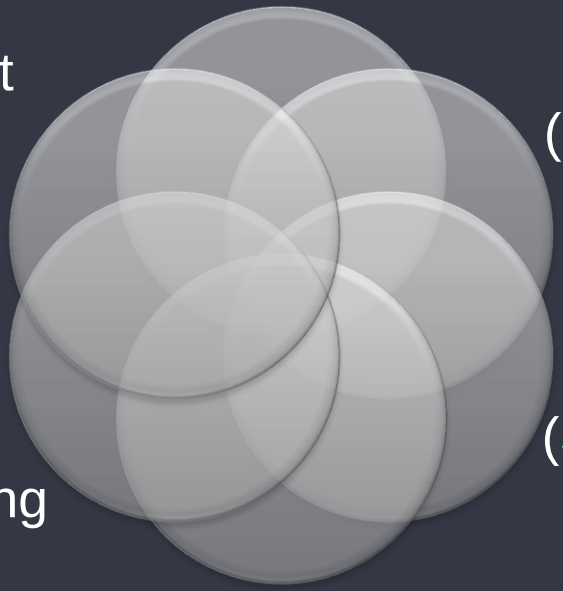


Multiple views /
Flexible sample positioning
(SPIM sample holder)

Low photo-damage
(SPIM-Lightsheet, fast acquisition)

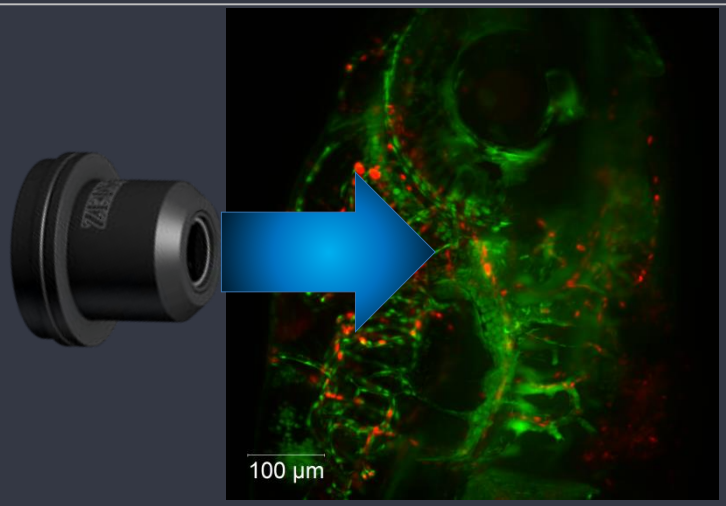
High Speed
(SPIM, fast acquisition)

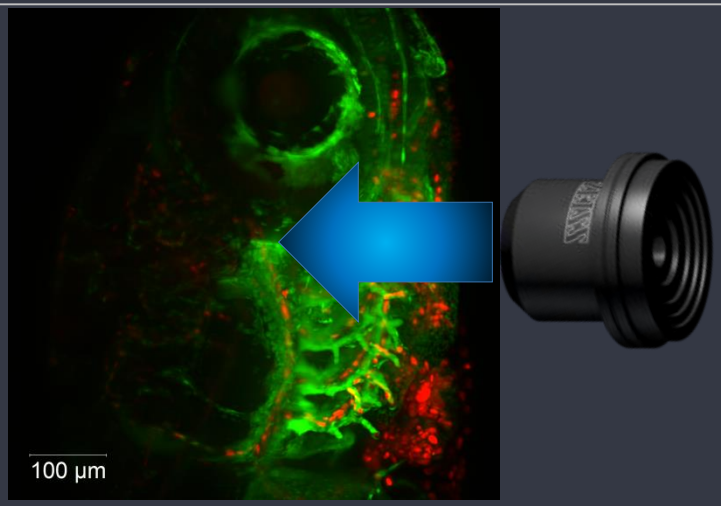
SPIM

: Selected Plane illumination Microscopy

Details to know I
Illumination Options: Dual Side vs. Single Side

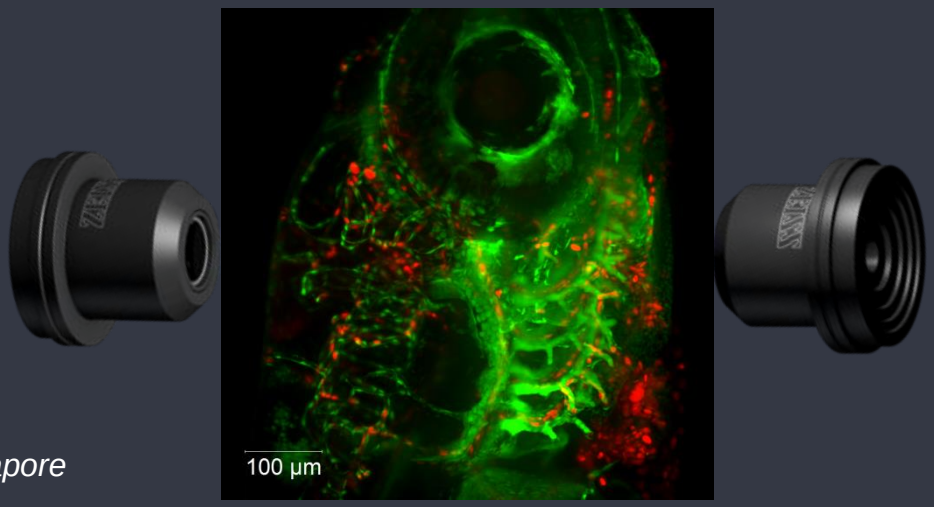
LEFT





RIGHT

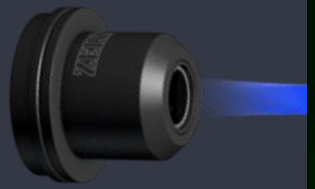
FUSED DUAL SIDE IMAGE

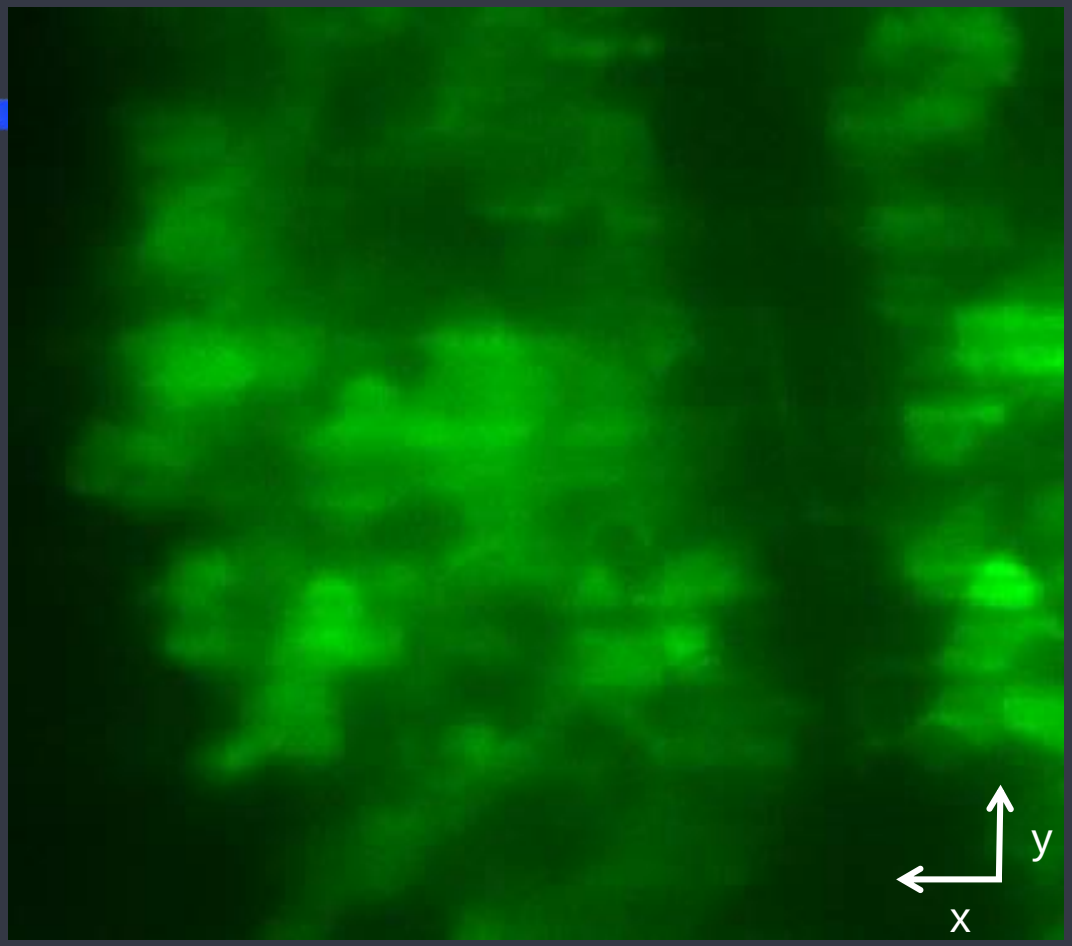


Sample by
Dr. Cathleen Teh, IMCB, Singapore

Details to know II : Pivoting light sheet
Where there is light, there are shadows (always!)
Shadow reduction

Left Side
Illumination



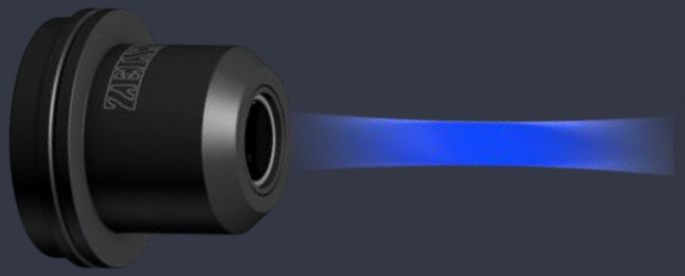


Shadow Stripes

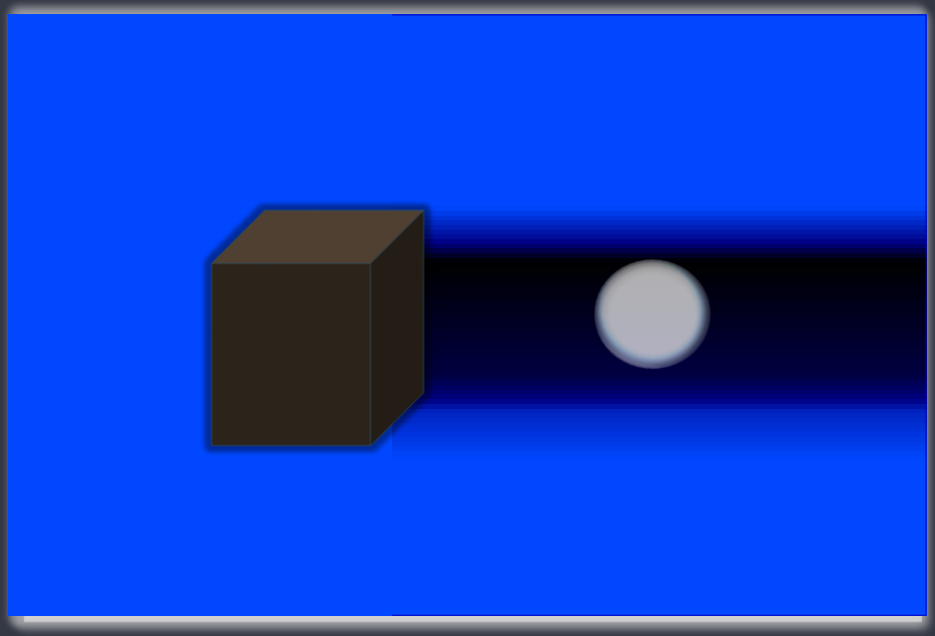
- Due to Scattering & Absorption of Illumination Light by Sample

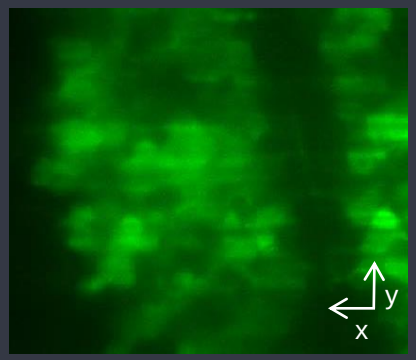
Details to know II : Pivoting light sheet
Where there is light, there are shadows (always!)
Shadow reduction

Without Pivot



Left Side
Illumination

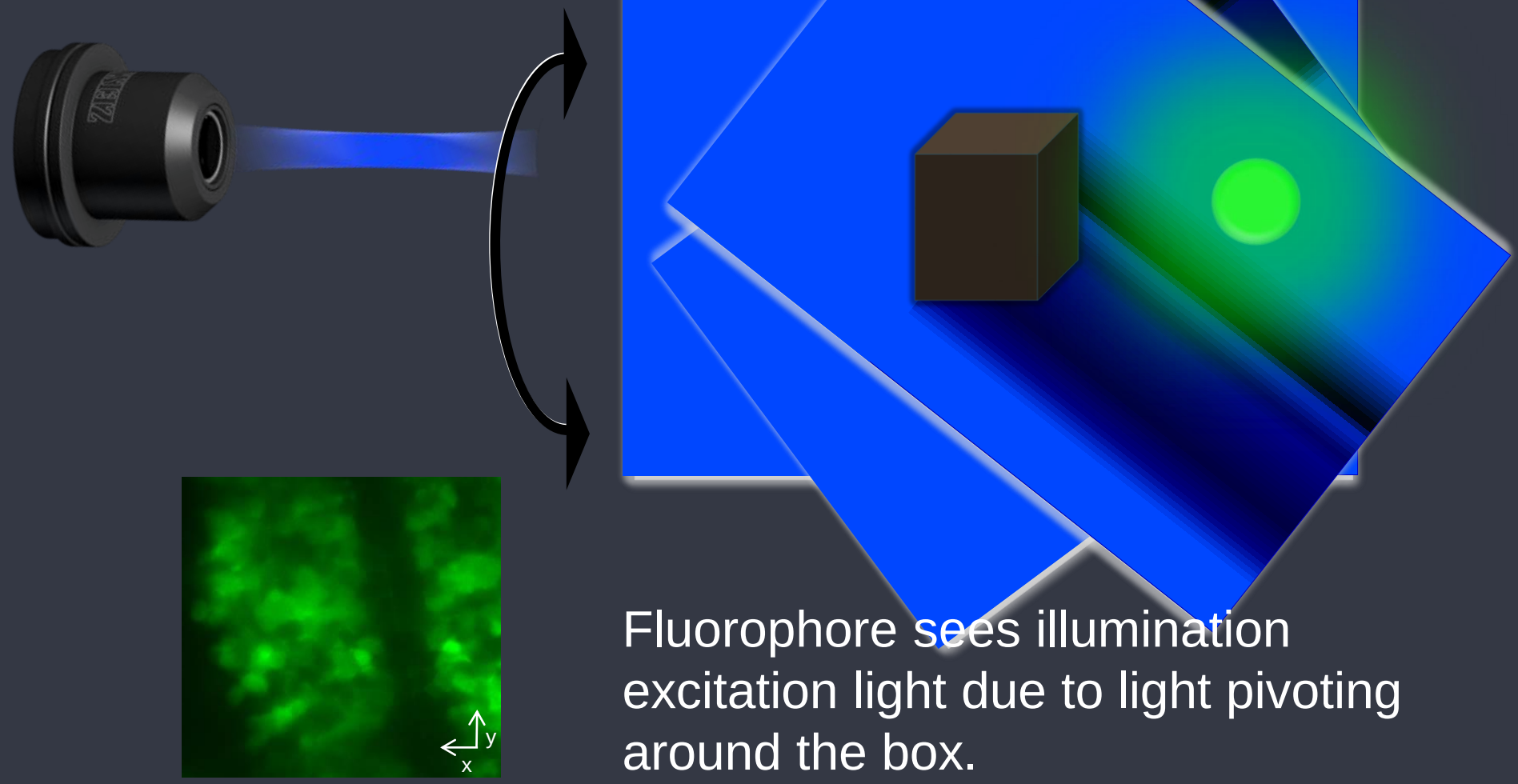




Fluorophore sees no illumination excitation light due to block's absorption & scattering.

Details to know II : Pivoting light sheet
Where there is light, there are shadows (always!)
Shadow reduction

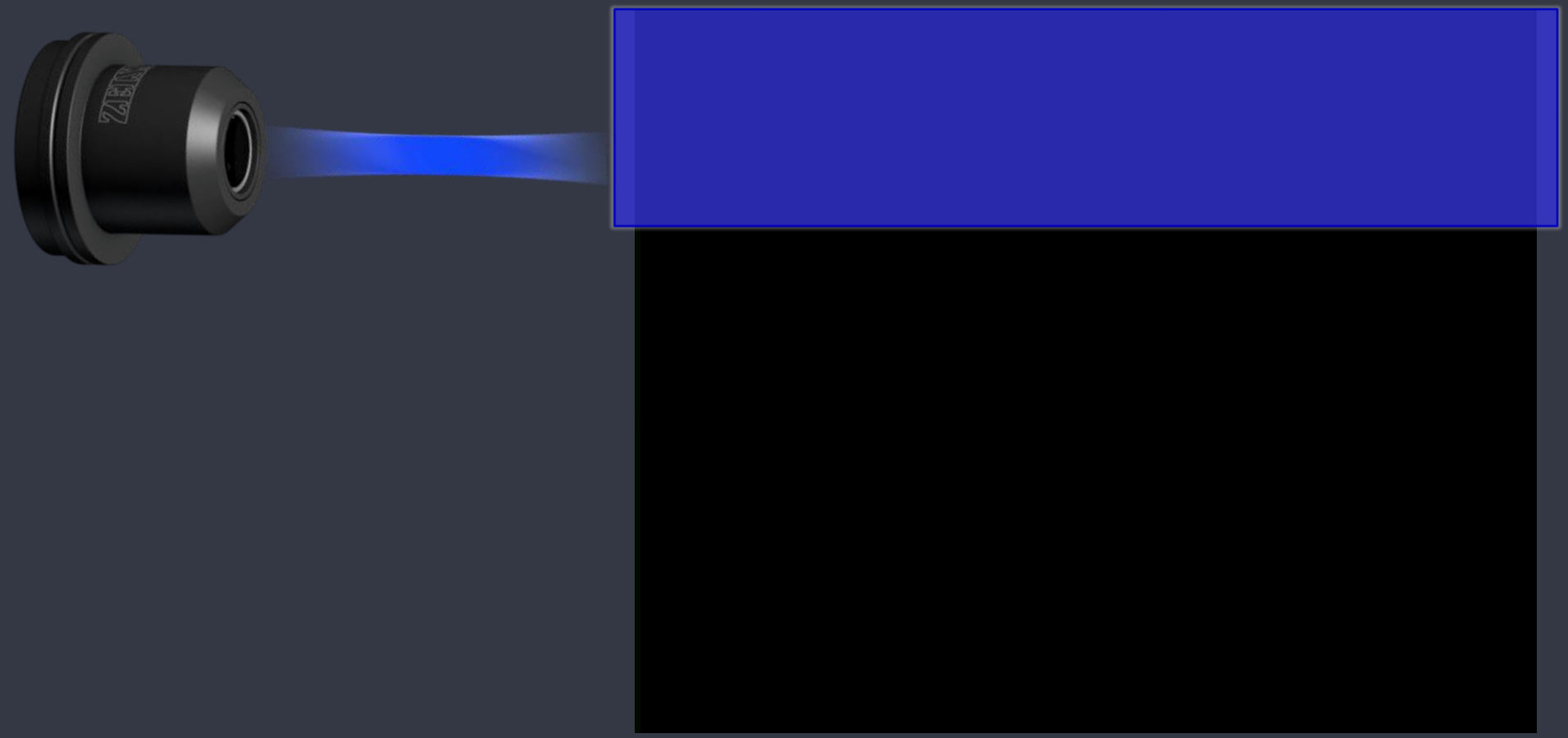
With Pivot



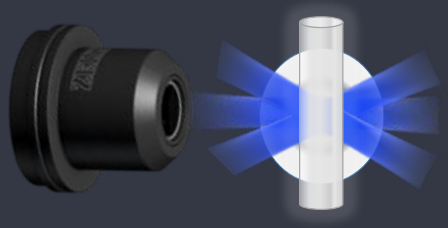
Fluorophore sees illumination excitation light due to light pivoting around the box.

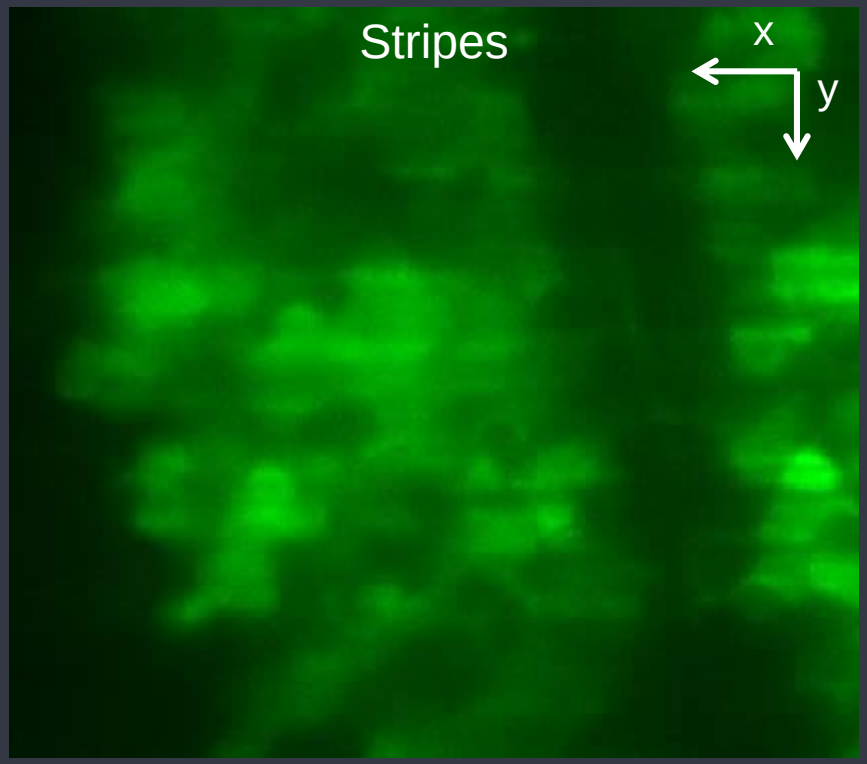
Details to know II : Pivoting light sheet
Where there is light, there are shadows (always!)
Shadow reduction

With Pivot

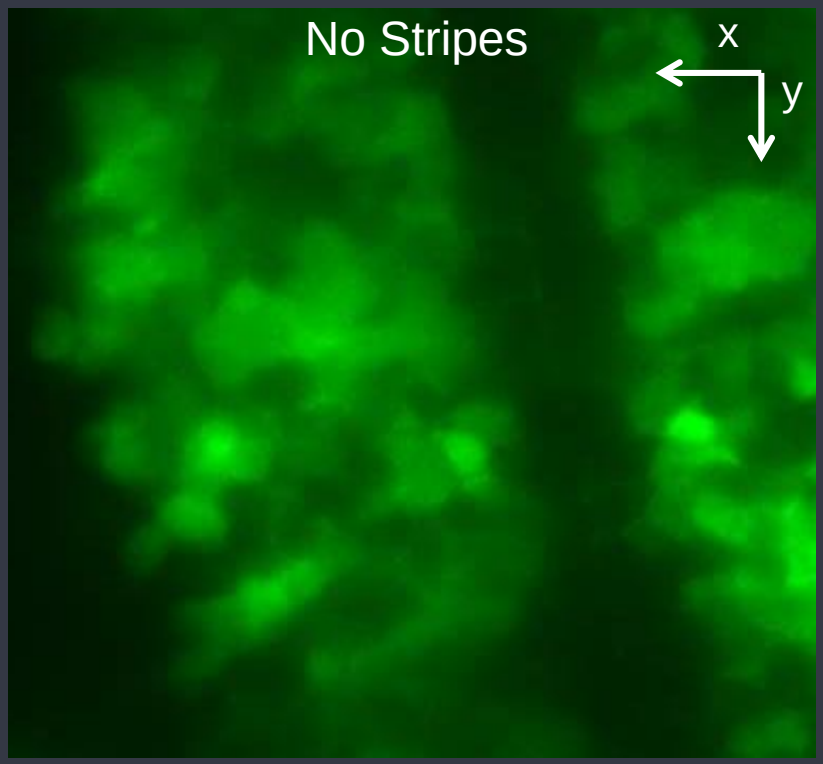


Details to know II : Pivoting light sheet
Where there is light, there are shadows (always!)
Shadow reduction





Without Pivot

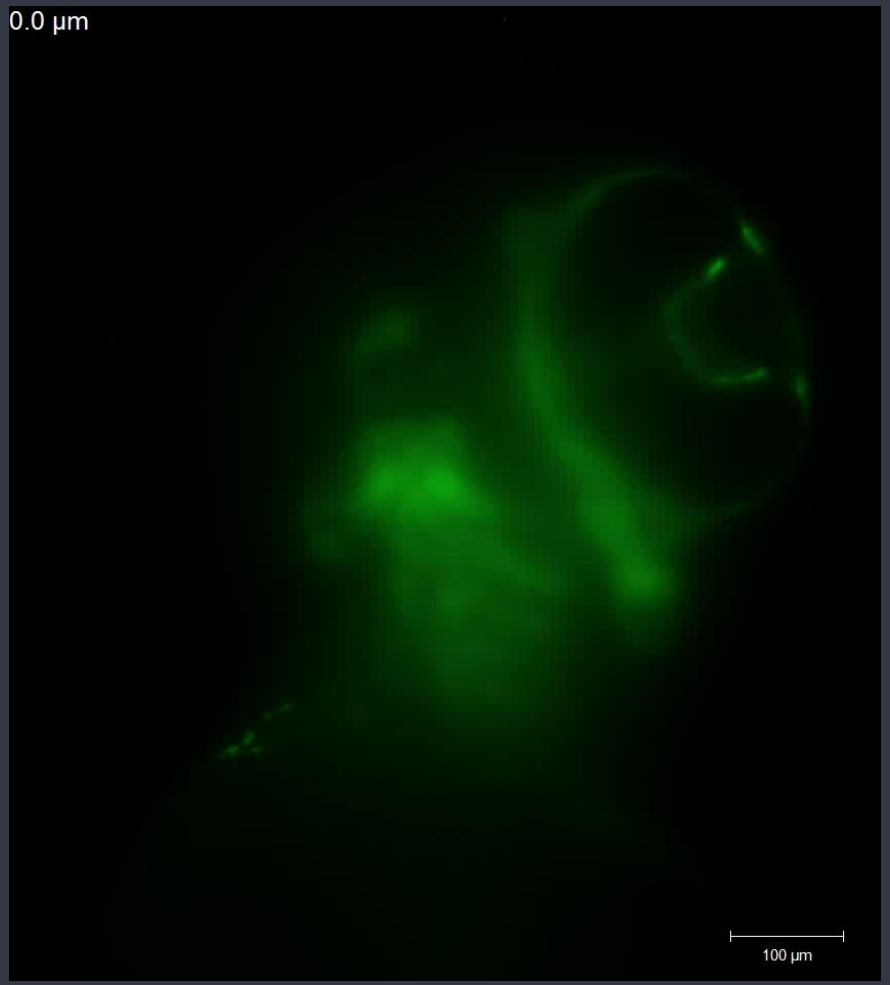


With Pivot

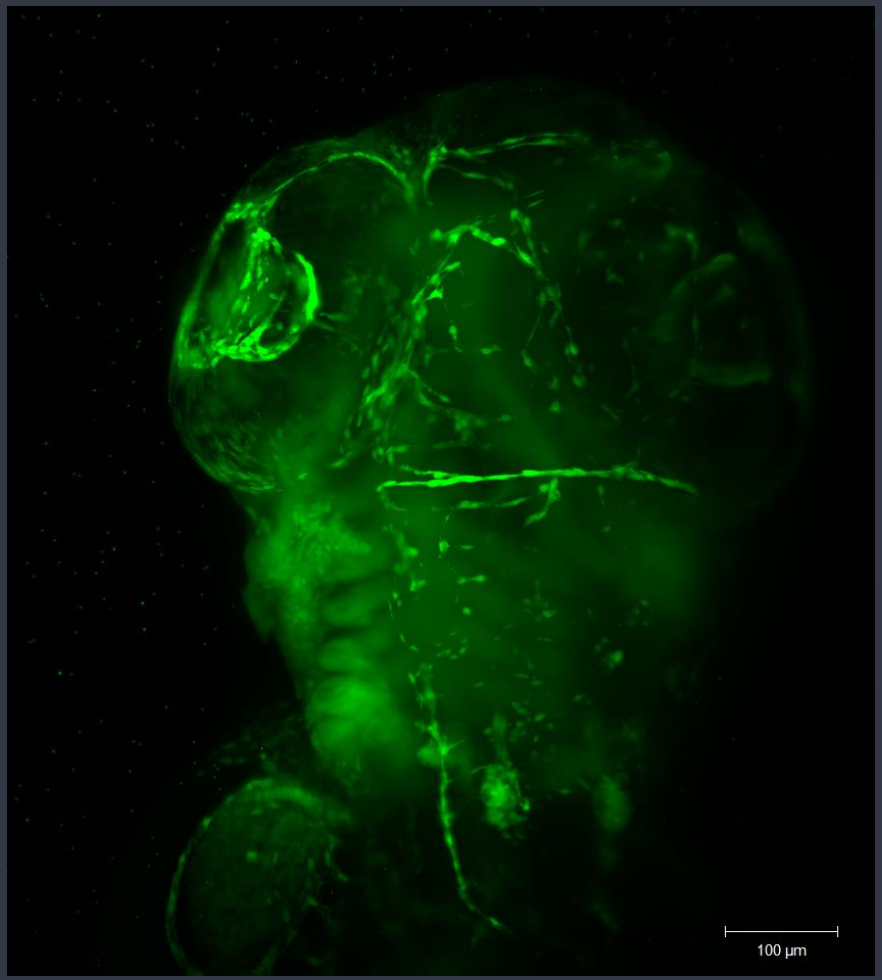
Light Sheet Fluorescence Microscopy (LSFM)

Fast Z-Stack Movie

Z- Stack (continuous drive) with 30 fps



Z- Stack maximum intensity projection



Details to know III:
Pick your Viewing Perspective(s): Rotation

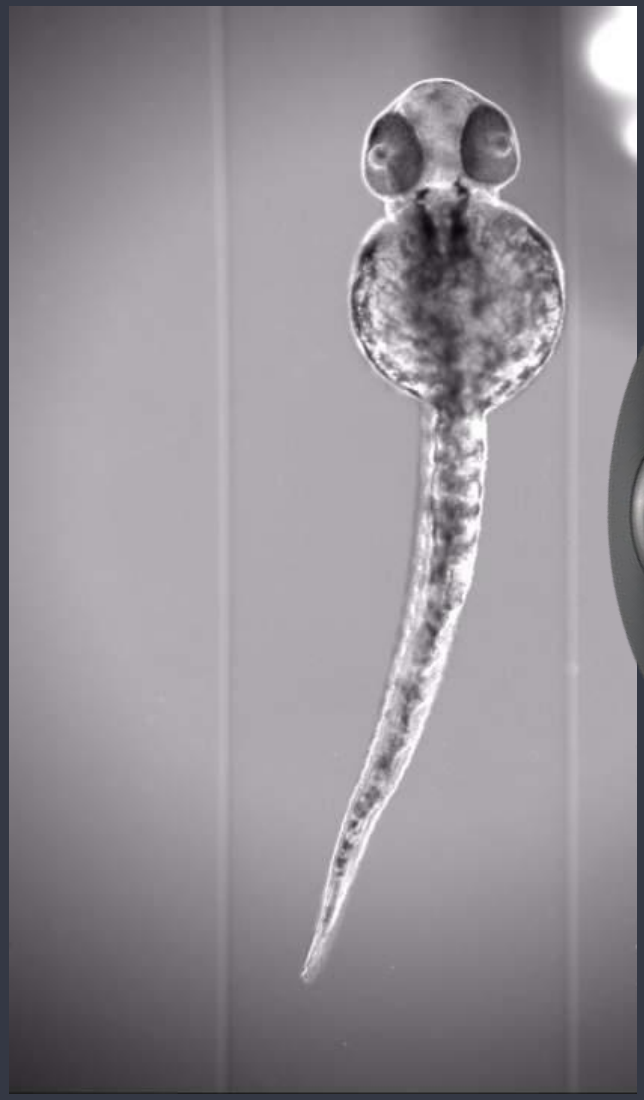
Your Sample:

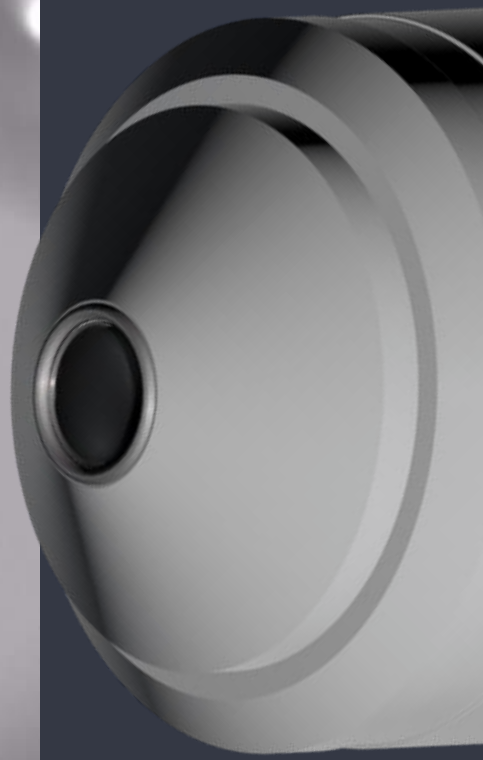
Gently embedded in physiological environment

Multiview Imaging via Rotation:

Sequentially acquired stacks (optical sections) from different directions.

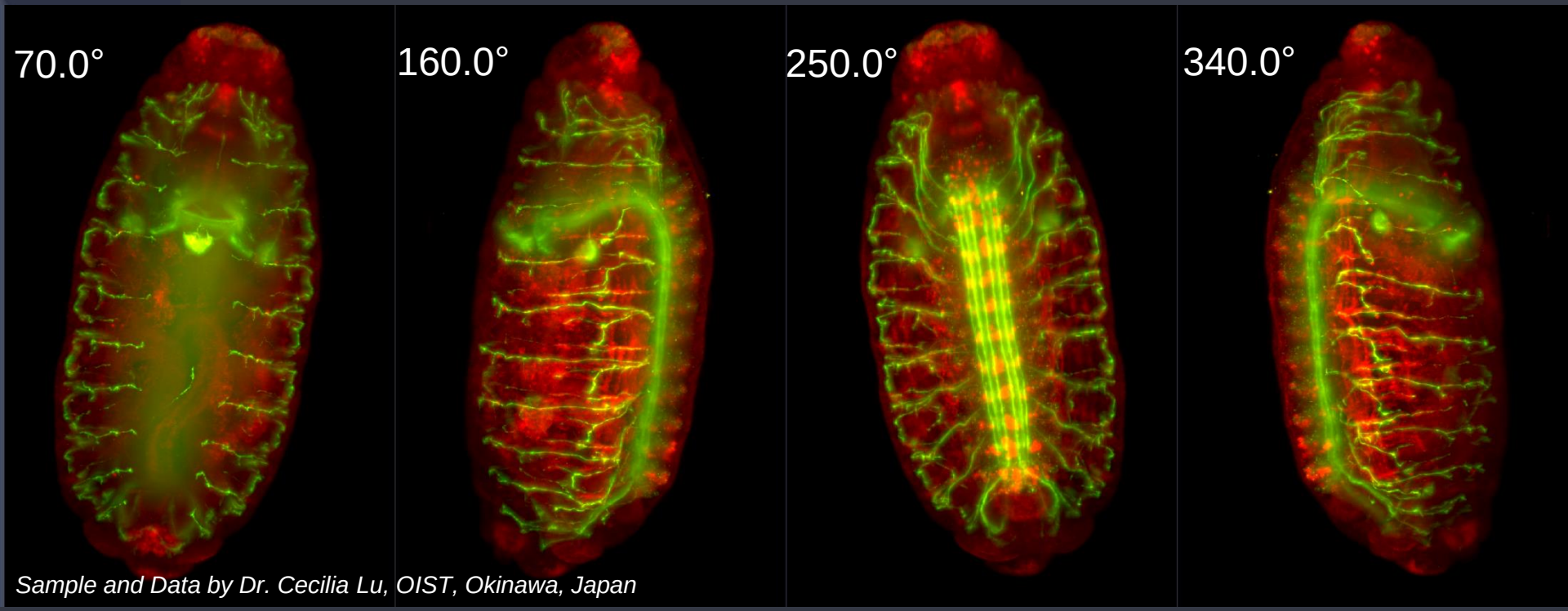
In Light Sheet Fluorescence Microscopy, stacks are taken from different rotation angles.





Details to know III:
Pick your Viewing Perspective(s): Rotation

Multiview Imaging

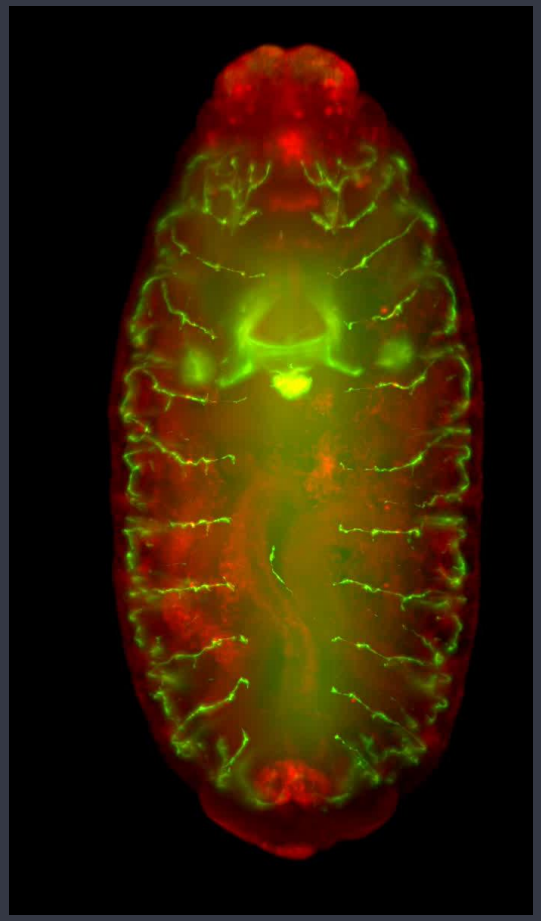


Sample and Data by Dr. Cecilia Lu, OIST, Okinawa, Japan

Benefit: Complementary information from different viewing angles.
Potential resolution improvement (sample dependent).

Details to know III:
Pick your Viewing Perspective(s): Rotation

Z-Stack View 1



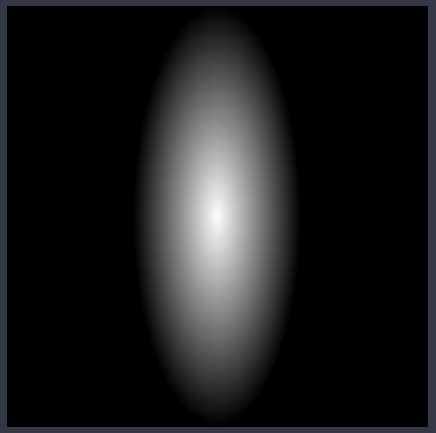
Registration & Fusion

Z-Stack View 3

Sample and Data by Dr. Cecilia Lu, OIST, Okinawa, Japan

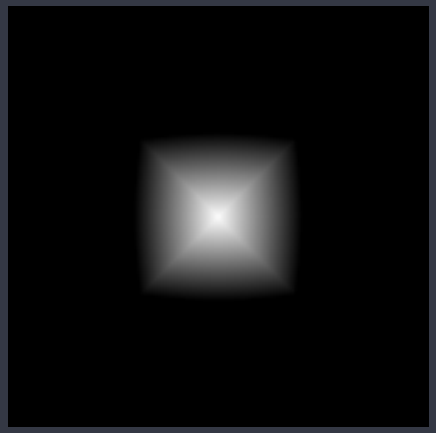
Details to know IV: MultiView Fusion Resolution
Achieving isotropic 3D resolution

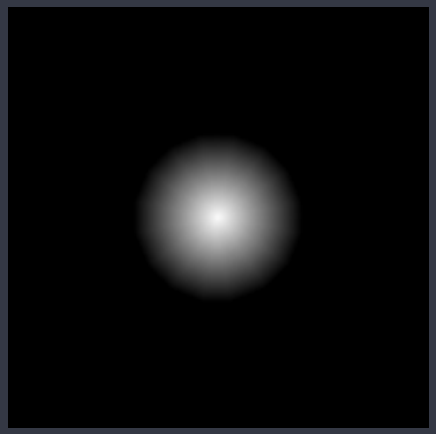
Single view



Orthogonal view

2 views fused





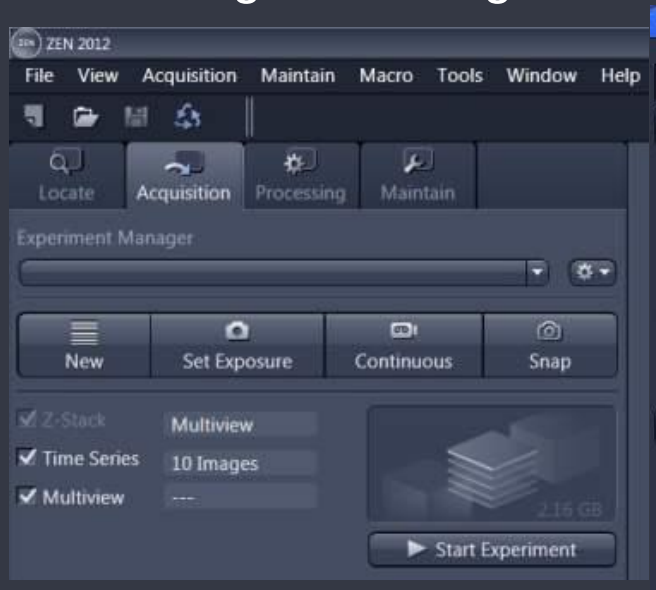
8 views

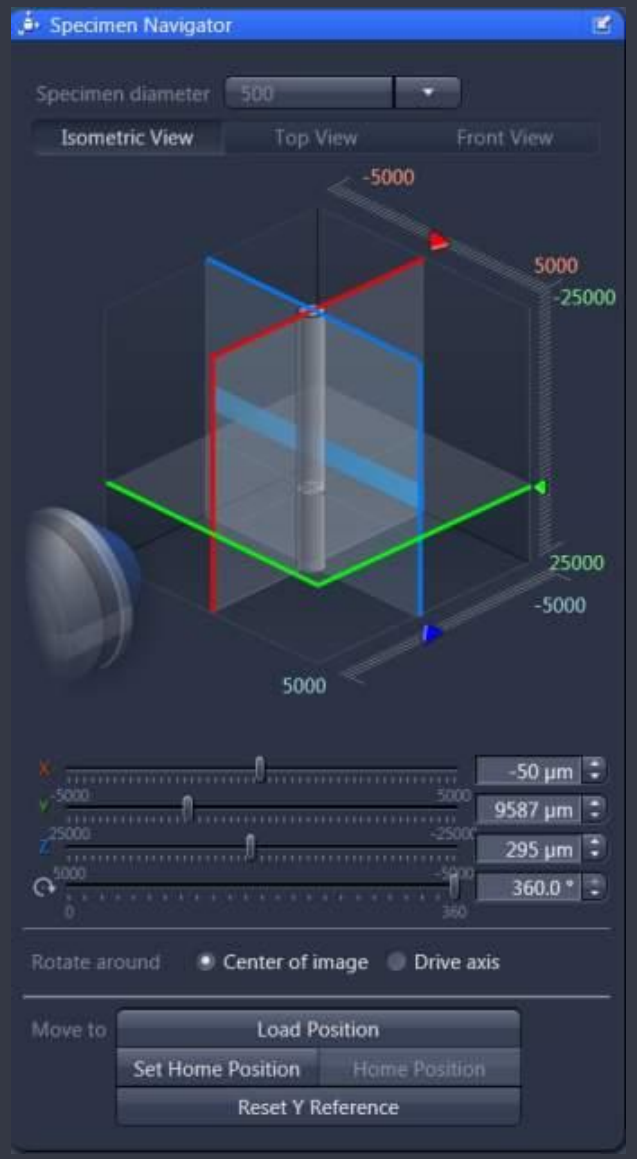
- Multiview Imaging can improve the resolution in z!
- If the Point Spread Function is of different quality from the different views, Multiview Imaging improves the images by combining complementary information.

For example:
Swoger, Huisken & Stelzer, Opt Lett, 2003
Verveer … Stelzer, Nat Meth 2007; Swoger … Stelzer, Opt Expr 2007

ZEN Software for Lightsheet Z.1
Easy to learn and use

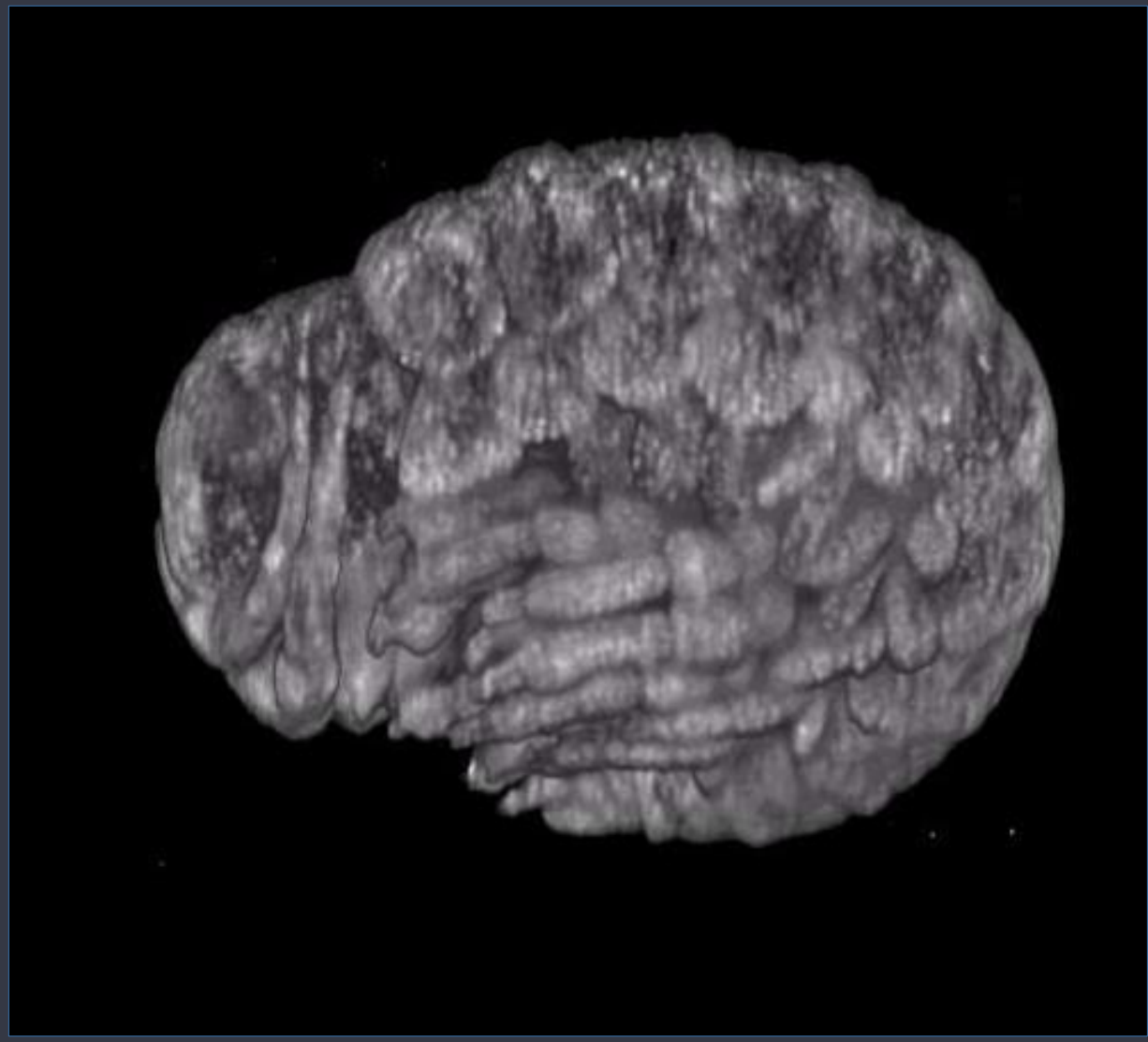
- Fully Integrated within the ZEN software platform
- Cross platform compatibility from stereomicroscopes to superresolution microscopes
- New, easy to use tools specifically designed for Lightsheet Z.1





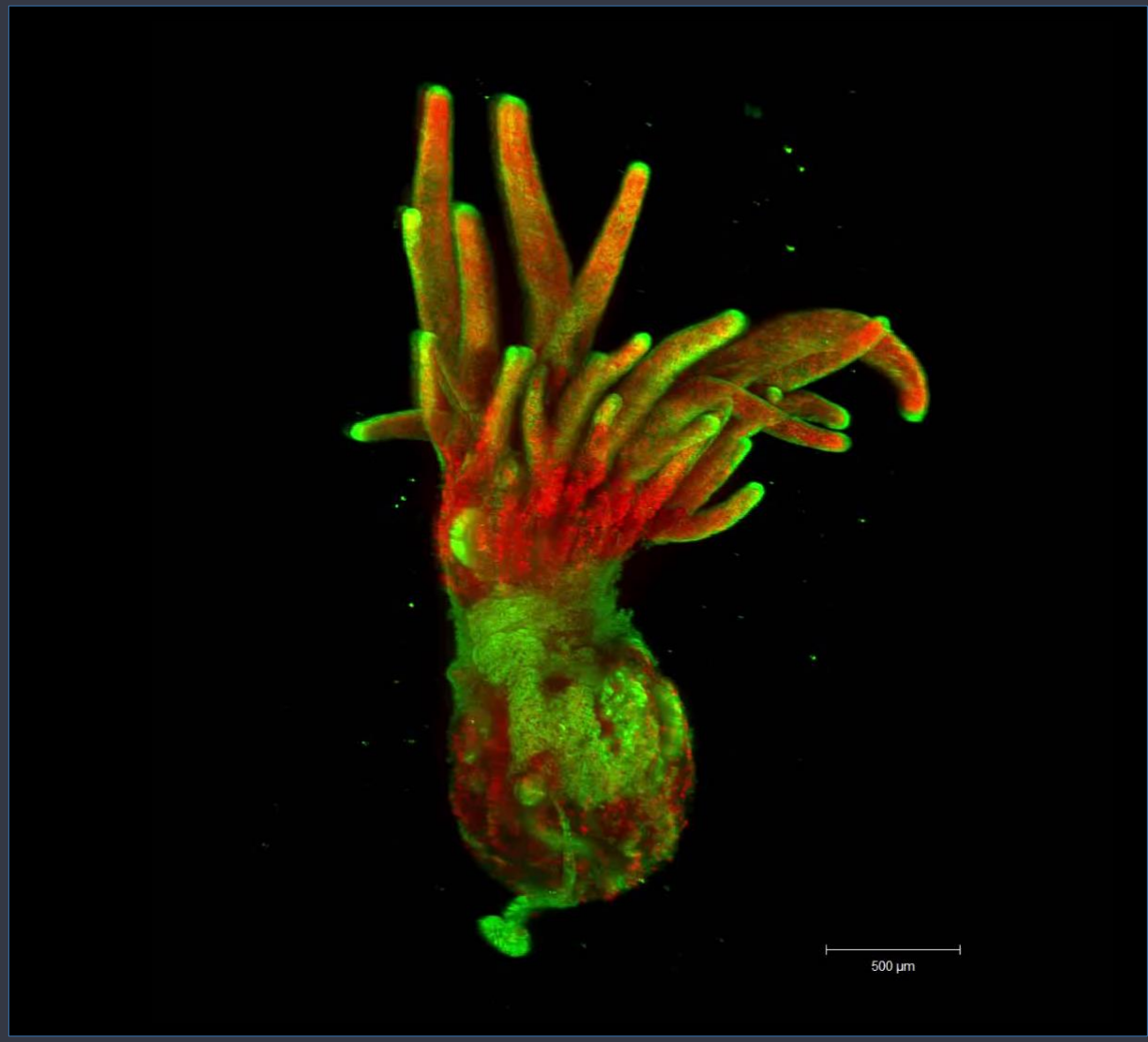
Pharhyale hawaiensis (live embryo)
3D Surface Rendering

- Nuclear red fluorescent protein (Histone2B-mRFPruby)
- Views: 4
- Multiview registration and fusion
- 3D Surface Rendering
- *Data by A. Pavlopoulos and P. Tomancak from MPI-CBG Dresden, Germany*



Aiptasia, marine anemone (coral)
Emerging model system for corals

- Nuclei in green endosymbiotic dinoflagellates in red by autofluorescence
- Views: 6
- Detection Optic: 5x
- *Data by Annika Guse, COS Heidelberg*
- Images were taken during the EMBO-MAMED course 2013

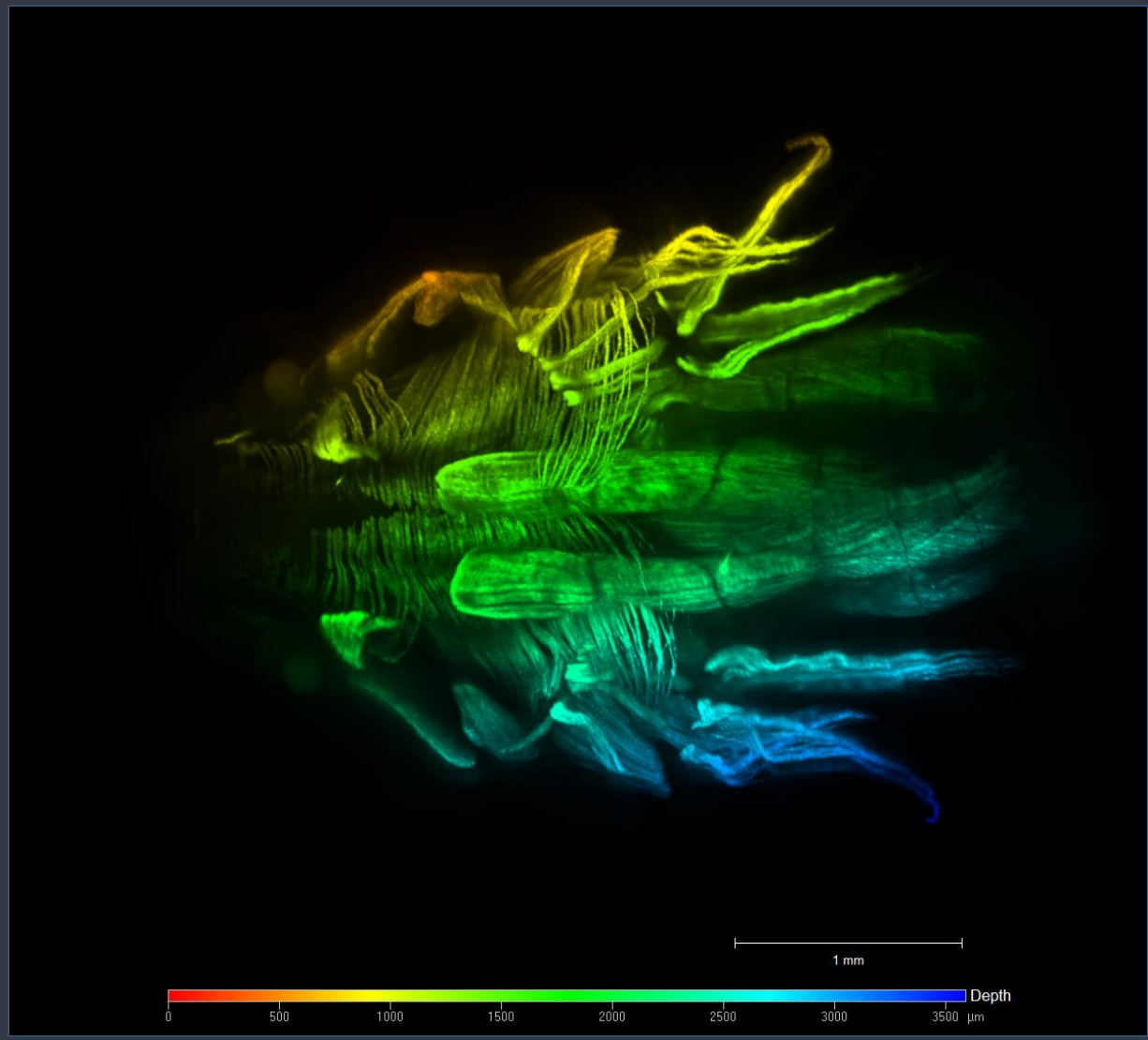


Cnidaria

- Immunostaining of
  - Microtubuli (green)
  - Myosin (red)
  - Nuclei (blue)
- Views: 4
- Maximum Intensity Projection
- *Data by H. Parra, Inst. de Biologia Evolutiva (CSIC-U Pompeu Fabra), Barcelona*
- Image taken during EMBO course on Marine Animal Models in Evolution & Development, Sweden 2013

Ambystoma mexicanum
Salamander larvae

- Larvae optically cleared by fructose (SeeDB)
- Immunostain for skeletal muscle, microtubule for detection of muscles and nerves.
- Views: 5
- Detection Optic: 5x/0.16
- *J.Schmidt and Prof. L. Olsson, Inst. of Sys Zool. & Evo. Biol. Friedrich-Schiller-University, Jena, Germany*



Tribolium castaneum
Flour Beetle embryo

- GFP in all nuclei

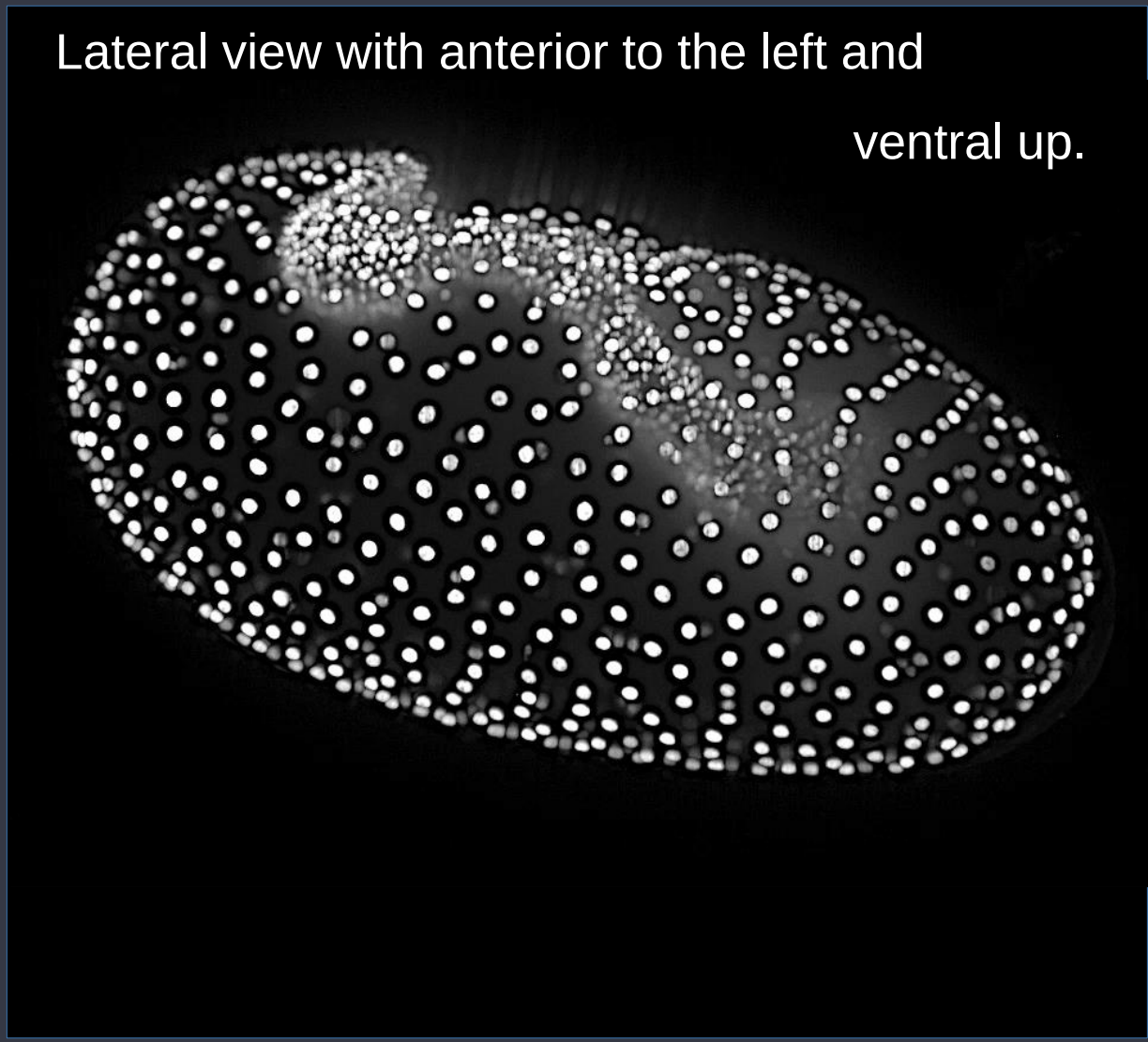
- germband extends into yolk, extra-embryonic membranes closing over ventral side. embryo grows, segments get visible germband wraps around ant. & post. end of egg

- 6:30 h all 5 min.

- Temperature @ 29°C

- *Data by Nipam Patel, UCBerkeley, Dep. MCB; Dep. of Int. Biol*

Lateral view with anterior to the left and ventral up.



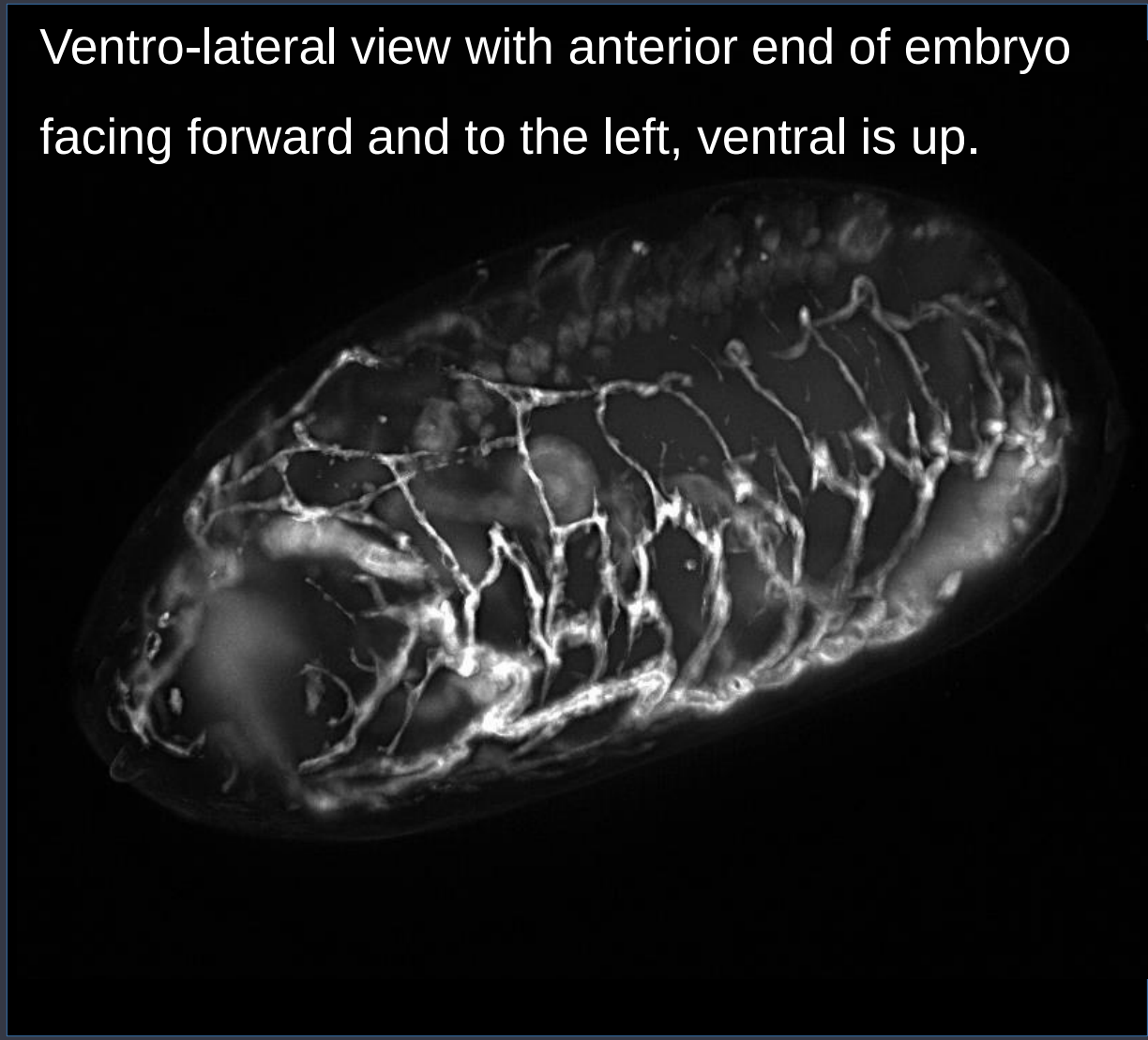
Drosophila melanogaster, emryo,
Trachea development

- 4:30 h, every 5 minutes

- Temperature @ 26°C
- Actin:GFP tracheal (respiratory) system incl ventral midline of CNS.
- Tracheal system develops into tubes with branches extending throughout the embryo

- *Data by Nipam Patel, UCBerkeley, Dep. MCB; Dep. of Int. Biol*

Ventro-lateral view with anterior end of embryo facing forward and to the left, ventral is up.



Drosophila melanogaster
Salivary Glands of third instar larvae

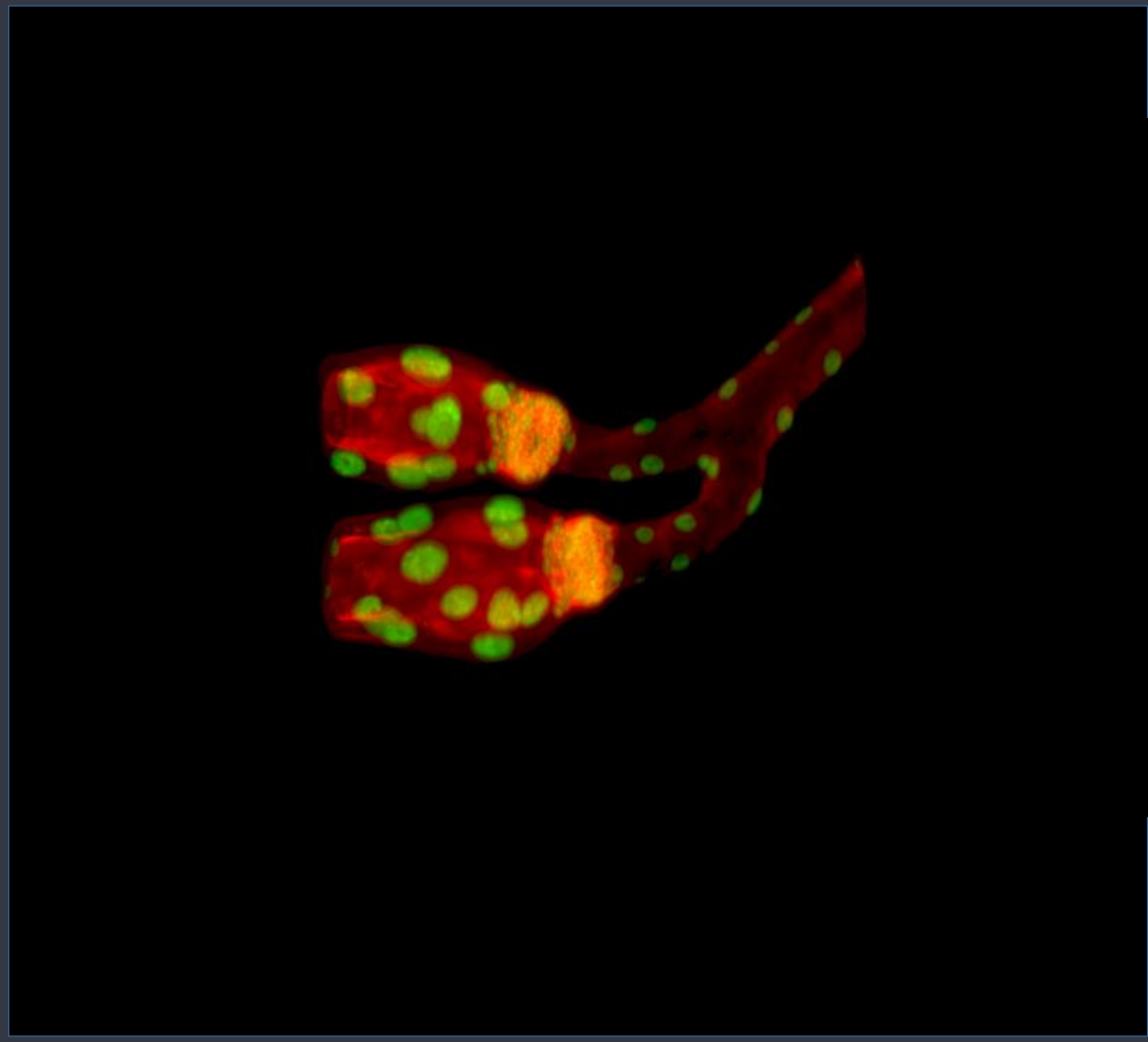
- Salivary glands of third instar larvae

- Nuclei: green (GFP)
- Actin : red (Phalloidin)

- Views: 3

- Dual side illumination

- Data by A. Pavlopoulos and P. Tomancak from MPI-CBG Dresden, Germany



Zebrafish Development, 2d embryo
Structural imaging of larger organisms

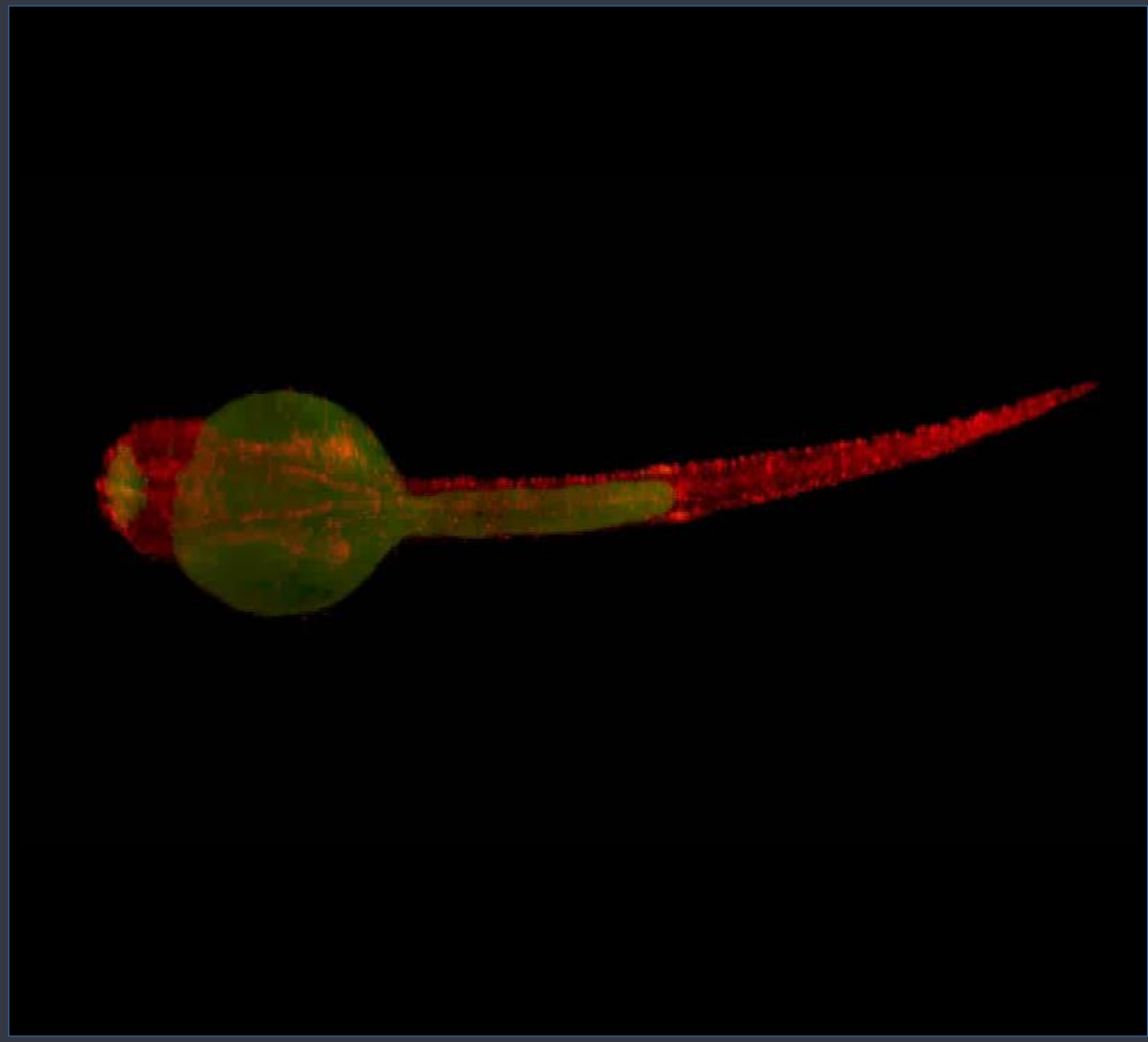
- Nuclei: Red
- Yolk: autoFL

- Views: 4

- Multiview registration and fusion

- Maximum Projection

- Data by Cornelia Hoppe, Gopi Shah (Huisken Lab, MPI-CBG, Dresden, Germany)



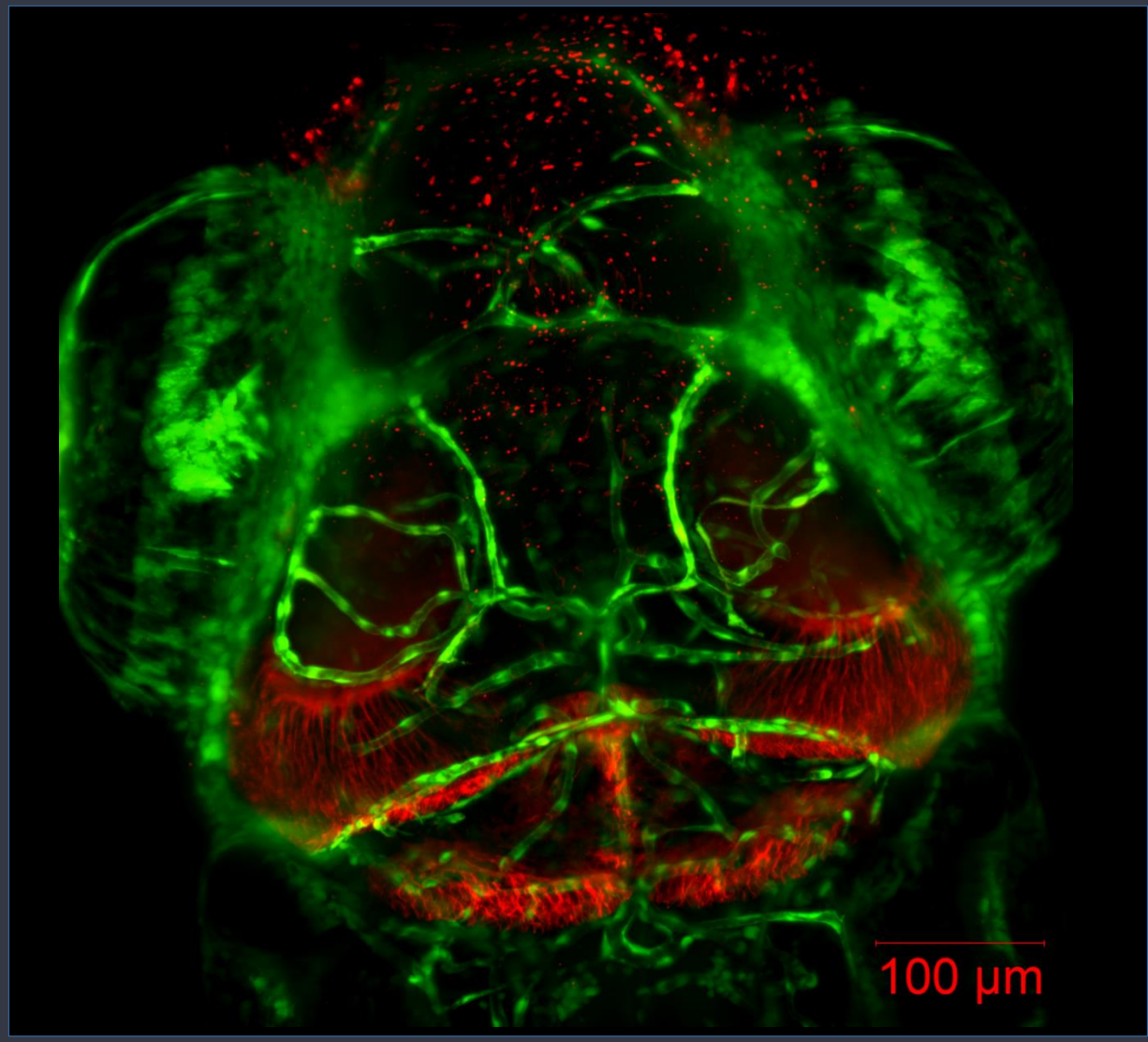
Zebrafish Head
Blood Vessels & Neurons

- Green: Blood vessel, Autofluorescence
  Red: neurons
- Views: 1

- Dual side illumination

- Objective: W Plan Apochromat 20x/1.0

- Sample by Dr. Cathleen Teh, IMCB, Singapore



Zebrafish Heart Development, 2d
Fast fluorescence imaging

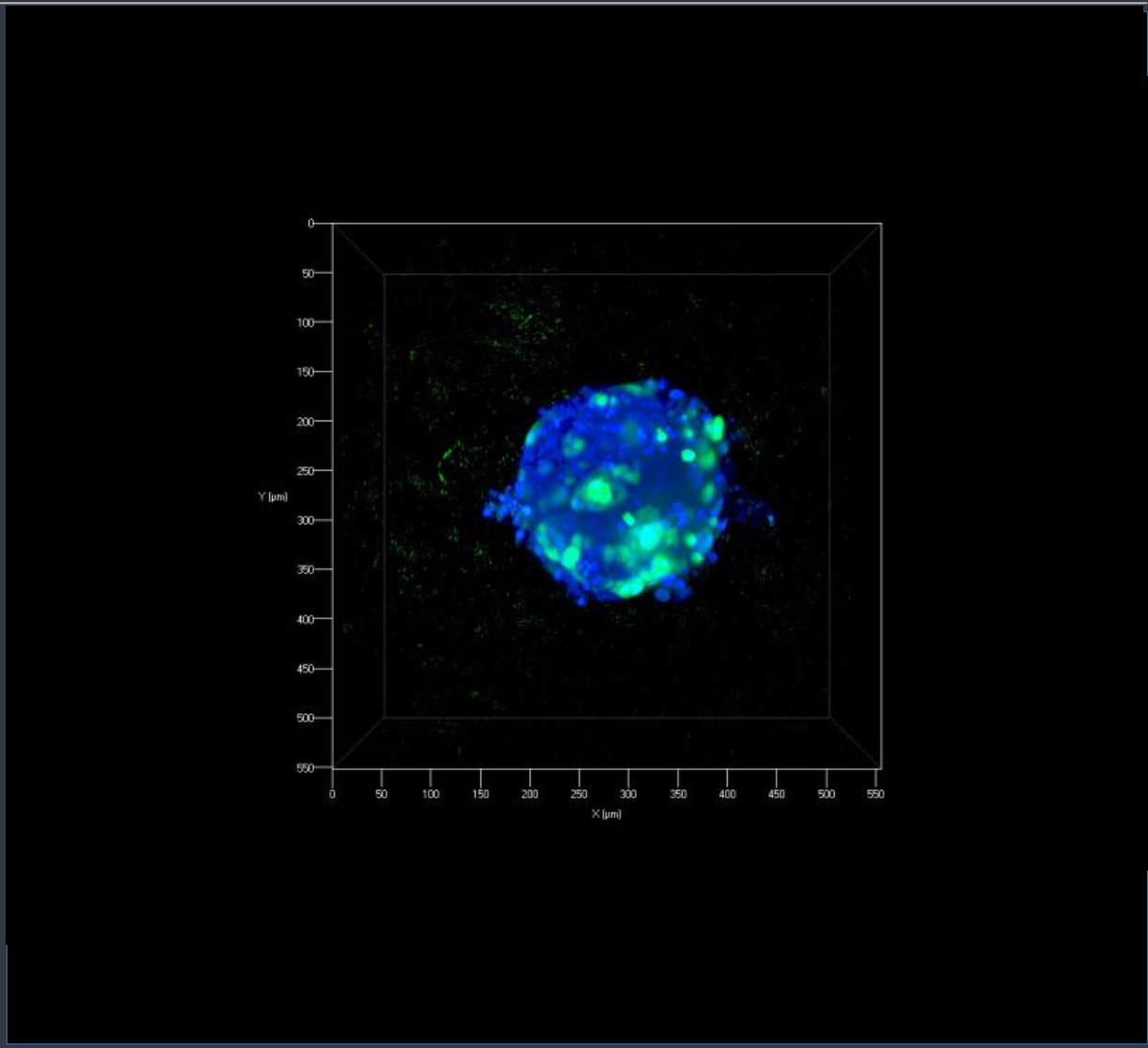
- Red: blood vessels, - endocardium
- Green : myocardium
- imaging:80 fps
- movie: **20 fps**

Imaging of beating heart with maximal frame rates (80 to 100 fps) for extended periods of time with minimal light exposure to the specimen.

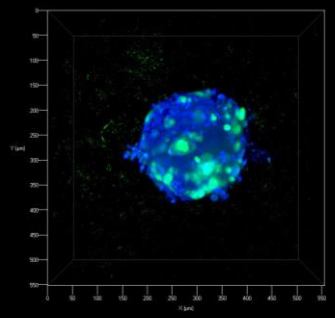
M. Mickoleit, M. Weber (Huisken Lab, MPI-CBG, Dresden, Germany)

Spheroid Imaging
Cell culture in three dimensions

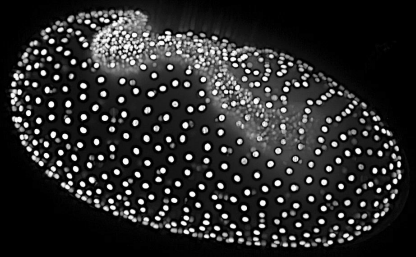
- HBE human derived cell lines cultured as micro-tissues (DAPI staining in blue; fixed with PFA)
- Infection with human Adenovirus type 2 expressing GFP
- Dual side illumination
- Views: 4 (90° rotation each)
- Landmark registration using fluorescent beads (small green dots surrounding the spheroid)
- Maximum Projection
- *Data by Vardan Andriasyan, Artur Yakimovich (University of Zürich, Switzerland)*



Lightsheet Z.1
The whole volume, every minute, for hours or days!



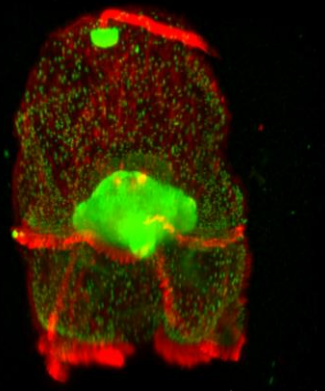
3D Cell Spheroids
(Vardan Andriasyan, Artur Yakimovich, University of Zürich, Switzerland)



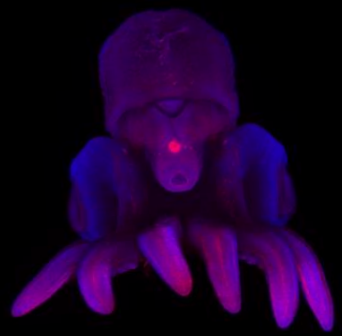
Tribolium castaneum
(Nipam Patel, University of California, Berkeley, Dept of Molecular & Cell Biology and Dept of Integrative Biology)

Aiptasia
(Annika Guse, COS Heidelberg, Germany)

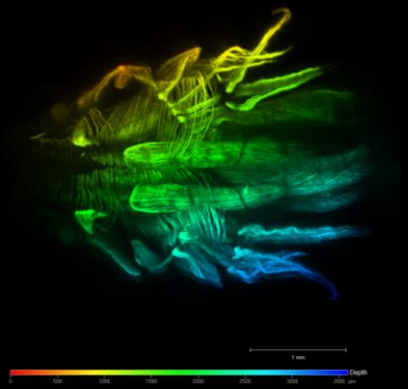
Drosophila melanogaster
(Ali Asgar Bohra & Prof. K Vijay Raghavan, National Center for Biological Sciences/NCBS, Bangalore, India)



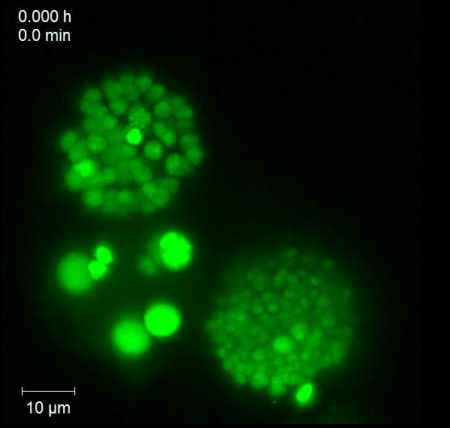
Nemertea
(Luis Bezares, Max Planck Institute for Developmental Biology, Germany)



Octopus bimaculoides
(Eric Edsinger & Daniel S. Rokhsar, Okinawa Institute of Science and Technology)



Ambystoma mexicanum
(Jennifer Schmidt & Prof. Dr. Lennart Olsson, Friedrich-Schiller-University, Jena, Germany)



Caenorhabditis elegans
*(*Dr Bruno Albuquerque, Institute of Molecular Biology, Mainz, Germany*)*

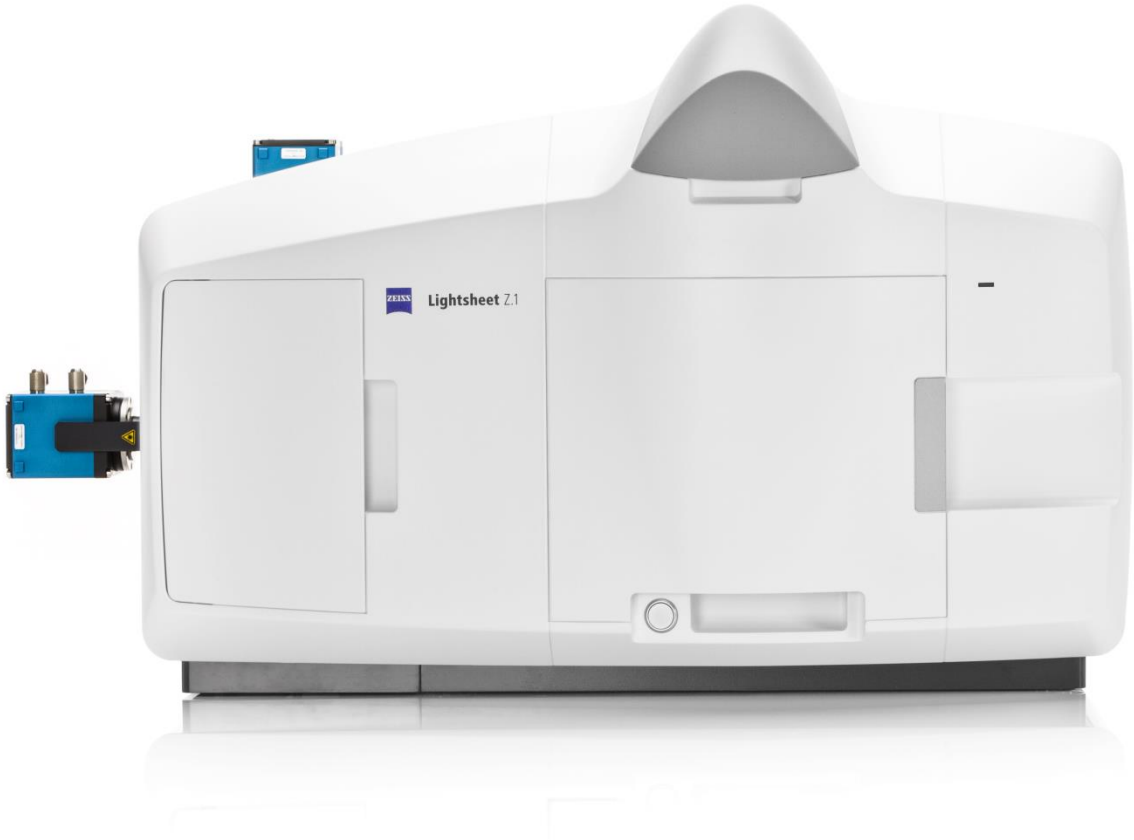
Good to remember
about the turn-key system Lightsheet Z.1

1. **Gentle:** Highest Sensitivity combined with virtually no photo-damage or photo-bleaching when performing long-term time-lapse imaging
2. **Fast:** Visualize dynamic processes with ultrafast optical sectioning
3. **Real life:** A special sample chamber to maintain the perfect environment for living specimens including heating, cooling, and CO_2
4. **Pick your viewing perspective(s)**: Best imaging possibilities for your specimen with multidirectional illumination and multiview imaging
5. **Stunning image quality:** Light sheet optics by ZEISS

The first microscope built around your sample

(and not vice versa).

Lightsheet Z.1
by ZEISS



We make it visible.