Light Sheet Fluorescence Microscopy
by Carl Zeiss

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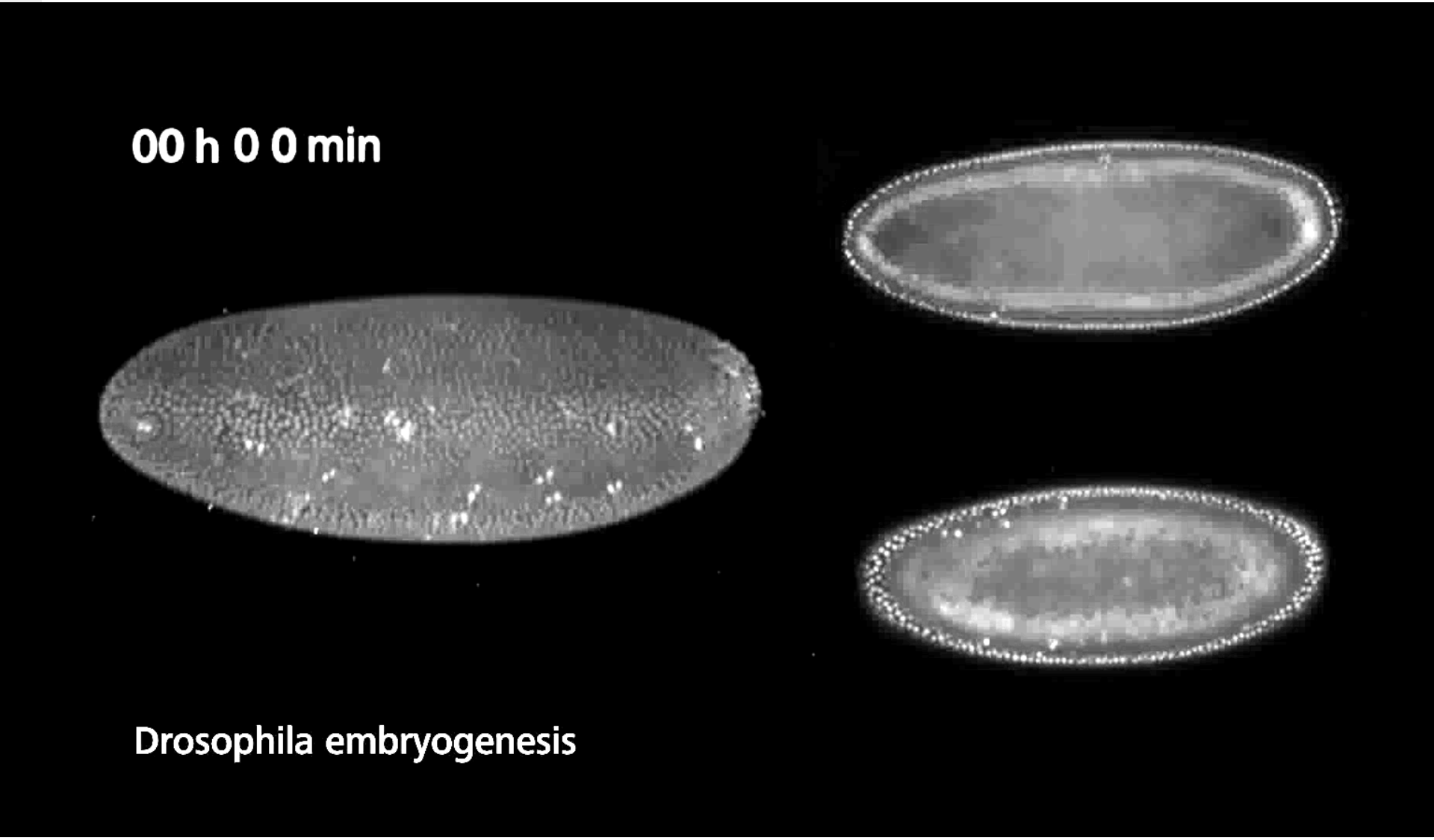
Dublin Workshop– 25/11/2013

Agenda

1. Requirements and challenges of live cell imaging
2. Light sheet fluorescence microscopy (LSFM) Technique
3. Samples and mounting
4. Applications
5. Hardware

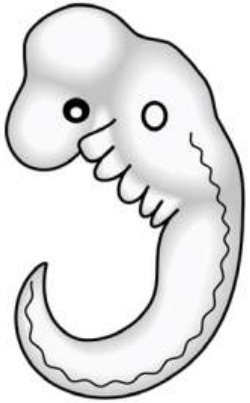
“Life is all about dynamic processes of complex multicellular organisms in a three-dimensional world.”

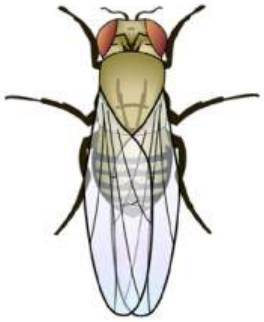


Imaging of living, multi-dimensional specimens
What are the challenges?

Challenges:

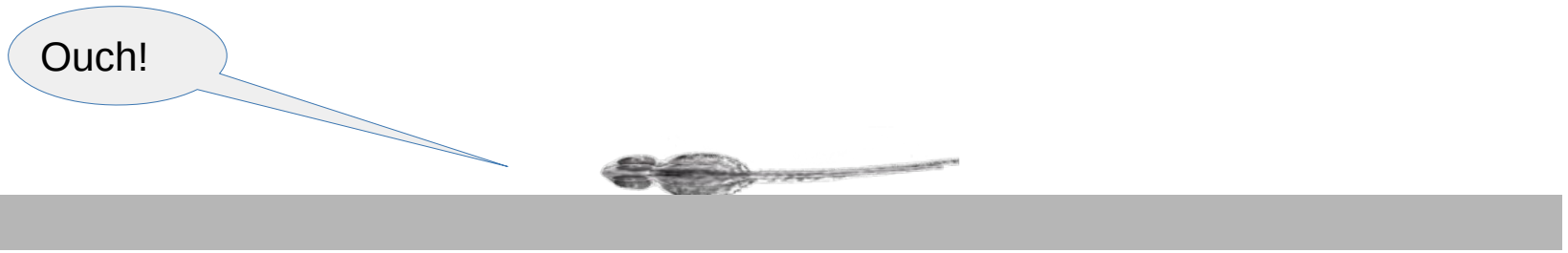
1. Bleaching and photo-damage
2. Capturing highly dynamic processes in 3D
3. Sample size
4. Sample positioning for viewing perspective
5. Out of focus fluorescence for optical sectioning





Challenges

A cover slip on a thicker, living sample flattens it.

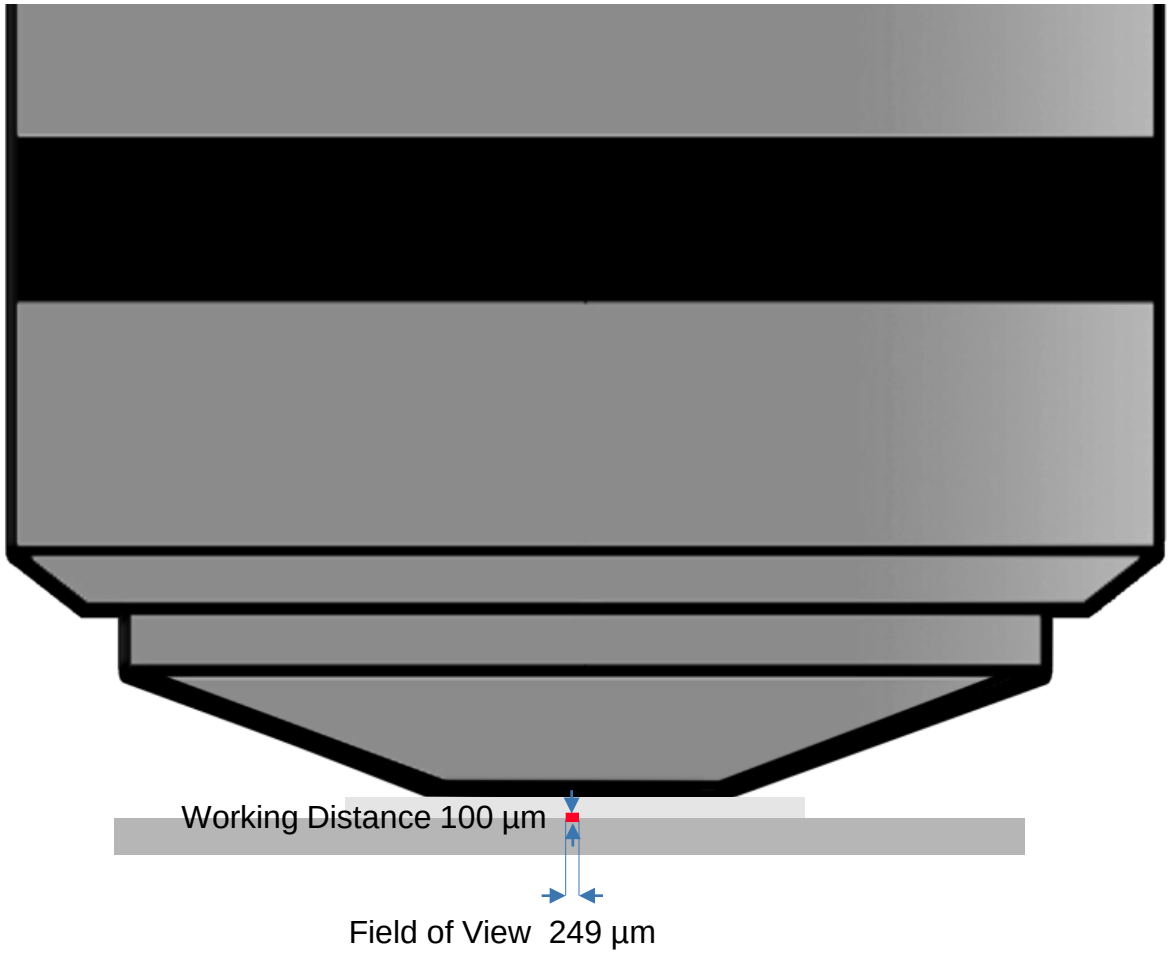


Challenges
Typical fluorescence experiments use an objective…

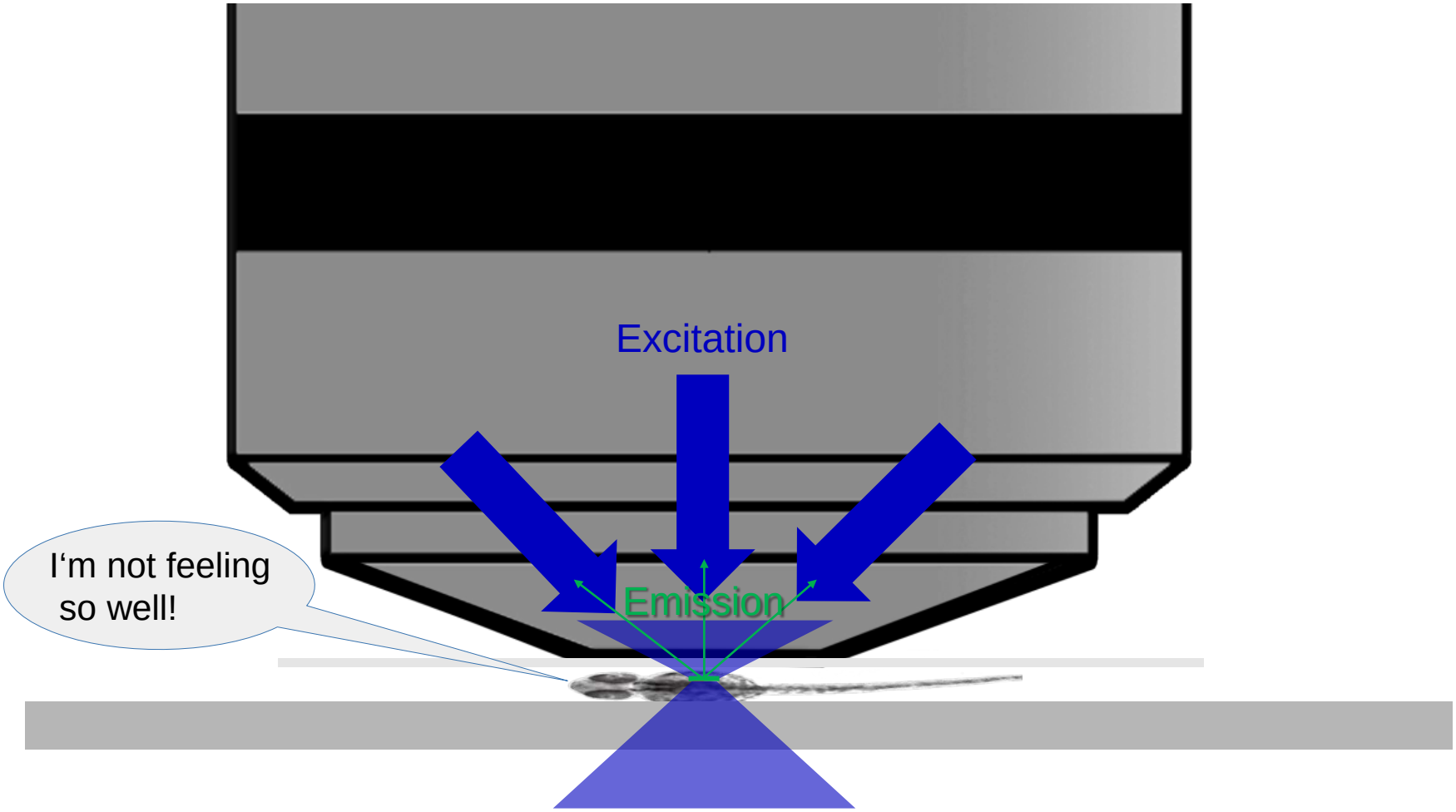
pupil Ø 24.9 mm

object field Ø 249 µm

Challenges
... to look at a small area of a flattened sample…



Challenges
leading to sample heating and photo-damage by imaging.



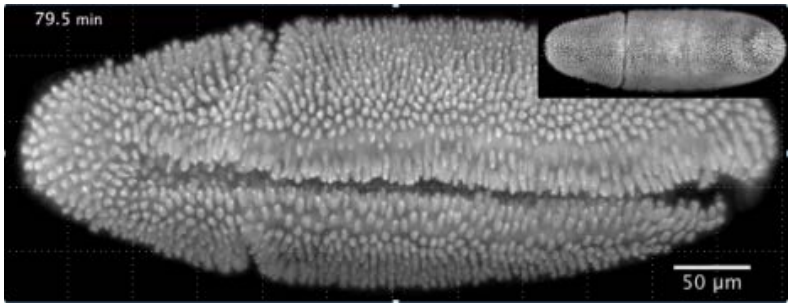
Live Cell Imaging of Large Specimens
What kind of microscope would you need?

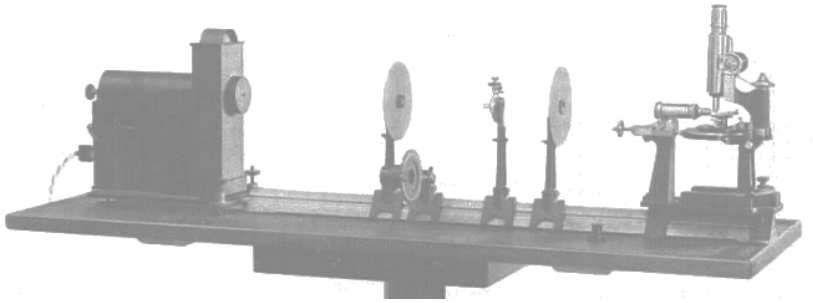
- Optical sectioning while minimizing photodamage
- Very sensitive fluorescence detection
- Image acquisition rate as fast as possible
- Imaging large volumes with high resolution and contrast

= Lightsheet Z.1 from Carl Zeiss

Light Sheet Fluorescence Microscopy (LSFM)
The evolution of the method

- **1903**: First Lightsheet by Siedentopf and Zsigmondy (Ultramicroscopy, Colloid Chemistry)
- **1964-1993**: Various Lightsheet Microscopy implementations, mostly for surface visualization, fluid dynamics, etc
- **2004**: Ground-breaking renaissance of the illumination technique by Huisken et al. (“SPIM”, MultiView, Live imaging in Developmental Biol.)
- **2005** commercialization announced by EMBL and Carl Zeiss
- **2005 – today**: a large number of innovative implementations of Light Sheet Fluorescence Microscopy for a wide range of applications

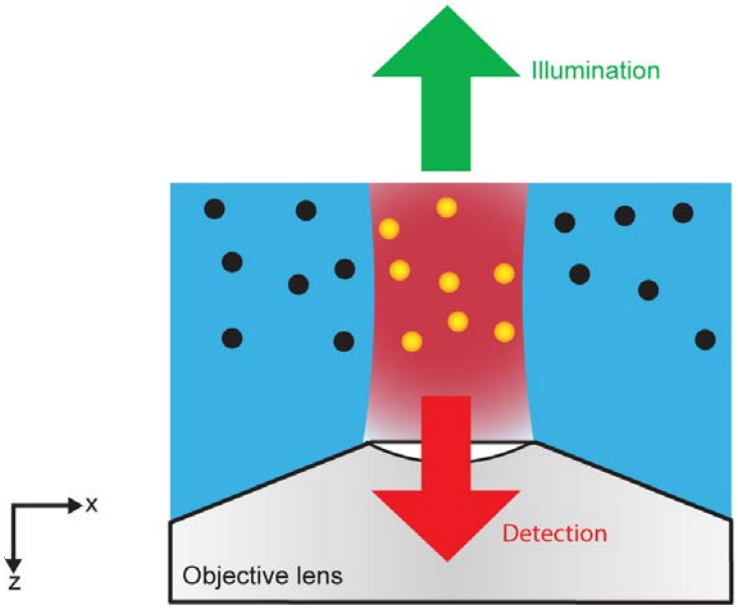




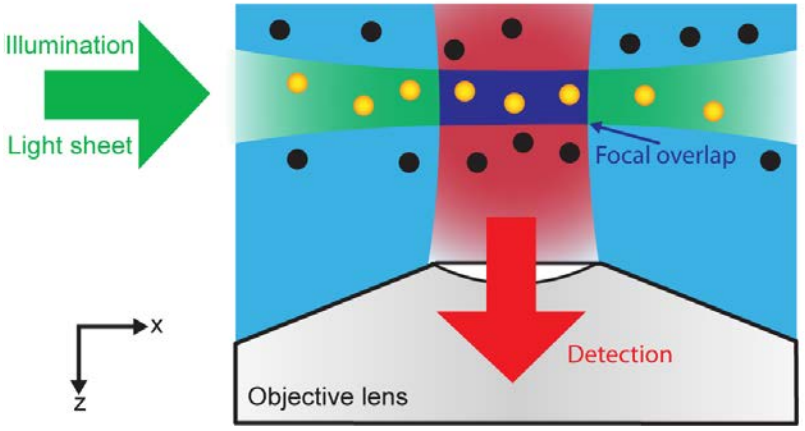
“Ultramikroskop”, 1903

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

Epi-Illumination



Light sheet illumination

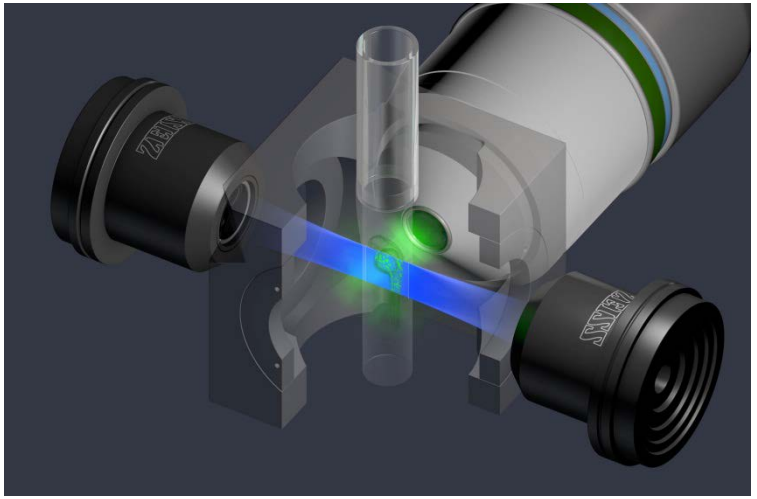


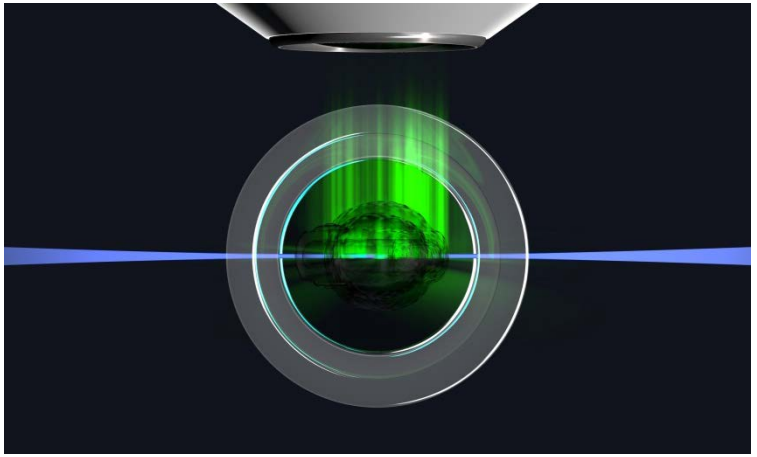
- Inherent optical sectioning capability of the illumination method
- No excitation of out-of-focus fluorescence

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

Light Sheet Fluorescence Microscopy
The principle

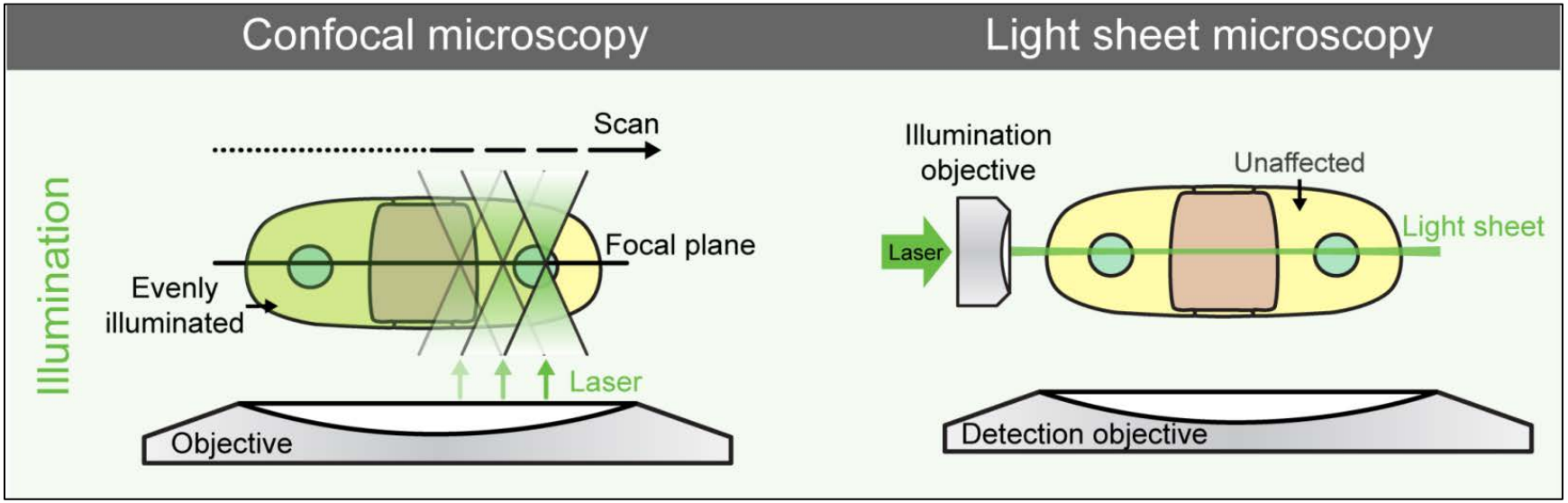
- Orthogonal light paths for Illumination and detection in a horizontal microscope
- Laser beam is shaped by a cylindrical lens
- Scanning mirrors move the beam along the focal plane (y-direction)





Lightsheet Z.1

The most gentle way of live 3D fluorescence imaging



Advantage of light sheet based fluorescence microscopes depends on ratio of object thickness over light sheet thickness !

Yeast →	3 – 6 x
Cells →	10 – 20 x
Drosophila M. →	100 – 150 x
Medaka / Zebrafish →	200 – 300 x

- Longer periods of observation !
- Less photo-damage
- + cameras ➔ up to 1000x less light exposure

Sample Mounting
Goodbye coverslips

Sample mounted vertically in hydrogel

- Translation & rotation: easy positioning, z-stacks & multiview
- Suspended in medium / buffer

Chamber for aqueous sample environment

- Physiological conditions
- Aqueous medium and minimized aberrations
- Compact and stable temperature control and incubation

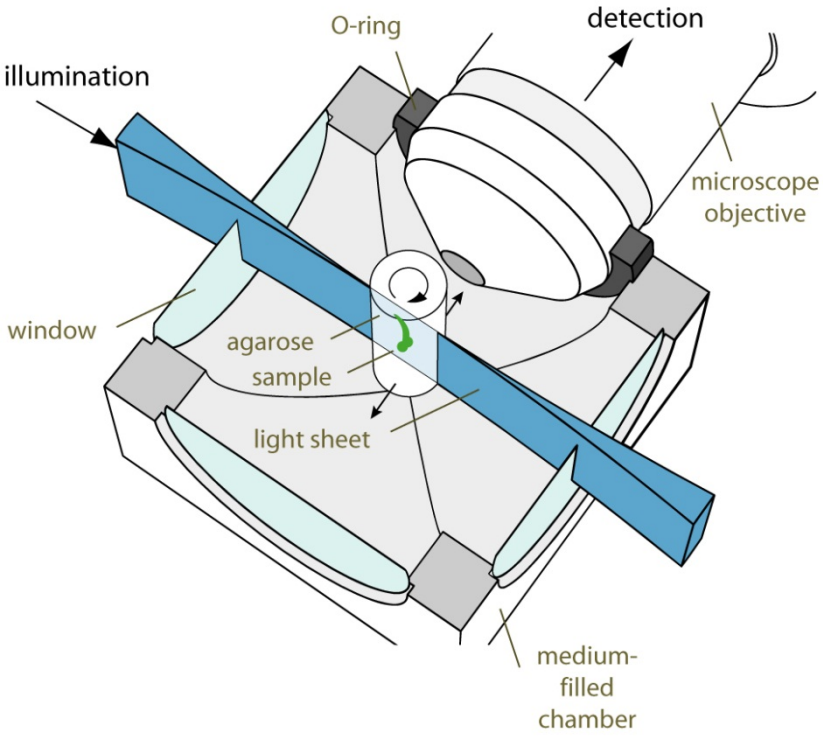


Figure: E.H.K. Stelzer, EMBL

Sample Mounting
Sample holder

- Suitable for large range of glass capillaries to mount specimen in hydrogel cylinders
- Precise yet easy to assemble
- Precision 3-point contact mount to insert and remove quickly from positioning motor

Sample Mounting
Sample chamber

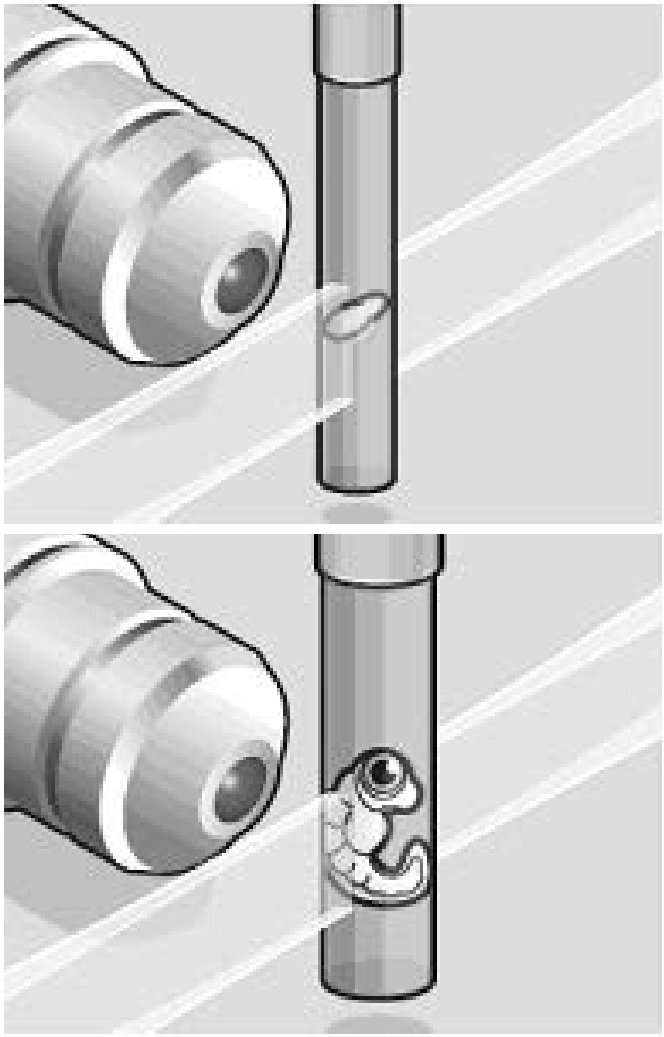
- Tubing connectors for medium change
- Precise yet easy to assemble coverslip windows
- O-ring sealing to allow the use dipping detection lenses
- Peltier-based heating and cooling block available

Sample Types and Preparation

- Ideal for medium-sized to **large living specimen** in aqueous solution!
- Easy switch from conventional

  slide to agarose embedding
- Fixed samples can be kept a few days at 4°C
- All necessary material present in any lab (chemicals)

  or part of the system (capillary, plunger etc.)
- Sample preparation chapter as part of the manual



Reynaud et al., HFSP J. (5) 2008

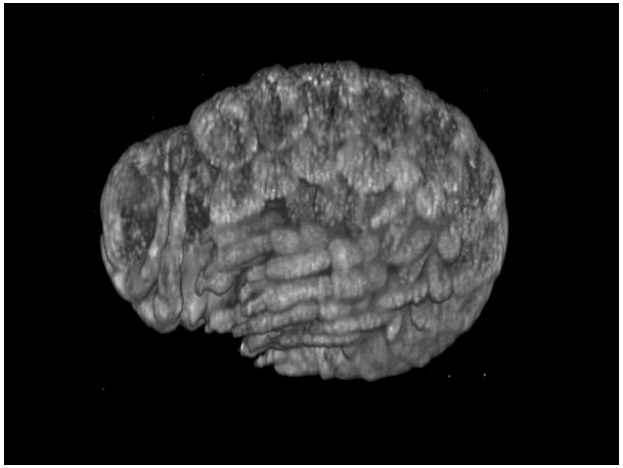
Any View: Rotation

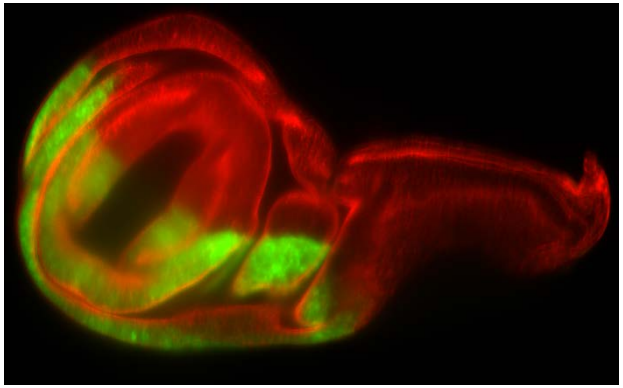
Rotateable specimen cylinder:

- Complementary information in different views (more info)
- Potentially improved resolution (depends on specimen)

Key applications today
what Lightsheet Z.1 is made for…

- **Morphogenesis and spatio-temporal patterns** of cells during embryogenesis of Drosophila, Zebrafish and other model organisms (“In toto” imaging)
- **Cellular dynamics** in embryos and small organisms
- **3D cell culture**, spheroids and cysts, tissue culture, organotypic cultures
- Fluorescence imaging of **marine organisms**
- Structural imaging of **larger (µm-mm) organisms**

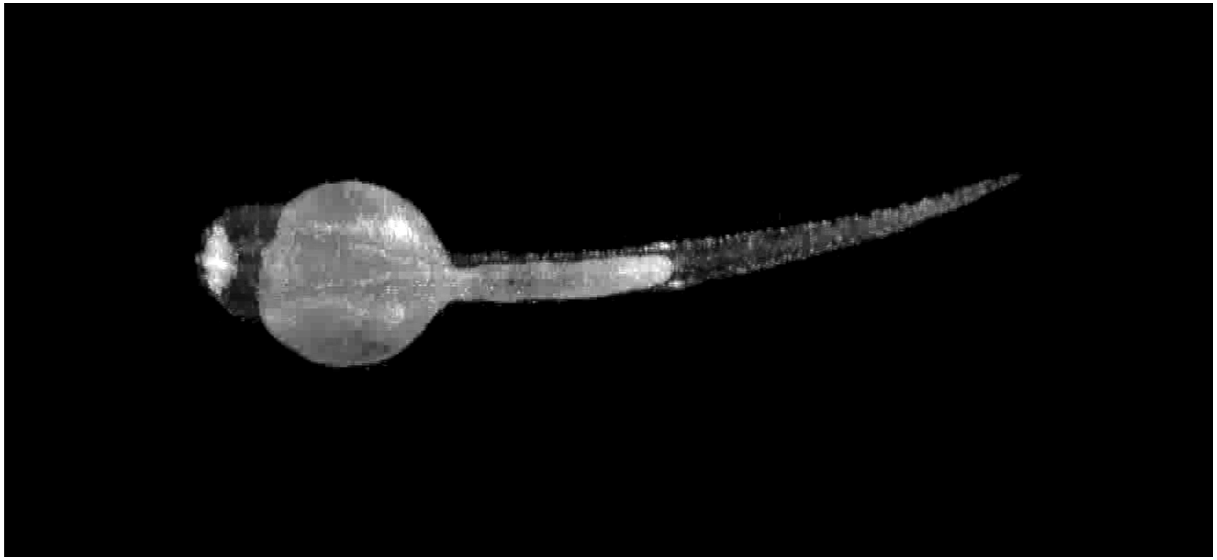




Data by A. Pavlopoulos and P. Tomancak from MPI-CBG Dresden, Germany

Zebrafish Development
Structural imaging of larger organisms

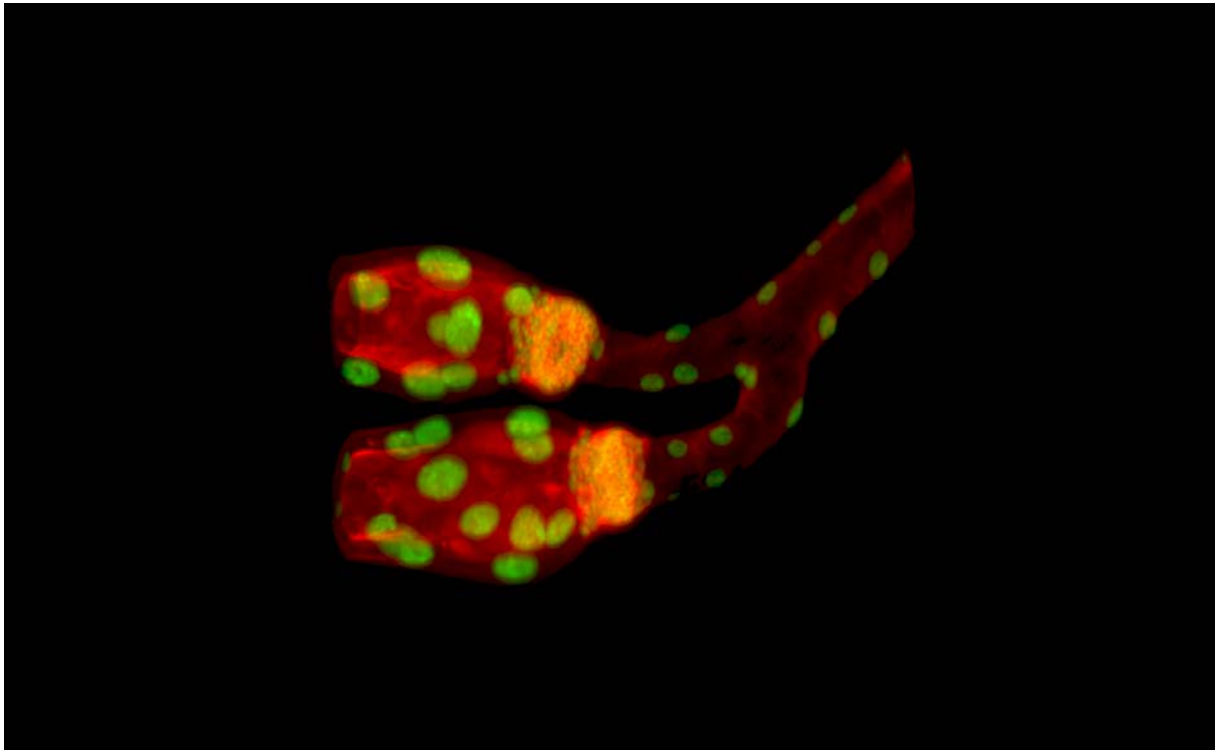
- Zebrafish, 2 day old embryo
- Views: 4
- Multiview registration and fusion
- Maximum Projection
- Tg(Bactin:H2A-mCherry) in the nuclei
- Yolk: autofluorescence



acquired using Carl Zeiss Light Sheet Fluorescence Microscope by M. Weber and J. Huisken (MPI-CBG Dresden, Germany)

Salivary Glands

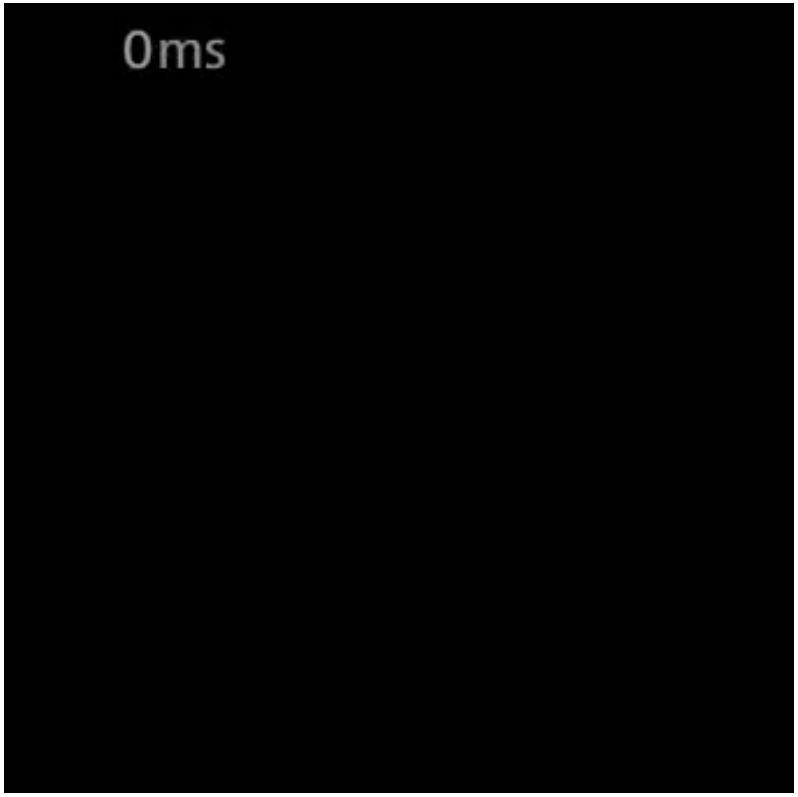
- Salivary glands of third instar larvae, *Drosophila melanogaster*
- Green: engrailed – GFP
  Red: actin labeling, phalloidin Alexa 561
- Views: 3
- Dual side illumination
- Objective: W Plan Apochromat 20x/ 1.0
- Data by A. Pavlopoulos and P. Tomancak from MPI-CBG Dresden, Germany



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

Zebrafish Heart Development
Fast fluorescence imaging

- Zebrafish heart of 2 day embryo
- Acquisition rate: 80 fps
- Movie: **20 fps**
- Red label: blood vessels, endocardium
- Green label: myocardium
- Light sheet fluorescence microscopy allows to image the beating heart with maximal frame rates (80 to 100 fps) for extended periods of time with minimal light exposure of the specimen.

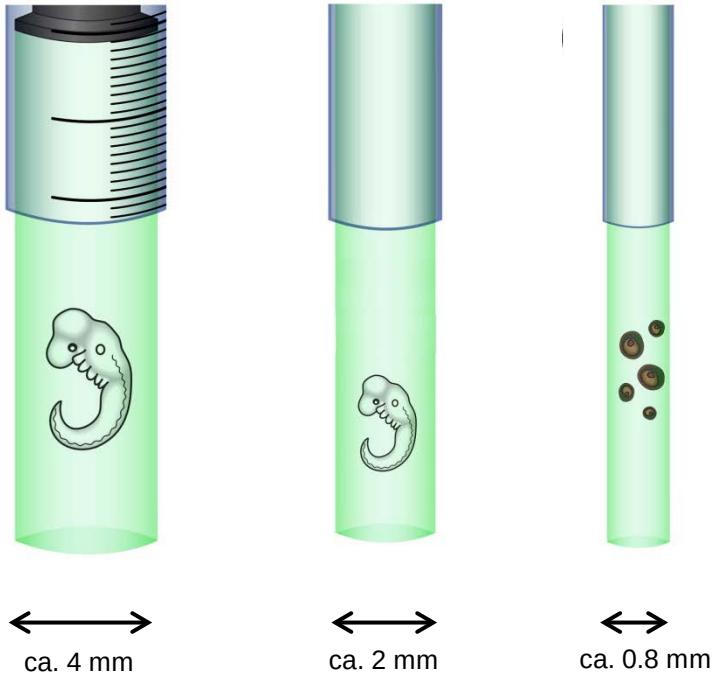


acquired using Carl Zeiss Light Sheet Fluorescence Microscope by M. Weber and J. Huisken (MPI-CBG Dresden, Germany)

Lightsheet Z.1

Introducing your model organism into our microscope

Sample Types and Preparation

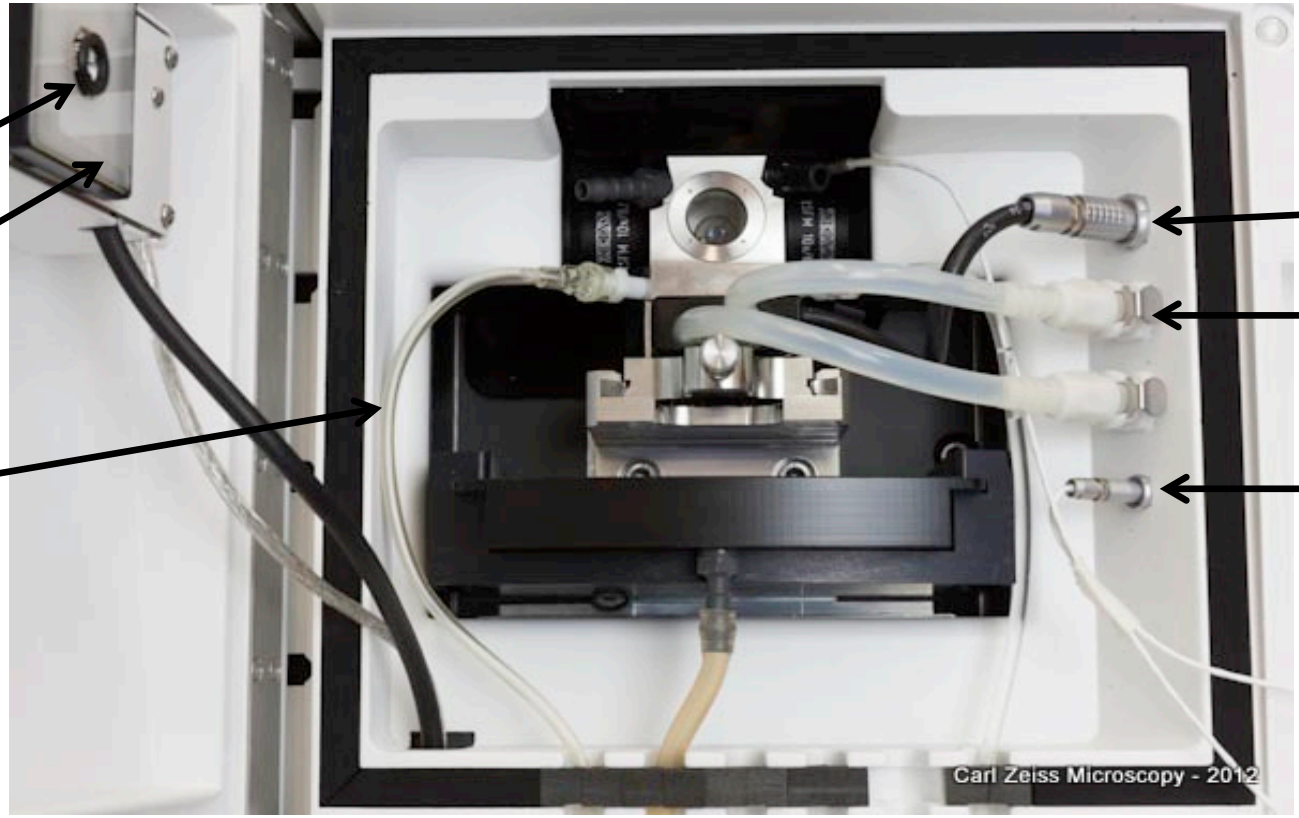


Ideal for medium-sized to **large living specimen** in aqueous solution!

- Easy switch from conventional slide to agarose embedding
- Sample prep Starter Kit is part of the system
- Additional material is common lab equipment
- Sample preparation chapter as part or the manual

Microscope Interior
System cavity

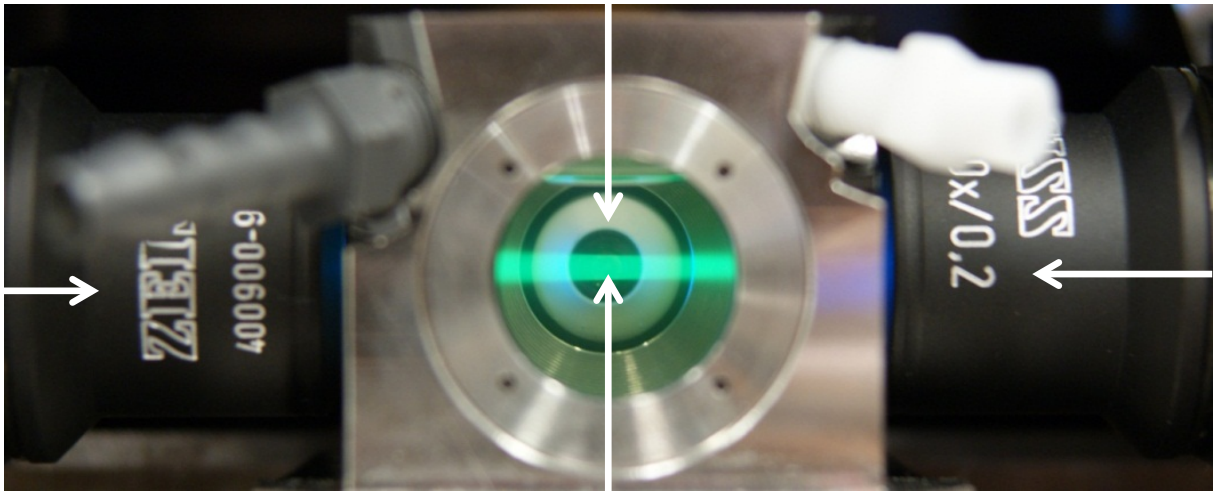
The system cavity for accessing the sample chamber with incubation, the detection optics and the illumination optics is located behind the front system door



Lightsheet Z.1
Sample Chamber Cavity

Detection
Objective

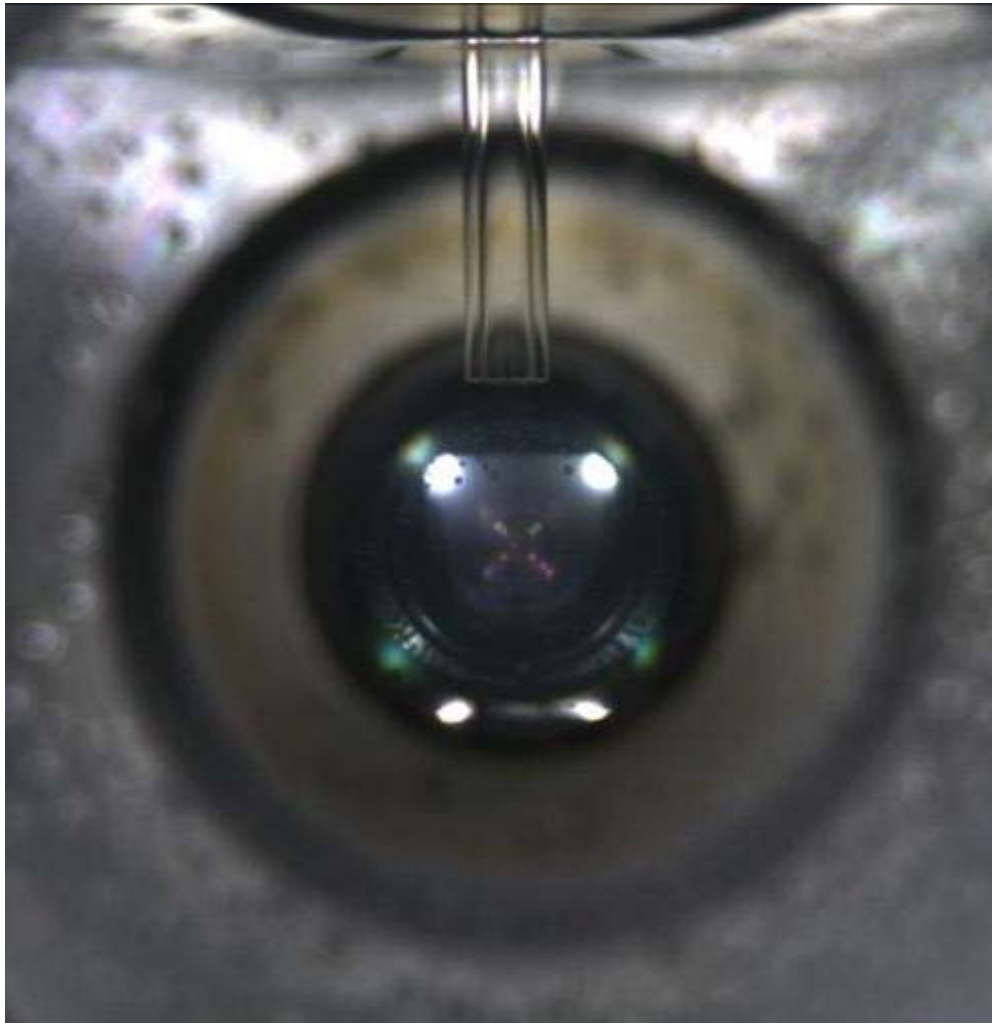
Illumination
Optic

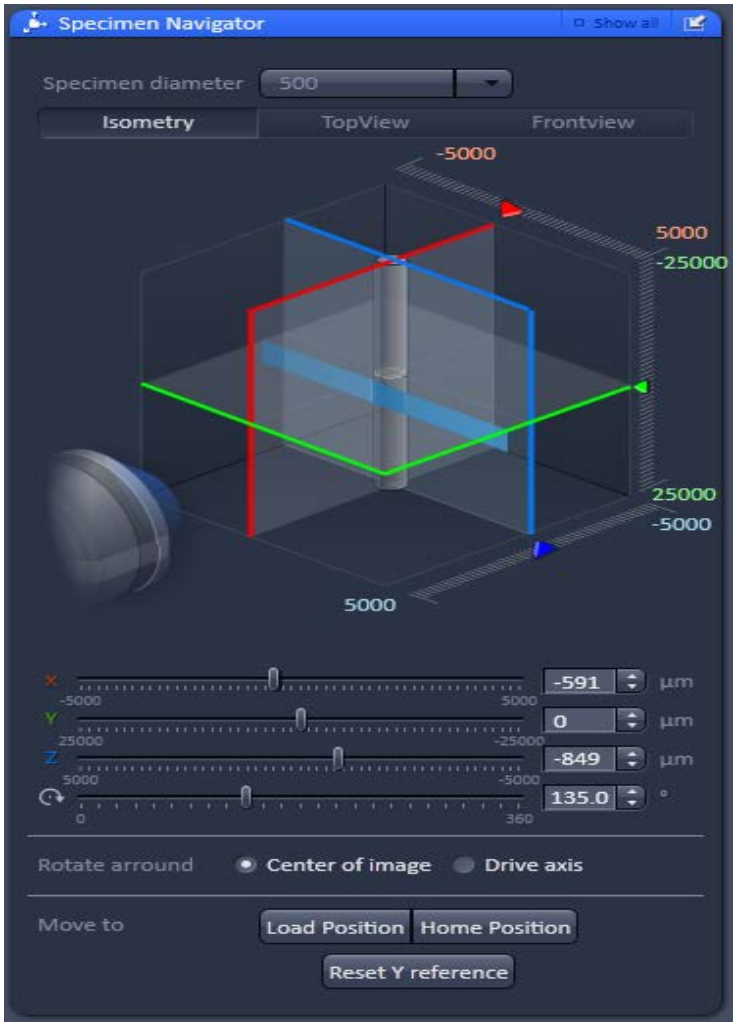


Illumination
Optic

Scanned
Light sheet

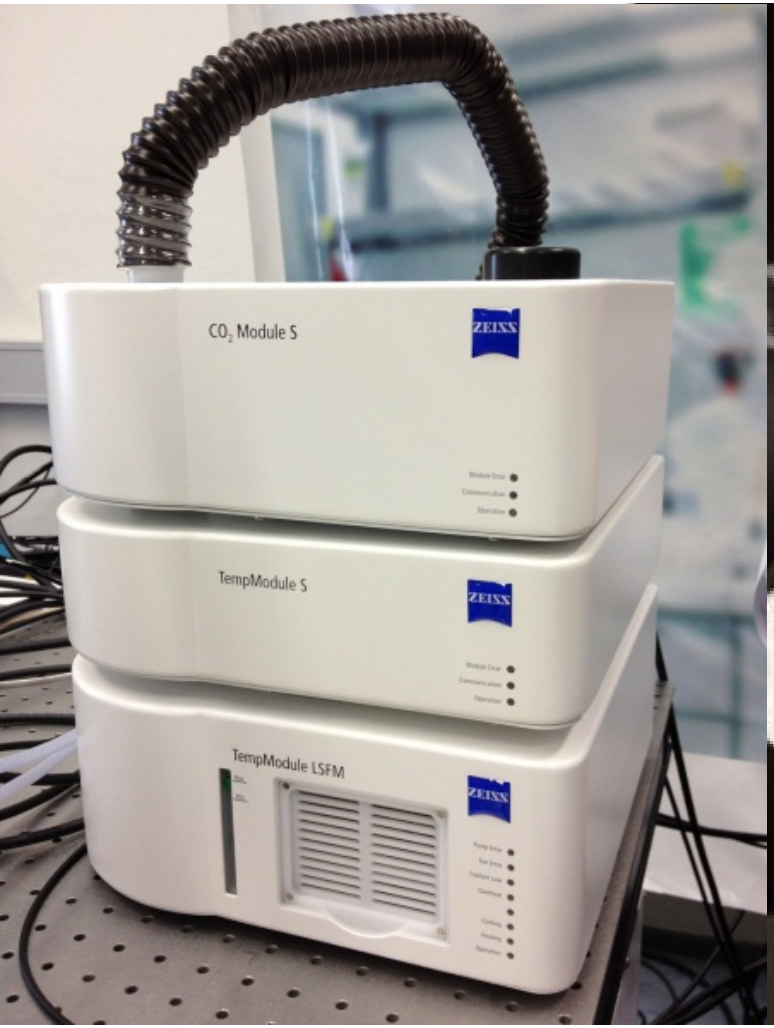
Lightsheet Z.1
Bringing your sample into focus

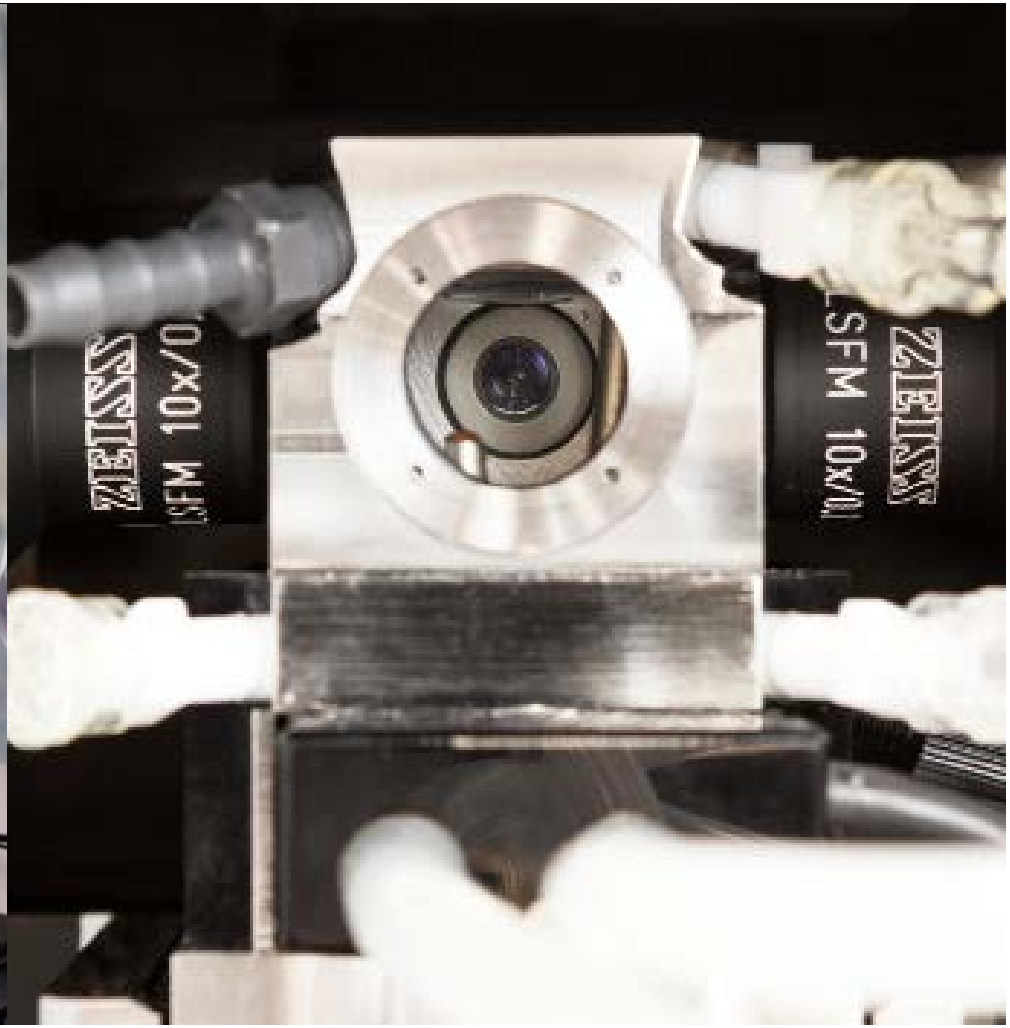




! Key feature: Environmental control

Keep your sample happy under physiological conditions





Why Do We Need Multidimensional Illumination?
Stripes, shadows and other limitations

Simple one sided illumination in light sheet fluorescence microscopy yields to different issues:

Absorption and scattering artifacts

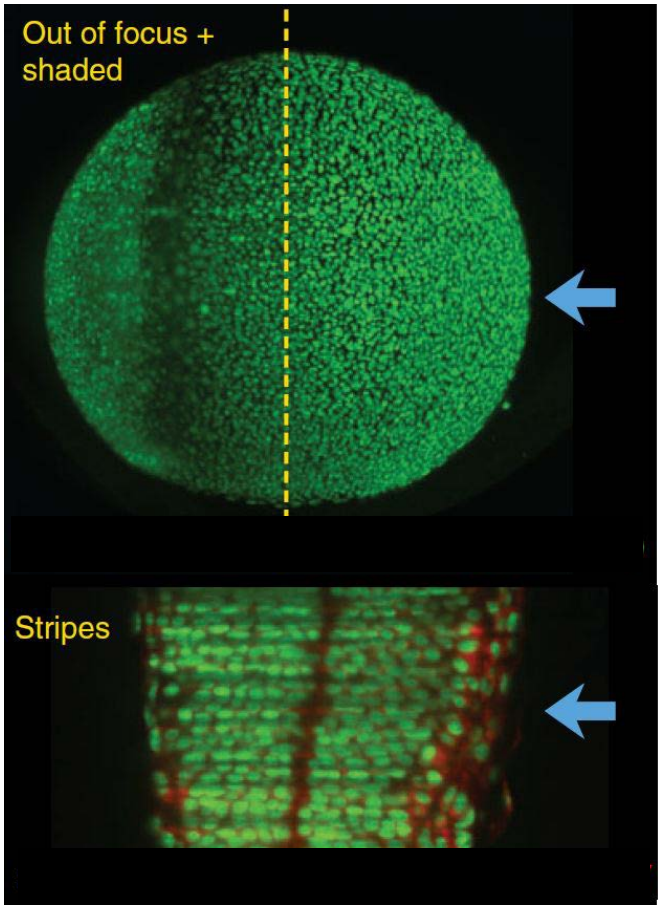
- depending on how long the light has to travel inside the sample and on the optical density of the sample, the light sheet and image quality decreases (dimmer, image blur)

Stripe artifacts (“Shadows”)

- Light sheet is “patterned” by granularity of the sample
- depending sample properties and NA of illumination

Anisotropy of resolution

- depending on sample orientation in relation to detection optics

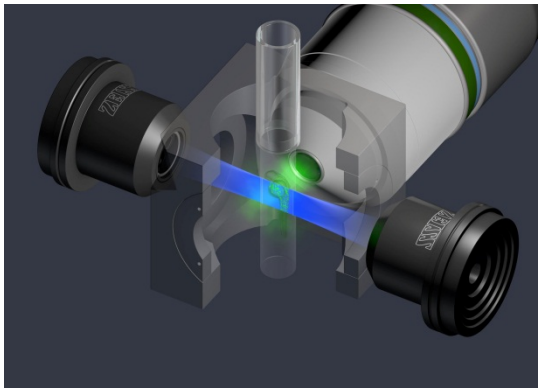


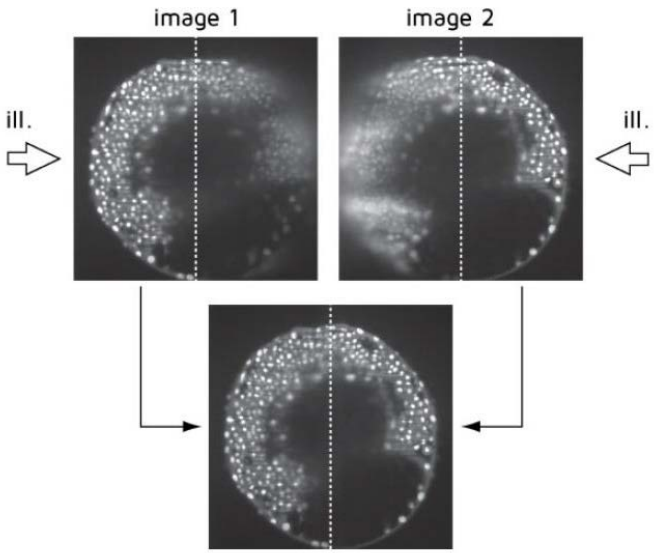
Modified from Huisken and Stainier, Devel. 2009

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 sequentially

- Scattering and absorption of excitation light largely compensated
- reduced anisotropy in x/y-dimension
- much higher penetration depth in thick specimen
- increased acquisition speed



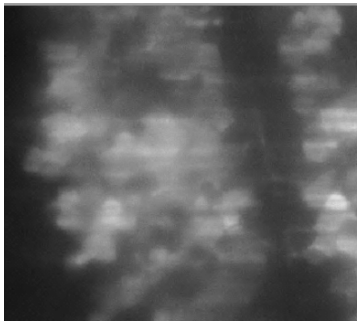


Adapted from Huisken, Bioessays, 2012

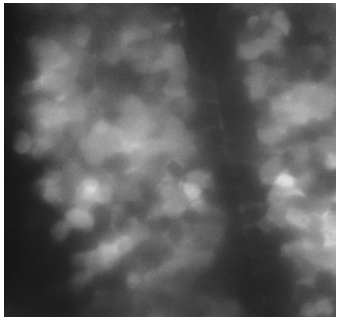
Pivot Scan
Shadow reduction

Any “obstacle” (absorbing or scattering) in the beam path which is hit by a light sheet will cast a shadow along the direction of illumination:

- Pivot scan can eliminate these shadows.
- 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”.



Without Pivot scan

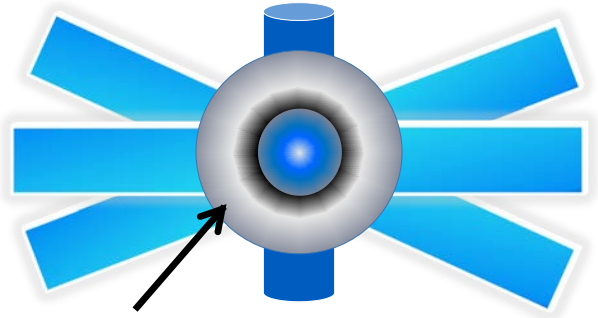


With Pivot scan

Lightsheet position: Pivot Scan 2

Lightsheet position: Pivot Scan 1

Lightsheet position: Pivot Scan 3

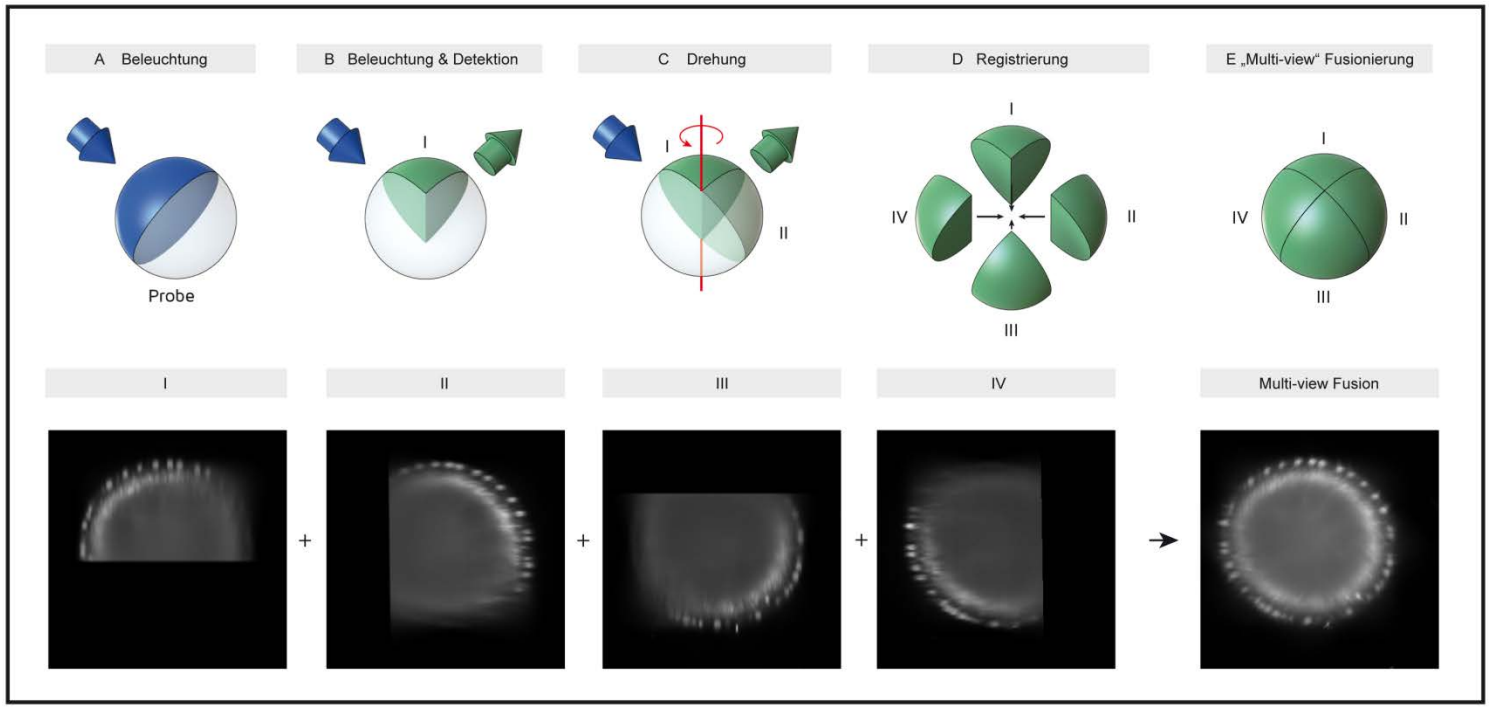


Adapted from Huisken and Stainier, Devel. 2009

Lightsheet Z.1 Key Feature:

Multiview Imaging

Multiview Imaging: Sequential acquisition of multiple stacks of optical sections from different directions. In LSFM they are usually taken from different rotation angles.



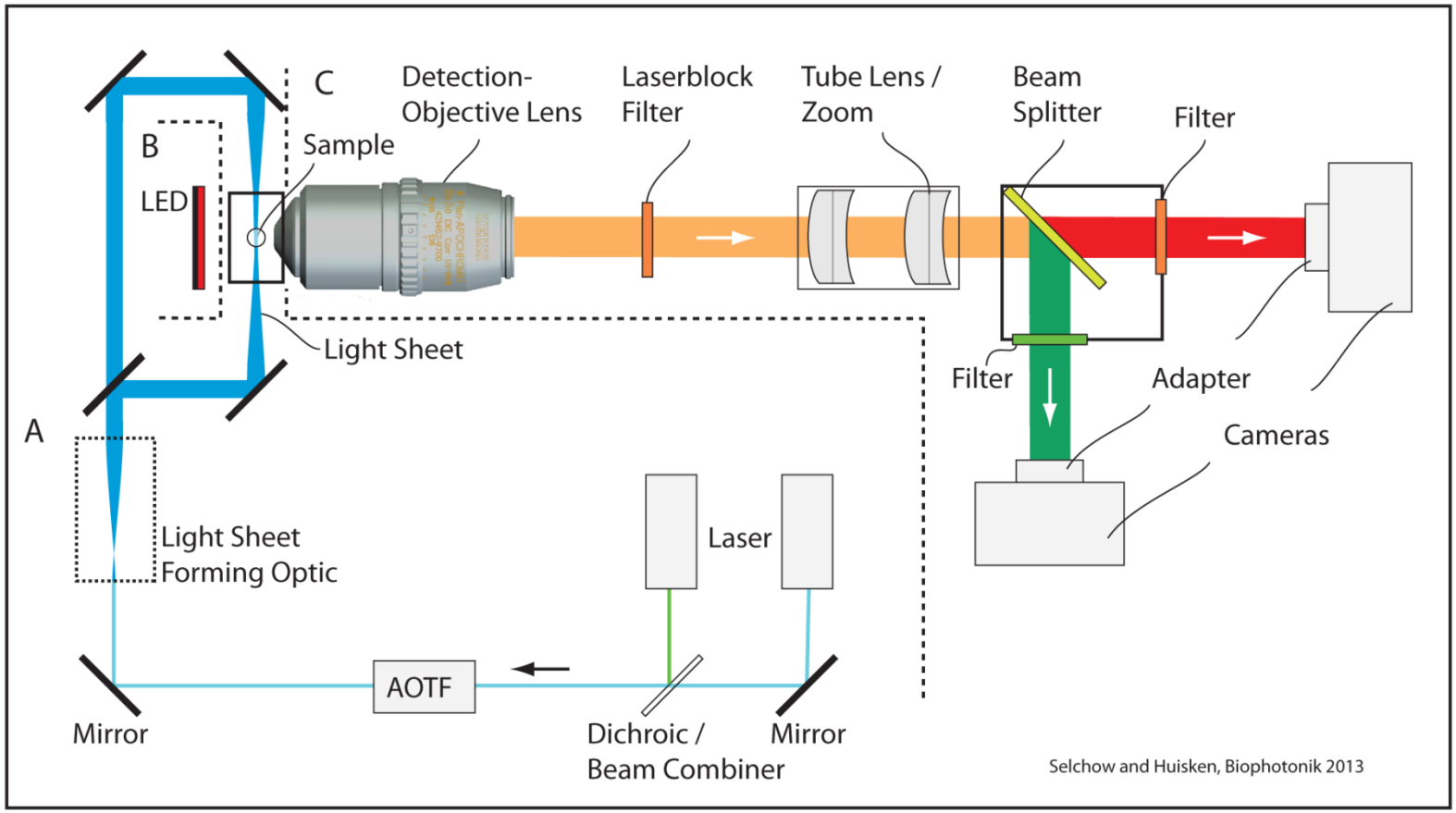
Selchow and Huisken
Biophotonik, 2013

Benefit:

- Complementary information in different views (more info)
- Potentially improved resolution (depends on specimen)

Lightsheet Z.1

Light Sheet Fluorescence Microscopy by Carl Zeiss



Lightsheet Z.1
Laser

Examples of Samples:

Antibody staining
Dyes

High FP expression
High QE (eGFP, eYFP)

Medium FP expression
High QE (eGFP, eYFP)

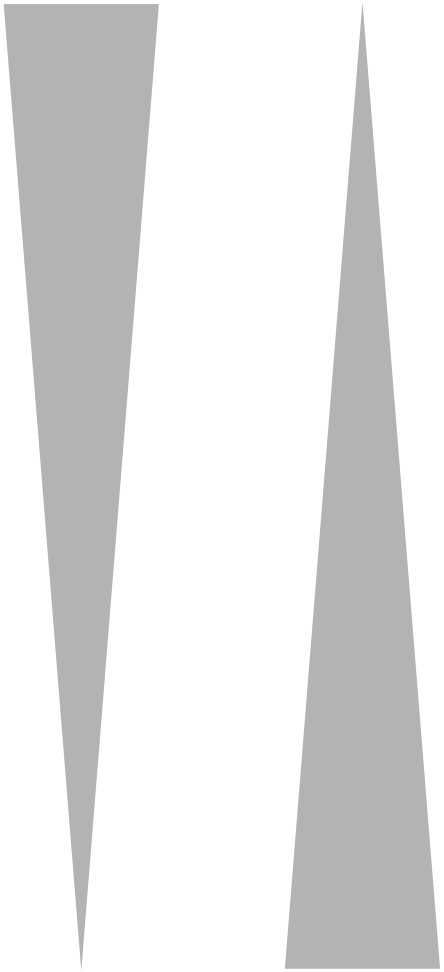
Medium FP expression
Medium QE (mCherry, Cerulean, RFP)

Medium FP expression
Low QE (CFP, BFP)

Low FP expression
physiological levels

Brightness

Laserpower



Laser	Power*
405	20 mW
405	50 mW
445	25 mW
445	40 mW
488	30 mW
491	50 mW
491	100 mW
515	20 mW
515	50 mW
561	20 mW
561	50 mW
561	75 mW
638	75 mW

* Typical laser fiber out ± 5 %

Lightsheet Z.1
Illumination Optics and Detection Objectives

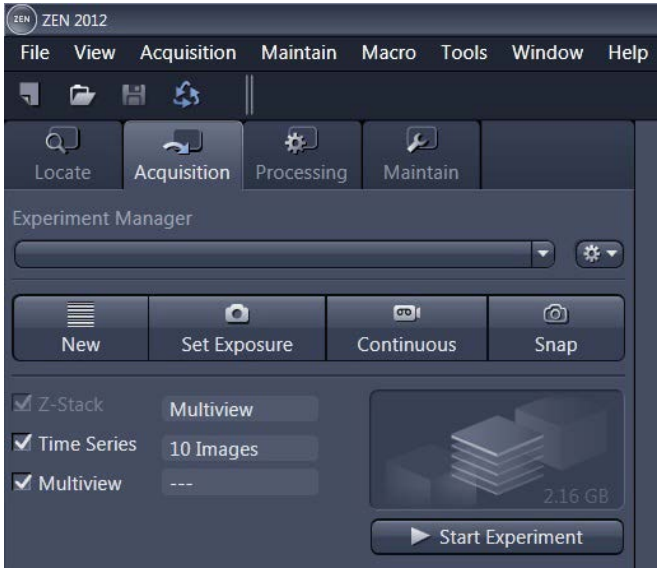
- Illumination optics and detection objective lenses covering the range of FOVs from 10s of µm to 2 or 3 mm
- **Additional zoom optics** → Adjustment of magnification and FOV

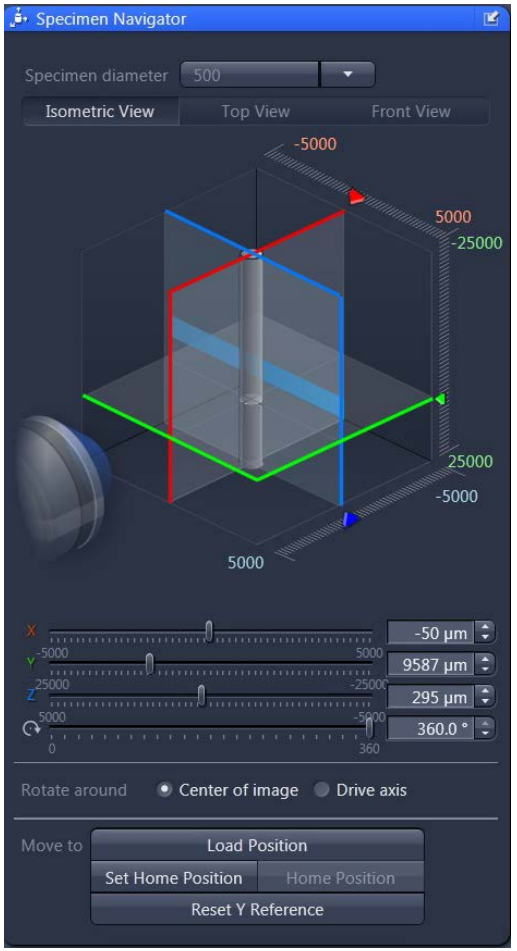
Detection Modules for Lightsheet Z.1
General informations

- CCD and CMOS detection technology are supported
- Detection modules are carefully integrated in the trigger-scheme of the system to ensure optimal performance and speed
- Detection modules come mounted and precisely adjusted on quick-swap adapters to ensure a pixel-precise alignment for dual camera applications

Lightsheet Z.1
Easy to use and learn

- Full Integration into ZEN 2012
- Cross platform compatibility from stereomicroscopes to superresolution microscopes
- New easy to use tools specific to Lightsheet Z.1





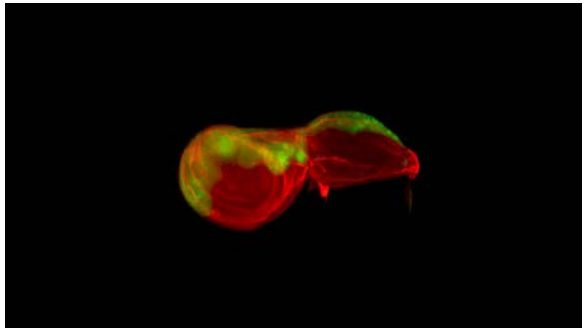
Good to remember
about the turn-key system Lightsheet Z.1

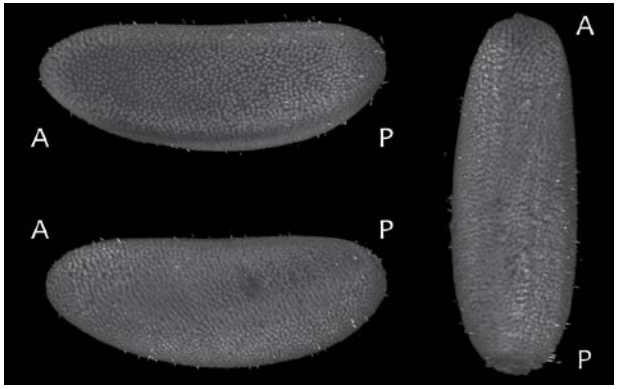
1. **Real life:** A special sample chamber to maintain the perfect environment for living specimens including heating, cooling, and CO_2
2. **Pick your viewing perspective(s)**: Best imaging possibilities for your specimen with multidirectional illumination and multiview imaging
3. **Stunning image quality:**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

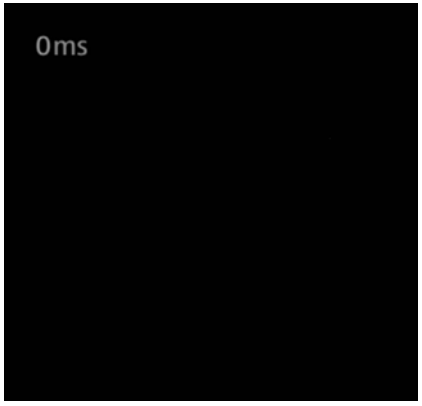
The first microscope built around your sample (and not vice versa).

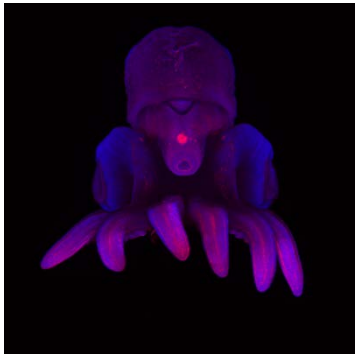
Taking Life Imaging to New Dimensions:

Lightsheet Z.1 opens a new Dimension for your Experiments

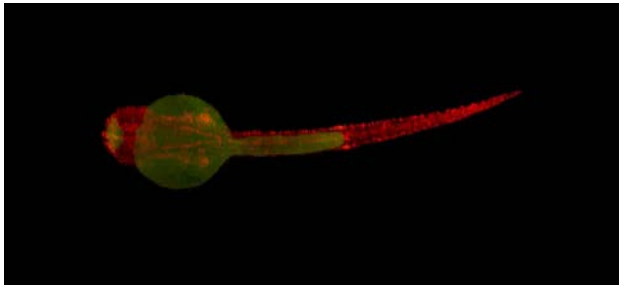




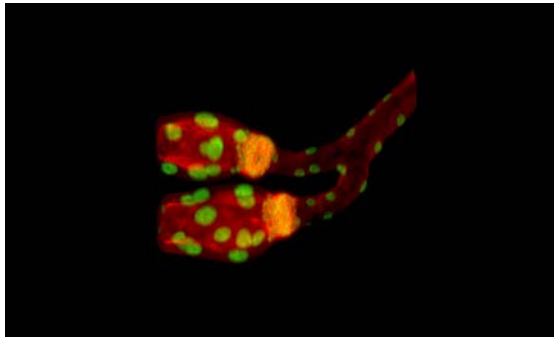




- Drosophila Melanogaster Wing Disc
- Drosophila Melanogaster Embryo – csp staining
- Drosophila Melanogaster Embryo – HIS- YFP
- Zebrafish heartbeat – 80 fps movie, 2 channels
- *Octopus bimaculoides* 1 month Alexa 546 phalloidin (actin/muscle) ToPro3-Alexa 642 (DNA)
- Pharhyale hawaiensis, live embryo
- Whole Zebrafish Embryo – 2 day old
- Drosophila Melanogaster - Salivary Glands



- **The whole volume**
- **Every few minutes**
- **For hours and days**



We make it visible.