Lightsheet Z.1

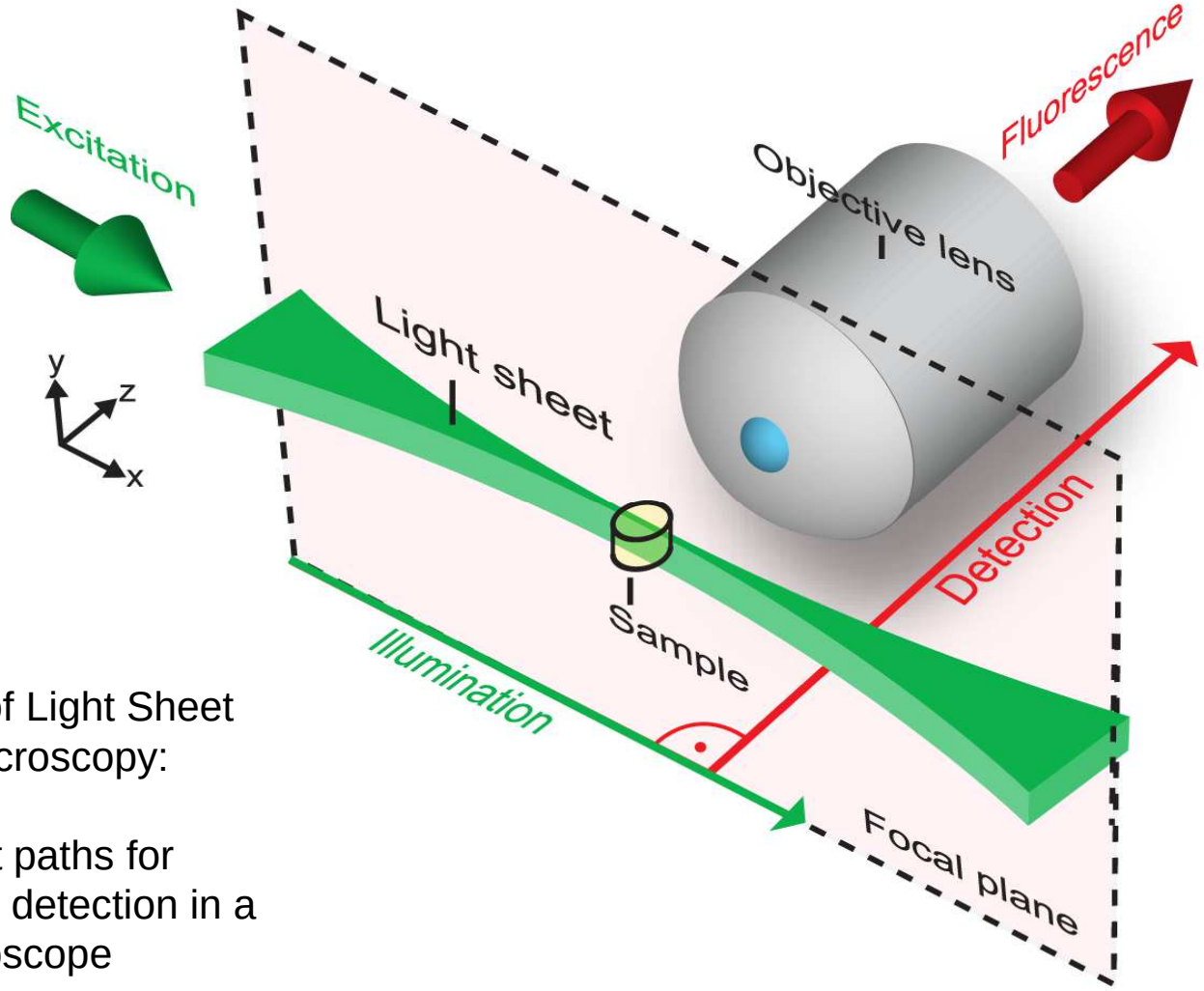
Light Sheet Fluorescence Microscopy by Carl Zeiss

Fabrice Schmitt, Sales Manager Carl ZEISS France

12.12.2012

Light Sheet Fluorescence Microscopy (LSFM)

Principle



The **Principle** of Light Sheet Fluoresence Microscopy:

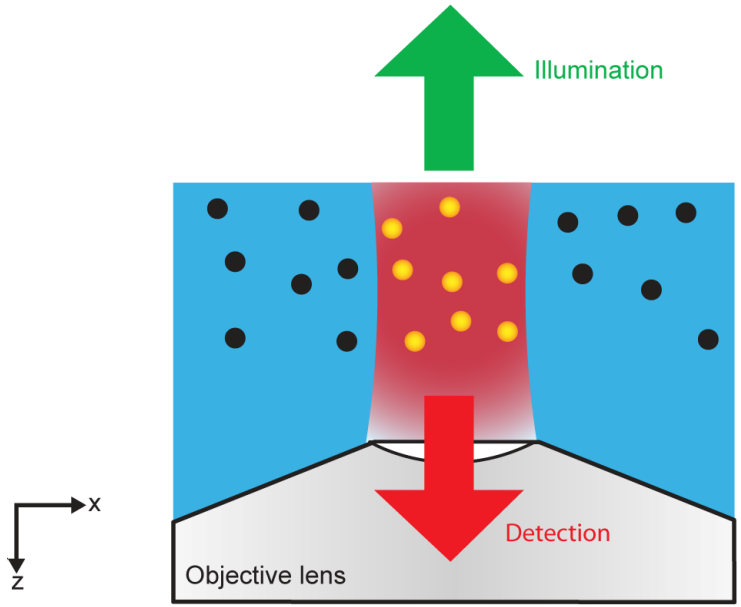
Orthogonal light paths for Illumination and detection in a horizontal microscope

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

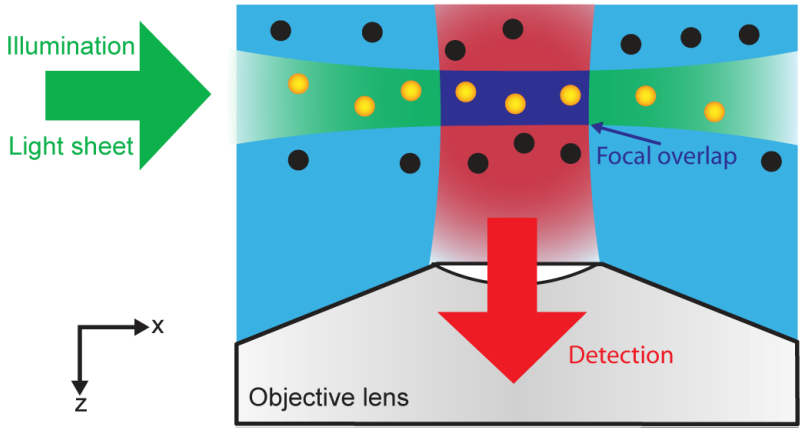
Light Sheet Fluorescence Microscopy (LSFM)

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)

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Light Sheet Fluorescence Microscopy by Carl Zeiss

Real Life in a Horizontal Microscope

Mounting Samples will change – for the better:
Unique Sample Chamber, Sample Holder and Microscope design

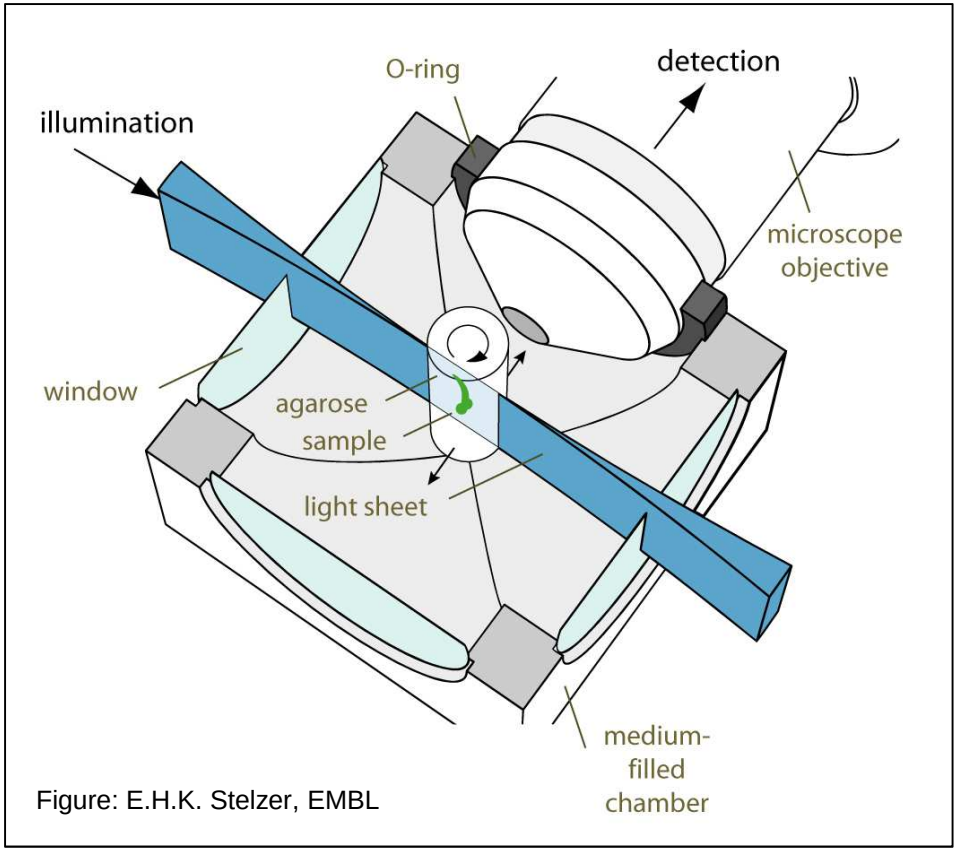

Figure: E.H.K. Stelzer, EMBL

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

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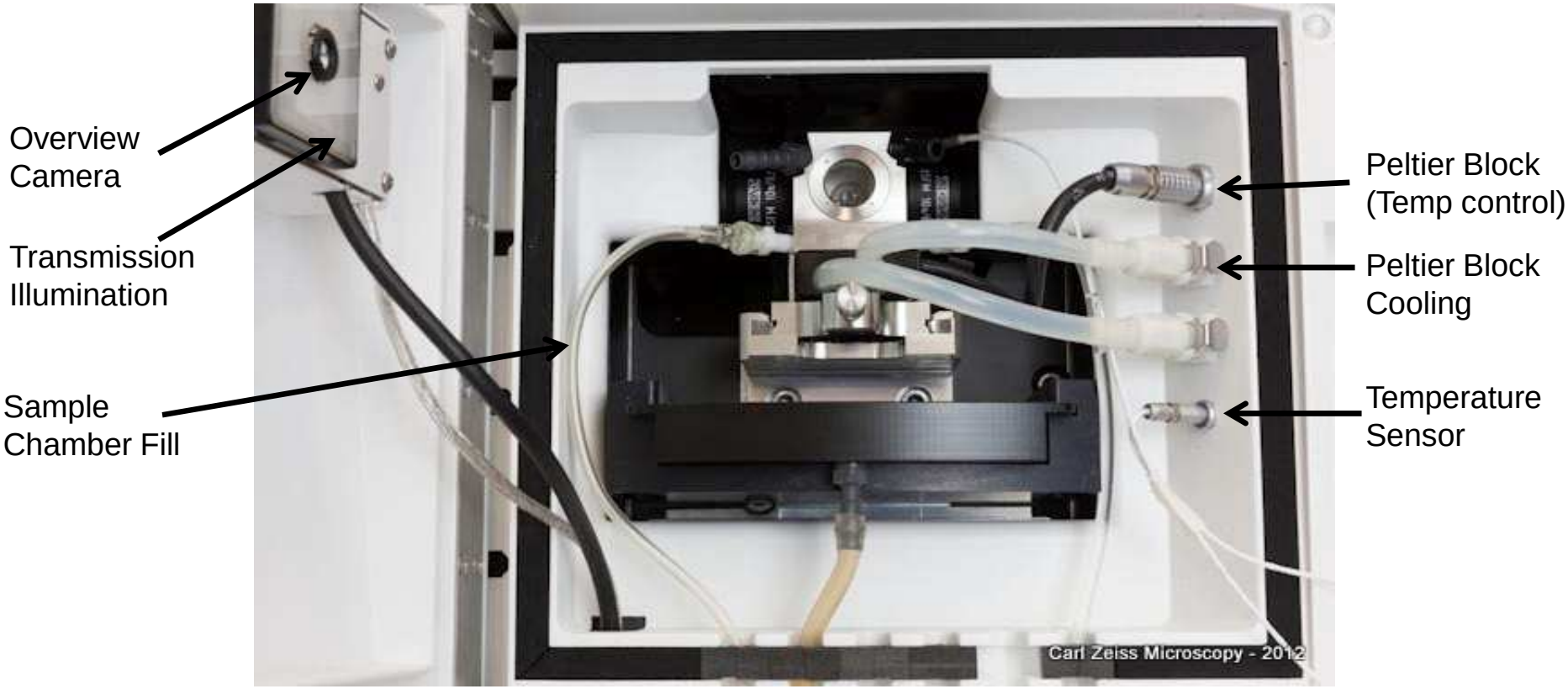
Microscope Interior: System cavity

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



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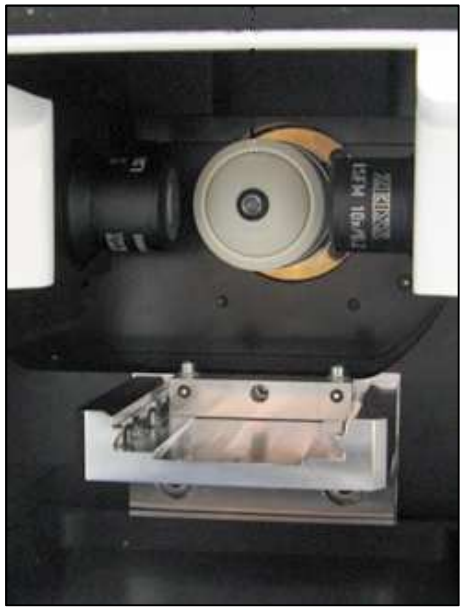
System Cavity: Sample Chamber Mounting

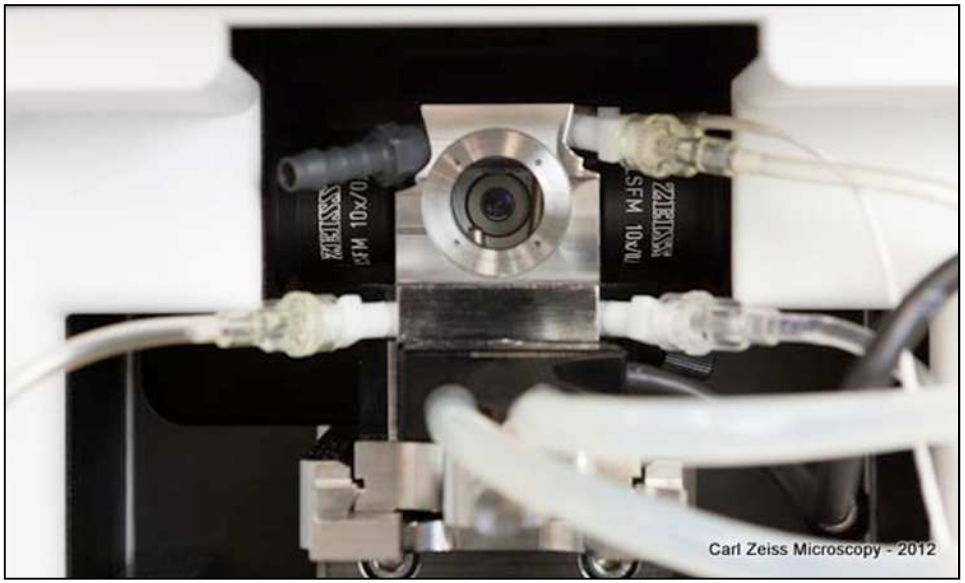
Sample Chamber

- Tubing connectors for medium change
- Precise yet easy to assemble coverslip windows
- Special Adapter ring to allow the use of water immersion dipping objective lenses
- Peltier-based heating and cooling block



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



Any View: Rotation and 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.

Benefit:

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

Lightsheet Z.1 Key Feature:

Multiview Imaging

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Benefit:

- Complementary information in different views (more info)
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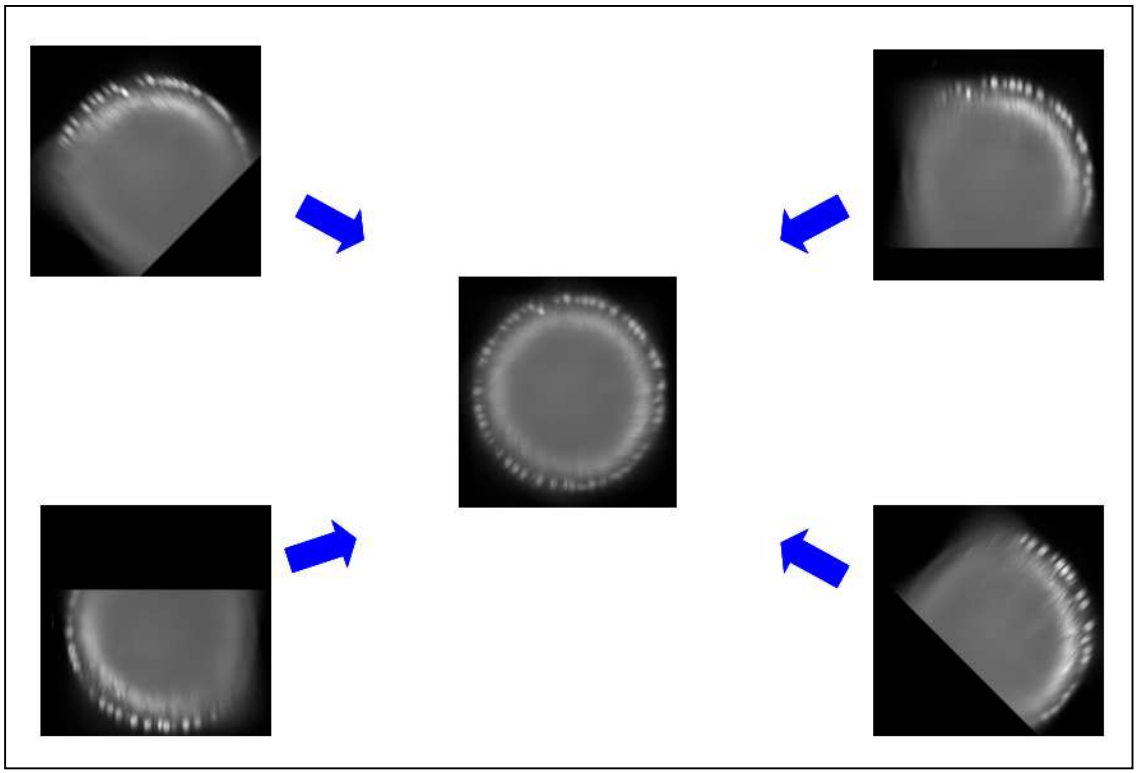
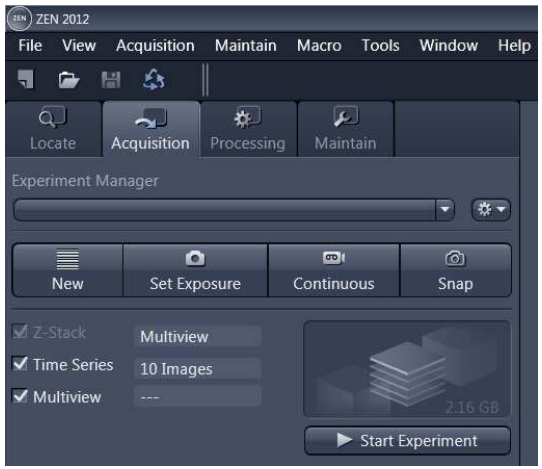
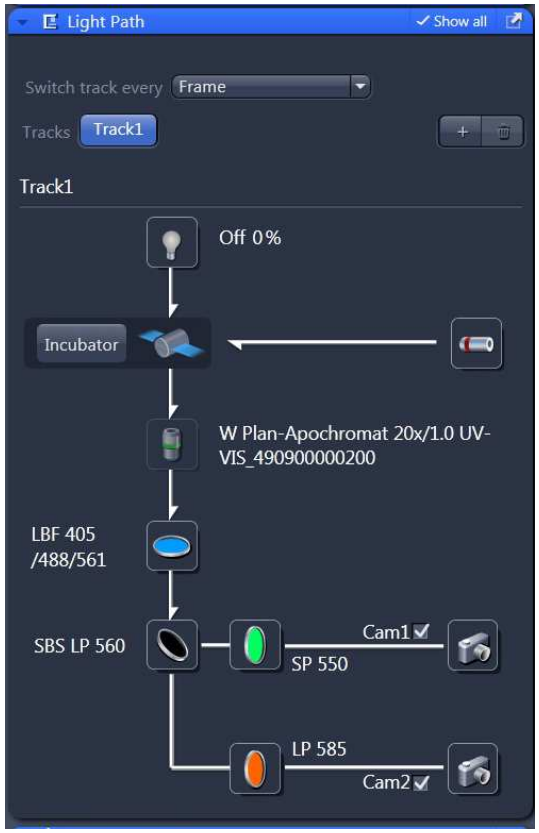


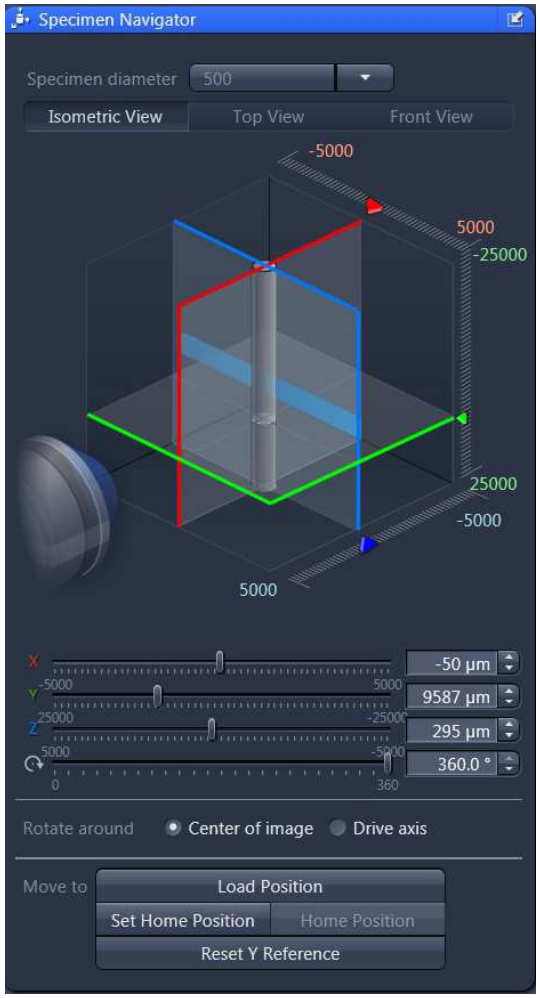
MultiView reconstruction of a Drosphila Embryo (Section),
(Schäfer et al, FOM 2009, data: Tomancak et al. MPI Dresden)

Lightsheet Z.1

Easy to Use and Learn







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

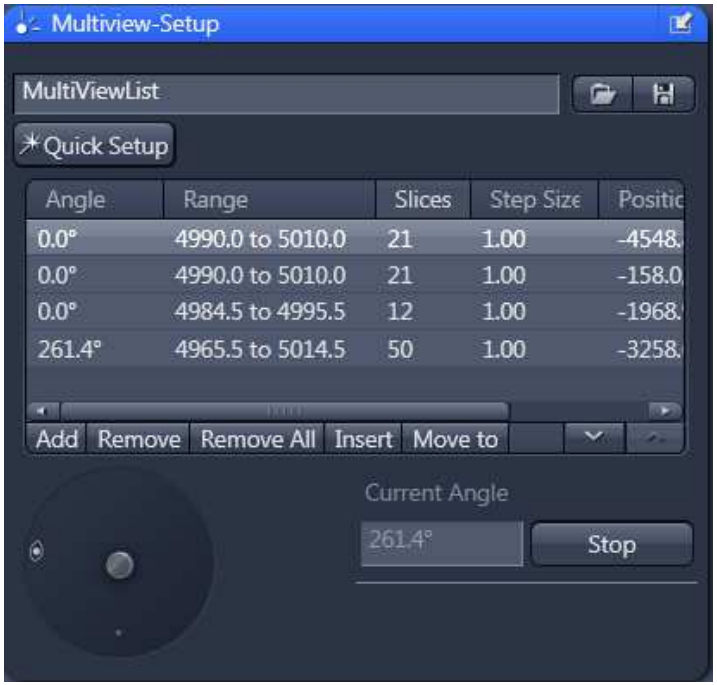
Lightsheet Z.1

Multiview Acquisition

Typical Multiview experiment

- 4 views at 1000 slices (≈ 500 µm³)
- Dual-side illumination
- 1 Excitation wavelength
- 30 FPS

Acquisition time ≈ 5 min



New software tool for convenient and fast setup of MultiView Experiments

Key Features of the Lightsheet Z.1

- Light sheet optics by Carl Zeiss: Excellent optics for **stunning image quality**
- **All the time:** Stability to perform long term time lapse imaging and observe the development of whole organisms in 3D for days.
- **Real life:** A special sample chamber to maintain the perfect environment for living specimen including heating, cooling and CO2
- **Any view**: Acquisition of entire large specimen by Multiview Imaging
- **Gentle:** Highest Sensitivity combined with virtually no phototoxicity or photobleaching
- **Fast:** Visualize dynamic processes with ultrafast optical sectioning

Zebrafish Heart Development

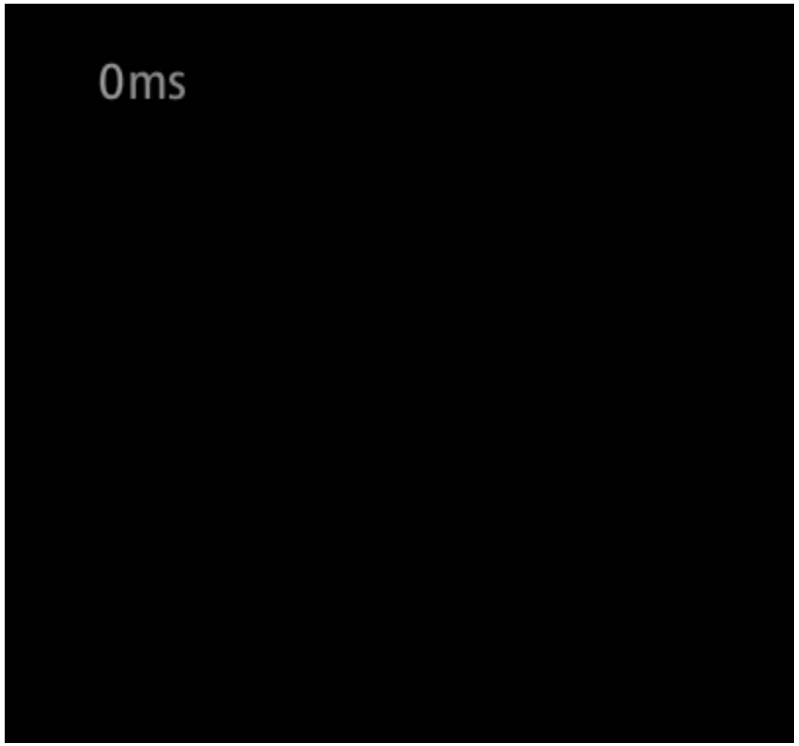
Zebrafish heart of 2 day embryo

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



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)

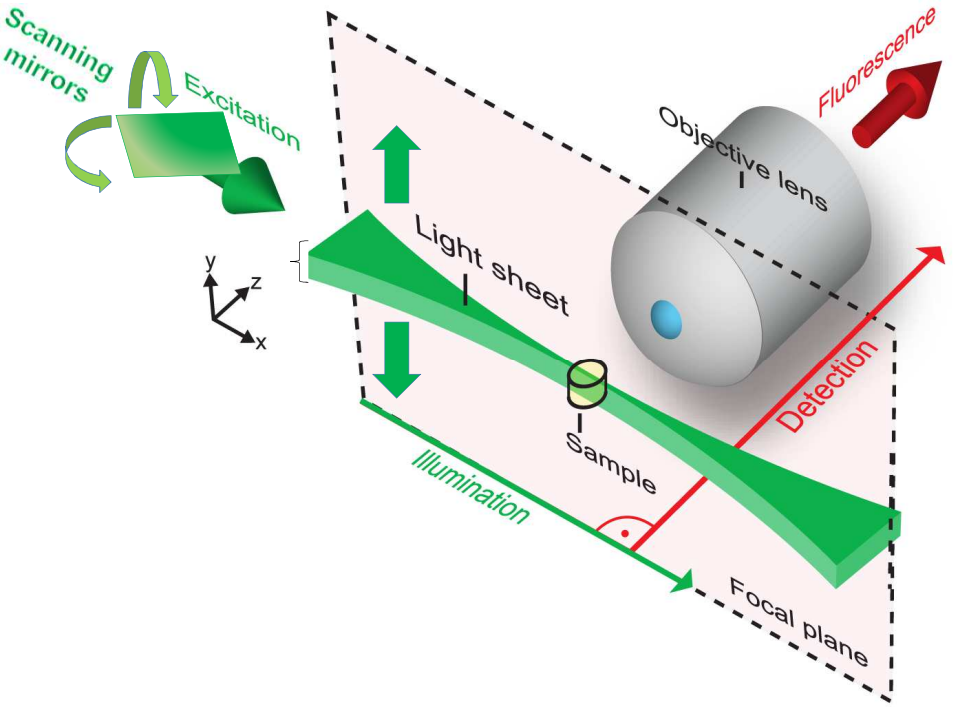


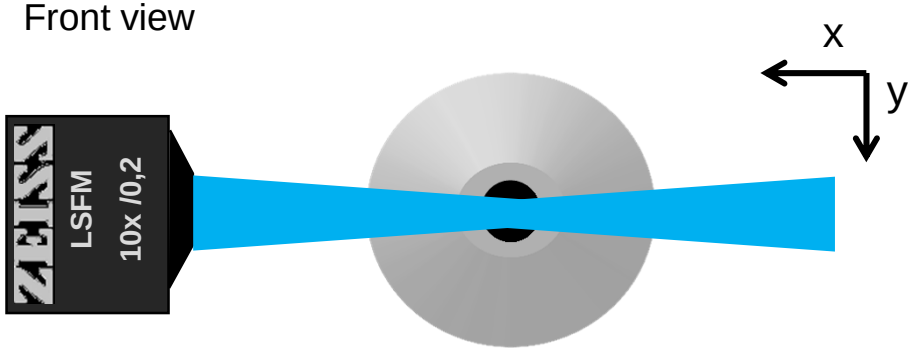
Figure from the PhD thesis of Jörg Ritter (2011), University of Bonn, Germany)

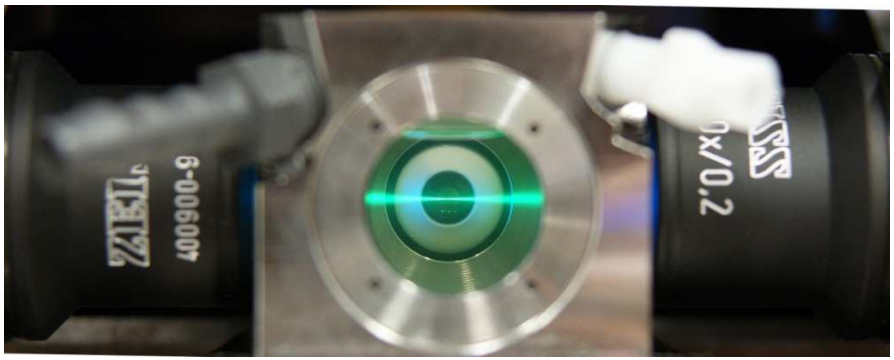
Lightsheet Z.1

The Scanned Light Sheet

Example: only right side illumination

Front view





Right side illumination, no scanning

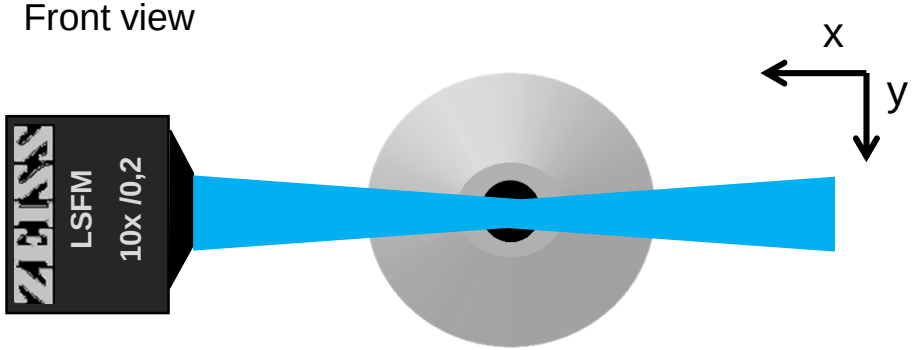
Homogenous light sheet covers about 1/3 to 1/4 of the field of view

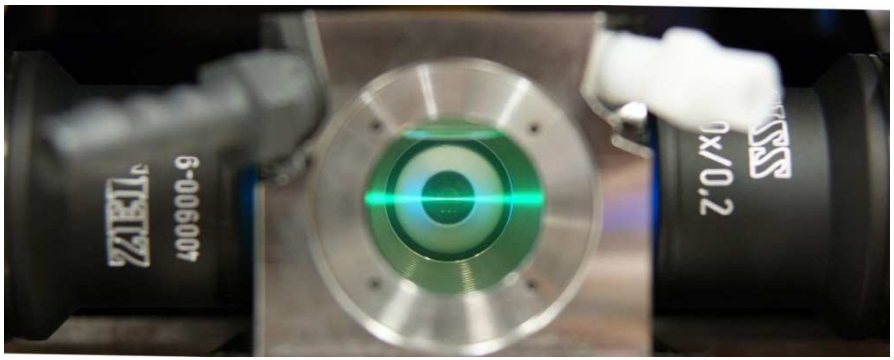
Lightsheet Z.1

The Scanned Light Sheet

Example: only right side illumination

Front view





Right side illumination, no scanning

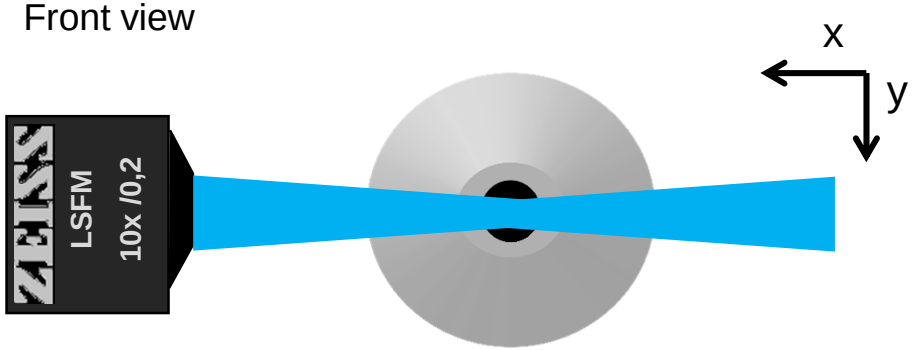
Scanning of light sheet to
illuminate the whole field of view

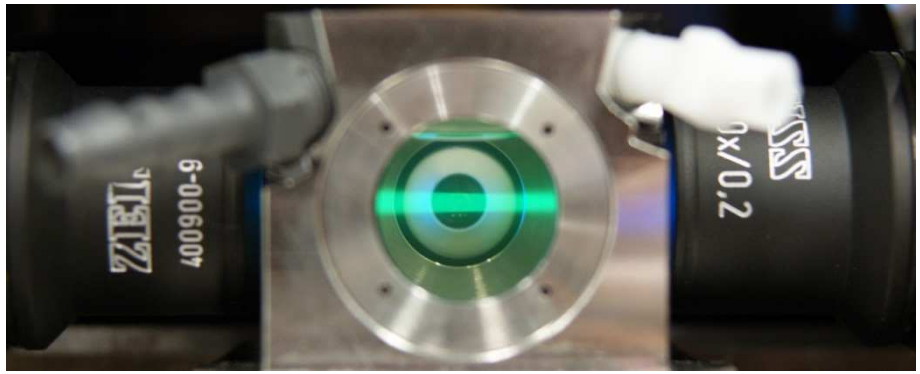
Lightsheet Z.1

The Scanned Light Sheet

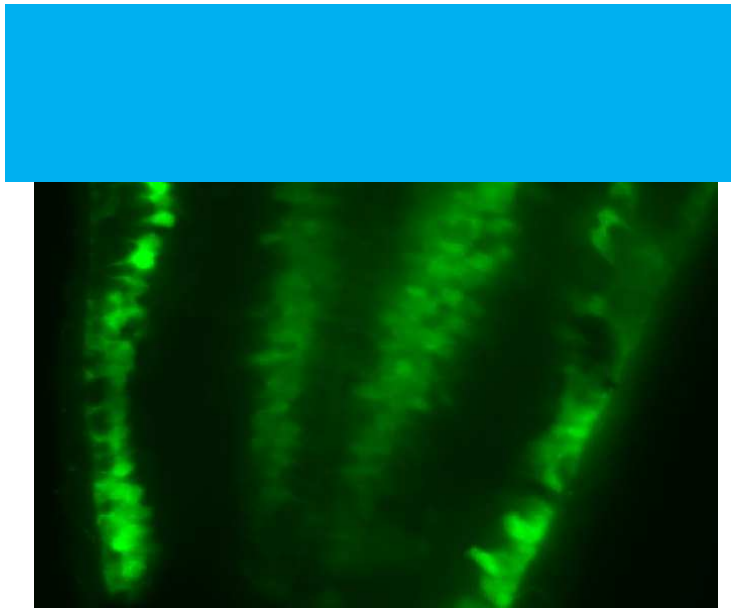
Example: only right side illumination

Front view





Right side illumination, scanning

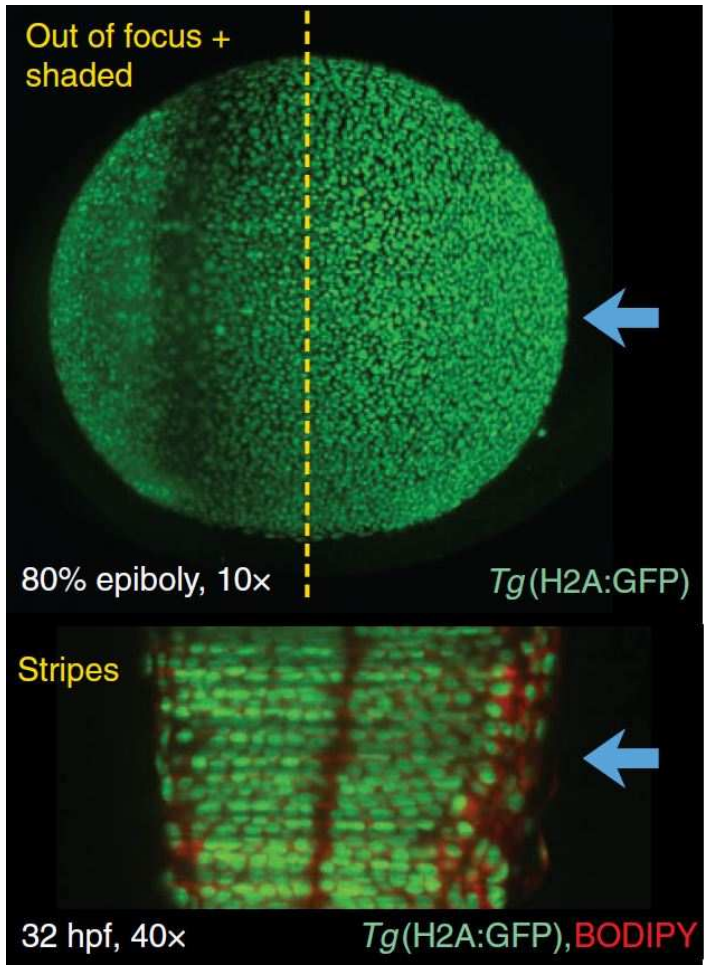


Whole number of scans per frame, synchronized with exposure time.

Multidimensional Illumination?

Stripes, shadows and other limitations

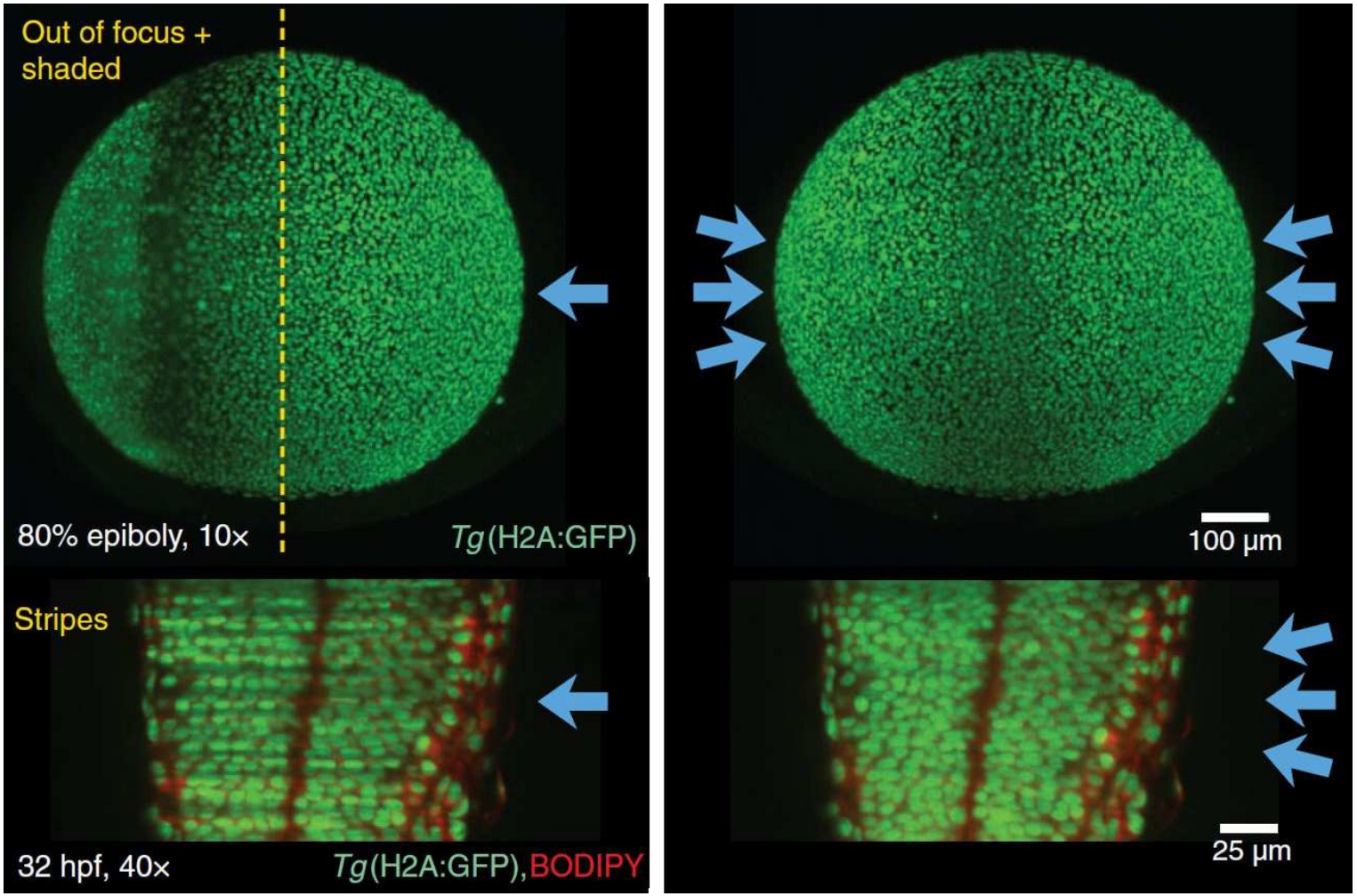
- Simple one sided illumination in light sheet fluorescence microscopy has different issues:
  - Absorption and scattering artifacts
  - ⇒ depending on how long the light has to travel inside the sample and on the optical density of the sample, the light sheet and image quality decreases (dimmer, image 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



Adapted from Huisken and Stainier, Devel. 2009

How to work on these limitations?

Dual side illumination, multi view detection / reconstruction and pivot scan of lightsheet



Adapted from Huisken and Stainier, Devel. 2009

Dual side illumination

Putting your sample in its true light

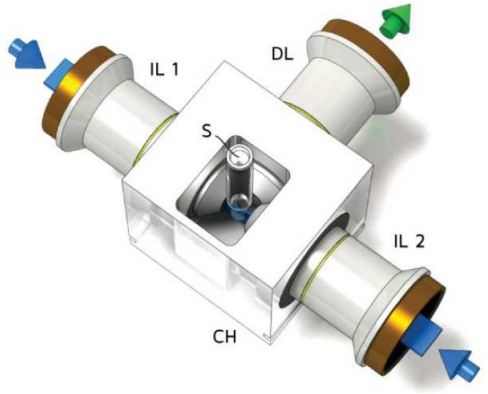
- Lightsheet Z.1 is equipped with two opposite illumination optics: Light Sheets can be generated from two sides

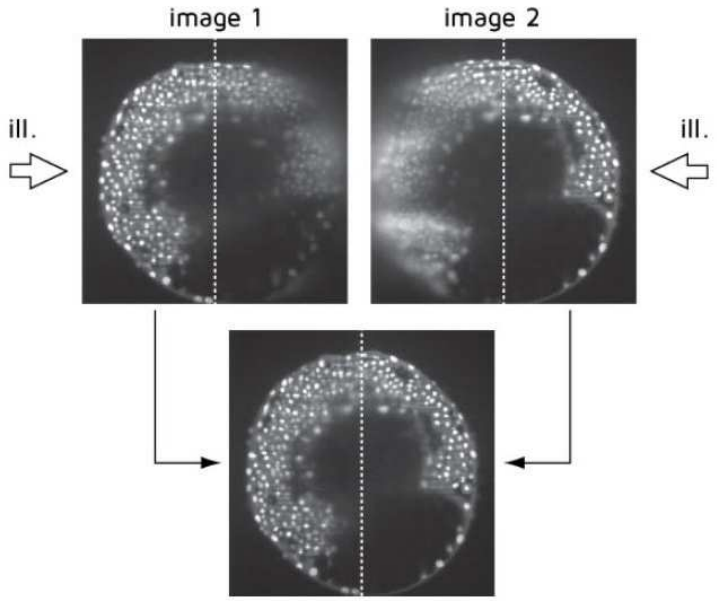
⇒ Scattering and absorption of excitation light largely compensated

⇒ reduced anisotropy in x/y-dimension

⇒ much higher penetration depth in thick specimen

⇒ increased acquisition speed

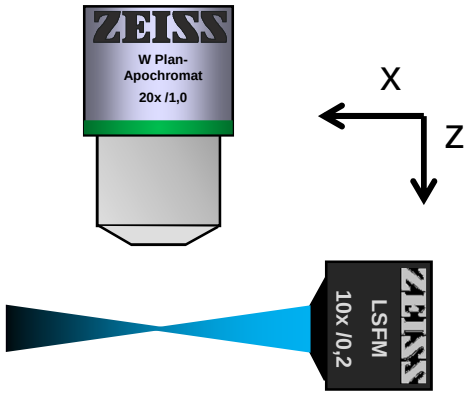


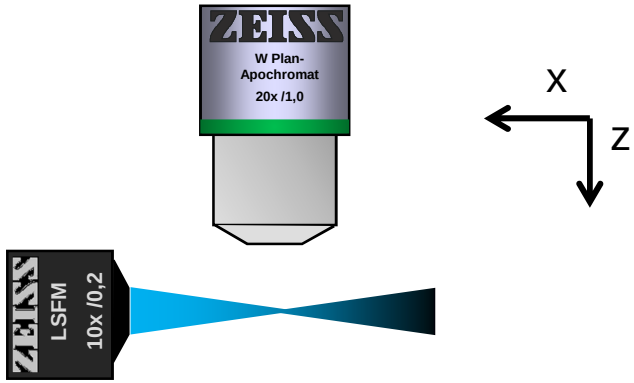


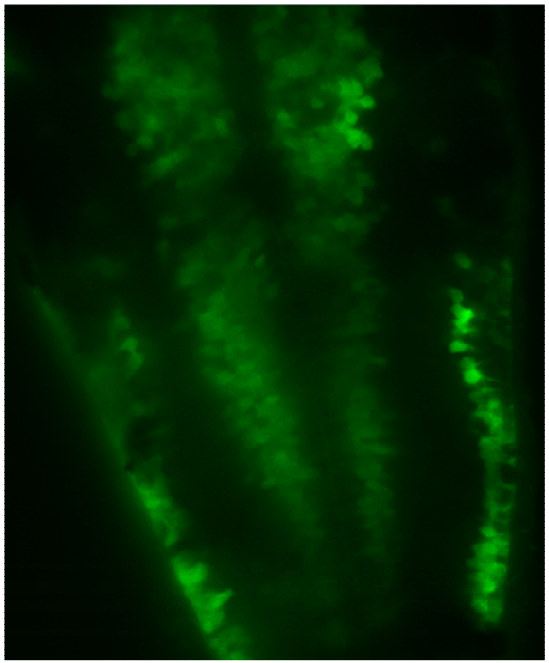
Adapted from Huisken, Bioessays, 2012

Lightsheet Z.1

Dual Side Illumination

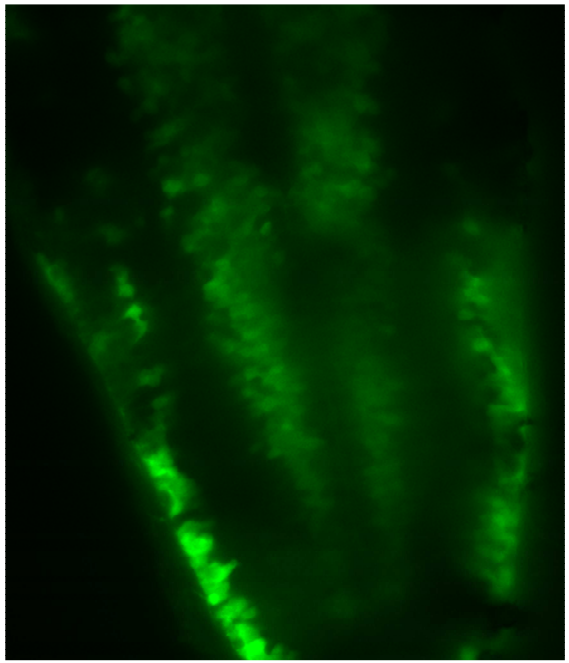






Light is absorbed, blocked and scattered along the axis of the light sheet.

- Illumination from both sides enhances the quality of information
- Dual side illumination is done sequentially



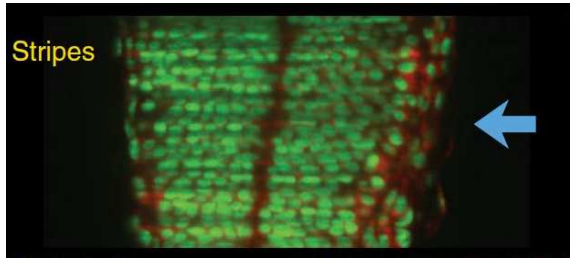
Pivot Scan

Shadow Reduction

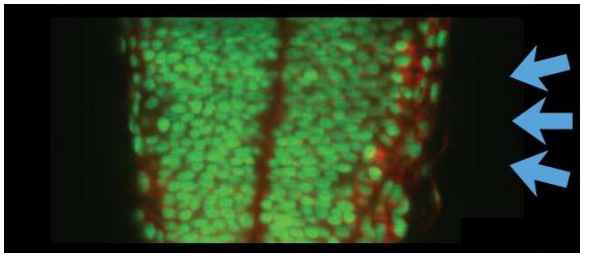
- Any “obstacle” (absorbing or scattering) in the beam path which is hit by a light sheet will cast a shadow along the direction of illumination
- Pivot scan can eliminate these shadows:

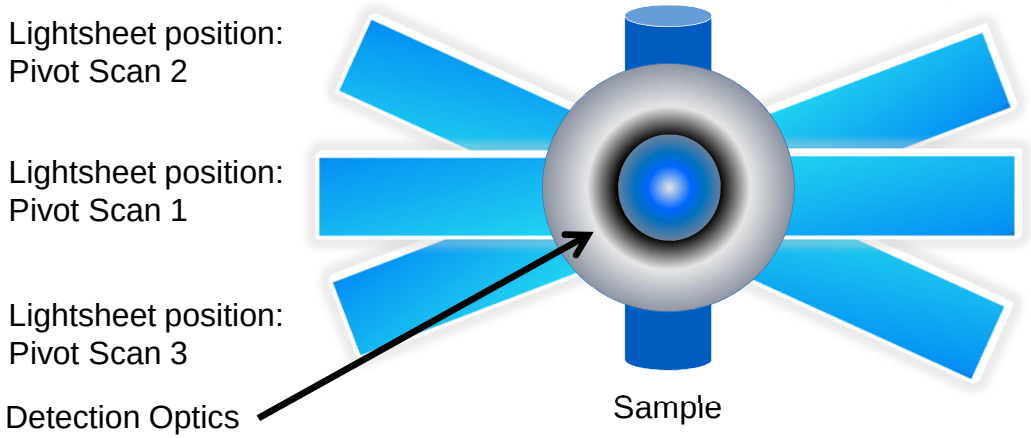
⇒ Light sheets are generated from different angles during the exposure time of the camera and thus cancel out the shadows by illuminating also “behind” the “obstacles”

Without Pivot scan



With Pivot scan



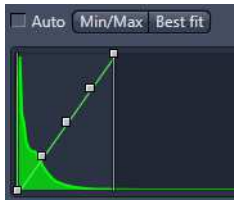


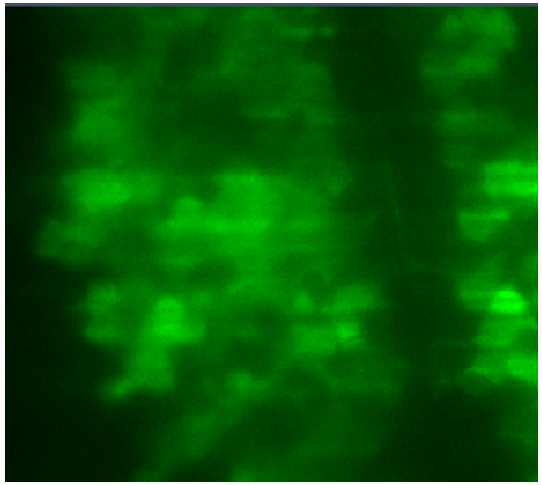
Adapted from Huisken and Stainier, Devel. 2009

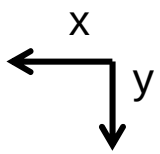
Lightsheet Z.1

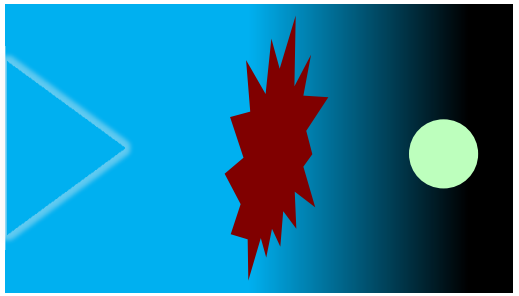
Pivot Scanner

Without Pivot

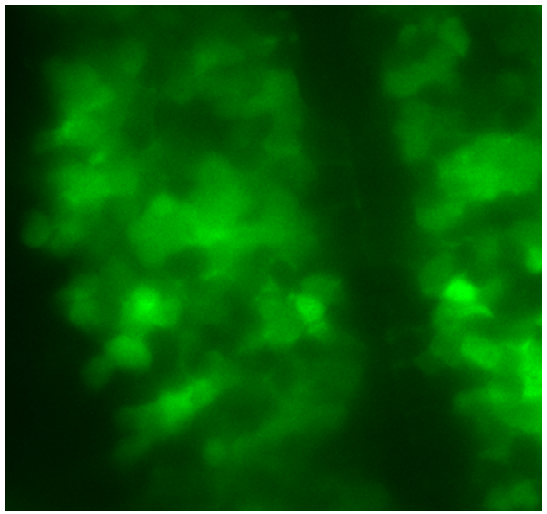


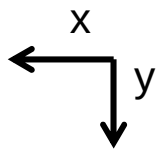


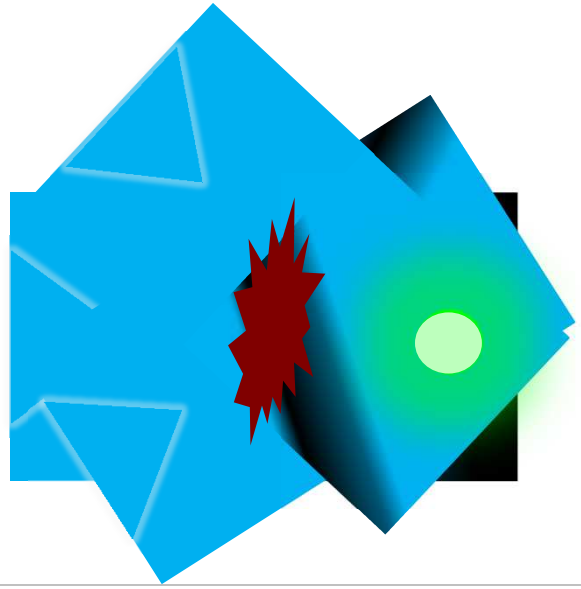




With Pivot



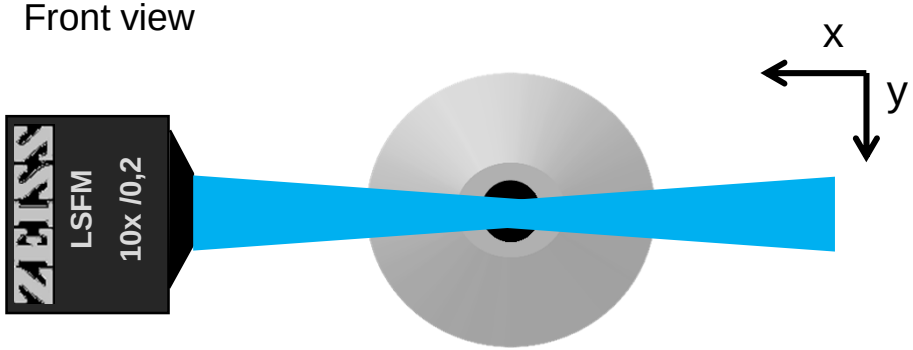


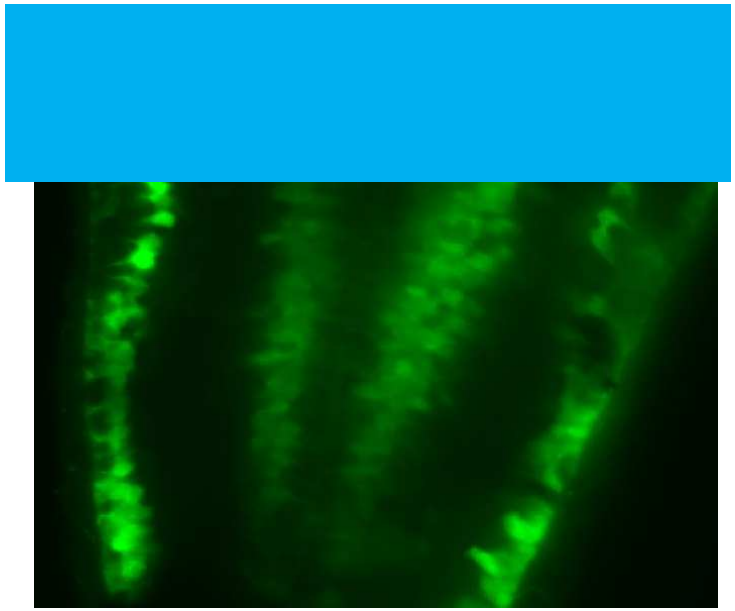


Lightsheet Z.1

Pivot Scanner

Front view





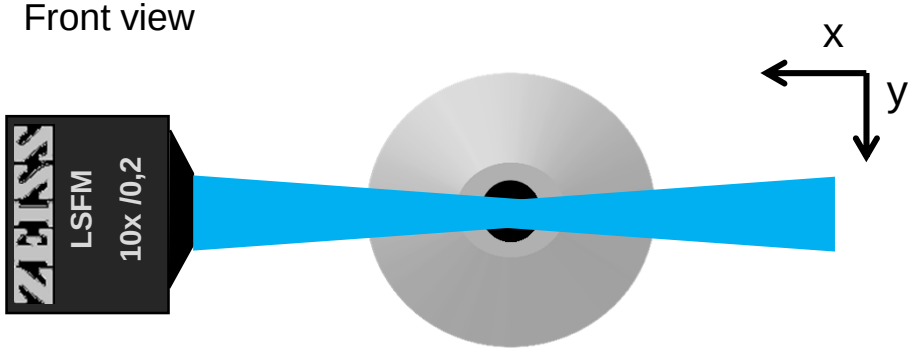
Both scanner motions in the **same** (x,y) plane

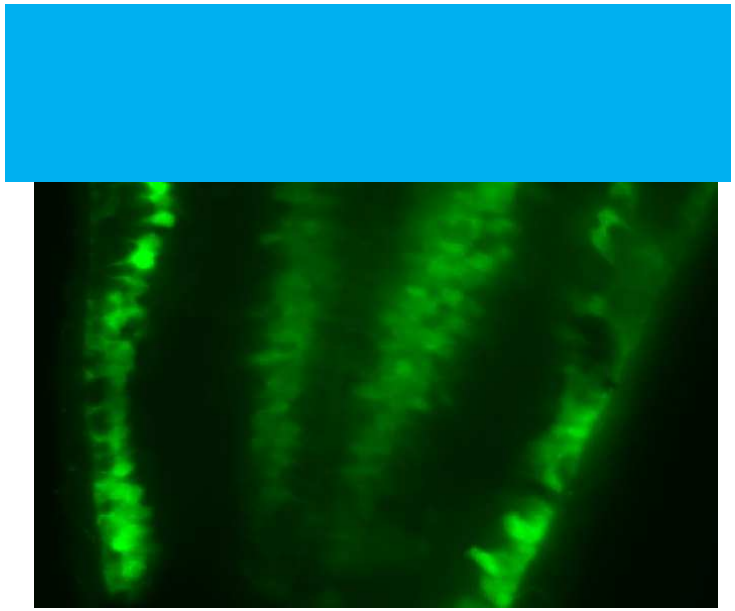
- Imaging scanner to illuminate the frame
- Pivot scanner to reduce shadows

Lightsheet Z.1

Pivot Scanner

Front view





Both scanner motions in the **same** (x,y) plane

- Imaging scanner to illuminate the frame
- Pivot scanner to reduce shadows