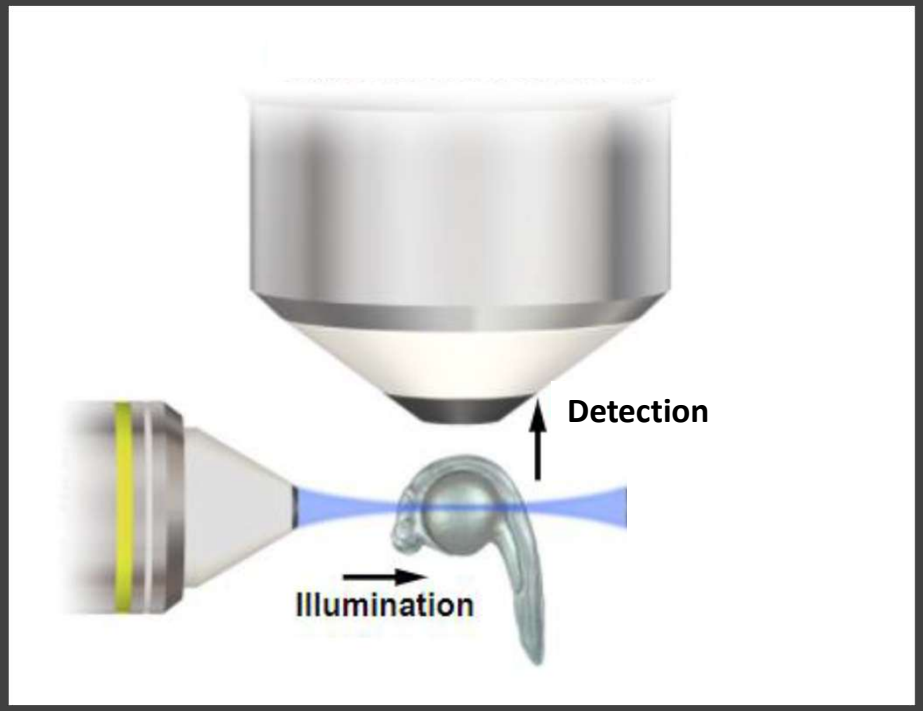
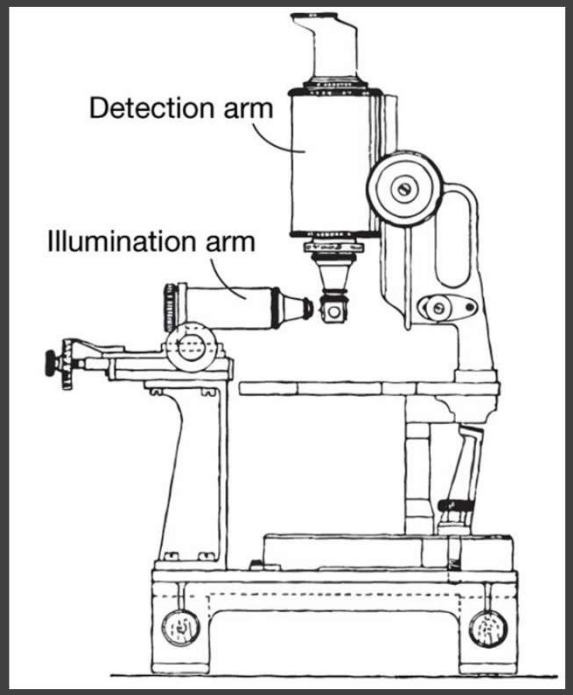
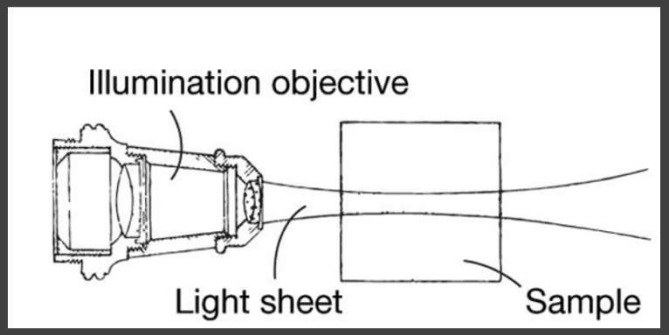
Lightsheet microscopy



Gert-Jan Kremers
11-11-2026

The “Ultramicroscope” – the 1st light sheet microscope (1903)





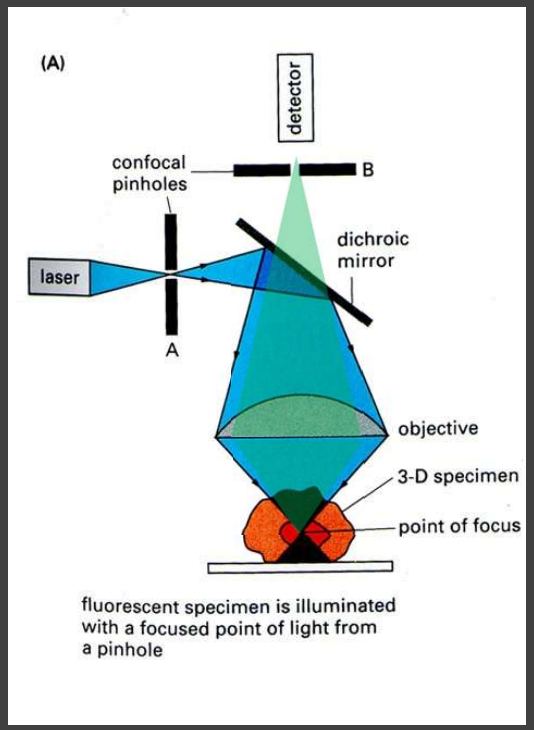
Siedentopf, H., and Zsigmondy, R. (1903), Annalen der Physik

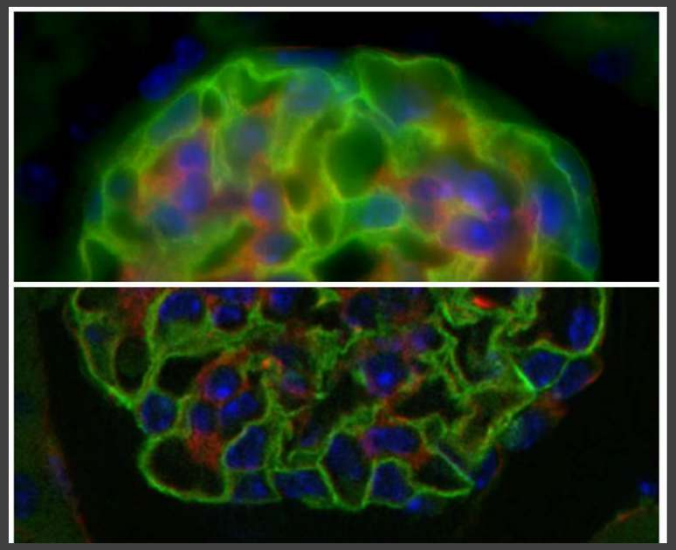
Zsigmondy was awarded Nobel Prize in chemistry in 1925

Lightsheet microscopy

- What's the benefit of using a lightsheet microscope?
  - Speed !!!
  - Optical sectioning capability
  - Less phototoxicity
- When is this useful?
  - Large 3D volume imaging
  - Fast (3D) biological processes

Laser scanning confocal microscopy

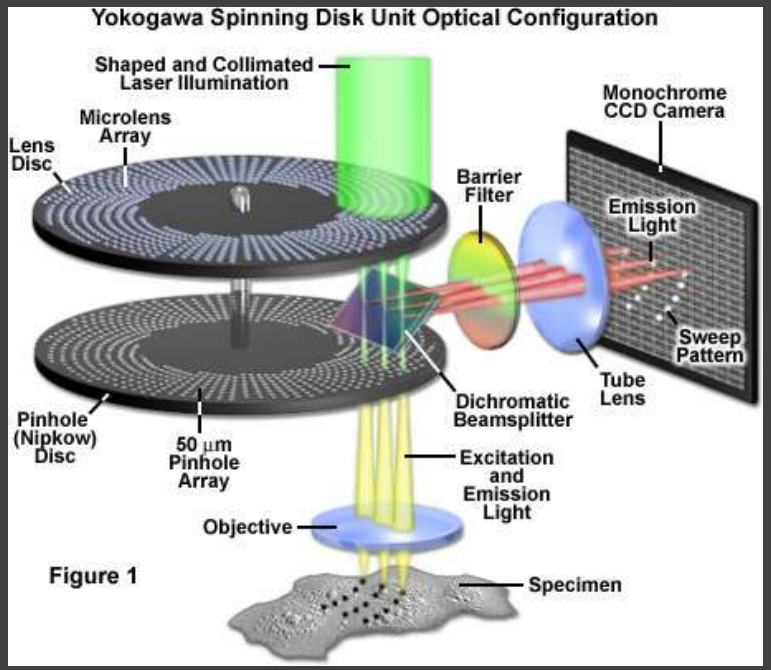


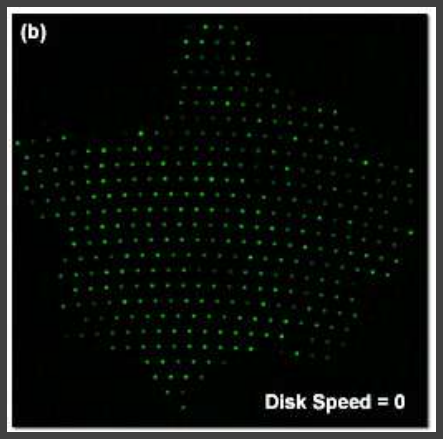


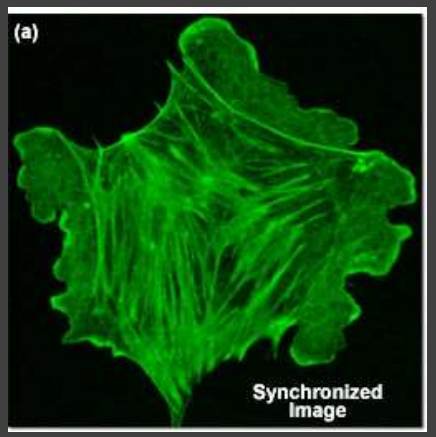
https://microscopysolutions.files.wordpress.com/2012/10/split--confocalconfocal--widefield.jpgwidefield.jpg

- Excellent optical sectioning!!!
- “Out focus” bleaching
- Pixel scanning is slow

Spinningdisc confocal microscopy





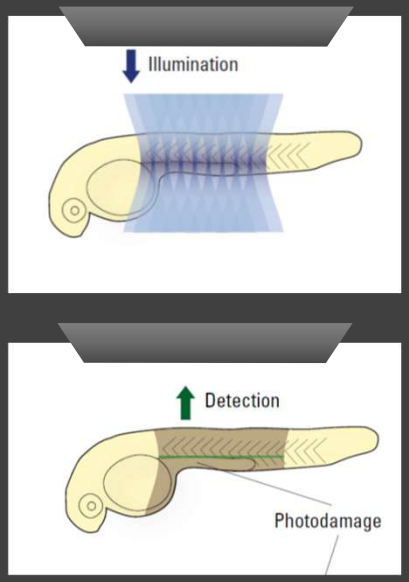


- Good optical sectioning
- Reduced photobleaching
- “Out of focus” bleaching
- Camera detection = fast (100fps)

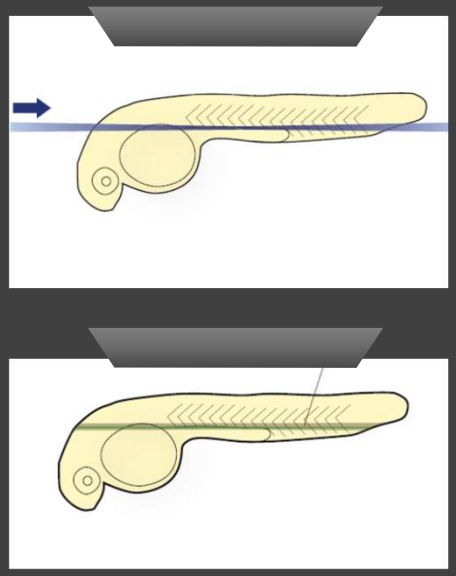
https://zeiss-campus.magnet.fsu.edu/

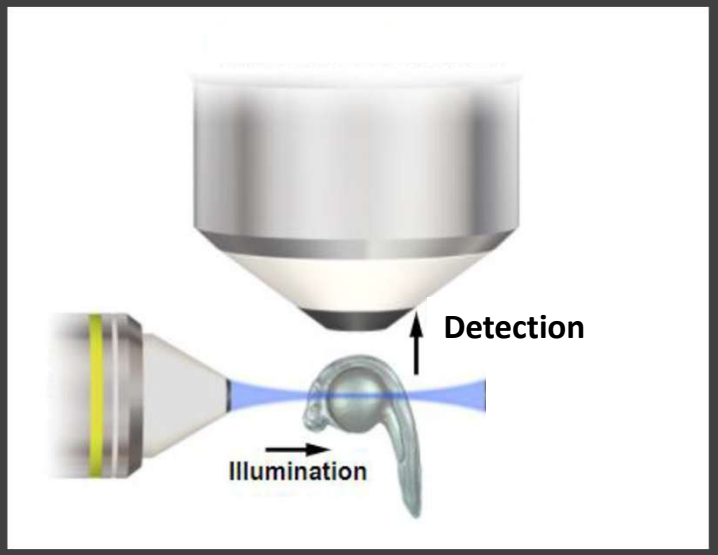
Principle of lightsheet imaging

Laser scanning confocal



Lightsheet imaging



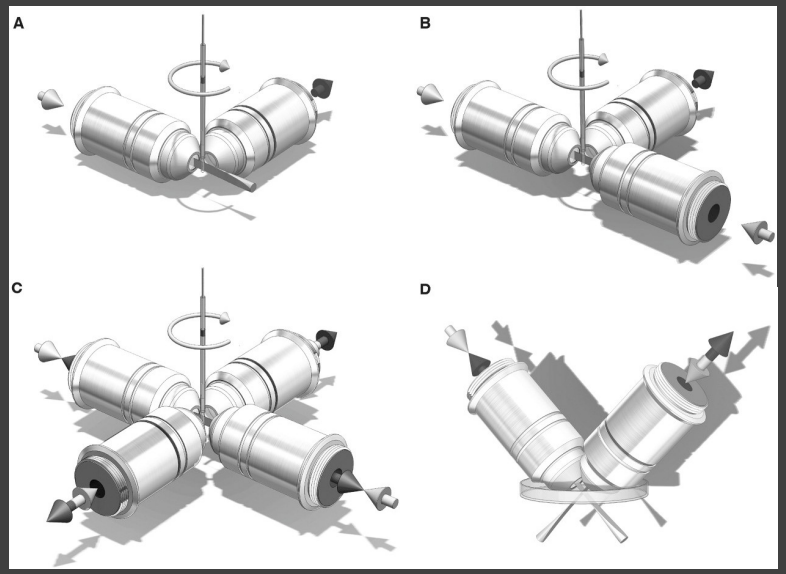


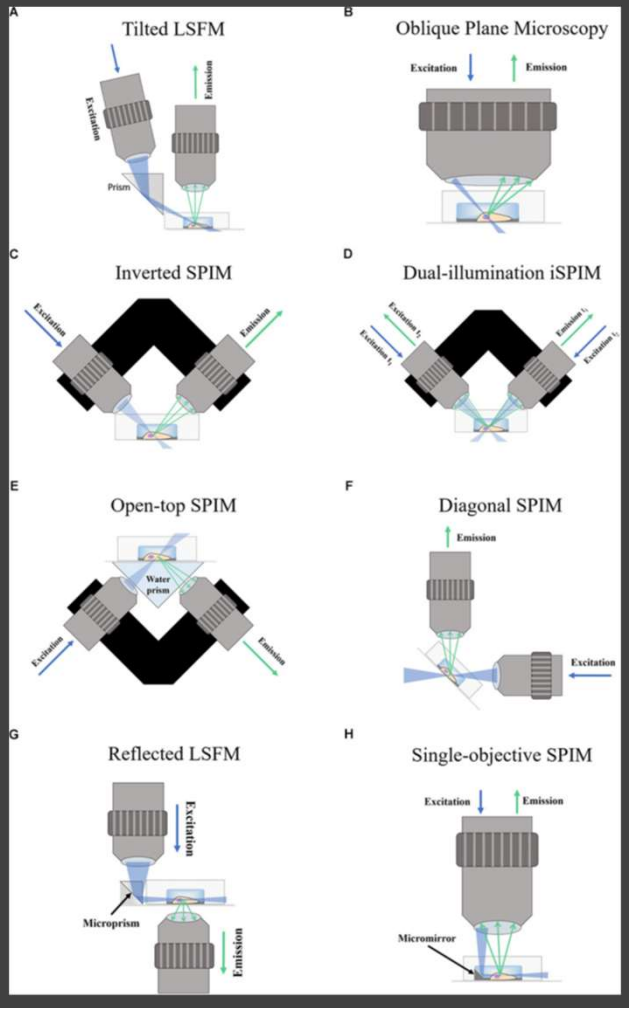
Camera captures whole image at once = fast

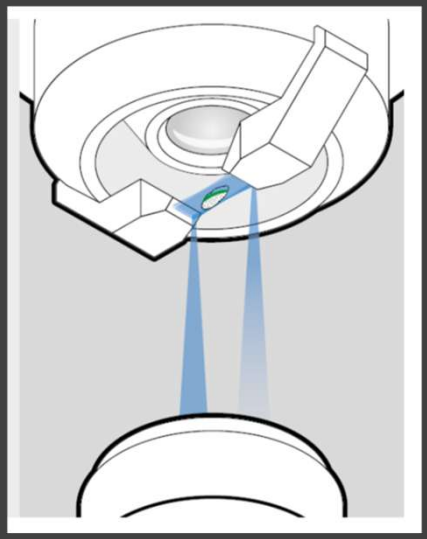
There’s no free lunch in microscopy

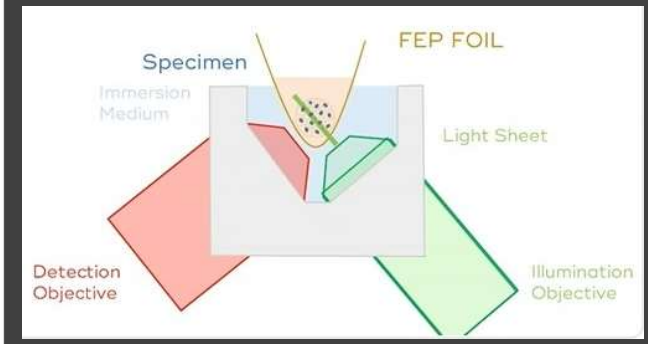
- Always a trade-off between:
  - Speed
  - Resolution (x,y,z)
  - Sensitivity and noise
  - Field of view or volume
  - Optical sectioning capability
  - Sample preparation !!!

Many different lightsheet configurations



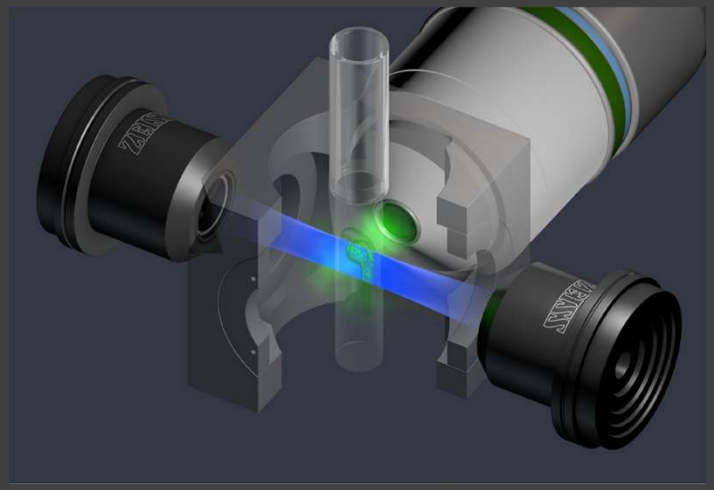




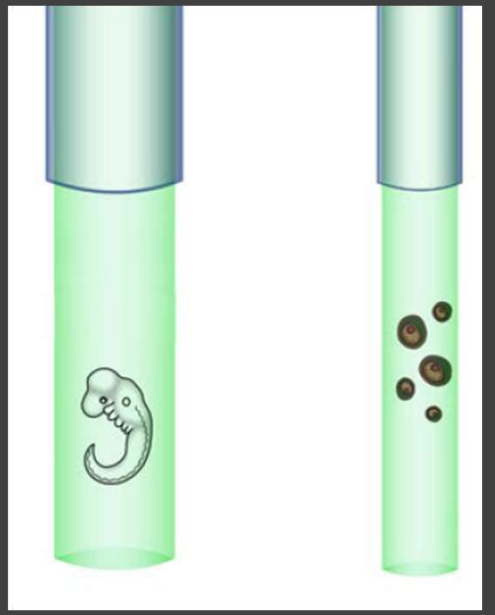


Lightsheet microscopes are often “build around a sample”

- Embryo’s & organoids

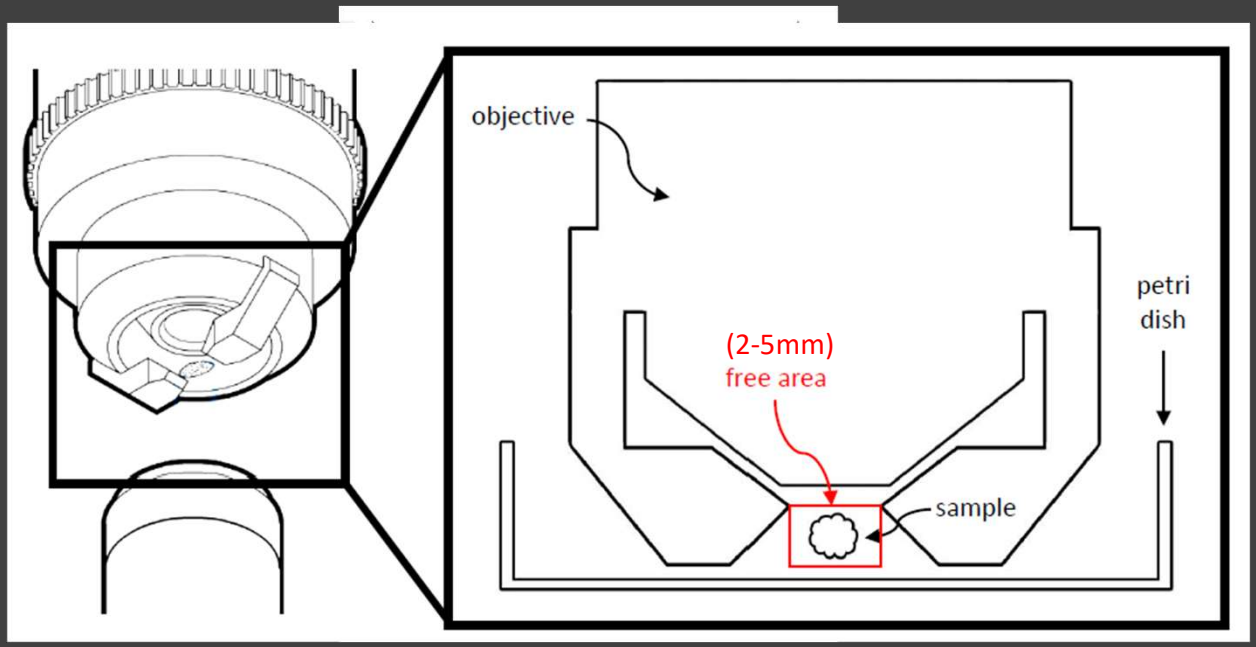






Zeiss lightsheet Z1

Embryo’s & organoids

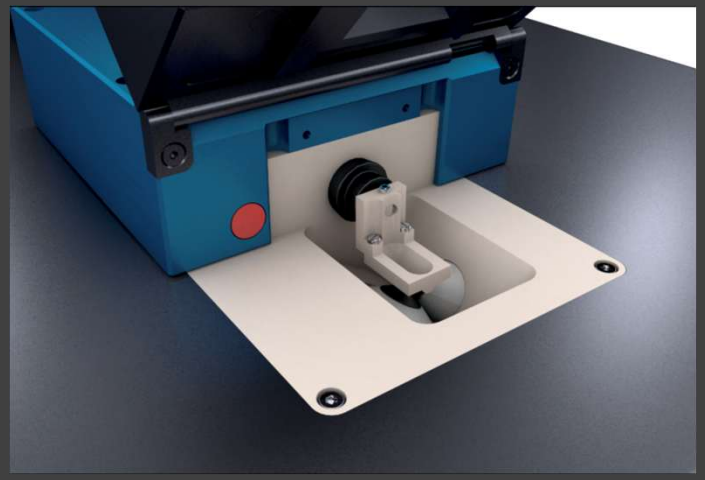


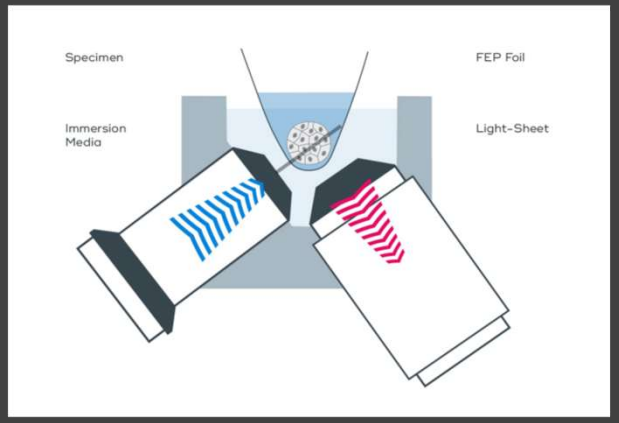
Leica Dynamic Lightsheet (DLS)



Embryo’s & organoids

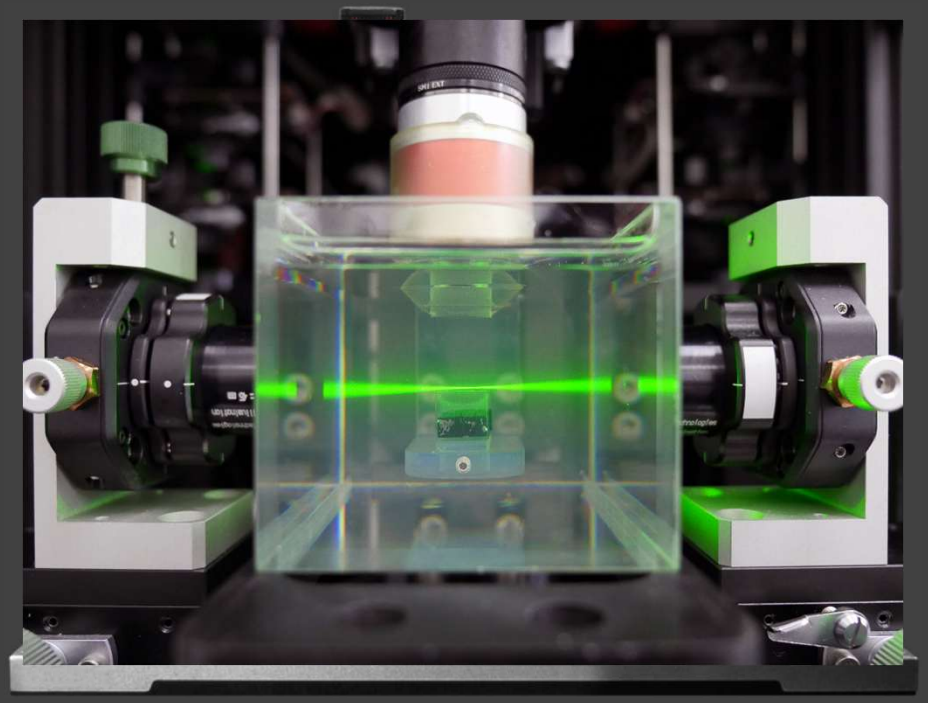




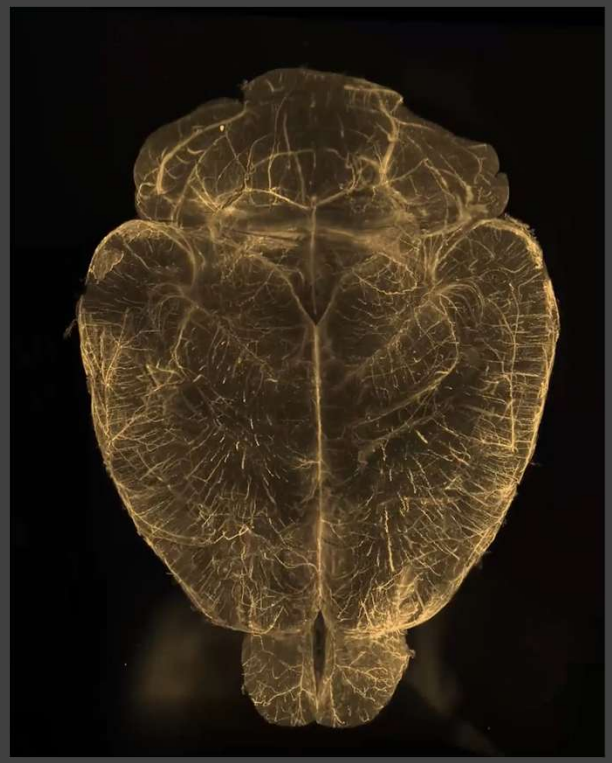


Bruker InviSPIM

Mouse brain and other large tissue samples

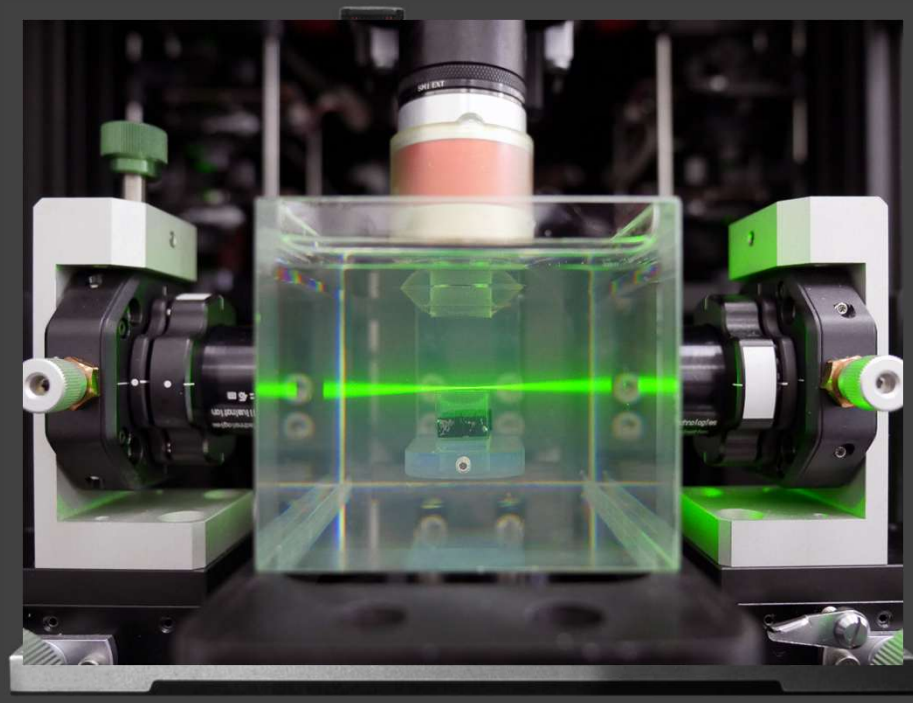


3i Cleared Tissue LightSheet

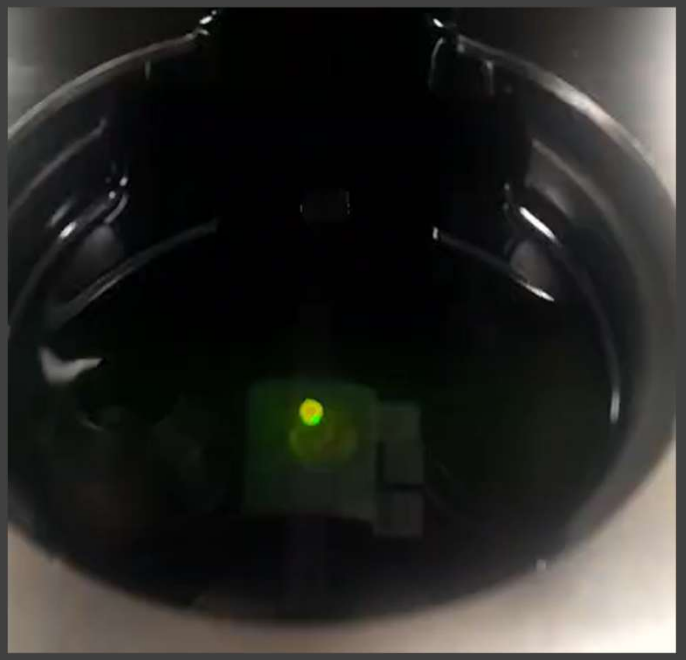


Smooth muscle cells in the arteries from veins and capillaries of cleared mouse brain reported by NG2BacDsRed.
https://www.intelligent-imaging.com/

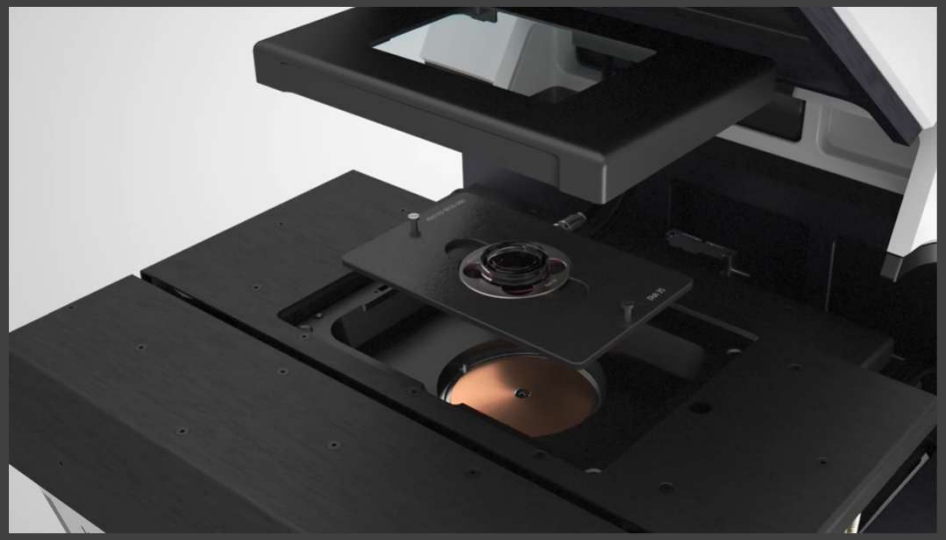
Mouse brain and other large tissue samples

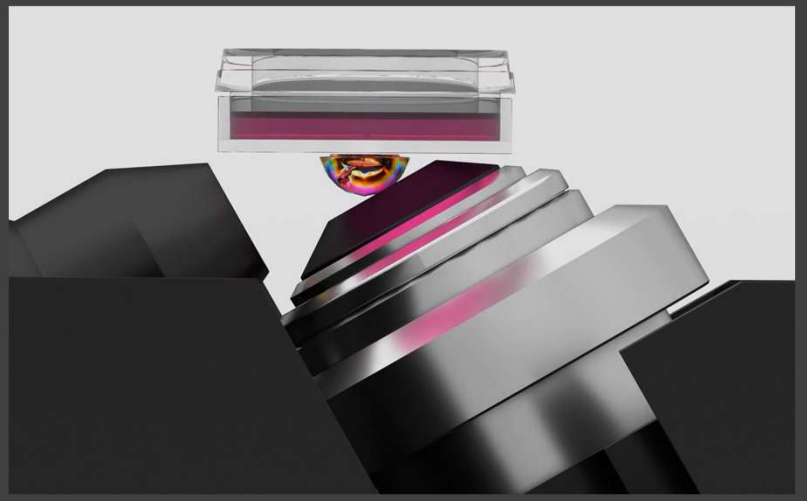


3i Cleared Tissue LightSheet



Cells and subcellular imaging

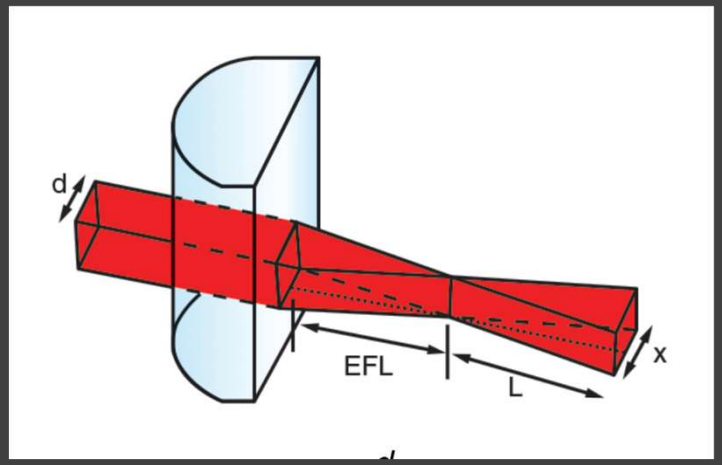




Zeiss lattice lightsheet

How to make a lightsheet?

Cylindrical lens



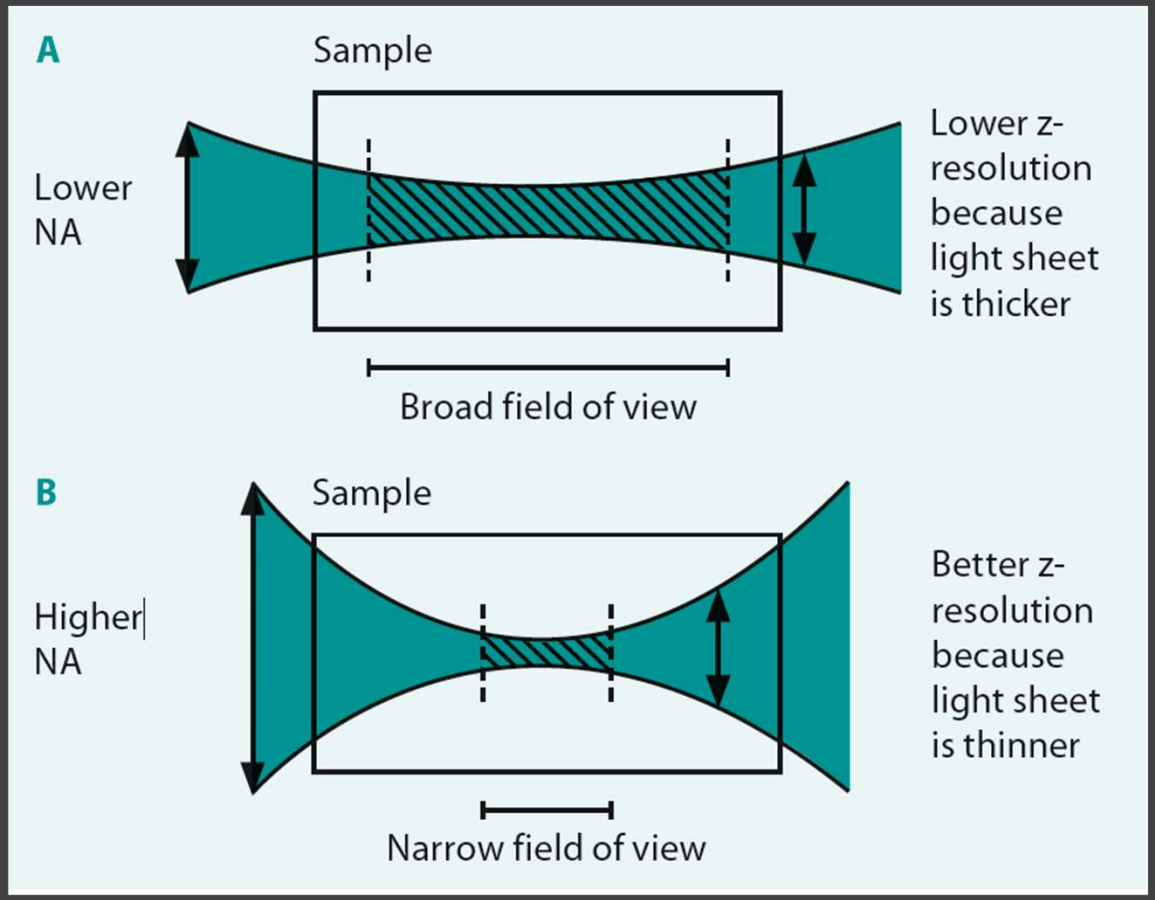
https://www.edmundoptics.com/campaigns/cylinder-lens/

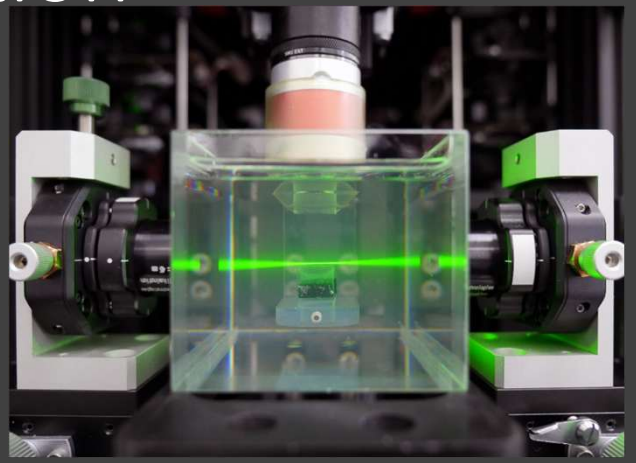
Scanned laserbeam



https://pangolin.com/

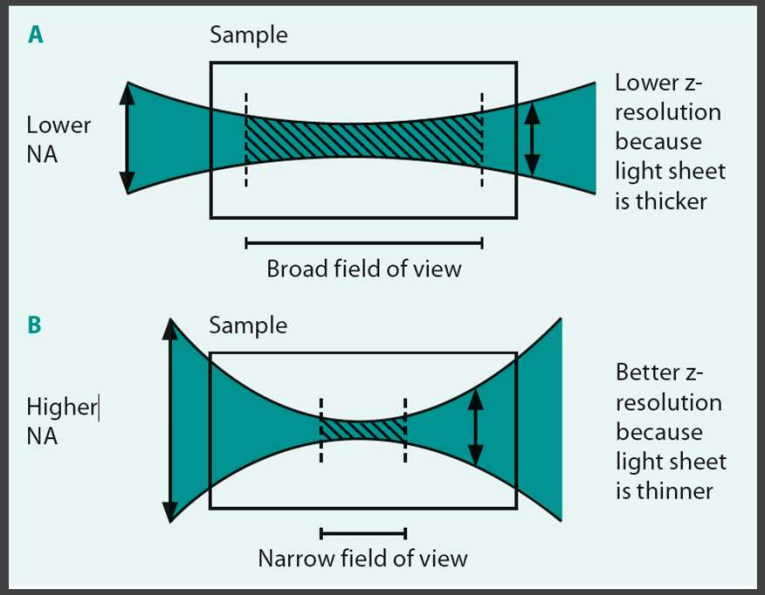
Lightsheet thickness = Z-resolution

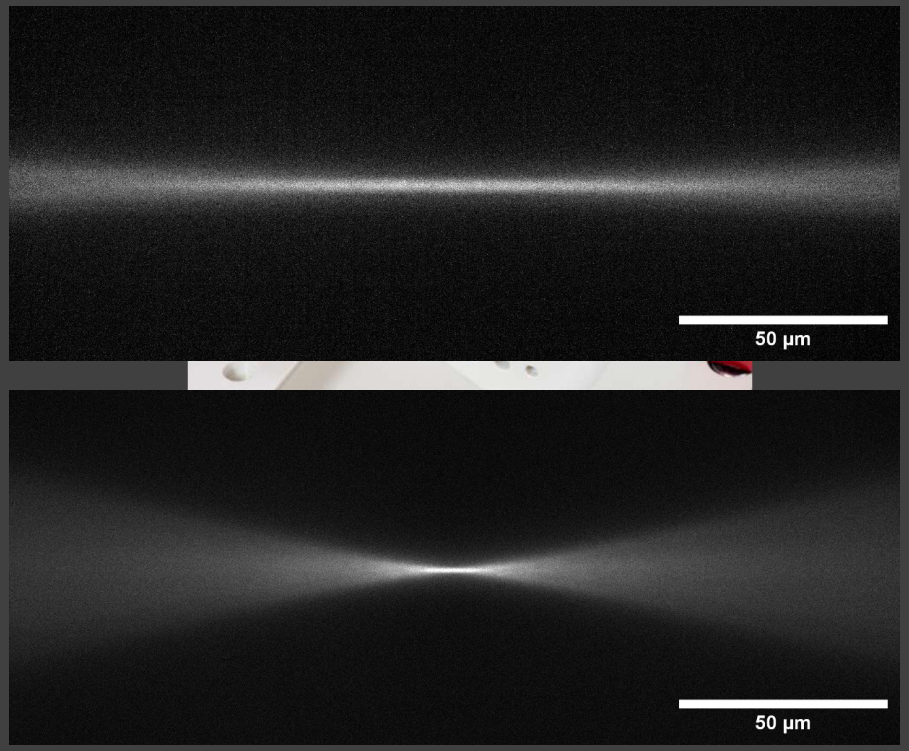






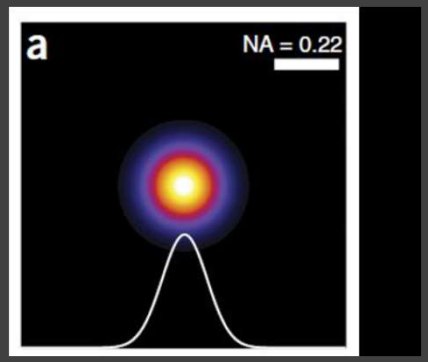
Beam shaping: Gaussian laser beam

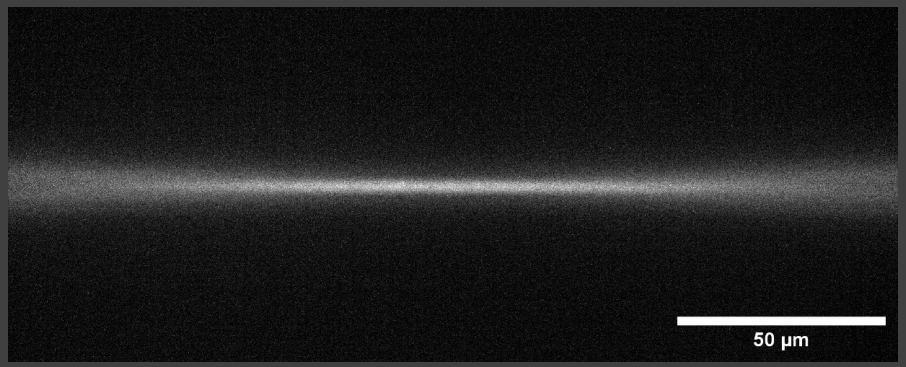




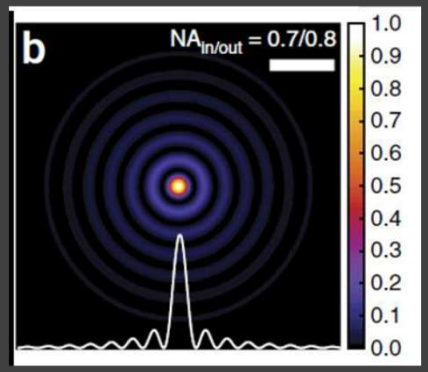
Beam shaping: Bessel laser beam

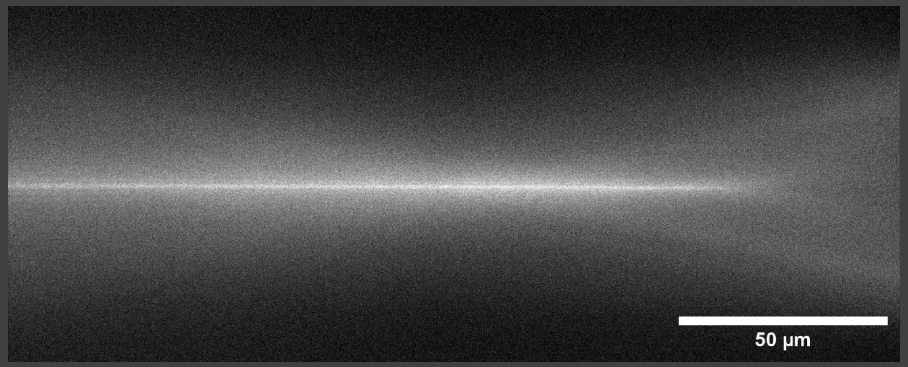
Gaussian beam



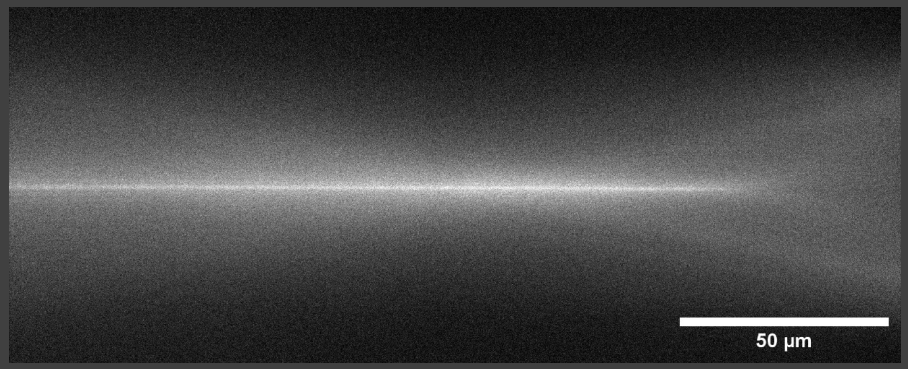


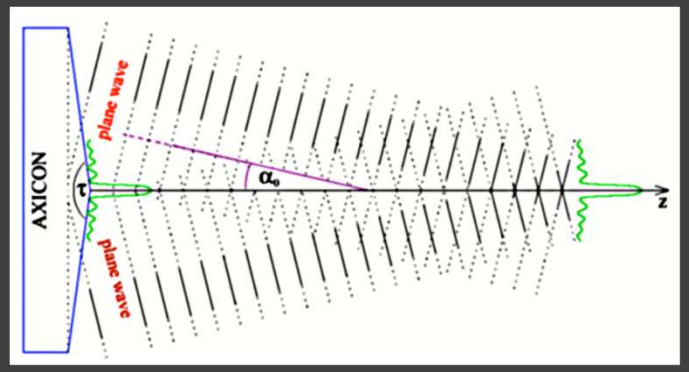
Bessel beam

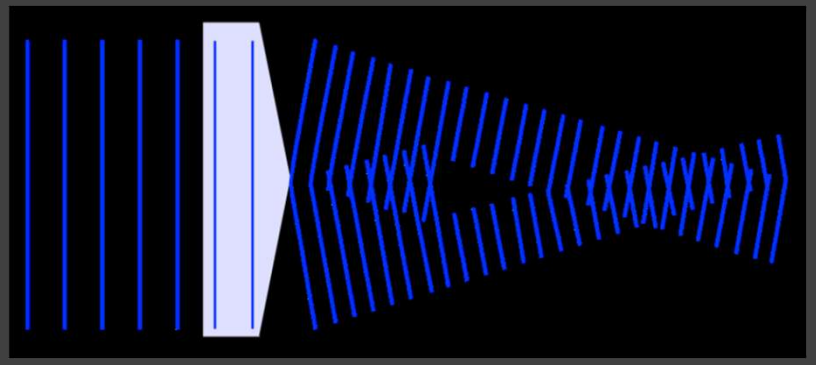




Shading effects



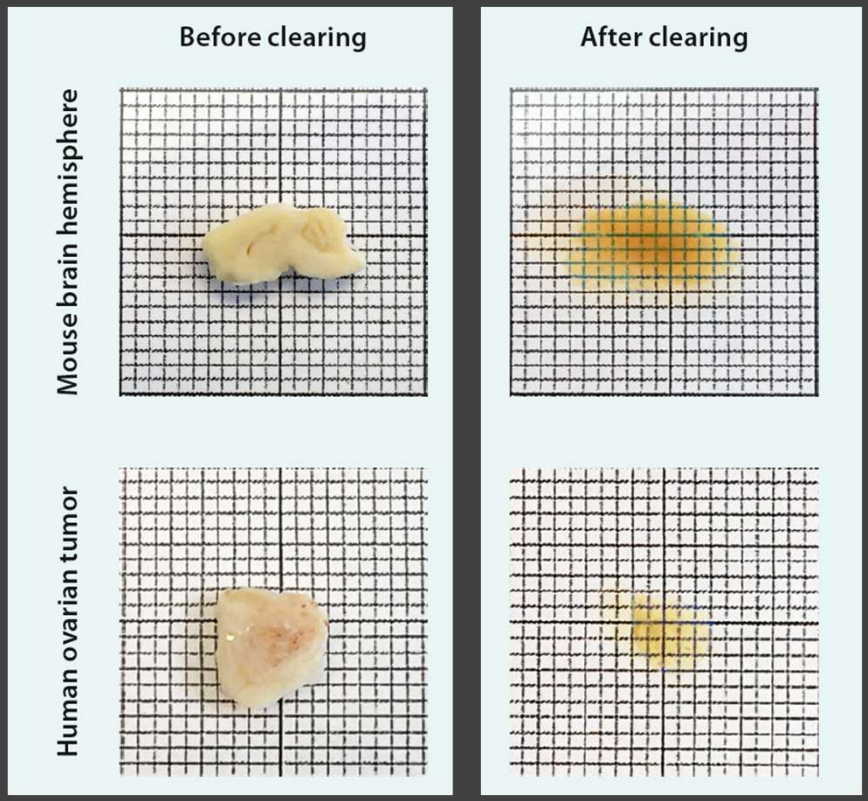




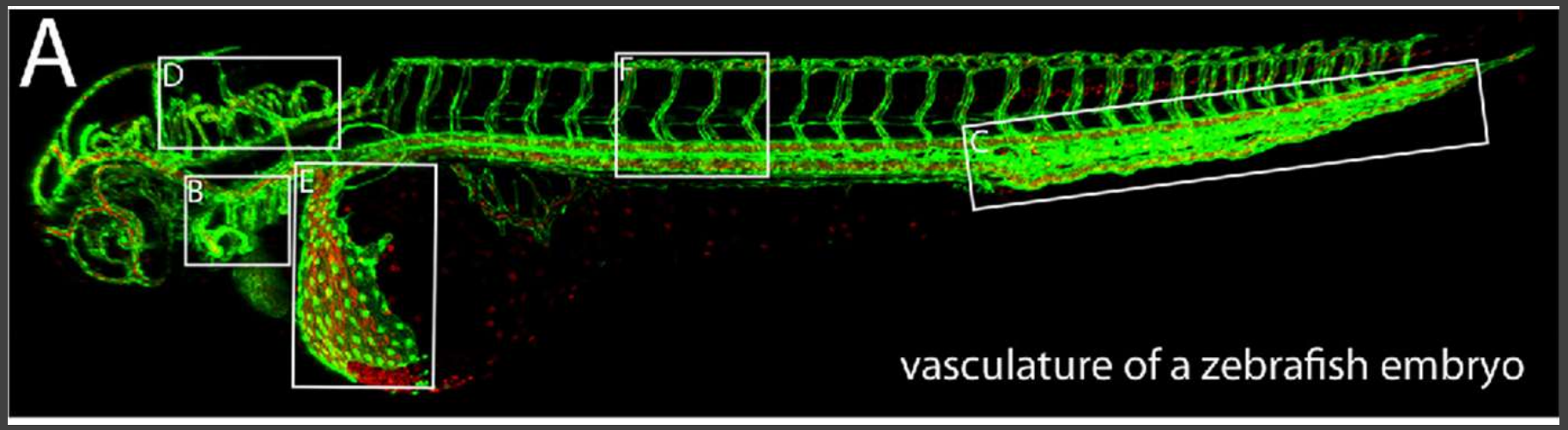
Sample transparency

Cells and embro’s are transparent

Tissues, organs and organisms typically are not



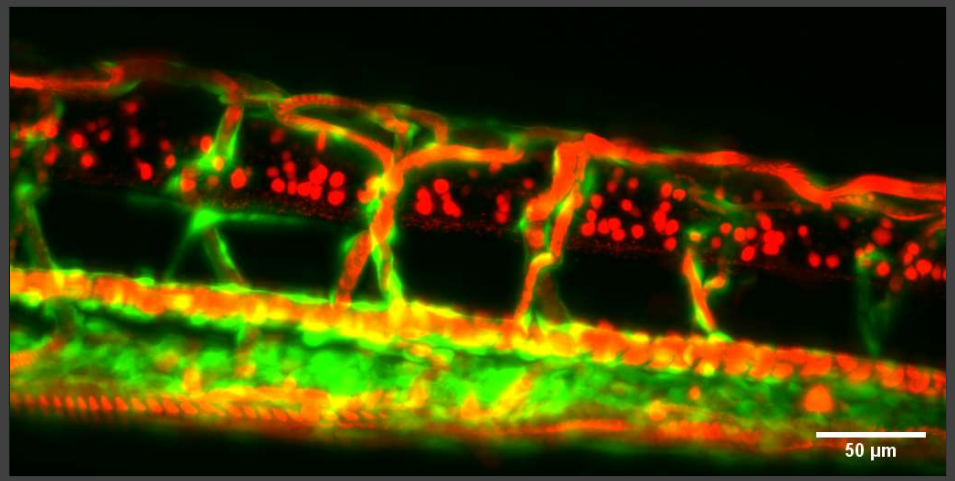
Zebrafish embryo imaging



Vascular system in the zebrafish embryo at 60 hours post-fertilisation (Schuermann et al. 2014).

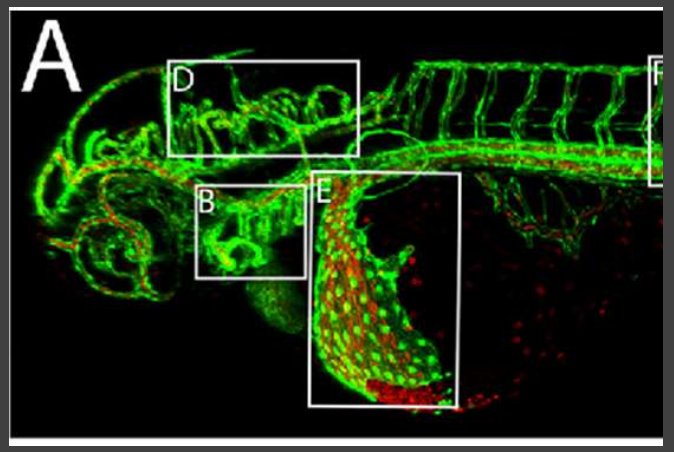
Tg(Flk:GFP/Gata:dsRed) = endotheel cellen groen en rode bloedcellen rood

3D imaging of vasculature

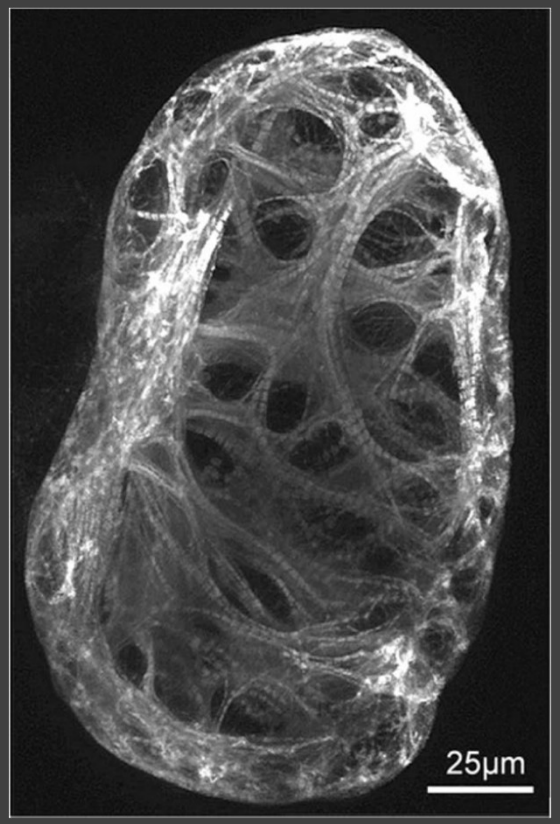


Claudine Ruijmbeek, Clinical Genetics

426 x 213 x 165um

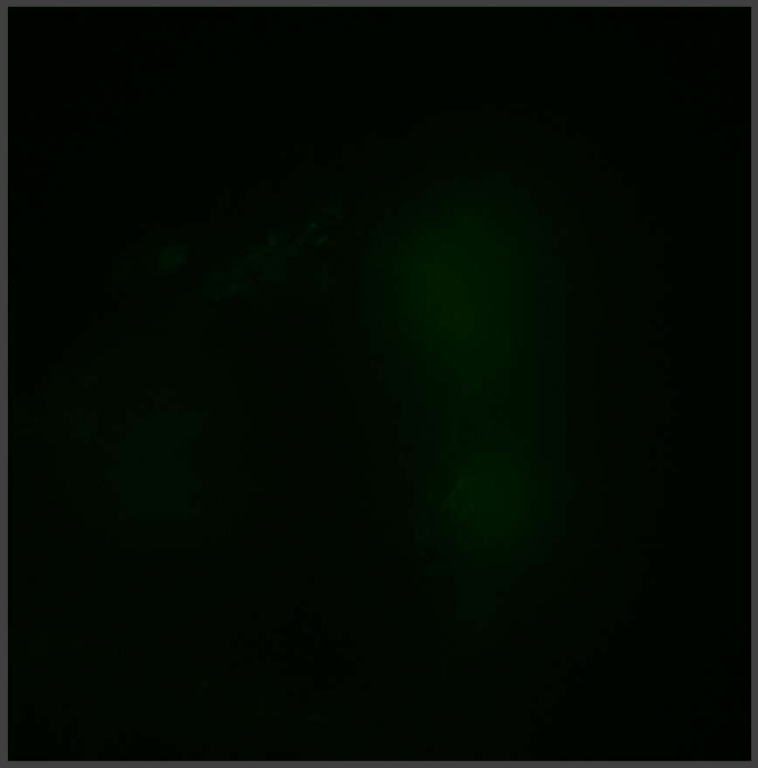


InViSPIM Lattice Pro vs SP5 intravital

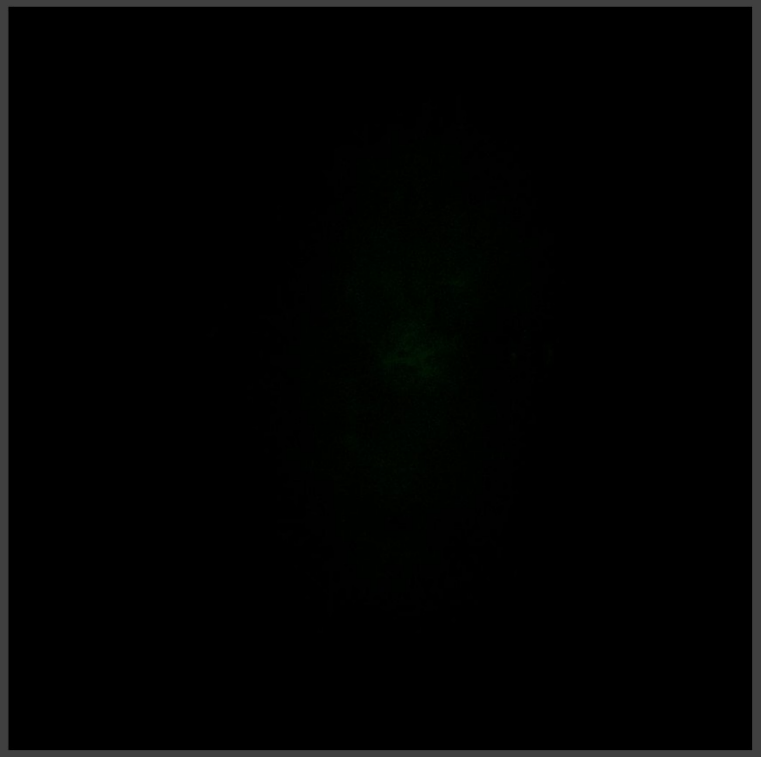


Ventricle Tg(myl7:LifeAct-GFP).
Confocal imaging of a 4.5dpf zebrafish ventricle, capable of resolving myofibril structures of the Tg(myl7:LifeAct-GFP) line. Figure was adapted from Reischauwer et al., 2014.

InViSPIM Lattice Pro vs SP5 intravital

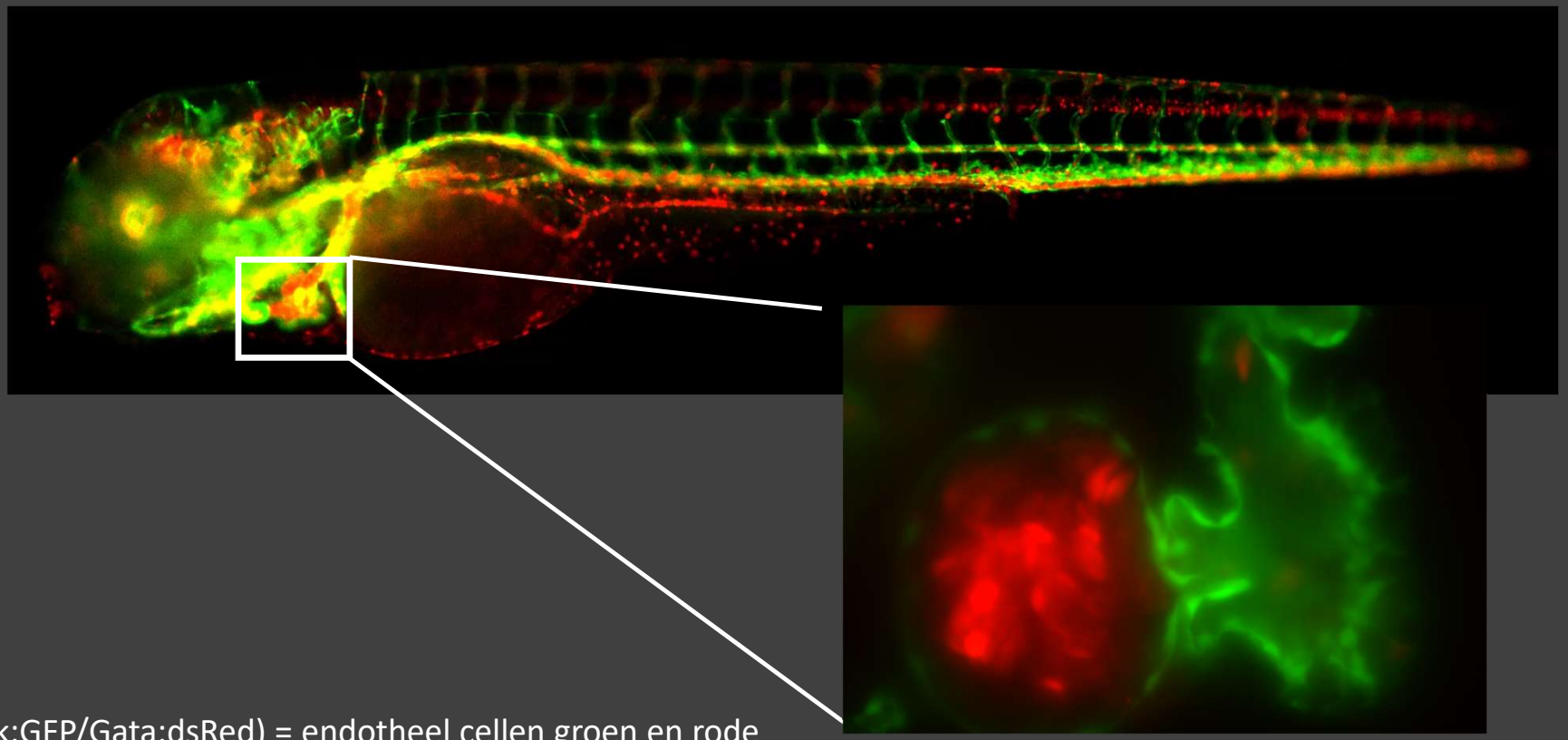


Live-act GFP



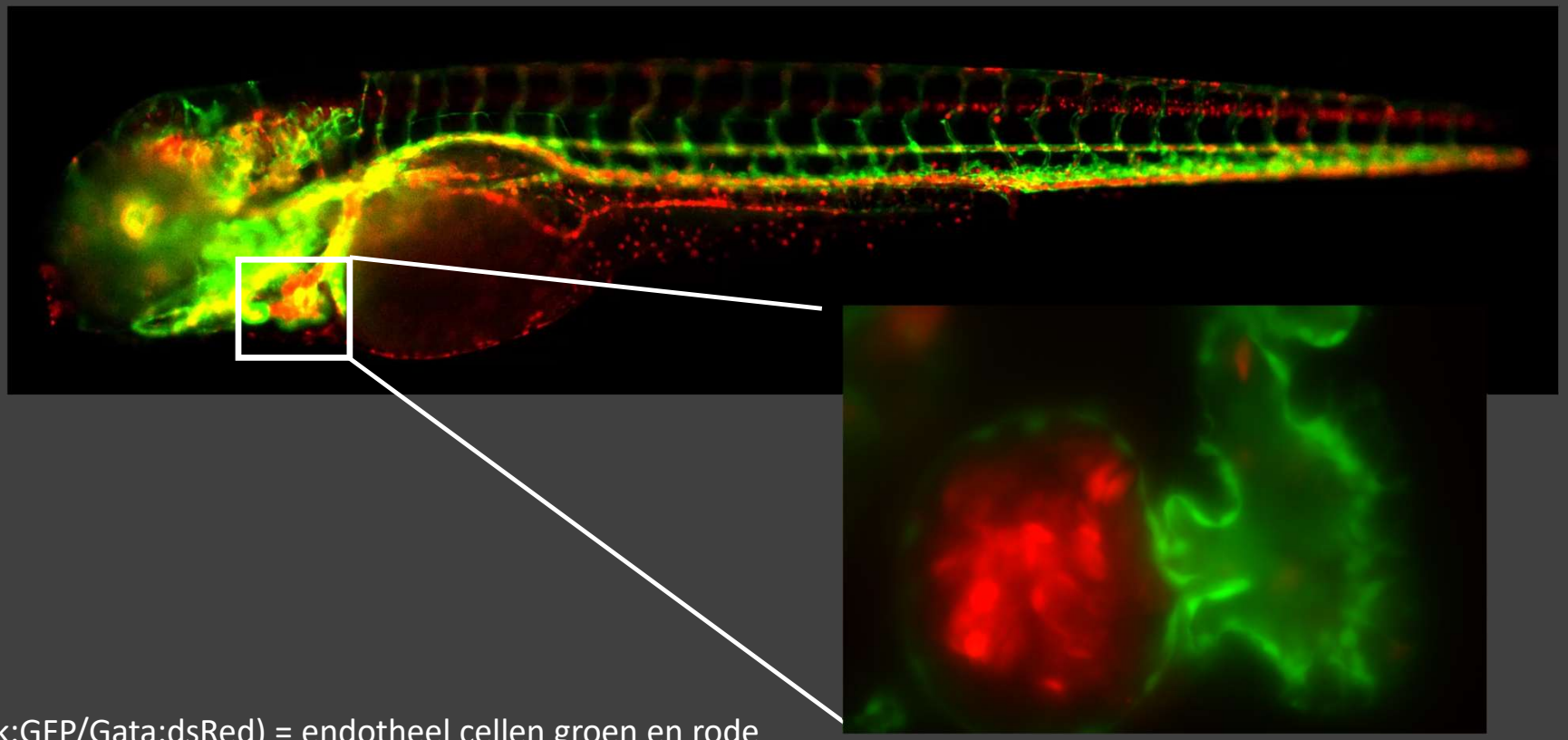
Floris Ophorst

Lightsheet microscopy



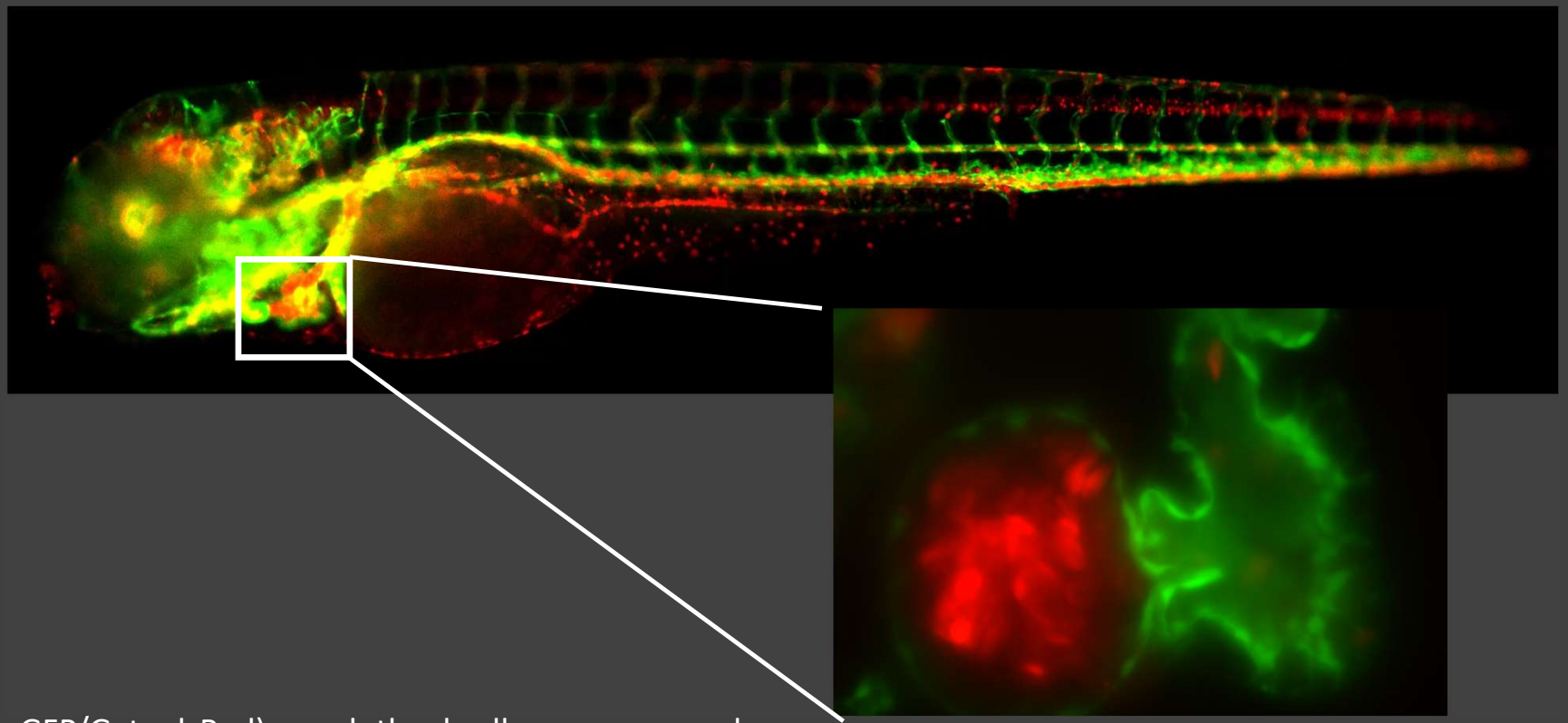
Tg(Flk:GFP/Gata:dsRed) = endotheel cellen groen en rode bloedcellen rood

Lightsheet microscopy



Tg(Flk:GFP/Gata:dsRed) = endotheel cellen groen en rode bloedcellen rood

Lightsheet microscopy



Tg(Flk:GFP/Gata:dsRed) = endotheel cellen groen en rode bloedcellen rood

20x slowed down

Conclusion

Lightsheet microscopy:

- Fast 2D/3D imaging (up to 100fps)
- 3D-cell culture, organoids, C. elegans, embryos, tissues, organs
- Fixed or live
- Optical sectioning capability depends on lightsheet thickness
- Special sample preparation!!!