



# Light Sheet Fluorescence Microscopy and some biological applications

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University Hospital Bonn

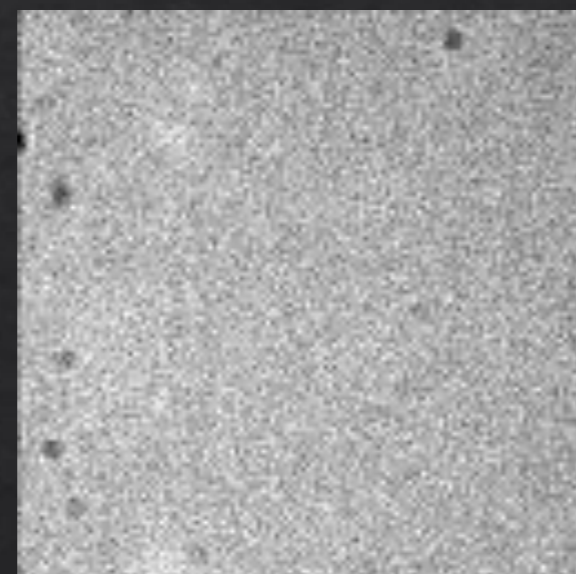
[je.rodriguez-gatica@uni-bonn.de](mailto:je.rodriguez-gatica@uni-bonn.de)

X @JuanEdoRG

*What is light-sheet microscopy?*

# Low contrast & bad axial resolution in epi-illumination microscopy

## Epi-fluorescence microscopy



500 kDa dextran-Atto633 in buffer  
40X, NA 1.2W objective lens  
Image field 19.2  $\mu\text{m}$   
Image acquisition 100 Hz, display 33 Hz

excitation

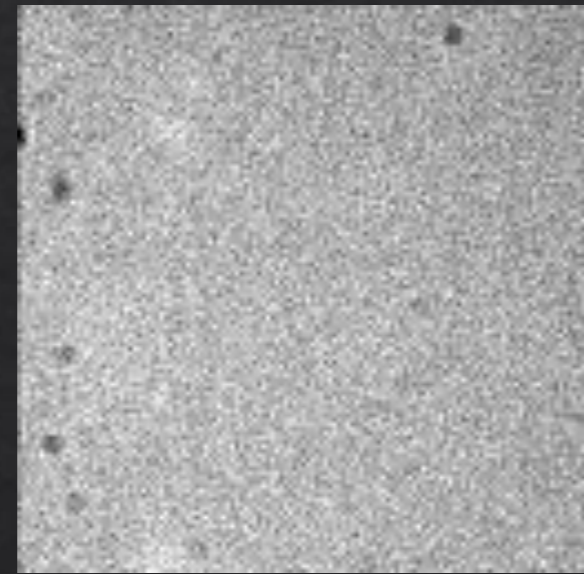
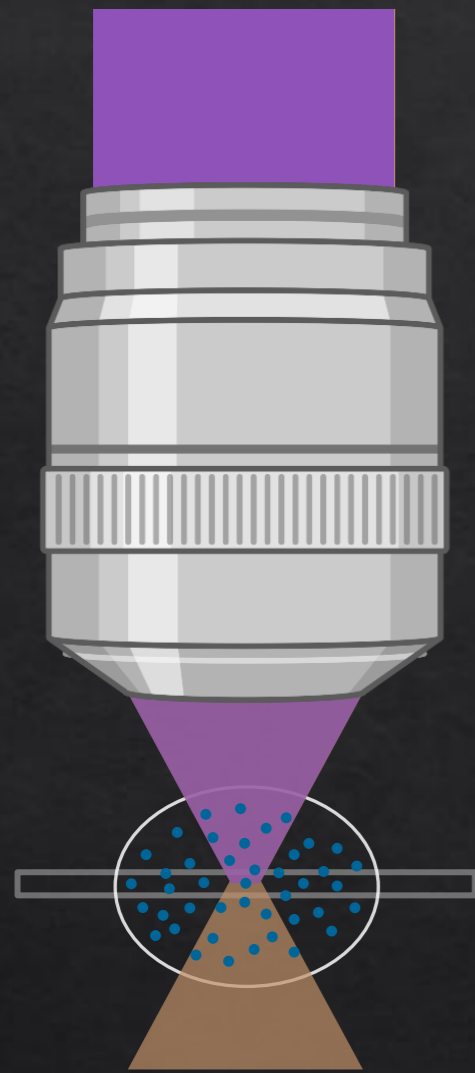


emission



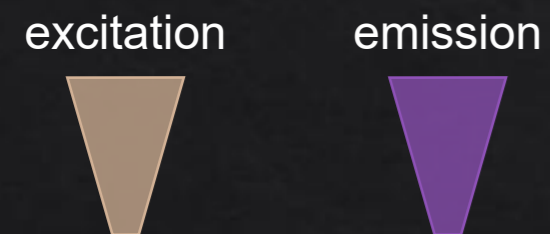
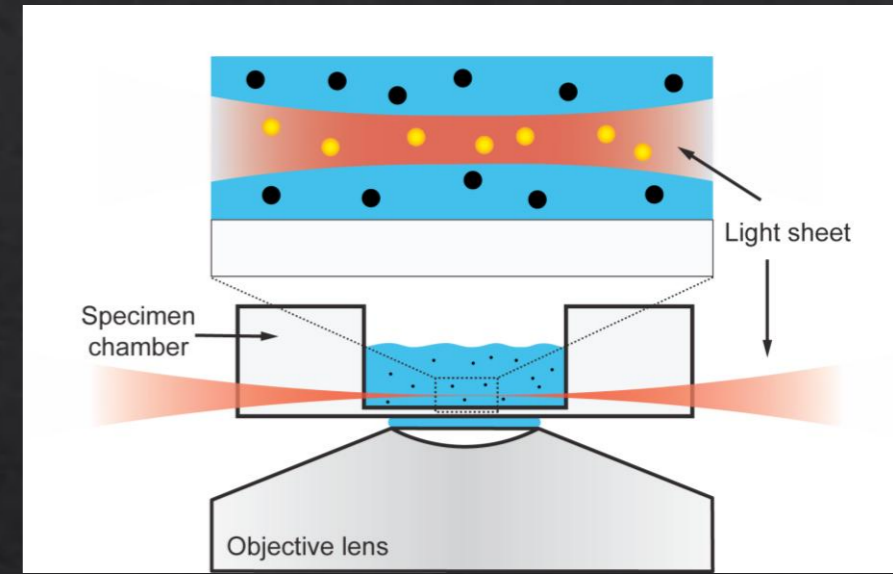
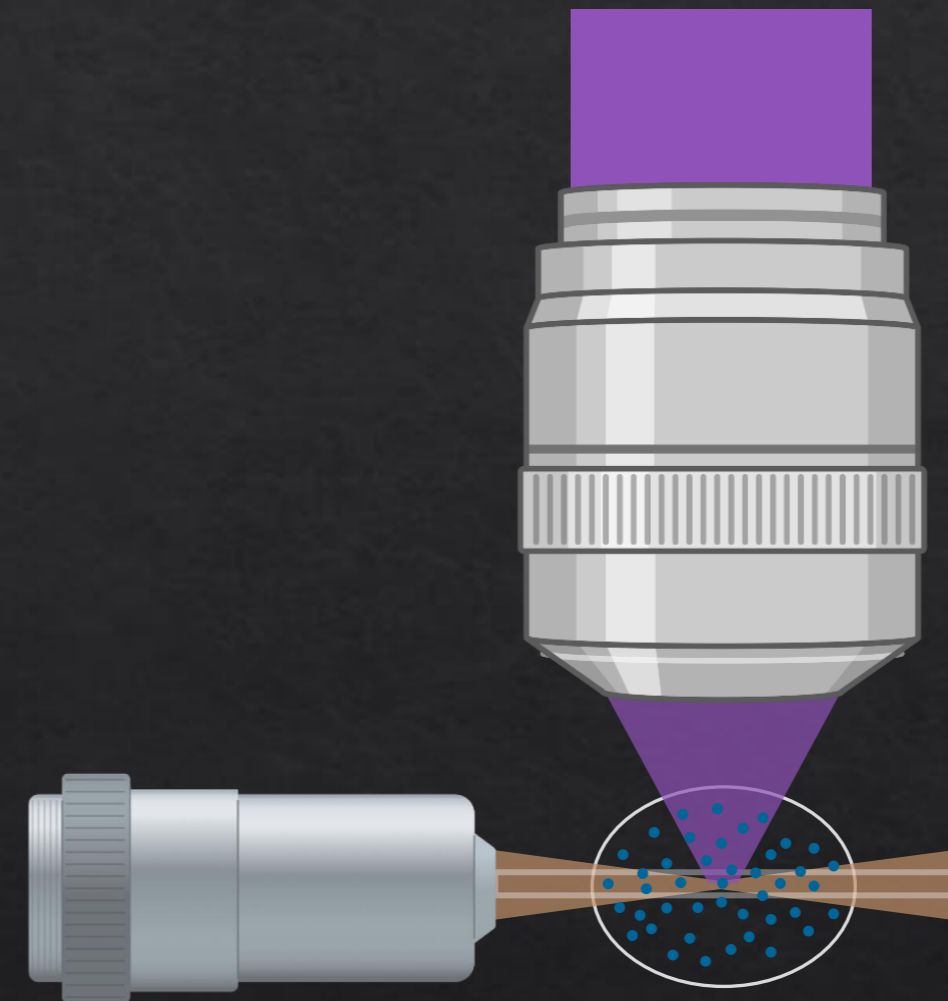
# High contrast by light-sheet based microscopy

## Epi-fluorescence microscopy



500 kDa dextran-Atto633 in buffer  
40X, NA 1.2W objective lens  
Image field 19.2  $\mu\text{m}$   
Image acquisition 100 Hz, display 33 Hz

## Light-sheet illumination



Zsigmondy, 1903  
Voie et al, 1993  
Huisken et al., 2004  
Dodt et al., 2007

.....

# Comparison between epi- and light sheet illumination

## Epi-fluorescence microscopy



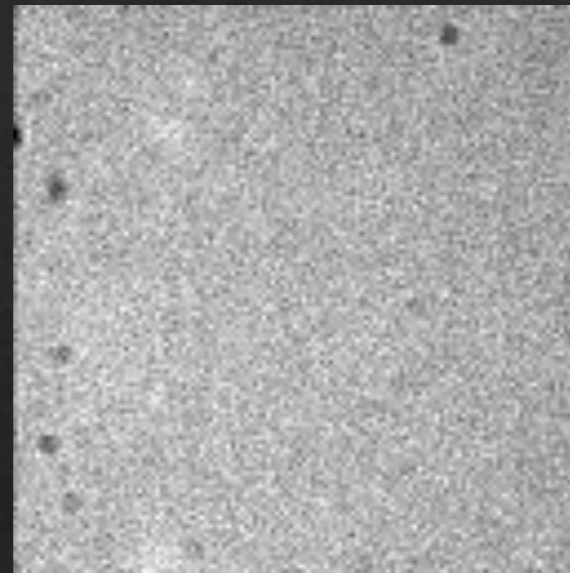
excitation



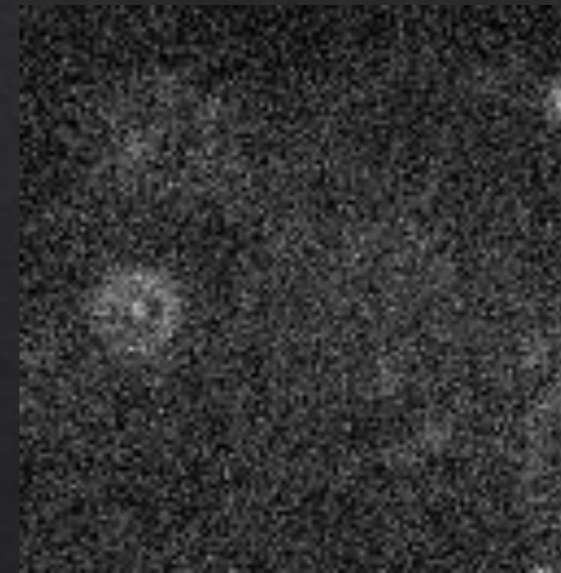
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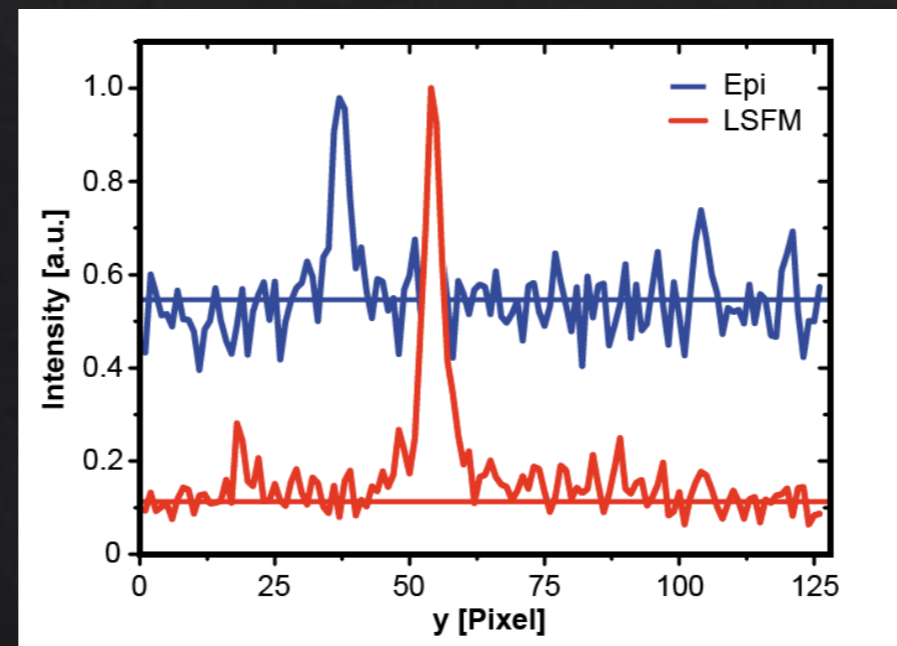
500 kDa dextran-Atto633 in buffer  
40X, NA 1.2W objective lens  
Image acquisition 100 Hz, display 33 Hz



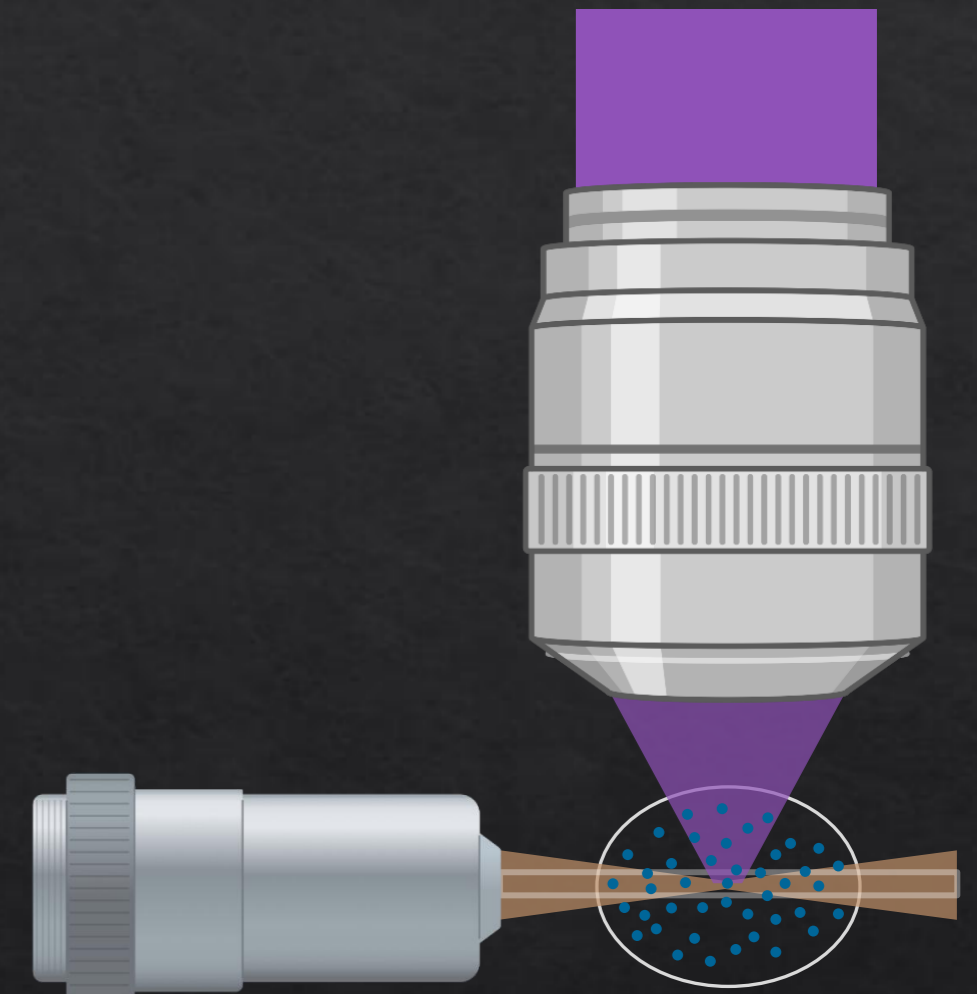
Epi-illumination  
Contrast 0.37



Sheet illumination  
Contrast 0.97

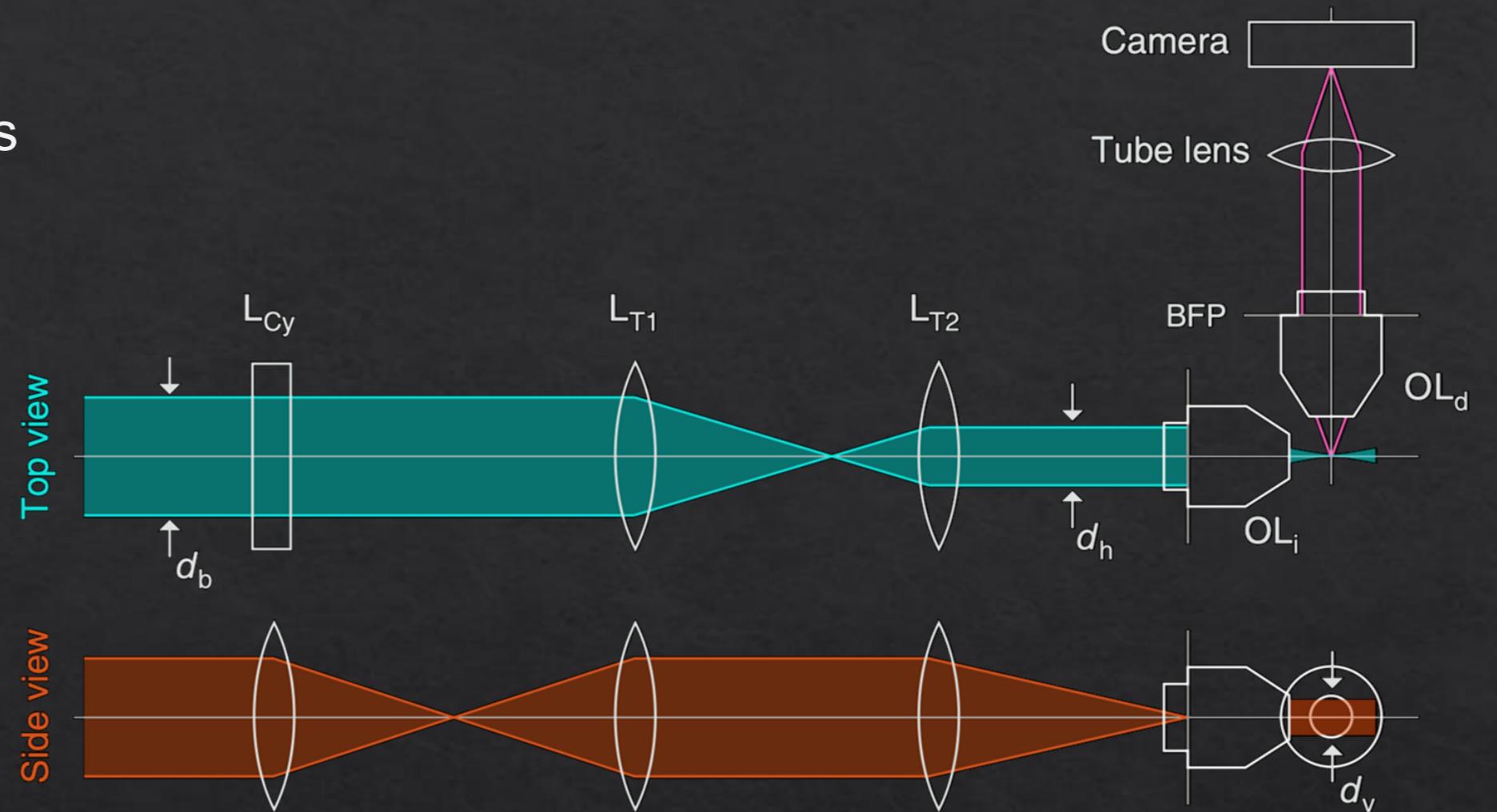
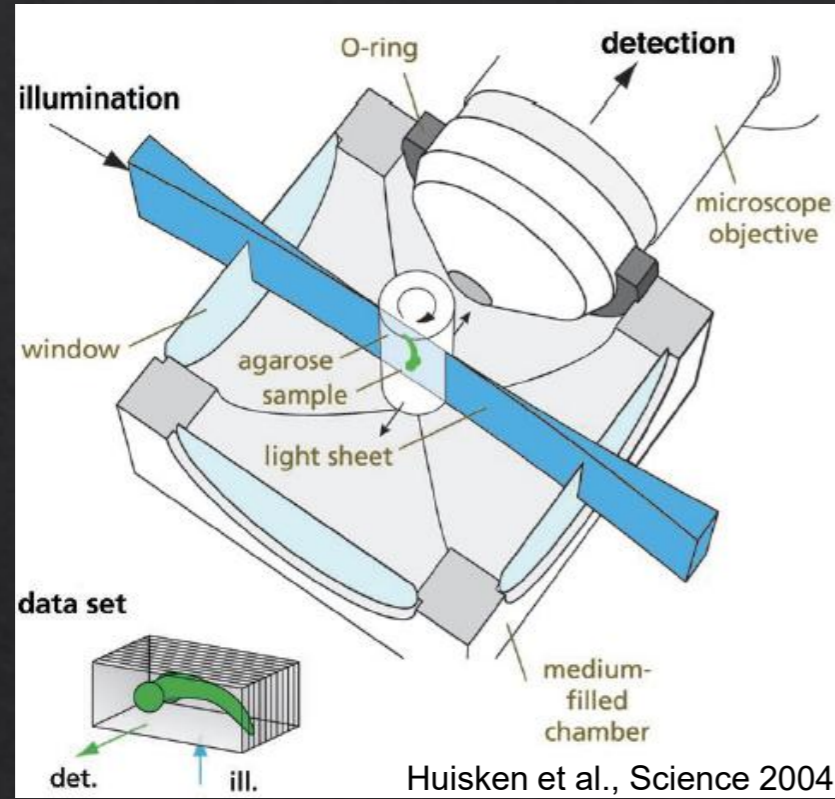


## Light-sheet illumination

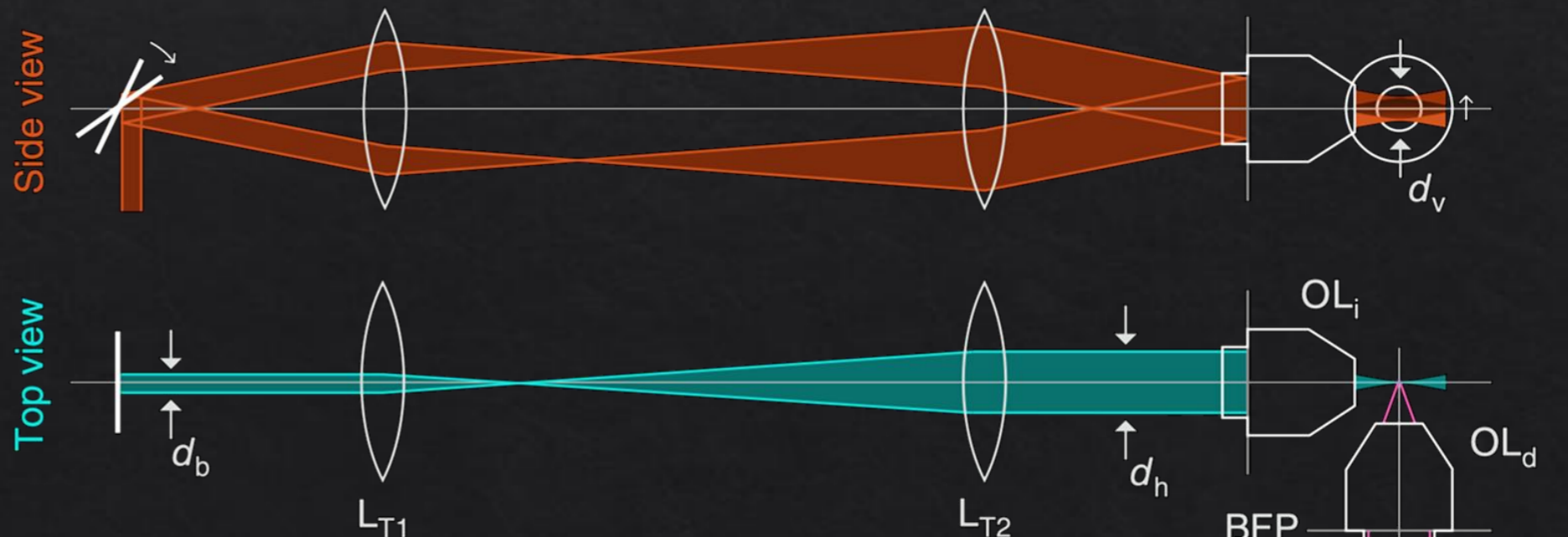
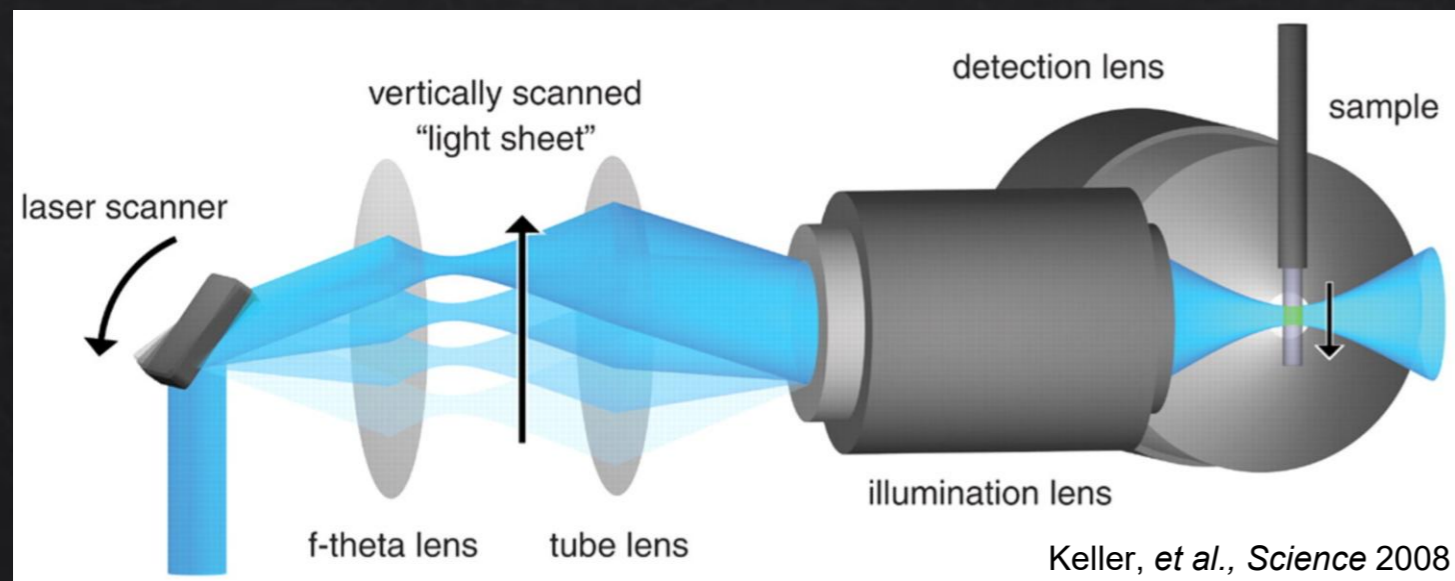


# Principle of light sheet microscopy

## Light Sheet Microscopy using a cylindrical lens

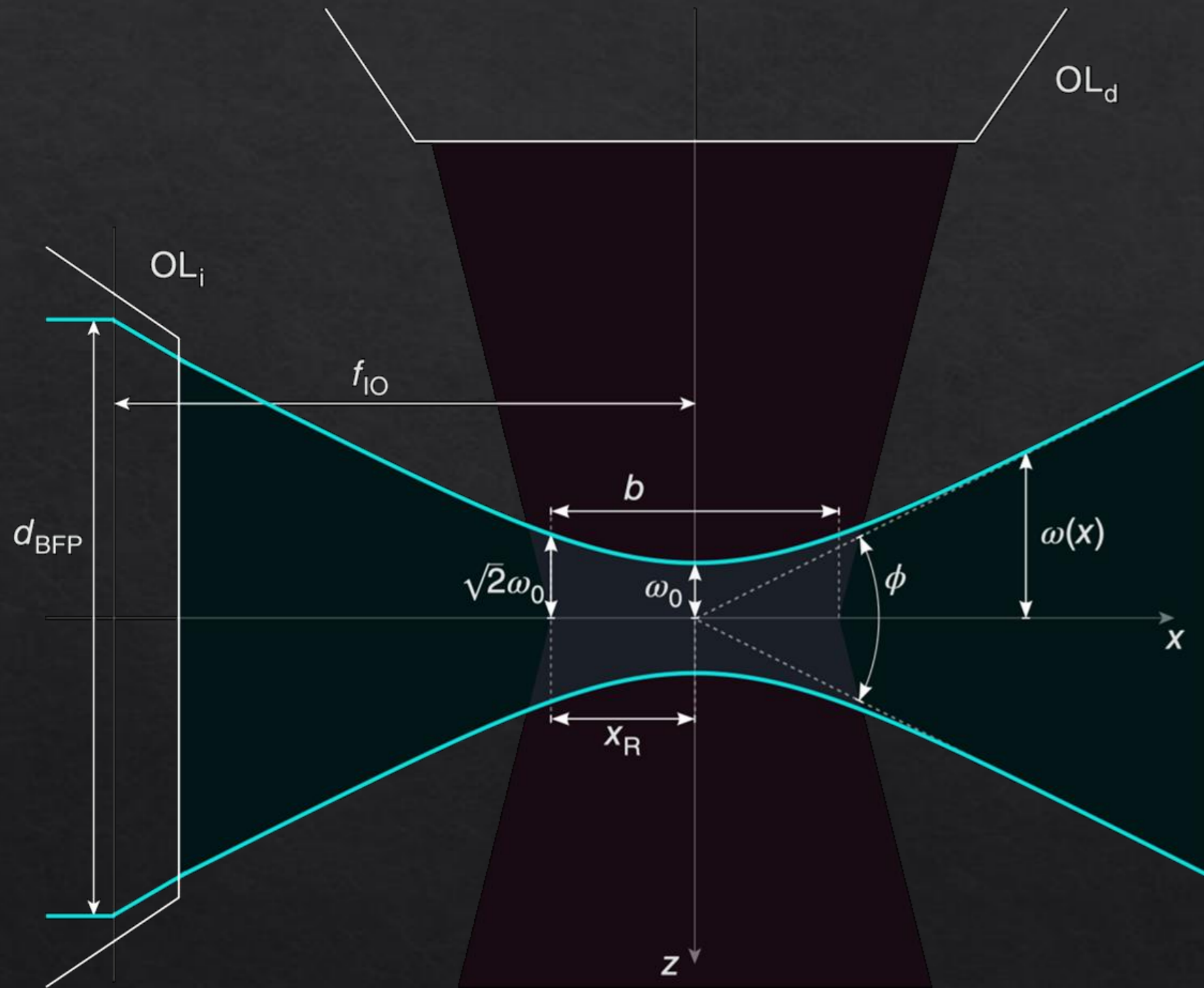


## Scanned Light Sheet Microscopy



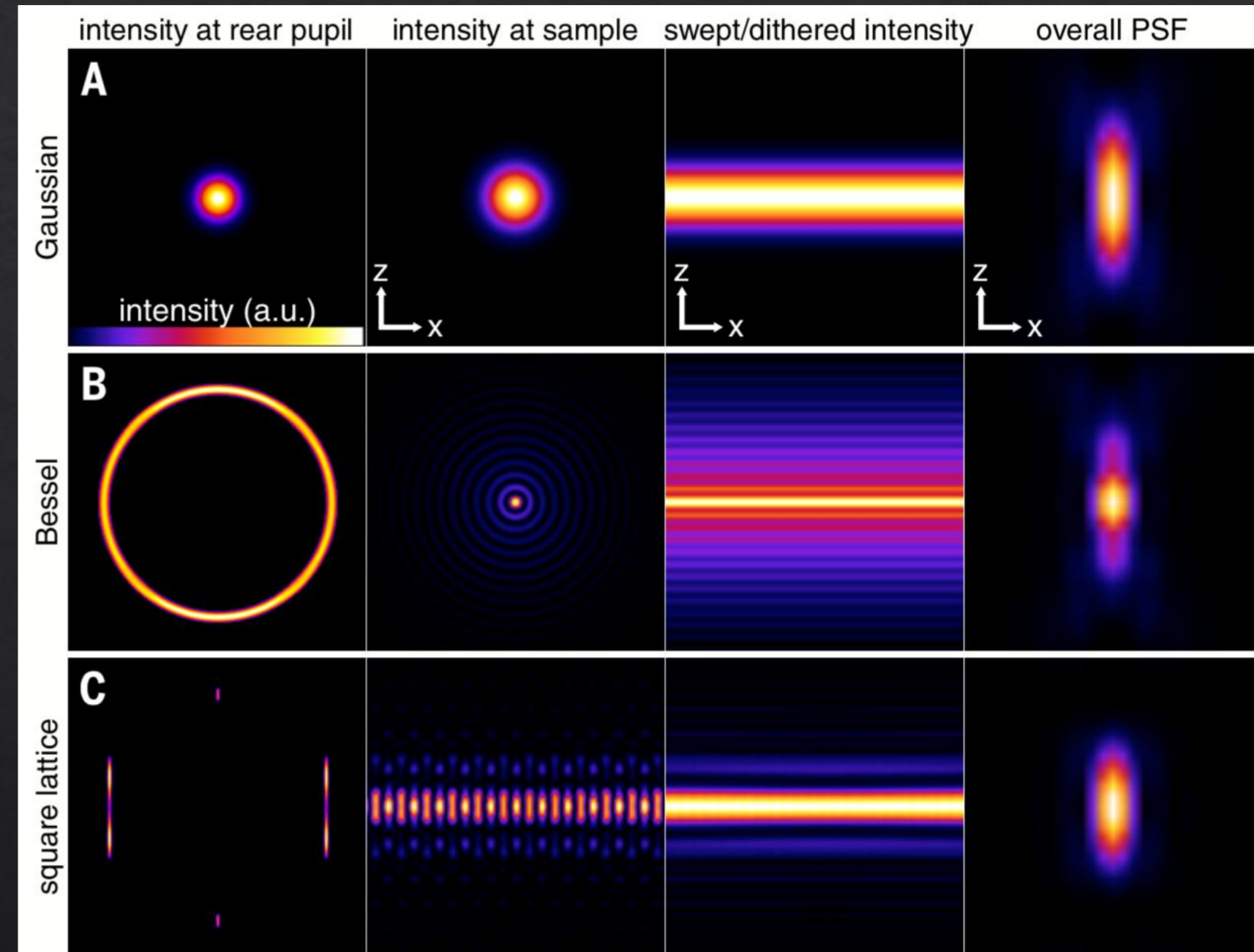
# Relationship between light sheet dimensions and field of view

## Gaussian Illumination



Gaussian beam illumination:  
 limited observation field size  
 Width and length are closely related to each other

## Different light-sheet illumination beams

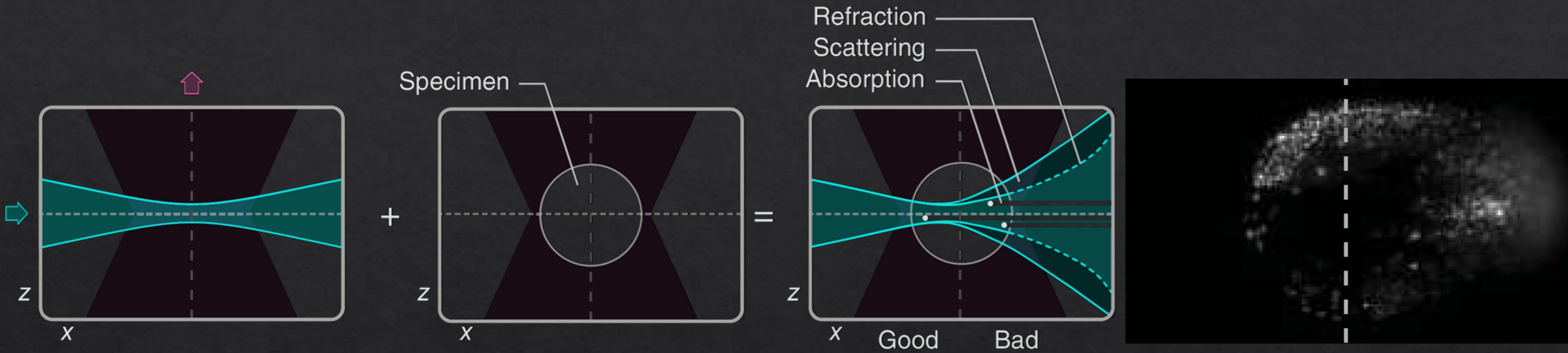


Chen, .... Betzig, Science 2014

Chang et al 2020 → Lattice resolution: 220 nm vs  
 Gaussian resolution 250 nm

# Light sheet interaction with tissue

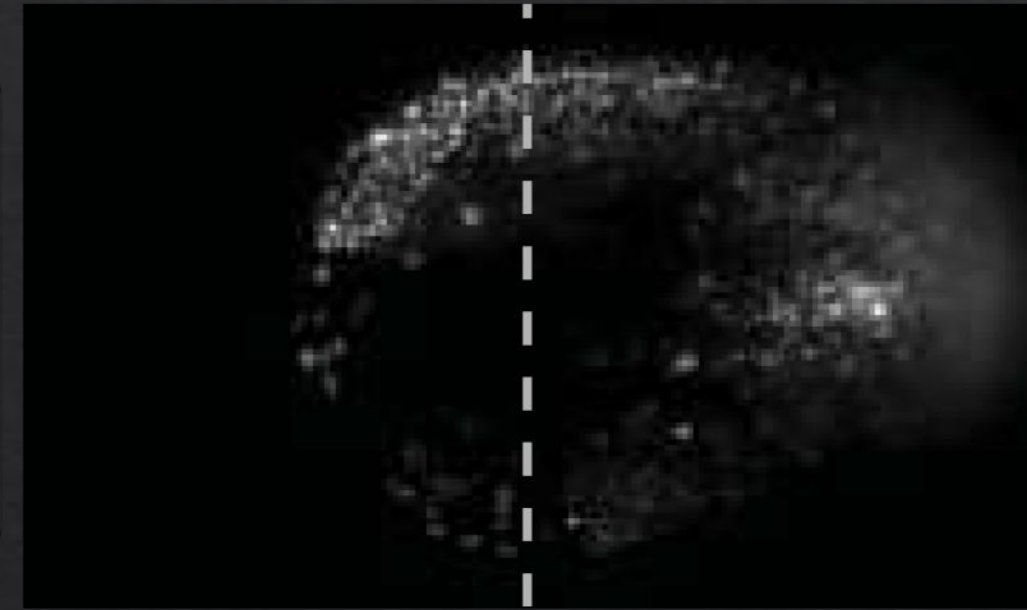
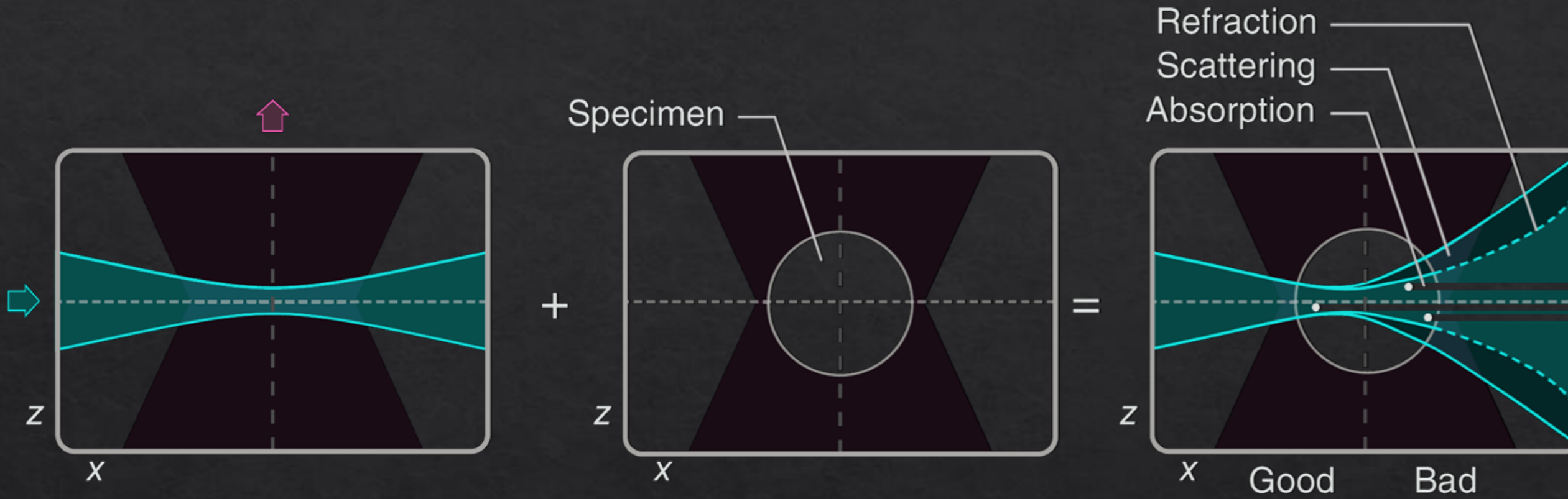
## Single-sided illumination



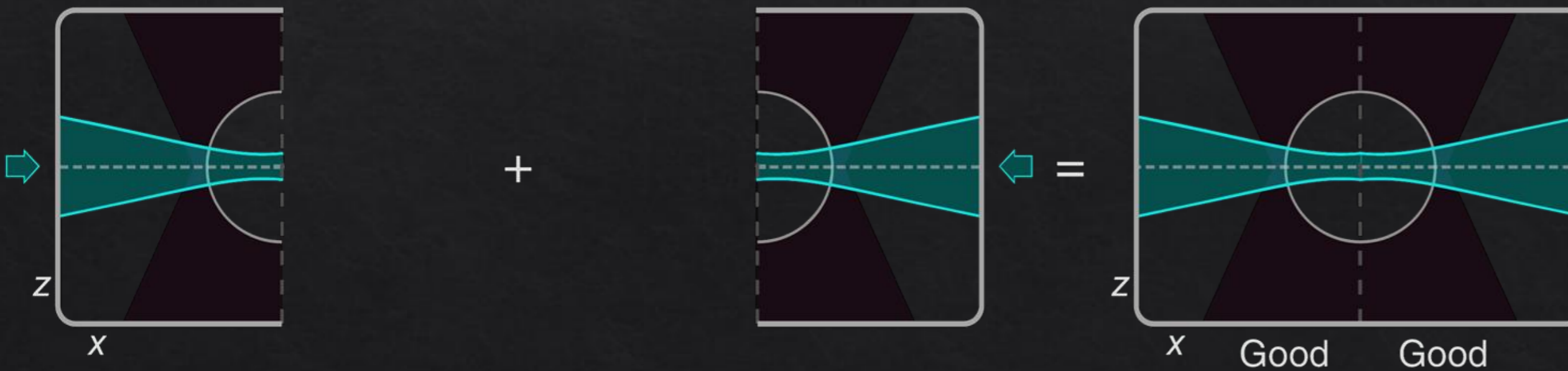


# Light sheet interaction with tissue

## Single-sided illumination

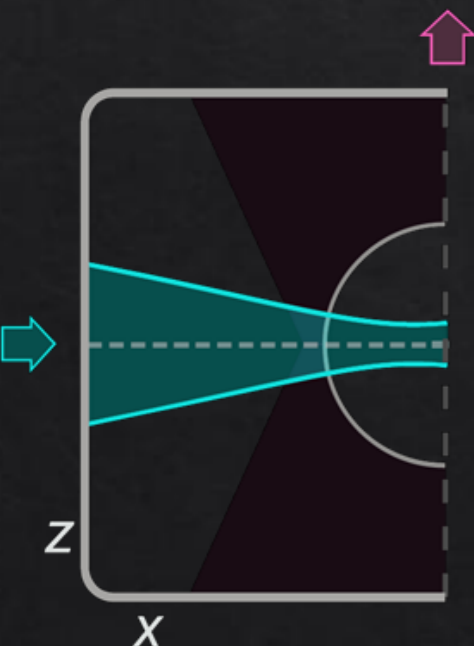
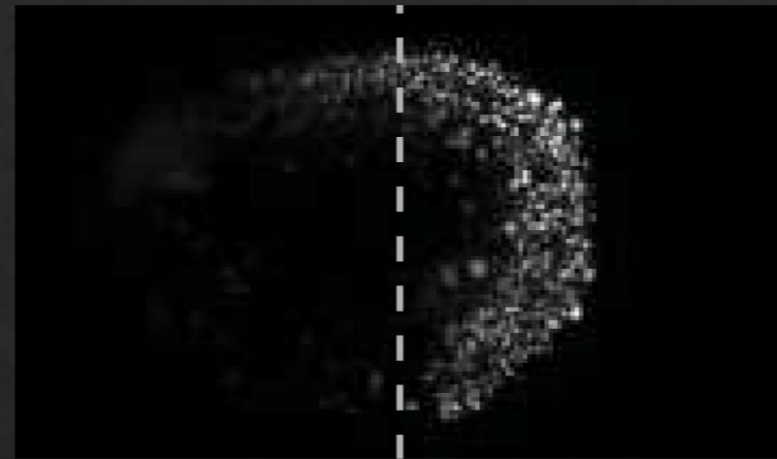
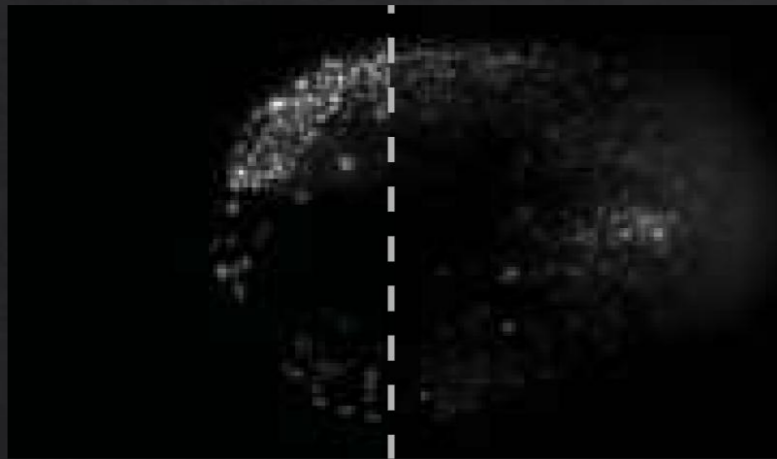


## Double-sided illumination

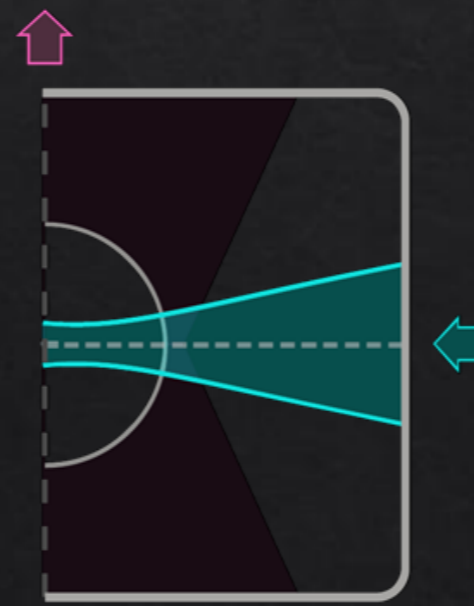


# Light sheet interaction with tissue

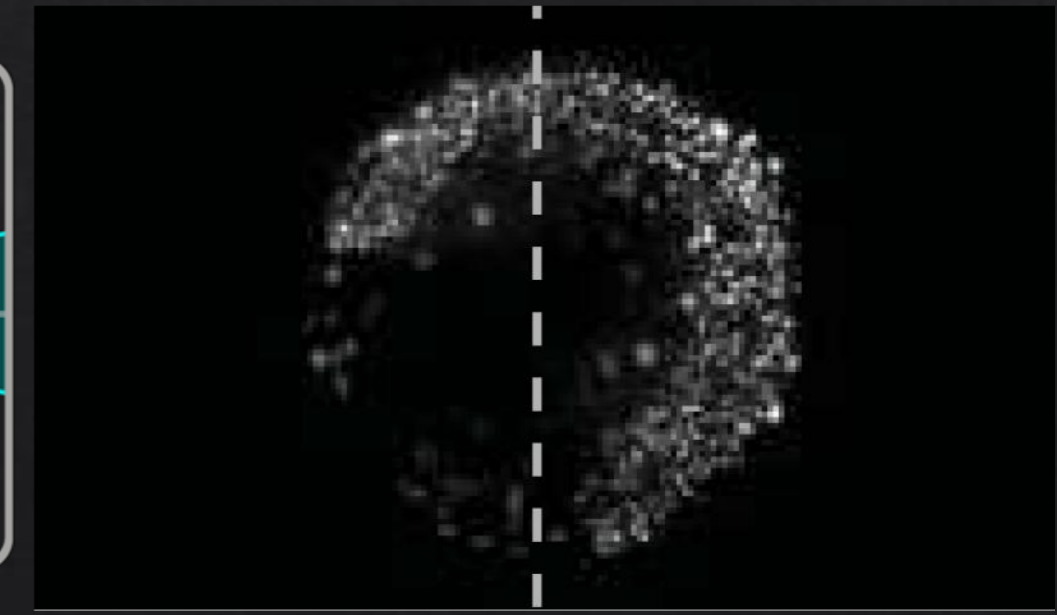
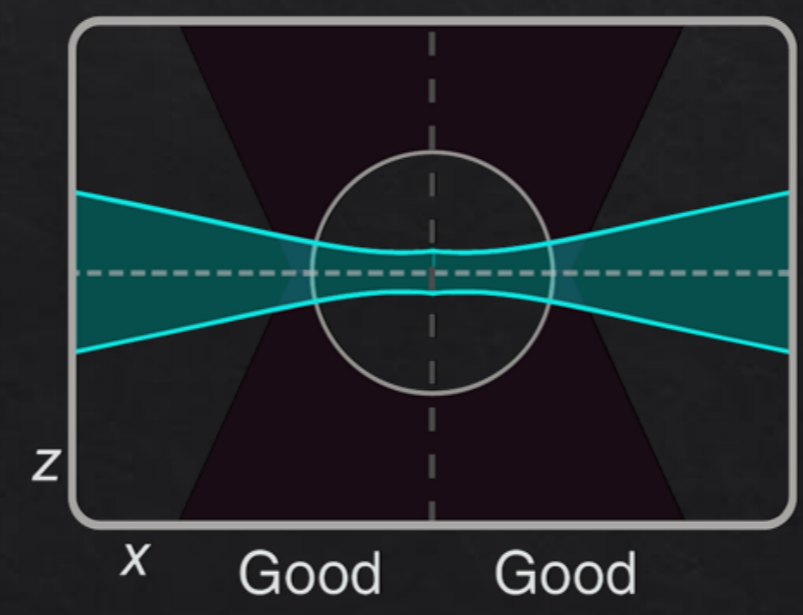
Double-sided illumination



+

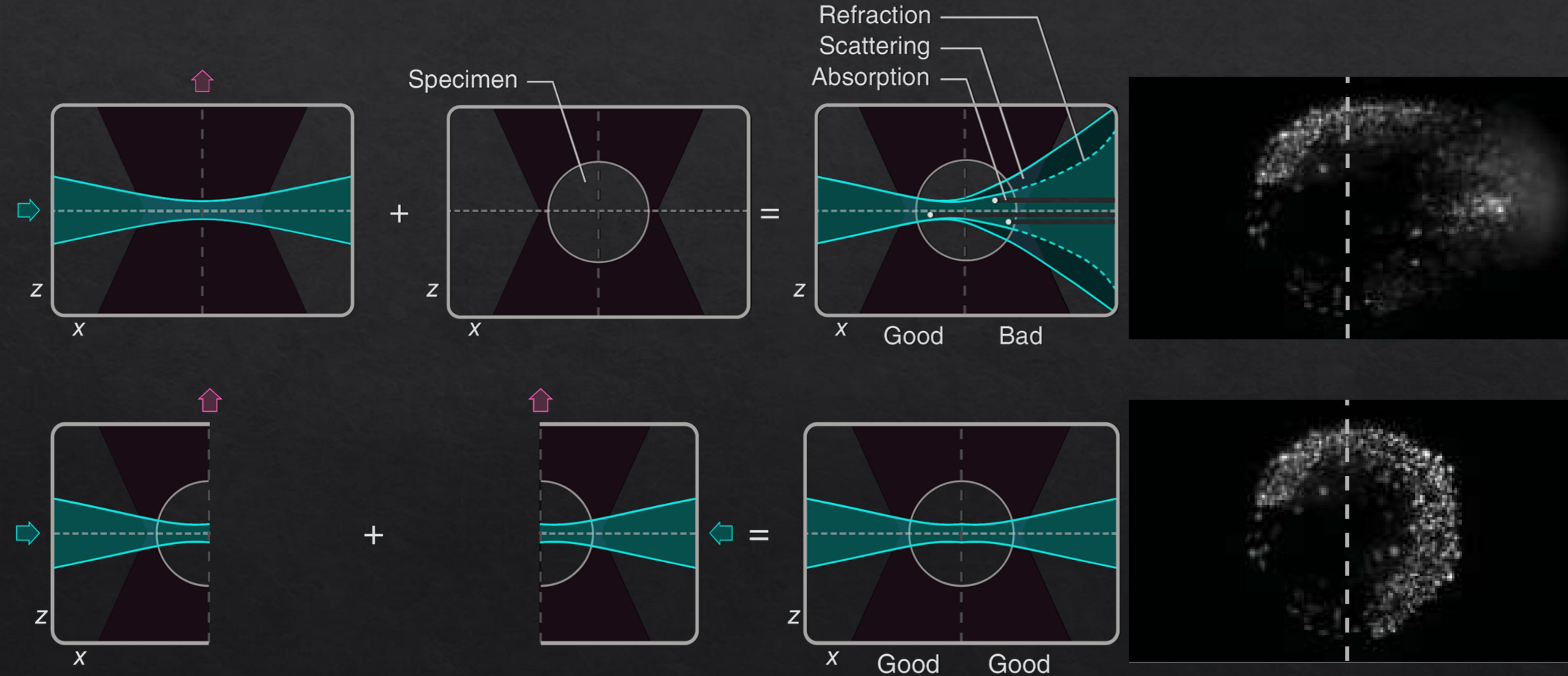


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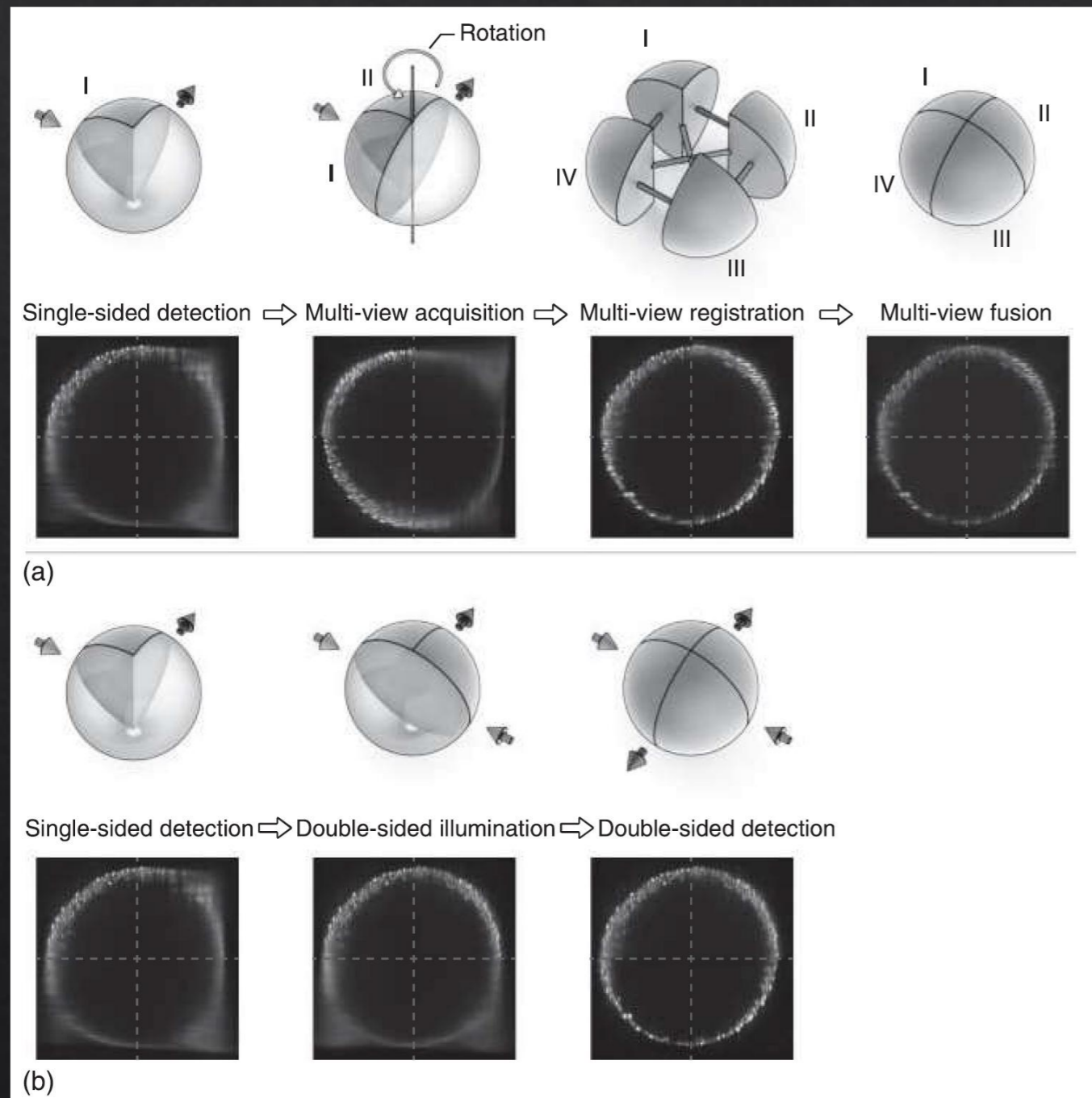
# Light sheet interaction with tissue

Correction of sample-induced effect on the light-sheet

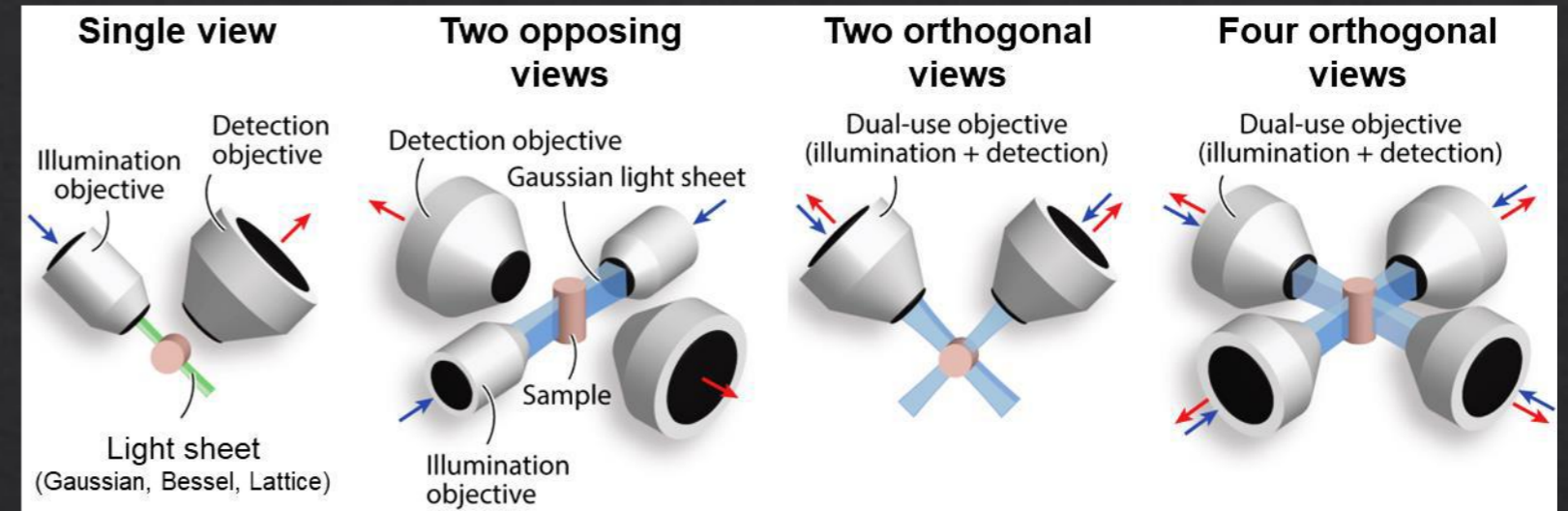


# Multiview imaging

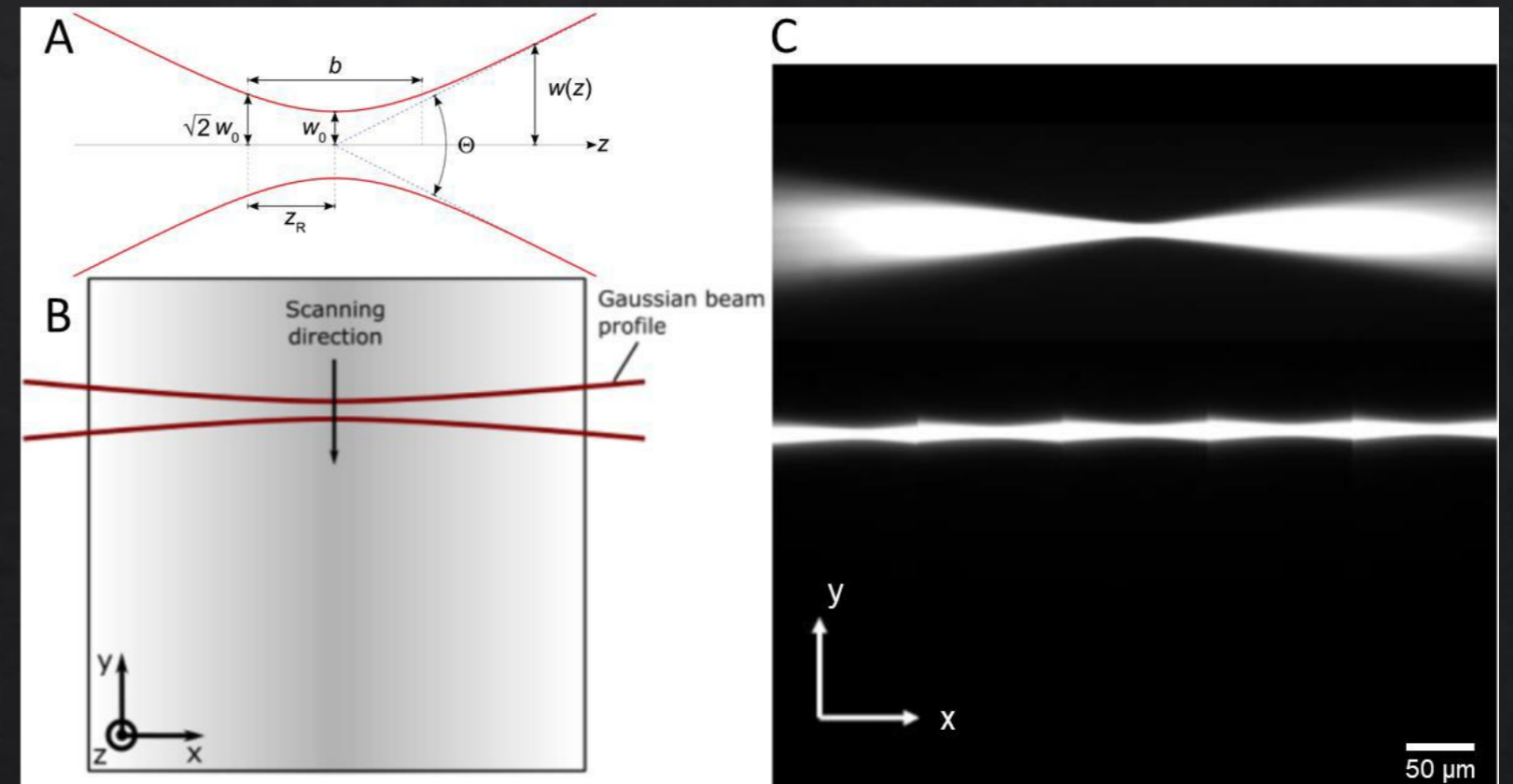
## Multiview acquisition strategies



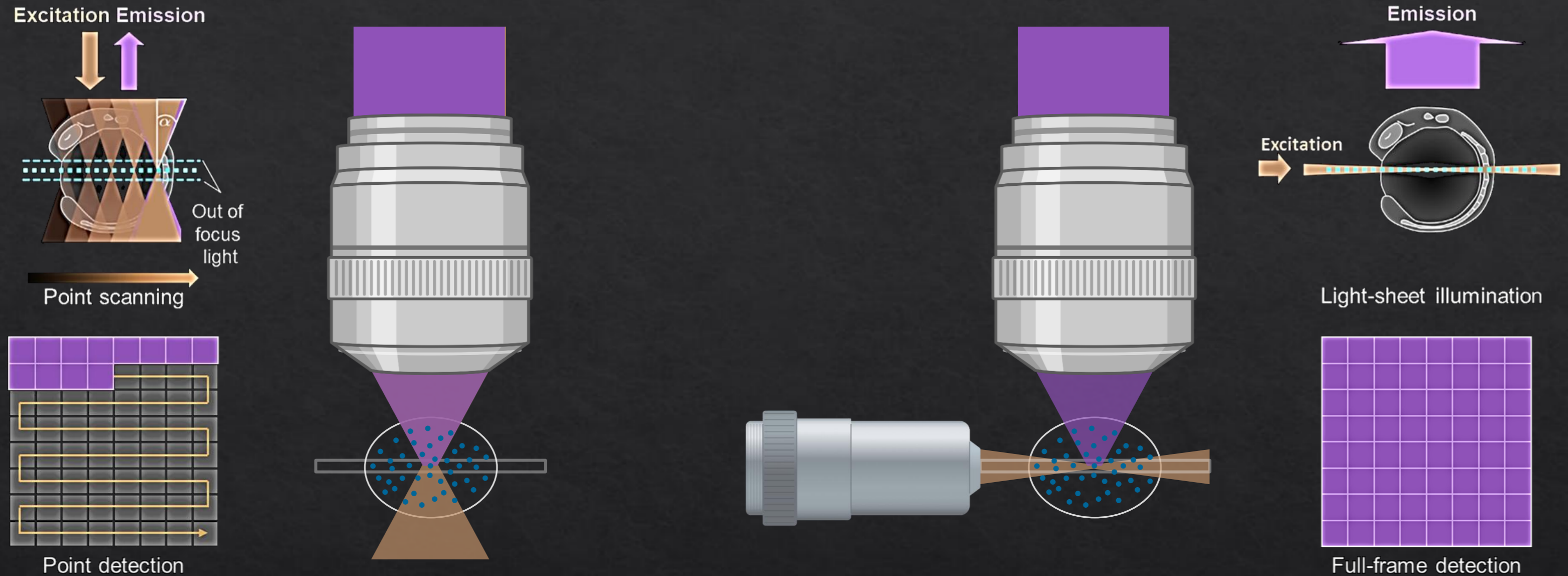
## Different Lens configuration



## Gaussian beam tiling



# Comparison with confocal scanning microscopy



## Confocal laser scanning microscopy

- Low frame rate
- High photodamage
- Out-of-focus fluorescence excitation
- Removal of out-of-focus and scattered light

## Light sheet fluorescence microscopy

- High frame rate
- Very low photodamage
- No out-of-focus excitation
- Sensitive to absorption and scattered light

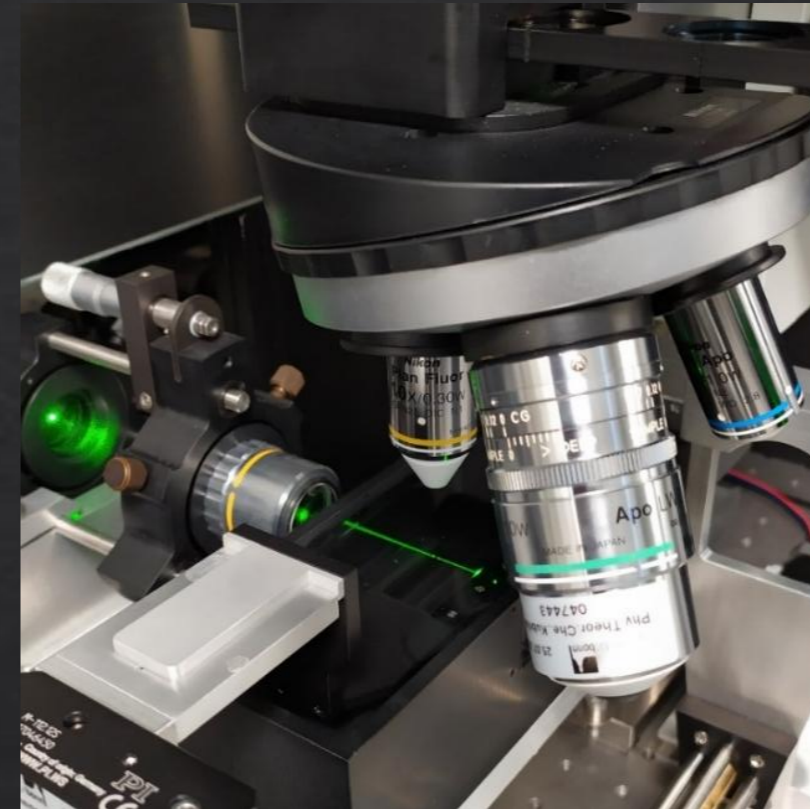
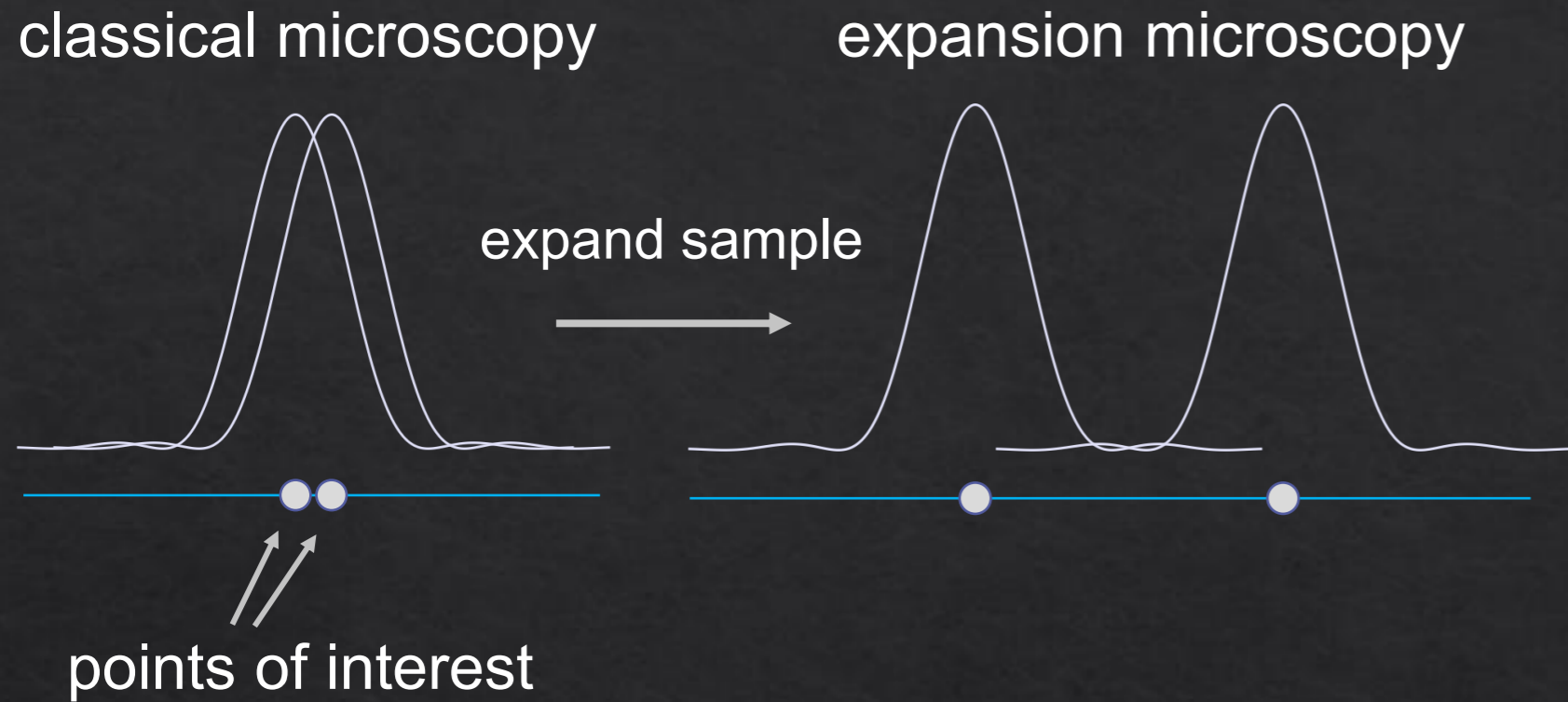
*Application:*  
*Whole sample imaging*

How to analyze the architecture of whole organoids in 3D, revealing also the position and orientation of cellular and subcellular structures?

**What do we need to achieve our aim?**

- 3D architecture preservation
- Whole-organoid imaging
- Super-resolution (<250 nm)

# LSFM + ExM = LSFEM (Light sheet fluorescence expansion microscopy)



- LSFM Characteristics:**
- ✓ Optical sectioning
  - ✓ High contrast
  - ✓ Low photo-bleaching
  - ✓ Fast acquisition rates
  - ✗ Big data generation



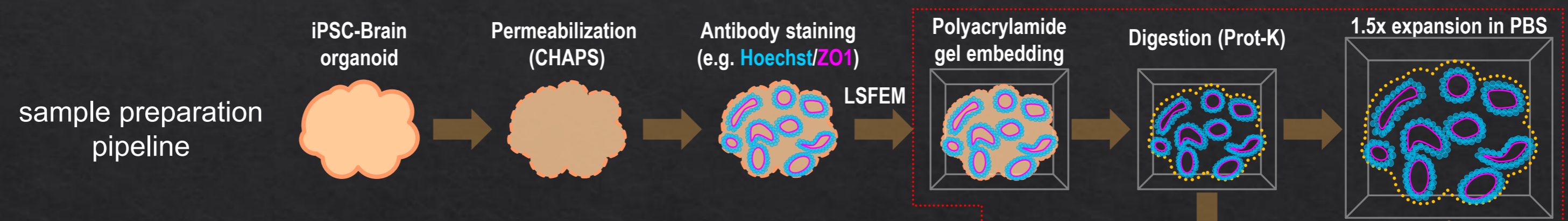
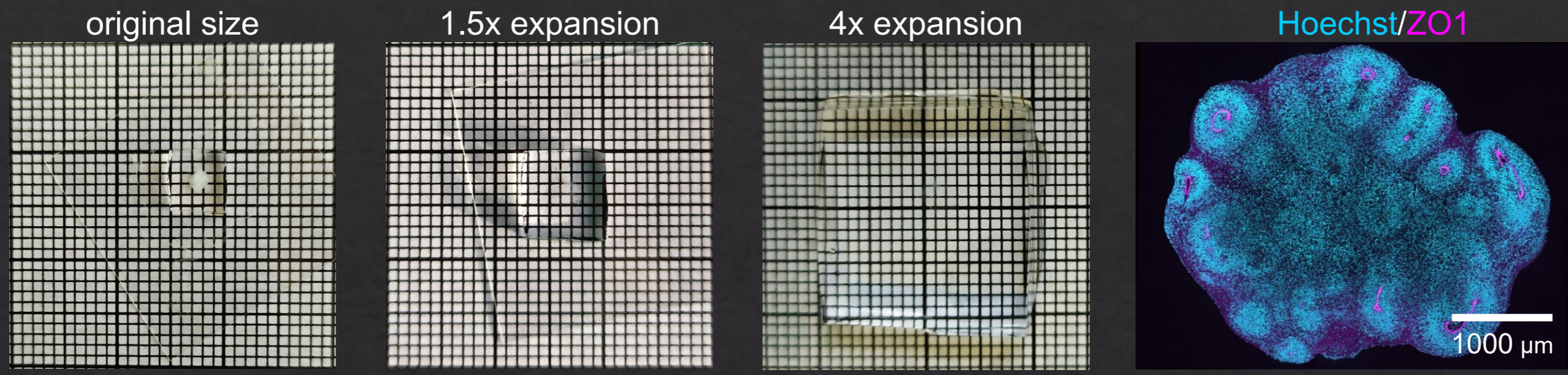
## ExM Characteristics:

- ✓ Sample transparent
- ✓ Refraction index like water
- ✓ Optimized protocol for thick samples
- ✓ Preservation of fluorescent proteins (FP)
- ✗ Larger sample size

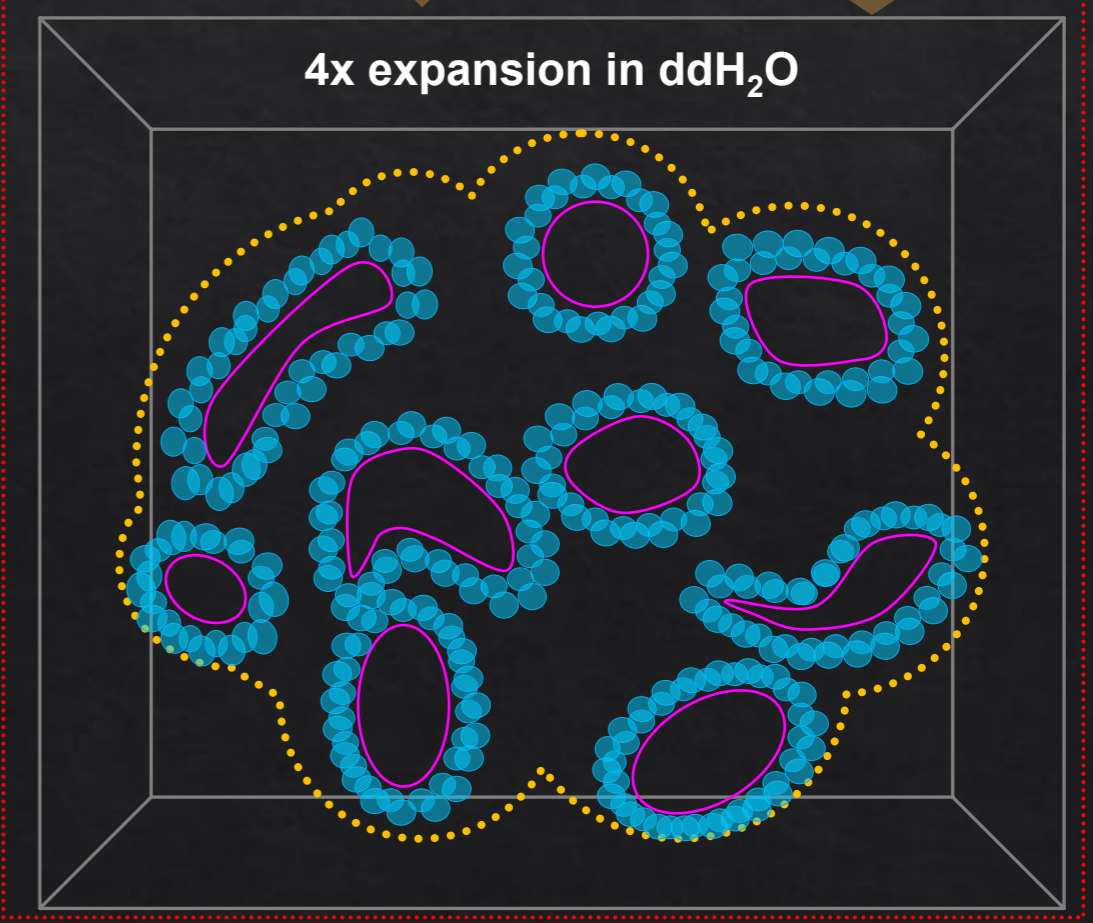
Scale	Objective lens	Effective optical resolution*		Data size per 1 mm <sup>3</sup> sample
		lateral	axial	
<b>Mesoscale</b>	10x, NA 0.3, WI	0.8 μm	11.8 μm	5.5 GB
<b>Microscale</b>	25x, NA 1.1 WI	200 nm	800 nm	500 GB
<b>Nanoscale</b>	37x, NA 1.1 WI	90 nm	300 nm	8 TB

\* considering 4x expansion



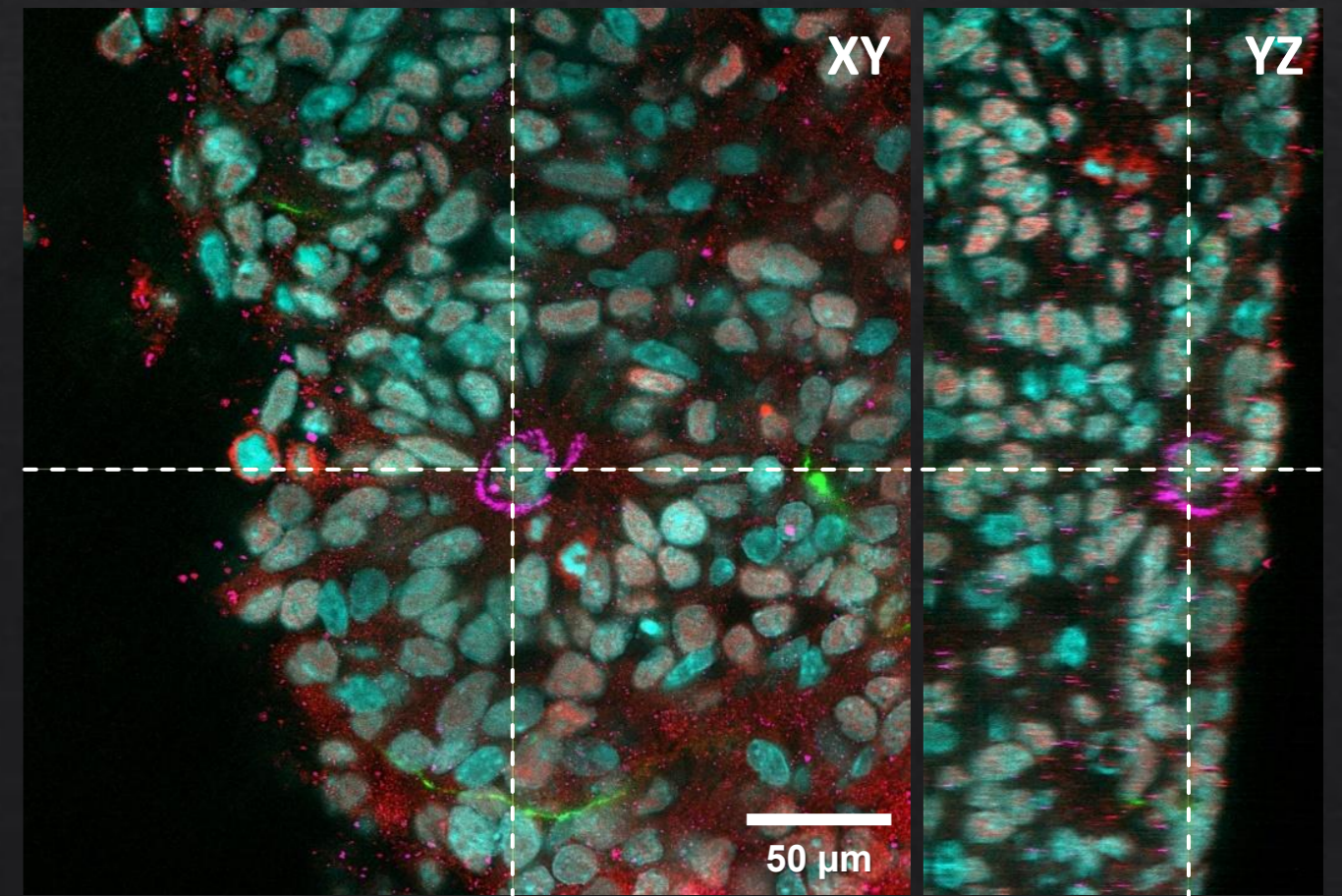
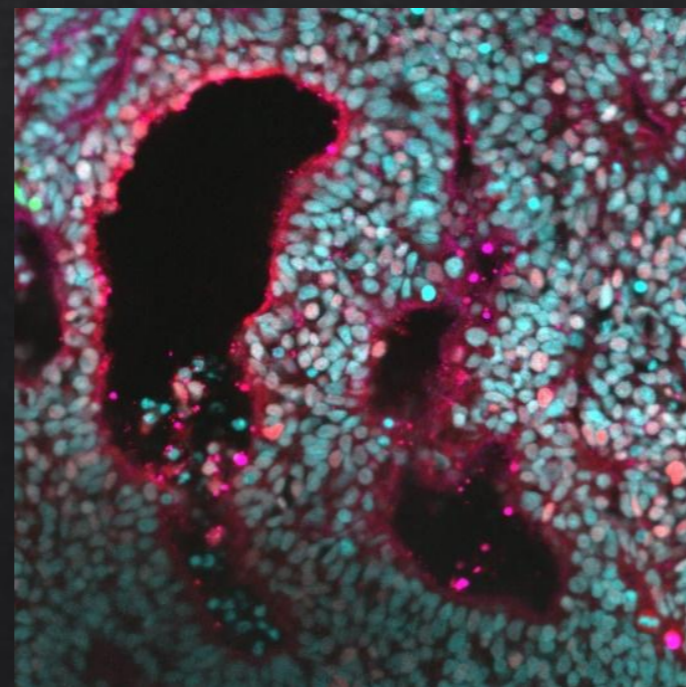
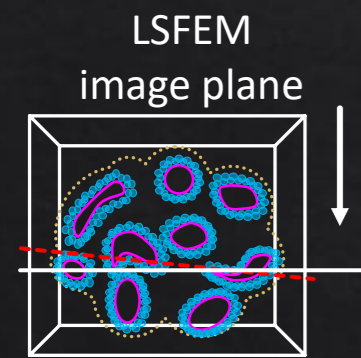
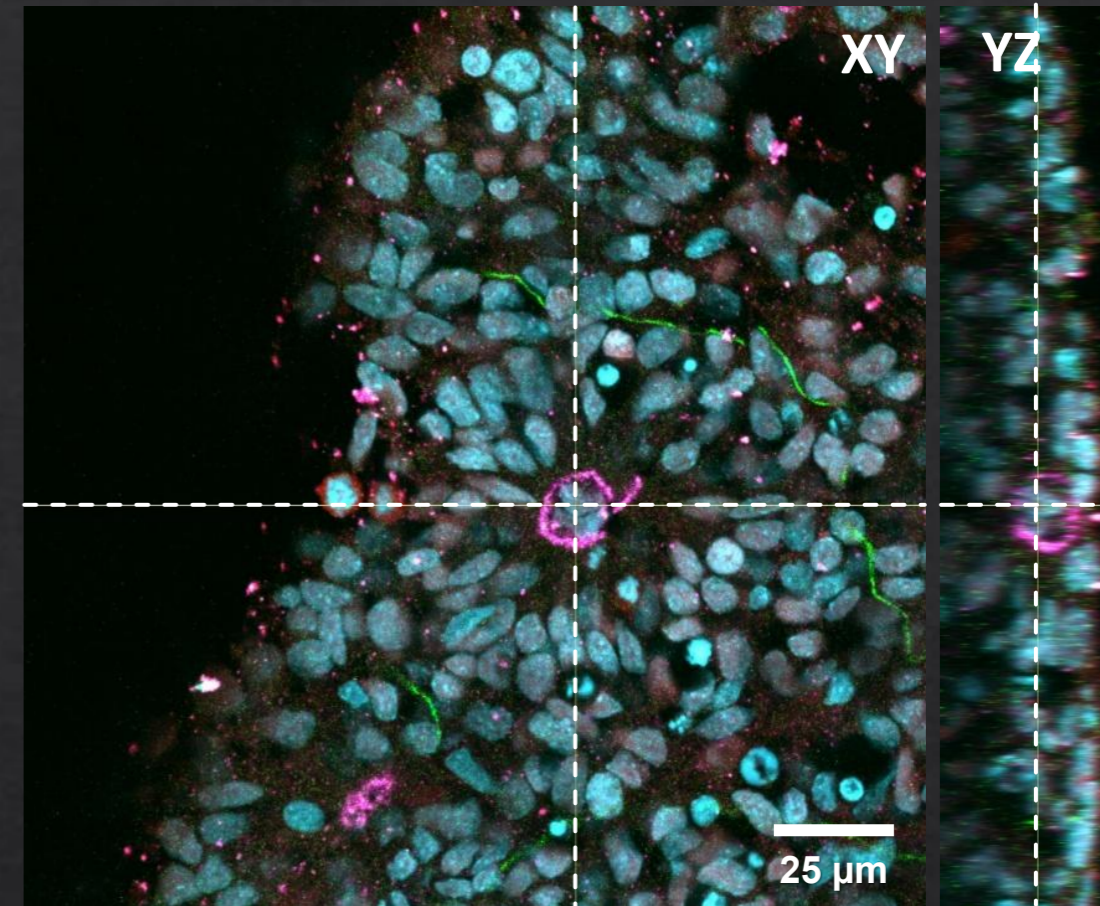
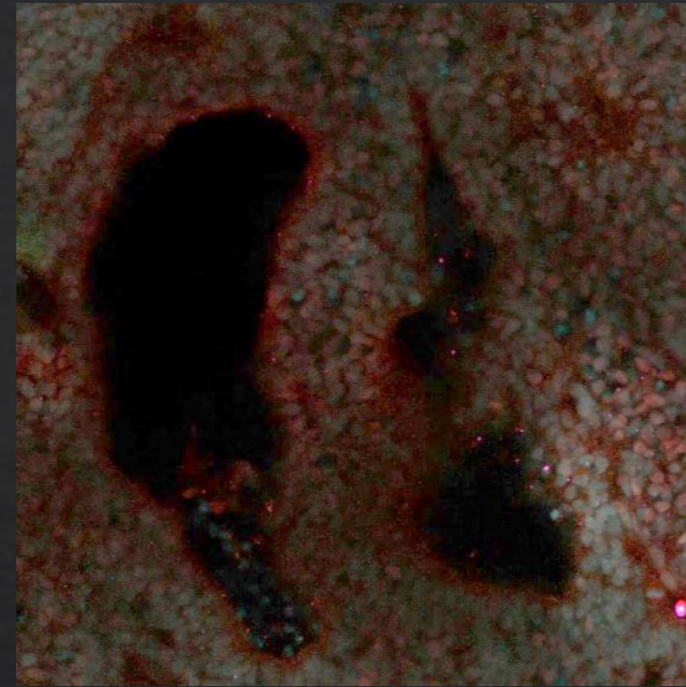
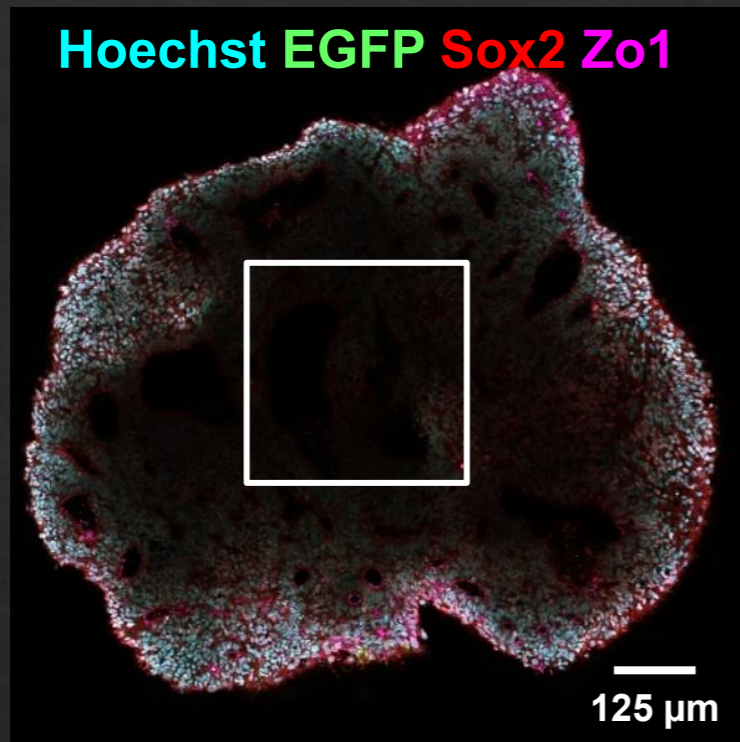
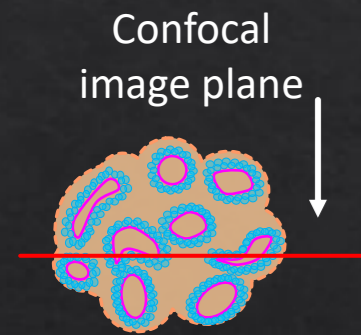


Light-sheet imaging		
Meso-scale	Micro-scale	Nano-scale
1.5x expansion		4x expansion
low mag/NA	high mag/NA	high mag/NA
Whole organoid	ROI (e.g. rosettes)	ROI (e.g. neural proj.)
<ul style="list-style-type: none"> <li>Organoid volumen</li> <li>Surface área</li> <li>Rosettes distribution</li> </ul>	<ul style="list-style-type: none"> <li>Cleavage planes</li> <li>TBR2+ cells/oRGs</li> </ul>	<ul style="list-style-type: none"> <li>Neurites and spines</li> <li>Synapses</li> </ul>



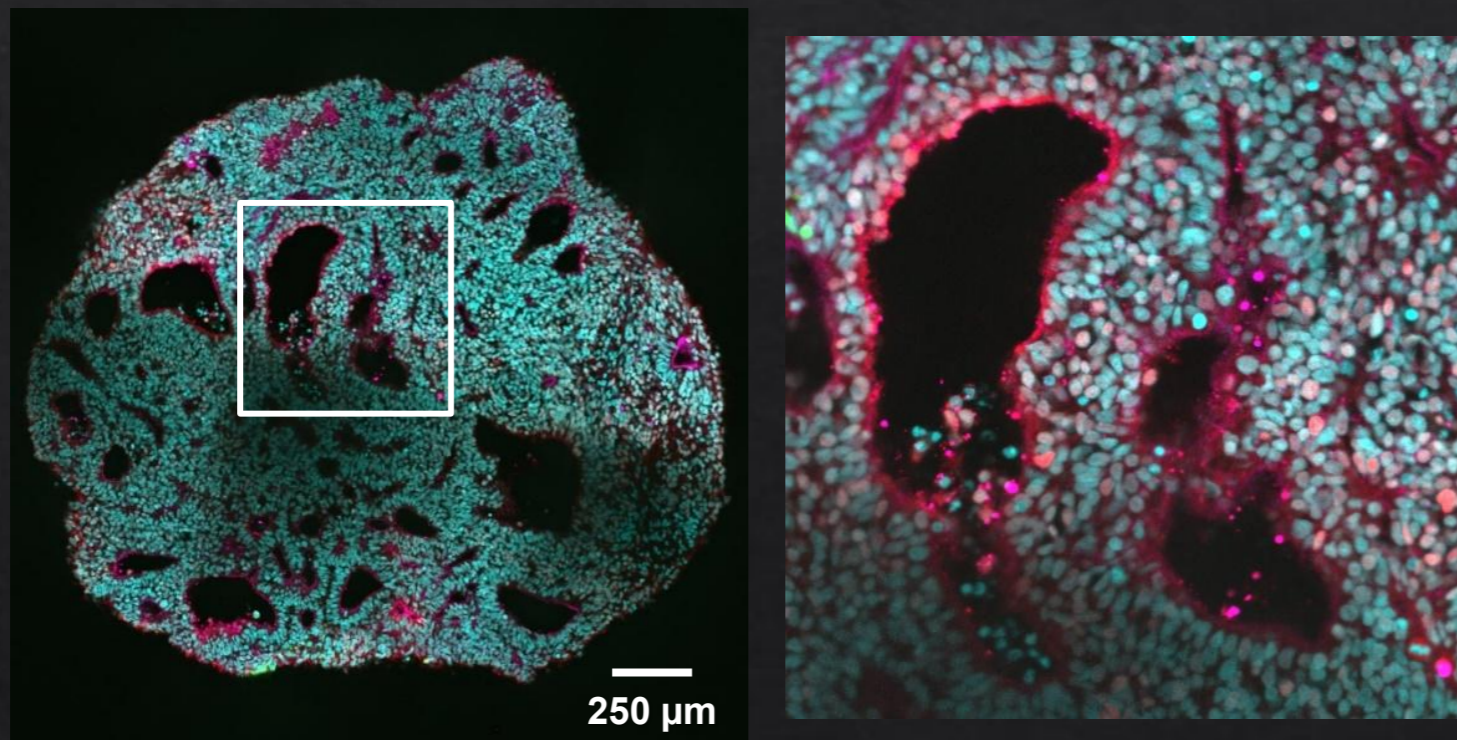
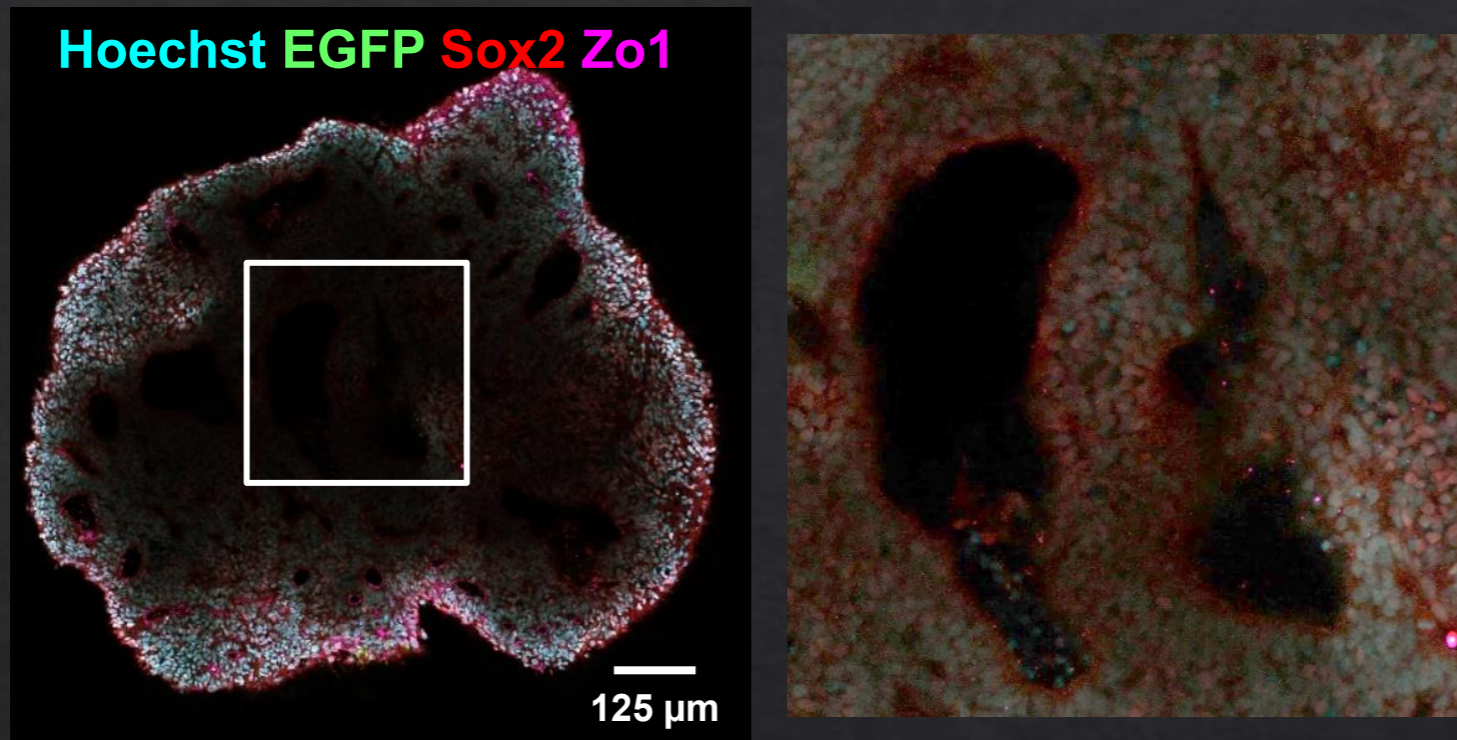
# Advantages: Clearing procedure's effect on both staining fidelity and structural integrity

Whole-organoid imaging capability



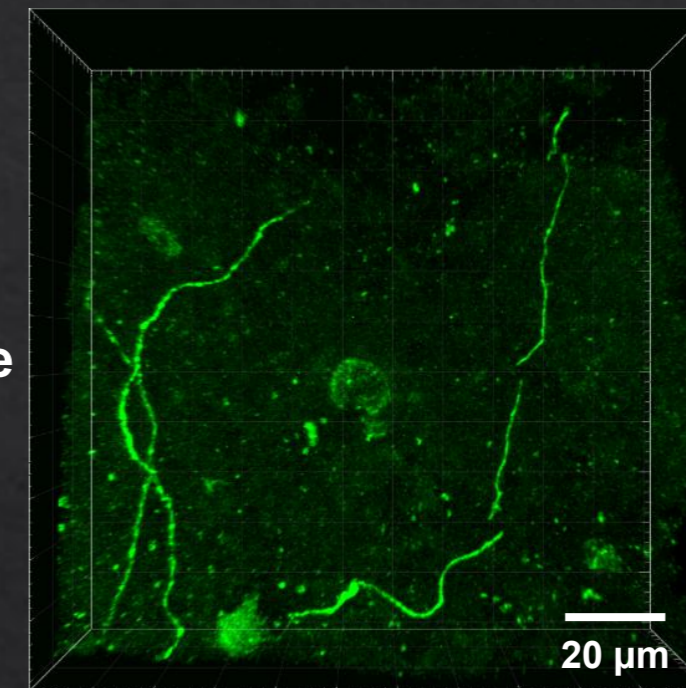
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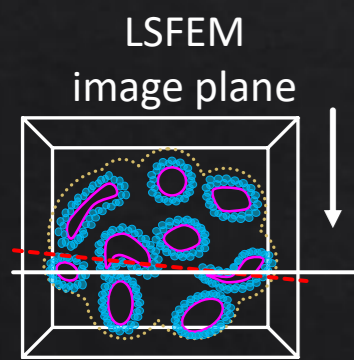
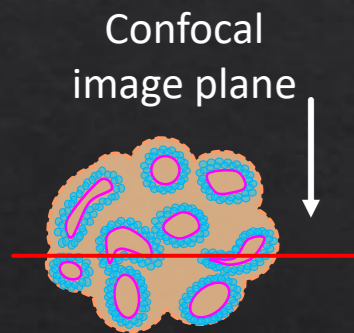
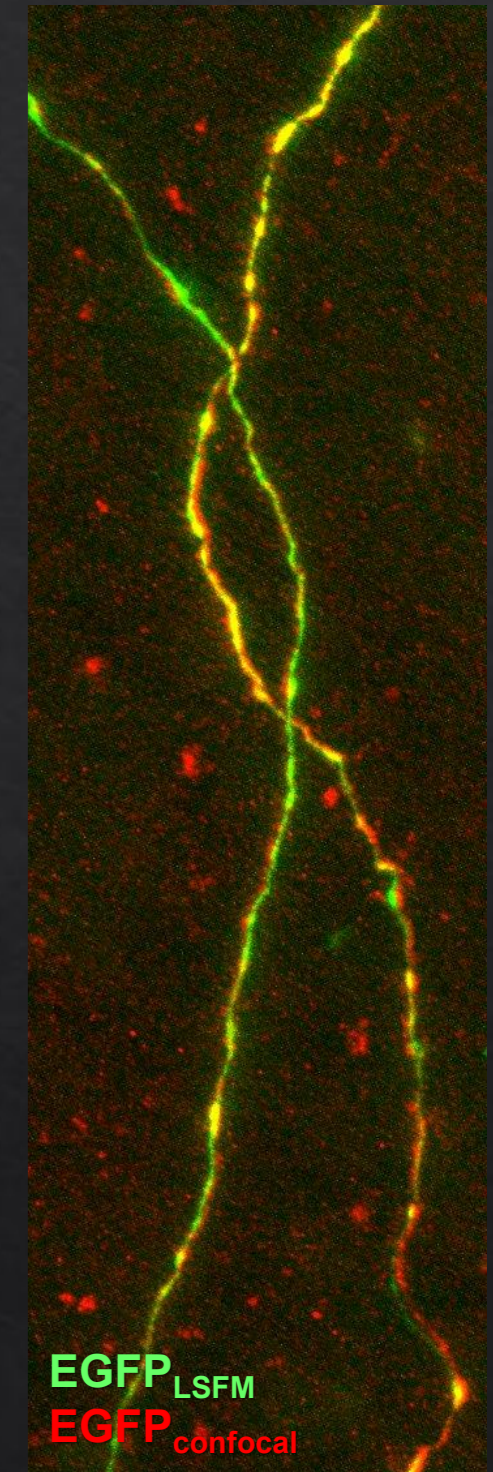
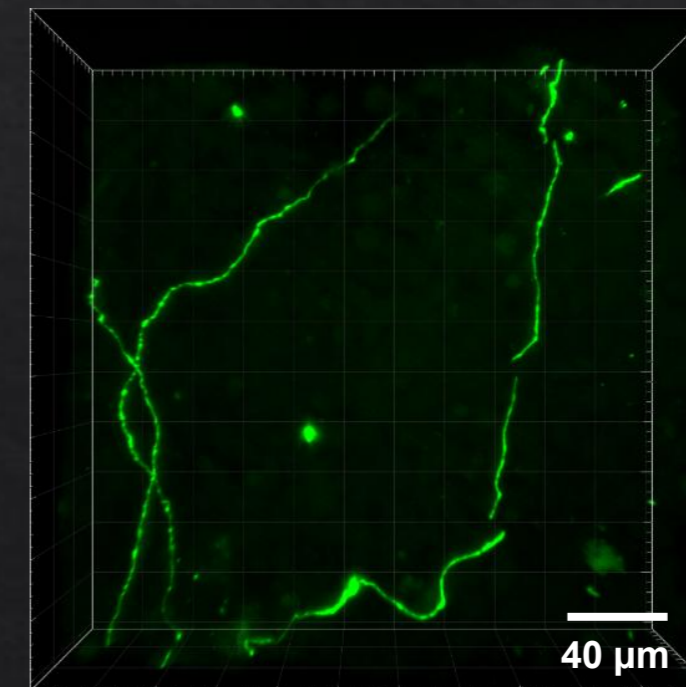


## Structural Integrity Post LSFEM Processing

### Pre-expansion Confocal image



### Meso-scale LSFEM image



*Application 2:*

*Assembloid analysis to validate it as a Parkinson's disease model*

# Modeling early phenotypes of Parkinson's disease by age-induced midbrain striatum assembloids

Collaboration project with Dr. Kyriaki Bampa (Schwamborn Lab)

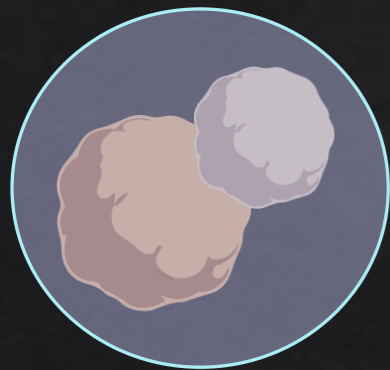
New model presented: A nigrostriatal pathway model based on midbrain-striatum assembloids with inducible aging to study the onset and progression of Parkinson's disease.

- What do we need to achieve our aim?**
- 3D architecture preservation
  - Whole-assembloid imaging
  - Focusing on distinct assembloid compartments in one imaging session

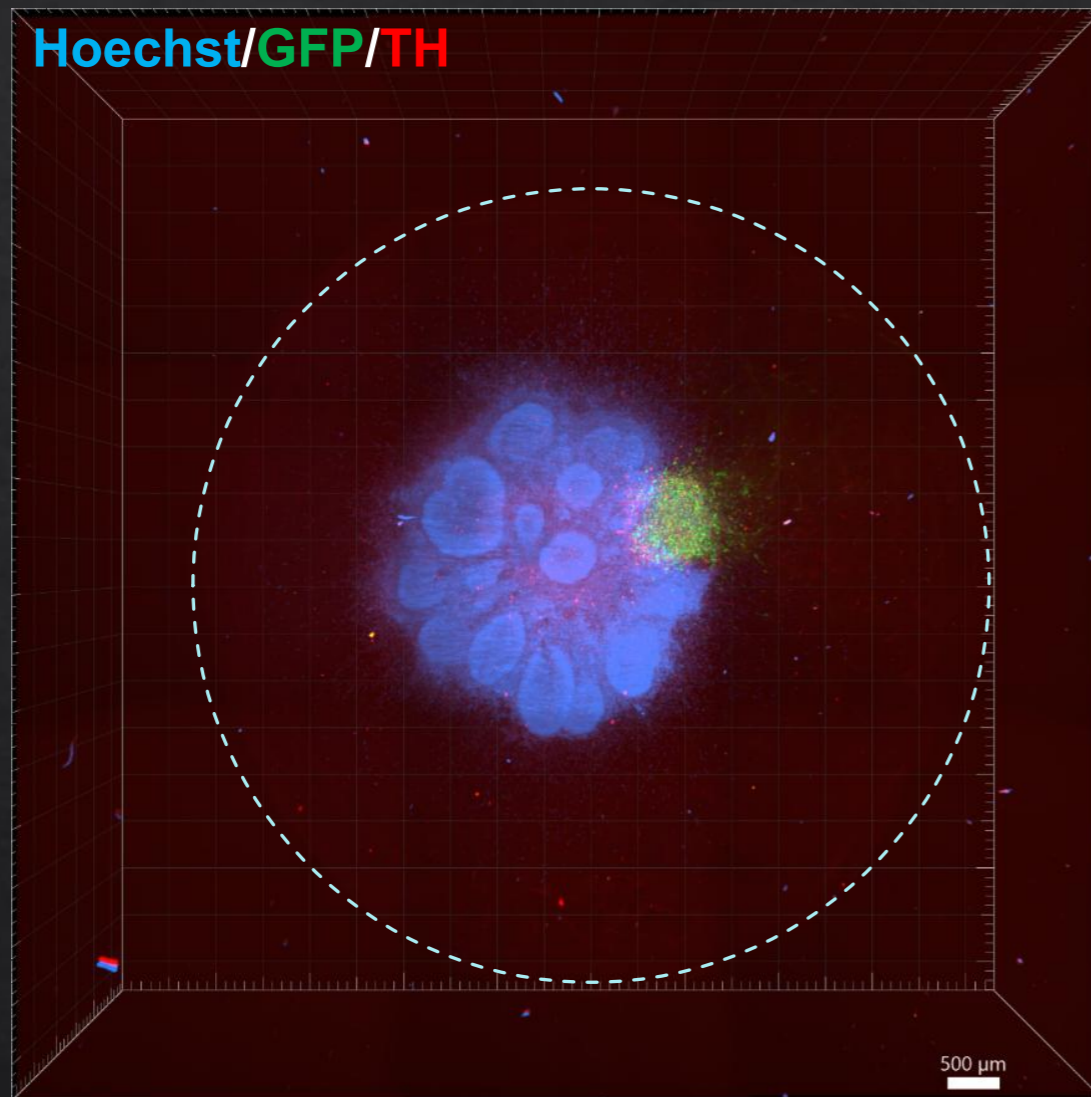
Striatum Organoid (StrO), D35



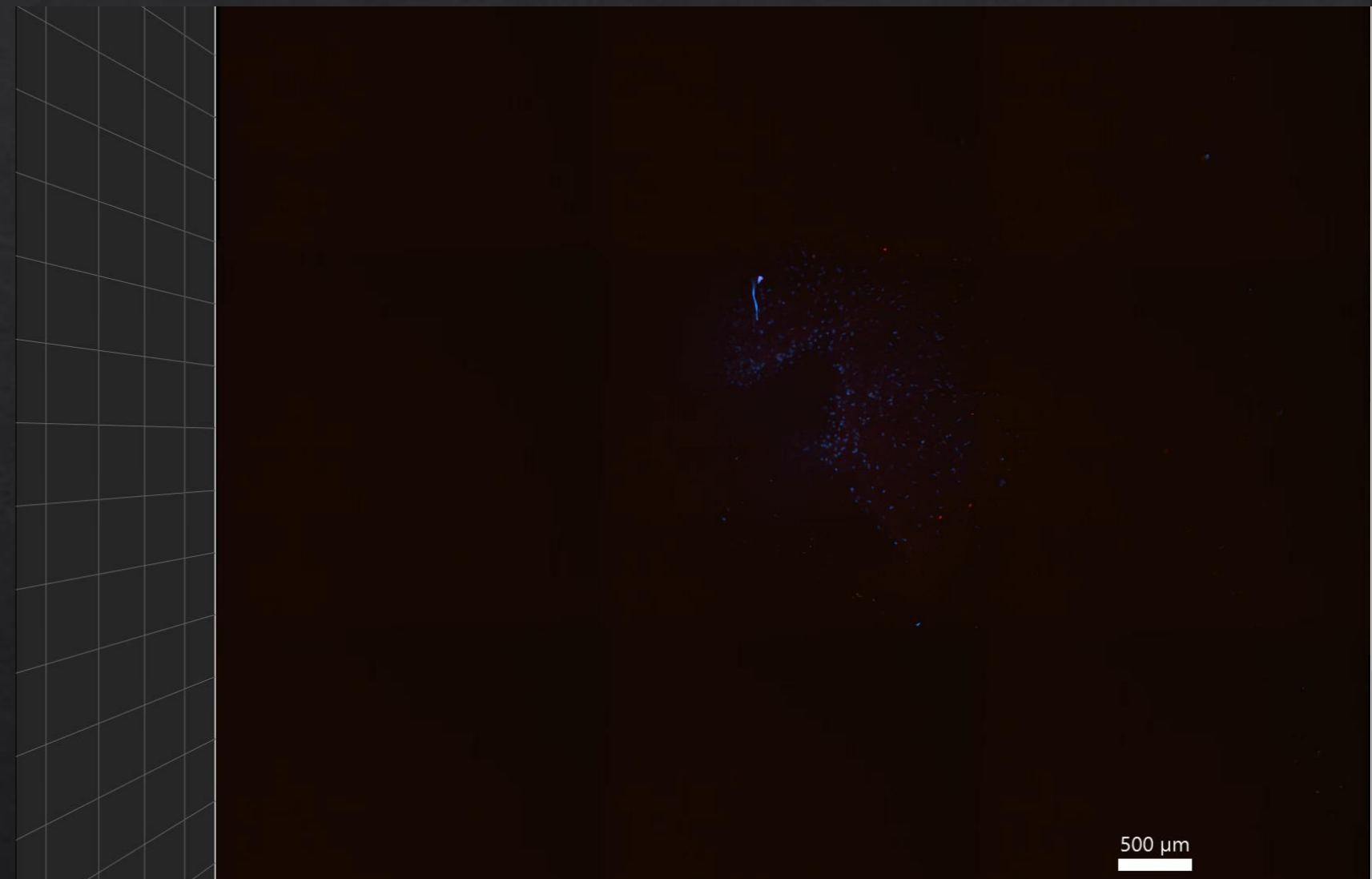
Midbrain Organoid (MO), D25, GFP+



Embedding in Geltrex



1.5-fold expanded  
4x / NA 0.35 WI  
Volume: 9298 x 9308 x 2860 μm<sup>3</sup>



Step size: 1 μm

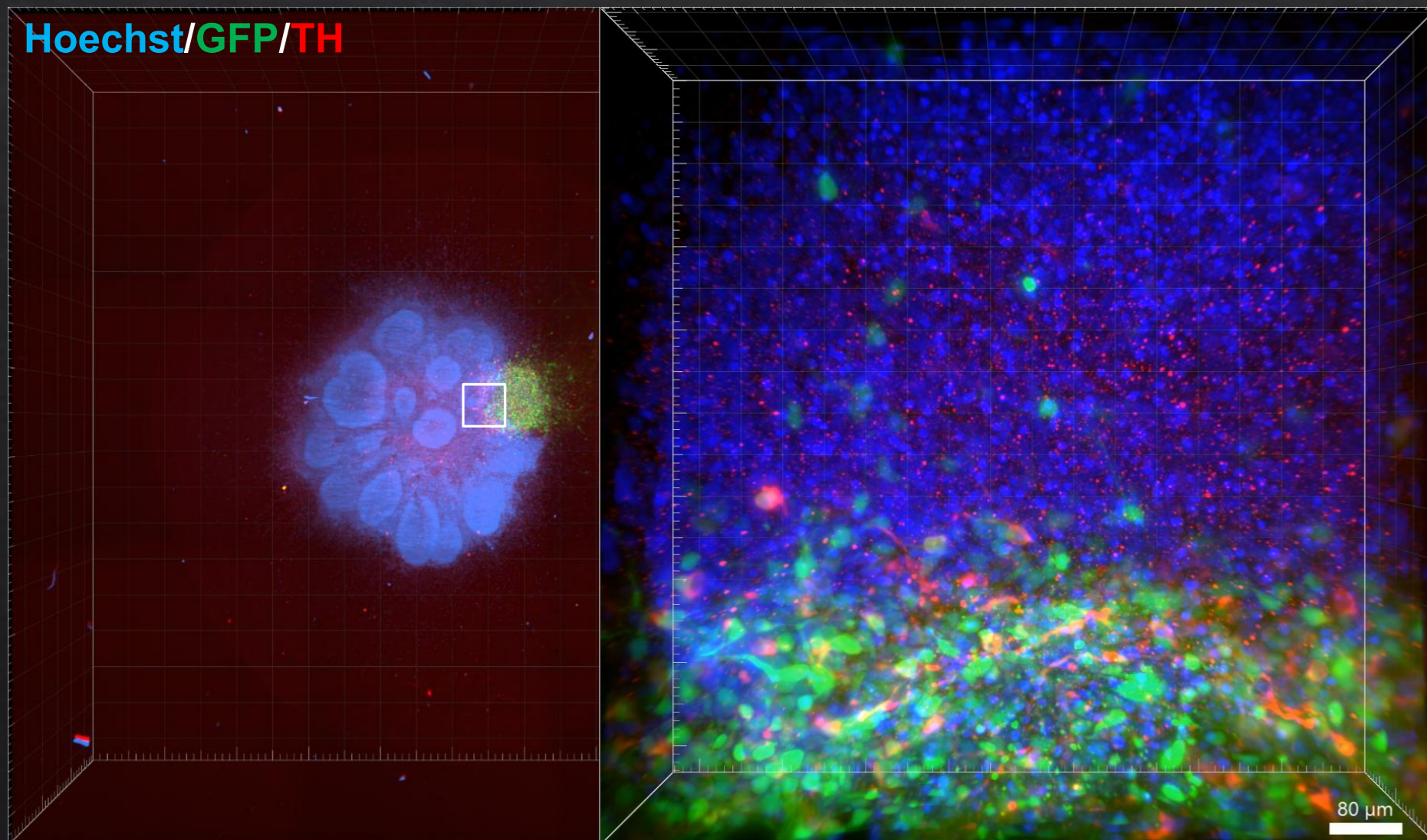
# Midbrain-Striatum assembloids develop nigrostriatal pathway connectivity

## Identified neurons (whole-mount imaging of D30 assembloids):

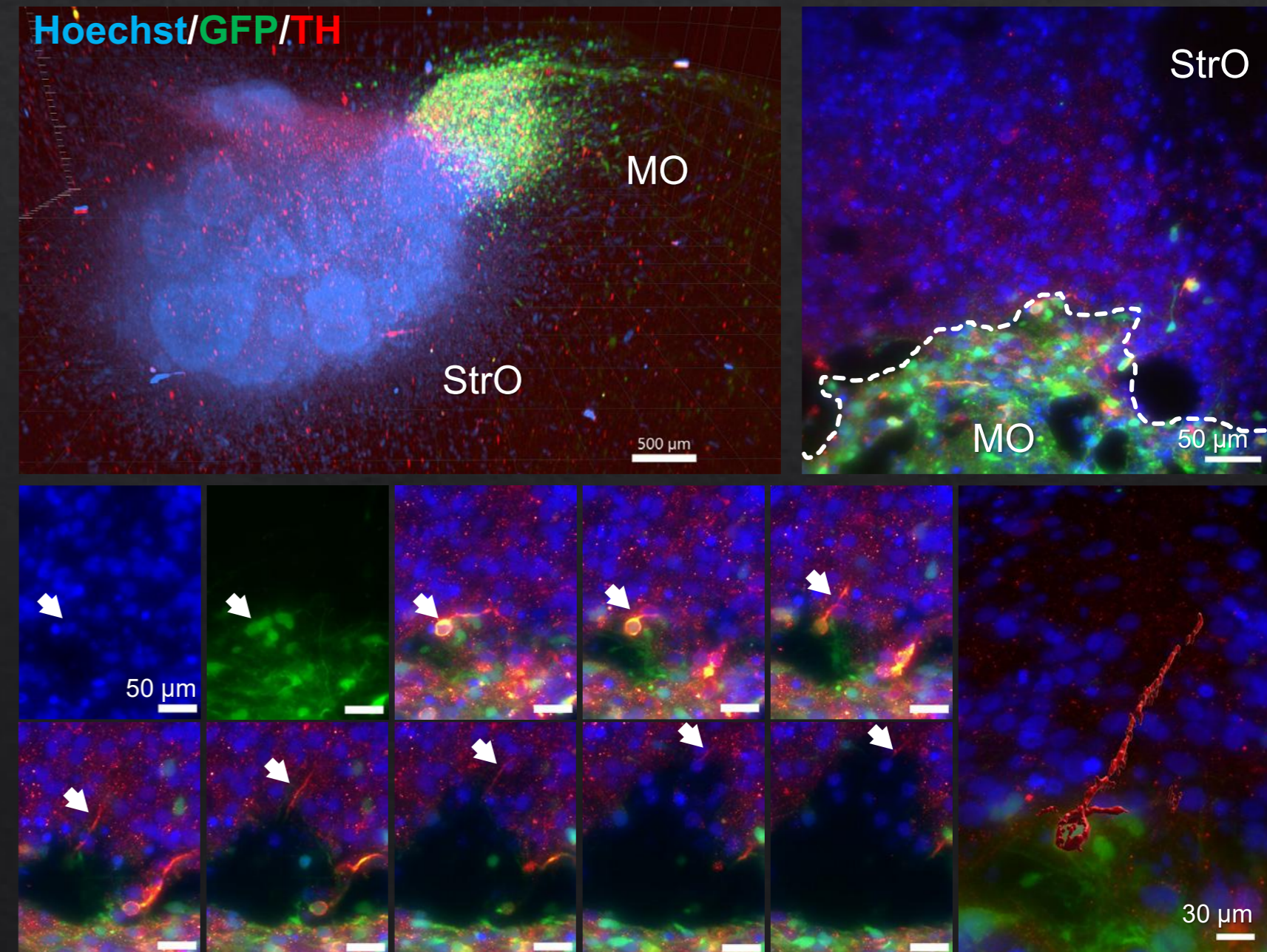
- Neurons with TH+/GFP+ soma, indicating their midbrain origin.
- TH+ axons projecting toward the striatum side of the assembloid.

## Nigrostriatal pathway connectivity validation:

- TH+ signal in the striatum of assembloids originates from the MOs (midbrain organoids) TH+ neuron innervation.



UltraMicroscope Blaze, Miltenyi  
25x / NA 0.95 WI  
Volume: 832 x 832 x 300  $\mu\text{m}^3$   
Step size: 0.5  $\mu\text{m}$



*Application 3:  
3D cell culture model in fibrin/collagen matrix*

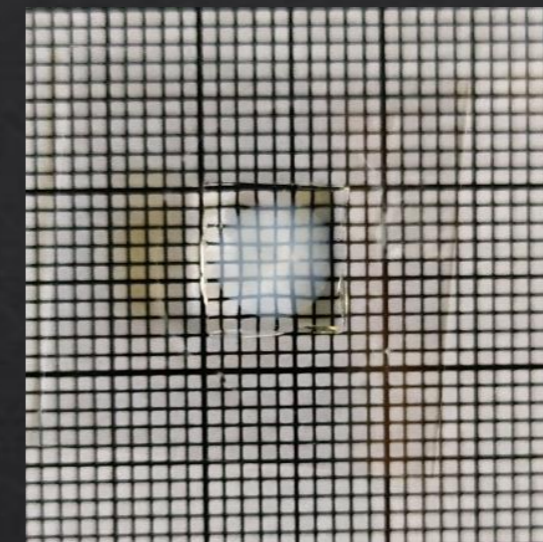
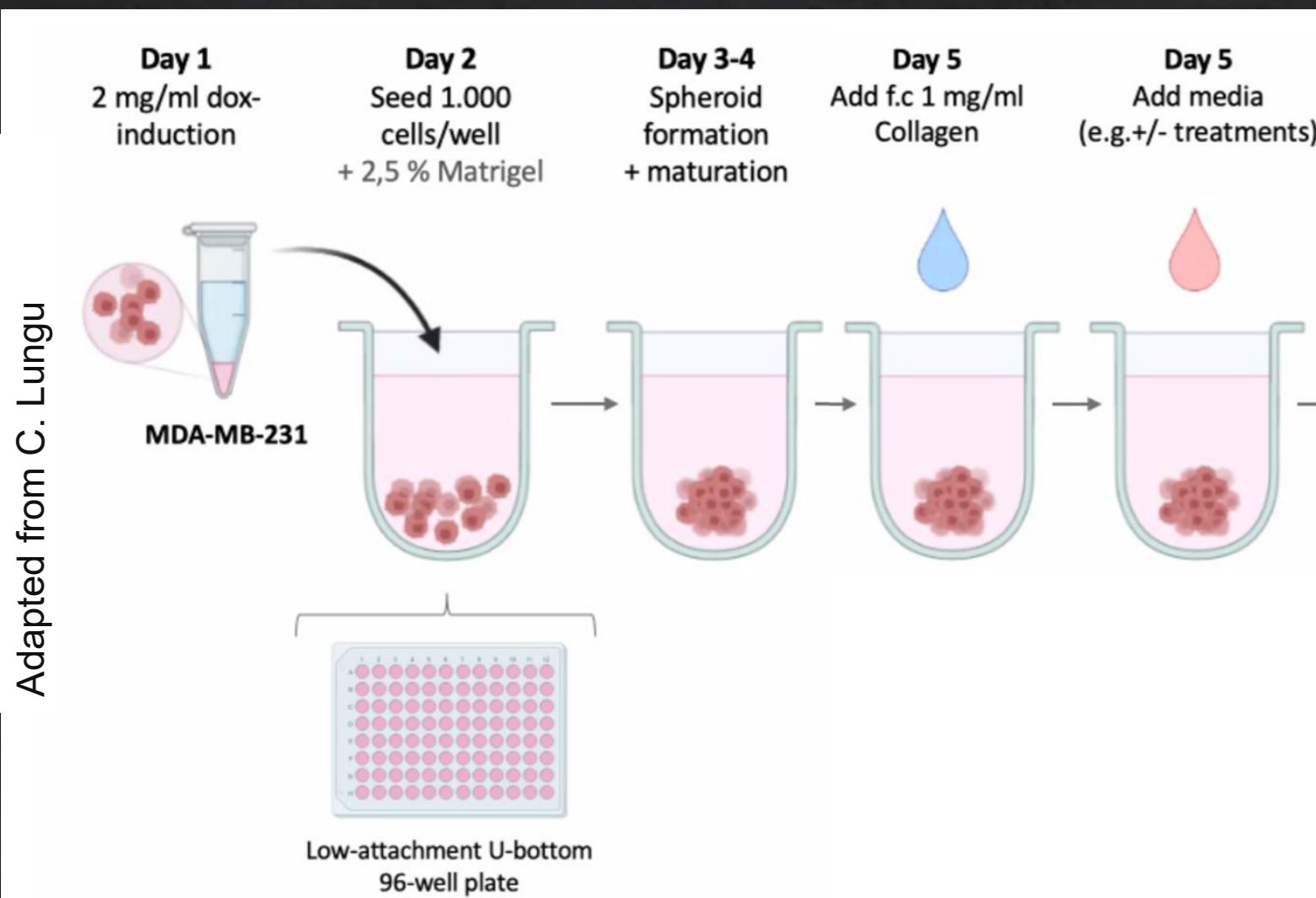
# Project: Breast Cancer Spheroids

Collaboration with **Dr. Cristiana Lungu**

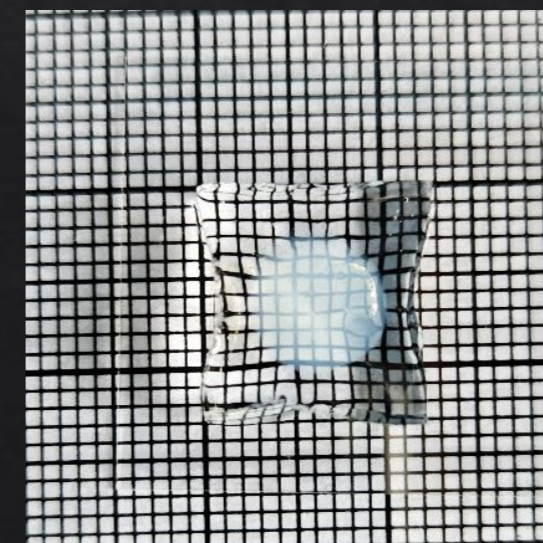
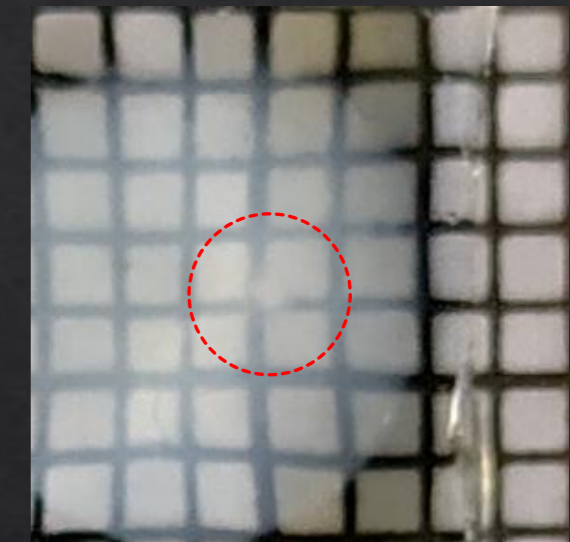
AG Olayioye, Institute of Cell Biology and Immunology, University of Stuttgart

Breast cancer cell line: **MDA-MB-231**

**Aim:** investigate invasiveness of the MDA-MB-231 cells after genetic modification



Collagen sample embedded in gel



After digestion Prot-k

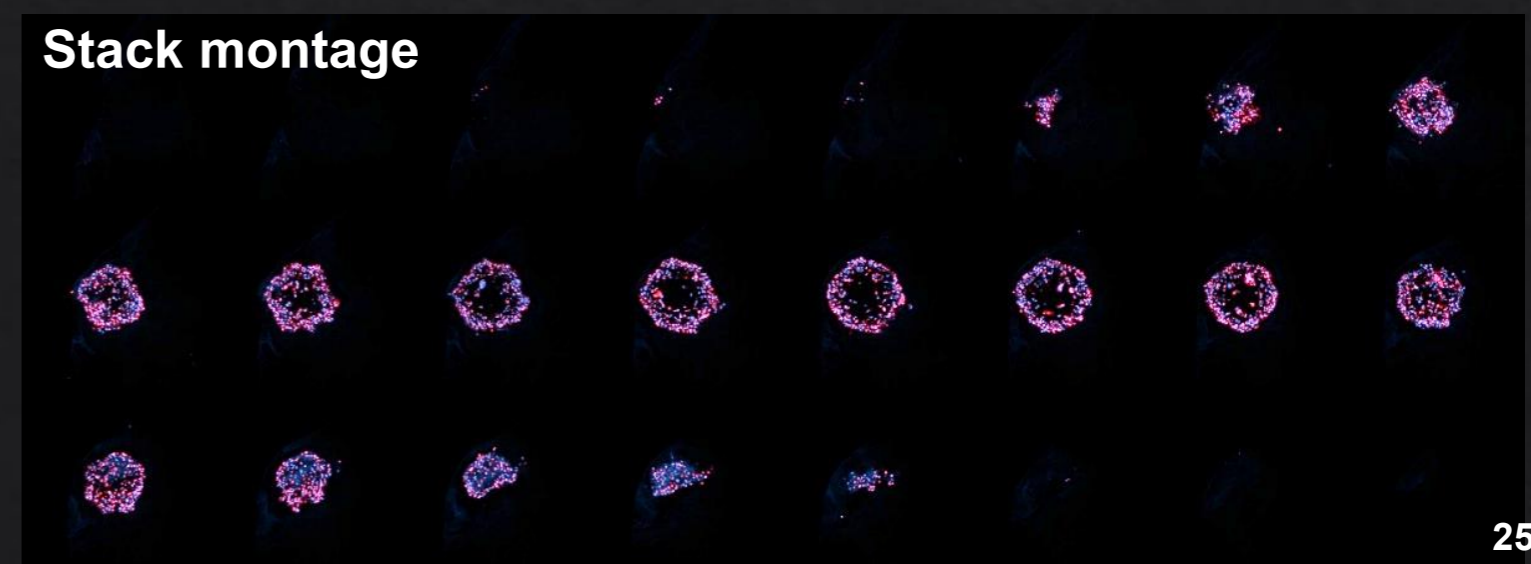
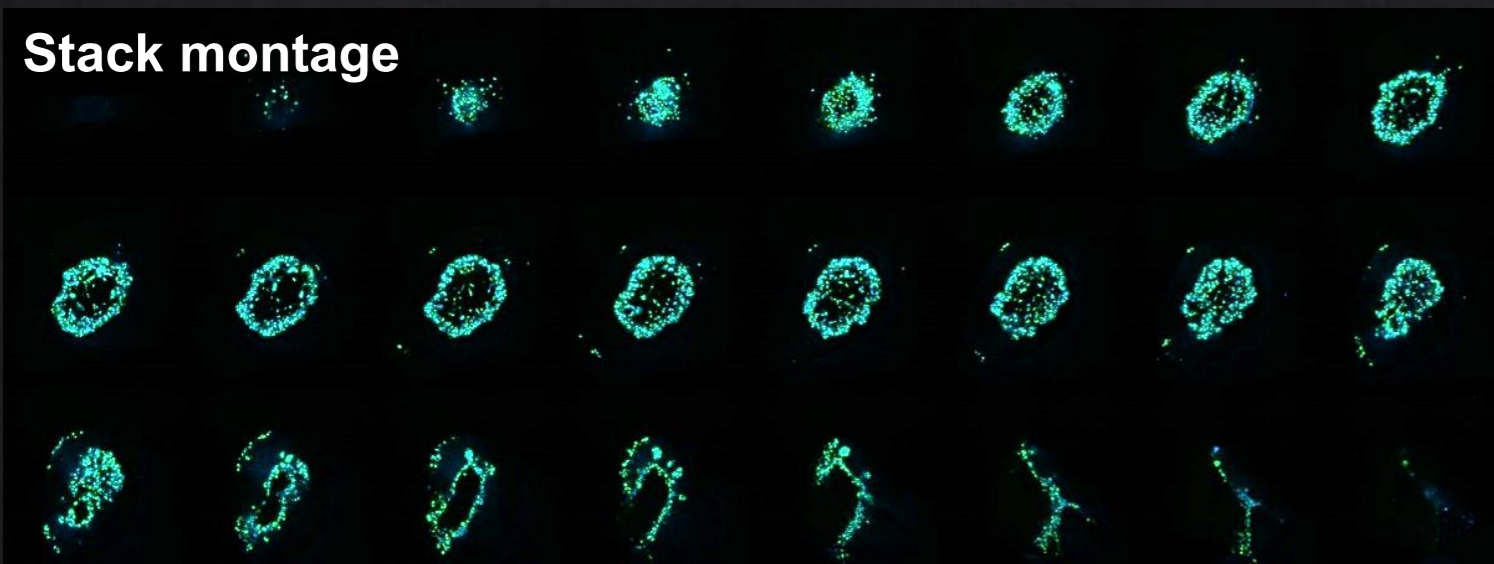
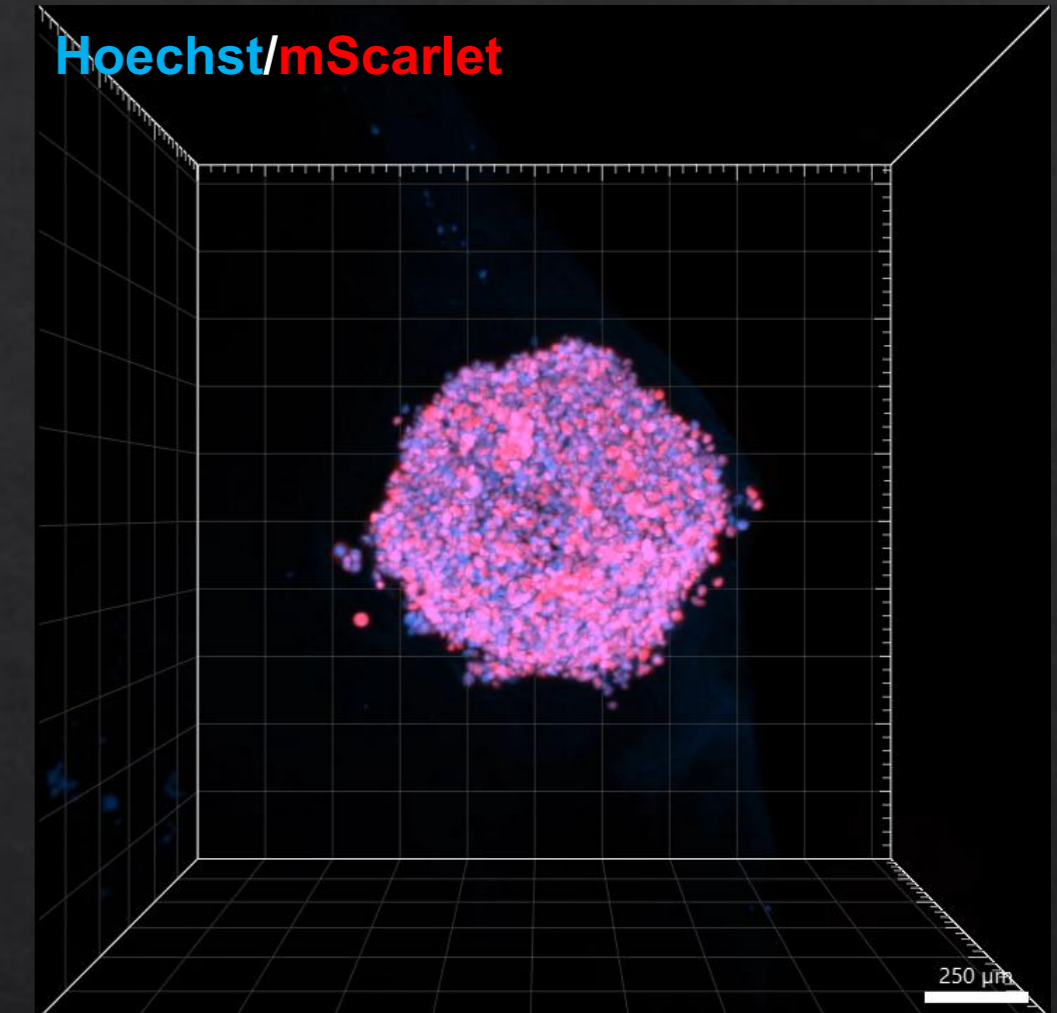
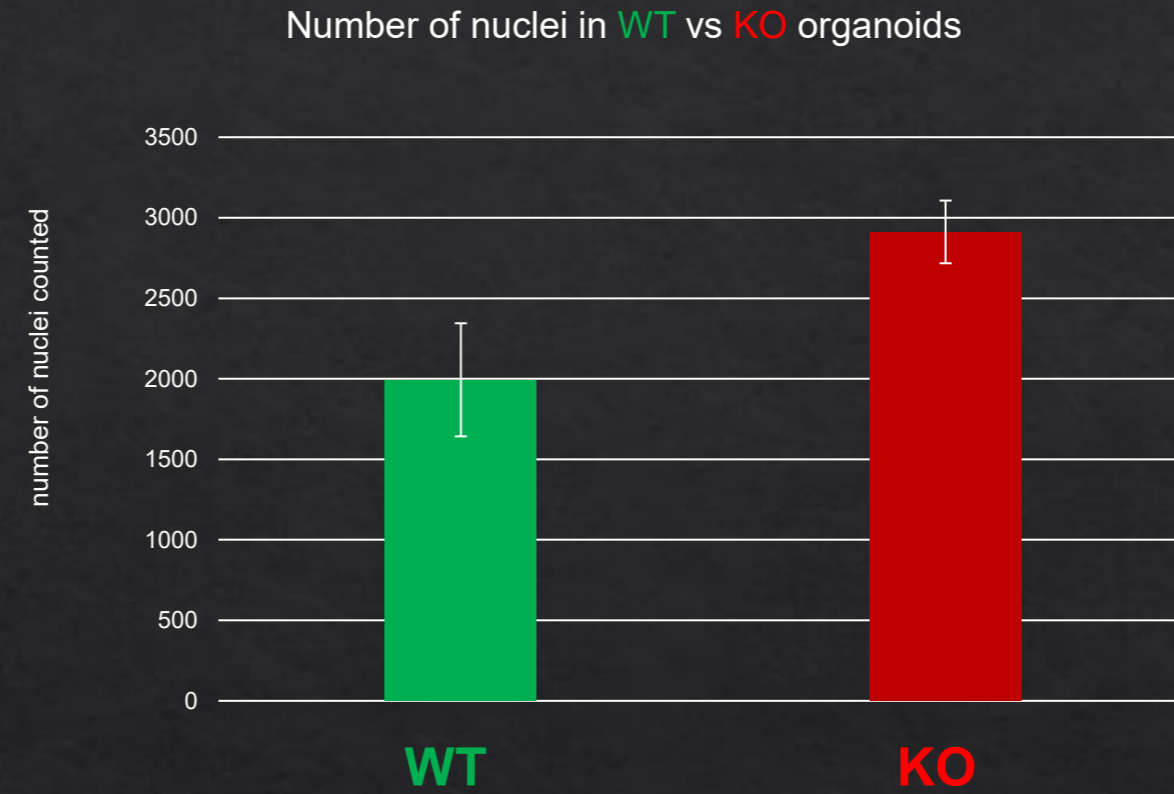
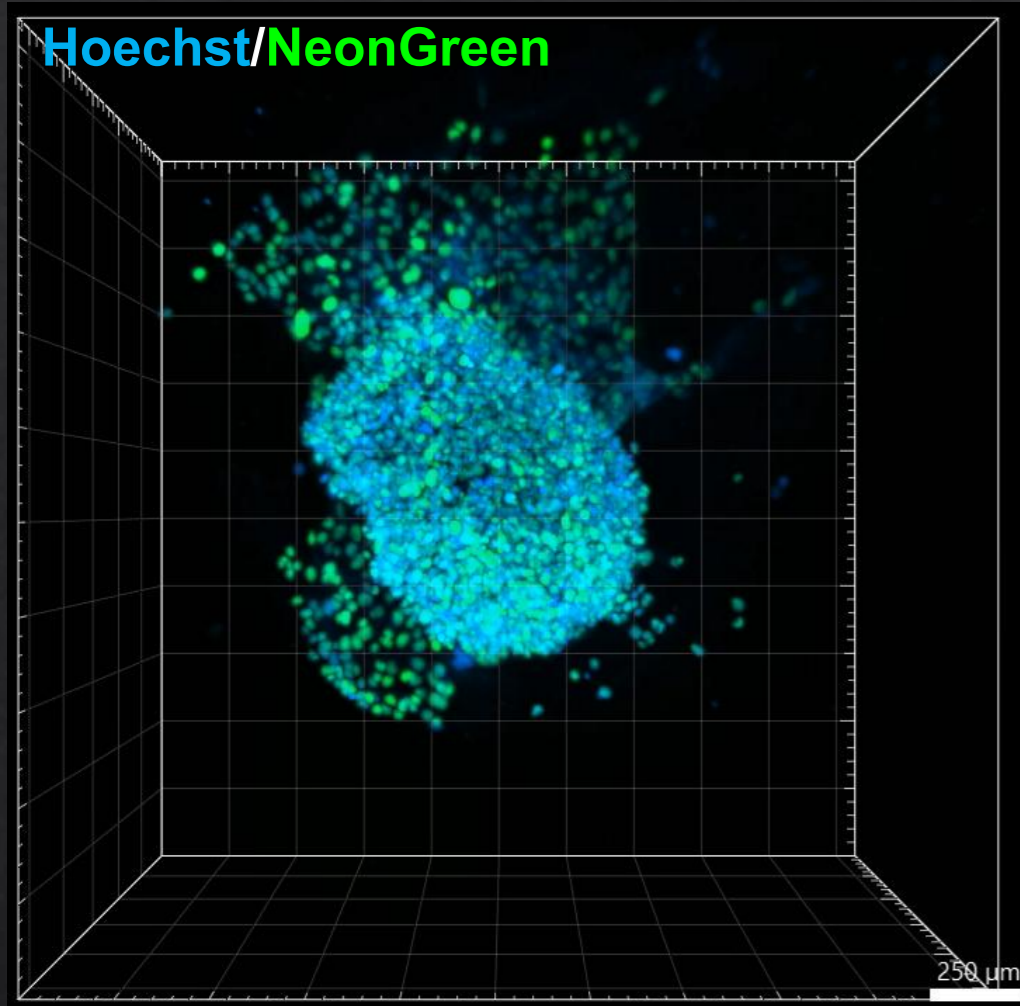


After collagen digestion



# Project: Breast Cancer Spheroids

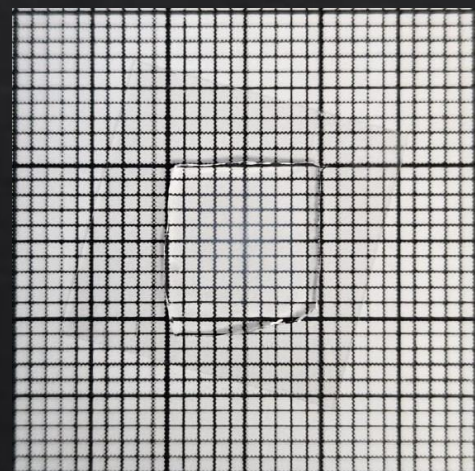
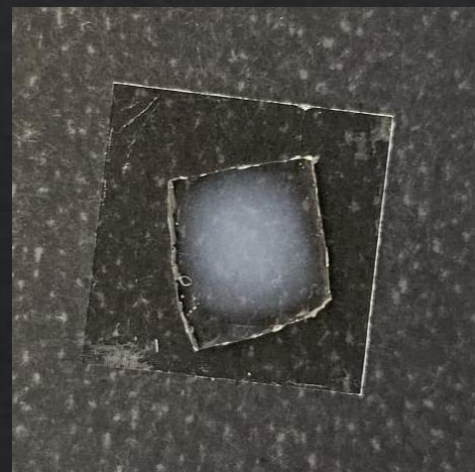
Video of the 3D data



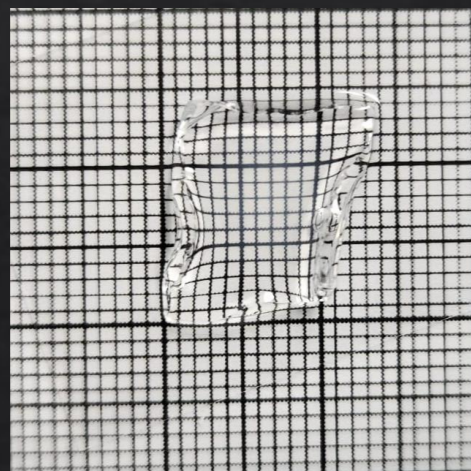
# Flexible protocol: possibility to be optimized for different samples

3D co-culture cell: primary human brain endothelial cells (HBMEC) and human brain vascular pericytes (HBVP)...

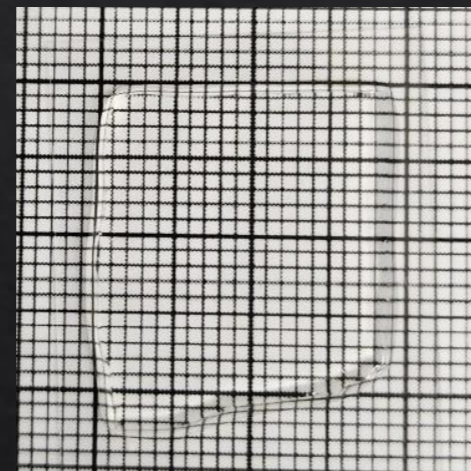
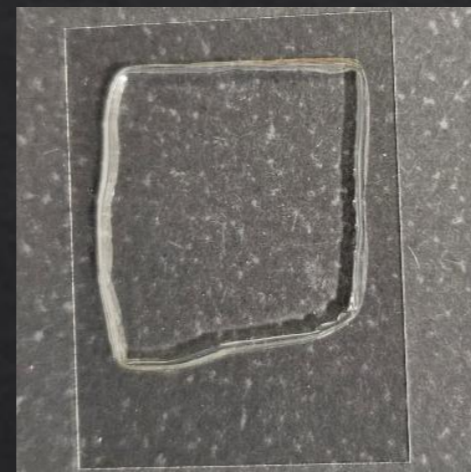
Samples embedded in fibrin/collagen and fixed the gels with 4% PFA



Collagen sample embedded in gel

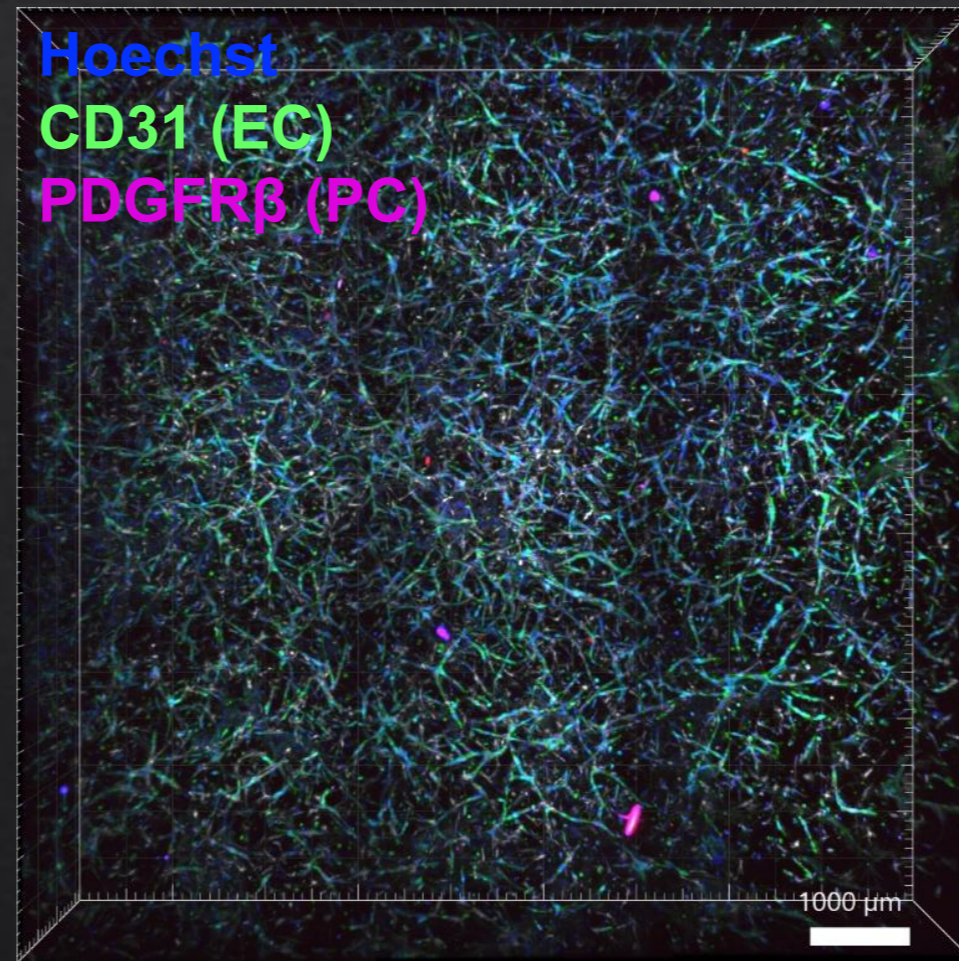


After digestion Prot-k

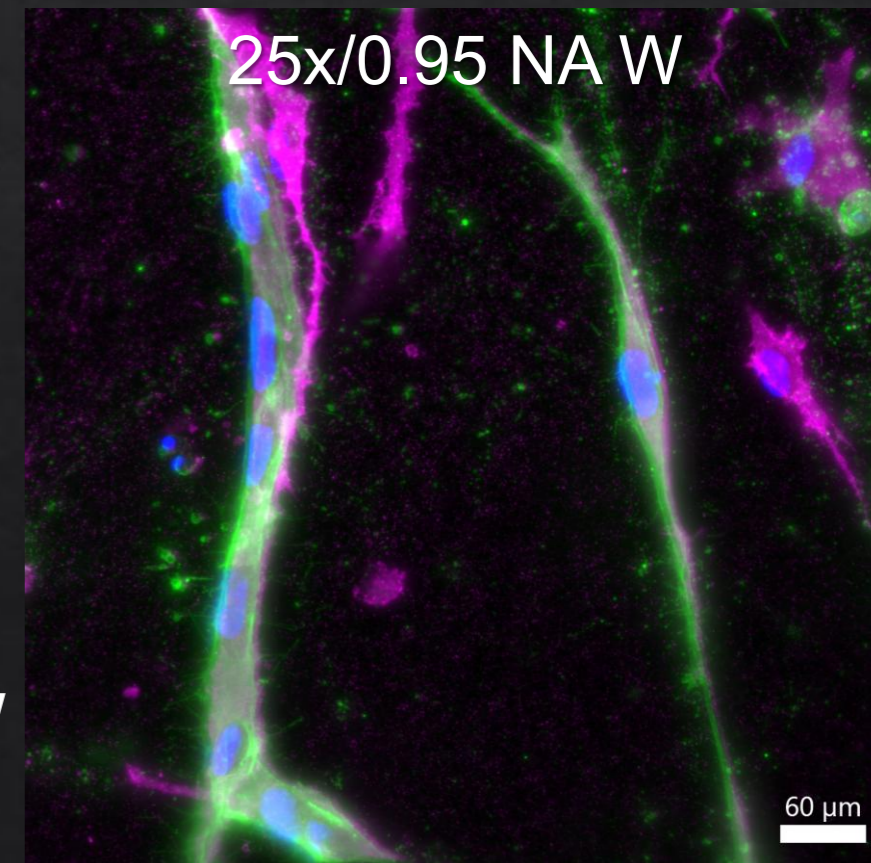
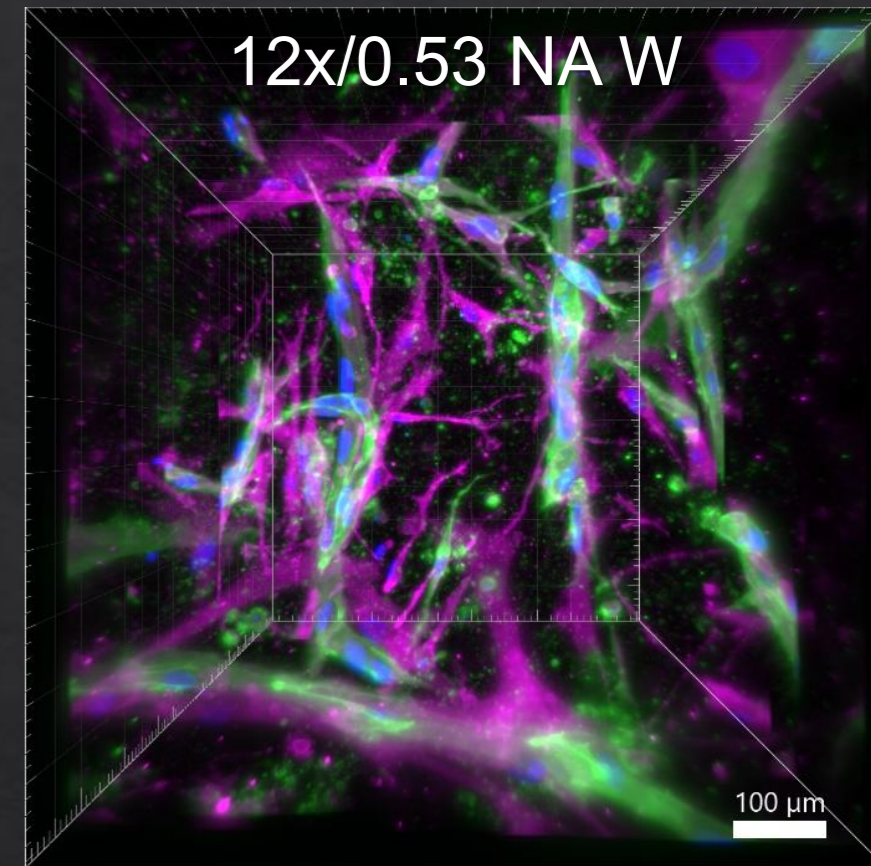


After optimized protocol

**Expansion of 1.8x in PBS**



LSFM z-stack  
Ultramicroscope Blaze, 4x/0.35 NA W  
Constructed using 9 tiles  
voxel:  $1.62 \times 1.62 \times 10 \mu\text{m}^3$   
Size:  $8978 \times 8960 \times 1640 \mu\text{m}^3$



## Conclusion and Outlook

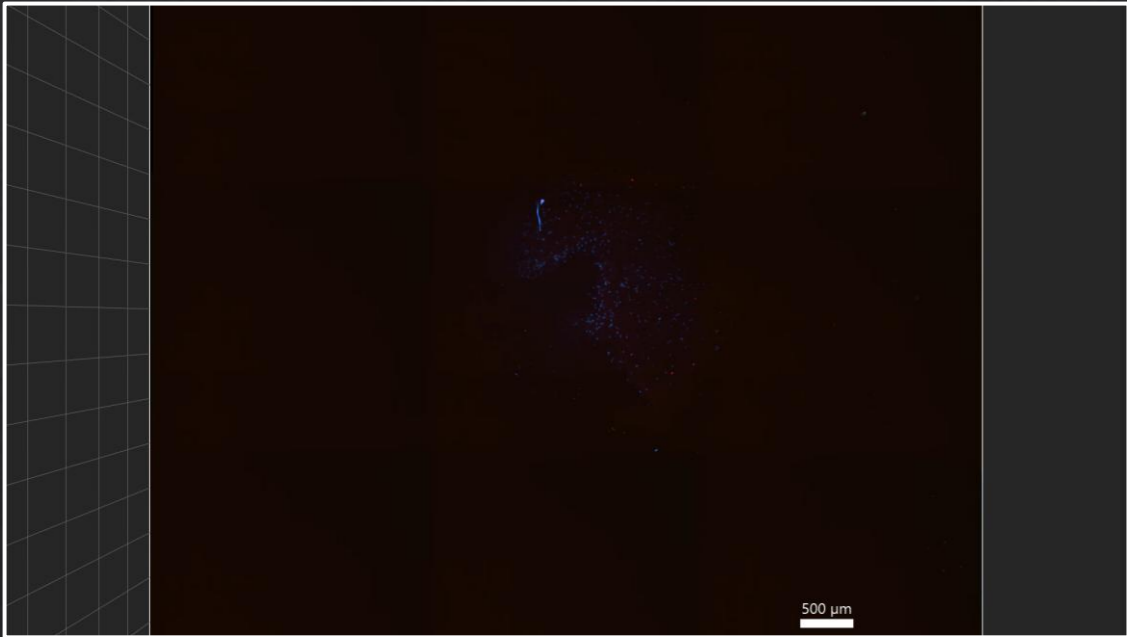
Light sheet fluorescence microscopy (LSFM) enables rapid, multicolor fluorescent imaging of extended nervous tissue samples

Light sheet fluorescence expansion microscopy (LSFEM) enables super-resolution

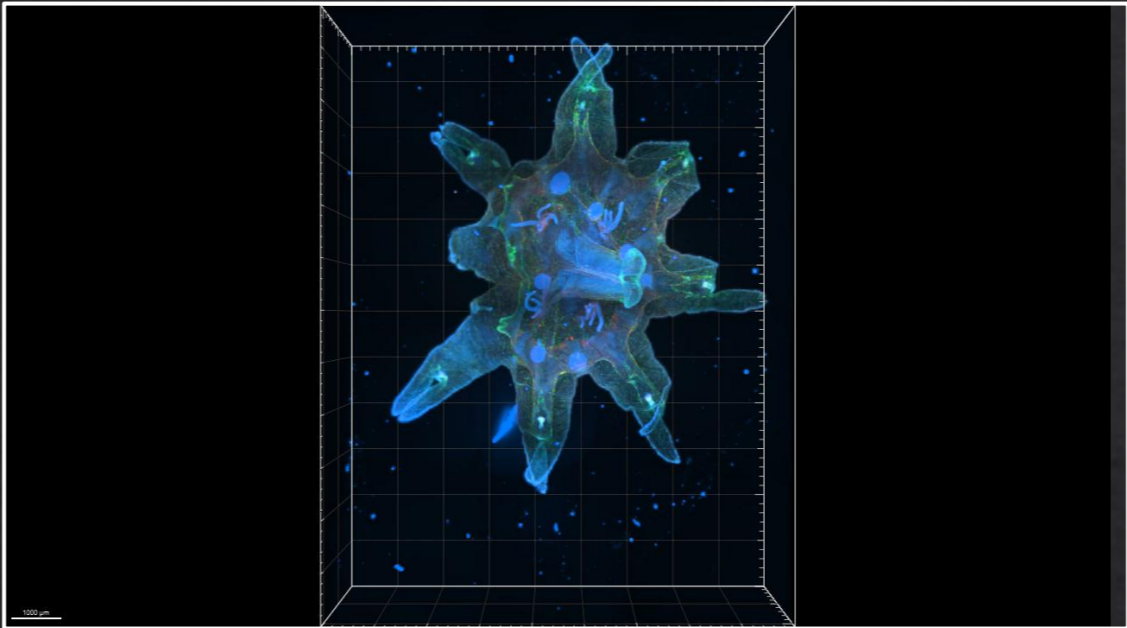
Extended the application of LSFEM beyond neuroscience to fields like developmental biology and cancer research

# Ongoing collaborations...

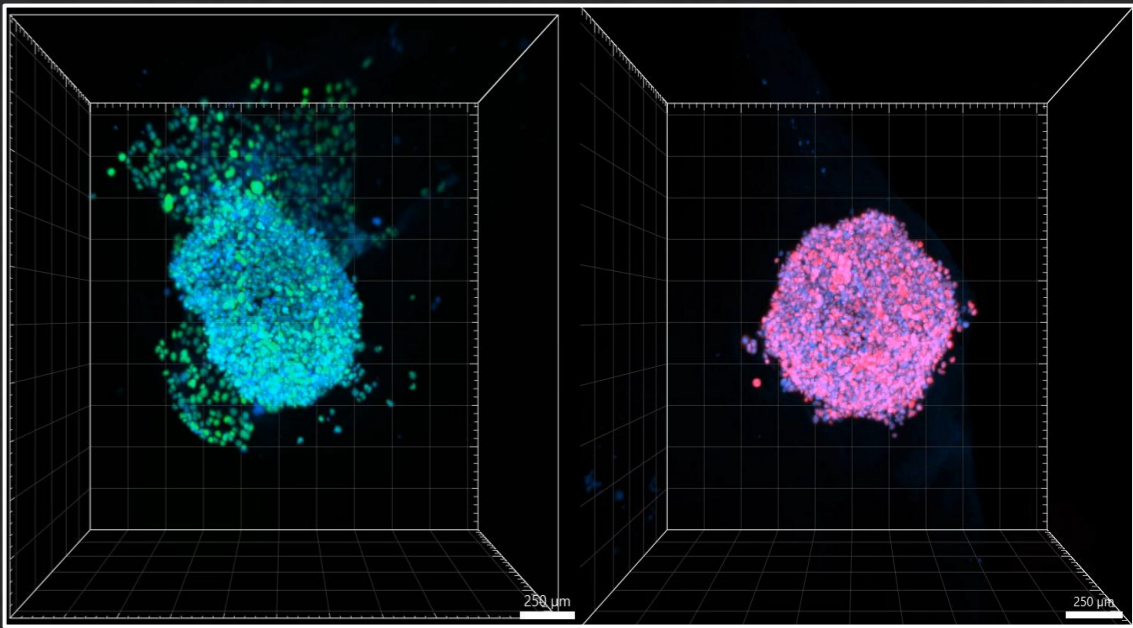
Midbrain-striatum assembloids  
Jens Schwamborn Lab, Luxembourg



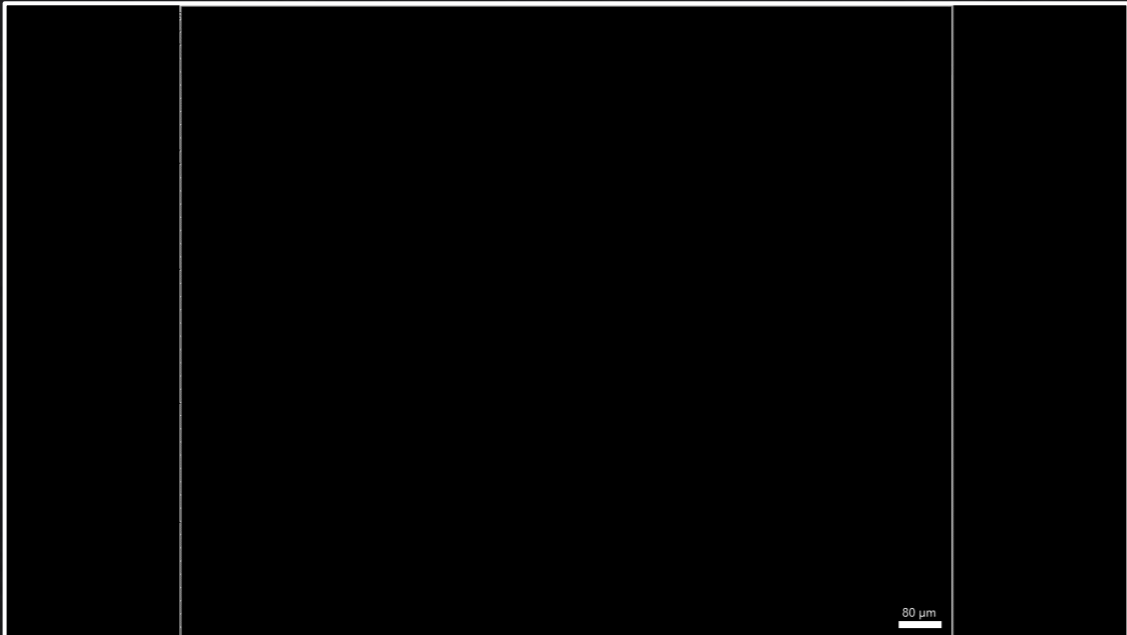
*Aurelia aurita*  
Raoul Memmesheimer Lab, Bonn



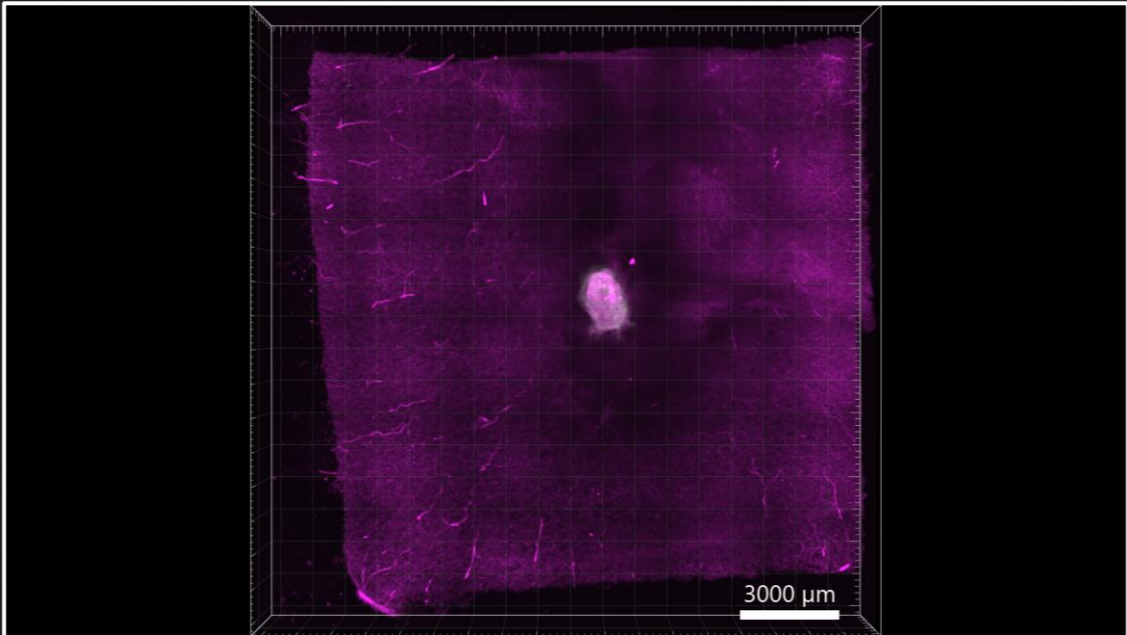
Breast cancer spheroids  
Cristiana Lungu Lab, Stuttgart



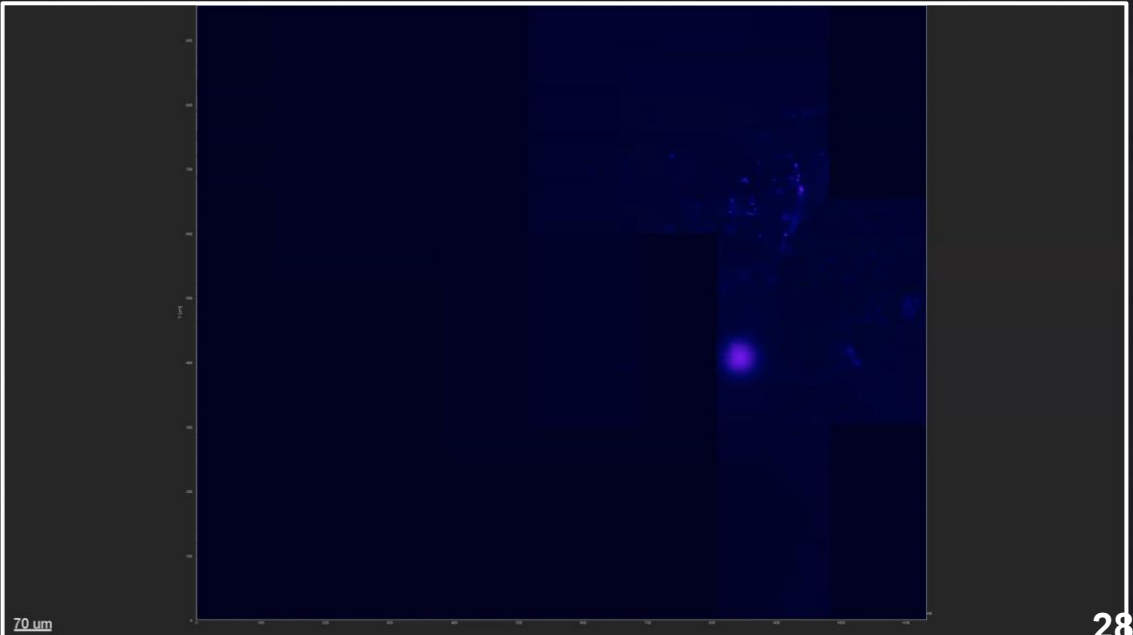
Mouse brain granule cells  
Heinz Beck Lab, Bonn



Human glioblastoma  
Matthias Schneider Lab, Bonn



Zebrafish (pineal gland)  
Steffen Härtel Lab, Chile



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# Light Sheet Fluorescence Microscopy and some biological applications

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