Light sheet fluorescence expansion microscopy
Imaging from Meso- to Nanoscale

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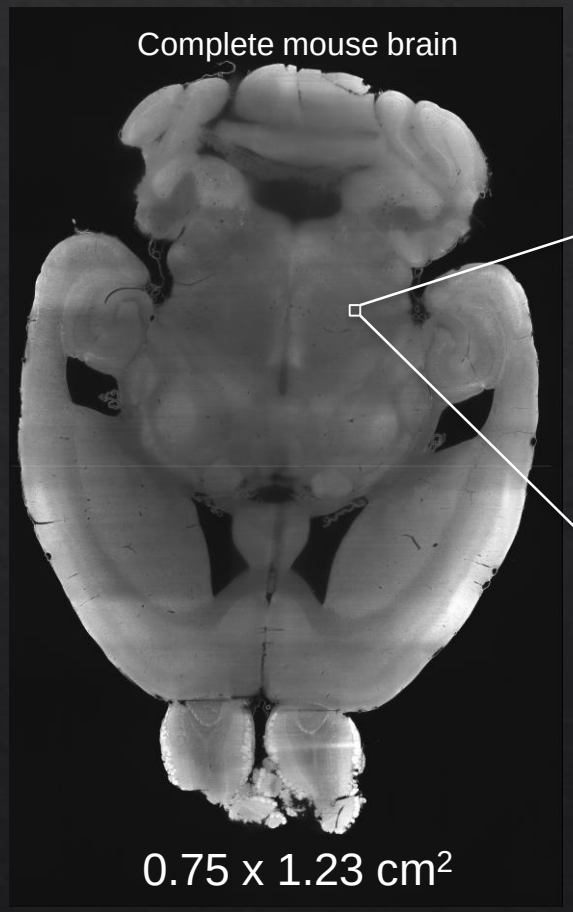
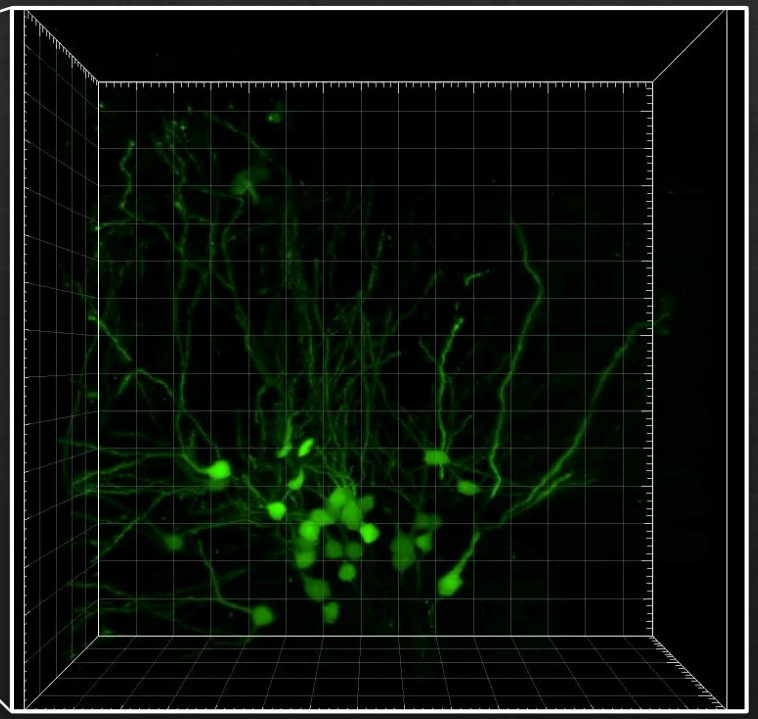
Motivation

Neuronal connections in the brain
have lengths of centimeters

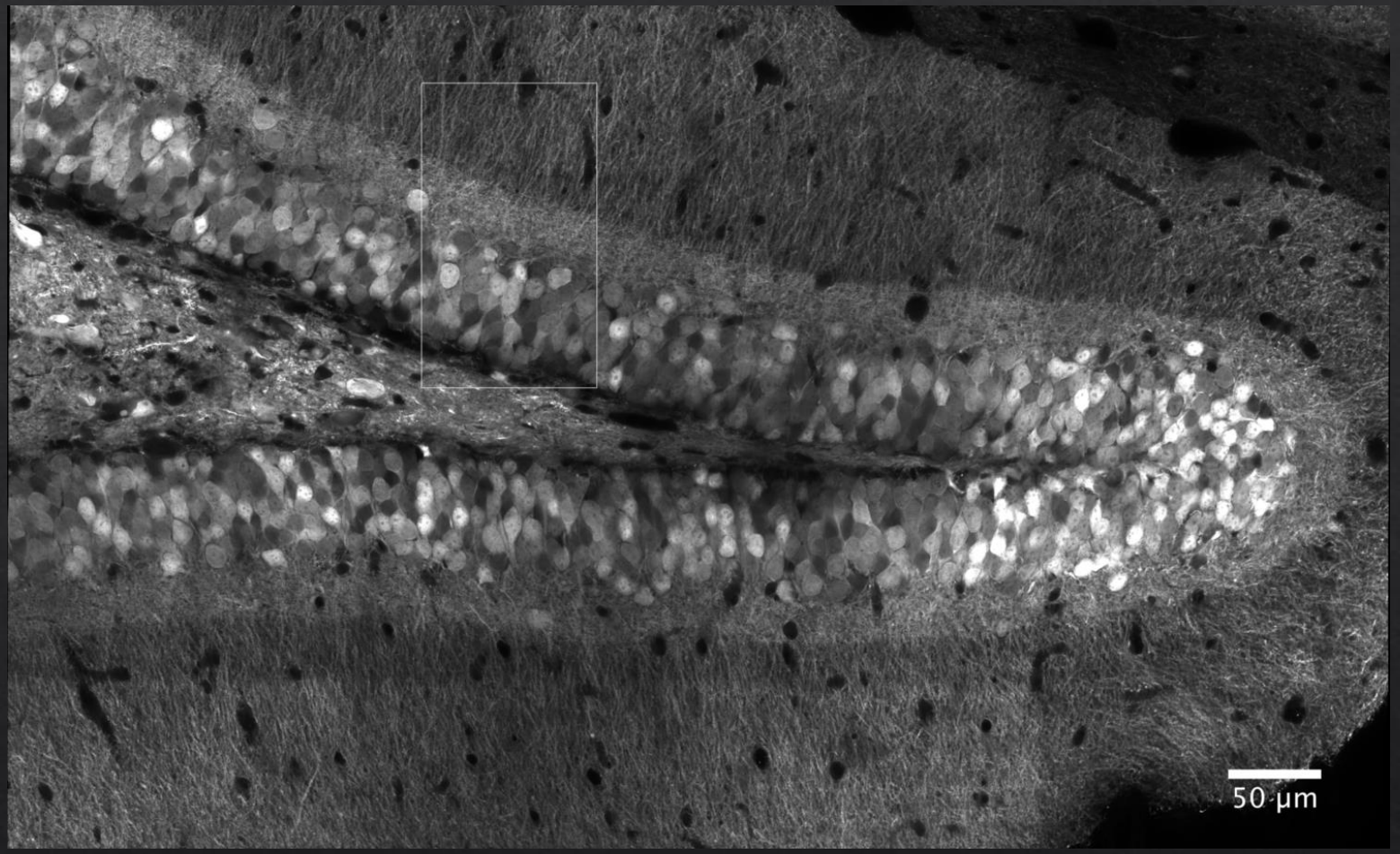


Spines and synapses occur at
length scales of tens of nanometers



How to achieve detailed imaging of neuronal connections
in mouse brains ranging from the nanometer to the centimeter scale?

Mouse dentate gyrus (DG) imaged by Airyscan confocal microscopy



Single confocal plane acquired using a 40x 1.2 NA WI objective lens and an Airyscan detector.

Total field size, 740x452 µm^2, achieved by stitching 8x4 stacks comprising 2048x2048 pixels in each frame using the algorithm by Preibisch et al. (2009).

Granule cells in the mouse DG imaged by Airyscan confocal microscopy

Magnification of the ROI marked in the previous figure.

Coronal section of a mouse DG containing EGFP-expressing granule cells. Endogenous EGFP fluorescence enhanced by antibody staining against EGFP.

Single confocal plane acquired using a 40x 1.2 NA water immersion objective lens and an Airyscan detector.

Super-resolution confocal microscopy does not provide the required image quality!

Light sheet fluorescence expansion microscopy

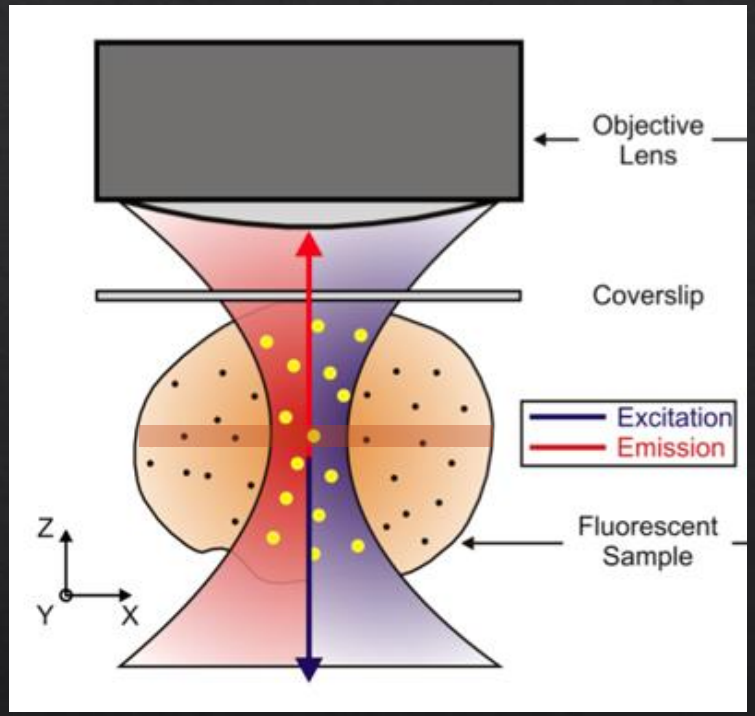
What is light sheet microscopy?

What is expansion microscopy?

UNI BONN

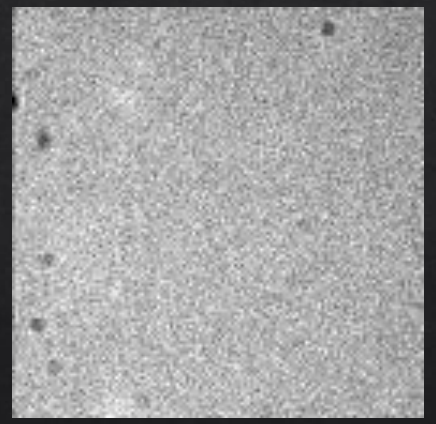
Part 1:
Light sheet fluorescence microscopy

Low contrast & bad axial resolution in epi-illumination microscopy

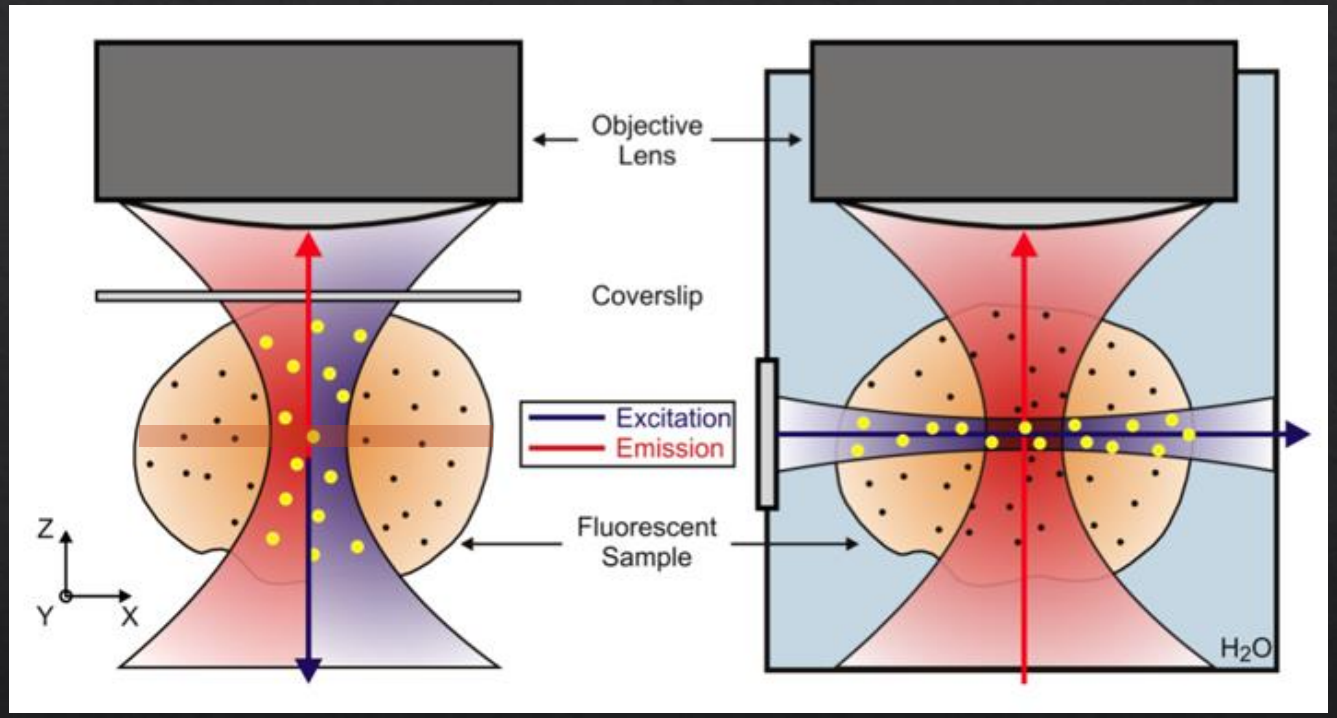


epi-illumination

500 kDa dextran-Atto633 in buffer
40X, NA 1.2W objective lens
Image field 19.2 µm
Image acquistion 100 Hz, display 33 Hz



High contrast by light-sheet based microscopy



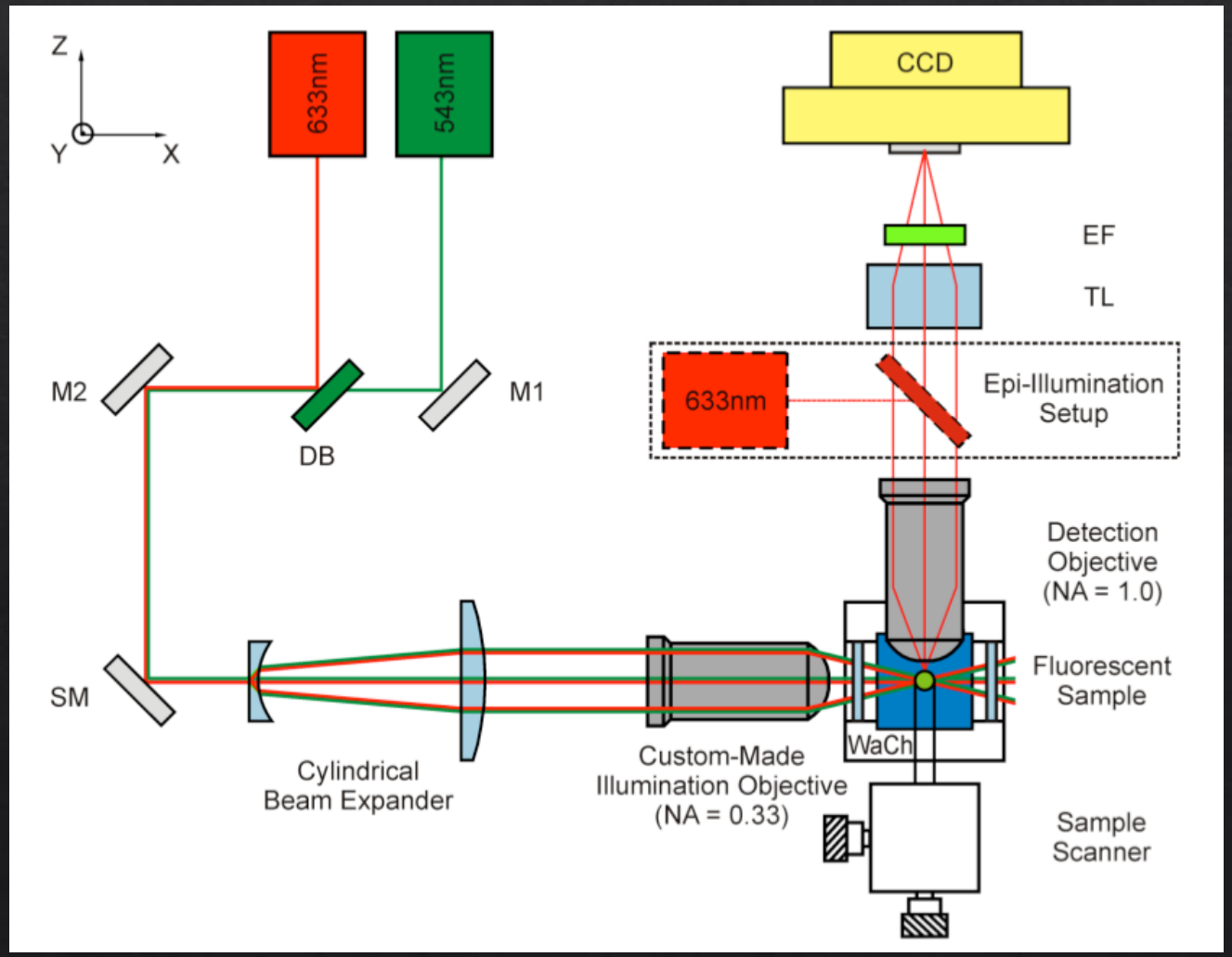
epi-illumination

focal plane illumination

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

Light sheet fluorescence microscopy

Jörg Ritter, 2006



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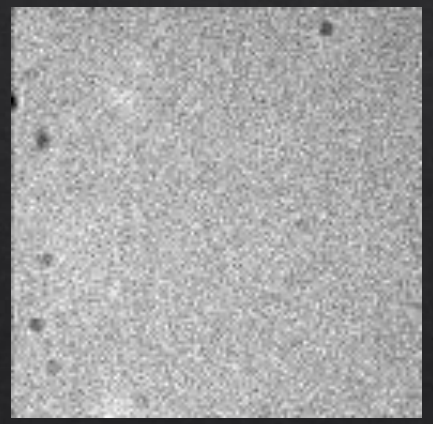
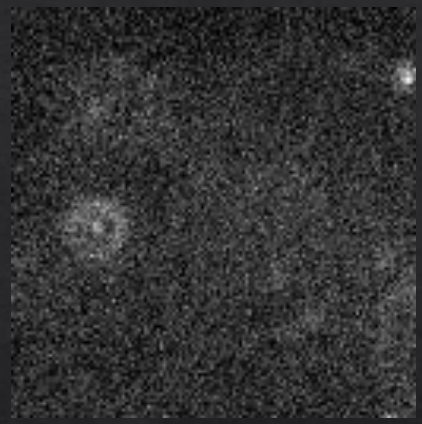
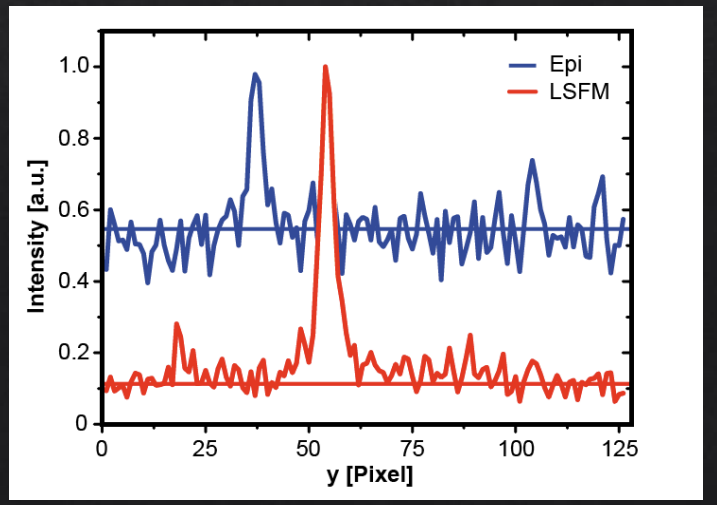
40X, NA 1.2W objective lens
Image field 19.2 µm
Image acquisition 100 Hz, display 33 Hz

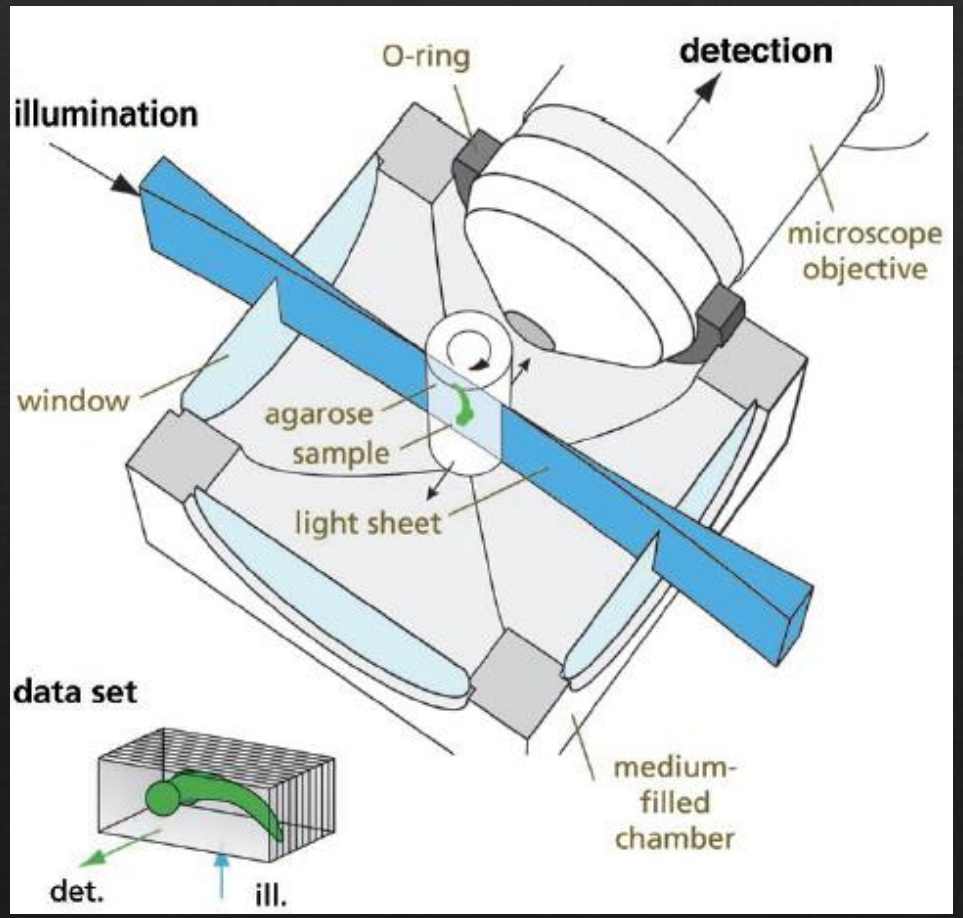






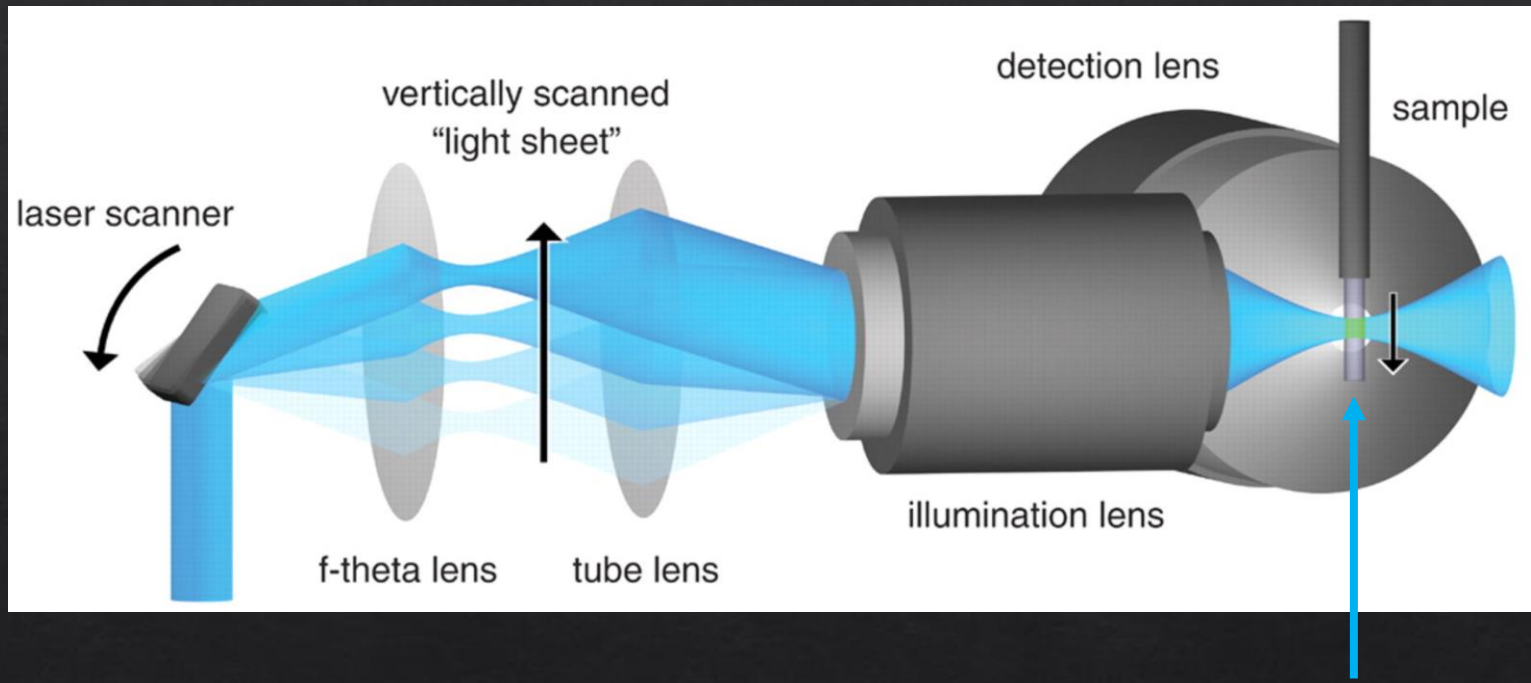
Principle of light sheet microscopy

Cilindrical lenses



Huisken et al., Science 2004

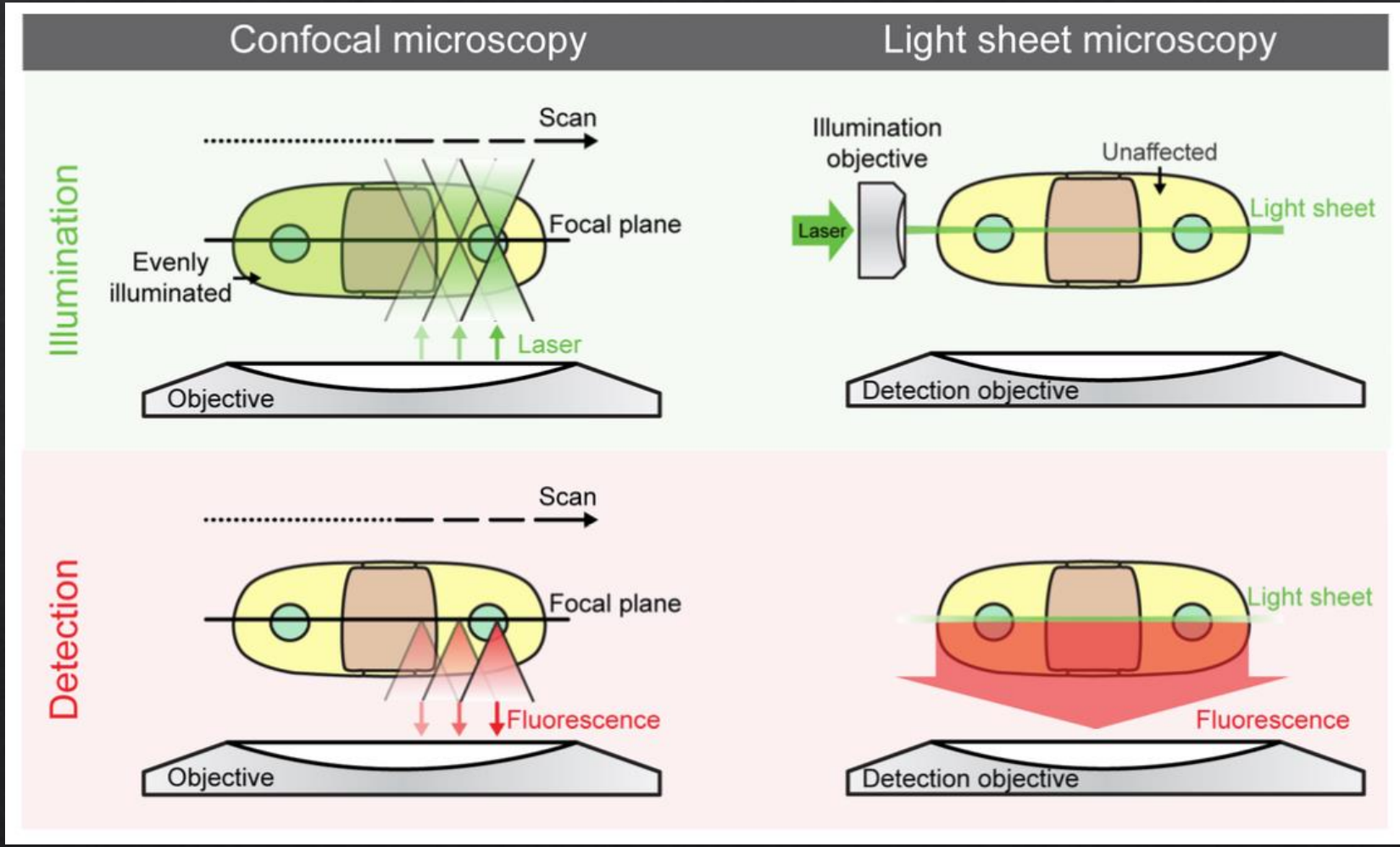
Scanned light sheet



Scan circular beam rapidly vertically across the object plane

Philipp J. Keller, *et al., Science* **322**, 1065 (2008)

Image generation in confocal and light sheet microscopy



Ritter, 2011

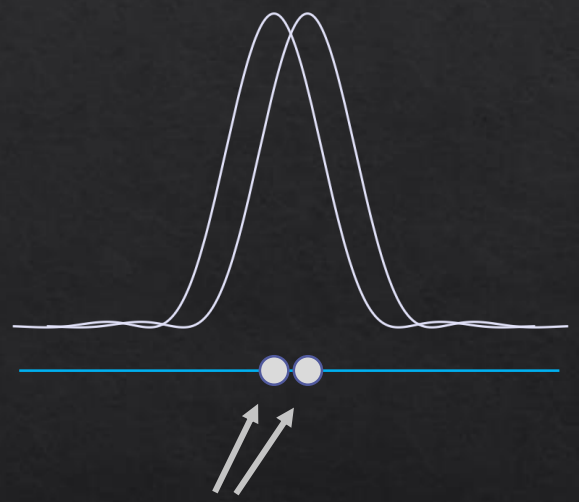
Pros and Cons

Confocal laser scanning microscopy	Light sheet fluorescence microscopy
Low frame rate	High frame rate
High photodamage	Very low photo damage
Out-of-focus fluorescence excitation	No out-of-focus excitation
Removal of out-of-focus and scattered light.	Detection of scattered light

Part 2:
Expansion microscopy

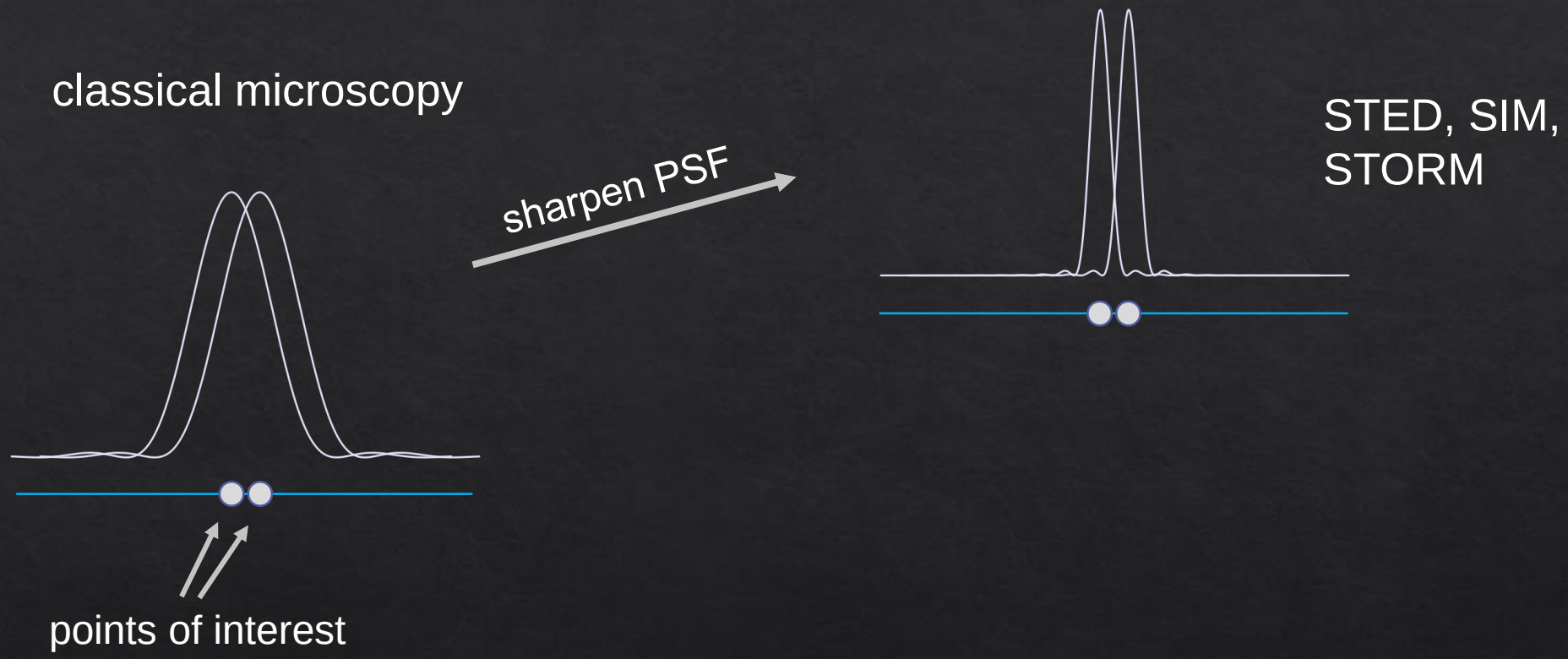
How to image a sample with sub-resolution features?

classical microscopy

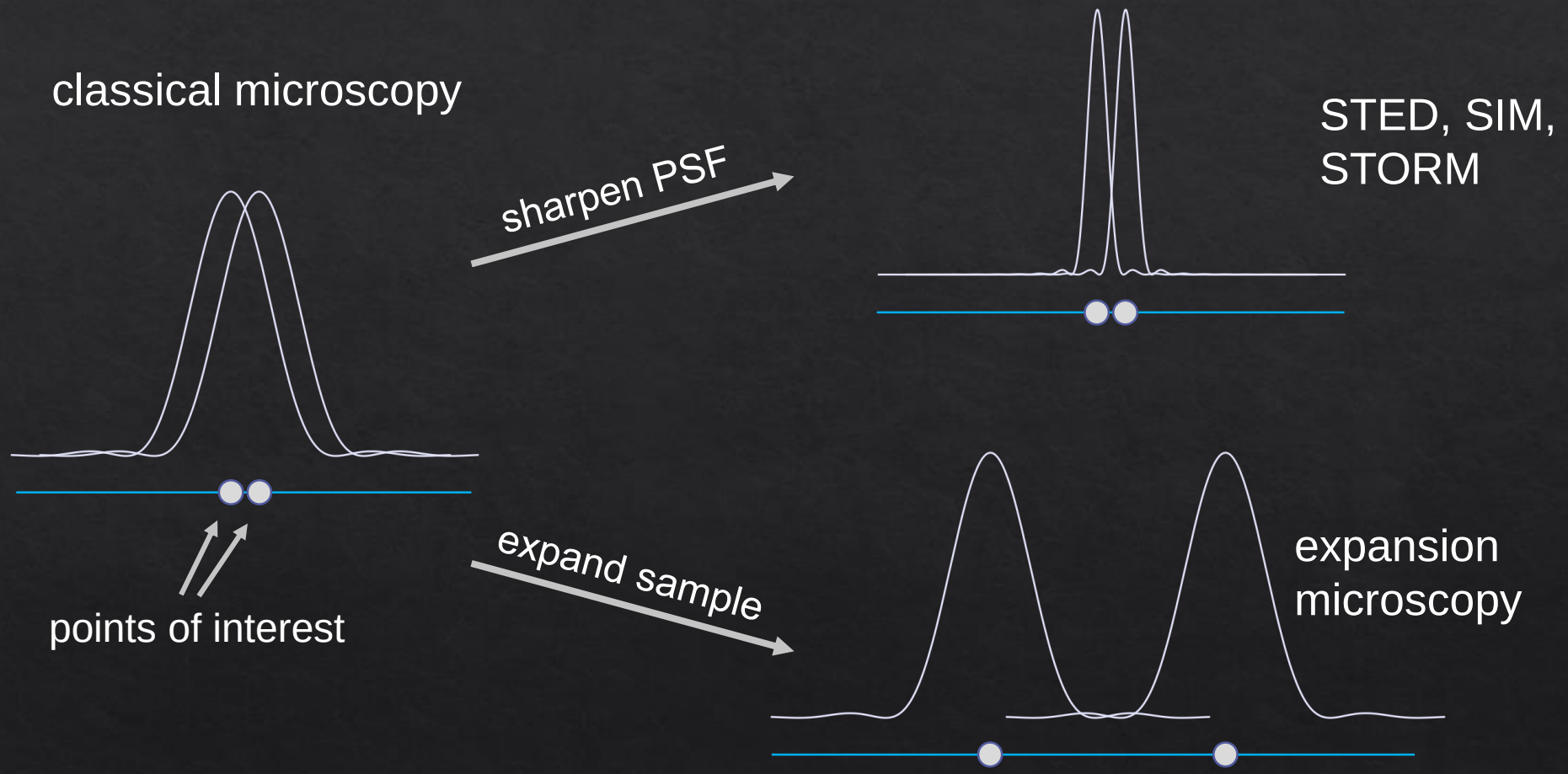


points of interest

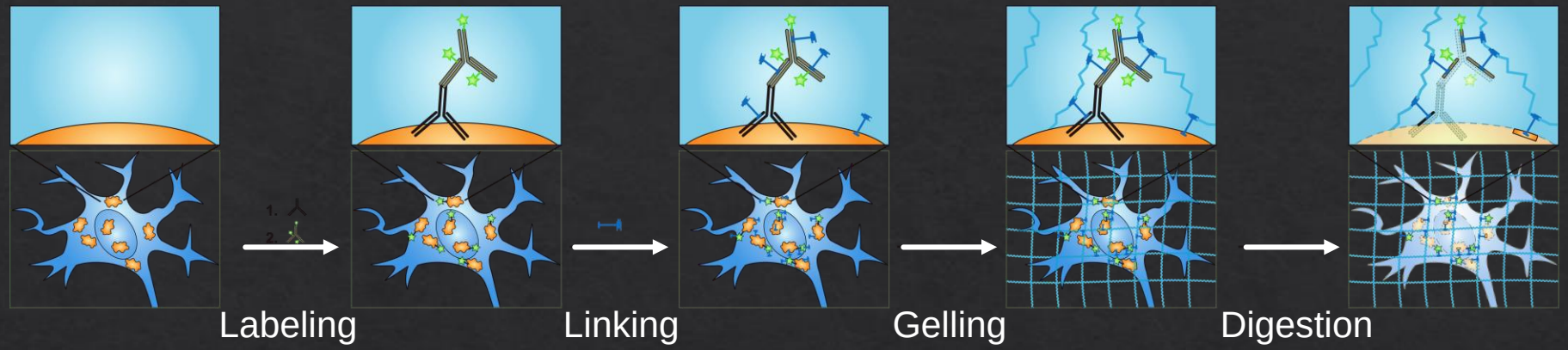
Increase optical resolution by „sharpening“ the point spread function

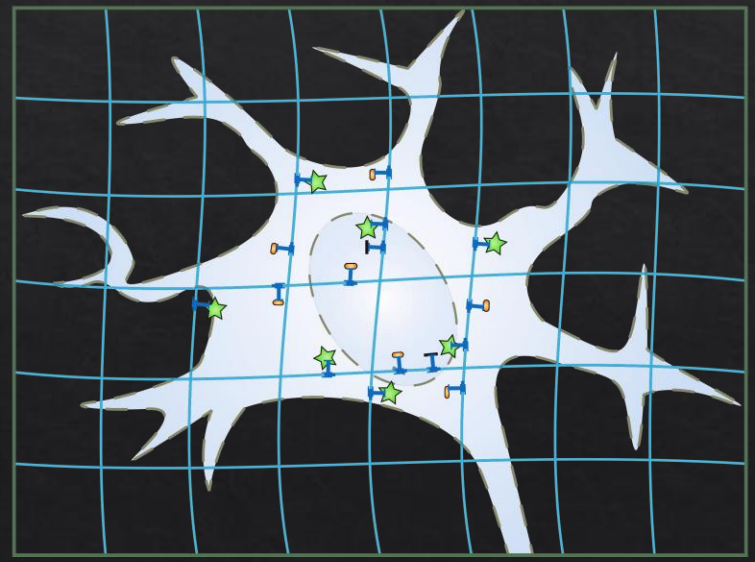


… or use expansion microscopy: much cheaper and faster!



Biochemical procedure of expansion





Expansion 4x

Advantages:

Transparent sample

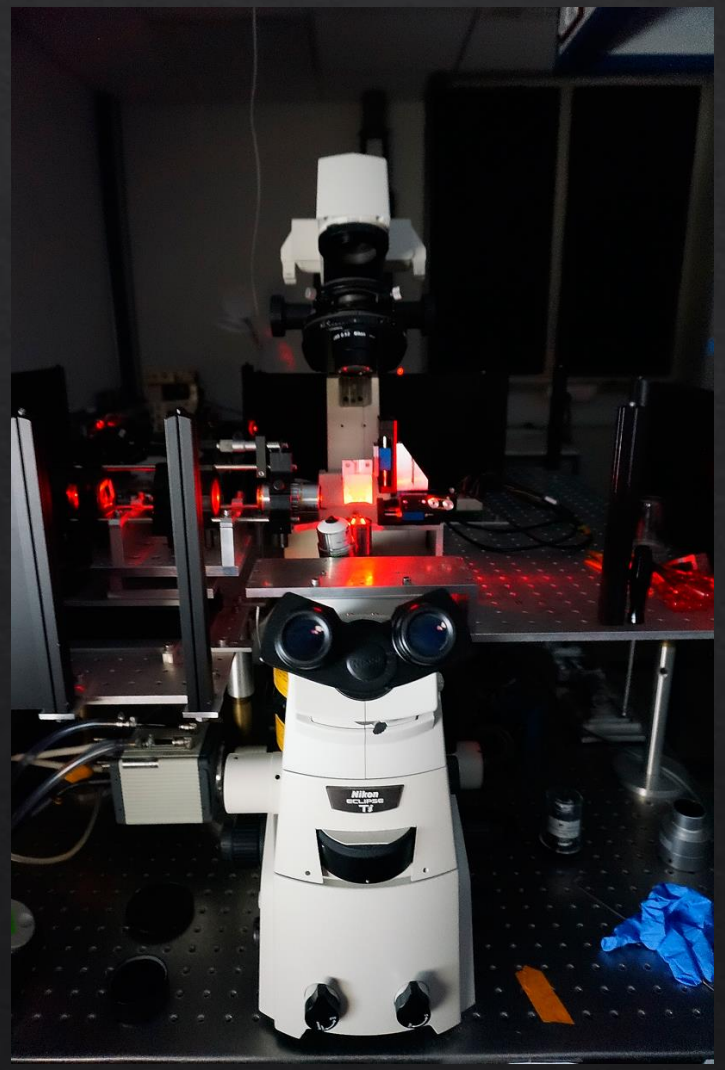
Refractive index of water

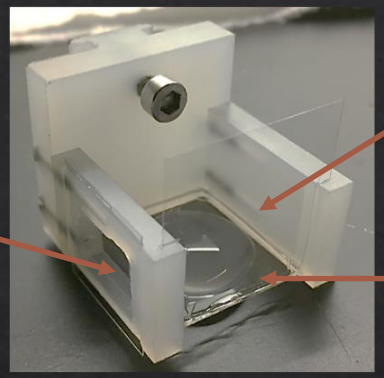
Expansions up to 20x

Expansion according to: Shen et. al. Science (2015) and Chozinski et. al. Nature Methods (2016)

Light sheet microscopy of expanded samples
- Mouse brain samples

Light sheet microscopy of expanded samples

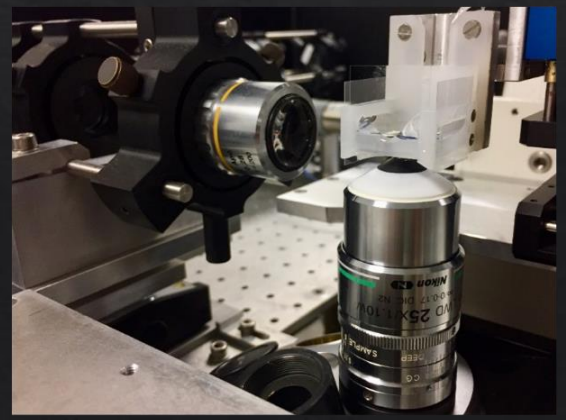


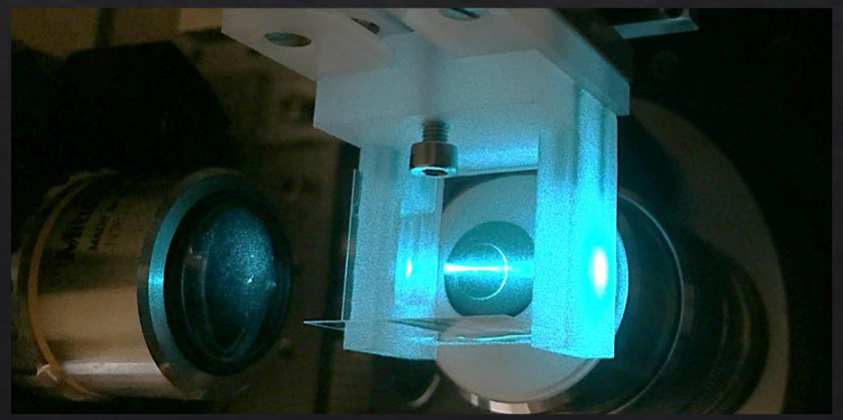


Water filled

Illumination window
24 x 24 mm
0.17 mm thick

Sample mounted on cover slip
of 24 x 24 mm, 0.17 mm thick





LFSM optical resolution: theory and experiment

PSF of the 25x, NA 1.1 WI objective from Nikon determined by Huygens

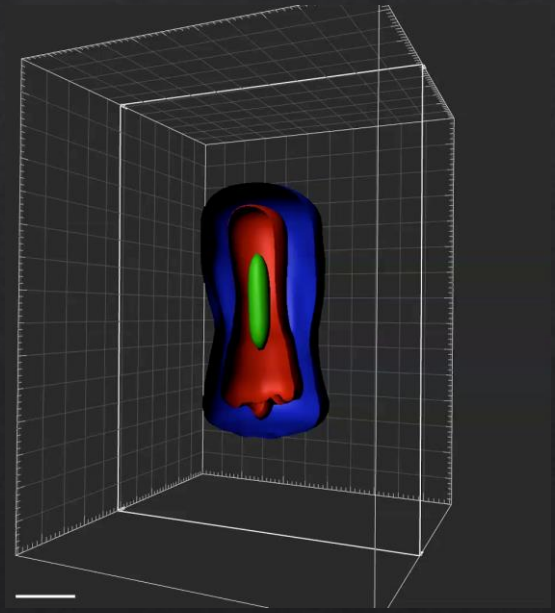


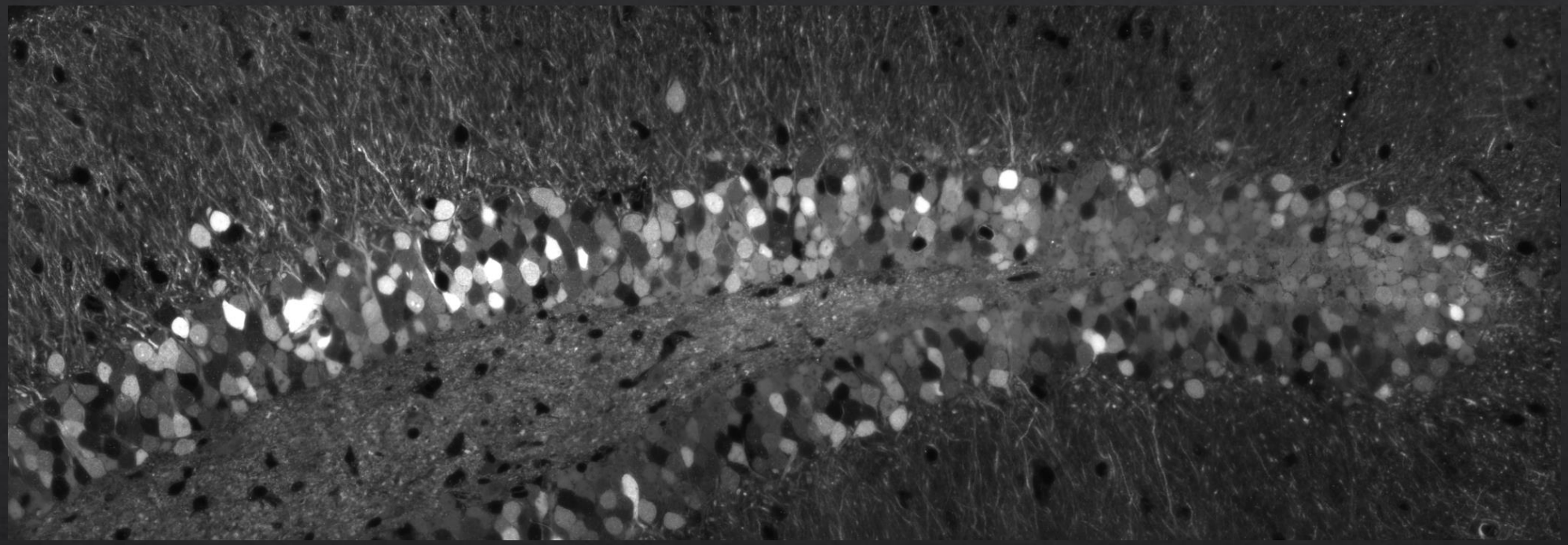
Table 1 Optical resolutions of the utilized microscopes.

	Theoretical FWHM resolution (nm)[a]		Experimental FWHM resolution (nm)[b]		Experimental virtual FWHM resolution (nm)[c]	
LSM/ excitation	Lateral	Axial	Lateral	Axial	Lateral	Axial
Airyscan 488 nm	—	—	160	810	—	—
LSFM 488 nm	242	790	380	1625	100	415
LSFM 640 nm	310	1010	520	2300	135	590

[a]The theoretical FWHM values were determined using the procedures given above and emission wavelengths of 520 and 665 nm for green and red excitation, respectively.
[b]All values have errors of maximally 5%.
[c]The virtual resolution was calculated taking the average expansion factor of 3.9 into account.

Bürgers, Rodriguez-Gatica, et al., *Neurophotonics* 2019

Mouse dentate gyrus imaged by LSFExpansionM: LSFEM



A single confocal plane acquired using a custom built LSFM and an 25x 1.1 NA WI objective lens plus 1.5x

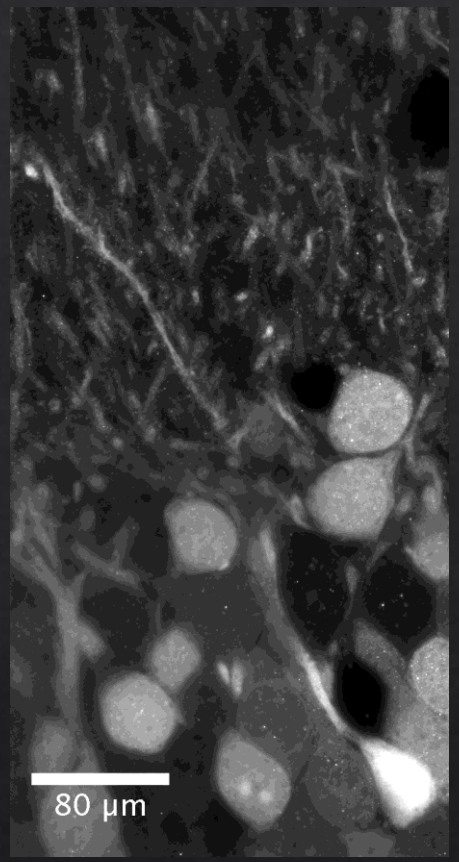
The total field size was 3600 x 1240 μm^3, achieved by stitching 80 stacks comprising 2048 x 2048 pixels in each frame.

Stitched using the algorithm by Preibisch et al. (2009).

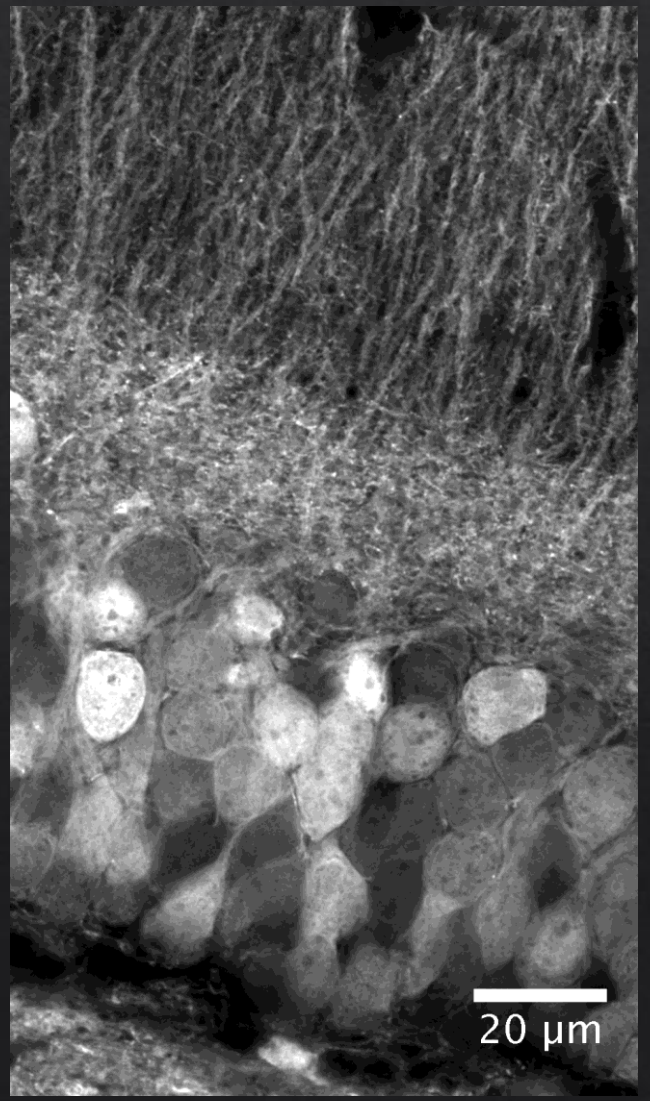
Bürgers, Rodriguez-Gatica, et al., *Neurophotonics* 2019

Comparison of LSFEM and confocal

LSFEM image



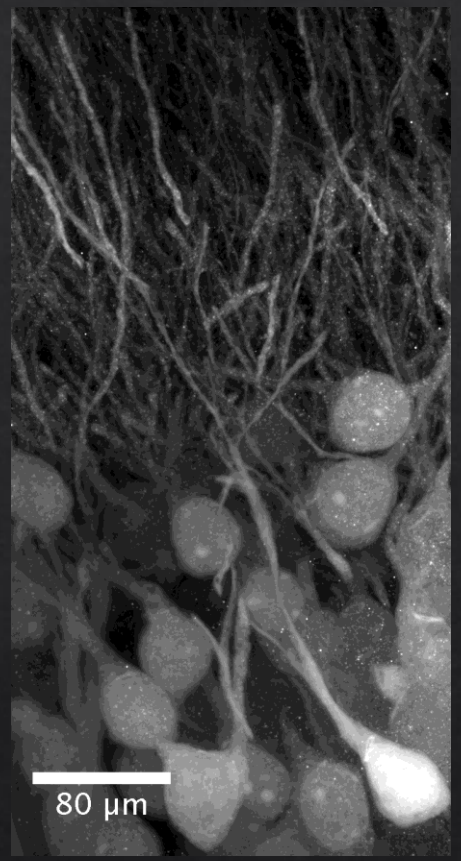
Confocal Airyscan image



Bürgers, Rodriguez-Gatica, et al., *Neurophotonics* 2019

Mouse dentate gyrus imaged by LSFEM

LSFEM image

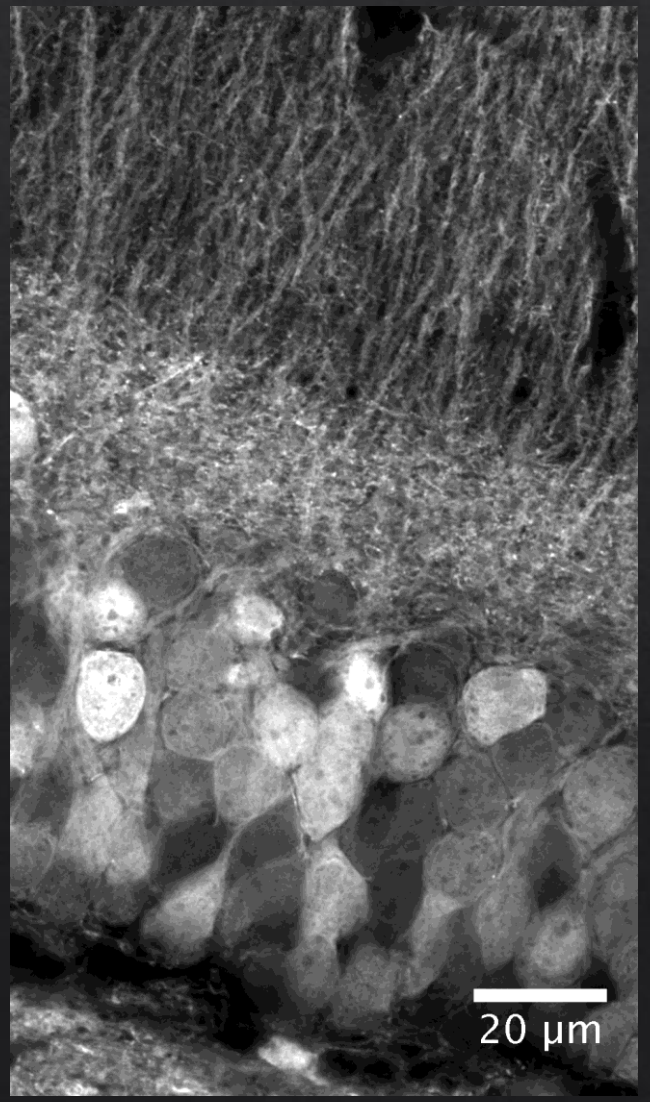


Maximum projection of the selected region shown above

comprising 75 µm of the stack

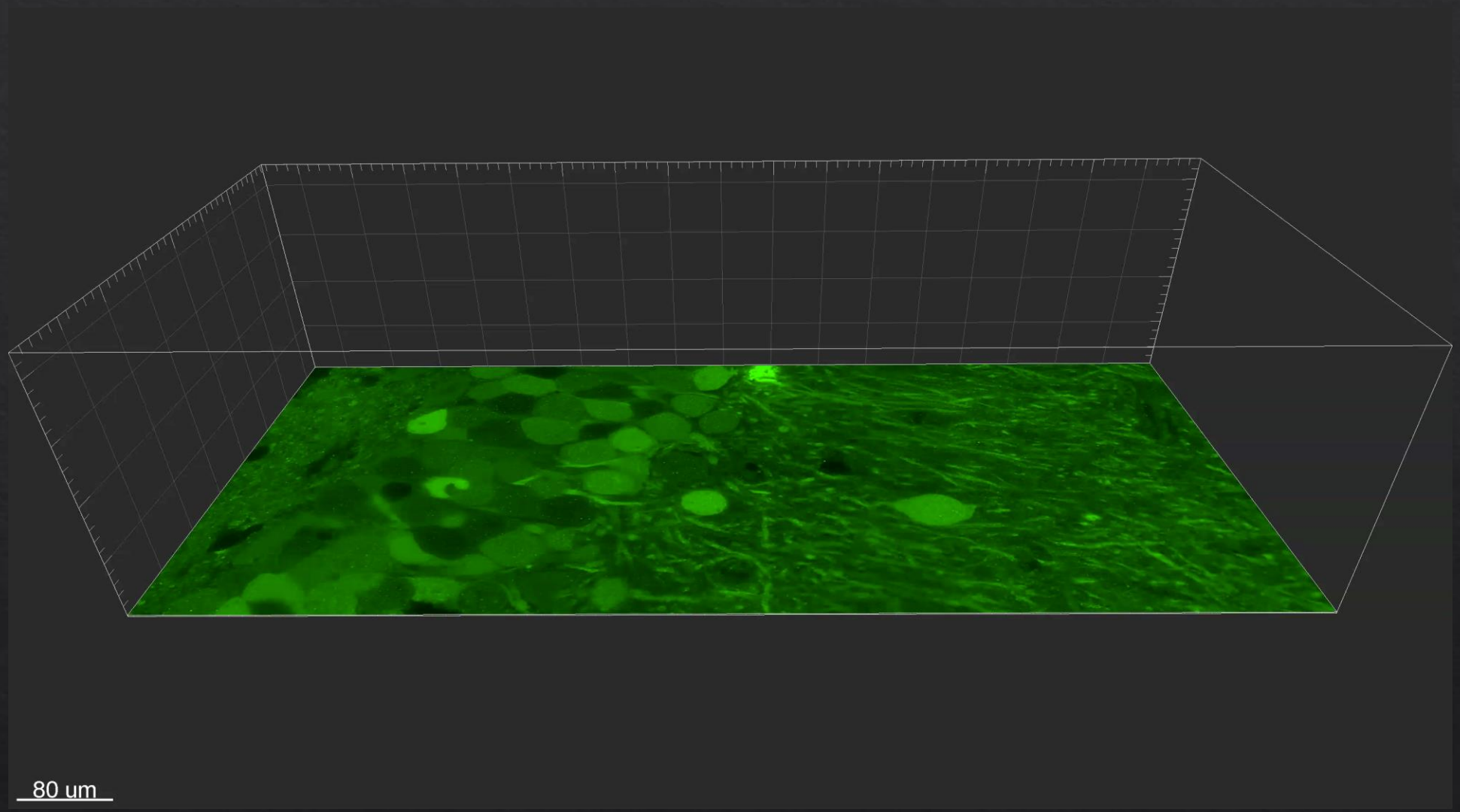
lateral field size 254 x 492 µm^2

Confocal Airyscan image



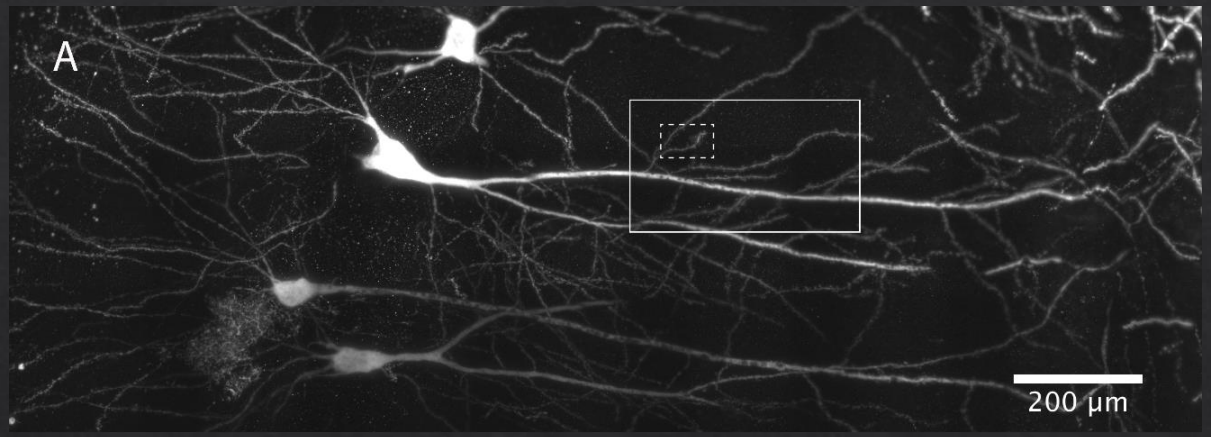
Bürgers, Rodriguez-Gatica, et al., *Neurophotonics* 2019

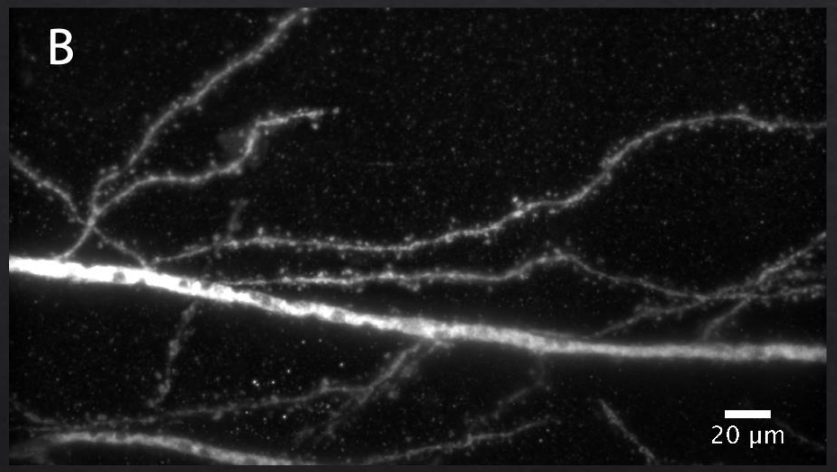
Neuronal network in super resolution following expansion

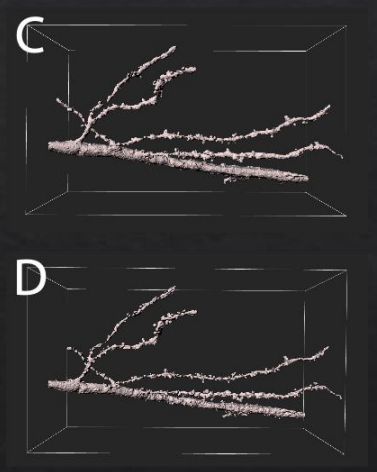


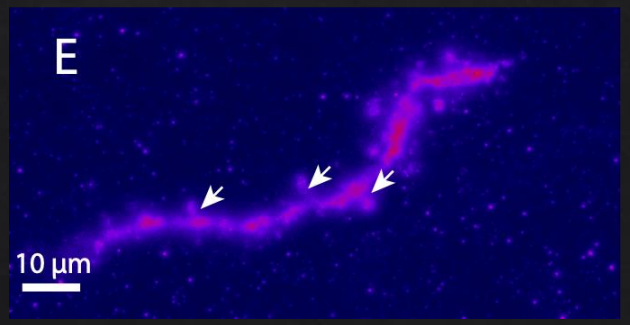
Segmentation of three arbitrarily selected cells of the previous data set.
Total volume shown: 3600 x 1240 x 275 µm³ (after expansion)

Resolving dendritic spines of sparsely labeled pyramidal neurons in CA1









(A) MIP of a total of 24 stacks with an axial step size of 0.3 µm covering a depth of 450 µm. The sparse labeling and small axial imaging step size allowed to reconstruct the labeled granule cells and dendrites over distances of 1.3 mm after expansion.

(B) Magnification of the large ROI marked in (A). (C) 3-D surface rendering of a region of the data shown in (B), the dimensions were 256 × 152 × 205 μm^3.

(D) Deconvolution of the image data using the experimental PSF yielded a significant increase in data quality.

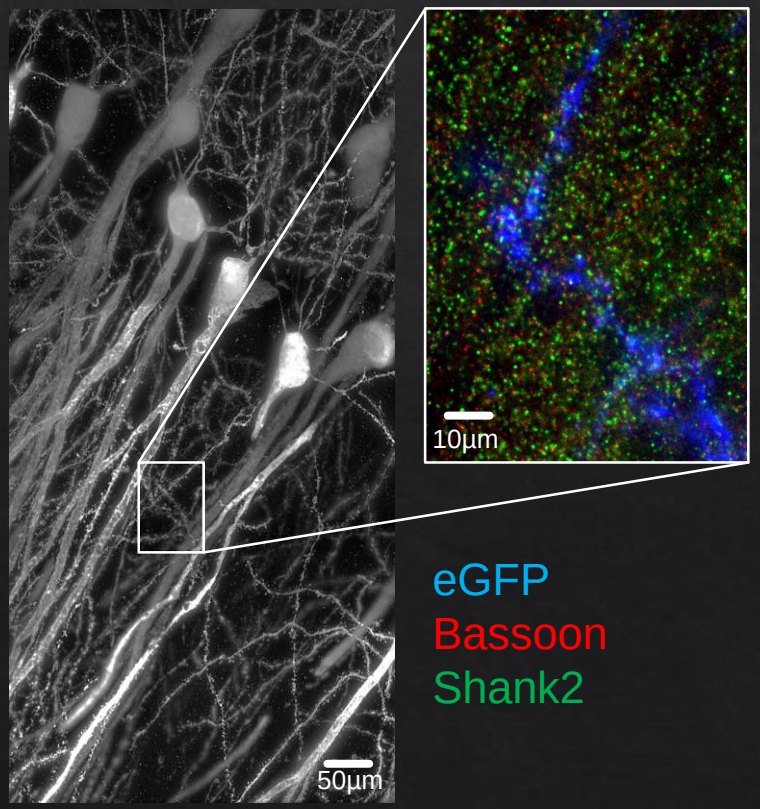
(E) A maximum projection of 250 frames of a magnified region of the image stack shown in (A) (see dashed ROI) containing a single dendrite demonstrates that even dendritic spine necks (arrows) can be recognized.

Bürgers, Rodriguez-Gatica, et al., *Neurophotonics* 2019

Synaptic connectivity: three-colour LSFEM

Sparsely labeled pyramidal neurons in CA1 expressing EGFP

Antibody staining against the
Presynaptic active zone protein bassoon
and the postsynaptic scaffold protein, shank2



eGFP
Bassoon
Shank2

Left:
MIP of an expanded mouse brain slice.
Size 456 x 945 x 390 µm³ - deconvolved data
(9 stitched z-stacks, step size of 300 nm, 15% lateral overlap)

Right:
magnification of the ROI, MIP of lateral field size 76 x 106 µm².

Bürgers, Rodriguez-Gatica, et al., *Neurophotonics* 2019

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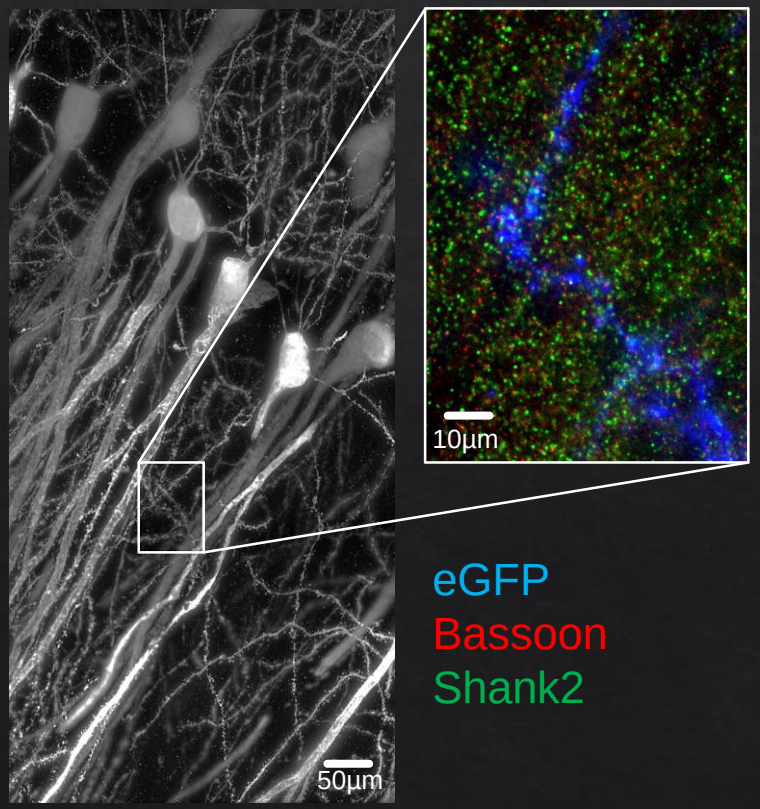

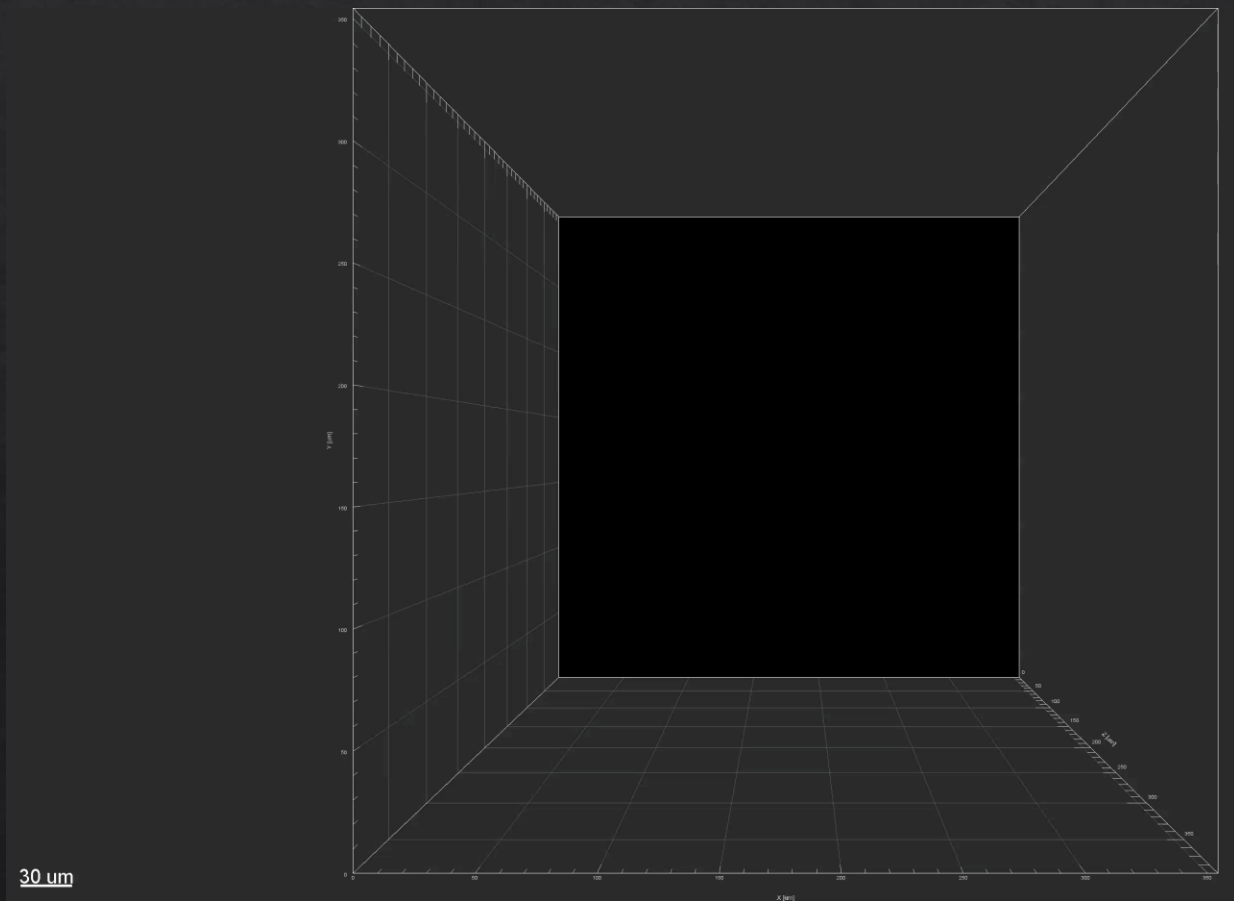

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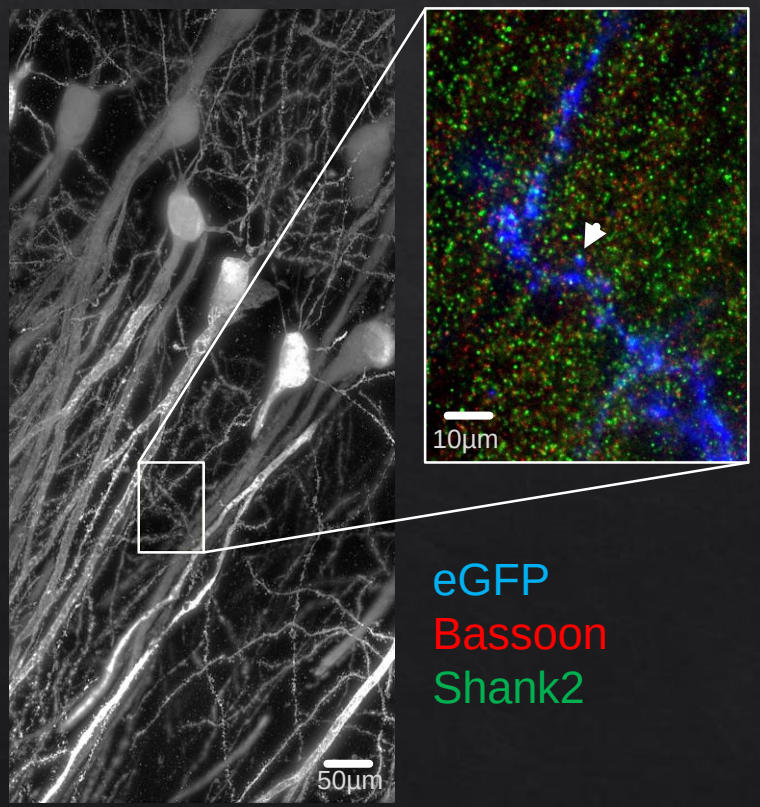
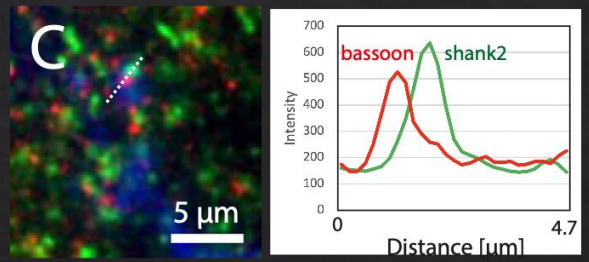
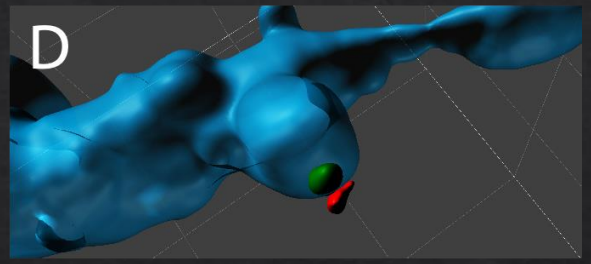


eGFP
Bassoon
Shank2





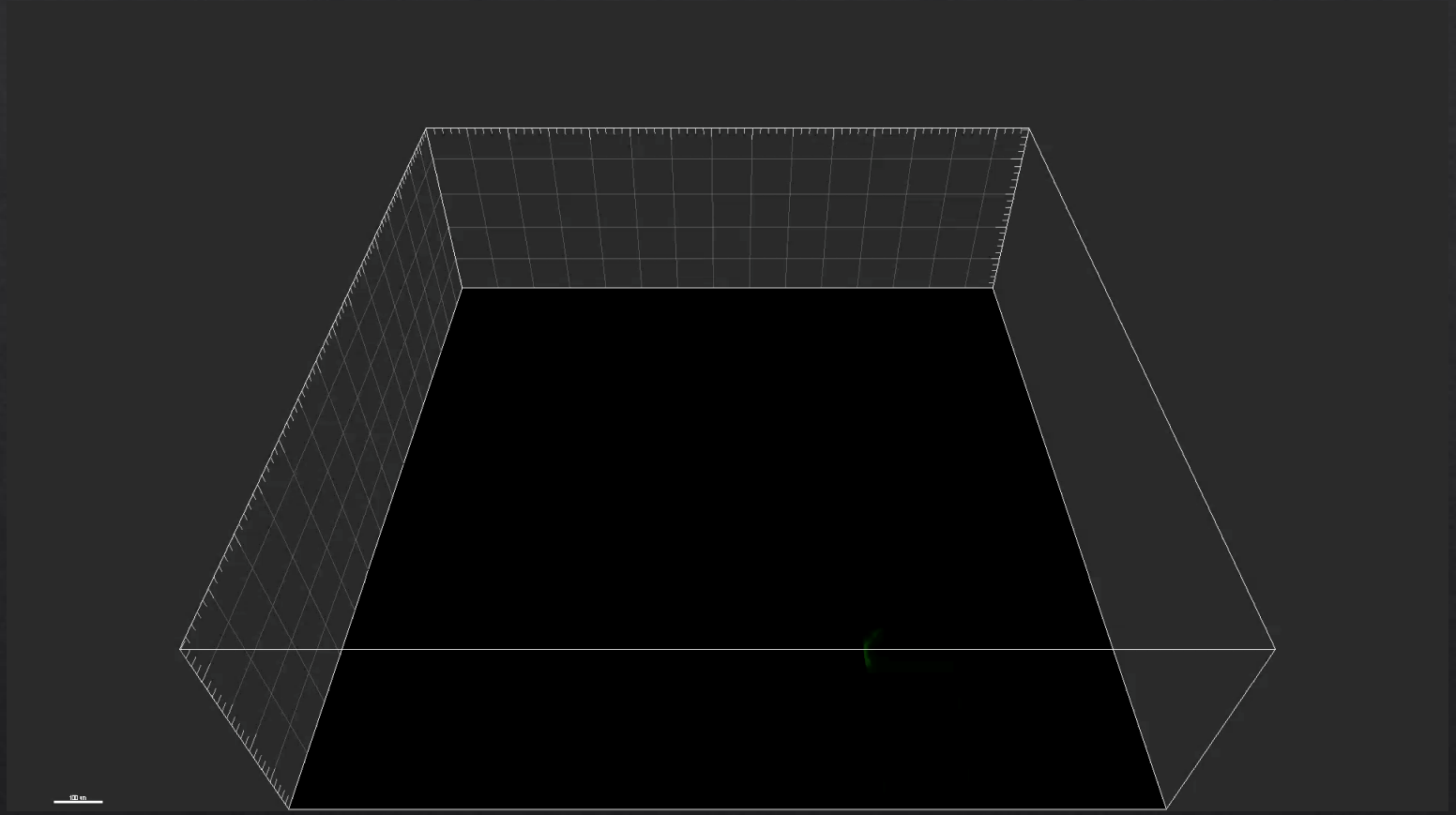
Distance of 160 ± 50 nm between pre and postsynaptic proteins

Capability of our approach to visualize details of synaptic connectivity

Bürgers, Rodriguez-Gatica, et al., *Neurophotonics* 2019

LSFME of thick and fluorescent protein labeled mouse brain slices

Modification of the expansion procedure allows to preserve FP and also to increase slice thickness

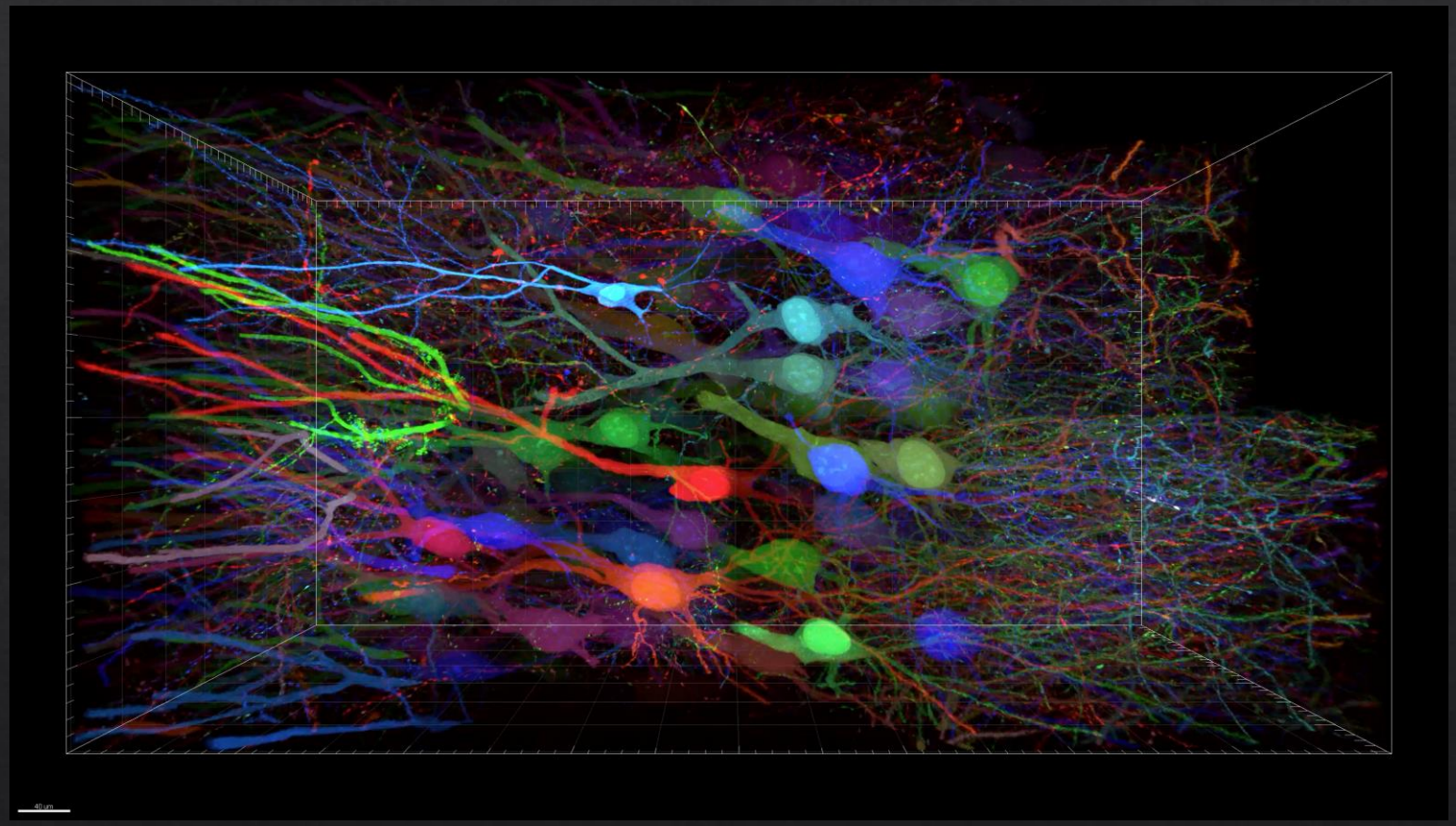


Mouse dentate gyrus granule cells expressing EGFP
Imaged using an objective 25x/NA 1.1 WI
40 z-stacks with 1610 slices each, 15% lateral overlap between stacks, axial step size 300 nm, volume 1150 x 1010 x 483 μm^3

Tetbow labeling of neurons in the CA3 region

Tetbow labeling: Sakaguchi et al., eLife 2018

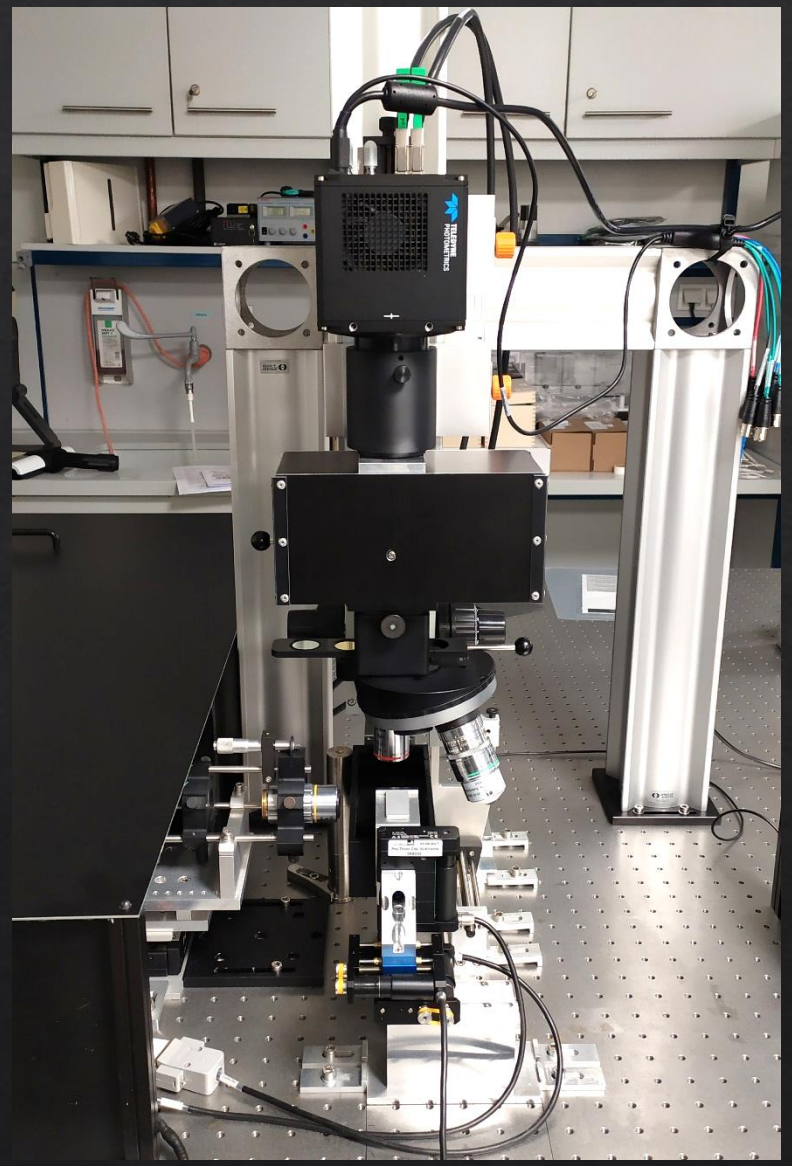
mTurquoise EYFP tdTomato

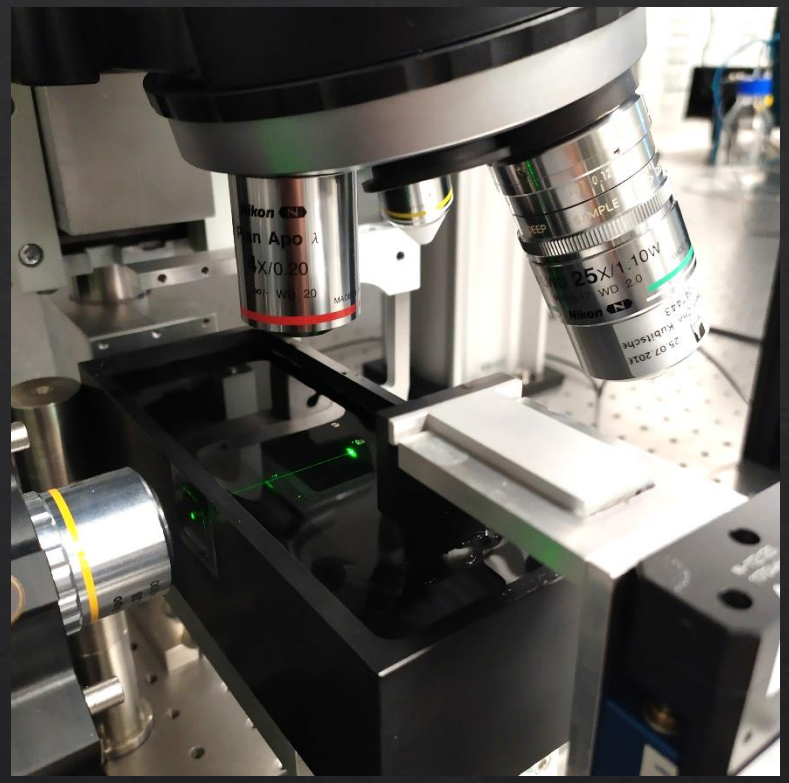


Mouse brain section, 200 µm thick - before expansion
3D view of the sample after digestion (sample in PBS)
Zeiss LSM 880, objective 40x/N.A. 1.1 WI,
6 tiles, step size 1µm, volume 788 x 406 x 354 μm^3

Light sheet microscopy of expanded samples
- Brain organoids

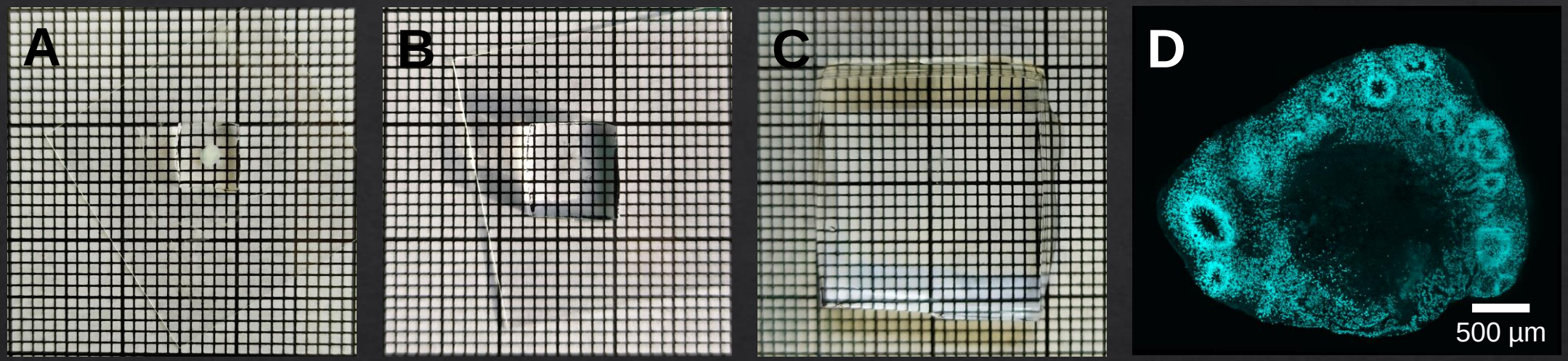
Improvement of the LFSM optical resolution





Scale	Objective lens	True optical resolution		Effective optical resolution		Data size per 1 mm³ sample
		Lateral	axial	Lateral	axial	
Mesoscale	10x, NA 0.3, WI	1.2 µm	17.8 µm	0.8 µm	11.8 µm	5.5 GB
Microscale	25x, NA 1.1 WI	300 nm	1100 nm	200 nm	800 nm	500 GB
Nanoscale	25x, NA 1.1 WI	300 nm	1100 nm	80 nm	300 nm	8 TB

Brain organoid before and after clearing and expansion



(A) Two-month-old (2mo) brain organoid embedded in a polyachrylamide gel. (B) sample after proteinase K digestion, which resulted in a clearing of the organoid and an approximately 1.5-fold expansion. (C) sample after expansion in bidistilled water yielding an approximately 4-fold expansion.
(D) Optical section of the cleared and 1.5-fold expanded organoid showing the cell nuclei of an optical section in a depth of 1 mm inside the organoid.

Rodriguez-Gatica, et al., *unpublished* 2021

Summary

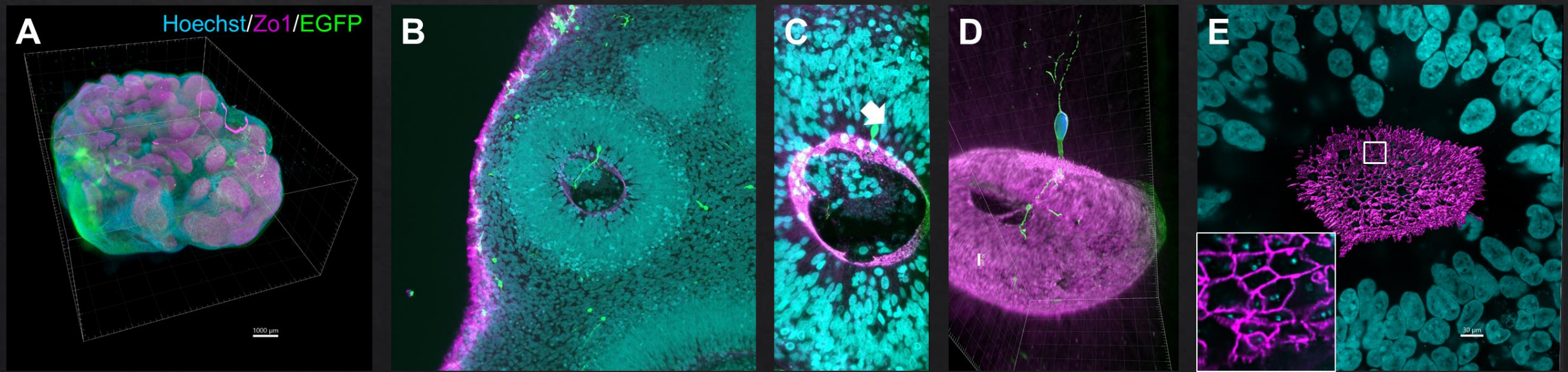
Confocal vs light sheet fluorescence microscopy

Imaging of extended brain slices (expanded size 2x2 cm, 2 mm thick)
using 20 ms image integration time

Super resolution achieved (laterally ≲ 80 nm, axially ≲ 300 nm)

Neuronal connectivity details (spines, pre- and postsynaptic structures) detectable

Imaging of Organoids on the Meso-, Micro- and Nanoscale
- topology of dense neural networks



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Thank you!

Light sheet fluorescence expansion microscopy
From Meso to Nanoscale

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