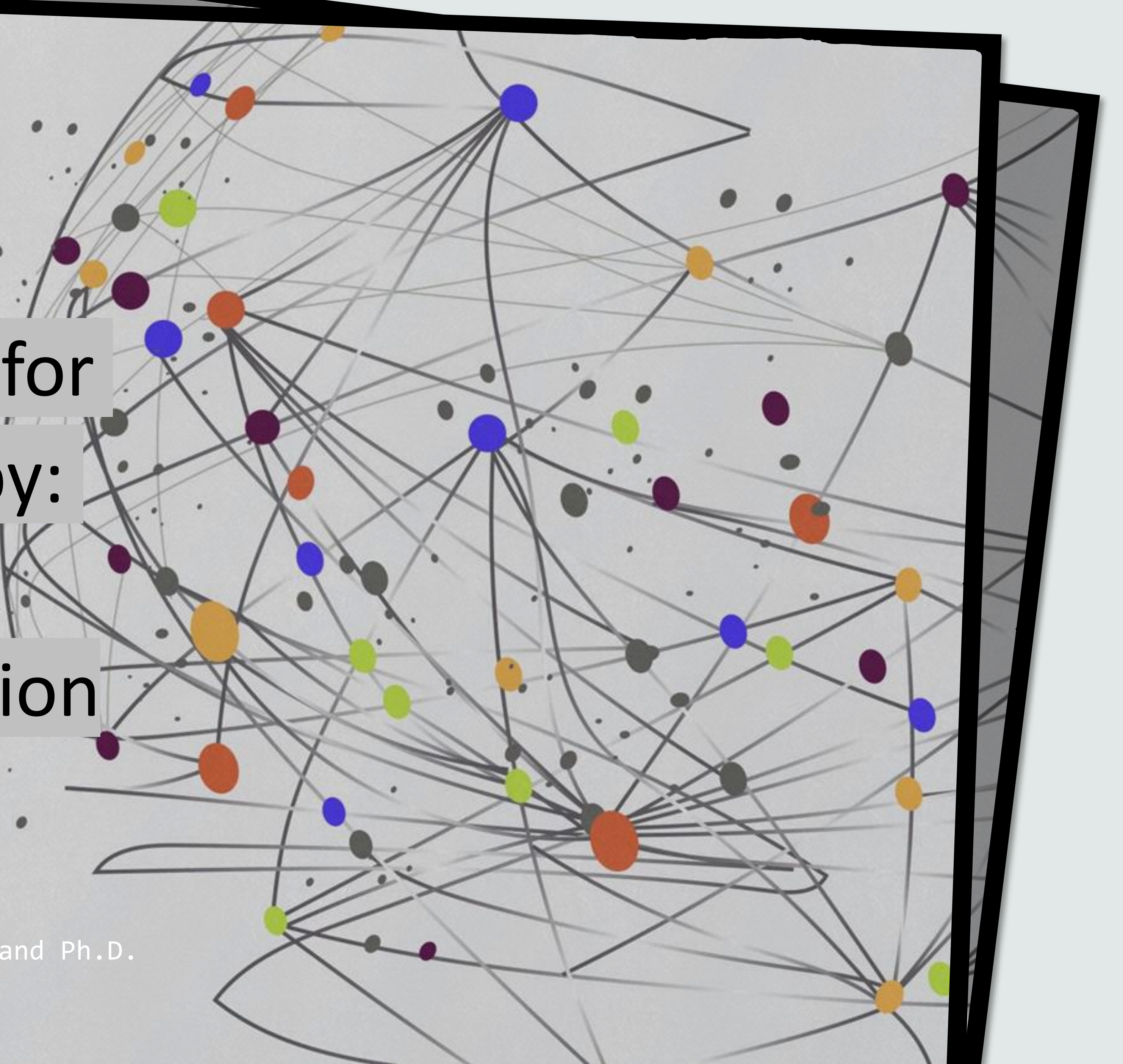
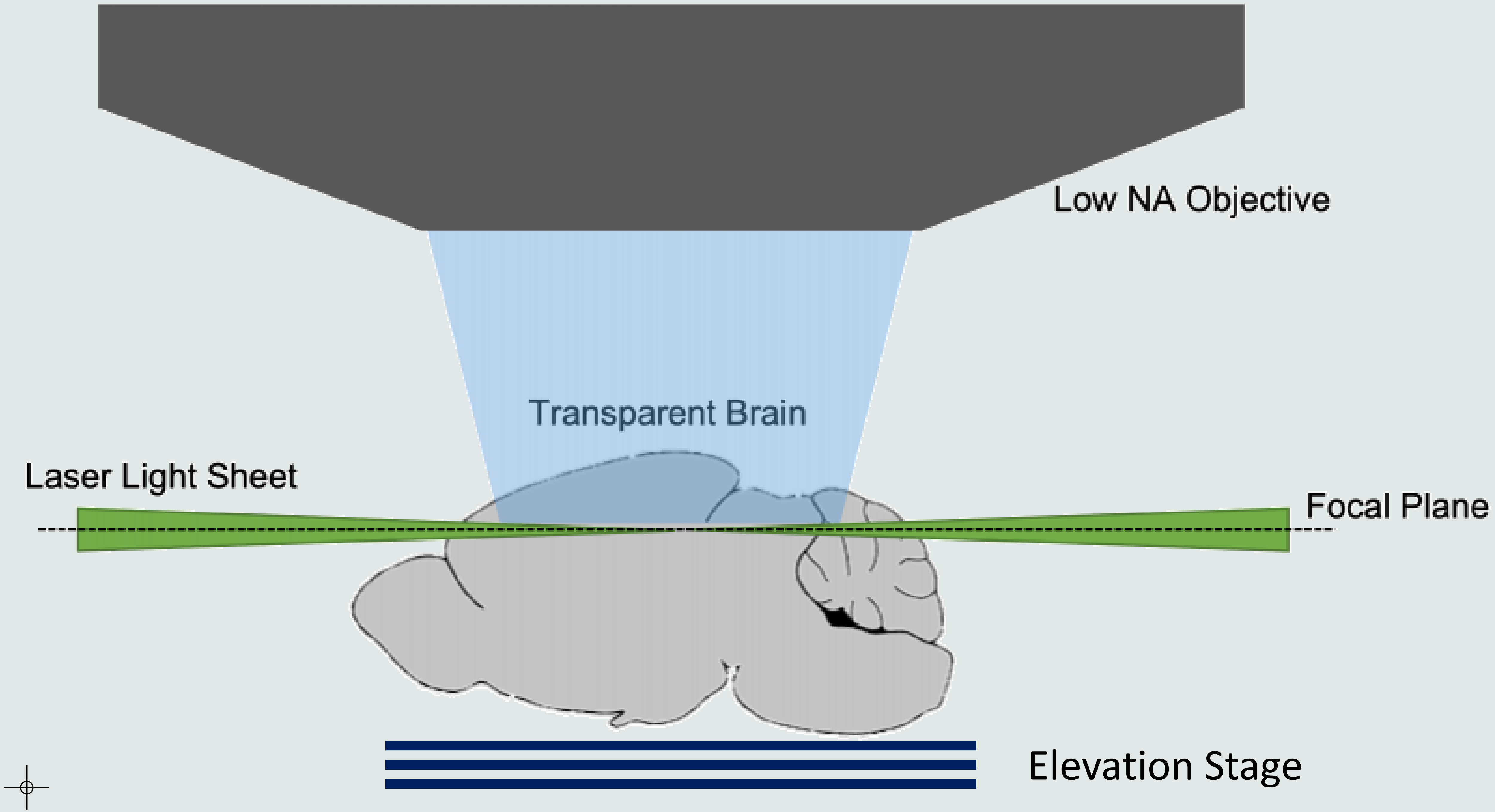
Image enhancement for light sheet microscopy: towards automated neuronal reconstruction

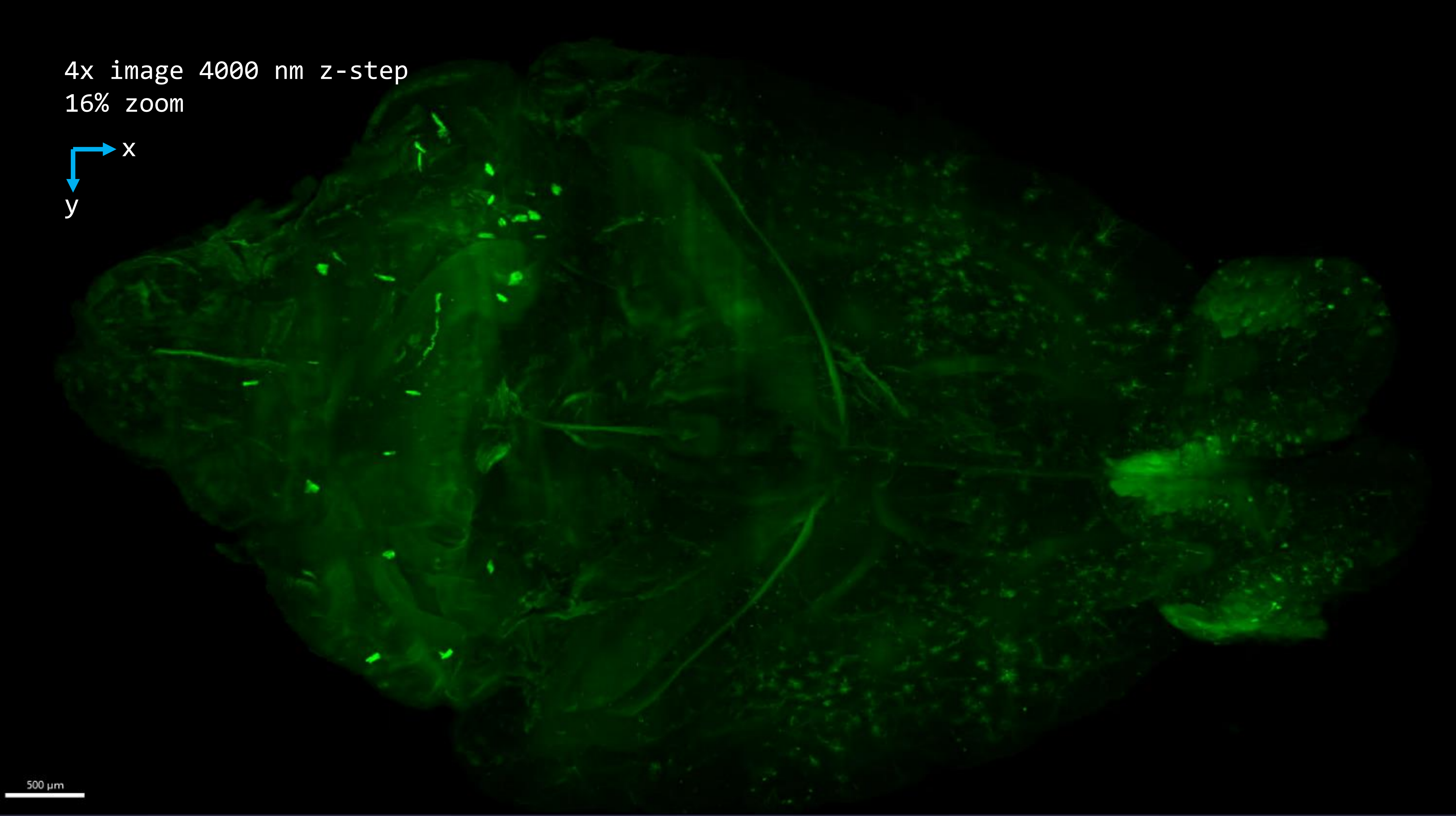


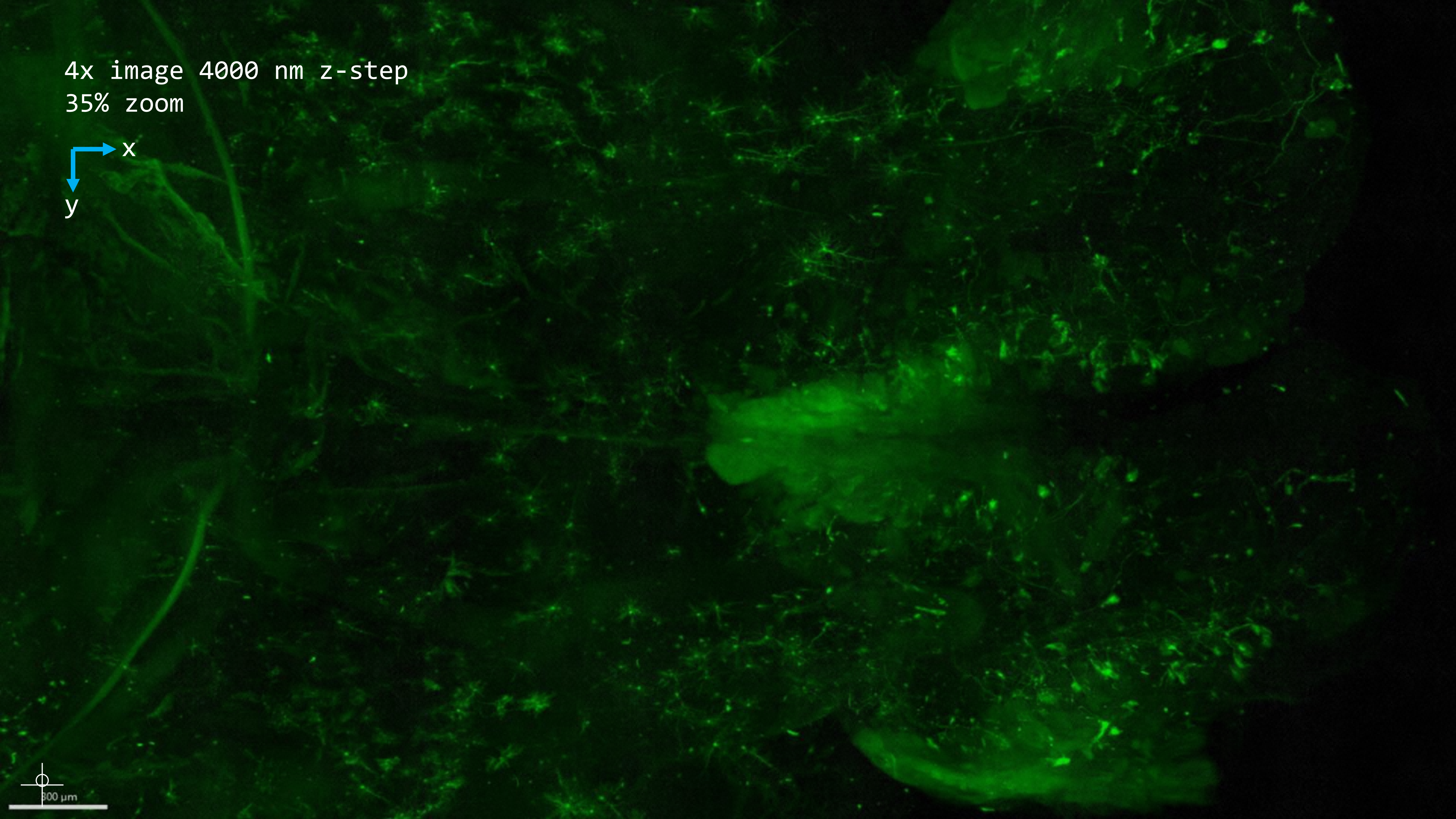
Keivan Moradi, M.D. and Ph.D.

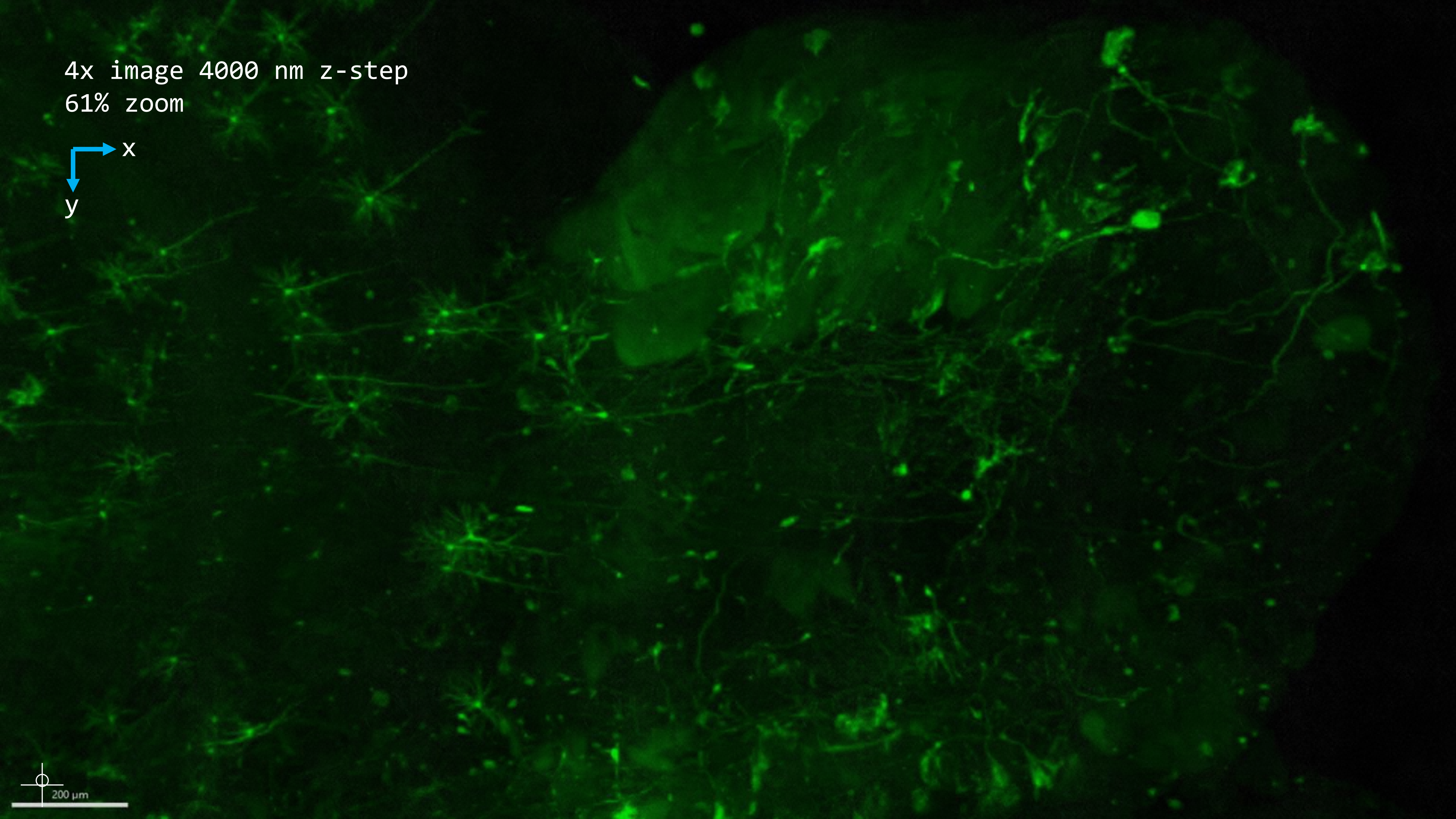
Research scientist and UCLA B.R.A.I.N

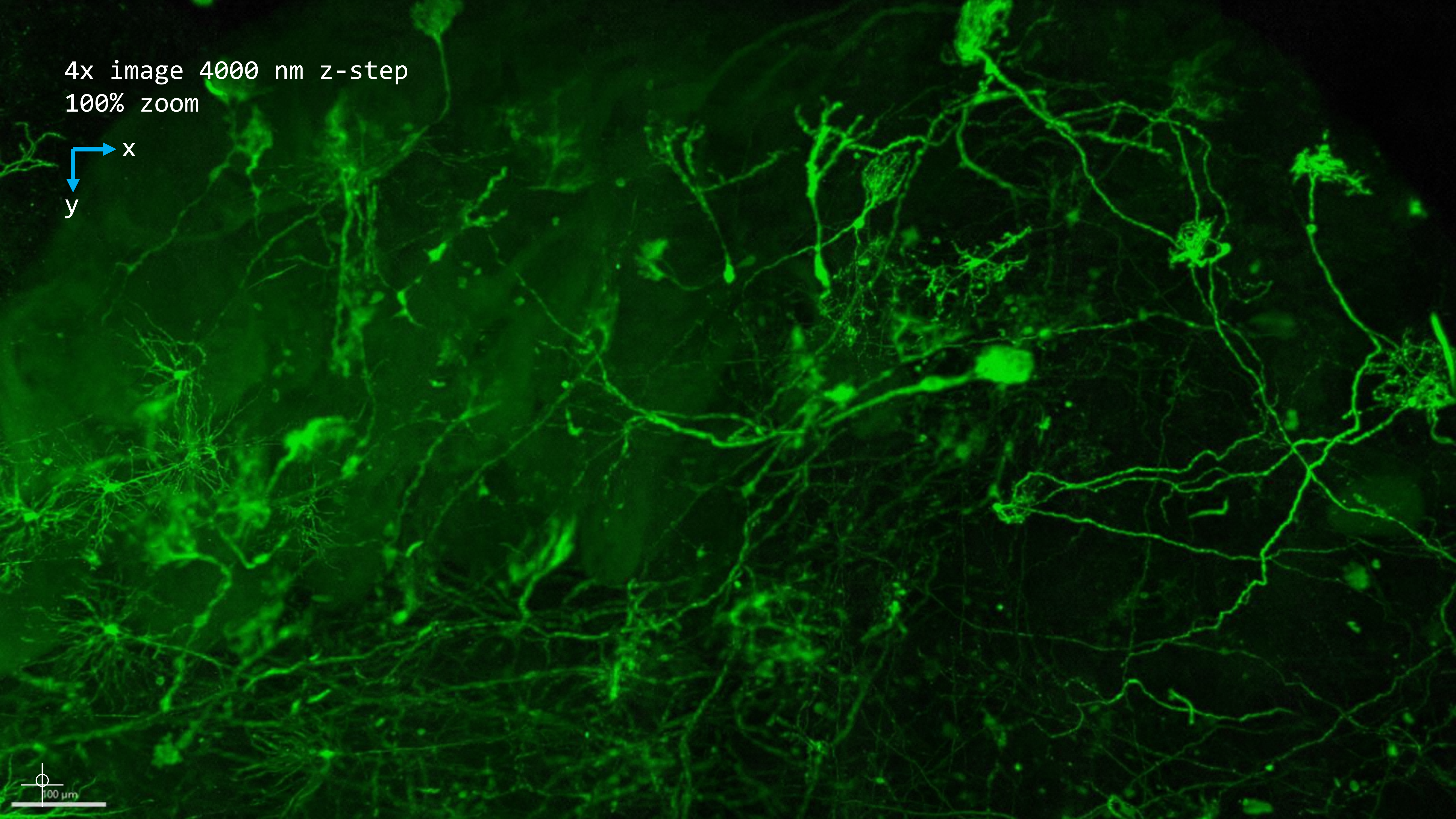
Under supervision of Dr. Hongwei Dong, M.D. and Ph.D.

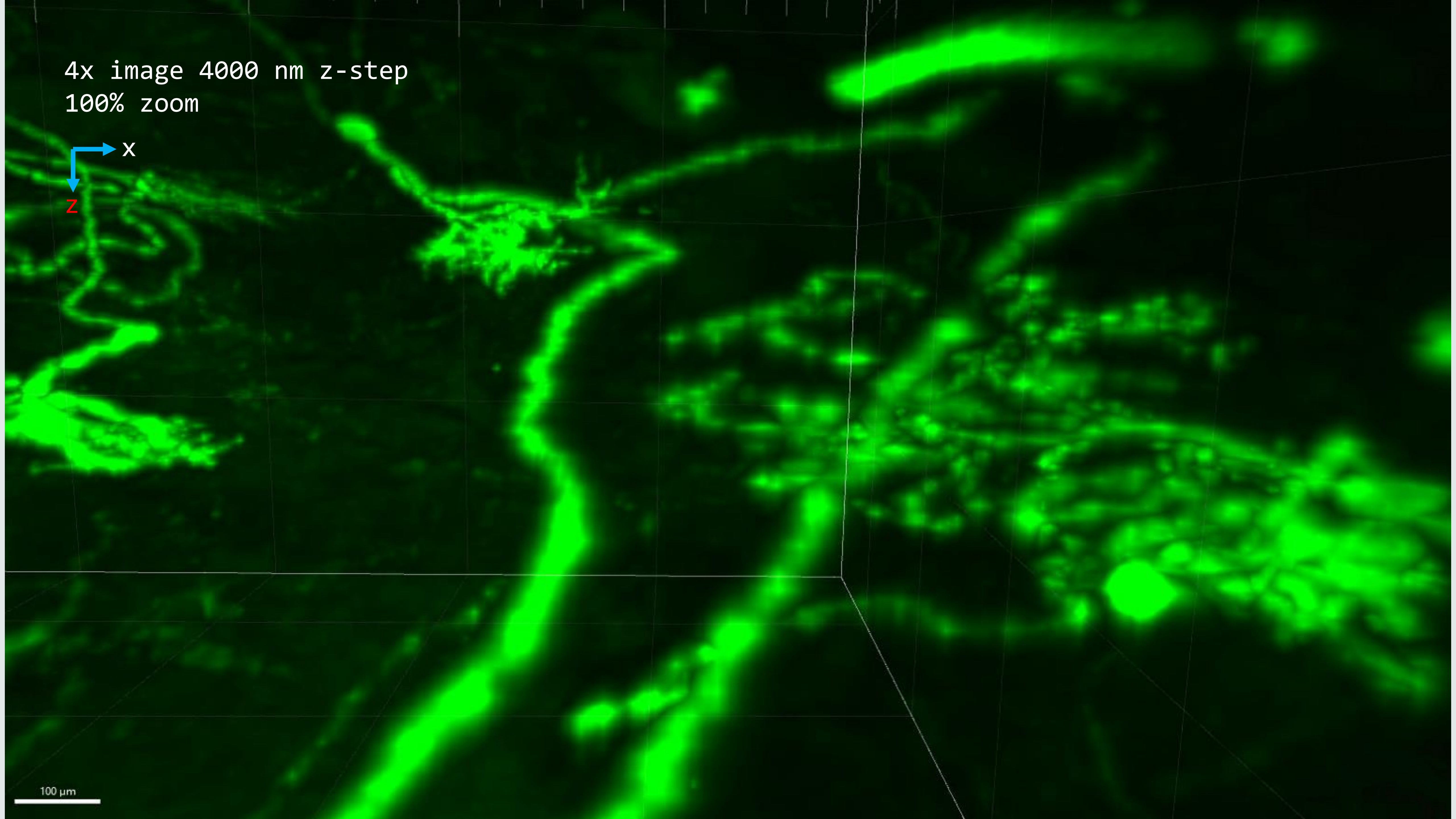

Low NA Objective
Transparent Brain
Laser Light Sheet
Focal Plane
Elevation Stage


4x image 4000 nm z-step
16% zoom
x
y
500 µm

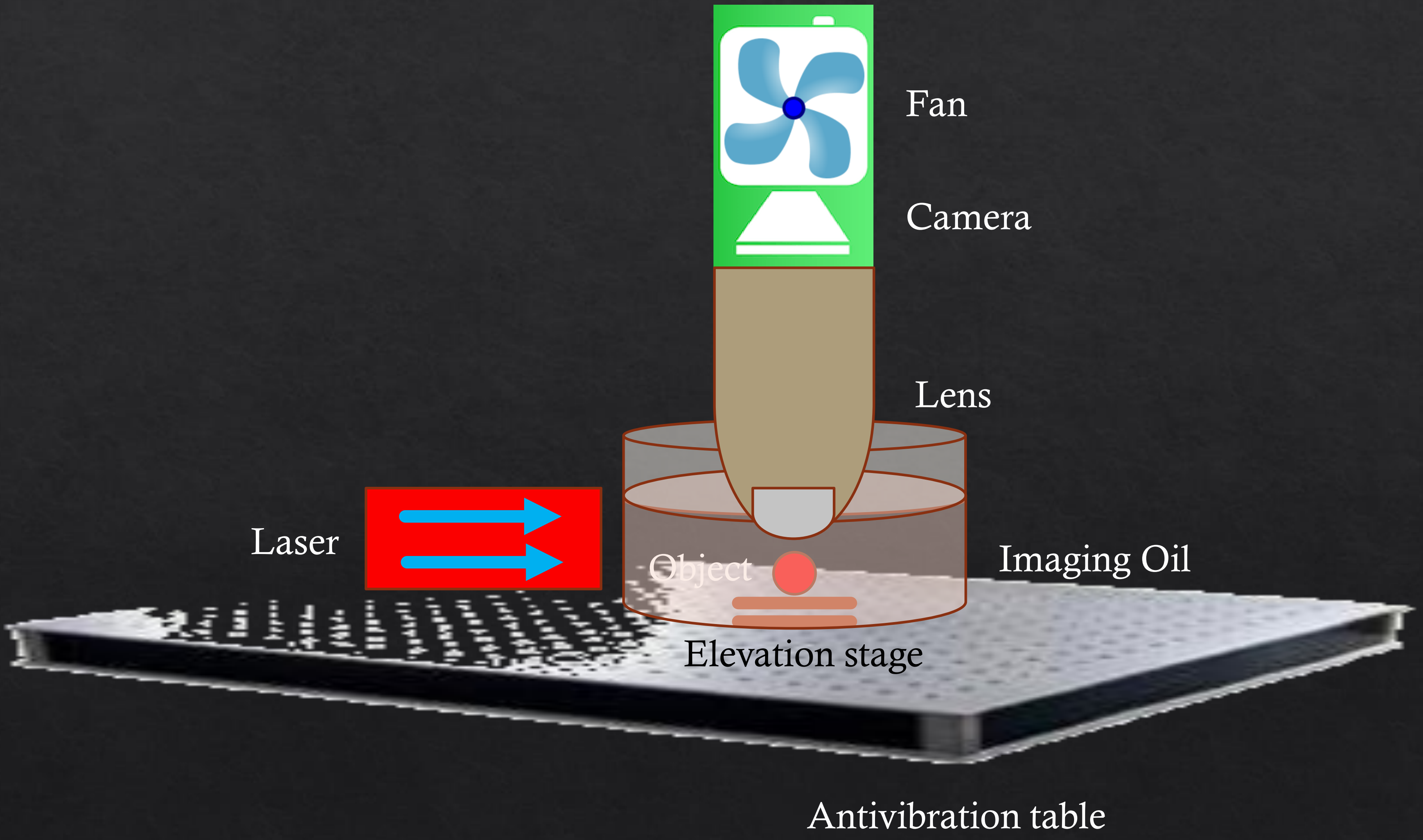

4x image 4000 nm z-step
35% zoom
x
y
300 µm


4x image 4000 nm z-step
61% zoom
x
y
200 µm

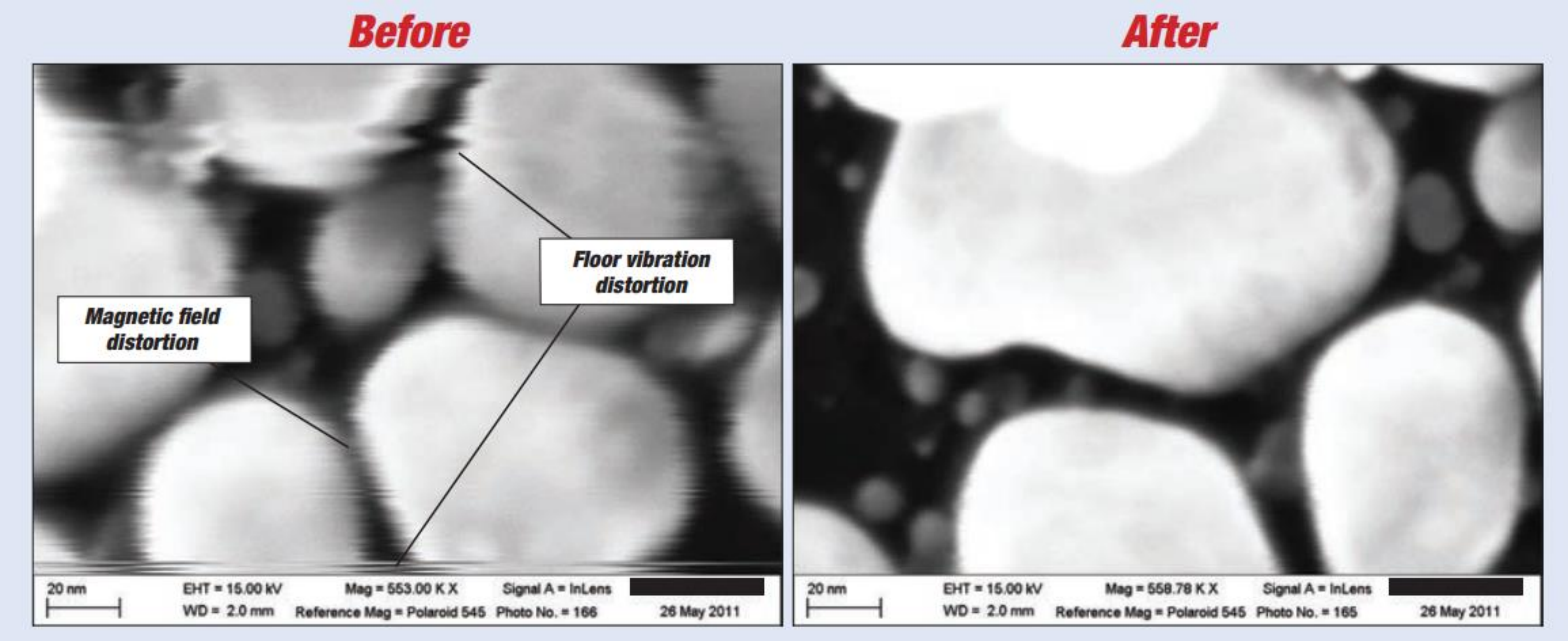

4x image 4000 nm z-step
100% zoom
x
y
100 µm


4x image 4000 nm z-step
100% zoom
x
z
100 µm

Problems: vibrations and the wobbly brain

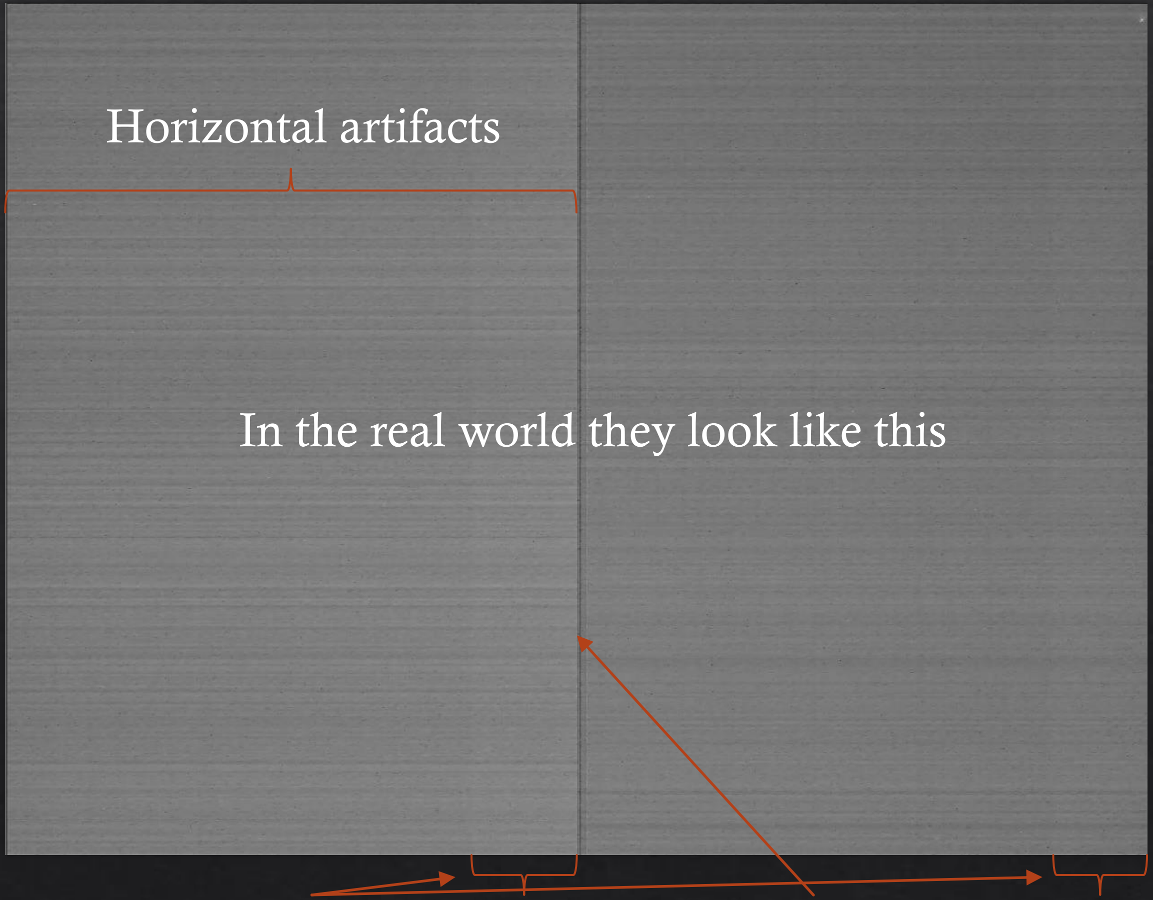


SEM Image



https://www.gmp.ch/pdf/catalogues/TMC_Resource_Guide_15.pdf

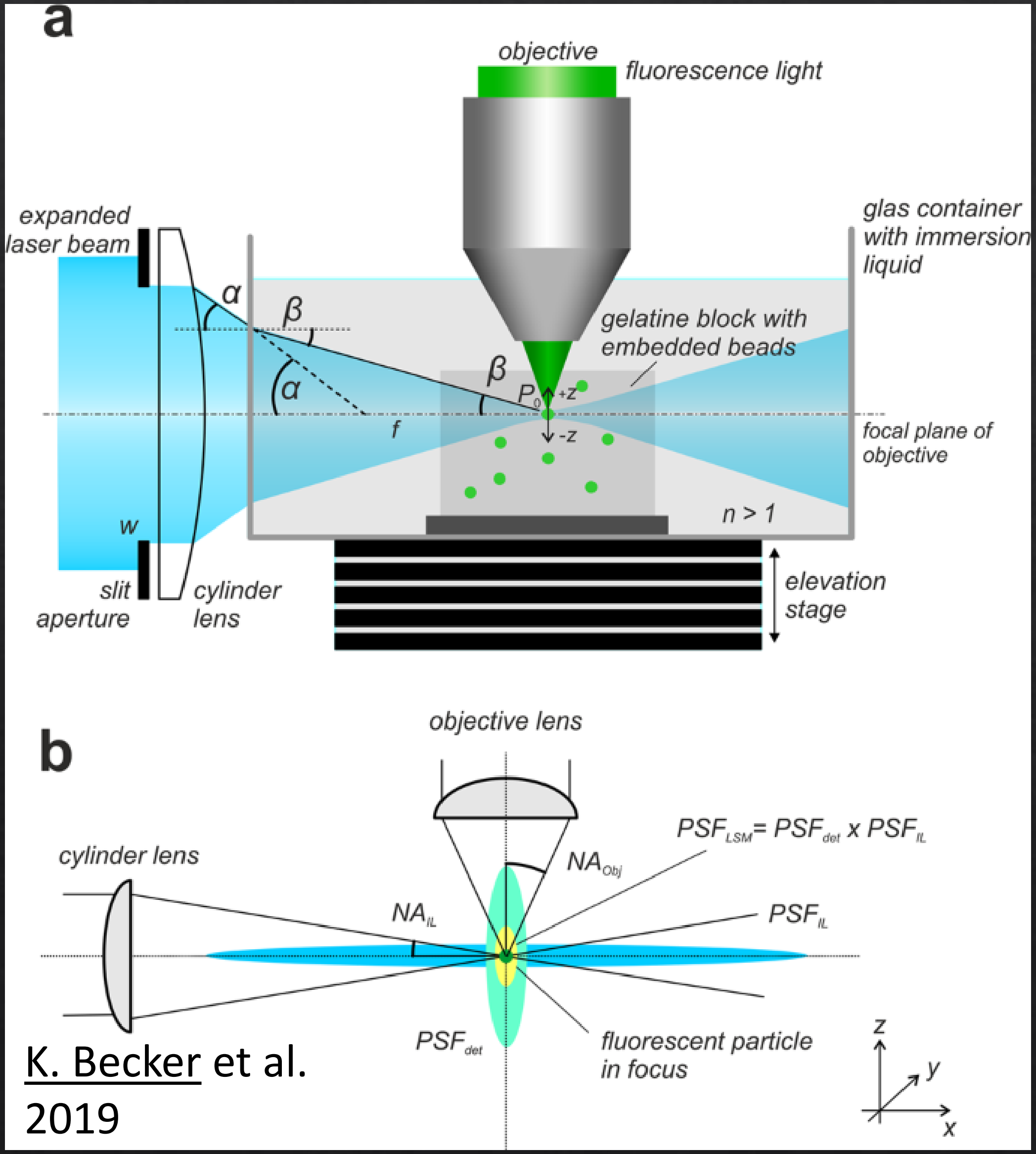
Problems: imperfect camera and tissue clearing



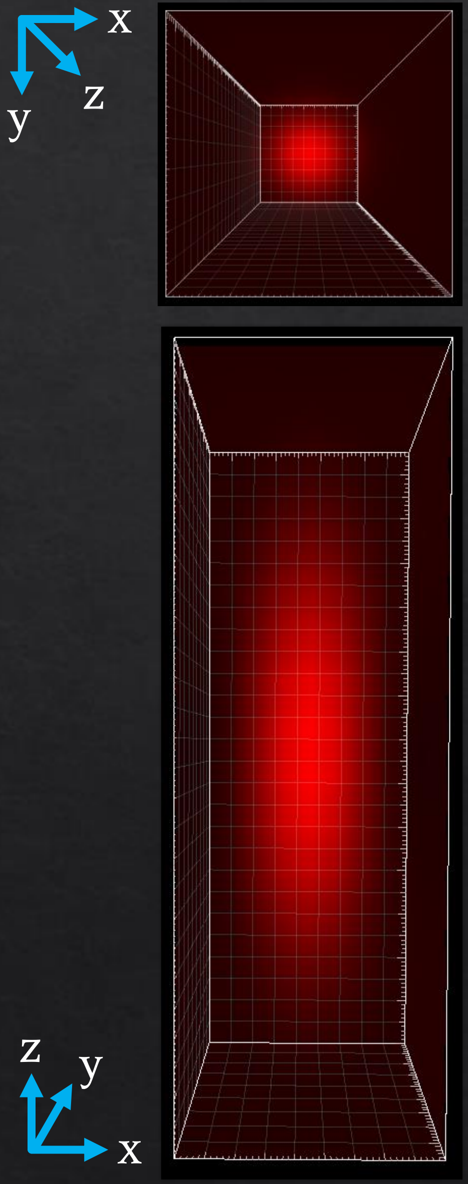
Probably vibration artifact
Wrong camera offset setting

Midline artifact

Problems: point spread function (PSF)

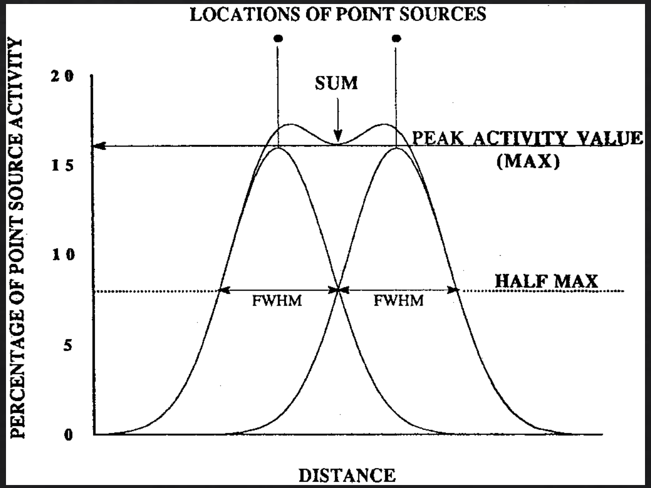


K. Becker et al. 2019

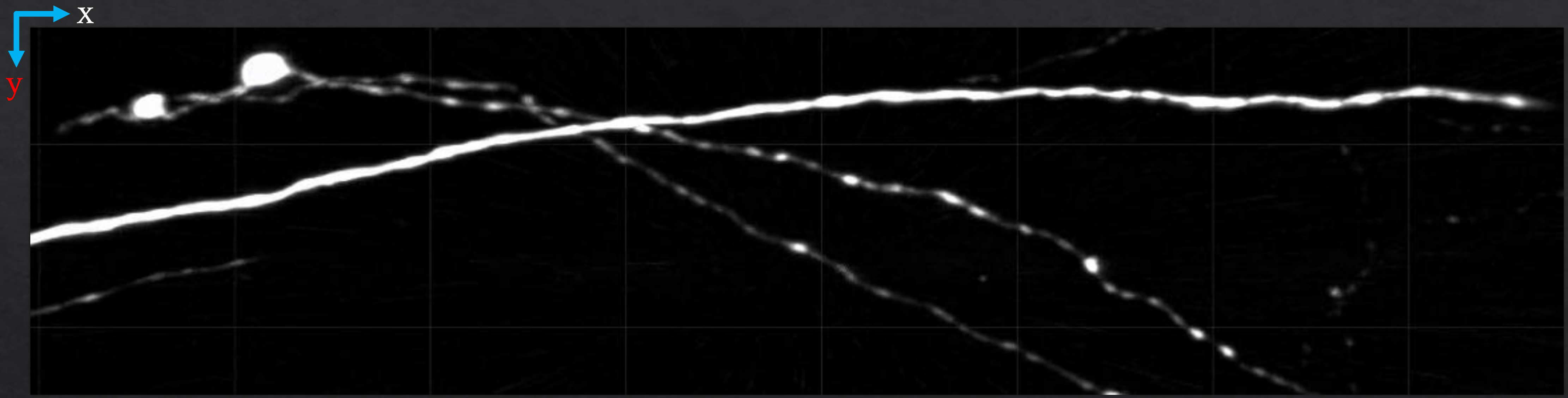


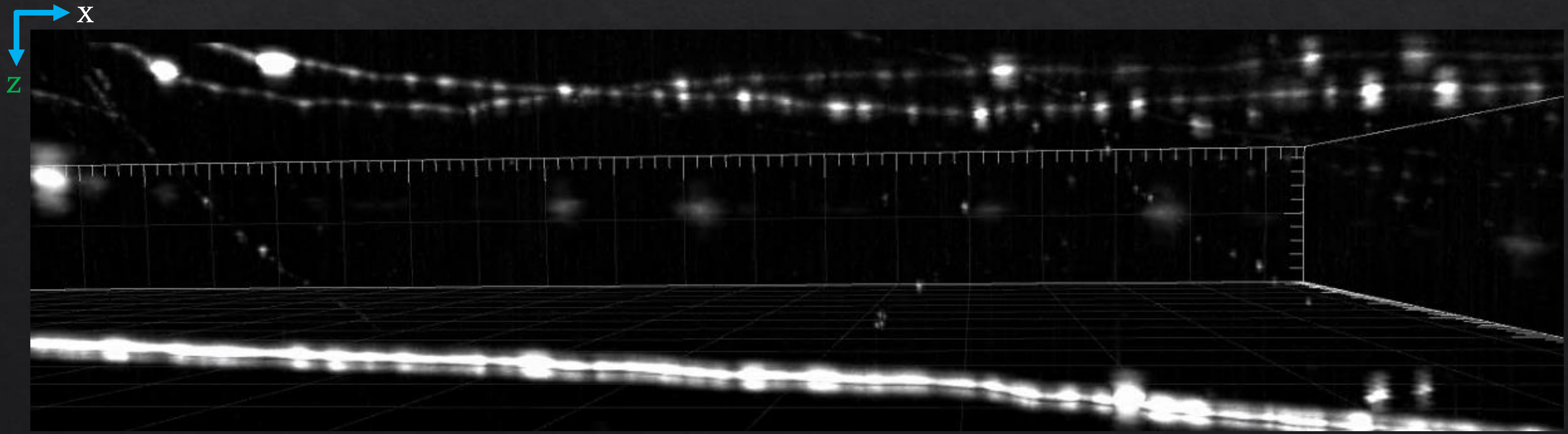
Slit width	= 12 mm
Wavelength excitation	= 680 nm
Wavelength emission	= 642 nm
Voxel size xy (camera and lens)	= 422 nm
Voxel size z (z-step)	= 1000 nm
Refractive index of the imaging oil	= 1.52
Numerical Aperture of the lens	= 0.4
F cylinder lens	= 240

full width half maxima of xy-plane	= 1255.2 nm
full width half maxima of z-axis	= 13204.8 nm

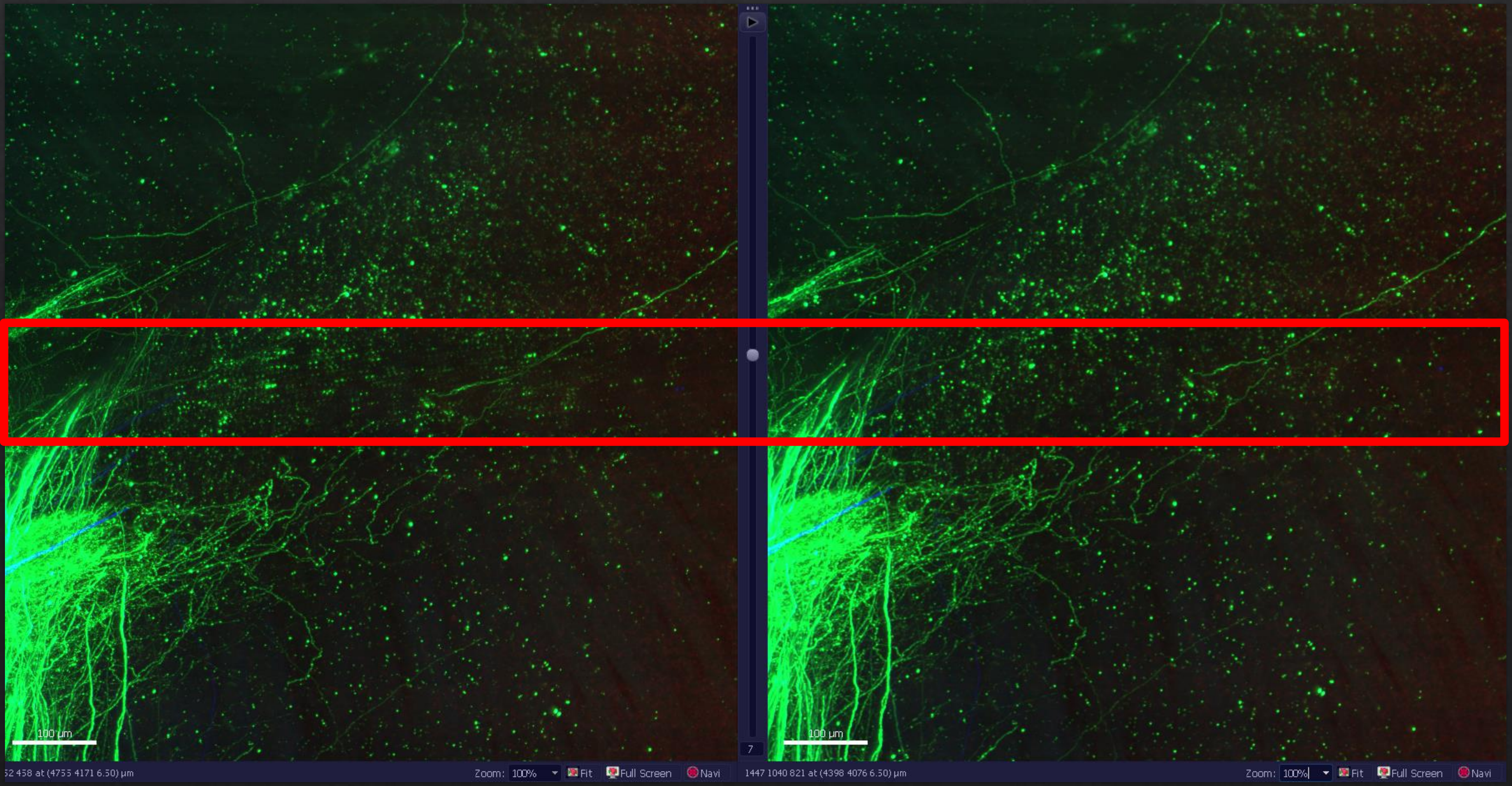


Sample image: 15x=422nm xy, 1000nm z-step





Problems: stitching artifact removal



Goal:
Stitch 2 TB images in 2 Hrs

Solutions

GUI to detect flat images

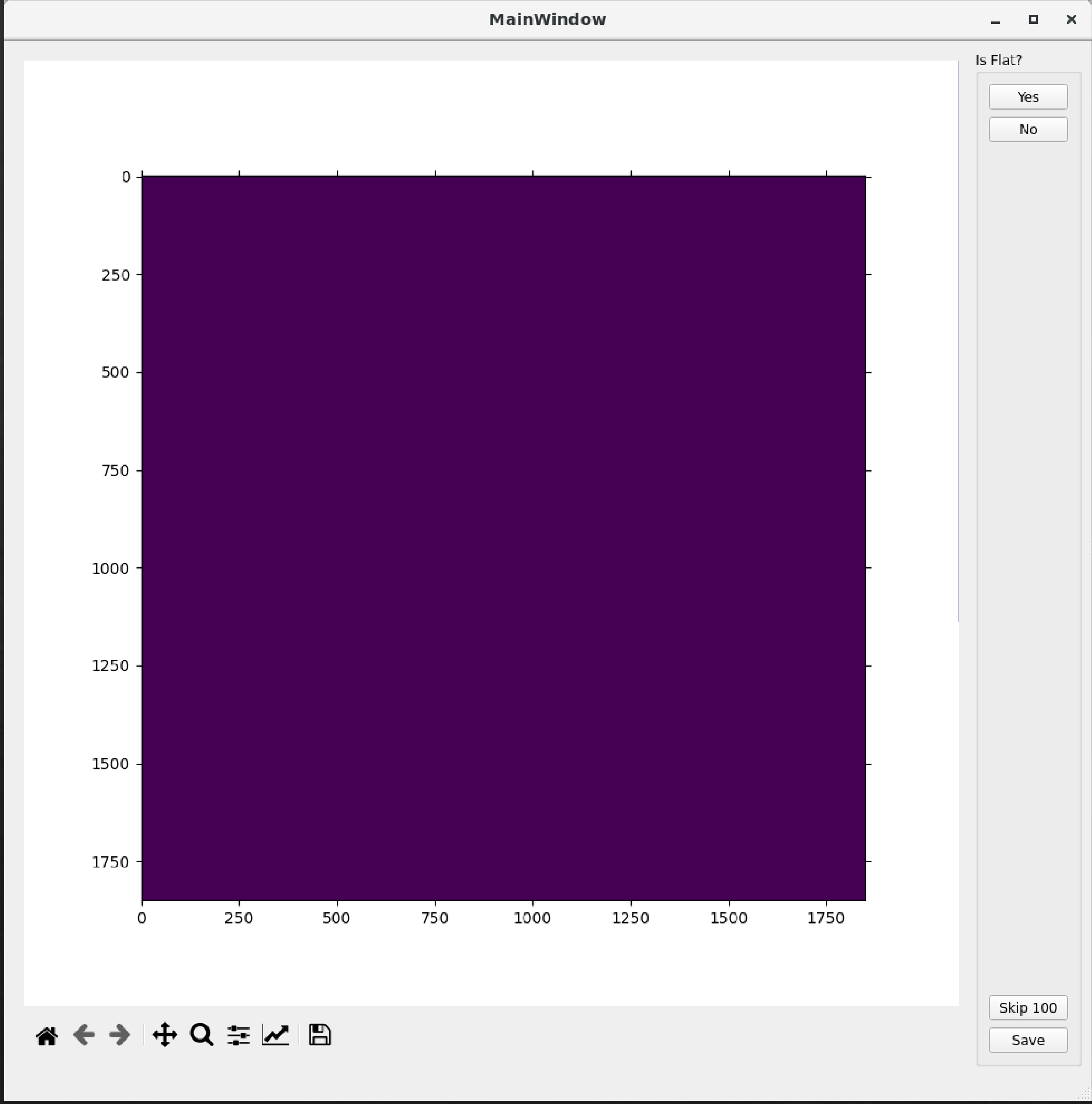
Uses parallel processing

Removes noises such as dust

Generates flat images

Generates training data to automate the flat image detection

(mean, max, min, sd, cv, skewness, kurtosis) vs (flat or non-flat)



Manually generated flat image

Automated flat image generation

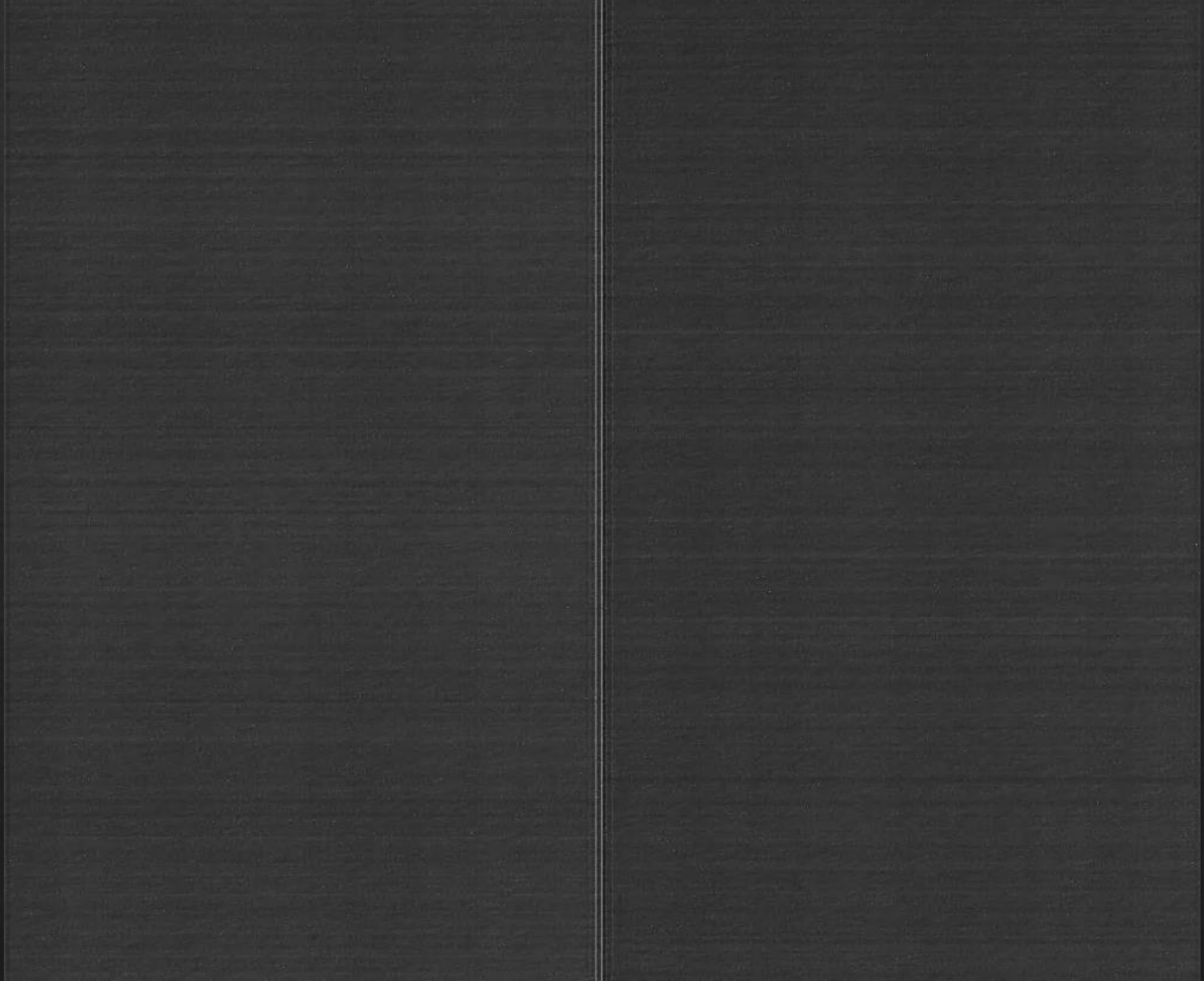
Use random forest method to detect flat images with 91% accuracy

Parallel processing

Denoising

```
def create_flat_img(
        img_source_path, flat_training_data_path, tile_size,
        max_images=256,
        batch_size=256,
        patience_before_skipping=200,
        skips=256,
        sigma_spatial=1,
        save_as_tiff=True):
```

Secondary flat image after flat image application



Enhanced PyStripe Package

New:

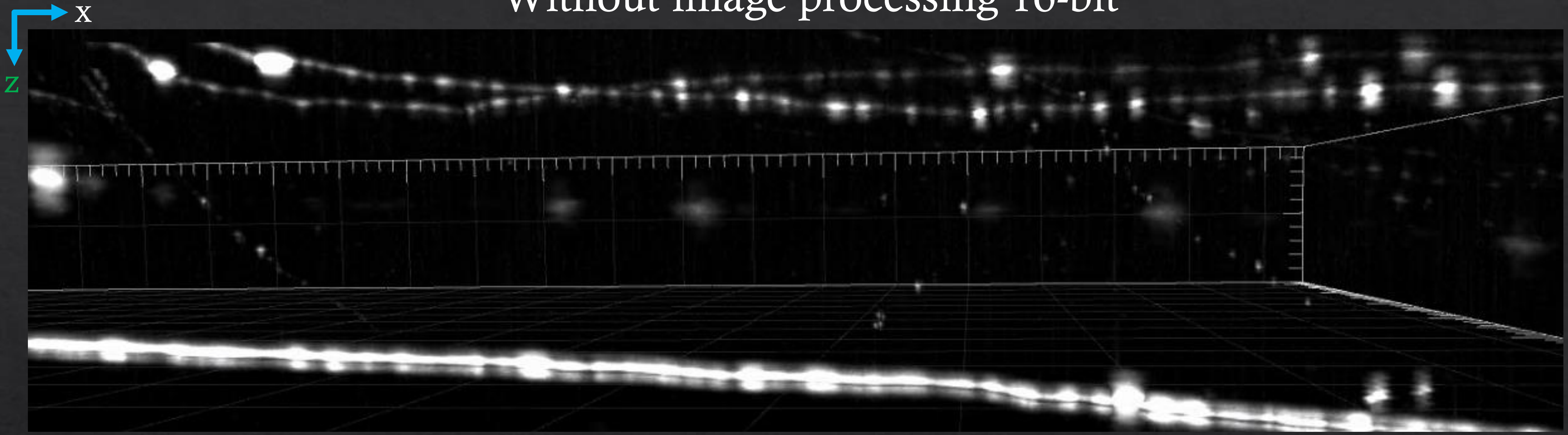
- Better flat image application.
- Corrected a bug in black level subtraction.
- Added 8-bit conversion and bit shifting + max thresholding support
- Tunning batch size to speed up the parallel processing speed.
- Resume support.
- Down-sampling and down-sizing support to make isotropic images.

Already was there:

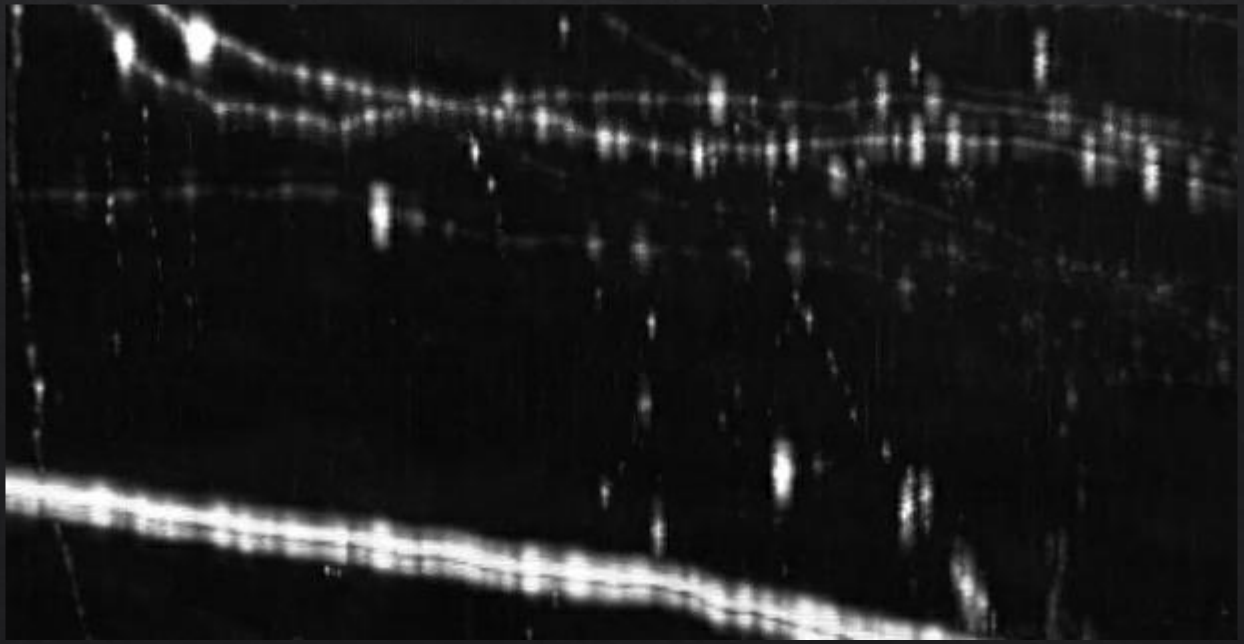
- Computational clearing of light-sheet images.
- Wavelet method for de-striping.

```
def read_filter_save(
        input_path,
        output_path,
        sigma,
        level=0,
        wavelet='db3',
        crossover=10,
        threshold=-1,
        compression=('ZLIB', 1),
        flat=None,
        dark=0,
        z_idx=None,
        rotate=False,
        lightsheet=False,
        artifact_length=150,
        background_window_size=200,
        percentile=.25,
        lightsheet_vs_background=2.0,
        dont_convert_16bit=False,
        convert_to_8bit=True,
        bit_shift_to_right=8,
        down_sample=(2, 2),
        new_size=None
```

Without image processing 16-bit

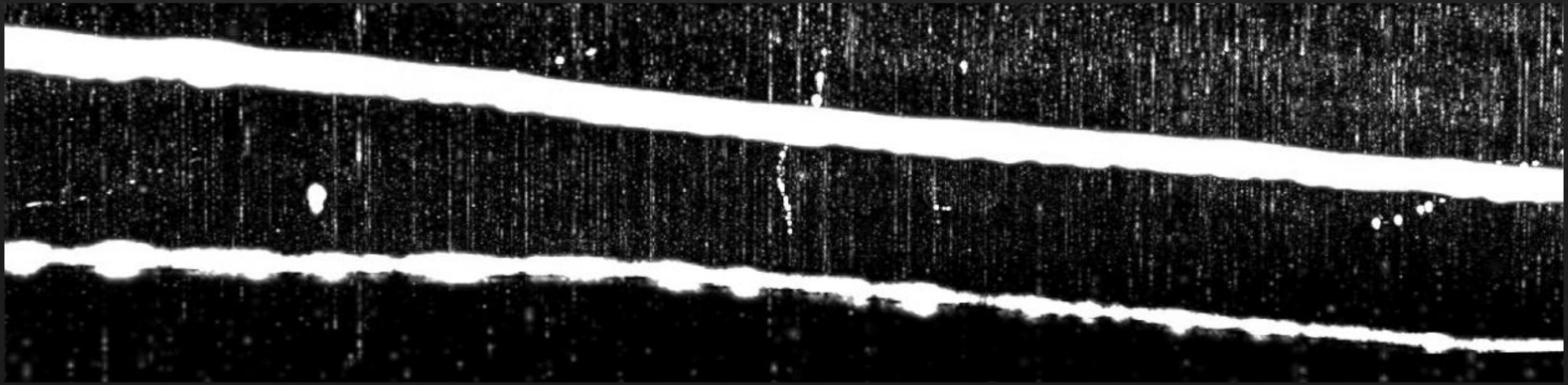


After
- flat image application,
- 8-bit conversion,
- thresholding,
- Light-sheet cleaning method,
- max down sampling and resizing → isotropic volume

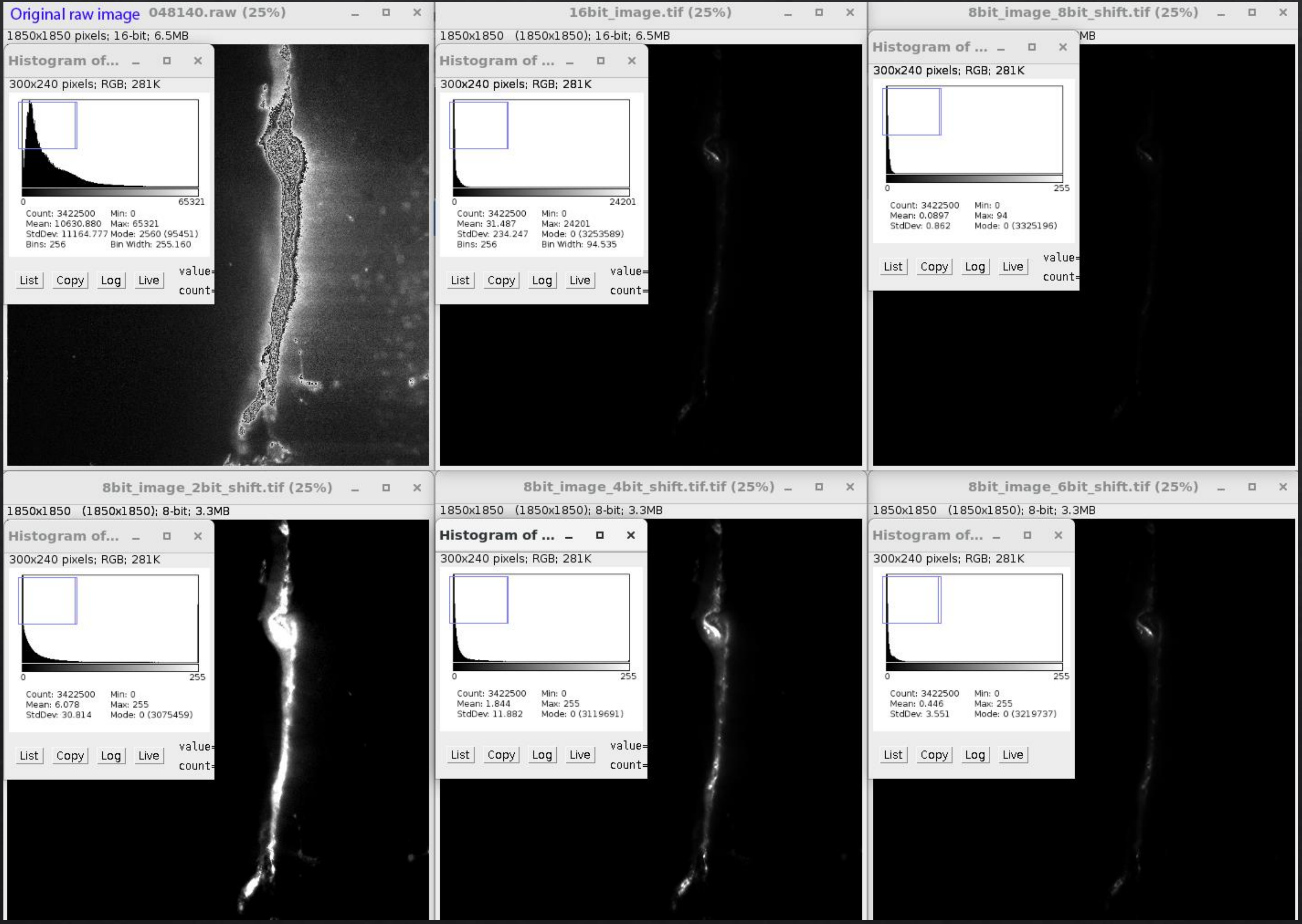


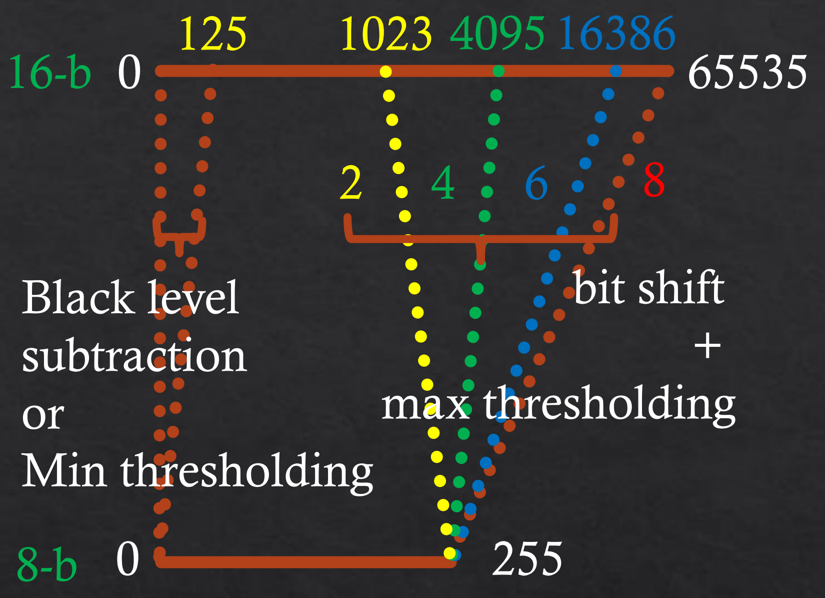
Deconvolution - dampening + black subtraction (PyCudaDeconvolve)

Deconvolution + dampening - black subtraction (Matlab)



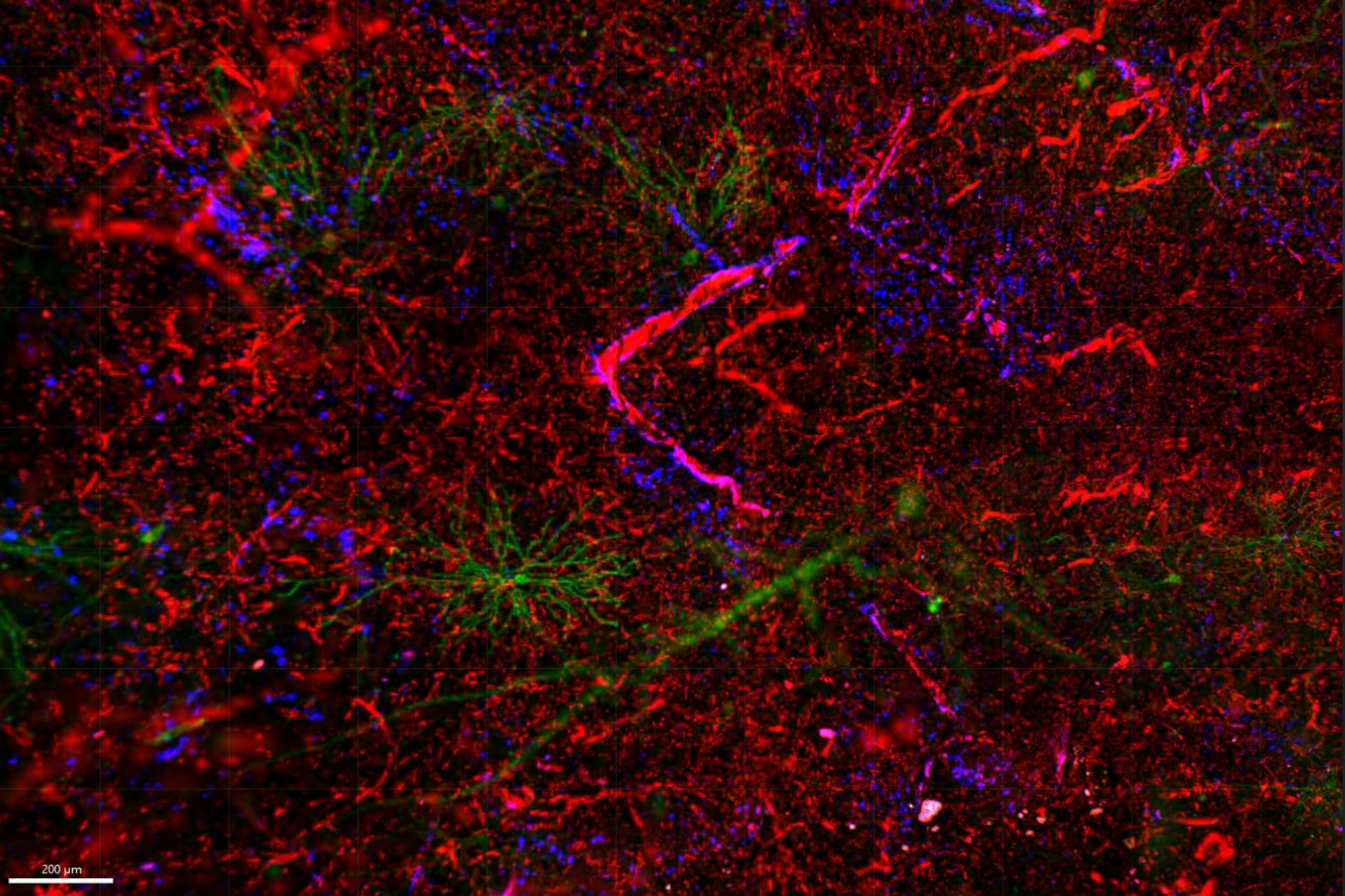
De-striping + Flat image subtraction + 16-bit to 8-bit conversion



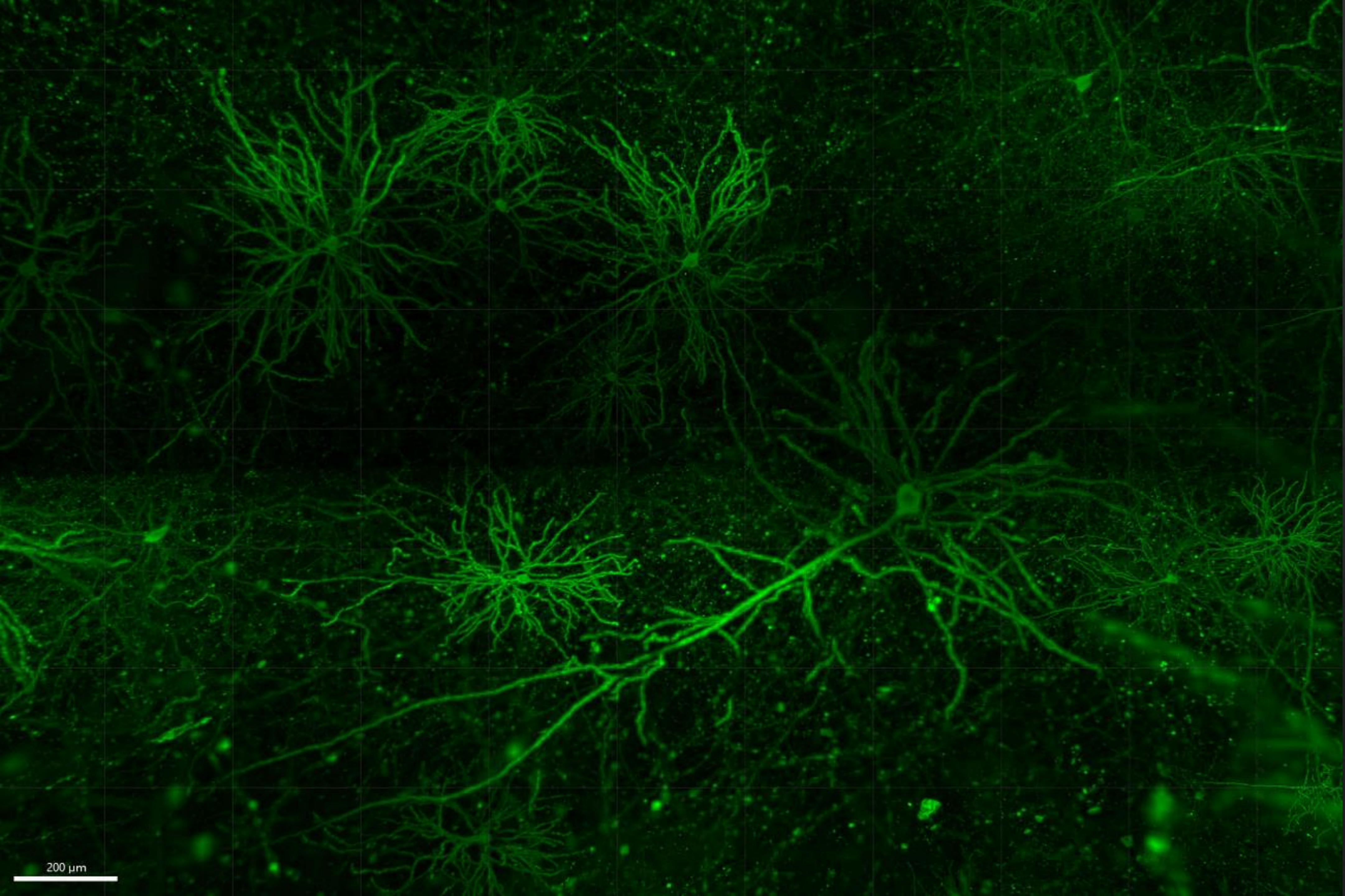


Bit shift	16bit max	8bit max
2	>1023	255
4	>4095	255
6	>16383	255

2000 GB single channel RAW → 5 GB compressed 8-bit IMS



- Down-sampled 15x.
- Stitch based on channel 1 for all channels
- Channel 1: nucleus
- Channel 2: axons
- Channel 3: neurons (morf labeling).
- No deconvolution.
- Original file size (compressed tiff) 4.56 TB
- Final file size: 42 GB



- Only Channel 3

Vaa3D

- The final stage (6, which is the most time-consuming part) of ParaConverter and ParaStitcher are not working in parallel in Windows.
- Stitching directly to TeraVR format (HDF5, for instance).
  - Using ZSTD as the compression method to speed up the compression.
  - Or fix problems regarding converting our tif files to a format supported by TeraVR.
- Trace neurons in the entire brain using TeraVR.
- Convert SWCs to NumPy format to allow generate training data for machine learning.

Our plans

- Segment voxels to soma, dendrites, axons and boutons.
- Convert segmentation to a mask to clear the tissue.
- Trace neurites across the entire brain automatically.
- Register neurons to common brain framework.
- Classify individual neuronal types for the entire brain in 3D space.