

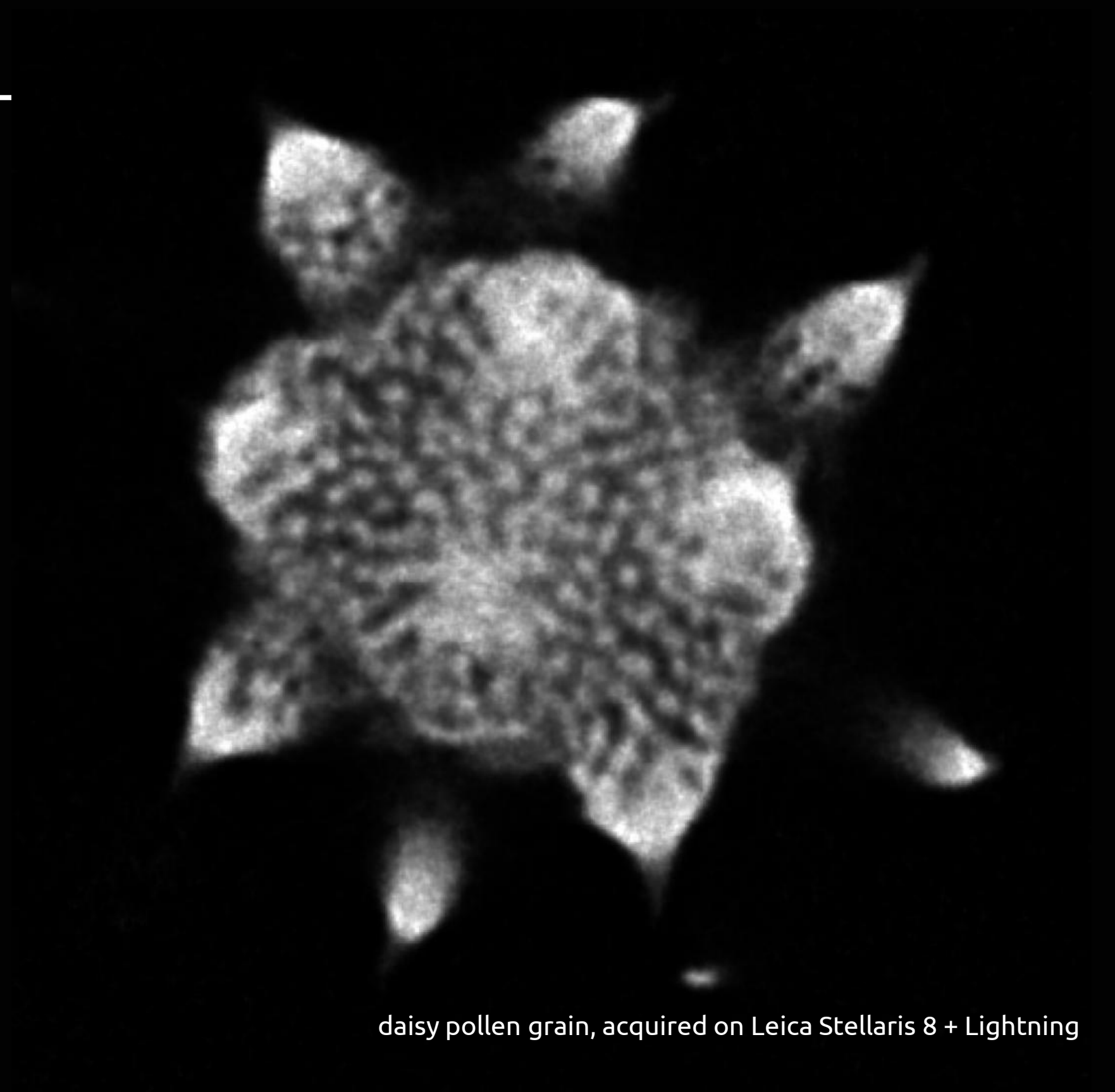
# Deconvolution in practical use

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MMIB  
2025

**Ivan Novotný**  
**Light microscopy core facility**

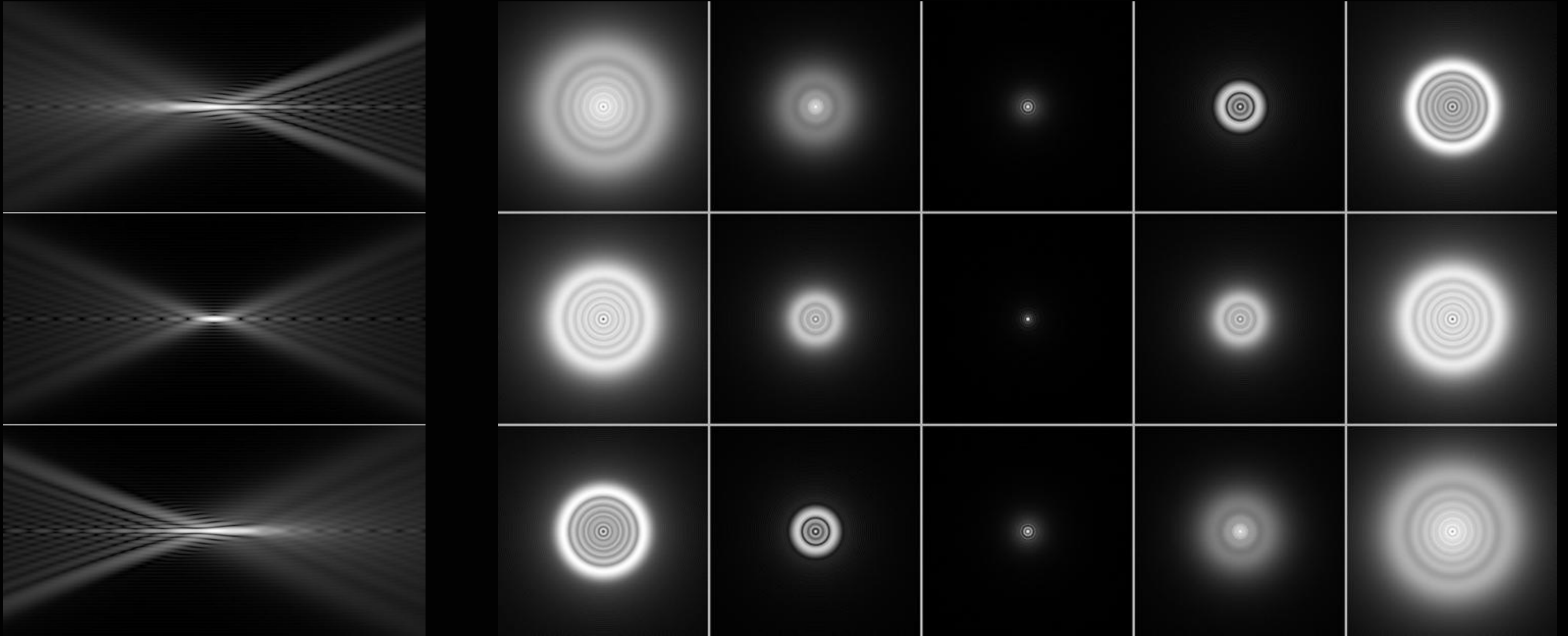
Institute of Molecular Genetics  
of the Czech Academy of  
Sciences  
Videnska 1083  
CZ-142 20, Prague 4



daisy pollen grain, acquired on Leica Stellaris 8 + Lightning

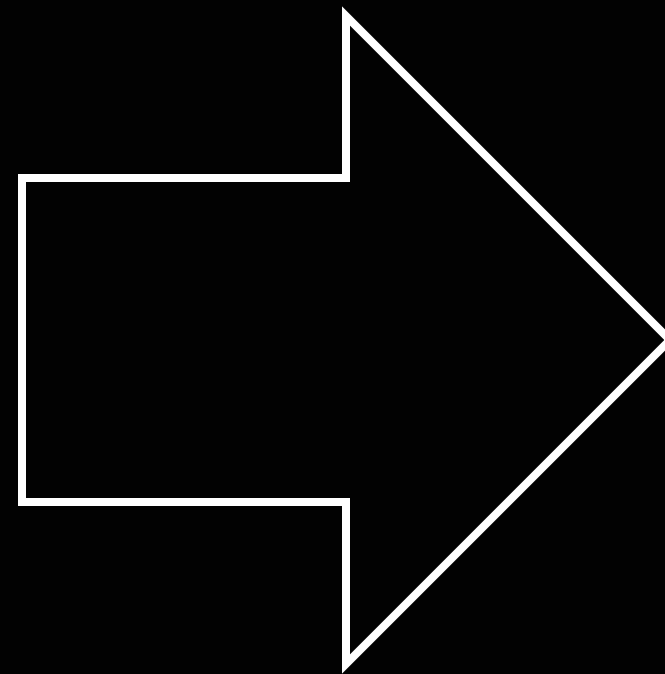
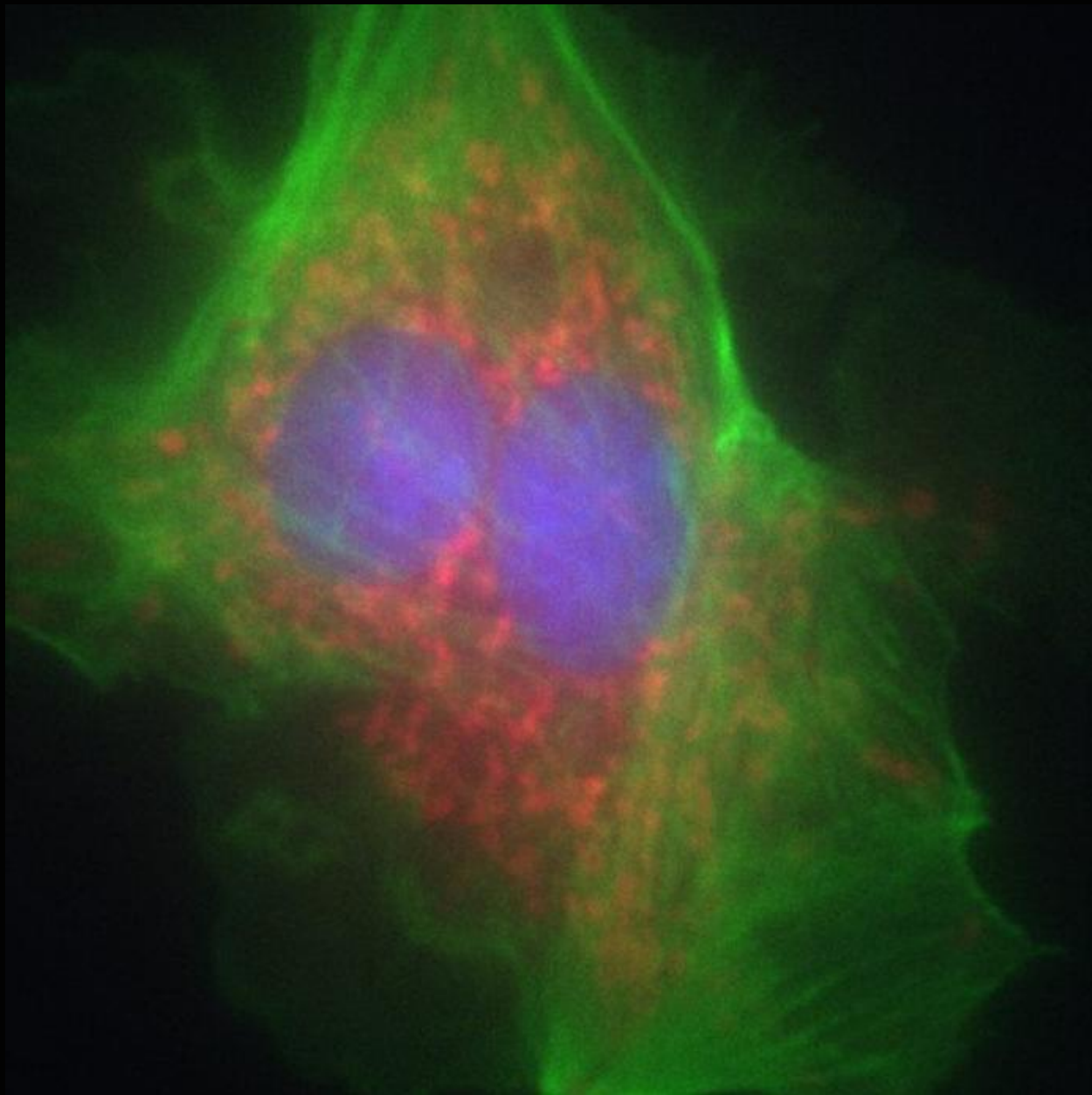
# Spherical aberration:

sudden death for high-resolution  
images

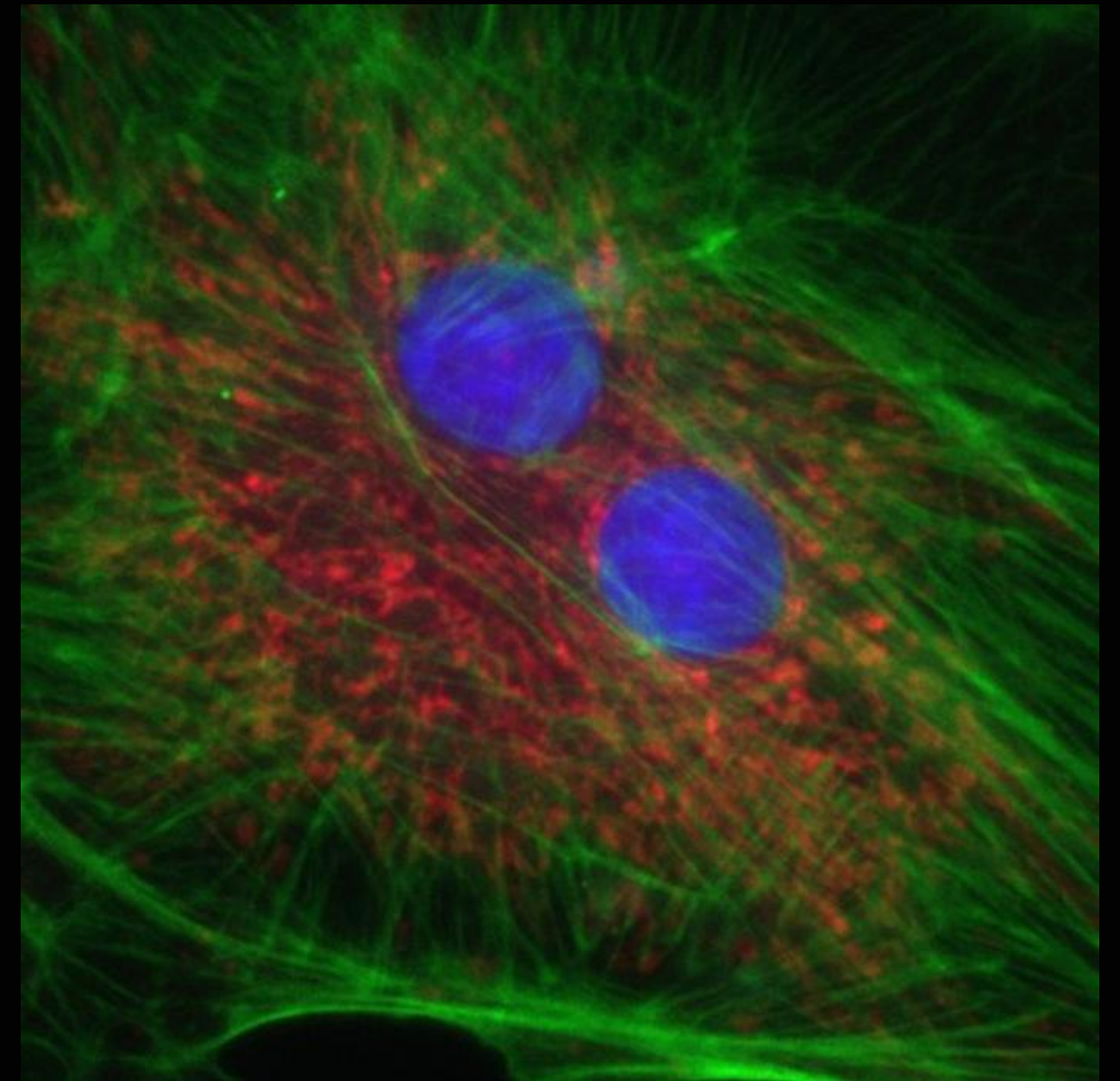


# Spherical aberration:

sudden death for high-resolution  
images

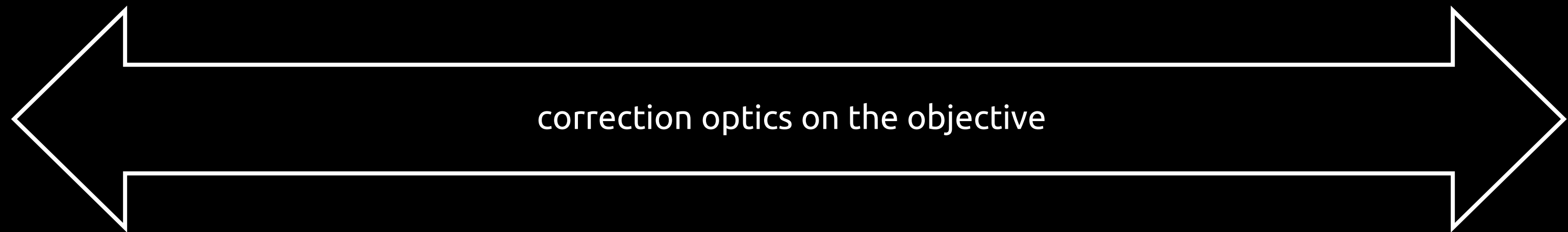
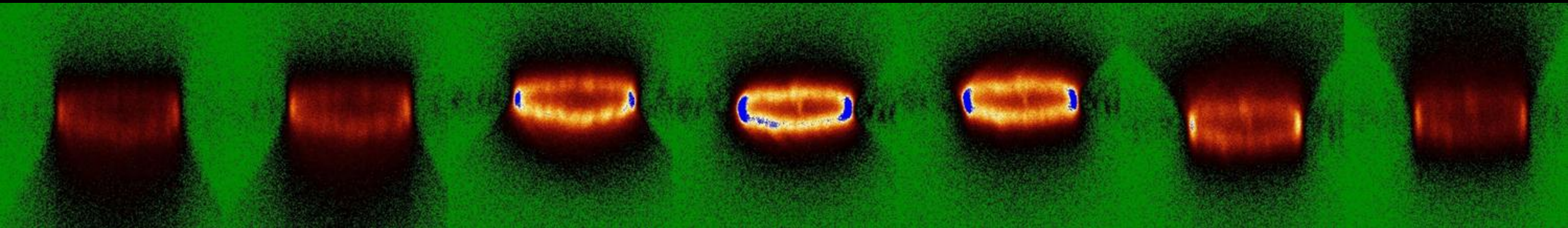


correction optics on  
the objective





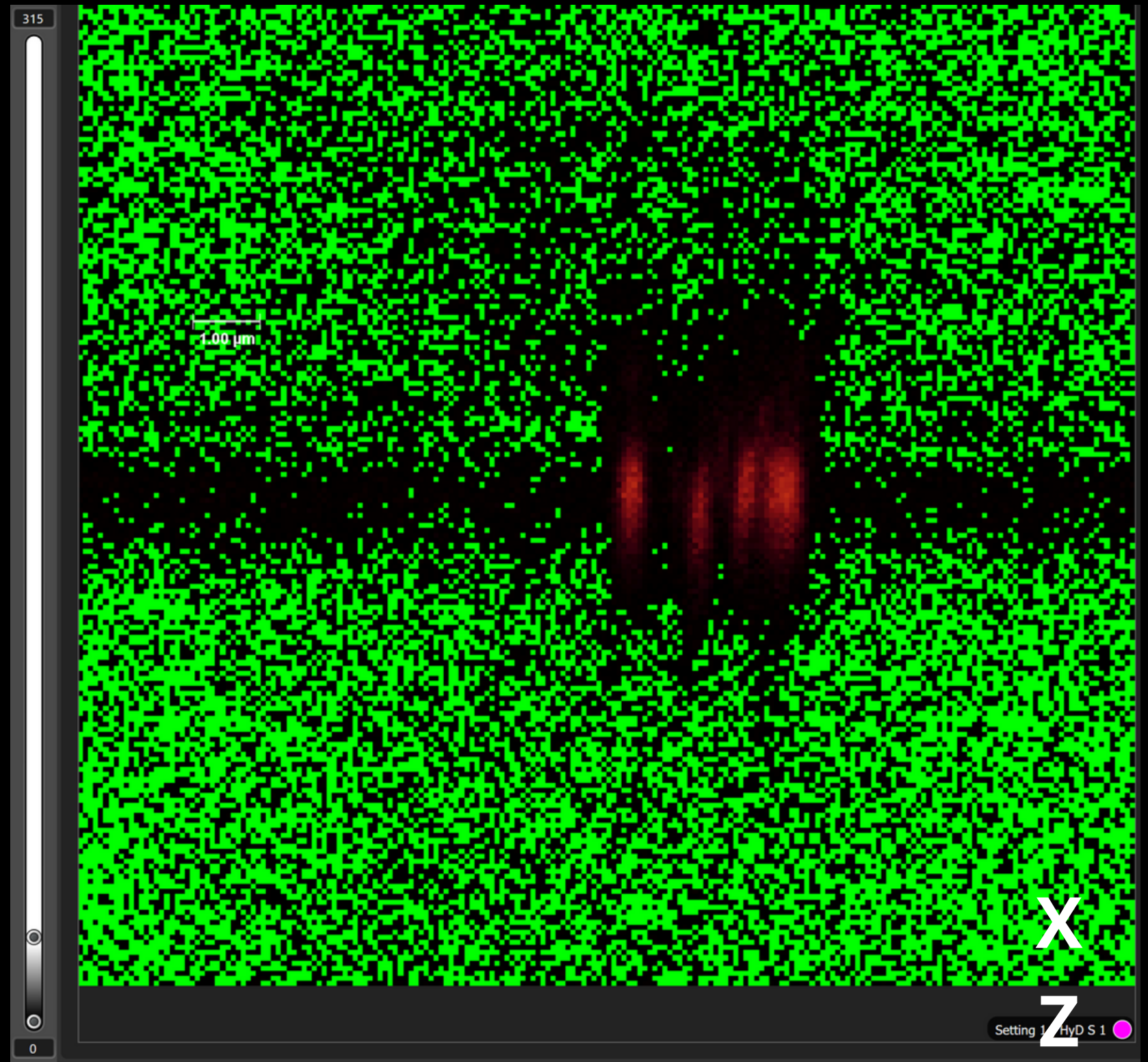
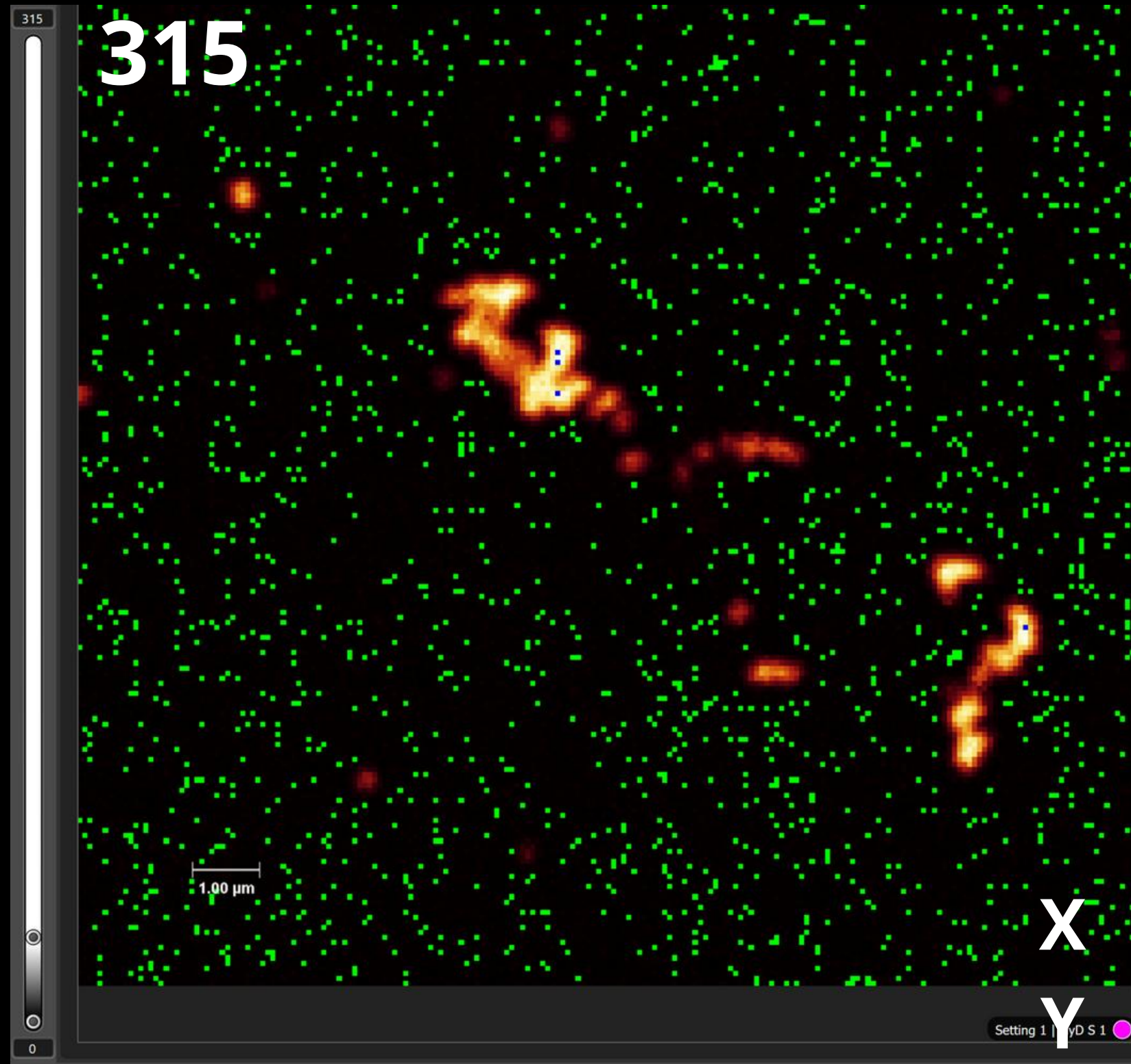
Spherical aberration:  
sudden death for high-resolution  
images





# Practical steps:

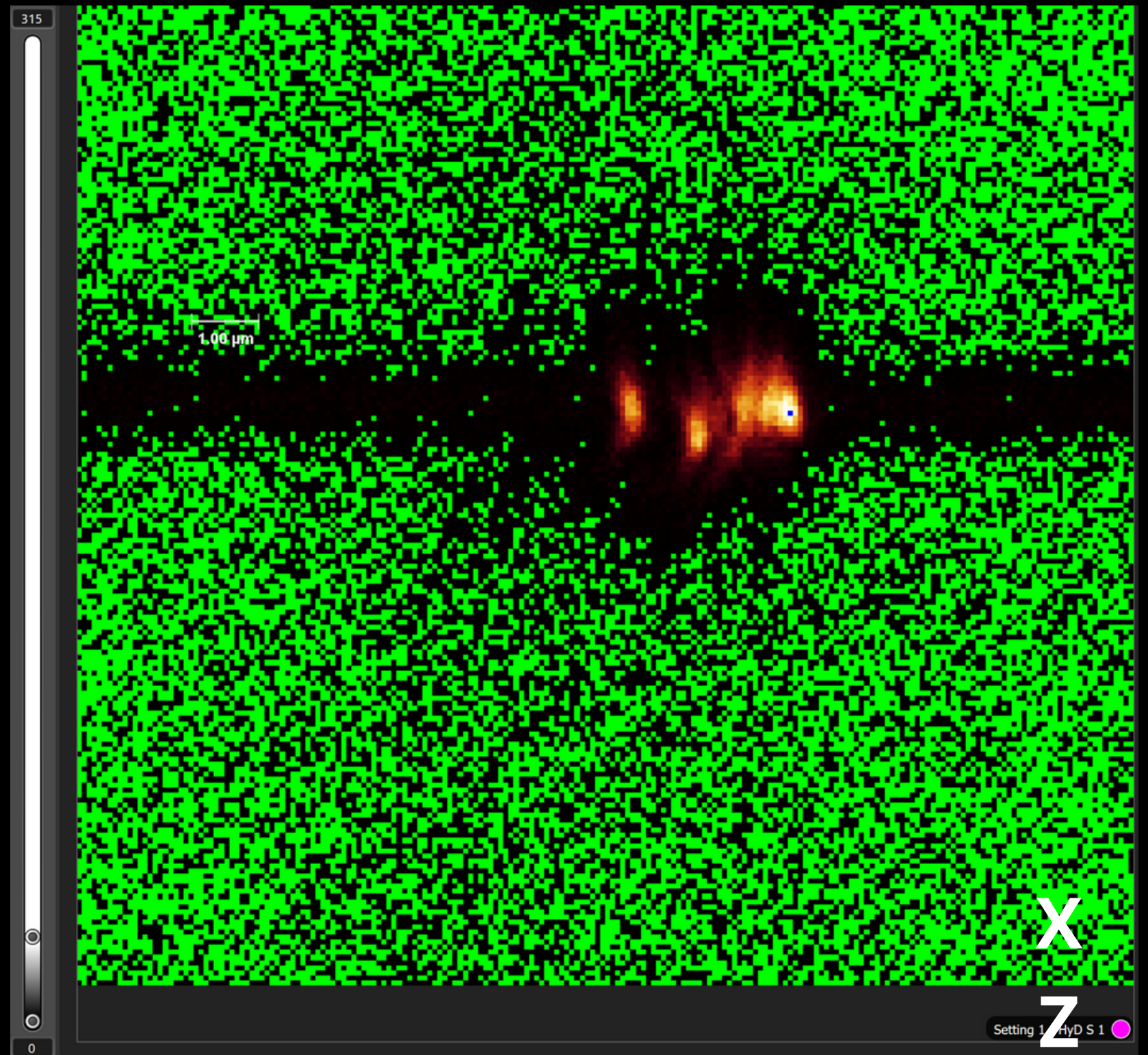
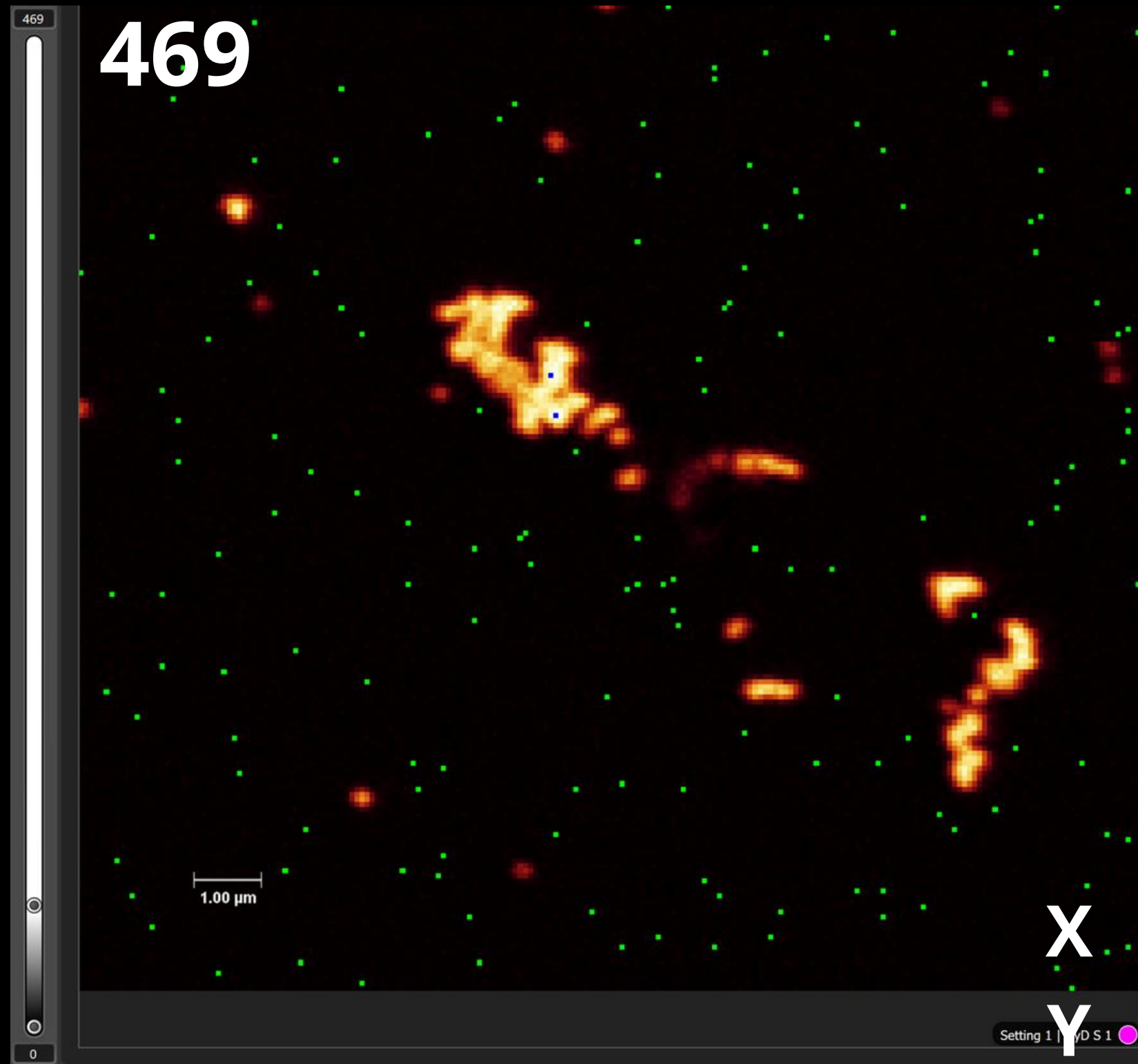
01 - check the signals, PSF shape and try to compensate spherical aberration by optical correction






# Practical steps:

01 - check the signals, PSF shape and try to compensate spherical aberration by optical correction



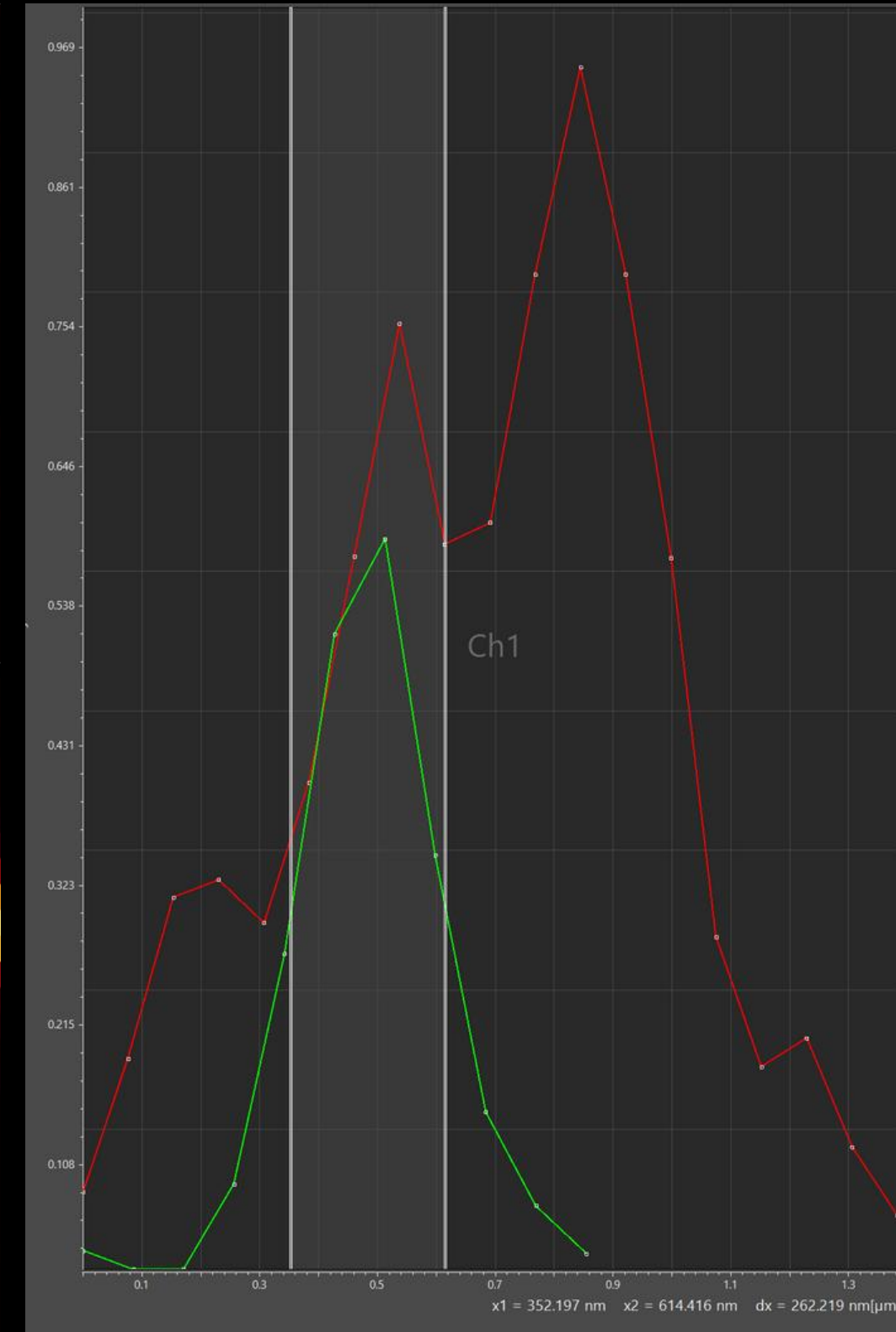
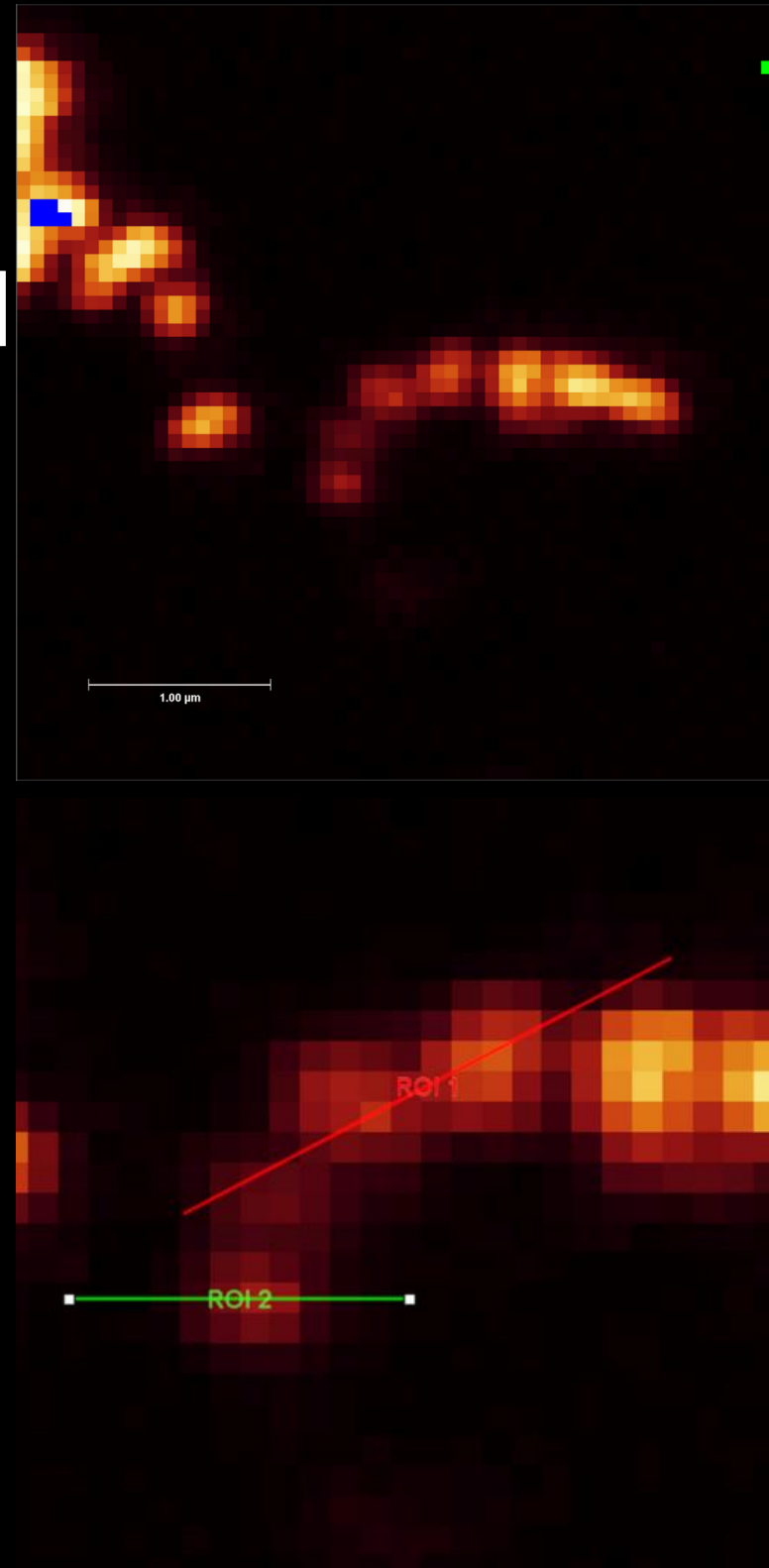
# Practical steps:

02 - Keep your pixel size Nyquist-friendly, or kiss your high-res goodbye

  75.6 nm



 0.33 μm

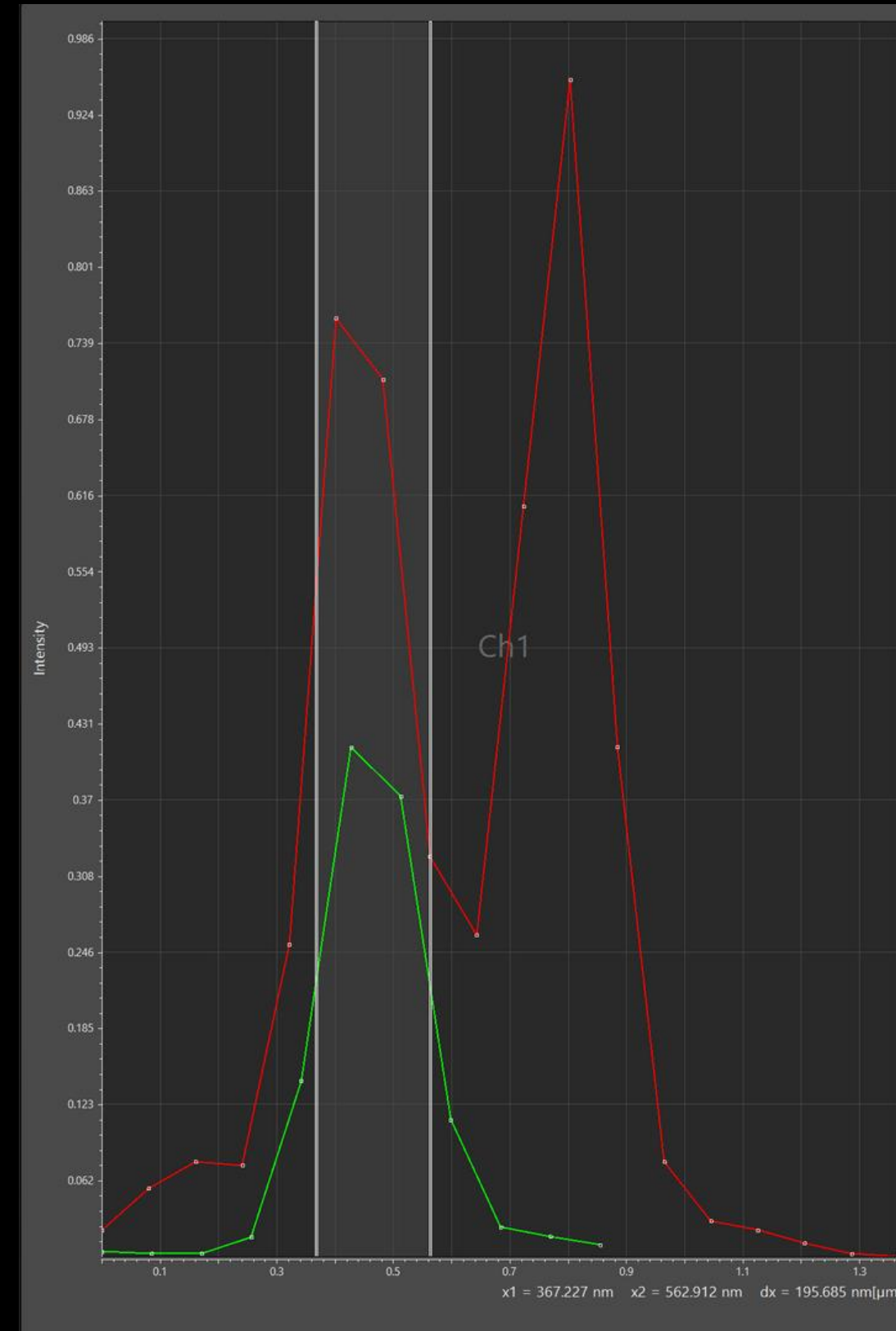
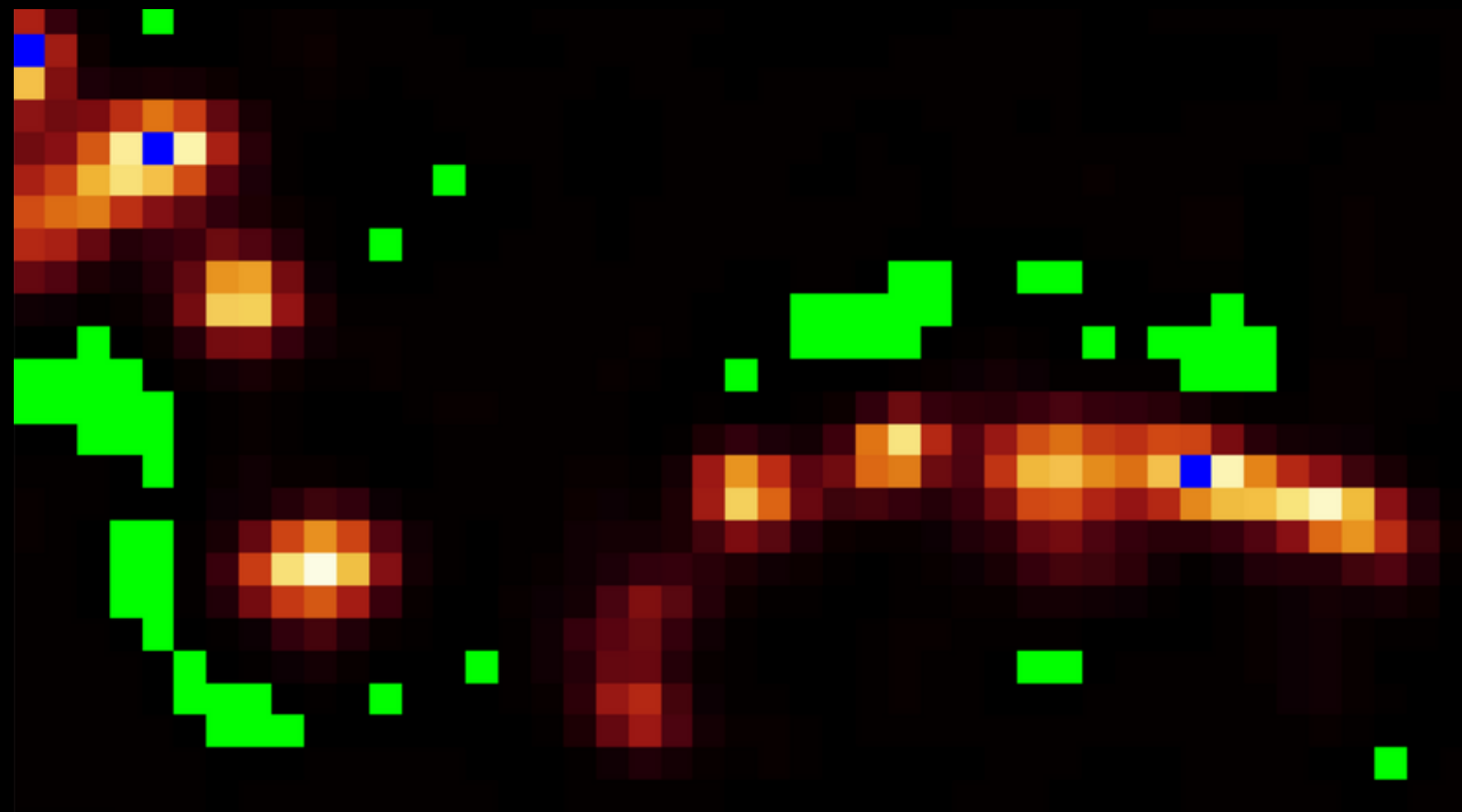


FWHM 262 nm

# Practical steps:

02 - Keep your pixel size Nyquist-friendly, or kiss your high-res goodbye

The deconvolution results




FWHM 195 nm



# Practical steps:

02 - Keep your pixel size Nyquist-friendly, or kiss your high-res goodbye

 Nyquist rate and PSF calculator

### Results

This is the parameter list used in this calculation:

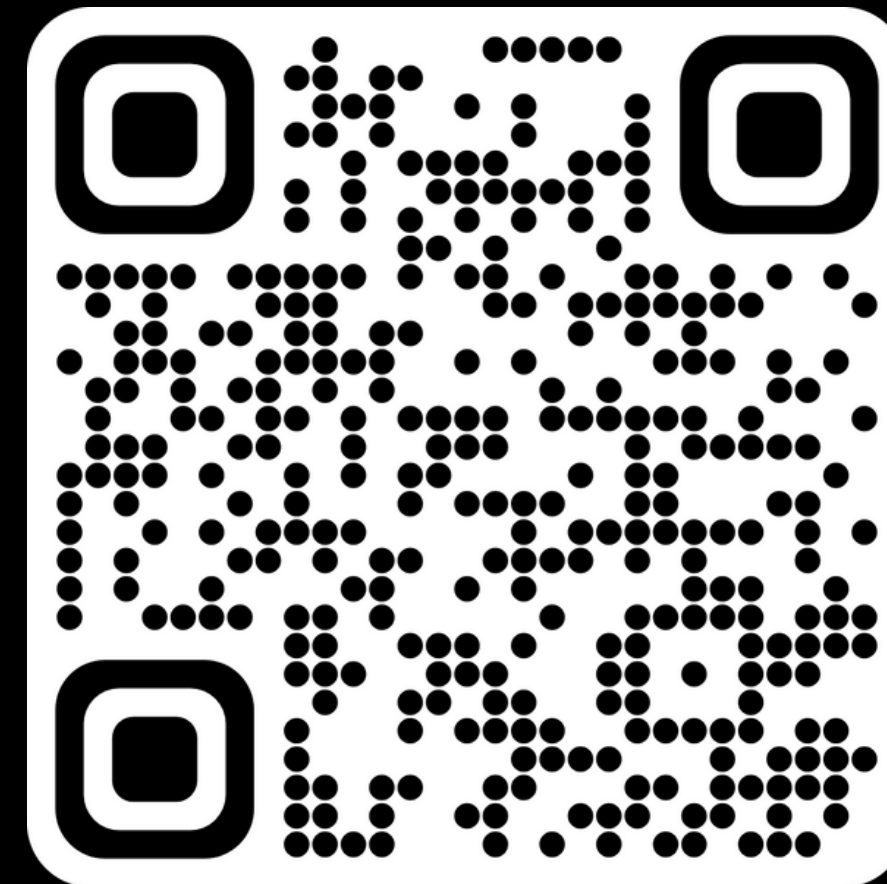
Parameter	Value
Microscope type	Confocal
Numerical aperture	1.3
Excitation wavelength	488
Emission wavelength	520
Number of excitation photons	1
Lens immersion refractive index	1.474

The optical axis lays along z. Your Nyquist sampling is:

x: 46 nm

y: 46 nm

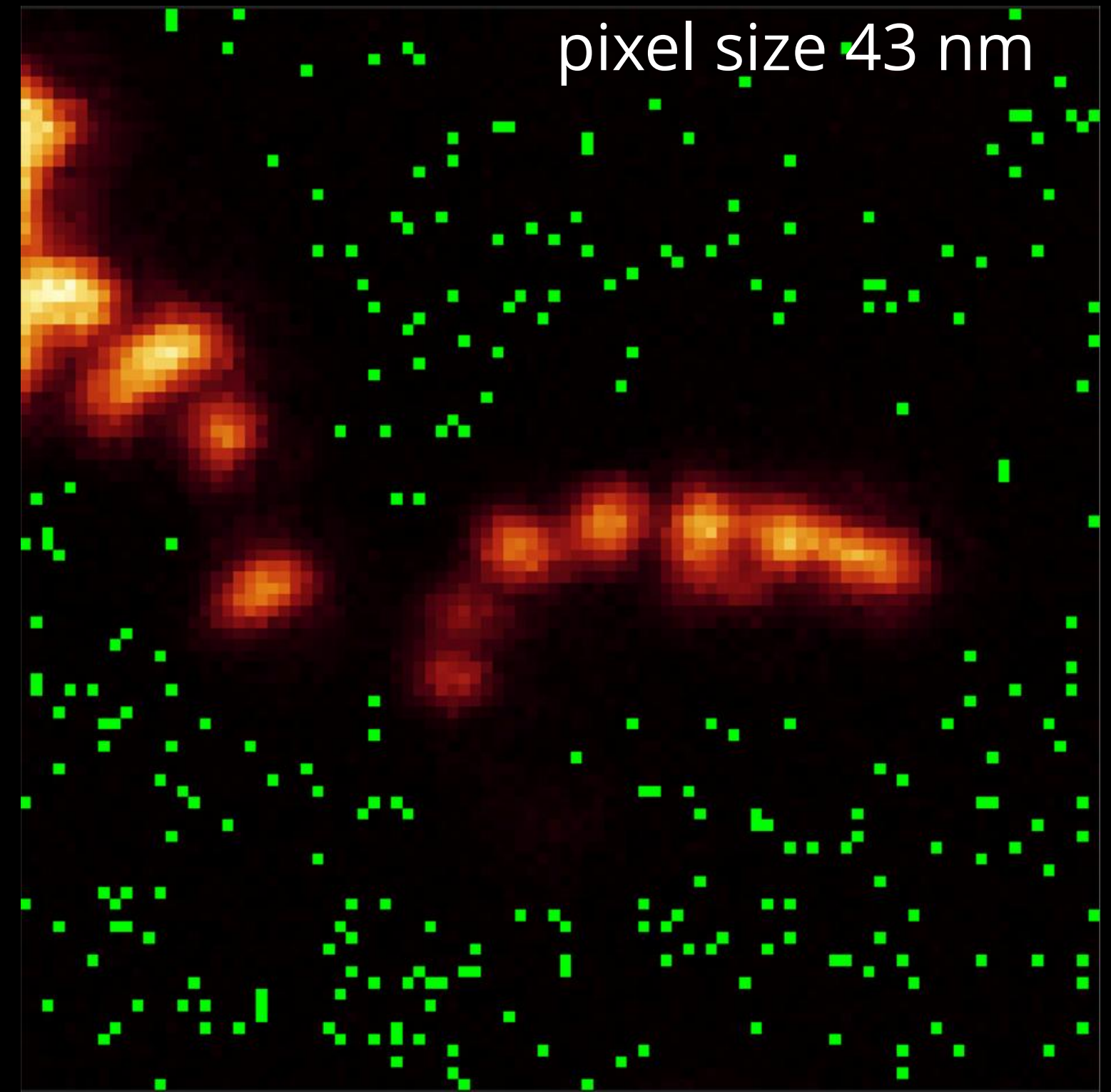
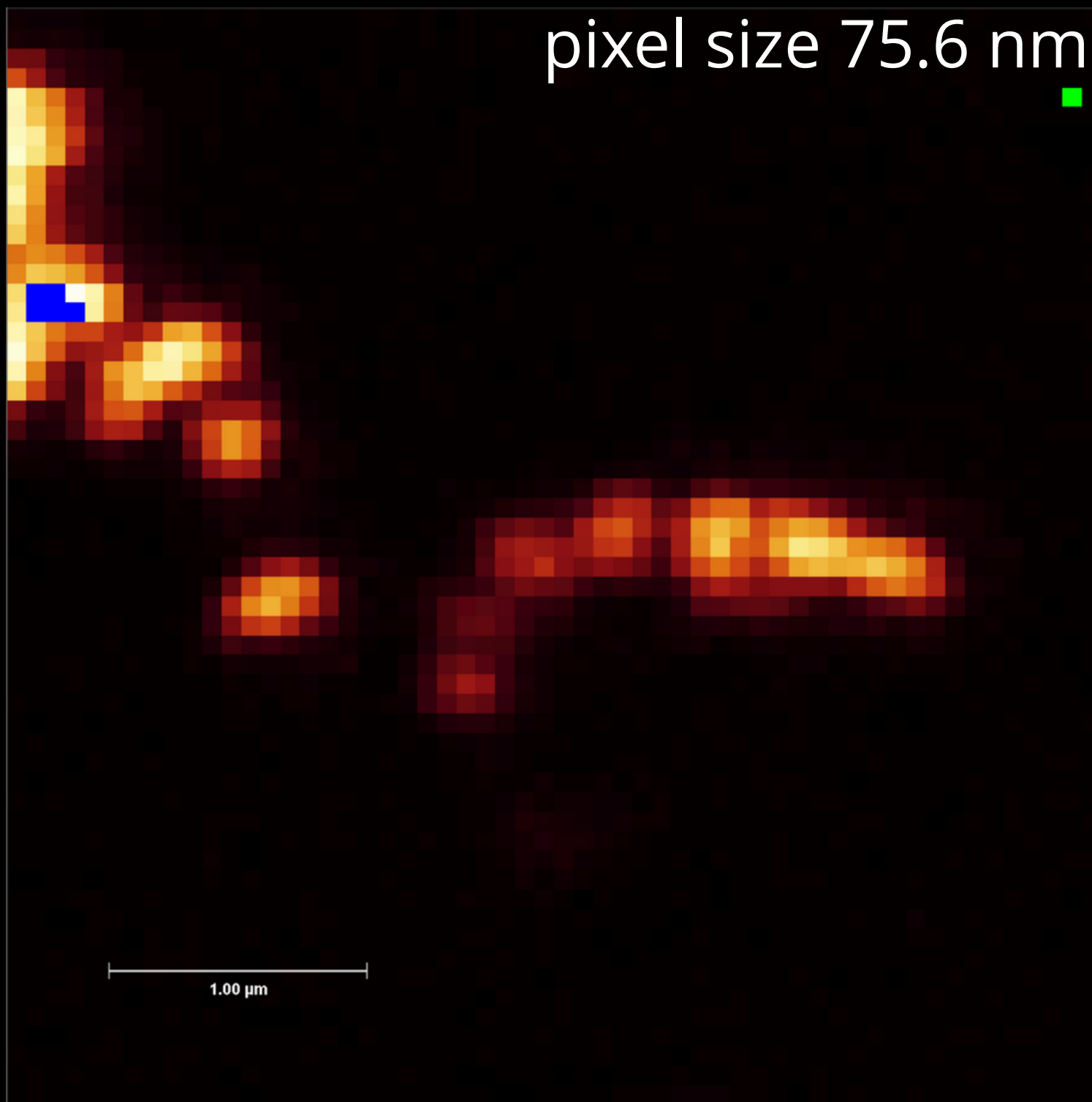
z: 156 nm



<https://svi.nl/Nyquist-Calculator>

# Practical steps:

02 - Keep your pixel size Nyquist-friendly, or kiss your high-res goodbye



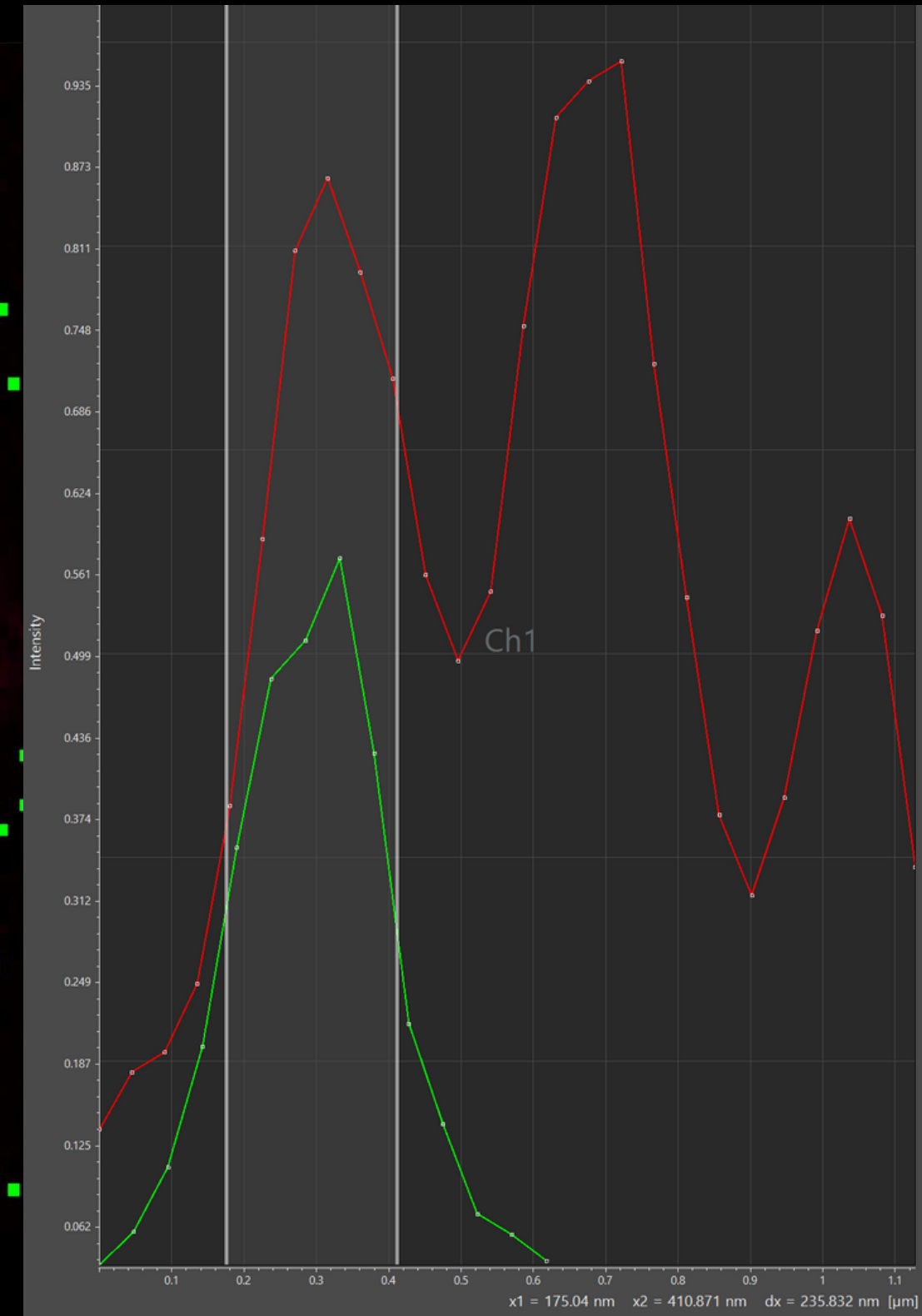
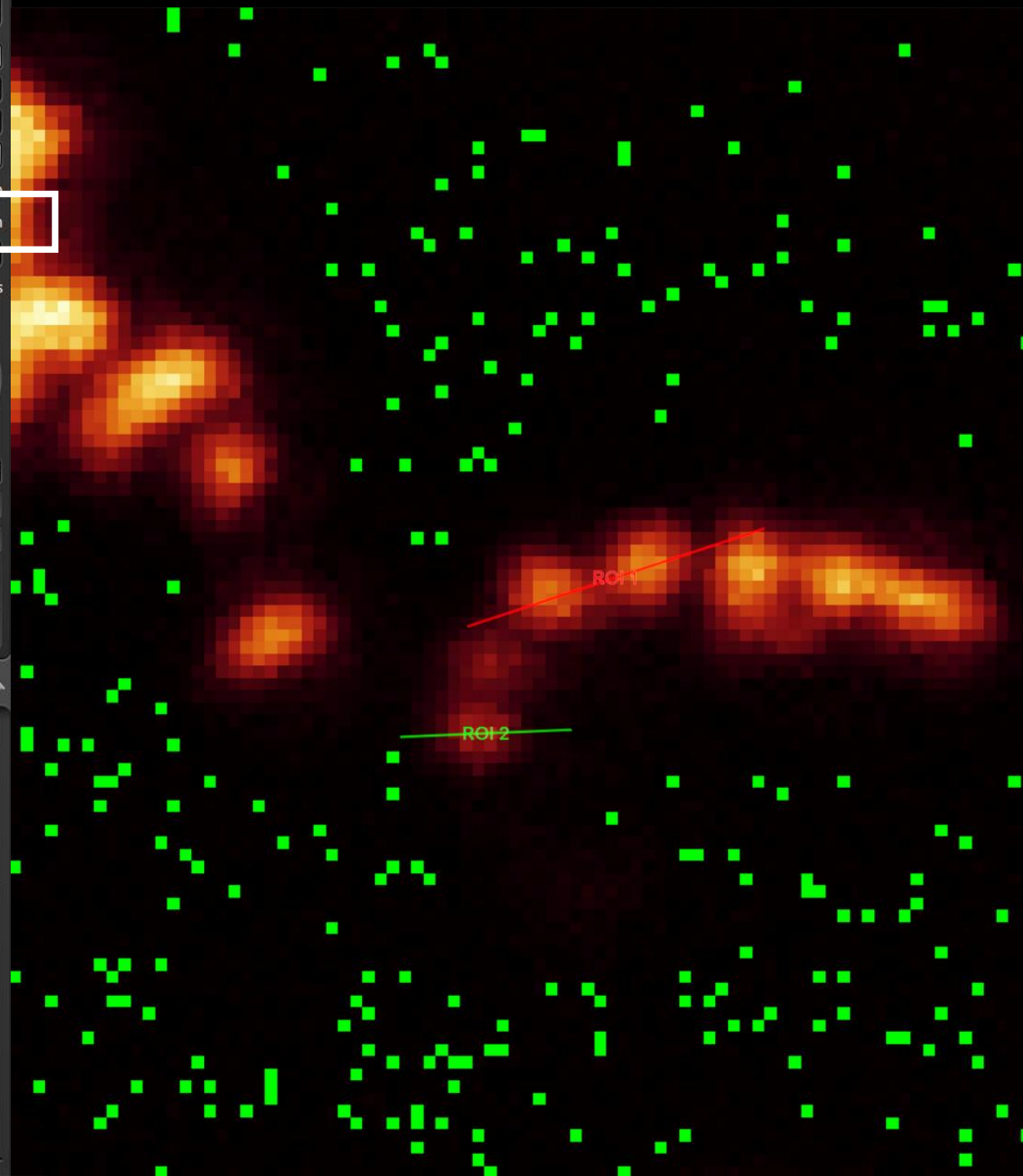


# Practical steps:

02 - Keep your pixel size Nyquist-friendly, or kiss your high-res goodbye

43,8 nm

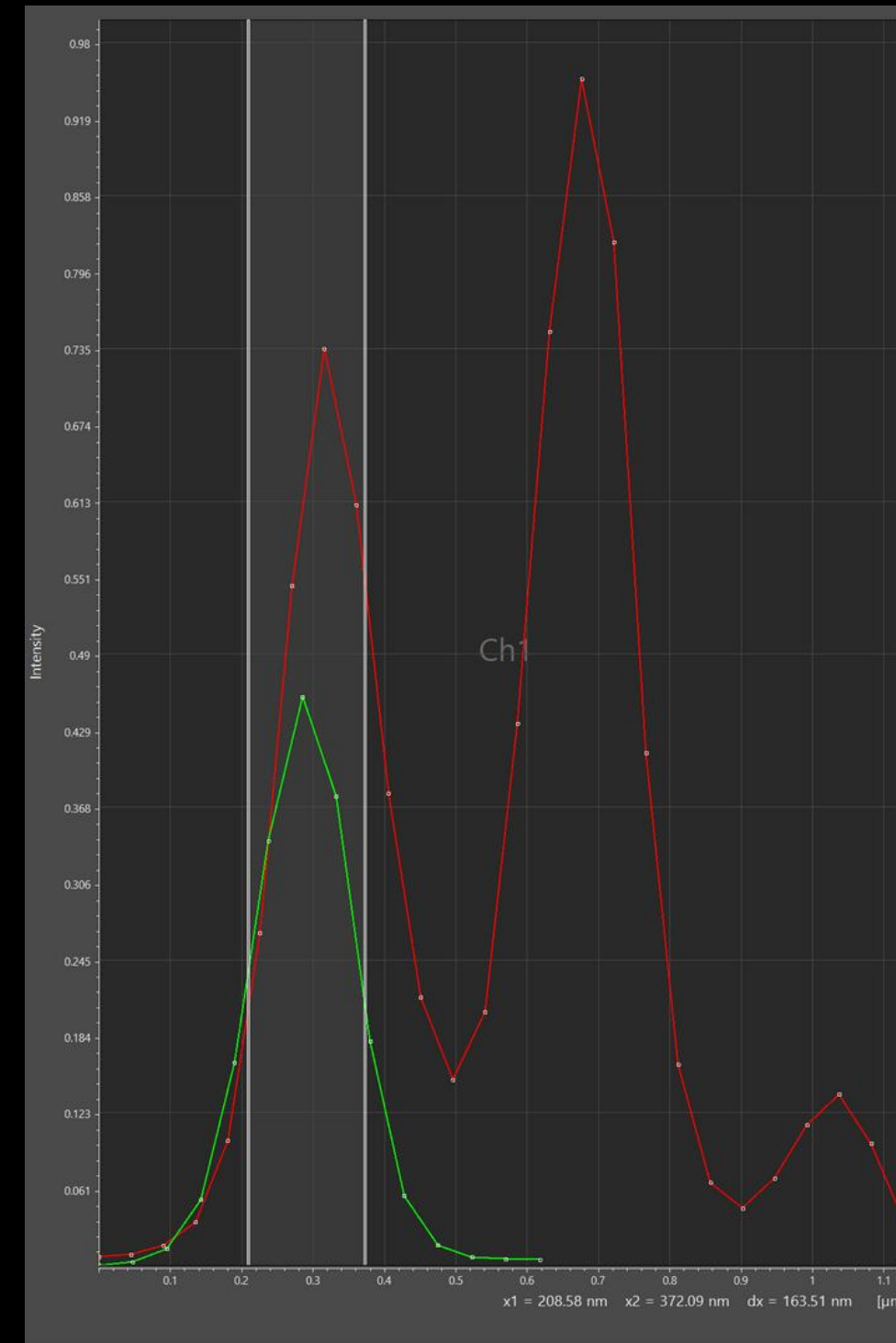
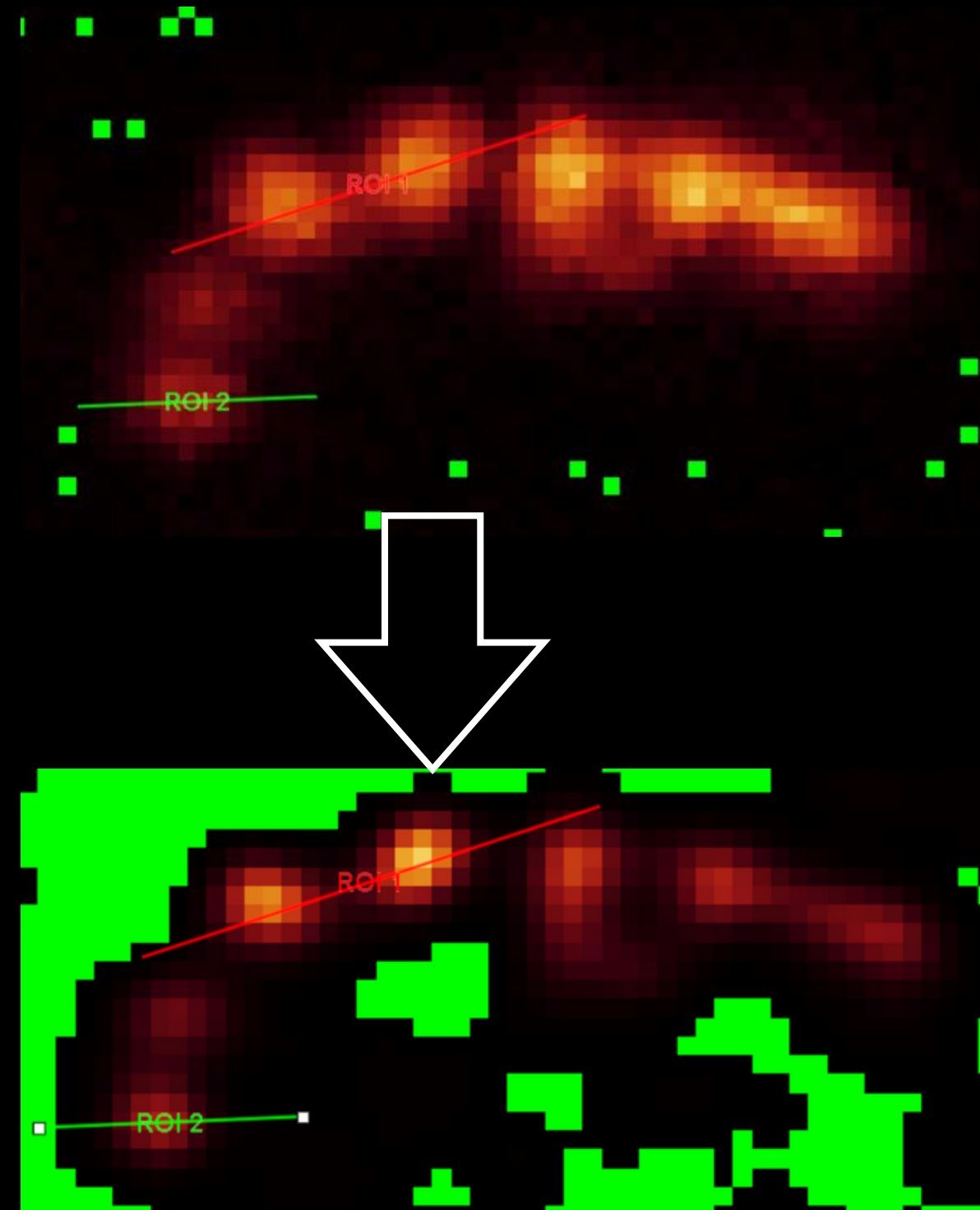
0.12  $\mu\text{m}$



FWHM  
235 nm

# Practical steps:

02 - Keep your pixel size Nyquist-friendly, or kiss your high-res goodbye



FWHM  
235 nm

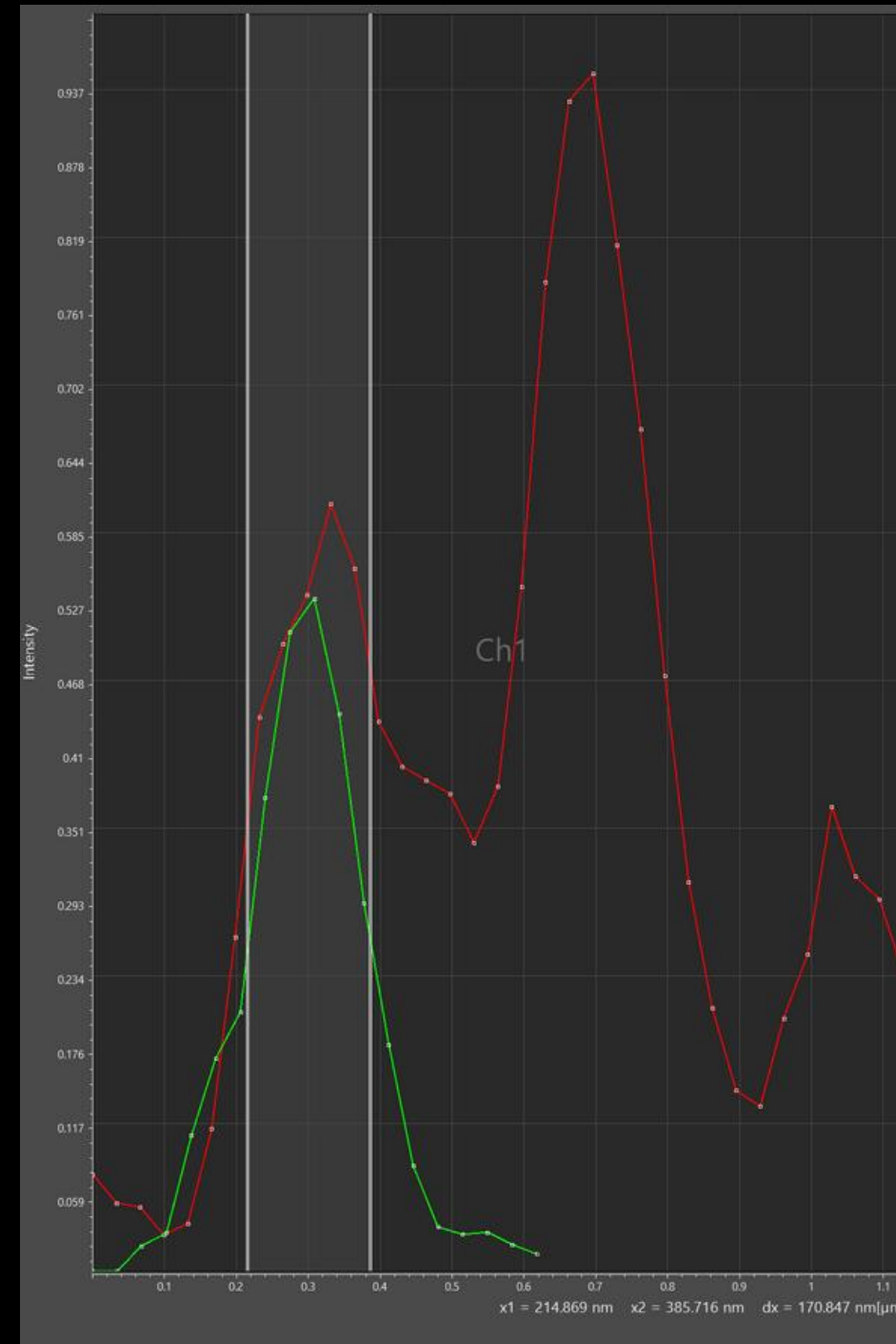
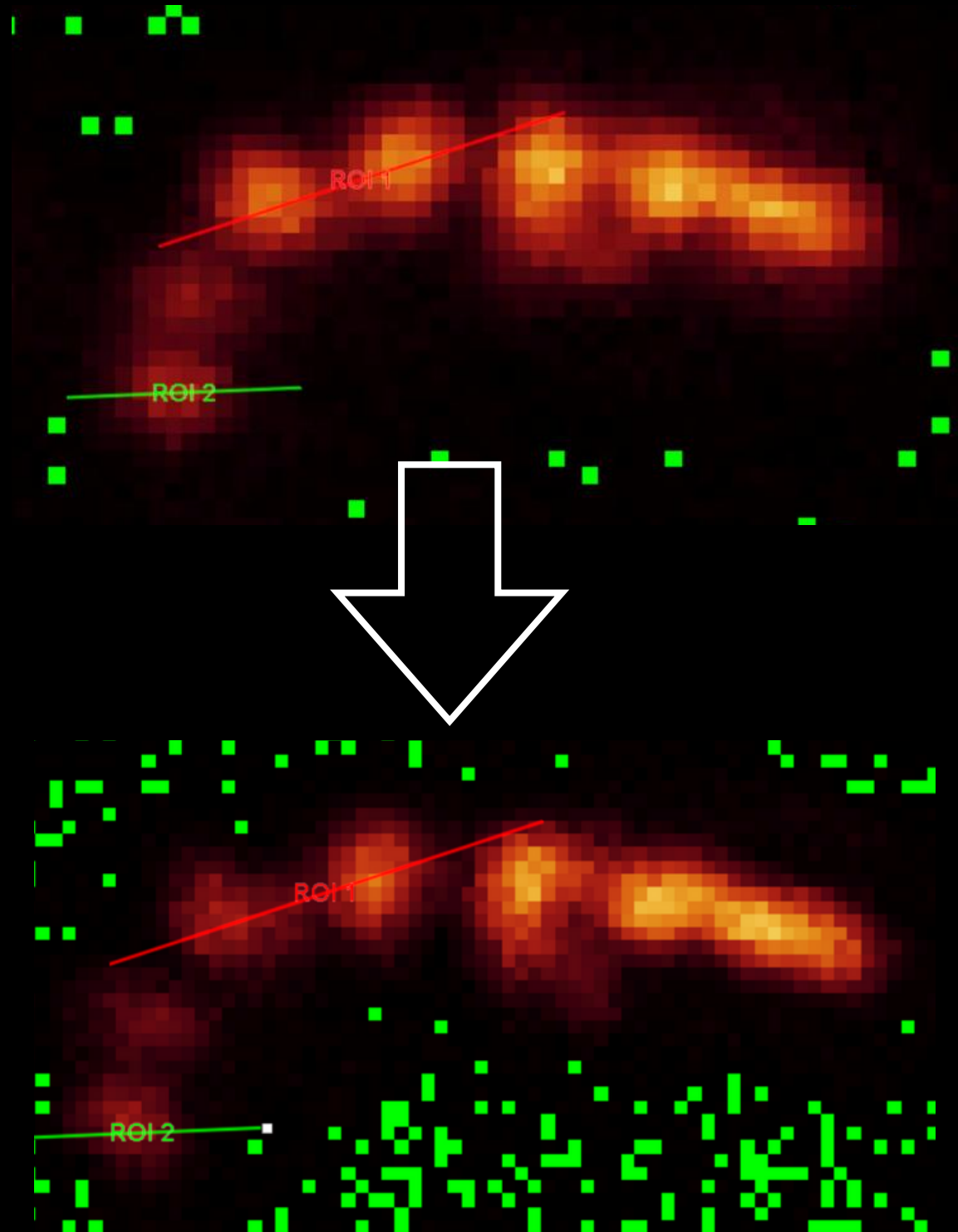
Deconvolution

FWHM  
163 nm



# Practical steps:

03 - Close the pinhole up to 0.6 AU (I would recommend to go down with pixel size to 35 nm)



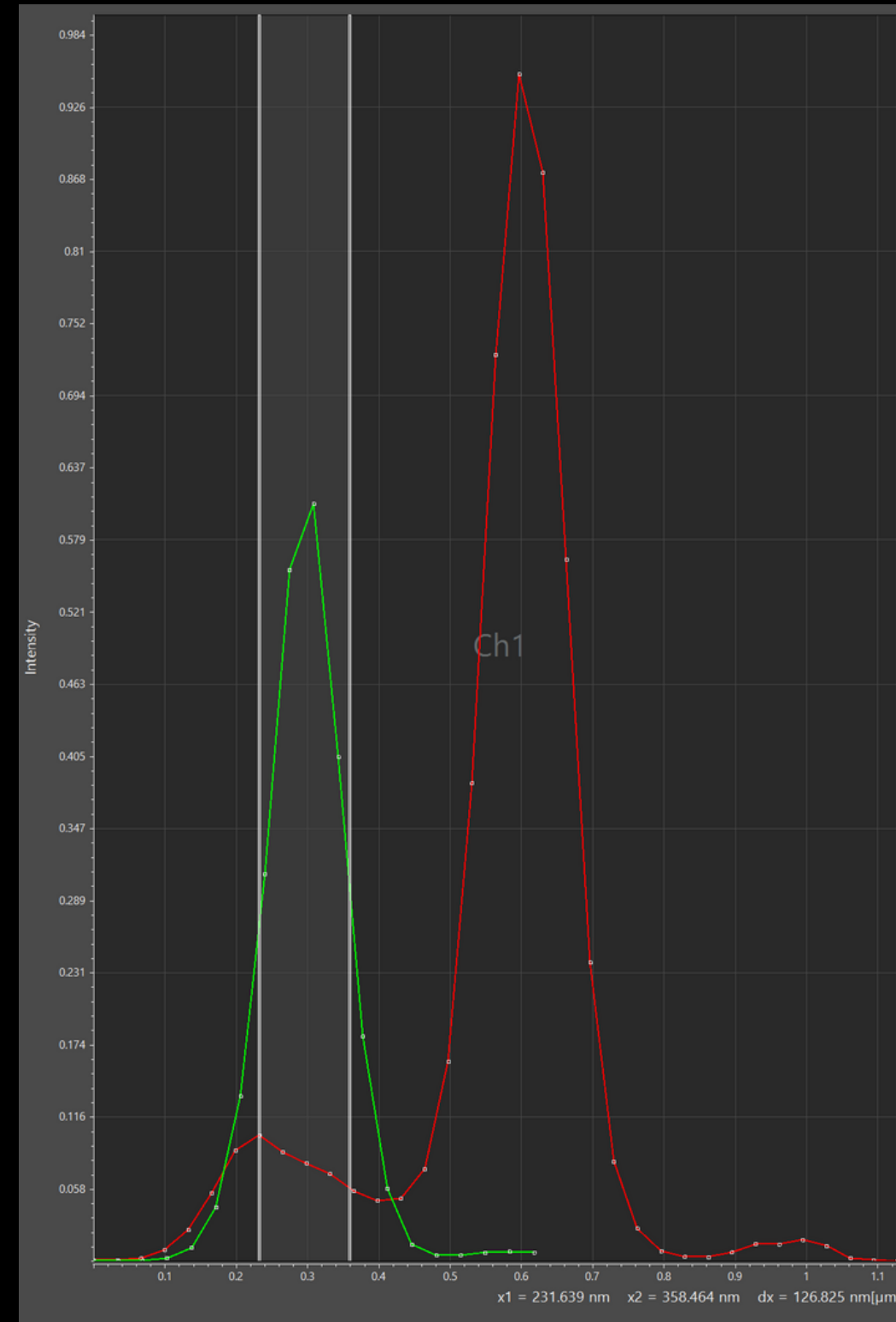
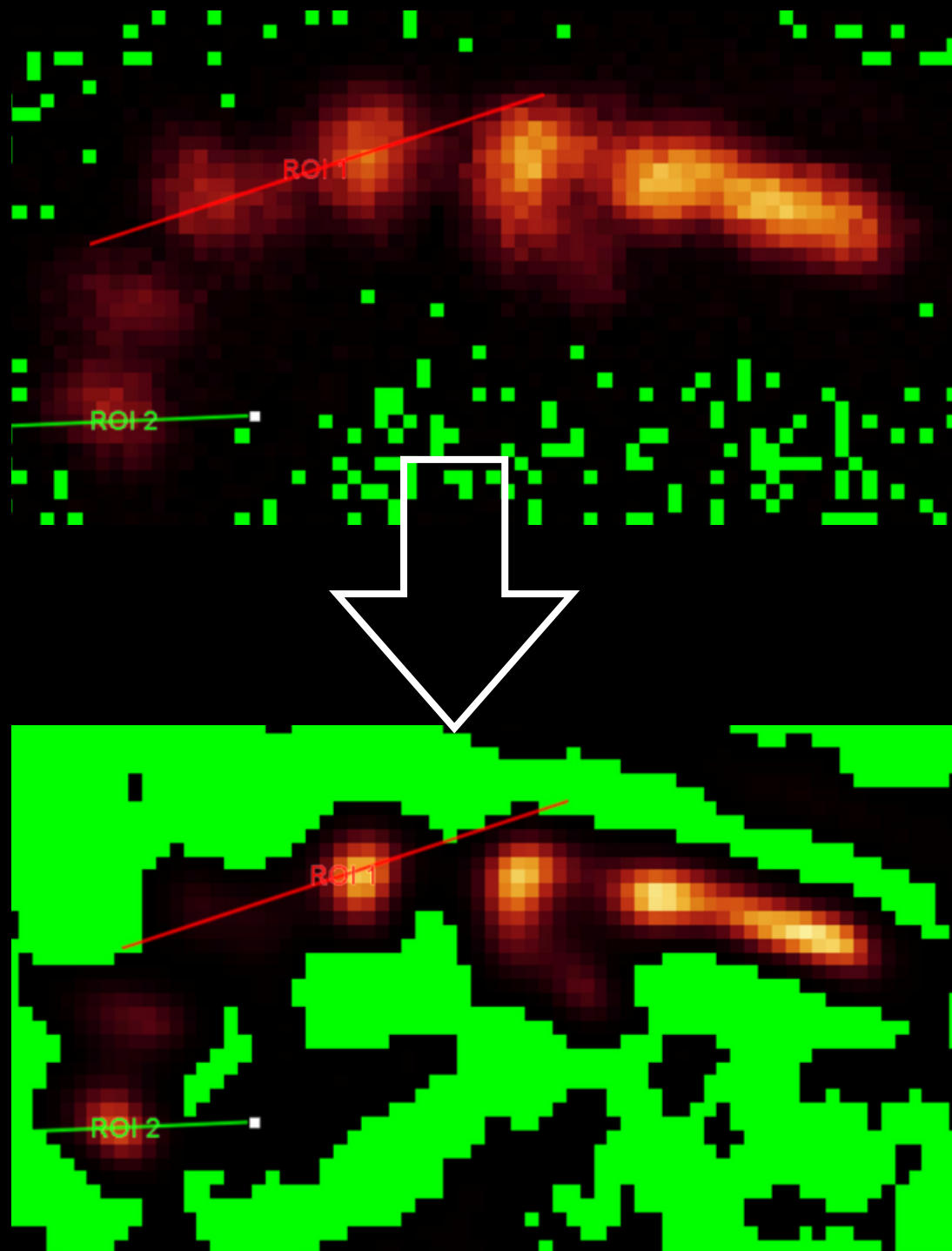
FWHM  
235 nm

pinhole effect

FWHM  
170 nm

# Practical steps:

03 - Close the pinhole up to 0.6 AU (I would recommend to go down with pixel size to 35 nm)



FWHM  
235 nm

pinhole effect

FWHM  
170 nm

deconvolution

FWHM  
~130 nm

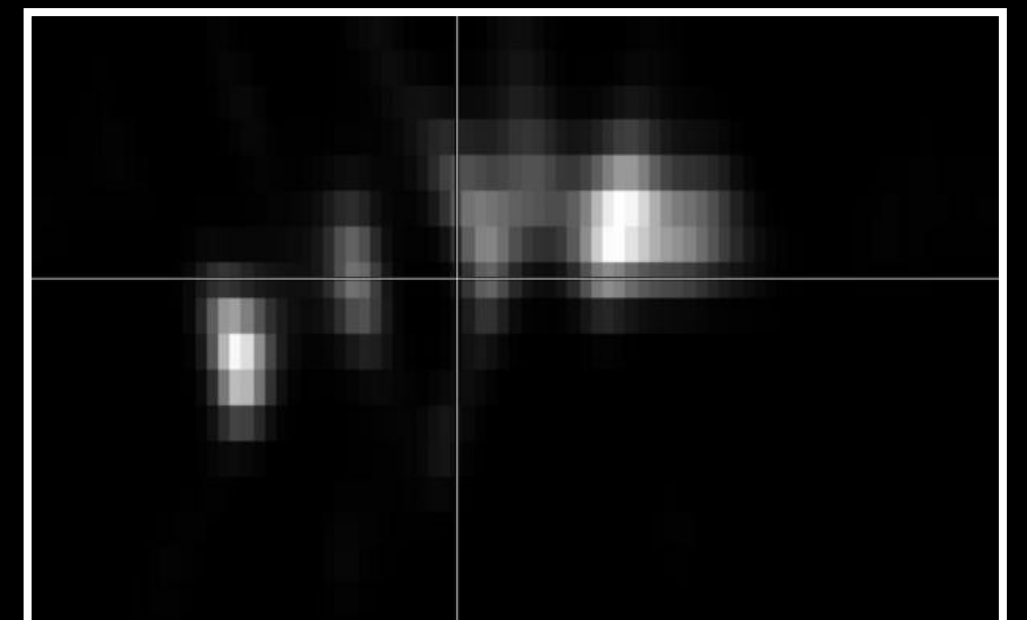
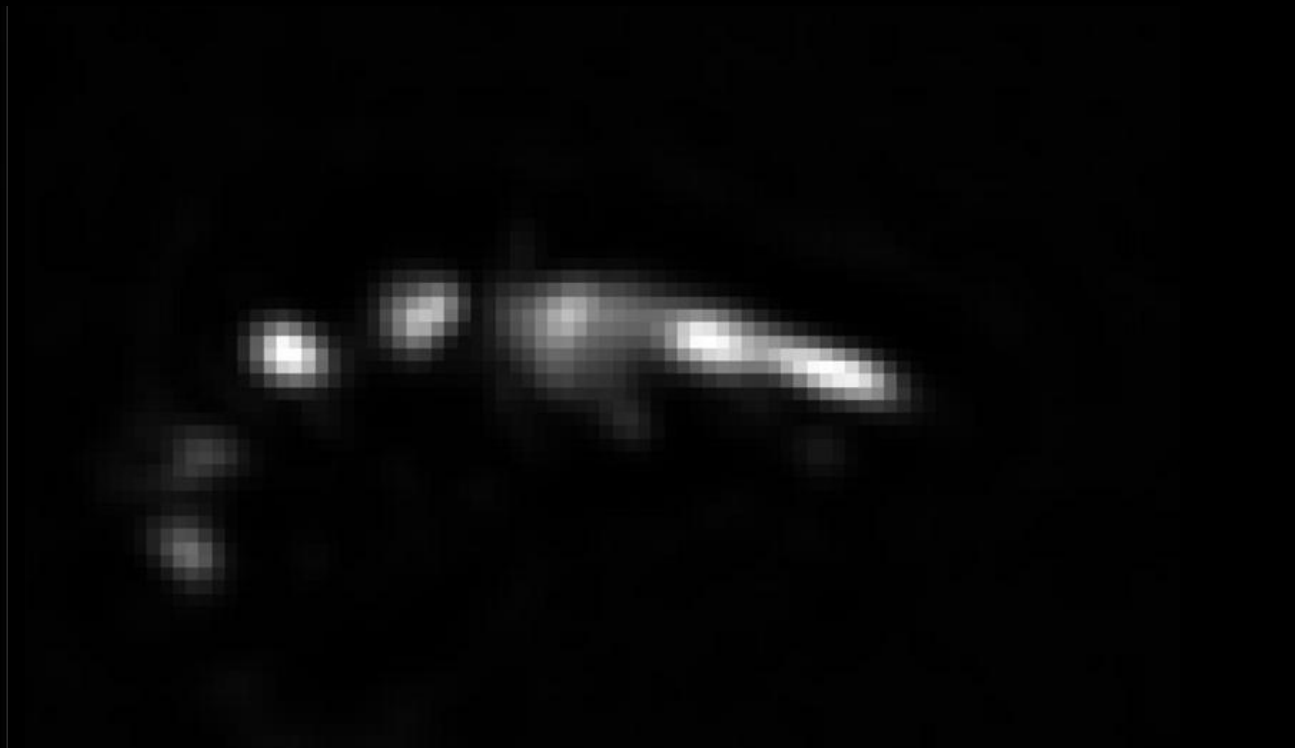
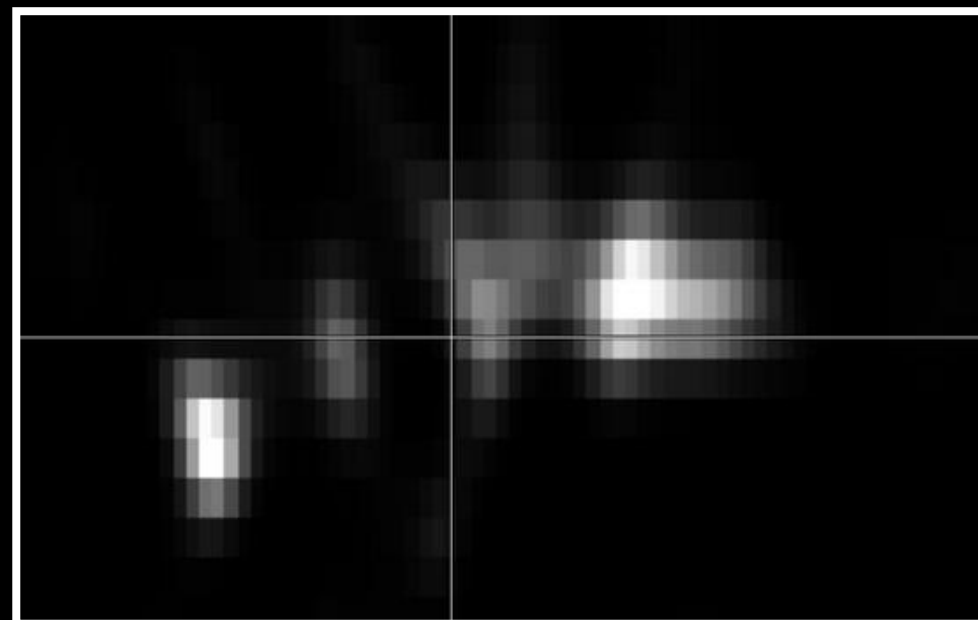
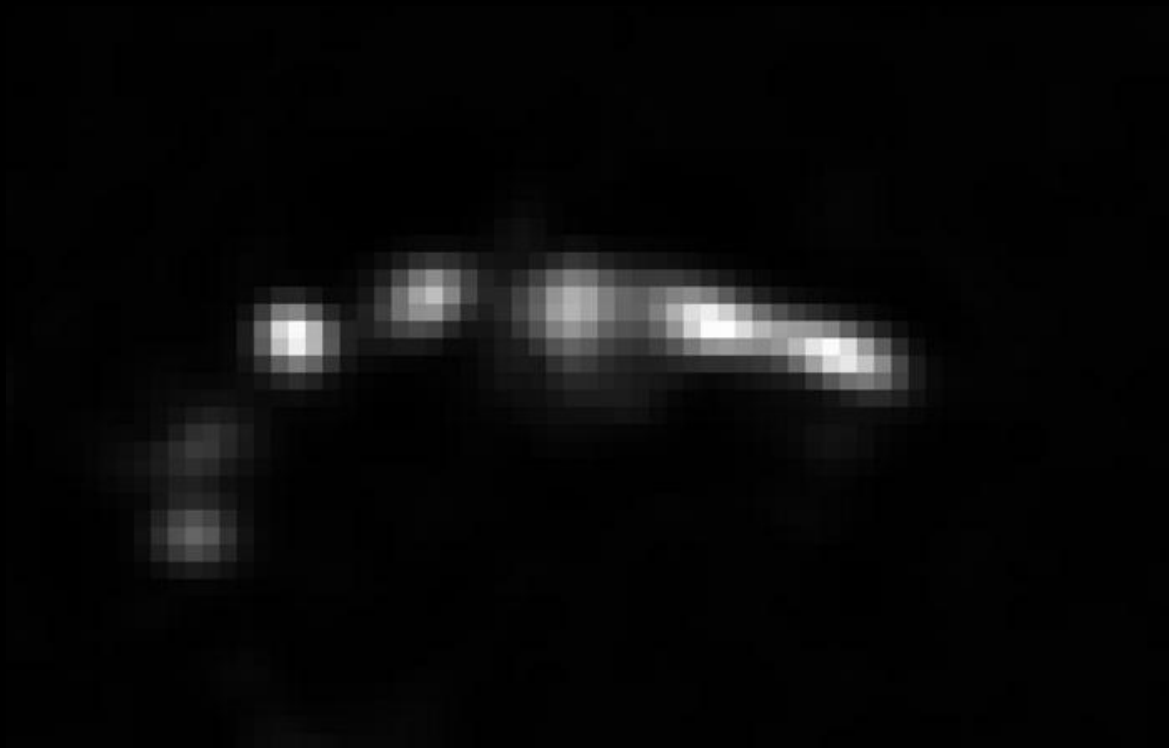
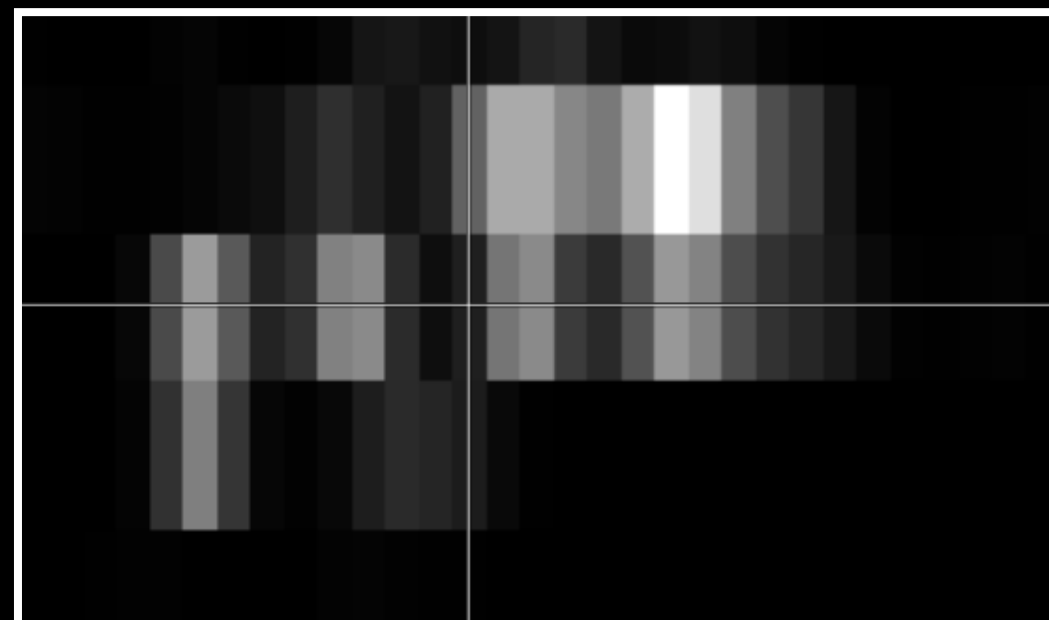
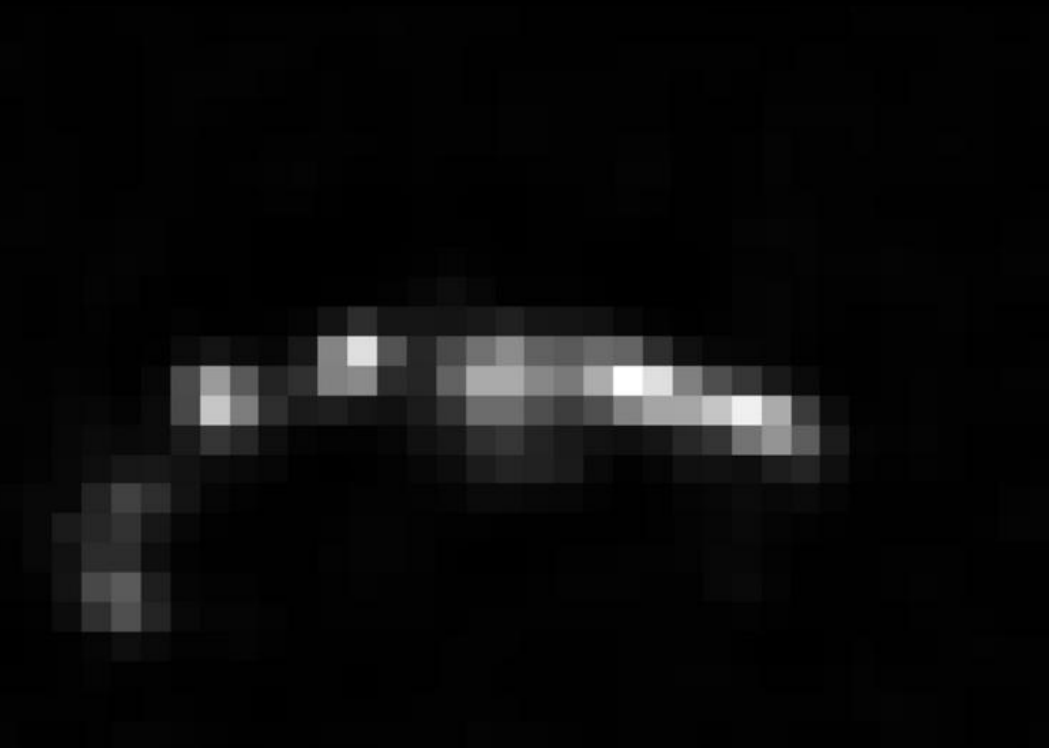


# Compare the results of the deconvolution

Pixel size 75 nm, pinhole 1AU

Pixel size 43 nm, pinhole 1AU

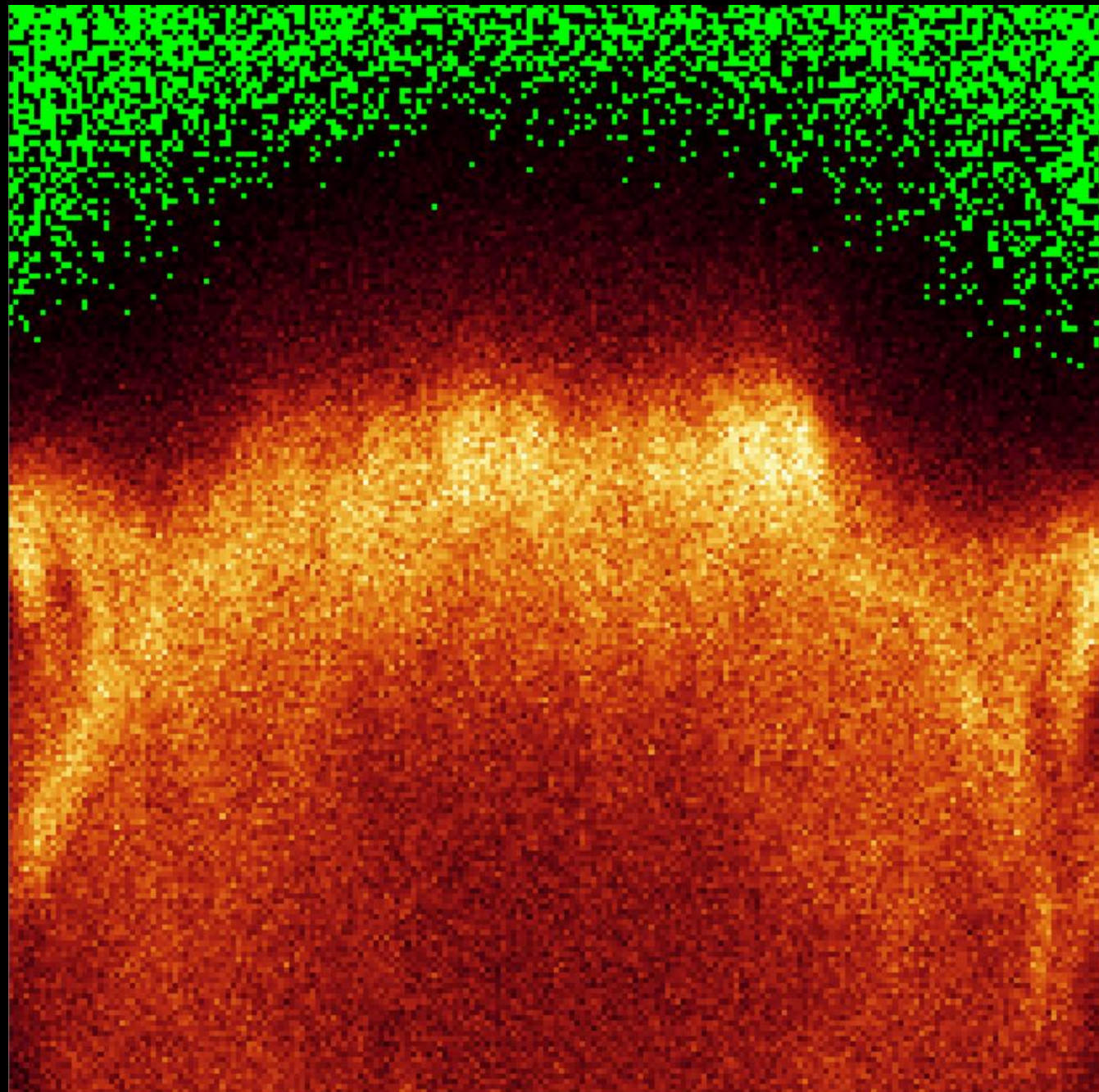
Pixel size 35 nm, pinhole 0.6 AU



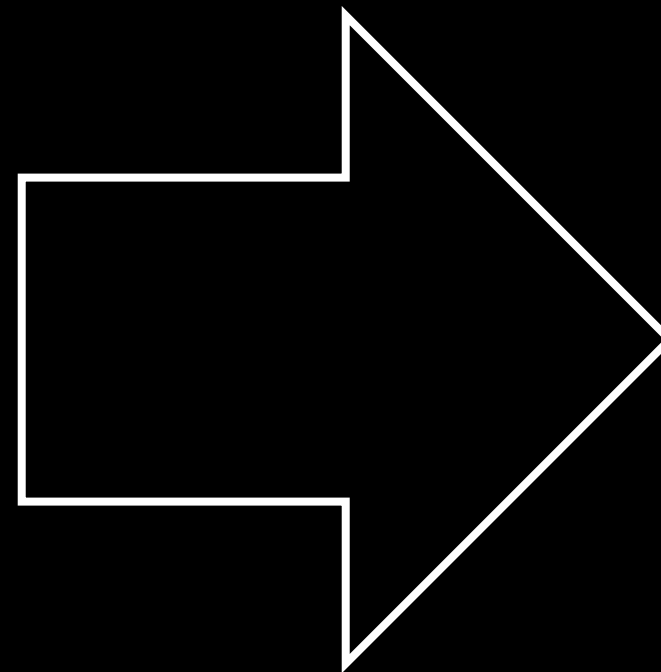


# Practical results on real structures - plant sample -daisy pollen grain

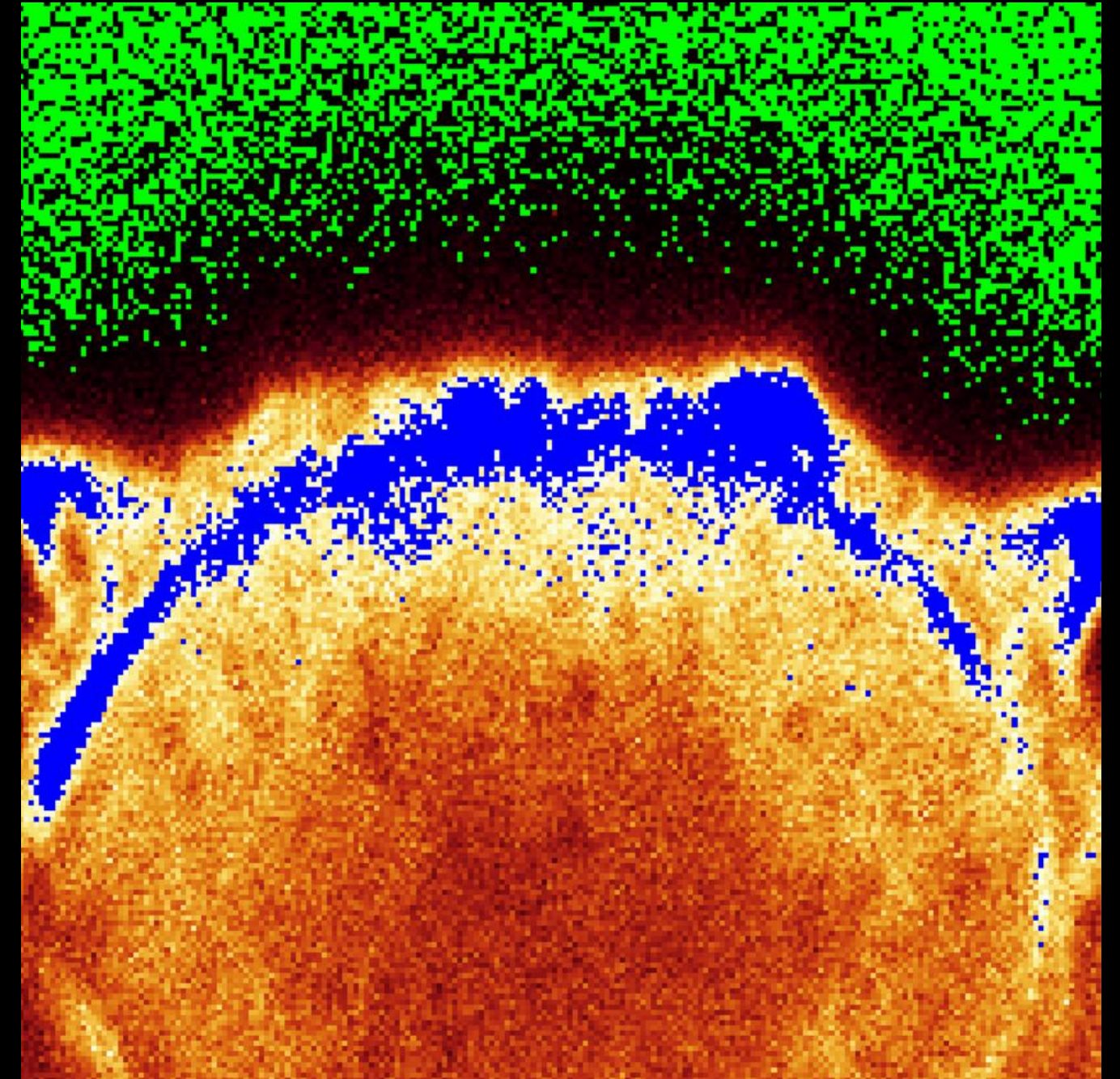
01 - check the signals, PSF shape and maximize signals by correction optics



XZ scan through  
the structures



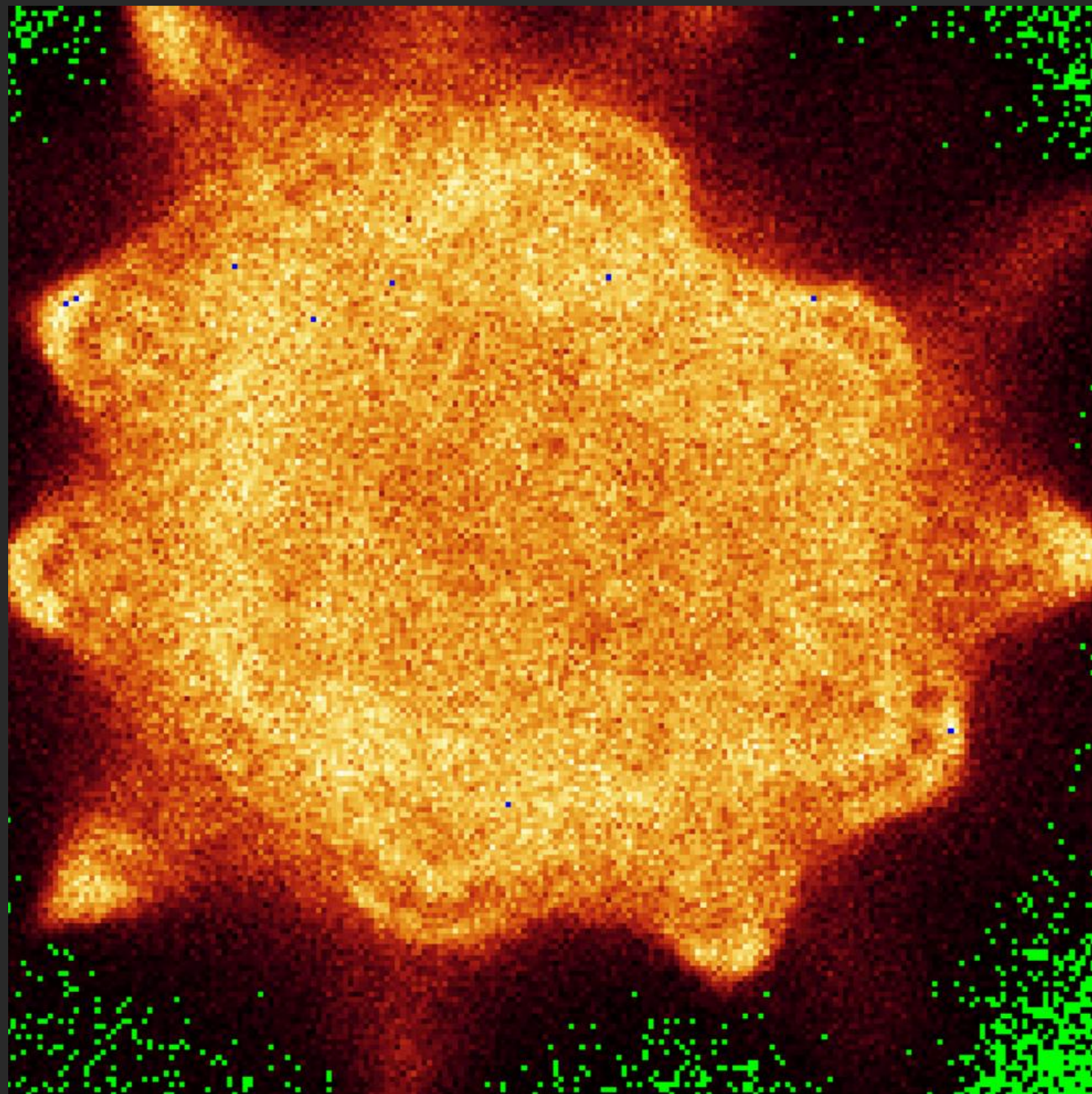
correction optics on  
the objective



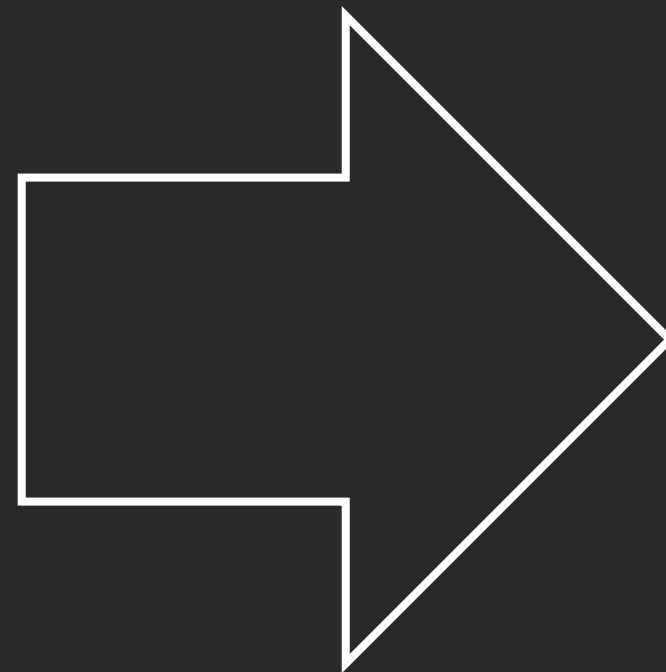


# Practical results on real structures - plant sample -daisy pollen grain

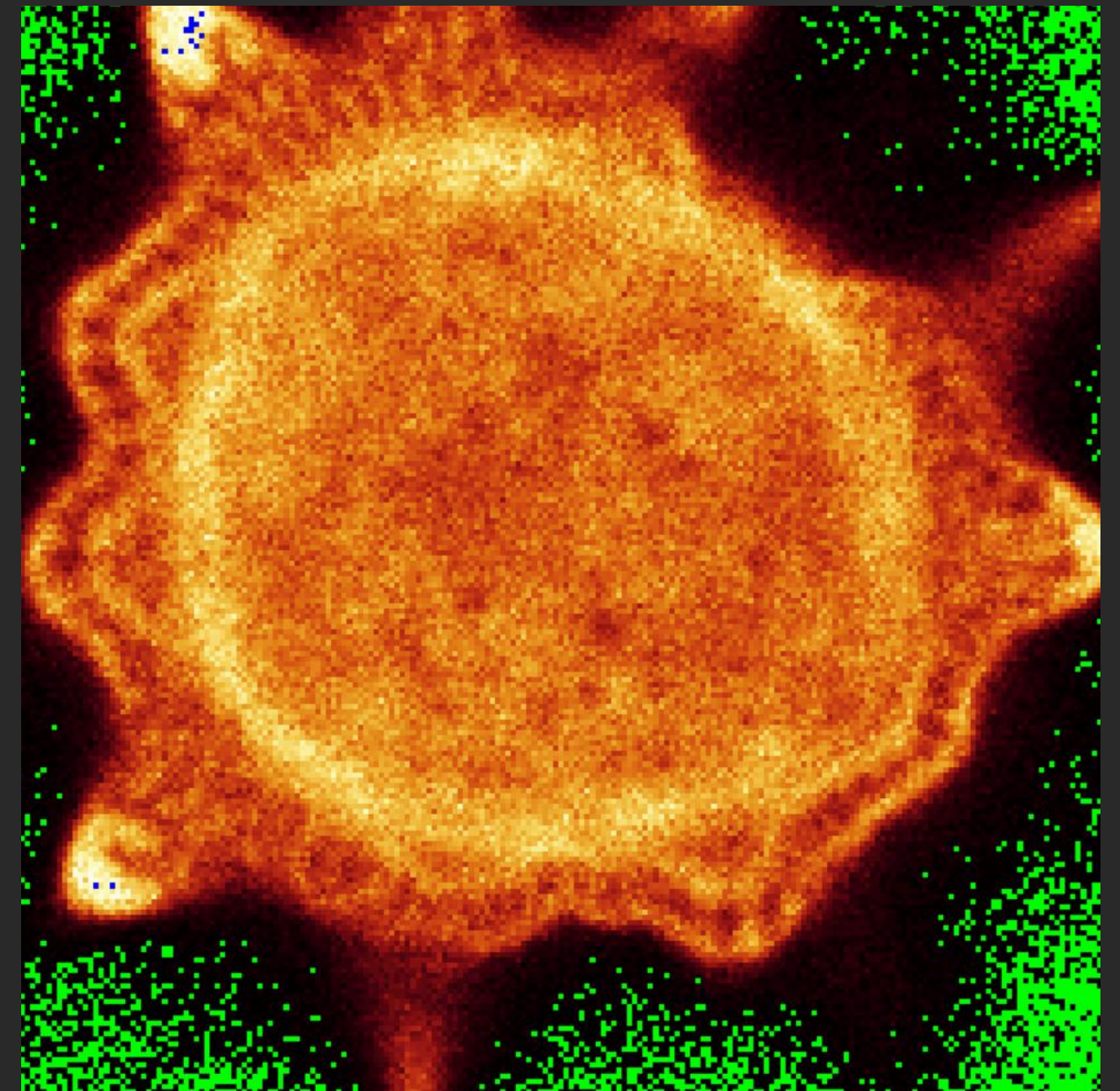
01 - check the signals, PSF shape and maximize signals by correction optics



XY scan through  
the structures



correction optics on  
the objective

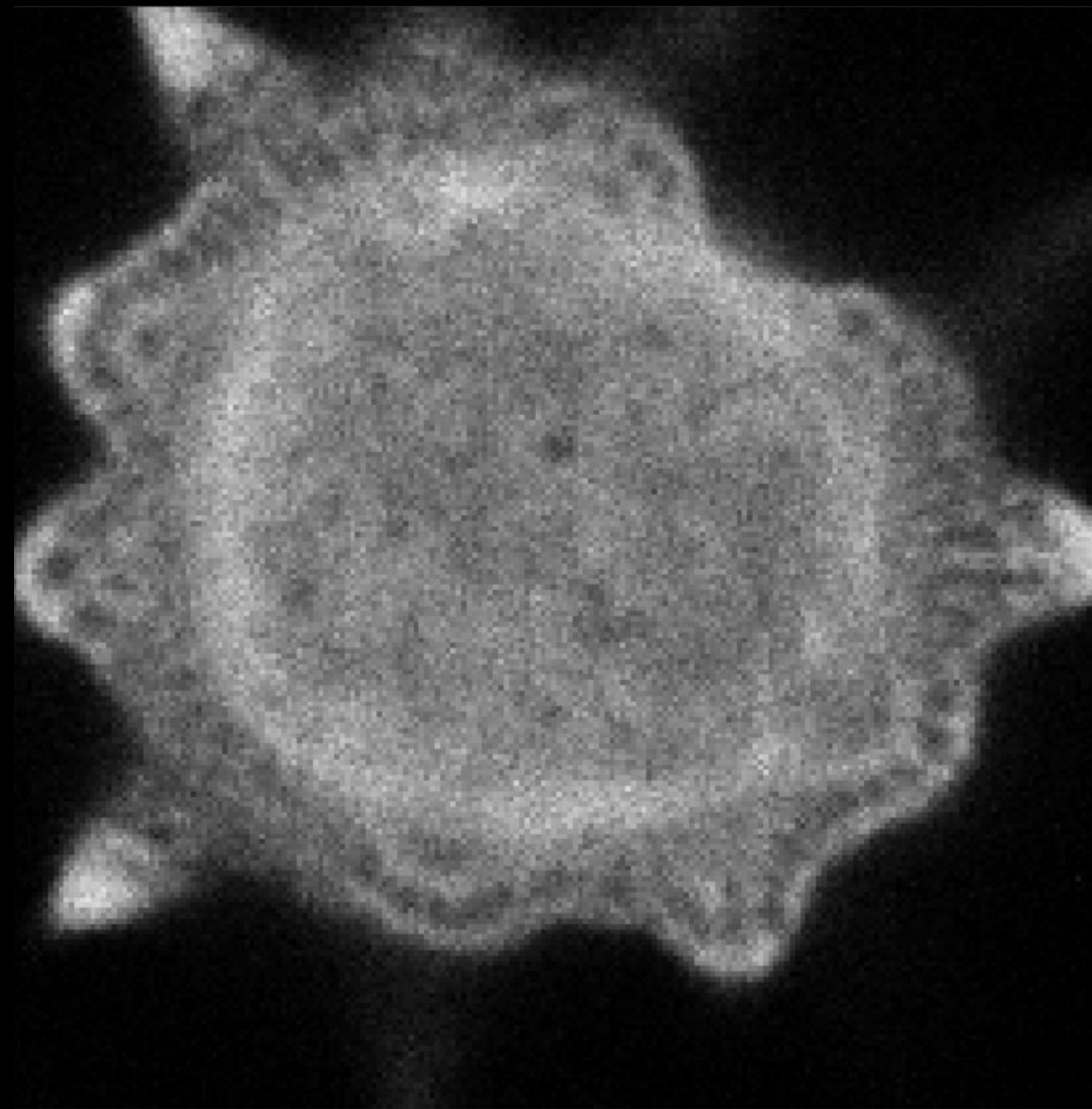
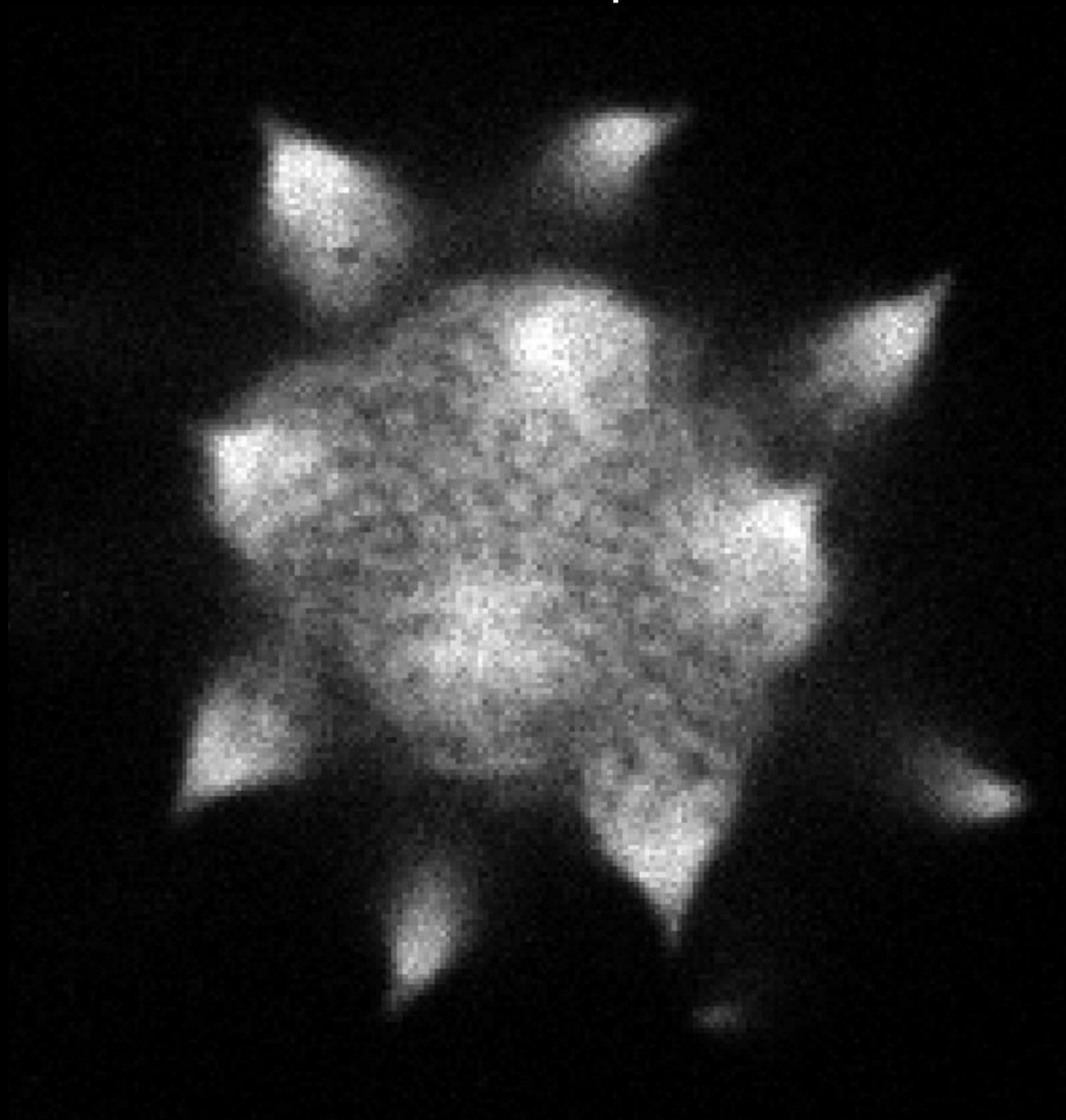




# Practical decon results on real structures

02 - precisely set the pixel size

Pixel size 75 nm, pinhole 1AU

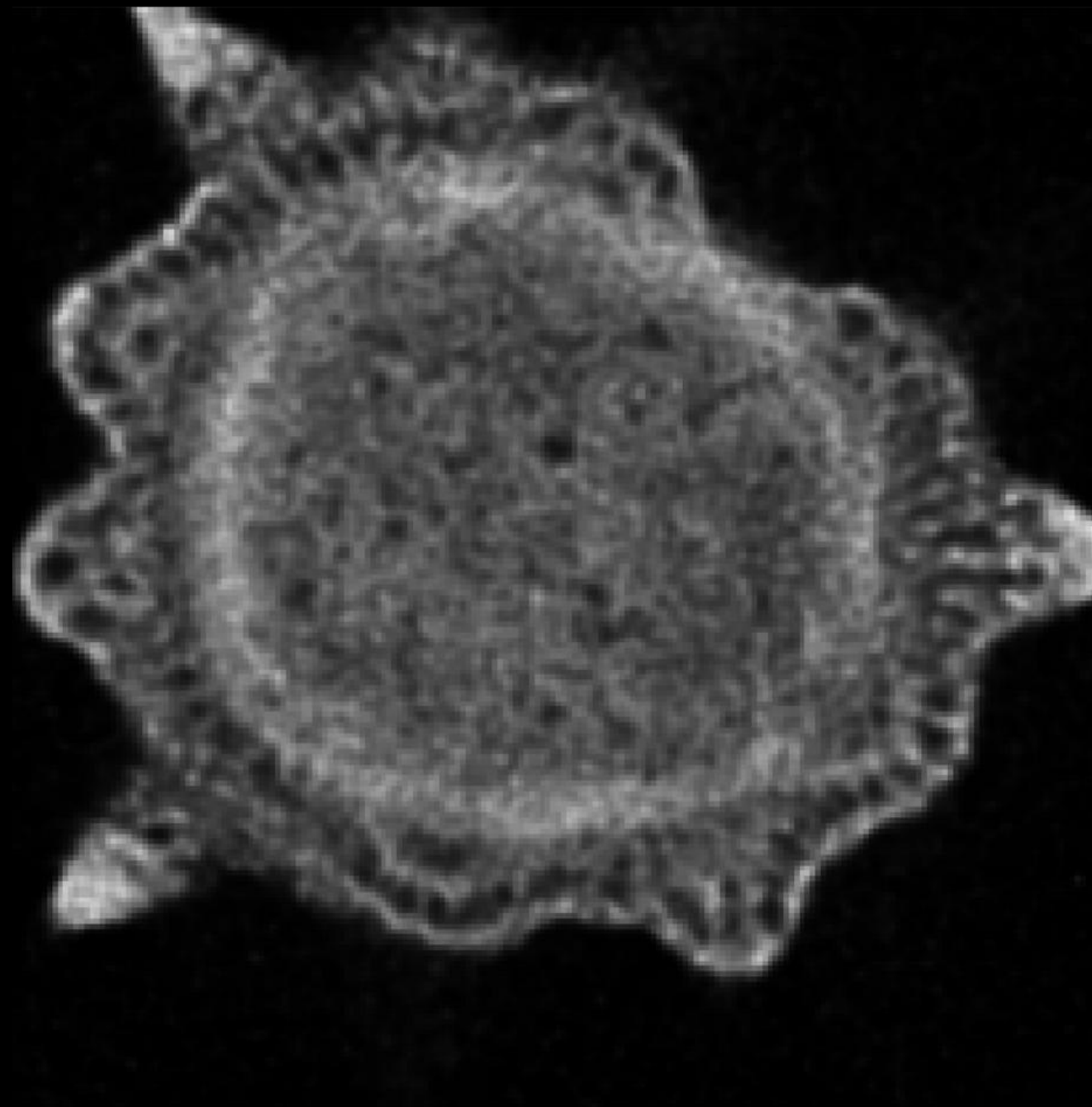
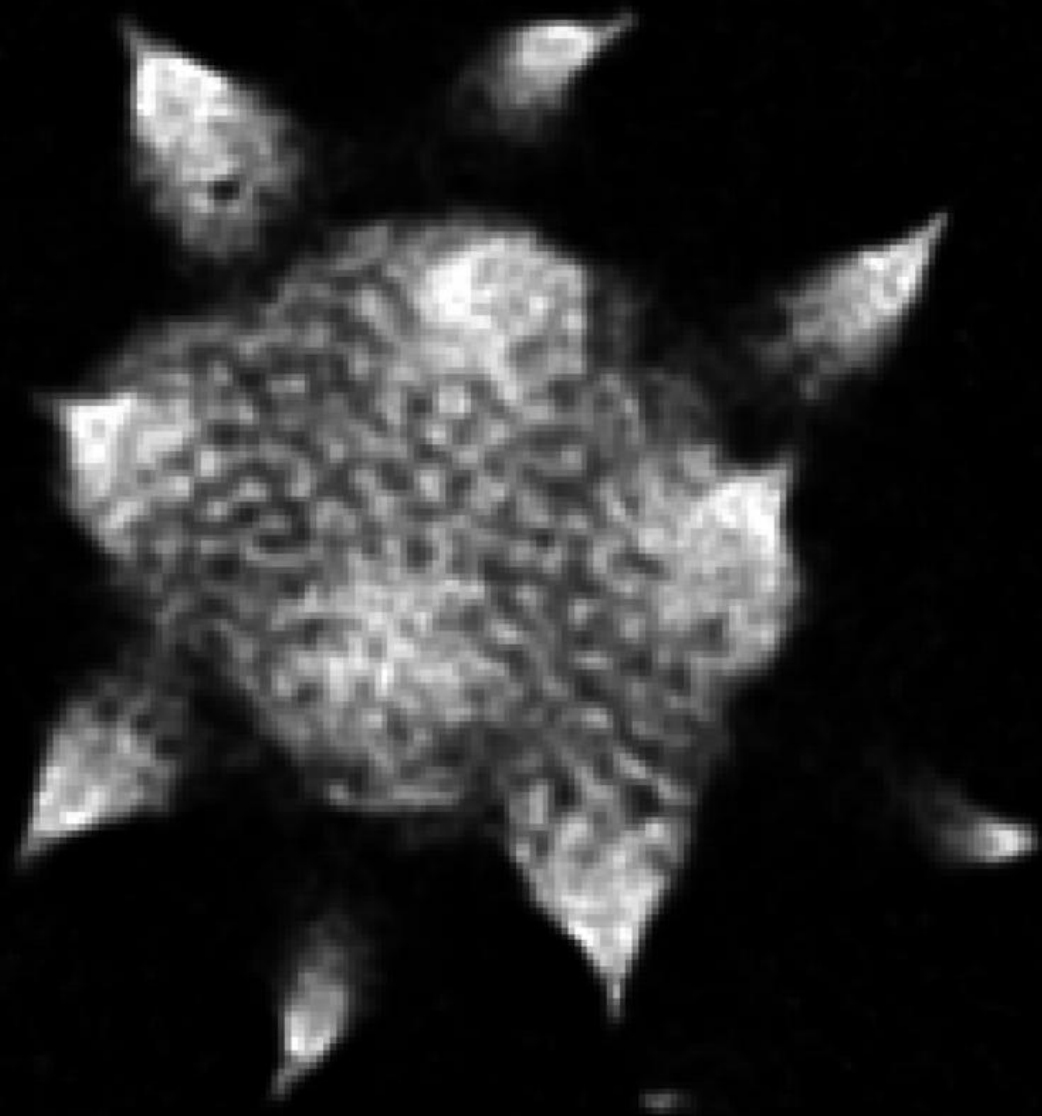




# Practical decon results on real structures

02 - precisely set the pixel size

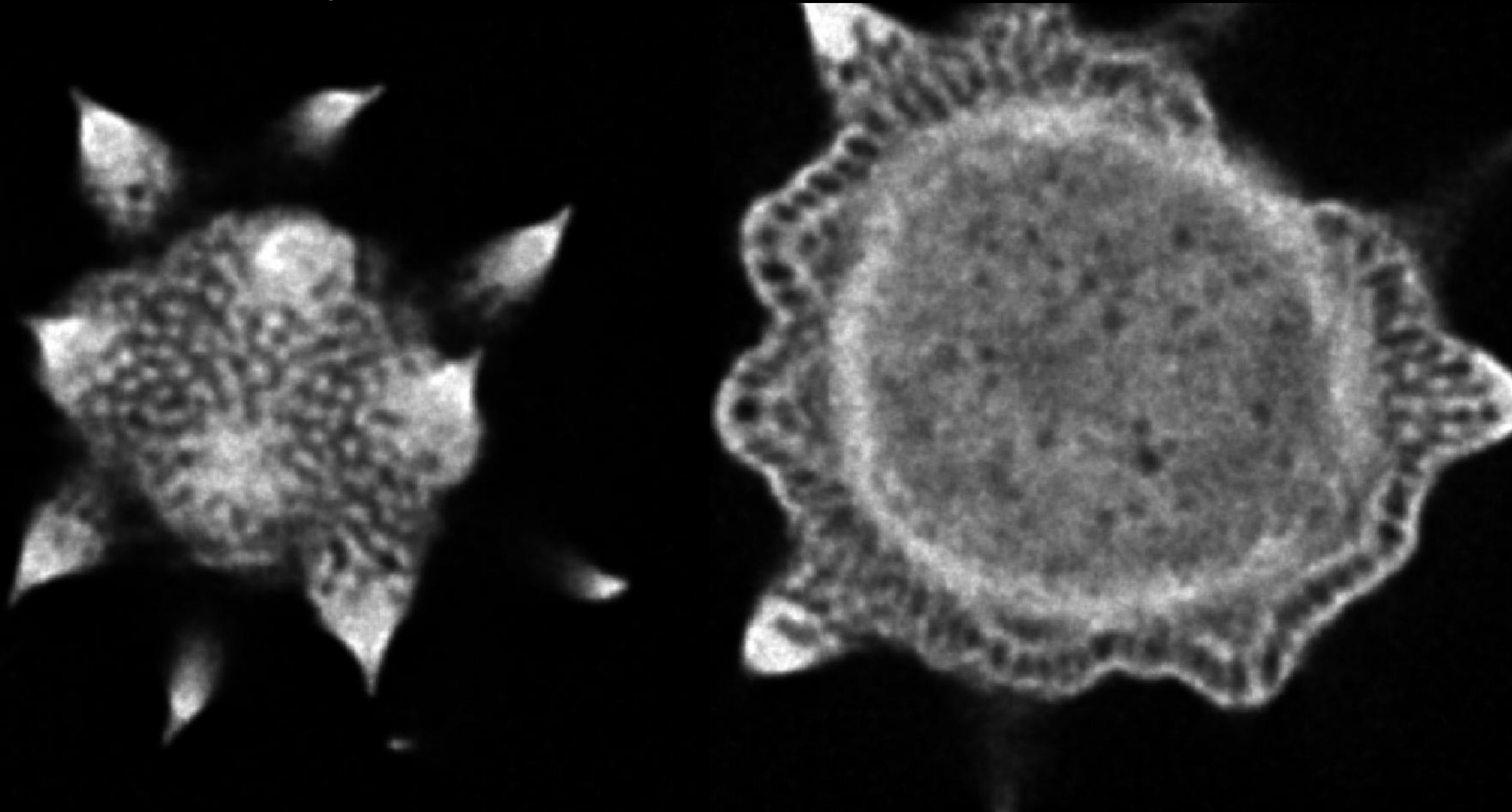
Pixel size 75 nm, pinhole 1AU



# Practical decon results on real structures

02 - precisely set the pixel size

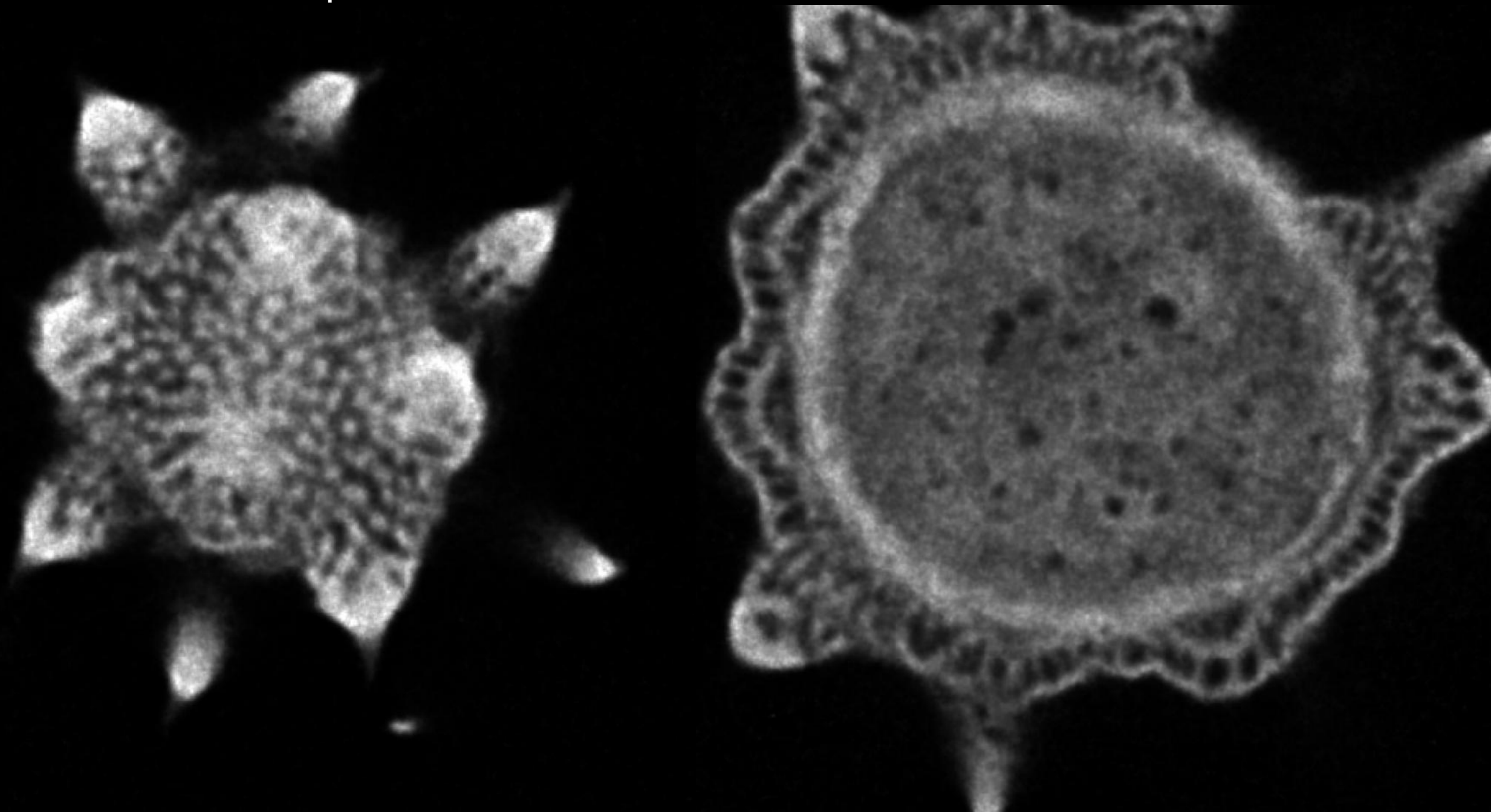
Pixel size 43 nm, pinhole 1AU



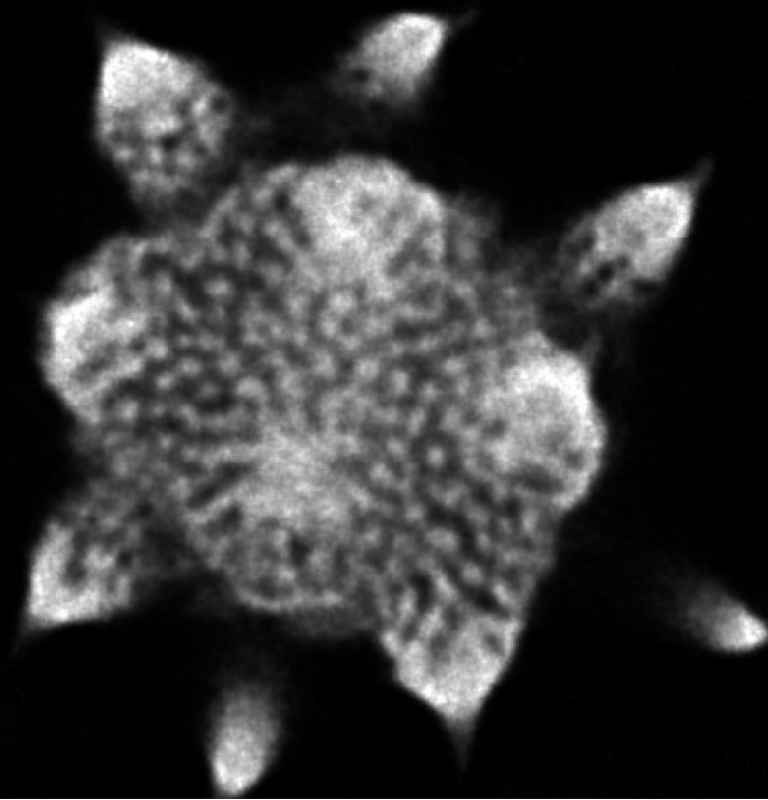
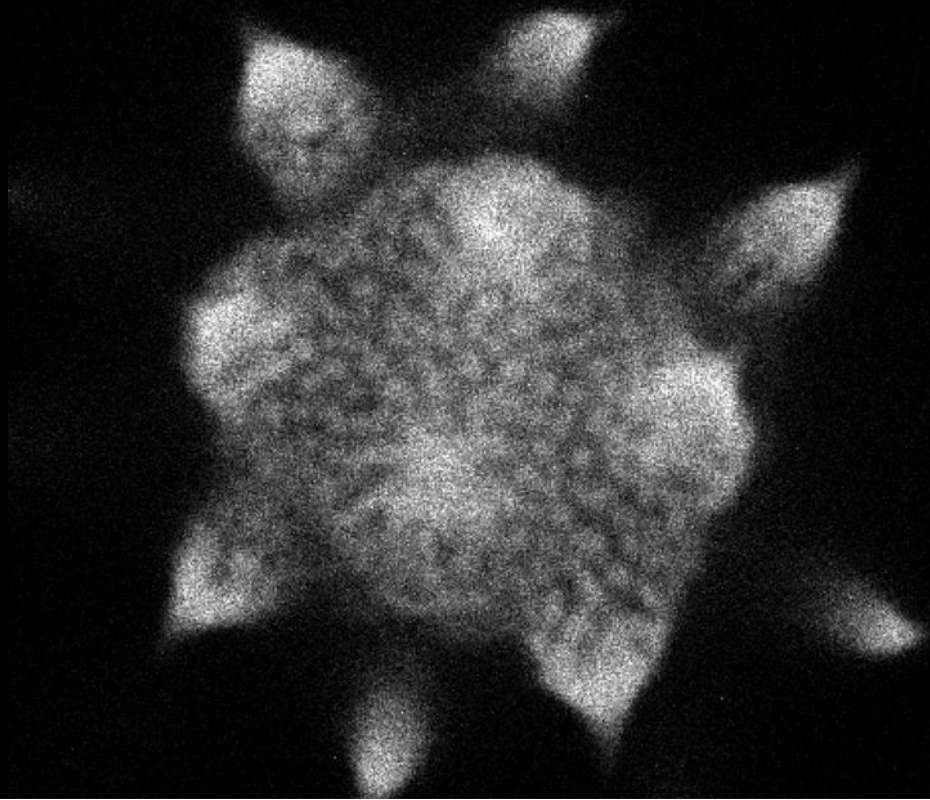
# Practical decon results on real structures

03 - close the pinhole

Pixel size 35 nm, pinhole 0.6 AU

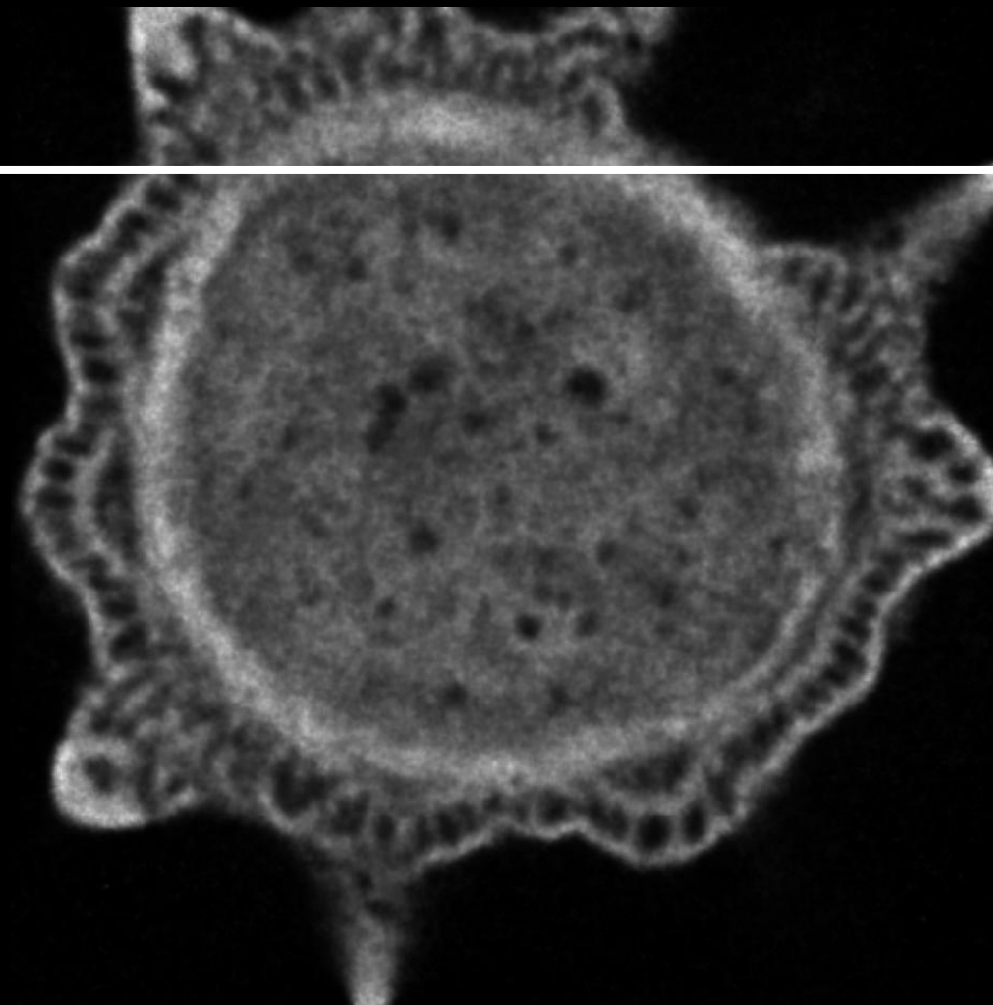
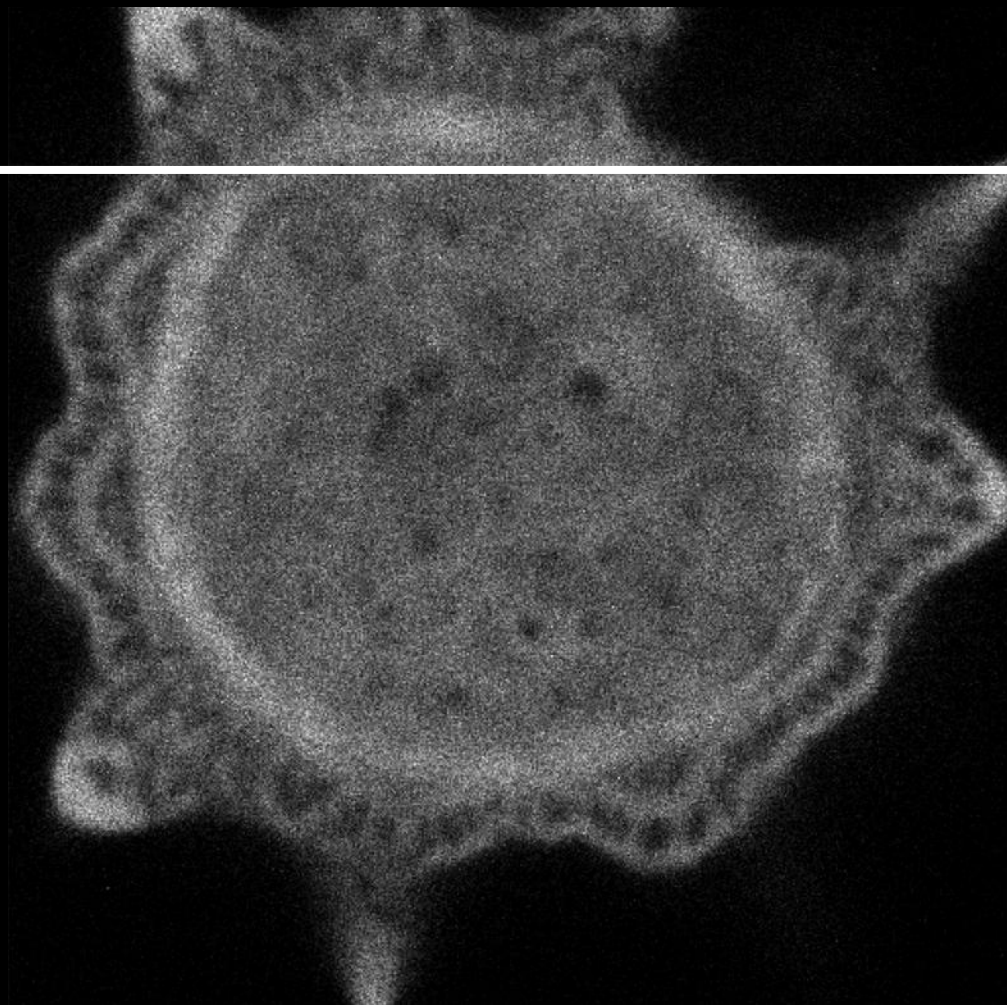






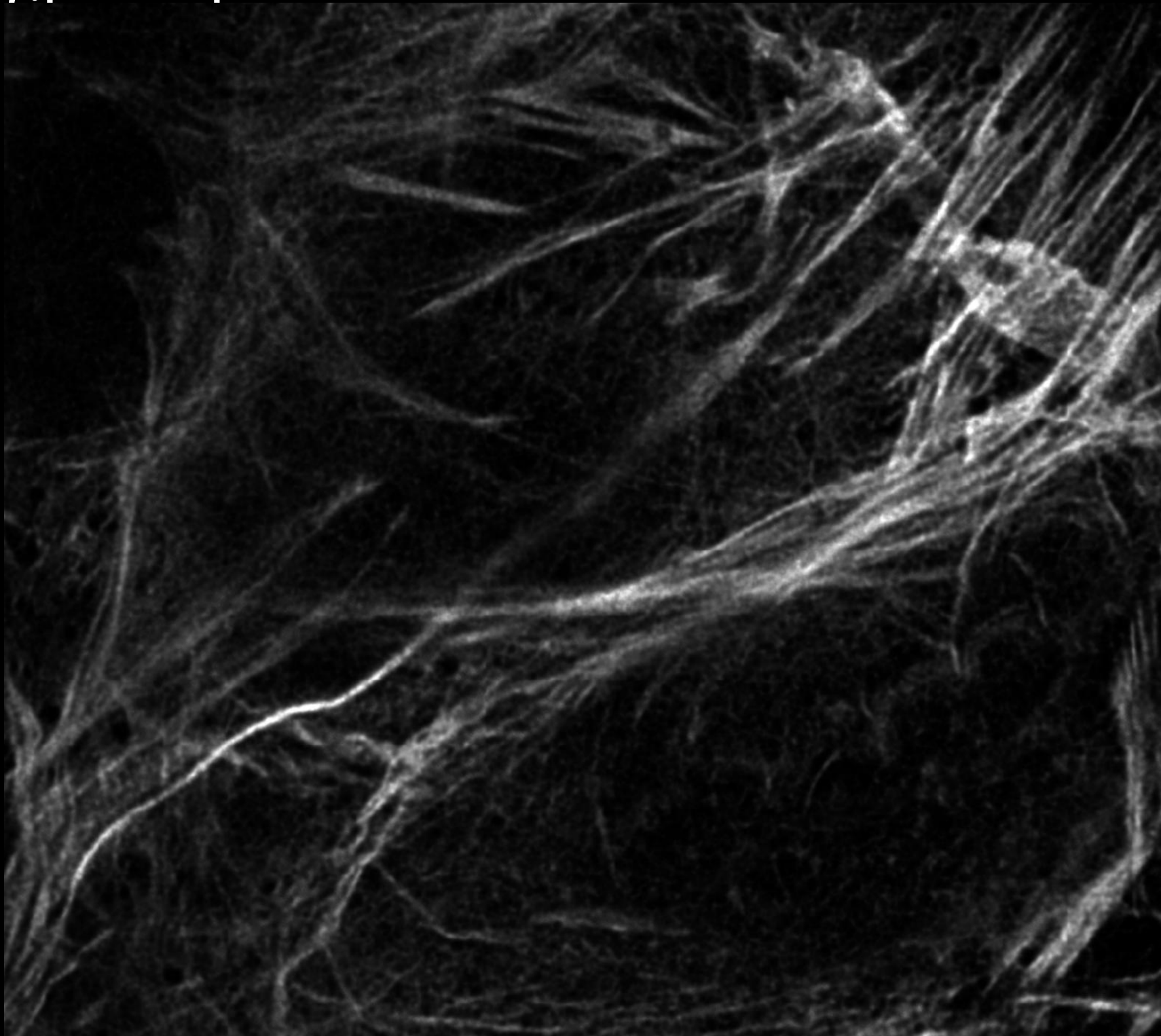
**Source data**

**Decon results**



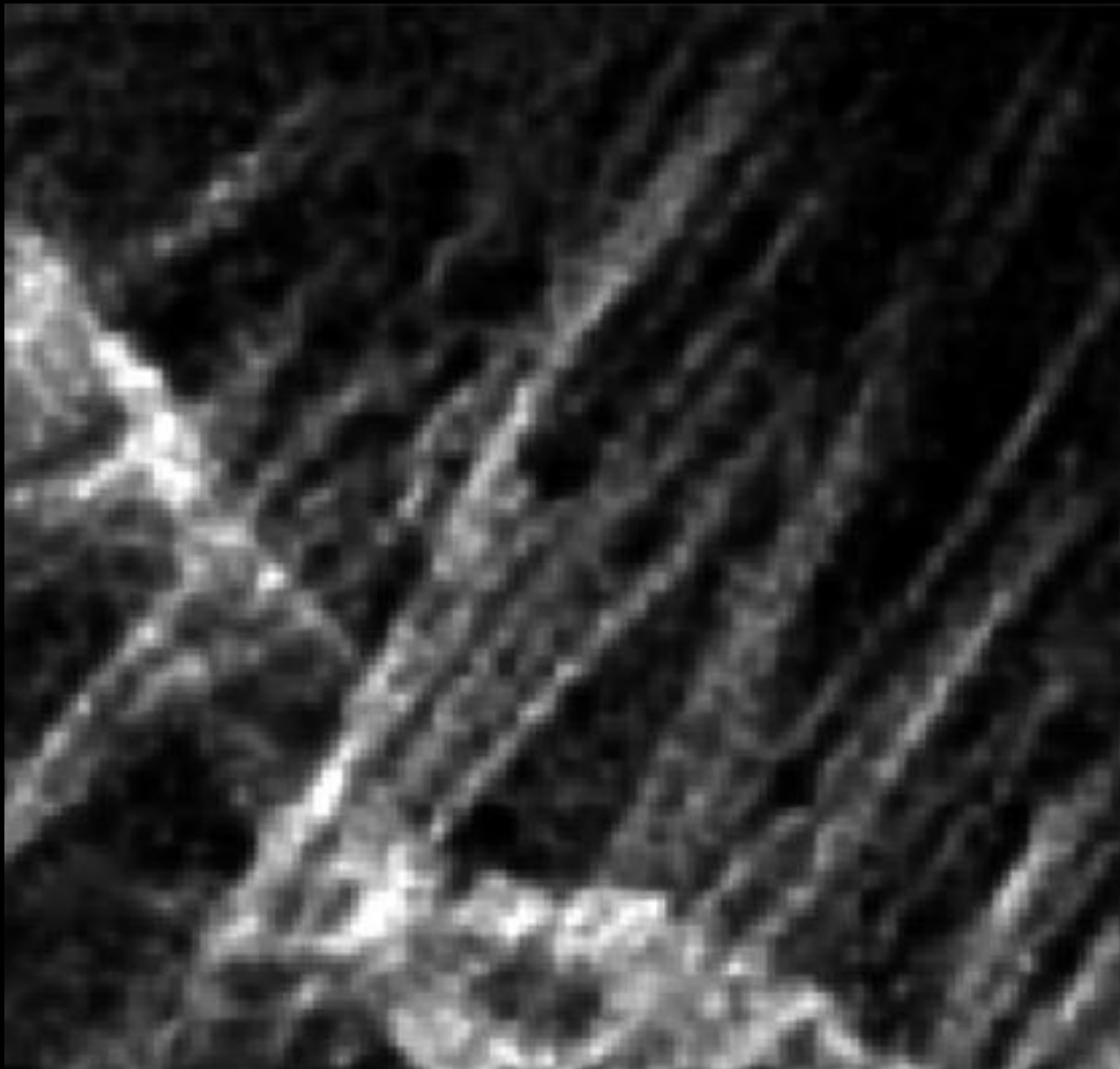
# Compare the results of the deconvolution

Actin

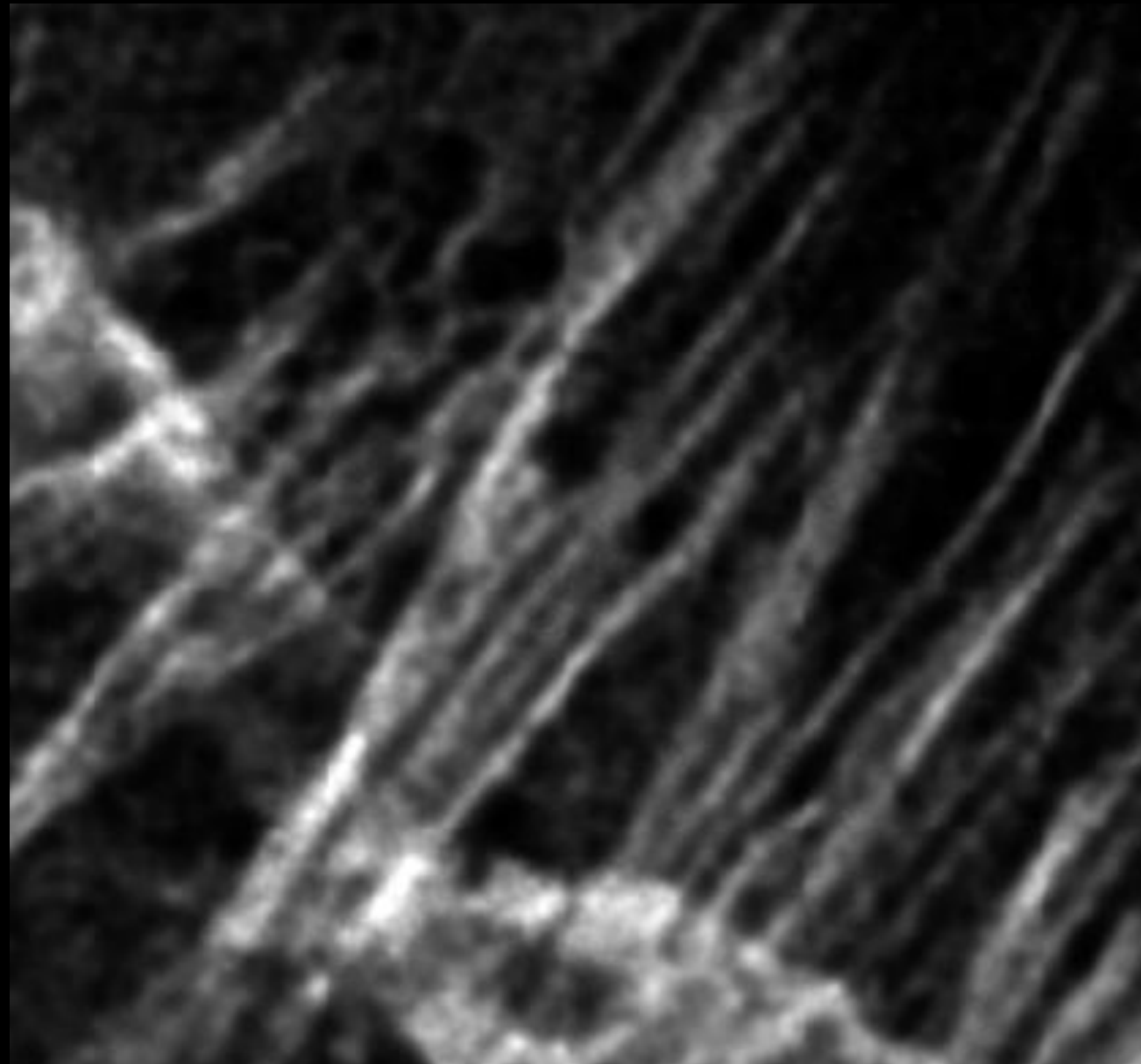


# Compare the results of the deconvolution filaments

Pixel size 75 nm, pinhole 1AU



Pixel size 43 nm, pinhole 1AU

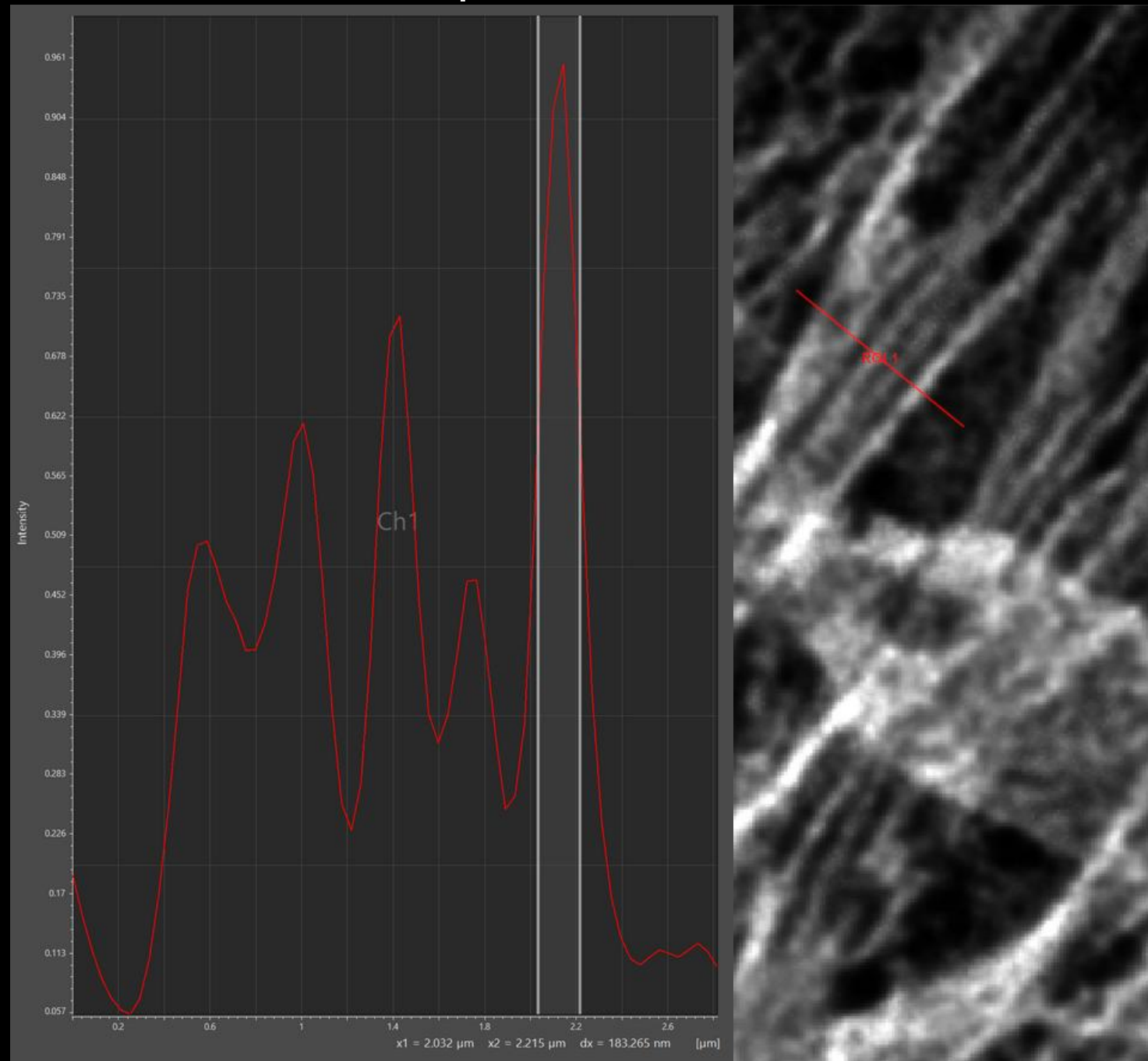




# Compare the results of the deconvolution

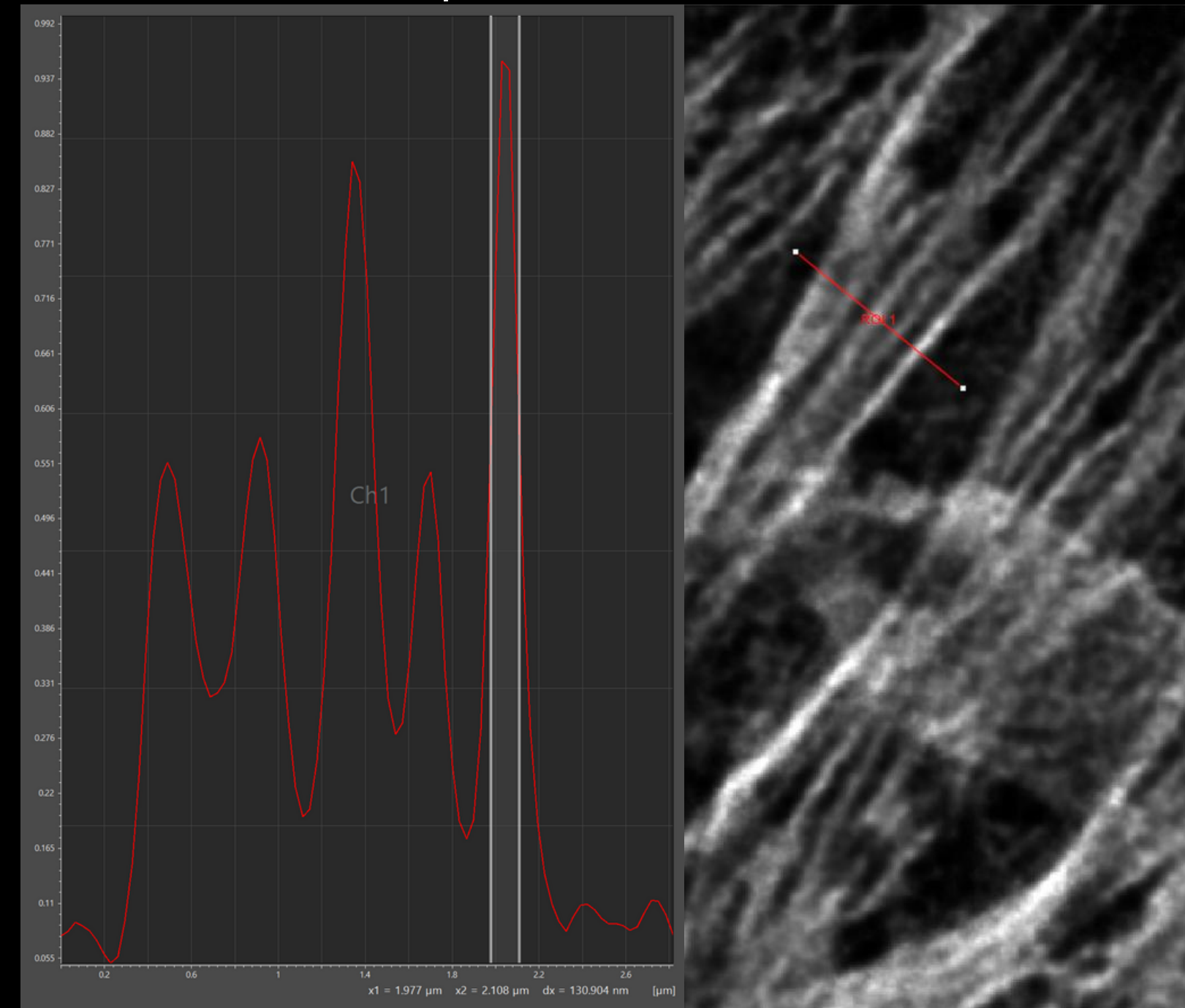
## Actin filaments

Pixel size 43 nm, pinhole 1AU



FWH ~ 180 nm

Pixel size 35 nm, pinhole 0.6AU

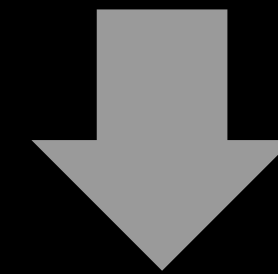


FWH ~ 130 nm

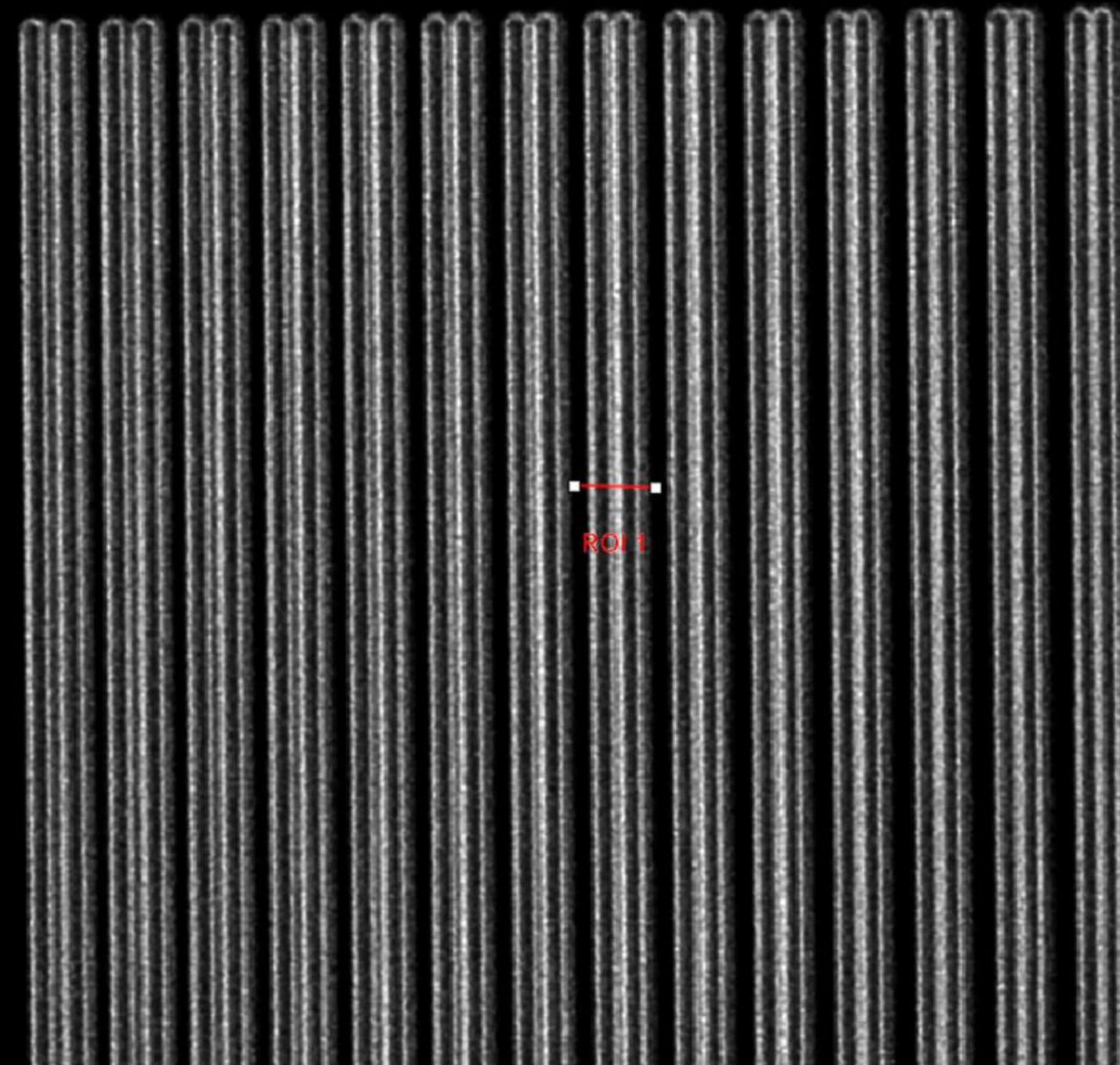
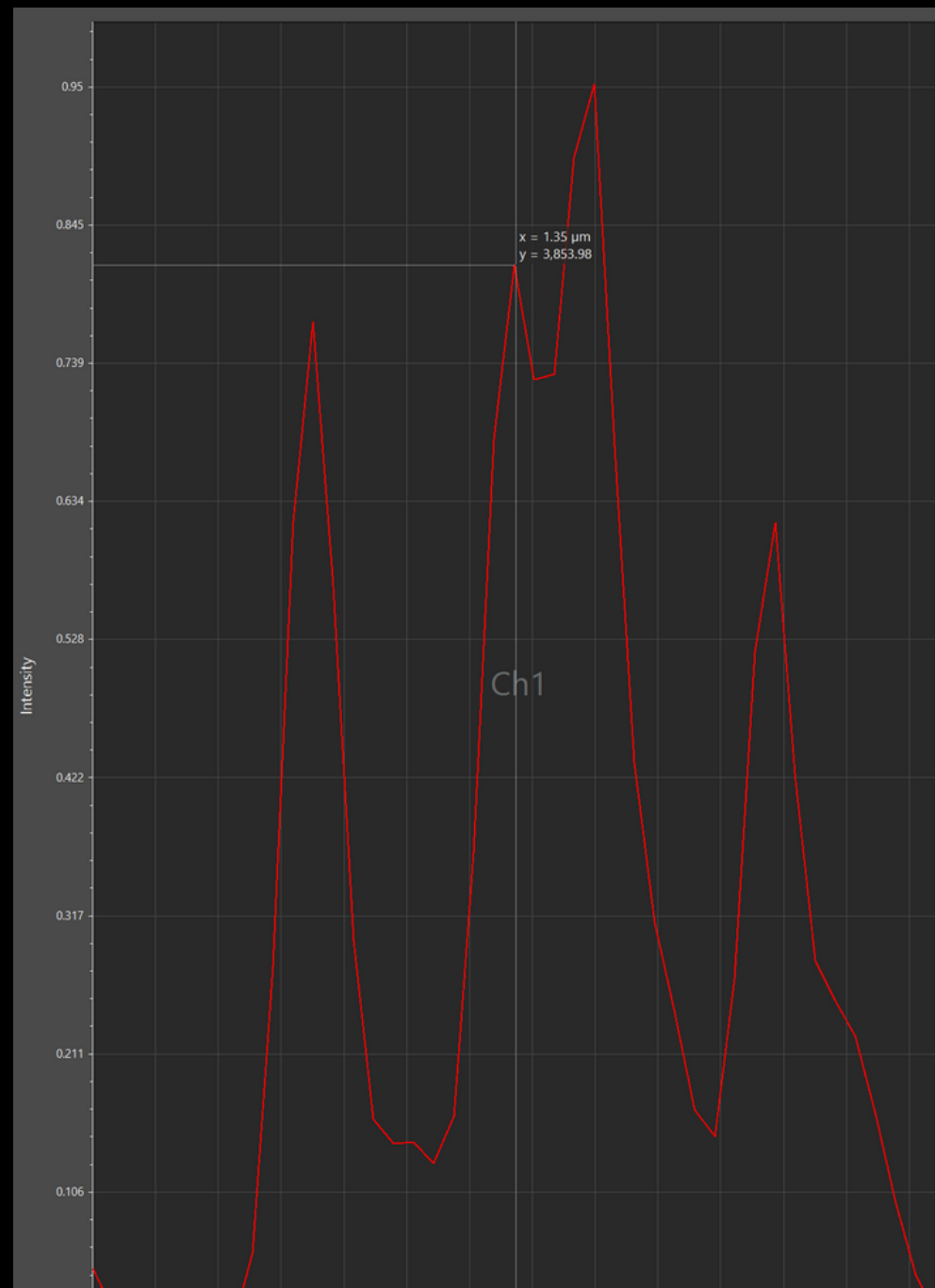
# The resolution estimation

Argolight slide SIM pattern

Pixel size 75 nm, pinhole 1AU



210 180 150 120 90 60 30 0 [nm]

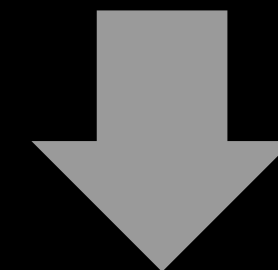




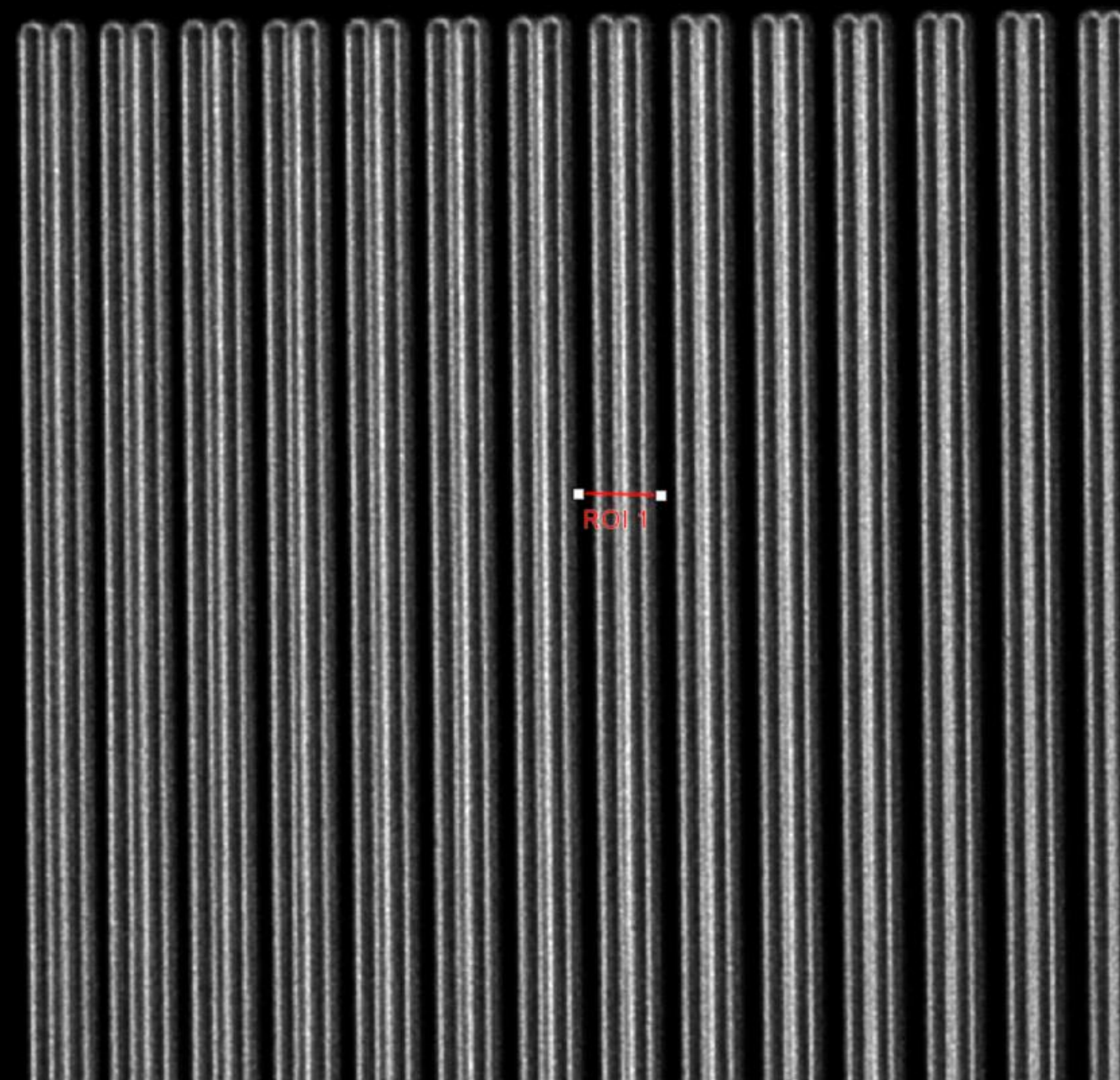
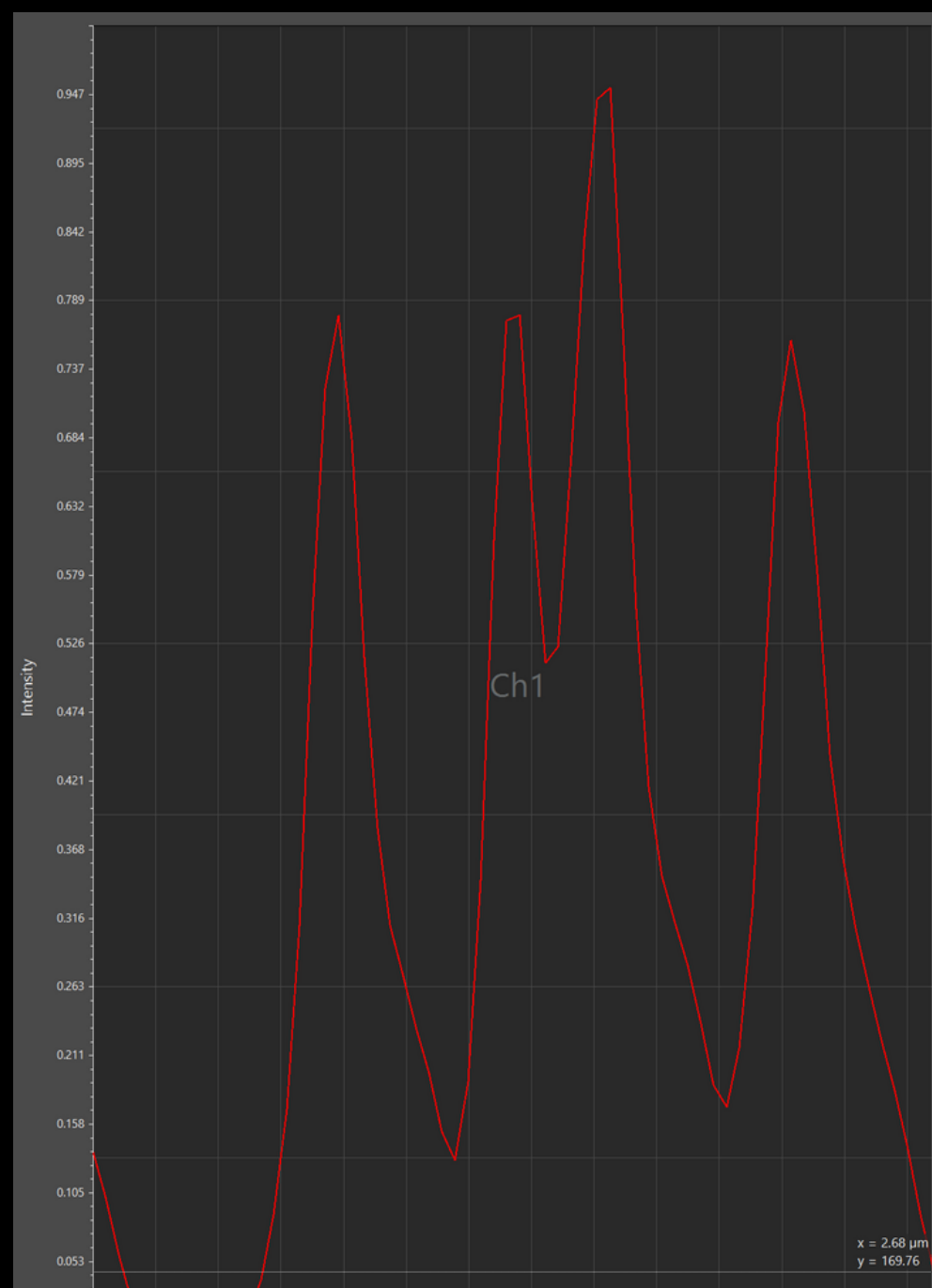
# The resolution estimation

Argolight slide SIM pattern

Pixel size 43 nm, pinhole 1AU

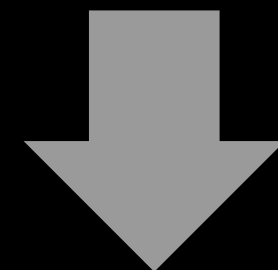


210 180 150 120 90 60 30 0 [nm]

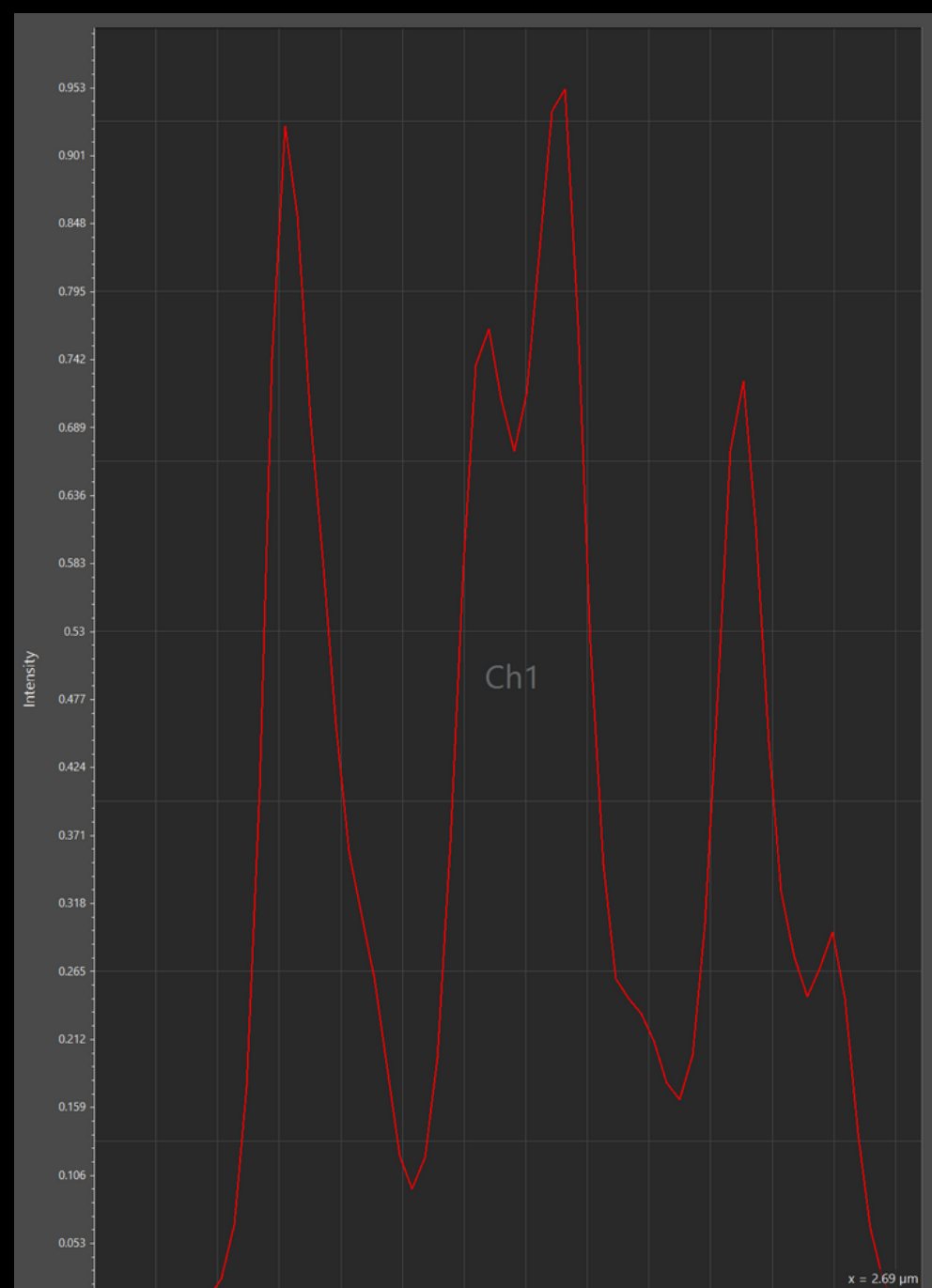


# The resolution estimation

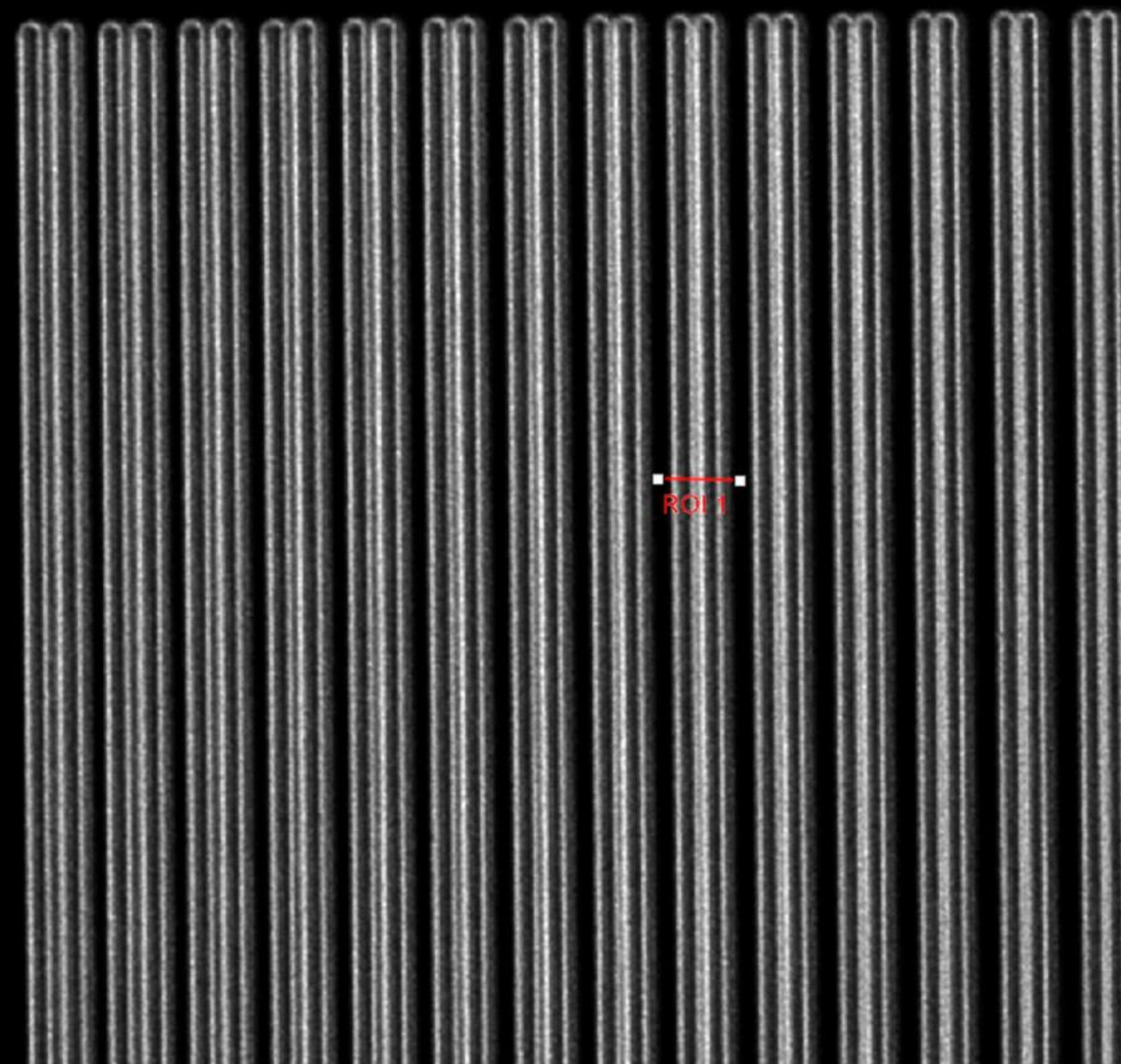
Argolight slide SIM pattern



Pixel size 43 nm, pinhole 1AU



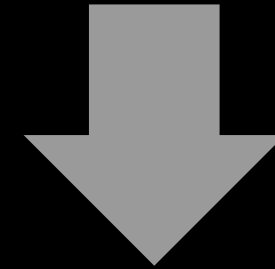
210 180 150 120 90 60 30 0 [nm]



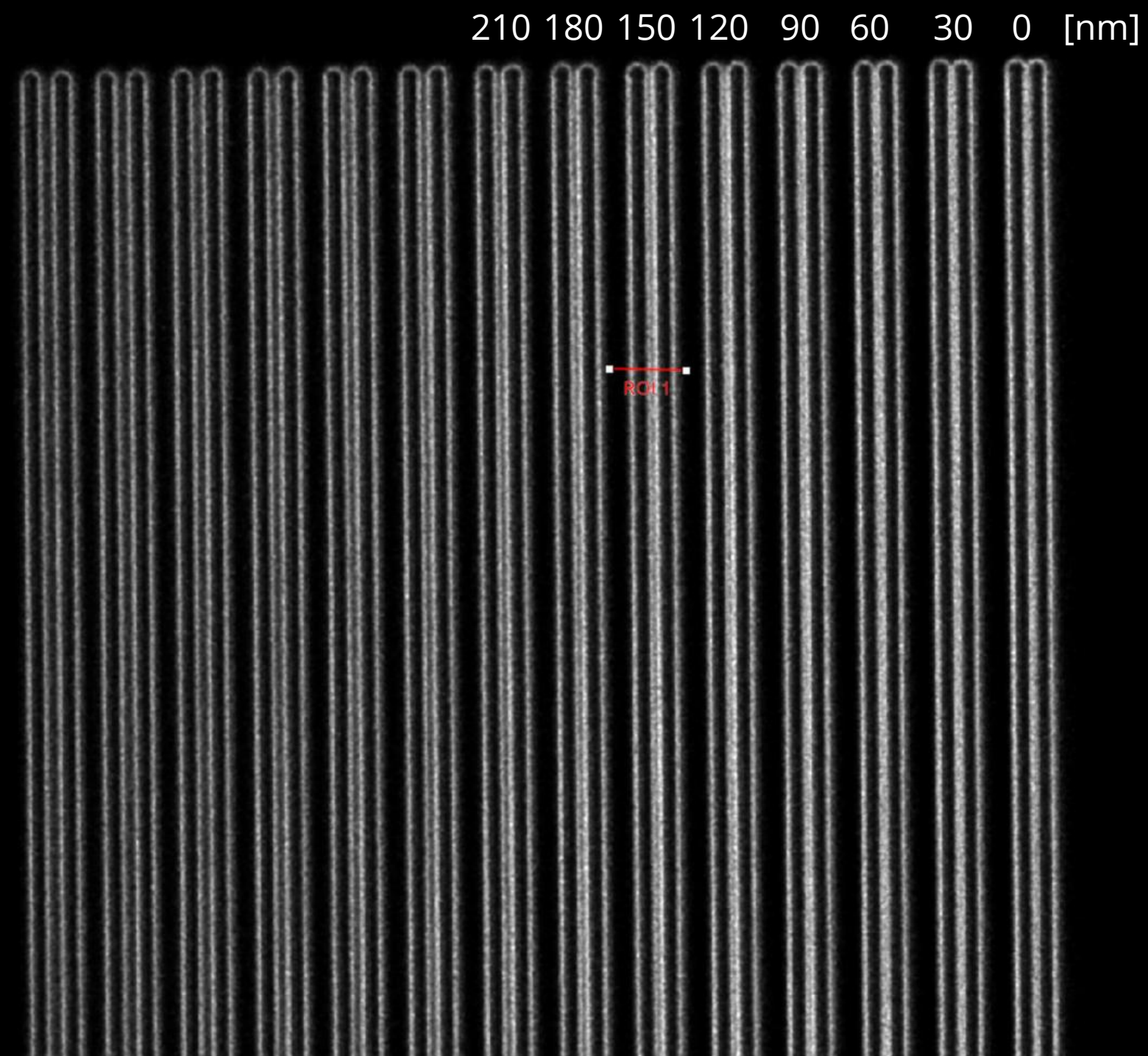
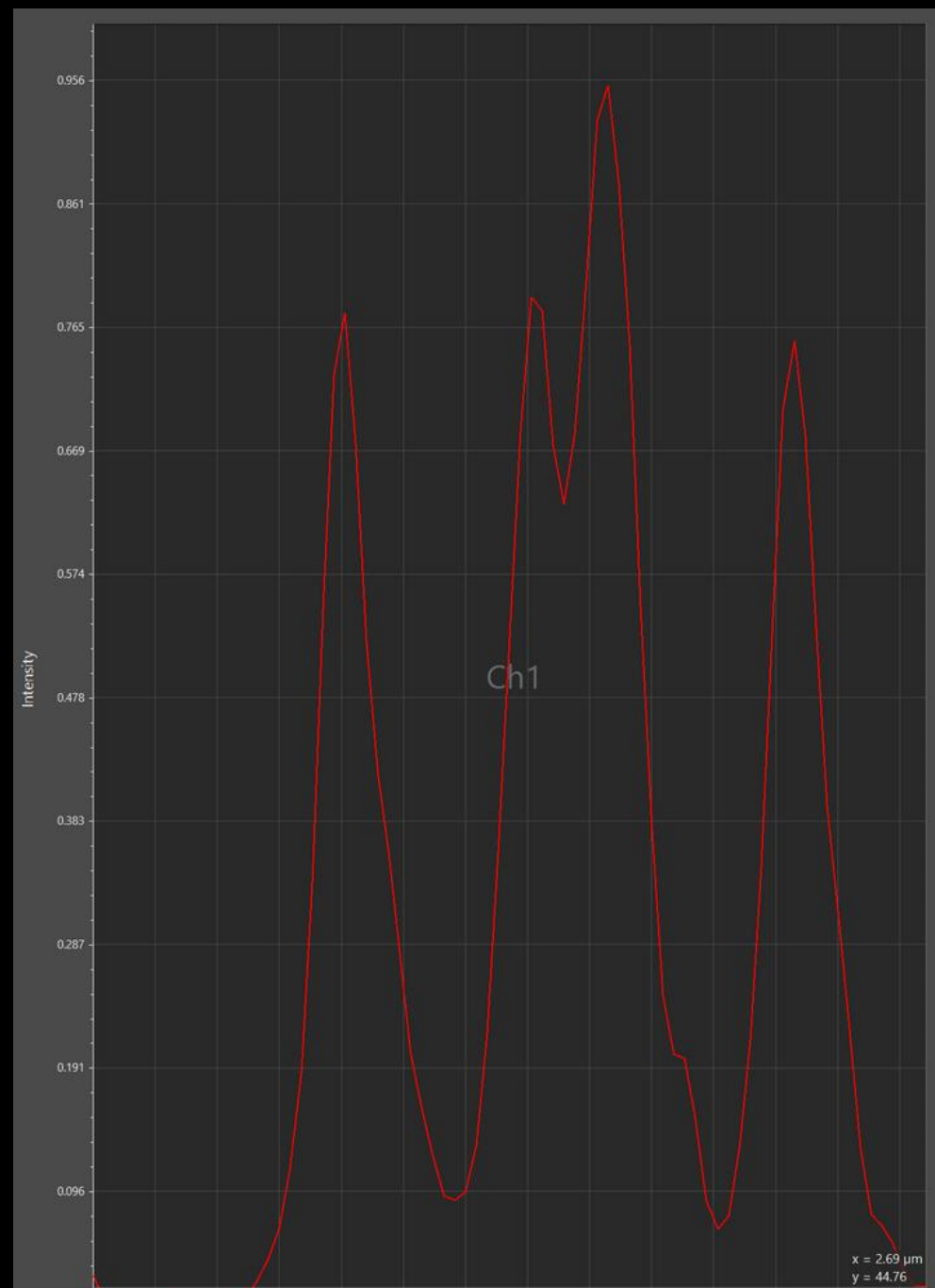


# The resolution estimation

Argolight slide SIM pattern



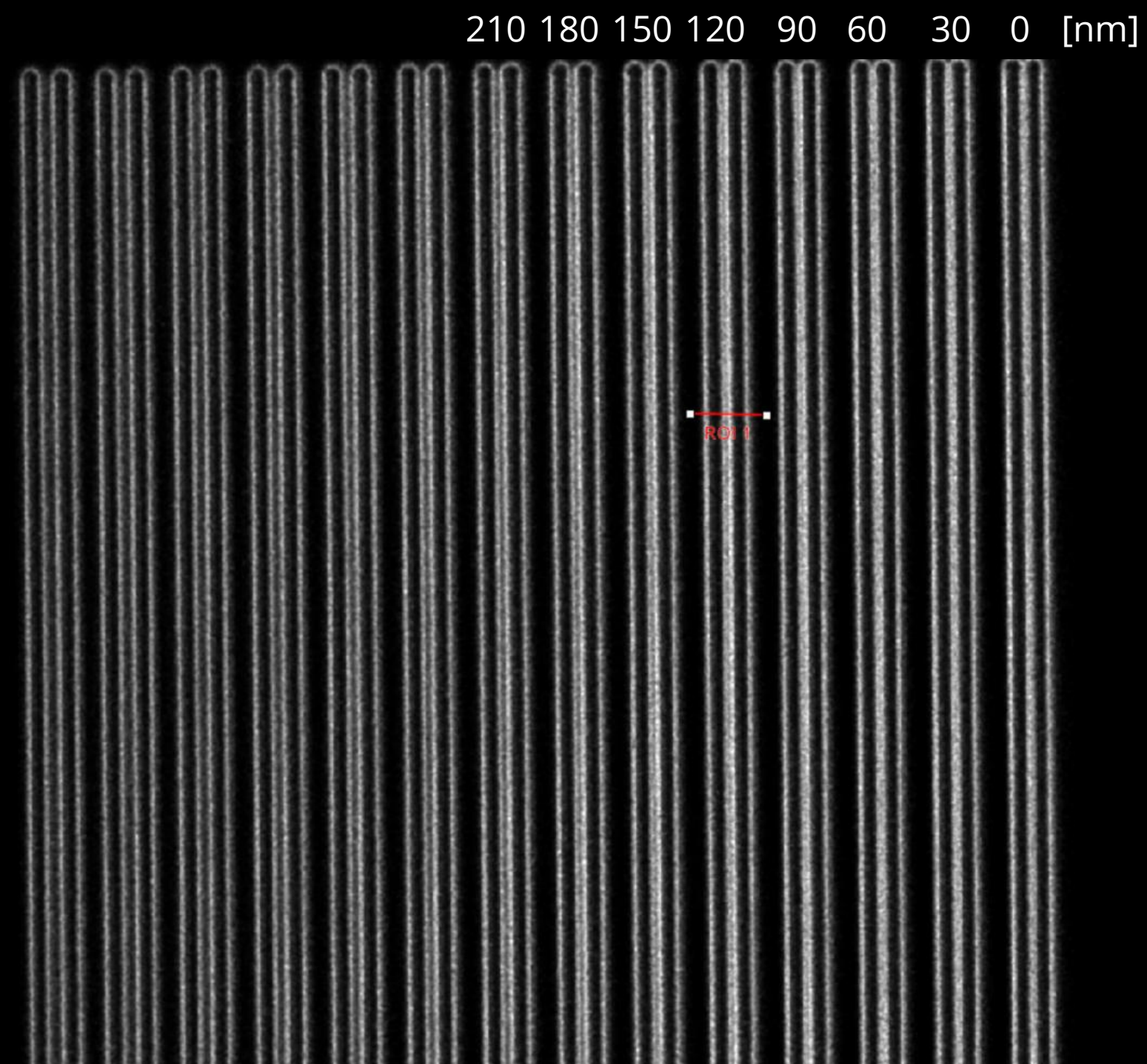
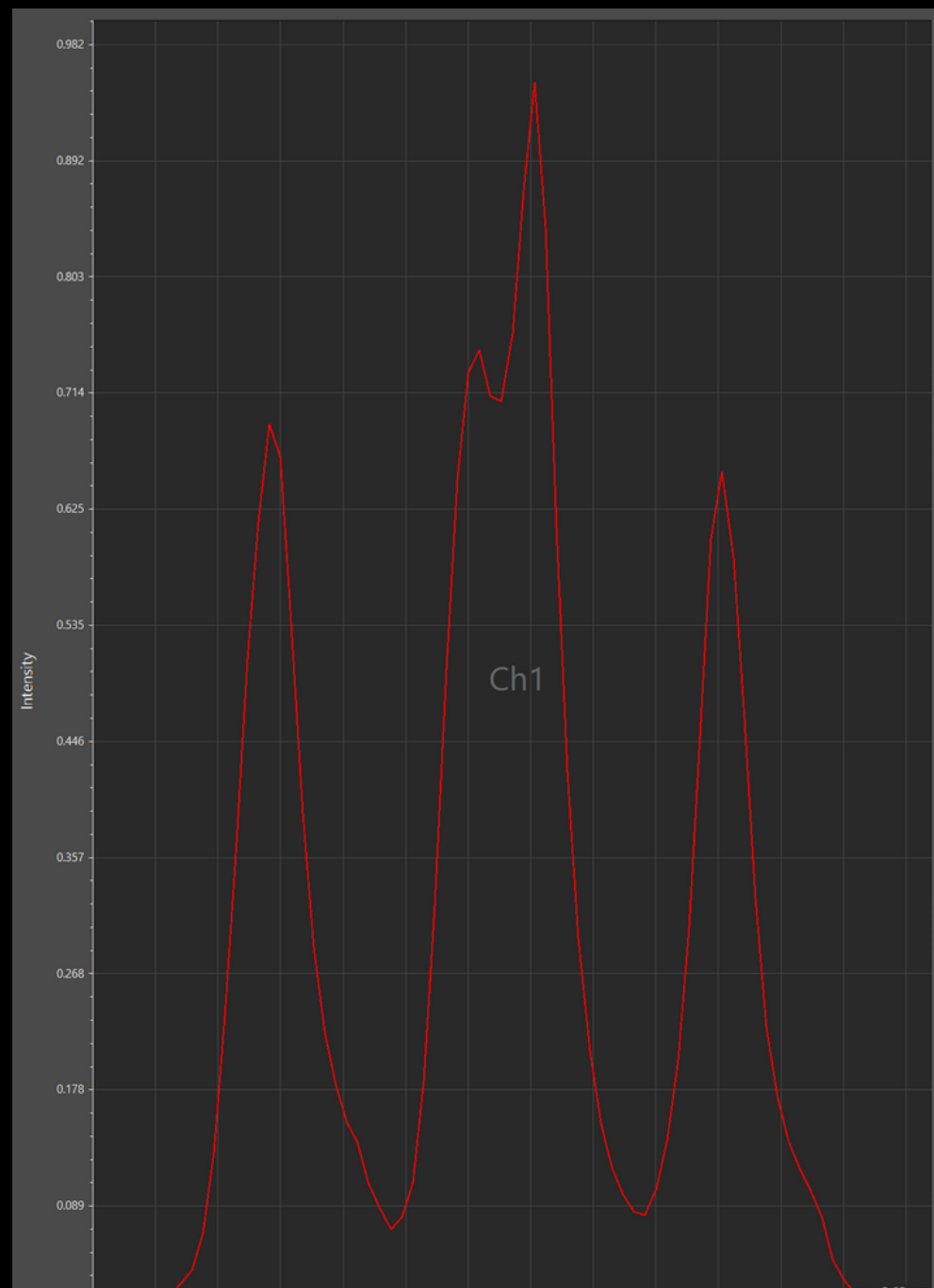
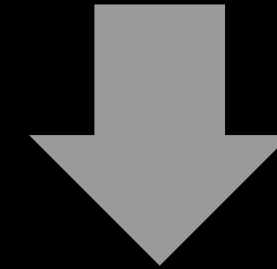
Pixel size 35 nm, pinhole 0.6 AU



# The resolution estimation

Argolight slide SIM pattern

Pixel size 35 nm, pinhole 0.6 AU







# Thank you for the attention

Pixel size 35 nm, pinhole 0.6 AU

Pixel size 35 nm, pinhole 0.6 AU,  
Huygens deconvolution