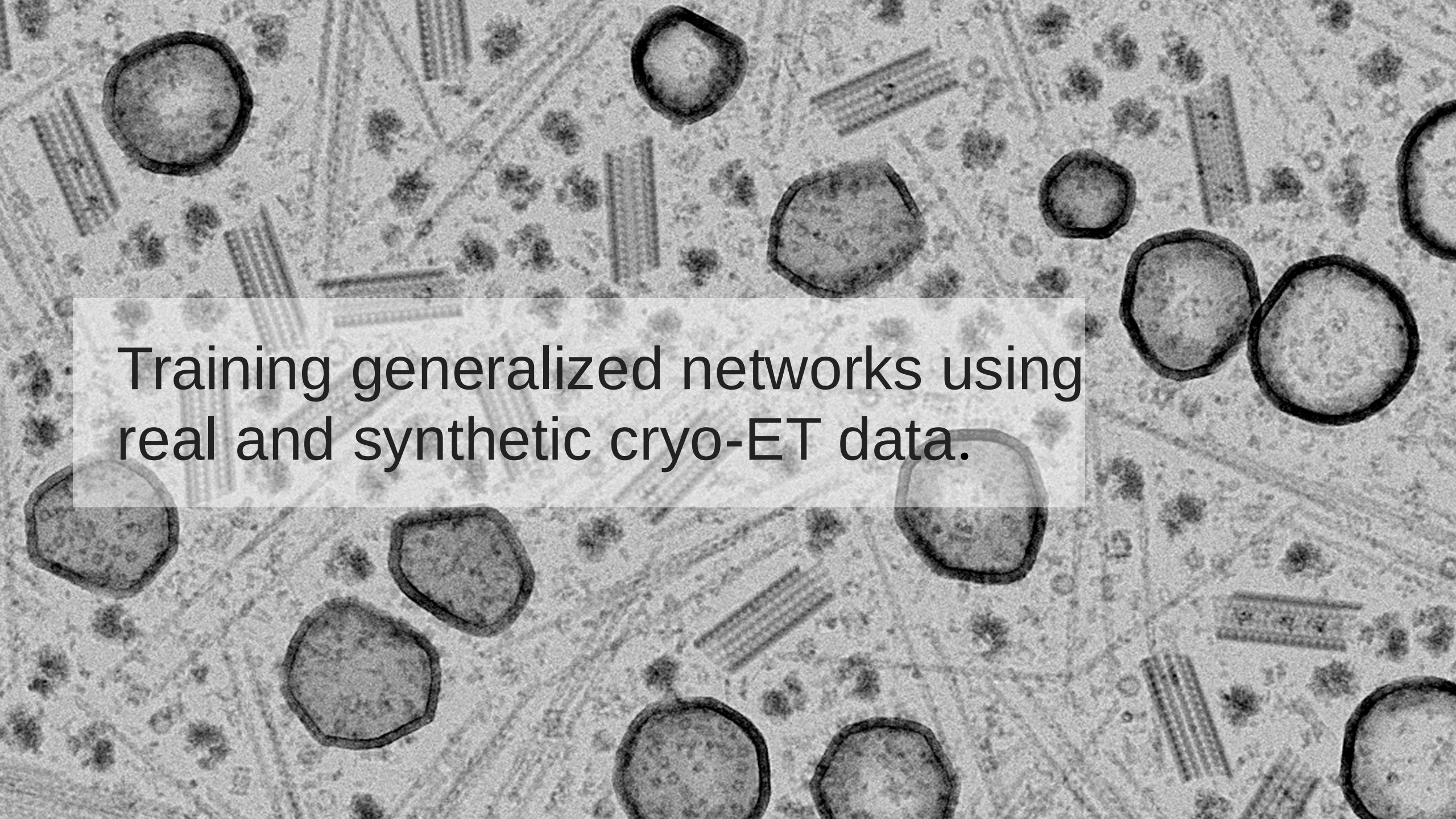
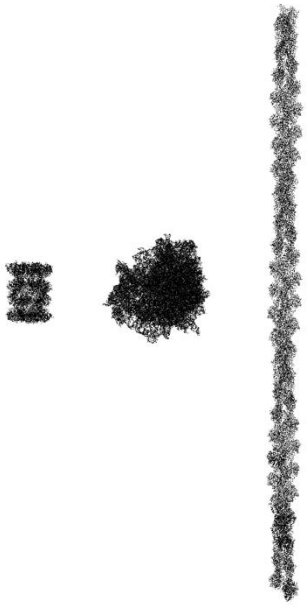
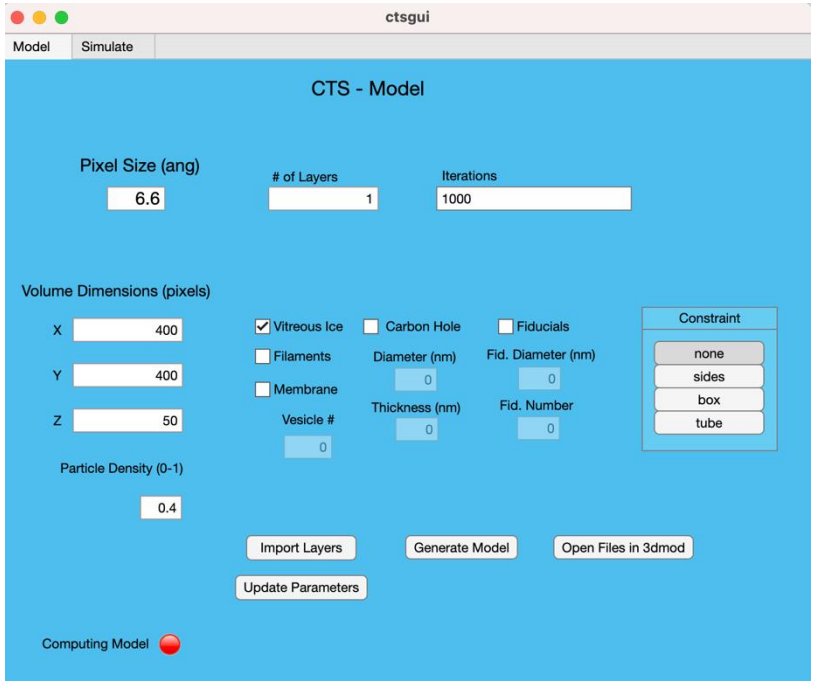

Training generalized networks using real and synthetic cryo-ET data.

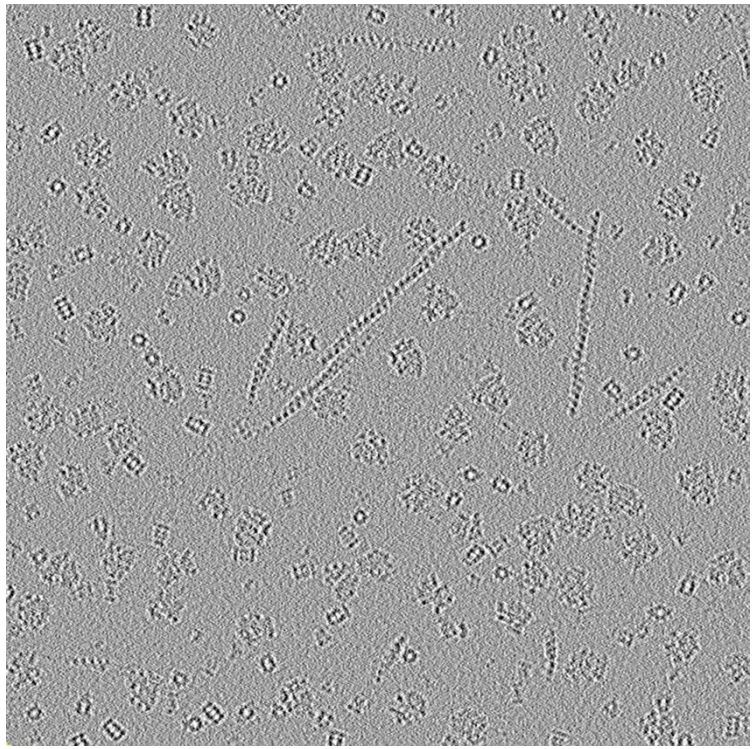
Atomic Models

CryoTomoSim

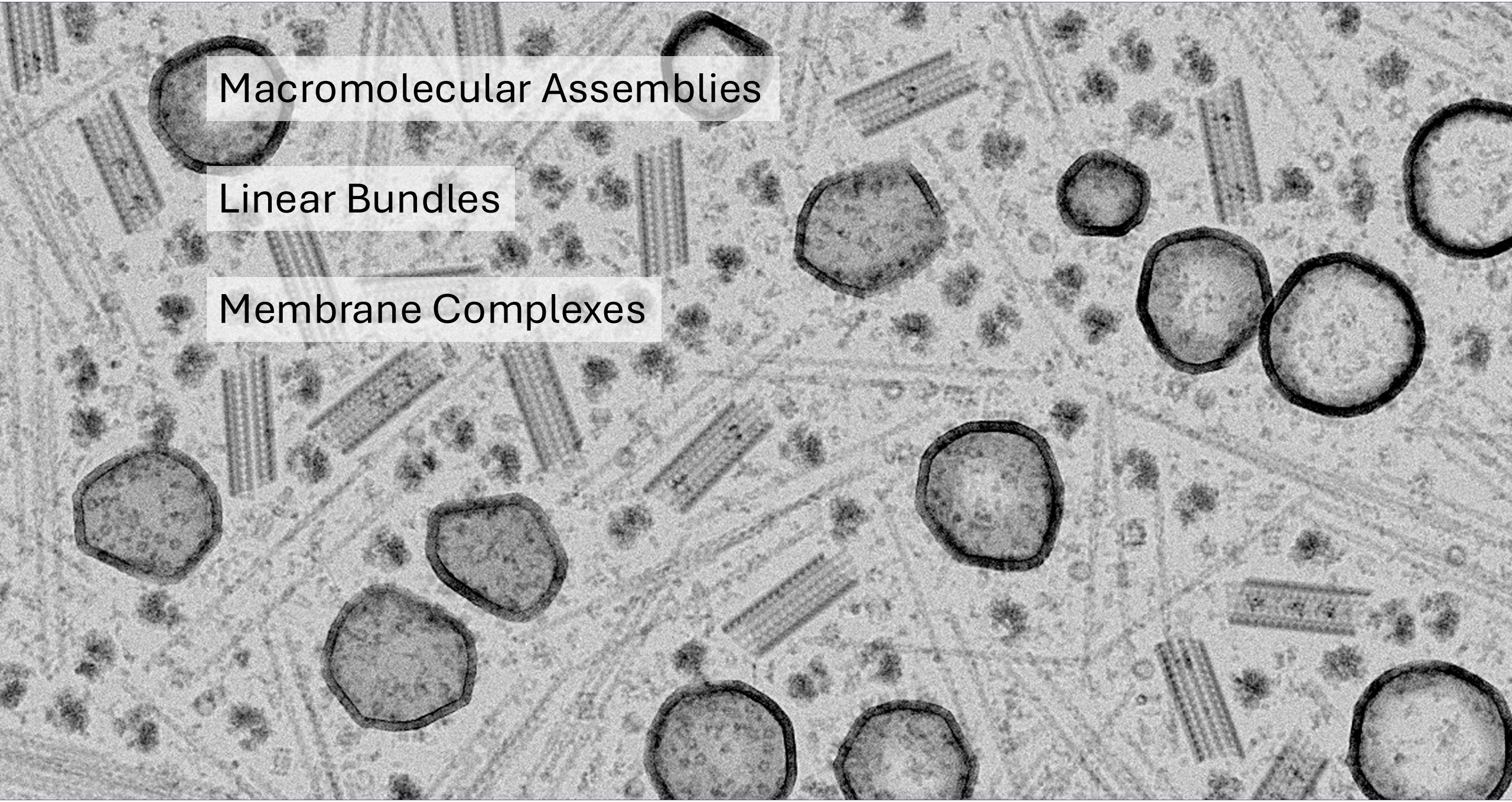
Synthetic Tomogram

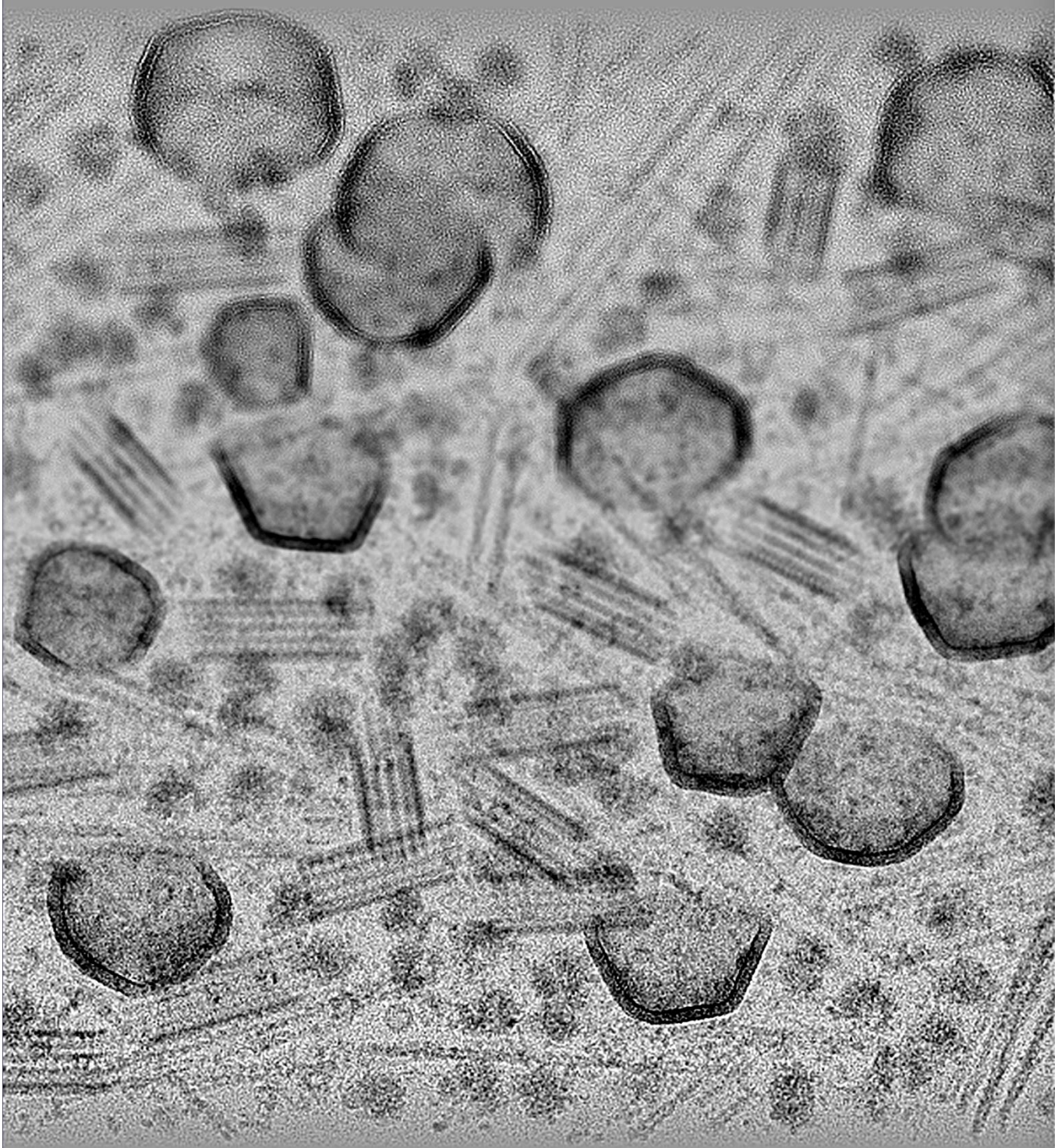


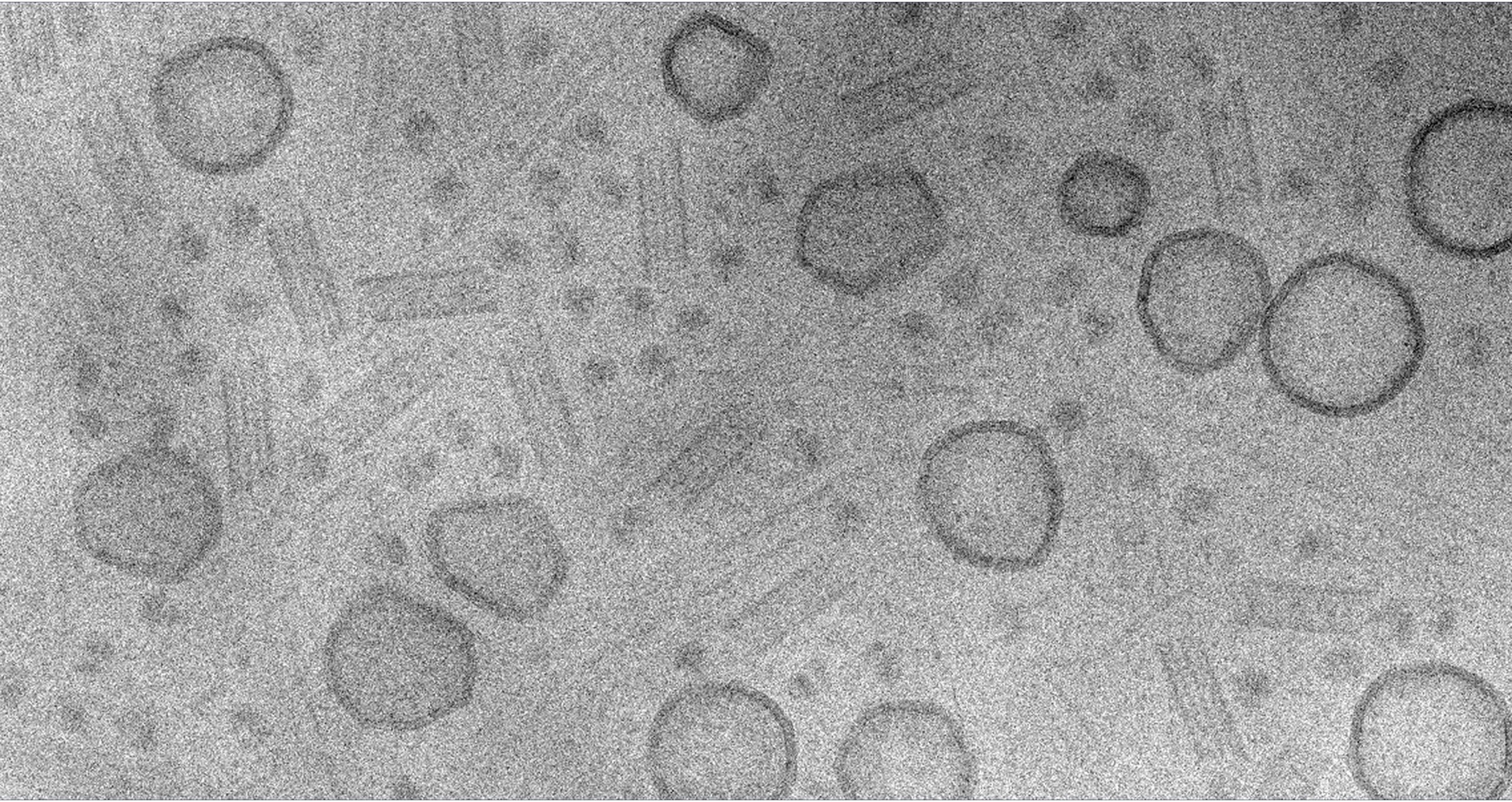


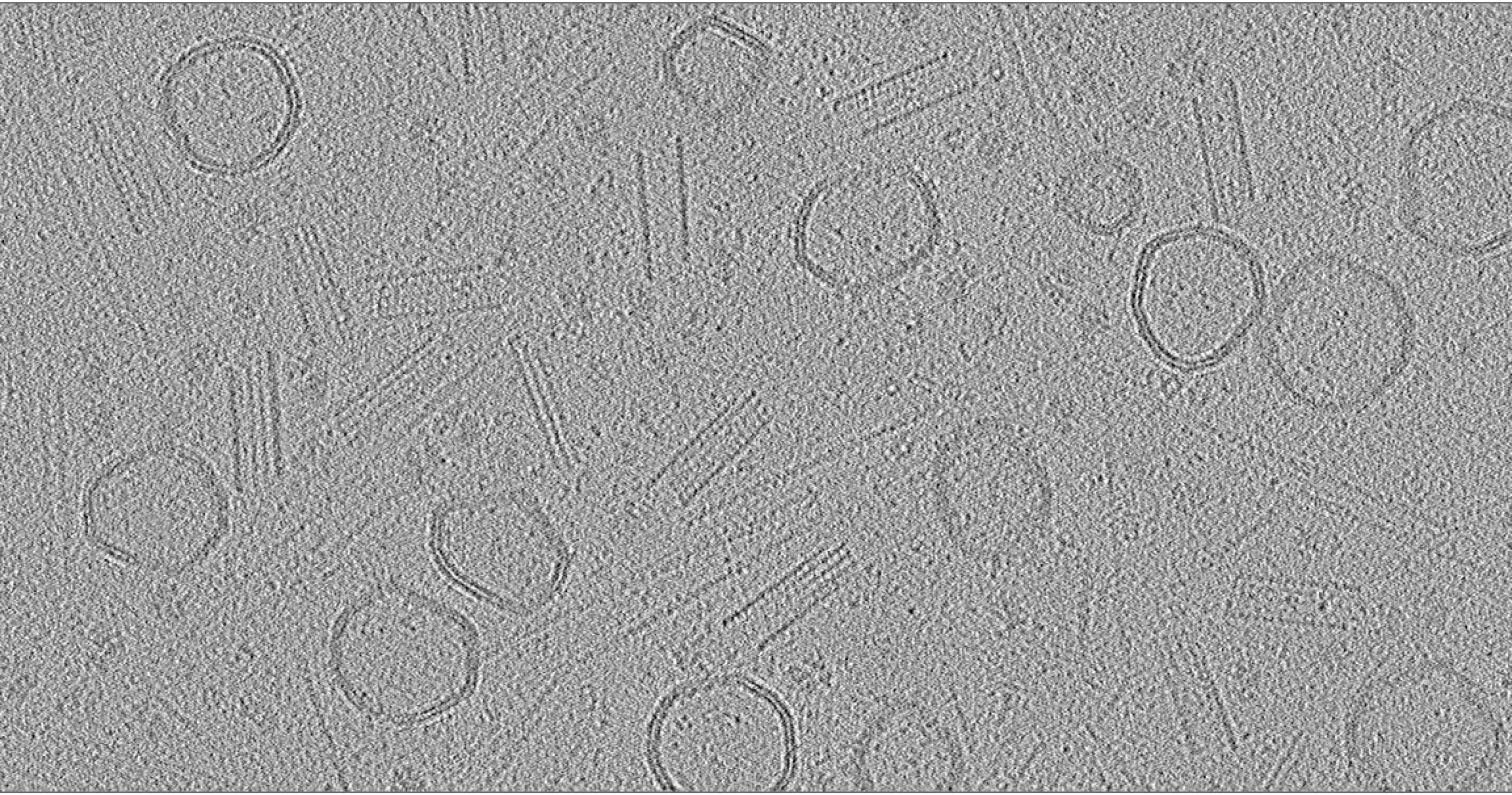


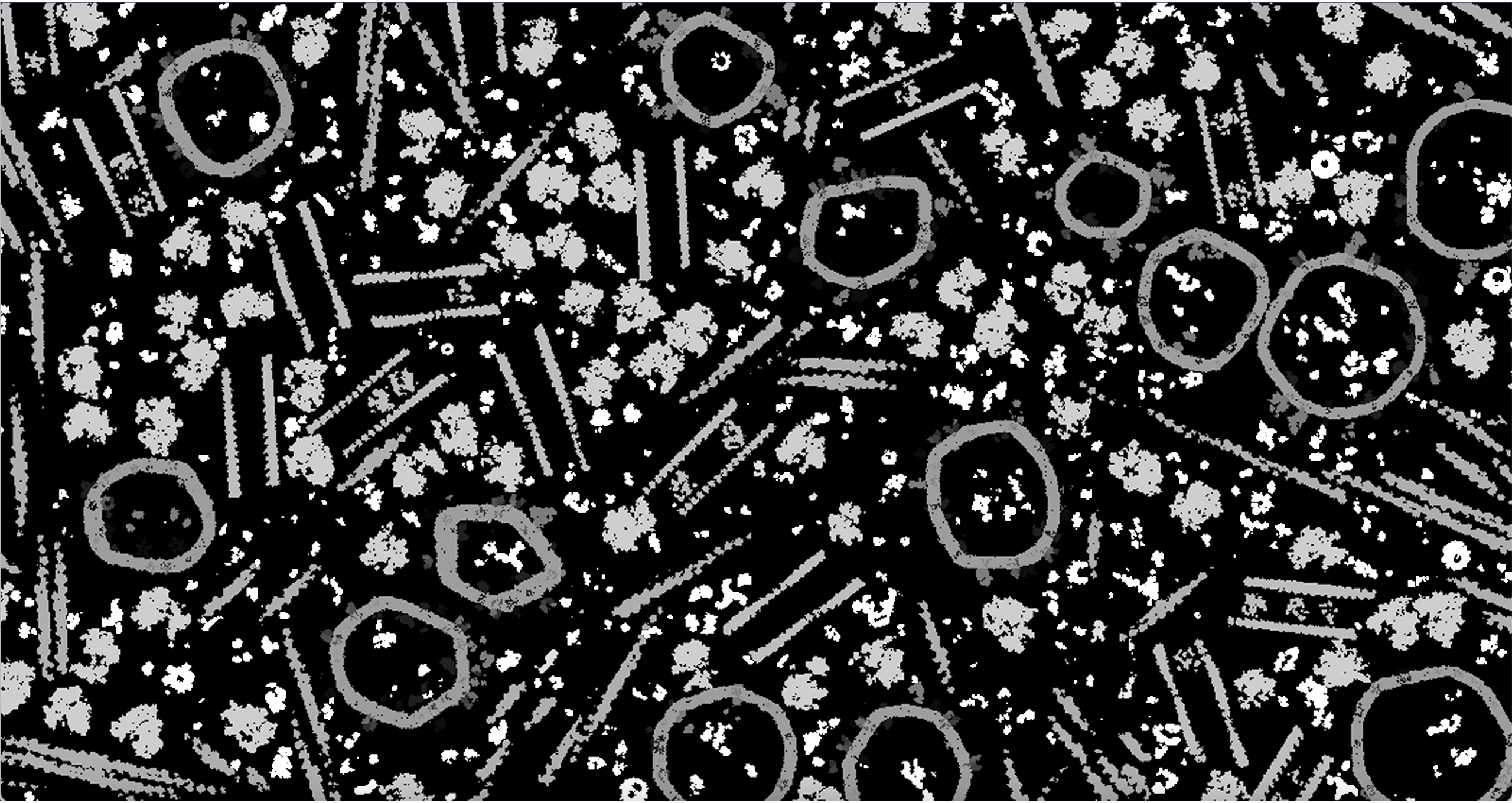
https://github.com/carsonpurnell/cryotomosim_CTS


Macromolecular Assemblies
Linear Bundles
Membrane Complexes









Segmentation

Typical Deep Learning Parameters

All models were trained in Dragonfly as either semantic segmentation or regression U-Nets

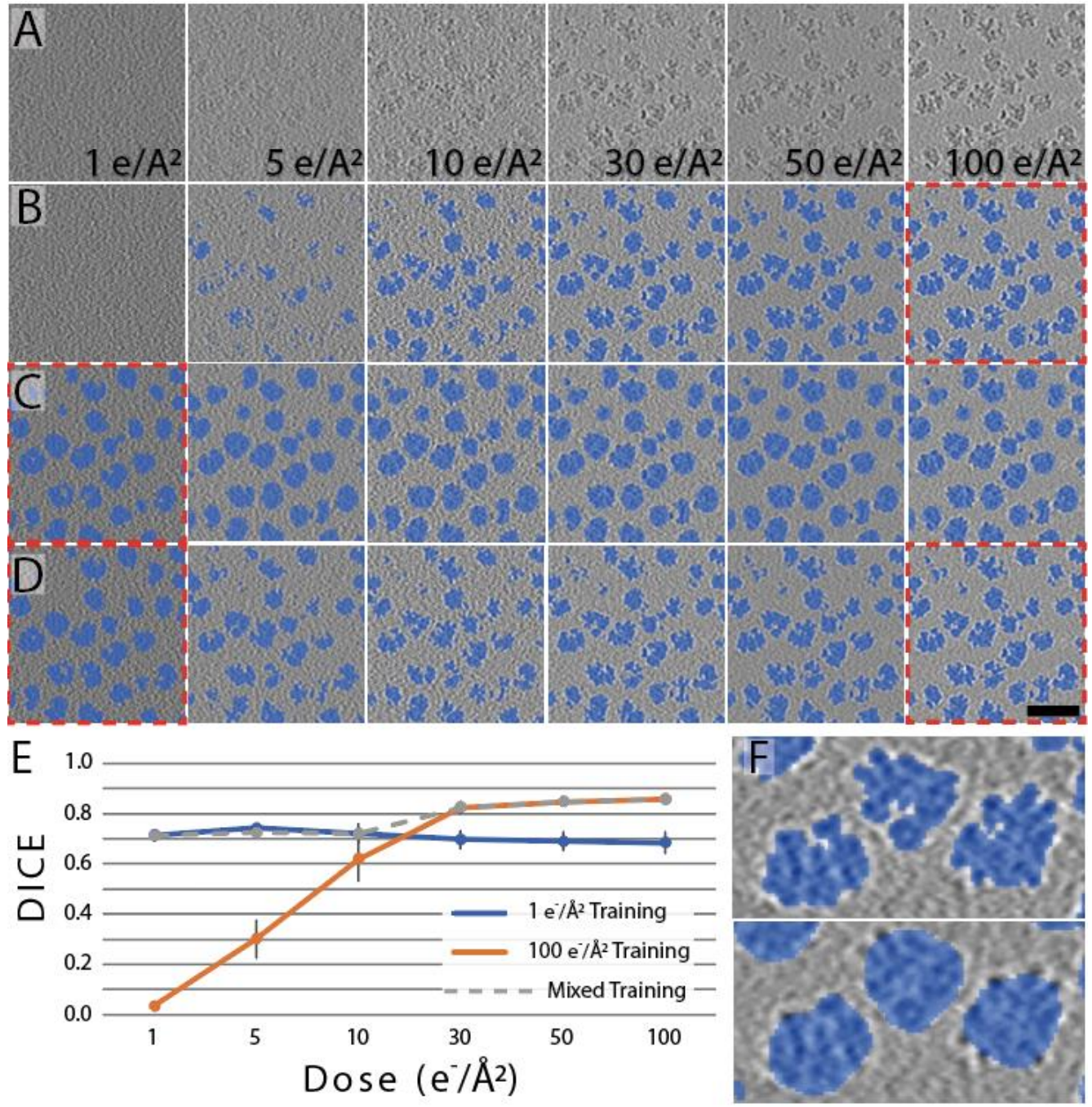
Patch Size: 128x128x11

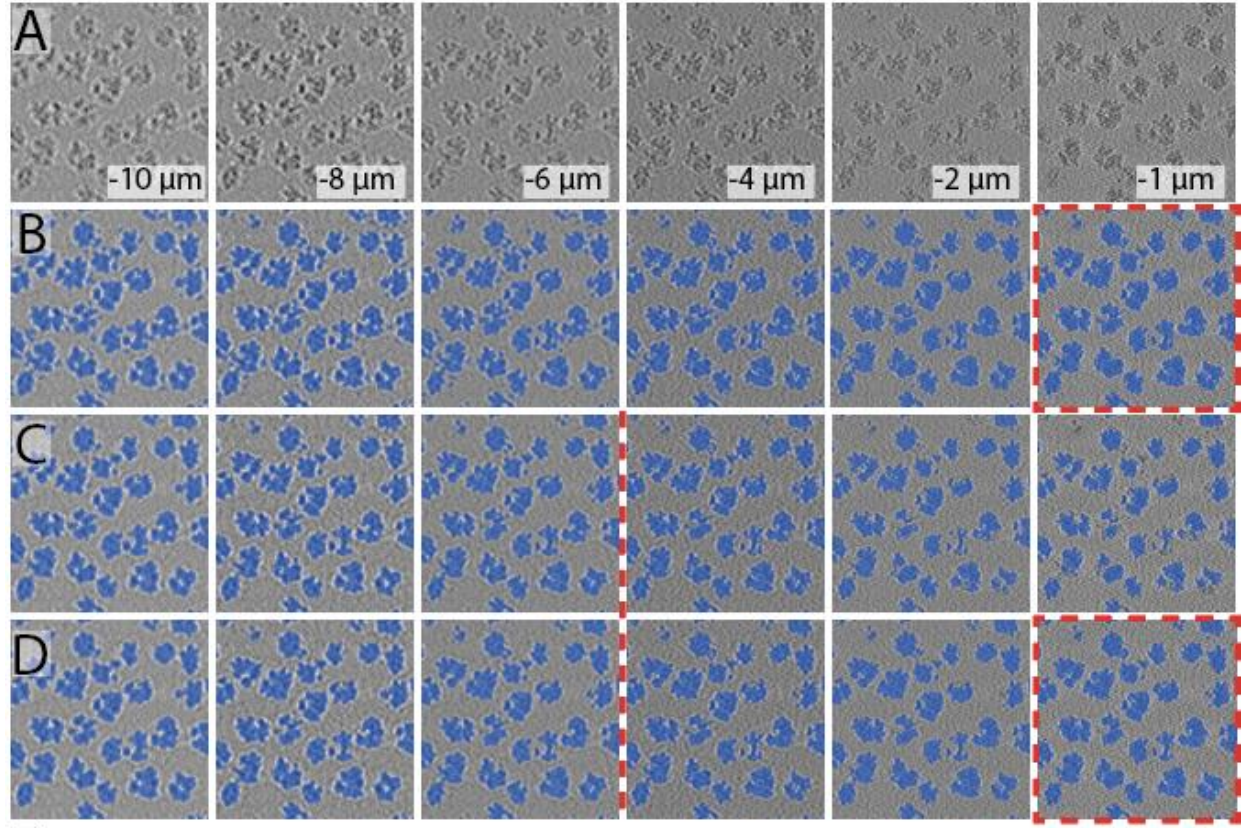
Batch Size: 8

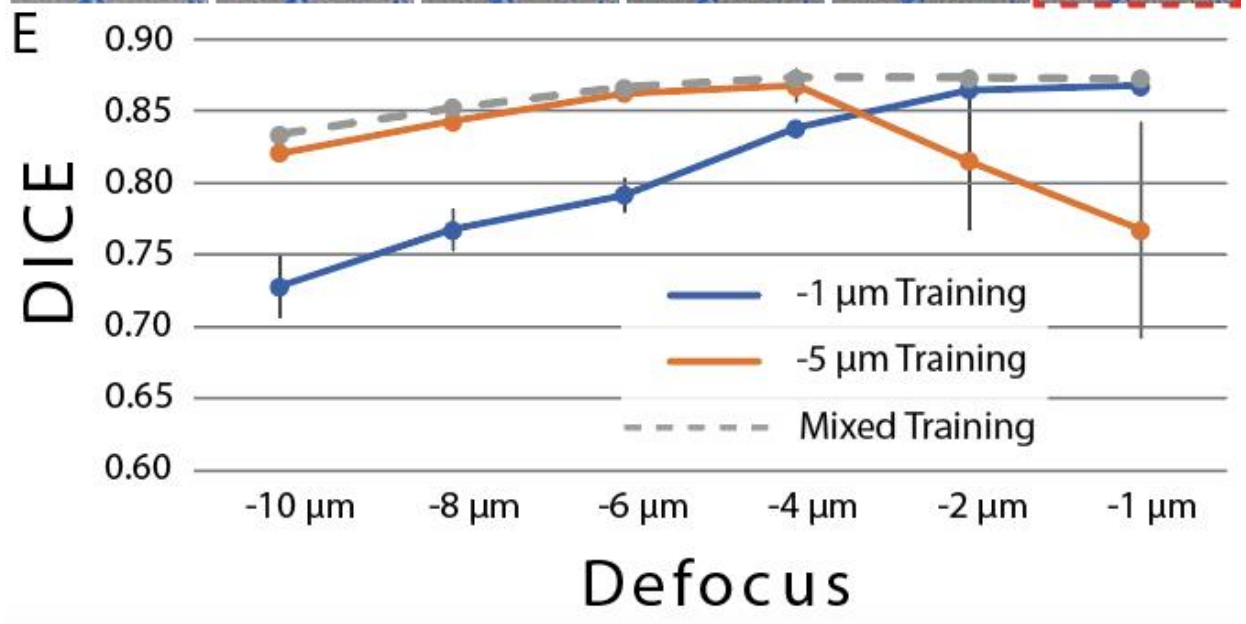
Loss: Categorical Cross Entropy (segmentation)
Mixed Gradient Loss (regression)

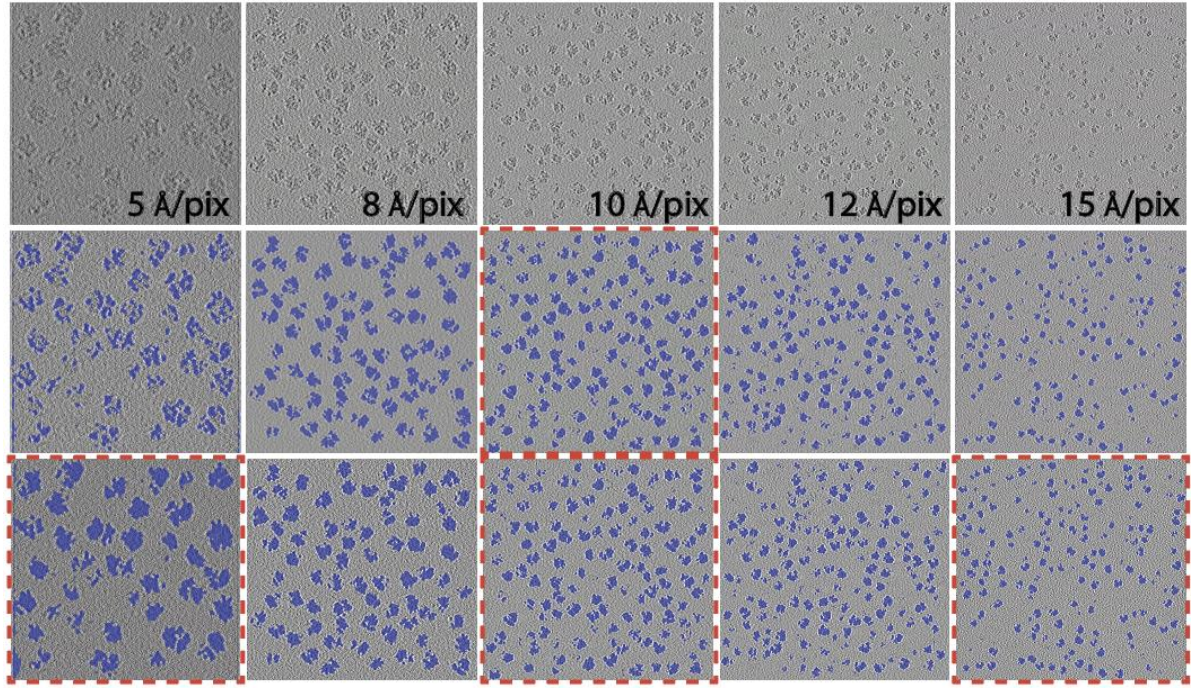
Optimization: Adadelta

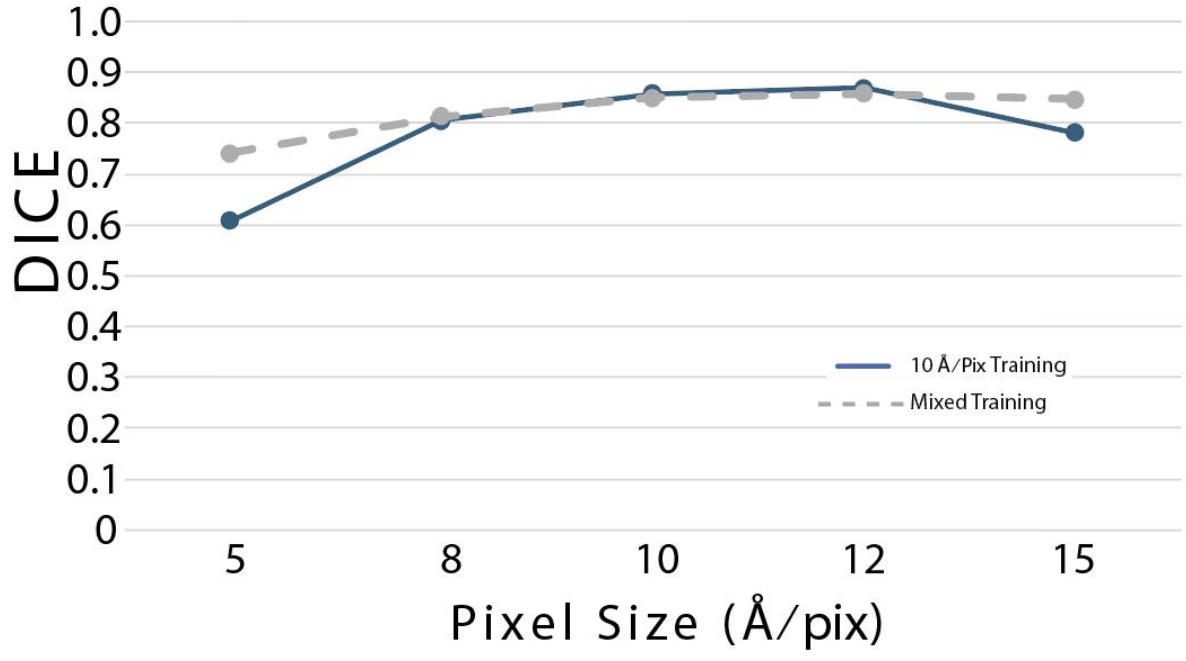
Patience: 15 epochs

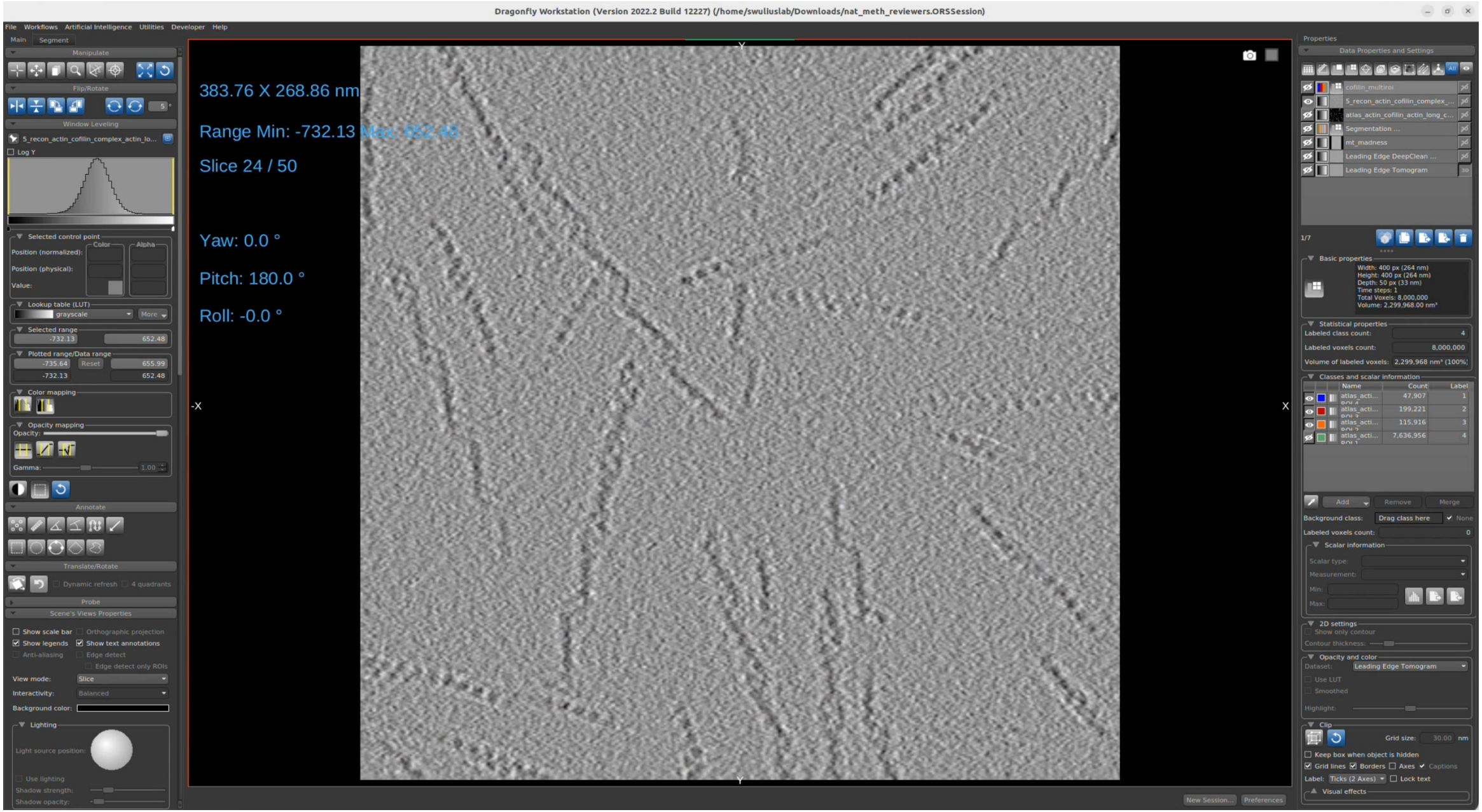

A
1 e/A²
5 e/A²
10 e/A²
30 e/A²
50 e/A²
100 e/A²
B
C
D
E
DICE
1.0
0.8
0.6
0.4
0.2
0.0
1 e⁻/Å² Training
100 e⁻/Å² Training
Mixed Training
1
5
10
30
50
100
Dose (e⁻/Å²)
F

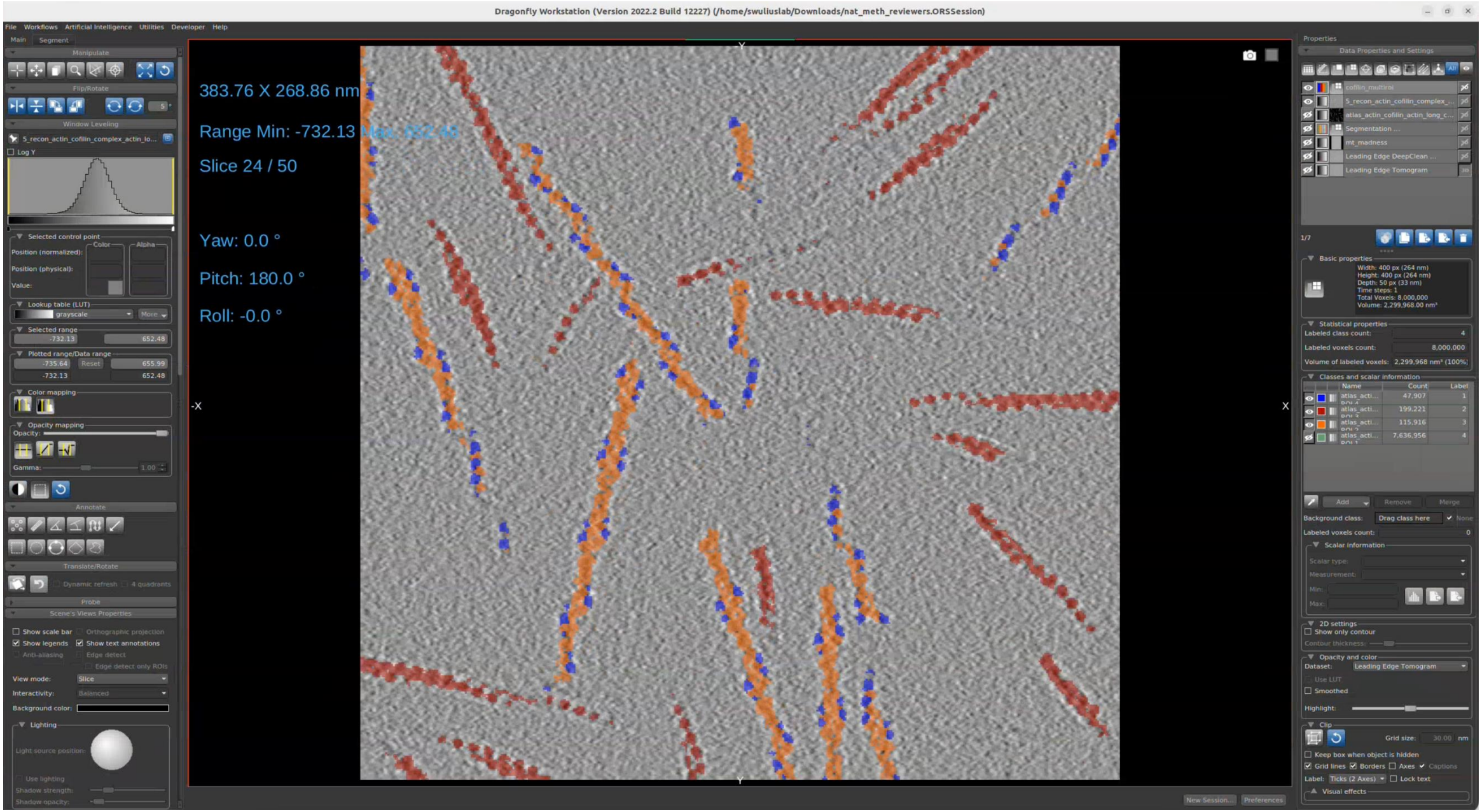

A
-10 µm
-8 µm
-6 µm
-4 µm
-2 µm
-1 µm
B
C
D

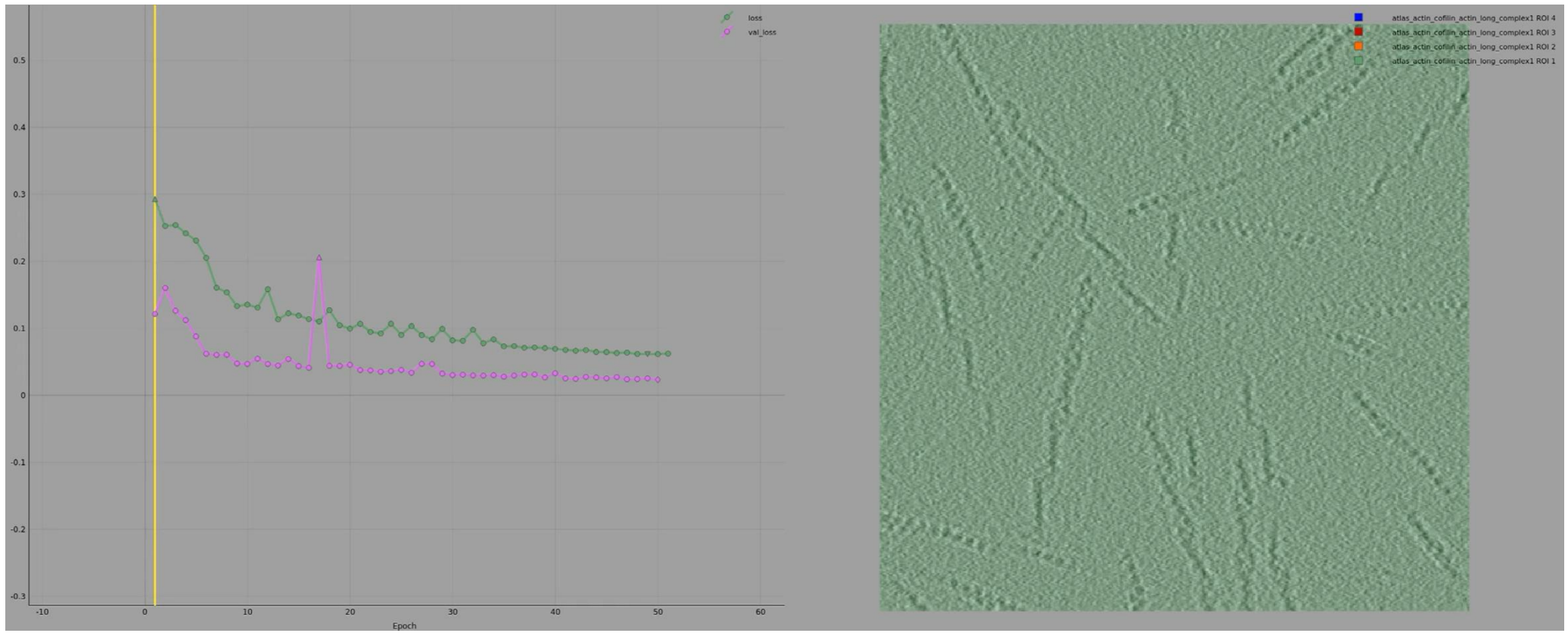

E
0.90
0.85
0.80
0.75
0.70
0.65
0.60
DICE
-1 µm Training
-5 µm Training
Mixed Training
-10 µm
-8 µm
-6 µm
-4 µm
-2 µm
-1 µm
Defocus

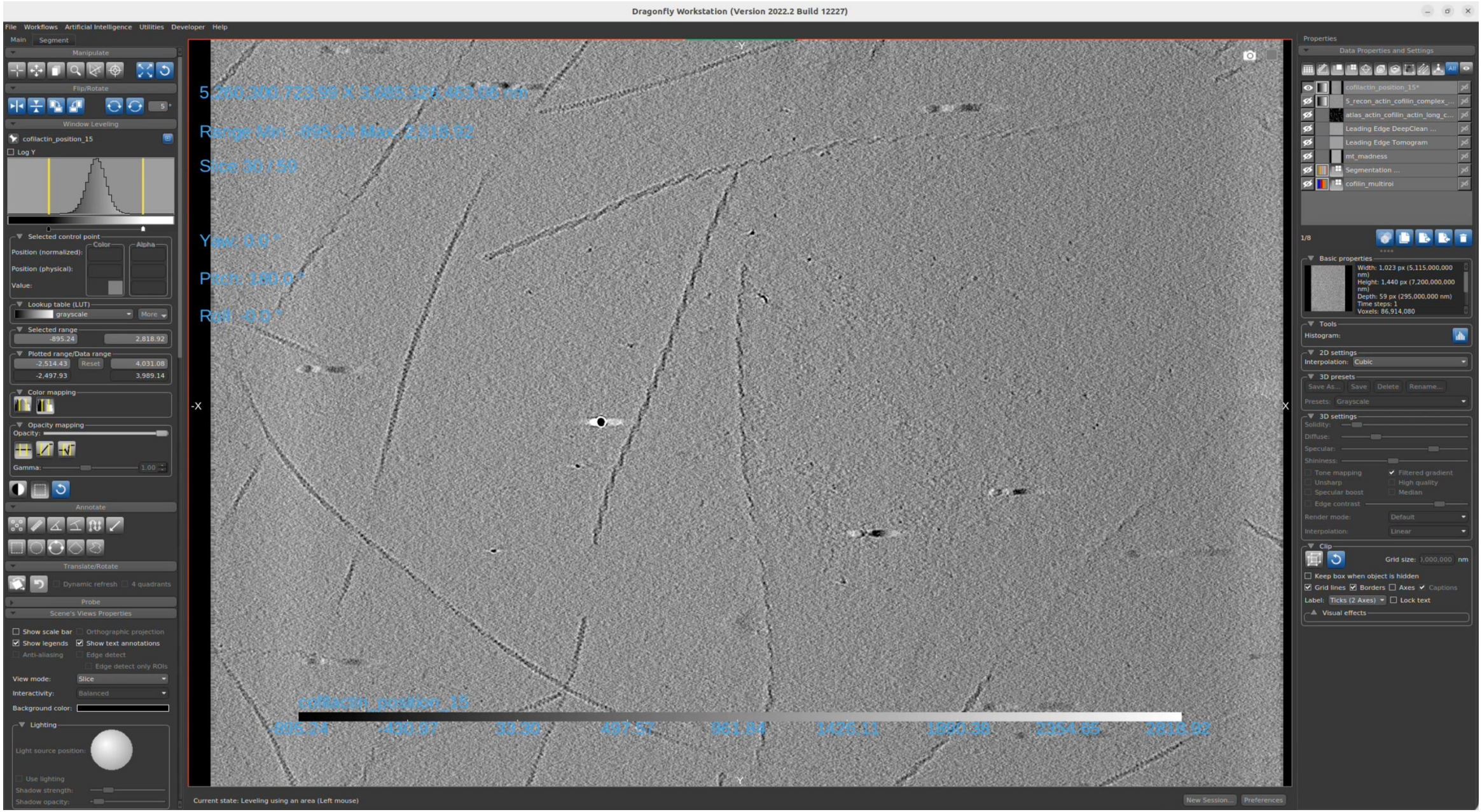

5 Å/pix
8 Å/pix
10 Å/pix
12 Å/pix
15 Å/pix

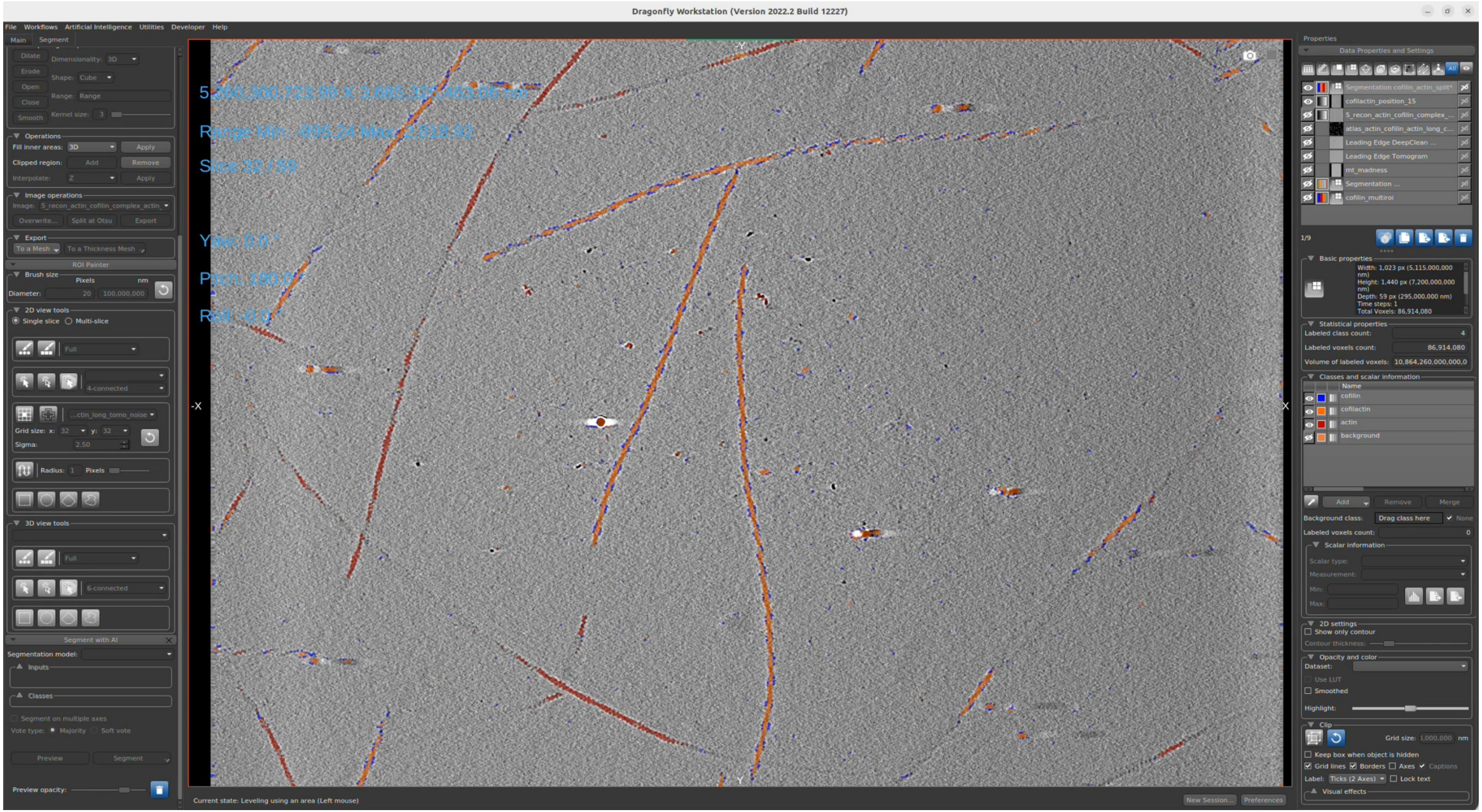

DICE
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
5
8
10
12
15
Pixel Size (Å/pix)
10 Å/Pix Training
Mixed Training

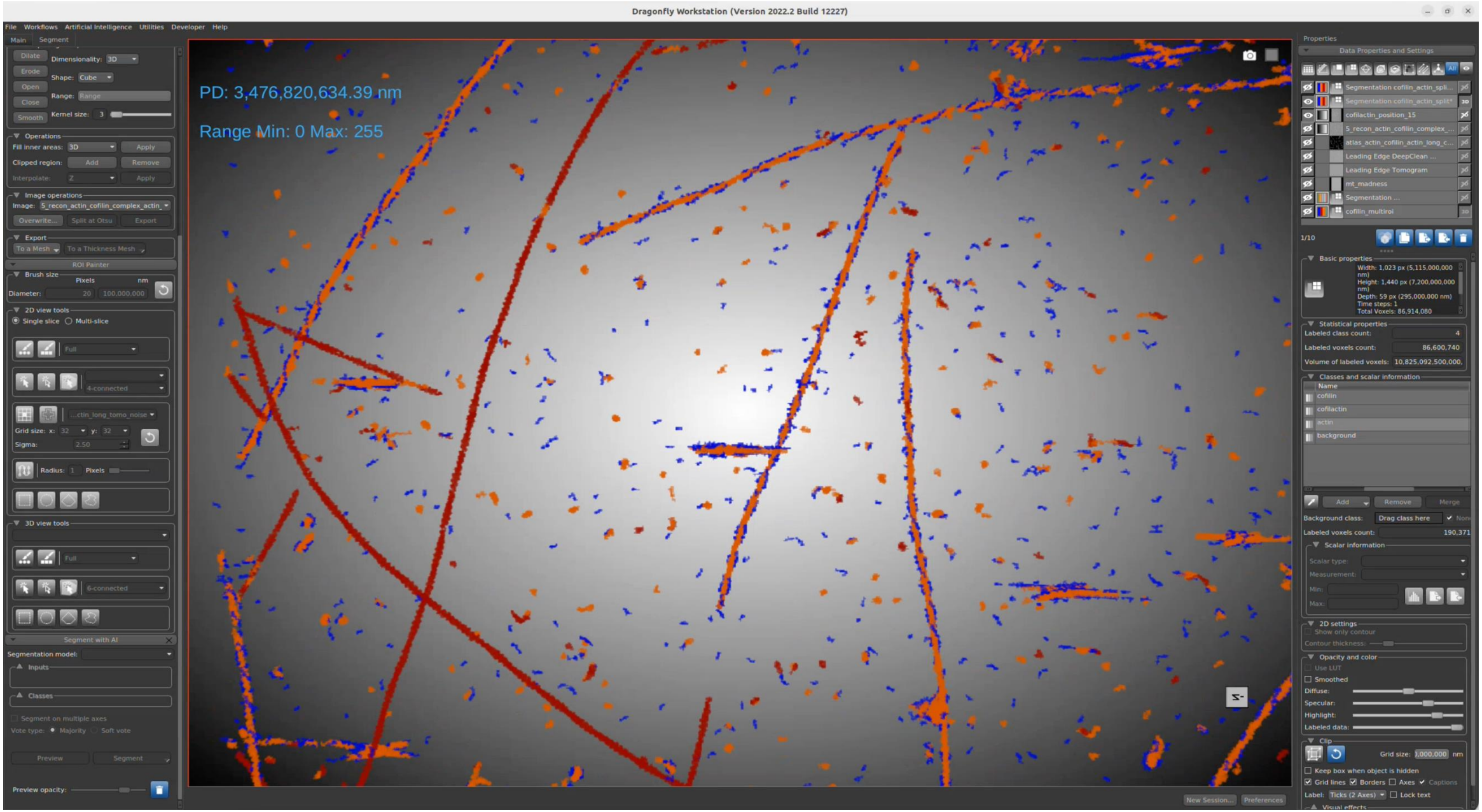

Dragonfly Workstation (Version 2022.2 Build 12227) (/home/swuliuslab/Downloads/nat_meth_reviewers.ORSSession)
File Workflows Artificial Intelligence Utilities Developer Help
Main Segment
Manipulate
Flip/Rotate
Window Leveling
5_recon_actin_cofilin_complex_actin_lo...
Log Y
Selected control point
Position (normalized):
Position (physical):
Value:
Color
Alpha
Lookup table (LUT)
grayscale
More
Selected range
-732.13
652.48
Plotted range/Data range
-735.64
Reset
655.99
-732.13
652.48
Color mapping
Opacity mapping
Opacity:
Gamma:
1.00
Annotate
Translate/Rotate
Dynamic refresh
4 quadrants
Probe
Scene's Views Properties
Show scale bar
Orthographic projection
Show legends
Show text annotations
Anti-aliasing
Edge detect
Edge detect only ROIs
View mode:
Slice
Interactivity:
Balanced
Background color:
Lighting
Light source position:
Use lighting
Shadow strength:
Shadow opacity:
383.76 X 268.86 nm
Range Min: -732.13 Max: 652.48
Slice 24 / 50
Yaw: 0.0 °
Pitch: 180.0 °
Roll: -0.0 °
Y
-X
X
Y
New Session...
Preferences
Properties
Data Properties and Settings
All
cofilin_multiroi
5_recon_actin_cofilin_complex_...
atlas_actin_cofilin_actin_long_c...
Segmentation ...
mt_madness
Leading Edge DeepClean ...
Leading Edge Tomogram
3D
1/7
Basic properties
Width: 400 px (264 nm)
Height: 400 px (264 nm)
Depth: 50 px (33 nm)
Time steps: 1
Total Voxels: 8,000,000
Volume: 2,299,968.00 nm³
Statistical properties
Labeled class count: 4
Labeled voxels count: 8,000,000
Volume of labeled voxels: 2,299,968 nm³ (100%)
Classes and scalar information
Name
Count
Label
atlas_acti...
47,907
1
atlas_acti...
199,221
2
atlas_acti...
115,916
3
atlas_acti...
7,636,956
4
Add
Remove
Merge
Background class:
Drag class here
None
Labeled voxels count: 0
Scalar information
Scalar type:
Measurement:
Min:
Max:
2D settings
Show only contour
Contour thickness:
Opacity and color
Dataset:
Leading Edge Tomogram
Use LUT
Smoothed
Highlight:
Clip
Grid size:
30.00
nm
Keep box when object is hidden
Grid lines
Borders
Axes
Captions
Label:
Ticks (2 Axes)
Lock text
Visual effects

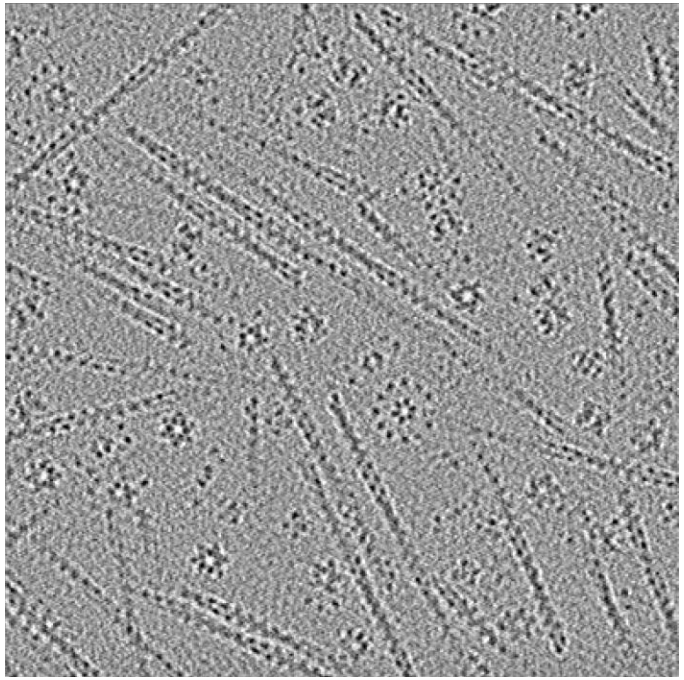

Dragonfly Workstation (Version 2022.2 Build 12227) (/home/swuliuslab/Downloads/nat_meth_reviewers.ORSSession)
File Workflows Artificial Intelligence Utilities Developer Help
Main Segment
Manipulate
Flip/Rotate
Window Leveling
5_recon_actin_cofilin_complex_actin_io...
Log Y
Selected control point
Position (normalized):
Position (physical):
Value:
Color
Alpha
Lookup table (LUT)
grayscale
More
Selected range
-732.13
652.48
Plotted range/Data range
-735.64
Reset
655.99
-732.13
652.48
Color mapping
Opacity mapping
Opacity:
Gamma:
1.00
Annotate
Translate/Rotate
Dynamic refresh
4 quadrants
Probe
Scene's Views Properties
Show scale bar
Orthographic projection
Show legends
Show text annotations
Anti-aliasing
Edge detect
Edge detect only ROIs
View mode:
Slice
Interactivity:
Balanced
Background color:
Lighting
Light source position:
Use lighting
Shadow strength:
Shadow opacity:
383.76 X 268.86 nm
Range Min: -732.13 Max: 652.48
Slice 24 / 50
Yaw: 0.0 °
Pitch: 180.0 °
Roll: -0.0 °
Y
-X
X
Y
New Session...
Preferences
Properties
Data Properties and Settings
cofilin_multiroi
5_recon_actin_cofilin_complex_...
atlas_actin_cofilin_actin_long_c...
Segmentation ...
mt_madness
Leading Edge DeepClean ...
Leading Edge Tomogram
1/7
Basic properties
Width: 400 px (264 nm)
Height: 400 px (264 nm)
Depth: 50 px (33 nm)
Time steps: 1
Total Voxels: 8,000,000
Volume: 2,299,968.00 nm³
Statistical properties
Labeled class count: 4
Labeled voxels count: 8,000,000
Volume of labeled voxels: 2,299,968 nm³ (100%)
Classes and scalar information
Name
Count
Label
atlas_acti...
47,907
1
atlas_acti...
199,221
2
atlas_acti...
115,916
3
atlas_acti...
7,636,956
4
Add
Remove
Merge
Background class:
Drag class here
None
Labeled voxels count: 0
Scalar information
Scalar type:
Measurement:
Min:
Max:
2D settings
Show only contour
Contour thickness:
Opacity and color
Dataset:
Leading Edge Tomogram
Use LUT
Smoothed
Highlight:
Clip
Grid size:
30.00
nm
Keep box when object is hidden
Grid lines
Borders
Axes
Captions
Label:
Ticks (2 Axes)
Lock text
Visual effects

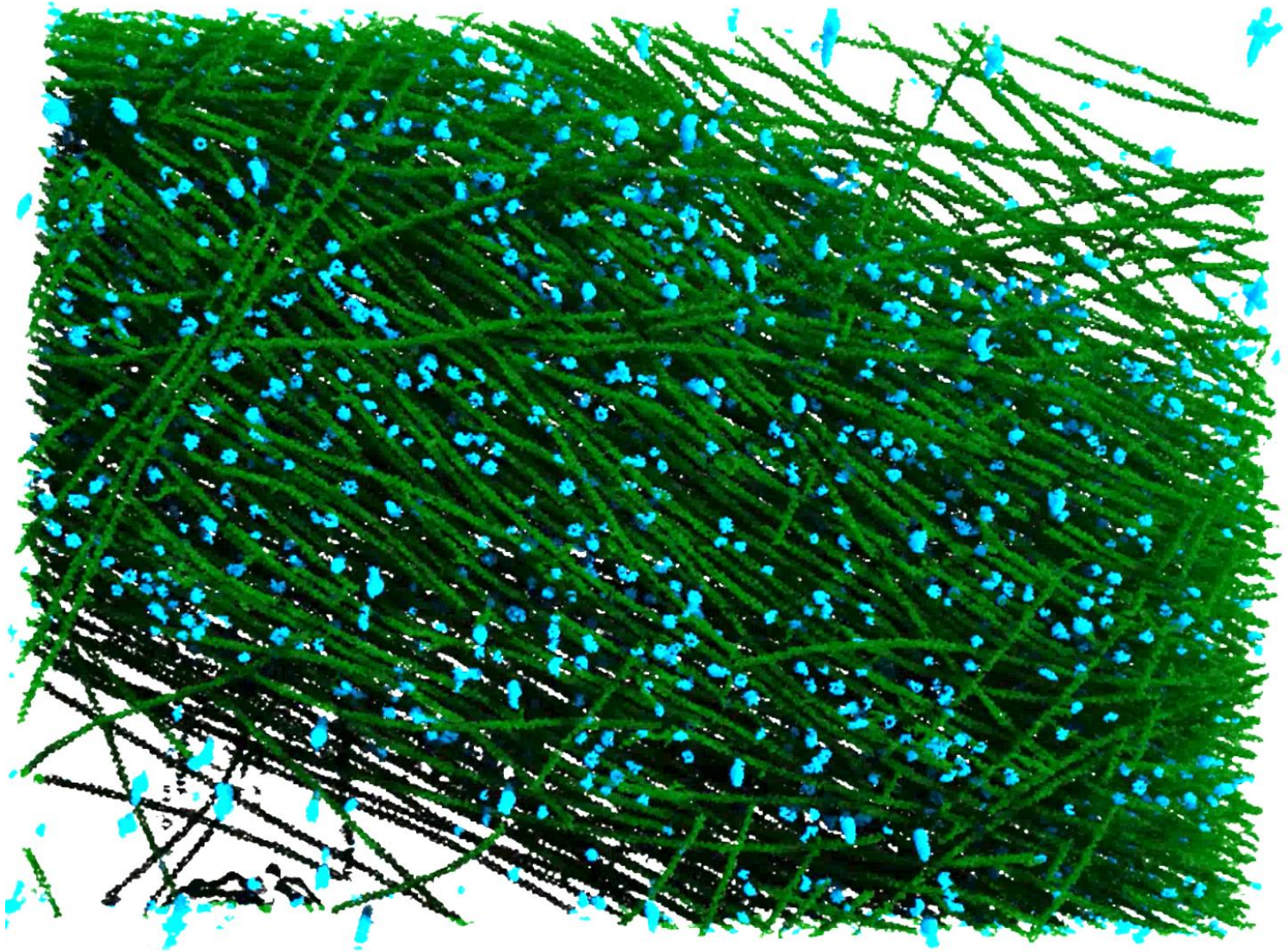

loss
val_loss
0.5
0.4
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
-10
0
10
20
30
40
50
60
Epoch
atlas_actin_cofilin_actin_long_complex1 ROI 4
atlas_actin_cofilin_actin_long_complex1 ROI 3
atlas_actin_cofilin_actin_long_complex1 ROI 2
atlas_actin_cofilin_actin_long_complex1 ROI 1

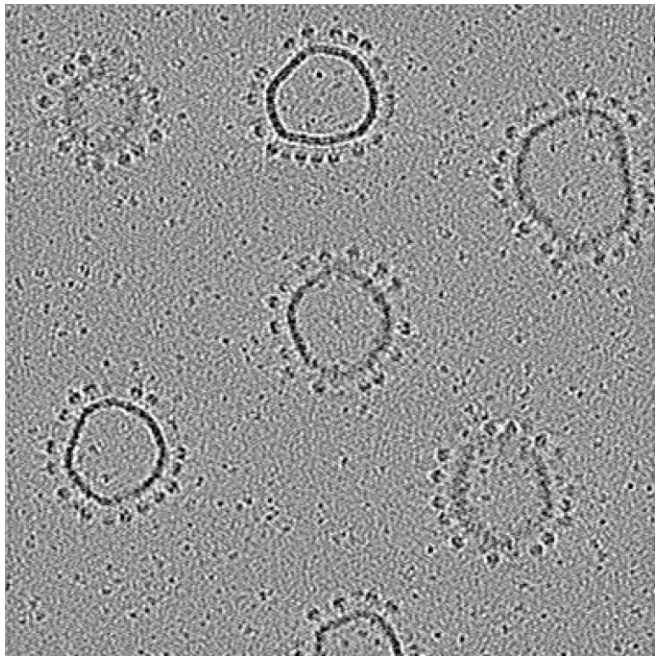

Dragonfly Workstation (Version 2022.2 Build 12227)
File Workflows Artificial Intelligence Utilities Developer Help
Main Segment
Manipulate
Flip/Rotate
Window Leveling
cofilactin_position_15
Log Y
Selected control point
Position (normalized):
Position (physical):
Value:
Color
Alpha
Lookup table (LUT)
grayscale
More
Selected range
-895.24
2,818.92
Plotted range/Data range
-2,514.43
Reset
4,031.08
-2,497.93
3,989.14
Color mapping
Opacity mapping
Opacity:
Gamma:
1.00
Annotate
Translate/Rotate
Dynamic refresh
4 quadrants
Probe
Scene's Views Properties
Show scale bar
Orthographic projection
Show legends
Show text annotations
Anti-aliasing
Edge detect
Edge detect only ROIs
View mode:
Slice
Interactivity:
Balanced
Background color:
Lighting
Light source position:
Use lighting
Shadow strength:
Shadow opacity:
5,280,300,723.89 X 3,605,320,463.06 nm
Range Win: -895.24 Max: 2,818.92
Slice 30 / 50
Yaw: 0.0 °
Pitch: 180.0 °
Roll: -0.0 °
Y
-X
X
Y
cofilactin_position_15
-895.24
-430.87
33.30
497.57
961.84
1426.11
1890.38
2354.65
2818.92
Current state: Leveling using an area (Left mouse)
New Session...
Preferences
Properties
Data Properties and Settings
cofilactin_position_15*
5_recon_actin_cofilin_complex_...
atlas_actin_cofilin_actin_long_c...
Leading Edge DeepClean ...
Leading Edge Tomogram
mt_madness
Segmentation ...
cofilin_multiroi
1/8
Basic properties
Width: 1,023 px (5,115,000,000 nm)
Height: 1,440 px (7,200,000,000 nm)
Depth: 59 px (295,000,000 nm)
Time steps: 1
Voxels: 86,914,080
Tools
Histogram:
2D settings
Interpolation: Cubic
3D presets
Save As...
Save
Delete
Rename...
Presets: Grayscale
3D settings
Solidity:
Diffuse:
Specular:
Shininess:
Tone mapping
Filtered gradient
Unsharp
High quality
Specular boost
Median
Edge contrast
Render mode: Default
Interpolation: Linear
Clip
Grid size: 1,000,000 nm
Keep box when object is hidden
Grid lines
Borders
Axes
Captions
Label: Ticks (2 Axes)
Lock text
Visual effects

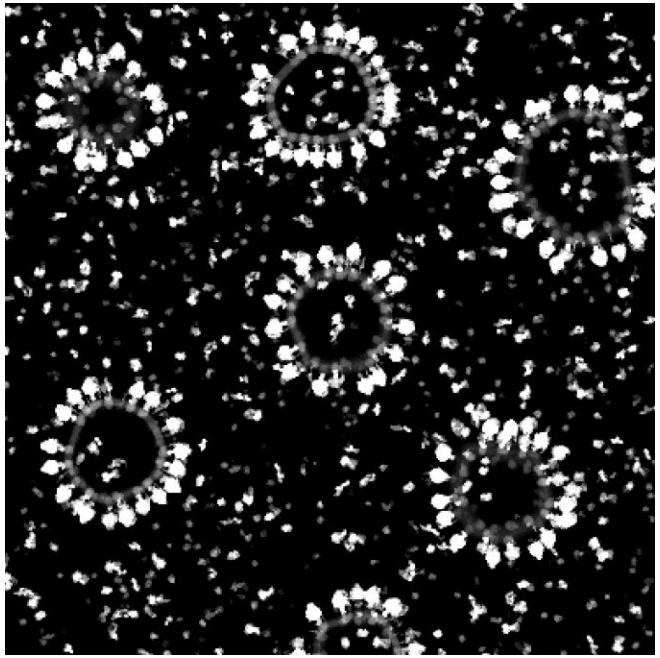

Dragonfly Workstation (Version 2022.2 Build 12227)
File Workflows Artificial Intelligence Utilities Developer Help
Main Segment
Dilate Dimensionality: 3D
Erode Shape: Cube
Open Range: Range
Close
Smooth Kernel size: 3
Operations
Fill inner areas: 3D Apply
Clipped region: Add Remove
Interpolate: Z Apply
Image operations
Image: 5_recon_actin_cofilin_complex_actin_
Overwrite... Split at Otsu Export
Export
To a Mesh To a Thickness Mesh
ROI Painter
Brush size
Pixels nm
Diameter: 20 100,000,000
2D view tools
Single slice Multi-slice
Full
4-connected
...ctin_long_tomo_noise
Grid size: x: 32 y: 32
Sigma: 2.50
Radius: 1 Pixels
3D view tools
Full
6-connected
Segment with AI
Segmentation model:
Inputs
Classes
Segment on multiple axes
Vote type: Majority Soft vote
Preview Segment
Preview opacity:
Range Min: -895.24 Max: 2,819.92
Slice: 32 / 59
Yaw: 0.0 °
Pitch: 180.0 °
Roll: -0.0 °
Y
-X
X
Current state: Leveling using an area (Left mouse)
New Session... Preferences
Properties
Data Properties and Settings
Segmentation cofilin_actin_split*
cofilactin_position_15
5_recon_actin_cofilin_complex_...
atlas_actin_cofilin_actin_long_c...
Leading Edge DeepClean ...
Leading Edge Tomogram
mt_madness
Segmentation ...
cofilin_multiroi
1/9
Basic properties
Width: 1,023 px (5,115,000,000 nm)
Height: 1,440 px (7,200,000,000 nm)
Depth: 59 px (295,000,000 nm)
Time steps: 1
Total Voxels: 86,914,080
Statistical properties
Labeled class count: 4
Labeled voxels count: 86,914,080
Volume of labeled voxels: 10,864,260,000,000,0
Classes and scalar information
Name
cofilin
cofilactin
actin
background
Add Remove Merge
Background class: Drag class here None
Labeled voxels count: 0
Scalar information
Scalar type:
Measurement:
Min:
Max:
2D settings
Show only contour
Contour thickness:
Opacity and color
Dataset:
Use LUT
Smoothed
Highlight:
Clip
Grid size: 1,000,000 nm
Keep box when object is hidden
Grid lines Borders Axes Captions
Label: Ticks (2 Axes) Lock text
Visual effects

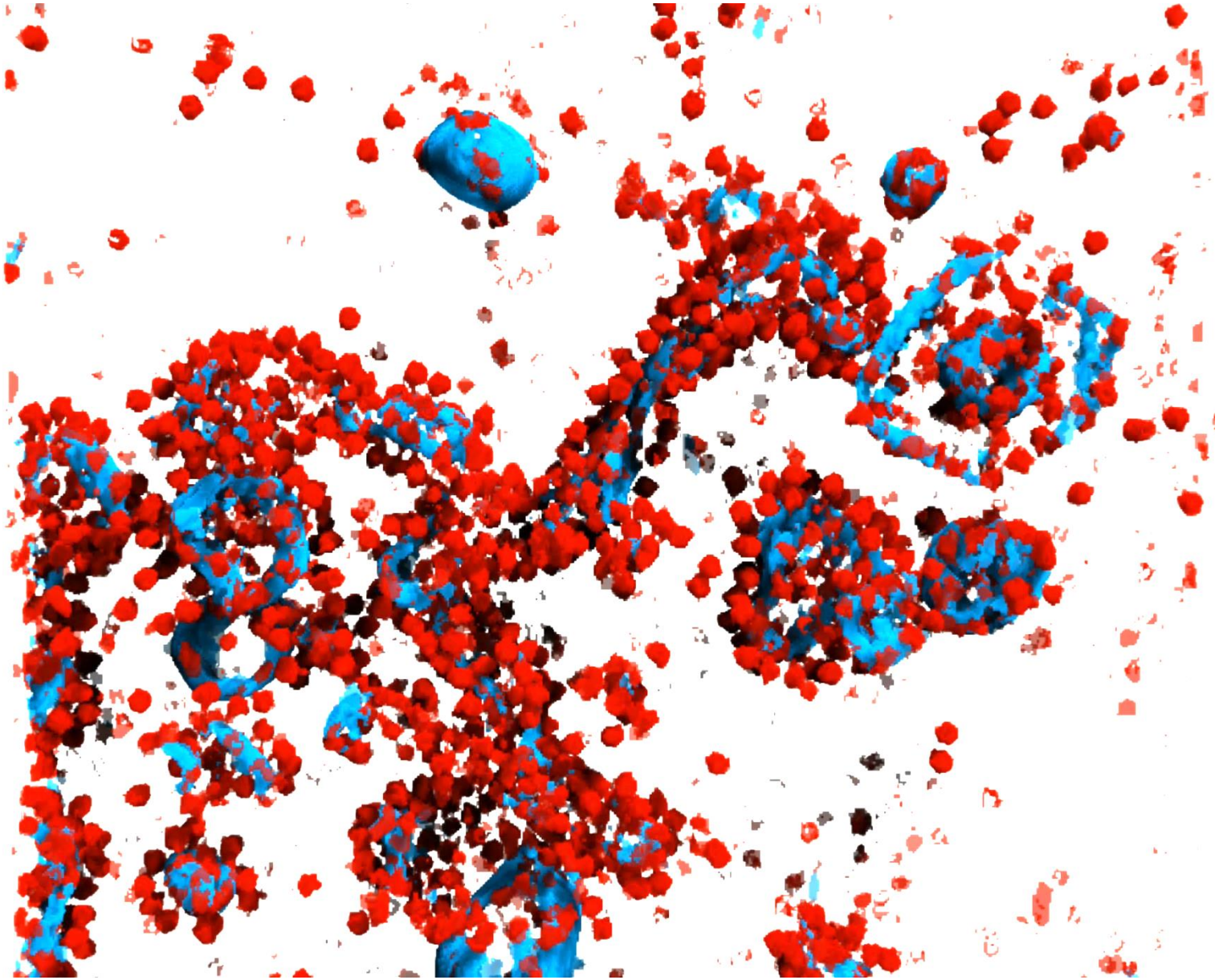

Dragonfly Workstation (Version 2022.2 Build 12227)
File Workflows Artificial Intelligence Utilities Developer Help
Main Segment
Dilate
Erode
Open
Close
Smooth
Dimensionality: 3D
Shape: Cube
Range: Range
Kernel size: 3
Operations
Fill inner areas: 3D Apply
Clipped region: Add Remove
Interpolate: Z Apply
Image operations
Image: 5_recon_actin_cofilin_complex_actin_
Overwrite... Split at Otsu Export
Export
To a Mesh To a Thickness Mesh
ROI Painter
Brush size
Pixels nm
Diameter: 20 100,000,000
2D view tools
Single slice Multi-slice
Full
4-connected
...ctin_long_tomo_noise
Grid size: x: 32 y: 32
Sigma: 2.50
Radius: 1 Pixels
3D view tools
Full
6-connected
Segment with AI
Segmentation model:
Inputs
Classes
Segment on multiple axes
Vote type: Majority Soft vote
Preview Segment
Preview opacity:
PD: 3,476,820,634.39 nm
Range Min: 0 Max: 255
New Session... Preferences
Properties
Data Properties and Settings
Segmentation cofilin_actin_spli...
Segmentation cofilin_actin_split*
cofilactin_position_15
5_recon_actin_cofilin_complex_...
atlas_actin_cofilin_actin_long_c...
Leading Edge DeepClean ...
Leading Edge Tomogram
mt_madness
Segmentation ...
cofilin_multiroi
1/10
Basic properties
Width: 1,023 px (5,115,000,000 nm)
Height: 1,440 px (7,200,000,000 nm)
Depth: 59 px (295,000,000 nm)
Time steps: 1
Total Voxels: 86,914,080
Statistical properties
Labeled class count: 4
Labeled voxels count: 86,600,740
Volume of labeled voxels: 10,825,092,500,000,
Classes and scalar information
Name
cofilin
cofilactin
actin
background
Add Remove Merge
Background class: Drag class here
Labeled voxels count: 190,371
Scalar information
Scalar type:
Measurement:
Min:
Max:
2D settings
Show only contour
Contour thickness:
Opacity and color
Use LUT
Smoothed
Diffuse:
Specular:
Highlight:
Labeled data:
Clip
Grid size:),000,000 nm
Keep box when object is hidden
Grid lines Borders Axes Captions
Label: Ticks (2 Axes) Lock text
Visual effects

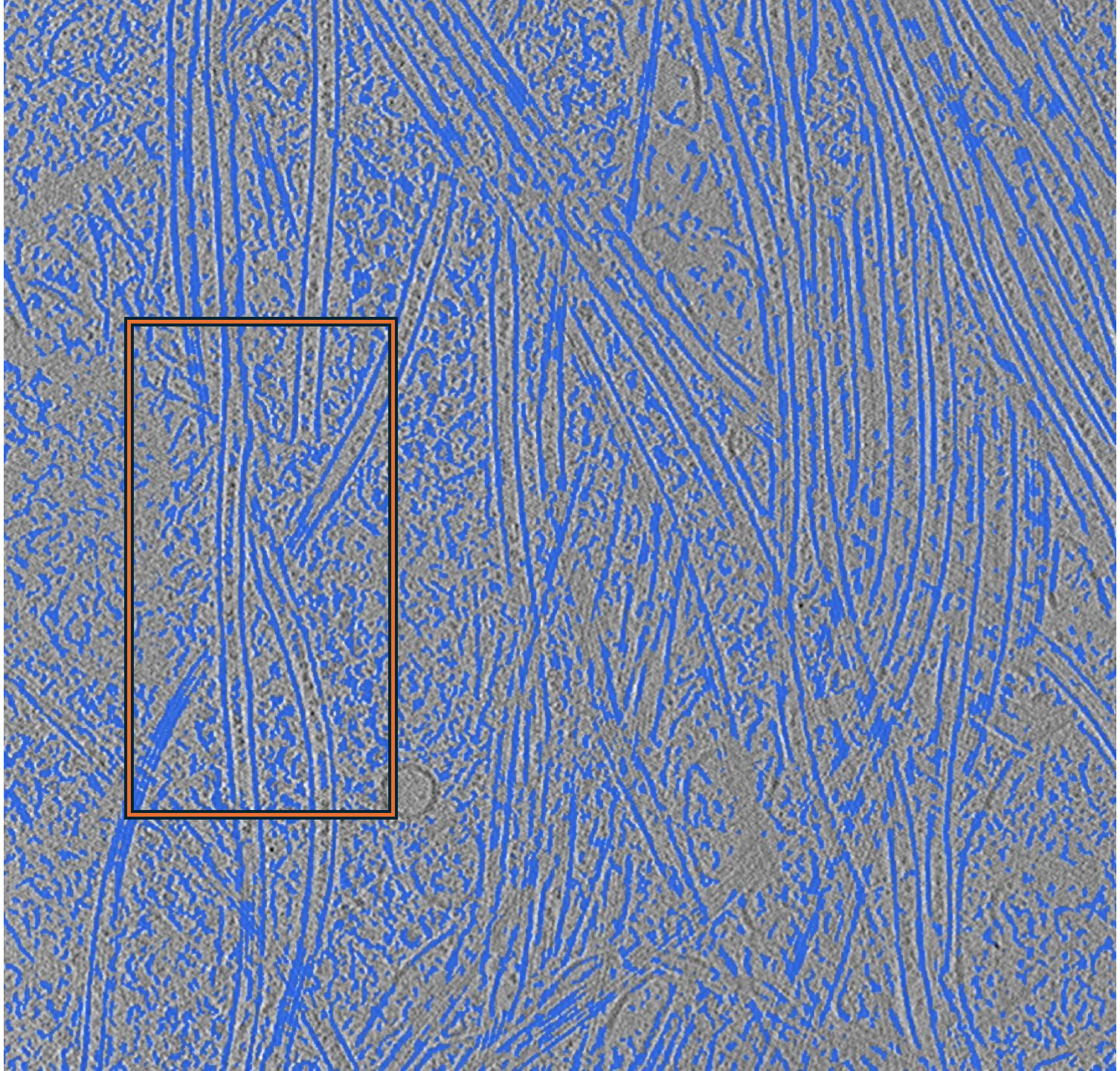




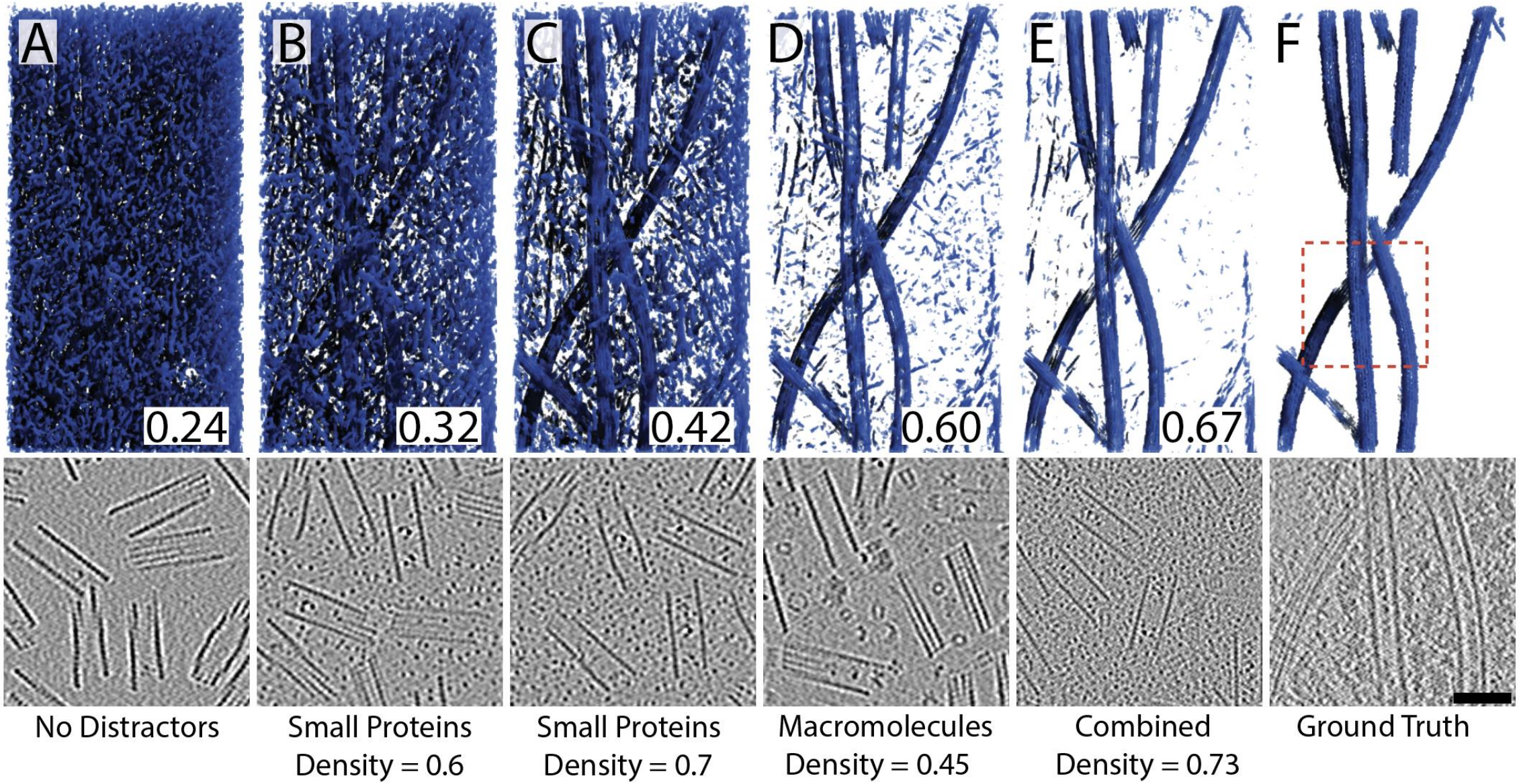


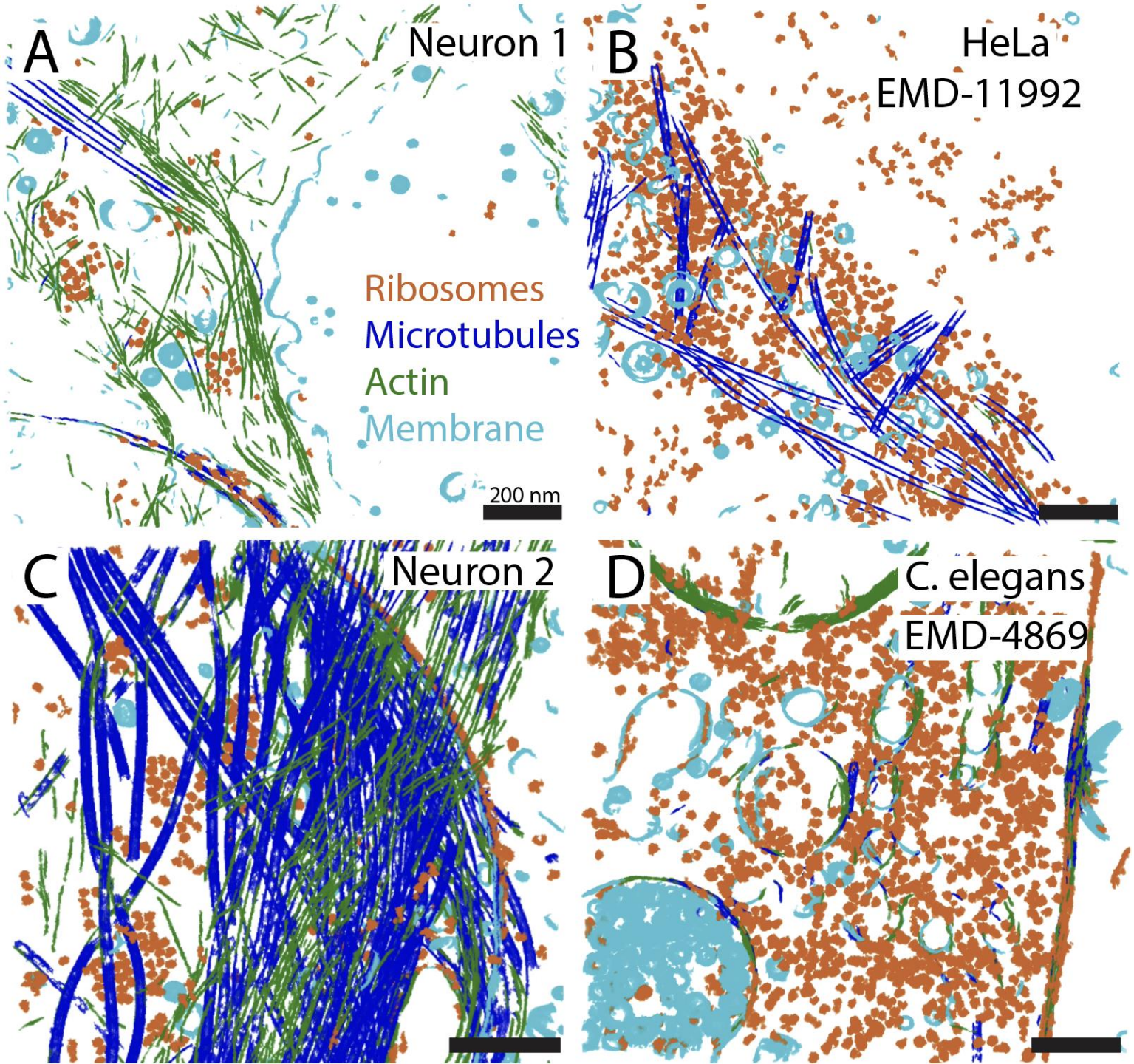


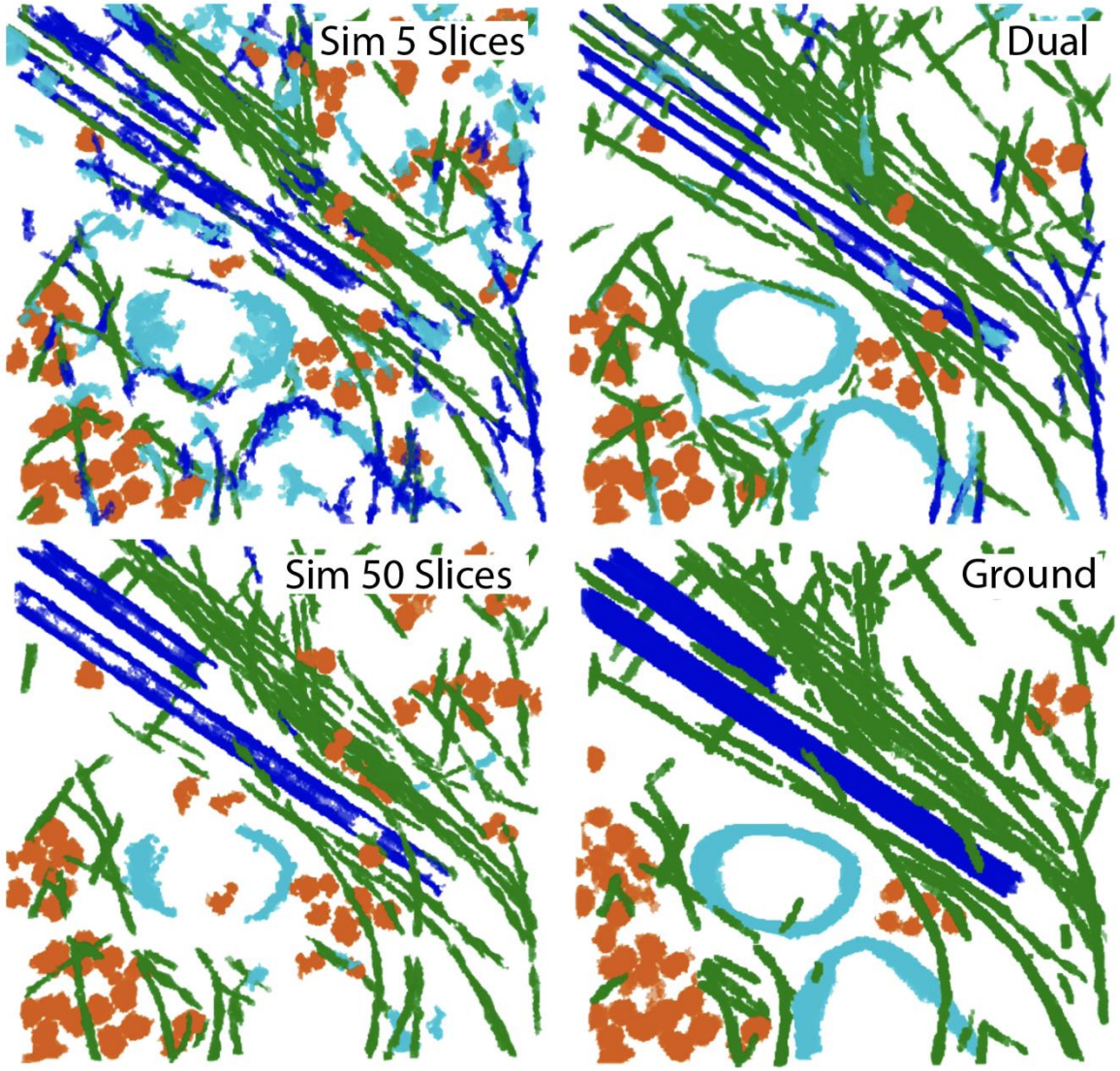


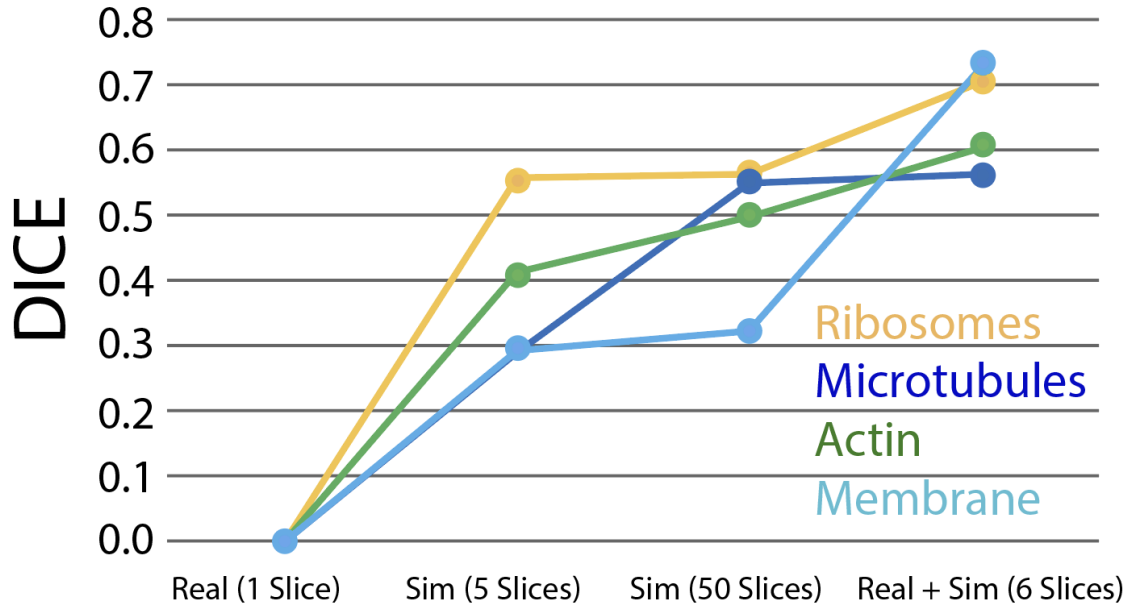


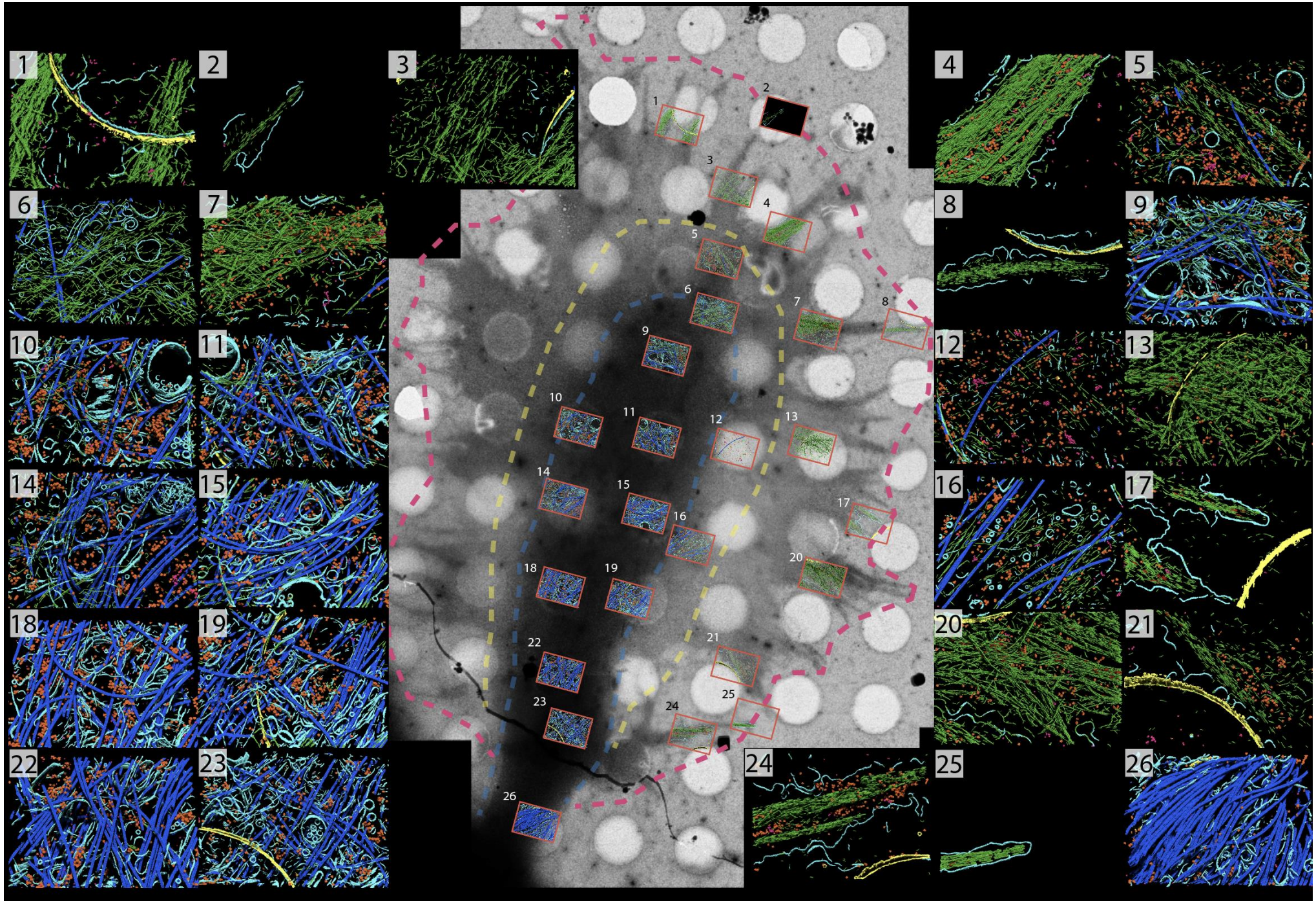
Diversify your crowding agents




A
Neuron 1
B
HeLa
EMD-11992
Ribosomes
Microtubules
Actin
Membrane
200 nm
C
Neuron 2
D
C. elegans
EMD-4869


Sim 5 Slices
Dual
Sim 50 Slices
Ground


DICE
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Real (1 Slice)
Sim (5 Slices)
Sim (50 Slices)
Real + Sim (6 Slices)
Ribosomes
Microtubules
Actin
Membrane


1
2
3
4
5
6
7
8
9
10
11
12
13
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16
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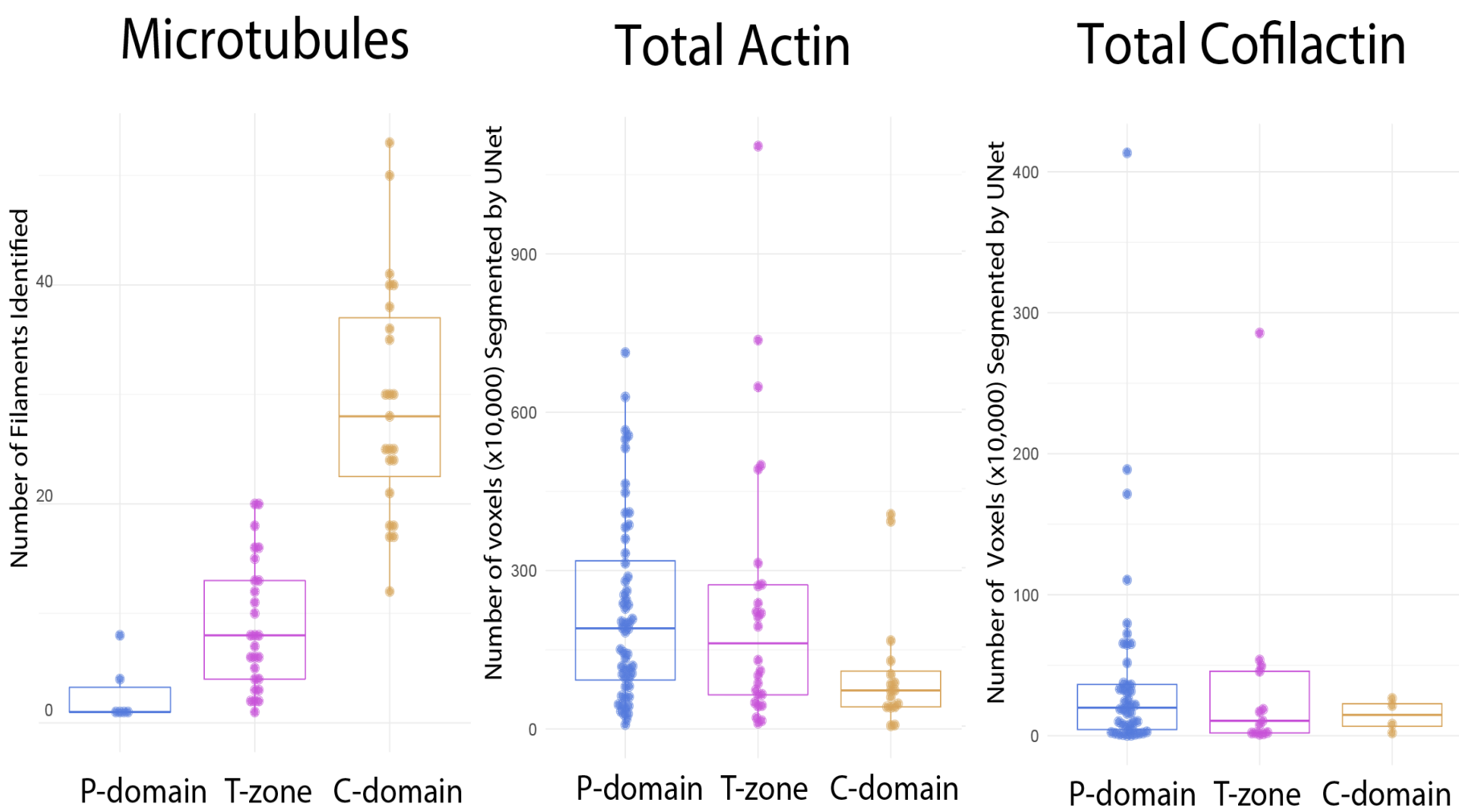
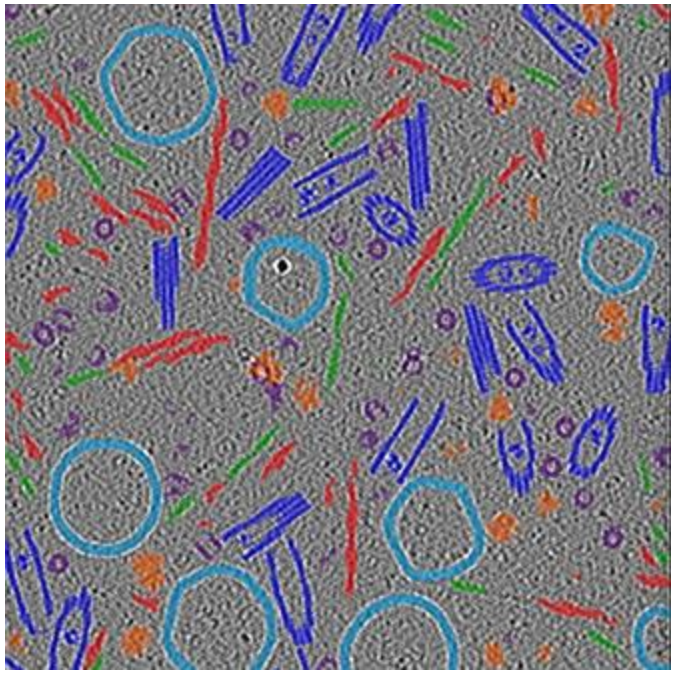
18
19
20
21
22
23
24
25
26


Microtubules
Number of Filaments Identified
0
20
40
P-domain
T-zone
C-domain
Total Actin
Number of voxels (x10,000) Segmented by UNet
0
300
600
900
P-domain
T-zone
C-domain
Total Cofilactin
Number of Voxels (x10,000) Segmented by UNet
0
100
200
300
400
P-domain
T-zone
C-domain

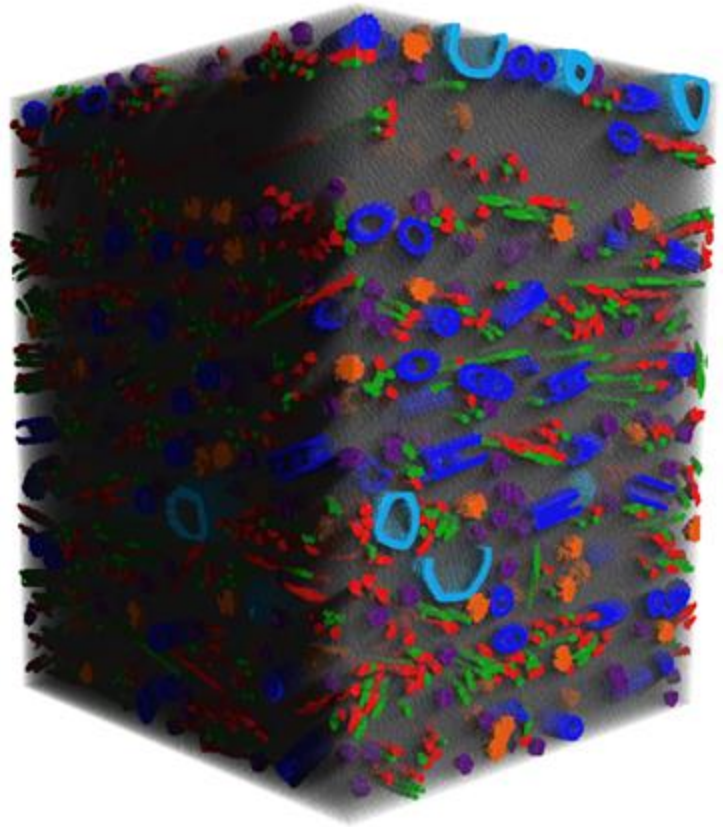
Training a generalized segmentation model

7 classes:
Membrane
TriC
Ribosome
Actin
Cofilactin
Microtubules
Background



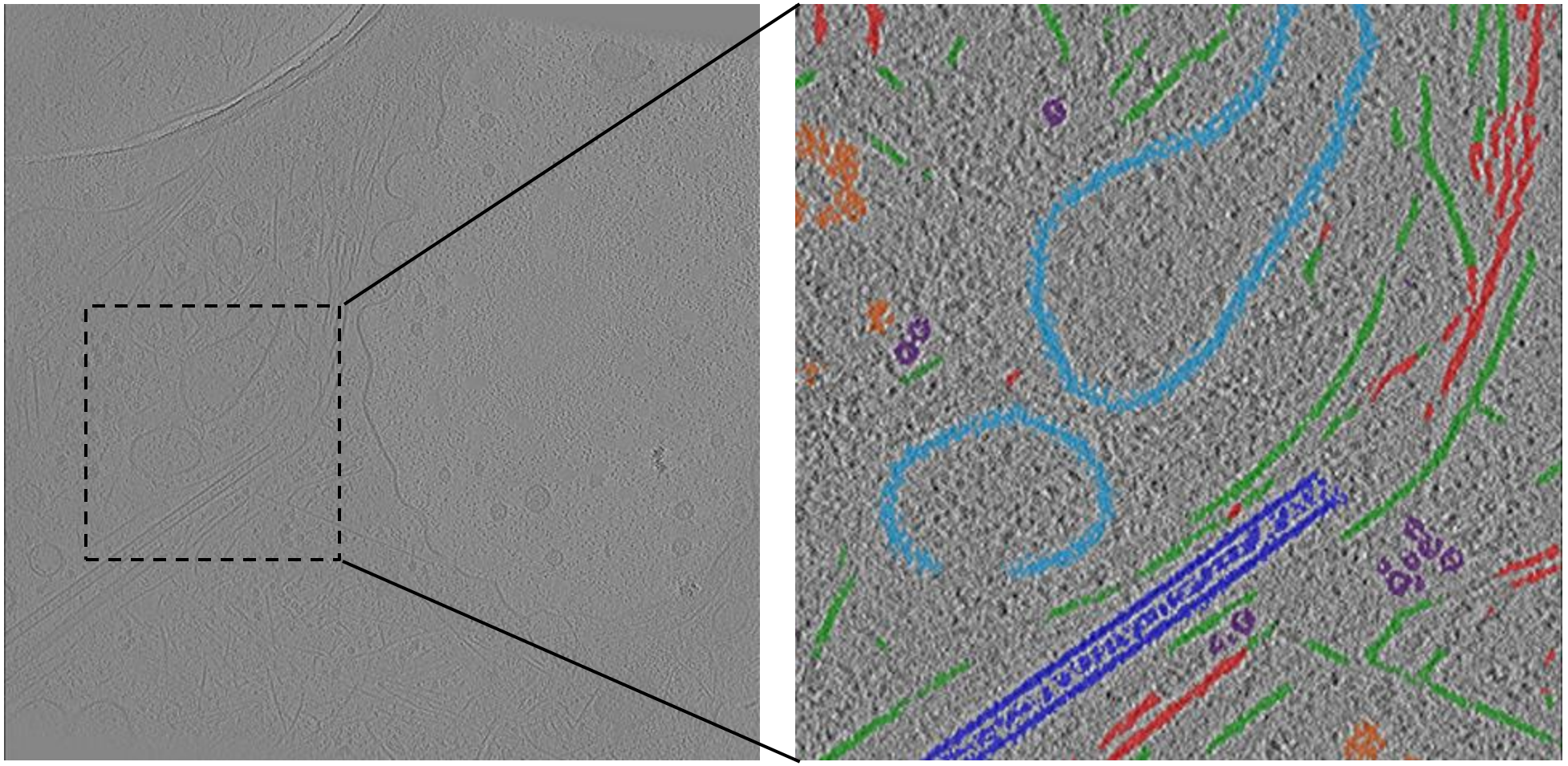
Slice through an example tomogram
of 400x400xZ voxels,
where Z=43-59

Concatenation



400x400x531
voxels

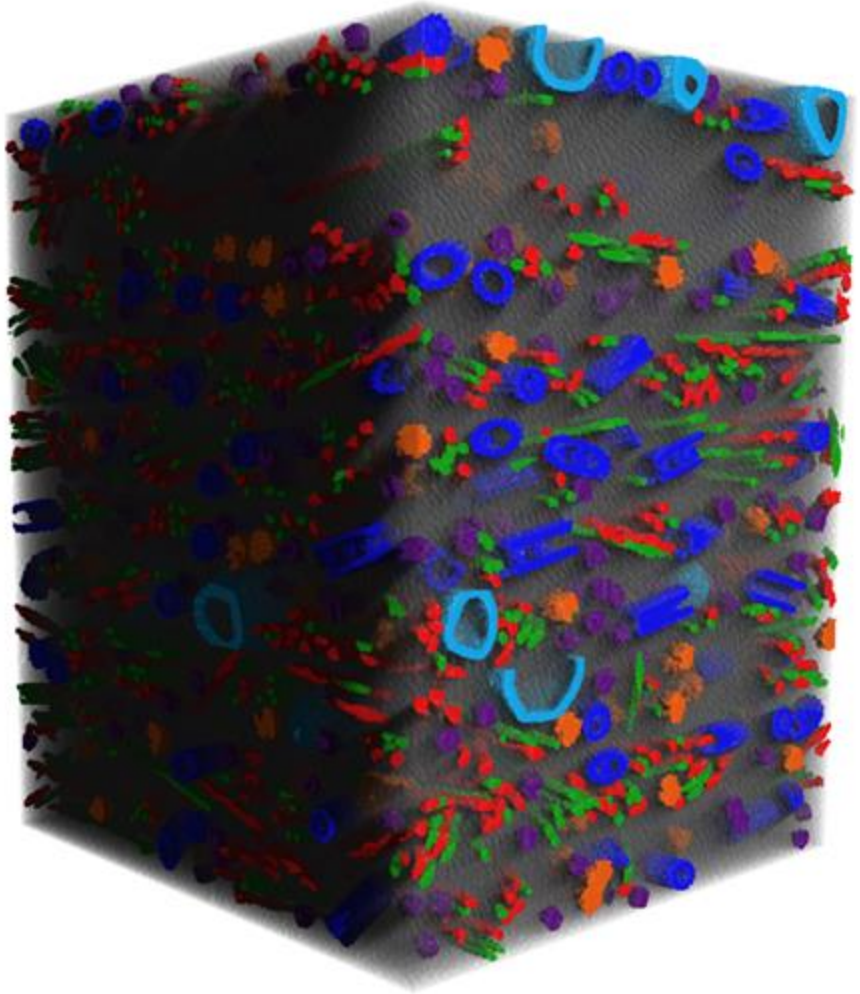
First iteration: Neuronal tomograms



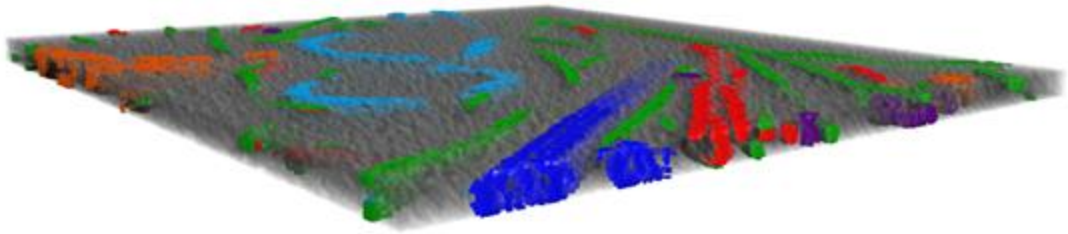
400x400x19 voxels

7 classes:
Membrane
TriC
Ribosome
Actin
Cofilactin
Microtubules
Background

Base network: Training data

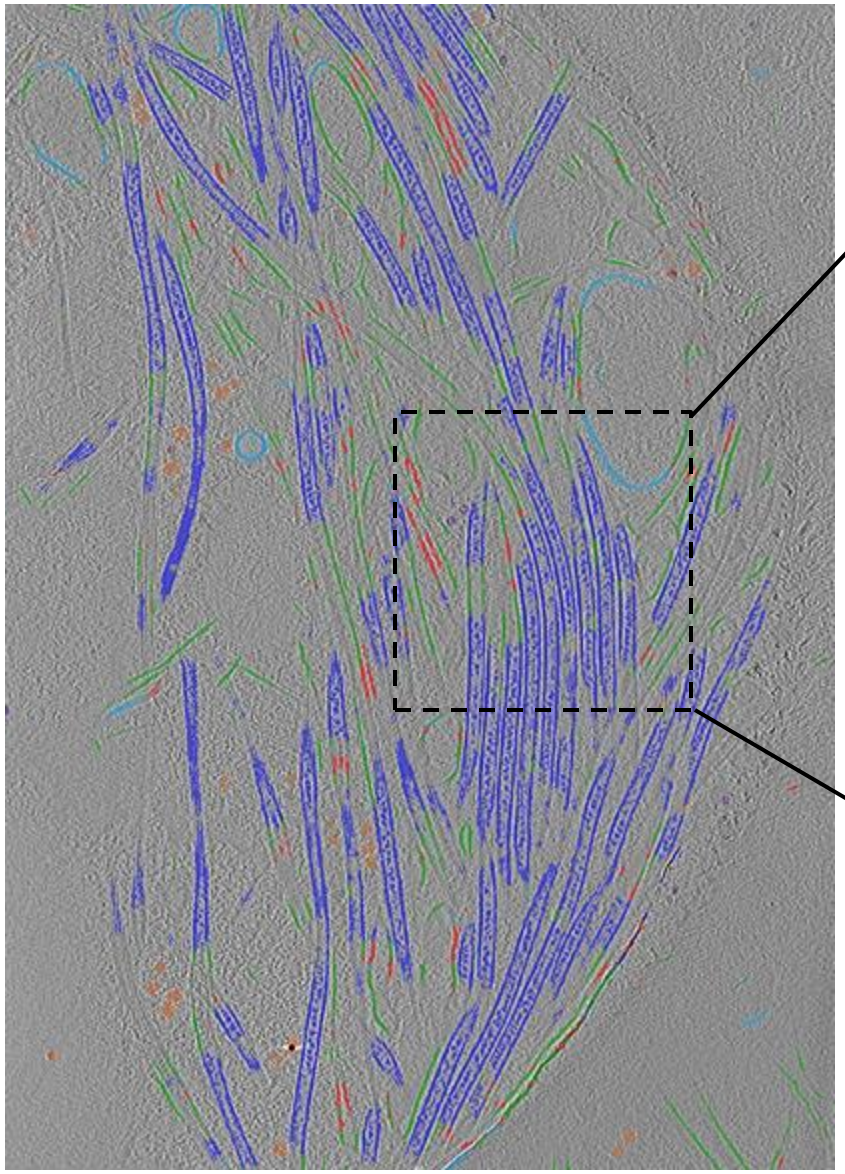


Synthetic block

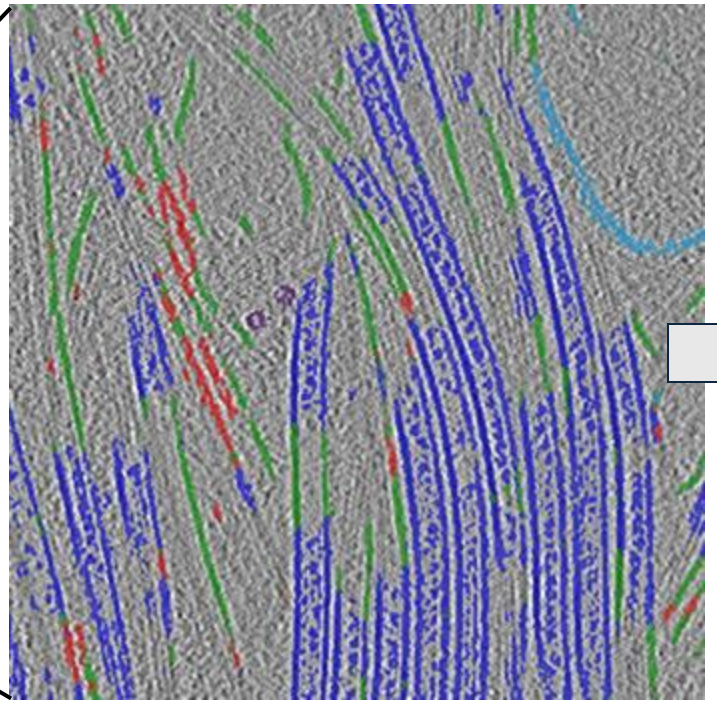


Real block

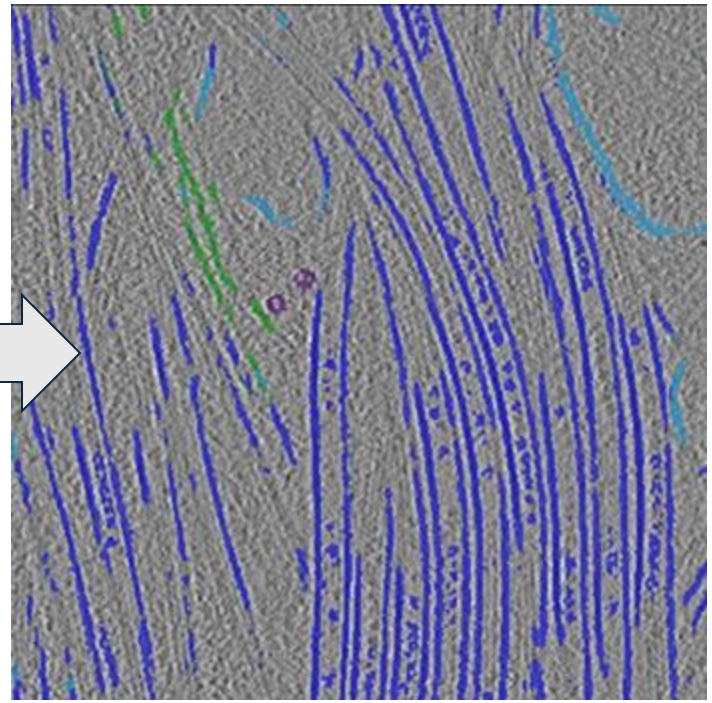
Result from first iteration & Hand-correction



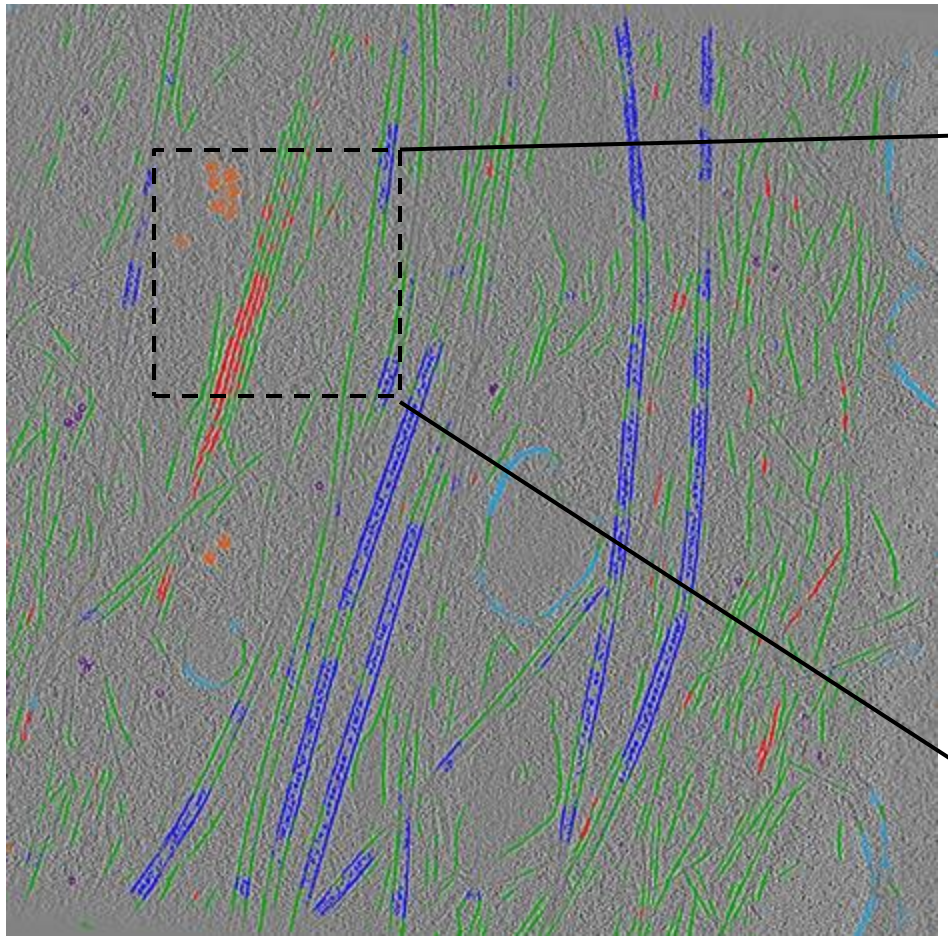
Overlapping microtubules



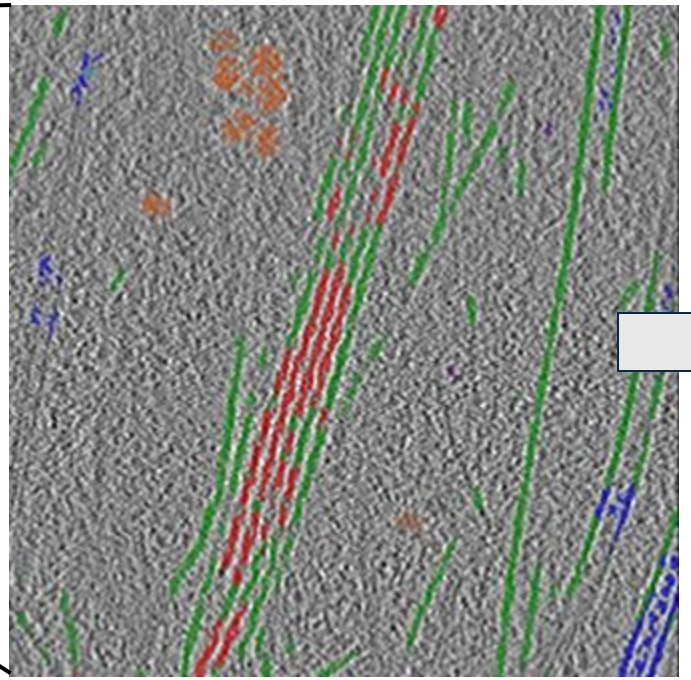
400x400xZ voxels,
where Z = 10-50

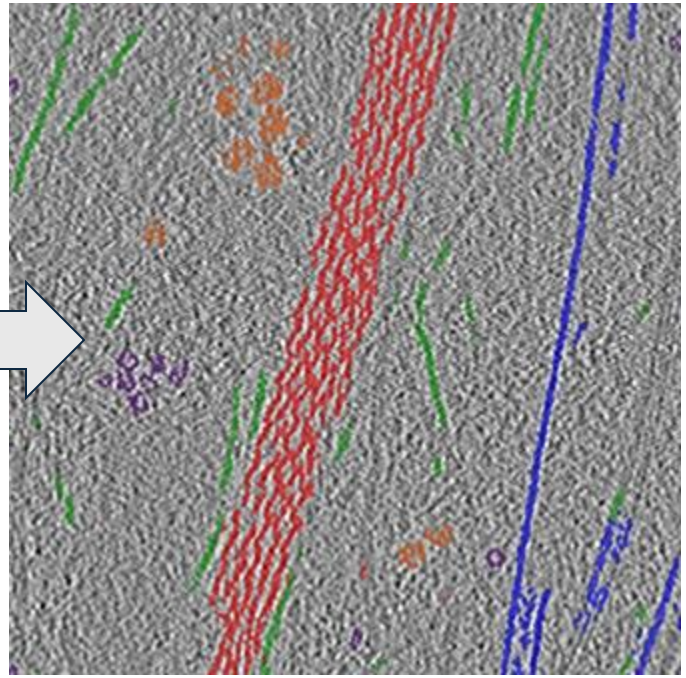


Result from first iteration & Hand-correction



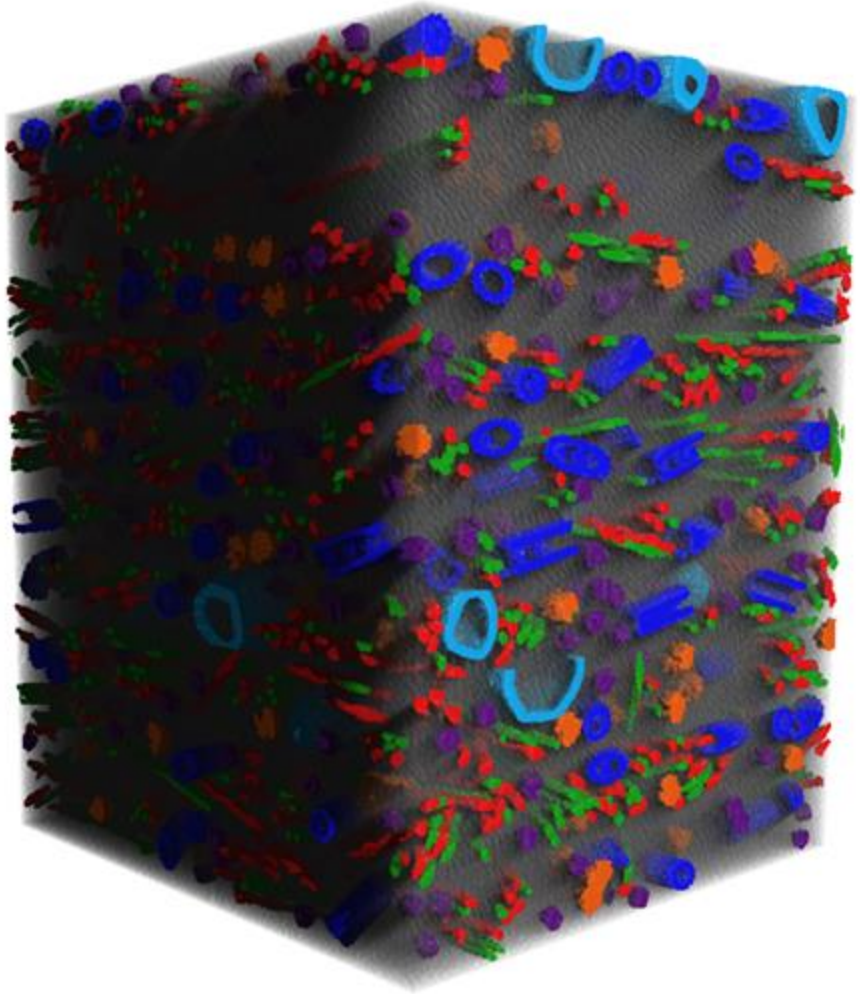
Cofilactin bundles



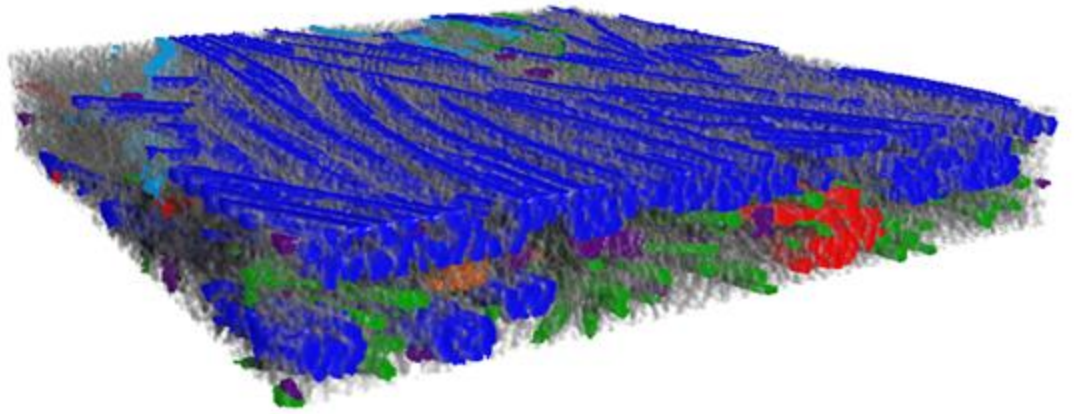


400x400xZ voxels,
where Z = 10-50

Concatenation to update training data

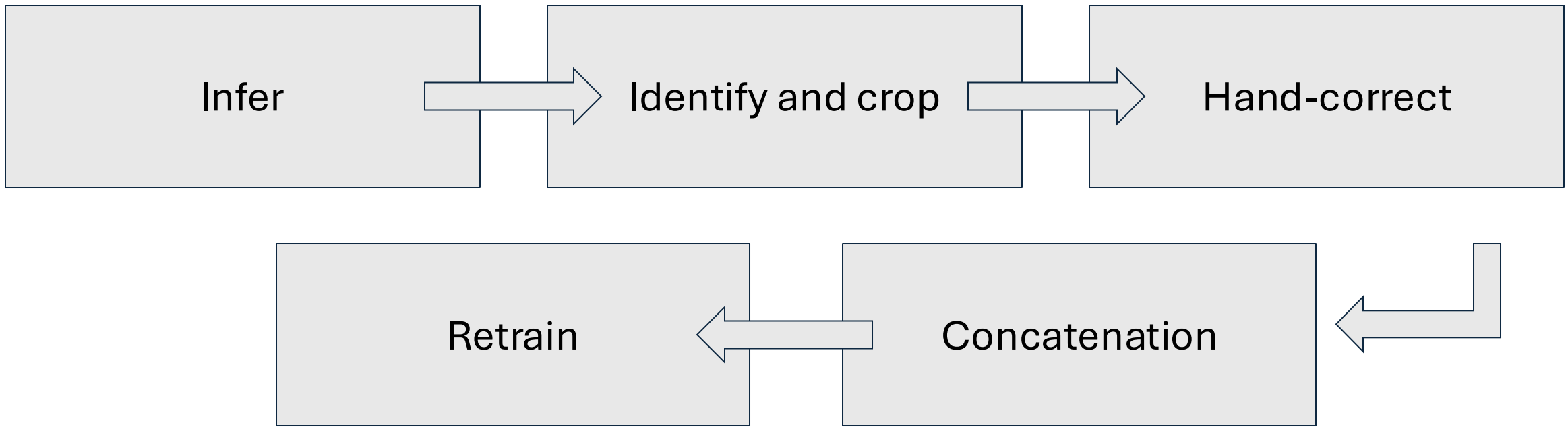


Synthetic block

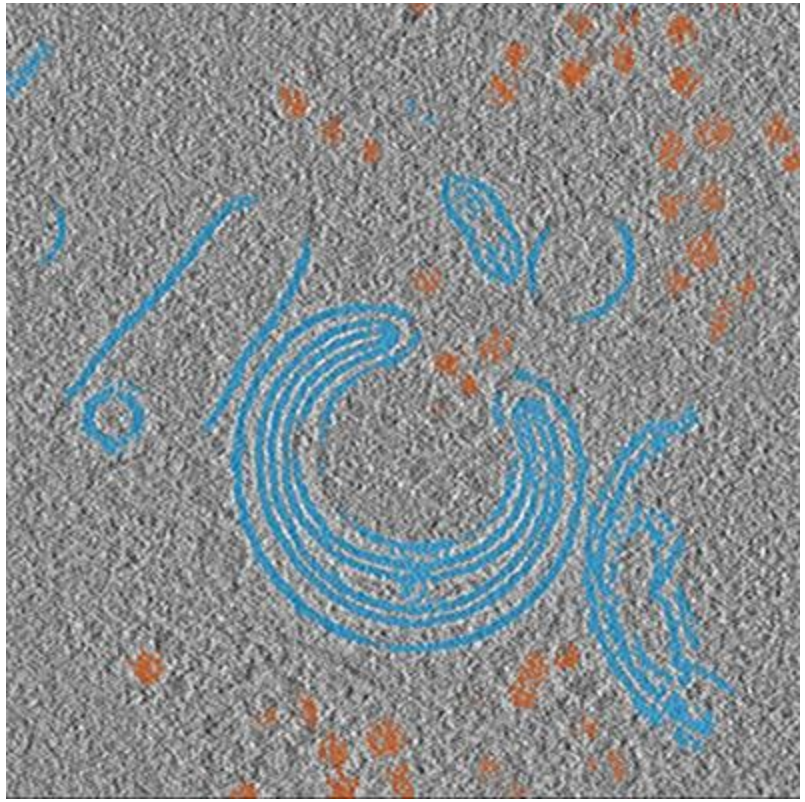


Real block

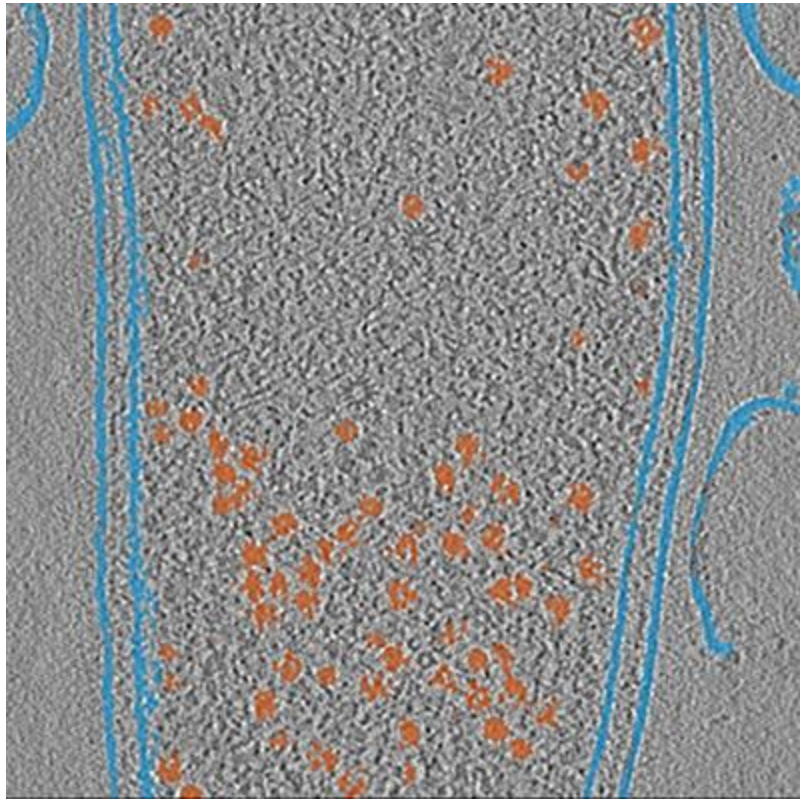
Basic workflow



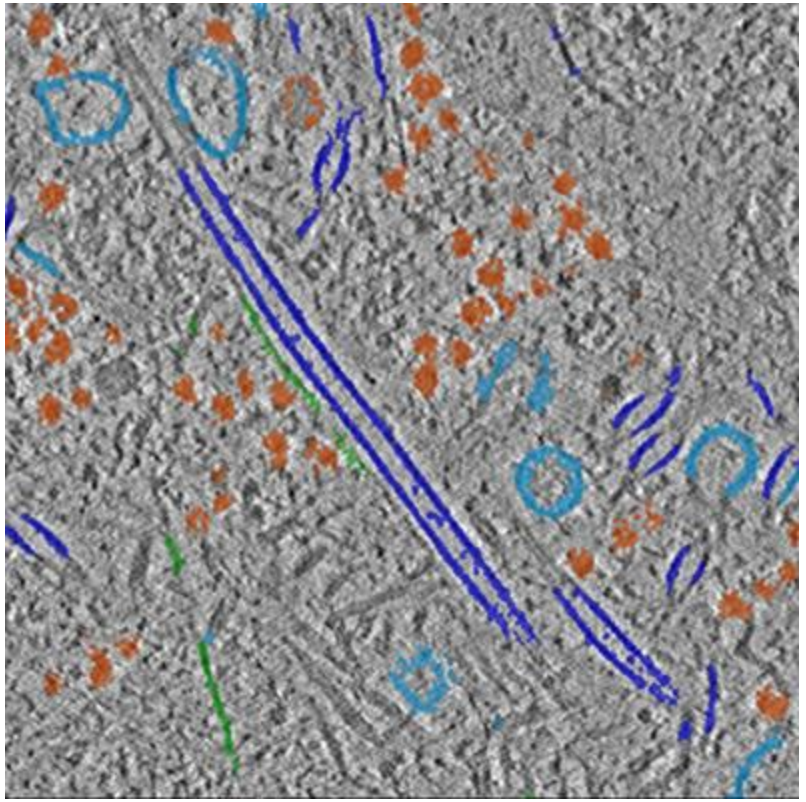
Second iteration: Non-neuronal tomograms



Eukaryotic multi-layer membrane

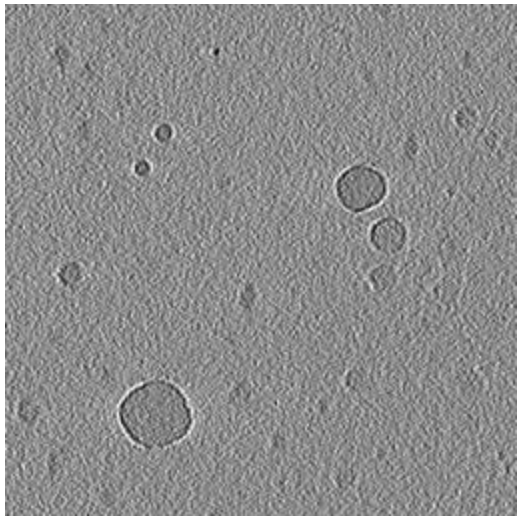


Bacterial double membrane



Eukaryotic Cytoplasm

Third iteration: Tomographic artifacts

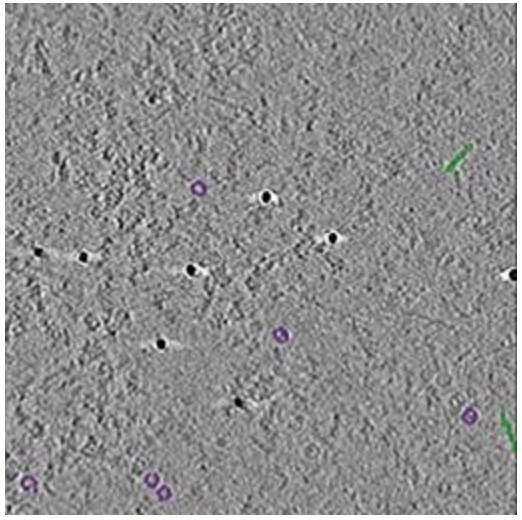


Ice

Carbon edge

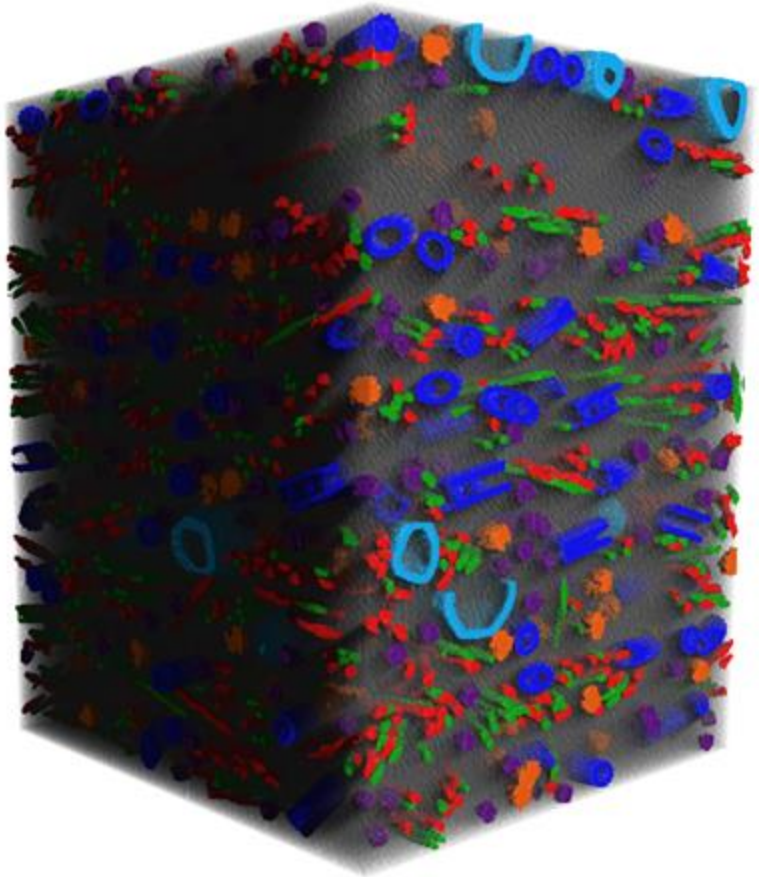
Carbon surface

Tomographic fringing

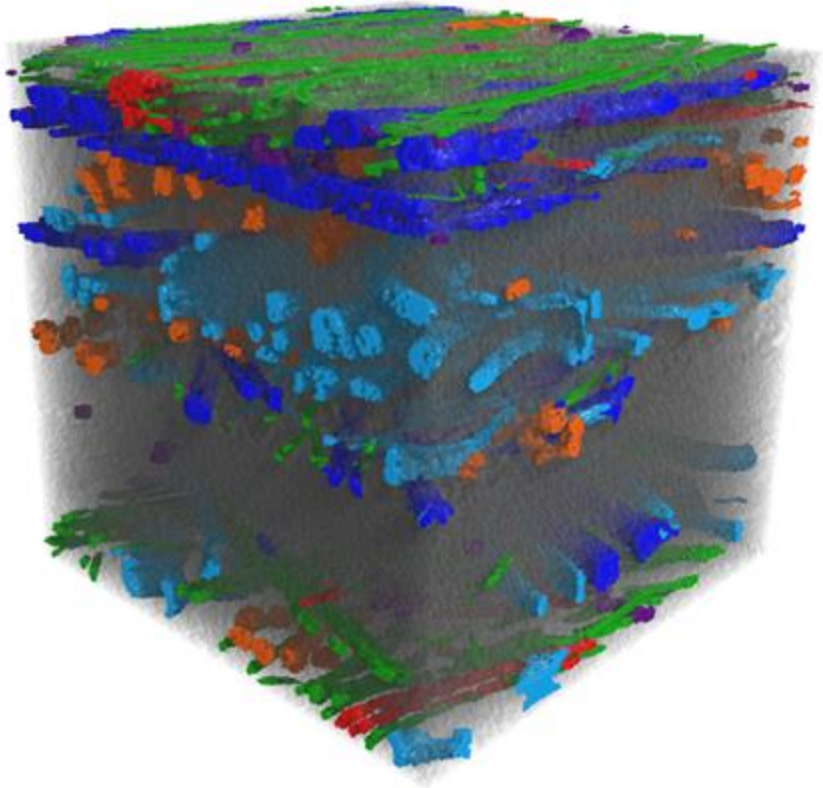


Gold fiducials

Final network: Training data



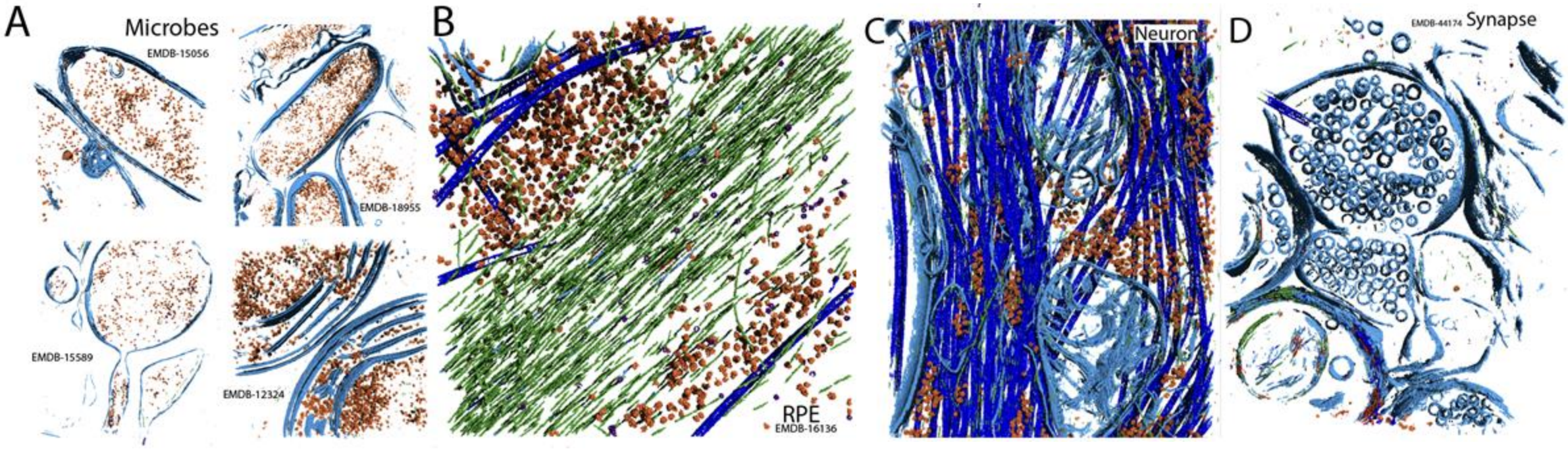
Synthetic data
(400x400x531)



Real data (400x400x395)

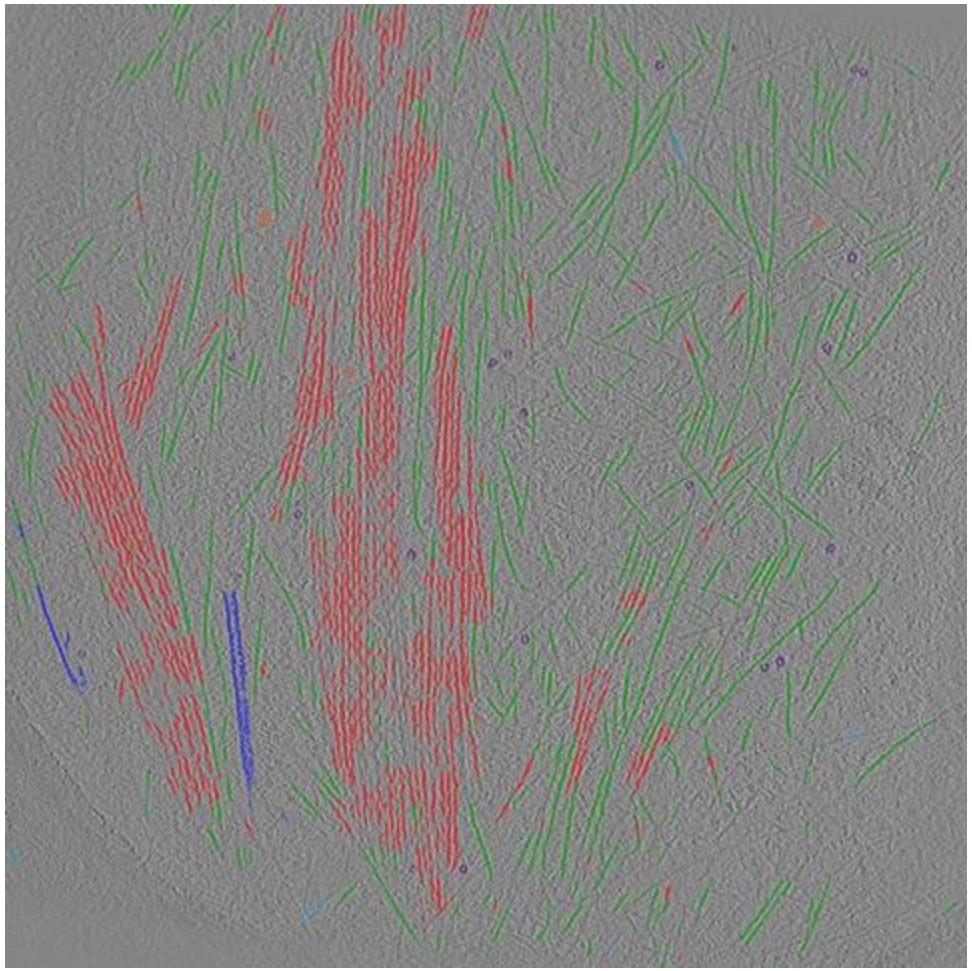
NeuralSeg

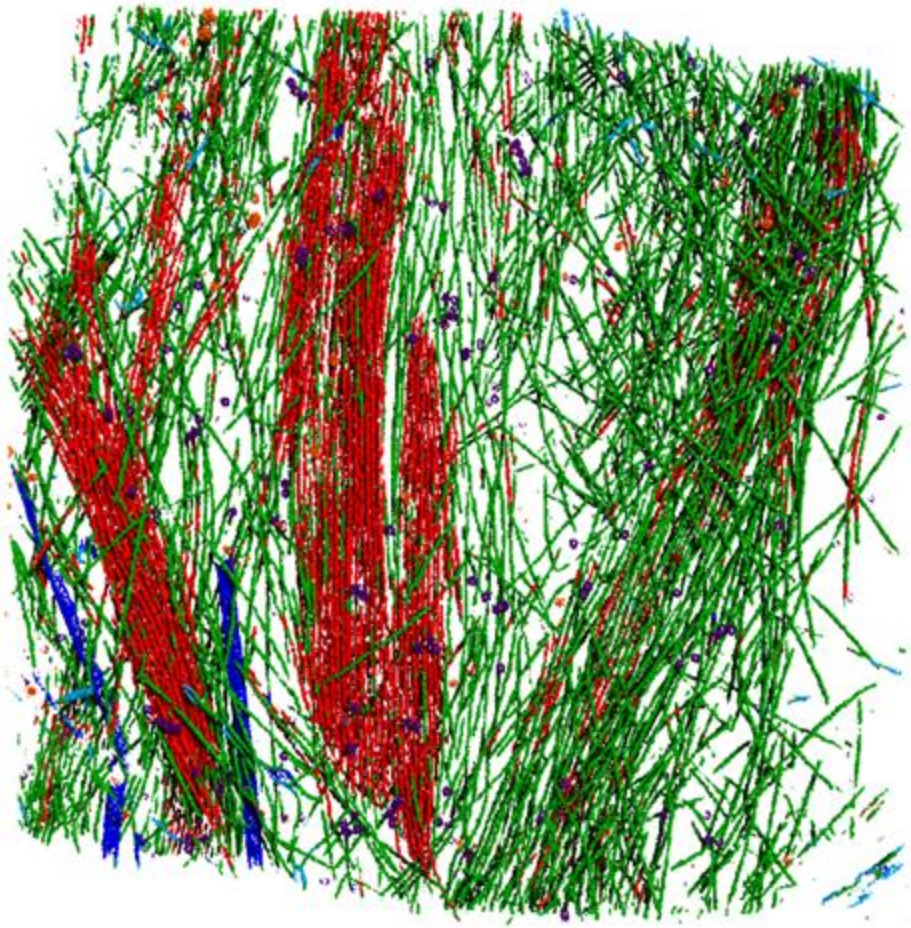
NeuralSeg can segment most cellular tomograms that meet its criteria



Ideal tomograms have 12-14 angstroms/pixel,
-4 to -10 μM defocus, and sufficient contrast.

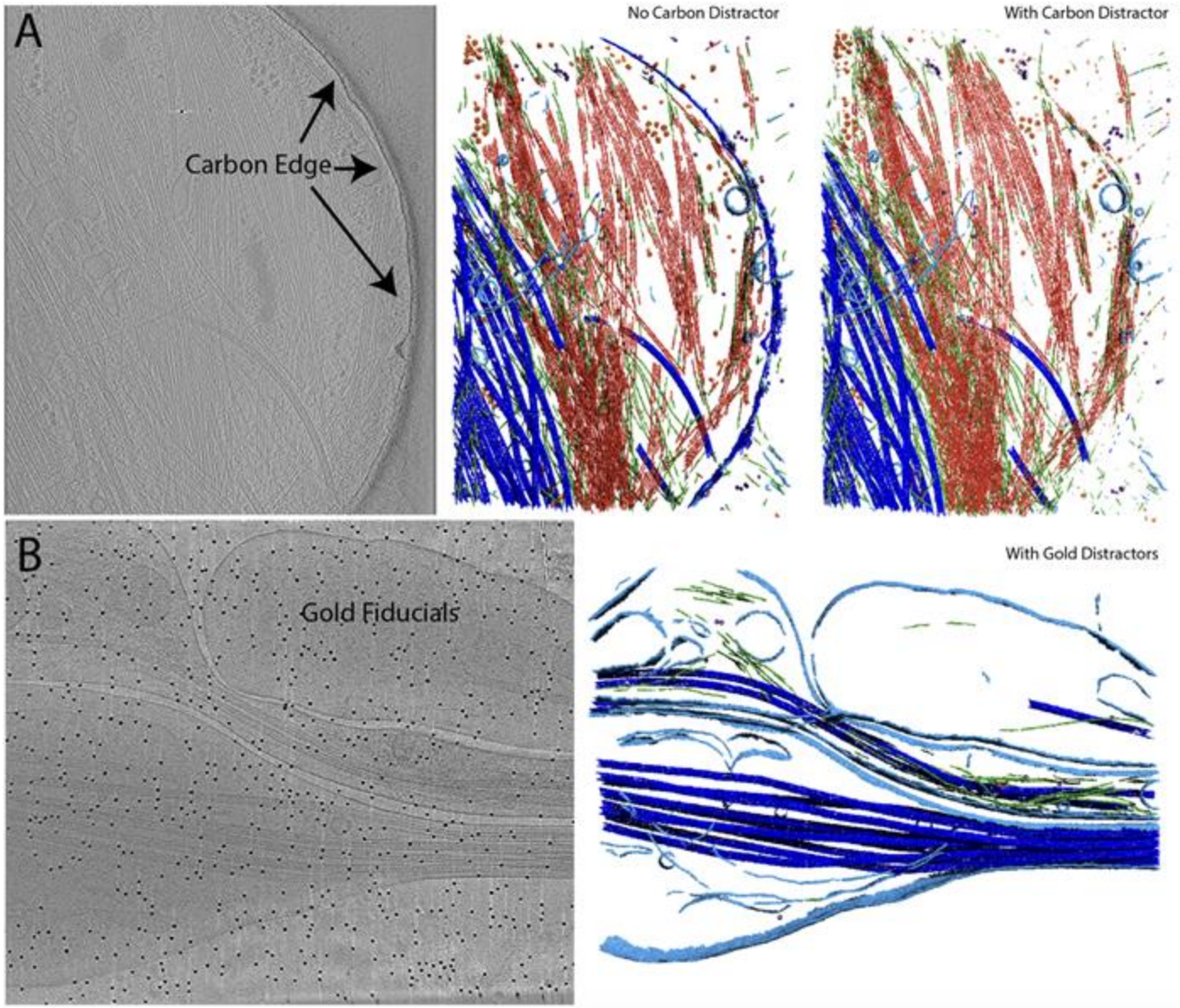
NeuralSeg can differentiate between actin and cofilactin

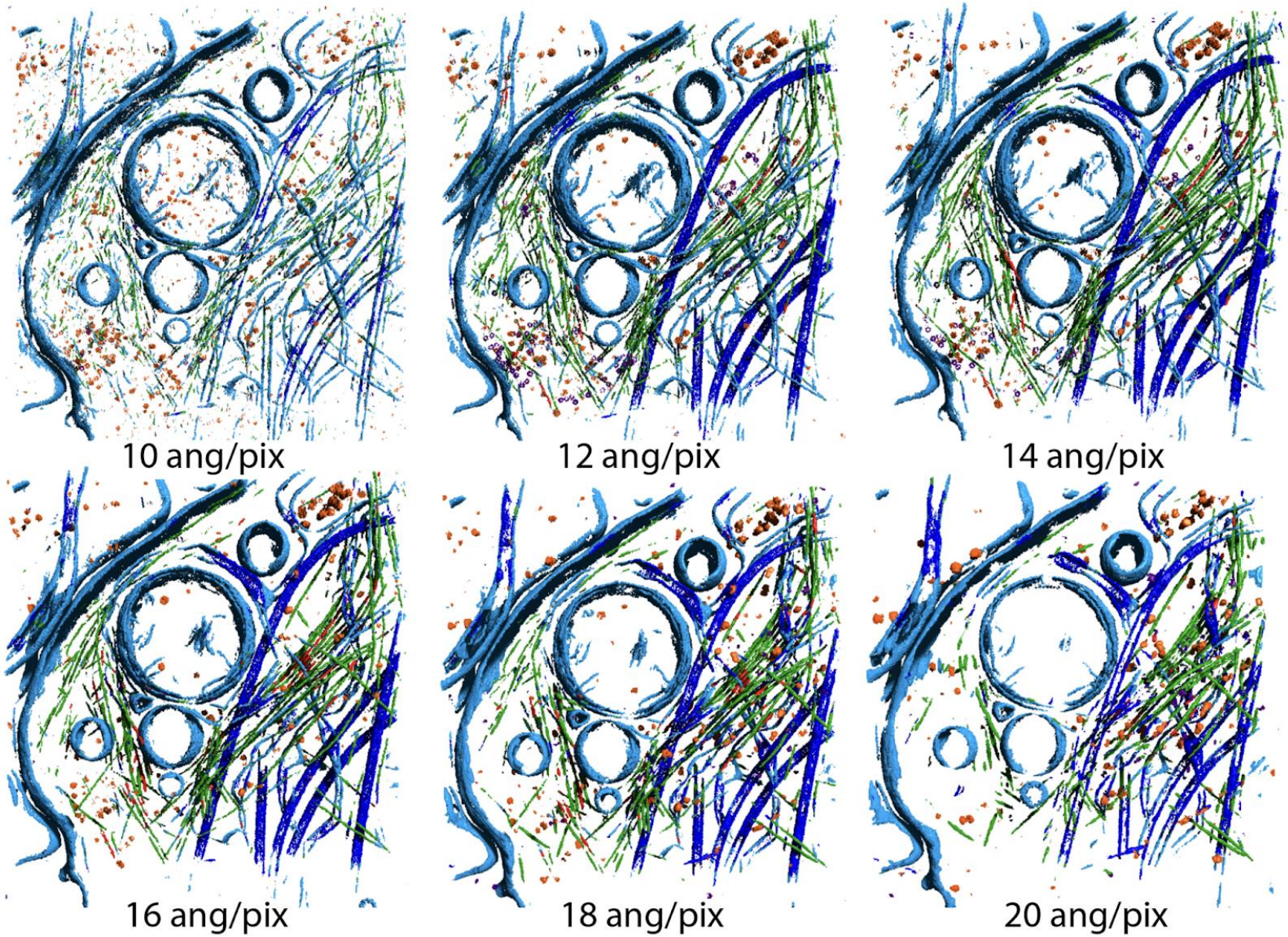




Bare actin and cofilin-decorated actin are distinguished as individual, bundles, or mixed bundles.

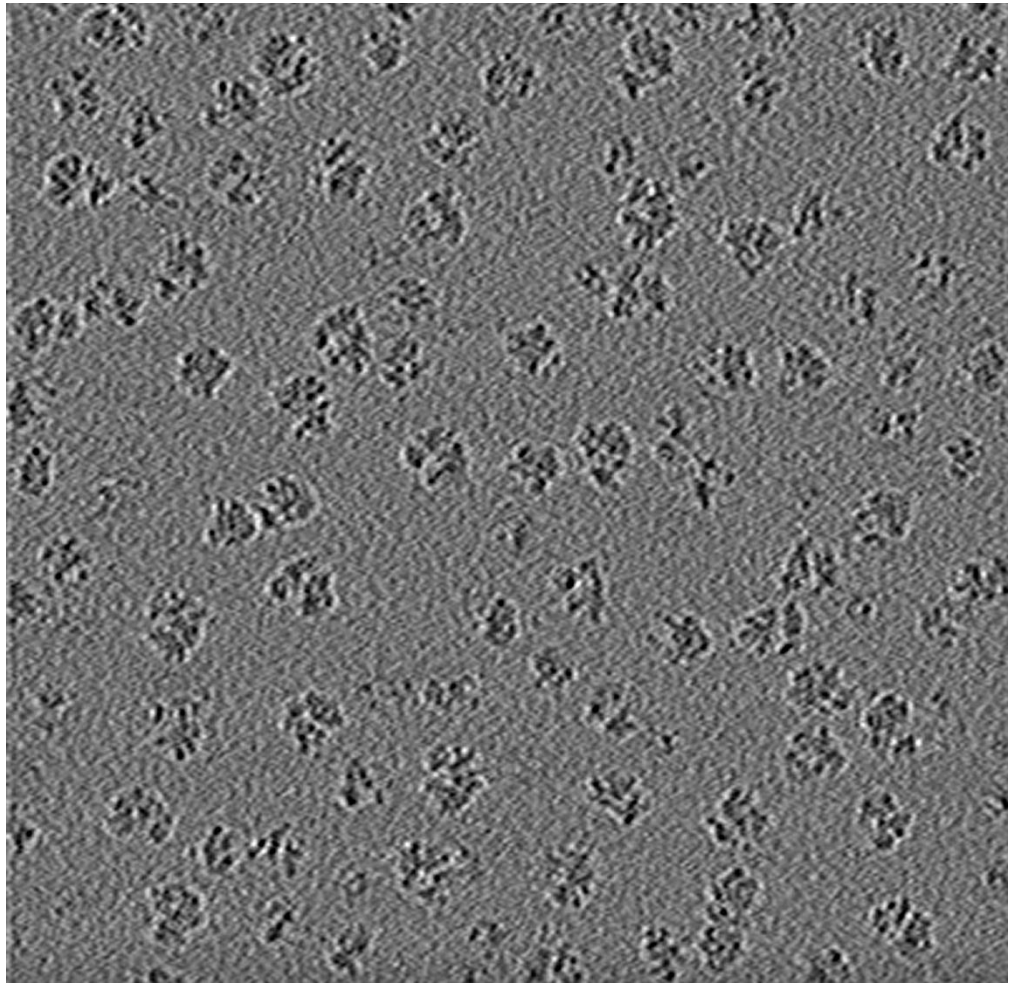
Typical tomographic artifacts are ignored



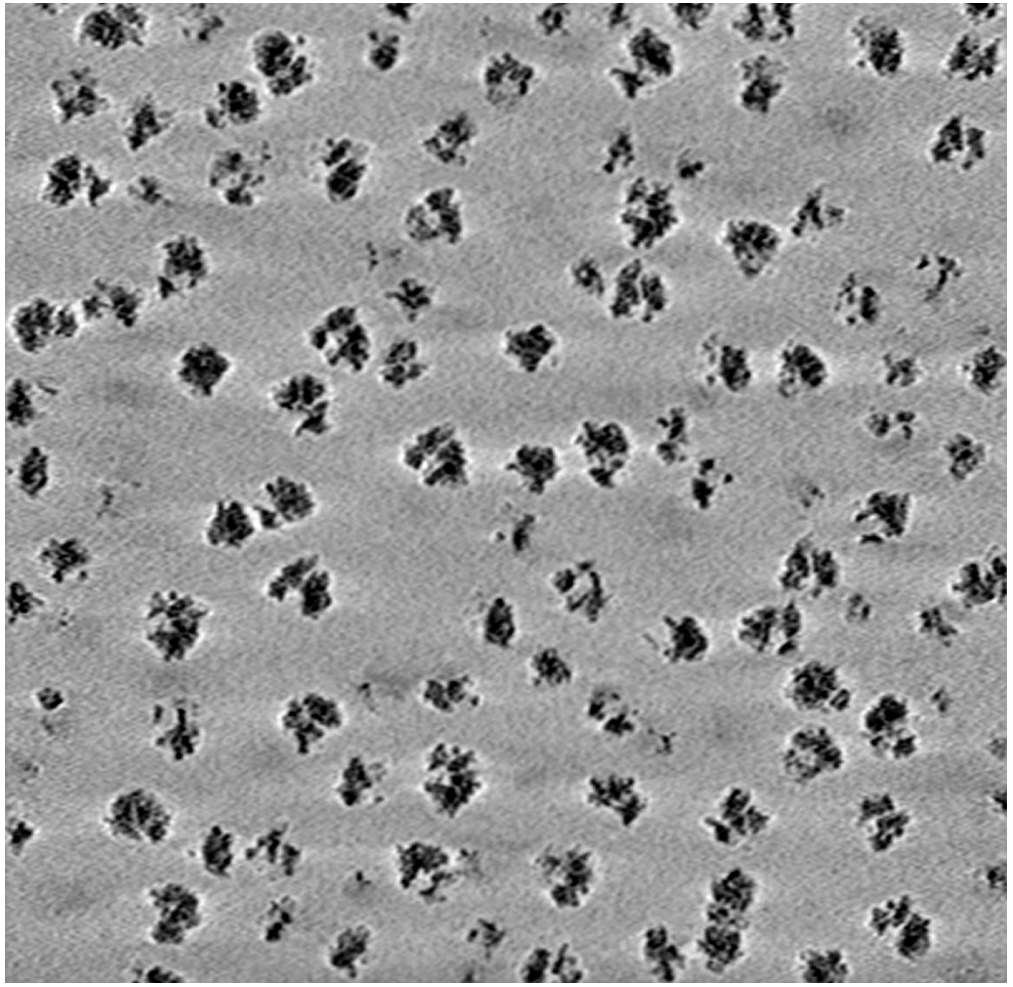

10 ang/pix
12 ang/pix
14 ang/pix
16 ang/pix
18 ang/pix
20 ang/pix

Denoising

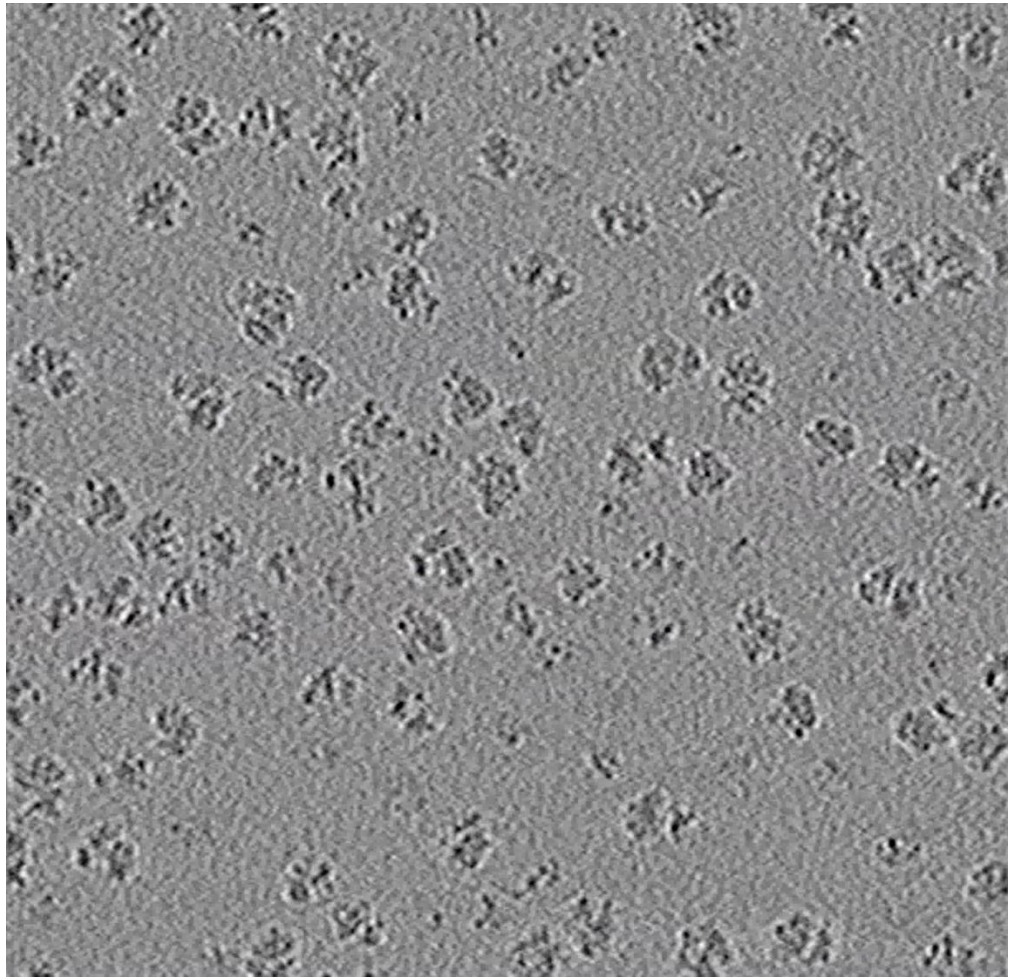
Tomo



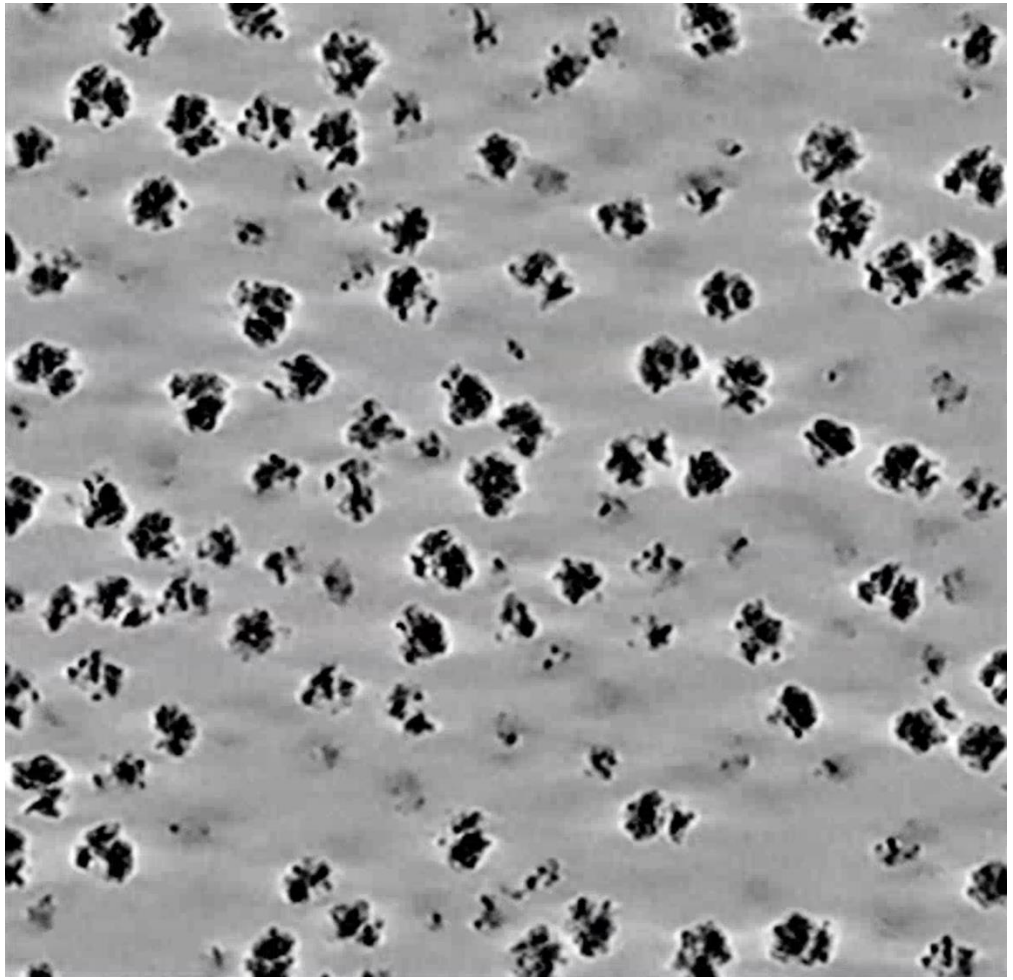
Ground Tomo



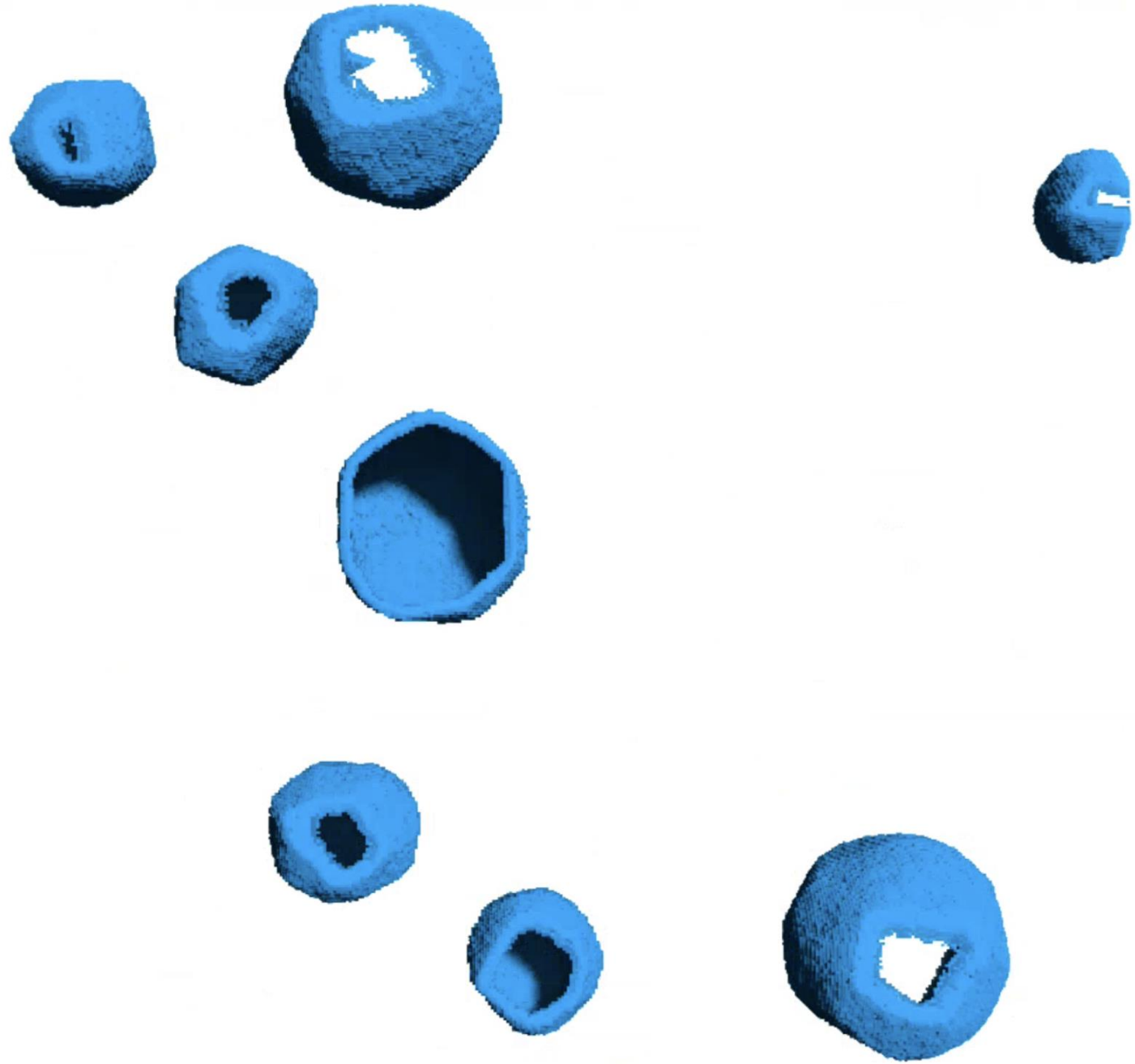
New Tomo



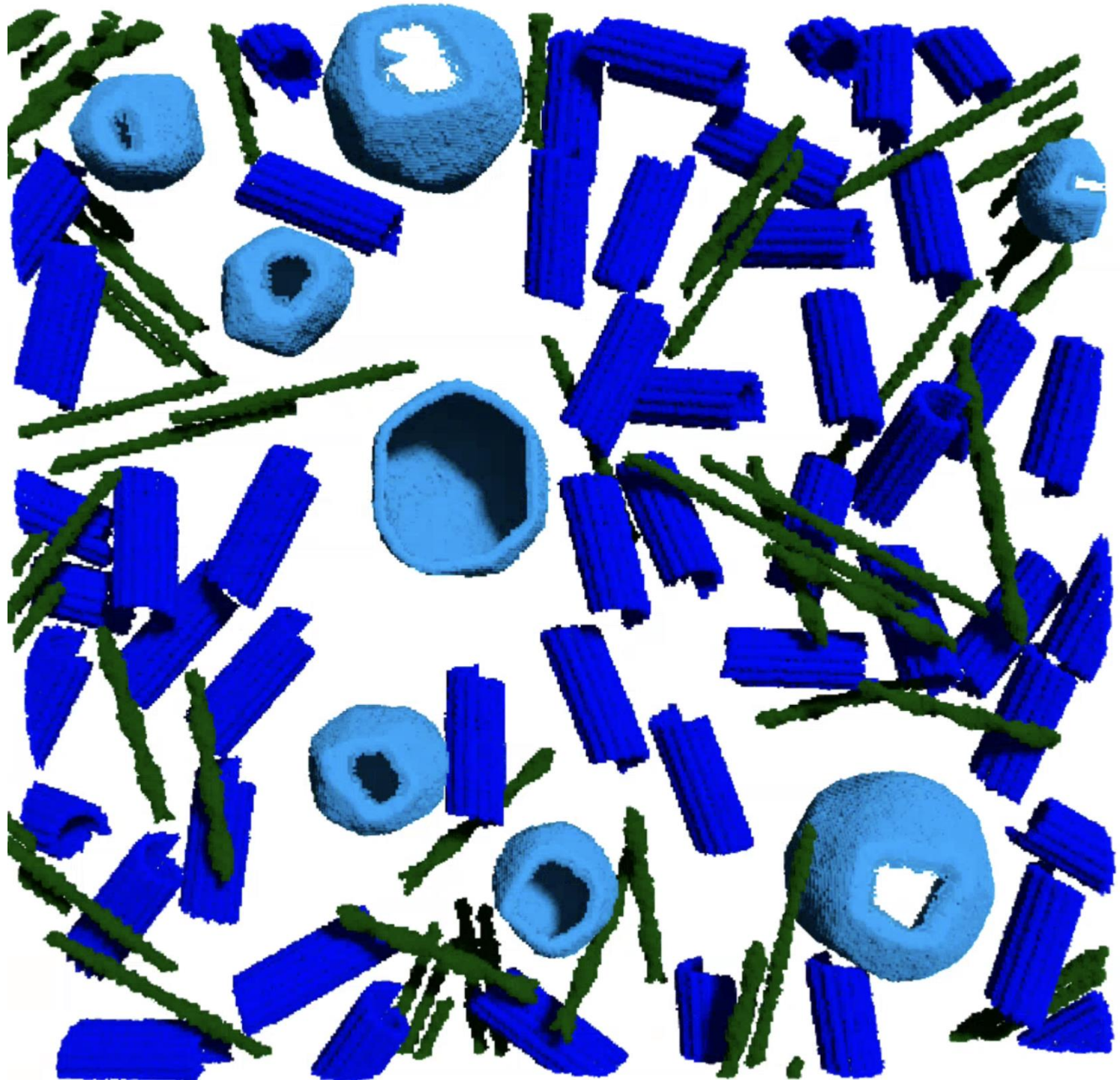
Denoised Tomo



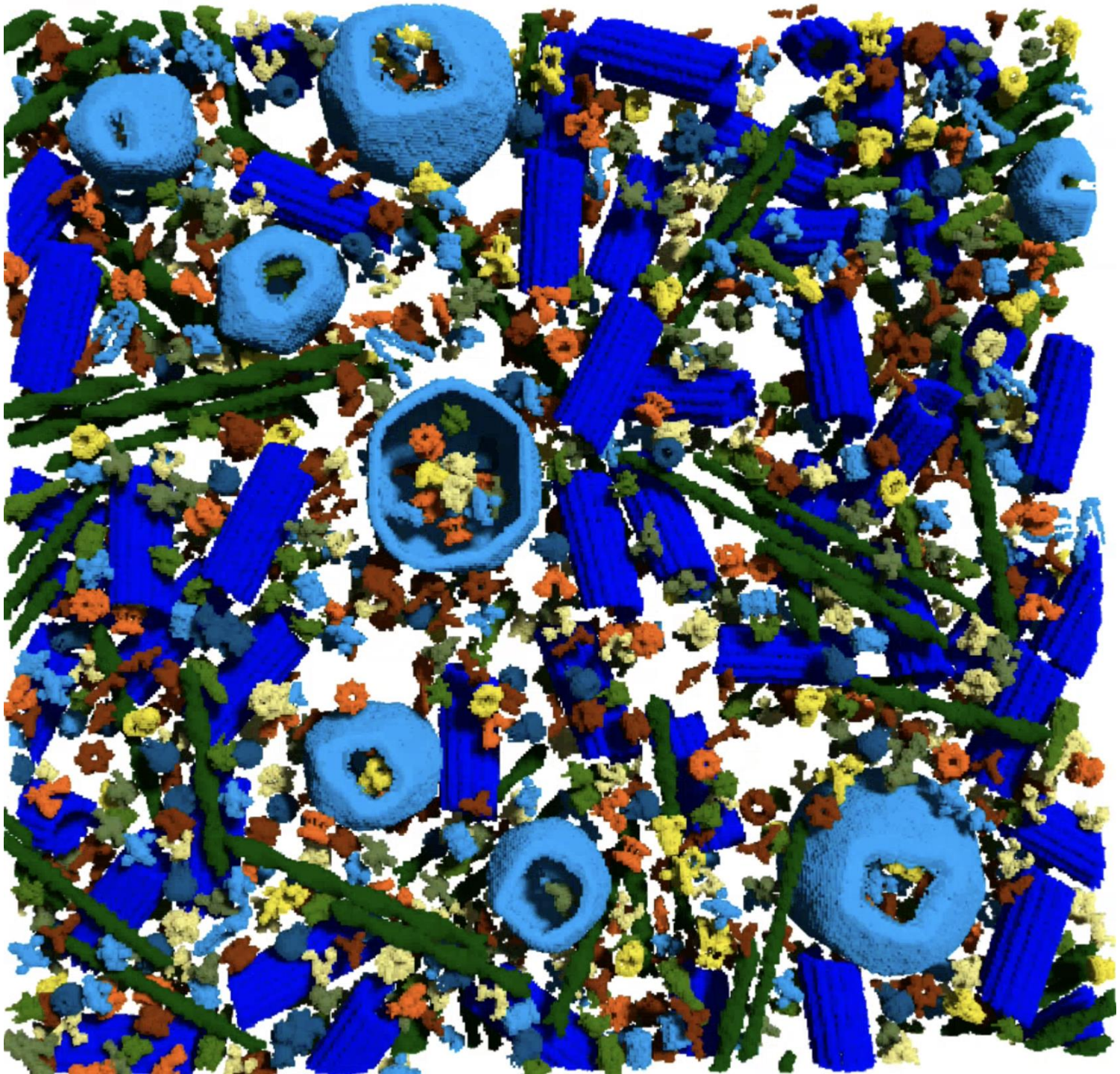
Membrane



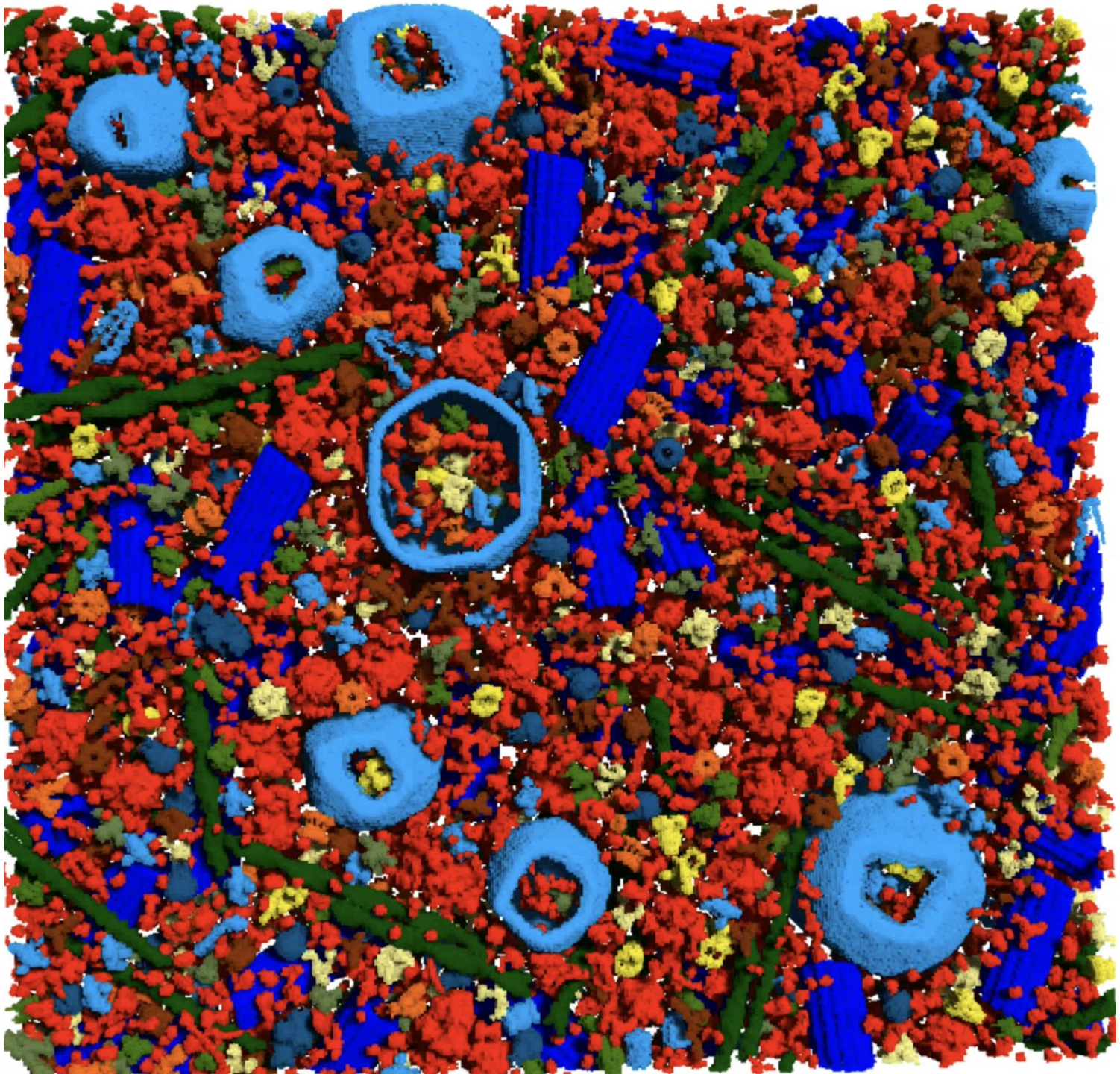
Cytoskeleton



Large Macromolecules
>200kDa

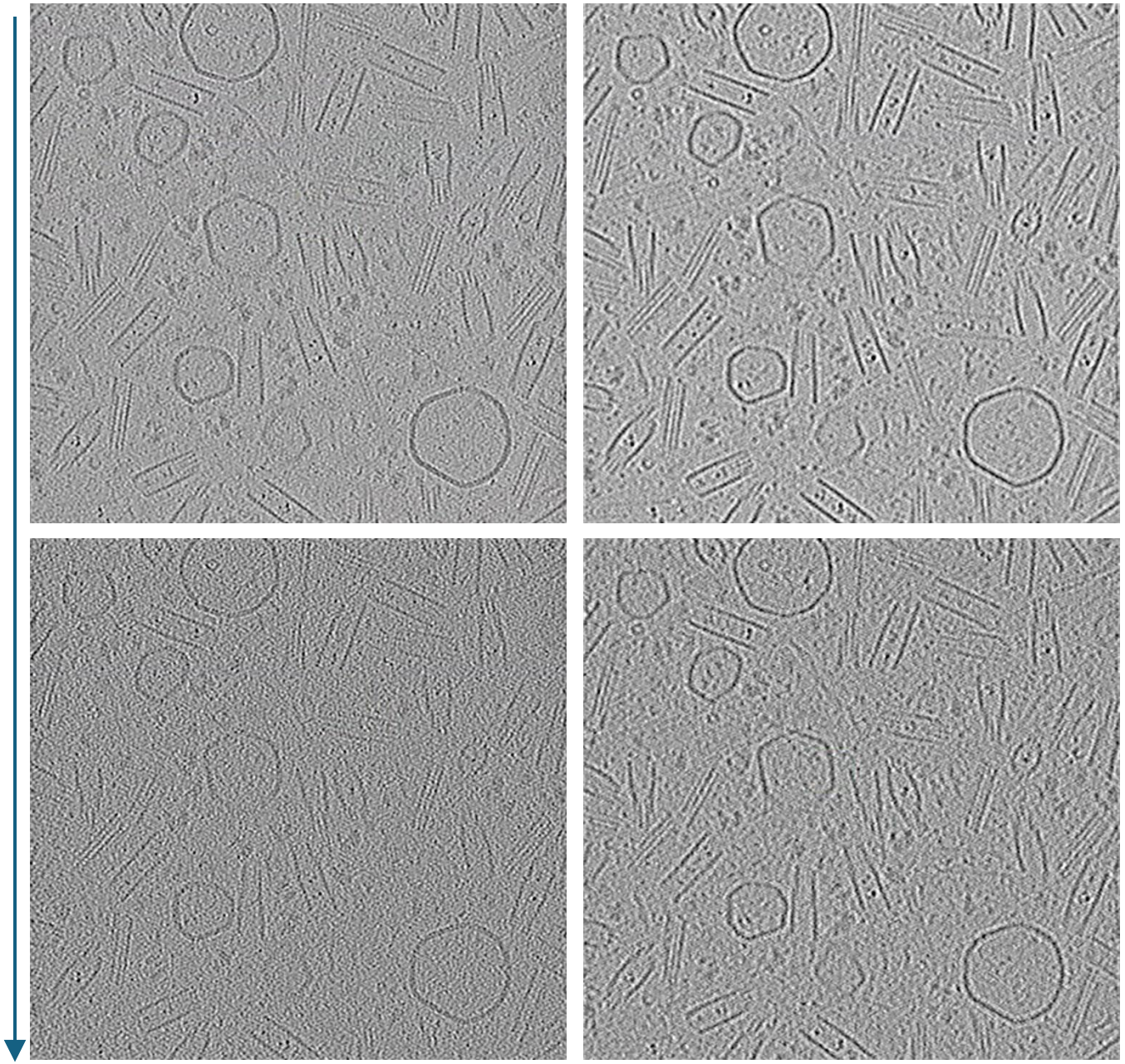


Small Macromolecules
<200kDa

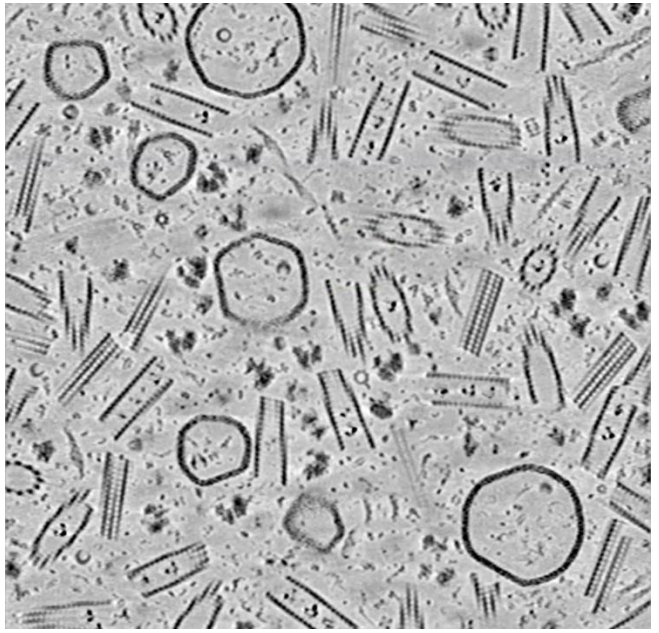


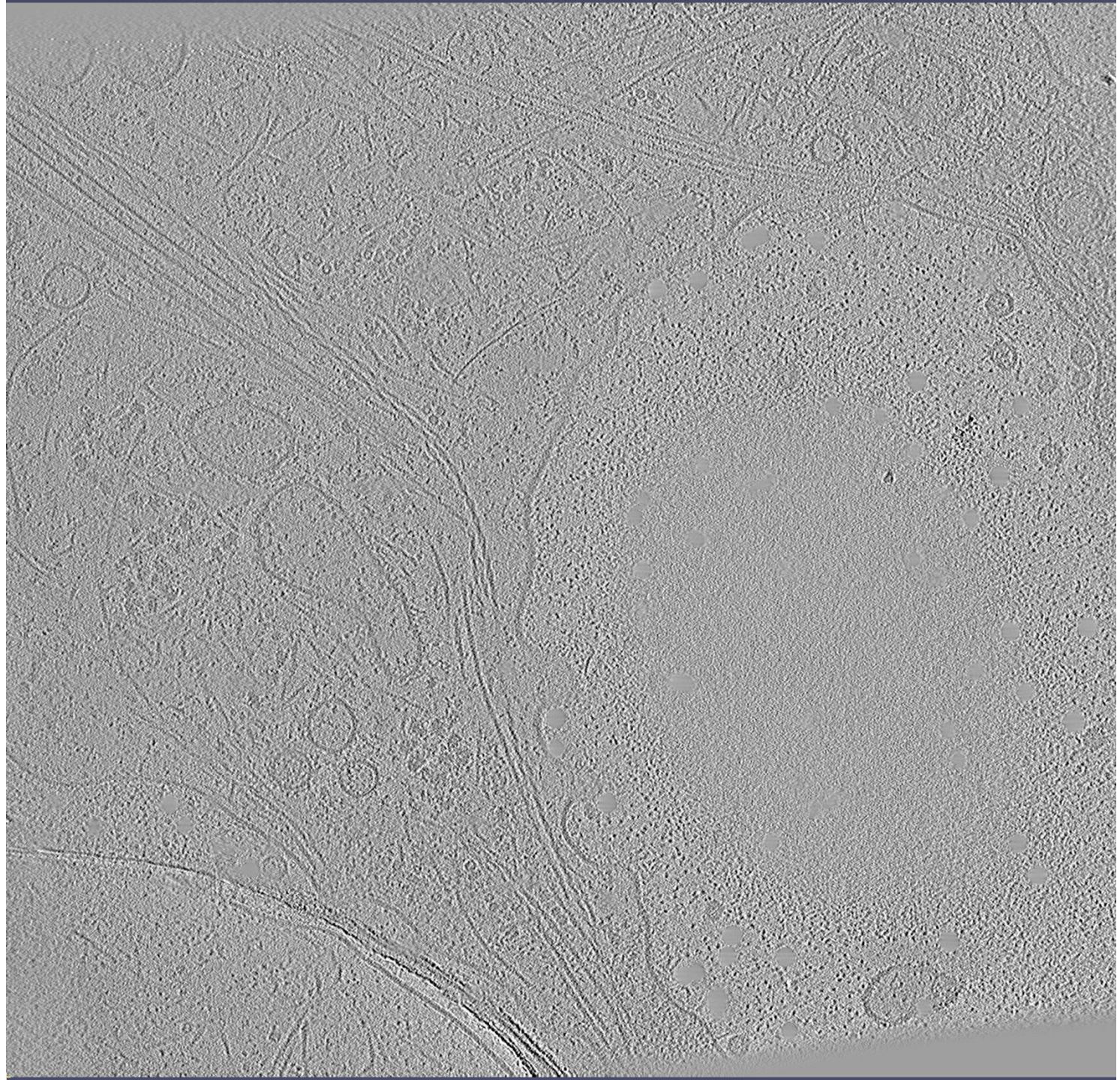
Defocus

SNR

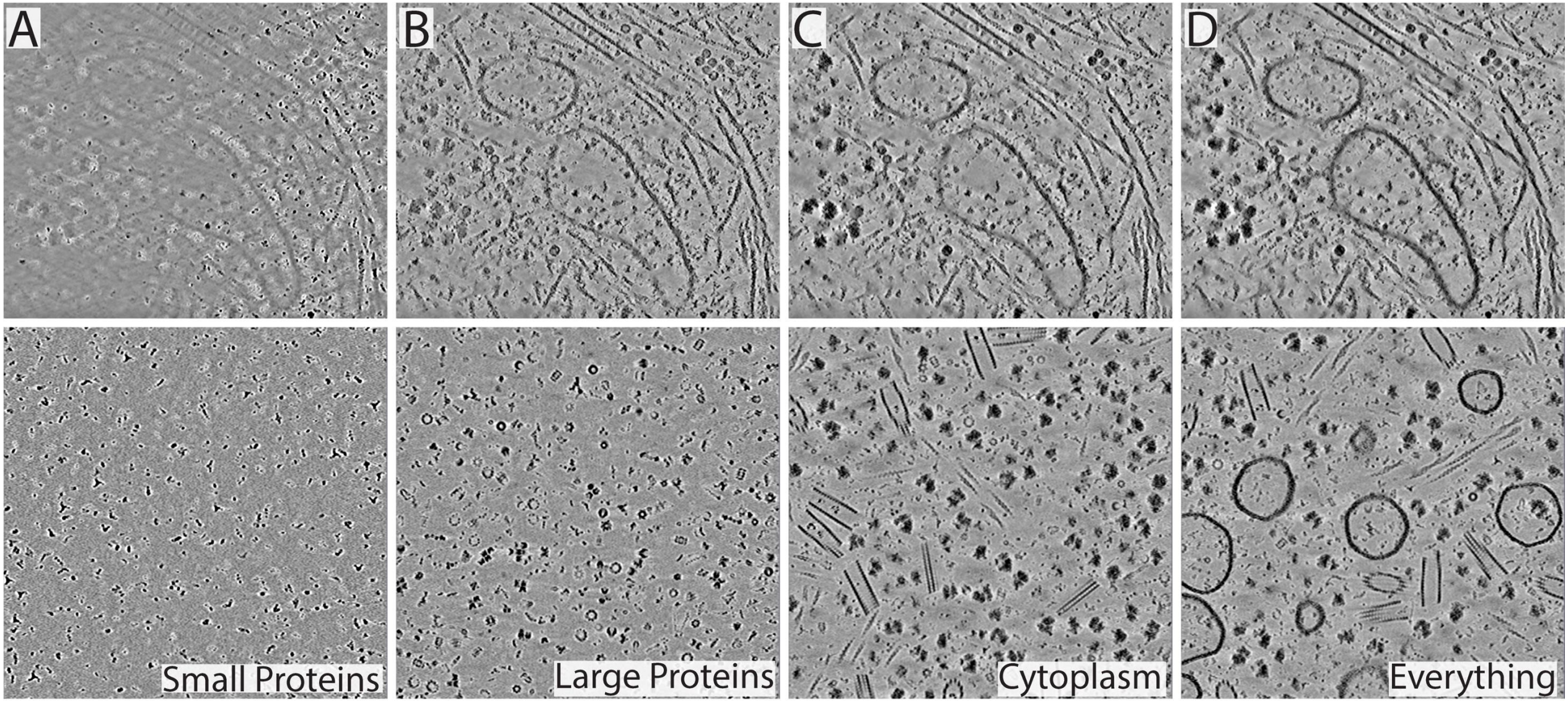


Ground



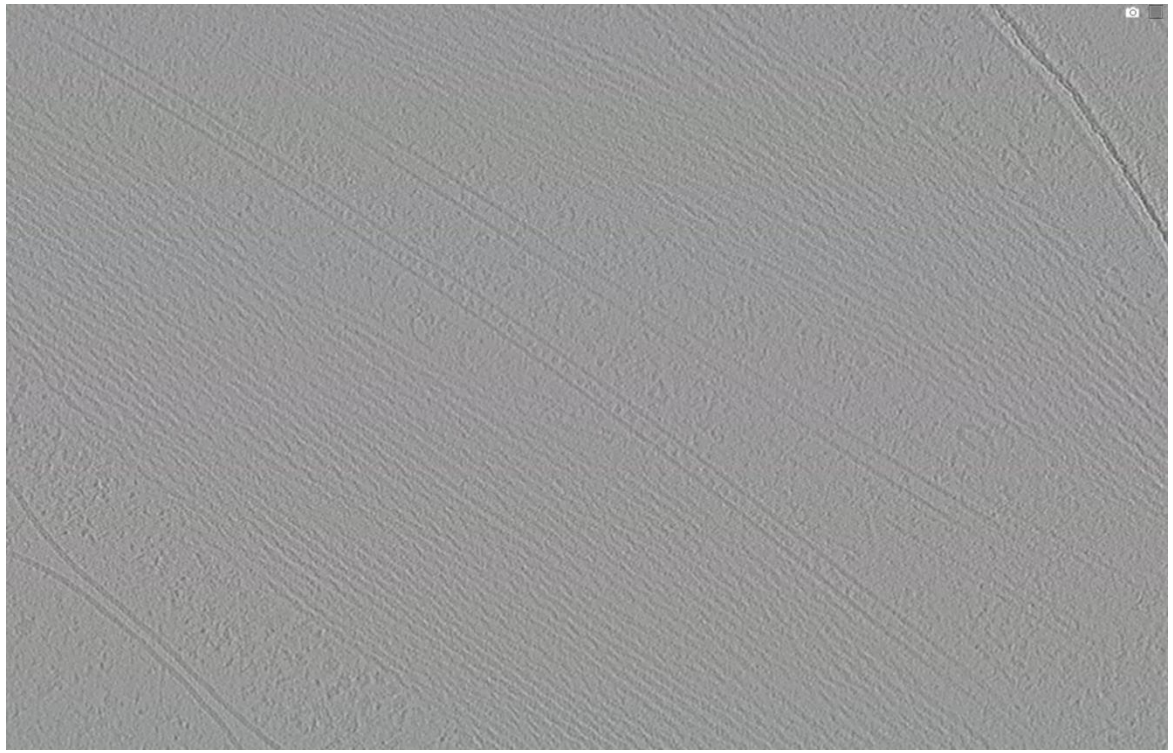


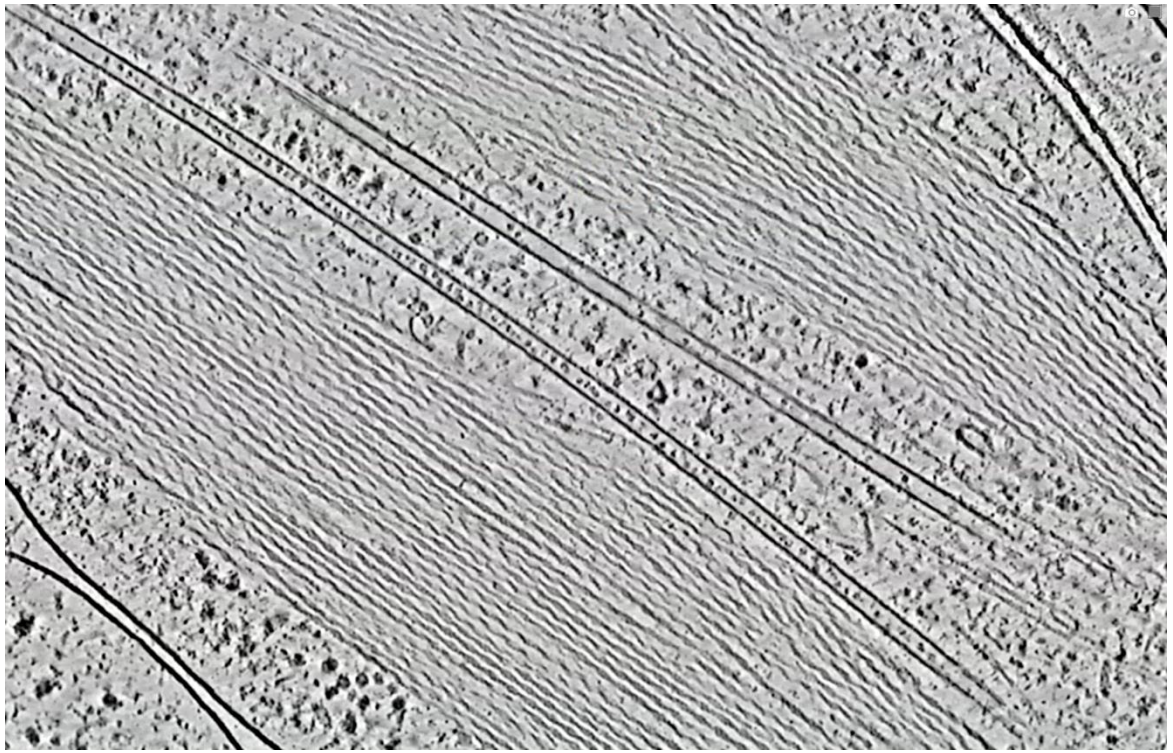
Diversify your inputs

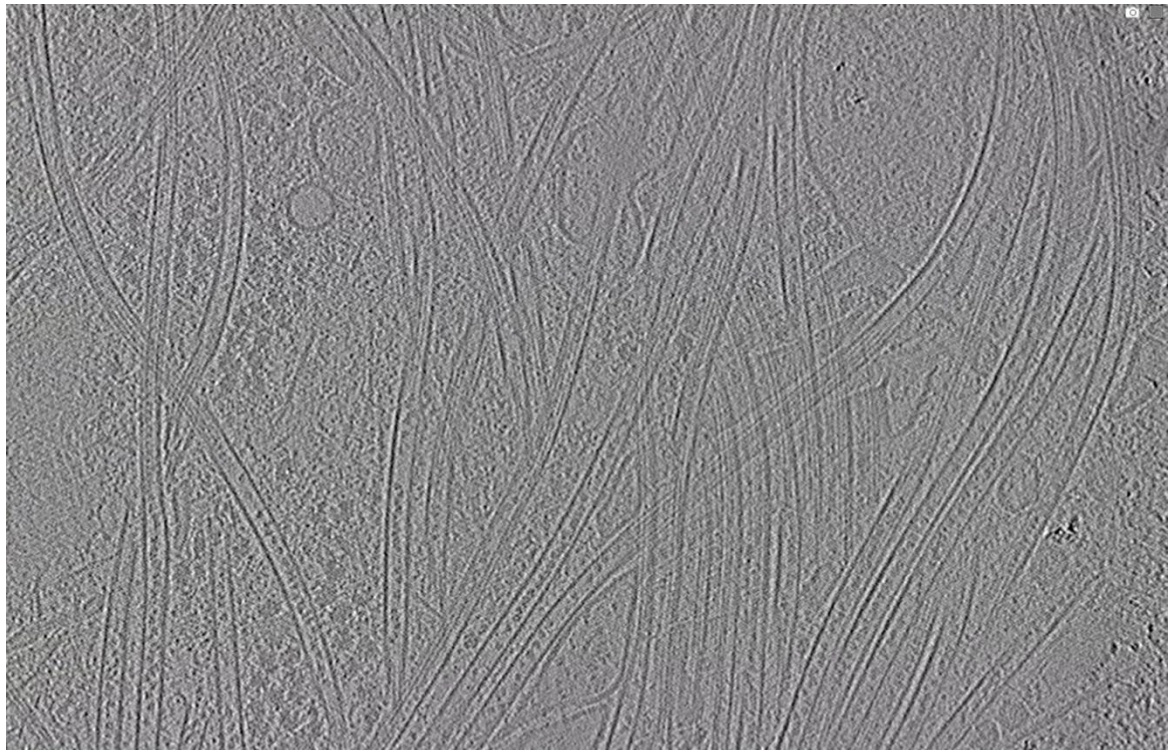


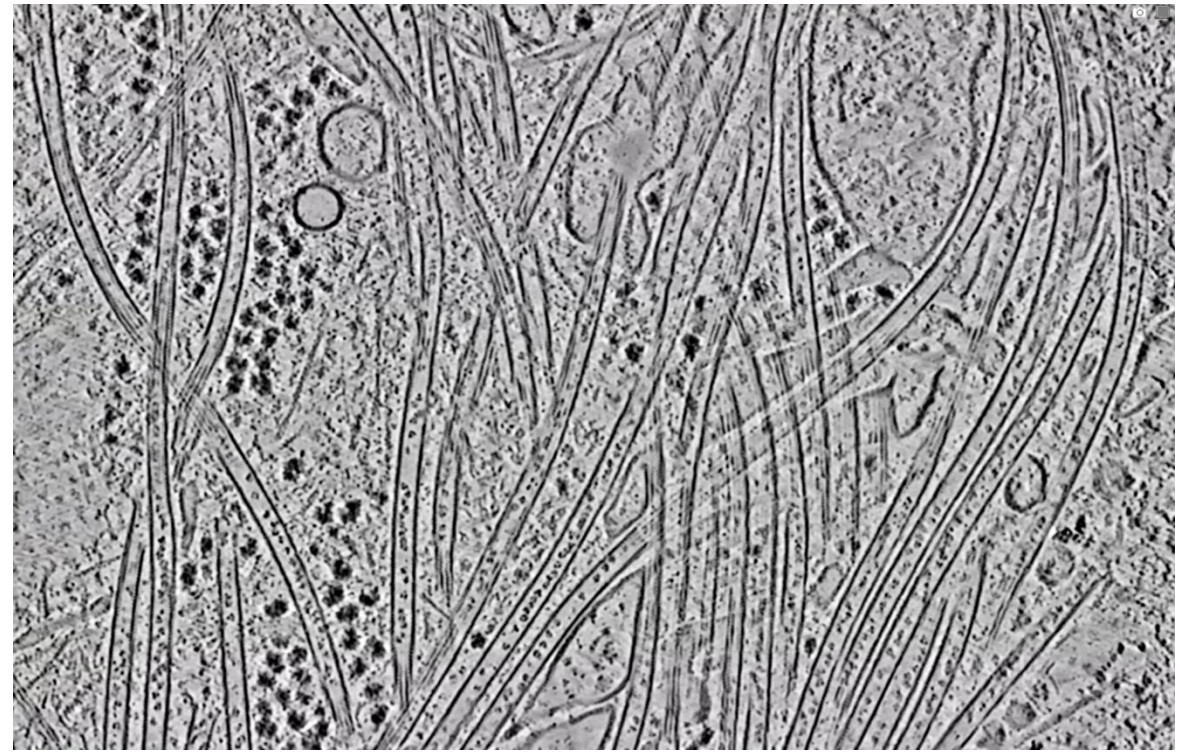
<200 kDa

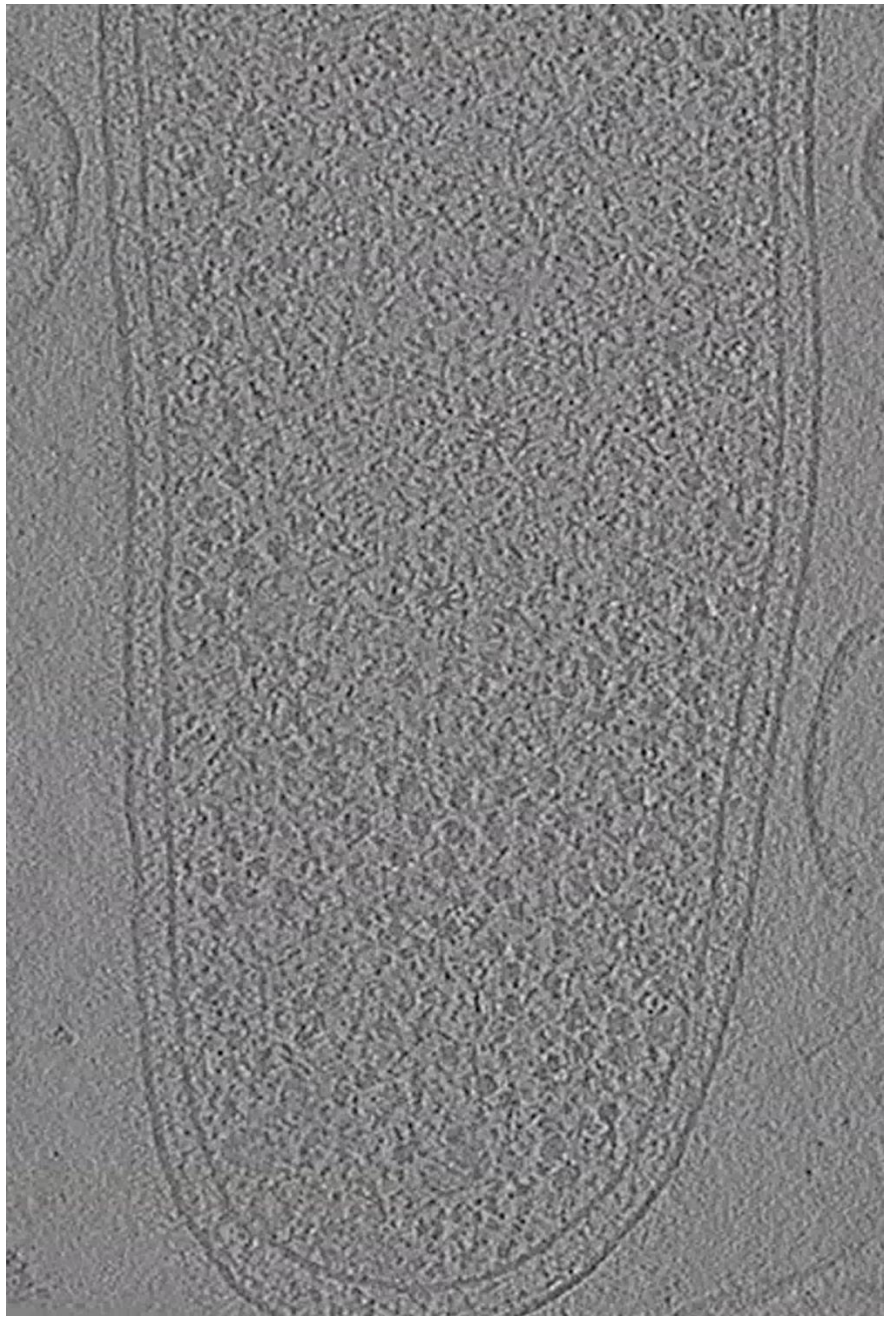
>200 kDa

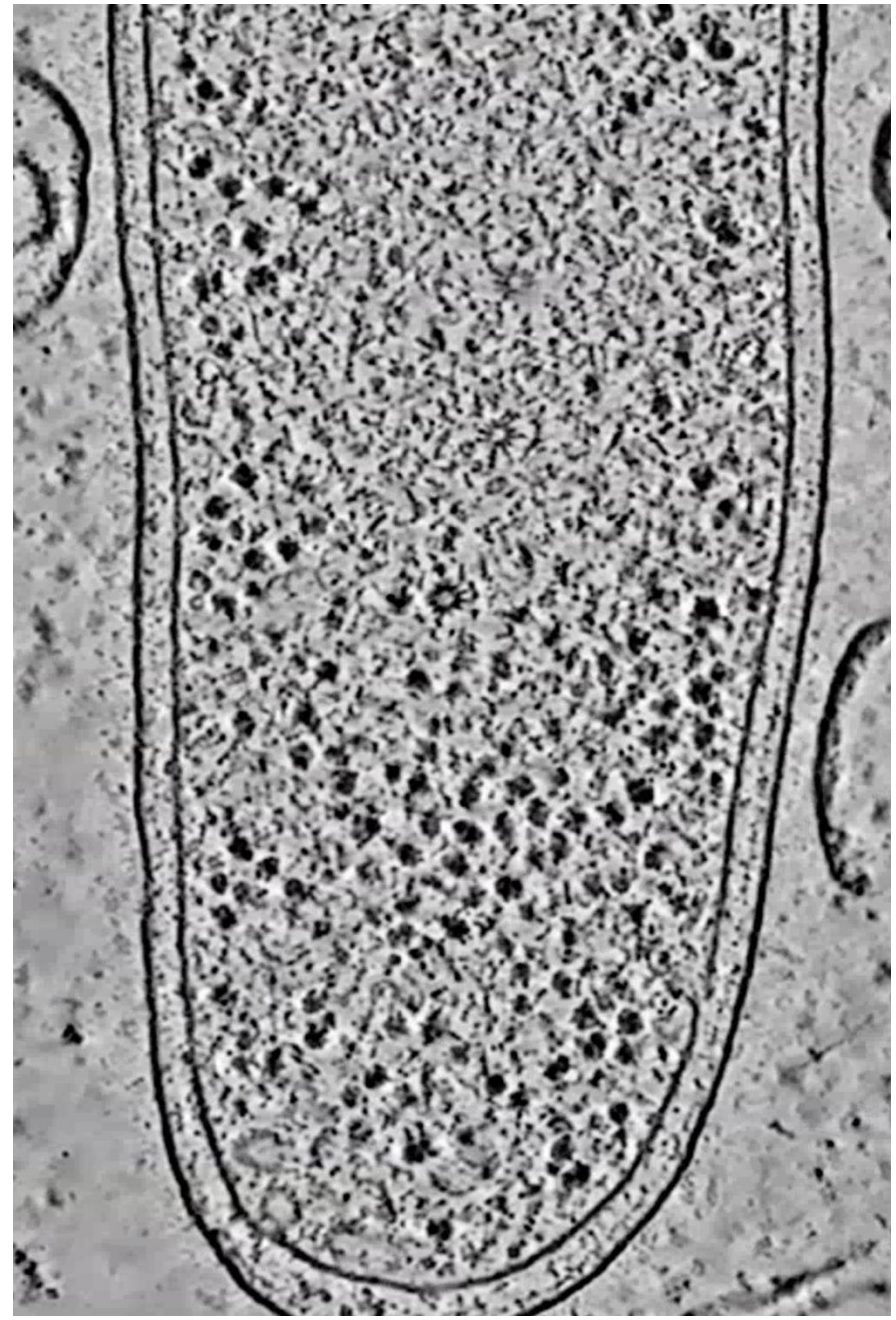


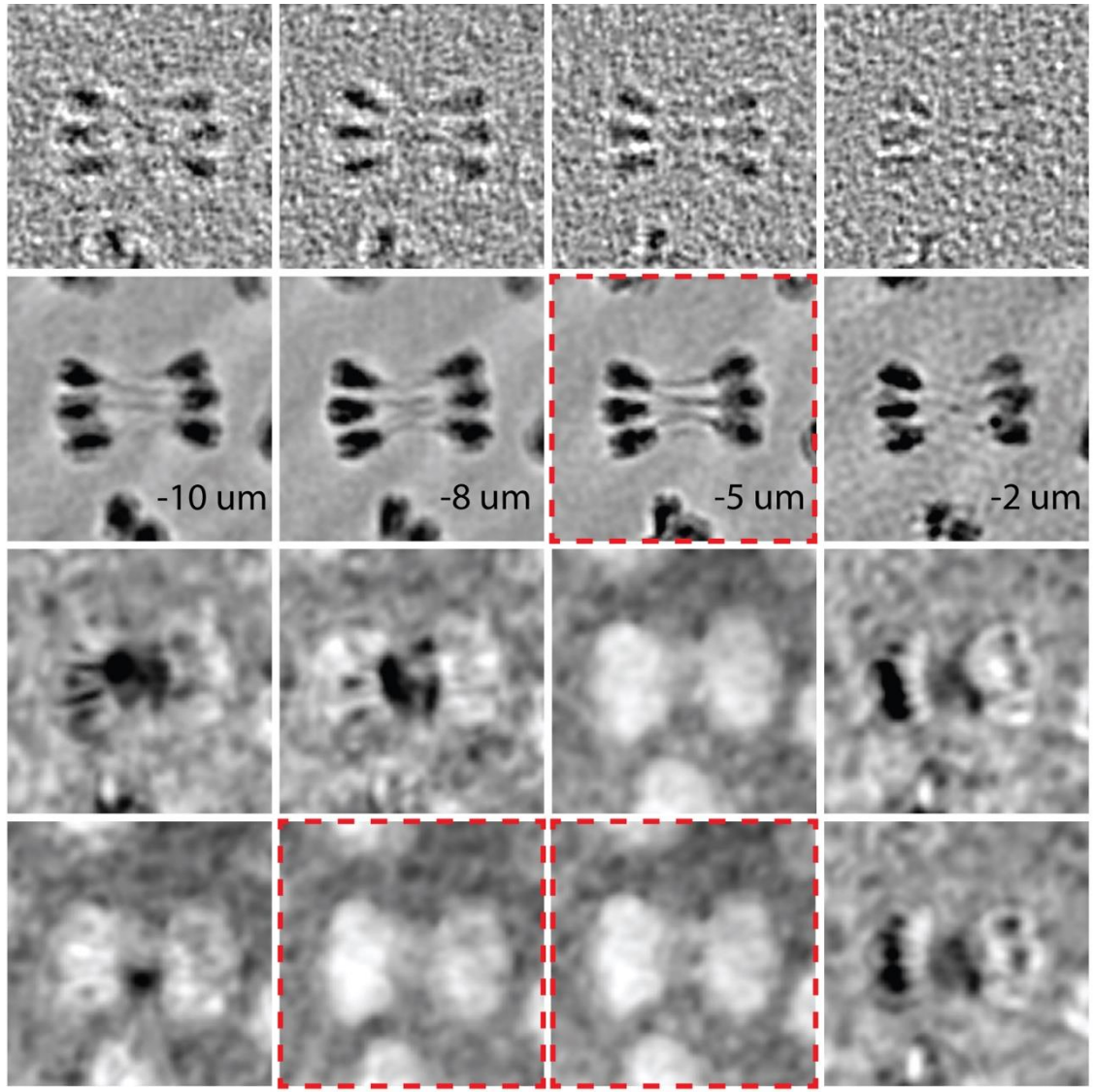


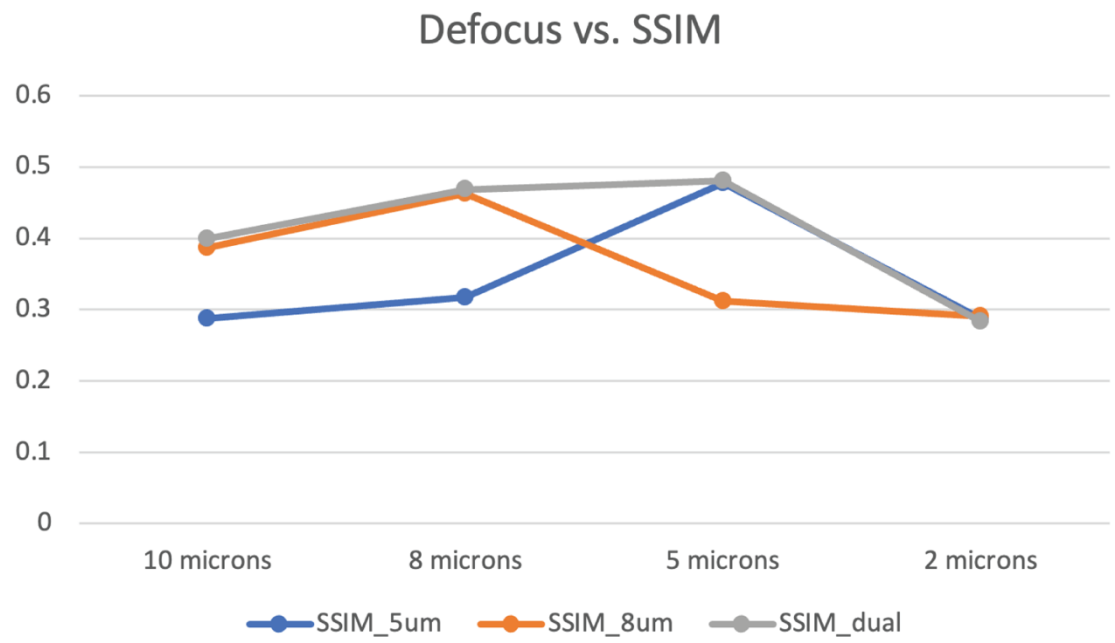









-10 um
-8 um
-5 um
-2 um


Defocus vs. SSIM
0.6
0.5
0.4
0.3
0.2
0.1
0
10 microns
8 microns
5 microns
2 microns
SSIM_5um
SSIM_8um
SSIM_dual

PennState
College of Medicine

Thanks for your attention!

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Carson Purnell
Michael Grillo
Steph Grillo
Linh Nguyen

Ex-Members
Ryan Hylton
Jessica Heebner

Collaborators
Neal Waxham
Sergei Grigoryev
Fred Heberle

DragonFly
Everyone

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