



(Shallow) Deep Learning for Cellular Tomogram Segmentation (and other things)

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Ogura, T. & Sato, C., **2001**, An automatic particle pickup method using a neural network applicable to low-contrast electron micrographs, *Journal of structural biology*, 136(3), pp. 227-38.

Pascual-Montano, A., Taylor, K.A., Winkler, H., Pascual-Marqui, R.D. & Carazo, J.M., **2002**, Quantitative self-organizing maps for clustering electron tomograms, *Journal of structural biology*, 138(1-2), pp. 114-22.

Hinton, G.E., Osindero, S. & Teh, Y.W., **2006**, A fast learning algorithm for deep belief nets, *Neural Comput*, 18(7), pp. 1527-54.

Wang, F., Gong, H., Liu, G., Li, M., Yan, C., Xia, T., Li, X. & Zeng, J., **2016**, DeepPicker: A deep learning approach for fully automated particle picking in cryo-EM, *Journal of structural biology*, 195(3), pp. 325-36.

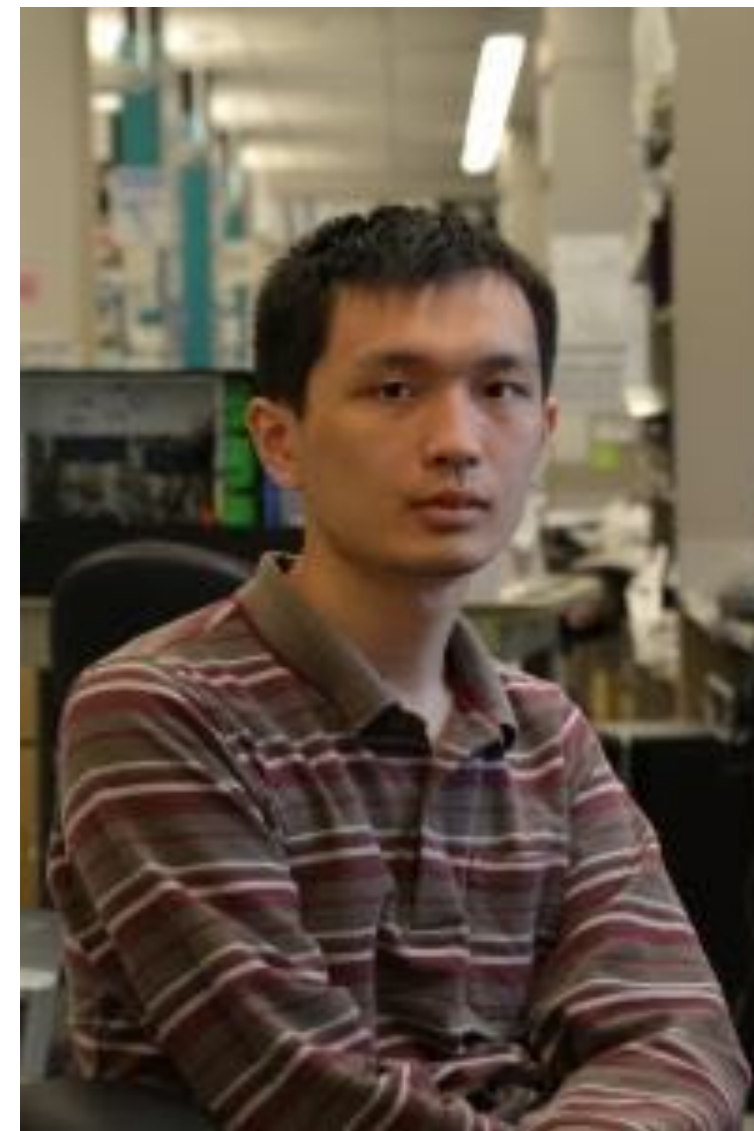
Zhu, Y., Ouyang, Q. & Mao, Y., **2017**, A deep convolutional neural network approach to single-particle recognition in cryo-electron microscopy, *BMC bioinformatics*, 18(1), p. 348.

Che, C., Lin, R., Zeng, X., Elmaaroufi, K., Galeotti, J., Xu, M. Improved deep learning based macromolecules structure classification from electron cryo tomograms, **2017**, arXiv:1707.04885.

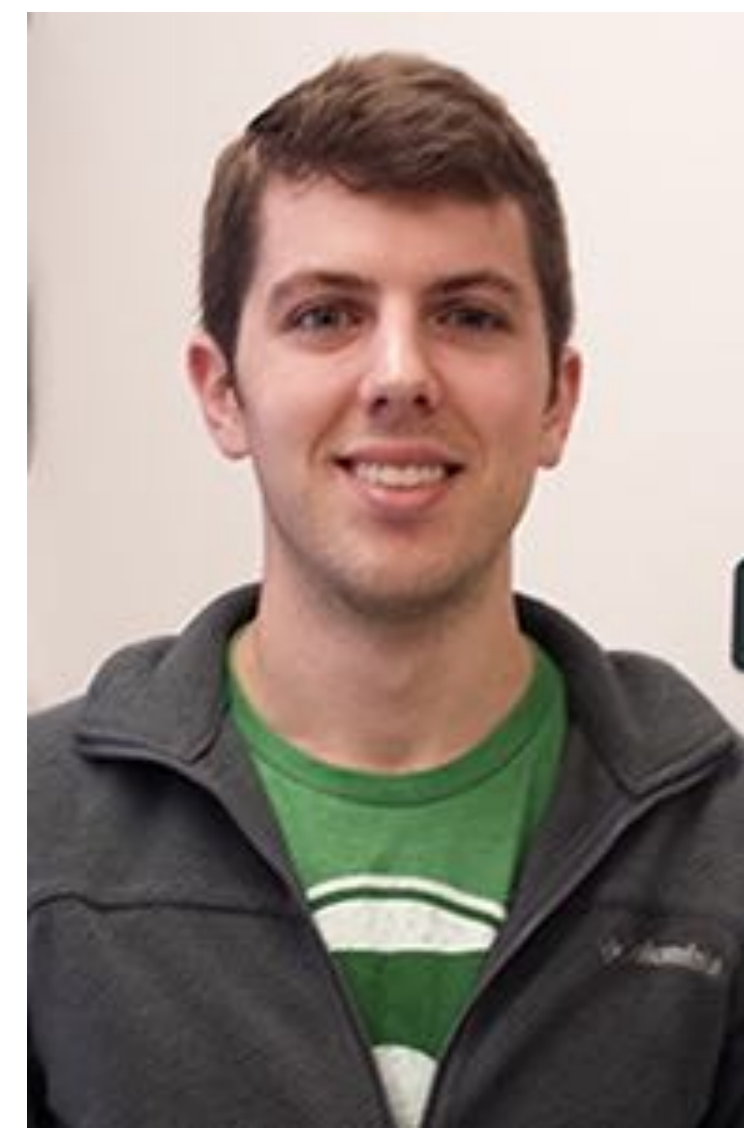
Chen, M., Dai, W., Sun, S.Y., Jonasch, D., He, C.Y., Schmid, M.F., Chiu, W. & Ludtke, S.J., **2017**, Convolutional neural networks for automated annotation of cellular cryo-electron tomograms, *Nature methods*.

Acknowledgements

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Muyuan Chen



James (Michael) Bell

Cyanobacteria
Wei Dai (Rutgers)

Trypanosome
Stella Sun (SLAC)
Cynthia He (NUS)

PC12 Neurite
Wei Dai (Rutgers)

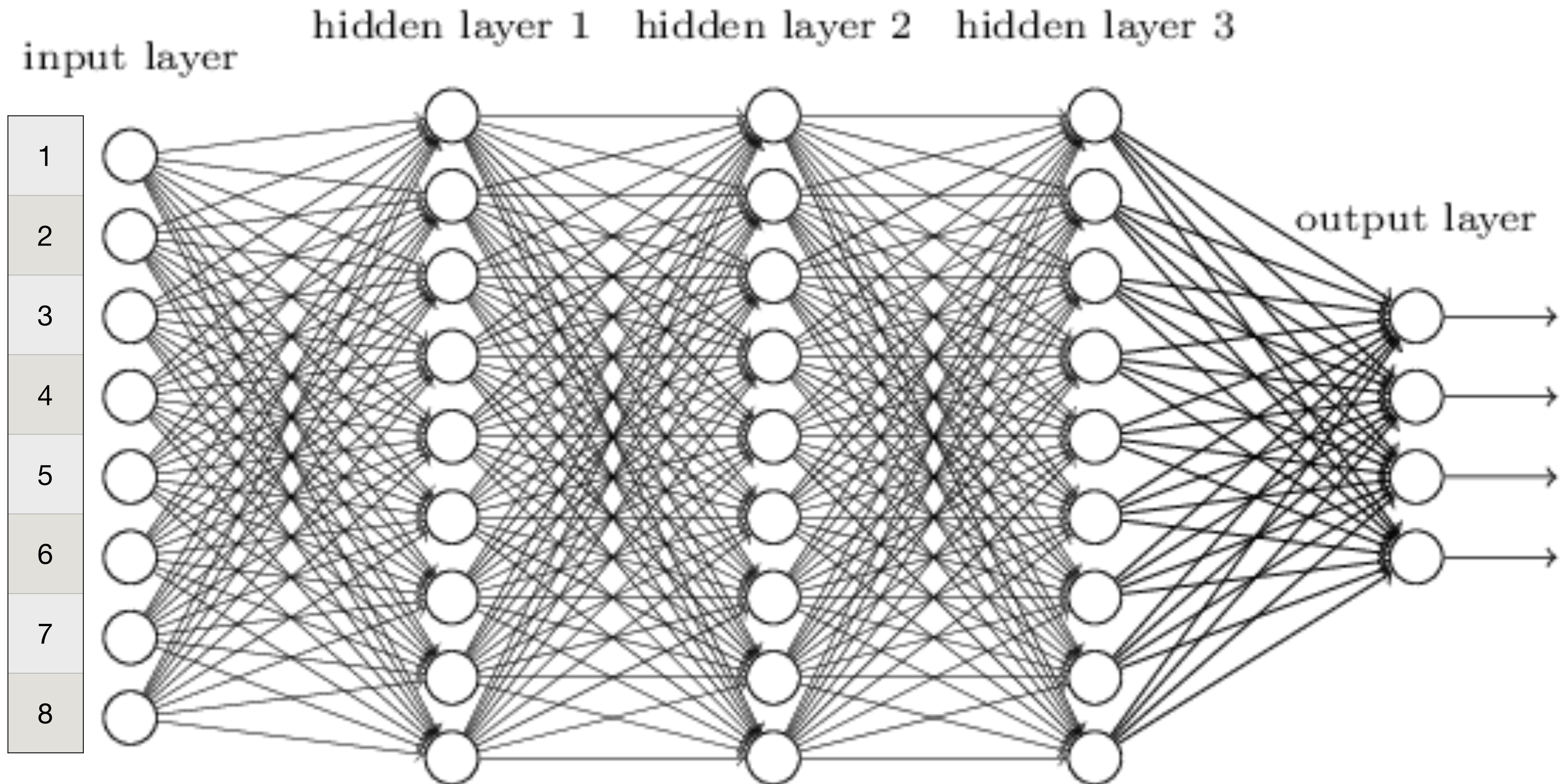
Mouse Neurite
Patrick Mitchell (SLAC)
Gong-her Wu (SLAC)

Wah Chiu, Mike Schmid (SLAC)

Chen, M., Dai, W., Sun, S.Y., Jonasch, D., He, C.Y., Schmid, M.F., Chiu, W. & Ludtke, S.J., 2017, Convolutional neural networks for automated annotation of cellular cryo-electron tomograms, *Nature methods*.

Movie of Tomogram Excluded

Feedforward Neural Network



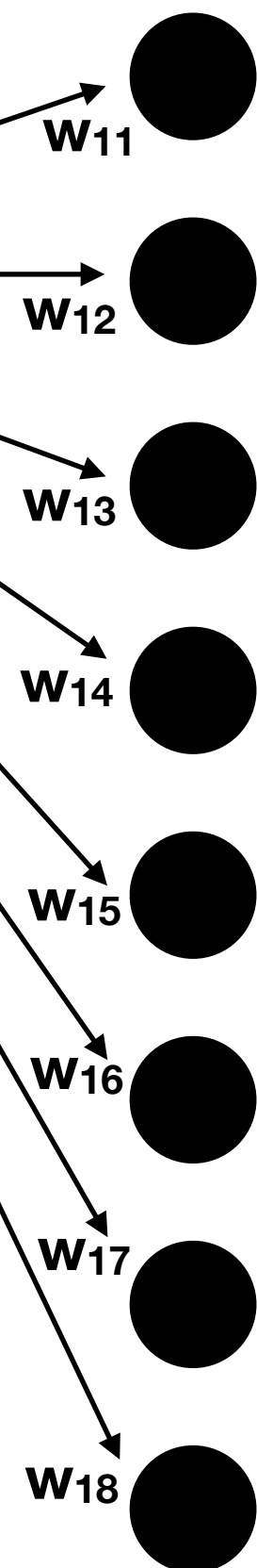
Feedforward Neural Network

input
layer

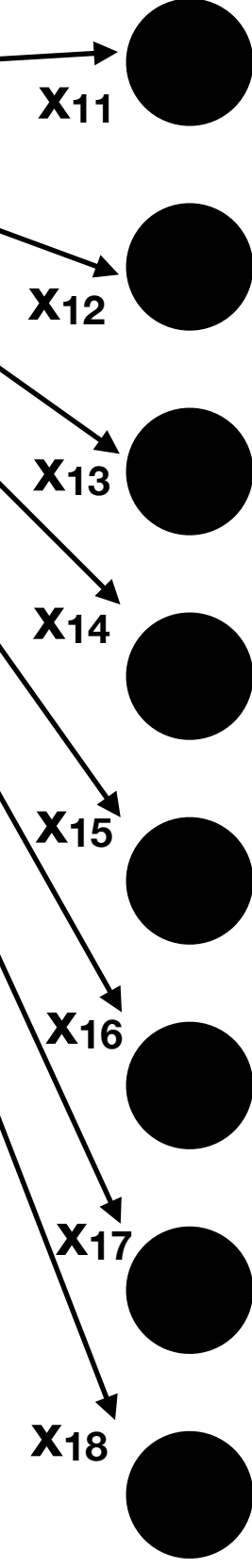
1
2
3
4
5
6
7
8

pixels

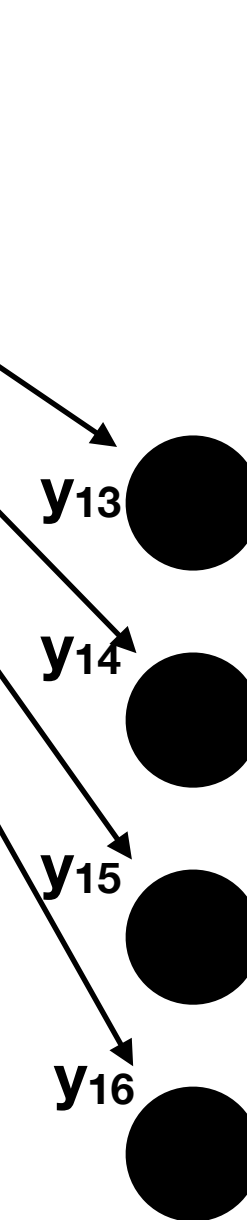
hidden
layer



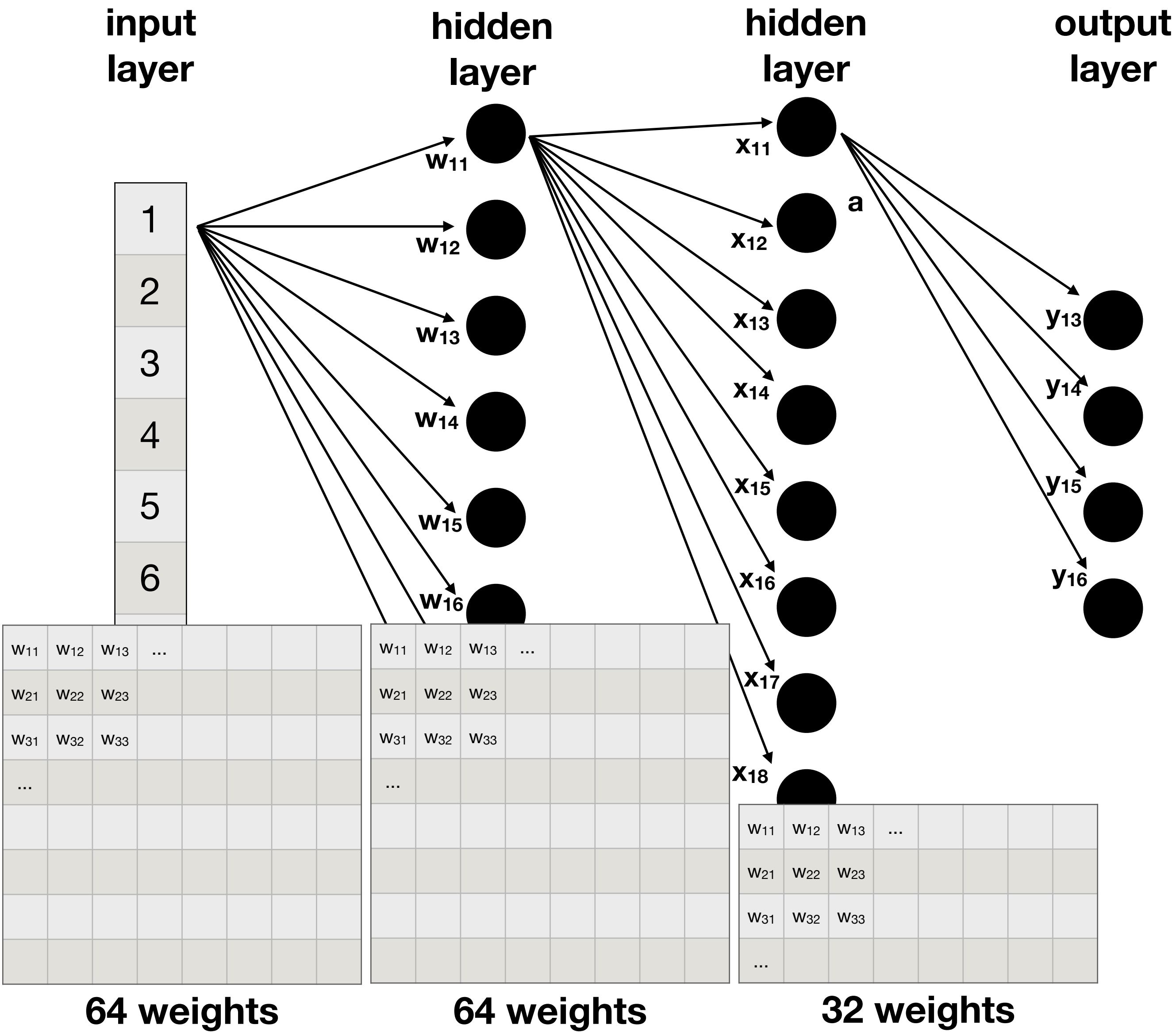
hidden
layer



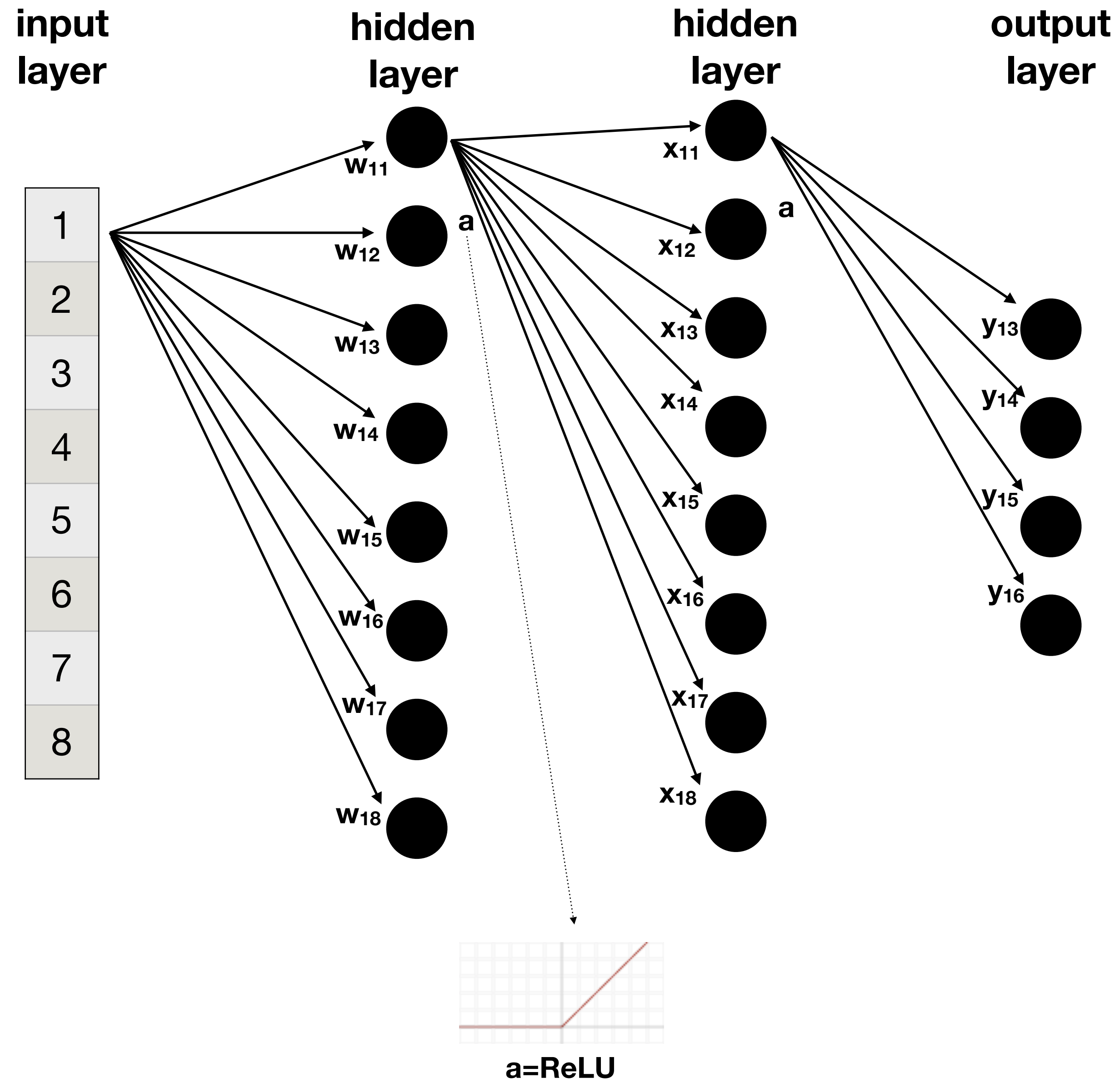
output
layer



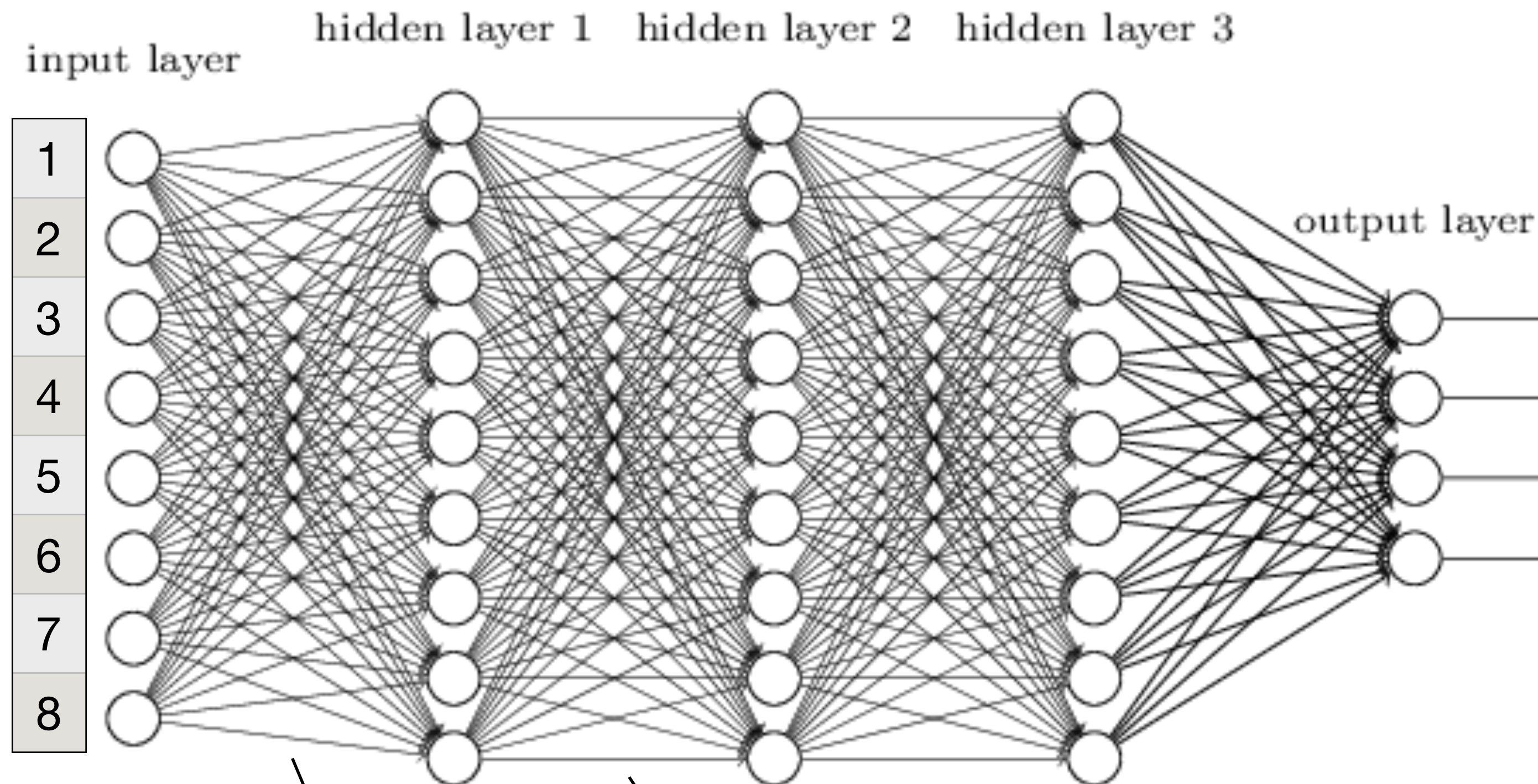
Feedforward Neural Network



Feedforward Neural Network



Feedforward Neural Network



100x100 pixel image = 10,000 neurons

$10,000^2$ connections = 10^8 weights

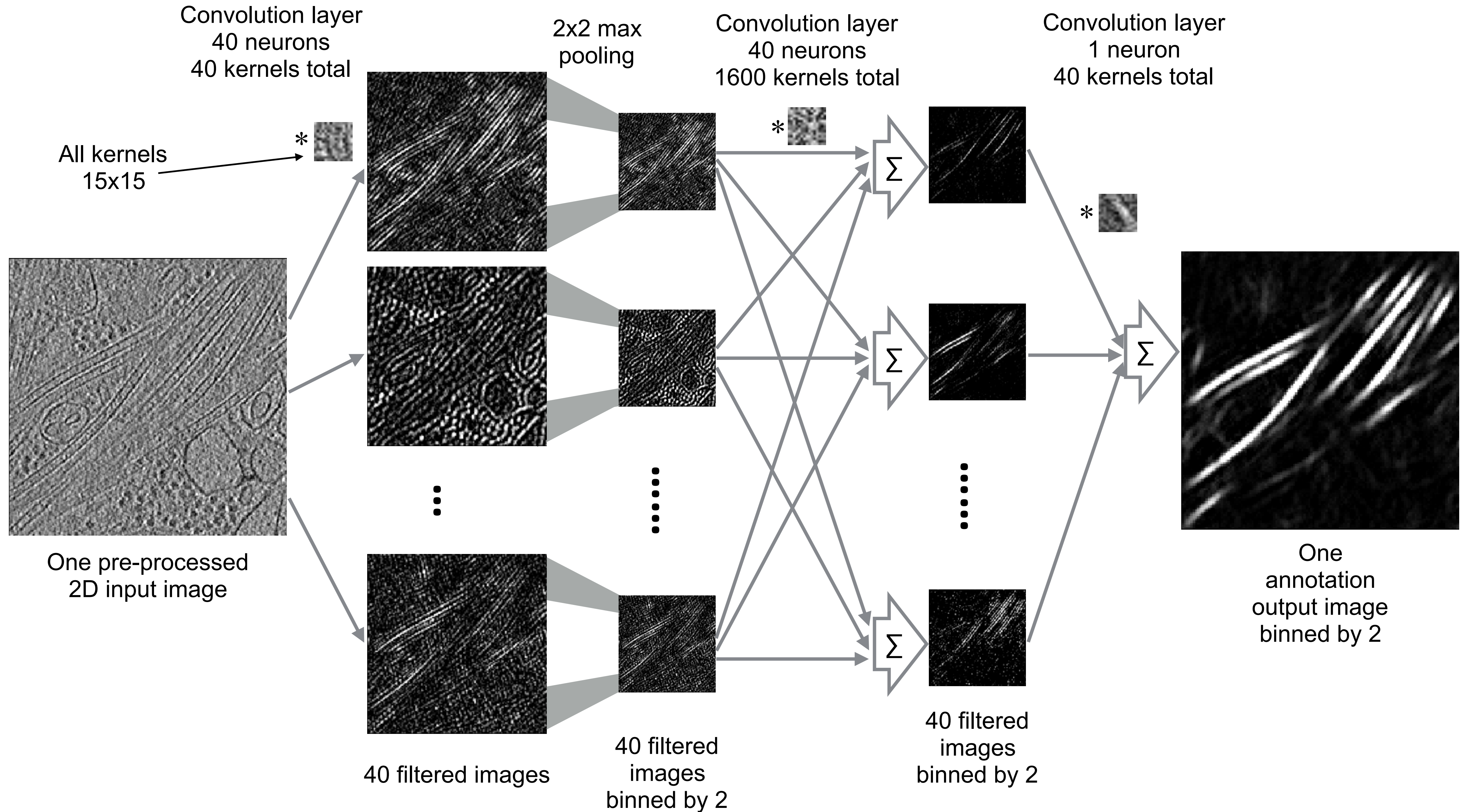
per layer

... and it's extremely inefficient (no translational equivalence)

Deep Learning

- Many different concepts
 - Deep networks
 - Convolutional networks
 - Modules
 - Autoencoders
 - Recursive Neural Networks
 - Long Short-Term Memory
 - ...

Convolutional Neural Network (CNN)

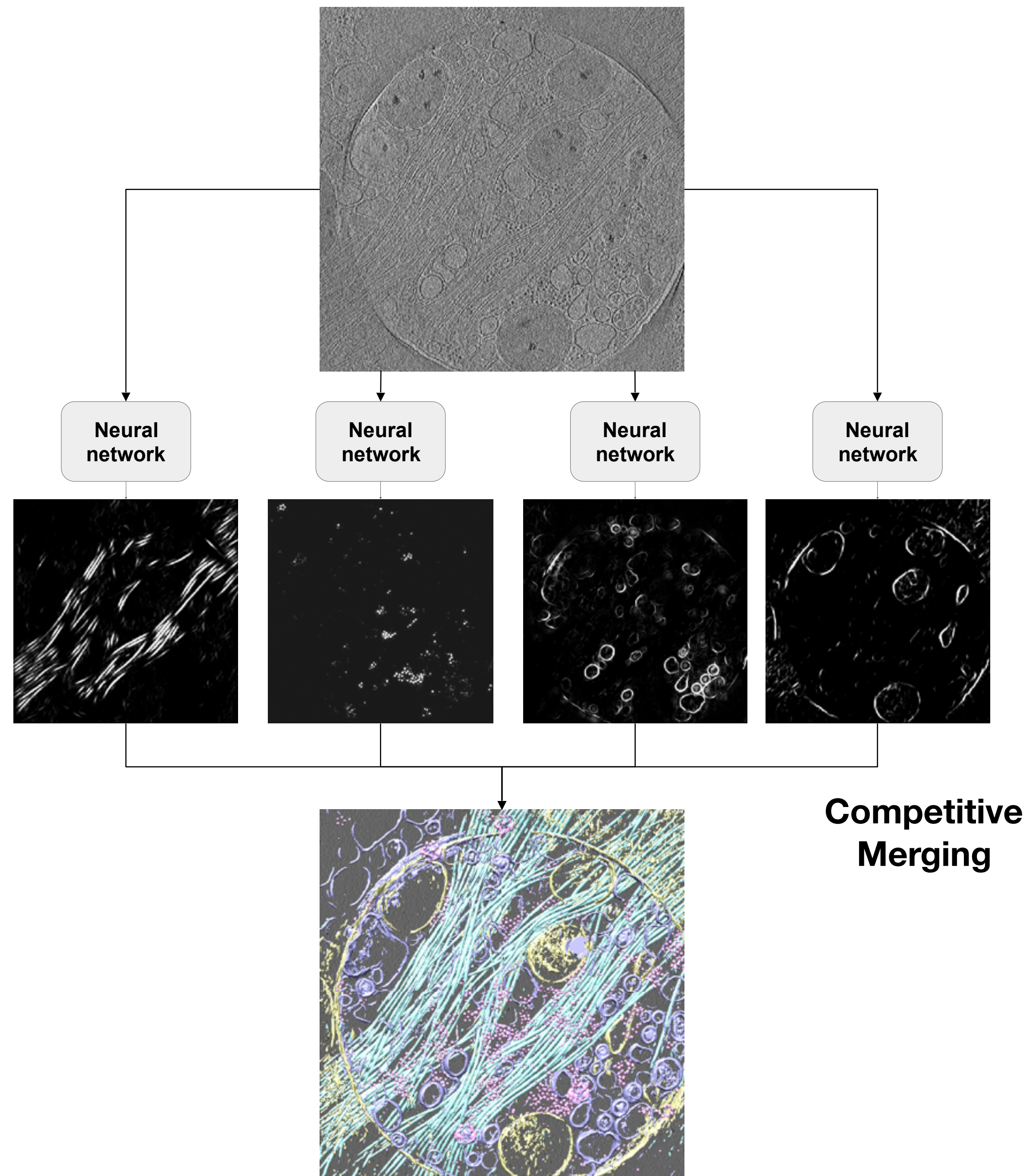


Deep or Shallow?

- Deep:
 - Extremely flexible, one network could handle many different features and cell types
 - Extremely expensive to train, may not converge
- Shallow:
 - Easy to train. Fixed network structure possible
 - Limited Scope, Focused on one user and one feature

Convolutional Neural Networks

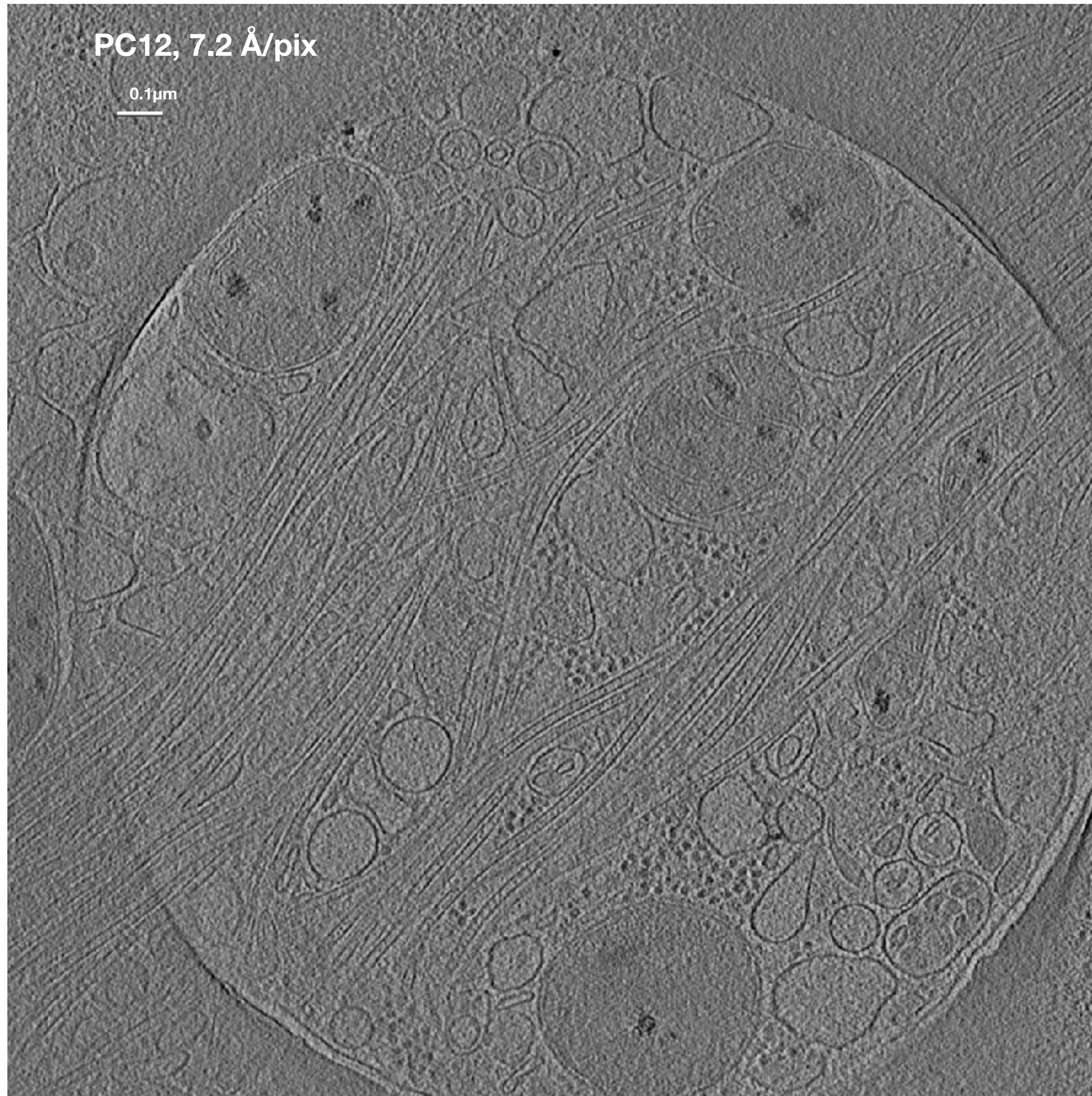
- Mimic a human annotator
- One network = 1 feature x 1 annotator
- Easily trained with a small number of regions

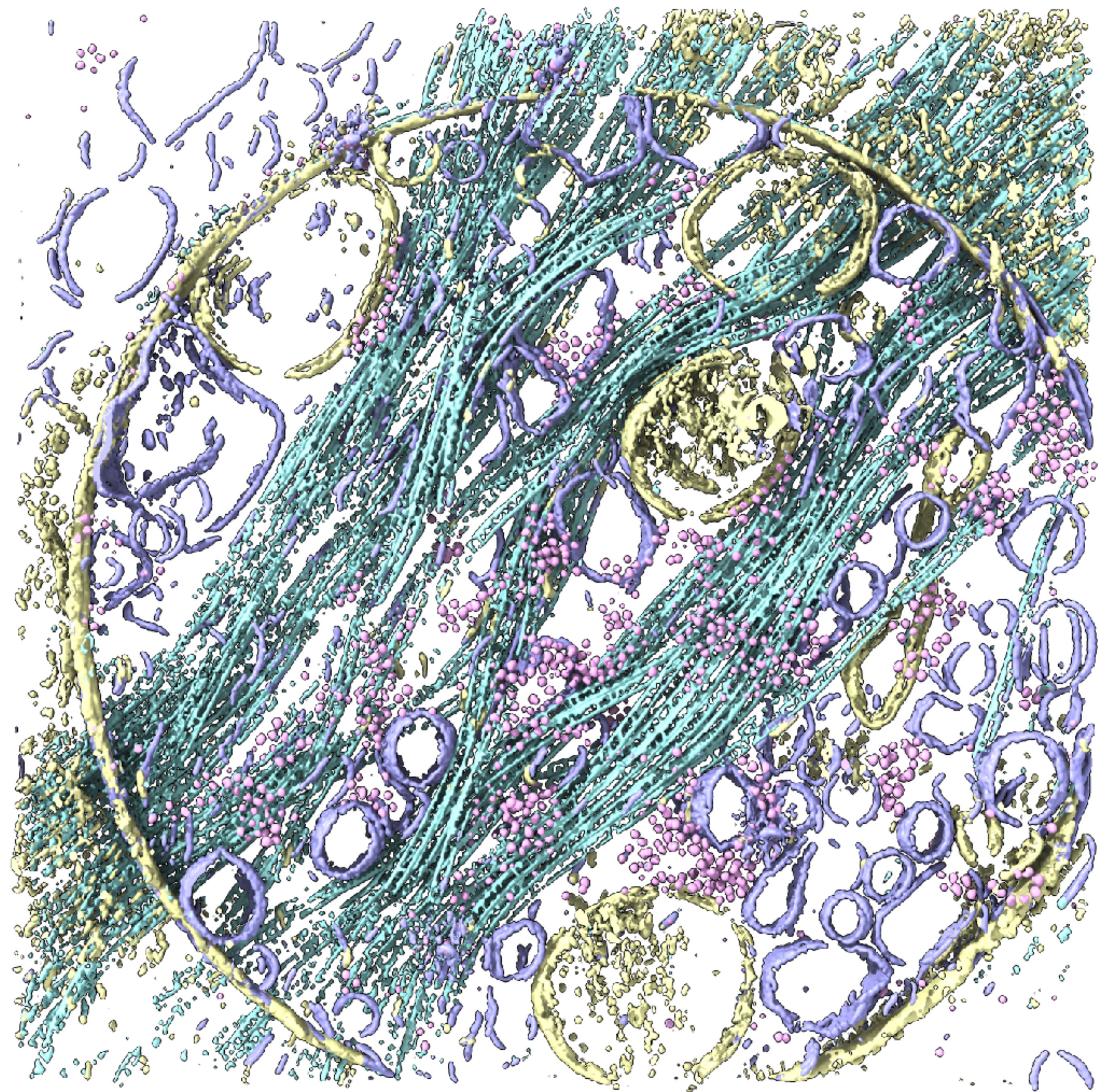


Movie showing usage deleted

PC12, 7.2 Å/pix

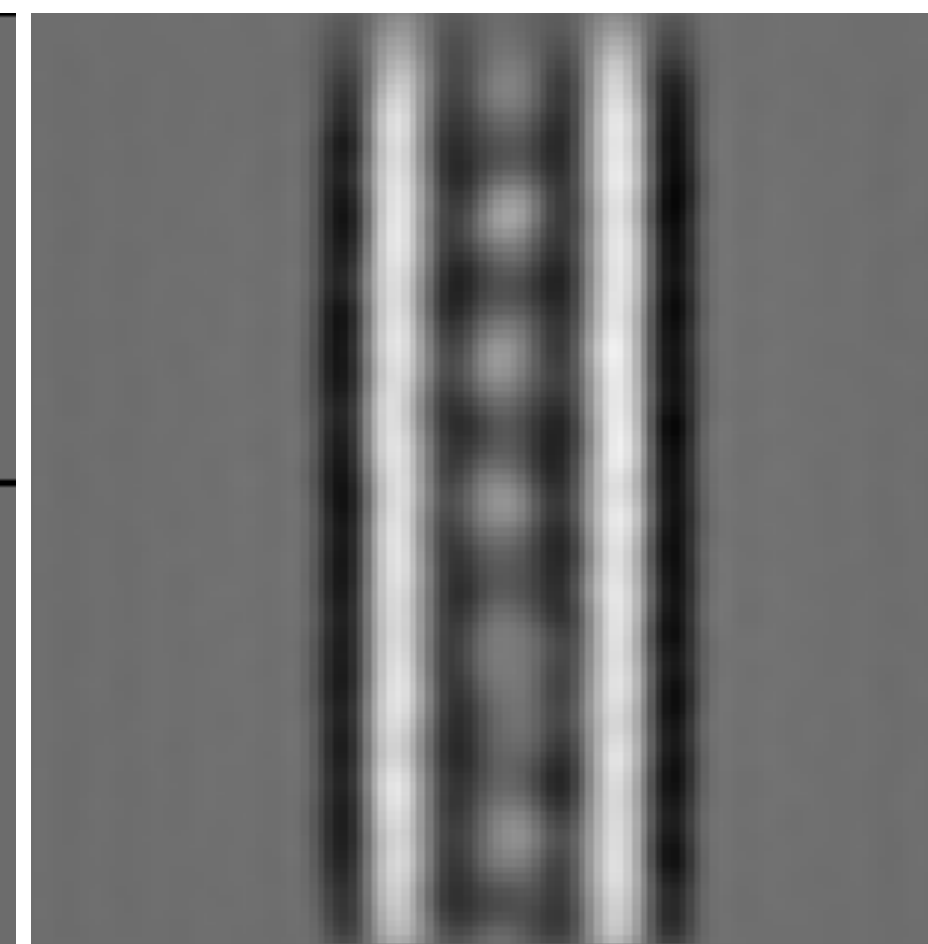
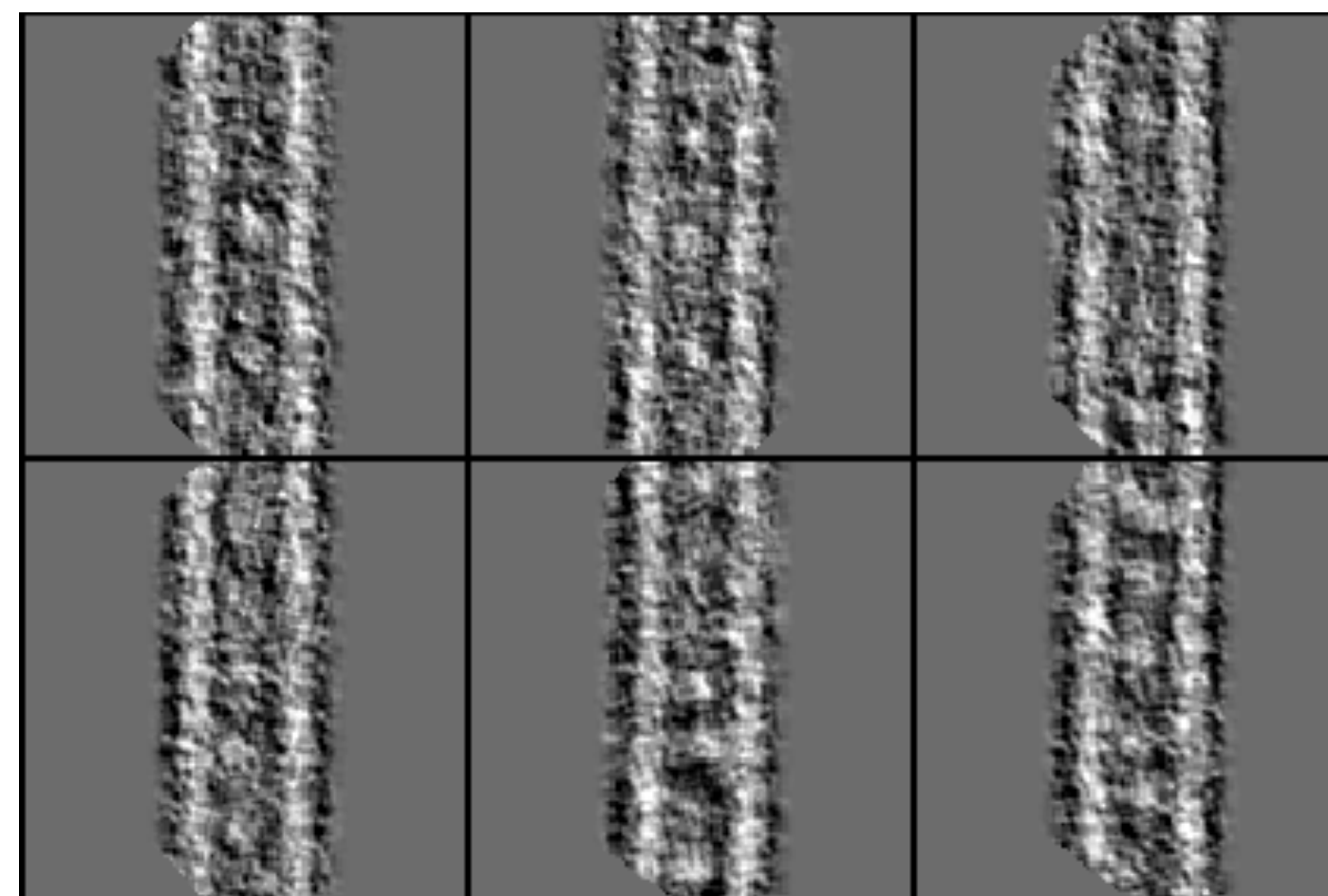
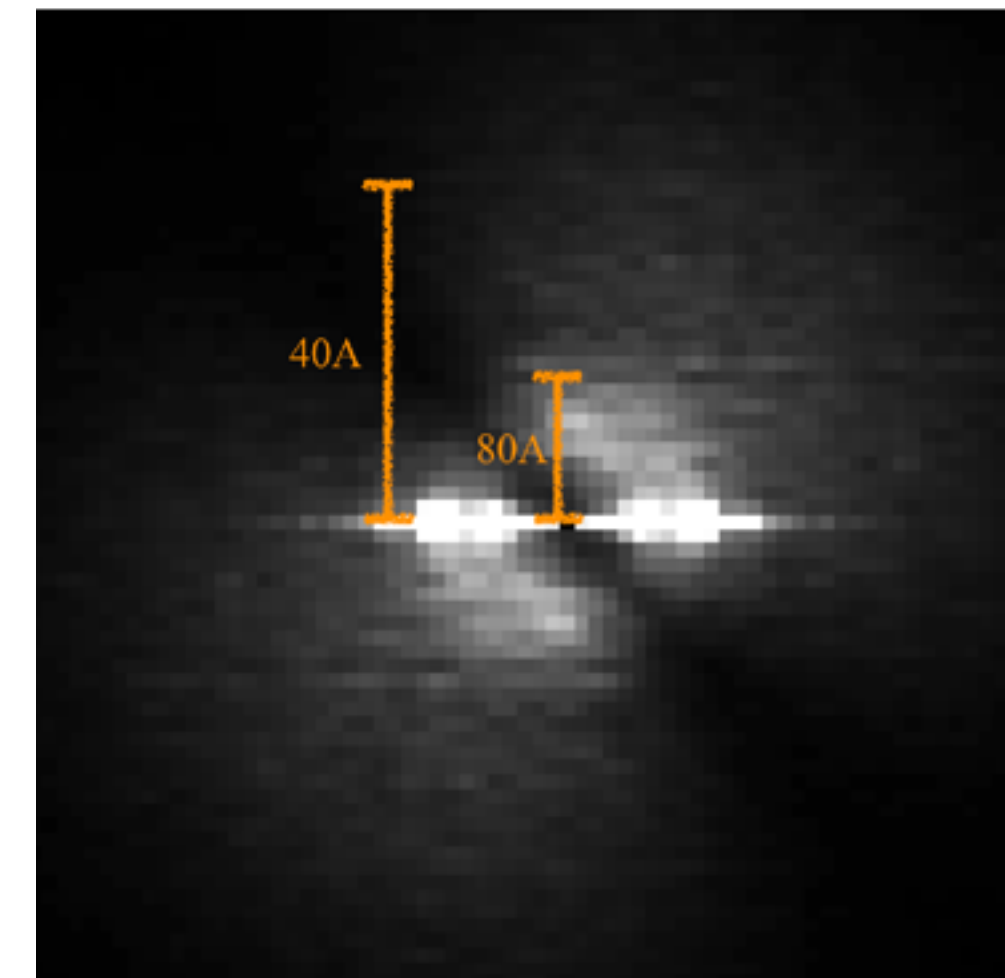
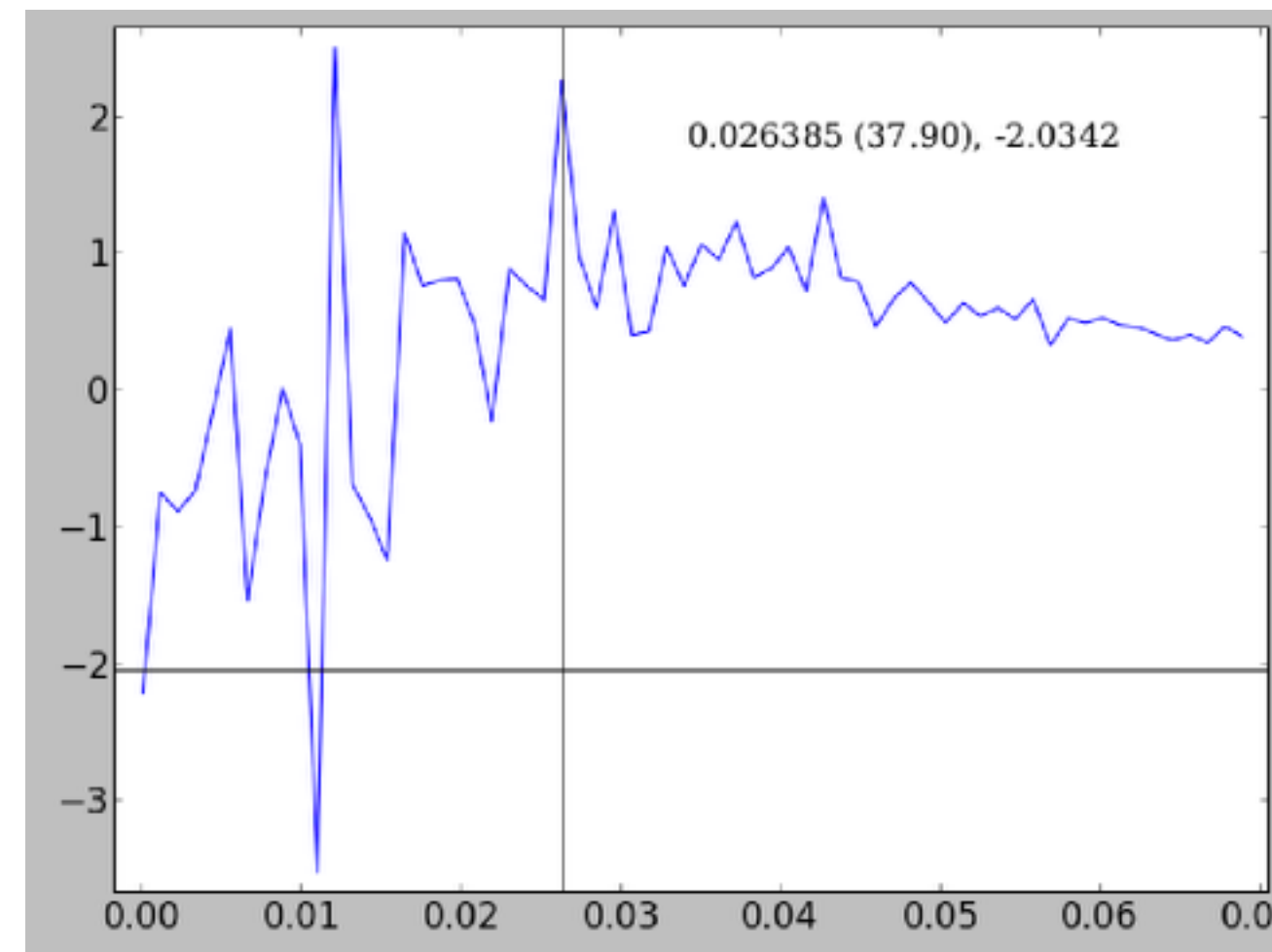
0.1μm



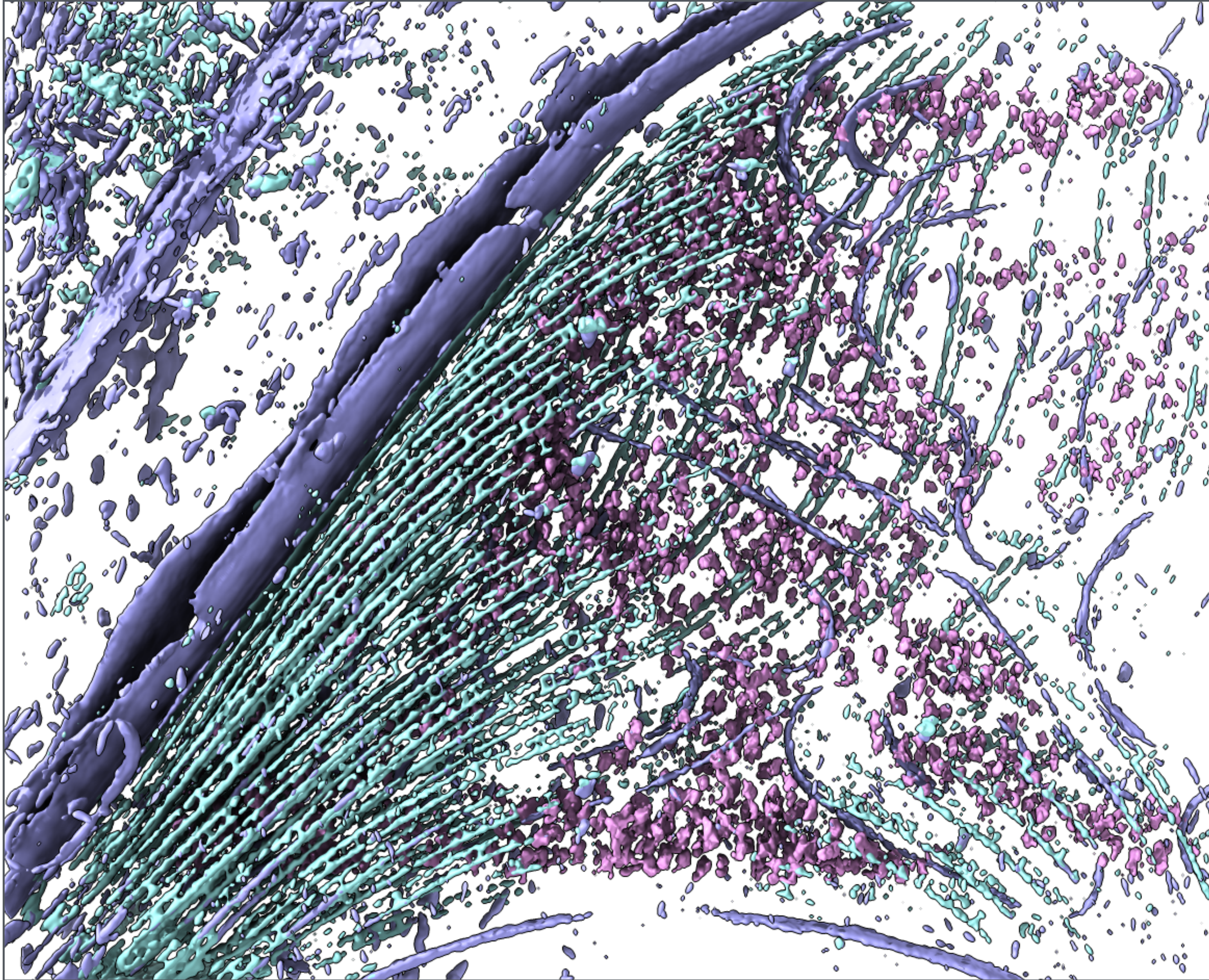


Microtubule

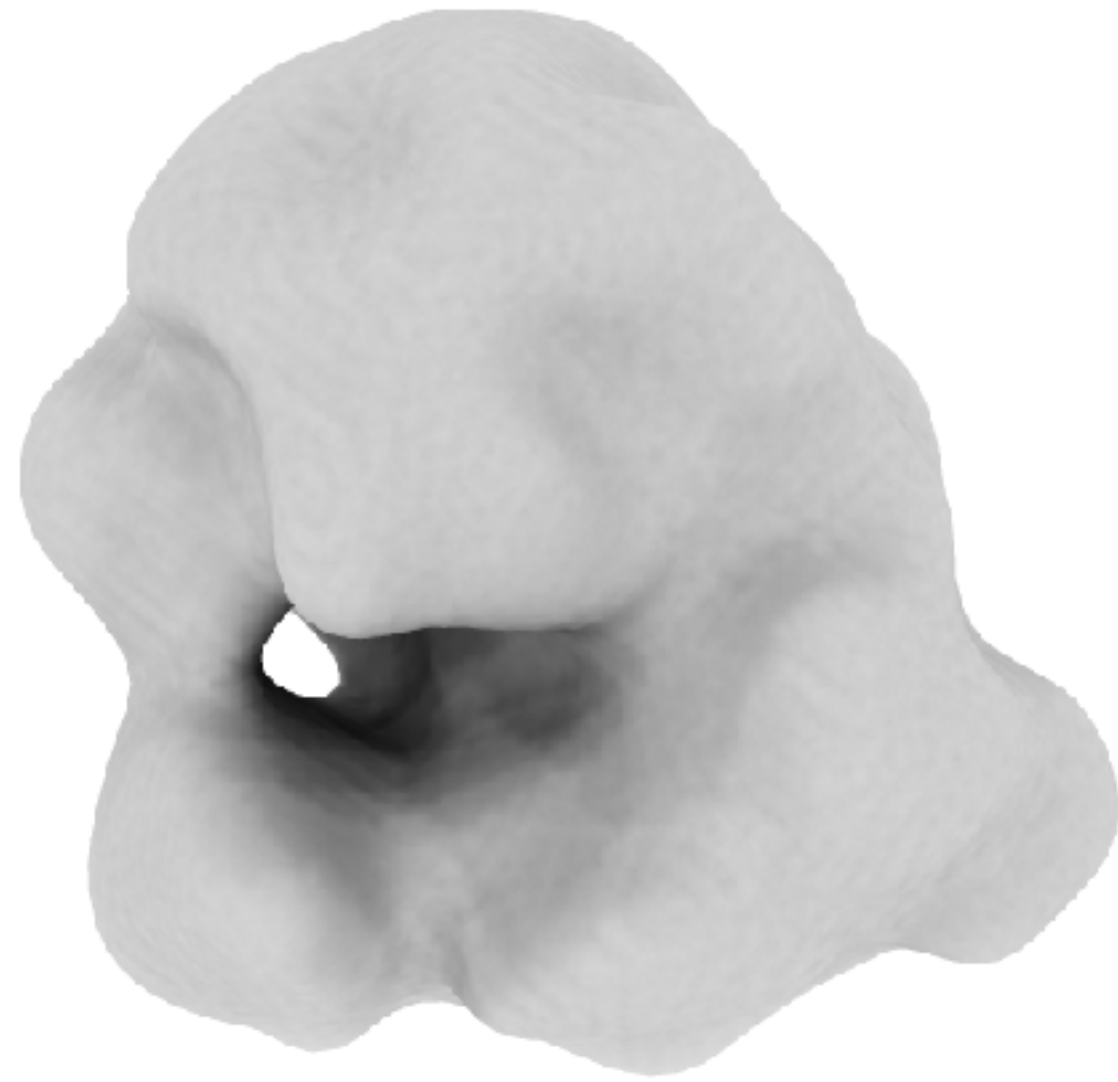
PC12,JEM2100, Apix=14.3



Trypanosoma Brucei



Ribosome



**EMDB-2239
(filtered)**



**Trypanosome
Subtomogram
Average**

Ribosome

Note:

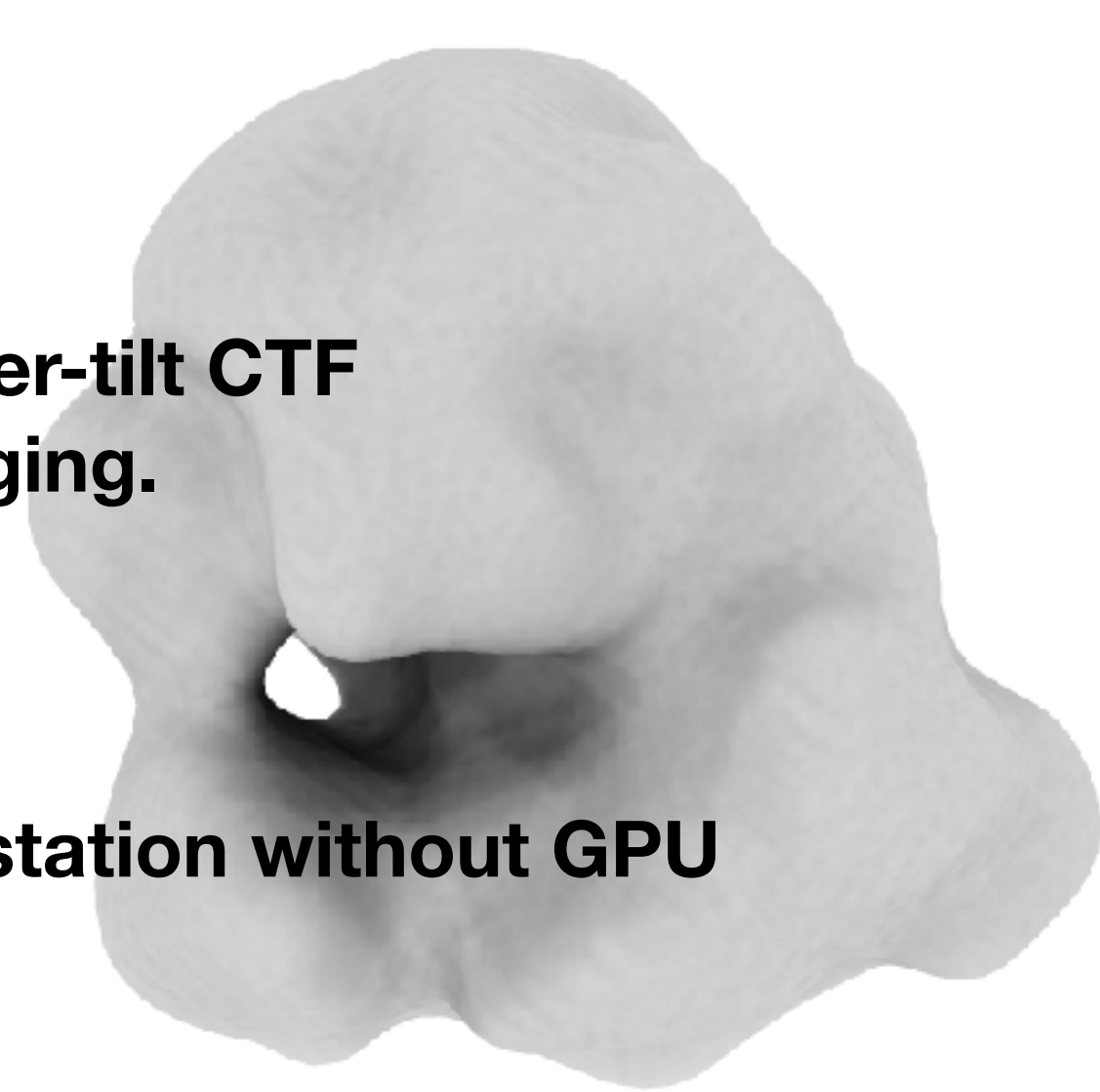
EMAN2.2 can do per-particle/per-tilt CTF corrected subtomogram averaging.

Wedge-aware 3-D alignment:

6000, 300x300x300 particles

<12 hours on a multicore workstation without GPU

avg ~7 sec/alignment

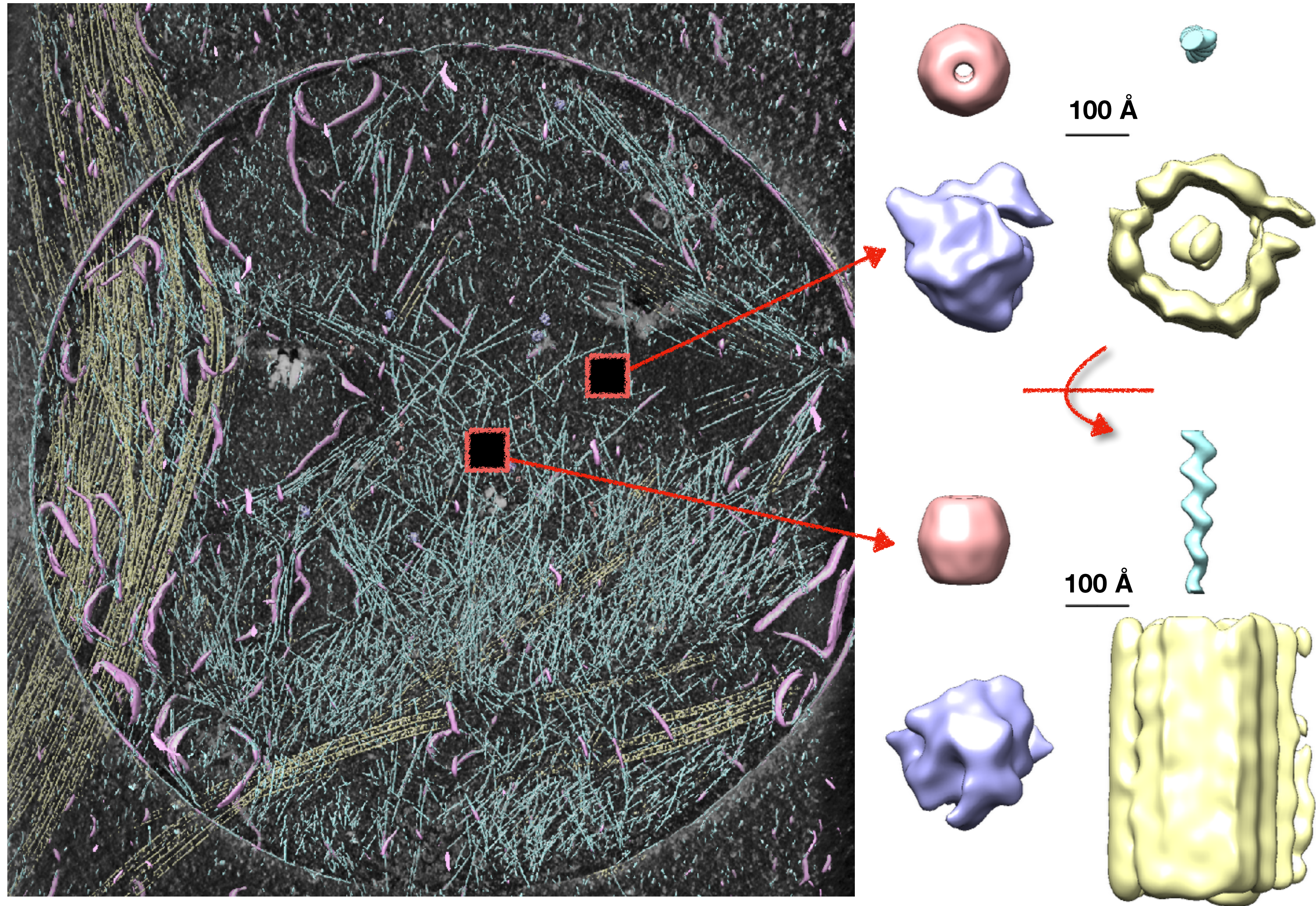


**EMDB-2239
(filtered)**

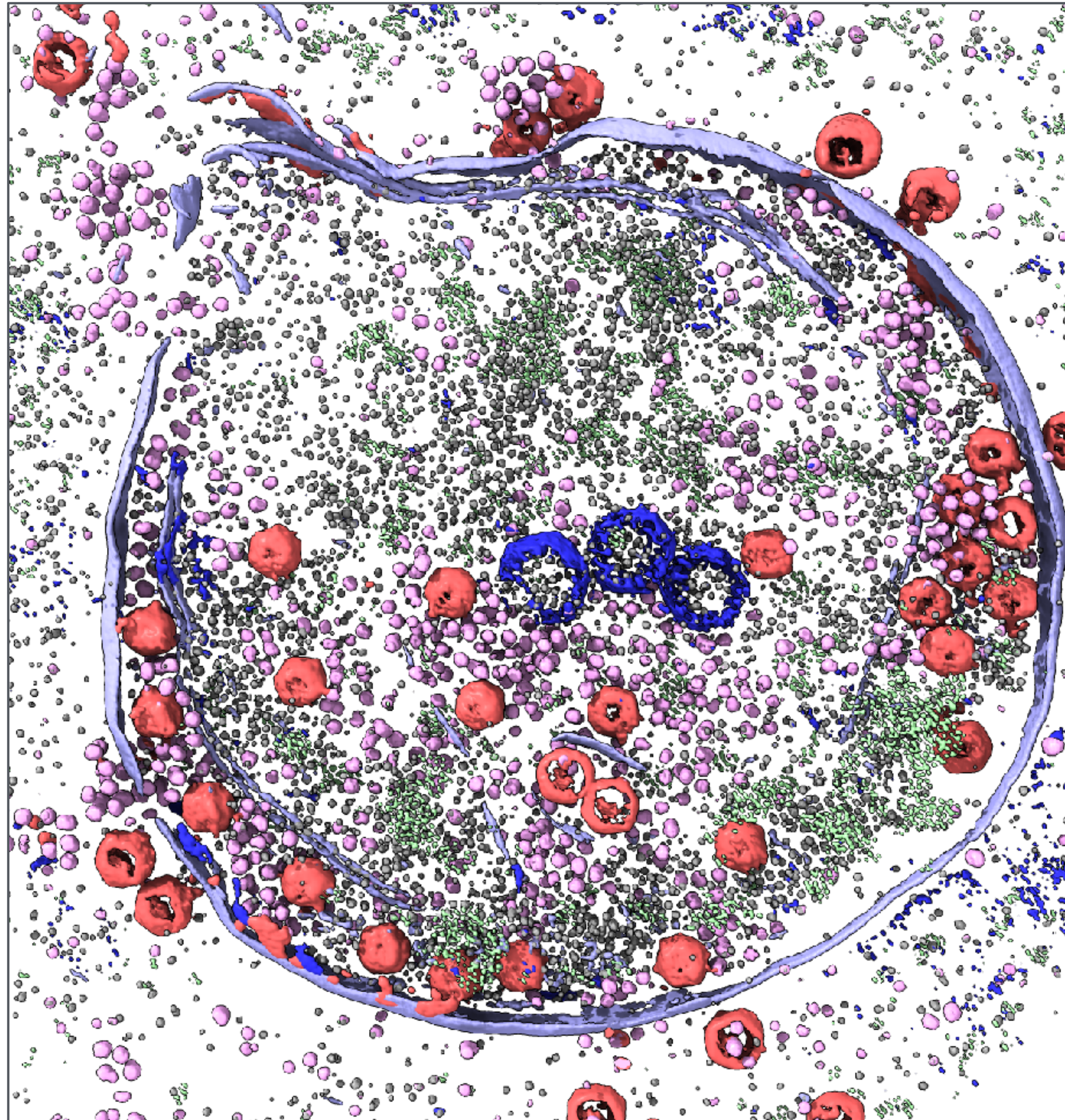


**Trypanosome
Subtomogram
Average**

Cultured Mouse Neuron, 6.6 Å/pix

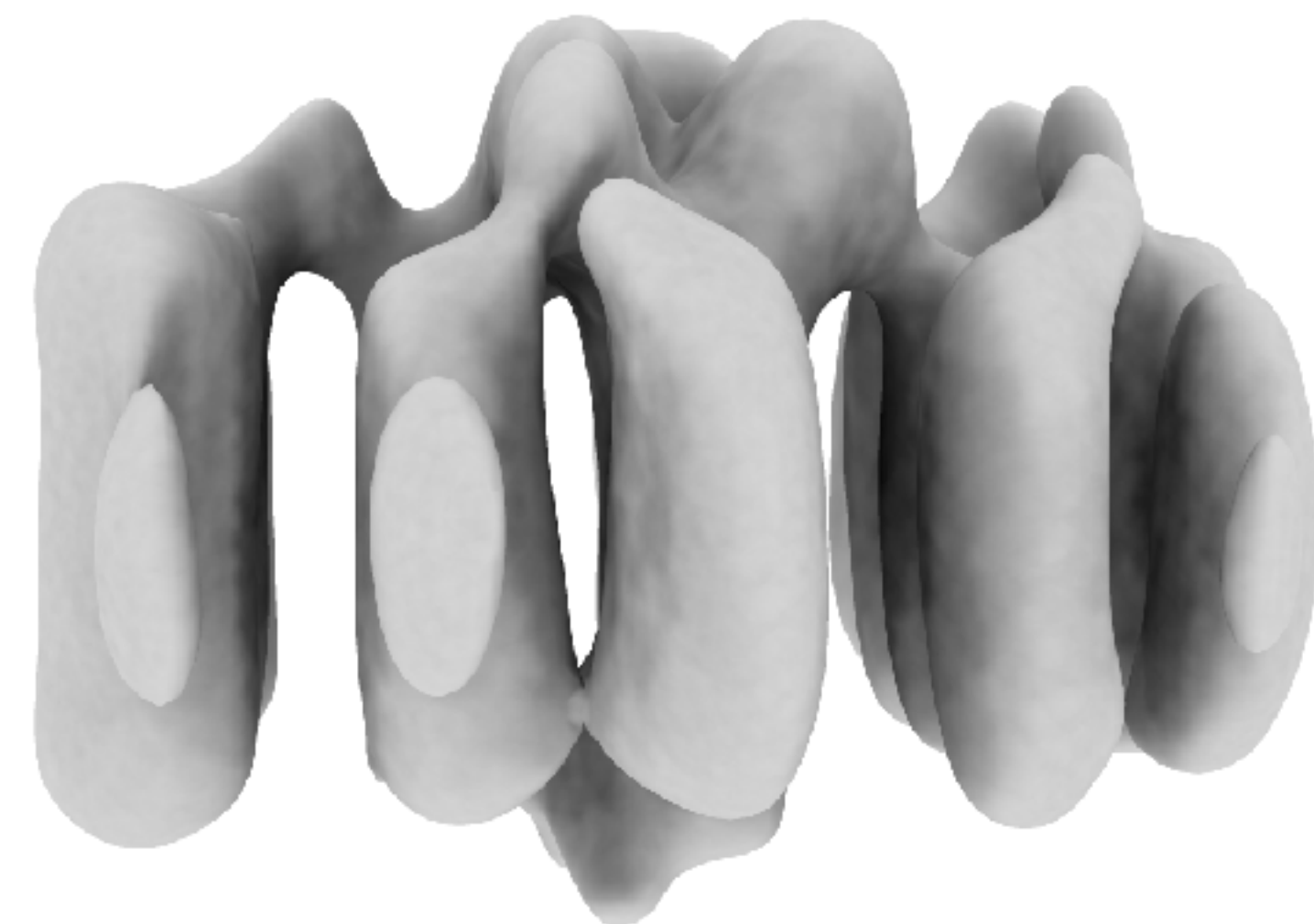
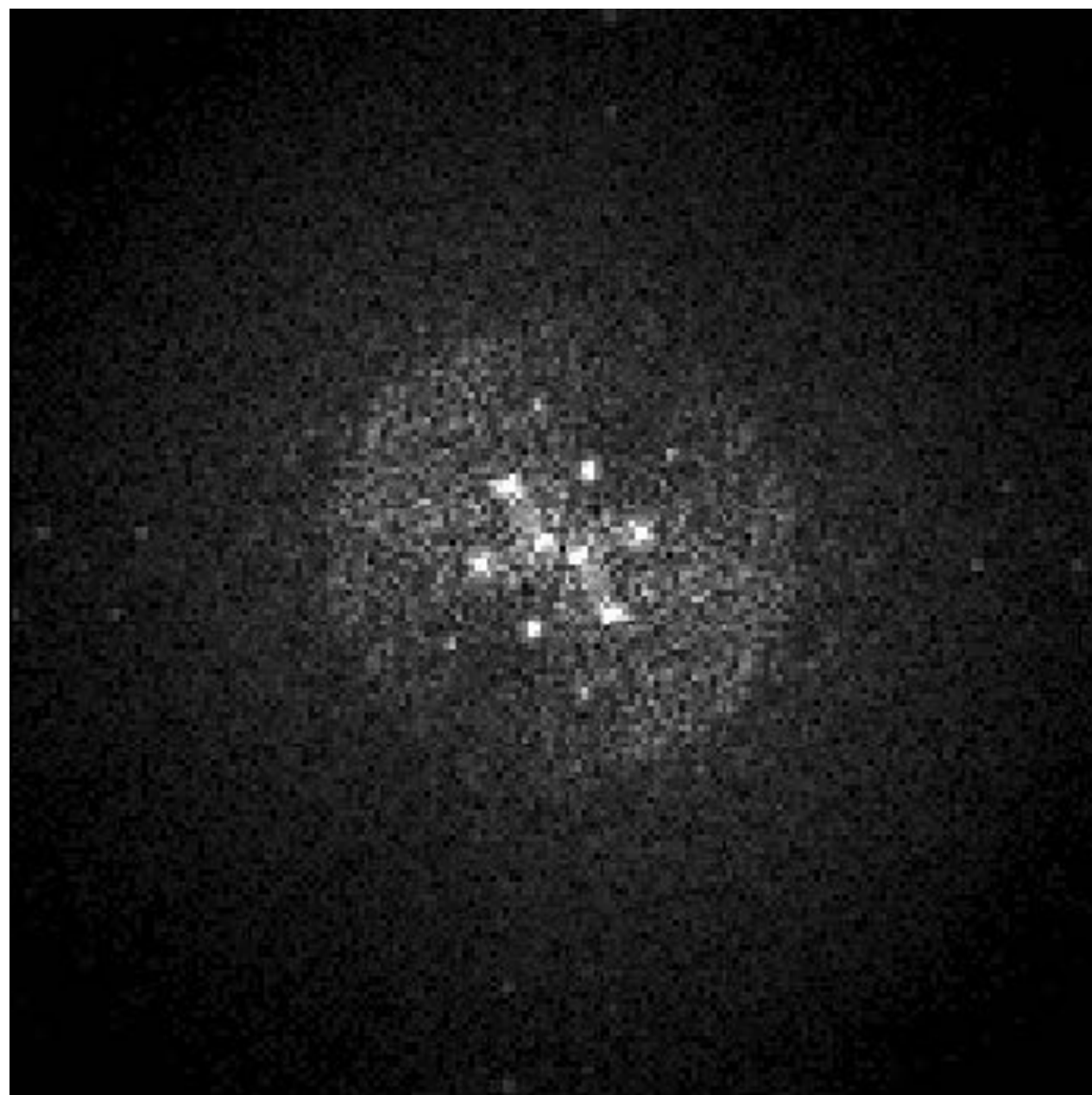
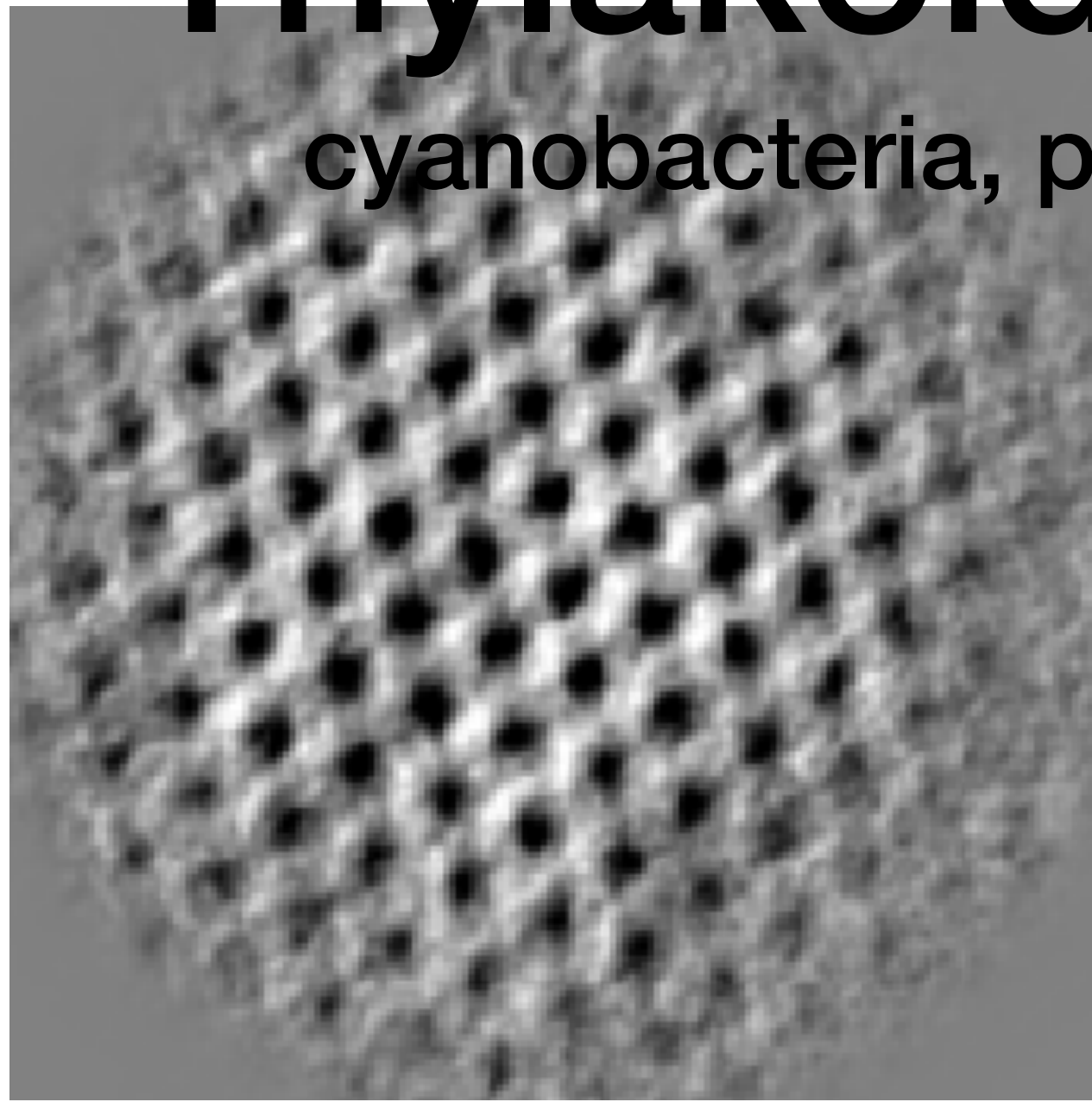


Cyanobacteria, late stage phage infection



Thylakoid membrane

cyanobacteria, phase plate, Apix=10.4

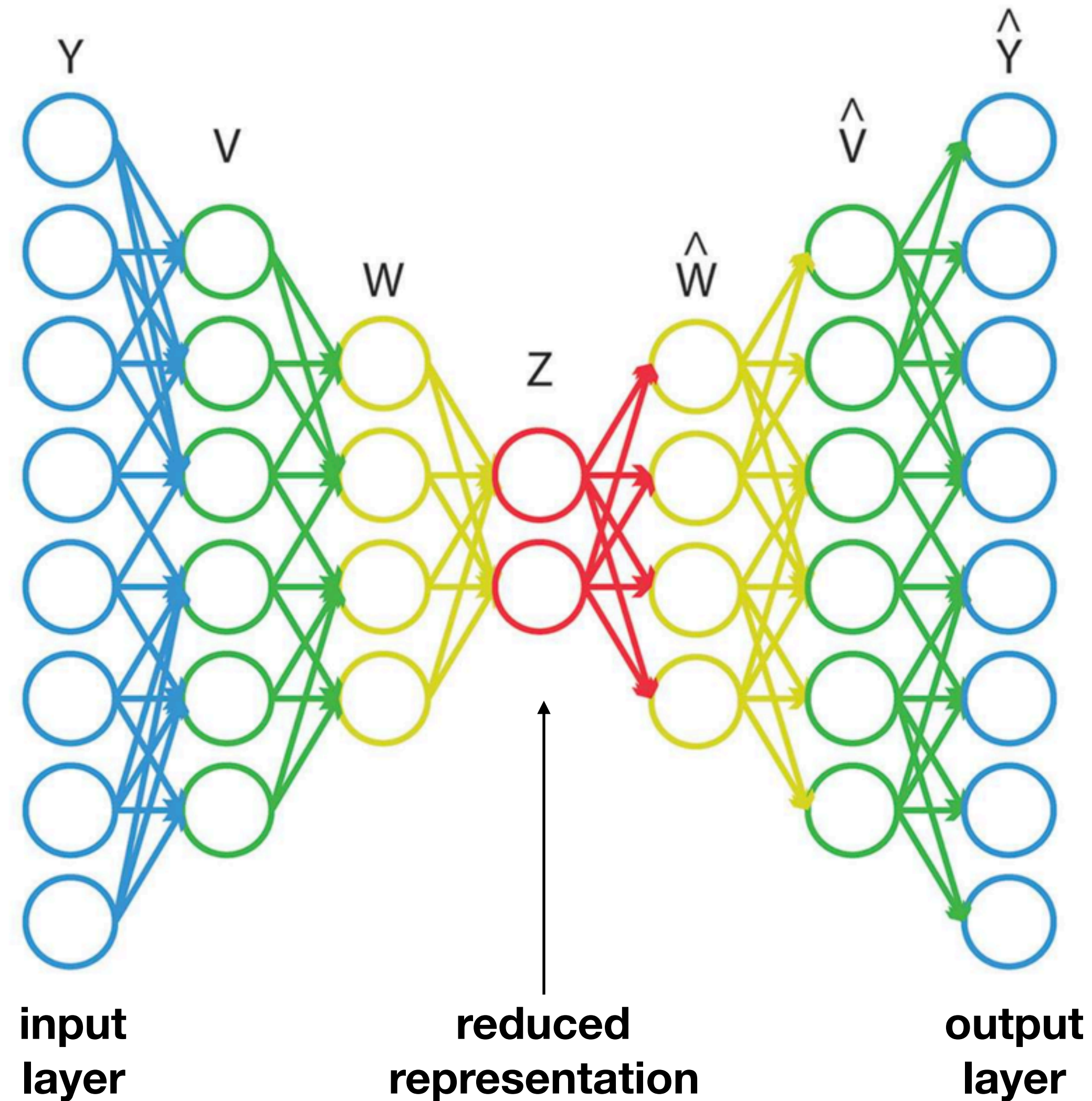


Using the Tools

- For each Feature of Interest (FOI):
 - Select ~10 tiles (64x64) with FOI
 - Select ~100 tiles without FOI
 - Draw the FOI manually on the ~10 tiles
 - Train the network (~3m on GPU)
- Apply all networks to each tomogram
 - 2k x 2k x 512 tomogram ~1hr/FOI on CPU (good workstation)
- Competitive merging for each tomogram

Autoencoder

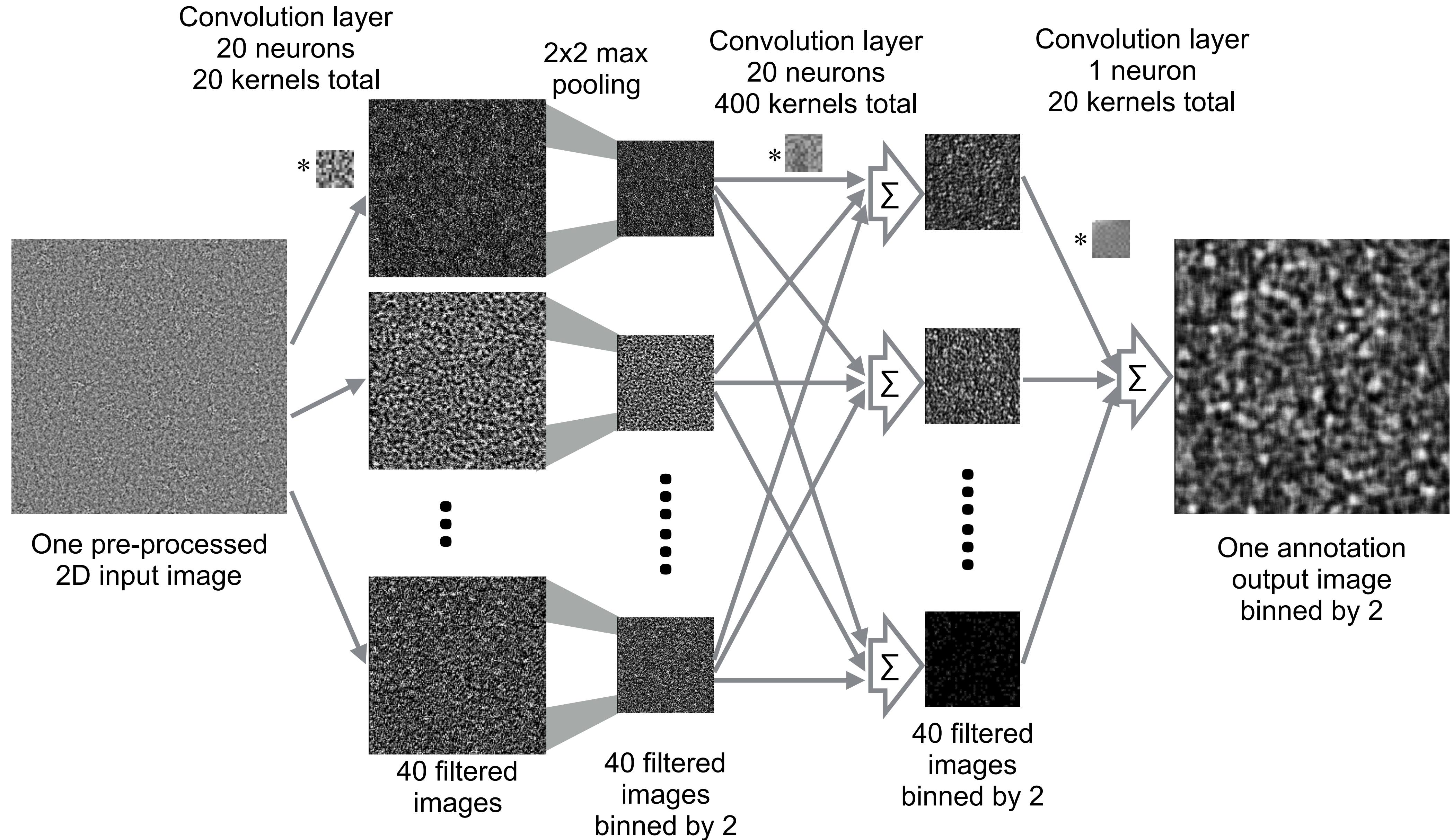
Che, C., Lin, R., Zeng, X., Elmaaroufi, K., Galeotti, J., Xu, M. Improved deep learning based macromolecules structure classification from electron cryo tomograms. arXiv:1707.04885.



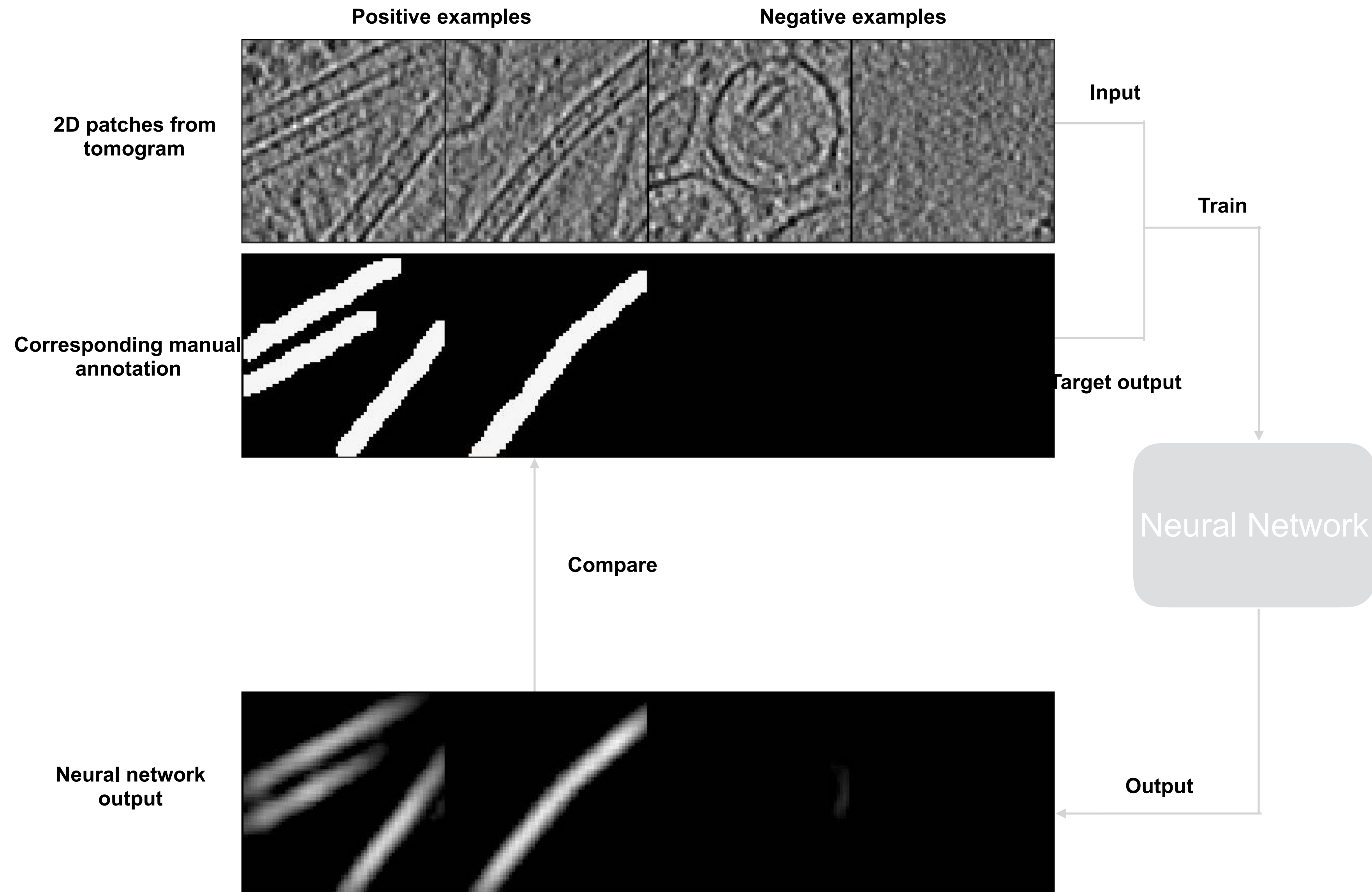
Particle Picking Using CNNs

- Why is this is a good idea?
 - Gold standard for particle picking is the human brain
 - Neural networks do a good job at mimicking humans
 - Particles look similar, but things that are NOT particles are diverse

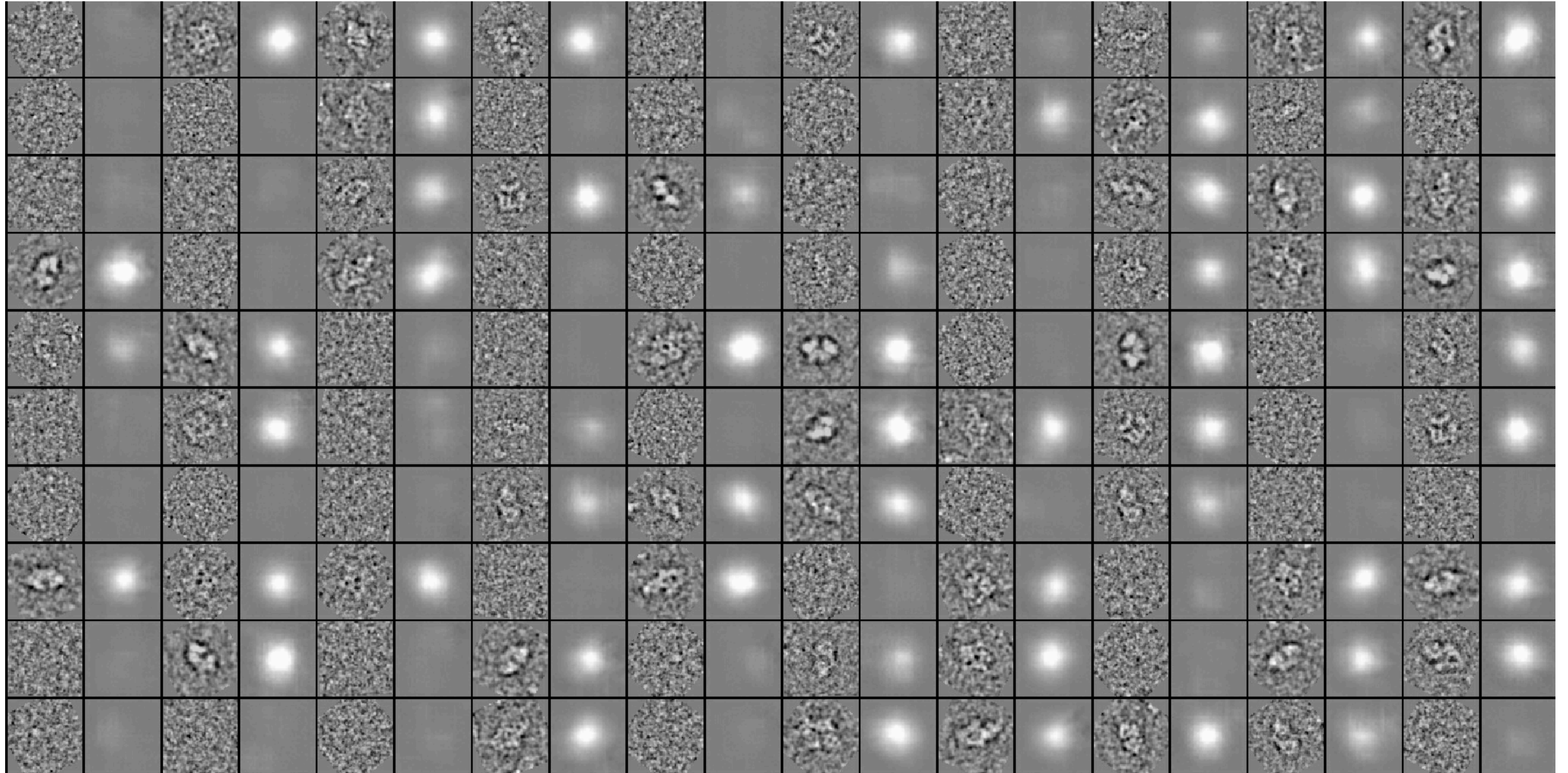
Particle Picking Using CNNs

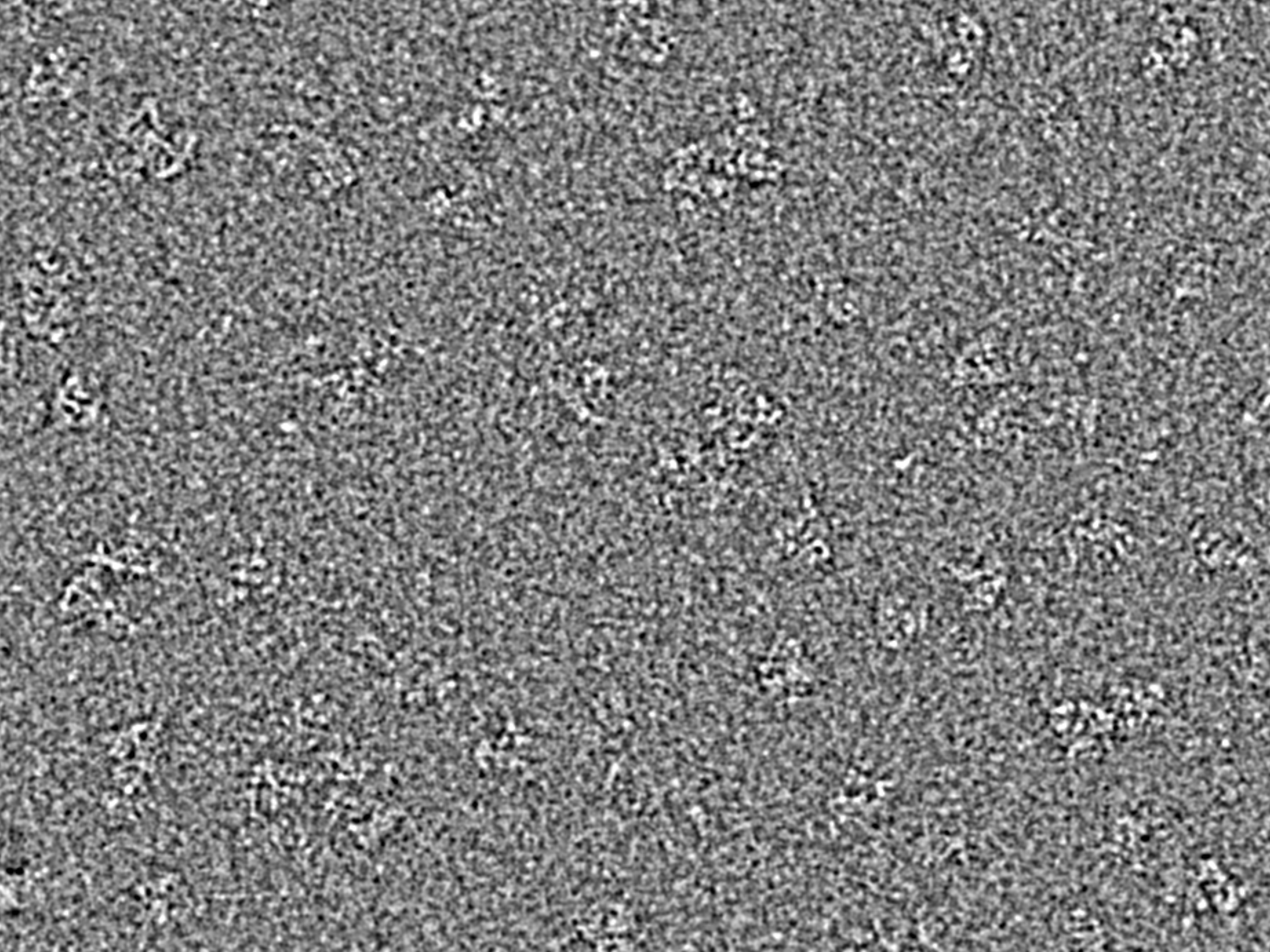


Tomogram Segmentation

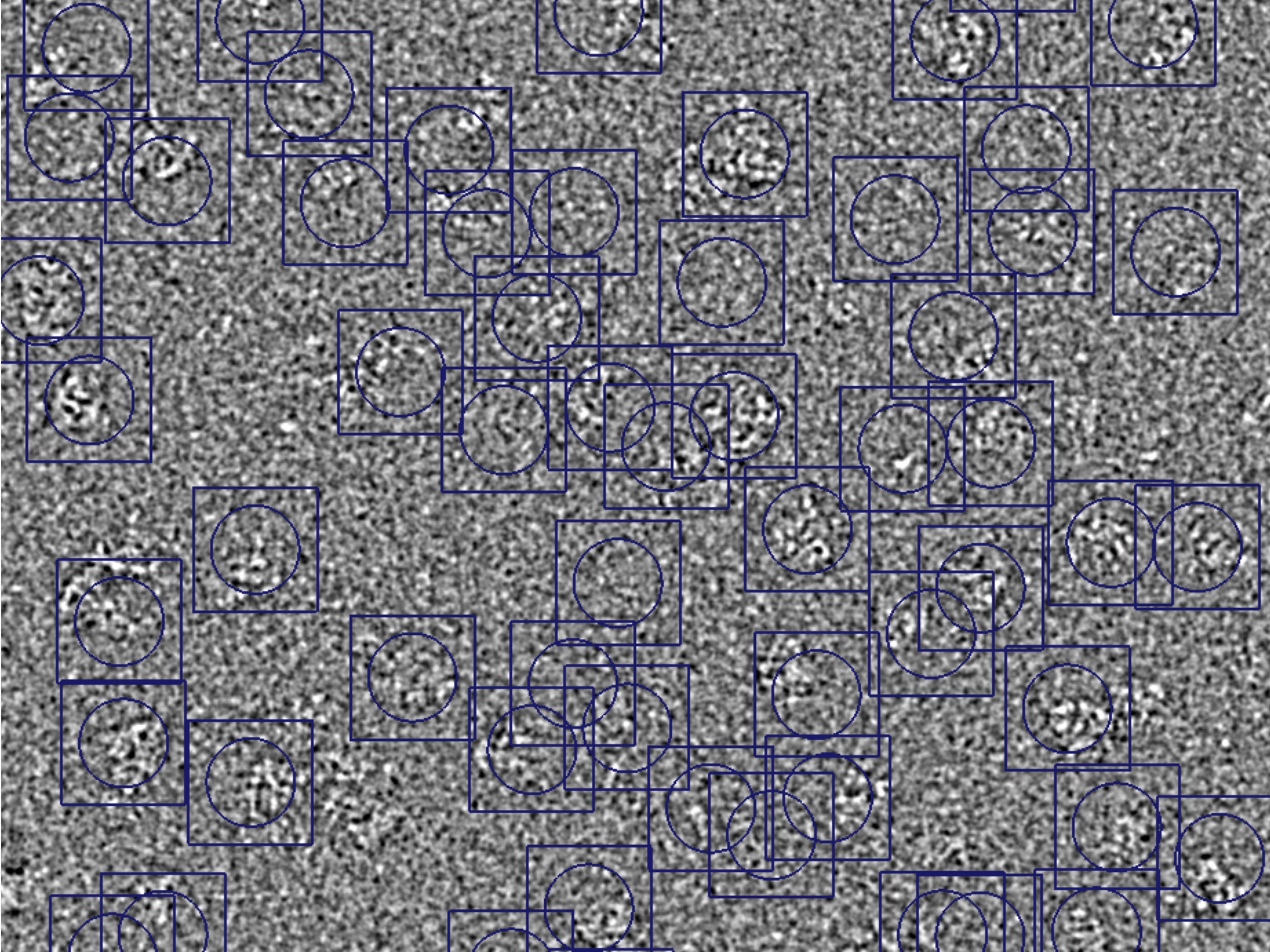


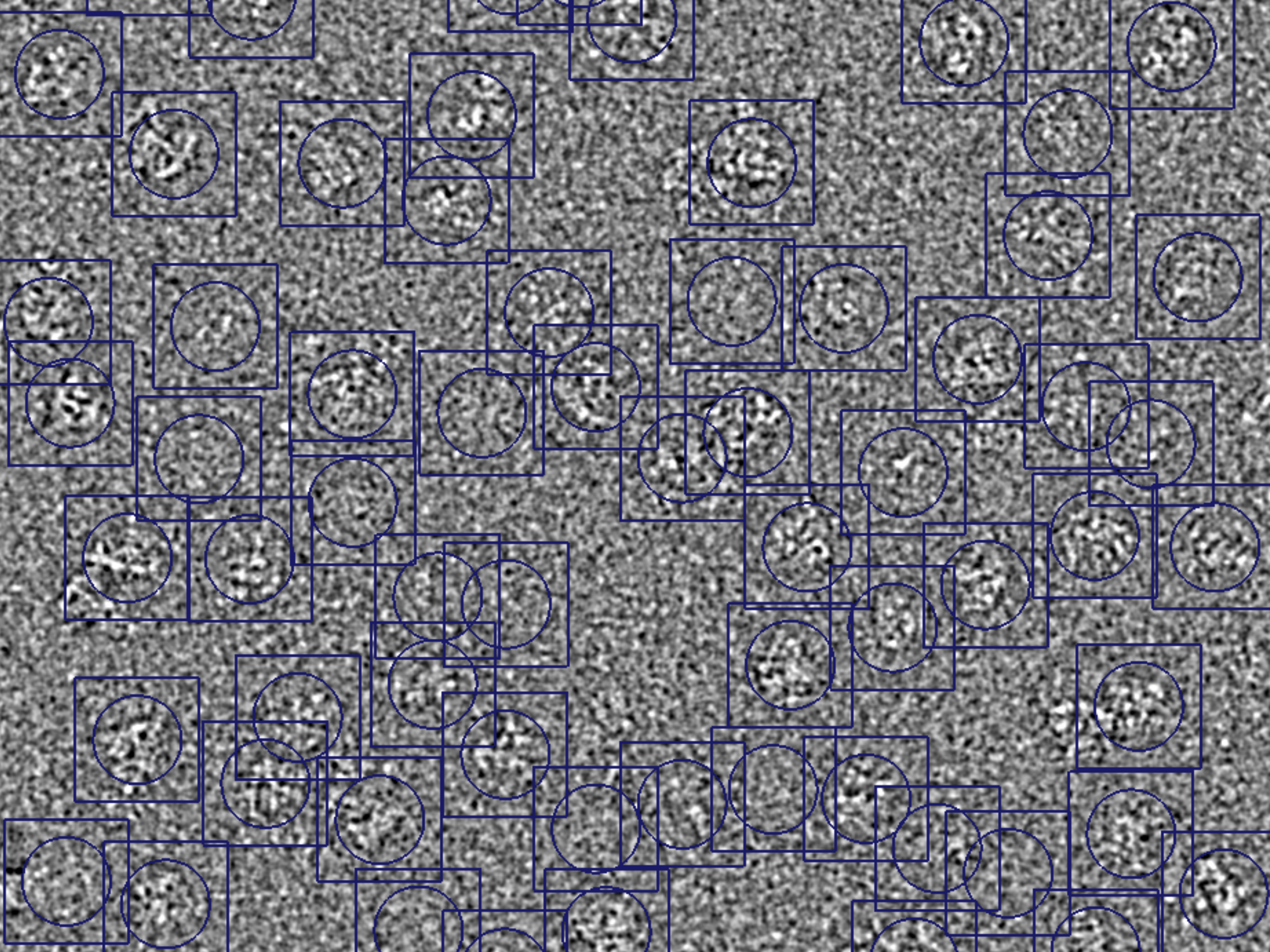
Particle Picking Using CNNs



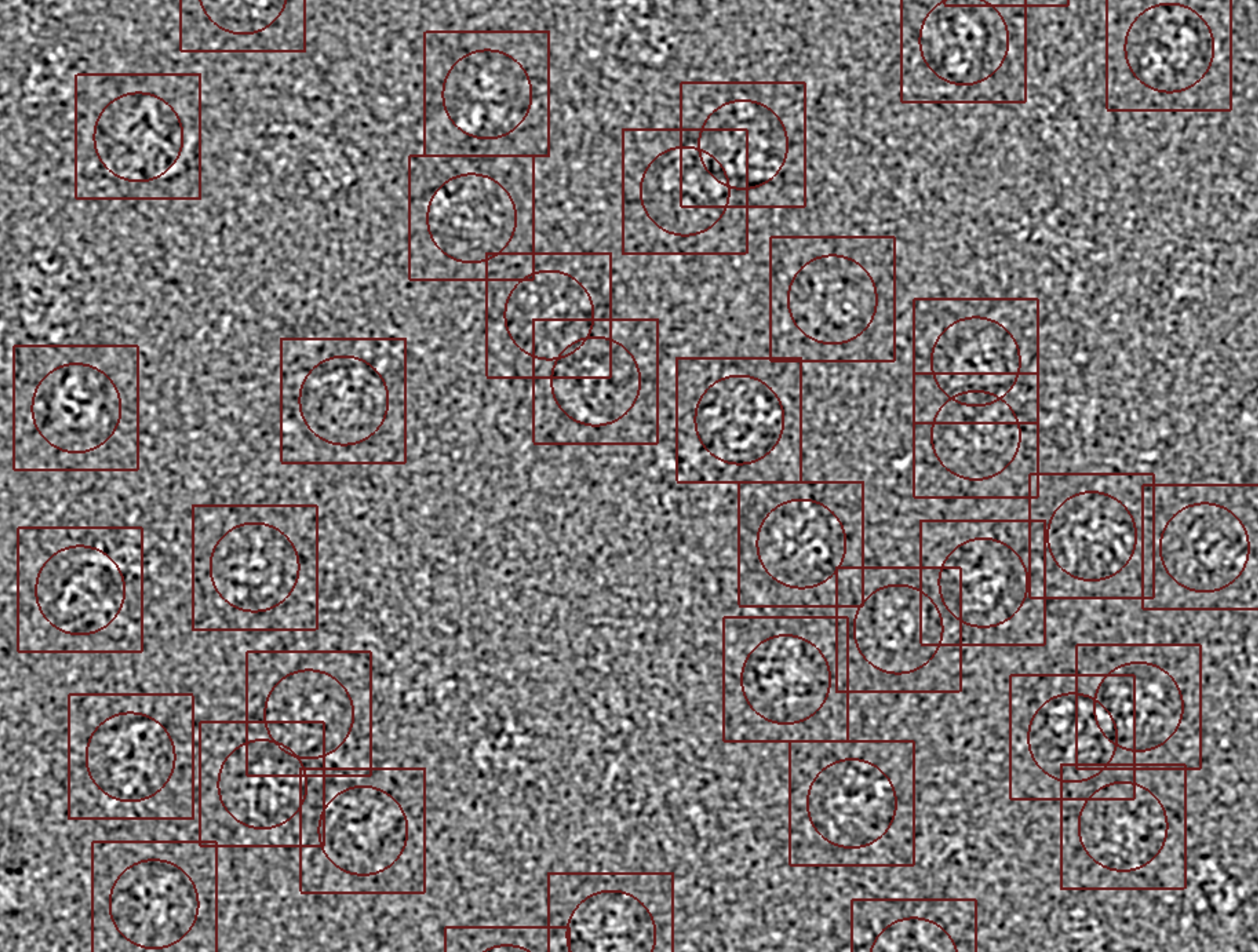


**Reference-based
autopicker
using 20 hand-picked
references**





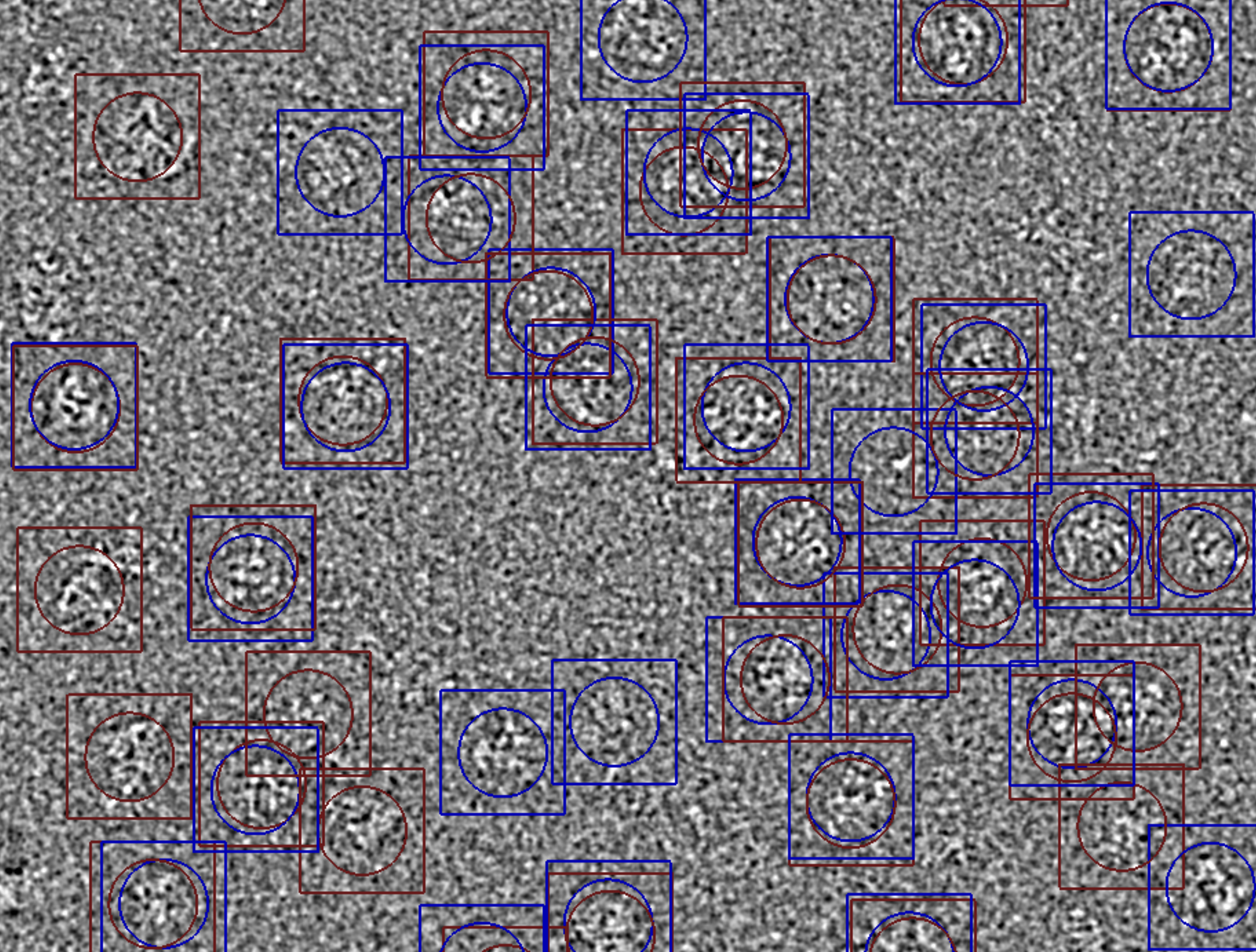
**Reference-based
autopicker
using autogenerated
projection references from
a good 3-D map**



**CNN Autopicker using the
same 20 manual
references and 60 "bad"
references.**

Training = 37s on 1 GPU

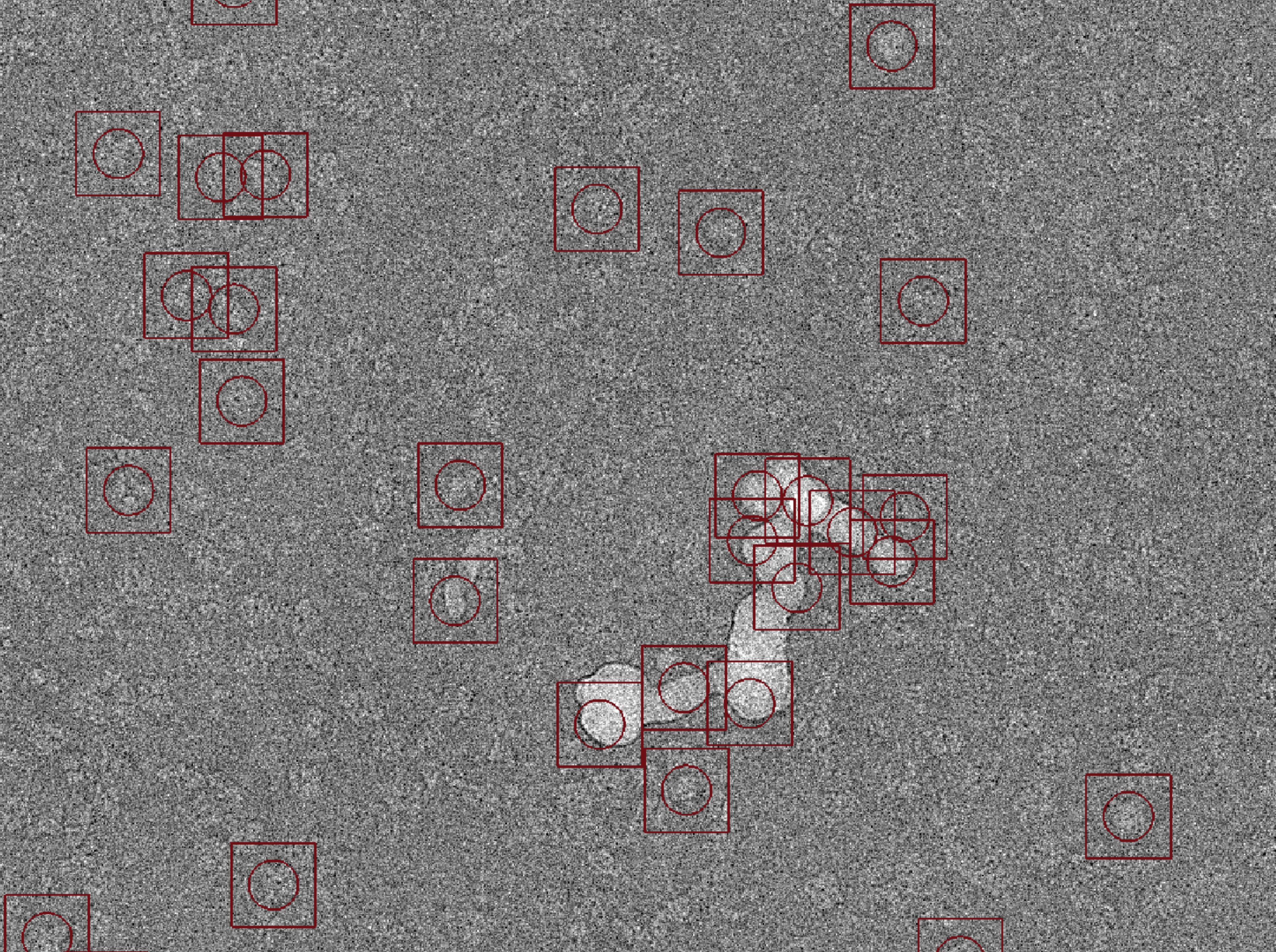
Autopicking (4k) =
10 s/image on 1 CPU

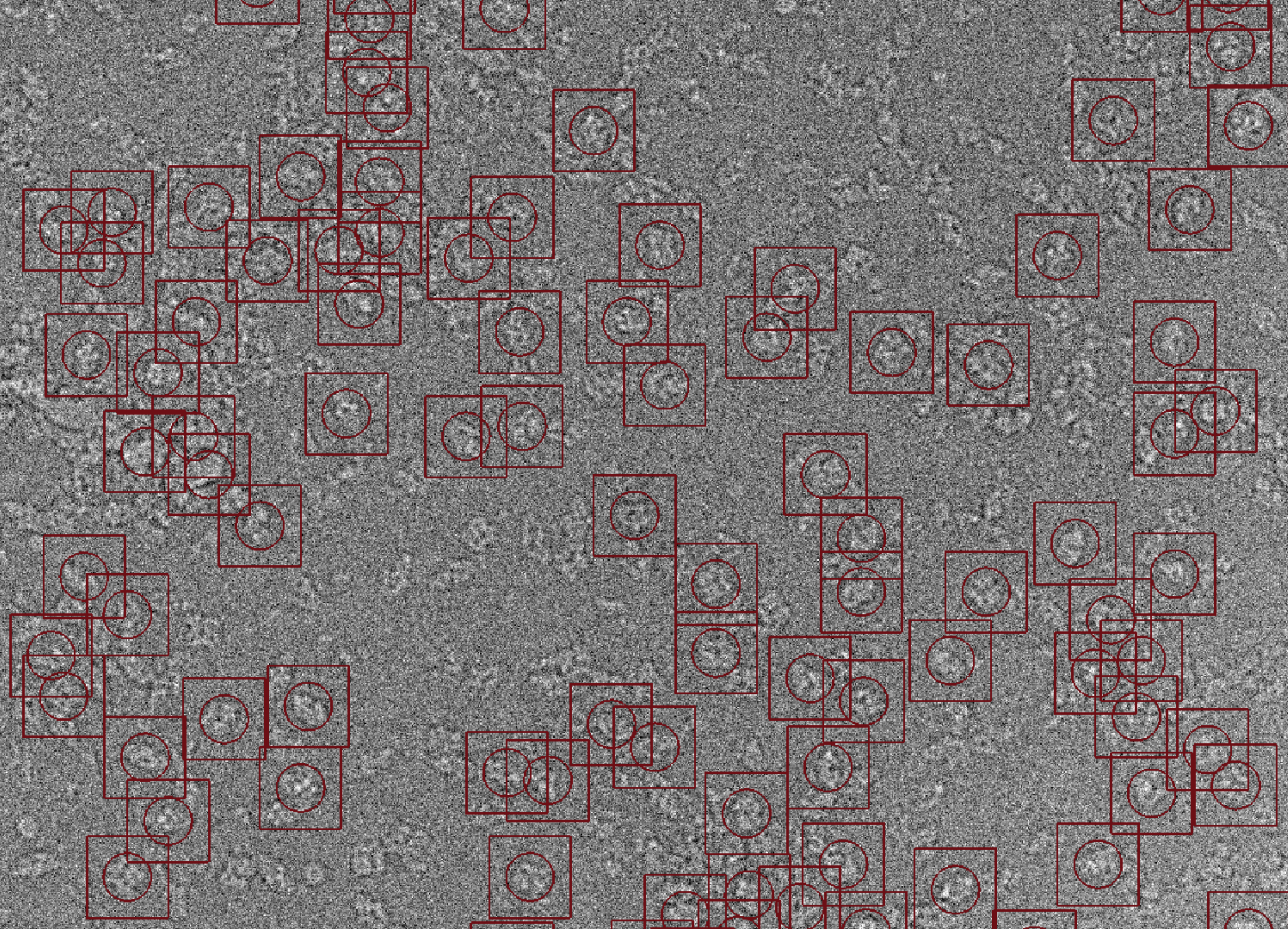


**CNN Autopicker (red) vs.
manual selection (blue)**

Problems Ice Contamination

(B-gal, empiar 10012)





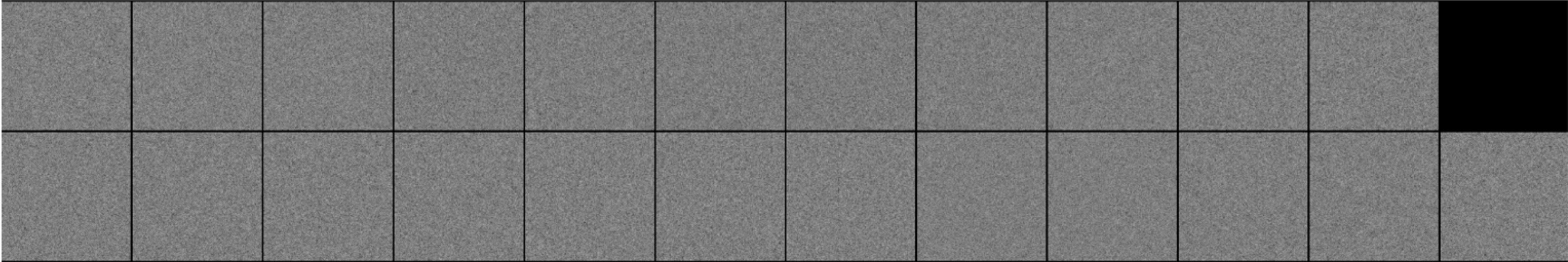
**Problems with
aggregated particles**

(B-gal, empiar 10012)

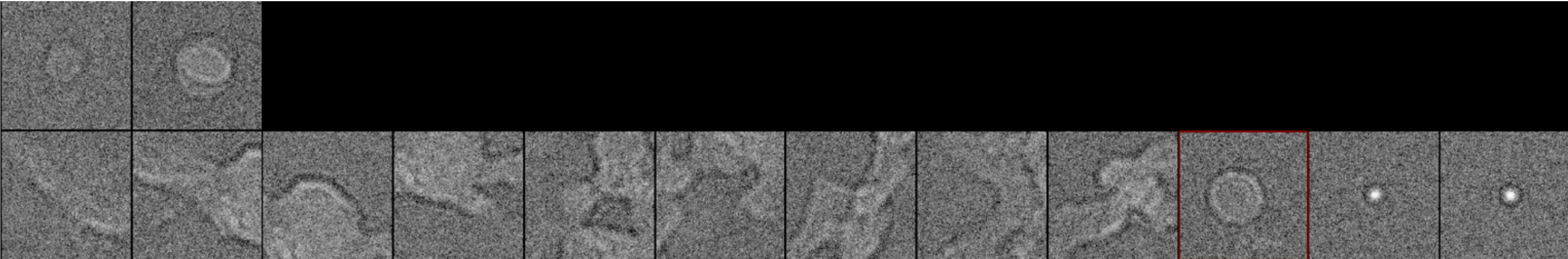
CNN Particle Picking v2

- 3 sets of references:
 - Good particles, preferably isolated
 - Empty ice/background
 - Contamination, or anything else which isn't a particle
- About 10-15 boxes in each category, preferably drawn from several images (different defocus)
- -> 2 Networks, particles vs. background and particles vs. contamination

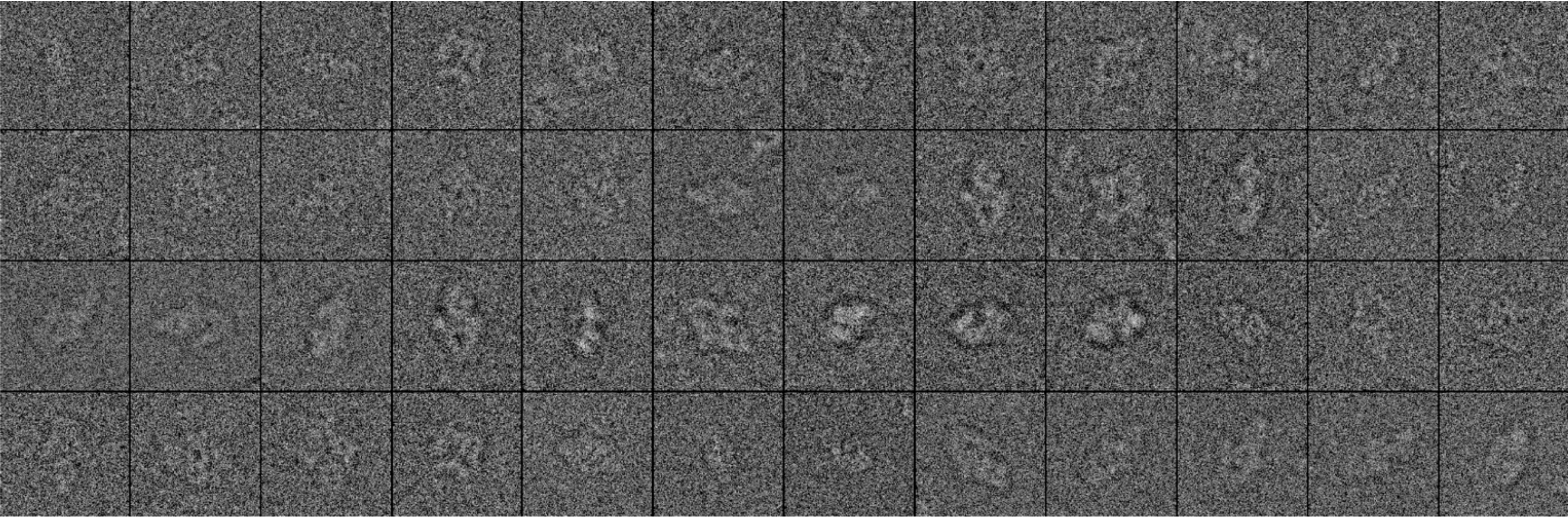
Background



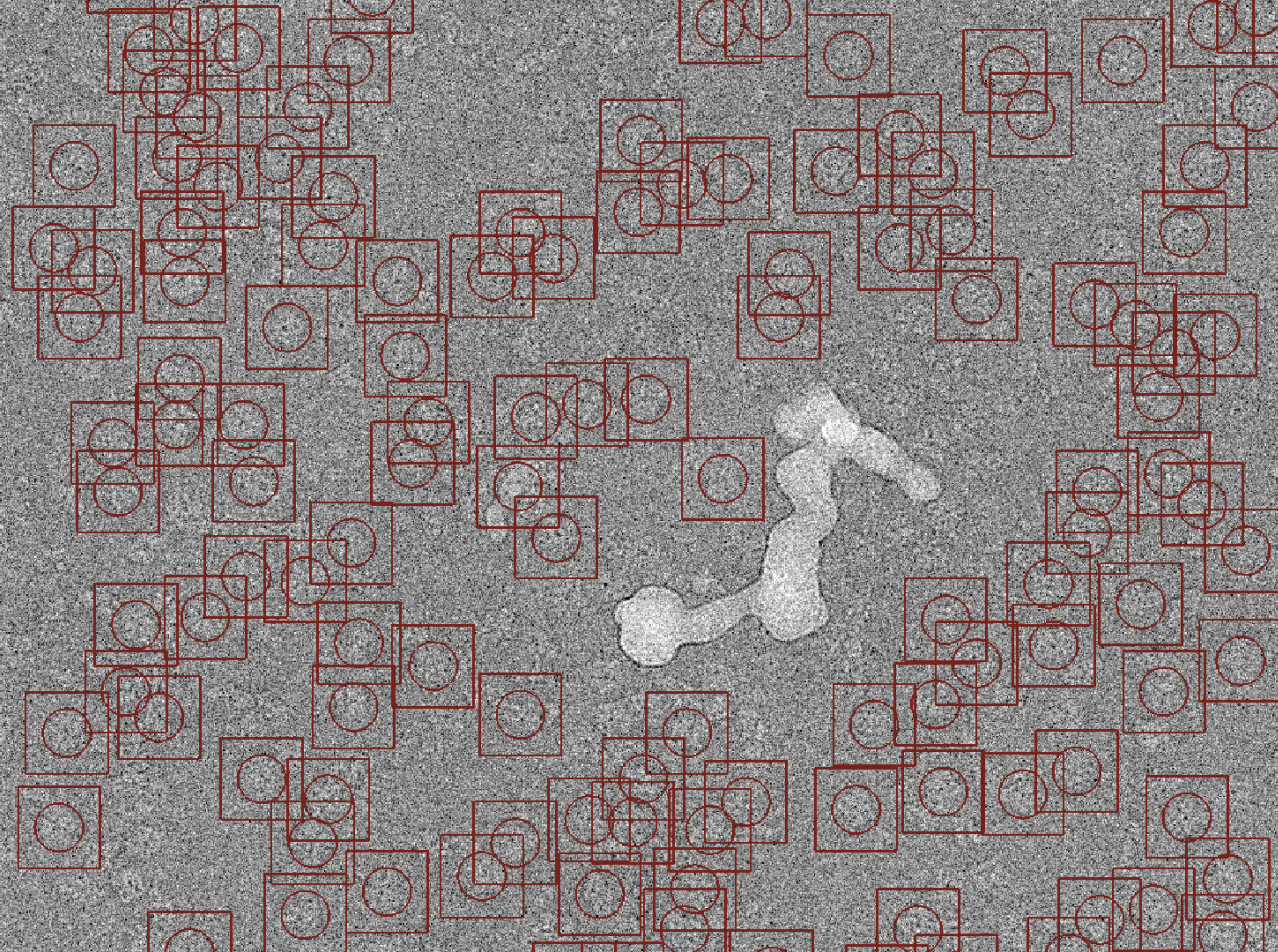
Bad Refs



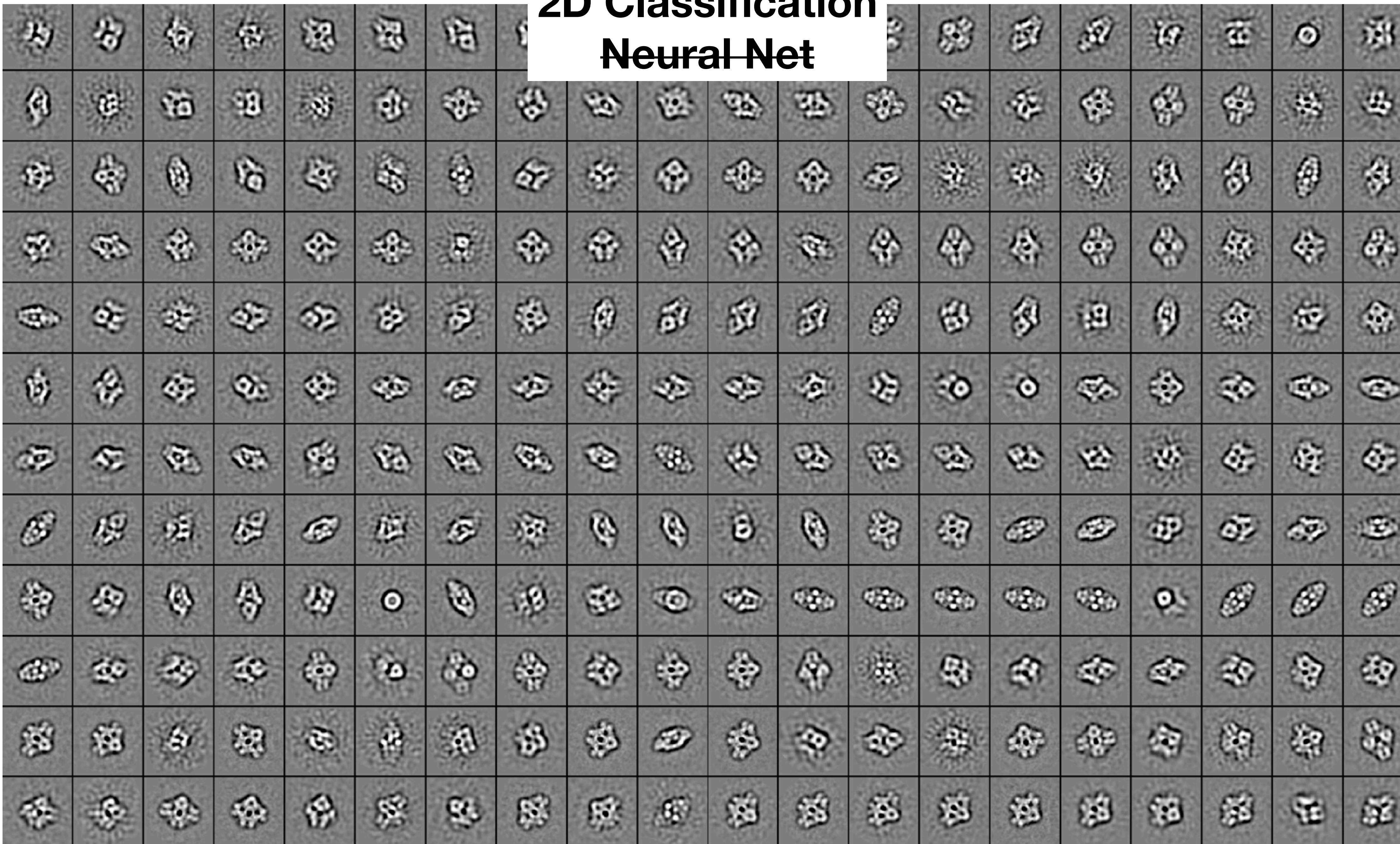
Good Refs



(B-gal, empiar 10012)

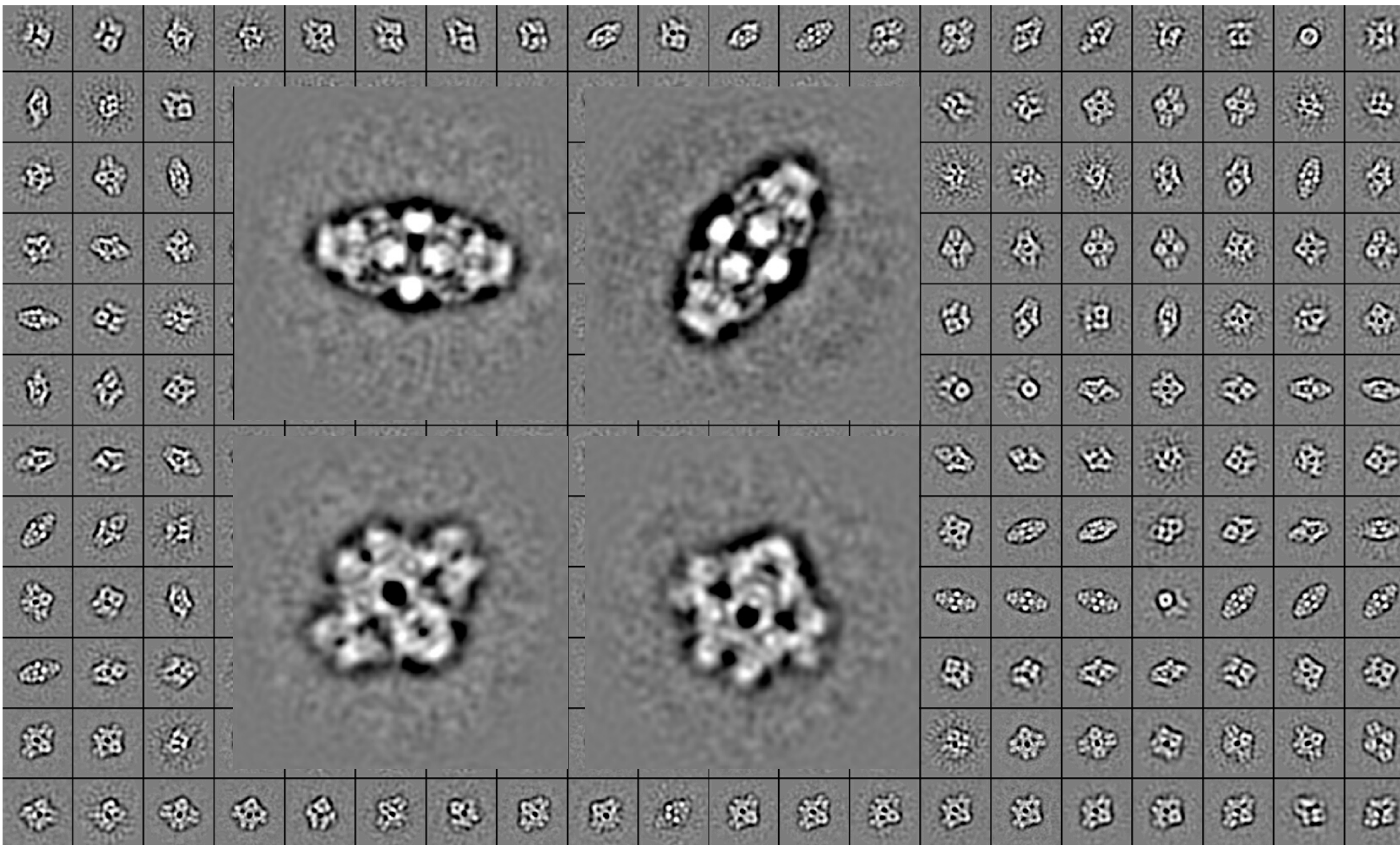


2D Classification Neural Net



70,000 particles
256 Classes
8 minutes
1 Workstation
(28 cores,
no GPU)

Old approach
~2-3 hours
same machine



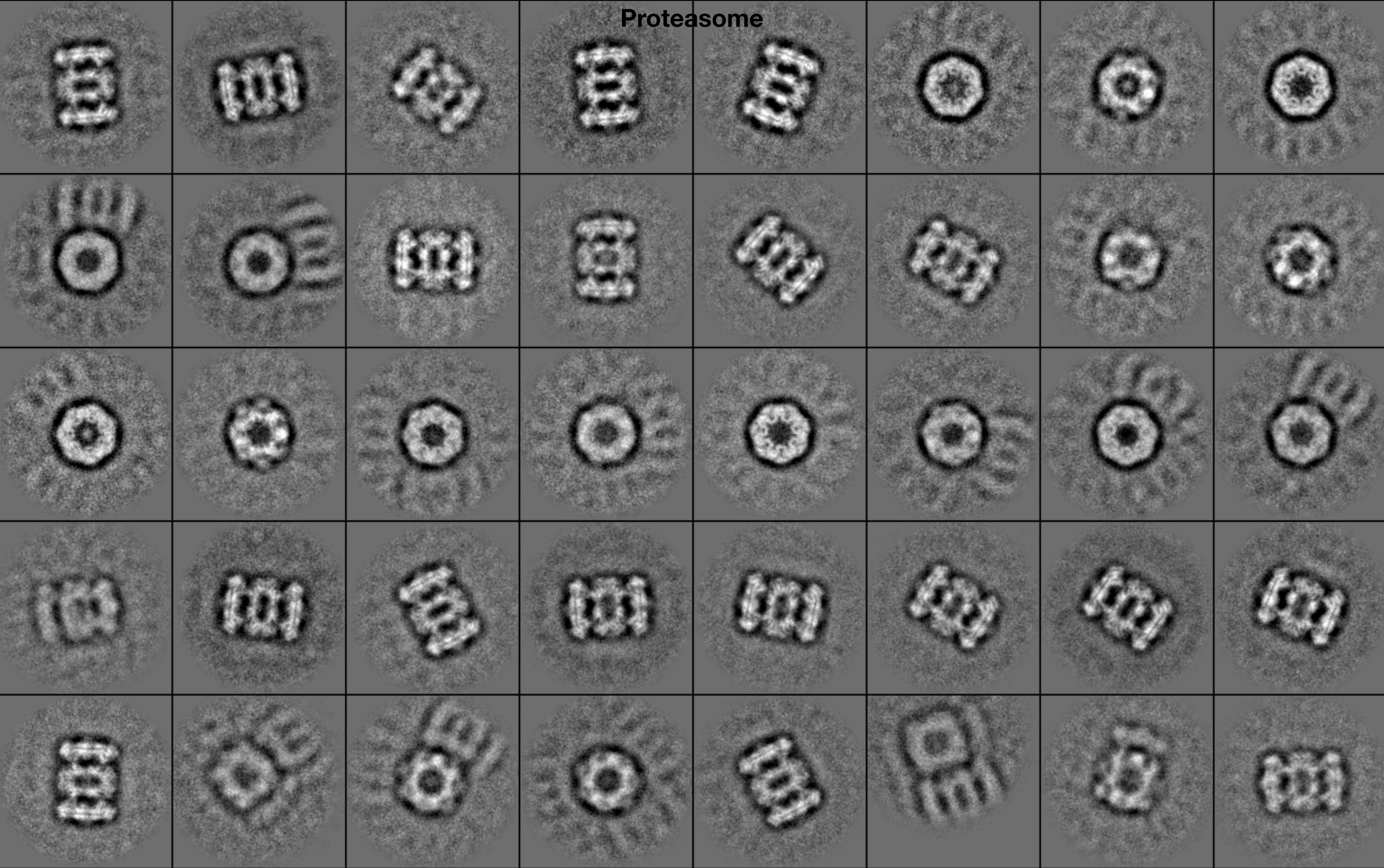
70,000 particles
256 Classes
8 minutes
1 Workstation
(28 cores,
no GPU)

Old approach
~2-3 hours
same machine

3-D Refine
to 5 Å
45 min
(same CPU)

to 3.5 Å
~3 hr

Proteasome



Warning

- EMAN2.2 uses Theano for GPU accelerated neural networks. Much slower on CPU
- After installing, you need to enable GPU support in Theano tied to your own CUDA installation. This is explained in the EMAN2.2 install instructions.
- Mac support is CPU only

Other Neural Net Applications

- Gaussian Mixture Model with Autoencoder for 3-D Dynamics from 2-D particles
- Missing Wedge Compensated Subtomogram Classification
- Dimension Reduction and Manifold Generation for Flexibility Analysis

The End