Near Atomic Resolution cryoEM: How Far Can We Go?

Melody Campbell & David Veesler
Automated Molecular Imaging
National Resource for automated Molecular Microscopy
The Scripps Research Institute

The revolution

2008

2012

2013

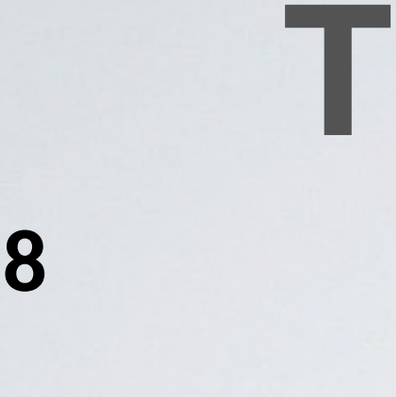
Single-particle EM

near-atomic resolution

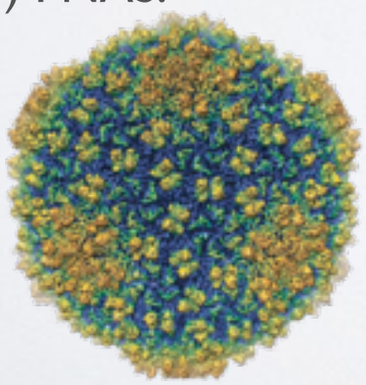
First reconstruction accounting for beam-induced motion

Small/asymmetric samples studied at near-atomic resolution

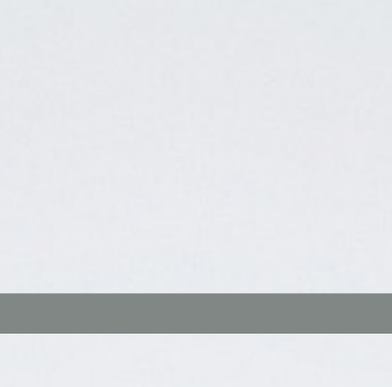
Yu X. et al.
(2008) Nature.



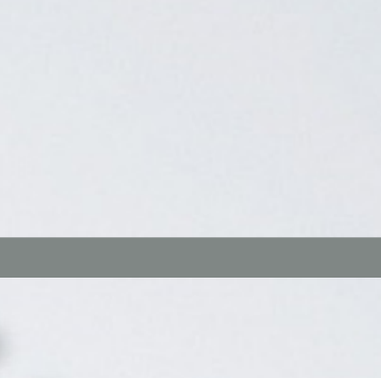
Zhang X. et al.
(2008) PNAS.



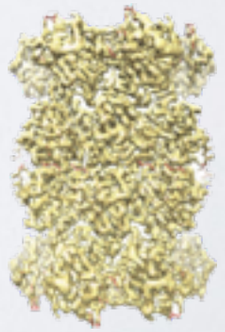
Jiang W. et al.
(2008) Nature.



Campbell M.G. et al.
(2012) Structure.



Bai X.C. et al.
(2013) ELife



Li X. et al.
(2013) Nat. Methods

Lu P. et al.
(2014) Nature.

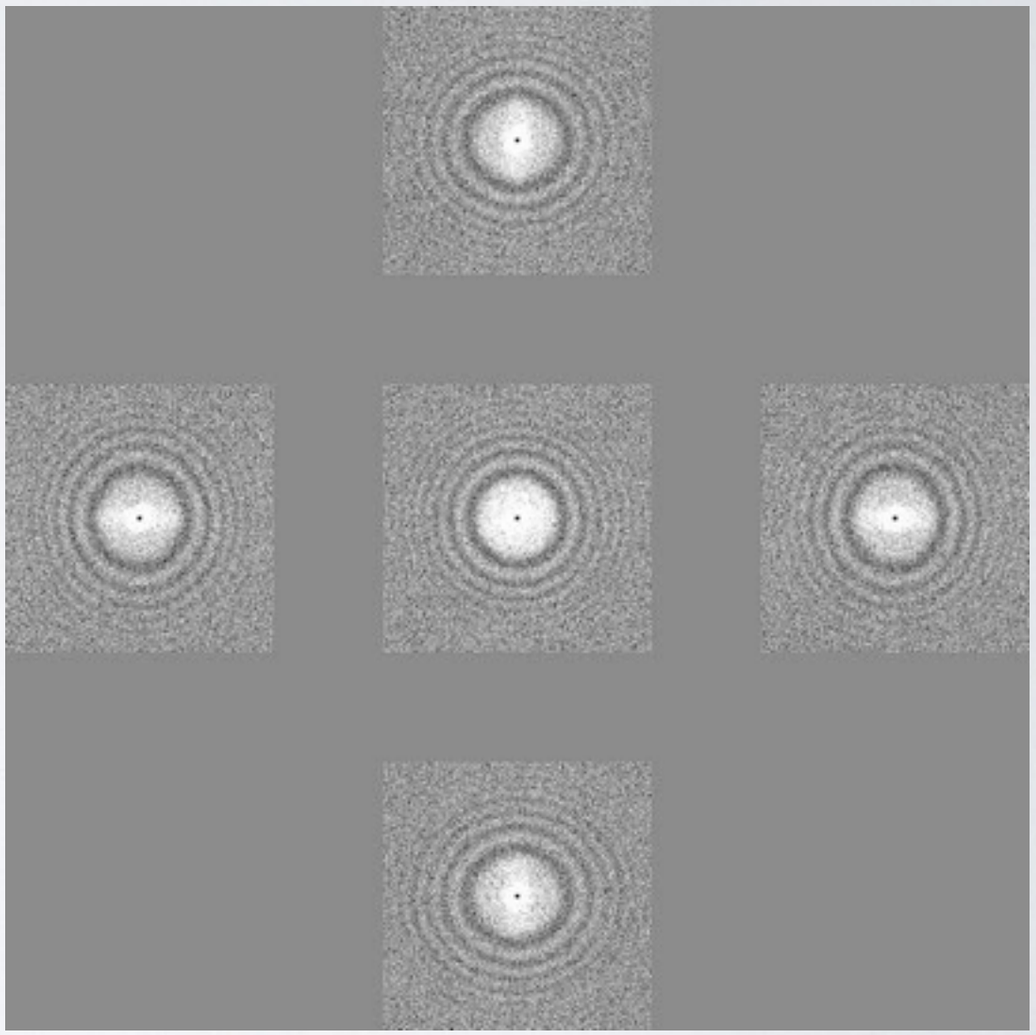
Testing the limit of our instruments

- Test specimen
  - *Thermoplasma acidophilum* 20S proteasome (T20S)
  - 700 kDa, D7 symmetry
  - Kind gift from Yifan Cheng

- FEI Titan Krios

- Different direct detectors
  - FEI Falcon 2
  - Gatan K2 Summit

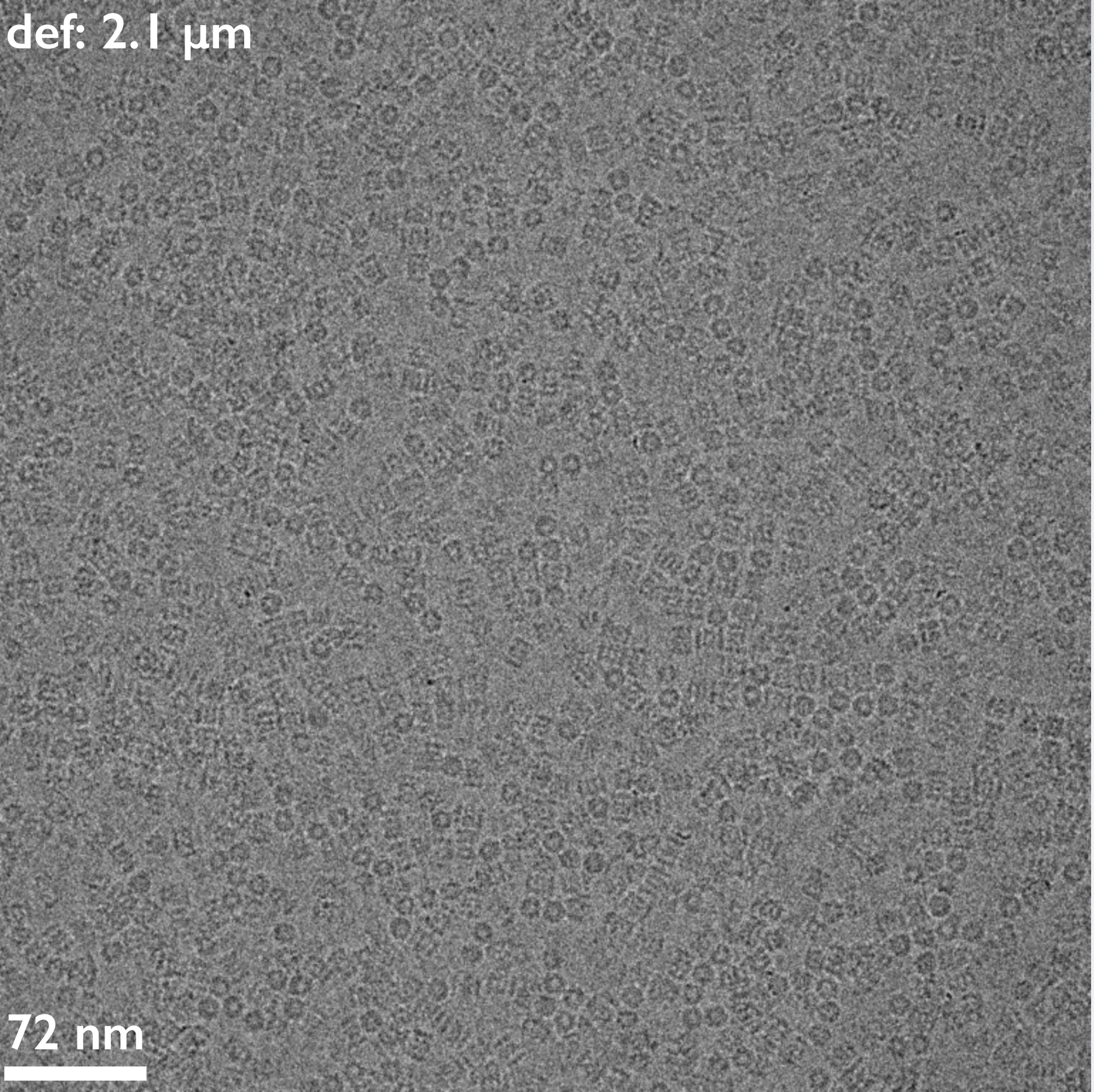
- Automated pipeline
  - Leginon
  - Appion/Relion



Coma-free alignment

Glaeser R.M. et al.
(2011) J Struct Biol.

T20S data set collected using Titan Krios/Falcon 2



Krios/Falcon 2

ext: 4500V

gun lens: 4

spotsize: 6

C2: 70 µm

Obj: 100 µm

beam: 0.9 µm

Microprobe

1sec - 7 frames

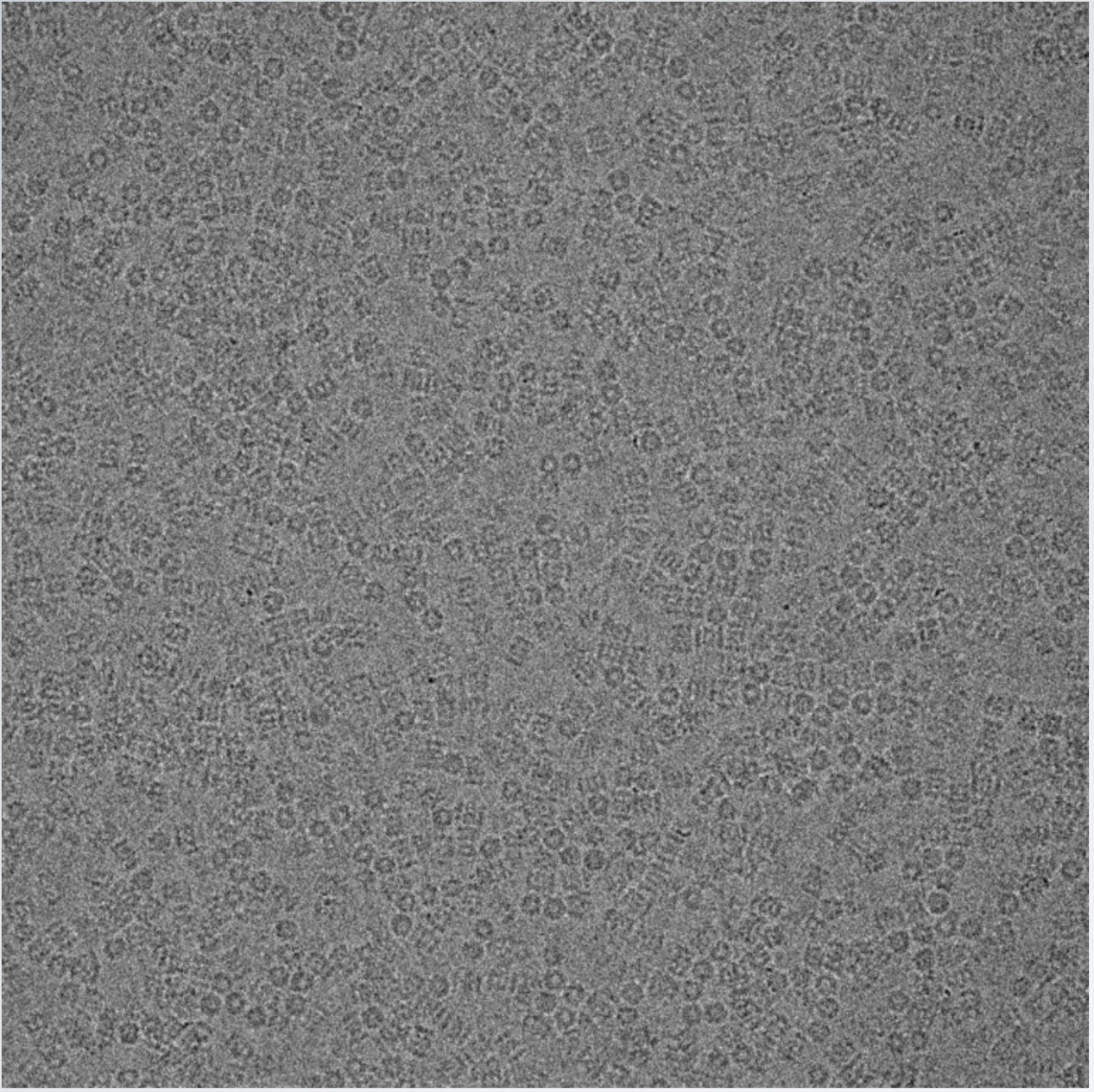
dose: 26 e/Å^2

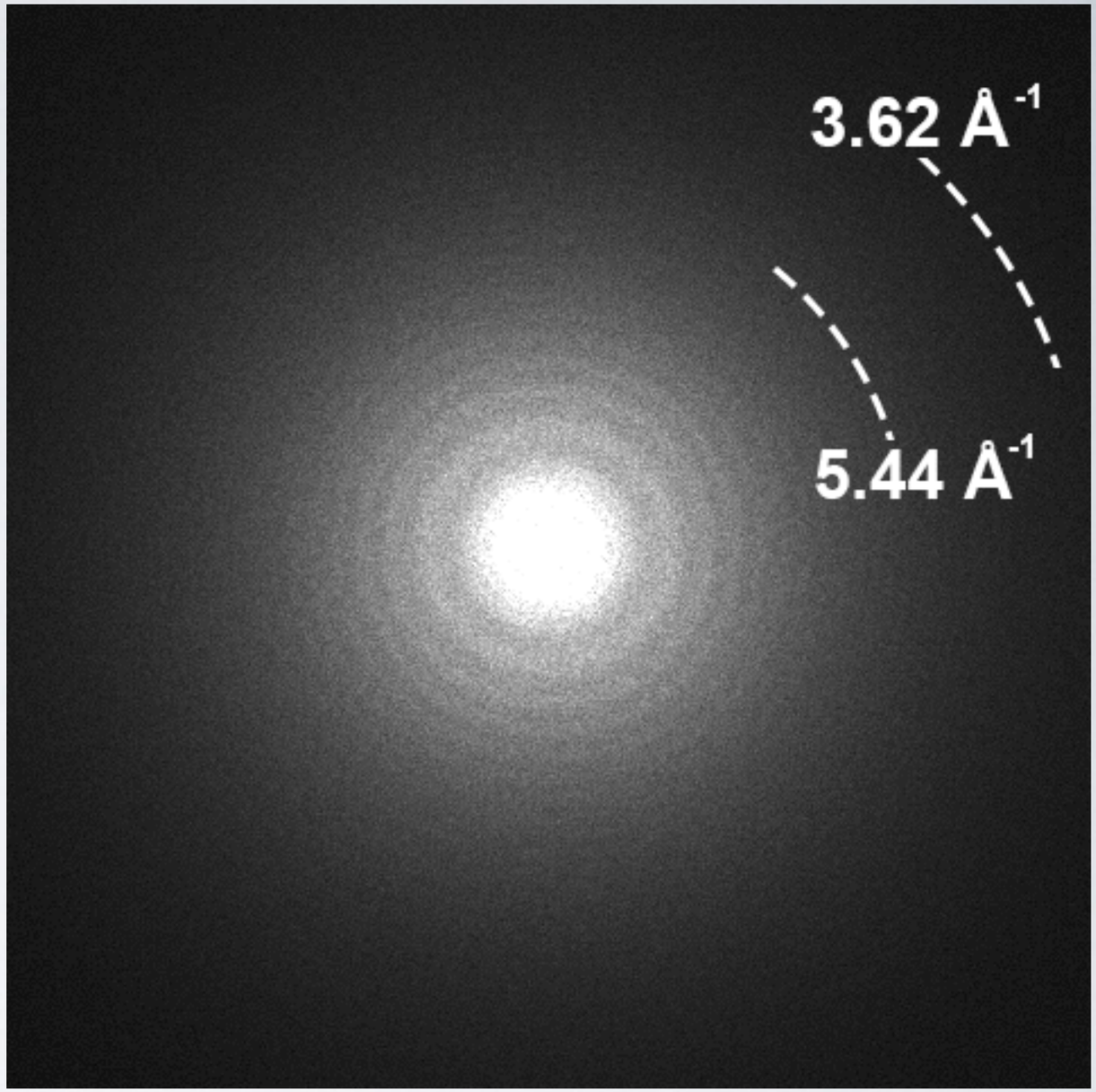
(~50e/pix/sec)

59,000x

(1.36 Å/pix)

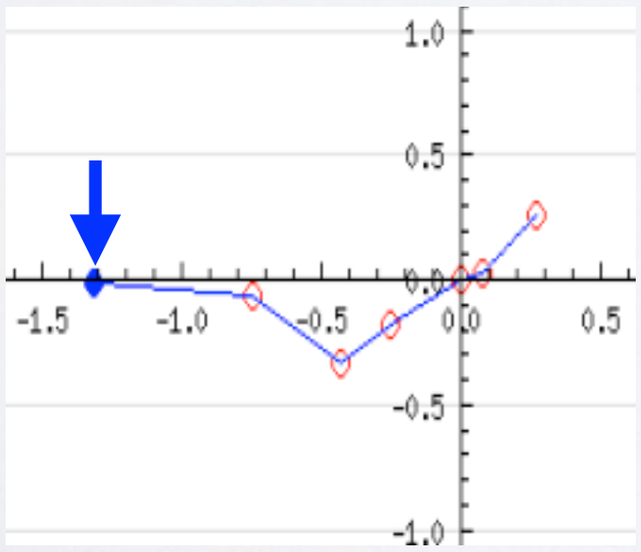
Wait 30 sec before each exposure

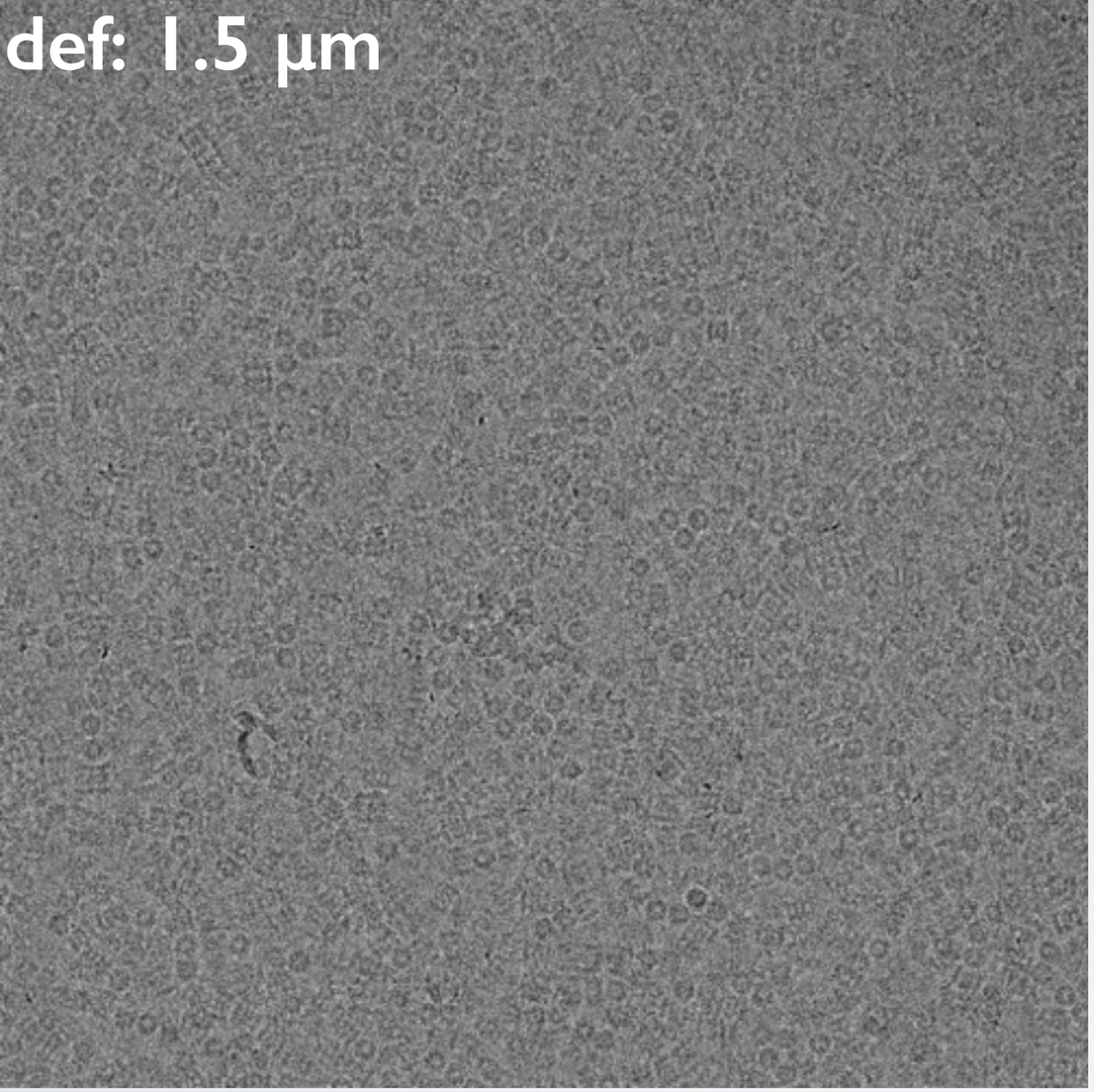


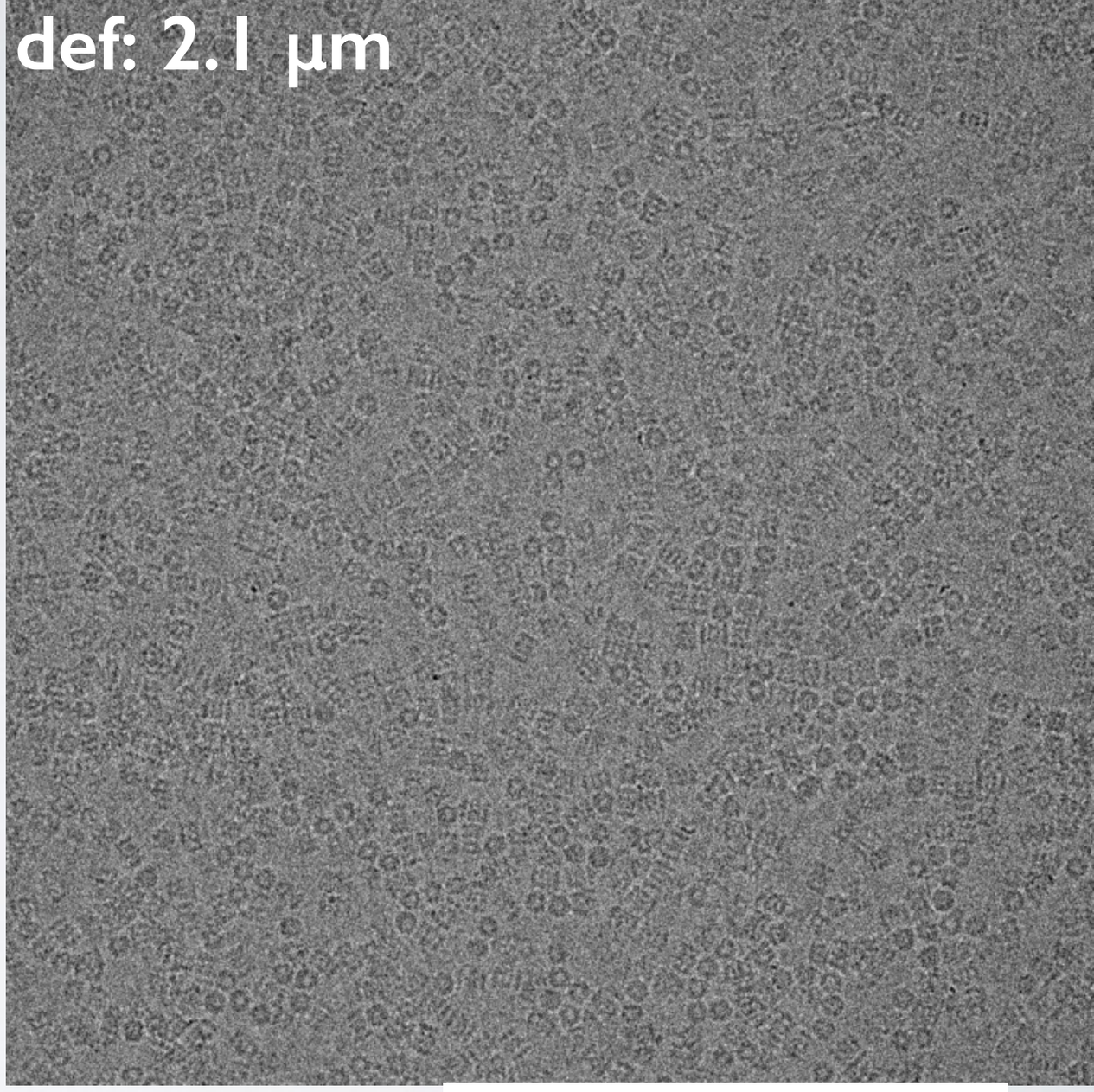


dosef_driftcorr

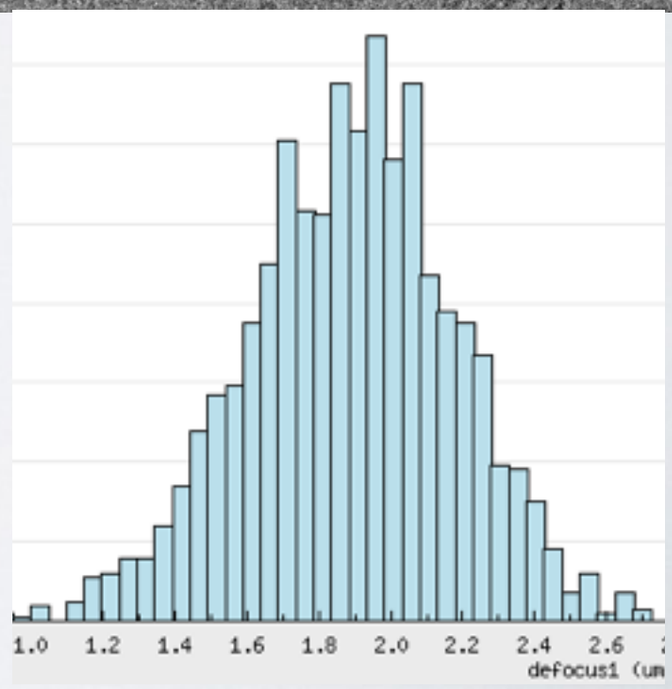
Li X. et al.
(2013) Nat. Methods





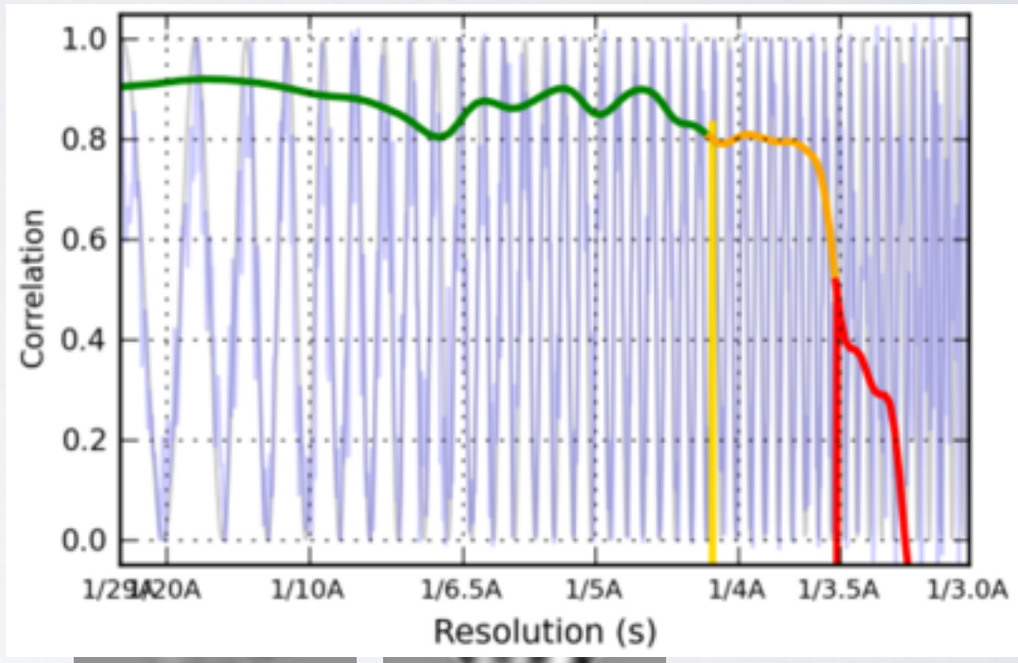


Data collected using a defocus spread comprised between 1.0 µm and 2.7 µm

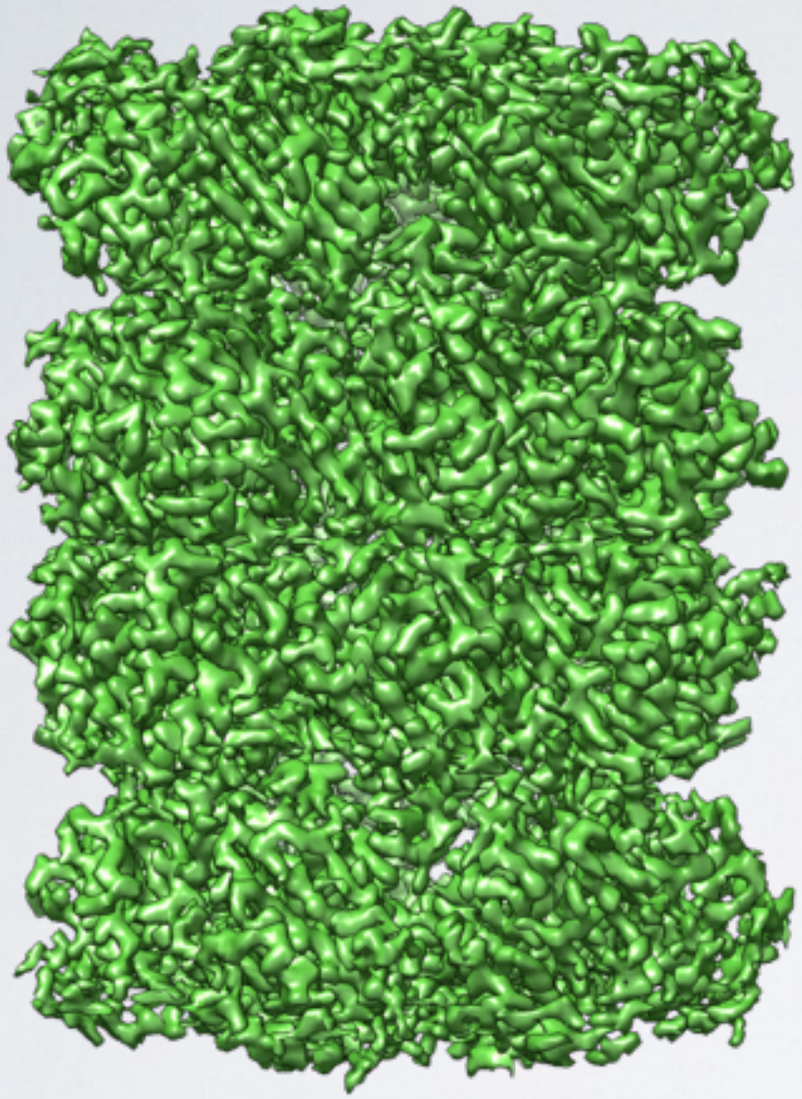


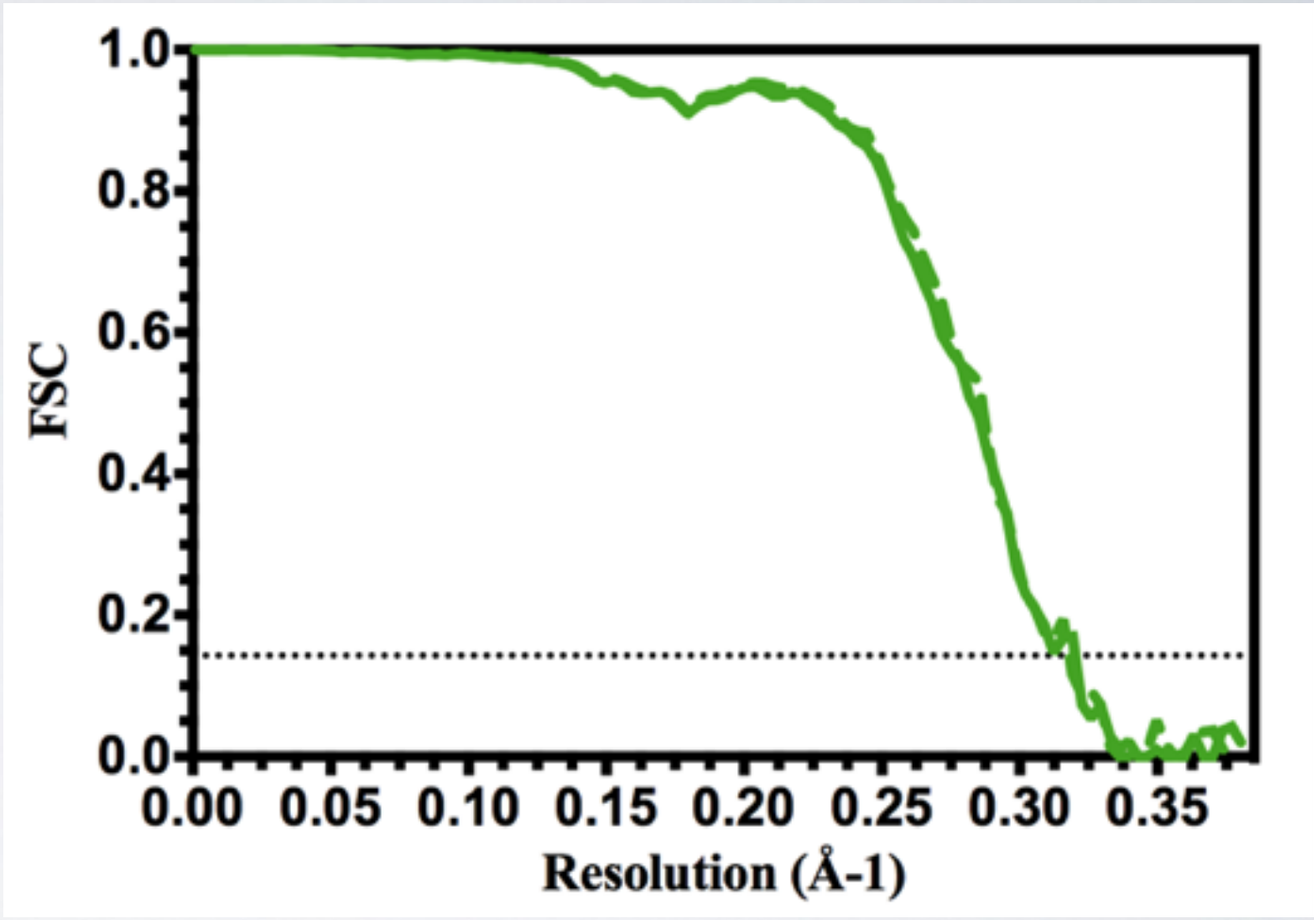
Krios/Falcon 2 statistics

- 1000 micrographs/487,184 particles picked
- Micrograph selection based on ice thickness: Thon rings 6Å resolution or better.
- 103 micrographs/48,023 particles
- Stack cleaning
  - *xmipp_mpi_classify_CL2D*
  - 45,945 particles
- Relion projection-matching & polishing

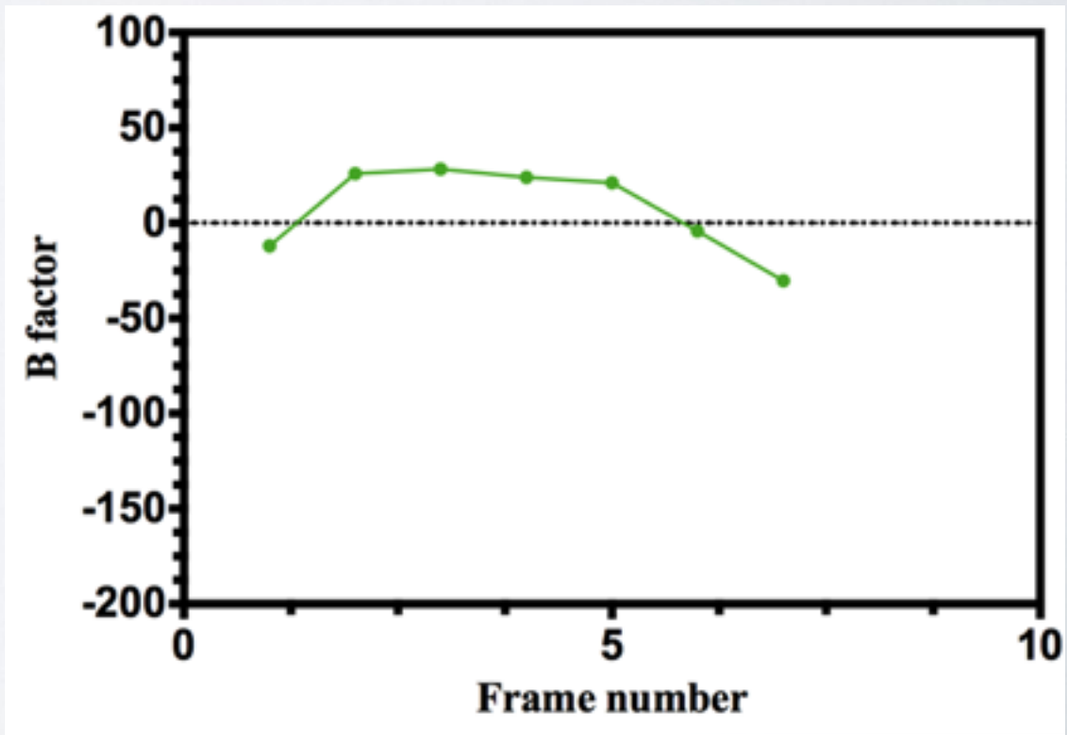


Krios/Falcon 2 reconstruction

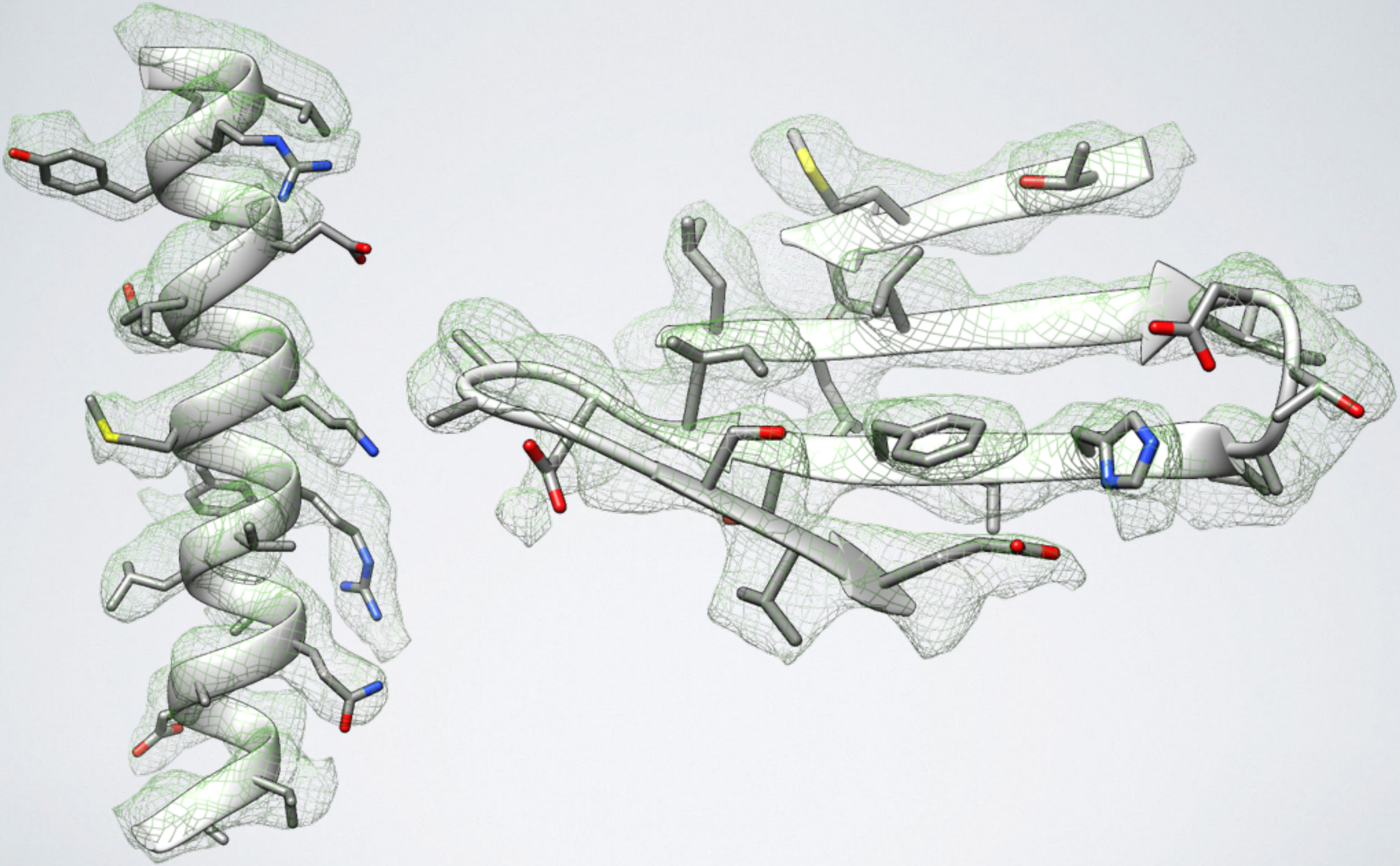




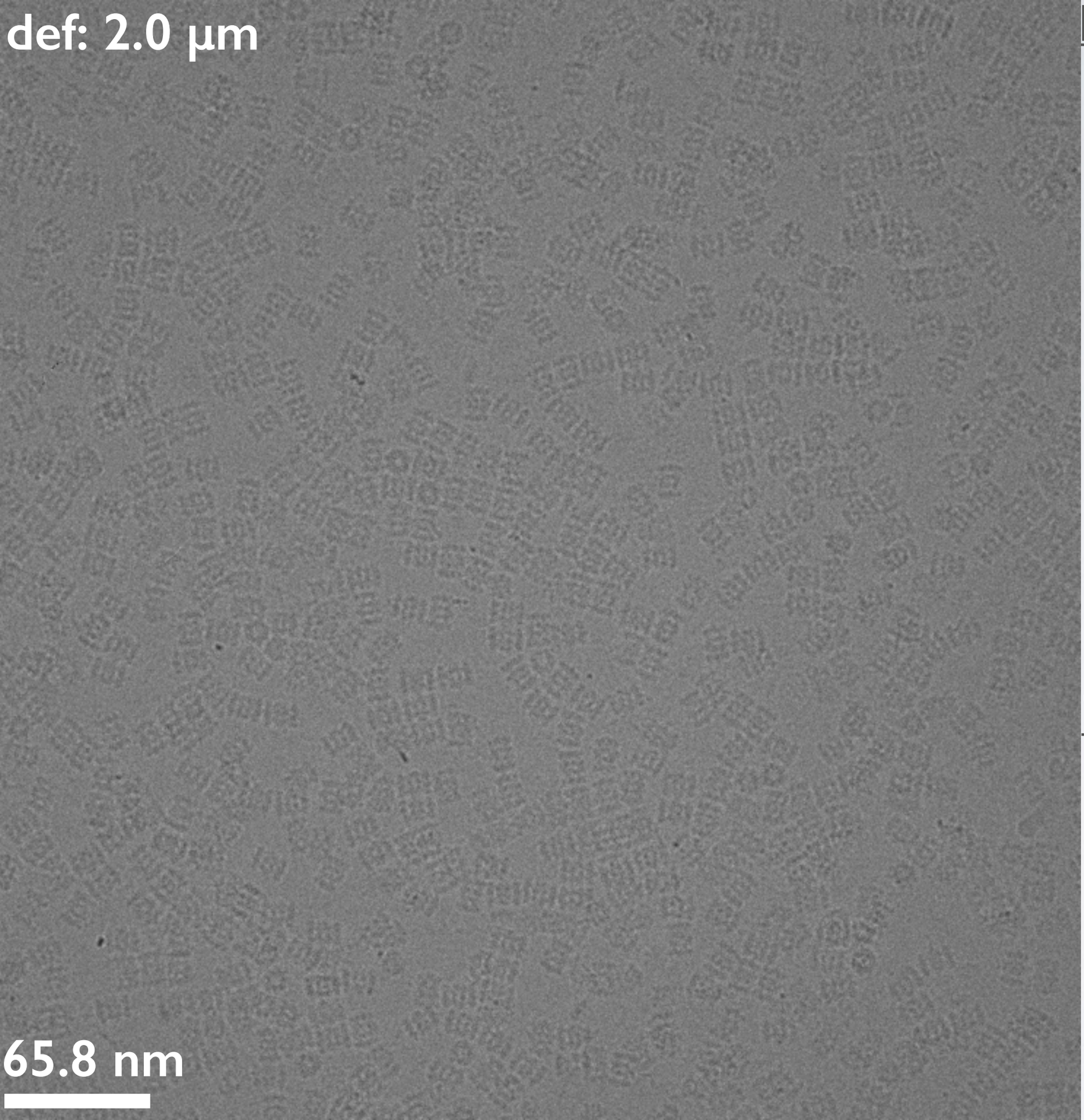
- - -	Relion 3D auto-refine	3.3 Å	82.4%
———	Particle polishing	3.26 Å	83.4%



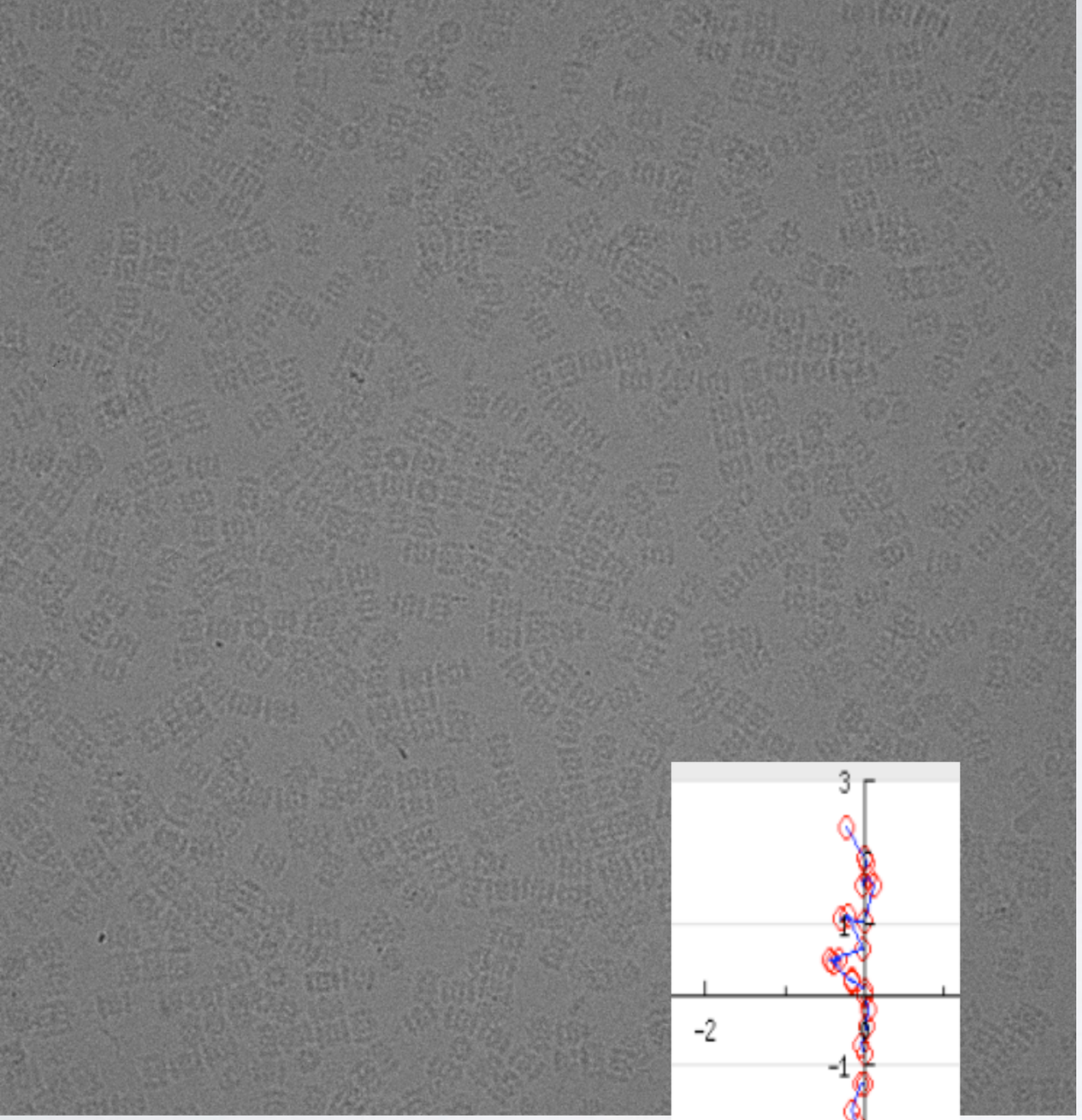
T20S at 3.26 Å resolution using a Falcon 2

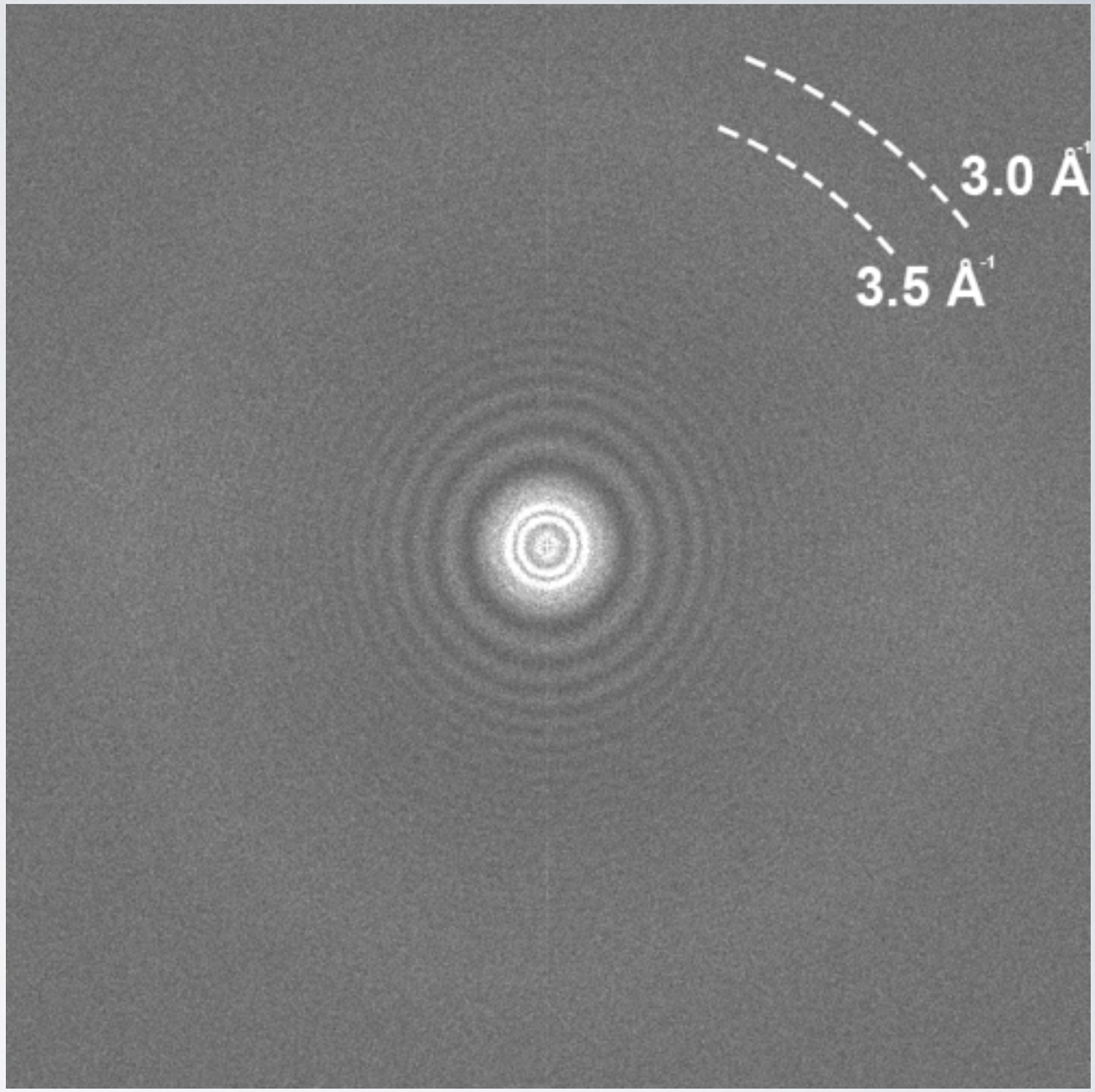


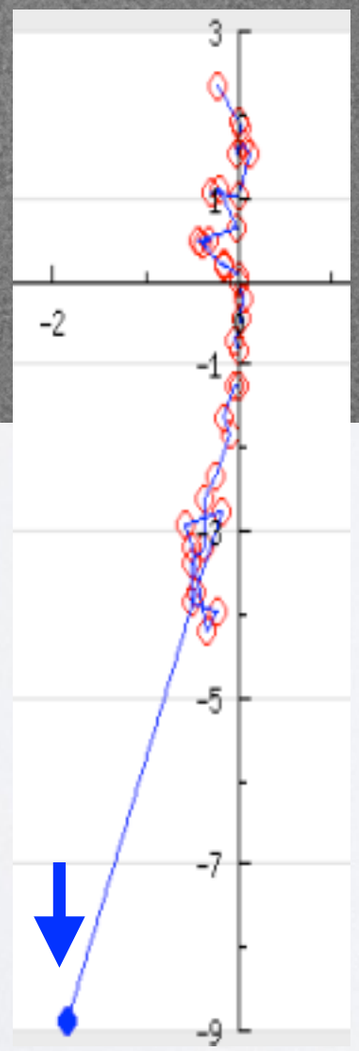
T20S data set collected using Titan Krios/K2 Summit



Krios/K2 (sup-res)
ext: 4500V
gun lens: 3
spotsize: 8
C2: 70 µm
Obj: 100 µm
beam: 1.9 µm
Microprobe
dose: 39 e/Å^2
~9cts/pix/sec
~12e/pix/sec
7.6sec - 38 frames
22,500x
(0.6575-1.315 Å/pix)
Wait 40 sec before each exposure





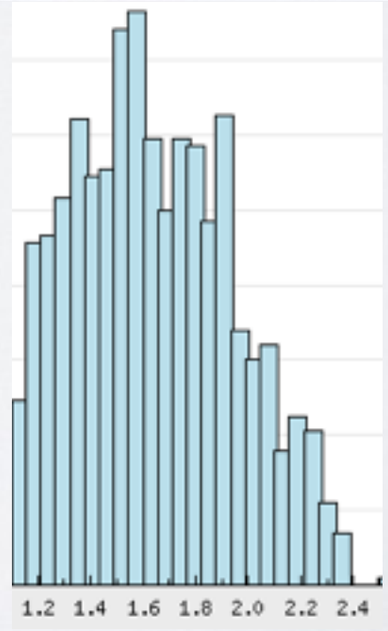


dosef_driftcorr

Li X. et al.
(2013) Nat. Methods

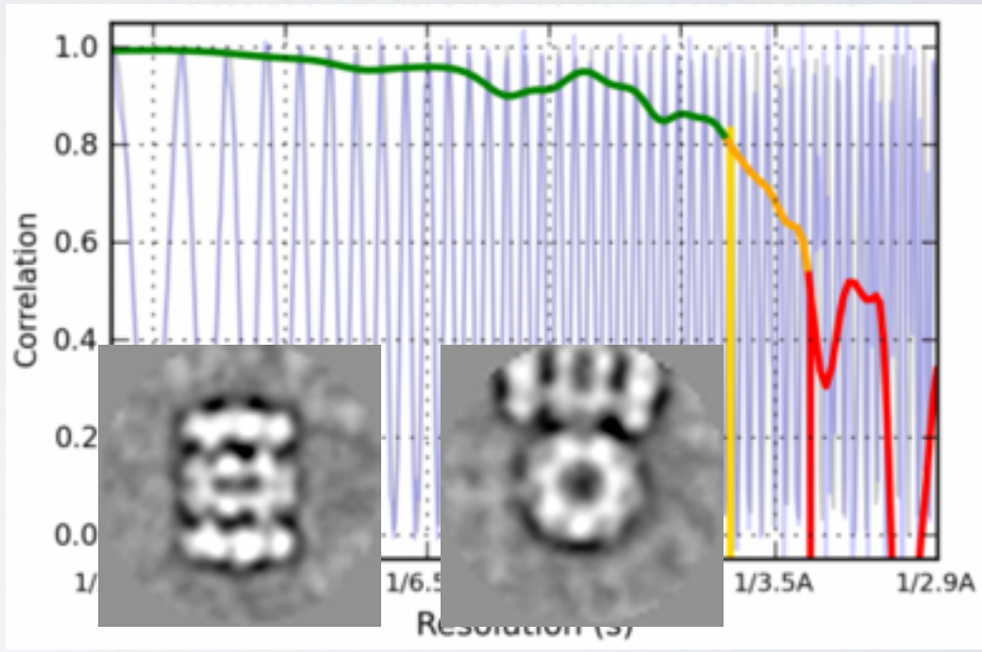
B=1000 pixel^2

Data collected using a defocus spread comprised between 1.1 µm and 2.4 µm

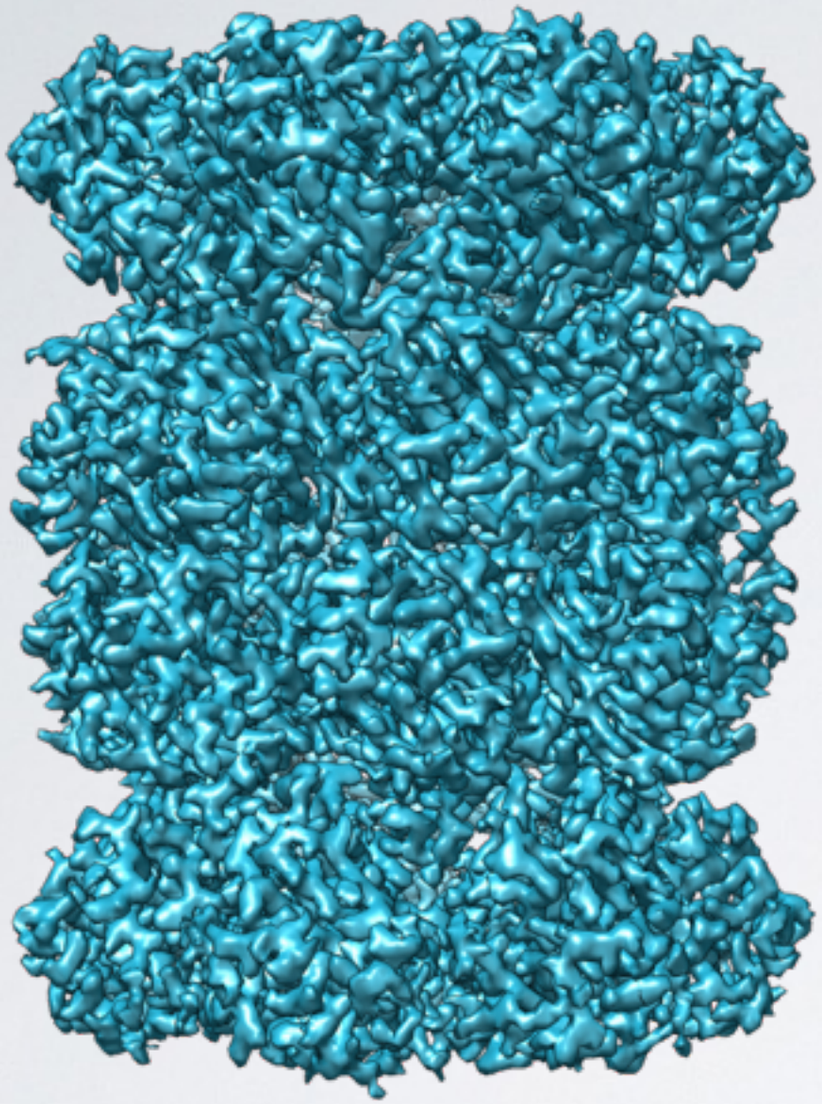


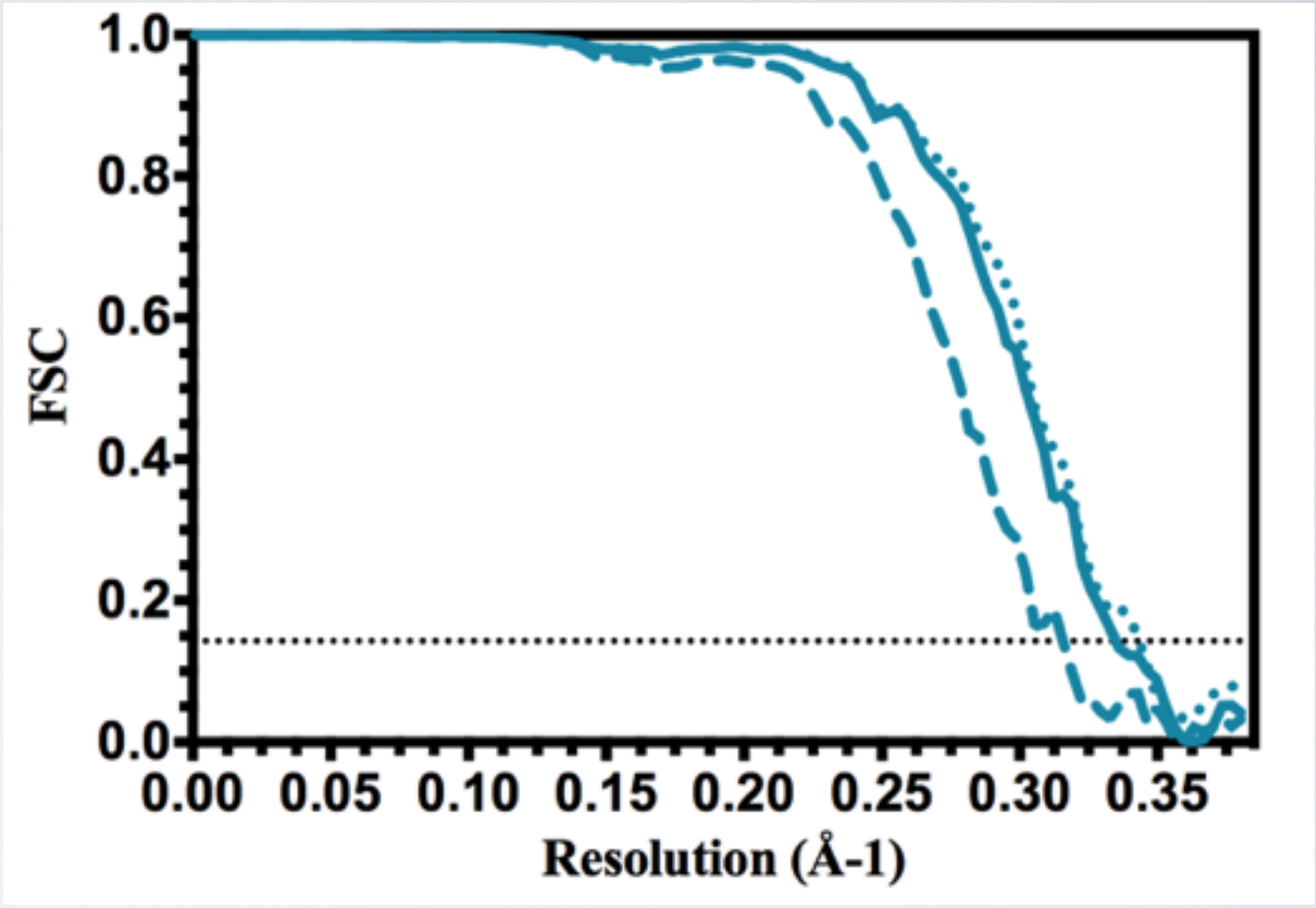
Krios/K2 statistics

- 868 micrographs/419,169 particles picked
- Micrograph selection based on ice thickness: Thon rings 4.5Å or better.
- 138 micrographs/62,551 particles
- Stack cleaning
  - xmipp_image_sort_by_statistics
  - *xmipp_mpi_classify_CL2D*
  - 51,218 particles
- Relion projection-matching & polishing

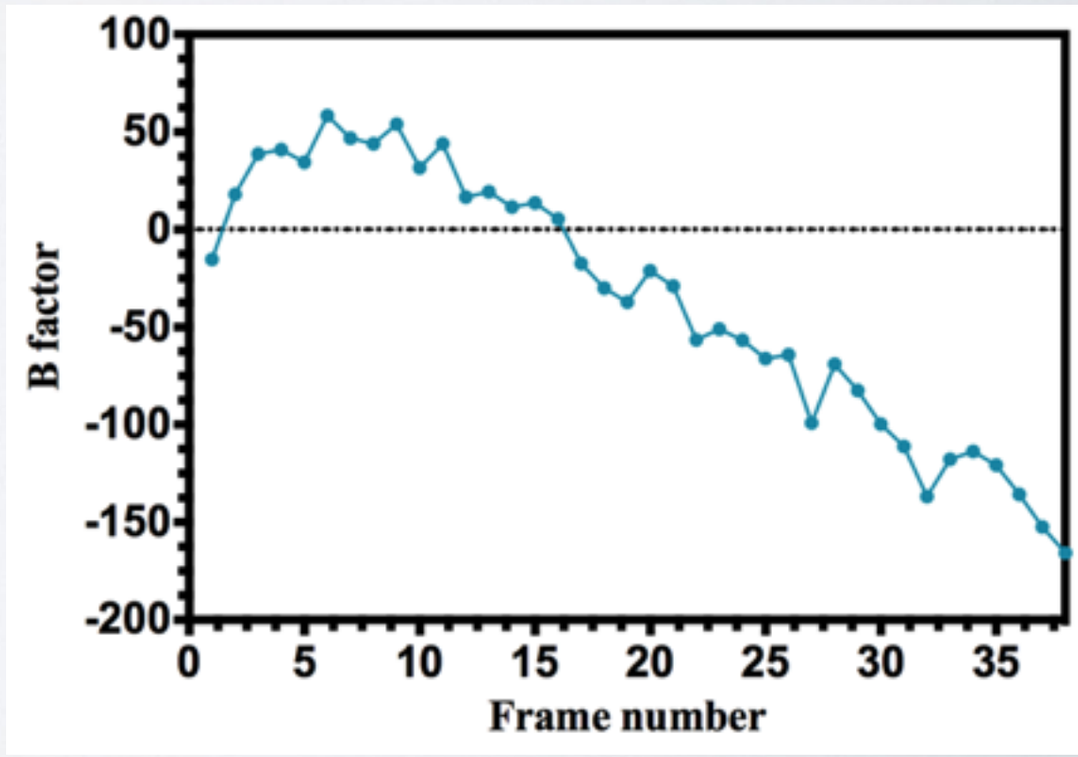


Krios/K2 reconstruction

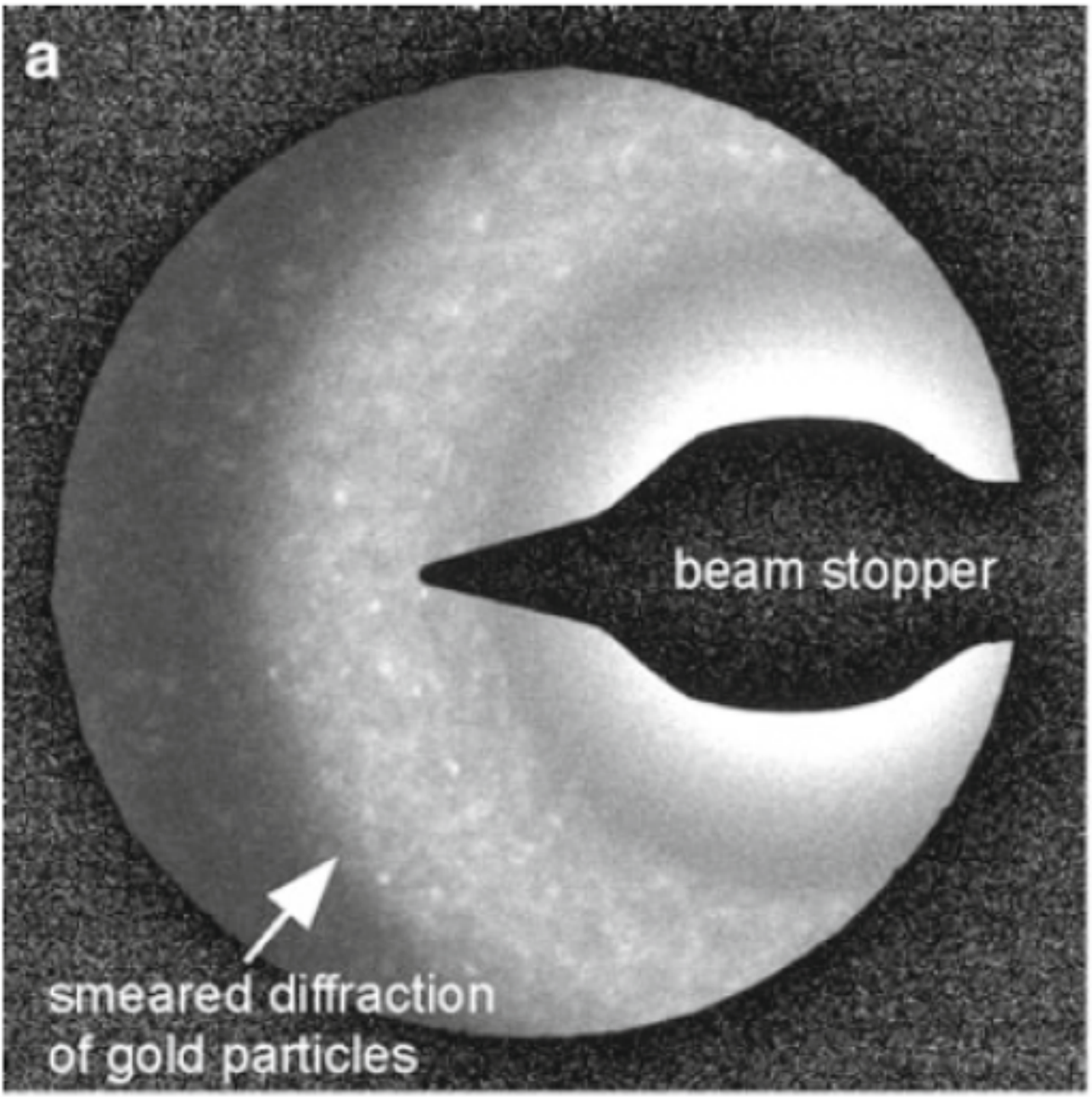


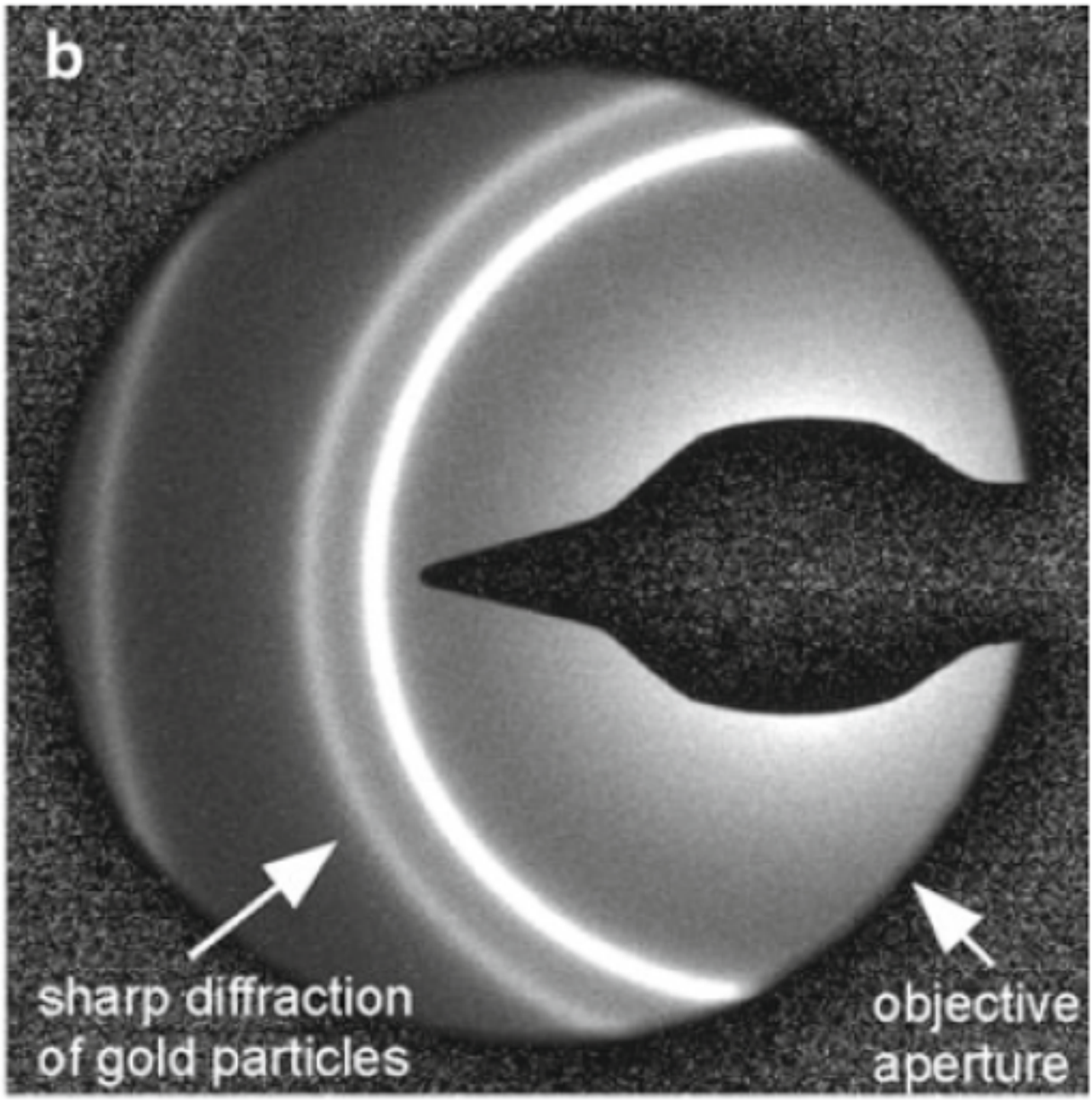


— — —	Relion 3D auto-refine	3.2 Å	82.0%
———	Particle polishing	3.0 Å	87.7%
·····	Particle polishing + MaxProb filter (37,005 out of 51,218 particles)	2.9 Å	90.7%



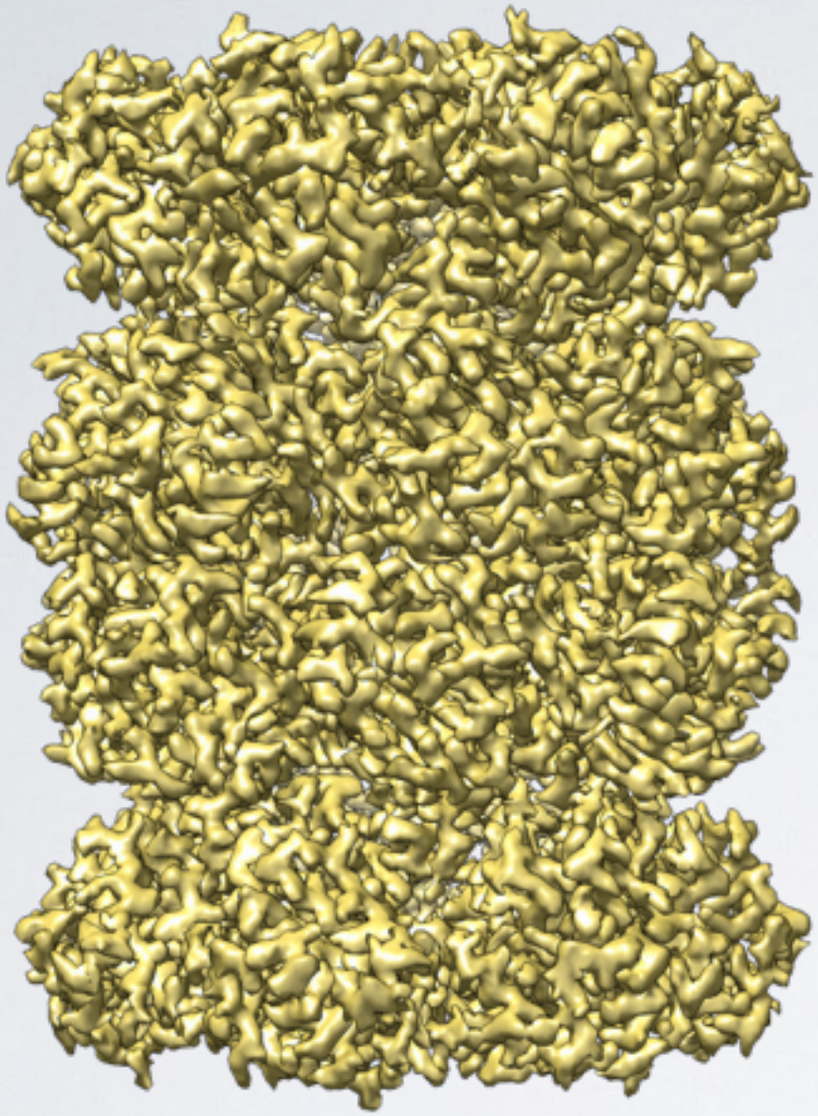
Is 2.9 Å resolution the best we can do?

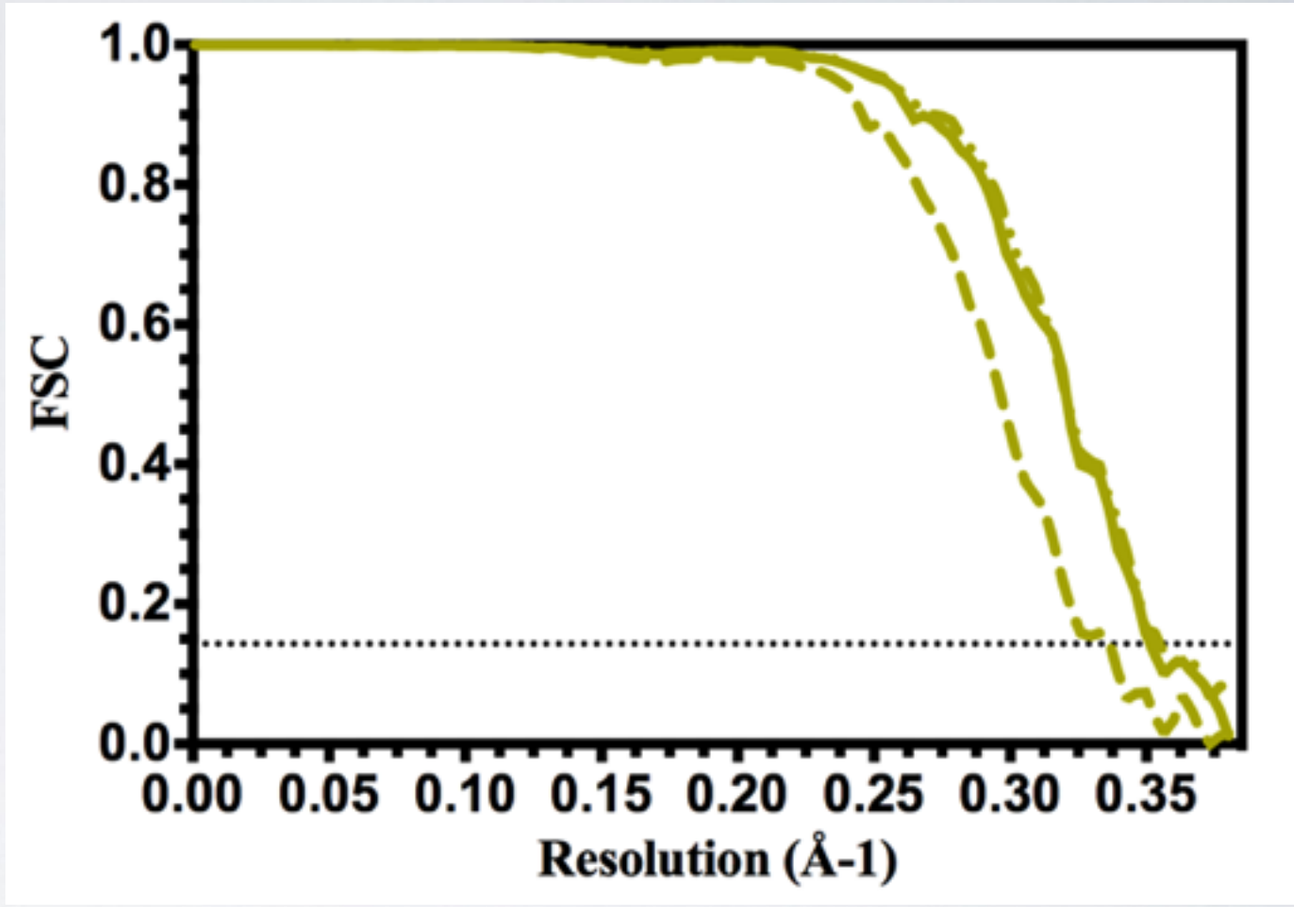




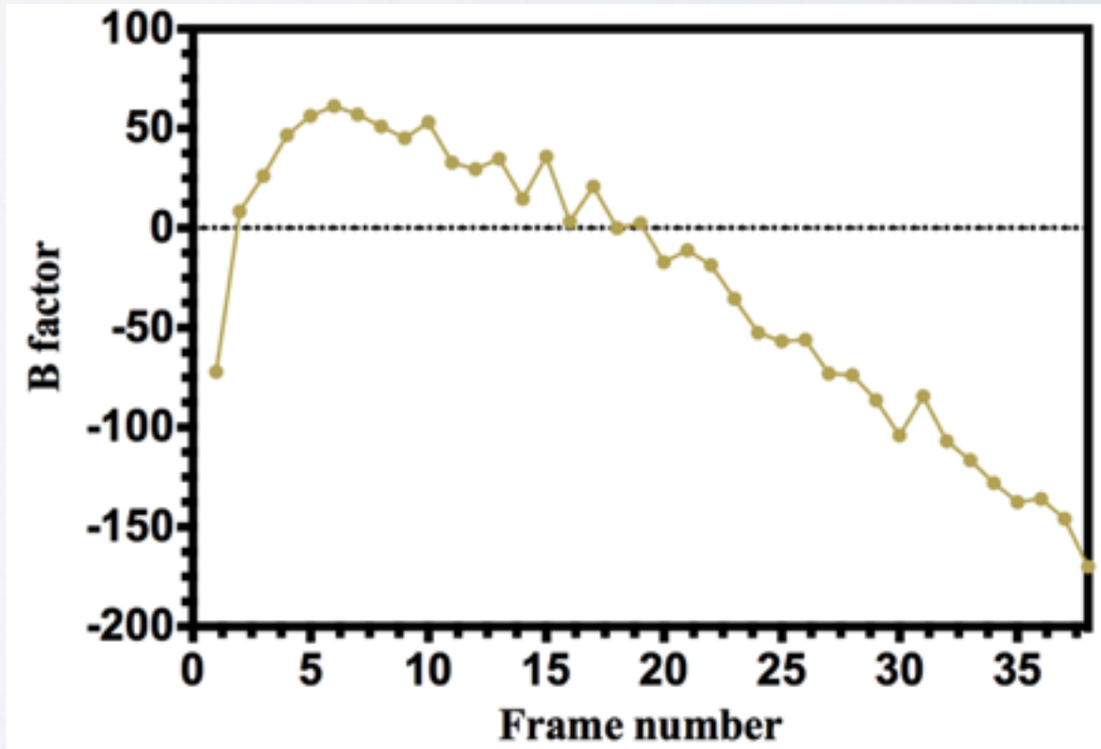
Avila-Sakar A. et al.
(2013) Methods Mol Biol.

A perfectly parallel illumination

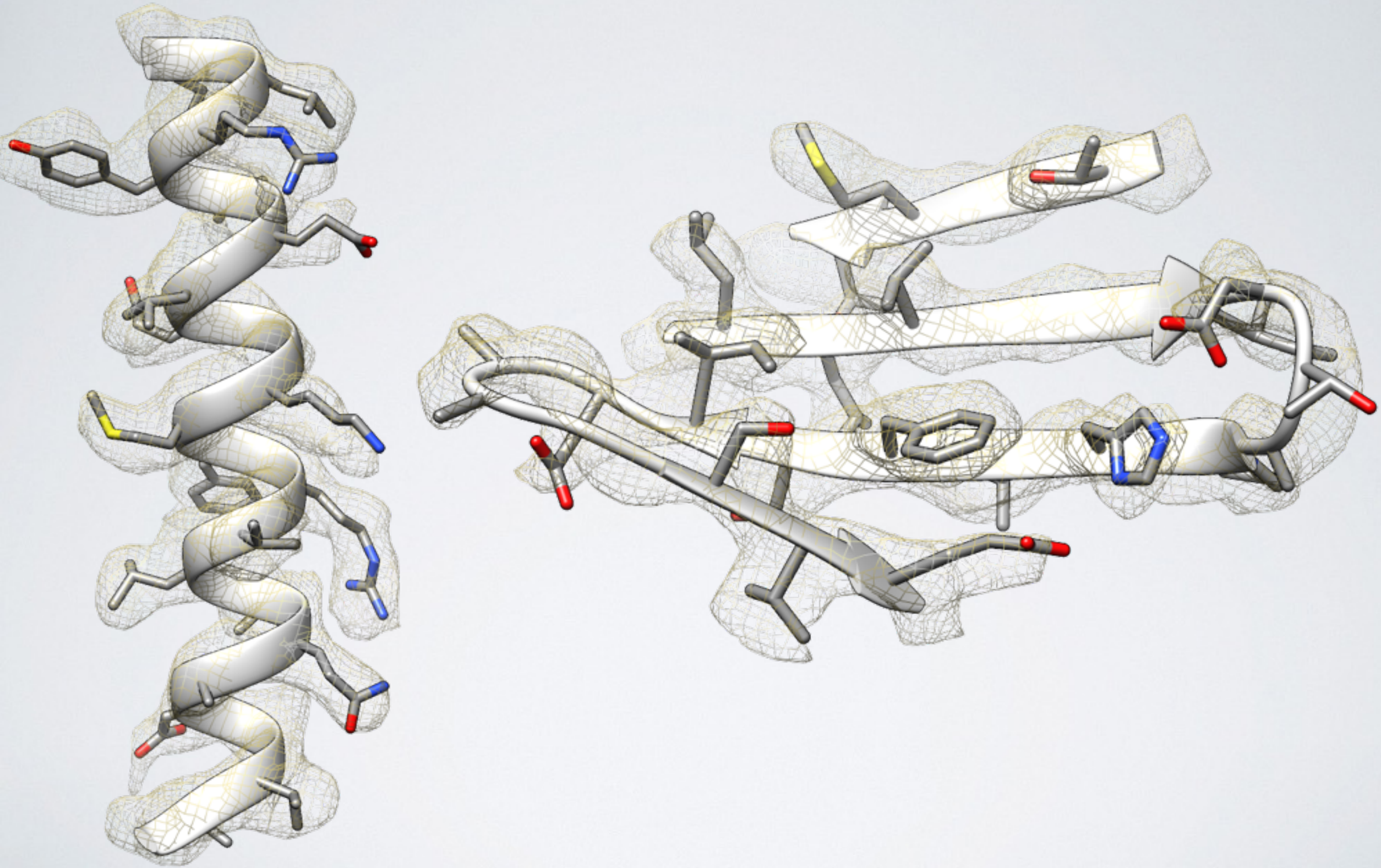




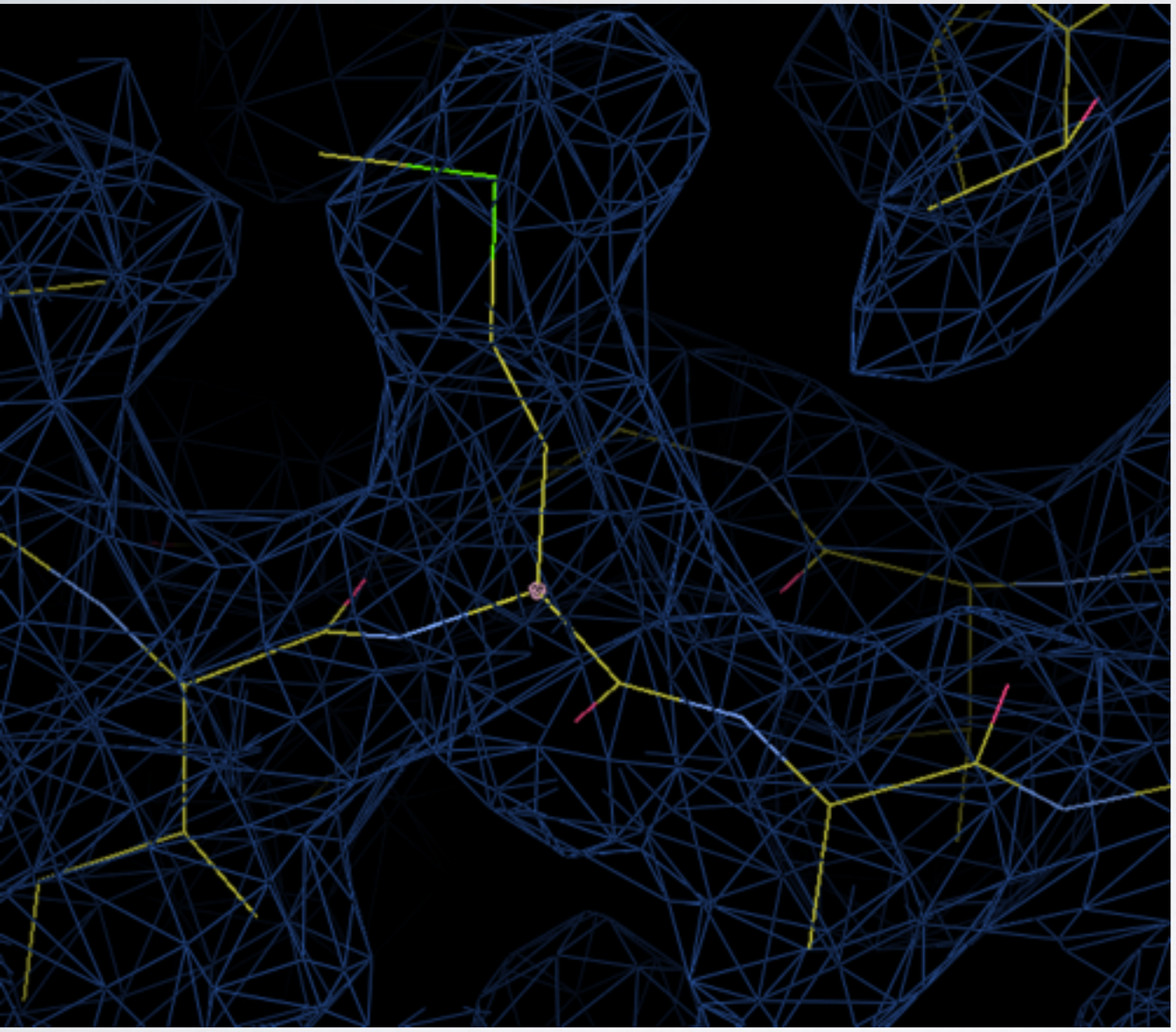
— — —	Relion 3D auto-refine	3.0 Å	87.7%
———	Particle polishing	2.86 Å	92.0%
—·· —·· —	Particle polishing 0.98 Å/pixel	2.83 Å	69.2%

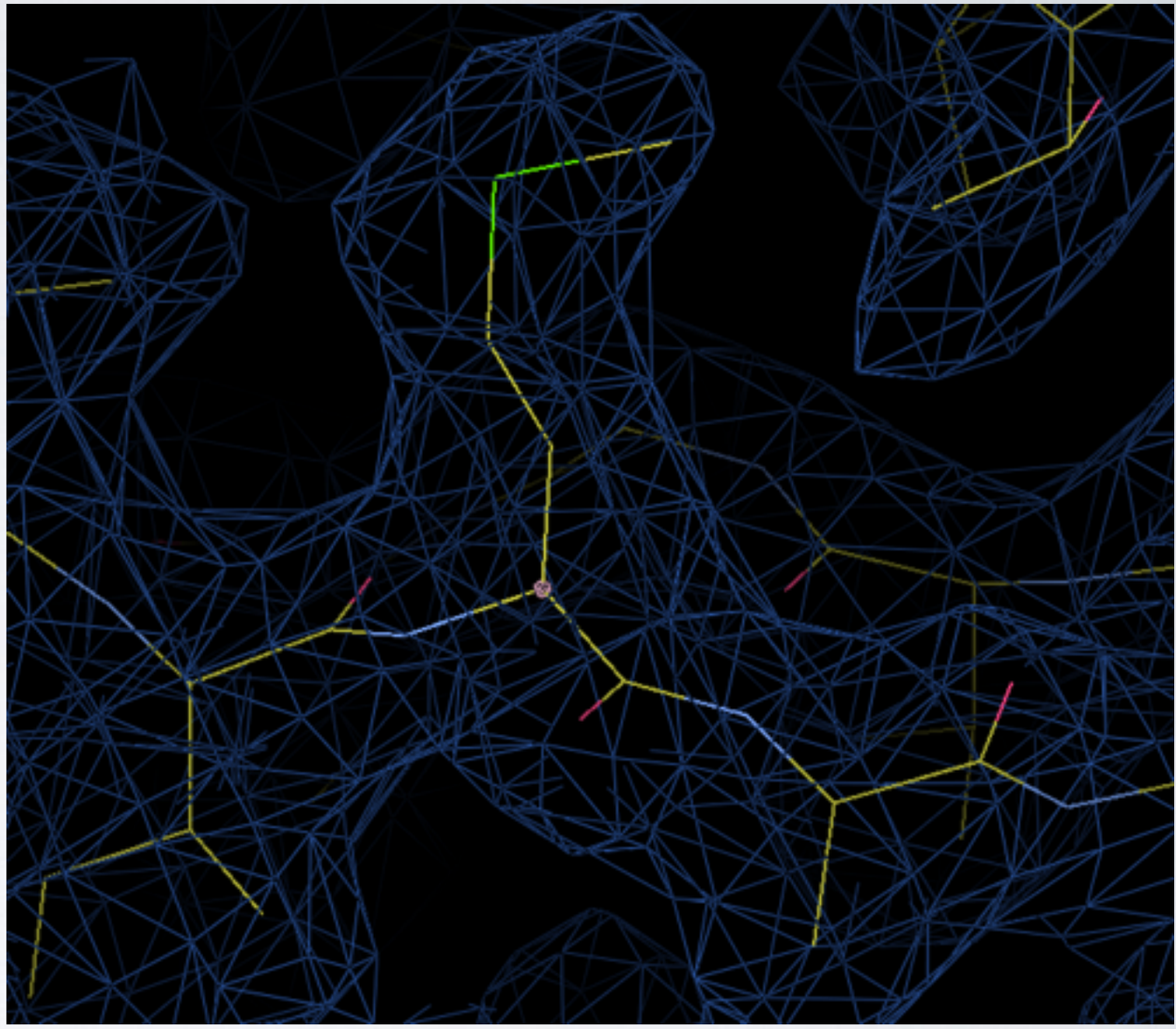


T20S at 2.8 Å resolution using a K2



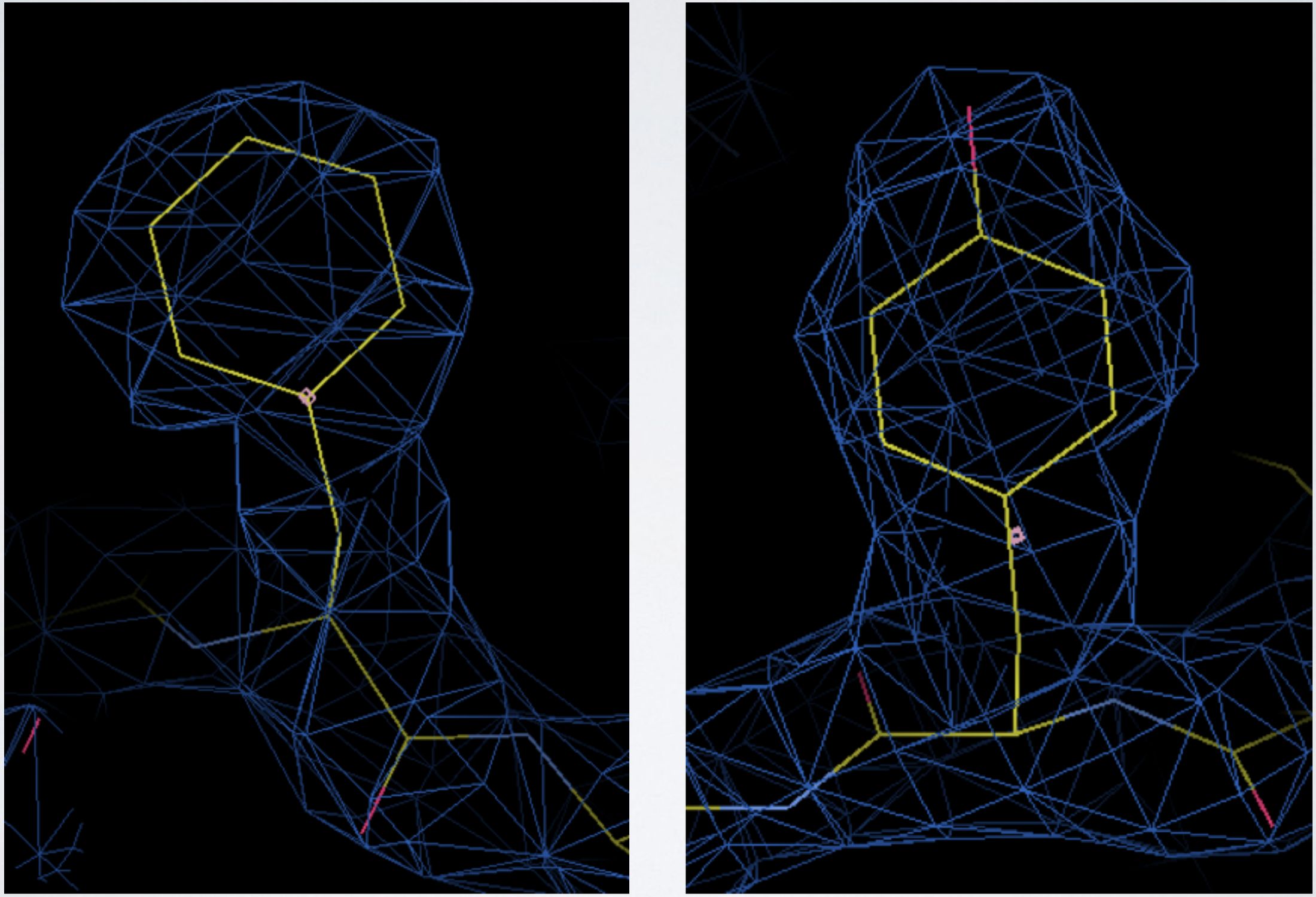
T20S at 2.8 Å resolution using a K2





Some side chain rotamers can be distinguished and adjusted

T20S at 2.8 Å resolution using a K2

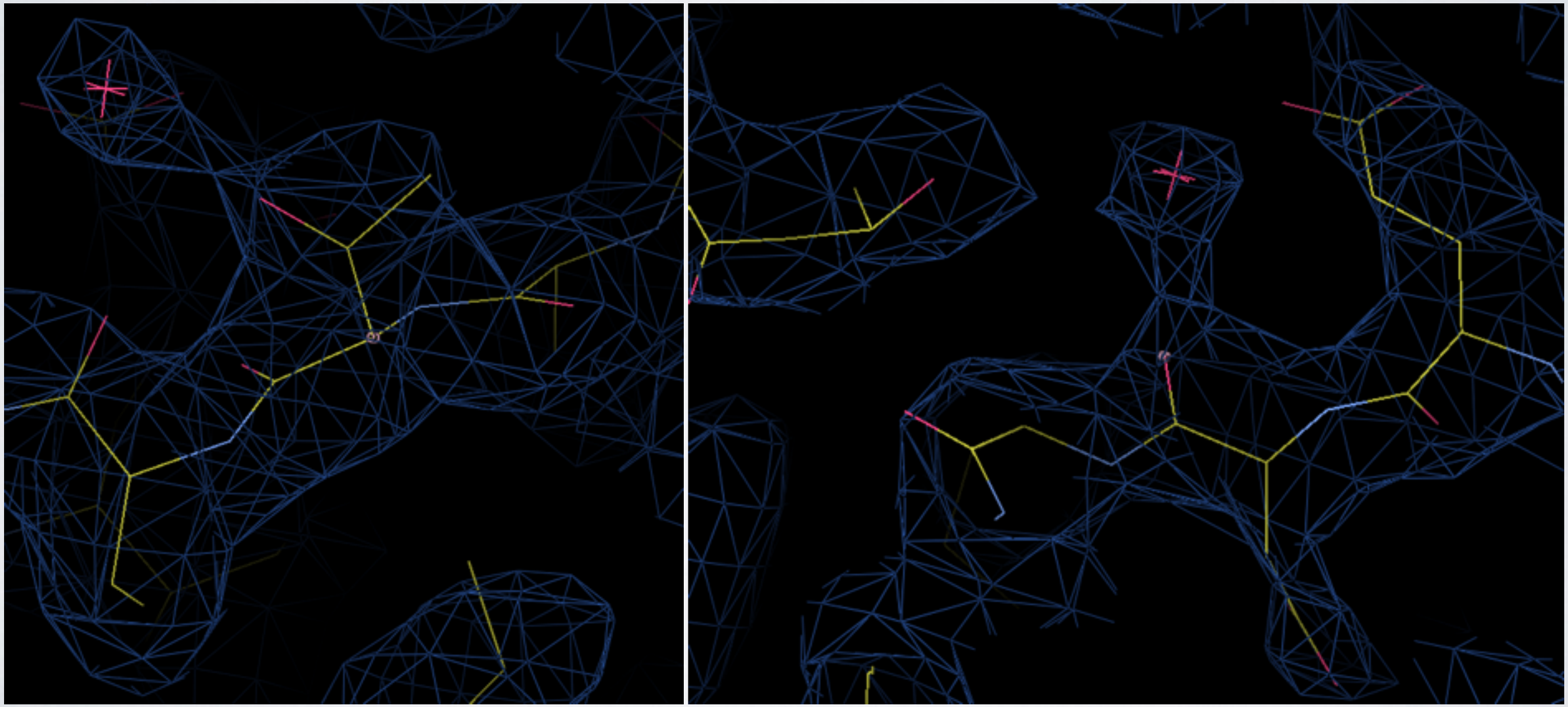


Distinguishing between Phe and Tyr start to become possible

How about water molecules?

As a rule of thumb, the number of water molecules expected to be visible in a structure solved by X-ray crystallography is:

(3-resolution) x number of residues

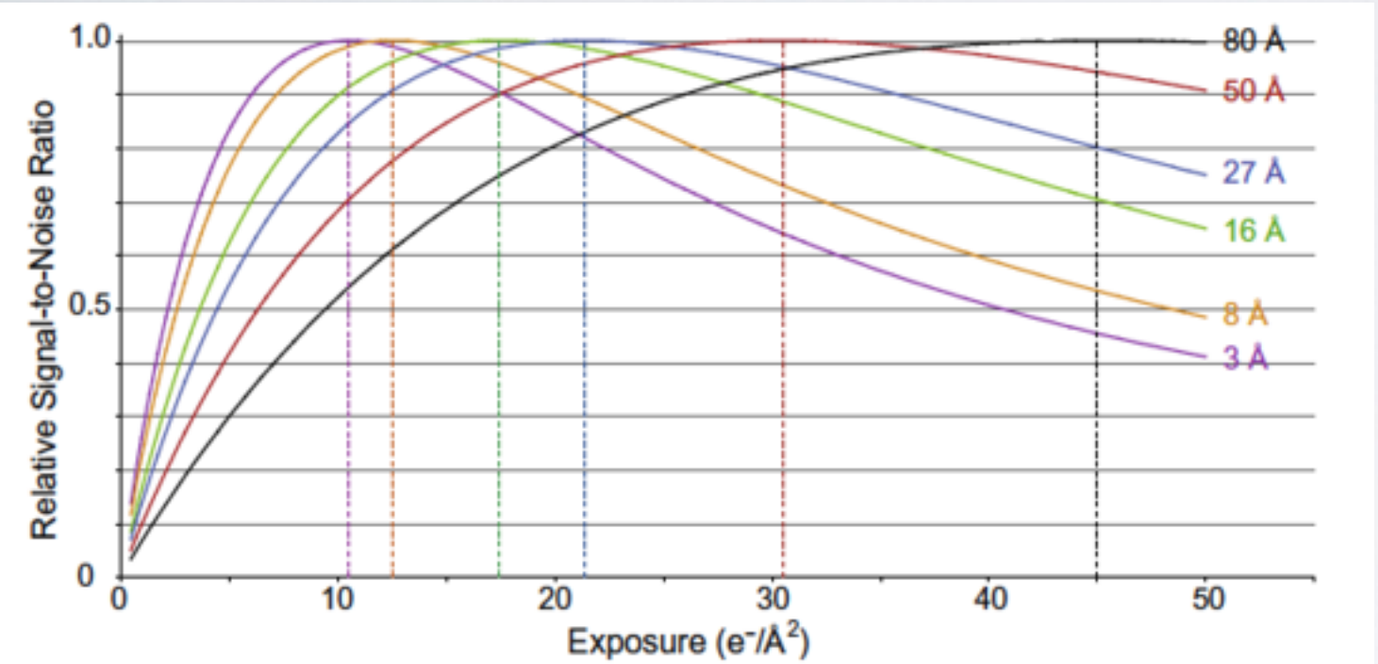


How do we know those are water molecules?

- Appropriate chemical environment
- Expected distances for H-bonding (2.8-3.5 Å)
- Visible in the two half maps produced by the gold-standard refinement procedure
- Locations cross-validated by looking at a 1.9 Å X-ray structure of the T20S (1YAR)

Optimal exposure for single-particle

Catalase crystals



Baker L.A. et al.
(2010) J Struct Biol.

Single particle

T20S-Krios/Falcon2	3.3 Å	26 e/Å^2
T20S-Krios/K2	3.0 Å	39 e/Å^2
T20S-TF20/K2	4.4 Å	38 e/Å^2
NwV-TF20/K2	3.7 Å	38 e/Å^2

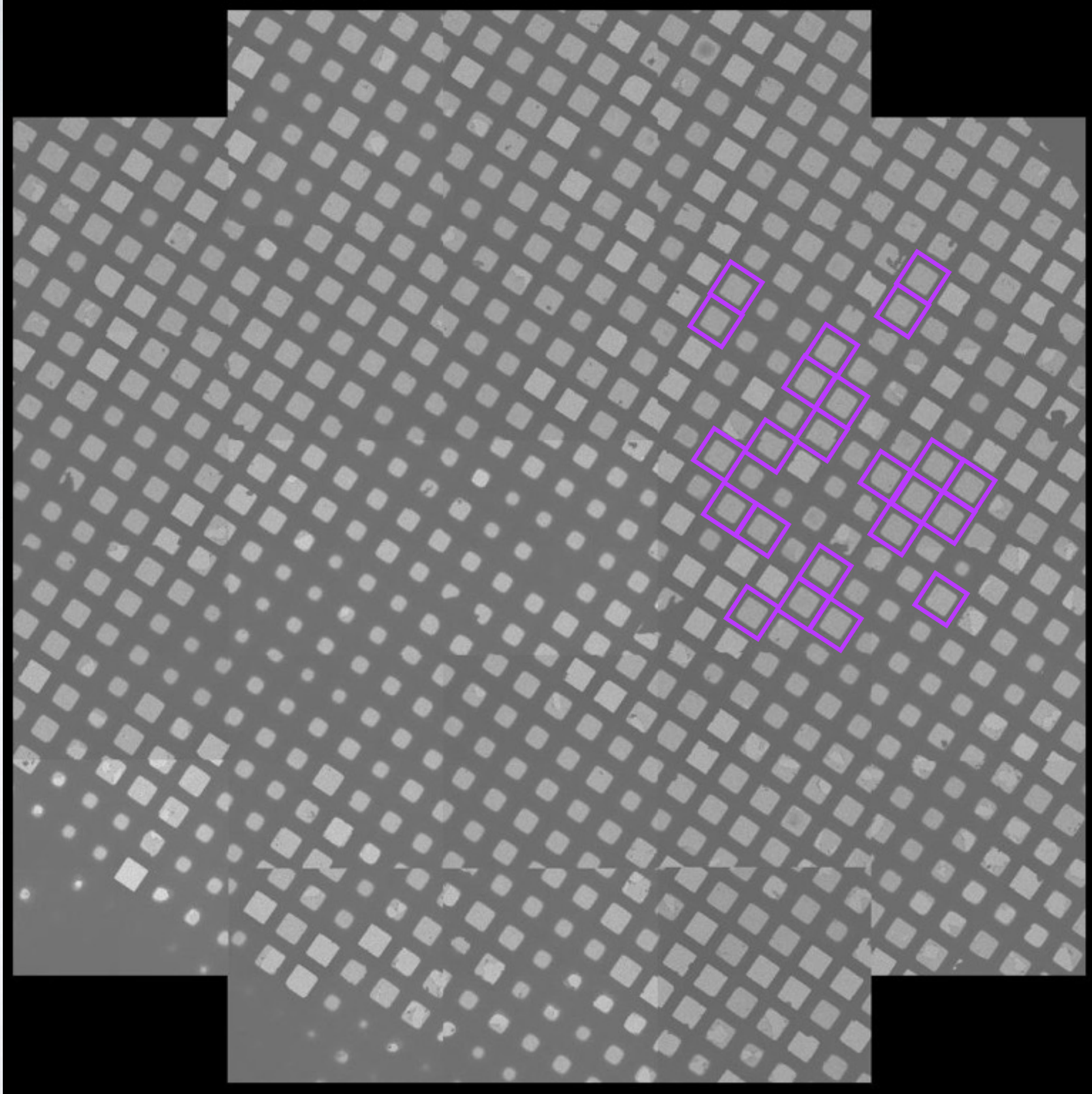
without frequency dependent weighting

Atlas

81x

Chose 21 grid squares to target

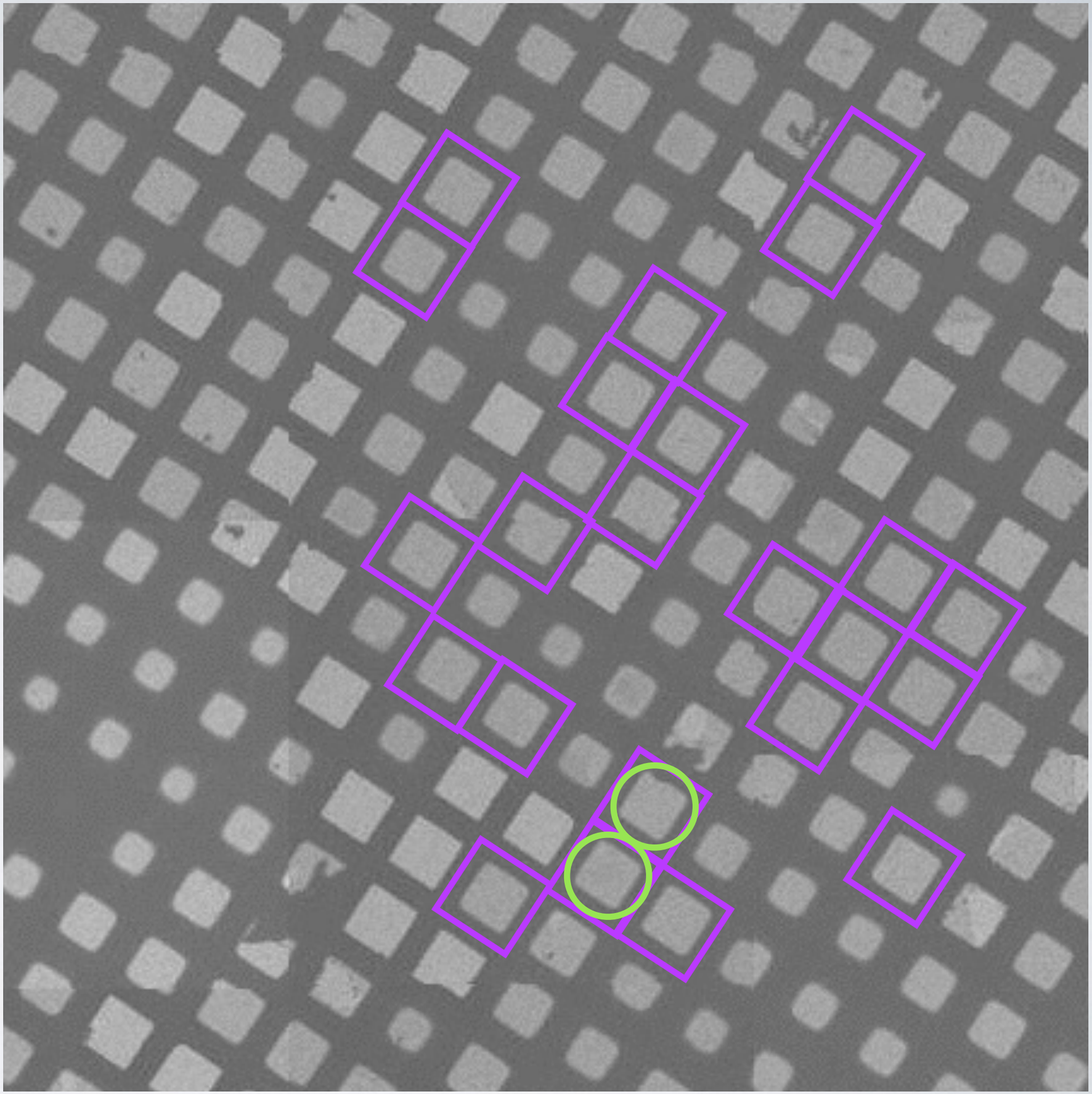
c-flat
1 µm holes
plasma cleaned
frozen with cp3



Atlas
(Zoom)

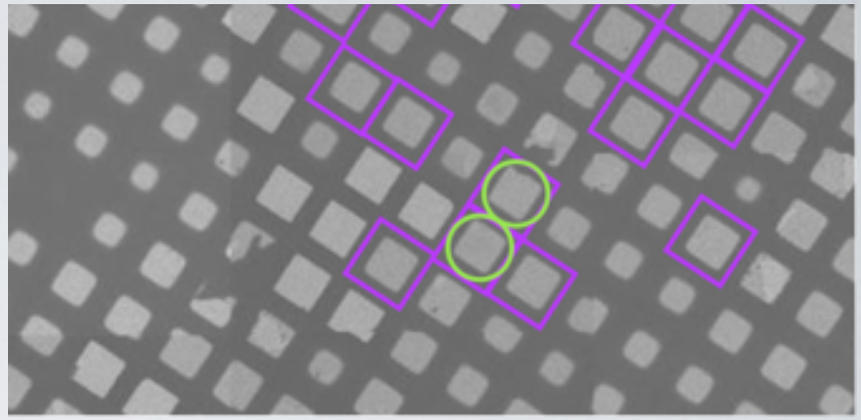
81x

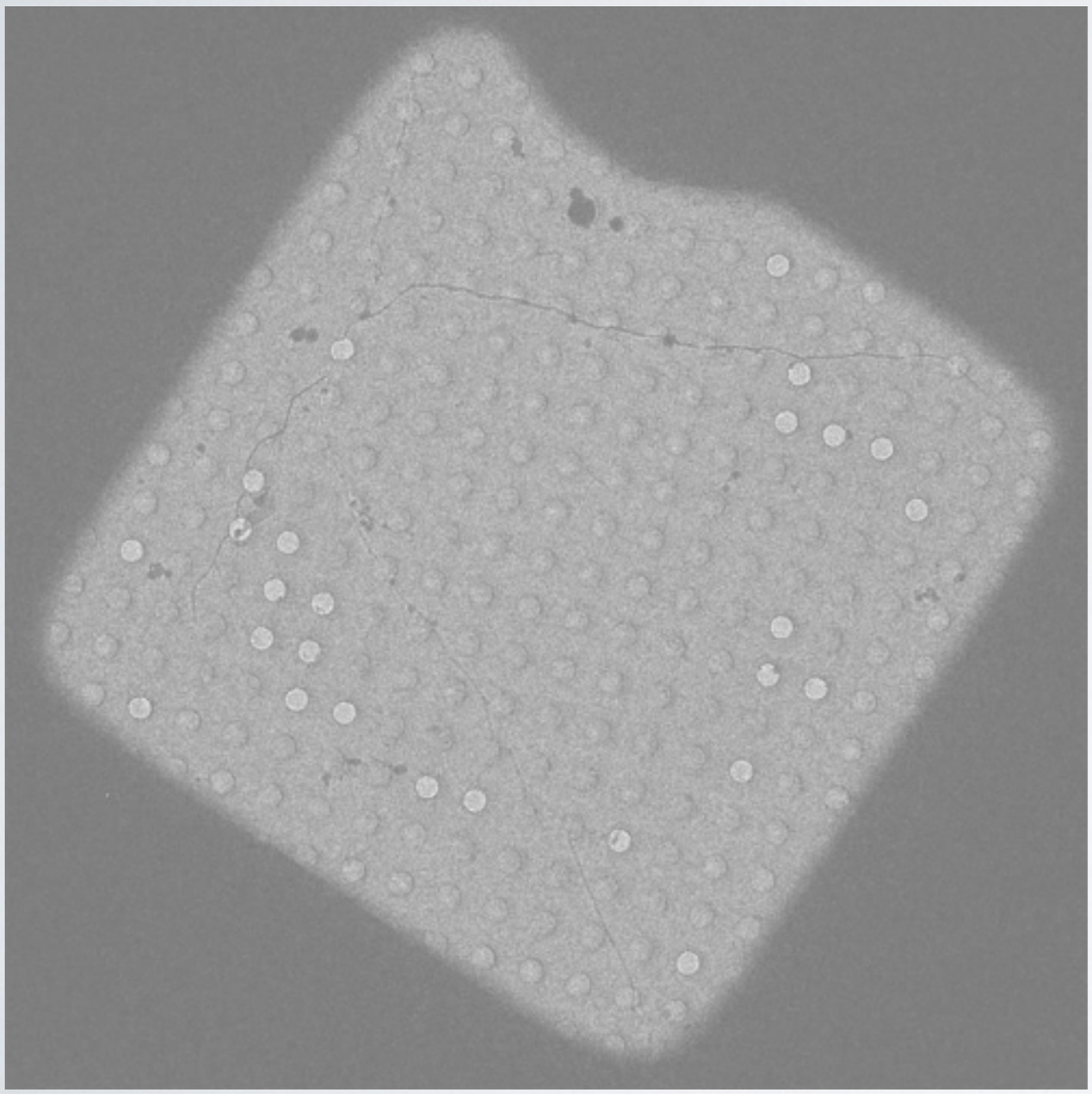
Chose 21 grid squares to target

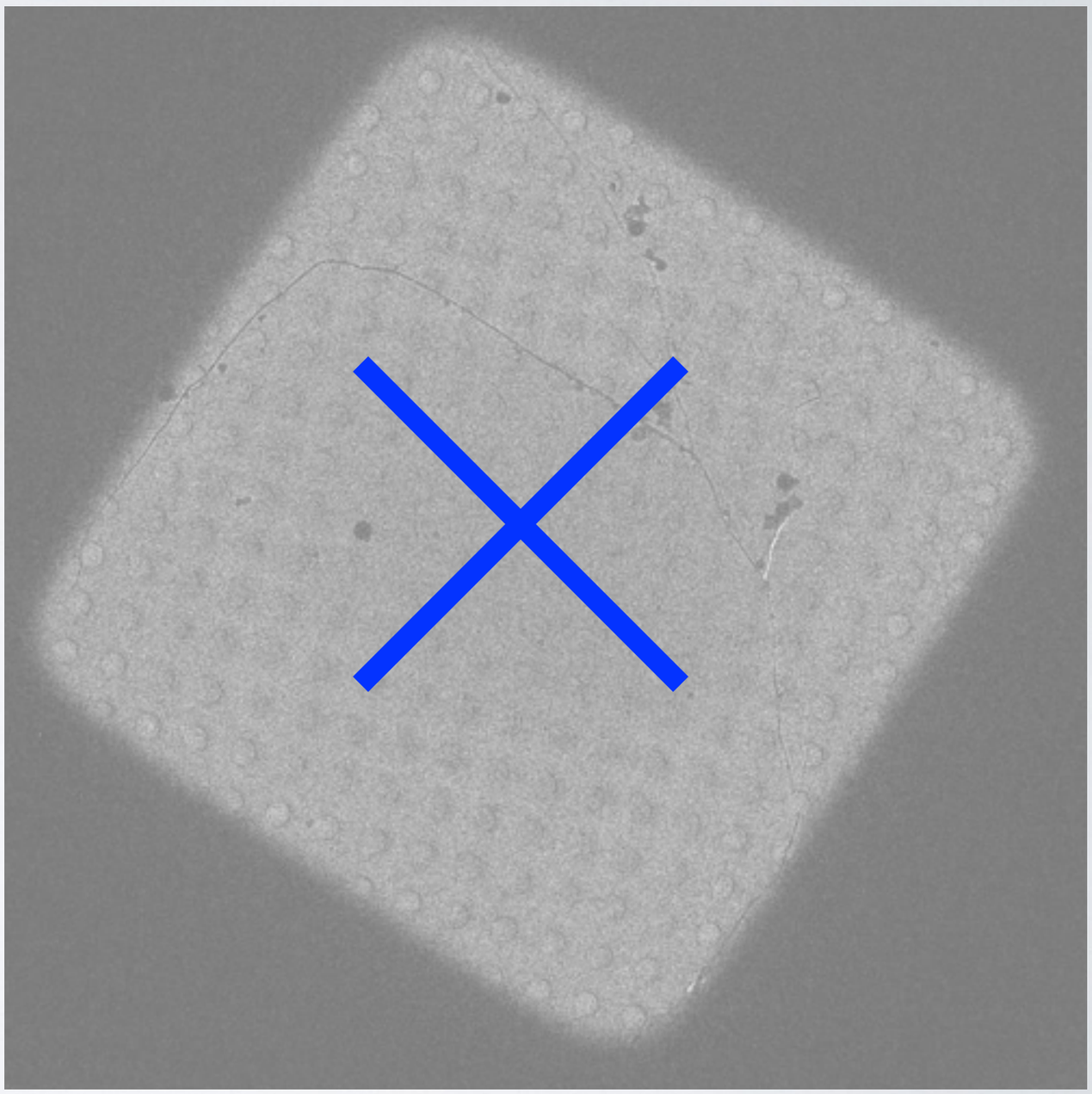


Thin vs Thick Ice

165x





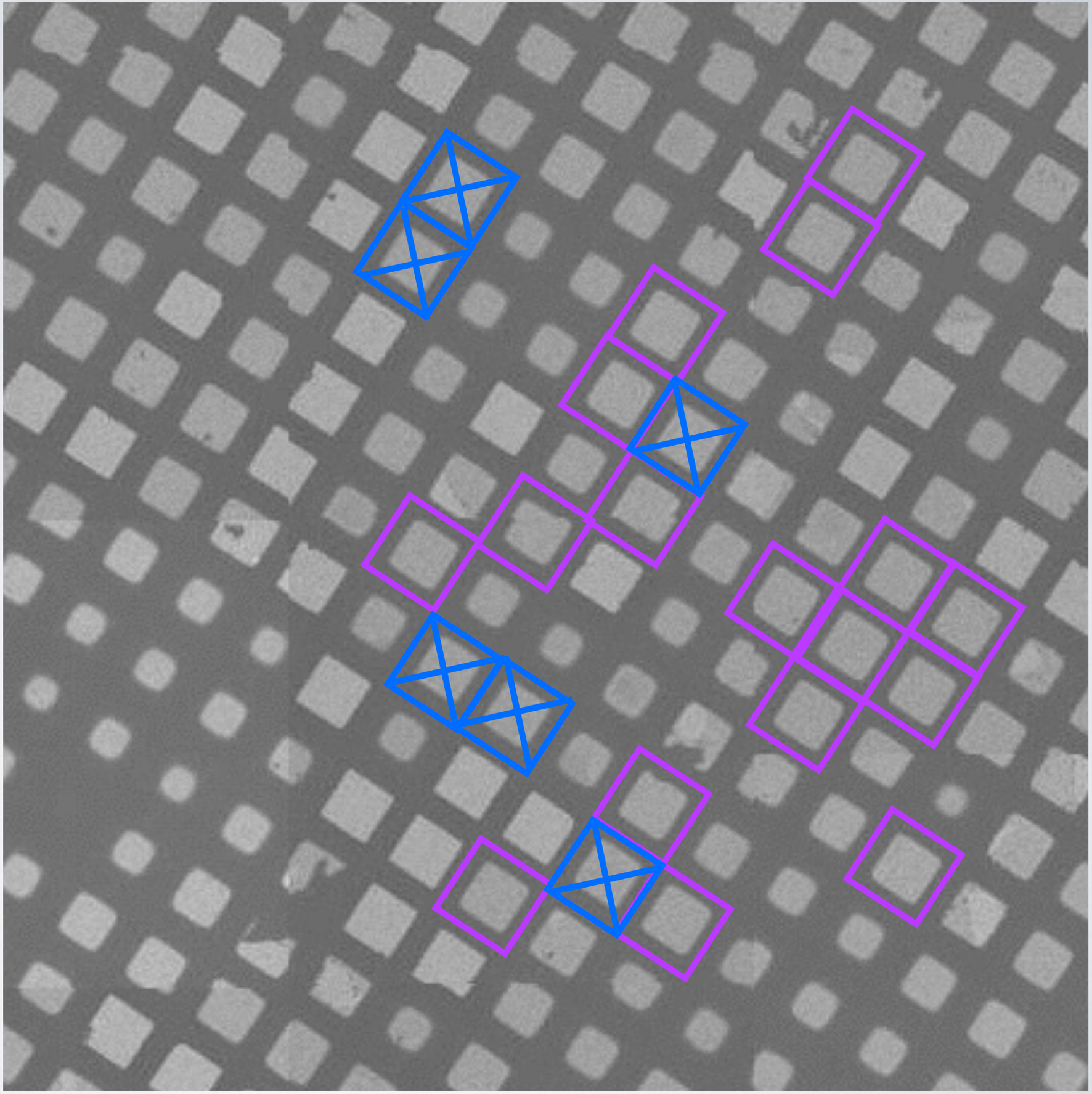


Atlas

81x

Rejected 6 squares by eye

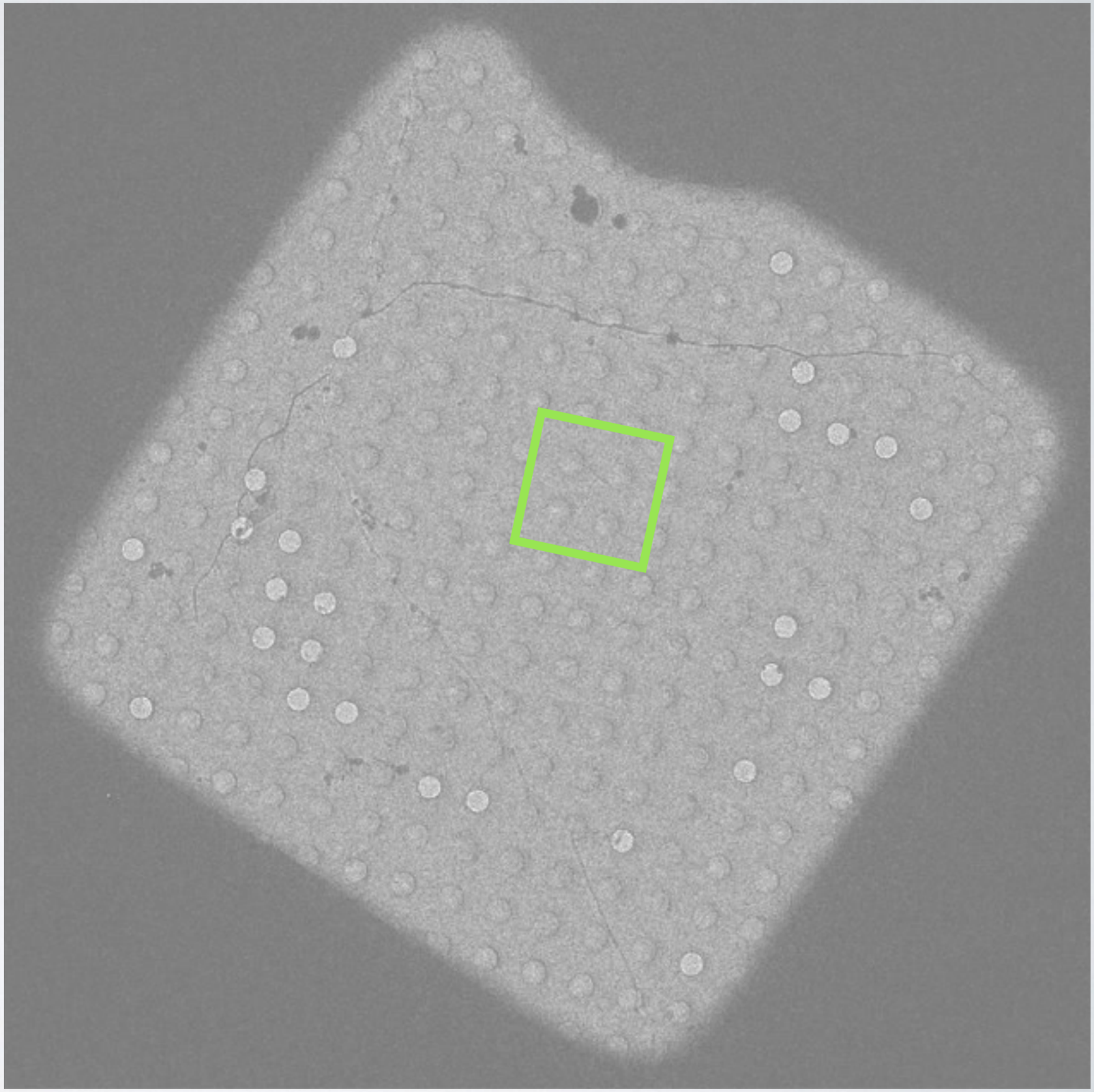
Collected high mag images of 17 squares



Square

165x

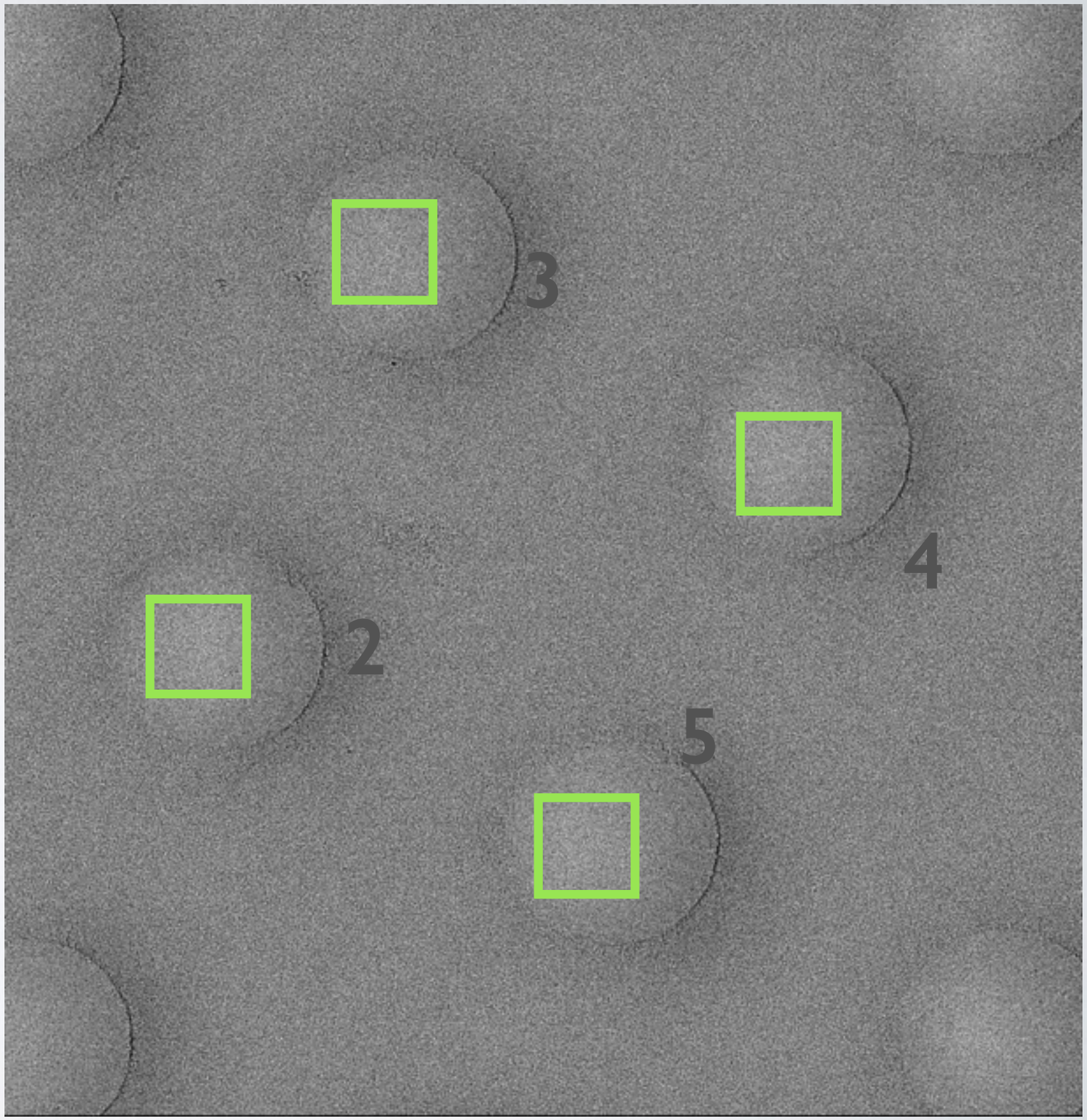
Find eucentric height
Manually target the most promising looking areas



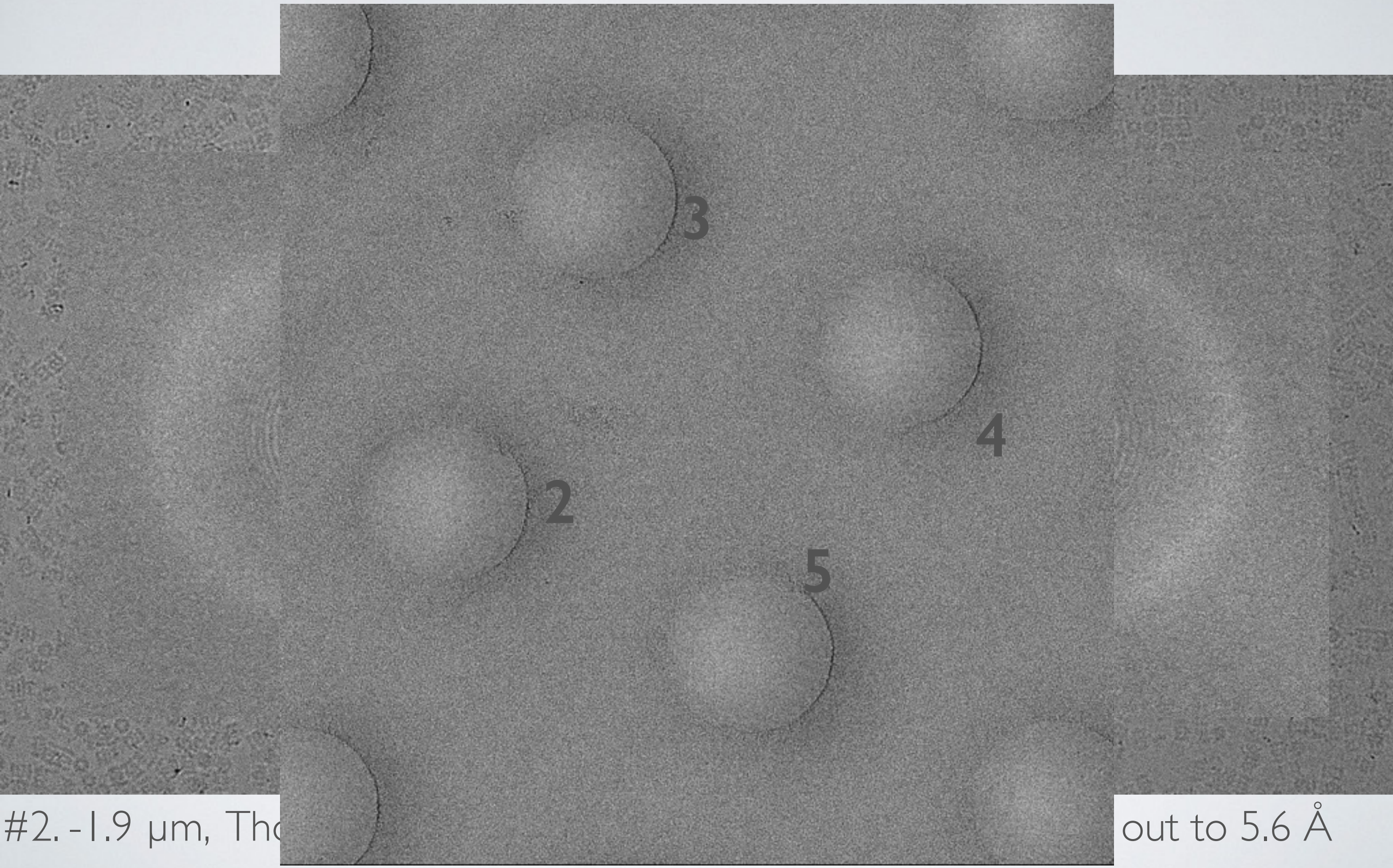
Target High Mag Images

1700x

Manually target exposures
Focus every 4 images
Move the stage for each image
Wait 40 seconds between each exposure



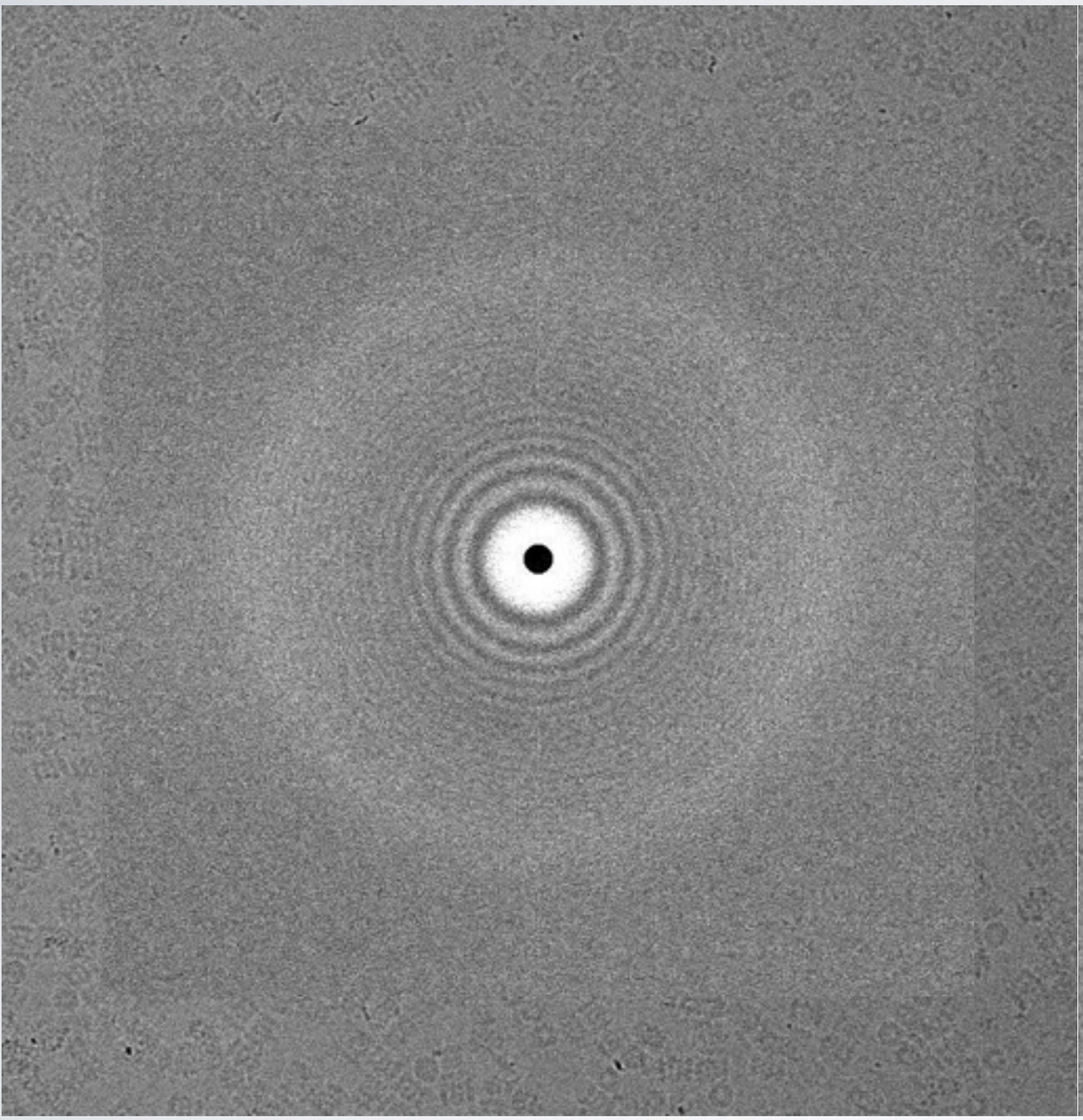
Adjacent Holes Give Different Quality Images



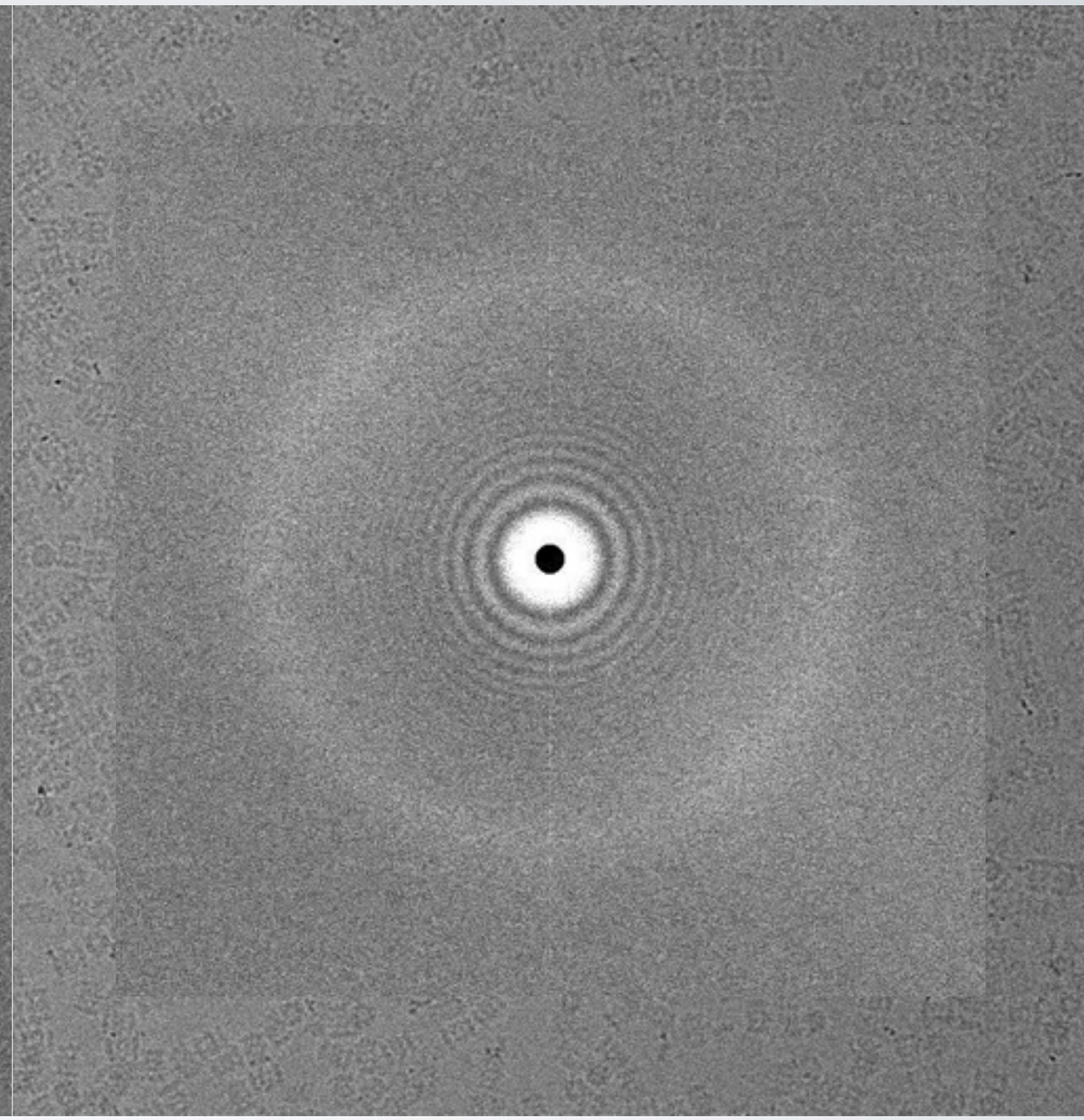
#2. -1.9 µm, Tho

out to 5.6 Å

Adjacent Holes Give Different Quality Images II

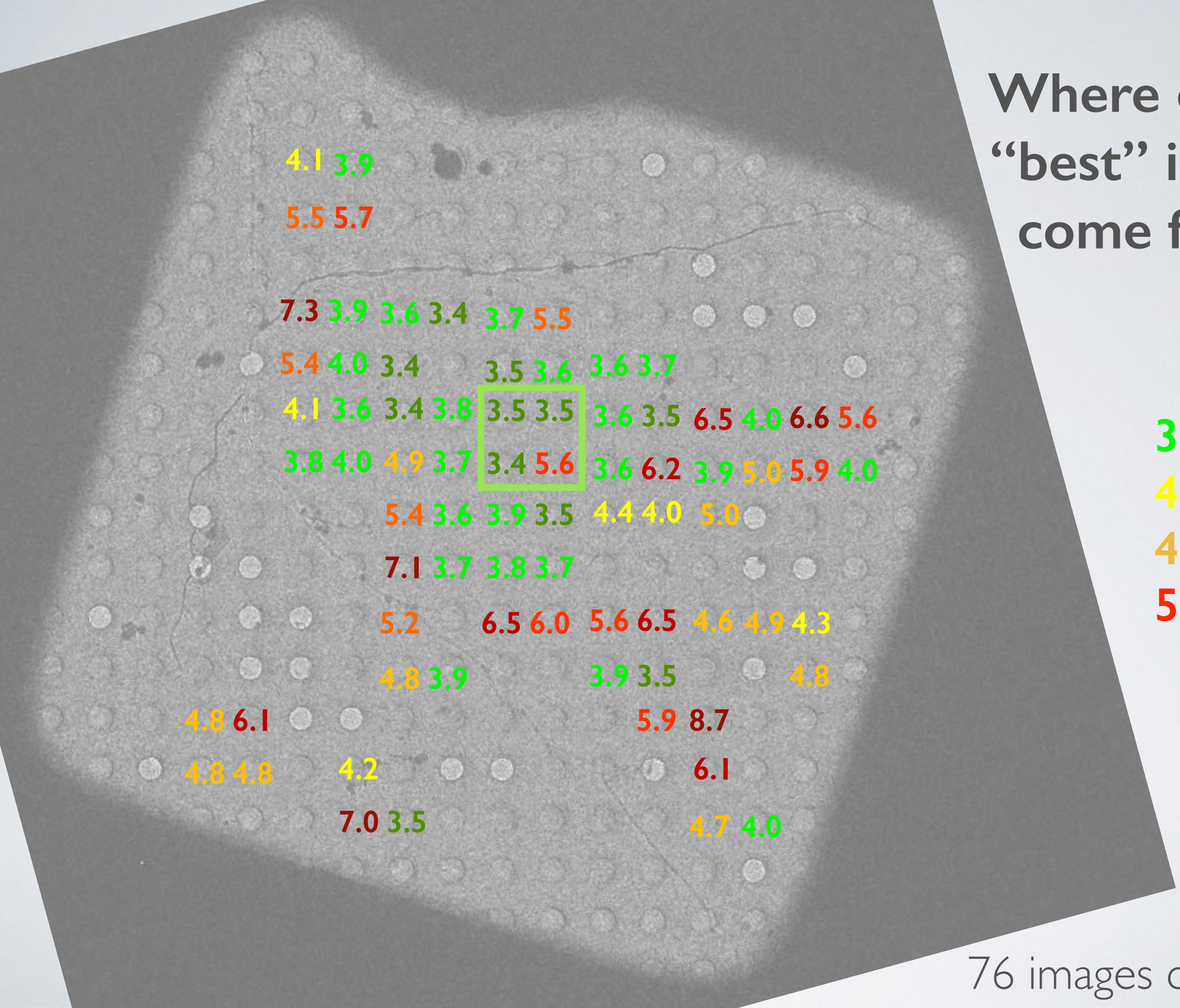


#4. -1.4 µm, Thon rings out to 3.5 Å



#5. -1.7 µm, Thon rings out to 5.6 Å

Where do the "best" images come from?



3-3.5Å
3.6-4.0Å
4.1-4.5Å
4.6-5.0Å
5.1-6.0Å
6.1Å+

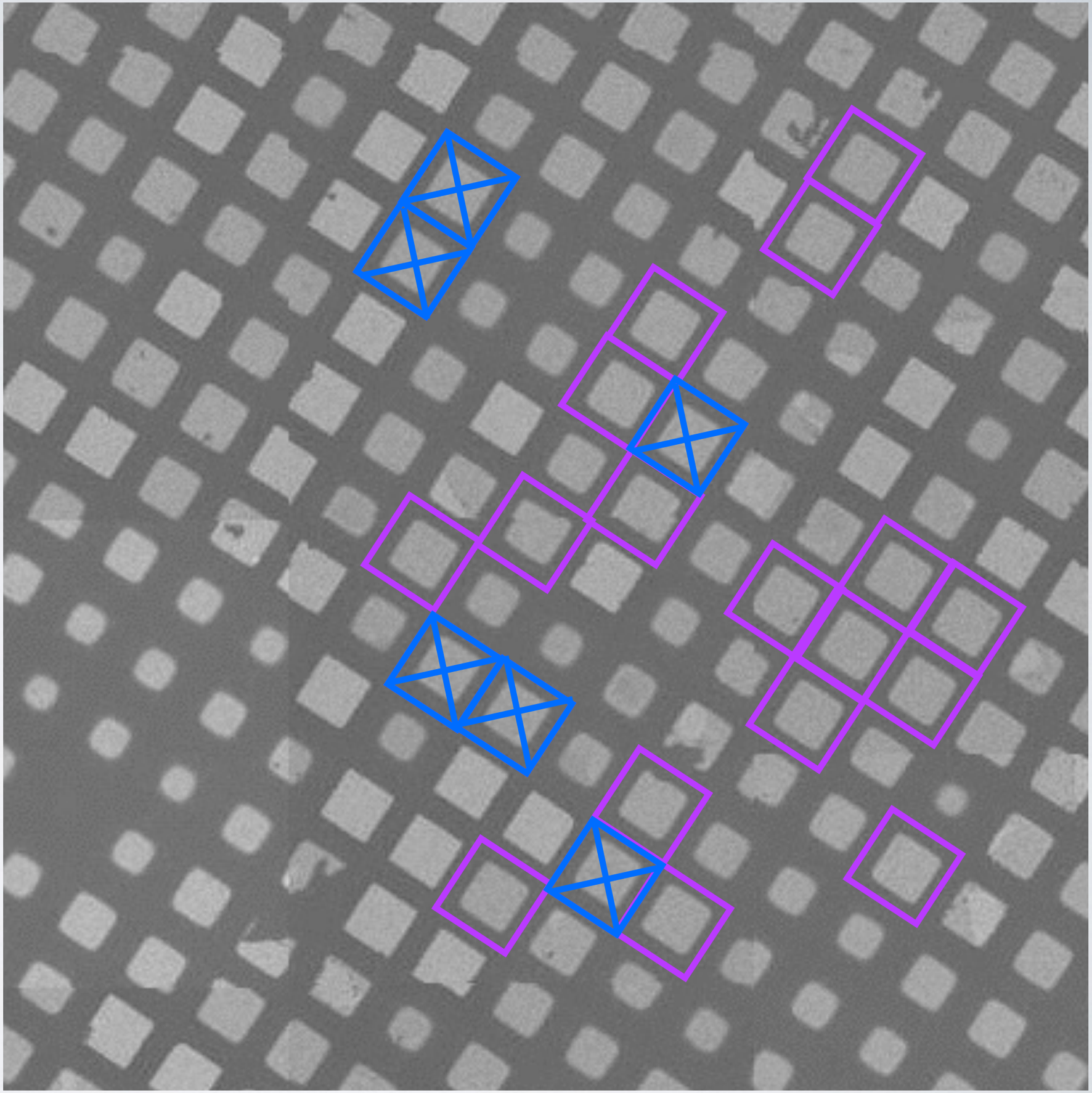
76 images collected

Atlas

81x

Collected high mag images of 17 squares

Rejected 80% of images (all images that didn't have Thon rings past 4.0 Å)

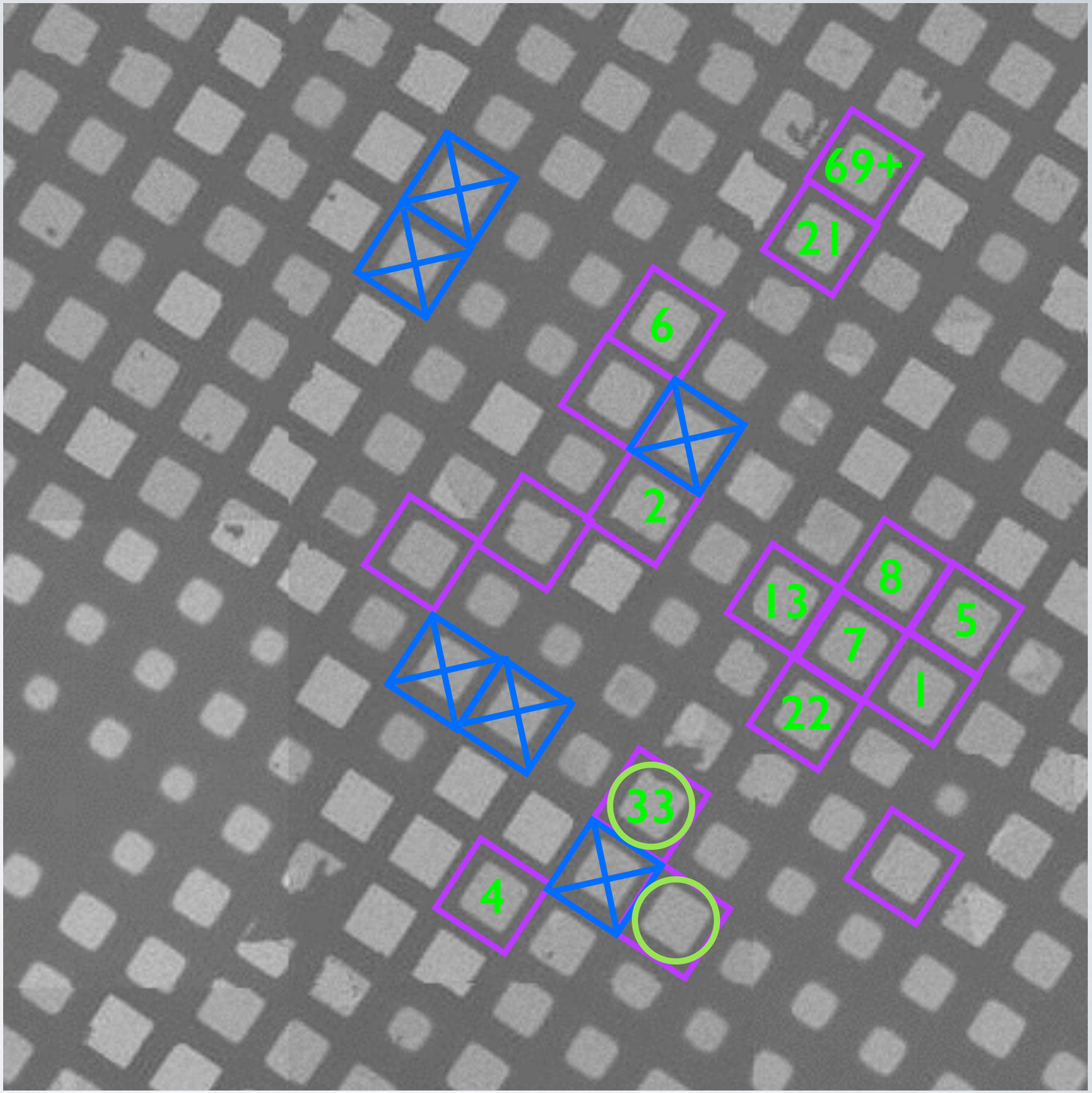


Atlas

81x

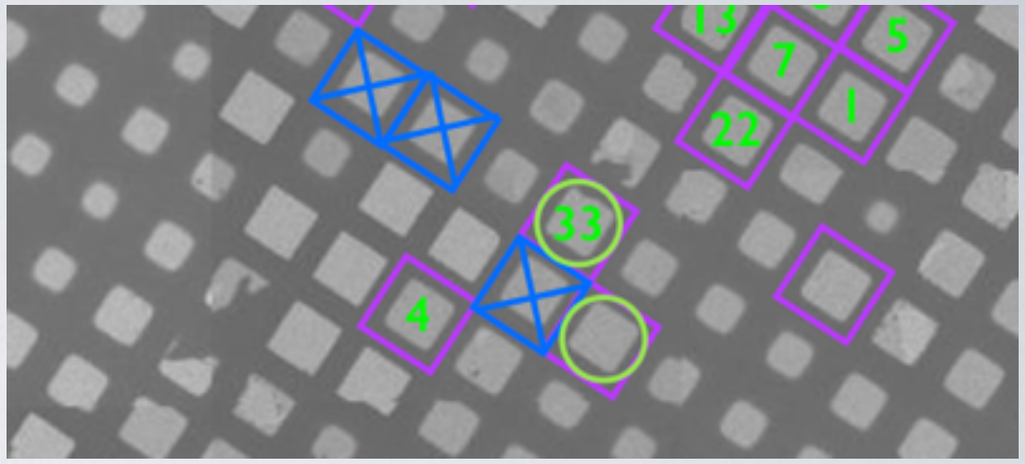
12 of the remaining 17 had the "best" ice

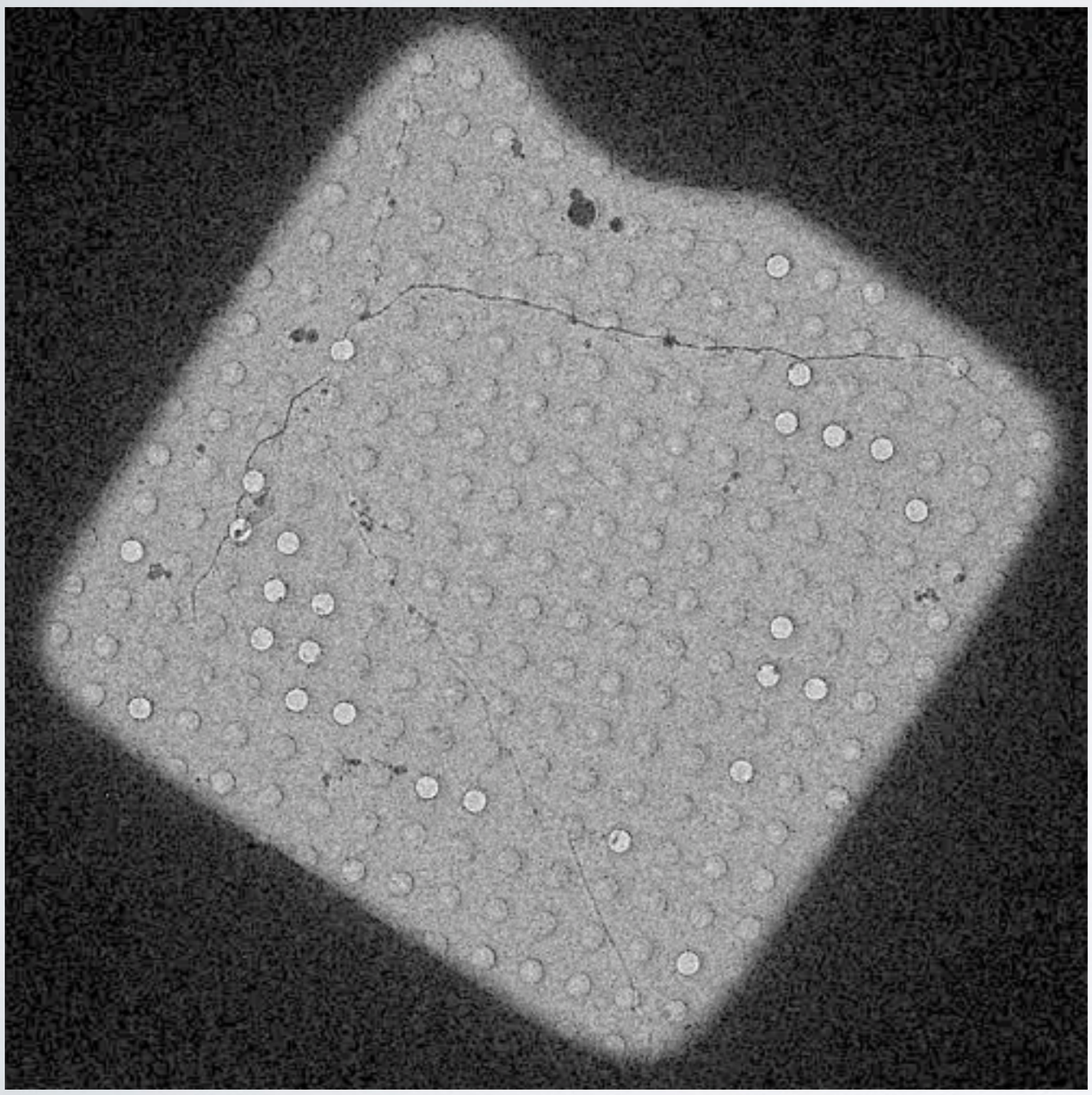
Number of high mag images contributing to "best" 20%



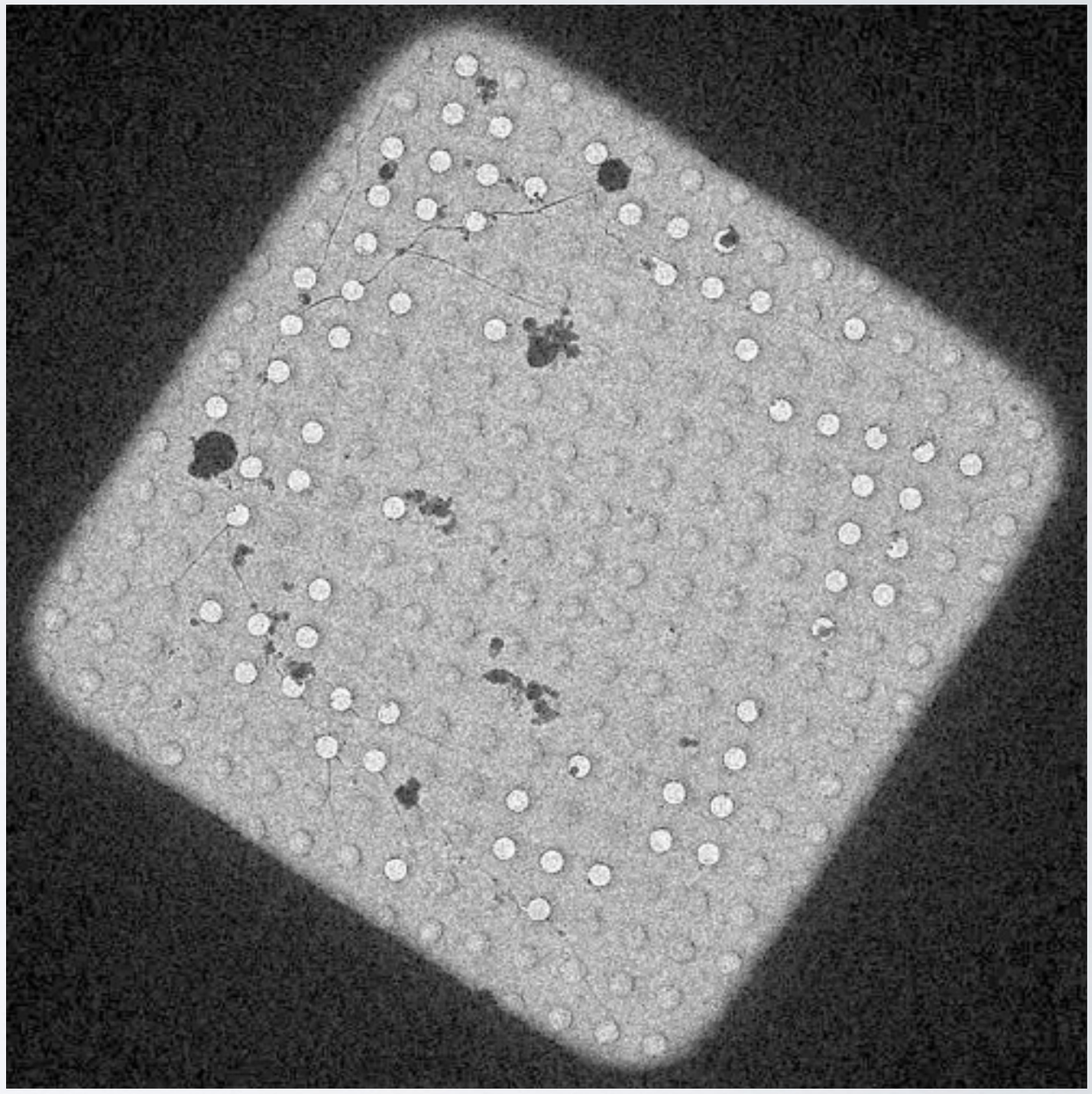
Good vs. Bad Ice

165x





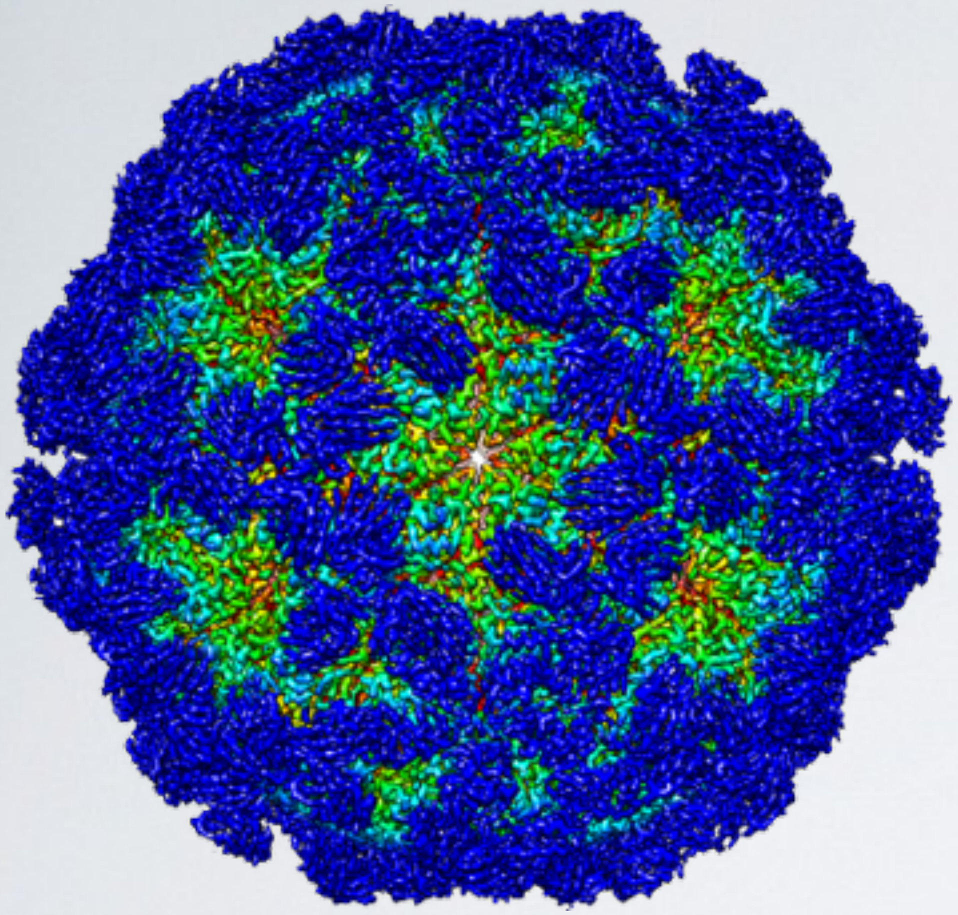
33 of 76 Images Contributed

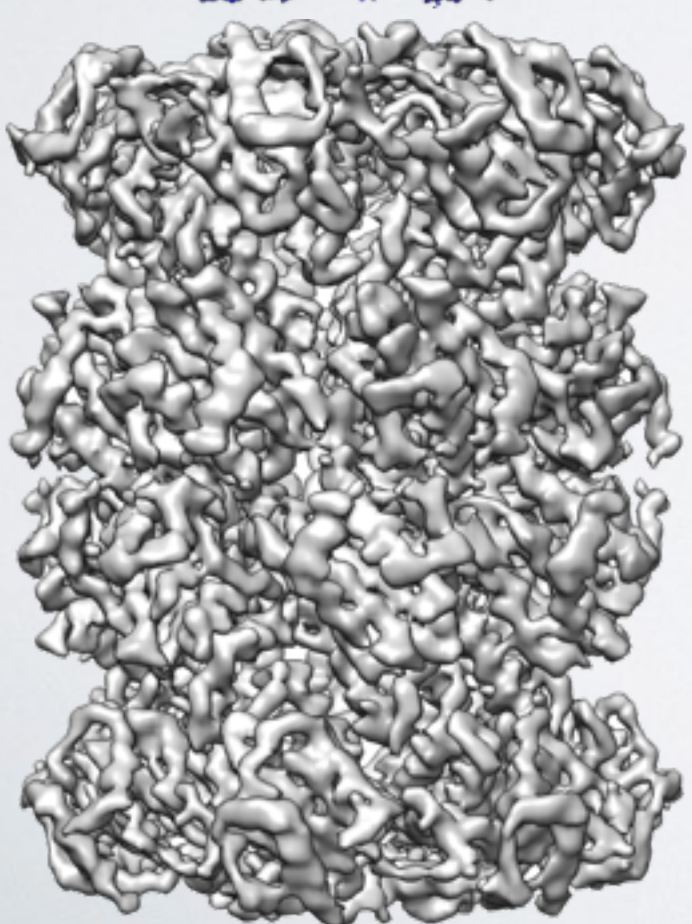


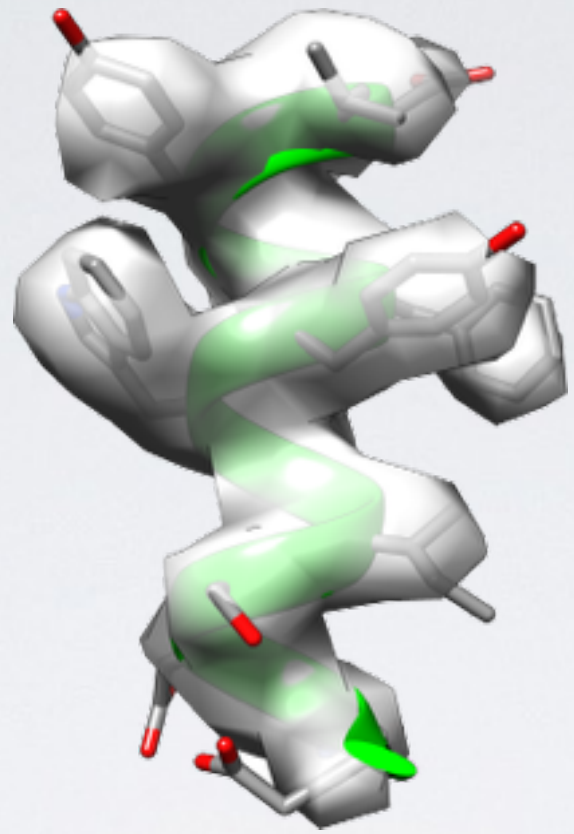
0 of 59 Images Contributed

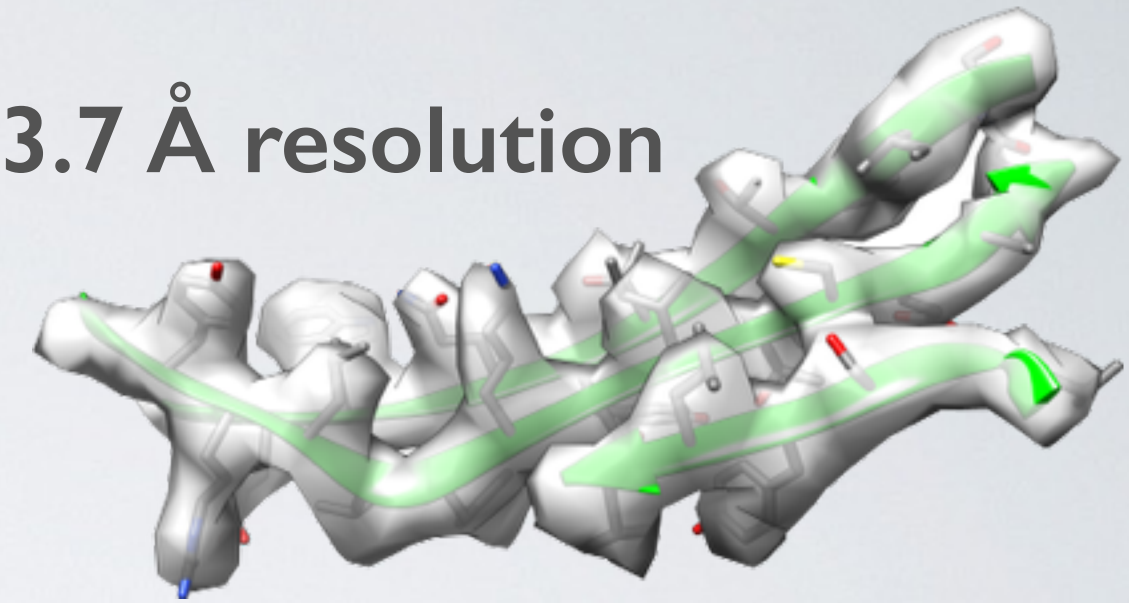
Number of Images Contributing to Best 20% of Images vs. Collection Order

Near-Atomic resolution is no longer reserved for Kings… …but is also accessible to lower-end workhorse electron microscopes

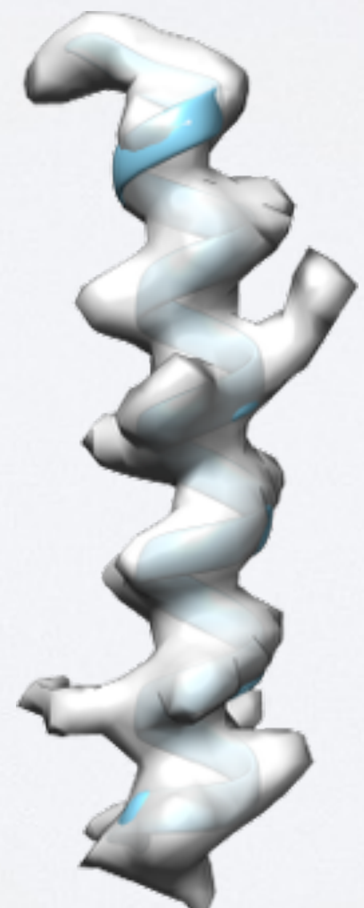


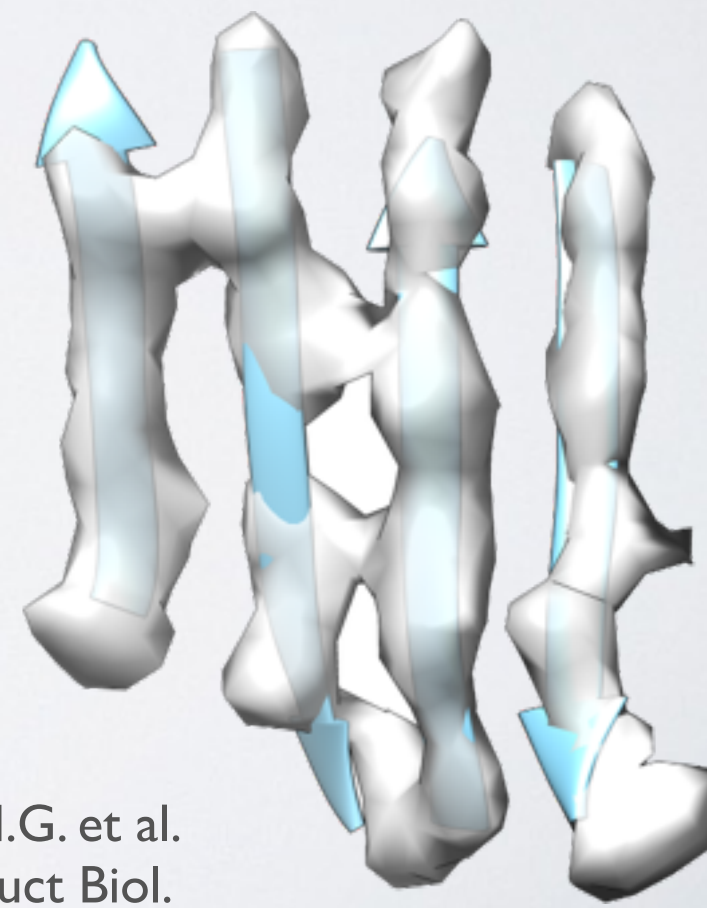






3.7 Å resolution





4.2 Å resolution

Campbell M.G. et al. (2014) J Struct Biol.

Cost of a structure

- Krios time ($1000/day): $2000

- Movie frame-alignment (6 cents/gpu hours): ~$6
- 1000 movies with 38 frames each

- Data processing (3 cents/cpu hours): $2437.5
  - Xmipp cl2d: ~$92
  - Relion preprocessing: ~$1.5
  - Relion auto-3D-refine: ~$281
  - Relion movie processing: ~$948
  - Relion particle polishing: ~$57
  - Relion auto-3D-refine: ~$828
  - Relion auto-3D-refine MaxProb: ~$230

- Fast disk access ($1,500/Tb/year): ~$2,750
  - Unaligned (2.1 Tb) + Aligned movies (2.1 Tb)+ Relion files (1 Tb)

- External USB drive ($129/4Tb): $258

((($7,451.5 x 3) + Labor) x 2

Acknowledgements

Bridget Carragher
Clint Potter
- *Anchi Cheng*
- **Sargis Dallakyan**

John Crum
- **Jeff Spier**
- **Emily Greene**
- **Jana Albrecht**
- **Yong Zi Tan**
- **Ivan Razinkov**

UCSF

Yifan Cheng
- **Kiyoshi Egami**

The Veesler Lab
Coming January 2015

David Veesler
- ***???***
- ***???***
- ***…you?***

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