Atomic resolution imaging with TEM

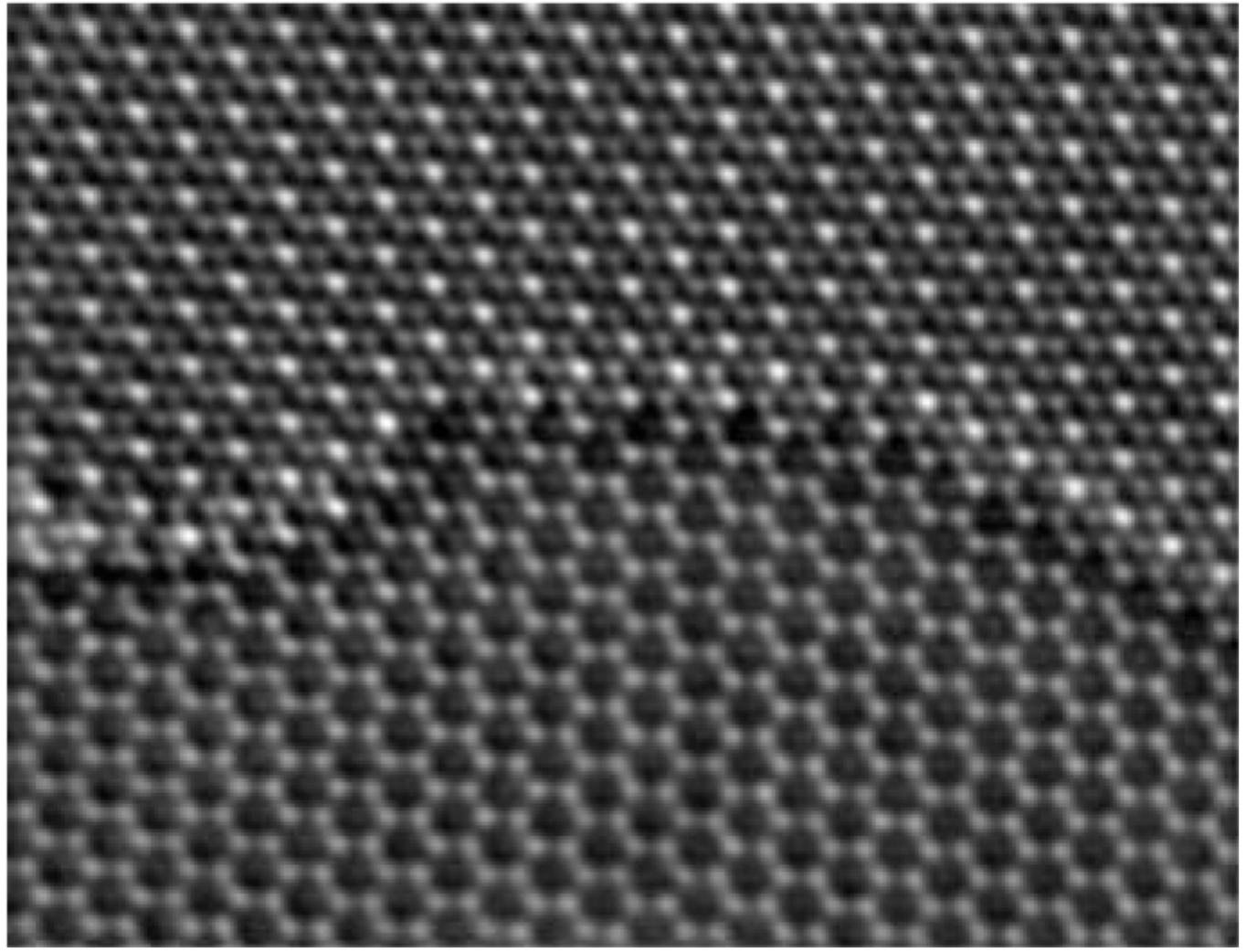
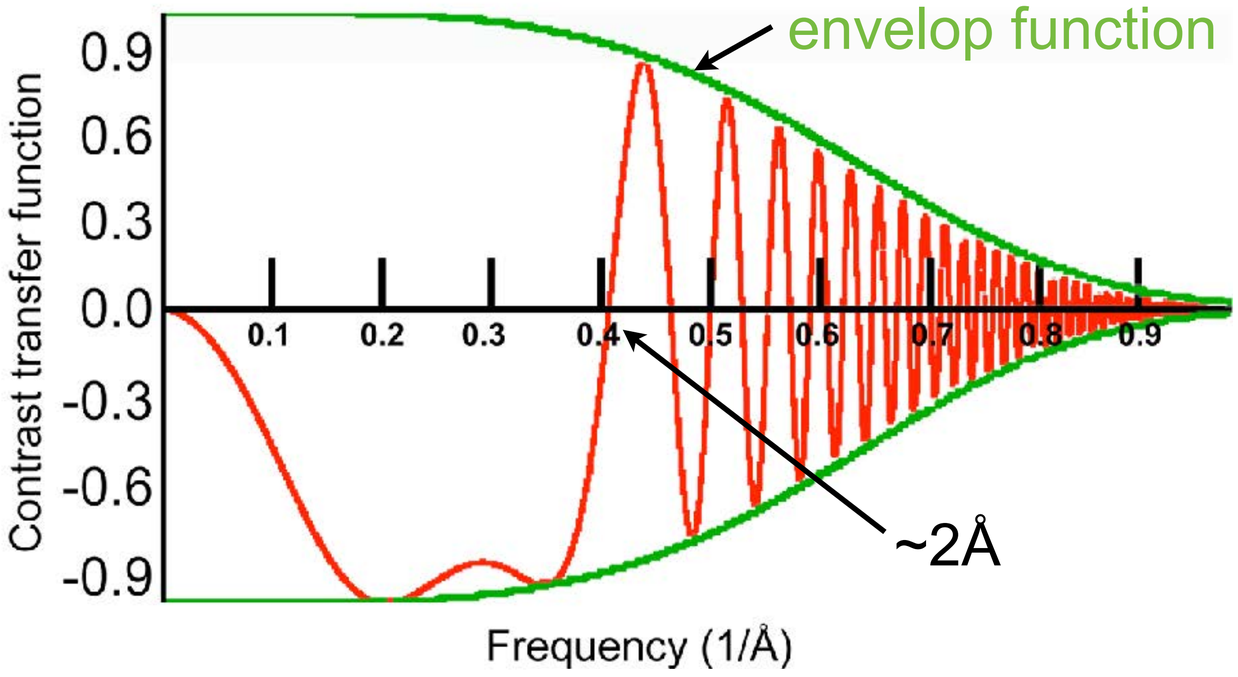


Image of graphene, Nature Mat, 2011, ***10***, 165

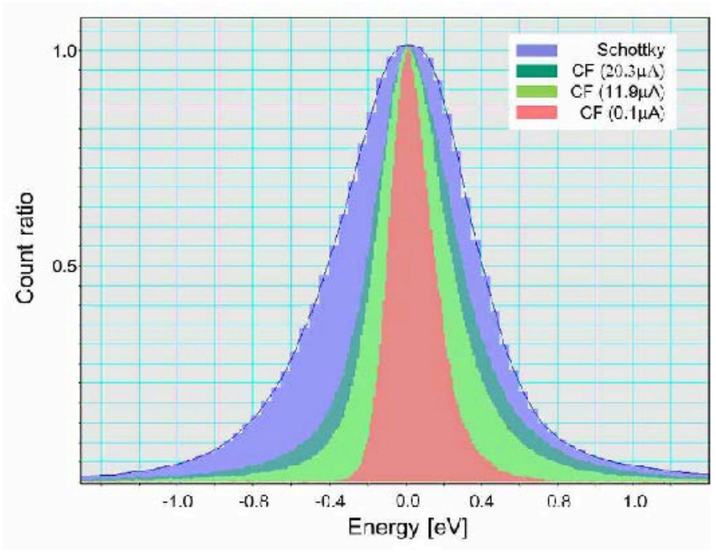
Electron optic system of a modern electron microscope is of sufficient quality to image radiation resistant material (typically inorganic) at atomic resolution (~2Å or better).

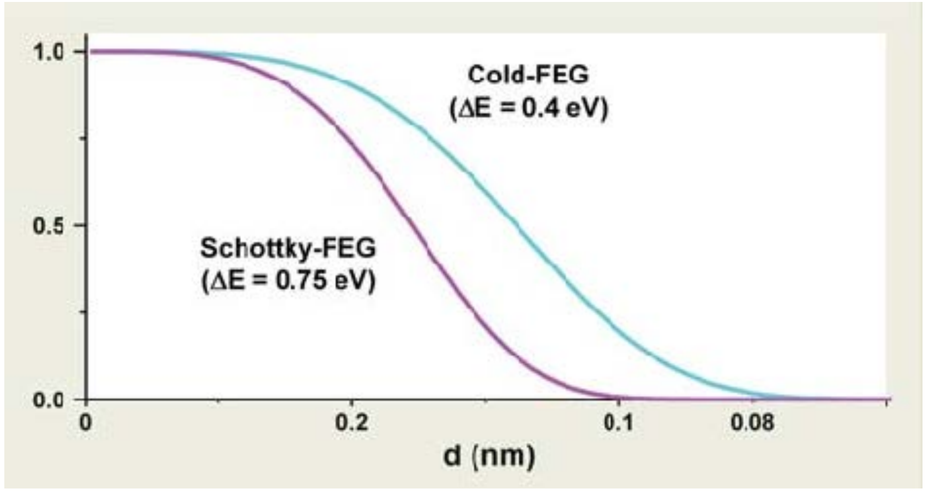
Determinants of resolution



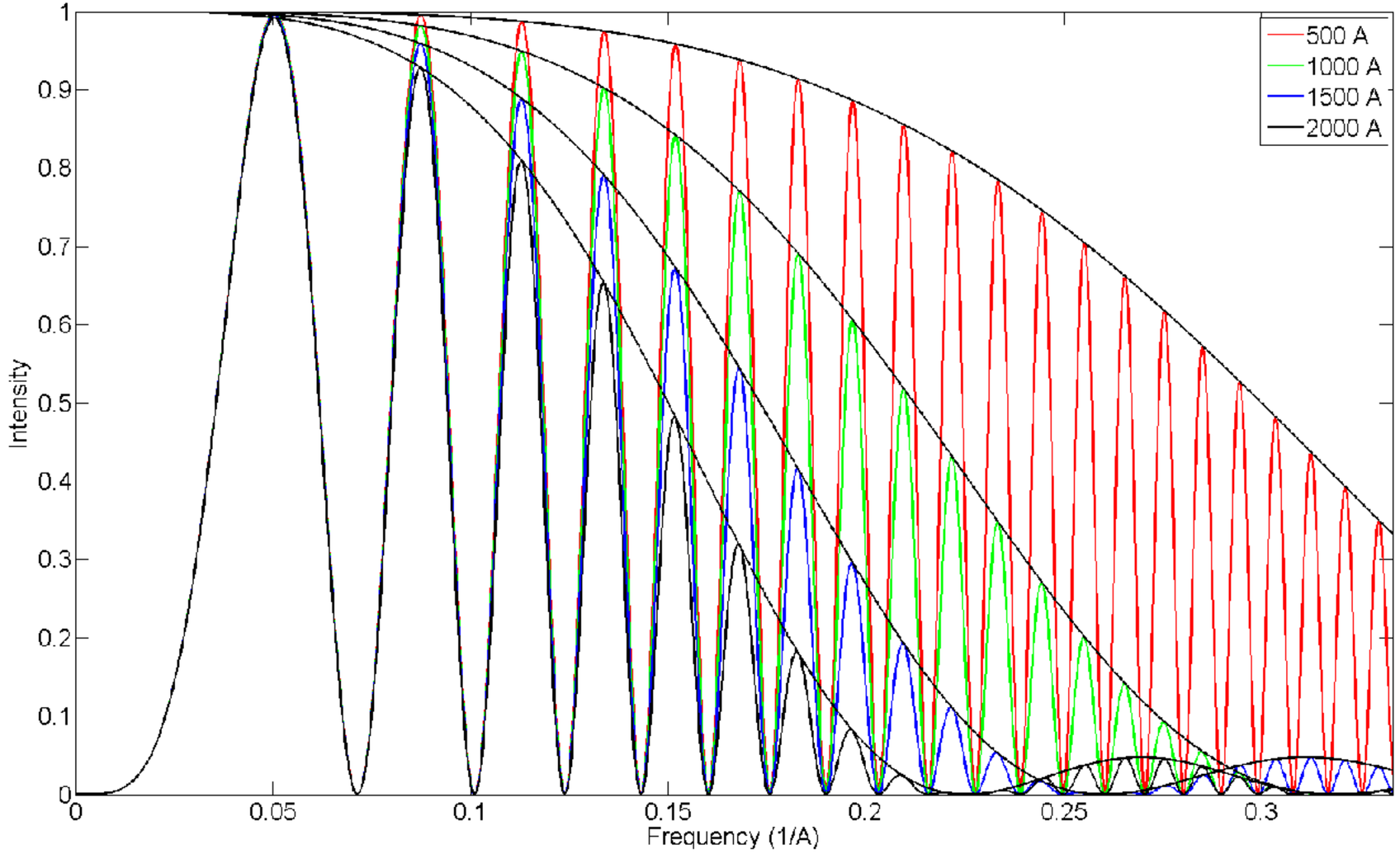
- Envelop function determines the information limit of a micrograph;
- Envelop function itself is shaped by defocus, beam spacial coherence,

$$D(\vec{k}) = e^{-\frac{1}{2}\pi^2 \Delta^2 \lambda^2 (\vec{k})^4} e^{-\pi^2 \alpha^2 (\vec{k})^2 \left[\varepsilon + C_S \lambda^2 (\vec{k})^2\right]^2}$$





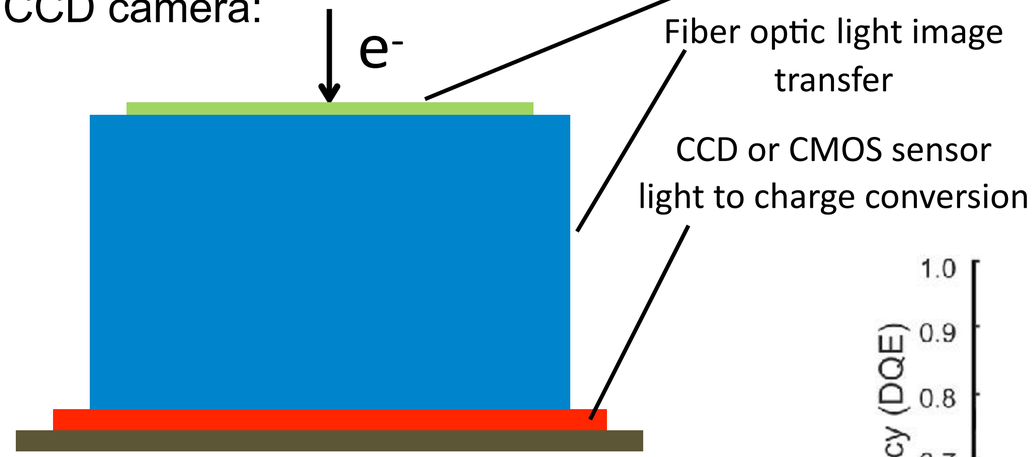
Influence of sample thickness

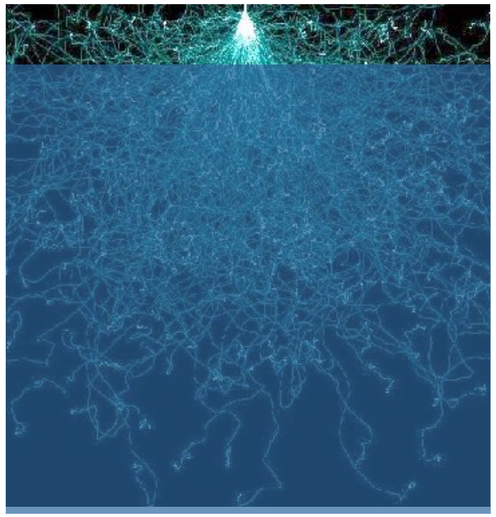


Scintillator based camera/photographic film

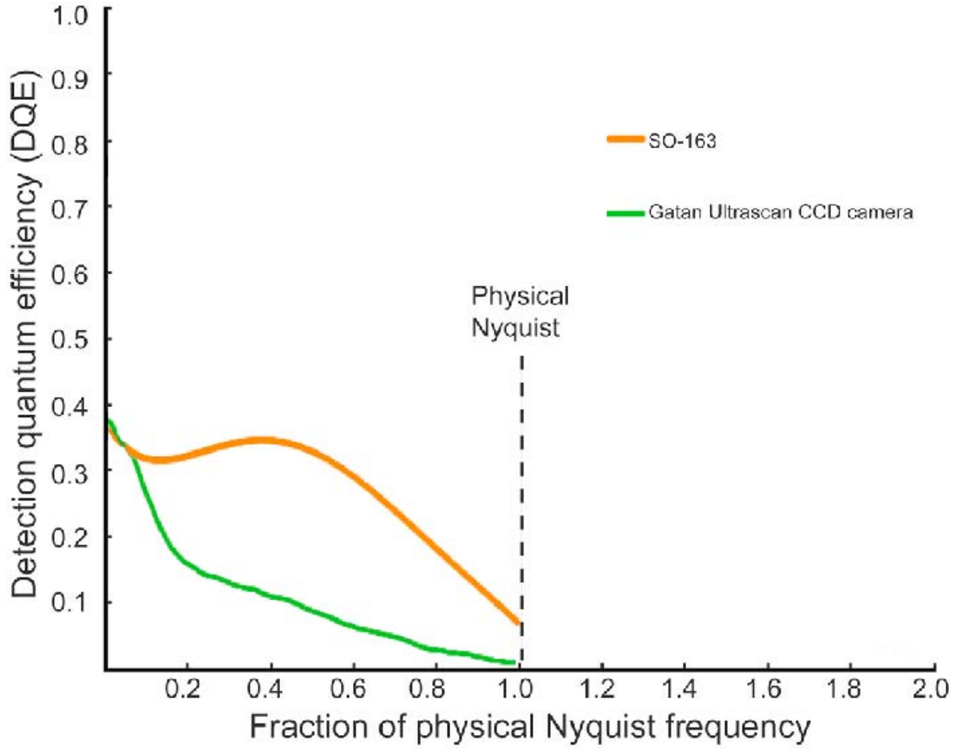
Scintillator based camera and photographic film are inadequate for high-resolution cryo-EM.

CCD camera:



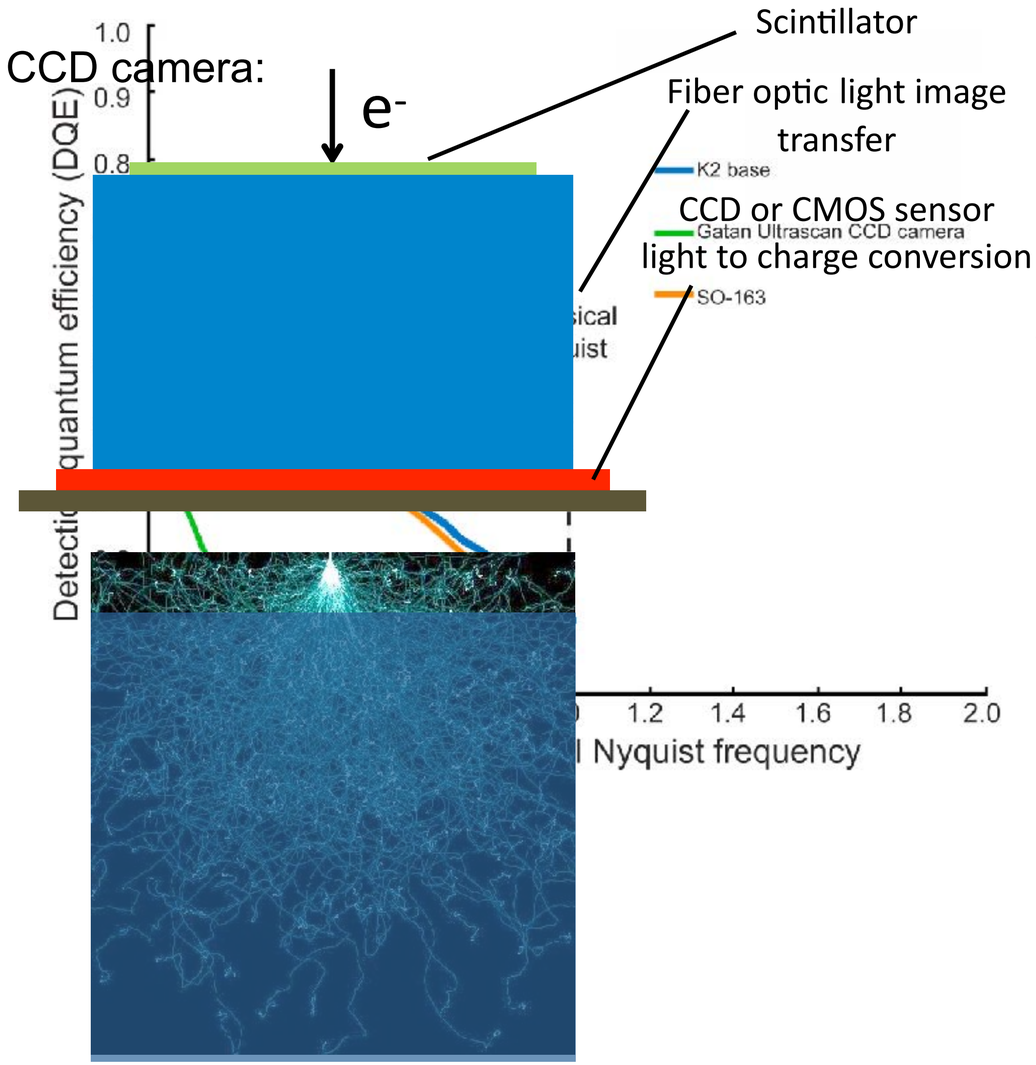


DQE:

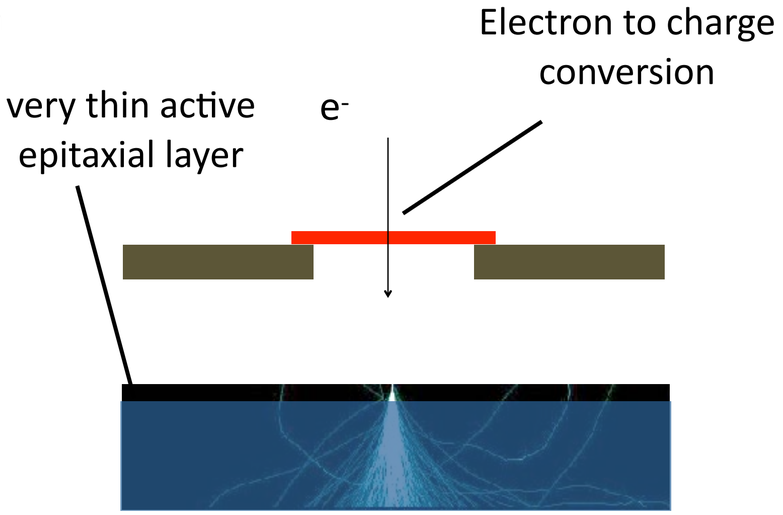


CMOS direct detection camera

Direct detection minimizes the point spread function, and improve camera performance at both low and high resolution.



CMOS direct detection

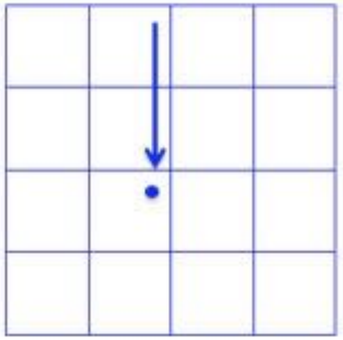


improved sensitivity

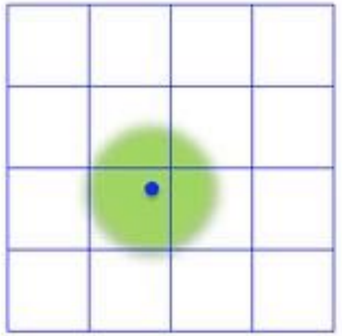
Single electron counting by the K2 Summit (UCSF, LBNL, Gatan)

* Counting and centroiding primary electron events.
* Counting removes Landau noise and further improves DQE;

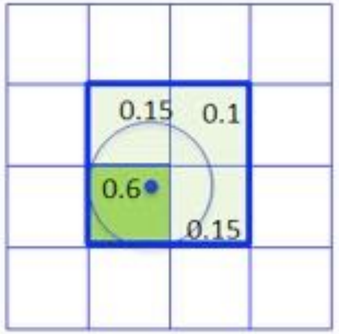
1. Electron enters detector



2. Signal Is Scattered



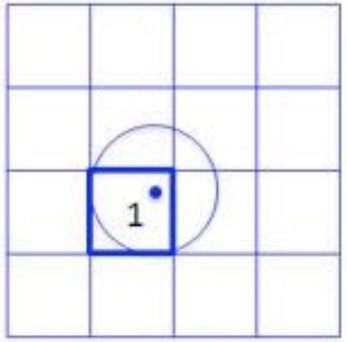
3. Charge collects in each pixel



K2 Base™: Charge Integration

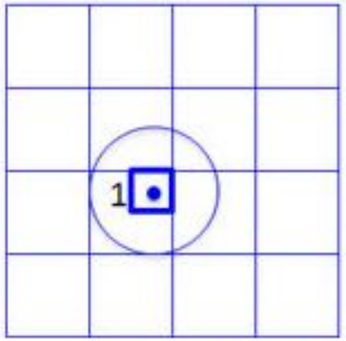
Improved DQE at high Frequency

4a. Events are reduced to the highest charge pixels

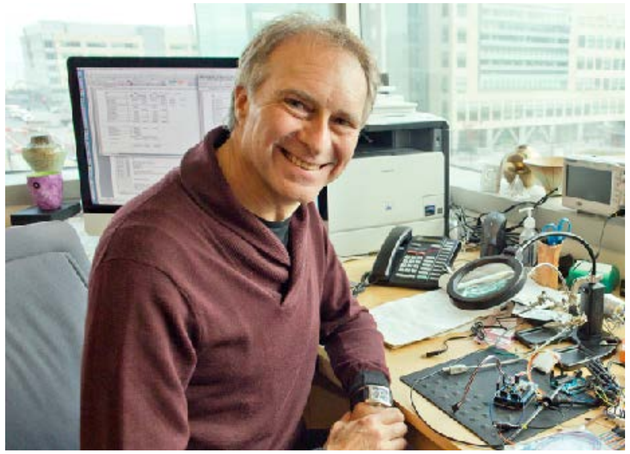


K2 Summit™ : Counting

4b. Events are localized with sub-pixel accuracy



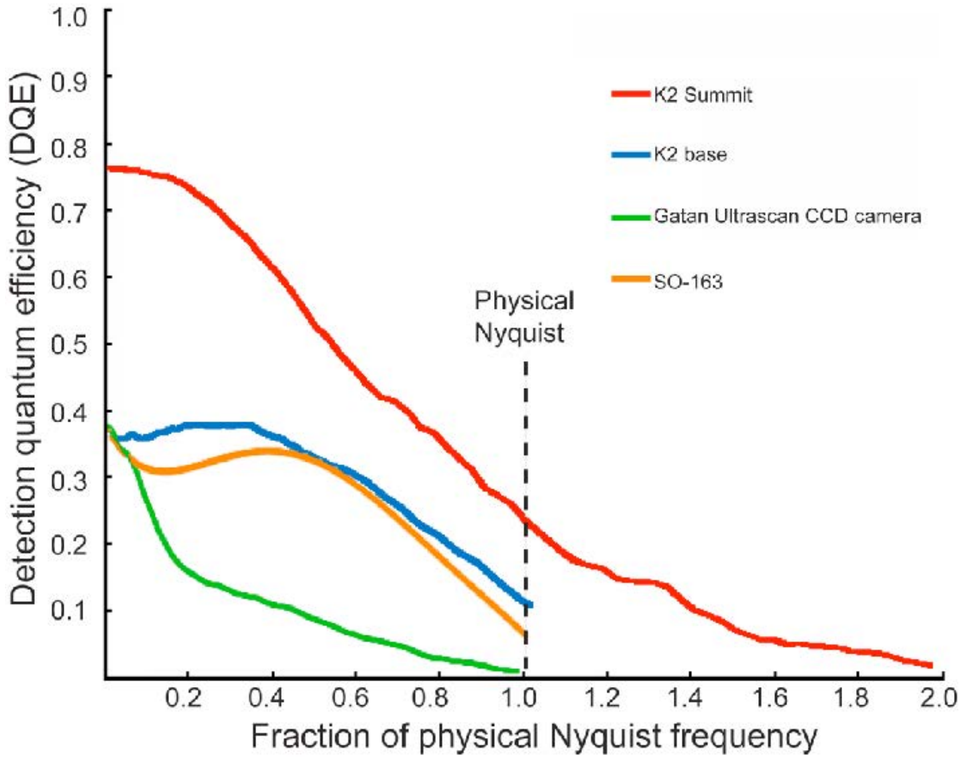
K2 Summit™: Super Resolution



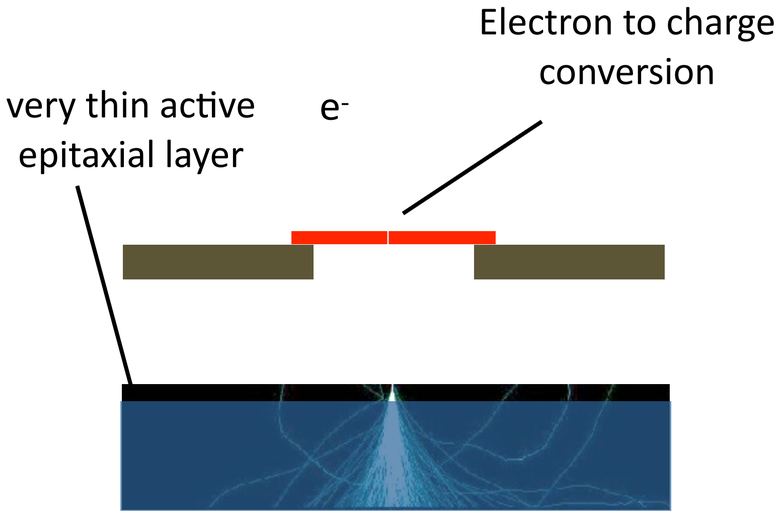
with David Agard (HHMI/UCSF)

Single electron counting improves DQE

- Direct detection of single electron remove read out noise
- Rapid read out enabled recording image as movie

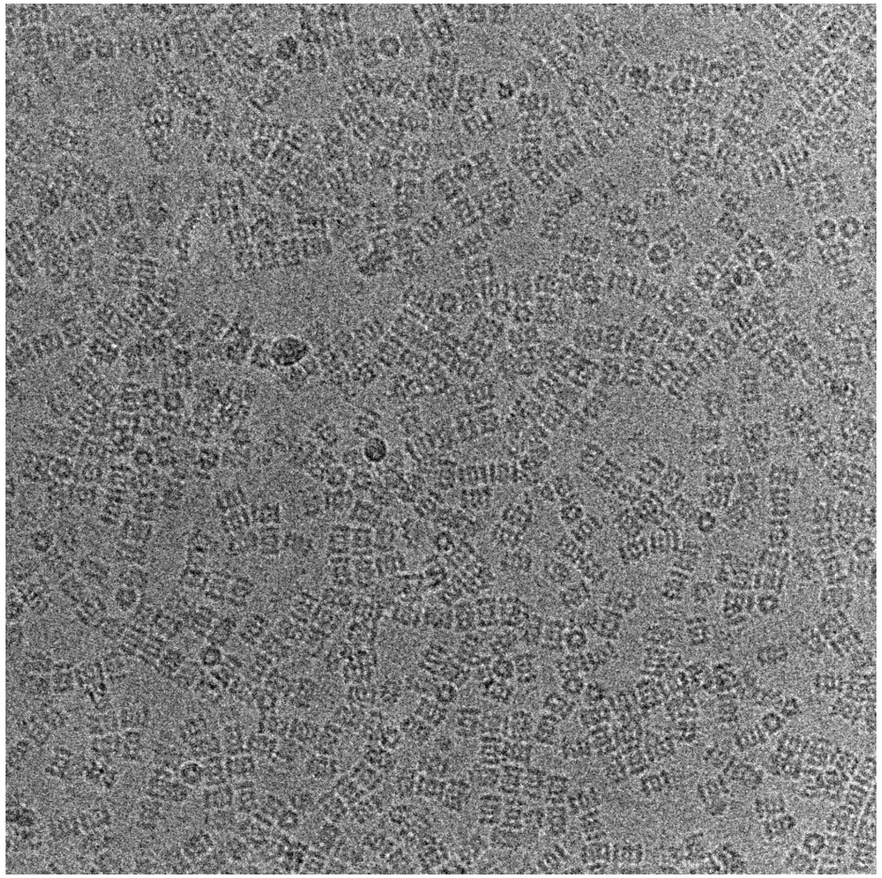


CMOS direct detection

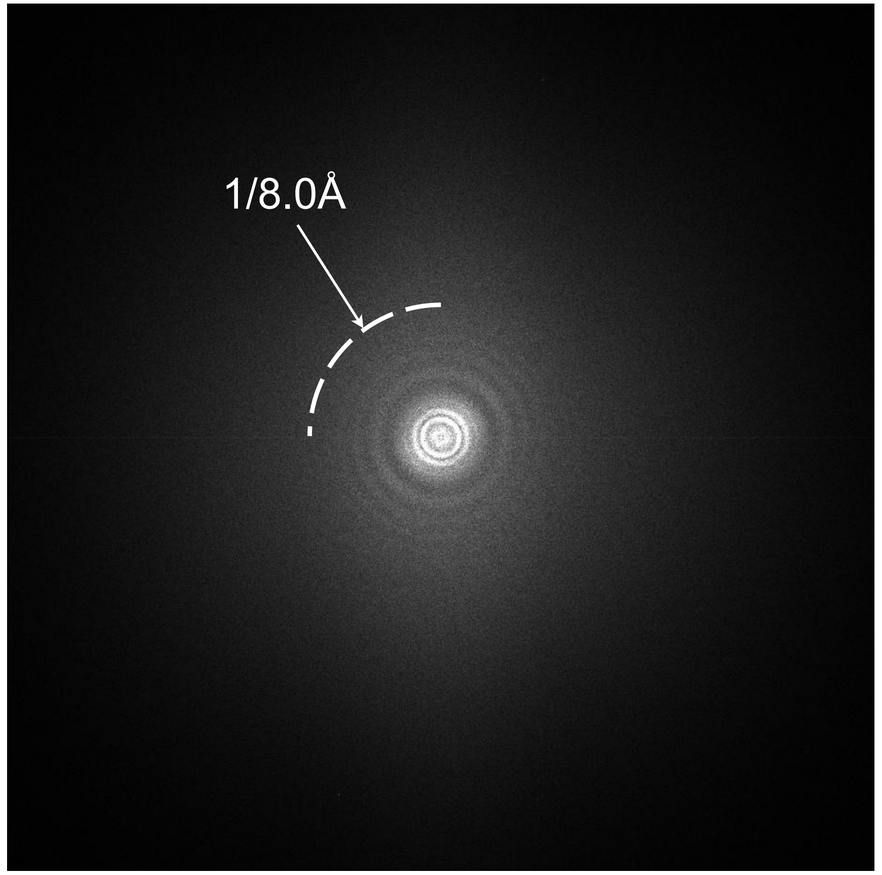


improved sensitivity

Image is further modified by recording devices



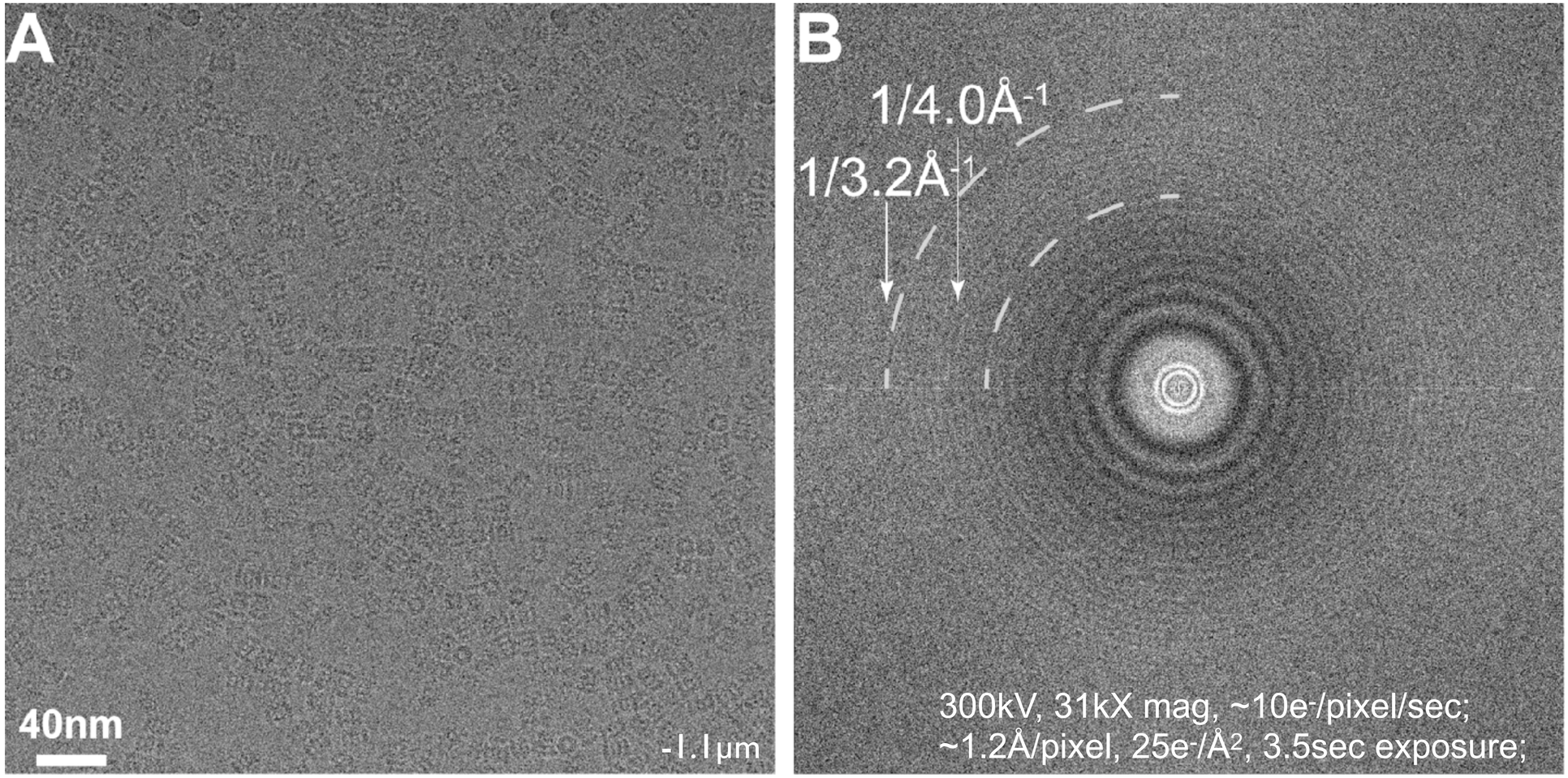
TVIPS F816 CMOS camera,
T20S proteasome, 700kDa,
200kV, -1.6µm (equivalent: 300kV, -2µm);



FFT: Thon ring visible to ~8.0 Å;

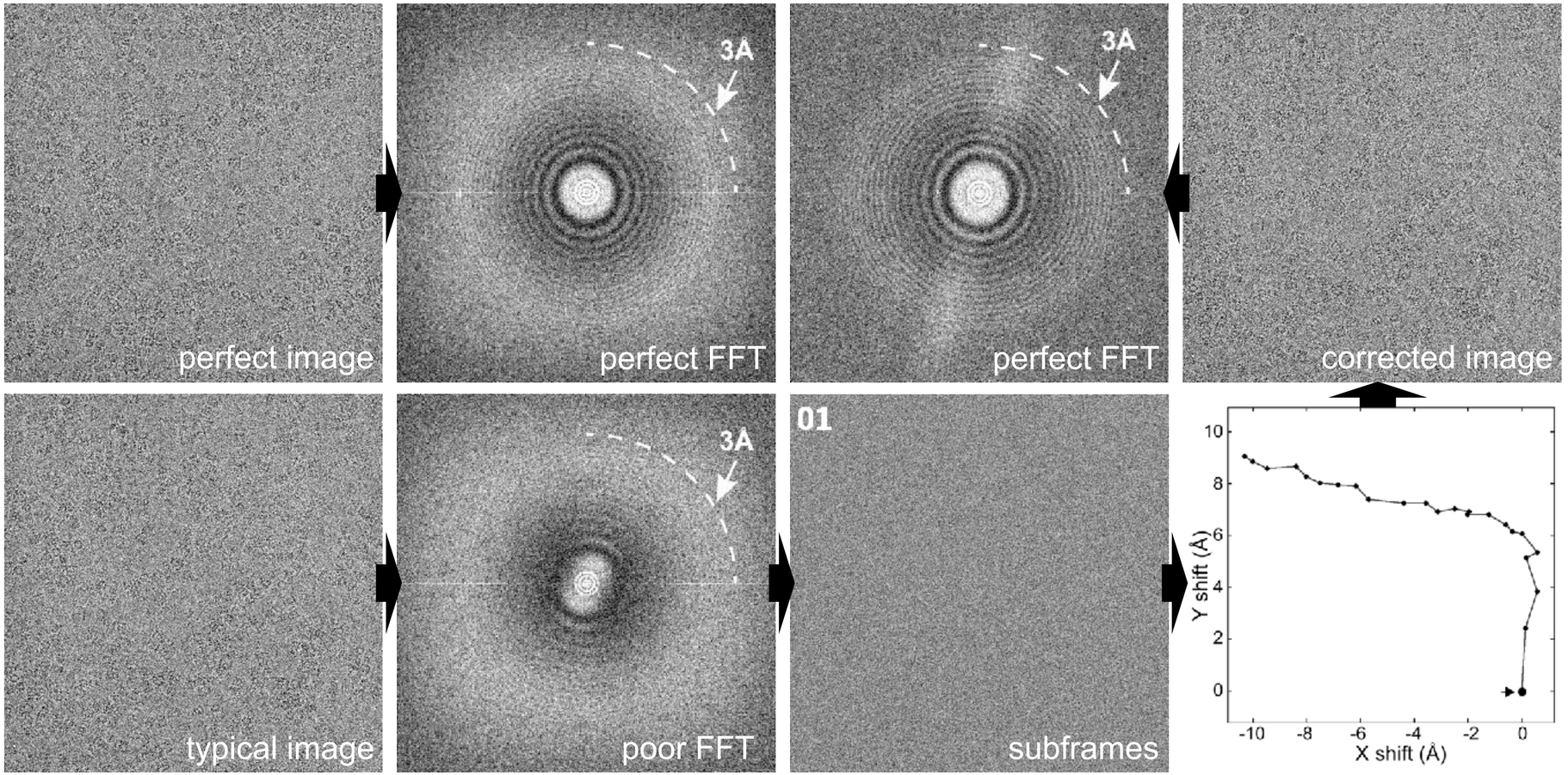
Xueming Li

K2 image of frozen hydrated protein samples, archaeal 20S proteasome



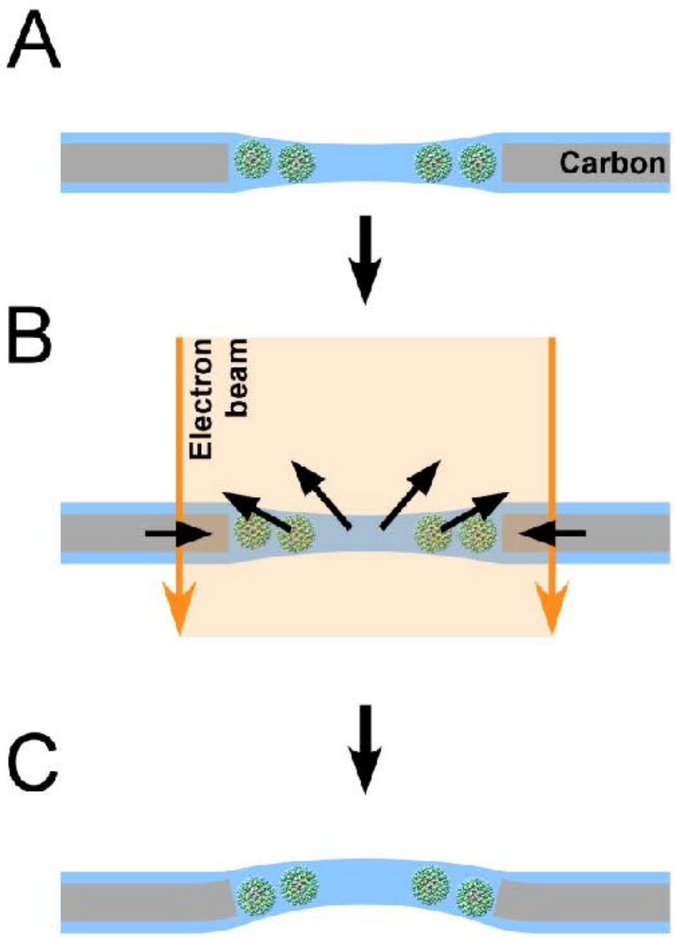
-

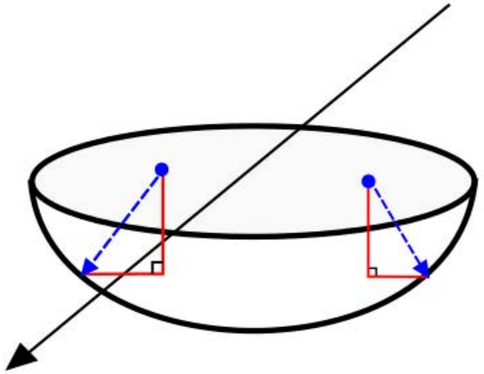
Li, Zheng, Egami, Agard and Cheng (2013) ***JSB***

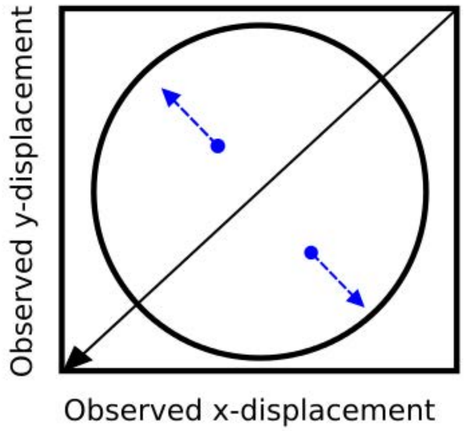


Li, Zheng, Booth, Braunfeld, Gubbens, Agard and Cheng (2013) ***Nature Methods***

Caveat: all significant motion is not global





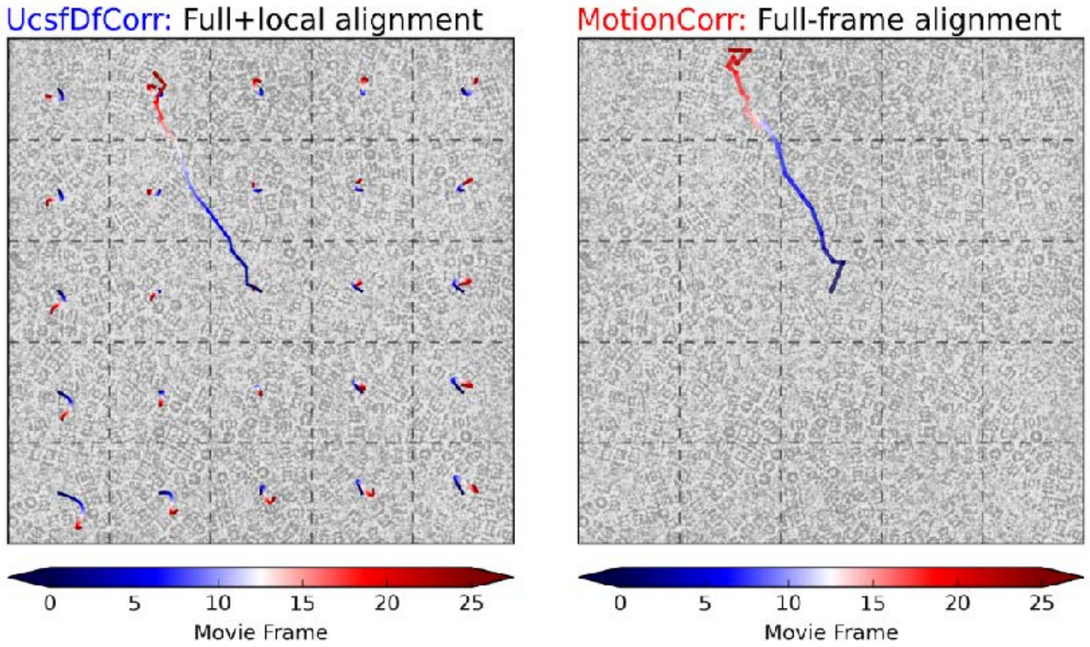


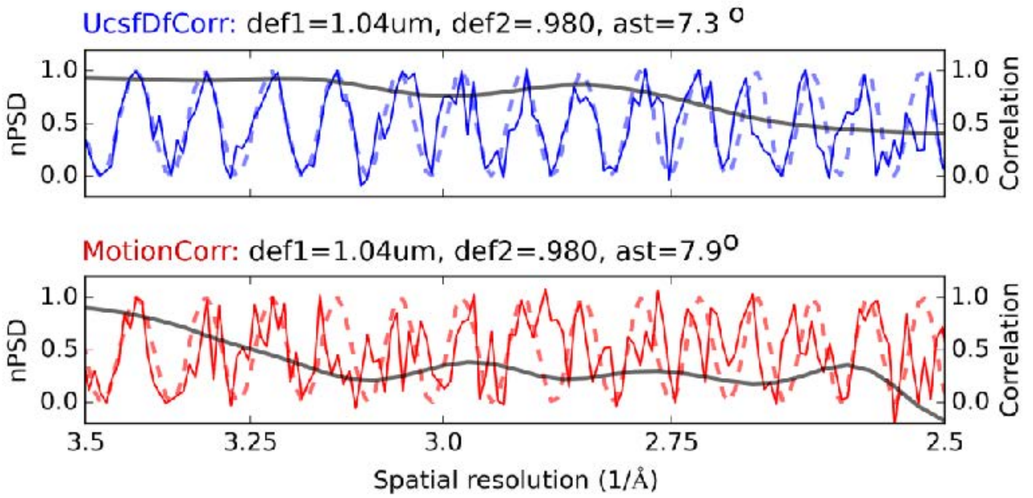
Axel Brilot (now in the Agard lab) discovered that vitrified viruses at the periphery of the sample hole move more than those in the center.

It was suggested that the electron beam causes the sample to ‘dome.’

Shawn Zheng (also Agard lab) wrote a new algorithm that takes such motion into account.

Caveat: all significant motion is not global



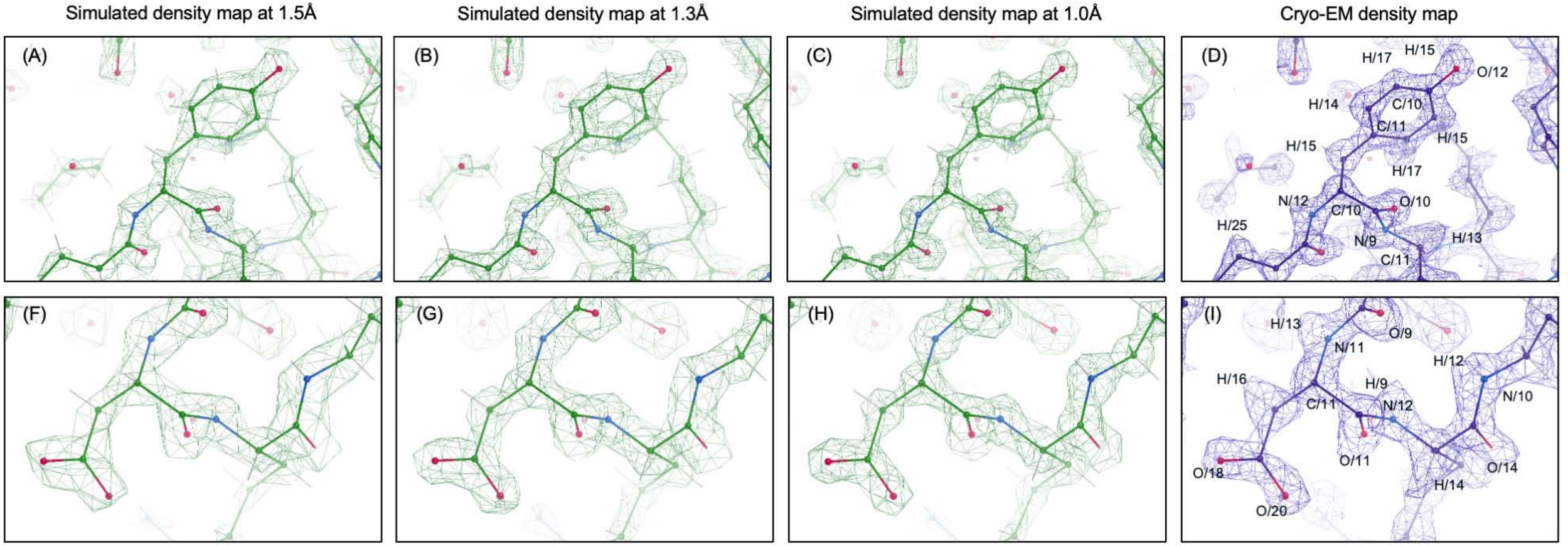


CTF Oscillations in the radially averaged Fourier Transform

Correcting local doming motions (interpolating each pixel of the image on the left with a time-varying vector field fitted by the trajectories in different patches of the image) improves the signal below 3A.

True atomic resolution by single particle cryo-EM

Apoferritin: an ideal test specimen for demonstrating the resolution high-resolution single particle cryo-EM; ~444kDa, with 24 fold symmetry.

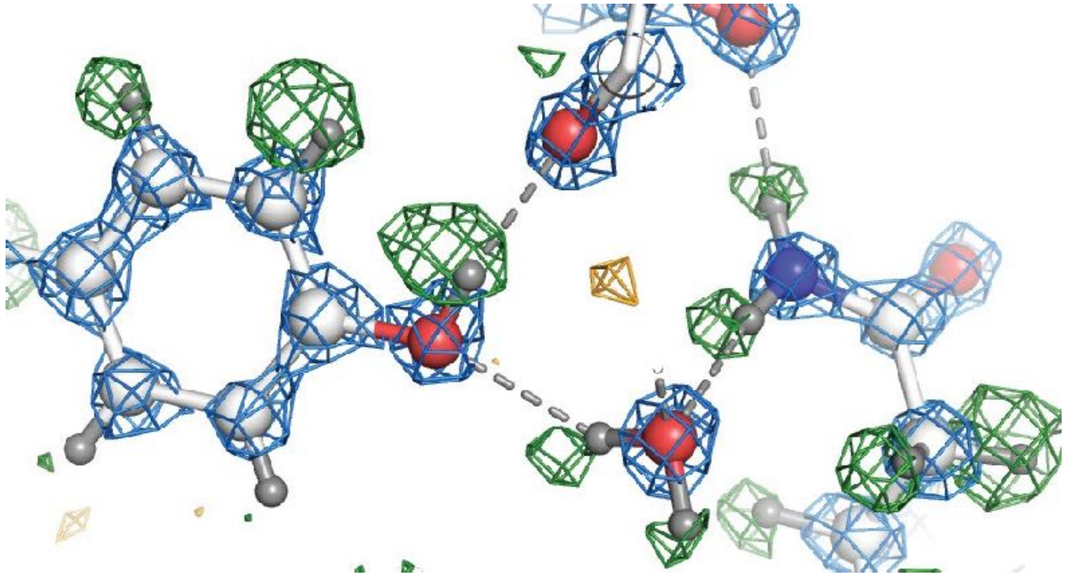


Titan Krios with standard configuration: XFEG, K3 camera, Bio-Quantum energy filter

Sun, Azumaya and Tse, et al (2020) *BioRxiv*

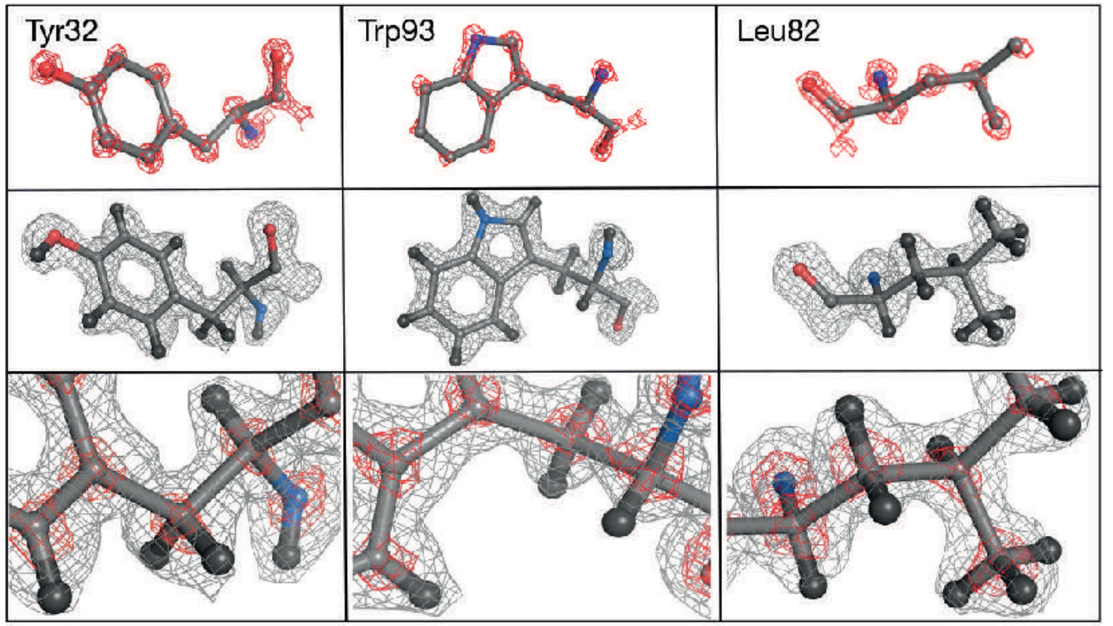
True atomic resolution by single particle cryo-EM

Apoferritin: an ideal test specimen for demonstrating the resolution high-resolution single particle cryo-EM; ~444kDa, with 24 fold symmetry.



“Single particle cryo-EM at atomic resolution:
Nakane, …, Scheres (2020) Nature

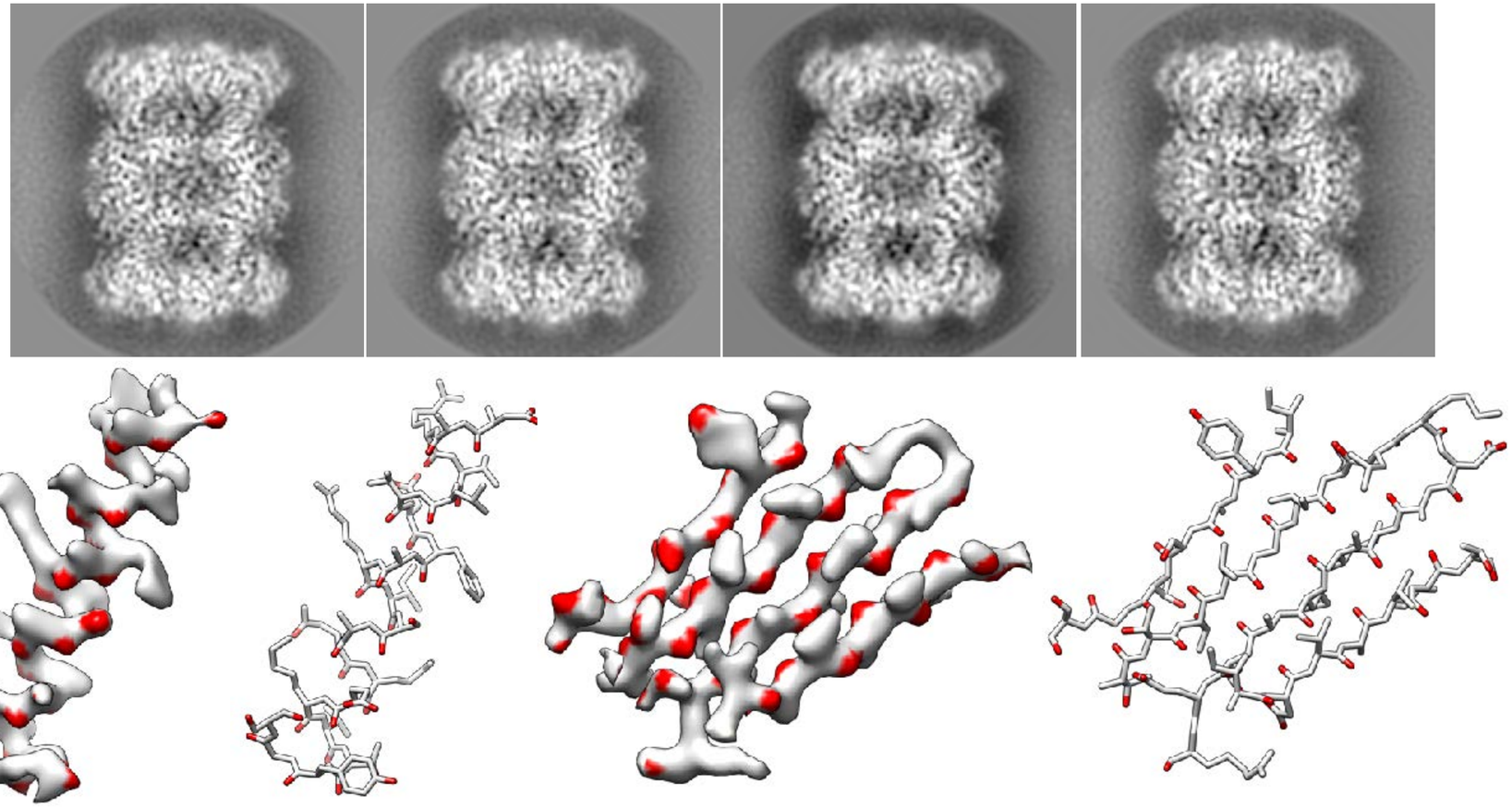
Cold FEG, Falcon 4, Energy filter



“Atomic-resolution protein structure determination by cryo-EM
Yip, …, Stark (2020) Nature

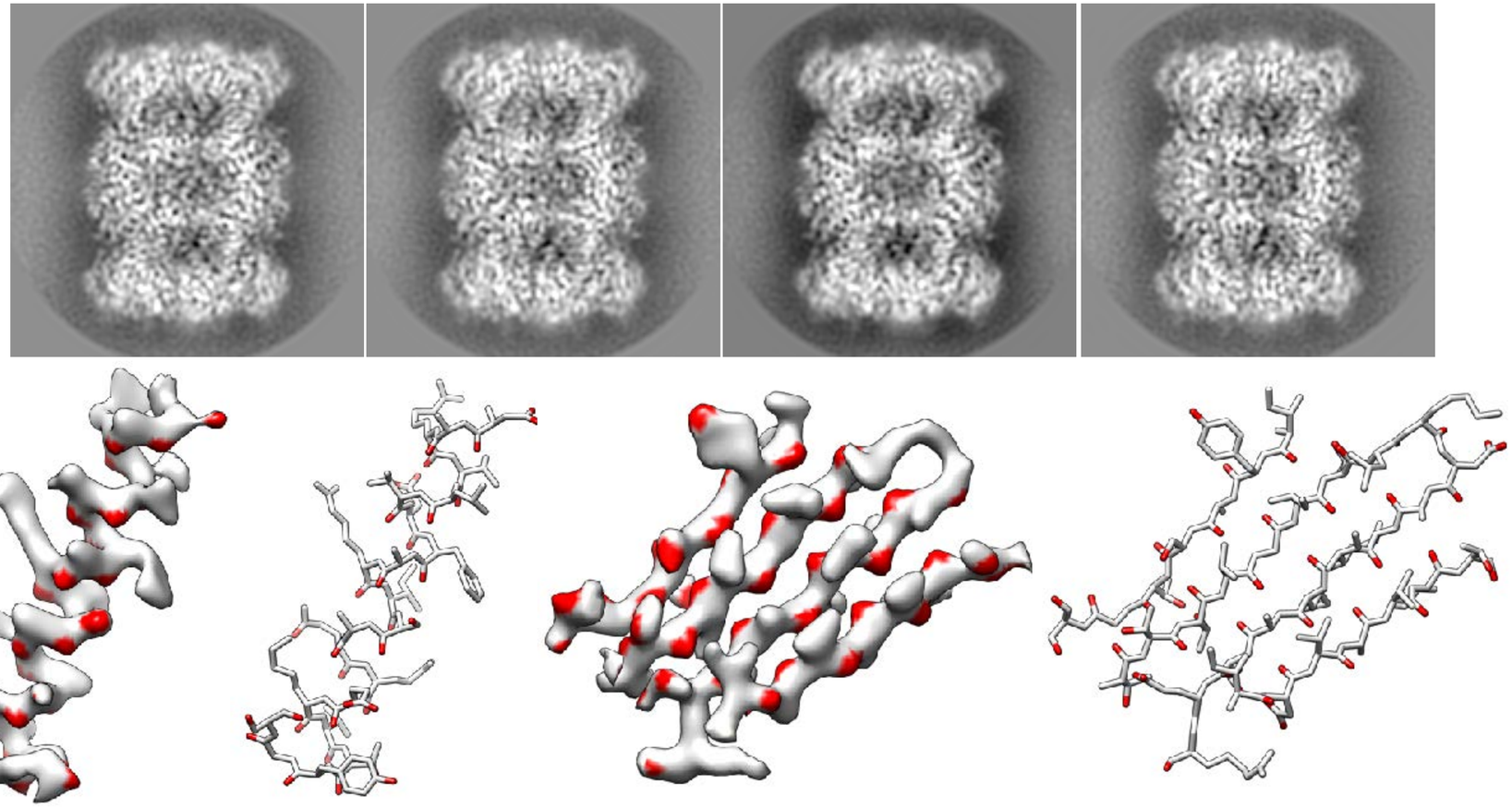
Monochromator, Cs corrector

Re-process with the algorithm: archaeal 20S proteasome at ~2.5Å resolution



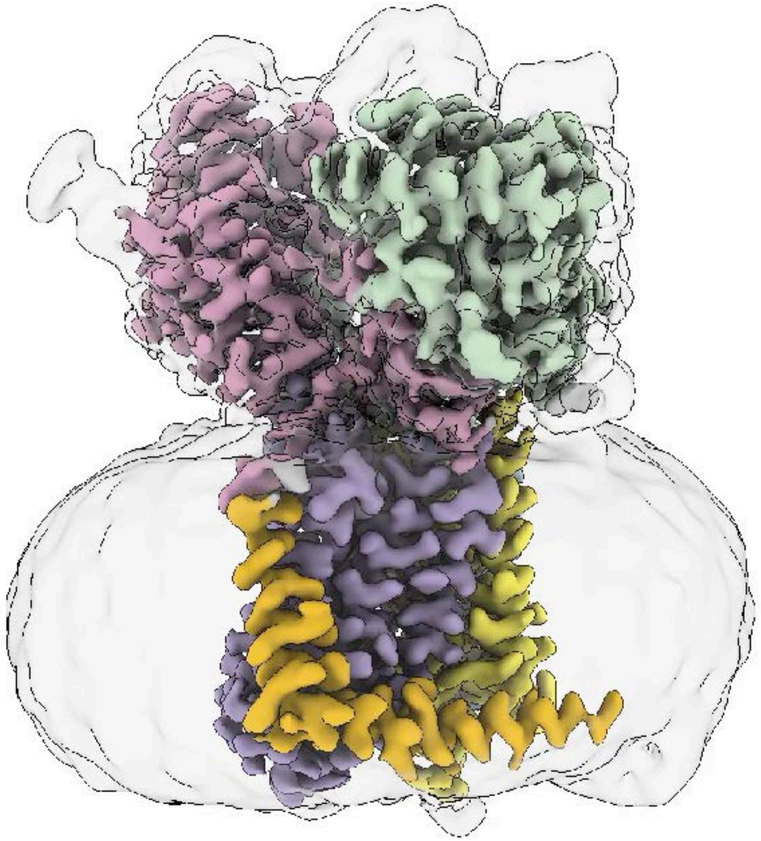
Zheng, Palovcak, Armache, Verba, Cheng and Agard (2017) ***Nature Methods***

Re-process with the algorithm: archaeal 20S proteasome at ~2.5Å resolution

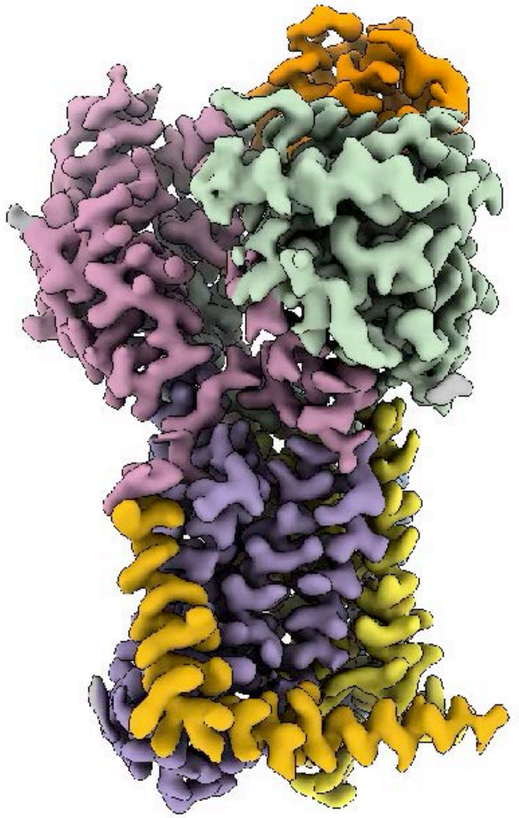


Zheng, Palovcak, Armache, Verba, Cheng and Agard (2017) ***Nature Methods***

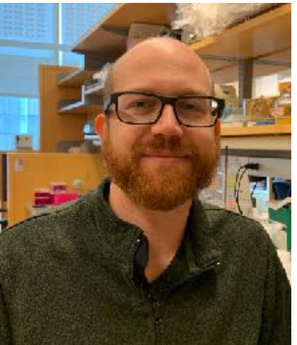
Dispatched structures



Structure of DISP1

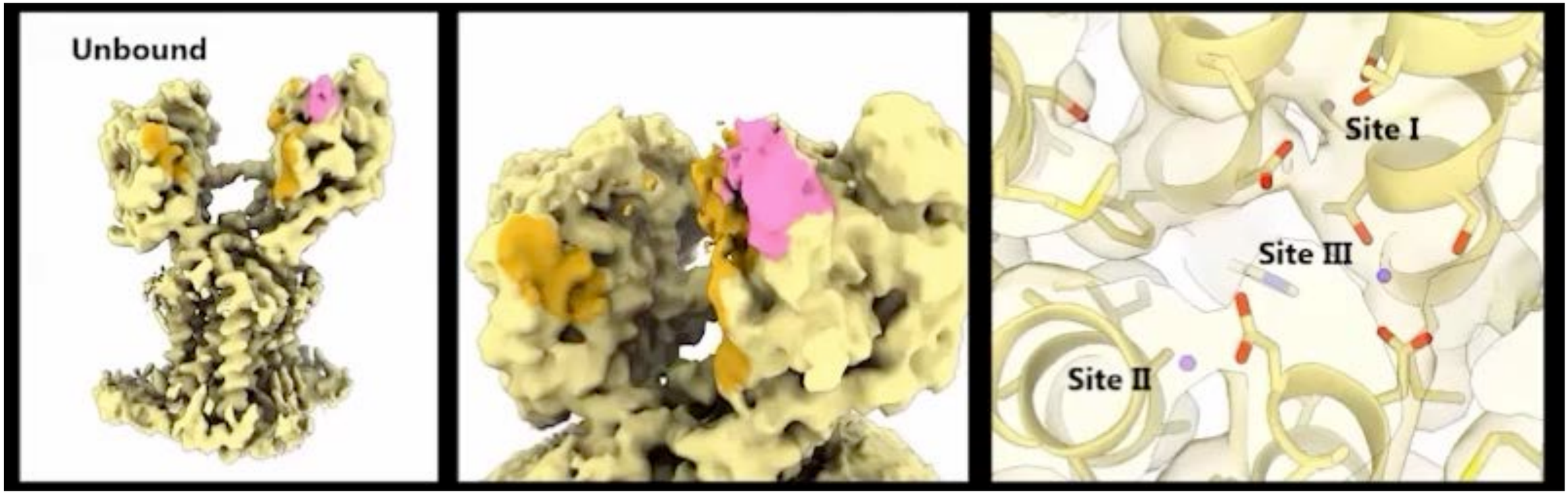


Structure of DISP1-ShhN complex



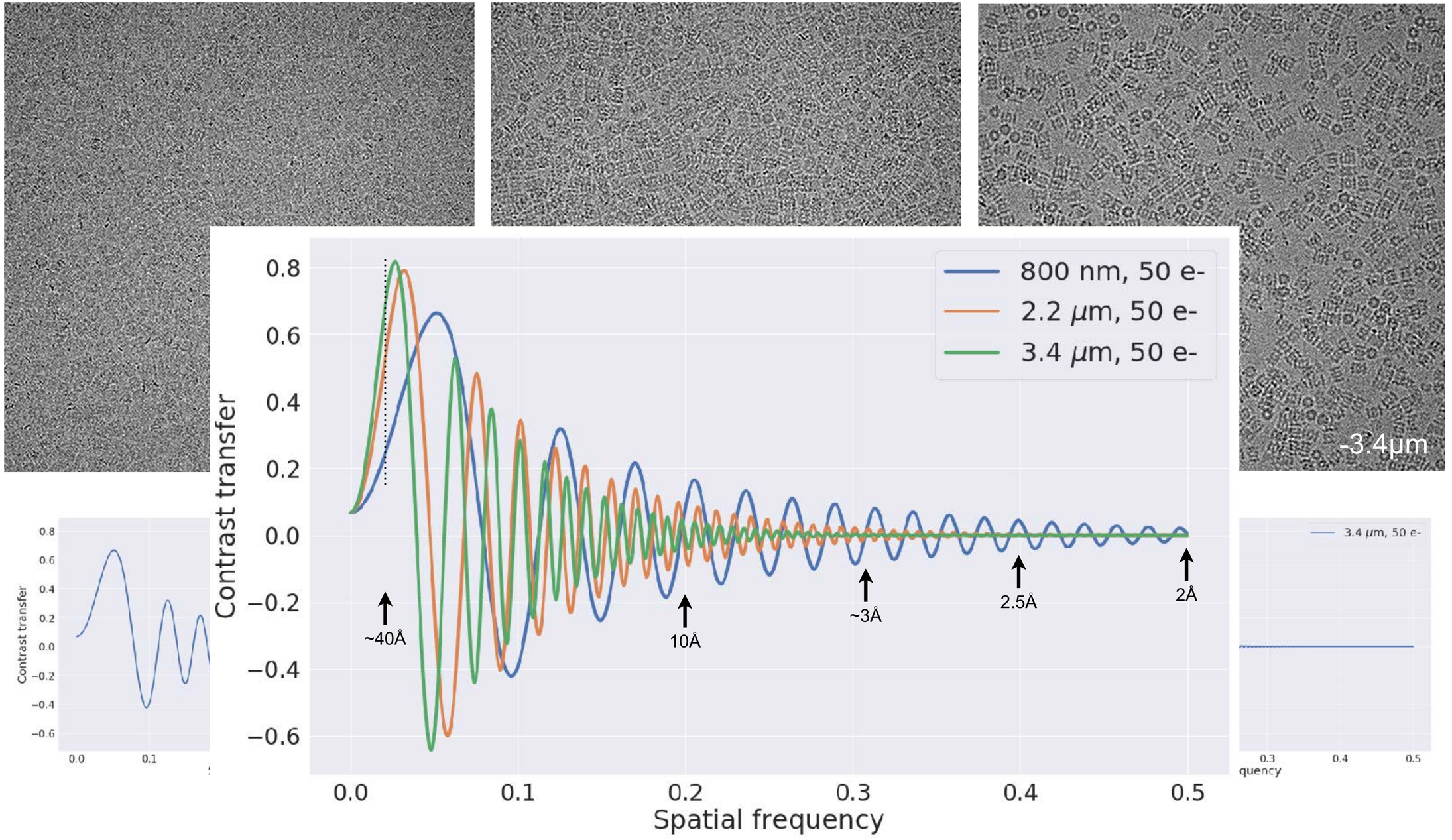
Daniel Asarnow

Dispatched structures

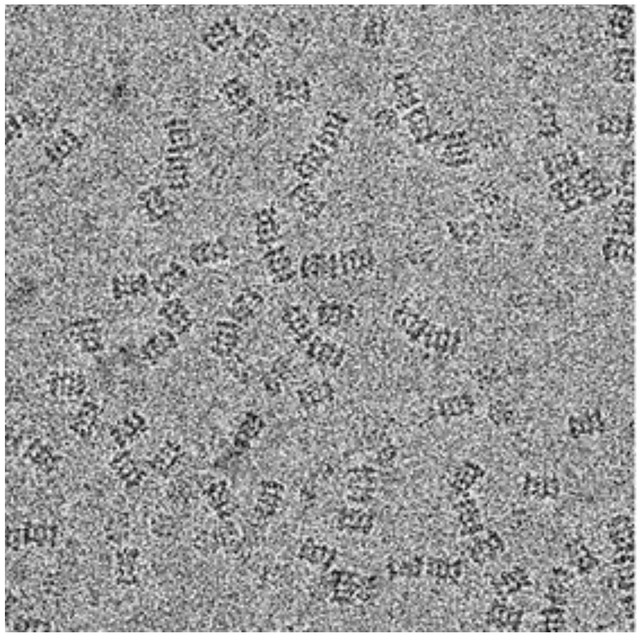


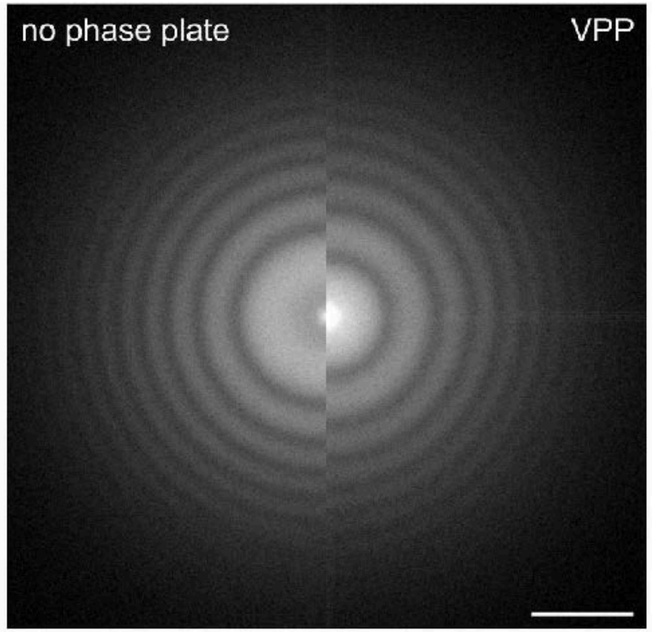
3D Variation Analysis (3DVA) reveal ShhN binding associated with conformation transitions and Na+ occupancies in the transmembrane domain;

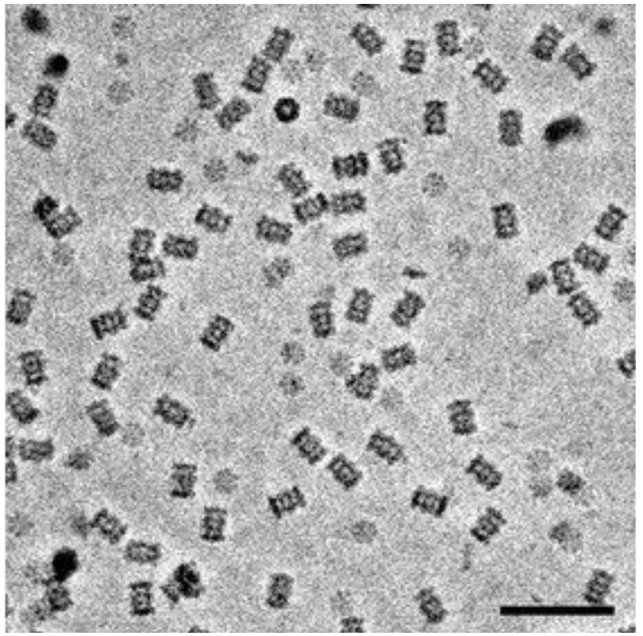
Influence of CTF on image

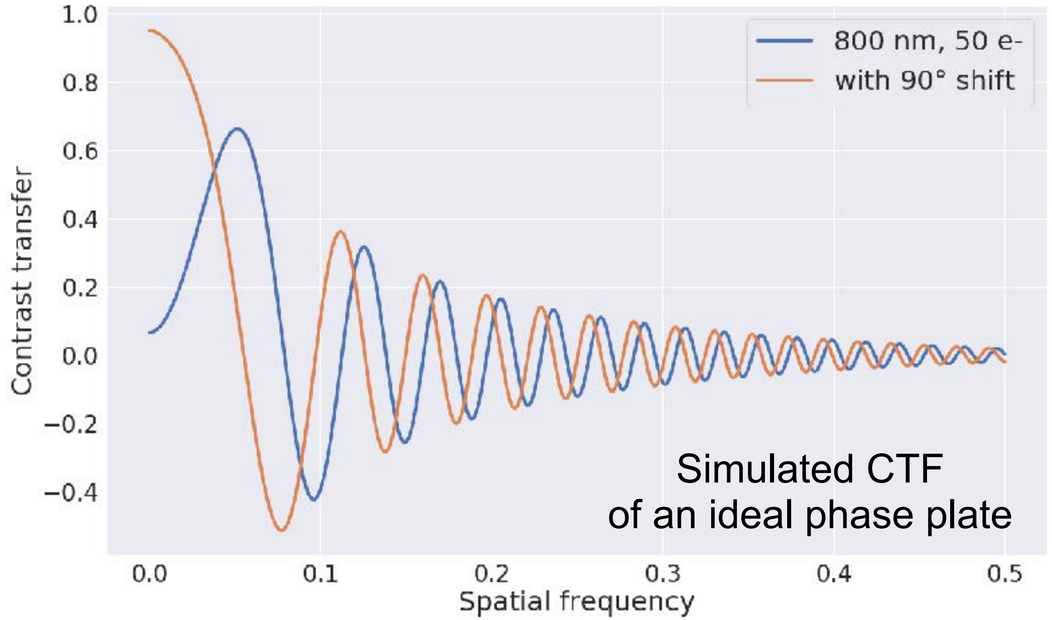


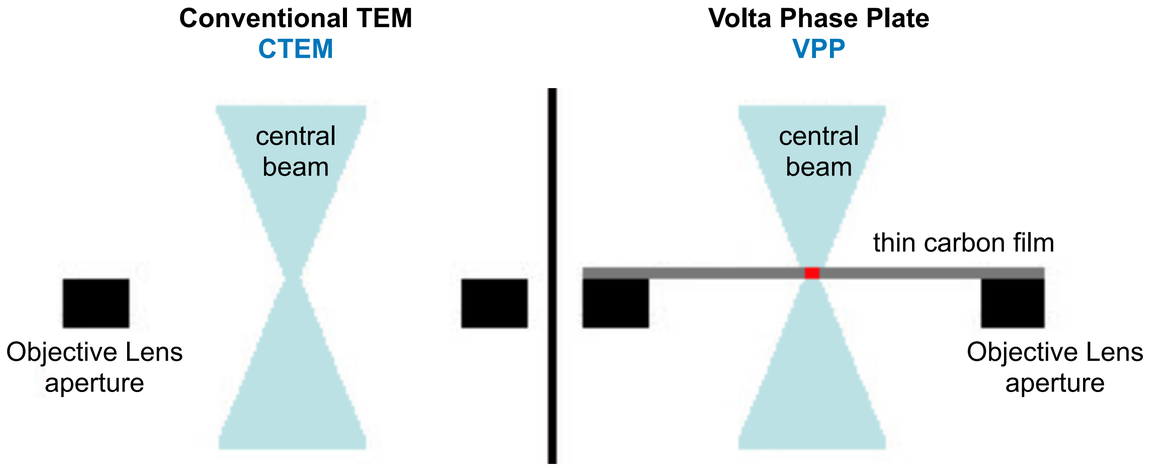
Phase plate: to enhance image contrast





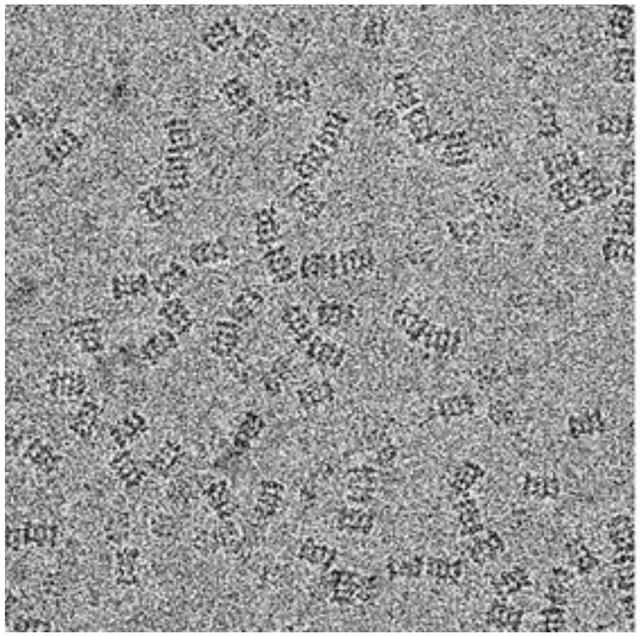


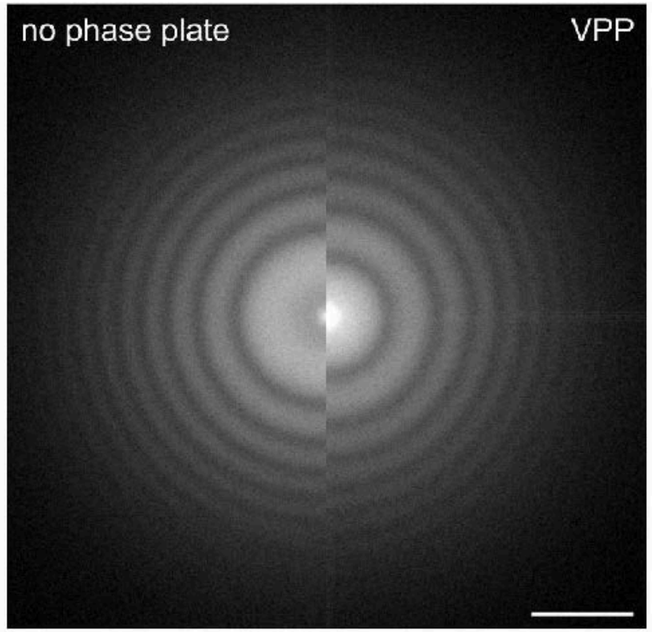


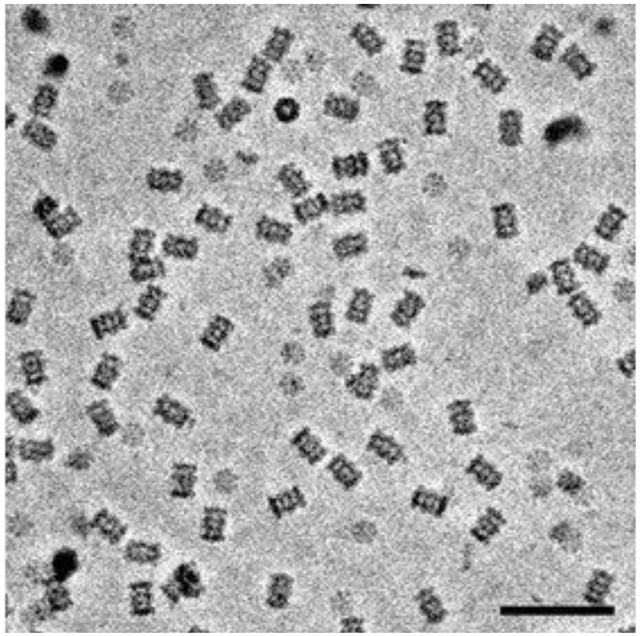


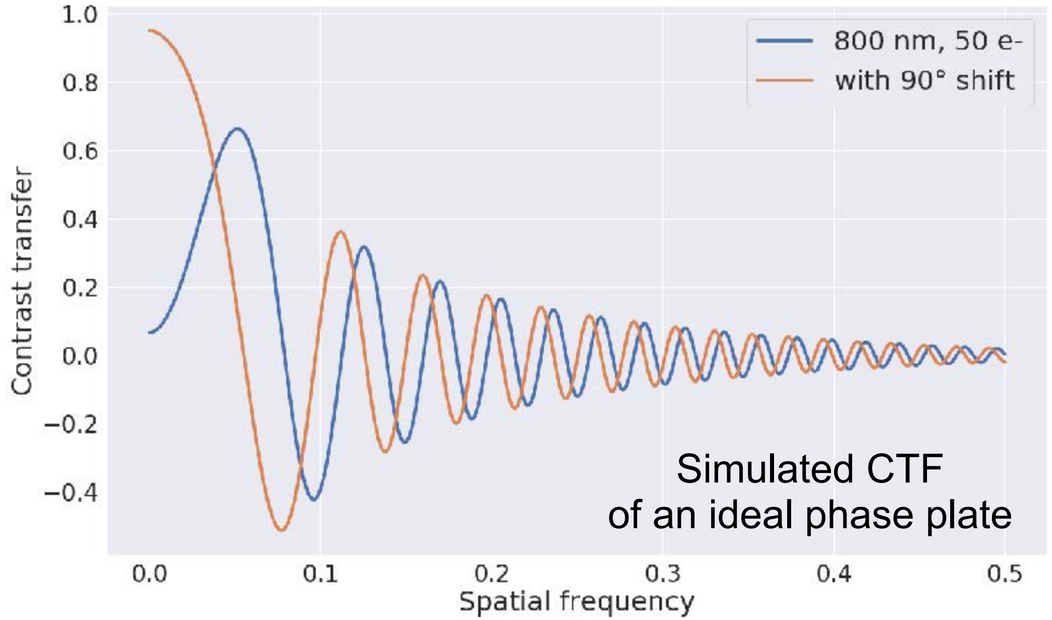
Thin carbon film (VPP) introduces phase shift,
but also absorb electron thus reduces signals.

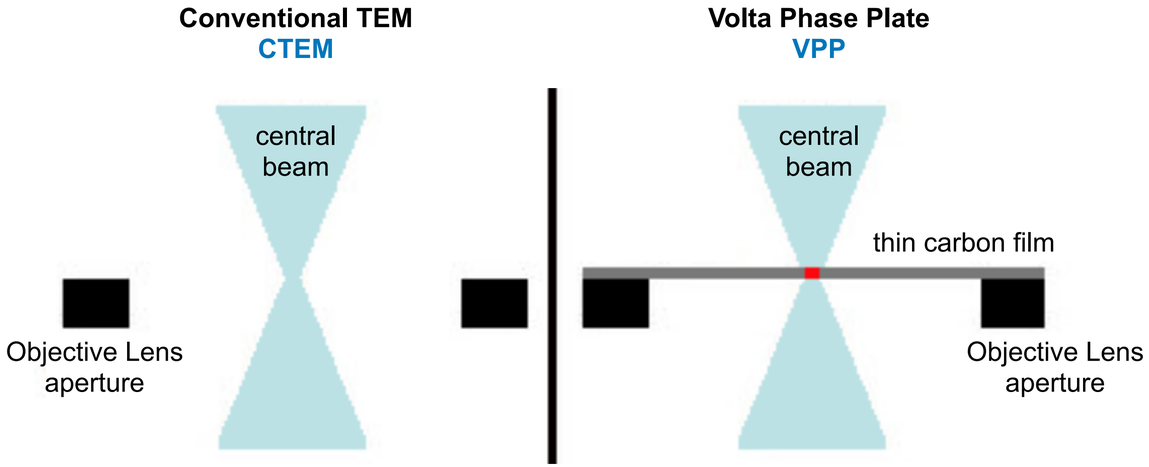
Phase plate: to enhance image contrast









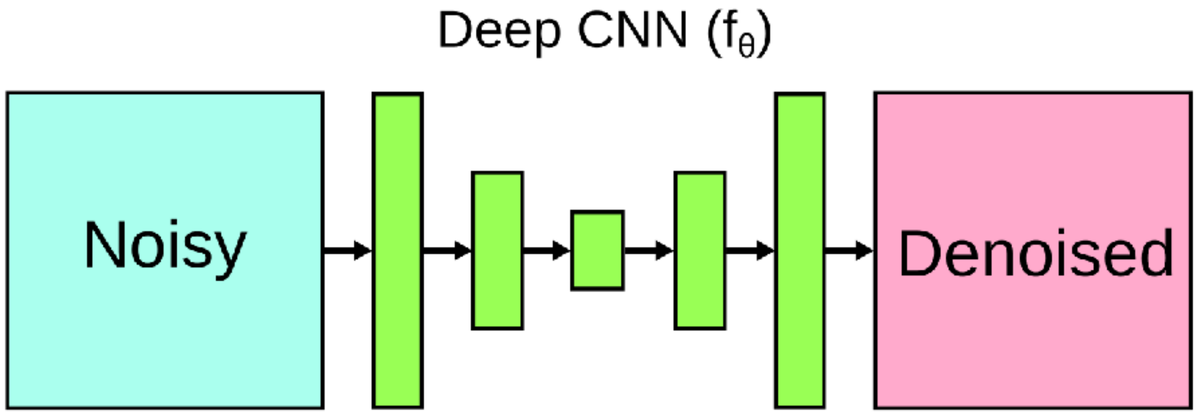


Thin carbon film (VPP) introduces phase shift,
but also absorb electron thus reduces signals.

Can image contrast enhanced computationally?

- Low-pass filter: boosting low-frequency signal by removing high-frequency signals;

Another approach: computational image restoration by deep learning



Noisy image (M) = signal (S) + noise (N)
A **denoiser** is a function such that:

$$f_\theta(M) = S$$

Deep convolutional neural networks

- Deep convolutional neural networks (CNNs) are parameterized **function approximators** for images;
- Deep CNNs are the state-of-the-art algorithms for many image processing tasks, including denoising;

U-Net: Convolutional Networks for Biomedical Image Segmentation

Olaf Ronneberger, Philipp Fischer, and Thomas Brox

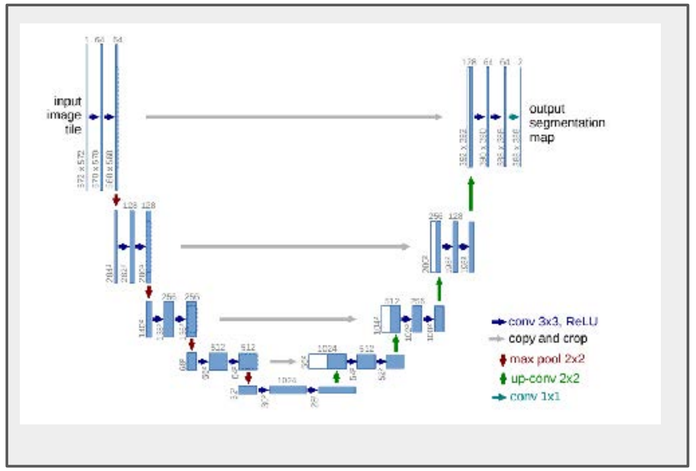
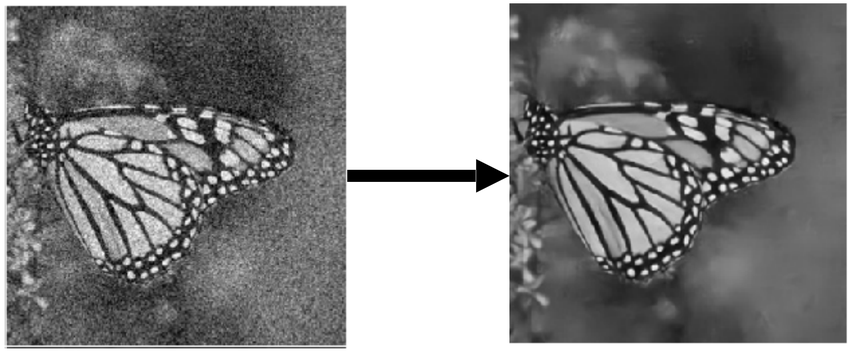


Image Restoration Using Convolutional Auto-encoders with Symmetric Skip Connections

Xiao-Jiao Mao, Chunhua Shen, Yu-Bin Yang



The CNN approach to denoise

We want a function (denoiser): $f_\theta(M) = S$

If we have noisy and noiseless image pairs M and S, we can change the parameters θ of f_θ to optimize:

$$\text{argmin}_\theta \, \mathbf{E}[\, |f_\theta(M) - S|^2 \,]$$

or to train CNN to produce the desired denoised images, where **E**[...] is the expectation over all pairs of training images, M and S

But, for cryo-EM images, we do NOT have such image pair!

Noise2noise scheme for training a neural network

Noise2Noise: Learning Image Restoration without Clean Data

Jaakko Lehtinen[1 2] Jacob Munkberg[1] Jon Hasselgren[1] Samuli Laine[1] Tero Karras[1] Miika Aittala[3] Timo Aila[1]

Arxiv. (2018)

With two noisy images of the same object:

$$M_1 = S + N_1$$
$$M_2 = S + N_2$$

Noise2noise algorithm trains a CNN
to turn one noisy image into another:

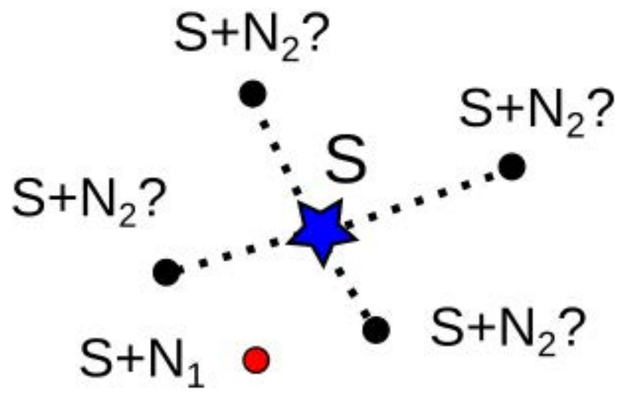
$$\text{argmin}_\theta \; \mathbf{E}[\, |f_\theta(M_1) - M_2|^2 \,]$$

$$= \text{argmin}_\theta \; \mathbf{E}[\, |f_\theta(S+N_1) - S+N_2|^2 \,]$$

If N_2 is uncorrelated with N_1 and zero-mean,
this training scheme approximates the function:

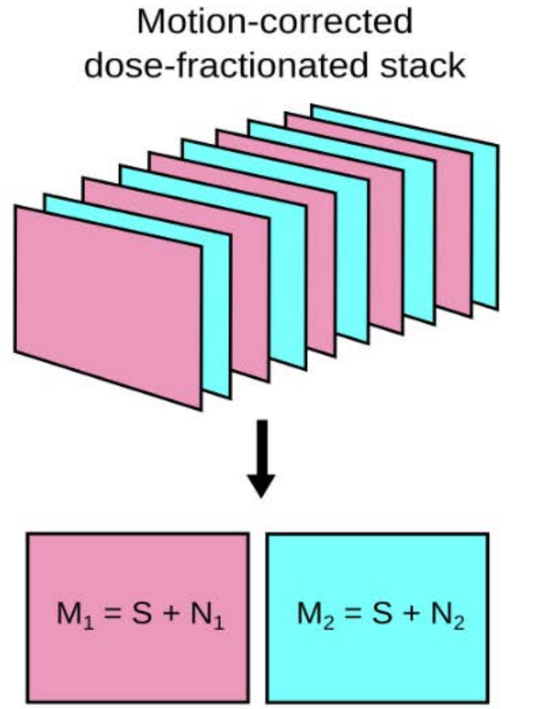
$$f_\theta(M_1) = \mathbf{E}[\, M_2 \,] = \mathbf{E}[\, S + N_2 \,] = S$$

and so f_θ approximates a **denoiser**



Obtaining two images of the same object

Direct electron detector =
Electron movie camera

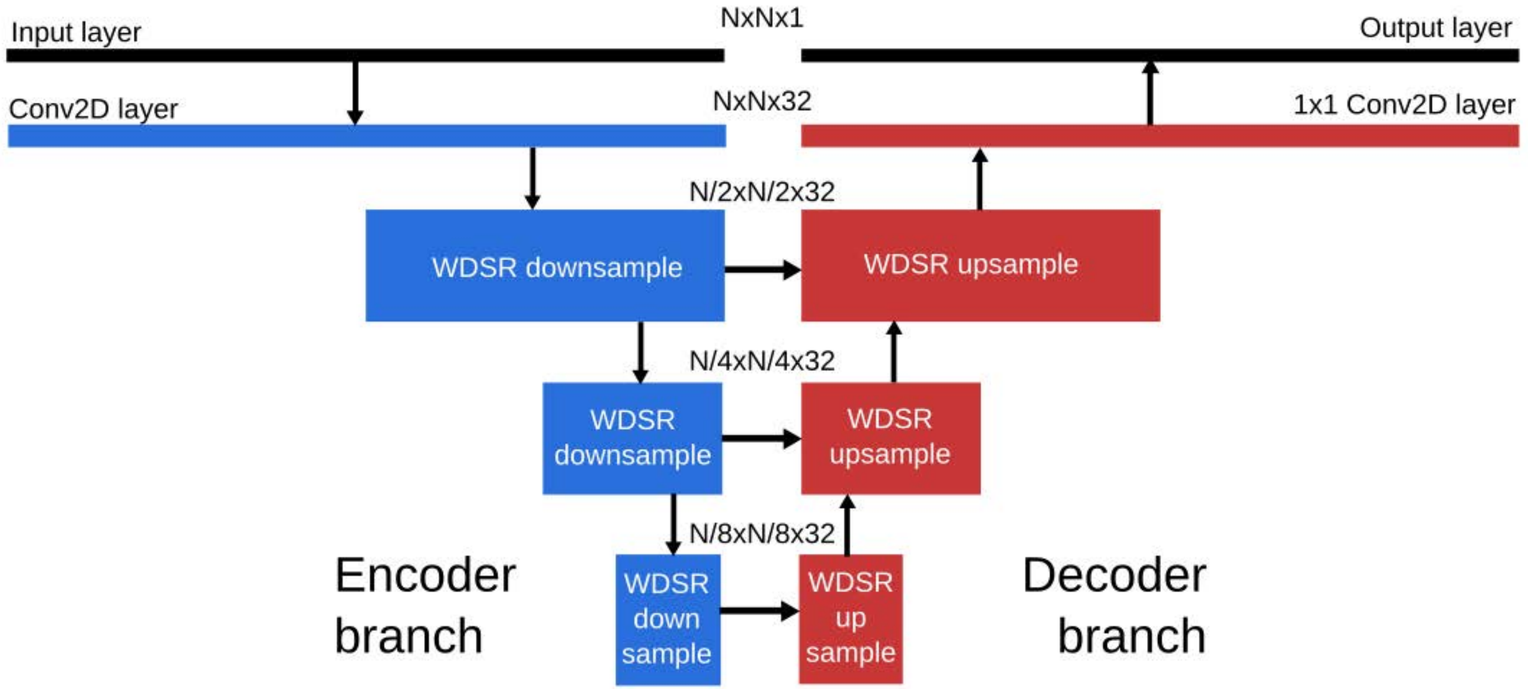


Even & odd sums have the same
signal but uncorrelated noise

- Training data can be obtained from cryo-EM *movie* images, by splitting the movie frames to two different sums of all frames of either odd or even frame numbers.

Implementing noise2noise for cryo-EM image denoise

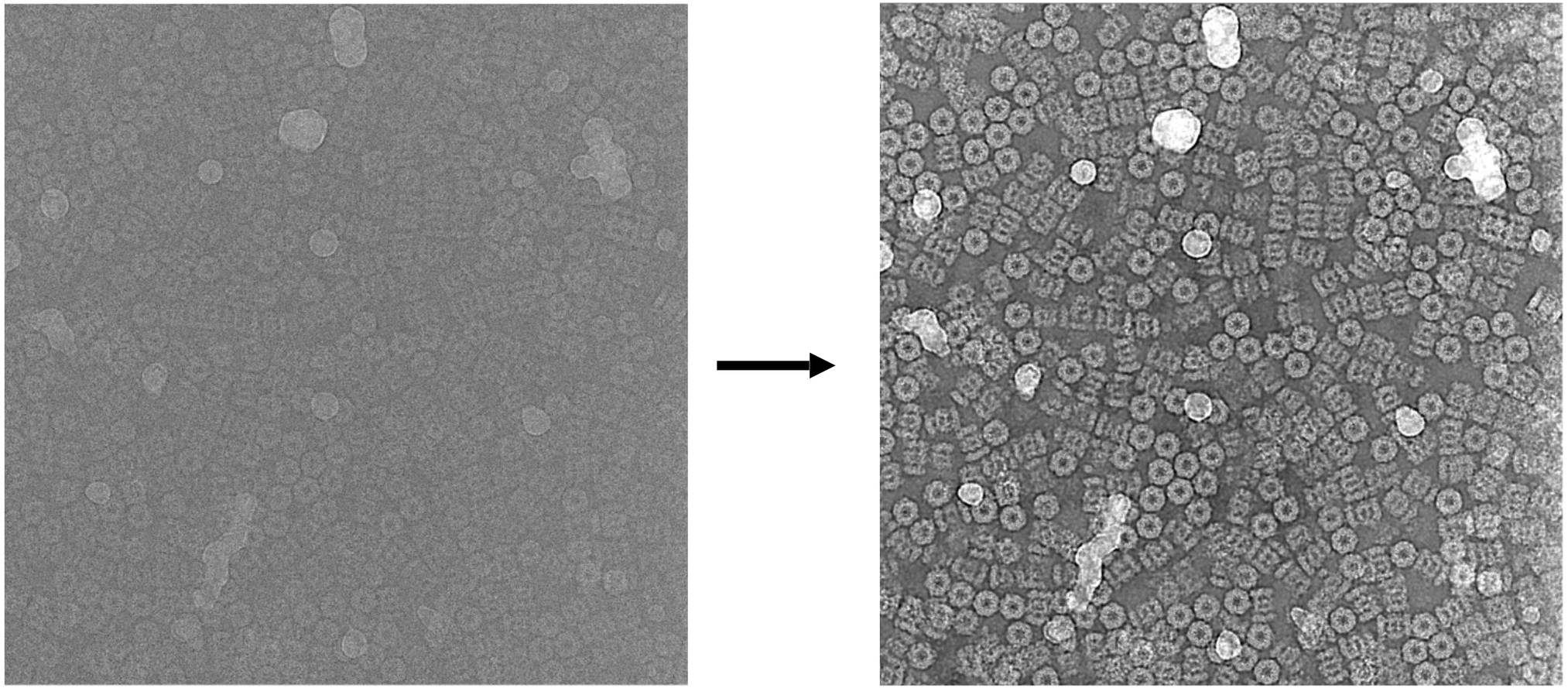
- Final CNN has an encoder-decoder structure with 96 convolutional layers and 2.3 million trainable parameters
- Python based program package **restore**: open-source, GPU-accelerated, and extremely fast (a K3 image takes only 6 seconds on a modern Nvidia GPU);



Palovcak, et al (2020) *IUCrJ*

Examples of cryo-EM image denoising

We tested denoising a number of different images and specimens: especially images recorded at low defocus and/or of small particles or oddly-shaped particles.

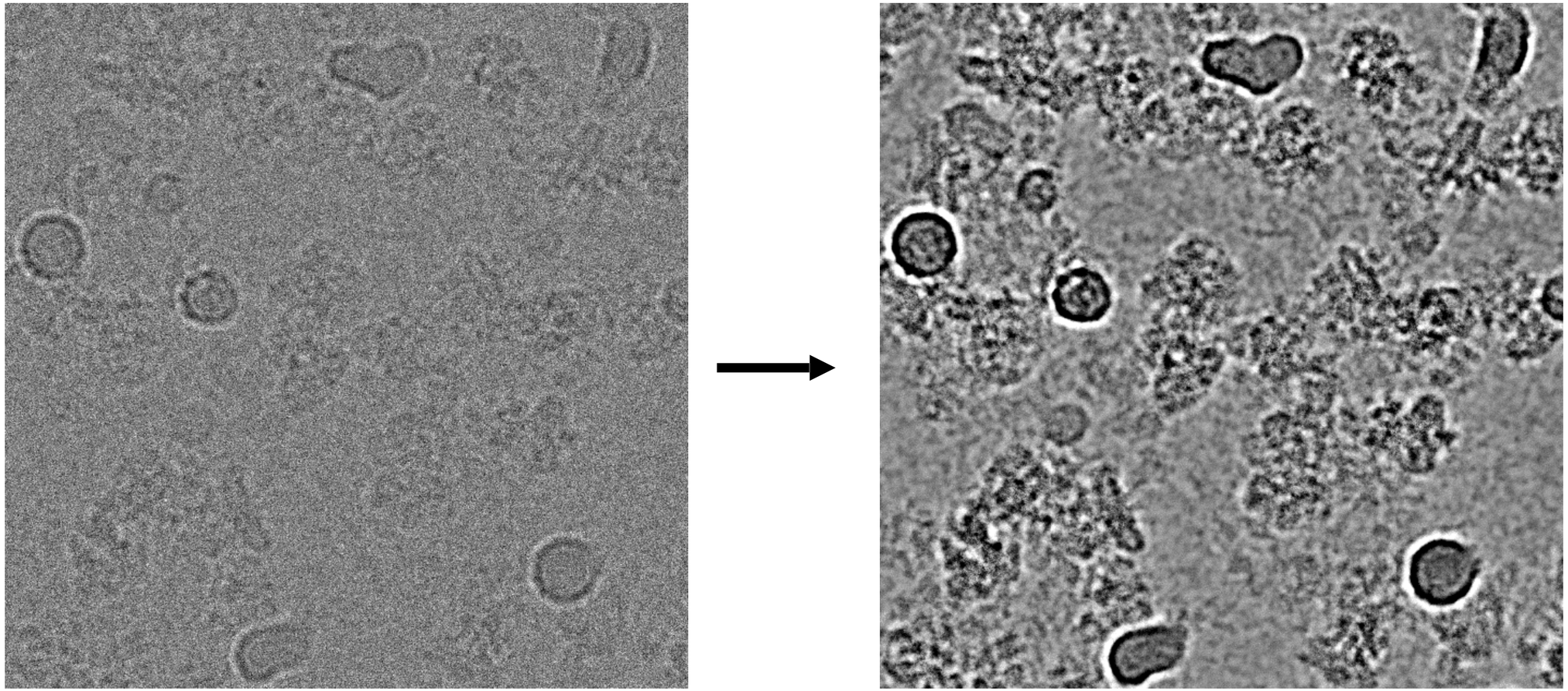


- T20S proteasome: ~700kDa complex | ~0.6um defocus;

Palovcak, et al (2020) *IUCrJ*

Examples of cryo-EM image denoising

We tested denoising a number of different images and specimens: especially images recorded at low defocus and/or of small particles or oddly-shaped particles.

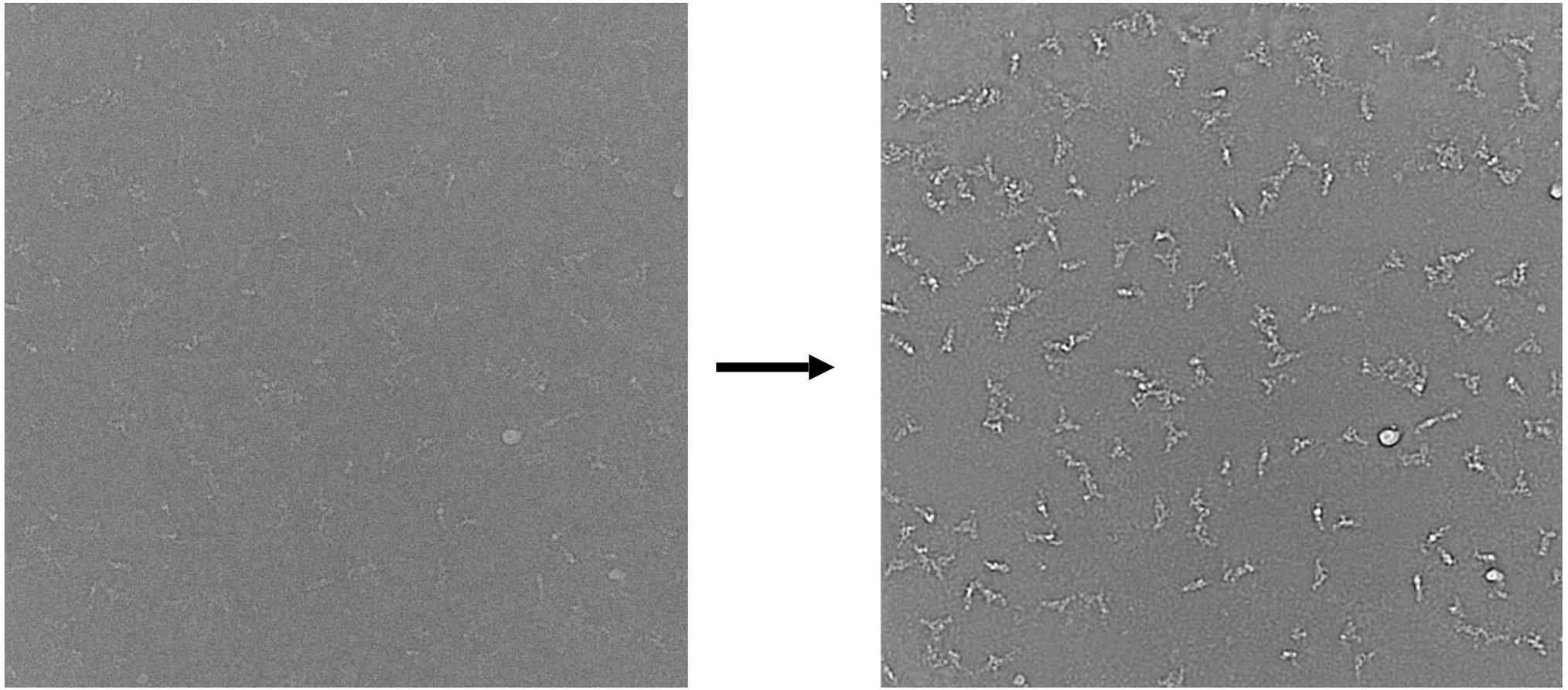


- TRPM4 ion channel: ~700kDa complex | ~1.4um defocus;
- in messy image, intact particles can be identified easier from “junk” particles;

Palovcak, et al (2020) *IUCrJ*

Examples of cryo-EM image denoising

We tested denoising a number of different images and specimens: especially images recorded at low defocus and/or of small particles or oddly-shaped particles.

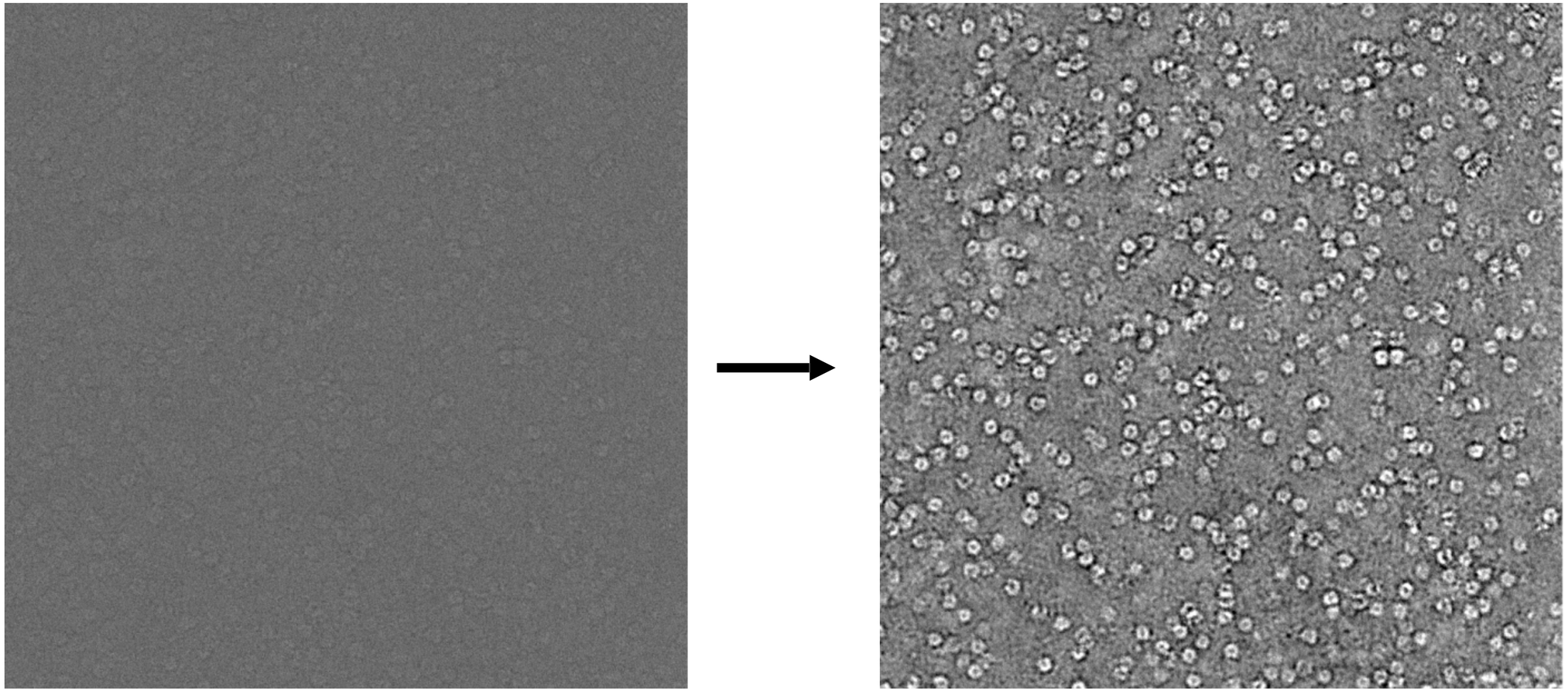


- integrin-Fab complex: ~380kDa complex | ~2um defocus;
- small and conformational heterogeneous particles can be easily identified;

Palovcak, et al (2020) *IUCrJ*

Examples of cryo-EM image denoising

We tested denoising a number of different images and specimens: especially images recorded at low defocus and/or of small particles or oddly-shaped particles.

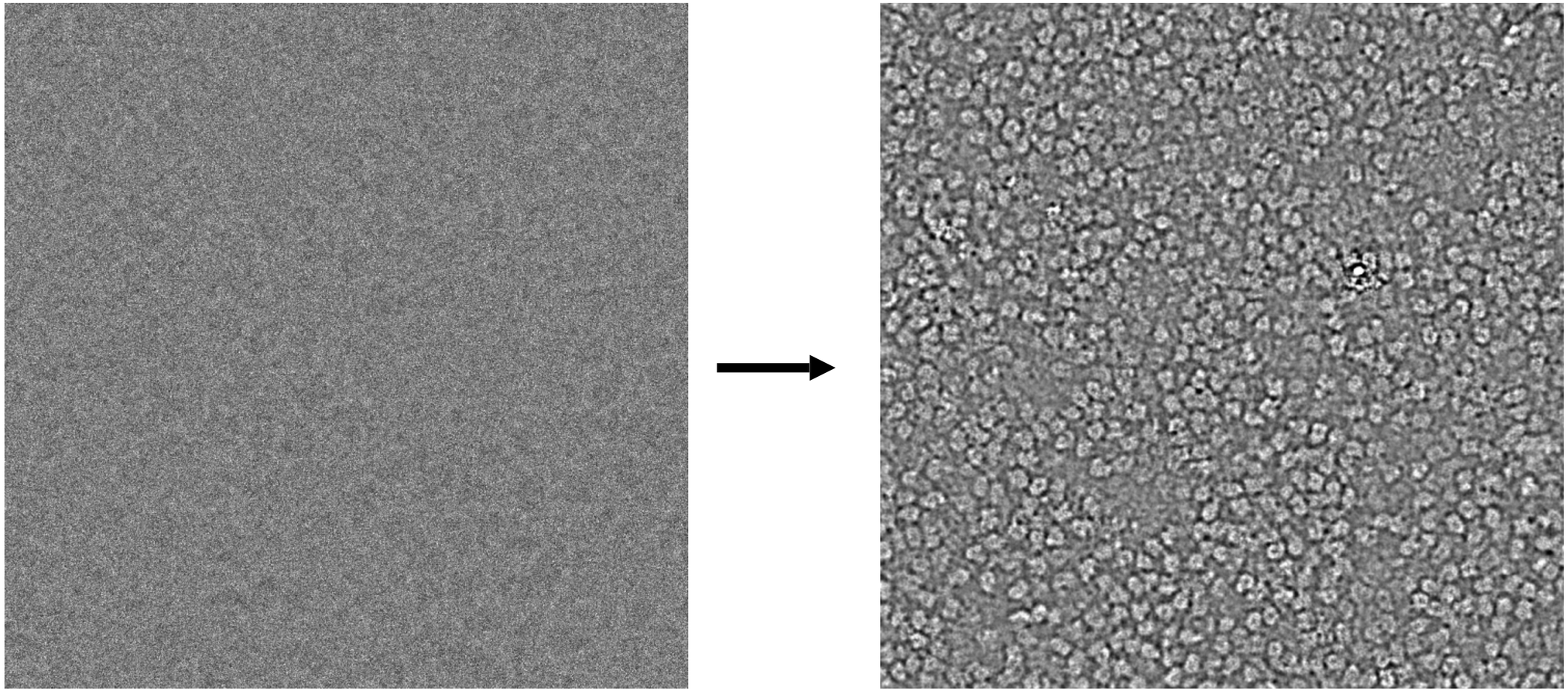


- streptavidin on GO: ~55kDa complex | ~0.8um defocus
- very small particles can be identified and picked easily;

Palovcak, et al (2020) *IUCrJ*

Examples of cryo-EM image denoising

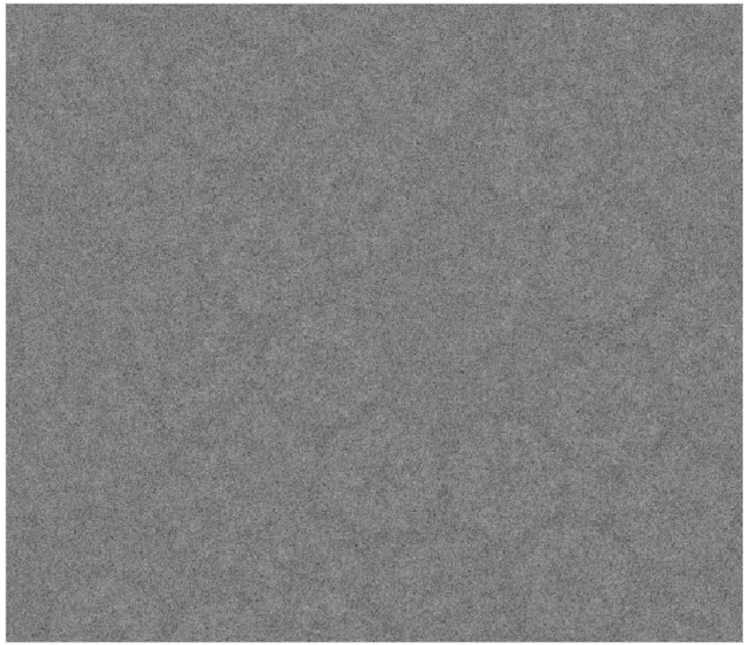
We tested denoising a number of different images and specimens: especially images recorded at low defocus and/or of small particles or oddly-shaped particles.



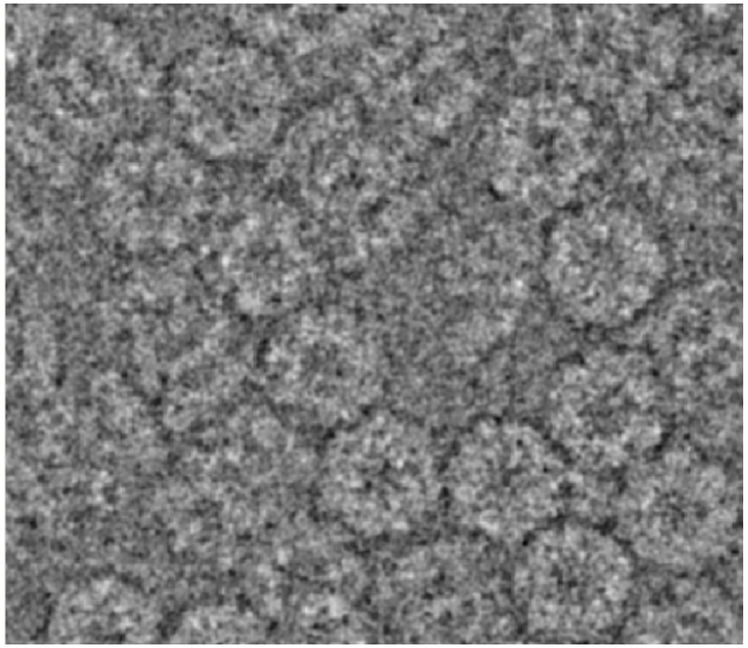
- protein kinase A catalytic domain (Lander lab): ~43kDa complex | ~1um defocus;
- very small particles can be identified and picked easily;

Palovcak, et al (2020) *IUCrJ*

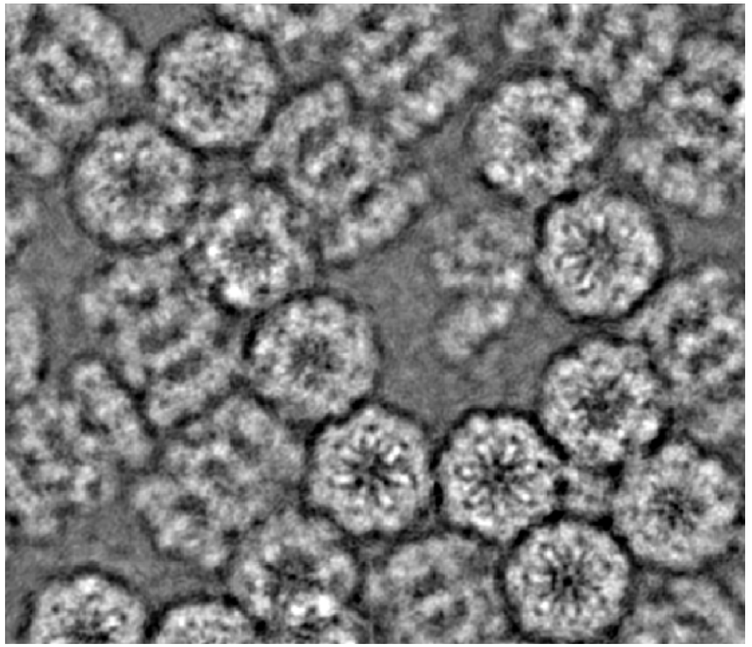
CNN denoising enhances cryo-EM image contrast



Raw image



Wiener filtered image
(optimal linear denoiser)

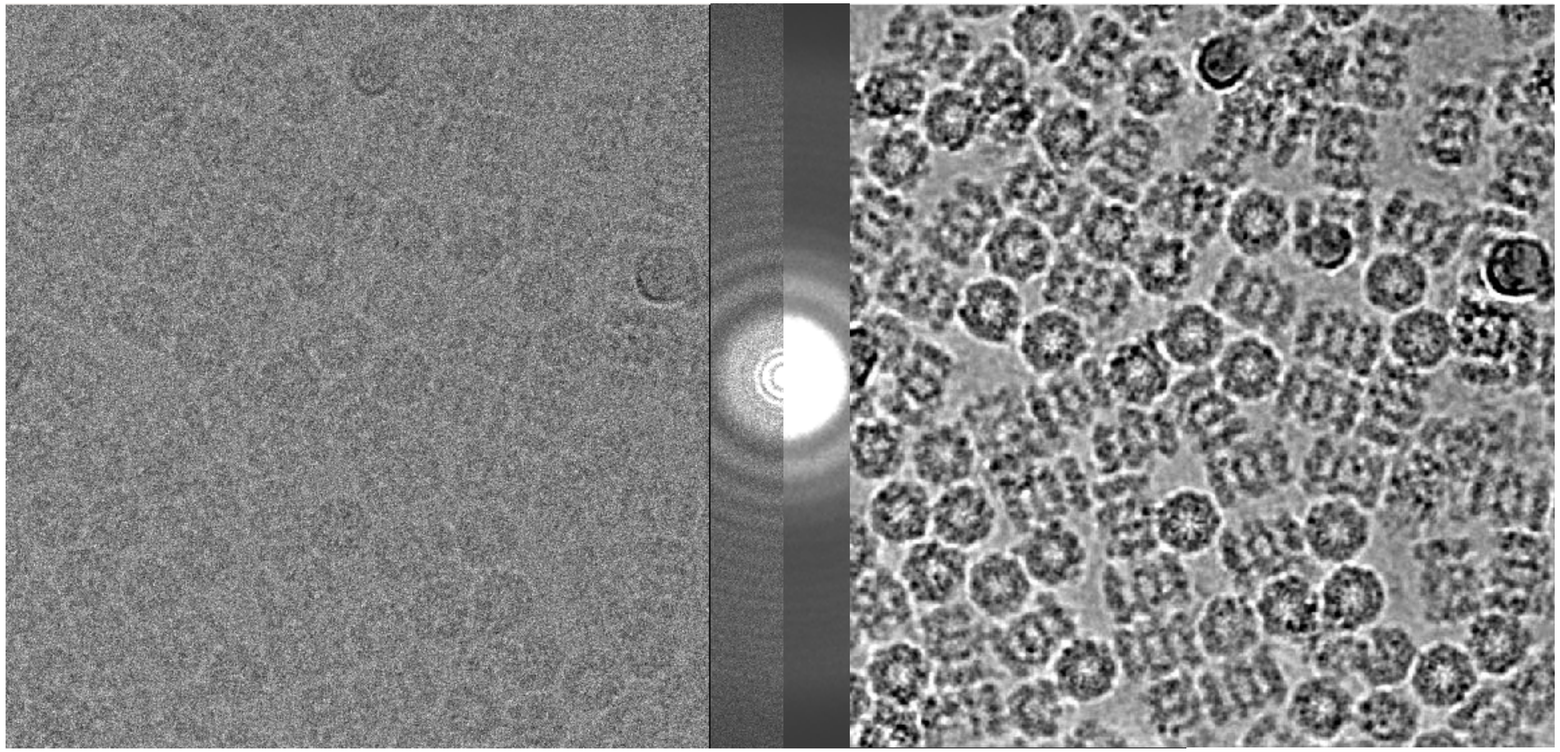


CNN denoised image

- CNN denoising produces better image contrast than commonly used Wiener filter;
- **It is a non-linear procedure;**

Palovcak, et al (2020) *IUCrJ*

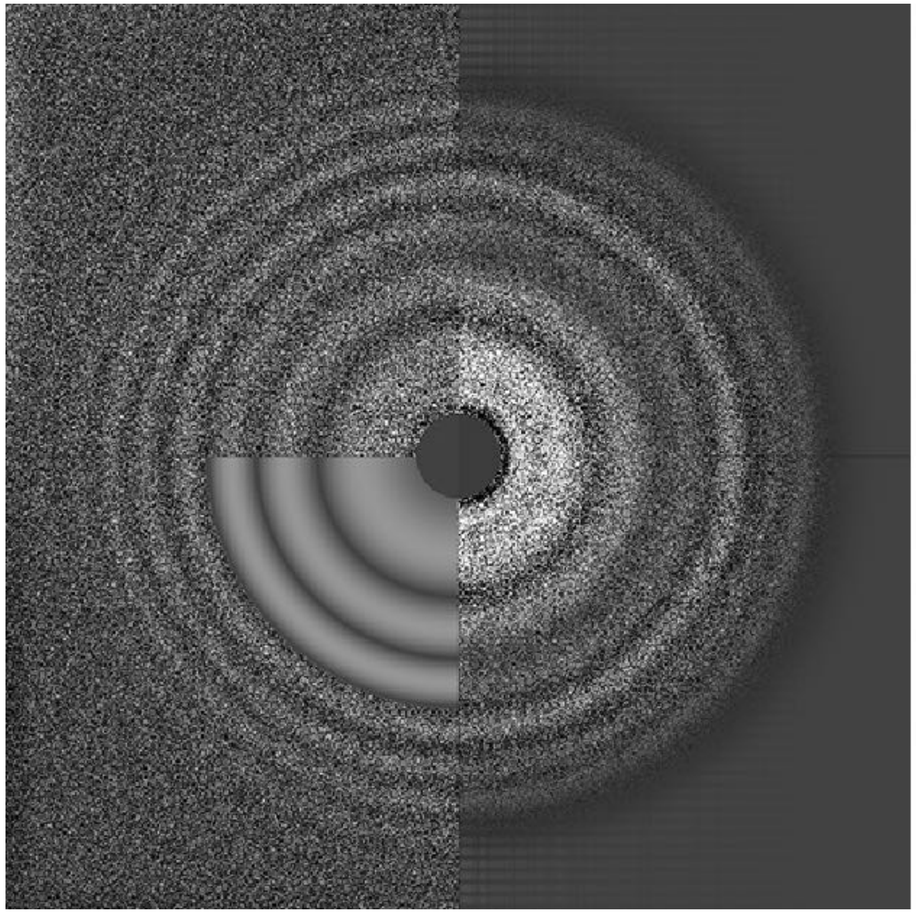
CNN denoising enhances cryo-EM image contrast

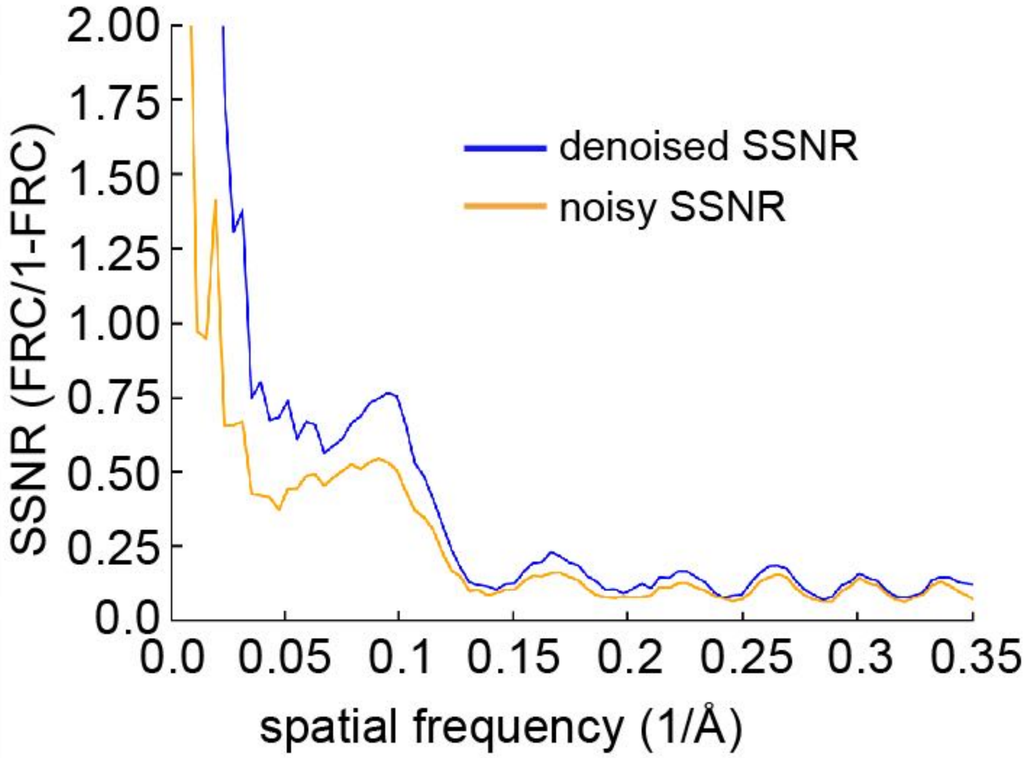


- Real space: low frequency signal is greatly enhanced in the denoised image.
- Fourier space: FFTs of raw and denoised images show significant enhancement in low-frequency signal,;
- Contrast enhancement is somewhat similar as VPP;
- Unlike VPP, positions of all Thon rings are not altered;

Palovcak, et al (2020) *IUCrJ*

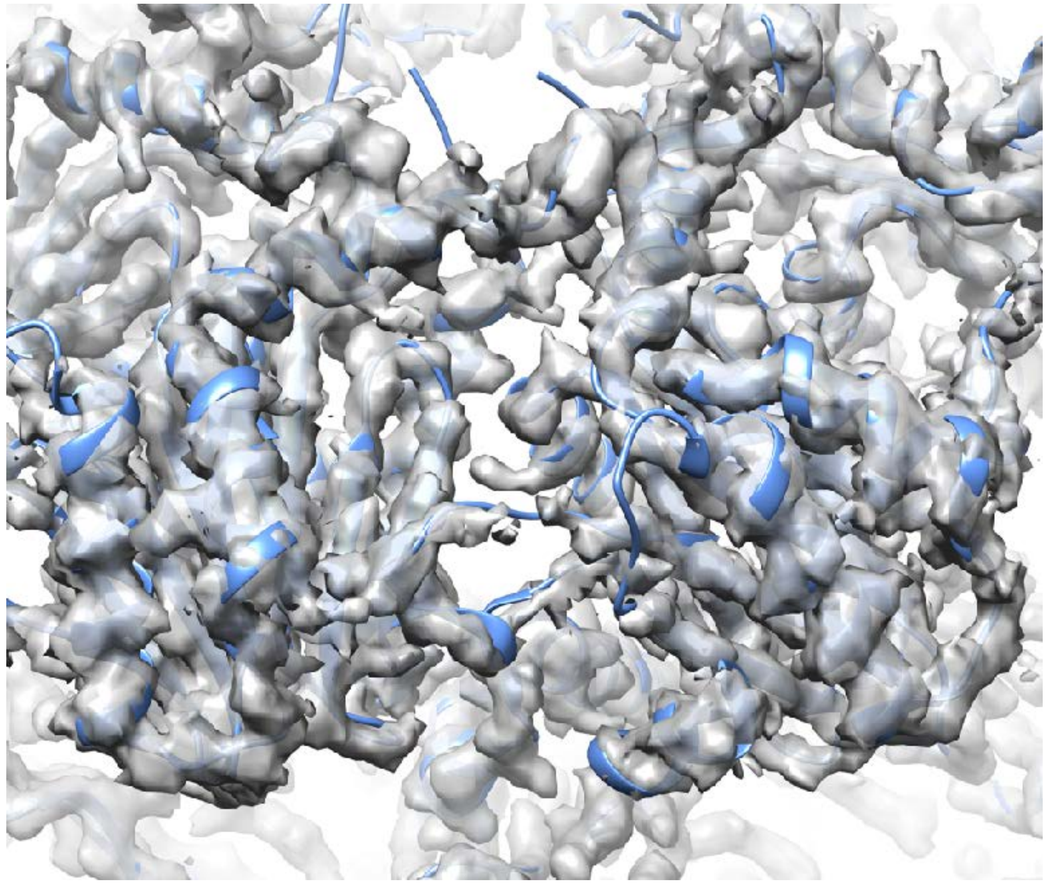
CNN denoising enhances SNR



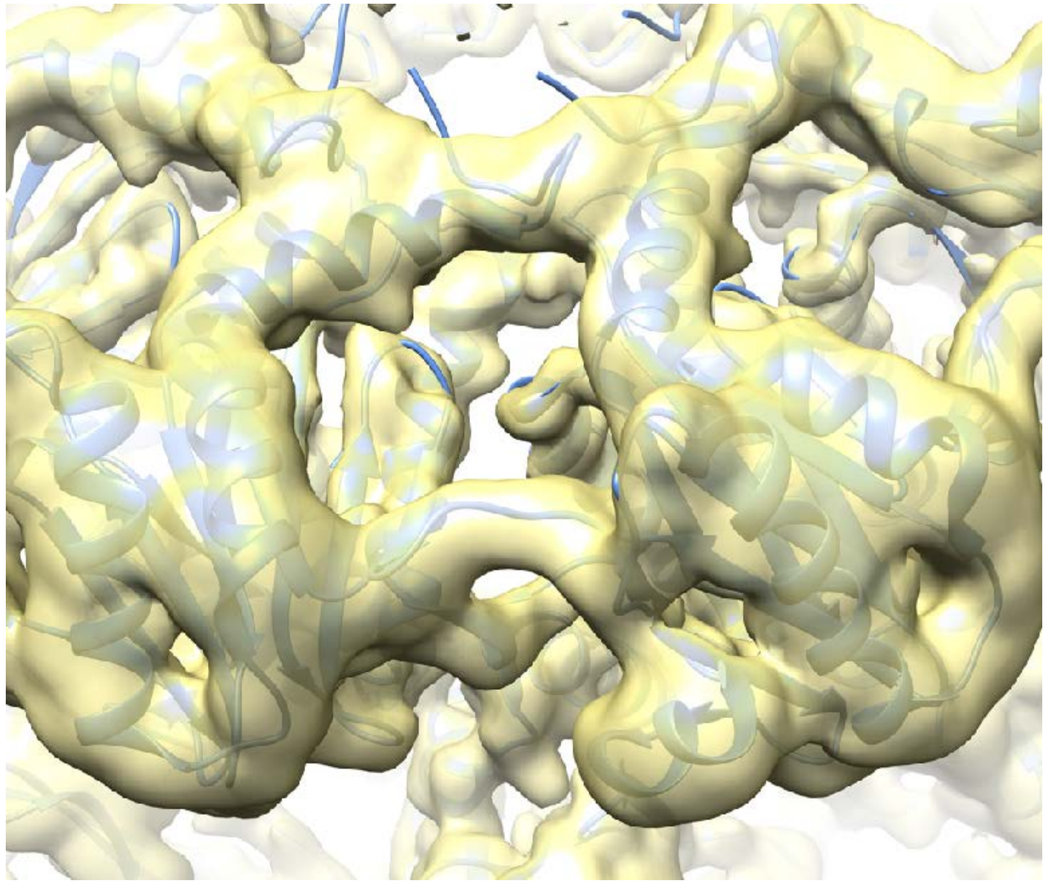


Palovcak, et al (2020) *IUCrJ*

Can denoised particle being used for recontruction?



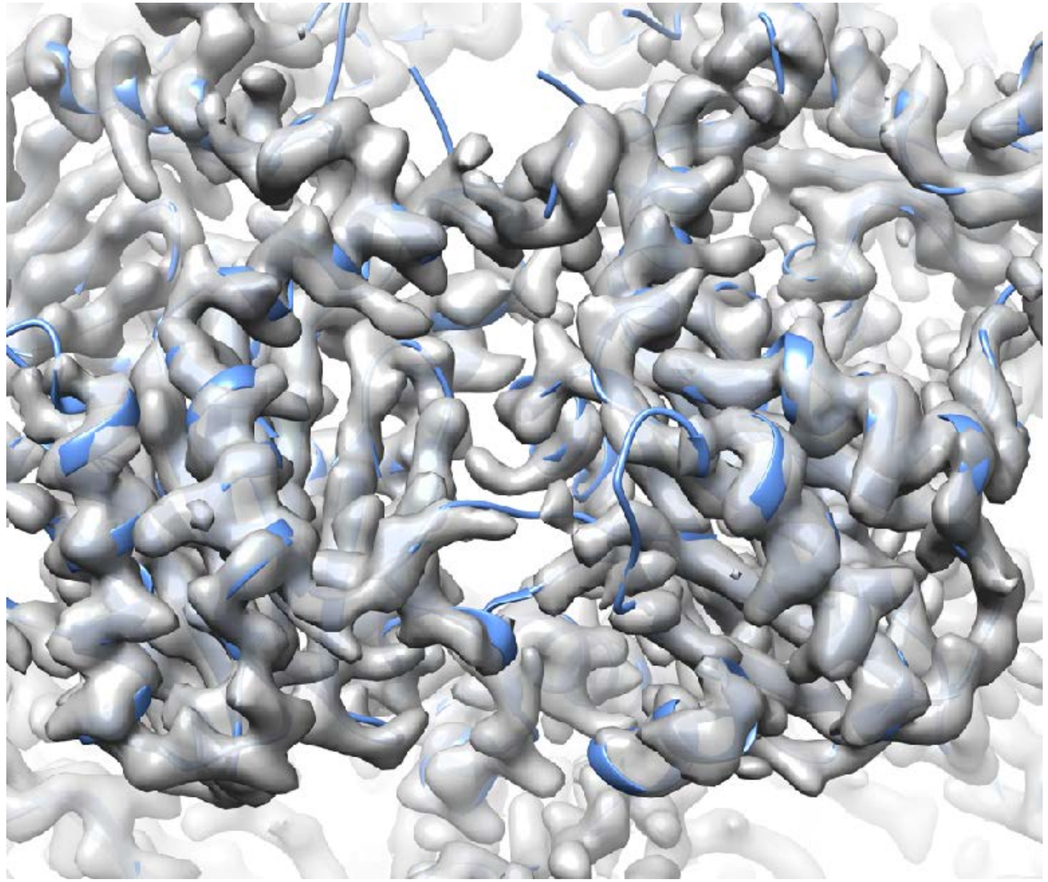
3D reconstruction from raw particles
(~3Å resolution, C1 symmetry)



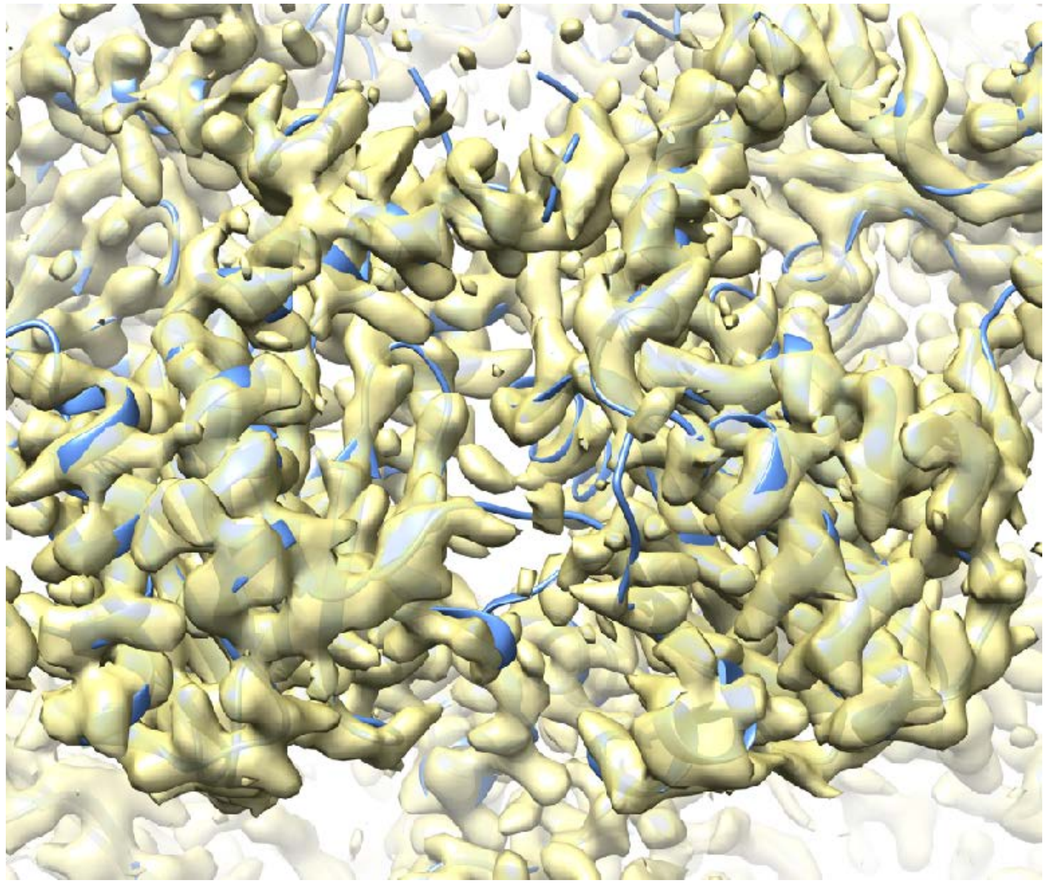
3D reconstruction from denoised particles, using orentation parameter determined from raw particles (~3Å resolution, C1 symmetry)

Palovcak, et al (2020) *IUCrJ*

Can denoised particle being used for recontruction?



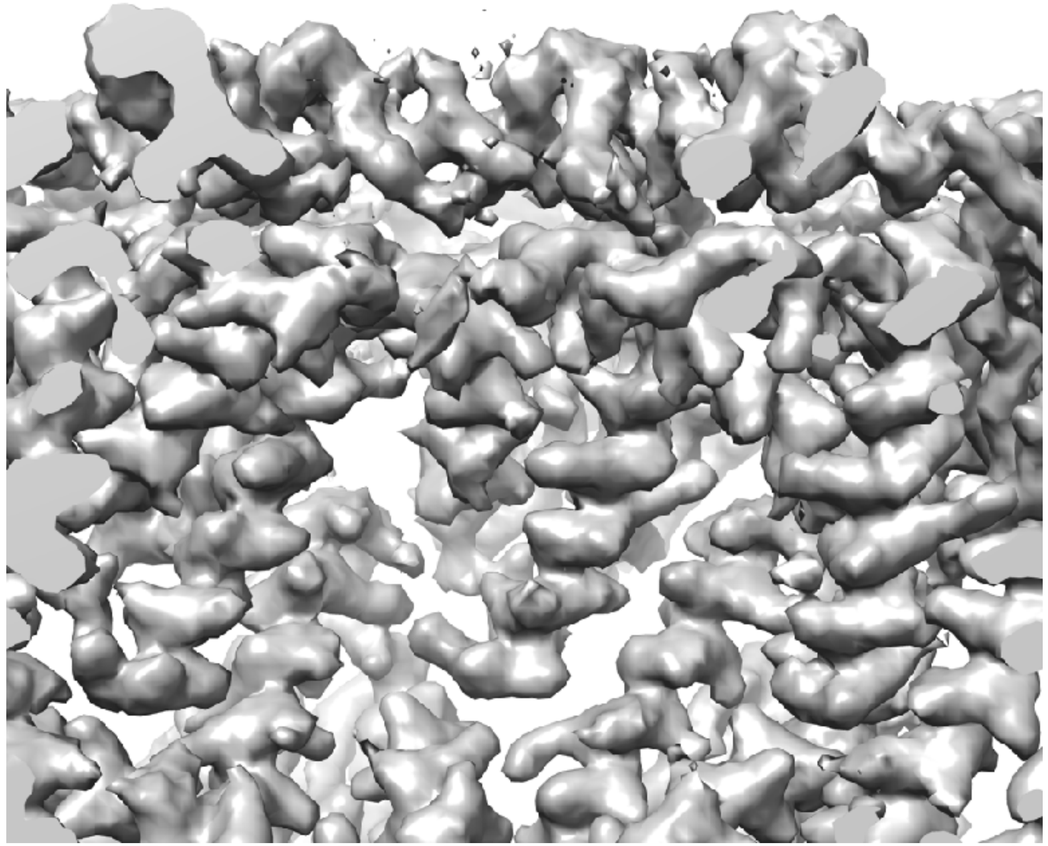
3D reconstruction from raw particles (~3Å resolution, C1 symmetry), sharpened by B-factor -40Å^2



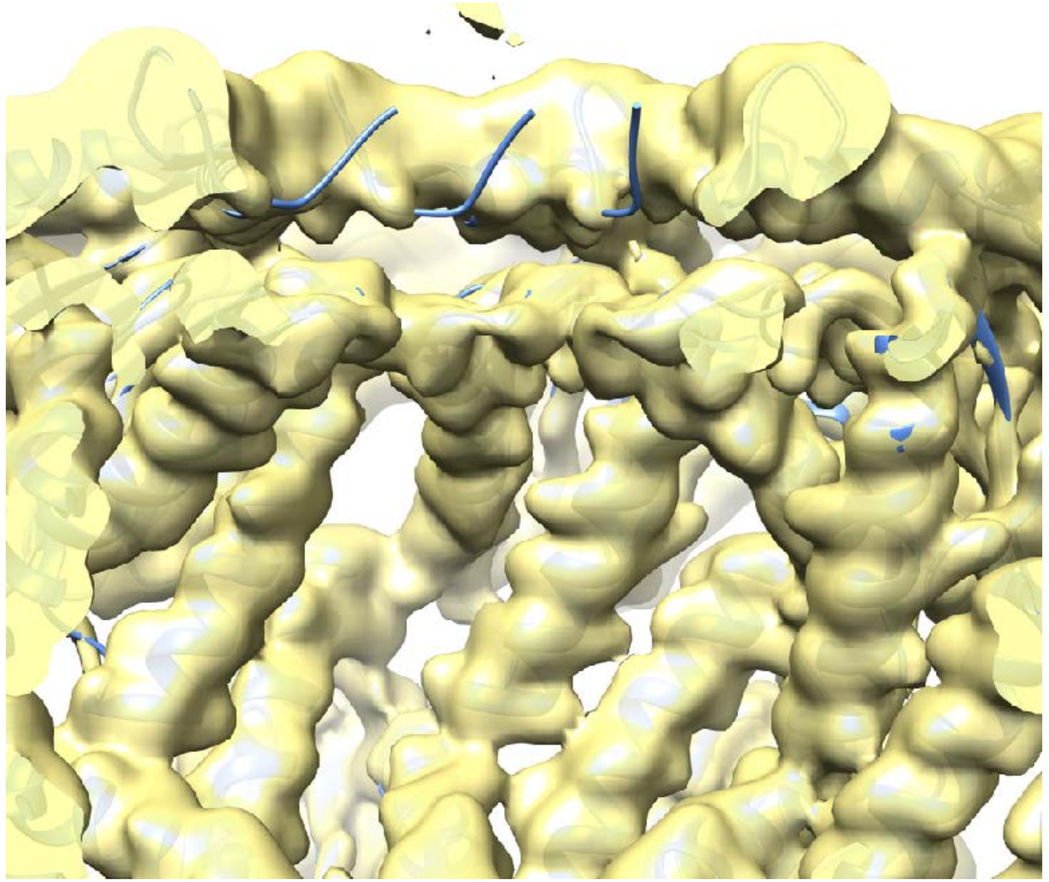
3D reconstruction from denoised particles, using orentation parameter determined from raw particles (~3Å resolution, C1 symmetry), sharpened by B-factor -180Å^2

Palovcak, et al (2020) *IUCrJ*

Can denoised particle being used for recontruction?



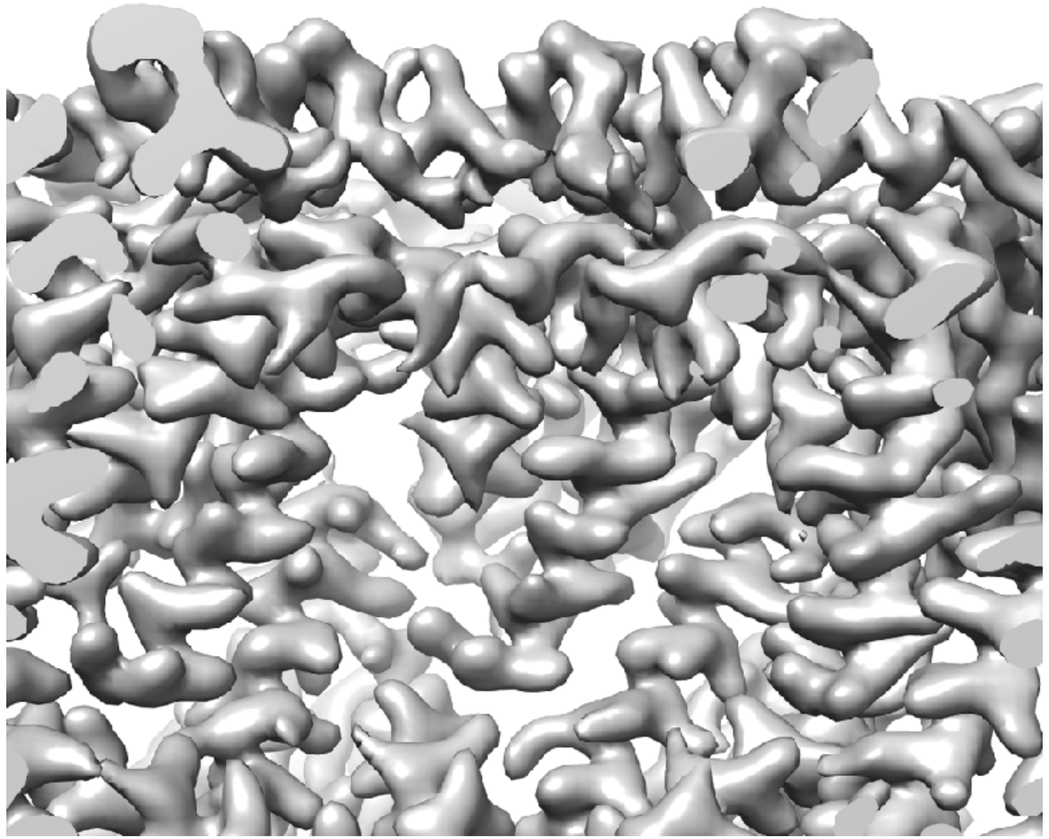
3D reconstruction from raw particles
(~3Å resolution, C1 symmetry)



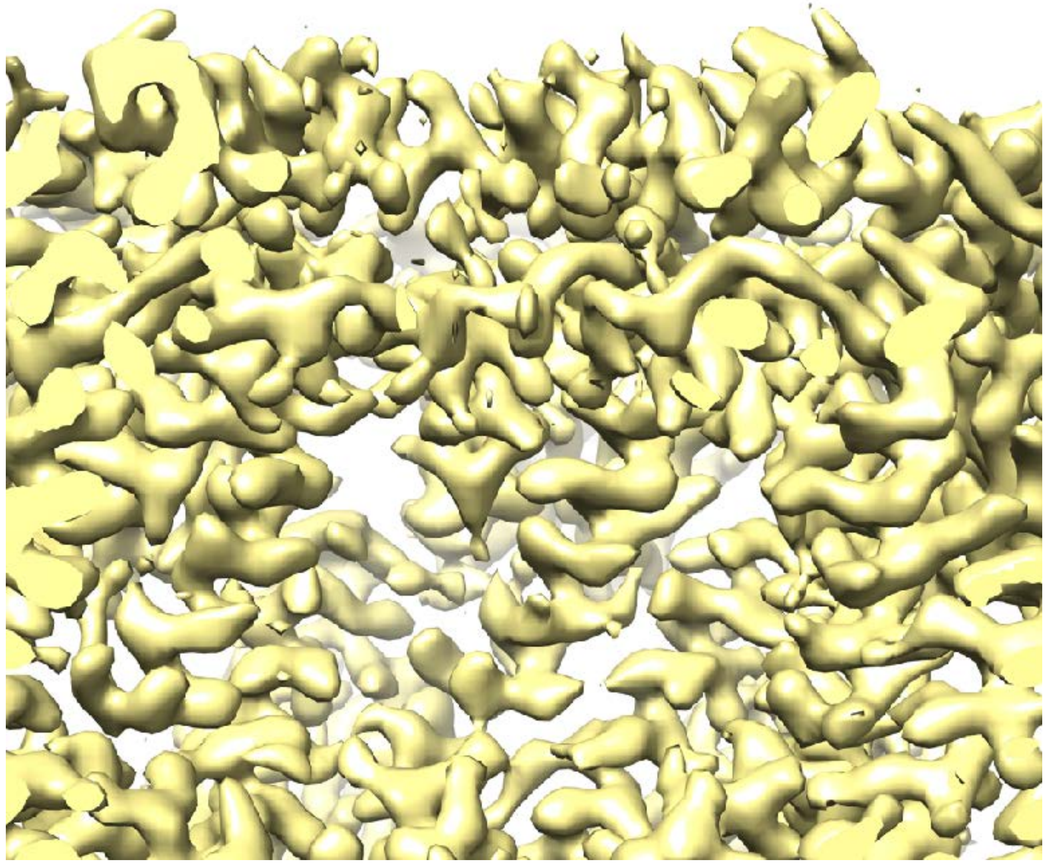
3D reconstruction from denoised particles, using orentation parameter determined from raw particles (~3Å resolution, C1 symmetry)

Palovcak, et al (2020) *IUCrJ*

Can denoised particle being used for recontruction?



3D reconstruction from raw particles
(~3Å resolution, C1 symmetry),
sharpened by B-factor -40Å^2



3D reconstruction from denoised particles,
using orentation parameter determined from
raw particles (~3Å resolution, C1 symmetry),
sharpened by B-factor -180Å^2

Palovcak, et al (2020) *IUCrJ*

Use molecular EM in your own research

• exam the quality of your purification
• verify your hypothesis
• obtain addition information about your proteins: such as oligomeric status of your protein, formation of complex, etc
• Or if you are really really serious, get a high resolution structure by cryoEM!

Facility at UCSF:
Keck Advanced Microscopy Laboratory
Yifan Cheng - S472B (ycheng@ucsf.edu)
David Agard - S412D (agard@msg.ucsf.edu)
Adam Frost - S472F (Adam.Frost@ucsf.edu)