Cryo-EM Movie-Mode Imaging with Direct Detection Cameras

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Outline

Introduction to Direct Detectors

Performance Comparison

Movie-Mode Processing

Motion Correction

Damage Compensation

Example Result

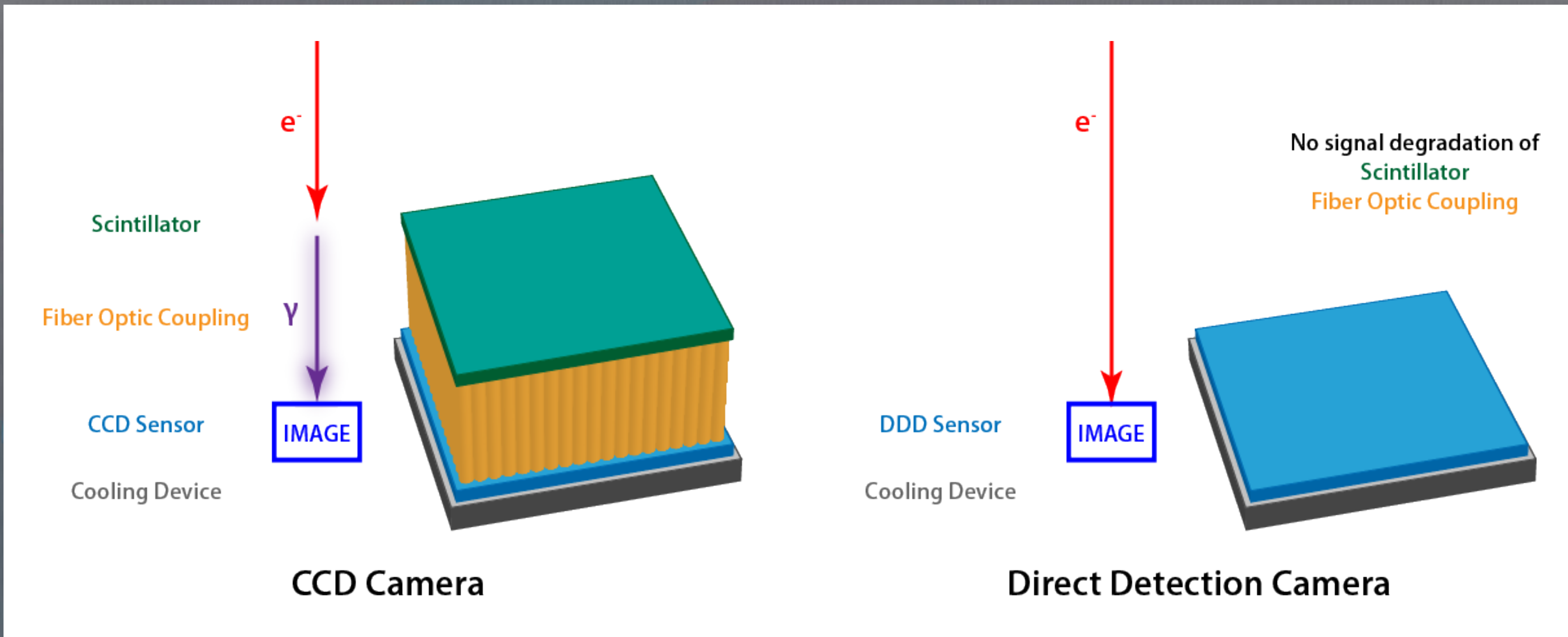
Introduction to Direct Detectors

We Need to Better Detectors

"Although photographic film has long been the standard to beat (especially for 300 keV electrons), it leaves much room for improvement in terms of detective quantum efficiency under low-exposure conditions. New types of area detectors that are currently being developed for EM not only improve on the readout speed of CCD cameras but also promise to improve the point-spread function (i.e., resolution) relative to the pixel size of the detector."

Glaeser & Hall, *Biophys J* **100** (2011), 2331-2337.

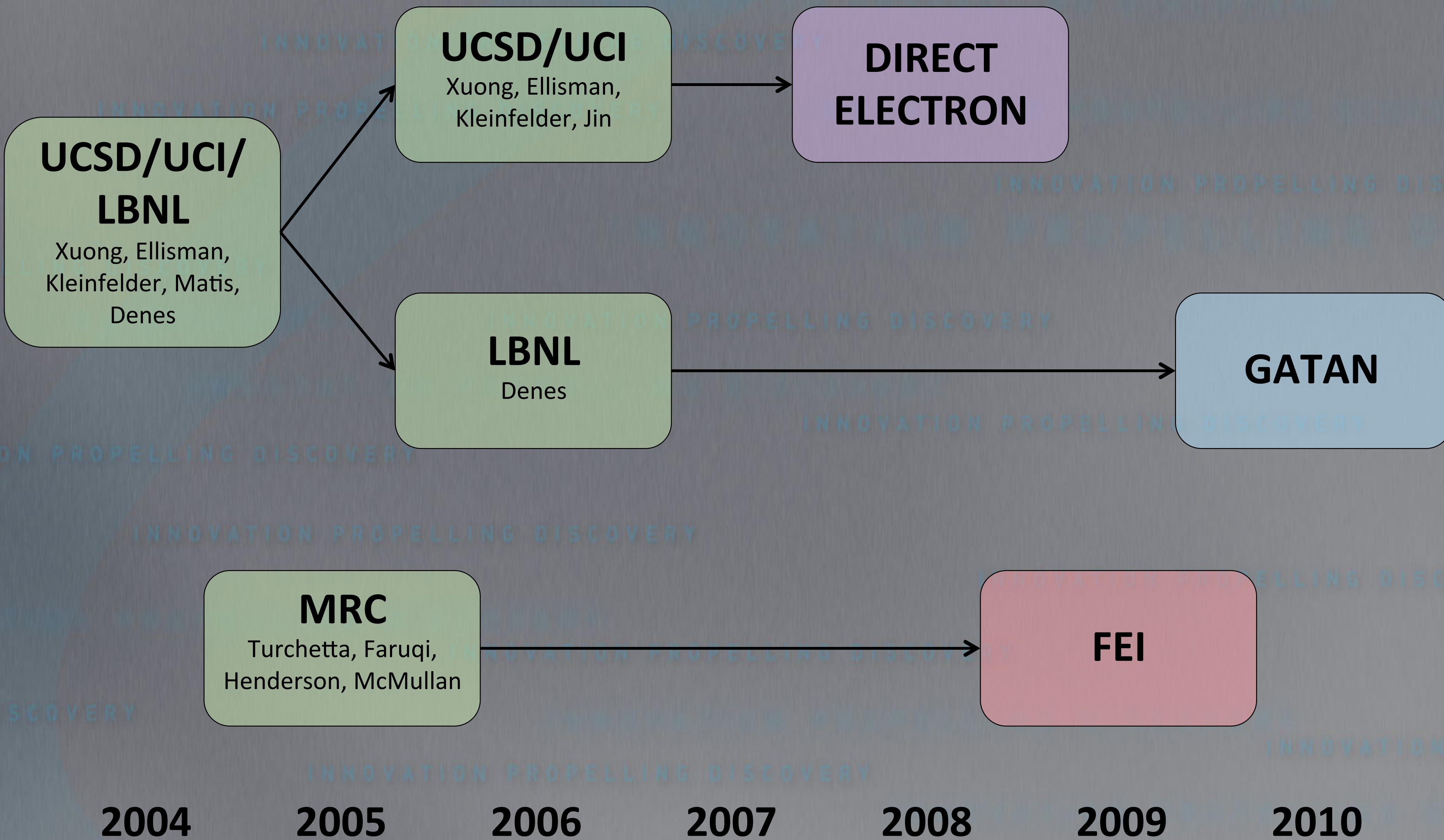
Principle of Direct Detection



TRADITIONAL TEM DIGITAL IMAGING.
Performance is limited by the scintillator and fiber optic coupling.

NEXT GENERATION TEM IMAGING.
High-performance direct detection of primary electrons.

TEM CMOS APS Development



Dates show first academic publications (green) or first commercial product announcements/release.

DDD Development History

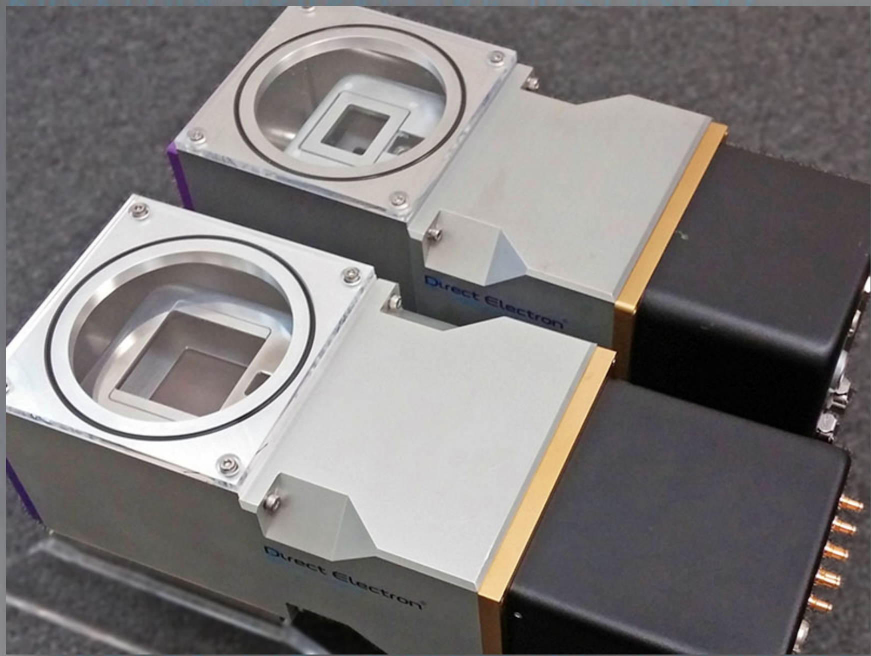
Over a decade of continuing development, in partnership with academia and the NIH.

Generation	1	2	3	4	5	6	7	8	9	10
Year	2002	2002	2003	2005	2006	2008	2010	2011	2012	2014
Pixel Size (µm)	20	5, 10, 20, 30	5	5	5	6	6	6	6.4	6.5
Array Size (pixels)	128 × 128	Various	550 × 512	1024 × 1024	560 × 460	4096 × 3072	4096 × 3072	4096 × 3072	5120 × 3840	8192 × 8192
Pixel Design	3T	3T	3T	3T	3T	> 3T	> 3T	> 3T	> 3T	> 3T
Feature/ Milestone	4 quad	4 sections	Single pixel design; MTF/DQE	Larger format; cryo-tomo	ADC per column; electron counting	Large format	Faster; more radiation hard	Thinned; improved at 200 kV	Larger; reduced noise; improved MTF	Ultra-large format; improved SNR, dynamic range, and radiation hardness
Developer/ Funding	University of California, San Diego NIH RR018841					Direct Electron, LP NIH RR024964 & NIH GM103417				

Overview of DE-Series Cameras

- Direct detection of primary electrons from 80 keV to 1.25 MeV
- Continuous streaming with user-adjustable frame rate and full access to raw data
- 6 – 6.5 µm pixel pitch, with >3T pixel design (CDS)

	DE-12	DE-20	DE-16	DE-64
Generation	8	9	10	10
Pixel Size (µm)	6.0	6.4	6.5	6.5
Array Size (pixels)	4096 × 3072	5120× 3840	4096 × 4096	8192 × 8192
Maximum Frame Rate (full frame)	40	32	60	40



Unique, Industry-Leading Features

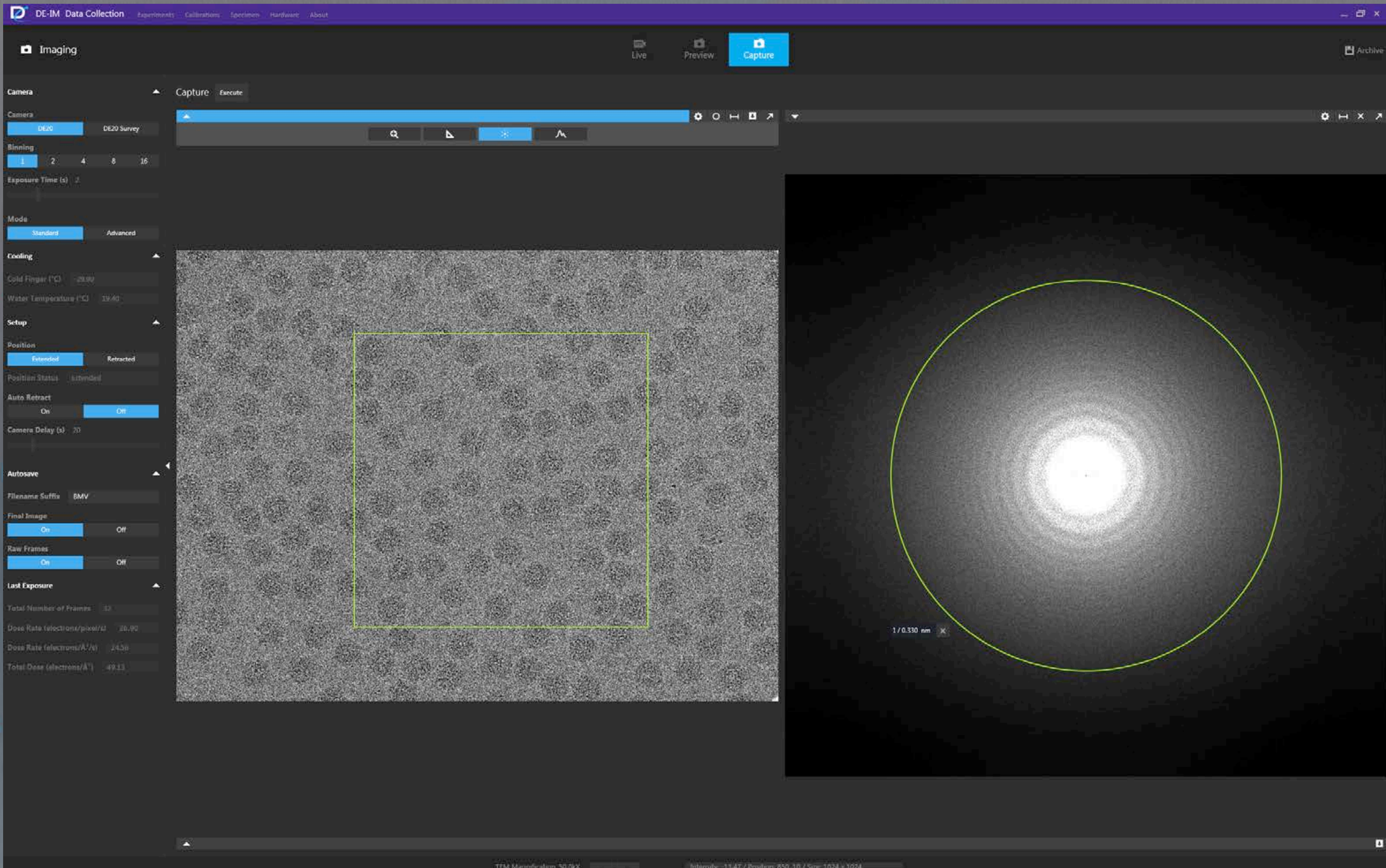
	Direct Electron	Alternative 1	Alternative 2
Pixel Size (μm)	6.0 – 6.5	14	5
Array Size (pixels)	Various sizes up to 8192 × 8192	4096 × 4096 (*17% smaller than DE-20; 300% smaller than DE-64*)	3838 × 3710 (*38% smaller than DE-20; 370% smaller than DE-64*)
Pixel Design	>3T (CDS to reduce noise)	3T (High noise)	3T (High noise)
Frame Shutter	Global or rolling	Rolling (Introduces gradients across images)	Rolling (Introduces gradients across images)
Backthinned	Yes	Yes	Yes
Radiation Hardness	Excellent	Excellent	Excellent
Optimal Exposure Rate	Consistent performance at any exposure rate	Worse performance at low exposure rates	Only very low exposure; < 3 e^-/pixel/s is optimal; e.g., at 10 e^-/pixel/s performance drops ~30%[1]
Dynamic Range	> 400 e^-/pixel/s	~ 160 e^-/pixel/s[2]	At ~60 e^-/pixel/s quantum efficiency decreases ~60%[3]

[1] *Ruskin et al., J Struct Biol 184 (2013).* [2] *McMullan et al., Ultramicrosc 147 (2014).* [3] *Li et al., J Struct Biol 184 (2013).*

Unique, Industry-Leading Features

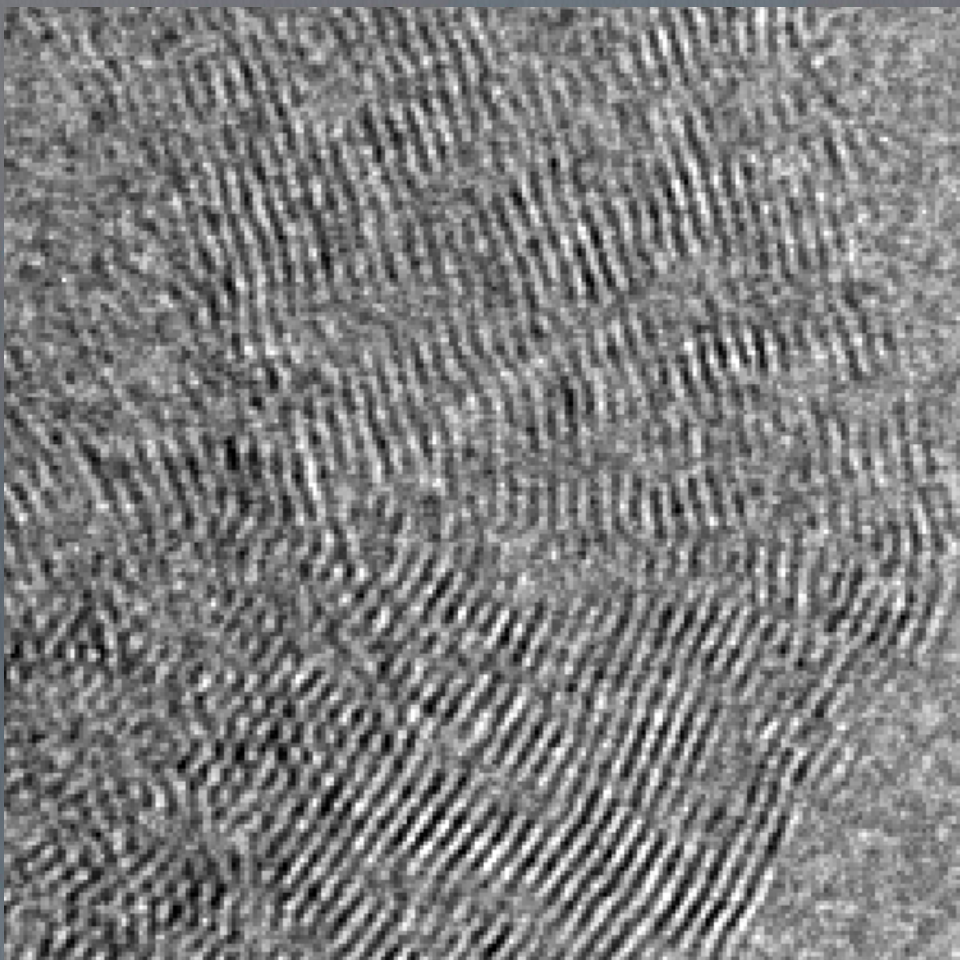
	Direct Electron	Alternative 1	Alternative 2
Frame Rate	Flexible; Up to 30 fps full frame; 1000+ fps for subarrays	18 fps; Typically users may only save 7 frames	40 fps transfer rate to computer
Survey Sensor	Integrated near-axis 2048 × 2048 survey sensor	Separately purchased and maintained	Separately purchased and maintained
Exposure Measurement	Integrated Faraday plate above the DDD sensor	None	None
Sensor Protection Shutter	Yes, patented	None	None
Retractable	Yes, mechanical	Yes	Yes, pneumatic
Mounting Position	Film chamber (JEOL) or standard bottom mount	Bottom mount	Bottom mount
Magnification Factor	Film chamber: 1.0× Bottom mount: ~1.4×	~1.4×	~1.4×
Data Acquisition Software	Multiple options; Open API for integration	Proprietary	Proprietary
Movie Processing Software	Flexible and open-source; patented algorithms	Minimal	Proprietary

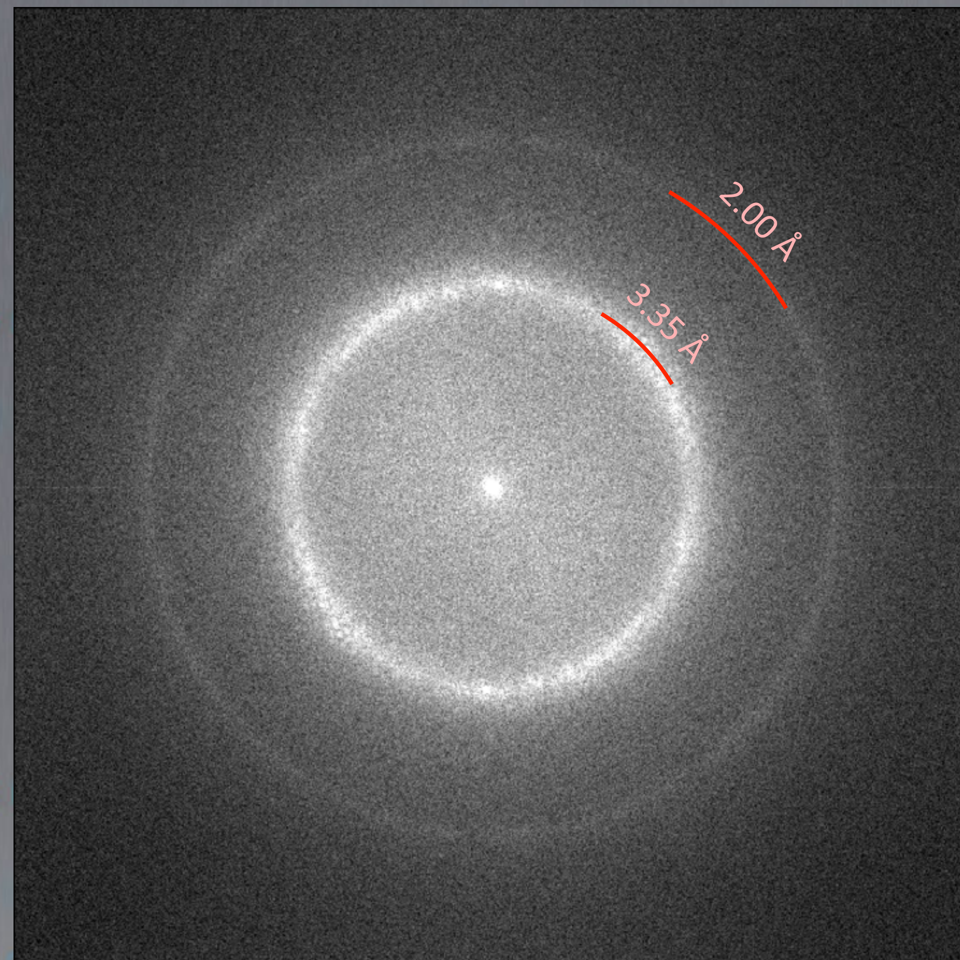
Direct Electron Imaging Manager (DE-IM)



Graphitized Carbon

56,000× nominal magnification (0.71 Å/pixel).

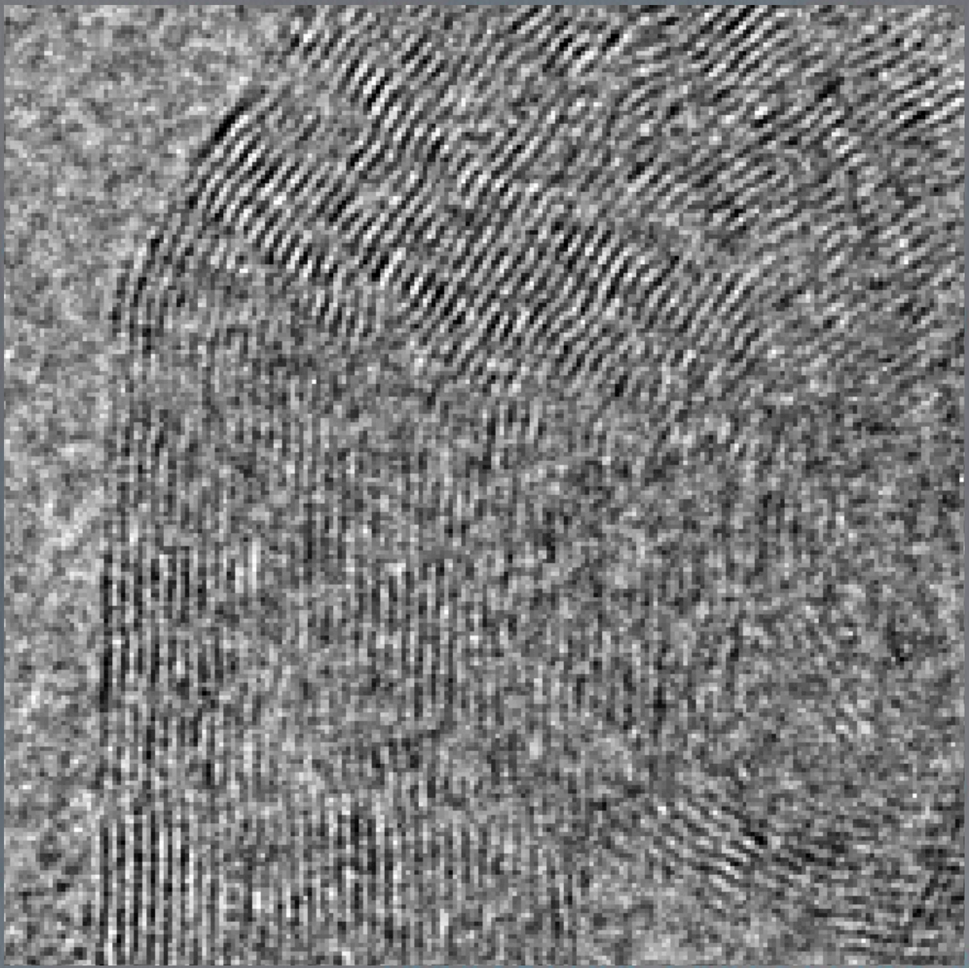


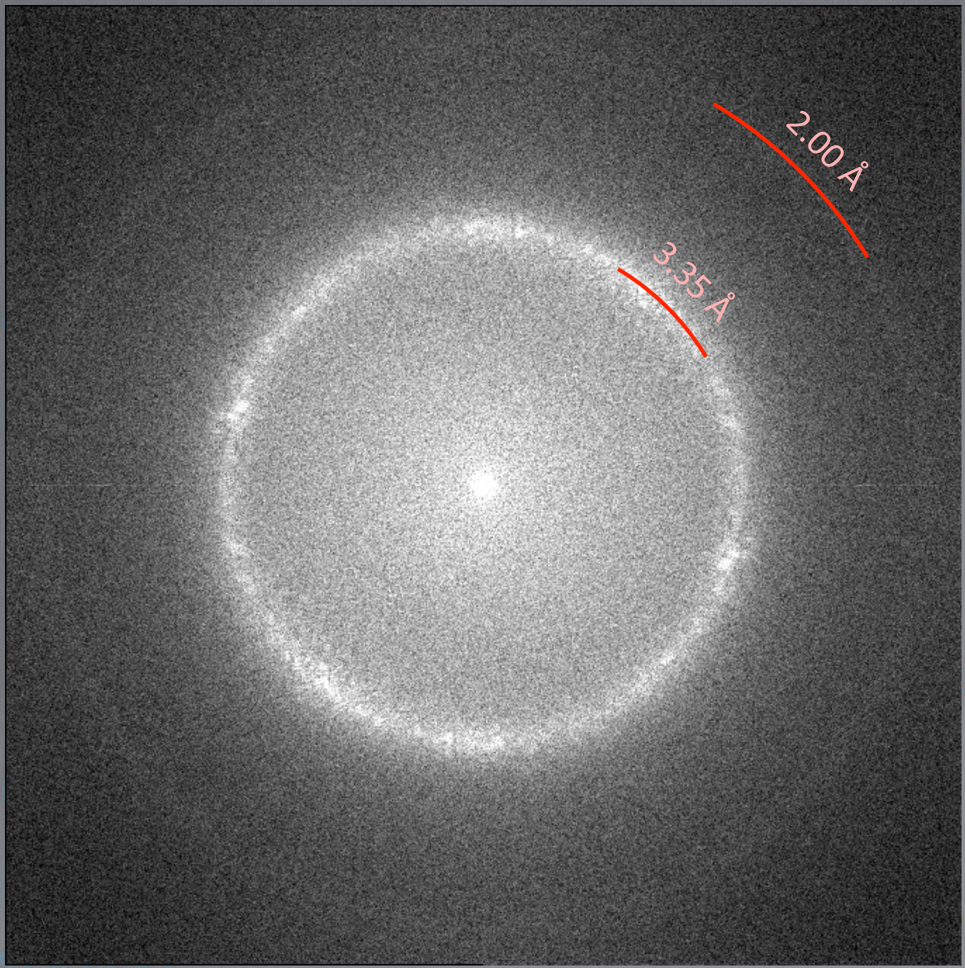


Collected on a FEI Titan with a DE-12 Camera.

Graphitized Carbon

43,000× nominal magnification (0.91 Å/pixel).

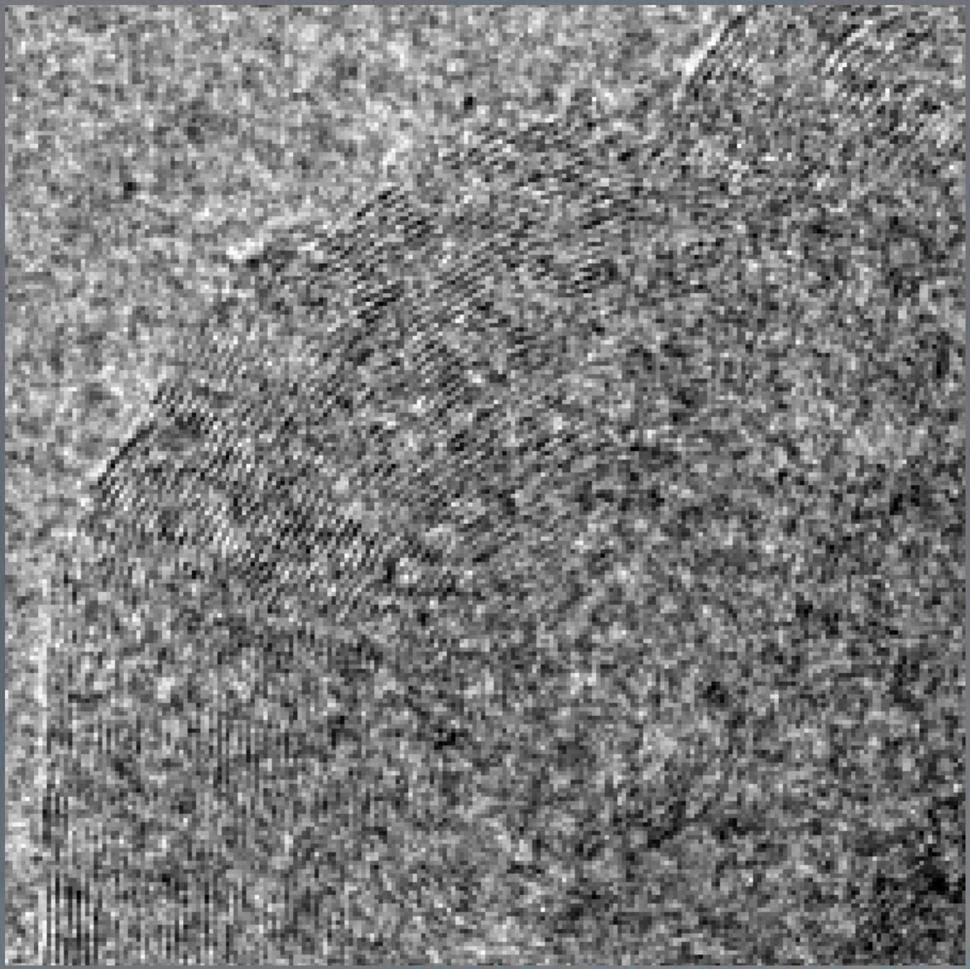


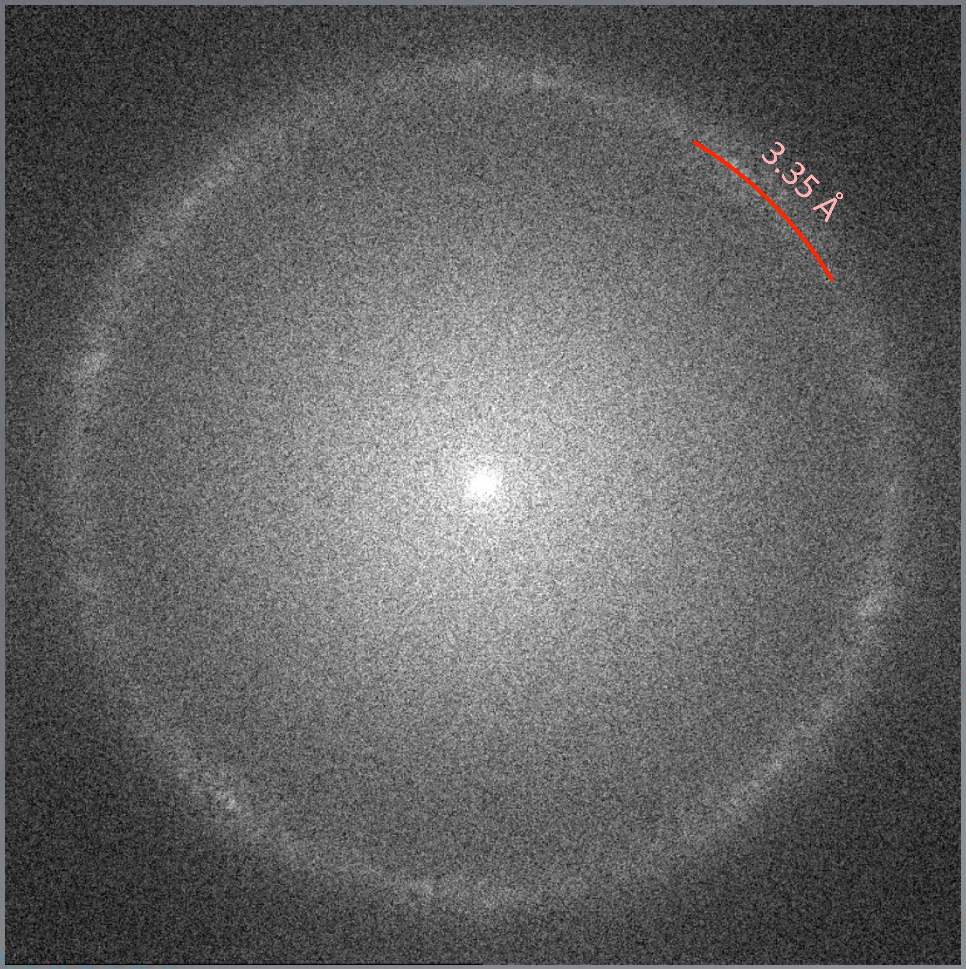


Collected on a FEI Titan with a DE-12 Camera.

Graphitized Carbon

27,000× nominal magnification (1.78 Å/pixel).

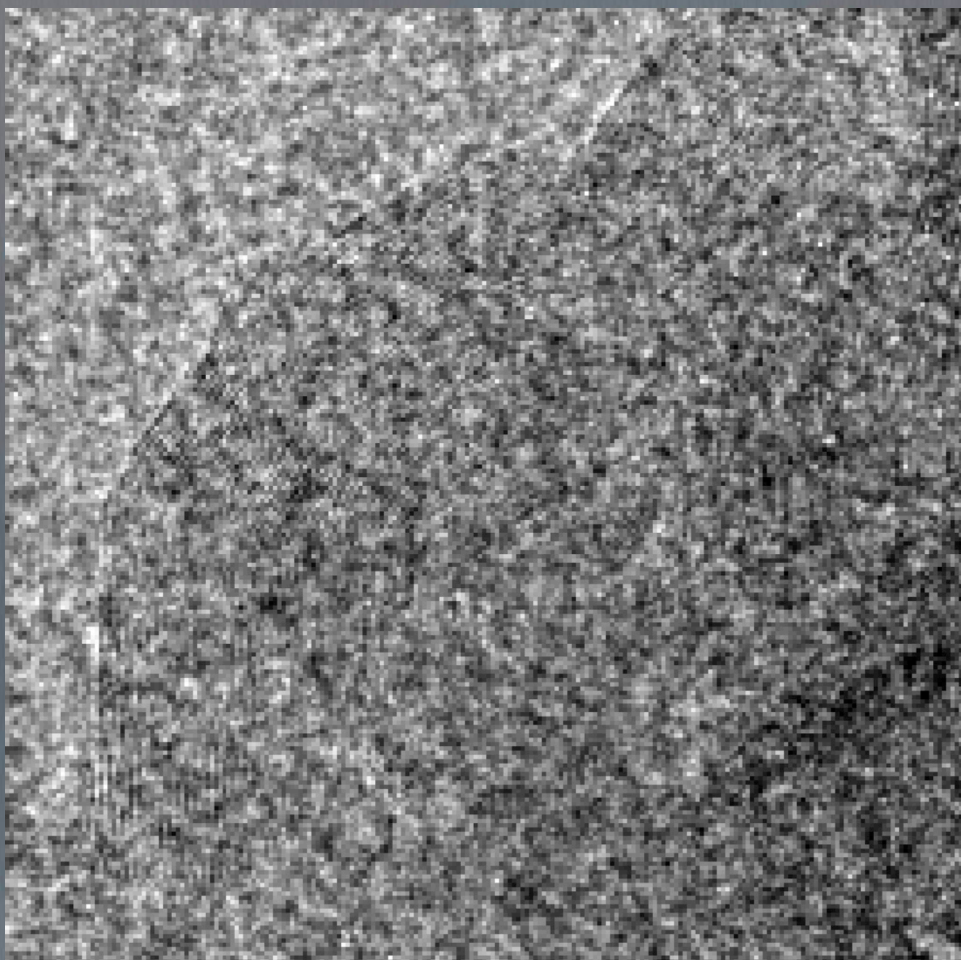




Collected on a FEI Titan with a DE-12 Camera.

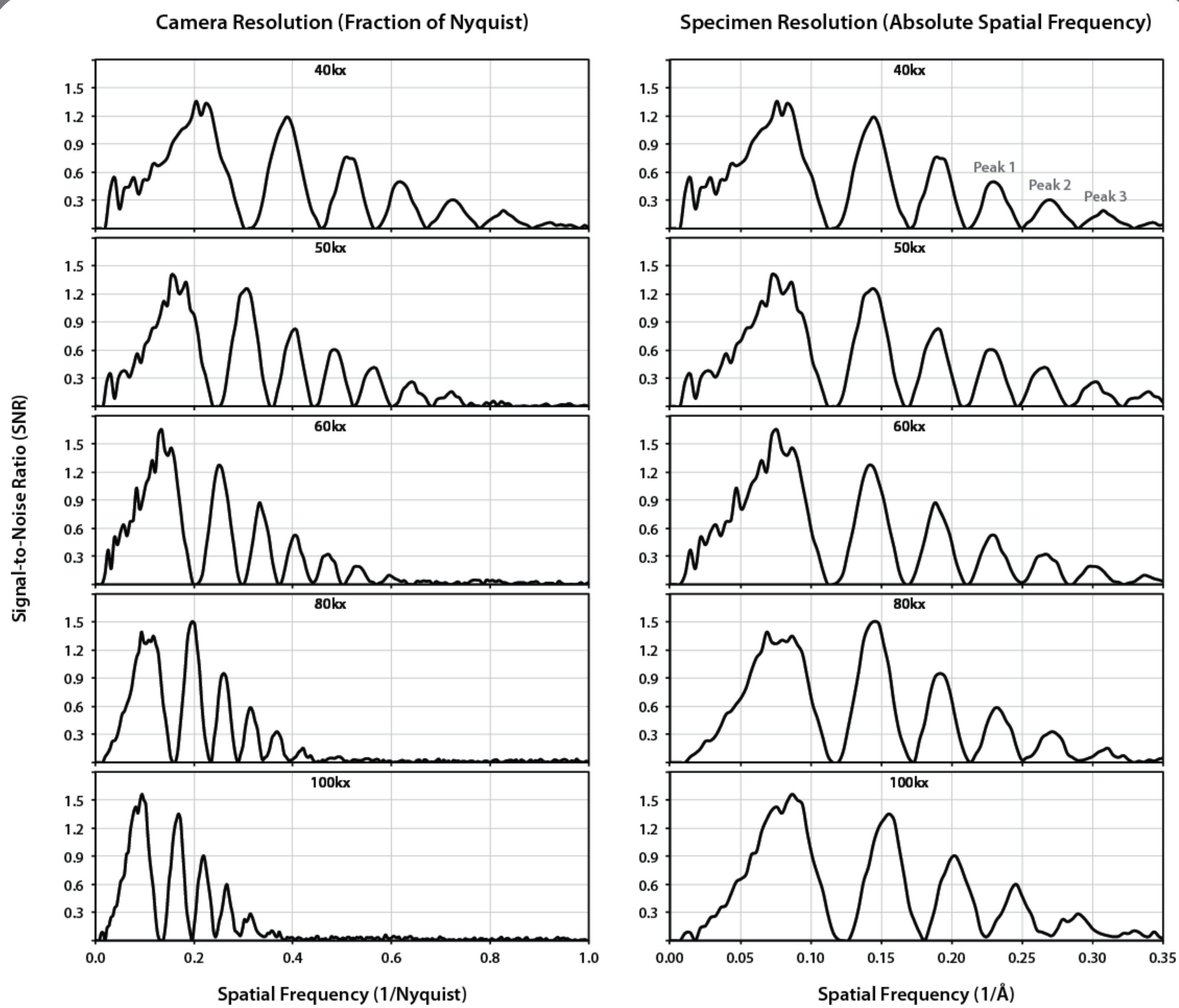
Graphitized Carbon

21,000× nominal magnification (1.82 Å/pixel).



Collected on a FEI Titan with a DE-12 Camera.

Consistent High-Resolution Performance



High-resolution (e.g., 3.5 Å) SNR is maintained at at nearly the same level at 40kx mag. (80% Nyquist) as it is at 100kx mag (30% Nyquist). This indicates a consistent high-resolution performance, meaning that you can image at lower magnification (for a larger field-of-view) *without* sacrificing high-resolution SNR.

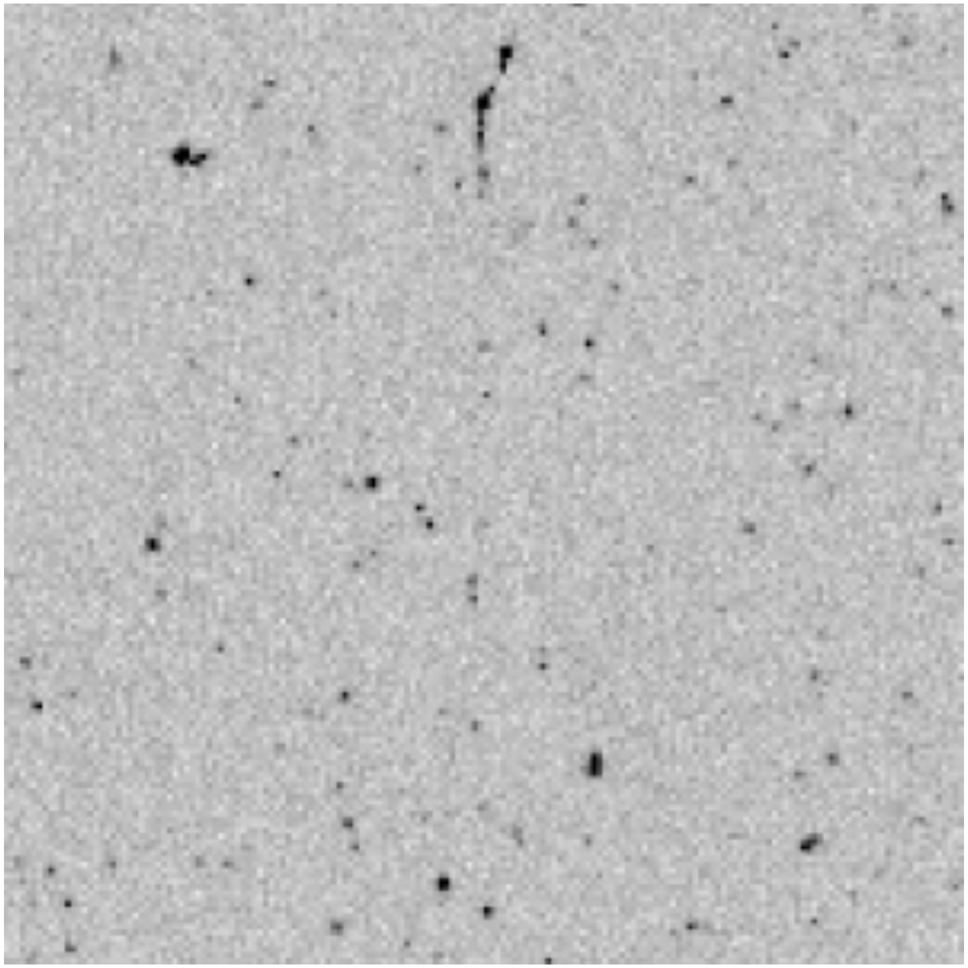
Lower magnification means a larger field-of-view!

Performance Comparison

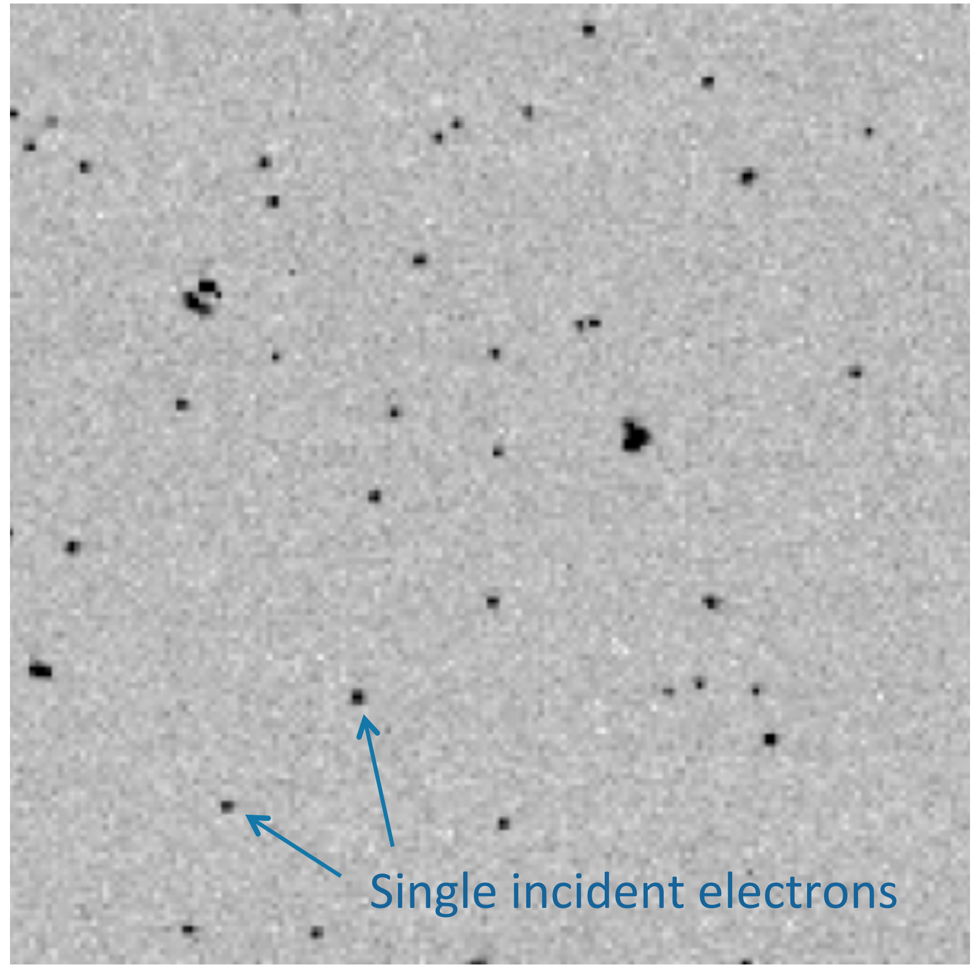
SNR from Each Incident Electron

2.5× improvement!

Competing Direct Detector (SNR=19.6)

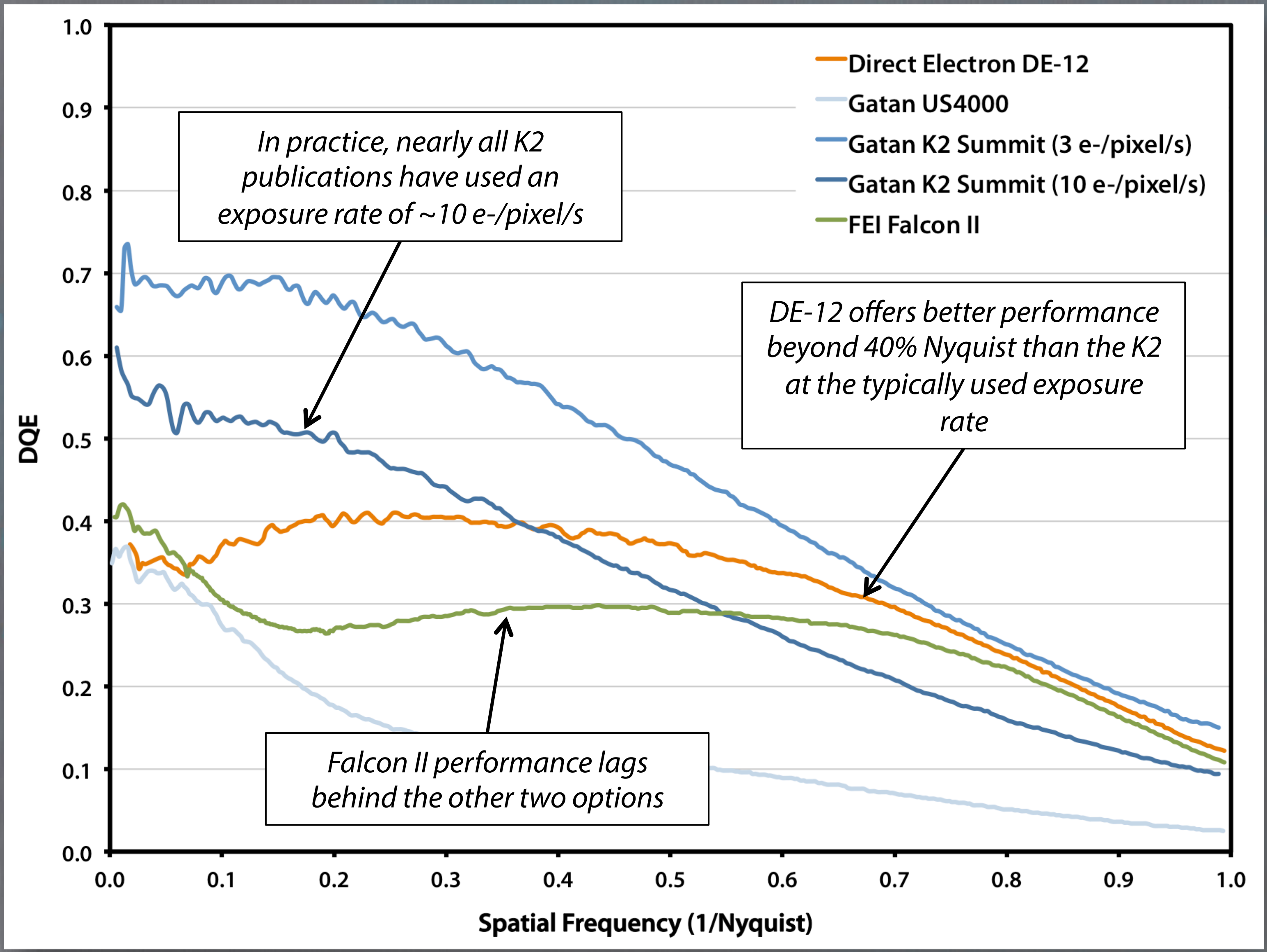


Direct Electron DE-20 (SNR=49.6)



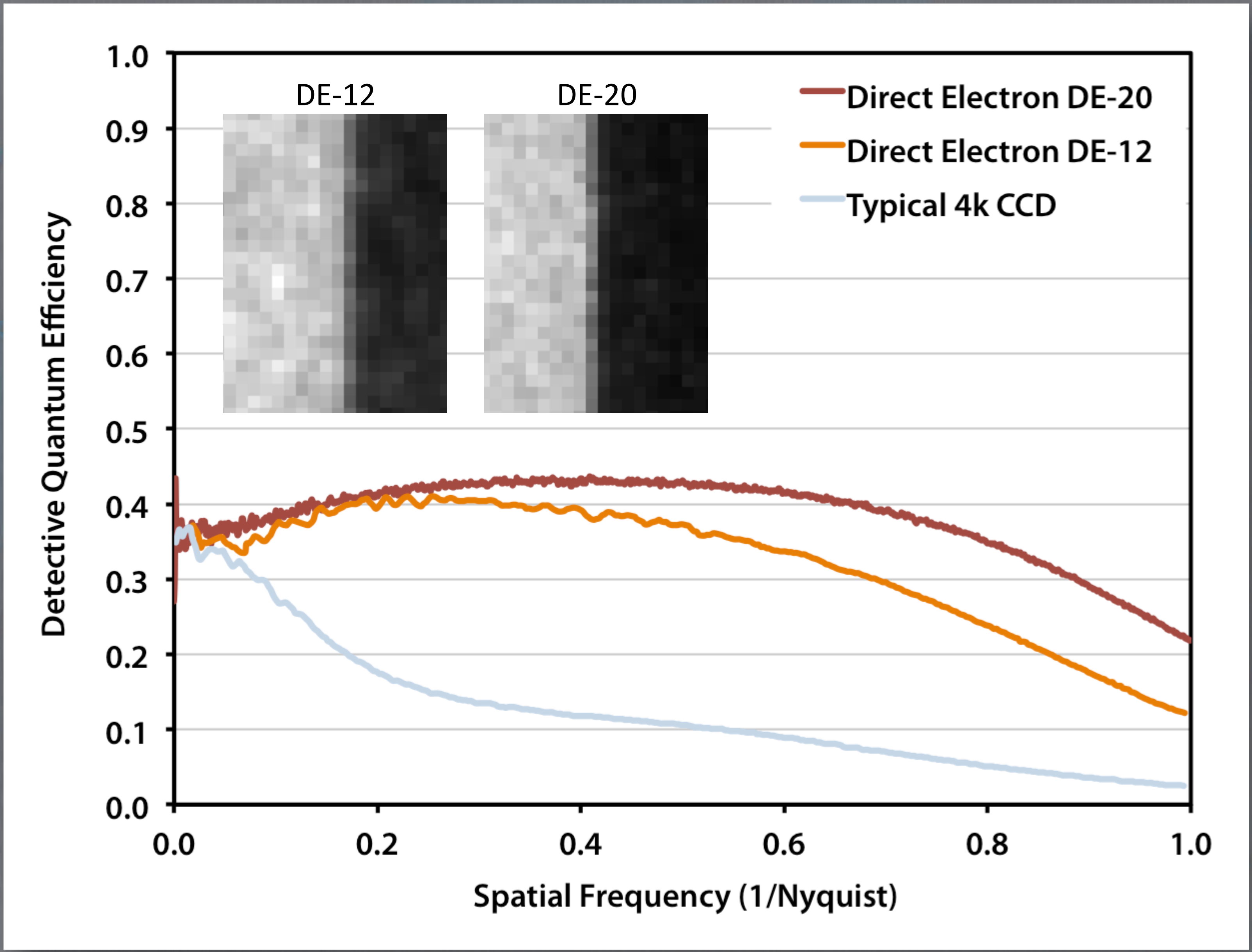
Courtesy of Greg McMullan (MRC) and Dan Clare (Birkbeck).

DQE Comparison (200 kV)



Curves from Ruskin et al., J Struct Biol 184 (2013).

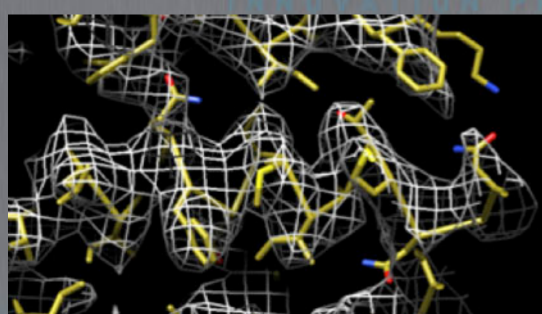
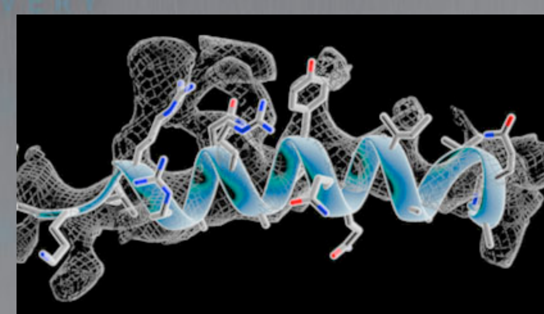
DQE Improvement from DE-12 to DE-20



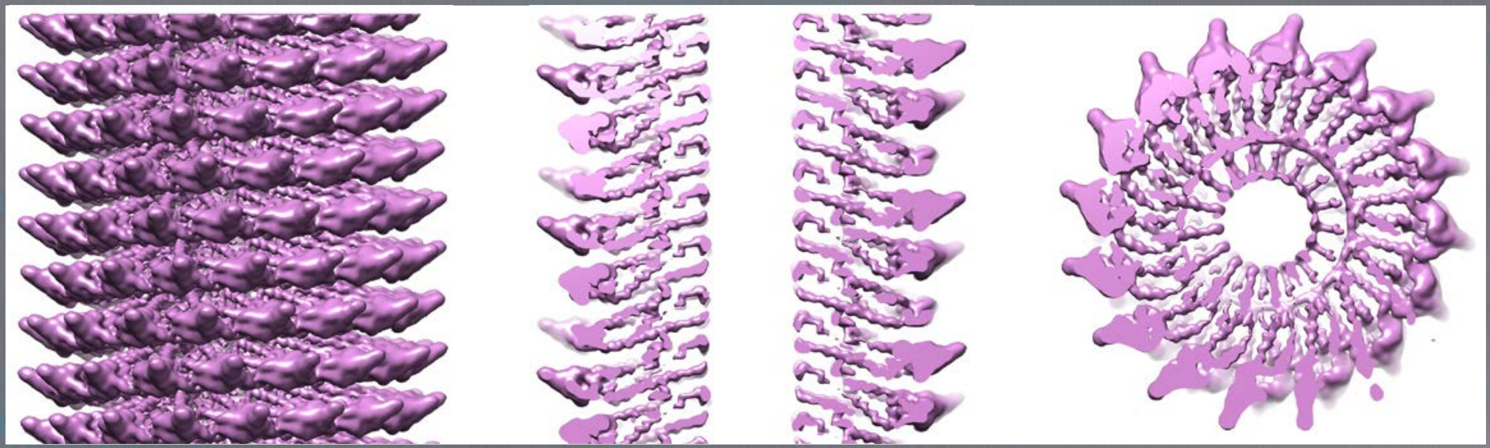
CCD and DE-12 curves from Ruskin et al., J Struct Biol 184 (2013). DE-20 curve calculated independently. Results for 200 keV electrons.

Published Virus Reconstructions (200 kV)

RED indicates differences between the two experiments, either worse results or experimental parameters that should have generated better results.

	Direct Electron DE-12 Campbell, et al. (2012) *Structure* 20, 1823-8.	**Gatan K2 Summit** Veesler, et al. (2013) *J. Struct. Biol.* 184, 193-202.
Sensor Size	4096 × 3072 with 6 µm pixels, (Note: DE-12 gen1 was NOT backthinned)	7424 × 7680 (super-resolution), backthinned binned by 2× yielding 3712 × 3840 with 5 µm pixels
Magnification	1.42 Å/pixel (42,135× magnification)	1.21 Å/pixel (41,322× magnification)
Exposure Time	0.64 s (saved 16 frames @ 25 fps)	4.00 s (saved 16 frames @ 4 fps)
Dose	32 e^-/Å^2 (rate = 99 e^-/pixel/s)	22 e^-/Å^2 (rate = 8 e^-/pixel/s)
Total Images	561 acquisitions (movies) 1915 particles originally extracted 807 particles used for the final reconstruction	754 acquisitions (movies) 4490 particles originally extracted 4446 particles used for the final reconstruction
Drift Corr.	Per particle drift correction	Drift correction of 2k × 2k regions
Refinement	CTFFIND3 & Frealign	CTFFIND3 & Frealign
Symmetry	Icosahedral (with 13-fold NCS averaging on the coat)	Icosahedral
Resolution	Virus = 6.2 Å (46% Nyquist) / Coat = 4.4 Å (65% Nyquist)	Virus = 6.1 Å (40% Nyquist) / Coat = 4.4 Å (55% Nyquist)
Specimen	Rotavirus DLP (70 MDa)	Sulfolobus turreted icosahedral virus (STIV) (75 MDa)
Example of Visible Side Chains		

Direct Comparison Between Direct Detectors



- Comparison between Direct Electron DE-20 and Gatan K2-Summit.
  - Identical cryo-EM experiment on two different cameras.
- Same microscope (FEI Polara 300 kV).
- Same specimen preparation (TMV).
- Similar imaging conditions and number of particles.
- Same image processing.

Courtesy of Helen Saibil, Elena Orlova, and Dan Clare (Birkbeck University of London).

Direct Comparison Between Direct Detectors

DE-20:

- FEI Polara (300 kV)
- Spot size 6, C2 52.7%
- 39,000x TEM
- Pre-GIF (no energy filter)
- 1.221 Å/pixel
- 25 $e^-/Å^2$ total exposure used for reconstruction
- 15 fps, at 2.1 $e^-/Å^2$/frame
- Exposure time for reconstruction 0.8 s
- DE_process_frames.py with whole frame alignment (quanta 1) and no damage compensation
- 11,000 particle segments boxed and used

K2-Summit:

- FEI Polara (300 kV)
- Spot size 8, C2 51.8%
- 160,000x EFTEM
- Post-GIF with 30 eV energy filter slit
- 0.695 Å/virtual pixel (super-resolution mode),
- 1.390 Å/physical pixel
- 25 $e^-/Å^2$ total exposure used for reconstruction
- 5 fps, at 0.59 $e^-/Å^2$/frame
- Exposure time for reconstruction 8.4 s

Required 10x longer exposure time for an equivalent image

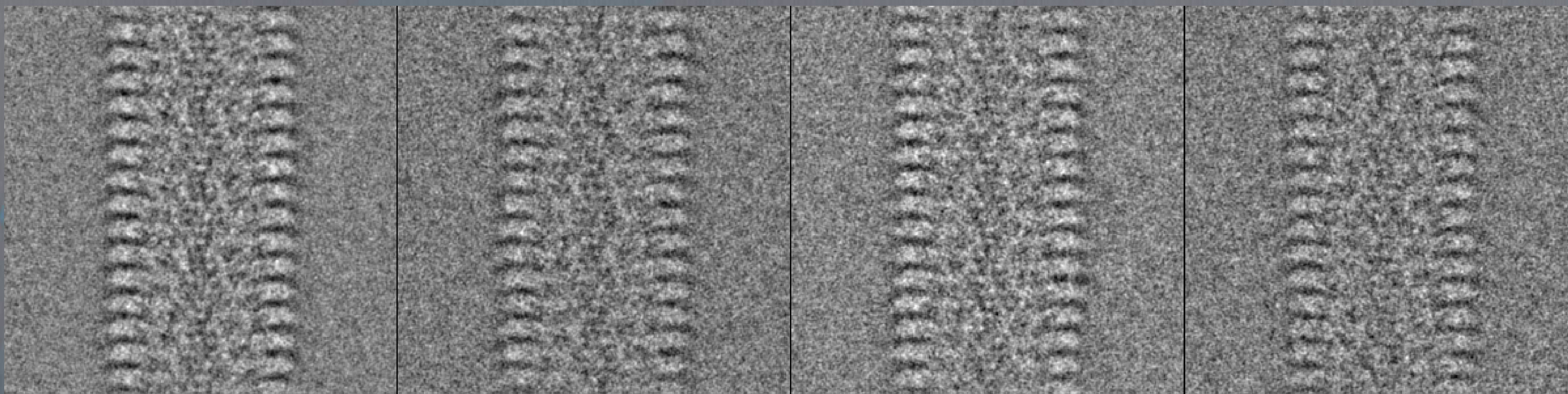
- Tried both IMOD for sub-frame alignment (similar to DE_process_frames.py) and MOTIONCORR from Yifan
- 11,000 particle segments boxed and used

Courtesy of Helen Saibil, Elena Orlova, and Dan Clare (Birkbeck University of London).

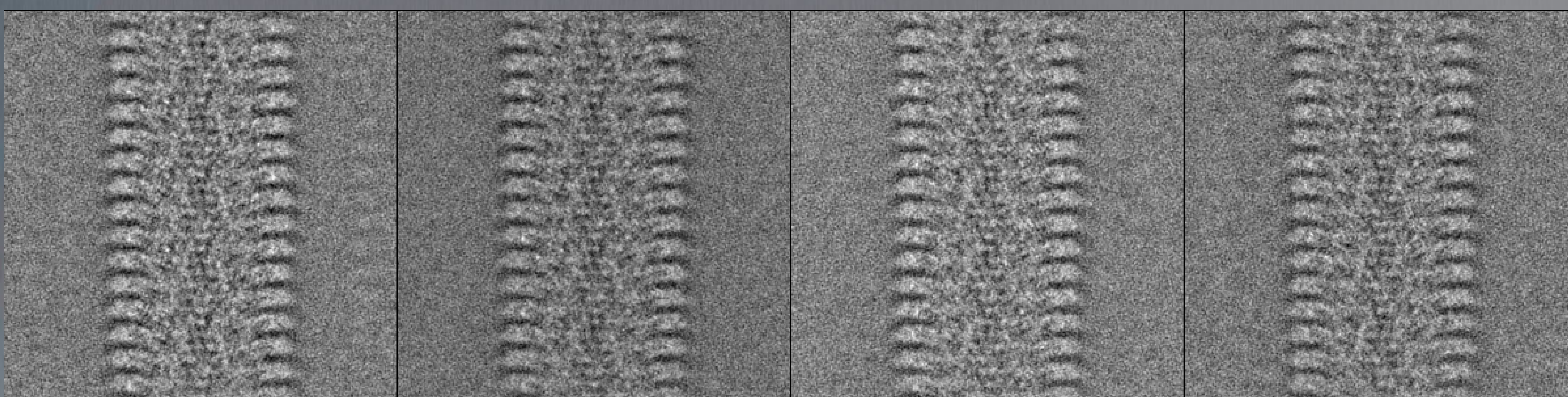
Class Averages

After 6 rounds of alignment

DE-20



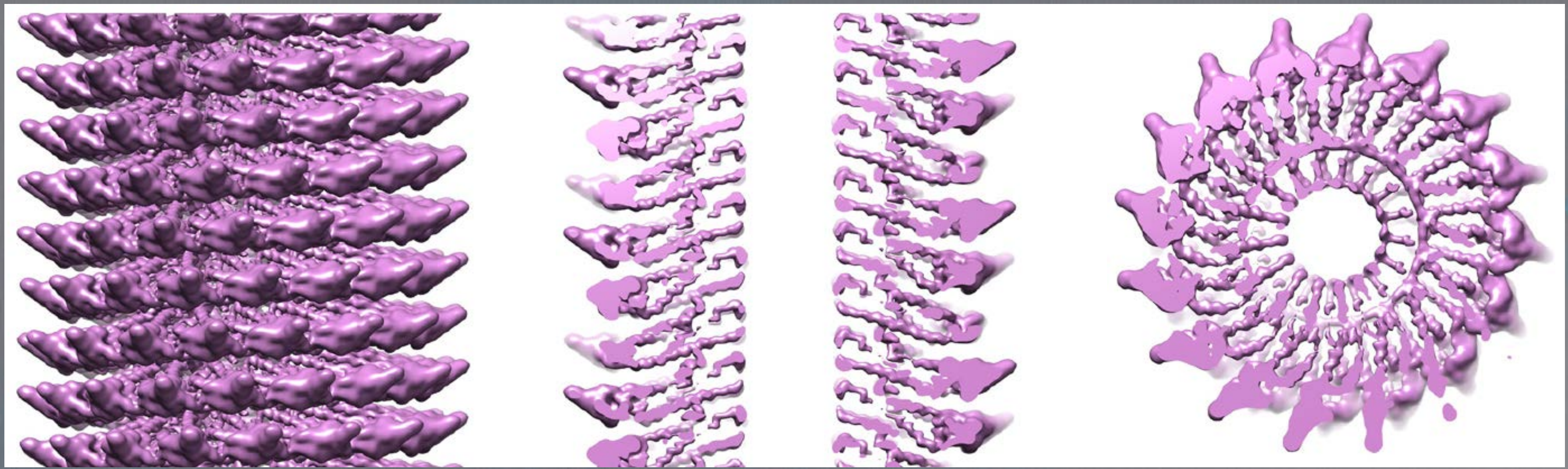
K2-Summit



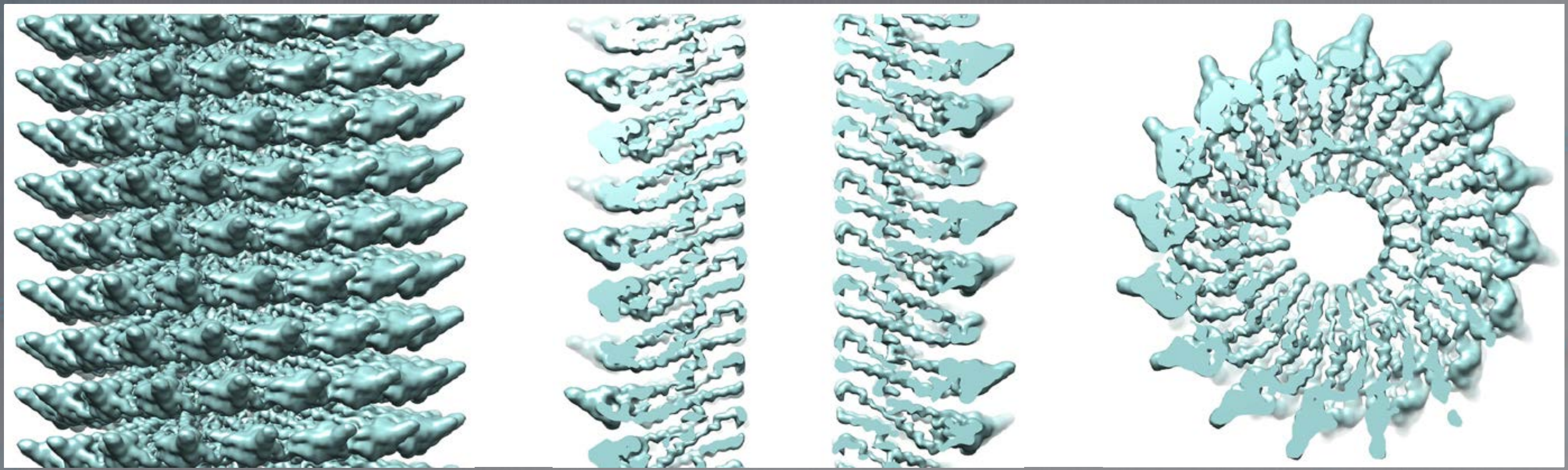
Courtesy of Helen Saibil, Elena Orlova, and Dan Clare (Birkbeck University of London).

3D Reconstructions (Unfiltered)

DE-20



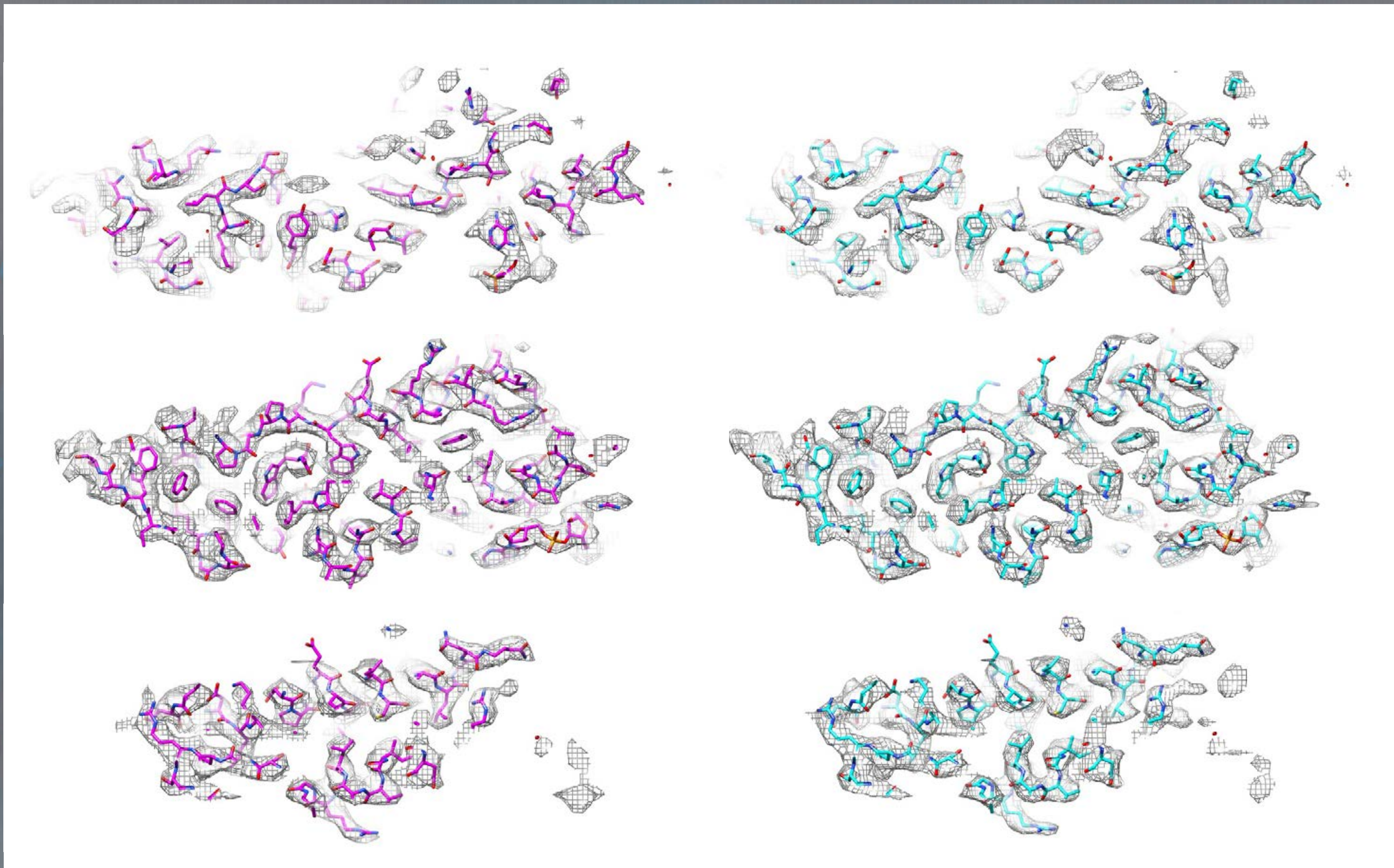
K2-Summit



Courtesy of Helen Saibil, Elena Orlova, and Dan Clare (Birkbeck University of London).

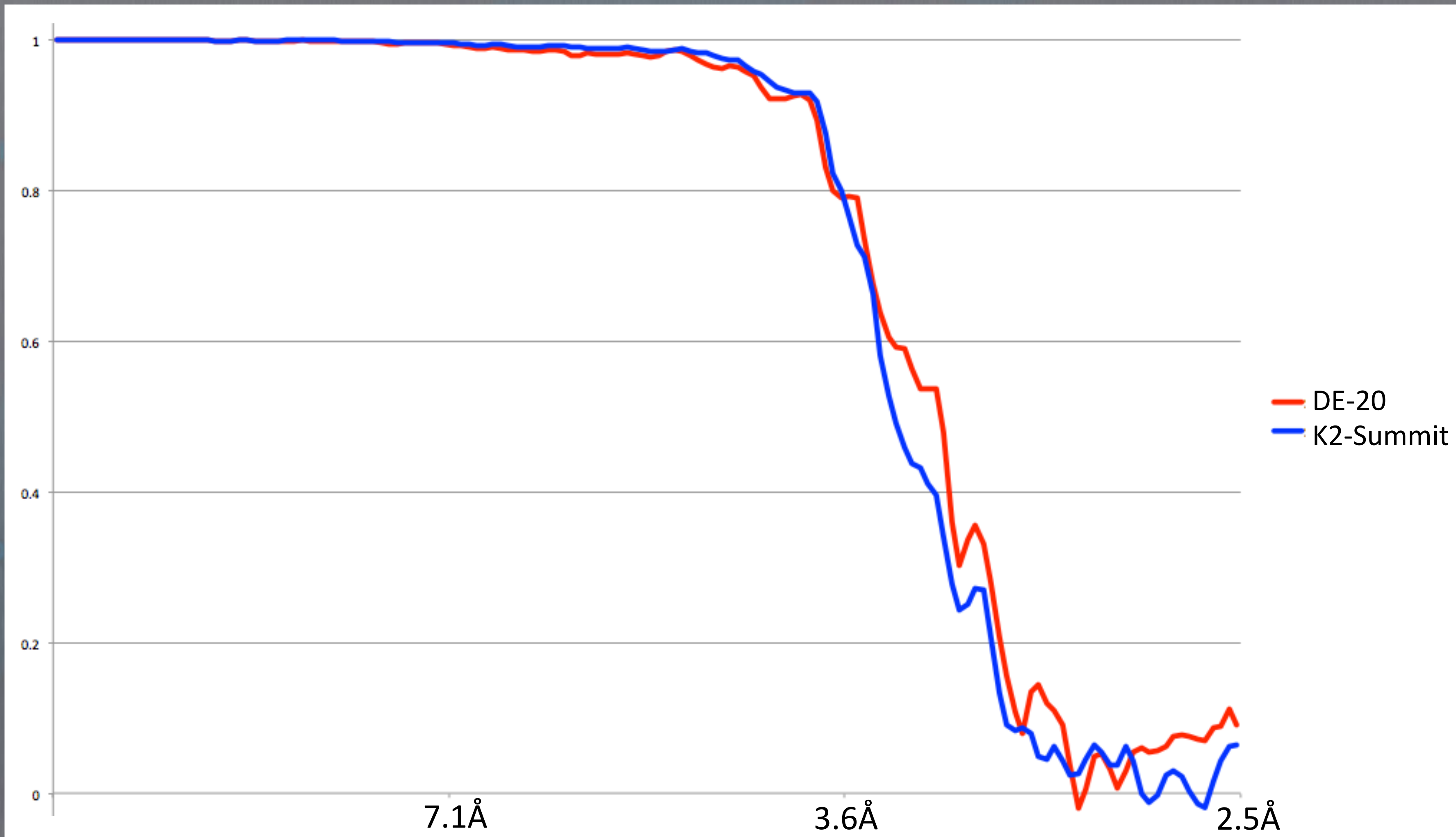
3D Reconstructions (Sharpened)

DE-20 K2-Summit



Courtesy of Helen Saibil, Elena Orlova, and Dan Clare (Birkbeck University of London).

Final Resolution (FSC)

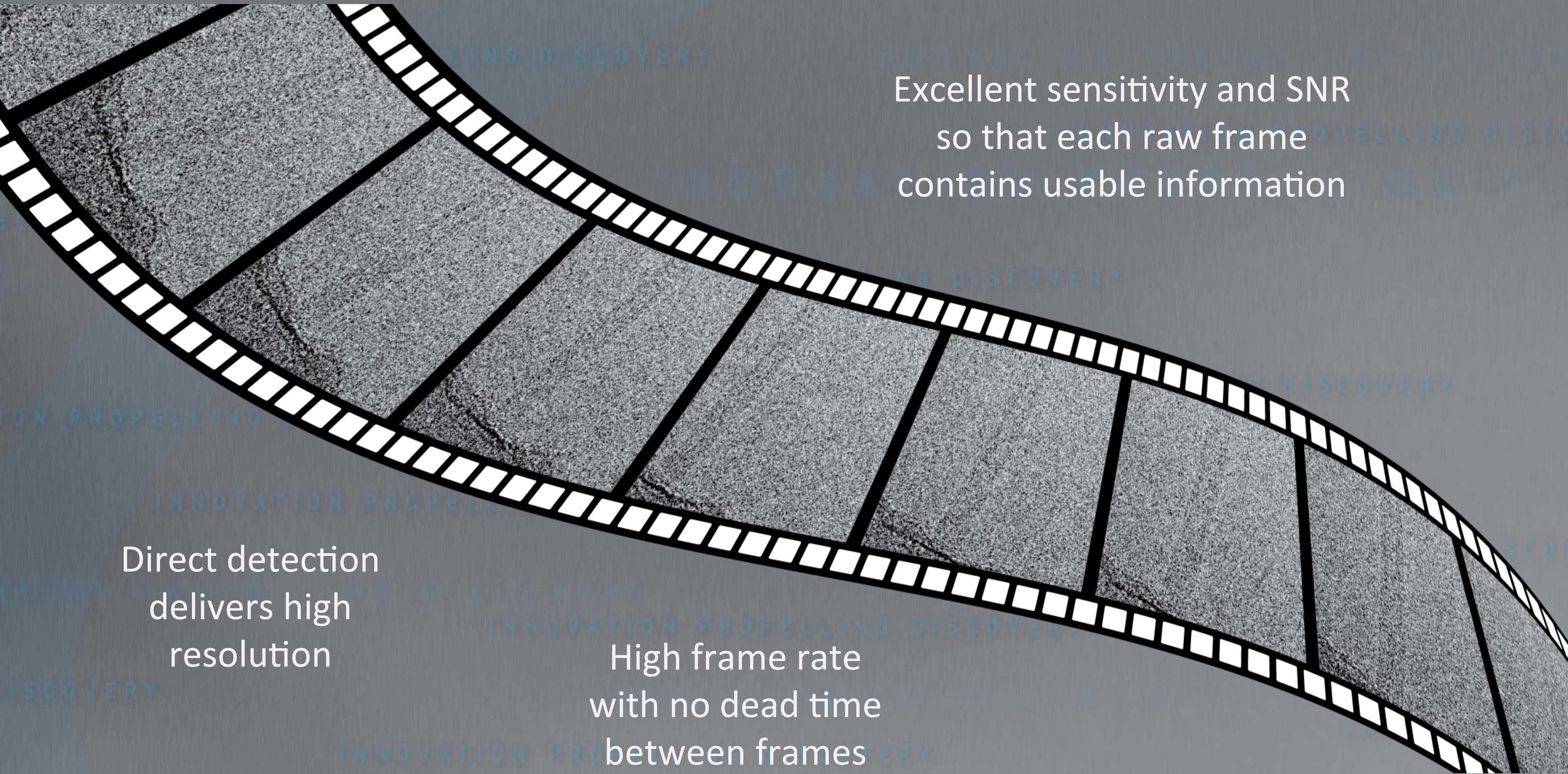


Red: DE-20 0.5 FSC = 3.3 Å (0.143 = 3 Å)
Blue: K2-Summit 0.5 FSC = 3.4 Å (0.143= 3 Å) (processed also at 0.695 Å/pix yielding 3.36 Å at 0.5)

Courtesy of Helen Saibil, Elena Orlova, and Dan Clare (Birkbeck University of London).

Movie-Mode Processing

Collect Movies Instead of Static Images

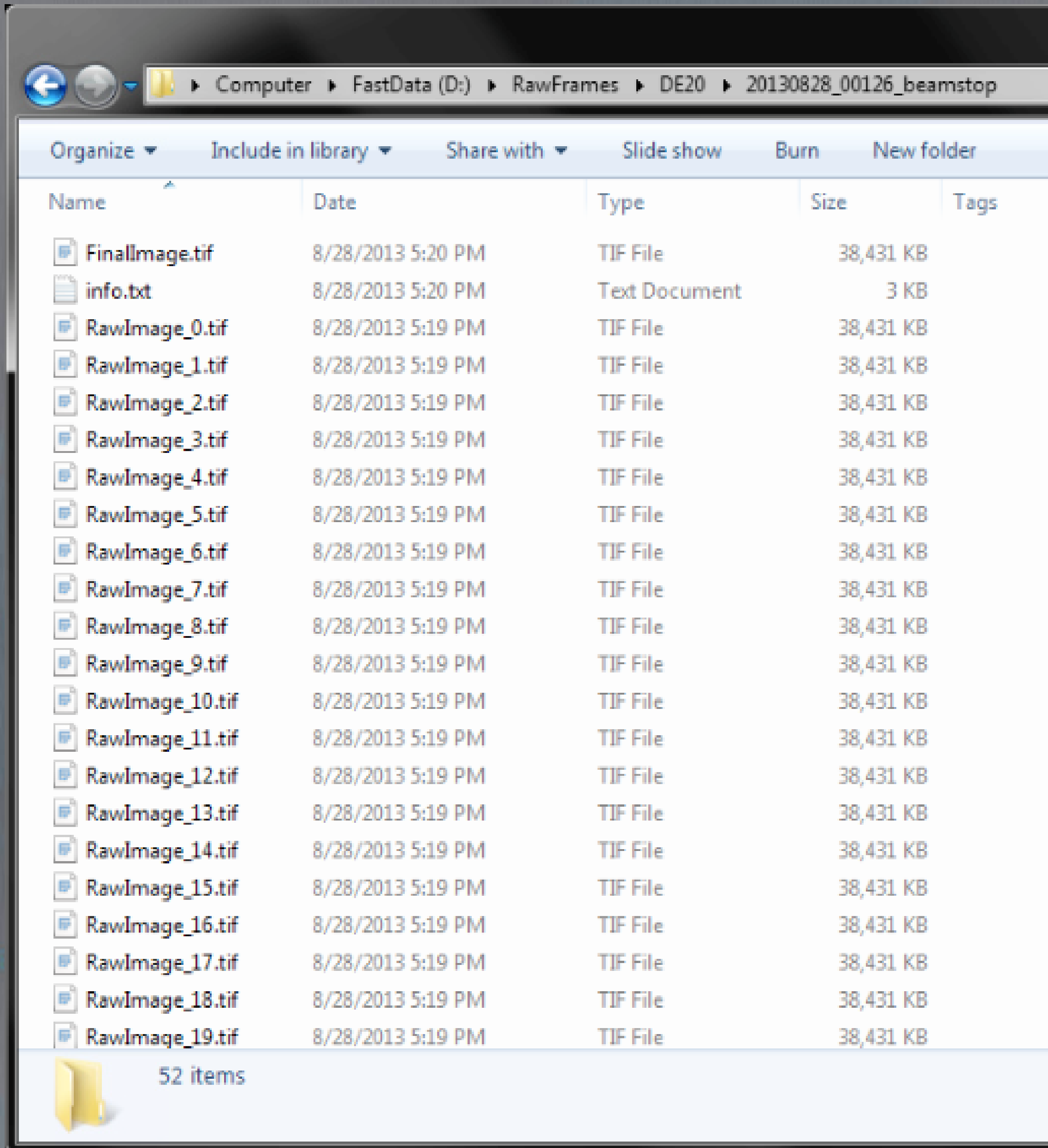


Excellent sensitivity and SNR so that each raw frame contains usable information

Direct detection delivers high resolution

High frame rate with no dead time between frames

Saving Raw Frames



DE-20

37.5 MB/frame

1.1 GB for 30 frames

DE-64

128 MB/frame

3.75 GB for 30 frames

The Impact of "Movie-Mode" for Imaging

- Motion correction
  - Correct the motion of the entire frame (best for tomography).
  - Correct the motion of individual particles (best for single-particle imaging since it corrects for both stage drift and beam-induced specimen motion).
  - *Dramatically increases data quality*.
- Choose your exposure *after* collecting data
- Damage compensation
  - Use a high dose while maintaining high-resolution features.
  - *Dramatically increases contrast.*

Motion Correction

We Need Stability, Not Motion

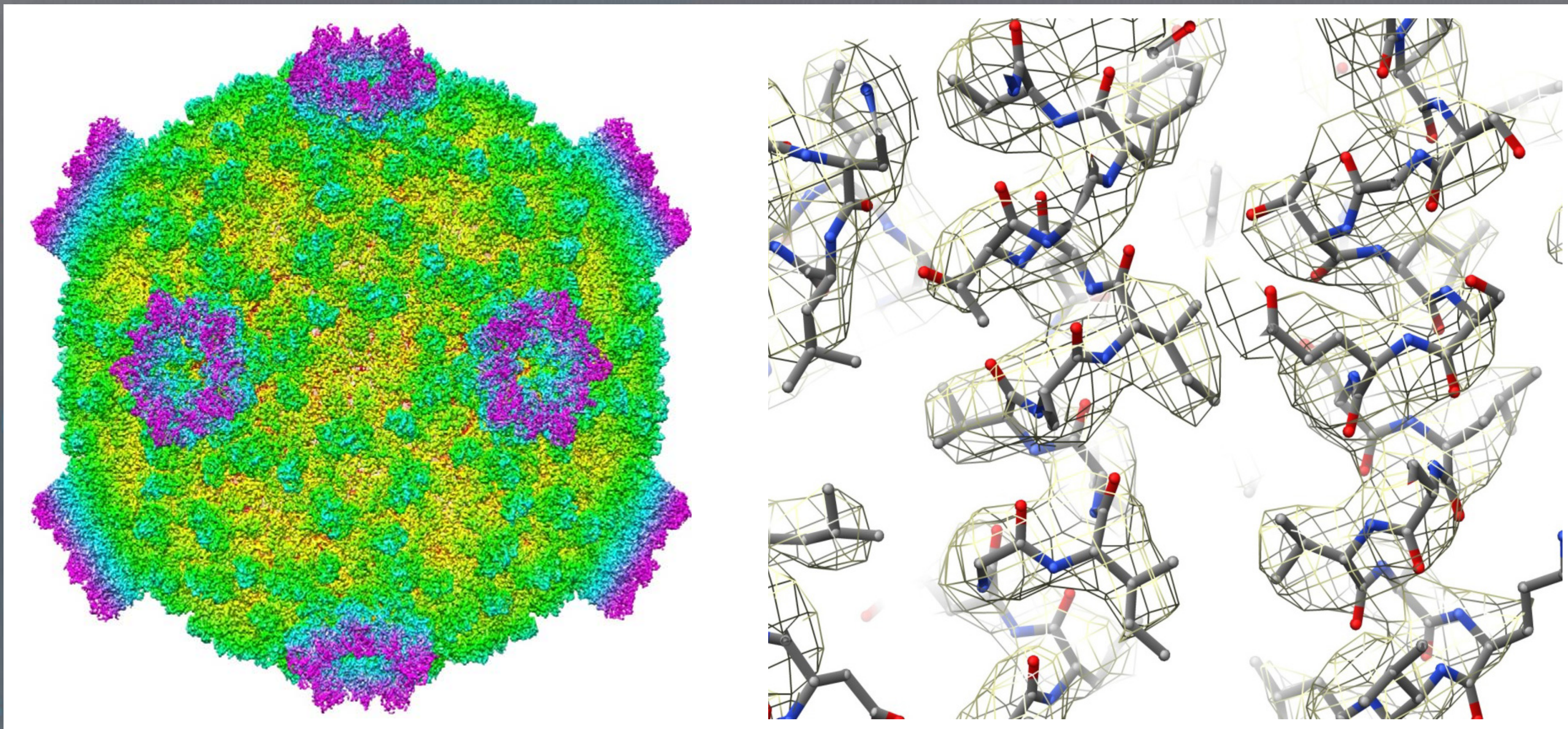
"Finally, we turn to the point that the signal in cryo-EM images shows a much steeper falloff at high resolution than is observed in the electron diffraction pattern of the same specimen... In any event, beam-induced movement that occurs while the image is recorded is responsible for the steep falloff of signal."

Glaeser & Hall, *Biophys J* **100** (2011), 2331-2337.

Motion Correction Development

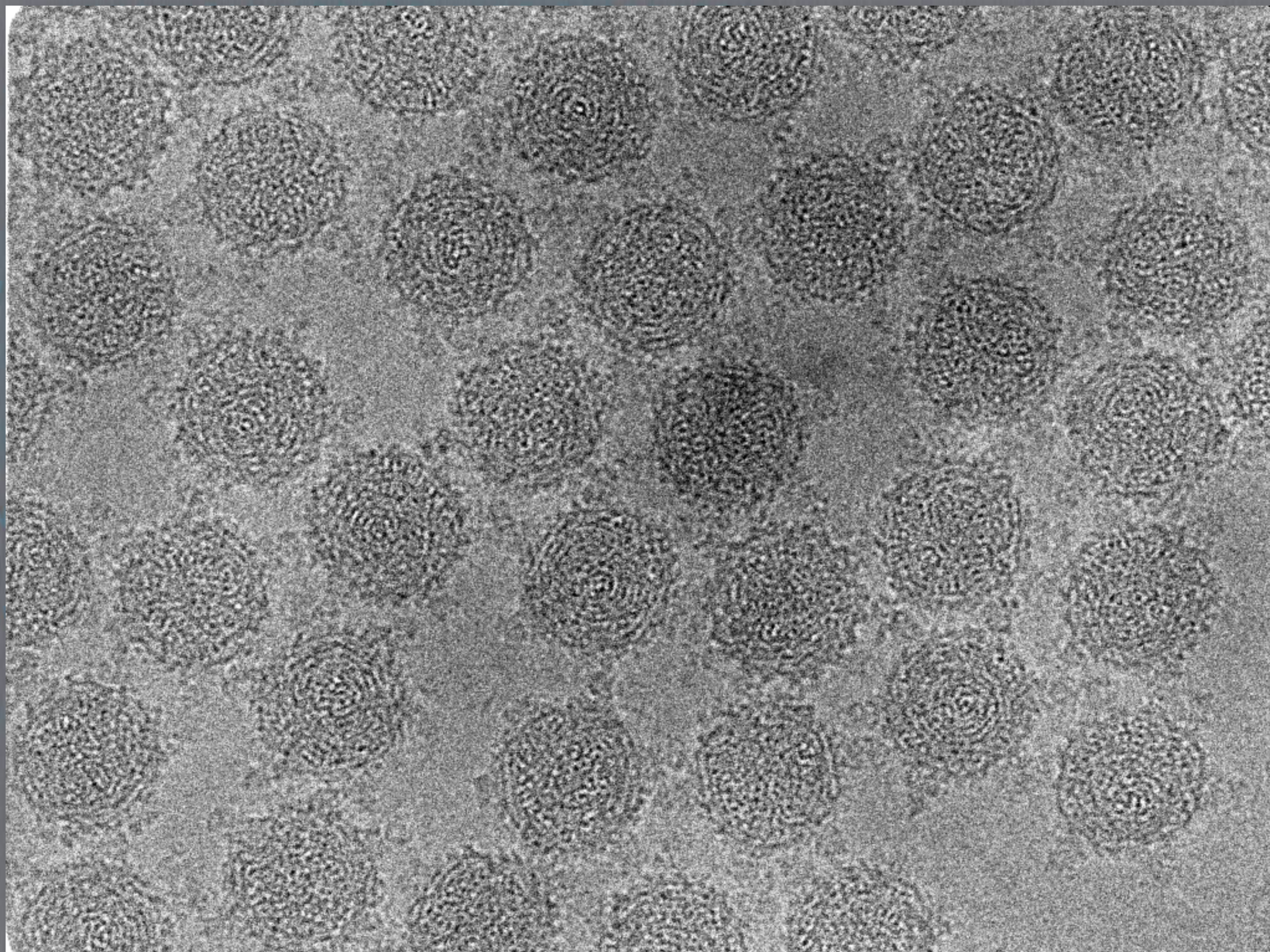
- Applications of direct detection device in transmission electron microscopy.
  Jin, et al. *J Struct Biol*. 2008 Mar;161(3):352-8.
- Beam-induced motion of vitrified specimen on holey carbon film.
  Brilot, et al. *J Struct Biol*. 2012 Mar;177(3):630-7.
- Movies of ice-embedded particles enhance resolution in electron cryo-microscopy.
  Campbell, et al. *Structure*. 2012 Nov 7;20(11):1823-8.
- Electron counting and beam-induced motion correction enable near-atomic-resolution single-particle cryo-EM.
  Li, et al. *Nat Methods*. 2013 Jun;10(6):584-90.
- Ribosome structures to near-atomic resolution from thirty thousand cryo-EM particles.
  Bai, et al. *Elife*. 2013 Feb 19;2:e00461.

CPV at 3.5 Å Resolution

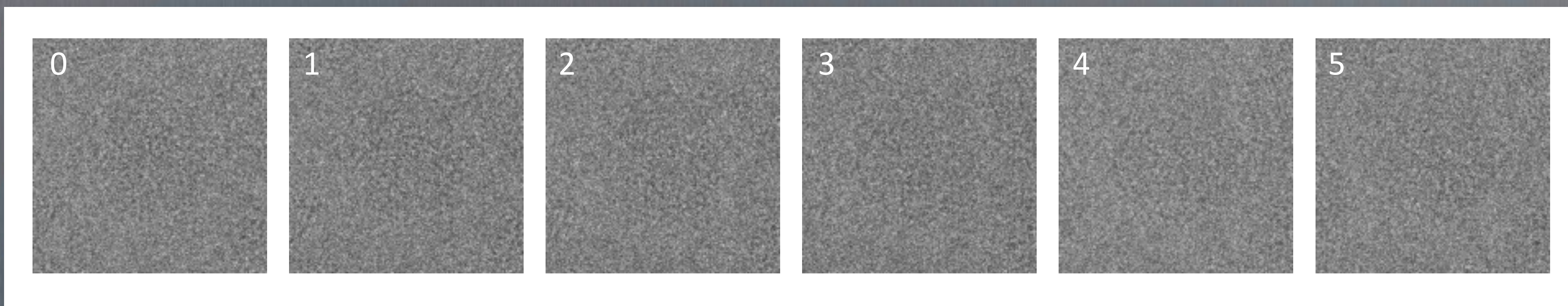


Courtesy of Hong Zhou (UCLA), unpublished.

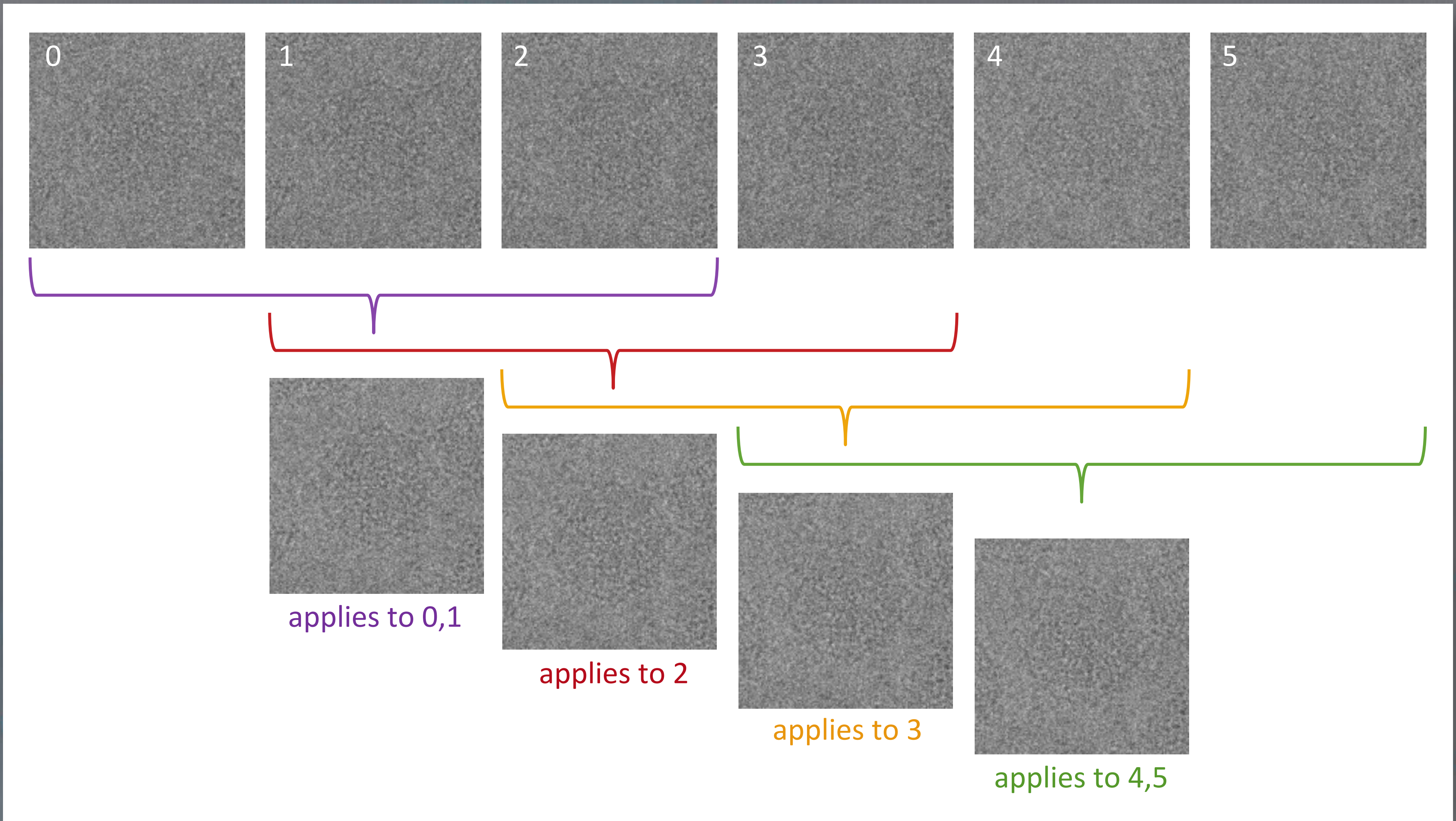
DE-12 Image (~2 micron defocus)



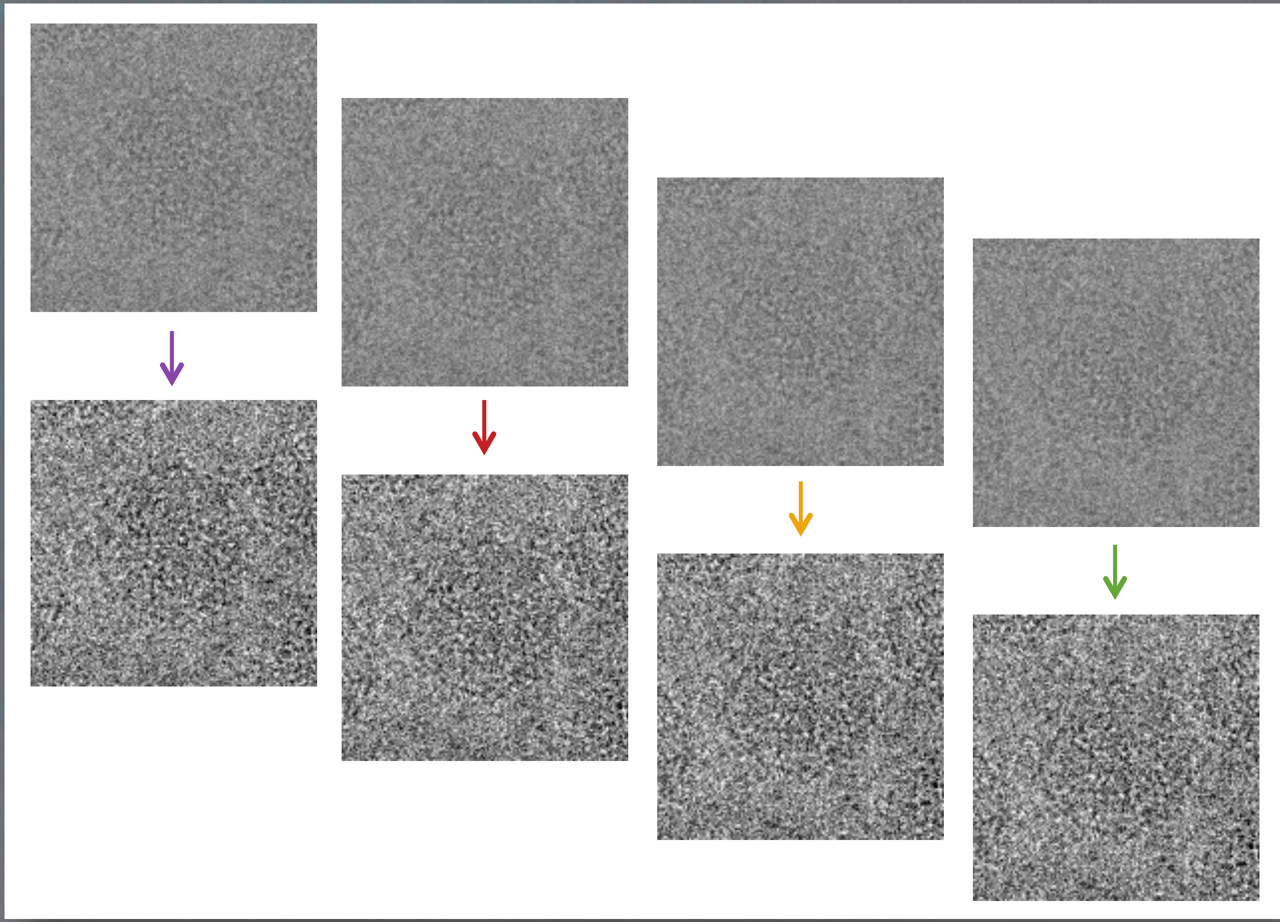
Aligning Frames



Rolling Averages



Filtering Alignment Images

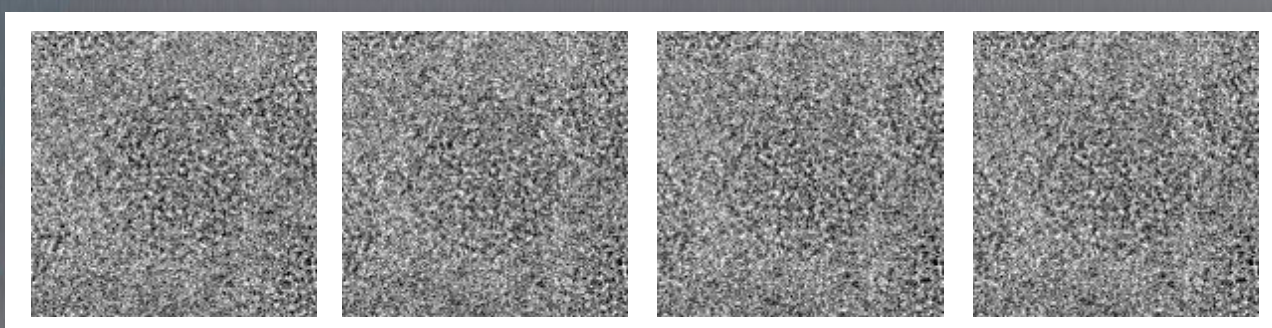


Bin 6× | Bandpass Filter | Normalize | Taper Edge

Alignment with Cross-Correlation

Align with IMOD (*tiltxcorr*)

-RotationAngle 0 -FirstTiltAngle 0 -TiltIncrement 0
-FilterRadius2 0.30 -FilterSigma1 0.01 -FilterSigma2 0.02
-ShiftLimitsXandY %i,%i -Iterate %i
-CumulativeCorrelation -ReverseOrder



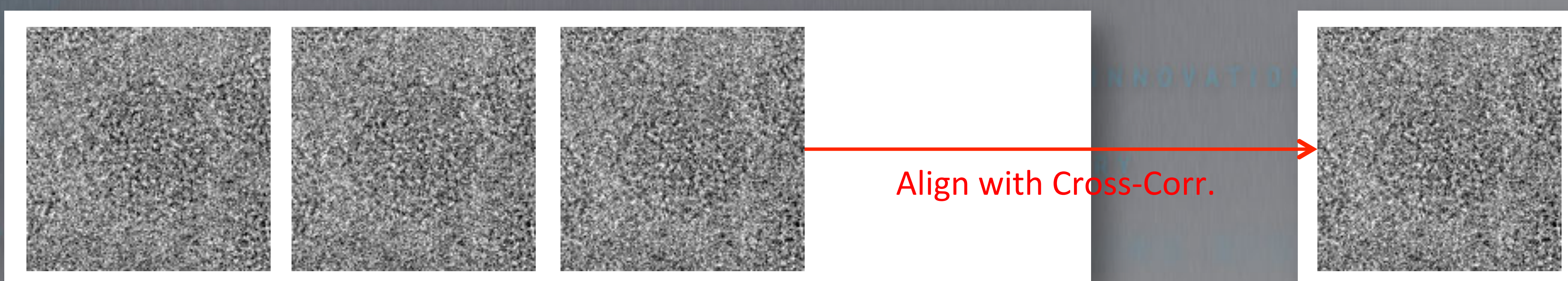
Frames to Align

Reference

Alignment with Cross-Correlation

Align with IMOD (*tiltxcorr*)

-RotationAngle 0 -FirstTiltAngle 0 -TiltIncrement 0
-FilterRadius2 0.30 -FilterSigma1 0.01 -FilterSigma2 0.02
-ShiftLimitsXandY %i,%i -Iterate %i
-CumulativeCorrelation -ReverseOrder



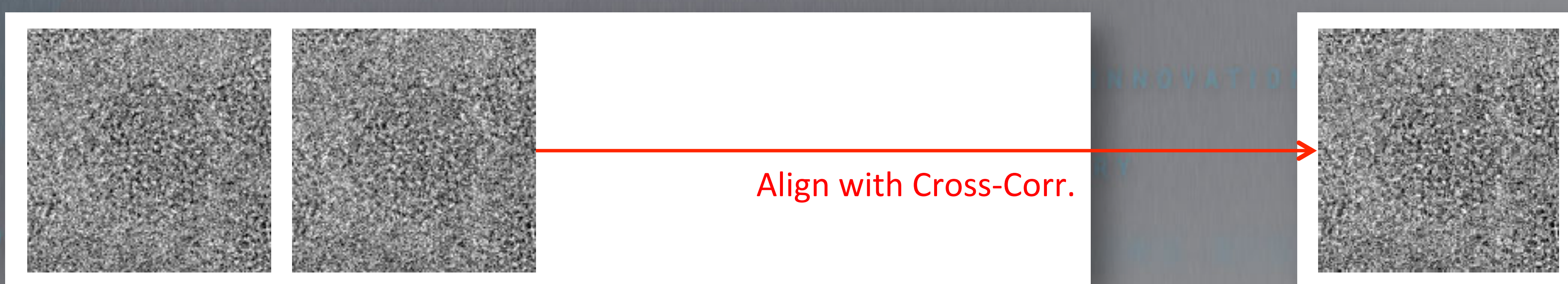
Frames to Align

Reference
(Image 4)

Alignment with Cross-Correlation

Align with IMOD (*tiltxcorr*)

-RotationAngle 0 -FirstTiltAngle 0 -TiltIncrement 0
-FilterRadius2 0.30 -FilterSigma1 0.01 -FilterSigma2 0.02
-ShiftLimitsXandY %i,%i -Iterate %i
-CumulativeCorrelation -ReverseOrder



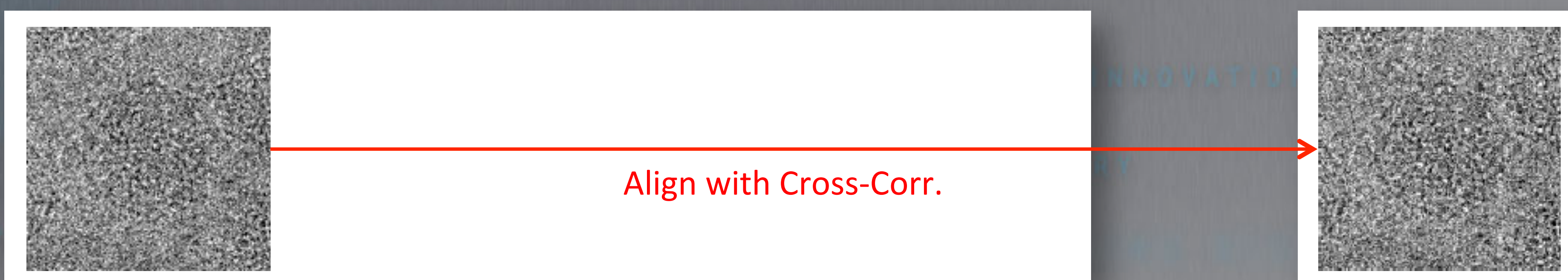
Frames to Align

Reference
(Sum of 4, 3)

Alignment with Cross-Correlation

Align with IMOD (*tiltxcorr*)

-RotationAngle 0 -FirstTiltAngle 0 -TiltIncrement 0
-FilterRadius2 0.30 -FilterSigma1 0.01 -FilterSigma2 0.02
-ShiftLimitsXandY %i,%i -Iterate %i
-CumulativeCorrelation -ReverseOrder

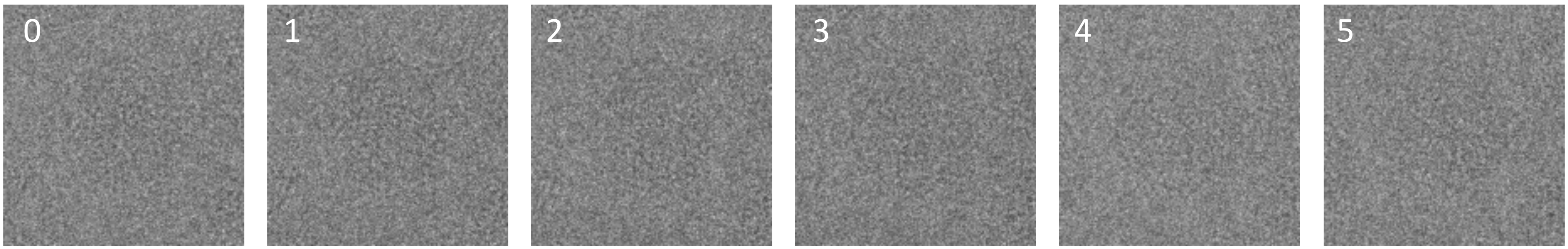


Frames to Align

Reference
(Sum of 4, 3, 2)

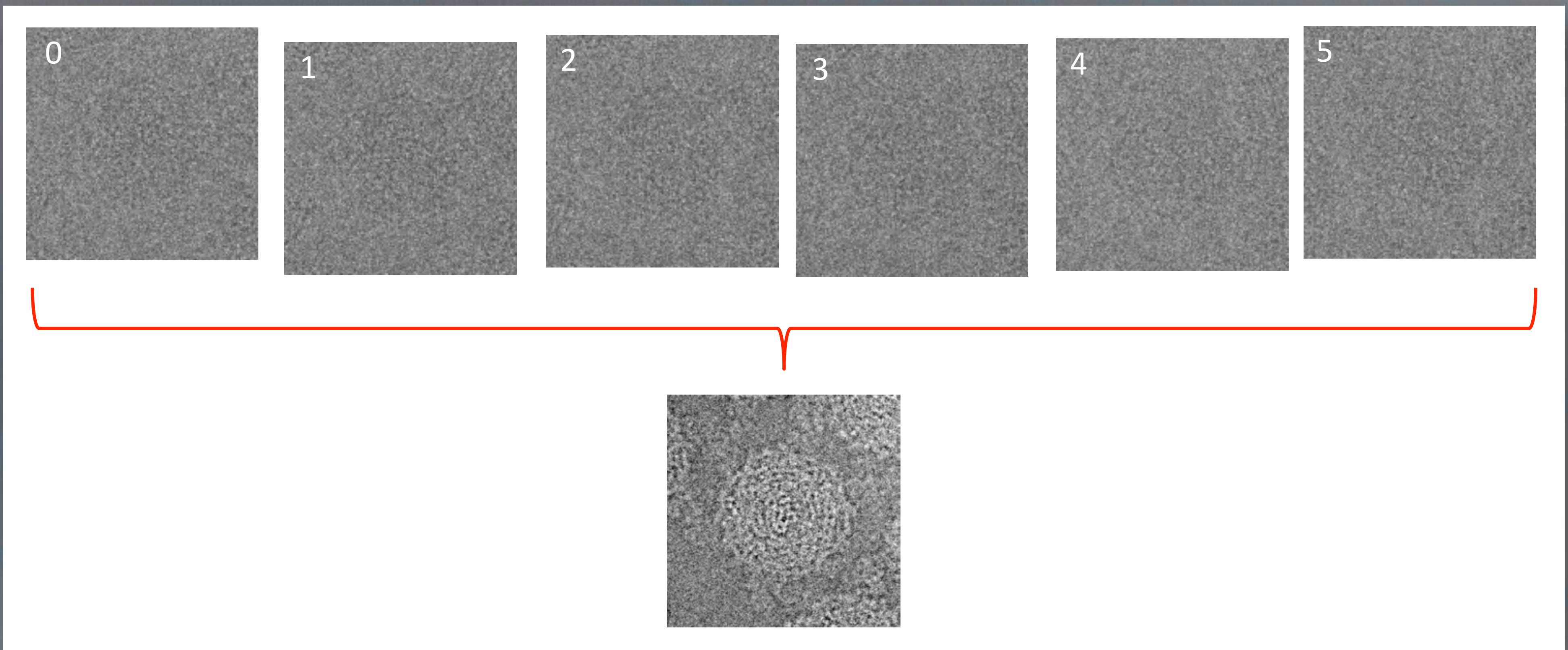
Applying Translations

Apply translations (subpixel) with EMAN2
Transform Class

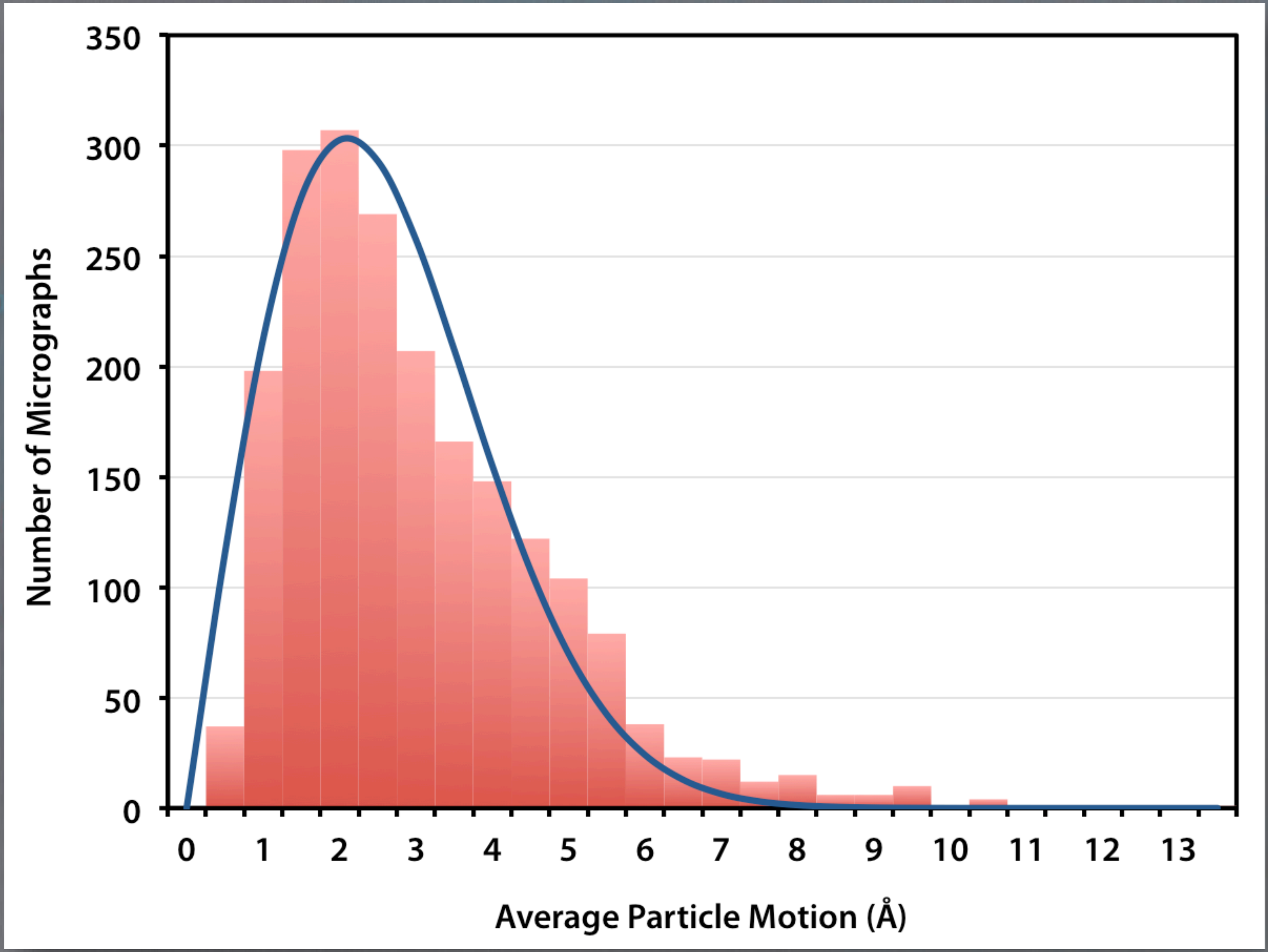


Applying Translations

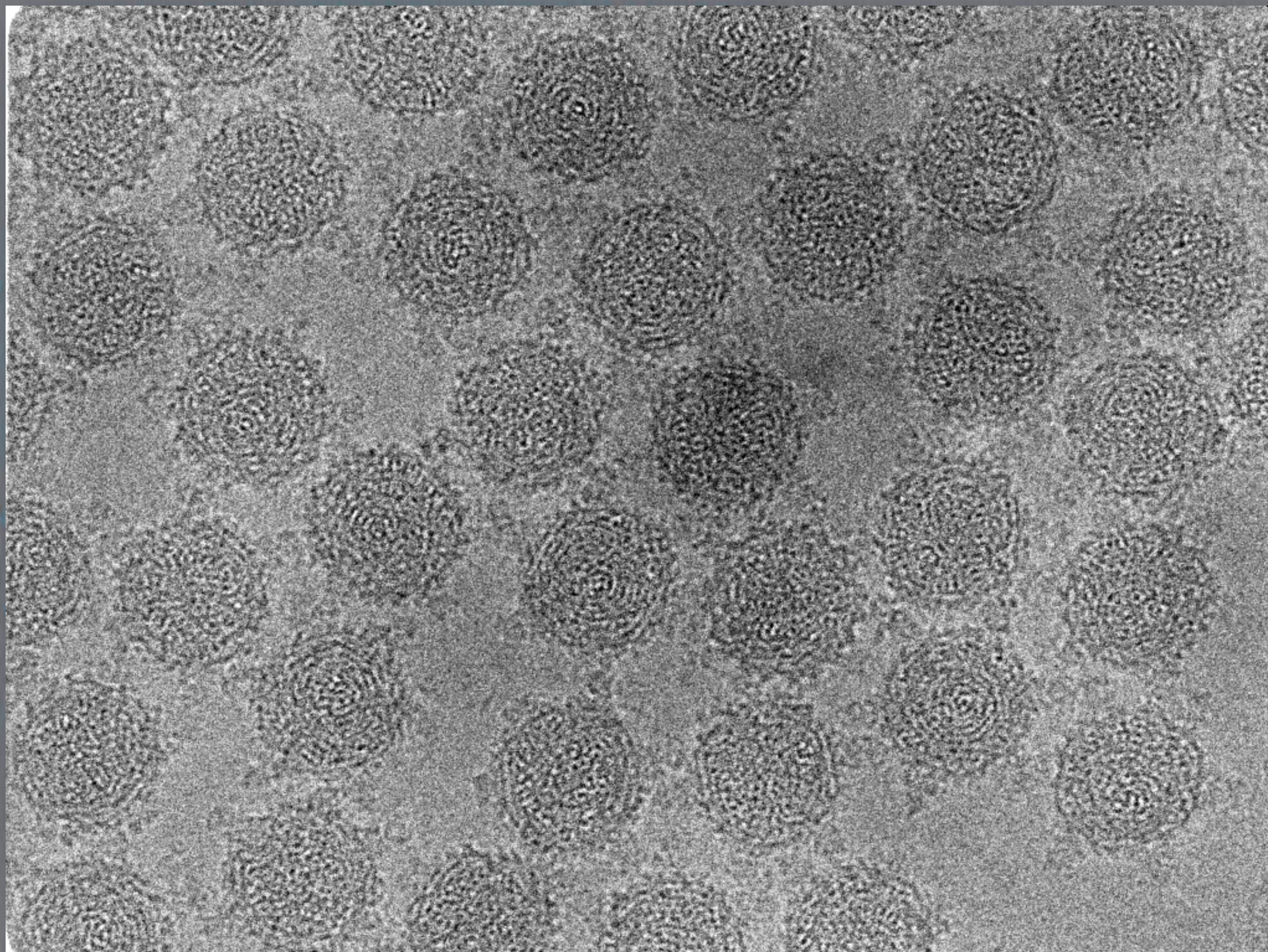
Apply translations (subpixel) with EMAN2
Transform Class



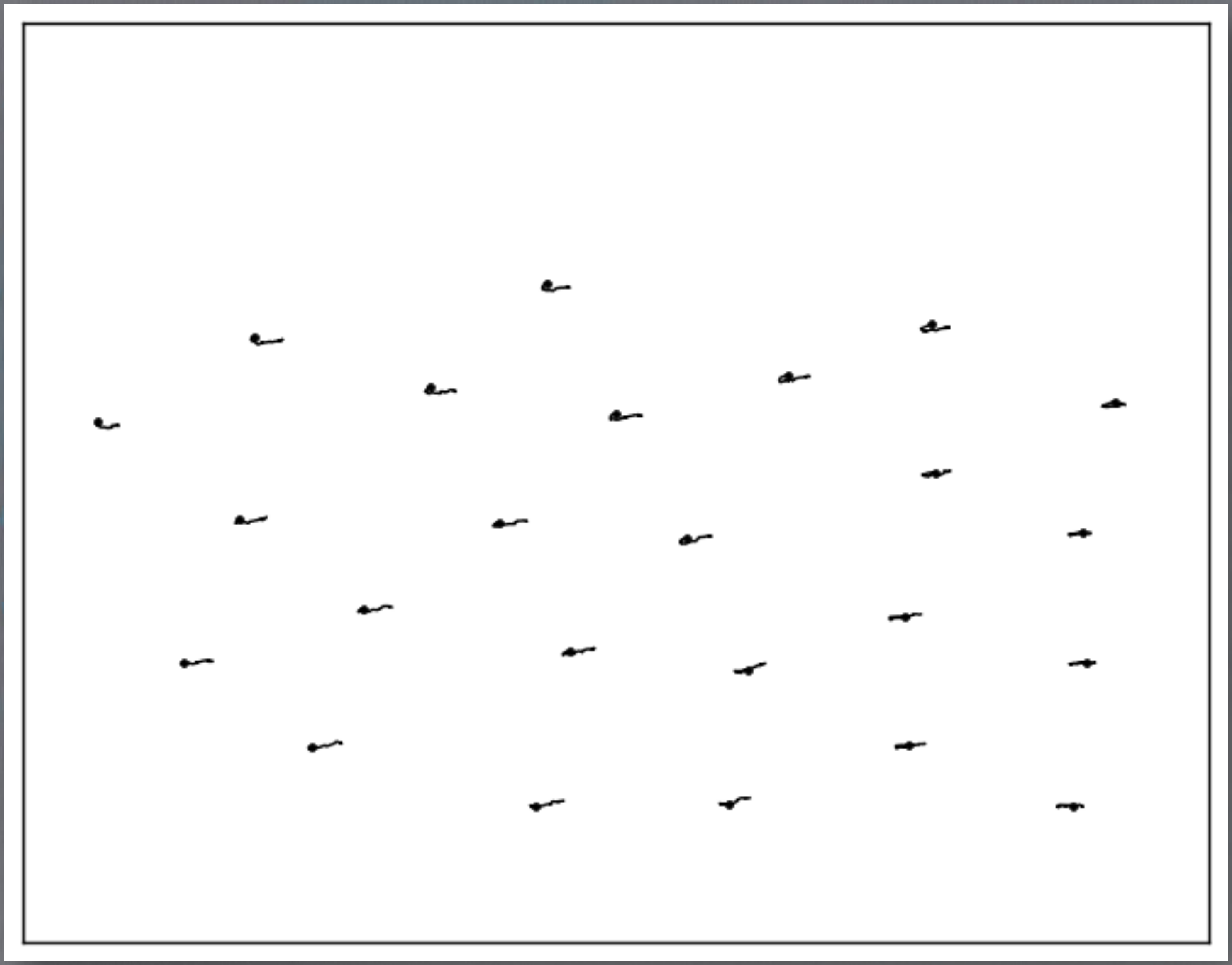
Specimen Motion



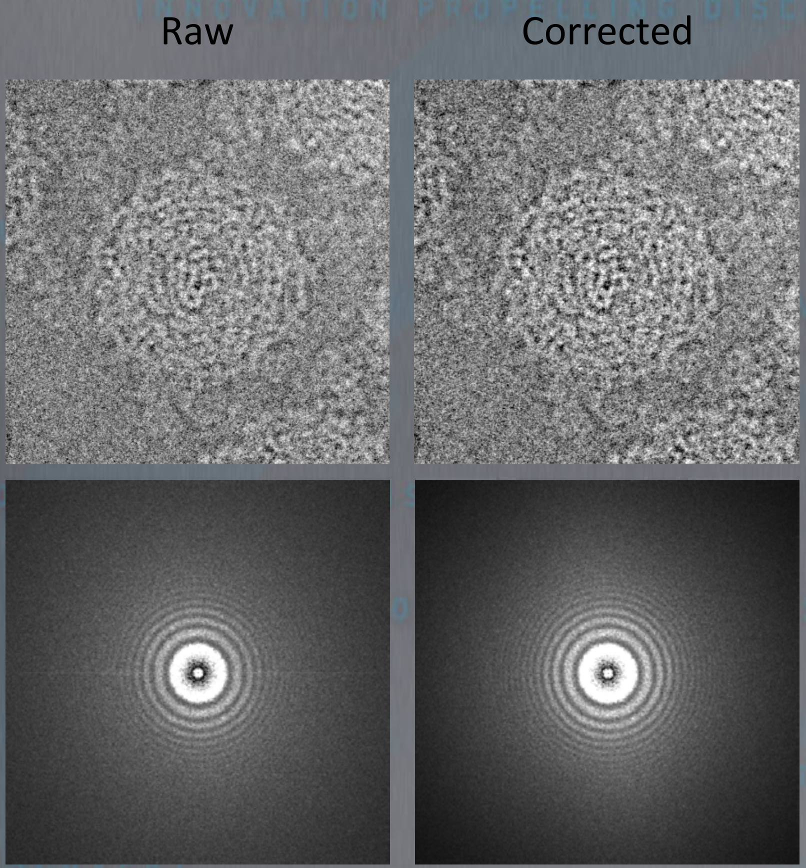
Typical Motion (2.5 Å Traveled)

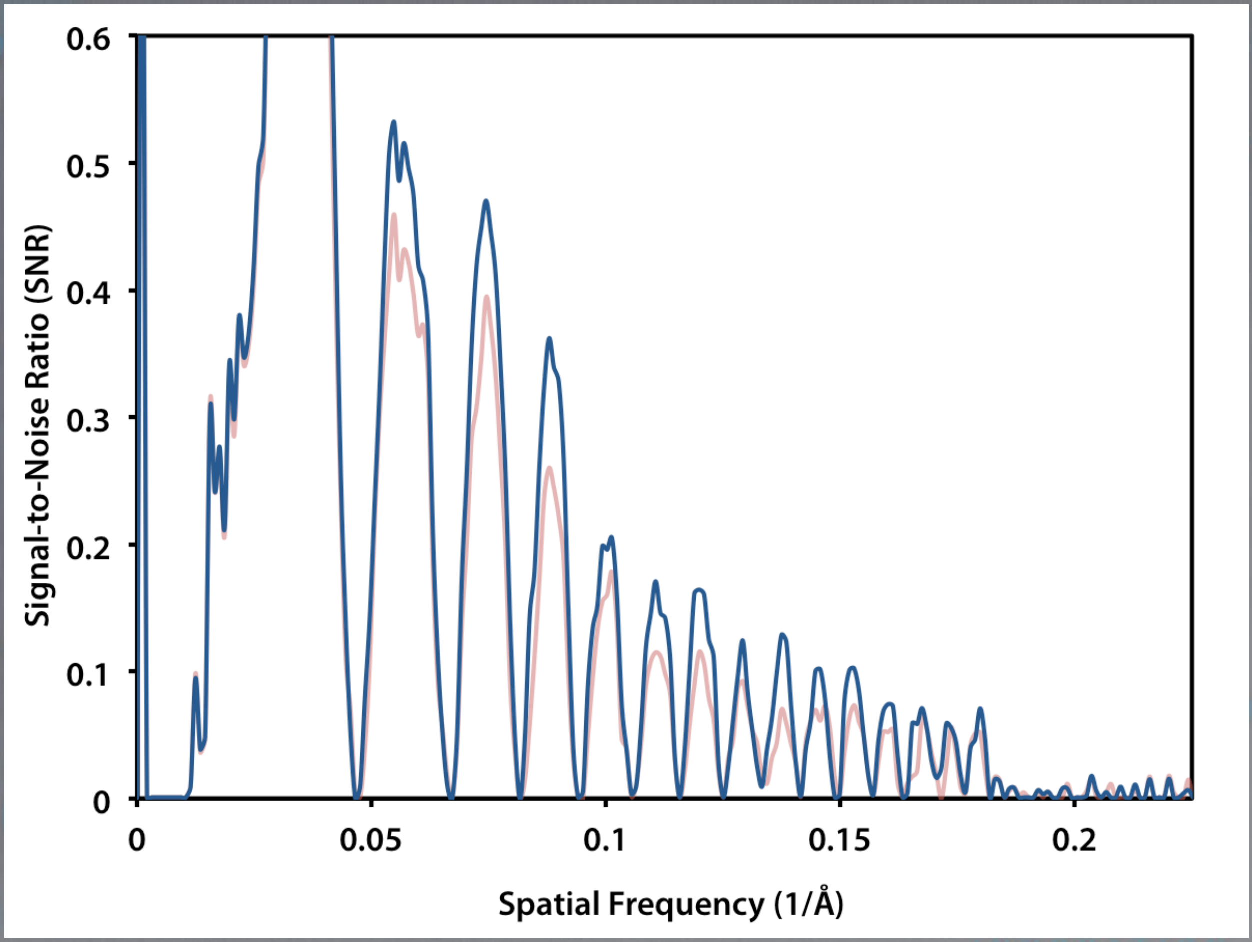


Typical Motion (2.5 Å Traveled)



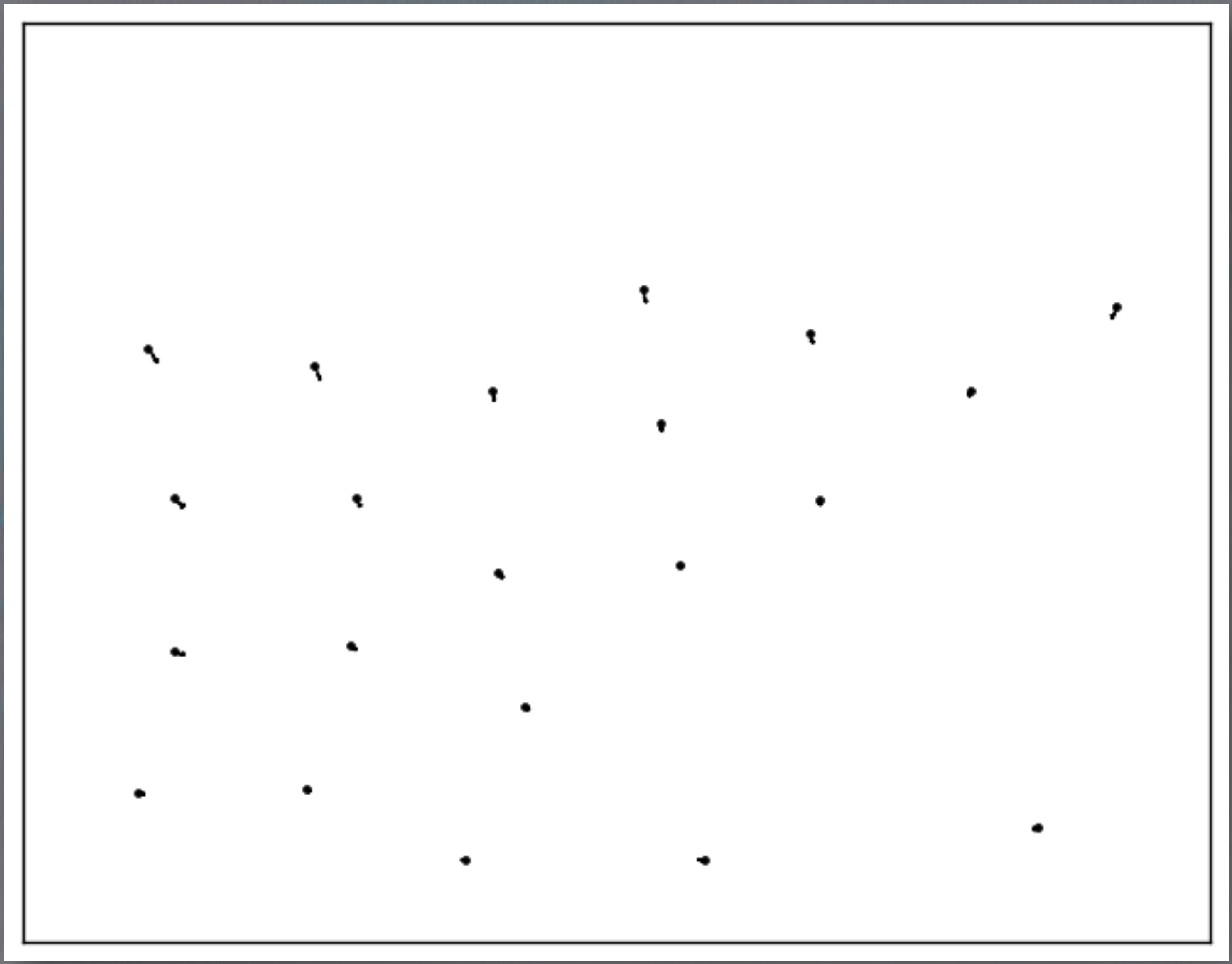
Typical Motion (2.5 Å Traveled)





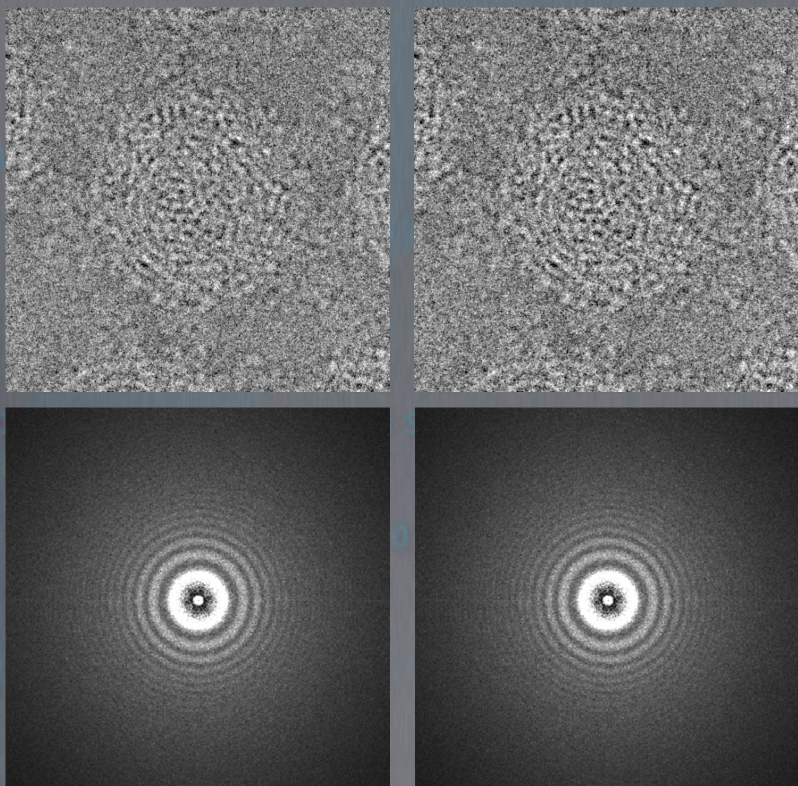
28% average SNR improvement

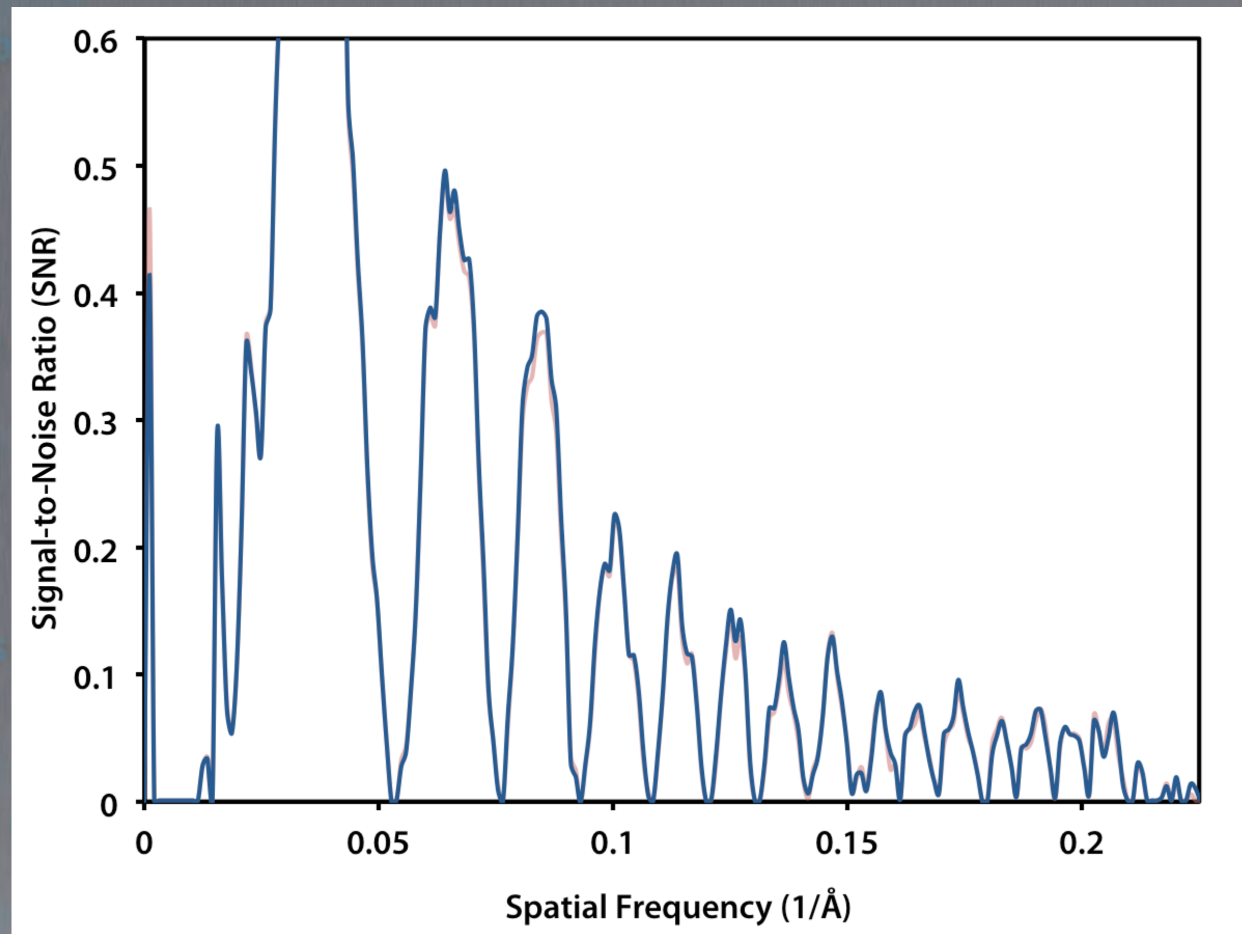
Small Motion (0.25 Å Traveled)



Small Motion (0.25 Å Traveled)

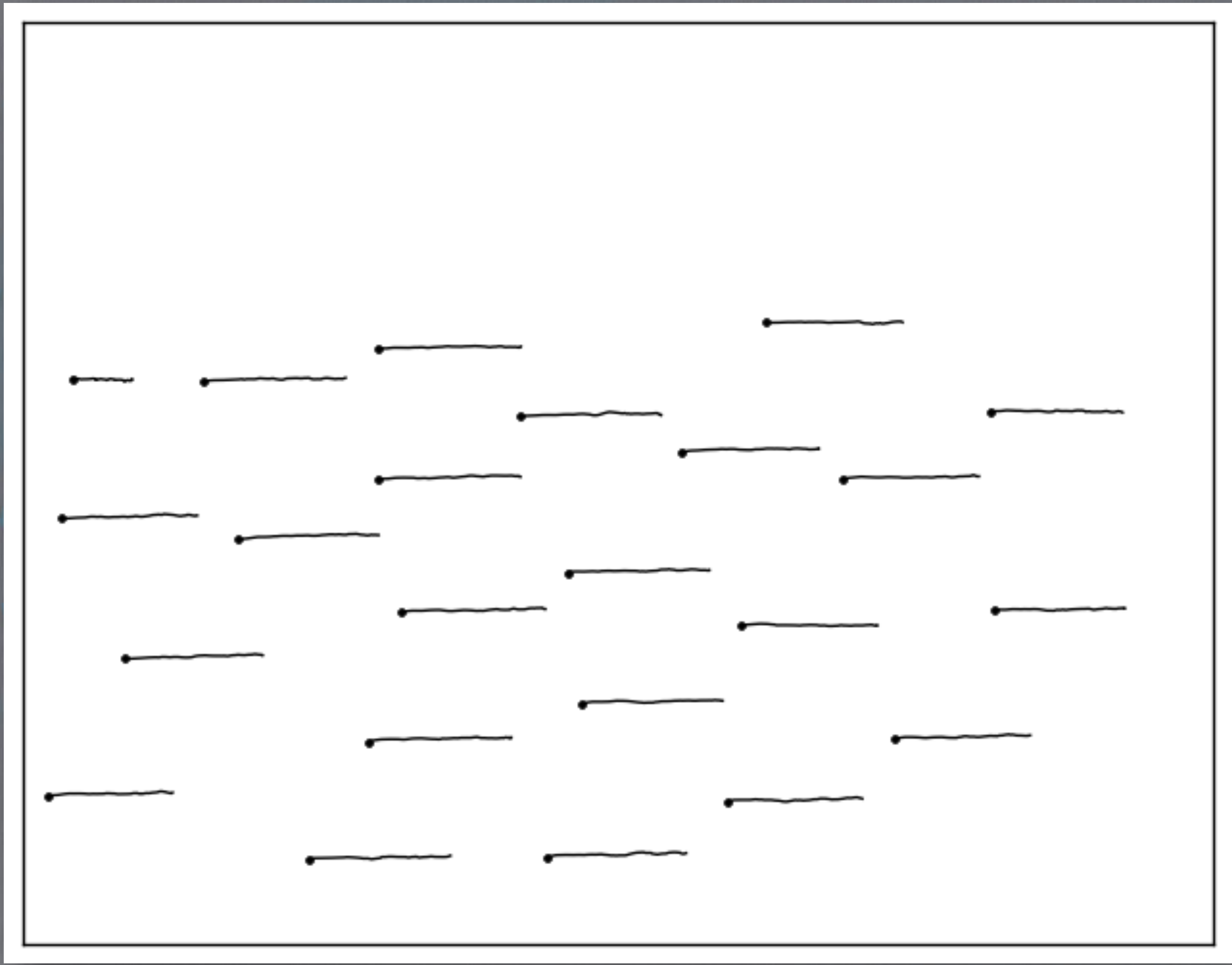
Raw | Corrected



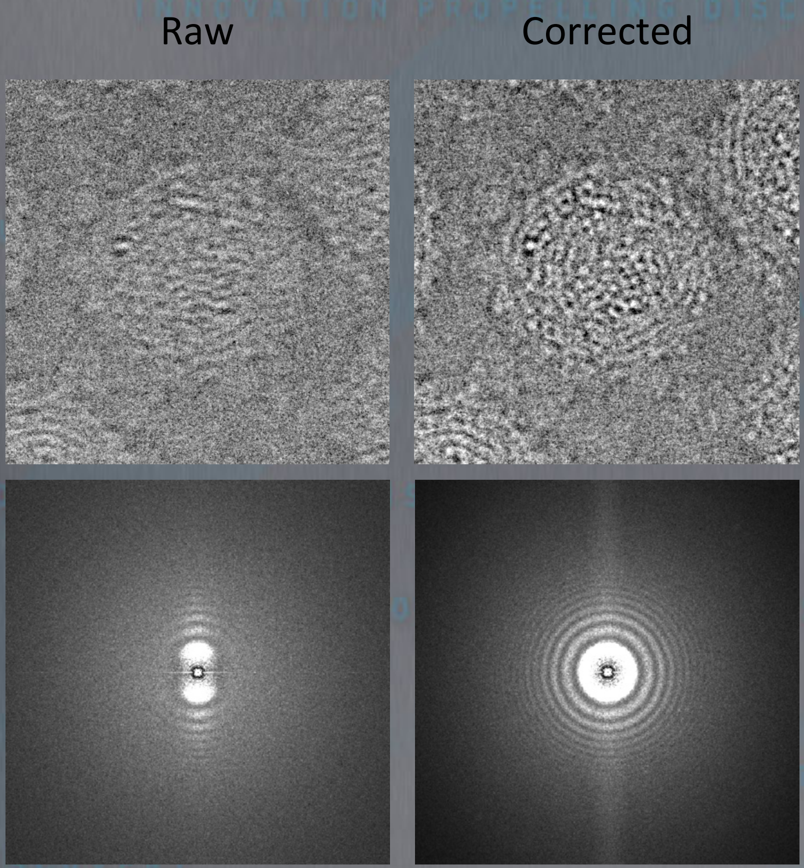


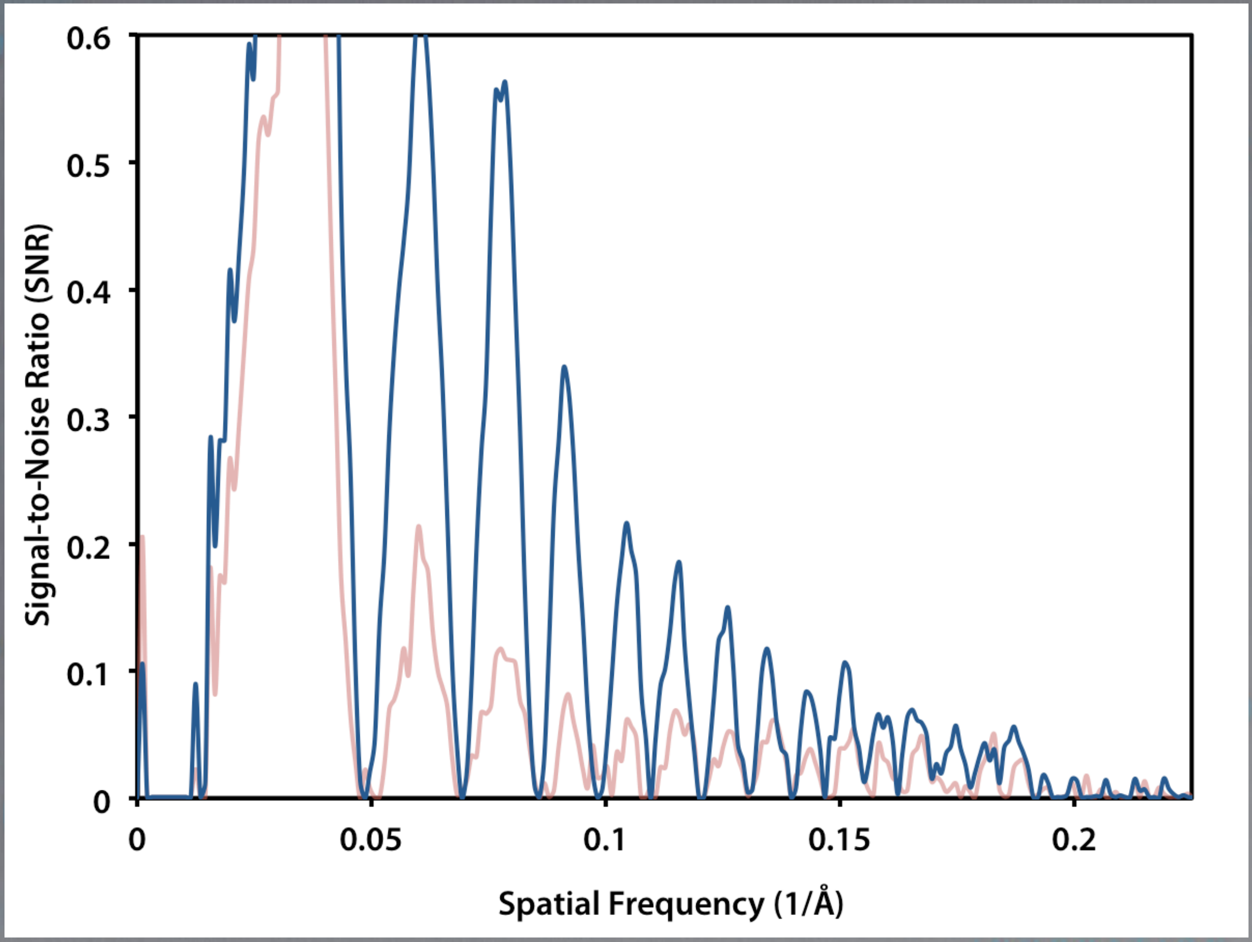
3% average SNR improvement

Large Motion (9.8 Å Traveled)



Large Motion (9.8 Å Traveled)

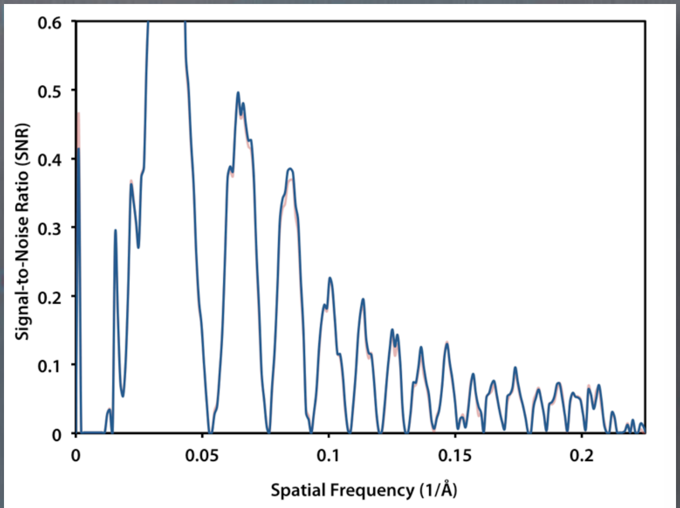




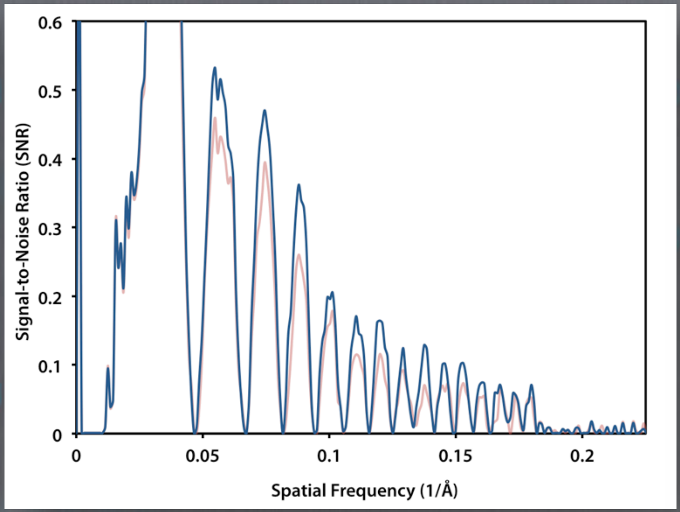
173% average SNR improvement

SNR Improvement Comparison

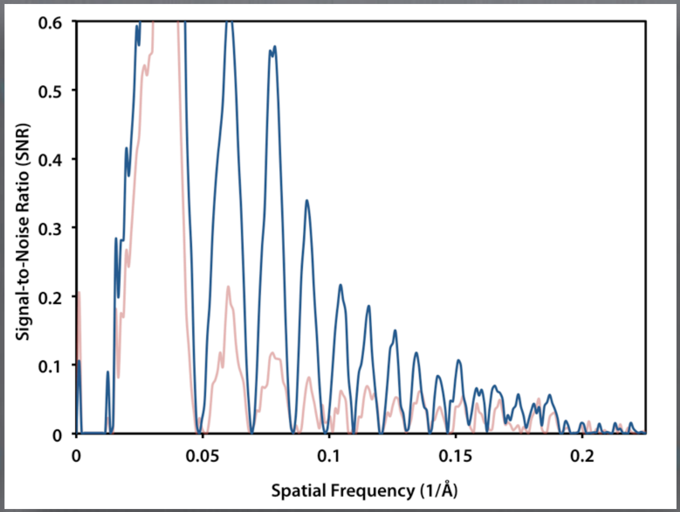
Small Motion



Typical Motion



Large Motion



Damage Compensation

We Need to Overcome Radiation Damage

"The primary limiting factor is radiation damage and, more specifically, the poor signal/noise ratio (SNR) in images recorded with optimal electron exposures... The optimal electron exposure that can be used for cryo-EM depends somewhat on the resolution. For example, it is counterproductive to use exposures (with 300 keV electrons) higher than 2000 electrons/nm^2 to image features at high resolution, whereas exposures five times larger than that can be used to image features at very low resolution."

Glaeser & Hall, *Biophys J* **100** (2011), 2331-2337.

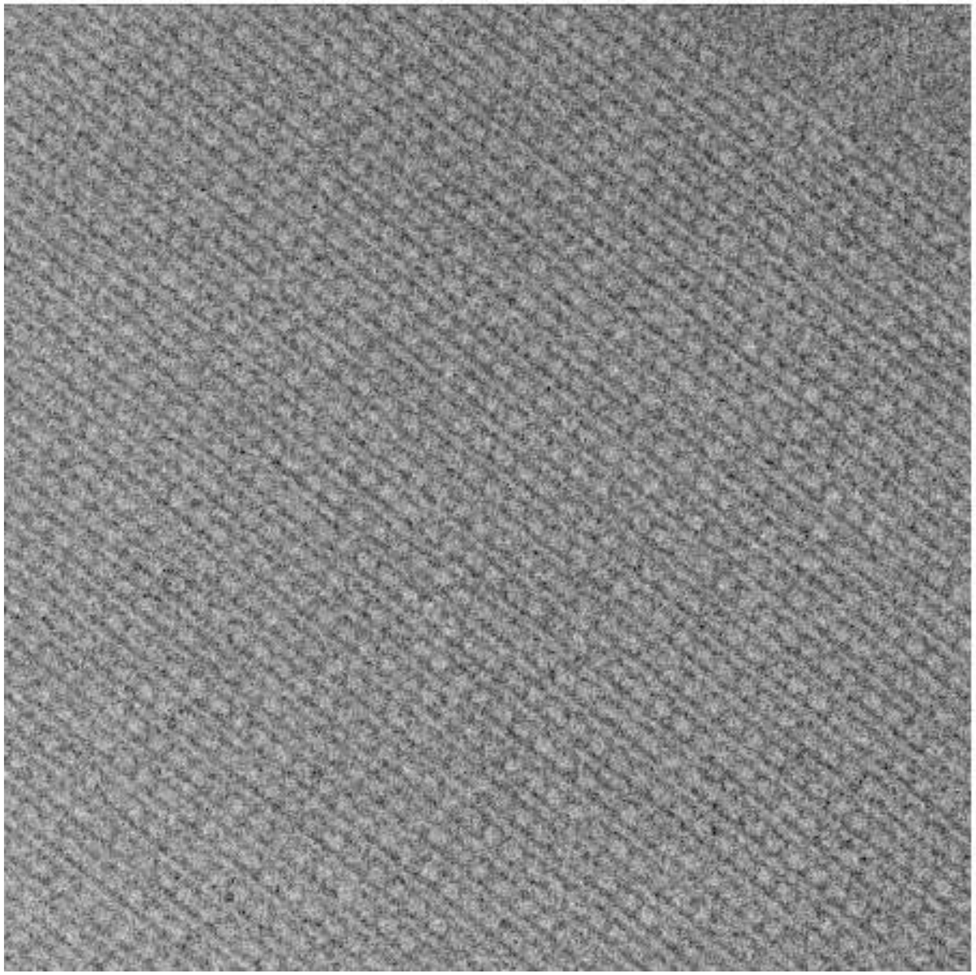
Damage Compensation Development

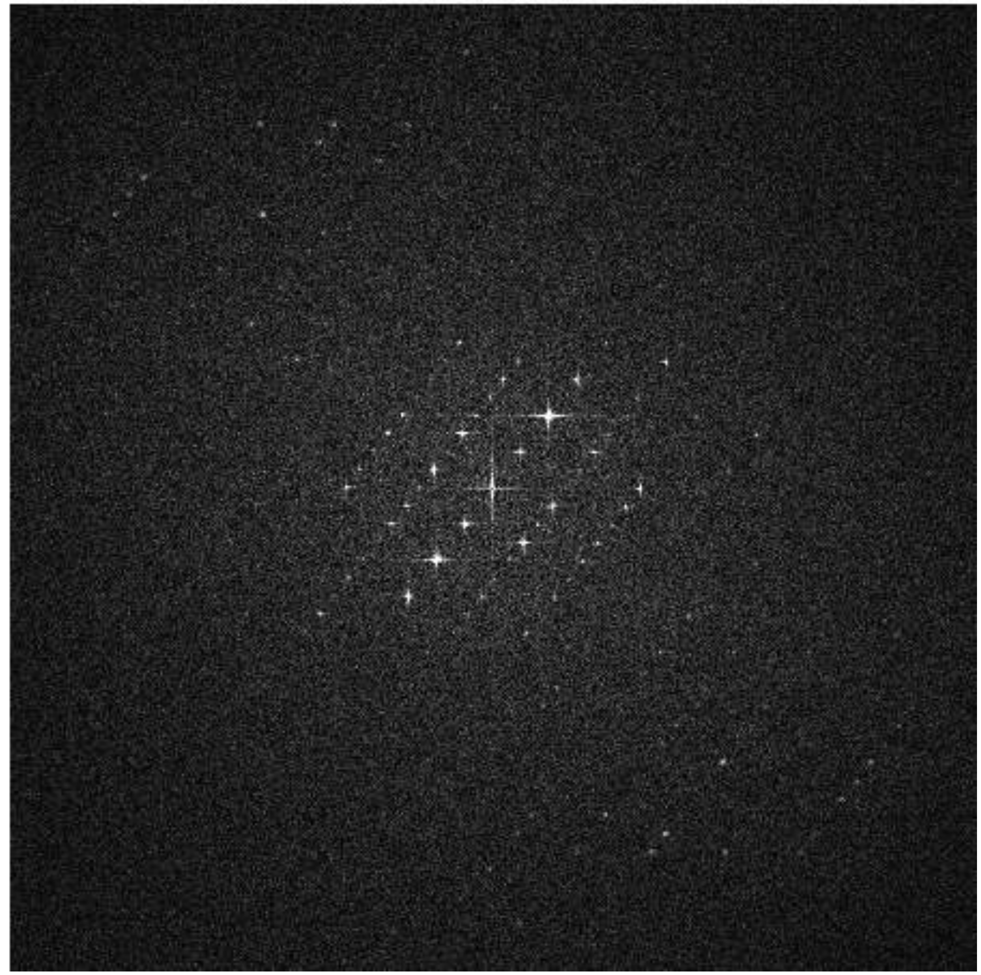
- The resolution dependence of optimal exposures in liquid nitrogen temperature electron cryomicroscopy of catalase crystals.
  Baker, et al. J Struct Biol. 2010 Mar;169(3):431-7.

- Visualizing and correcting dynamic specimen processes in TEM using a Direct Detection Device.
  Bammes, et al. Microsc Microanal. 2013 Aug;19(S2):1320-1.

Cumulative Exposures on Catalase

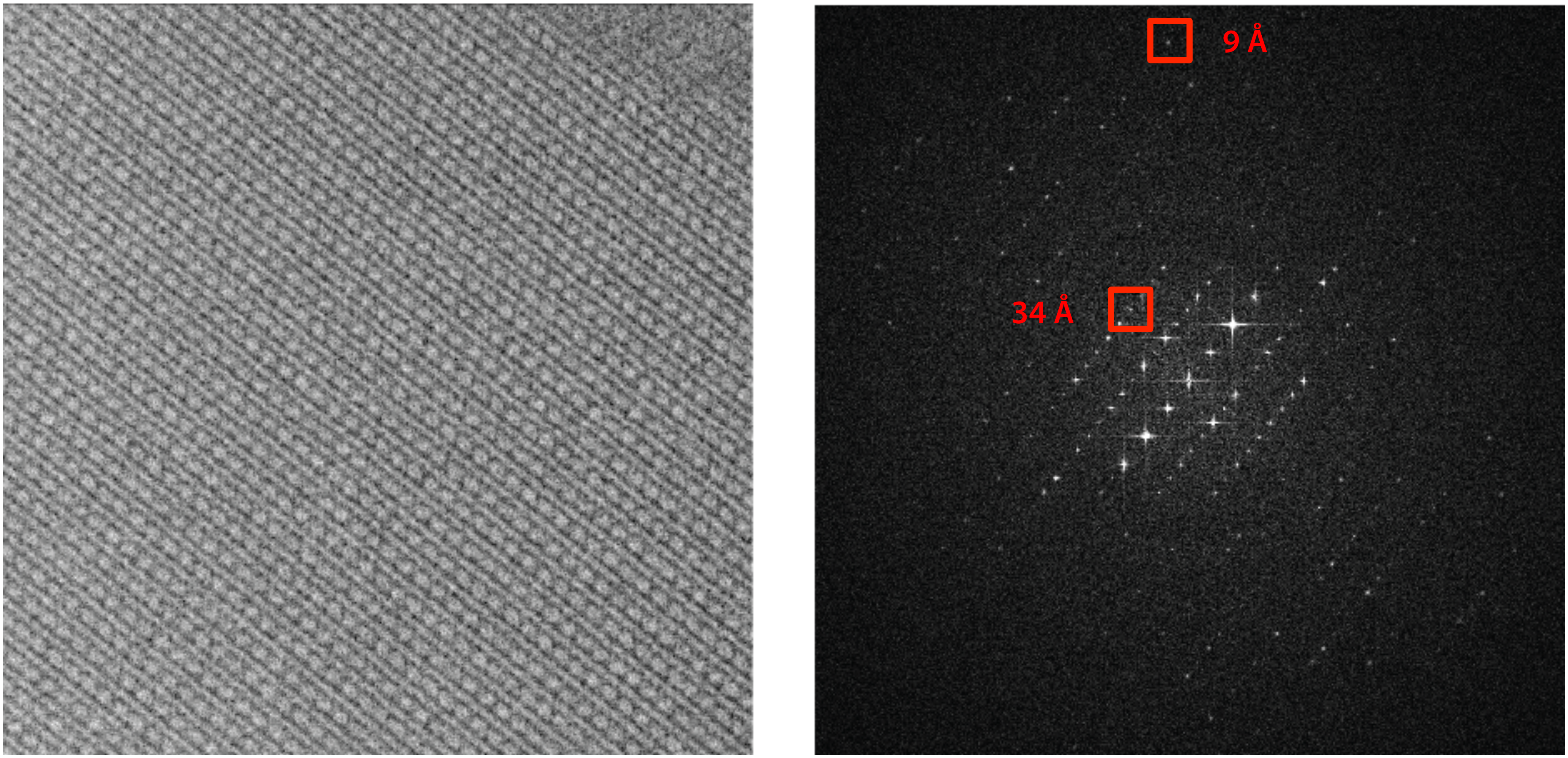
3 e⁻/Å²



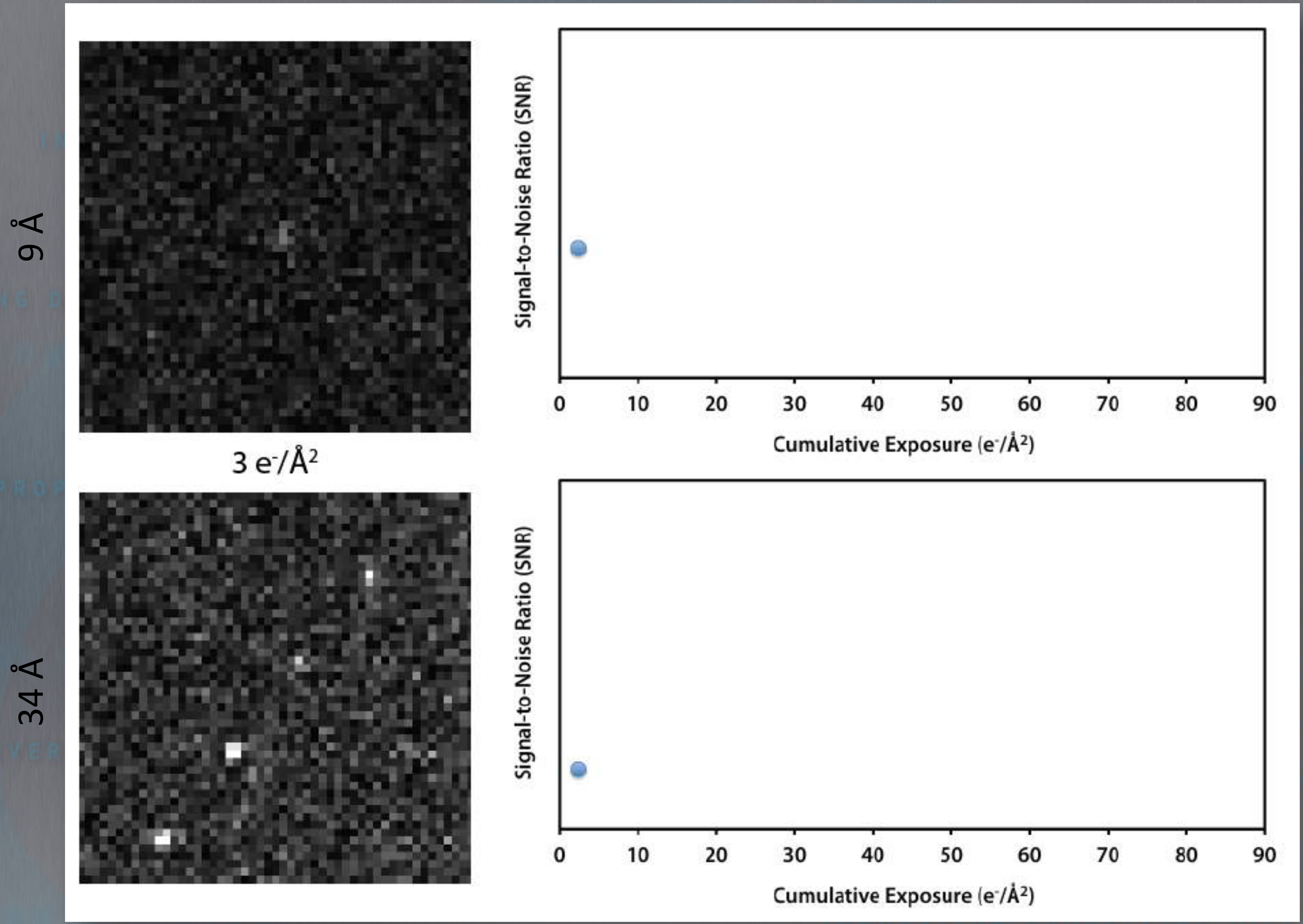


Cumulative Exposures on Catalase

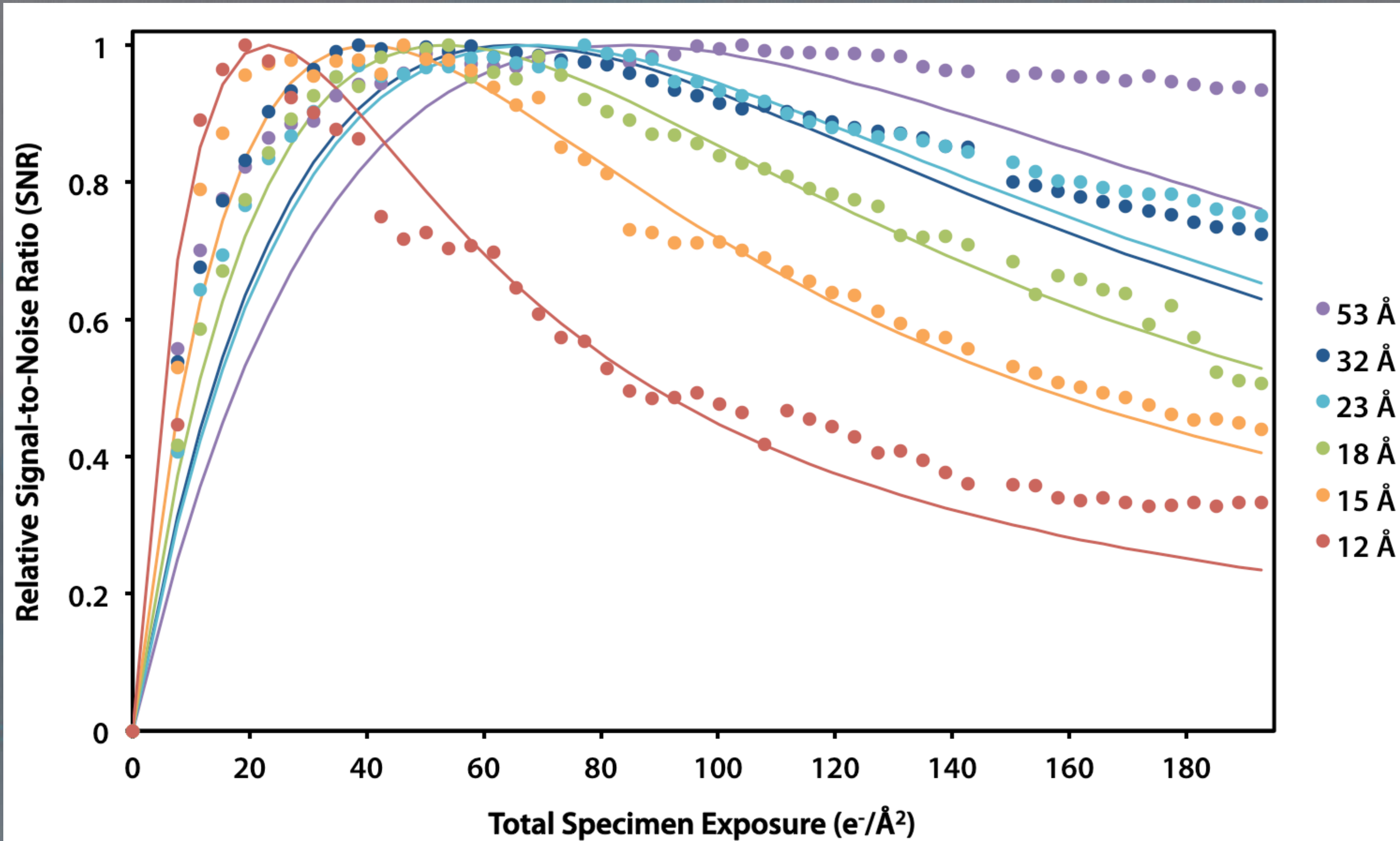
9 e⁻/Å²



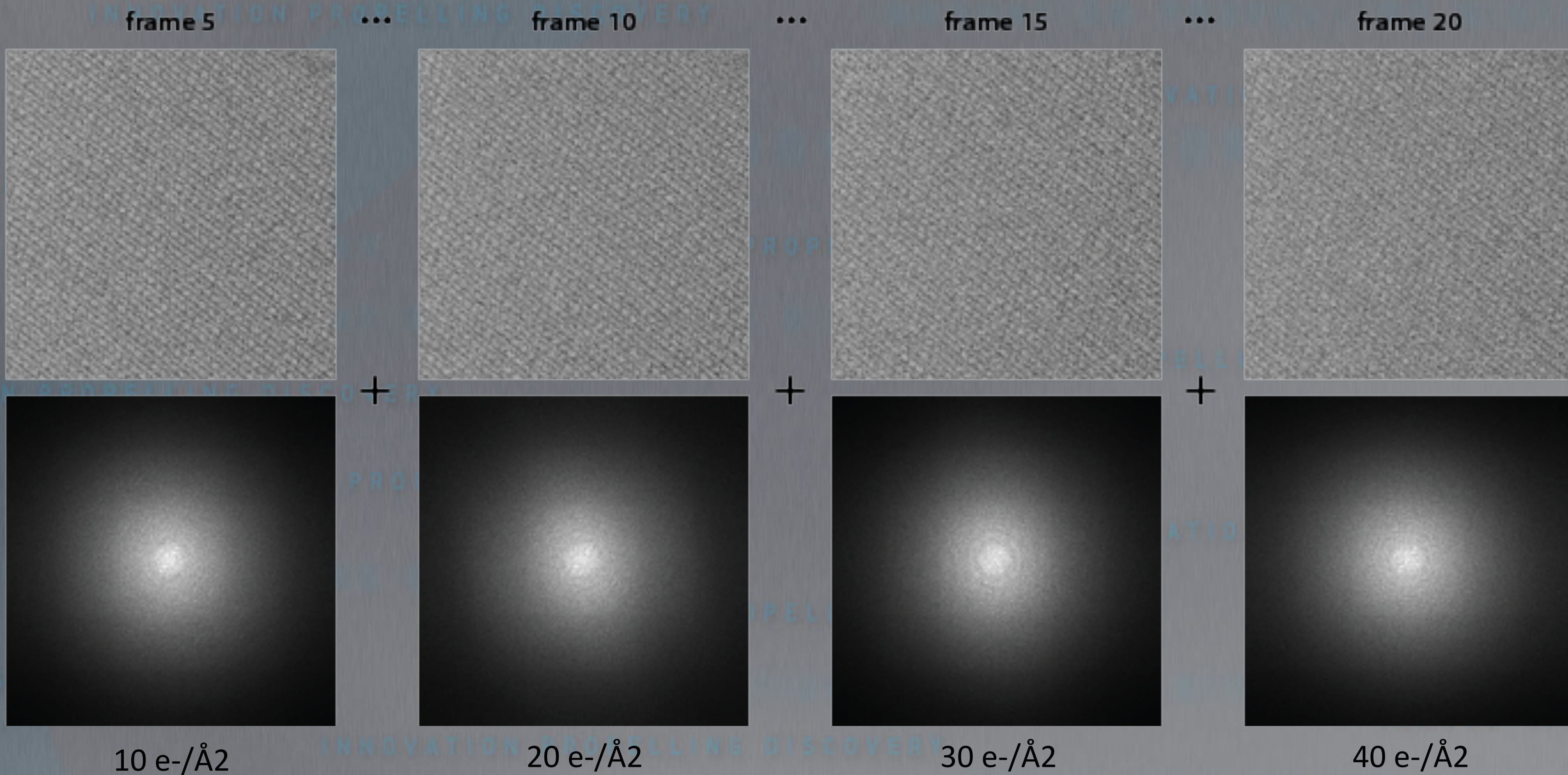
Cumulative Exposures on Catalase



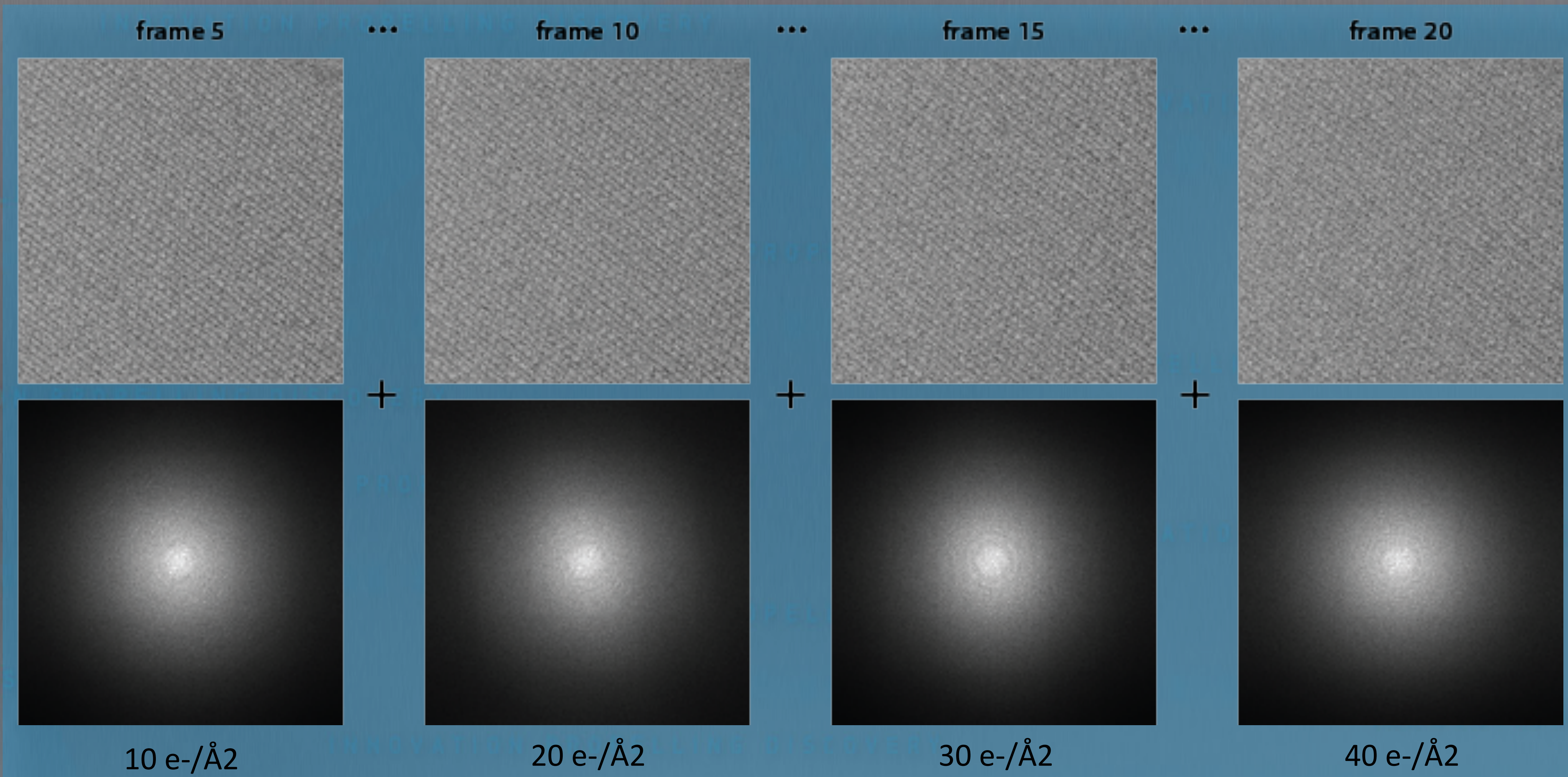
Optimal Exposure vs. Resolution



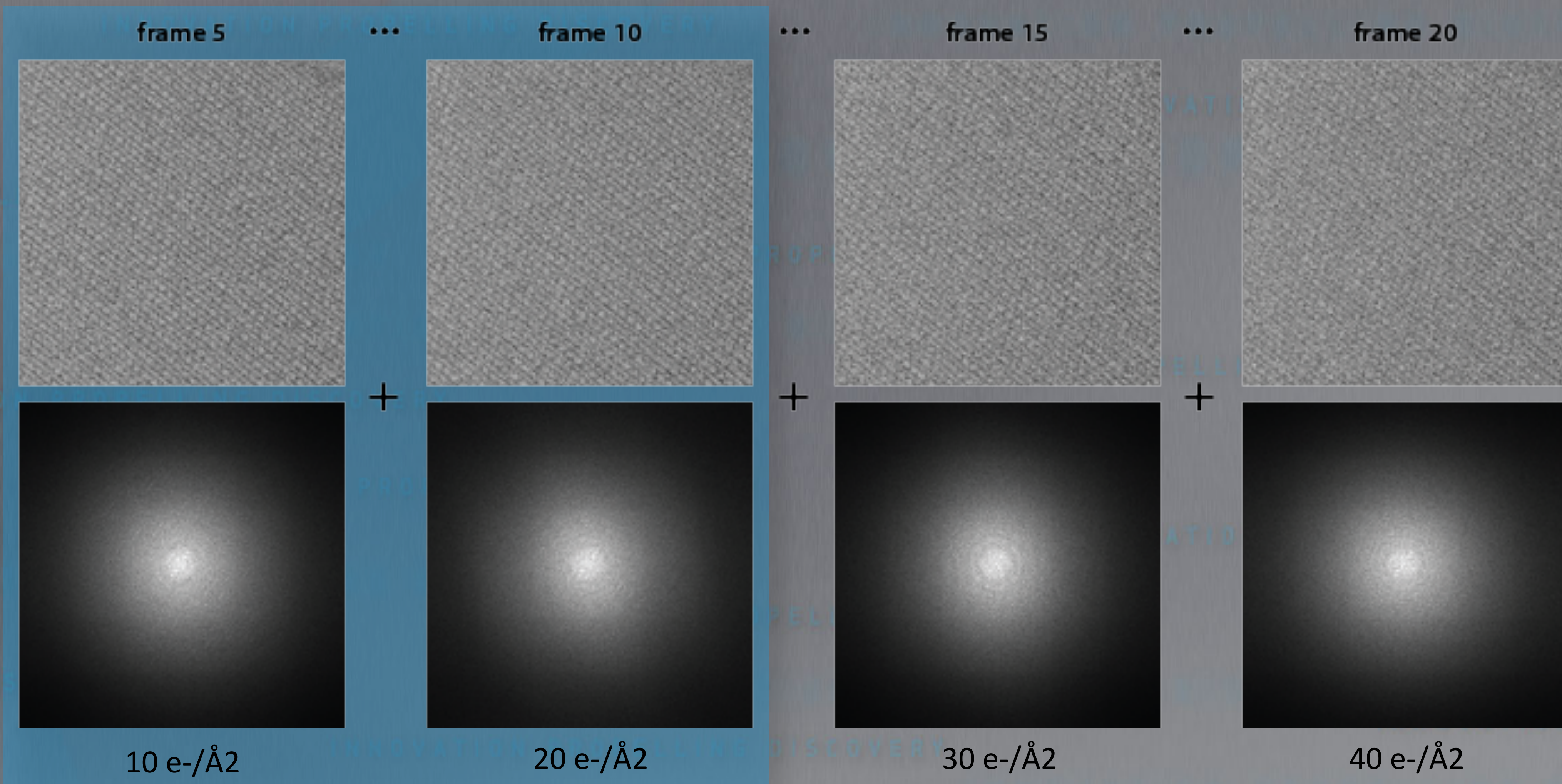
Movie-Mode Dose Fractionation



Movie-Mode Dose Fractionation

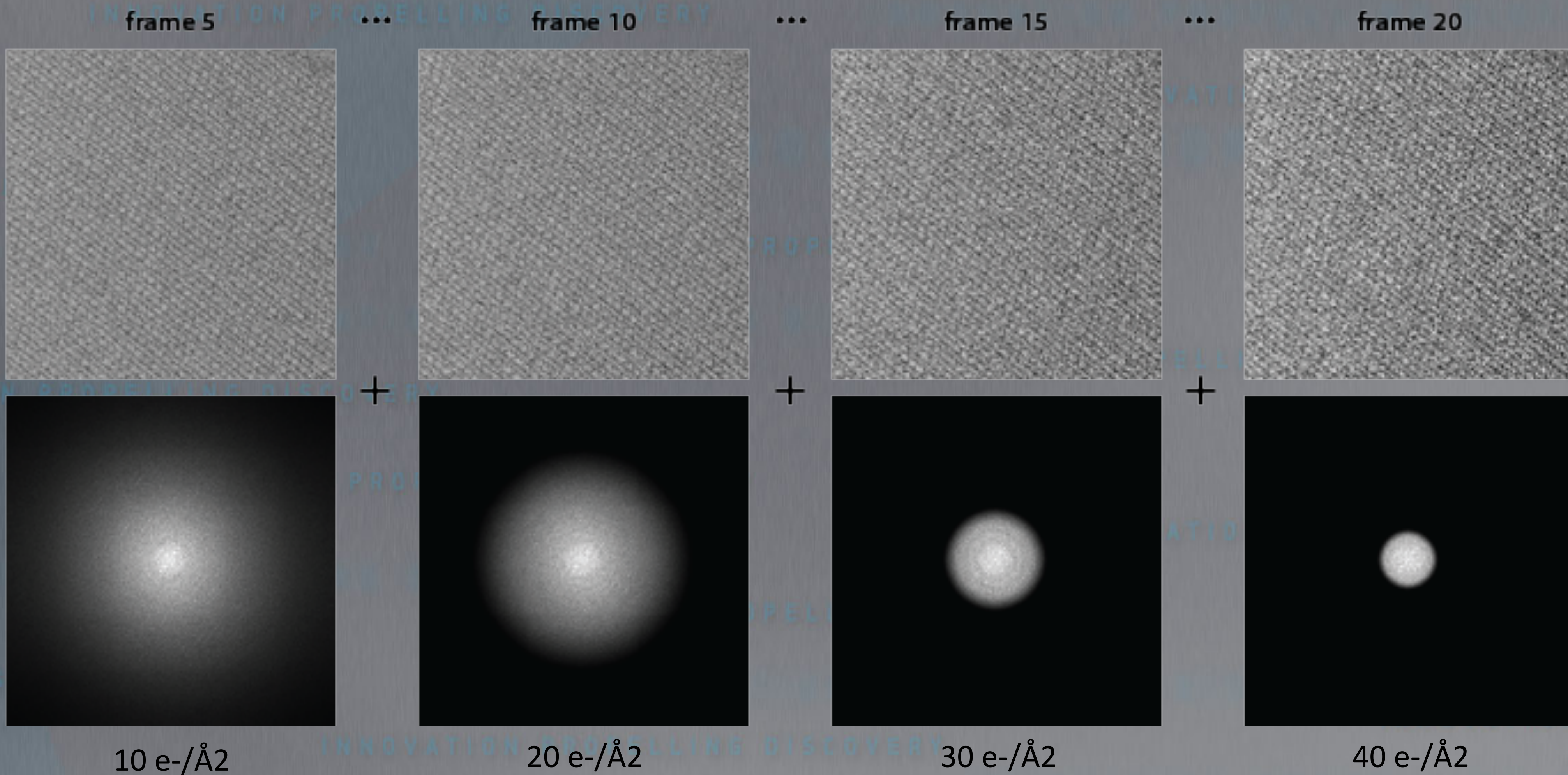


Movie-Mode Dose Fractionation

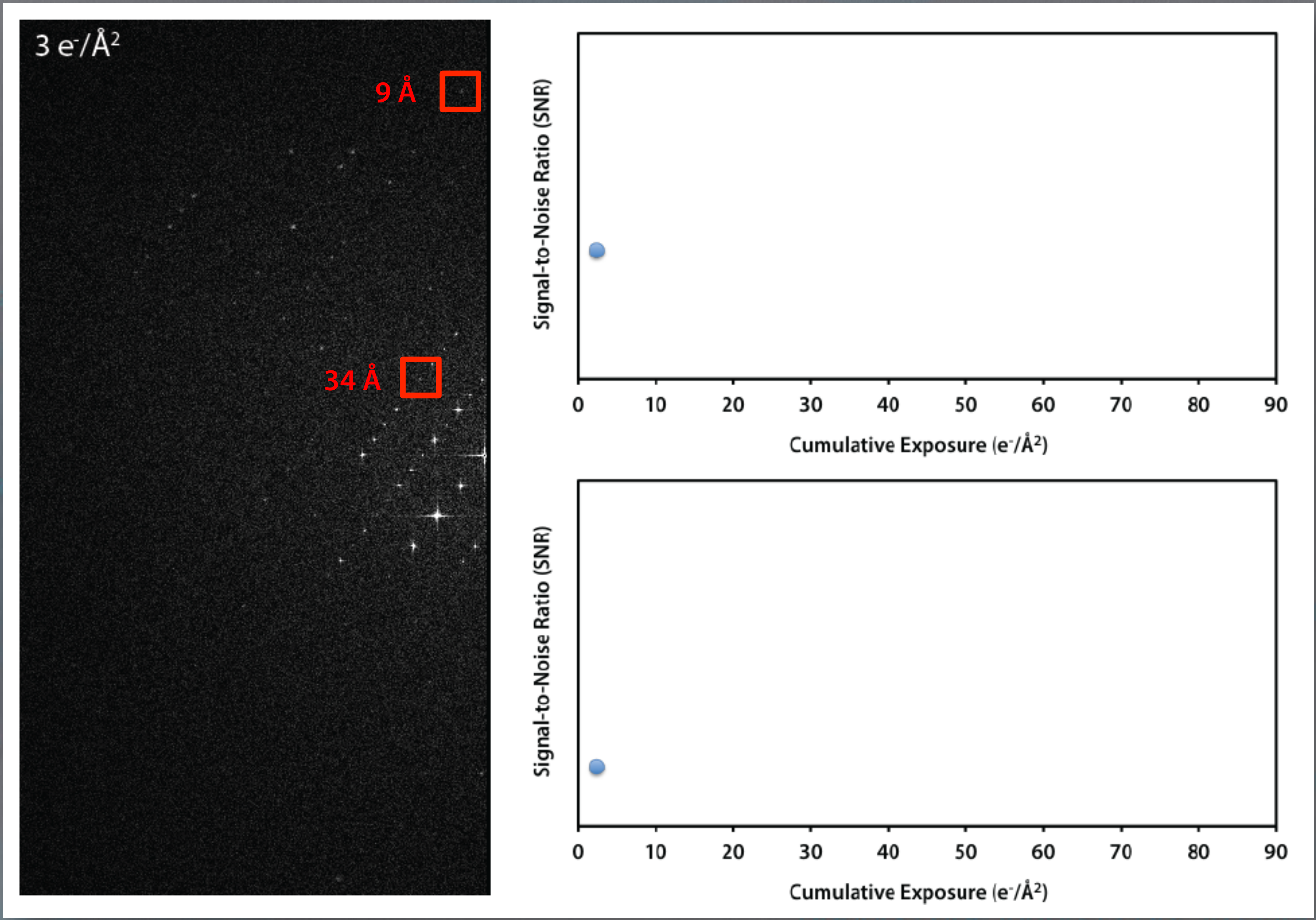


Direct Electron

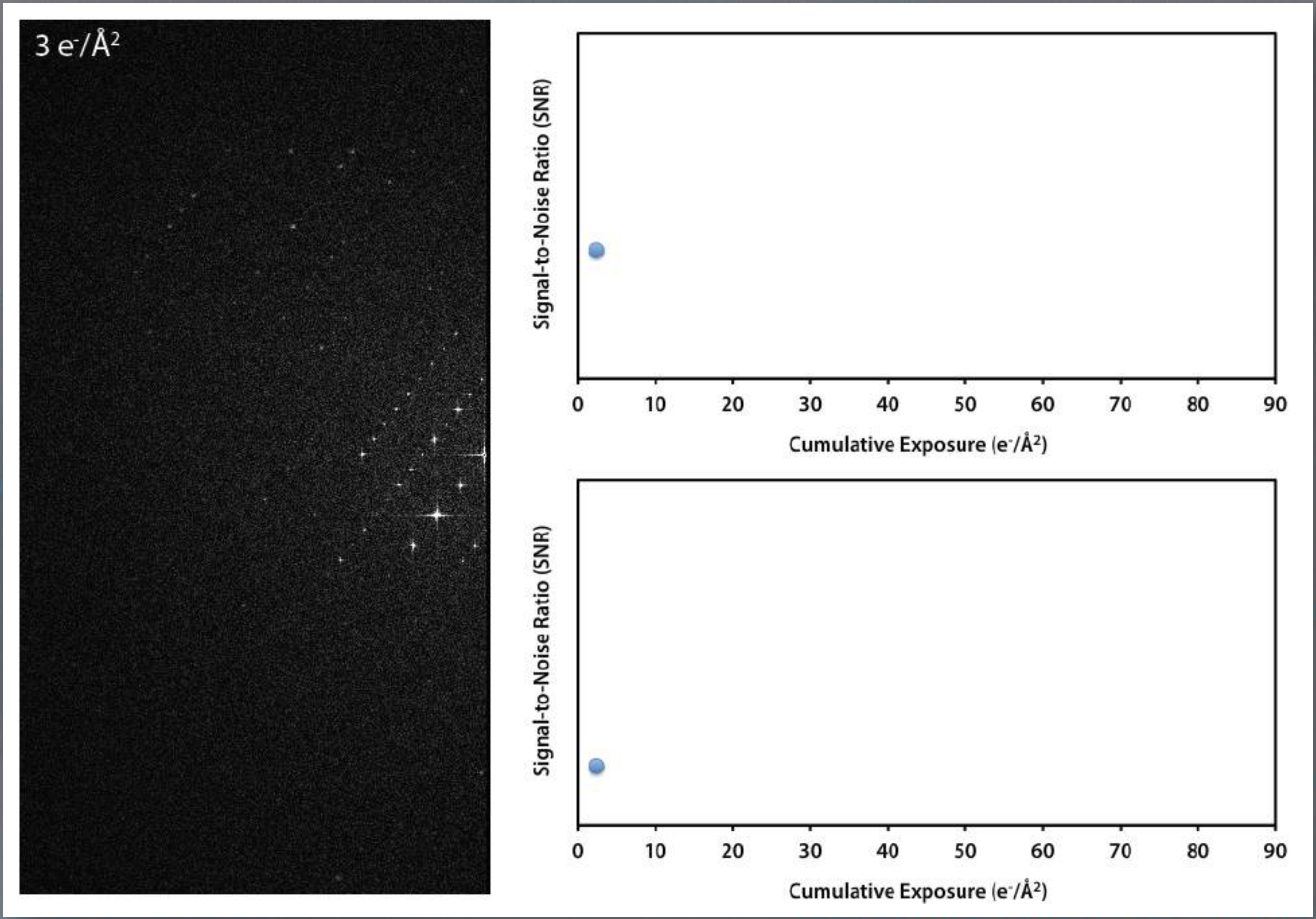
Movie-Mode Dose Fractionation



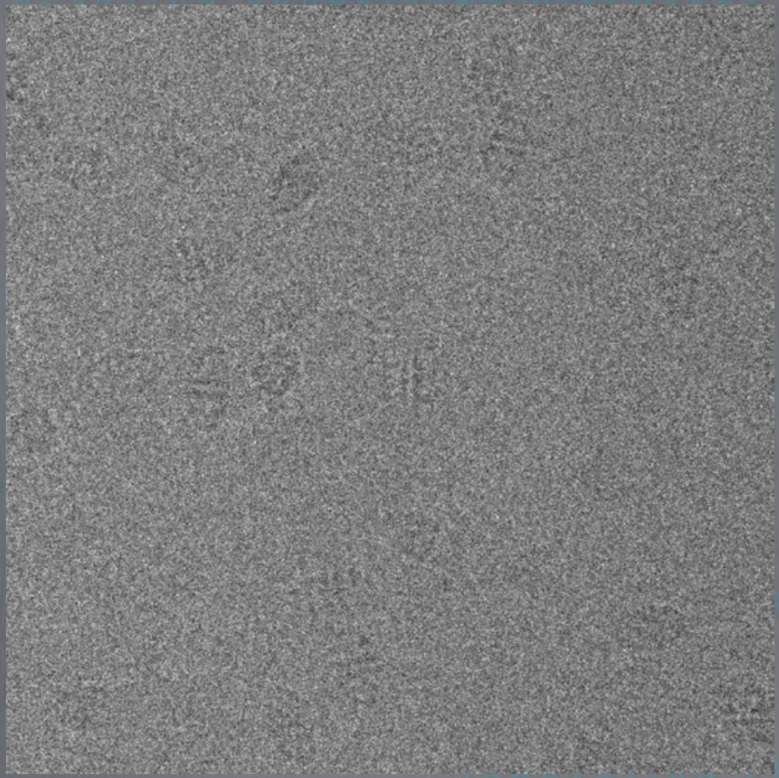
Damage Compensation on Catalase



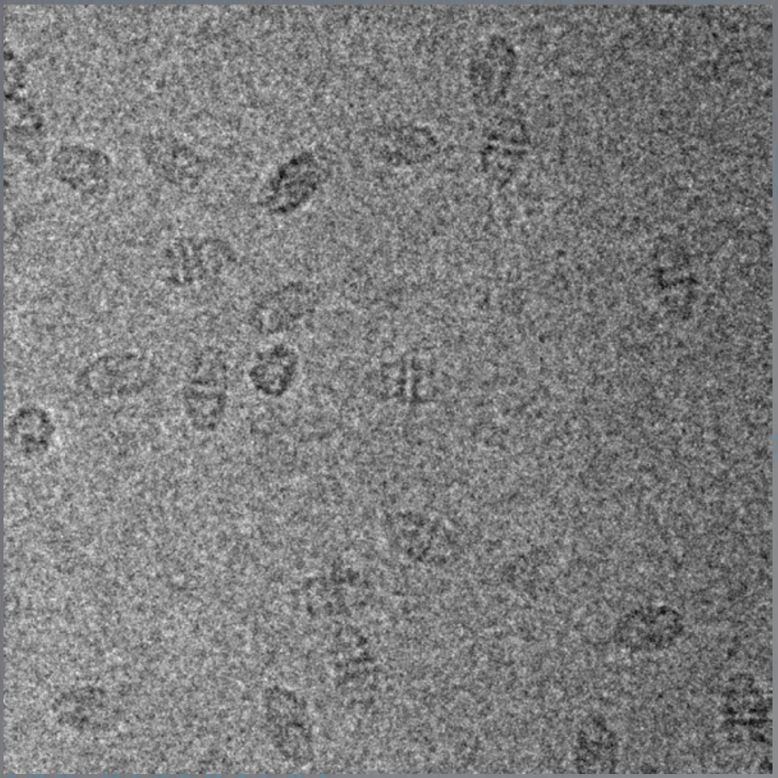
Damage Compensation on Catalase



Contrast Enhancement for GroEL

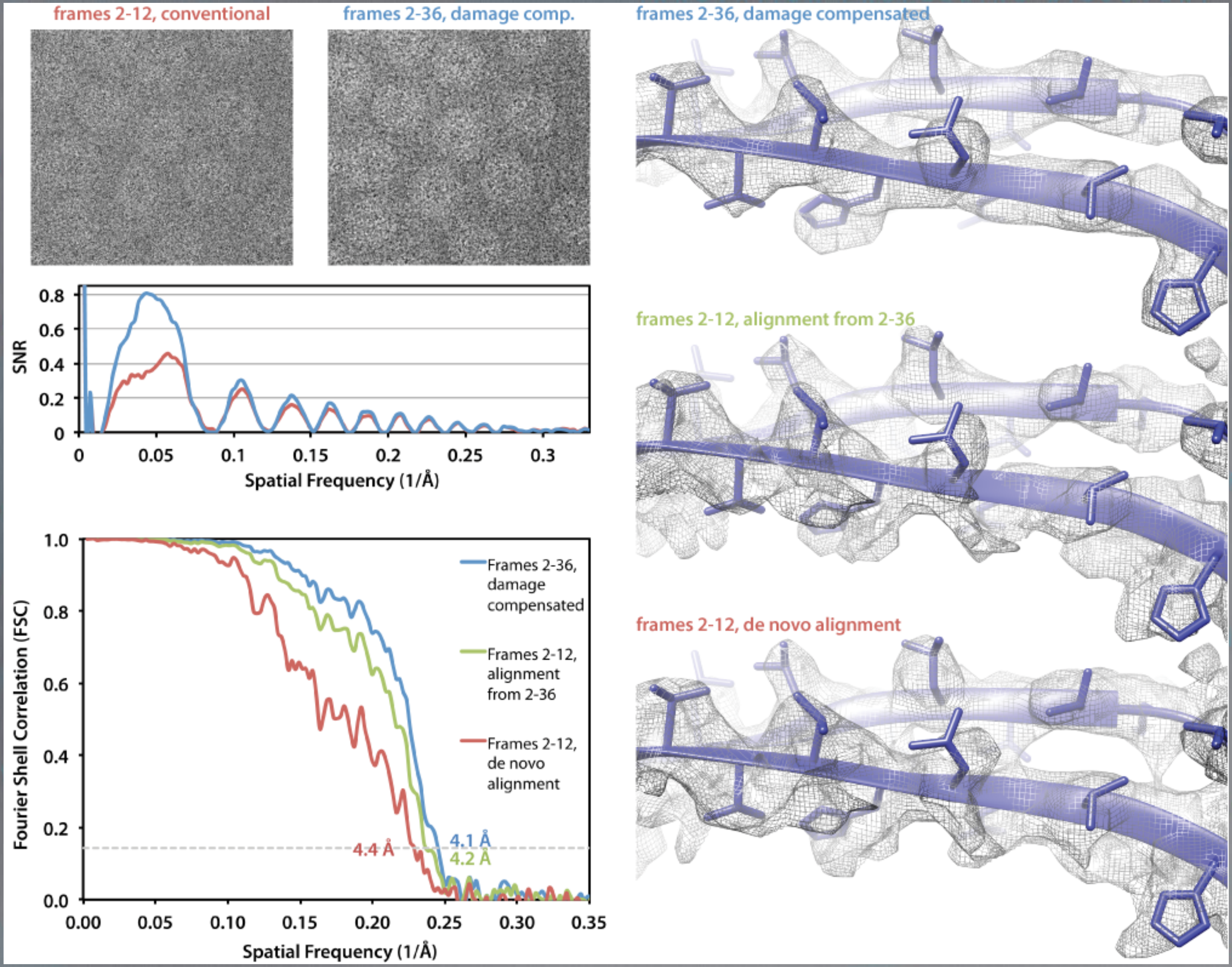


25 $e^-/Å^2$



100 $e^-/Å^2$

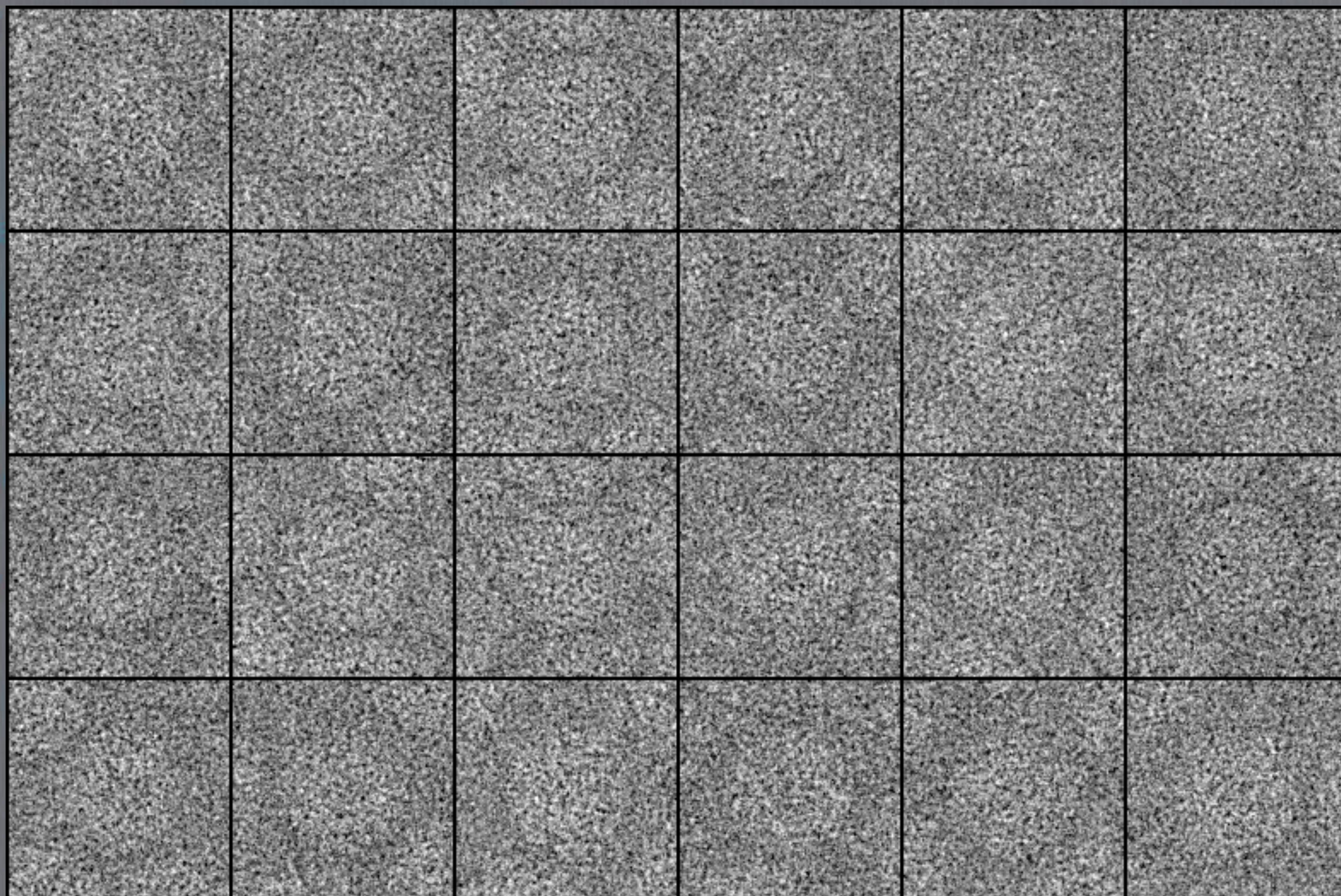
Example: BMV


frames 2-12, conventional
frames 2-36, damage comp.
SNR
0.8
0.6
0.4
0.2
0
0
0.05
0.1
0.15
0.2
0.25
0.3
Spatial Frequency (1/Å)
Fourier Shell Correlation (FSC)
1.0
0.8
0.6
0.4
0.2
0.0
0.00
0.05
0.10
0.15
0.20
0.25
0.30
0.35
Spatial Frequency (1/Å)
Frames 2-36, damage compensated
Frames 2-12, alignment from 2-36
Frames 2-12, de novo alignment
4.4 Å
4.1 Å
4.2 Å
frames 2-36, damage compensated
frames 2-12, alignment from 2-36
frames 2-12, de novo alignment

Example Result

Application: Single-Particle Cryo-EM

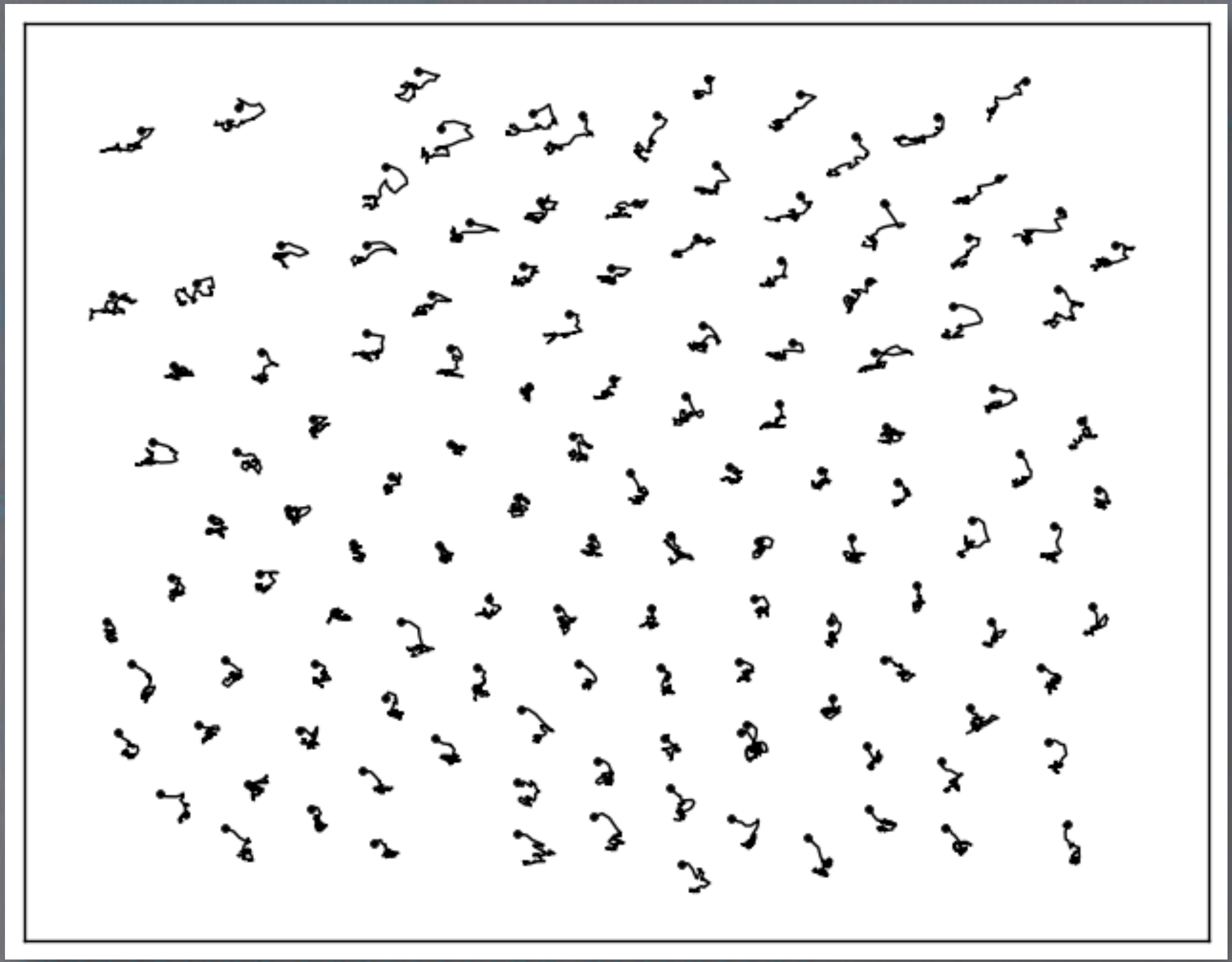
Brome mosaic virus (BMV) collected at 50k× magnification (1.07 Å/pixel) with a 2 s exposure and 50 $e^-/Å^2$, at ~0.7 µm defocus



Courtesy of Wah Chiu (BCM) and Wen Jiang (Purdue).

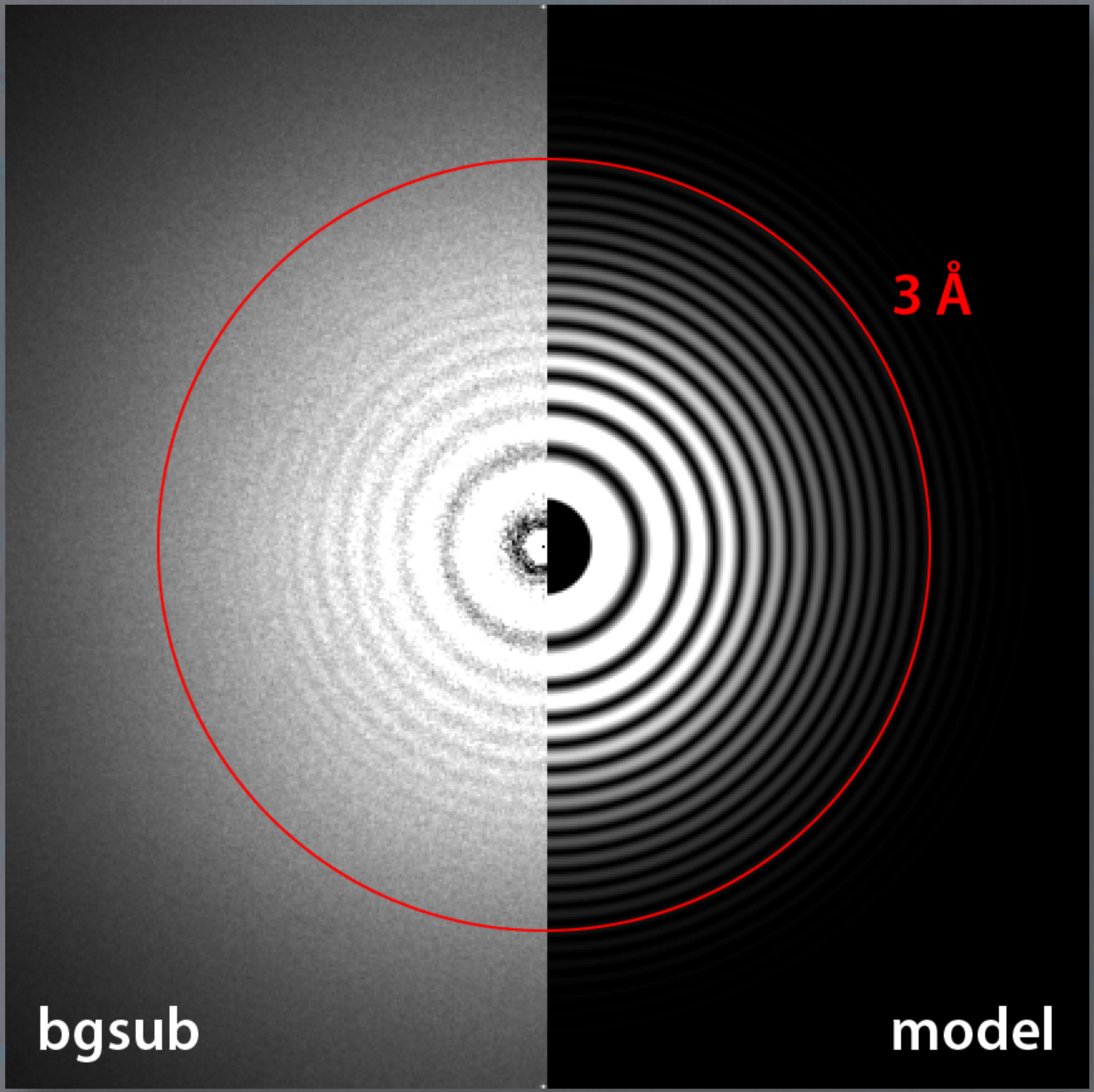
Per-Particle Motion Correction of BMV

Vector plot of the motion of each BMV particle in one image.
Vectors are magnified by 50× to improve visualization.



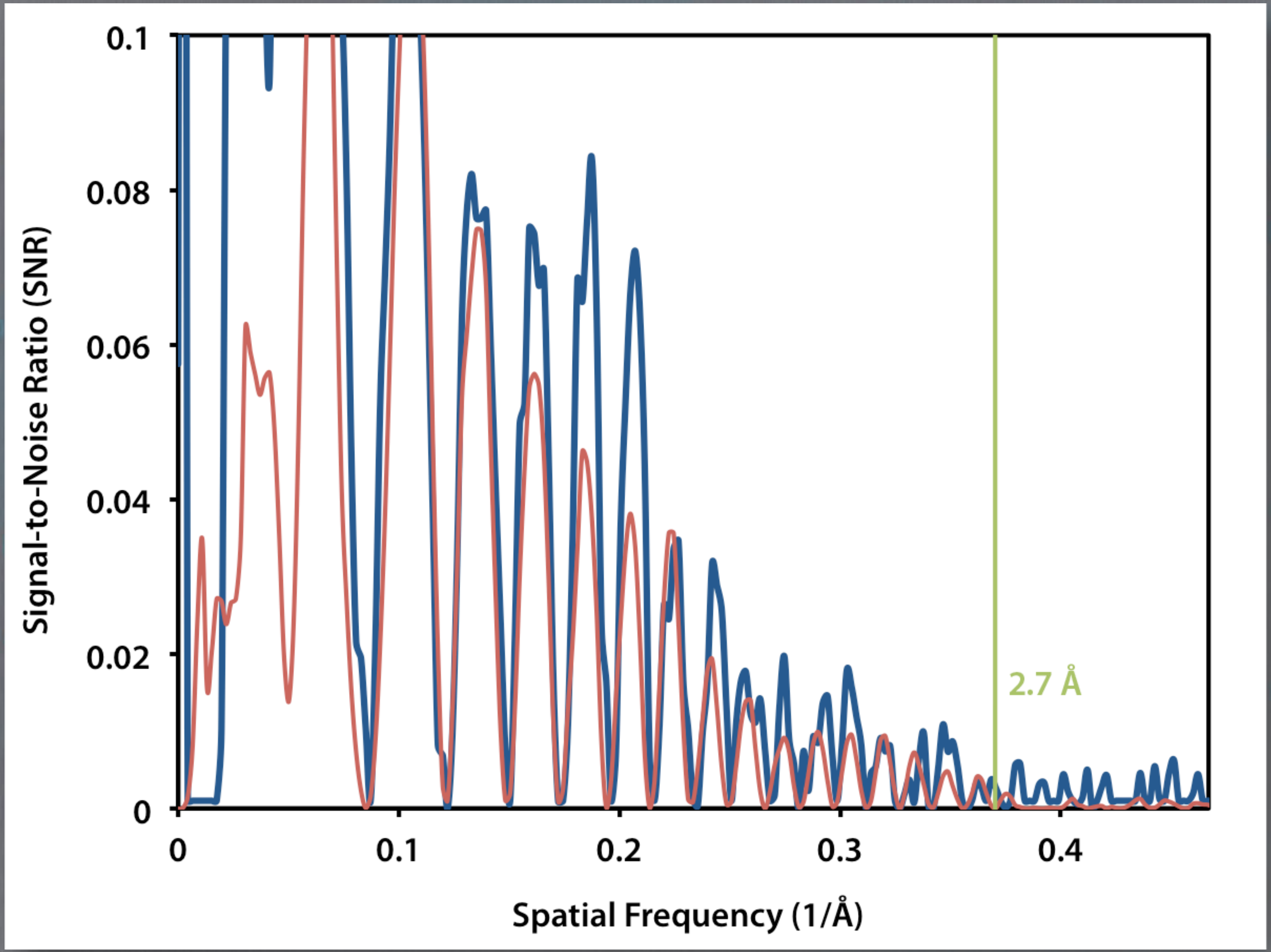
Courtesy of Wah Chiu (BCM) and Wen Jiang (Purdue).

Thon Rings for a Stack of BMV Particles



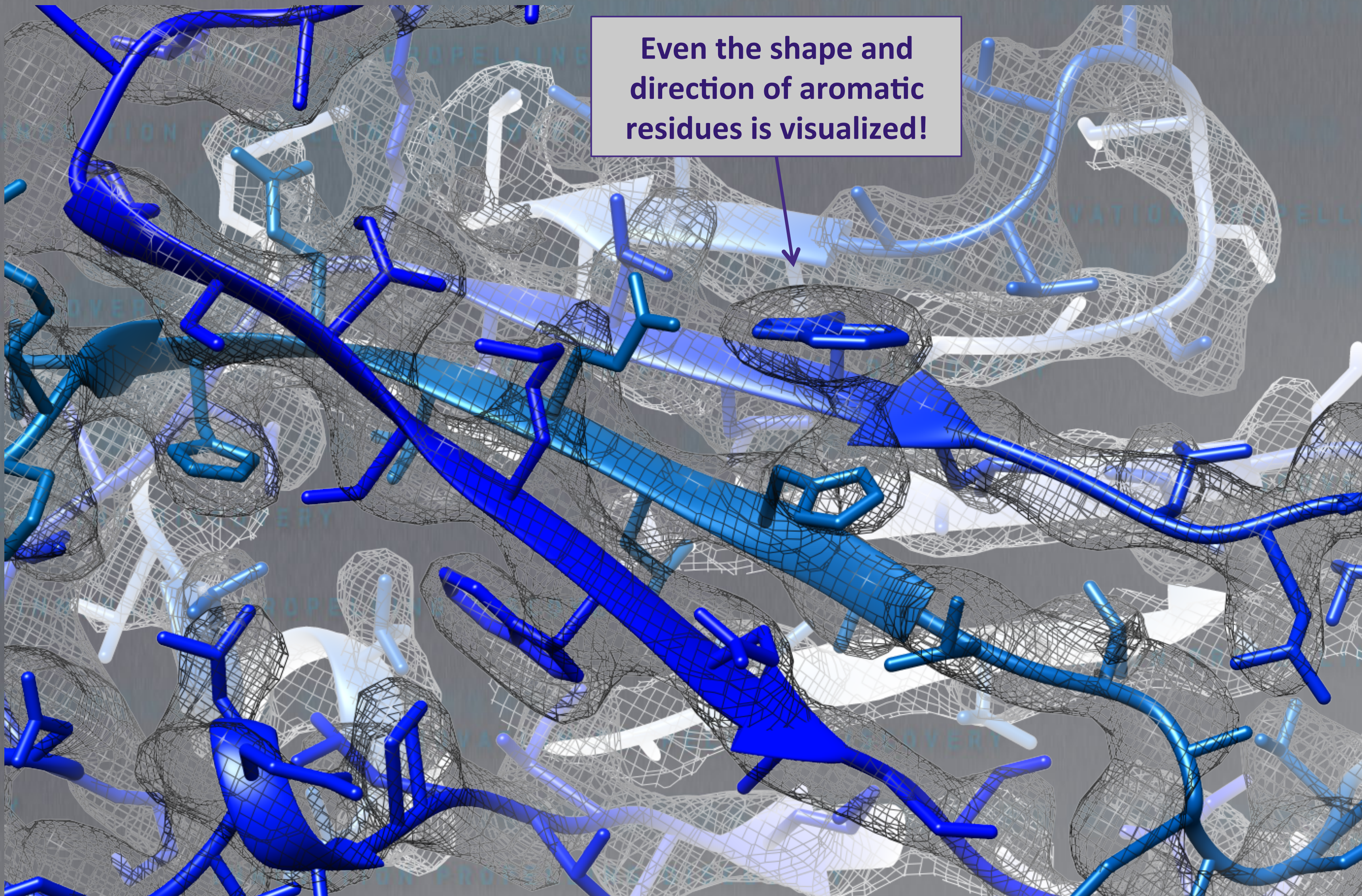
Courtesy of Wah Chiu (BCM) and Wen Jiang (Purdue).

SNR Plot Has Signal Up to 2.7 Å!



Courtesy of Wah Chiu (BCM) and Wen Jiang (Purdue).

De Novo 3D Reconstruction



Courtesy of Wah Chiu (BCM) and Wen Jiang (Purdue).

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