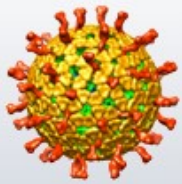
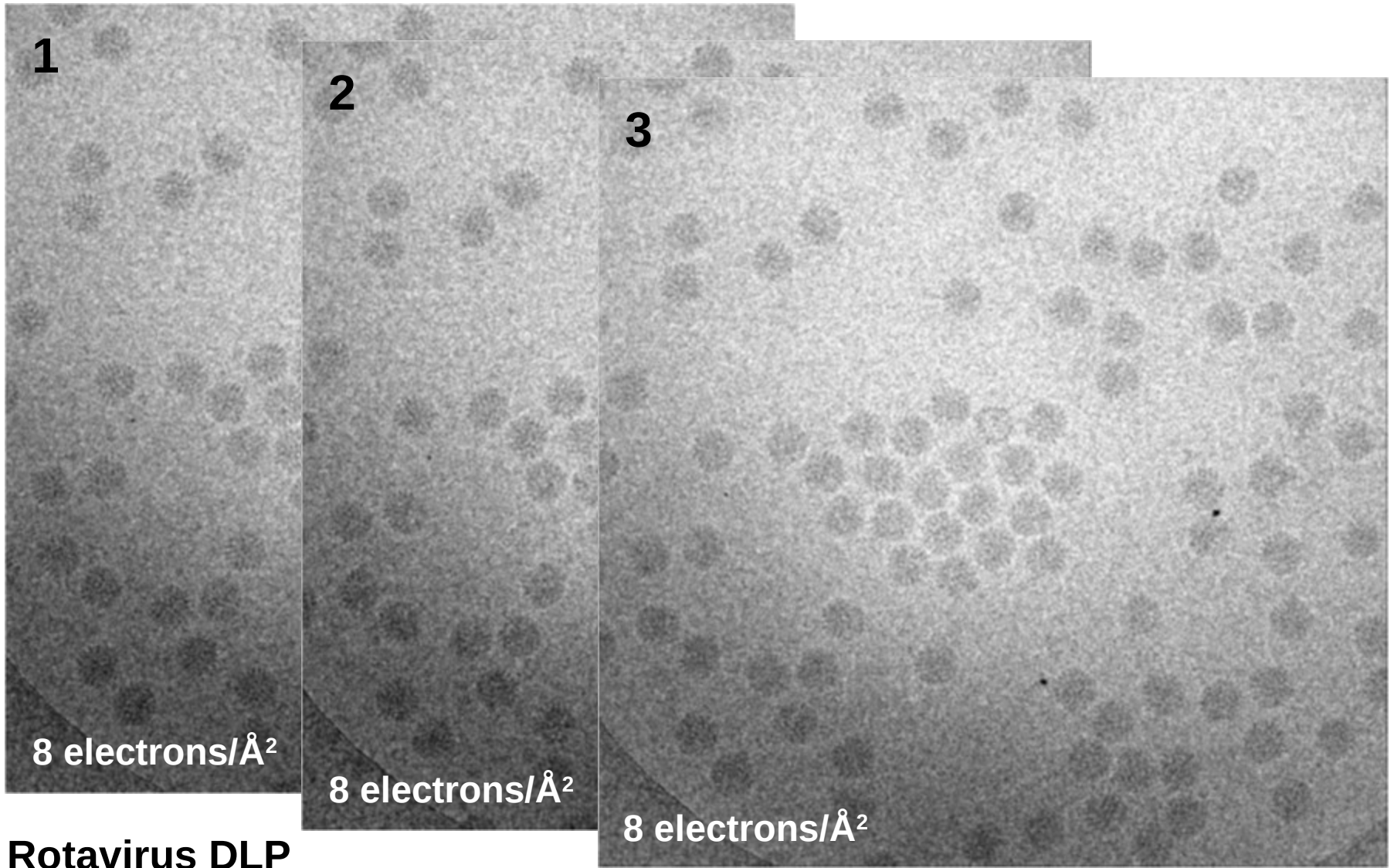


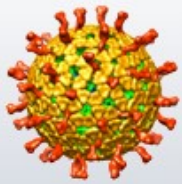
Data Collection with the DE-12 Camera

**Nikolaus Grigorieff
Brandeis University & TSRI**

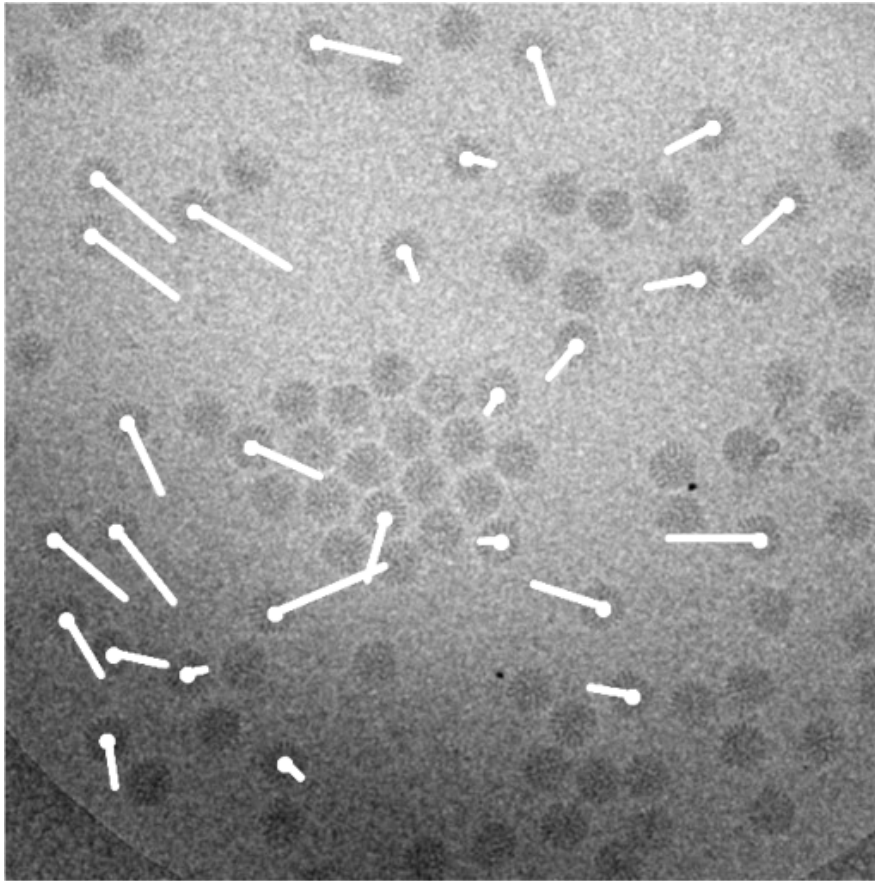


Exposure Series



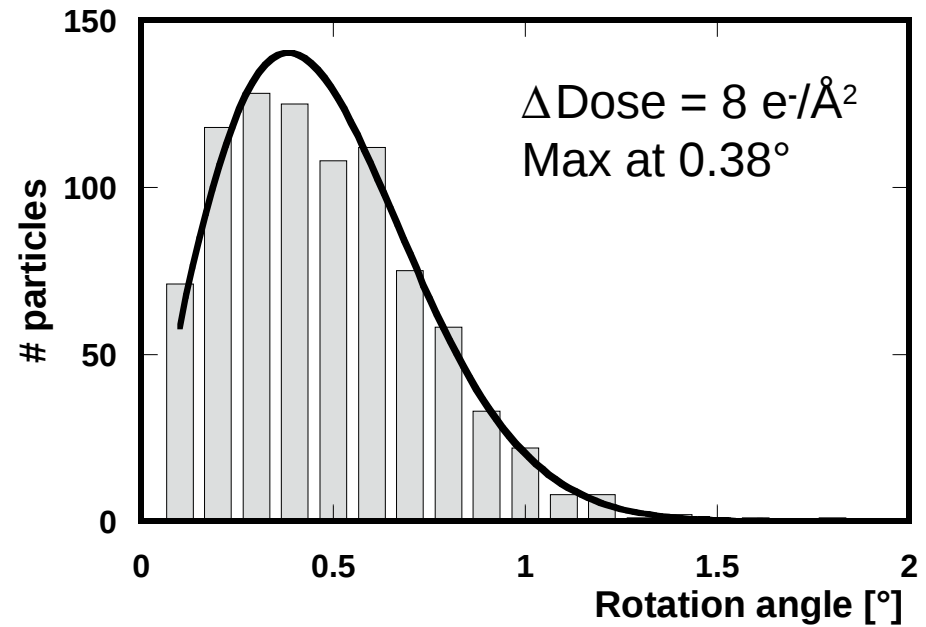


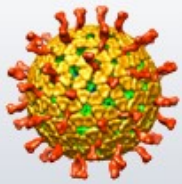
Mapping Rotations



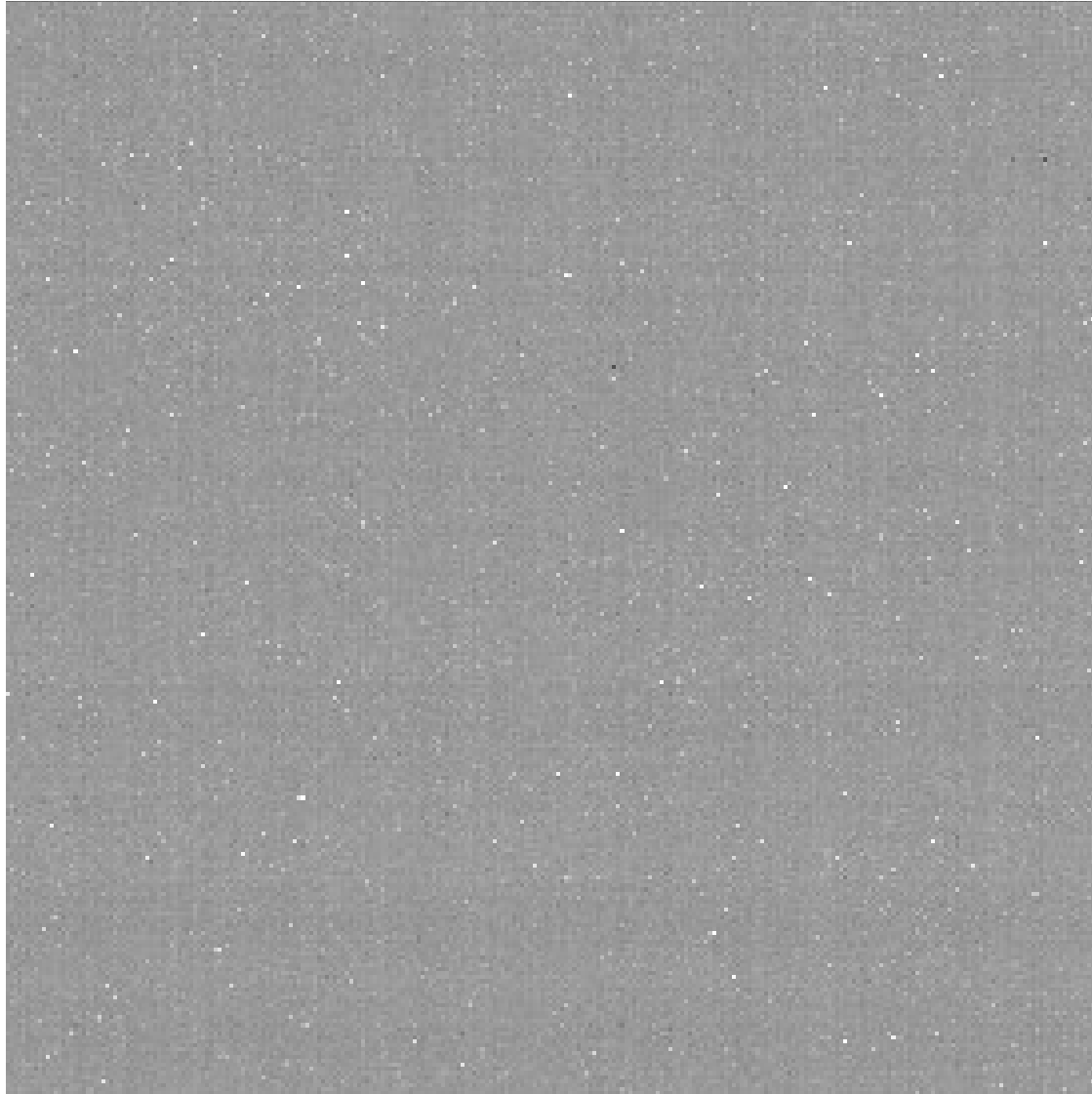
Exposure 1 → 2

Longest vectors
correspond to
~2 deg rotations.





Movies

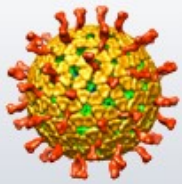


Recorded with
**direct electron
detector DE-12**
(Direct Electron)

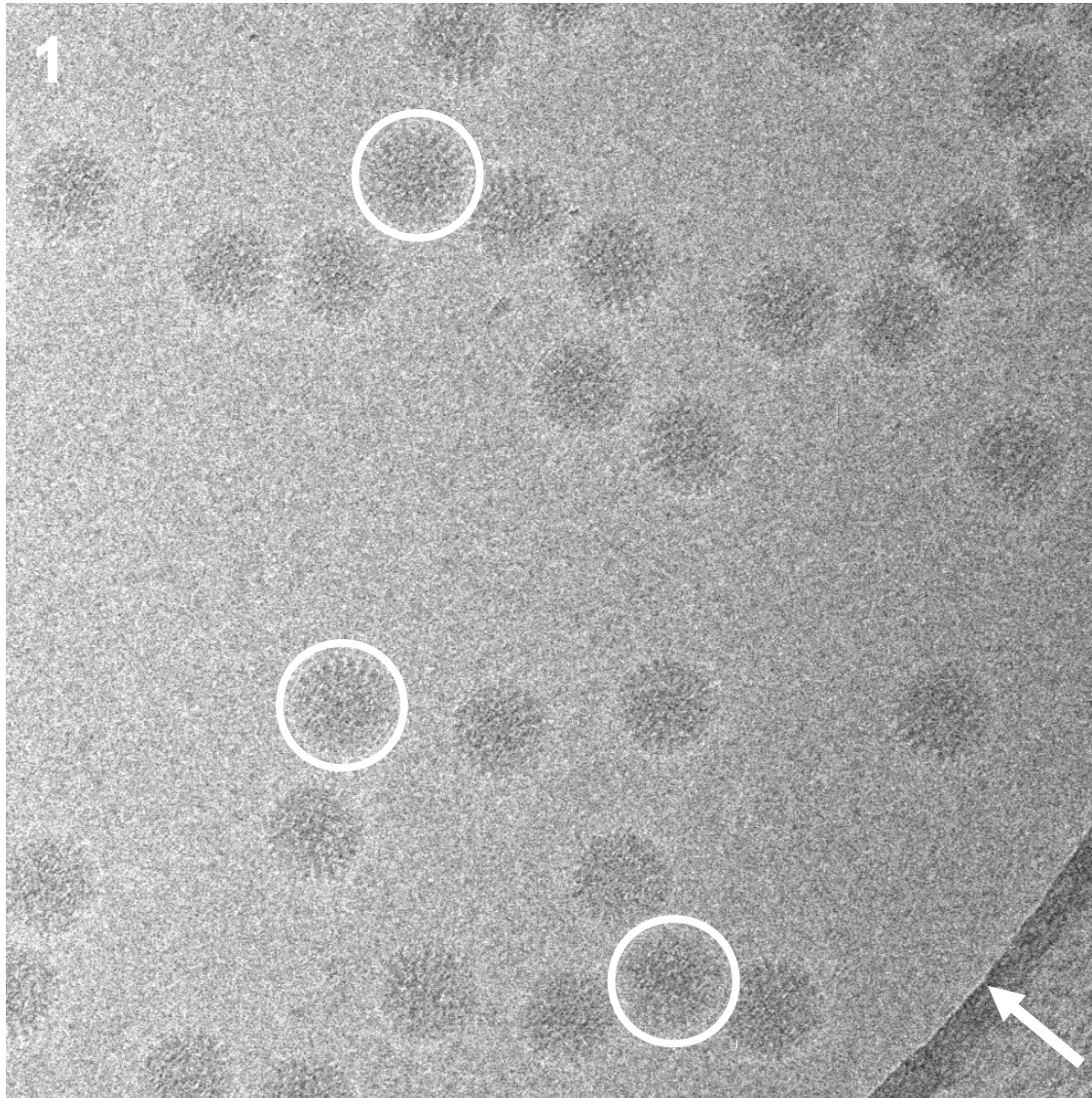
Frame rate = 40 fps
Dose/frame = $0.5 \text{ e}^-/\text{\AA}^2$
Duration = 1.5 s
No. of frames = 60
Total dose = $30 \text{ e}^-/\text{\AA}^2$

1 movie = 720 MB
(1 byte/pixel)

→ **Data Tsunami!**

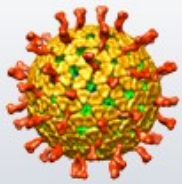


10-Frame Averages

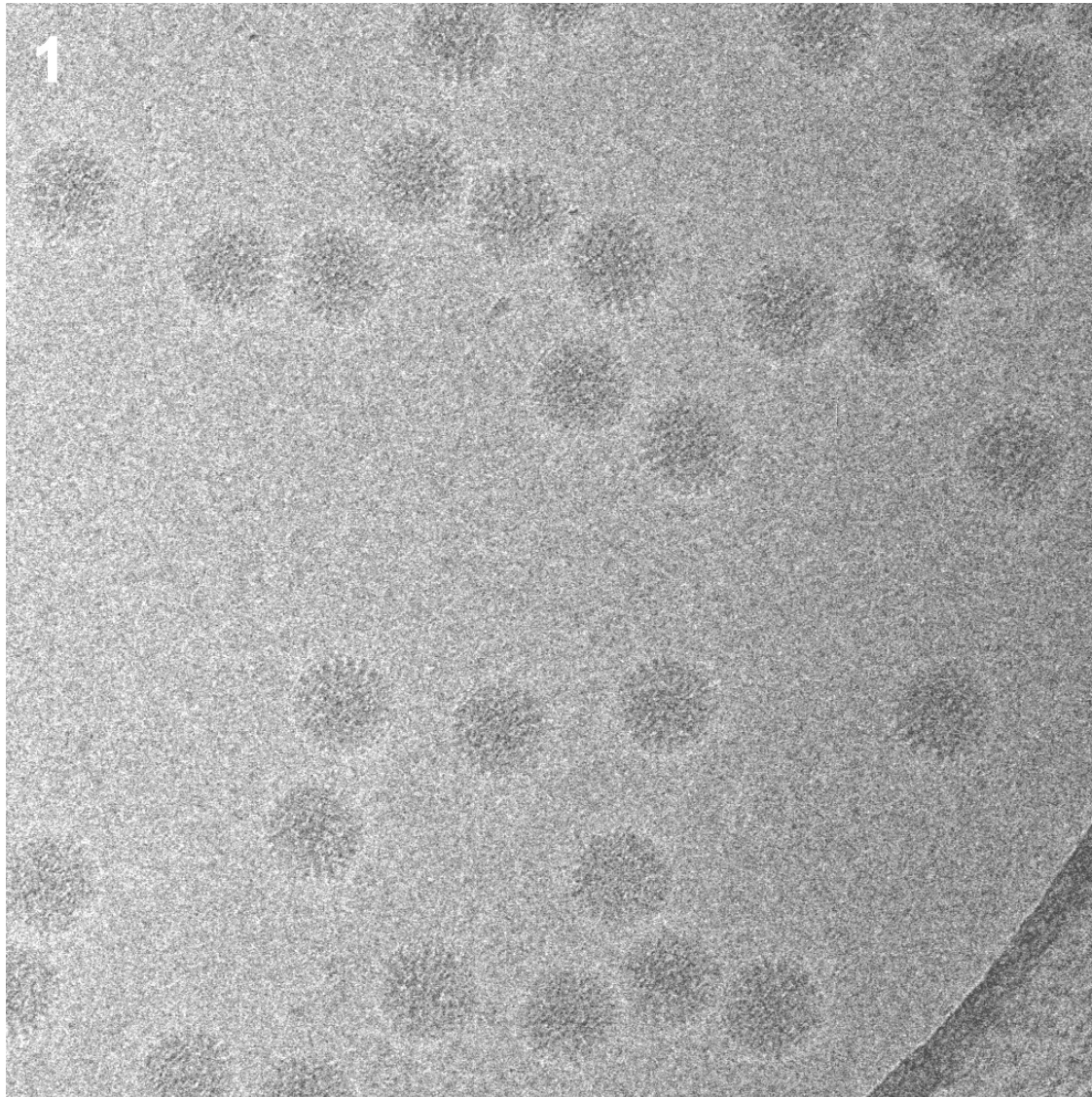


Each averaged frame
corresponds to 0.25 s.

Dose/frame = $5 \text{ e}^-/\text{\AA}^2$



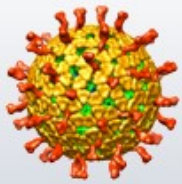
Rotations Over Time



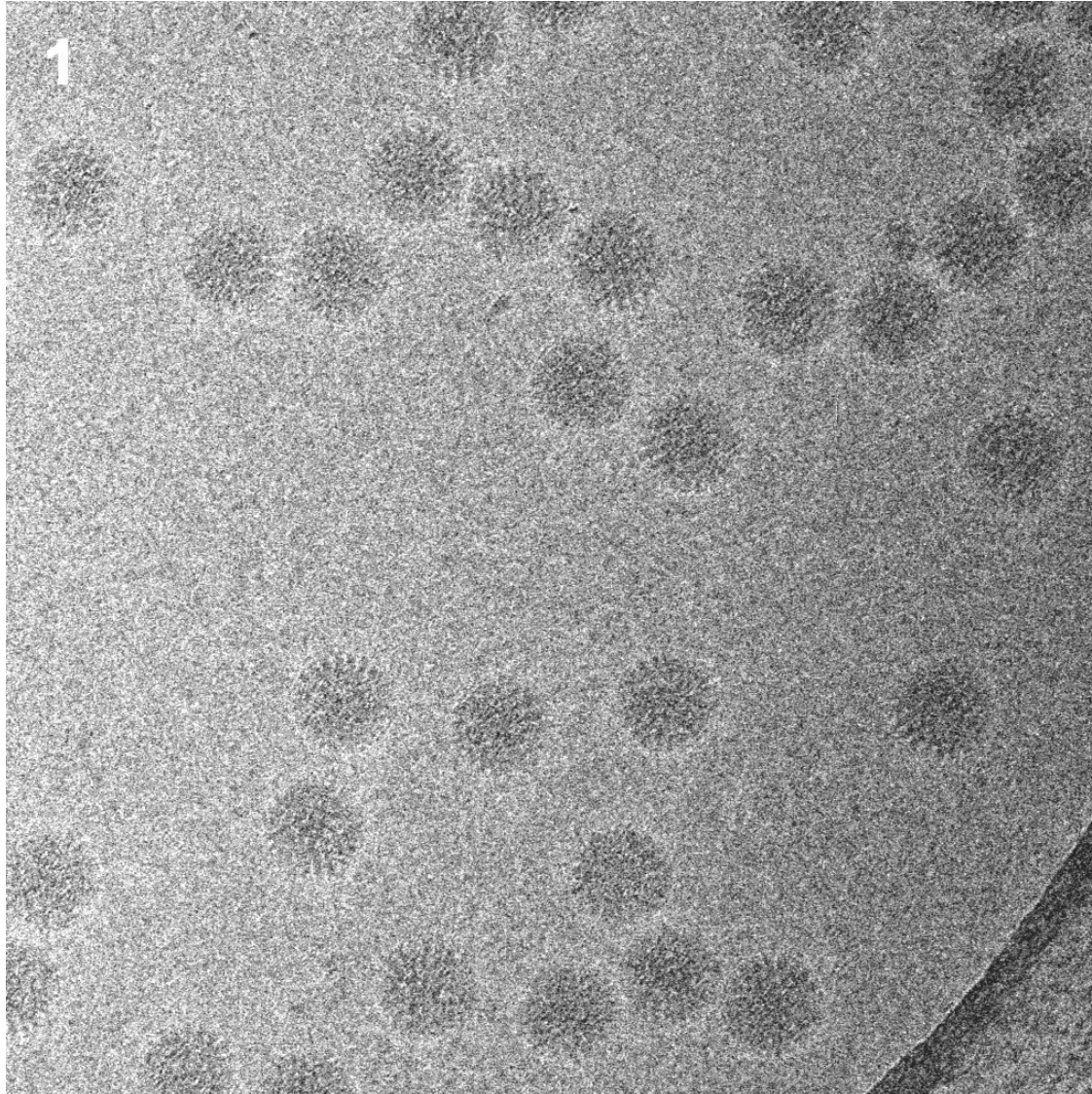
Vectors

2.5° deg





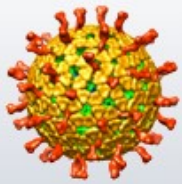
Translations Over Time



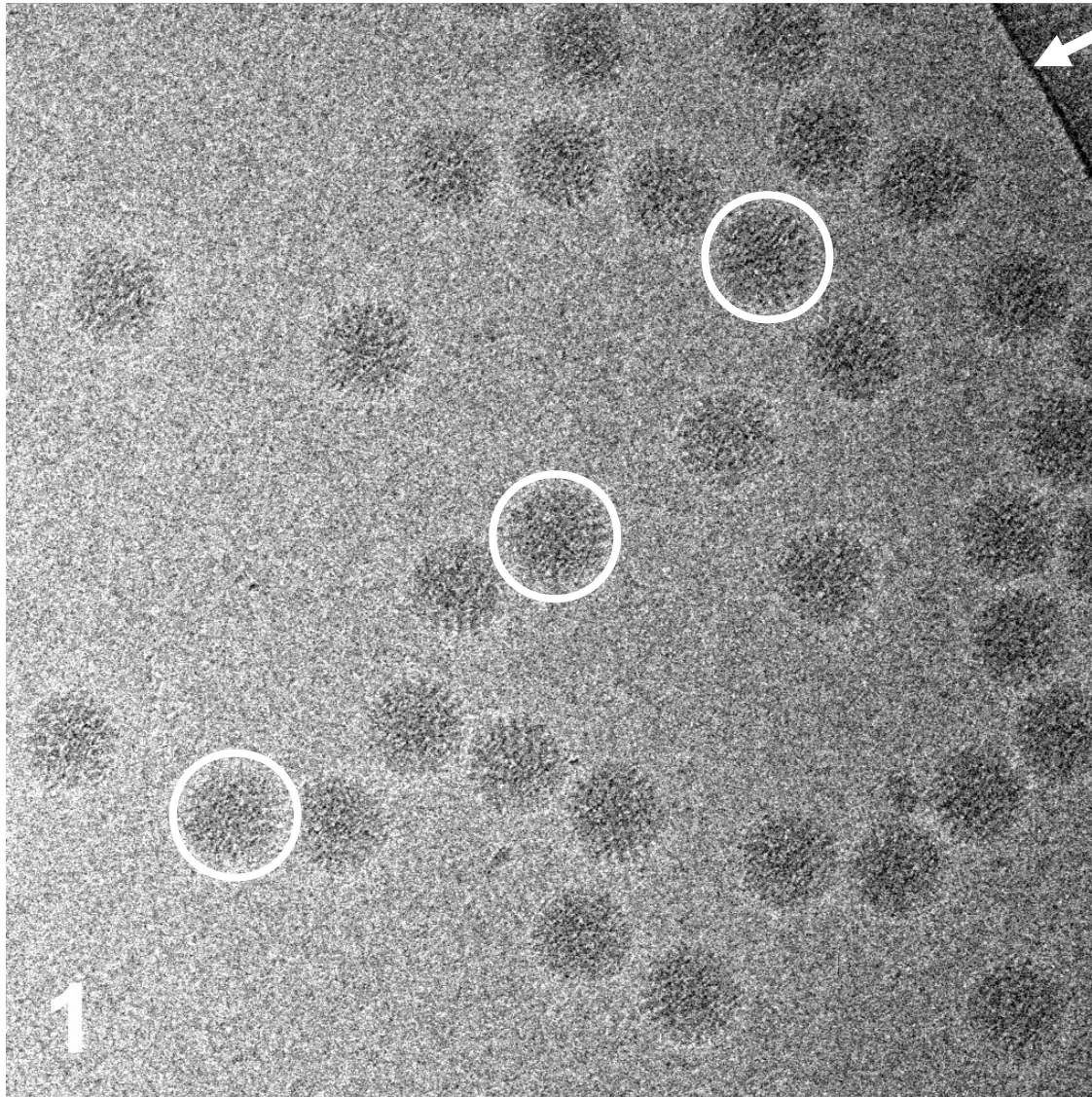
Vectors

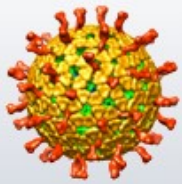
25 Å





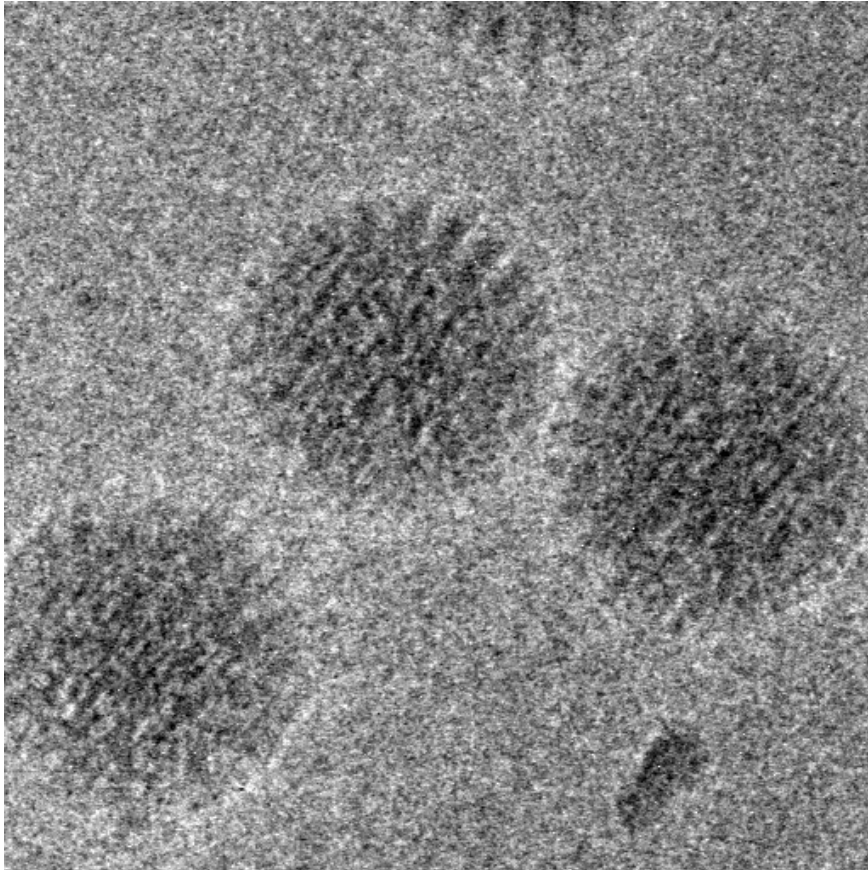
Different Corner



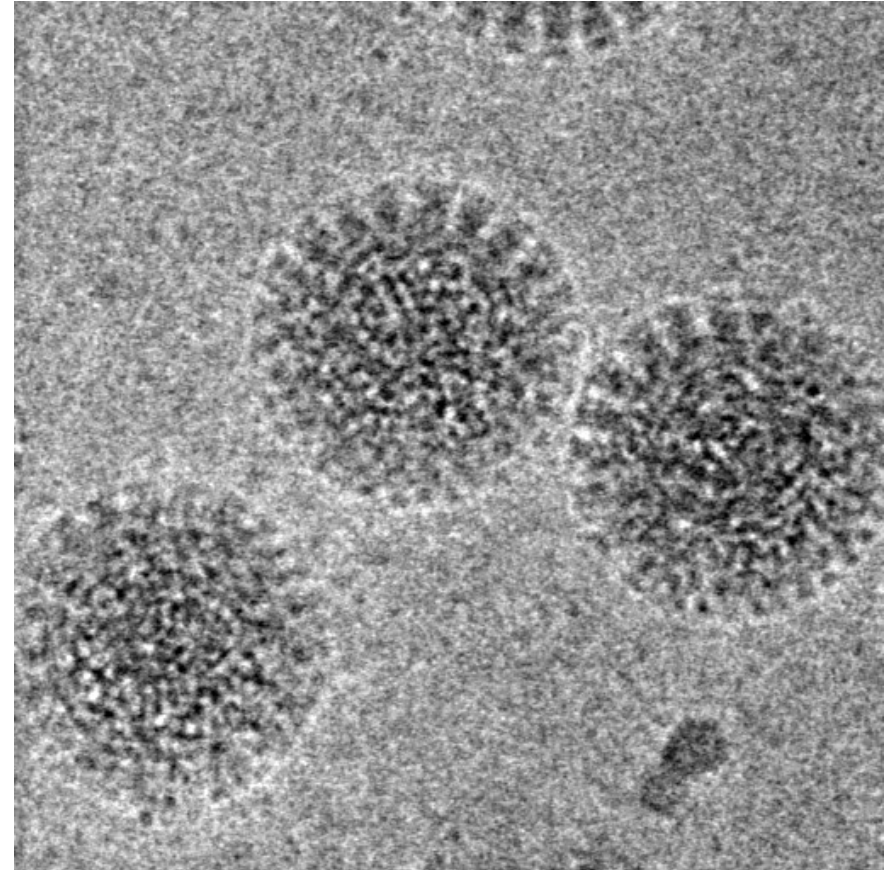


Frame Alignment

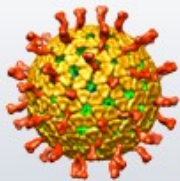
60-frame average
(no alignment)



60-frame average
(translational alignment)

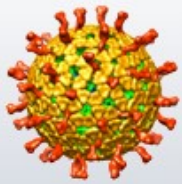


Rotation: ~ 1 deg in this area \rightarrow signal at 10 \AA attenuated by 70% ($B\text{-factor} = 500 \text{ \AA}^2$)

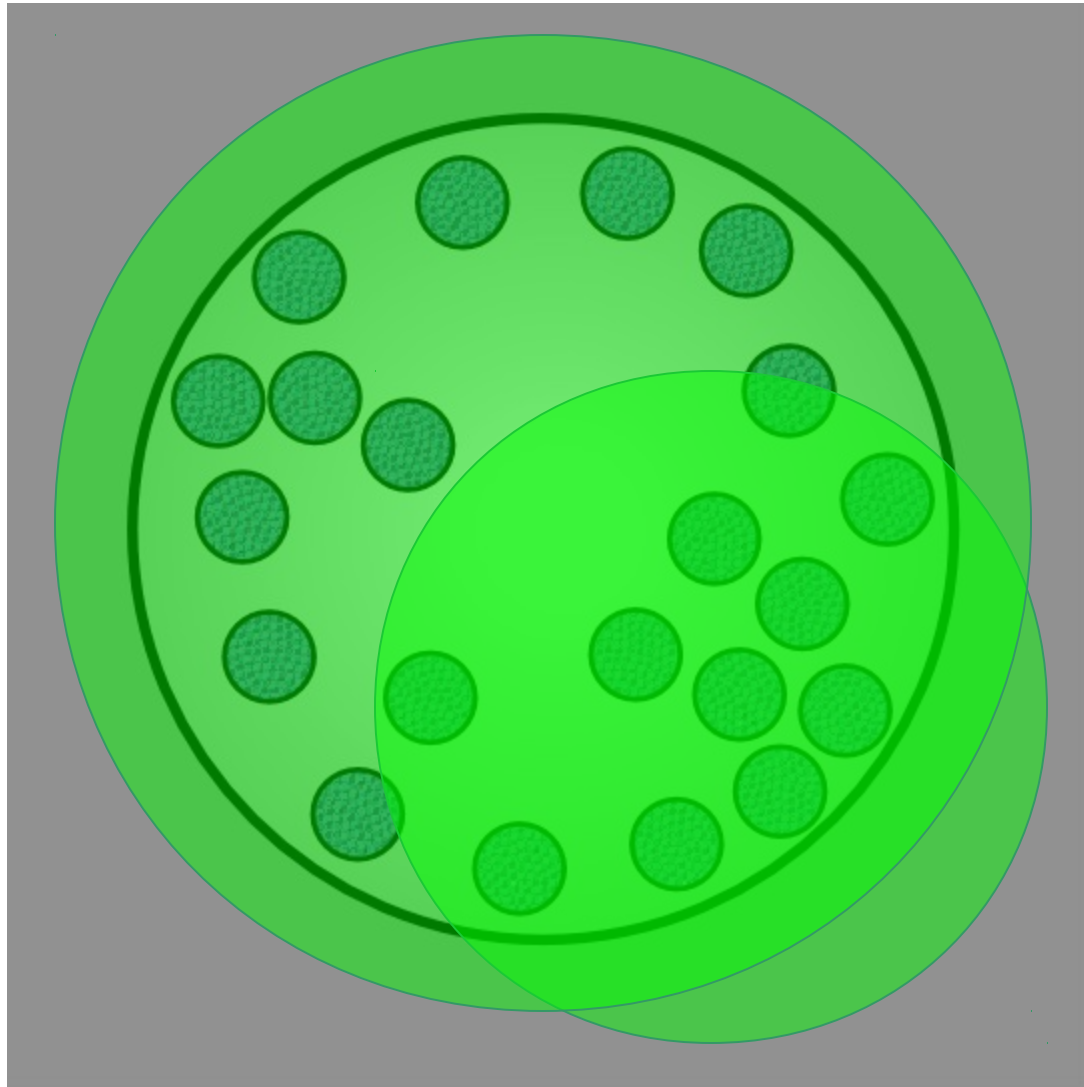


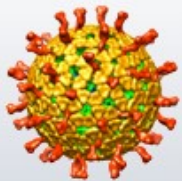
Larger Data Set

- Microscope: FEI TF20 (200 kV)
- Detector: DE-12 (4k x 3k, direct)
- Frame rate: 25 fps
- Frames: 18 (2 dark + 16 light)
- Dose/frame: 2 electrons/Å²
- Total dose: 32 electrons/Å²
- Grids: C-flat 1.2/1.3 μm
- Pixel size: 1.42 Å
- Movies collected: 501
- Particles: 1915 total, 807 used



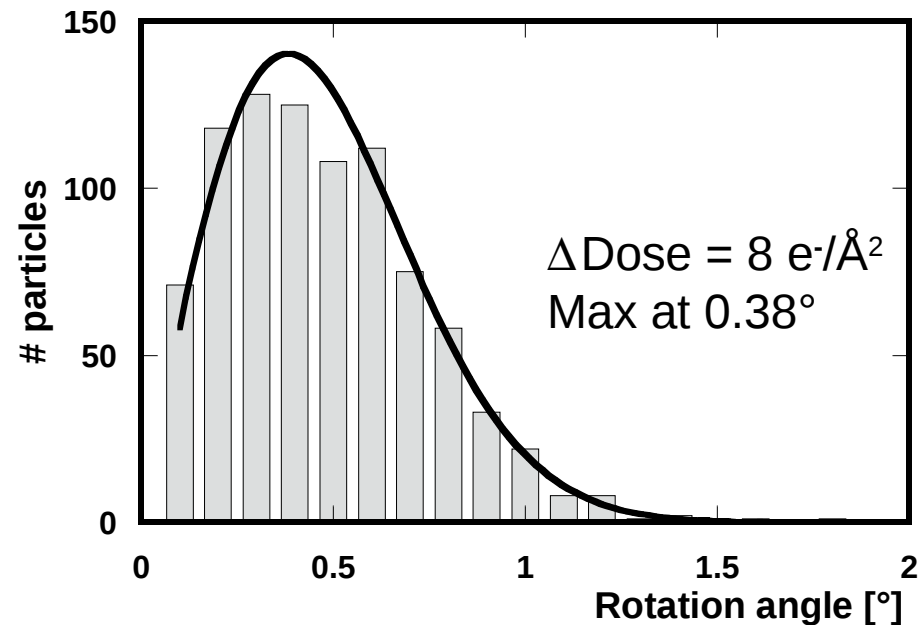
Rotavirus DLP Data Set



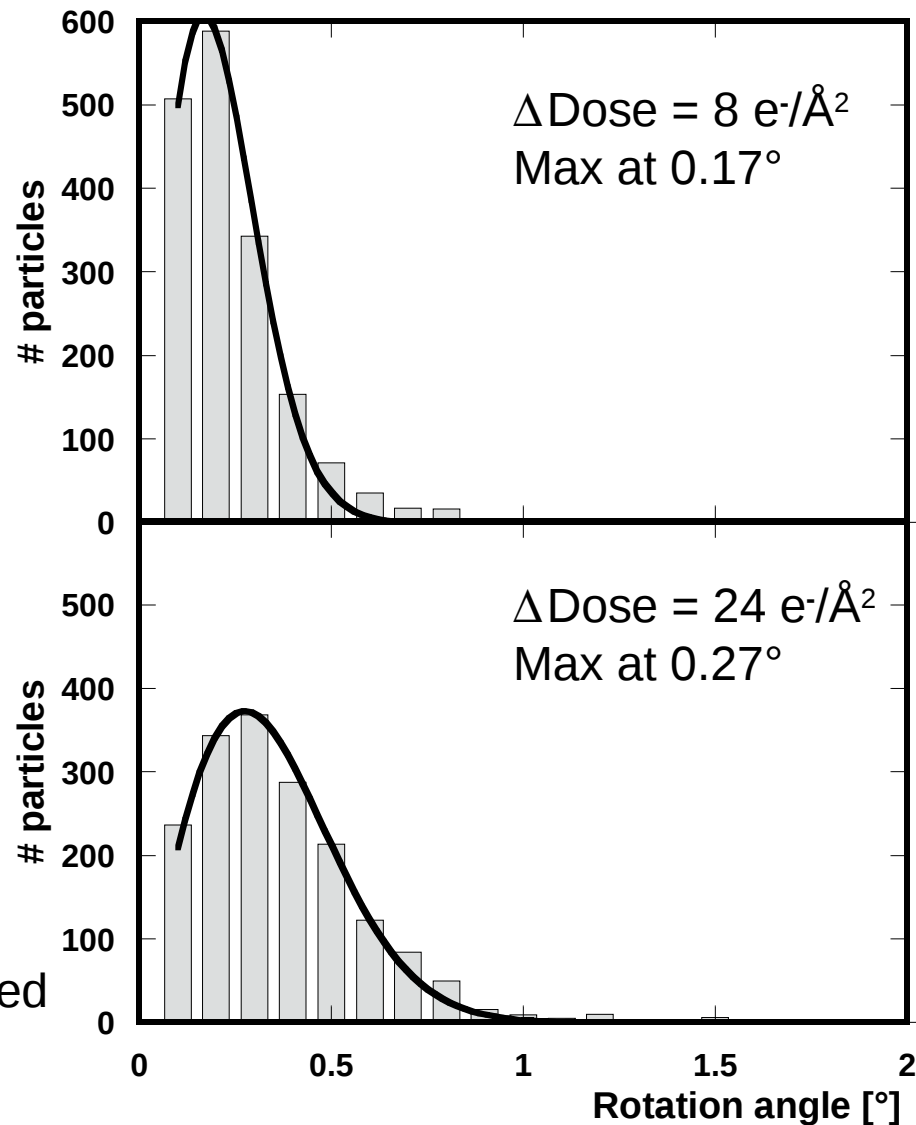


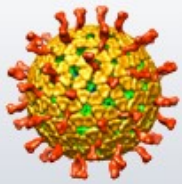
Rotation Histograms

Entire hole exposed

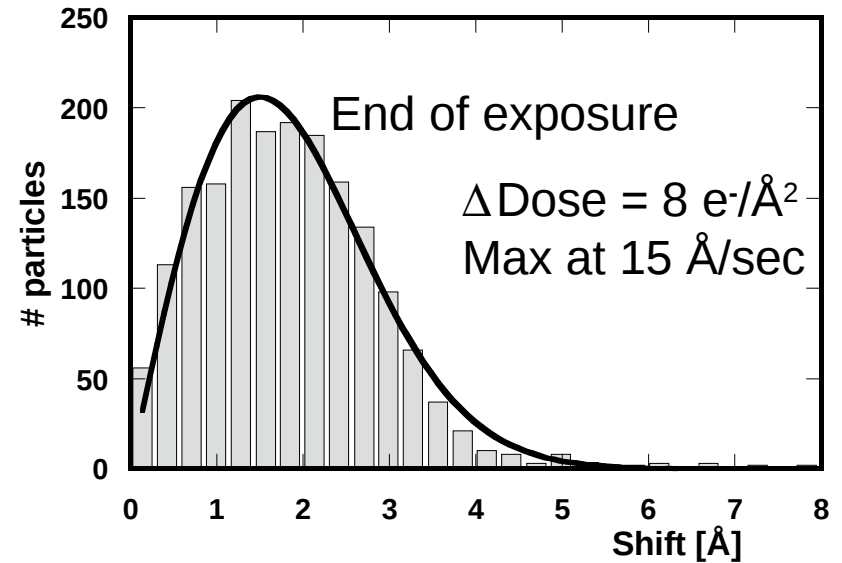
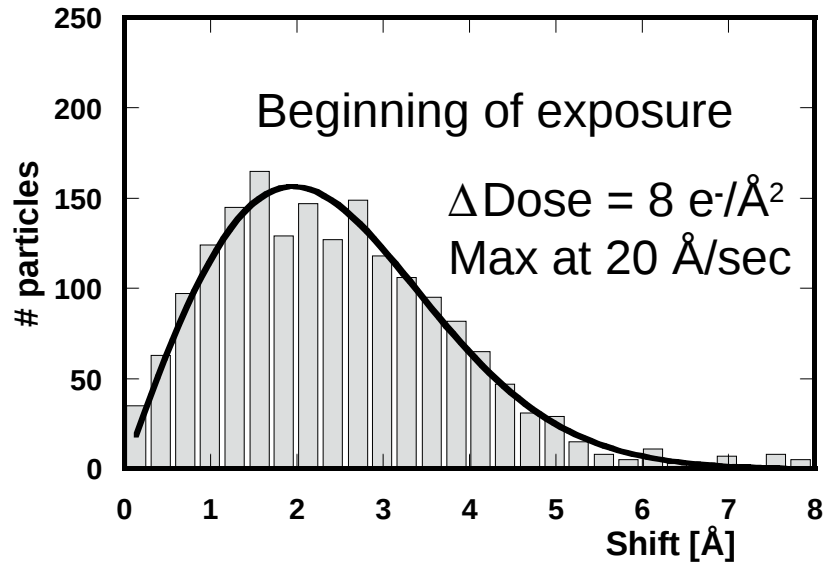


One side of hole exposed

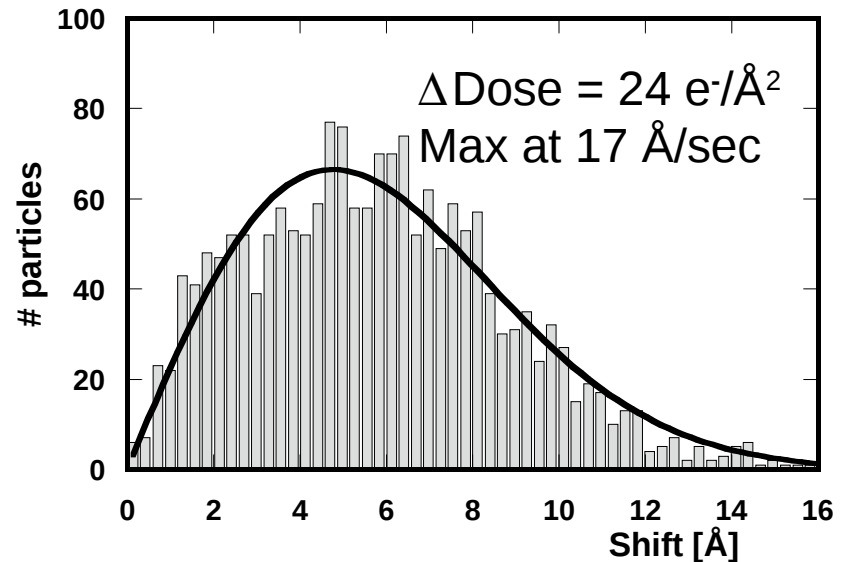


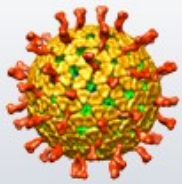


Translation Histograms



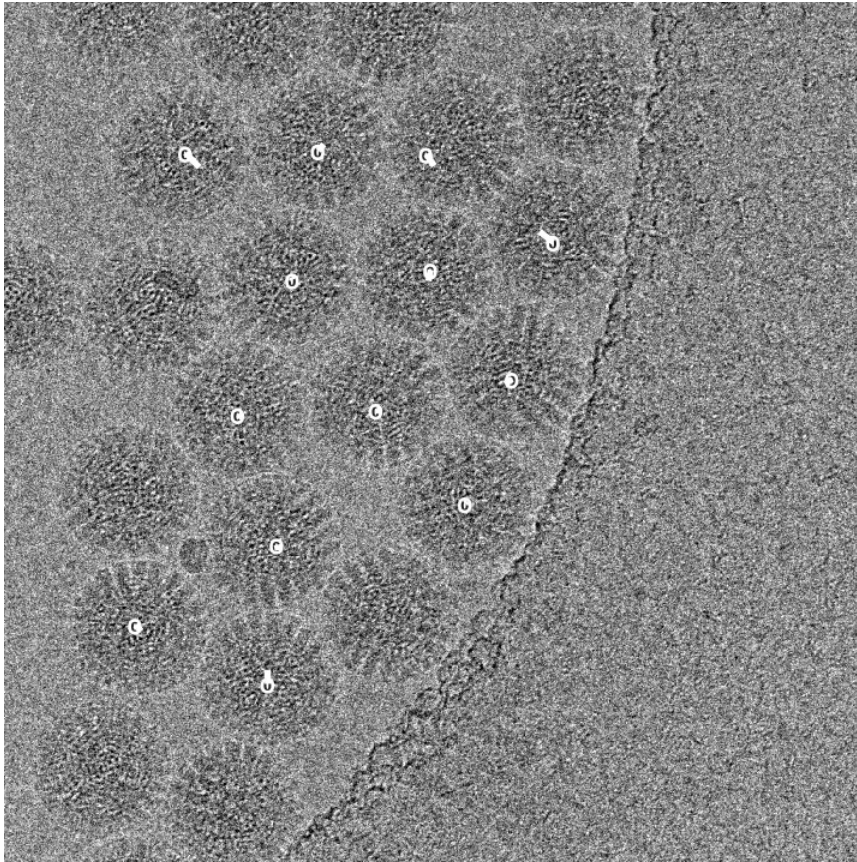
Sample drift $\sim 2 \text{ \AA}/\text{sec}$ (Leginon)





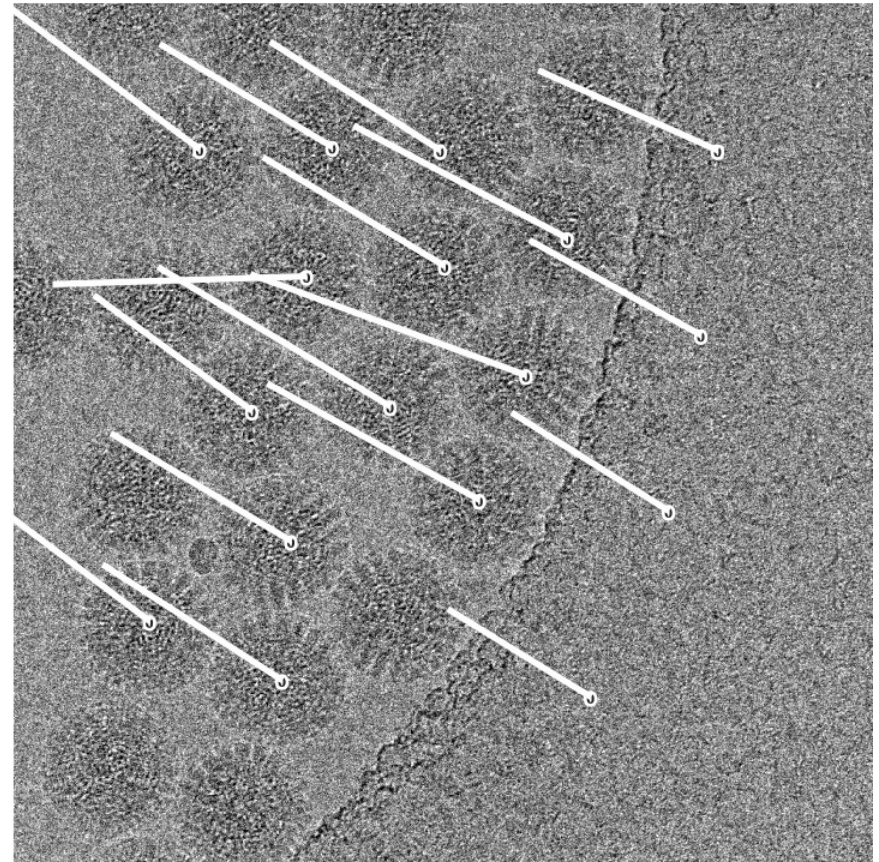
Rotations and Translations

$$\Delta\text{Dose} = 24 \text{ e}^-/\text{\AA}^2$$



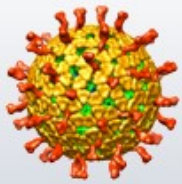
Rotations

2.5° deg

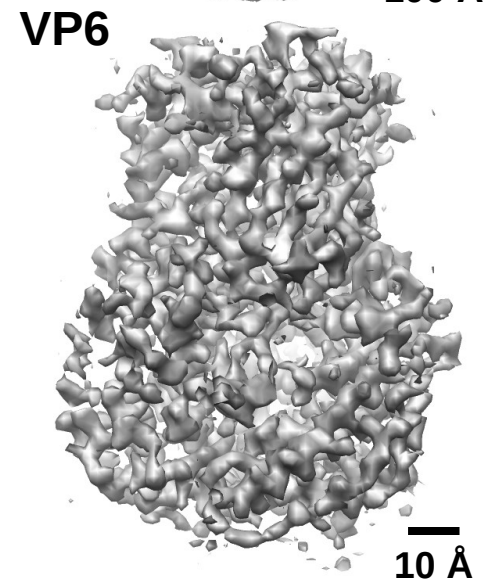
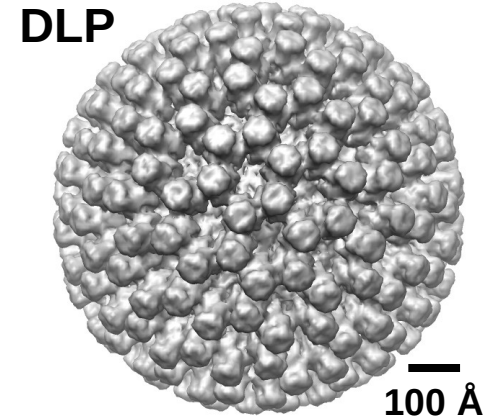
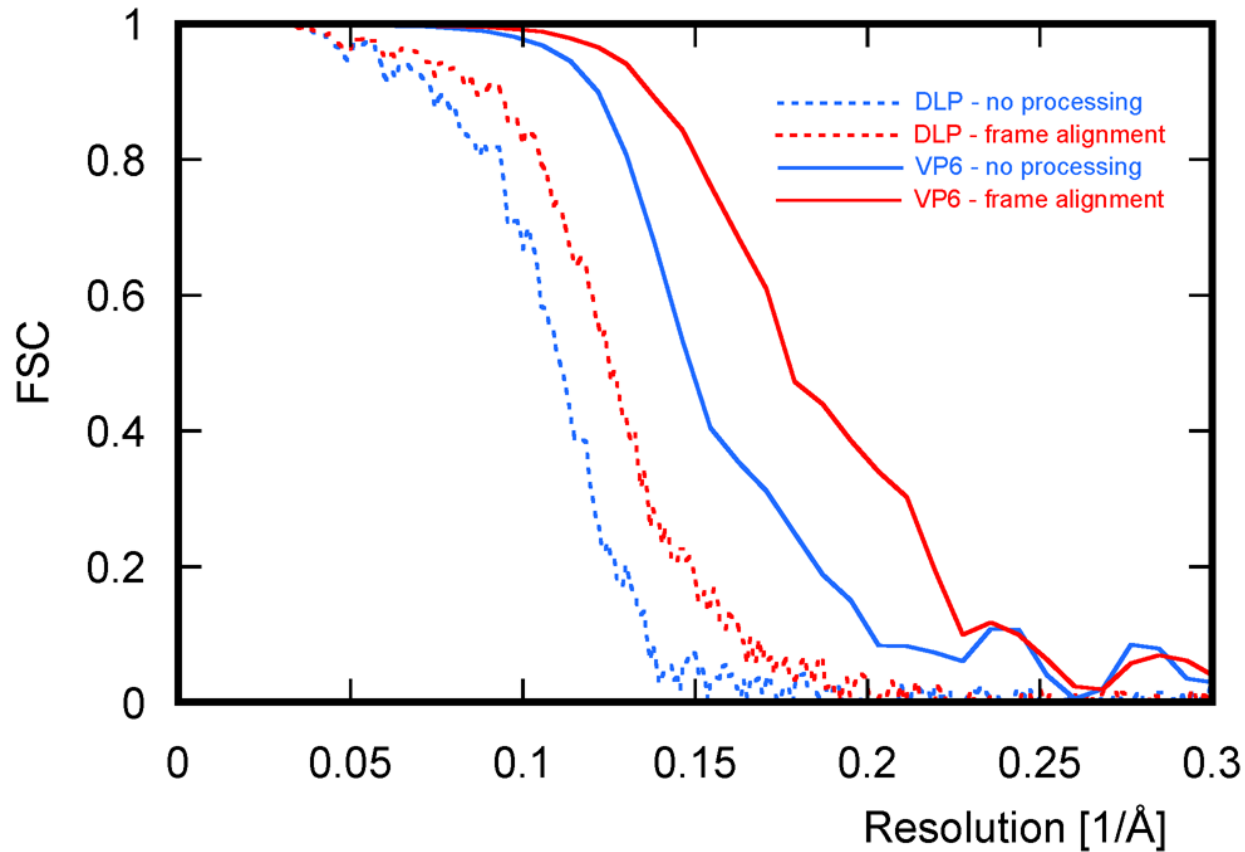


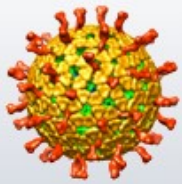
Translations

10 Å

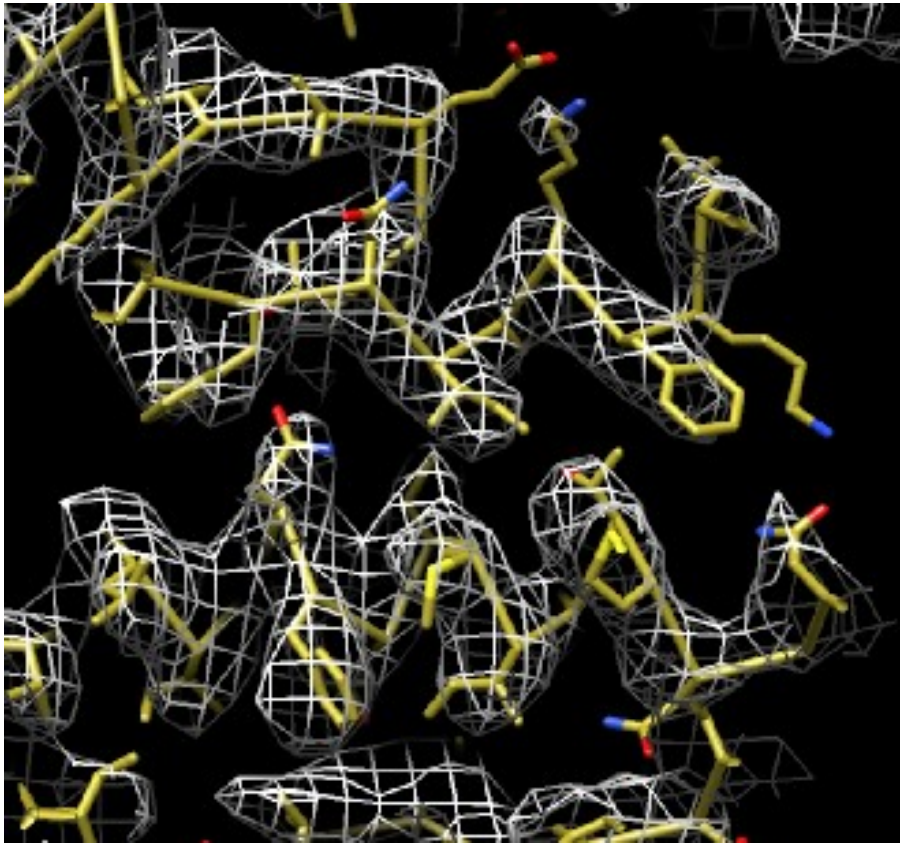


Increased Resolution

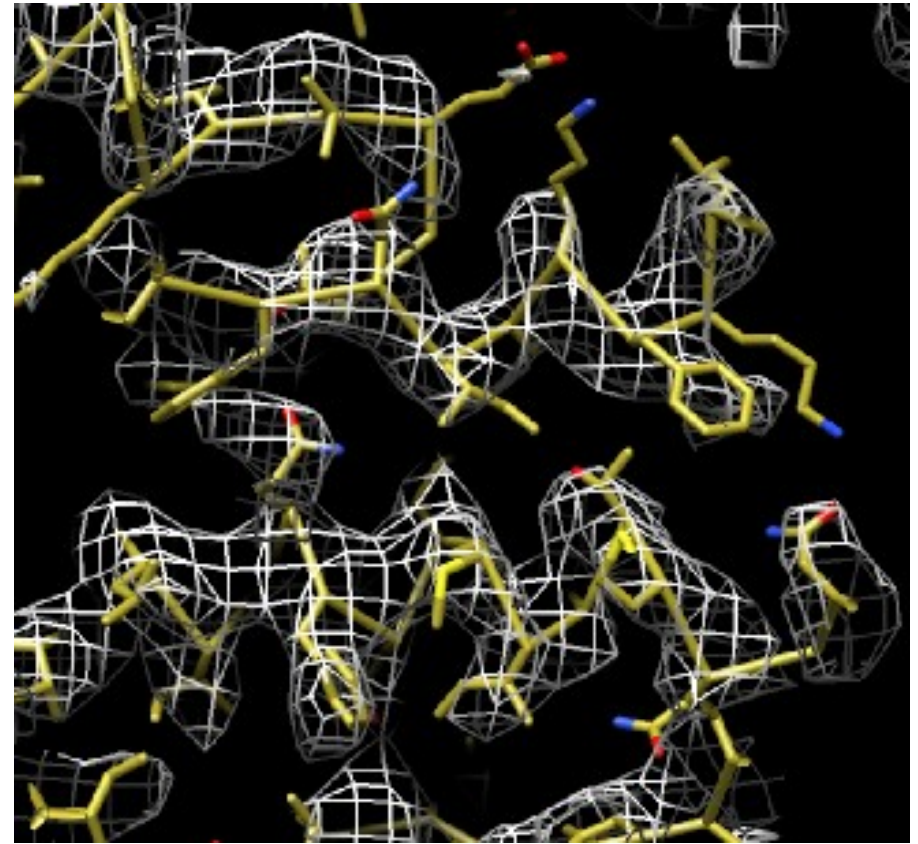




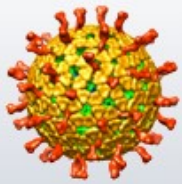
Improved Density



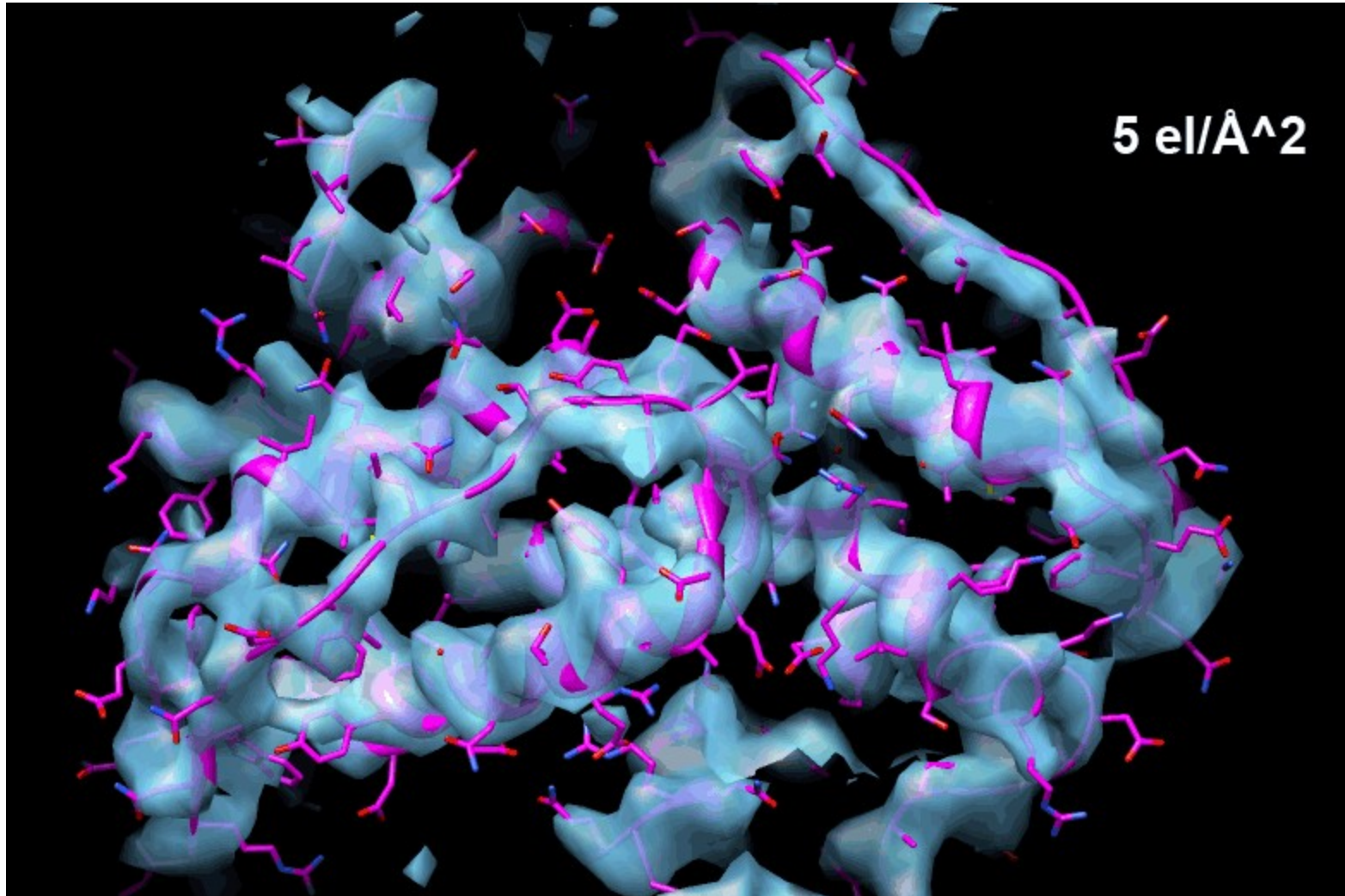
2008 map (~8,000 particles)

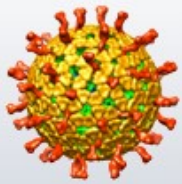


With frame alignment (~800 particles)



Beam Damage





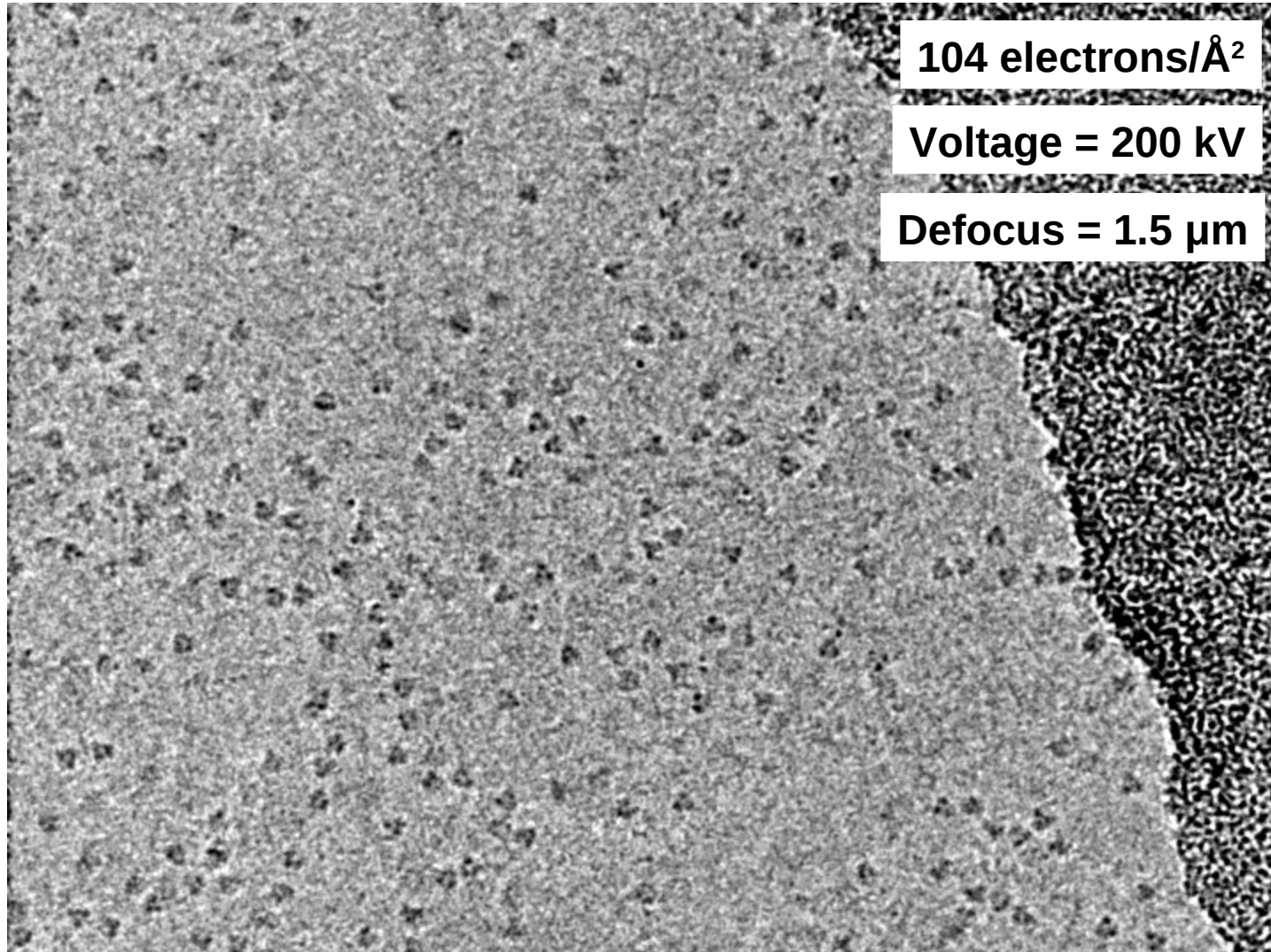
High-Dose Imaging

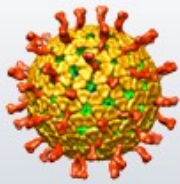


Flu hemagglutinin
(2FK0, Stevens et al. 2006)

Trimer = 180 kDa

Melody Campbell
Peter Lee
(unpublished)





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- Brandeis **Axel Brilot**, James Chen
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