# Pros and cons of direct $e^-$ detection with an integrating camera

Warts and all

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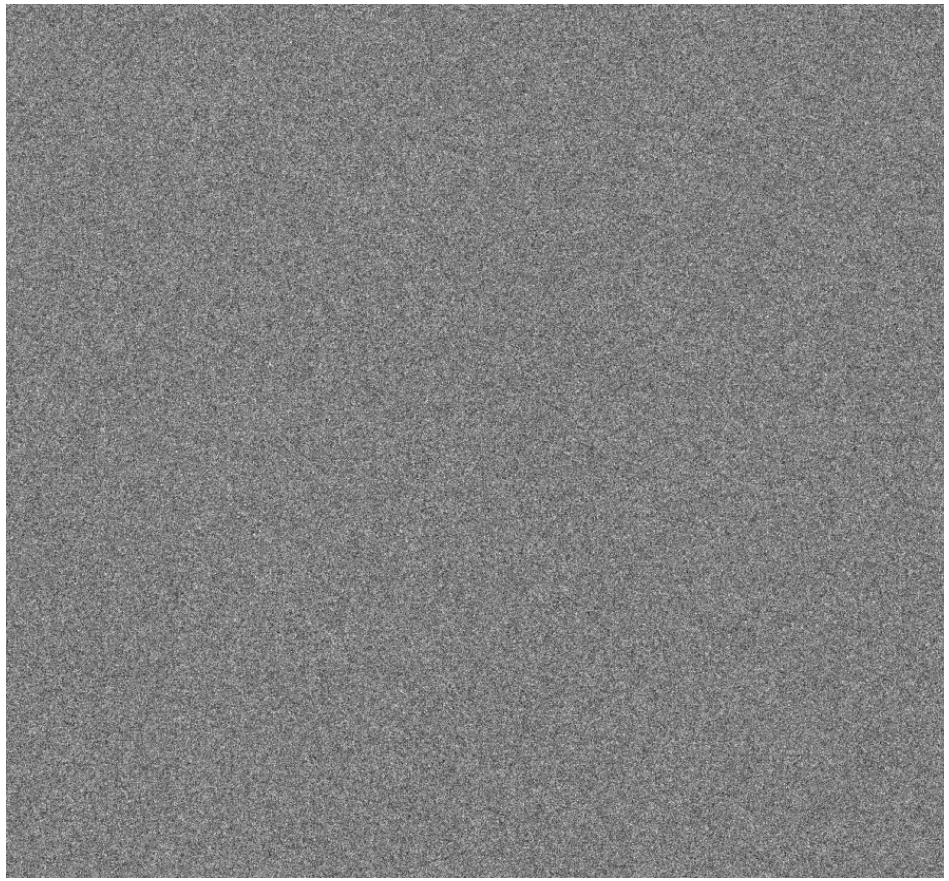
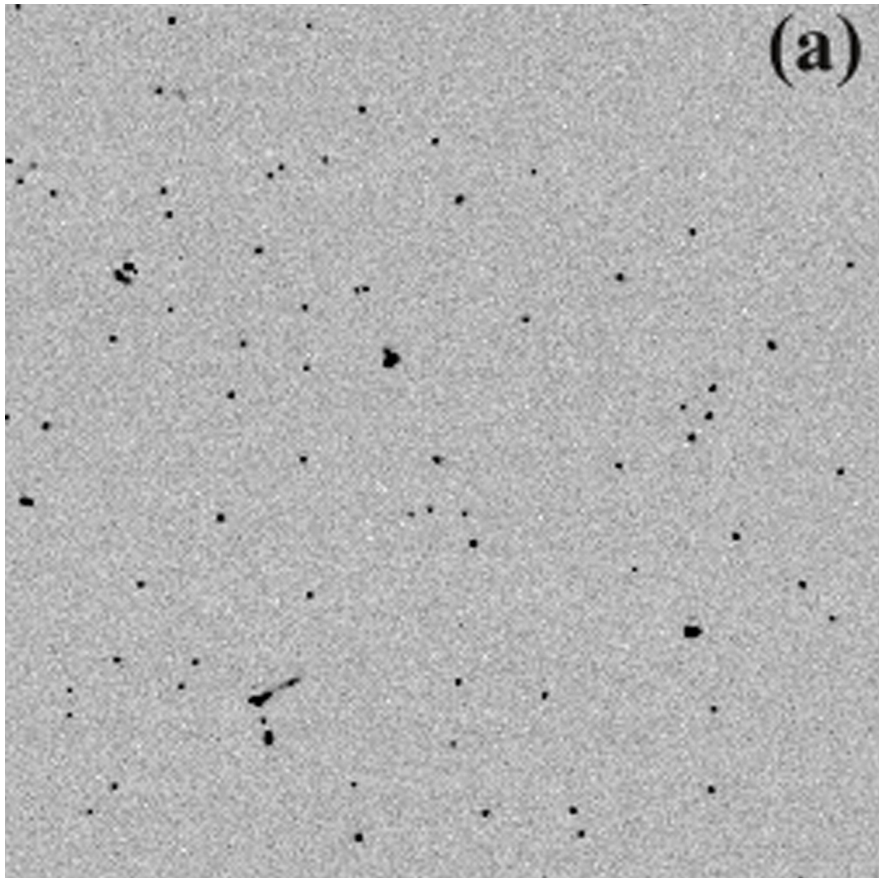
# Outline

- Comparison of integrating vs counting detectors
- Potential advantages of integrating cameras
  - Importance of throughput
- Our experiences with DE cameras
- The DE64
- A fair comparison of detectors

# Counting vs. integrating



- $e^-$ detection results in a certain number of counts
- Frames are summed up

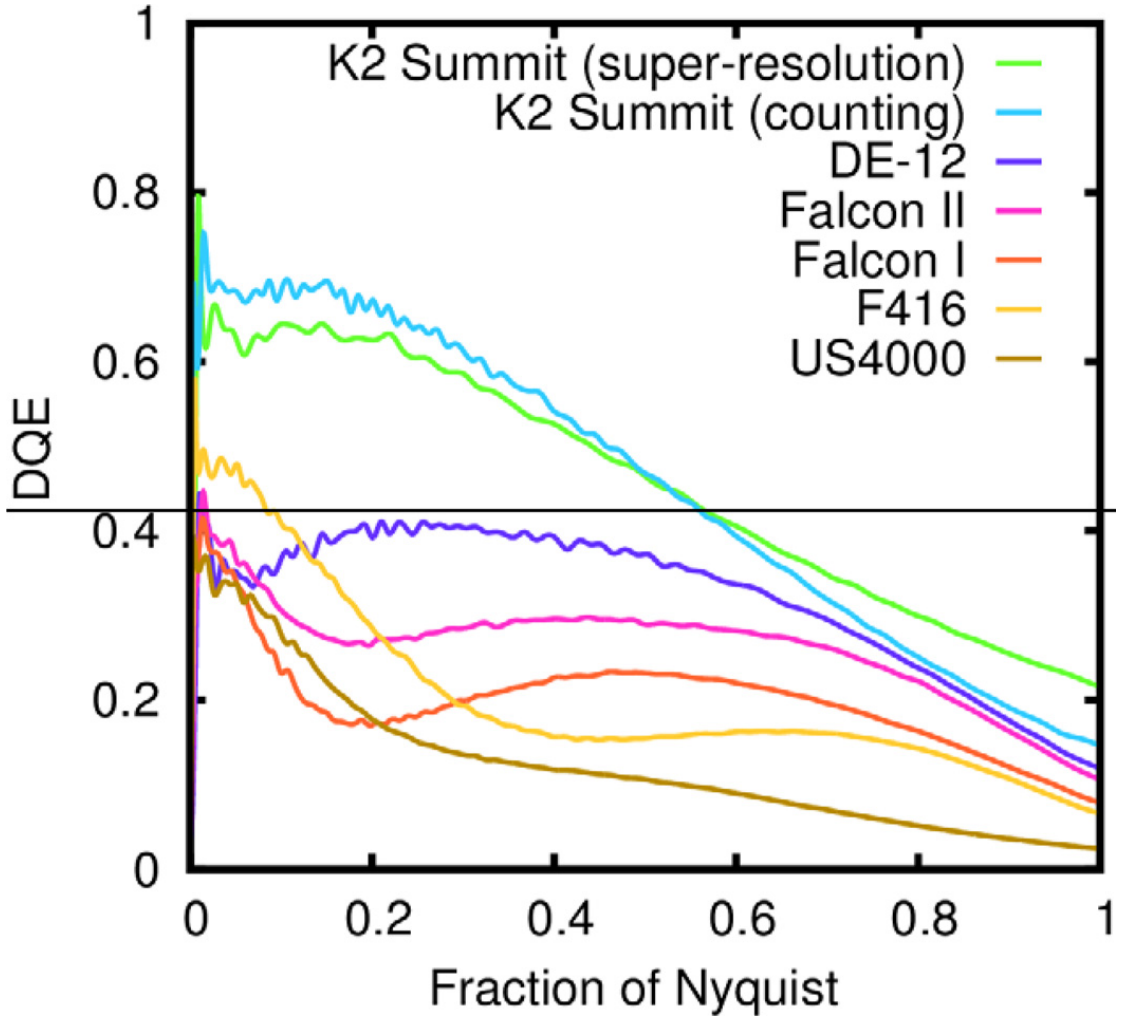



- $e^-$ hits are ”counted”
- Removes Landau noise due to $e^-$ depositing different amounts of energy
- Counted frames sorted into bins then whole set of frames summed

McMullan et al., JSB, 2014

# DQE comparison for various detectors

- Due to reduction in Landau noise, DQE for counting is dramatically better than integrating




Ruskin *et al.*, JSB, 2013

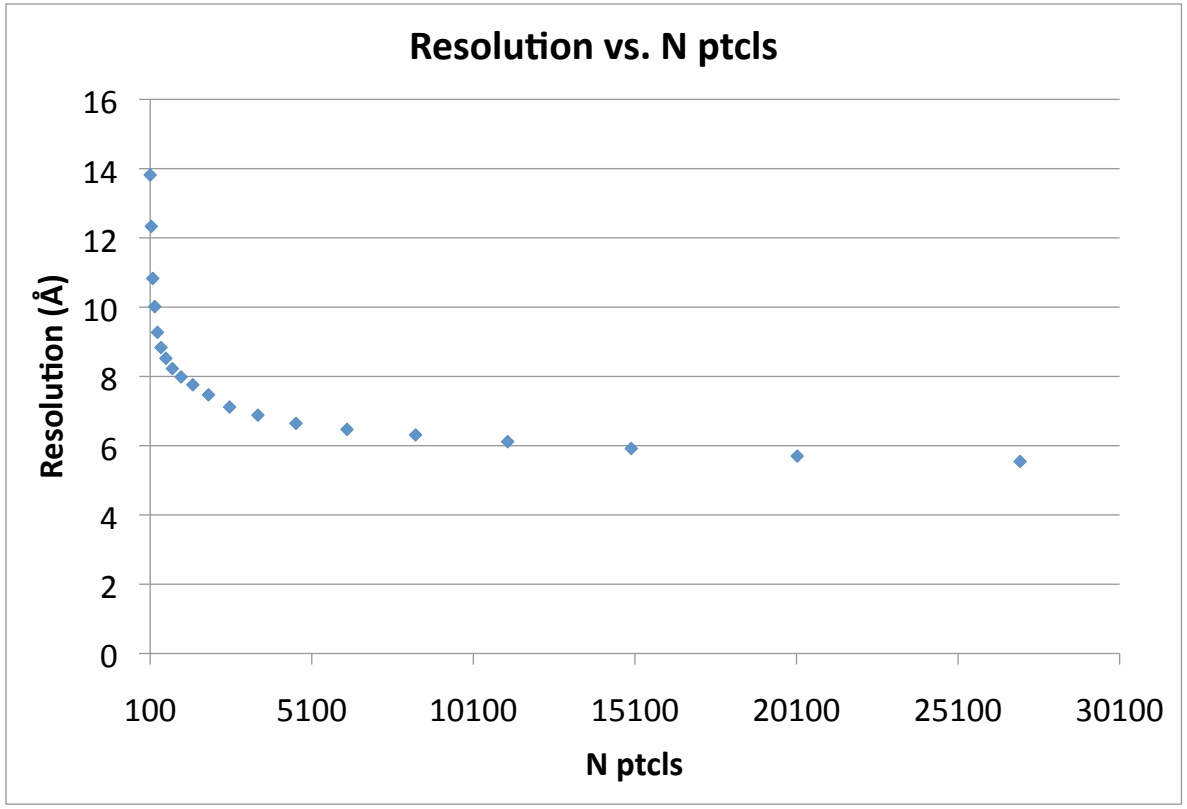
# Other key differences between integrating and counting

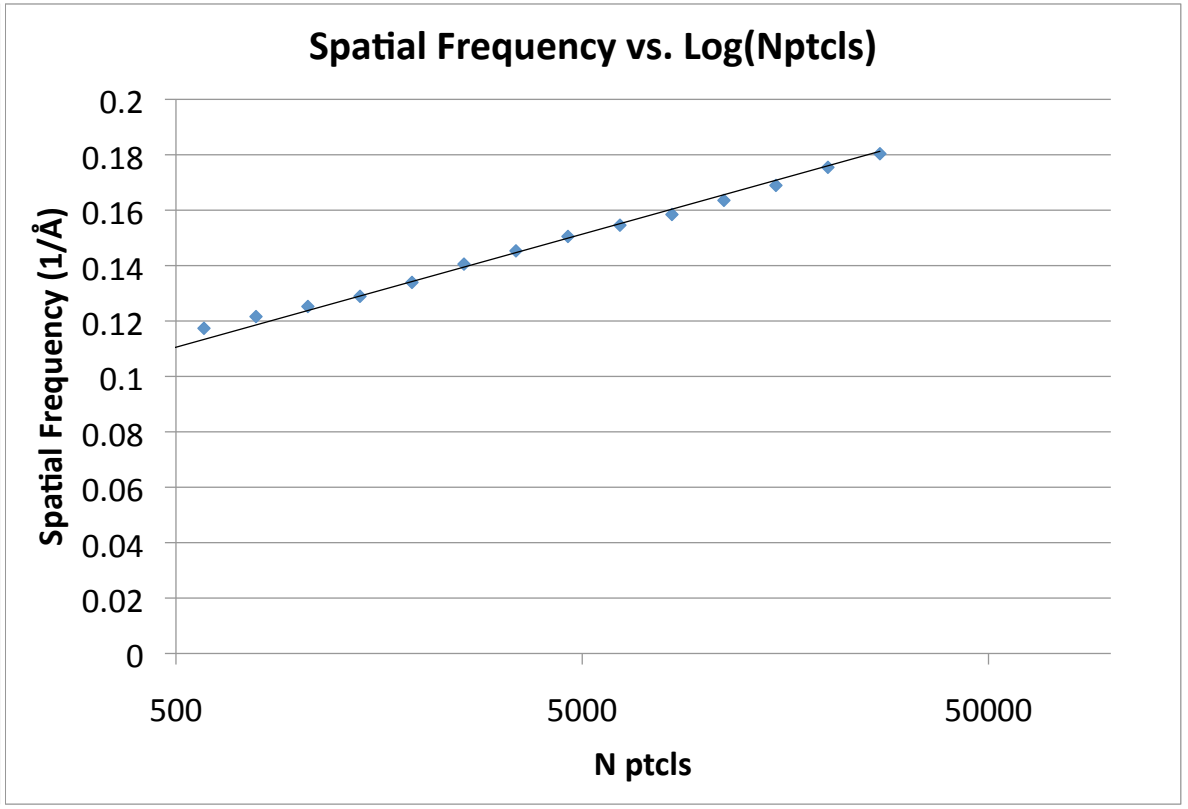
- 1 second exposure time for integrating vs 10 s exposure for K2 counting for the same dose
  - Gives the integrating mode potential for higher throughput
- Much brighter beam used for integrating compared to counting
  - ~ 60 e-/Å$^2$/s integrating
  - ~ 6 e-/Å$^2$/s counting
  - So beam induced motion will be different for the two modes of data collection

# Potential advantages of integrating

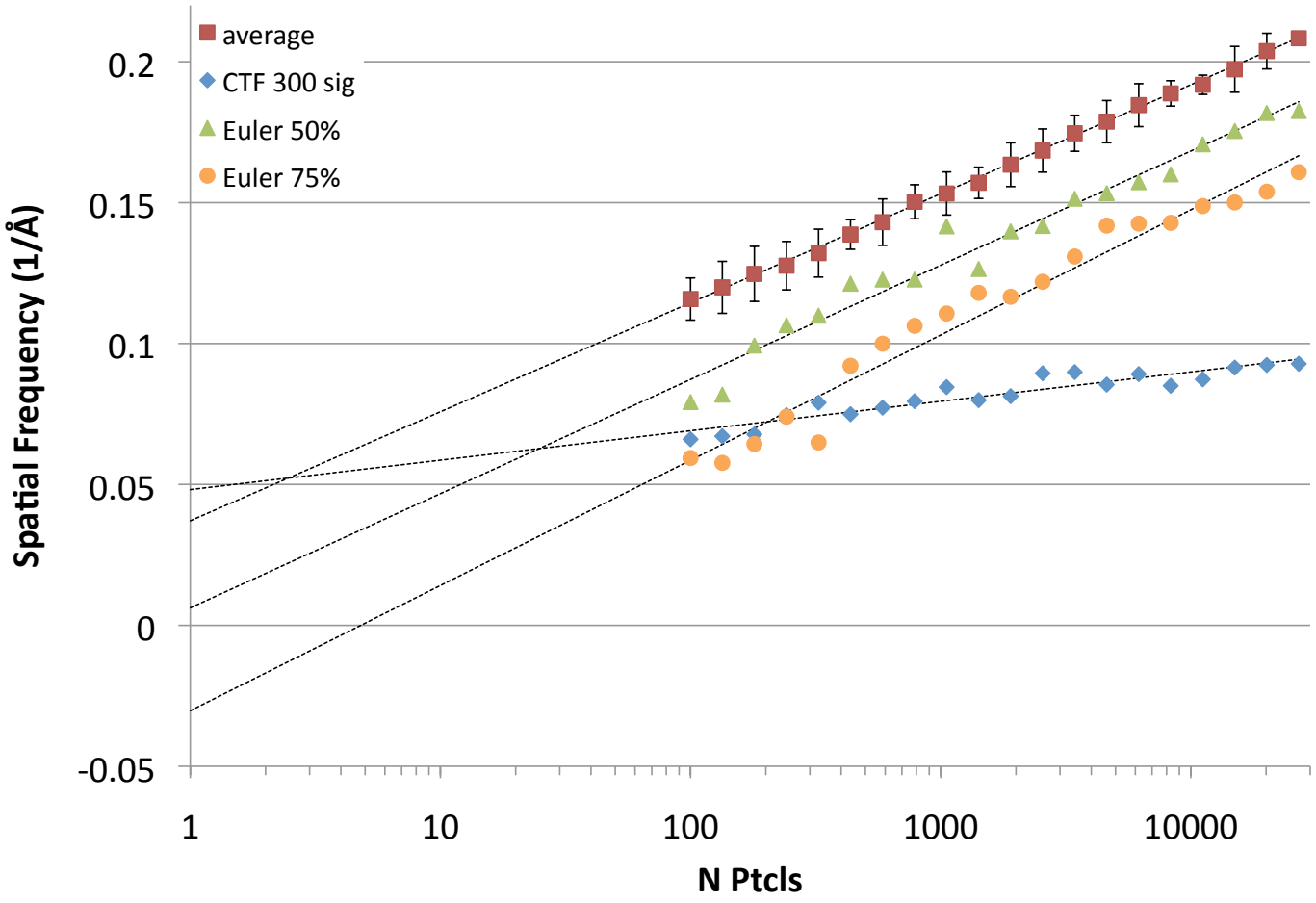
- Potentially higher throughput
  - Depending on what is rate limiting step
    - Data collection dependent
  - This can be important because as a field, we're throwing away up to 90% of our data
- Potentially better beam induced motion
  - We have observed less motion than others have reported
  - This has not been systematically tested

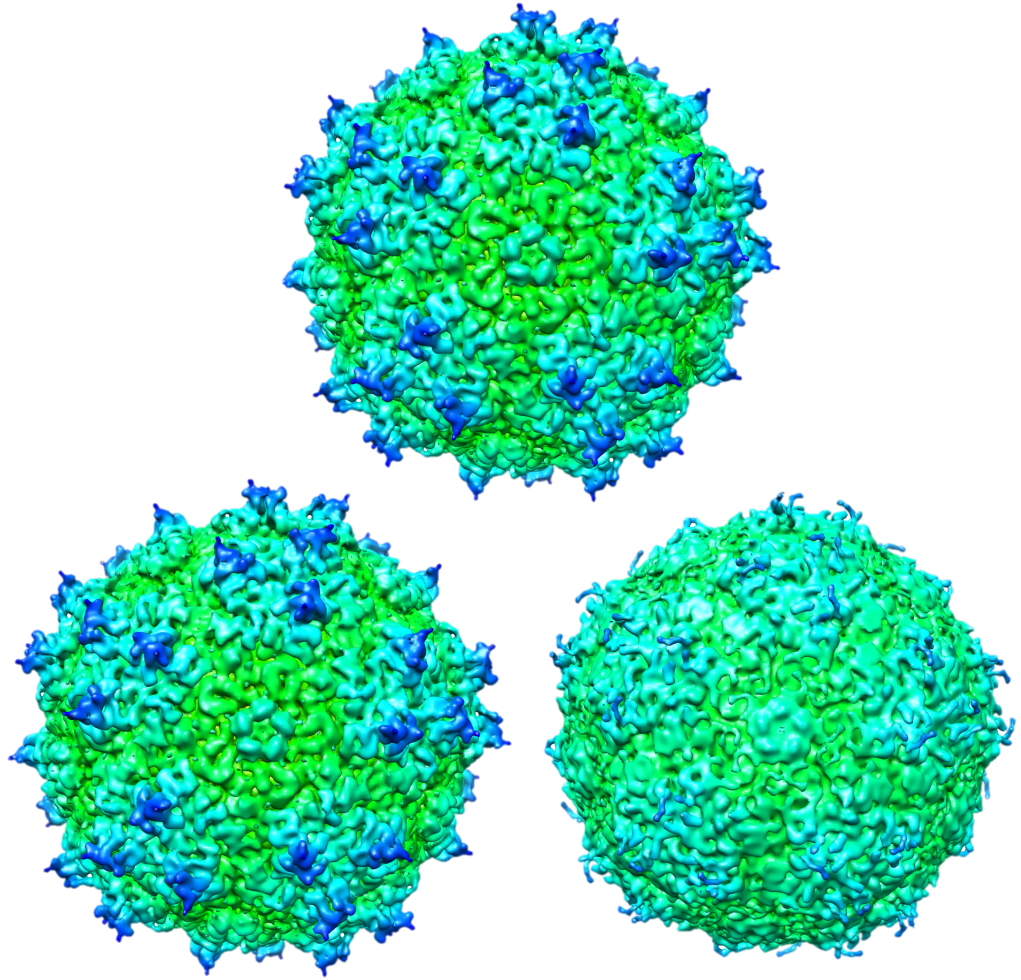
# Plots of spatial frequency vs. log(N) particles are linear



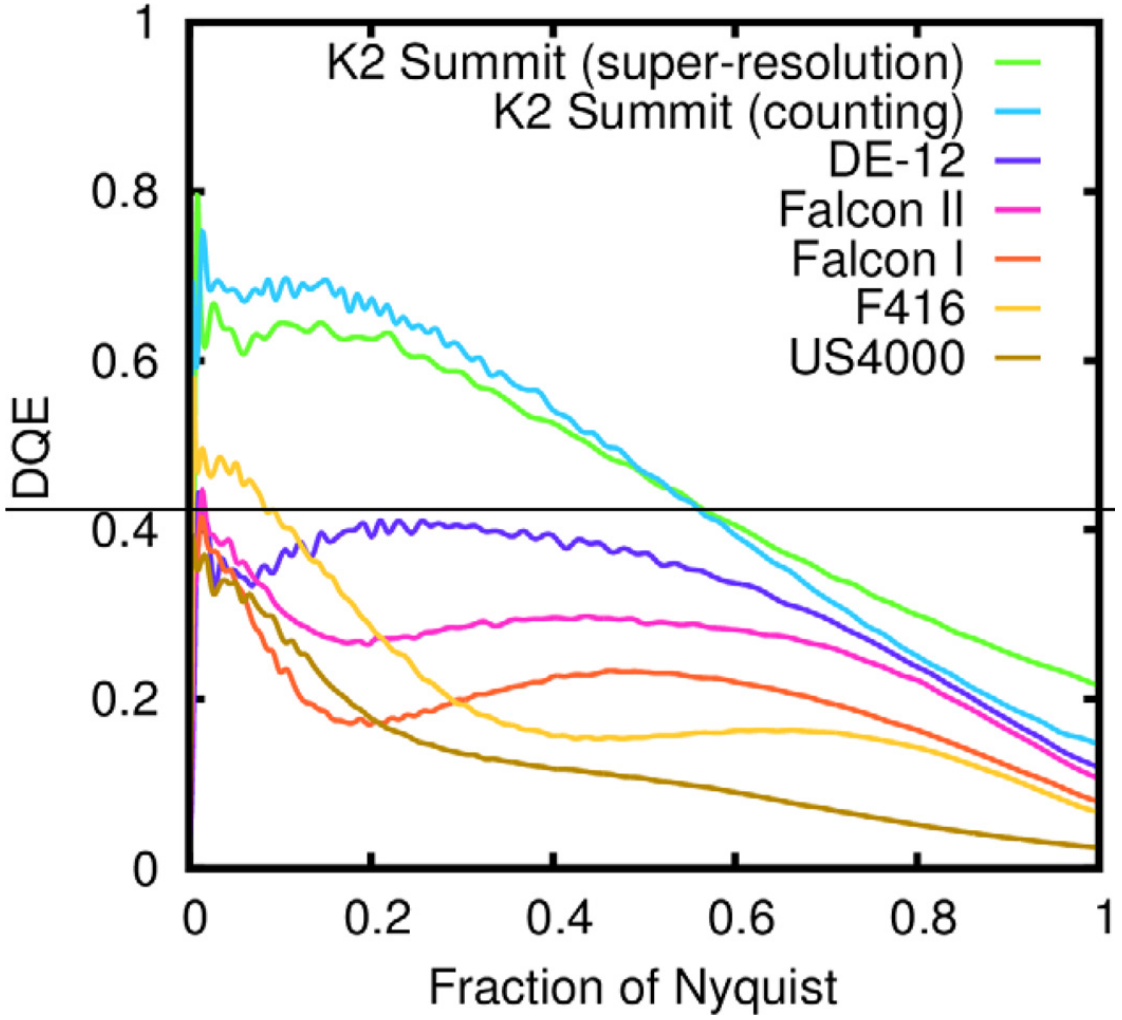
# ResLog slope and intercepts are indicators of quality of data/reconstruction



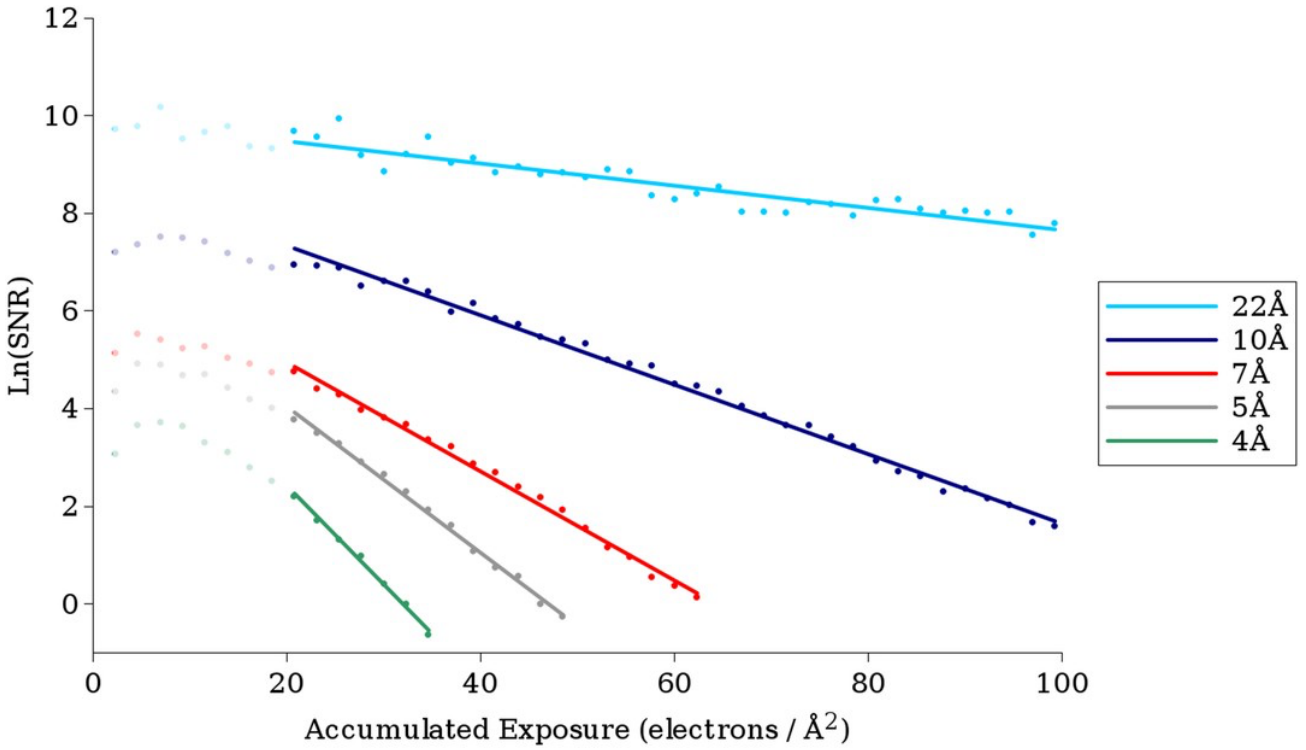



Stagg *et al.*, JSB, 2014

# Lower DQE at low frequency can be compensated by higher dose

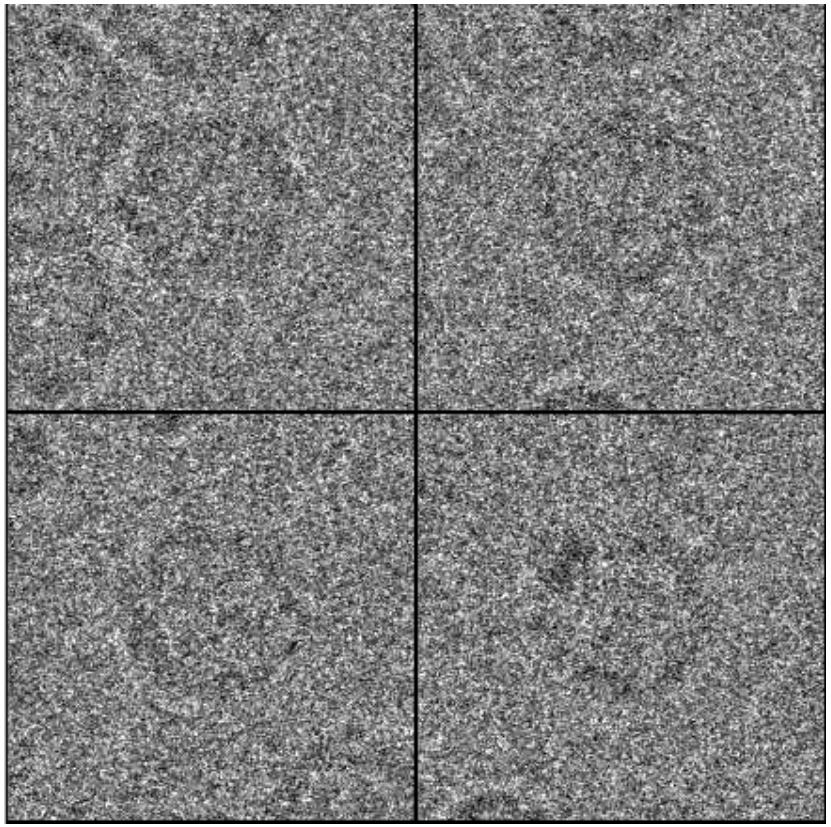



Ruskin *et al.*, JSB, 2013

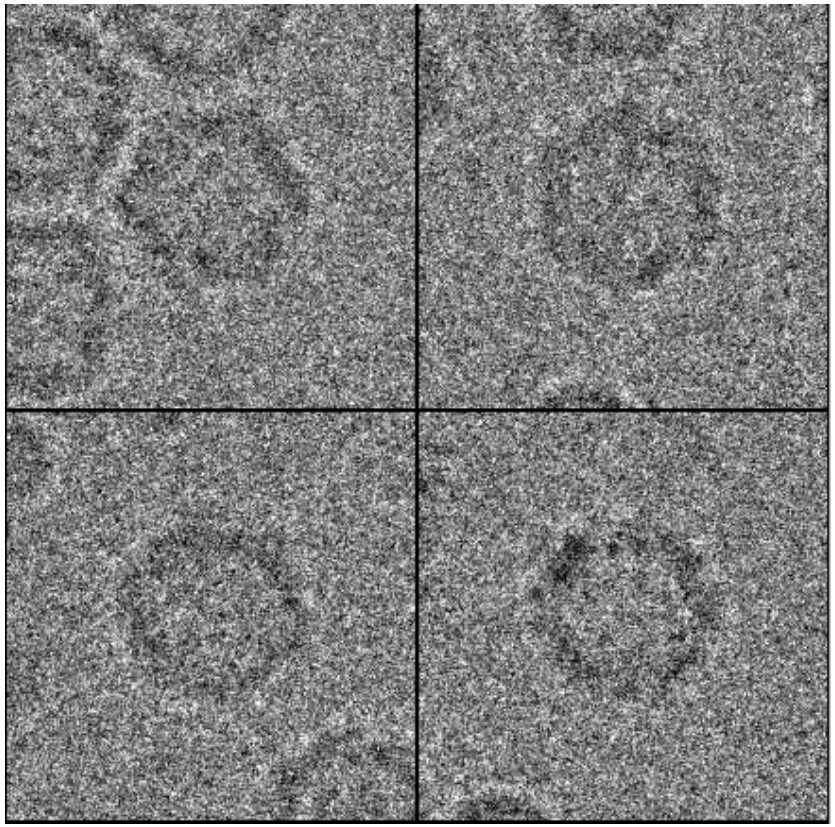



Grant *et al.*, eLife, 2015

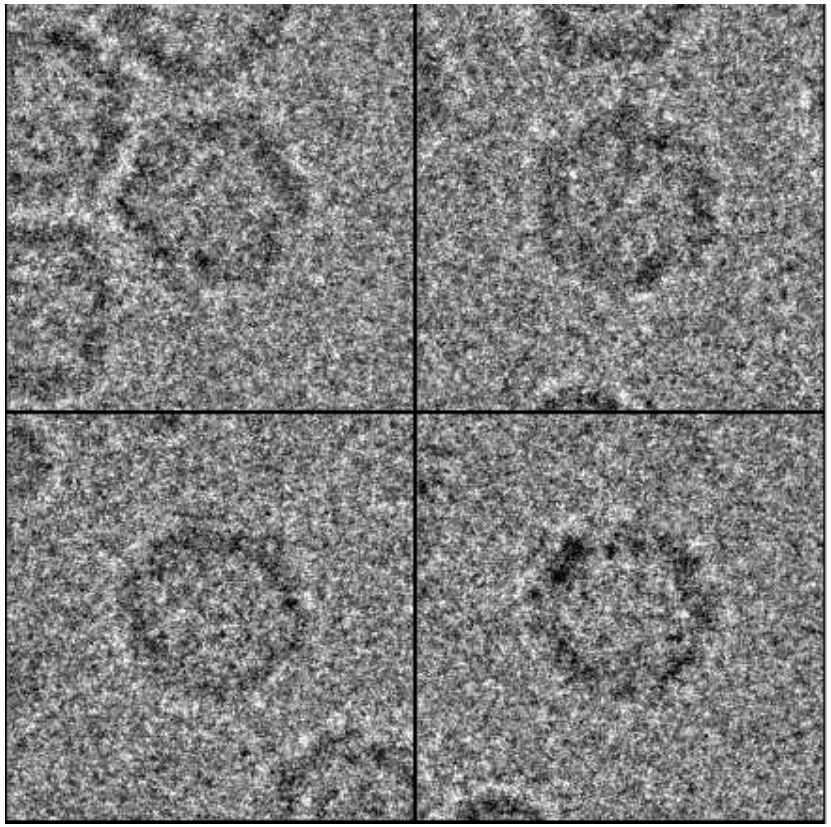
# Low frequency contrast improves with higher dose



15 $e^-/Å^2$
Aligned/summed



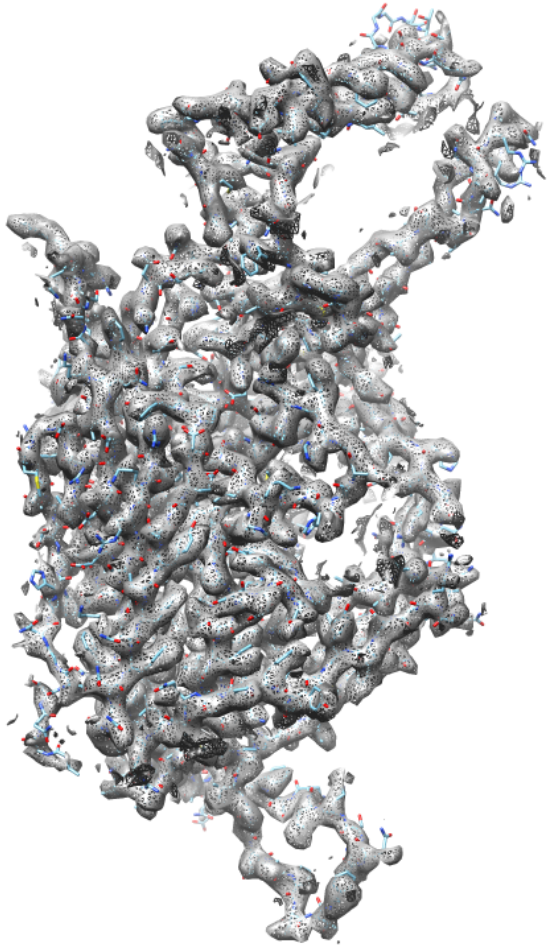
52 $e^-/Å^2$
Aligned/summed



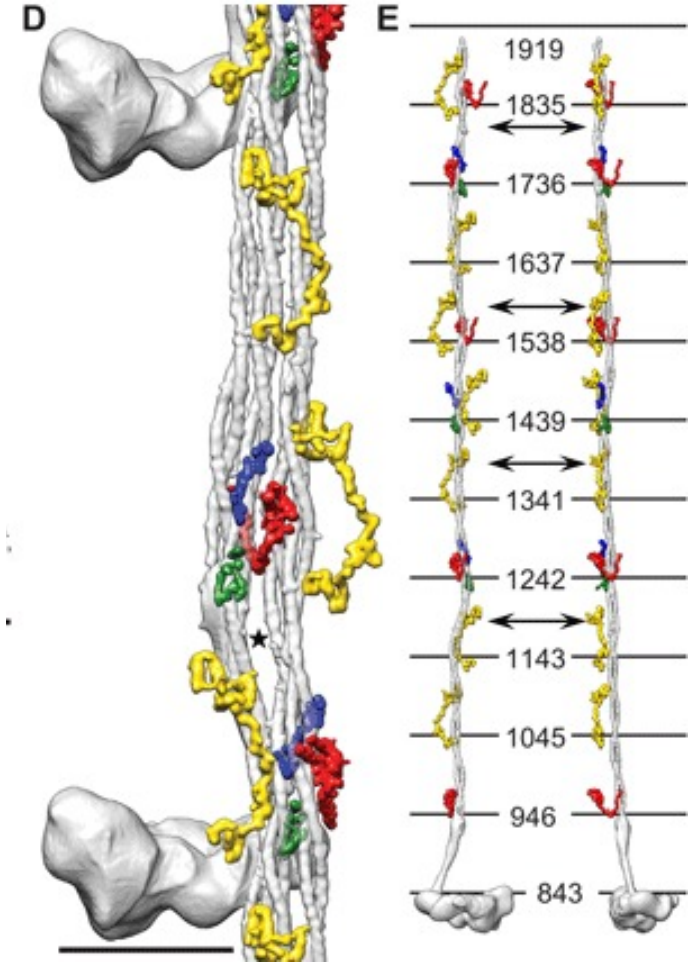
52 $e^-/Å^2$
Aligned/compensated

# Our experience with DE cameras

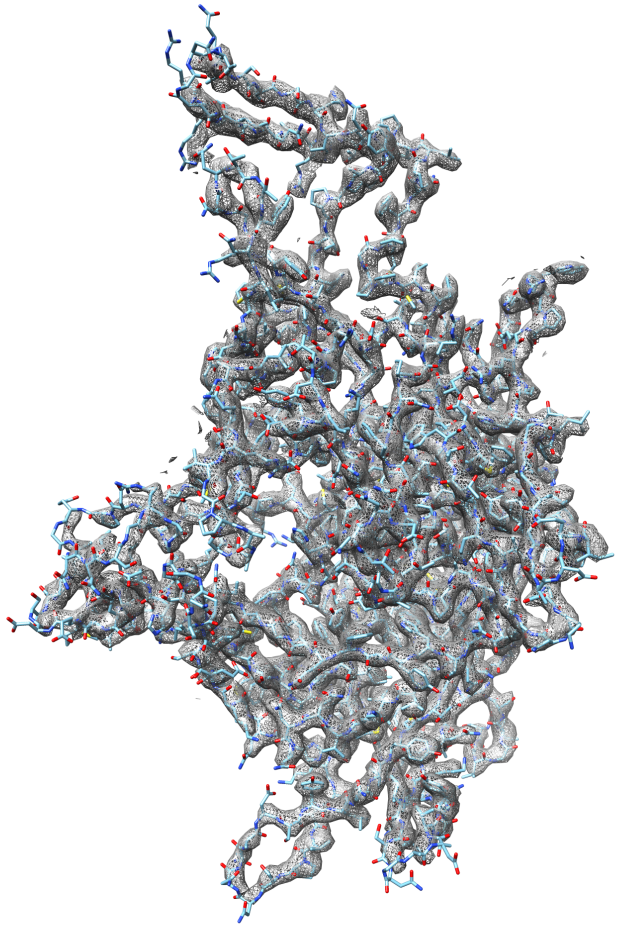
# Successes



2.8 Å AAV
Spear *et al.,* JSB, 2015




Full length myosin filaments
Hu *et al.*, Science Advances, 2016

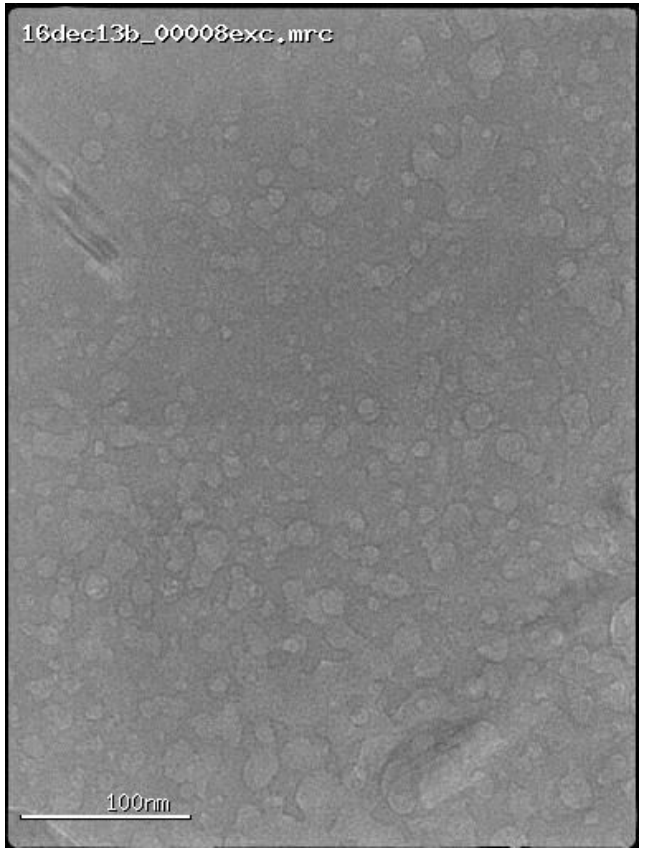


2.8 Å Human bocavirus
Mietzch *et al.,* J. Virol, 2017

# A call for objectivity

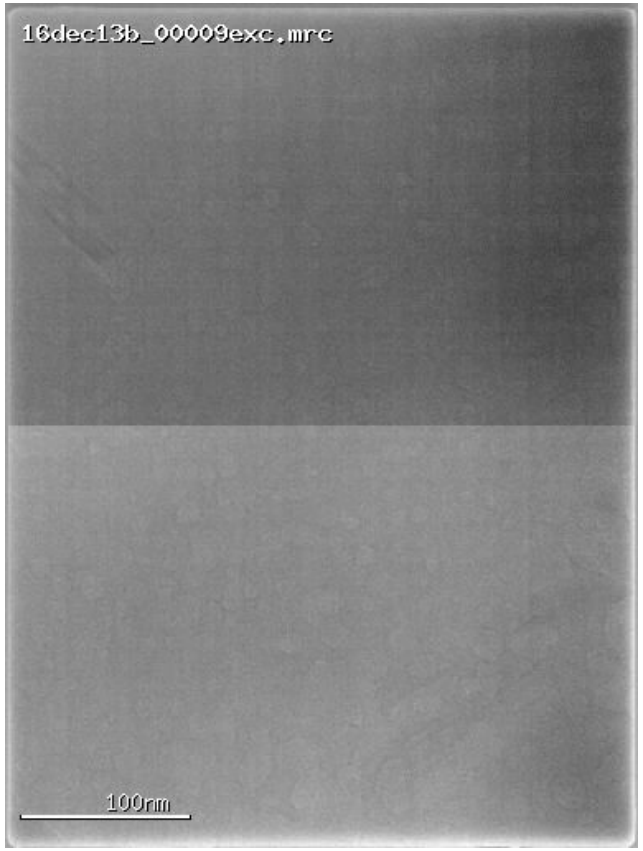
- Let us endeavor to not be victims of confirmation bias
  - A criticism on a recent grant application suggested essentially “you can’t do that without a K2”

# Importance of achieving sufficient counts per frame



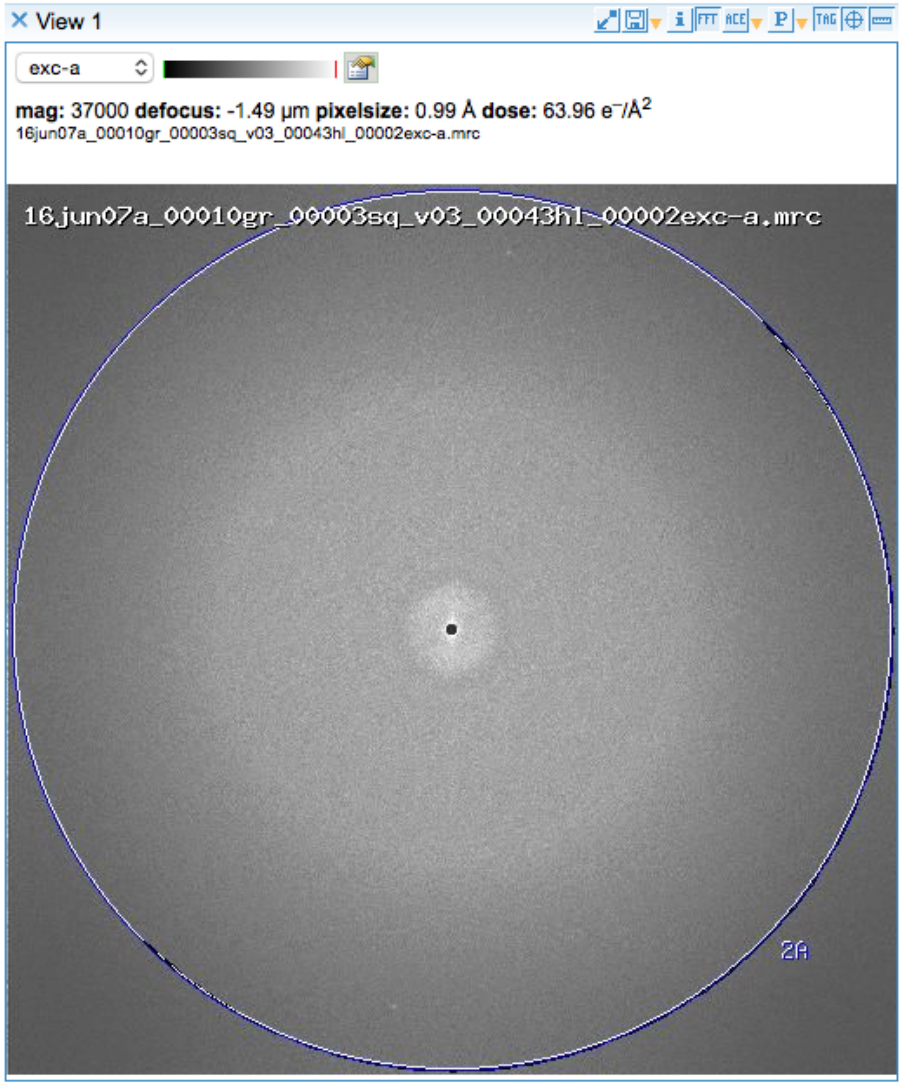

Spot 4
1s exposure
32 fps

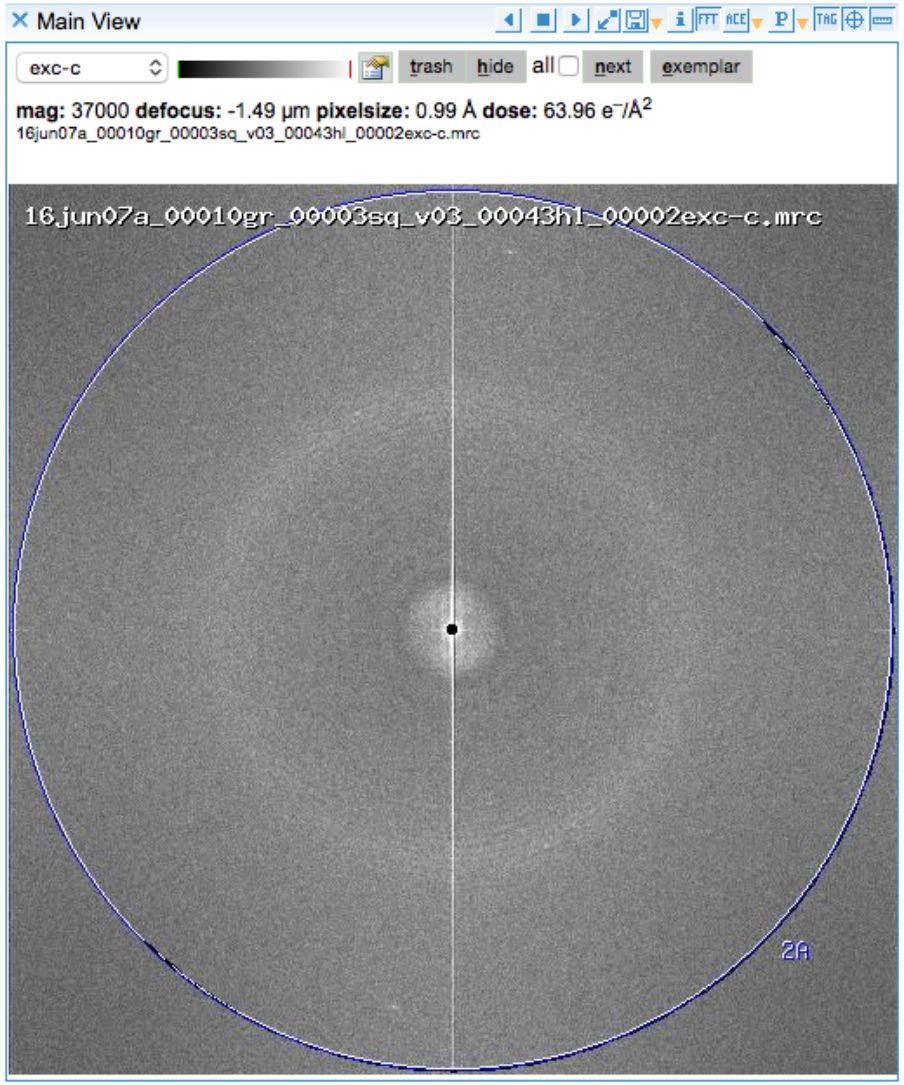
← Same mean →




Spot 8
4s exposure
32 fps

# Progress toward counting




80 s exposure
2560 frames

80 counted/summed frames

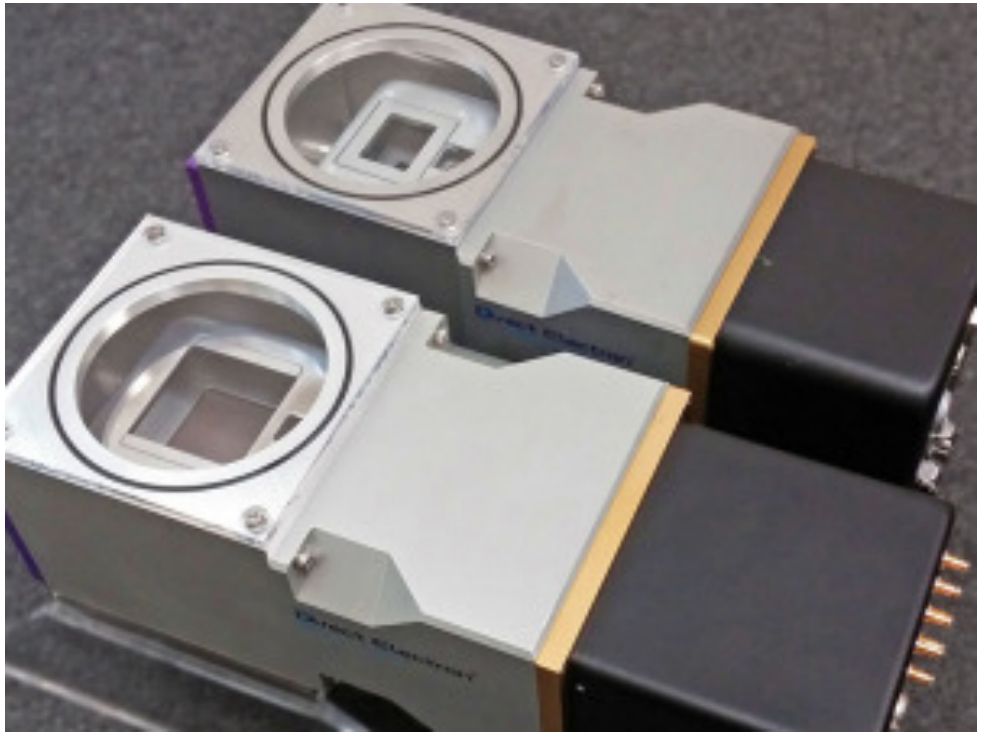
160 counted/summed frames

# Counting on DE20 with Leginon

- Frame rate increased by using only central 1/3 of pixels
- Abandoned because insufficient area to do targeting and autofocusing in Leginon

# DE64 at FSU

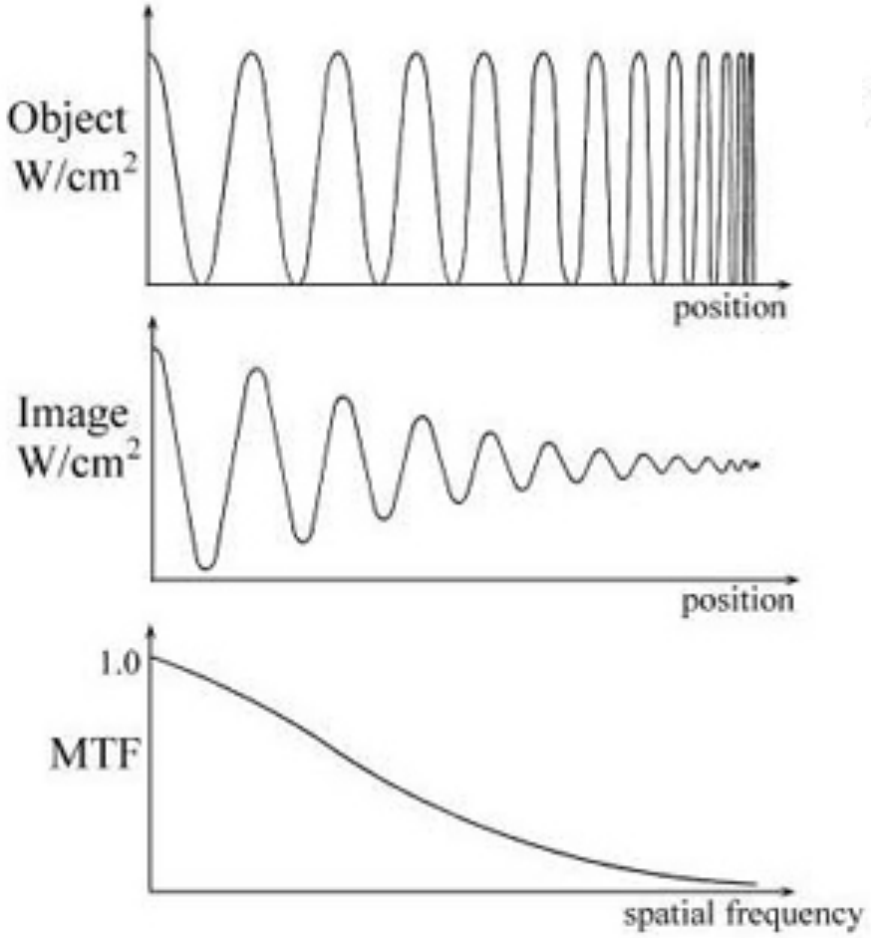
- Installed in May 2017
- One week afterwards
  - Hose failure gave the camera a bath
  - Also killed the chiller for the Titan
- After camera reinstalled
  - Shutter got stuck
- Unsticking the shutter showered chip in dust
  - Factory serviced shutter and rinsed the chip in acetone
- Working fine now
- S#@t happens



# DE64 technical specs

- 6.5 um pixels (as compared to 5 um K2, or 14 um Falcon II)
- Variable frame rate up to 45 fps for 8K x 8K images
  - This can be useful for accumulating sufficient counts per frame with different dose rates
- 146 fps with 2x hardware binning
  - 4K x 4K counting mode

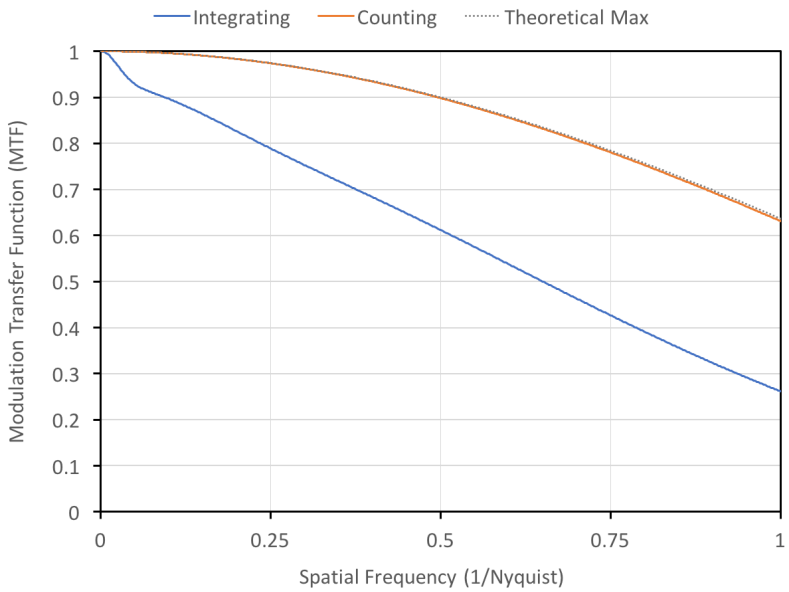
# Modulation Transfer Function

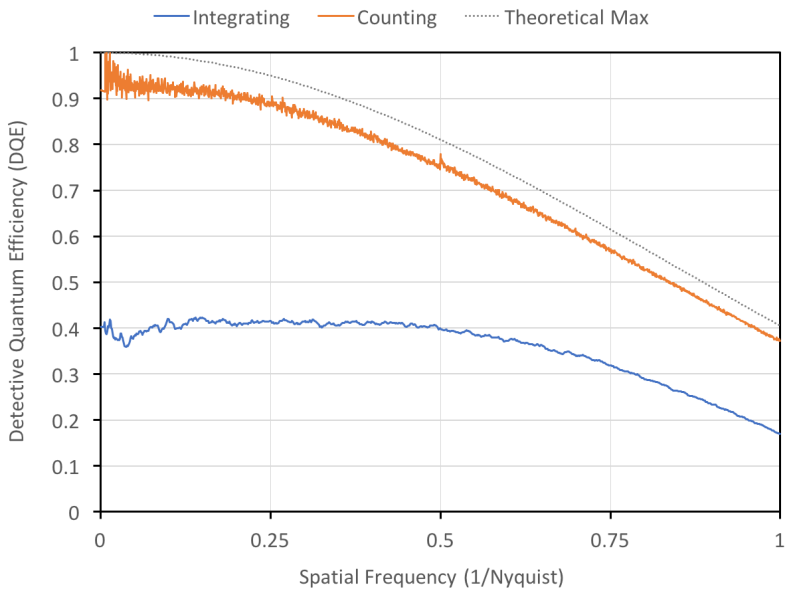



$$\mathrm{MTF}(\xi) \equiv \mathcal{M}_{\mathrm{image}}(\xi)/\mathcal{M}_{\mathrm{object}} .$$

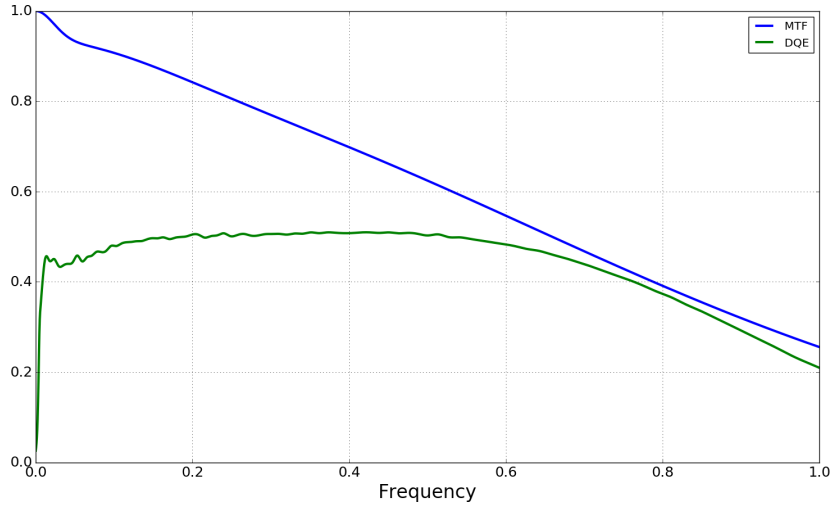
G. D. Boreman, *Modulation Transfer Function in Optical and Electro-Optical Systems*, SPIE Press, Bellingham, WA (2001).

# DE64 $e^-$ detection performance



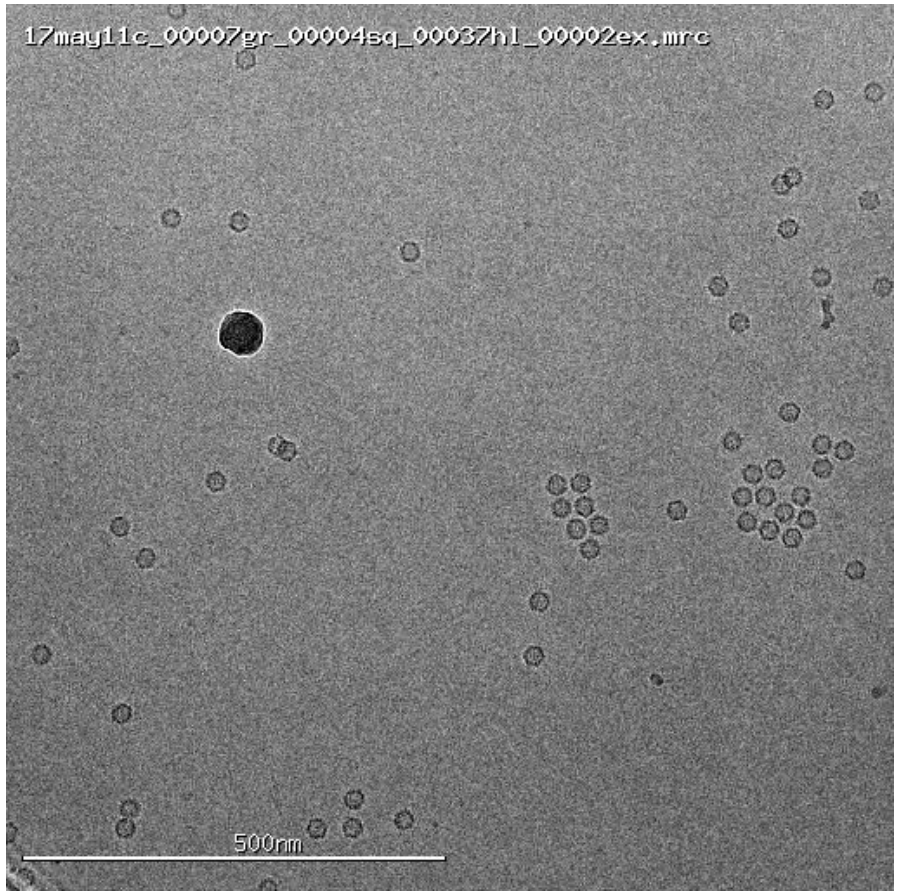



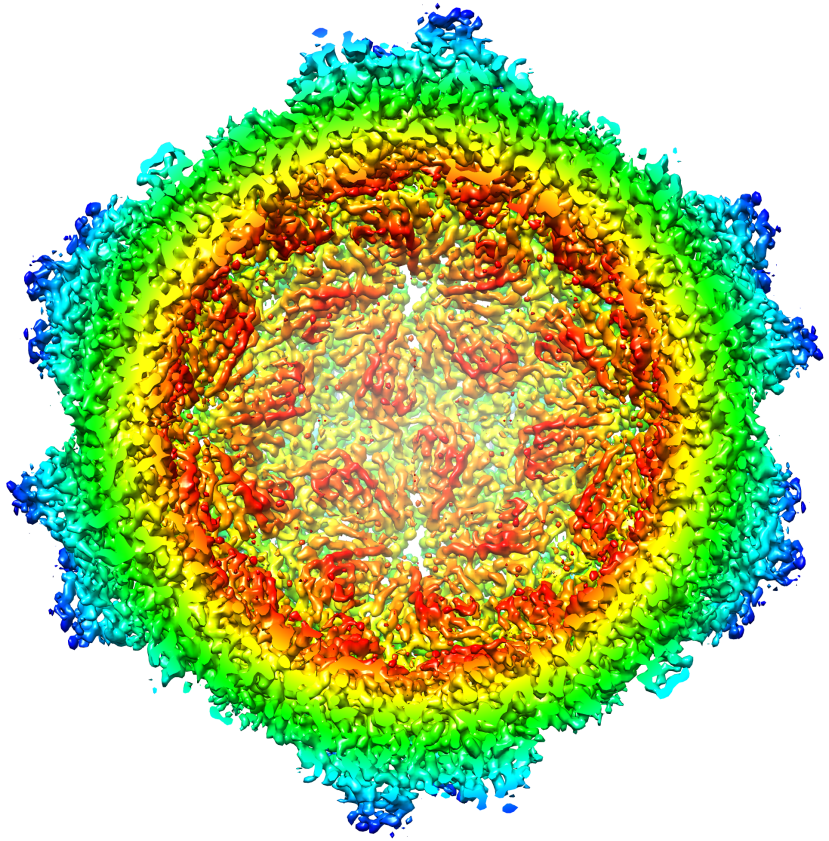

From Direct Electron




Calculated using FindDQE from Grigorieff lab

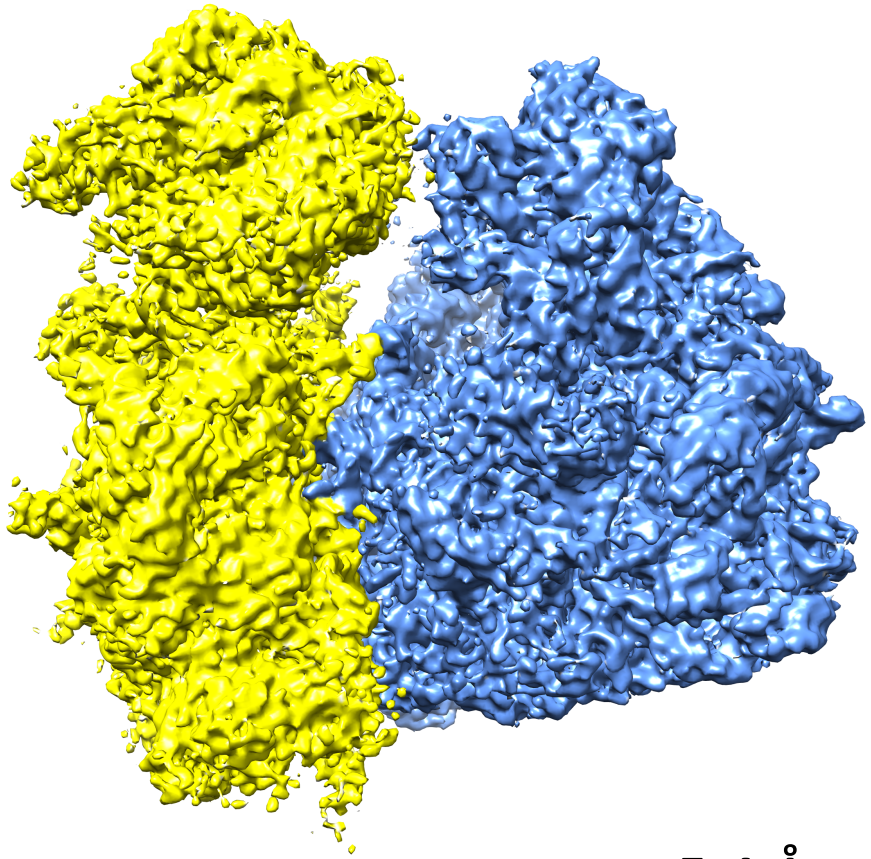
# Results so far with the DE64



2688 ptcls
4.3 Å

# 80S ribosome with preferred orientation

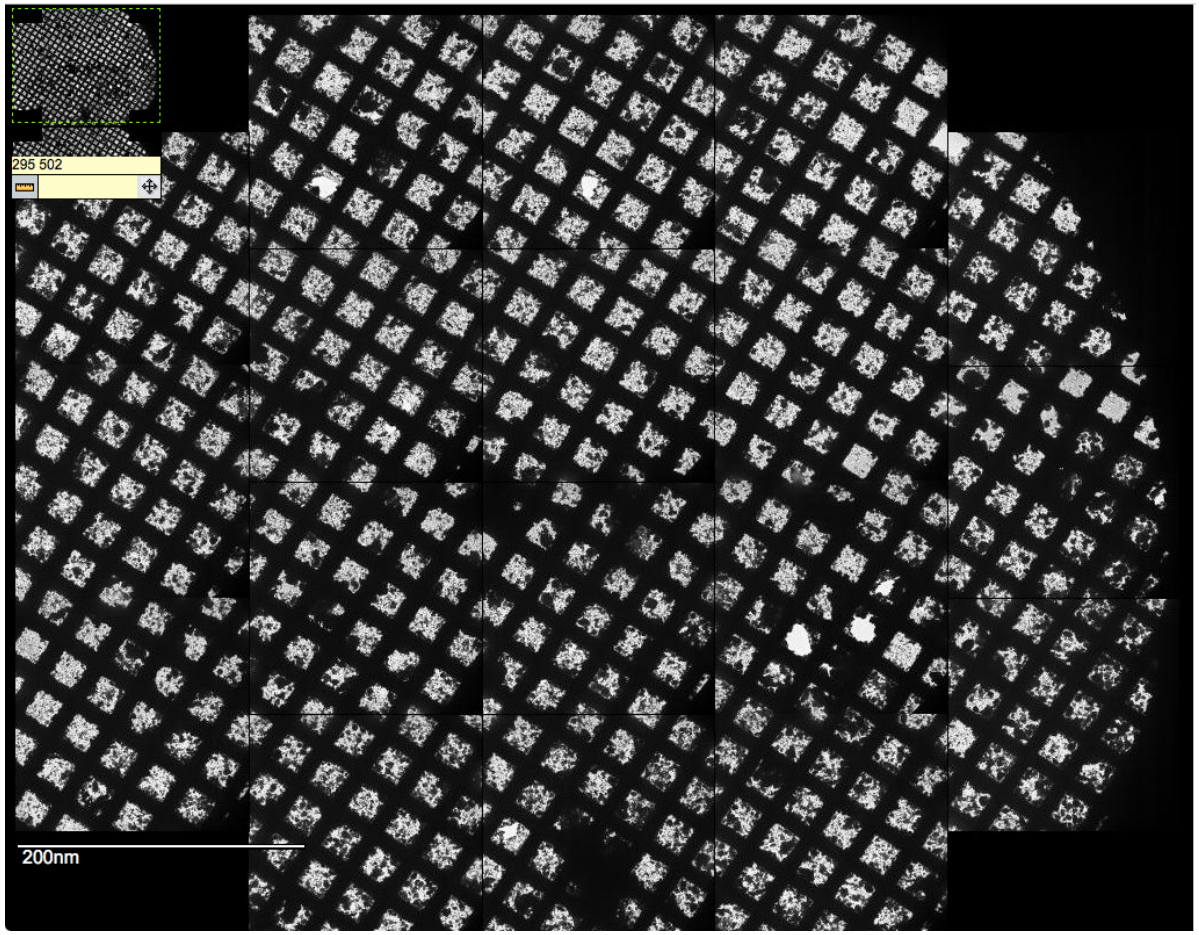


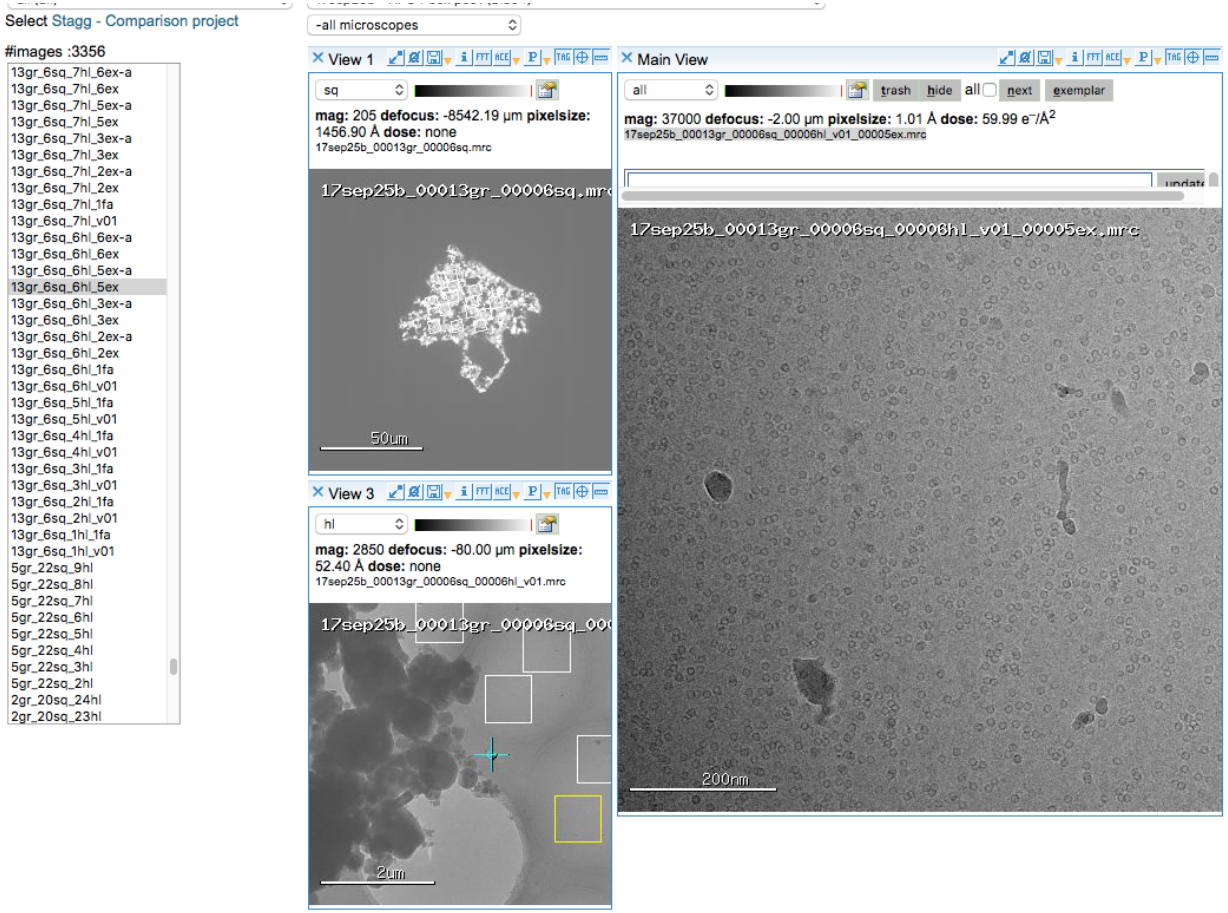
5.4 Å resolution

# Towards a fair comparison

- The goal: compare reconstructions from same sample on same grid on different cameras
- Determine resolution as function of time on the scope
  - Clearly on a per particle basis particles will be better with counting, but one can collect more particles per unit time with integrating
- Endeavor to take sample preparation variability out of the equation
  - Collect on Apoferritin
    - High symmetry but hard to align
  - Samples prepared with Spotiton

# First attempt at Apoferritin

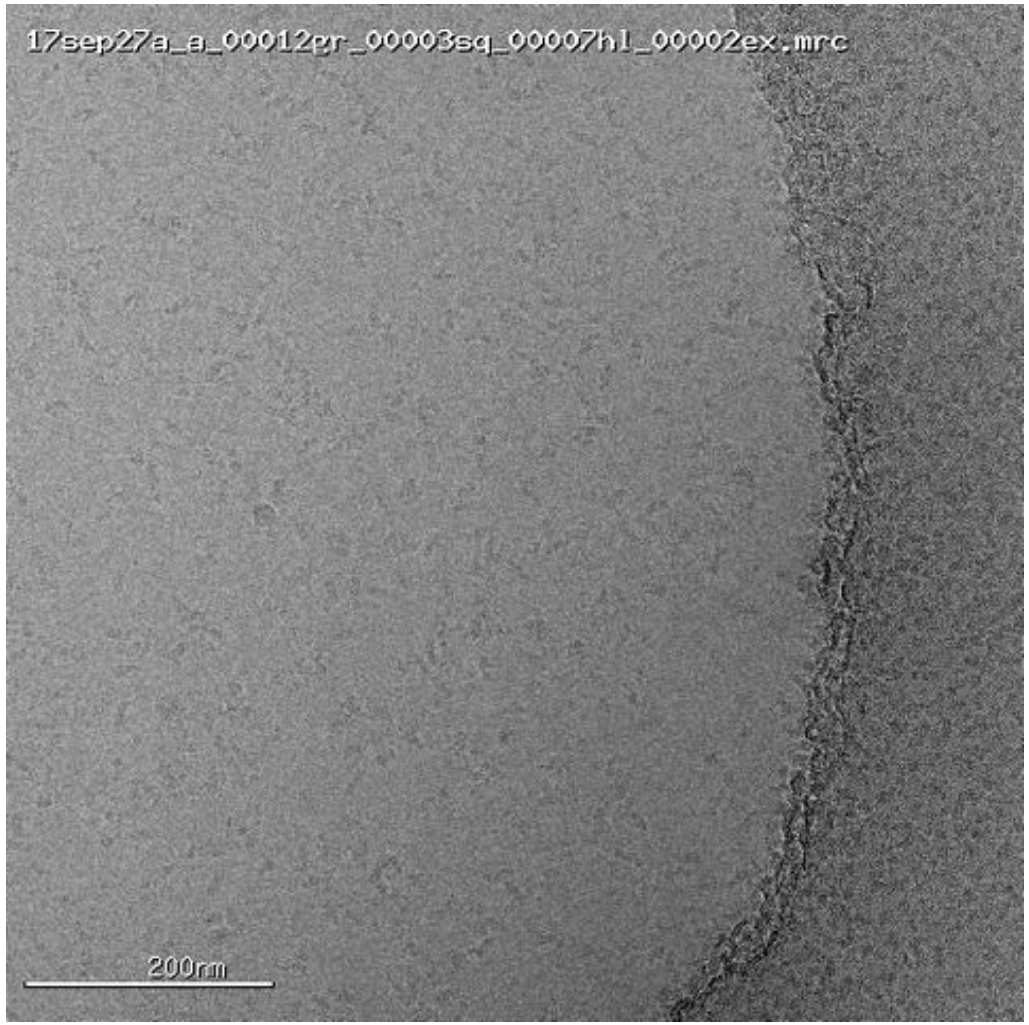


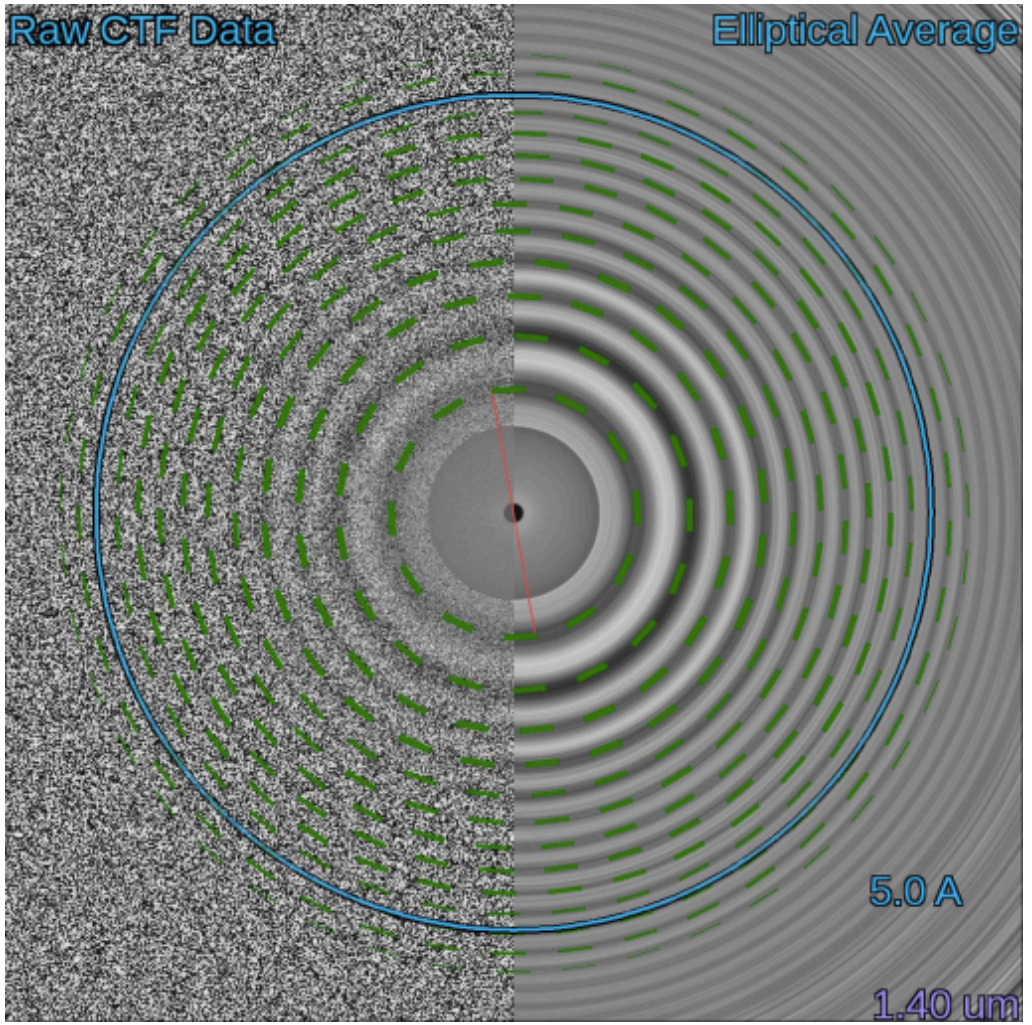



# Throughput

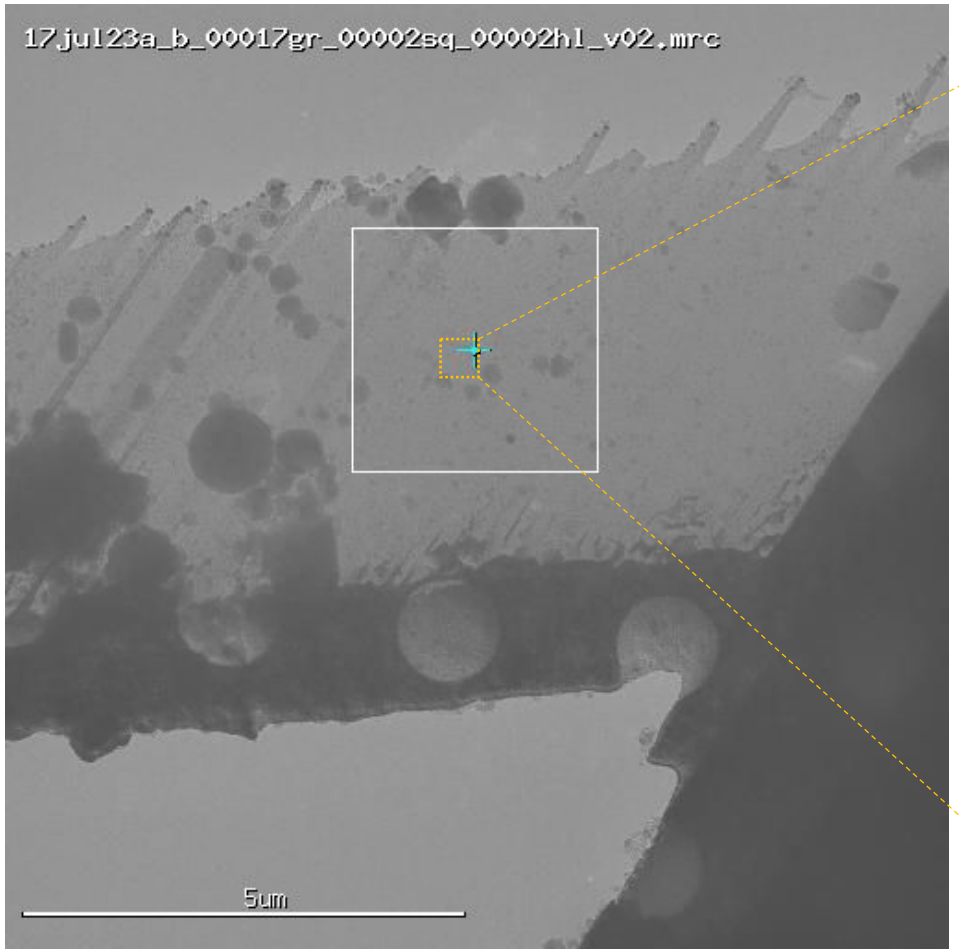
- 1421 images in ~12 hours
- 801,000 particles
- ~8 TB of data
- Did not get anywhere with reconstruction
  - There is some problem with the data
  - Thon rings are poor on carbon

# Compared to good dataset

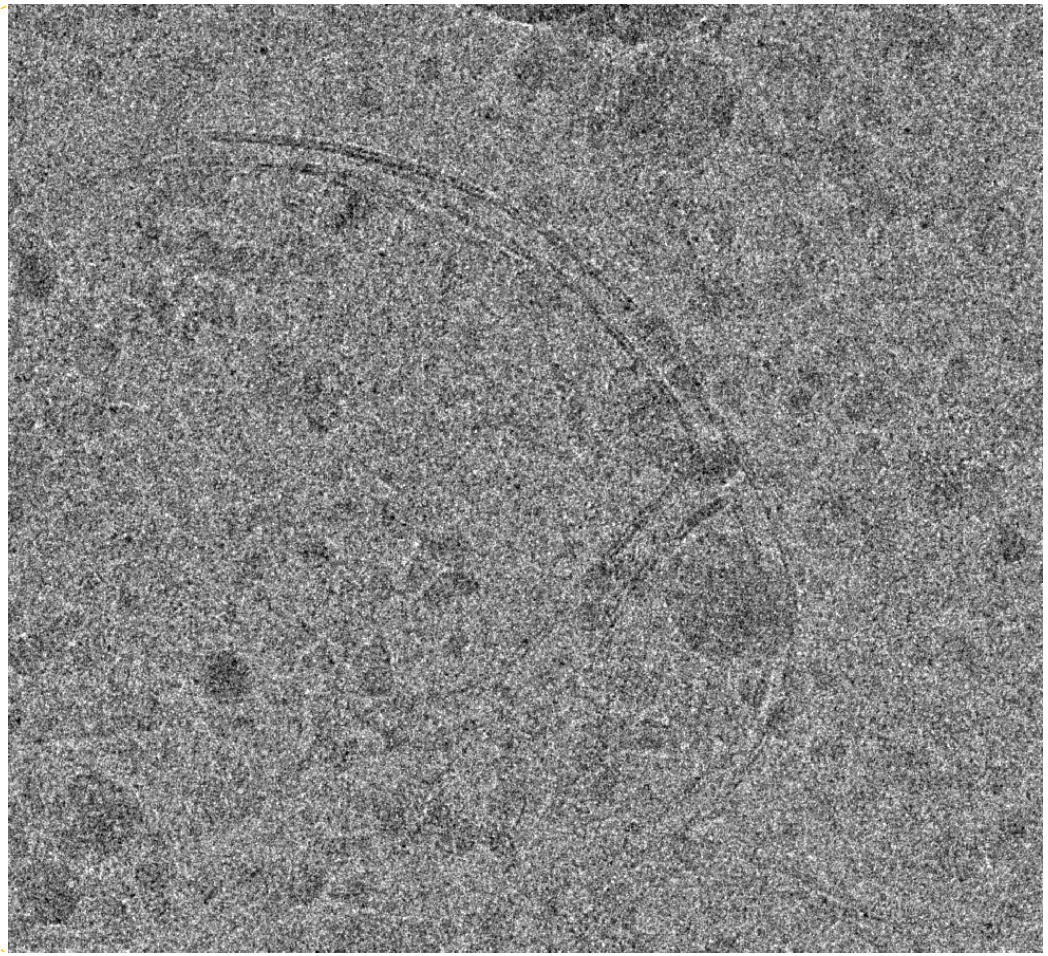


# Tomography of lamella

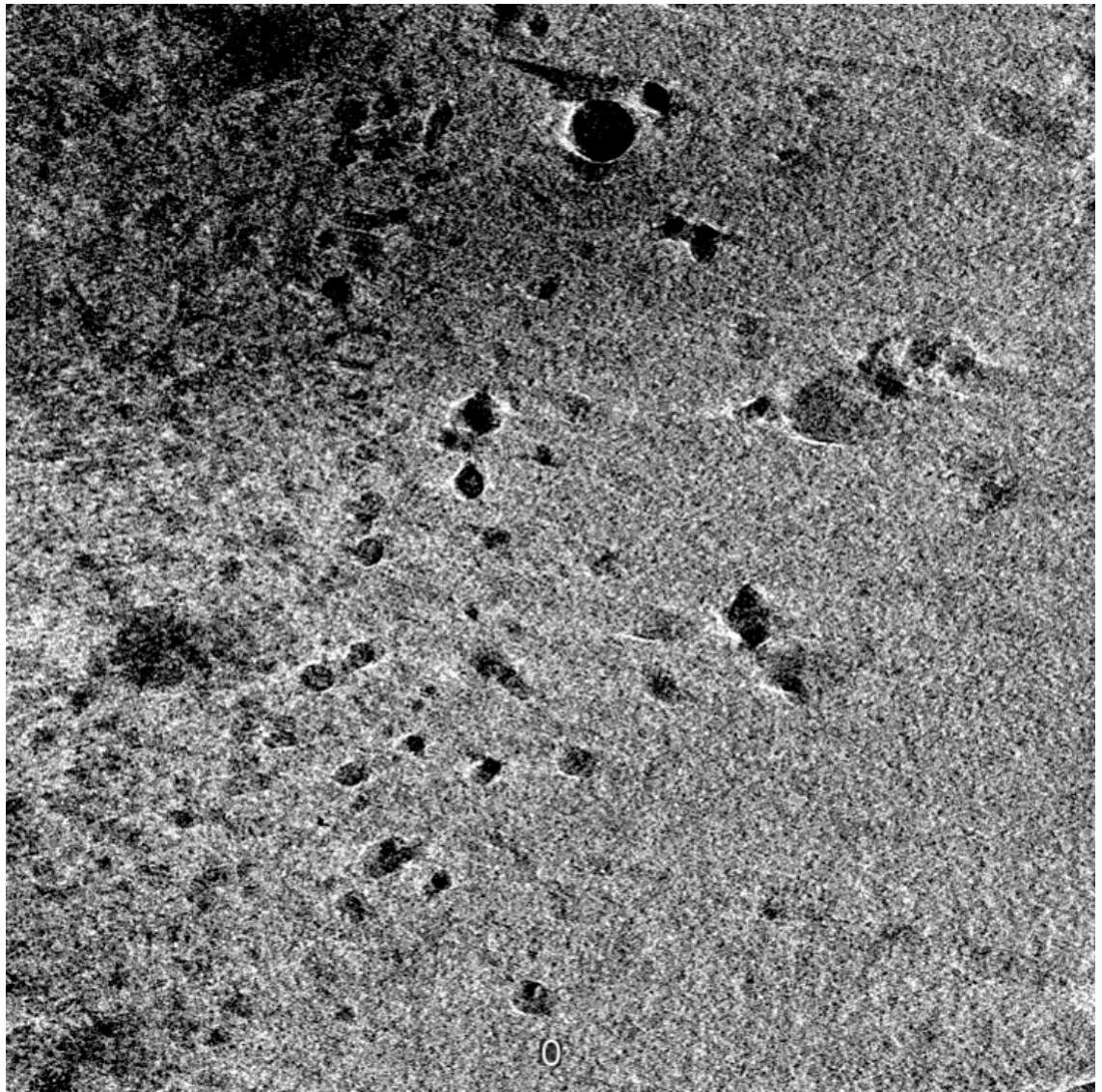


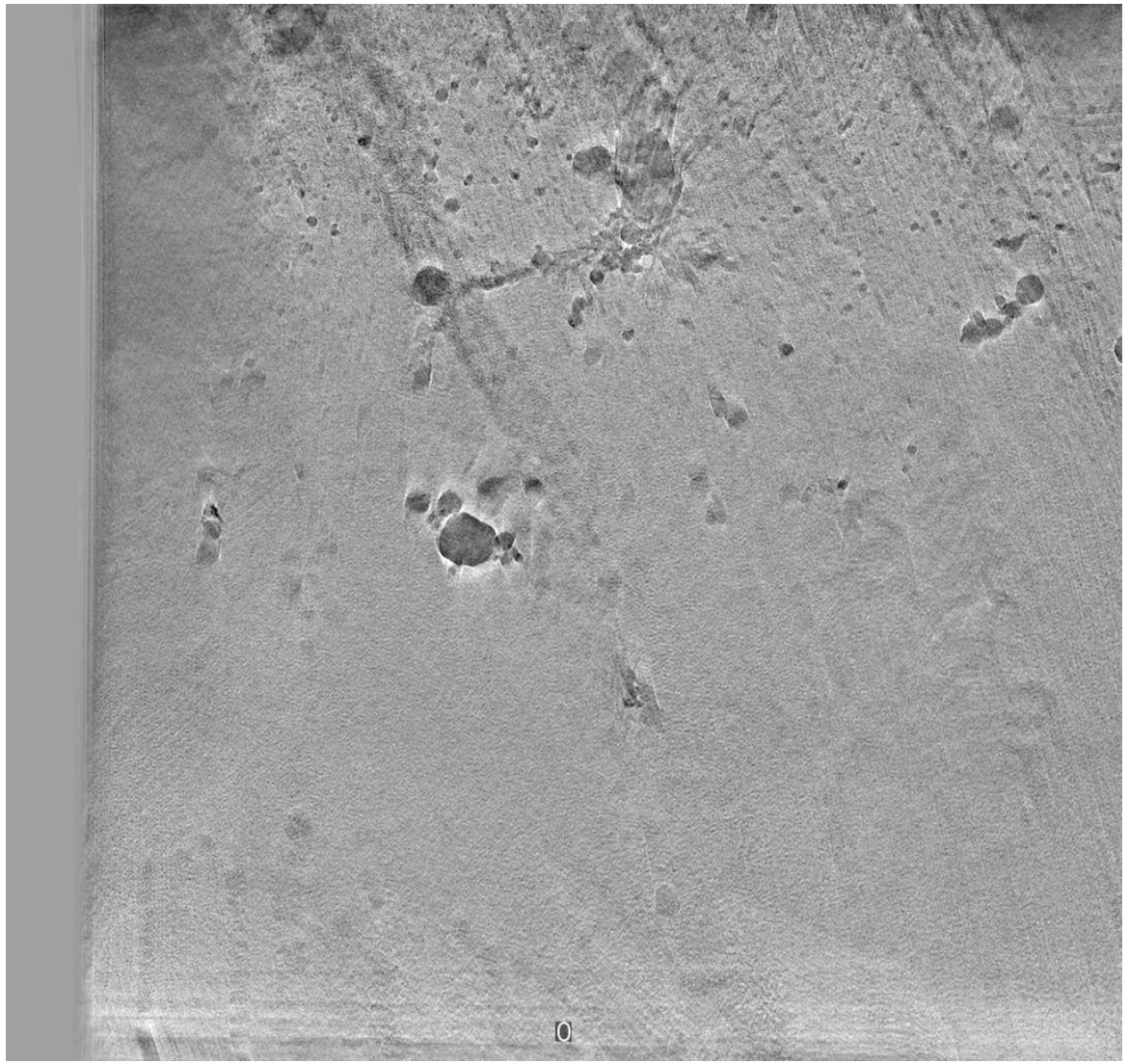

Imaging area of 2.8 um at sampling of 3.4 Å/pix



Small cutout showing bilayer

# Movie of lamella





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- Direct Electron
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