Technical Challenges

Nikolaus Grigorieff
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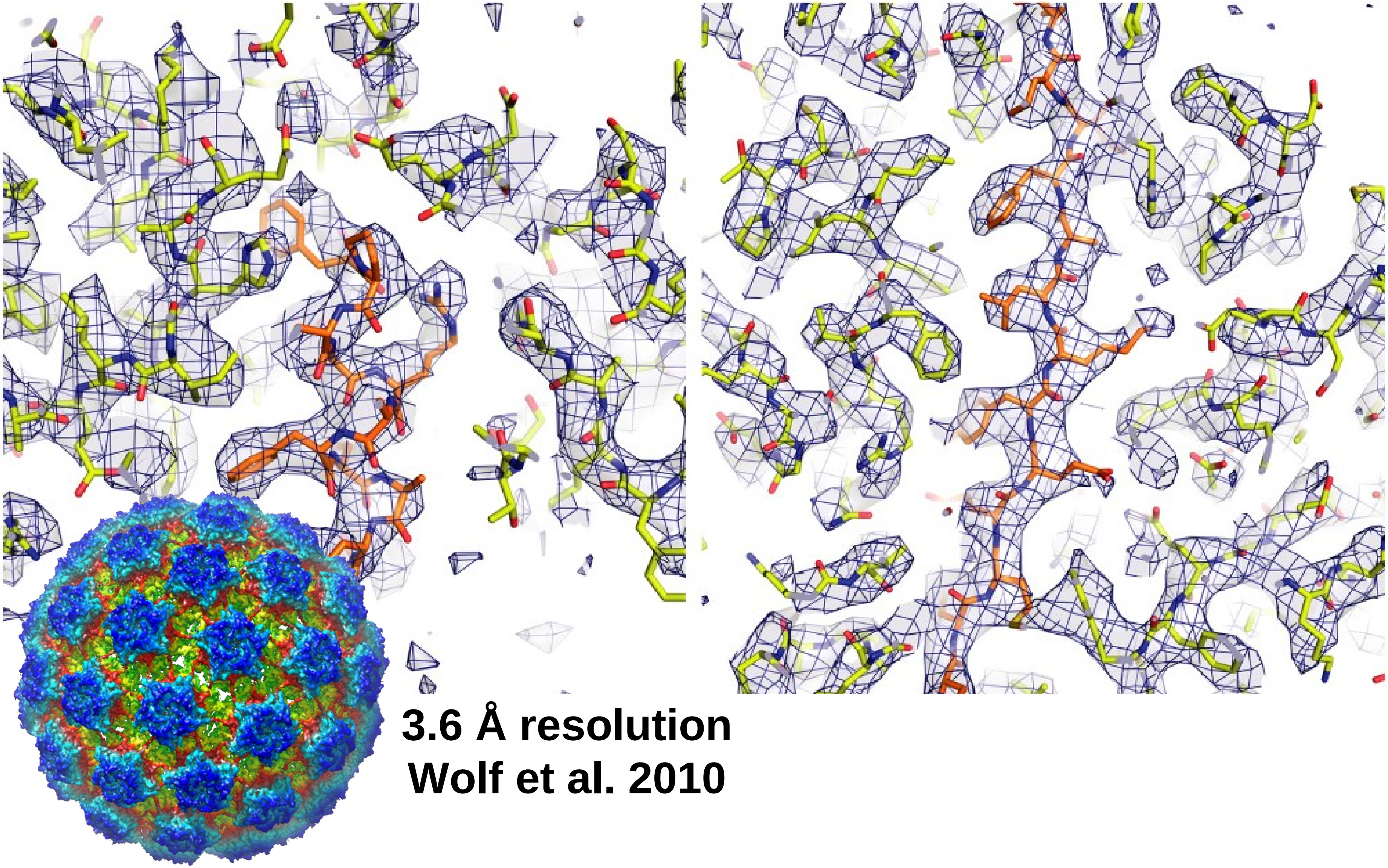
Knowing how it could change the lives of canines everywhere, the dog scientists struggled diligently to understand the Doorknob Principle.

Larson, The Far Side

What Technical Challenges?



3.6 Å resolution
Wolf et al. 2010

An Old Prophecy

How many images must be averaged to reach near-atomic resolution?

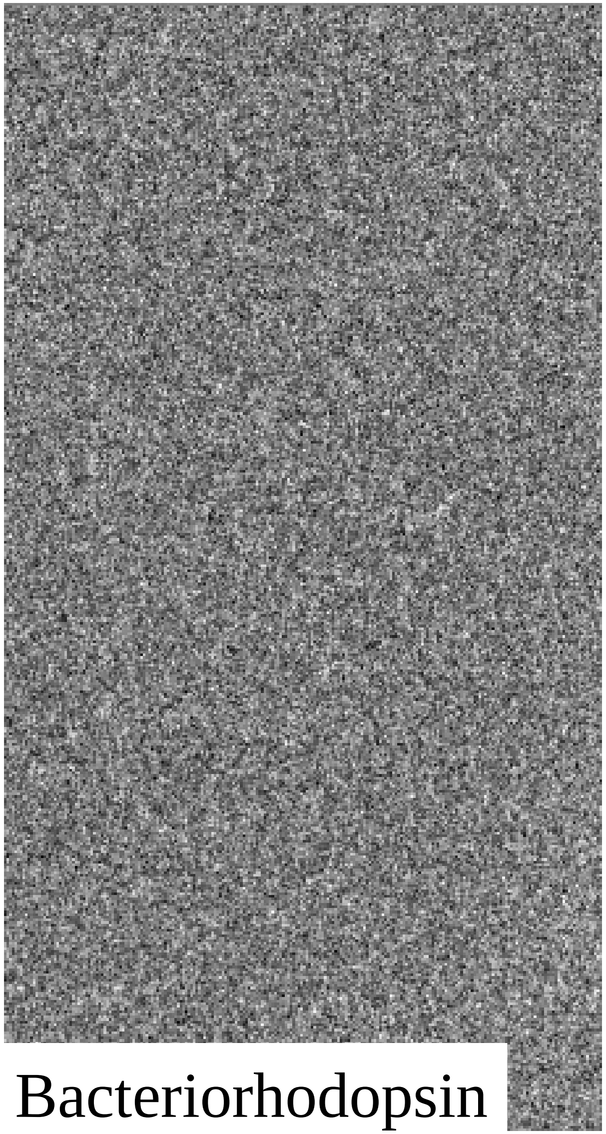
Theoretical prediction (Henderson, Glaeser):

a few thousand

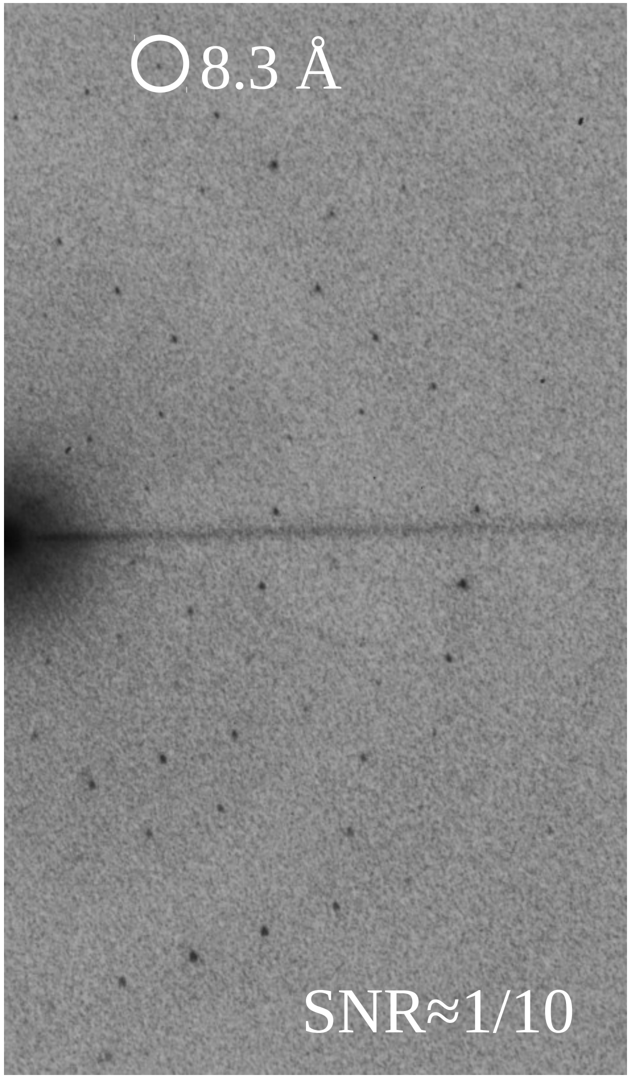
Papillomavirus L1 subunits averaged to reach 3.6 Å:

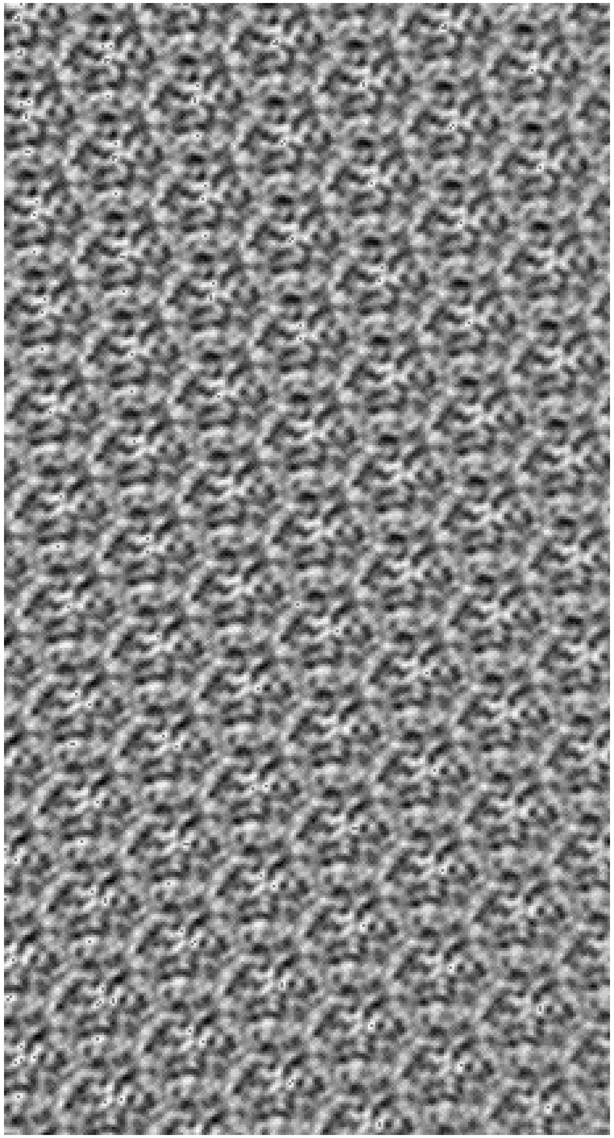
1.5 million!

Contrast and Noise

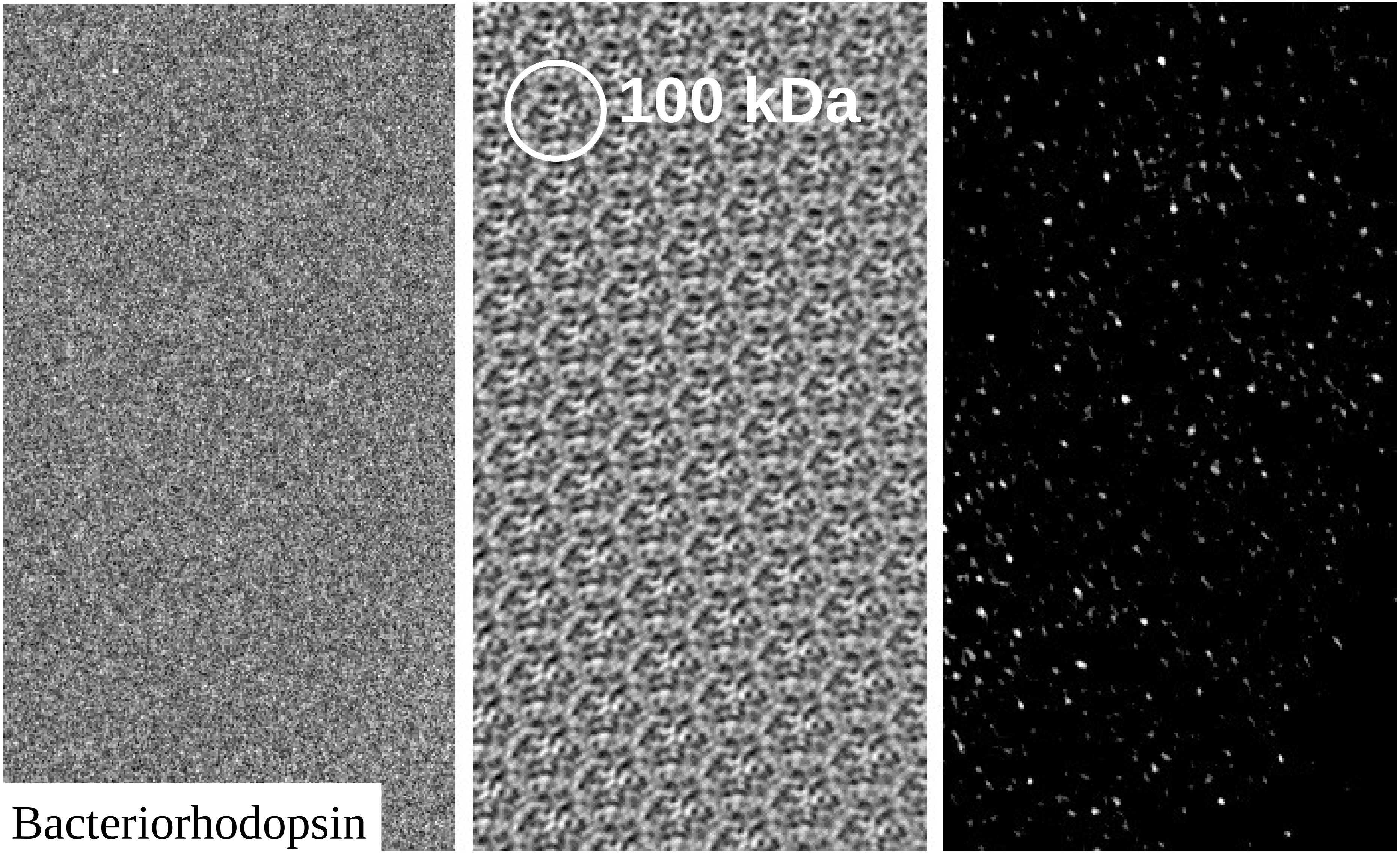


Bacteriorhodopsin

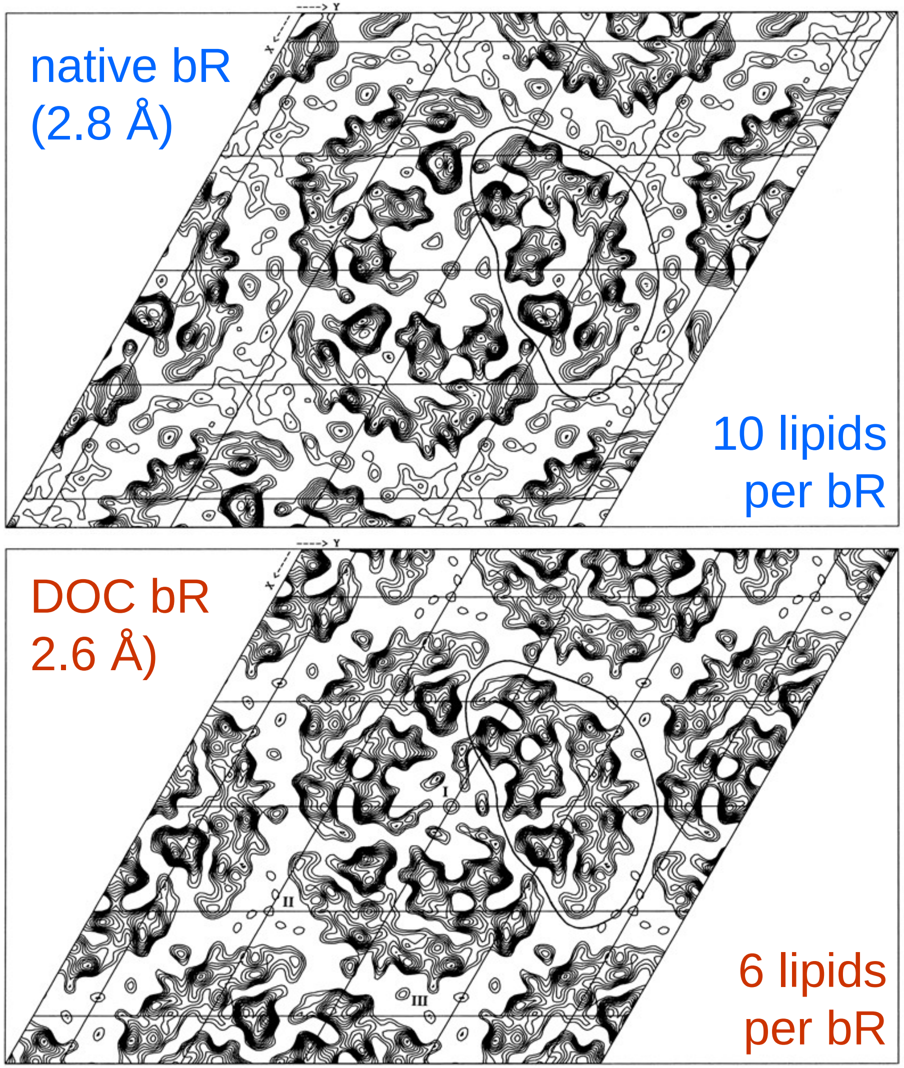


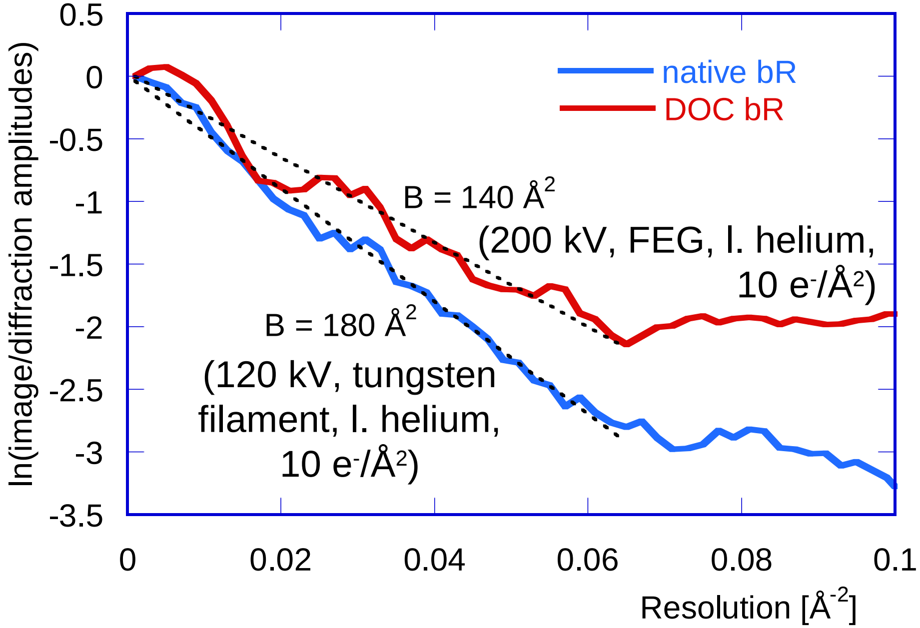


Aligning Small Particles



B-Factor Analysis





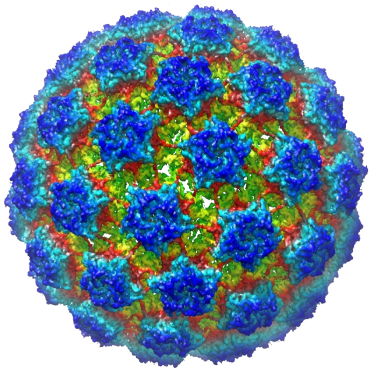
B_{damage} = 40 Å^2 (liquid helium)

$B_{detector}$ = 70 Å^2 (film)

→ B_{total} = 110 Å^2

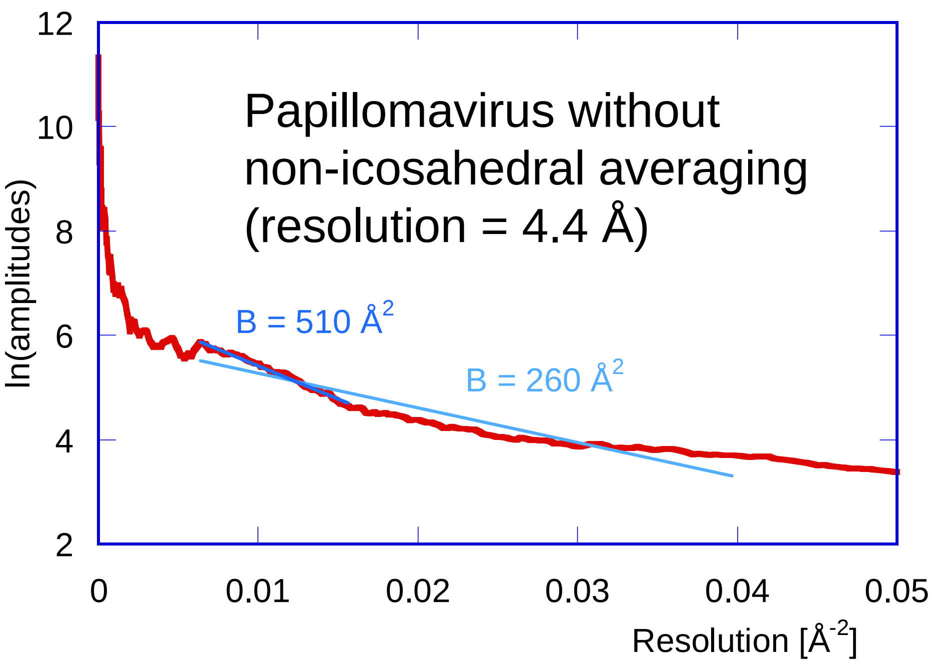
Grigorieff et al. 1995

B-Factor Analysis



3.6 Å resolution
3,977 particles
60-fold icos. sym.
6-fold non-icos. sym.

Wolf et al. 2010



B_{damage} = 60 Å^2 (liquid nitrogen)

$B_{detector}$ = 70 Å^2 (film)

B_{motion} = 160 Å^2 (Campbell et al. 2012)

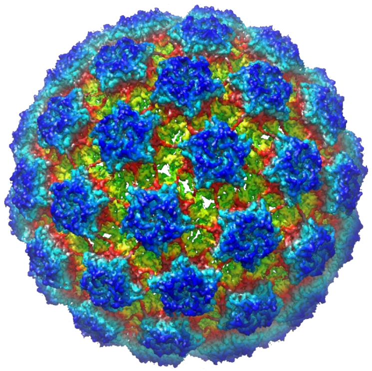
$B_{alignment}$ = 90 Å^2 (σ_{shift} = 0.2 Å, σ_{rot} = 0.2°, $\sigma_{defocus}$ = 200 Å)

→ B_{total} = 380 Å^2

$B_{observed}$ = 510 Å^2

→ $B_{unexplained}$ = 130 Å^2

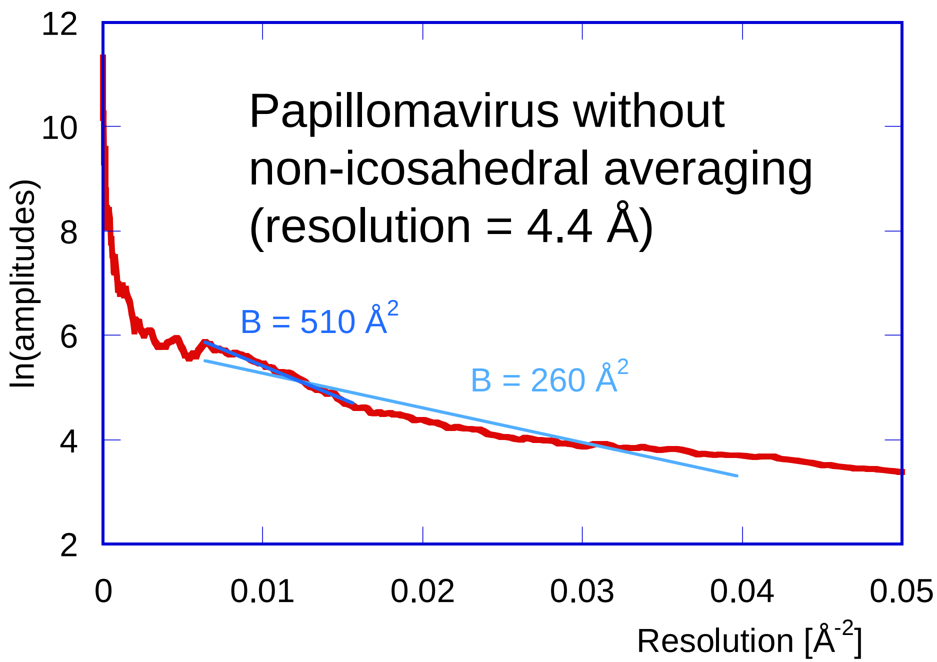
B-Factor Analysis

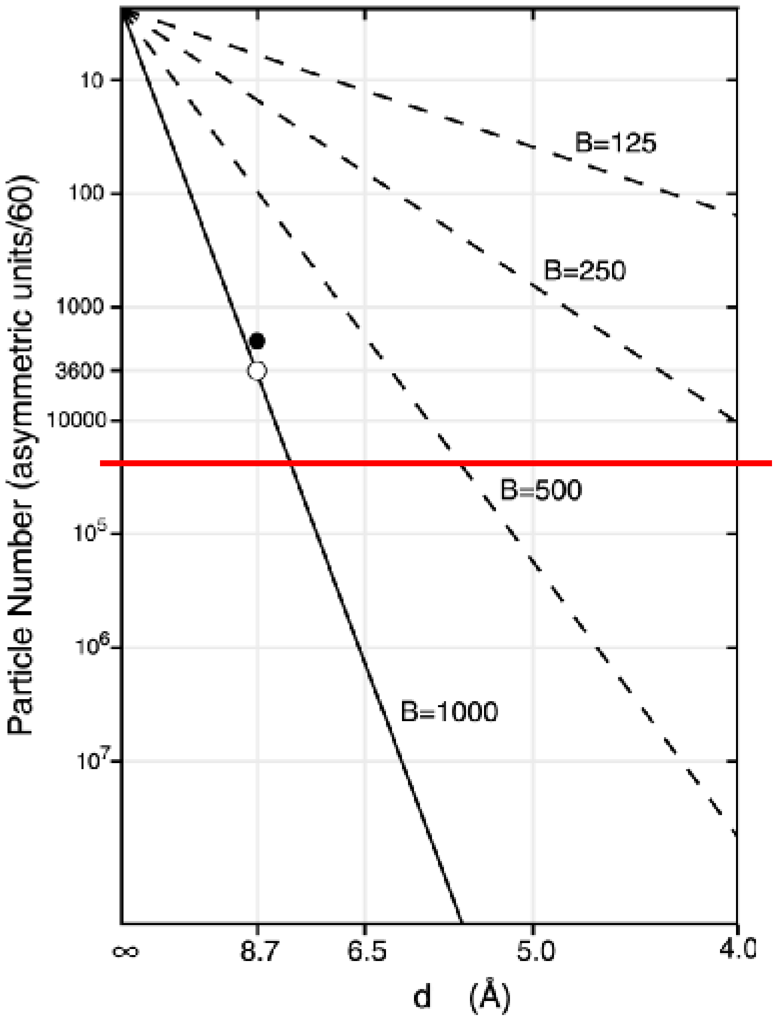


Wolf et al. 2010

3.6 Å resolution
3,977 particles
60-fold icos. sym.
6-fold non-icos. sym.

→ 24,000 "60-fold" particles

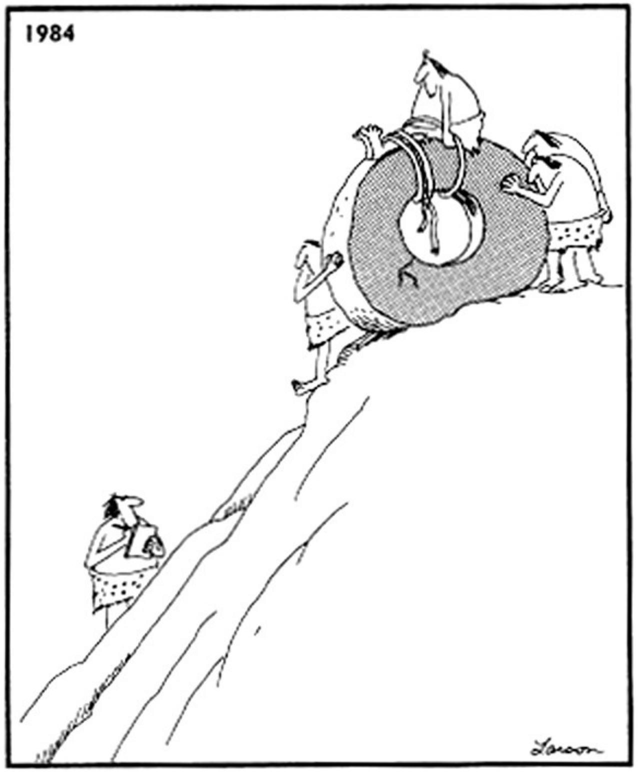




Rosenthal & Henderson 2003

The Challenges

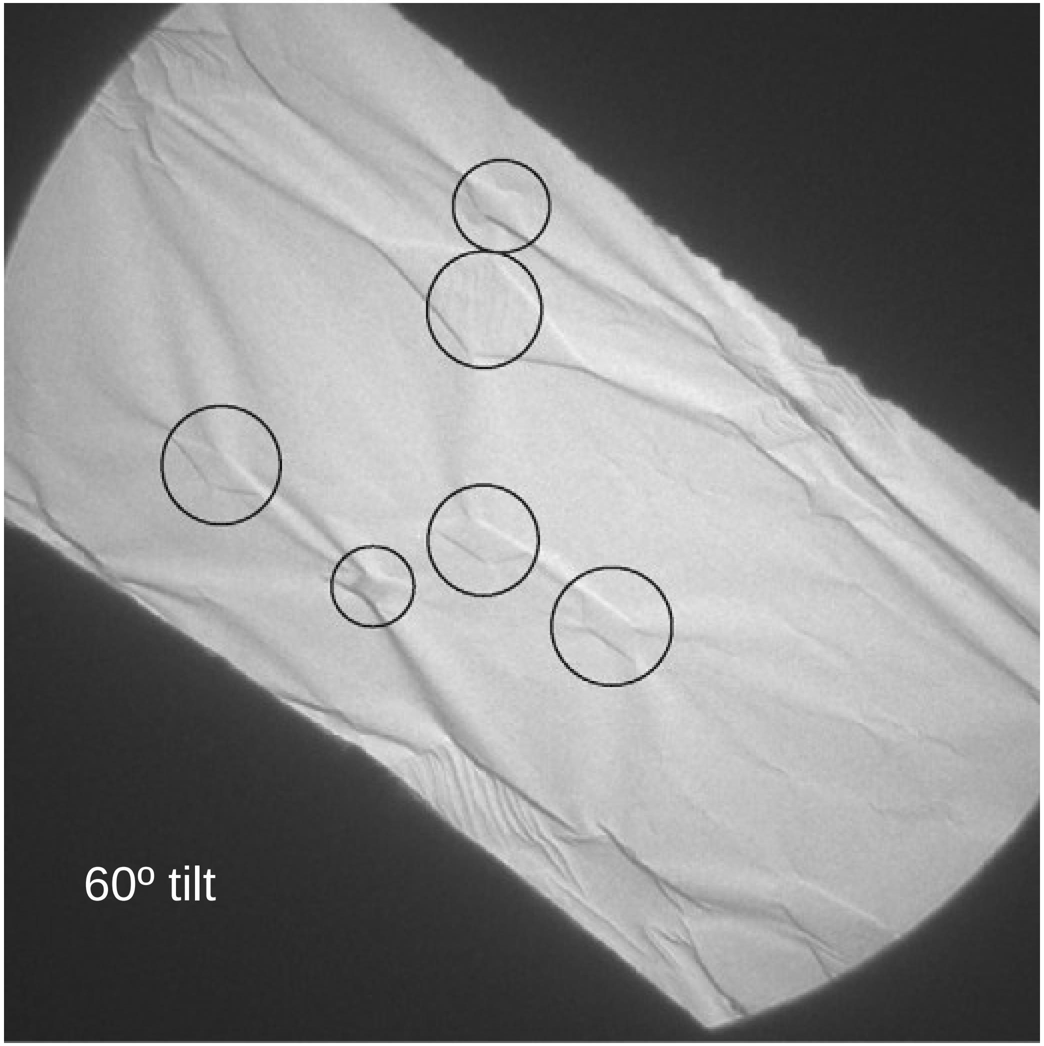
- Beam-induced motion & charging
- Detector DQE
- Beam damage
- Alignment errors



Early experiments in transportation

Larson, The Far Side

Better Sample Support



Thin carbon support shows crinkling due to shrinking of the copper grid (0.3%) and paraffin crystals (1% – 2%) at liquid nitrogen temperature.

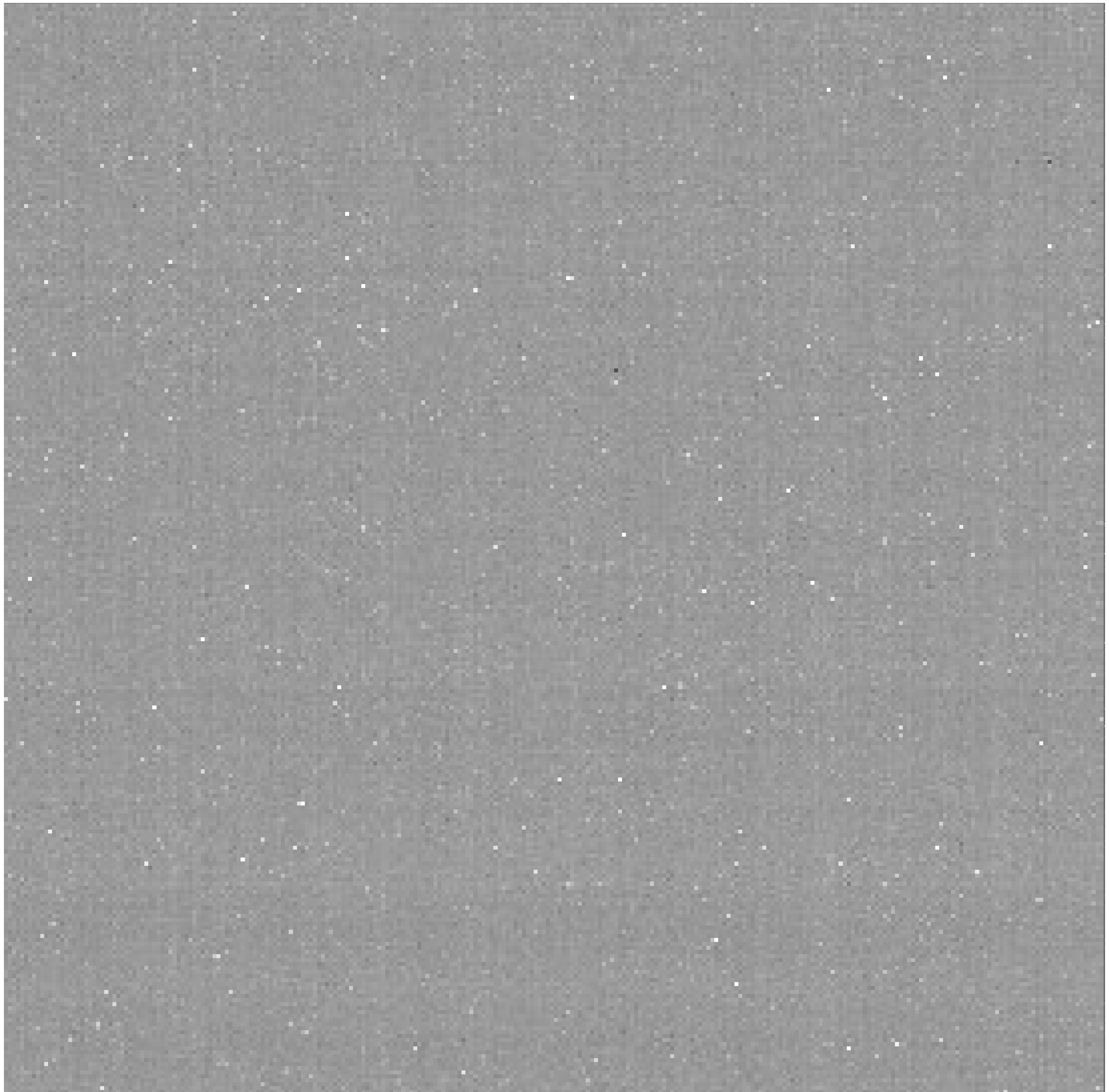
Thick carbon (350 Å) reduces or eliminated movement.

→ Flatness and mechanical strength of the support film are important.

Molybdenum grids may help.

Glaeser et al. 2011

Movies



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

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

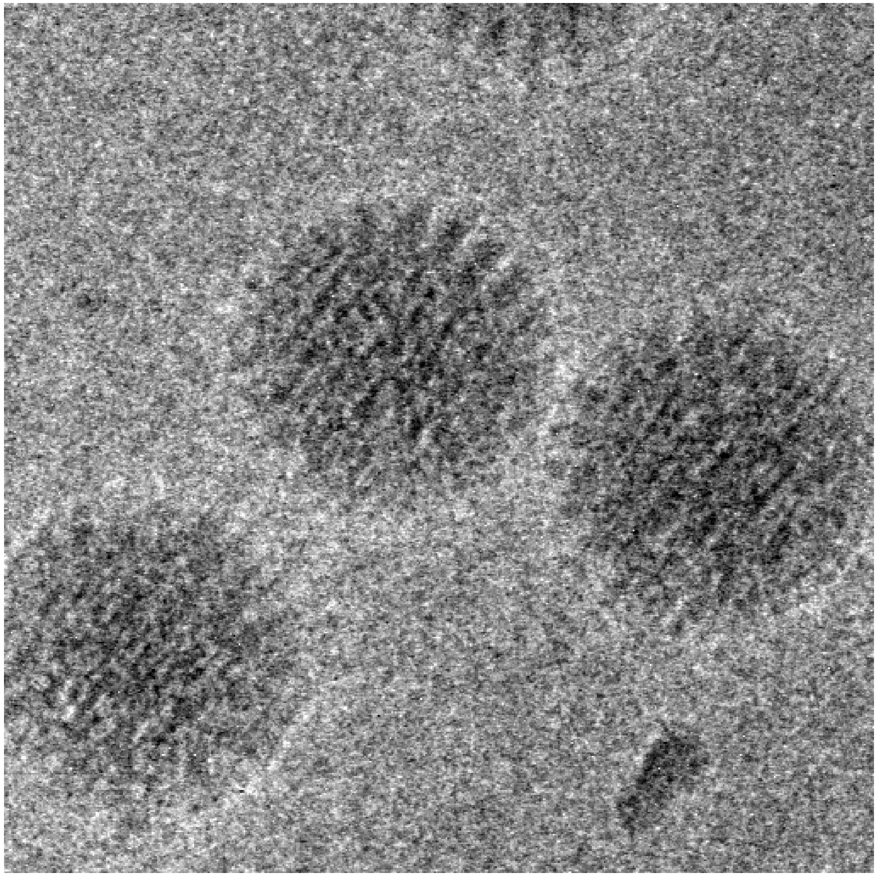
1 movie = 720 MB
(1 byte/pixel)

→ Data Tsunami!

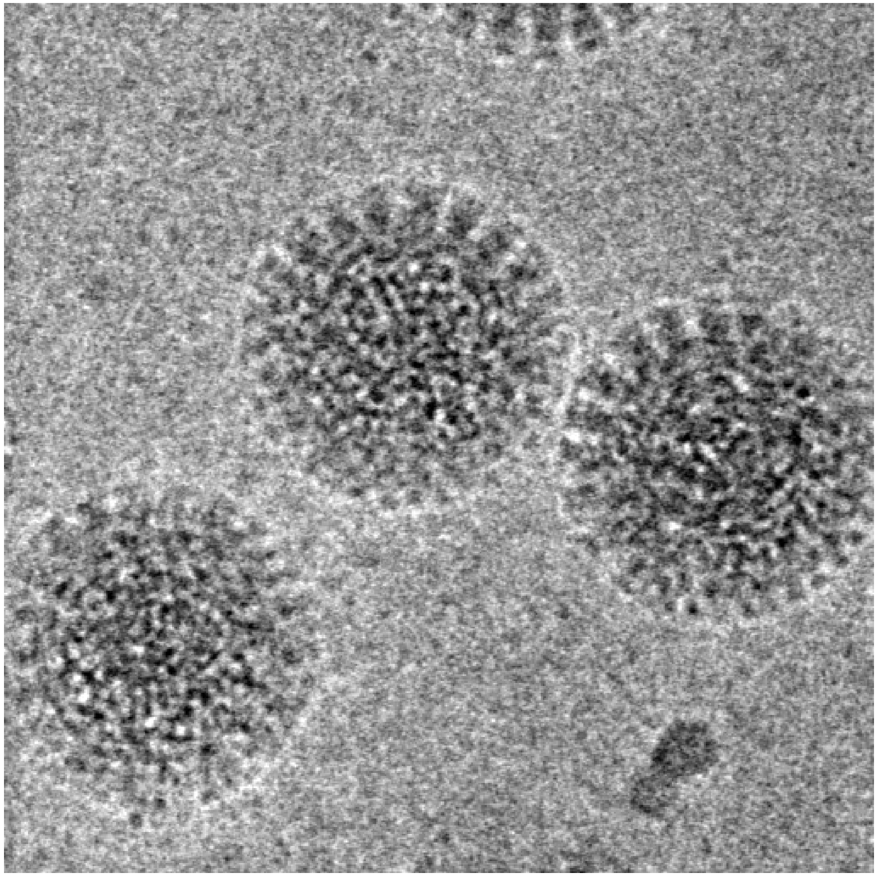
Brilot et al. 2012

Frame Alignment

60-frame average
(no alignment)

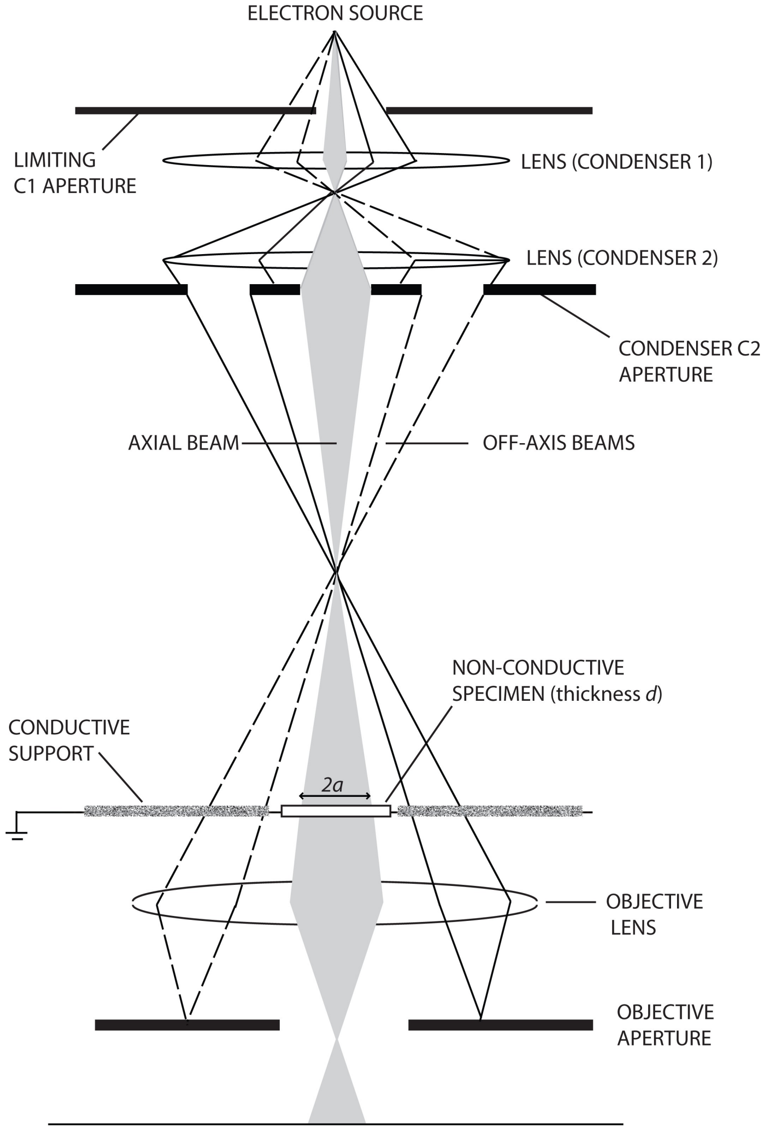


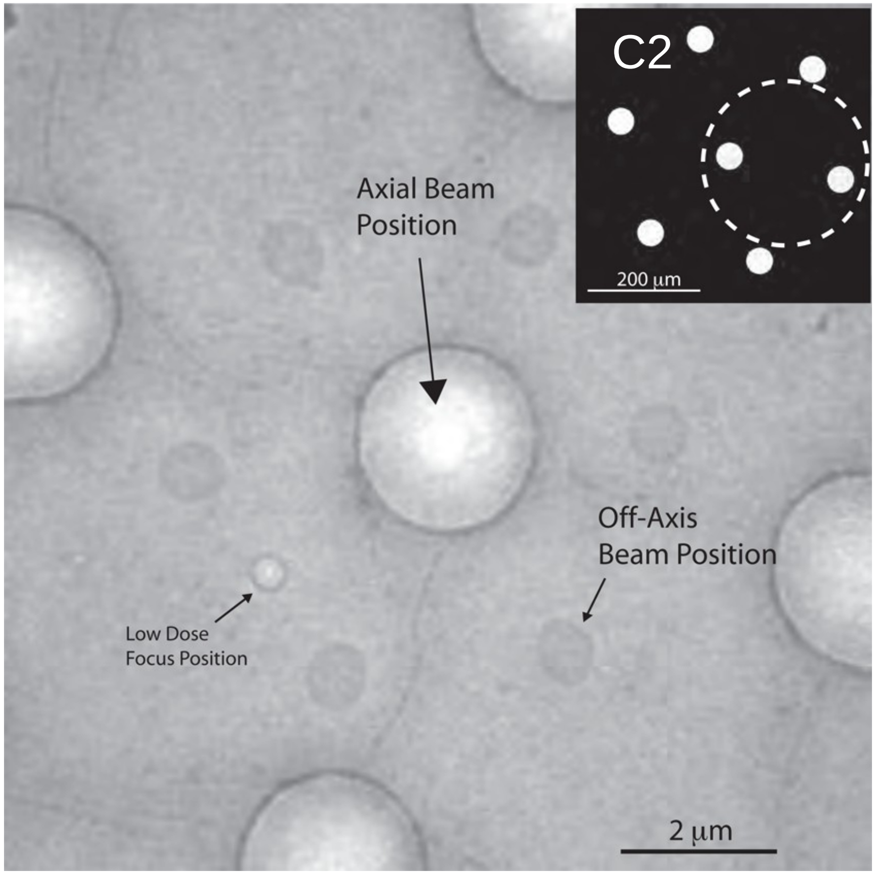
60-frame average
(translational alignment)



Brilot et al. 2012

Paraxial Charge Compensation





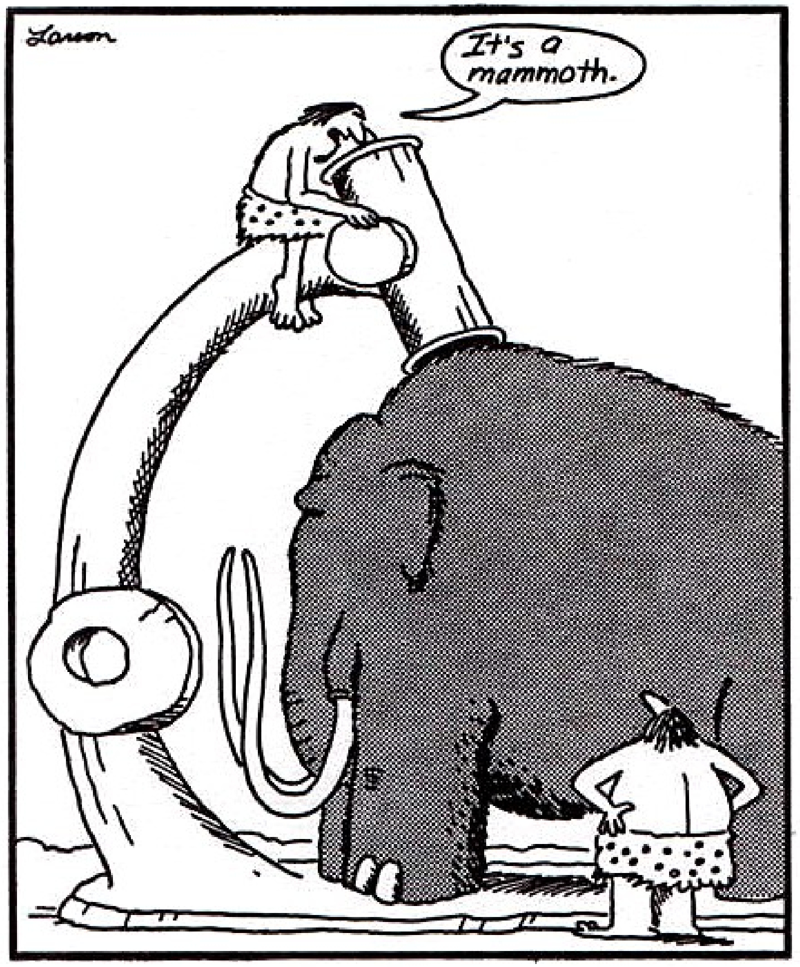
Berriman & Rosenthal 2012

The Challenges

- Beam-induced motion & charging
- Detector DQE → Contrast
- Beam coherence
- Alignment errors

Improving Contrast

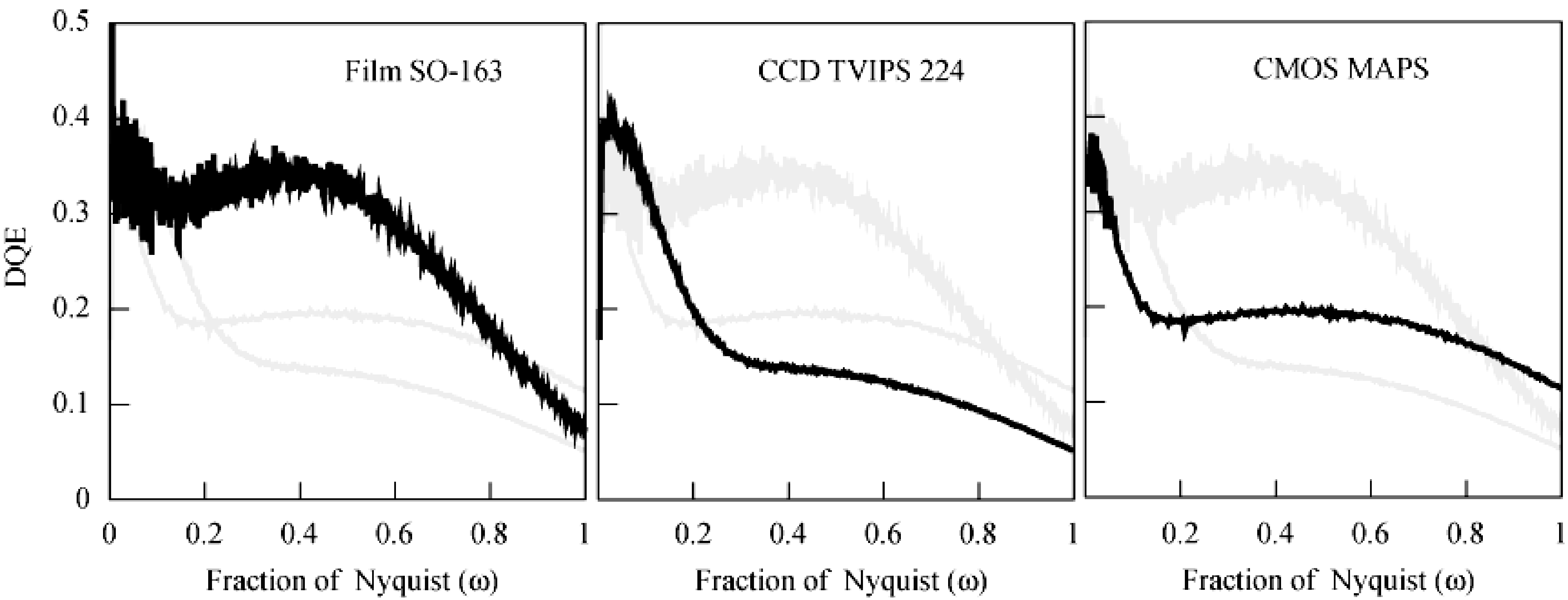
- Better detectors
- Low voltage
- Phase plate
- Inelastic scattering
- Astigmatism



Early microscope

Larson, The Far Side

Direct Electron Detectors

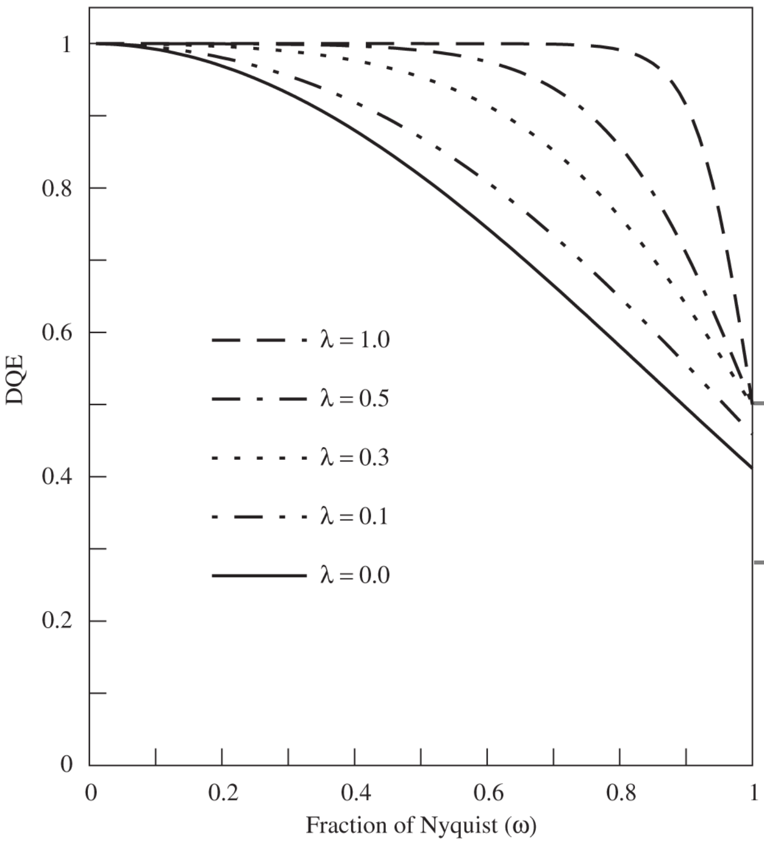


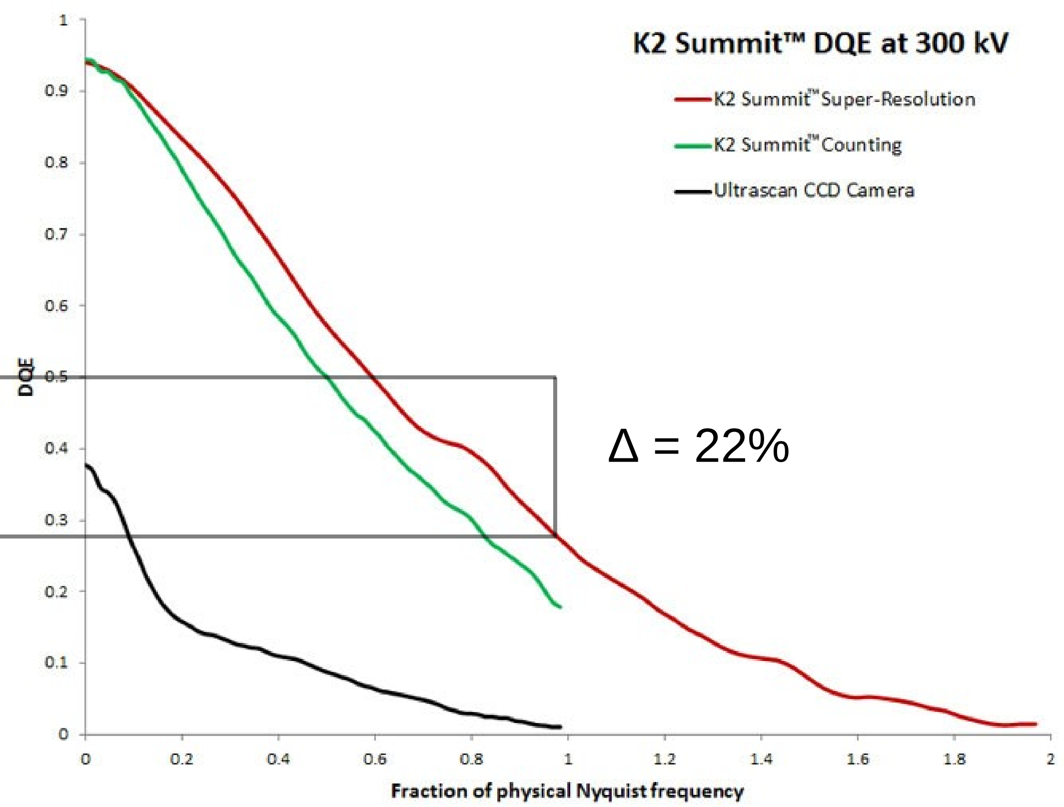
300 kV

Chris Booth (Gatan)
Benjamin Bammes (Direct Electron)
Panel discussion (David Agard)

McMullan & Henderson, 2009

Perfect Detector





McMullan & Henderson, 2009

Gatan webpage, 2012

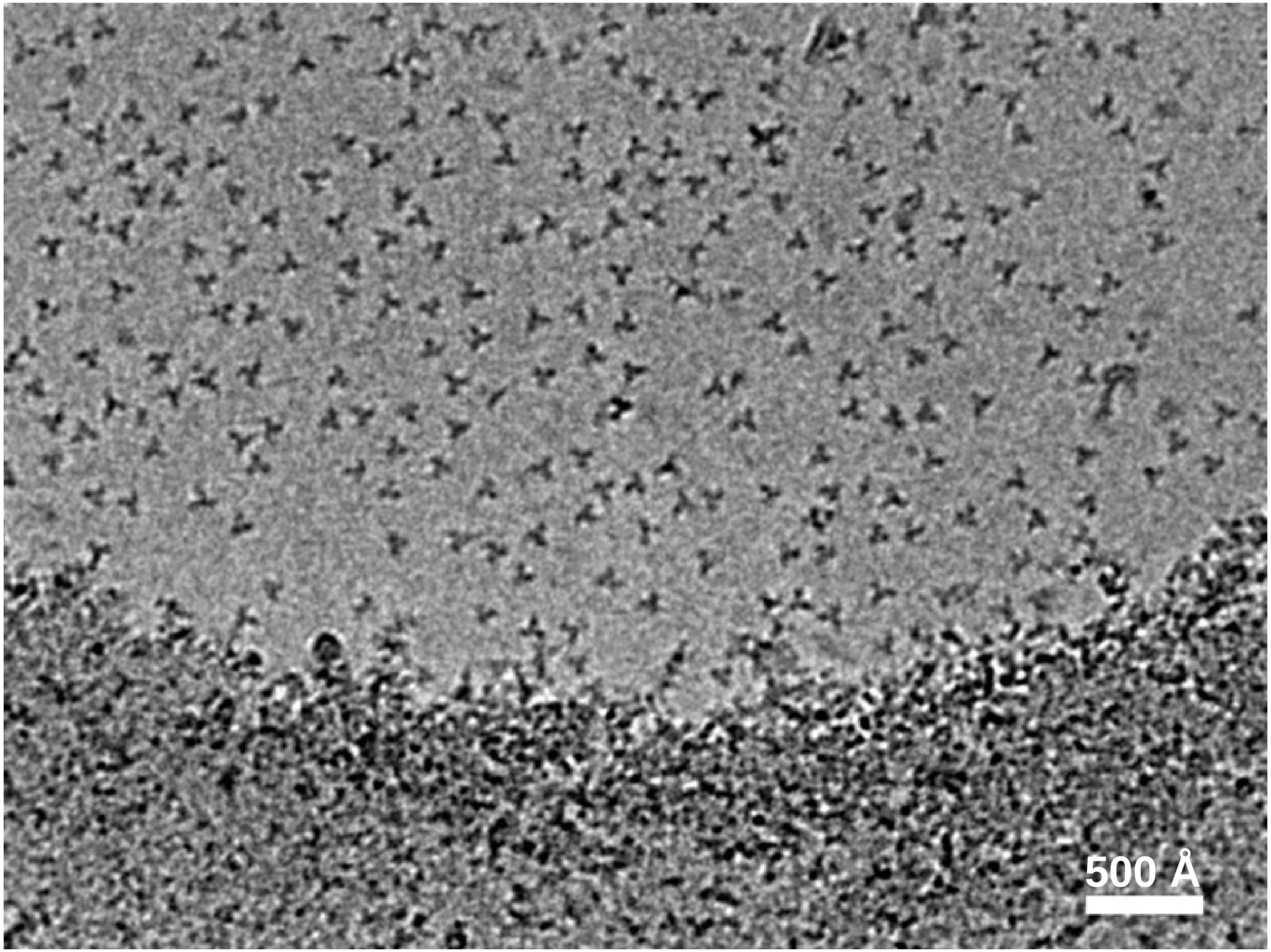
Low Voltage

Trimers of HIV gp140 in ice

M = 420 kDa

80 kV
20 $e^-/Å^2$

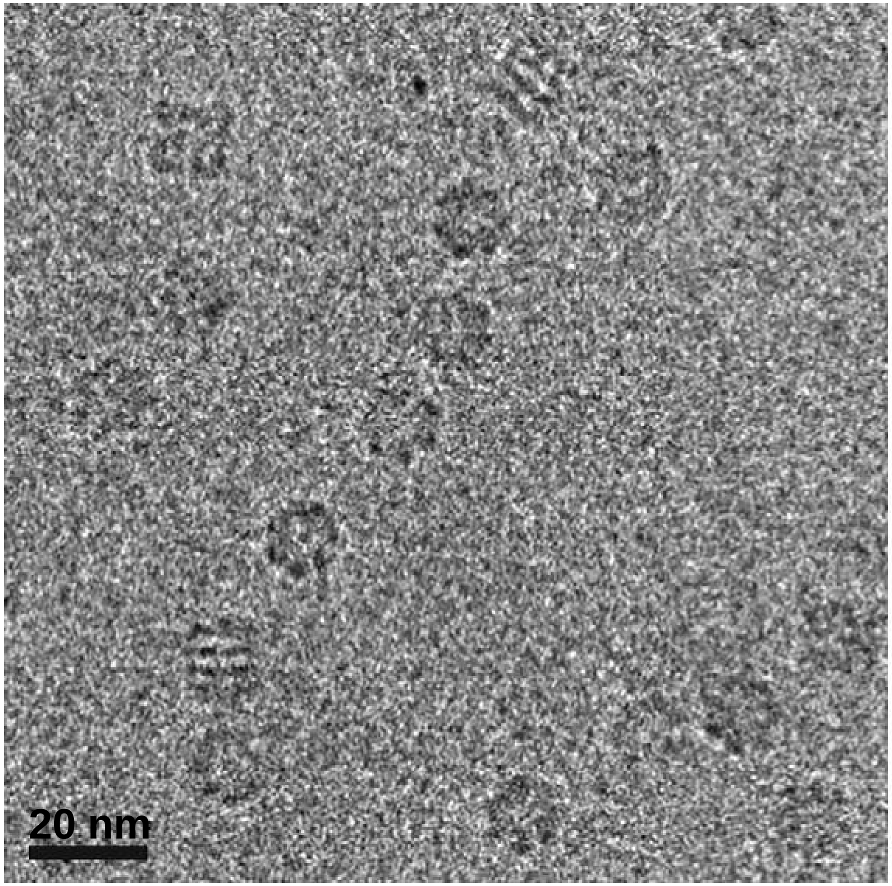
DQE of film and scintillator-based cameras improved at lower voltage



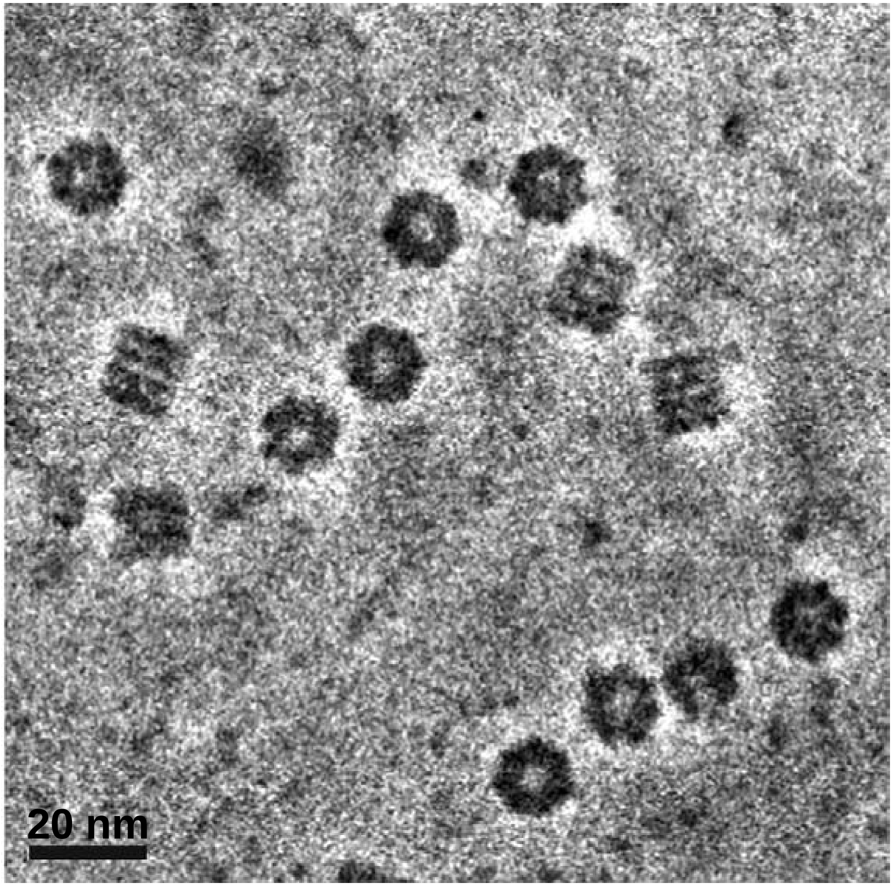
Harris et al 2011

Phase Plate

GroEL in ice



Defocus contrast

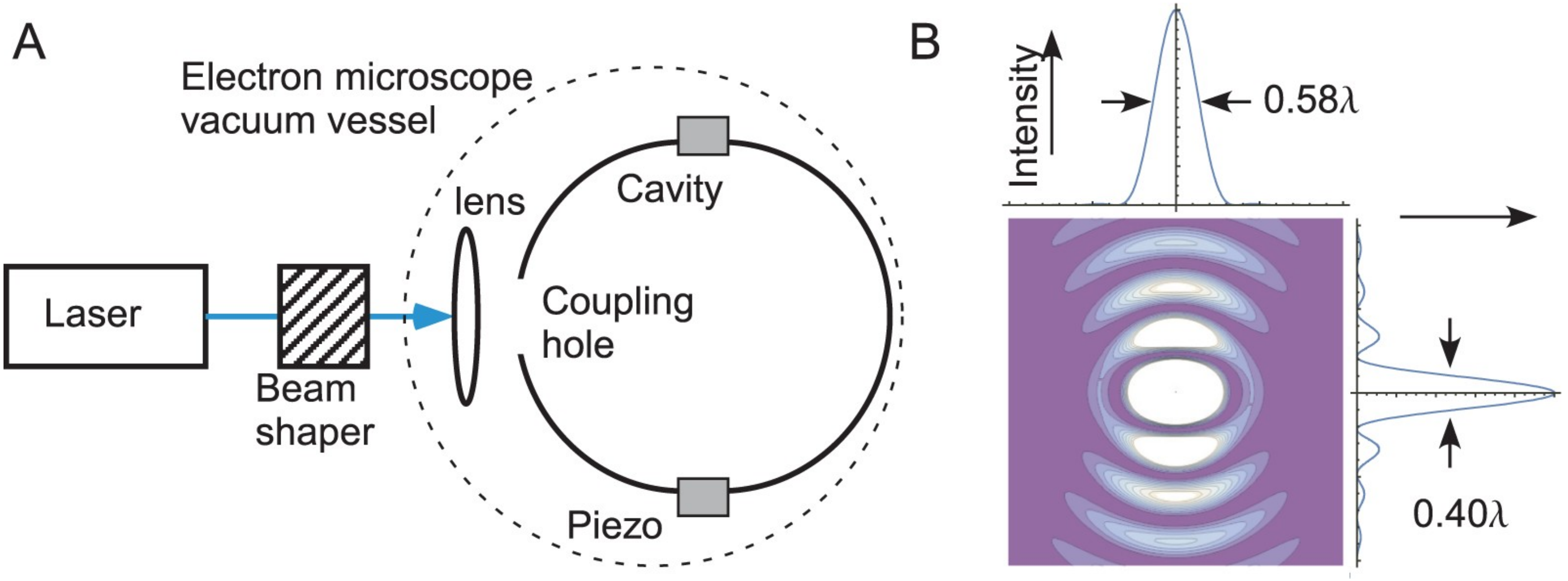


Zernike phase plate

Wah Chiu, Bob Glaeser

Danev & Nagayama 2008

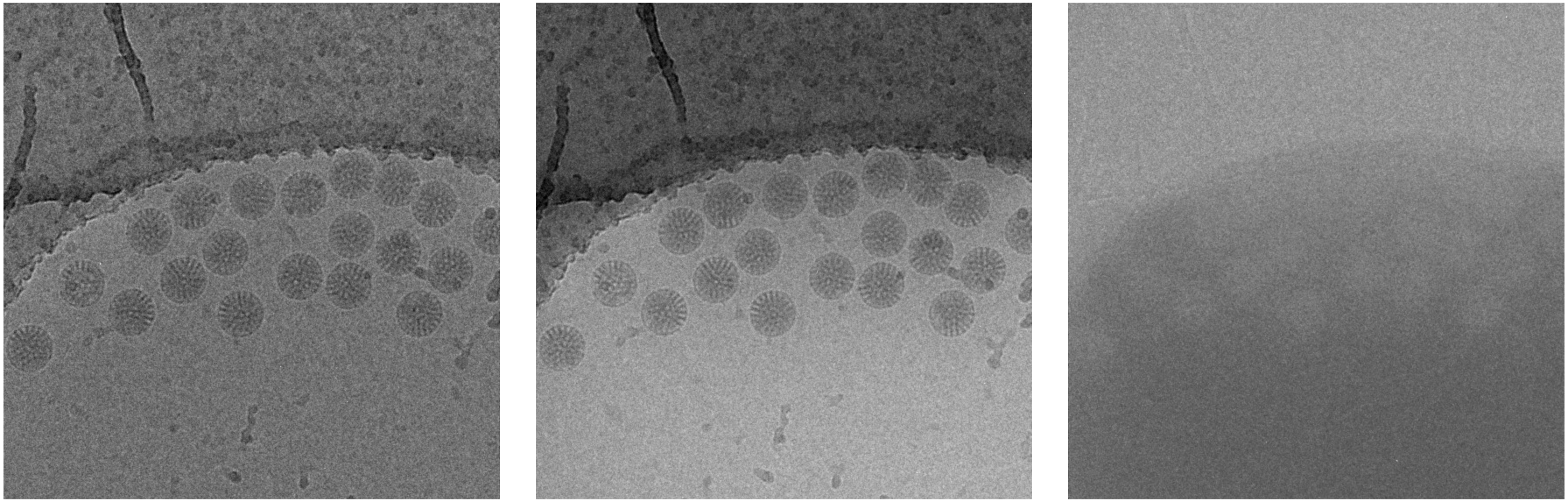
Phase Plate with Lasers



Elastic Compton scattering
Spherical resonant cavity
40 W laser with λ = 2 μm

Müller et al. 2010

Inelastic Scattering



Unfiltered 0 eV 25 eV

300 kV, 6 µm underfocus, 15 eV energy window

Assuming 700 Å sample thickness:
Electrons scattered elastically: 9%
scattered inelastically: 18%

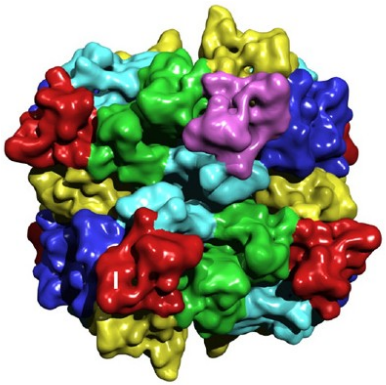
→ C_c correctors will increase image contrast.

Chen Xu (unpublished)

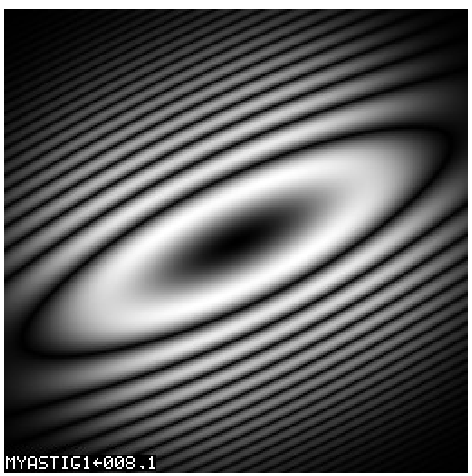
Astigmatic CTF

Hemocyanine

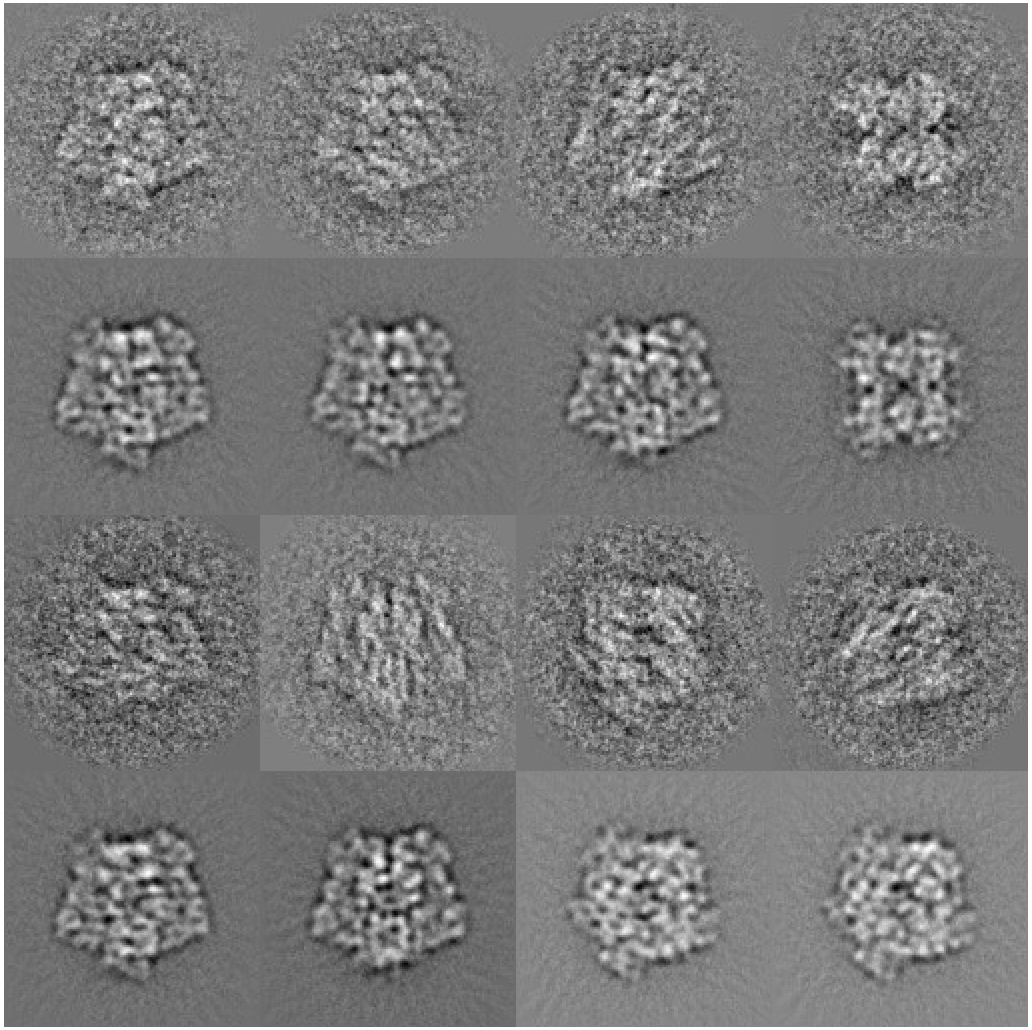
3.8 MDa
D_2 symmetry



Martin et al. 2007

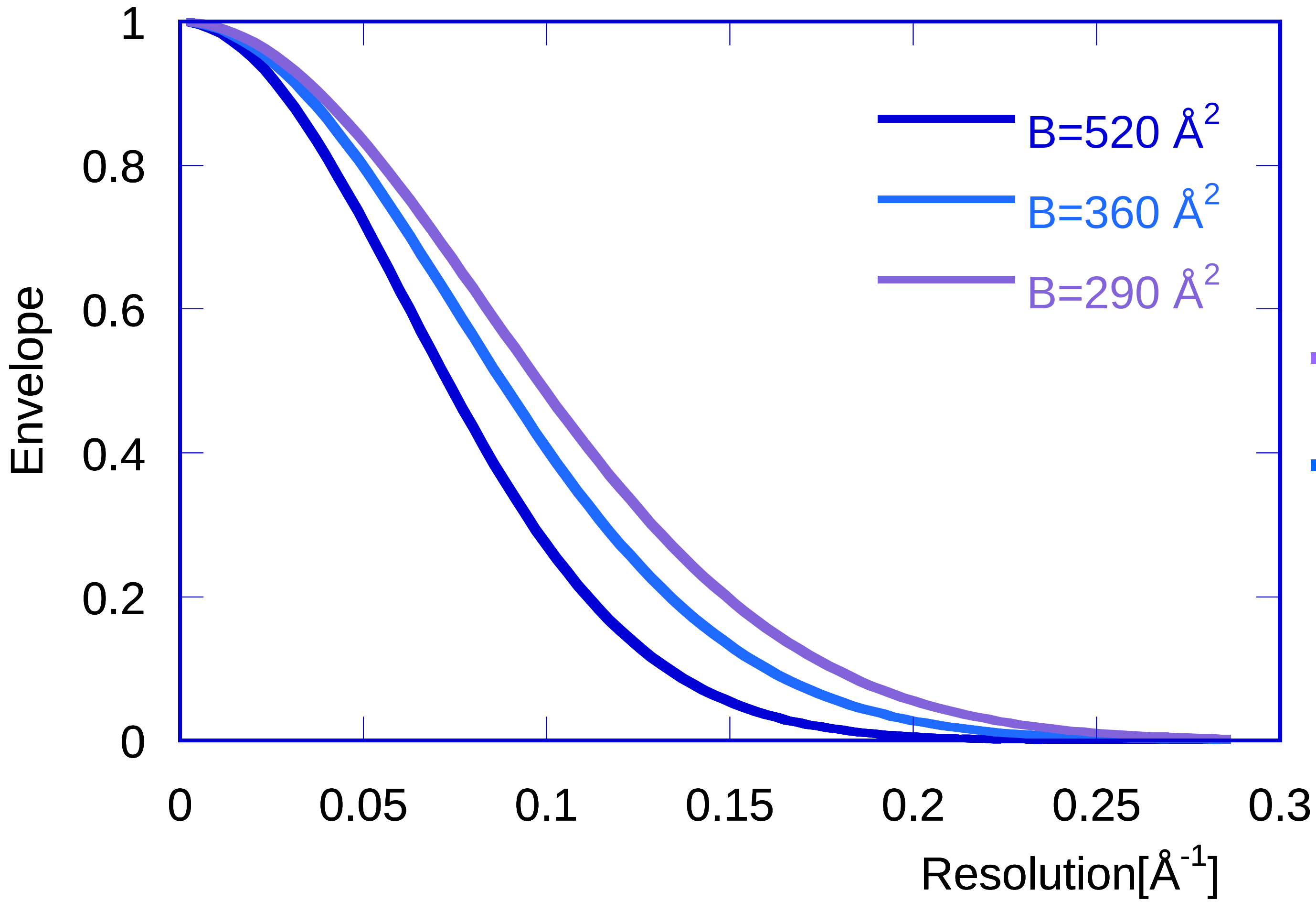


DF_1 = 1000 Å, DF_2 = 14000 Å



Grant & van Heel (unpublished)

Potential Improvements



B_{damage} = 60 Å^2

~~$B_{detector}$ = 70 Å^2~~

~~B_{motion} = 160 Å^2~~

$B_{alignment}$ = 90 Å^2

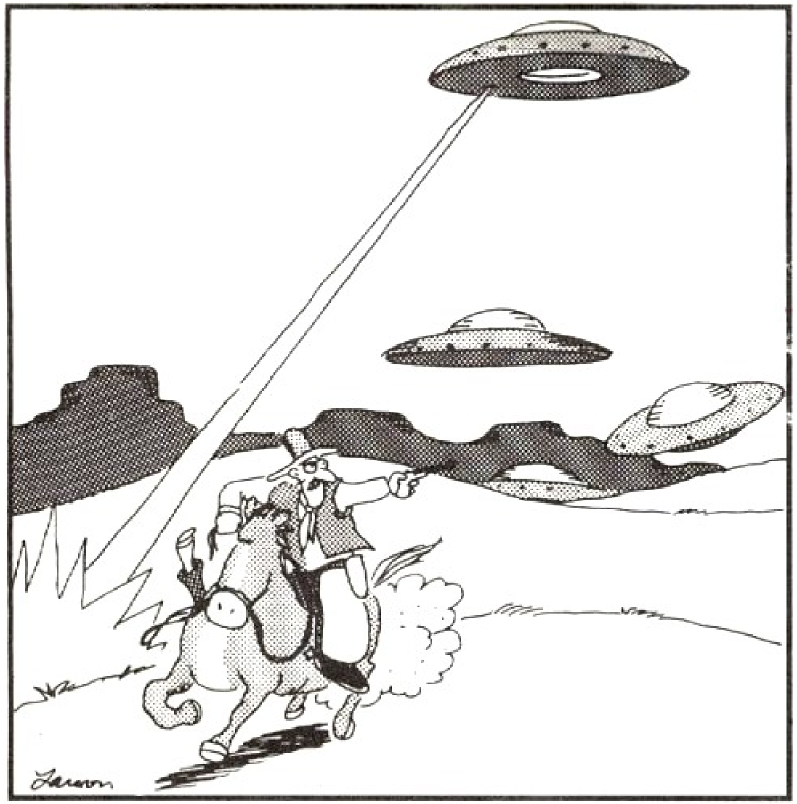
Factor @ 3.5 Å:

110

12,000 x signal

The Challenges

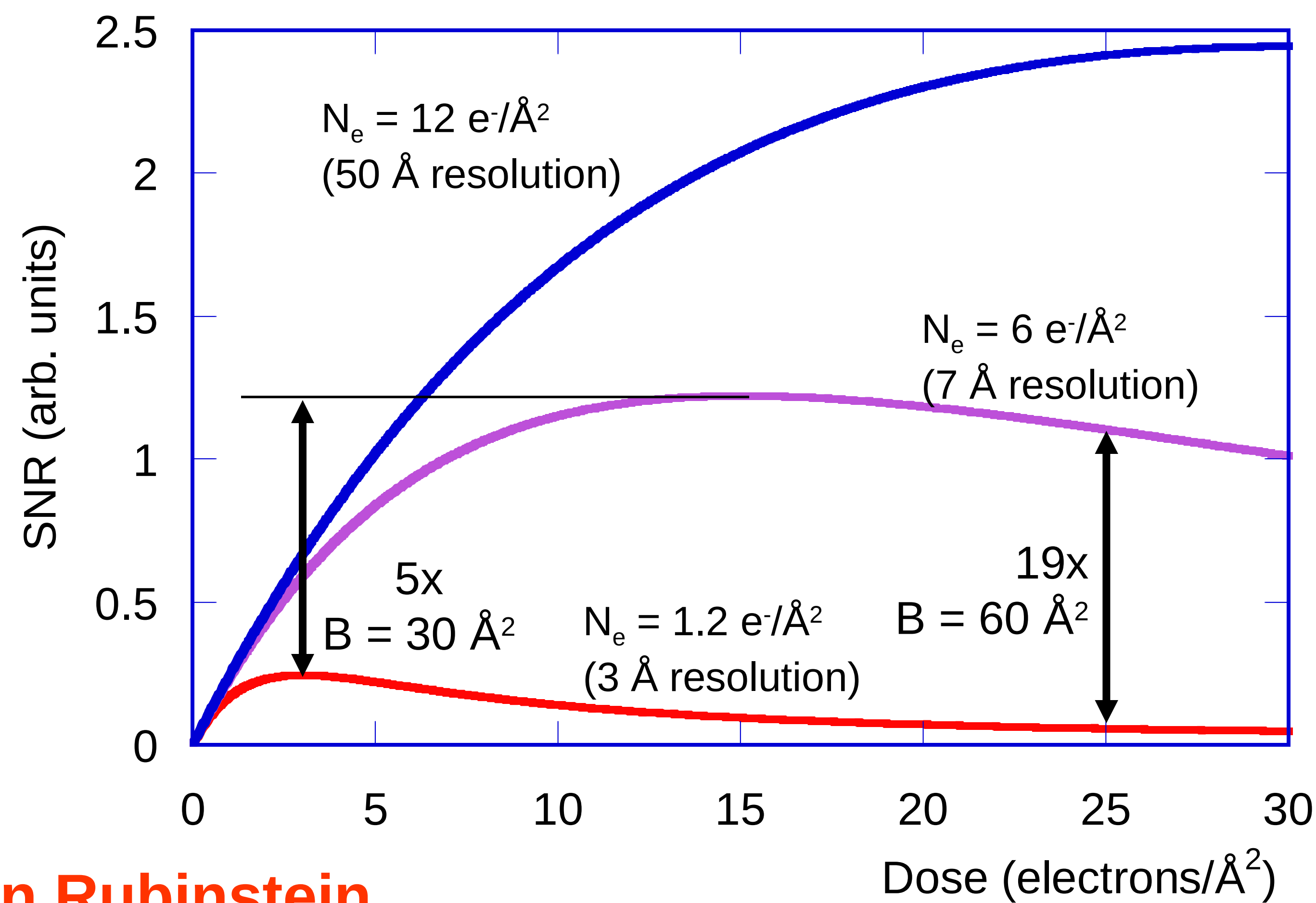
- Beam-induced motion & charging
- Detector DQE
- Beam damage
- Alignment errors



The often romanticized image of cowboys and aliens

Larson, The Far Side

Optimal Dose



John Rubinstein

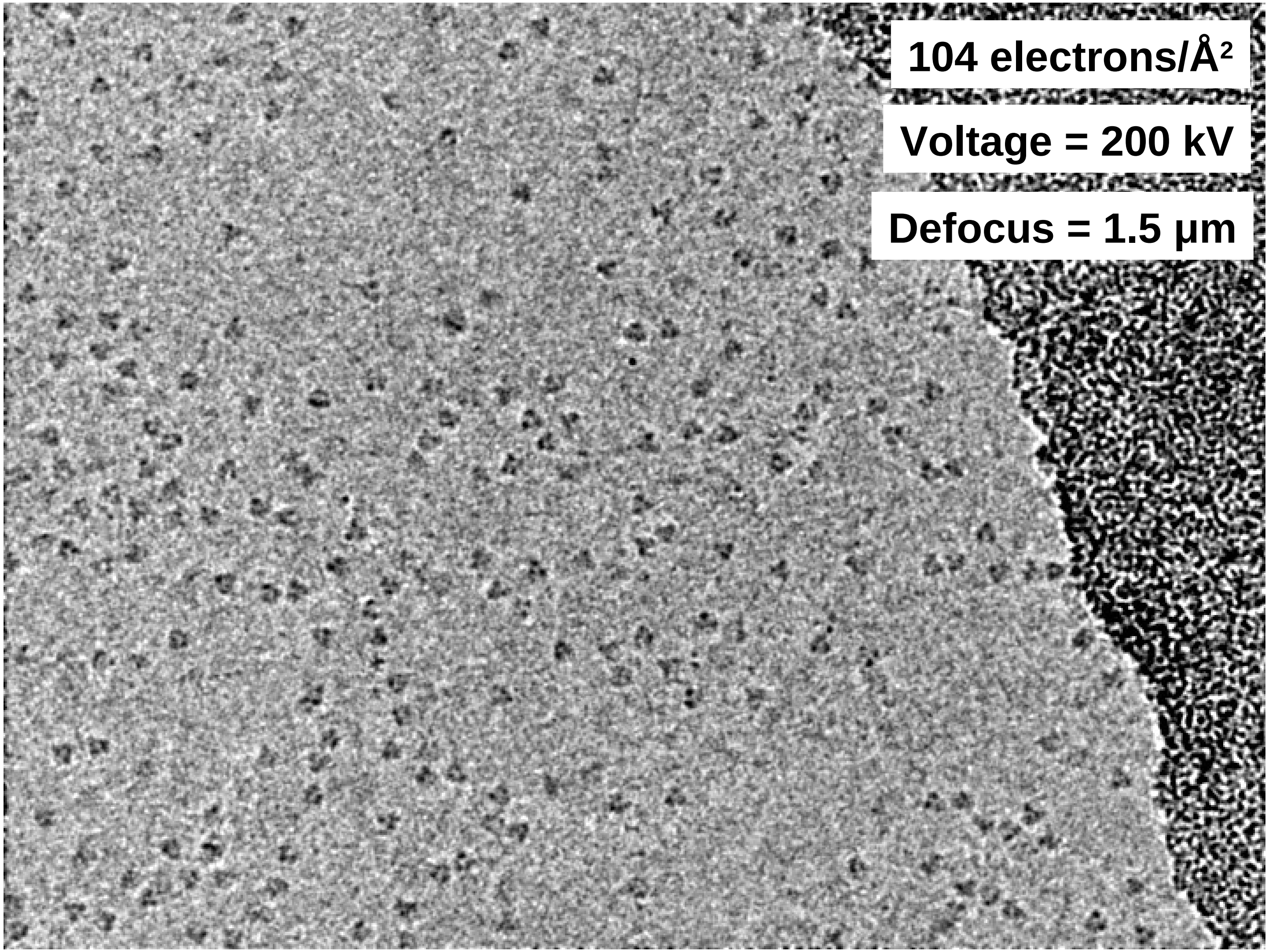
Unwin & Henderson 1975; Hayward & Glaeser 1979; Stark et al. 1996, Baker et al. 2010

High-Dose Imaging

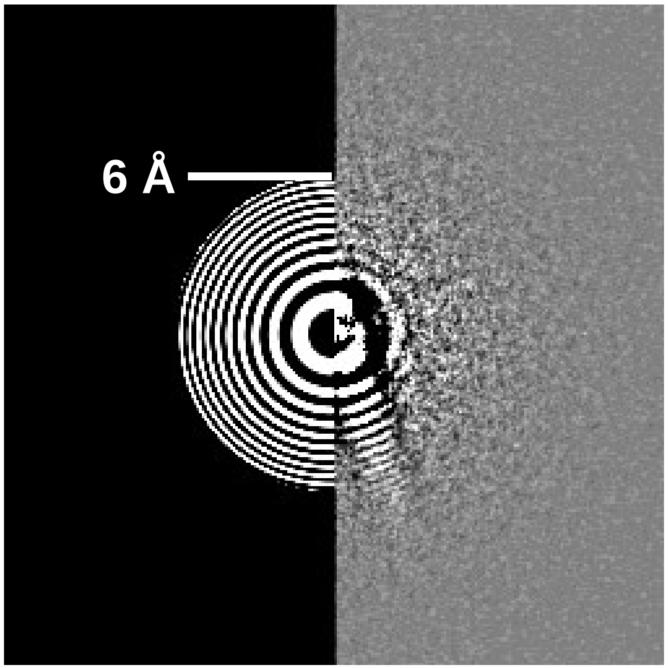
Flu hemagglutinin
(2FK0, Stevens et al. 2006)

Trimer = 180 kDa

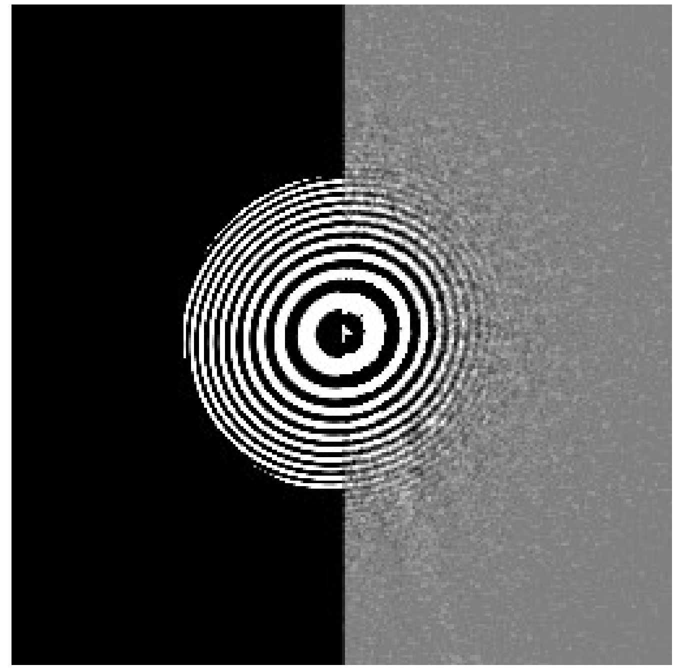
Melody Campbell
Peter Lee
(unpublished)



Thon Ring Patterns



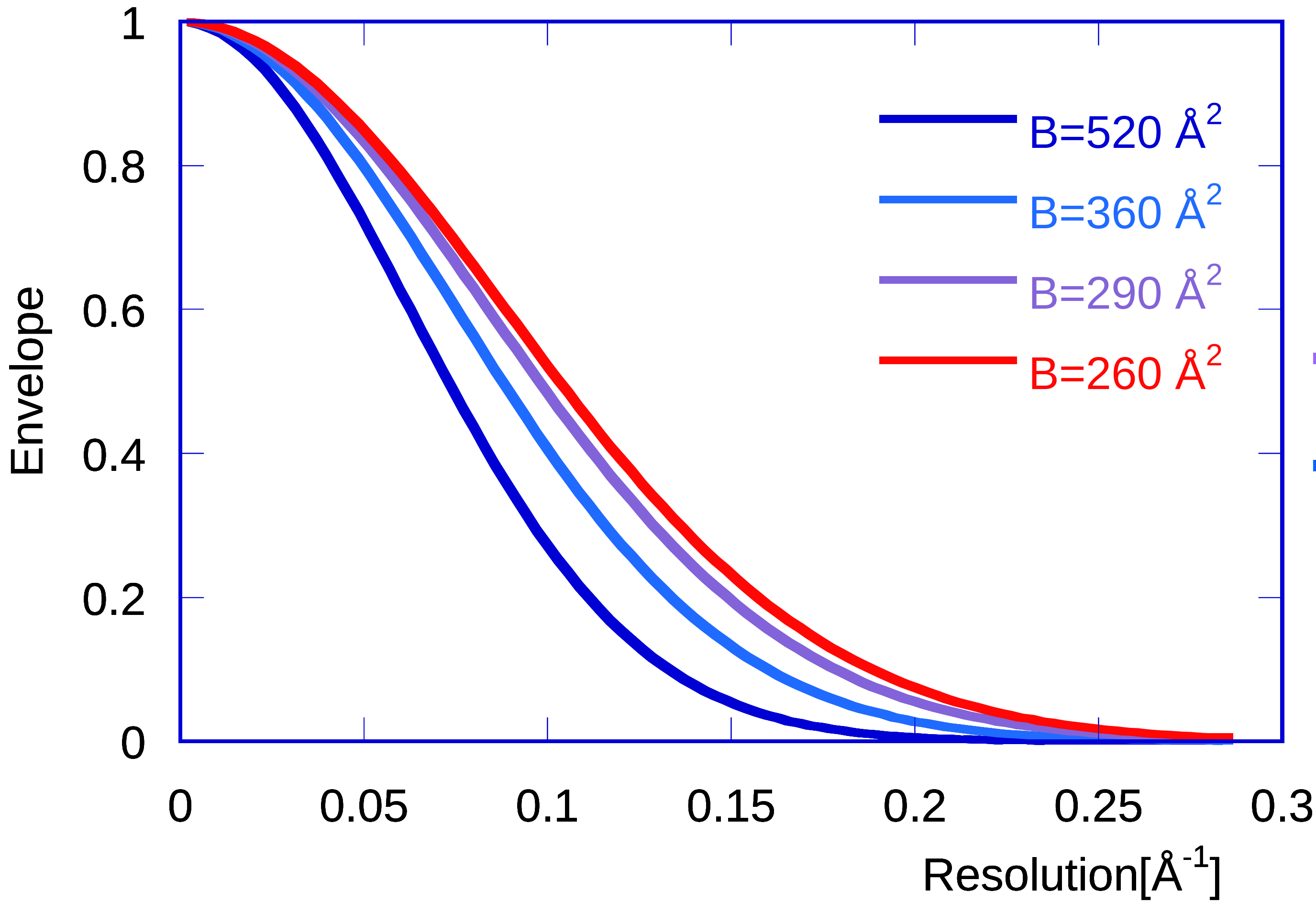
No frame alignment



With frame alignment

Melody Campbell
Peter Lee
(unpublished)

Potential Improvements



B_{damage} = 30 Å²

~~$B_{detector}$ = 70 Å²~~

~~B_{motion} = 160 Å²~~

$B_{alignment}$ = 90 Å²

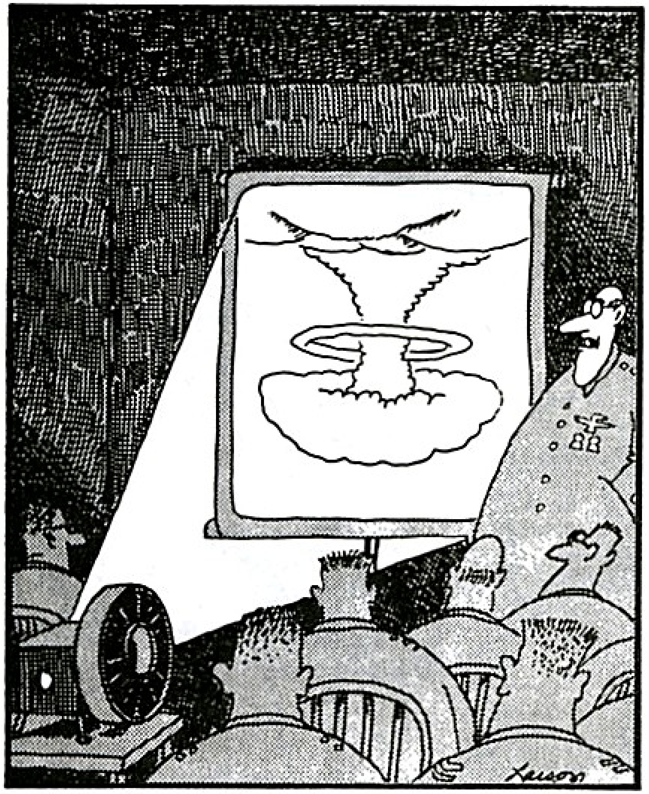
Factor @ 3.5 Å:

200

40,000 x signal

The Challenges

- Beam-induced motion & charging
- Beam damage
- Detector DQE
- Alignment errors

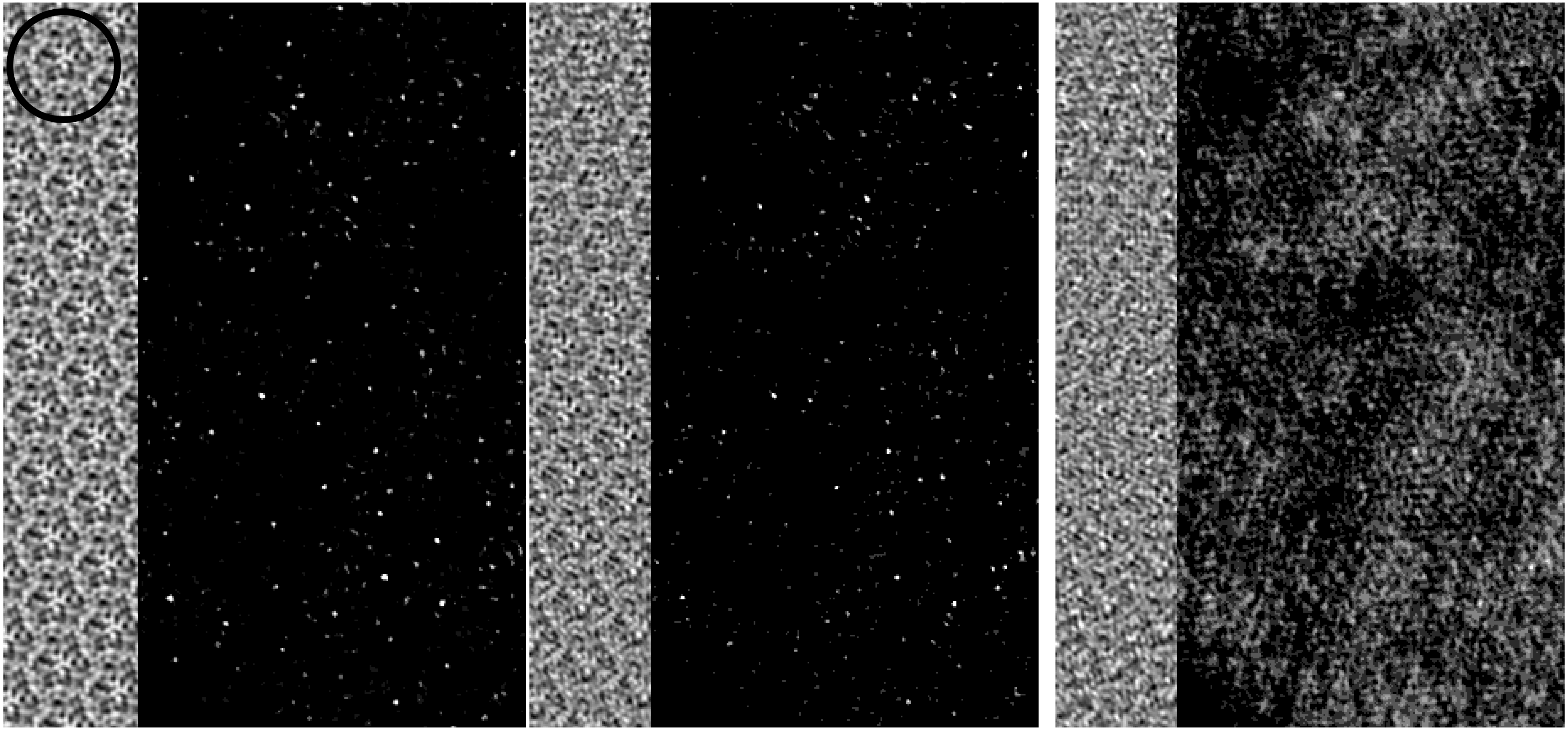


"Now this next slide, gentlemen, demonstrates the awesome power of our twenty megaton . . . For crying out loud! Not again!"

Larson, The Far Side

Dealing With Noise

100 kDa



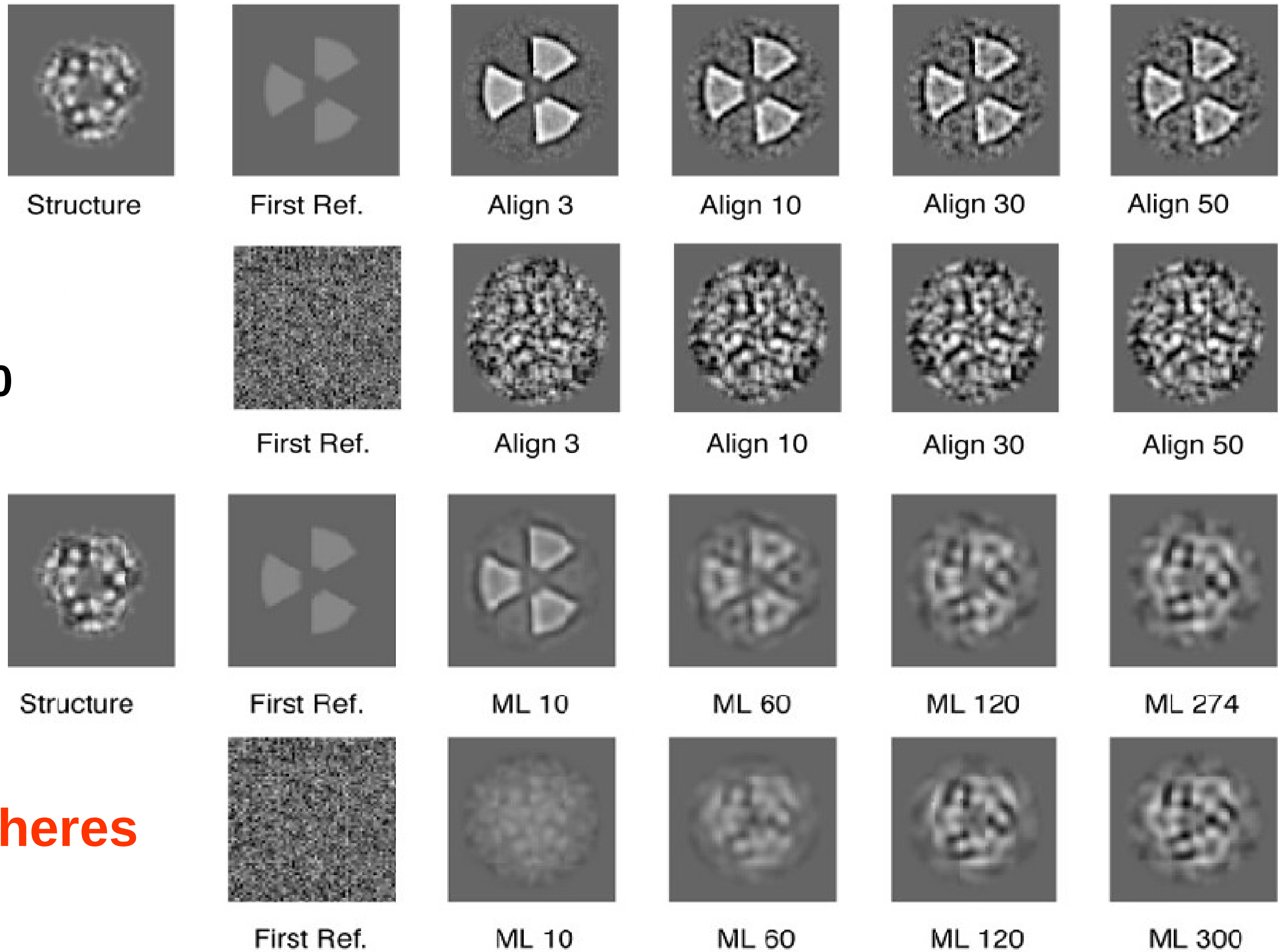
Bacteriorhodopsin

Maximum Likelihood

Correlation alignment

N = 4000
SNR = 1/200

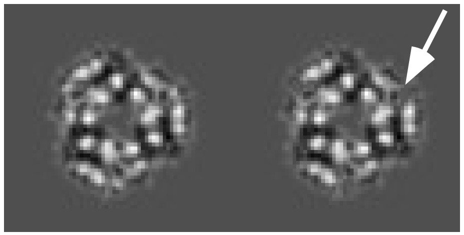
ML estimation

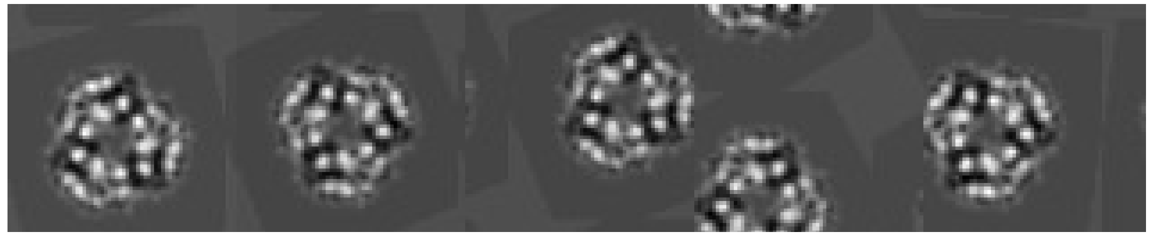


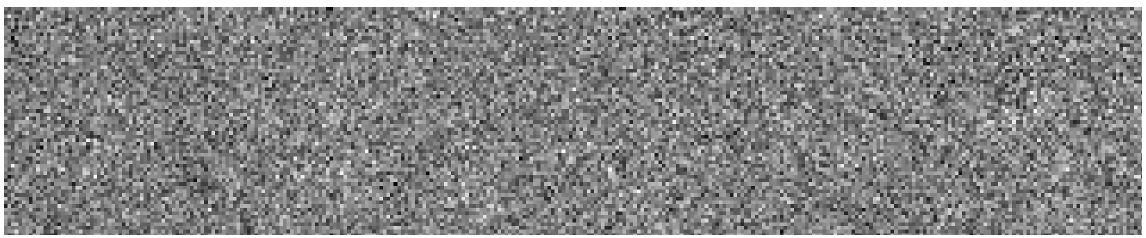
Sjors Scheres

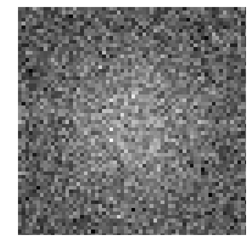
Sigworth 1998

ML Classification



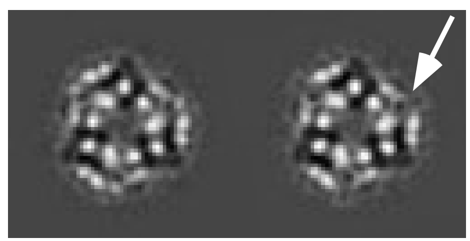


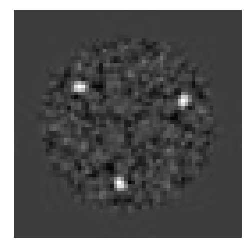




SNR = 1/50
N = 2000

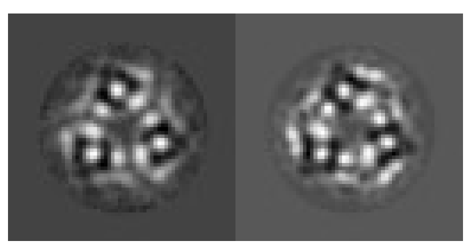
Sjors Scheres
Pawel Penczek

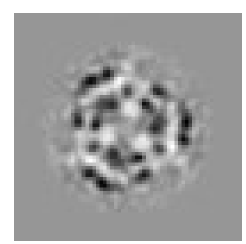




Difference map

Correlation alignment





All Things Considered

- Accurate modeling of all parameters
  - CTF, envelope, variability …
- Statistical models
  - noise models, parameter distributions, weighting …
- Score dependent on all known facts/data
  - cross-linking, homology, total mass …
- Reproducibility tests
  - multiple starts, consistency checks …
- Large data sets
  - automation

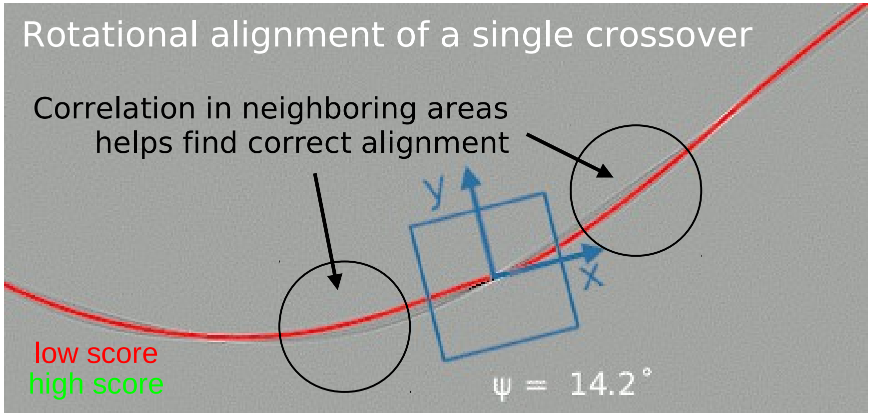
Helical Processing with Frealix

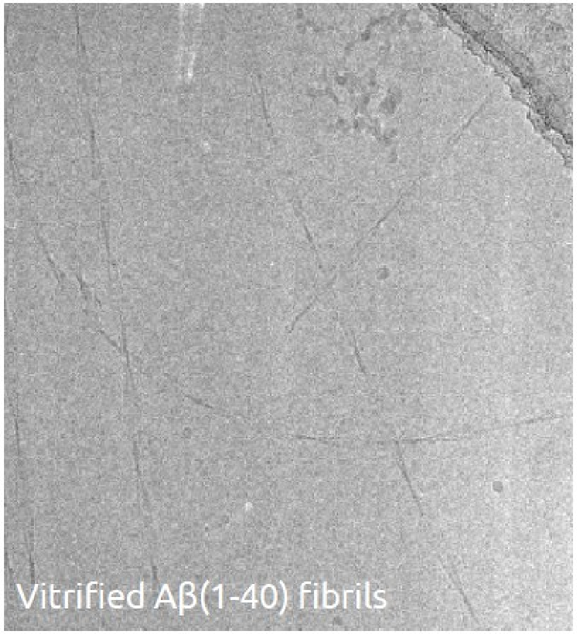
Emphasis on flexible filaments
(amyloid fibrils)

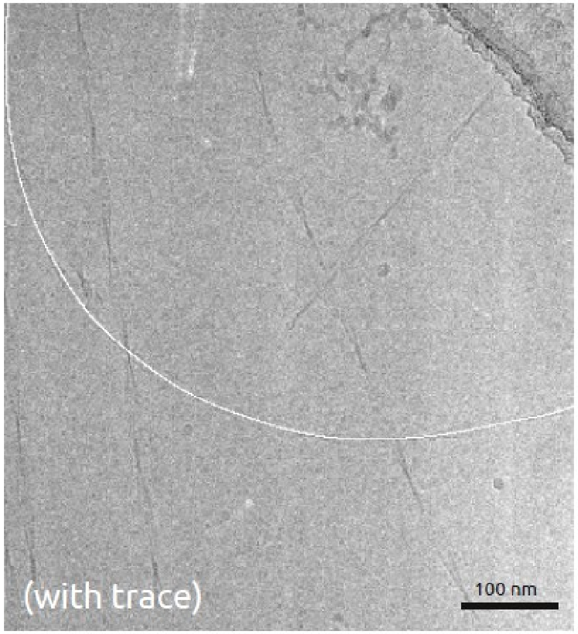
Full-filament processing
(no segment boxing)

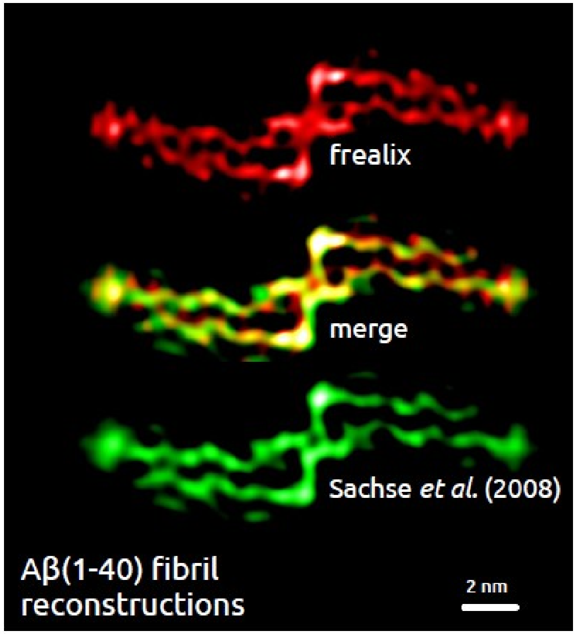
Other filament types
(TMV, microtubules)

Constraints during image processing
(persistence length...)



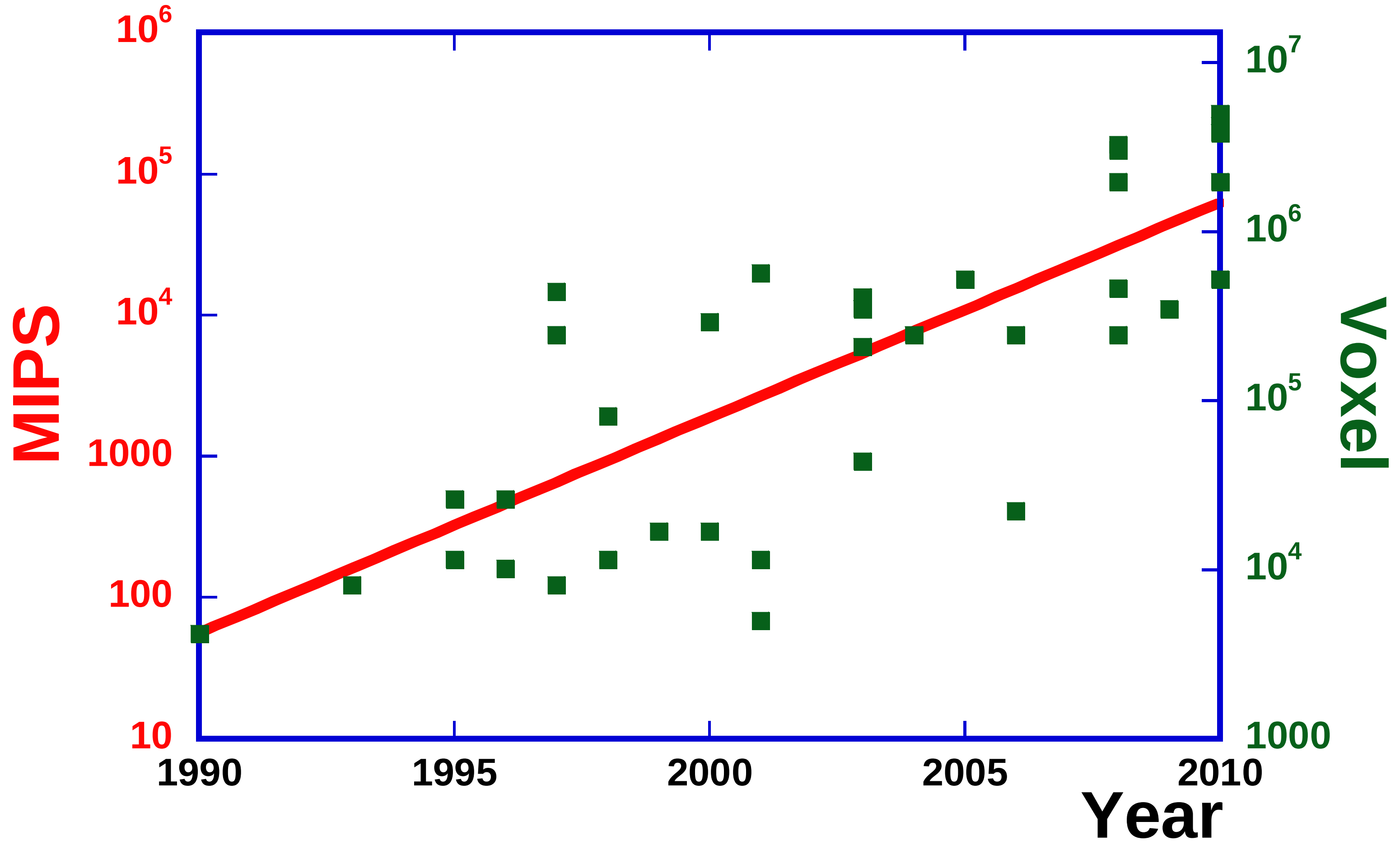






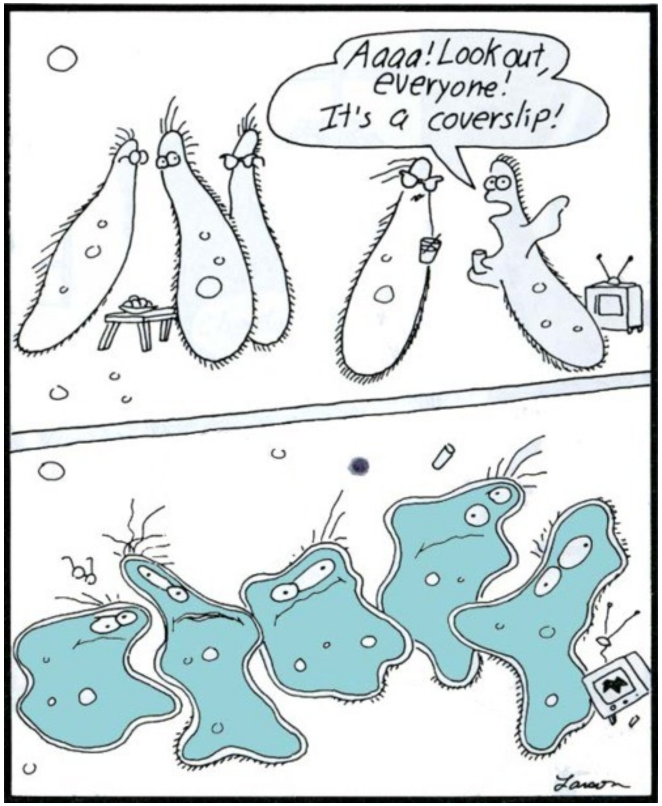
Alexis Rohou, unpublished

Computer Games (Doom et al.)



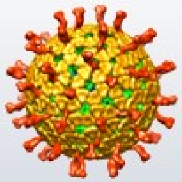
Sample Limitations

- Sample heterogeneity/stability
  - biochemistry
  - new algorithms
- Transient complexes
  - affinity grids, streptavidin crystals
- Detergent and lipid
  - amphipol, GLC/GDN
  - amphiphilic β-strand peptides
- Low molecular weight



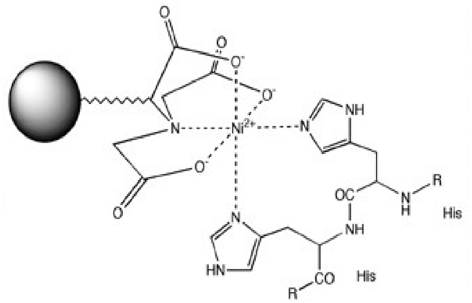
Larson, The Far Side

Holger Stark
Debbie Kelly

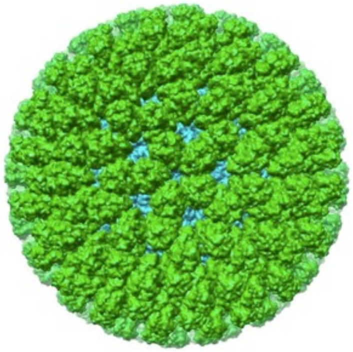


Scaffolds

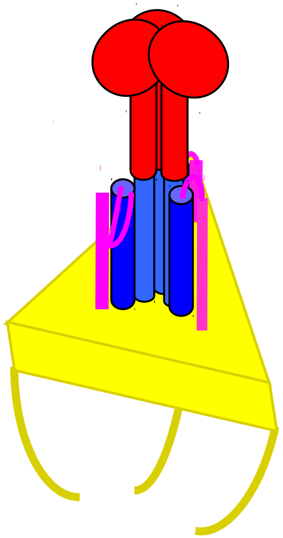
Yifan Cheng

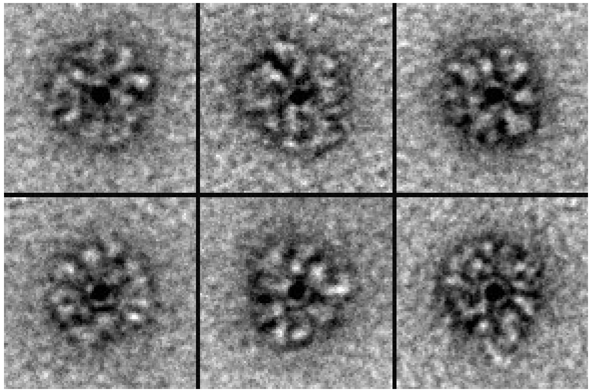


Ni-NTA nanogold



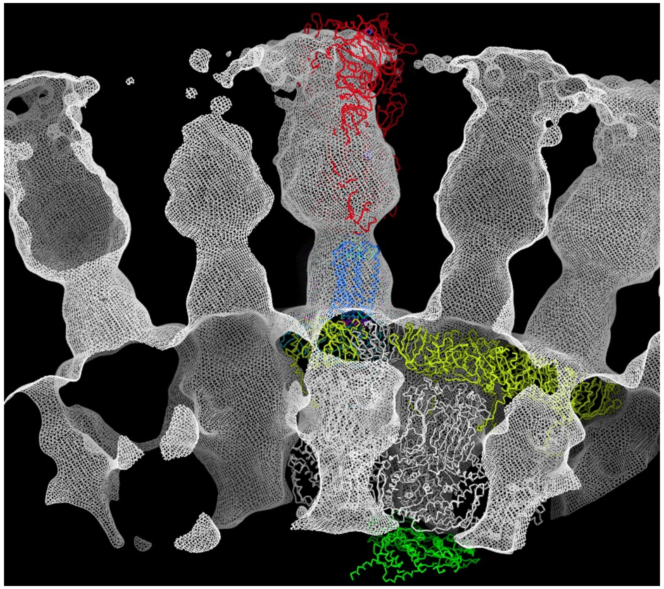
Rotavirus DLP





His-tagged flu hemagglutinin

Yuhang Liu, unpublished



Junhua Pan, unpublished

Thank You!