NCMI

National Center for Macromolecular Imaging

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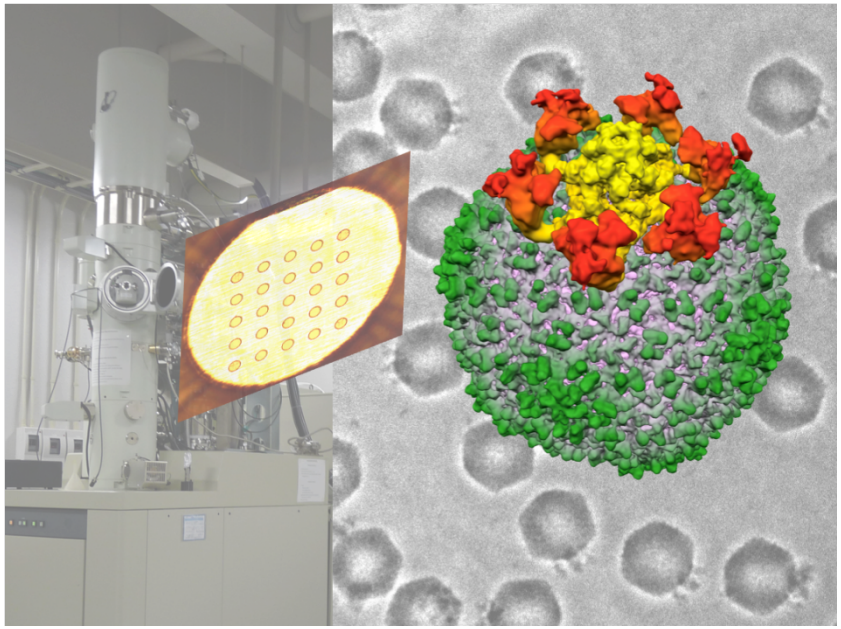

Questions

- What are the pros and cons of using a high-end microscope (a JEOL or a Krios) ?
- Are 200kV instruments just as good if we use the right imaging conditions?
- What progress has been made with using phase plates? What are the advantages and disadvantages of phase plates per se? What are the advantages and disadvantages of the various phase plate solutions that are out there?
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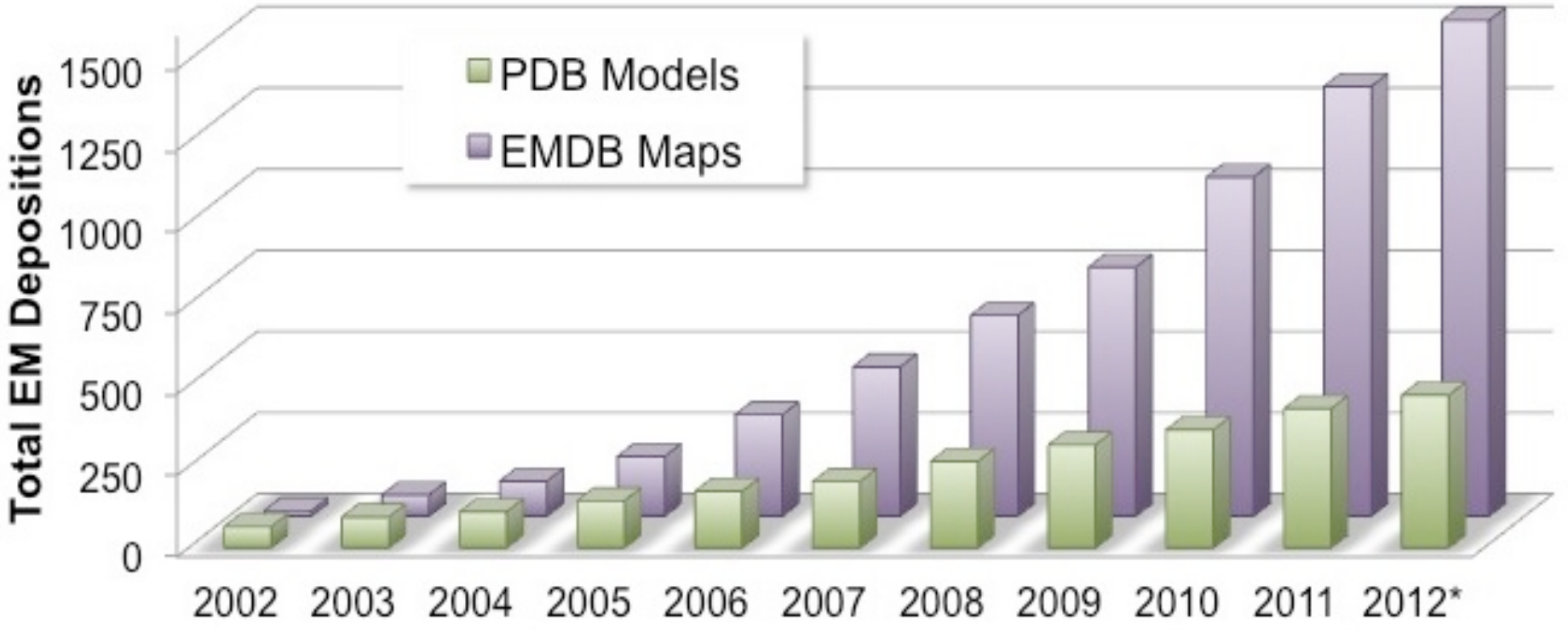
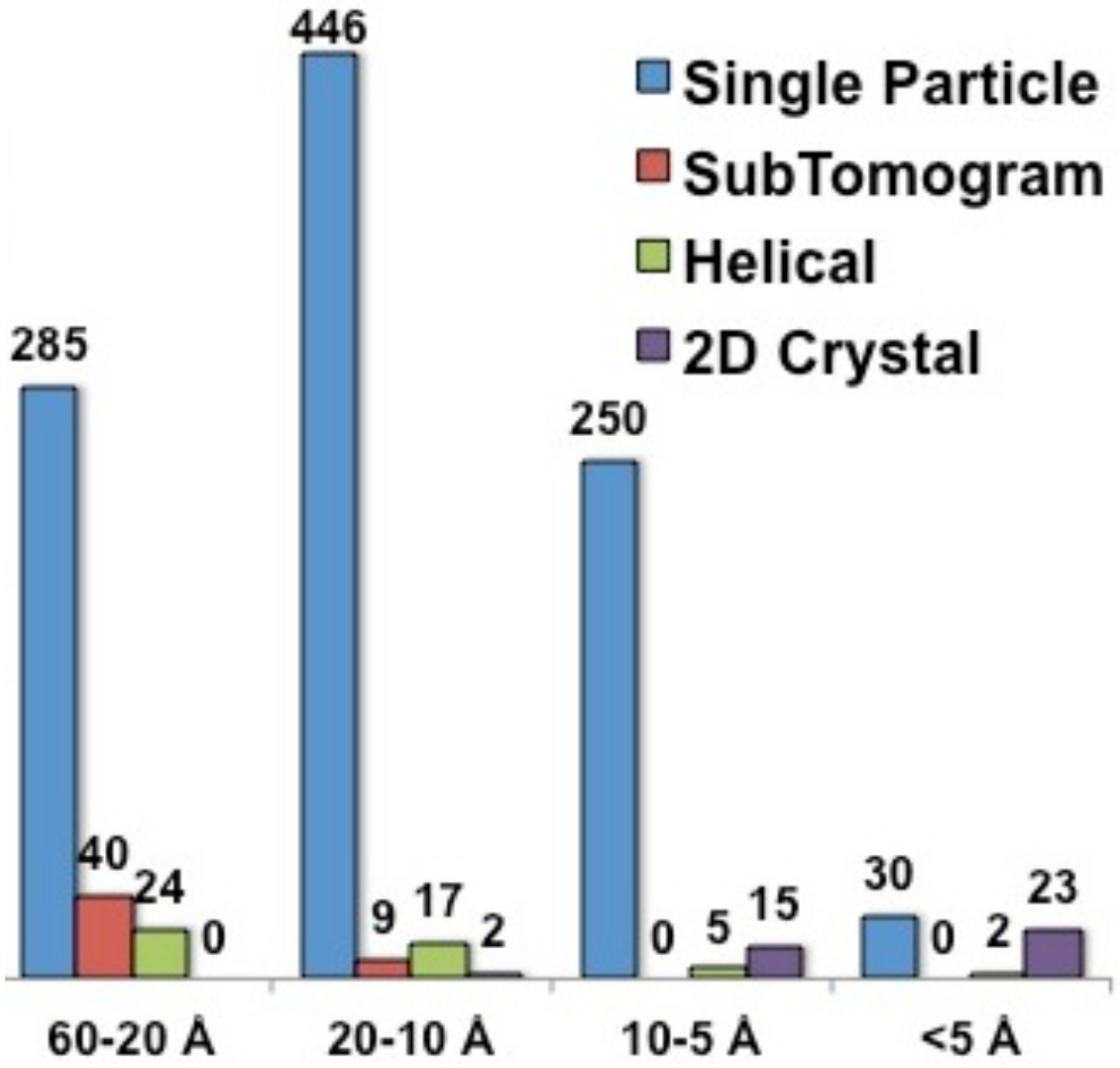
3DEM Structure Deposition Statistics



http://www.ebi.ac.uk/pdbe/emdb/index.html

Courtesy of Cathy Lawson, PDB, Rutgers University

EMDB Data Statistics



Courtesy of Cathy Lawson, PDB, Rutgers University

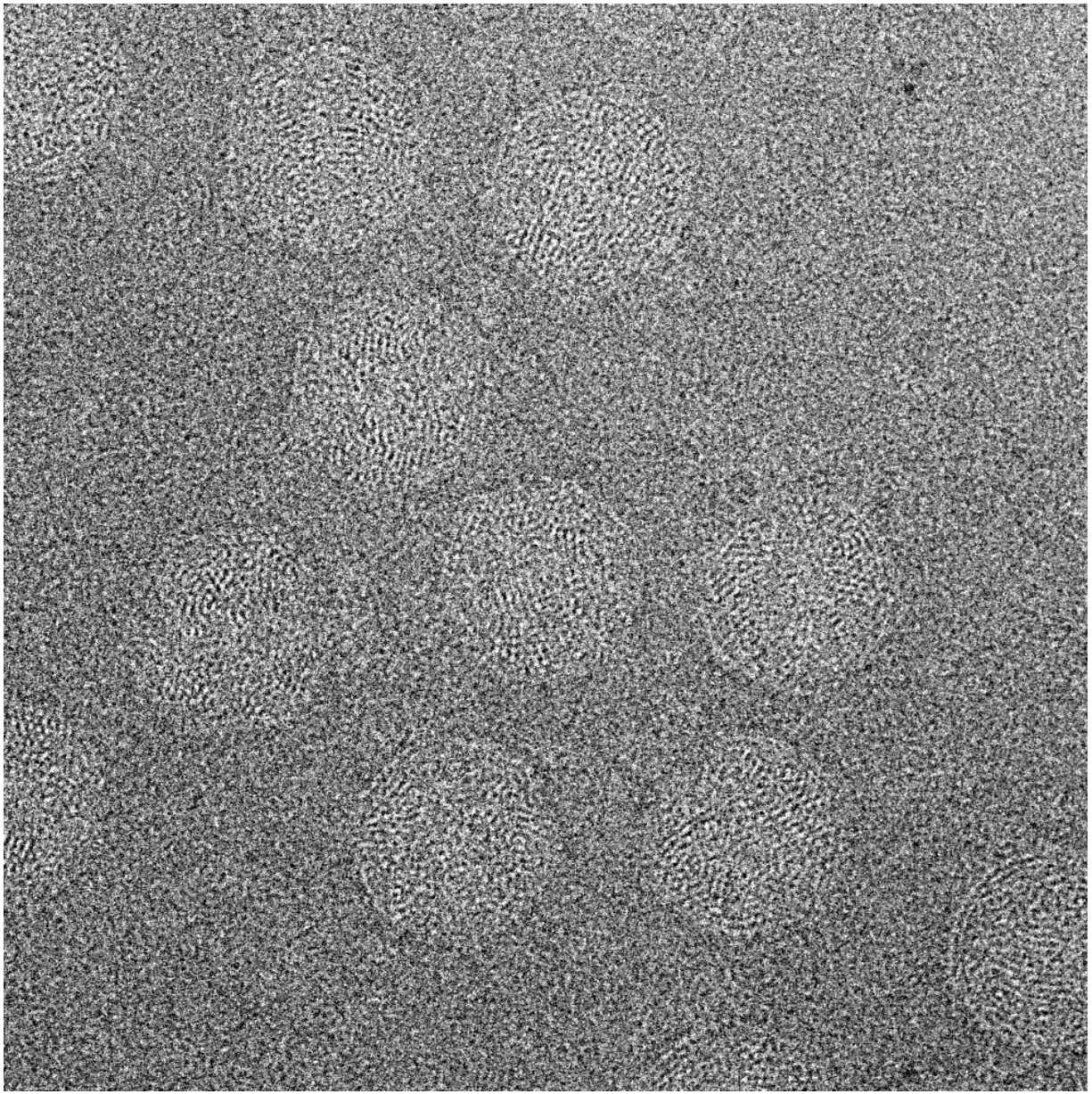
Instrument Options

- 300 kV Microscopes
  - JEOL: JEM3000, JEM3200, JEM3100
  - FEI: Krios, Tecnai F30, Polara
- Specimen Temperature
  - Liquid helium vs. Liquid nitrogen
- Detector
  - Photographic film
  - CCD
  - Direct electron detection (DE, Gatan, FEI)
- Energy Filter
  - In-column filter
  - Post-column filter

Examples of High Resolution Maps

Specimens	**Resolution (Å)**	**Microscope**
Epislon-15 phage	4.5 (FSC 0.5)	JEM3000
P-SSP7 phage	4.6 (FSC 0.5)	JEM3200
P22 procapsid	3.8 (FSC 0.5)	JEM3000
P22 phage	4.0 (FSC 0.5)	JEM3000
VEE virus	4.4 (FSC 0.5)	JEM3200
GroEL chaperonin	4.2 (FSC 0.5)	JEM3000
Mm-Cpn chaperonin	4.3 (FSC 0.5)	JEM3200
CP Virus	3.9 (FSC 0.5)	FEI Polara
Sputnik virus	3.5 (FSC 0.143)	FEI Krios
Adenovirus	3.6 (FSC 0.5)	FEI Krios
Aquareovirus	3.3 (FSC 0.143)	FEI Krios
Papilloma Virus	4.2 (FSC 0.5)	Tecnai F30
Rotavirus	3.8 (FSC 0.143)	Tecnai F30

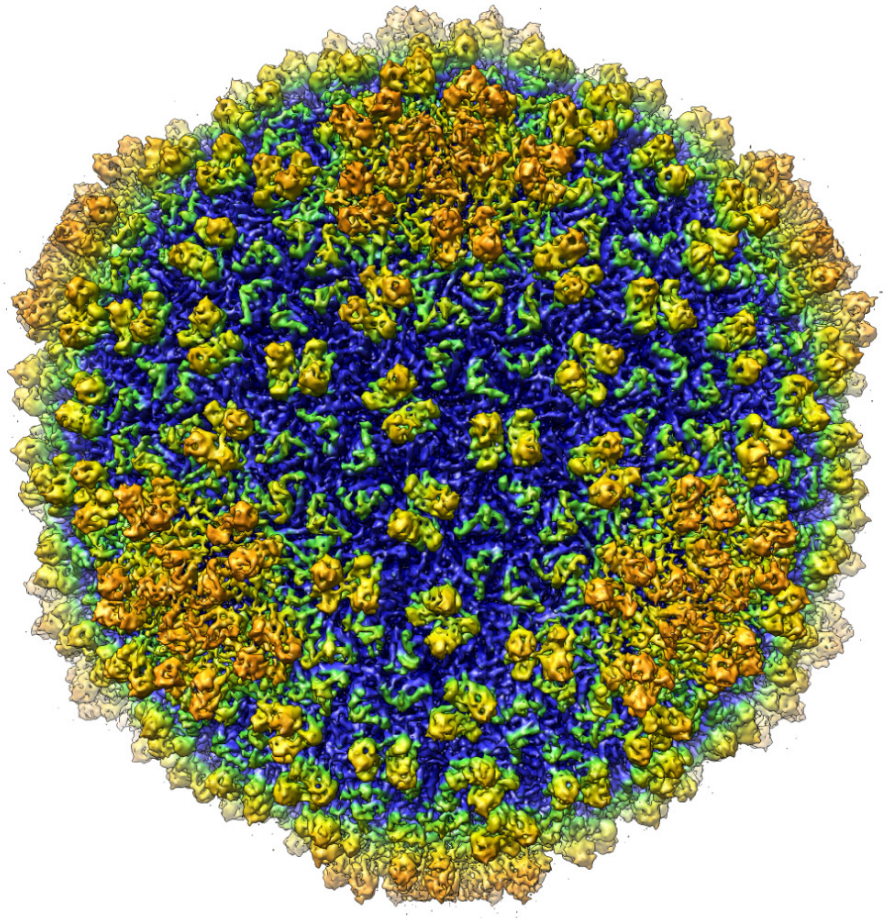
Imaging ε15 Phage at 4°K

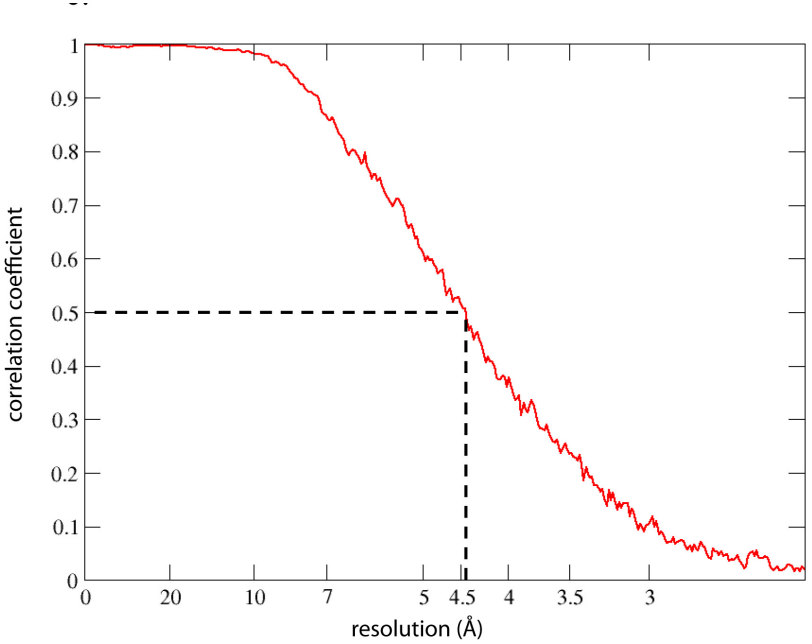


- JEM3000FSC
- 300kV
- Liquid helium
- 60,000x mag
- 28 e/Å^2
- Film data

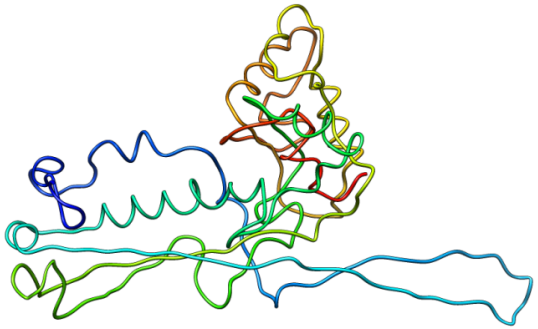
Joanita Jakana

4.5 Å Map of ε15 Phage Based on EMAN1





20,000 particle images



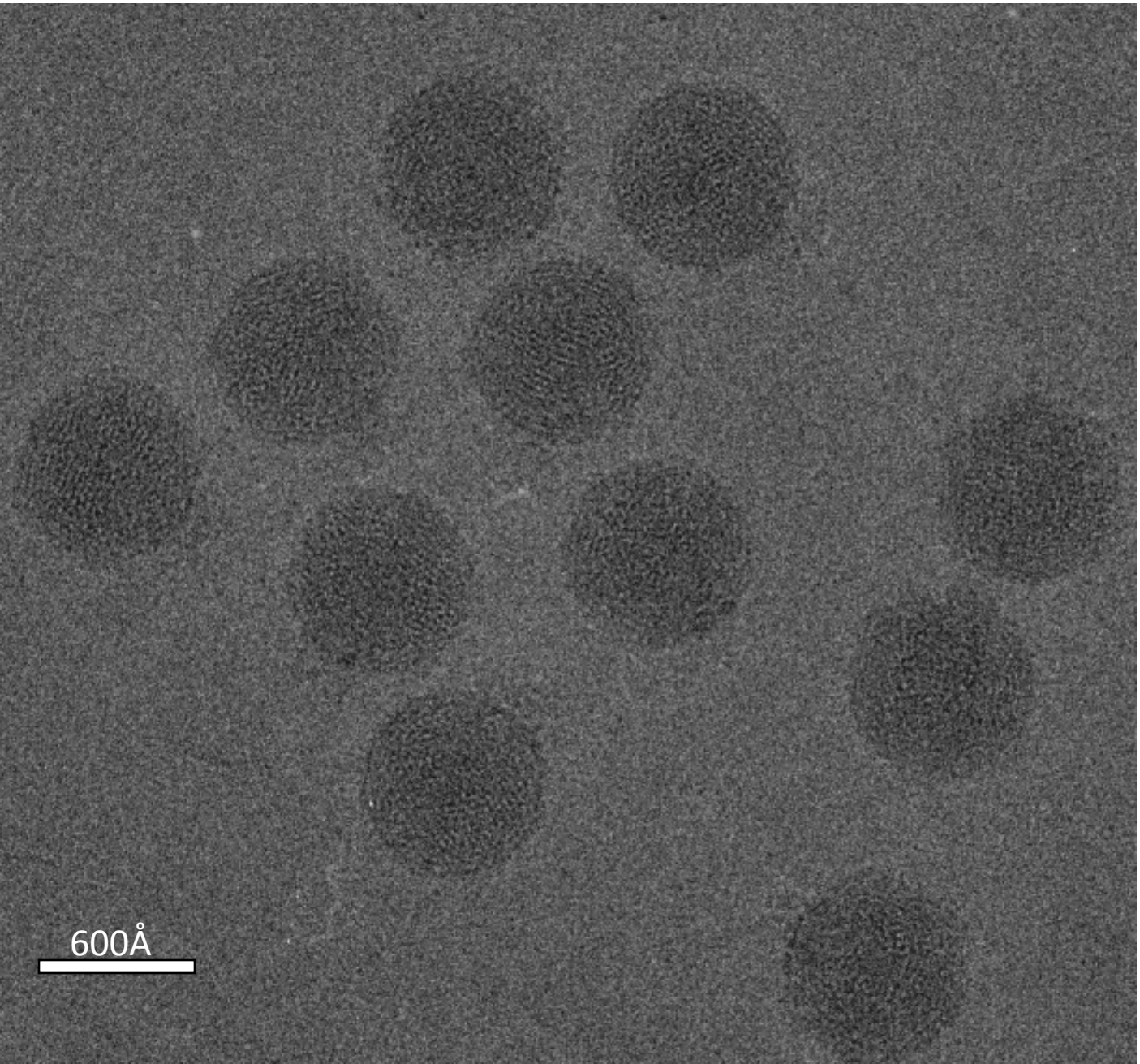
Even-Odd with split data
(10,000 particles per set)

Jiang et al (2008) *Nature*

Resolution Criteria

$$\mathrm{FSC} \equiv \frac{\Sigma(F_1 \cdot F_2^*)}{\sqrt{(|F_1|)^2 \cdot (|F_2|)^2}}$$

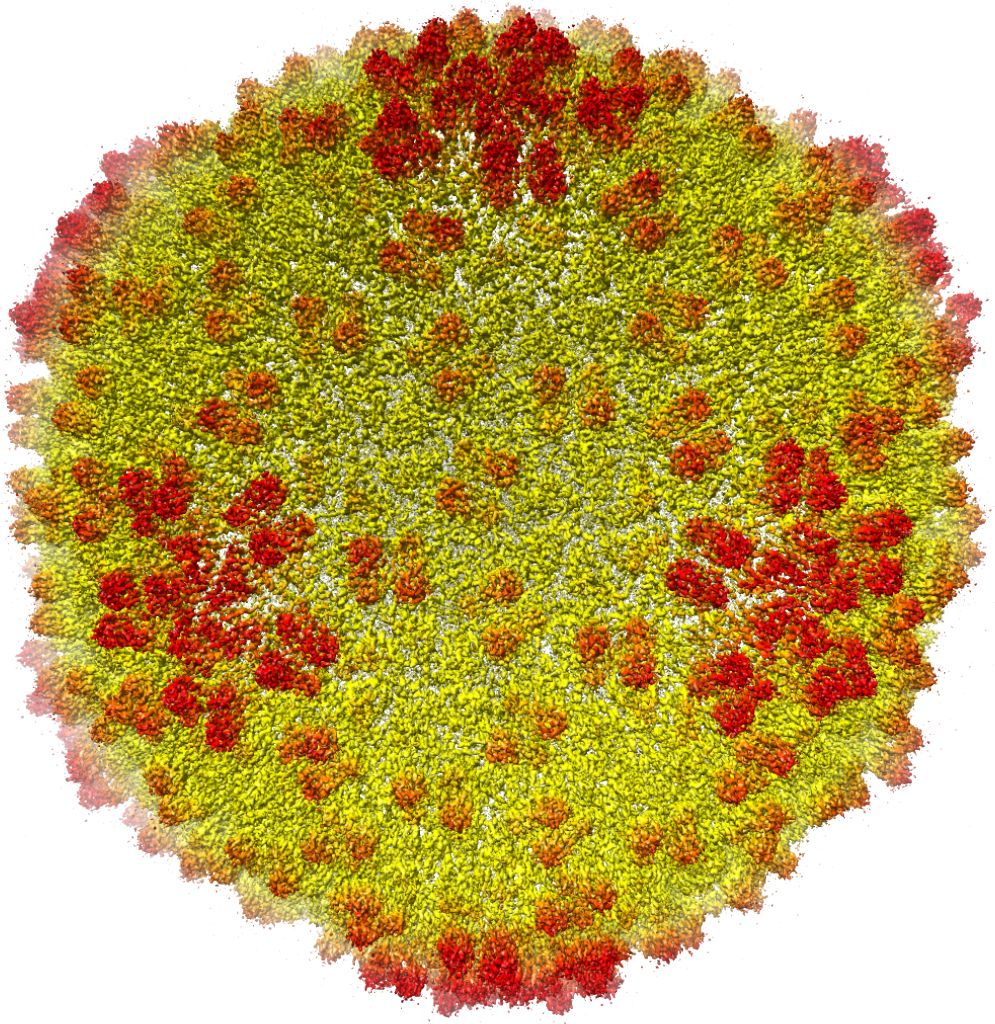
A Second Set of ε15 Phage Images



- JEM3200FSC
- Liquid Nitrogen
- 300 kV
- Energy filter
- 50,000x mag
- Films data
- Scanner: Nikon

Qinfen Zhang

ε15 Phage 2012 Map II Using Modified EMAN1



W. Jiang, Purdue U. unpublished

Questions and Answers

Q: What are the pros and cons of using a high-end microscope (a JEOL or a Krios) ?

A: Need more rigorously defined specimens, data processing protocols, resolution tests, map and model validations.

Questions

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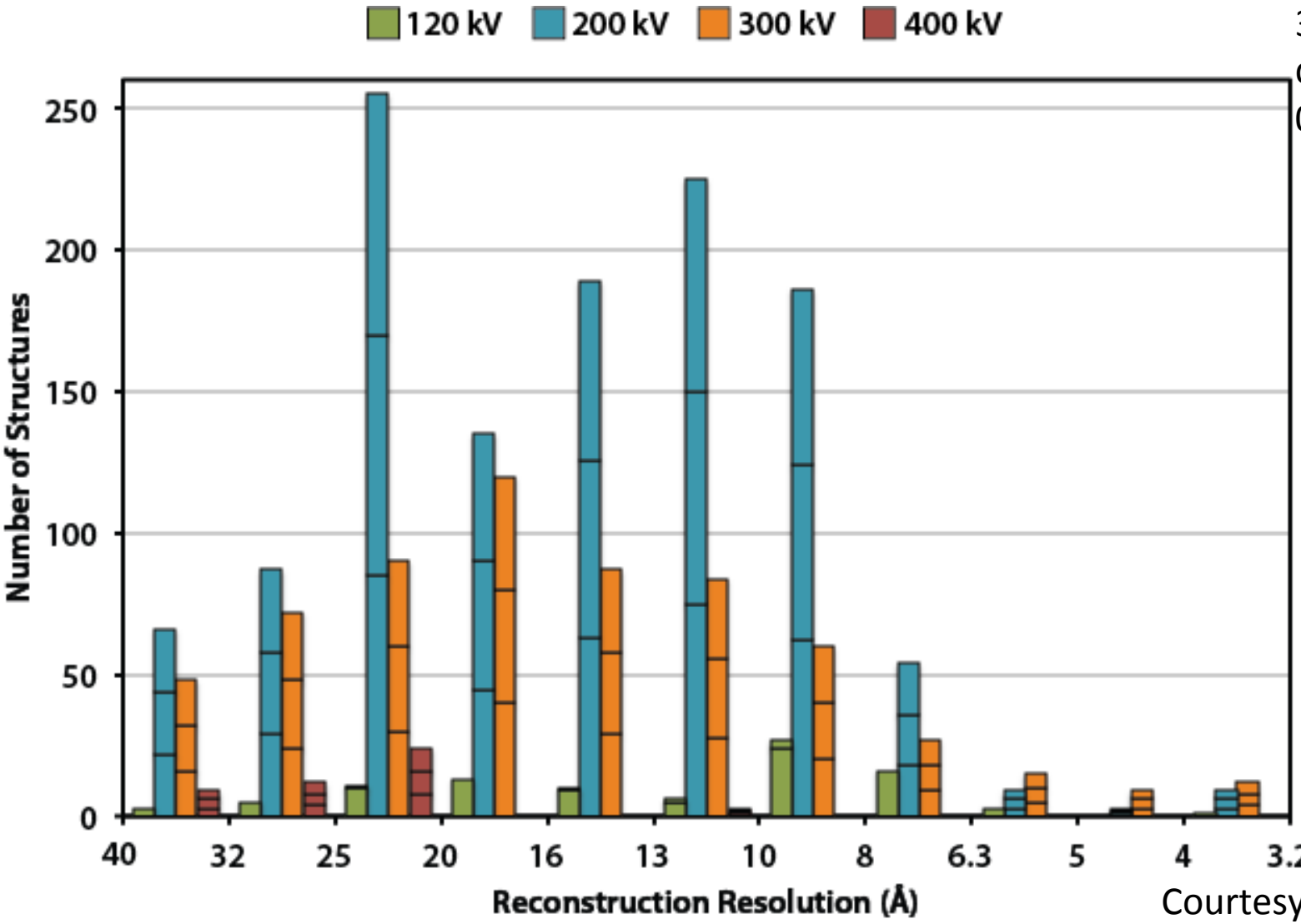
Single Particle Maps Deposited in EMDB with Different Resolutions and Electron Voltages



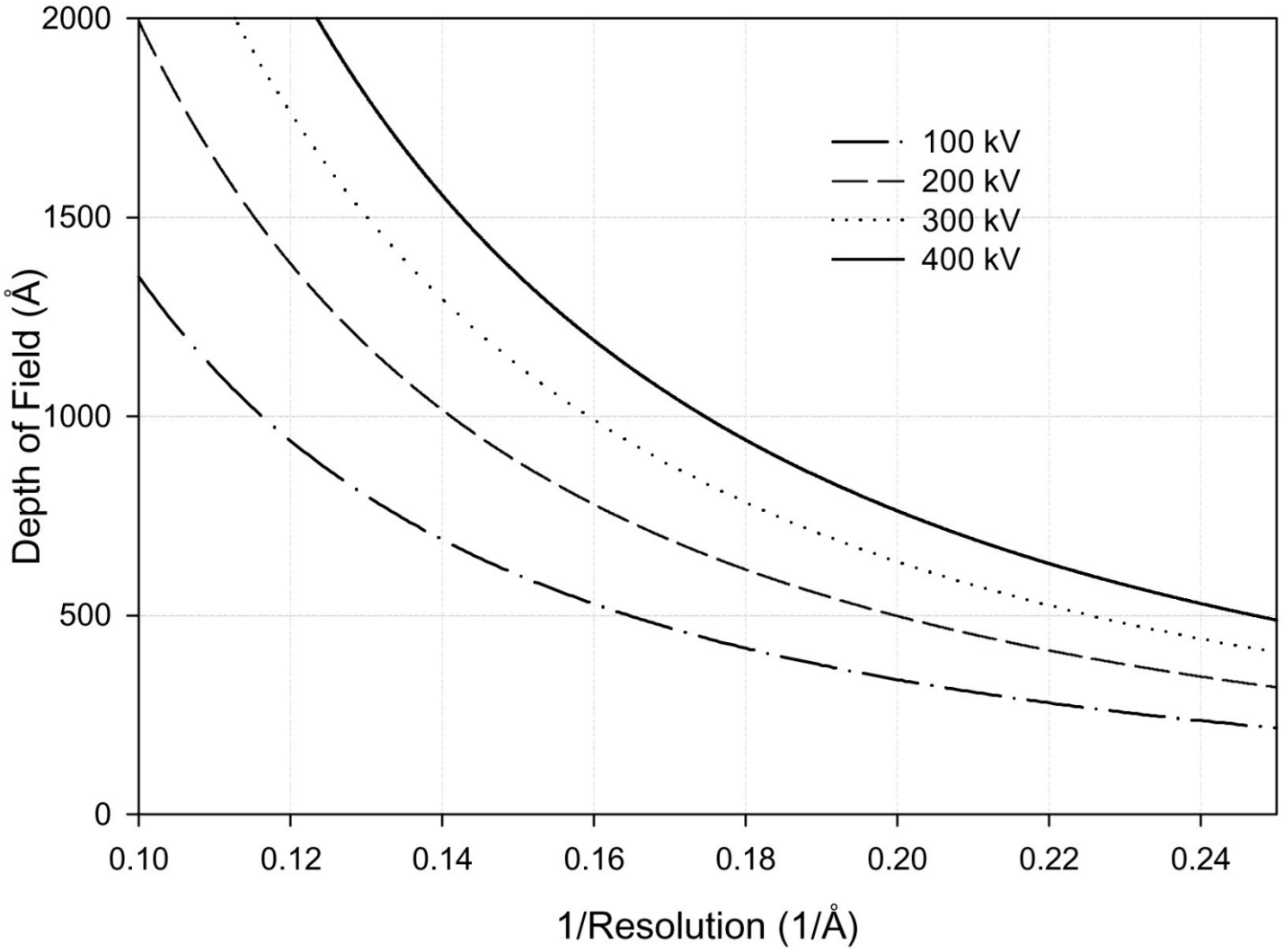
3 different FSC definitions: 0.5, 0.143 and ?

Courtesy of B Bammes

Examples of 200 kV Data for Subnanometer Resolution Maps

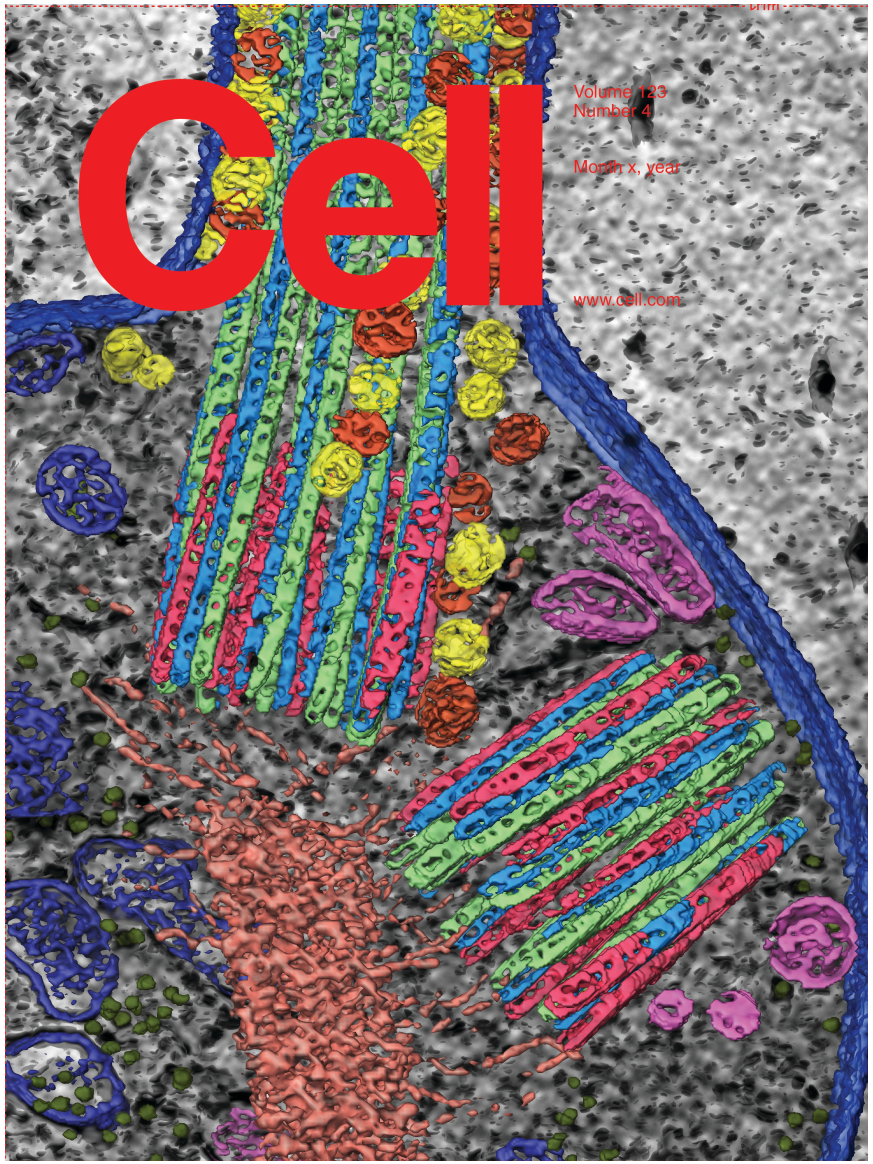
- Böttcher, B., S. A. Wynne and R. A. Crowther (1997). "Determination of the fold of the core protein of hepatitis B virus by electron cryomicroscopy." *Nature* **386**(6620): 88-91.
- Ludtke, S. J., D. H. Chen, J. L. Song, D. T. Chuang and W. Chiu (2004). "Seeing GroEL at 6 Å resolution by single particle electron cryomicroscopy." *Structure* **12**(7): 1129-1136.
- Campbell, M. G., A. Cheng, A. F. Brilot, A. Moeller, D. Lyumkis, D. Veesler, J. Pan, S. C. Harrison, C. S. Potter, B. Carragher and N. Grigorieff (2012). "Movies of Ice-Embedded Particles Enhance Resolution in Electron Cryo-Microscopy." *Structure*.

Depth of Field Dependence on Resolution and Sample Thickness



Zhou and Chiu, *Adv Prot Chem* **64**: 93-130 (2003)

200 kV Electron Cryo-Tomography of Cells



Sensory Cilia in Rod Inner Segment

Data from
JEM2100 LaB_6 gun

J. Gilliam et al. *Cell*
in press (Cover)

Questions and Answers

Q: Are 200kV instruments just as good if we use the right imaging conditions?

A: Yes for most specimens

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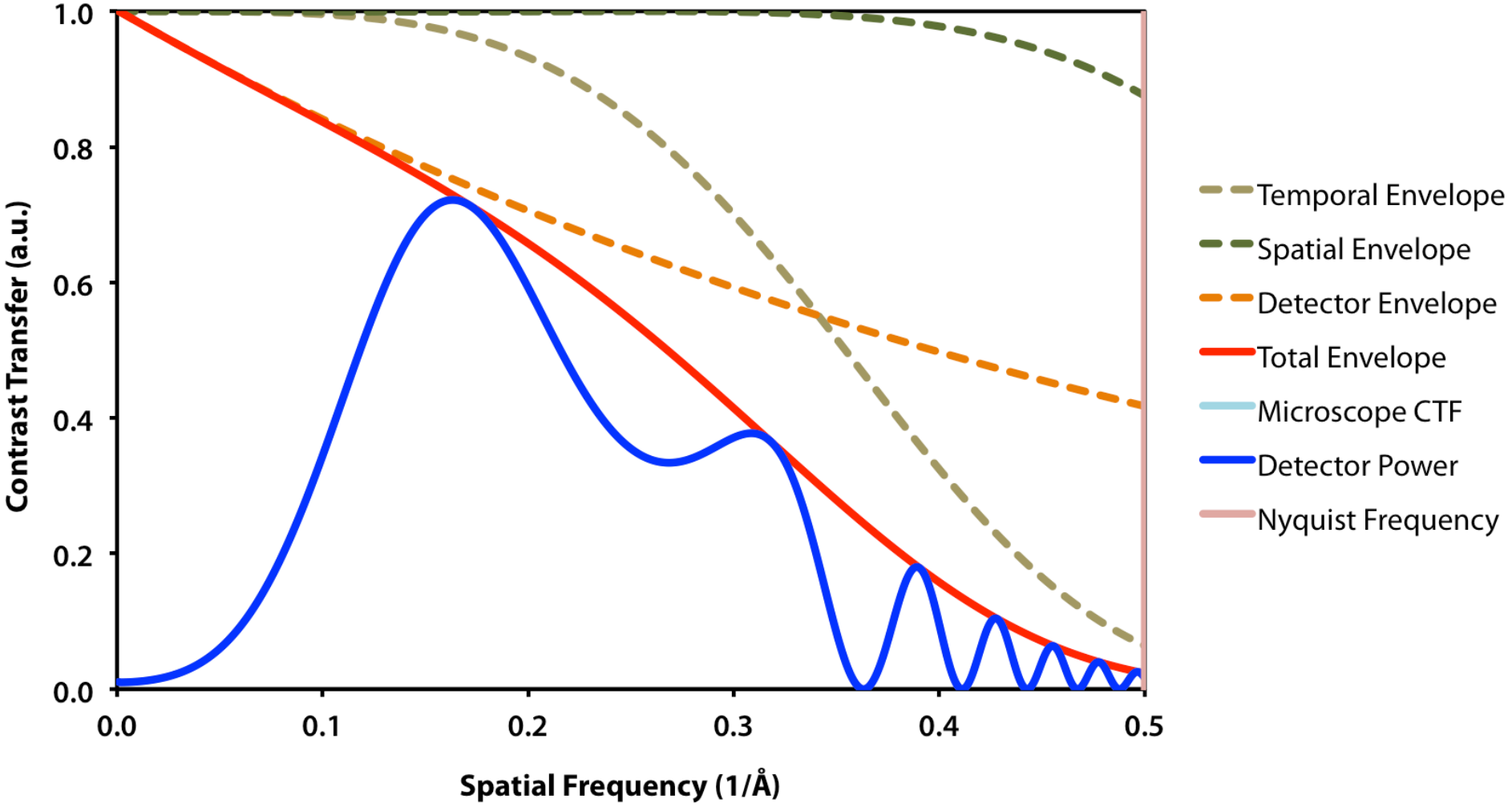
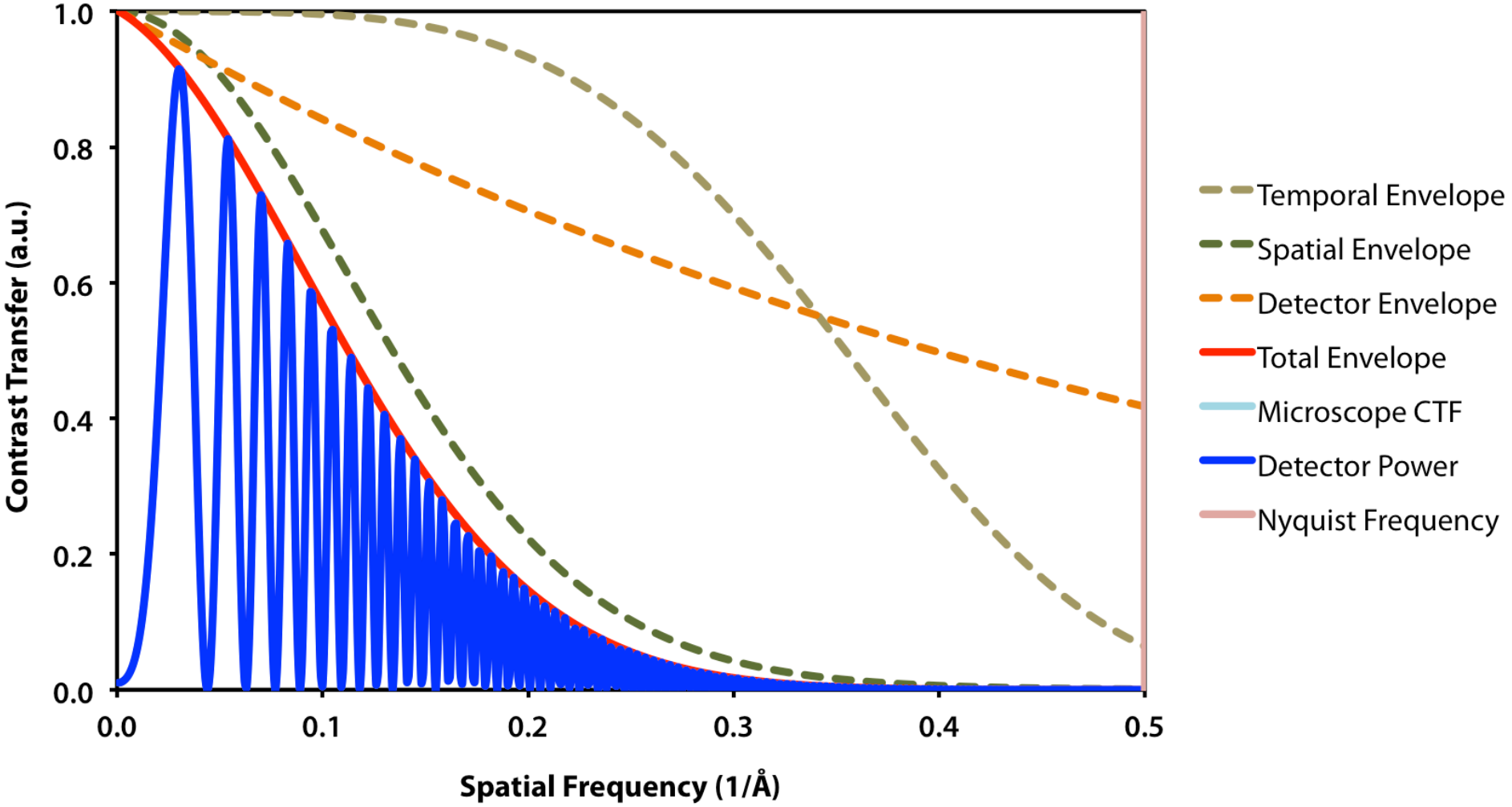
CTF in Conventional In-Focus Images



Courtesy B Bammes

CTF in Conventional Out-Of-Focus Images



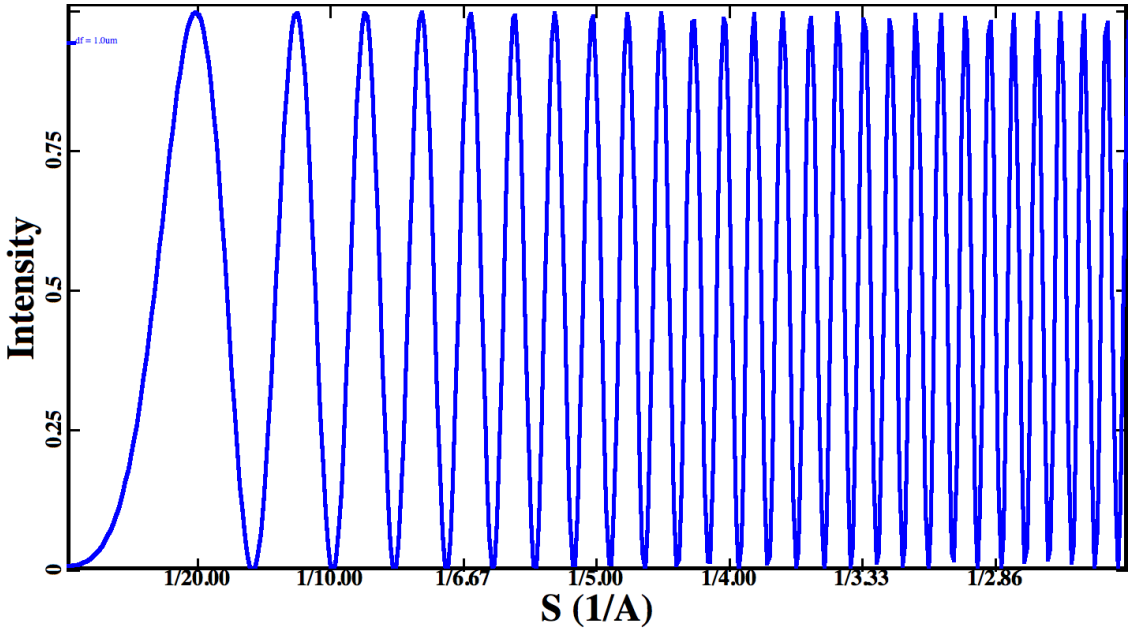
Courtesy B Bammes

Various Types of Phase Modulating Devices

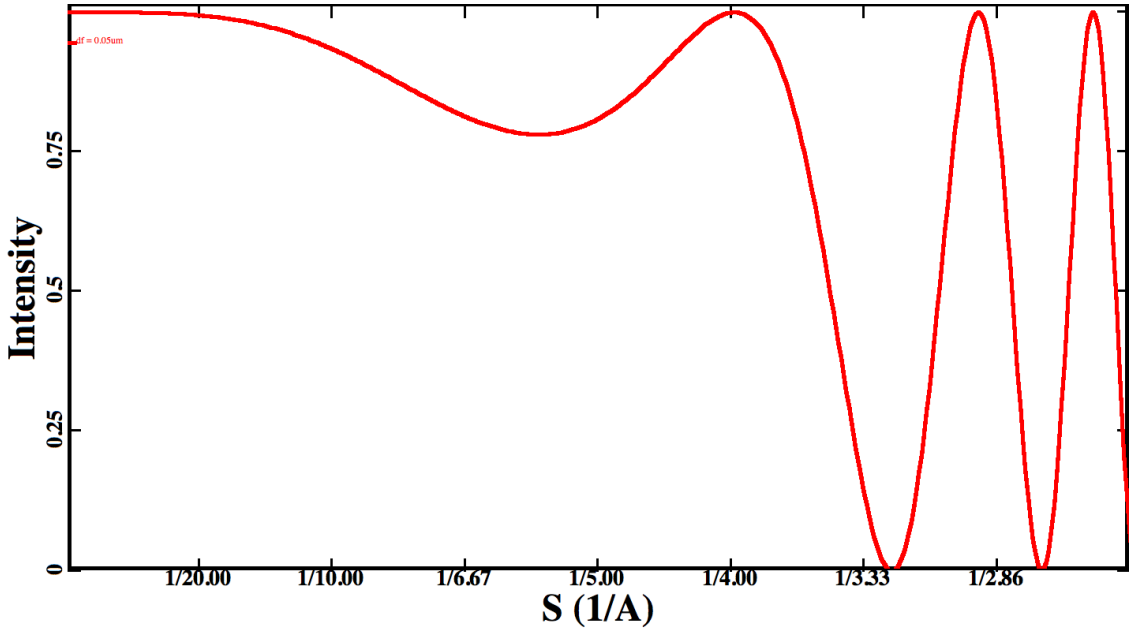
- **SINGLE-SIDEBAND IMAGING (FOUCAULT KNIFE-EDGE APERTURE)**
- **ON-AXIS CHARGES IN THE BACK FOCAL PLANE (UNWIN; MALAC)**
- **THIN-FILM PHASE PLATE (TWO VERSIONS)**
  - **CARBON FILM WITH A HOLE: "EXACT" ANALOG OF ZERNIKE OPTICS**
  - **PHASE ANALOG OF FOUCAULT KNIFE EDGE (HILBERT CONTRAST)**
- **MICROFABRICATED ELECTROSTATIC DEVICES**
- **MICROFABRICATED MAGNETIC DEVICES (VECTOR POTENTIAL DEVICES)**
- **PIXELATED ELECTROSTATIC MIRROR**
- **LASER (ELECTRODYNAMIC/PONDEROMOTIVE) PHASE PLATE**

Courtesy of Bob Glaeser

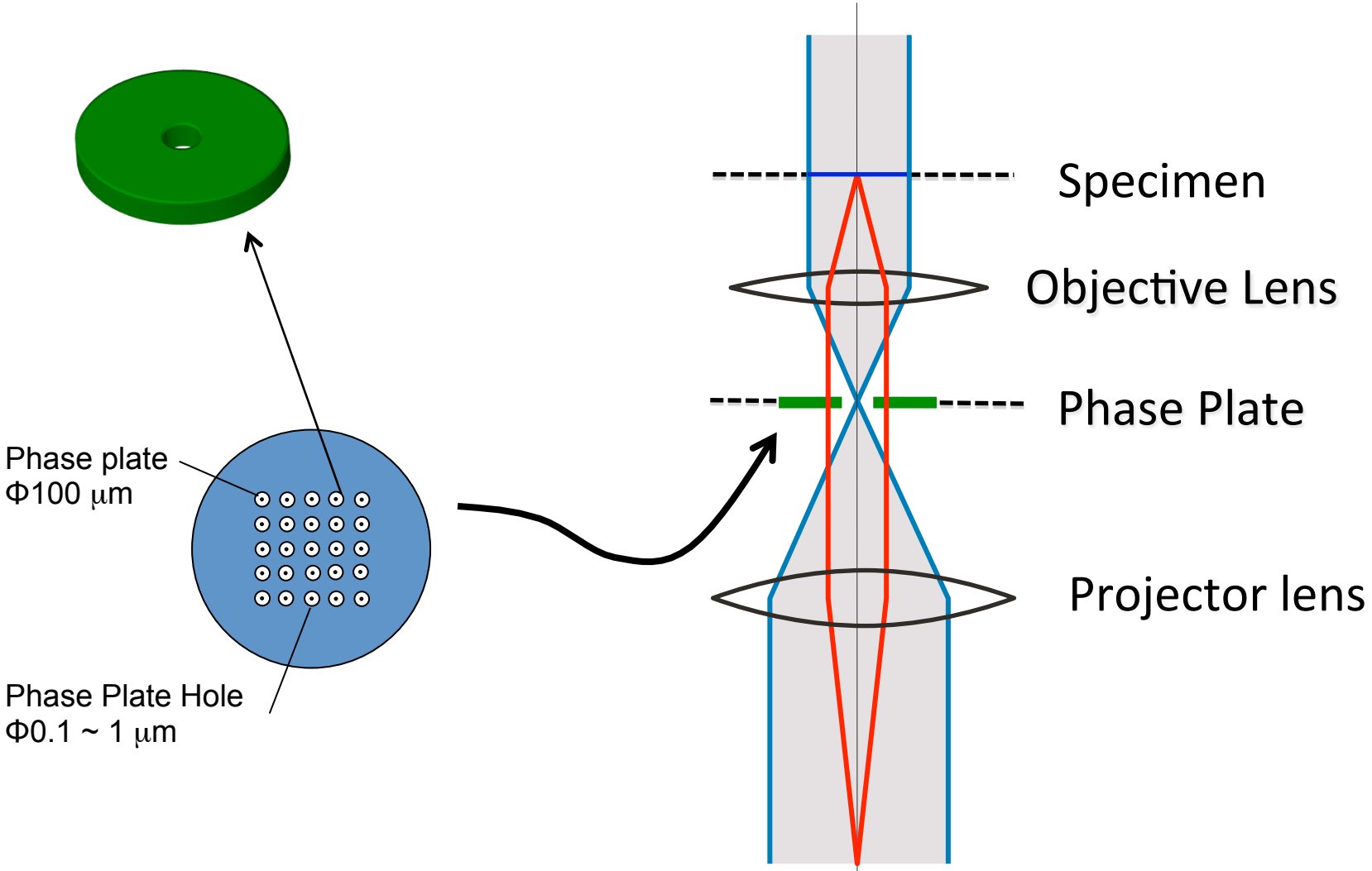
CTEM


Df = 1.0um
Intensity
0
0.25
0.5
0.75
1/20.00
1/10.00
1/6.67
1/5.00
1/4.00
1/3.33
1/2.86
S (1/A)

ZPCTEM

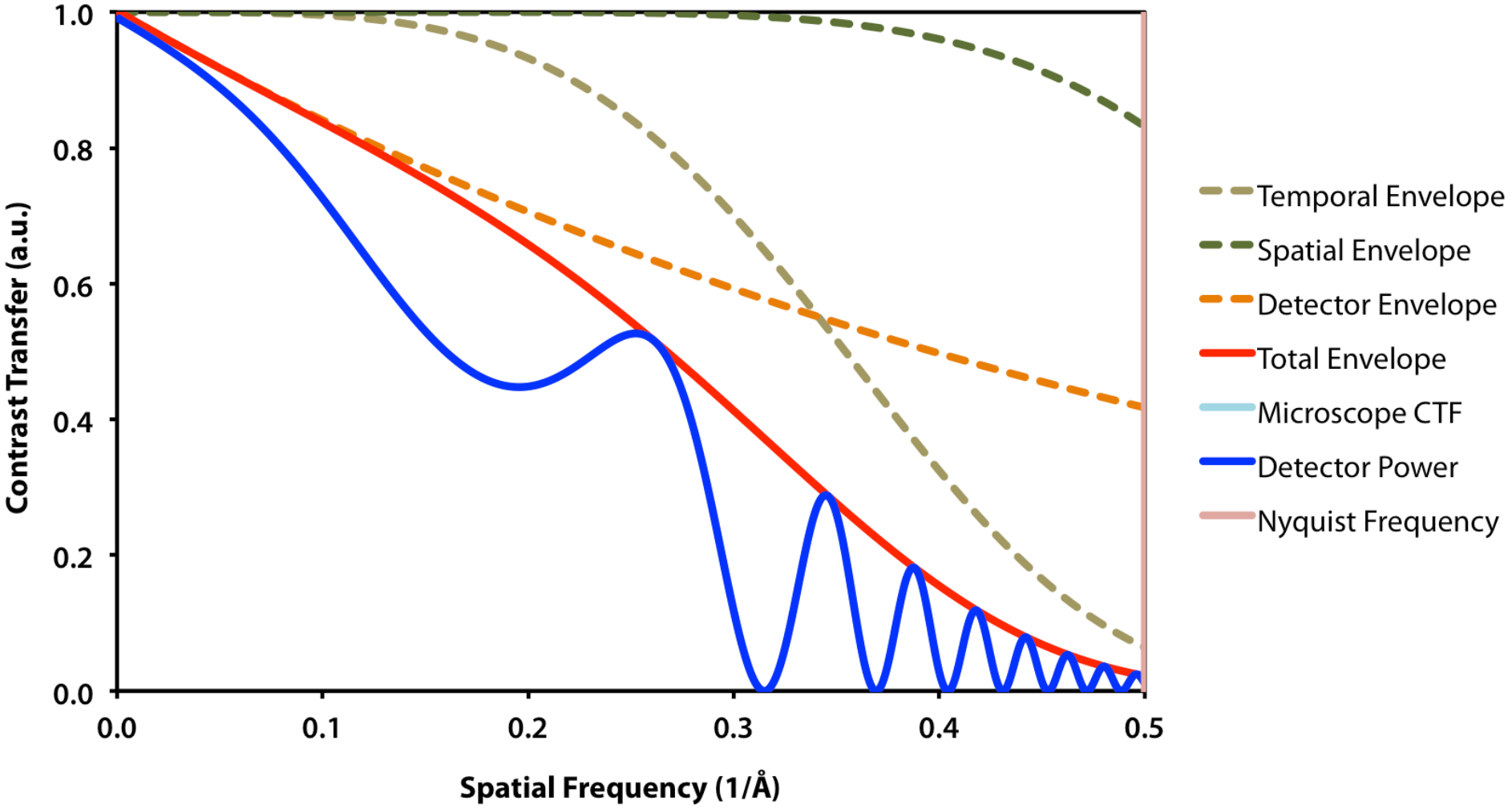

Df = 0.05um
Intensity
0
0.25
0.5
0.75
1/20.00
1/10.00
1/6.67
1/5.00
1/4.00
1/3.33
1/2.86
S (1/A)

Zernike Phase Contrast Optics



Nagayama & Danev, *Phil. Trans. R. Soc. B* (2008) 363, 2153
Hall, Nogales, Glaeser *Biophysics J* (2011)

CTF in Zernike Phase In-Focus Images



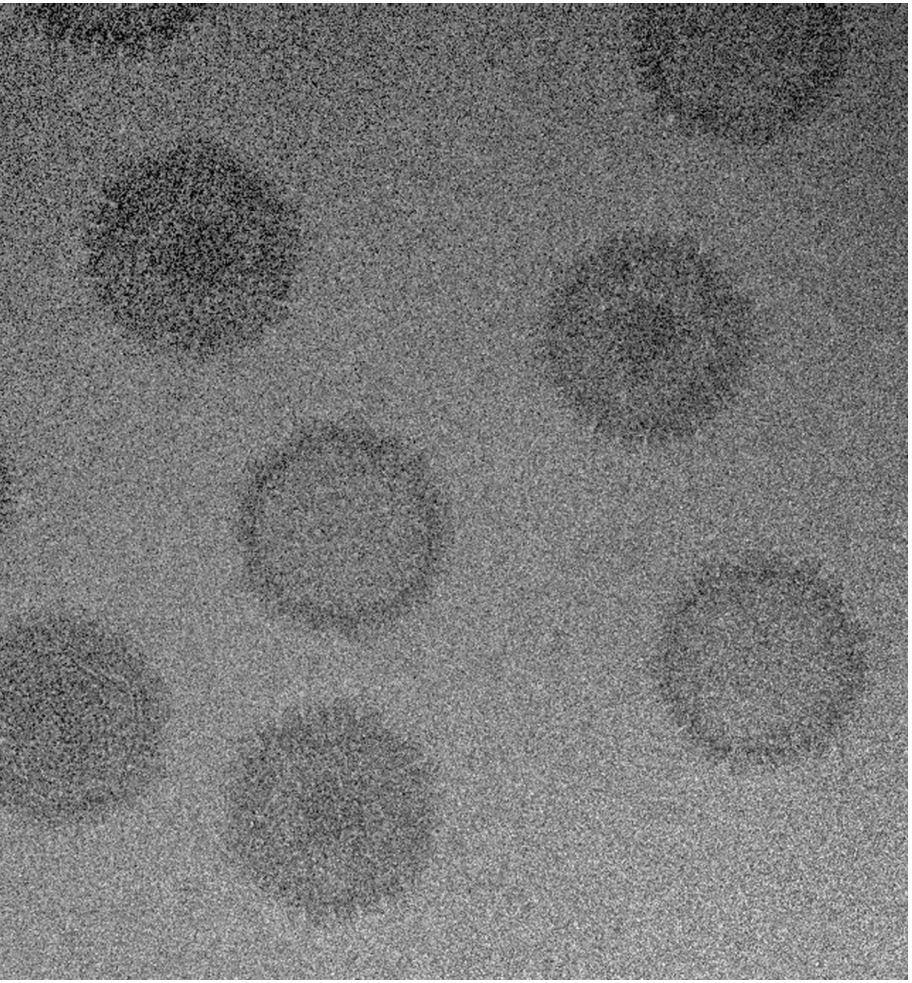
Courtesy B Bammes

Zernike Phase Contrast Applications in Biological Cryo-EM & Cryo-ET

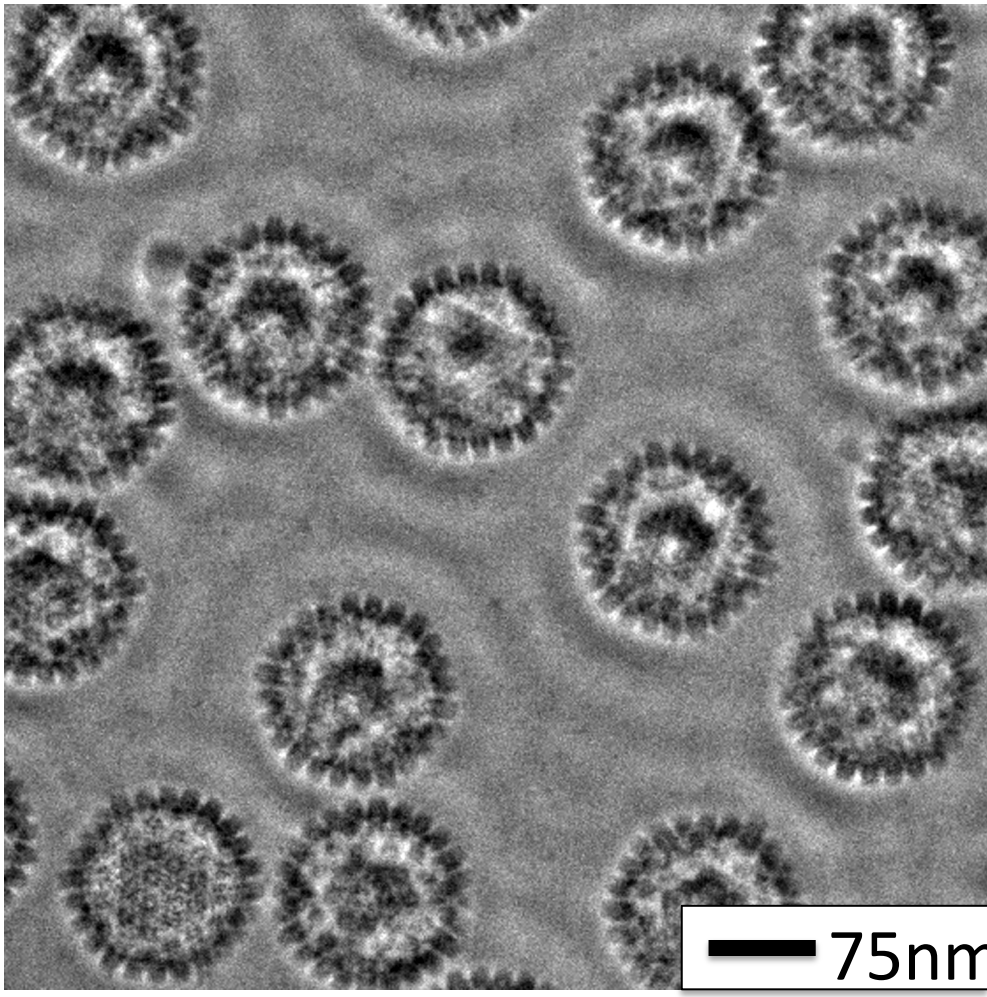
- Small molecular components in a large assembly
- Small molecules
- Conformational and/or compositional heterogeneous samples
- Molecular machines inside the cell

1. Cryo-EM of Herpes Virus Type-1 Capsid

Conventional

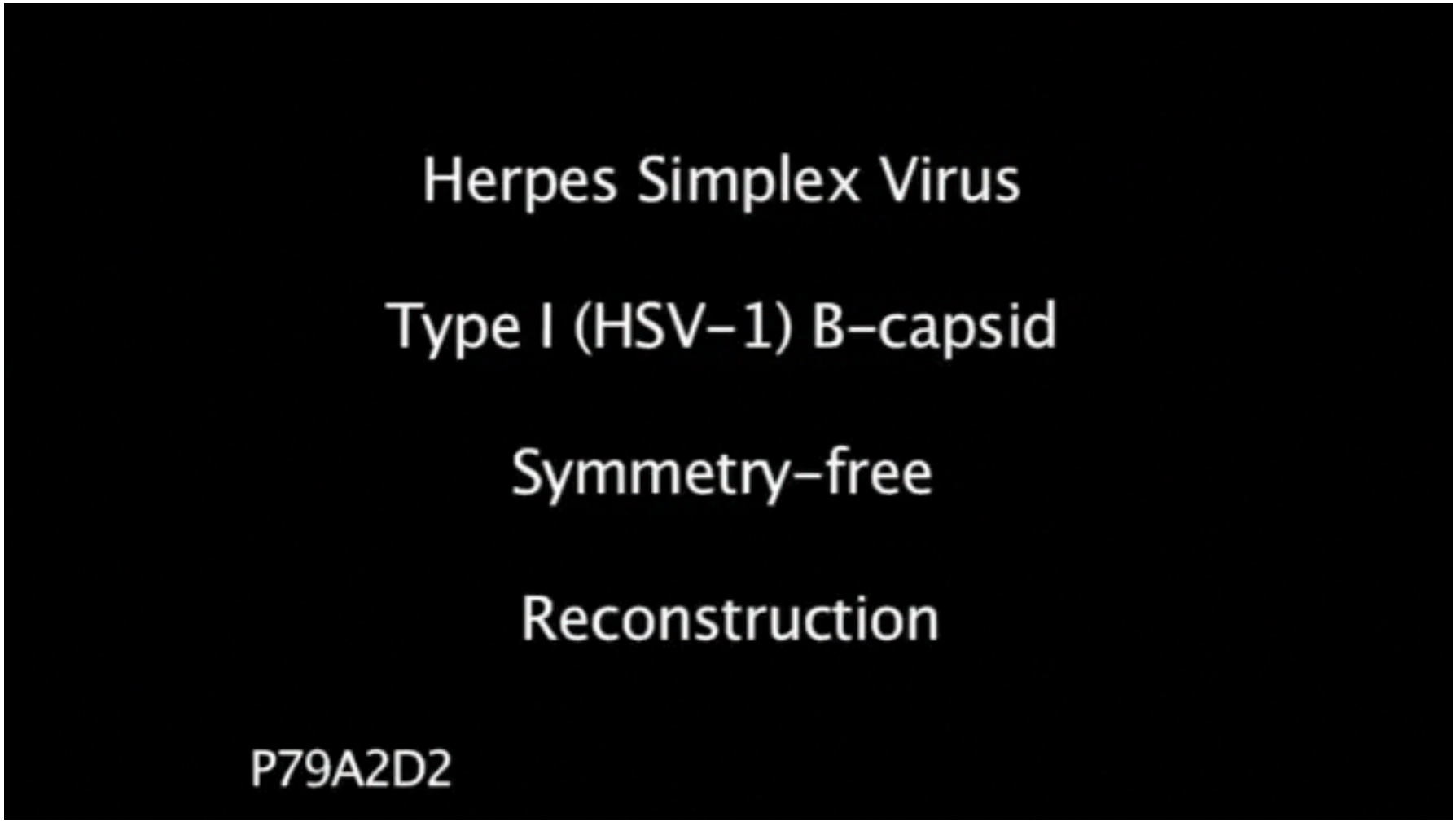


Zernike Phase Plate



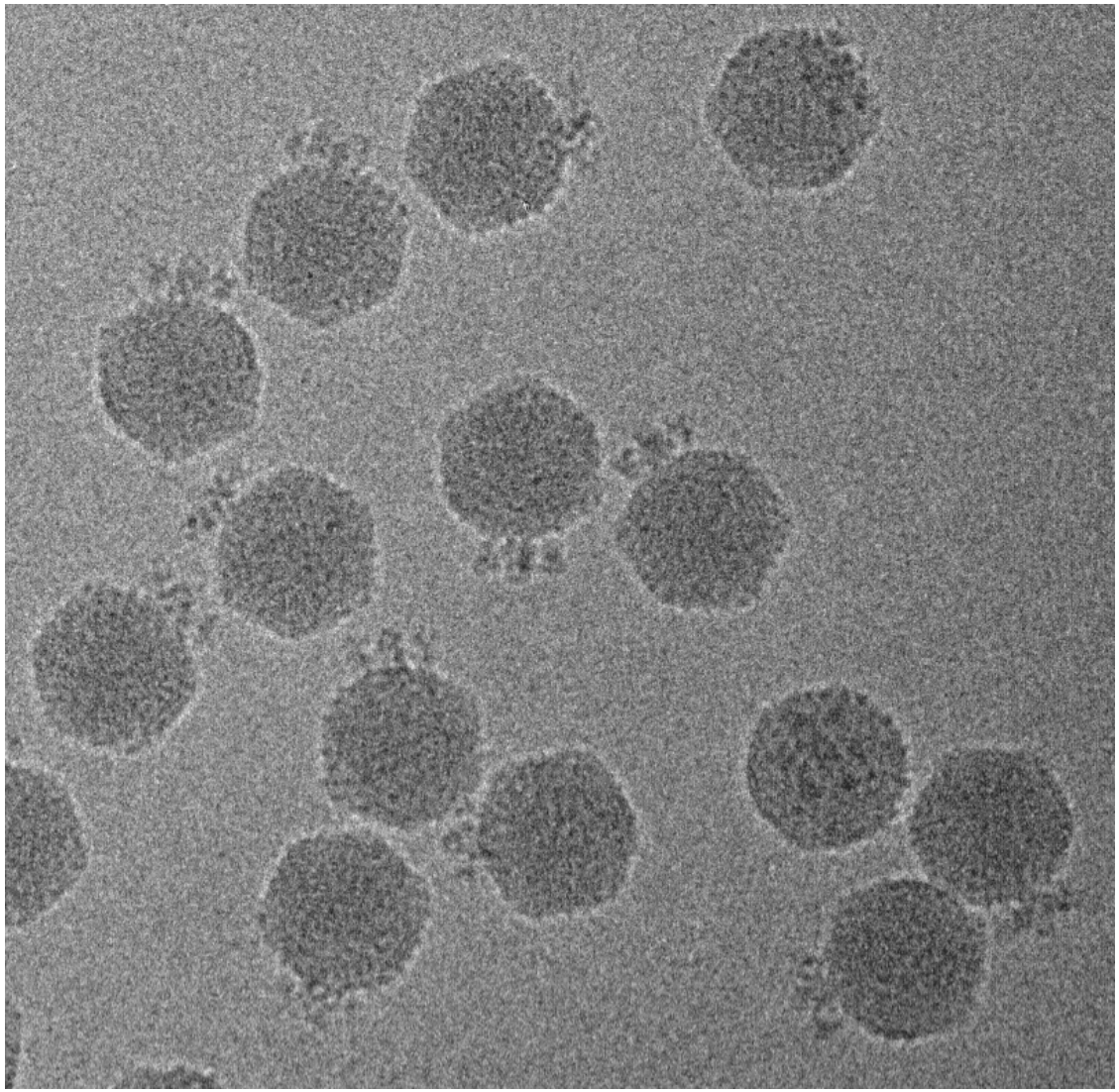
Rochat, R. (2011) *J Virol* **85**(4): 1871-1874.

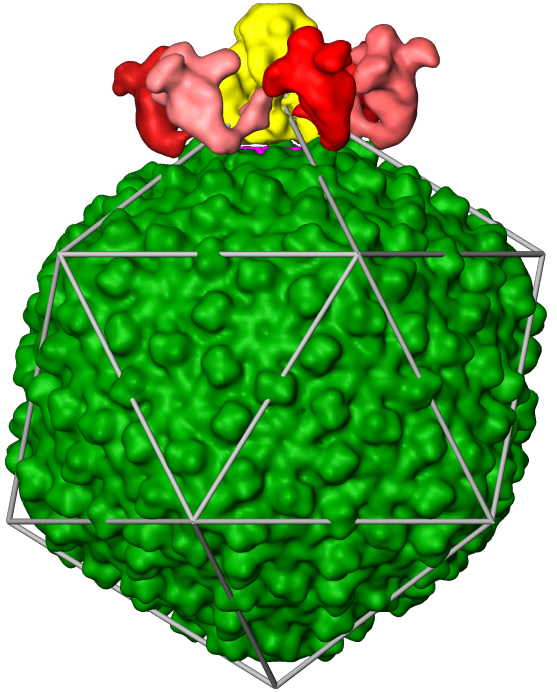
1. Seeing a Portal Vertex in HSV-1 Capsid

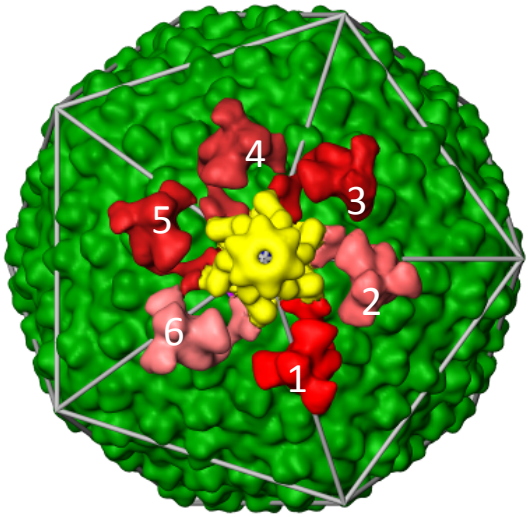


Rochat, R. (2011) *J Virol* **85**(4): 1871-1874.

2. Conventional Single Particle Cryo-EM of ε15 Phage



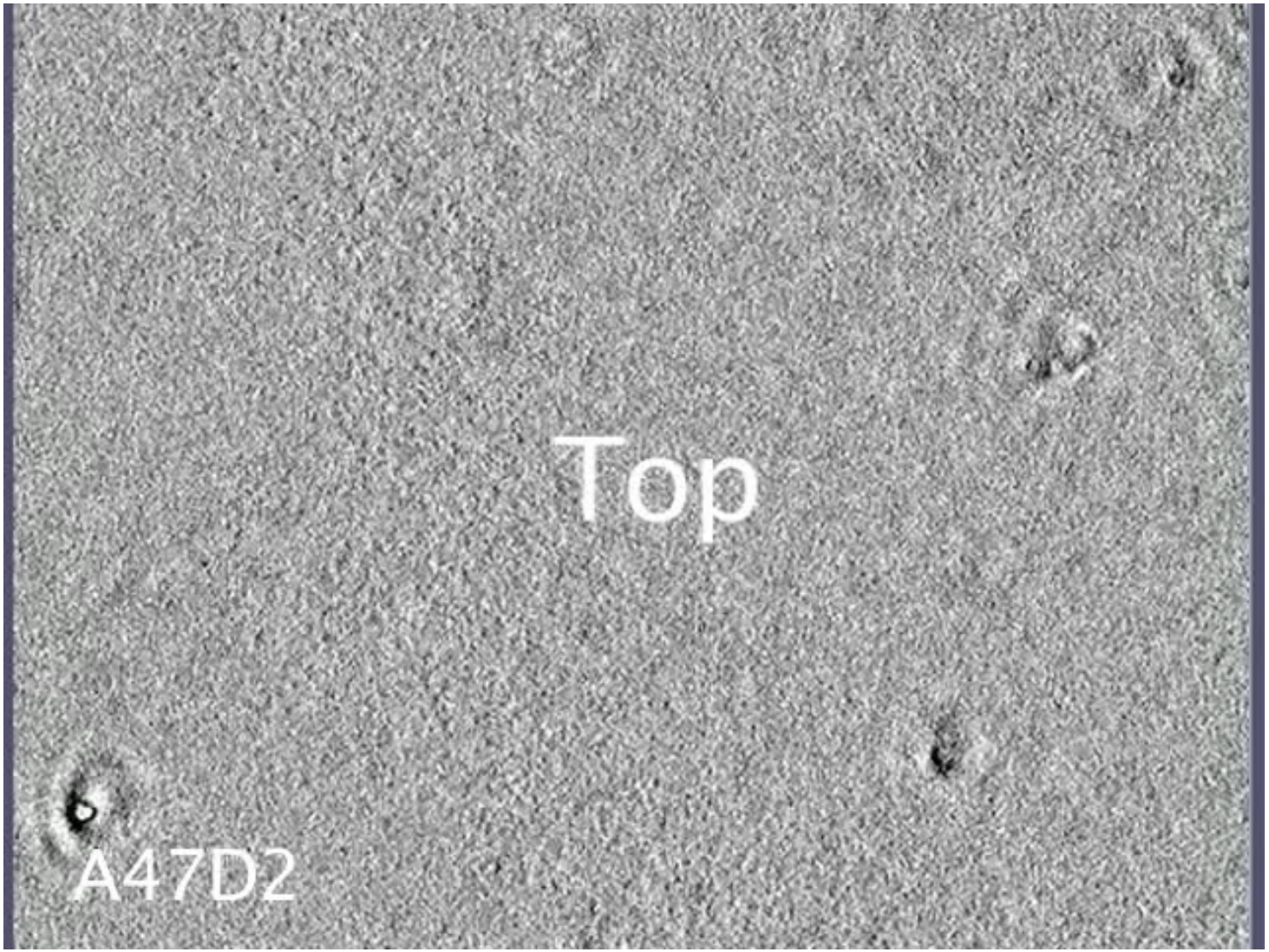




Jiang et al. Nature 2006

Over 25,000 particles

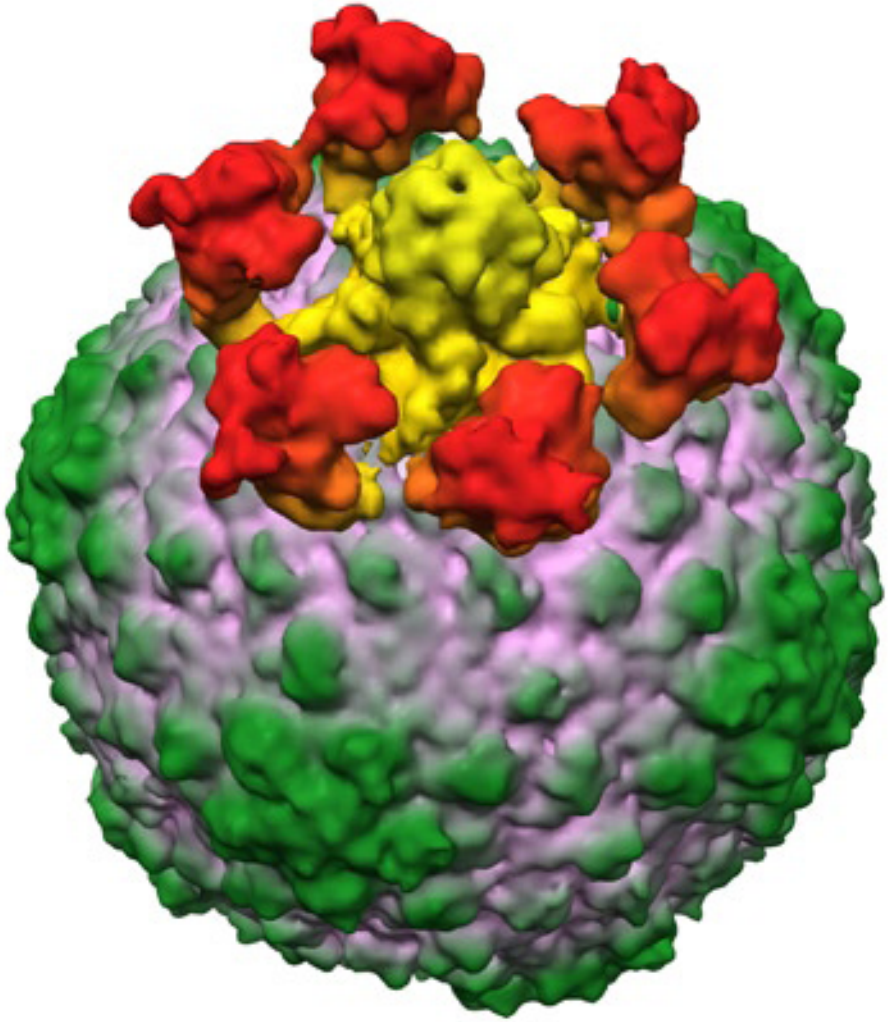
2. Zernike CET of ε15 Phage



2. Sub-Tomogram Average of ε15 Phage from Zernike CET

31.5 Å resolution

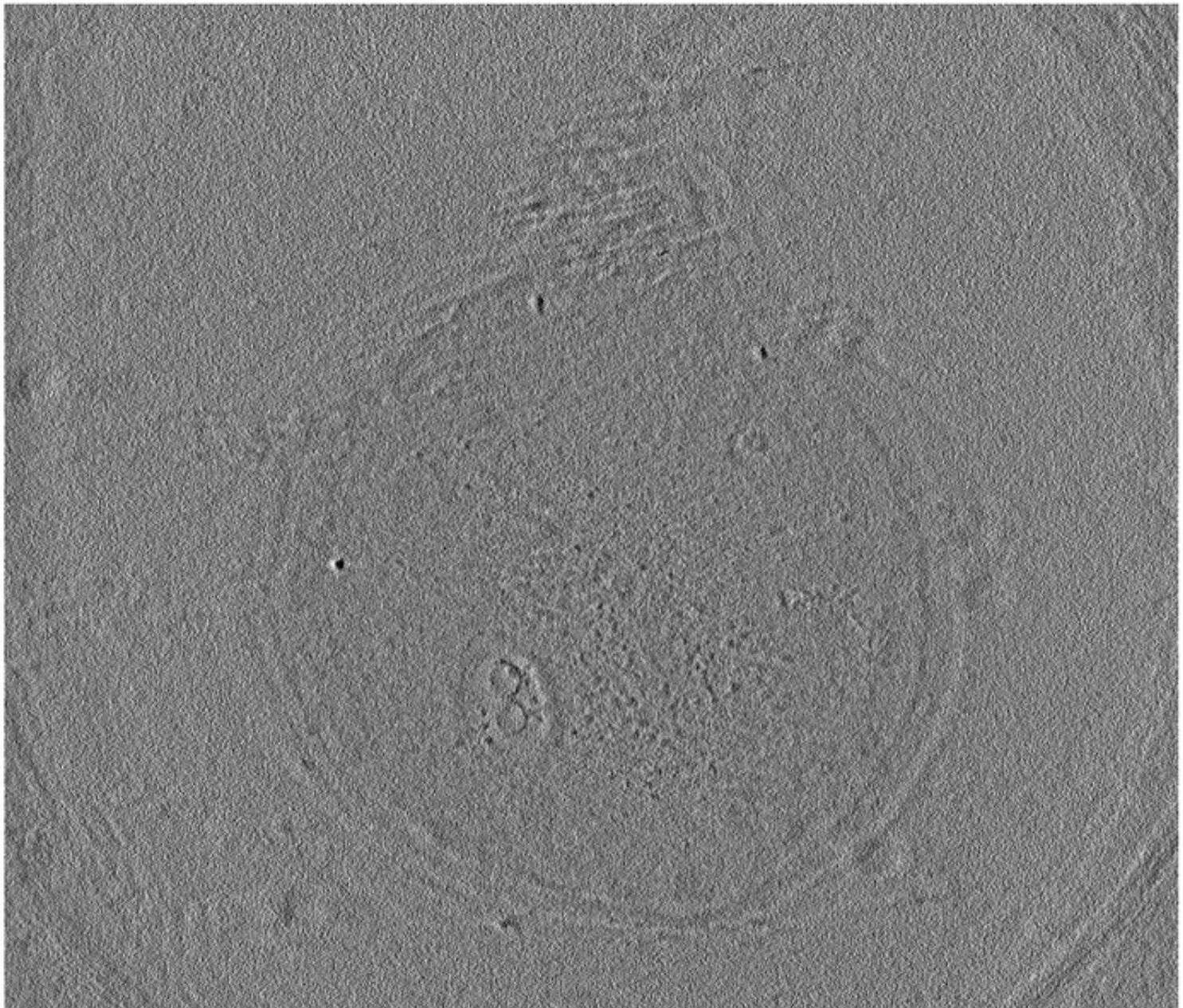
130 subtomograms in a single tomogram



Murata, Liu et al (2010) *Structure* 18:903-912

Irobalieva, Fu, Schmid, Summers Unpublished

4. ZCET of Cyanobacterium Infected with Phages



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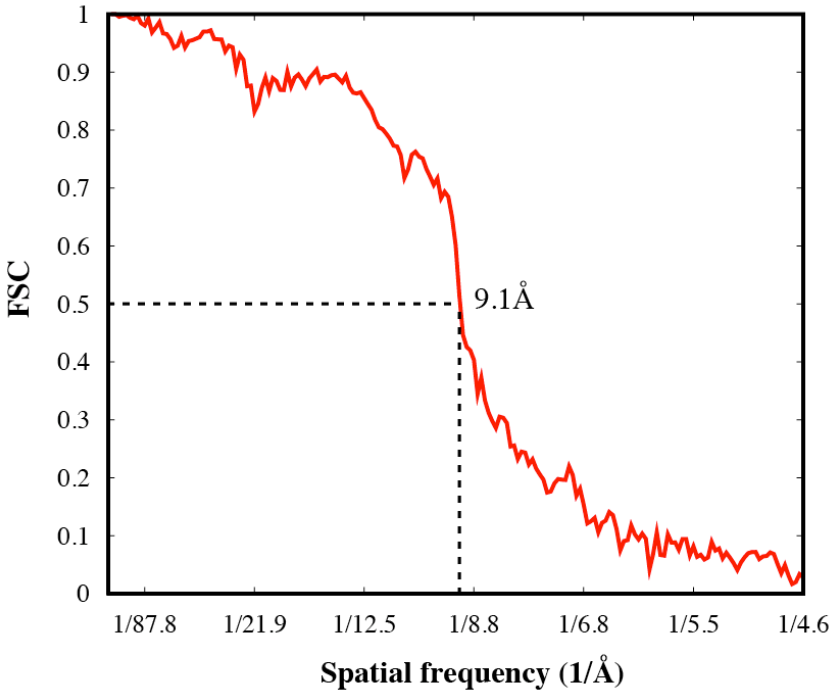
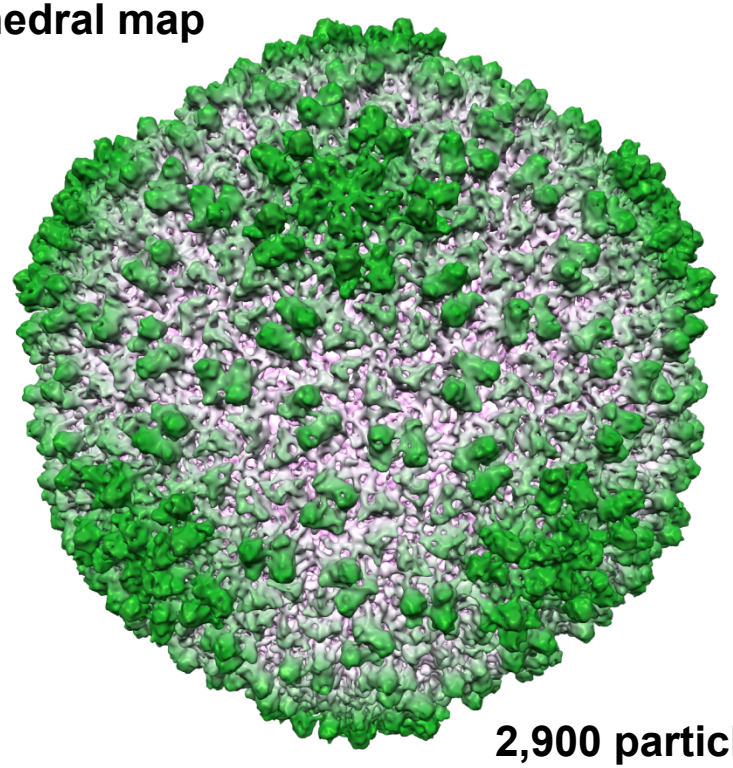
ε15 Phage Icosahedral Map at 9 Å Resolution

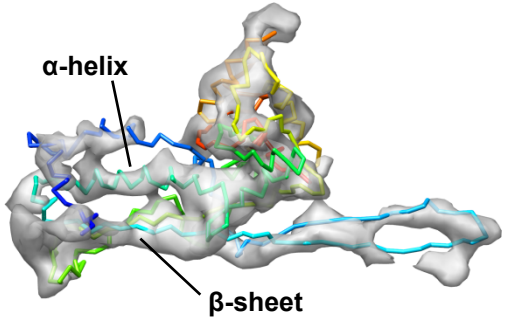


Icosahedral map



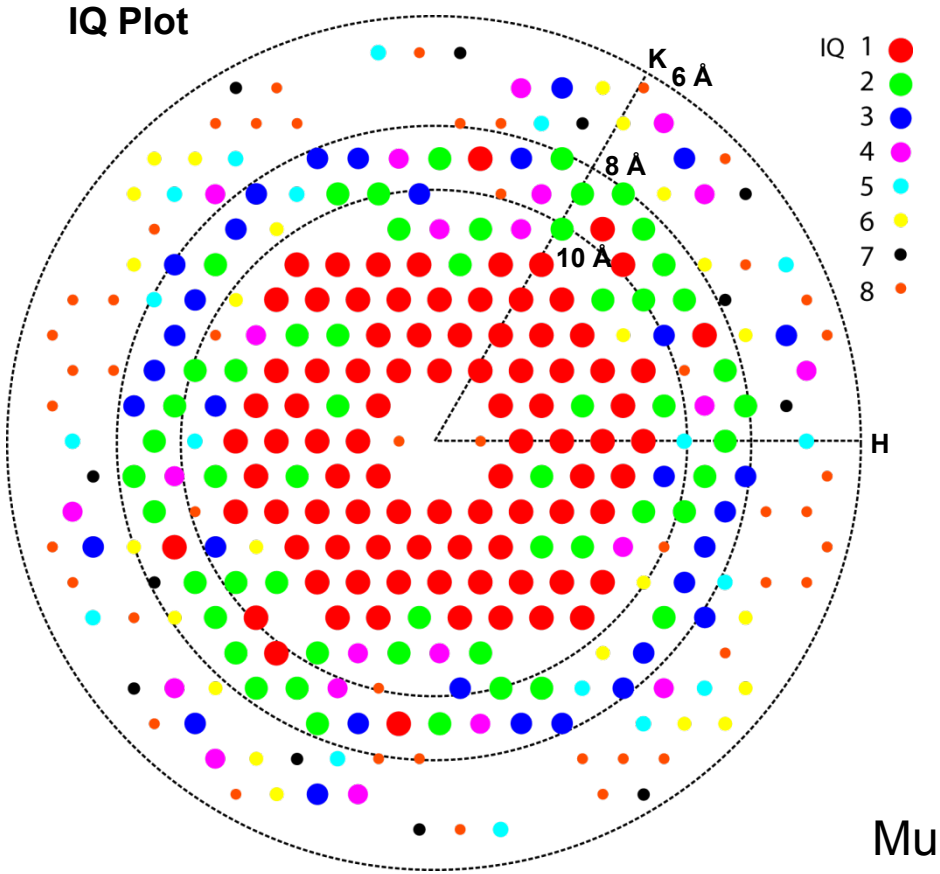
2,900 particles

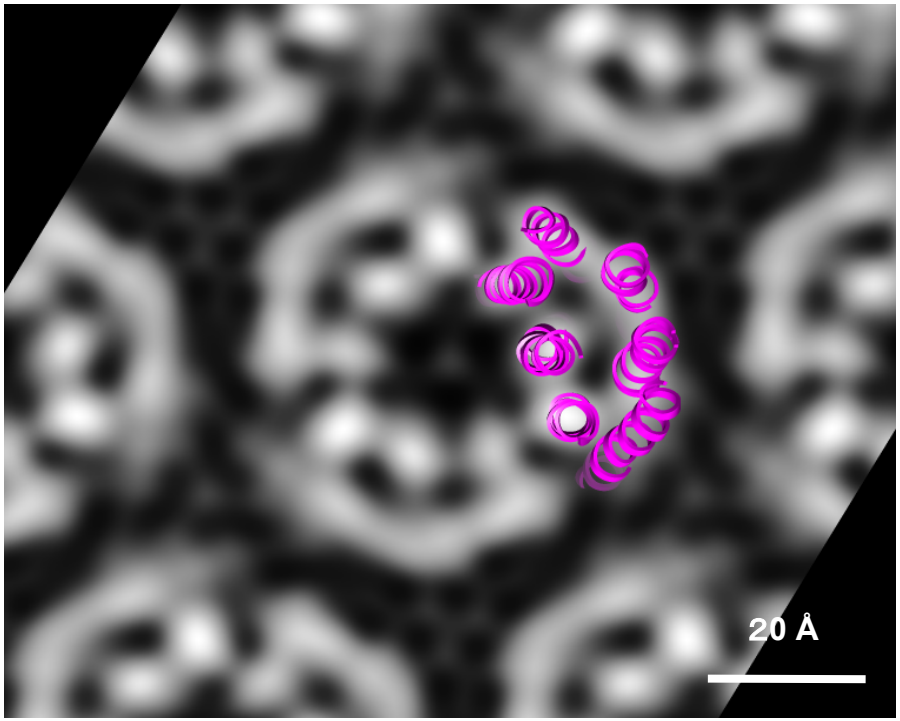
Asymmetric unit



Murata, Liu et al (2010) *Structure* 18:903-912

8-Å Map of Bacteriorhodopsin





Murata, Liu et al (2010) *Structure* 18:903-912

Phase error from the Henderson's projection map

Resolution range (Å)	100-15	15-10	10-8	100-8
Phase error	7.2°	10.2°	11.2°	9.7°
S.D.	6.7°	9.3°	7.8°	8.1°

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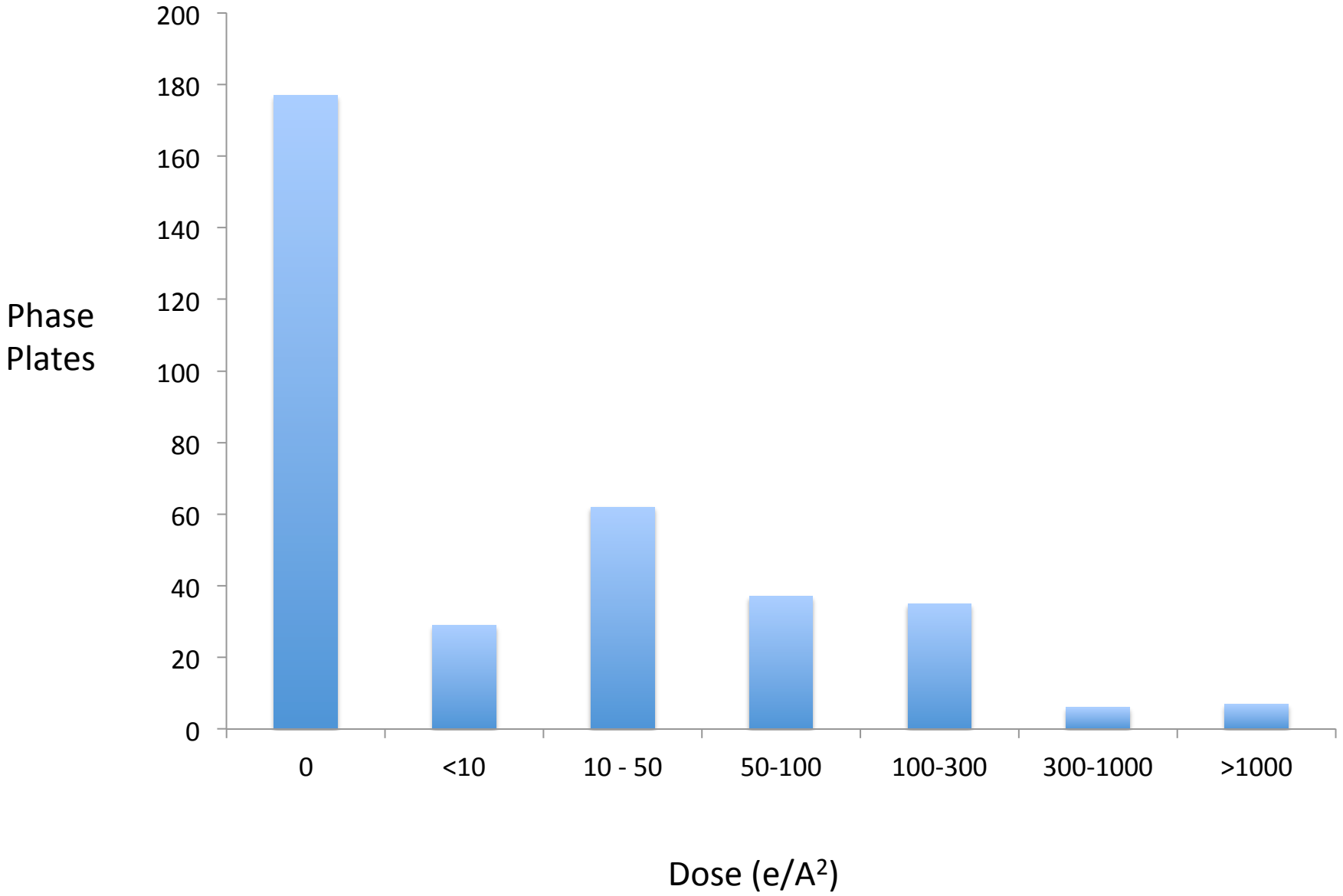
Phase Plate Durability



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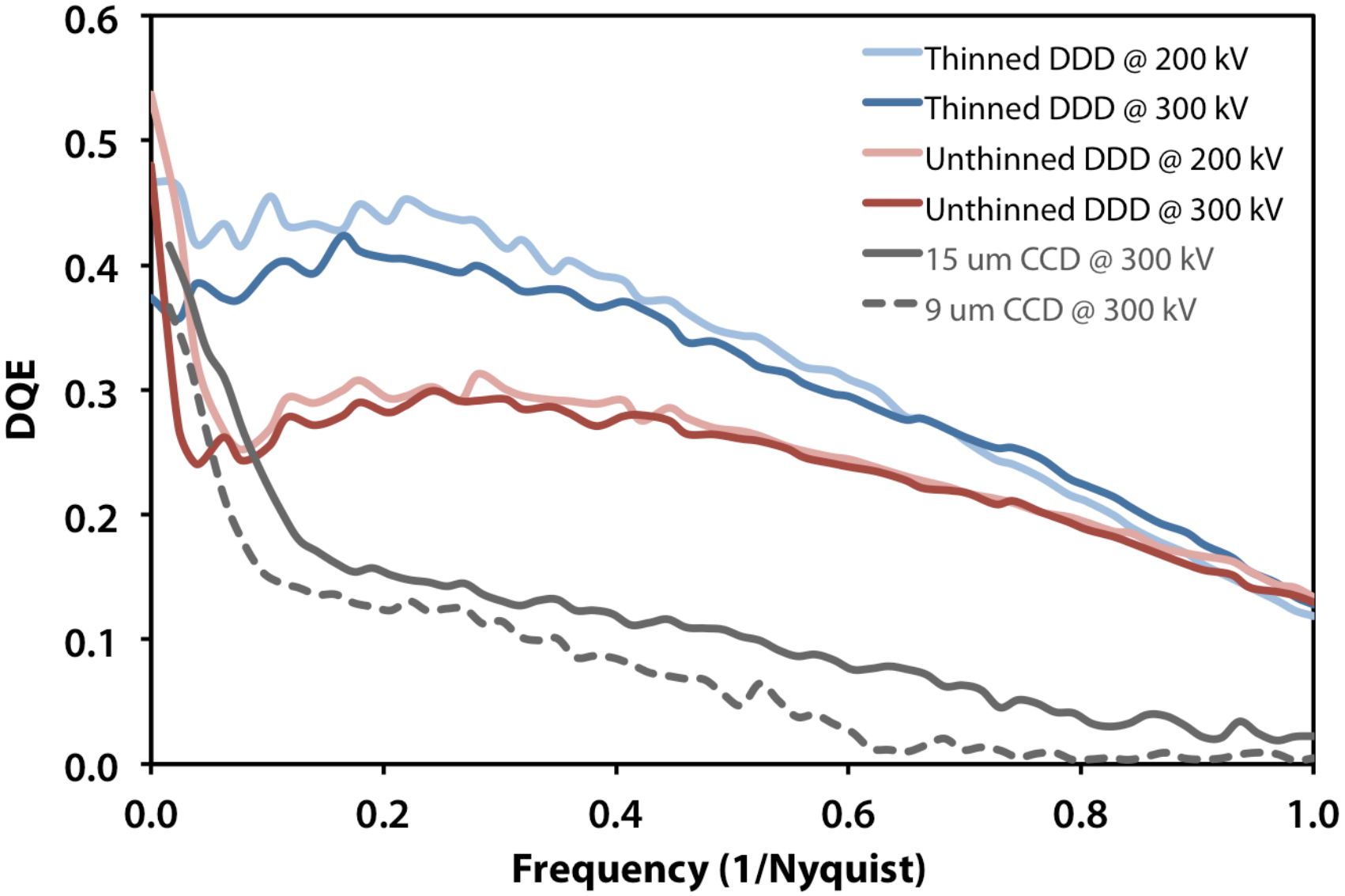
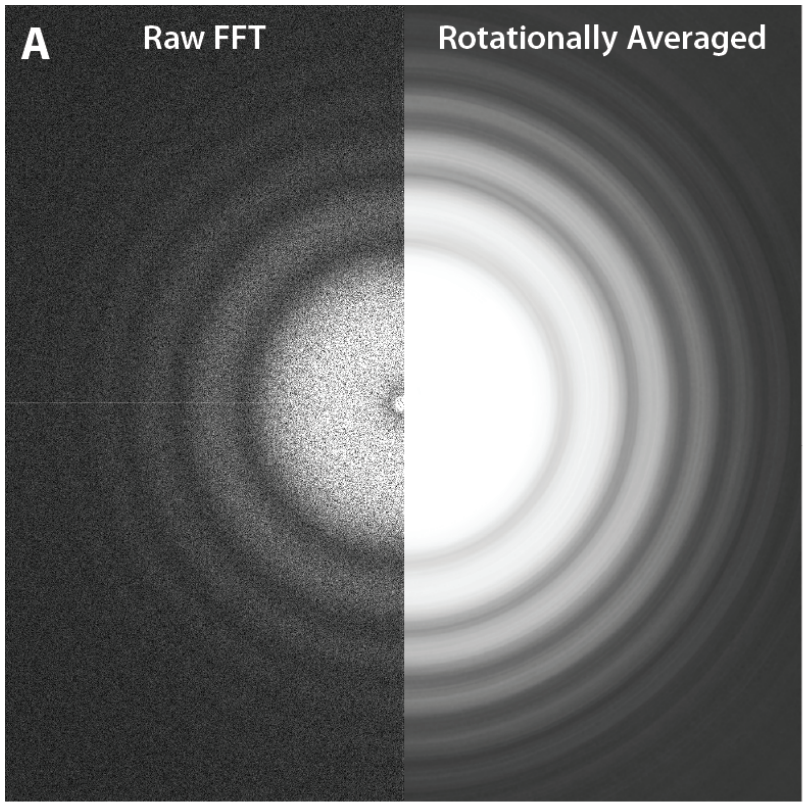
Detective Quantum Efficiency (DQE)

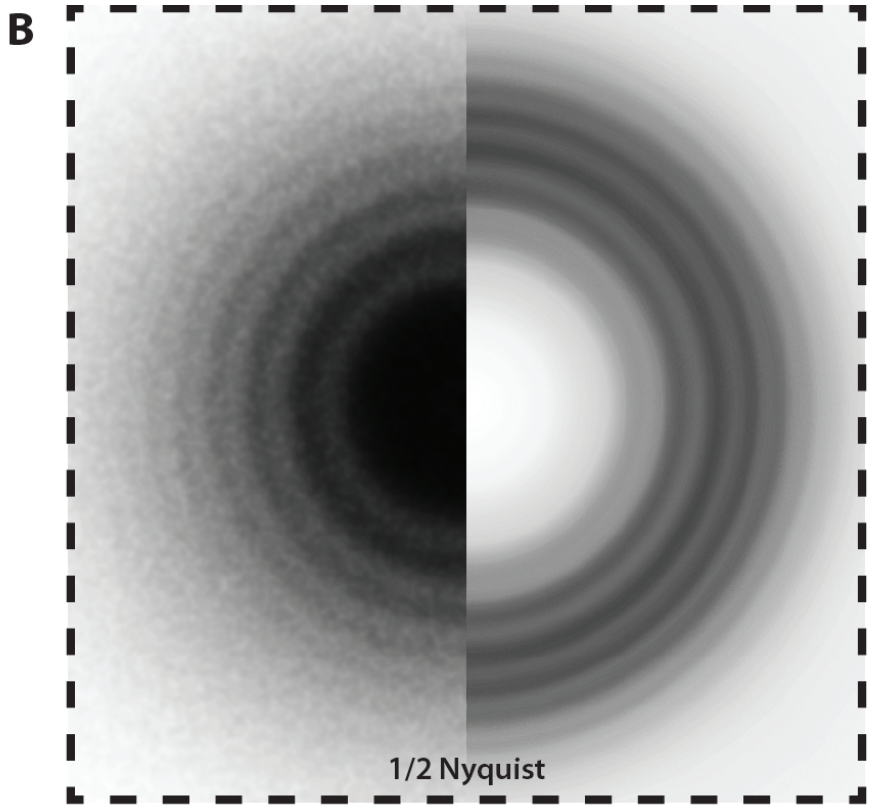


Ben Bammes, DDD

FFT Signals from 300 kV C-Film Image

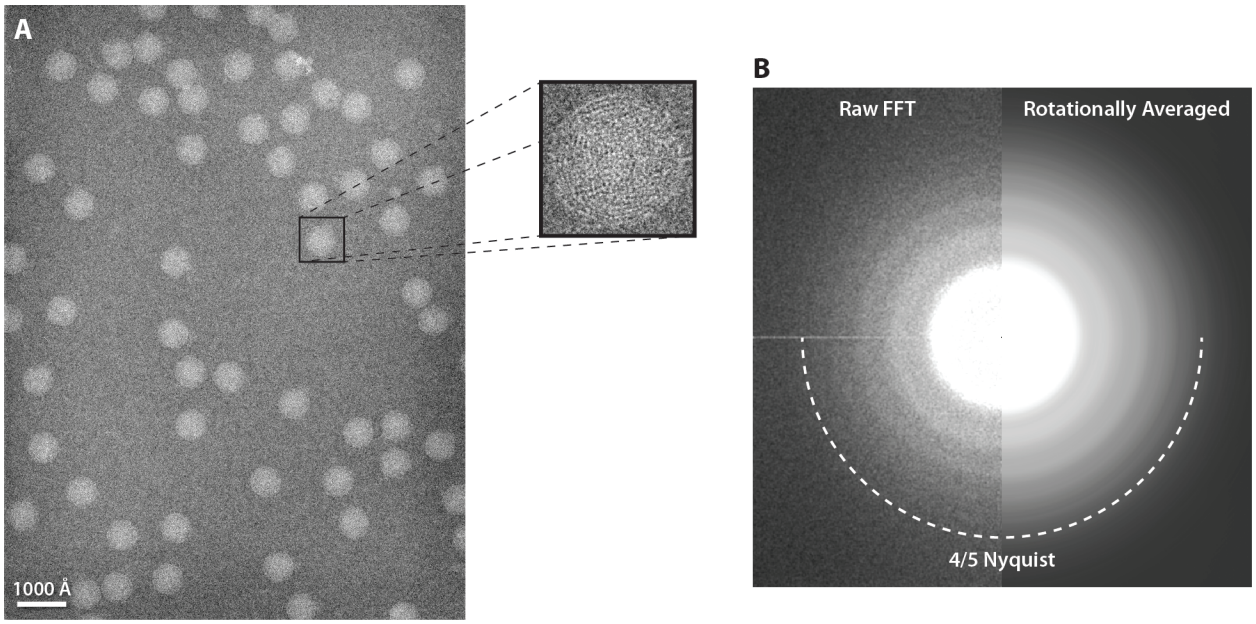


3kx4k DDD
10,800 x mag
Bammes et al (2012) *JSB* **177**:589-601

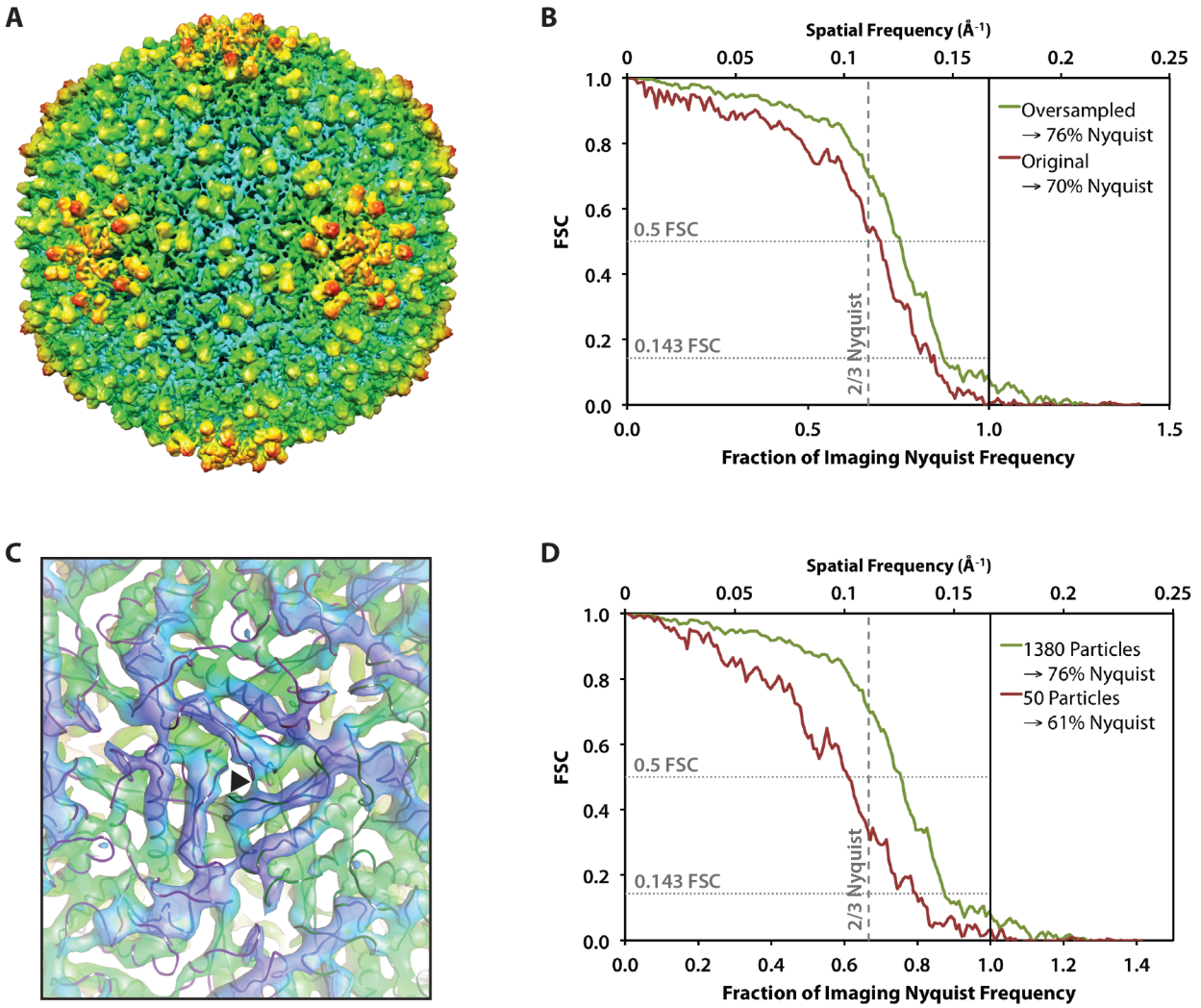


10kx10 K CCD
70,000x mag
Bammes et al. (2011)
JSB 175: 384-93

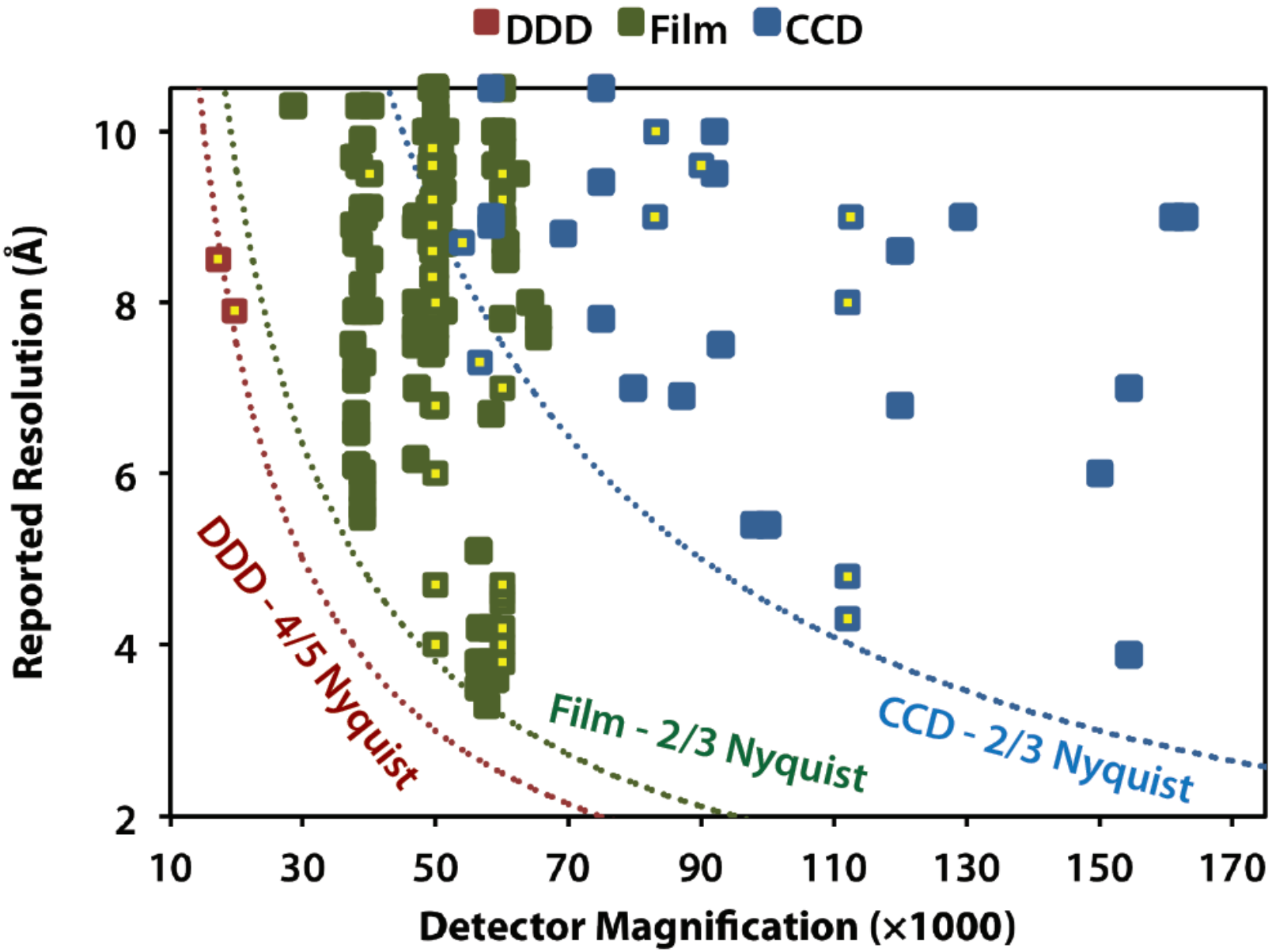
DDD performance with virus particle



DDD performance with virus particle



Bammes et al. (2012) *JSB*


DDD
Film
CCD
Reported Resolution (Å)
10
8
6
4
2
10
30
50
70
90
110
130
150
170
Detector Magnification (×1000)
DDD - 4/5 Nyquist
Film - 2/3 Nyquist
CCD - 2/3 Nyquist