Journal club articles in detail:

1. A comparison of original and modern plunge freezing techniques

Adrian et al. 1984 & Razinknov et al. 2016

These papers are focused on sample preparation methodology.

2. The best voltage for biological cryo-EM

Peet et al. 2019 & Naydenova et al. 2019

These papers deal with the microscopes and cover details of optics and information loss in EM.

3. The beginning of the resolution revolution

Liao et al. 2013

Seminal work on TRPV1 by Yifan Cheng's lab. Widely considered to be the "beginning" of routine high resolution cryo-EM. The discussion should focus on th technological and scientific advancements that made this work possible. There are far too many to explicitly assign, so it is left more open-ended.

4. HIV trimer controversy and Einstein from noise

Mao et al. 2013 & Henderson 2013 & van Heel 2013

The first paper is a cryo-EM structure of the HIV trimer, and the second two are criticisms of that paper's EM data processing. It is a significant case study in what can go wrong in EM.

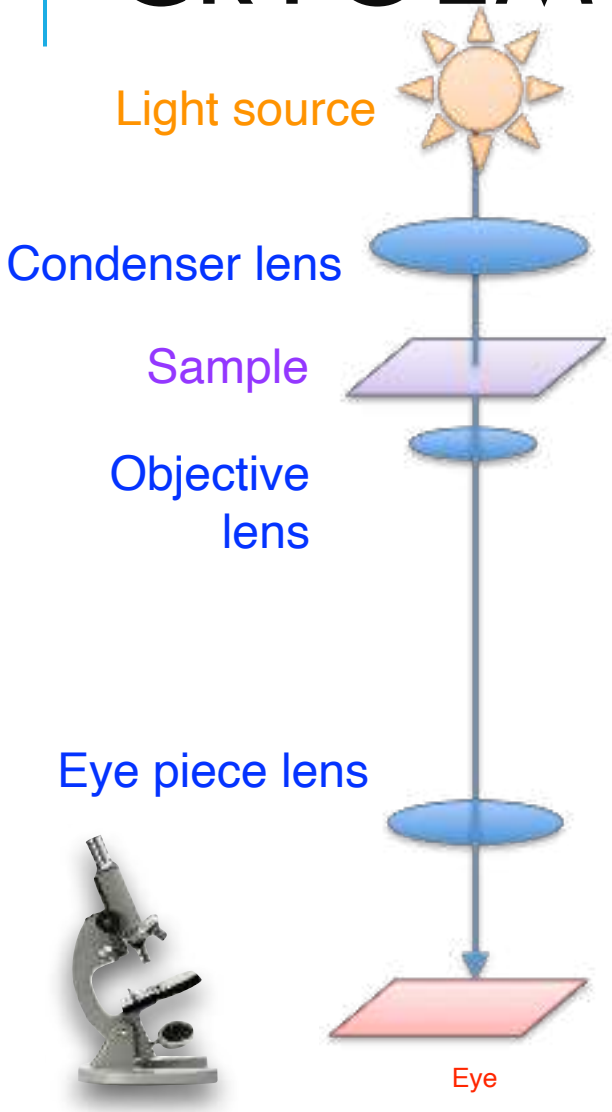
5. Challenges in and recommendations for validating cryo-EM data

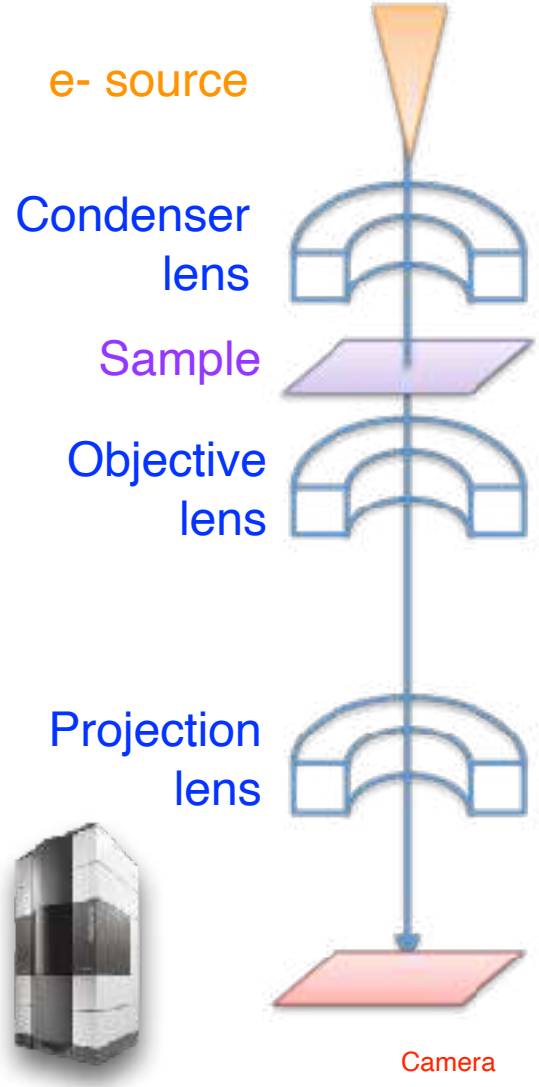
Henderson et al. 2012 & Neumann et al. 2018

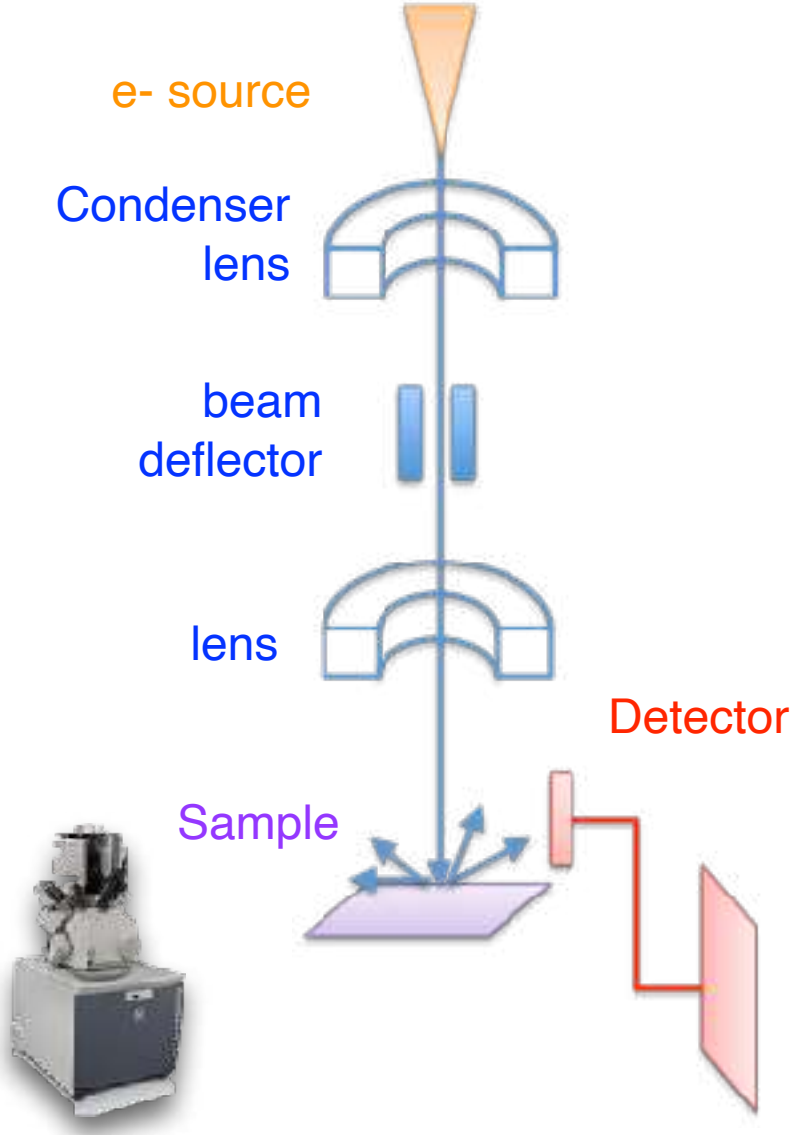
The first paper is the set of recommendations from the first Electron Microscopy Task Force Meeting, and the second is a more recent approach to validatio light of the resolution revolution.

6. TBD

CRYOEM TOOLS

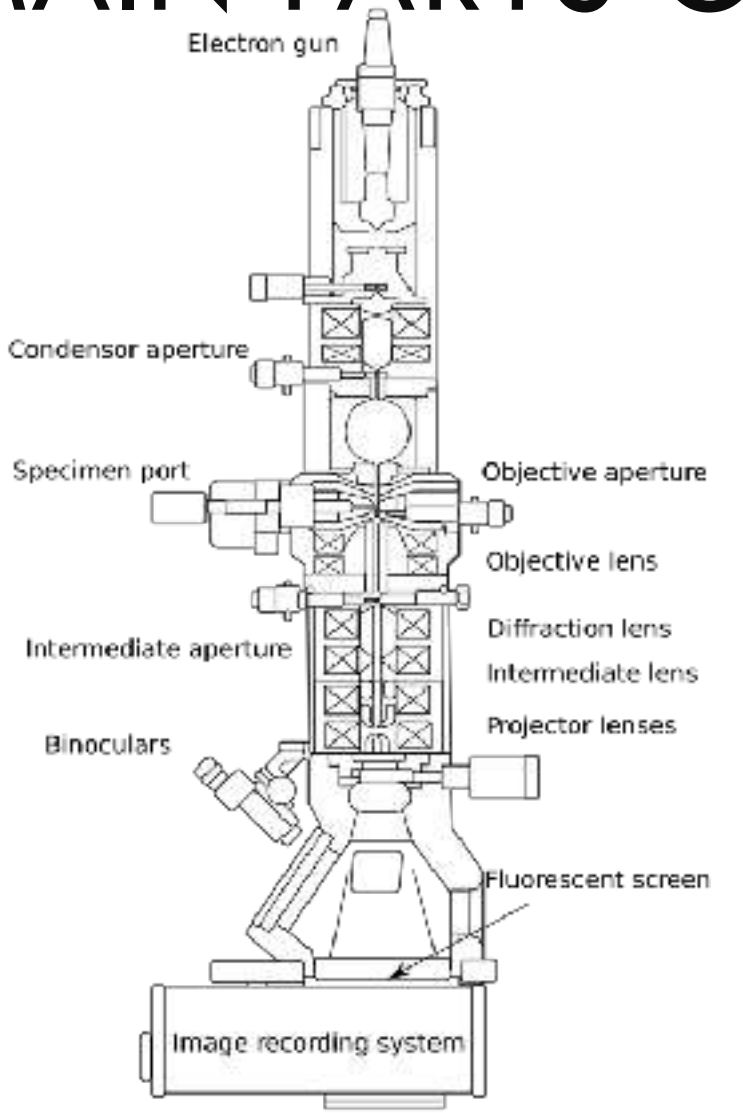







F20

MAIN PARTS OF AN EM



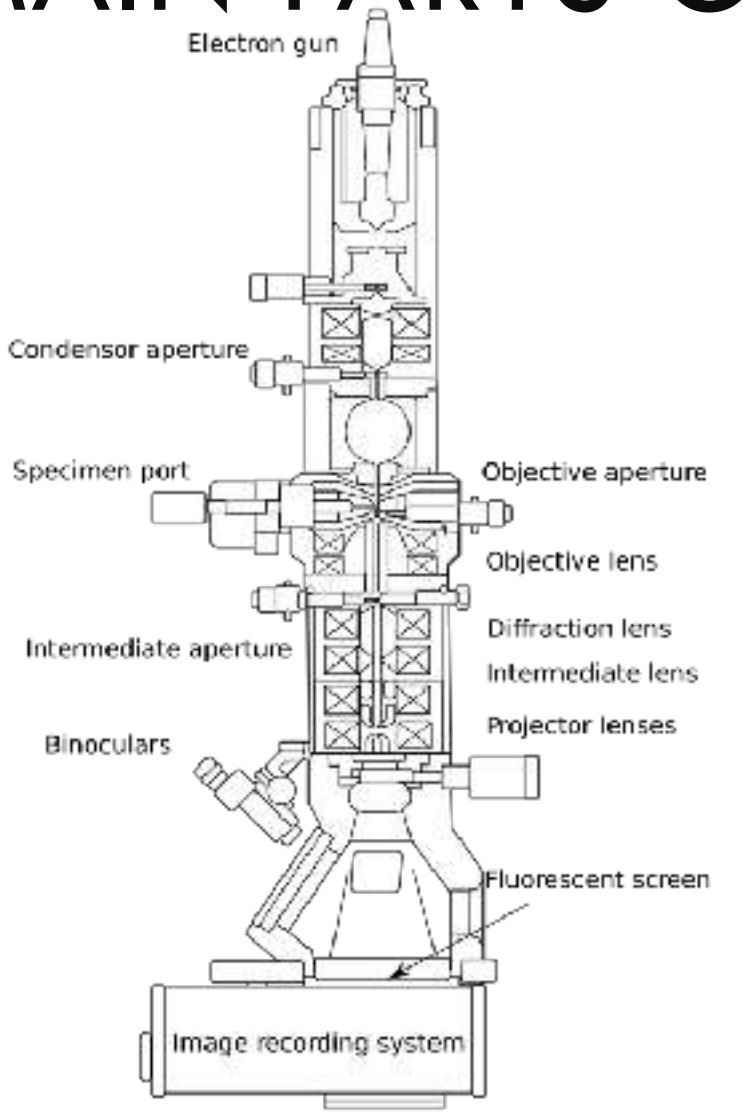
Electron sources

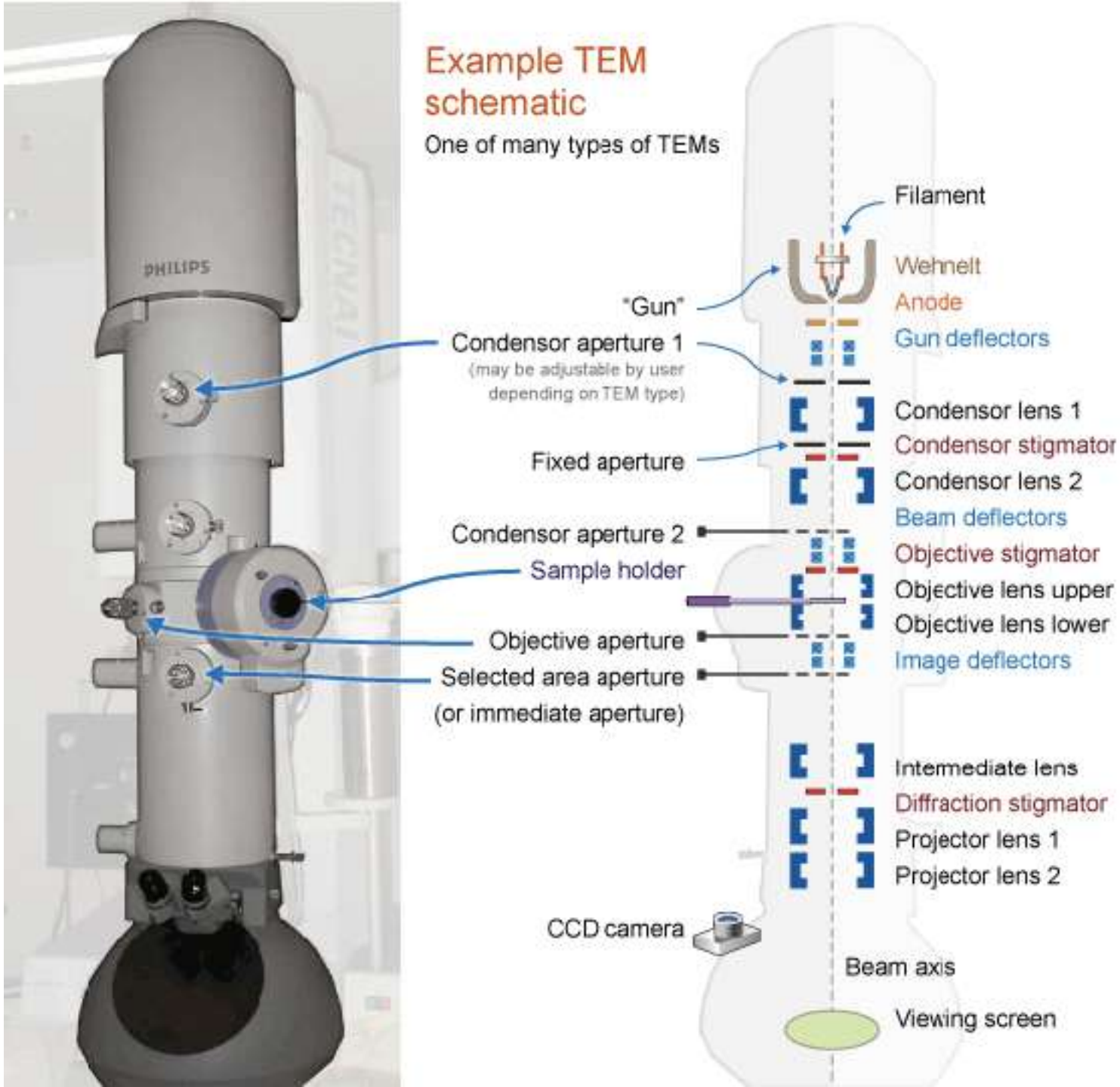
Vacuum systems

Lenses

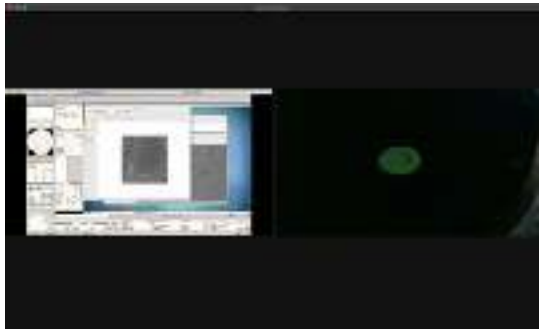
Detectors

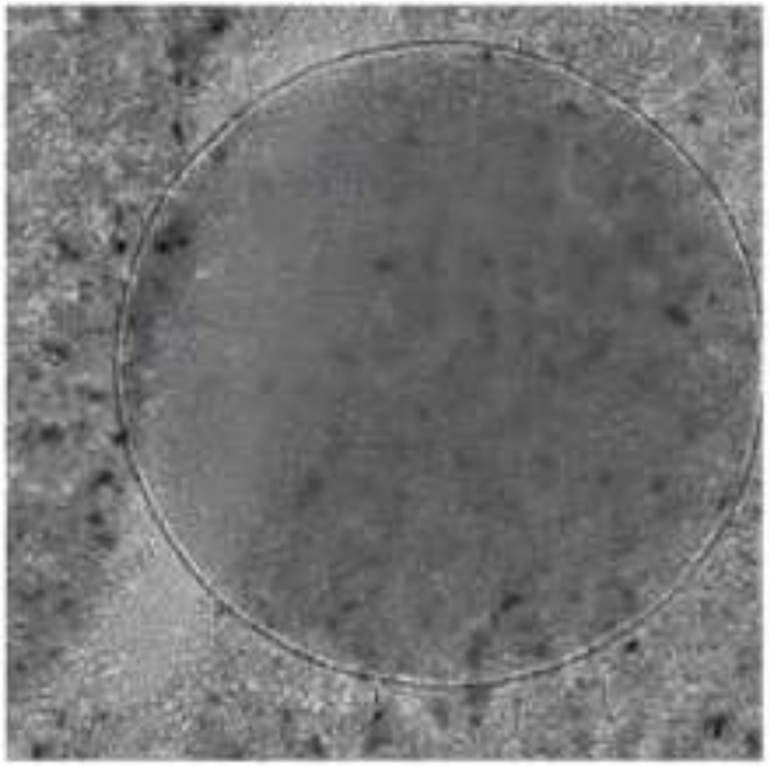
MAIN PARTS OF

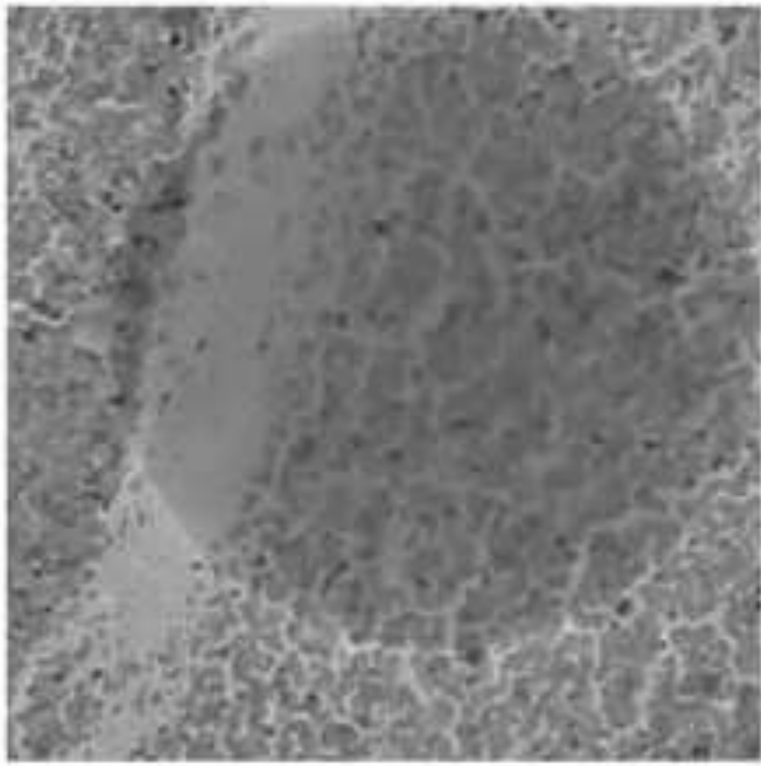


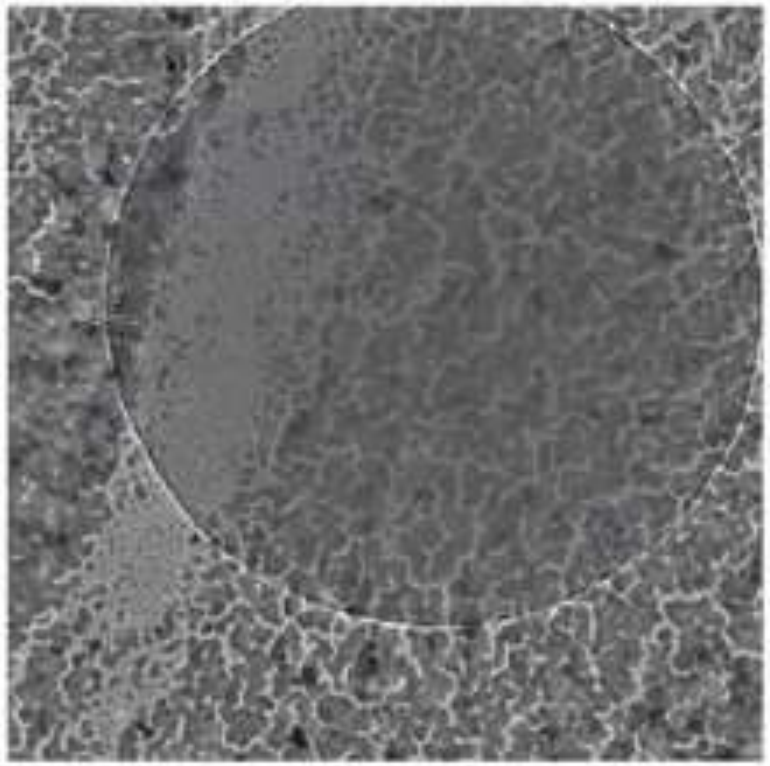

Example TEM schematic
One of many types of TEMs
PHILIPS
Filament
Wehnelt
Anode
"Gun"
Gun deflectors
Condensor aperture 1
(may be adjustable by user depending on TEM type)
Condensor lens 1
Condensor stigmator
Fixed aperture
Condensor lens 2
Beam deflectors
Condensor aperture 2
Objective stigmator
Sample holder
Objective lens upper
Objective lens lower
Objective aperture
Image deflectors
Selected area aperture
(or immediate aperture)
Intermediate lens
Diffraction stigmator
Projector lens 1
Projector lens 2
CCD camera
Beam axis
Viewing screen

FOCUS









K3 specs

https://www.gatan.com/K3

Specifications

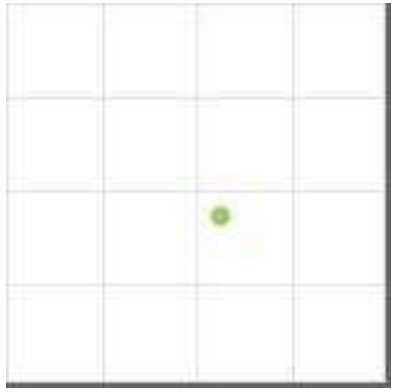
	K3	K3 Base
TEM operating voltage (kV)	200 / 300	
Sensor size (pixels)	5,760 x 4,096	3,456 x 4,096
Readout modes	Counting Super-resolution	Counting
Max. image size (pixels)	11,520 x 8,184 Super-resolution	3,456 x 4,096
Performance relative to physical Nyquist (DQE)		
Peak	>0.87 / >0.83	>0.8
0.5	>0.53 / >0.53	>0.5
Sensor read-out (full fps)	>1500	
Transfer speed to computer (full fps)	>75	>25
Motion correction	Inline	
Gatan Microscopy Suite® software	Included	
Automation support	Latitude and other third-party software	

Specifications are subject to change without notice.

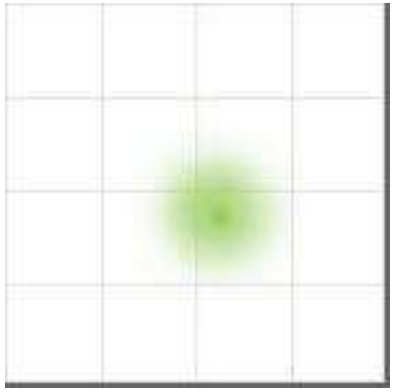
Counting mode

5,760 x 4,096 px

11,520 x 8,184 px

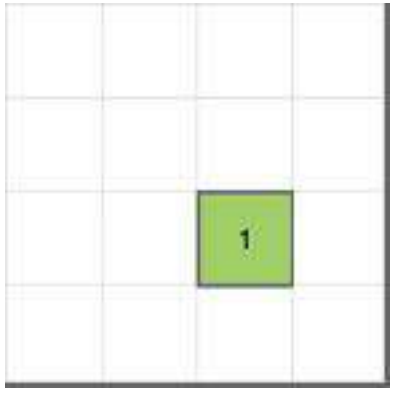


Electron enters detector.



Electron signal is scattered.

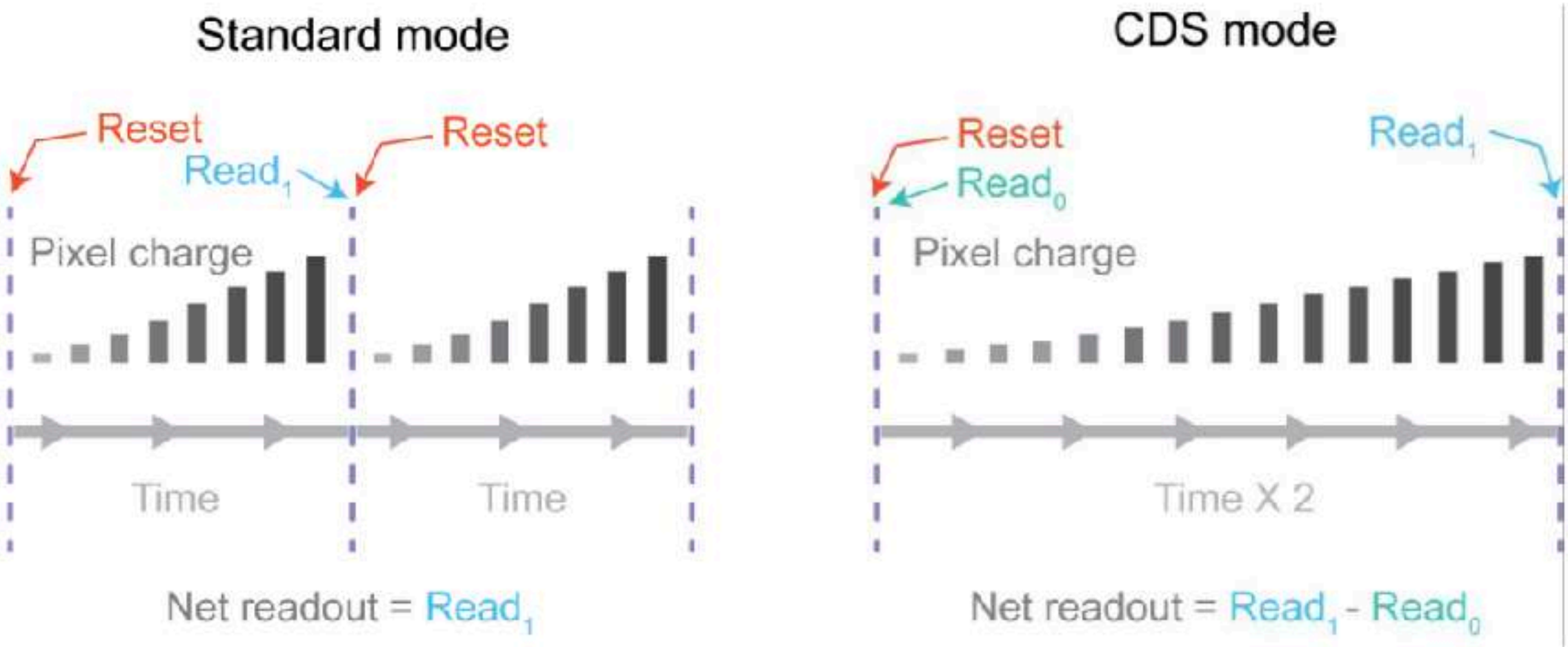
Charge collects in each pixel.



Events reduced to highest charge pixels.

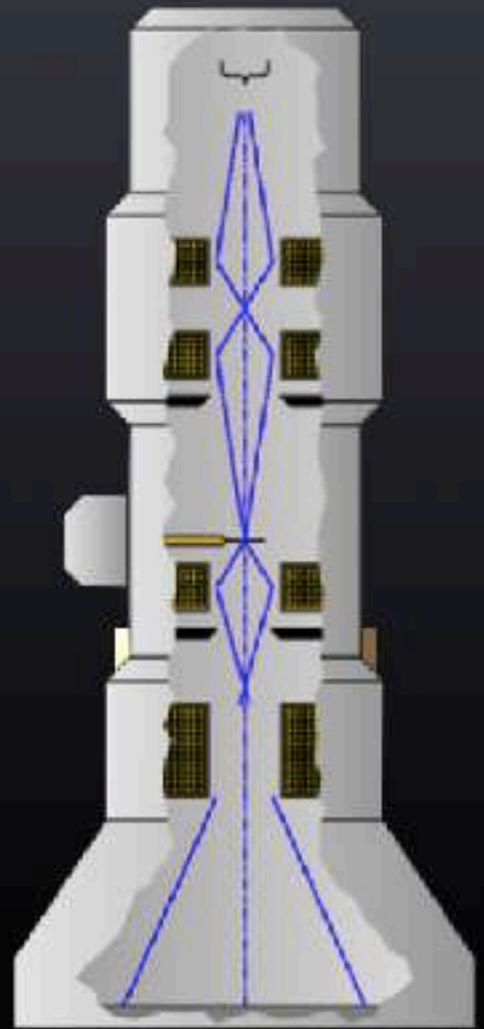
https://www.gatan.com/improving-dqe-counting-and-super-resolution

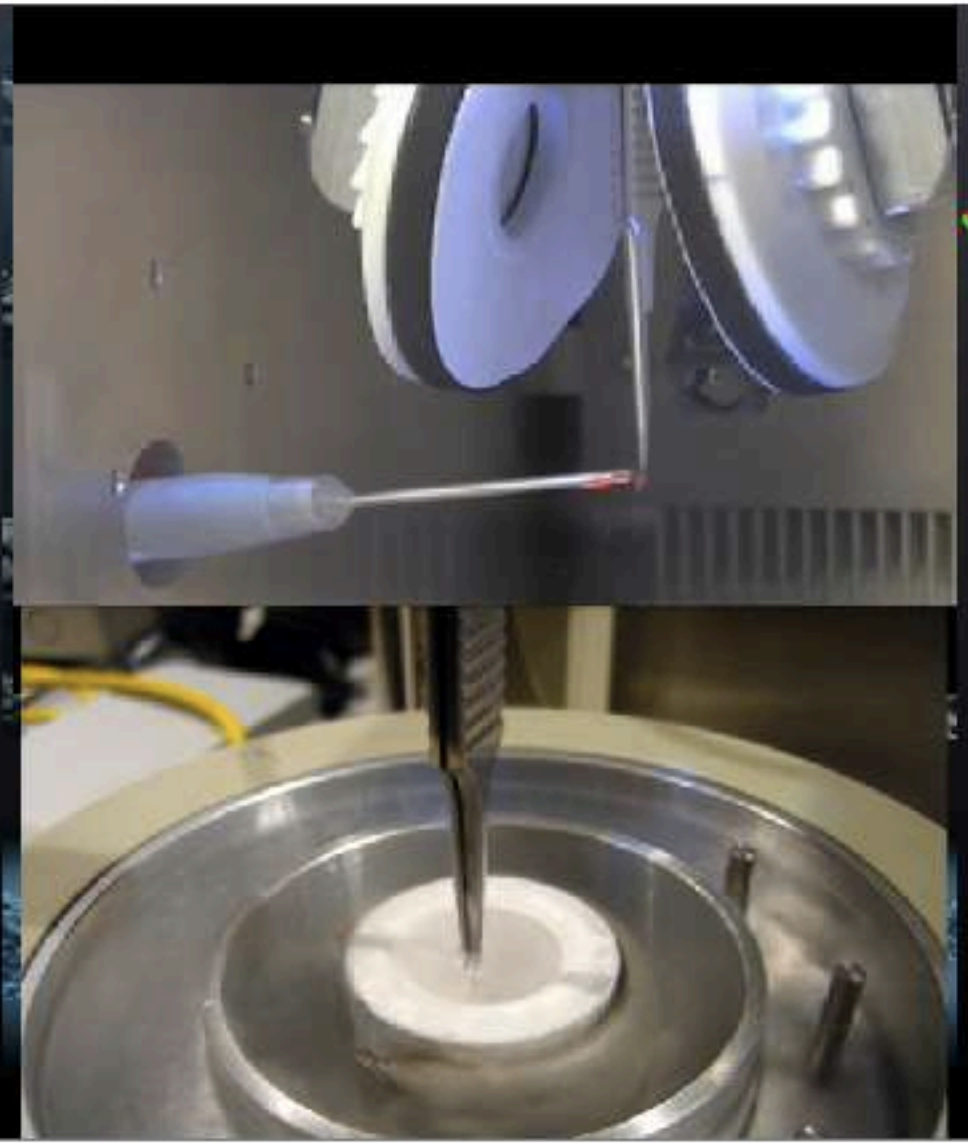
K3 lowers Read Noise with Correlated Double Sampling (CDS)



https://www.gatan.com/

Vitrifying a biological sample





>99.999%

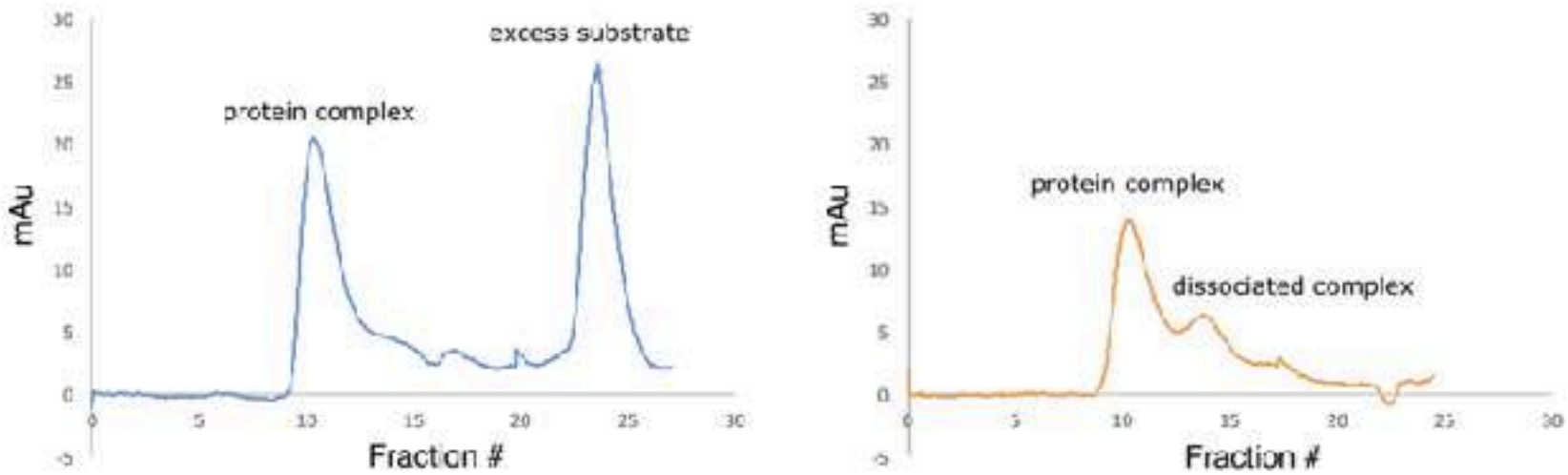
<0.001%

~3µl

<3nl

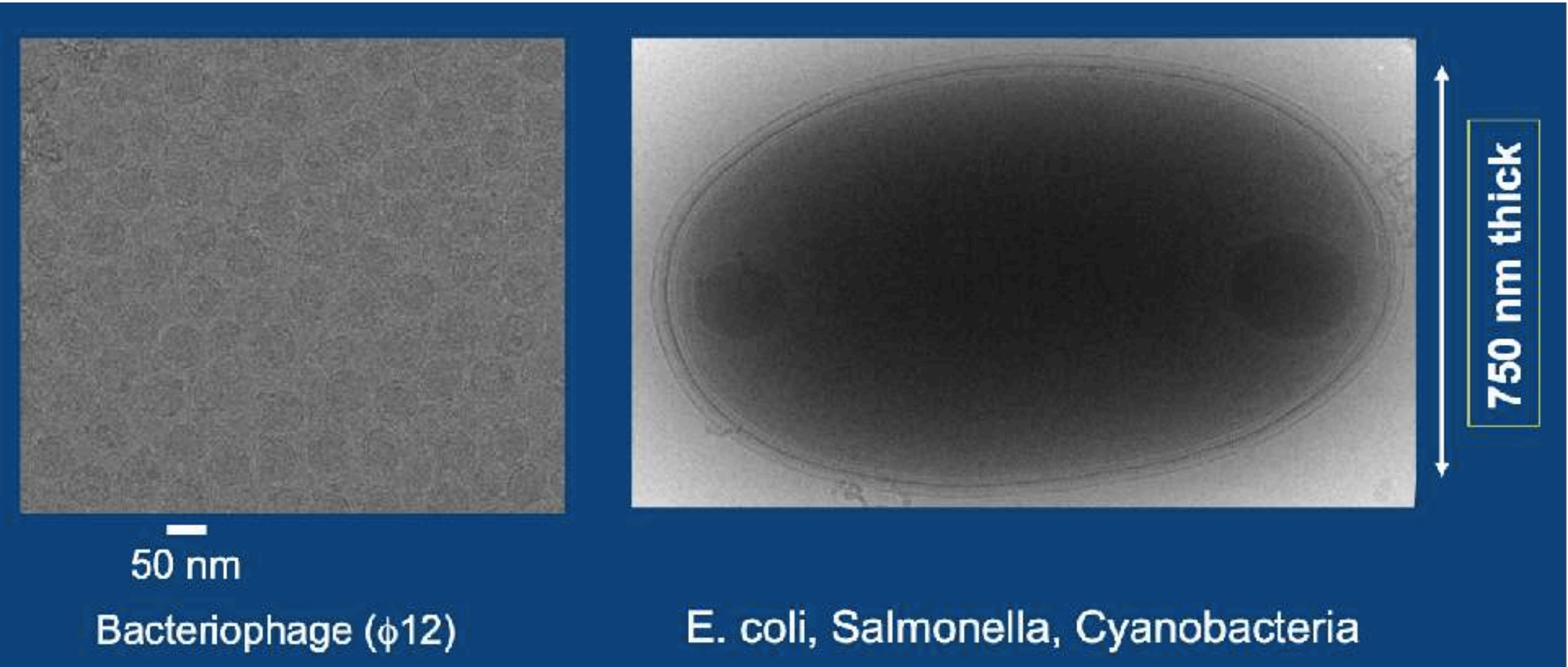
>100,000 potential imaging targets; most of them are not usable.

cryoem101.org

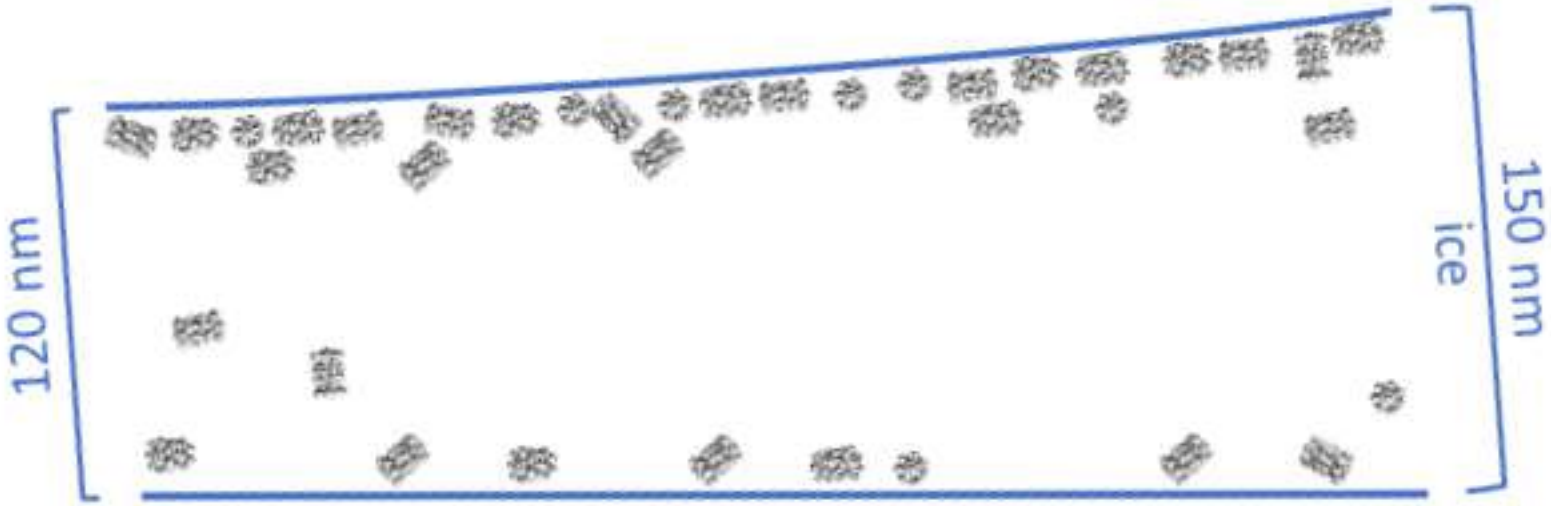


An example of an optimal gel filtration profile is shown on the left. The protein complex is enriched and abundant, and well separated from other peaks (e.g., excess substrate). On the right, a less optimal gel filtration profile for the same protein complex is shown (prepared in the absence of its substrate). Note that there are multiple peaks that are not well resolved, and the major complex species is less abundant.

How thin do I need my sample?

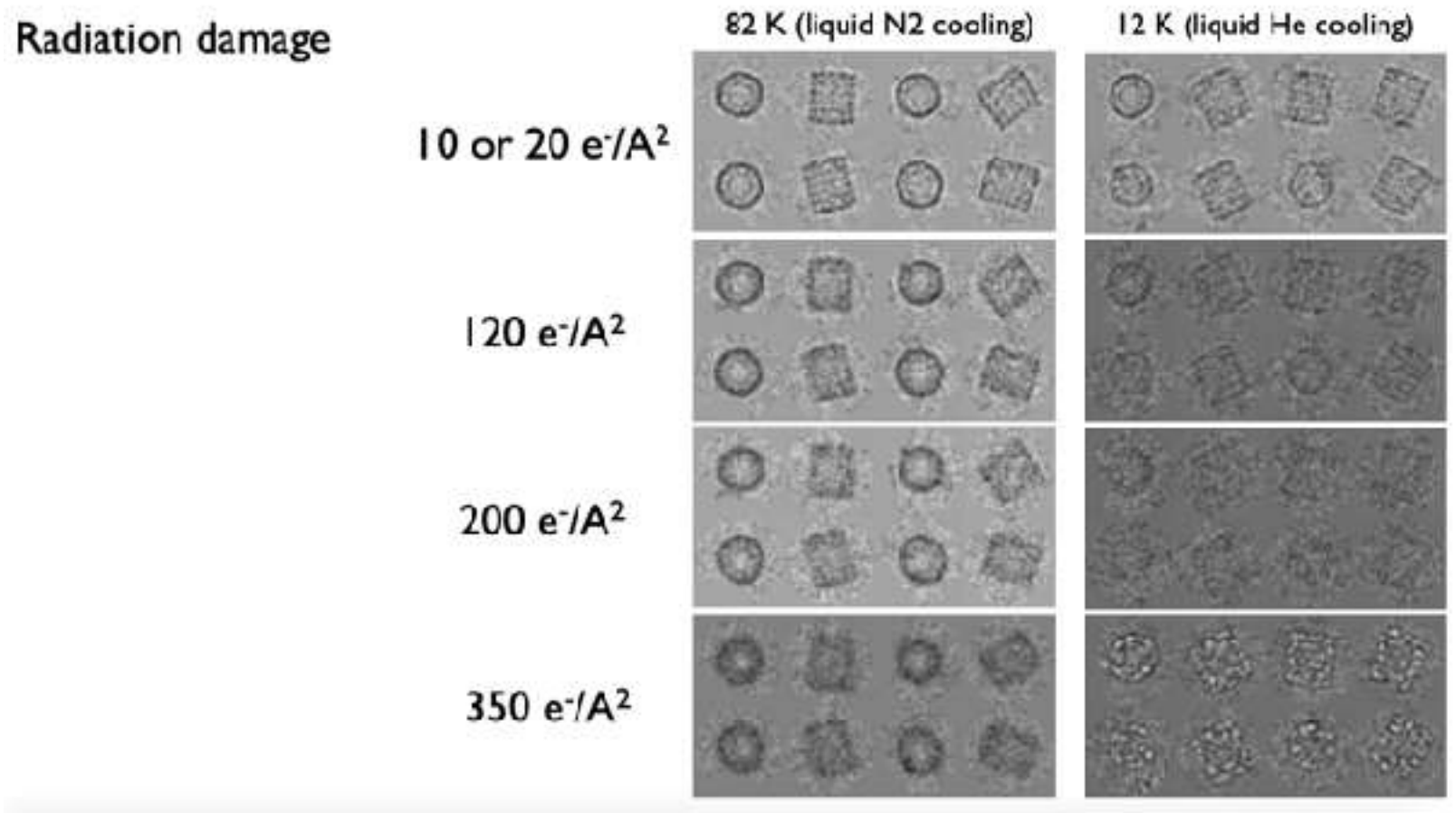


How thin do I need my sample?



Electron cryo-tomography analysis of single particles show that the overwhelming majority of particles adhere to the air-water interface and adopt a limited range of views. Image from Noble et al. eLife 2018.

https://cryo-em-course.caltech.edu/



Specimen Behavior in the Electron Beam

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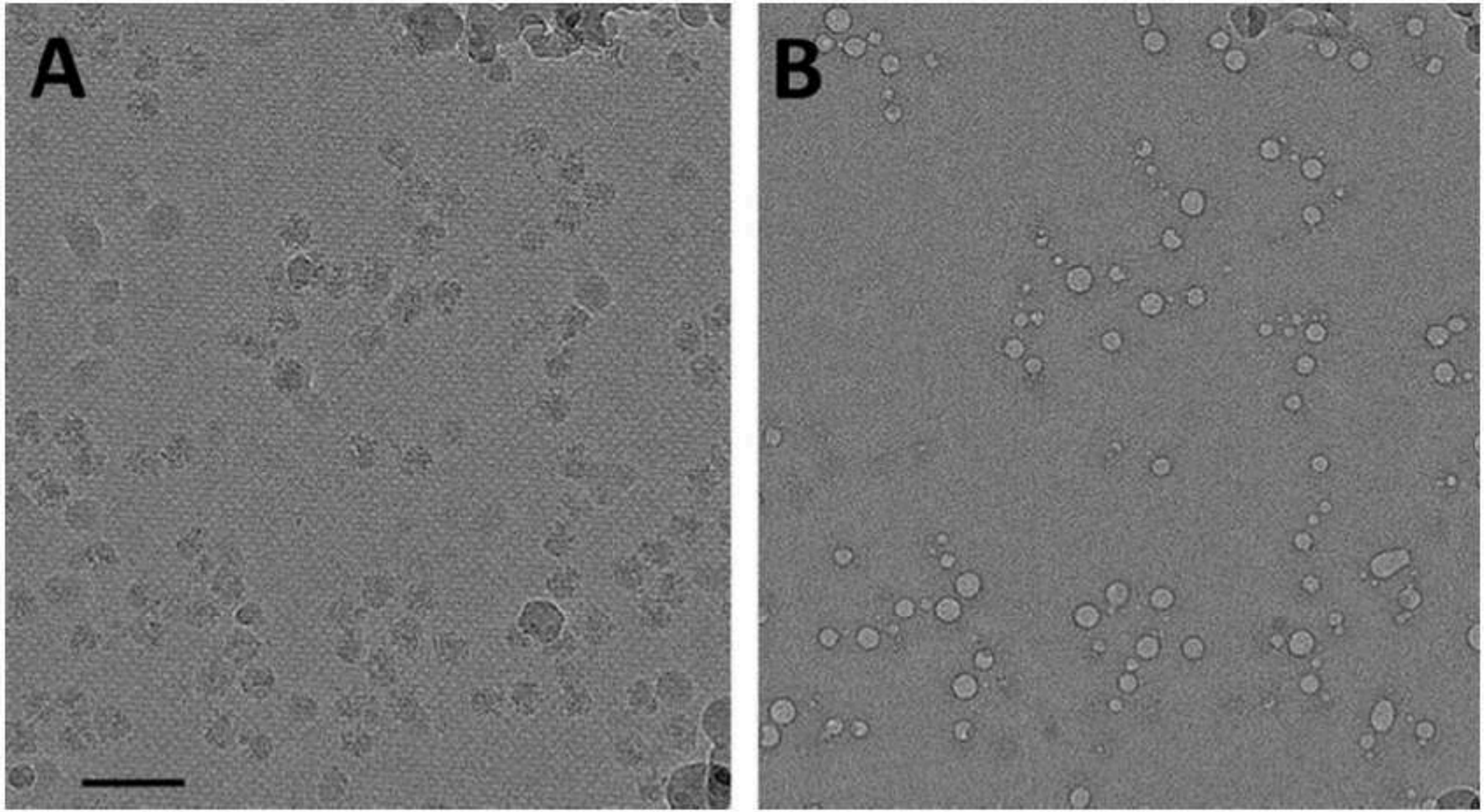
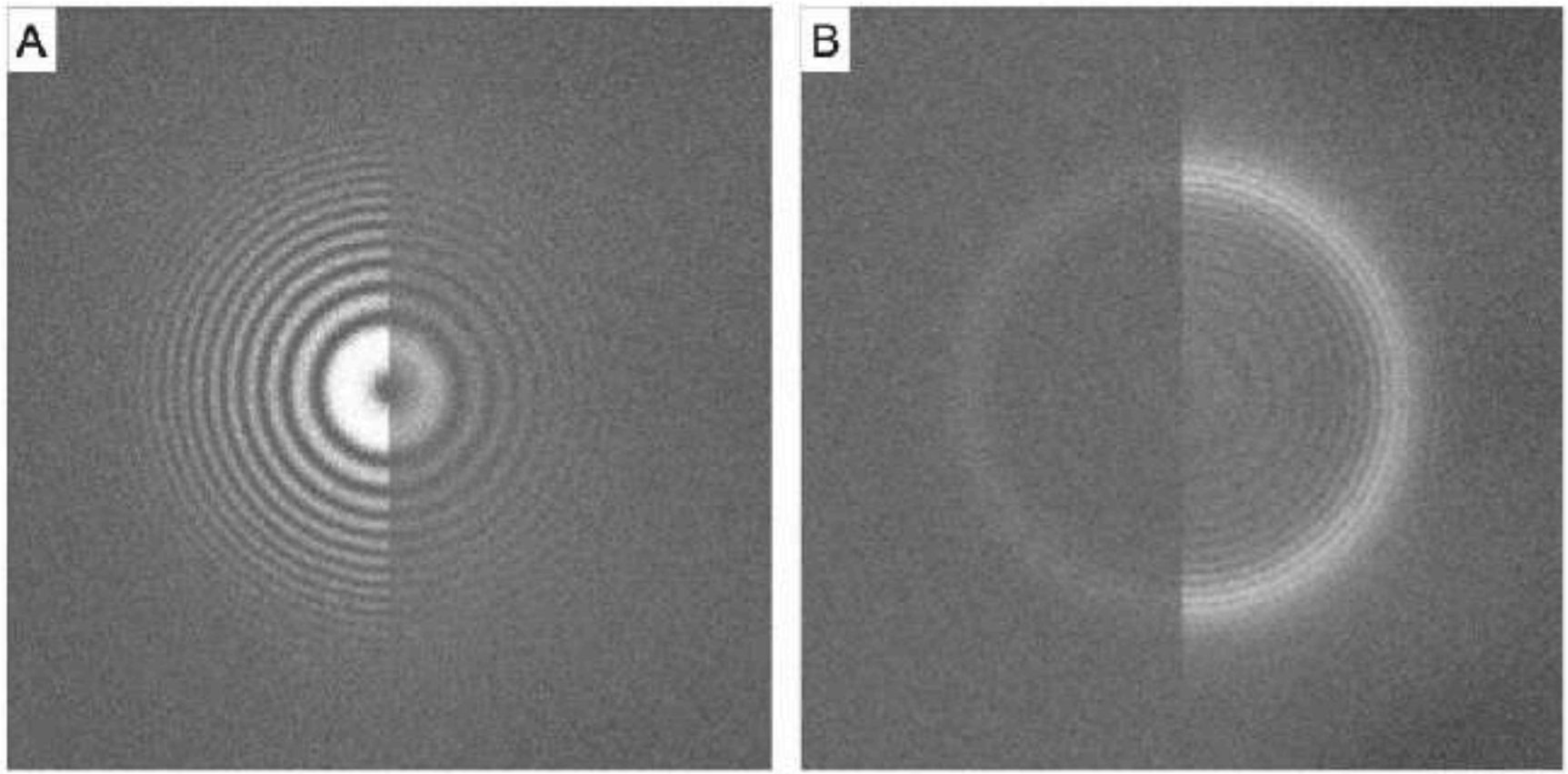
Lawrence Berkeley National Laboratory, University of California, Berkeley, CA, United States

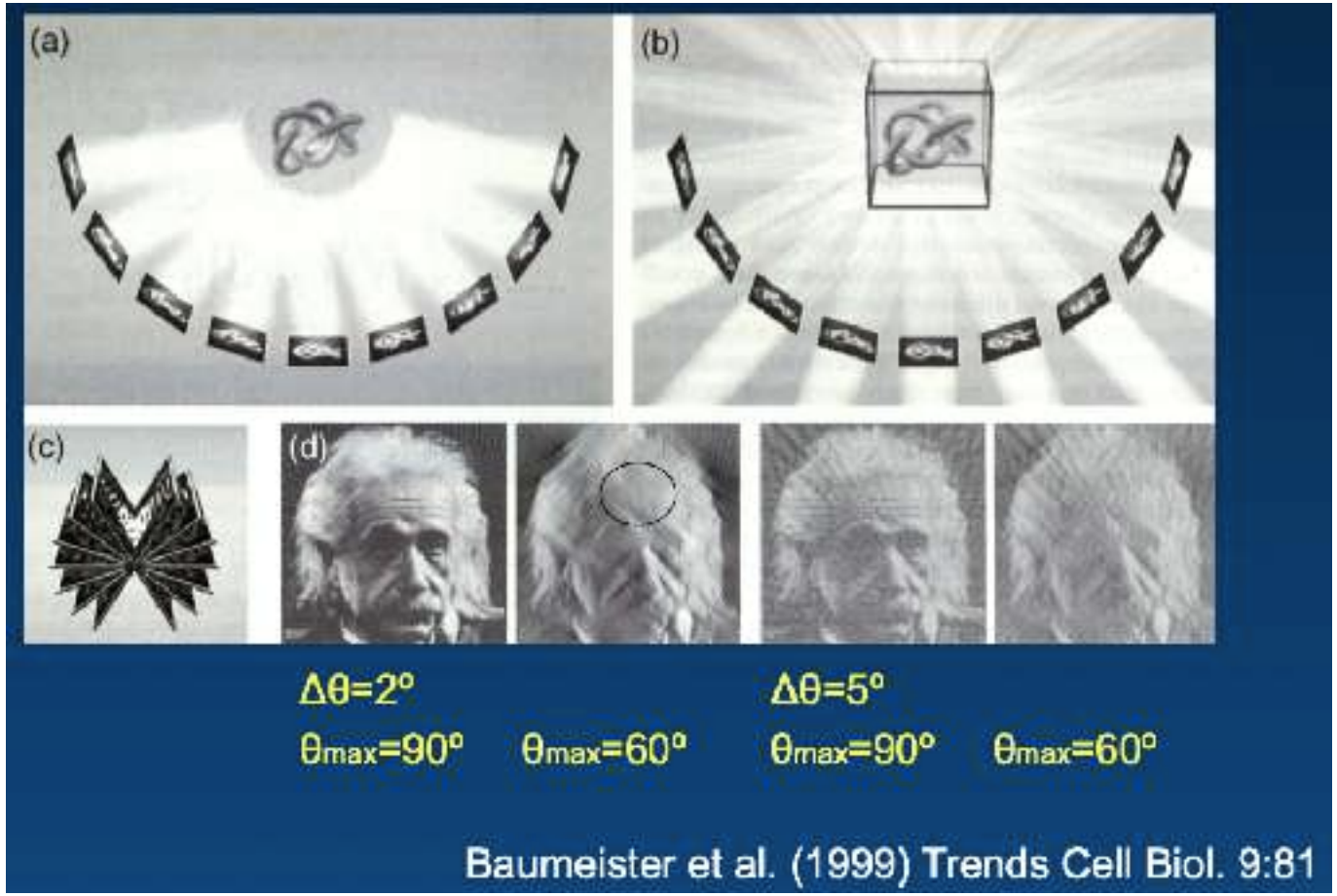
[1]Corresponding author: e-mail address: rmglaeser@lbl.gov

- The first notice- able bubbles appear after the accumulated exposure (for 300 keV electrons) is approximately 150 e/A . At this high exposure, high-resolution features would long since be destroyed, of course, but the macromolecular particles might still be visible.


A
B


A
B

Directional information loss



https://3dfsc.salk.edu