Study Meeting 6: Electron-specimen interactions

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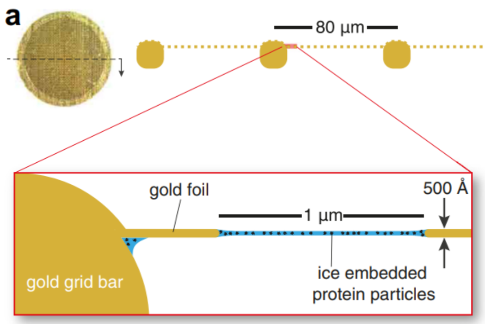
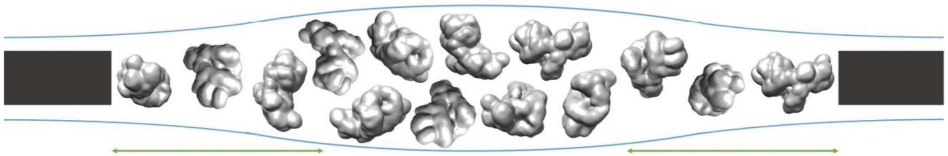
Three interrelated topics

- The specimen support
  - Gold grids
  - Nanowire grids



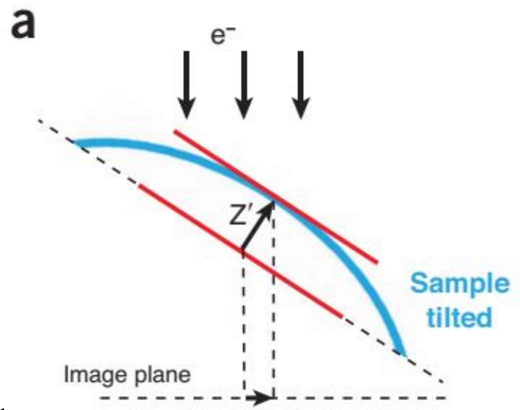
Russo & Passmore (2016)
J. Struc. Bio. 193:33-44

- The sample in ice
  - Description
  - Air-water interface



Noble *et al.* (2018)
elife, 7:e34257

- Electron-specimen interactions



Zheng *et al.* (2017)
Nat. Meth. 14(4):331

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Summary from last meeting

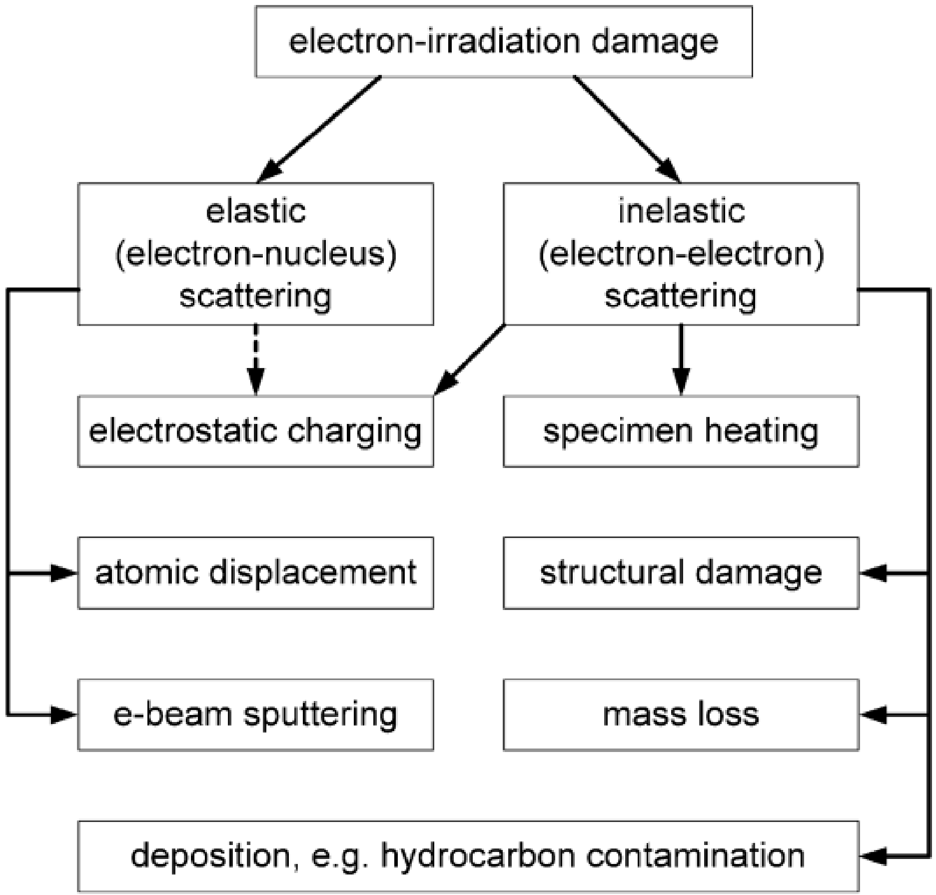
- Au-Au best grid (or nanowire grid)
- Most proteins (90%) are at the AWI
- Majority of proteins damaged by this?
  - At least FAS is (90%)
- Can we use graphene & 1-pyrCA to improve stability & get some nice structures?

Goals for today

- Electrons are ionizing radiation
- Biological samples are damaged by electrons
  - Proteasome
  - Rotavirus VP6
- Damage to the vitreous ice
- Methods to reduce damage
- Specimen charging

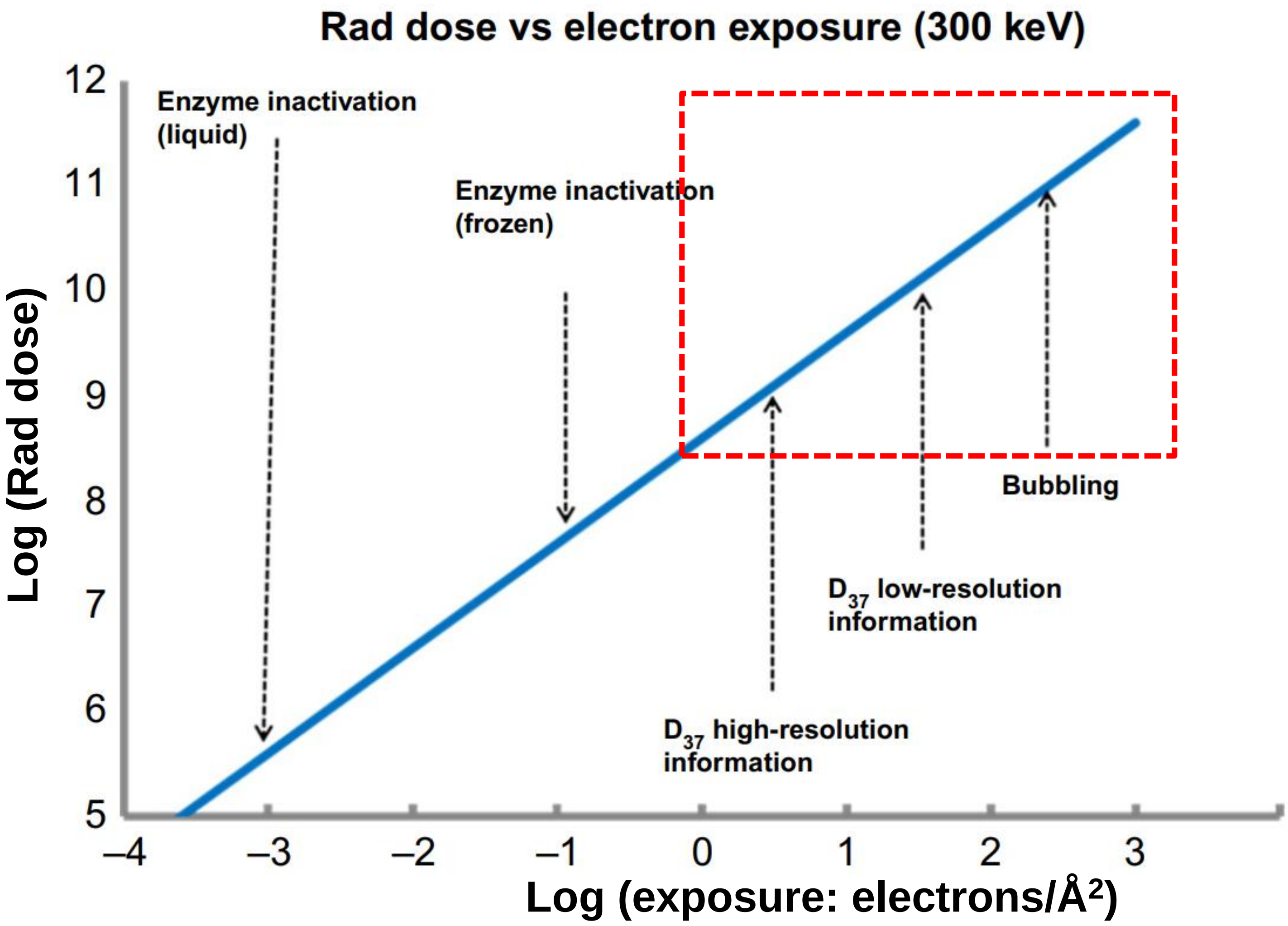
Electrons interact elastic or inelastically

- Elastic scattering
  - No change in incident electron energy*
- Inelastic scattering
  - Transfer of energy to sample



Egerton & Malac (2004)
Micron 35:399-409

Electron exposure & radiation damage



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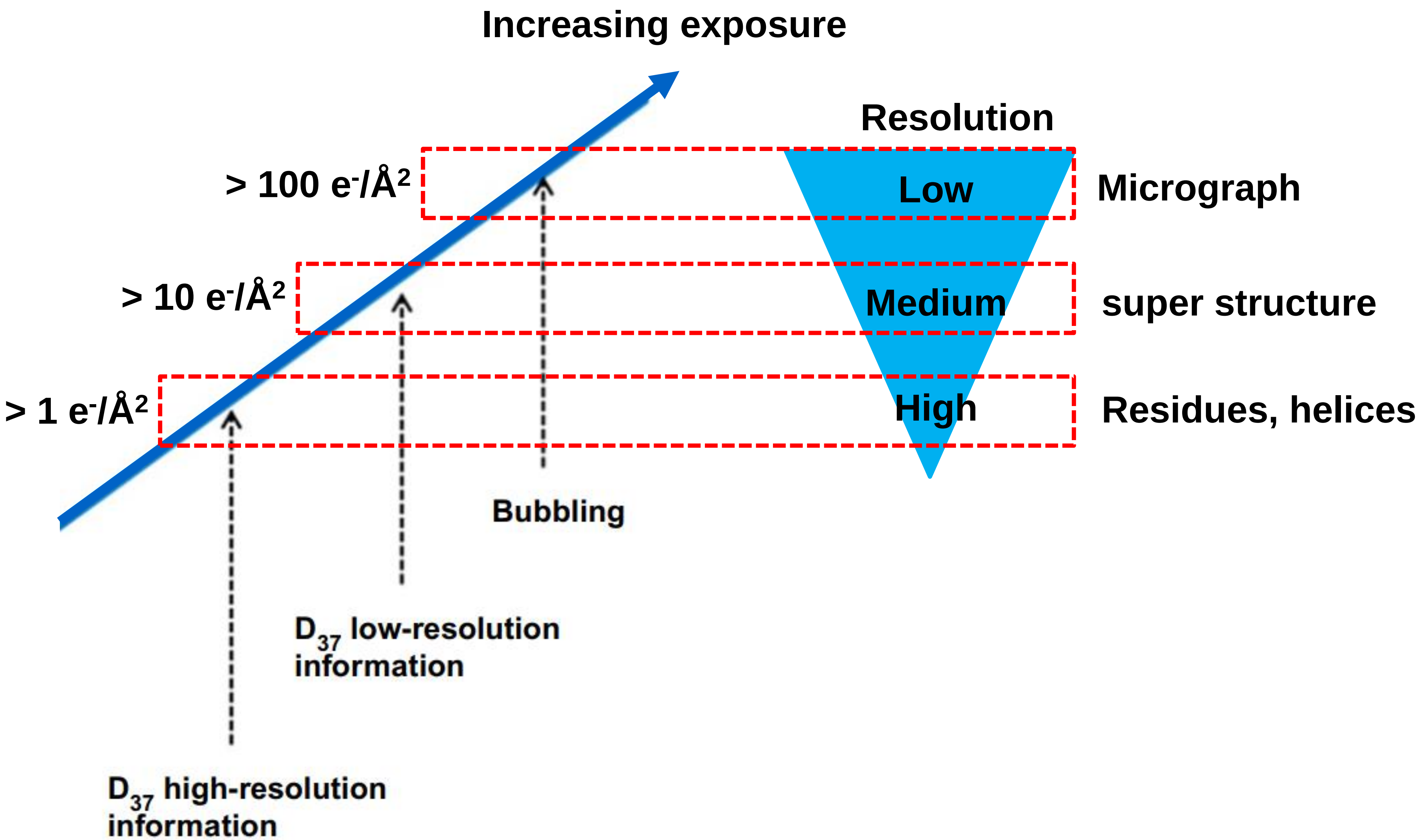
Glaeser (2016)
Meth. In Enzy. 579:19-50

When can we see radiation damage?



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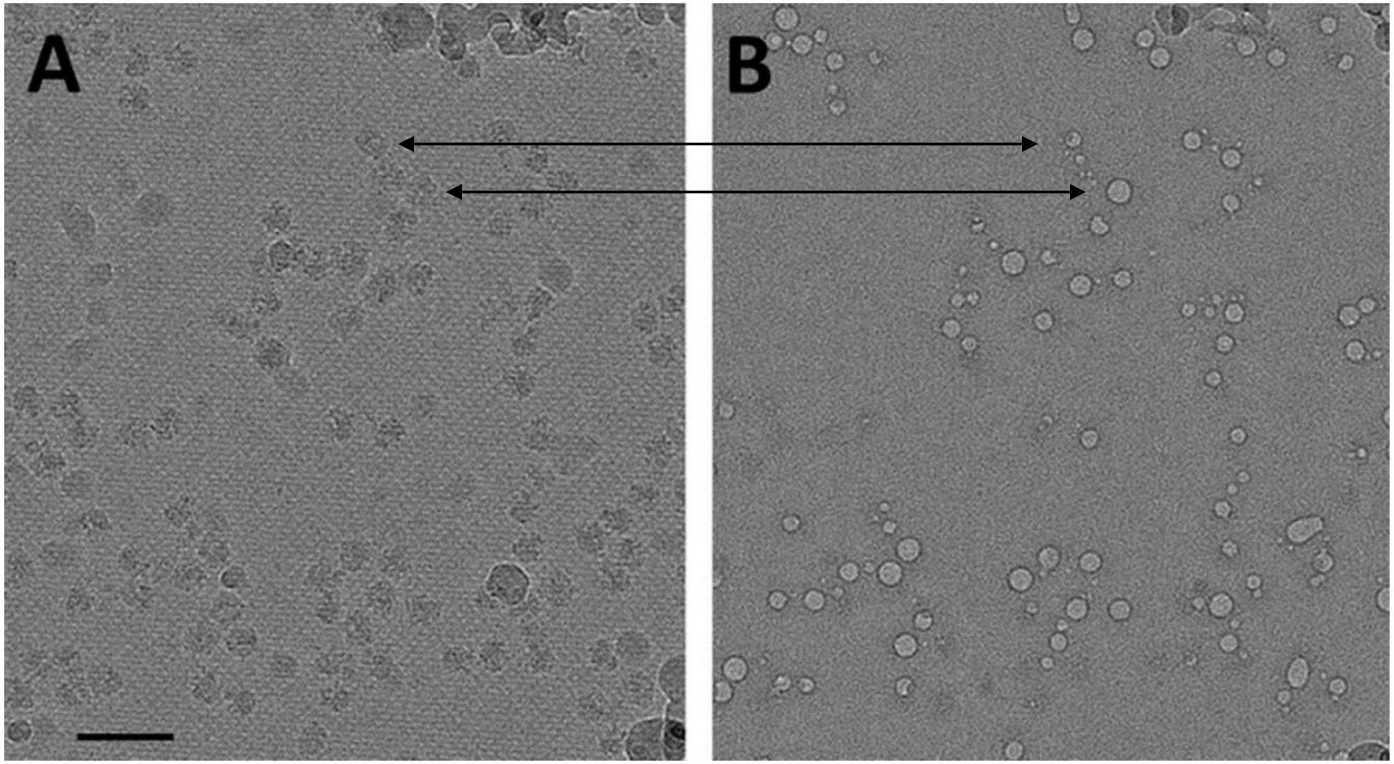
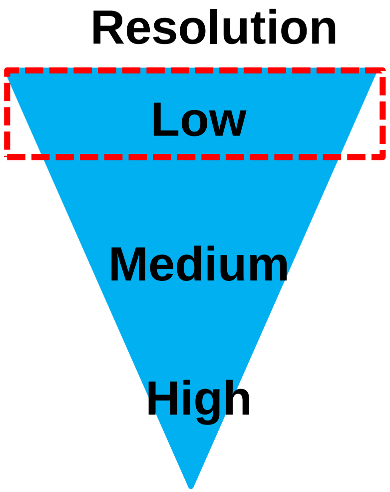
Glaeser (2016)
Meth. In Enzy. 579:19-50

Low resolution: bubble formation



$25e^{-}/Å^{2}$ ‘excess’ exposure



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Glaeser (2016)
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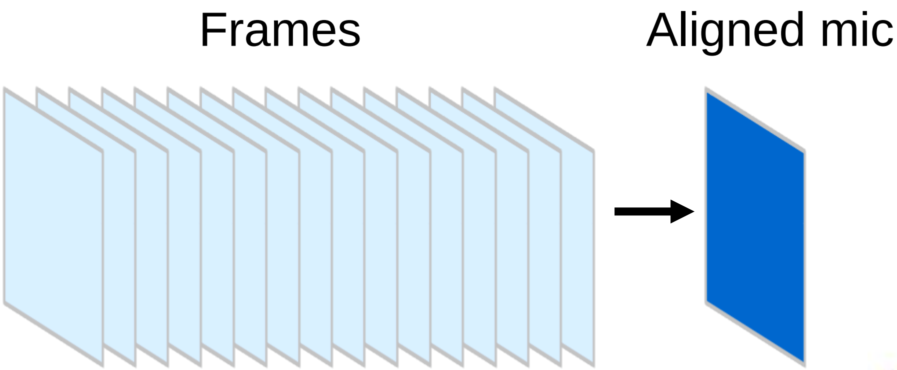
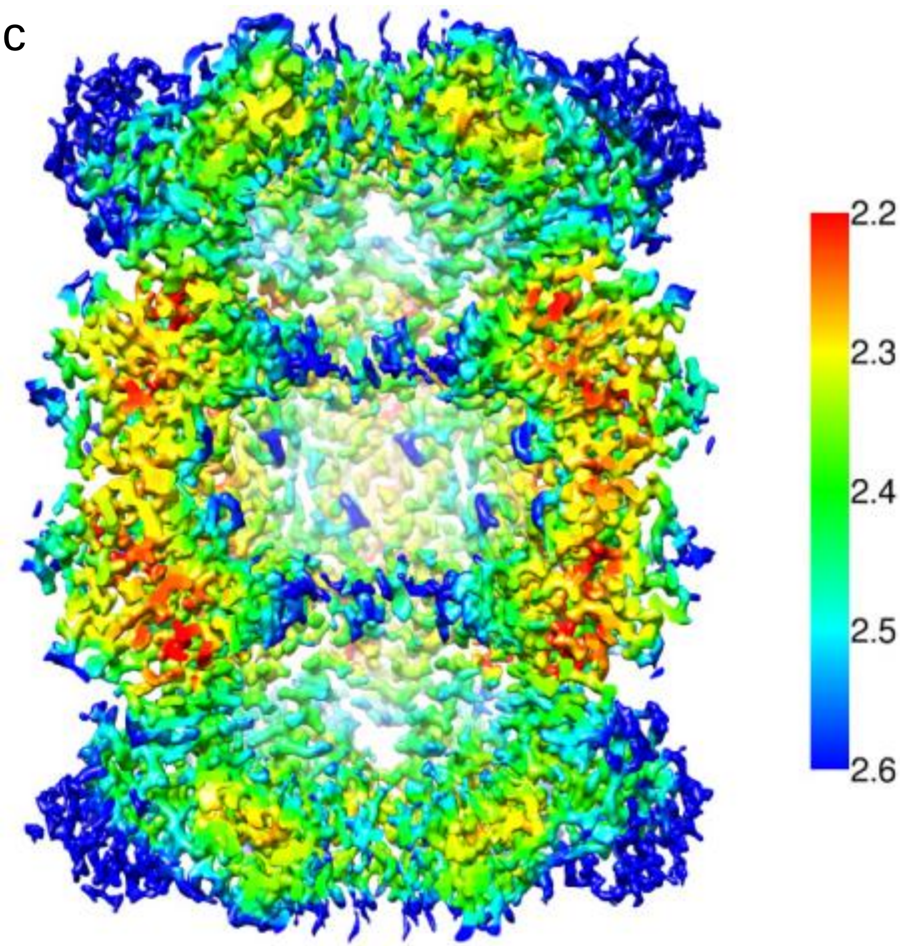
Proteasome data





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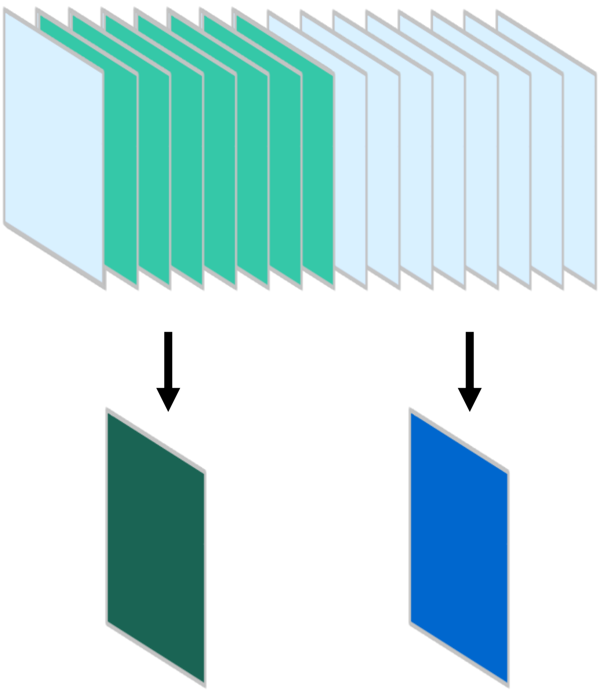
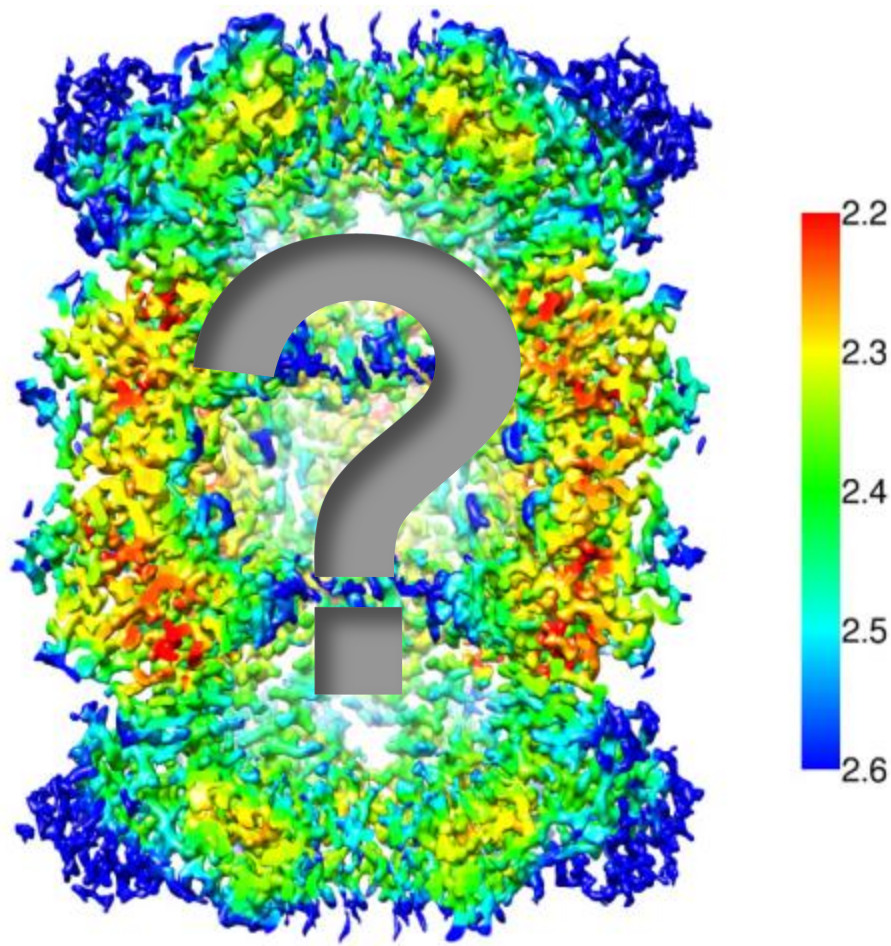
Danev *et al.* (2017)
eLife, 6:e23006

Proteasome data

Frames



T1: Frames 3-12 **T2**: Frames 11-24



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Danev *et al.* (2017)
eLife, 6:e23006

FSC

- Randomly assigned particles to either even (E) or odd (O) groups
- Compute FSC

Unpublished results

(T1)	Black	Frames: 3-12	2.56 Å
(T2)	Blue	Frames: 11-24	3.77 Å
(T12)	Green	Frames: 3-24	2.46 Å

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Wang *et al.* (2018)
Protein Sci. Unpublished

High and low threshold

T1: Frames 3-12 | **T2**: Frames 11-24

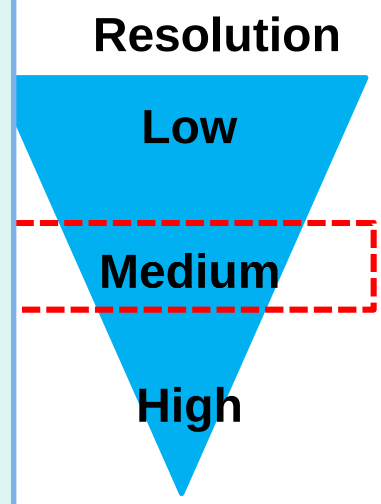
Low

Unpublished results

High

total: $16e^{-}/Å^{2}$

Total: $39e^{-}/Å^{2}$
Frame: $(22.75e\text{-}/Å^{2})$



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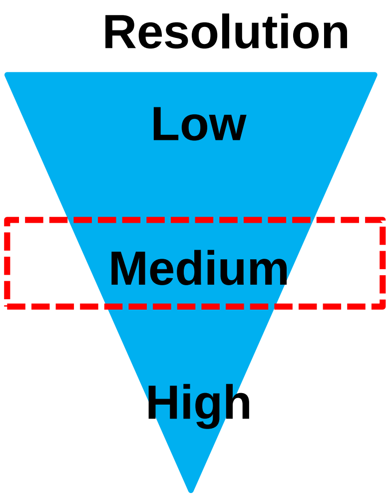
Wang *et al.* (2018)
Protein Sci. Unpublished

Electron radiation increase heterogeneity

- E.g., T2 rotates relative to T1
- Increased structural heterogeneity



Unpublished results

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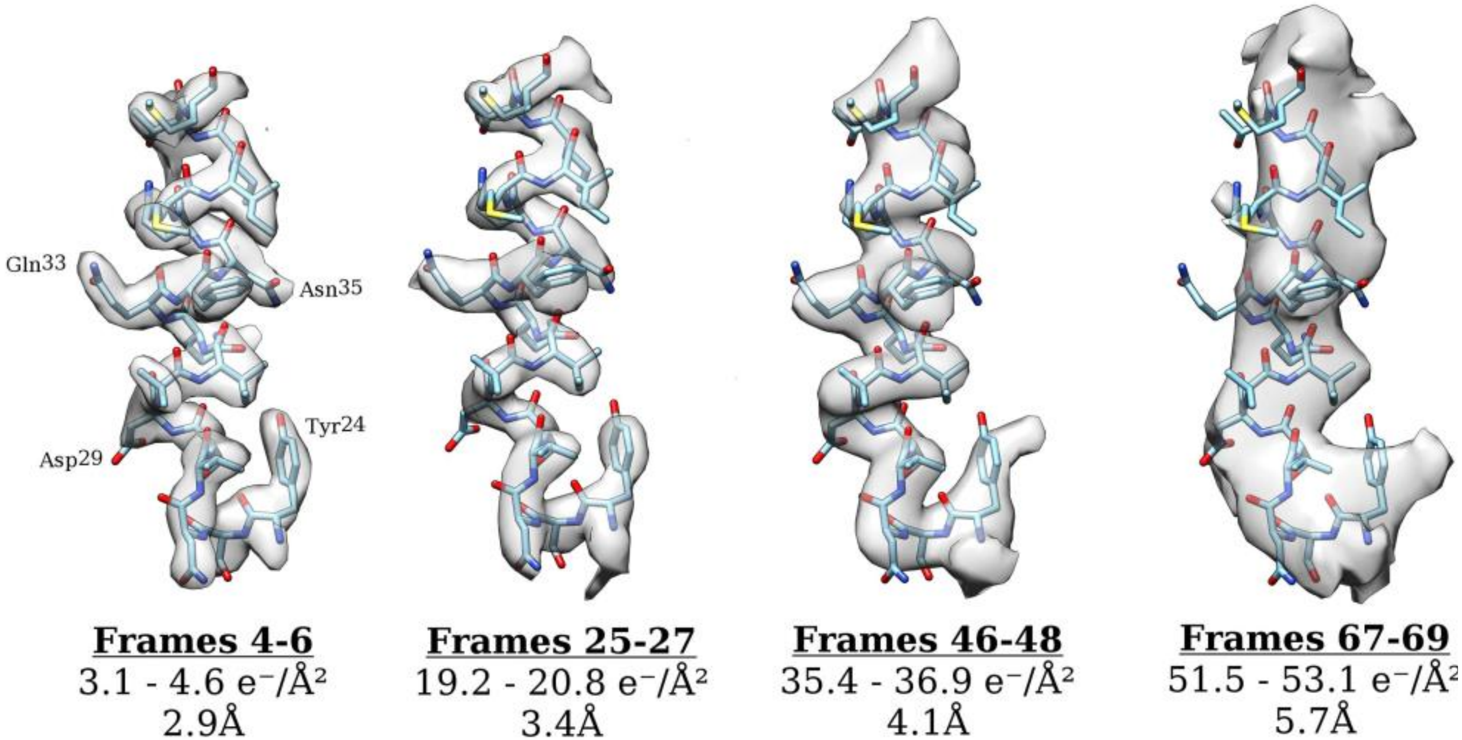
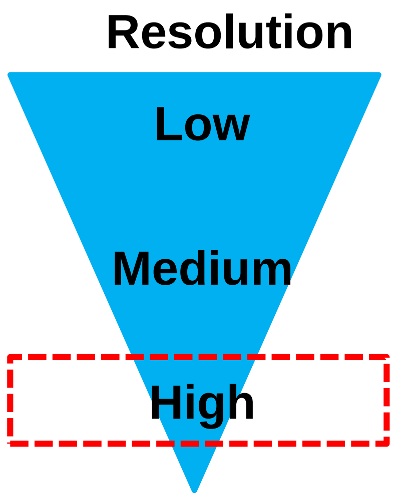
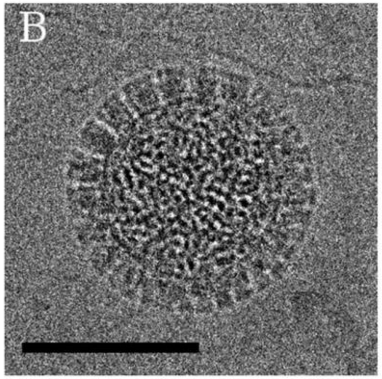
Wang *et al.* (2018)
Protein Sci. Unpublished

Other examples





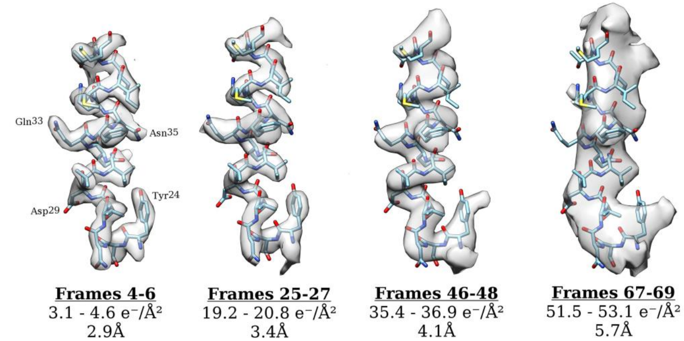


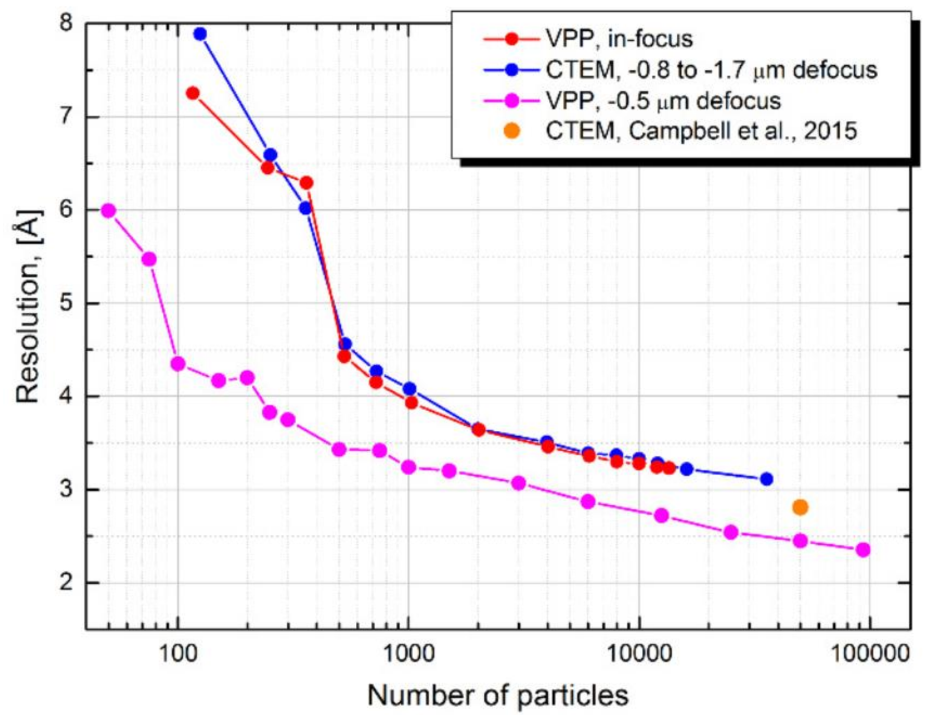
Rotavirus VP6

Grant & Grigorieff (2015)
eLife

Should we only use the first frames?

- Early frames have less damage & are less heterogeneous but number of particles (frames) is a very important factor for high resolution





Danev *et al.* (2017)
eLife, 6:e23006

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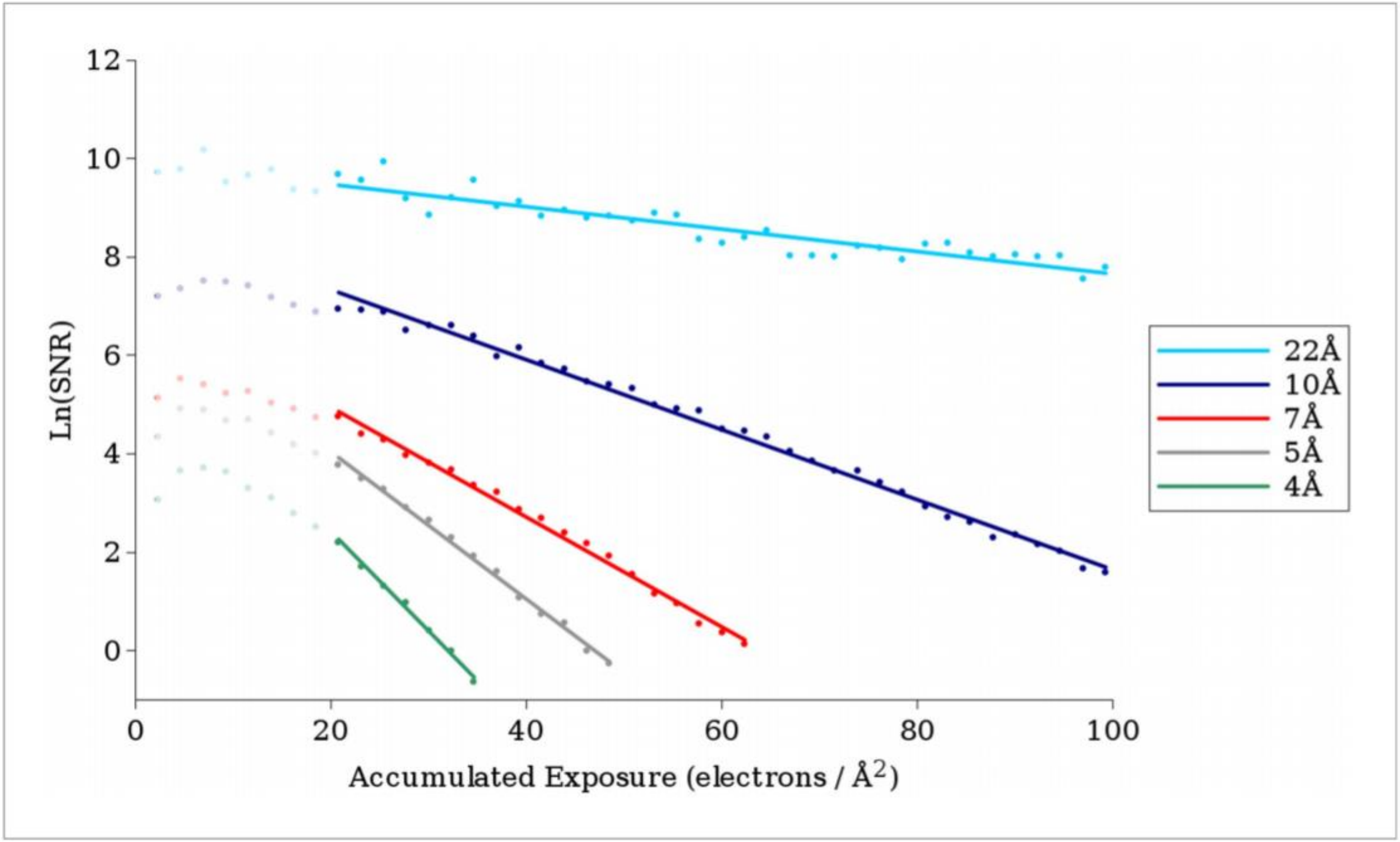
Decrease in SNR due to damage



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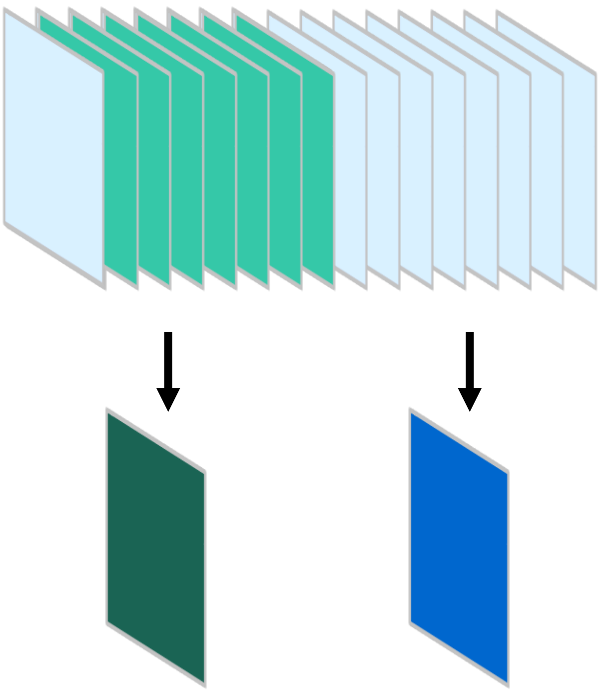
Grant & Grigorieff (2015)
eLife

Changes between early and late

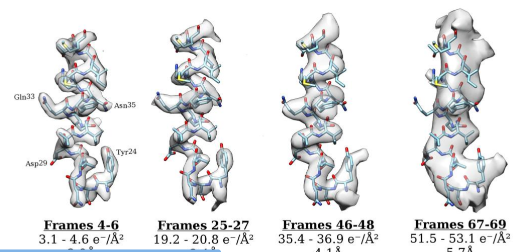
Frames



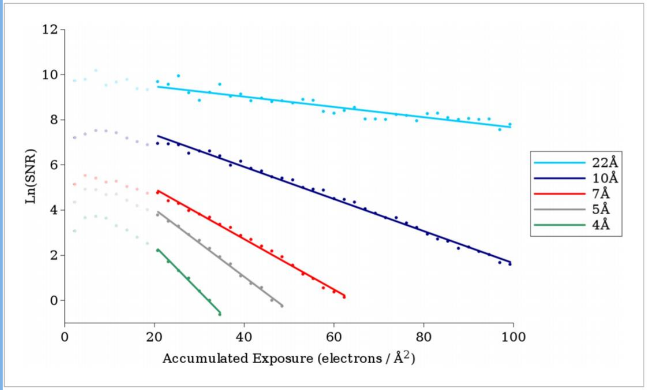
Early frames

Late frames

- Damage to residues
- Increased heterogeneity
- Lower SNR



Unpublished results



Goals for today

- Electrons are ionizing radiation
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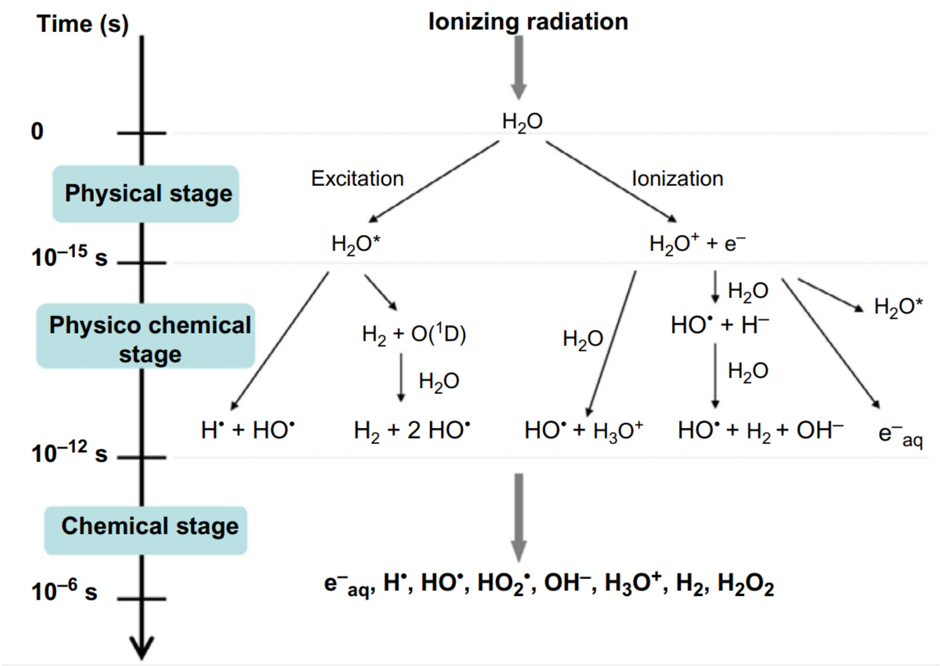
  - Proteasome
  - Rotavirus VP6
- Damage to the vitreous ice
- Methods to reduce damage
- Specimen charging

Water molecules are damaged by radiation

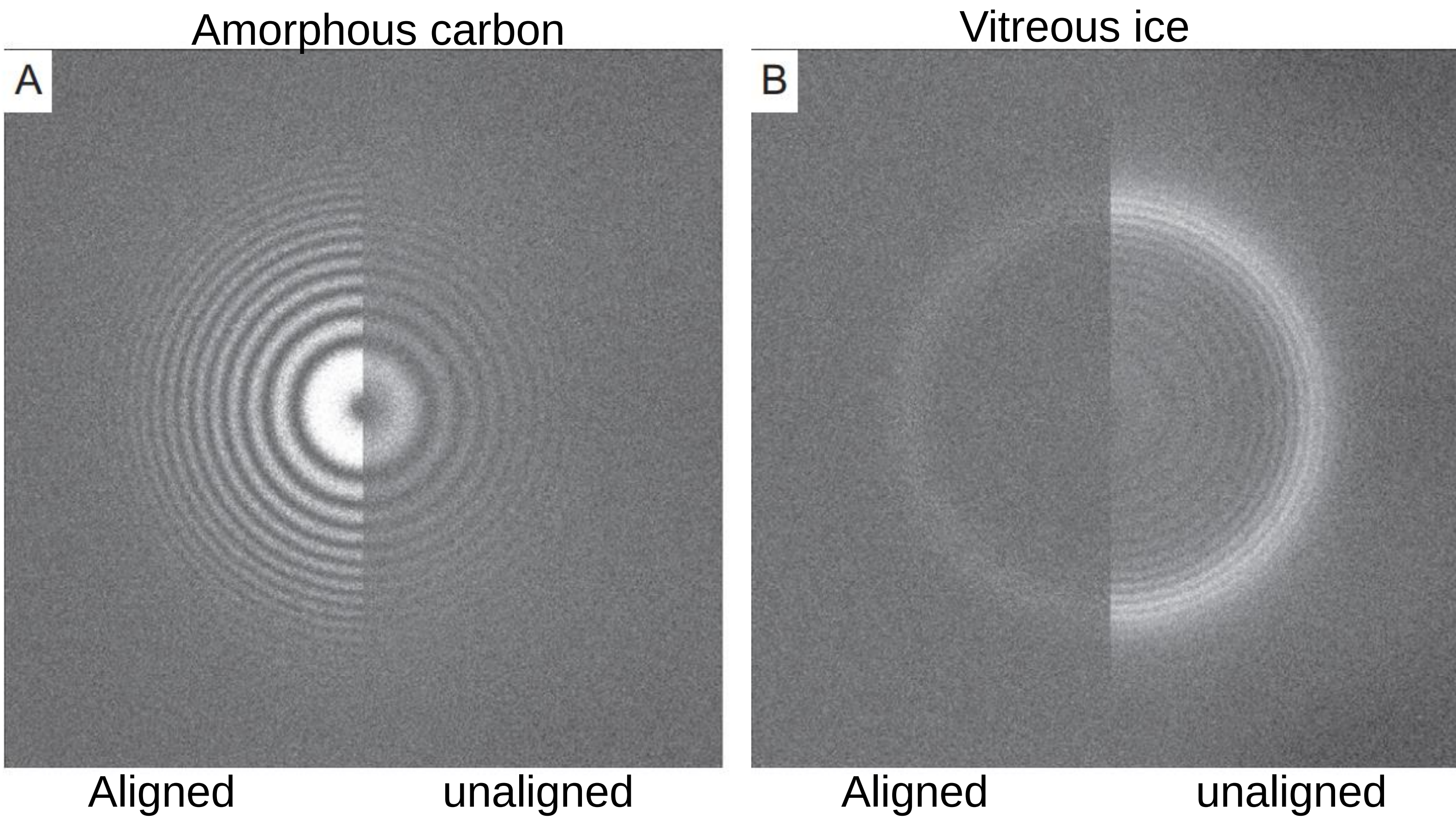


In pure water, many of the chemical intermediates shown in Fig. 3 have no alternative but to react with each other, ultimately returning nearly everything back to molecules of water. It is even believed that hydrogen radicals and hydroxyl radicals can convert hydrogen gas and hydrogen peroxide back to water (Le Caër, 2011). This very likely is why pure ice may appear to be unchanged when irradiated in the electron microscope.

Glaeser (2016)
Meth. In Enzy. 579:19-50

Vitreous ice is rearranged in the beam

- Structure factor of vitreous ice changes between each frame → **unaligned** frames have strong Thon ring for ice



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Glaeser (2016)
Meth. In Enzy. 579:19-50

Ways to overcome specimen damage:

- Small probe size
- Cooling the specimen
- Increase kV
- Dose-weighting
- Gold grids?

Ways to reduce specimen damage

- *Small probe size*
- Cooling the specimen
- Increase kV
- Dose-weighting
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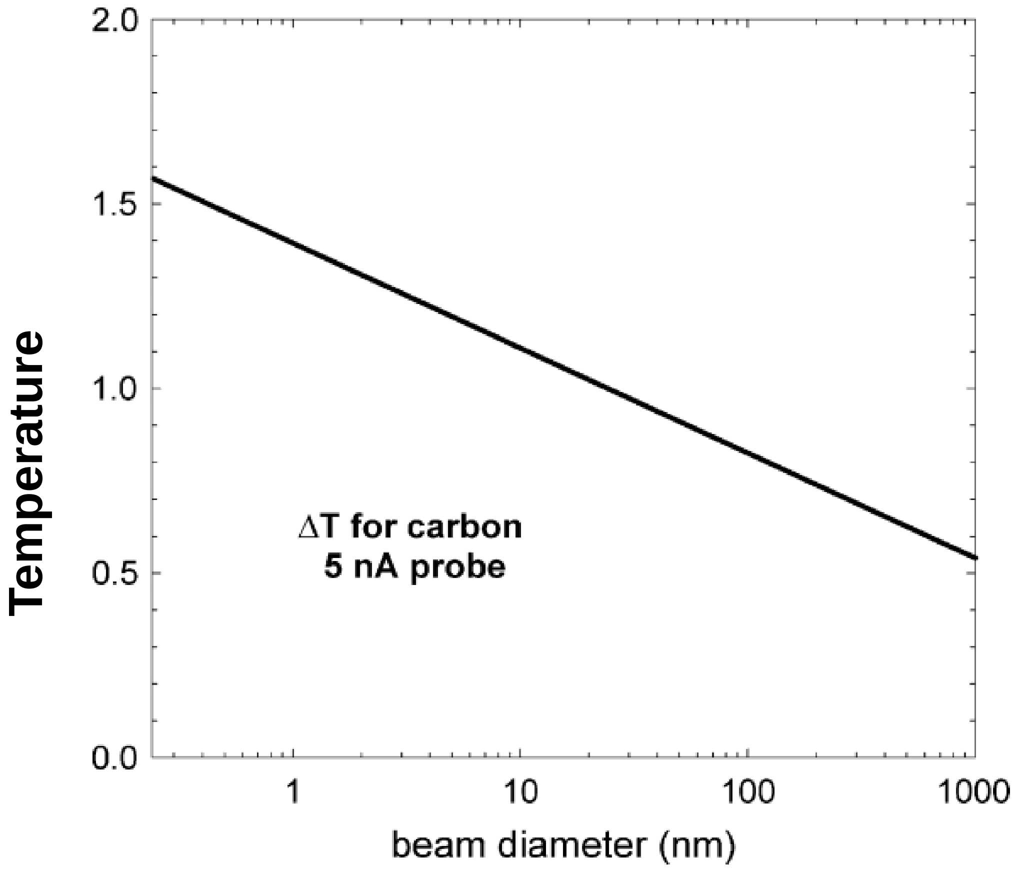

Egerton & Malac (2004)
Micron 35:399-409

Ways to reduce specimen damage

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Table 3
Characteristic dose for the removal of specified elements from organic specimens irradiated with 80 keV electrons, determined by EELS (Egerton et al., 1987)

Material	Element	D_e (C/cm^2) at 300 K	D_e (C/cm^2) at 100 K
Collodion	N	0.002	0.25
	O	0.006	0.5
	C	0.06	0.3
Formvar	O	0.03	~1
PMMA	O	0.06	0.5
	C	0.5	0.8
Polycarbonate	O	0.5	>5
$Cl_{16}Cu$ Pc	Cl	~3	~10

Egerton & Malac (2004)
Micron 35:399-409

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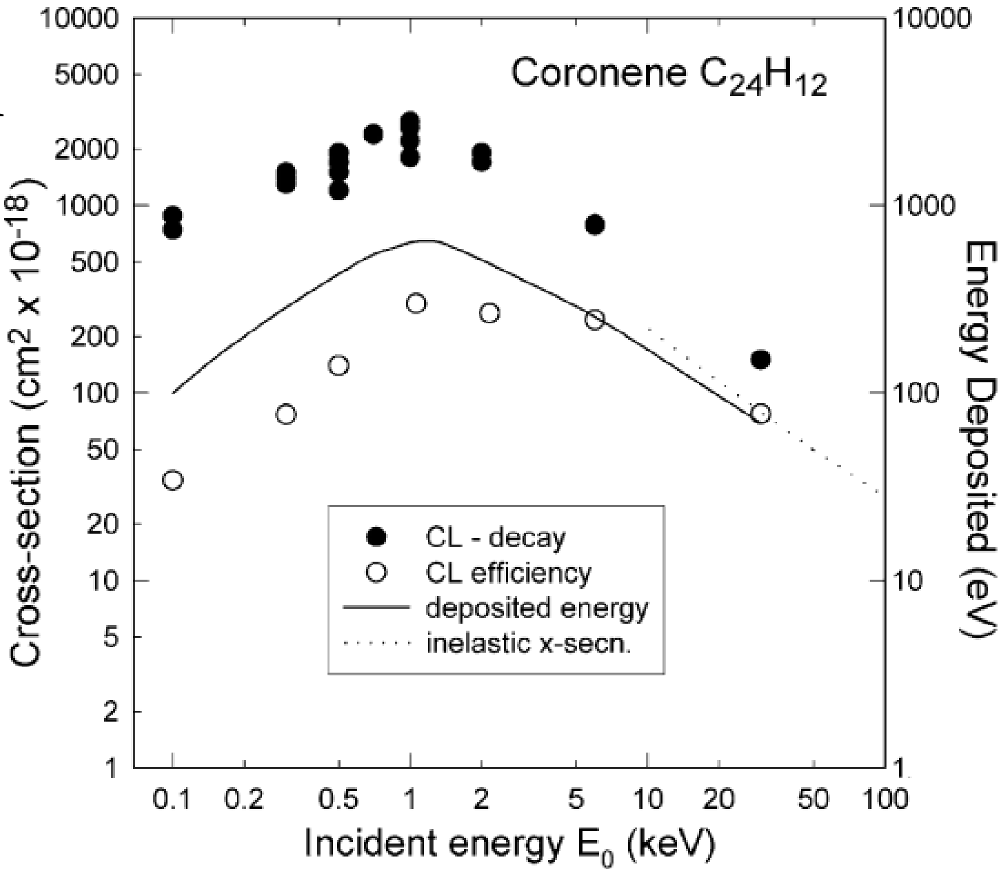

Egerton & Malac (2004)
Micron 35:399-409

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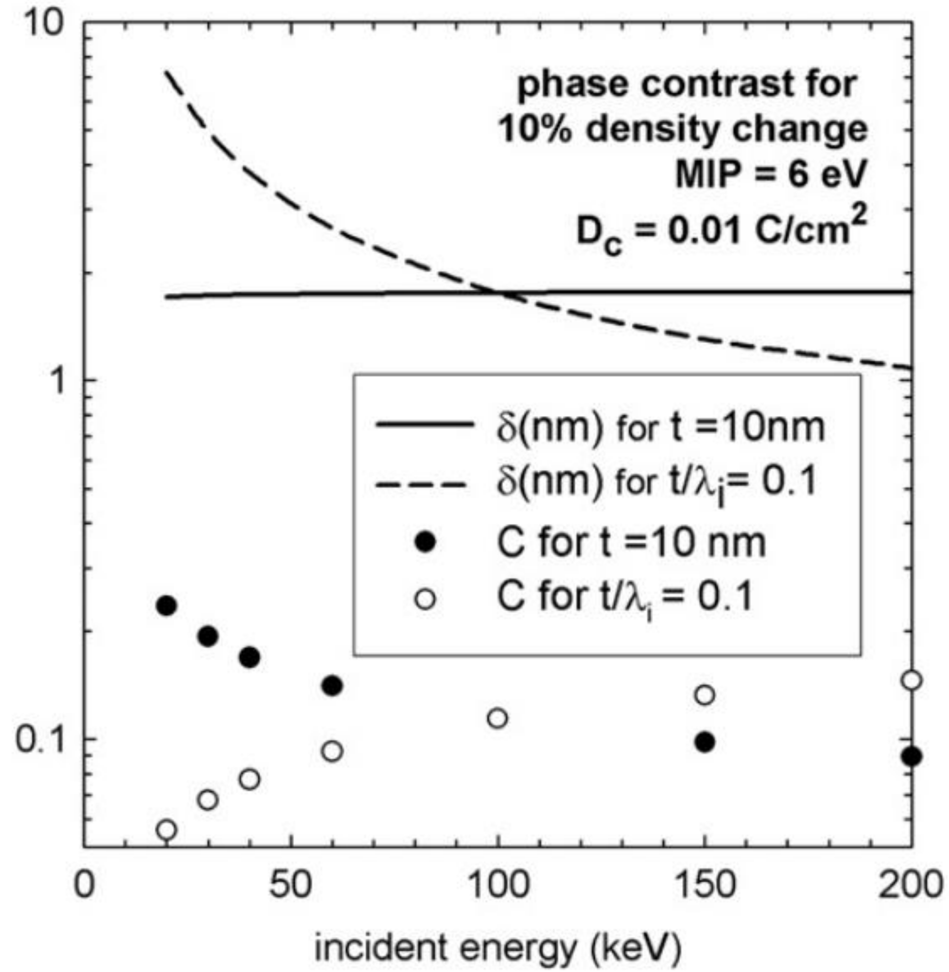


Egerton (2013)
Ultramicroscopy 127:100-108

Everything is a trade off: lower contrast

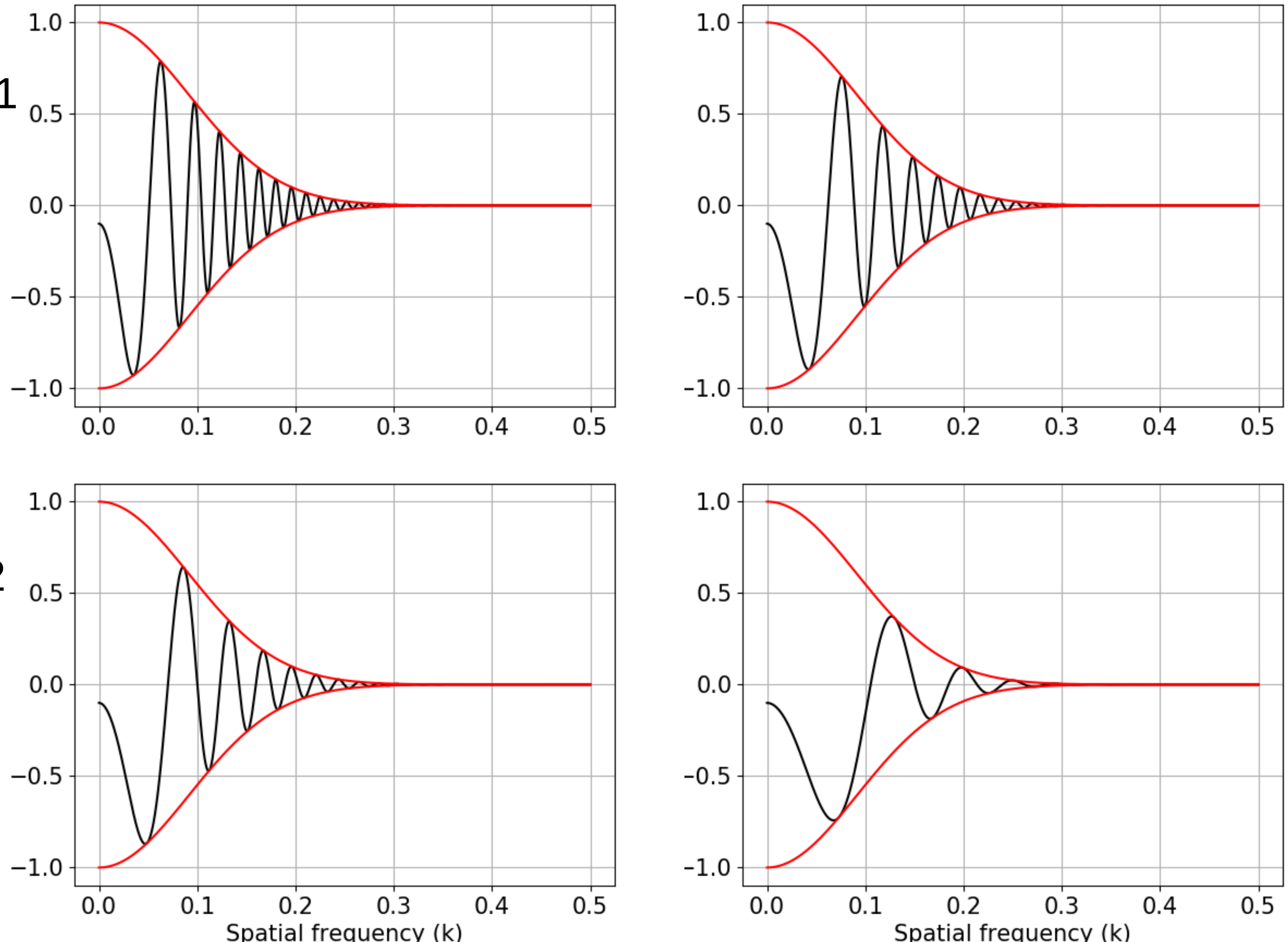
$$H(k) = 1[\sin\gamma(k) - W\cos\gamma(k)] \qquad \gamma(k) = 2\pi(-0.5\Delta z\lambda k^2 + 0.25Cs\lambda^3k^4)$$

100 kV
Area= 681

200 kV
Area=670

300 kV
Area=662

1000 kV
Area=650



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Ways to reduce specimen damage

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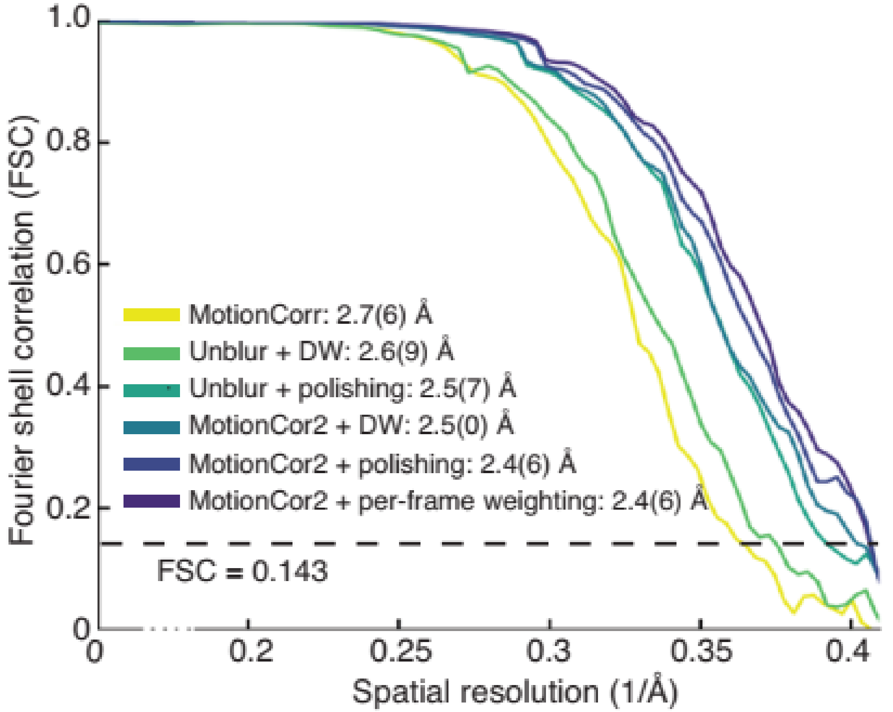

Zheng *et al.* (2017)
Nat. Meth. 14(4):331

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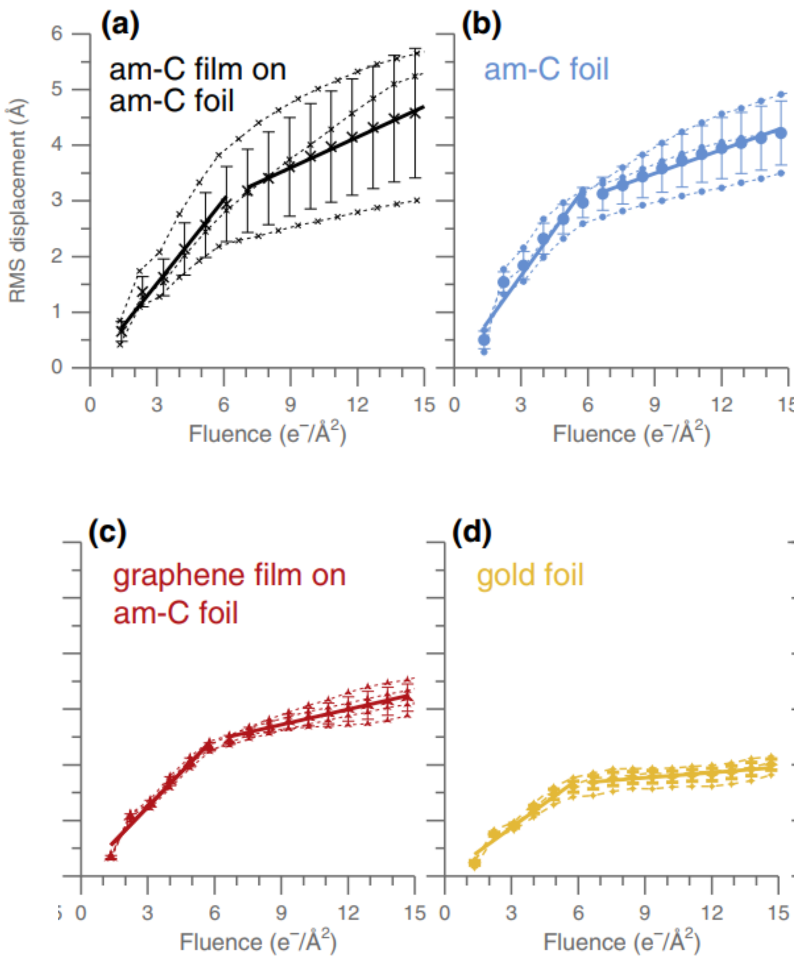
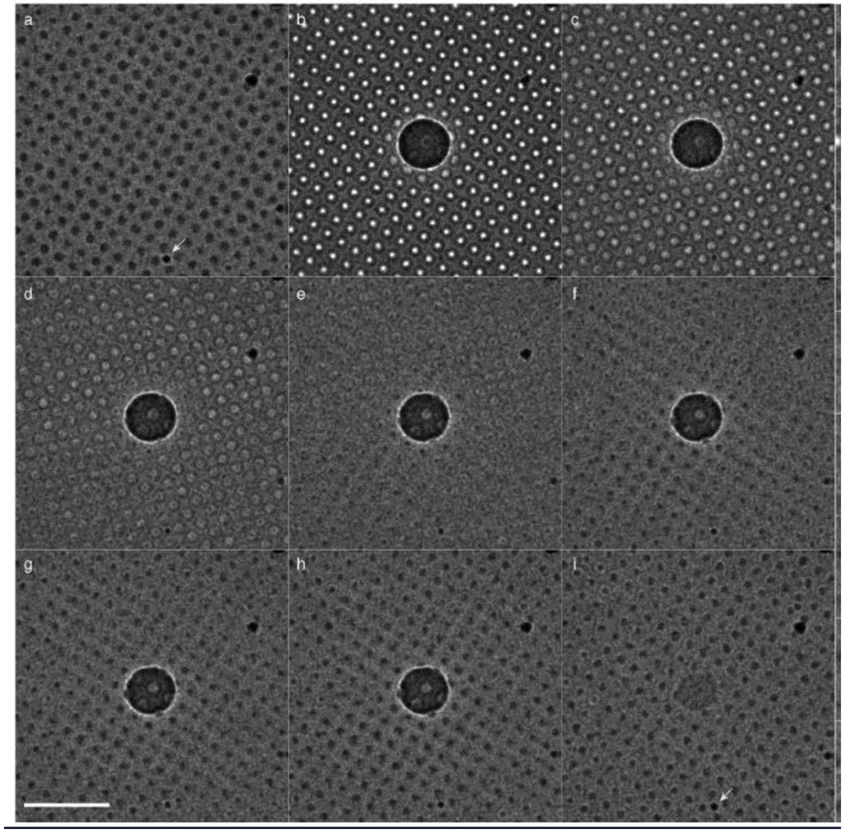


Russo & Passmore, (2016)
Curr. Opin. Struc. Bio. 37:81-89

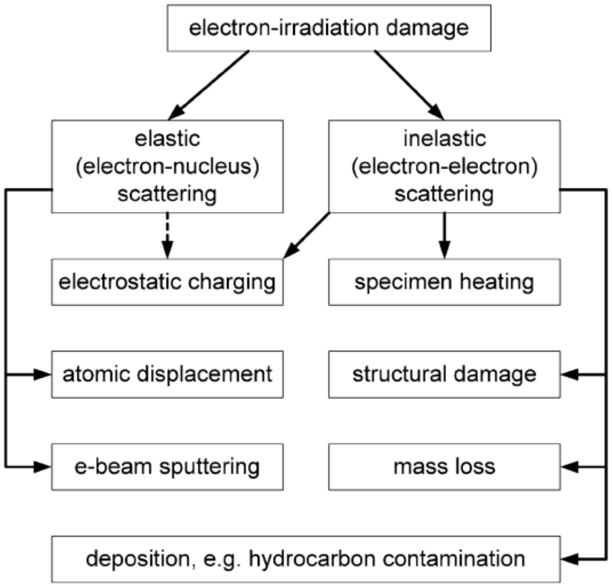
Specimen charging

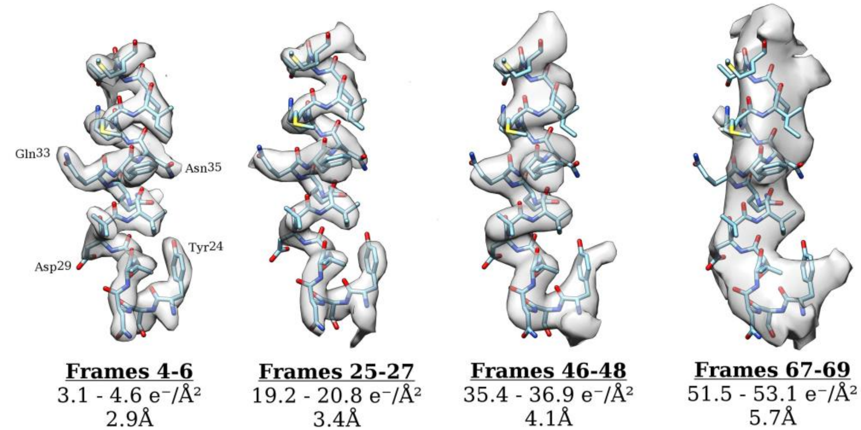
- Illumination results in charging
- Charge changes the path of incident electrons.



Russo & Henderson (2018)
Ultramic. 187:43-49

Summary





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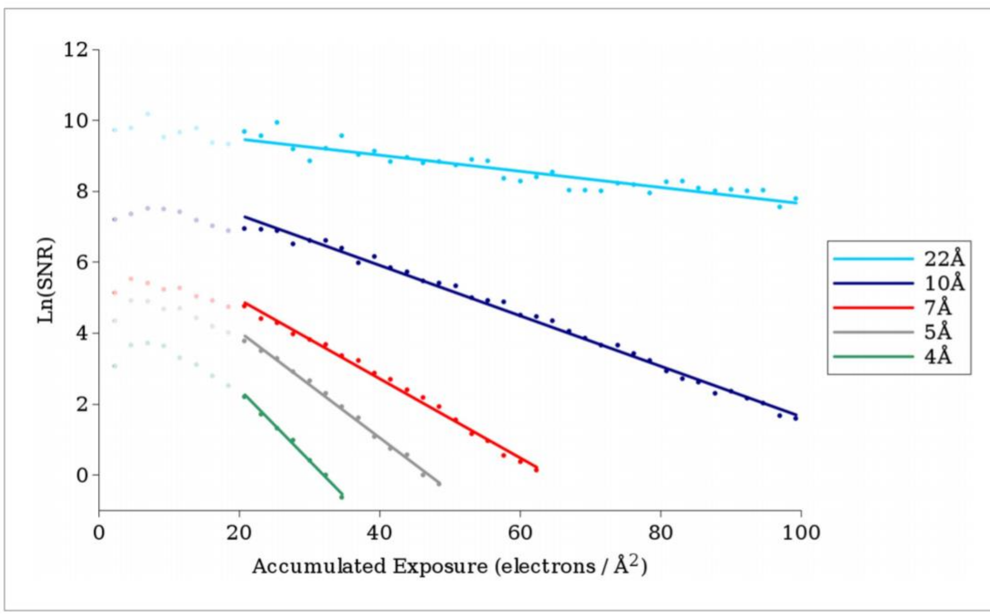

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