Cryo-electron tomography

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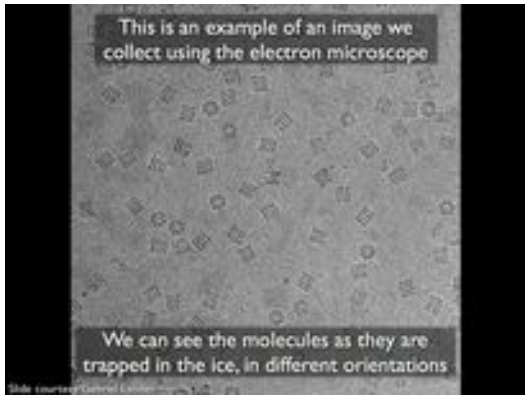
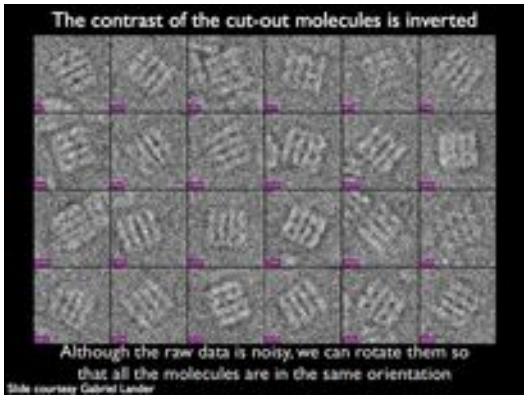
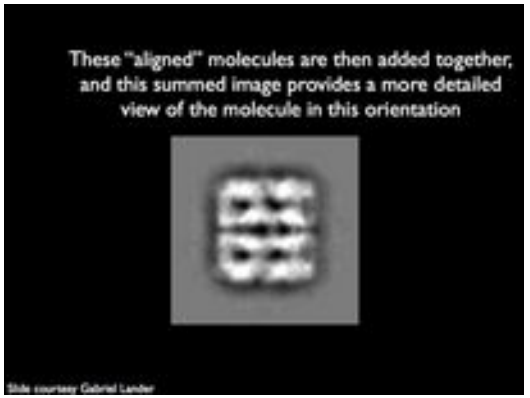
Leicester cryo-EM workshop

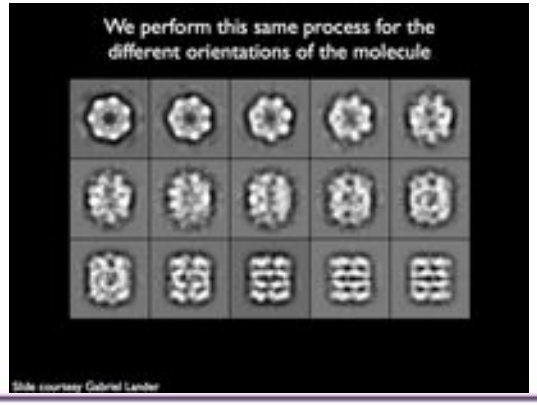
16 March 2021

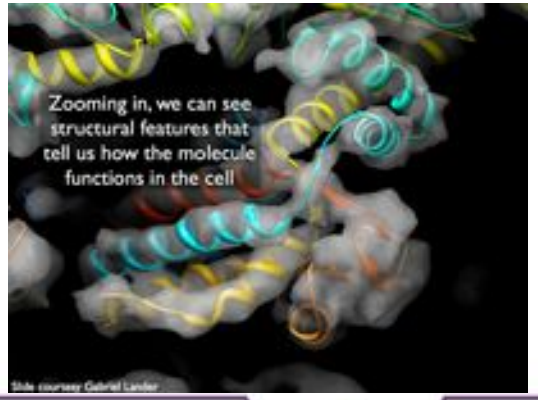
3D cryo-EM structure







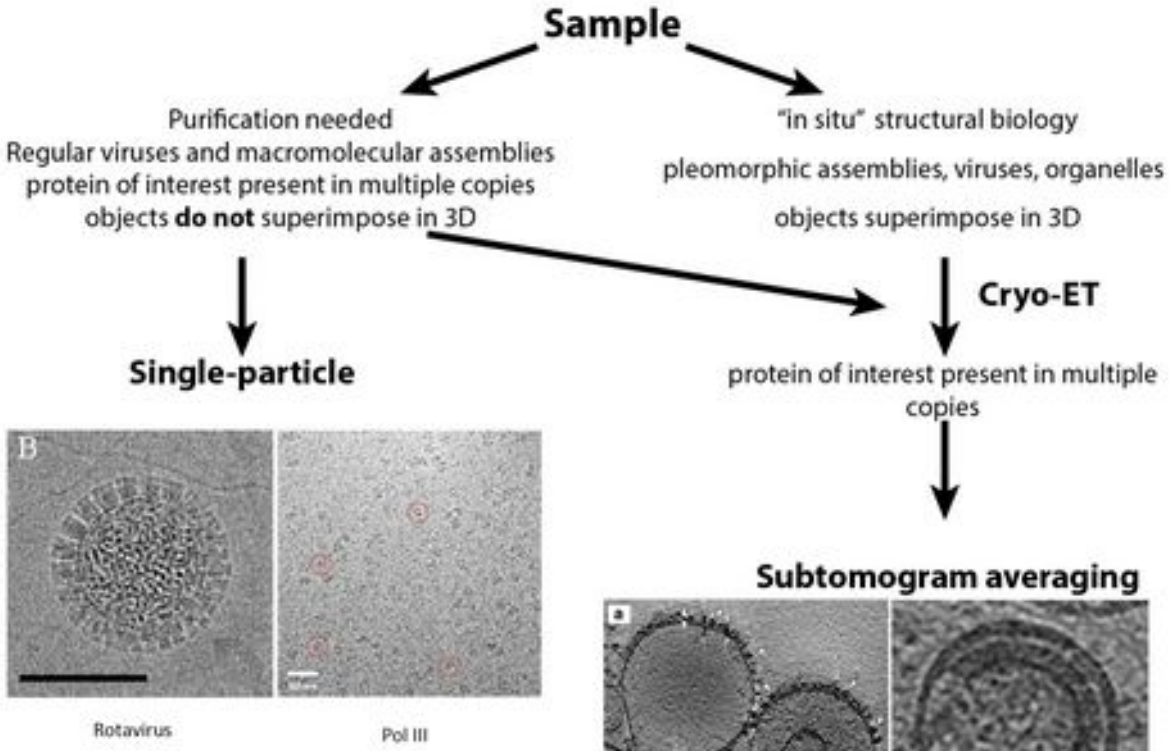




Electron tomography

- Allows 3D images of complex samples to be obtained
- No need for averaging procedures so can image a unique sample in 3D
- Helps to bridge the gap between light microscopy and protein structure determination methods

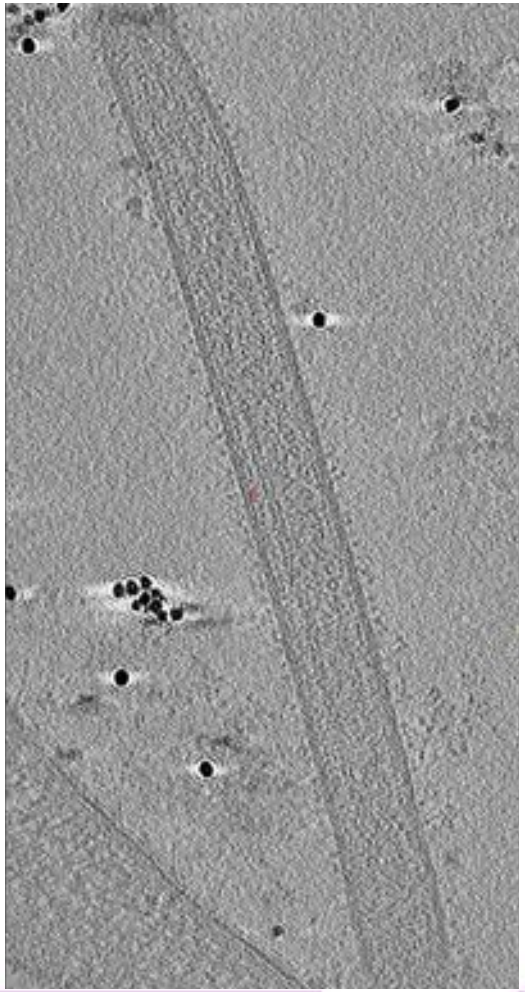
When to use tomography?



Grant T & Grigorieff N, 2015, elife
Hoffmann N et al, 2015, Nature
Pfeffer S et al, 2015, Nat.Comm.
Briggs JAG & Kraeusslich HG, 2011, J. Mol. Biol

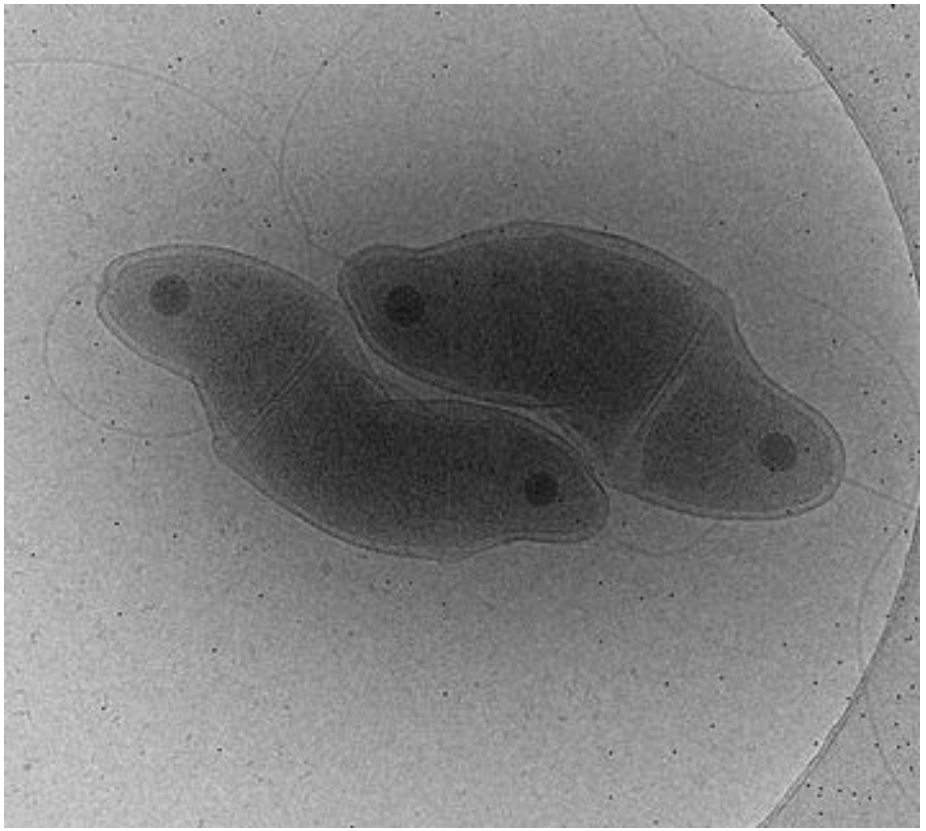
Cryo-electron tomography

- Thin samples (< 300 nm)
  - Edges of cells
  - Micro-organisms
  - Sections of cells/tissue



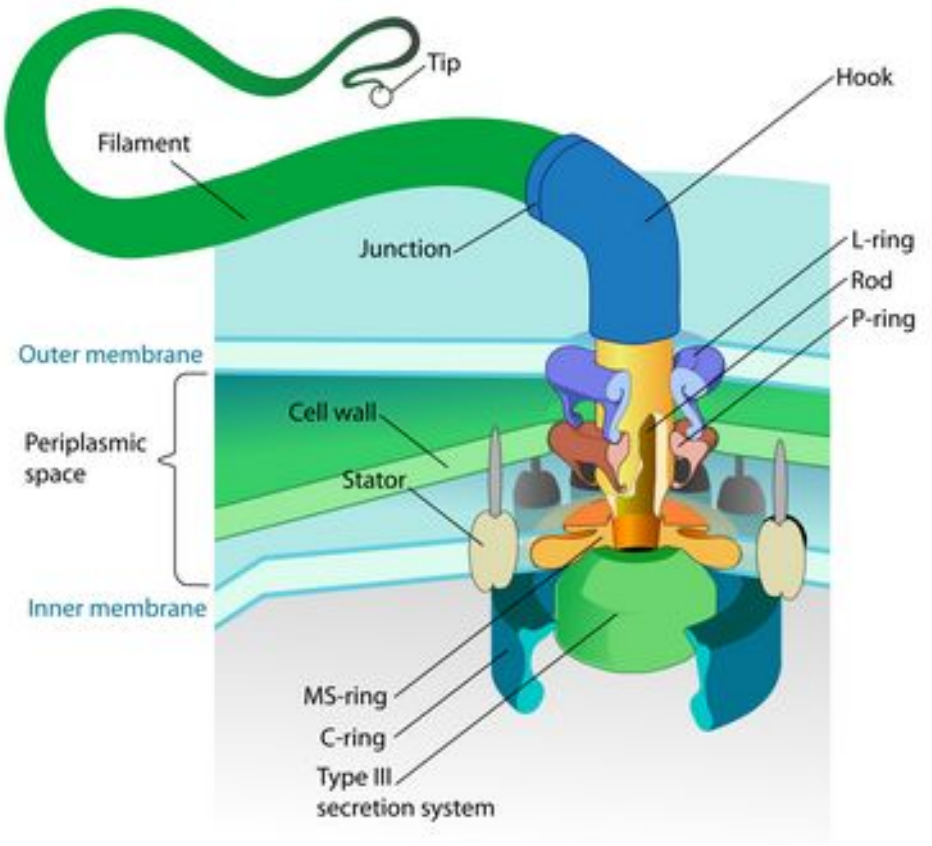
Campylobacter jejuni

- Causes food poisoning, possibly paralysis if untreated
- Spiral shape with flagellum at each end



Bacterial flagella

- Long filamentous assembly of protein
- Attached by a motor complex
- Motor rotates to move flagella
- Different species have different structure



Principle of tomography

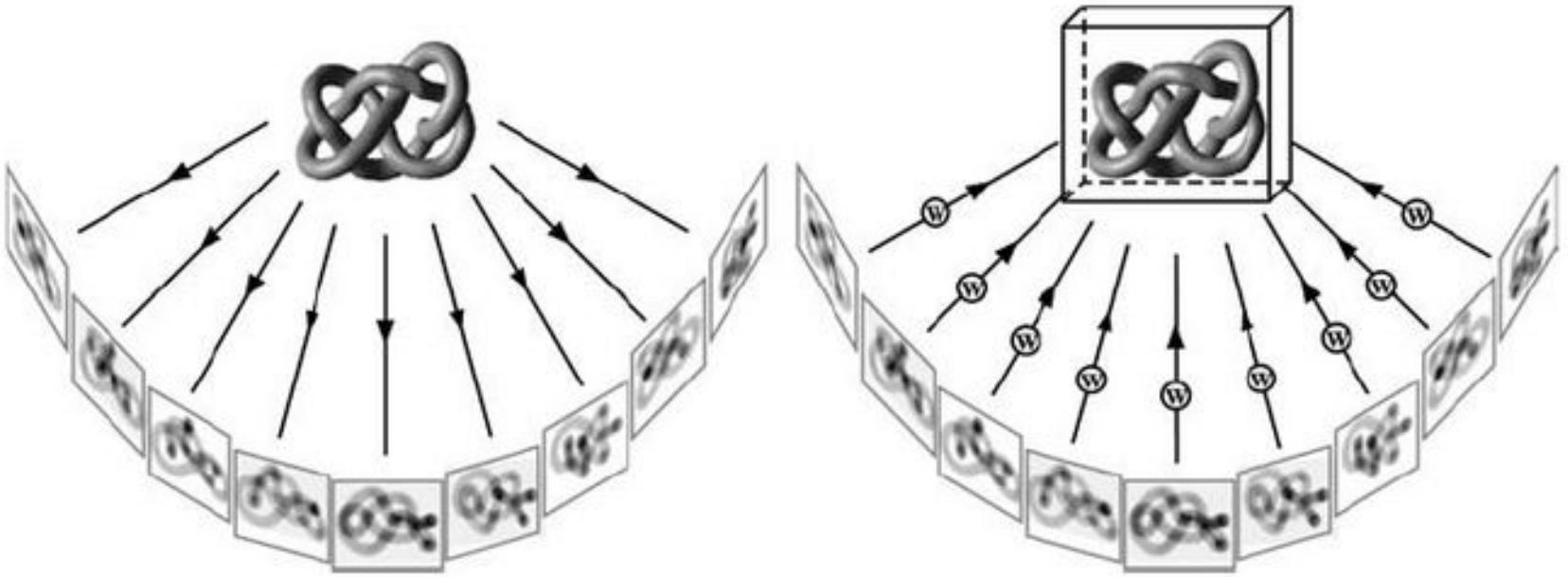
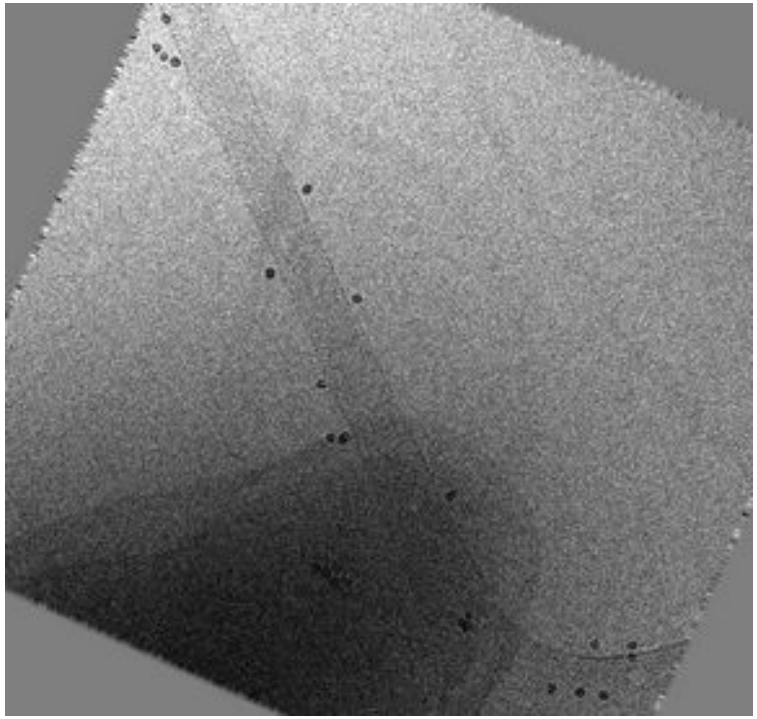


Figure 2 Principle of tomography. (*left*) Projections of the specimen were recorded from different directions by tilting the specimen holder. (*right*) The three-dimensional reconstruction of the sample is obtained most commonly by backprojection into a common three-dimensional reconstruction body.

Tilt series



Principle of tomography

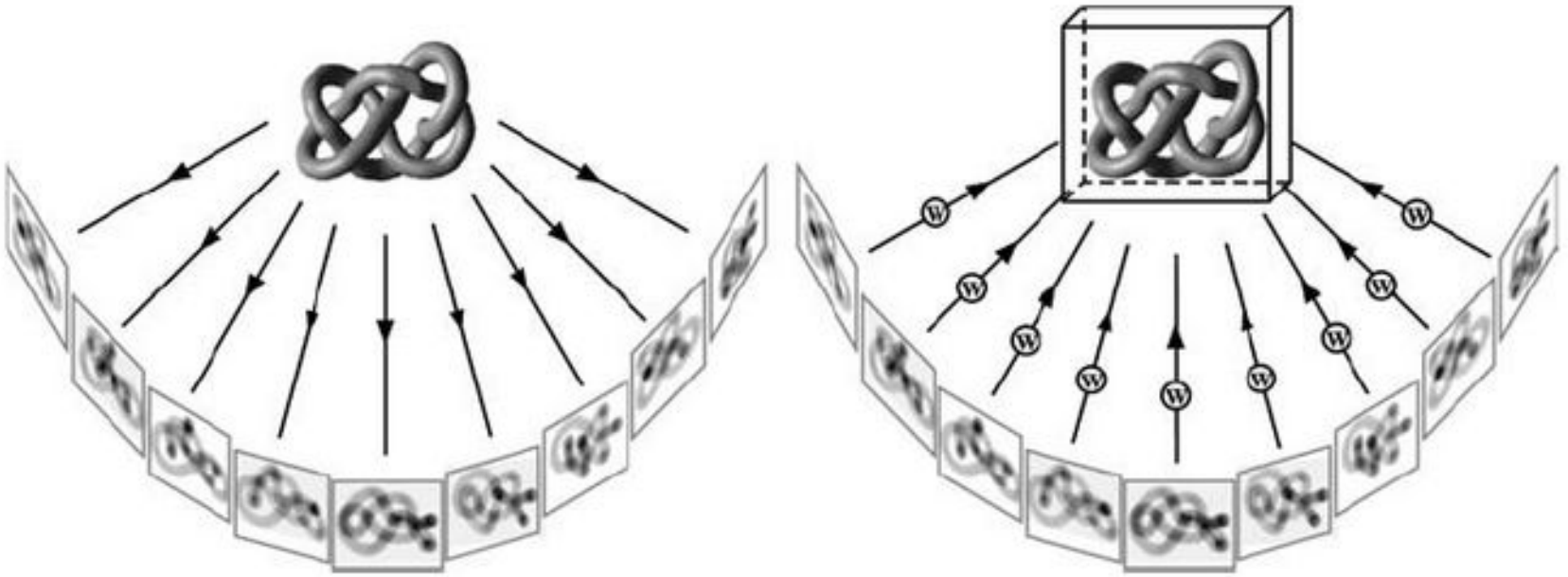
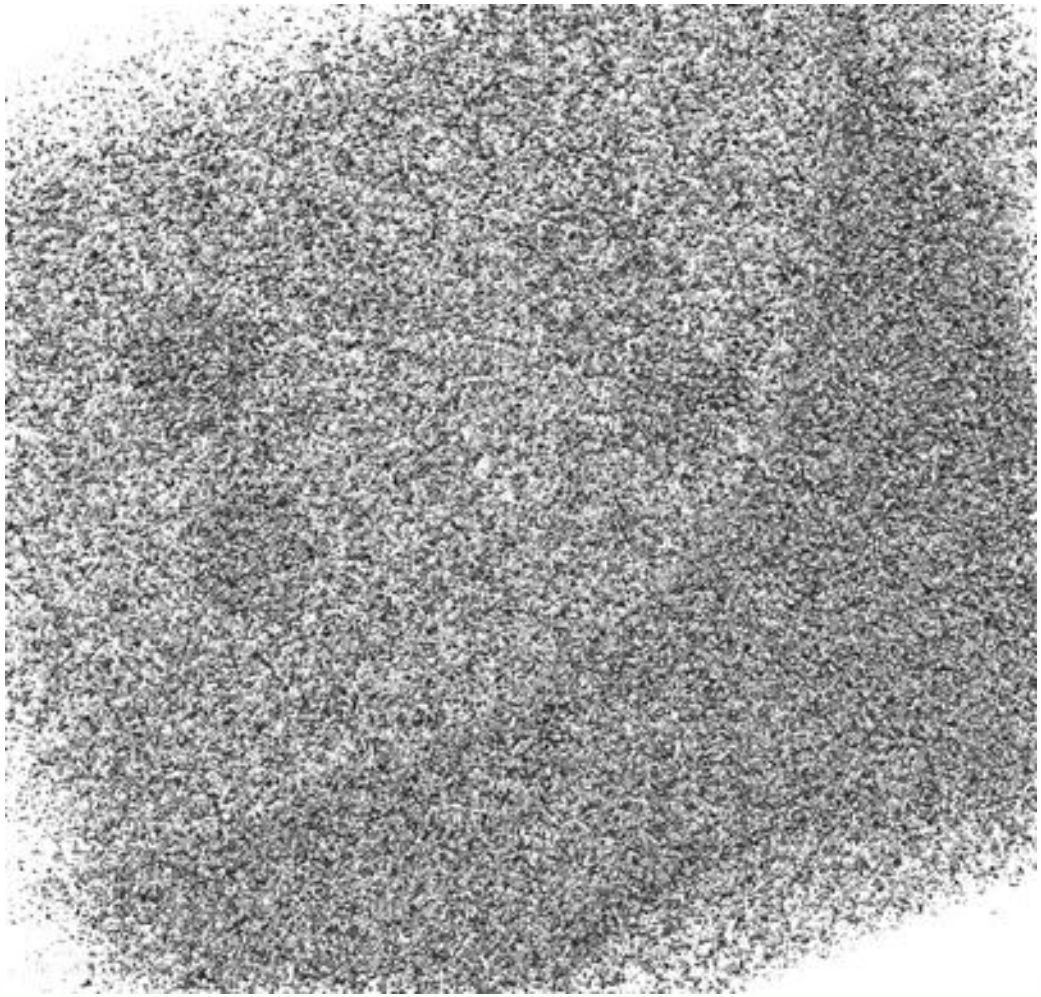


Figure 2 Principle of tomography. (*left*) Projections of the specimen were recorded from different directions by tilting the specimen holder. (*right*) The three-dimensional reconstruction of the sample is obtained most commonly by backprojection into a common three-dimensional reconstruction body.

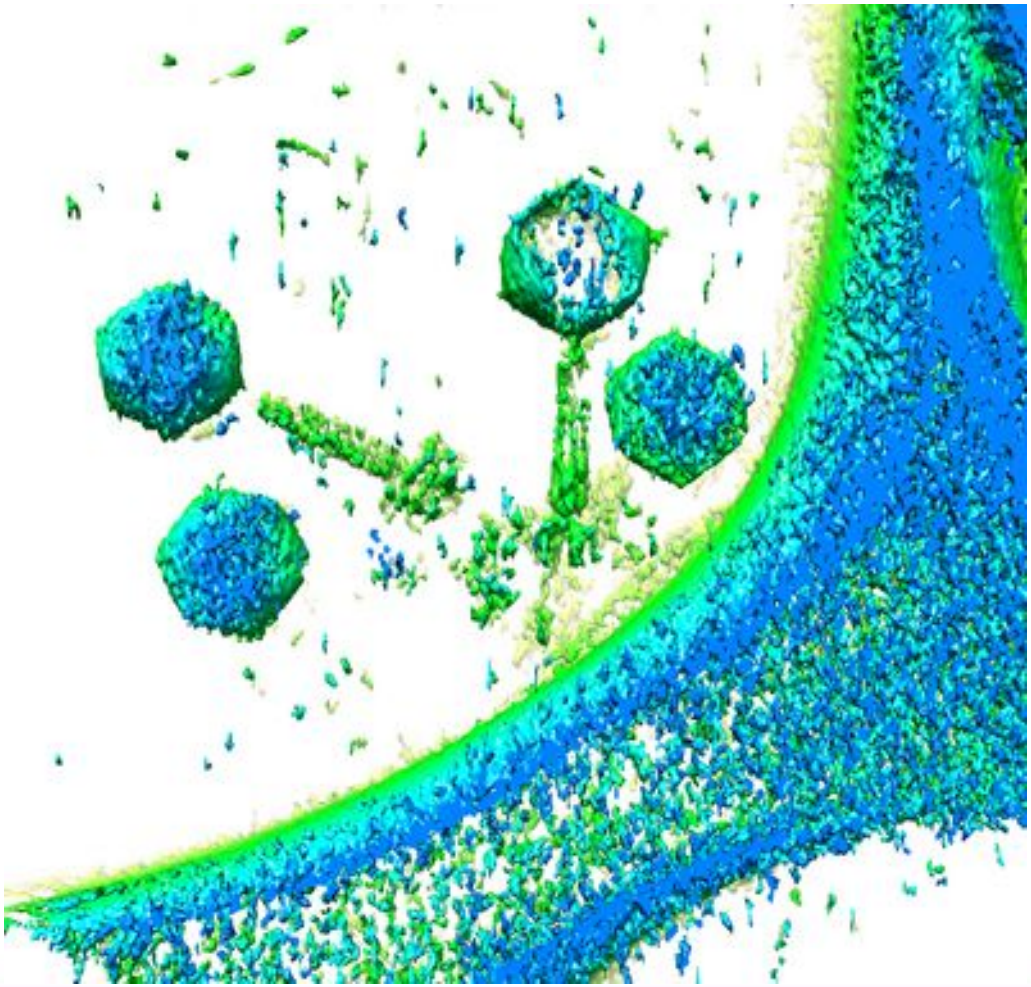
Tomogram

Flagella!

Not so pretty…



Pretty!



Data acquisition considerations

- Dose
  - 40-200 electrons/Å^2 has to be split over all tilts
  - Higher contrast versus accumulative damage
- Magnification/pixel size
  - Higher mag = smaller field of view = tracking harder during data acquisition
- Defocus

Low dose data acquisition

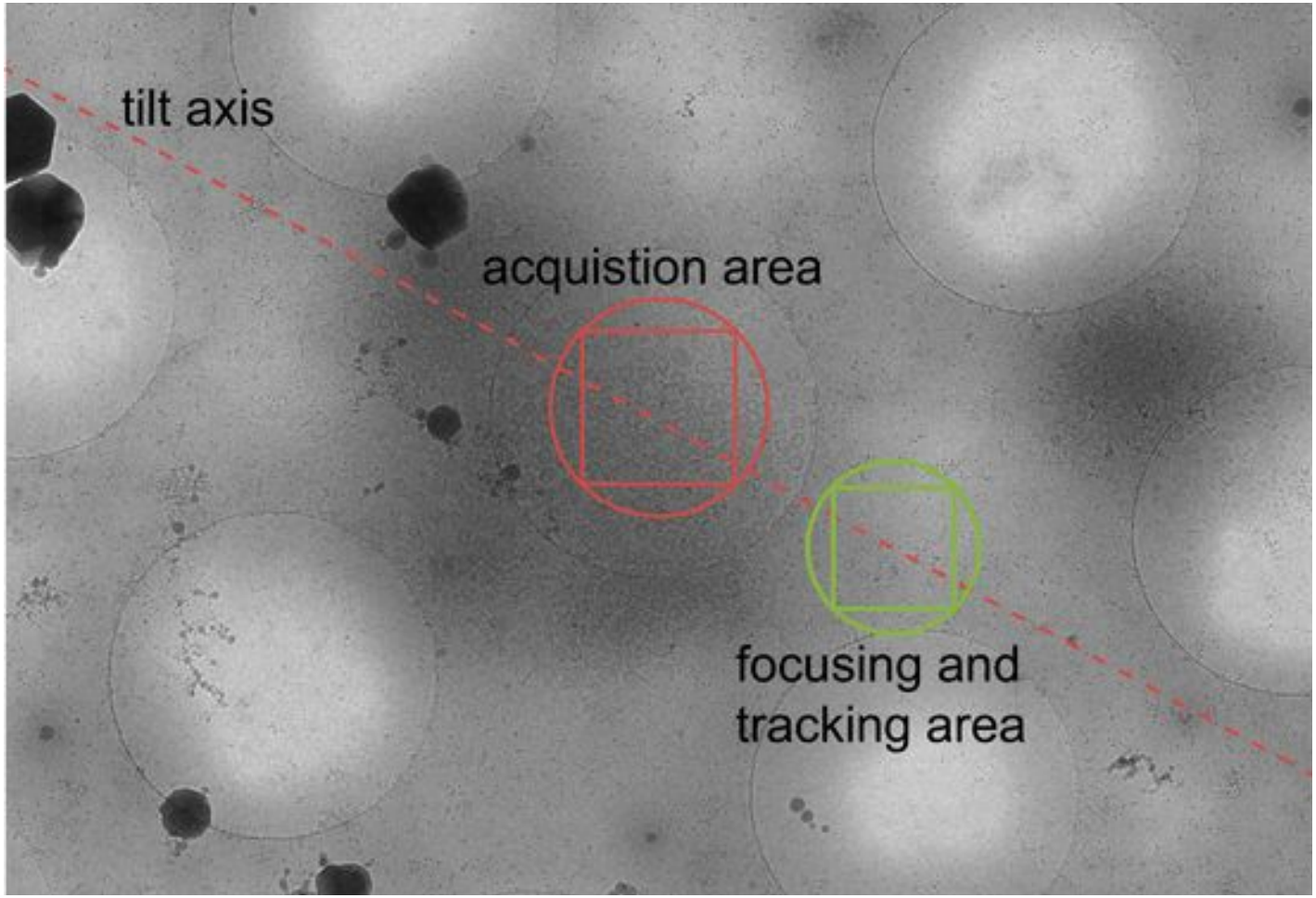
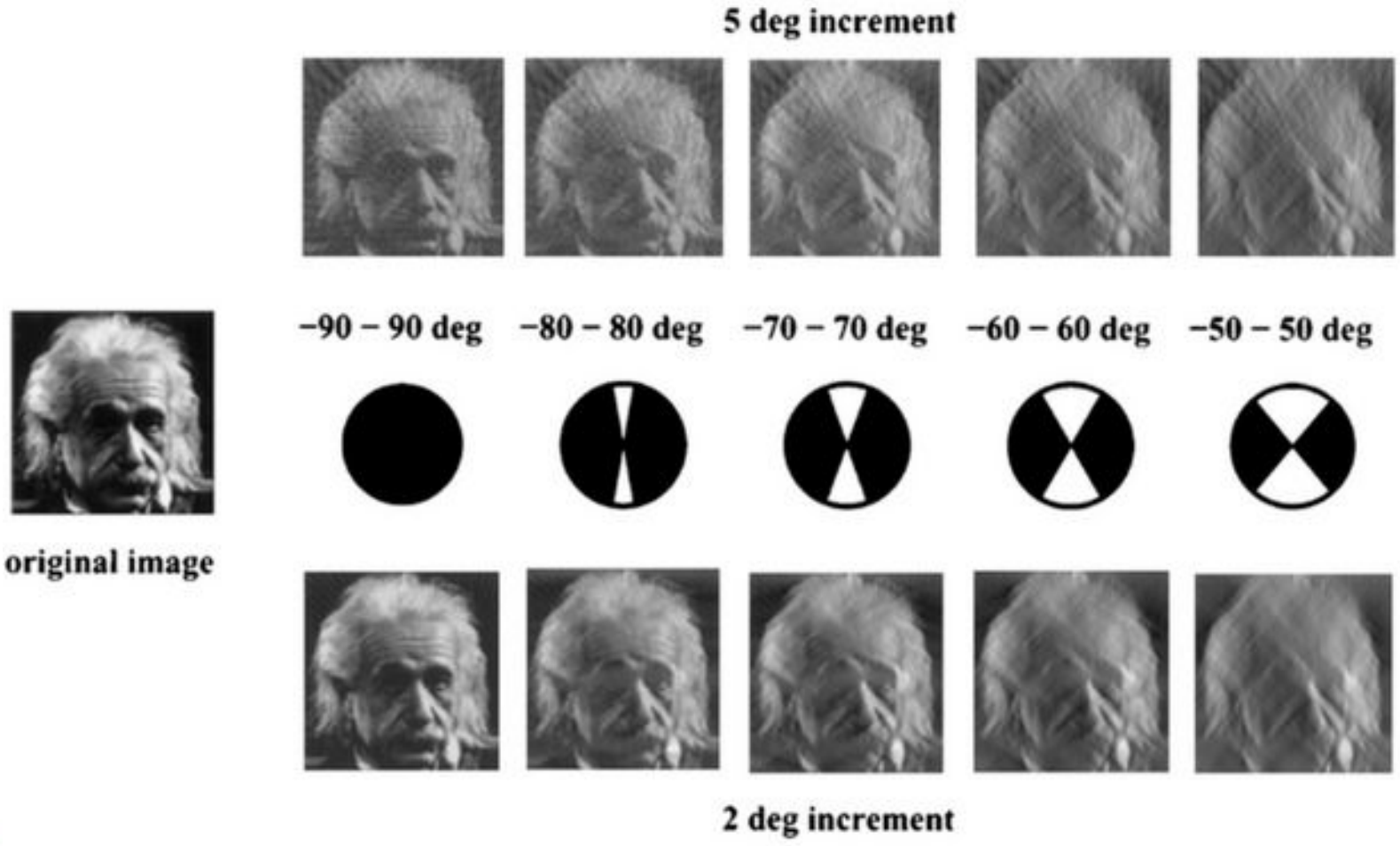


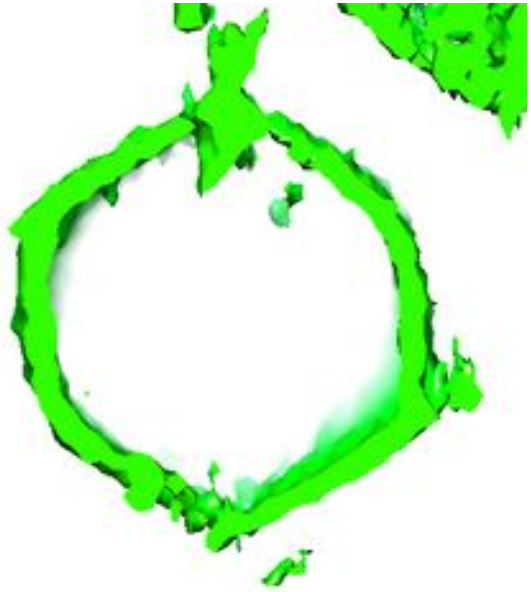
Image from Florian Schur

Missing wedge

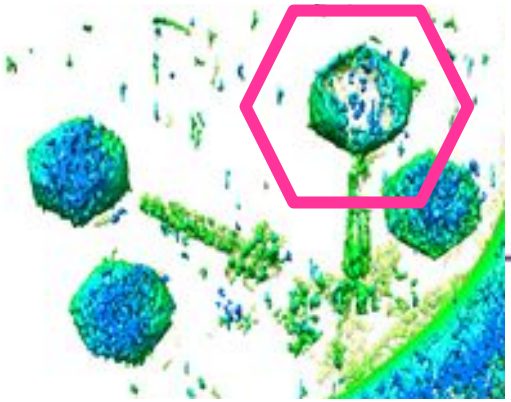


Nudelman F, de With G, Sommerdijk NAJM ,Cryo-electron tomography: 3-dimensional imaging of soft matter, 2011, Soft Matter (7)

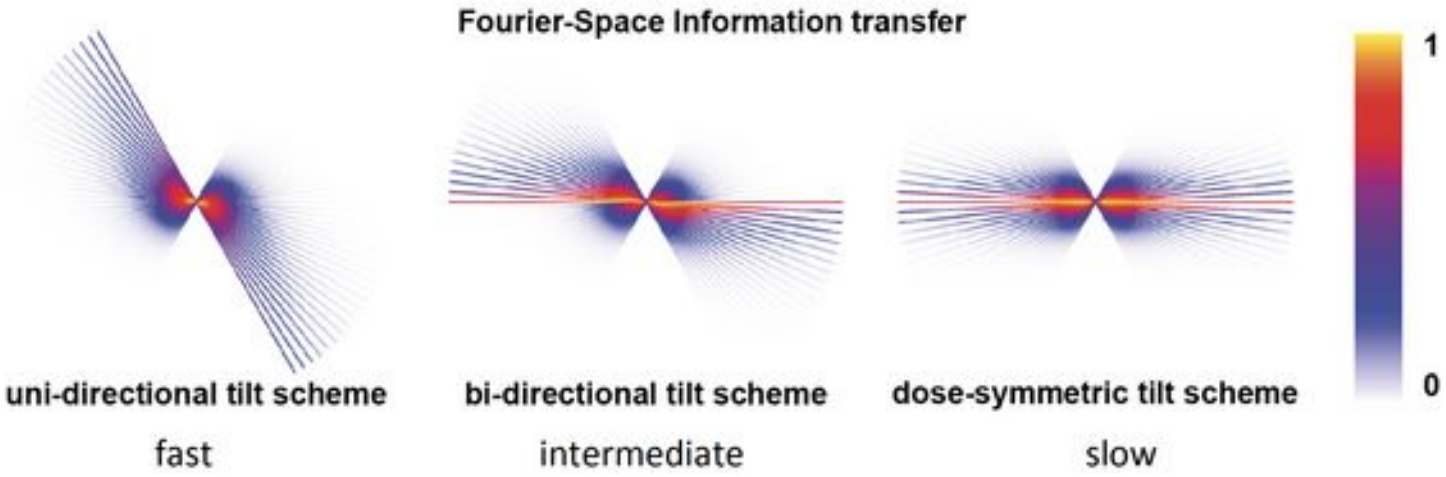
Missing wedge



Rotate
90˚



Tilt schemes

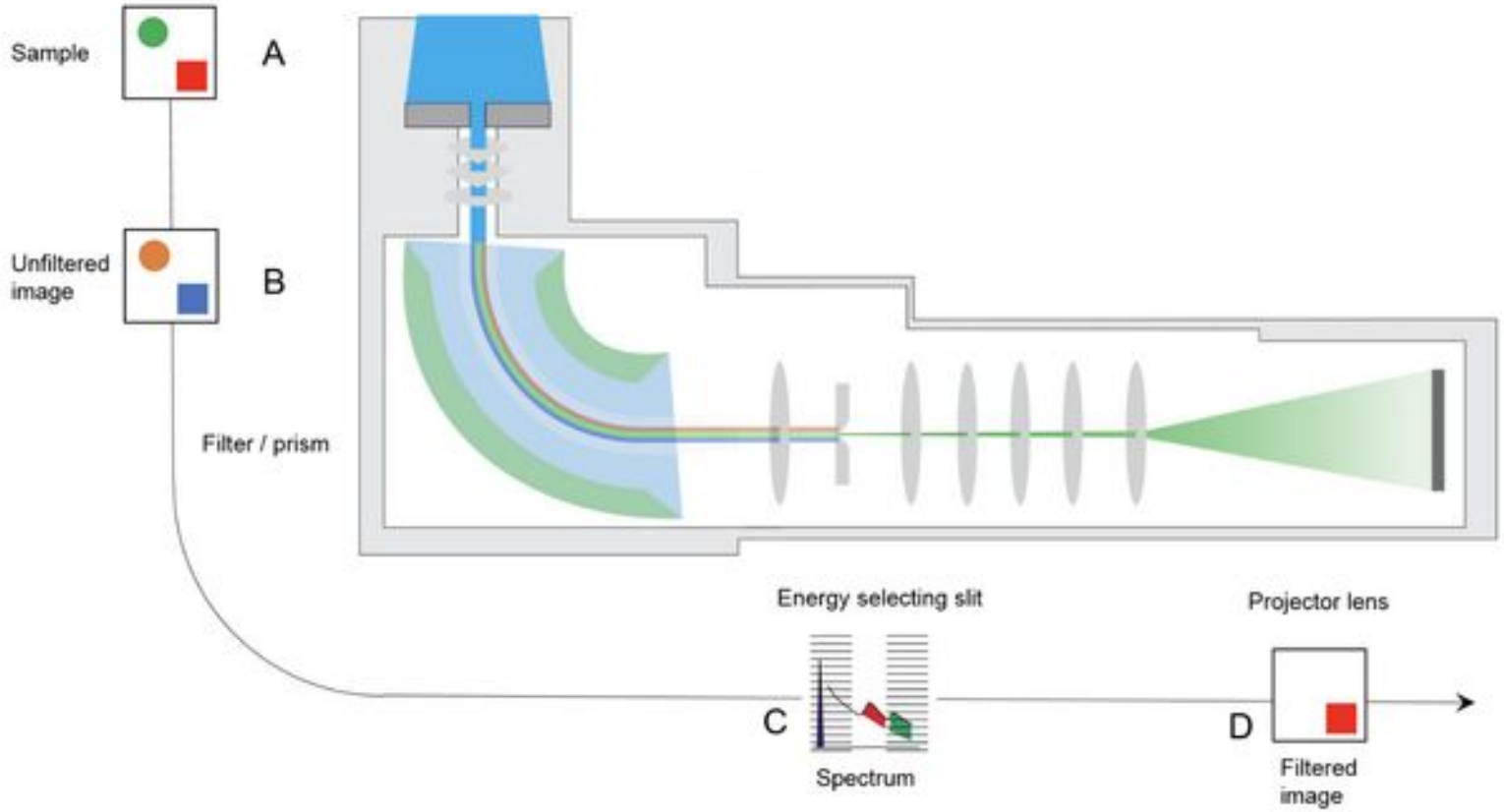


+60 to -60 Start at highest tilt	+20 to -60, then +20 to +60 Start at medium tilt	0, +3, -3, -6, +6, +9, -9, -12, +12, +15 etc Start at ZERO tilt

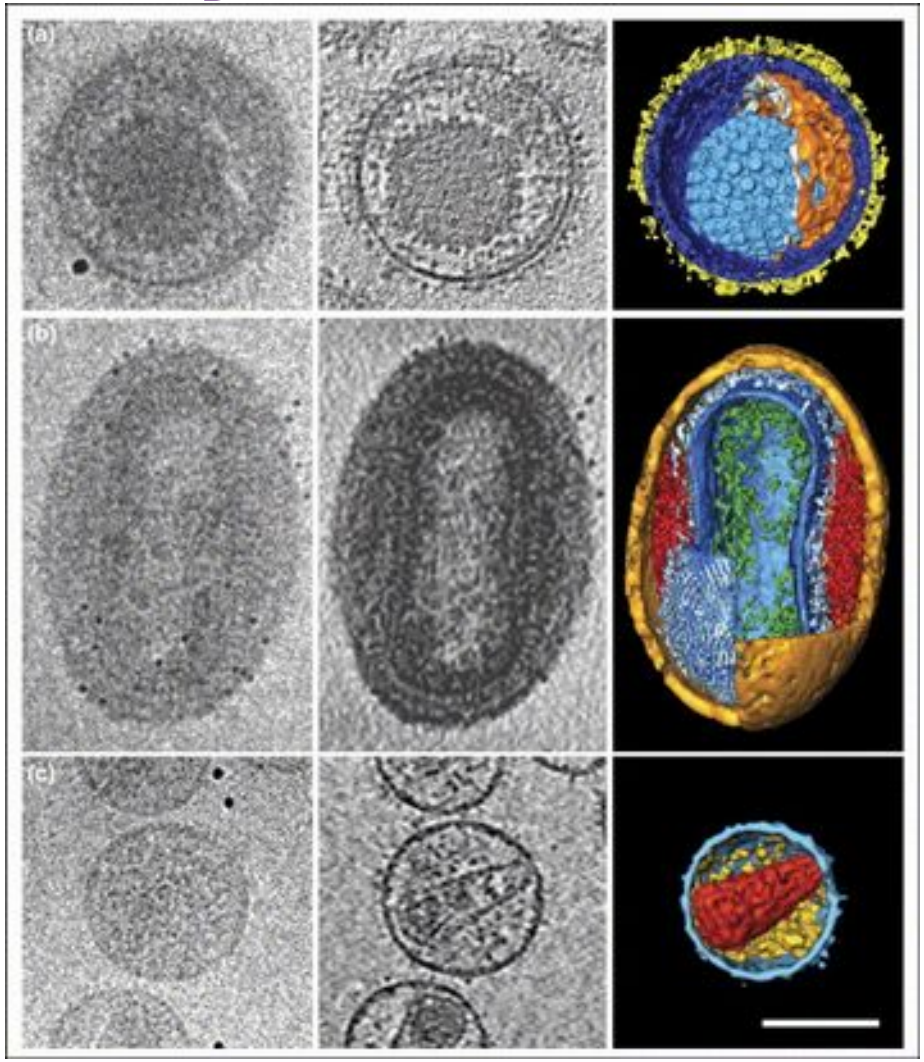
Do you need low dose at low tilt, or would more tomograms be better?

J Struct Biol. 2017 Feb; 197(2): 191–198.

Energy filter



Cryo-ET of viruses



(a) Herpes simplex virus was studied by Grünewald *et al.* . The virus is bound by an envelope membrane (dark blue) studded with a number of different glycoprotein spikes (yellow), enclosing the proteinaceous tegument layer (orange) and the off-centre icosahedral capsid (blue) harbouring the DNA.

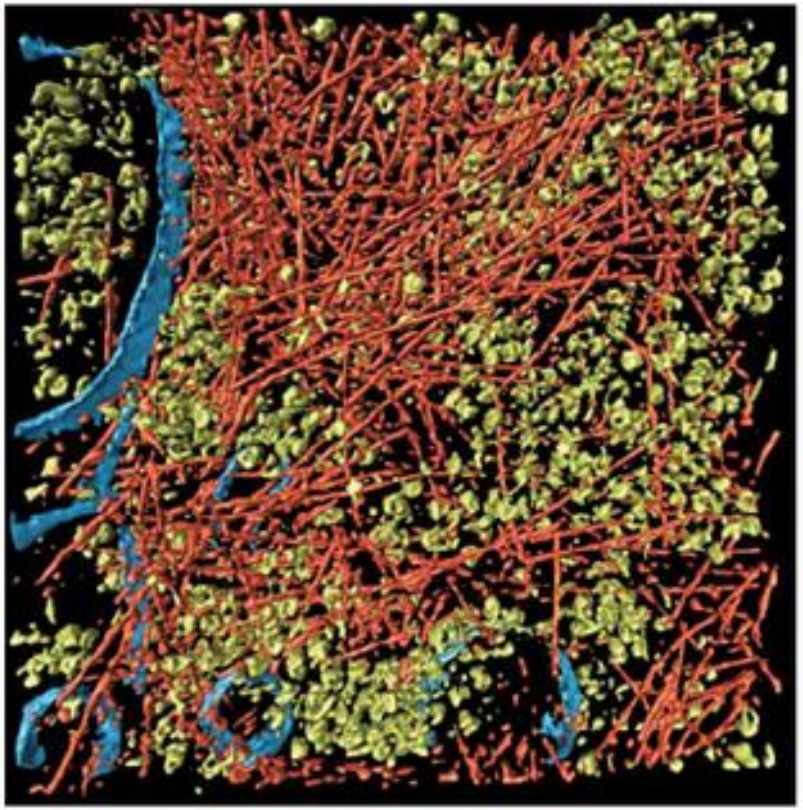
(b) VV IMV was studied by Cyrklaff *et al*. The virion is composed of multiple layers: outer membranes (orange), enclosing the lateral bodies (red) and the dumbbell shaped core (dark blue), with a remarkably compact distribution of DNA (green) and a crystalline spike layer (light blue).

(c) Human immunodeficiency virus studied by Briggs *et al.* consists of an envelope (blue), and an envelope-spanning core (red) of fullerene architecture, harbouring the RNA viral genome. The density marked yellow is probably Gag-derived protein not incorporated into the core. Scale bar of 100 nm applies to all panels.

Structure of complex viruses and virus-infected cells by electron cryo tomography. Curr Opin Microbiol. 2006 Aug;9(4):437-42. **Grünewald K et al.**

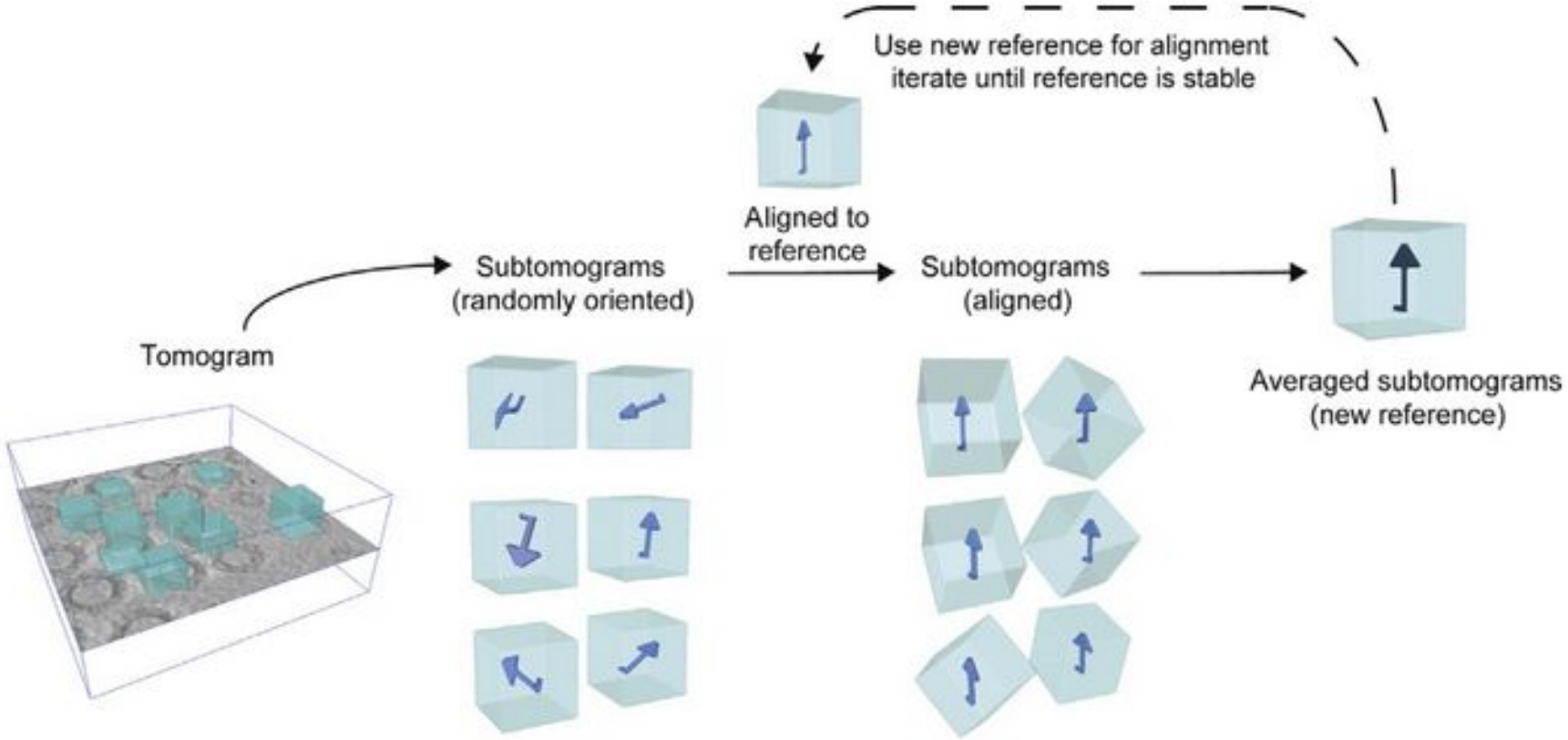
Actin filaments in a cell

- Actin filaments were trapped using millisecond freezing methods and identified using structural information

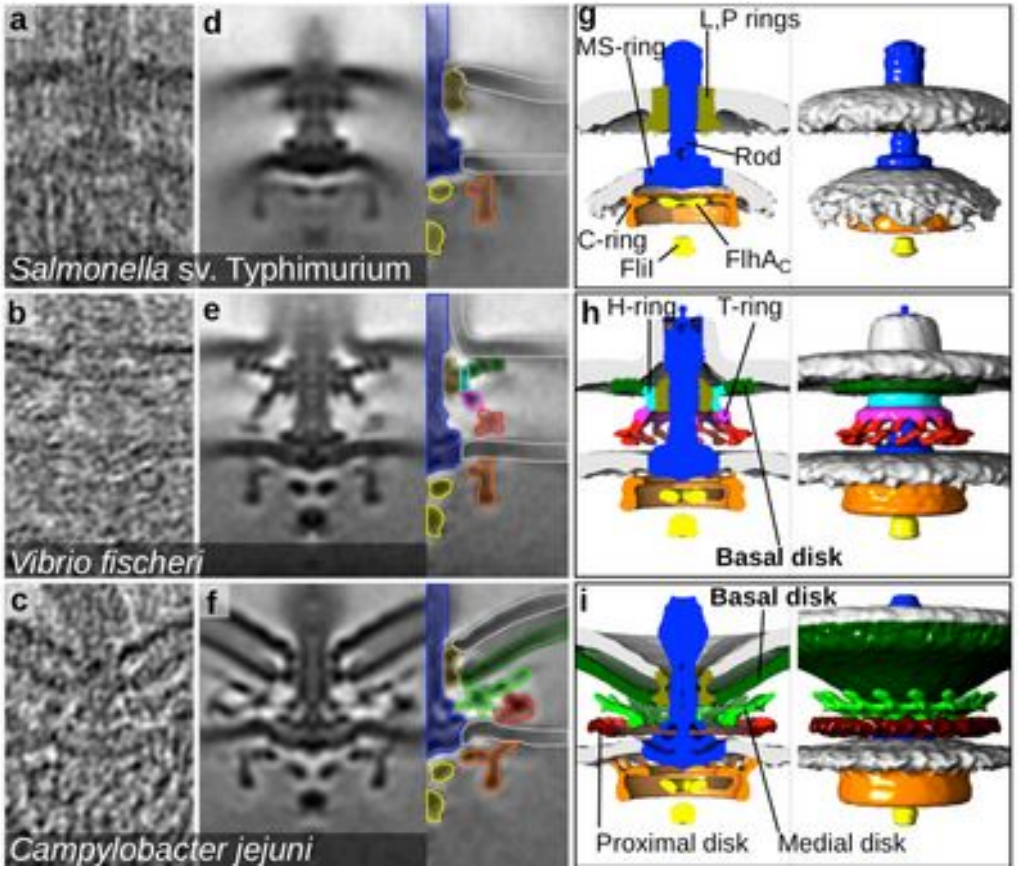


56. Medalia O, Weber I, Frangakis AS, Nicastro D, Gerisch G, Baumeister W. 2002. *Science* 298(5596):1209–13

Sub-tomogram averaging

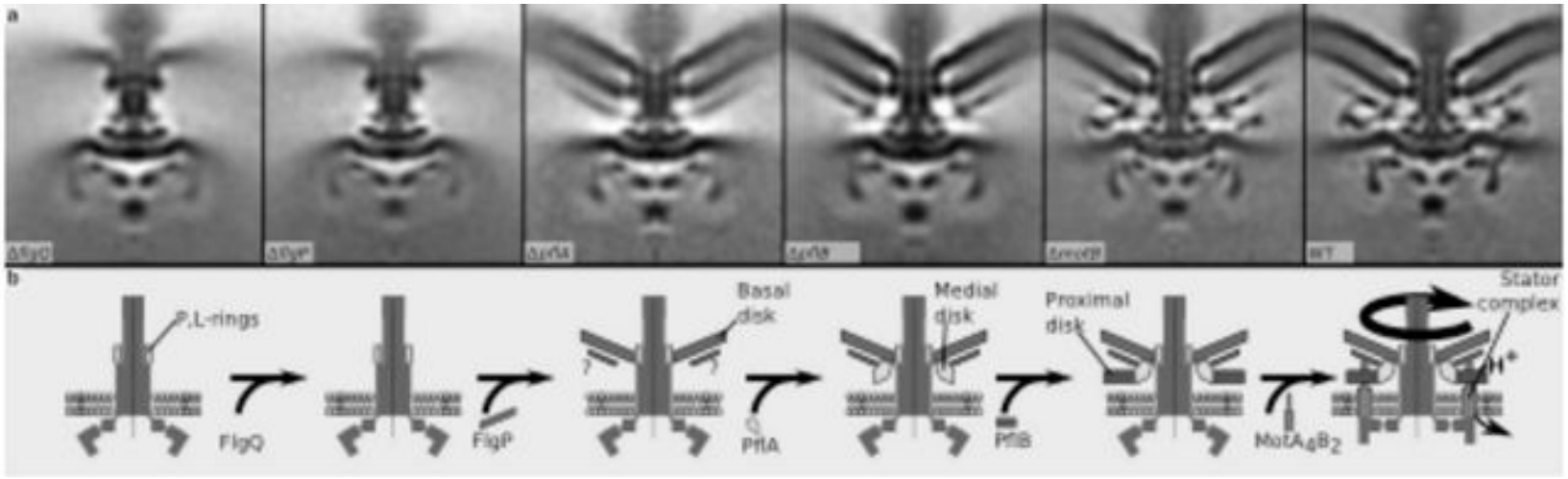


Flagellar motors



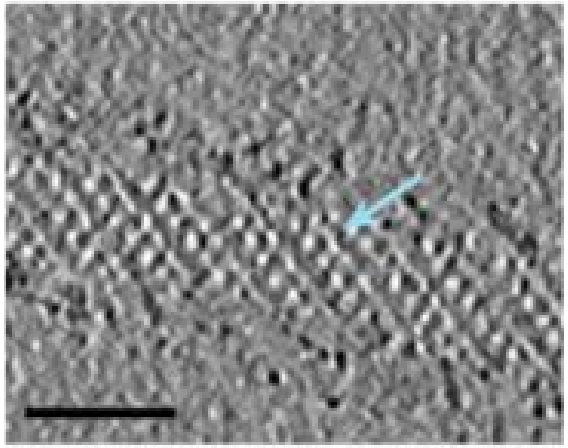
Beeby et al 2016 https://doi.org/10.1073/pnas.1518952113

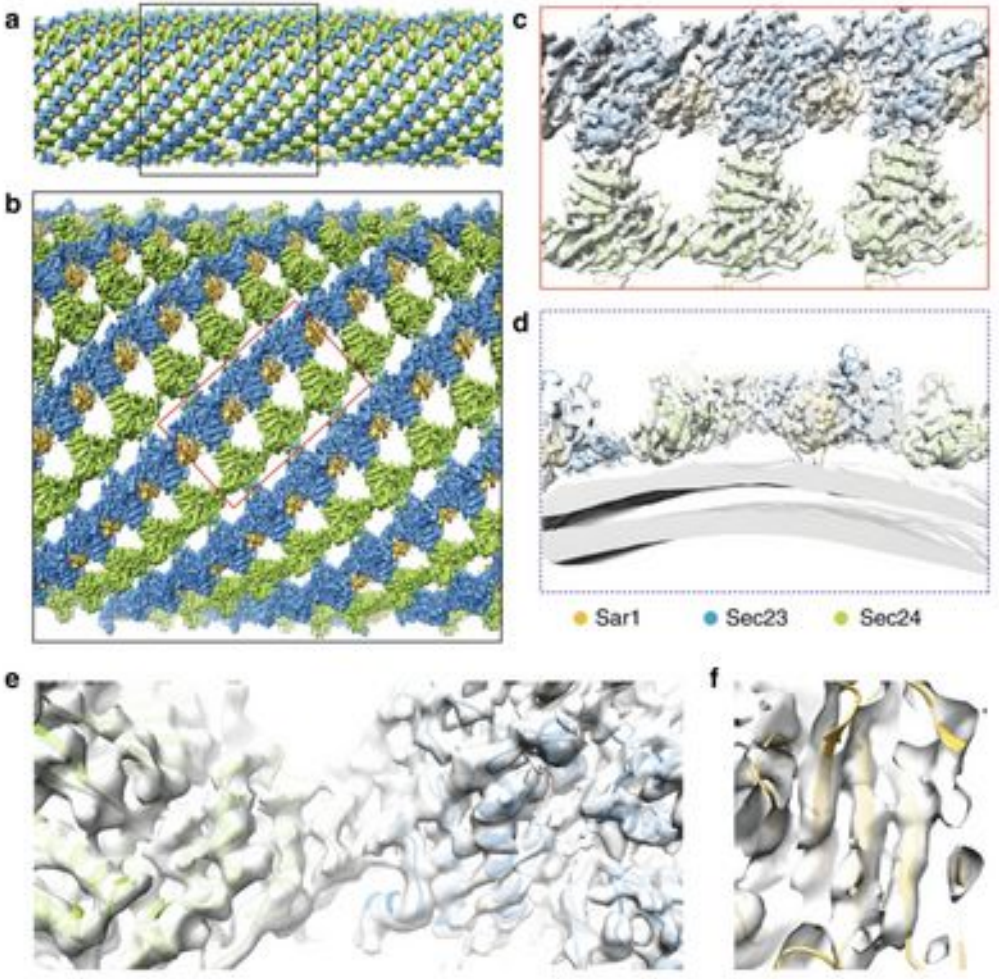
Deletion mutants



Beeby et al 2016 https://doi.org/10.1073/pnas.1518952113

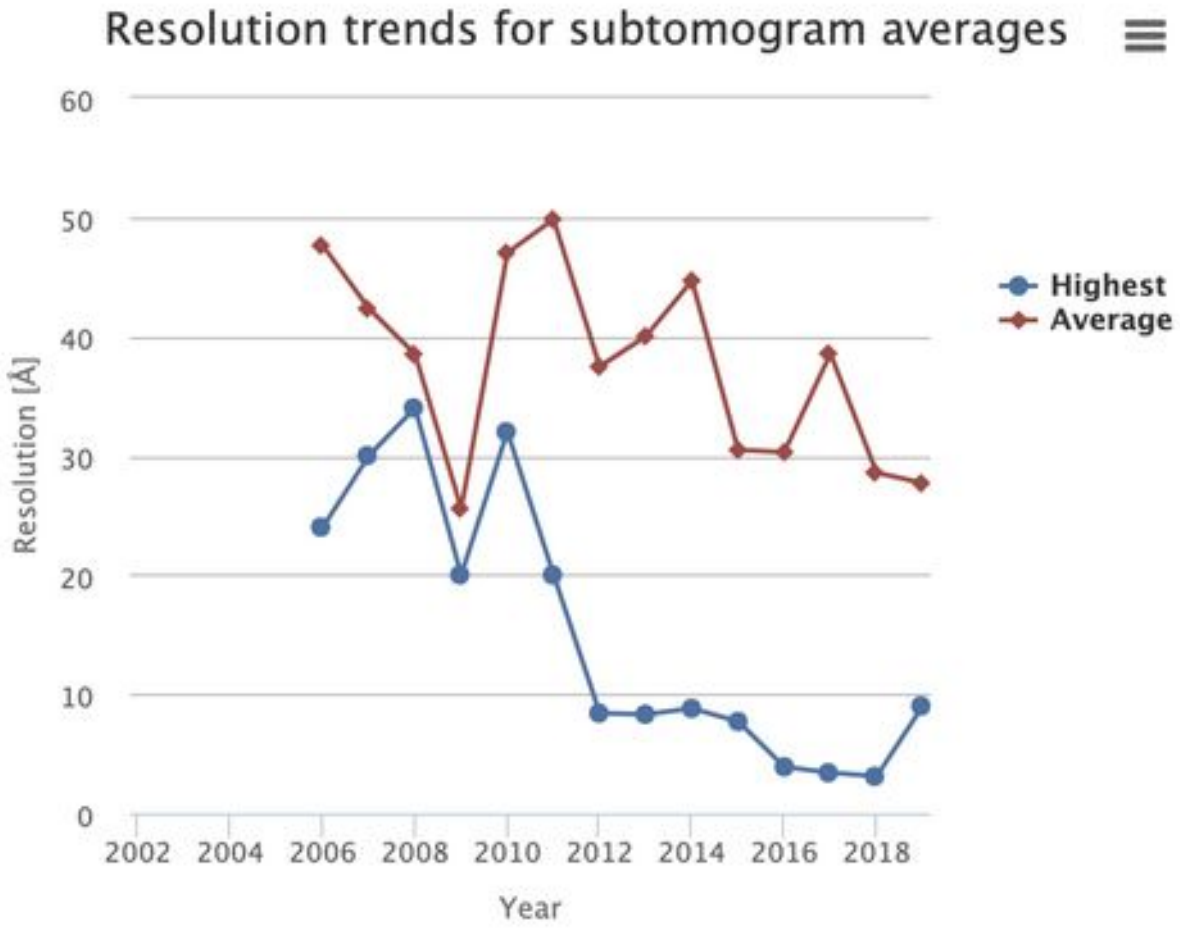
4.9Å sub-tomogram average...



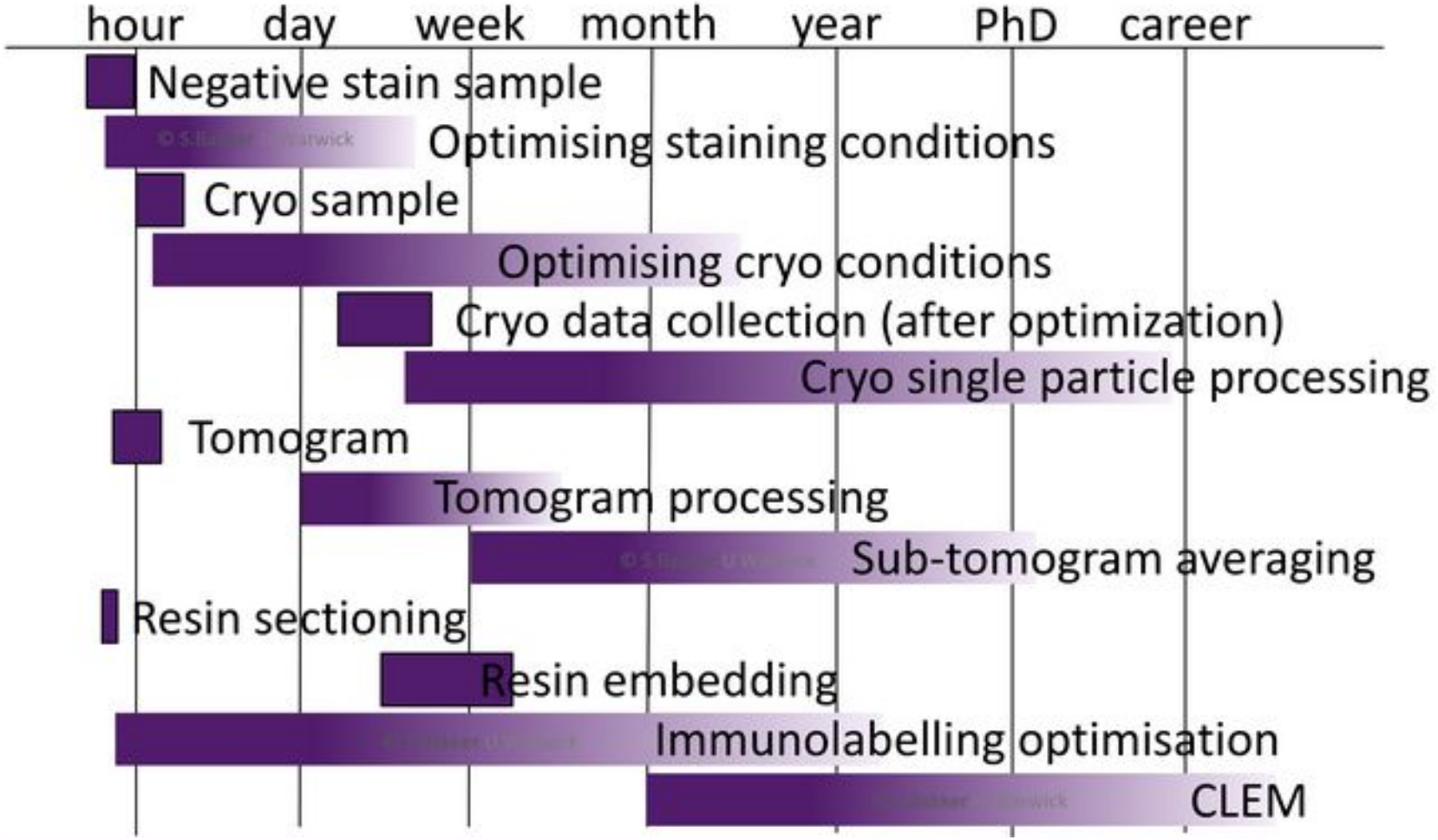


tchings et al 2018 https://doi.org/10.1038/s41467-018-06577-4

Not easy…


Resolution trends for subtomogram averages
60
50
40
30
20
10
0
Resolution [Å]
2002
2004
2006
2008
2010
2012
2014
2016
2018
Year
Highest
Average

How long is a piece of string?



References

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- Macromolecular architecture in eukaryotic cells visualised by cryoelectron tomography. O Medalia et al., Science (2002) 298, 1209-1213.
- Visualising cells at the nanoscale. A Leis et al., TIBS (2008) 34, 60-70
- Studying intracellular transport using high-pressure freezing and correlative light electron microscopy. E. Brown et al., Seminars in Cell and Developmental Biology (2009), 20, 910-919