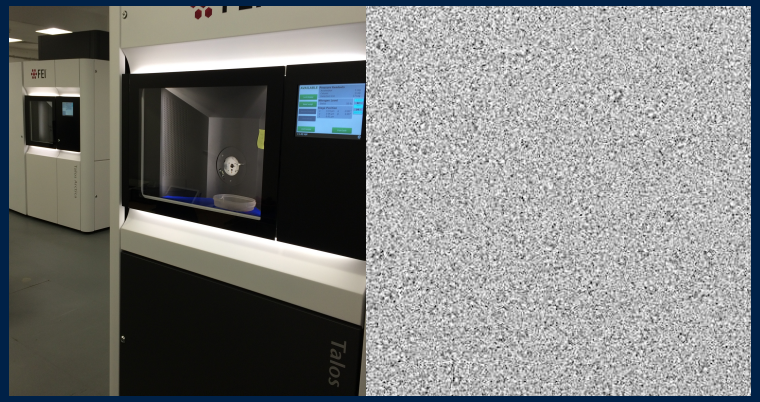
SIR WILLIAM DUNN
SCHOOL OF PATHOLOGY

UNIVERSITY OF OXFORD

Cryo-TEM

for MICRON EM course



Joanne Lo
Dunn School Bioimaging Facility

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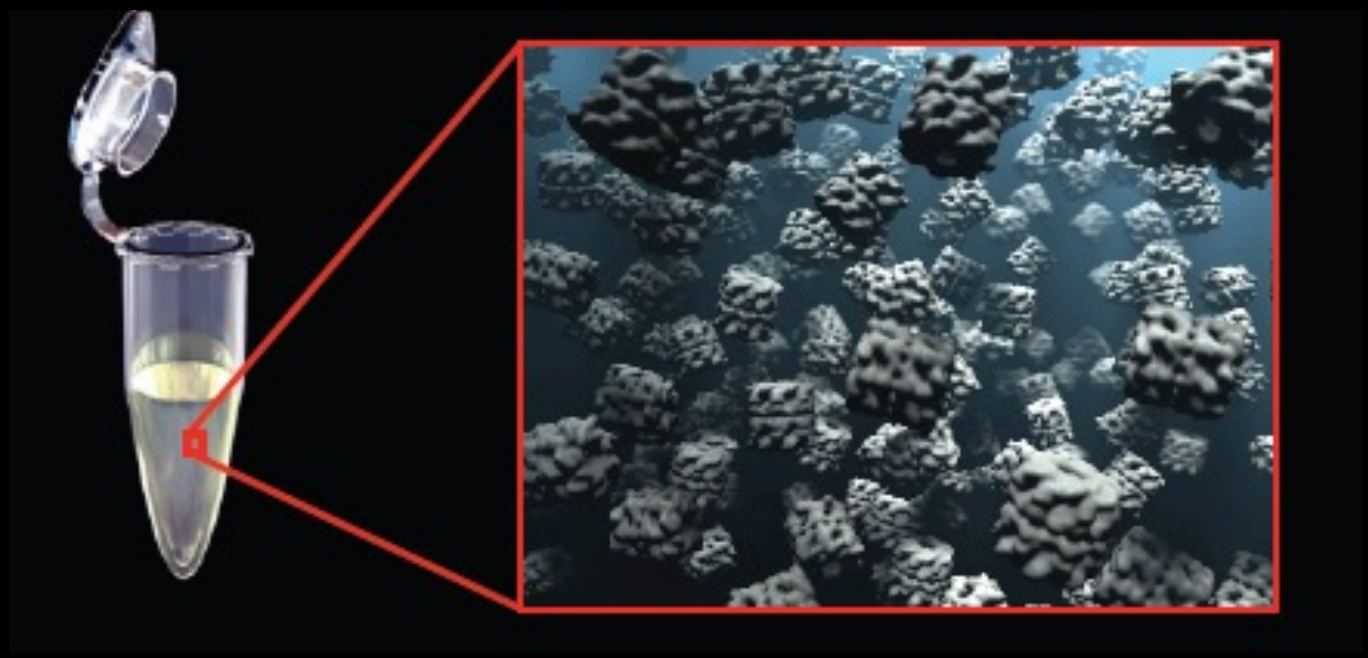
18 Nov 2016

Slide courtesy Gabriel Lander



Slide courtesy Gabriel Lander

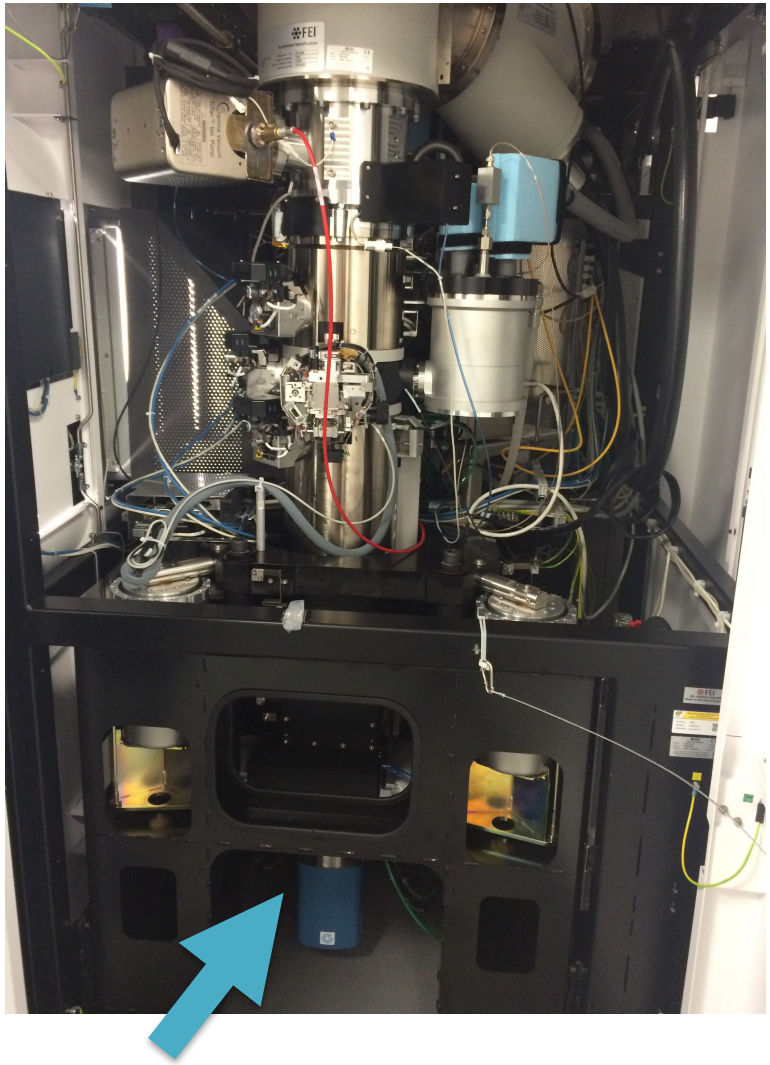
Cryo-EM.

Why and When?

Cryo-EM: Why and When?

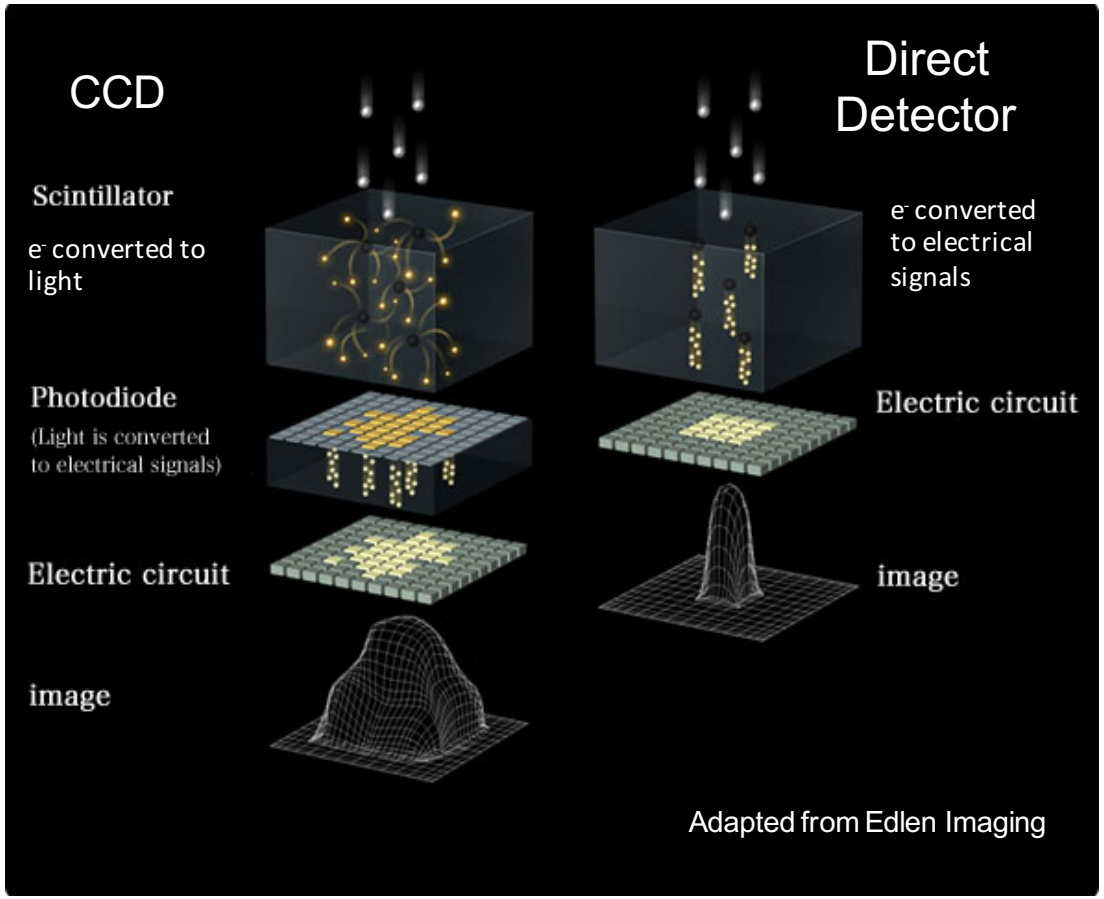
- native state of sample (✖ fixed, ✖ stained)
- for specimen difficult to be converted to 2D crystals
- specimens are observed in vitreous ice
- Cryo-fixation (e.g. cryo-plunging)
- thin enough for preservation & imaging
- low dose parameters required
- origin in 1980s (Bruggeller & Mayer; Dubochet & McDowall)

Cryo-EM



Principles of direct electron detectors

- Detect electron directly on the chip (no scintillator)



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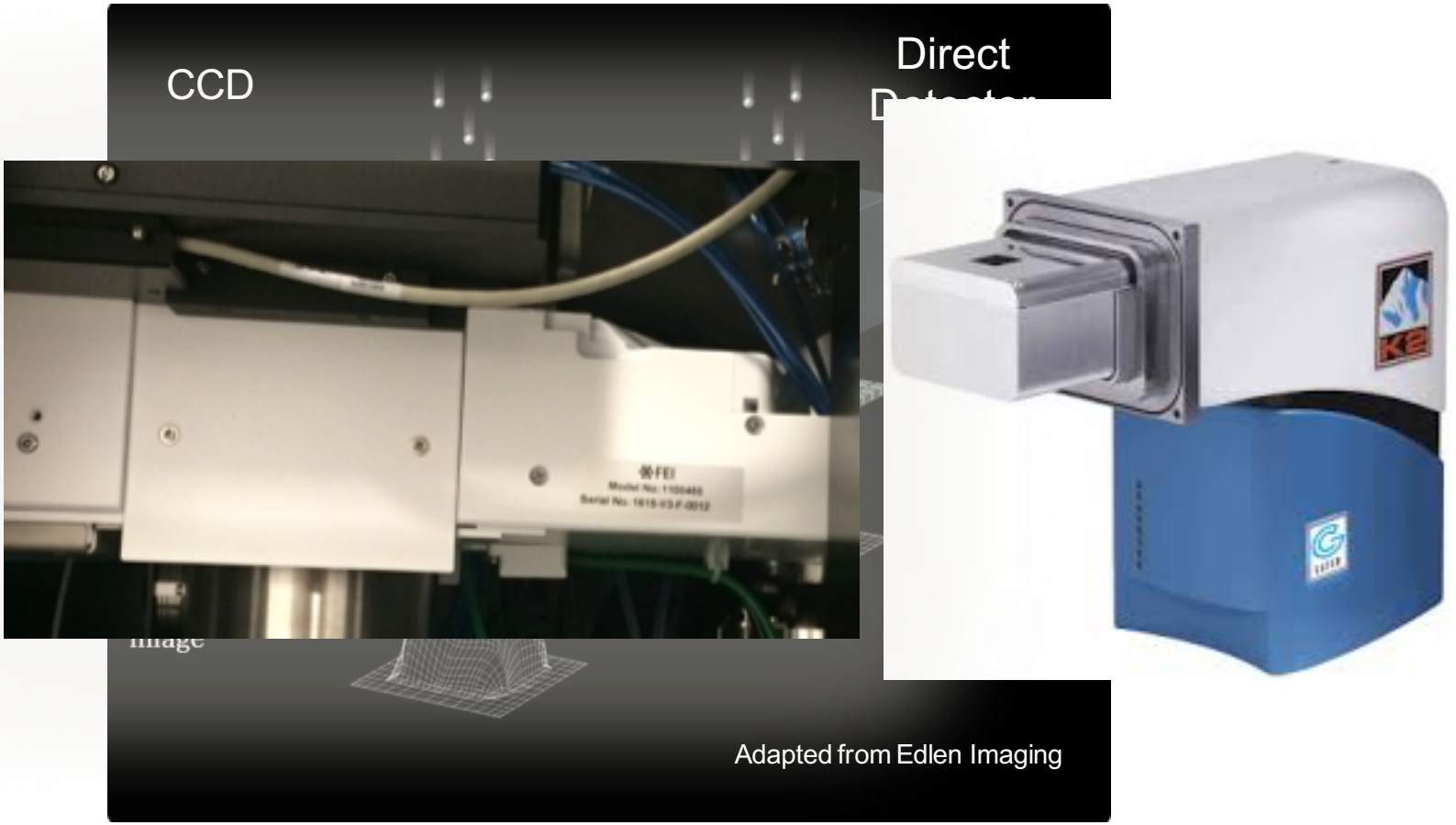
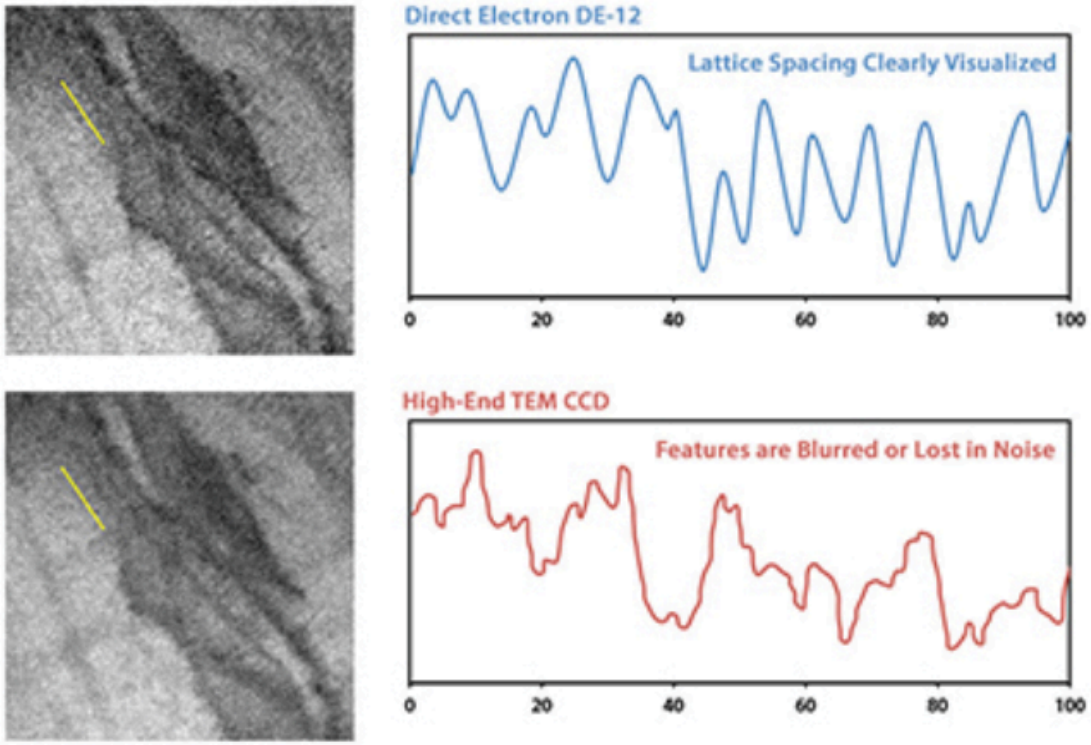


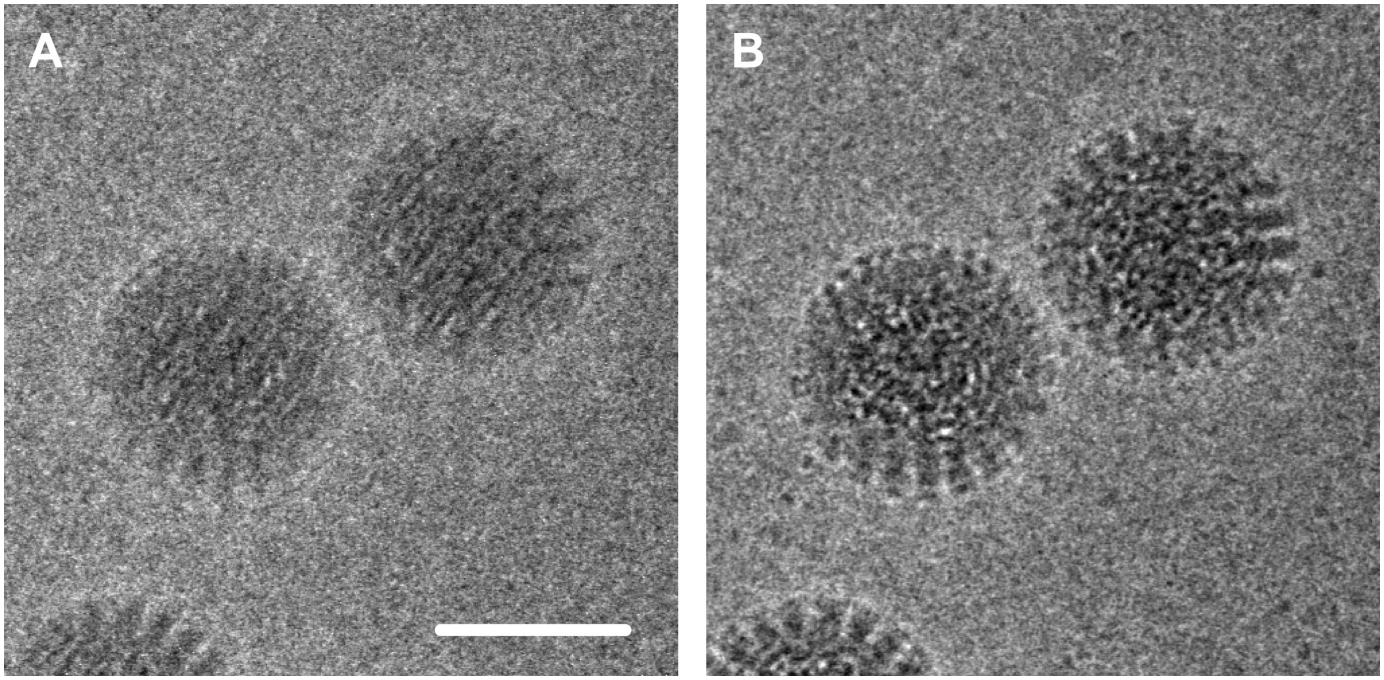
Direct electron detectors

- played a key role in the recent increase in the power of single particle electron cryomicroscopy (cryo-EM)



Ref: Direct Electron

Direct electron detectors



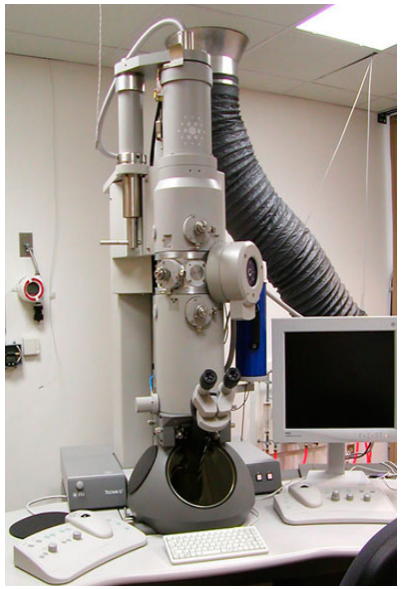
raw image

motion corrected

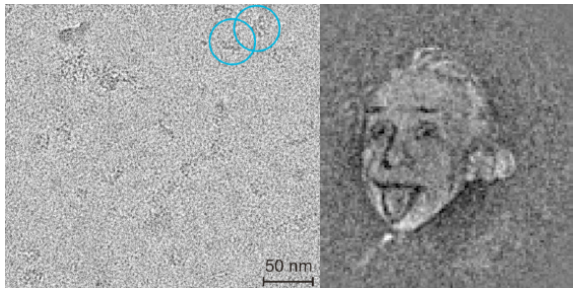
Brilot et al., 2012

Fundamental challenges in biological samples

- High vacuum damage
- Radiation damage
- Low signal-to-noise ratio (poor electron scattering, dose limitation)



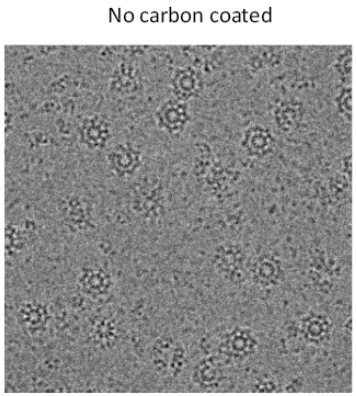
Cryo electron microscopy

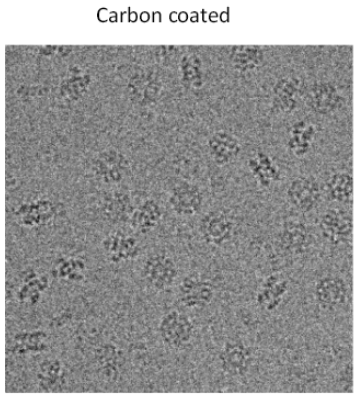


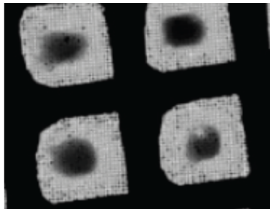
Henserson PNAS 2013

What else prevents us from achieving atomic resolution with biological samples

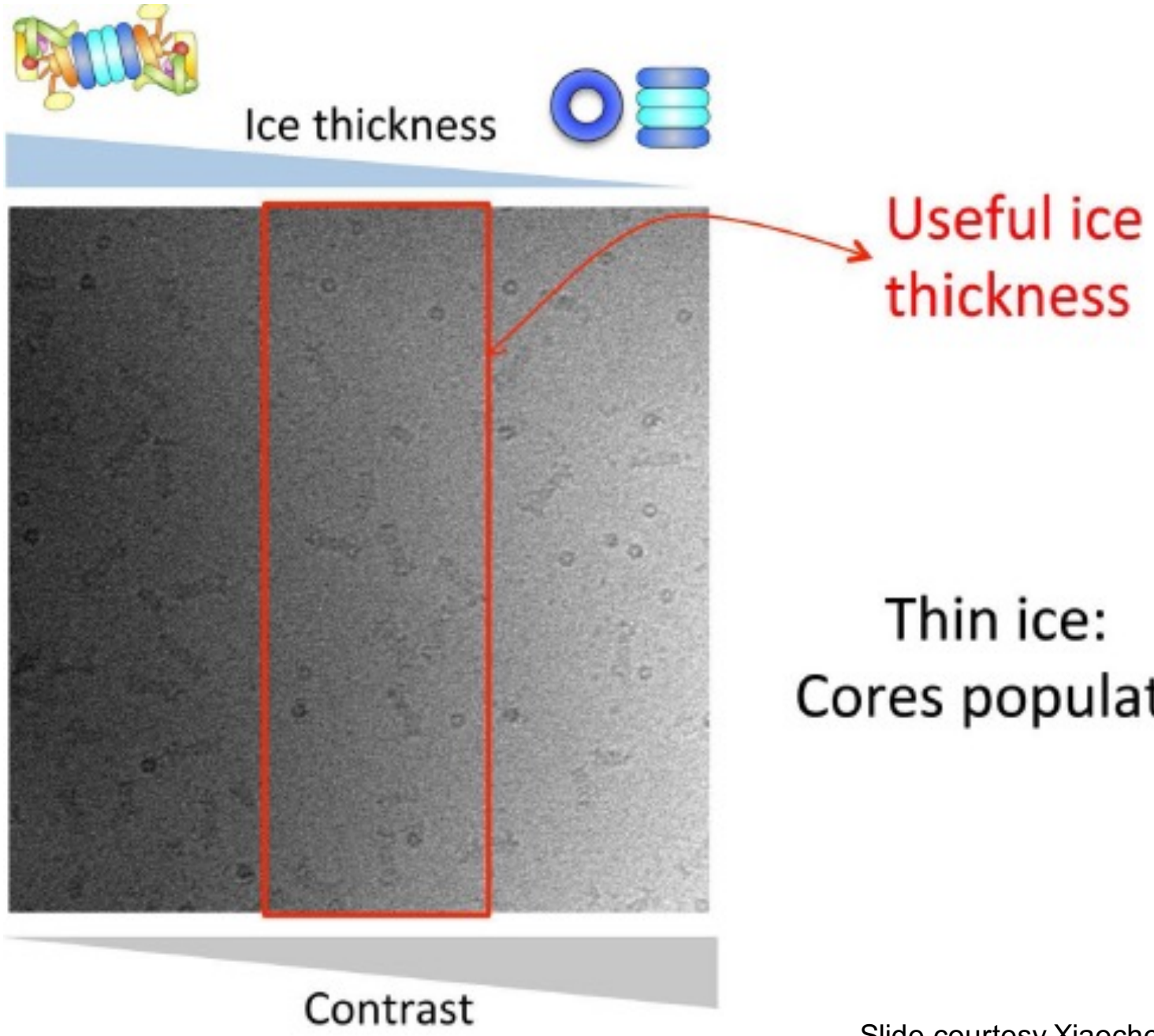
- Imperfect detectors
- Data analysis/software
- Image blurring
- Suboptimal samples
  - Sample purity & concentration
  - Particle density
  - Orientation preference
  - Ice thickness
  - Structural flexibility
  - Conformational heterogeneity
  - Compositional heterogeneity





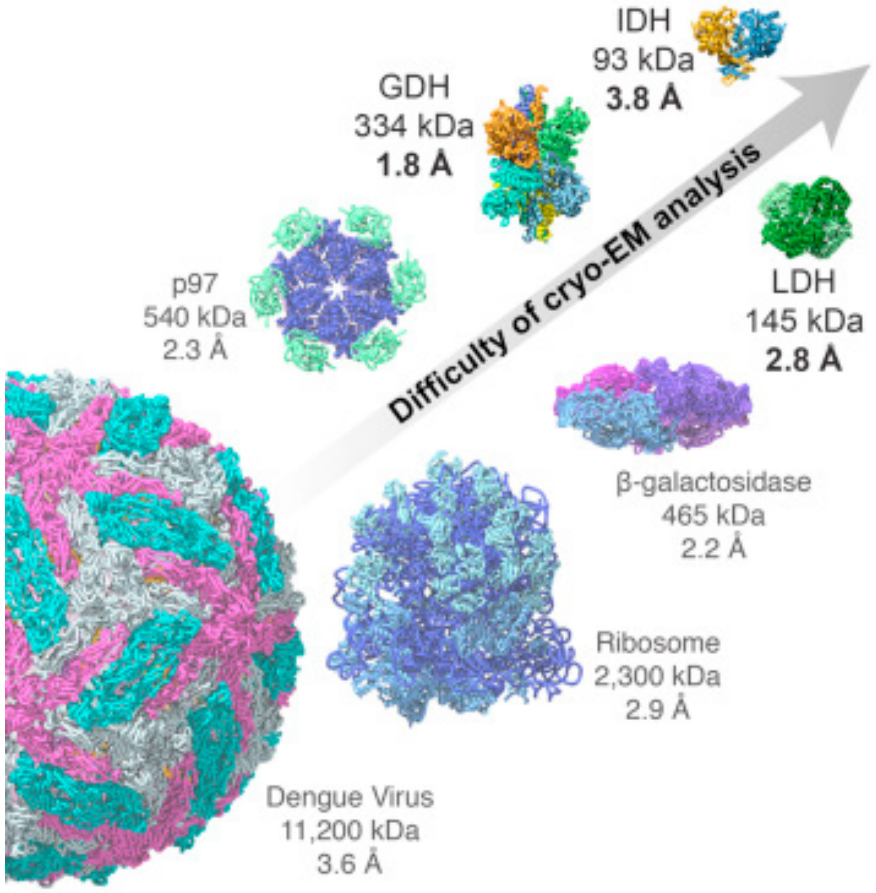


Slide courtesy Xiaochen Bai



Slide courtesy Xiaochen Bai

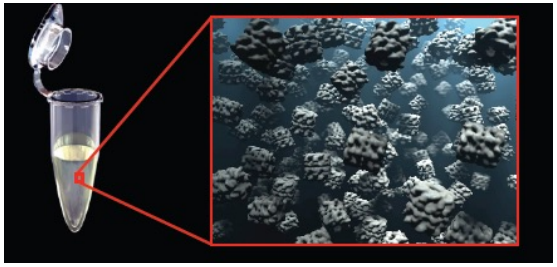
How small can my protein be?



Merk *et al.*
Cell 2016

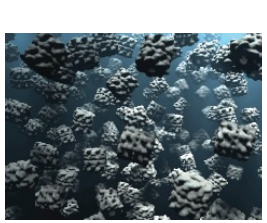
- Depends on what you want to achieve!

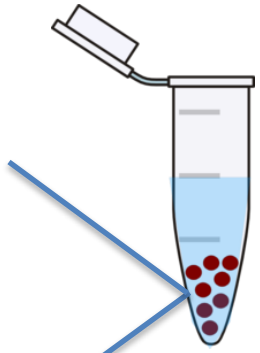
Ideal Sample



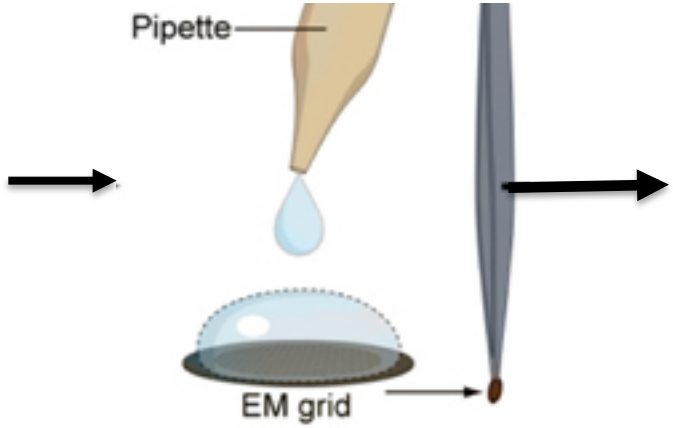
- Homogenous
- Stable (If not, e.g. GraFix)
- Good concentration
- Right thickness

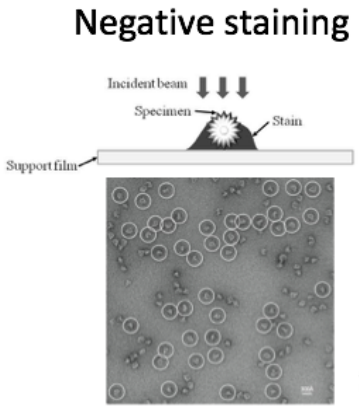
Cryo-EM workflow

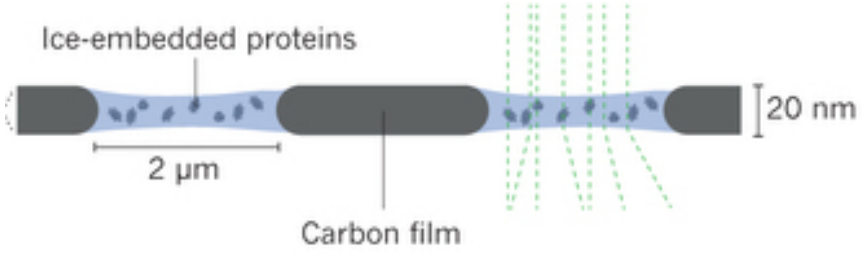


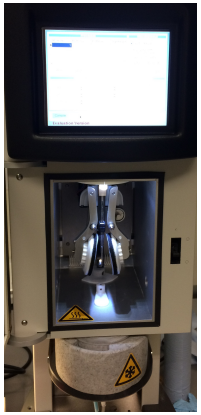


Purified Sample (protein, virus, thin cells etc.)



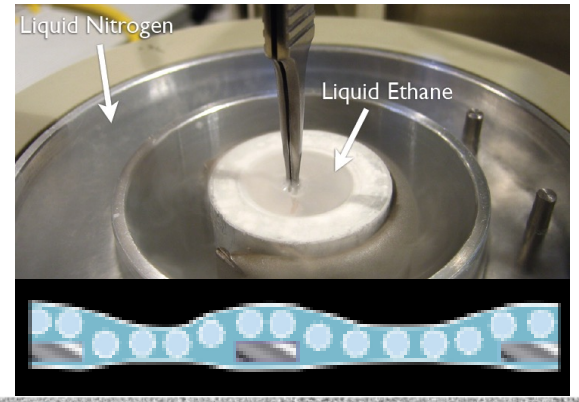


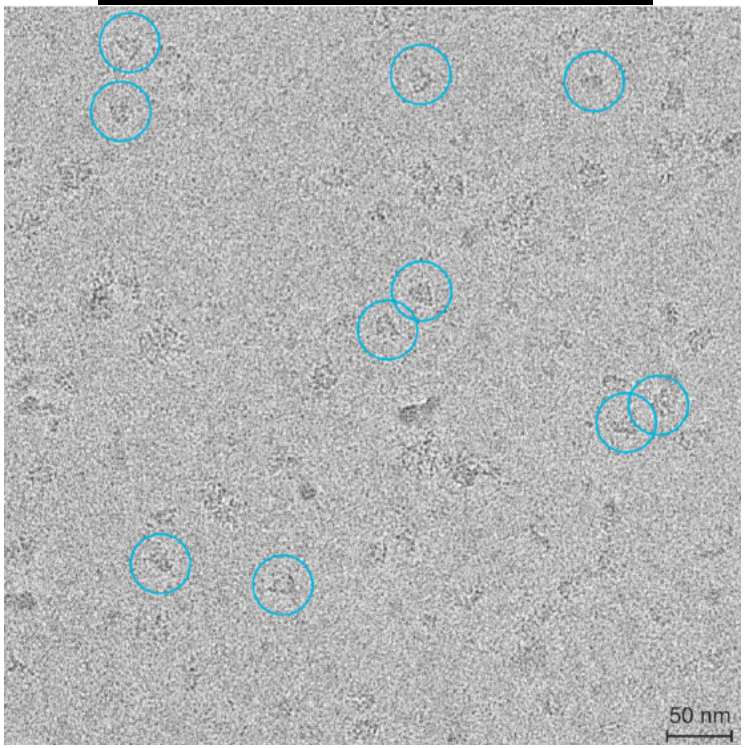


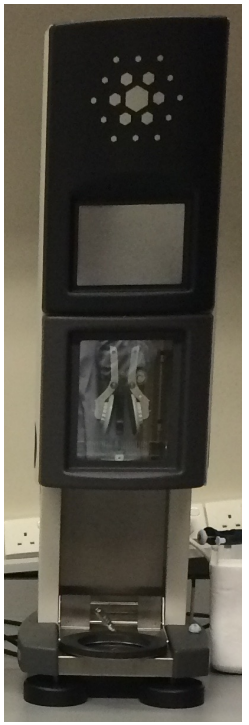


Plunge-freezing (<5um)

Plunge freezing



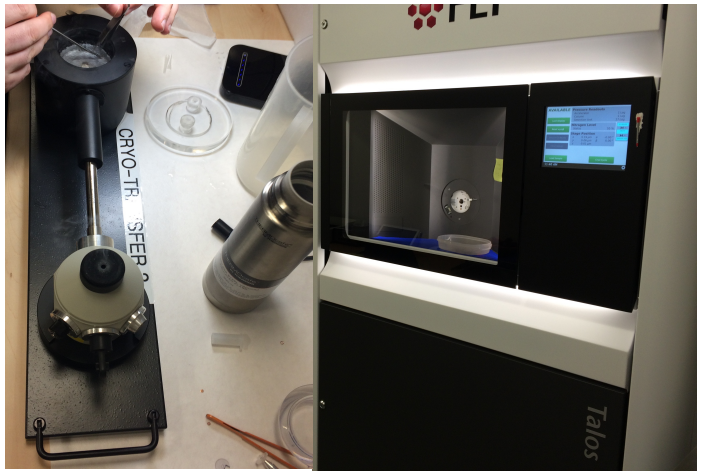




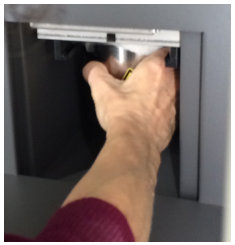
Vitrobot Mark IV

Schreiber *et al*. Nature 2011
De Fonseca *et al*. Nature 2011

Cryo-EM workflow

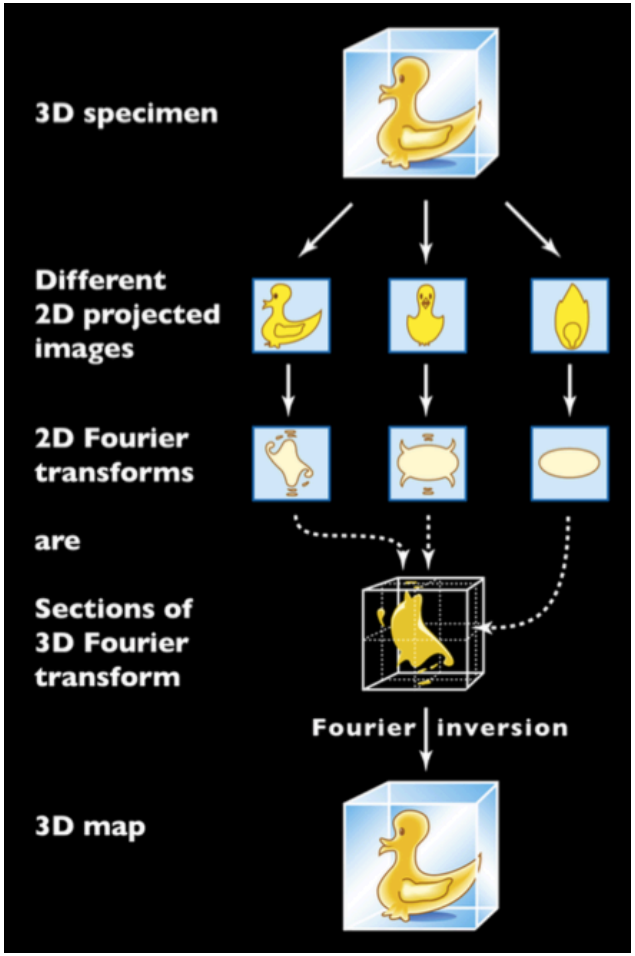


200C



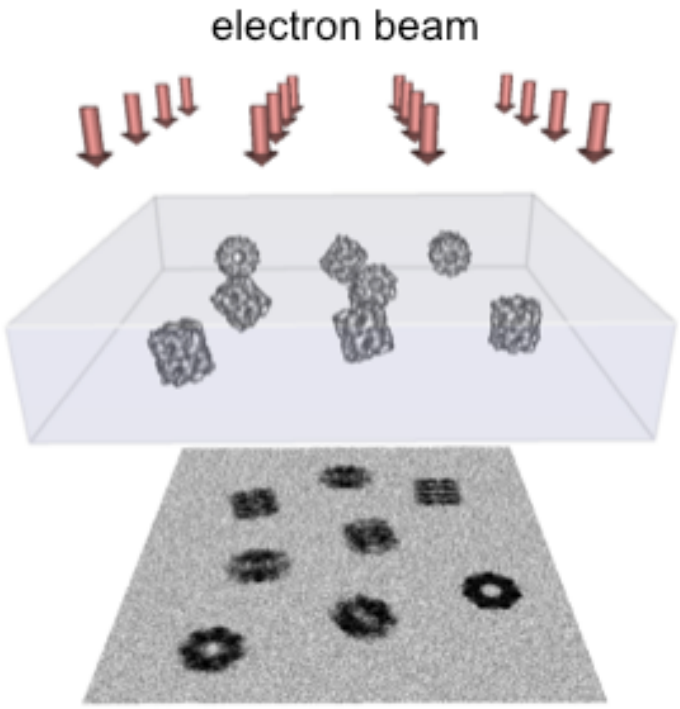
Arctica

Single particle analysis

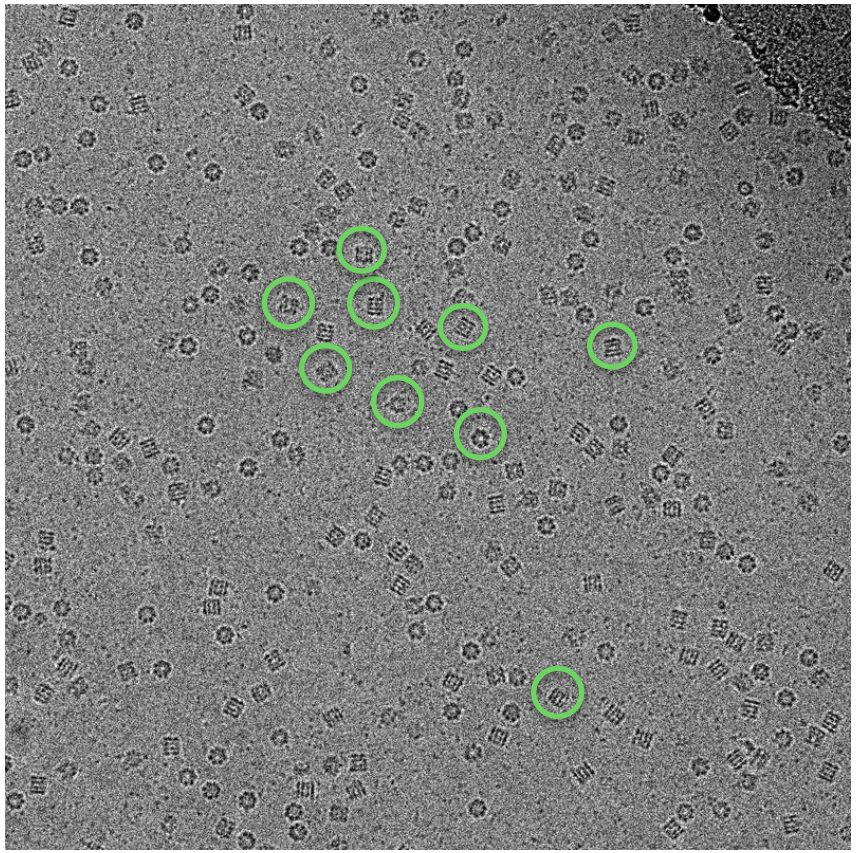


Slide courtesy Tony Crowther

Single particle analysis



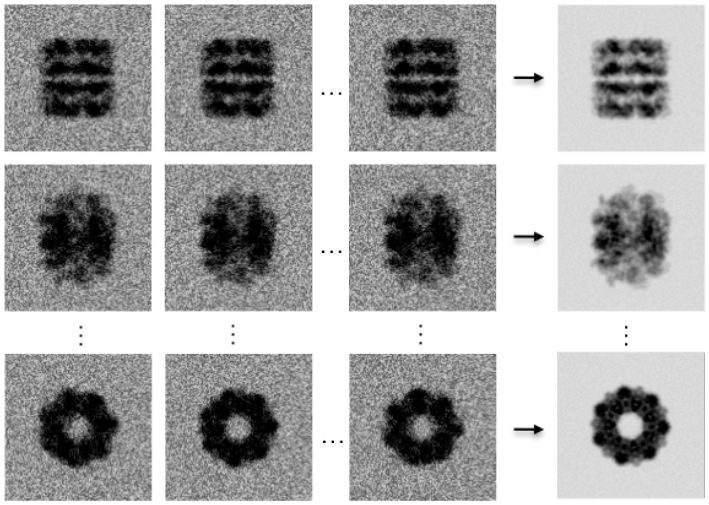
2D projection



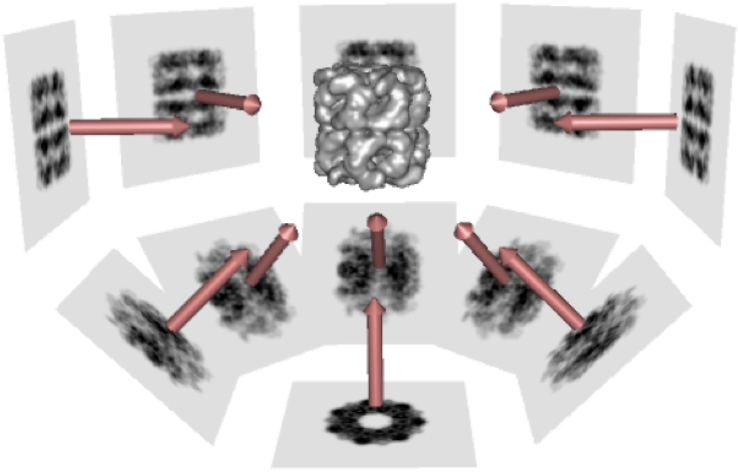
Particle picking

Slide courtesy Greg Pintilie

Single particle analysis



2D class averaging



3D reconstruction

Slide courtesy Greg Pintilie

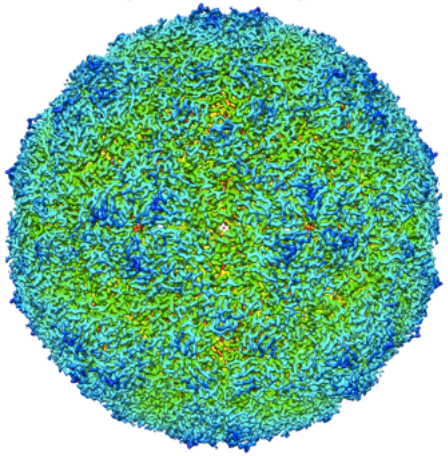
Application of single particle

nature
structural &
molecular biology

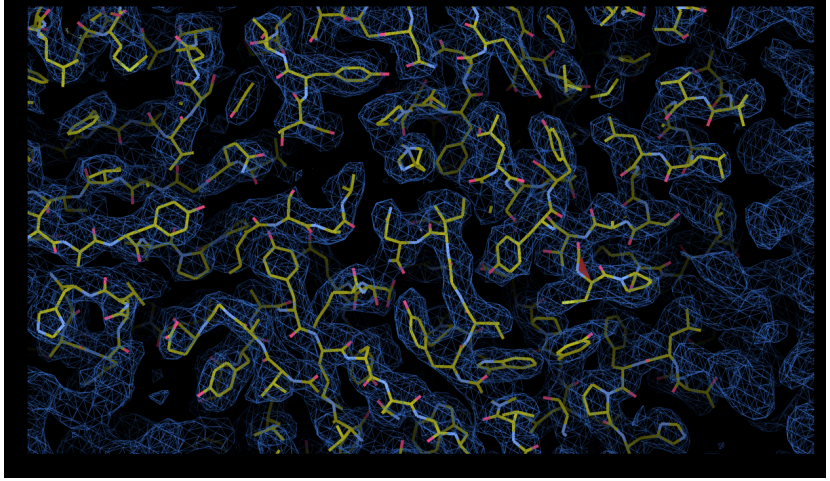
Structure-based energetics of protein interfaces guides foot-and-mouth disease virus vaccine design

Abhay Kotecha[1,8], Julian Seago[2,8], Katherine Scott[3], Alison Burman[2], Silvia Loureiro[4], Jingshan Ren[1], Claudine Porta[1,2], Helen M Ginn[1], Terry Jackson[2], Eva Perez-Martin[2], C Alistair Siebert[1], Guntram Paul[5], Juha T Huiskonen[1], Ian M Jones[4], Robert M Esnouf[1], Elizabeth E Fry[1], Francois F Maree[3,6], Bryan Charleston[2] & David I Stuart[1,7]

2.8Å CryoEM Map of FMDV



High resolution CryoEM Density with FMDV model – 2.8Å



Slide courtesy Abhay Kotecha

Cryo-electron tomography

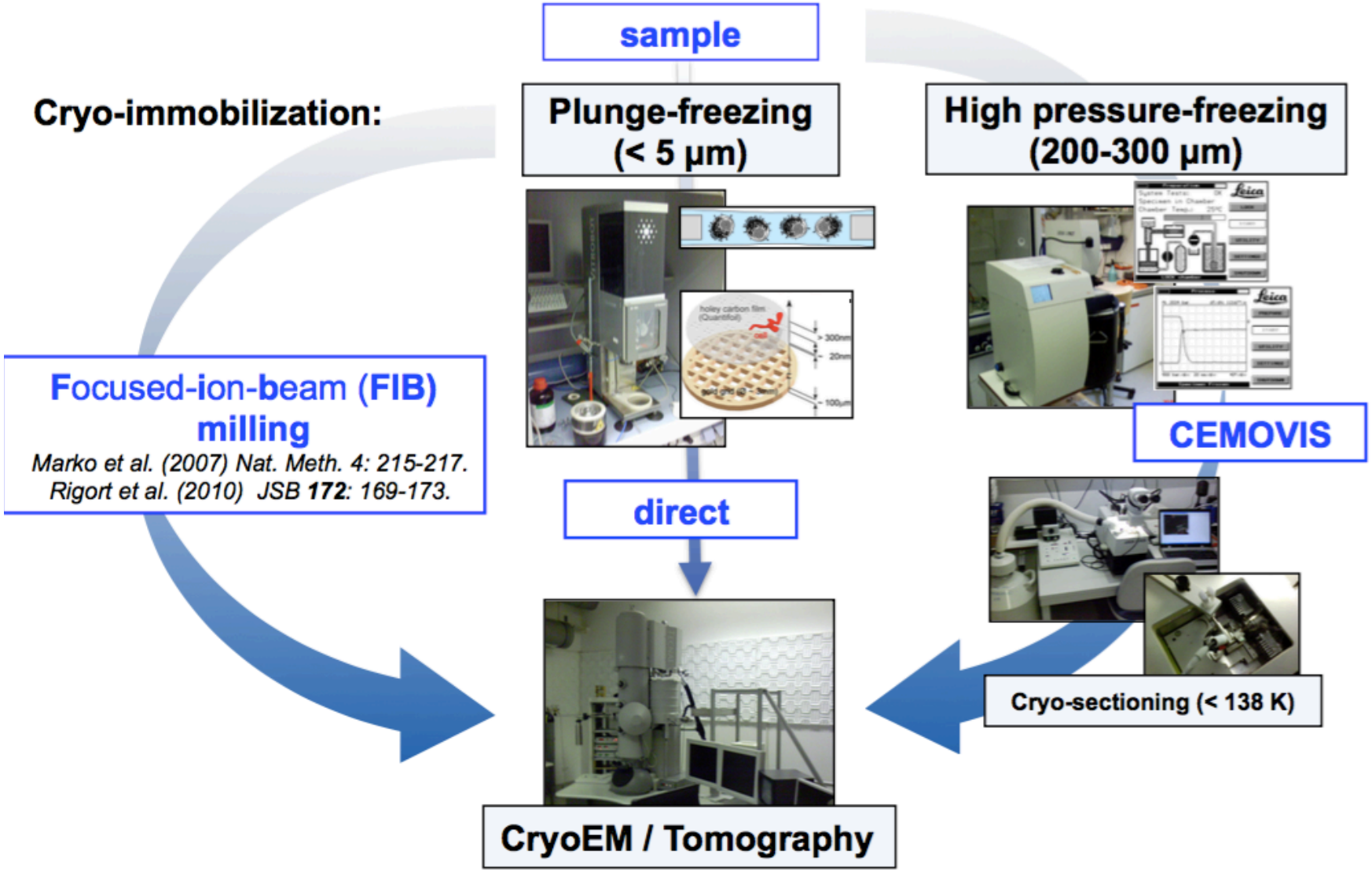
https://www.jove.com/video/1943/electron-cryotomography-of-bacterial-cells

LMB lecture-
Tanmay Bharat

Take home message…

- Cryo-EM can be used to determine structures at native state
- Prepare best sample possible before EM
- Use negative staining: initial screening, homogeneity assessment
- Best use the technologies e.g. microscope, camera & softwares

Be persistent! And you'll get the high resolution information with cryo-EM 😉



sample thickness limit for cryoET: **~ 1 µm** *(Lučić et al. (2005) Ann. Rev. Biochem. 74: 833-865)*

Slide courtesy Tzviya Zeev