Introduction to Electron Microscopes and Imaging Artifacts

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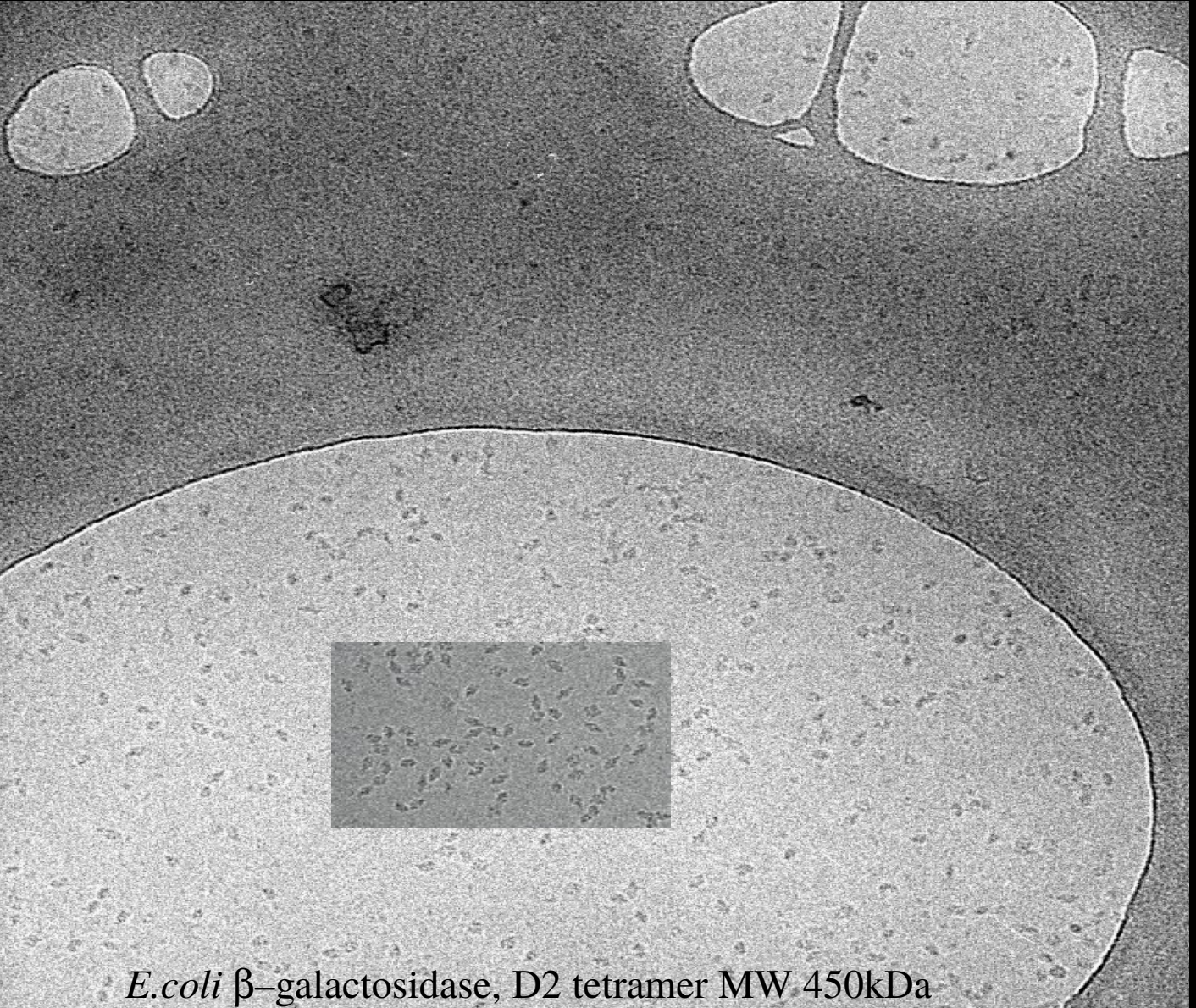
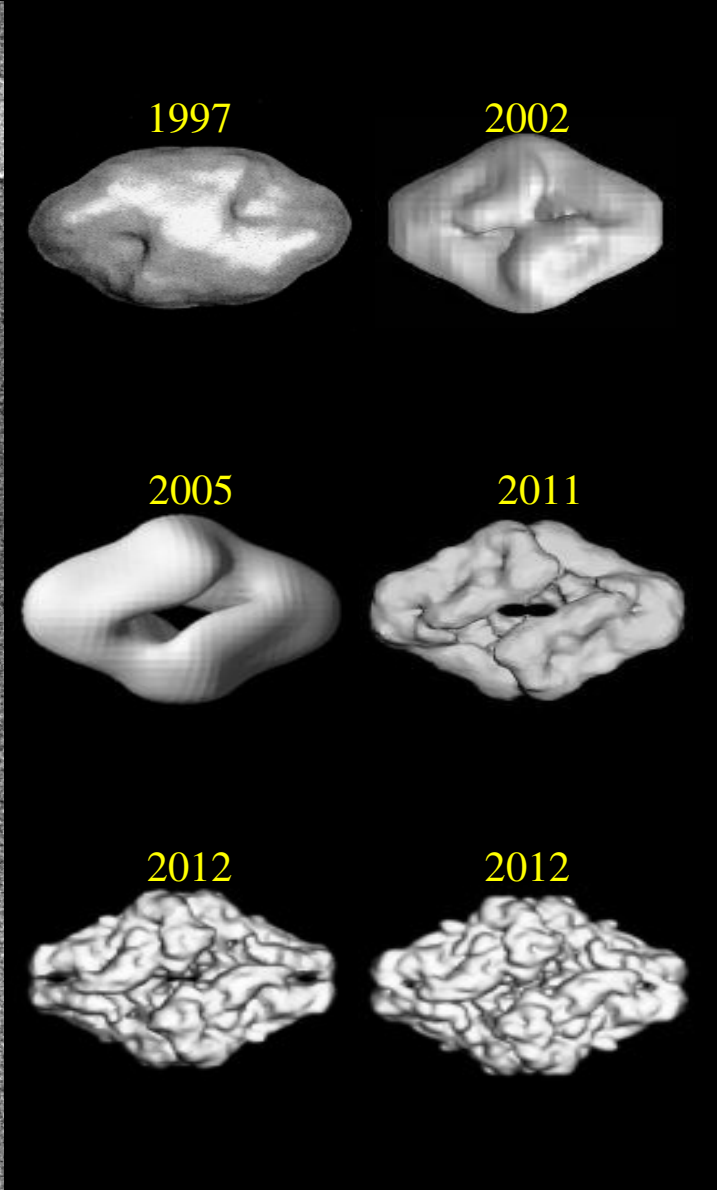
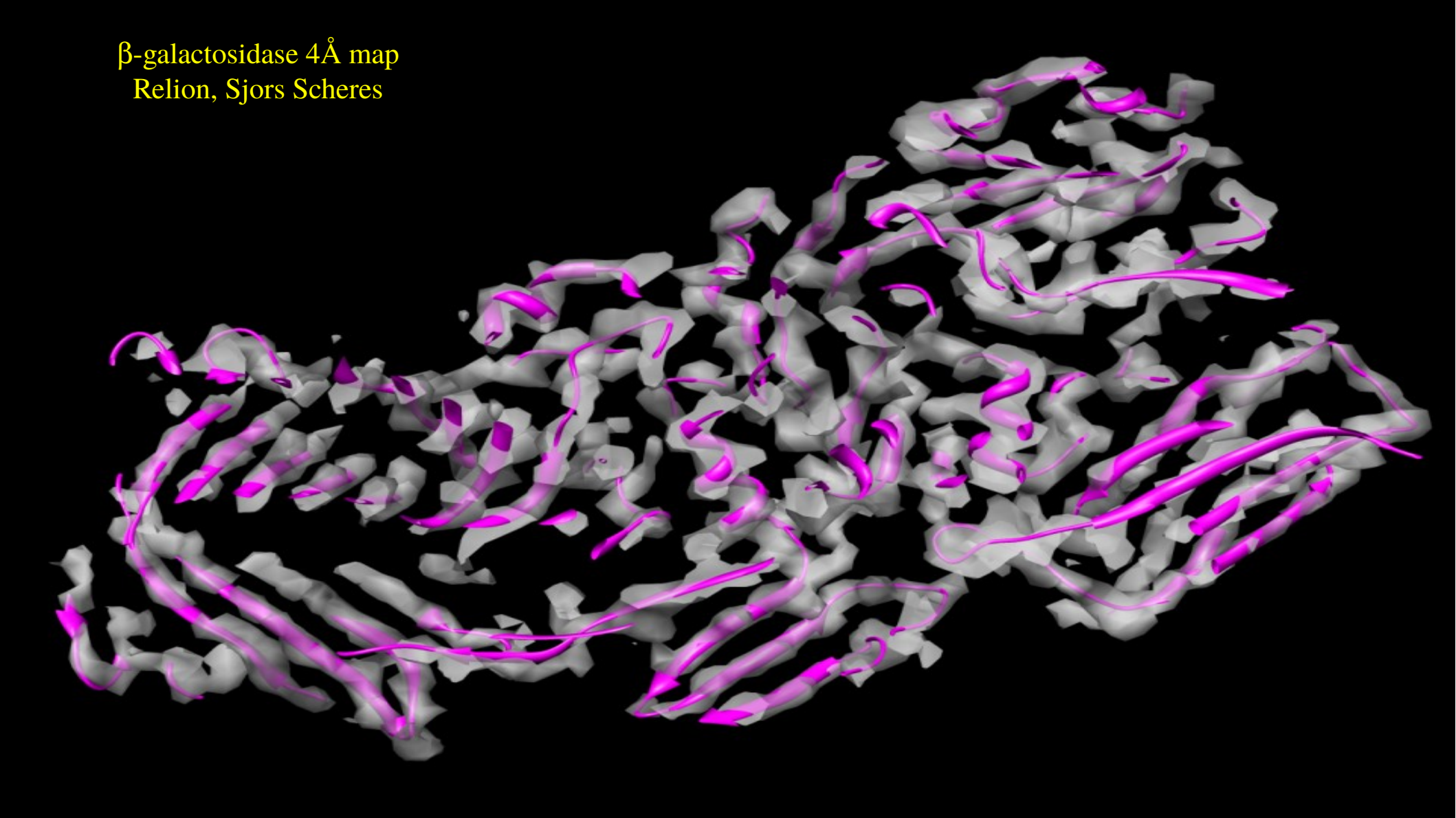


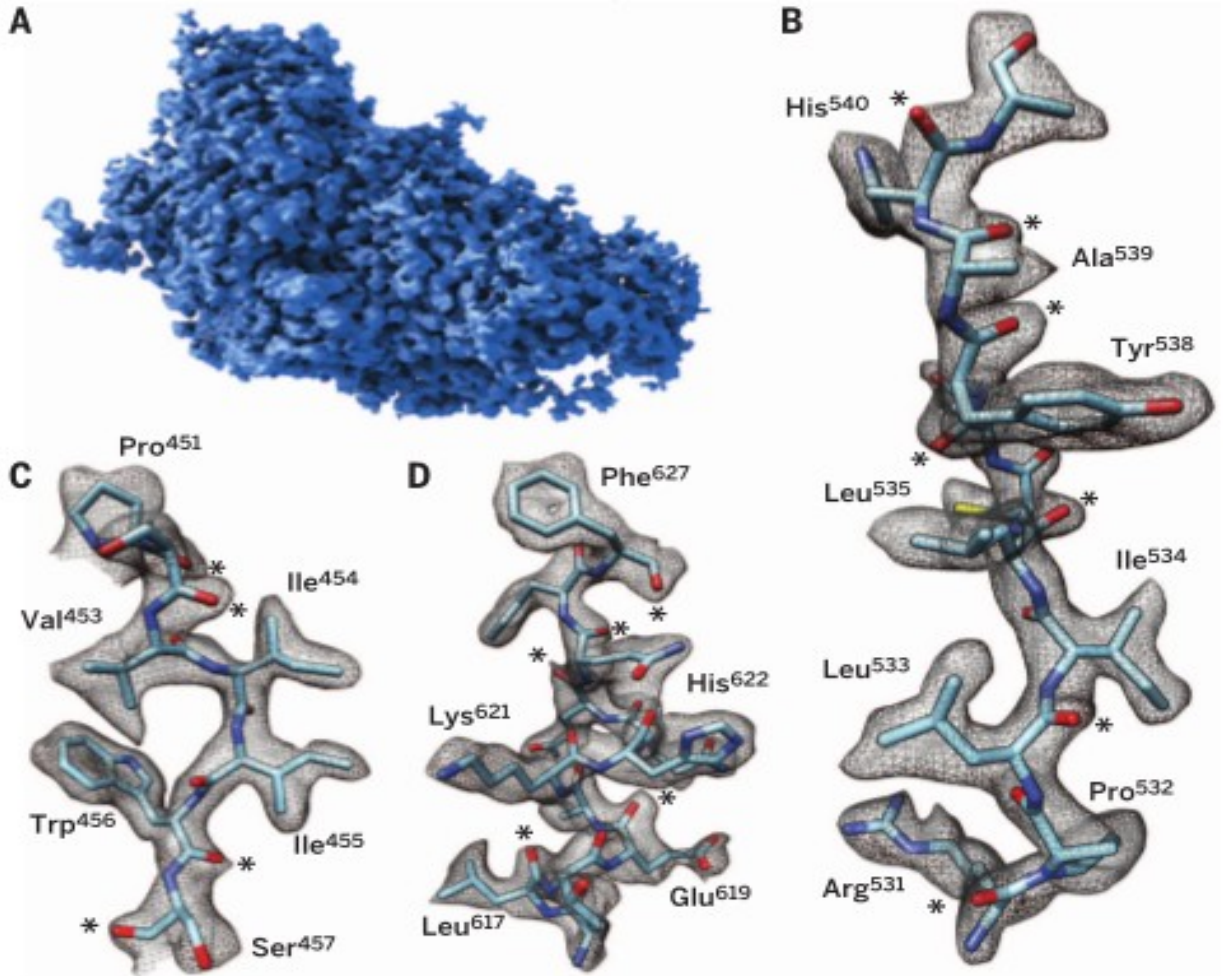
E.coli β–galactosidase, D2 tetramer MW 450kDa




β-galactosidase 4Å map
Relion, Sjors Scheres

β –galactosidase

450kDa, tetrahedral 2.2 Å map,
Bartesaghi et al, Science (2015)



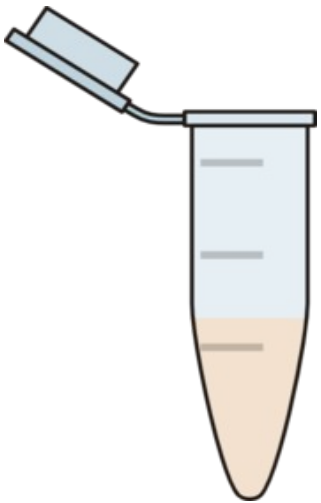
ELECTRON MICROSCOPY

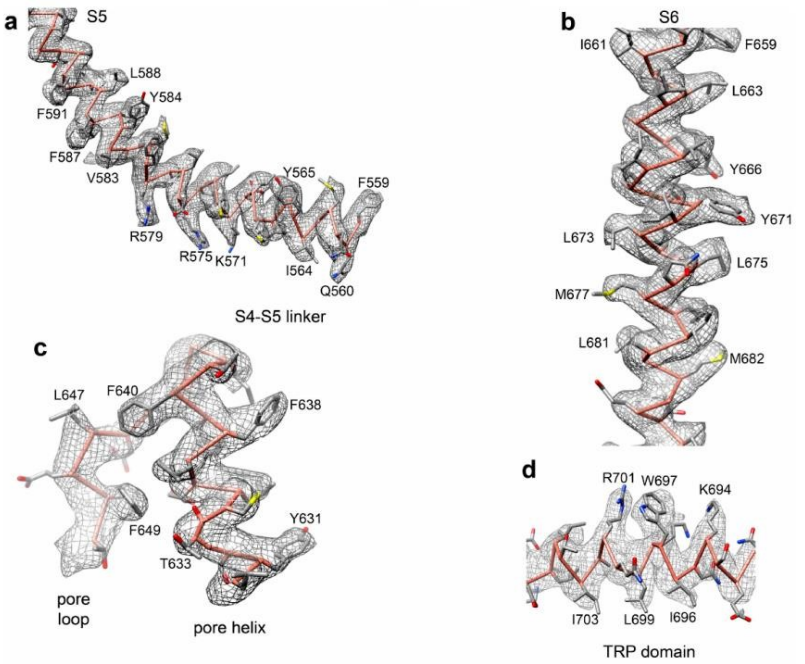
2.2 Å resolution cryo-EM structure of β-galactosidase in complex with a cell-permeant inhibitor

Alberto Bartesaghi,[1]* Alan Merk,[1]* Soojay Banerjee,[1] Doreen Matthies,[1] Xiongwu Wu,[2] Jacqueline L. S. Milne,[1] Sriram Subramaniam[1]†

Cryo–electron microscopy (cryo-EM) is rapidly emerging as a powerful tool for protein structure determination at high resolution. Here we report the structure of a complex between *Escherichia coli* β-galactosidase and the cell-permeant inhibitor phenylethyl β-D-thiogalactopyranoside (PETG), determined by cryo-EM at an average resolution of ~2.2 angstroms (Å). Besides the PETG ligand, we identified densities in the map for ~800 water molecules and for magnesium and sodium ions. Although it is likely that continued advances in detector technology may further enhance resolution, our findings demonstrate that preparation of specimens of adequate quality and intrinsic protein flexibility, rather than imaging or image-processing technologies, now represent the major bottlenecks to routinely achieving resolutions close to 2 Å using single-particle cryo-EM.

Sample → Microscope → Structure





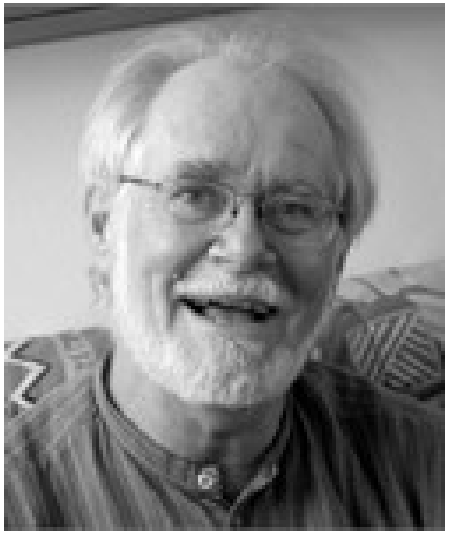
Structure of TRPV1 channel
Liao et al, Nature (2013) **504**, 107-112

Quarterly Review of Biophysics **21**, 2 (1988), pp. 129–228
Printed in Great Britain

Cryo-electron microscopy of vitrified specimens

JACQUES DUBOCHET[1], MARC ADRIAN[2], JIIN-JU CHANG[3], JEAN-CLAUDE HOMO[4], JEAN LEPAULT[5], ALASDAIR W. McDOWALL[6] AND PATRICK SCHULTZ[4]

European Molecular Biology Laboratory (EMBL), Postfach 10. 2209, D-6900 Heidelberg, FRG



NATURE VOL. 308 1 MARCH 1984

Cryo-electron microscopy of viruses

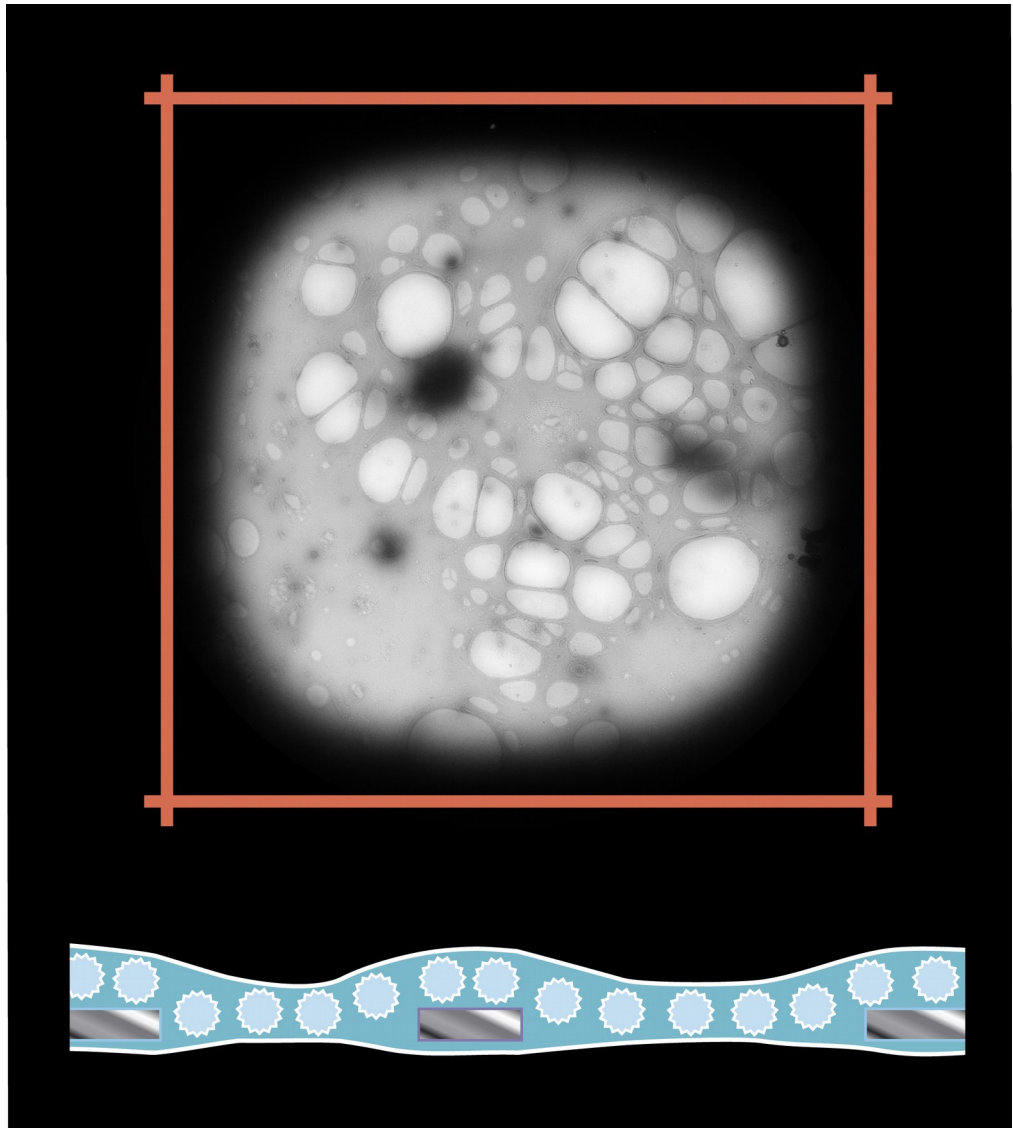
Marc Adrian, Jacques Dubochet, Jean Lepault & Alasdair W. McDowall

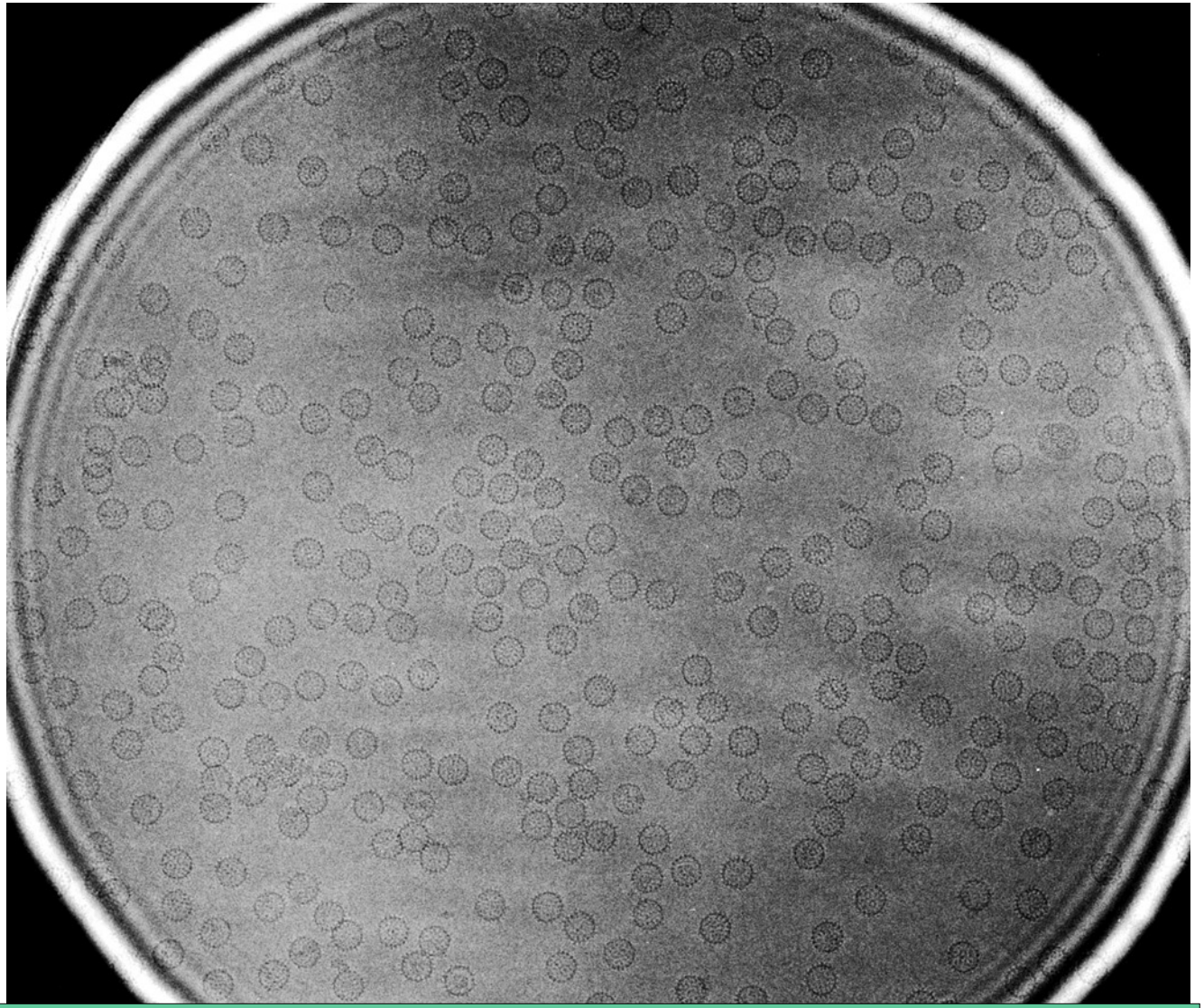
European Molecular Biology Laboratory, Postfach 10.2209, D-6900 Heidelberg, FRG

Thin vitrified layers of unfixed, unstained and unsupported virus suspensions can be prepared for observation by cryo-electron microscopy in easily controlled conditions. The viral particles appear free from the kind of damage caused by dehydration, freezing or adsorption to a support that is encountered in preparing biological samples for conventional electron microscopy. Cryo-electron microscopy of vitrified specimens offers possibilities for high resolution observations that compare favourably with any other electron microscopical method.


INOX.


TESTED FOR ELECTRICAL SAFETY
DC 27/04
SERIAL NO.
MYC
13880
Water Top Up Indicator
the de-ion water
ONLY!





Boettcher, Wyne and Crowther *Nature* **386** (1997) 88-91

Why use electrons?

- Photon wavelengths are too short and interactions too weak to see individual molecules.
- Electron wavelengths are ~10^5 times smaller e.g. 300 keV electron has a wavelength of 0.02Å.
- Strong lens aberrations
- Short wavelength peaks scattering in forward direction

Problems with electrons

- Radiation damage to sample
- Charging
- Expensive machines

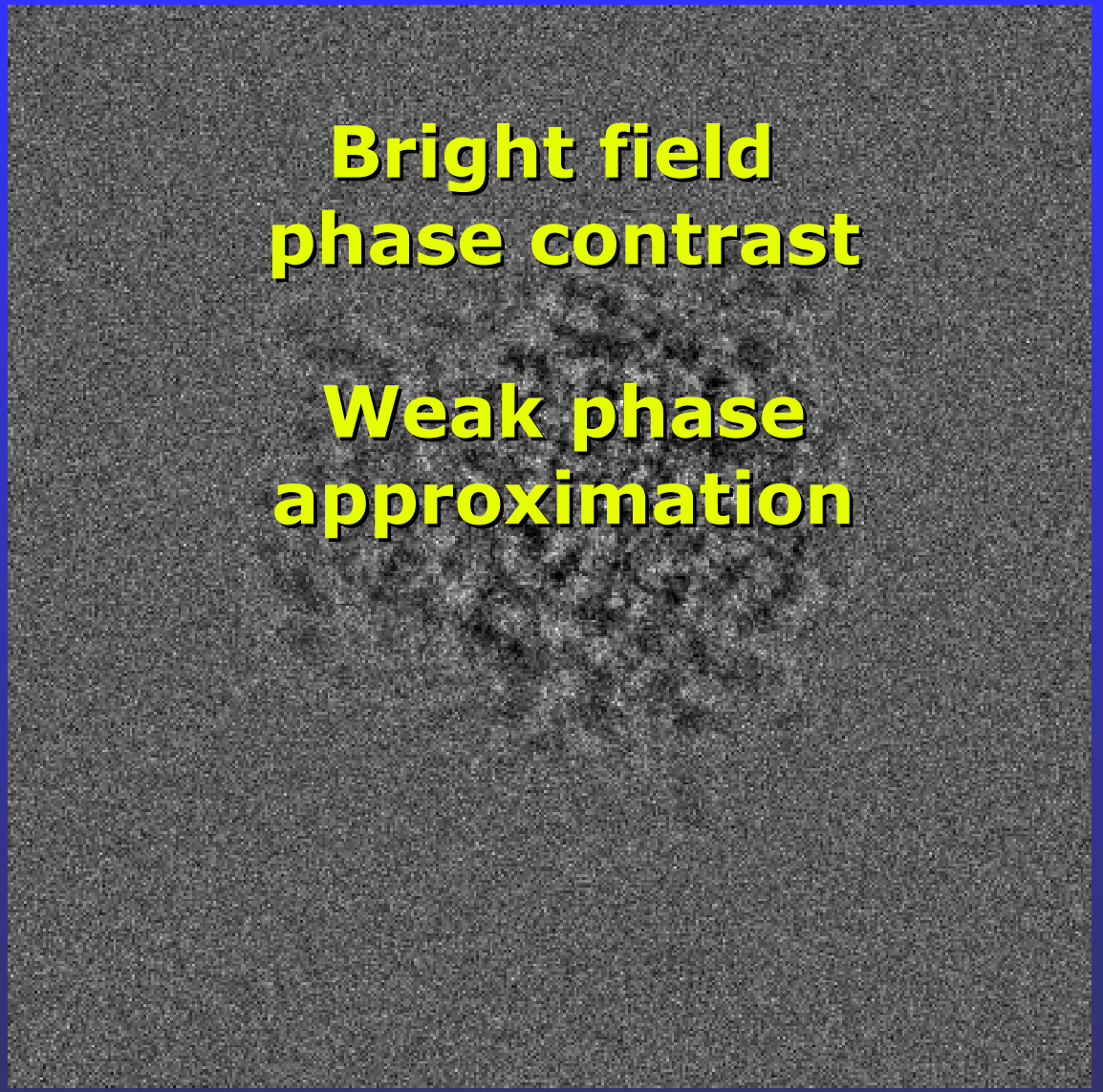
Perfect image

Perfect detector

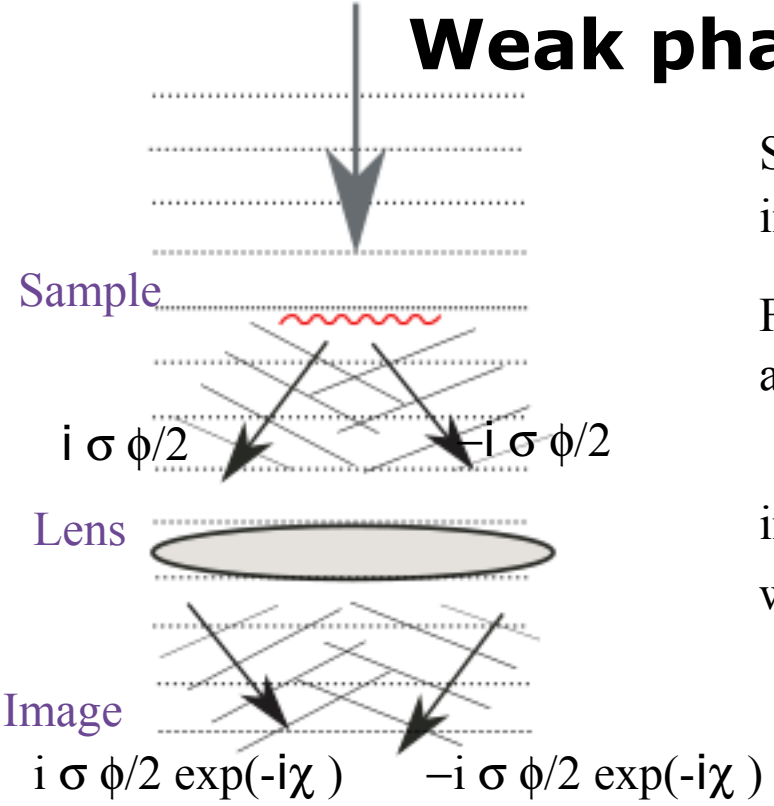
Perfect sample

20 el/pixel

2 μm defocus



Weak phase approximation (simplified)



Scattered component of wavefunction is small and given by $i\sigma\phi$ in which ϕ is the projected potential and $\sigma=2\pi me\lambda/h^2$.

Focusing wavefunction back to image plane introduces a phase shift of $\exp(-i\chi)$ where:

$$\chi(\theta) = 2\pi (\Delta_f\theta^2/2 - C_s\theta^4/4)/\lambda$$

in which Δ_f is the defocus, C_s the spherical aberration, λ the wavelength and θ the scattering angle ($\theta= \lambda/d$).

Wavefunction at image plane is

$$\Psi \sim 1 - i\sigma\phi \exp(-i \chi)/2 + i\sigma\phi \exp(-i \chi)/2$$

so image i.e, probability of an electron arrival, is

$$|\Psi|^2 \sim 1 - 2\sigma\phi \sin(\chi)$$

Contrast Transfer Function

Recorded image is “almost” the projection of the atomic potential.

Simplest approxiamtion the spatial frequencies are mutiplied by

$$-\sin(2\pi (\Delta_f \theta^2/2 - C_s \theta^4/4)/\lambda)$$

Note: 4Å with 300 keV electrons λ=0.0197Å and $\theta = 0.0197/4.0 = 4.9$ mrad = 0.28 degrees.

Erickson Klug, *Phil. Trans. Roy. Soc. London.* B. **261** (1971) 105-118.

Wade, *Ultramicroscopy* **46** (1992) **134**-156.

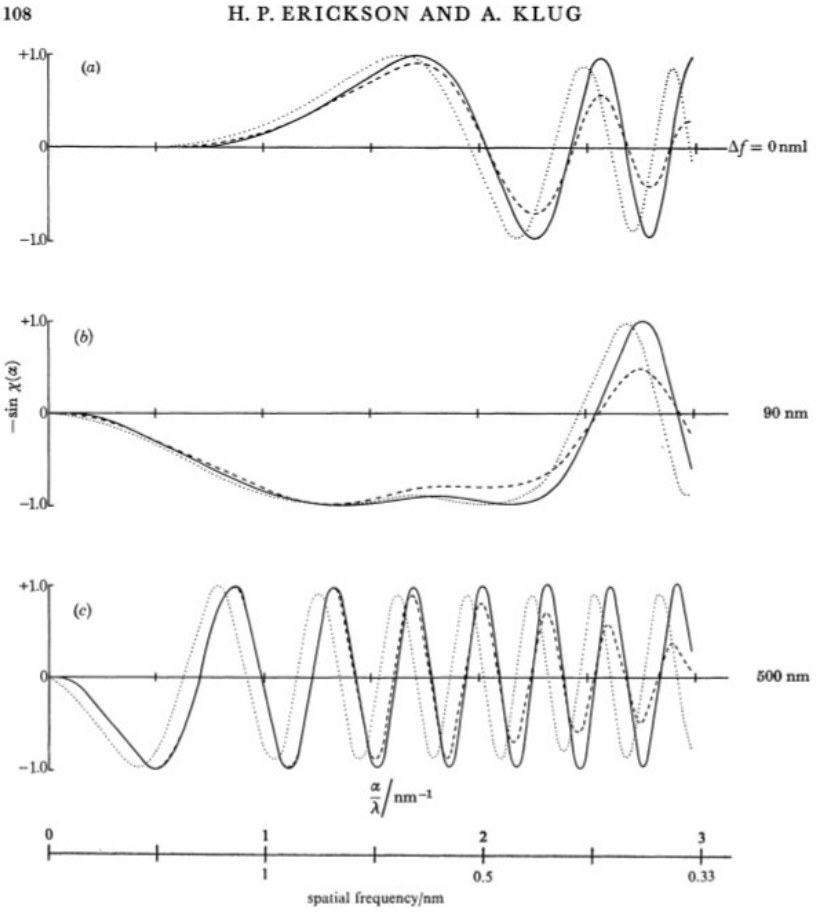
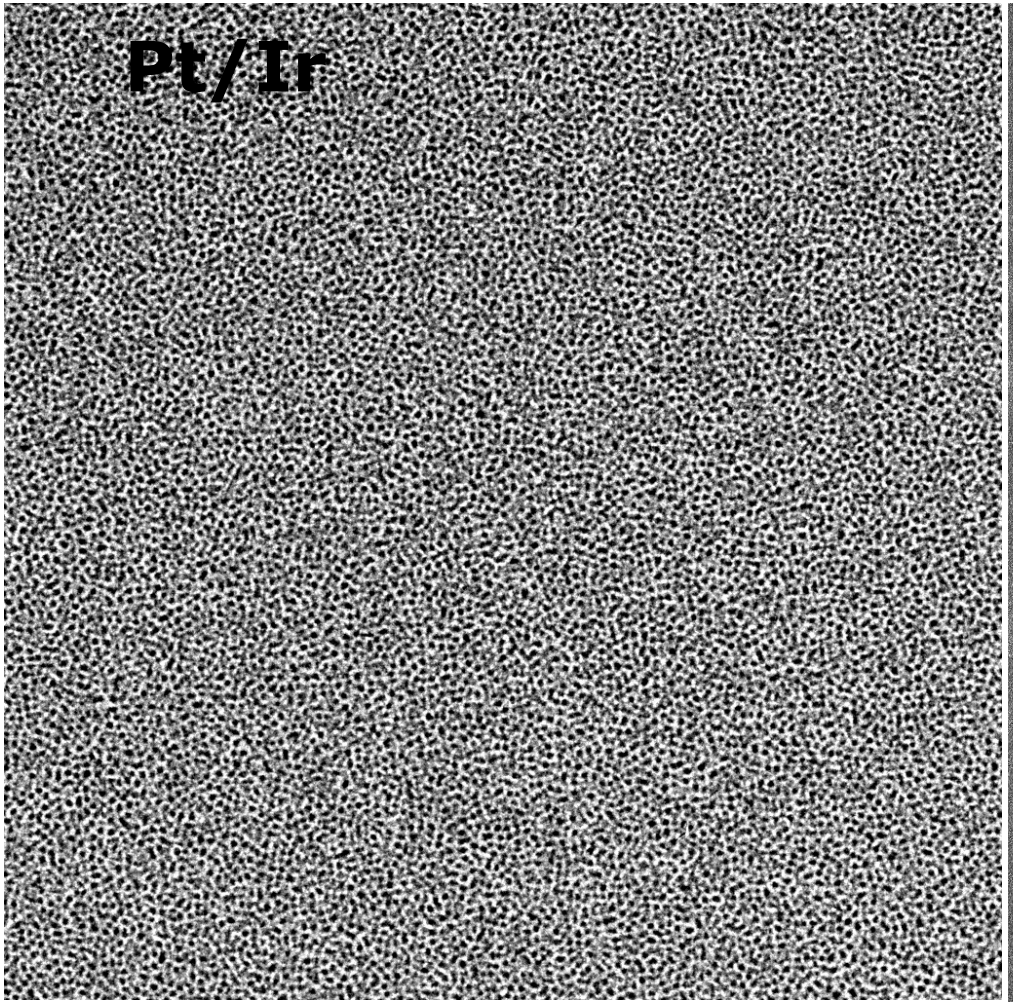
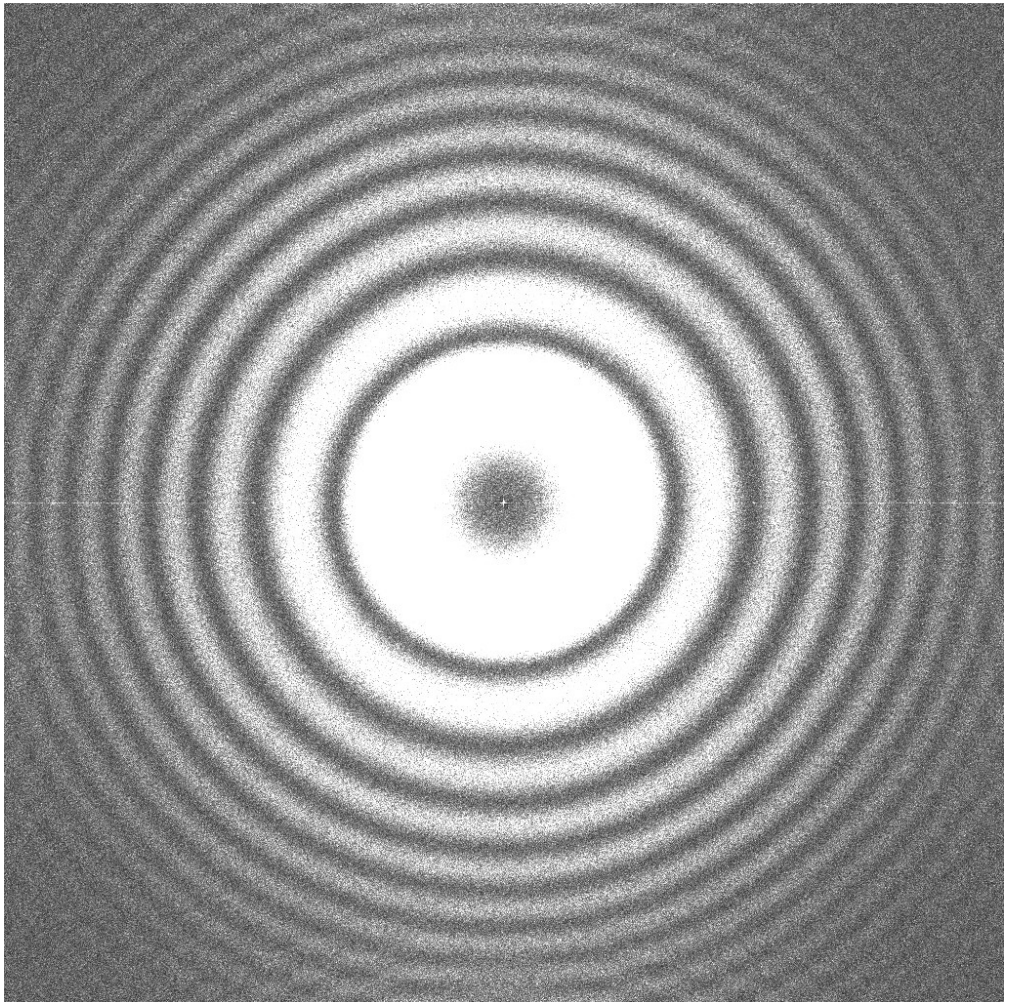


FIGURE 1. The phase-contrast transfer function, $-\sin\chi(\alpha)$, plotted as a function of α/λ, in nm^{-1}, for $\lambda = 0.0042$ nm, $C_s = 1.3$ mm, and for the indicated values of Δf. A negative value of this function implies that the corresponding region of the object transform is contributing to the image with normal contrast, i.e. a subtraction from the background electron intensity over regions of high mass density. The solid curves are for pure phase contrast. The dashed curves are corrected for the effects of chromatic aberration with normal electrica instabilities, by averaging over a range of Δf of ± 20 nm. The dotted curves are corrected for the effects of the partial coherence of the electron source, assuming a 100 μm diameter condenser aperture. These corrections are discussed by Erickson (1971).

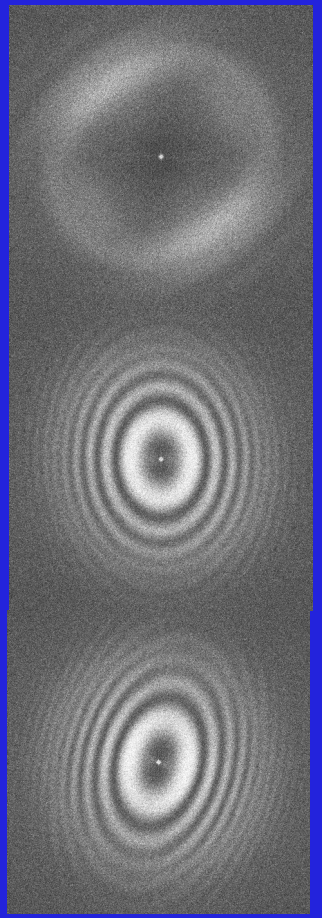




Falcon III, 128 e/pixel

Noise whitened power spectra

Daily use of Thon Rings



Alignment:

Pivot points

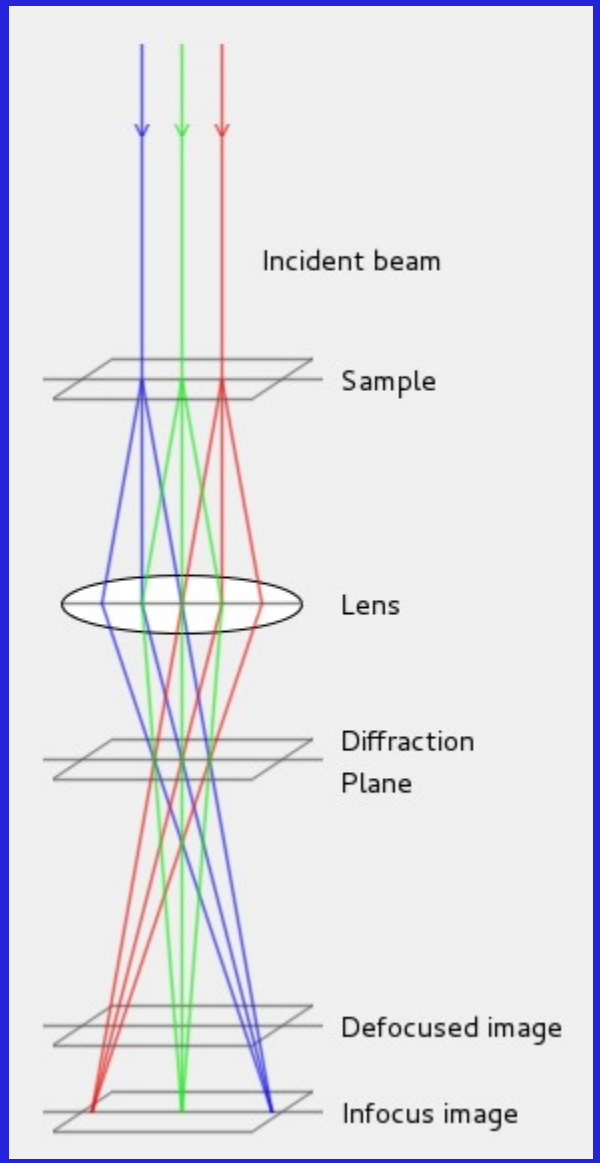
Astigmatism

Beam tilt

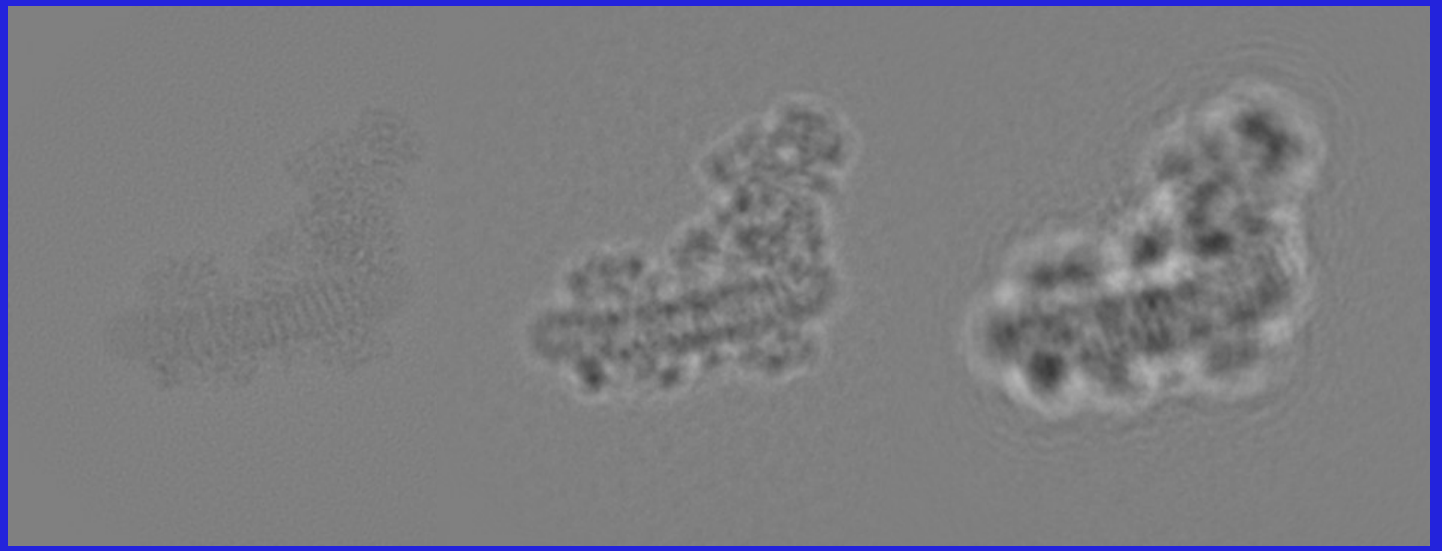
Is the weak phase approximation valid?

- To date it works (at least to 3Å).
- Single scattering assumed, but if there is enough contrast to see the particle there will be multiple scattering.
- Problems such as Ewald sphere (difference in defocus through the sample) or convergent illumination, don't seem to hurt much (given enough particles).

CryoEM Image Formation



Bright field phase contrast



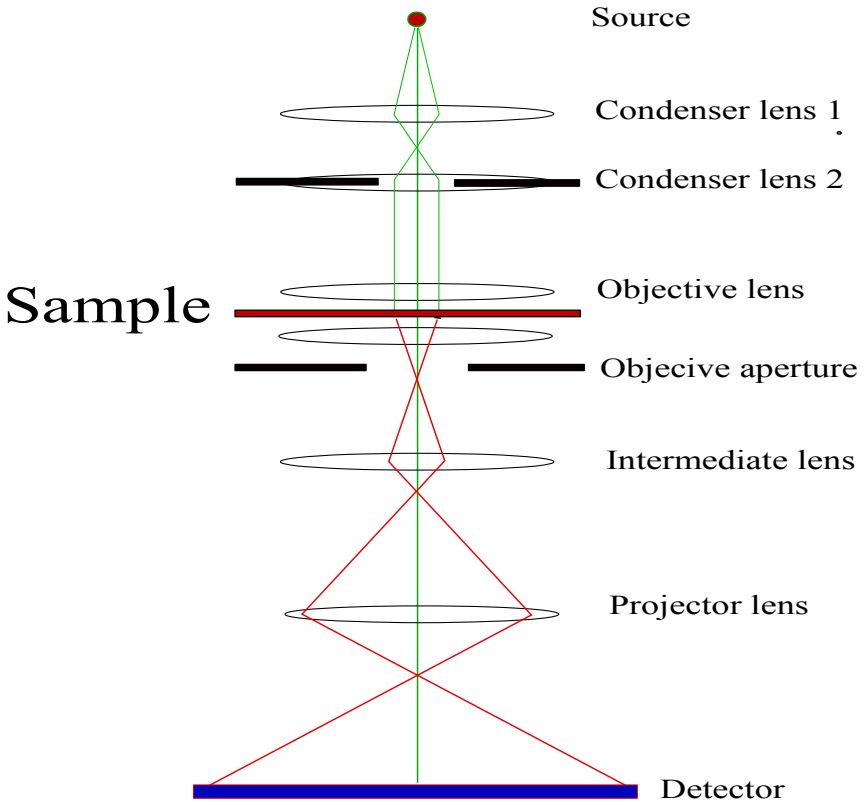
In focus *1 um* *3 um*

Computationally correct for CTF

- Known functional form
- Know C_s, set defocus.
- Fill in zeros with images of different defocus.
- Problem: real defocus is not what is says on the screen.

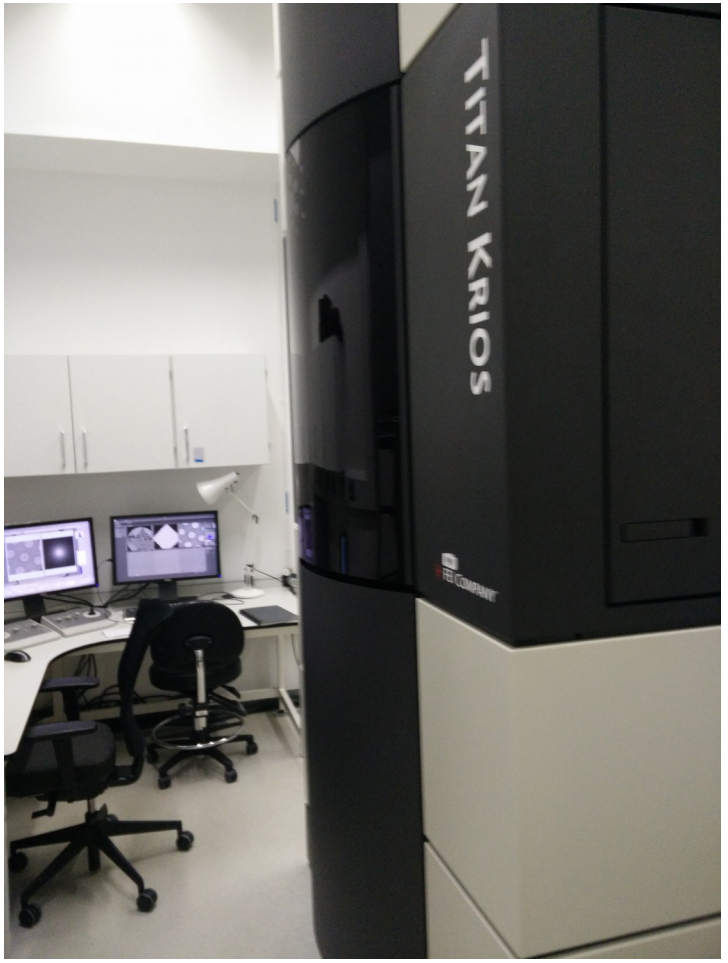
- Measure actual defocus from the images themselves.
- With new detectors you can do movie collection with long exposures and determine the CTF accurately.
- Sample height variation effects the magnification.

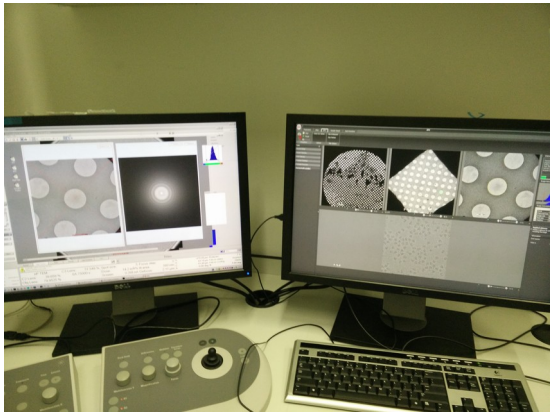
Transmission Electron microscope (bright field phase contrast)



- Condenser lens combination sets virtual source position.
- Condenser aperture set illumination angle
- Objective aperture provides a high frequency cut-off.
- Objective aperture provides a source of secondary electrons to help neutralise charge. build up on sample.

Which microscope?

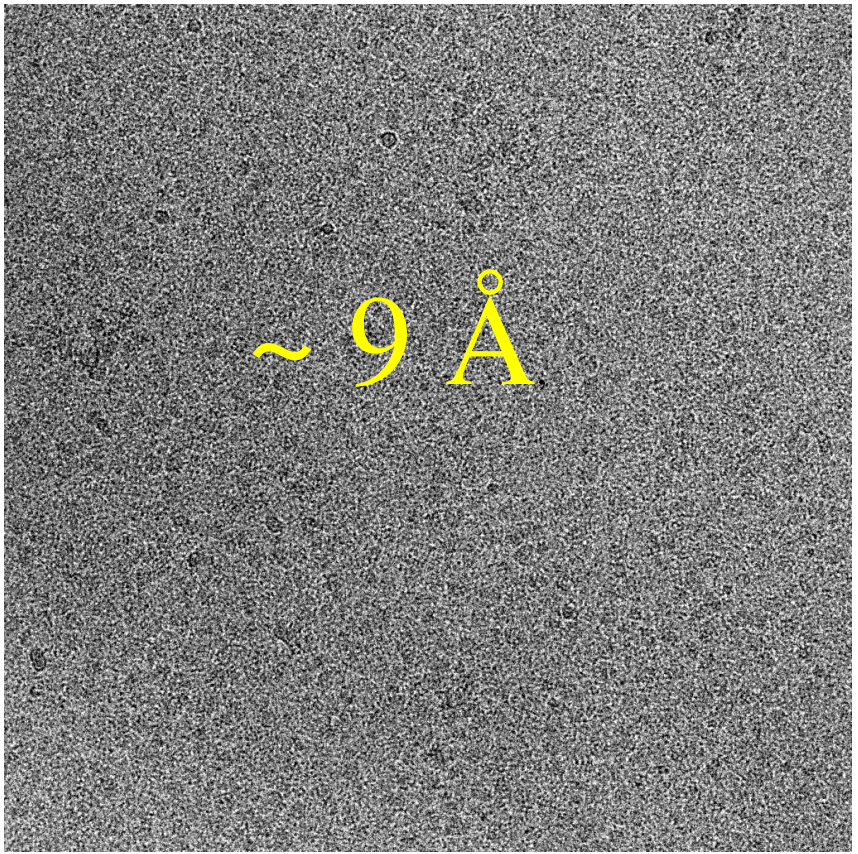


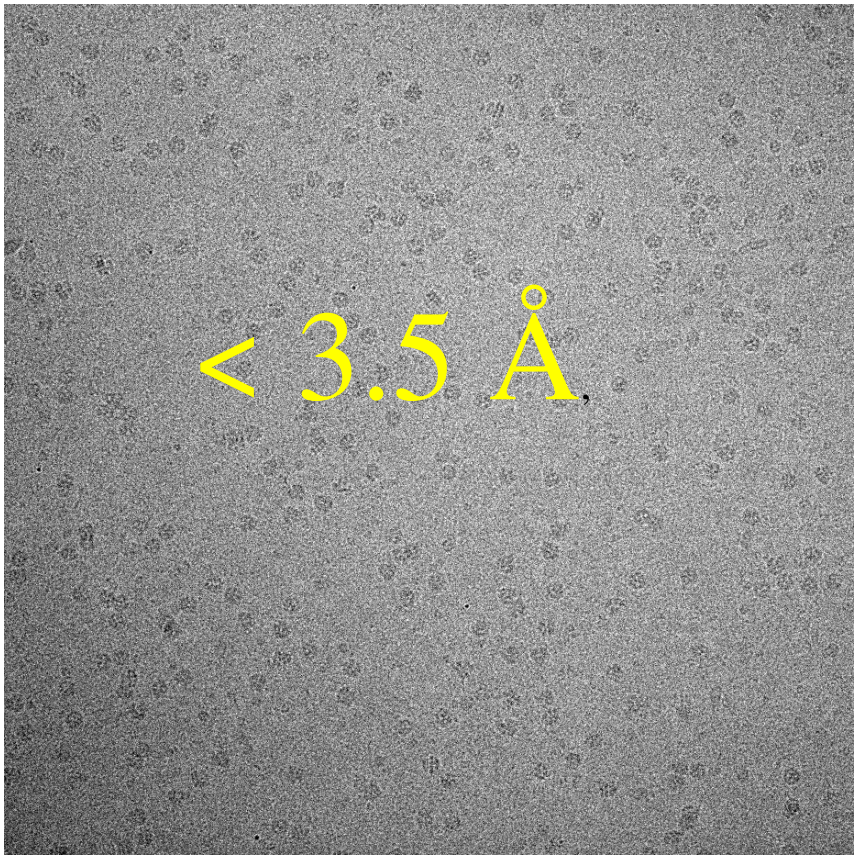


What matters in a microscope?

- Coherence (FEG)
- Contamination rate
- Drift
- Ease of use:
  - Constant power lens
  - Triple condenser lens
  - Integration
  - Automation

Sample, Sample, Sample, Sample, Sample

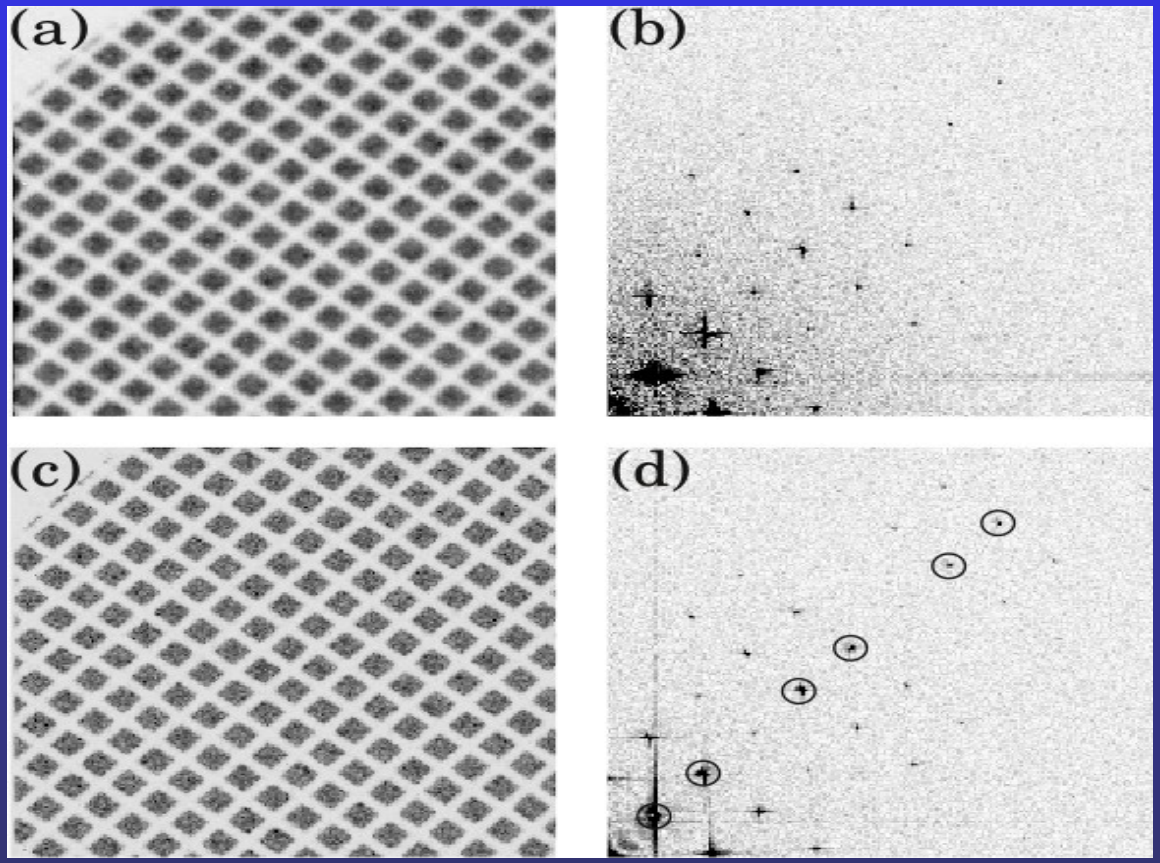




Christos Savva, MRC-LMB

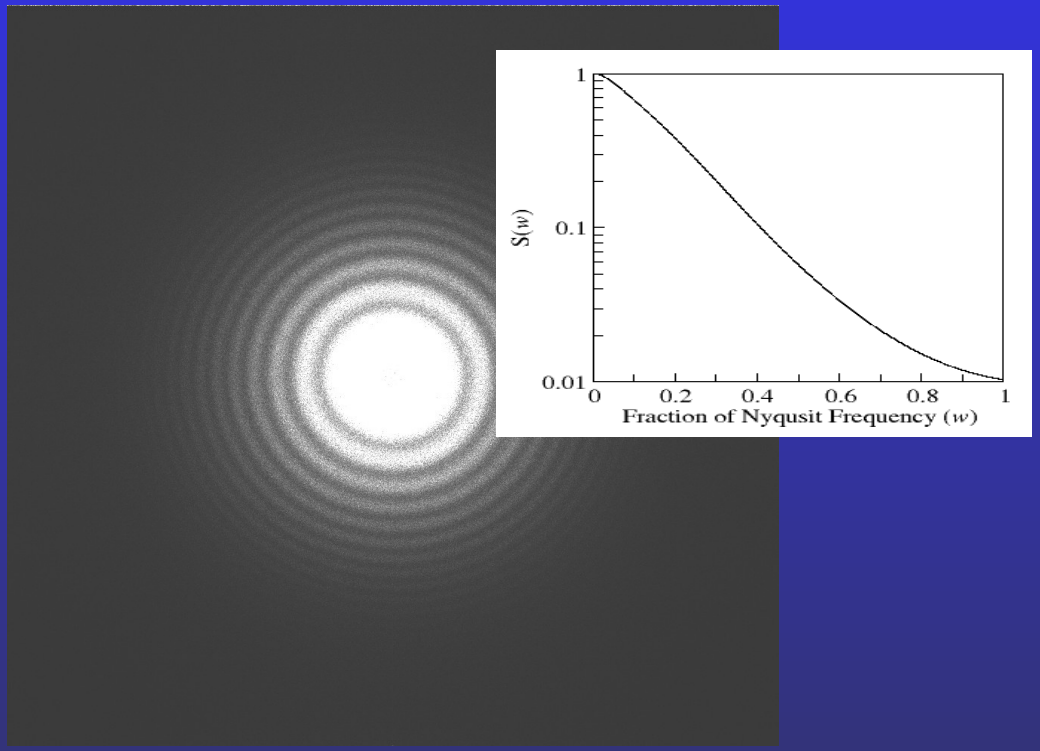
Detectors: DQE and MTF

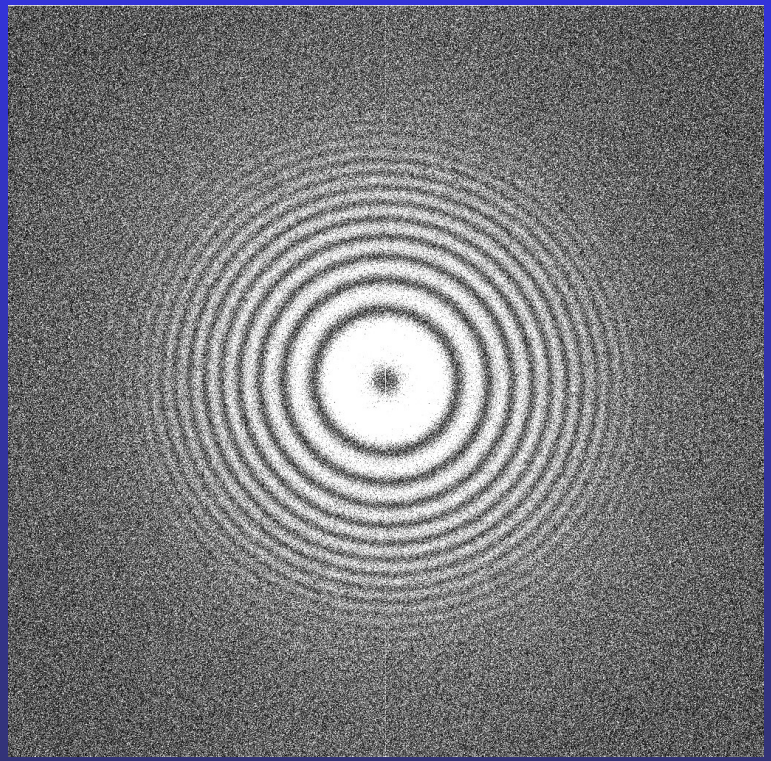
- Shadow image of a grid
- Same Detector
- (a)-(b) Integrating
- (c)-(d) Counting



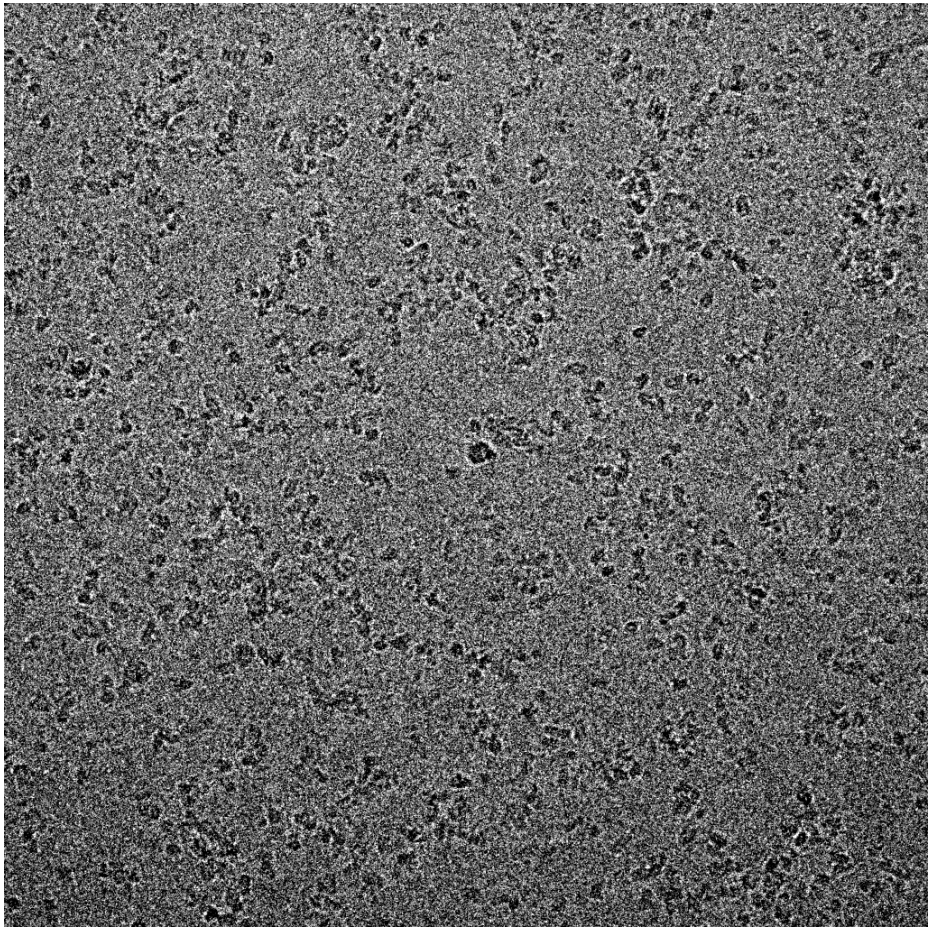
McMullan, Clark, Turchetta, Faruqi, *Ultramicroscopy* **109** (2009) 1411-16

Noise whiten Falcon II

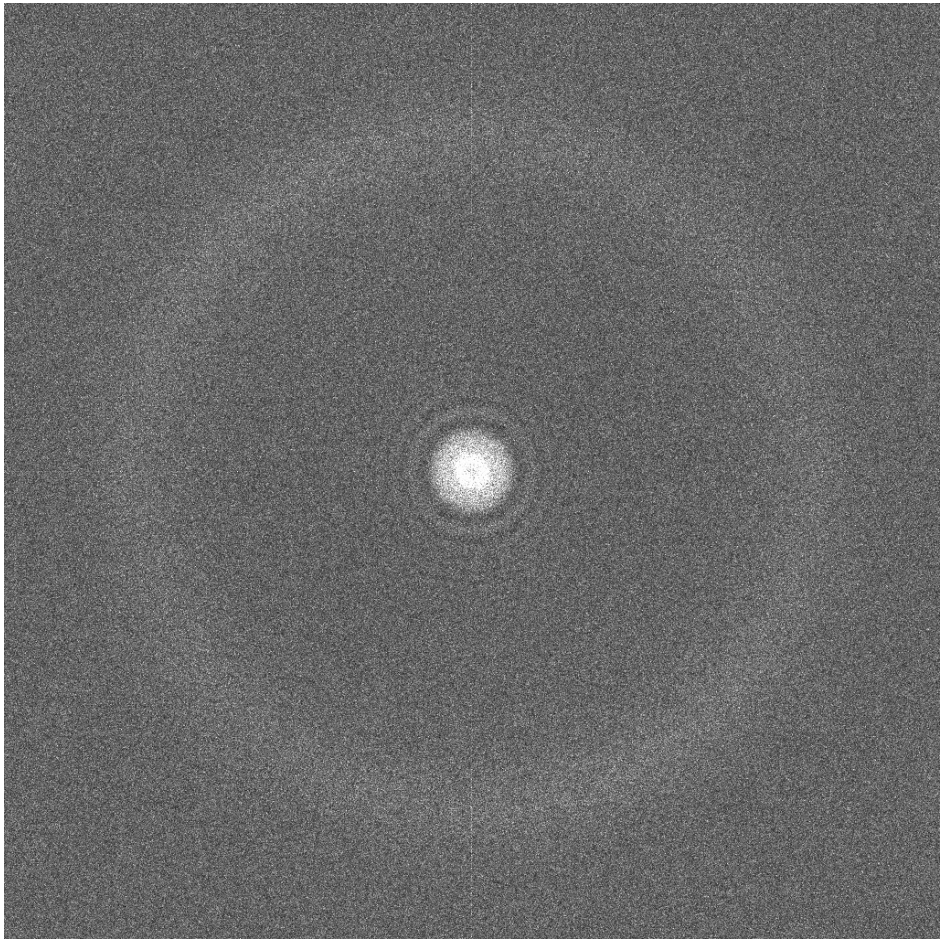




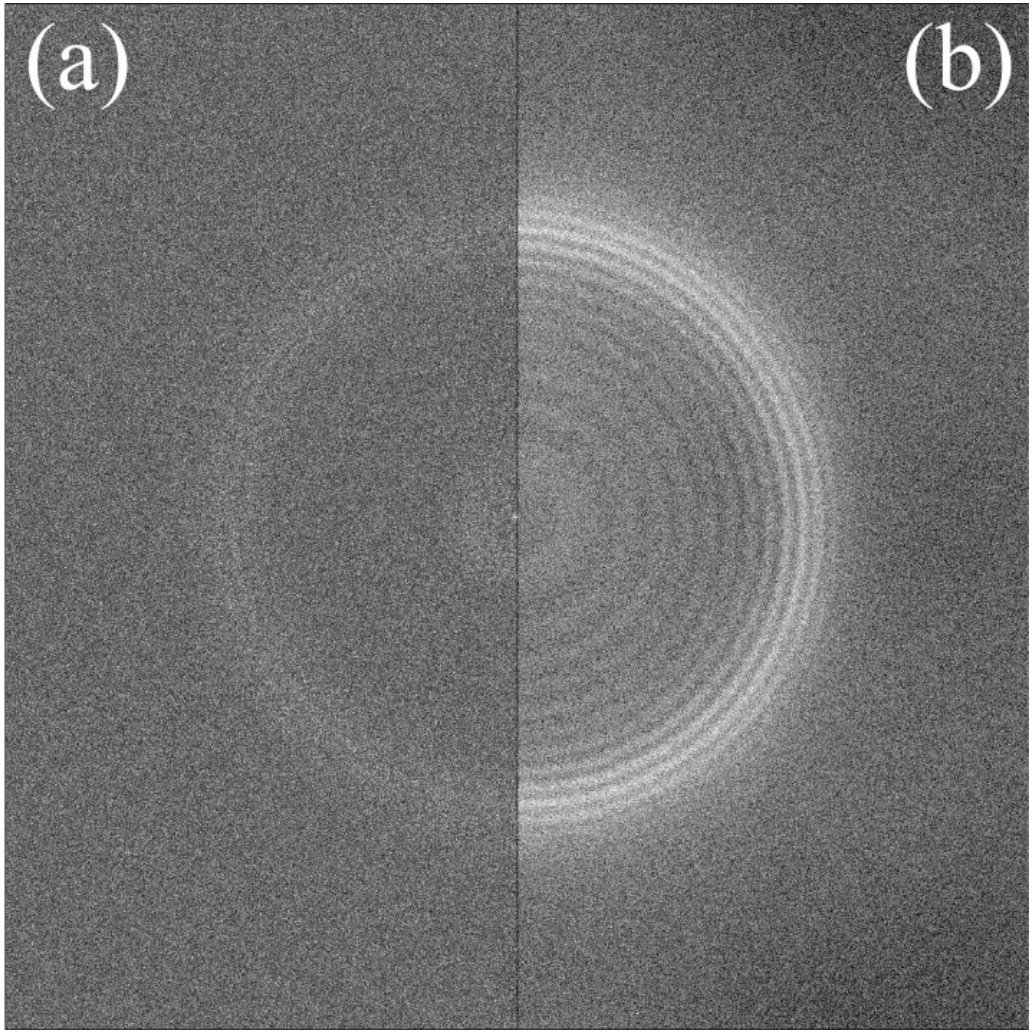
Should also work with DE, no need with Gatan K2



Falcon II, ~100 e/pixel



Noise whitened power spectra



Thon rings from double distilled water

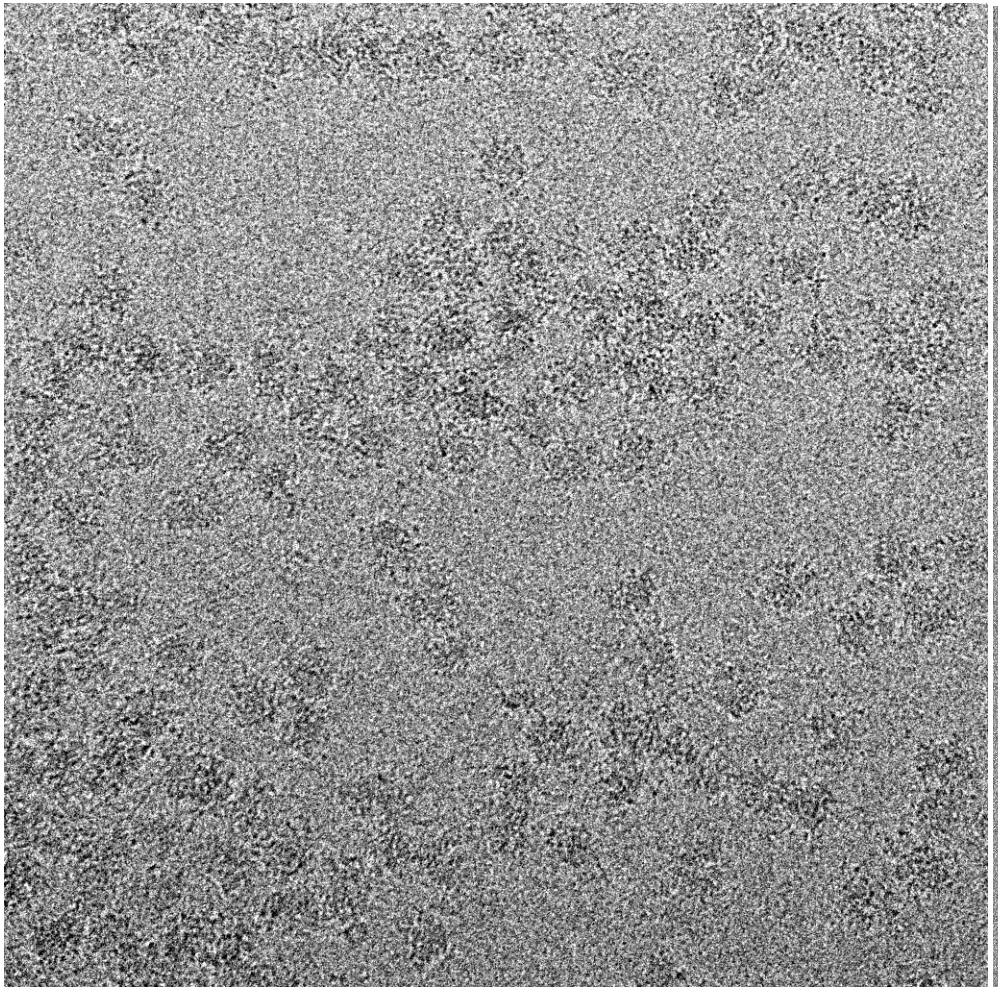
Noise whitened power spectra

Falcon II, ~328 e/pixel, 141 frames

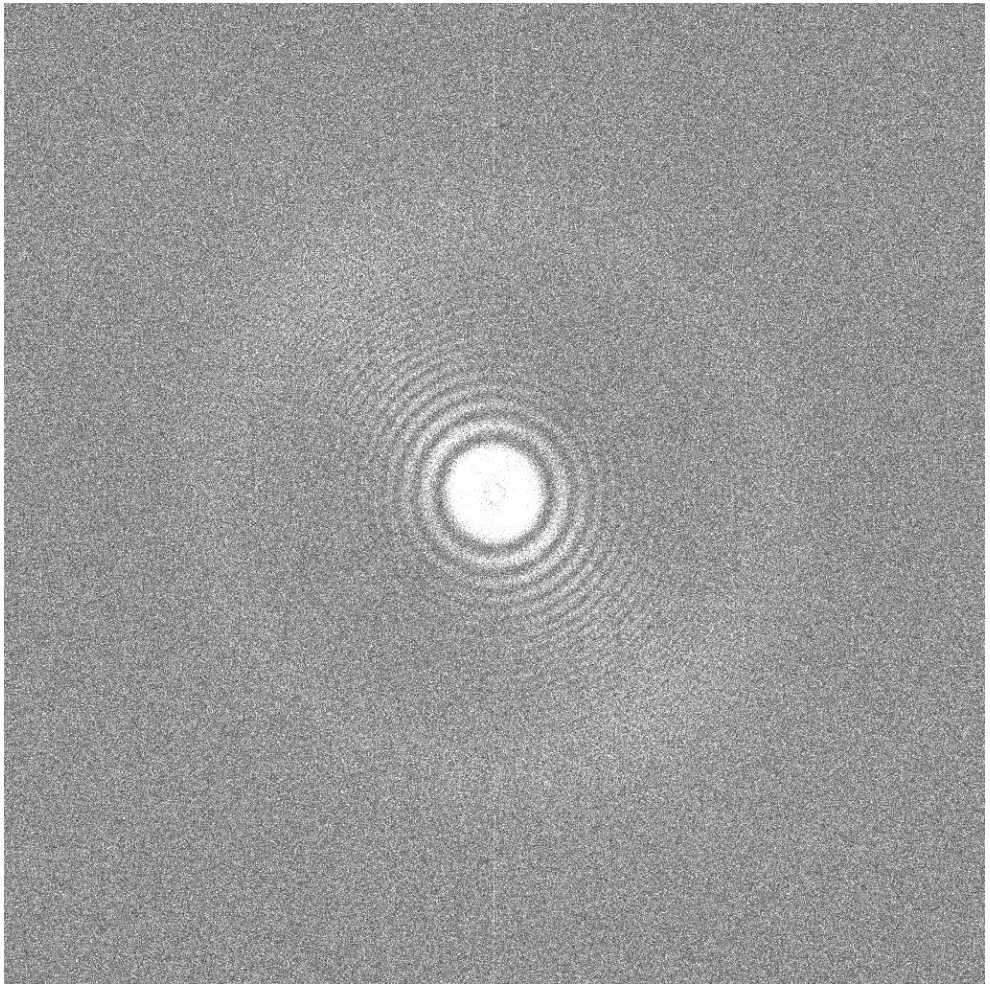
(a) Power spectra of summed image

(b) Sum of power spectra of individual frames

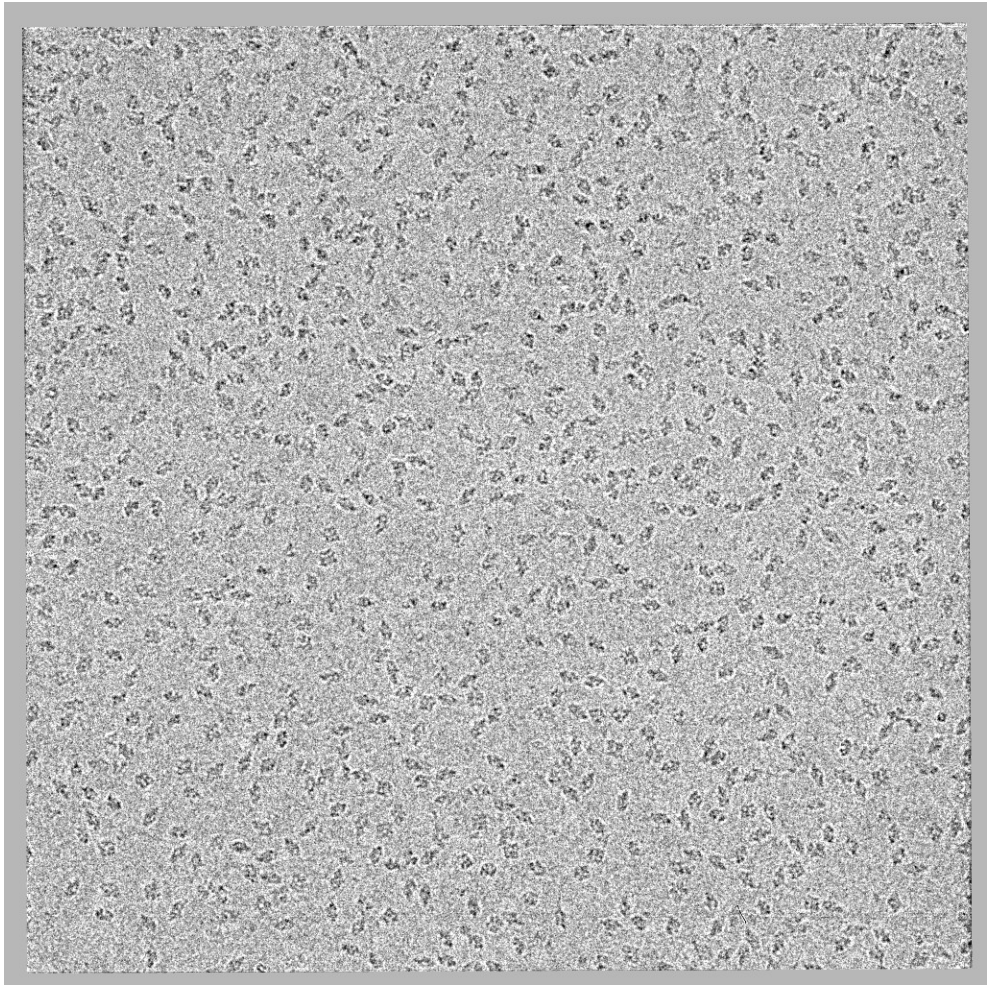
McMullan, Vinothkumar, Henderson *Ultramicroscopy* **158** (2015) 26-32



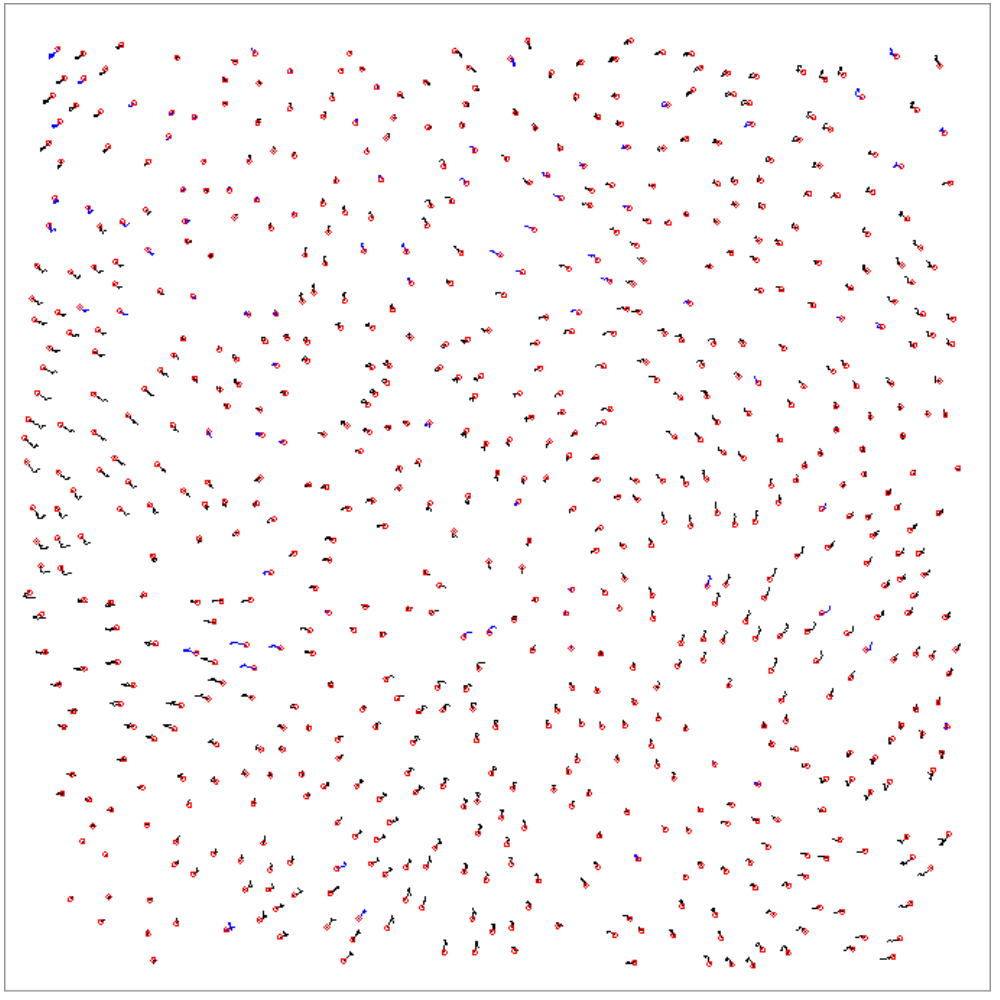
Falcon II, 28 e/pixel



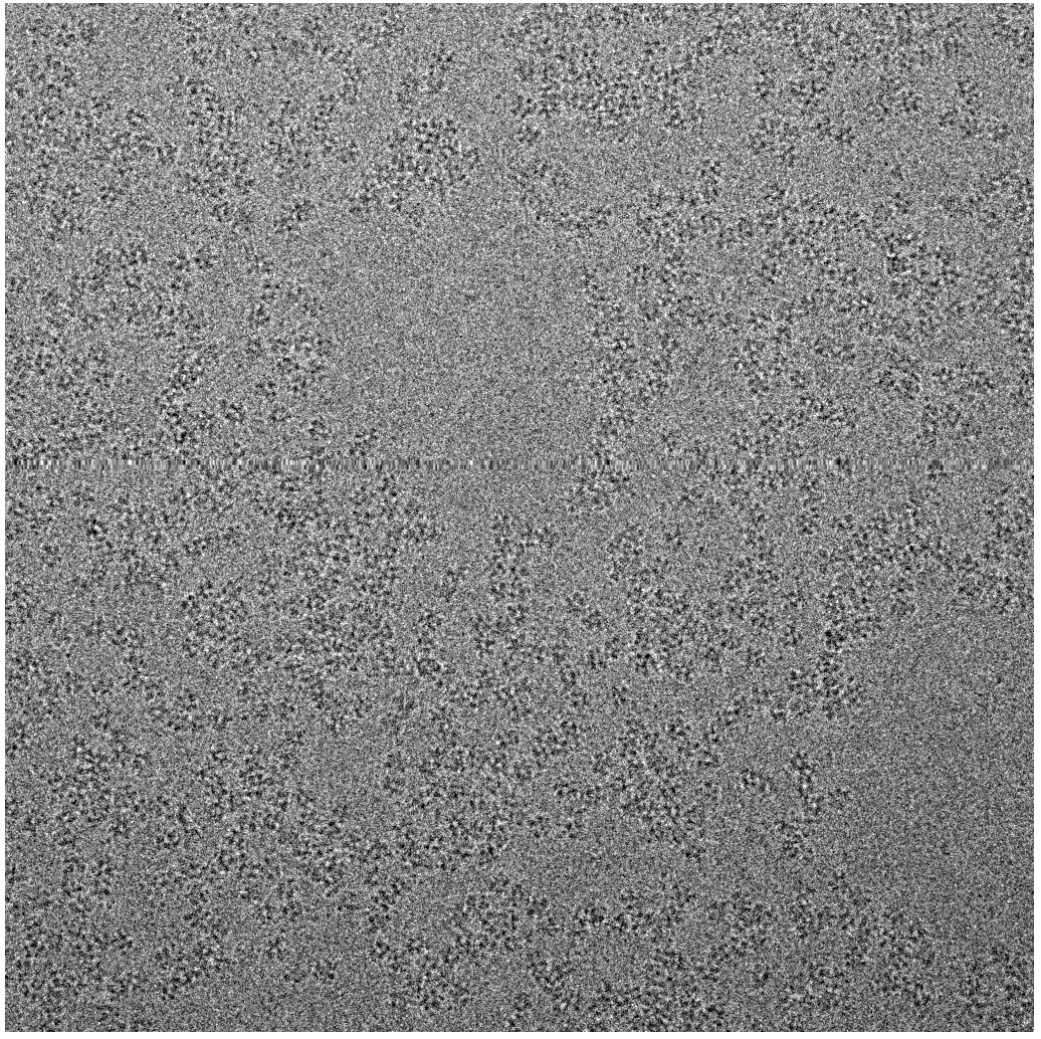
Noise whitened power spectra

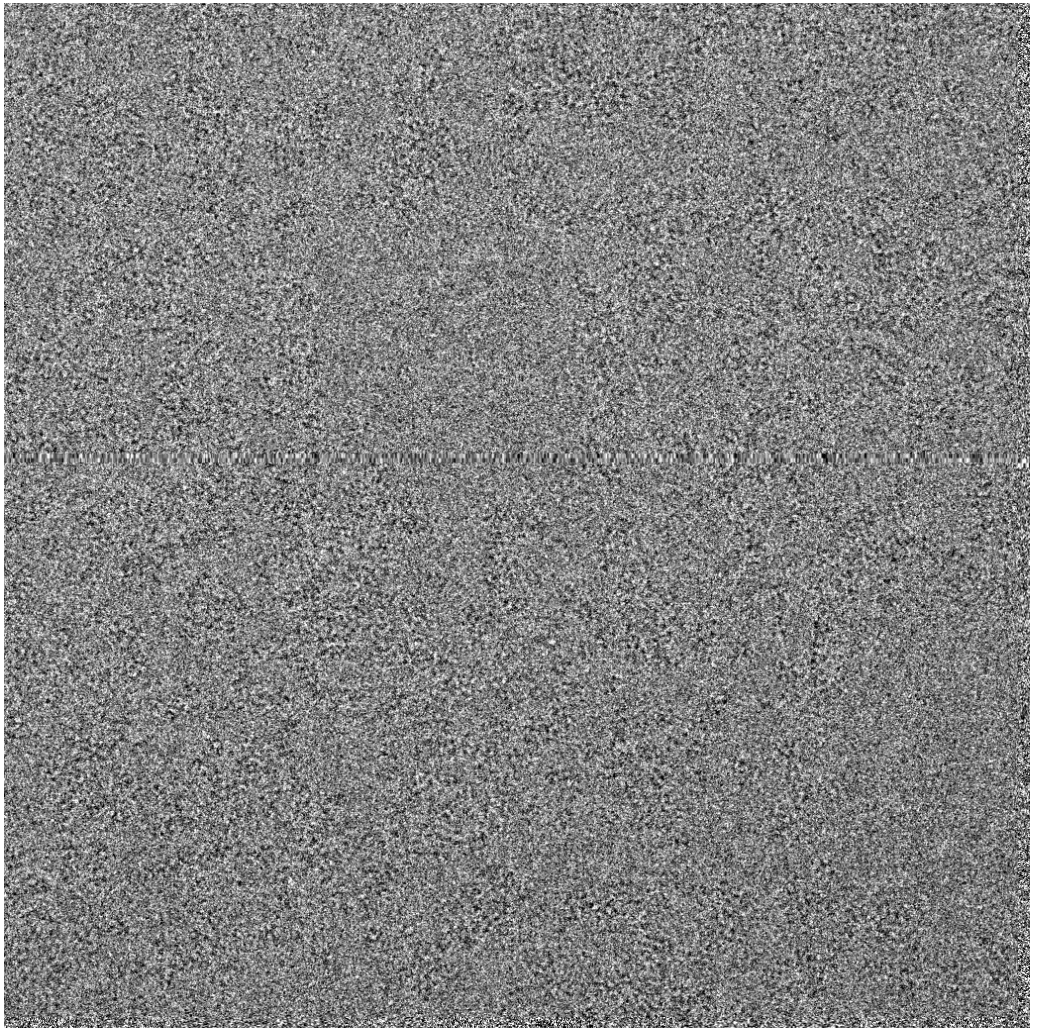


Drift corrected image



Motion of selected particles



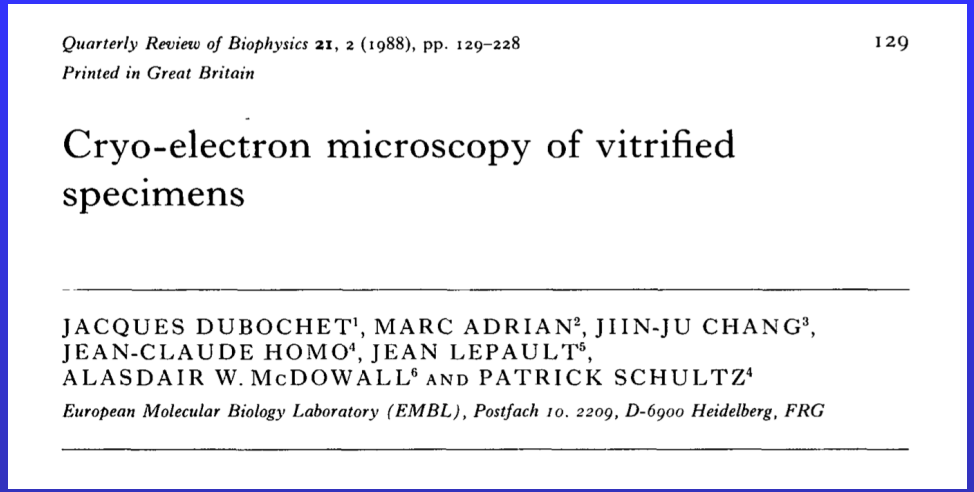


Movie processing

- Why?
  - Correct for drift
  - Allow radiation damage weighting
- Frame alignment has to be done carefully
  - Fixed pattern in detector
  - Low signal to noise in frames
  - Images in frames is changing with radiation damage and charging
  - Motion might not be simple

What's changed?

- Microscopes are better
  - Field emission guns
  - More stable stages
  - Lower contamination
  - Automation
- Imaging detectors are better
  - Higher DQE
  - Movie capture
  - Faster (more data)



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Cryo-electron microscopy of vitrified specimens

JACQUES DUBOCHET[1], MARC ADRIAN[2], JIIN-JU CHANG[3], JEAN-CLAUDE HOMO[4], JEAN LEPAULT[5], ALASDAIR W. McDOWALL[6] AND PATRICK SCHULTZ[4]

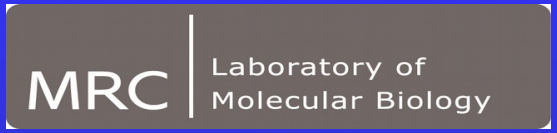
European Molecular Biology Laboratory (EMBL), Postfach 10. 2209, D-6900 Heidelberg, FRG

- Reconstruction Programs are better
  - More accurate less noise bias
  - Easier to use
  - Bigger computers

Future

- Improved microscopes
  - Automation
  - Easier to use
- Better detectors
  - Higher DQE
  - Faster
  - Bigger
- Reconstruction Programs
  - Easier to use
  - Faster (CPU + GPU)
  - Integrated pipelines
- Improved samples
  - Charging and initial movement

Thanks



- Richard Henderson
- Wasi Faruqi
- Shaoxia Chen
- Christos Savva
- Vinoth Kumar
- Sjors Scheres
- ….

- Jake Grimmett
- Toby Darling

- Alan Boswell
- Mark Crabb
- …