Principles of Cryo-electron tomography and Subtomogram Averaging

Methods and Techniques in structural biology: beyond black boxes

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07/10/2016

Outline

- Principles: Why cryo-ET?
- Sample preparation for cryo-EM/cryo-ET
- Data acquisition
- Data Pre-processing
- Subtomogram averaging

When to use cryo-electron tomography?

Sample

Purification needed
Regular viruses and macromolecular assemblies
protein of interest present in multiple copies
objects **do not** superimpose in 3D

"in situ" structural biology

pleomorphic assemblies, viruses, organelles

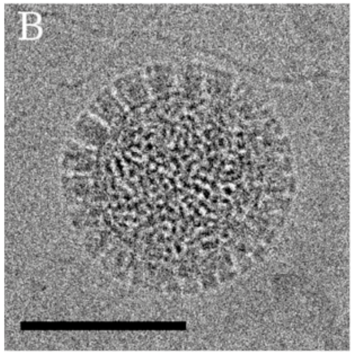
objects superimpose in 3D

Cryo-ET

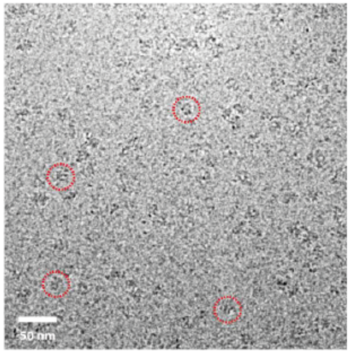
Single-particle

protein of interest present in multiple copies

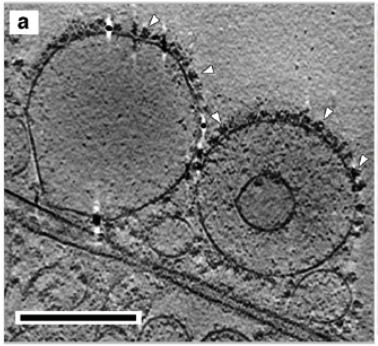
Subtomogram averaging



Rotavirus



Pol III



ER-membrane associated Ribosome

Immature HIV-1

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

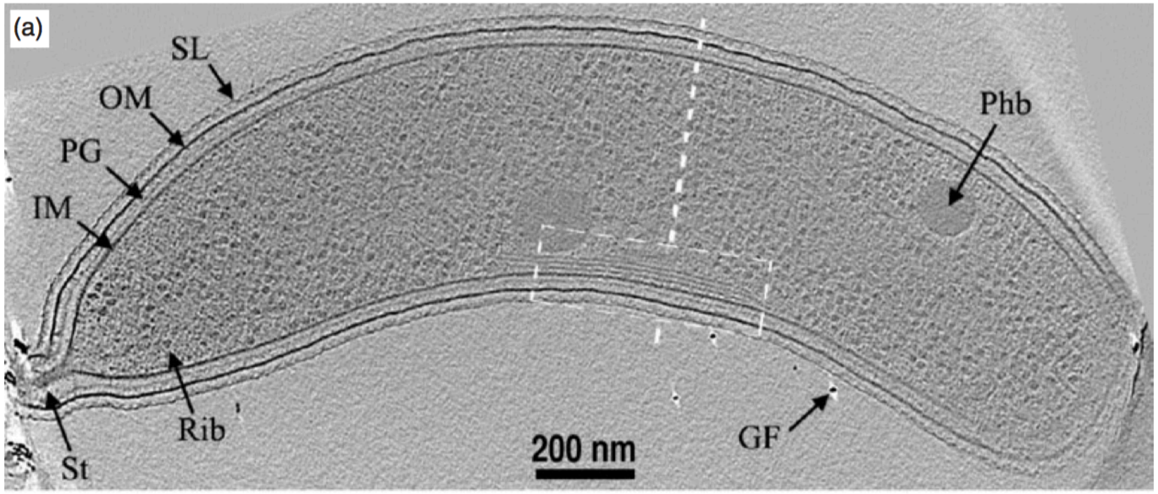
When to use cryo-electron tomography and subtomogram averaging?

- Purified proteins → Why not single-particle?
- Purified viruses → if icosahedral → why not single particle?

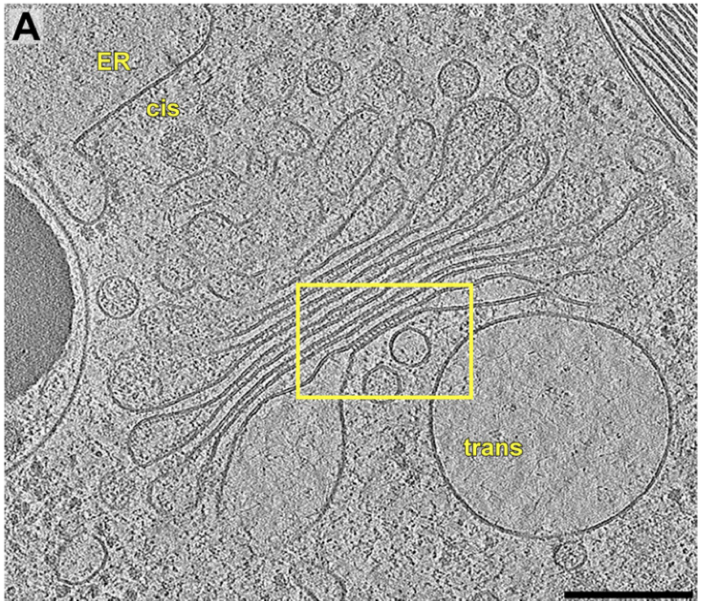
- Pleomorphic viruses → Tomography
- Whole mounts → Tomography
- Thin sections → Tomography
- FIB-lamella → Tomography

Not many people do cryo-ET and subtomogram averaging

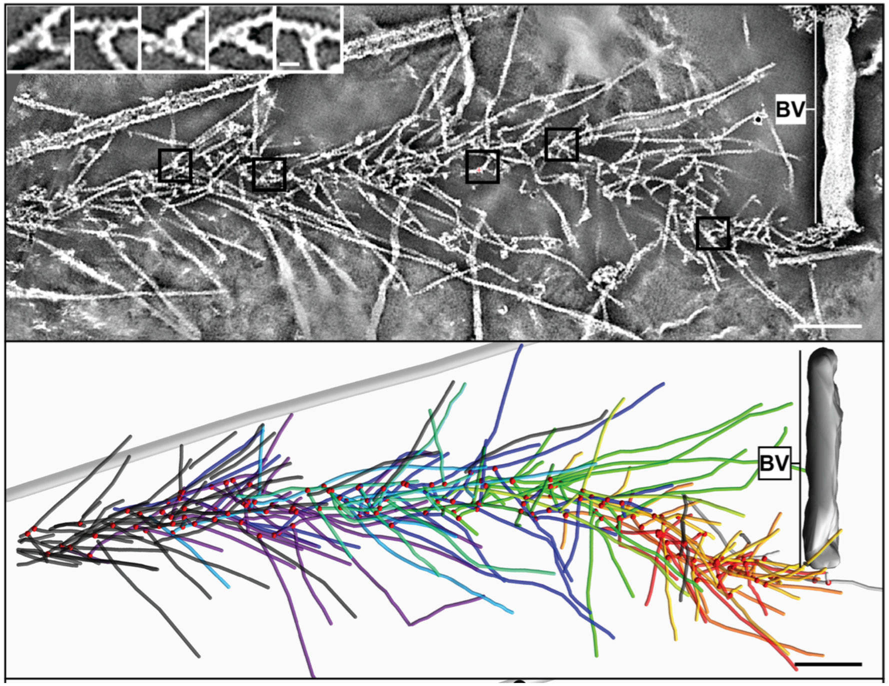
- Complicated data acquisition
- Increased sample thickness
- The missing wedge
- Defocus determination & Contrast transfer function correction
- Low-dose



L Gan, GJ Jensen, Electron tomography of cells, 2012, Q Rev Biophys (45)

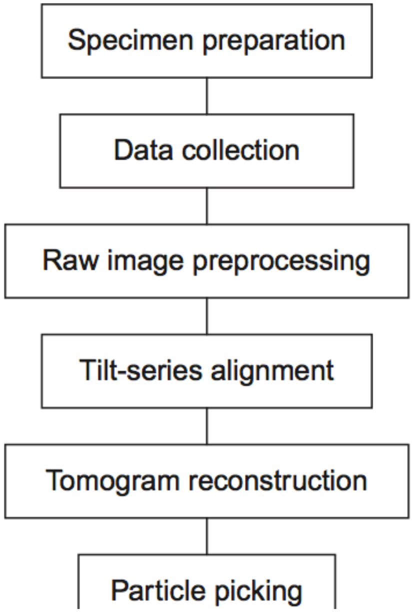


BD Engel ,M Schaffer, S Albert, S Asano, JM Plitzko, W Baumeister, In situ structural analysis of Golgi intracisternal protein arrays, 2015, PNAS (112)



J Mueller, J Pfanzelter, C Winkler, A Narita, C Le Clainche, M Nemethova, MF Carlier, Y Maeda, MD Welch, T Ohkawa, C Schmeiser, GP Resch, JV Small, Electron tomography and simulation of baculovirus actin comet tails support a tethered filament model of pathogen propulsion, 2014, Plos Path (12)

A “typical” cryo-electron tomography and subtomogram averaging workflow



Specimen preparation

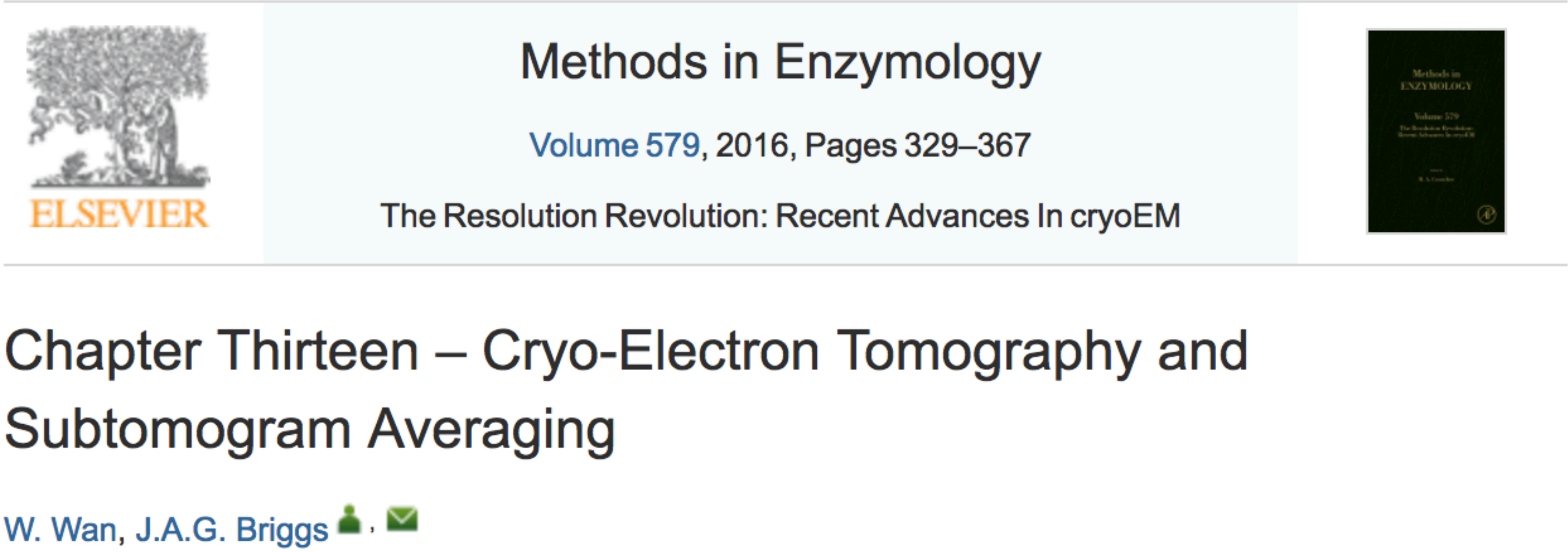
Data collection

Raw image preprocessing

Tilt-series alignment

Tomogram reconstruction

Particle picking



ELSEVIER

Methods in Enzymology

Volume 579, 2016, Pages 329–367

The Resolution Revolution: Recent Advances In cryoEM

Chapter Thirteen – Cryo-Electron Tomography and Subtomogram Averaging

W. Wan, J.A.G. Briggs

Sample preparation

Specimen preparation

Thinned samples

Plunge freezing
High-pressure freezing
CEMOVIS
FIB-SEM

↓

Organisms, tissues, cells, organelles

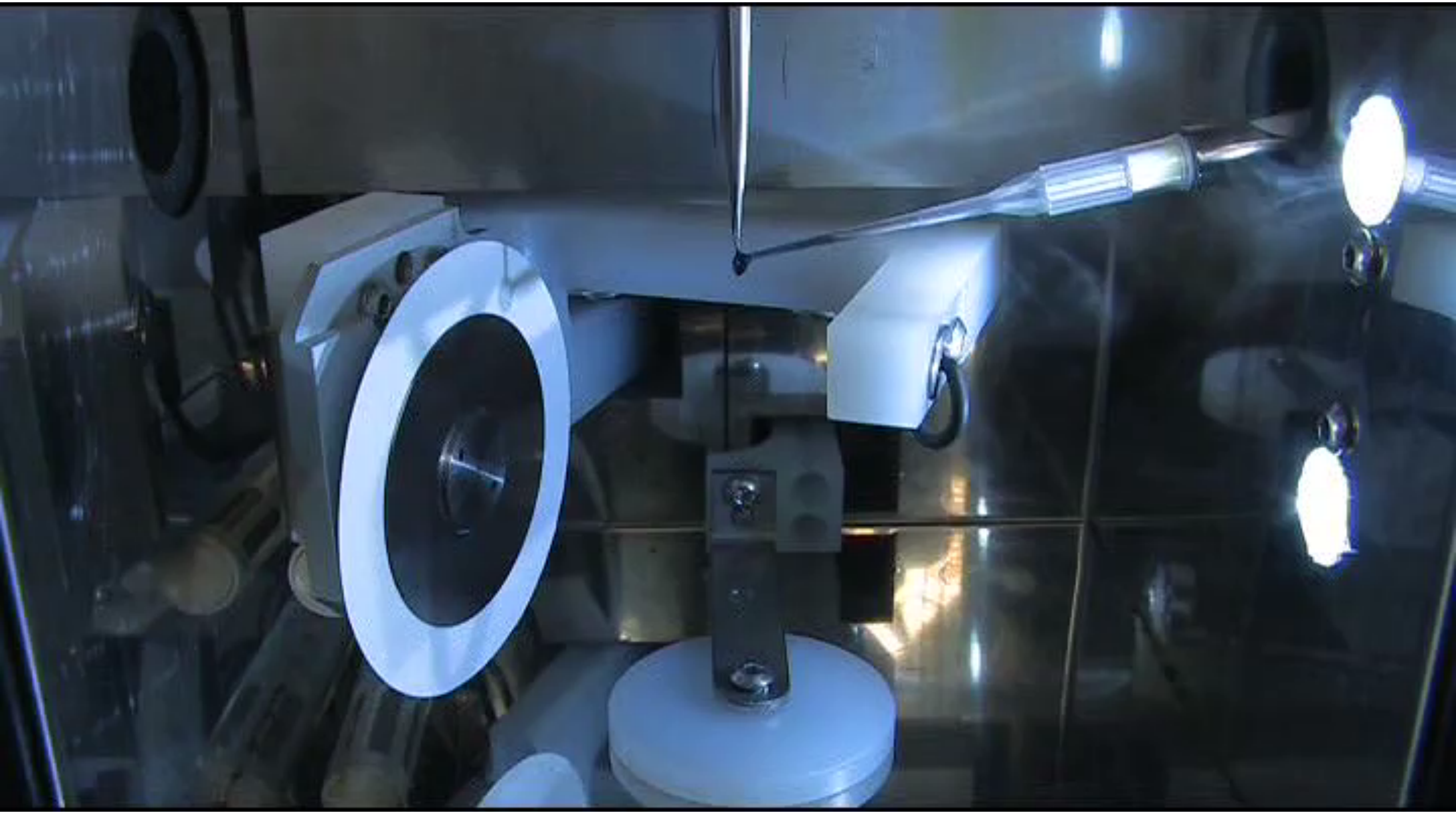
Whole mounts

Plunge freezing

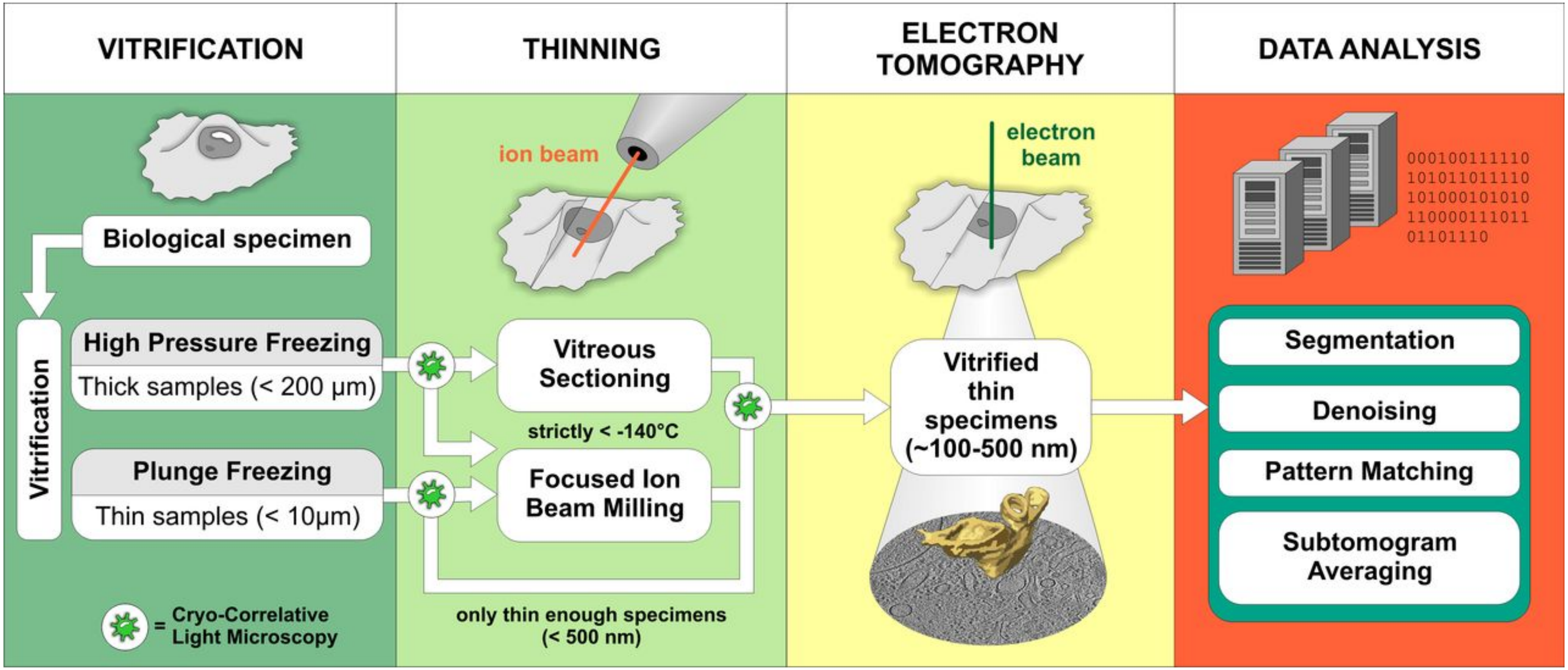
↓

Cells, Viruses, purified organelles, protein complex

Plunge freezing



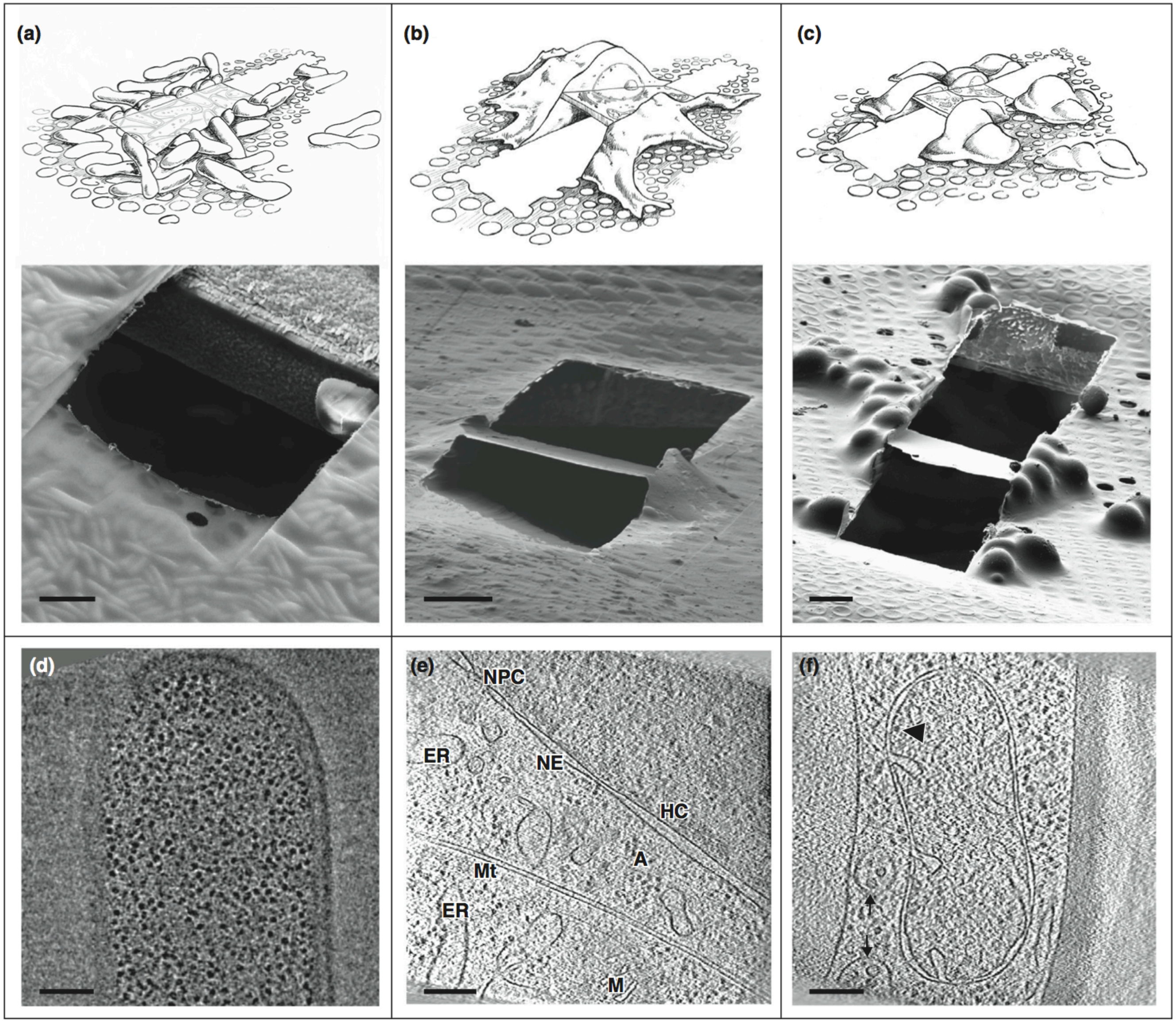
Cellular specimen preparation



Vladan Lučić et al. J Cell Biol 2013;202:407-419

Focused Ion beam (FIB)

- "Opening a window into the cell"



E Villa, M Schaffer, JM Plitzko, W Baumeister, 2013Opening windows into the cell: focused-ion-beam milling for cryo-electron tomography, Curr Opin Struct Biol (23)

Data acquisition

Microscope requirements

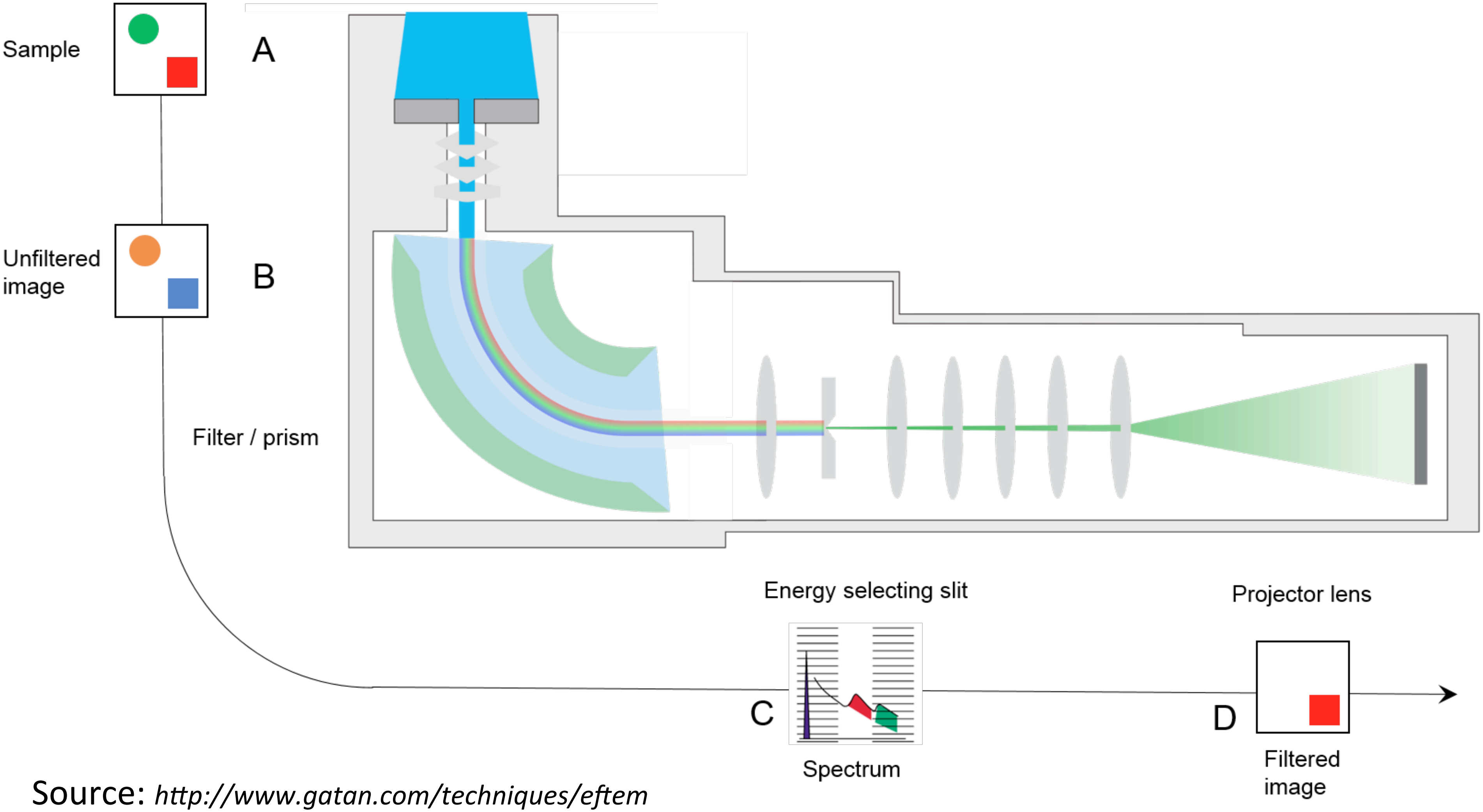
- High voltage
  - 300 keV
  - Necessary for thick specimens
- Stage stability
- Specimen holder
- Long-term acquisitions
- Automated acquisition
  - Software for low-dose acquisition
- Direct electron detector with **energy filter**

Energy filter

- Key concepts of electron scattering
  - Elastic scattering: electrons conserve energy
  - Inelastic scattering: energy loss
  - More inelastic scattering → Lower SNR
- Sample thickness in tomography
  - Much thicker than single-particle CEM
  - Can be up to several 100 nm
  - Increased sample thickness at higher tilt angles
  - $D_1=D_0/\cos\alpha$ (400 nm thickness of a 200 nm thick sample at 60 degrees)
  - More inelastic scattering
- Higher voltage → longer mean free path
  - average distance the electrons travel between scattering events

Energy filter

- Energy filter removes inelastic scattered electrons by removing electrons that are outside the 0-loss peak (0-30 eV)



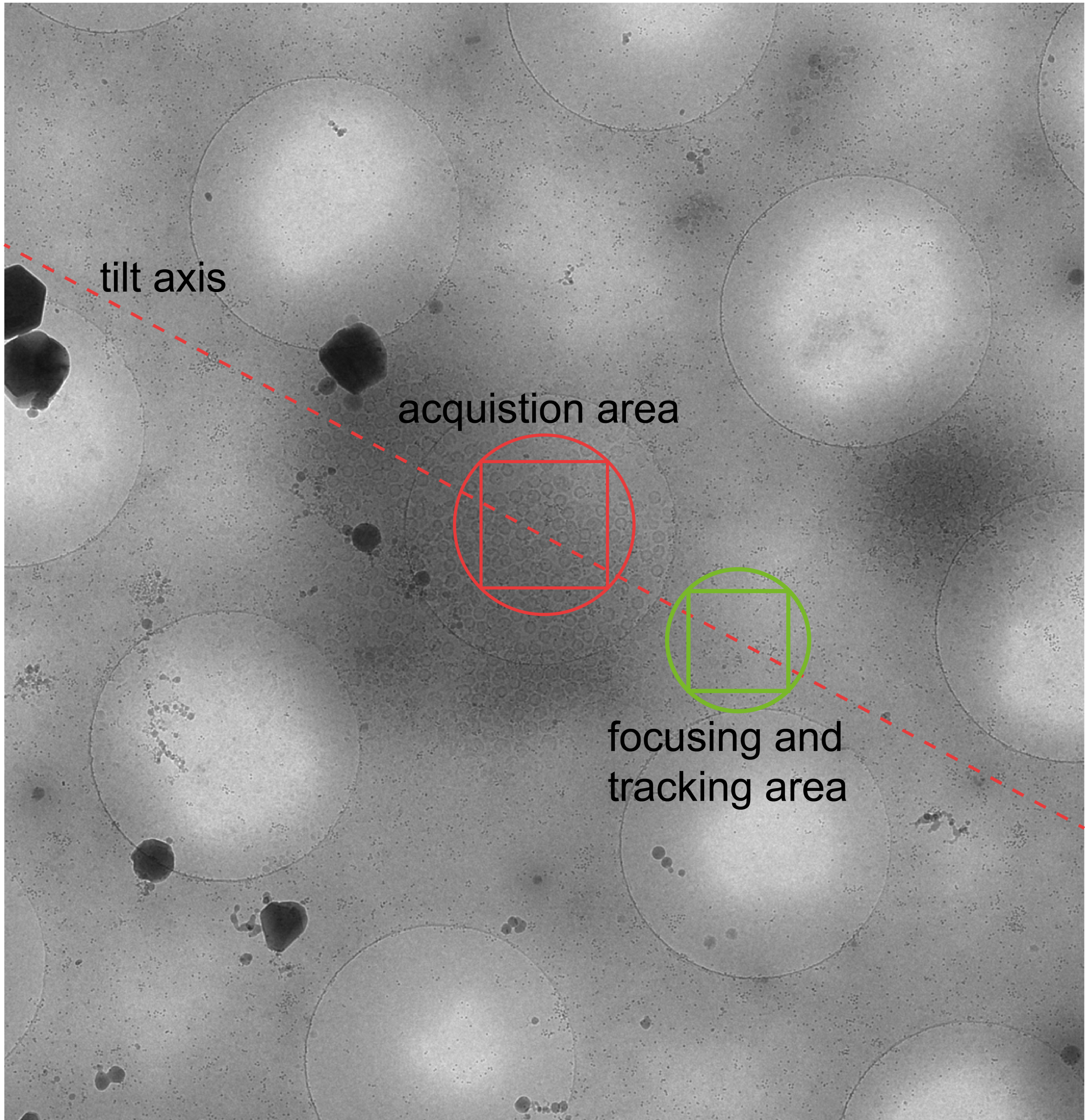
Source: *http://www.gatan.com/techniques/eftem*

Data acquisition parameters

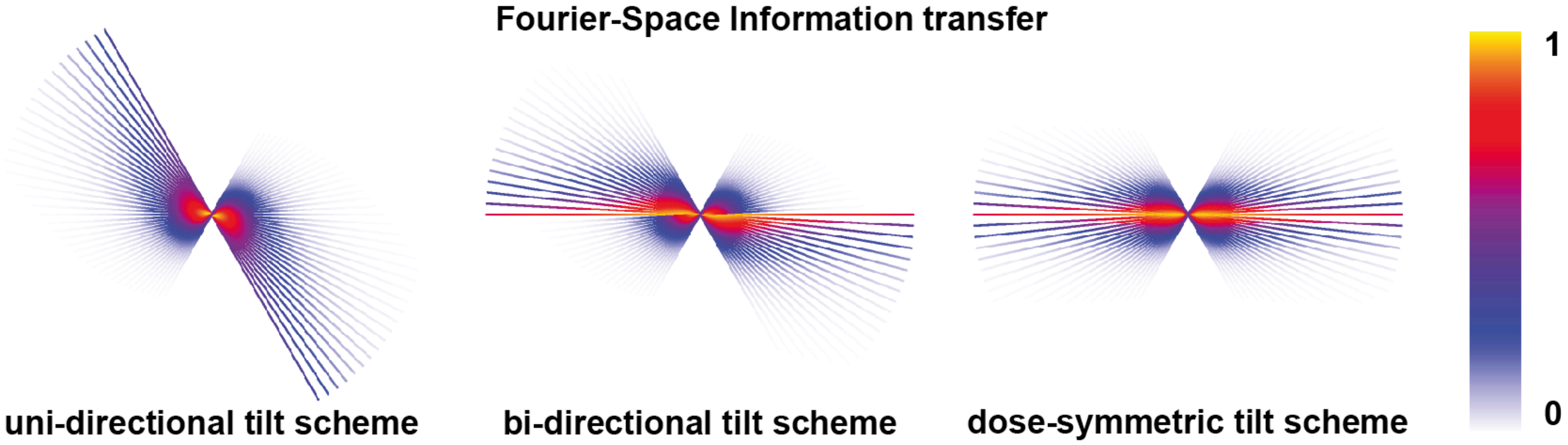
- How much dose?
  - Dose fractionation problem: Dose (40-200 electrons/Å^2) has to be split over all tilts
  - Higher contrast versus accumulative damage
- Which magnification/pixel size?
  - Smaller pixelsize for high-resolutions, but smaller field of view, harder tracking during data acquisition
  - If possible do not change magnification during acquisition
- Defocus?
  - High-resolution or high contrast
- Which tilt scheme?
  - How many tilts, which tilt interval?
  - Dual-axis?
  - The tilt scheme effects the missing wedge

Low dose data acquisition

- Iterative cycle of tracking → focusing → acquisition → tracking
- Focus and acquisition area should be as close to each other as possible
- Beam as small as possible



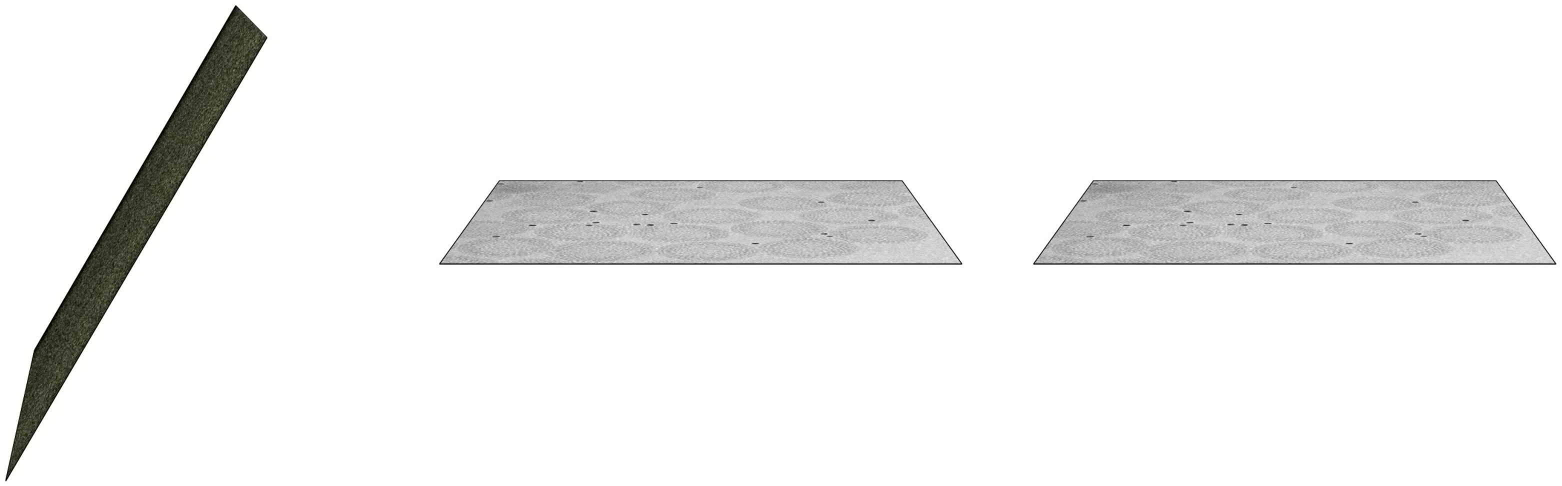
Tilt schemes



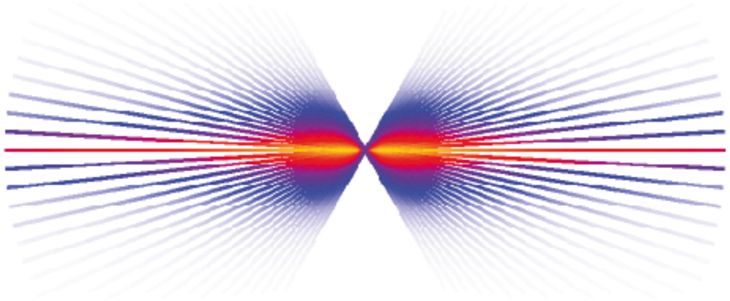
fast

intermediate

slow



Dose-symmetric tilt scheme



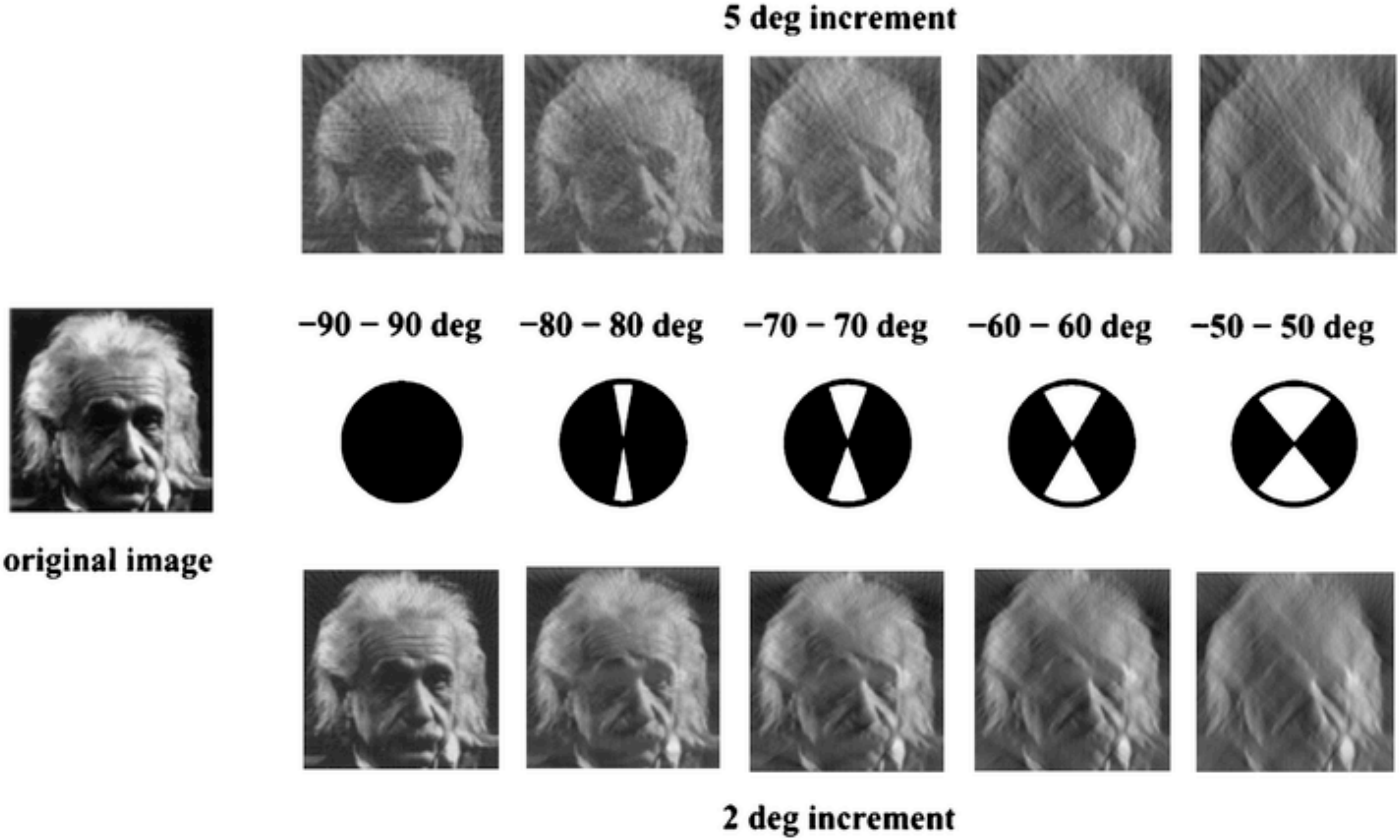
dose-symmetric tilt scheme

- Removes jumps between different parts of the tilt series
- Improves tilt series alignment
- Allows use of higher dose compared to uni or bidirectional tilt scheme

Hagen WHJ, et al, 2016, J. Struct. Biol., in press

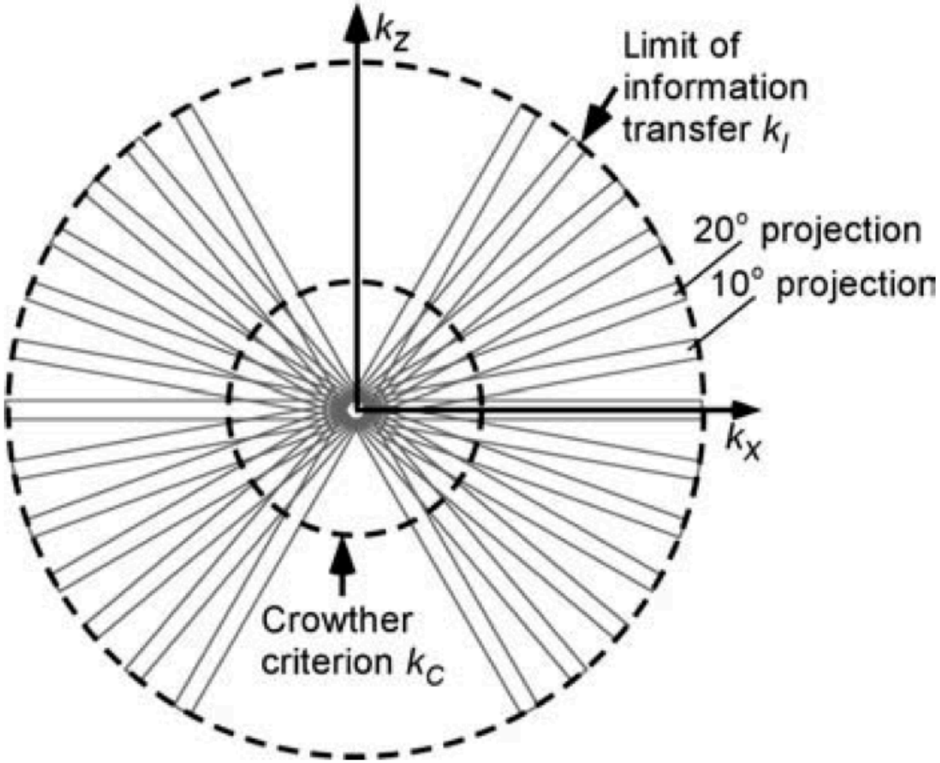
Missing information in cryo-ET

By Yury Bykov



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

The Crowther criterion: the resolution of a tomographic reconstruction



Crowther criterion

$Kc=(\Delta\alpha D)^{-1}$

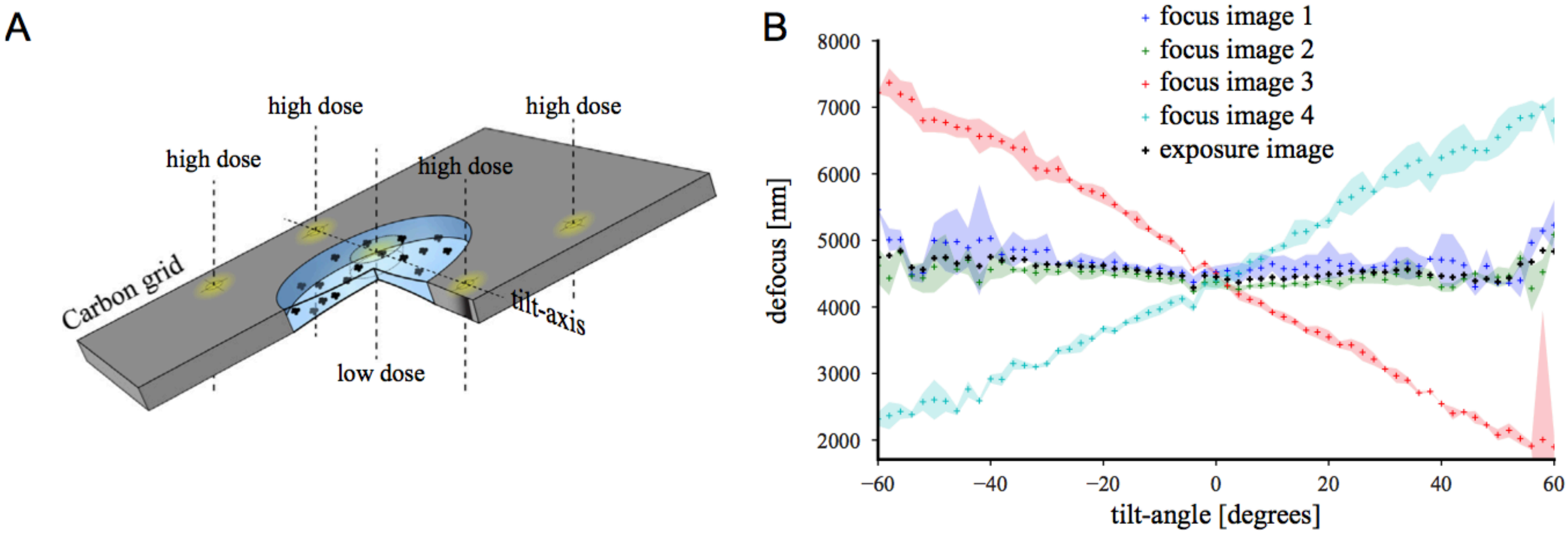
Δα = angular increment

D = object thickness

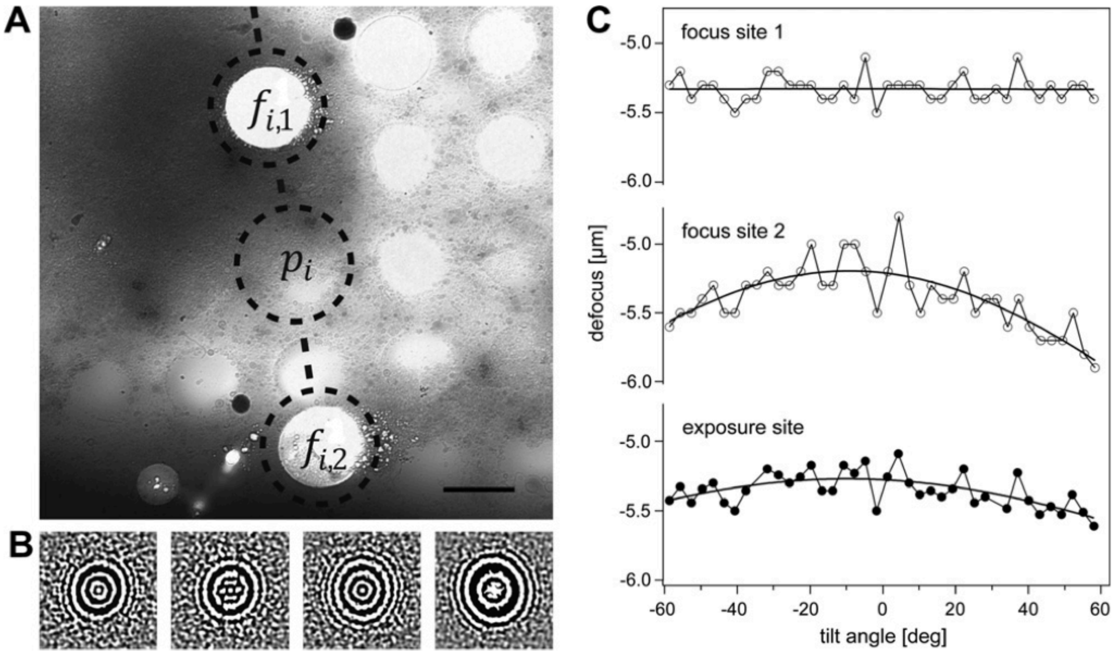
- Describes the resolution up to which fourier-space is fully sampled
- Is not considering the missing wedge or signal to noise

Schematic from: Lucic V, Förster F, Baumeister W., Annu. Rev. Biochem (2005)

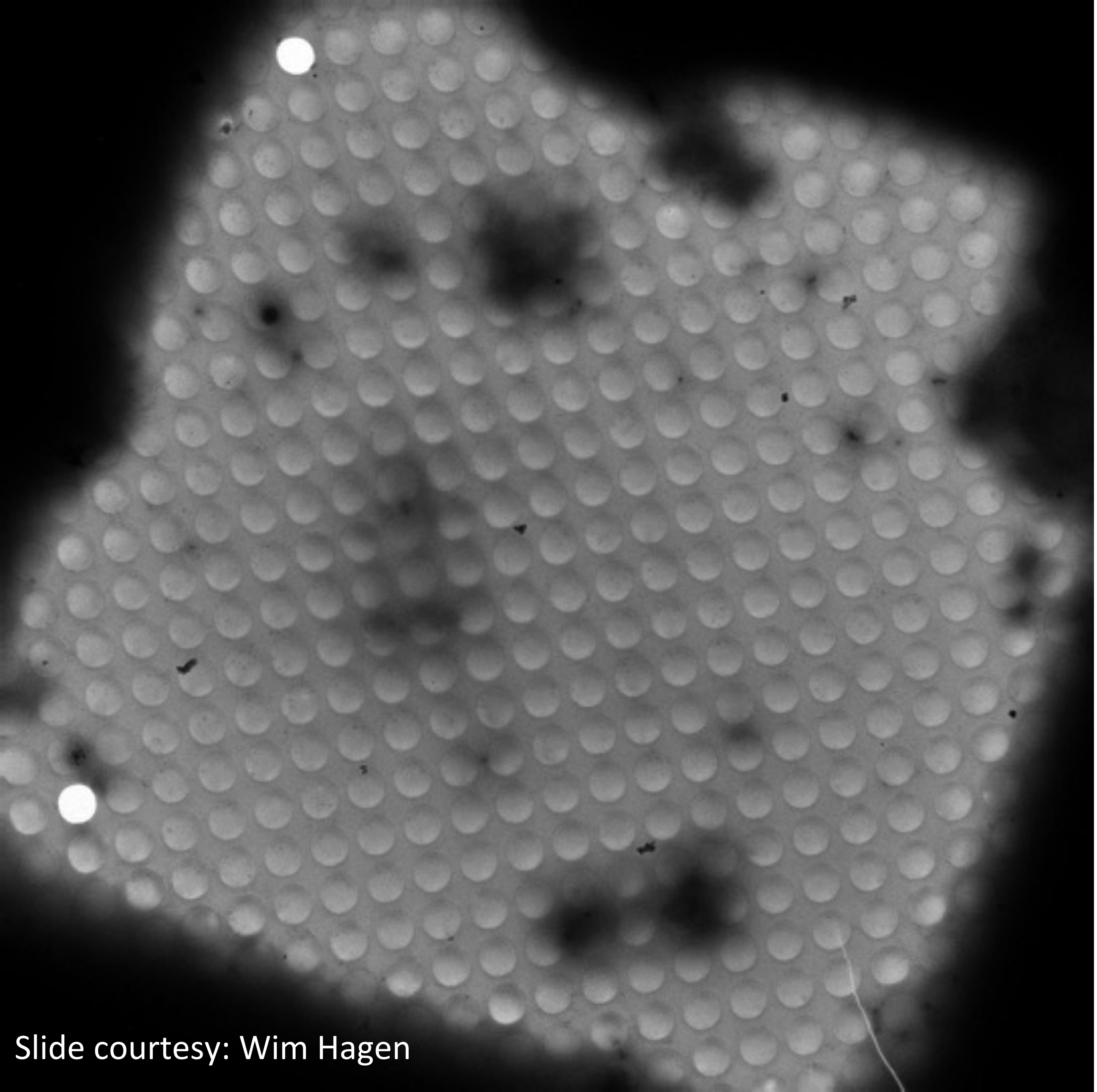
Defocus estimation and CTF-correction



Voortman, Lenard M., Vulović, Miloš, Maletta, Massimiliano, Voigt, Andreas, Franken, Erik M. , Simonetti, Angelita, Peters, Peter J., van Vliet, Lucas J., Rieger, Bernd, Quantifying resolution limiting factors in subtomogram averaged cryo-electron tomography using simulations, 2014, J. Struct. Biol. 187



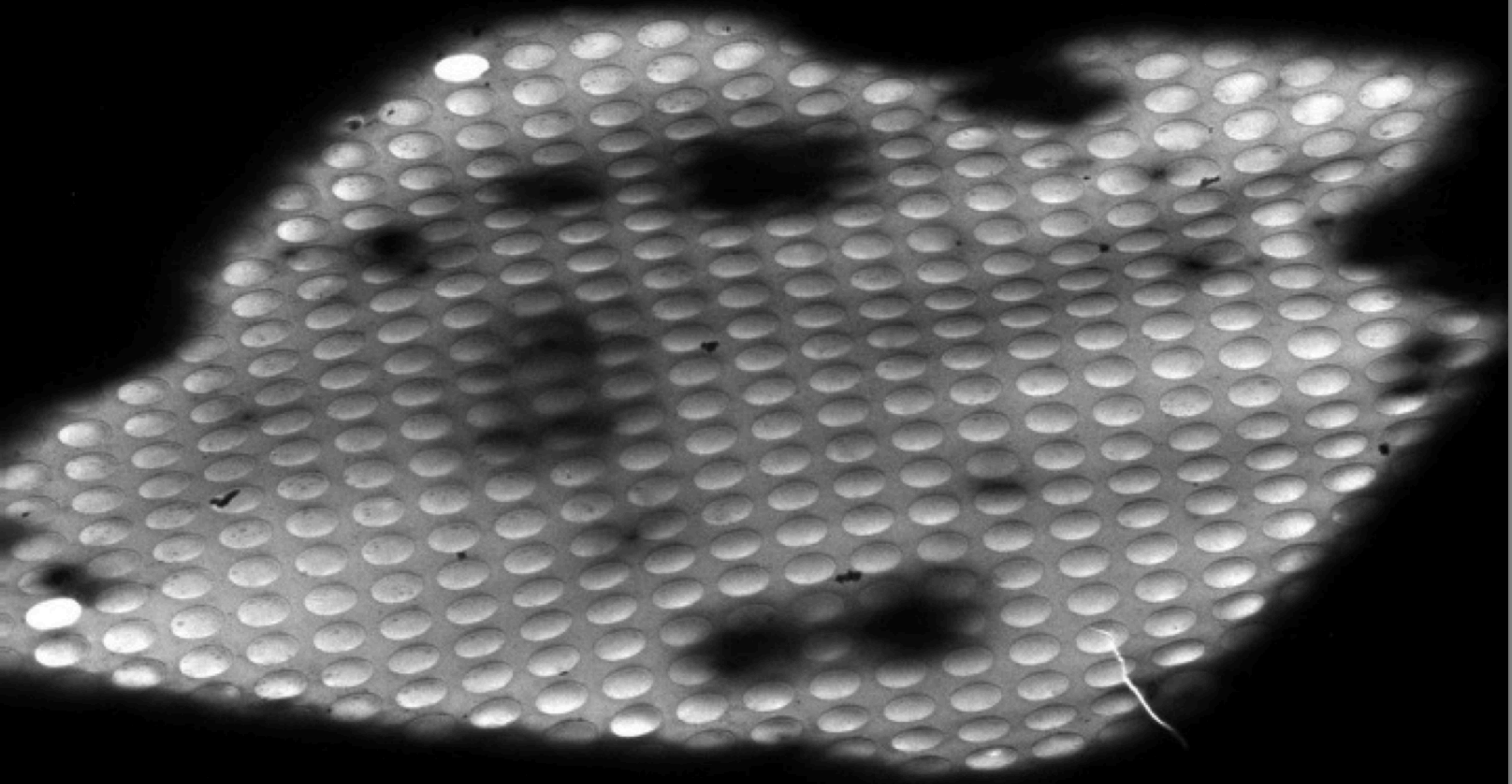
Eibauer, M., Hoffmann, C., Plitzko, J. M., Baumeister, W., Nickell, S., Engelhardt, H., Unraveling the structure of membrane proteins in situ by transfer function corrected cryo-electron tomography, 2012, J. Struct. Biol. 180



Slide courtesy: Wim Hagen

Flat?

EMBL

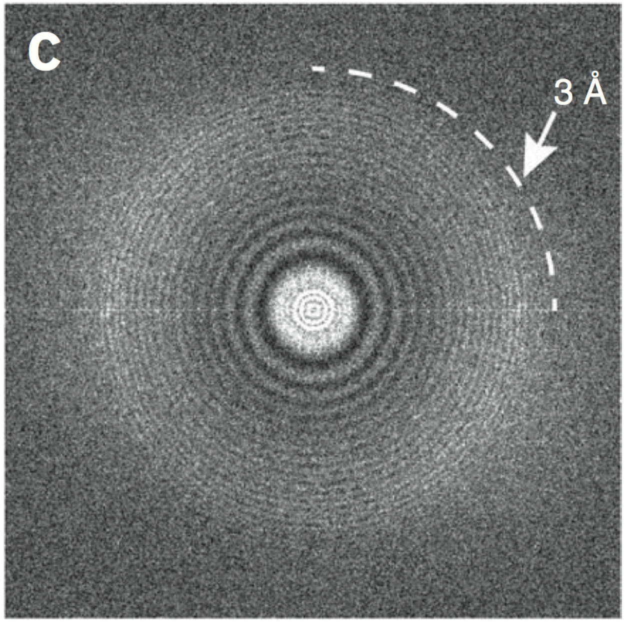


Slide courtesy: Wim Hagen

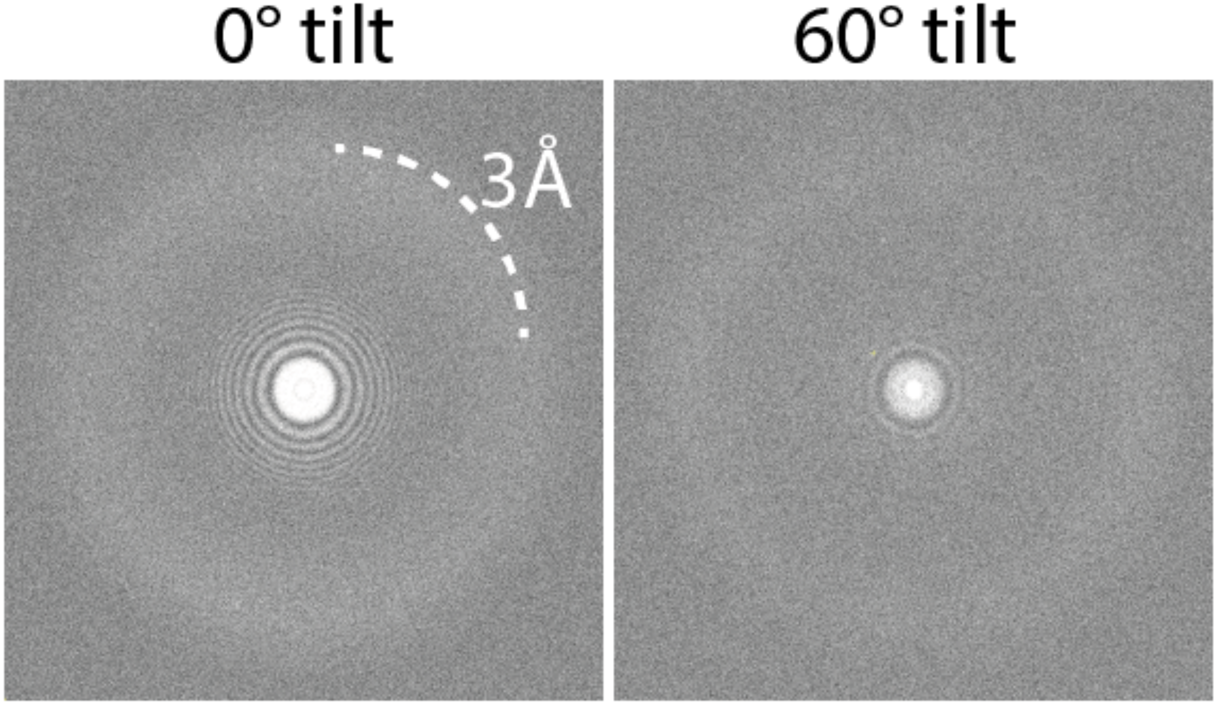
Defocus estimation should be done directly on the acquisition area

- Dose-fractionation in Cryo-ET results in very low signal in individual tilts
- Aggravated defocus determination

Single-particle cryo-EM



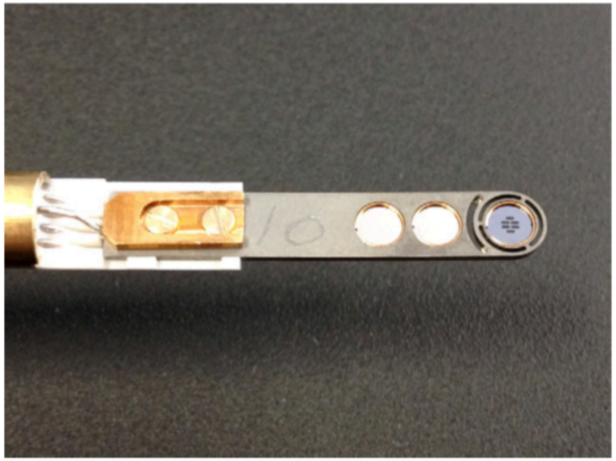
Cryo-electron tomography

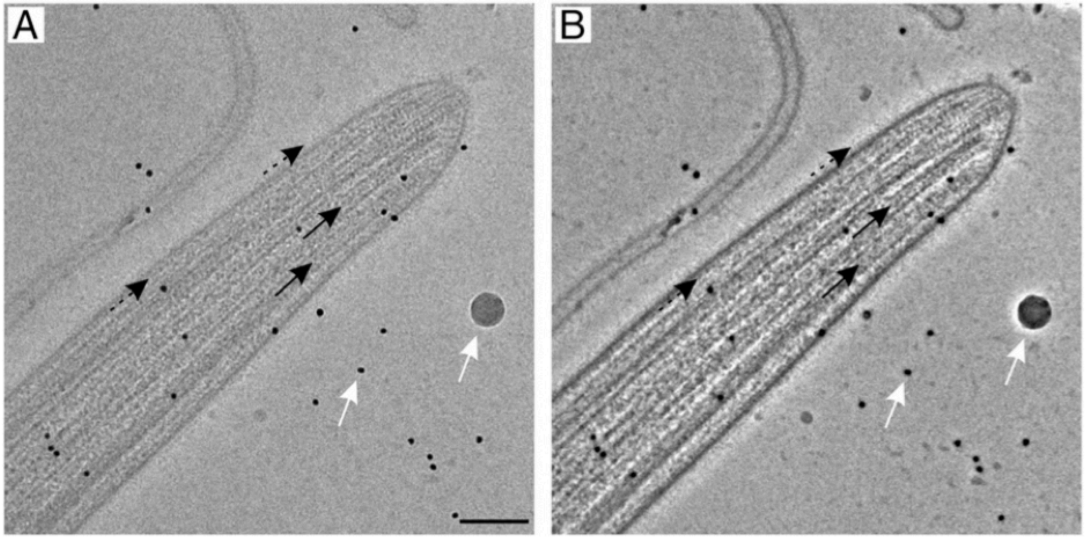


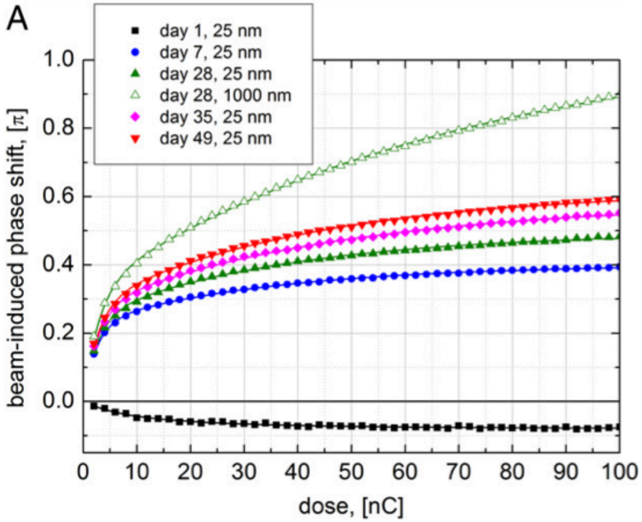
Li X, et al, 2013, Nat. Methods, 10

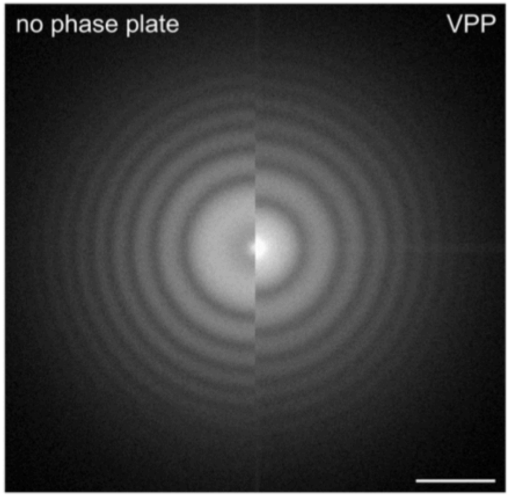
Slide courtesy William Wan

Volta Phase Plate







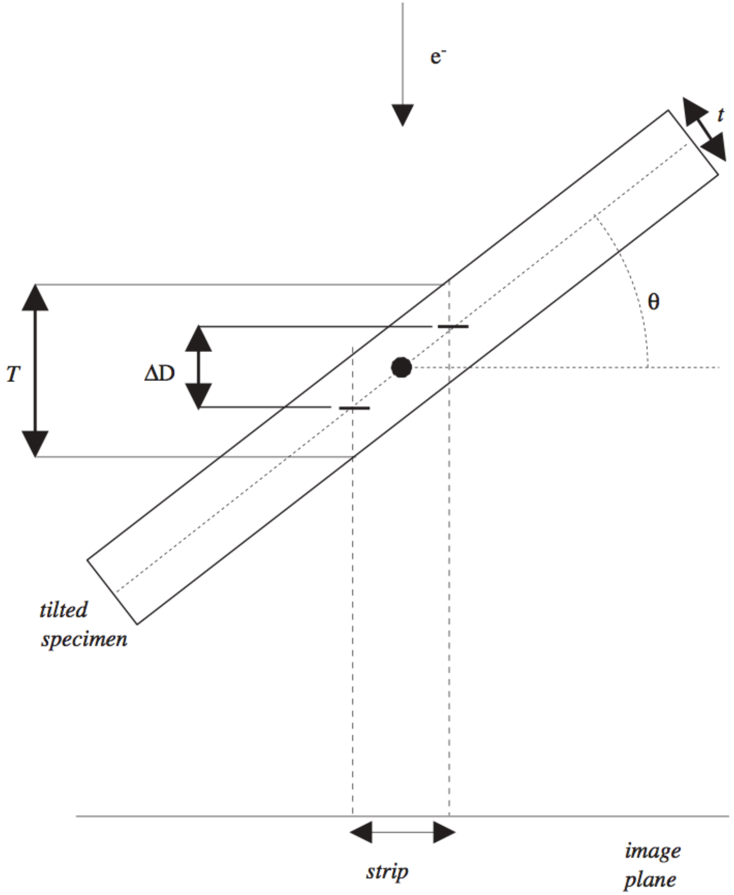


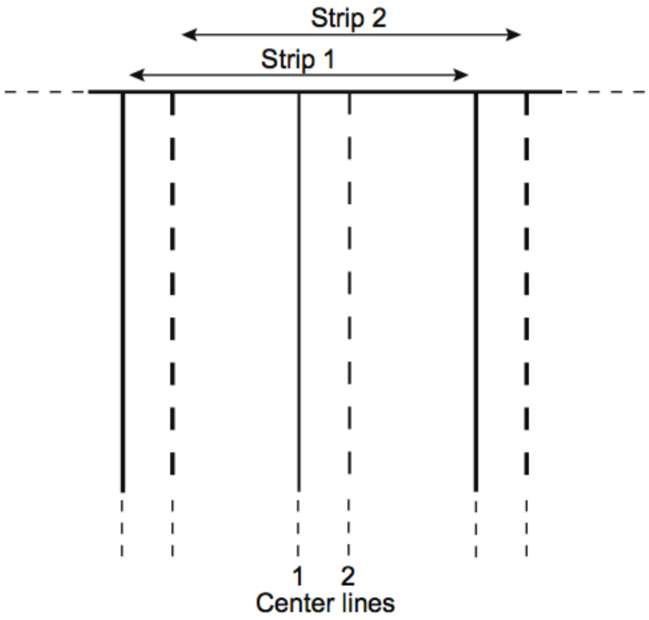
- Very low cut-on effect
- Phase shift is constantly evolving
- Relies on stable alignment

From: Danev et al (2014), Proc Natl Acad Sci USA

Data processing

Strip-based CTF-correction



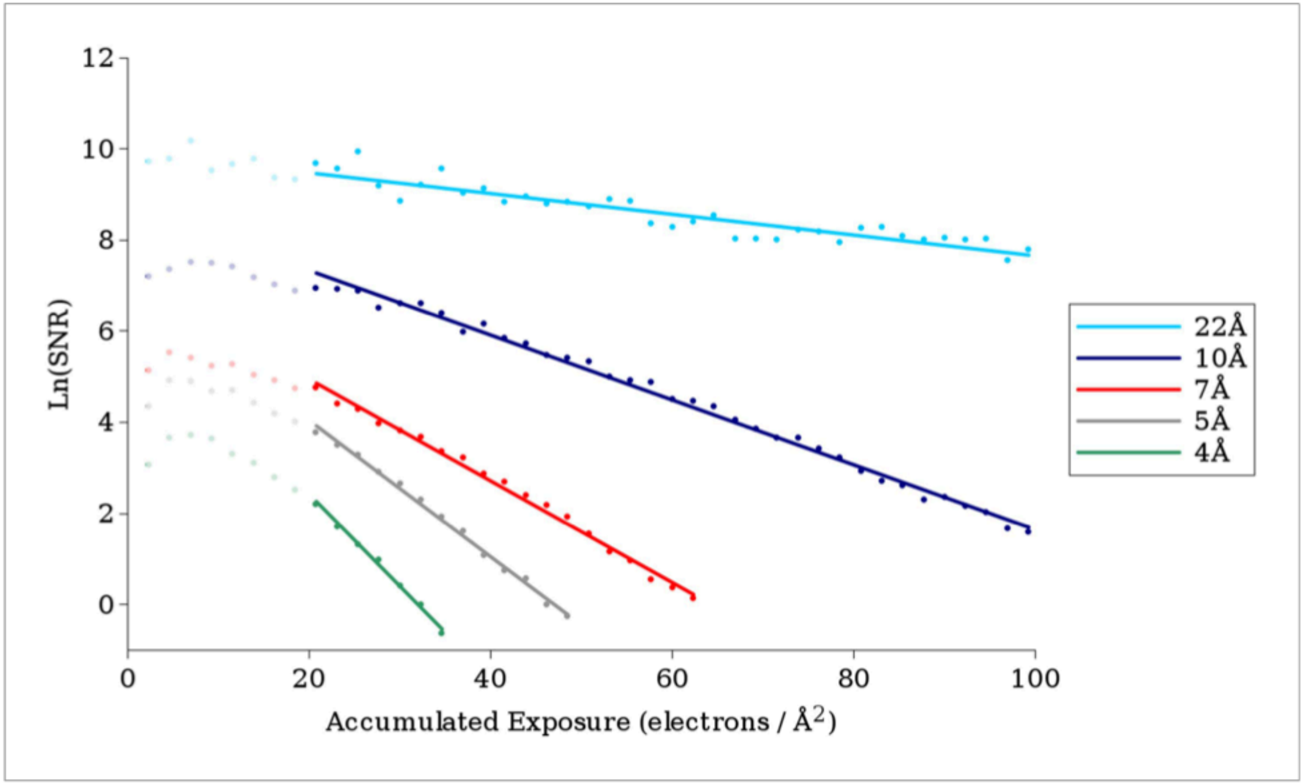


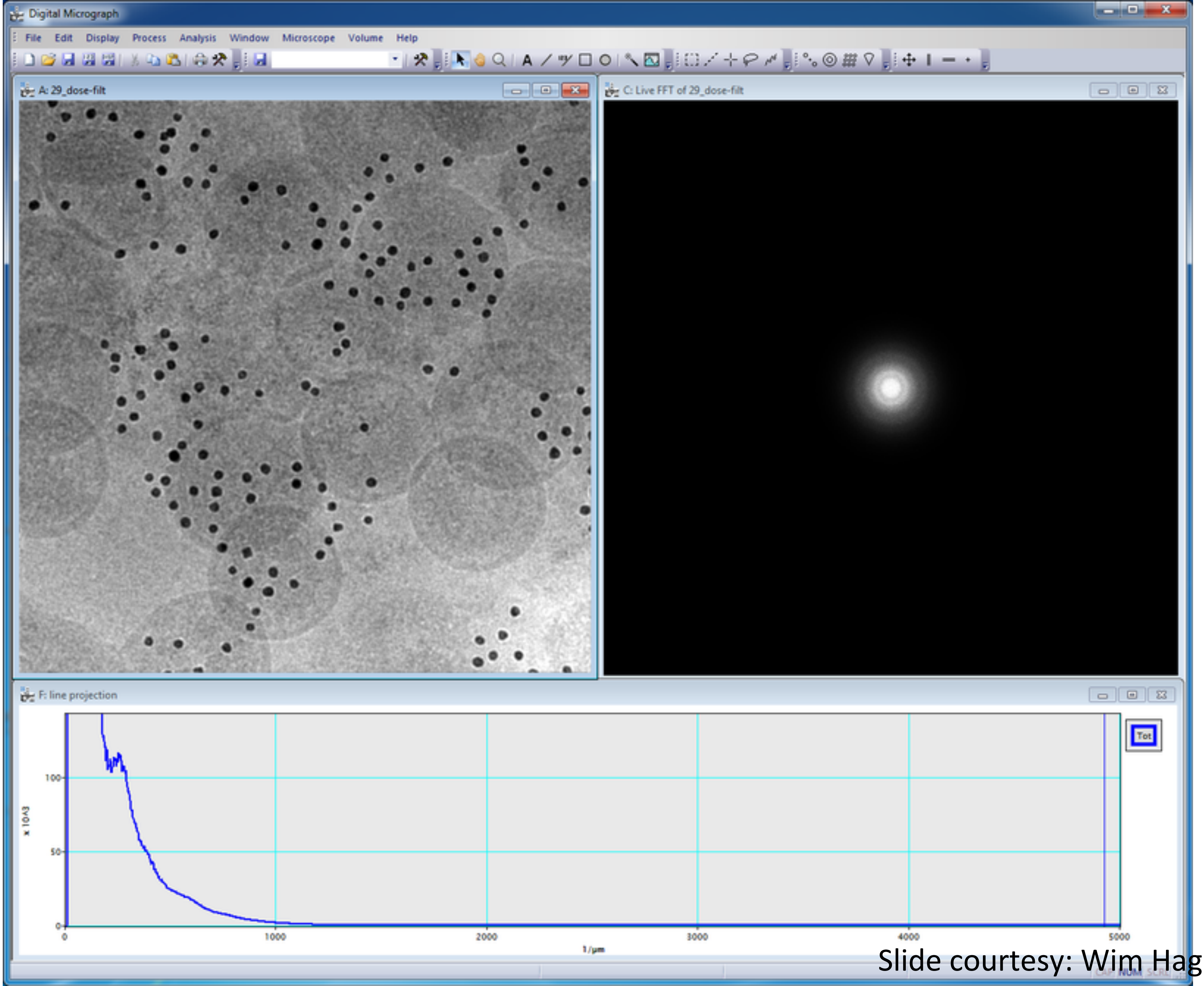
Fernández, J. J., Li, S., & Crowther, R. A. (2006). CTF determina;on and correc;on in electron cryotomography. *Ultramicroscopy*, *106*(7), 587–596.

Xiong Q, Morphew MK, Schwartz CL, Hoenger AH, Mastronarde DN(2009), CTF determination and correction for low dose tomographic tilt series, J.Struct.Biol (168)

Exposure filtering

- Grant & Grigorieff, elife, 2015,
- Filtering of tilt series for accumulative dose during acquisition
- Remove of high-resolution noise at higher tilt angles
- Improvement of SNR
- Improved alignment during subtomogram averaging



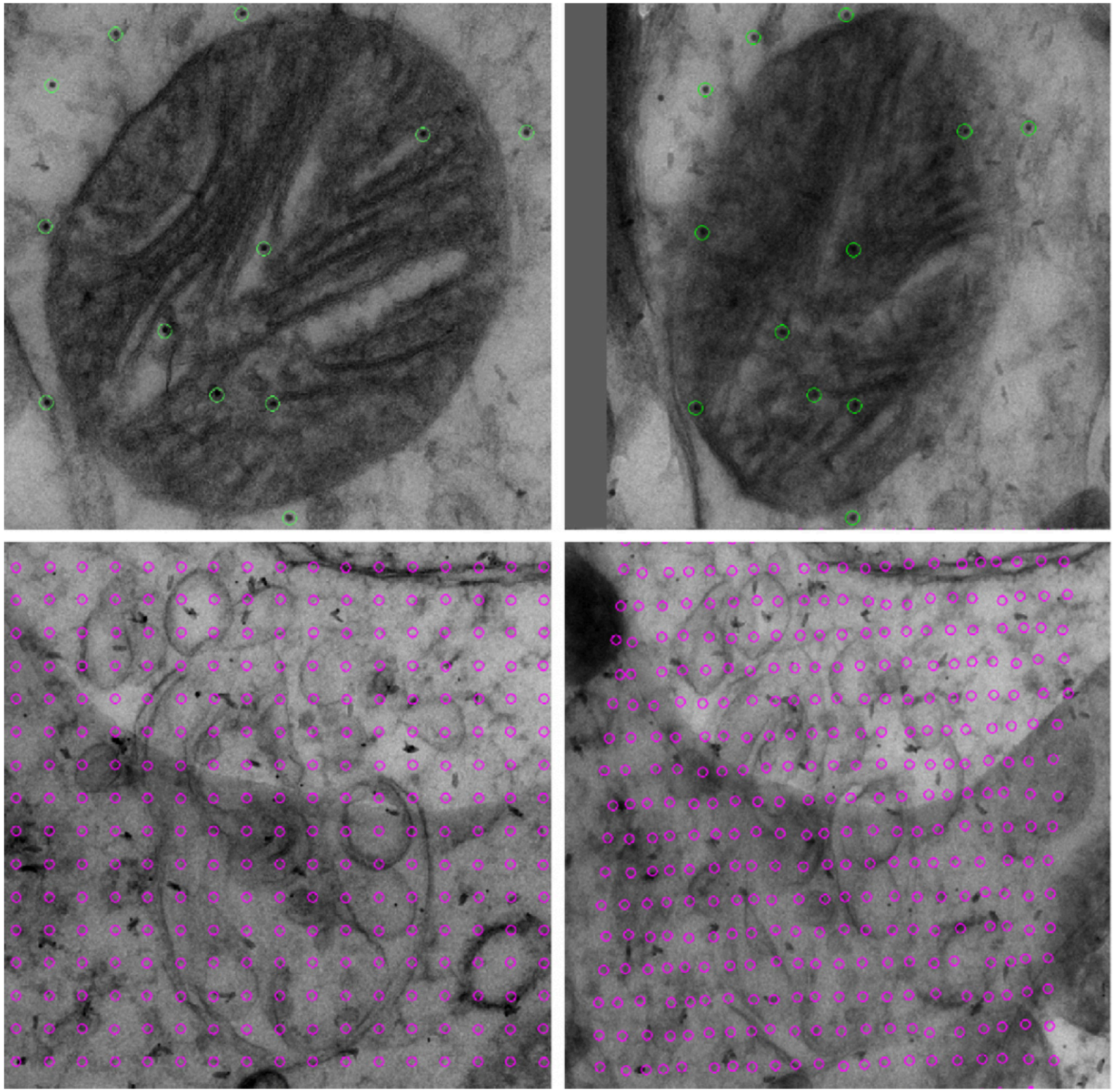


Slide courtesy: Wim Hagen

Tilt series alignment

- Fiducial based alignment (e.g. colloidal gold)

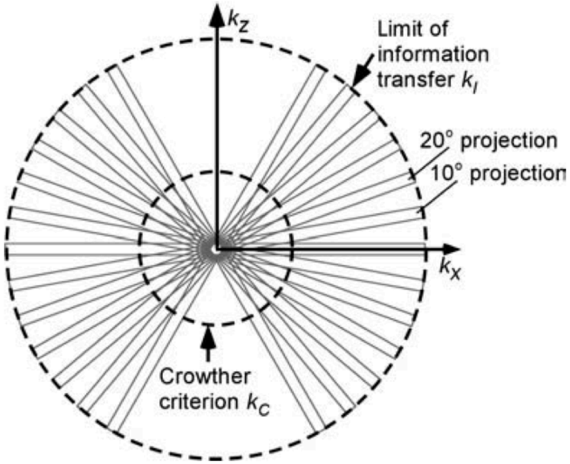
- Marker less alignment (feature-based cross-correlation)



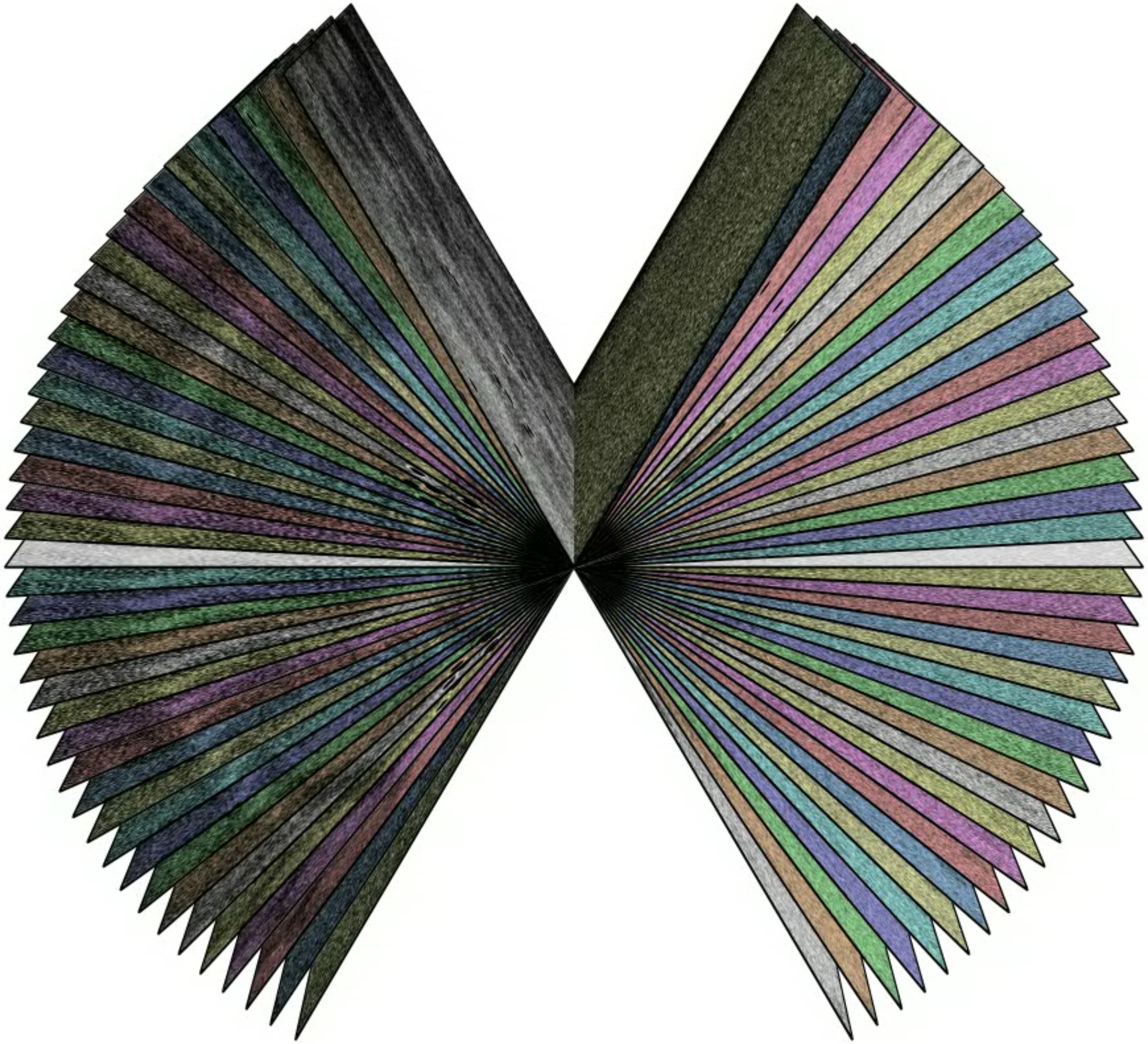
Fernandenz JJ, 2012, Micron ((43)

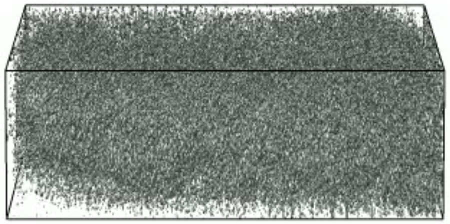
Tomogram generation

- Weighted back-projection (WBP)
  - Most widely used
  - High-resolution structures obtained in cryo-ET and subtomogram averaging are from WBP-tomograms
  - Reweights over-represented frequencies



- Iterative reconstruction algorithms
  - Algebraic reconstruction technique (ART)
  - Simultaneous iterative reconstruction technique (SIRT)
  - Simultanous algebraic reconstruction technique (SART)
  - Give improved contrast over WBP and are more often used in cellular tomography

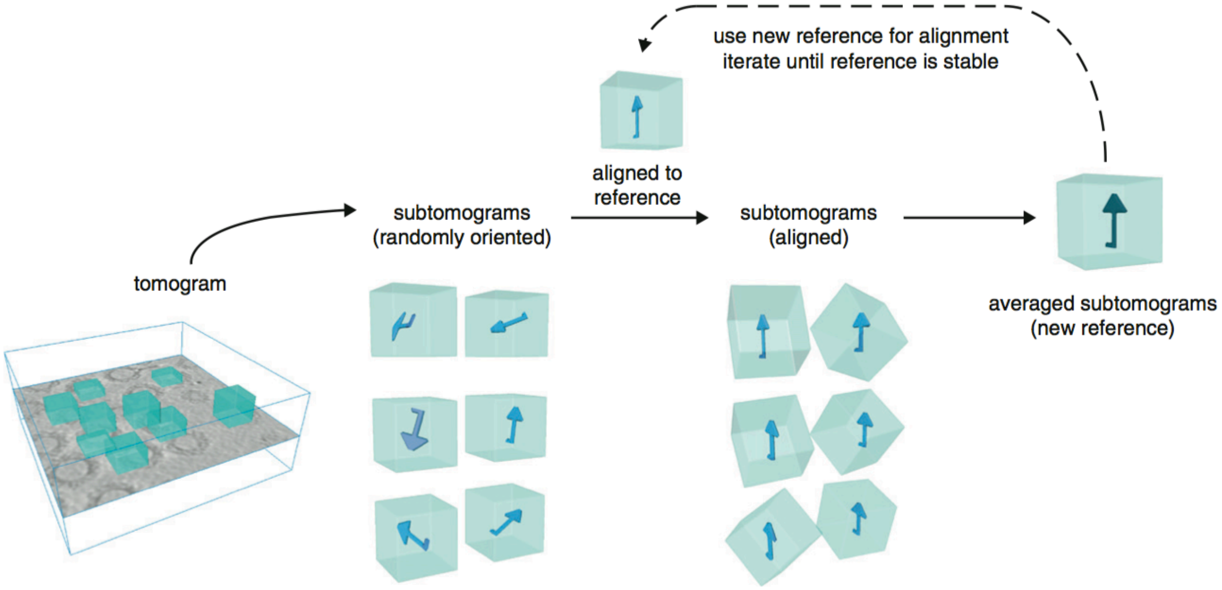




Subtomogram averaging

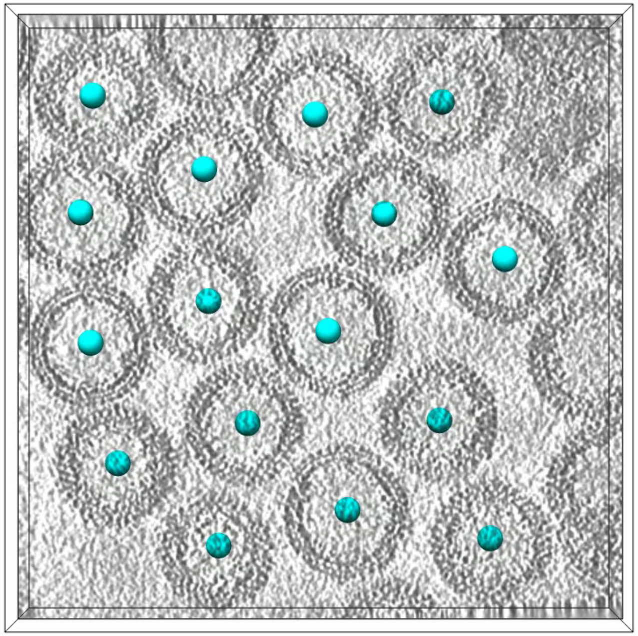
Subtomogram averaging

- Tomogram contains multiple copies of the same protein/ structure
- Helps to increase the resolution and fill the missing wedge
- Reveals three-dimensional arrangement of proteins
- Orientation and location determination of subtomograms via alignment against a reference

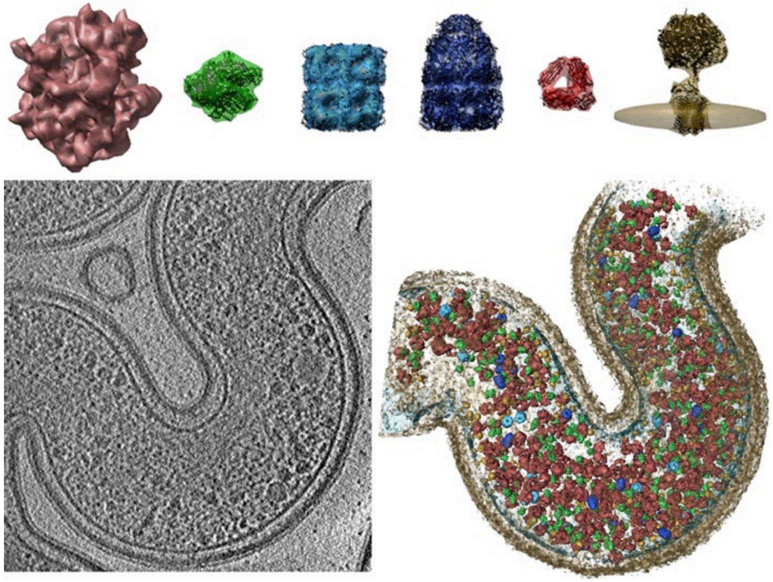


Particle picking

Use of geometrical information

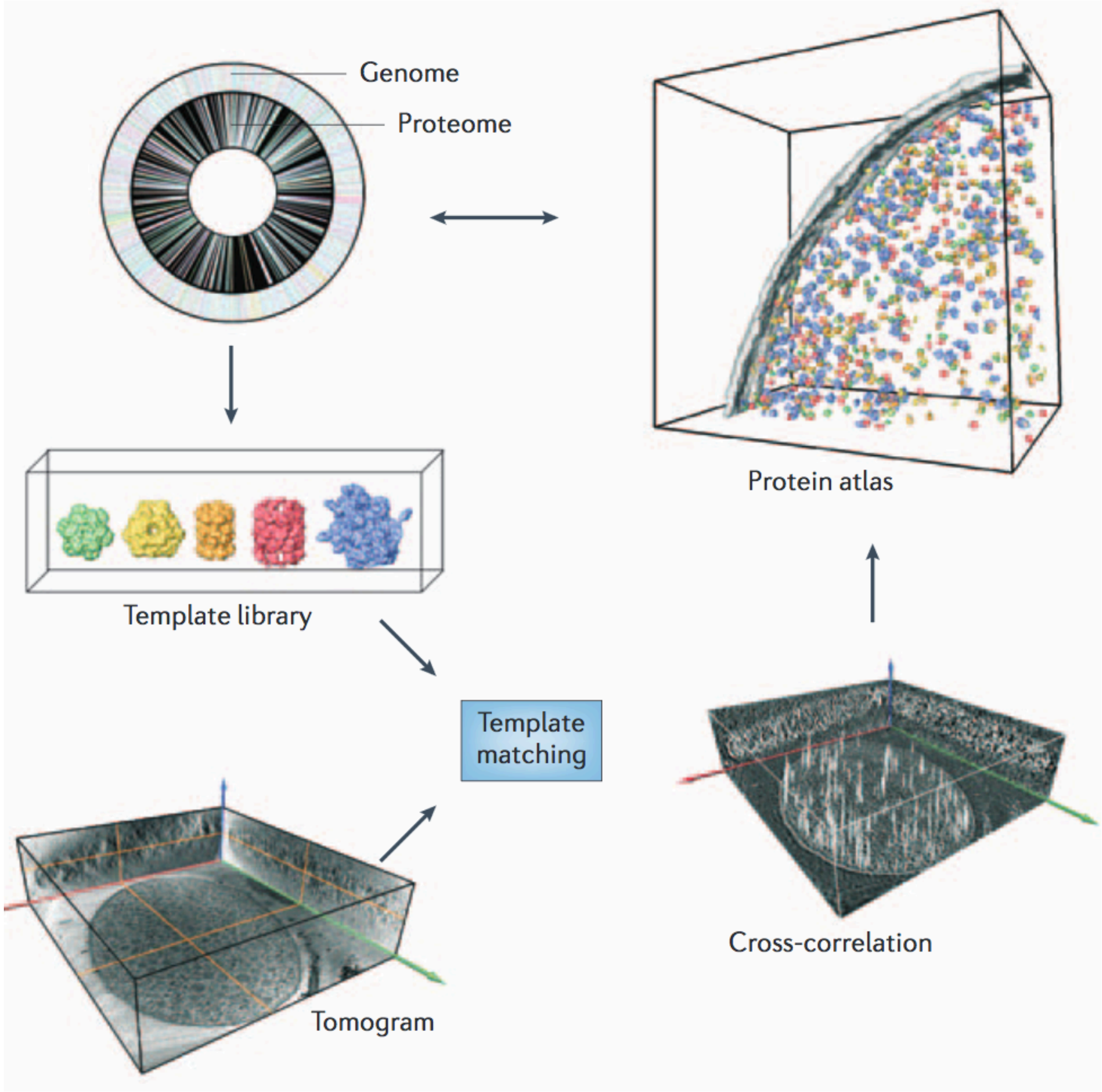


Template matching



Förster F, Han BG, Beck M, Visual proteomics, 2010, Meth Enzymol (483)

Visual Proteomics

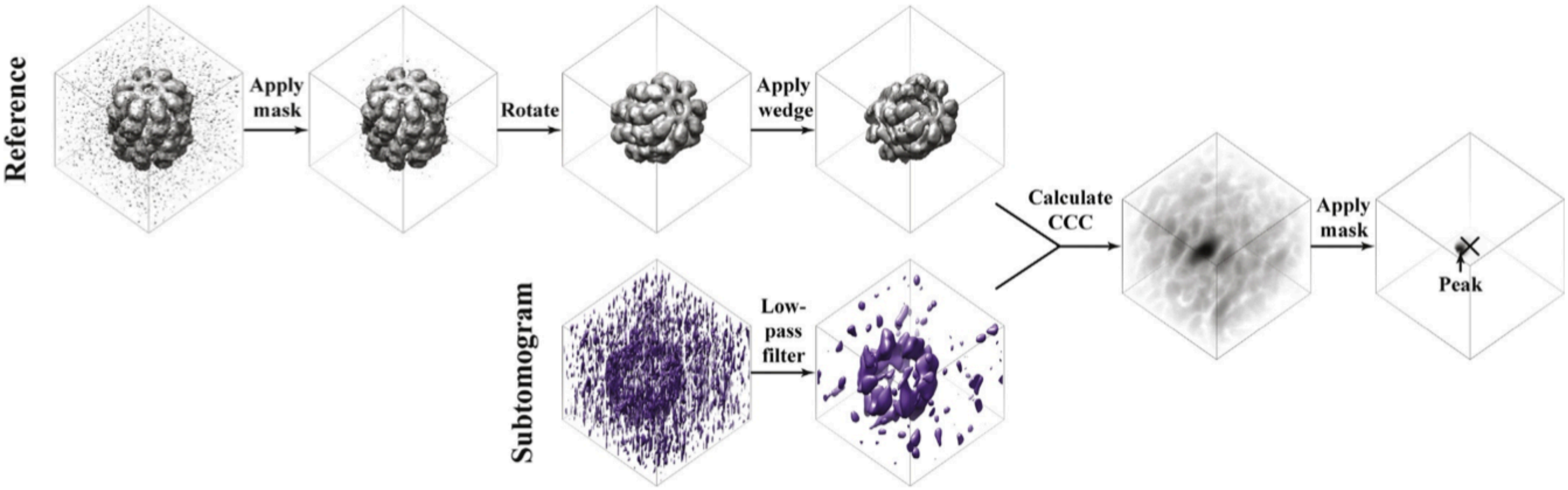


"The aim of visual proteomics is to map, in a comprehensive manner, all of the macromolecular complexes that are found inside a cell. The structures of individual macromolecular complexes are determined by X-ray crystallography, single-particle electron-microscopy analysis or hybrid approaches. Selected structures that can be resolved reliably using current cryo-electron-tomography methods are shown in the 'template library' "

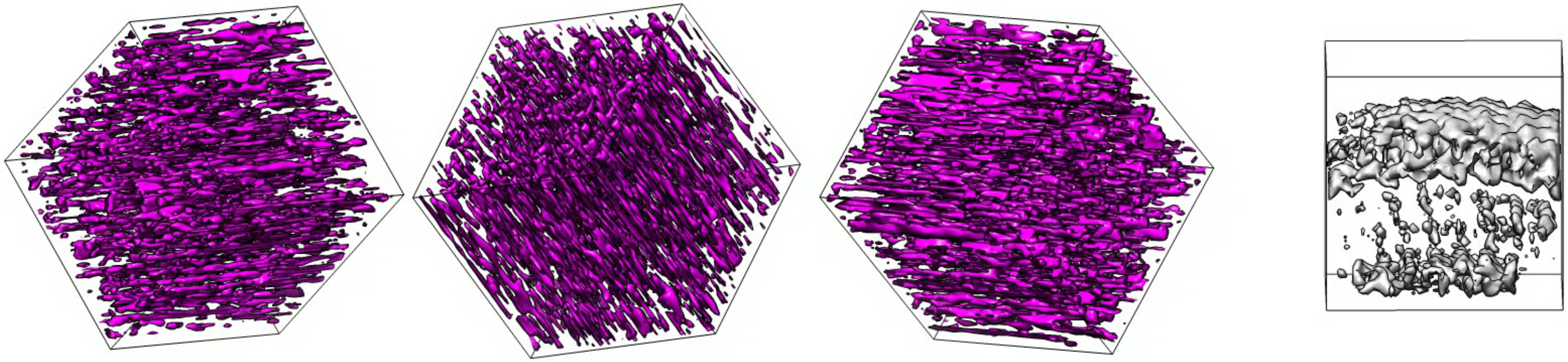
Quote and figure from:

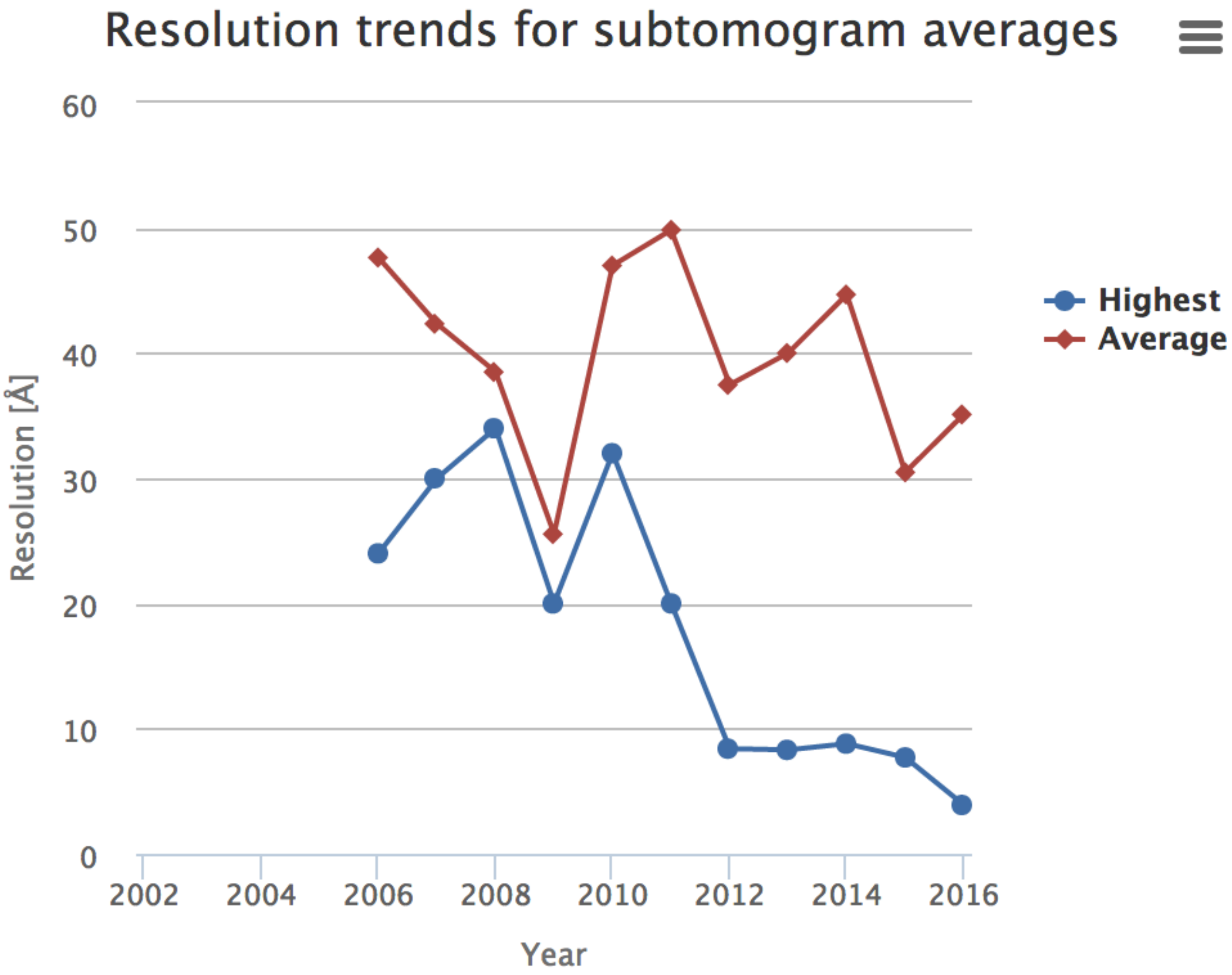
Stephan Nickell, Christine Kofler, Andrew P. Leis and Wolfgang Baumeister, 2006, A visual approach to proteomics, Nat. Rev. Mol. Cell. Biol. (7)

Subtomogram averaging



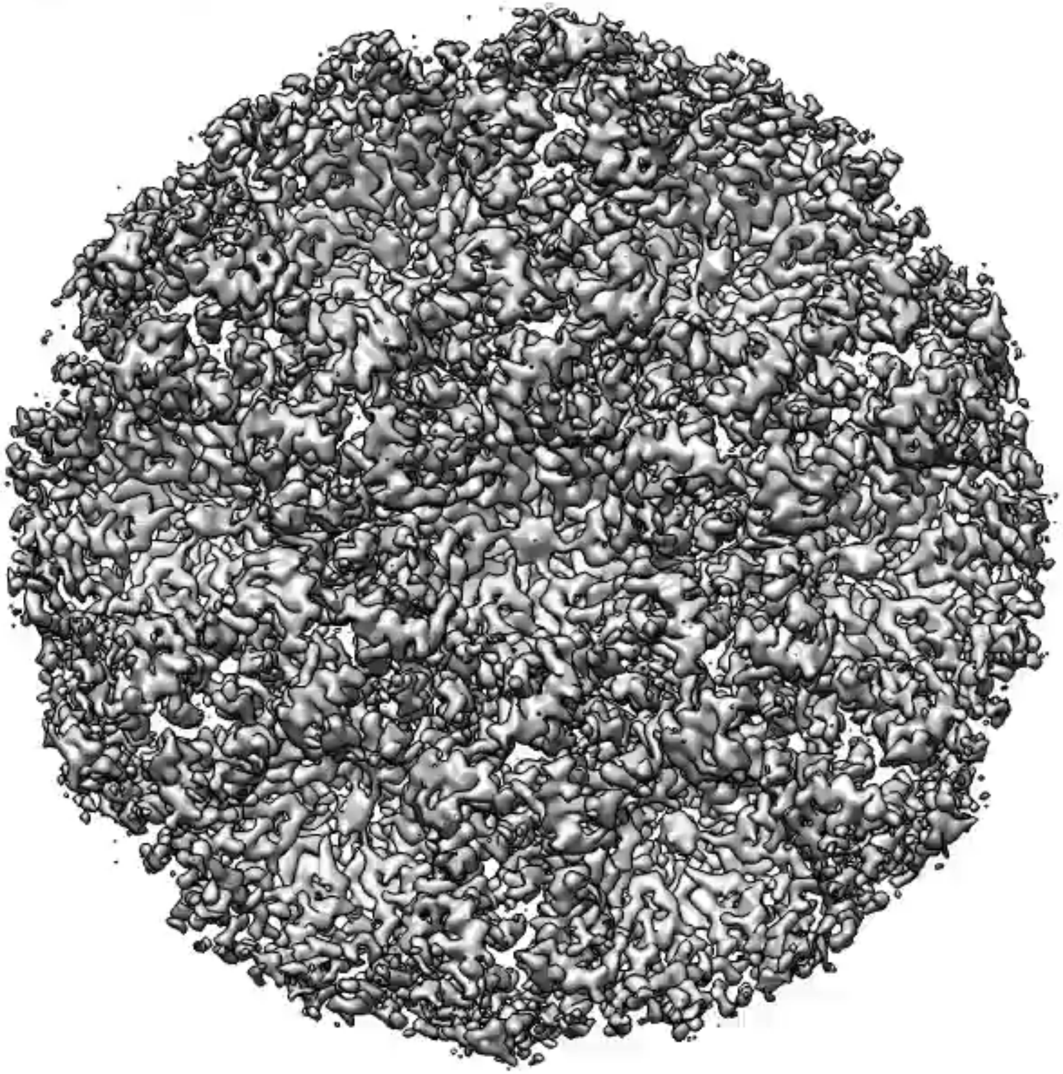
Iteration 1

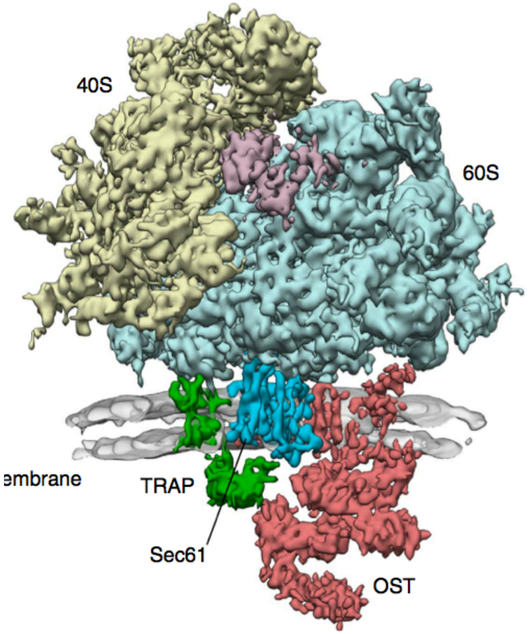




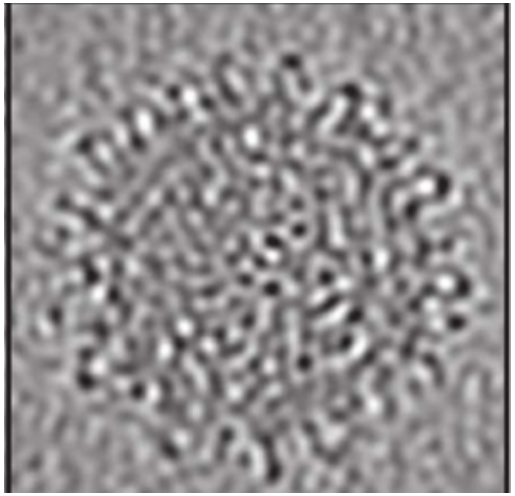
http://www.ebi.ac.uk/pdbe/emdb/statistics_tom_res.html/ (05/10/2016)

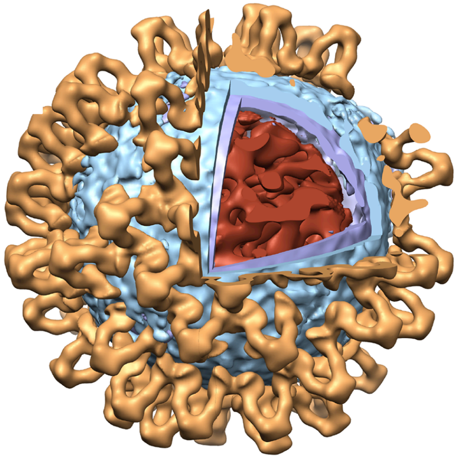
An atomic model of HIV-1 capsid-SP1 reveals structures regulating assembly and maturation
Schur F.K.M, Obr M., Hagen W.J.H, Wan W., Jakobi A.J., Kirkpatrick J.M., Sachse C., Kräusslich H-G., Briggs J.A.G



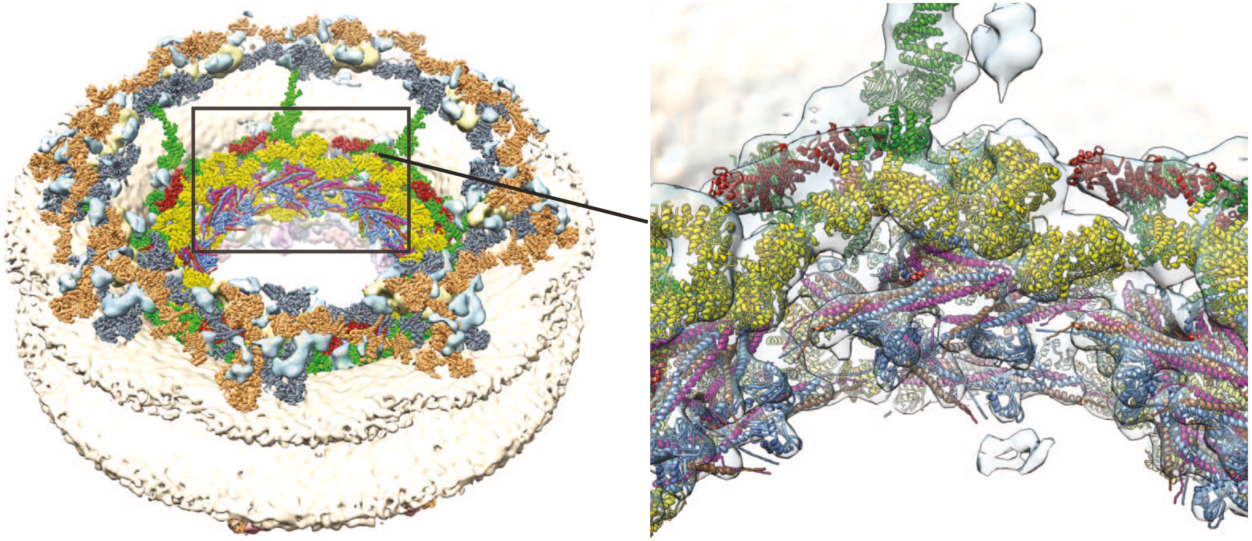


Pfeffer S et al, 2015, Nat.Comm.





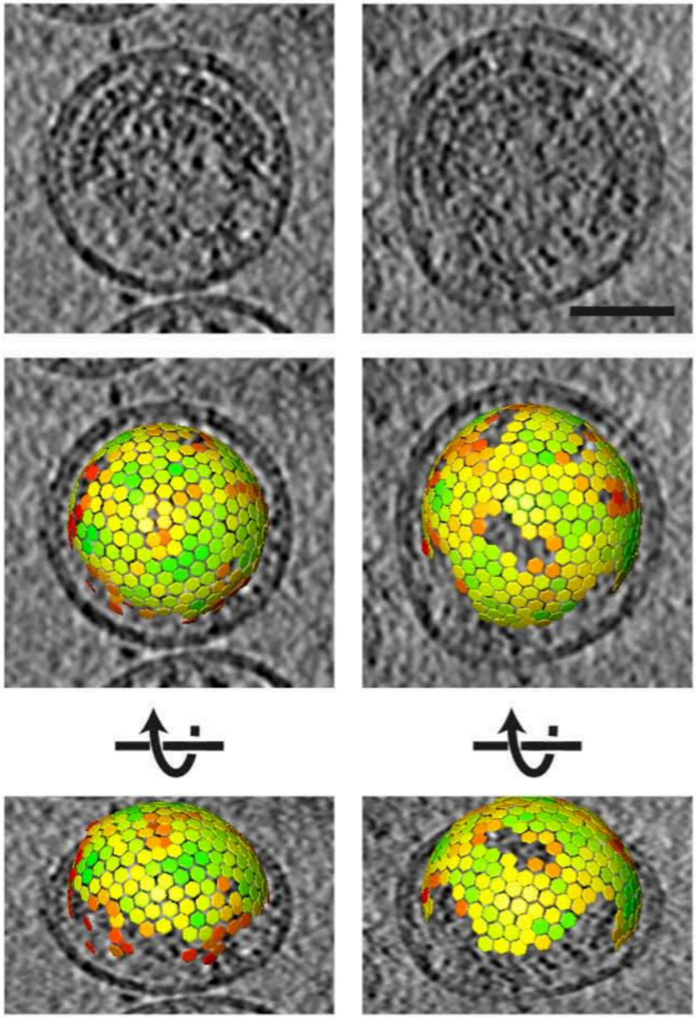
Bowden TA, et al, 2013, PLOS Pathogens (9)

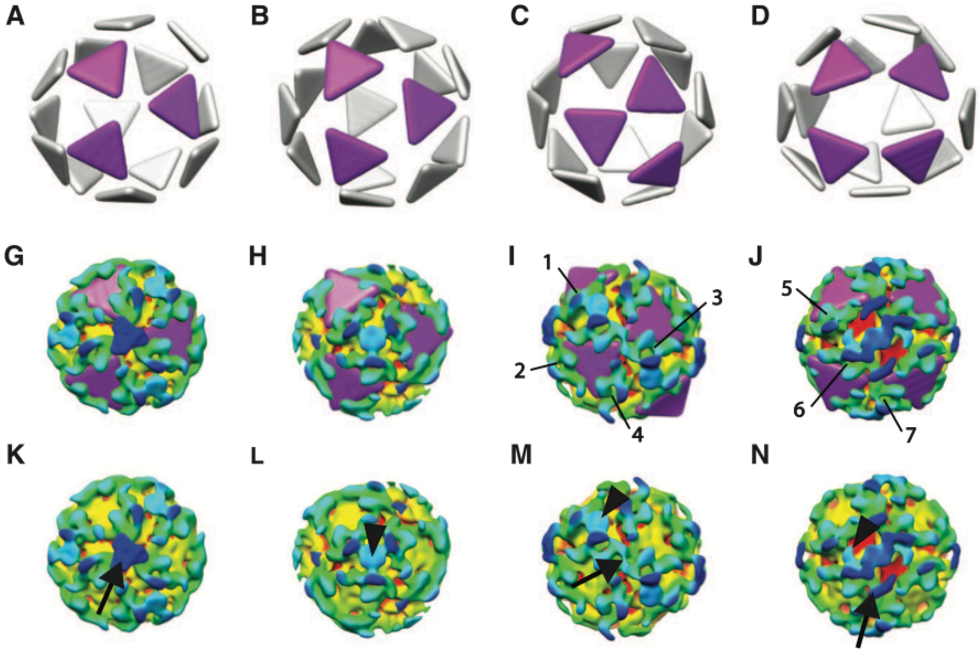


Kosinski J, et al, 2016 ,Science (352)

Three-dimensional Meta-Information

- Subtomogram averaging also reveals spatial distribution and relative spatial orientation of the proteins within the tomogram

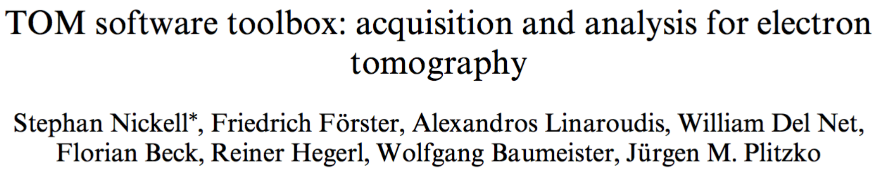




Faini M, et al, 2012, Science (336)

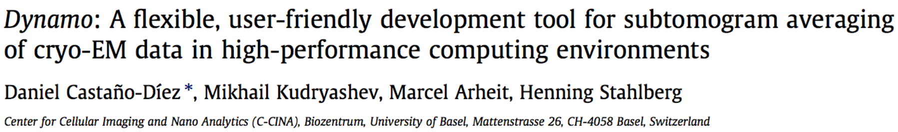
Software packages for subtomogram averaging

TOM/AV3	http://www.biochem.mpg.de/308752/AV31
Dynamo	https://wiki.dynamo.biozentrum.unibas.ch/w/index.php/Main_Page
PEET	http://bio3d.colorado.edu/PEET/
Relion	http://www2.mrc-lmb.cam.ac.uk/relion/index.php/Sub-tomogram_averaging
PyTOM	http://pytom.org/
Jsubtomo	http://www.opic.ox.ac.uk/wiki/index.php/Jsubtomo



TOM software toolbox: acquisition and analysis for electron tomography

Stephan Nickell*, Friedrich Förster, Alexandros Linaroudis, William Del Net, Florian Beck, Reiner Hegerl, Wolfgang Baumeister, Jürgen M. Plitzko



Dynamo: A flexible, user-friendly development tool for subtomogram averaging of cryo-EM data in high-performance computing environments

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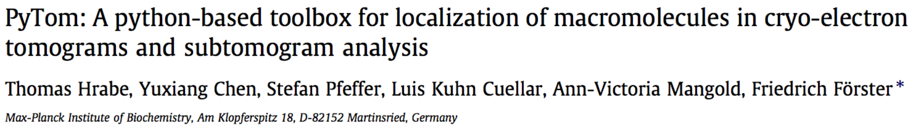

PyTom: A python-based toolbox for localization of macromolecules in cryo-electron tomograms and subtomogram analysis

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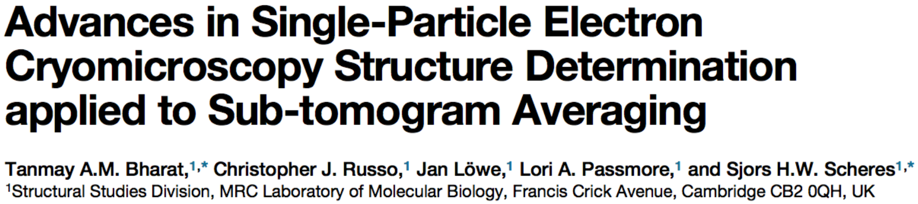

Advances in Single-Particle Electron Cryomicroscopy Structure Determination applied to Sub-tomogram Averaging

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Acknowledgements

- John Briggs
- Wim Hagen
- Yury Bykov
- William Wan
- Juha Huiskonen
- Misha Kudryashev