MAX-PLANCK-GESELLSCHAFT

MAX-PLANCK-INSTITUT FÜR BIOCHEMIE

General introduction to three-dimensional cryo-microscopy

Winter School on Structural Cell Biology

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Introduction

Three-dimensional cryo-microscopy

A concept that encompasses leading methods in **electron microscopy** for **three-dimensional structure** determination of biological samples, from macromolecules to cells, **in close to native conditions** (vitrified samples).

Cryo-EM: The challenges

- Samples that have to be studied in their hydrated state to ensure structural preservation

VITRIFICATION

converts liquid water into amorphous ice

- Suitable sample thickness to obtain molecular resolution

SAMPLE PREPARATION
ADVANCE MICROMACHINING
ADVANCE 3D CORRELATION

- Low-contrast due to weakly scattering building blocks

POOR IMAGING CONDITIONS
OPTIMIZE INSTRUMENTATION
E.G. DETECTORS & PHASE PLATES

- Sensitivity to ionizing radiation

LOW DOSE METHODS
ADVANCE AUTOMATION

TEM HISTORY: An Anecdote…

"You know," Szilard told Gabor over a café table in 1927, now that it is possible to make electron lenses, "why do you not make a microscope with electrons?" At smaller and smaller wavelengths, you would achieve much more detailed resolution than is possible with microscopes using light. Gabor and Szilard pondered his idea for a few minutes, then agreed it would serve no useful purpose. After all, you could not put living matter into the kind of vacuum tube needed to control electron beams. Besides, they concluded, so much power would be focused in the electron beam that it would incinerate any sample.

But as Gabor later realized, with that idle suggestion Szilard had grasped the possibility of an electron microscope at least a year before anyone else. And of the incinerated sample, Gabor later wrote, "Who would have dared to believe that the cinder would preserve not only the structure of microscopic bodies but even the shapes of organic molecules?" Gabor would be remembered years later as the inventor of holography, for which he received the 1971 Nobel Prize in physics.[15]

William Lanouette. Genius in the Shadows, A Biography of Leo Szilard, the Man Behind the Bomb

WHY?: Microscopy with electrons

At smaller and smaller electron wavelengths, one can achieve much more detailed resolution than with light.
The wavelength of an electron is given by the de Broglie equation:

$$\lambda_{electrons} = \frac{h}{mv} \approx \frac{h}{\sqrt{2m_0E(1+\frac{E}{2m_0c^2})}}$$

energy E [keV]	wavelength λ [Å]	velocity v [km/sec]
20	0.086	76000
40	0.06	107000
60	0.059	131000
80	0.042	152000
100	0.037	170000
300	0.019	233000
1000	0.009	282000

→ *Transmission electron microscopes can resolve details down to 1 Å!*

Some highlights in TEM history

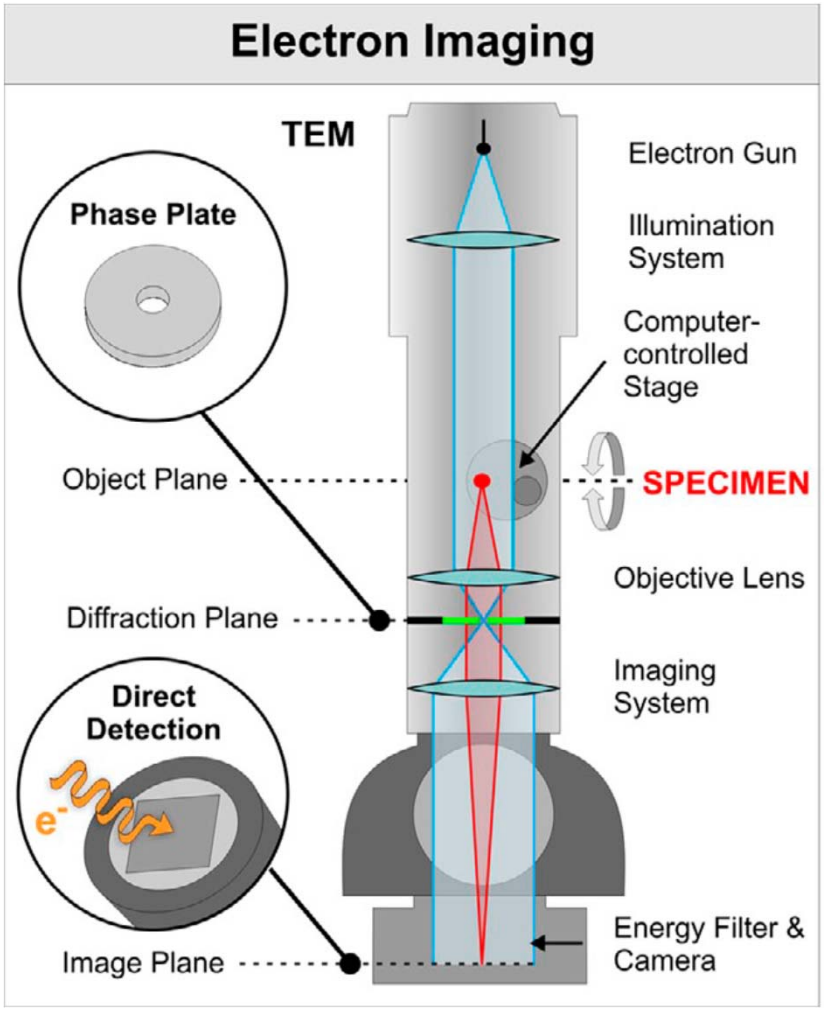
1931 Technical University, Berlin

1897	**J.J. Thomson**	Discovered the electron (as a *'corpuscle'*)
1924	**Louis de Broglie**	Predicted the wavelength for the electron $\lambda = h/mv$
1926	**H. Busch**	Magnetic and electric fields act as lenses for electrons
1927	**G.P. Thomson/ C.J. Davisson and L.H. Germer**	Observed the wave properties by electron diffraction
1931	**M. Knoll & E. Ruska**	Built the 1st electron microscope (EM)
1936	**Metropolitan Vickers**	Manufactured the 1st industrial EM (EM1)
1938	**B. von Borries & E. Ruska**	Produced the 1st practical EM (Siemens) – 10 nm resolution
1939	**Siemens&Halske**	Started their commercial EM production
1962	**R. Castaign & L. Henry**	Built the 1st energy filter
1982	**P.T.E Roberts et al.**	Used the 1st CCD's for EM
1986	**E. Ruska/ G. Binning and H. Rohrer**	Received the Nobel Prize for TEM and STM

E. Ruska und M. Knoll: Die magnetische Sammelspule für schnelle Elektronenstrahlen. *Z. techn. Physik*. Band 12, 1931, 389–400 und 448

M. Knoll und E. Ruska: Das Elektronenmikroskop. *Z. Physik* 78, 1932, 318-339.

Electron Microscope - basics

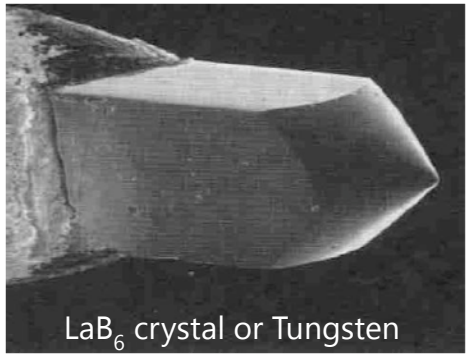


1	The “GUN”	Thermionic Emission
		Field Emission (FEG)
2	Condenser System	2 Condenser lens system
		3 Condenser lens system
3	Objective System	Twin lens
		Bio-Twin / Super-Twin / Ultra-Twin
		Phase Plates
4	The “STAGE”	Micro-Mechanical Motorized Stages
		Piezo-Controlled Motorized Stages
5	Sample Holders	Side-entry Holders
		Multispecimen Holders
6	Electron Detectors	Negative Plates/Film
		CCD Cameras
		Direct Electron Detectors
7	Energy Filters	In-column Energy Filters
		Post-Column Energy Filters

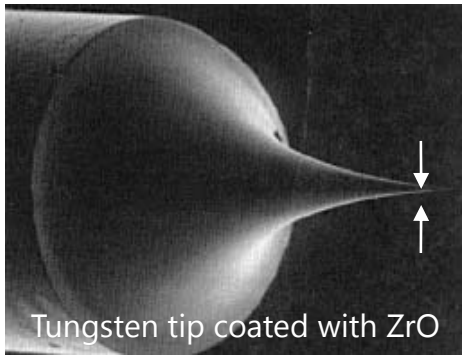
Lučić et al. *JCB* (2014)

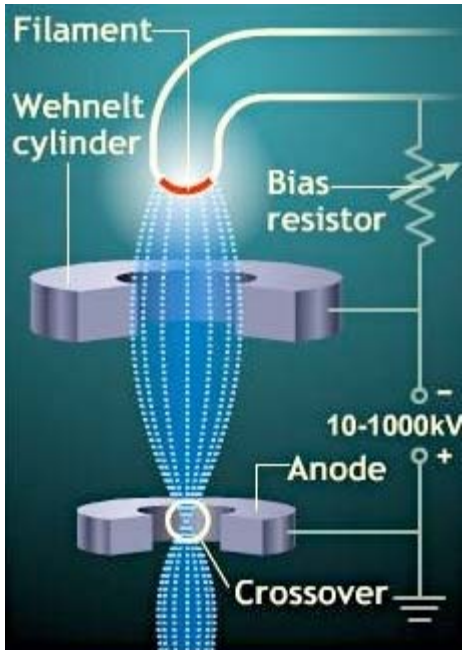
The Gun: Types of Emitters

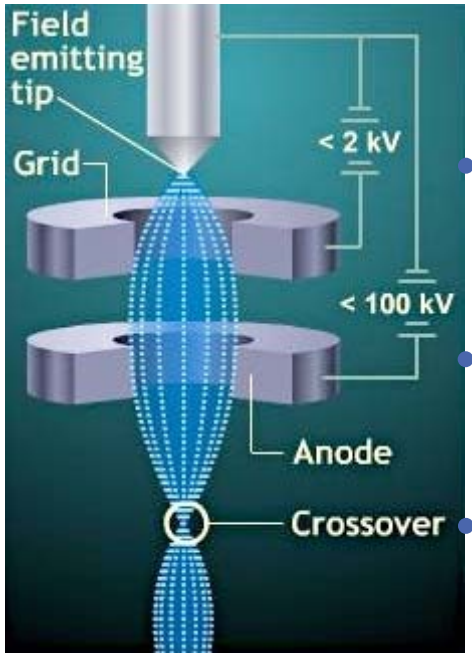
Thermionic Gun



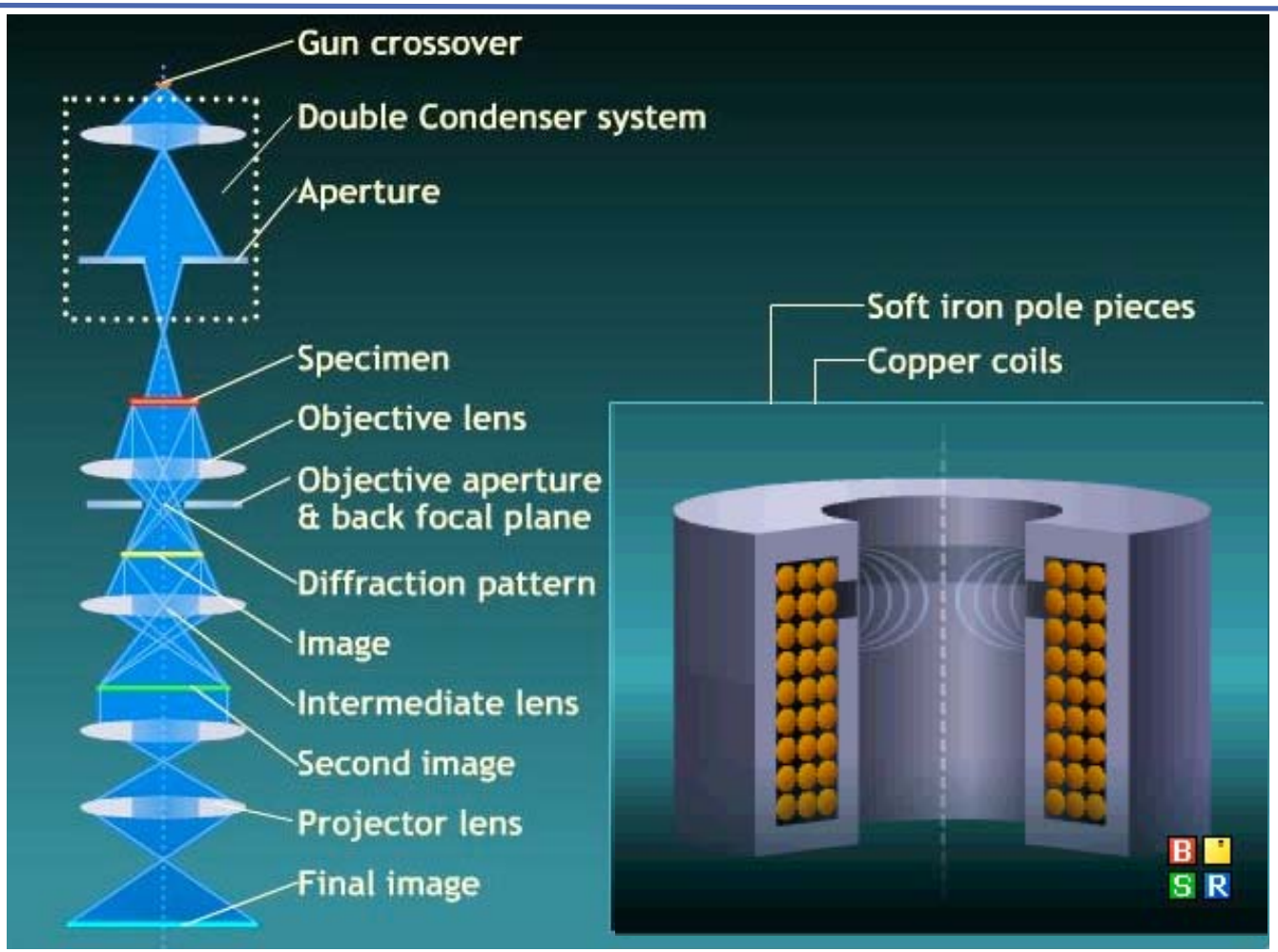
Field emission Gun (FEG)







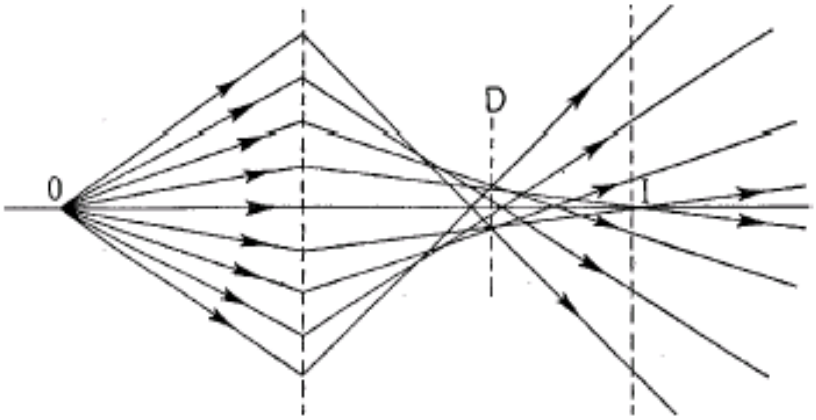
THE LENSES: Electron Optics



Electrons are manipulated using electromagnetic lenses.
The electron beam coming from the gun is focused and shaped with the help of condenser lenses and apertures.
The objective lens and the projection system are used to obtain and magnify a diffraction pattern or the real image.

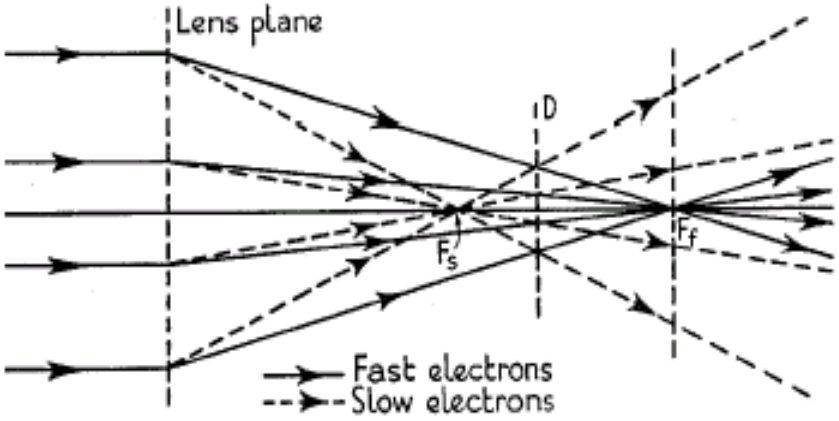
Optical Aberrations

spherical aberration



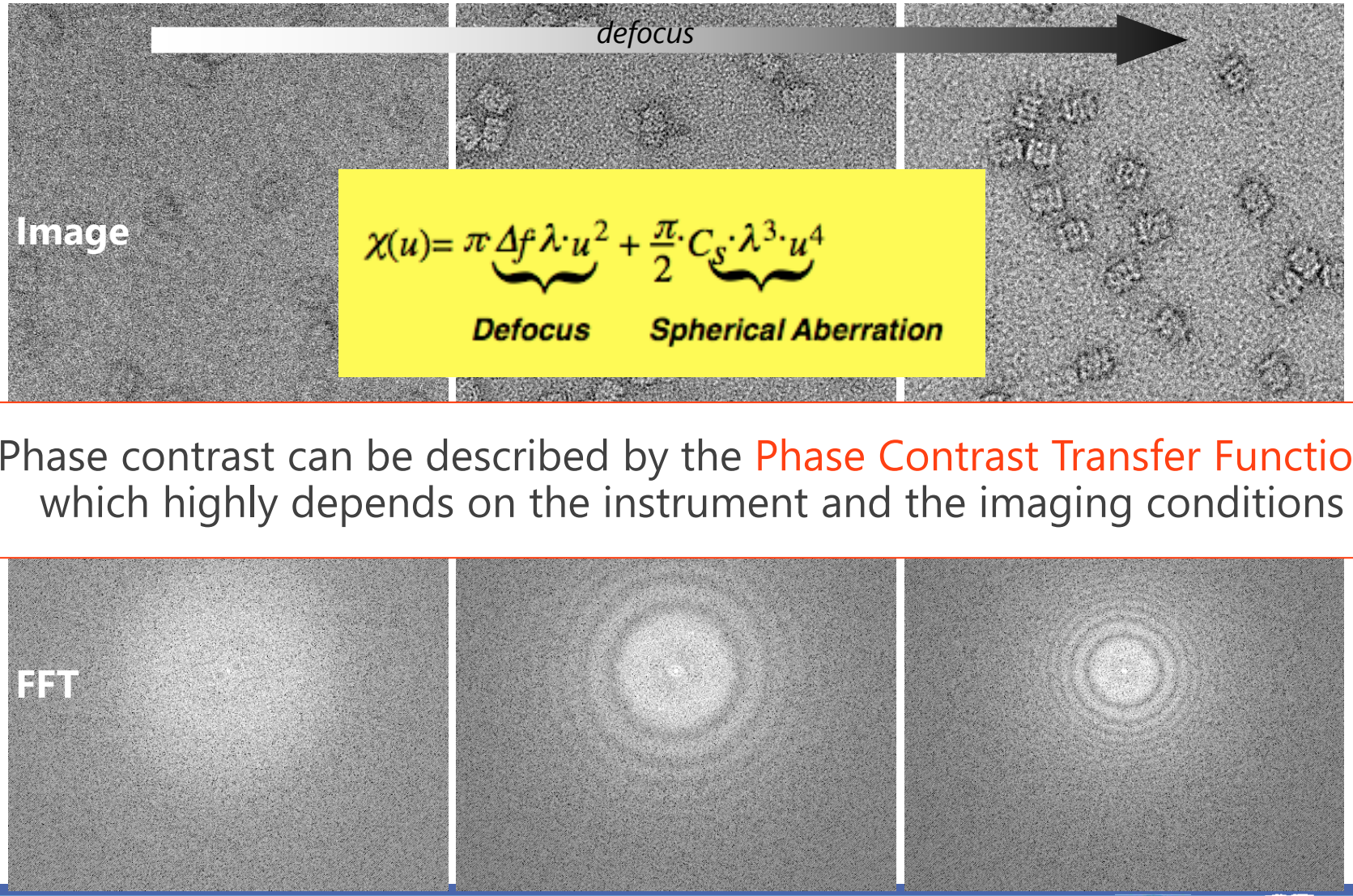
$$r_{sph} = C_s \beta^3$$

chromatic aberration



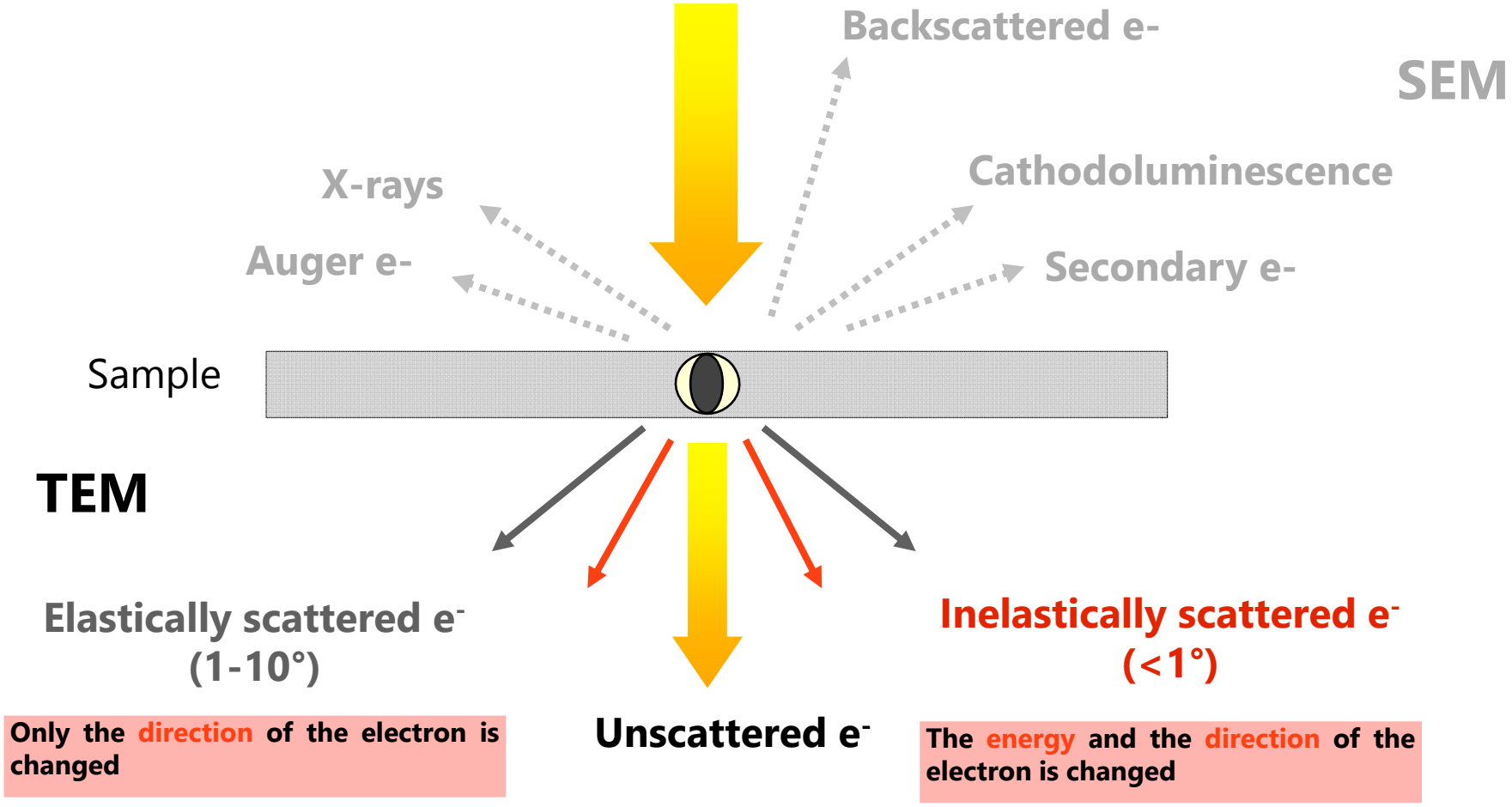
$$r_{chr} = C_c \frac{\Delta E}{E} \beta$$

Objective Lens



Phase contrast can be described by the Phase Contrast Transfer Function, which highly depends on the instrument and the imaging conditions

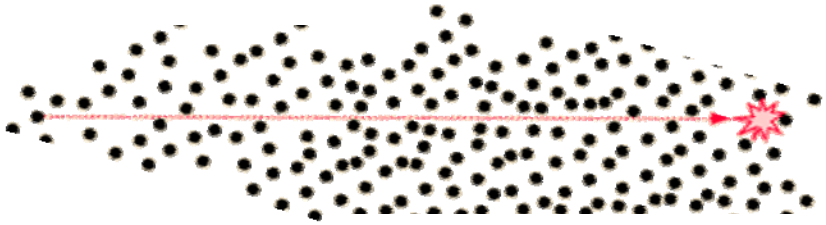
Electron-sample interactions



Inelastic Scattering

- The amount of inelastic scattering increases with specimen thickness.

- Inelastic **Mean Free Path (MFP)**, which is a dimension that indicates the path-length inside the specimen wherein all electrons (statistically speaking) will have undergone one inelastic scattering event.

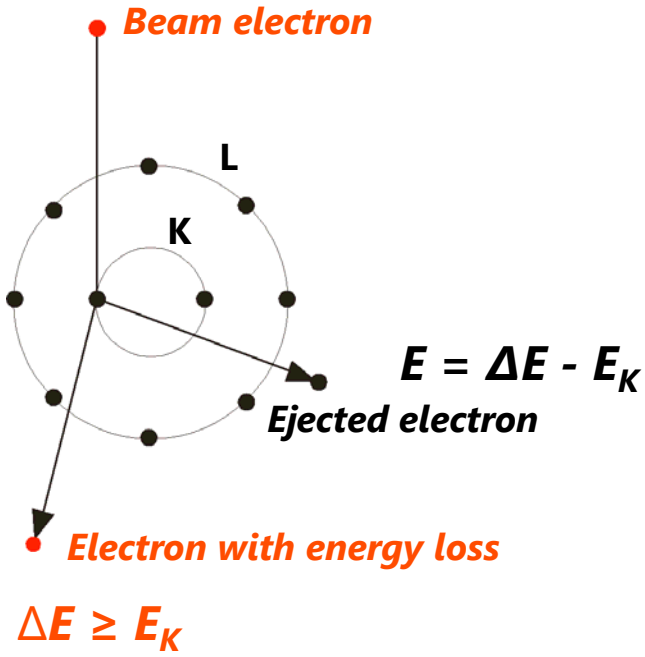


- Typical mean free paths are of the order of :

  050-100 nm for 120kV
  100-200 nm for 200kV
  150-300 nm for 300kV.

Inelastic Scattering *...& magnetic prism!*

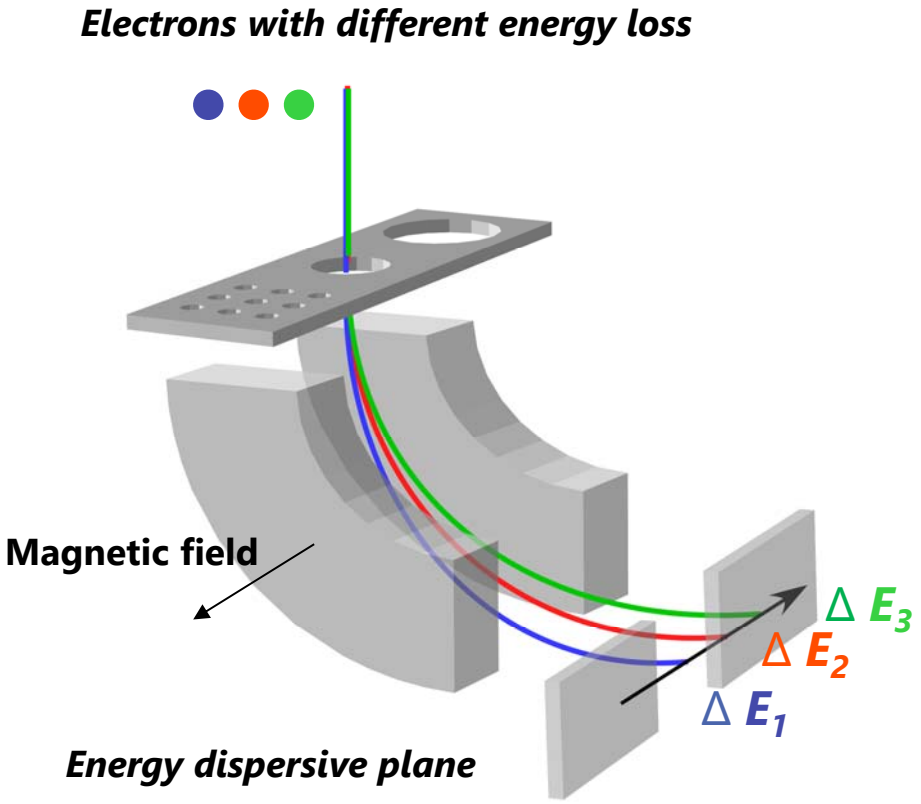
Inelastic scattering



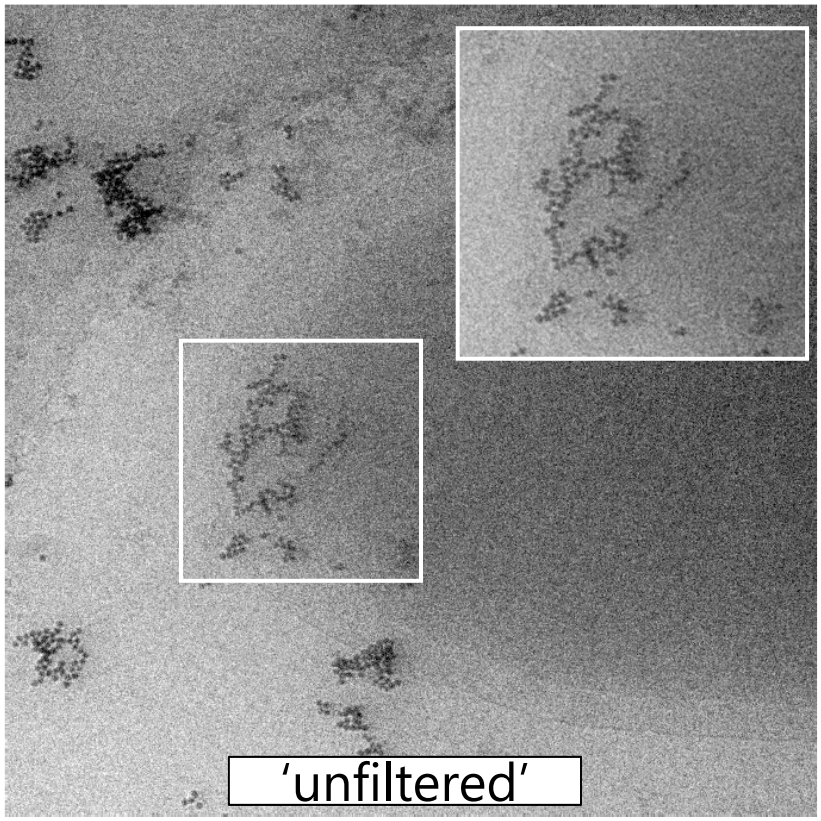
$E = \Delta E - E_K$

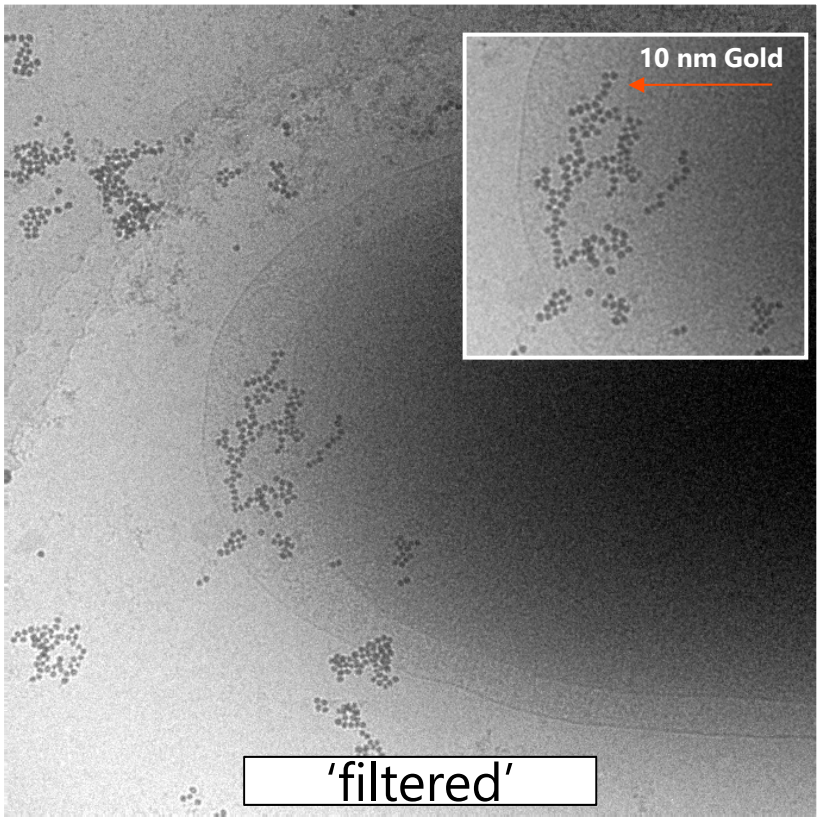
$\Delta E \geq E_K$

Magnetic Prism

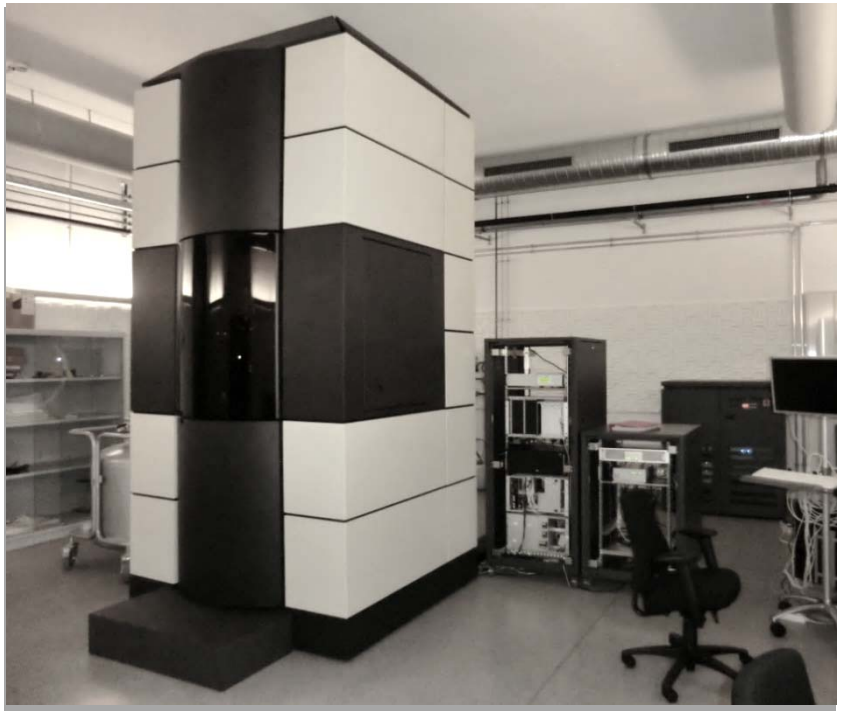


‘Zero-loss’ filtering





Methods: Instrumentation



FEI Titan Krios

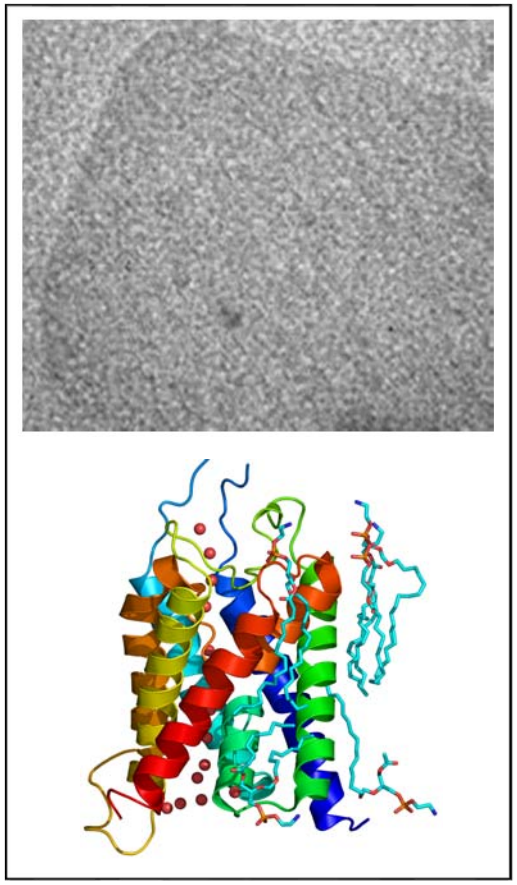
Transmission Electron Microscope

FEI™ Titan Krios

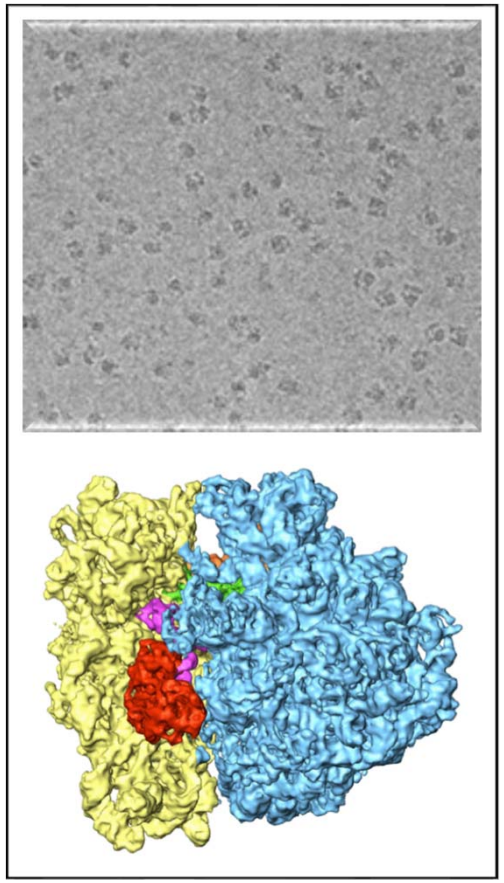
Some Properties:

- Electron Gun: FEG operated at 300 KeV
- Automated & robotic sample transfer
- Automated data acquisition/ remote controlled
- Stable goniometer with capabilities for dual-axis tilt tomography

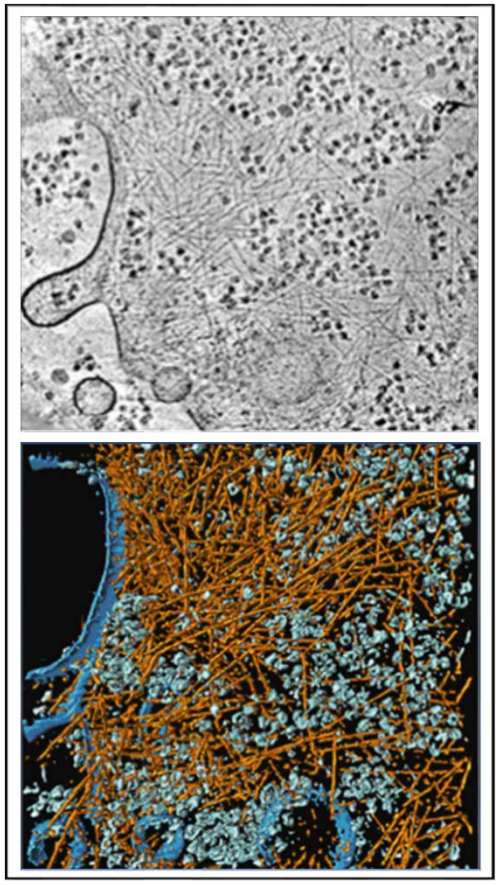
Three Branches of Tridimensional Electron Microscopy



Electron crystallography

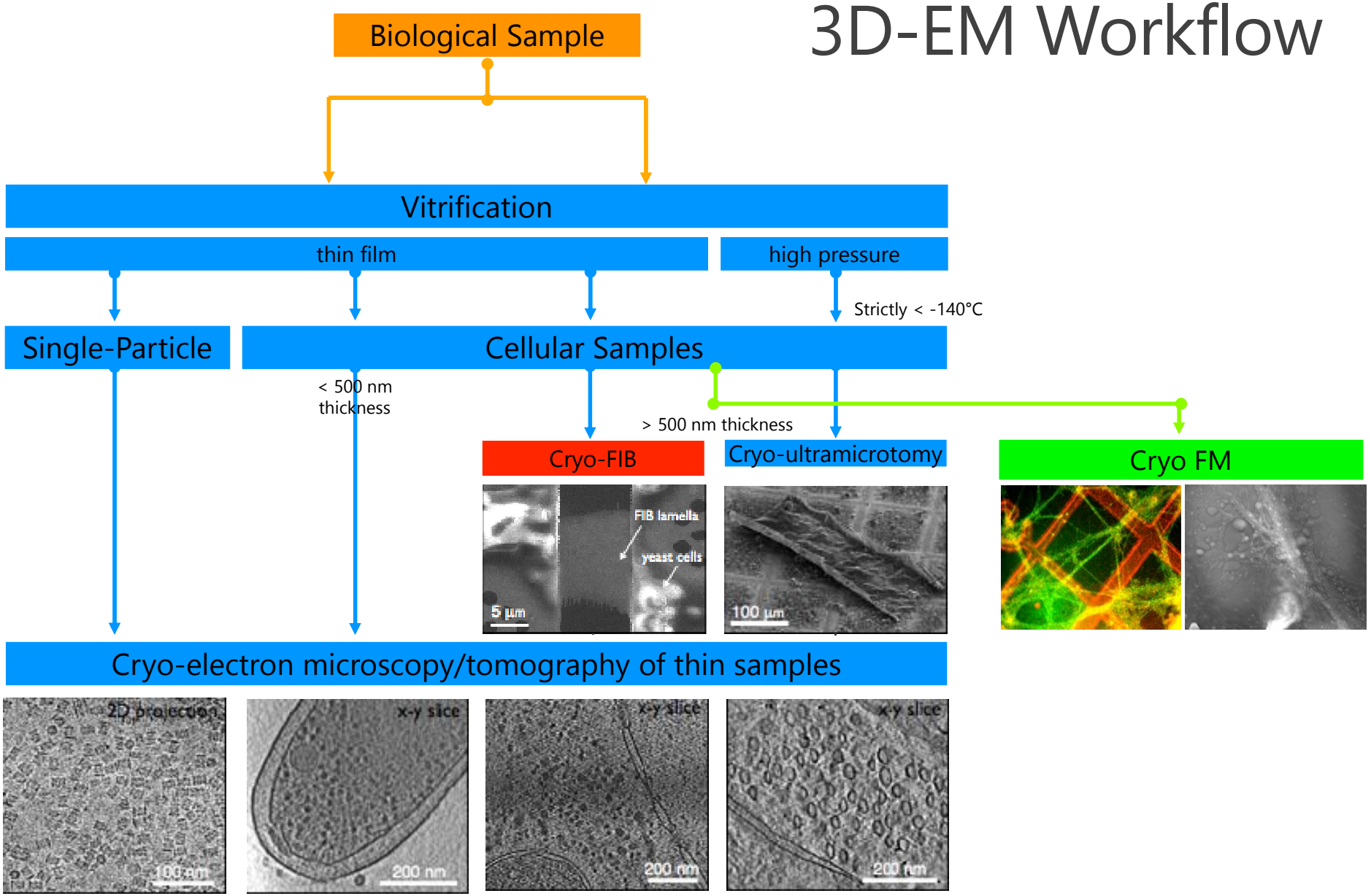


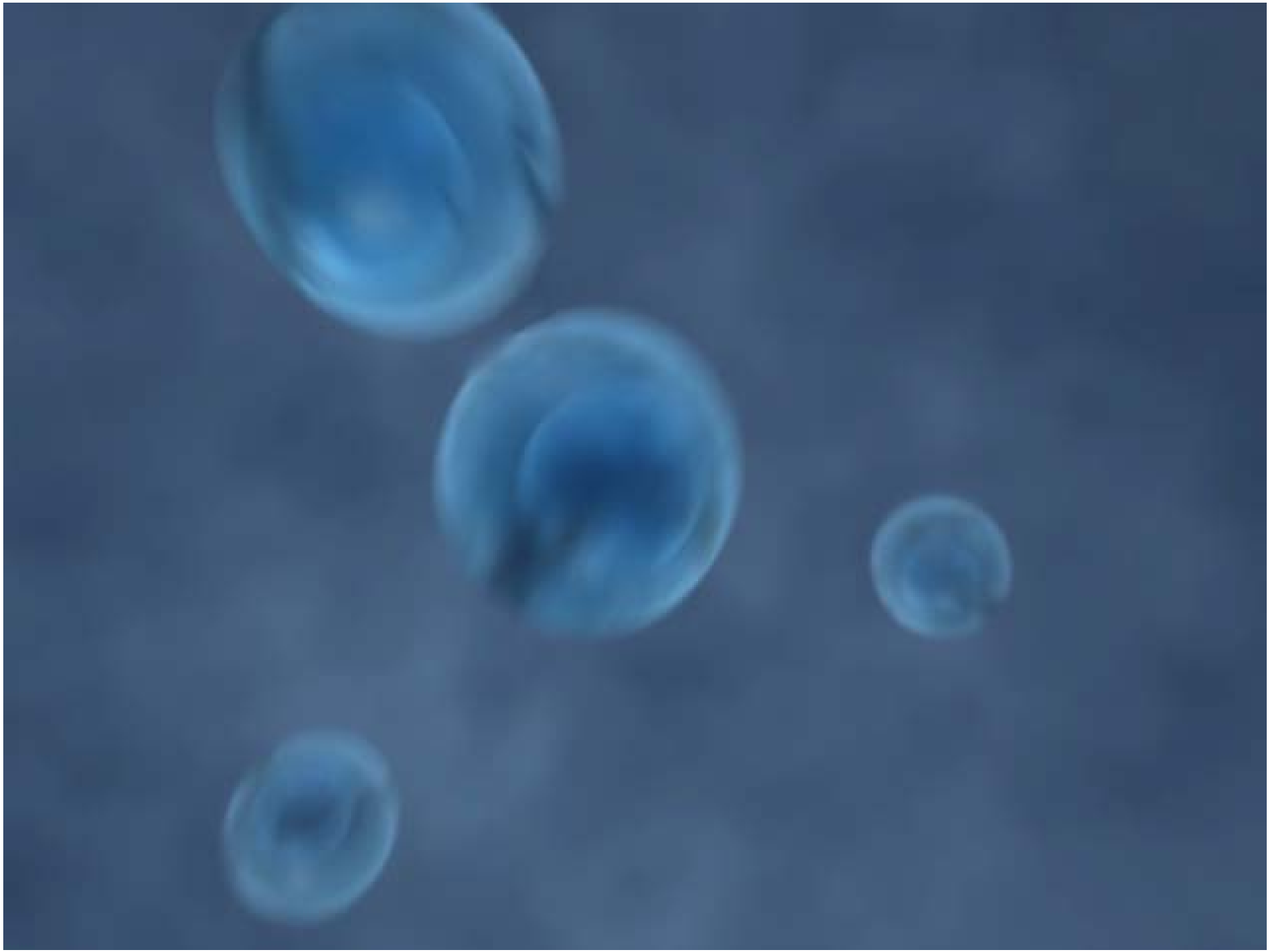
Single-particle analysis (SPA)



Cryo-Electron Tomography (CET)

3D-EM Workflow





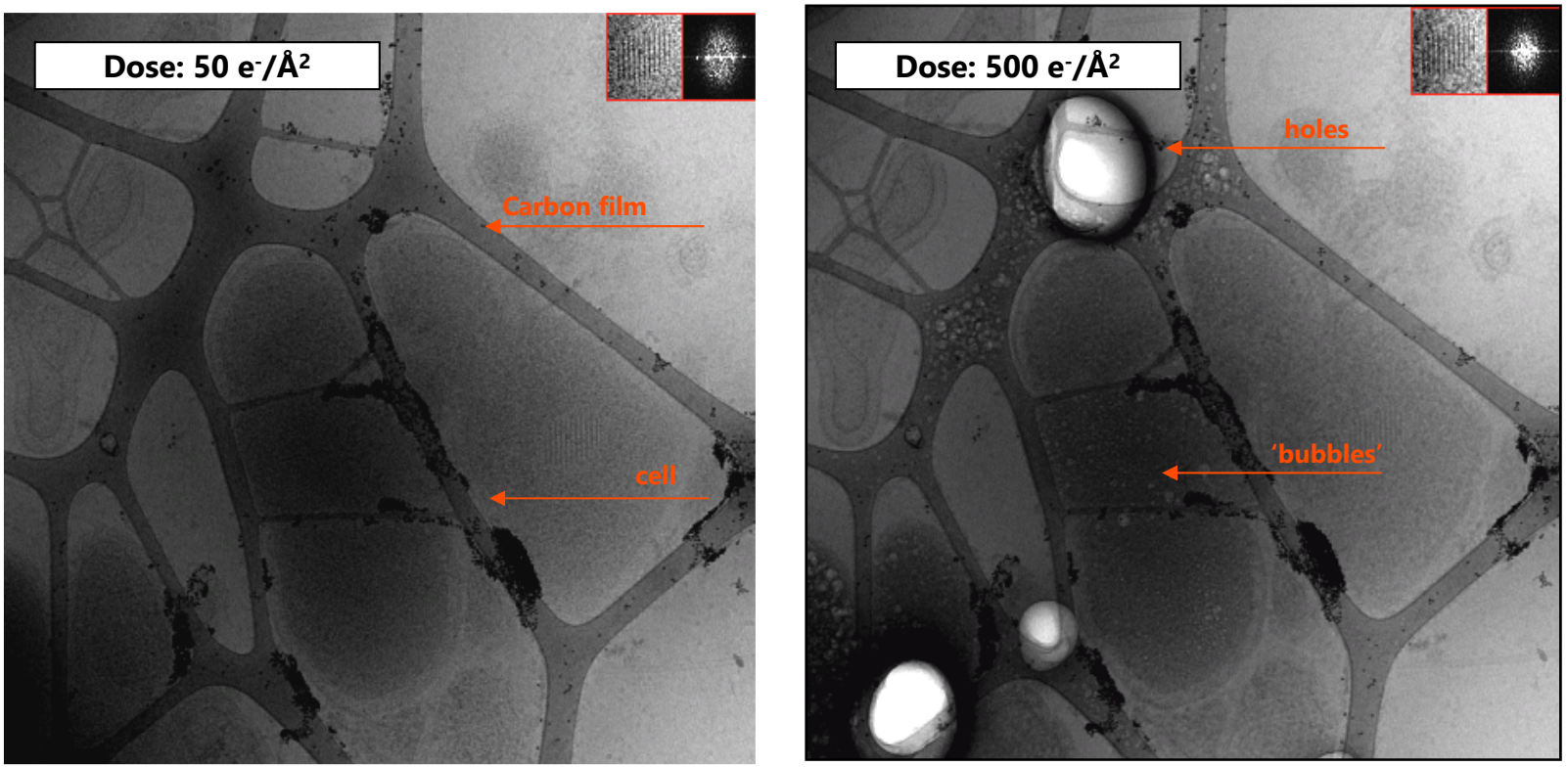
One Dilemma in Cryo-ET

Acquire as many images as possible (>= 100-200)

Over the largest possible angular range (+/- 70°)

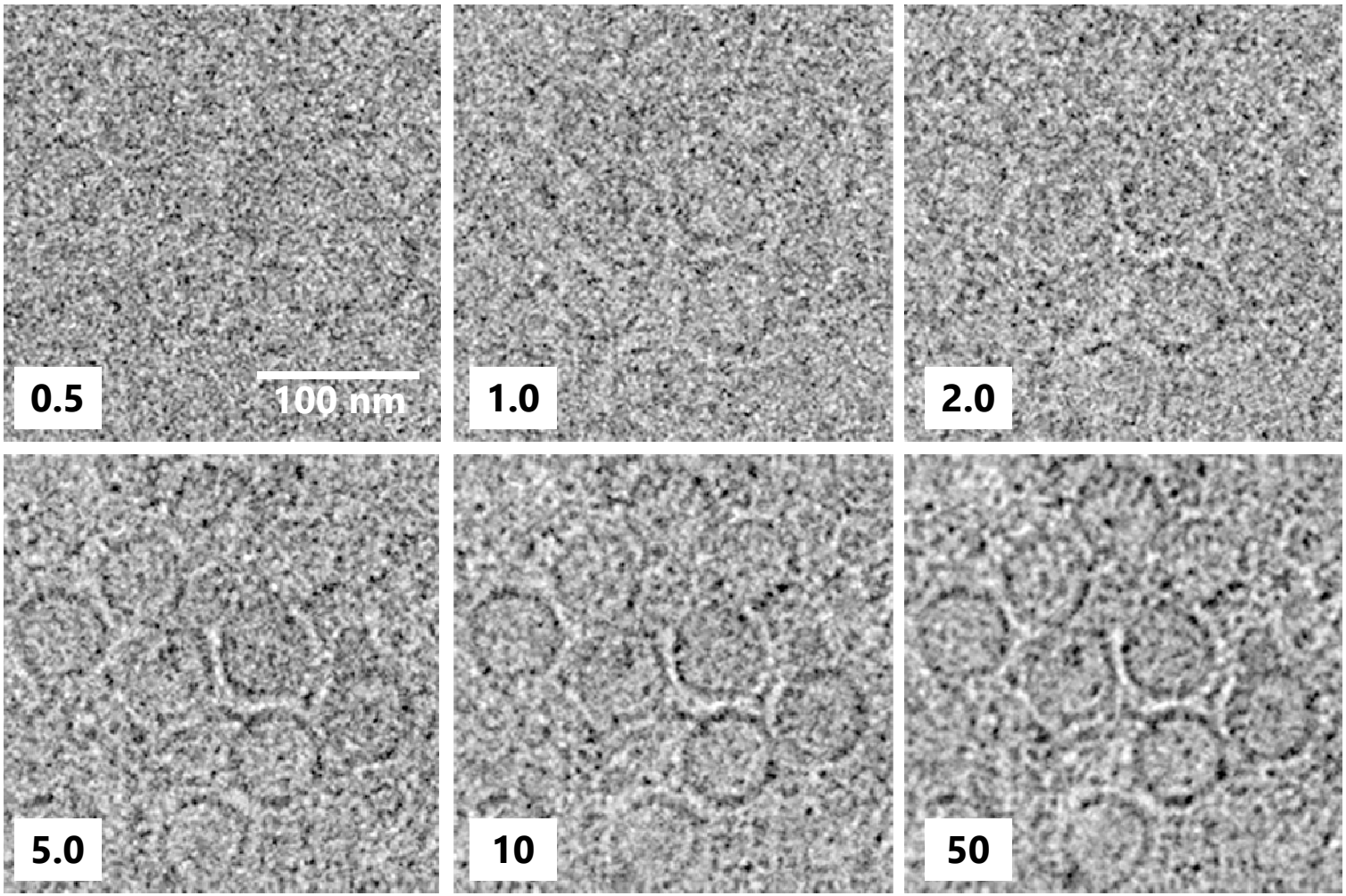
At the 'lowest' possible dose (~ 50-100 e^-/A^2)

Radiation damage



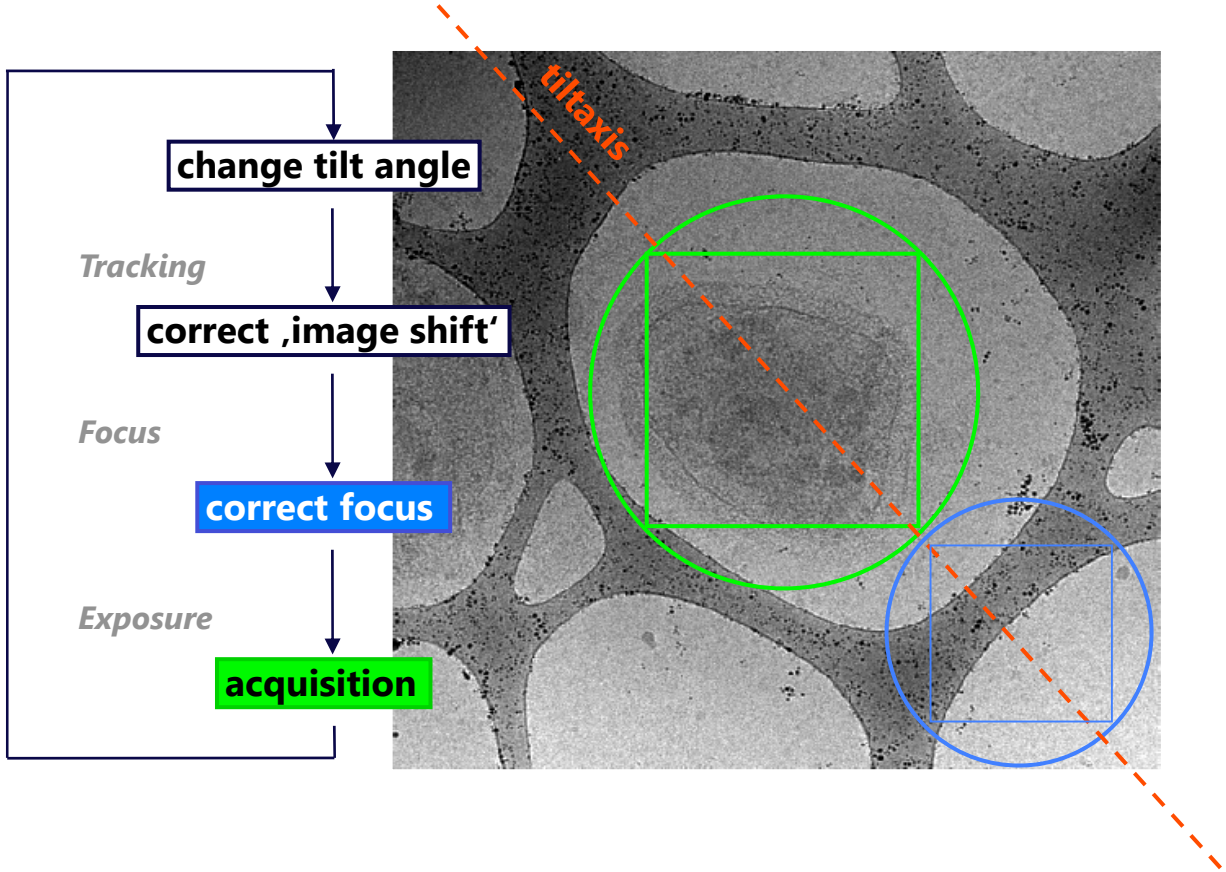
Dose = current density per unit area *j* (A/cm^2) multiplied by the exposure time *t* (s)
(C/cm^2, or e$^-$/Å^2, e$^-$ = electrons)

Electron Dose: Signal&Noise



→ vitrified chromatophore vesicles in *Rhodobacter sphaeroides (Falcon I)*

Low Dose Acquisition scheme



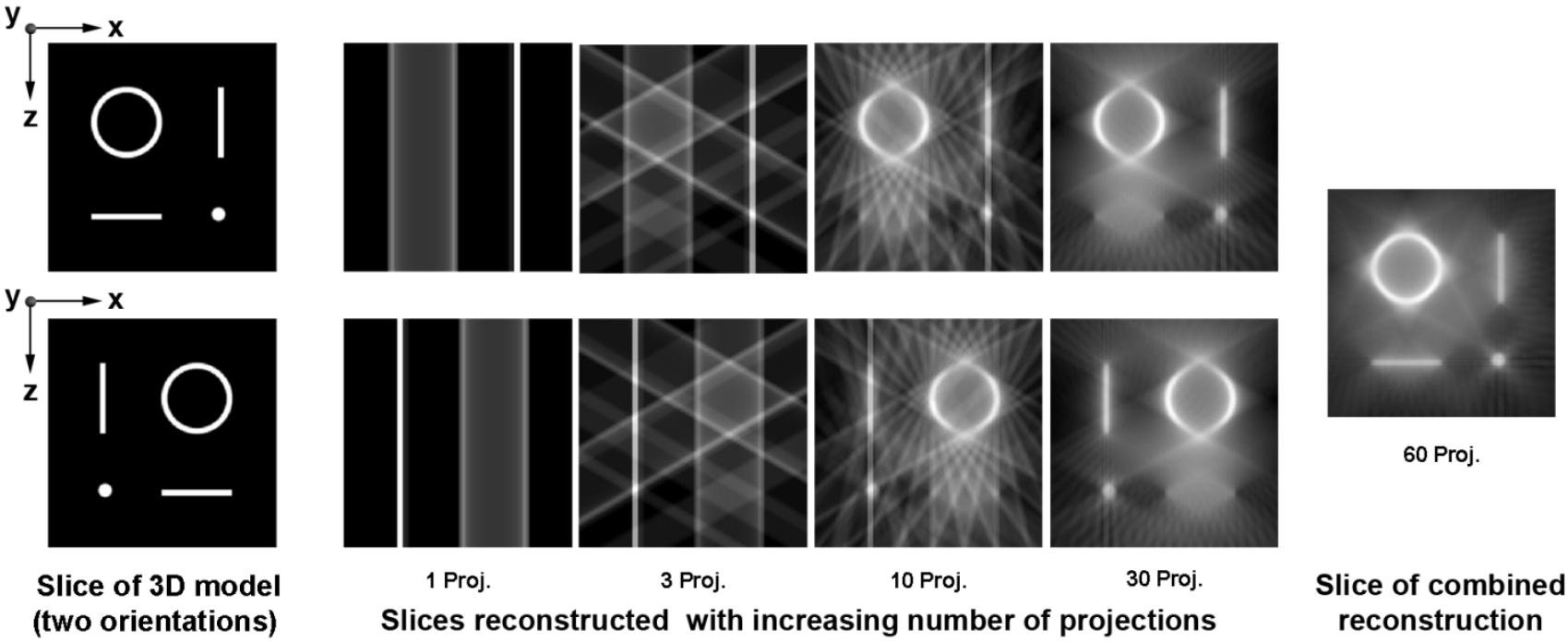
Cryo-electron tomography

Single-axis tilt Electron Cryotomography

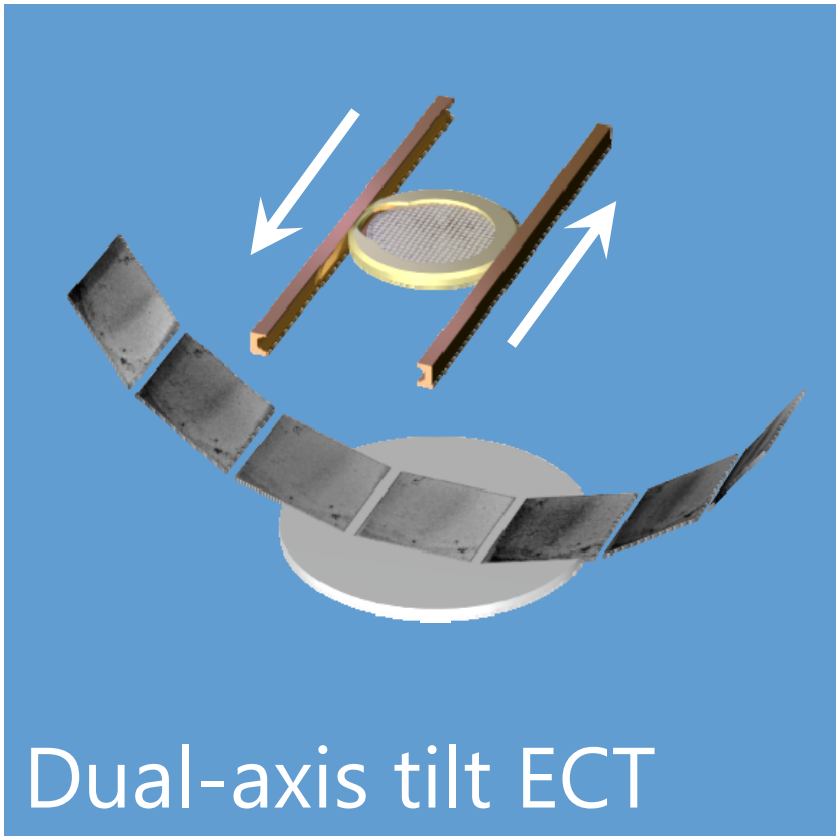
A tilt series is recorded in a TEM by rotation of the sample holder around one axis, usually ca. 120 degrees.

Aim:
3D reconstruction of the imaged object.

Missing Wedge Problem



Cryo-electron tomography



Dual-axis tilt Electron Cryotomography

Two orthogonal tilt series from the same object are recorded in a TEM.

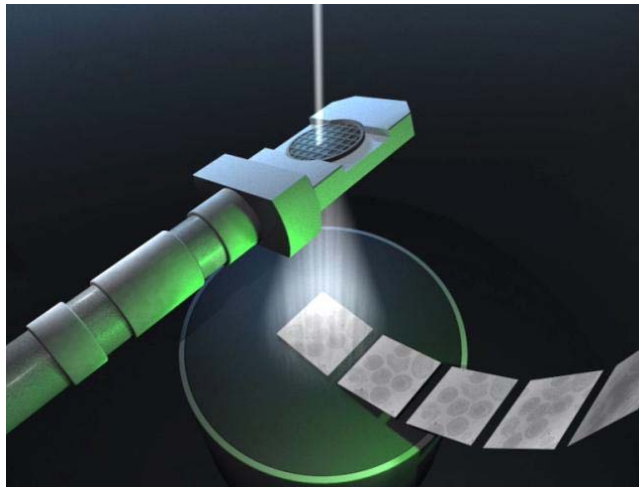
Aim:

To gain isotropic resolution for higher fidelity of macromolecular complexes recognition.

Missing wedge

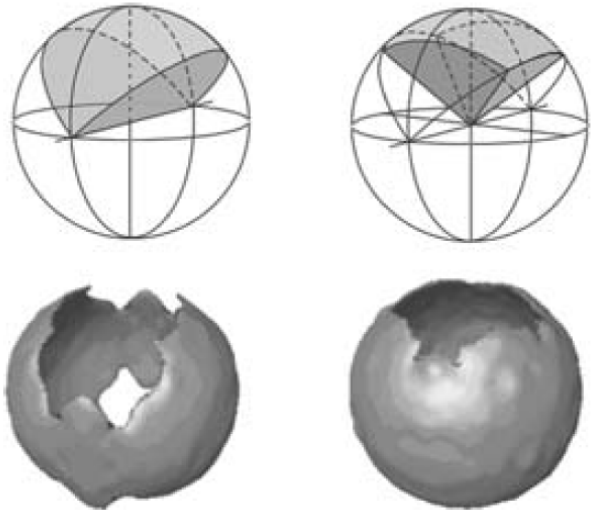
Limited tilt range implies an unsampled region

(information is missed)



Fourier space

Real space



Tilt range	Single	Double
±70°	78%	93%
±60°	67%	84%
±45°	50%	67%

(Lucić, V.,et al, Annual Review of Biochemistry, 2005)

“Cryo-Electron Microscope” concept

NEW APPROACHES IN CORRELATIVE STUDIES OF
BIOLOGICAL ULTRASTRUCTURE BY
HIGH-RESOLUTION ELECTRON MICROSCOPY

By

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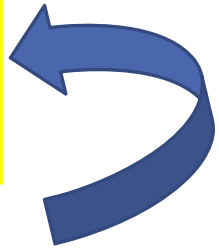
Committee on Biophysics
University of Chicago
Chicago 37, Illinois

Paper presented at
ROYAL MICROSCOPICAL SOCIETY'S
Celebration of the "Tercentenary of the
Microscope in Living Biology"
April 9, 1963
Bethesda, Maryland

Concept also in:
Fernandez-Moran, H. (1965) *PNAS* 53: 445-451

“Cryo-Electron Microscope” concept

These "cryo-electron microscopes", operating at temperatures of 1 to 4 degrees Kelvin, would embody the following significant features: (a) highly stable superconducting electromagnetic lenses, with very ripple-free magnetic fields of a persistent current in the optimum case; (b) operation in ultra-high vacuum and low temperatures resulting in decisive advantages of minimized specimen contamination, specimen damage and thermal noise; (c) optimum conditions for both low voltage (i.e. 1 to 10 kV) and high voltage electron microscopy. In addition, the use of high-efficiency image viewing (single-crystal fluorescent screens) and recording devices operating at optimum low temperatures would make it possible to use high-speed cinematography and stroboscopic recording (e.g. obtained through pulsed T-F emission from pointed filaments) for attainment of high temporal resolution combined with high spatial resolution.



Complementary metal–oxide–semiconductor (CMOS) based detector (i.e, *K2 Summit*™)

Methods: Instrumentation

Direct Electron Detectors

Direct Detection Cameras

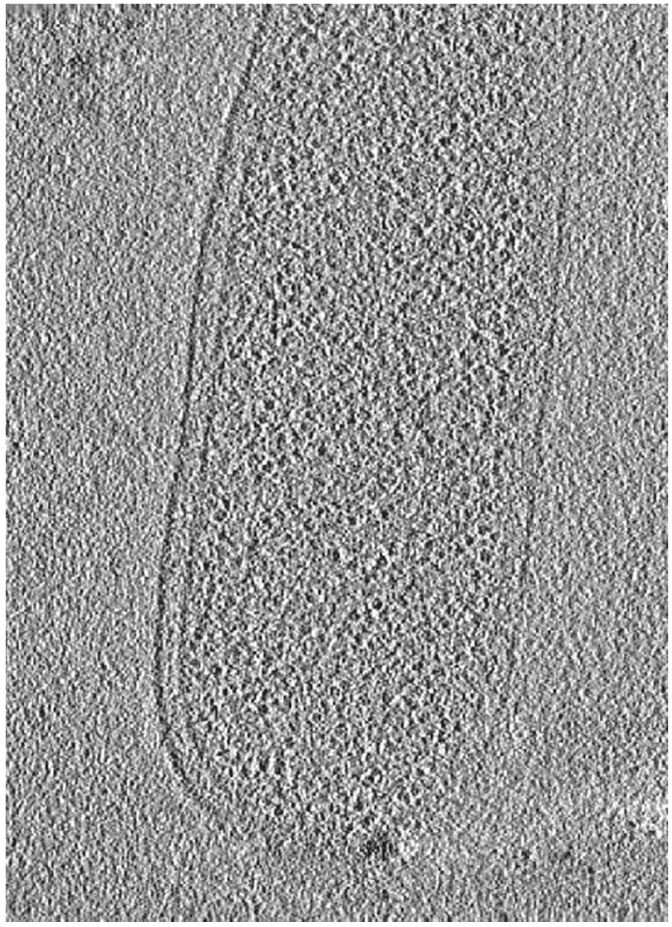
- Falcon (FEI™)
- K2 Summit™ (Gatan INC).

General Properties:

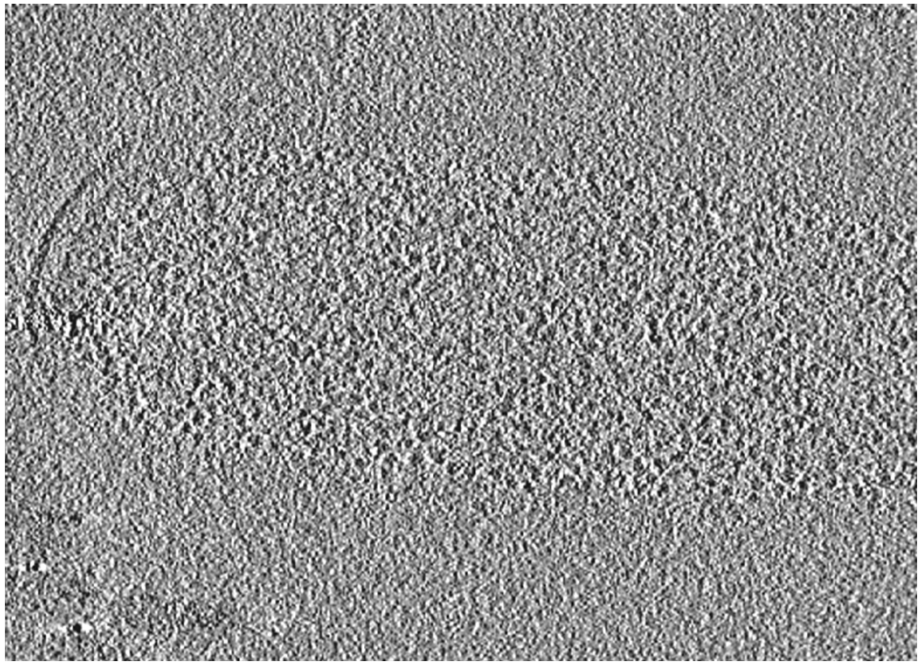
- High spatial resolution
- High Detective Quantum Efficiency (DQE)
- Higher sensibility than a CCD camera. Less electron dose required.

Dual-axis tilt tomography - FIB wedge *E. coli* BL21

Single-axis Tilt Tomogram A



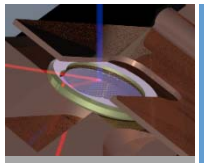
Single-axis Tomogram B



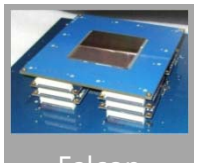
- FEI TITAN Krios
- Direct electron detection (Falcon)
- Mag. 22.5kx

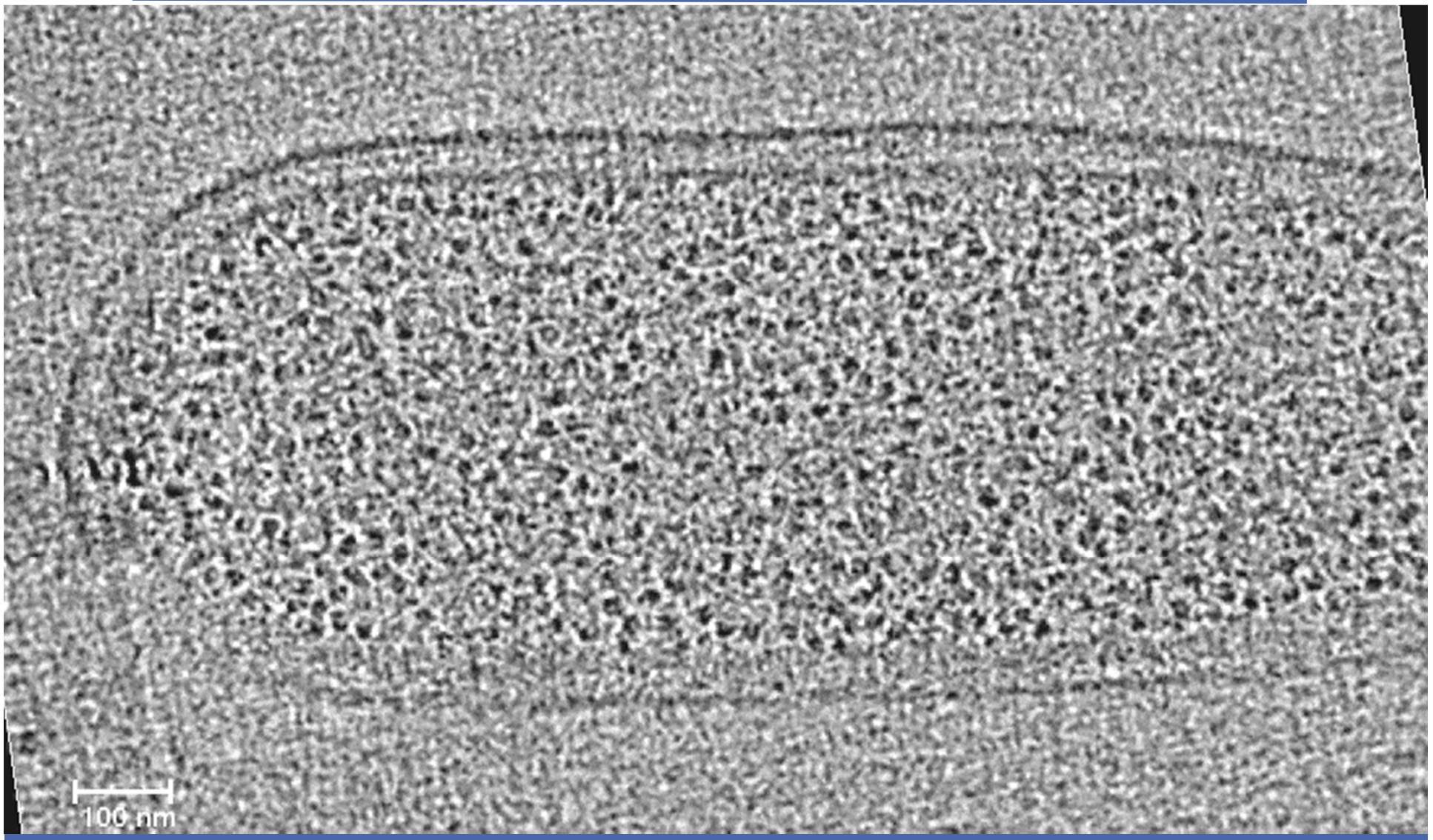
- Angular increment 3°
- Total Dose 2x ~50 $e^-/Å^2$
- Pix size: 0.372 nm/p
- Def. -9 µm

E. coli BL21 MtlA385-SecM


FIB

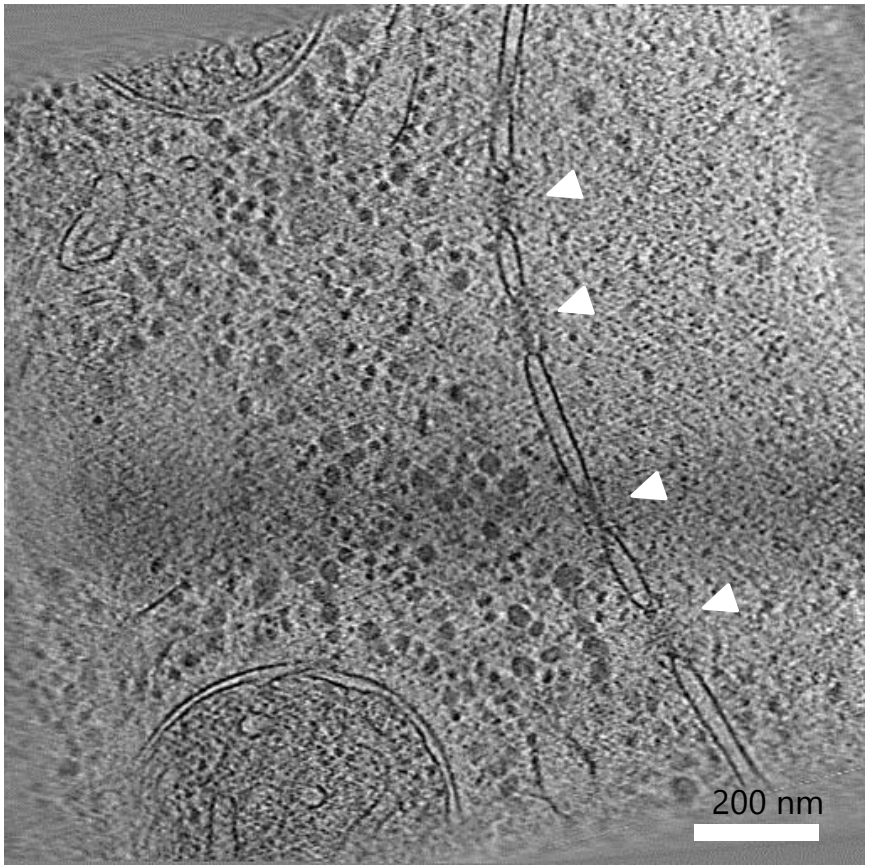

Dual-axis tilt


Falcon

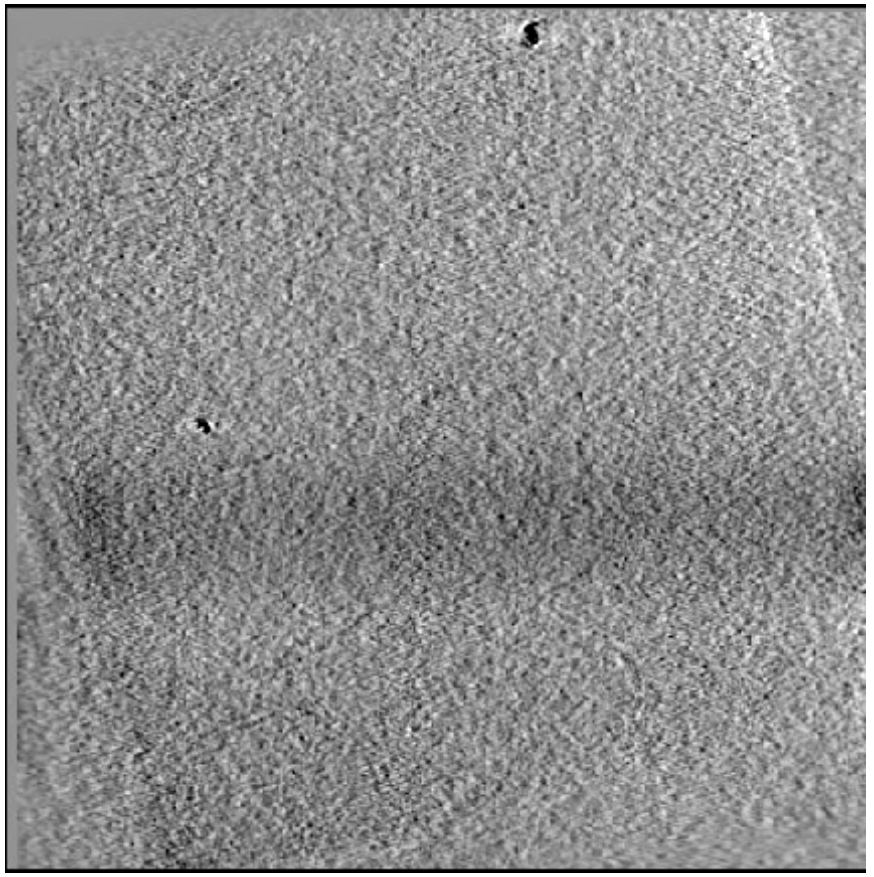


Cryo-FIB: Yeast

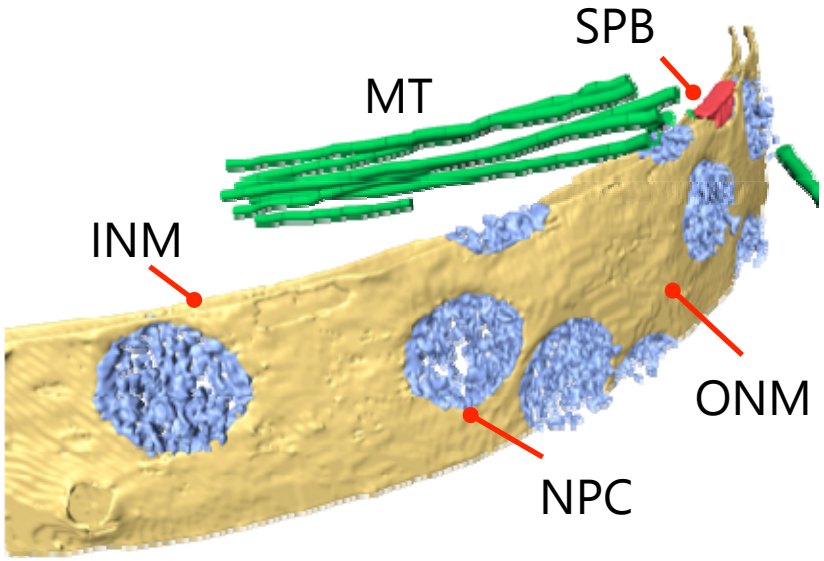
xy-slice from
3D reconstruction



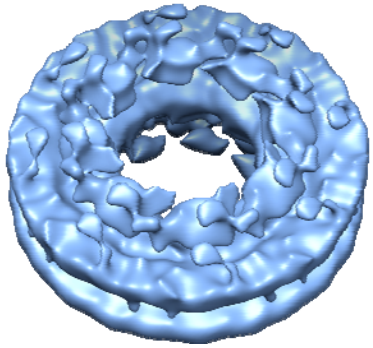
3D-segmentation



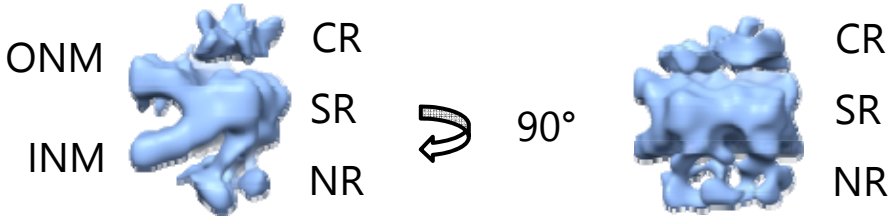
Cryo-FIB: Molecular architecture of NPCs



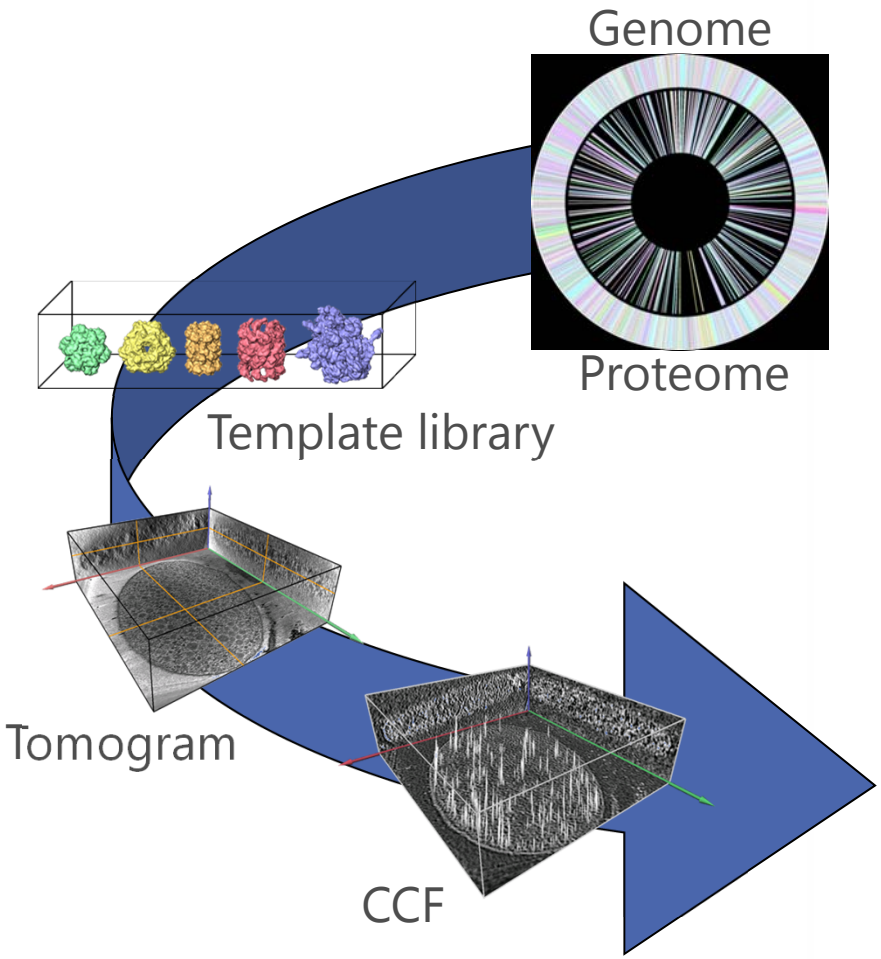
Average structure

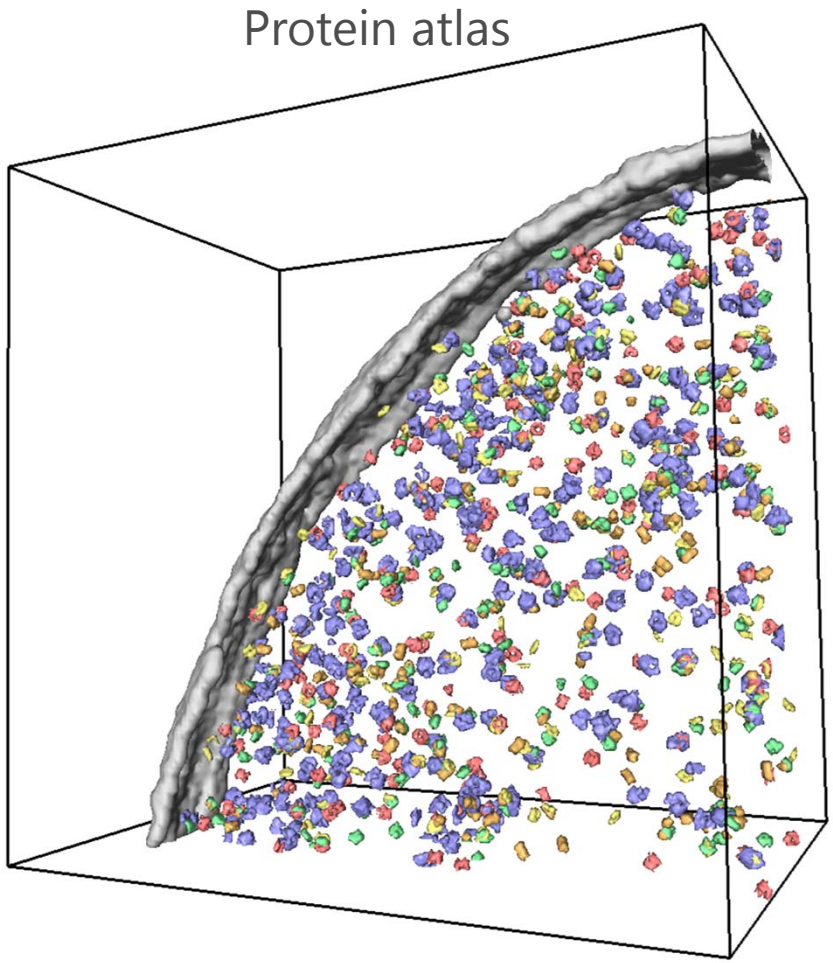


50 nm



From proteomic inventory to architecture





S. Nickell, C. Kofler, A. Leis, W. Baumeister: Nature Reviews Molecular Cell Biology 7 (2006) 225-230

More information…

Videos and Presentations:

- "Getting started in cryoEM" G. Jensen's Lab:

http://cryo-em-course.caltech.edu

- Sjors Scheres' Lab at the MRC:

http://www2.mrc-lmb.cam.ac.uk/groups/scheres/impact.html

Book: