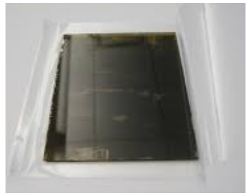
Microscopes for Automated Data Collection

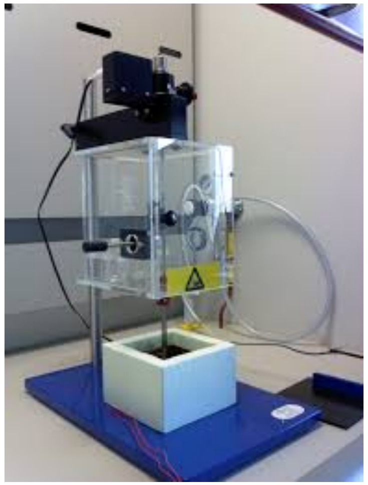
Rishi Matadeen

From Manual to Automation

Manual

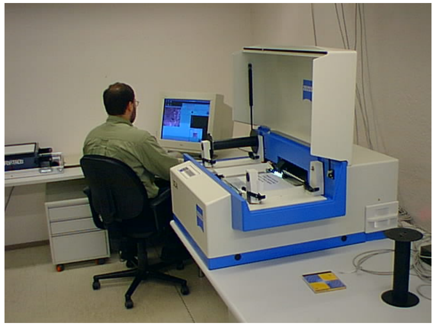


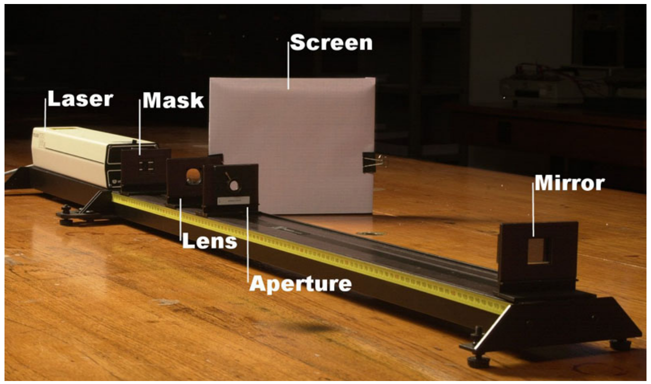
Film

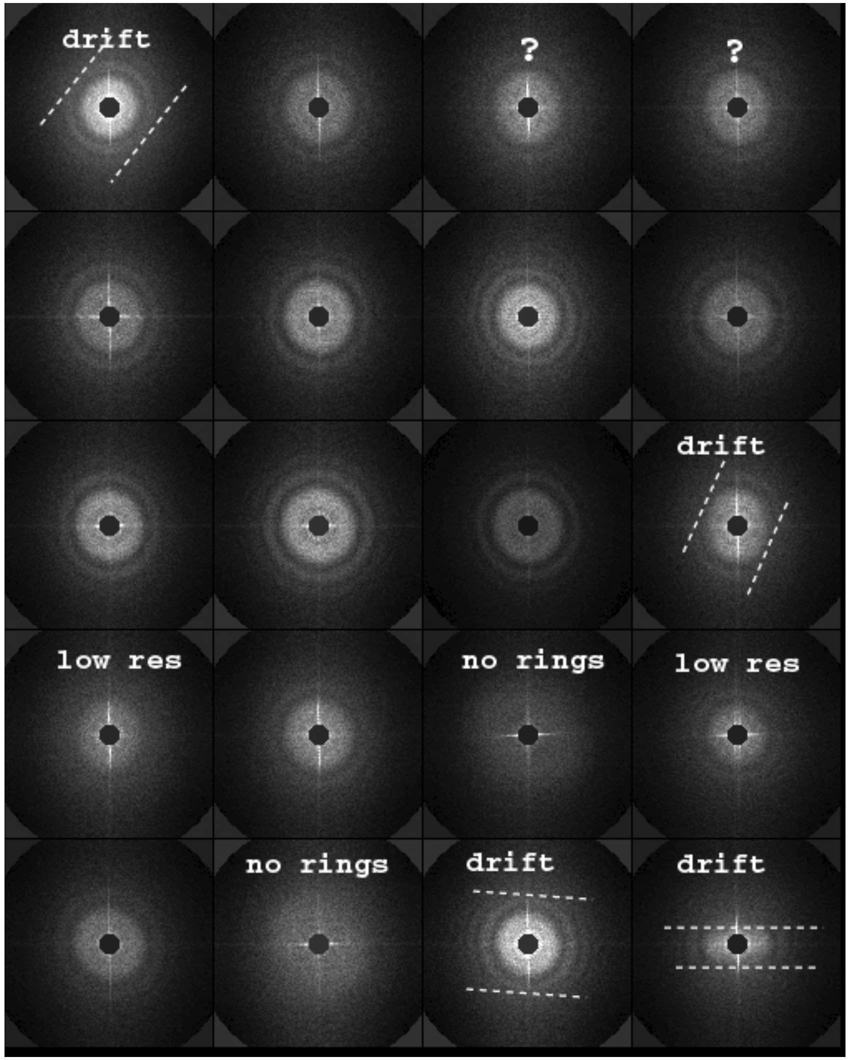


scanner

“Manual Data Collection”



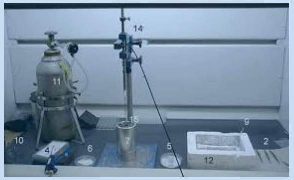


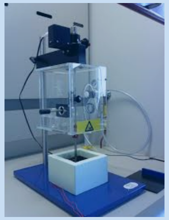


Manual to Automation

Krios

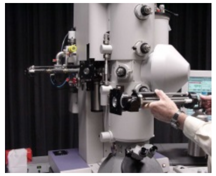
Manual



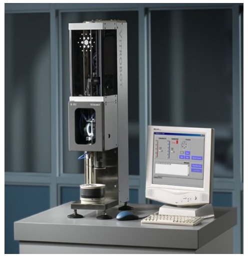


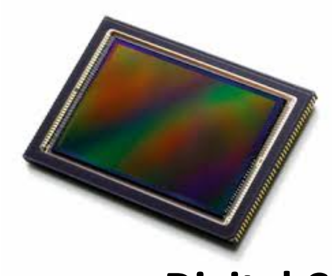
Film

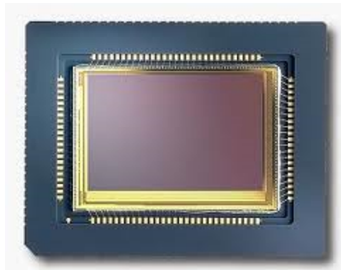
scanner



Automation



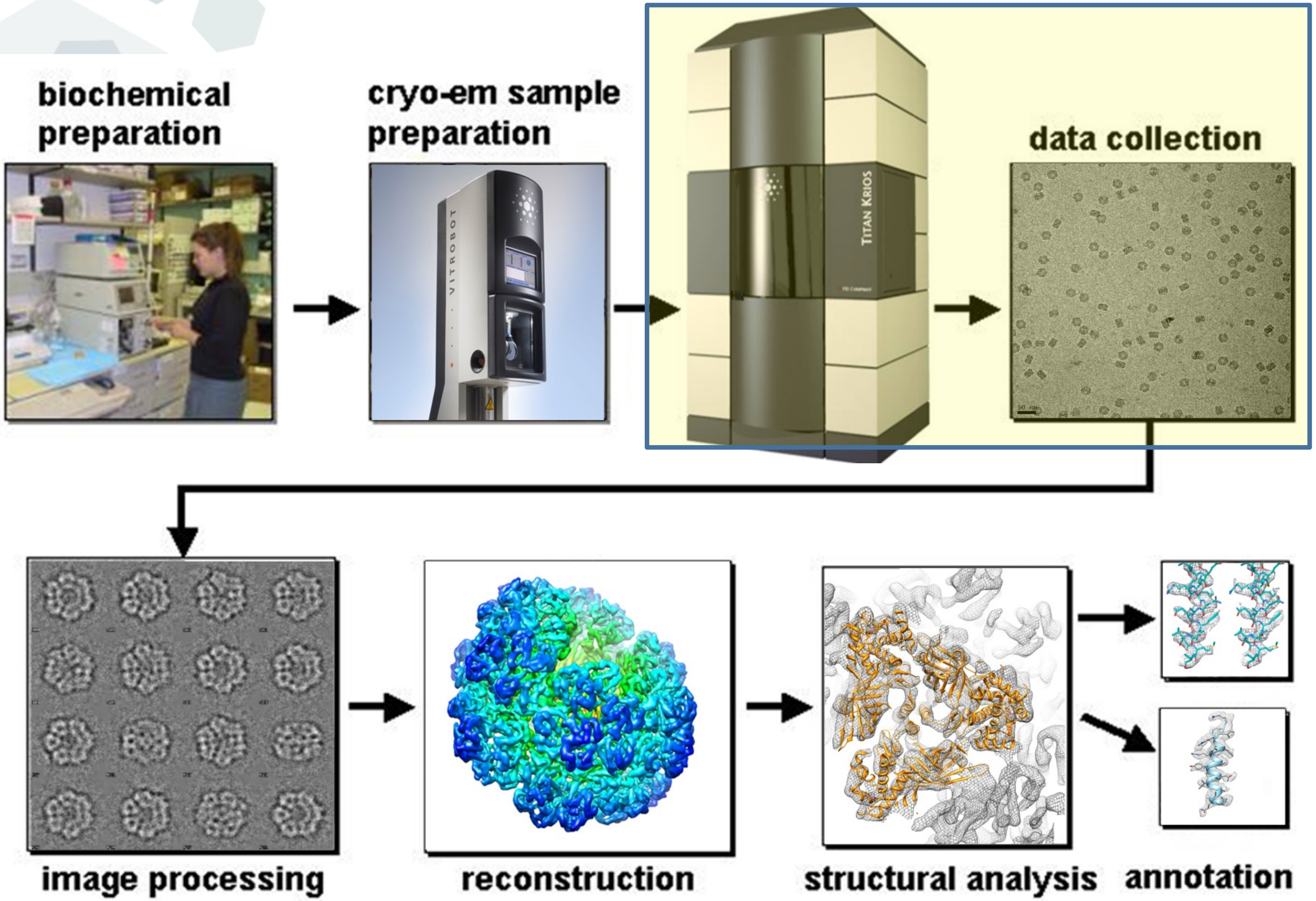




Digital CCD

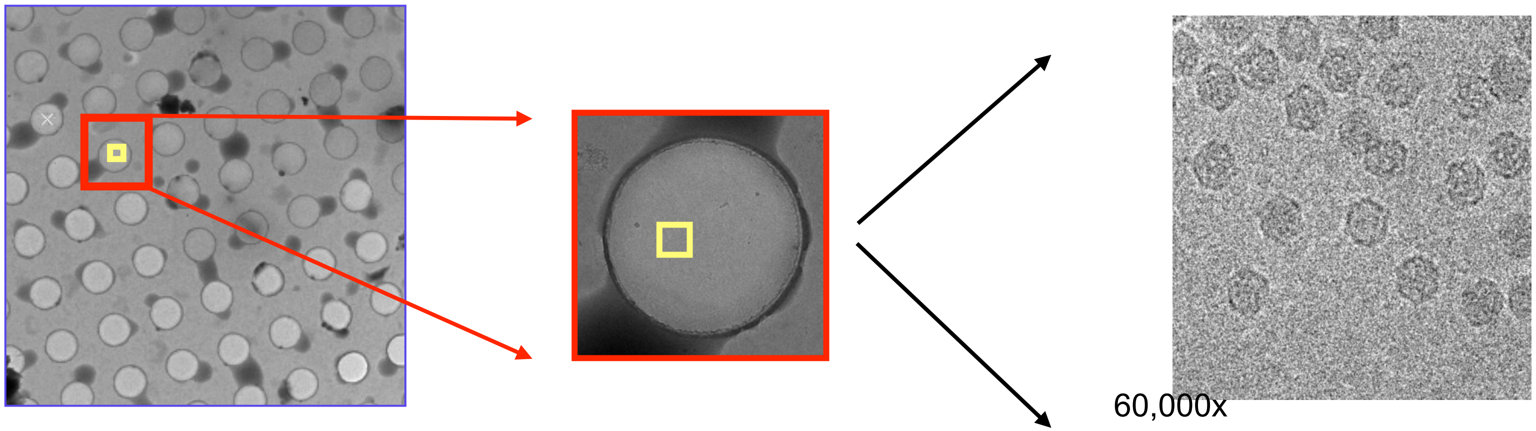
CMOS

From Sample to Structure

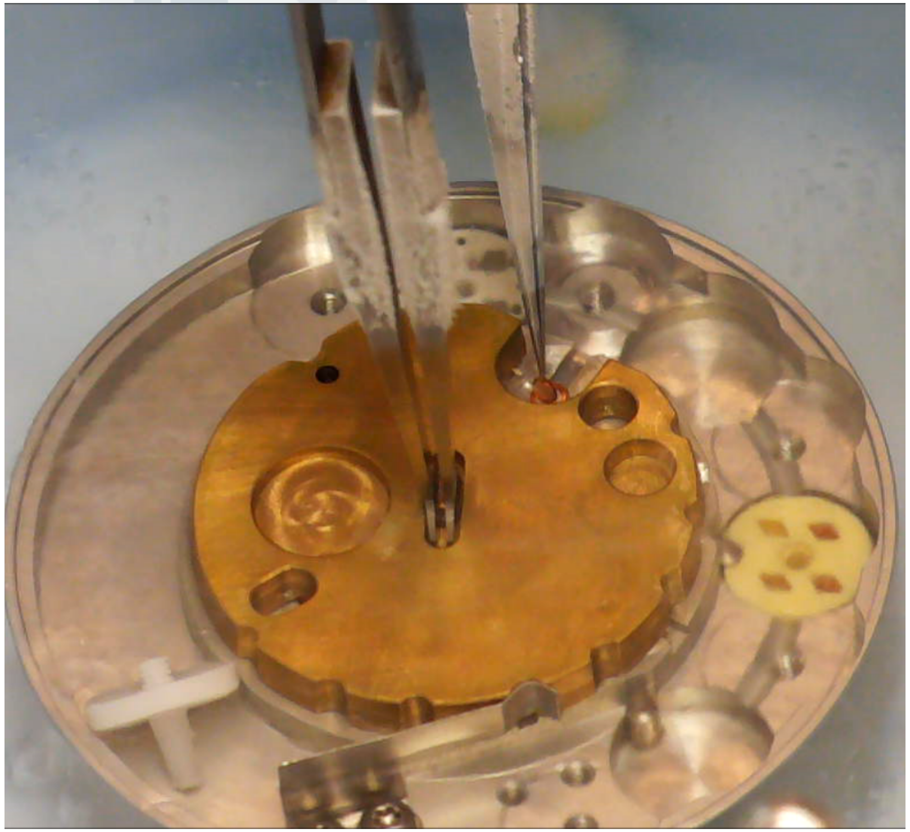


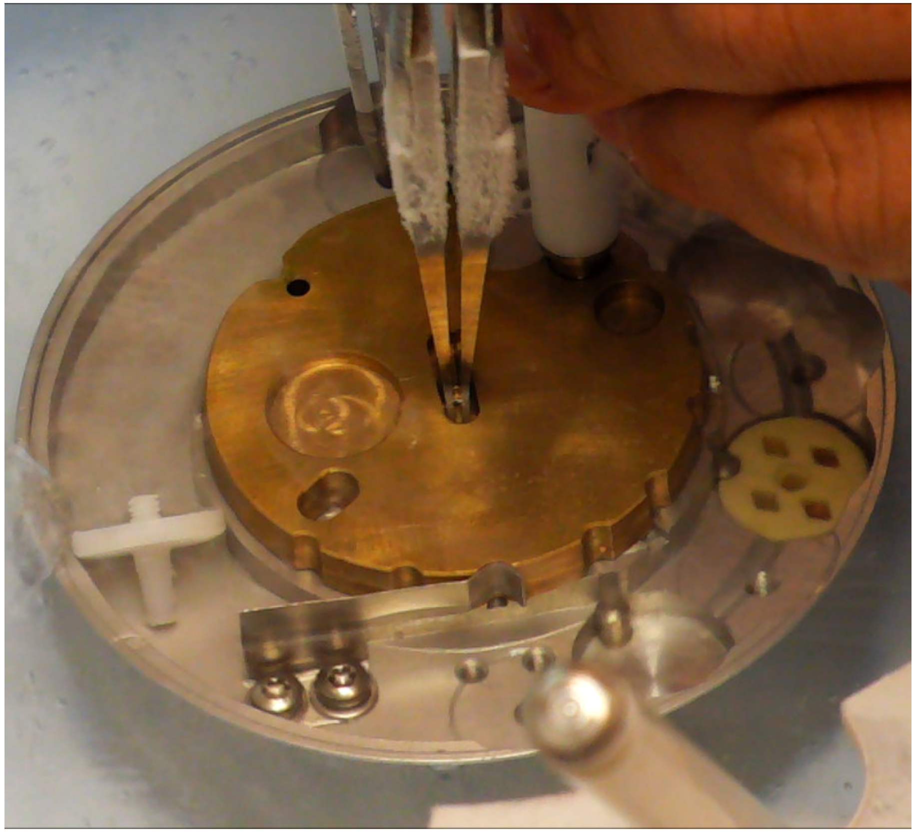
Slide adapted from De Carlo & Rémigy, Biophysics in drug discovery, Wiley (July 2015)

Titan Krios - Cryo TEM acquisition

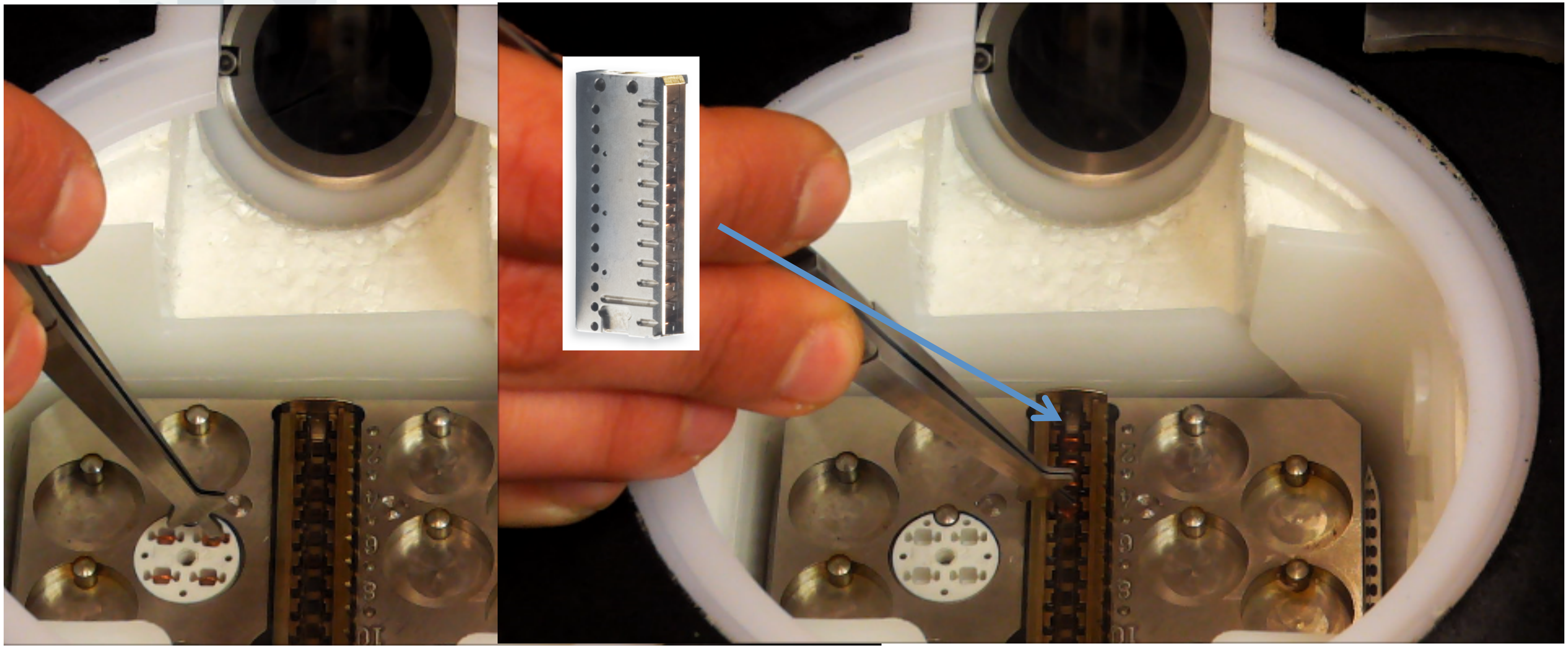


Autoloader – Grid Mounting

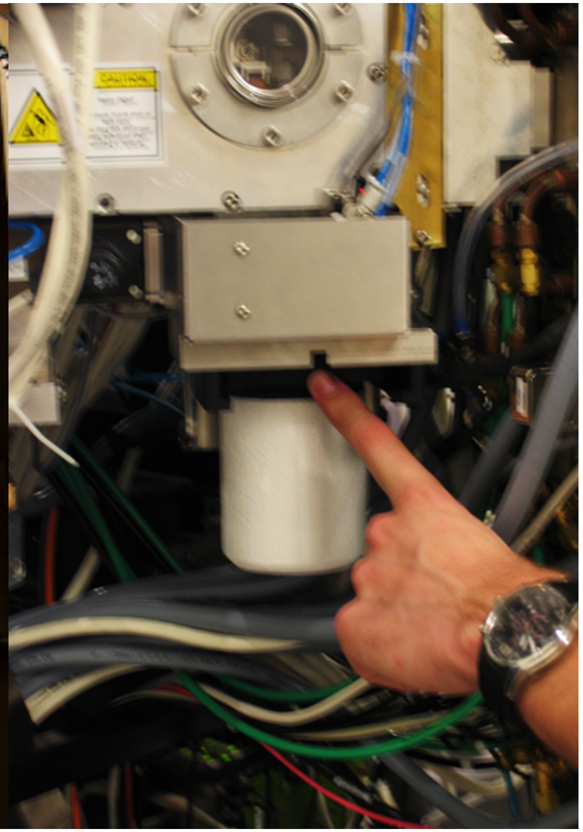




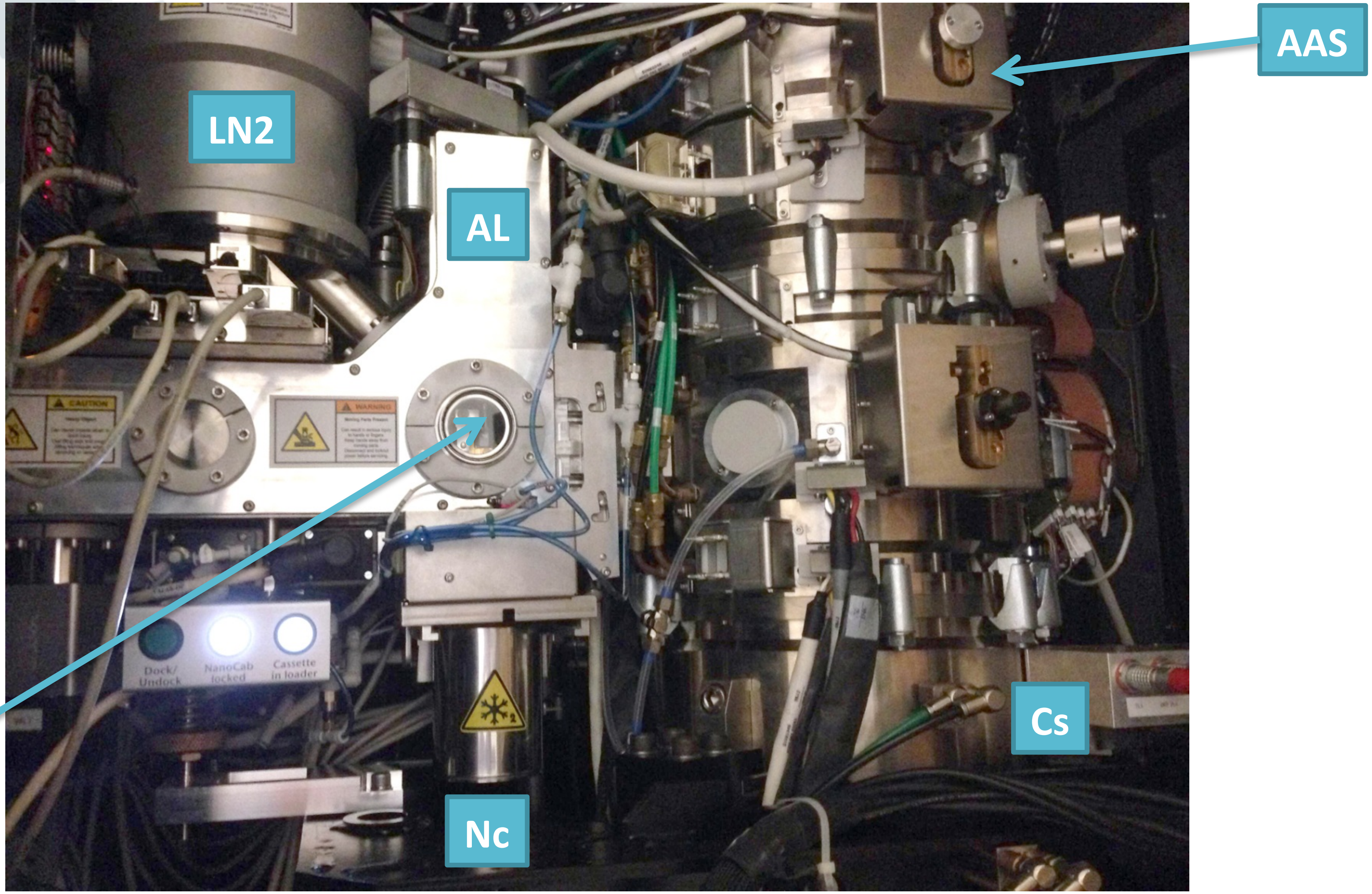
Autoloader – Grid Transfer

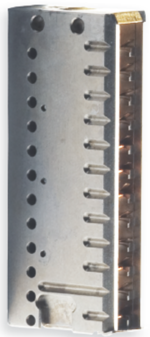


Autoloader – Cassette Docking



FEI Titan Krios (NeCEN#2, Leiden)





Talos Arctica

- Adopting Krios technology onto the Talos Platform

- Unattended High data throughput, Reduced Time-to-Result
  - Robotic sample handling, Auto-loading up to 12 samples
  - Auto filling of LN2 for continuous platform operation
  - Automated data acquisition through tailor made applications

- Excellent data quality
  - Optimized for 80-200kV
  - C-TWIN objective lens
  - Contamination 'free' sample loading
  - Increased sample life time (>24 hours)
  - Upgradeable with upcoming developments

Talos

- Tomography holder
- Gatan 70 degree cryo holder
- EPU
- Ceta 16Mpx 200kV camera
- Remote Operation

Dedicated Software Applications

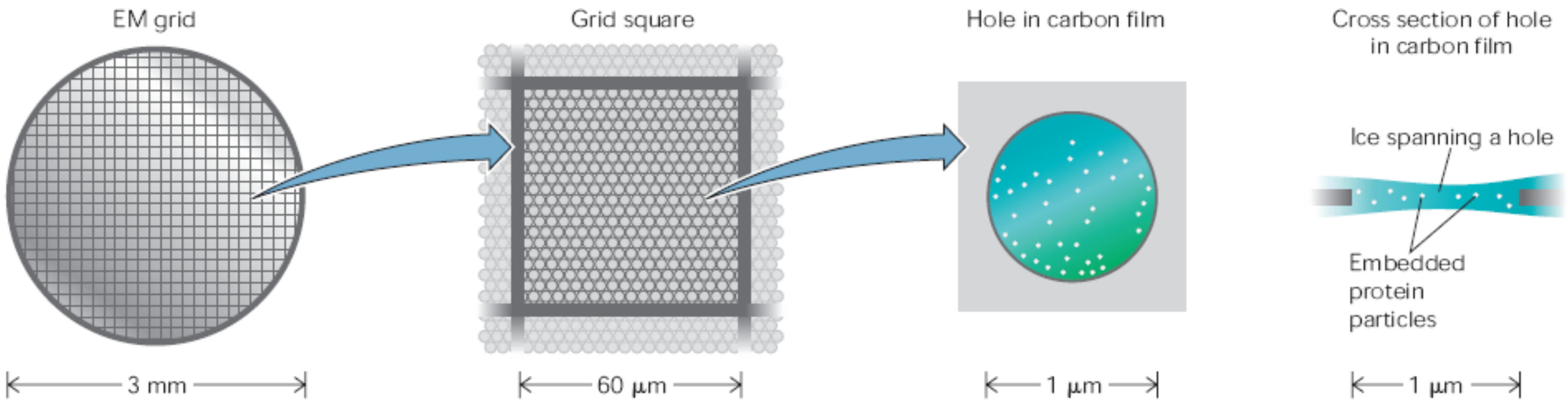
-EPU

('e pluribus unum' - 'out of many; one')

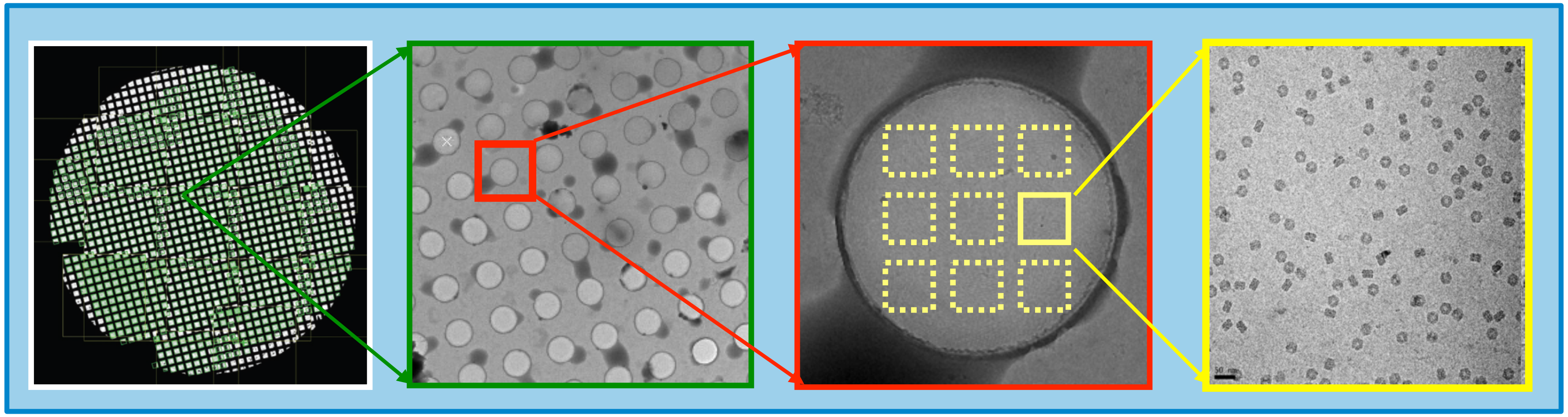
Automated single particle data acquisition

-Tomo 4

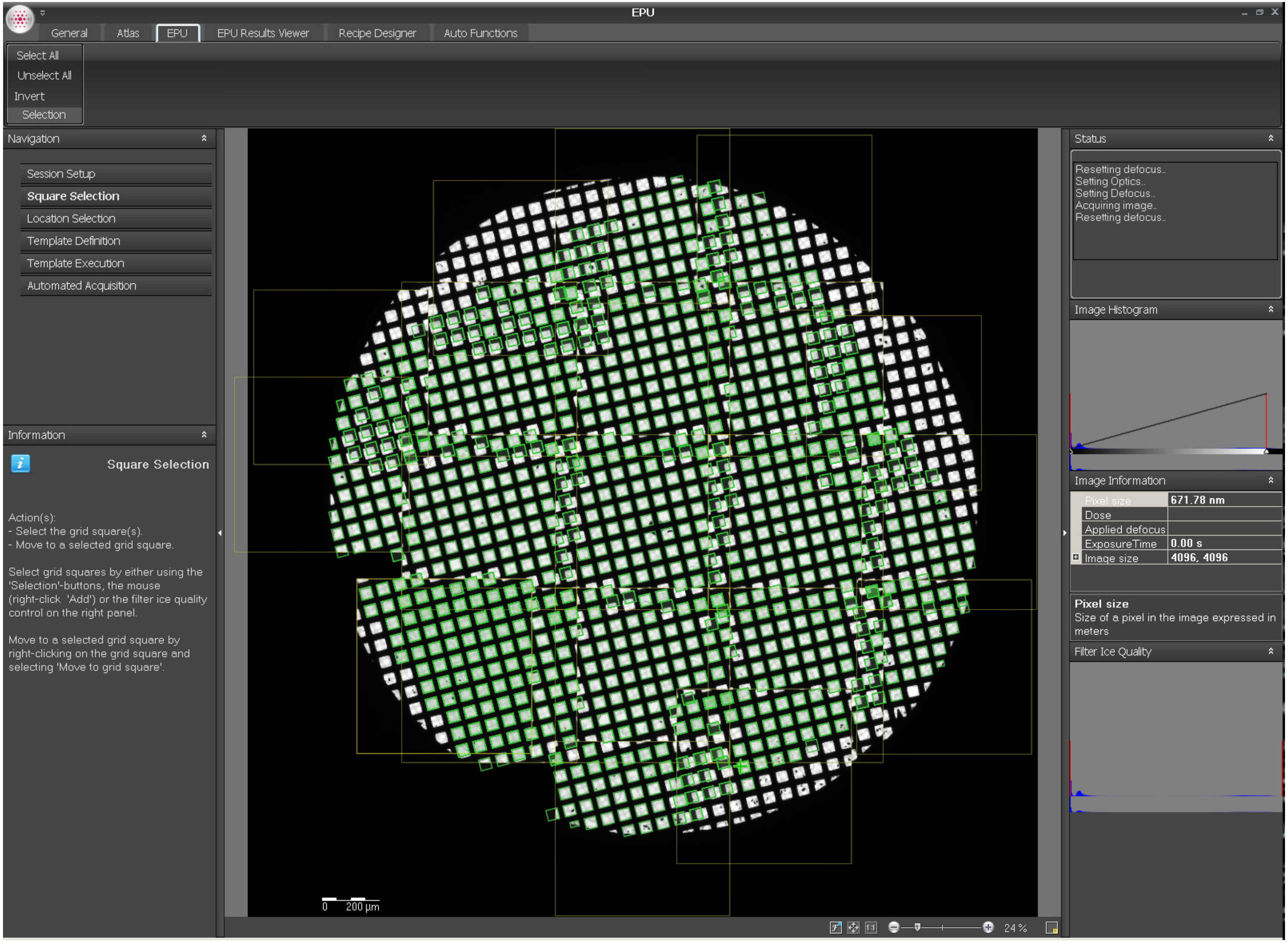
Automated Tomography data acquisition

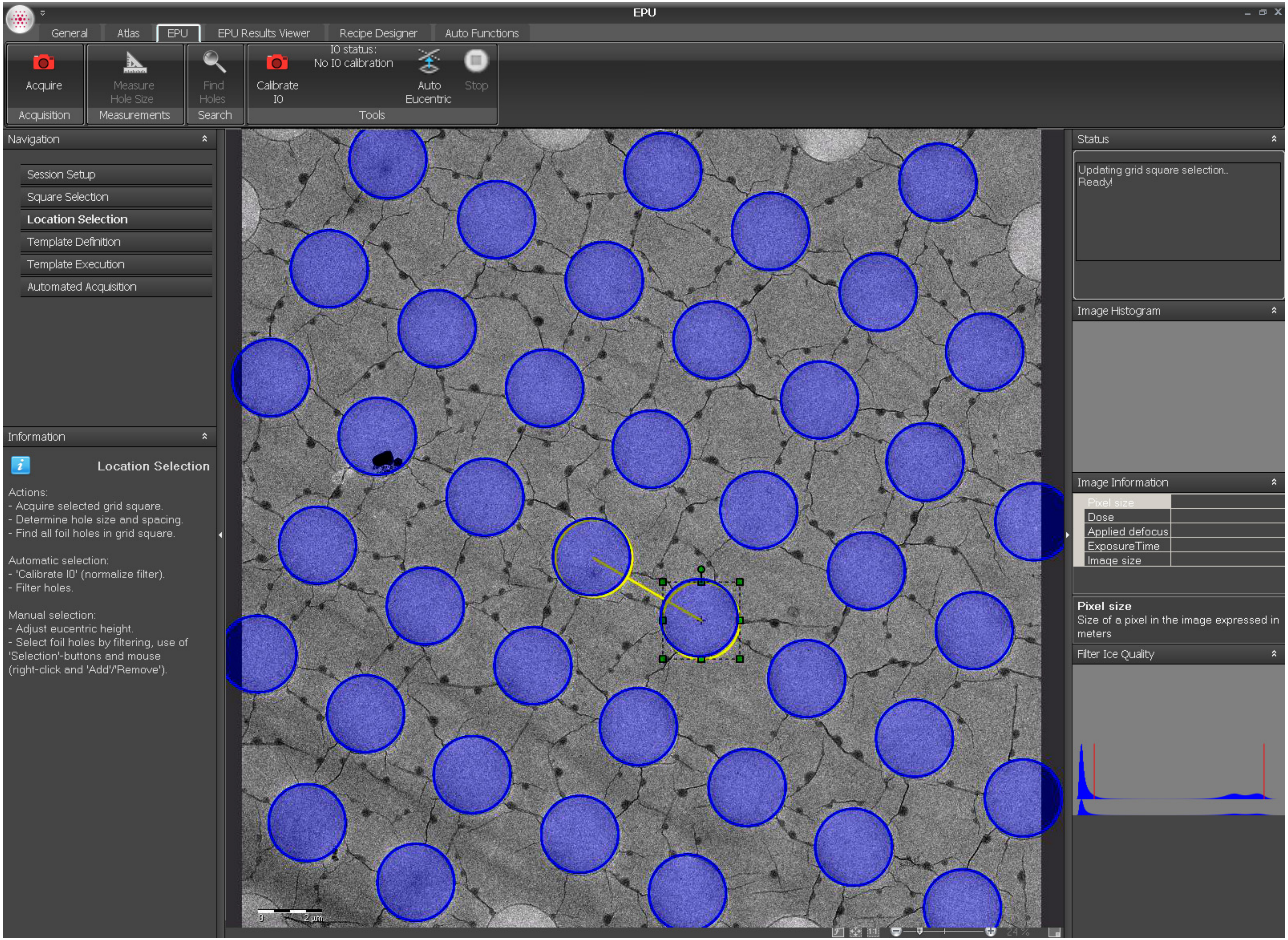


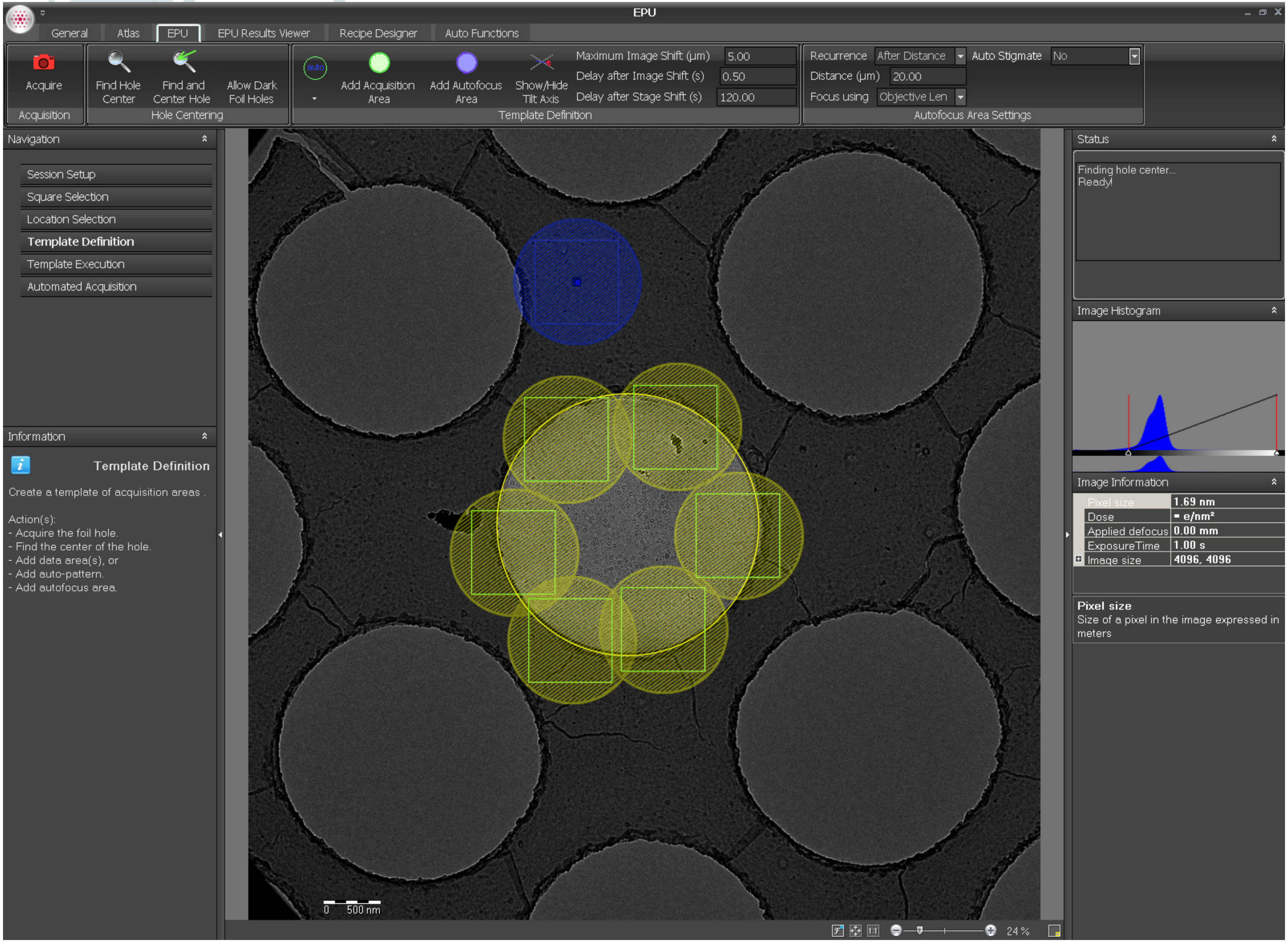
Automated image acquisition software – FEI EPU™



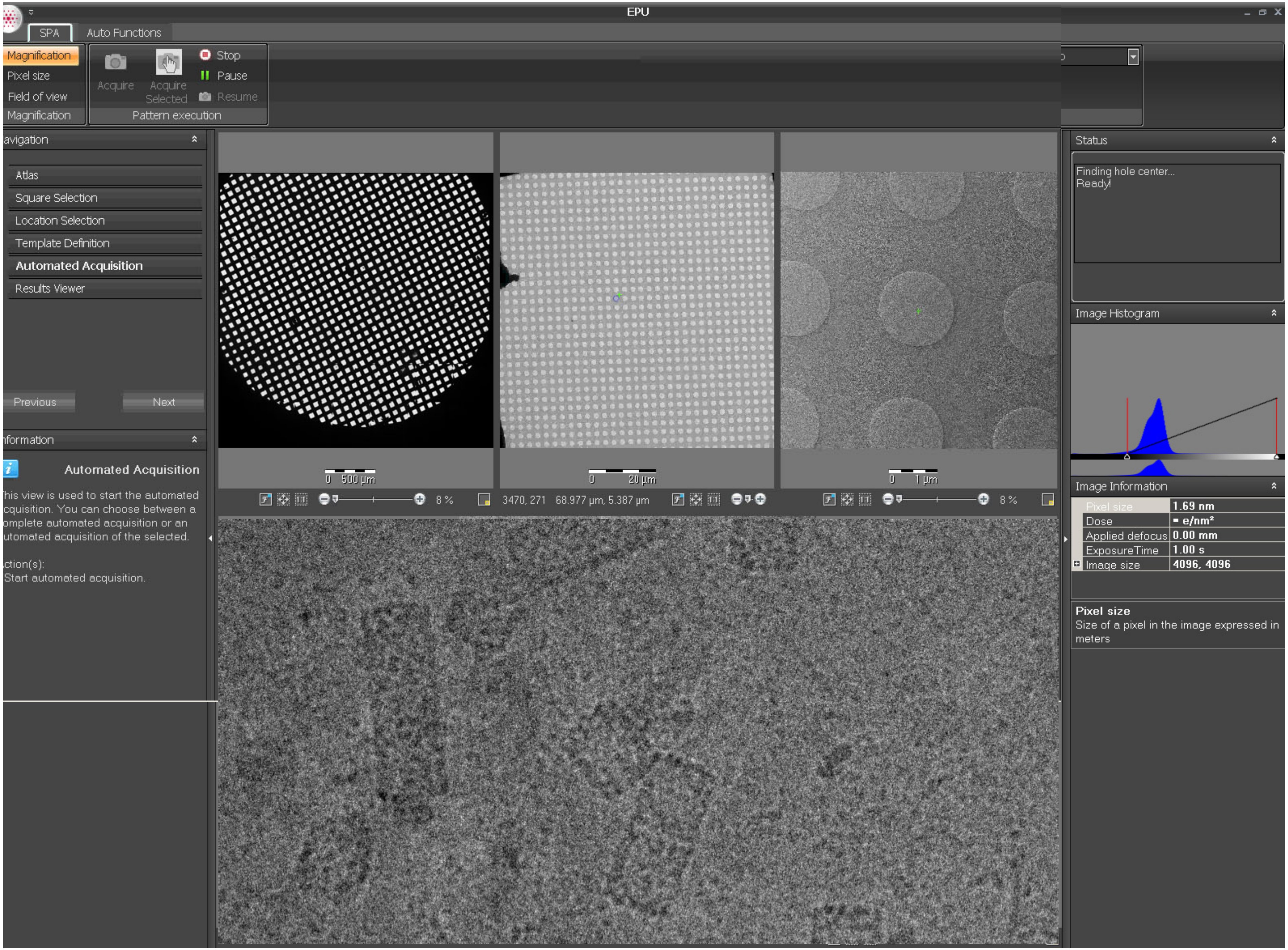
Potter *et al.* (1999). Leginon: … 1000 images a day. *Ultramicroscopy* **77**, 153-161


EPU
General
Atlas
EPU
EPU Results Viewer
Recipe Designer
Auto Functions
Select All
Unselect All
Invert
Selection
Navigation
Session Setup
Square Selection
Location Selection
Template Definition
Template Execution
Automated Acquisition
Information
Square Selection
Action(s):
- Select the grid square(s).
- Move to a selected grid square.
Select grid squares by either using the 'Selection'-buttons, the mouse (right-click 'Add') or the filter ice quality control on the right panel.
Move to a selected grid square by right-clicking on the grid square and selecting 'Move to grid square'.
0 200 µm
24 %
Status
Resetting defocus..
Setting Optics..
Setting Defocus..
Acquiring image..
Resetting defocus..
Image Histogram
Image Information
Pixel size 671.78 nm
Dose
Applied defocus
ExposureTime 0.00 s
Image size 4096, 4096
Pixel size
Size of a pixel in the image expressed in meters
Filter Ice Quality

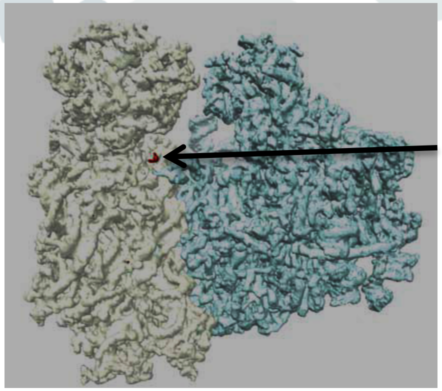

EPU
General
Atlas
EPU
EPU Results Viewer
Recipe Designer
Auto Functions
Acquire
Acquisition
Measure Hole Size
Measurements
Find Holes
Search
Calibrate I0
I0 status:
No I0 calibration
Auto Eucentric
Stop
Tools
Navigation
Session Setup
Square Selection
Location Selection
Template Definition
Template Execution
Automated Acquisition
Information
Location Selection
Actions:
- Acquire selected grid square.
- Determine hole size and spacing.
- Find all foil holes in grid square.
Automatic selection:
- 'Calibrate I0' (normalize filter).
- Filter holes.
Manual selection:
- Adjust eucentric height.
- Select foil holes by filtering, use of 'Selection'-buttons and mouse (right-click and 'Add'/'Remove').
Status
Updating grid square selection...
Ready!
Image Histogram
Image Information
Pixel size
Dose
Applied defocus
ExposureTime
Image size
Pixel size
Size of a pixel in the image expressed in meters
Filter Ice Quality
0
2 µm

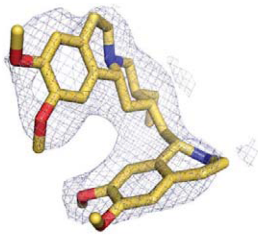


EPU
General
Atlas
EPU
EPU Results Viewer
Recipe Designer
Auto Functions
Acquire
Acquisition
Find Hole Center
Find and Center Hole
Allow Dark Foil Holes
Hole Centering
auto
Add Acquisition Area
Add Autofocus Area
Show/Hide Tilt Axis
Maximum Image Shift (µm) 5.00
Delay after Image Shift (s) 0.50
Delay after Stage Shift (s) 120.00
Template Definition
Recurrence After Distance
Auto Stigmate No
Distance (µm) 20.00
Focus using Objective Len
Autofocus Area Settings
Navigation
Session Setup
Square Selection
Location Selection
Template Definition
Template Execution
Automated Acquisition
Information
Template Definition
Create a template of acquisition areas .
Action(s):
- Acquire the foil hole.
- Find the center of the hole.
- Add data area(s), or
- Add auto-pattern.
- Add autofocus area.
Status
Finding hole center...
Ready!
Image Histogram
Image Information
Pixel size 1.69 nm
Dose = e/nm²
Applied defocus 0.00 mm
ExposureTime 1.00 s
Image size 4096, 4096
Pixel size
Size of a pixel in the image expressed in meters
0 500 nm
24 %

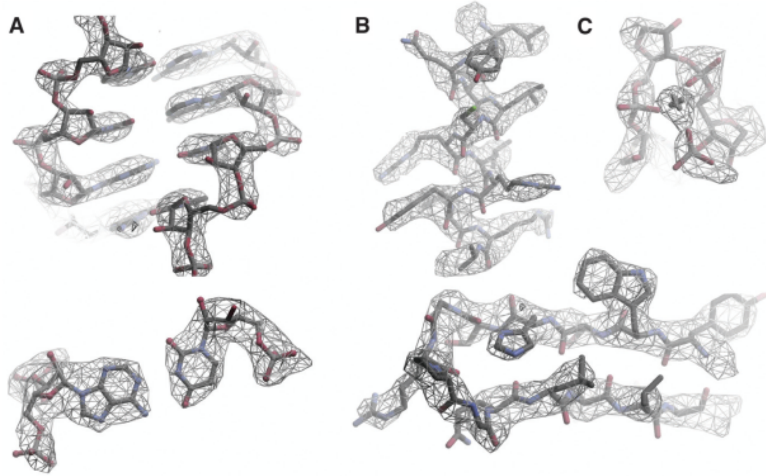

EPU
SPA
Auto Functions
Magnification
Pixel size
Field of view
Magnification
Acquire
Acquire Selected
Stop
Pause
Resume
Pattern execution
avigation
Atlas
Square Selection
Location Selection
Template Definition
Automated Acquisition
Results Viewer
Previous
Next
nformation
Automated Acquisition
his view is used to start the automated cquisition. You can choose between a omplete automated acquisition or an utomated acquisition of the selected.
ction(s):
Start automated acquisition.
0 500 µm
8 %
3470, 271 68.977 µm, 5.387 µm
0 20 µm
0 1 µm
8 %
Status
Finding hole center...
Ready!
Image Histogram
Image Information
Pixel size 1.69 nm
Dose = e/nm²
Applied defocus 0.00 mm
ExposureTime 1.00 s
Image size 4096, 4096
Pixel size
Size of a pixel in the image expressed in meters

Ribosomes



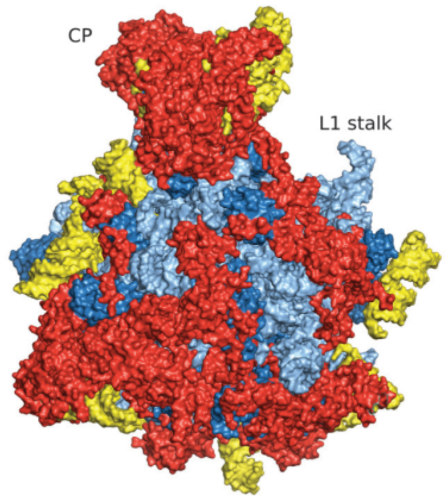


Emetine drug



Hussain T, et al. **Cell** (2014) 159 pp. 597-607 (Falcon)
Bischoff L, et al. **Cell Rep**. (2014) 9 pp. 469-475 (Falcon)
Arenz S, et al. **Molecular Cell** (2014) (Falcon)
Brown A, et al. **Science** (2014) 346 pp. 718-722 (Falcon)
Greber BJ, et. Al. **Nature** (2014) (Falcon)
Shao S, et al. **Molecular Cell** (2014) 55 pp. 880-890 (Falcon)
Voorhees RM, et al. **Cell** (2014) 157 pp. 1632-1643 (Falcon)
Wong W, et al. **eLife** (2014) 3 (Falcon)
Fernandez IS, **Cell** (2014) 157 pp. 823-831 (Falcon)
Amunts A, **Science** (2014) 343 pp. 1485-1489 (Falcon)
Greber BJ, et al. **Nature** (2014) 505 pp. 515-519 (cover) (Falcon)

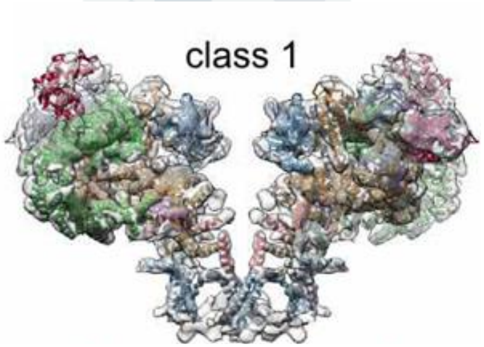
Full *de novo* model building



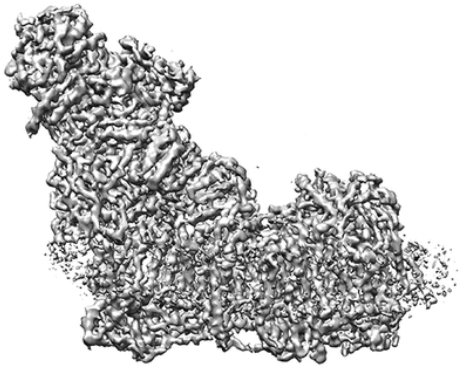
Many high impact publications on ribosomal complexes!

Routinely ≤4Å

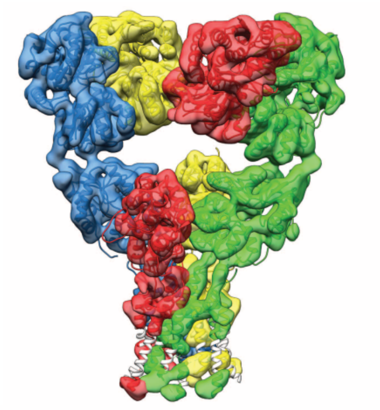
Membrane proteins



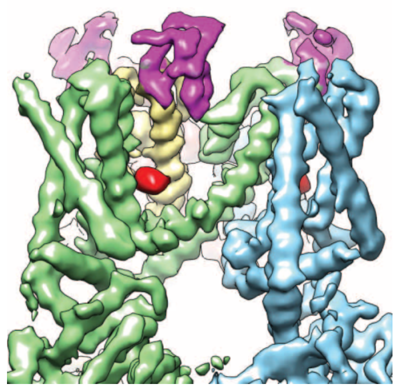
Ryanodine receptor (2.2MD)



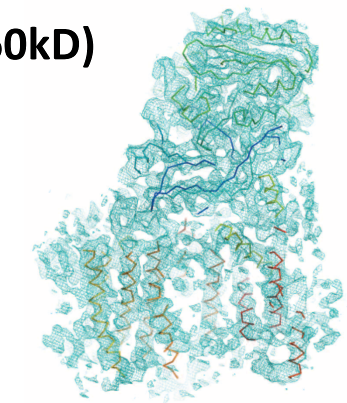
Complex I (1MD)



Glutamate receptor (460kD)

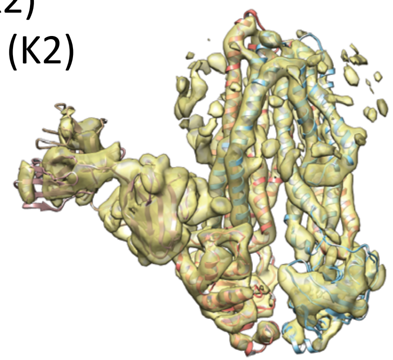


TRPV1 (300 kD)



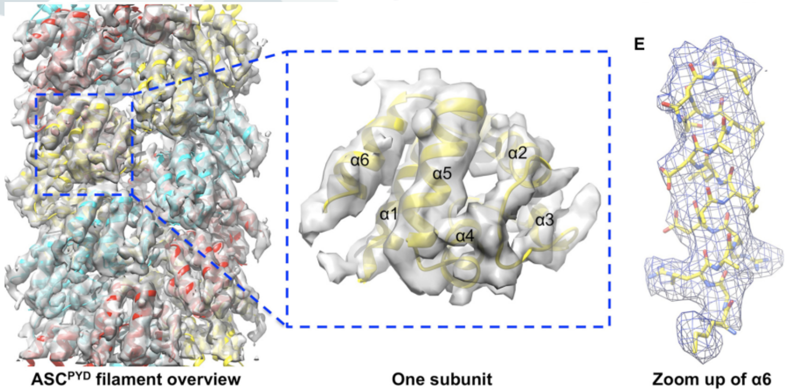
γ-secretase (170 kD)

Kutti R. Vinothkumar, et al. **Nature** (2014) 515 pp. 80-84 (Falcon)
Erhu Cao, et al. **Nature** (dec 2013) 504 pp. 113-118 (K2)
Maofu Liao, et al. **Nature** (dec 2013) 504 pp. 107-112 (K2)
JungMin Kim, et al. **Nature** (2014) (K2)
Joel R. Meyerson, et al. **Nature** (2014) (Falcon)
Peilong Lu, et al. **Nature** (2014) (K2)
Rouslan G. Efremov, et al. **Nature** (2014) (Falcon)

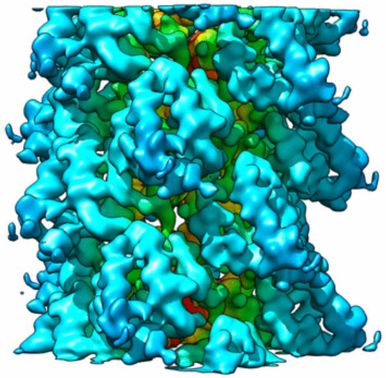


ABC-transporter (135 kD)

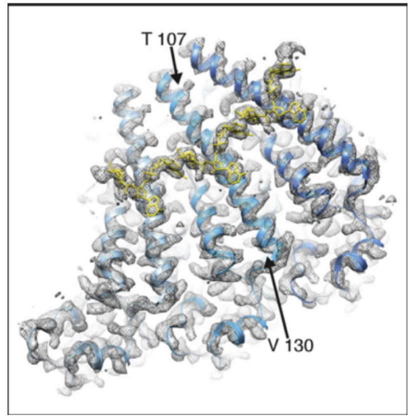
Filaments



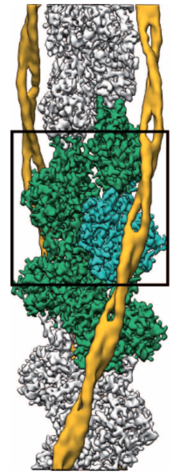
Inflammasomes



MAVS filament

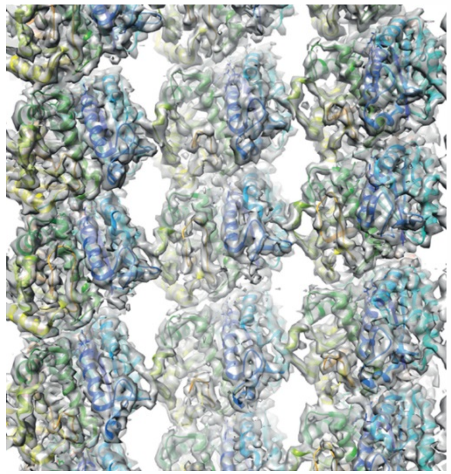


TMV



F-Actin

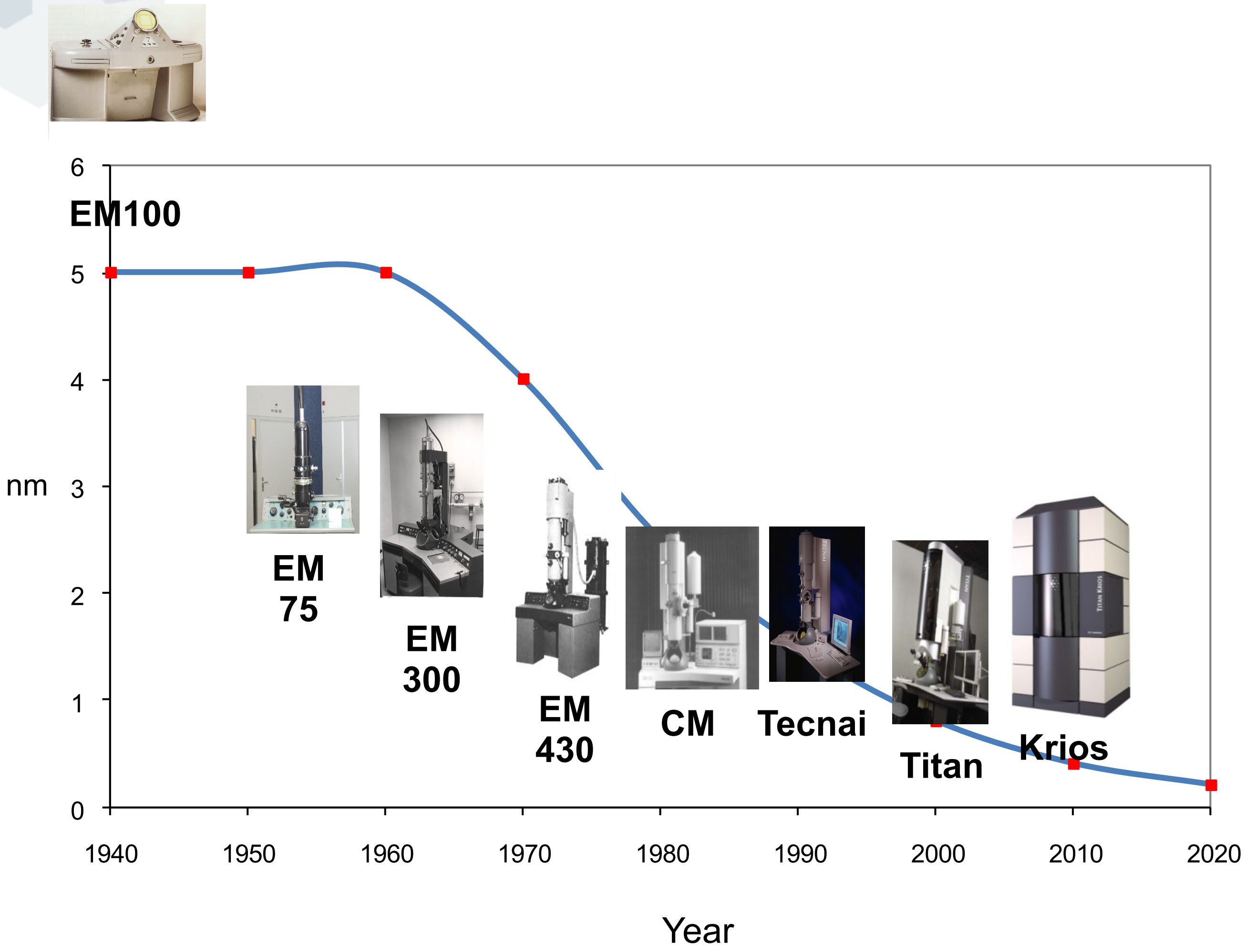
Fromm S. *et al*. **JSB** (2014) (Falcon, K2)
Lu A, *et al*. **Cell** (2014) 156 pp. 1193-1206 (Falcon)
Wu B, *et al*. **Molecular Cell** (2014) 55 pp. 511-523 (K2)
Von der Ecken J, *et al*. **Nature** (2014) (Falcon)
Galkin VE, *et al*. **Structure** (2014) (Falcon)
Alushin GM *et al*. **Cell** (2014) 157 pp. 1117-1129 (film)



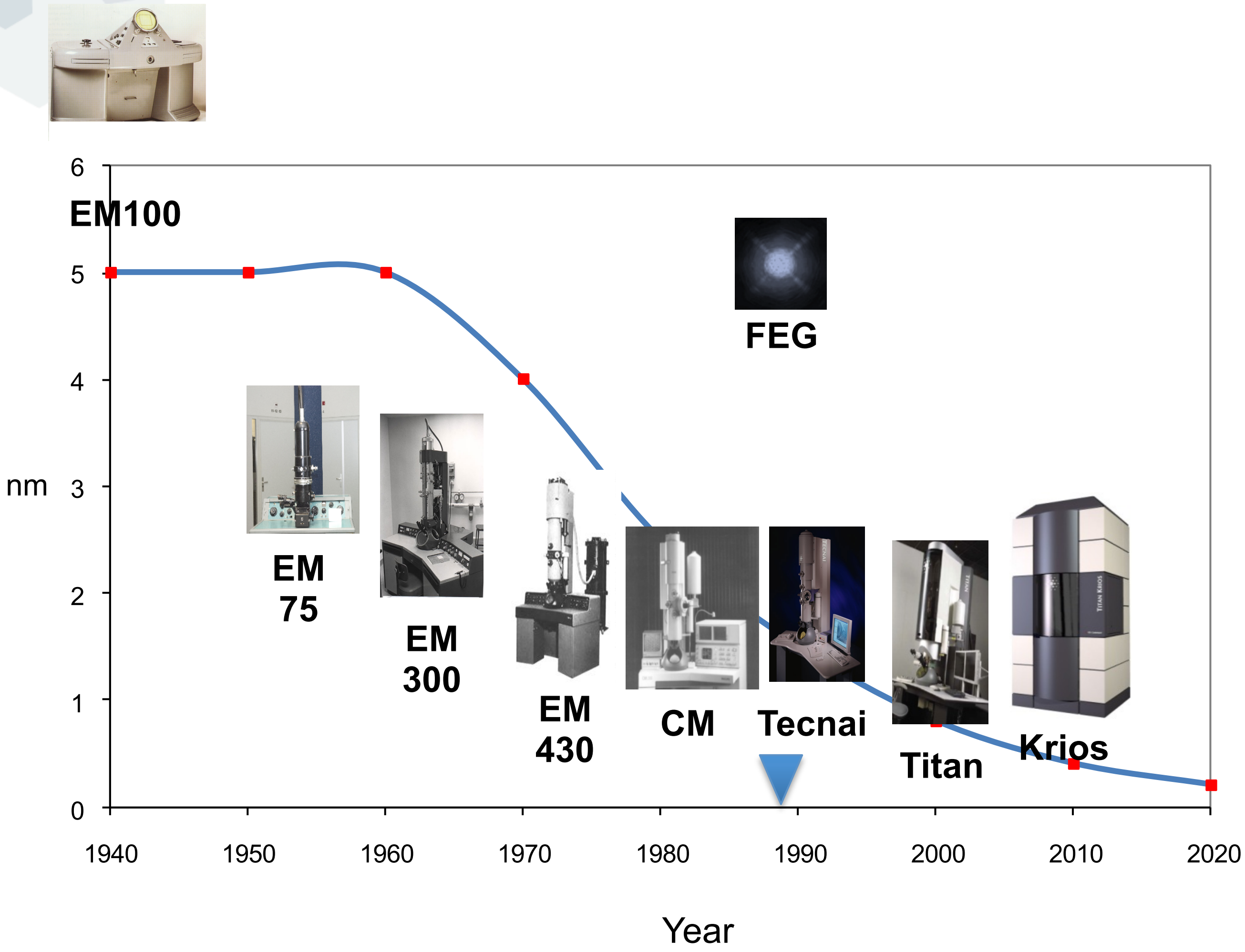
Microtubules

(M)any high resolution structure(s) seems to be a ticket for a high impact publication!

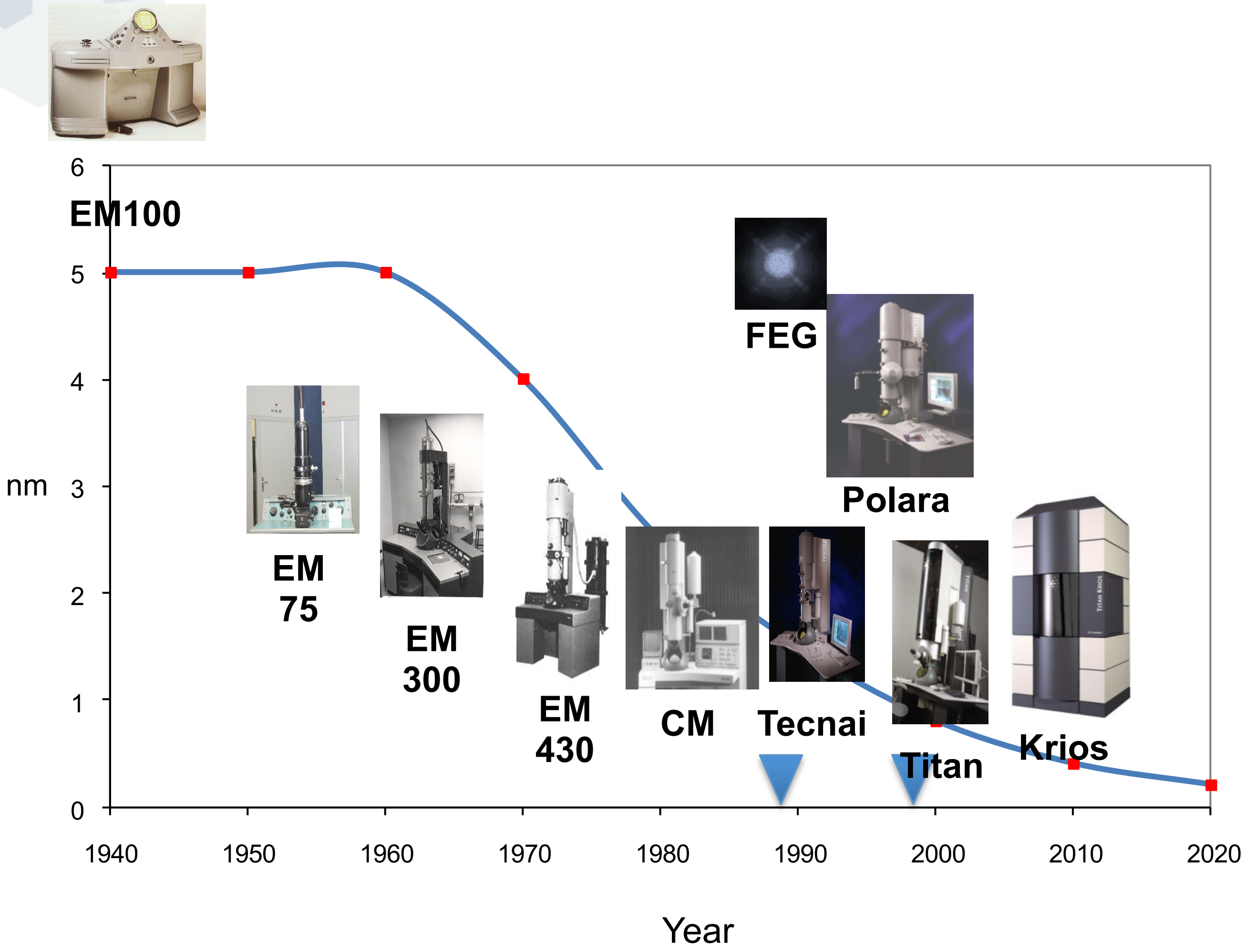
Cryo-TEM resolution (Single particle reconstruction)



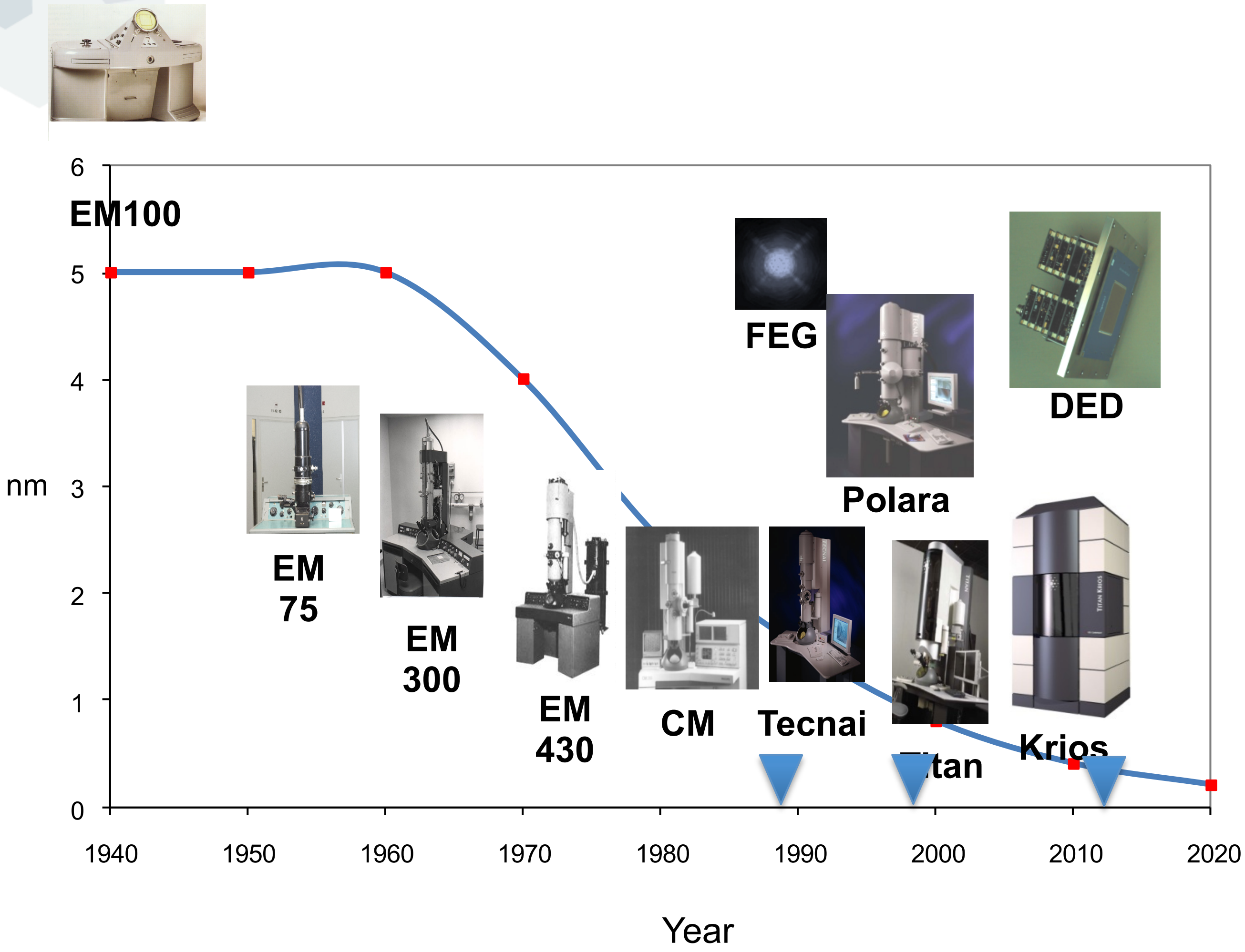
Cryo-TEM resolution (Single particle reconstruction)



Cryo-TEM resolution (Single particle reconstruction)



Cryo-TEM resolution (Single particle reconstruction)



Thank You

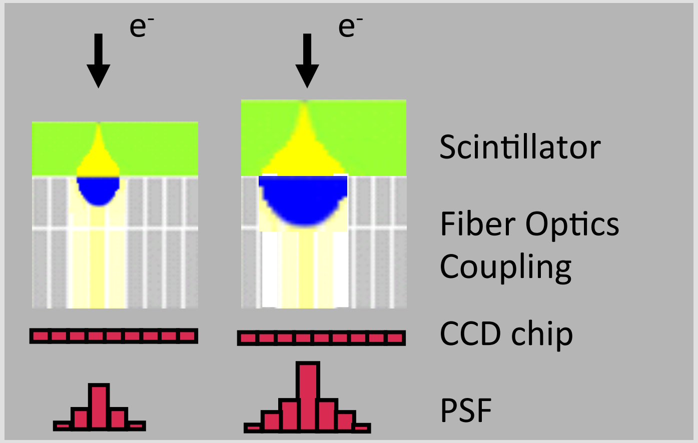
http://www.fei.com/life-sciences/

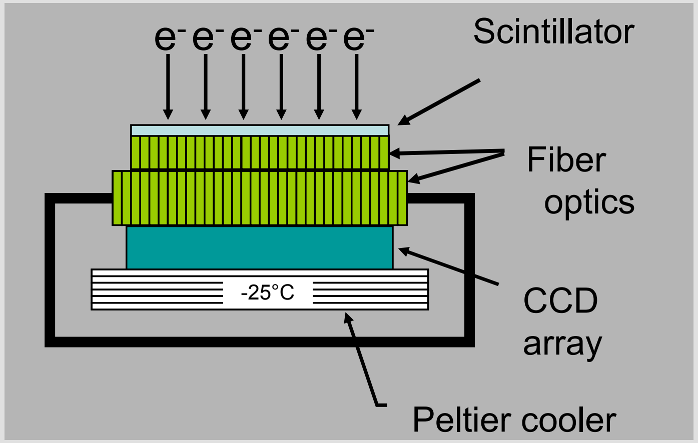
FEI™

Improving detection: “seeing electrons”

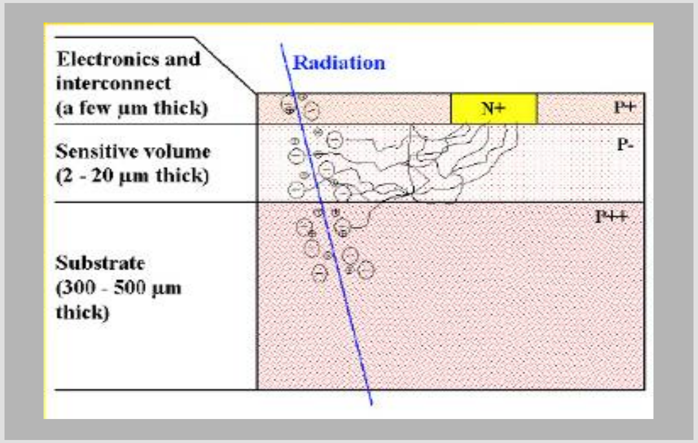
Improved Detective Quantum Efficiency (DQE)

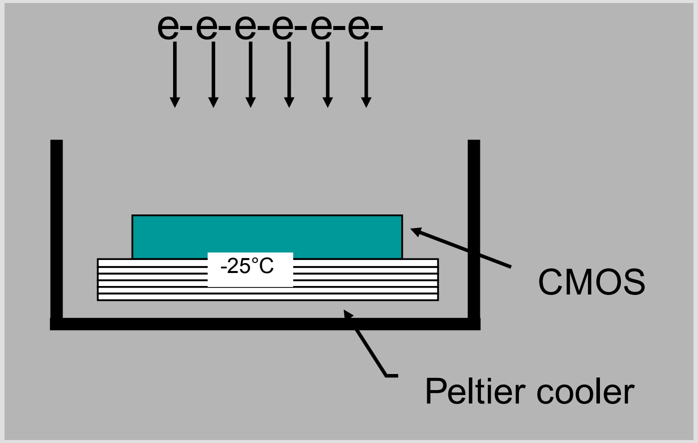
CCD: multi stage conversion of electron energy via fiber or lens optics



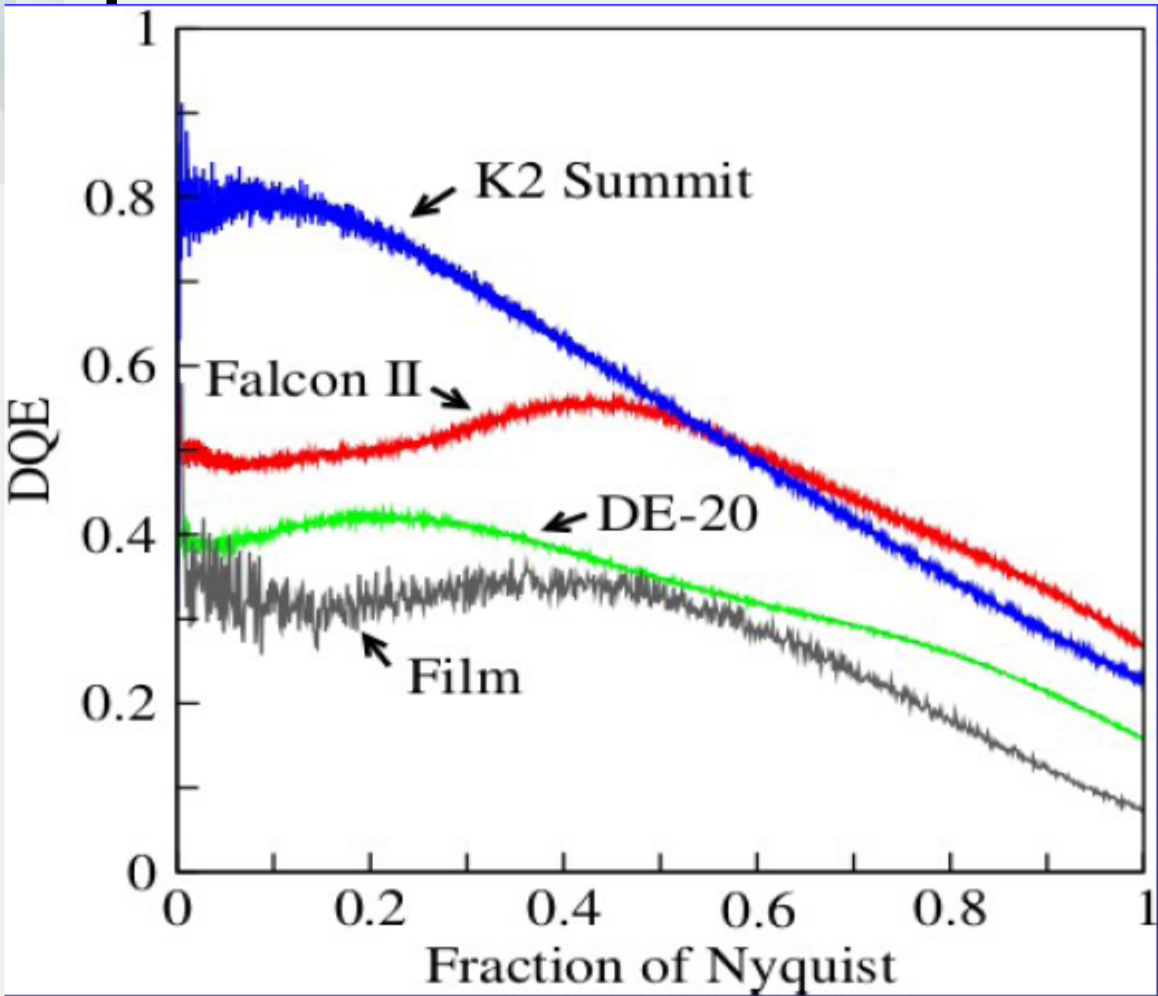


CMOS: direct conversion of electron energy without fiber or lens optics



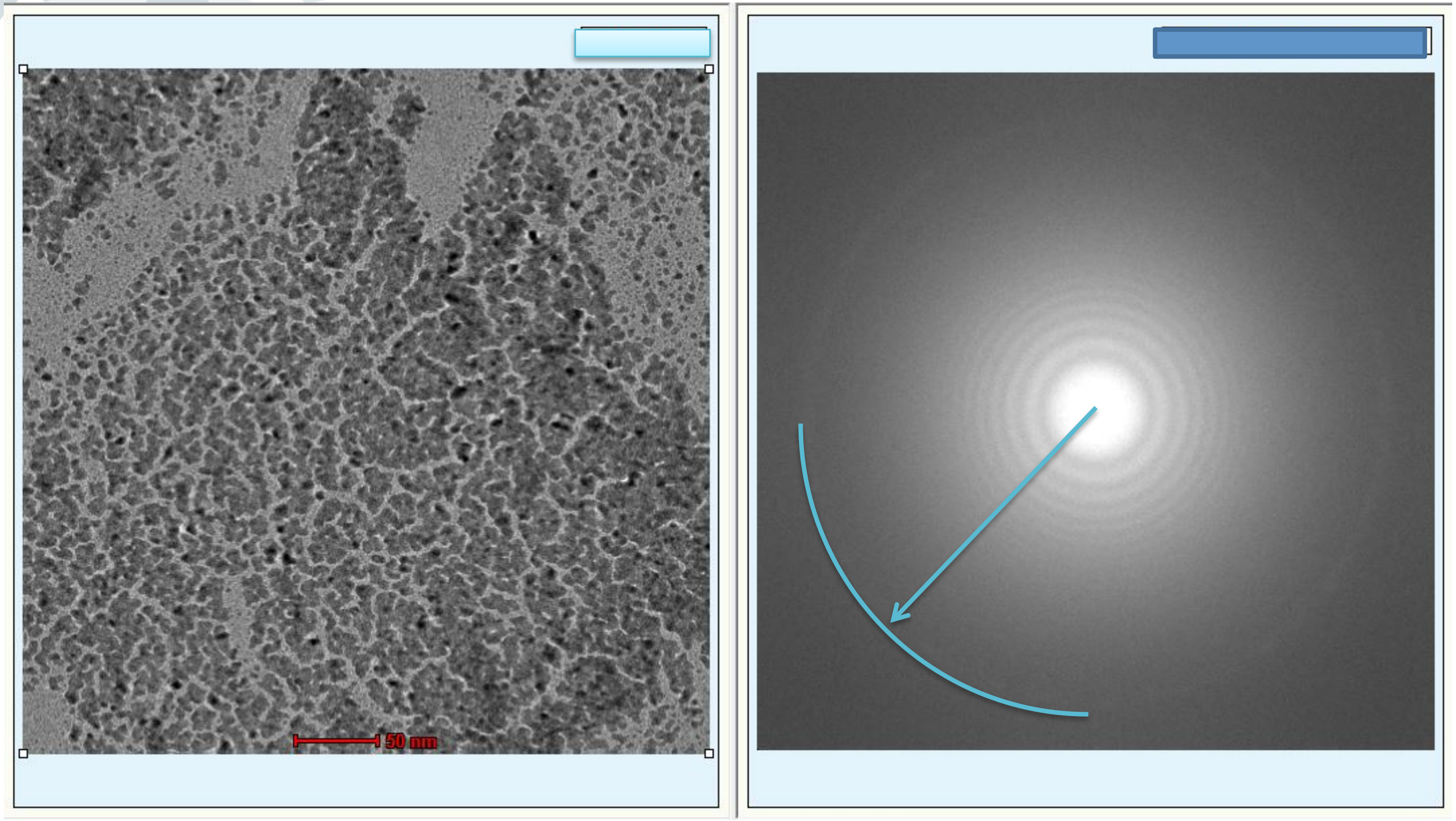


DQE comparison for various detectors at 300kV



McMullan *et al.* (2014) *Ultramicroscopy* **147**, 156-163

Cross-grating gold replica: Falcon2, low-dose



100kX nominal mag (1 Å pix size); Nyquist = 2Å

2.3Å gold reflection line still visible at <10 el./Å^2

Movie processing

Review
Trends in Biochemical Sciences January 2015, Vol. 40, No. 1

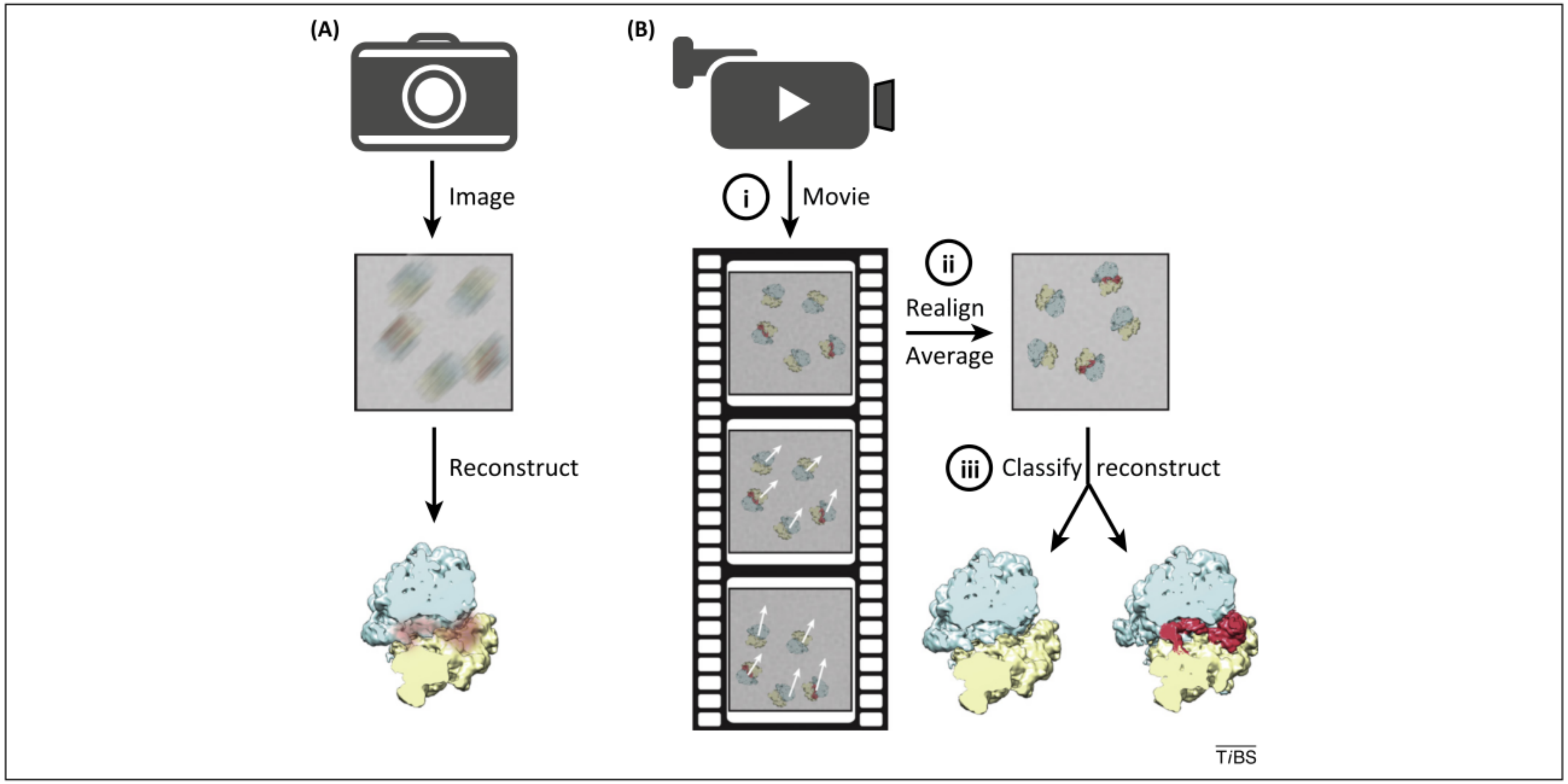
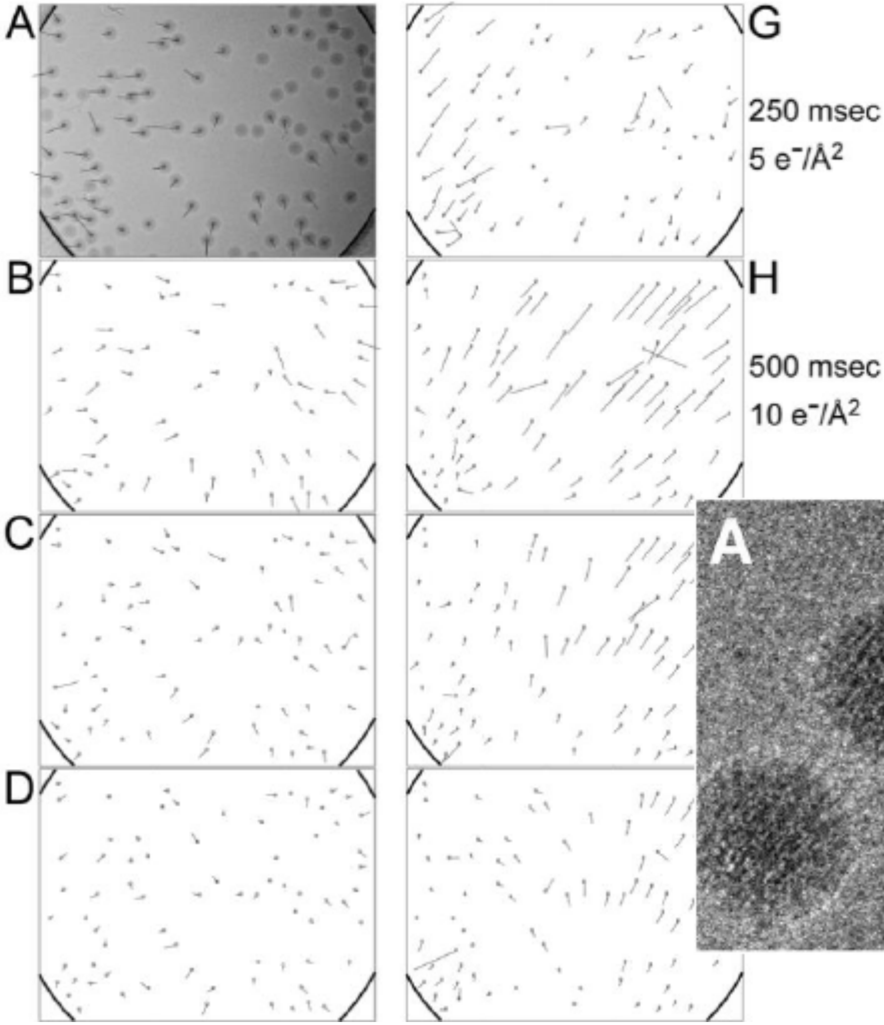
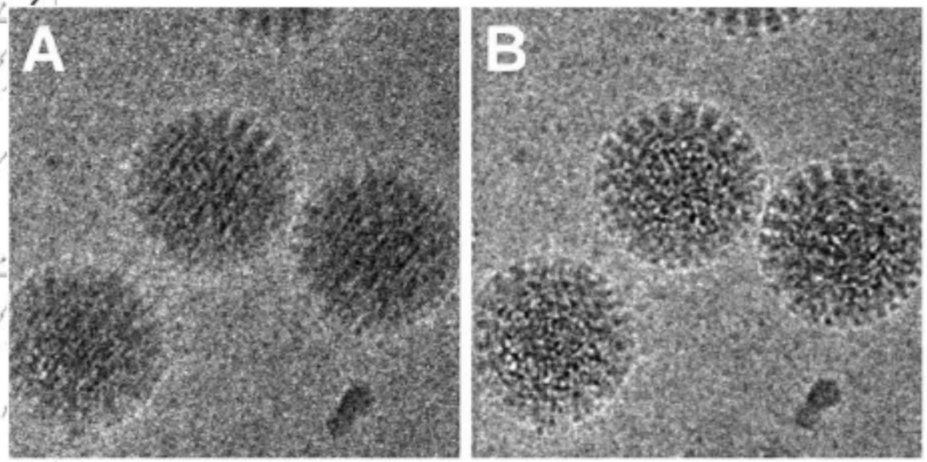


Figure 2. Recent technological advances. **(A)** Previously, noisier images were recorded on photographic film, beam-induced sample motion led to image blurring, and structurally different particles were often mixed in a single reconstruction. **(B)** Three recent advances yield better reconstructions: (i) digital direct-electron detectors yield data of unprecedented quality and allow recording movies during exposure; (ii) computer programs to realign the movie frames may correct for sample movements that are induced by the electron beam; and (iii) powerful classification methods lead to multiple structures from a sample mixture.

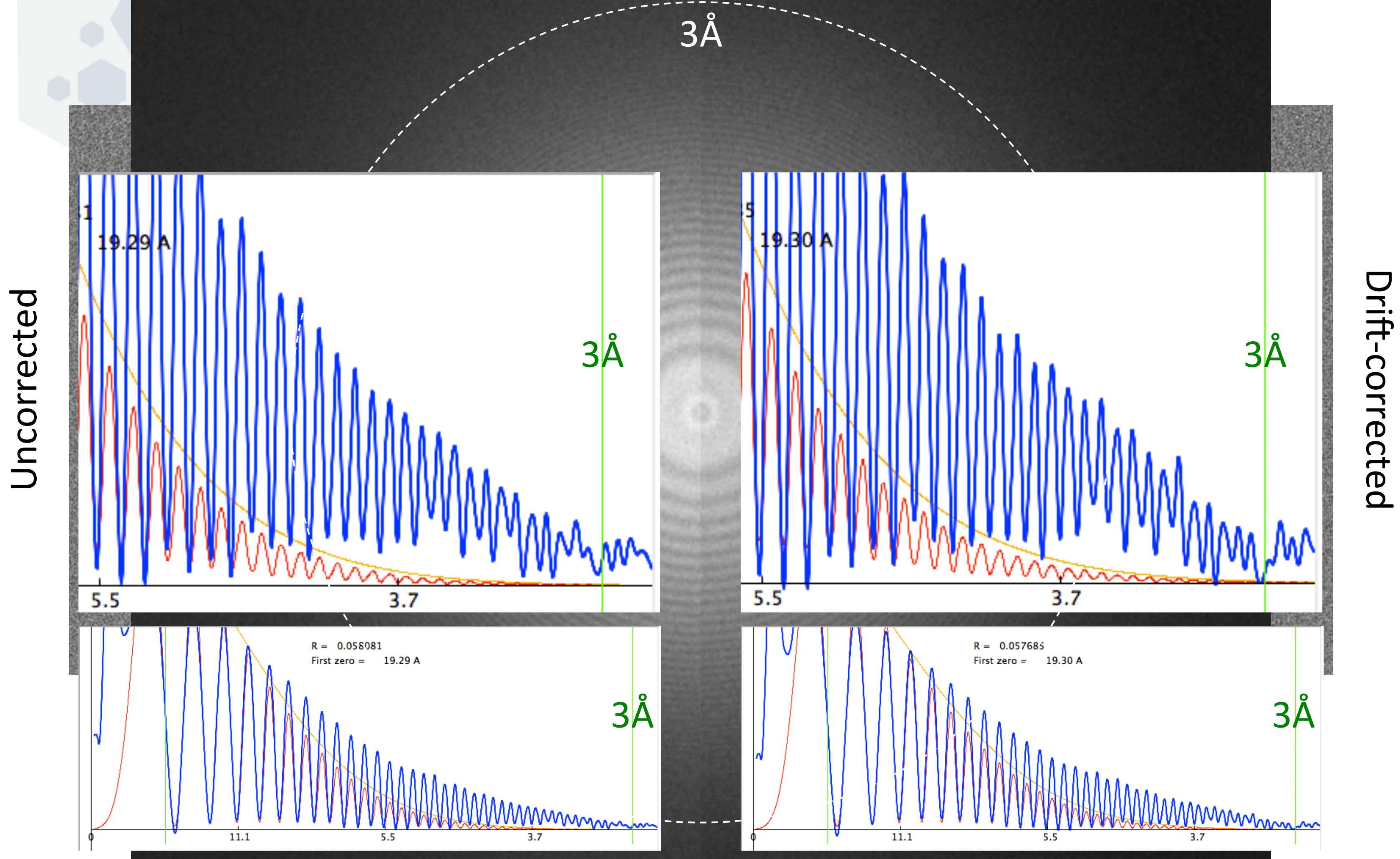
Motion correction: Rotavirus particles



Brilot, … , Potter,
Carragher, … , Grigorieff
(2012) *J.Struct.Biol.*

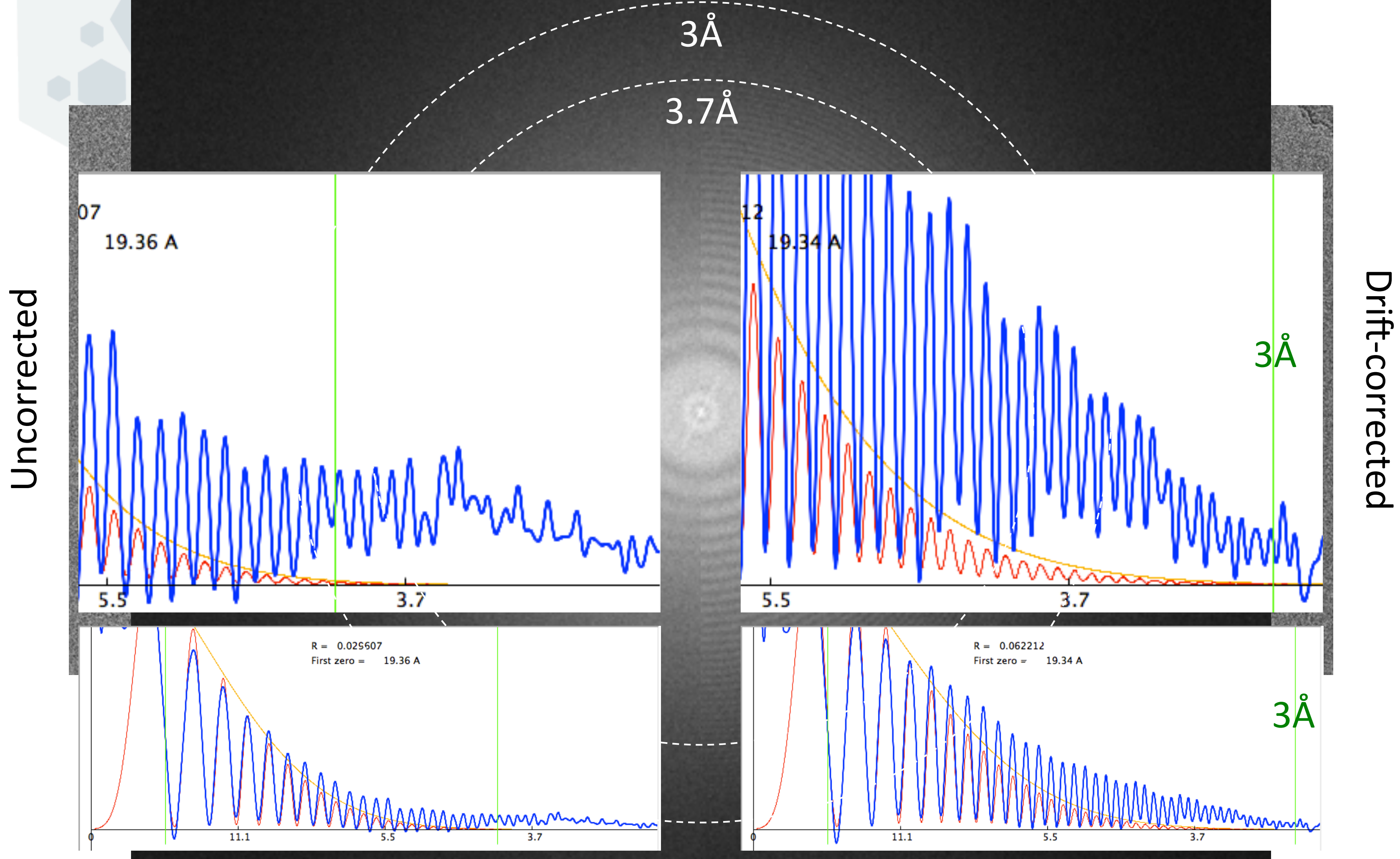


DED Cameras – FEI Falcon 2
1-little movement



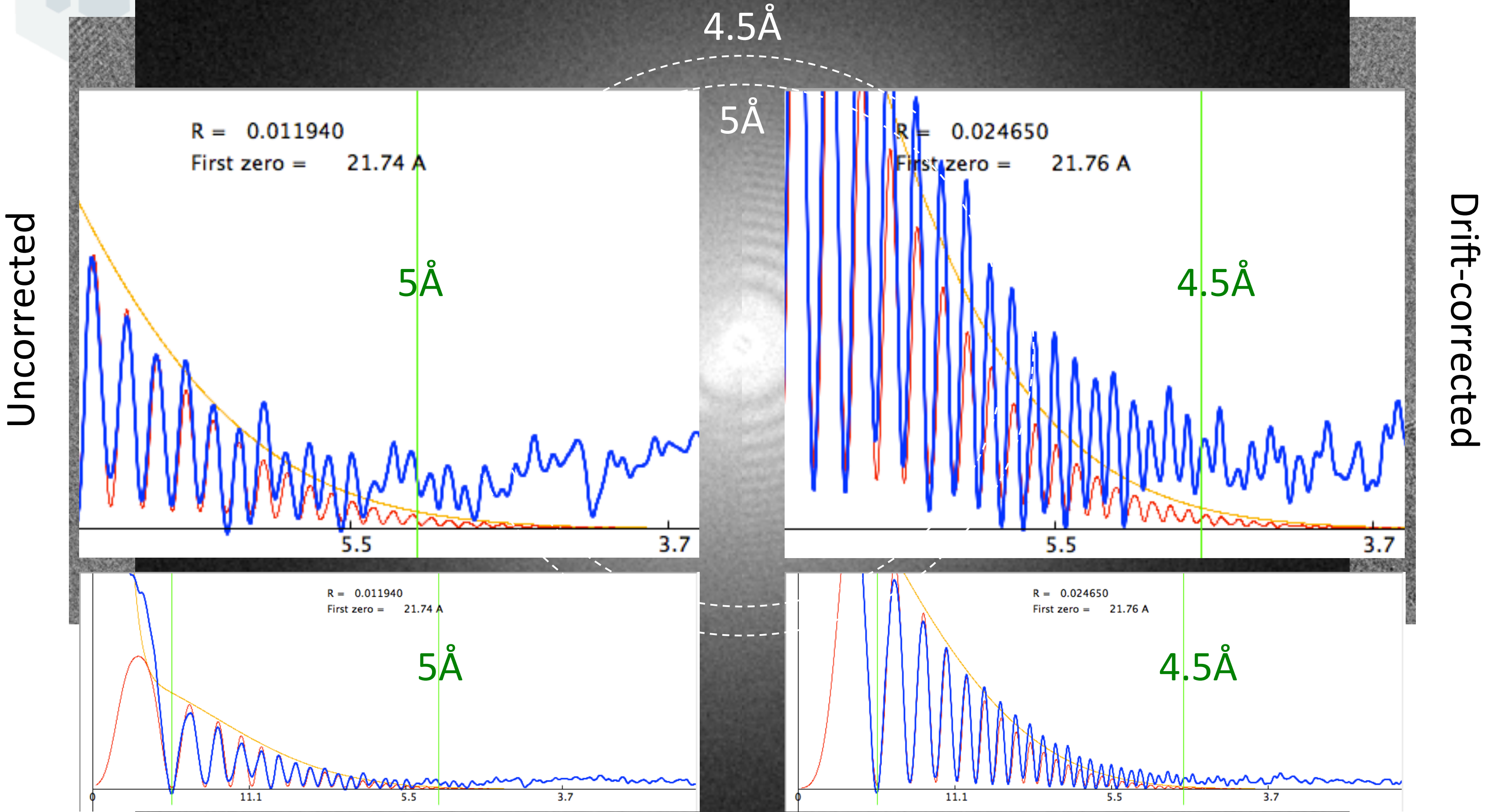
FEI™

DED Cameras – FEI Falcon 2
2-modest movement



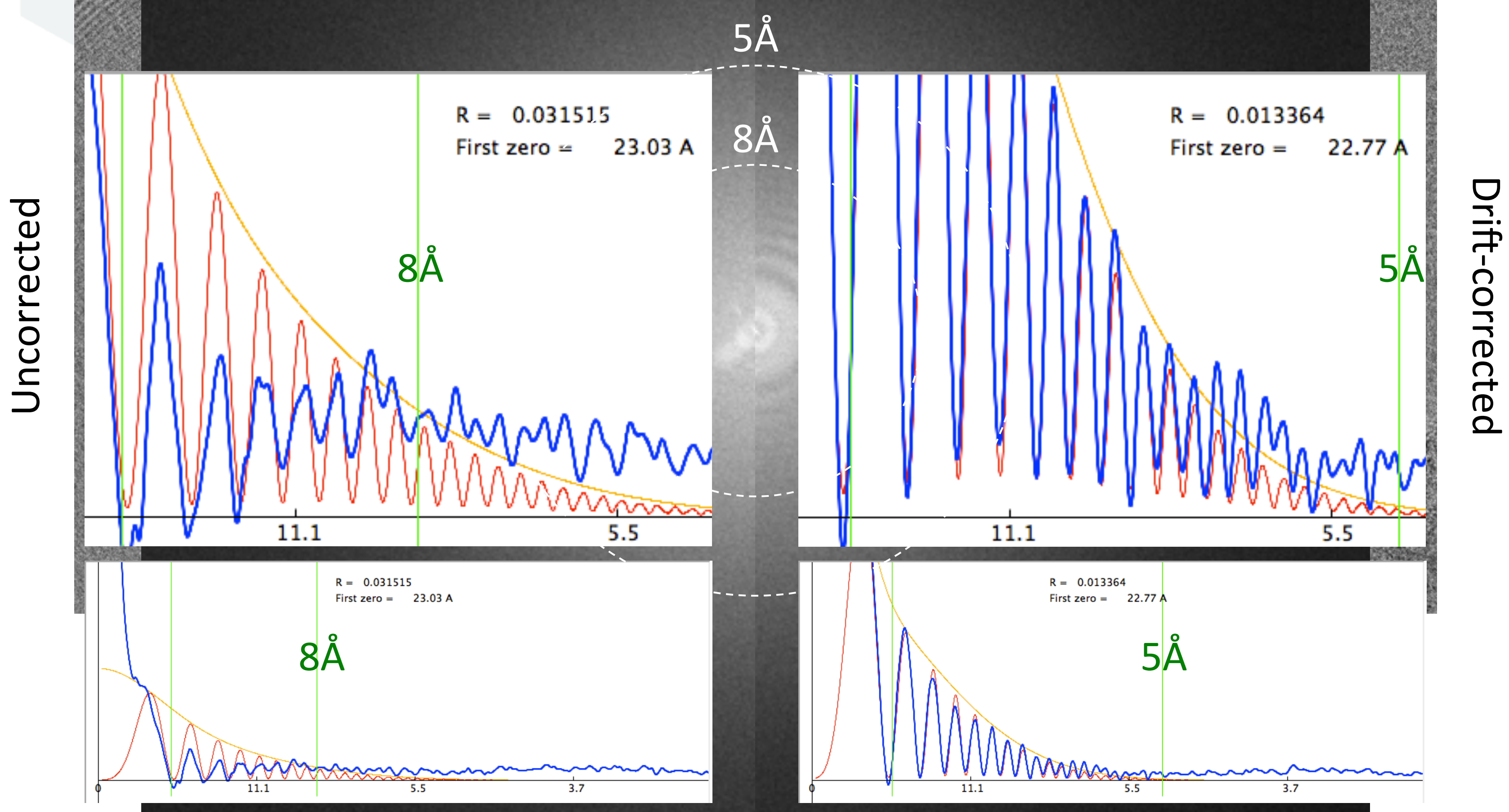
Explore. Discover

DED Cameras – FEI Falcon 2
3-large movement

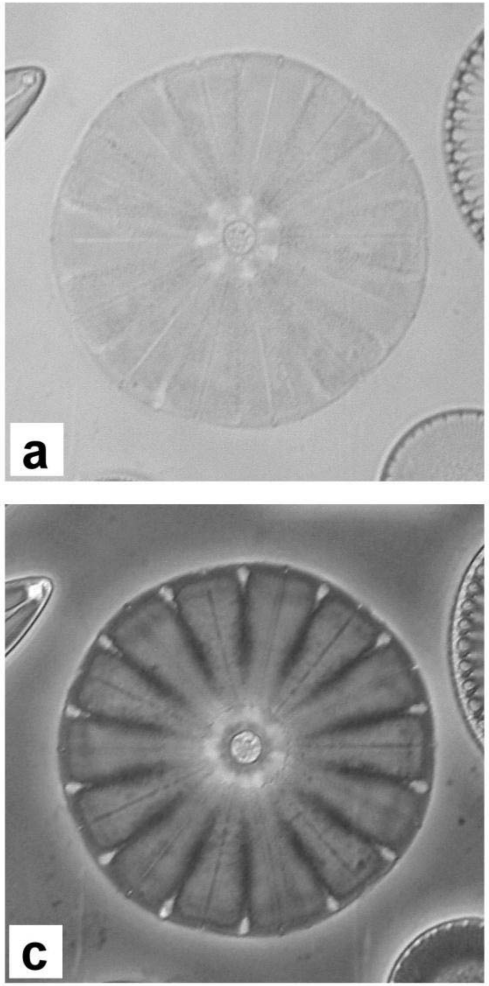


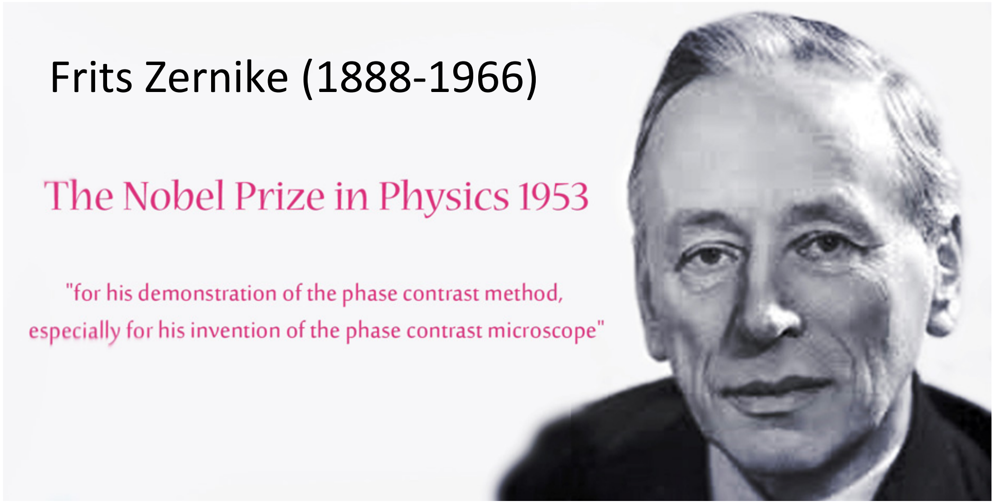
FEI™

DED Cameras – FEI Falcon 2
4-largest movement



Zernike Phase Contrast

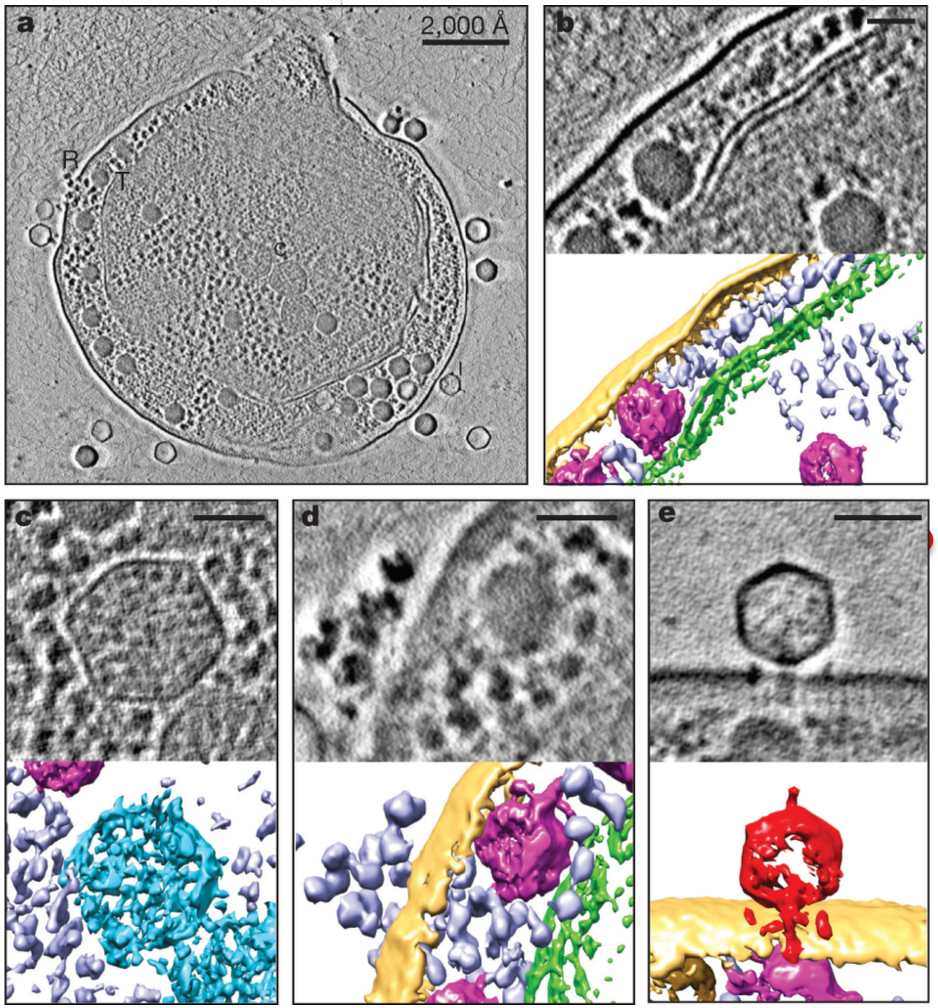




The basic principle to make phase changes visible in phase contrast microscopy is to separate the illuminating background light from the specimen scattered light, which make up the foreground details, and to manipulate these differently

Zernike Phase Plate in TEM

Dai… & Chiu (2014) *Nature Protocols* **9**, 2630–2642



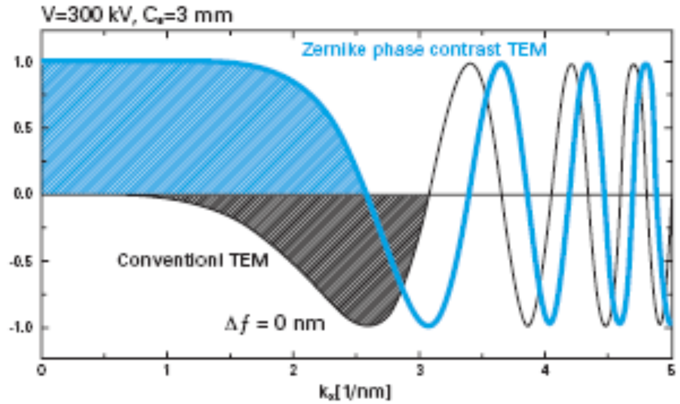


Figure 2

Posted: April 7, 2010

JEOL Offers First Commercially-Available Thin Film Phase Plate Technology for TEM

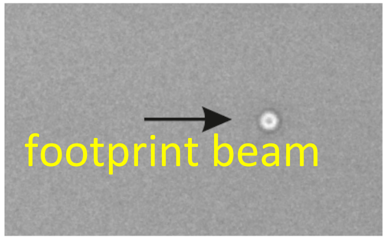
(*Nanowerk News*) The imaging performance of today's generation of Transmission Electron Microscopes (TEMs) is improved dramatically through the use of a novel technique, the thin film phase plate.

JEOL is the only electron microscope supplier to offer commercially-available thin film phase plate technology to its Life Sciences customers, in particular those involved in cryo-electron microscopy and cryo-electron tomography. The phase contrast imaging capability of a phase-

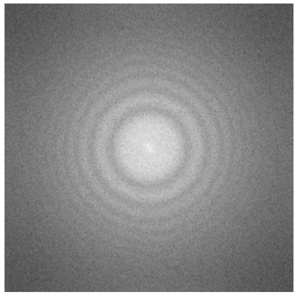
Volta Phase Plate

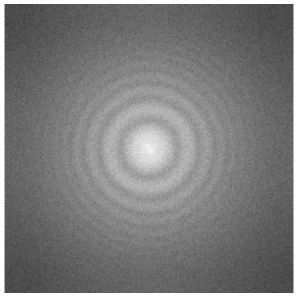
When an electron beam passes a continuous heated carbon film:

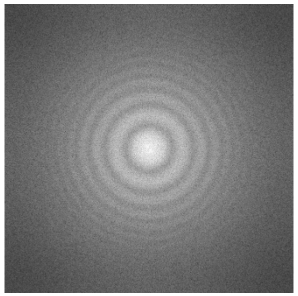
1. The beam leaves a white footprint (underfocus)



2. A phase shift occurs of the central beam relative to the diffracted beam







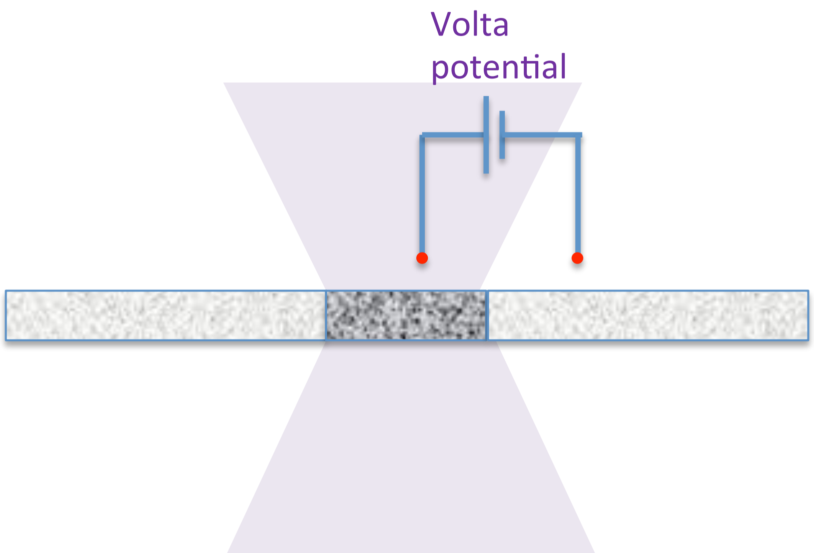
π/2

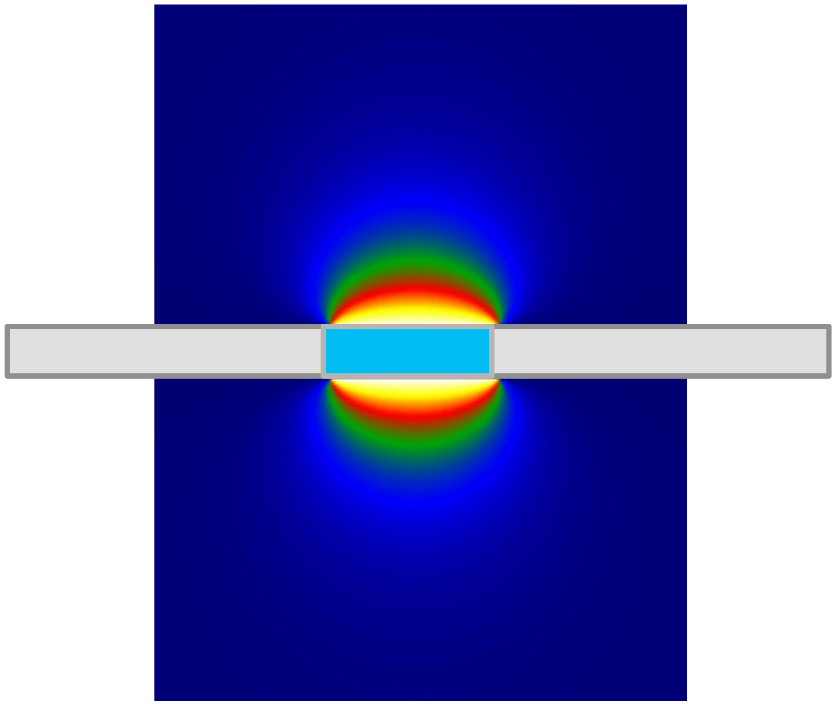
Principle

Phase shift not due to charging, but due to negative vacuum potential, called Volta potential

This potential can be caused by

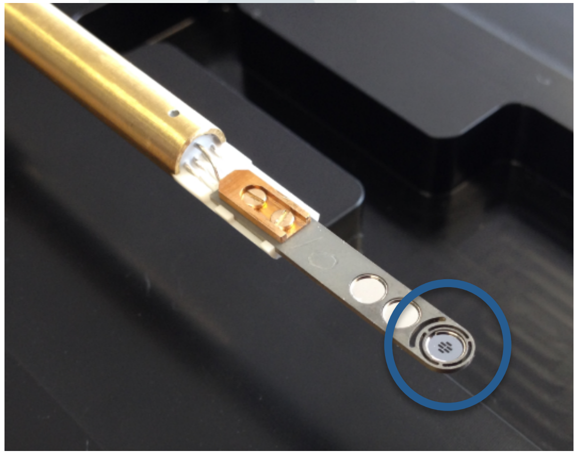
- changes in electronic structure of bulk
- changes in surface properties

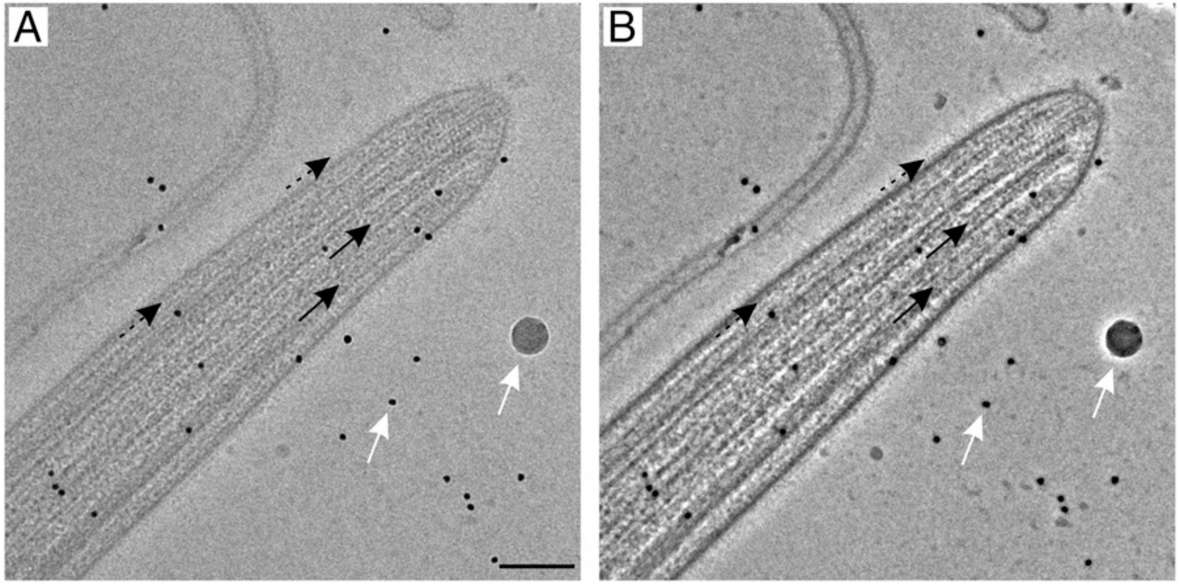




The undiffracted beam creates the phase shift

Contrast improvement for tomography





Danev R, *et al*. (2014), **PNAS** 111, p. 15635

- The FEI Volta Phase Plate provides “high defocus” contrast with in-focus imaging
- The FEI VPP has a long lifetime, regenerates itself, does not require an airlock nor frequent replacements, does not need a centering mechanism and is contamination free
- The FEI VPP is fully automated, easy and simple to use, embedded in the TEM UI and Explore3D tomography software (version 4.1)