

# Tomography Acquisition

-or-

## The First “R” (of 3)

Sharon Grayer Wolf

# Tomography workflow

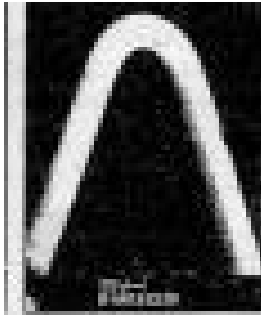
1. Sample preparation (Eyal, Katya)
2. Correlative Light-Electron  
Microscopy -CLEM (Tali)
3. Data acquisition (Sharon)
4. Image processing (Nadav)



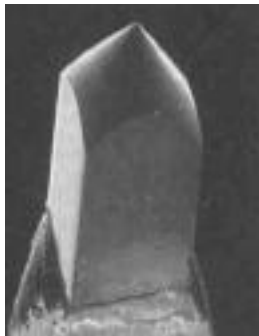
# Data Acquisition

1. Microscope Components important for Tomography workflow (EVERYTHING)
  - a) Electron guns
  - b) Higher Voltage for thick specimens
  - c) Sample exchange system
  - d) Excellent vacuum systems
  - e) Stable optics
  - f) Energy filter
  - g) Phase plates
  - h) Cameras/detectors
  
2. Tomography Acquisition
  - a) Tilt schemes
  - b) Dose considerations
  - c) Defocus, magnification choices that affect resolution
  - d) Data collection software

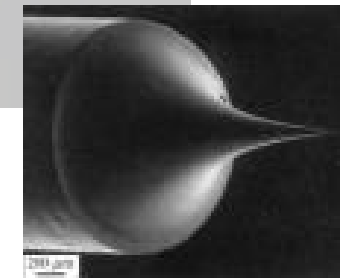
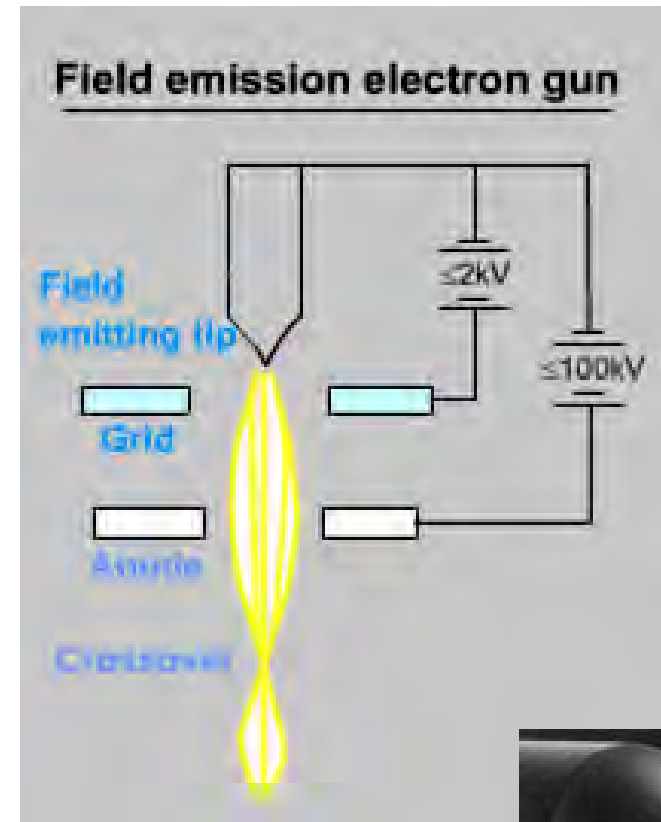
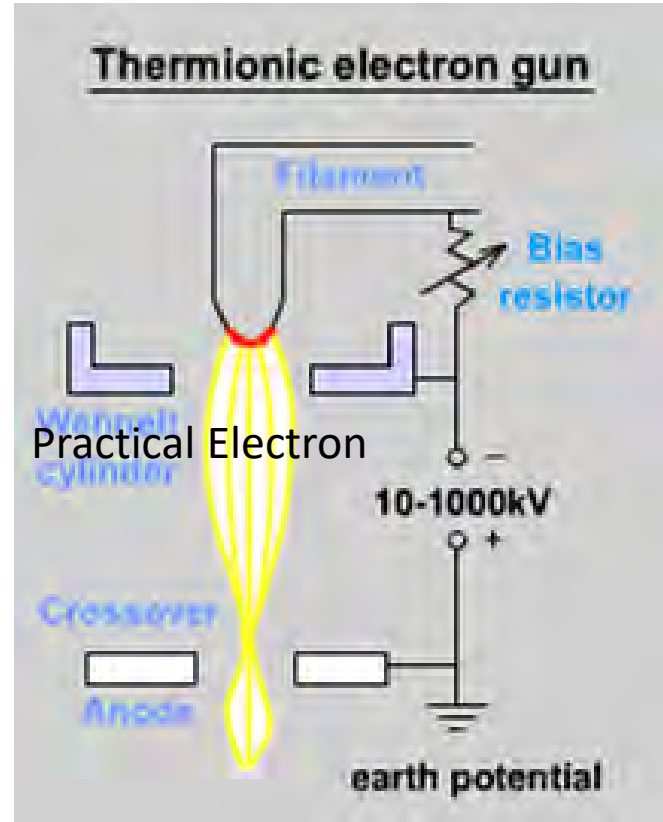
# Electron Sources



Tungsten



LaB6



FEG

# Electron Sources

- Thermionic guns: Electron emission through heating.
- Field Emission Guns (FEG): Electron emission through applying an extraction voltage.

Properties	Tungsten	LaB6	FEG
Work function / eV	4.5	2.4	4.5
Temperature / K	2700	2000	1800
Energy spread / eV	3-4	1.5-3	0.4-1.5
Source size / nm	30000	5000	3-20
Maximum current / nA	1000	500	300
Brightness / A/m <sup>2</sup> sr	10 <sup>9</sup>	5x10 <sup>10</sup>	10 <sup>13</sup>
Lifetime / h	100	500	> 1000

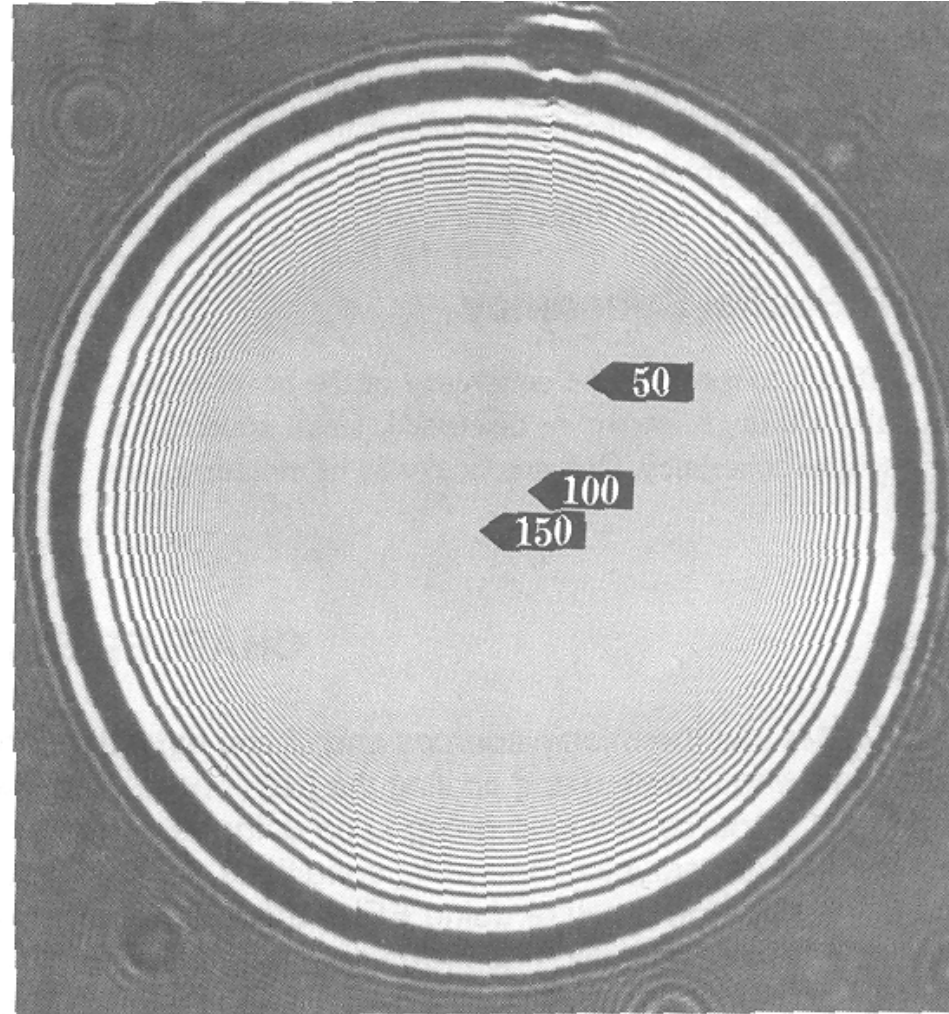
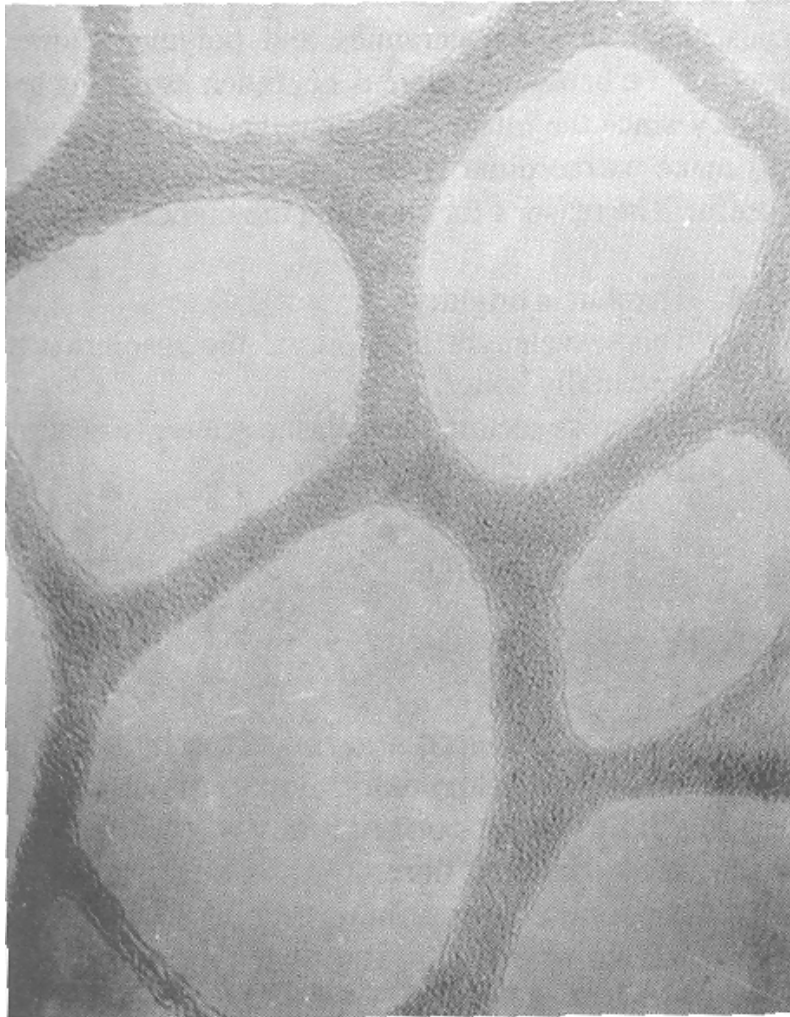


# Electron Sources

Frensel fringes

A Thermionic Source  
(poor coherency)

A FEG  
(high coherency)



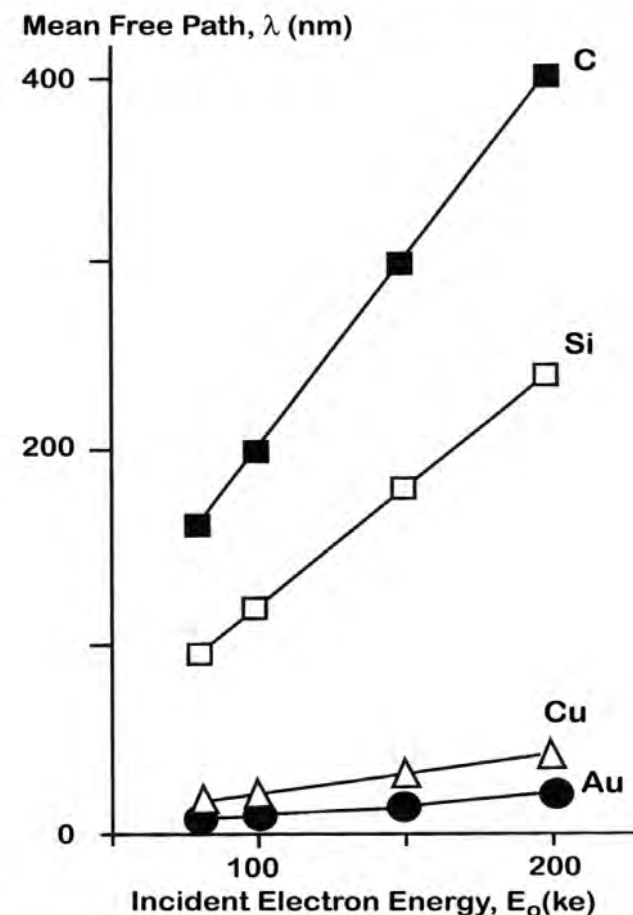
Increasing voltage increases mean free path

## Elastic scattering

### Screened relativistic Rutherford cross section

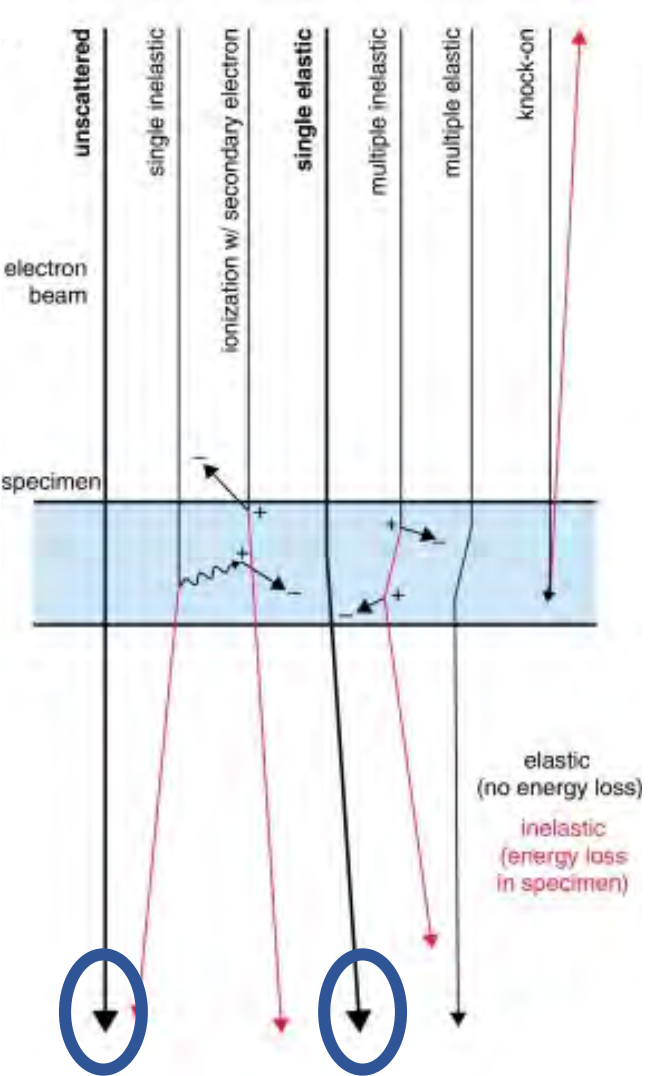
Can be plotted as an equivalent mean free path vs. incident energy

This gives you a good sense on allowable sample thickness!



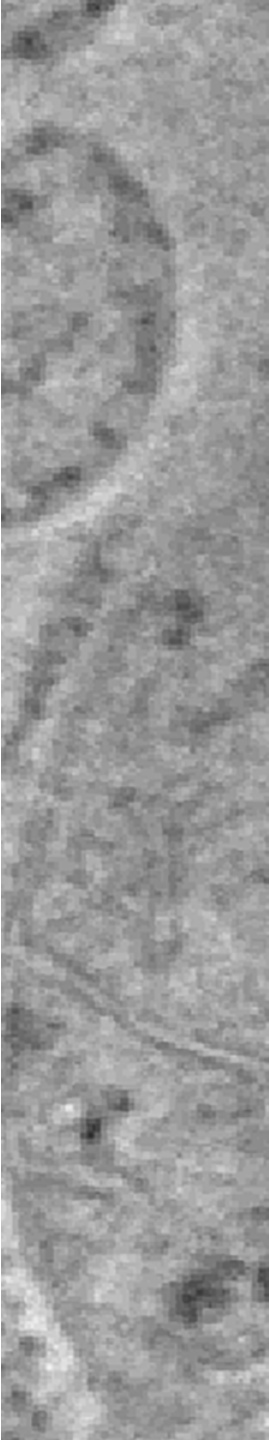


# Phase contrast in TEM images

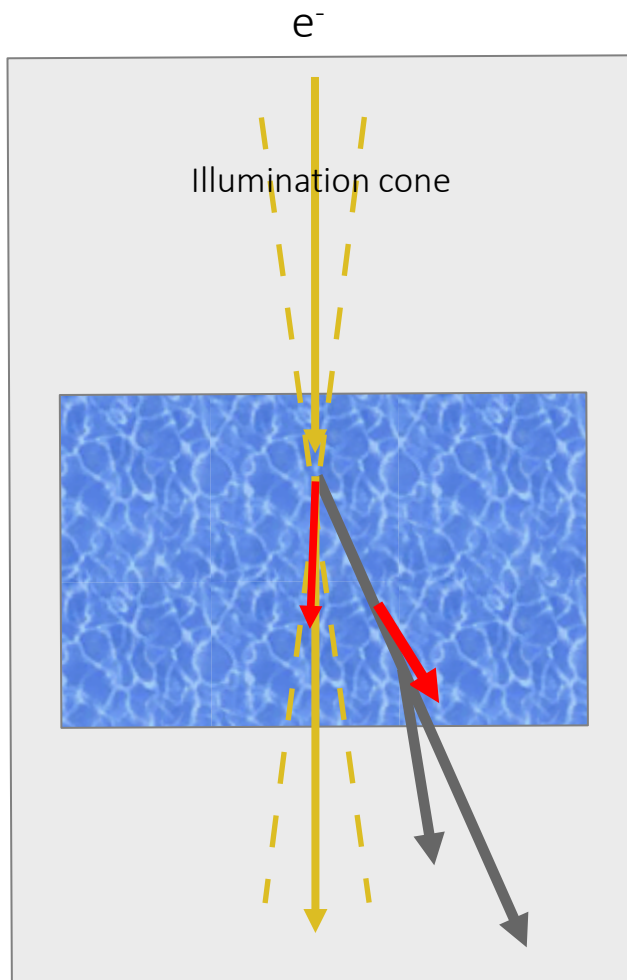


CryoTEM relies on *Phase Contrast* which requires:

**COHERENT, ELASTIC SCATTERING**



## CryoTEM: Sample thickness is limited by the INELASTIC MEAN FREE PATH



- Elastic scattering
- Inelastic scattering

Cross-section: elastic  $\sim Z^{3/2}$   
inelastic  $\sim Z^{1/2}$

Ratio inelastic/elastic  $\sim 20/Z$

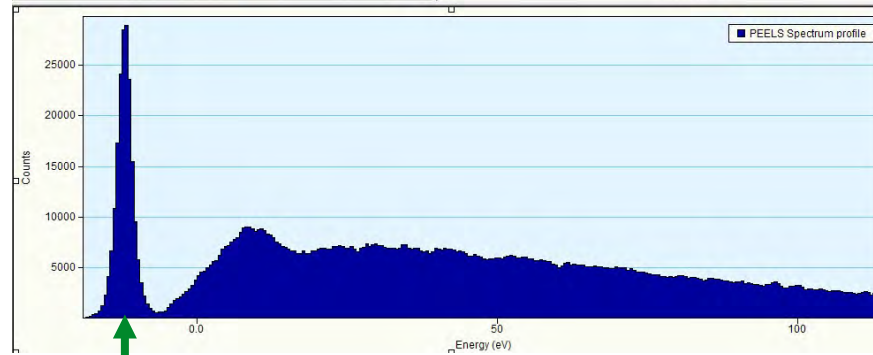
Inelastic mean free path in vitrified water for 200kV  
 $\sim 250 \text{ nm}$

The “Natural” limit for cryoTEM  
Tomography



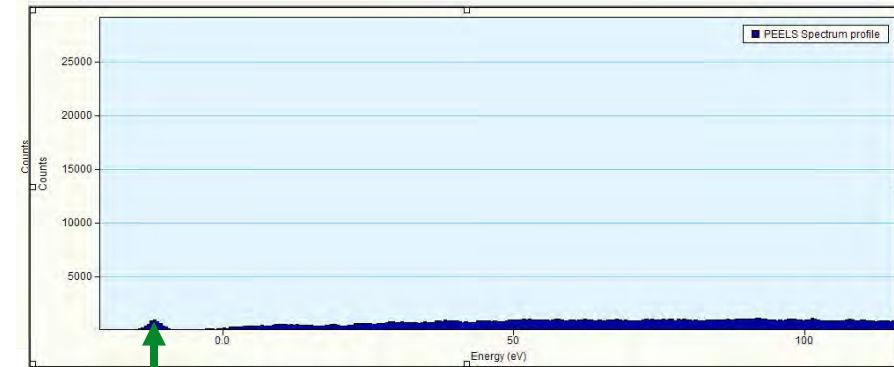
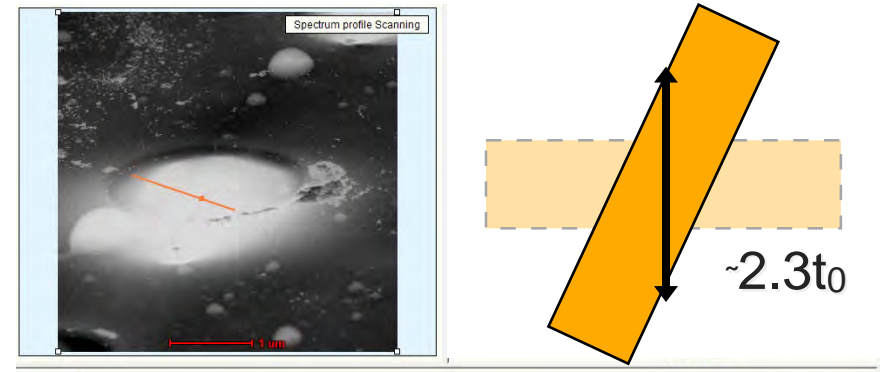
For thick samples, few “useful” (elastic) electrons remain:

0 deg



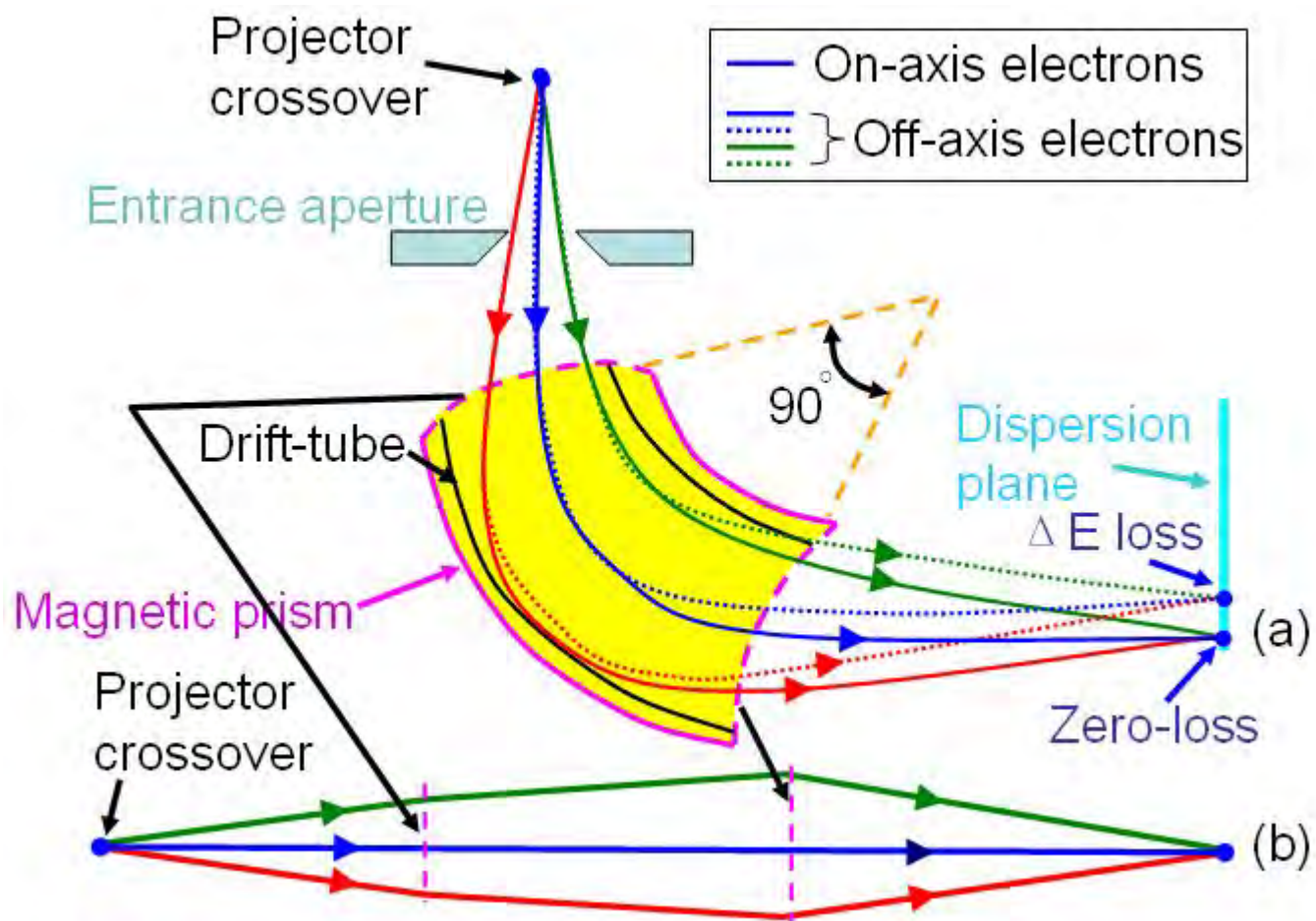
elastic scattering

65 deg tilt

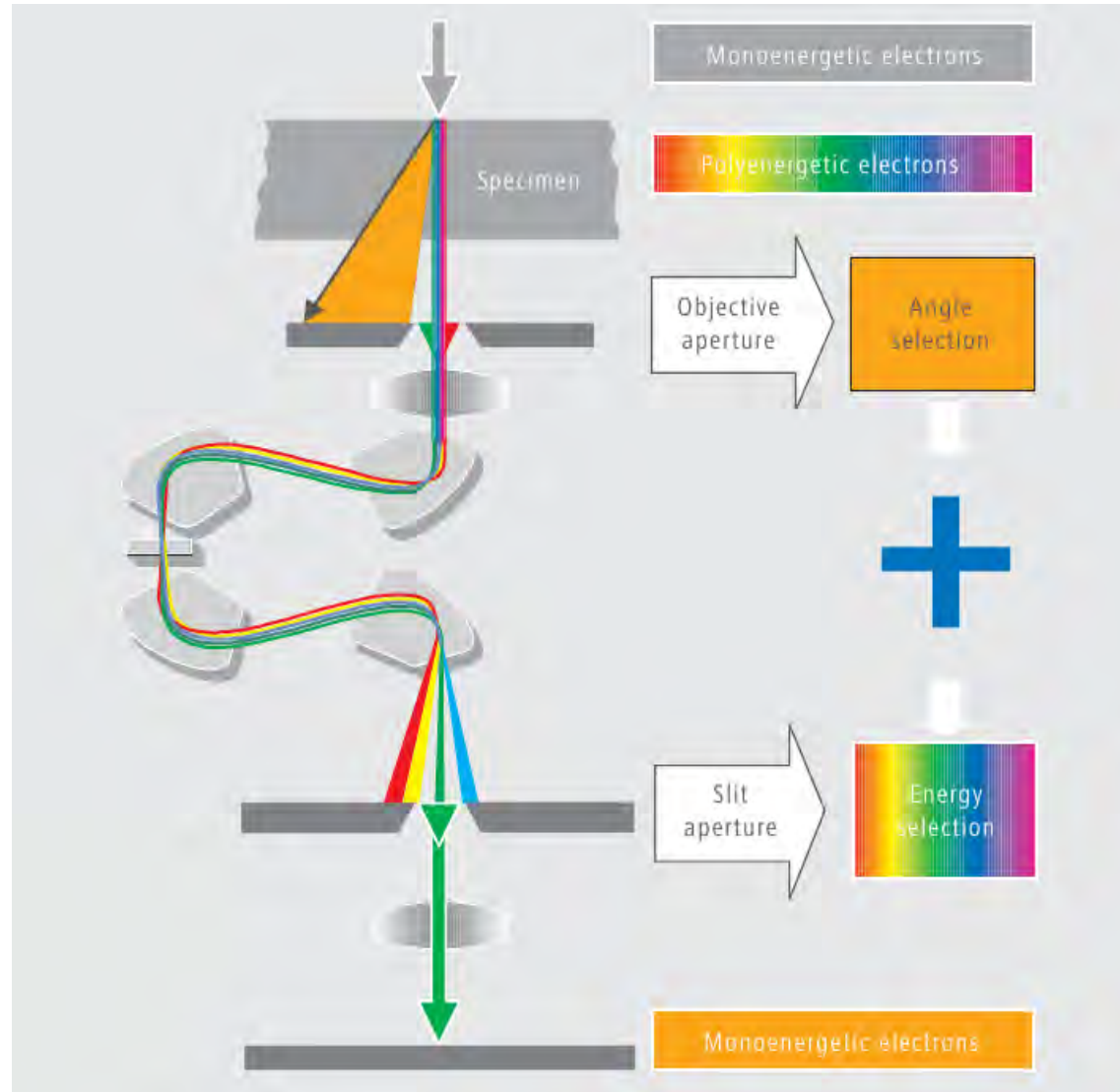


EELS SPECTRA

# Energy Filter

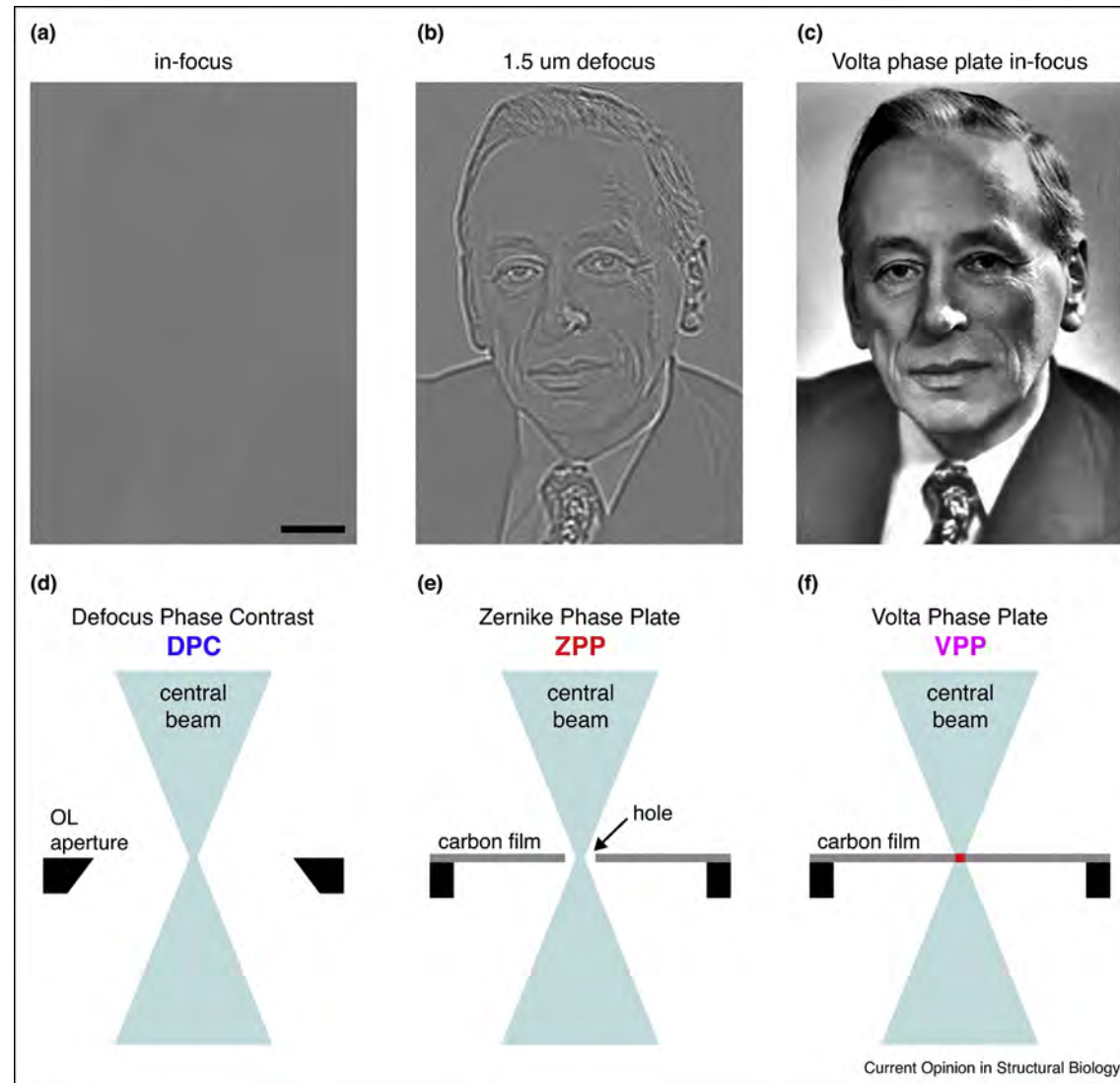


# Energy Filter

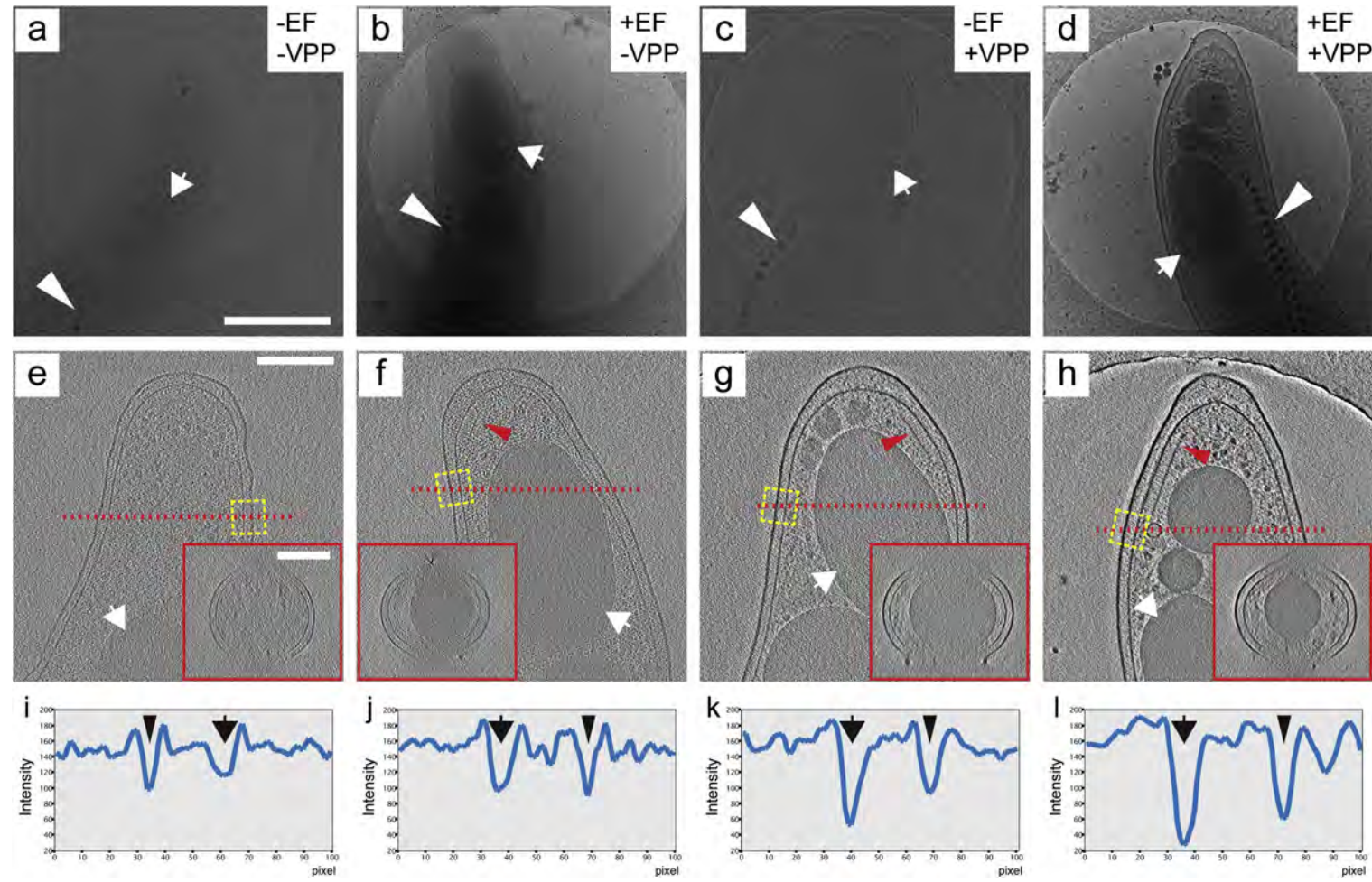




# Why Phase Plates? Enhanced Contrast

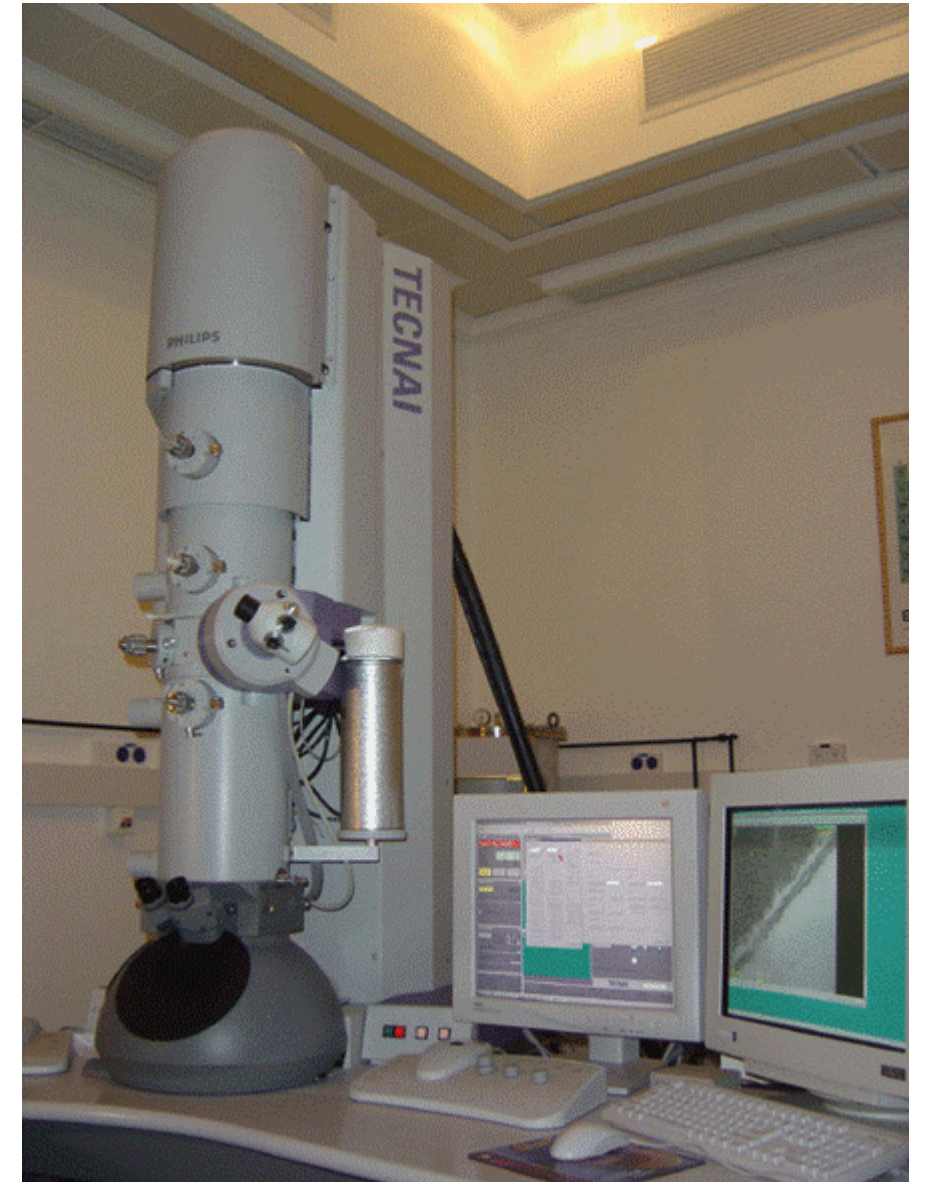
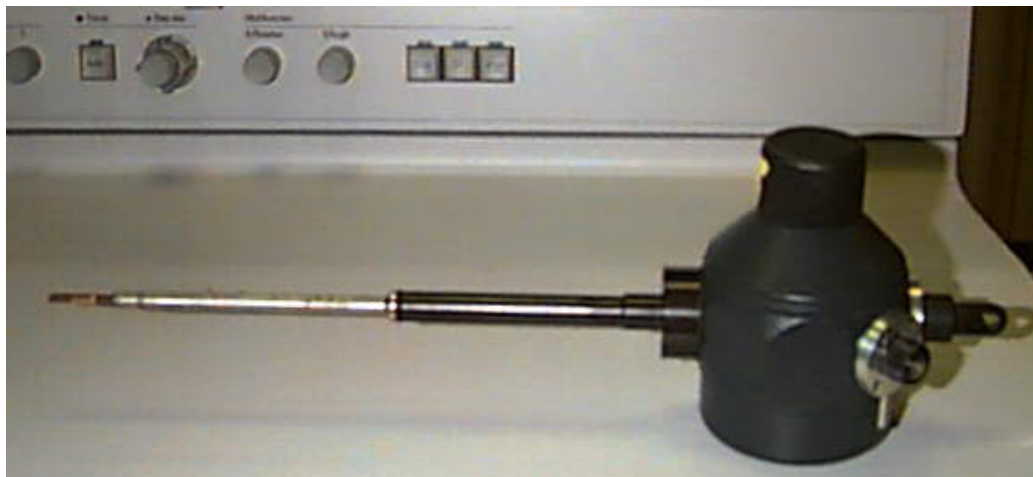
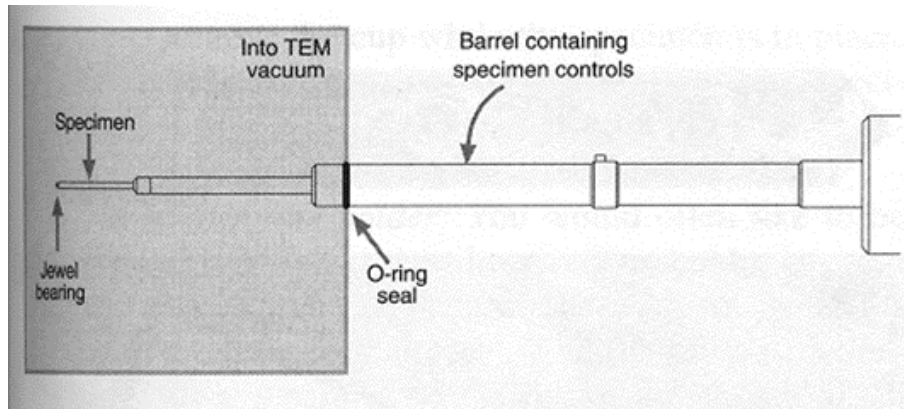


# Why Phase Plates? Enhanced Contrast



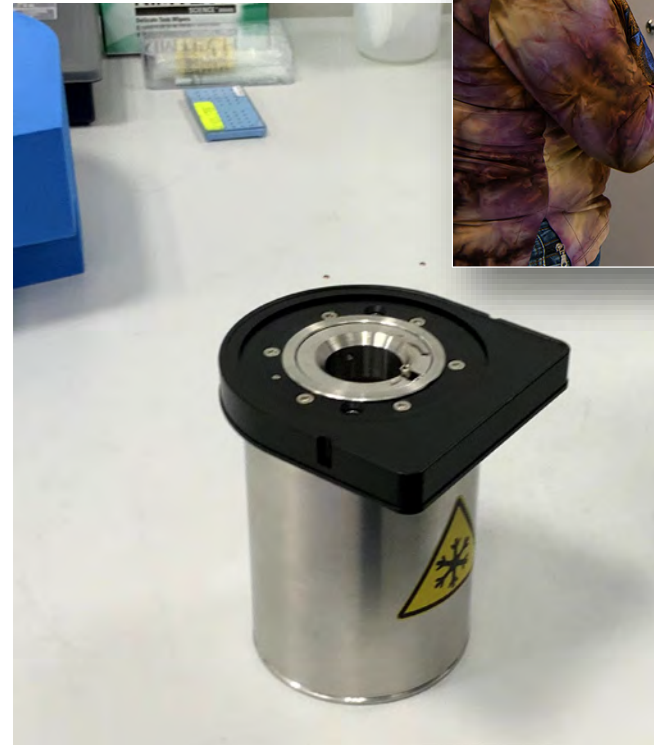
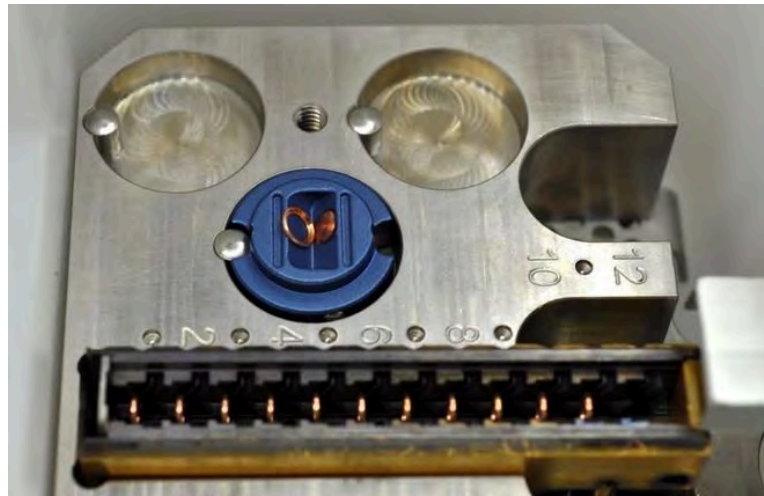
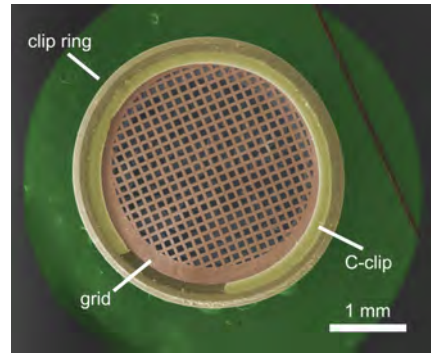


# Cryo-Specimen holders - side entry





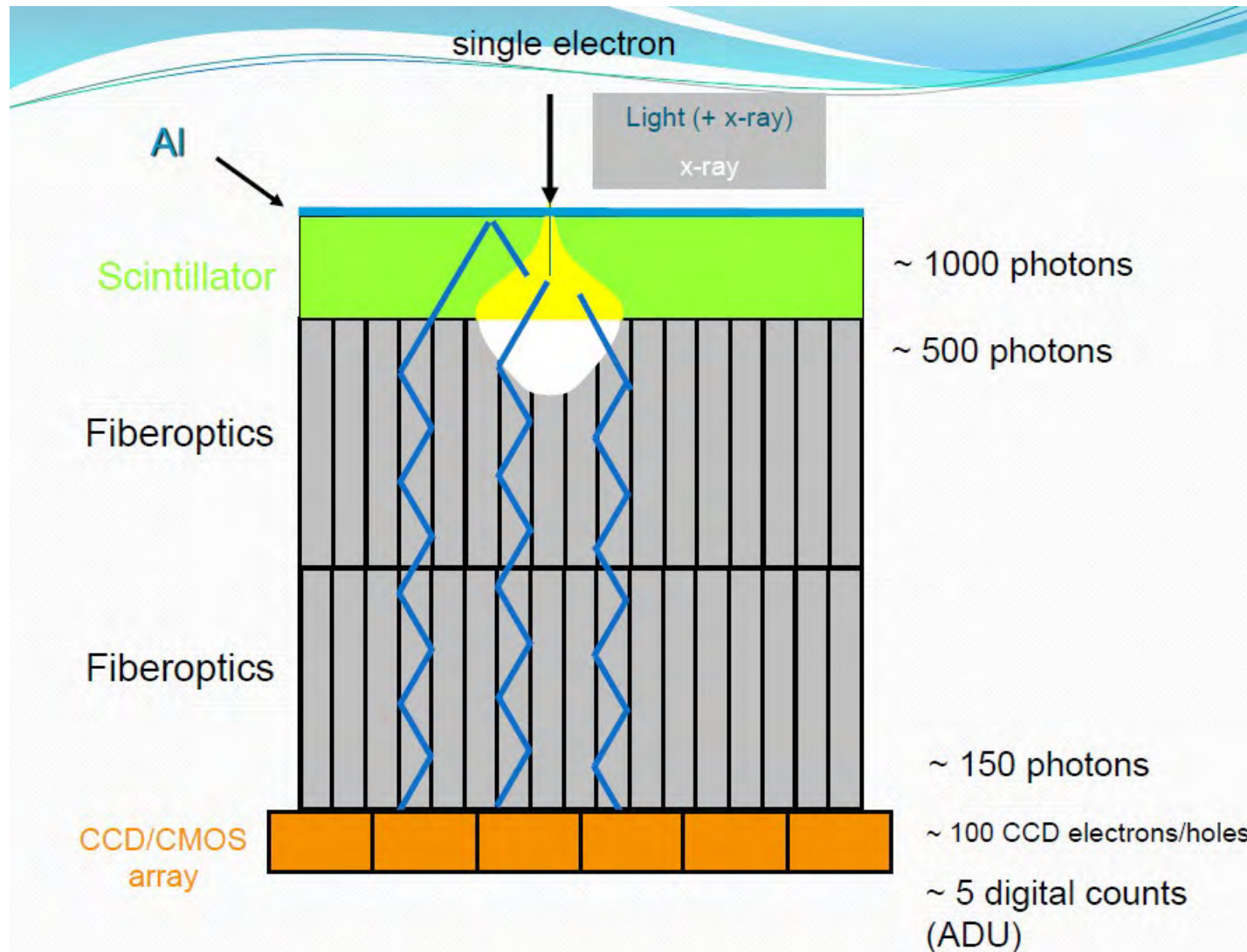
# Cryo-Specimen holders – cartridge systems



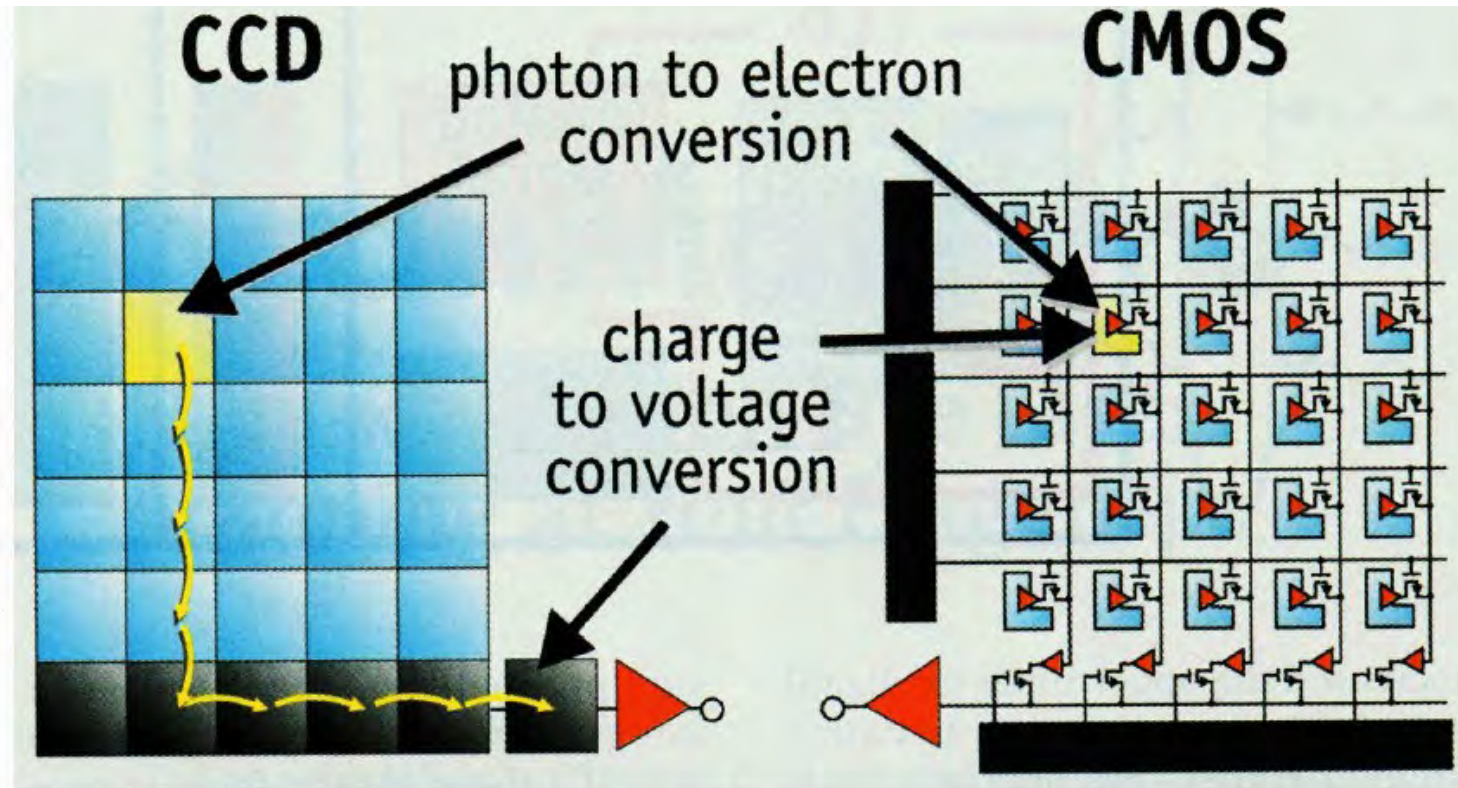


# Charge-Coupled Device (CCD) cameras

Electrons are converted into photons of light before being detected.



# CMOS (complementary metal-oxide semiconductor) cameras



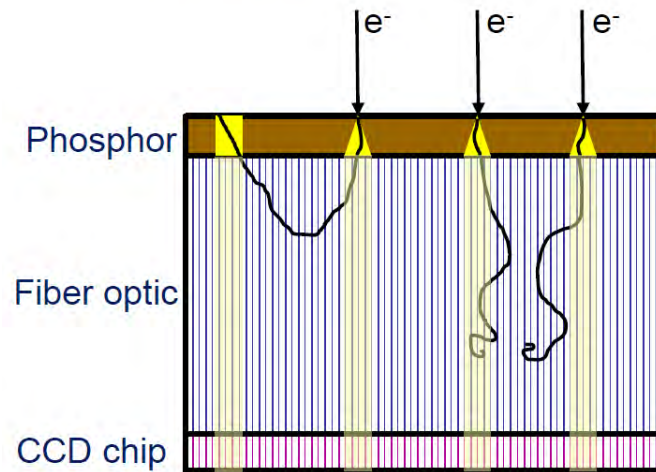
CCDs move photogenerated charge from pixel to pixel and convert it to voltage at an output node. CMOS imagers convert charge to voltage inside each pixel.



# Why Direct Electron Detection?

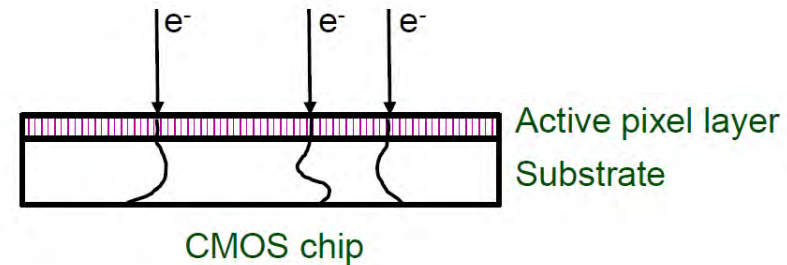
## CCD Camera

- Signal from one electron spreads in the scintillator and is recorded in several pixels
- At higher voltages, 20-30% of electrons scatter back into phosphor in the wrong place
  - Spurious signals ruin resolution and efficiency

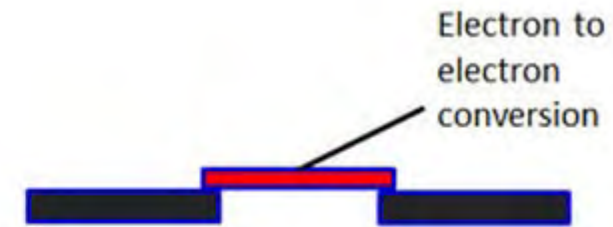
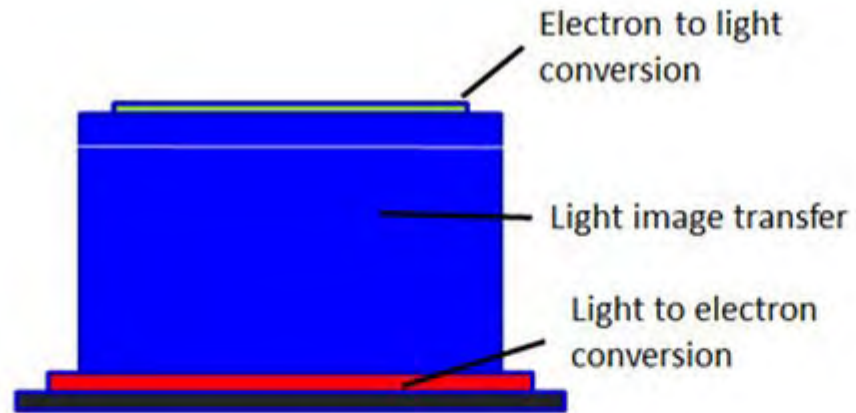


## Direct Detector

- Signal from one electron is generated mostly within one pixel
- Backscatter is much less if the substrate is thin enough



# Why Direct Electron Detection?



**Indirect (left) and Direct (right) detection and Monte Carlo simulations of electron tracks within the device**

From Gatan Inc. website



## Electron Counting

- Single electron events can be distinguished if frames are read out fast enough and number of electrons in each is low enough
- Electron counting helps by about a factor of 2 because it:
  - Reduces blurring from signals spreading to more than one pixel
  - Eliminates effects of variable sized packets of signal and of readout noise, which impair the signal-to-noise ratio



Single electron events in one frame from an active pixel sensor

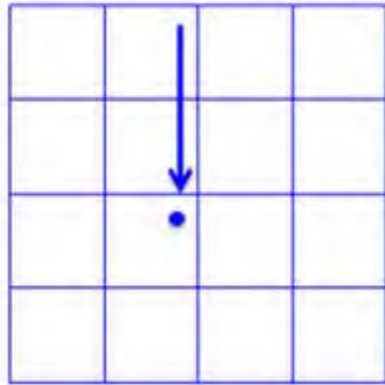
McMullan et al., 2009, Ultramicroscopy 109: 1411–1416

# Why Direct Electron Detection?

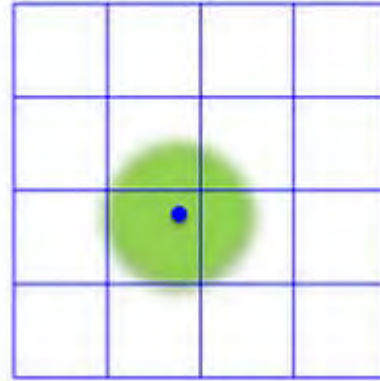
Single electron counting brings noise down to practically zero

## Standard Integrated Mode

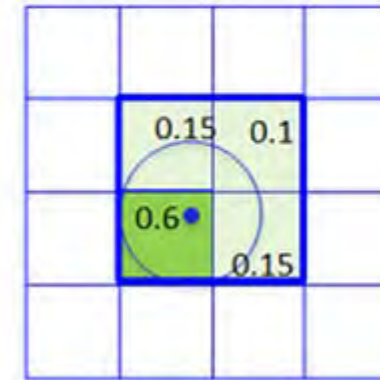
1. Electron enters detector



2. Signal Is Scattered

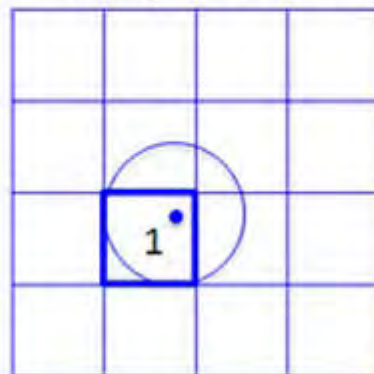


3. Charge collects in each pixel

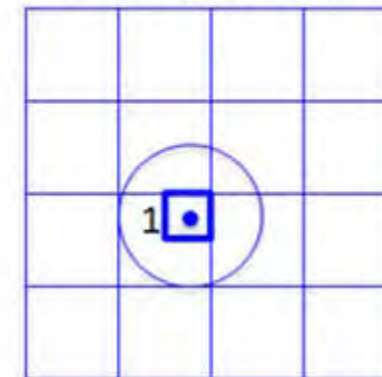


## Single Electron Counting Mode

4a. Events are reduced to the highest charge pixels



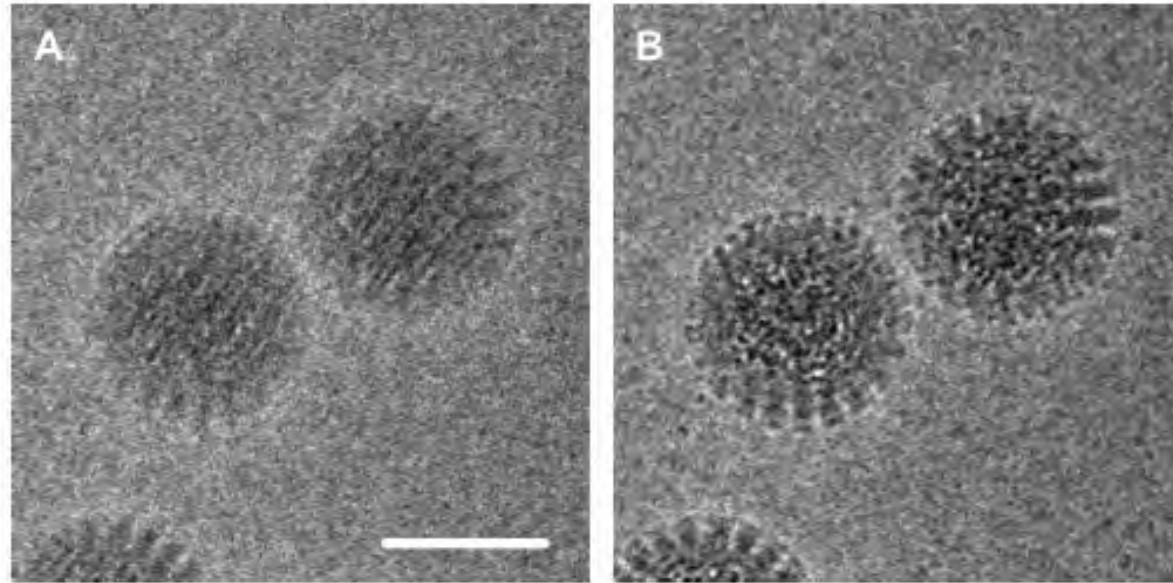
4b. Events are localized with sub-pixel accuracy



From Gatan Inc. website



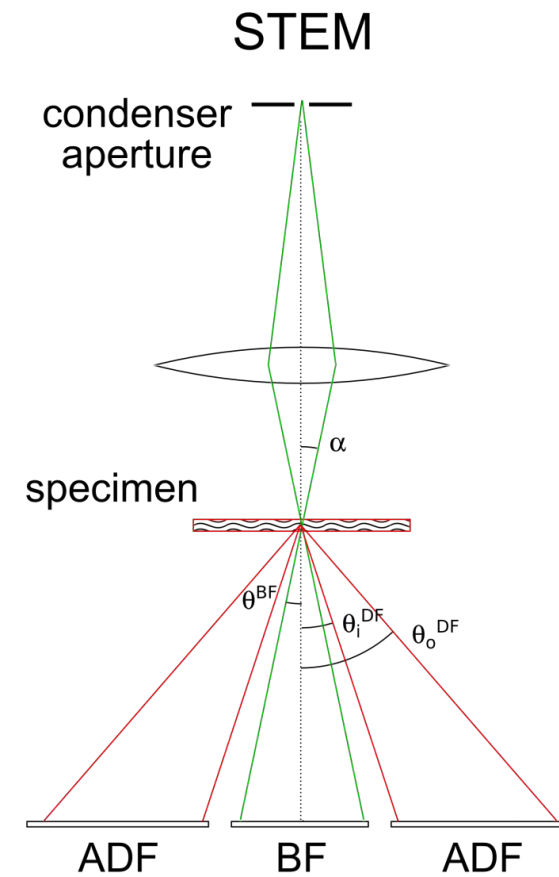
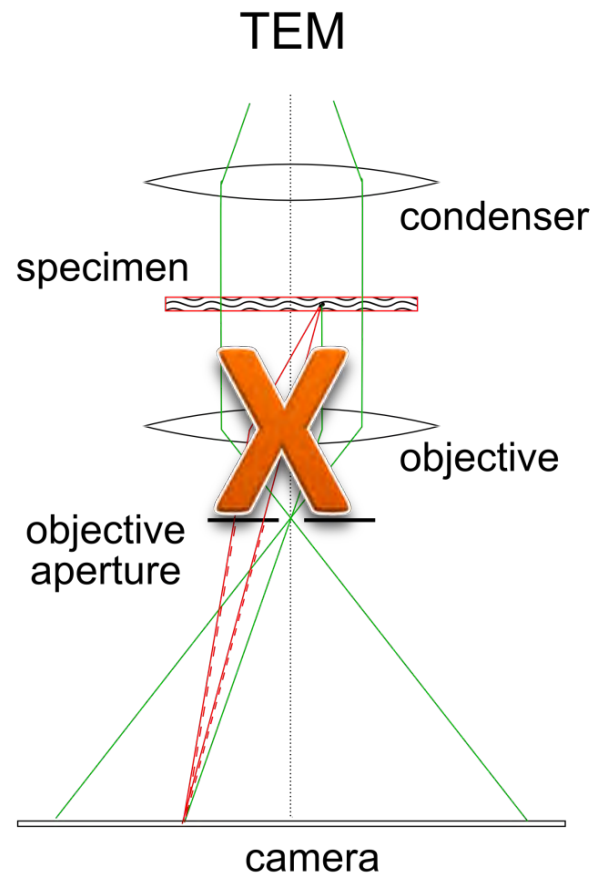
## Direct Electron Detector (DED) cameras

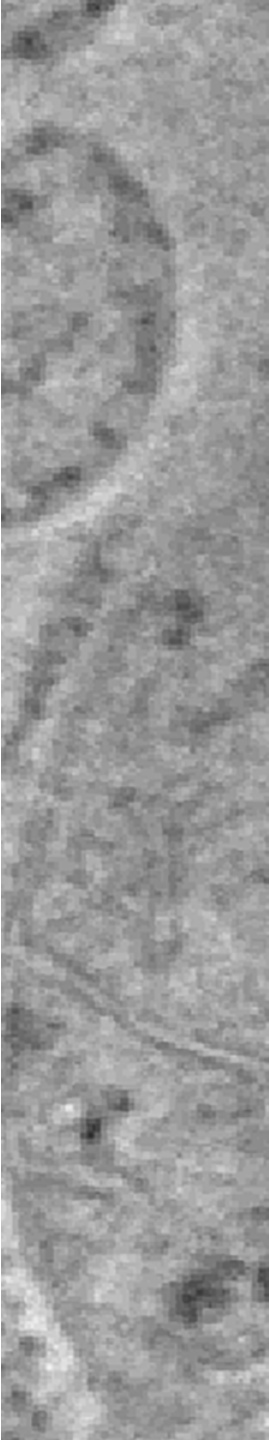


**Figure 1.** Electron micrograph of double-layered rotavirus particles frozen in a thin layer of amorphous ice. The image was recorded by the author and co-workers using the DE-12 direct electron detector (Direct Electron, San Diego, United States) in movie mode at 40 frames per second. In panel A, 60 frames have been averaged without alignment, resulting in an image that is blurred due to beam-induced movement. In panel B the frames have been aligned to compensate for this movement, which results in an image with significantly reduced blurring and improved contrast. The alignment method used here involved tracking the movement of the particles (*Brilot et al., 2012*); the alignment method used by Bai et al. used additional statistics to predict the movement of the particles caused by the electron beam. Scale bar = 50 nm.

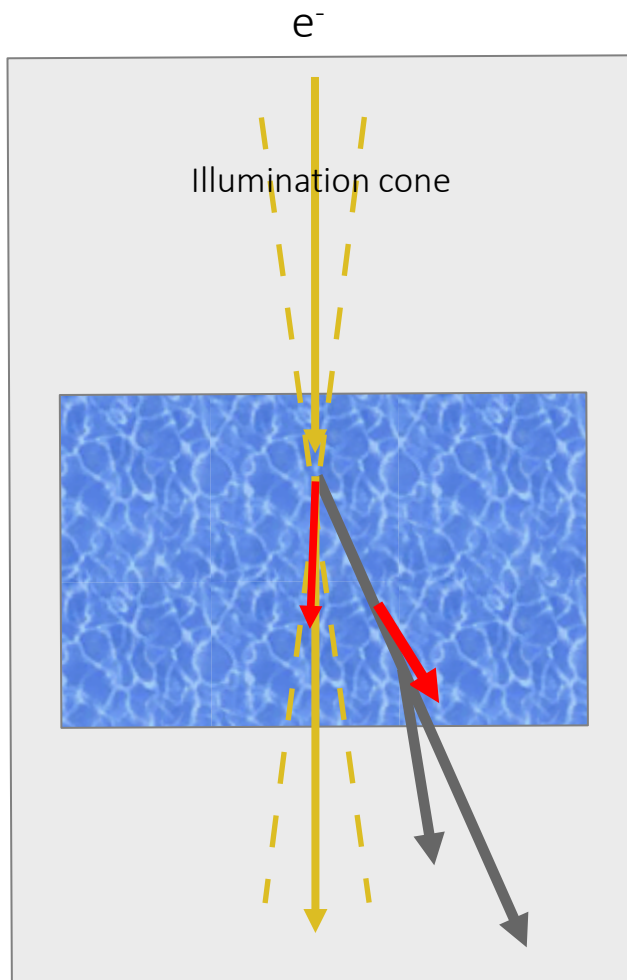


# Image formation in STEM – incoherent detection, particle nature





## CryoTEM: Sample thickness is limited by the INELASTIC MEAN FREE PATH



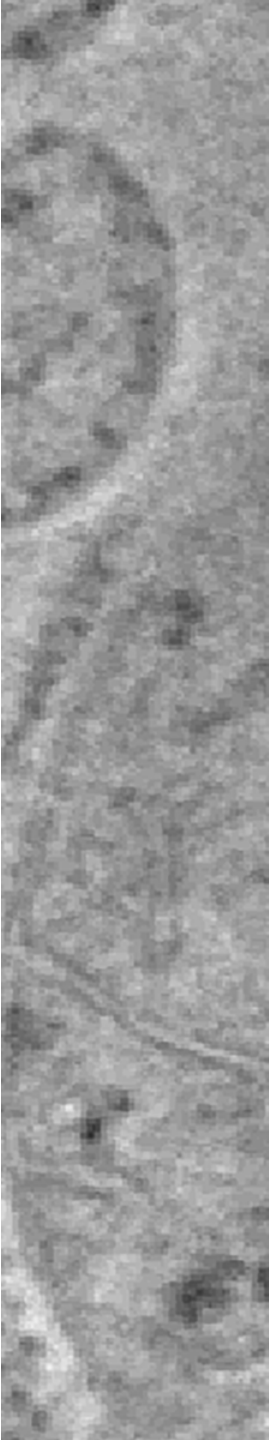
- Elastic scattering
- Inelastic scattering

Cross-section: elastic  $\sim Z^{3/2}$   
inelastic  $\sim Z^{1/2}$

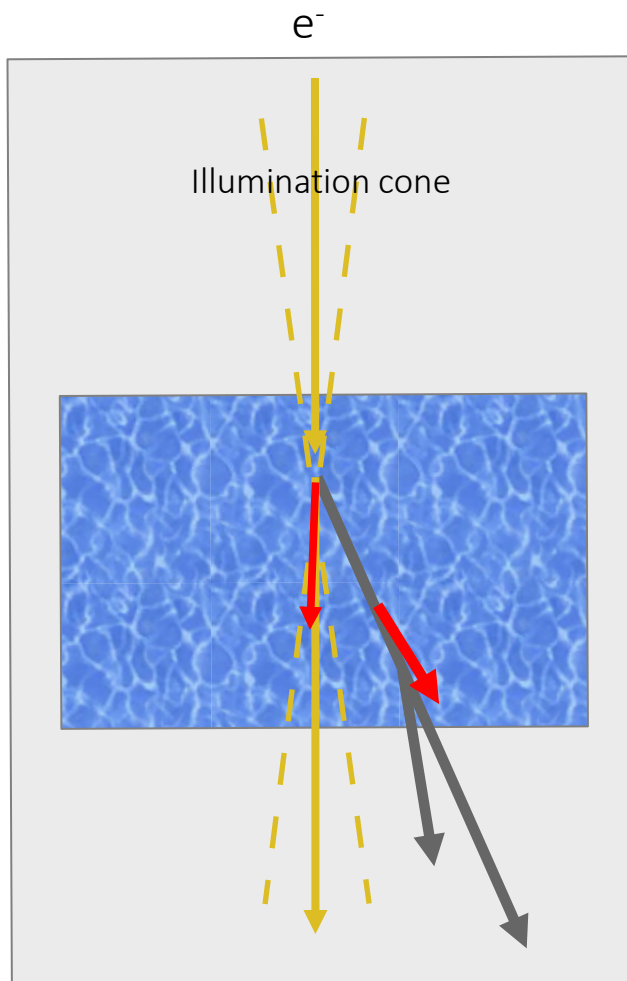
Ratio inelastic/elastic  $\sim 20/Z$

Inelastic mean free path in vitrified water for 200 kV  
 $\sim 250$  nm

The “Natural” limit for cryoTEM  
Tomography



## CryoSTEM: Sample thickness is limited by the ELASTIC MEAN FREE PATH



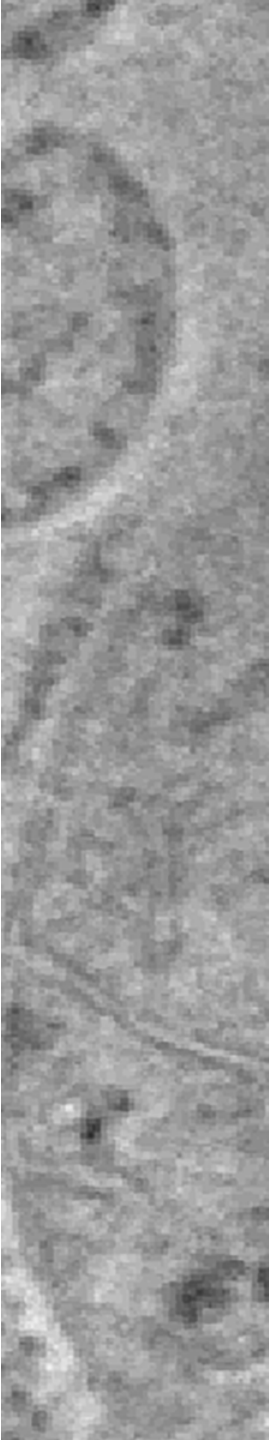
- Elastic scattering
- Inelastic scattering

Cross-section: elastic  $\sim Z^{3/2}$   
inelastic  $\sim Z^{1/2}$

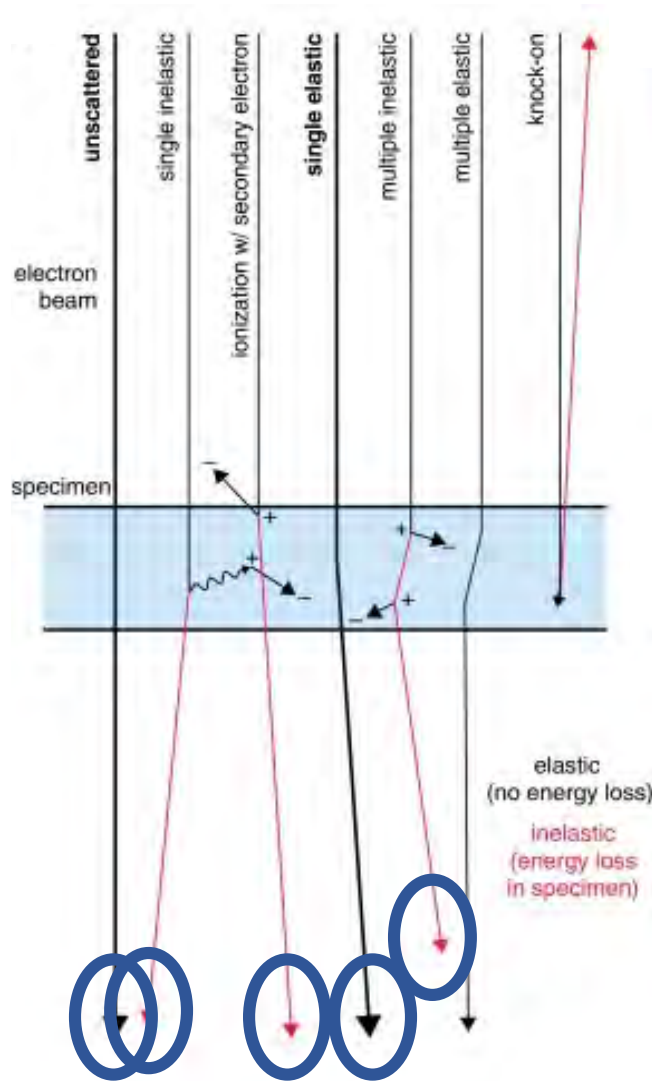
Ratio inelastic/elastic  $\sim 20/Z$

Elastic mean free path in vitrified water for 200 kV  
 $\sim 750$  nm

The “Natural” limit for cryoSTEM  
Tomography



## CryoSTEM: Efficient use of electrons

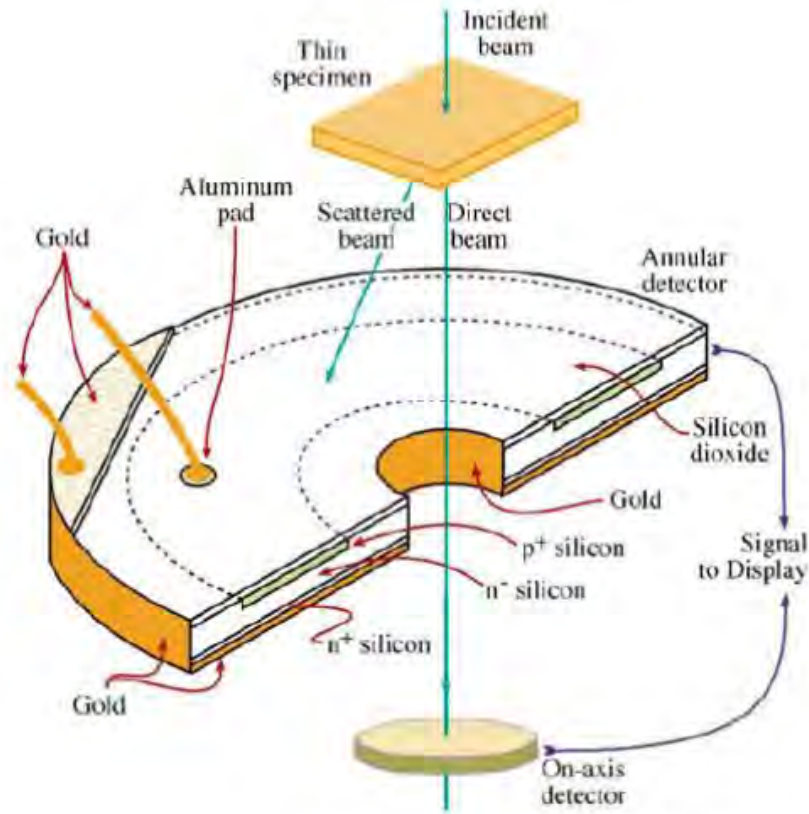


CryoSTEM relies on *Amplitude Contrast* which  
requires:

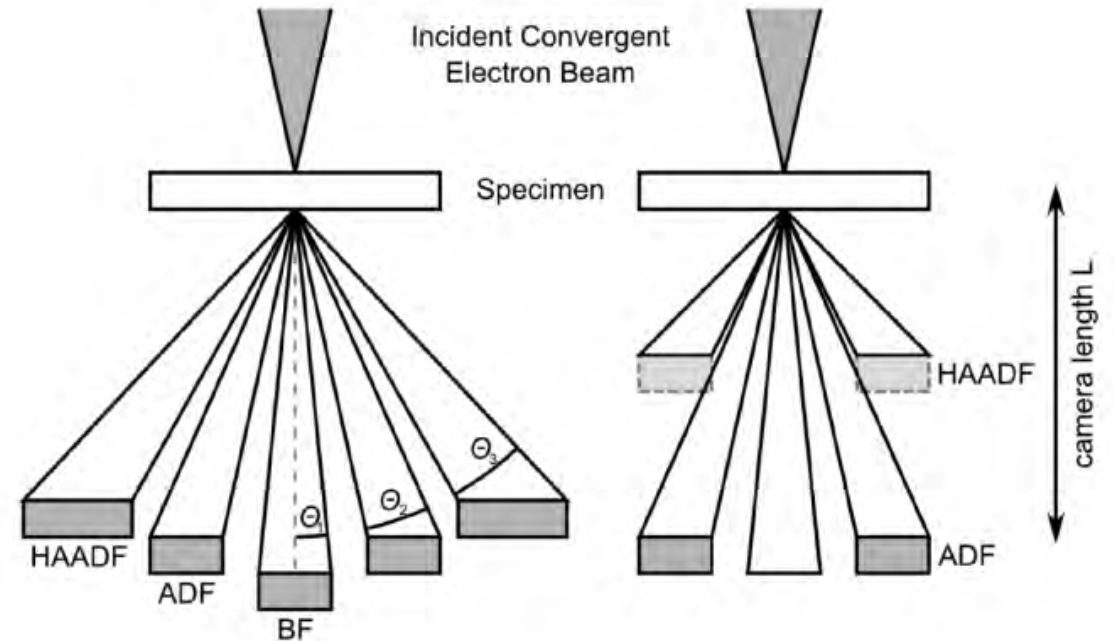
*INELASTIC or ELASTIC SCATTERING (but only  
one elastic scattering event)*



# STEM Detectors



<http://www.globalsino.com/EM/page4996.html>



# STEM Phase-contrast Detectors

4 quadrant (Krios)

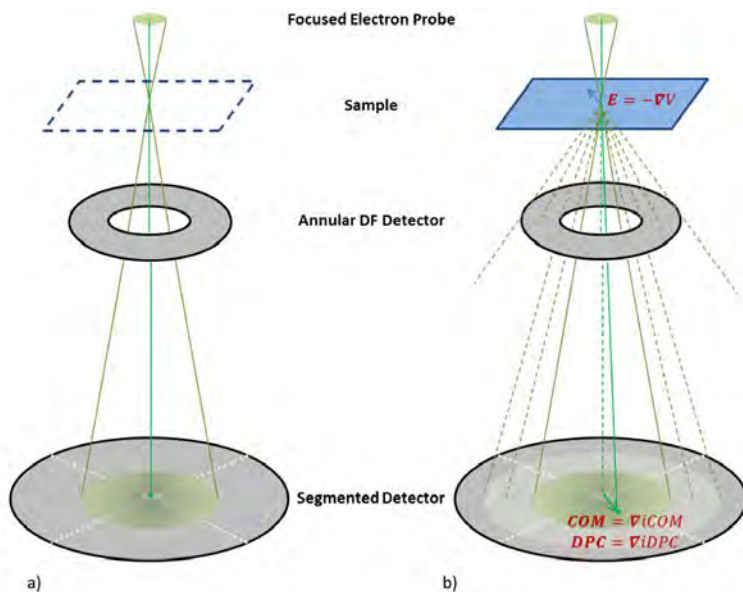
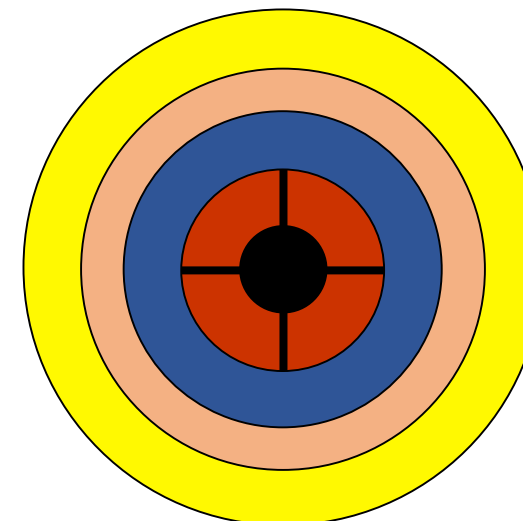


Figure 1. Schematic representation of the experimental setup. (a) Focused probe without sample. (b) Focused probe with a sample.

“integrated Differential Phase Contrast (iDPC)  
Yücelen et al, Sci Rep 2017

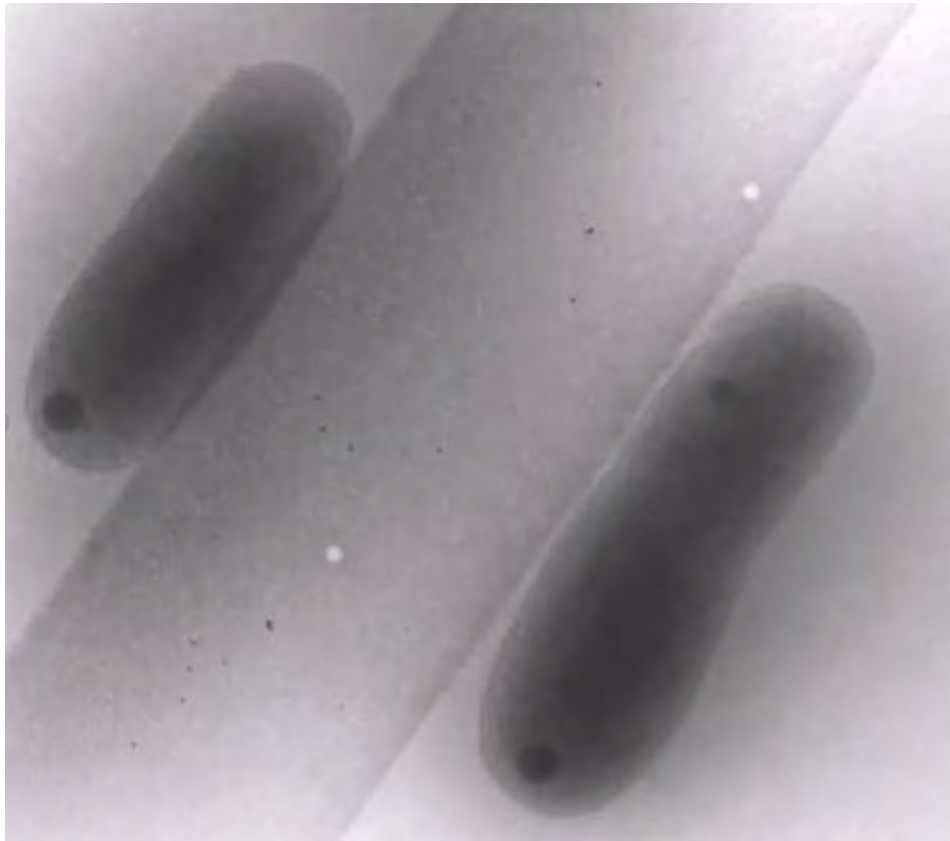
4-quadrant and annular rings (F20)



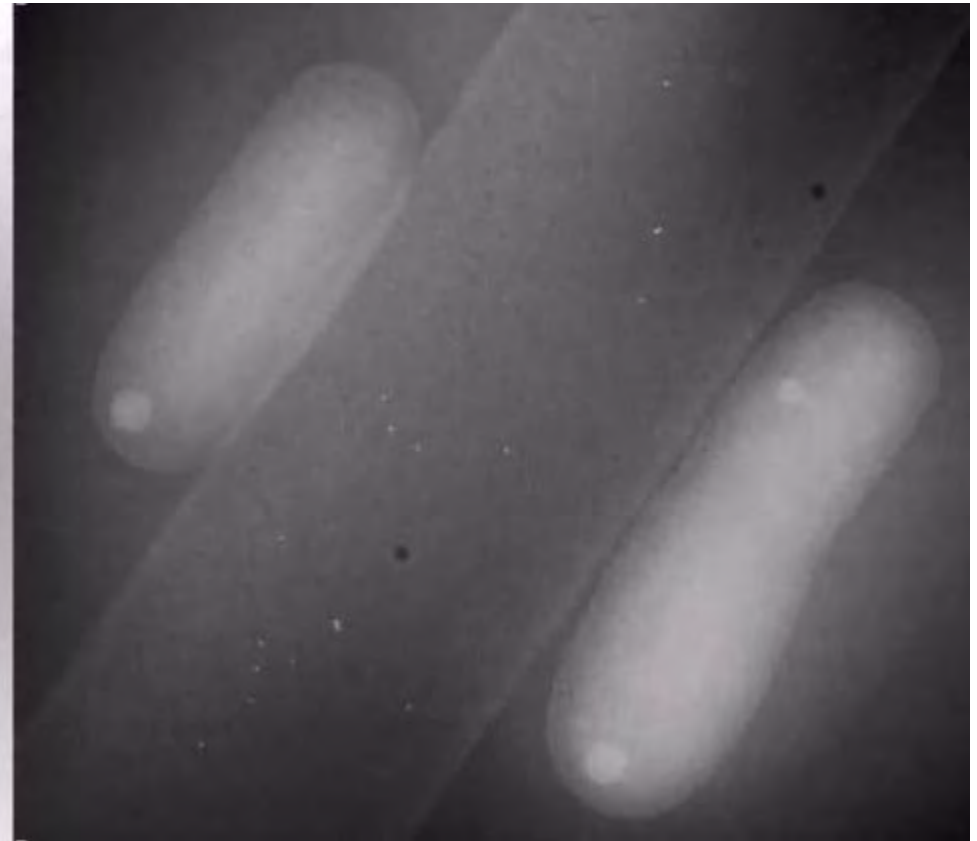
Under development  
Michael Elbaum, Lothar Houben, El Mul company

**Simultaneous BF and DF data collection.  
Dynamic Focus  
Agrobacteria**

bright field



dark field

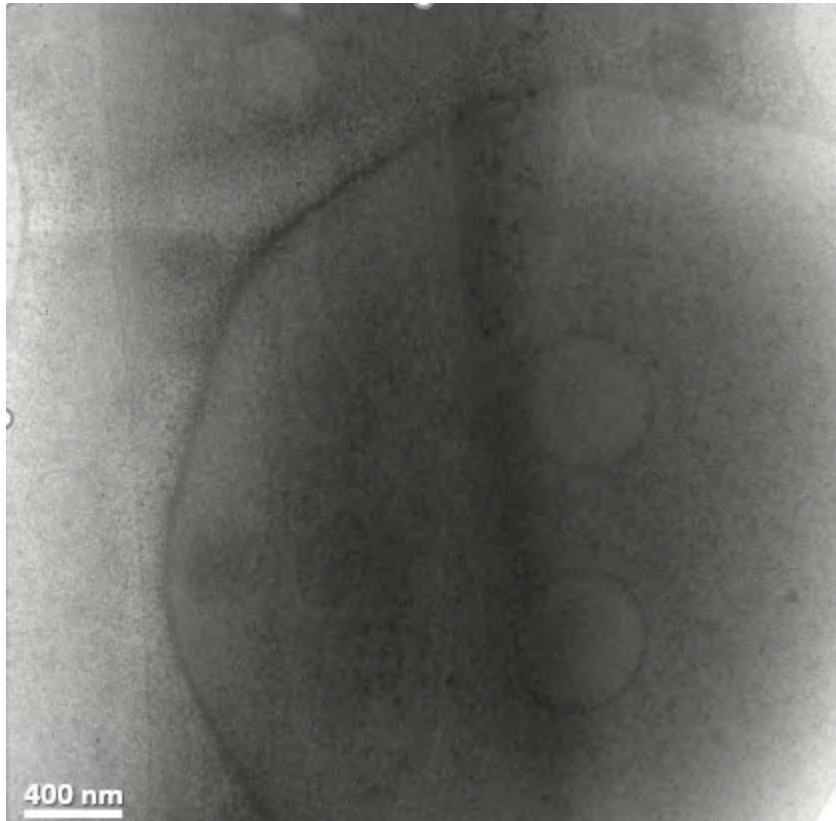




# Human WI-38 fibroblasts, ECM, 750 nm thick

Deborah Fass, Michael Elbaum

CSTET BF tilt series



CSTET BF reconstruction, 30 nm thick slices

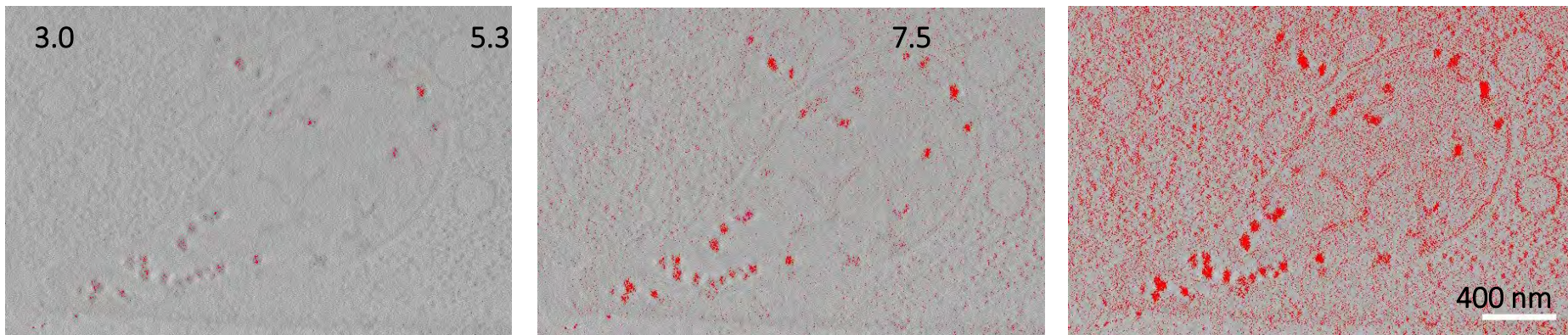
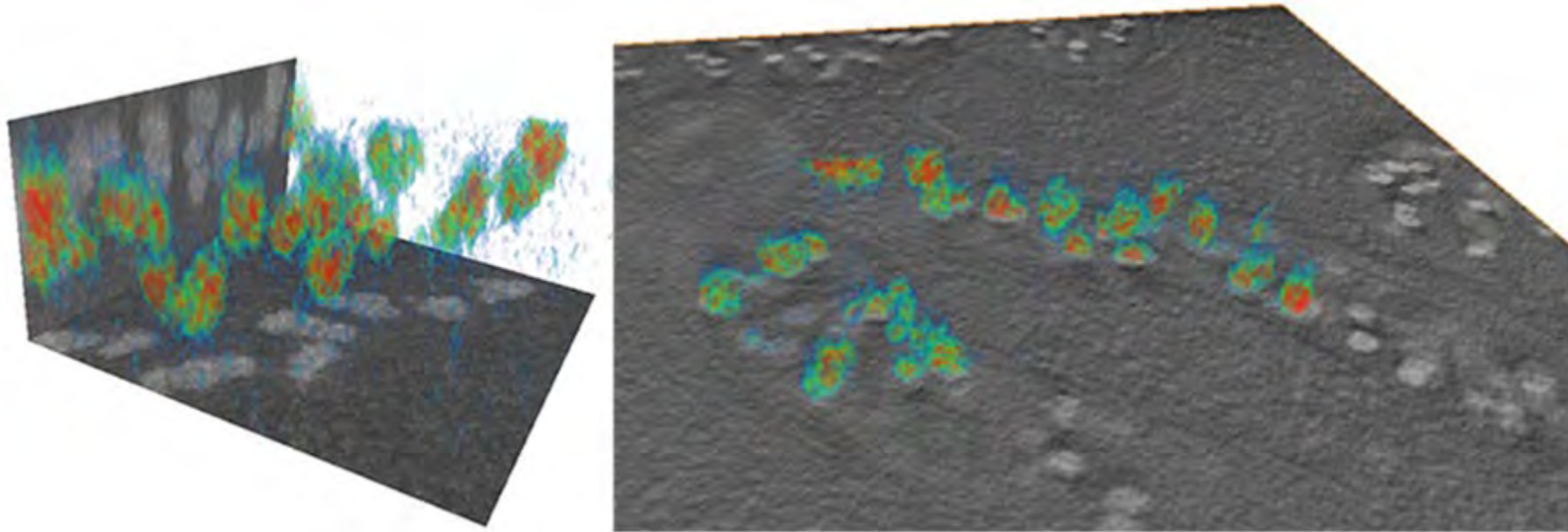


$-65^\circ \rightarrow +70^\circ$ , every  $2^\circ$ , every  $1.5^\circ$  above  $50^\circ$



# Granule Heterogeneity

Typical density 1.0 to 1.3 gm/cm<sup>3</sup> (ACP = 2.0)

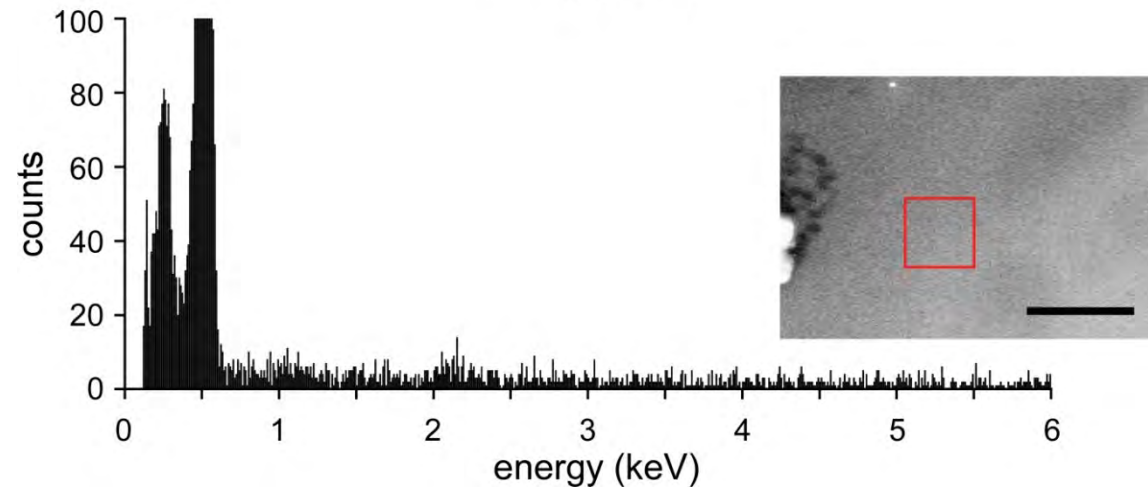
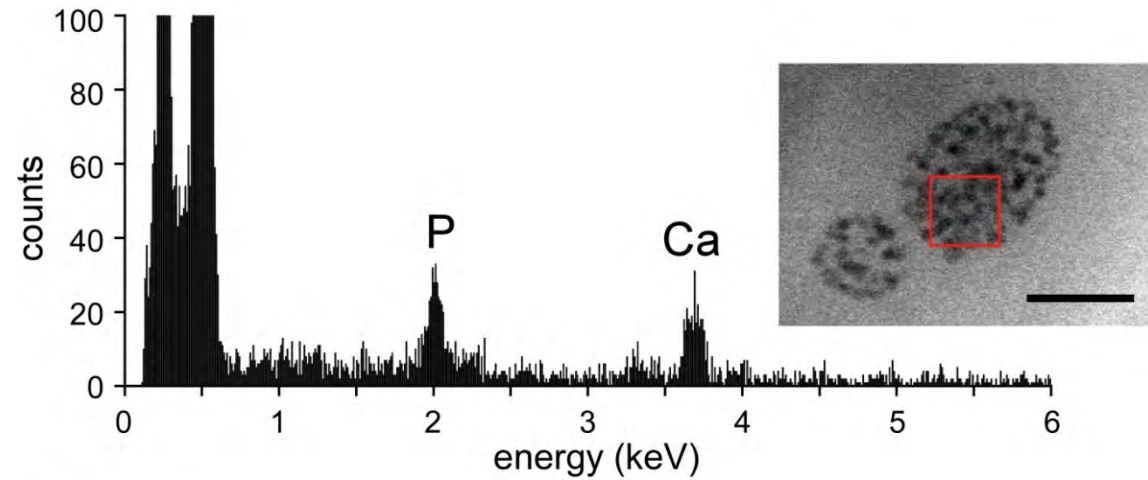
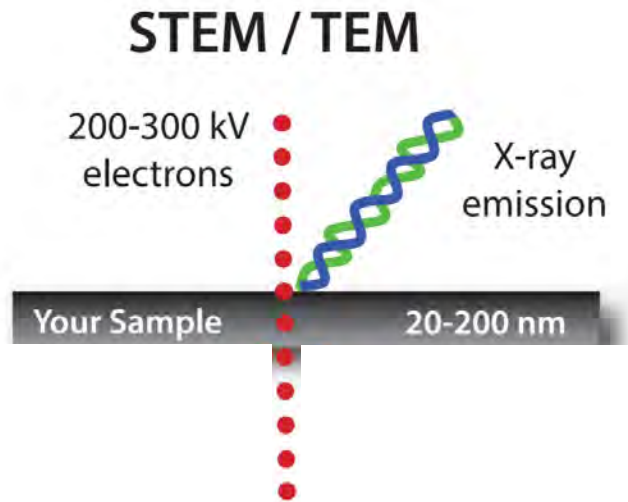


Dense parts of granules

Densest regions of ribosomes

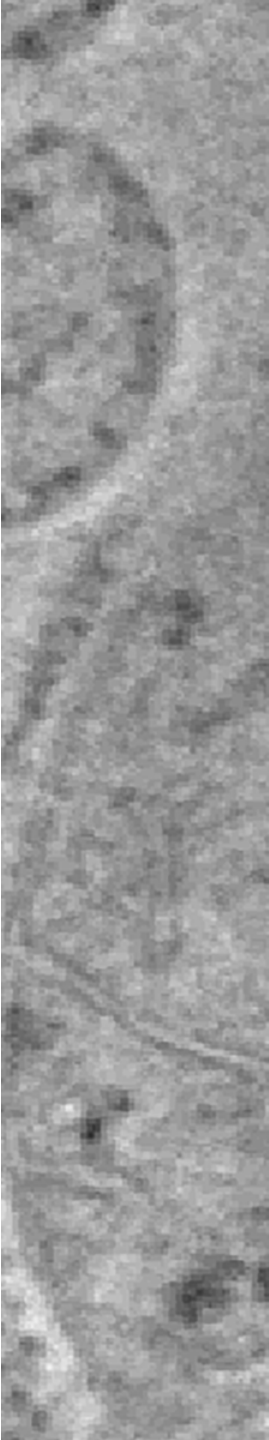
All ribosomal volumes

# EDX shows Ca, P





# Microscopes – which one to use?



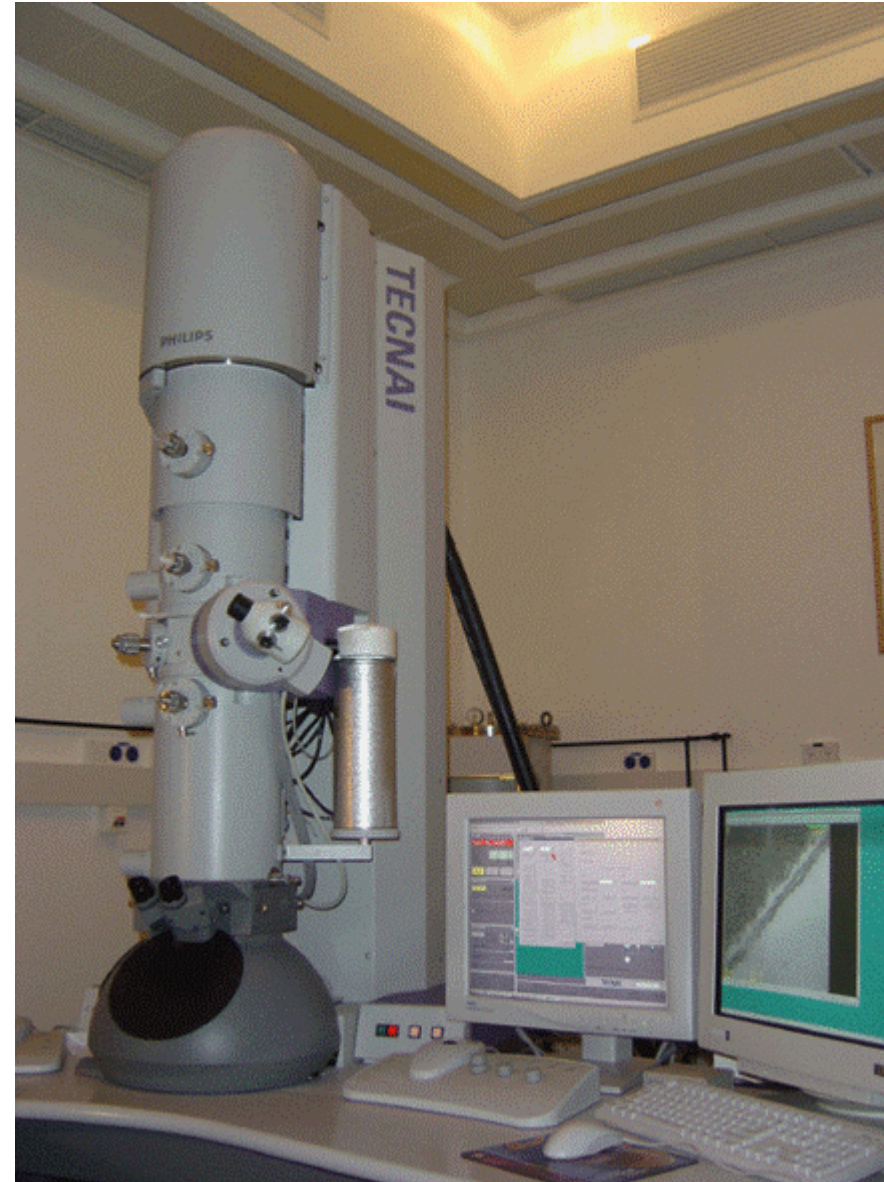
# Tecnai T12

## Good:

- LaB6 source
- Decent vacuum system
- Very sensitive CMOS camera (TVIPS)
- Cryo-blades

## Bad:

- LaB6 source
- Only 100 kV

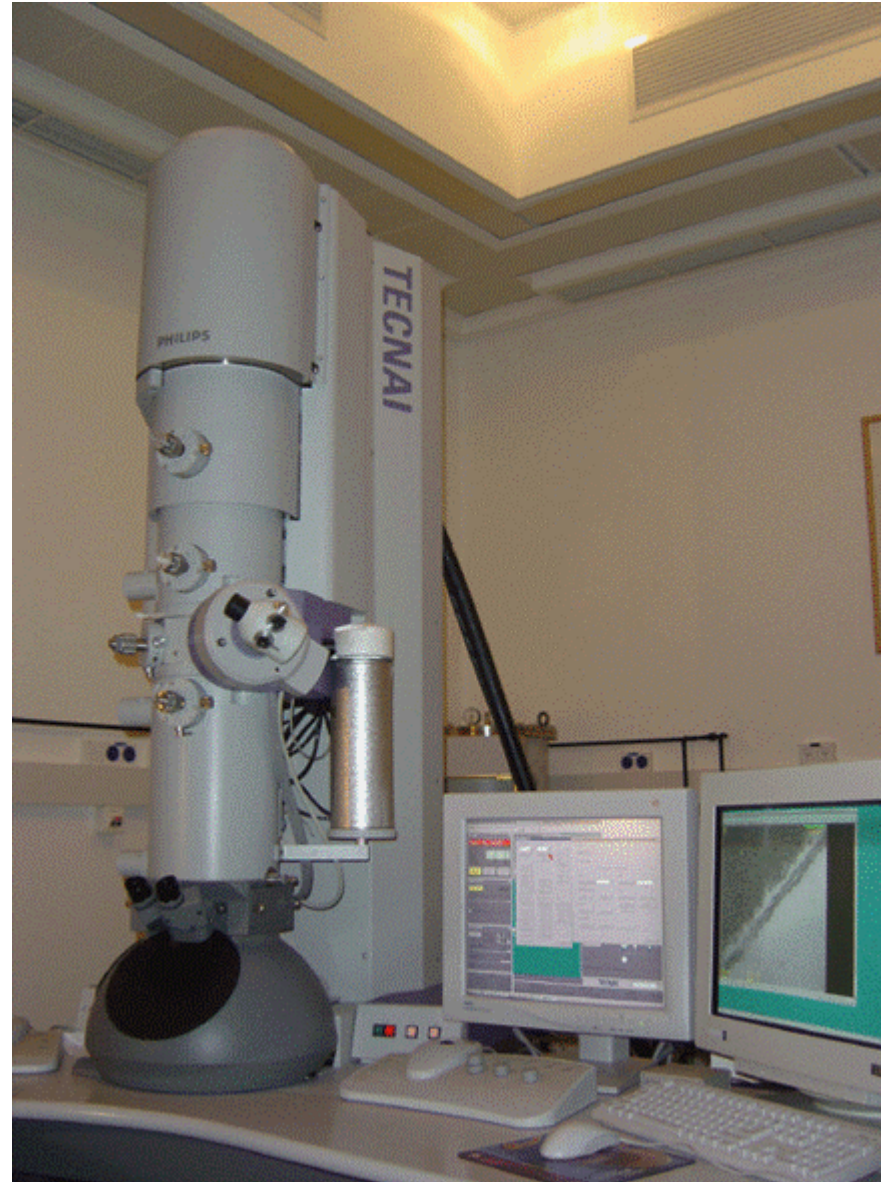




# Tecnai T12

In practice:

Used for screening and high quality TEM imaging of cryo-samples, negative stain samples, general biological TEM





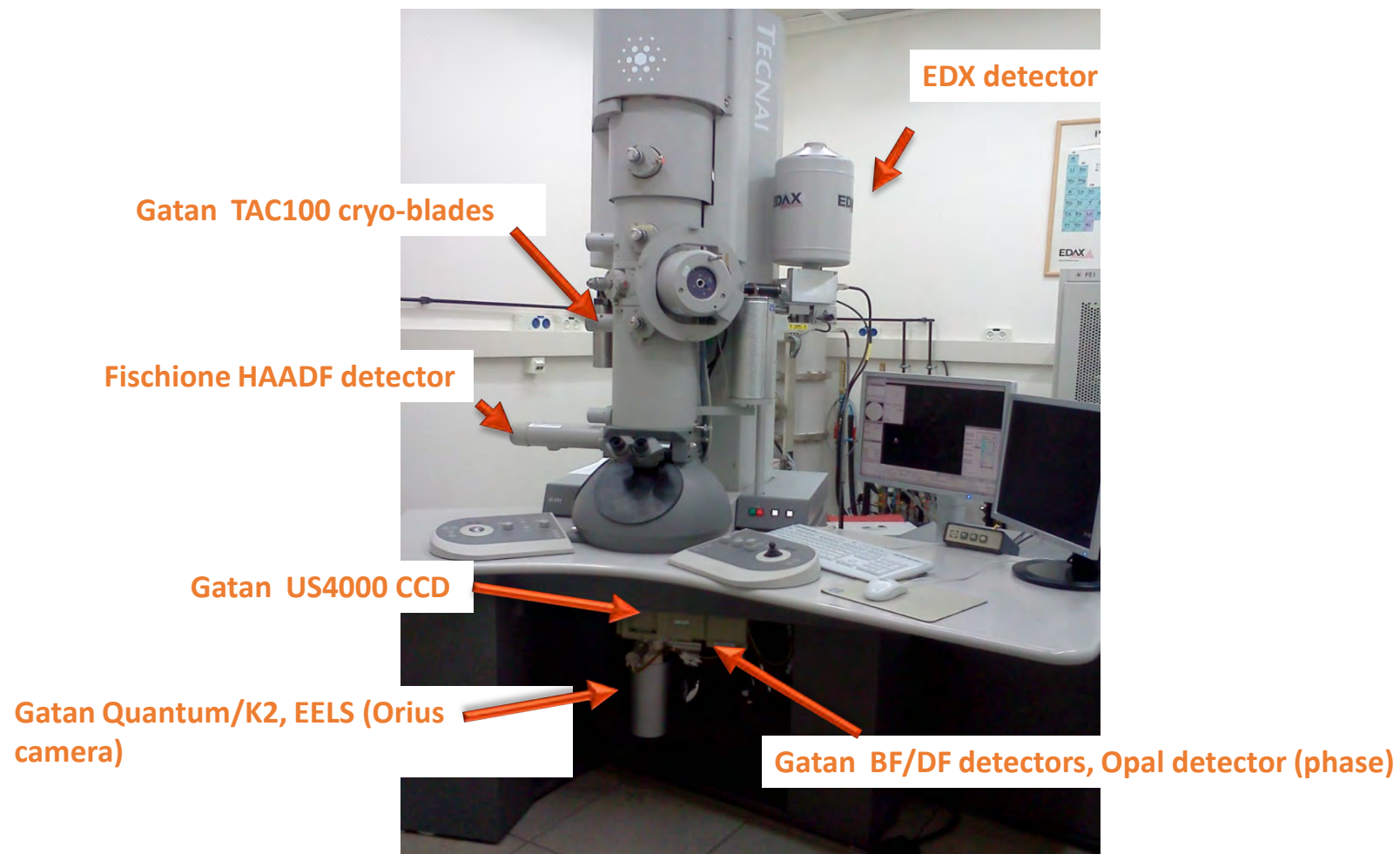
# Tecnai F20

## Good:

- FEG source
- Decent vacuum system
- Direct Electron Detector for TEM
- Easy Switching from TEM to STEM
- Multiple STEM detectors
- Stable optics
- 200 kV
- Energy filter for tomography

## Bad:

- Side entry holder
- Cryoblades and vacuum system not adequate enough for long-term cryo-sessions
- No convenient automated liquid N2 filling system
- Complicated interfaces with multiple computers
- Communication issues

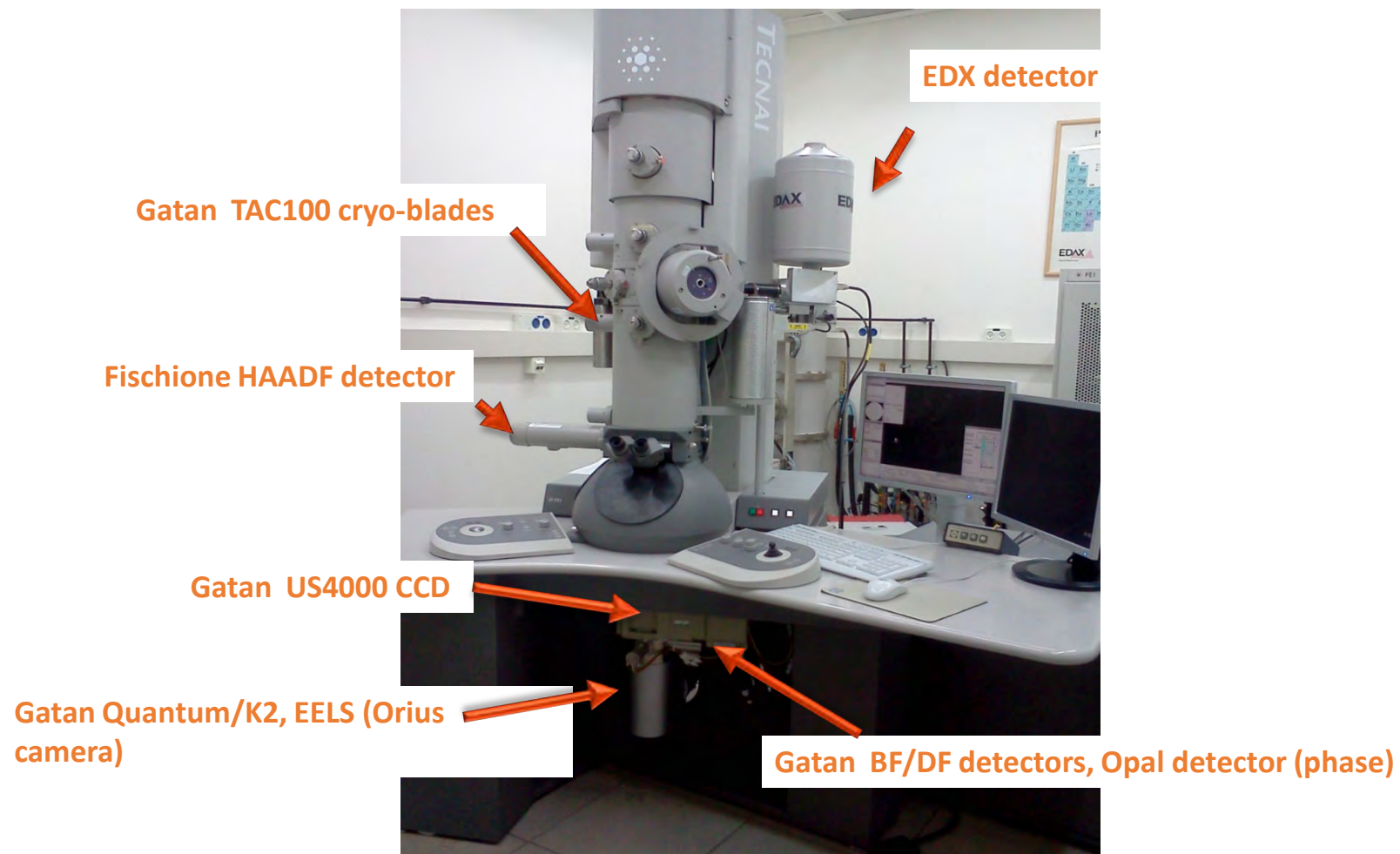


# Tecnai F20

In practice:

Not usually used for data collection for cryo-samples.

Used for data collection for plastic sections, TEM and STEM.





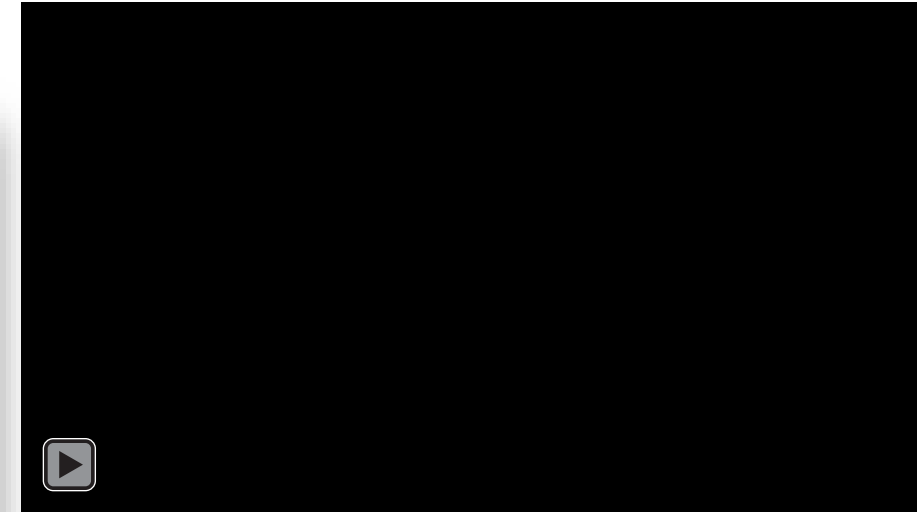


## Talos Arctica

- FEG
- Stable Optics
- 2-condenser lens system
- 200 kV
- Gatan CMOS camera (Oneview)
- STEM imaging



## Adjacent operator room



## Titan Krios

- FEG
- Super Stable Optics – power lenses
- 3-condenser lens system
- 300 kV
- Two DED's (Falcon III and GIF/K3)
- Energy Filter (with K3)
- STEM imaging



# Data Collection - Considerations

What total dose? Resolution vs signal-to-noise

What tilt range? Completeness of information vs dose and speed

What angular increment? Resolution vs dose and speed

What order to collect the images? Speed, reliability, optimal dose, sample distortion...

What magnification? Resolution and DQE vs field of view

What defocus? High-frequency information vs low frequency information

# What Magnification?

## The Shannon Theorem

If a function  $x(t)$  contains no frequencies higher than  $B$  [hertz](#), it is completely determined by giving its ordinates at a series of points spaced  $1/(2B)$  seconds apart.

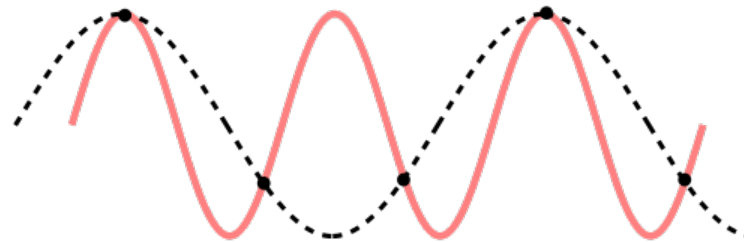
## The Nyquist Limit

$$B \leq \frac{f(s)}{2}$$

$f(s)$  is the sampling rate

### Example of undersampling:

Two different sine waves can accurately describe the sampled data. So data is not adequately sampled for this frequency.



## How much do we have to sample to get a desired resolution?

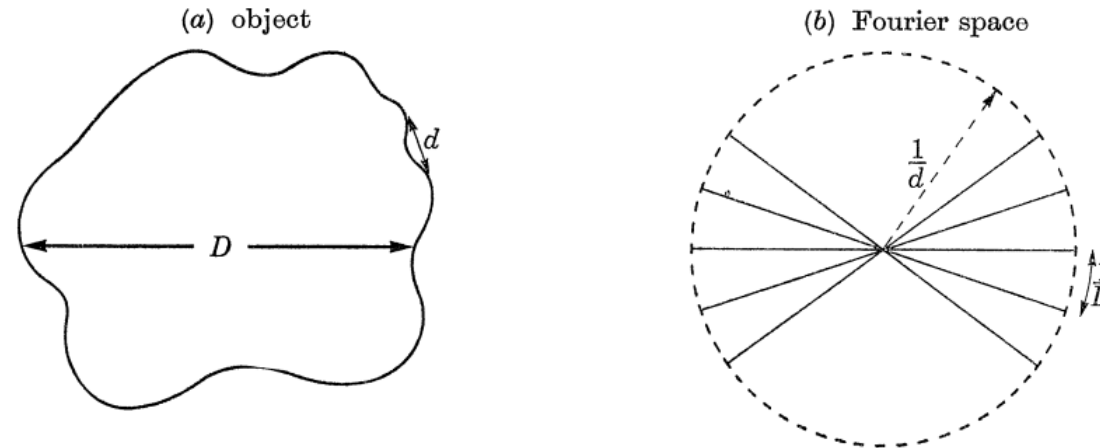


FIGURE 1. (a) An asymmetric particle of maximum diameter  $D$  containing features on a scale  $d$ . (b) Two-dimensional illustration of the 'filling-up' of Fourier space by central sections. Data must be collected to a radius of  $1/d$  if the reconstruction is to show detail to a resolution  $d$ . The angular separation is decided as follows. The Fourier transform of a particle of diameter  $D$  does not, on the average, change appreciably over a distance  $1/D$  in a Fourier space. There will thus be a sufficient number of sections if their intersections with a circle of radius  $1/d$  are spaced at intervals at least as small as  $1/D$ . The minimum number of views,  $n$ , is therefore equal to  $n = \pi(1/D)/(1/d) = \pi D/d$ . For a particle of diameter 20 nm, to be solved to a resolution of 2 nm,  $n \approx 30$ . The value of  $n$  will in practice need to be greater since the calculation assumes that all the data can be collected by a set of tilts about a single axis covering a range of  $\pm 90^\circ$ . This is impossible in practice. Limited ranges of tilts about more than one axis will have to be employed, and the strategy of data collection can be worked out by the methods given by Crowther, DeRosier & Klug (1970).

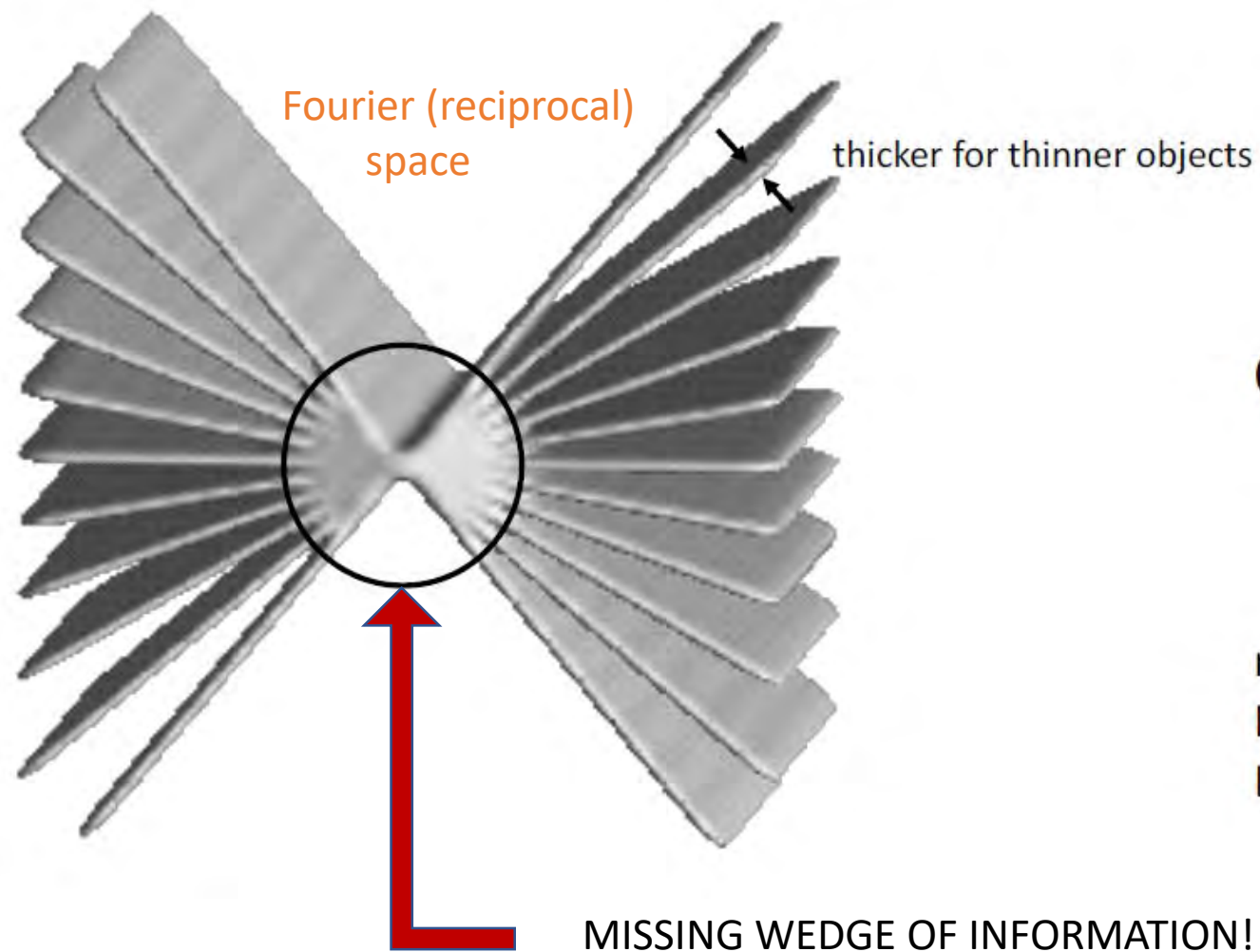
Optical Diffraction and Filtering and Three-Dimensional Reconstructions from Electron Micrographs

Author(s): A. Klug

Source: *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, Vol. 261, No. 837, A Discussion on New Developments in Electron Microscopy with Special Emphasis on their Application in Biology, (May 27, 1971), pp. 173-179



# How much do we have to sample to get a desired resolution?



## Crowther Criterion

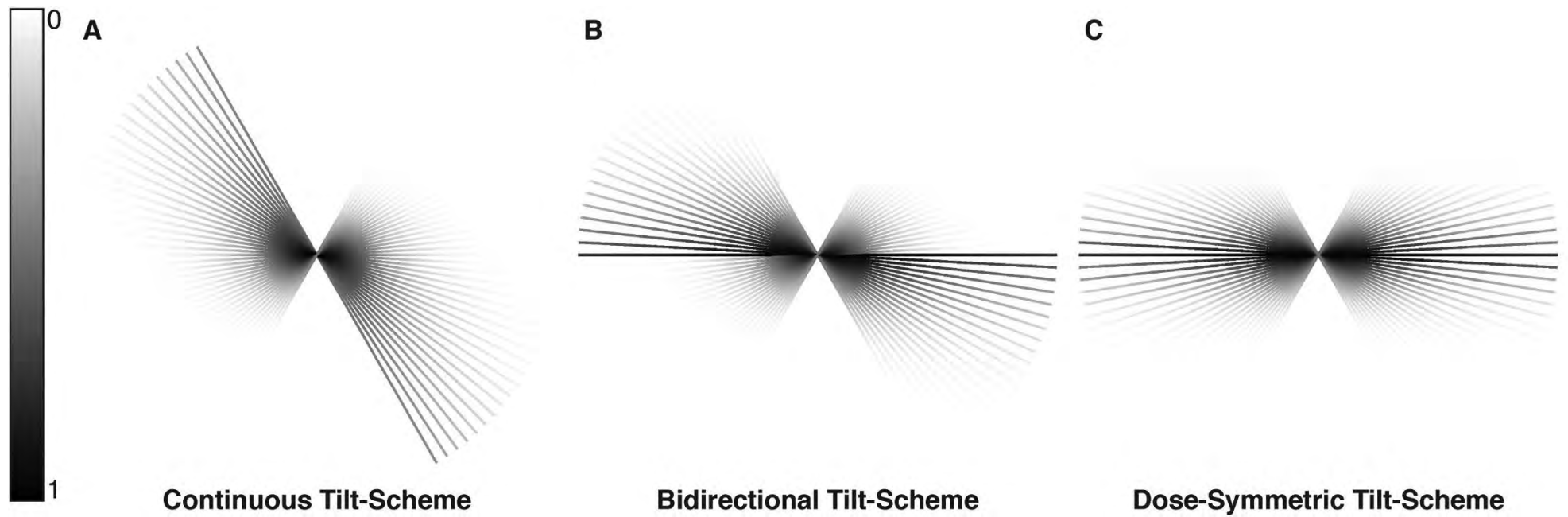
$$r = \pi D / N$$

r: Resolution limit

N: Number of projections

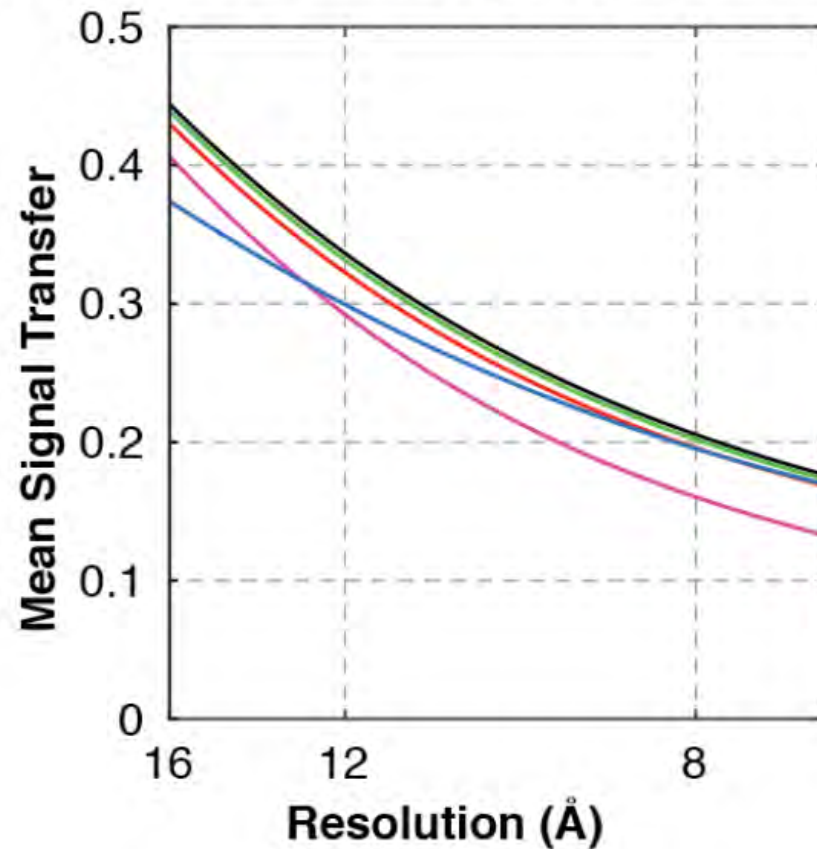
D: Object thickness

# Tilt Schemes



Hagen WJH, Wan W and Briggs JAG, J. Struct Biol. 197(2), 191-198 (2017)

# Tilt Schemes – signal transfer



Continuous

Bidirectional from 0

Bidirectional from -21

Bidirectional from -21  
deleting second branch

Dose-symmetric



# Data Collection - Considerations

	Strategy to increase contrast	Strategy to increase resolution	Strategy to deal with thick samples
Voltage	Low  (200 keV) as  thickness  allows	High  (300 keV)	High  (300 keV)
Total electron dose	High (>100e-/A <sup>2</sup> )	Low (<50e-/A <sup>2</sup> )	High (>100e-/A <sup>2</sup> )
Defocus	High (-5um to -15um)	Low (-2um to -4um)	High (-5um to -15um)
Magnification	Low (more e- per pixel)	High (pixel size half Nyquist)	Low (more e- per pixel)
Tilt range	Low  (maximise e- dose per projection image)	Higher  (decrease missing wedge)	Low  (extreme tilts will be even thicker)

	Strategy to increase contrast	Strategy to increase resolution	Strategy to deal with thick samples
Tilt increment	Large  (more e- per image)	Small  (sample fine details but not important for subtomogram averaging)	Large  (facilitates higher SNR, enabling alignment of tilt series)
Tilt scheme	Not relevant	Dose symmetric	Dose symmetric
Phase plates	Always use  if possible	<del>Always use</del> <del>if possible</del>	Always use  if possible
Energy filter	Always use  if possible	Always use  if possible	Always use  if possible

Imaging mode: STEM

TEM

STEM

# TFS Tomography Interface - ATLAS



The screenshot displays the TFS Tomography Interface - ATLAS software. The main window shows a grid of images with a red crosshair in the center. A context menu is open over the grid, listing options: Zoom 1:1 (Ctrl+Home), Zoom to fit (Home), Export image (Ctrl+S), Export image with overlay (Ctrl+L), and Move stage here. The interface includes a top menu bar with tabs for Preparation (STEM), Atlas, Auto Functions, Tomography Calibrations, Tomography, and Visualizer. On the left, there are panels for Acquisition (with Stop, Acquire, Pause, Resume, and Configuration buttons) and Tasks (with Session Setup and Atlas Acquisition sub-sections). On the right, there are panels for Messages (0 Errors, 0 Notifications), Status (Moving stage to -304.230 µm, -55.607 µm... Ready!), Image Histogram (with a graph and an 'Enable auto update' checkbox), and Image Information (5.00 µm, Image size: 4096, 4096, Pixel size: 436.904582 nm, Statistics: Min=0.00, Max=34009.00, Mean = 24047.6).



# TFS Tomography Interface

## Presets – Search/Template



IMAGING LIFE  
FROM MOLECULES  
TO CELLS

Resolution: 1024 x 1024  
Dwell Time (µs): 4.00  
Est. Frame Time (s): 5.97  
NanoProbe [BF]  
Set: 1 (µm) 26.799

Messages: 2 Errors, 0 Notifications  
Image shift Calibration missing for specified imaging context. Magnification: 4525 (x)  
Image shift Calibration missing for specified imaging context. Magnification: 4525 (x)

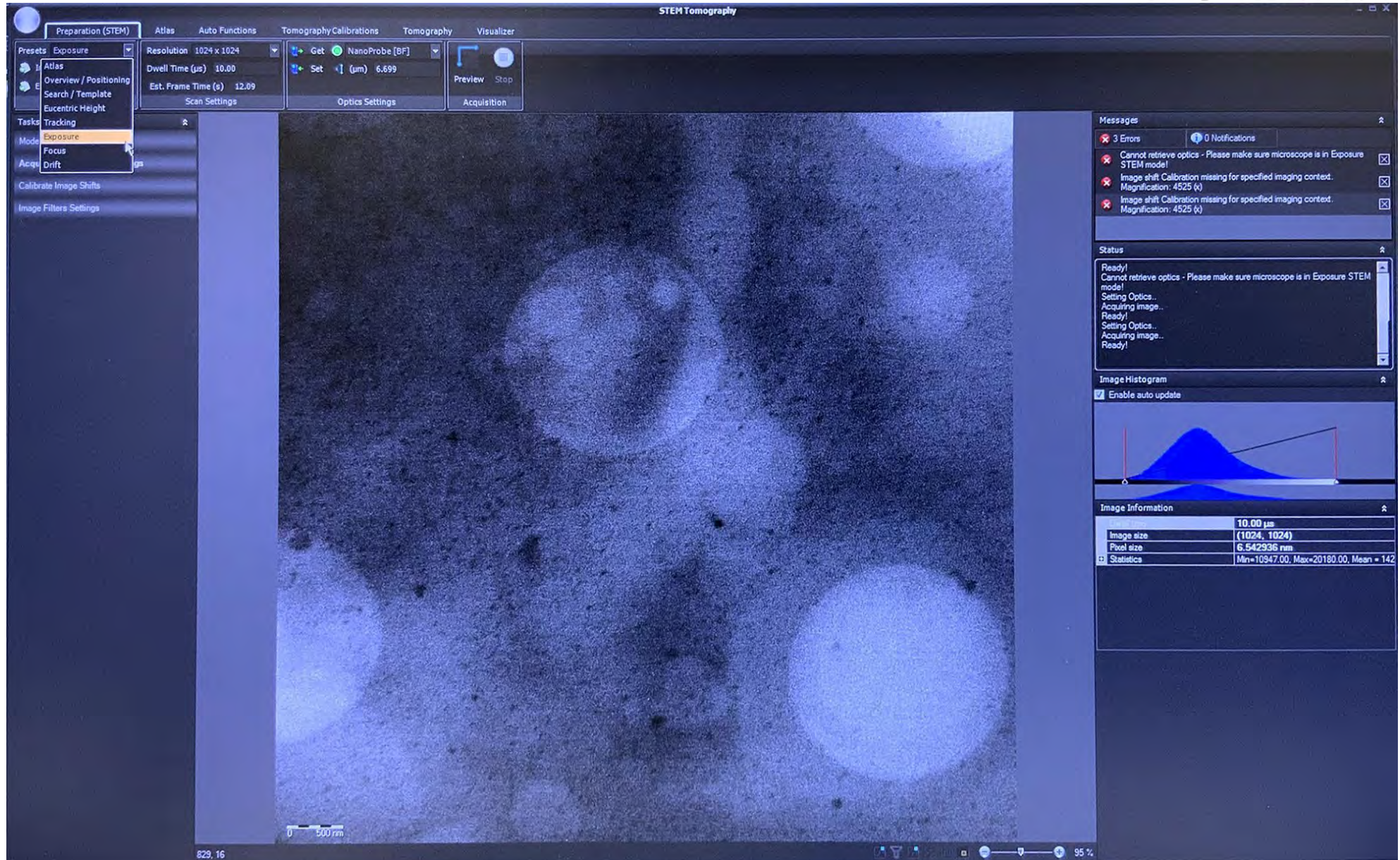
Status: 4525 (x)  
Acquiring image... Ready!  
Setting Optics... Ready!  
Acquiring image... Ready!  
Setting Optics... Ready!  
Acquiring image... Ready!

Image Histogram: Enable auto update

Image Information	
Acquire time	4.00 µs
Image size	(1024, 1024)
Pixel size	26.171744 nm
Statistics	Min=6470.00, Max=22051.00, Mean = 1553



# TFS Tomography Interface Presets – Exposure



The screenshot displays the TFS Tomography Interface with the 'Preparation (STEM)' tab selected. The 'Presets' dropdown menu is open, showing 'Exposure' as the selected option. The interface includes several panels:

- Resolution:** 1024 x 1024
- Dwell Time (µs):** 10.00
- Est. Frame Time (s):** 12.09
- Get:** NanoProbe [BF]
- Set:** (µm) 6.699

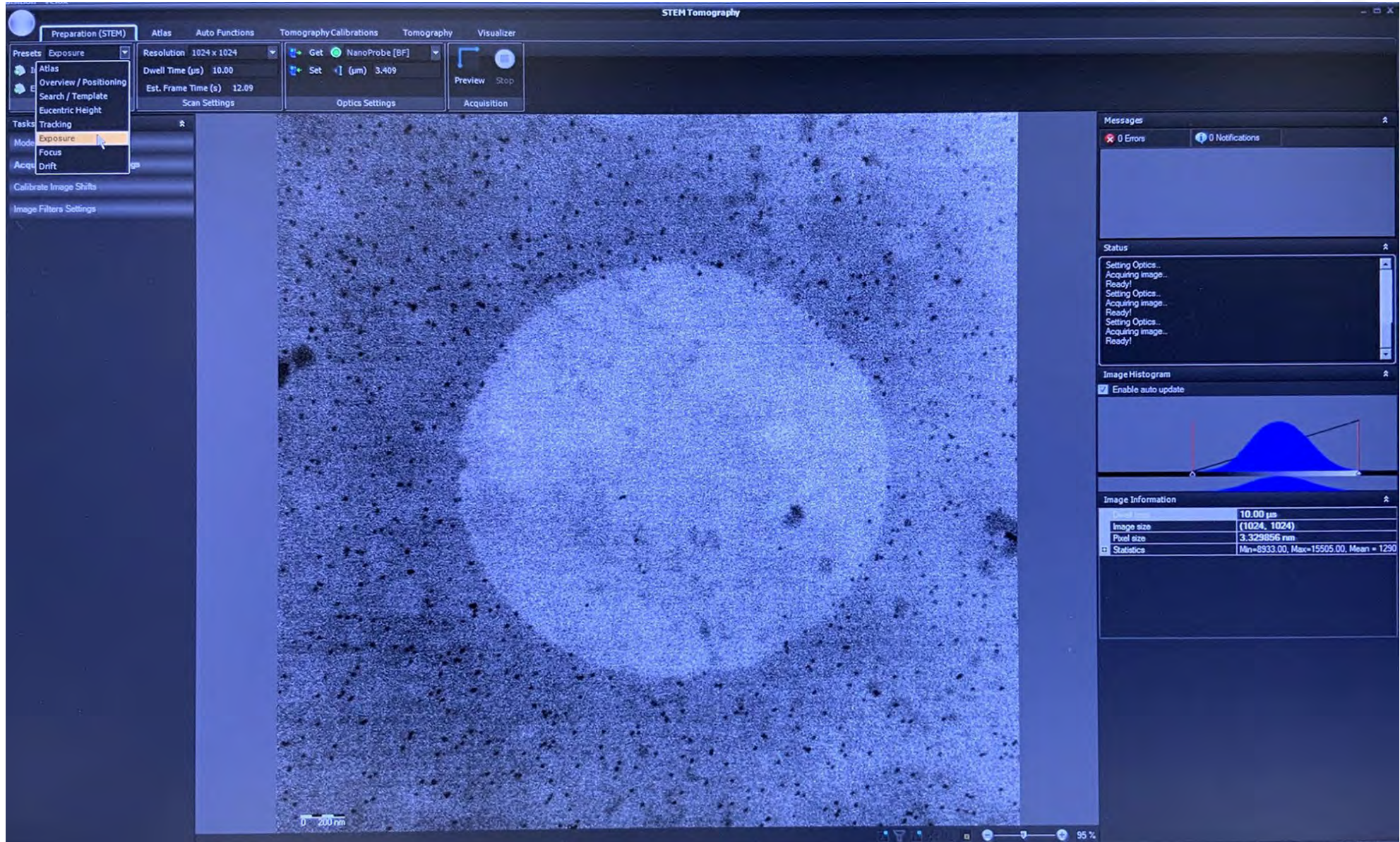
The central area shows a grayscale image of a cell with a 500 nm scale bar at the bottom. The right sidebar contains:

- Messages:** 3 Errors, 0 Notifications. Error messages include: 'Cannot retrieve optics - Please make sure microscope is in Exposure STEM mode!', 'Image shift Calibration missing for specified imaging context. Magnification: 4525 (x)', and 'Image shift Calibration missing for specified imaging context. Magnification: 4525 (x)'.
- Status:** Ready! Cannot retrieve optics - Please make sure microscope is in Exposure STEM mode! Setting Optics... Acquiring image... Ready! Setting Optics... Acquiring image... Ready!
- Image Histogram:** Enable auto update checked. A histogram plot is shown below.
- Image Information:**

Exposure	10.00 µs
Image size	(1024, 1024)
Pixel size	6.542936 nm
Statistics	Min=10947.00, Max=20180.00, Mean = 142



# TFS Tomography Interface Presets – Exposure



The screenshot displays the TFS Tomography software interface. The main window is titled "STEM Tomography" and features a top navigation bar with tabs: Preparation (STEM), Atlas, Auto Functions, Tomography Calibrations, Tomography, and Visualizer. The "Preparation (STEM)" tab is active, showing a "Presets" dropdown menu with "Exposure" selected. Other menu items include "Atlas", "Overview / Positioning", "Search / Template", "Eucentric Height", "Tasks", "Tracking", "Mode", "Exposure", "Focus", "Acq", "Drift", "Calibrate Image Shifts", and "Image Filters Settings".

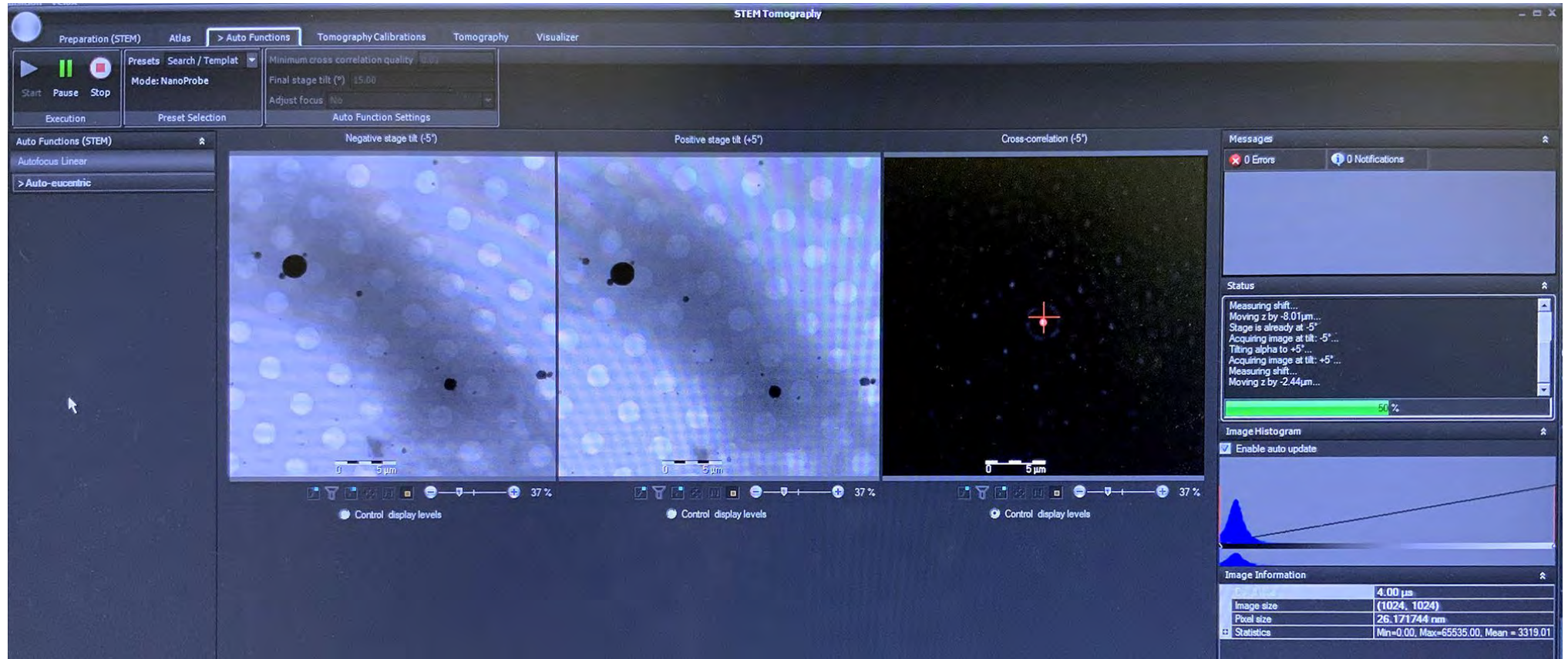
The central area shows a large, blue-tinted micrograph of a biological specimen. A scale bar at the bottom left indicates 200 nm. The right side of the interface contains several panels:

- Messages:** Shows 0 Errors and 0 Notifications.
- Status:** Displays a sequence of status messages: "Setting Optics...", "Acquiring image...", "Ready!", "Setting Optics...", "Acquiring image...", "Ready!", "Setting Optics...", "Acquiring image...", "Ready!".
- Image Histogram:** Includes a checkbox for "Enable auto update" and a histogram plot showing the distribution of pixel intensities.
- Image Information:** A table providing technical details:

Property	Value
Exposure time	10.00 $\mu$ s
Image size	(1024, 1024)
Pixel size	3.329856 nm
Statistics	Min=8933.00, Max=15505.00, Mean = 1290



# TFS Tomography Interface    Autofxns – Eucentric height



STEM Tomography

Preparation (STEM)    Atlas    > Auto Functions    Tomography Calibrations    Tomography    Visualizer

Start    Pause    Stop

Execution    Preset Selection    Auto Function Settings

Presets    Search / Templat

Mode: NanoProbe

Minimum cross correlation quality 0.97

Final stage tilt (°) 15.00

Adjust focus No

Auto Functions (STEM)

Autofocus Linear

> Auto-eucentric

Negative stage tilt (-5°)    Positive stage tilt (+5°)    Cross-correlation (-5°)

0 5 μm    0 5 μm    0 5 μm

Control display levels    Control display levels    Control display levels

37%    37%    37%

Messages

0 Errors    0 Notifications

Status

Measuring shift...  
Moving z by -8.01 μm...  
Stage is already at -5°...  
Acquiring image at tilt: -5°...  
Tilting alpha to +5°...  
Acquiring image at tilt: +5°...  
Measuring shift...  
Moving z by -2.44 μm...

50%

Image Histogram

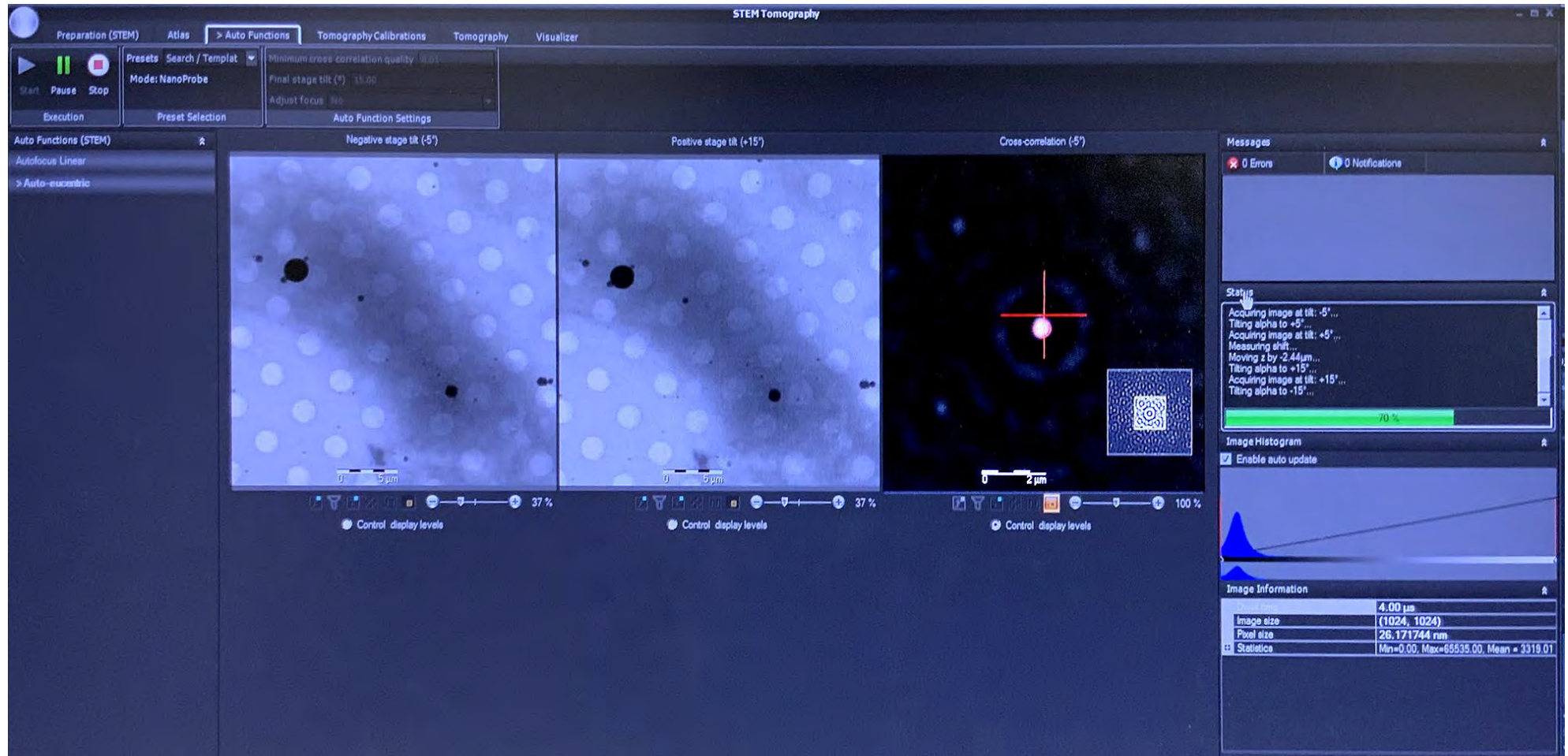
Enable auto update

Image Information

Exposure	4.00 μs
Image size	(1024, 1024)
Pixel size	26.171744 nm
Statistics	Min=0.00, Max=65535.00, Mean = 3319.01



# TFS Tomography Interface    Autofxns – Eucentric height



STEM Tomography

Preparation (STEM)    Atlas    > Auto Functions    Tomography Calibrations    Tomography    Visualizer

Start    Pause    Stop

Presets    Search / Templat

Mode: NanoProbe

Execution    Preset Selection    Auto Function Settings

Minimum cross correlation quality: 0.01

Final stage tilt (°): 15.00

Adjust focus: No

Auto Functions (STEM)

Autofocus Linear

> Auto-eucentric

Negative stage tilt (-5°)    Positive stage tilt (+15°)    Cross-correlation (-5°)

0 5µm    0 5µm    0 2µm

Control display levels    Control display levels    Control display levels

37%    37%    100%

Messages

0 Errors    0 Notifications

Status

Acquiring image at tilt: -5°...  
Tilting alpha to +5°...  
Acquiring image at tilt: +5°...  
Measuring shift...  
Moving z by -2.44µm...  
Tilting alpha to +15°...  
Acquiring image at tilt: +15°...  
Tilting alpha to -15°...

70%

Image Histogram

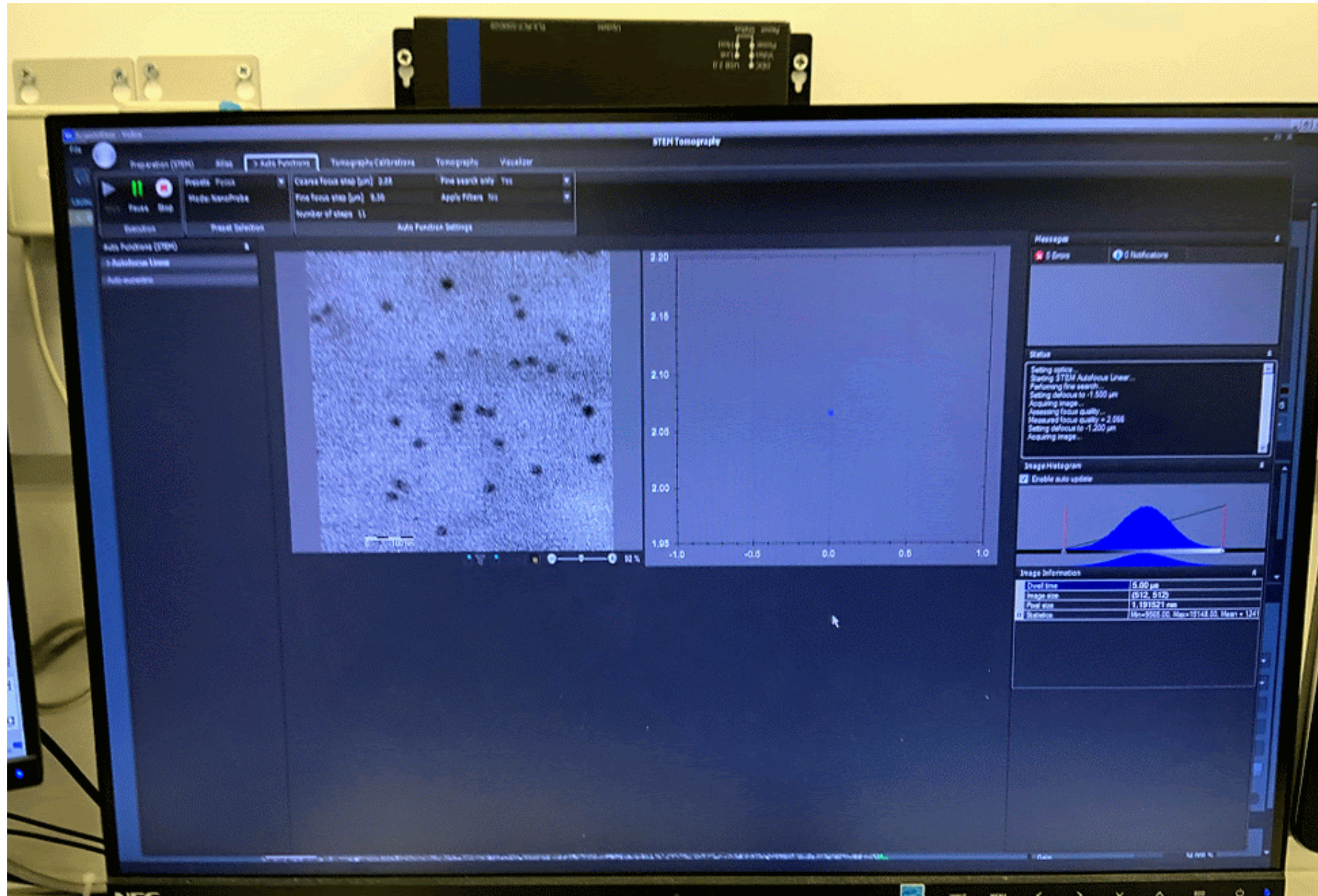
Enable auto update

Image Information

Pixel size	4.00 µm
Image size	(1024, 1024)
Pixel size	20.171744 nm
Statistics	Min=0.00, Max=65535.00, Mean = 3319.01

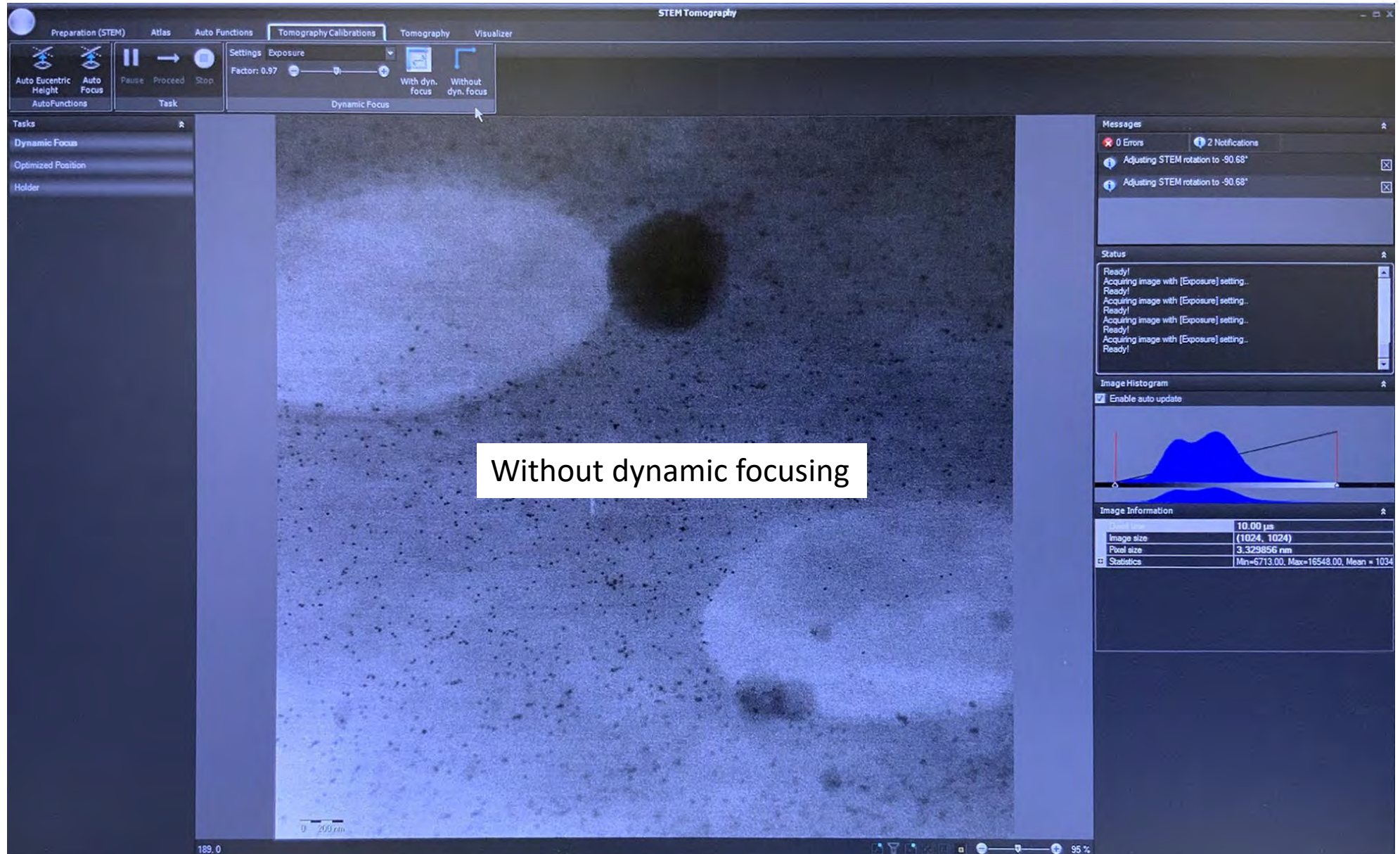


# TFS Tomography Interface    Autofxns – focusing





# TFS Tomography Interface STEM dynamic focusing



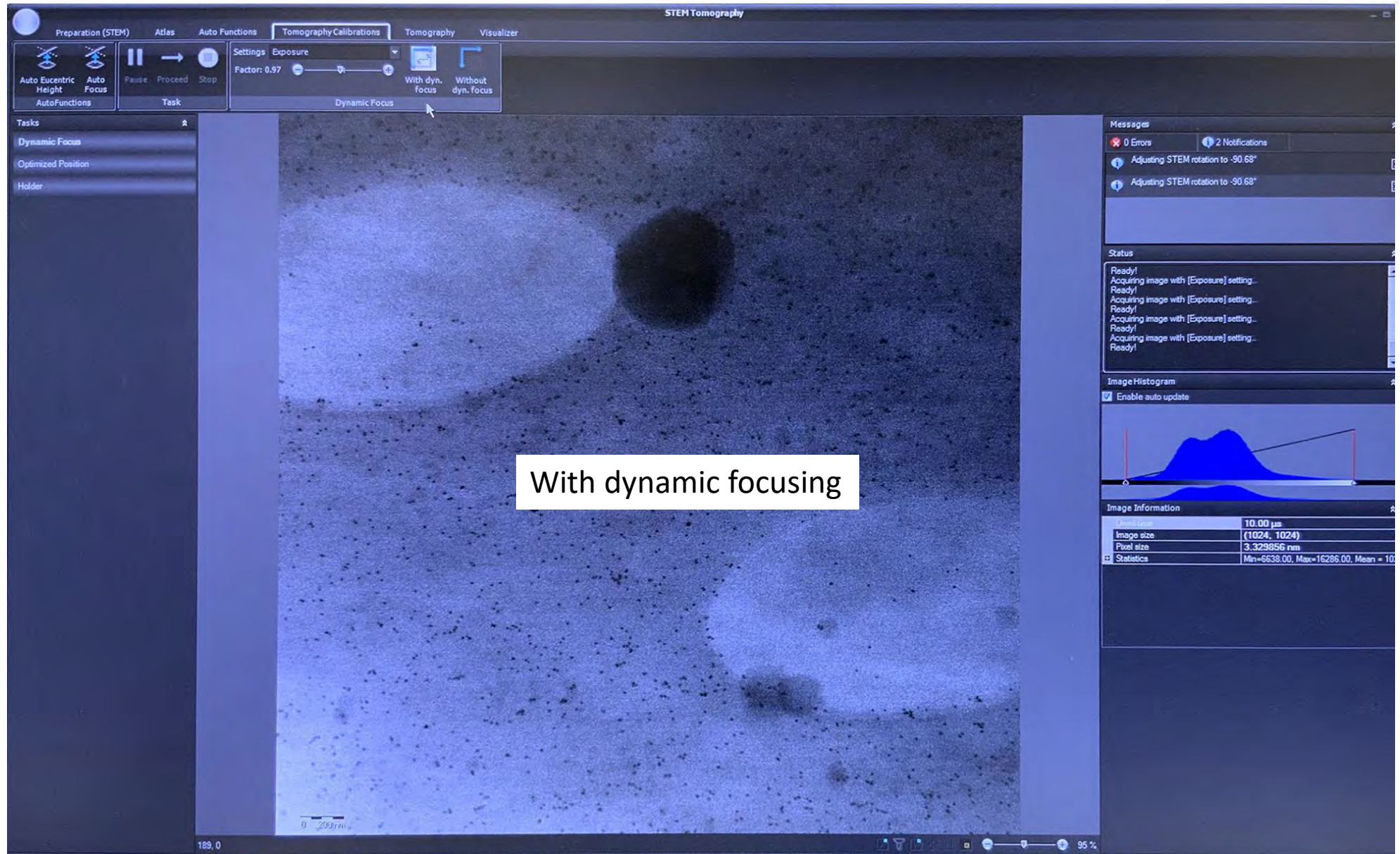
The screenshot displays the TFS Tomography Interface. The main window shows a STEM image of a biological specimen. A white text box is overlaid on the image with the text "Without dynamic focusing". The interface includes a top menu bar with tabs: Preparation (STEM), Atlas, Auto Functions, Tomography Calibrations, Tomography, and Visualizer. Below the menu bar is a control panel with buttons for "Auto Eucentric Height", "Auto Focus", "Pause", "Proceed", "Stop", "Settings", "Exposure", "Factor: 0.97", "With dyn. focus", and "Without dyn. focus". The "Without dyn. focus" button is highlighted. On the left side, there is a "Tasks" panel with "Dynamic Focus" selected. On the right side, there is a "Messages" panel showing two notifications: "Adjusting STEM rotation to -90.68°". Below the messages is a "Status" panel with a list of messages: "Ready!", "Acquiring image with [Exposure] setting...", "Ready!", "Acquiring image with [Exposure] setting...", "Ready!", "Acquiring image with [Exposure] setting...", "Ready!", "Acquiring image with [Exposure] setting...", "Ready!". Below the status panel is an "Image Histogram" panel with a checkbox for "Enable auto update" and a histogram plot. At the bottom right, there is an "Image Information" panel with a table:

Image Information	
Exposure time	10.00 $\mu$ s
Image size	(1024, 1024)
Pixel size	3.329856 nm
Statistics	Min=6713.00, Max=16548.00, Mean = 1034

The bottom status bar shows "189.0" and "95 %".



# TFS Tomography Interface STEM dynamic focusing



STEM Tomography

Preparation (STEM) Atlas Auto Functions Tomography Calibrations Tomography Visualizer

Auto Eucentric Height Auto Focus Auto Functions

Pause Proceed Stop

Settings Exposure

Factor: 0.97

Dynamic Focus

With dyn. focus Without dyn. focus

Tasks

Dynamic Focus

Optimized Position

Holder

Messages

0 Errors 2 Notifications

Adjusting STEM rotation to -90.68°

Adjusting STEM rotation to -90.68°

Status

Ready!

Acquiring image with [Exposure] setting...

Ready!

Acquiring image with [Exposure] setting...

Ready!

Acquiring image with [Exposure] setting...

Ready!

Acquiring image with [Exposure] setting...

Ready!

Image Histogram

Enable auto update

Image Information

Grid size	10.00 $\mu\text{m}$
Image size	(1024, 1024)
Pixel size	3.329856 nm
Statistics	Min=6638.00, Max=16286.00, Mean = 10...

189.0

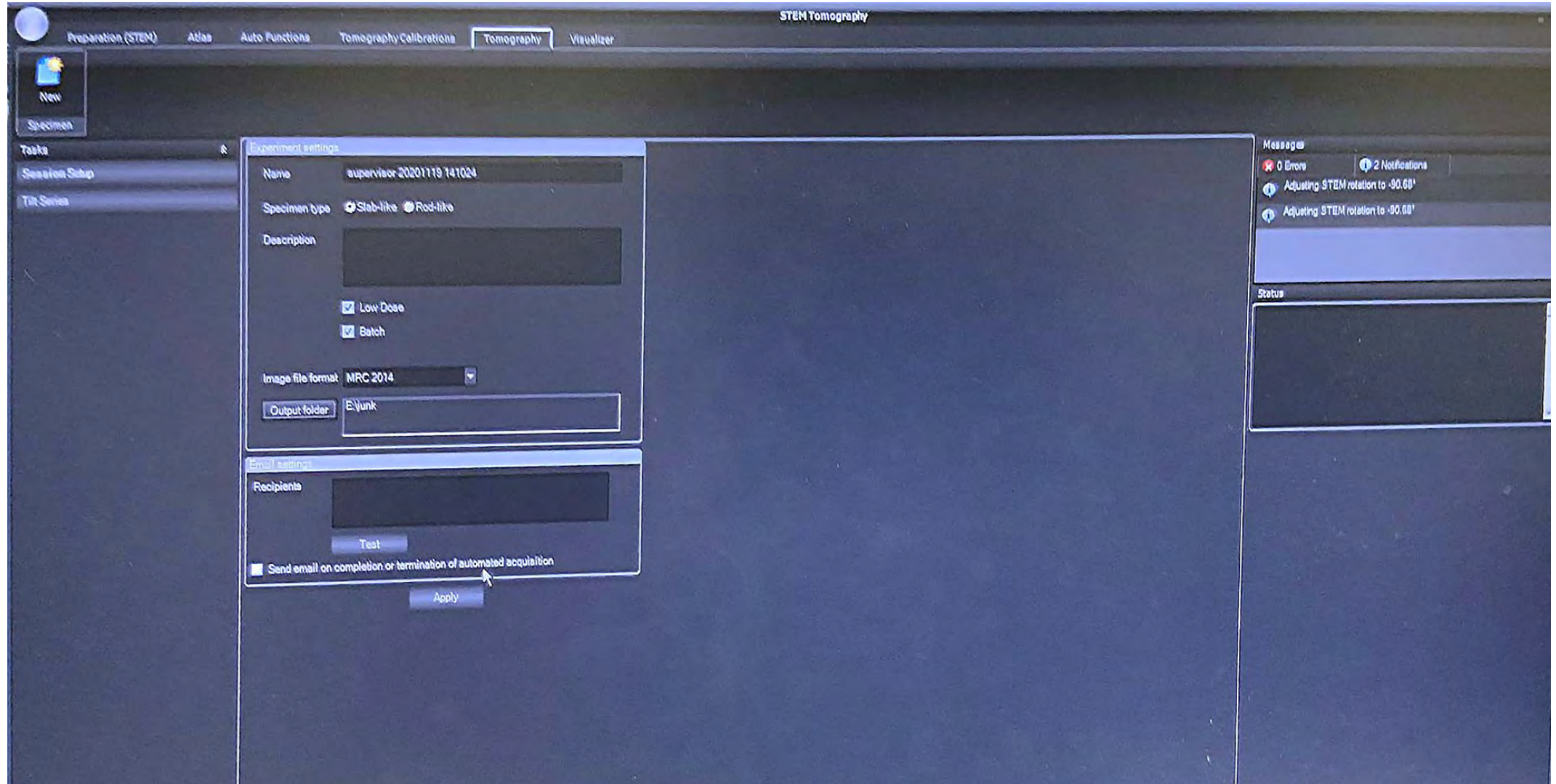
0 200nm

95%

With dynamic focusing



# TFS Tomography Interface - Set up Tomography

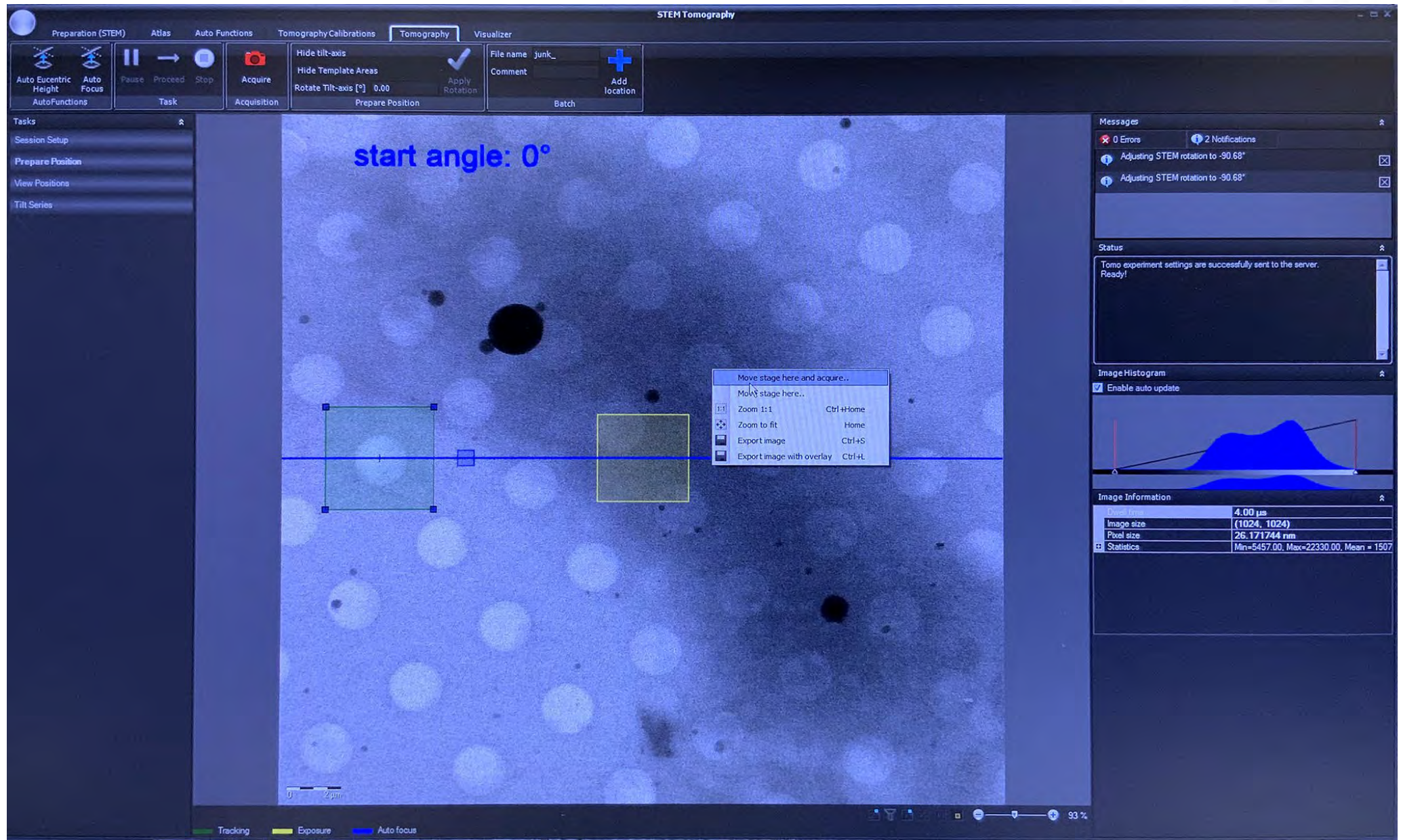


The screenshot displays the TFS Tomography Interface with the 'Tomography' tab selected. The interface includes a top navigation bar with 'Preparation (STEM)', 'Atlas', 'Auto Functions', 'Tomography Calibrations', 'Tomography', and 'Visualizer'. A 'New' button is visible in the top left. The main area is divided into several panels:

- Task Panel:** Contains 'Session Setup' and 'Tilt Series'.
- Experiment settings Panel:**
  - Name: supervisor\_20201119\_141024
  - Specimen type:  Slab-like  Rod-like
  - Description: [Empty text box]
  - Low Dose
  - Batch
  - Image file format: MRC 2014
  - Output folder: E:\junk
- Email settings Panel:**
  - Recipients: [Empty text box]
  - Test: [Button]
  - Send email on completion or termination of automated acquisition
  - Apply: [Button]
- Messages Panel:**
  - 0 Errors
  - 2 Notifications
  - Adjusting STEM rotation to -90.00°
  - Adjusting STEM rotation to -90.00°
- Status Panel:** [Empty area]



# TFS Tomography Interface - Prepare Position



STEM Tomography

Preparation (STEM) Atlas Auto Functions Tomography Calibrations Tomography Visualizer

Auto Eucentric Height Auto Focus  
AutoFunctions

Pause Proceed Stop  
Task

Acquire

Hide tilt-axis  
Hide Template Areas  
Rotate Tilt-axis [°] 0.00  
Prepare Position

Apply Rotation

File name junk\_  
Comment  
Batch

Add location

Tasks

- Session Setup
- Prepare Position
- View Positions
- Tilt Series

start angle: 0°

Move stage here and acquire..  
Move stage here..  
Zoom 1:1 Ctrl+Home  
Zoom to fit Home  
Export image Ctrl+S  
Export image with overlay Ctrl+L

Messages

- 0 Errors
- 2 Notifications
- Adjusting STEM rotation to -90.68°
- Adjusting STEM rotation to -90.68°

Status

Tomo experiment settings are successfully sent to the server. Ready!

Image Histogram

Enable auto update

Image Information

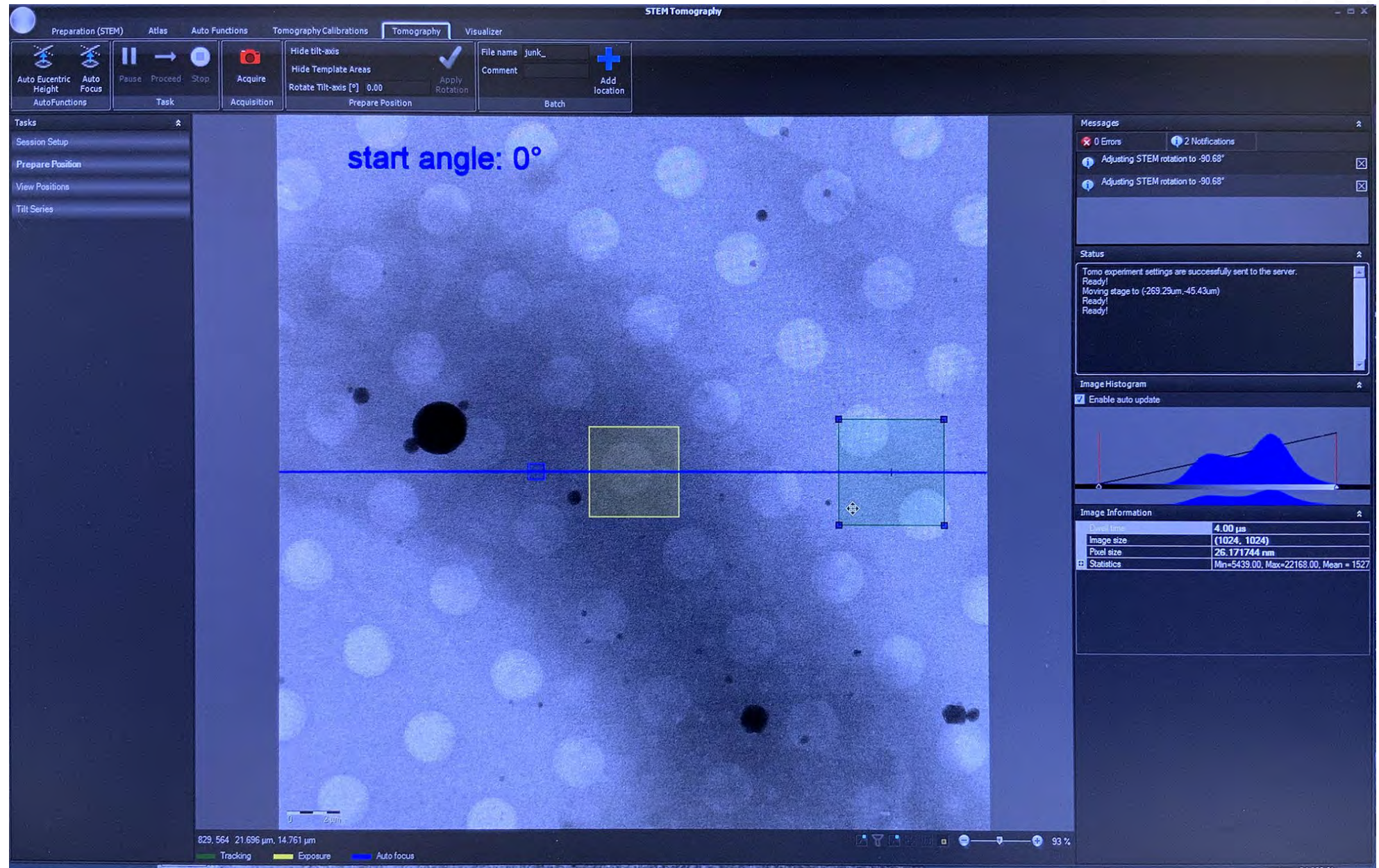
Exposure time	4.00 $\mu$ s
Image size	(1024, 1024)
Pixel size	26.171744 nm
Statistics	Min=5457.00, Max=22330.00, Mean = 1507

Tracking Exposure Auto focus

93 %



# TFS Tomography Interface - Prepare Position



Preparation (STEM) Atlas Auto Functions Tomography Calibrations Tomography Visualizer

Auto Eucentric Height Auto Focus  
AutoFunctions

Pause Proceed Stop  
Task

Acquire  
Acquisition

Hide tilt-axis  
Hide Template Areas  
Rotate Tilt-axis [°] 0.00  
Prepare Position

File name junk\_  
Comment  
Add location  
Batch

Tasks

- Session Setup
- Prepare Position
- View Positions
- Tilt Series

start angle: 0°

Messages

- 0 Errors 2 Notifications
- Adjusting STEM rotation to -90.68°
- Adjusting STEM rotation to -90.68°

Status

Tomo experiment settings are successfully sent to the server.  
Ready!  
Moving stage to (-269.29um, -45.43um)  
Ready!  
Ready!

Image Histogram

Enable auto update

Image Information

Dwell time	4.00 μs
Image size	(1024, 1024)
Pixel size	26.171744 μm
Statistics	Min=5439.00, Max=22168.00, Mean = 1527

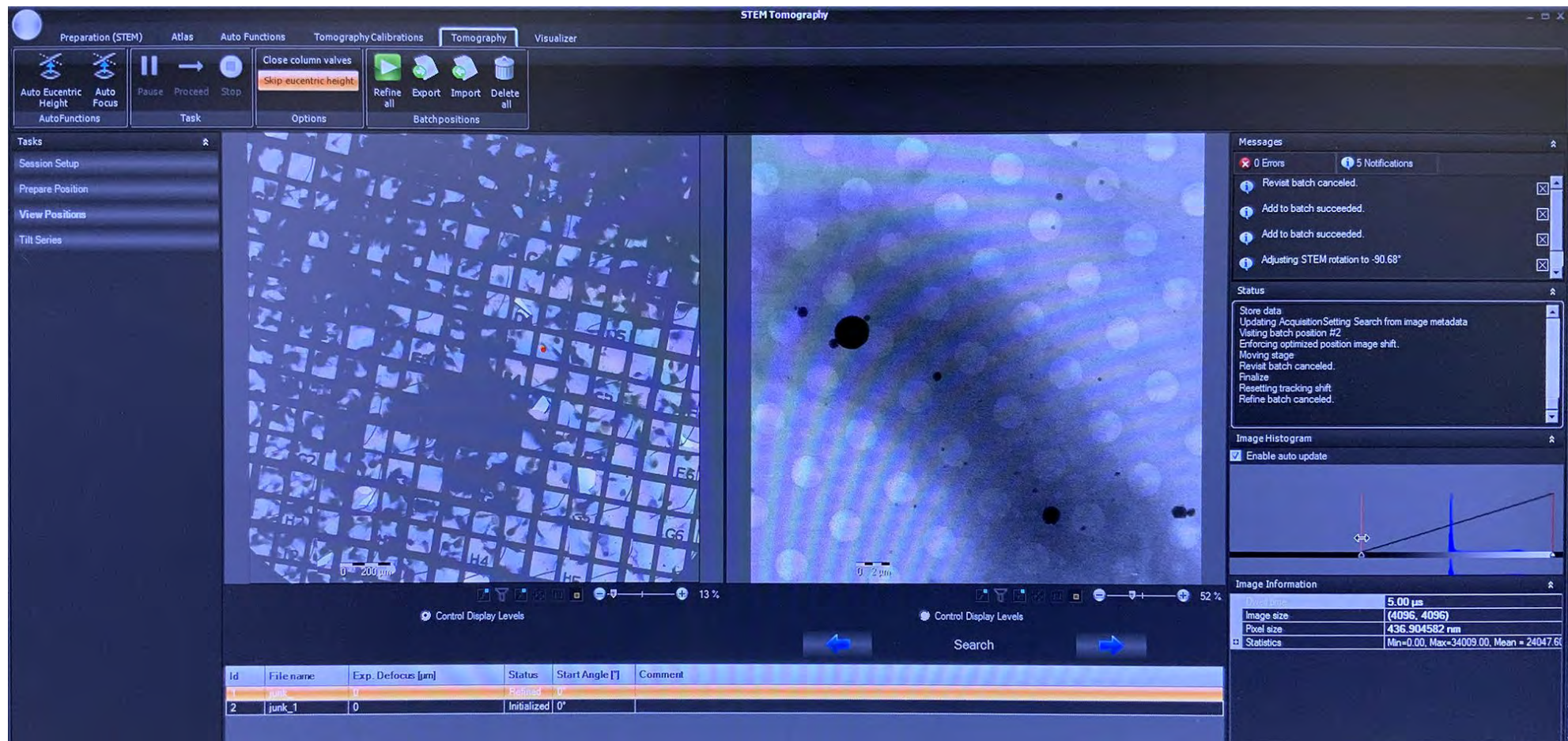
829, 564 21.696 μm, 14.761 μm

Tracking Exposure Auto focus

93%



# TFS Tomography Interface - Refine Positions

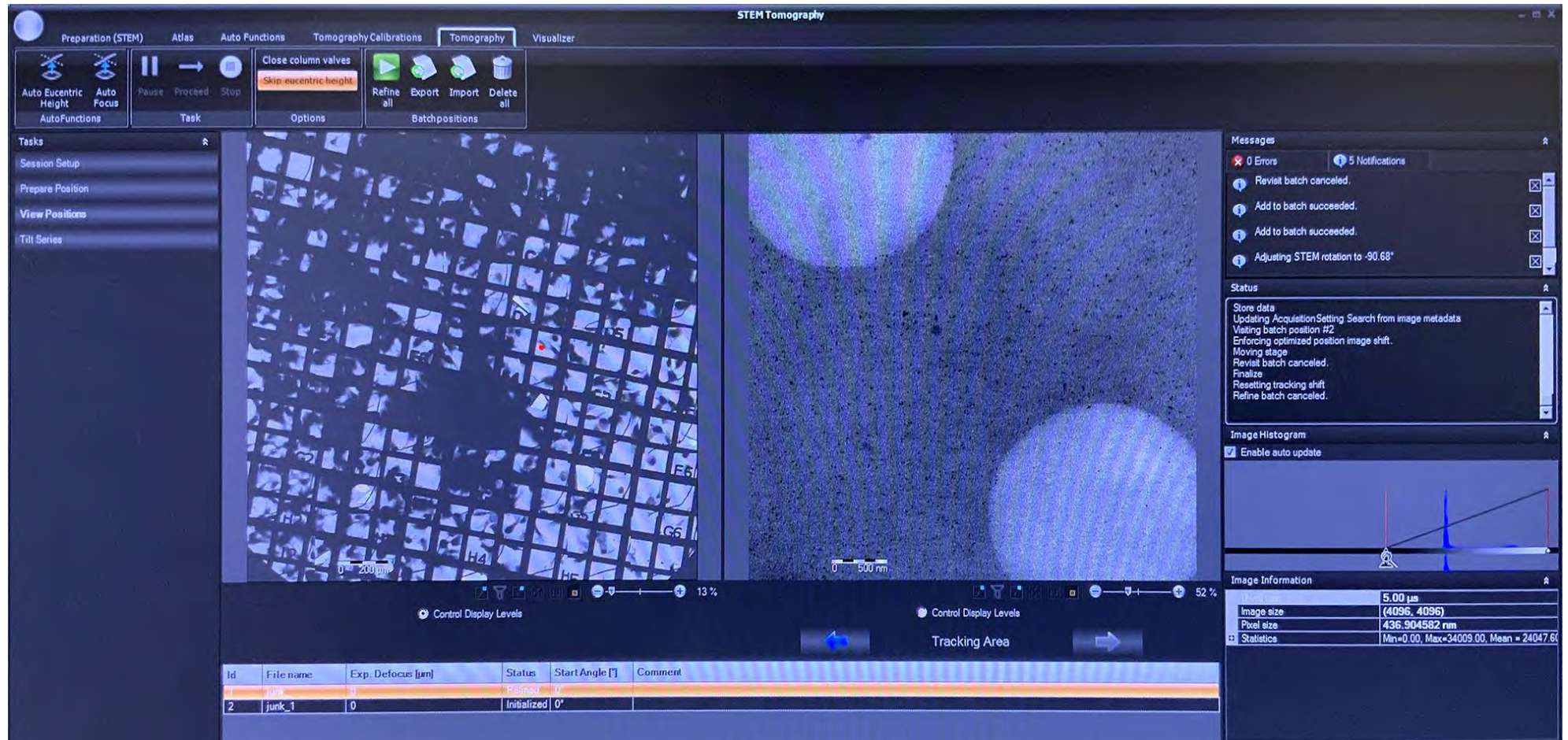


The screenshot displays the TFS Tomography software interface. The main window is titled "STEM Tomography" and features several tabs: Preparation (STEM), Atlas, Auto Functions, Tomography Calibrations, Tomography, and Visualizer. The "Tomography" tab is active, showing a grid of acquisition positions on the left and a corresponding image on the right. The interface includes a toolbar with buttons for "Auto Eucentric Height", "Auto Focus", "Pause", "Proceed", "Stop", "Close column valves", "Skip eucentric height", "Refine all", "Export", "Import", and "Delete all". A "Tasks" panel on the left lists "Session Setup", "Prepare Position", "View Positions", and "Tilt Series". A "Messages" panel on the right shows a list of system messages, including "Revisit batch canceled", "Add to batch succeeded", and "Adjusting STEM rotation to -90.68°". An "Image Histogram" panel is also visible, showing a graph of the image data. At the bottom, a table displays the current batch of positions.

Id	File name	Exp. Defocus [µm]	Status	Start Angle [°]	Comment
1	junk	0	Released	0°	
2	junk_1	0	Initialized	0°	



# TFS Tomography Interface - Refine Positions



The screenshot displays the TFS Tomography software interface. The main window is titled "STEM Tomography" and features a top navigation bar with tabs for Preparation (STEM), Atlas, Auto Functions, Tomography Calibrations, Tomography, and Visualizer. The "Tomography" tab is active, showing a toolbar with buttons for "Auto Eucentric Height", "Auto Focus", "Pause", "Proceed", "Stop", "Close column valves", "Skip eucentric height", "Refine all", "Export", "Import", and "Delete all".

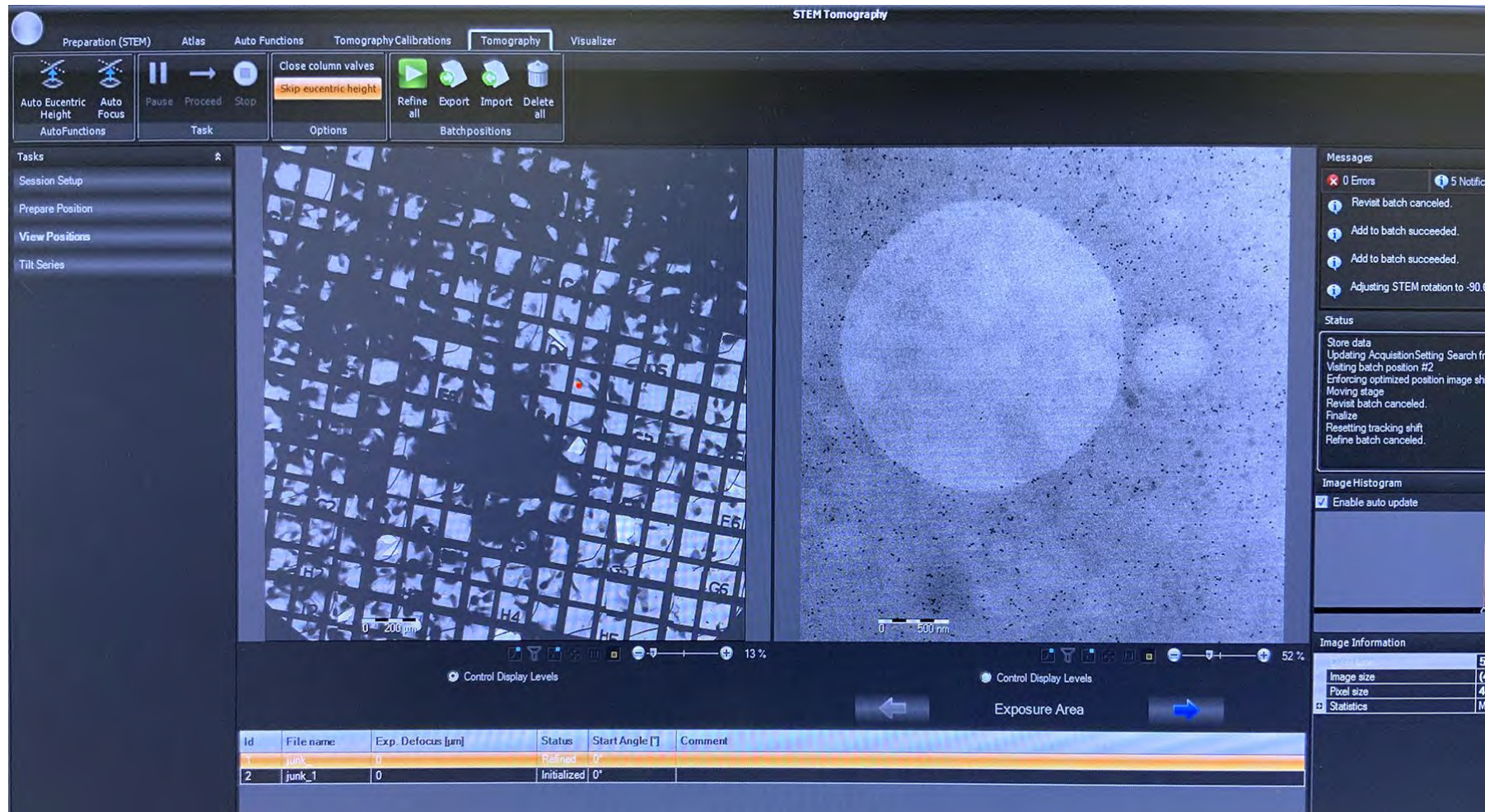
The interface is divided into several panels:

- Tasks:** A sidebar on the left lists tasks such as "Session Setup", "Prepare Position", "View Positions", and "Tilt Series".
- Main View:** The central area is split into two panels. The left panel shows a grid of acquisition positions labeled H4, H5, E5, and G6. The right panel shows a high-resolution image of a sample with a scale bar of 500 nm.
- Messages:** A panel on the right displays system messages, including "Revisit batch canceled", "Add to batch succeeded", and "Adjusting STEM rotation to -90.68°".
- Status:** A panel below the messages shows the current status of the acquisition, including "Store data", "Updating AcquisitionSetting", "Waiting batch position #2", "Enforcing optimized position image shift", "Moving stage", "Revisit batch canceled", "Finalize", "Resetting tracking shift", and "Refine batch canceled".
- Image Histogram:** A panel below the status shows a histogram of the image data with a scale of 52%.
- Image Information:** A panel at the bottom right provides technical details: "Acquisition Time: 5.00 µs", "Image size: (4096, 4096)", "Pixel size: 436.904582 nm", and "Statistics: Min=0.00, Max=34009.00, Mean = 24047.66".
- Tracking Area:** A table at the bottom of the interface lists the current batch of positions.

Id	File name	Exp. Defocus [µm]	Status	Start Angle [°]	Comment
1	junk	0	Finalized	0°	
2	junk_1	0	Initialized	0°	



# TFS Tomography Interface - Refine Positions



The screenshot displays the TFS Tomography Interface with the 'Tomography' tab selected. The interface includes a top navigation bar with tabs for Preparation (STEM), Atlas, Auto Functions, Tomography Calibrations, Tomography, and Visualizer. Below the navigation bar are control panels for 'Task' (Auto Eucentric Height, Auto Focus, Pause, Proceed, Stop), 'Options' (Close column valves, Skip eucentric height), and 'Batch positions' (Refine all, Export, Import, Delete all). A left sidebar lists tasks: Session Setup, Prepare Position, View Positions, and Tilt Series. The main area shows two images: a grid-patterned image on the left (0-200 μm scale) and a circular region of interest on the right (0-500 nm scale). A right sidebar contains a Messages panel (0 Errors, 5 Notifications), a Status panel with a list of actions, an Image Histogram panel (Enable auto update checked), and an Image Information panel. At the bottom, a table lists exposure data, and a central 'Exposure Area' control is visible.

Id	File name	Exp. Defocus [μm]	Status	Start Angle [°]	Comment
1	junk	0	Refined	0°	
2	junk_1	0	Initialized	0°	



# TFS Tomography Interface - Tilt Series Parameters

Tilt Series Acquisition	Tilt Scheme	Focus
Start Angle [°] <input type="text" value="-30.00"/>	<input checked="" type="radio"/> Linear <input type="radio"/> Saxton	<input checked="" type="checkbox"/> Use Dynamic Focus
Max. Negative Angle [°] <input type="text" value="-65.00"/>	Low Tilt Step [°] <input type="text" value="2.00"/>	Defocus (at 0°) [μm] <input type="text" value="0.000"/>
Max. Positive Angle [°] <input type="text" value="65.00"/>	High Tilt Switch [°] <input type="text" value="80.00"/>	
Stage Relaxation Time [s] <input type="text" value="3.00"/>	High Tilt Step [°] <input type="text" value="1.00"/>	
<input type="checkbox"/> Partial tilt series		<b>Detectors</b>
		<input checked="" type="checkbox"/> Use Additional Detectors
		<input checked="" type="checkbox"/> BF <input type="checkbox"/> DF2 <input type="checkbox"/> DF4
		<input checked="" type="checkbox"/> HAADF
<b>Acquisition Output</b>		
Output File Name <input type="text" value="Dummy"/>		
<input type="button" value="Apply"/> <input type="button" value="Reset"/>		

# TFS Tomography Interface - Tilt Series Corrections

<b>Auto focus</b> <input checked="" type="checkbox"/> Check focus before acquisition  Auto focus periodicities: High tilt <input type="text" value="3"/> Low tilt <input type="text" value="3"/> Switch angle [°] <input type="text" value="10.00"/>	<b>Tracking</b> <input checked="" type="checkbox"/> Track after acquisition <input checked="" type="checkbox"/> Track before acquisition  'Tracking before' periodicities: High tilt <input type="text" value="100"/> Low tilt <input type="text" value="1"/> Switch angle [°] <input type="text" value="20.00"/>
<b>Holder predictions</b> XY and Focus <input type="text" value="XY and Focus"/>	<b>Auto zero loss</b> <input checked="" type="checkbox"/> Adjust Periodicity <input type="text" value="100"/>
<input type="button" value="Apply"/> <input type="button" value="Reset"/>	



# TFS Tomography Interface - Acquisition



# References – emphasis on cellular tomography



- Chapter 10, Biomedical Electron Microscopy, “Microscopy”, Maunsbach and Afzelius  
<https://doi.org/10.1016/B978-012480610-8/50012-3> (1999)  
*Old book with great images demonstrating various optical phenomena, using biological sections as examples.*
- Transmission Electron Microscopy (textbook), DB Williams & CB Carter (2009)  
*Excellent general chapters for electron optics.*
- Cellular Imaging: Electron Tomography and Related Techniques, Ed. E Hanssen, Springer (2018).  
*Recent book covering the field.*
- Methods in Cell Biology, Volume 152: Three-Dimensional Electron Microscopy, Ed. T Muller-Reichert & G Pigino (2019).  
*Another recent book including practical guidelines in many chapters.*
- Electron Tomography Methods for Three-Dimensional Visualization of Structures in the Cell, 2<sup>nd</sup> Edition, Ed. J Frank, Springer (2006).  
*The basic tomography principles*