MRC Laboratory of Molecular Biology

Data Collection

Cryo-EM Workshop

University of Leicester

16th March 2021

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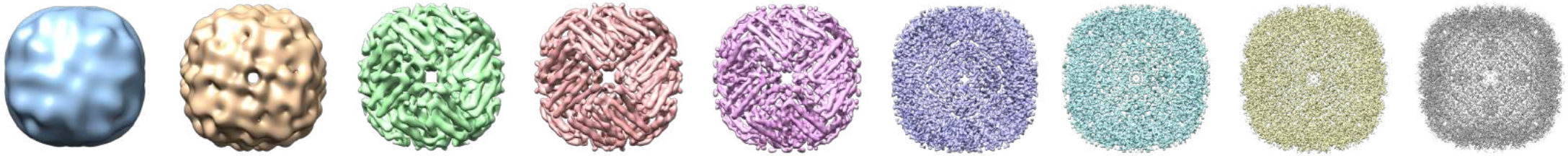
Senior EM Support Scientist

Data Collection Workflow

- Load your sample
- Find a good grid
- Tune the microscope
- Choose a detector
- Setup data collection
- Run

What Resolution Do I Need?

- Resolution target shall be based on the biological question you are trying to answer.
- 3Å target is enough in most cases (unless you are part of Center For Apoferritin Research #CARF).

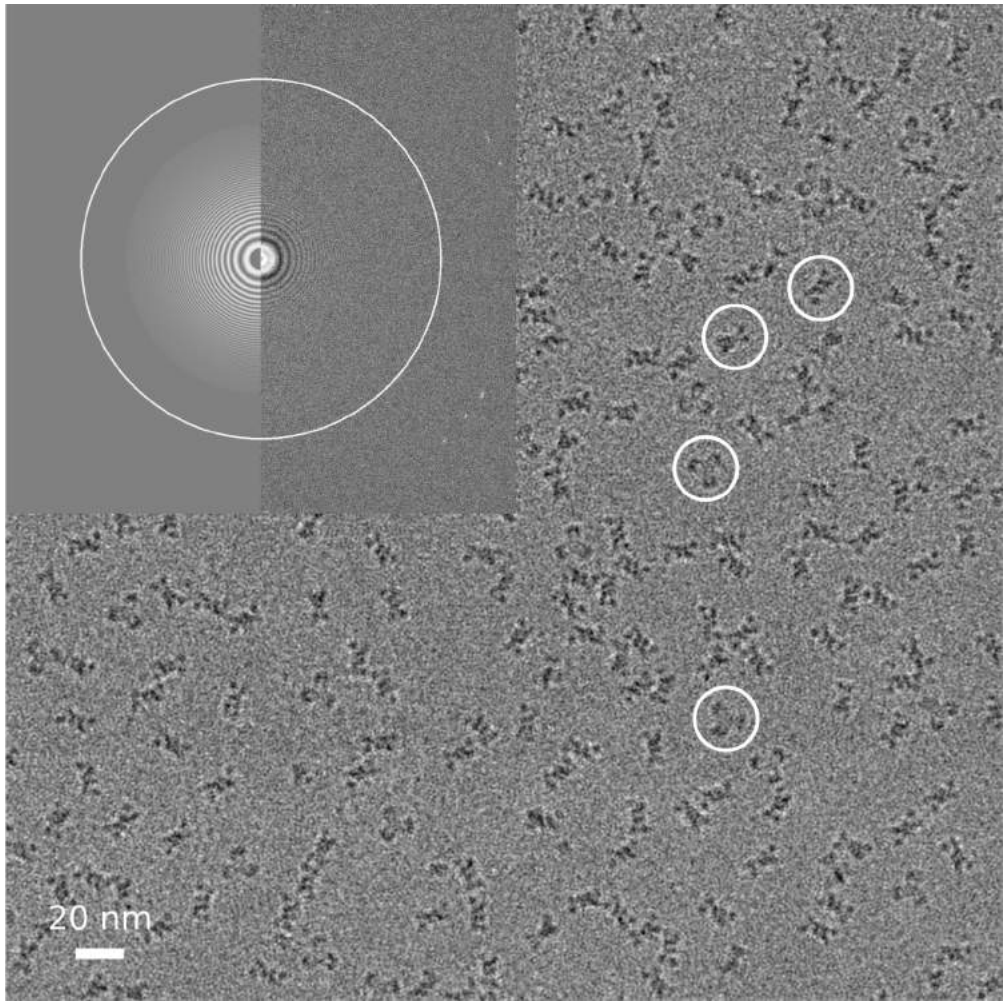


Find a Good Grid

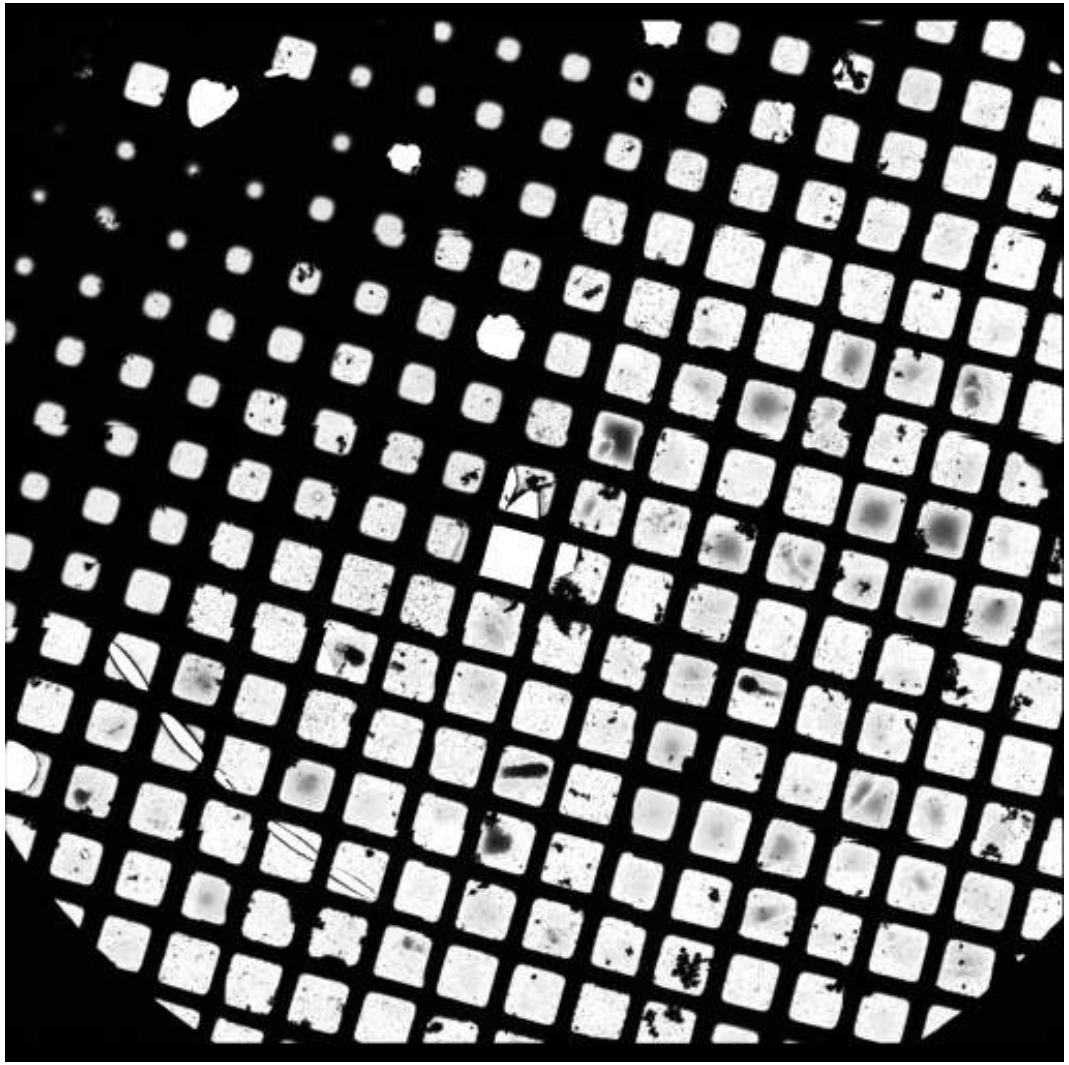
Choosing the Right Grid

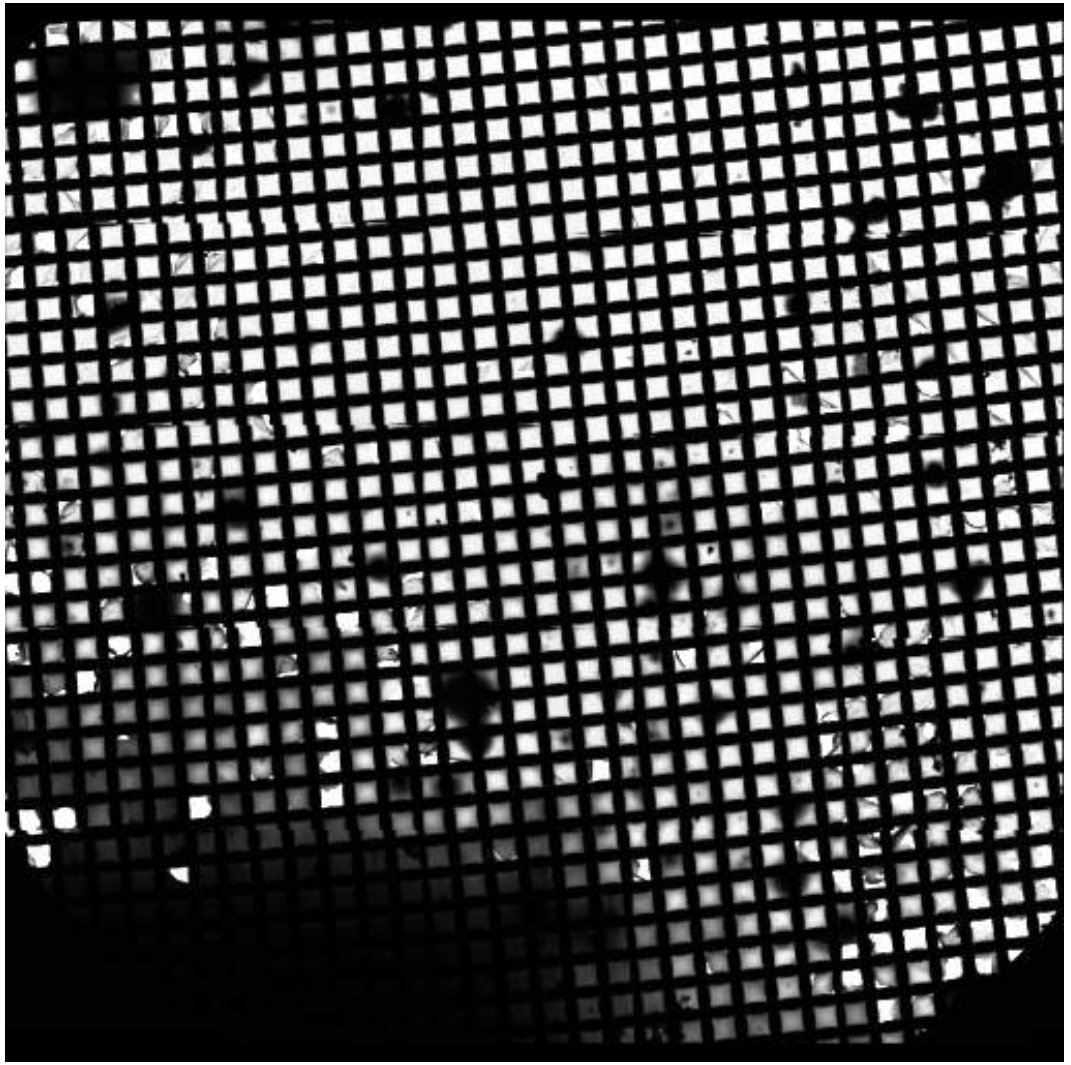
A good cryo-EM image:

- Mono-disperse and homogeneous particles embedded in vitreous ice
- Randomly oriented particles
- High SNR
- Strong isotropic Thon rings (the more the better!)

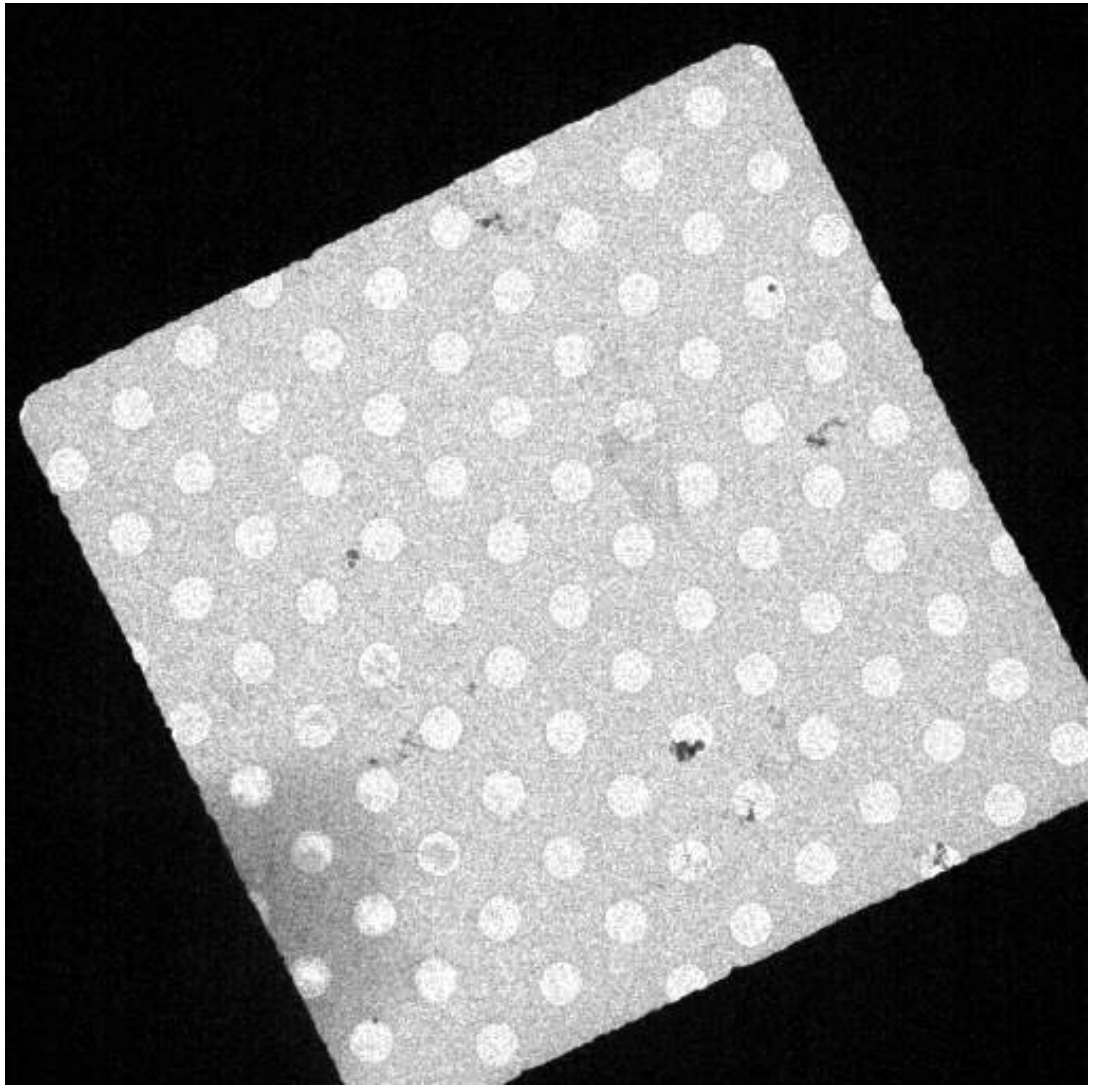


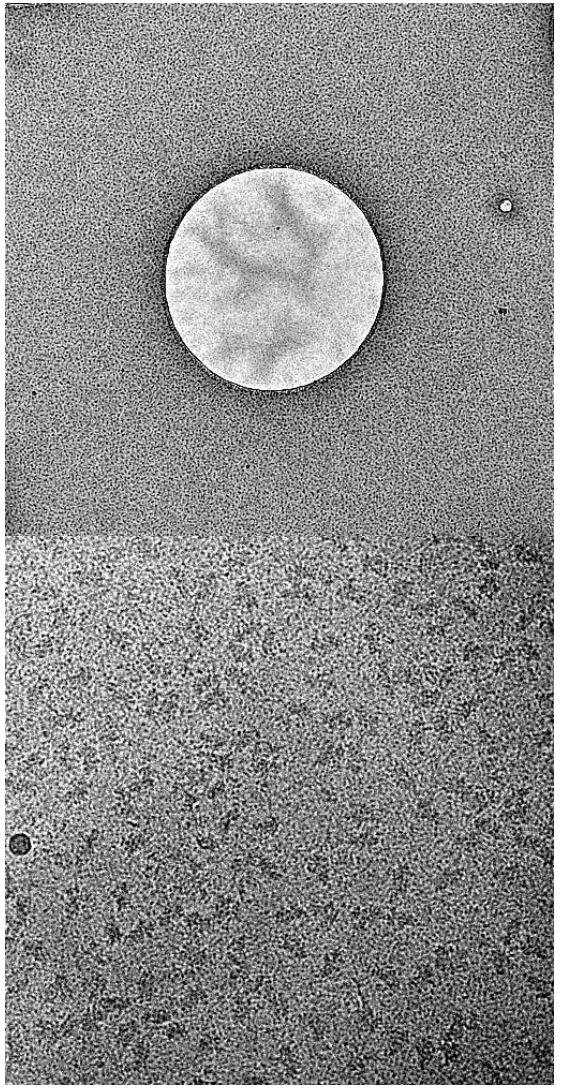
Initial screening: Atlas





Initial screening: Grid Square – Holes





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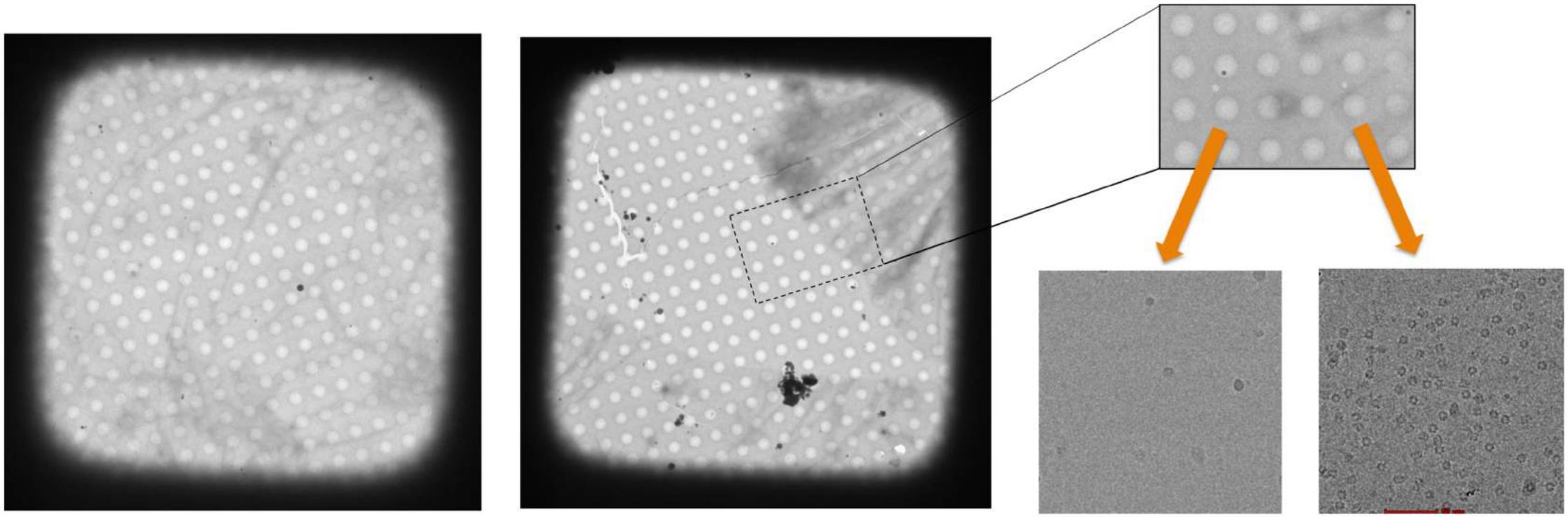
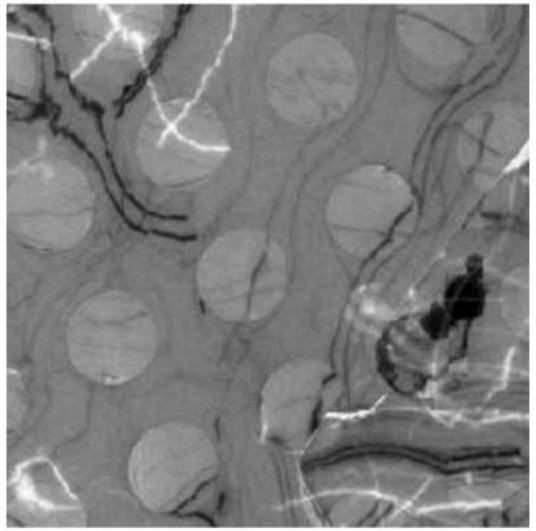


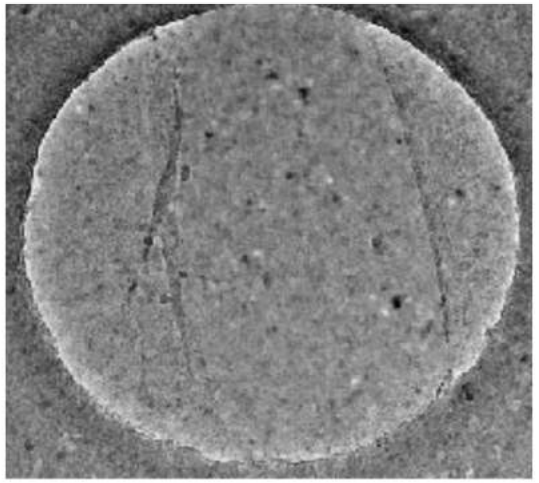
Fully covered

Partially covered

Christos Savva, 2017

Ice Pathology





Russo, 2016

Causes:

- Liquid ethane not enough cold < -150 ^{o}C
- Slow freezing
- Warm-up during transfer
- Vacuum crash

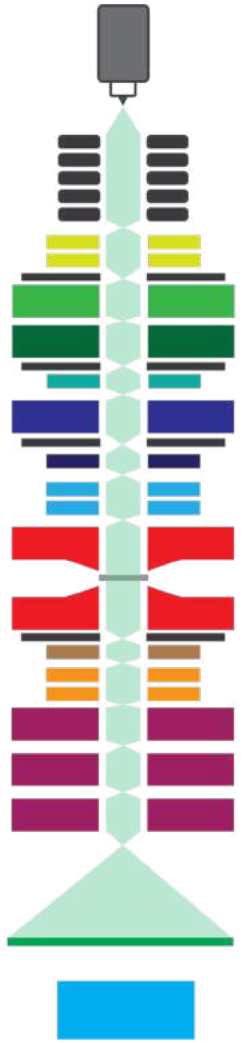
How to check:

- White and black appearance at low mag
- Tilt stage ±10^{o} and pattern change
- Avoid edges of the grid square

Tune the Microscope

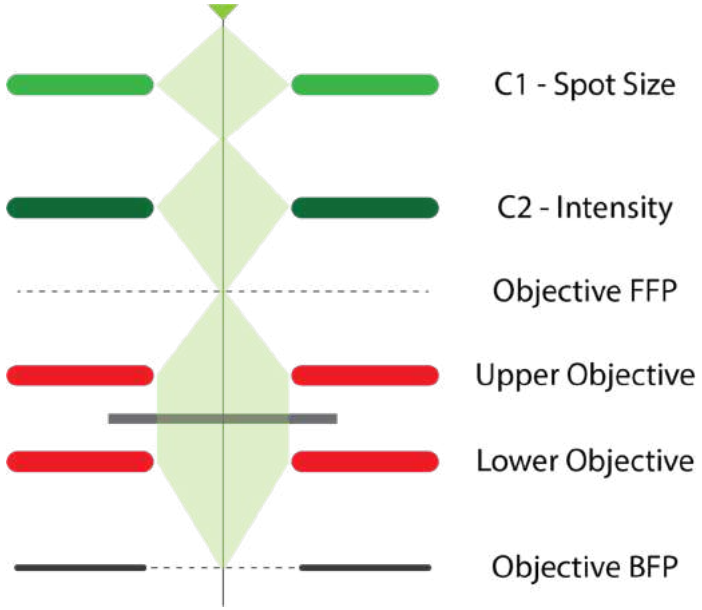
Golden Rules for TEM Operations

- Specimen at eucentric height
- Work close to eucentric focus
- Centered C2 aperture
- Parallel illumination
- Correct objective lenses astigmatism
- Centered objective aperture
- Beam tilt (coma-free)
- Make beam bigger than the hole
- Quality over quantity when possible



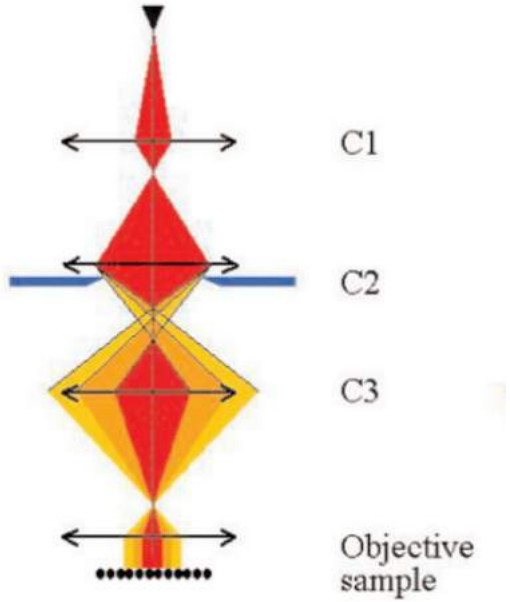
Choosing the Right Illumination

2 Lens condenser system



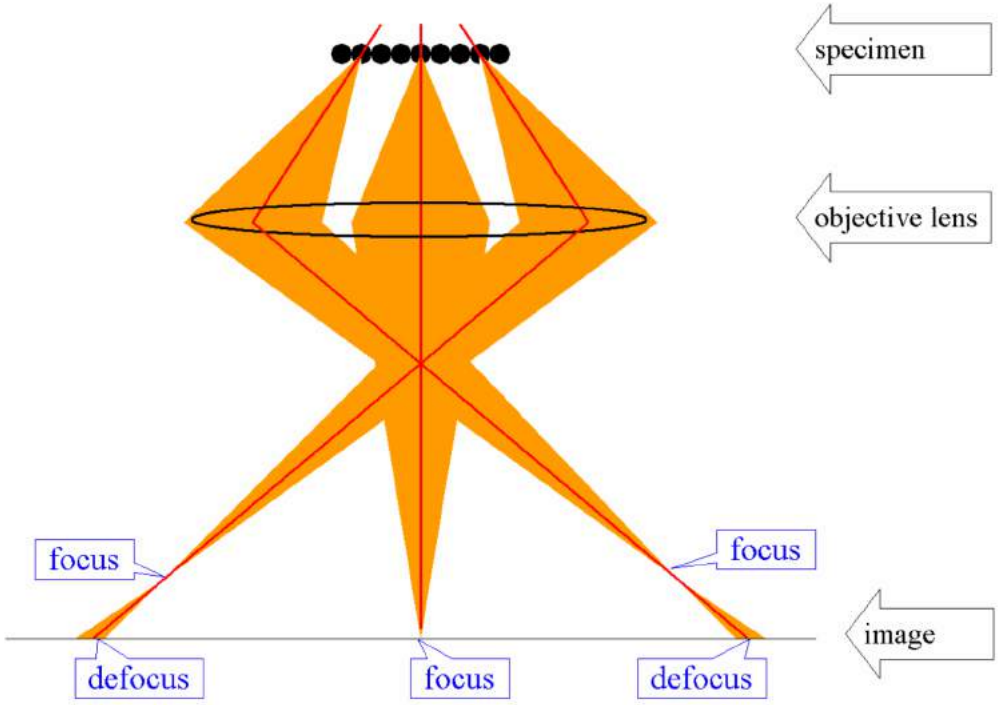
- Switch to diffraction (“Diffraction” button)
- Insert Objective aperture
- Focus Objective aperture (“Focus” Knob)
- Focus diffraction pattern (“Intensity” knob)

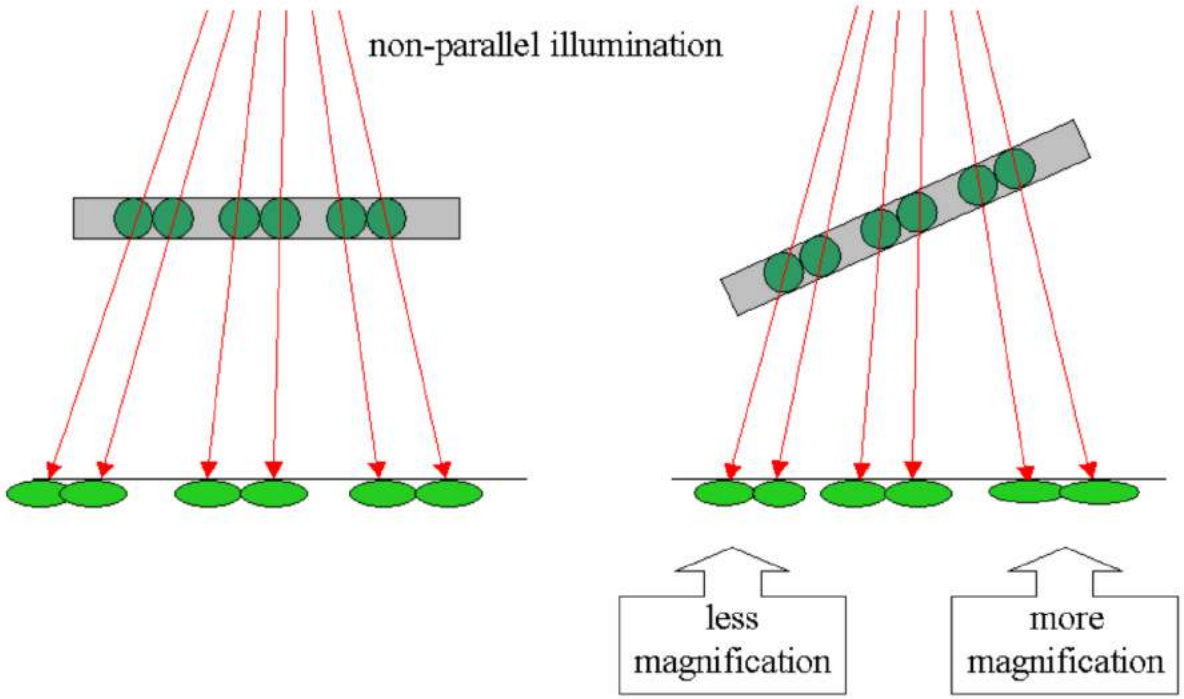
3 Lens condenser system



Check Beam setting panel for parallel range

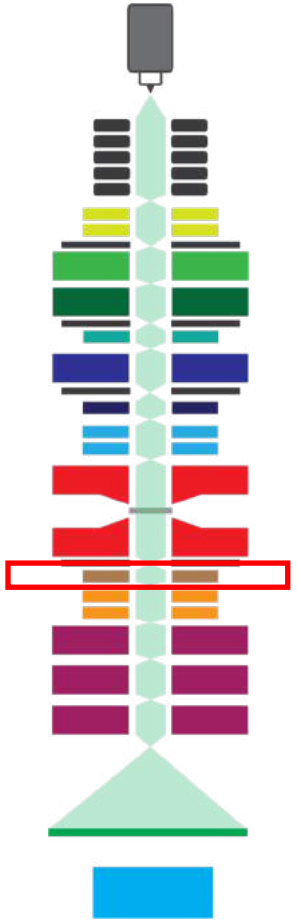
Problems with Non-Parallel Illumination





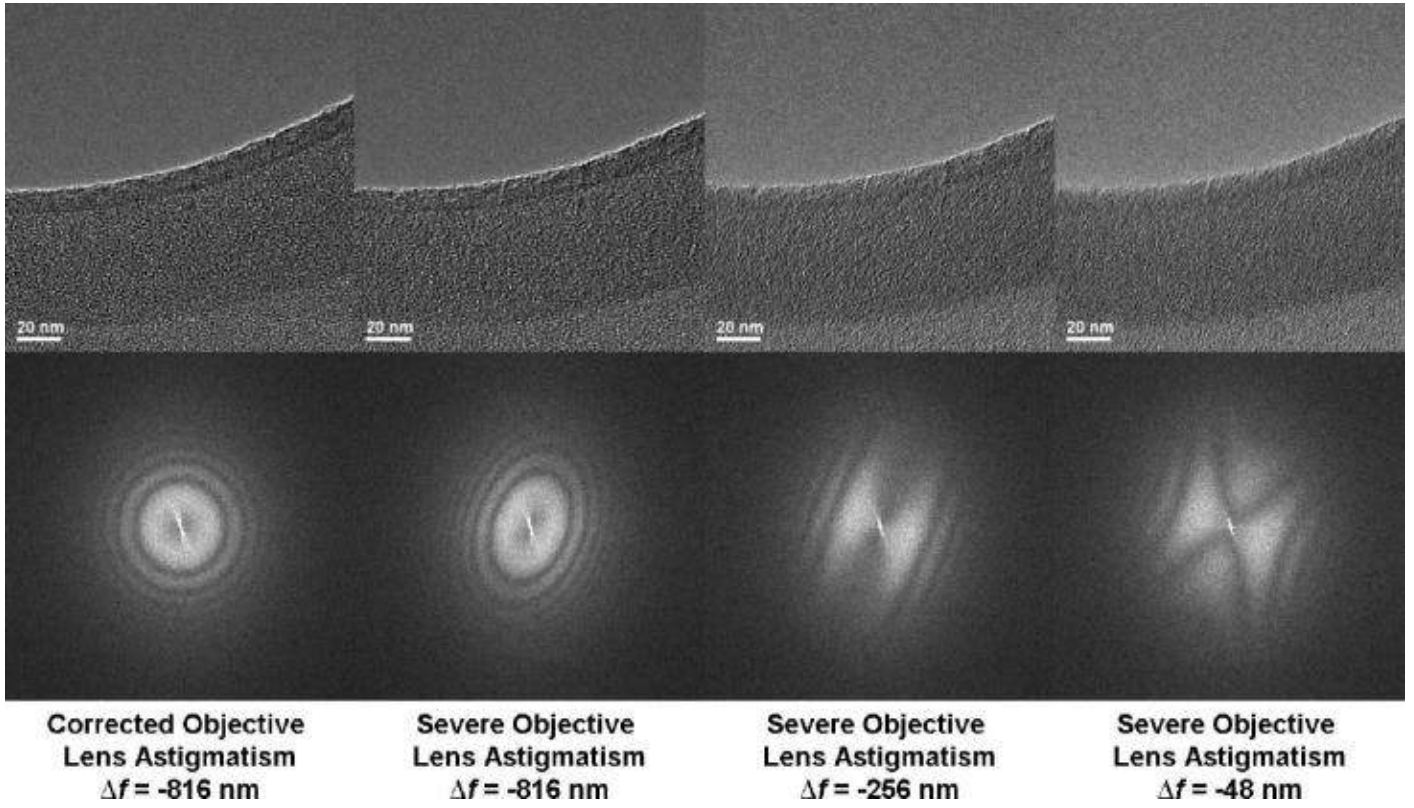
TFS Manual

Objective Lens Astigmatism



Manually: change objective stigmator until FFT rings are circular

Automatic: EPU, AutoCTF, SerialEM (more accurate than eyes)



Corrected Objective Lens Astigmatism Δf = -816 nm

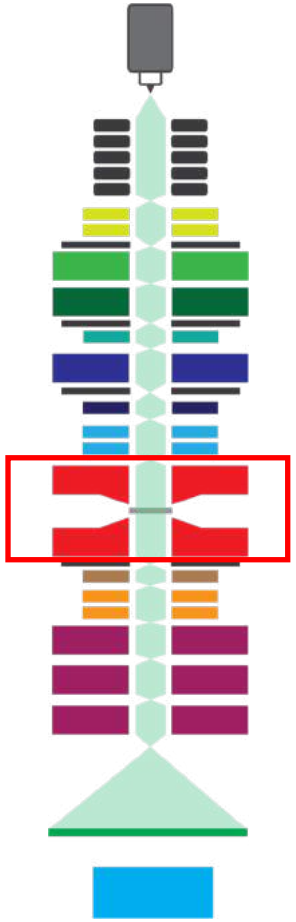
Severe Objective Lens Astigmatism Δf = -816 nm

Severe Objective Lens Astigmatism Δf = -256 nm

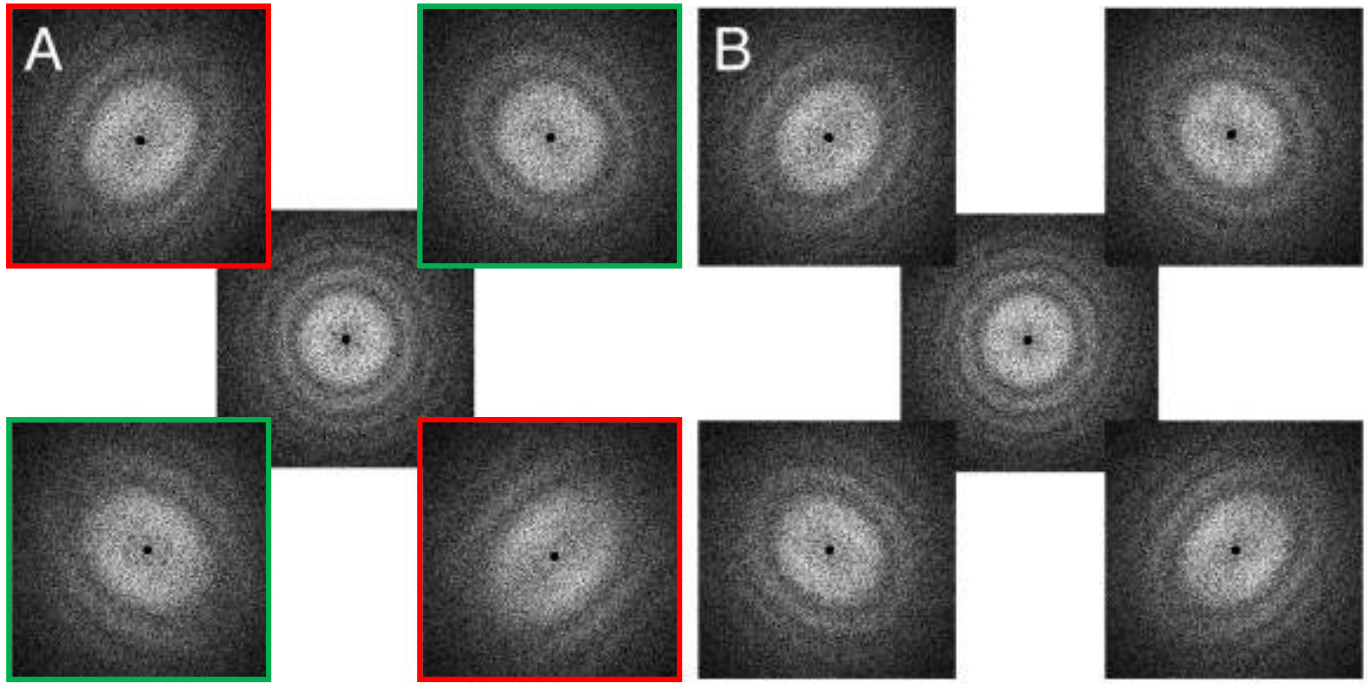
Severe Objective Lens Astigmatism Δf = -48 nm

Langmuir, Vol. 24, No. 20, 2008

Beam Tilt – Coma-free



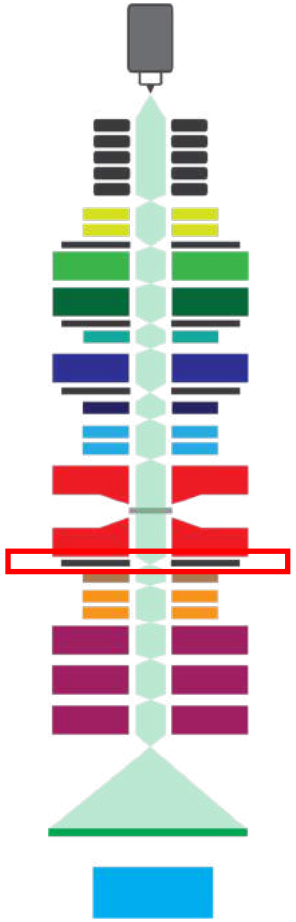
AutoCTF, EPU, SerialEM, Leginon



Before

After

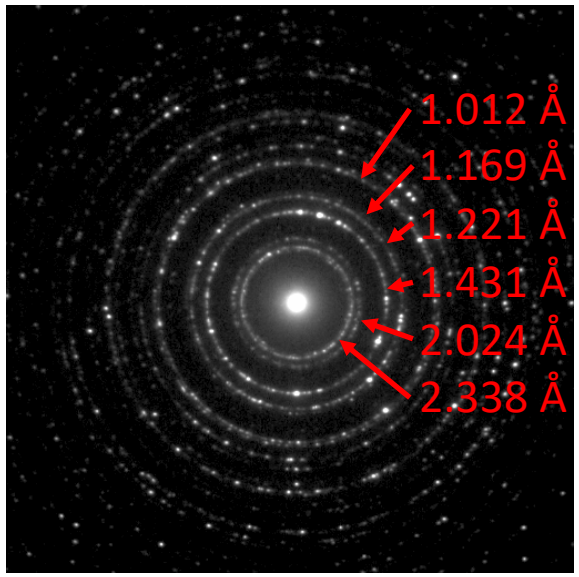
Objective Aperture



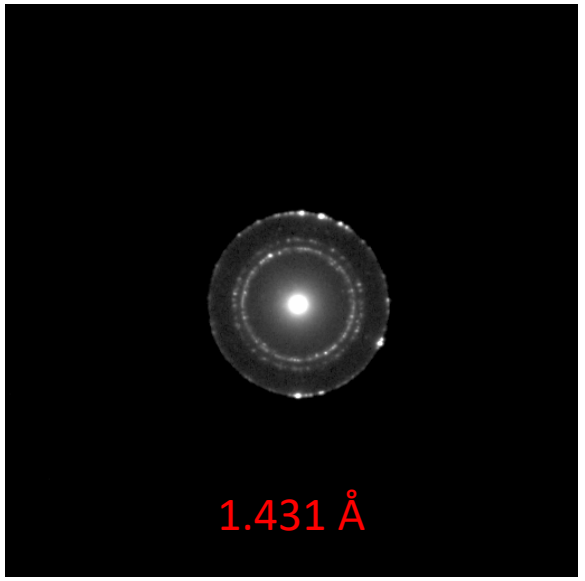
On-the-fly low pass filter

Removes high spatial frequencies -> ”more contrast”

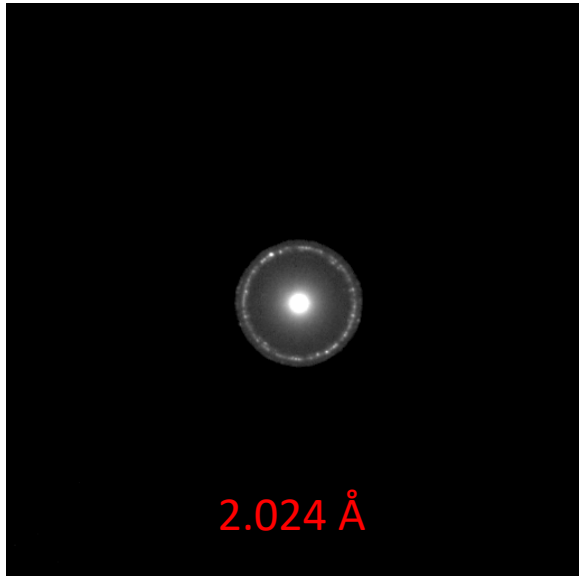
Nyquist = 2 * Pixel Size



No Obj.



100 µm



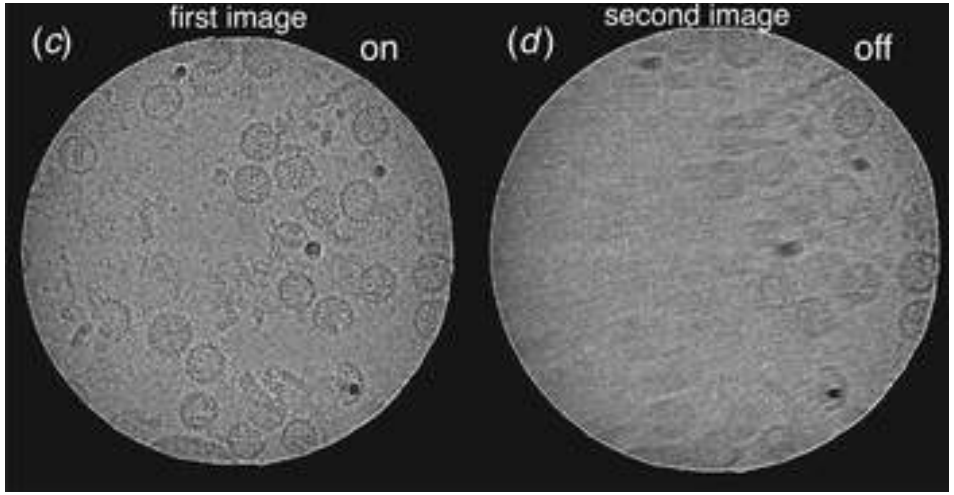
70 µm

Charging

- Irradiation of an insulating thin film of the type studied by TEM leads to positive charge build-up due to the ejection of secondary
- Charge on the sample acts as an electrostatic lens

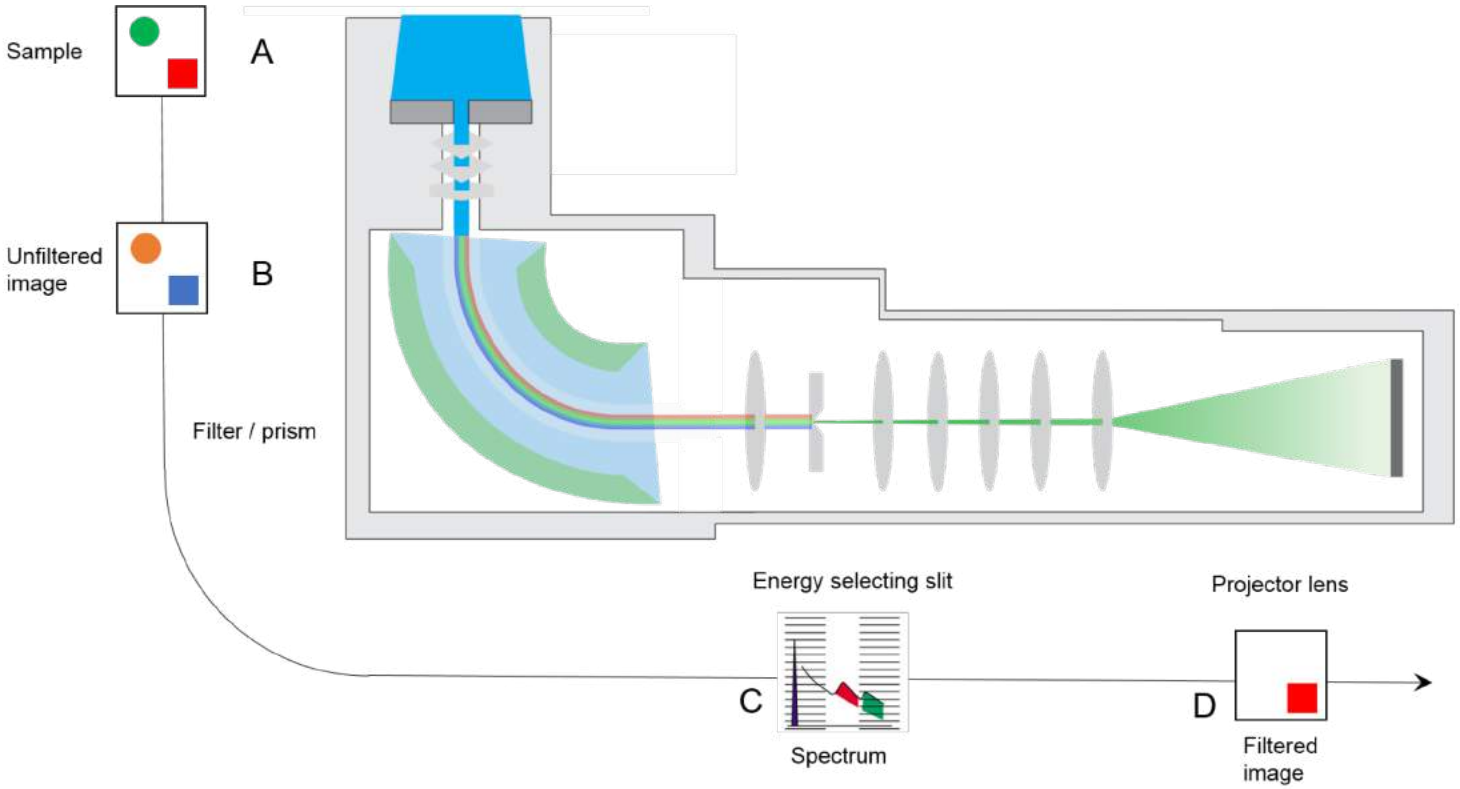
How to avoid charging?

- Illumination bigger than the hole
- Illuminate some carbon on the edge
- Use supports: graphene, carbon
- Use Objective aperture

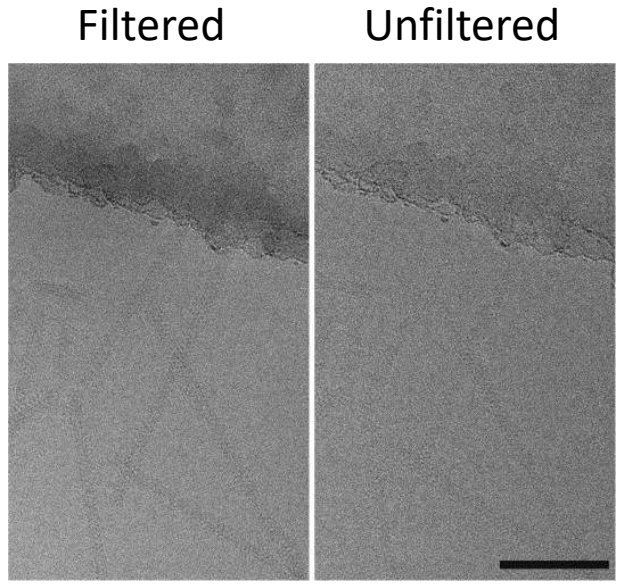


VinothKumar & Henderson, QRB, 2016

Energy Filter Imaging



Gatan.com



Yonekura et.al. JSB 2006

Contrast enhancement – Removes inelastically scattered electrons that produce background noise -> Improves contrast in images and diffraction patterns

Choosing the Detector

Choosing the most appropriate detector for an experiment

- Detectors available (F3, F4, K2, K3, Energy Filter)
- Your sample (size, heterogeneity, dispersion)
- The resolution goal
- Detector Quantum Efficiency (DQE)
- Time available (24h, 48, 72h, weeks!)
- Number of micrographs (600 -> 50000)
- Storage available

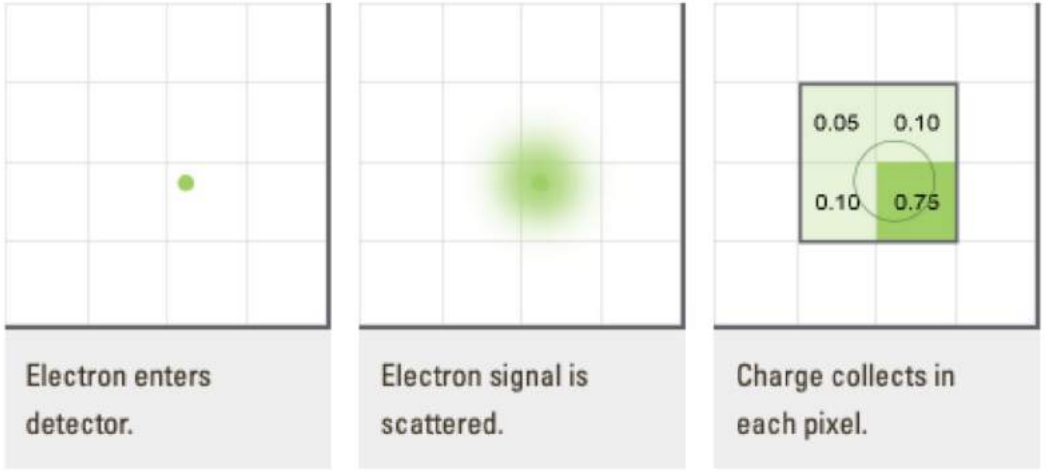
Different Types of Direct Electron Detectors

Detector	Sensor size (px)	Pixel size (um)	Readout speed (fps)	Throughput (No AFIS)	Throughput (AFIS)
DE-64	8192x8192	6.5	42	N/A	N/A
TFS Falcon II	4096x4096	14	17	N/A	N/A
Gatan K2	3838x3710	5	400	35-70	70-150
FEI Falcon III	4096x4096	14	40	24-27	35-40
TFS Falcon IV	4096x4096	14	250	125	250
Gatan K3	5760x4092	5	1500	150-180	300-700
Apollo	4096x4096	8	60	N/A	N/A

Throughput depends on: exposure time, number of frames, binning, software

Integrating (Linear) vs Counting Mode

Linear



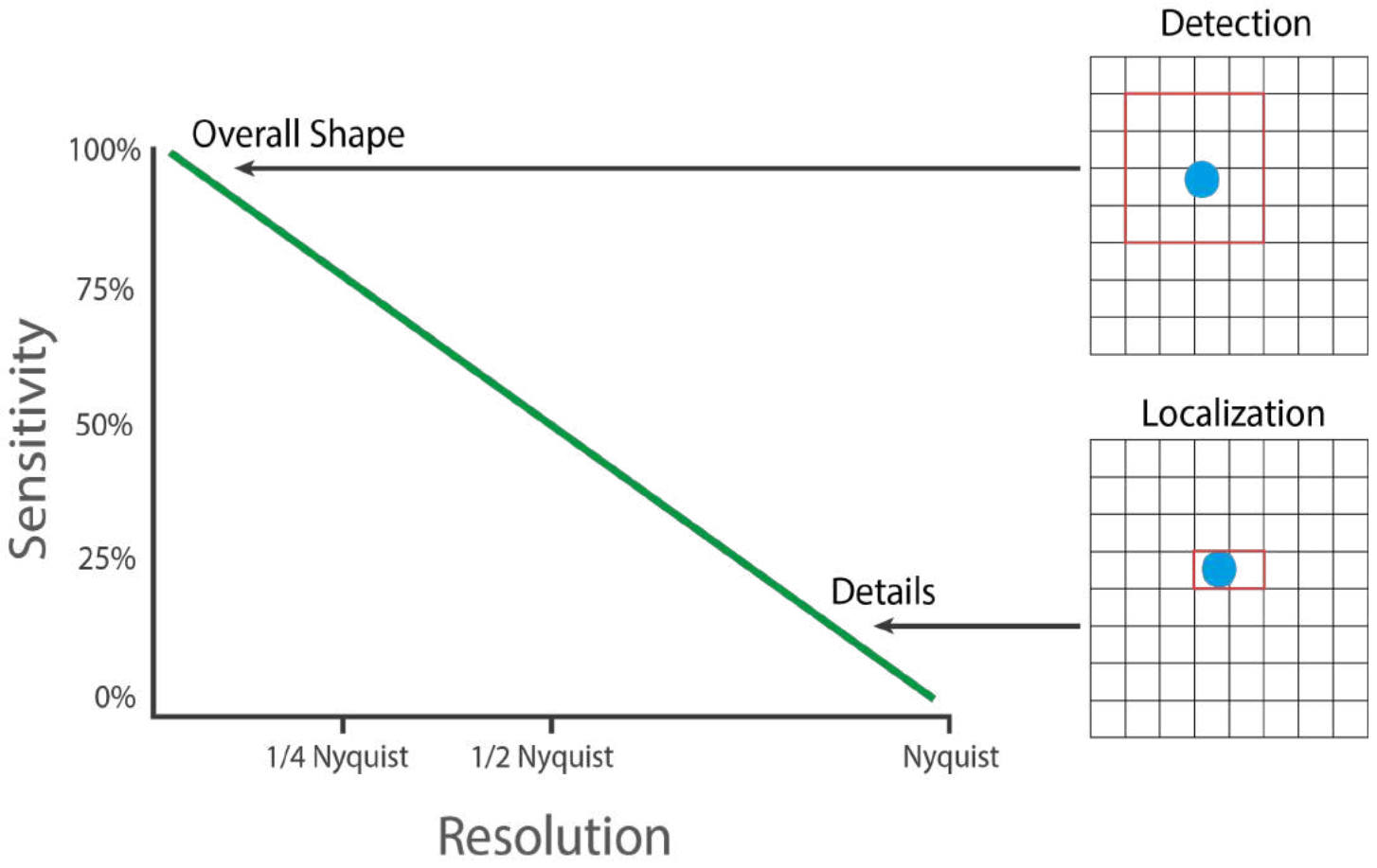
Counting

Gatan.com

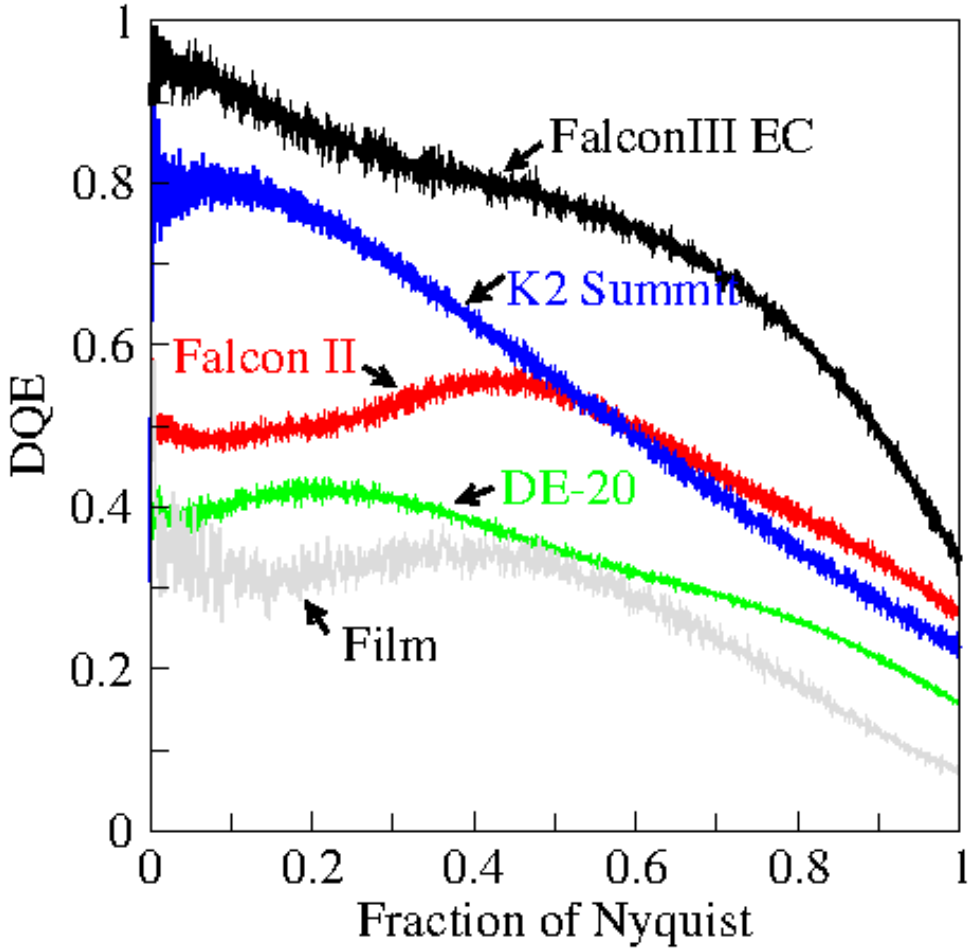
Detector Quantum Efficiency (DQE)

Pixel Size:
smallest information
you wish to record

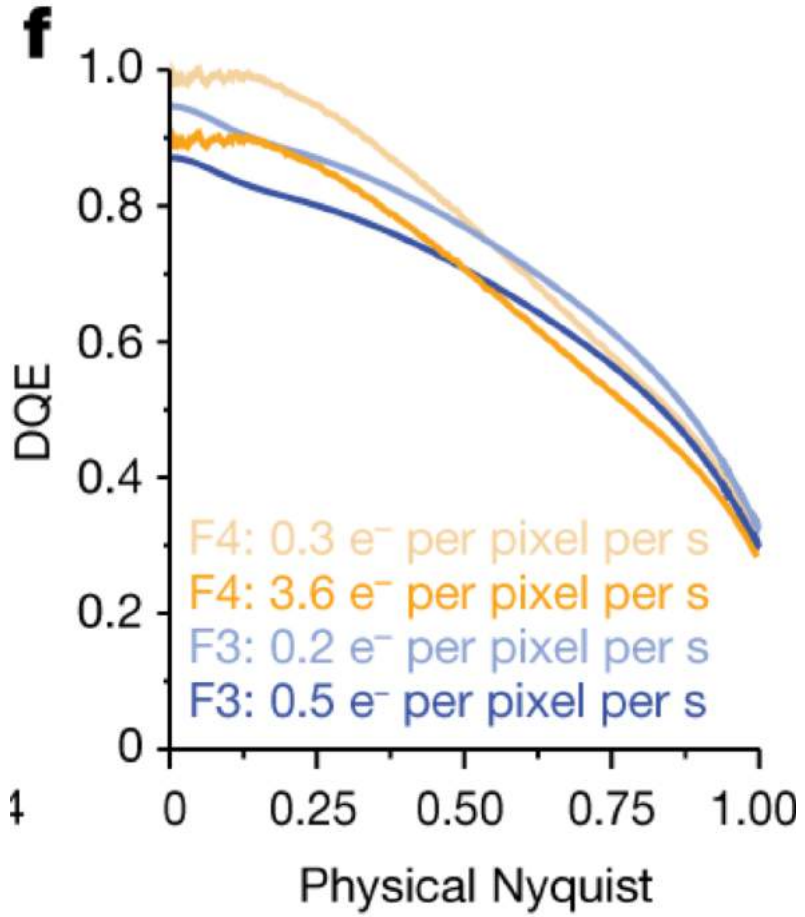
Nyquist Frequency:
smallest information
you are able to
resolve -> 2 * PS



Detector Quantum Efficiency



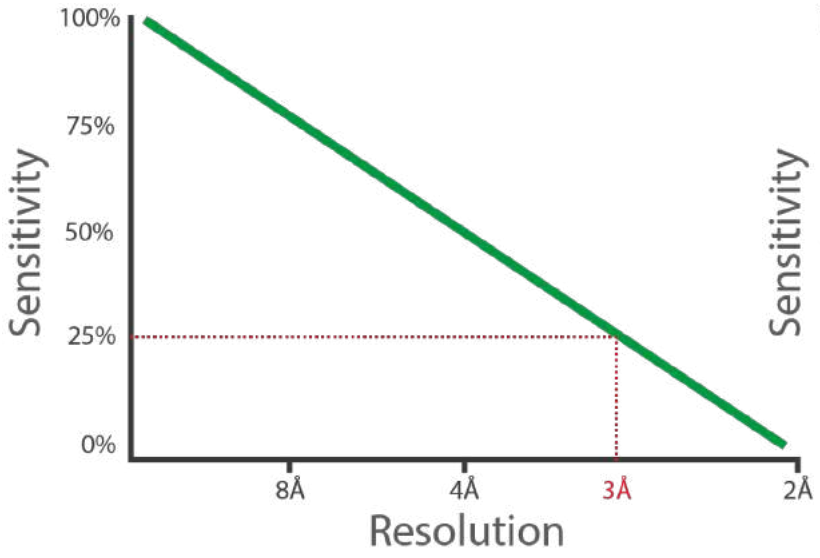
McMullan et. al., 2014



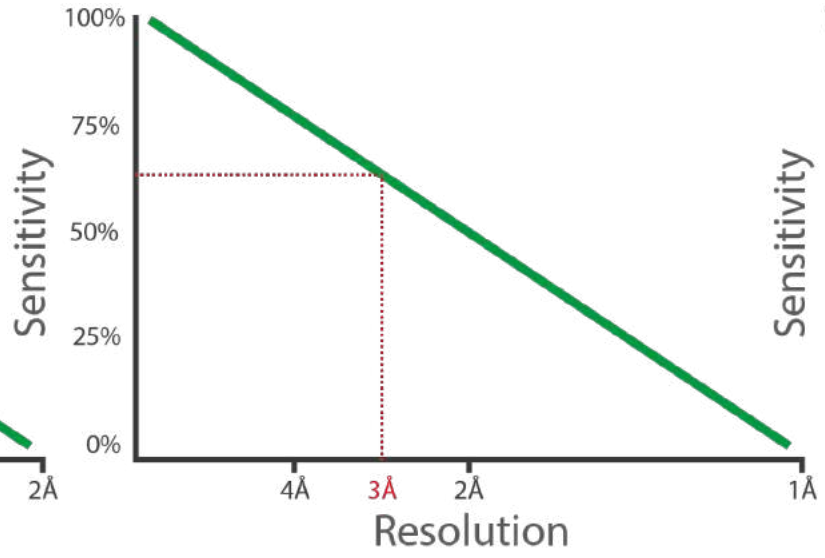
Nakane et. al., 2020

How to Use the DQE

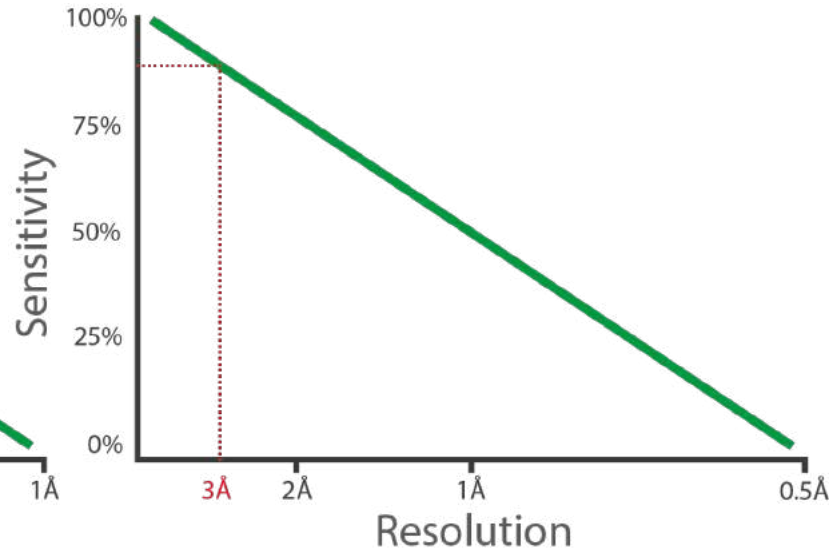
Pixel size 1Å



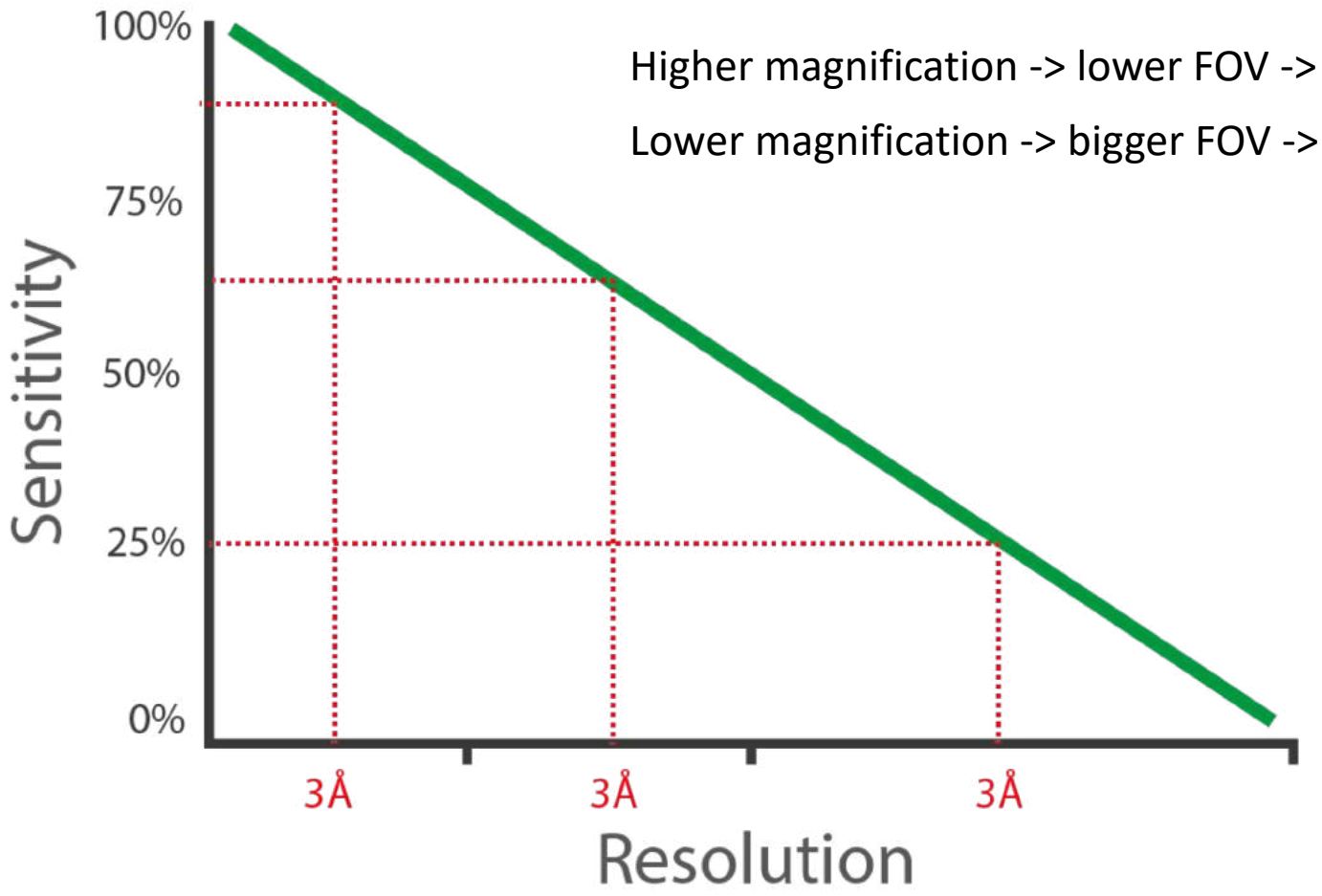
Pixel size 0.5Å



Pixel size 0.25Å



How to Use the DQE



Higher magnification -> lower FOV -> less particles/micrograph

Lower magnification -> bigger FOV -> more particles/micrograph

Guidelines

- Particles < 0.5 MDa
  - Energy filter, embrace defocus, embrace objective aperture, longer exposure
  - F3EC better images low throughput , K2 not as good as F3EC but faster, K3 same as K2 but faster, F4 better images faster detector.
  - Volta phase plate as last resource
- Particles 0.5 – 1 MDa
  - Falcon 3EC better images low throughput , K2/K3 not as good as F3EC and F4EC but higher throughput
- Particles > 1 MDa
  - F3L, K3 higher throughput than F3L

Setup Data Collection

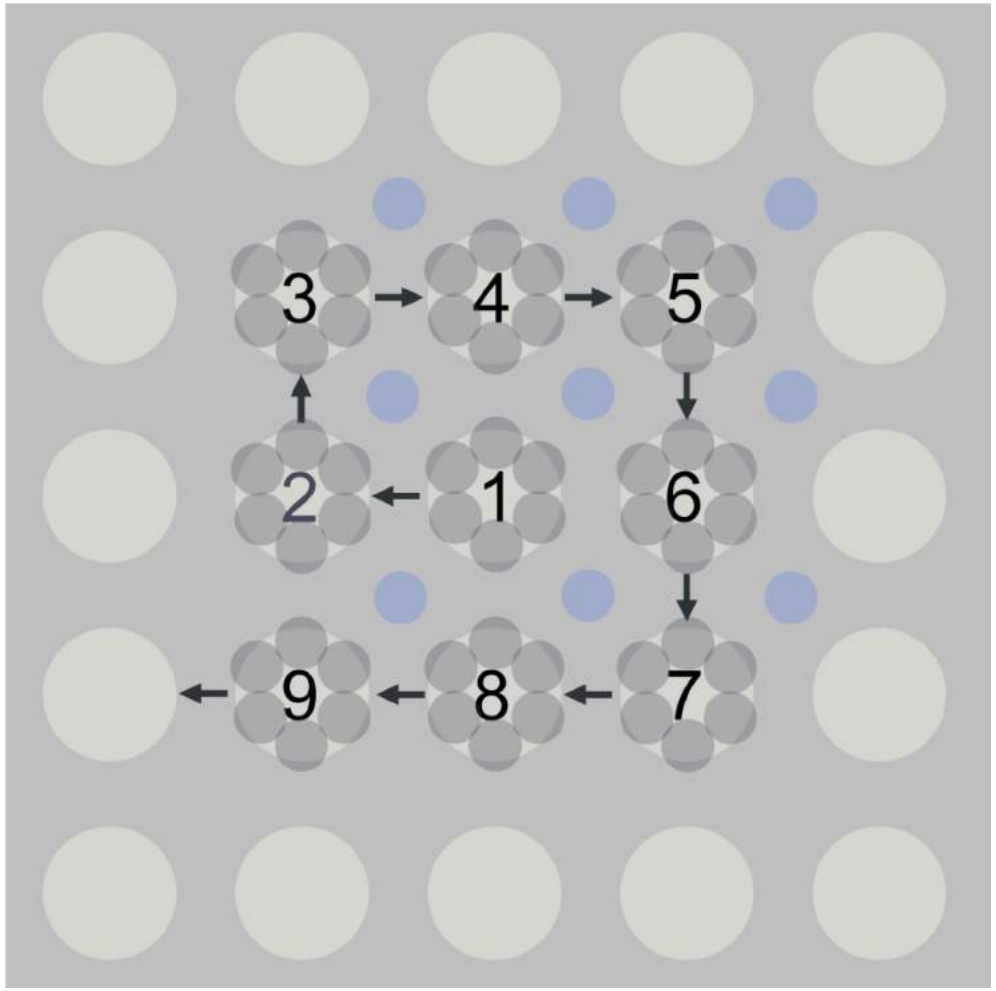
Data Collection Strategies

- Acquire an atlas
- Select grids squares
- Select holes within the selected grids squares
- Estimate how many exposures per hole
- Decide: exposure time, dose rate, fractionation, defocus values, defocus area, drift threshold
- Pick your strategy:
  - Single hole per stage movement
  - Multiple holes per stage movement
  - Single tilted hole per stage movement
  - Multiple tilted holes per stage movement
- Start

“Classic” Data Collection

For each selected hole:

- Move compustage to selected hole (wait for compustage to relax)
- Centre selected hole (compustage movement)
- Autofocus?
- Check drift?
- Acquire one or more image per hole



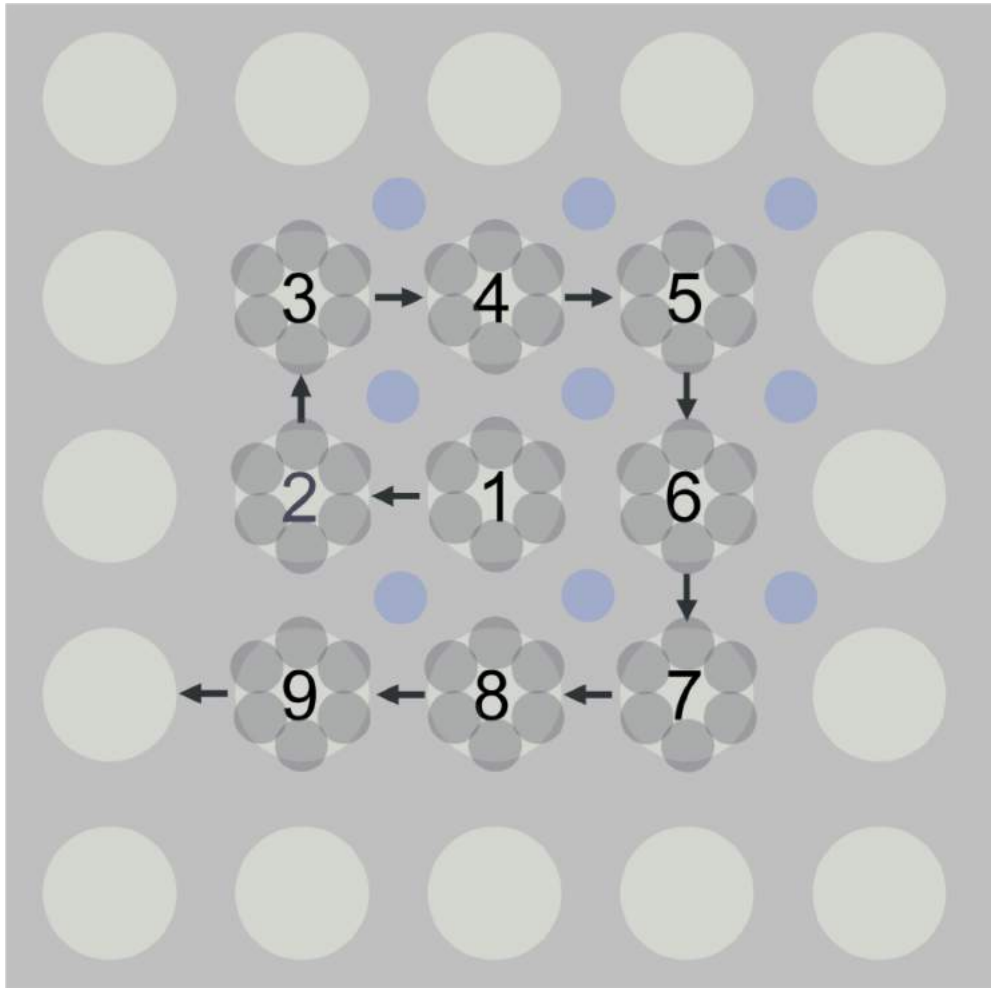
“Beam-Image Shift” Data Collection

For each selected hole:

- Move compustage to selected hole (wait for compustage to relax)
- Centre selected hole (compustage movement)
- Autofocus?
- Check drift?
- Apply beam-image shift to acquire one or more images per holes

*Compustage only moves every *n-th* holes

*This strategy is also known as AFIS (Aberration-Free Image Shift) in EPU and Active Beam Tilt Compensation in Serial EM



Beam-Image Shift

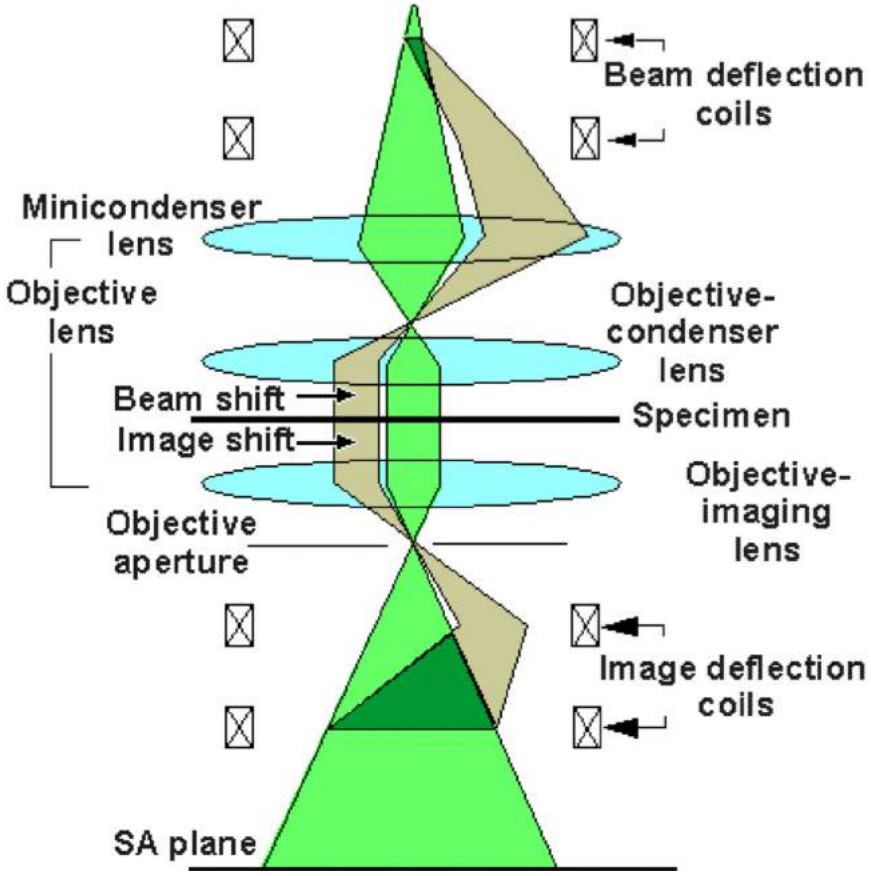
Axial coma is an image aberration that is introduced when the illumination is not parallel to the optical axis of the objective lens. This aberration is minimised with rotation center or coma-free alignments.

Up to 3A, beam tilt is negligible within ±3 to 4.5 µm of beam-image shift (*Chen A. et. al., JSB 2018*).

Coma vs Beam-Image is linear -> more image shift more coma

Serial EM can compensate for beam tilt (active beam tilt compensation) when using beam-image shift. Compensation is illumination depended.

EPU has also implemented an Aberration Free Image Shift (AFIS) method. Field service engineers to calibrate the beam tilt vs beam-image shift.



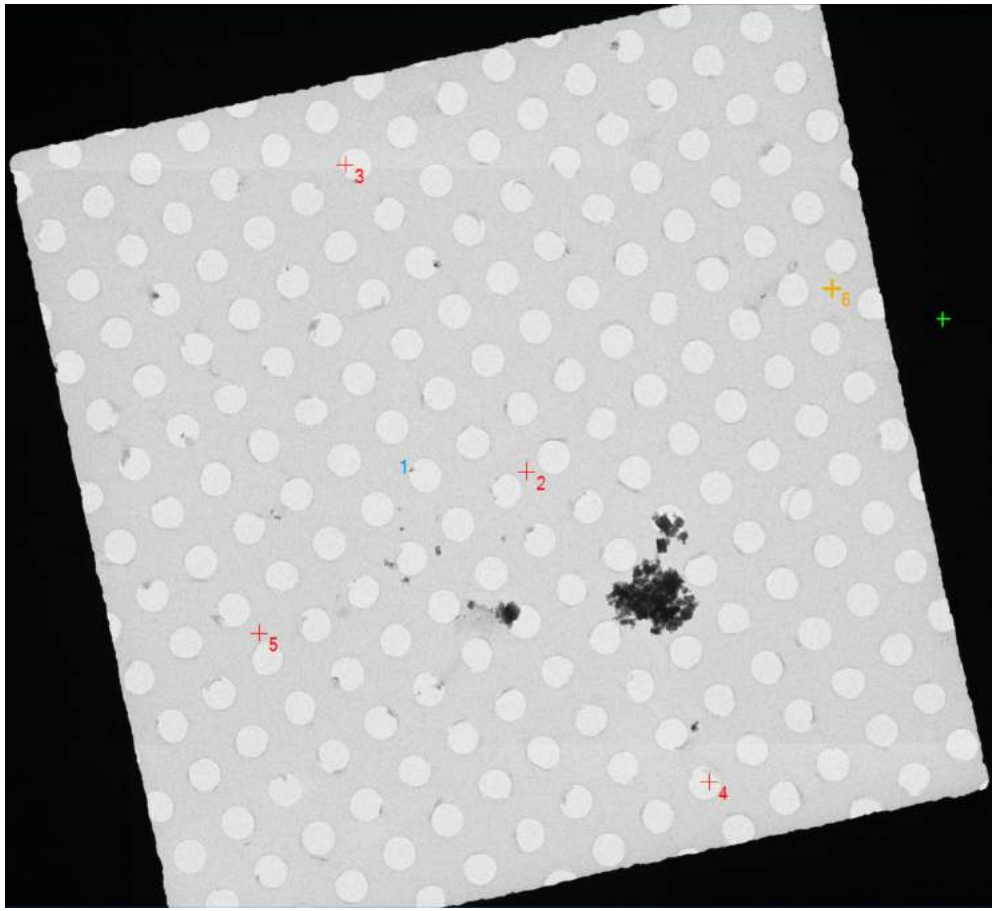
From FEI TEM Manual

How Much Beam-Image Shift?

EPU: TFS decides for you
SerialEM: you decide

Max image shift: ±24 um
Max holes per compustage movement:

- Quantifoil 0.6/1 = 21 x 21 = 441
- Quantifoil 1.2/1.3 = 14 x 14 = 196
- Quantifoil 2/2 = 8 x 8 = 64



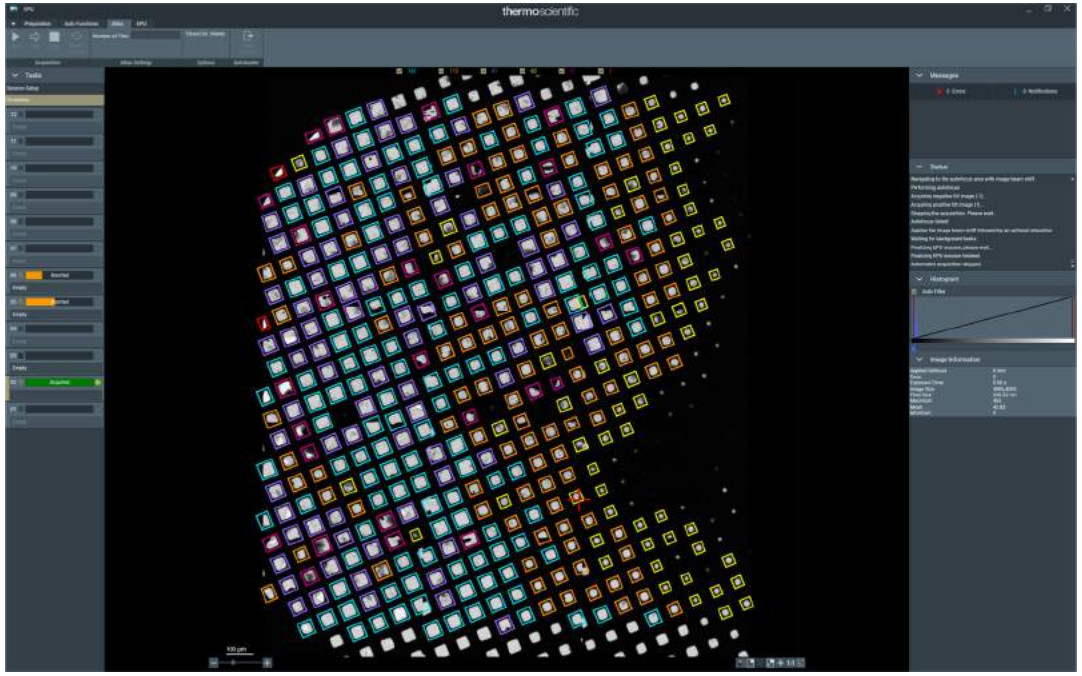
Avoid to shift more than 10 um

EPU (*E Pluribus Unum* – Out of many, one)

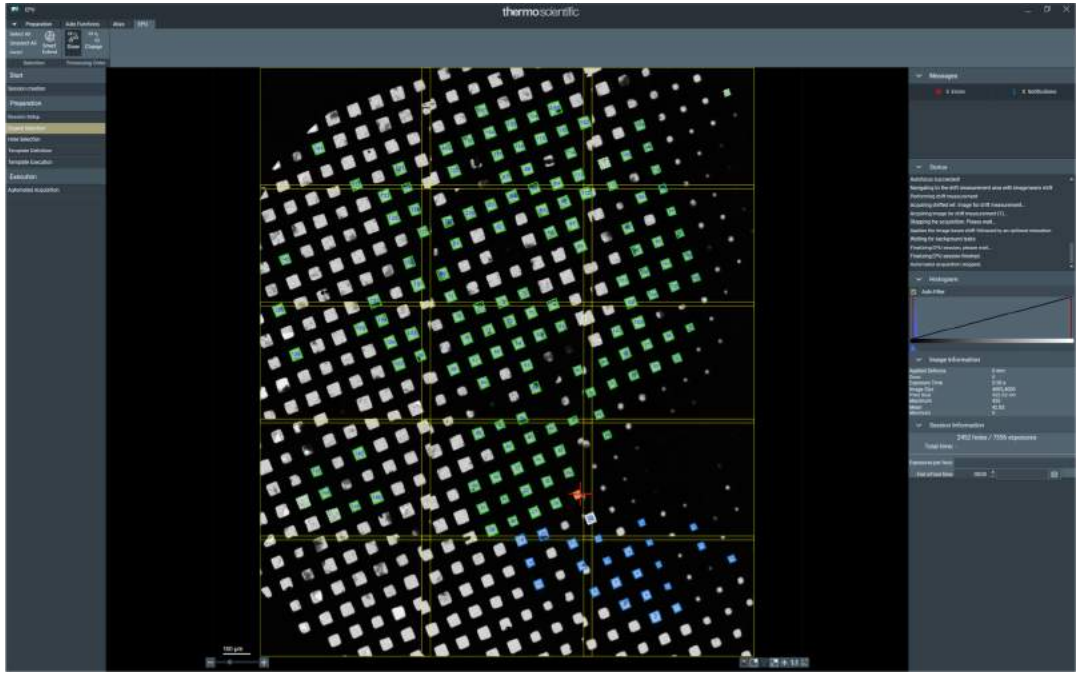
- Very user friendly
- Not open source
- Plug and play
- Only used for SPA (Tomo5 for tomography)
- Coma-free and astigmatism correction
- Active beam tilt compensation (Aberration Free Image Shift - AFIS)
- Data compression (tiff, eer)
- Volta Phase Plate
- EPU Multi-Grid (you can setup multiple data collection)
- EPU Quality Monitor (on-the-fly procesing)

EPU (*E Pluribus Unum* – Out of many, one)

Atlas-Screening

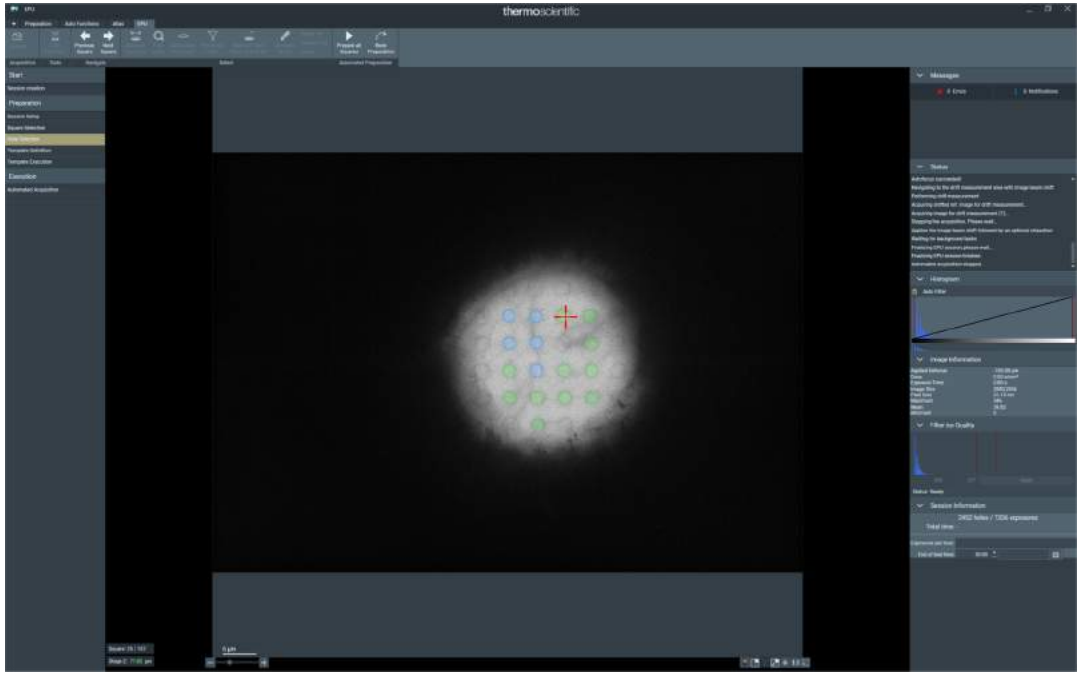


Grid Square Selection

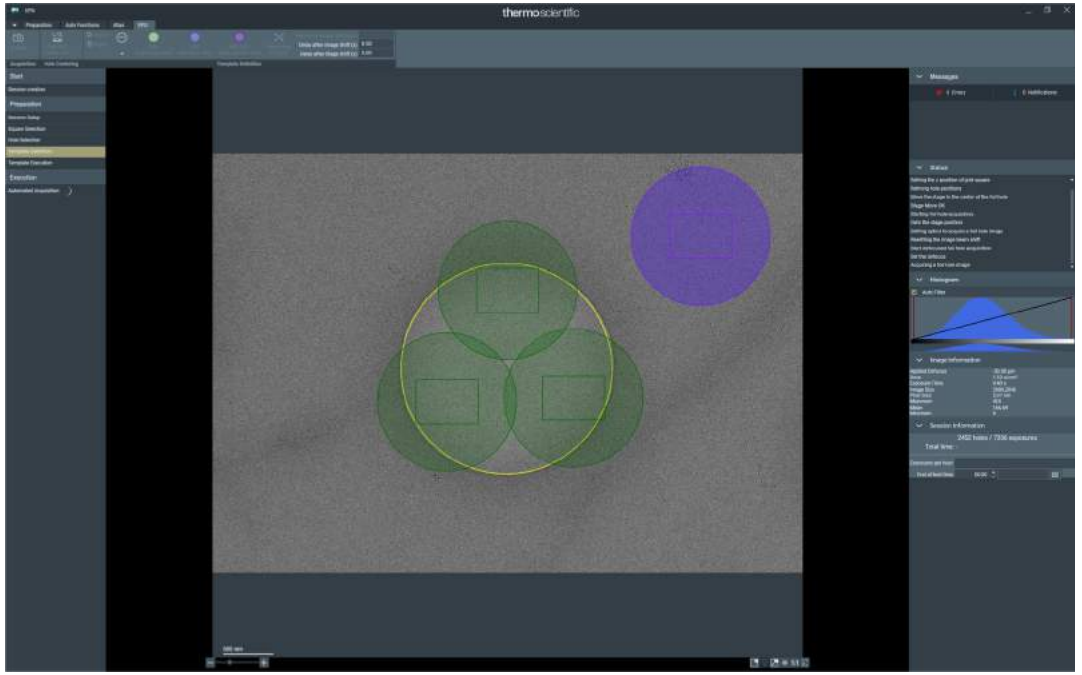


EPU (*E Pluribus Unum* – Out of many, one)

Holes Selection

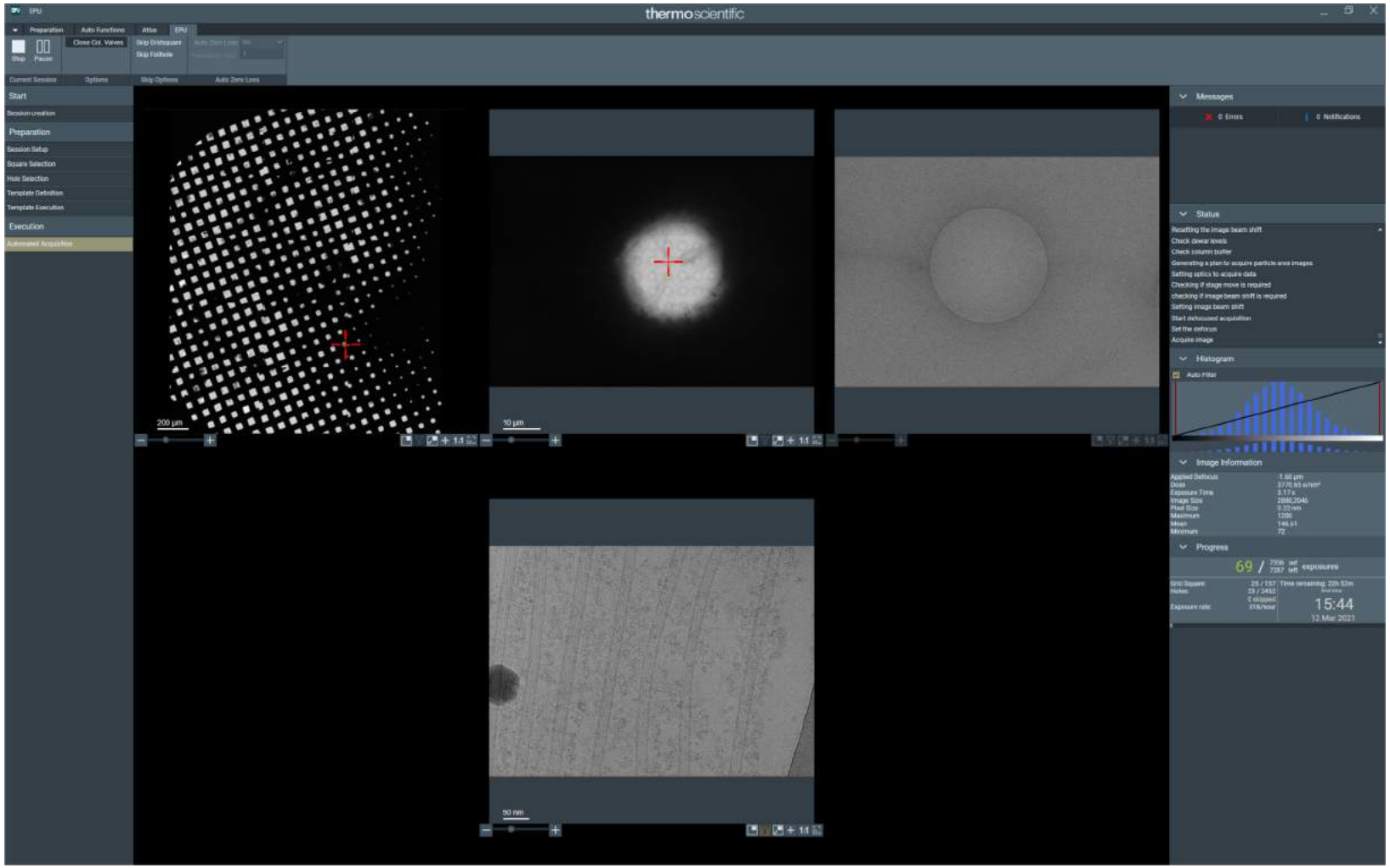


Data Acquisition Scheme



EPU (*E Pluribus Unum* – Out of many, one)

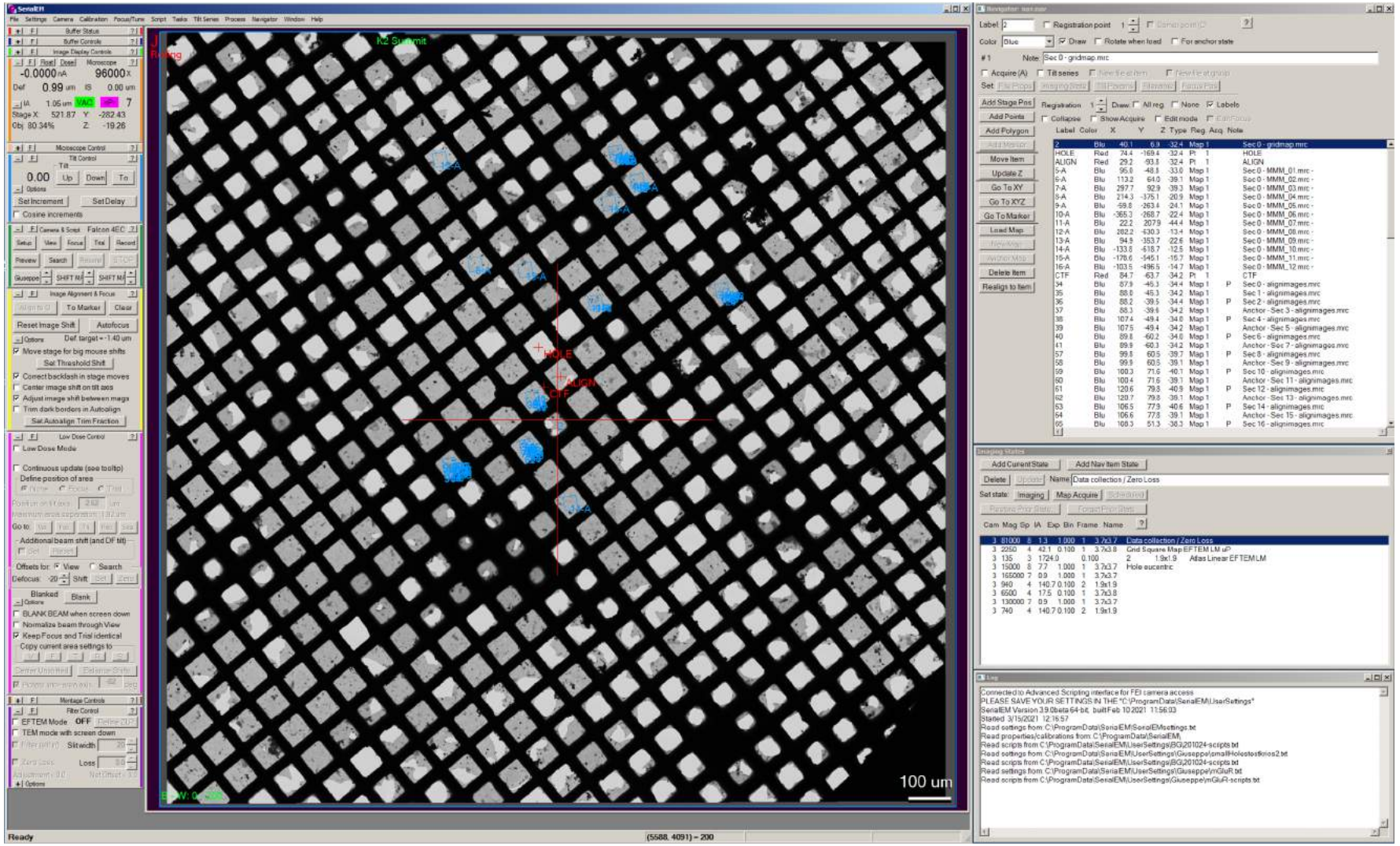
RUN!



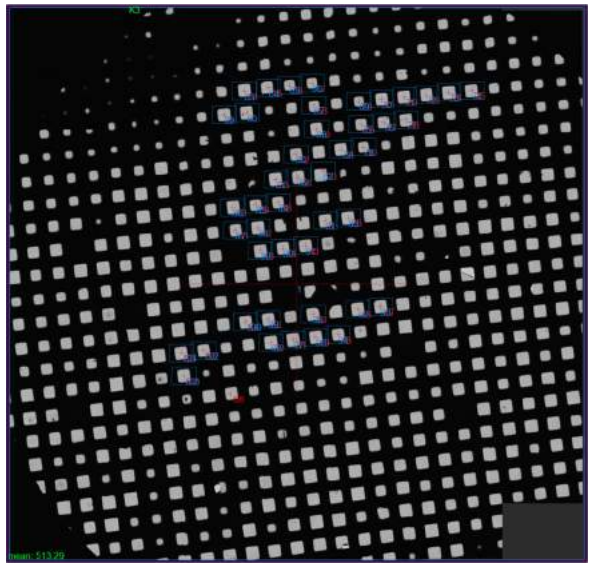
SerialEM

- Not user friendly, but can now be customized to be
- Very flexible
- Open source
- Very fast development & support
- Nearly full control of your microscope
- Repository of macros (https://serialemscripts.nexperion.net/)
- Coma-free and astigmatism correction
- Active beam tilt compensation
- Data compression (tiff, eer)
- Fully customizable metadata
- Support various microscopes and cameras
- You can develop your own plugin

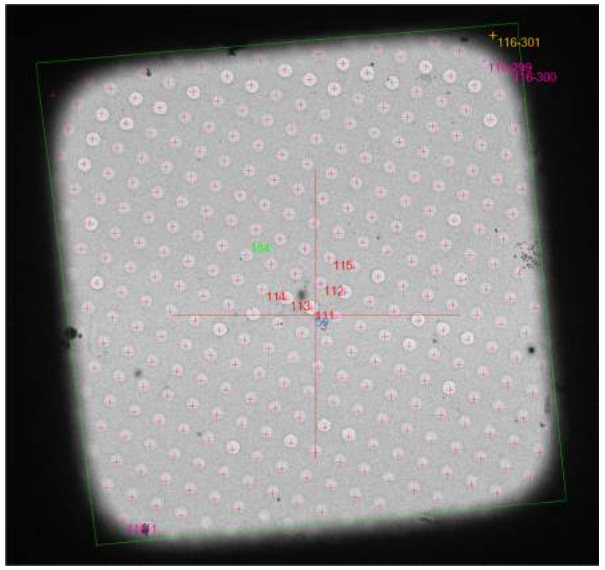
SerialEM



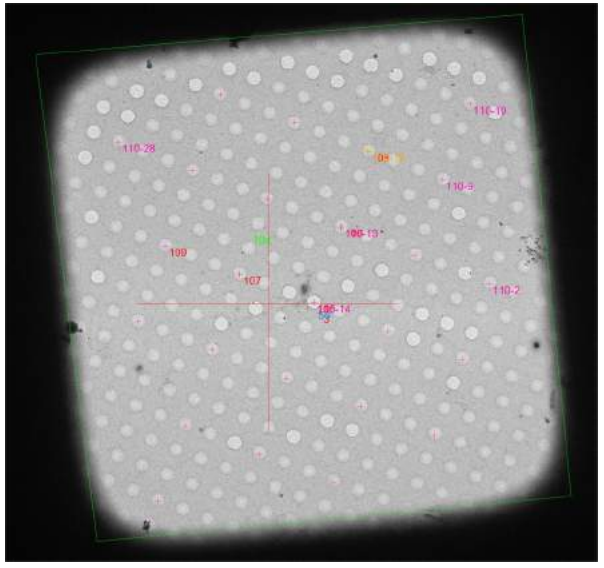
Atlas & Grid Square



135x

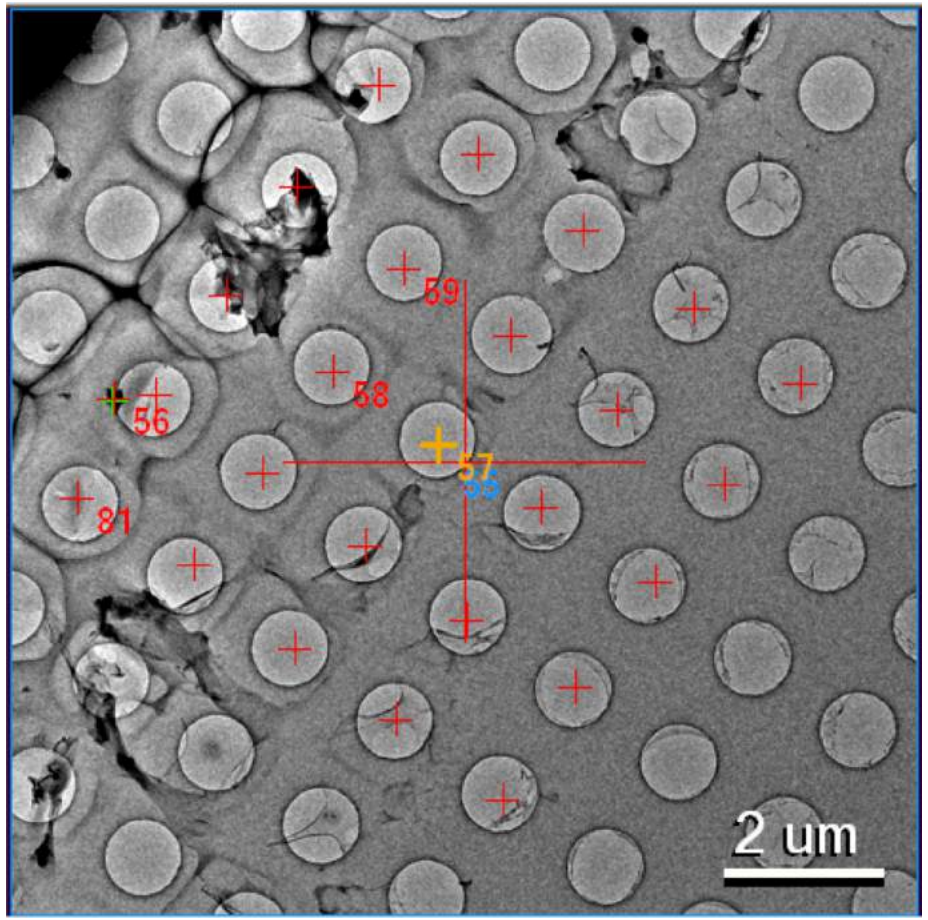


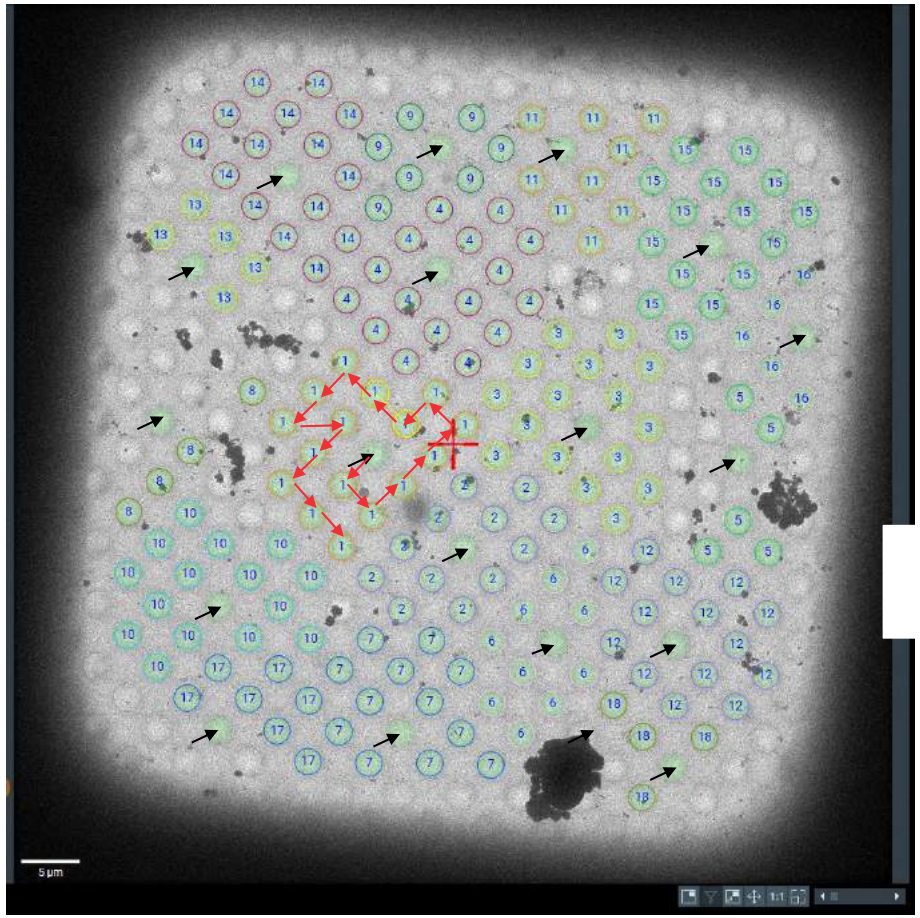
560x



560x

Image Shift and Relion _rlnOpticsGroup

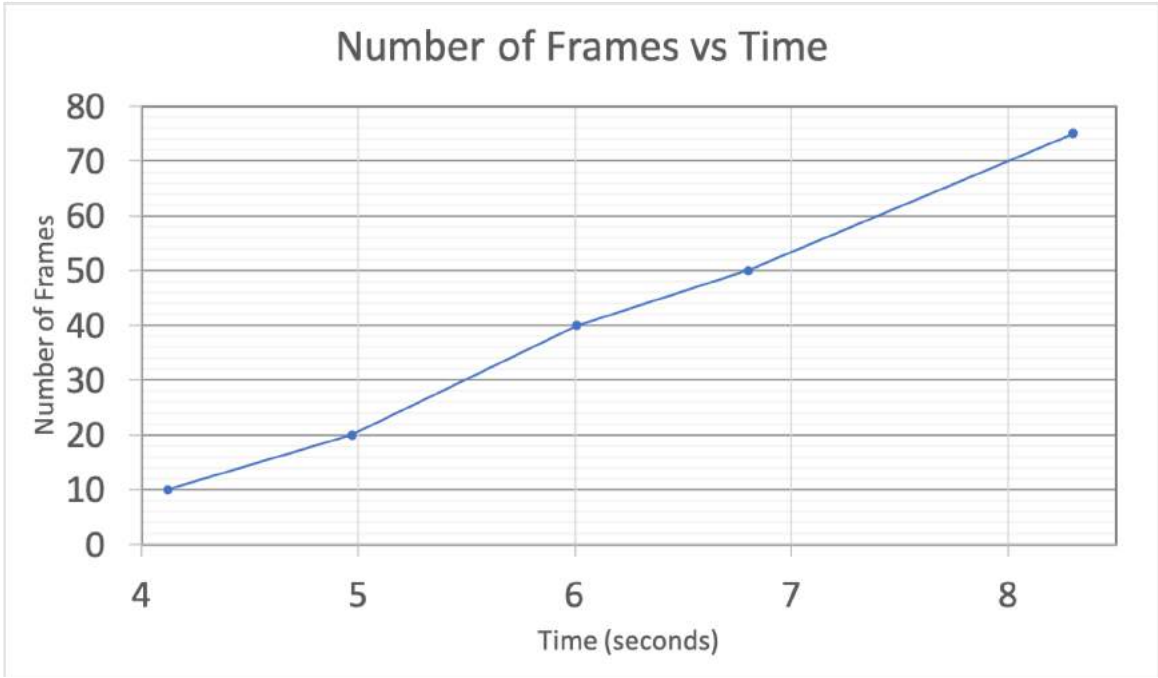




Thermo Fischer Scientific

How Fast?

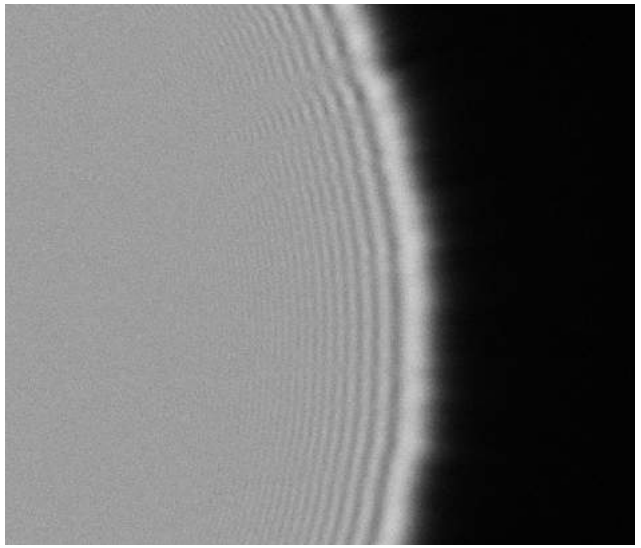
- Exposure time
- Number of frames
- Number of shots per hole
- Number of holes
- Stage stability
- Strategy for focusing and drift



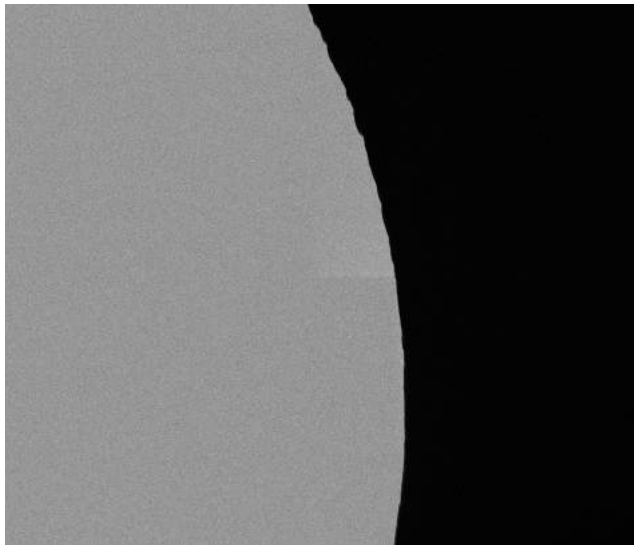
Fringe-Free Imaging

Beam fringes are due to the electron scattered by the C2 aperture

C2 out of focus



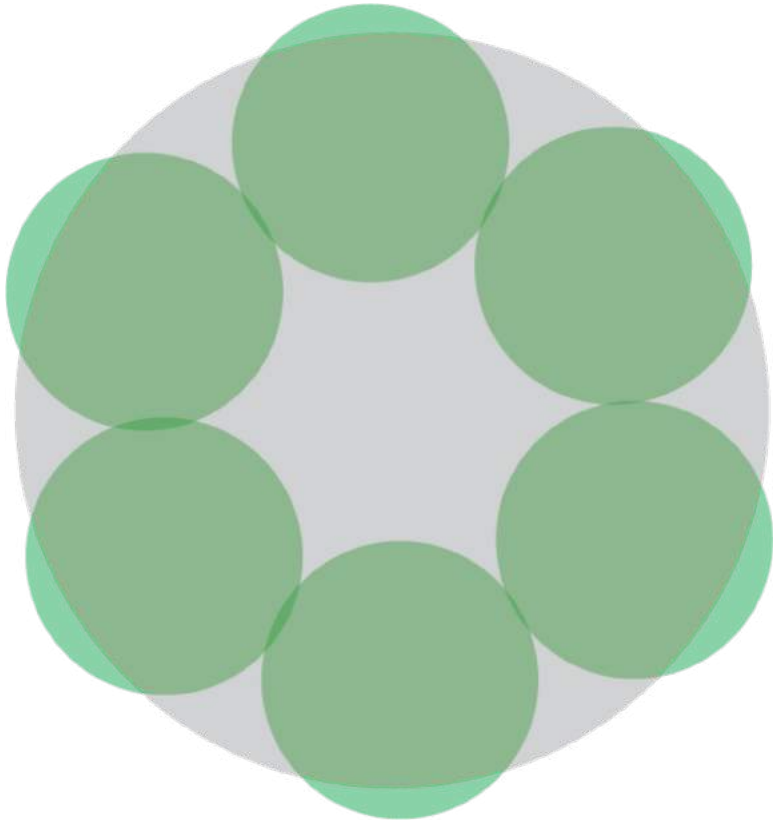
C2 in focus



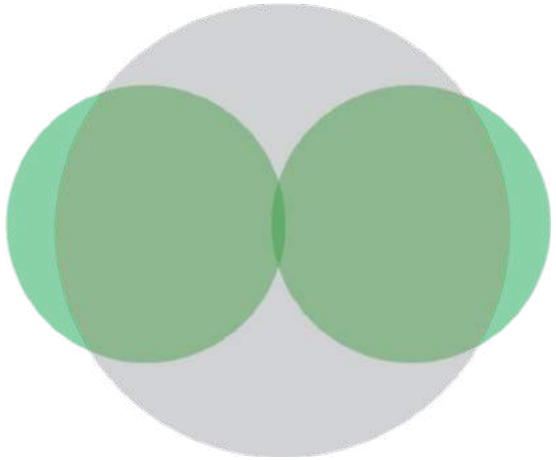
- Change “Focus” until a sharp edge is visible
- Sample no longer at eucentric, adjust Z to bring your specimen close to eucentric

- TFS can adjust compustages so that the beam is fringeless. (Paid upgrade)

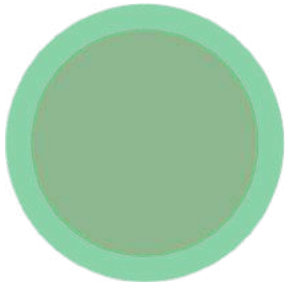
Some Examples: Beam Size 700 nm



2/2 um



1.2/1.3 um



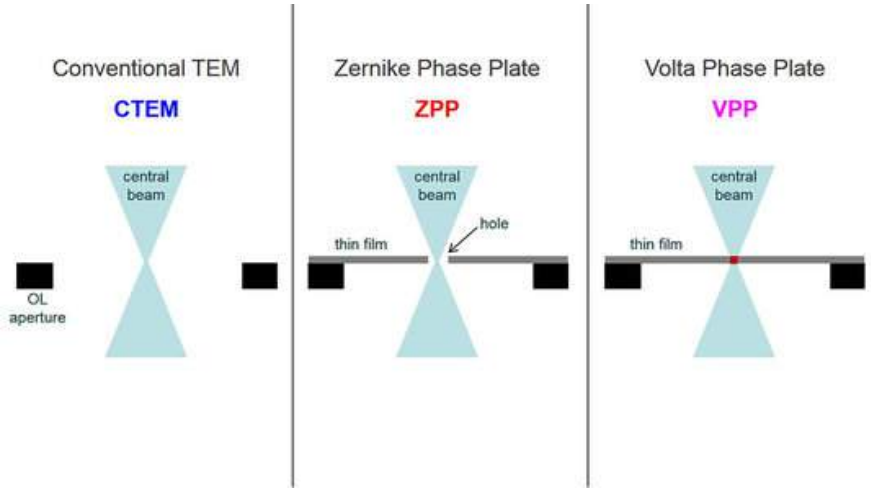
0.6/1 um

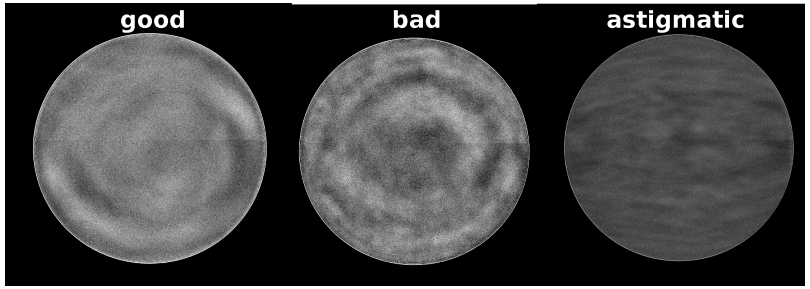
Volta Phase Plate

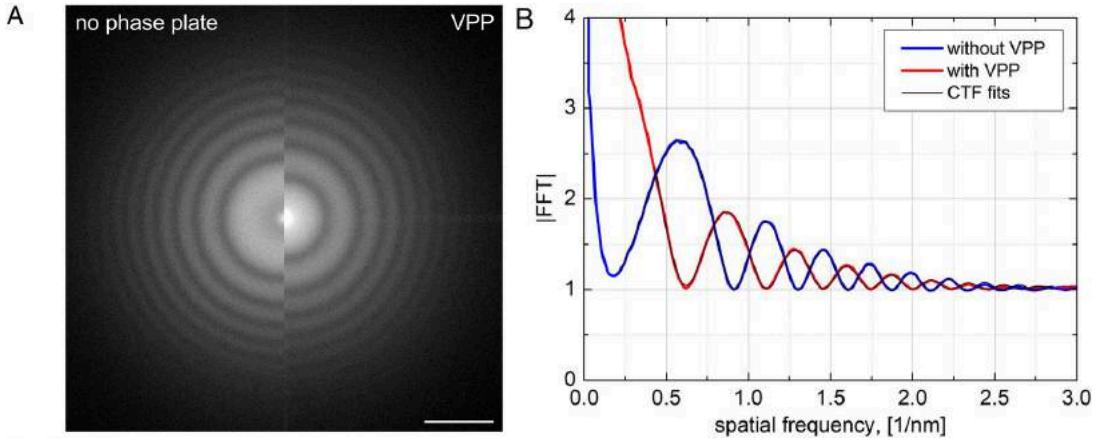
Thin (20 nm) continuous carbon film positioned in the back focal plane

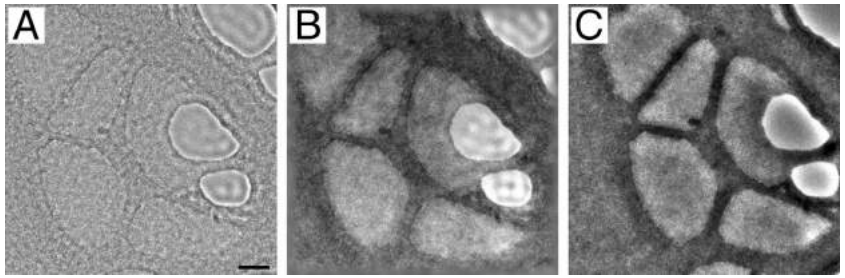
Electron beam passing through it create a Volta potential

Volta potential change the phase of the unscattered beam









Radostin Danev et al. PNAS 2014;111:44:15635-15640

Data Collection Settings

- Dose rate: K2 4-8 e/p/s; K3 10-24 e/p/s; F3L 80-100 e/p/s; F3C 0.5 e/p/s; F4EC 3 e/p/s
- Total dose: 30 – 60 e^-/Å (don't worry about radiation damage, polishing will make particles look shiny)
- Number of frames: 0.5 – 1 e^-/Å/frame (doesn't matter if using eer)
- Number of shots per hole: 1 if using AFIS
- Defosus: -0.6 to -2.8 µm (depends on the school)
- Autofocus recurrence: every 8 – 10 µm; every hole with VPP

Image Format and Data Compression

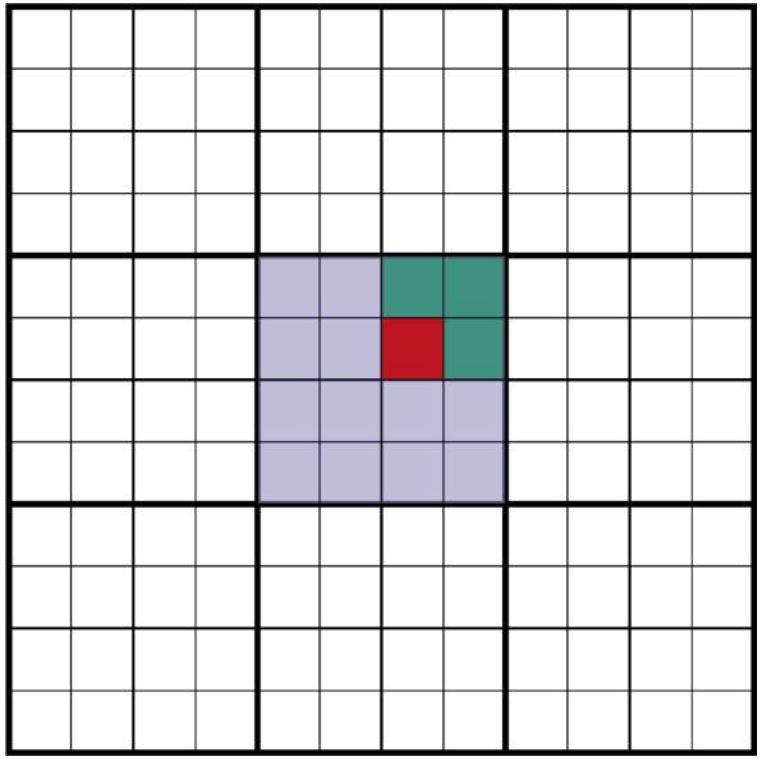
Format	Detector	EPU	SerialEM	Post-acquisition
MRC	K2, K3, F3, F4	Yes	Yes	IMOD, Relion (up to 68%)
TIFF	K2, K3	Yes*	Yes	No need
EER	F4	Yes	Yes	IMOD, Relion (30 – 75%)**

**W7 MPC only K3 can save tiffs*
*** Depends on eer fractions*

Compression rates depend on dose. Fewer electrons typically lead to better compression.

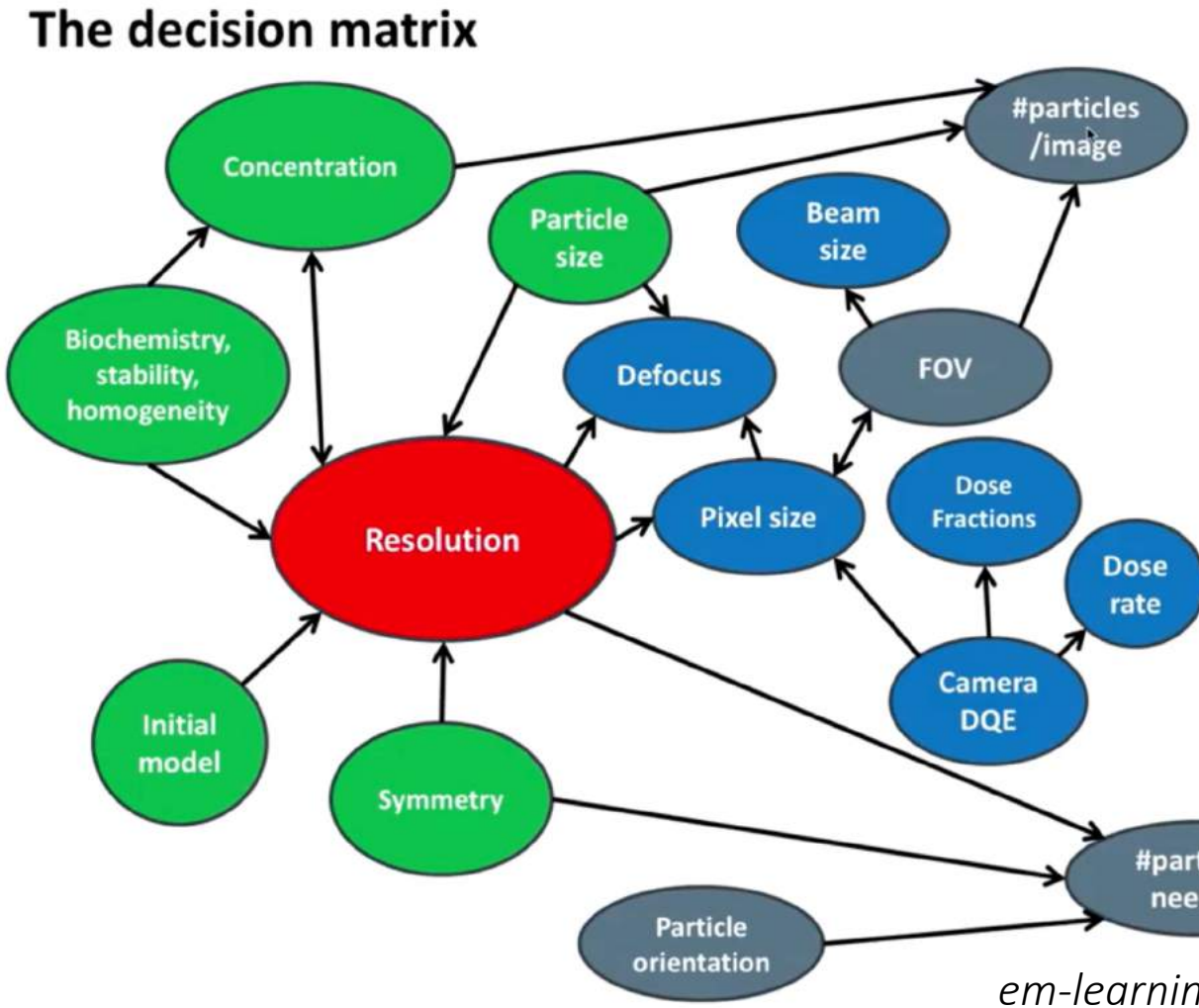
Taknori Nakane https://www3.mrc-lmb.cam.ac.uk/relion/index.php/Image_compression

EER

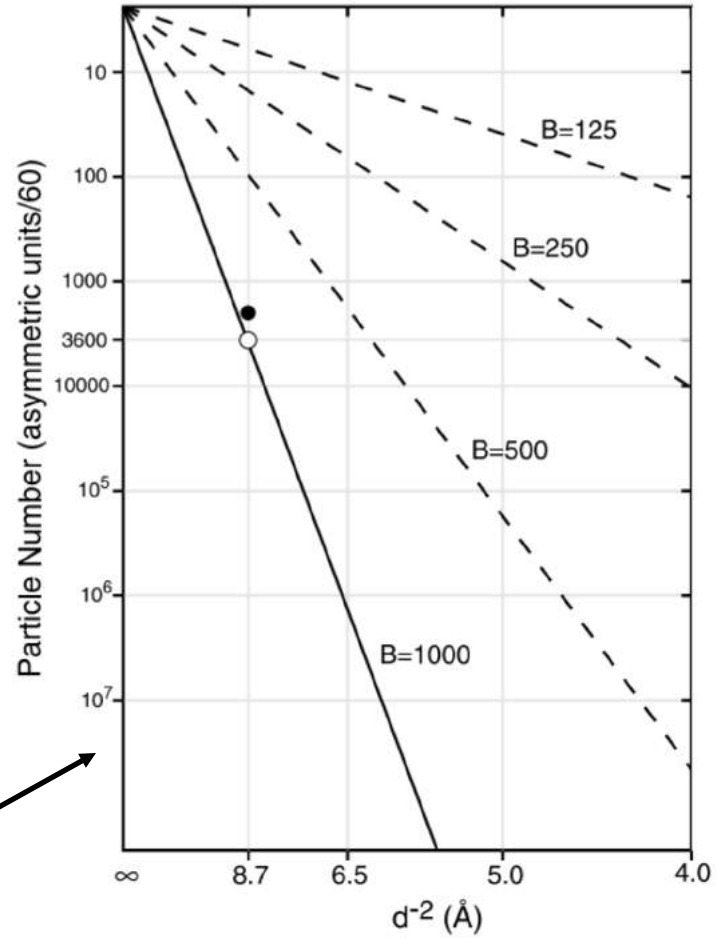


Sampling	Pixel Size
3x3 (2, 2, 1)	1Å
6x6 (4, 3, 1)	0.5Å
12x12 (7, 6, 1)	0.25Å

What to Consider?



em-learning.com

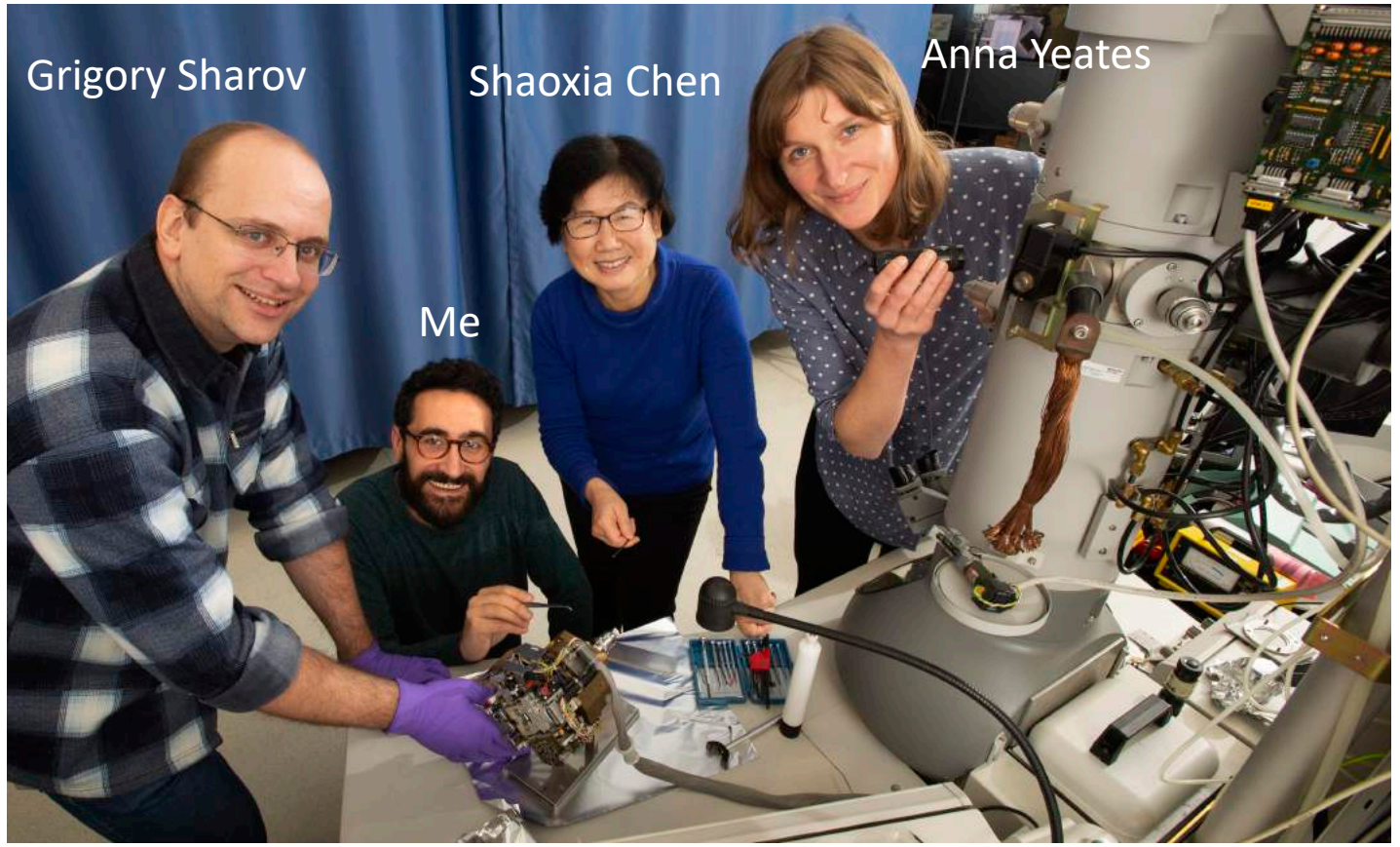


Rosenthal & Henderson

Resources for Learning

- MRC-LMB cryo-EM course (https://www.youtube.com/user/LMBCambridge/videos)
- Grant Jensen (https://www.youtube.com/playlist?list=PLhiuGaXlZZenm7lu5qv_A59zEWkRKkBn5)
- EM-Learning (https://em-learning.com)

Acknowledge

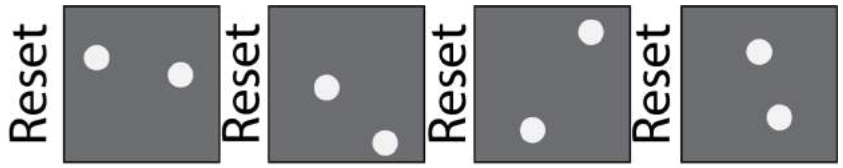


Greg McMullan

Thanks

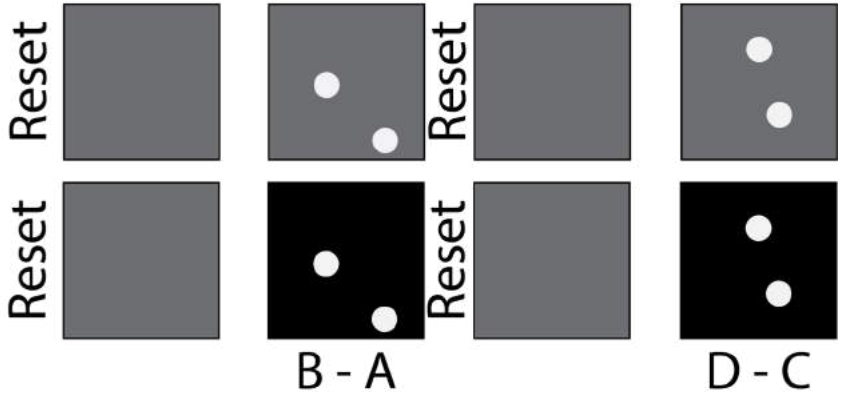
Counting – Correlated Double Sampling

NoCDS K2, K3



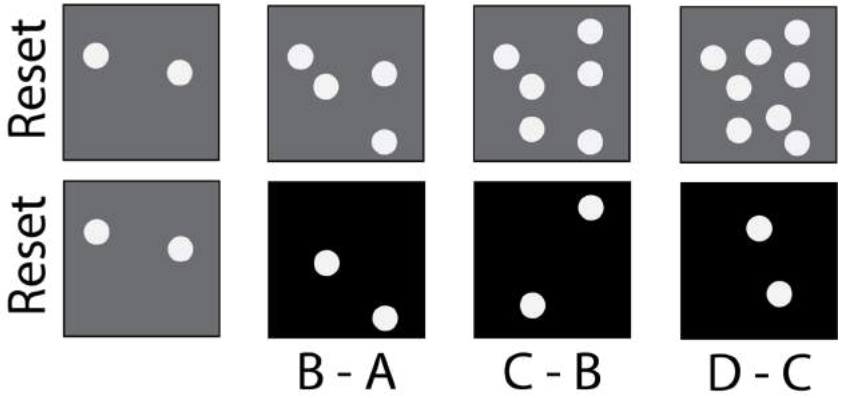
Full speed; No optimal DQE

CDS K3



½ Speed; Improved DQE

mfCDS F3, F4?



Full Speed; Improved DQE

Integration vs Counting

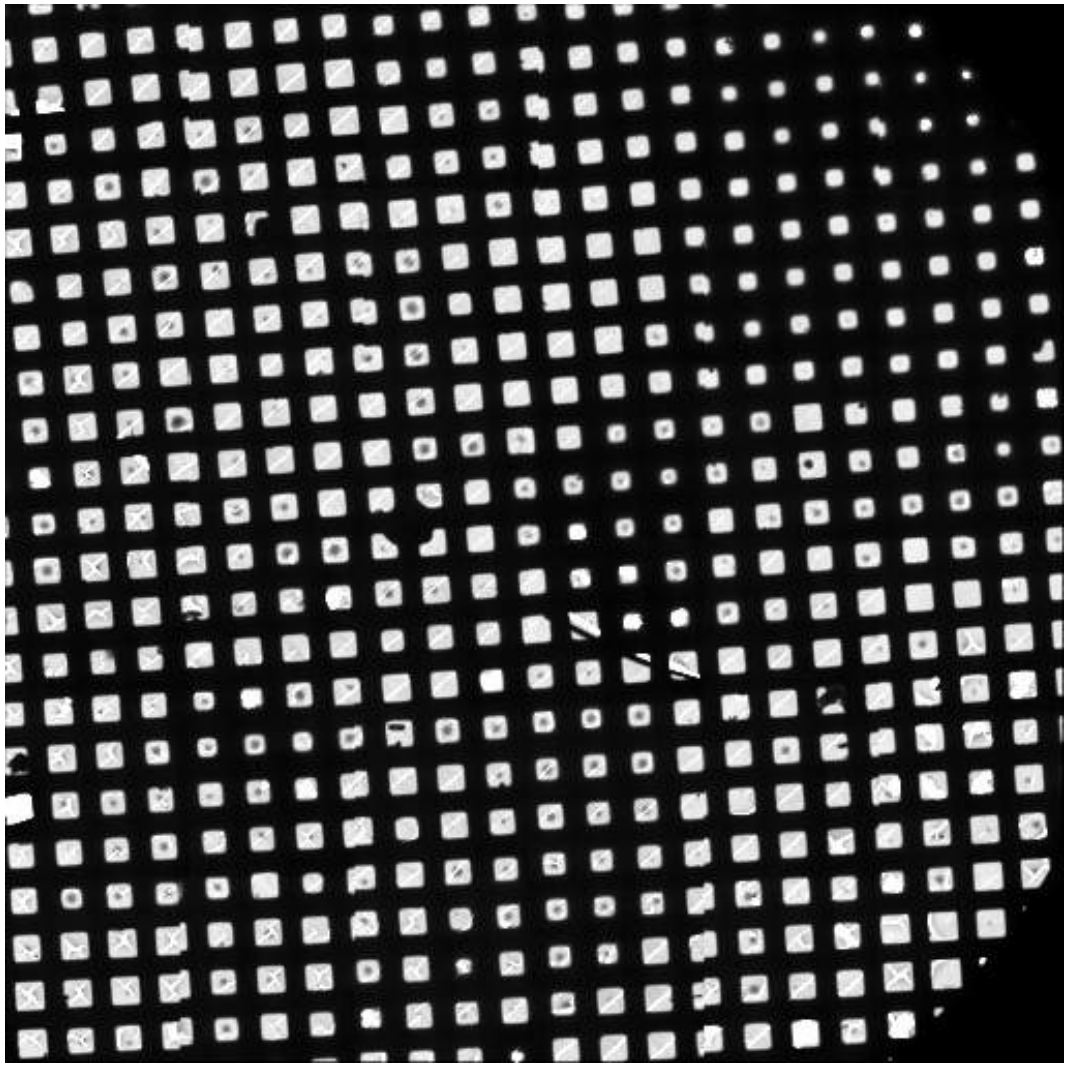
Integrating

- Lower DQE
- Higher dose rate (80 – 120 e/p/s)
- Shorter exposure
- Higher throughput

Counting

- Higher DQE
- Lower dose rate (0.5 – 32 e/p/s)
- Exposure: detector-dependent
- Throughput: detector-dependent

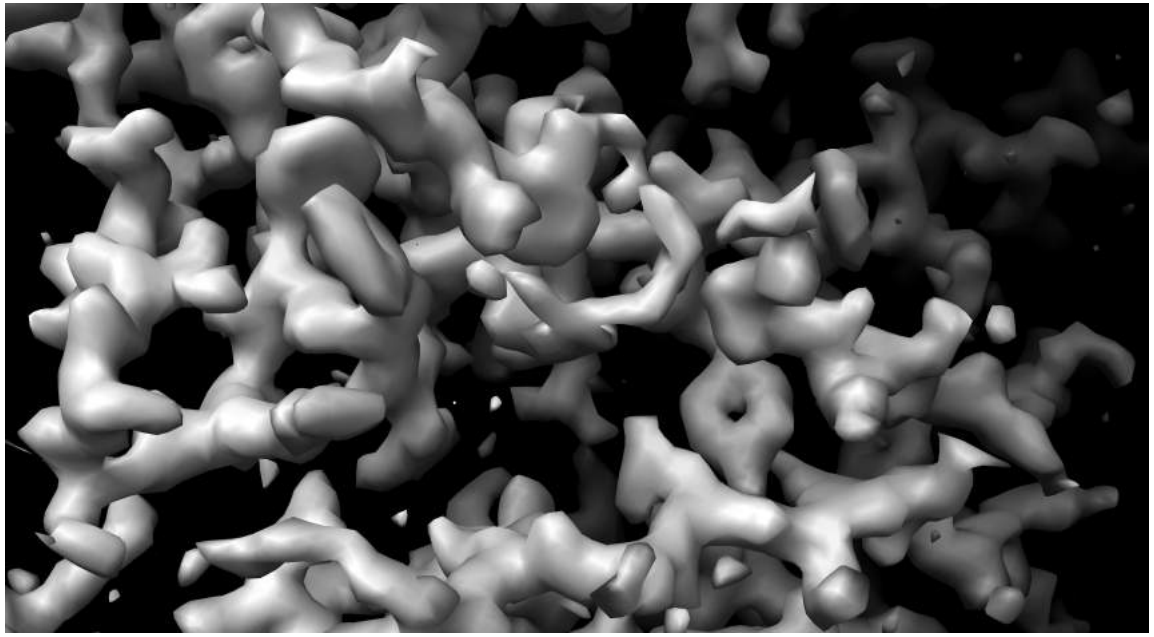
Initial screening: Atlas (SerialEM, EPU)



Does it Work?

3.0	2.9	3.1	2.7	2.7	2.8
2.9	2.8	3.0	2.7	2.8	2.7
3.1	3.1	3.2	2.9	2.7	2.9

Before After



2.3 no Polish…

1.8 After Polishing (95k particles)…

How Fast?

- Exposure time
- Number of frames
- Number of shots per hole
- Stage stability
- Strategy for focusing and drift

One Image per hole:

- 30 images/hour for K2 in counting
- 26 images/hour for Falcon III counting
- 45 images/hour for Falcon II/III linear

Detectors: TFS Falcon 4?

Key specifications		
Camera architecture	Direct electron detection	
Sensor size	4,096 × 4,096 pixels ~ 5.7 x 5.7 cm	
Pixel size	14 x 14 μm^2	
Operating voltage	200 kV, 300 kV	
Mounting position	On-axis, bottom mounted, retractable	
Frame rate	250 fps	
Lifetime	5 years in normal use (1.5 Gpe yields <10% DQE degradation)	
Detection modes	EC	Electron counting
	Linear	Integration mode

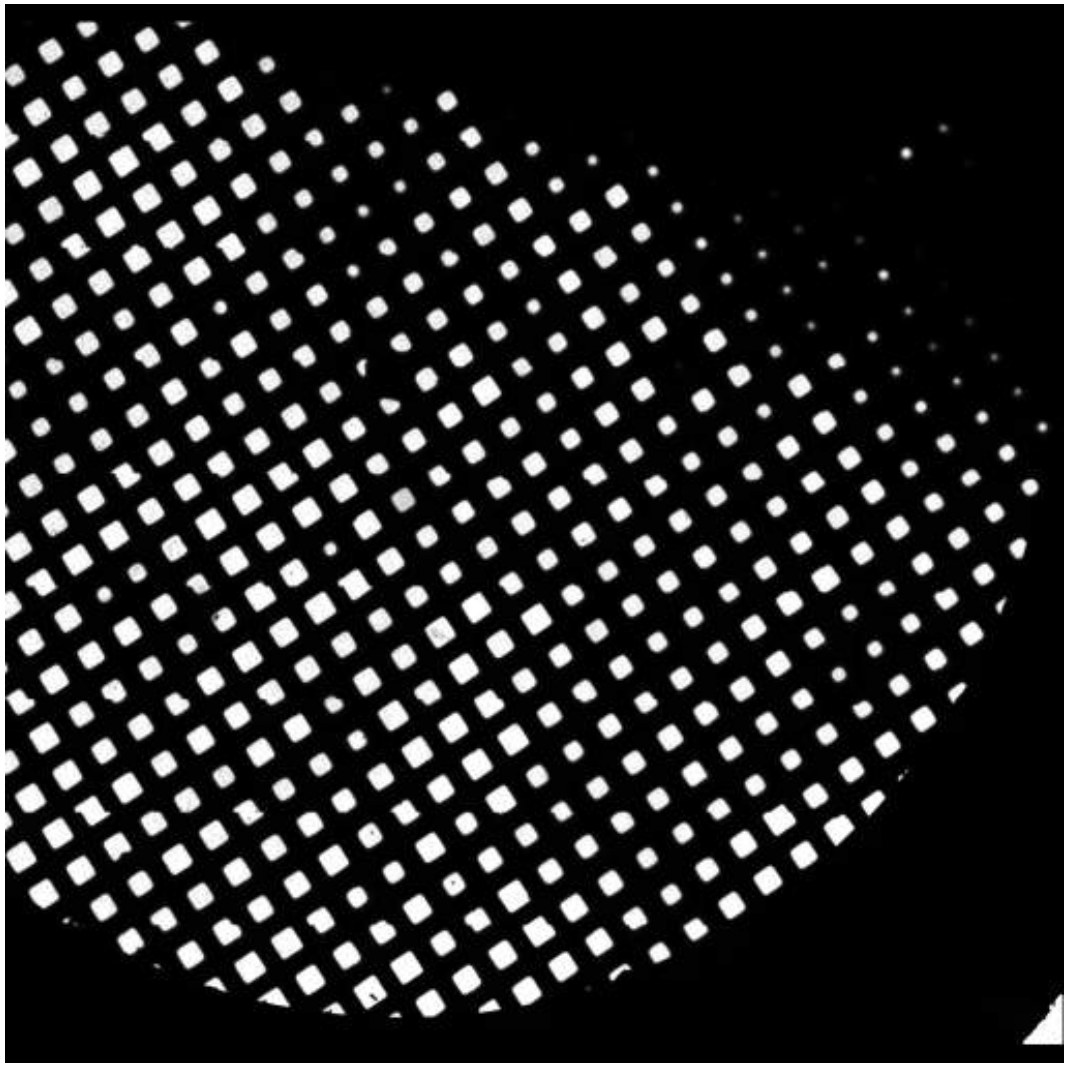
Imaging performance 4k x 4k DQE
Pixel size: 0.75 Å; Total dose: 40 e-1/$Å^2$

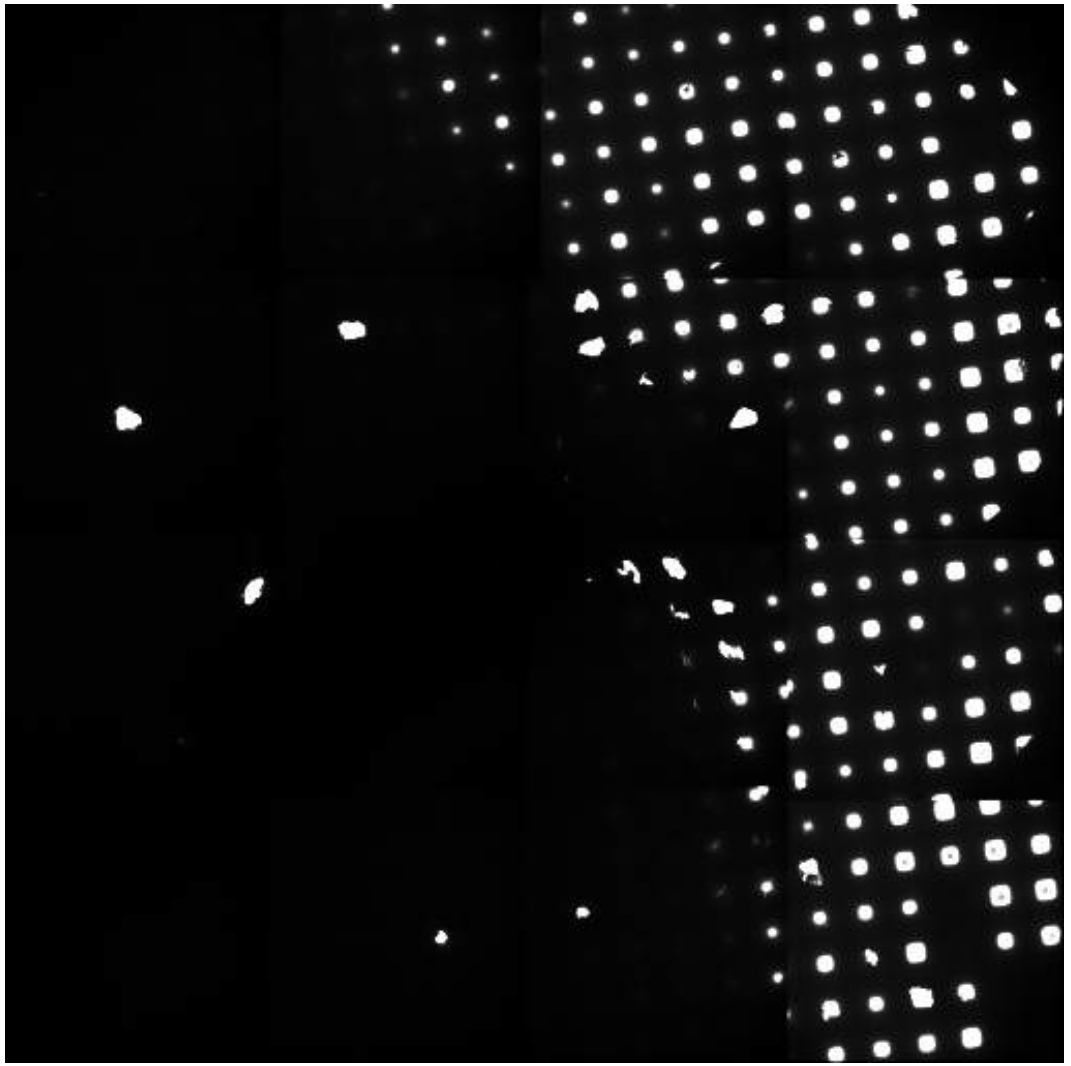
Mode	Dose rate (e/p/s)	Exp time (s)	DQE (0)	DQE (1/2 Nq)	DQE (1 Nq)
EC mode	2	11	0.90	0.75	0.35
EC mode	5	4.5	0.80	0.65	0.30
Linear mode.	20–100	0.2–1.0	0.50	0.40	0.25

Direct Electron Detector

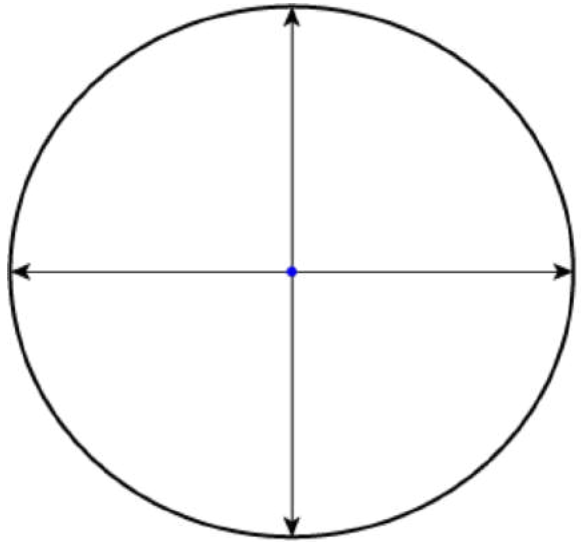
- Record Movies ->
  - reduce beam induced movement
  - Dose is not a limiting factor -> Polishing

Initial screening: Atlas (SerialEM, EPU)

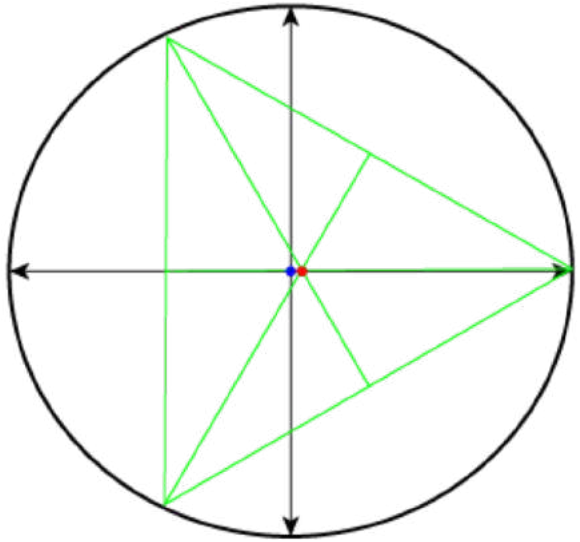




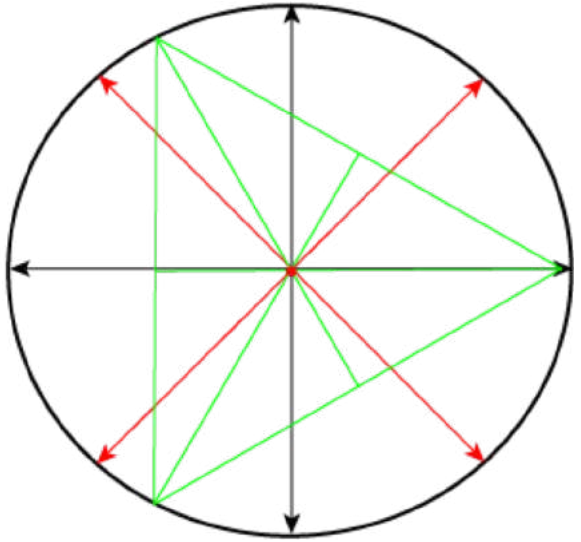
Alignments: Beam Tilt



AutoCTF



Rotation Center

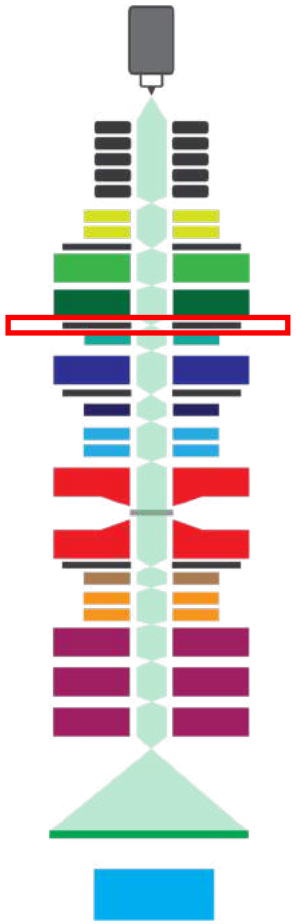


SerialEM (3x3)

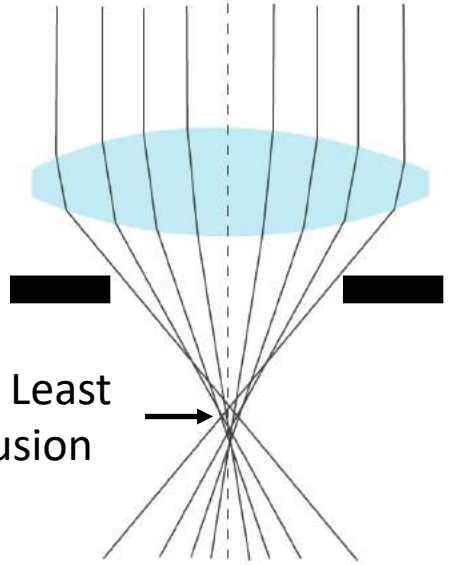
Outline

- Microscope
- Detectors
- Software
- Strategies

C2 Aperture Centering



Beam limiting aperture



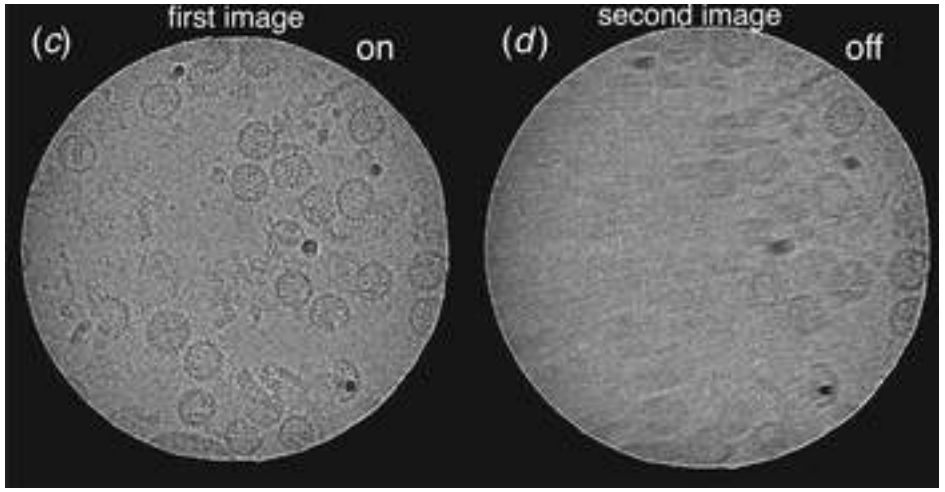
Beam expand symmetrically with respect to the screen -> does not move center when changing ”Intensity”

Beam fringes are due to the scattering from C2 aperture:

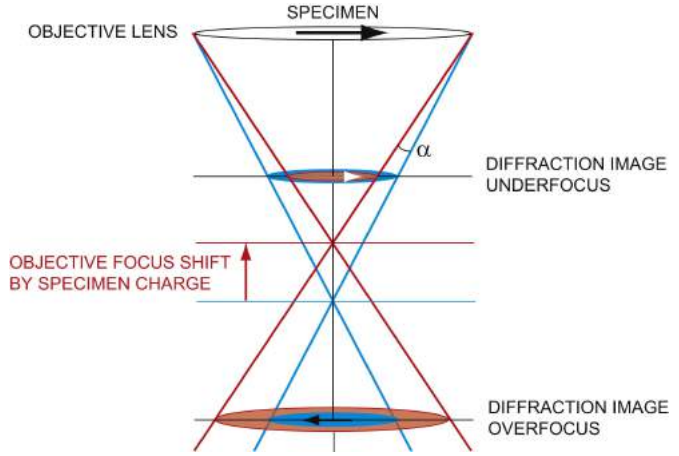
- Smaller aperture more fringes -> better image quality (more coherent illumination)
- Bigger aperture less fringes -> poorer image quality (less coherent illumination)

Charging

- Irradiation of an insulating thin film of the type studied by TEM leads to positive charge build-up due to the ejection of secondary and Auger electrons
- Charge on the sample acts as an electrostatic lens
- Conductive support can supply a current of electrons which may neutralize the positive charge
- Electrons scattered by the objective aperture or support film

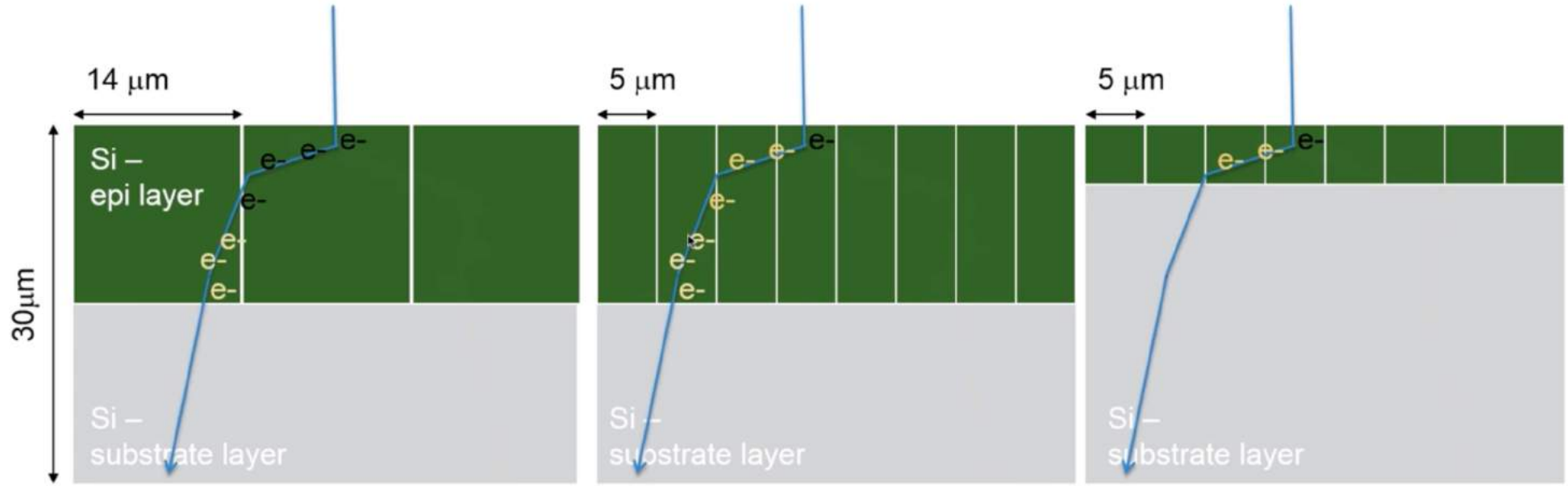


VinothKumar & Henderson, QRB, 2016



Berriman & Rosenthal, Ultramicrocopy, 2012

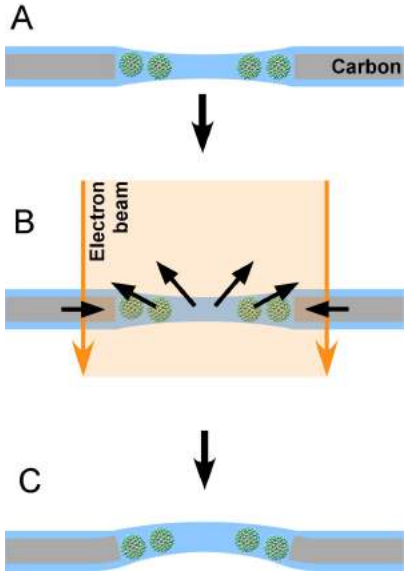
Direct Electron Detector (DED)

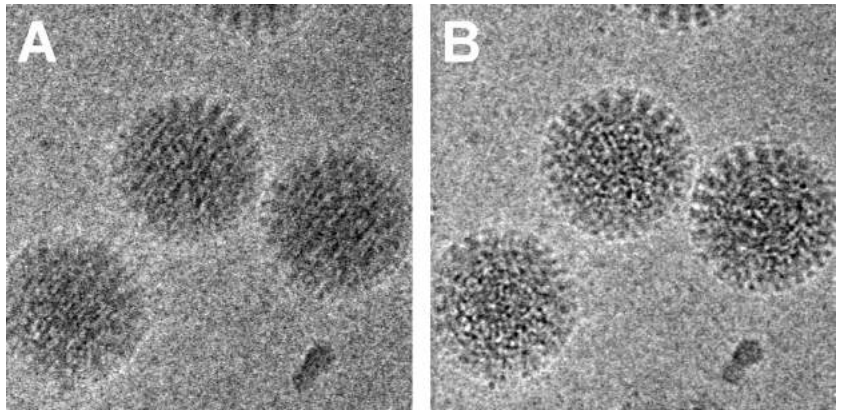


A large pixel with a thick epi layer enables better electron counting by

- more signal = higher likelihood of event detection
- more signal = less relative variation of signal
- more accurate impact location detection

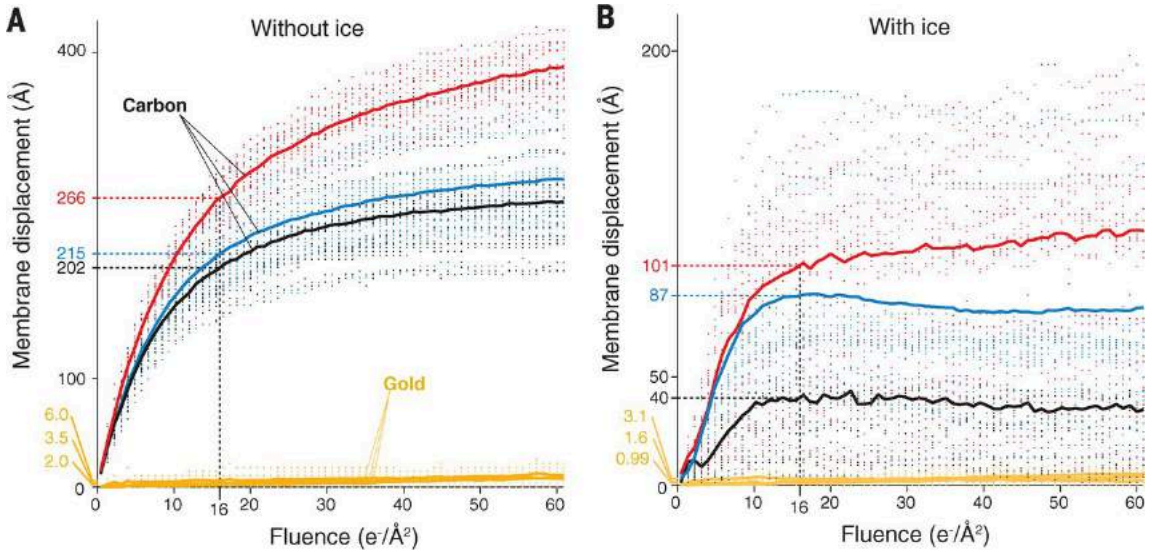
Beam Induced Movement





Brilot, et. al., JBS, 2012

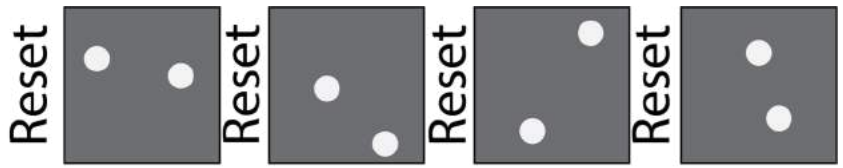
- Smaller holes appear to reduce motion on average
- BIM is proportional to the total dose



Russo & Passmore, Science, 2014

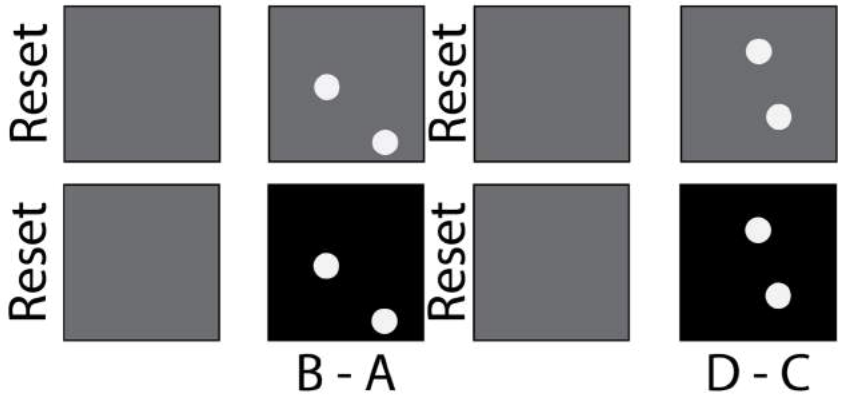
Counting – Correlated Double Sampling

NoCDS
K2 – K3



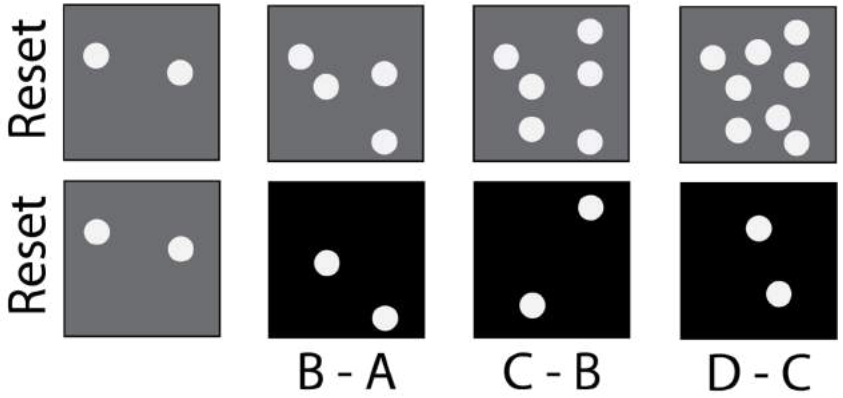
Full speed; No optimal DQE

CDS
K3



½ Speed; Optimal DQE

mfCDS
Falcon3

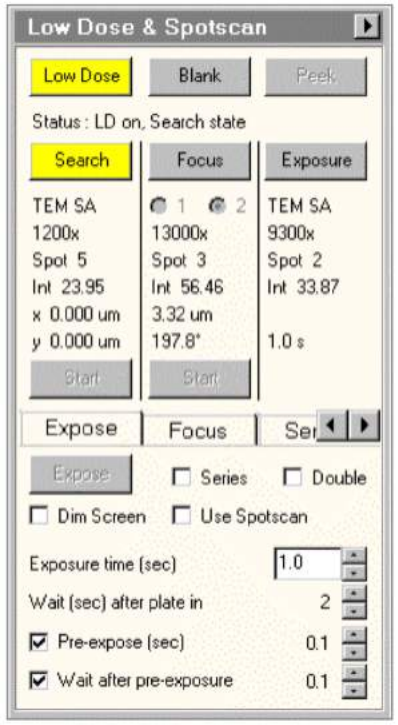


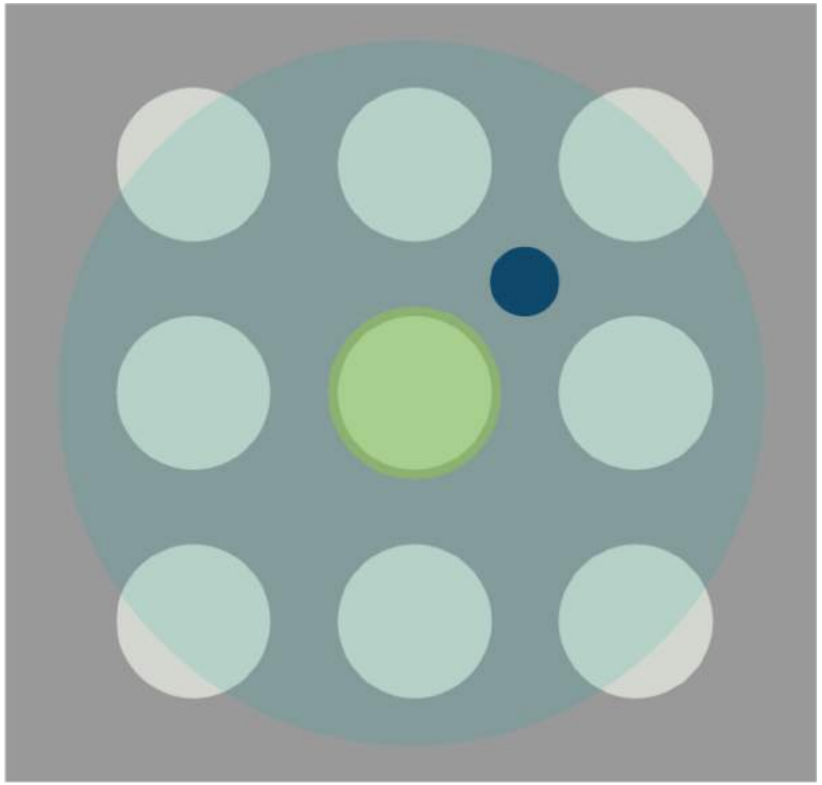
Full Speed; Optimal DQE

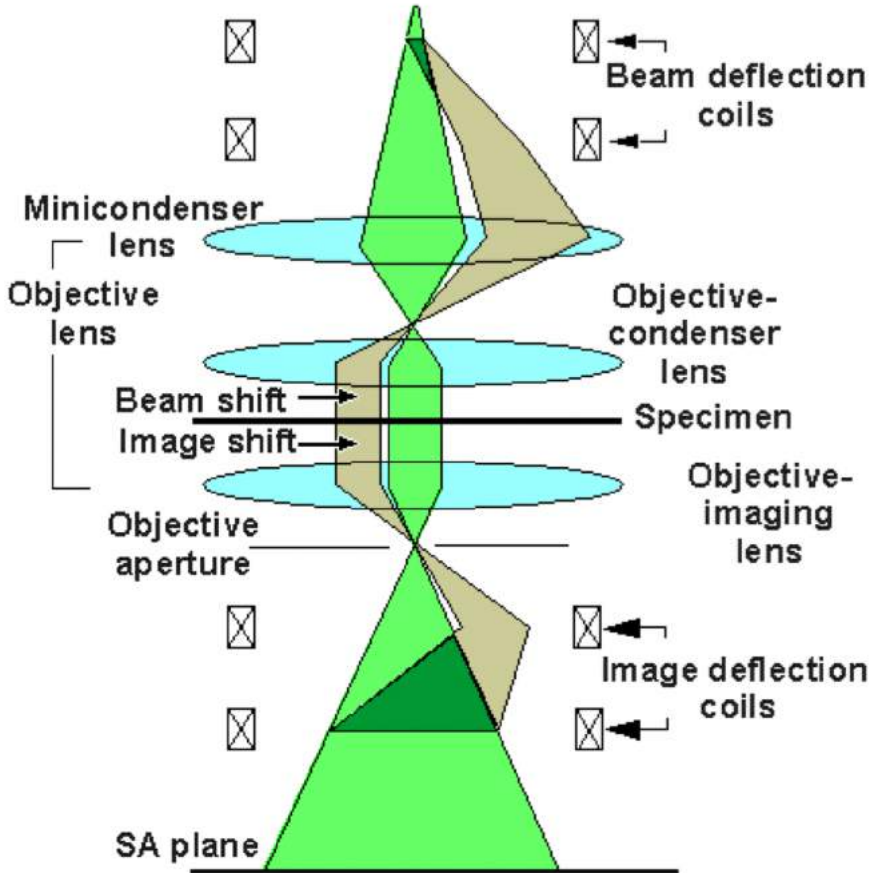
Low Dose Imaging

- Low dose imaging is a software and not a physical device controlling the dose rate.
- User controls dose rate when choosing data collection settings.
- Software records optics settings and controls beam blanking while collecting data.
- Either the software (SerialEM, EPU etc) or the user (manual data collection) controls the microscope and cycle through the settings in order to collect data.

Manual Low Dose Imaging



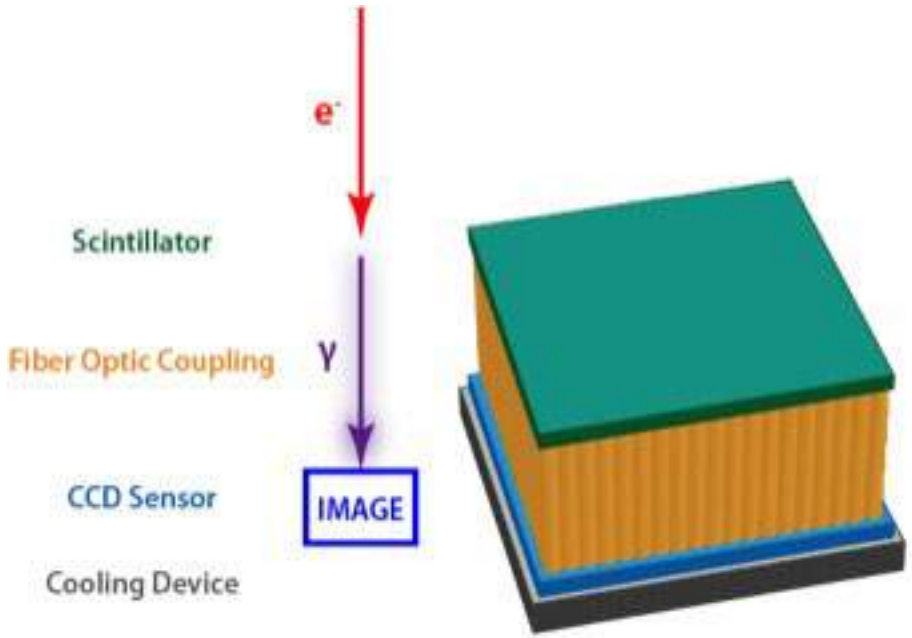




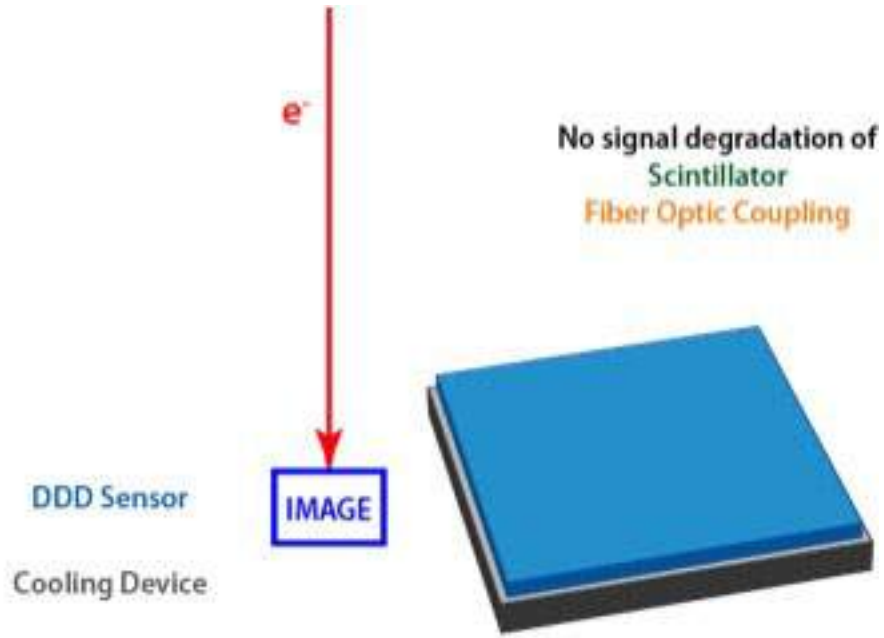
Tips

- Try to keep same spot size Hole Centering, Focus, and Data Acquisition -> Prevent beam from shifting too much
- Try to keep same illumination size for Focus and Data Acquisition settings > avoid to cycle C2 lens to much
- Use binning for Hole Centering and Focus > shorter exposure

Coupled Charged Device vs Direct Electron Detector



CCD Camera



Direct Detection Camera

https://www.directelectron.com

High-Throughput Data Collection

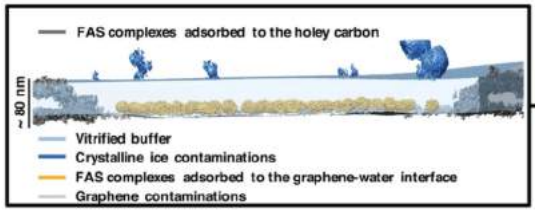
- Larger number of micrographs in allocated period of time (very large with K3 or F3L)
- It can be done with both SerialEM and EPU
- SerialEM allows to correct for beam tilt while collecting data (active beam tilt compensation) -> No need to process in groups
- EPU 2.5 will have this option
- Older versions of EPU -> Relion 3.0 will handle this for you
- Data must be saved in compressed TIFs for K2/K3
- Serial EM can save Falcon 3 frames (Krios 3 only)
- Not always compatible with VPP

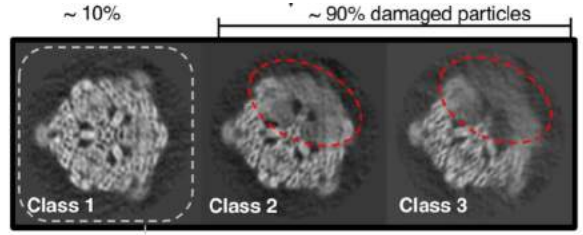
Choosing the Right Grid - Reality

There is no magic recipe, but simply try and error.

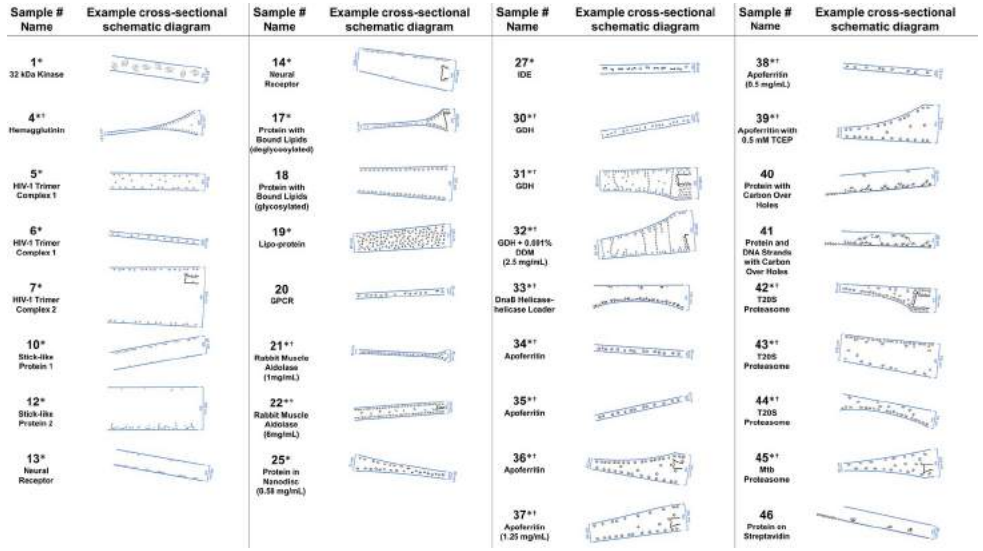
Common challenges:

- Air-water interphase -> graphene, graphene oxide or carbon support
- Preferential orientations -> functionalized graphene support
- Sub-optimal ice thickens -> blotting time, blotting force, smaller holes
- Ice pathologies (cubic, exagonal) -> improve handling.



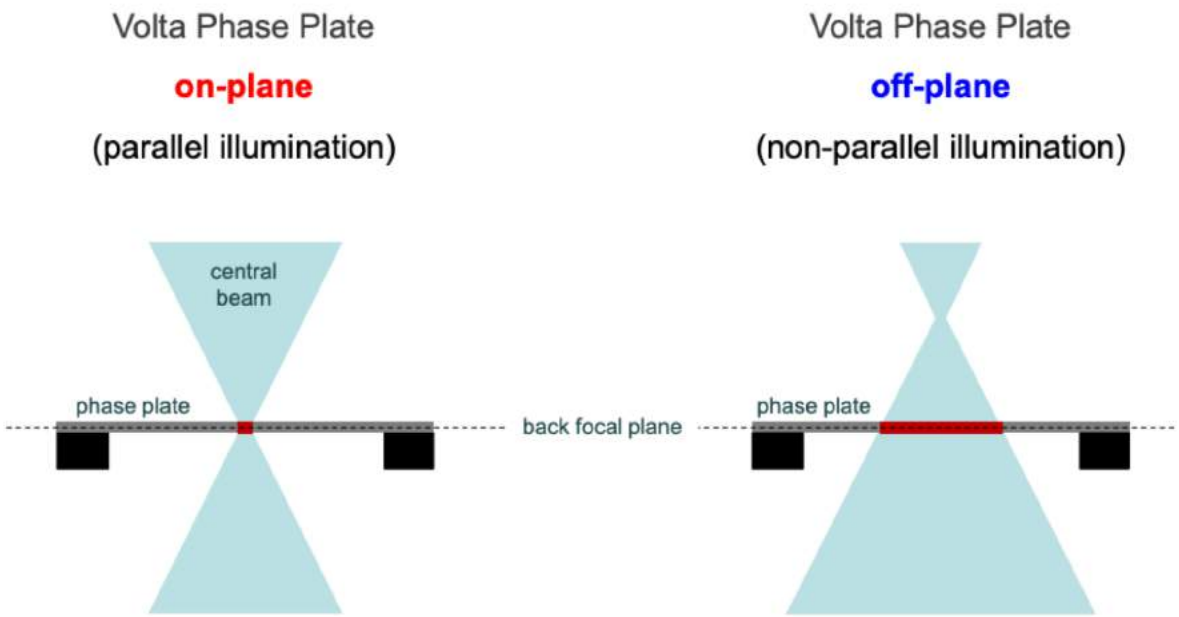


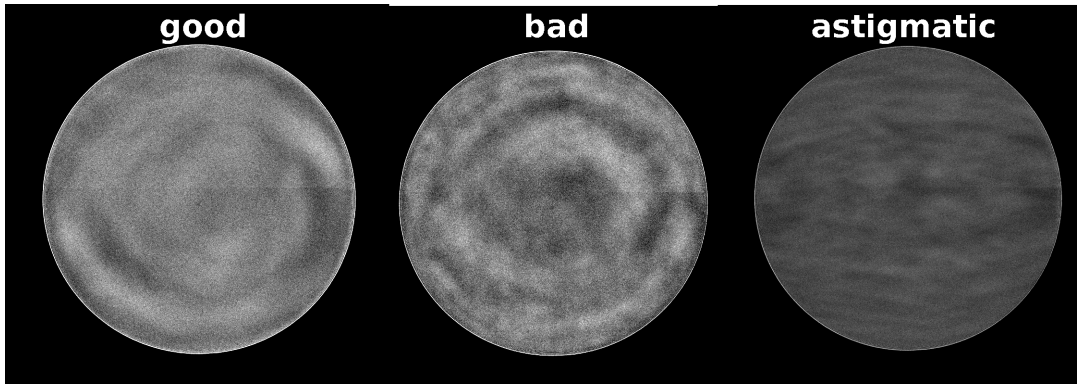
D'Imprima *et. al.*, eLife, 2019



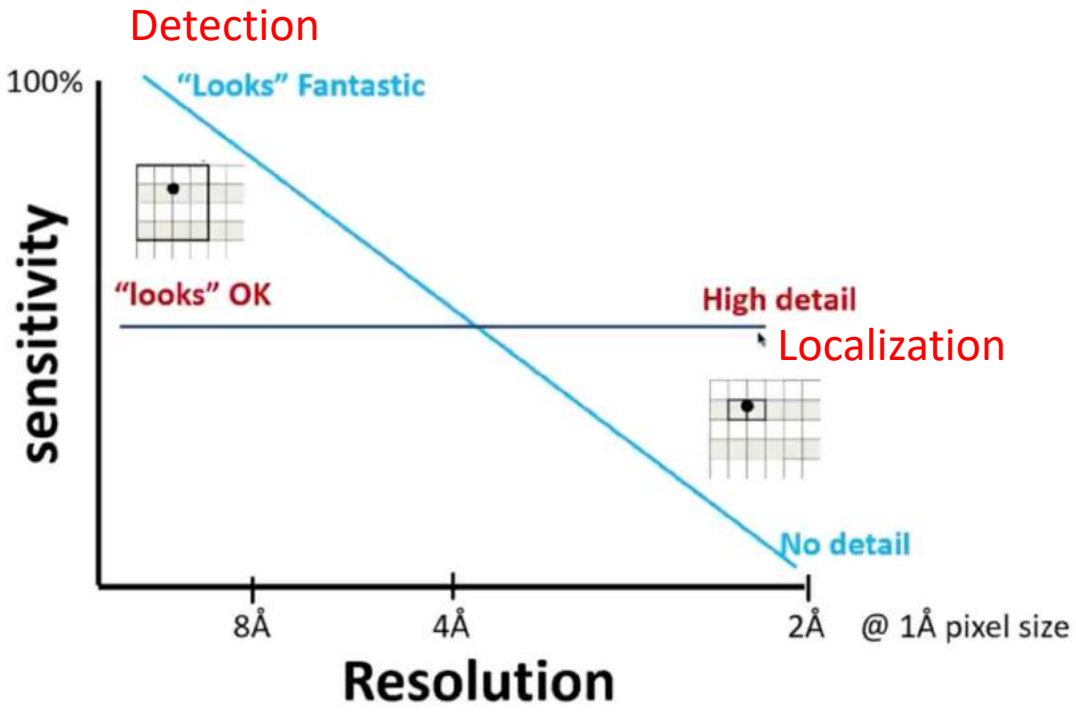
Noble *et. al.*, eLife, 2018

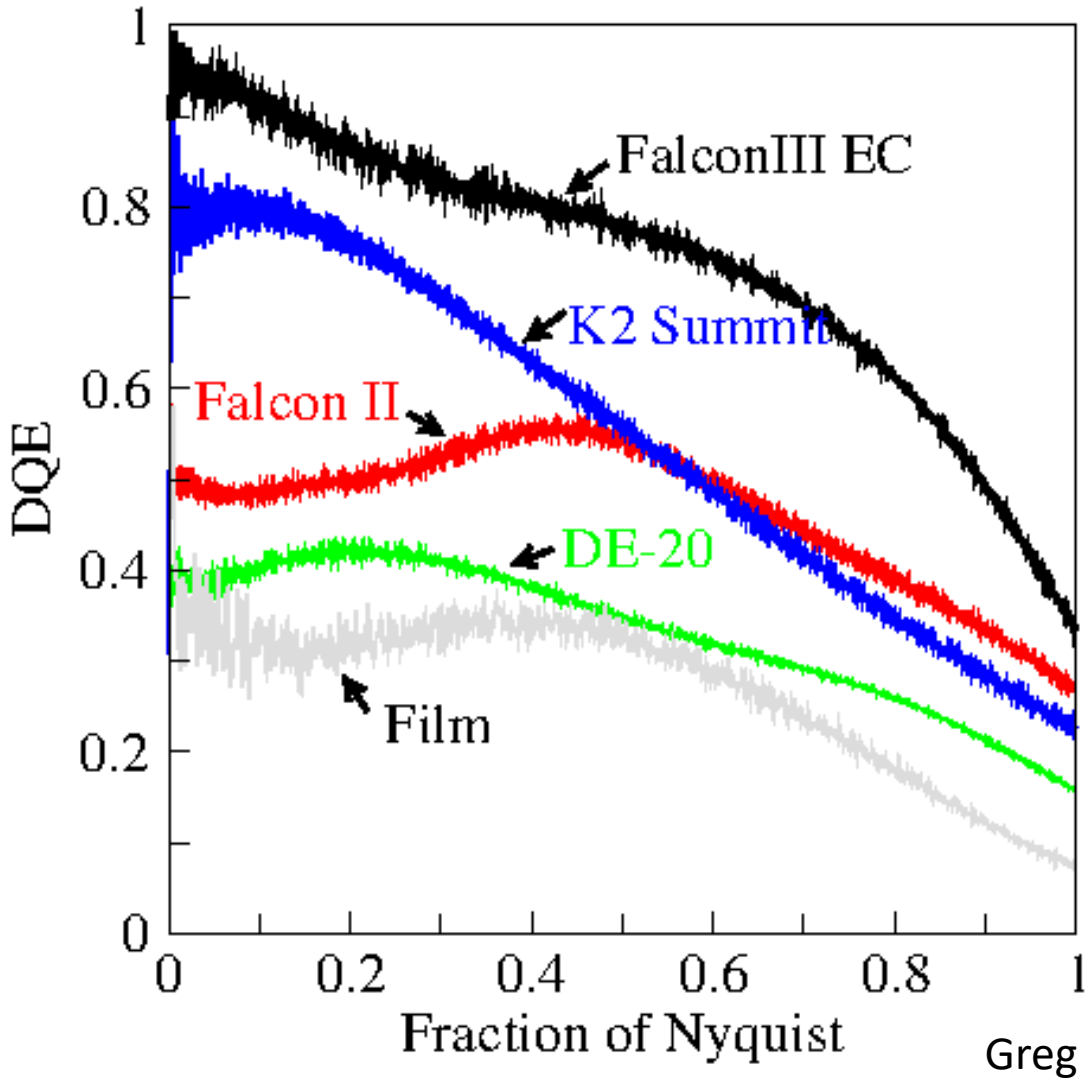
Volta Phase Plate





Detector Quantum Efficiency



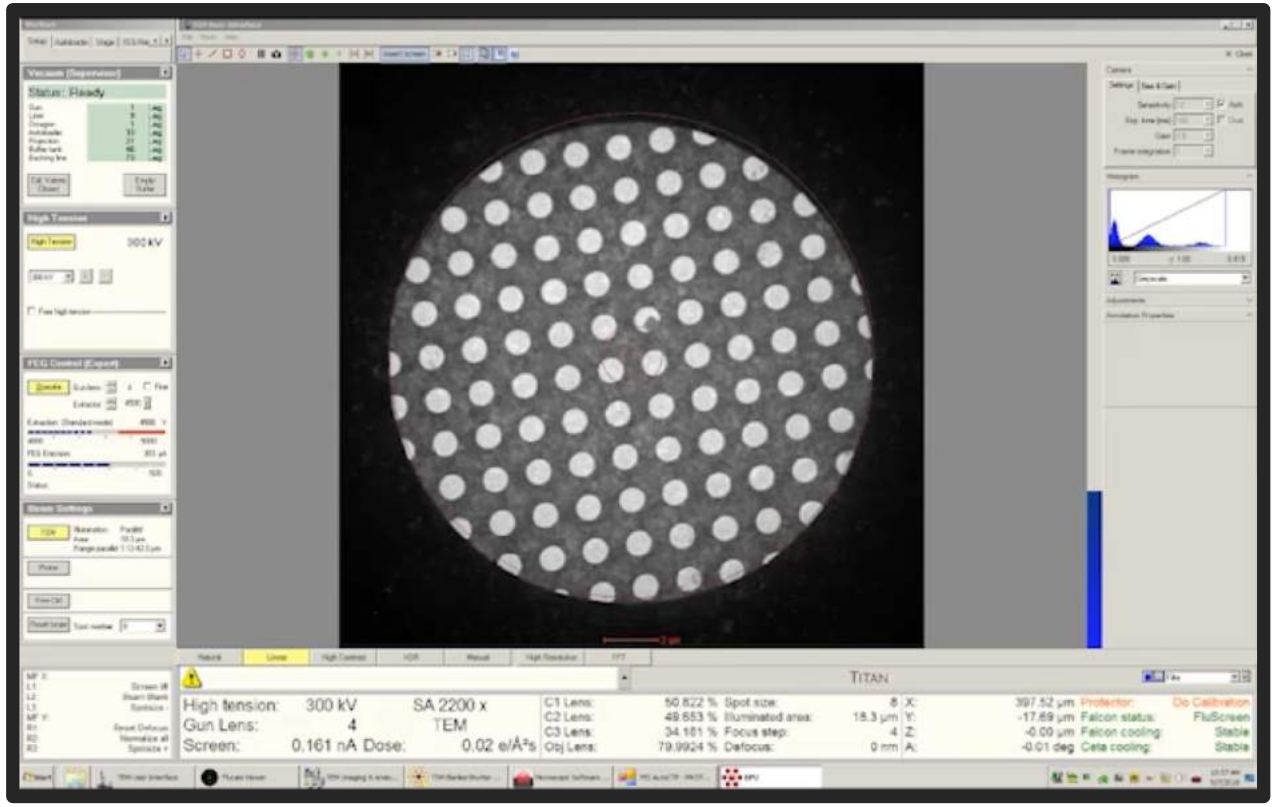


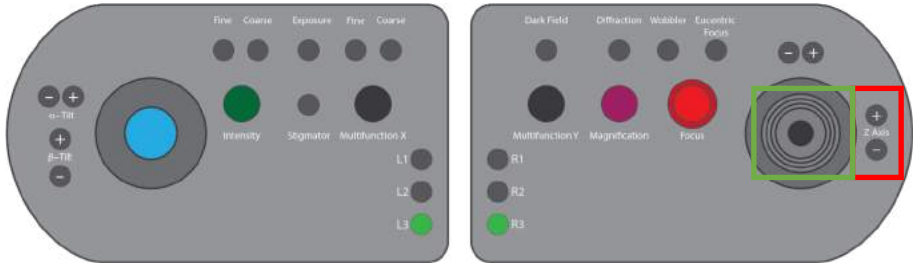
Pixel Size: smallest information you wish to collect -> magnification

Nyquist Frequency: smallest information you are recording and may be able to resolve -> 2 * PS

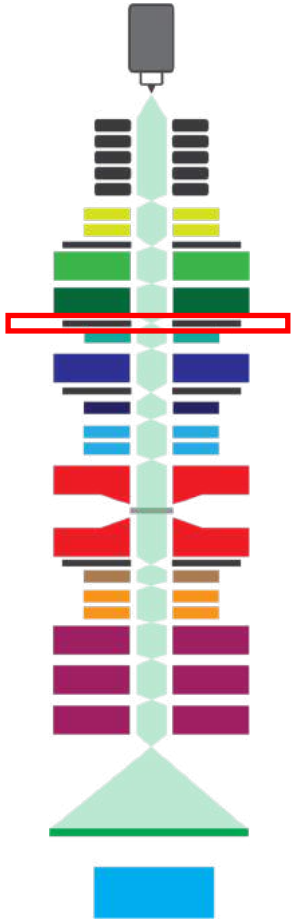
Greg McMullan

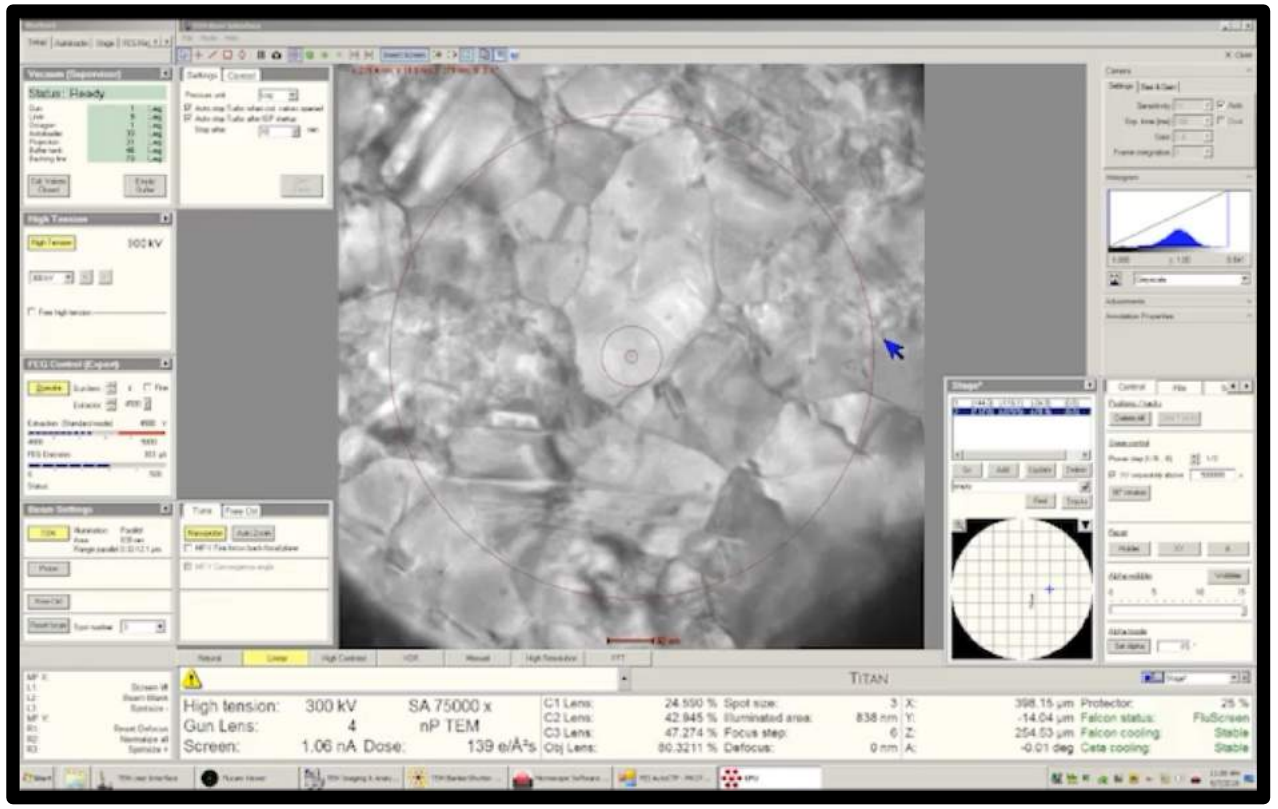
Eucentricity

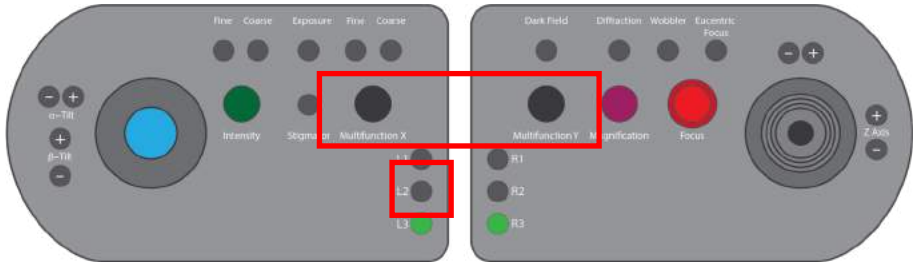




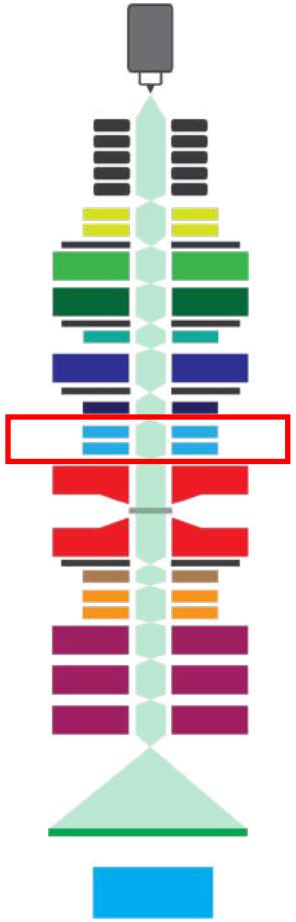
C2 Aperture Centering

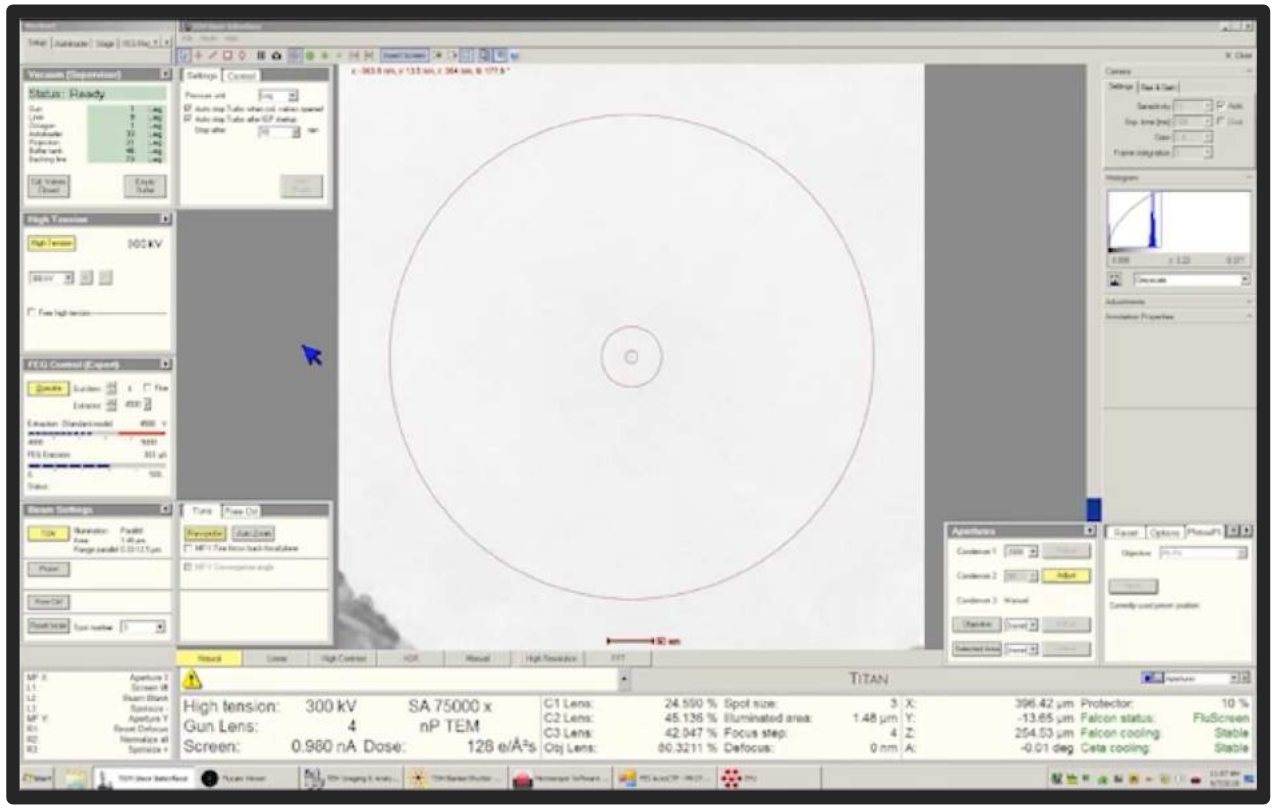


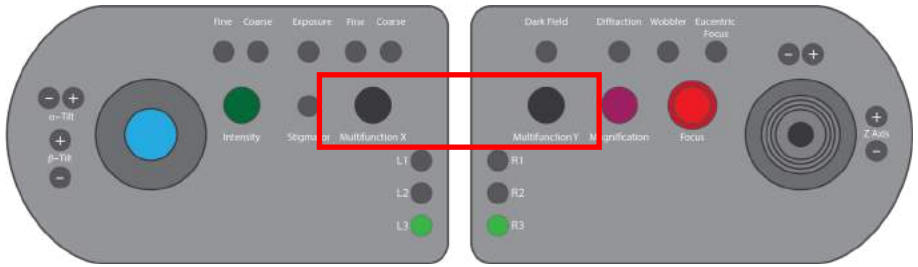




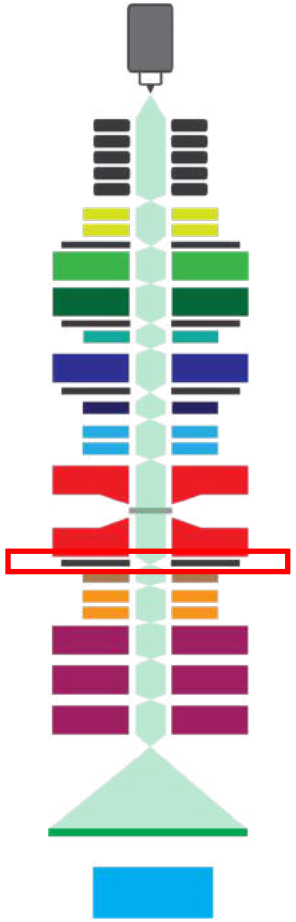
Beam Pivots Points

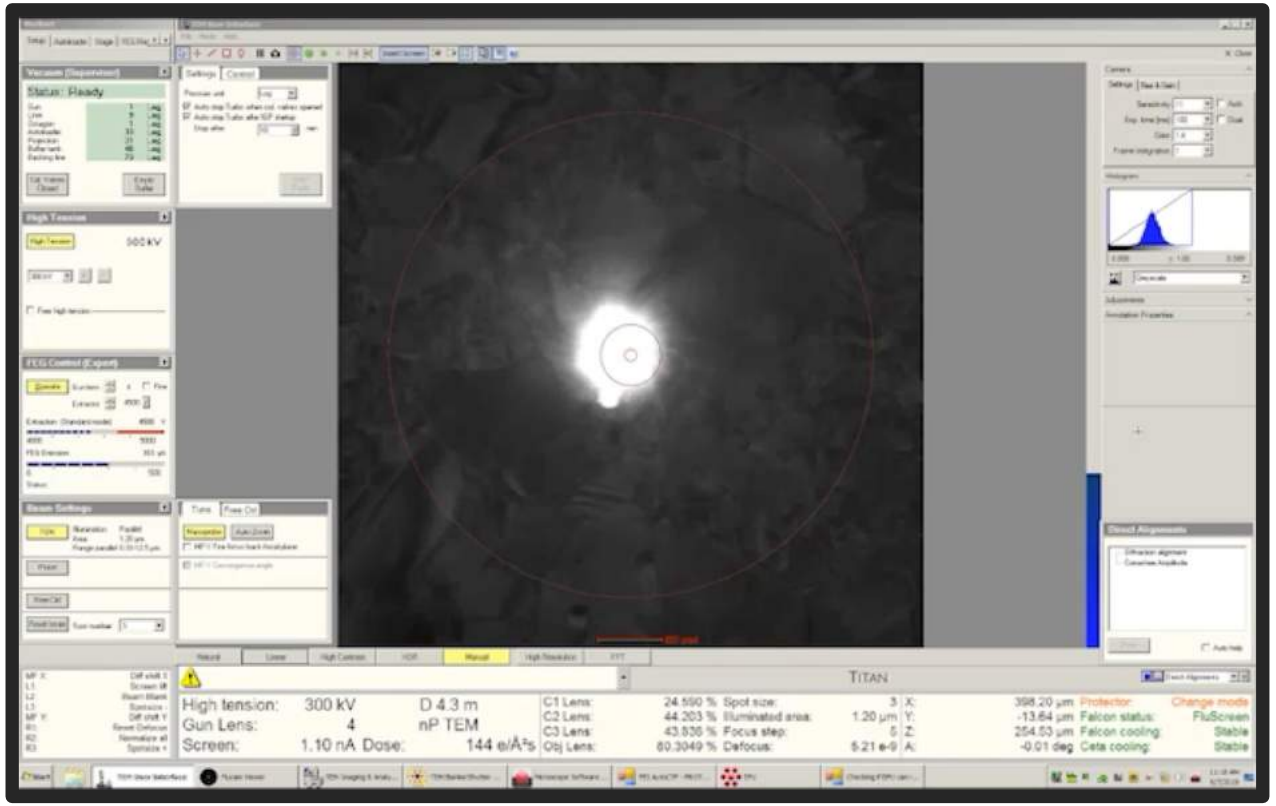


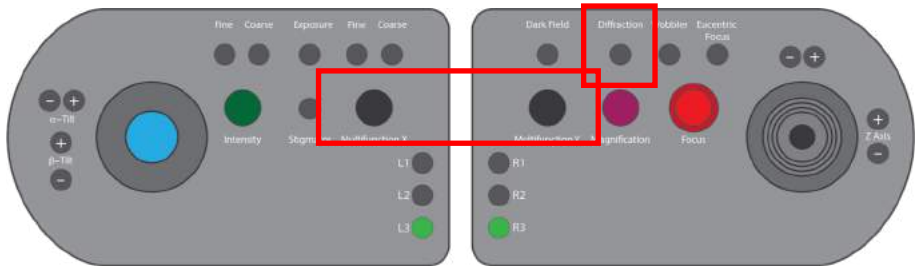




Objective Aperture





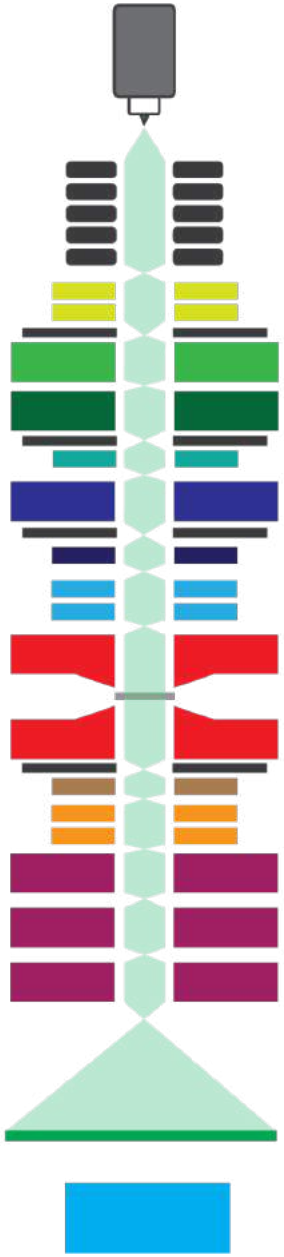


Volta Phase Plate

“Often, researchers believe that the phase plate will solve their experimental issues, while in actuality they are obstructed by the quality of their sample and not by the contrast in the images. This leads to wasted time trying to collect phase plate data and process it, which would have been much better spent on optimizing the sample.”

Danev, R., et. Al., TBS, 2019

TEM Microscope



Electron Source

Gun: Electron Source (W, LaB6, FEG) + Accelerator Stack (200 kV, 300 kV)

Accelerator Stack

Gun Tilt & Shift: MF X, Y

Gun deflection coils

C1 Aperture

C1 - Spot Size

First Lens Condenser

C2 – Intensity

Second Lens Condenser

C2 Aperture

C3 – Intensity - Parallel Range

Condenser Stigmator

Third lens Condenser

C3 Aperture

Condenser Stigmator – MF X,Y

Condenser Stigmator

Beam Deflection Coils

Beam Shift: MF X, Y

Focus

Objective Lens

Objective Stig. – MF X, Y

Objective Stigmator

Obj. Aperture

Image Deflection Coils

Beam-Image Shift: MF X, Y

Intermediate Lens

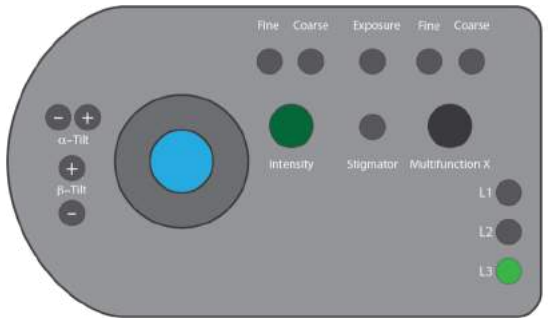
Magnification – MF X, Y

First Projection Lens

Second Projection Lens

Viewing Screen

Detector



Microscope Alignments

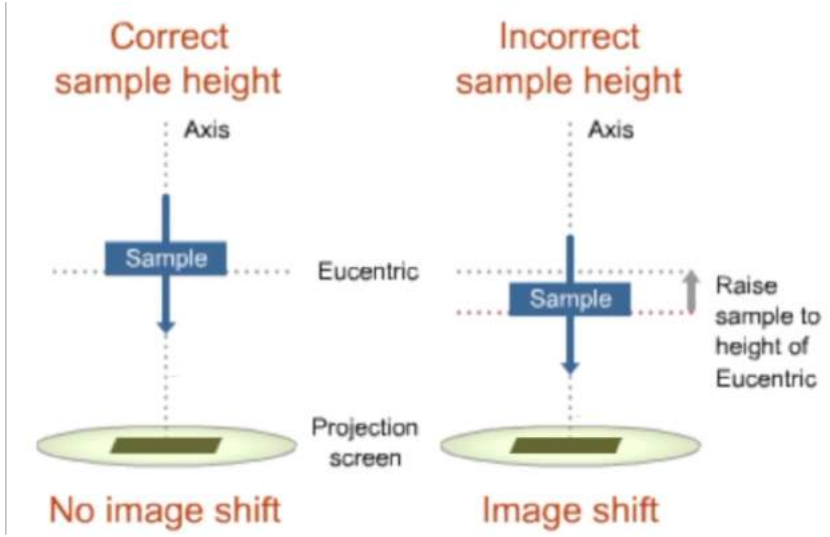
Alignments should be performed at the data collection settings

Check with your Facility Manager which ones you are allowed to perform

- C2 aperture centering
- Condenser lens astigmatism
- Beam tilt pivots points
- Beam shift
- Beam tilt (coma-free)
- Objective aperture
- Objective lens astigmatism

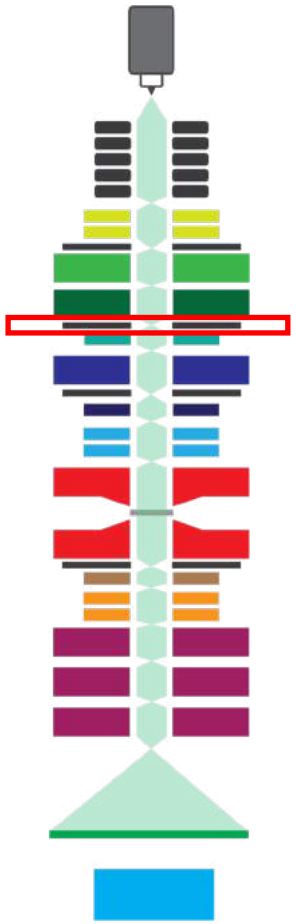
Eucentricity

- The eucentric position is the sample height (in the Z-direction) at which, one can tilt the sample around its axis without the image of the sample moving across the projection screen.
- Eucentric position is the reference point for many alignments and calibrations.
- Wrong eucentricty -> change in magnification; autofocus failure.

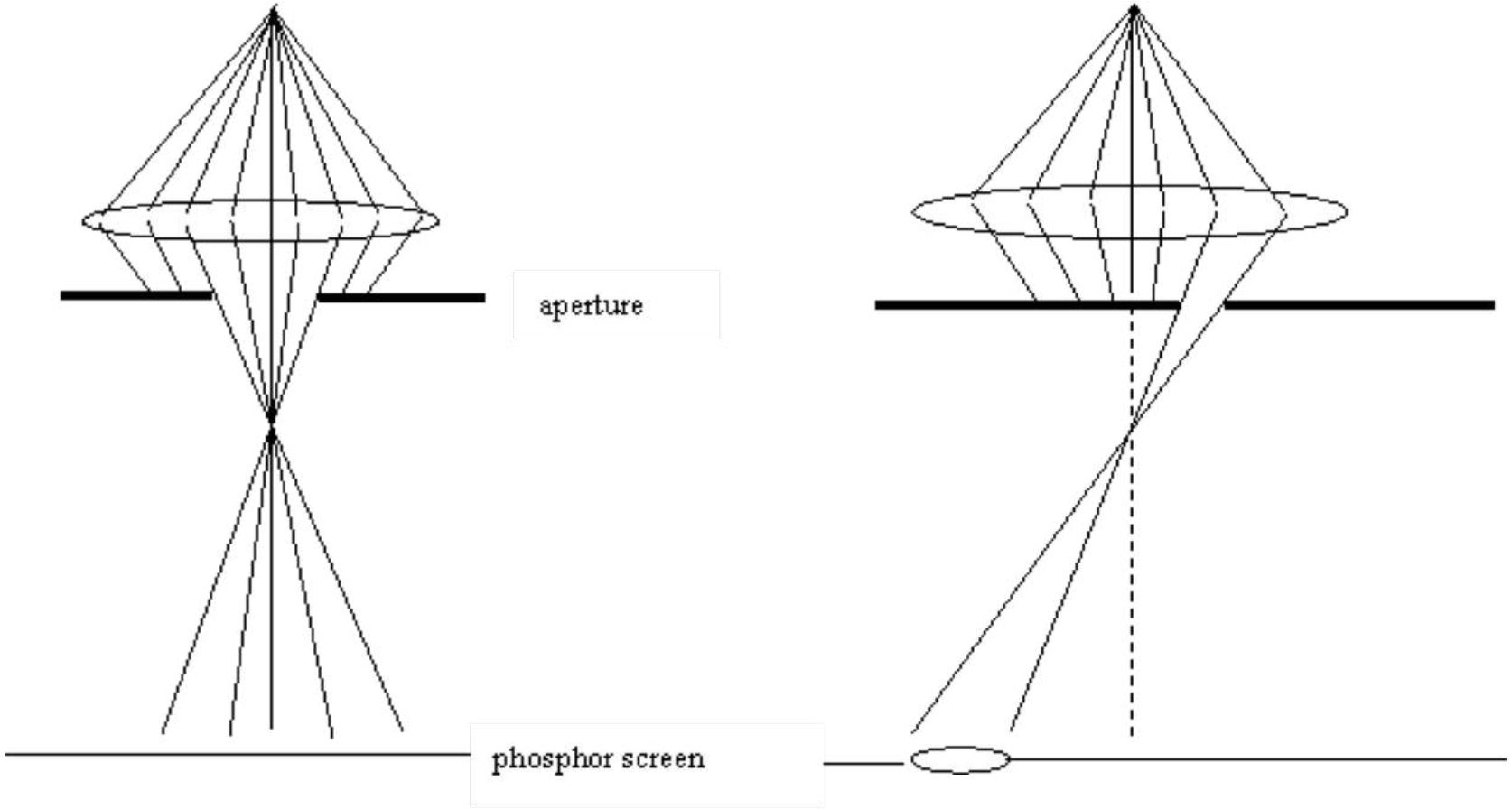


https://myscope.training

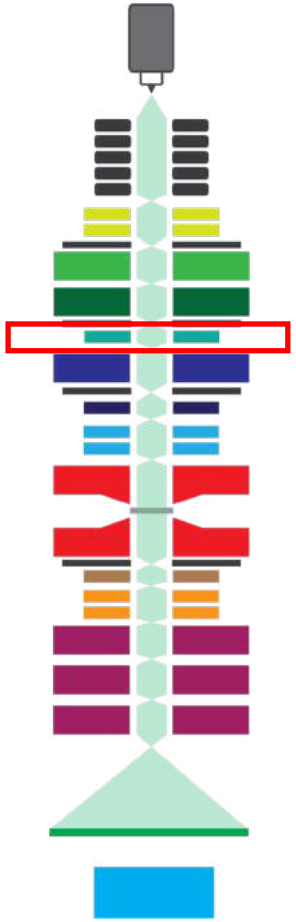
C2 Aperture Centering

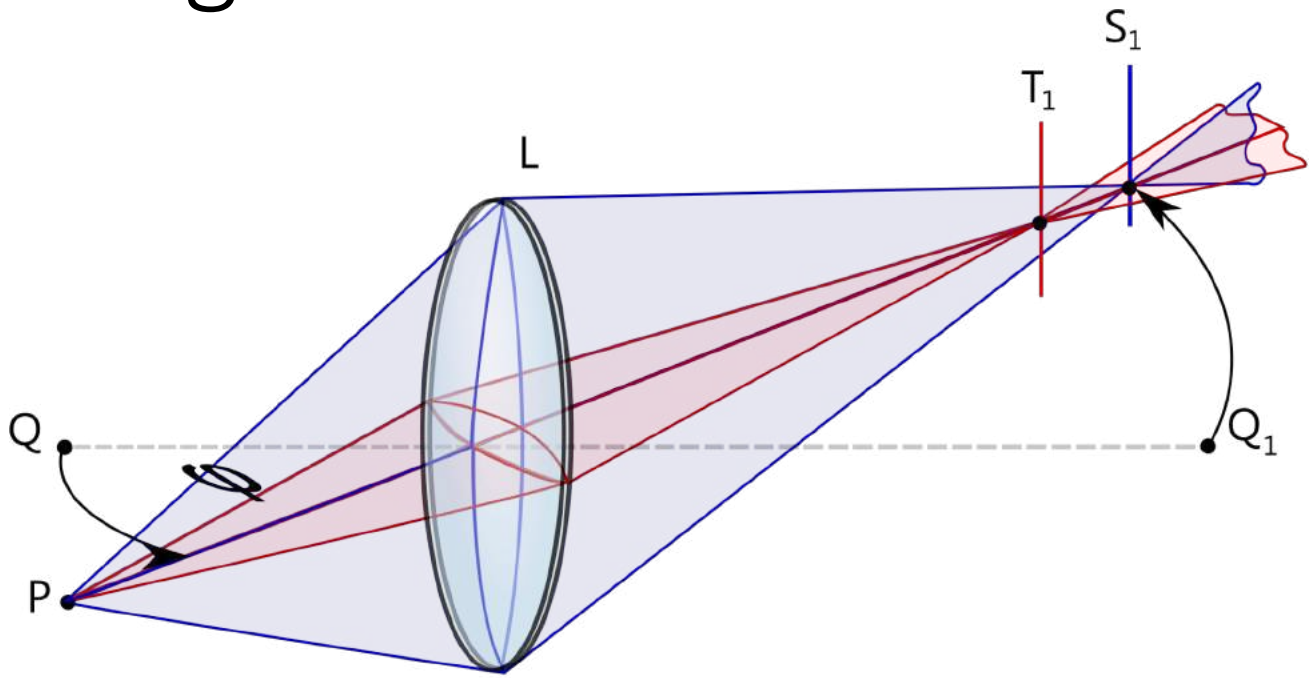


Beam expand symmetrically with respect to the screen -> does not move center when changing ”Intensity”

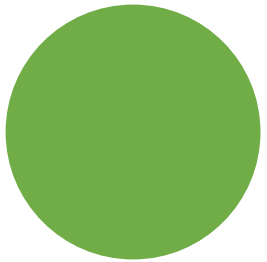


Condenser Astigmatism

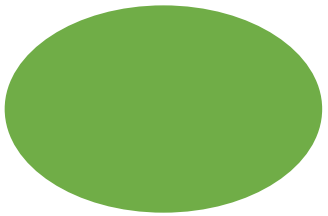




Beam Shape

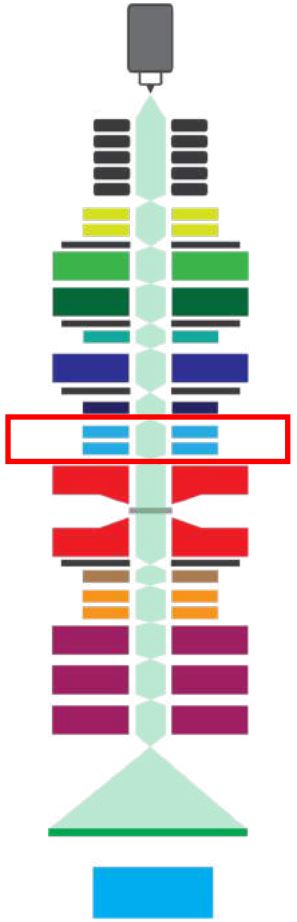


Good



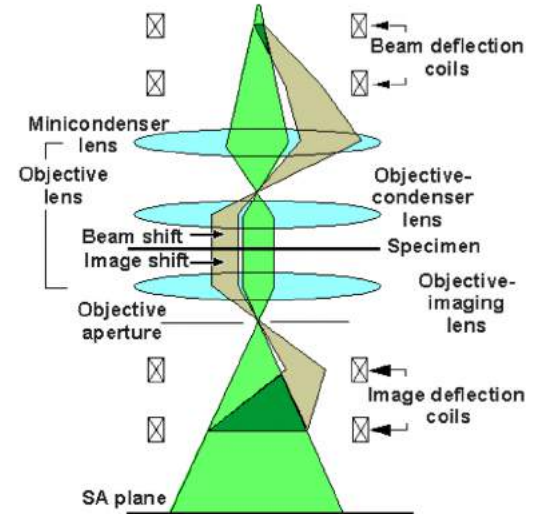
Bad

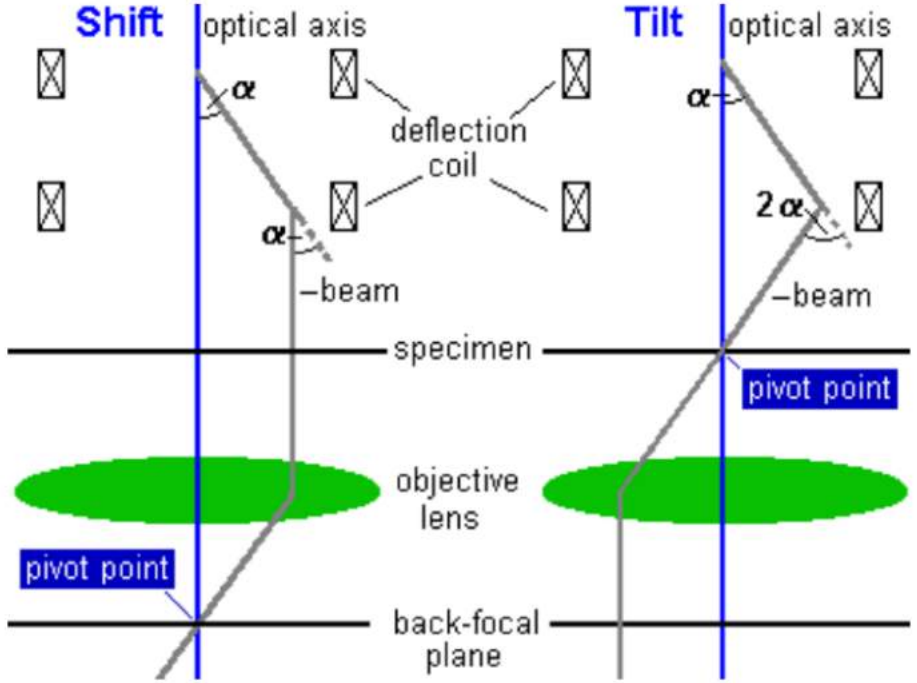
Beam Pivots Points



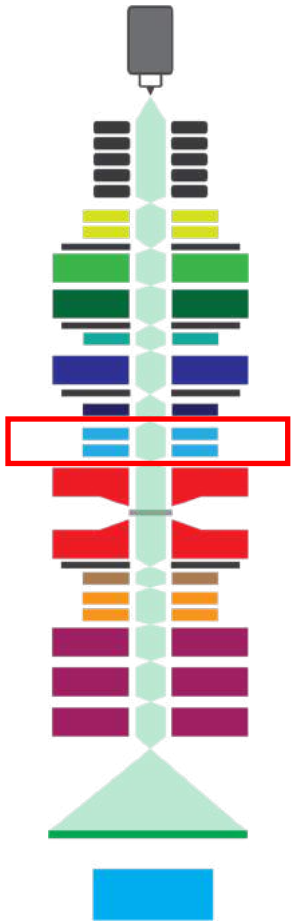
Beam Tilt Pivot Points (Direct Alignments):

- Beam does not shift when tilted -> Autofocus, Coma-free
- Beam tilt pivot points are set in image mode - where a beam shift will be visible





Beam Pivots Points

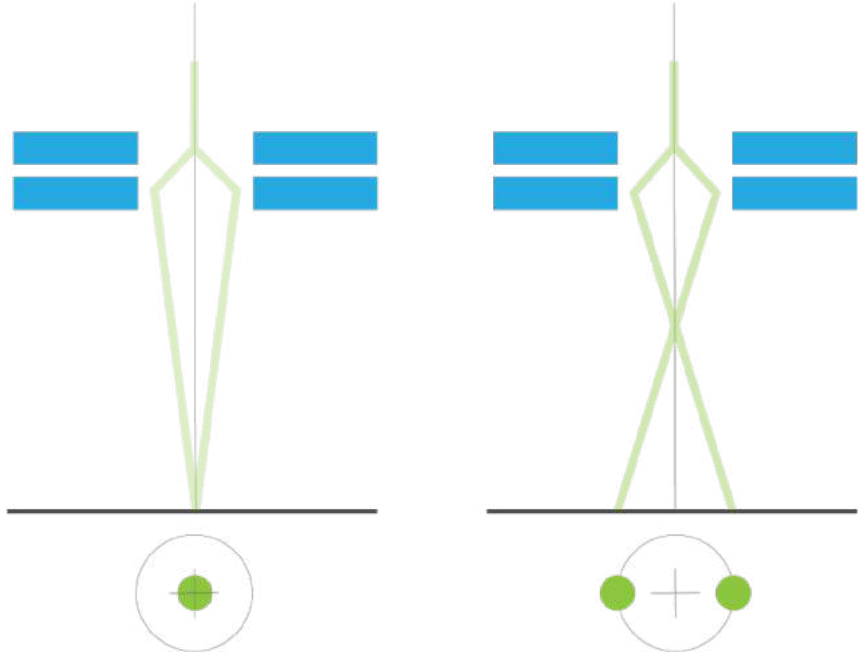


Beam Tilt Pivot Points :

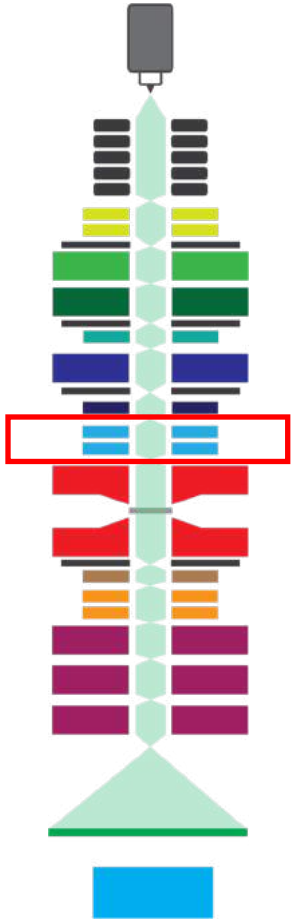
- Work at eucentric focus
- Avoid adjusting perpendicularity (multi-function Y) -> Beam Tilt Pivot Points share the perpendicularity with the Beam Shift Pivot Points.

Beam Shift Pivot Points (Column Alignments):

- Beam does not tilt when shifted -> hence less chances of beam tilt when doing beam-image shift



Beam Shift



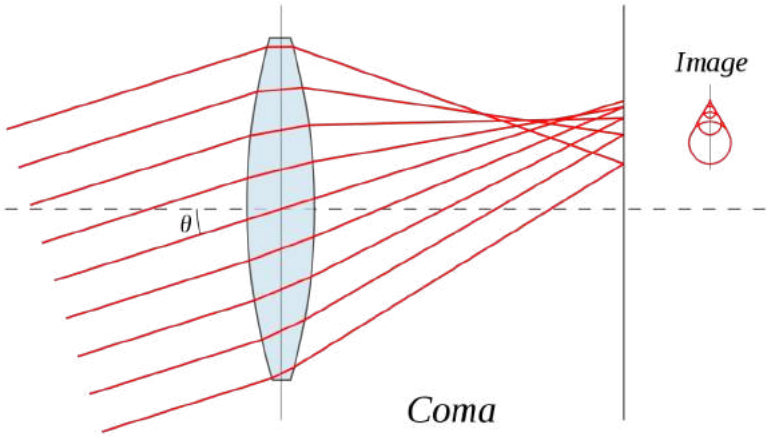
- Reset the beam deflection coils to a reference center, in this case the fluorescence screen or center of the detector (GIF entrance).
- Always center the beam with the desired beam diameter to minimize movement due to C2-C3 swing
- If you cannot see the edge of the beam, center the beam at lower mag

Beam Tilt

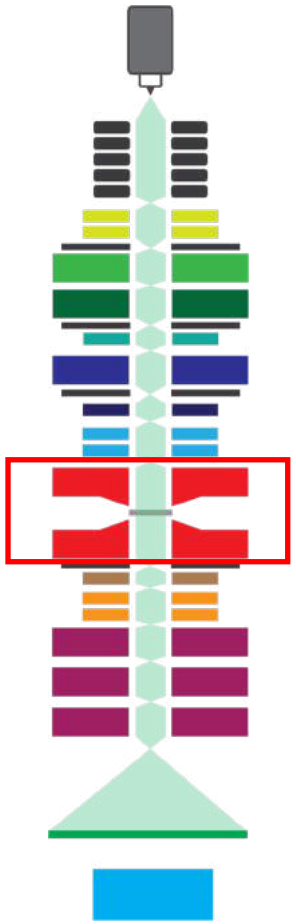
Axial coma is an image aberration that is introduced when the illumination is not parallel to the optical axis of the objective lens.

This aberration is minimised with rotation centre or coma-free alignments:

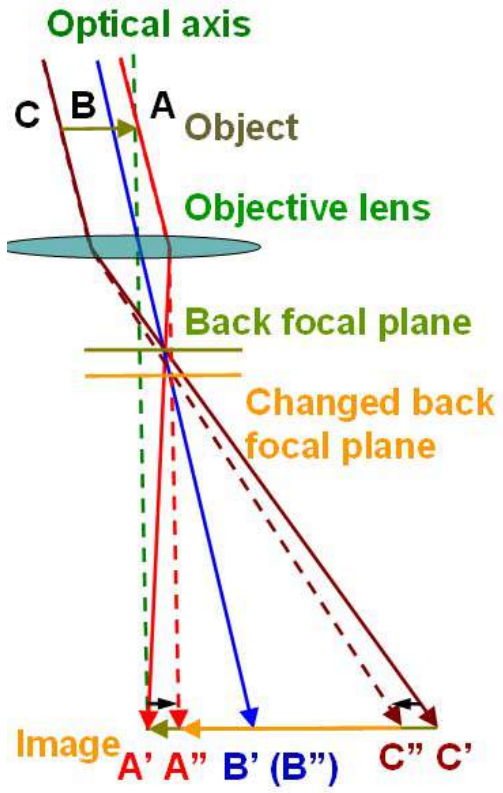
- Rotation center
  - Caustics rings
  - Reference point
- Coma-free
  - Zemlin tablou (SerialEM, AutoCTF, EPU)



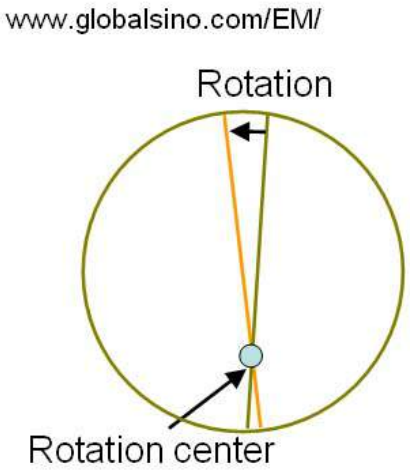
Beam Tilt - Rotation Centre



Microscope wobble the objective lens -> Change defocus

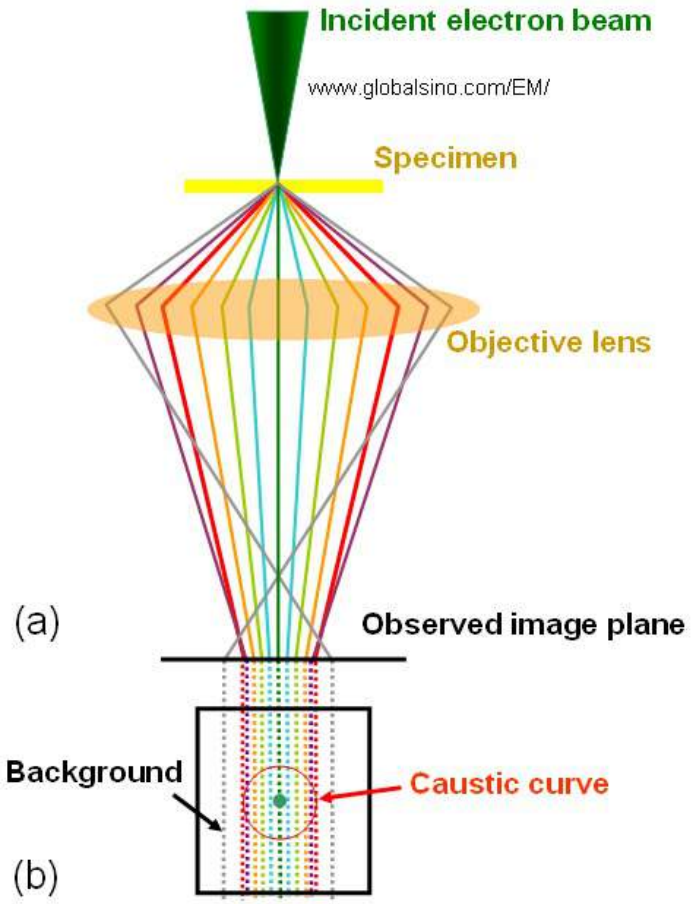


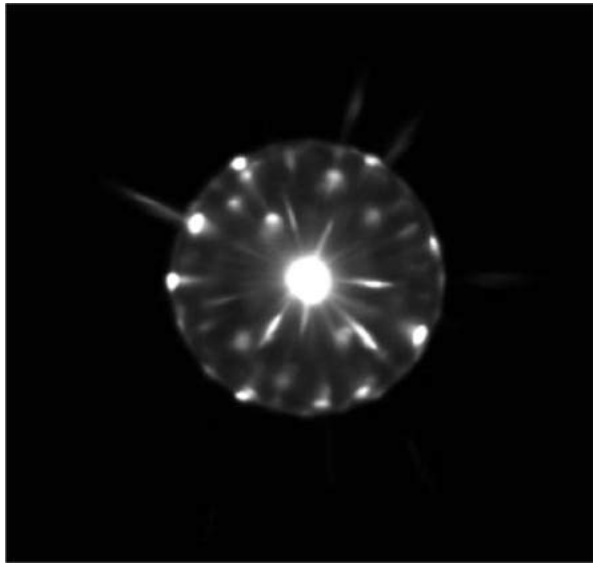
(a)

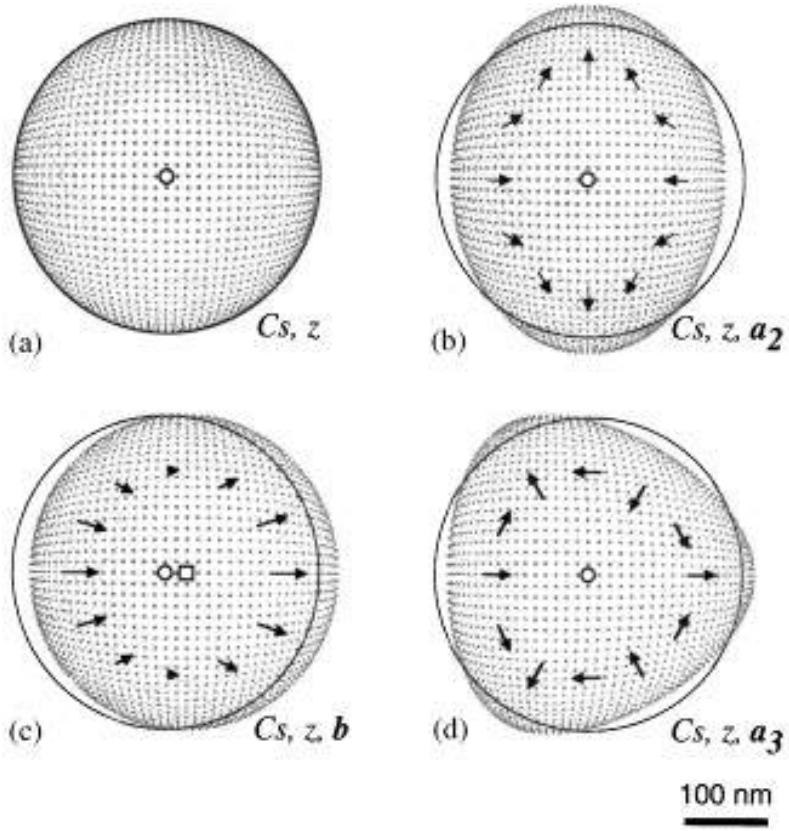


(b)

Beam Tilt - Rotation Centre



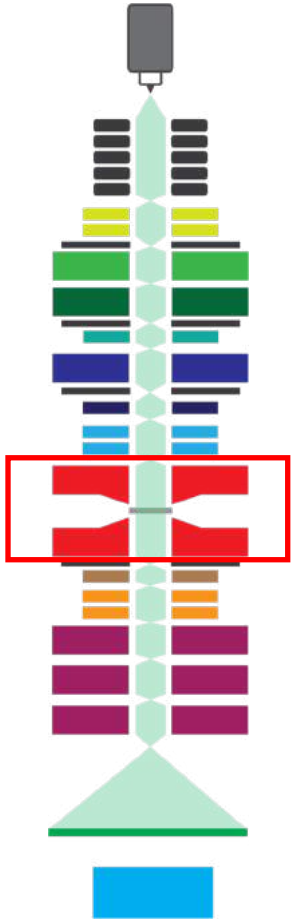


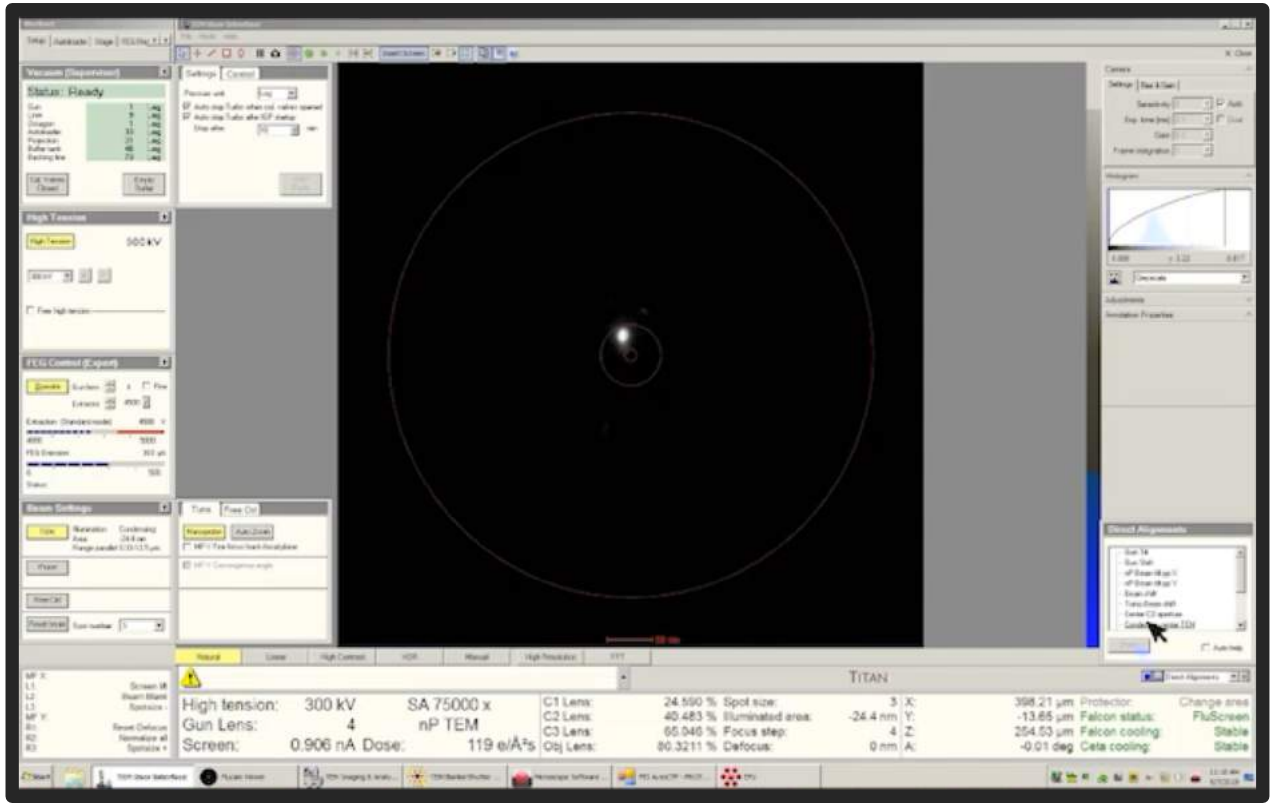


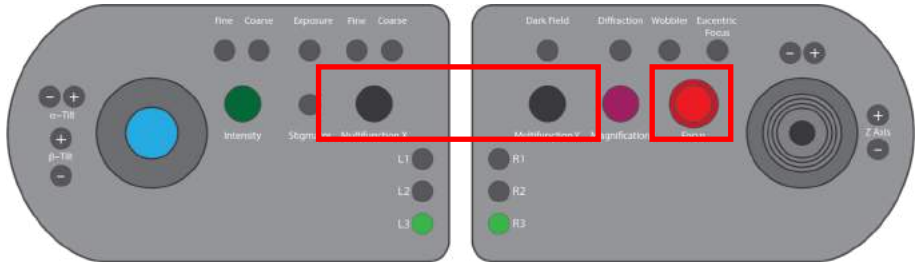
globalsino.com

Kimoto et al. Ultramicroscopy, 2003

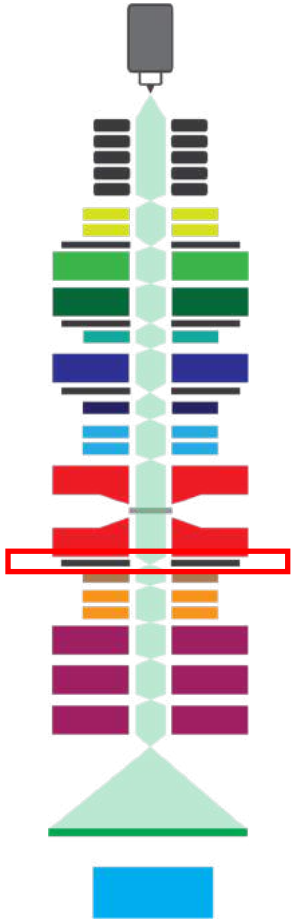
Beam Tilt - Rotation Centre



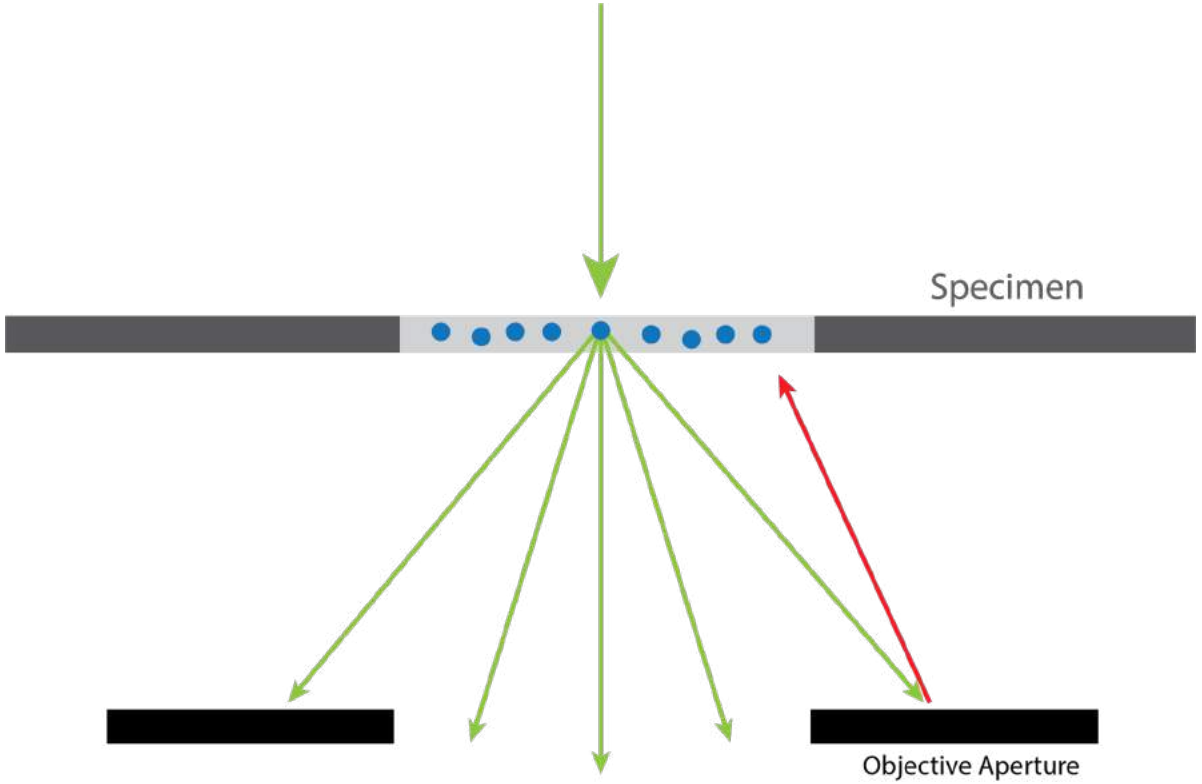




Charging



If I want very high resolution shall I remove the Obj? -> It helps with reducing charging of the specimen



ThermoFisher Falcon3

- 40 frames/sec
- Linear and Counting mode
- Optimal dose rate:
  - Linear: 80 - 120 e/p/s
  - Counting: 0.5 – 1 e/p/s
- Exposure:
  - Linear: 1 - 2 s
  - Counting: 30 – 60 s

Linear					
Mag	Dose	Exposure	Total Dose	Fractions	Dose/Frame
75Kx	80	1	74	40	1.85
96Kx	80	1	108	40	2.7
Counting					
Mag	Dose	Exposure	Total Dose	Fractions	Dose/Frame
75Kx	0.5	60	28	75	0.37
96Kx	0.5	60	43	75	0.6

Gatan K2

- 400 frames/sec (hardware)
- 40 frames/sec output
- Counting and Super-Res mode
- Optimal dose rate:
  - Counting: 4 – 8 e/p/s
- Exposure:
  - 8 – 16s

Counting					
Mag	Dose	Exposure	Total Dose	Fractions	Dose/Frame
81Kx	4	28	50	50	1
105Kx	4	15	50	50	1
130Kx	4	10	50	50	1

Gatan K3

- 1500 frames/sec (hardware)
- 75 frames/sec output
- Counting and Super-Res mode
- Optimal dose rate:
  - Counting: 15 – 32 e/p/s
- Exposure:
  - 1 – 3s
- Correlative Double Sampling
  - 750 frames/sec (hardware)
  - <7.5 e/p/s

Counting NoCDS					
Mag	Dose	Exposure	Total Dose	Fractions	Dose/Frame
81Kx	15	11	50	50	1
105Kx	15	6.5	50	50	1
130Kx	15	4	50	50	1
Counting CDS					
Mag	Dose	Exposure	Total Dose	Fractions	Dose/Frame
81Kx	7.5	22	50	75	1
105Kx	7.5	13	50	50	1
130Kx	7.5	8	50	50	1