How to best use your microscope

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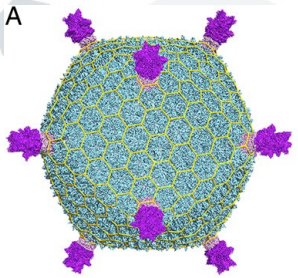
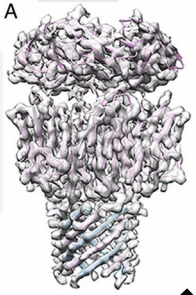
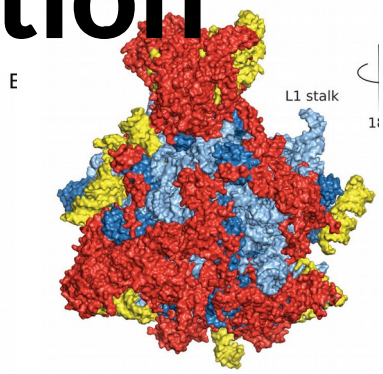
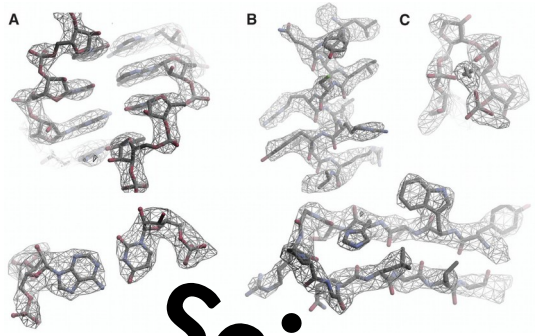
7th of November 2014

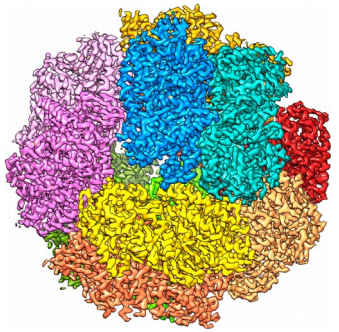
SPA revolution





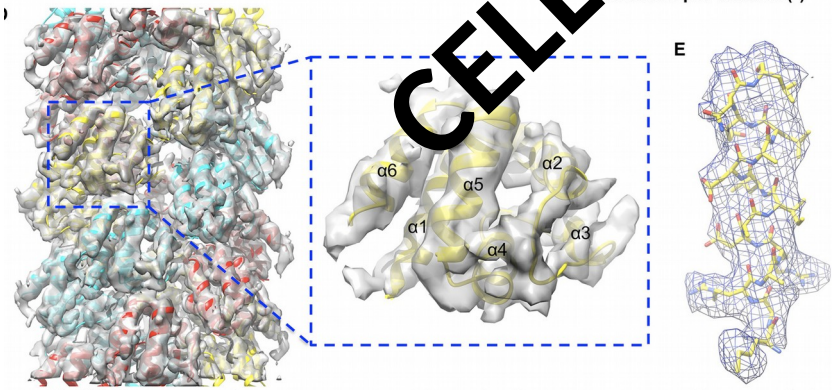


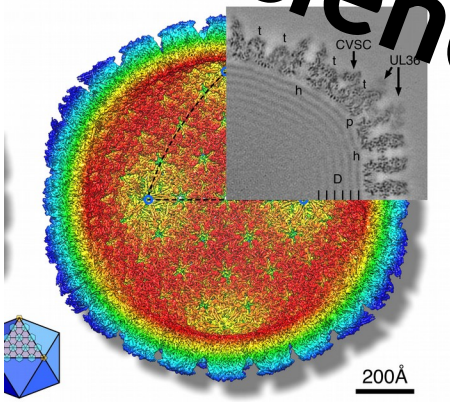


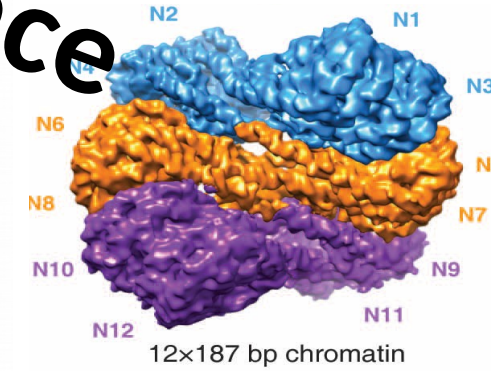


CELL

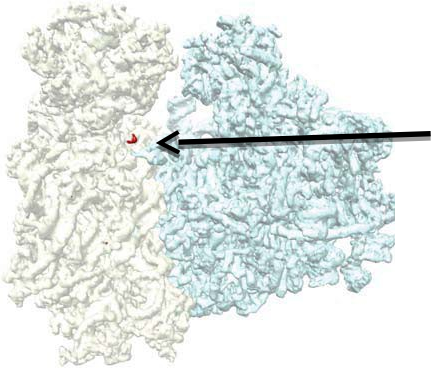
Science

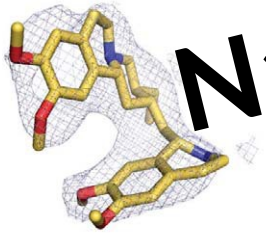


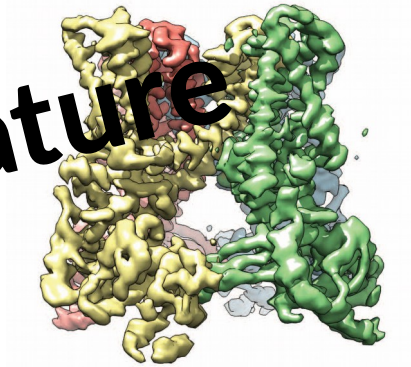


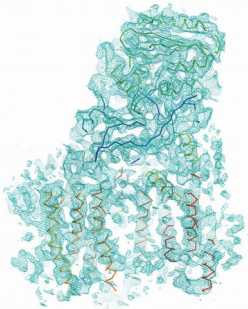


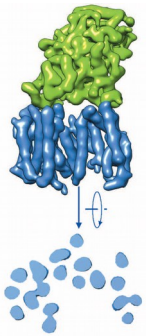
Nature











What NOT to do:

-You do NOT need to realign your microscope daily just out of routine!

-If you do not EXACTLY know what an alignment step does: DON'T DO ALIGNMENTS!

-The user is often more unstable than the microscope or the room.

Some more facts

- A Titan is NOT a Tecnai! (FEI is not ZEISS or JEOL or Hitachi): the optics are different!

- COLUMN alignments are not the same as DIRECT alignments

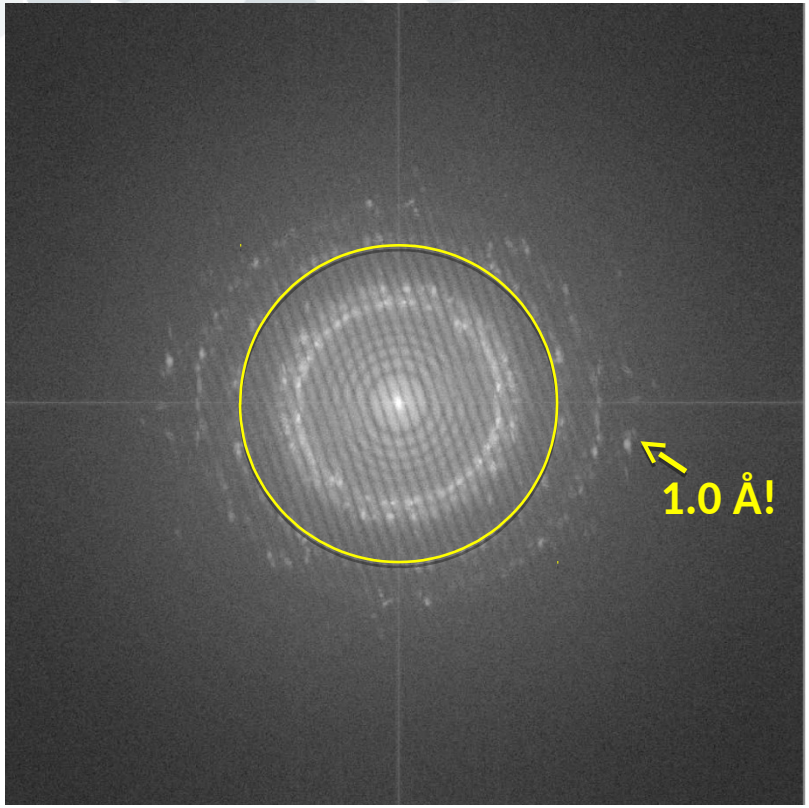
- Realign a COLUMN always completely, NEVER do individual steps

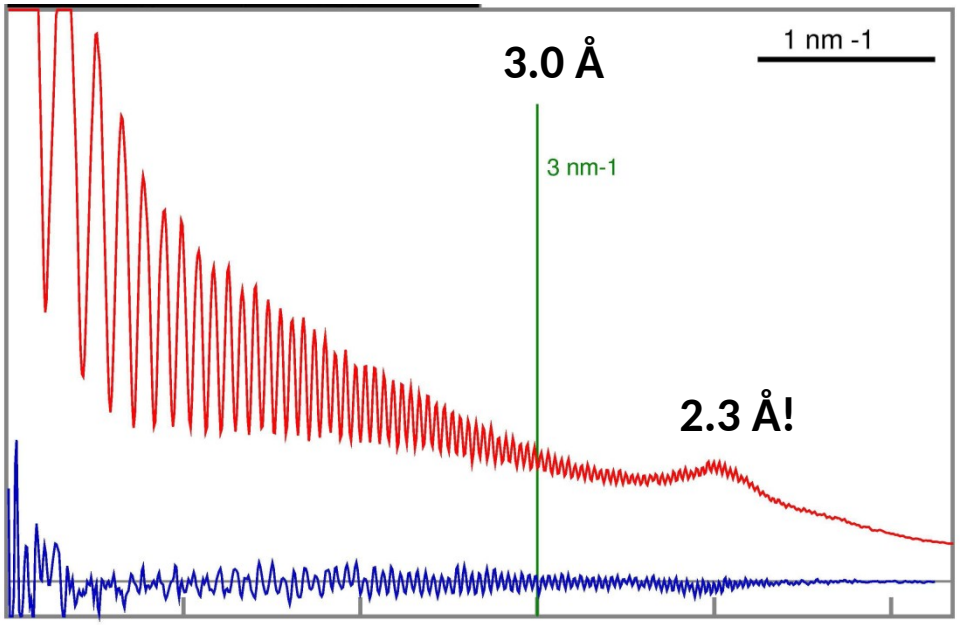
How to setup you're scope

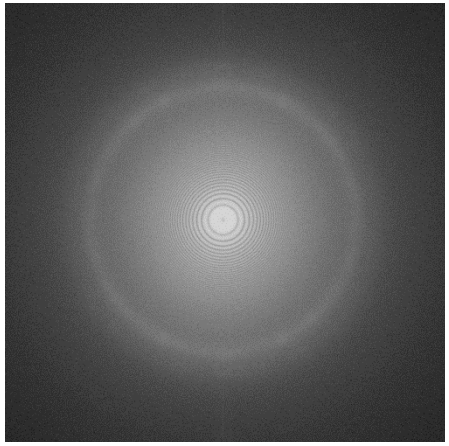
You should be:

-At Eucentric height
-At Eucentric focus
-Parallel
-Obj stigmated
-Coma free
-Dose rate

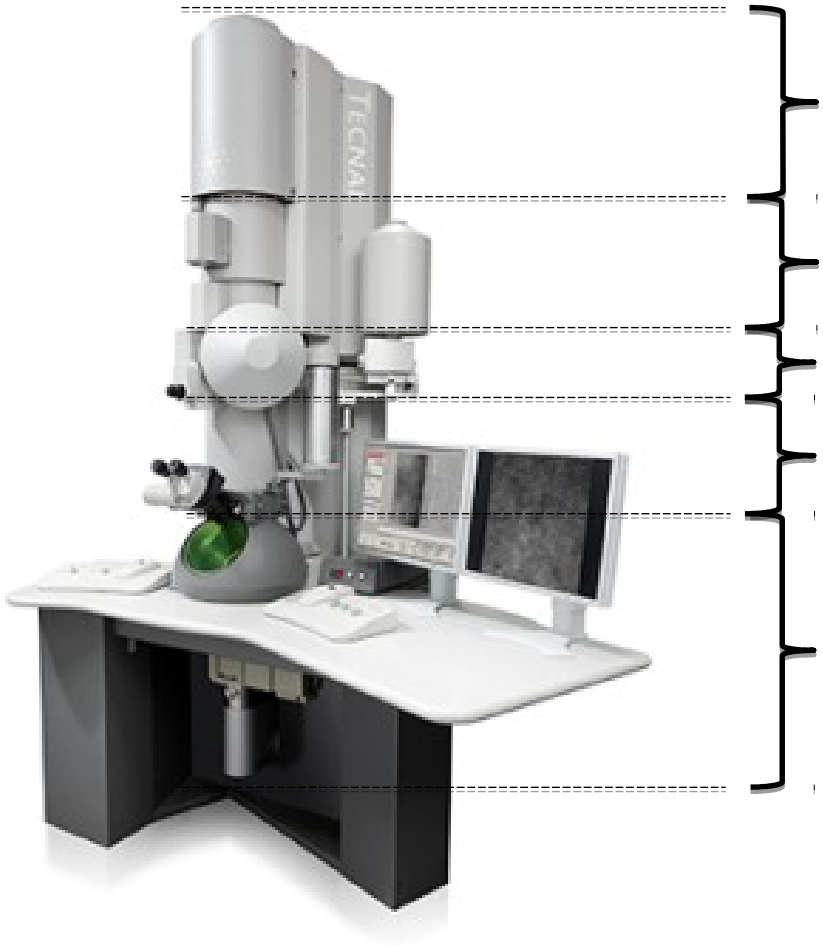
You should see something like this (Krios):







Microscope From Top to Bottom



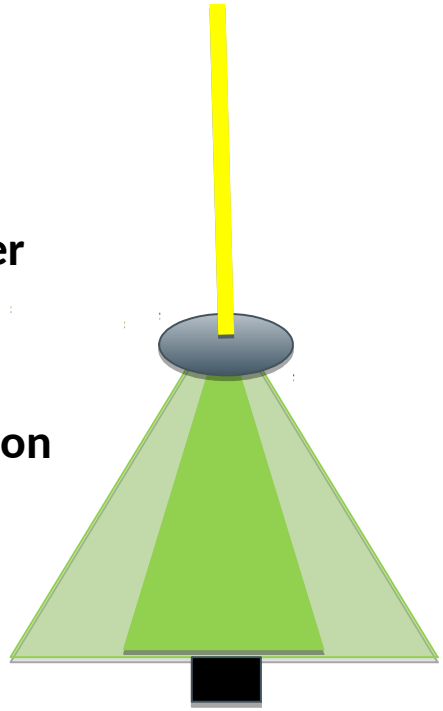
Source: gun

Illumination: condenser

Imaging: objective

magnification: projection

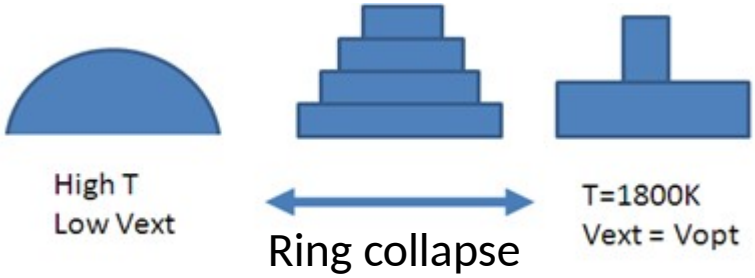
detection: camera's



Gun: stability, brightness, dE

If the temperature is low, and the extraction voltage is high
→ The tip end form will evolve in a single facet. This end form is stable resulting in stable

If the temperature is too high or if the extraction voltage is too low
→ The tip end form will evolve into a blunt round shape (low brightness)



emission characteristics with high brightness

- → But too high extraction voltage increases energy spread of electrons
- → Too low temperature makes the emission sensitive for pressure variations
- → Compromise: Vextr=Vopt (I'=0.2mA/sr) @ T=<1800K
  - → acceptable dE & good Brightness & reasonable stability

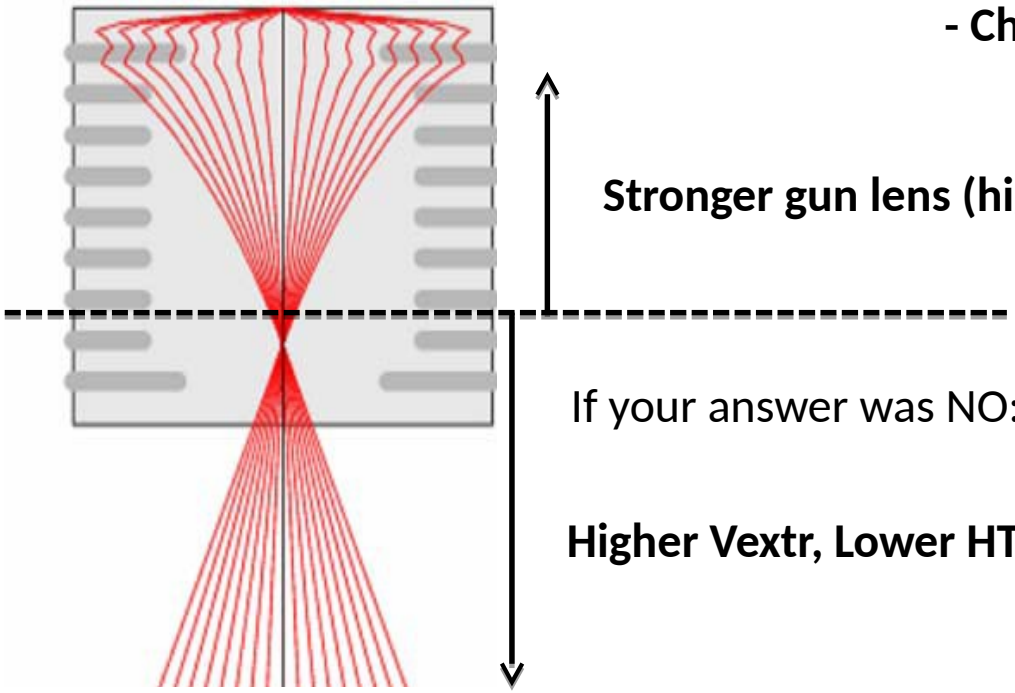
Gun settings

Who knows what happens when you:

- Change the HT?

- Change the extractor?

- Change the gun lens?



Stronger gun lens (higher number)

If your answer was NO: **DON'T TOUCH THE GUN SETTINGS**

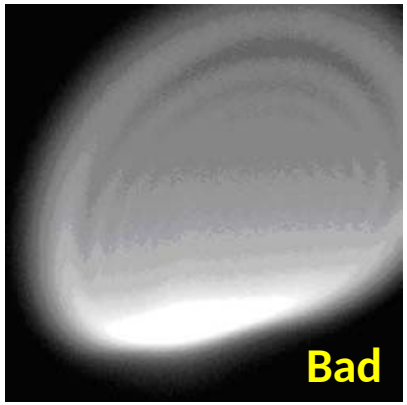
Higher Vextr, Lower HT

-Do not work with the cross-over (XO) outside of the gun lens: increase in Vextr and decrease in HT need to be compensated by a stronger GL

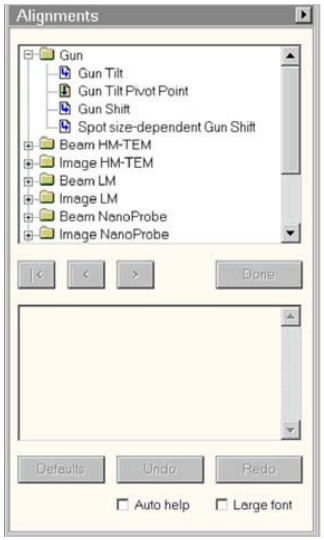
-If you do not do STEM: **DON'T TOUCH THE GUN SETTINGS**, Vextr as high as possible depending on the HT you work at, GL not to the max.

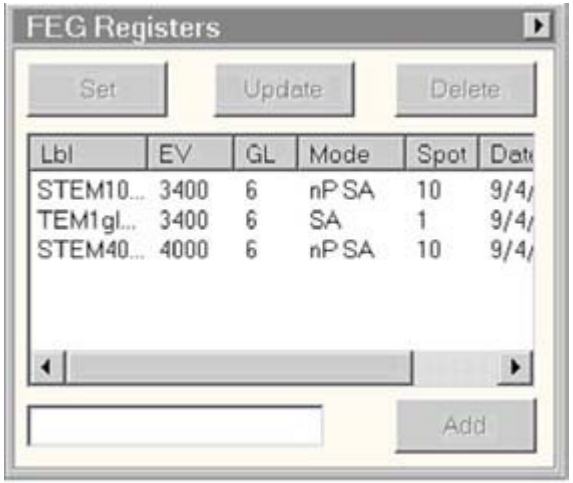
Gun alignment

-It is not that unstable that you need to do this daily!
-Try to avoid spot 1
-Align only when needed: whole gun alignment
-The Gun XO is the most important step, therefore only do a complete gun alignment
-Avoid direct gun alignments

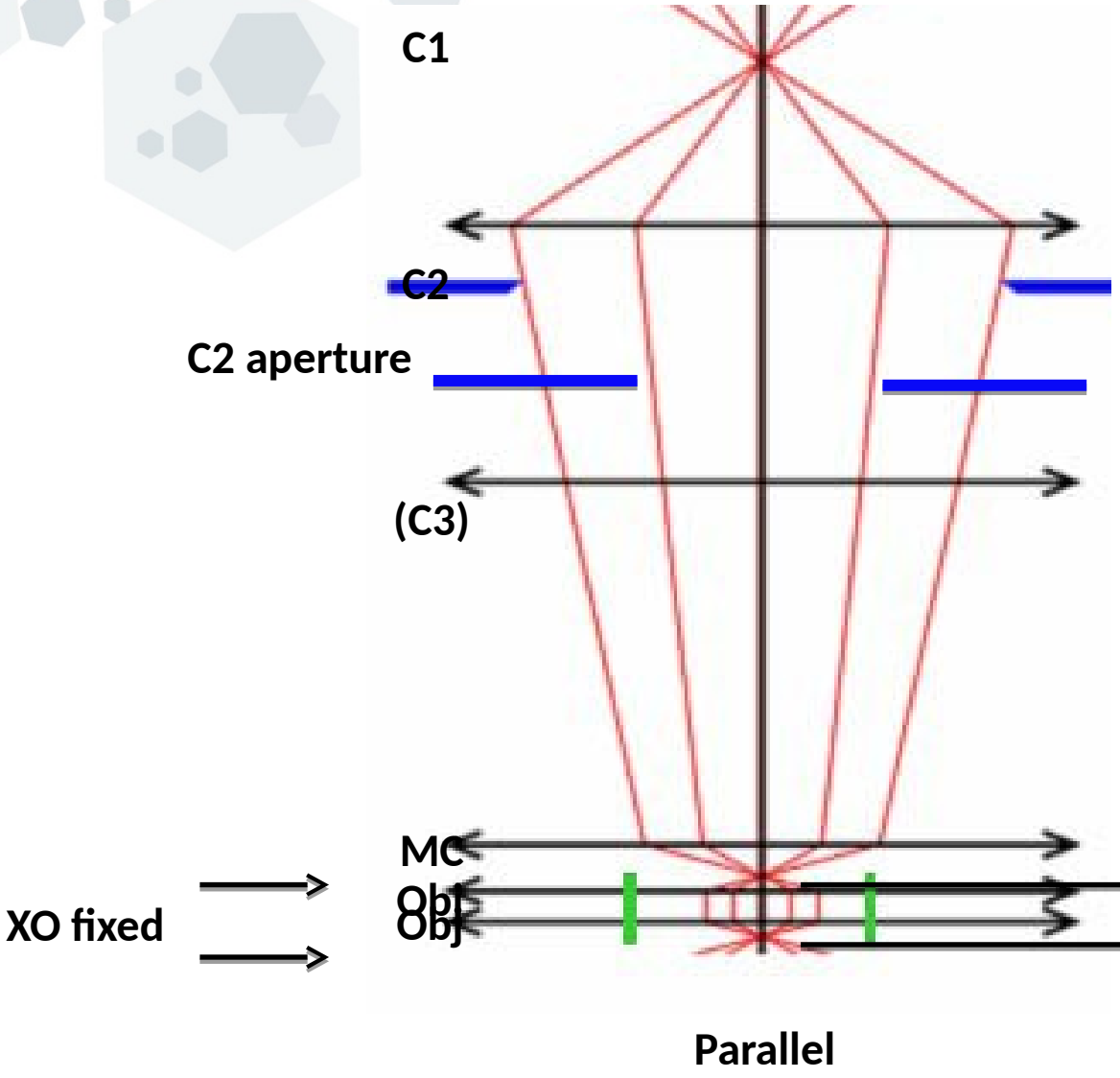


-Save a gun alignment in the FEG registers! That is what they are for! (DELETE THE OLD ONE!)





Condenser



IF the **last condenser XO** is at the **front focal plane** of the obj lens THEN the **diffraction pattern** is focused at the **back focal plane** (obj XO) AND we have parallel illumination by definition.

All is fixed!!! Only the C2 aperture determines illuminated area.

2 Condenser system

How to find parallel illumination?

IF the **last condenser XO** is at the **front focal plane** of the obj lens THEN the **diffraction pattern** is focused at the **back focal plane** (obj XO) AND we have parallel illumination by definition.

Where is the back focal plane? Where the diffraction pattern lies.

How to put your eye site at the back focal plane? Switch to diffraction.

How do you know you are focused at the back focal plane (BPF)? Find something fixed in the BFP

What is also in the back focal plane? The objective aperture.

How to focus the back focal plane? Sharpen the edge of the Obj aperture with FOCUS.

How to find the front focal plane? Change INTENSITY until the diff pattern is focused

When am I parallel? When SIMULTANEOUSLY the Obj aperture and diff pattern are focused!

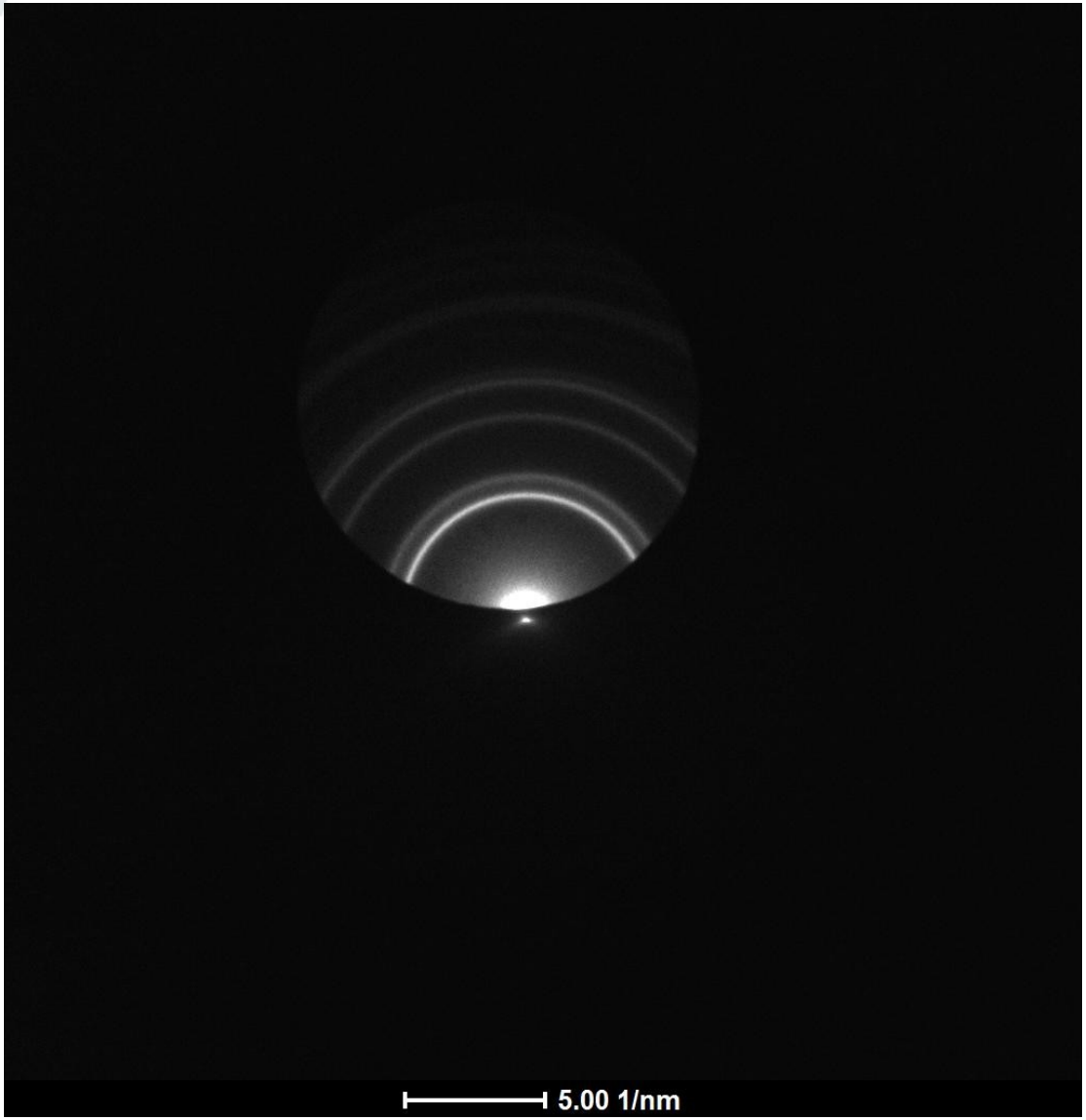
Parallel Illumination

-With FOCUS sharp obj aperture

-with INTENSITY sharp diff pattern

-DO NOT TURN THE TWO BUTTONS AT THE SAME TIME

-Sharp diffraction pattern with blurry obj aperture is NOT parallel!



Rules of Parallel Illumination

Parallel settings should not change when:

-changing spot size only
-changing magnification only
-changing C2 aperture

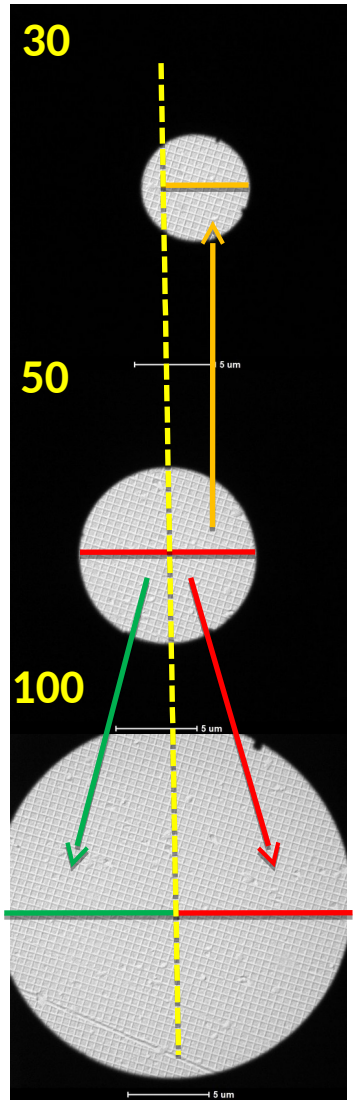
While being parallel beam size can only change when:

-changing C2 aperture

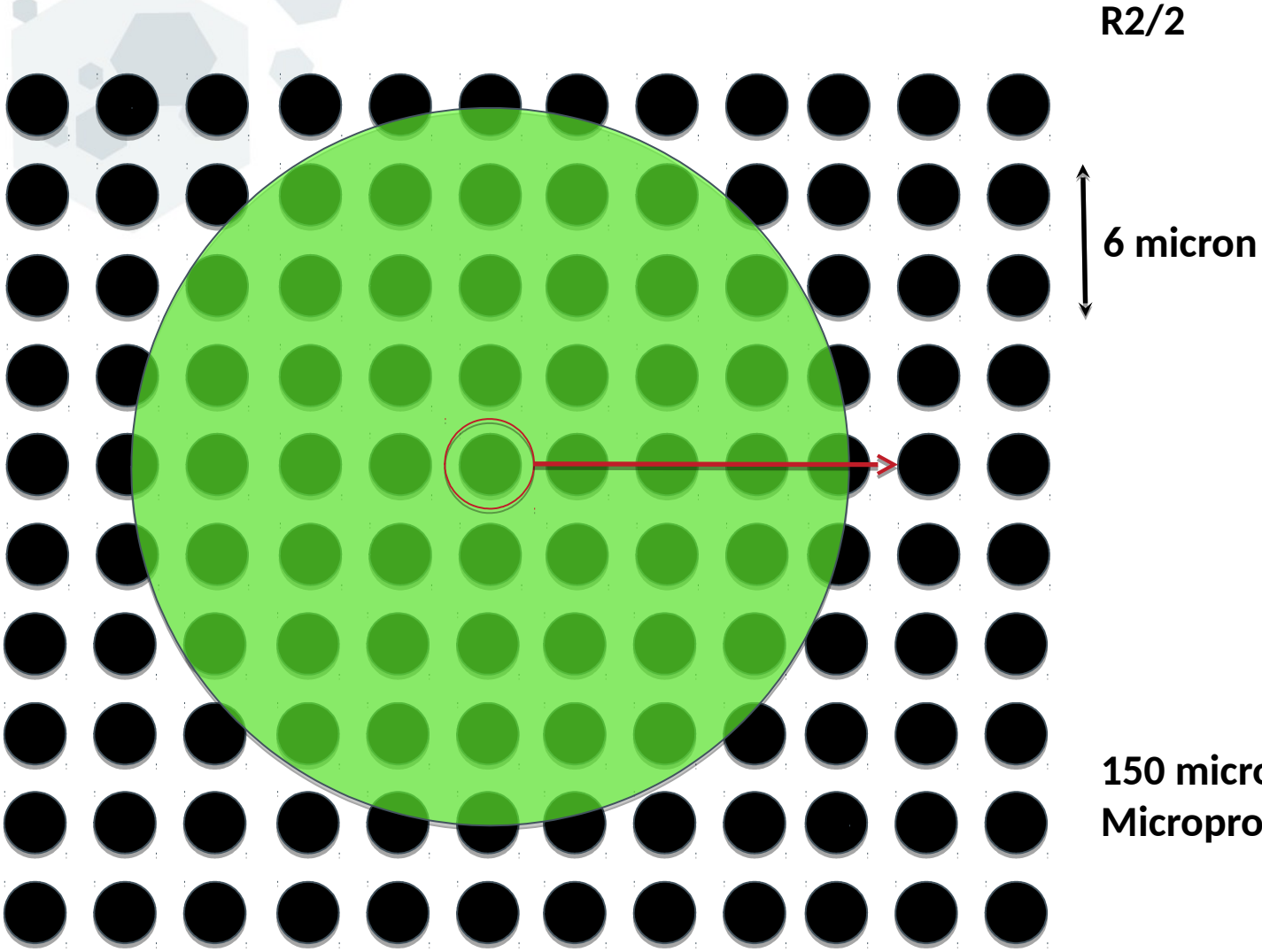
-and should be consistent (50->100 micron should be 2x larger beam)

Beam sizes should be constant for each C2 aperture when:

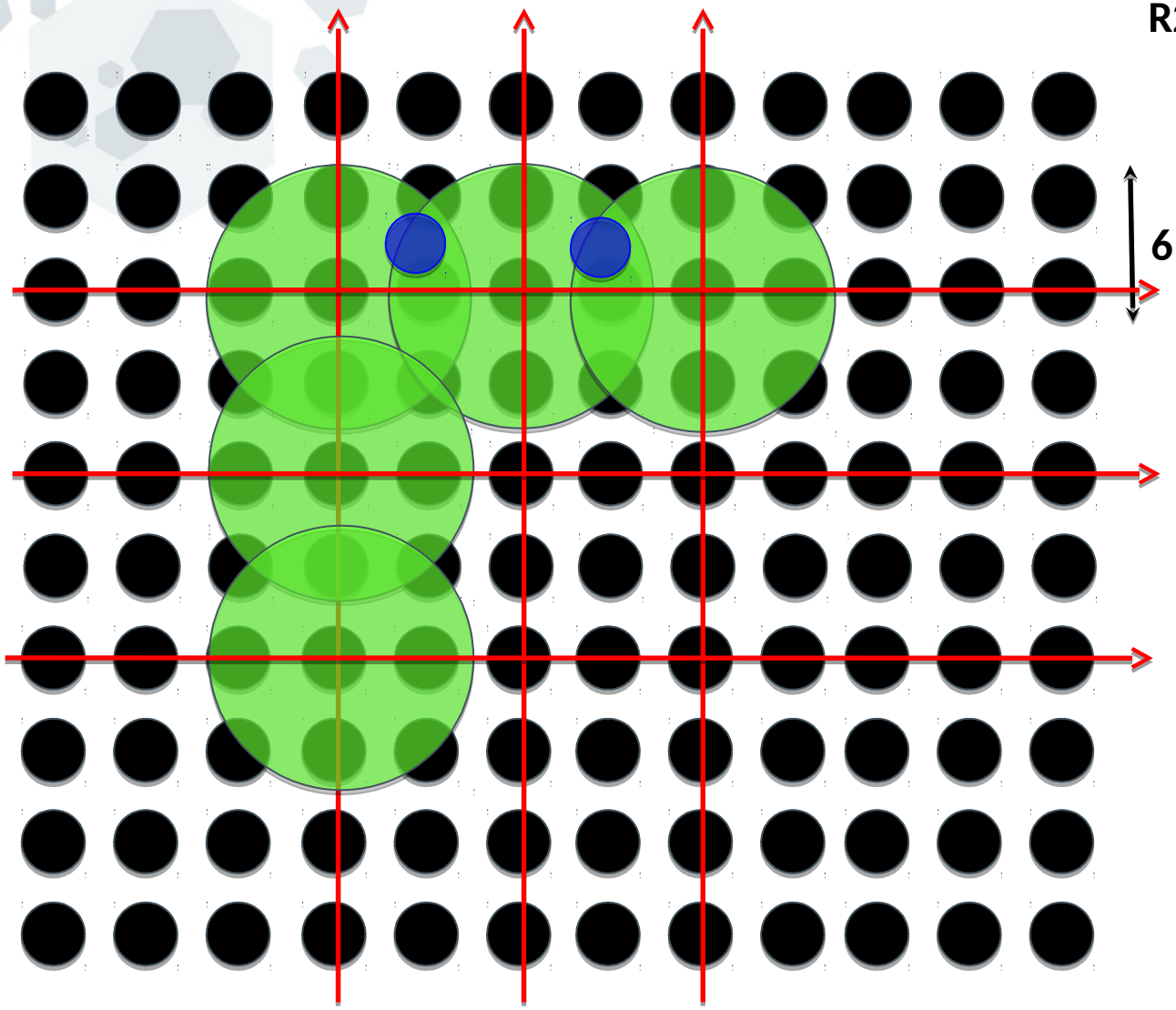
-changing spot size
-changing magnification



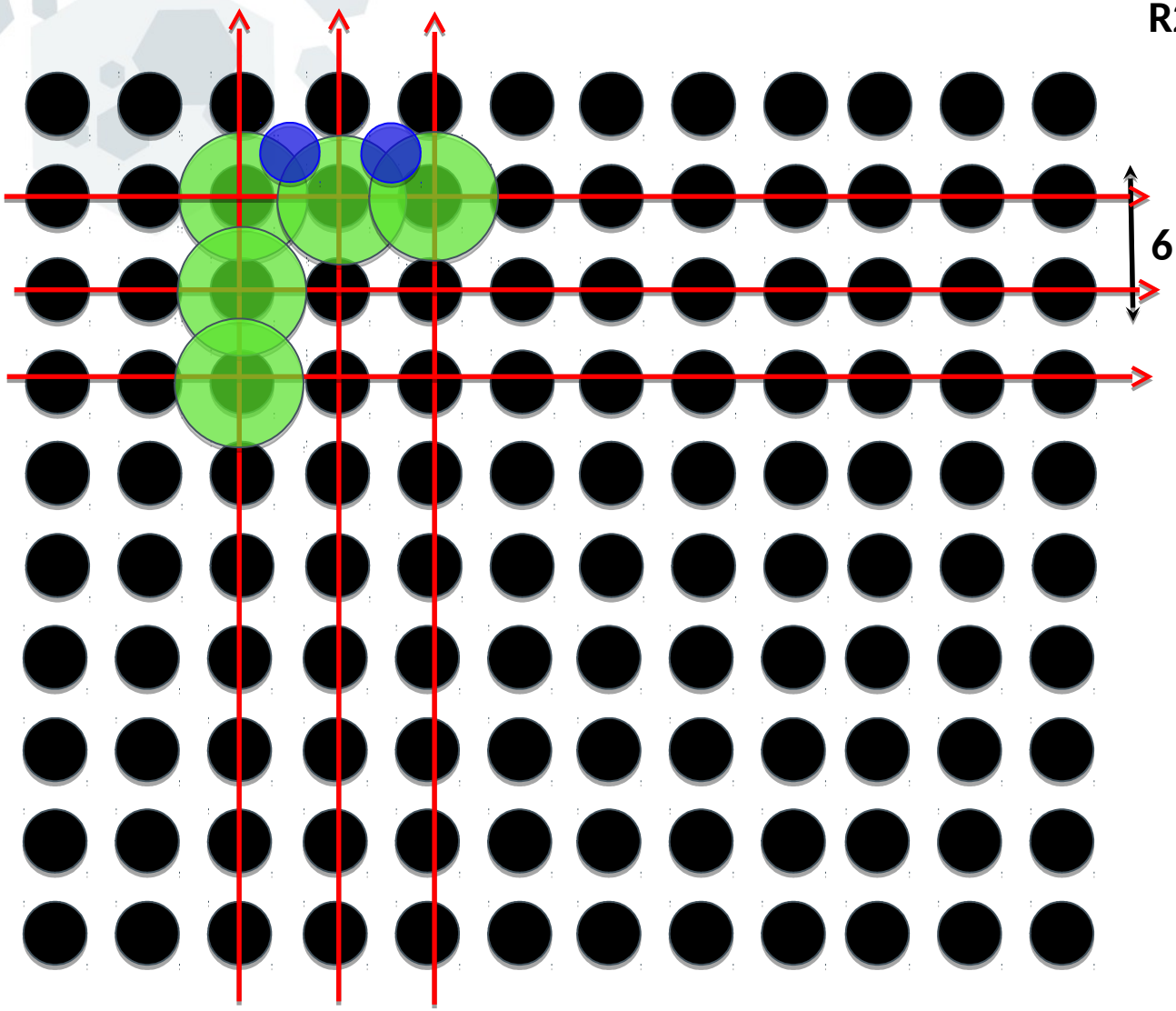
Microprobe (Tecnai)



Microprobe (Tecnai)



Nanoprobe (Tecnai)



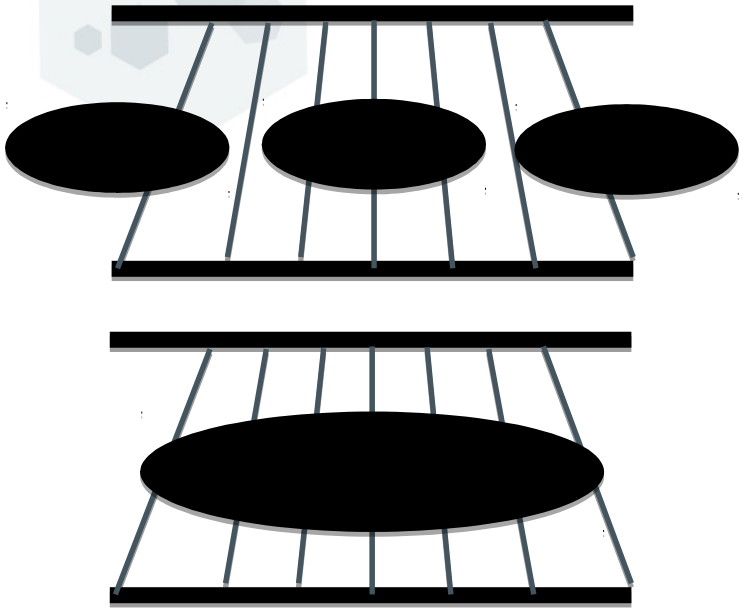
R2/2

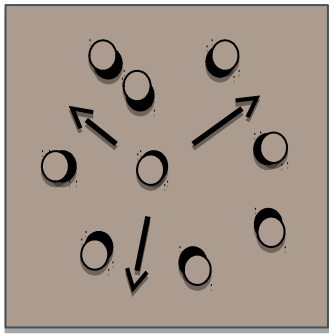
6 micron

150 micron C2 Nanoprobe

Image every hole

What if you are not perfect?





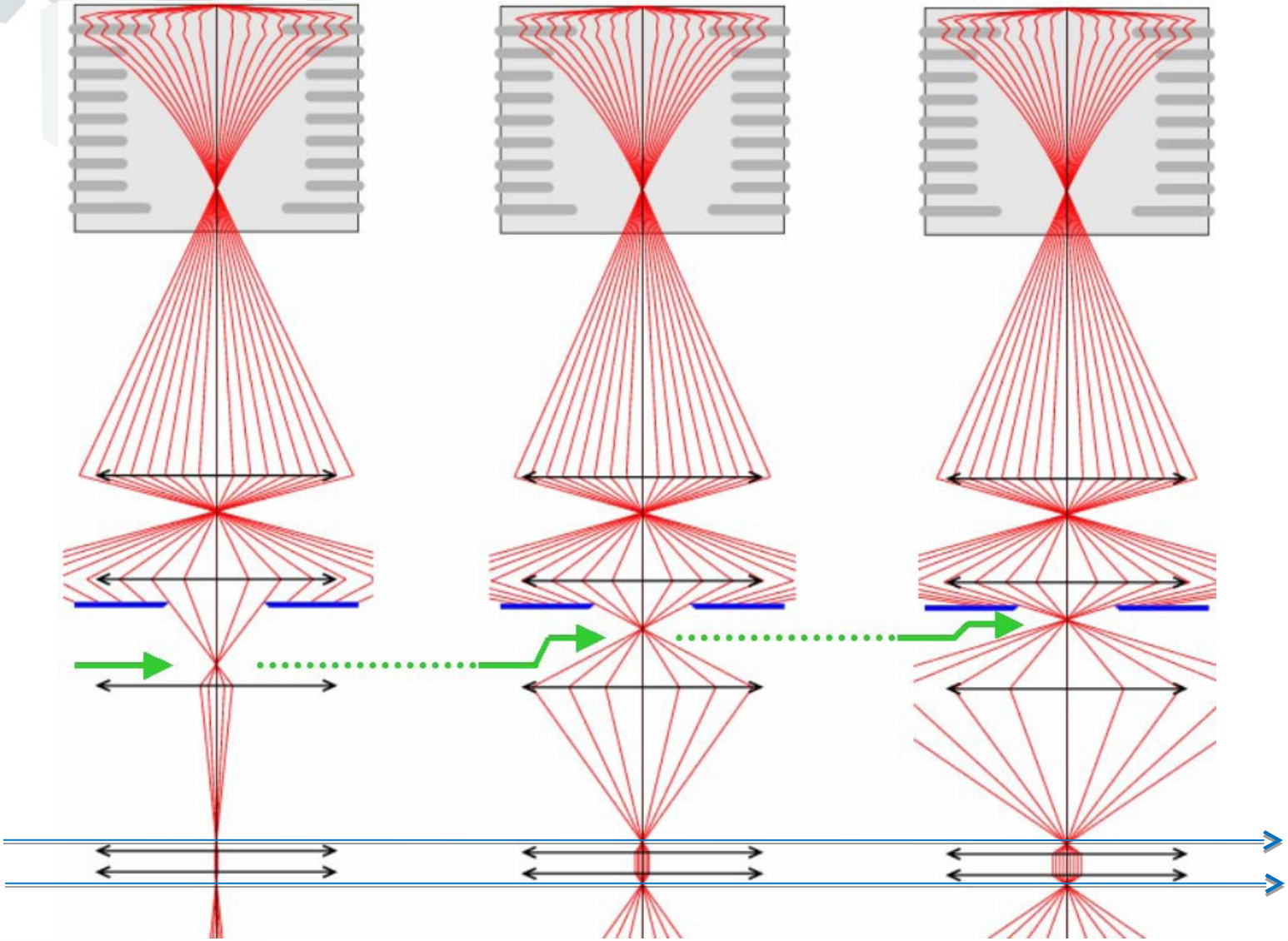
Not parallel

When you are not perfect on a Tecnai, you are better of in microprobe with a larger beam as the center of the beam is more parallel than the outside.

Better use a beam that illuminates 3x3 holes and image almost parallel in the center hole (sacrifice area/throughput for quality) then to image every hole and have mag changes at the edge of your image.

A test is to take an image with gold particles at -5 and -15 micron defocus. Then compare the two images and see if the gold is overlapping. If the images seems to “blow-up” and the gold does not overlap you are not parallel enough in that focus range.

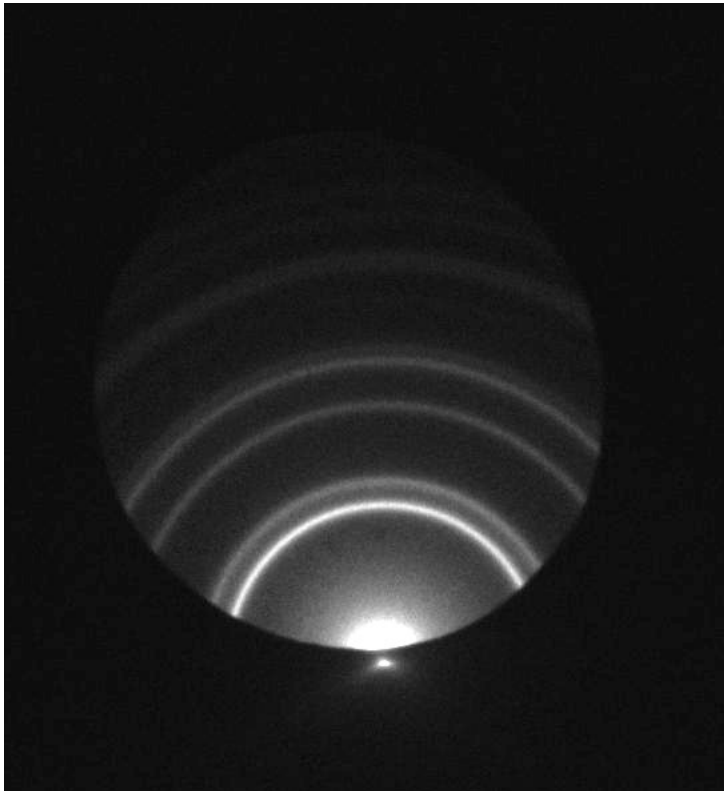
3 Condenser zoom system (Titan)



3 Condenser zoom system (Titan)

-Whenever in the parallel range on a titan,
- -pressing eucentric focus
- -inserting objective aperture
- -pressing diffraction

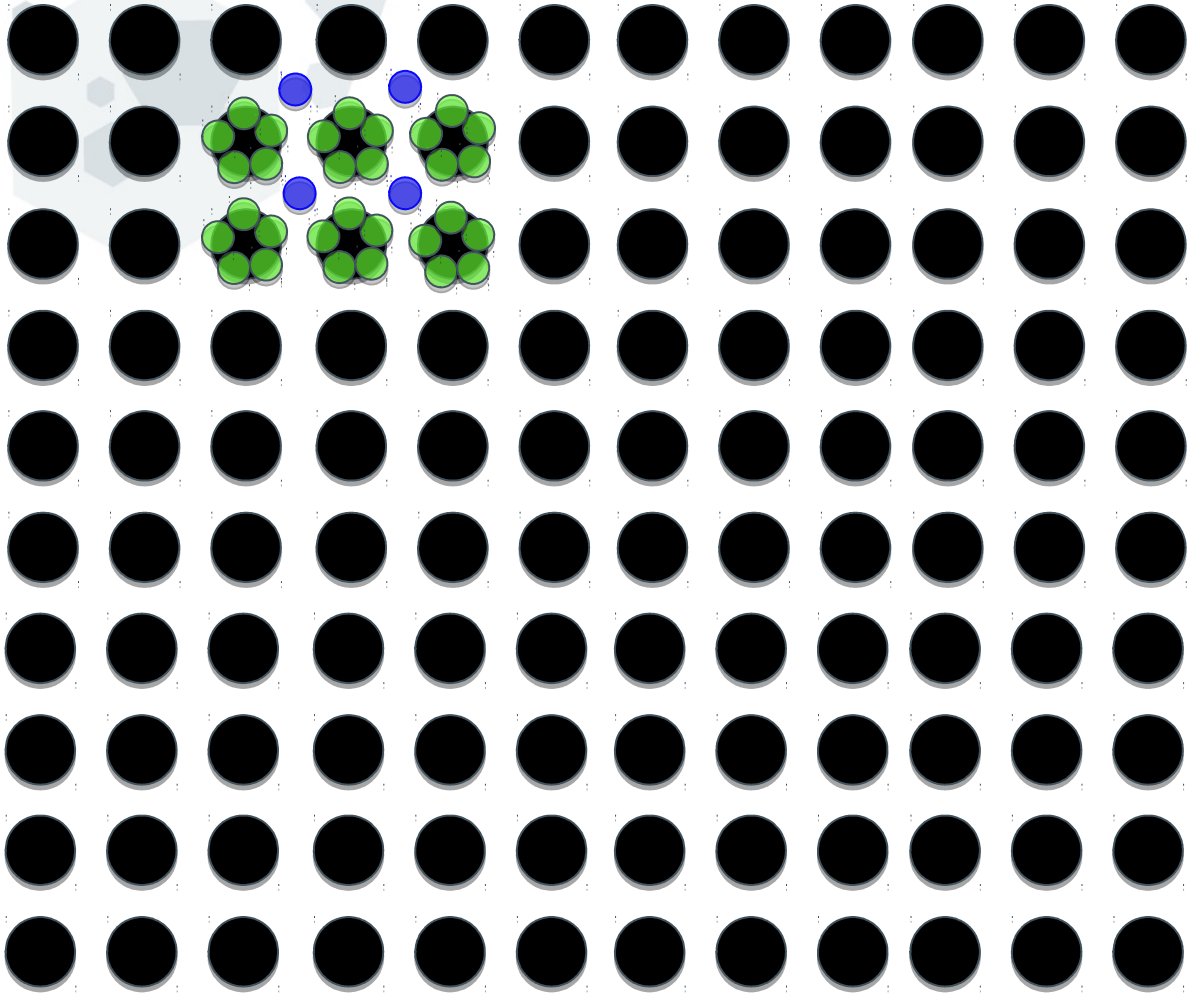
MUST result in the right image! (or very close to)



The image **MUST** remain focused in diffraction AND on the objective aperture when changing intensity and/or spotsize (apart from becoming more intense).

IF NOT: IT DID NOT LEAVE THE FACTORY LIKE THAT!

Nanoprobe (Krios)

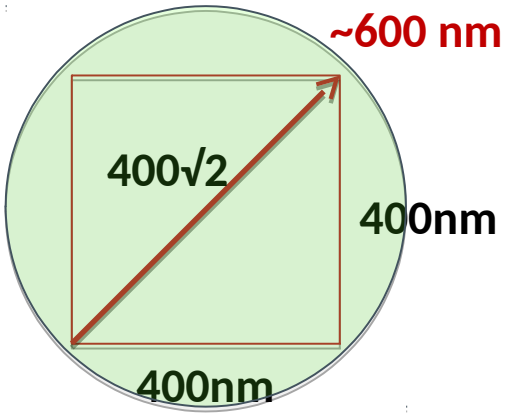


R2/2

6 micron

Any beam size:

4K camera: 1Å pixel

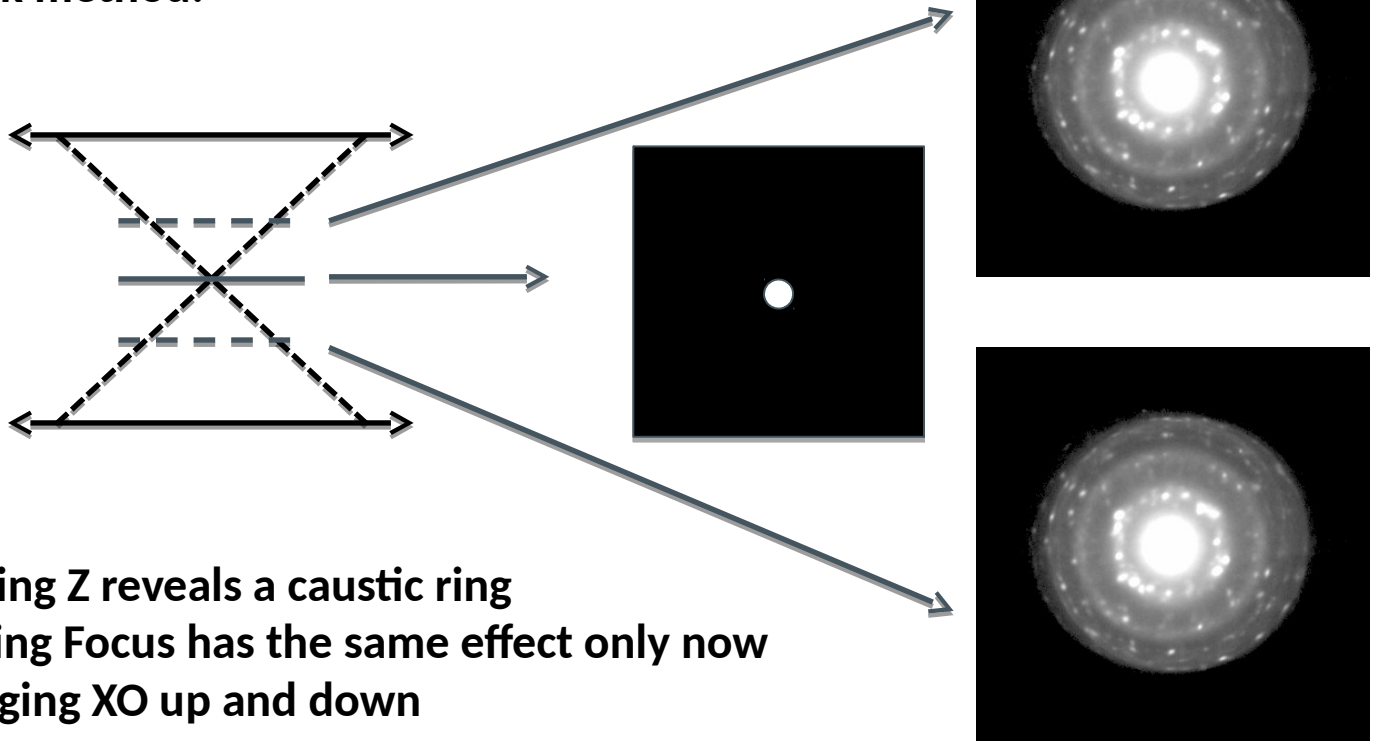


No compromise on dose rate, no wasted area, perfectly parallel, less strain on grid quality (one good grid square is enough)

Daily operation

-Press Eucentric focus
-Always set the sample at Eucentric height (ALPHA-wobbler or quick method)

Quick method:

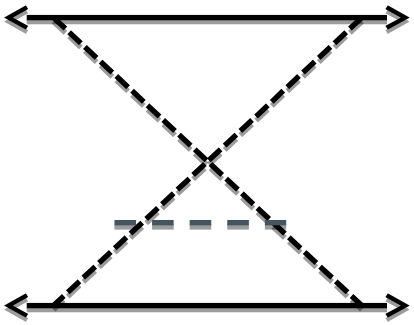


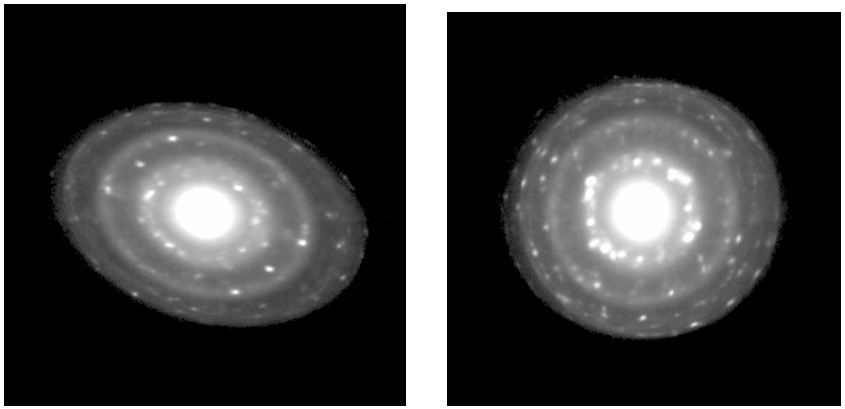
-Moving Z reveals a caustic ring
-Turning Focus has the same effect only now changing XO up and down

Daily operation

-When at Eucentric height and Eucentric focus lower Z to reveal the caustic
-Adjust the caustic ring to be round with objective stigmator
-Stigmator values should not be larger than 0.1. at intermediate mag 30-60kx it should not be much off from zero!

Quick method:



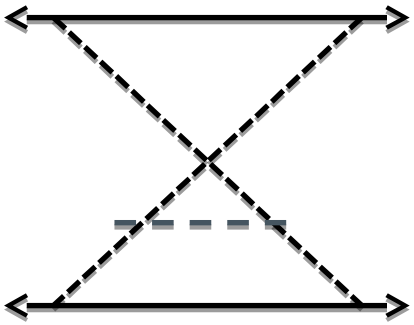


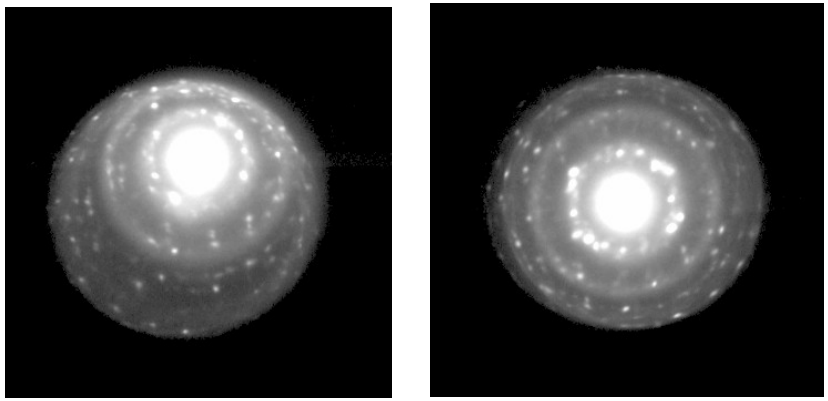
Objective Stigmation

Daily operation

-When at Eucentric height and Eucentric focus lower Z to reveal the caustic
-Activate Rotation center and stop the wobbler using the course focus button
-Center the central spot in the middle of the caustic using the MF buttons (rot center)
-Coma free alignment and Rotation center both use the same button! You can not optimize both!

Quick method:

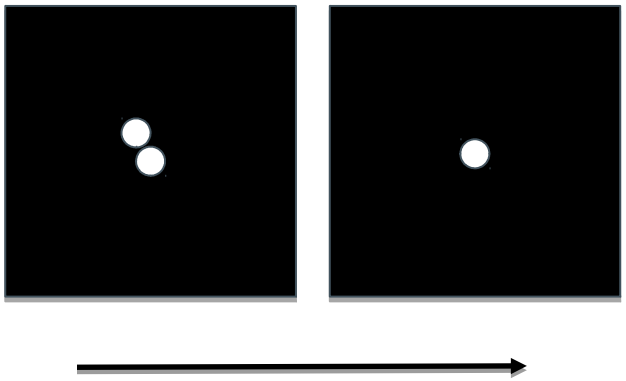




Rotation center/Coma

Daily operation

-Bring the sample back to Eucentric height with Z-height (caustic back to a spot)
-Check pivot points at Eucentric focus
-Be aware that the PP are focus dependent!
-As for SPA you change the focus the PP will change! (Unavoidable), therefore never correct PP at a specific focus other than Eucentric focus
-Since PP are focus dependent, the beam will move while imaging at different defocus settings! This is not beam instability, it's a fact of life.
-Your parallel beam size should therefore be chosen a bit larger than the size of the diagonal of the camera surface!



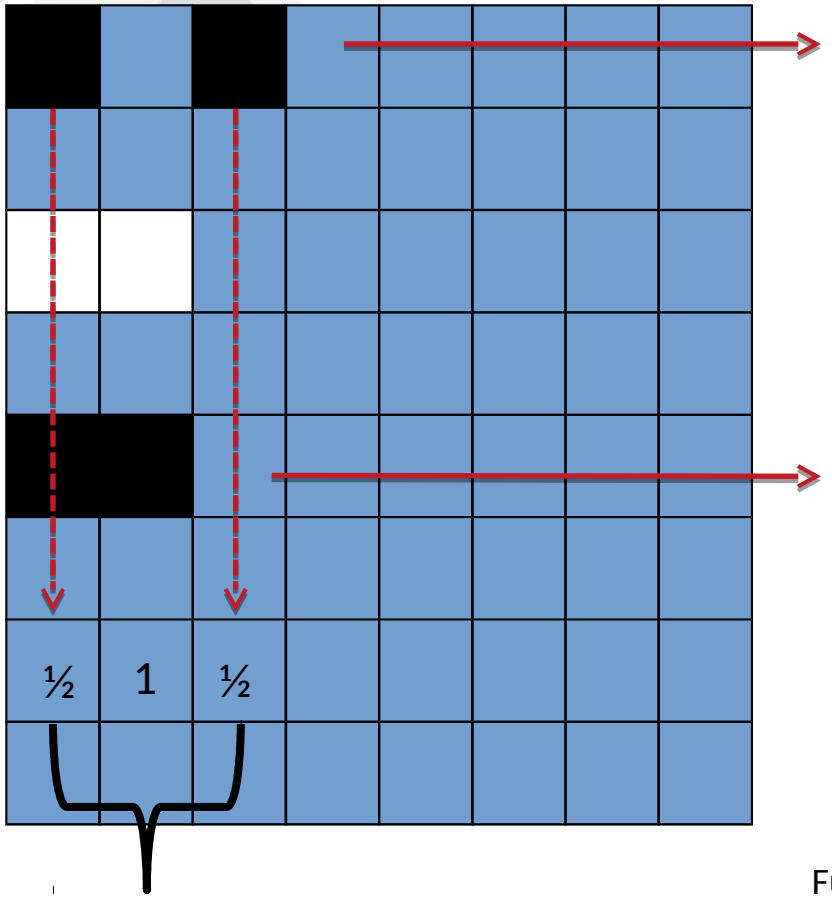
Pivot points

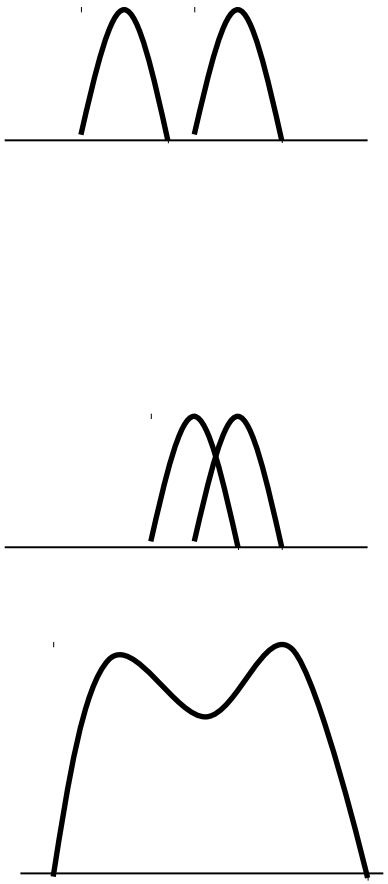
Now to choose the magnification

Depends on 3 Factors:

-The desired resolution
-The chosen defocus range and the sampled CTF
-Oversampling needed during image processing
-Sensitivity of the camera (DQE curve)

Resolution





Resolved

Not-Resolved

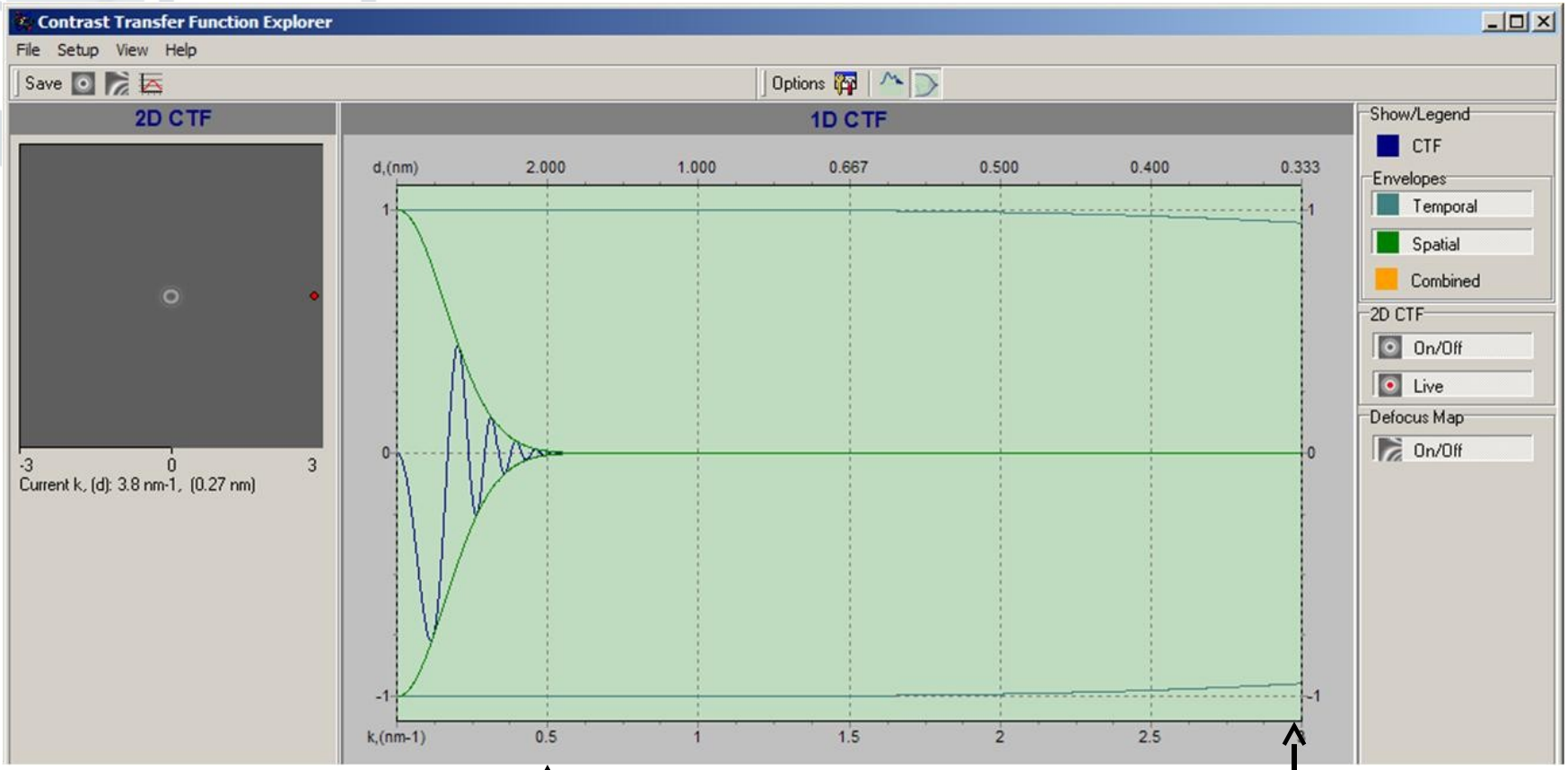
Magnified

2 pixels = smallest distance to be resolved = Nyquist

Full Nyquist = 1 (normalized) = highest resolution at given magnification

Example: 75000 mag, pix size = 1Å:

Ny = 2Å, ½ Ny = 4Å, ¼ Ny 8Å

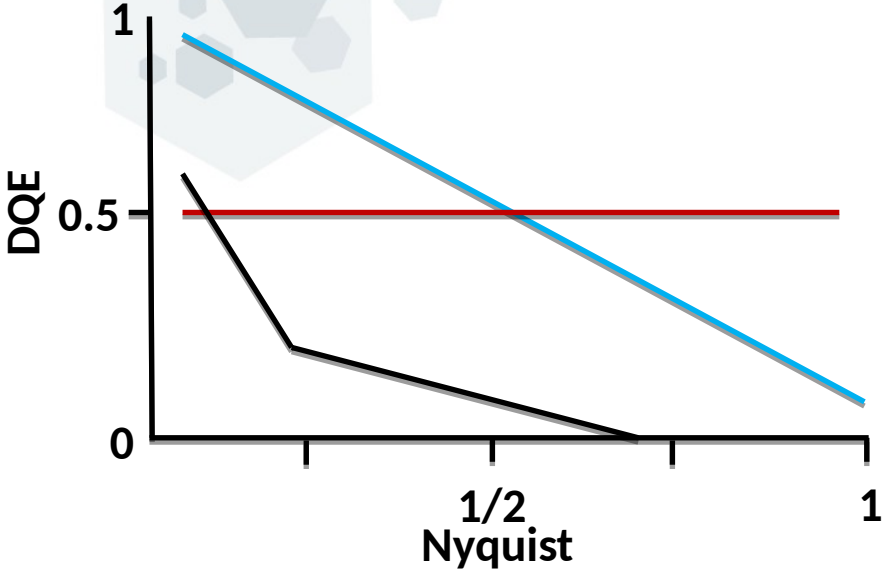


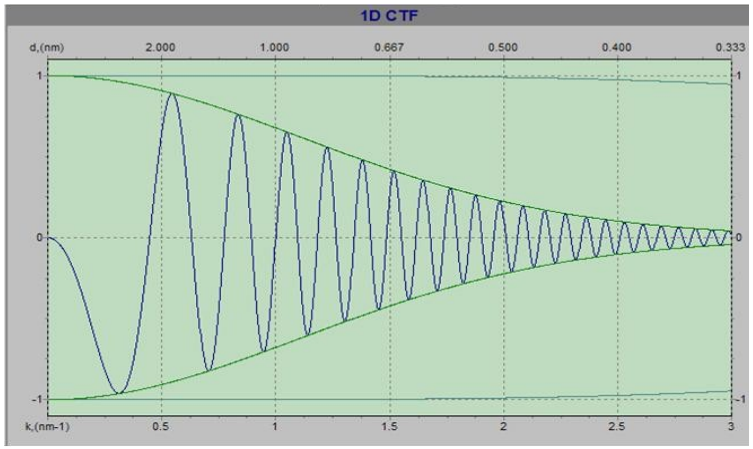
1.5Å pixelsize

1/0.5 = 2 nm resolution ⇒ 1 nm pixelsize is enough

Choose your magnification in relation to your defocus and to what you need to see!!

Magnification vs DQE



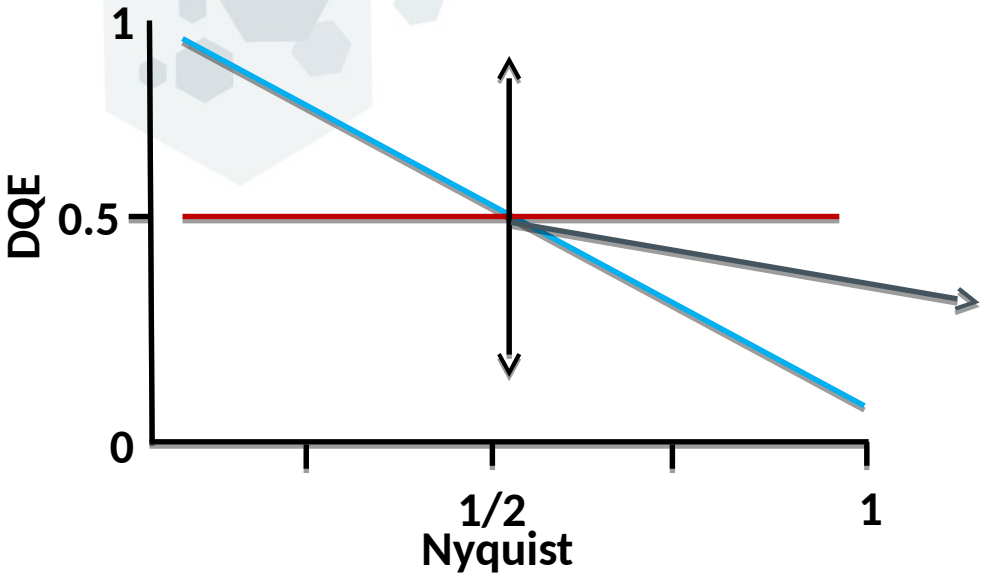


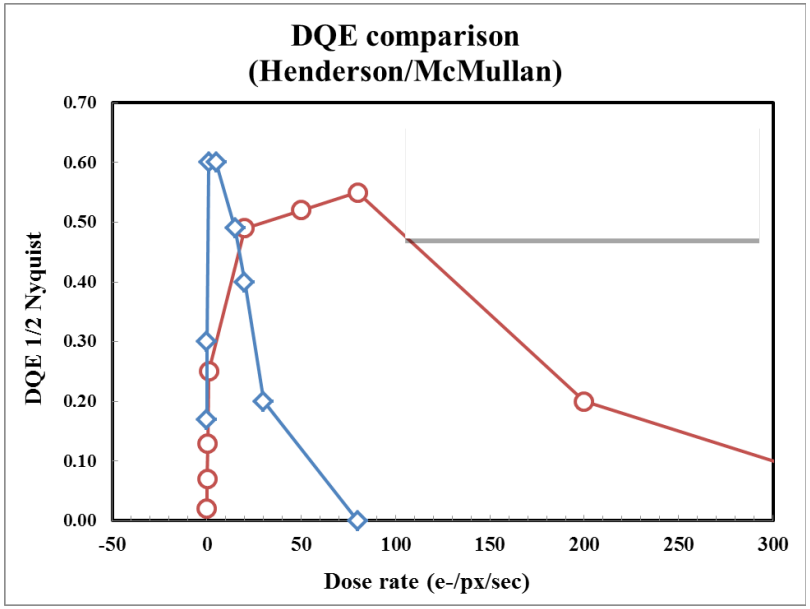
Integration based DED
Sensitive (enough) at Nyquist
Pixelsize = resolution/2
No point in binning
Large field of view (FOV)

Counting based DED
Very high DQE below ½ Nyquist
Pixelsize= resolution/4 or more
Binning makes sense to do
Small FOV

CCD
DQE pretty bad, no signal beyond ½ nyquist!
Pixelsize= resolution/4 or more
Binning almost necessary
Small FOV

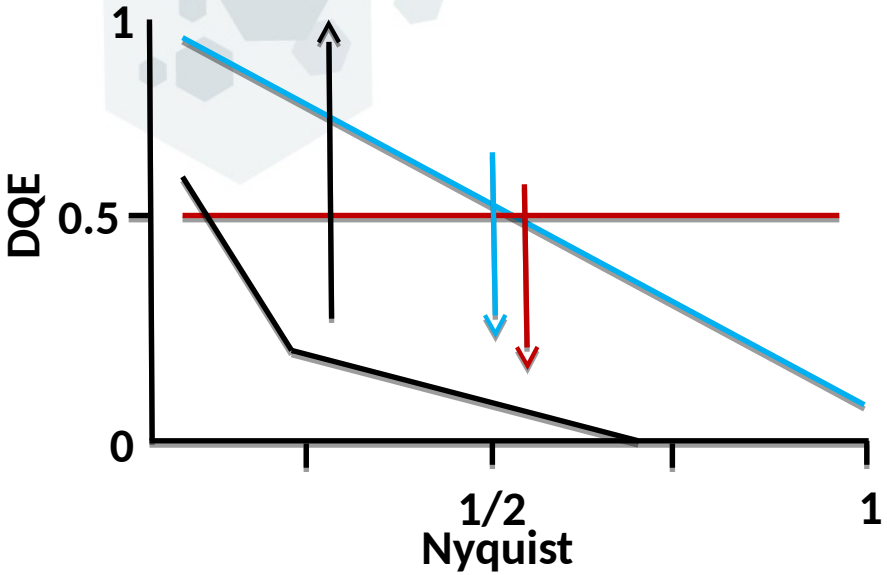
The effect of dose rate





-Integration based DED get better when increasing dose rate! (Short exposure times)
-Counting based detectors get better when decreasing dose rate! (long exposure times)
-Both have their optimum working conditions
-Don't use a counting based detector at high dose rate
-Don't use a integration based detector at low dose rate

The effect High Tension



All DED in general perform better at higher HT and worse at lower HT

All CCD camera's perform better at lower HT and is dominating performance.

How much worse or better depends on the design, or is not known yet

However, realize also that:

Beam damage and charging are worse at lower kV
Aberrations are worse at lower kV, thus resolution (uncorrected) is worse at lower kV
Penetration power is less at lower kV (more scattering and blurring)
Contrast, WITH THE SAME OBJ APERTURE, is better at lower kV

How to setup your scope

-This should not take more than 15 minutes:

-Determine the desired resolution, oversampling, sensitivity of the camera: pixelsize
-Pixelsize determines the magnification (example 1Å on Titan is 60kx), set mag!

-Insert cross-grating
-Press Eucentric Focus (~80% titan, 90% Tecnai)
-Set Eucentric Height using Alpha-wobbler for course and then using a focused spot (Intensity C2) for fine by minimizing caustic ring

-Lower Z Height to show caustic ring
-Center spot in the middle of caustic ring: rotation center
-Make caustic ring round: objective astigmatism
-Bring Z-height back to a spot
-Check Pivot Points

How to setup your scope

-Find parallel illumination condition
-Switch to diffraction
-Insert objective aperture
-Focus objective aperture with focus
-Focus diffraction pattern with intensity
-Measure beam size for each C2 aperture
-Choose the C2 aperture closest to camera size that is still workable

-ON A TITAN, simply set the beam size slightly larger than the camera diameter in the parallel range, then press diffraction and insert objective aperture: BOTH should be focused by definition!!!!

-You should now be:
-At Eucentric height
-At Eucentric focus
-Parallel
-Illuminating the detector fully
-Roughly stigmated
-Roughly coma free

How to setup your scope

Finishing touch:

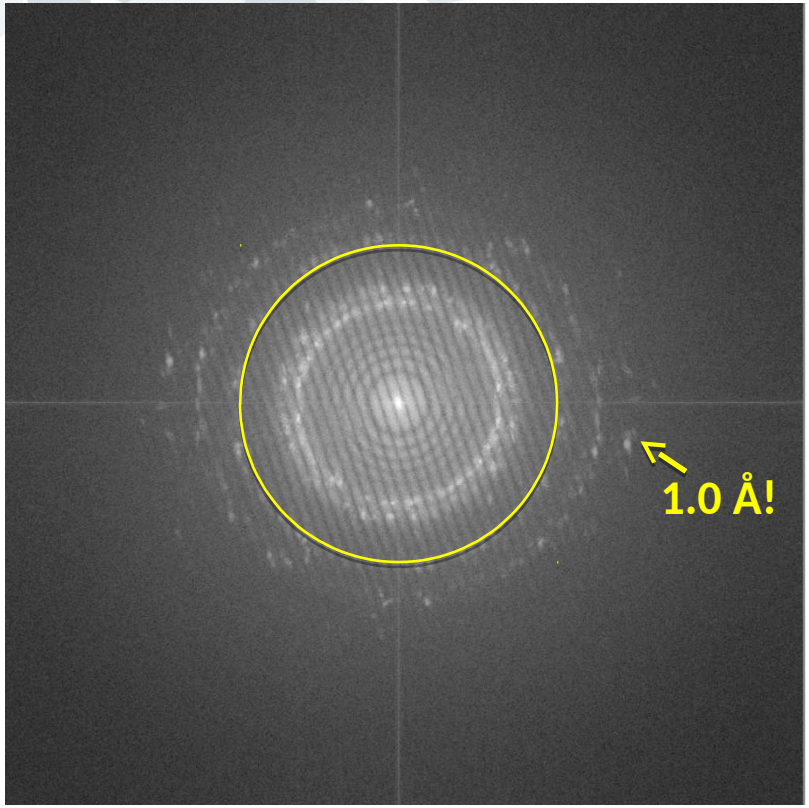
-At the desired mag now at all settings roughly right, switch to live camera mode
-Live FFT (use binning if necessary)
-Stigmate and focus accurately
-Defocus ~700nm
-Activate coma Free alignment and make both tilts at the same defocus (don't look at astigmatism in this step)
-Stigmate again if needed
-Set focus 1 micron

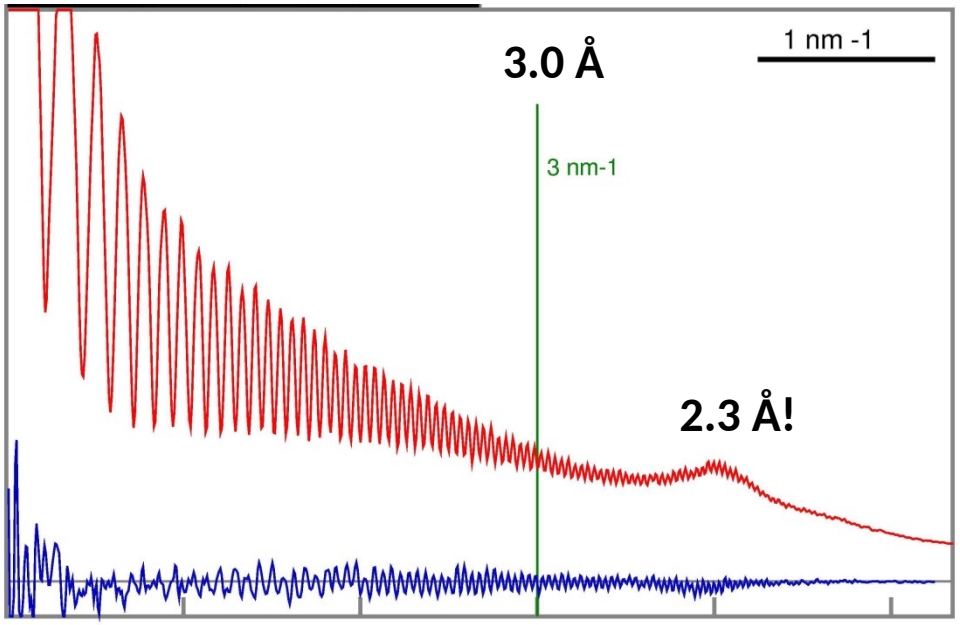
-Set desired dose rate using spotsize (optimal dose rate for camera)
-Calculate exposure time

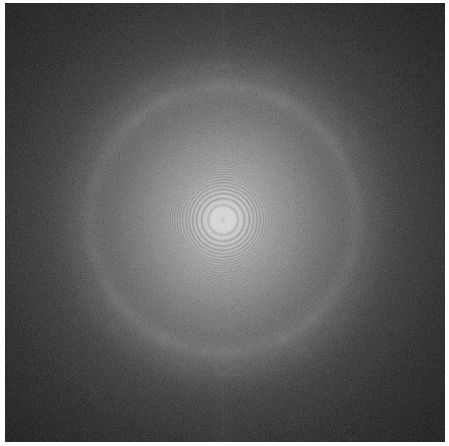
-Take image

When the thon rings run out beyond the first ring of gold you are already at 2.3Å, given a 1Å pixel size this should be visible if the X-grating allows, otherwise use more dose!

You should see something like this (Krios):







Thank you!

good luck!