Microscope Basics

Midlands Cryo-EM Workshop
2021

Christos Savva

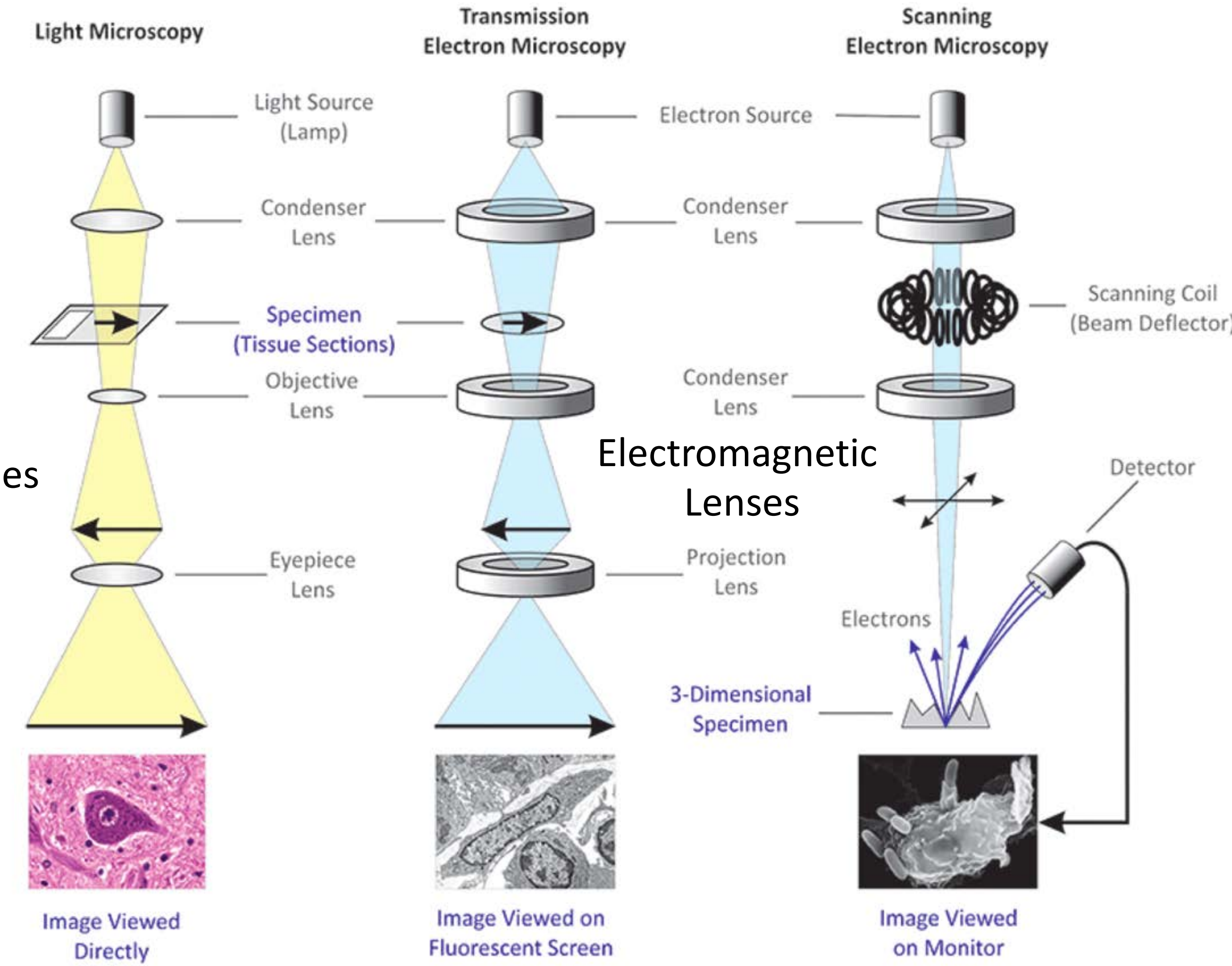
1931

2021

Electron Microscopy

Glass Lenses

Electromagnetic Lenses

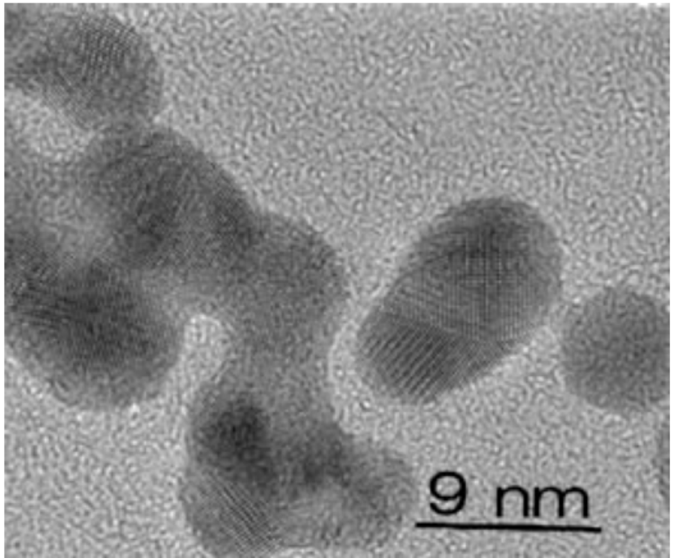


MicrobiologyInfo.com

Transmission Electron Microscopy

- The Transmission electron microscope is required for high-resolution structural studies
- Requires ultra-thin specimens <0.2 µm
- 100 kV electrons λ=0.037 Å
- Lens aberrations limit resolution to ~1 Å (0.5Å in some cases)

Electron Micrograph: Picture taken with an EM

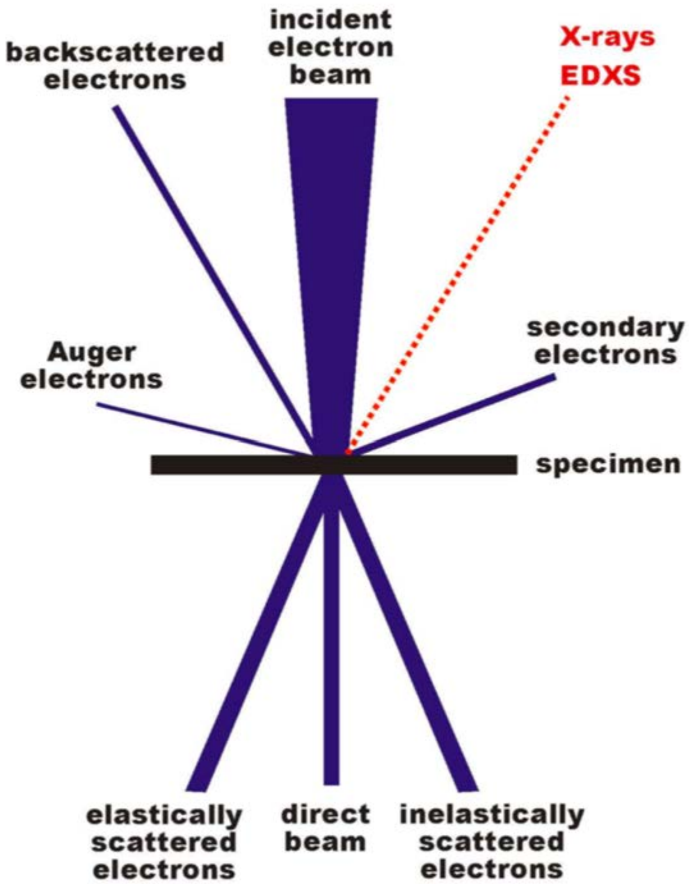


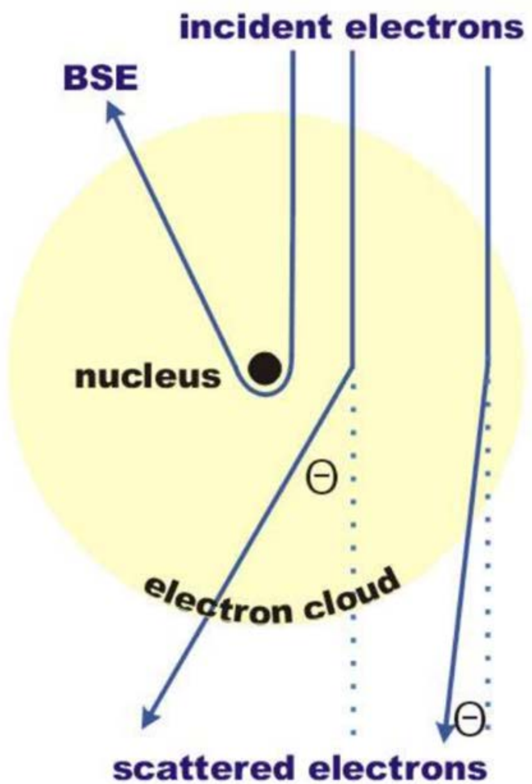
Gold particles on carbon film. Lattice spacing of 2.04 Å

Image:
Electron Microscopy Sciences

UNIVERSITY OF LEICESTER

Transmission Electron Microscopy



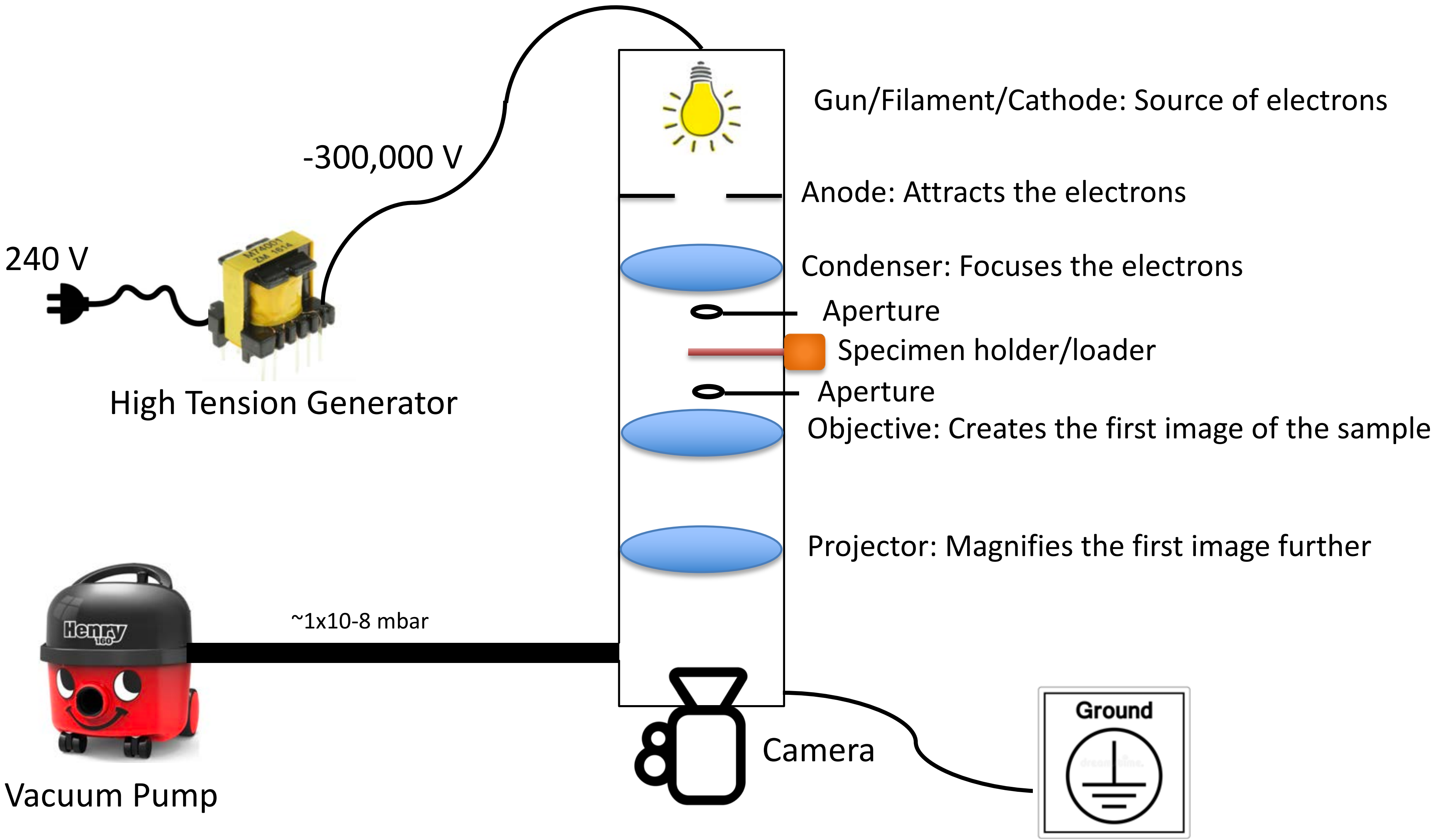


Electrons attracted to nucleus of atom. The higher the atomic number (Z) the higher the scattering.

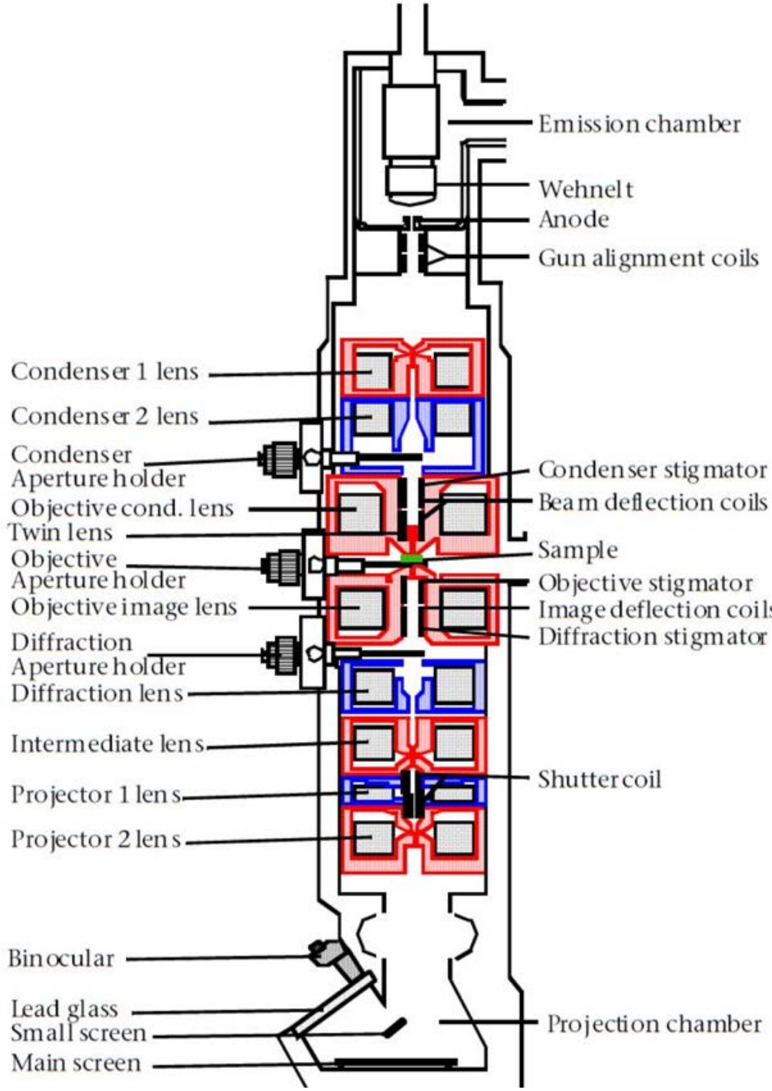
Figures 4 and 5 Frank Krumeich

A very Basic TEM

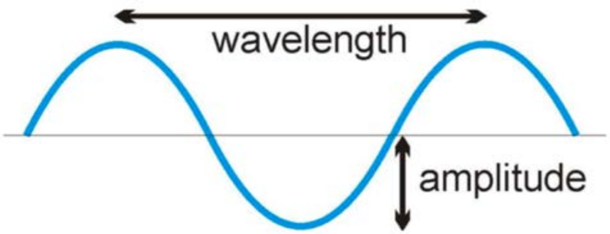
(that probably wont work)



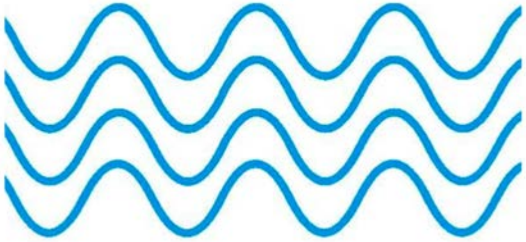
The Transmission Electron Microscope



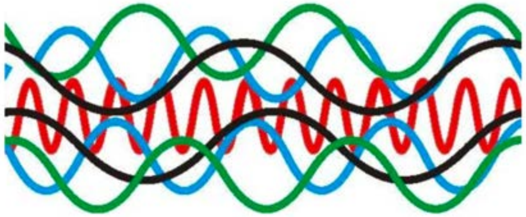
Wave Coherence



- Spatial Coherence: Are the electrons coming from the same direction and in phase?
- Temporal coherence: Do the electrons have the same energy/speed (wavelength)?



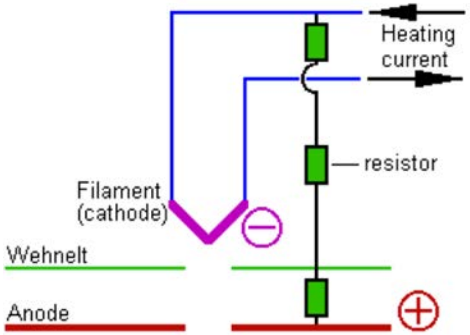
Coherent Waves

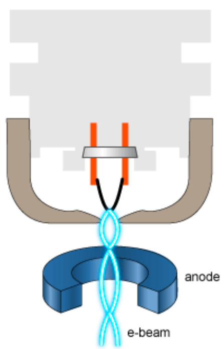


Incoherent Waves

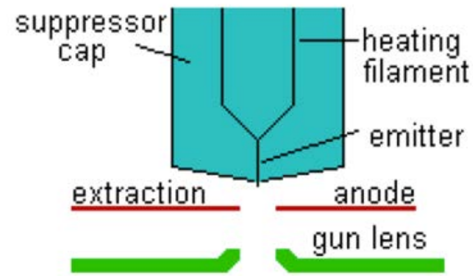
TEM Gun types

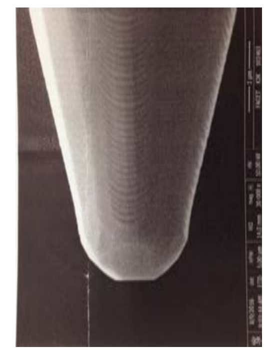
Thermionic: W or LaB6





Field Emission Gun (Schottky)

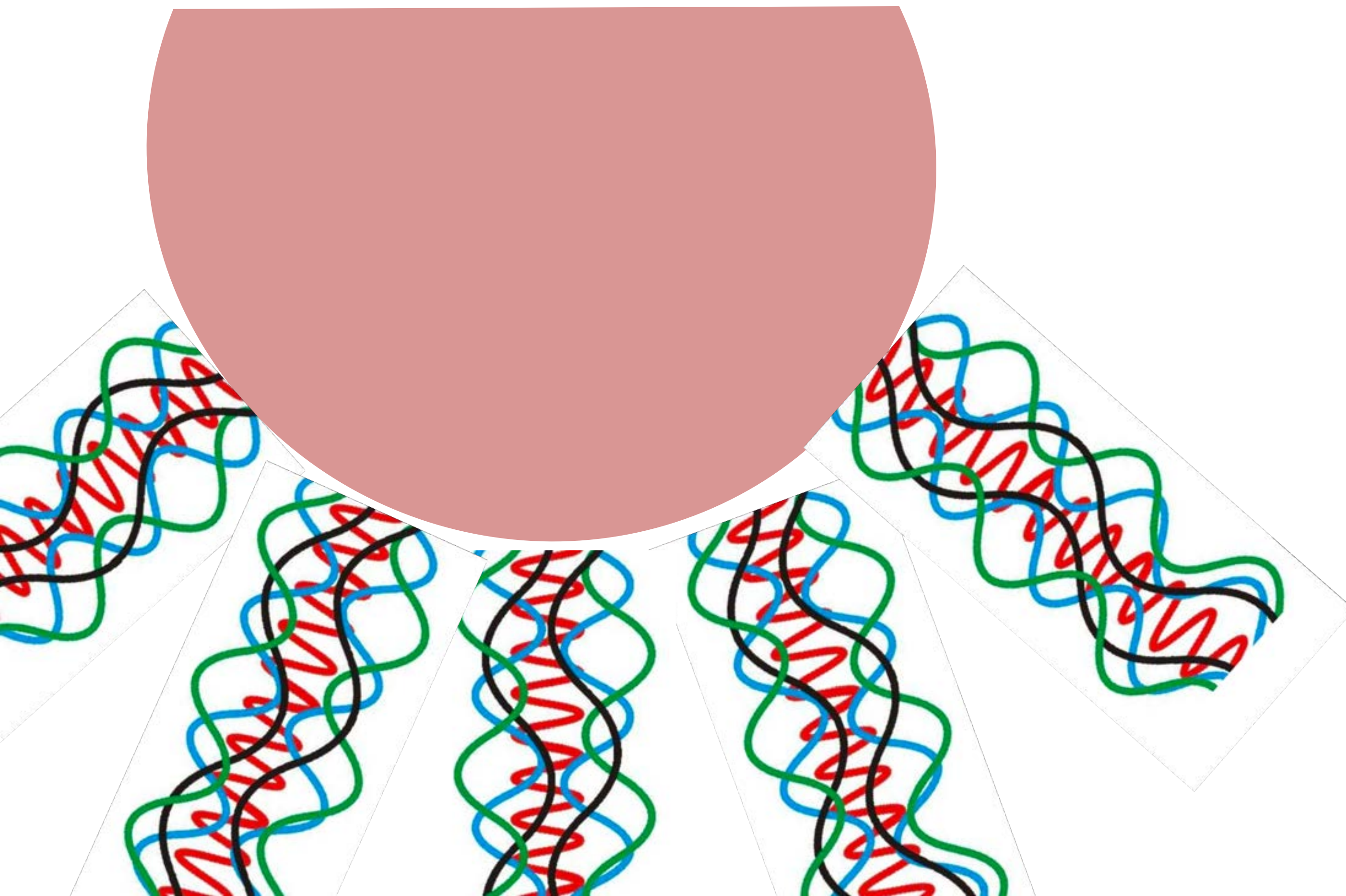




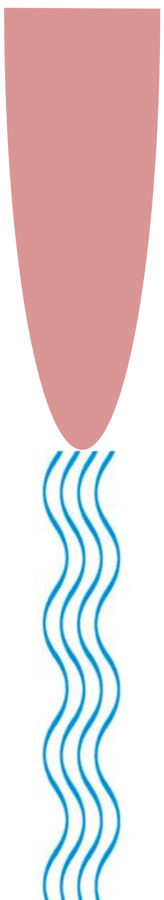
Temporal	1.0-2.0	Energy Spread (eV)	0.2-0.3
Spatial	$>10^4$	Source Size (nm)	<30
	10^5	Brightness (A/cm^2xsr)	10^8
	10^{-3}	Vacuum required (Pa)	10^{-8}
	100-300	Life Span (hrs)	~8000

Electron Beam Coherence

Thermionic gun

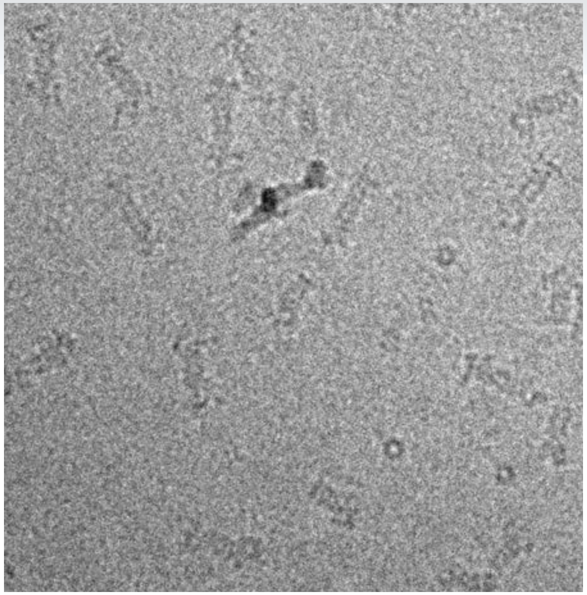


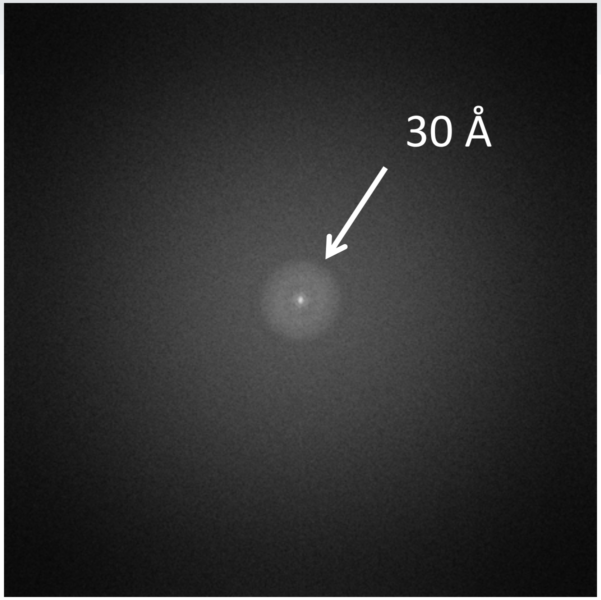
Field Emission Gun



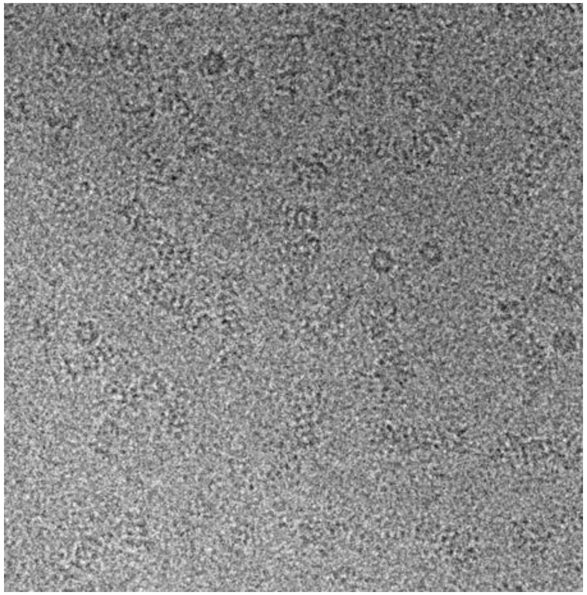
Gun Dependent Resolution

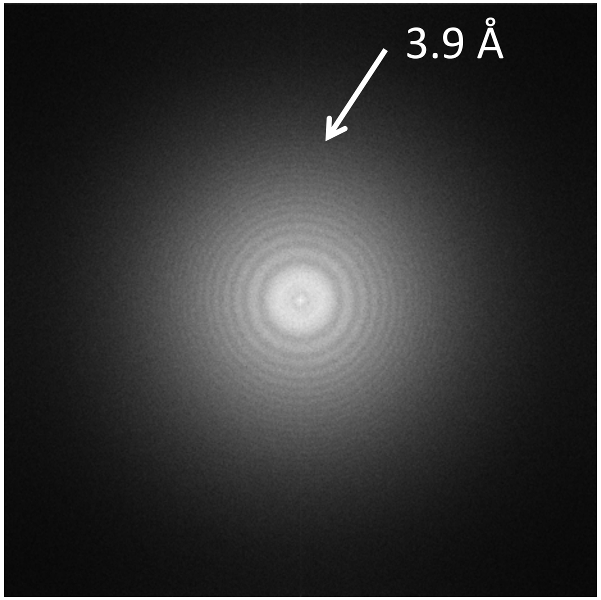
Thermionic Gun





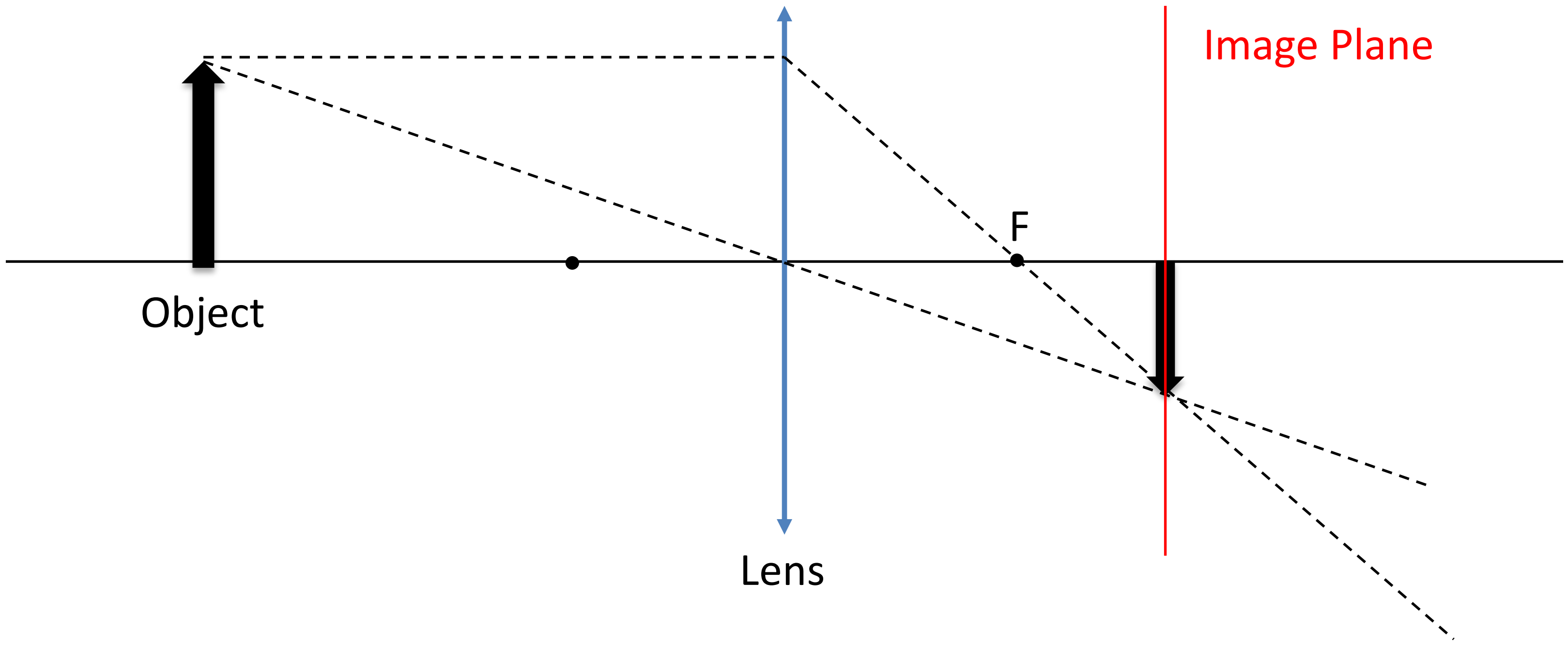
Field Emission Gun





(Alice Clark, 2014)

Lenses



F=Focal point

Lenses

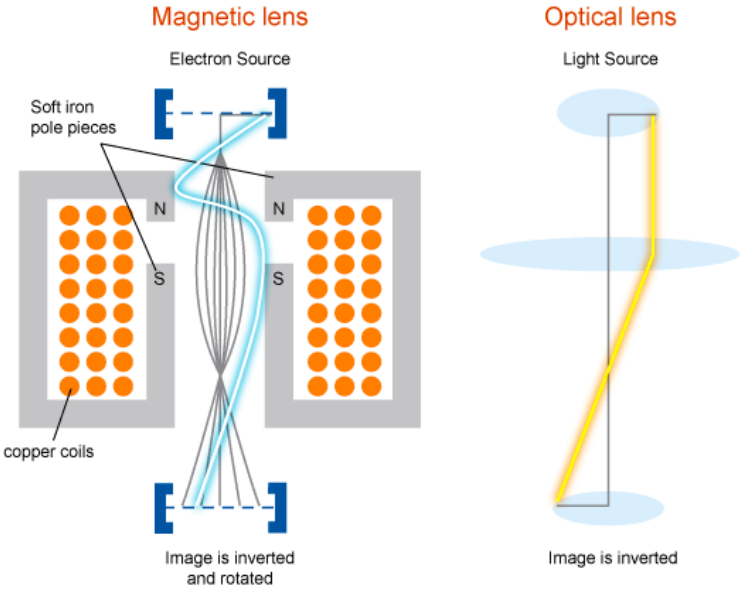
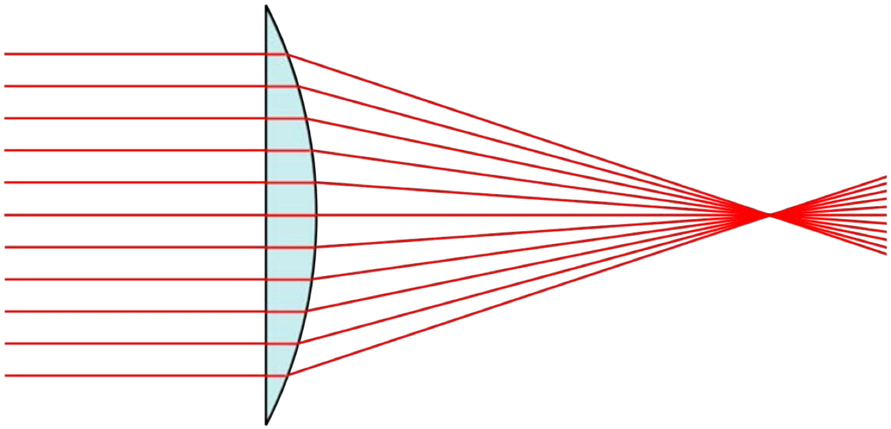


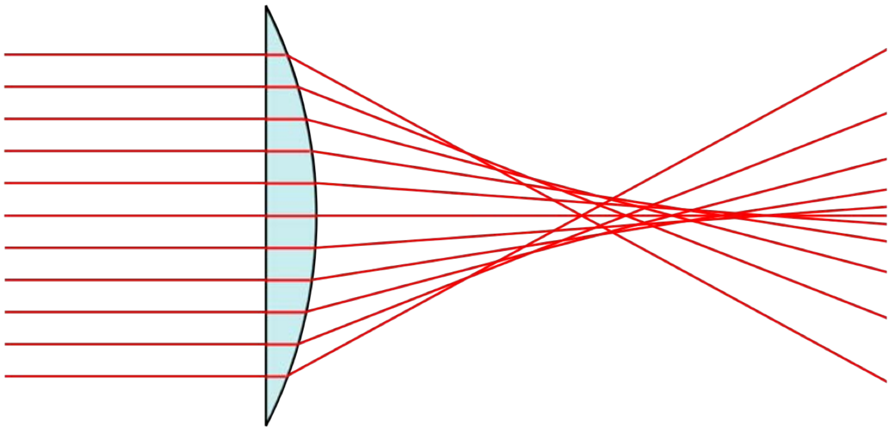
Image:
myscope.training

Lens aberrations

Lenses are not perfect and suffer from aberrations



Perfect Lens

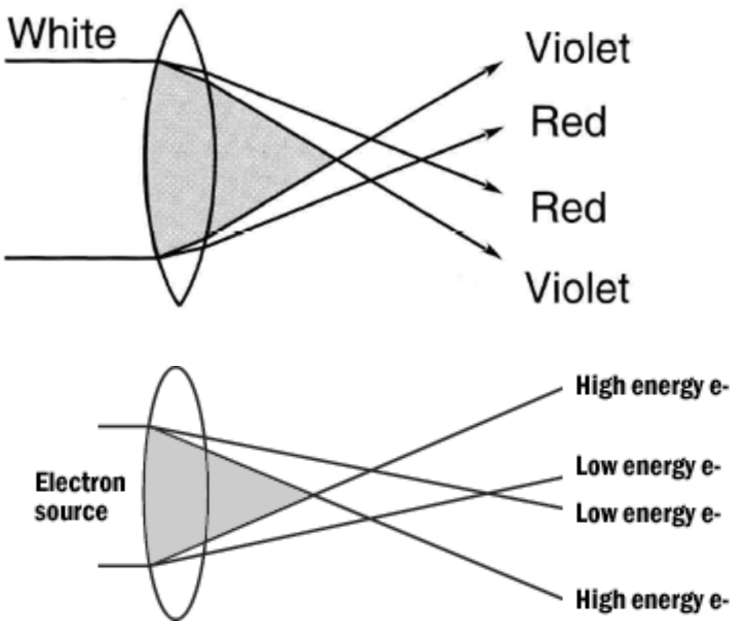


Spherical Aberration (C_s)

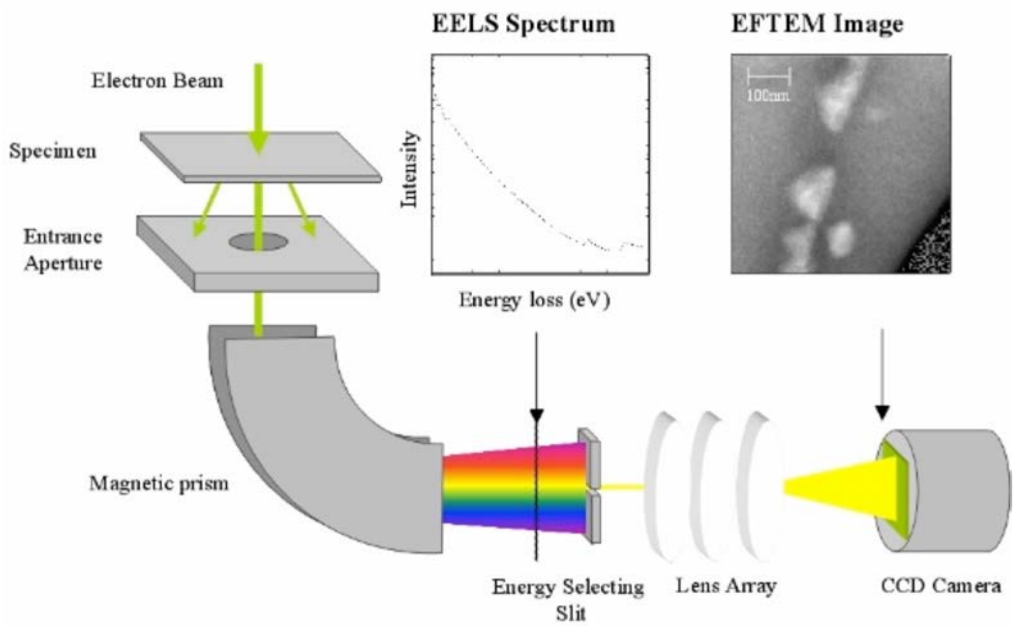
Requires expensive hardware to correct

Chromatic aberration (C_C)

- Energy spread of electrons due to gun temporal coherence
- Thick samples also result in many electrons with lost energy
- Cs correctors available but expensive
- Energy filters can remove energy loss electrons
- More important for tomography of thick specimens



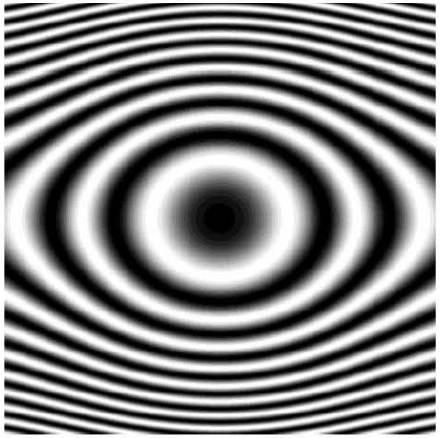
Chromatic Aberration of the objective lens

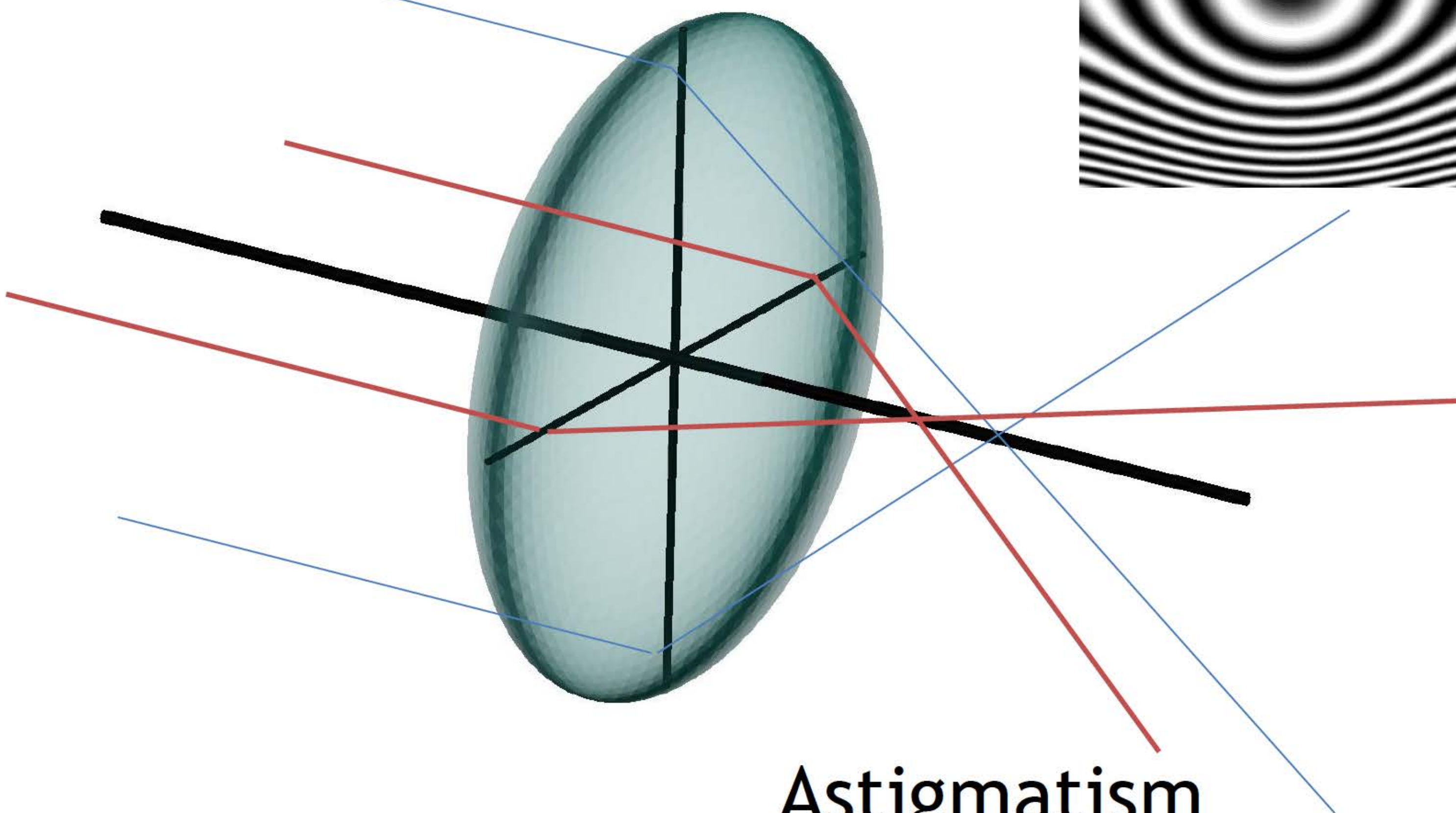


Objective Lens Astigmatism

FFT of sample

Astigmatic

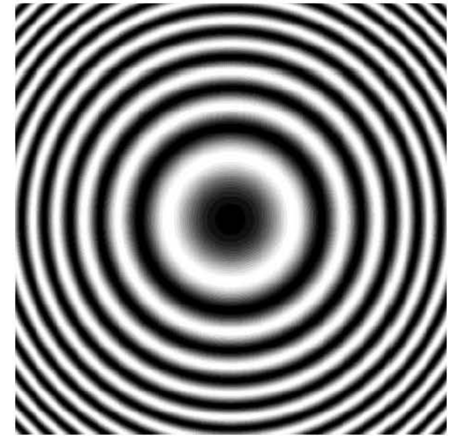


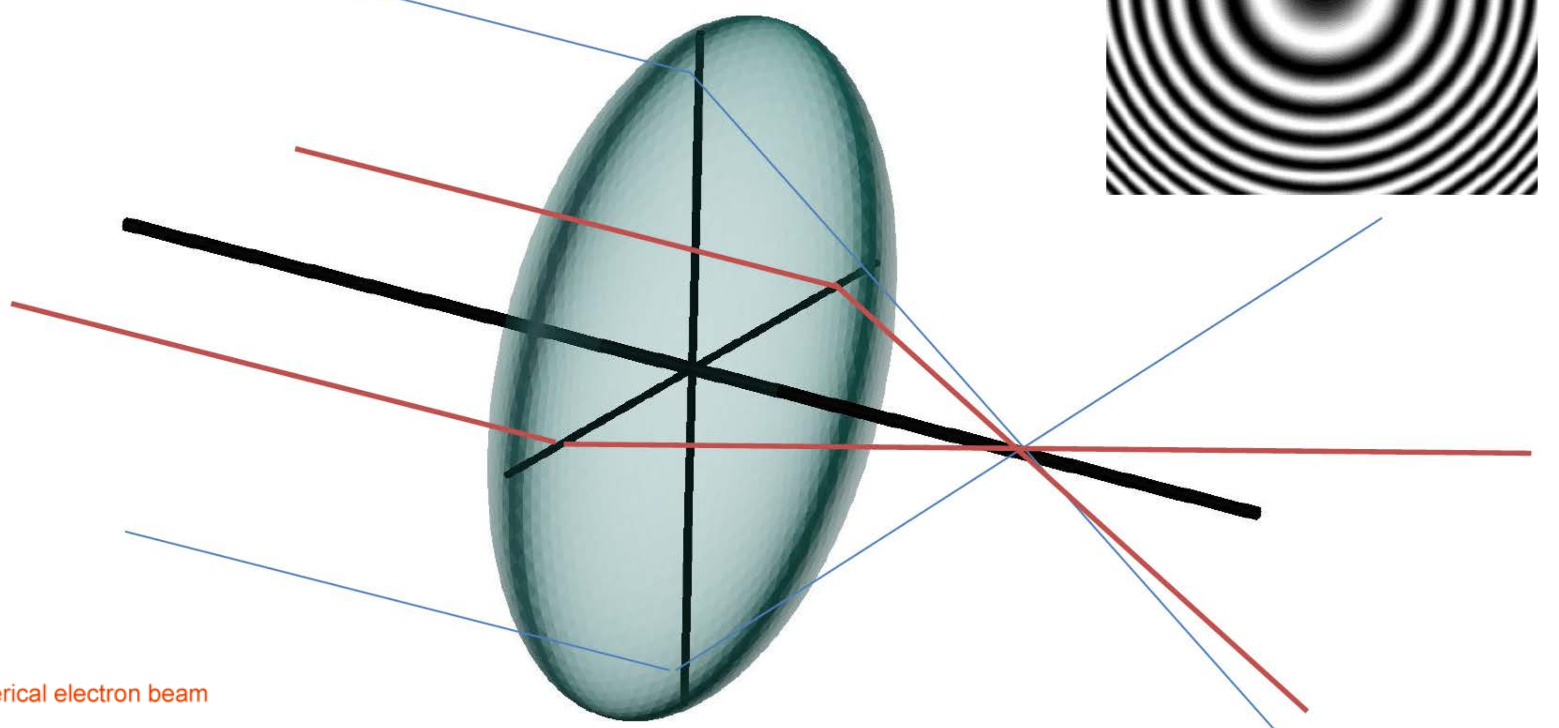


Astigmatism

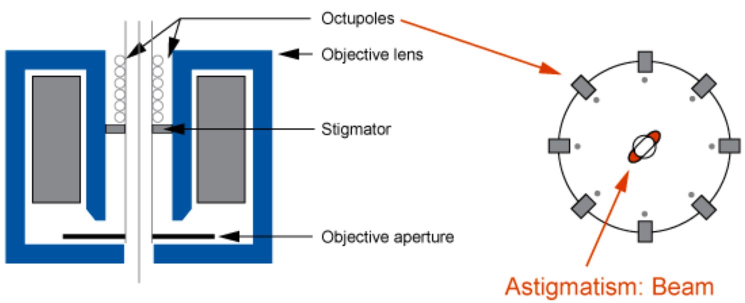
Astigmatism is easy to correct on modern microscopes

Corrected

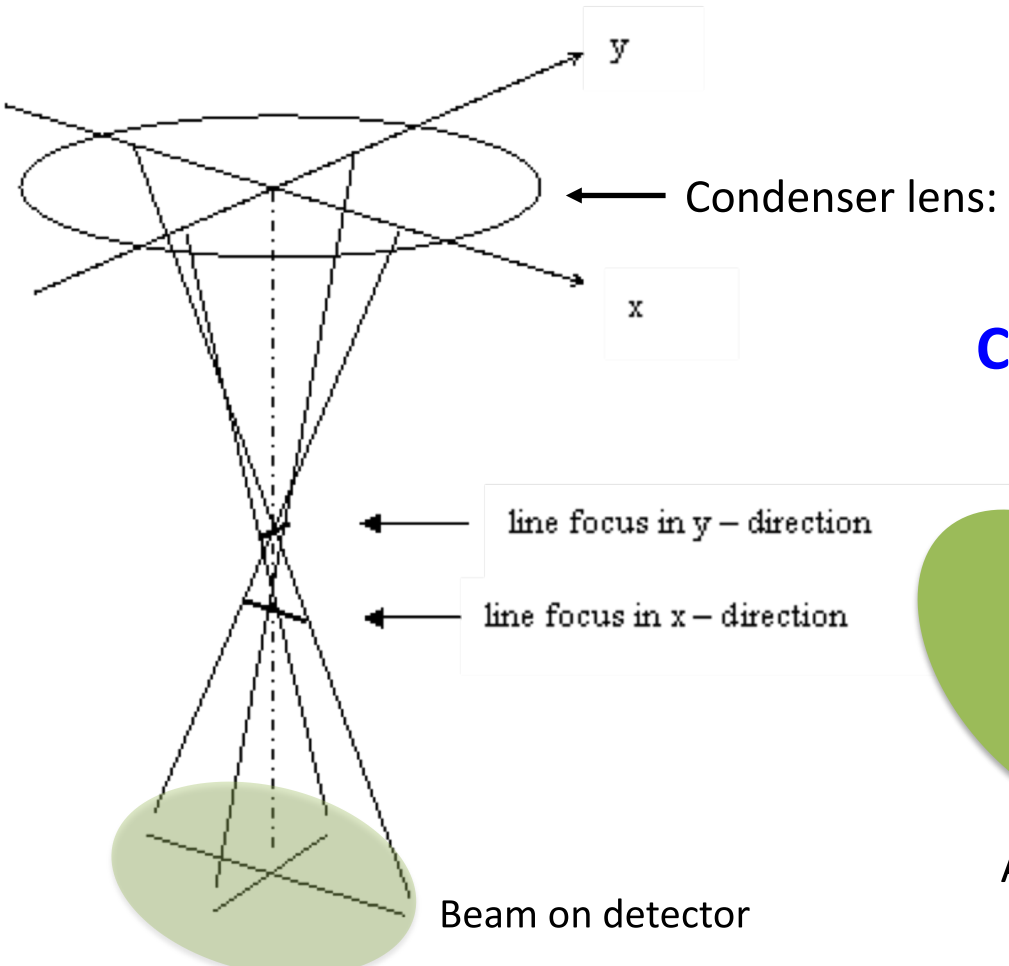



Astigmatism

Astigmatism: Non-spherical electron beam



Condenser Lens Astigmatism



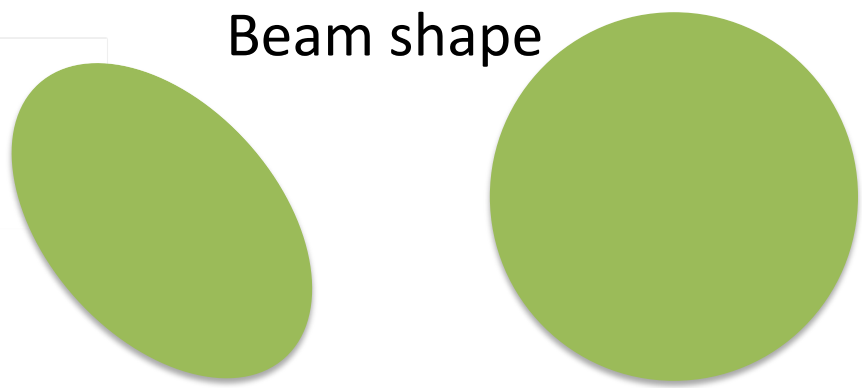
Condenser lens: produces an image of your electron source

Beam on detector

www.rodenburg.org

Check for a circular beam

Beam shape



Astigmatic

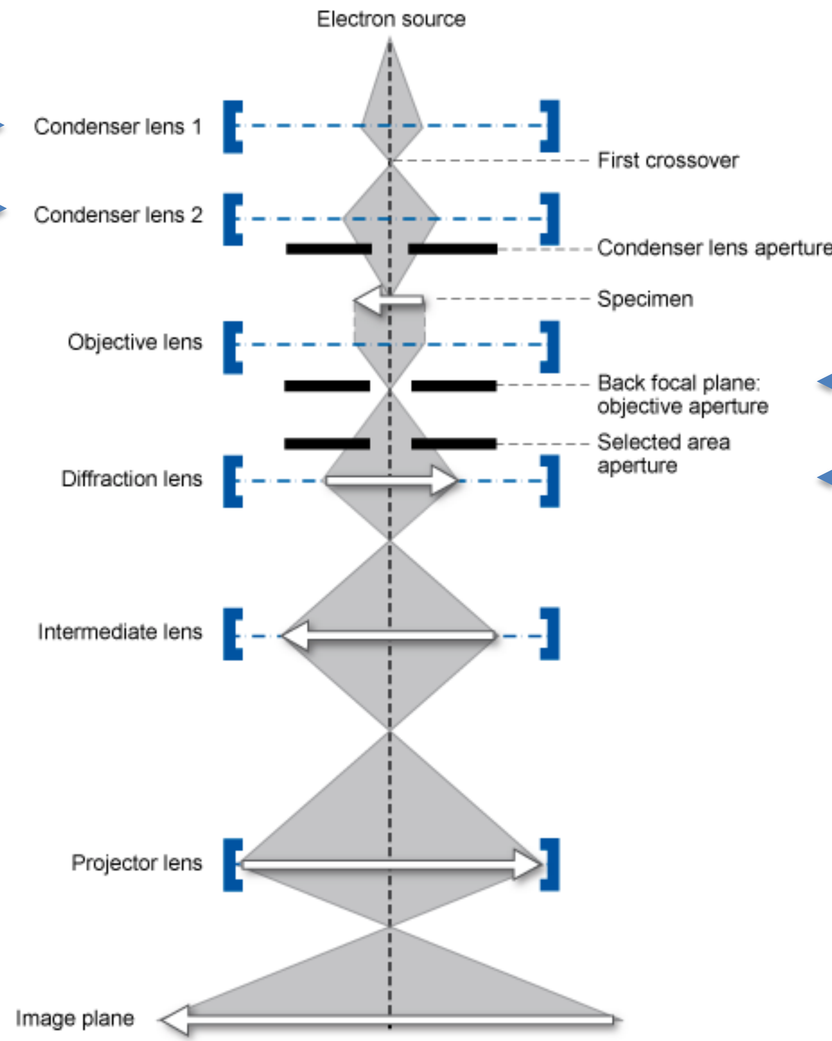
Non Astigmatic

Electron beam path through the lenses

Microscopes with 2 condenser lenses

Spot Size

Beam diameter



Diffraction plane

Image plane

Image: myscope.training

Choice of Accelerating Voltage

What kV should I use? What to consider:

- Resolution: Wavelength at 300 kV vs smaller that at 100 kV
- Aberrations: higher effect of lens aberrations higher at lower kV
- Radiation damage: 1.5 x less dam age at 300 kV vs 100 kV
- Useful information: 25% higher elastic/inelastic ratio at 100 kV
- Detector performance: DDD perform worst at lower kV. Detectors for lower kV scopes have reduced field of view
- Cost: 100 kV much cheaper than 200 or 300 kV
- Sample thickness: Can image thicker samples at higher kV

Peet et al. 2019 and Naydenova et al. 2020

A 100 kV FEG TEM

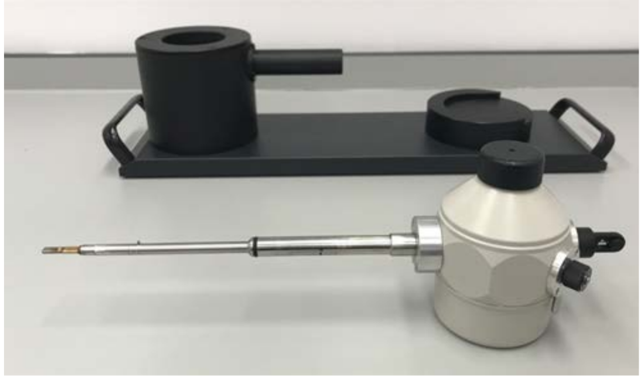
Tundra Cryo-TEM

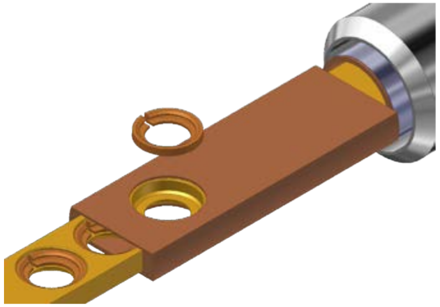
Specimen Holders: Side entry

Samples kept at ~-175°C

Dubochet MKI

Gatan 626: 1 grid





Gatan 910: 3 grids at once

Simple Origin 200: Takes 2 autogrids

Specimen Holders: Side entry

Advantages

- Cheap (£40-80K…yes that's cheap!!)
- Can be used on almost any side-entry TEM (Anti-contaminator required)
- Was the only option until recent years

Disadvantages

- Fiddley to use
- Fragile/easy to break
- Require pumping/heating for optimal performance (Pumping station and Heater)
- Low throughput (Sample exchange takes ~45-60min)
- Each load cycle introduces moisture to the column
- Stability not great: Drift, vibration prone
- Require manual LN2 top-up

Autoloaders

TFS Glacios 200 kV

TFS Krios 300 kV

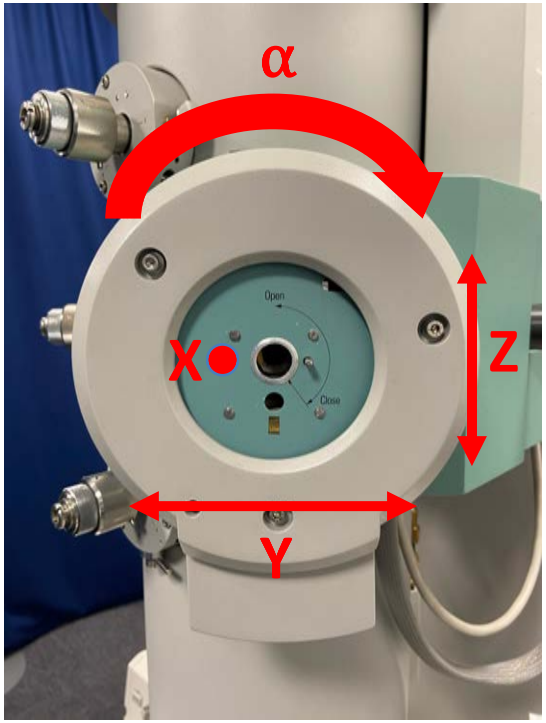
JEOL Cryo-ARM 200

JEOL Cryo-ARM 300

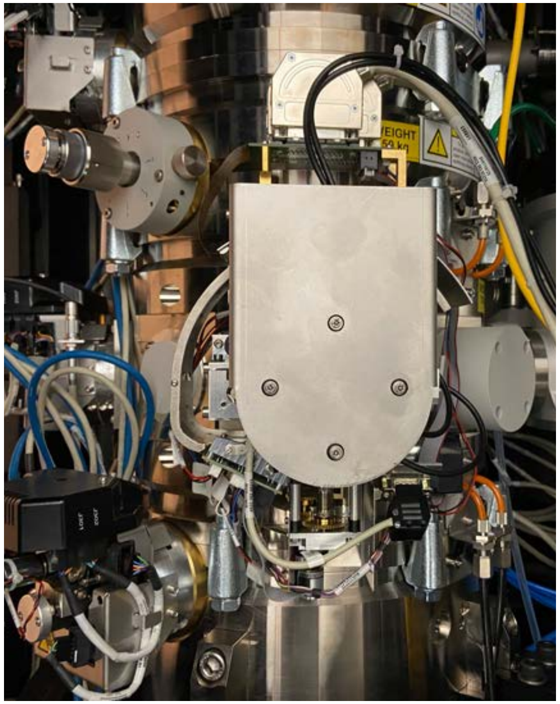
Microscope Stage

- The stage or goniometer supports the specimen holder
- On Autoloader systems the holder is always inserted
- Moves along X, Y and Z directions and tilts along α (and does the same to your sample)

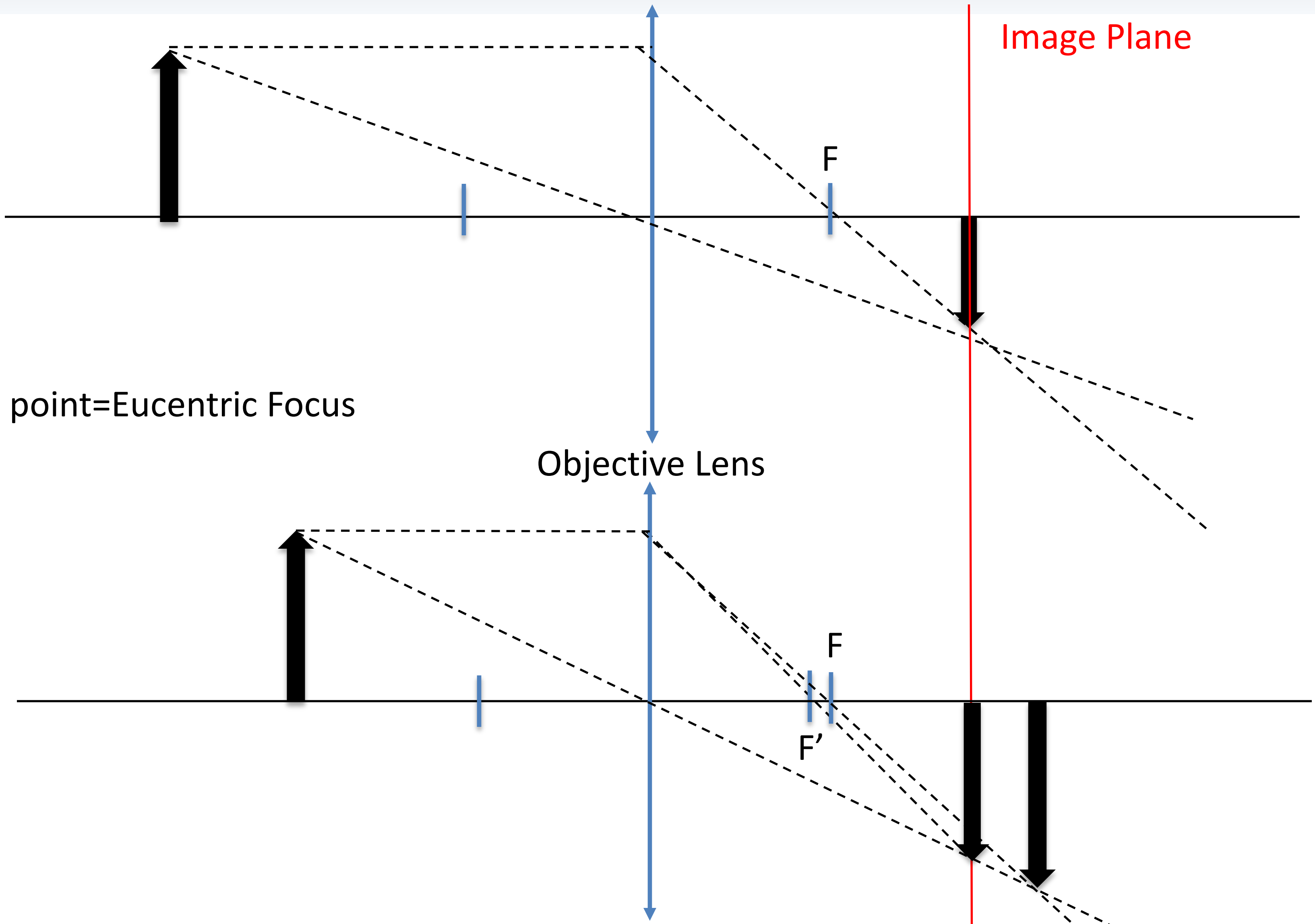
Philips CM200 Stage



Krios Stage



Setting the sample to Eucentric Position



F=Focal point=Eucentric Focus

UNIVERSITY OF LEICESTER

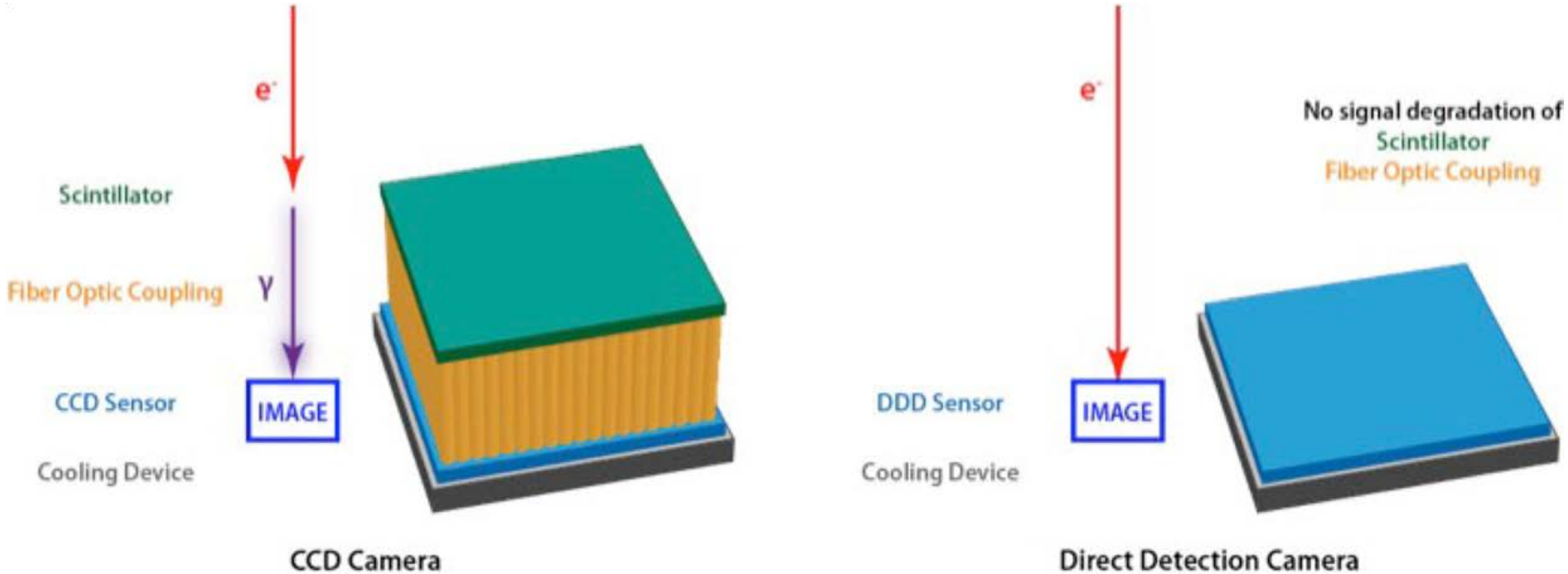
Detectors for the TEM

Detective Quantum Efficiency (DQE) $=SNR^2o/SNR^2i$
A measure of the signal to noise ratio degradation
Perfect detector has DQE of 1

Detector	Advantages	Drawbacks
Film	- Large area - Descent DQE	- Limited to 50 exposures - Needs developing-Scanning - Adds moisture to microscope
CCD	- Easy to use - Instant	- Low DQE (0.1)
Direct electron Detectors	- High DQE - Fast frame rates-Movies	- Expensive

McMullan et. al.
Ultramicroscopy, 2014

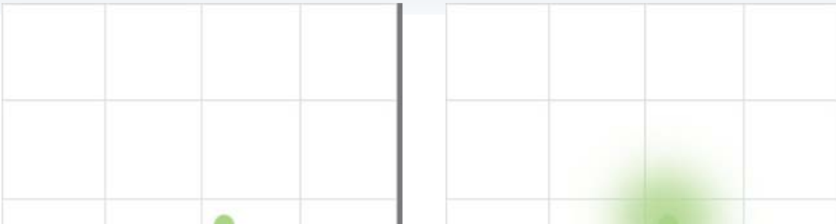
CCD vs DED

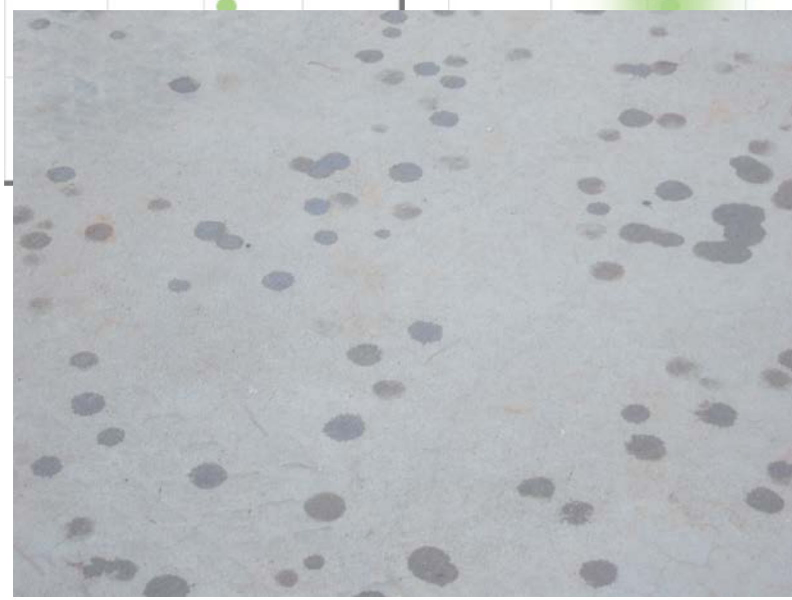


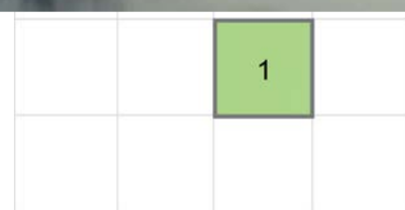
Complementary metal-oxide semiconductor (CMOS)

www.directelectron.com

Integration vs Counting







Events reduced to highest charge pixels.

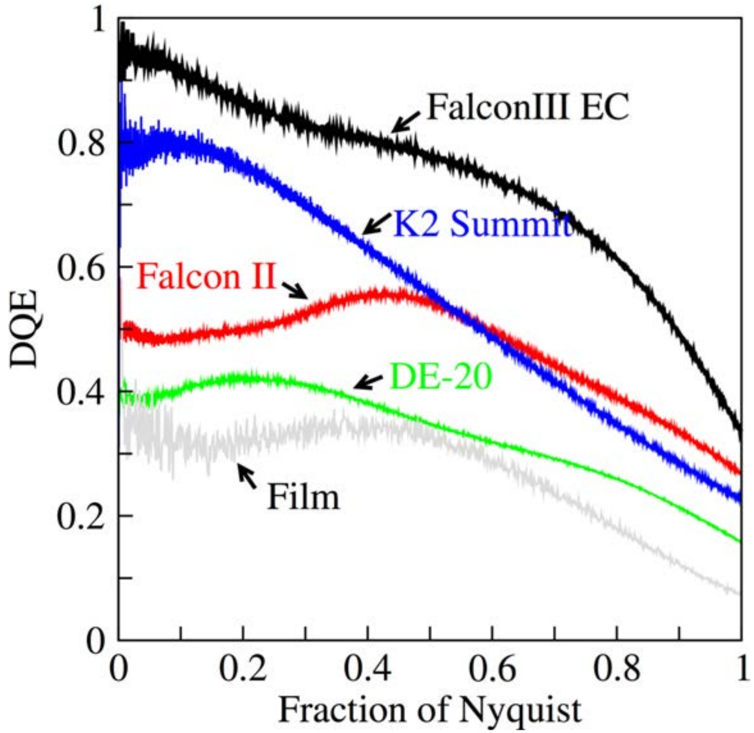
Integration
- Short exposures
- High Dose-rates

(0.5-15 e-/pixel/sec)
- Fast frame rates
- Long exposures
- Higher DQE

www.gatan.com

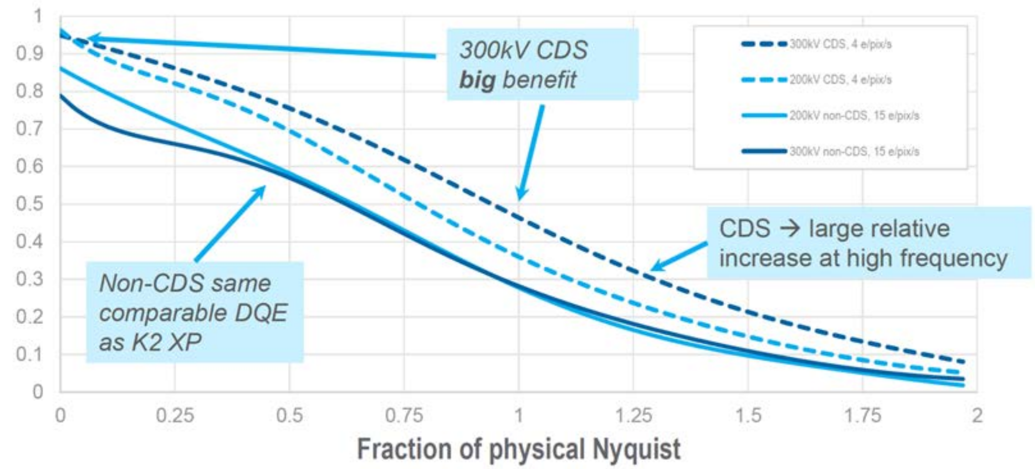
DQE comparison of some detectors

Previous Generation DDD



McMullan et. al.
Ultramicroscopy, 2014

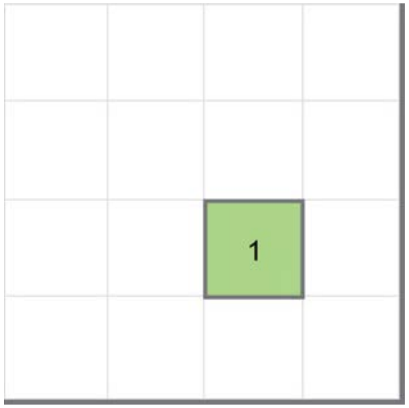
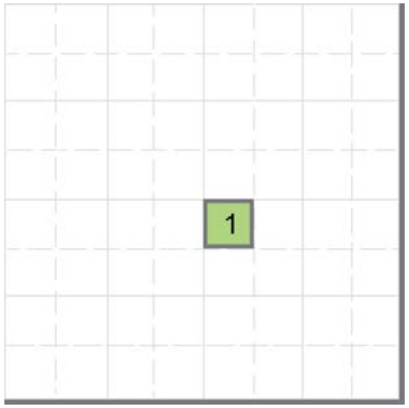
The GATAN K3 (Paul Mooney, GATAN)



Nyquist: 2X the magnified pixel size

UNIVERSITY OF LEICESTER

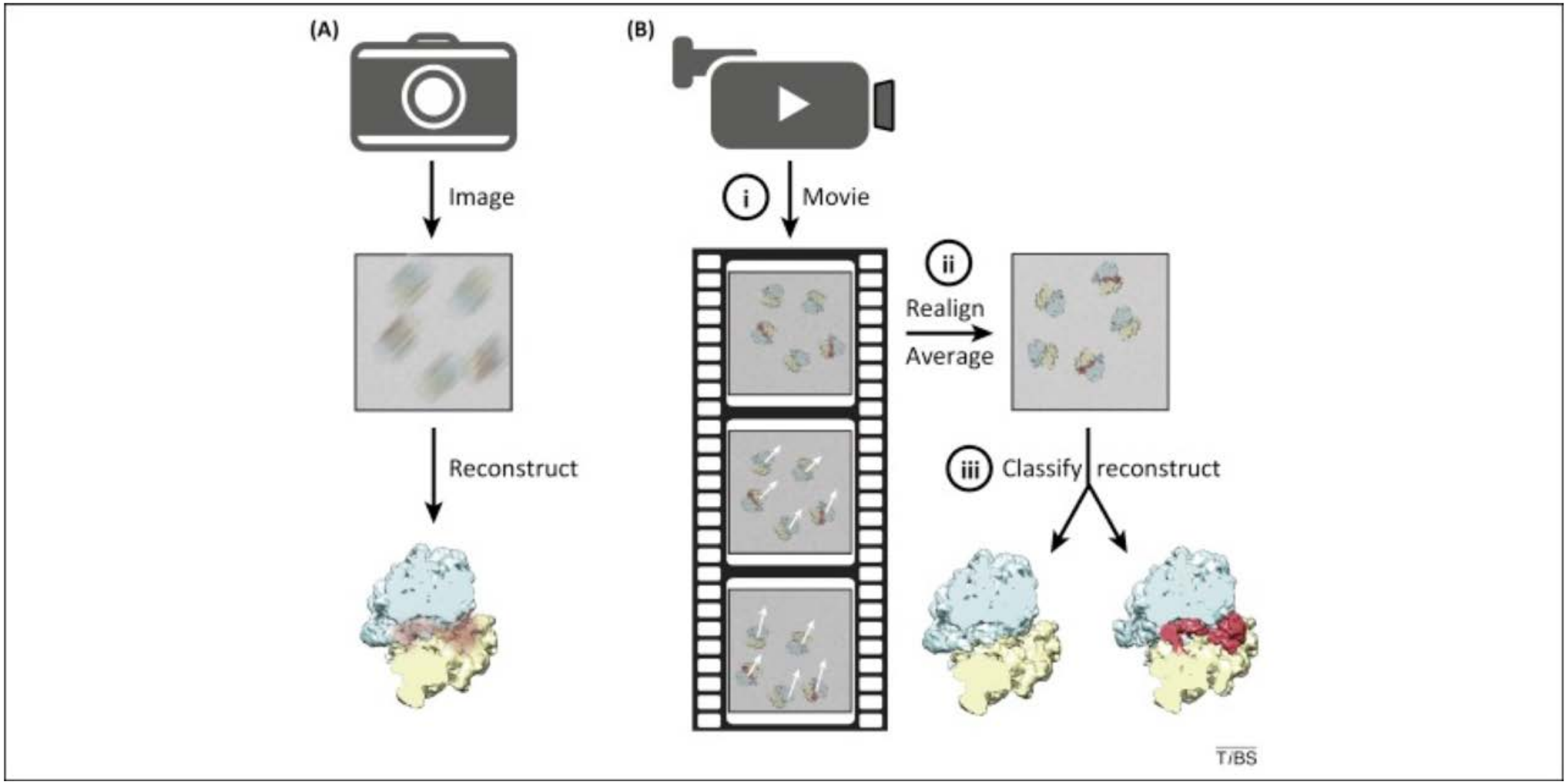
Super-resolution

Counting	Counting with Super-resolution
	
Events reduced to highest charge pixels.	Events Events localised to sub-pixel accuracy.

- Super-resolution with Fourier cropping “binning” increases DQE
- Increases disk space requirement
- Even allows one to go beyond physical Nyquist:
  - Recent example at our facility: Data at 81K (Pixel 1.09Å). Collected at SR bin1
  - Resolution reached physical Nyquist (~2.2Å)
  - Re-extracted SR movies during polishing step > Reached 1.9Å

Movies instead of snapshots

- CCD and film limited to one exposure
- Fast frame rate of DED allows movie collection

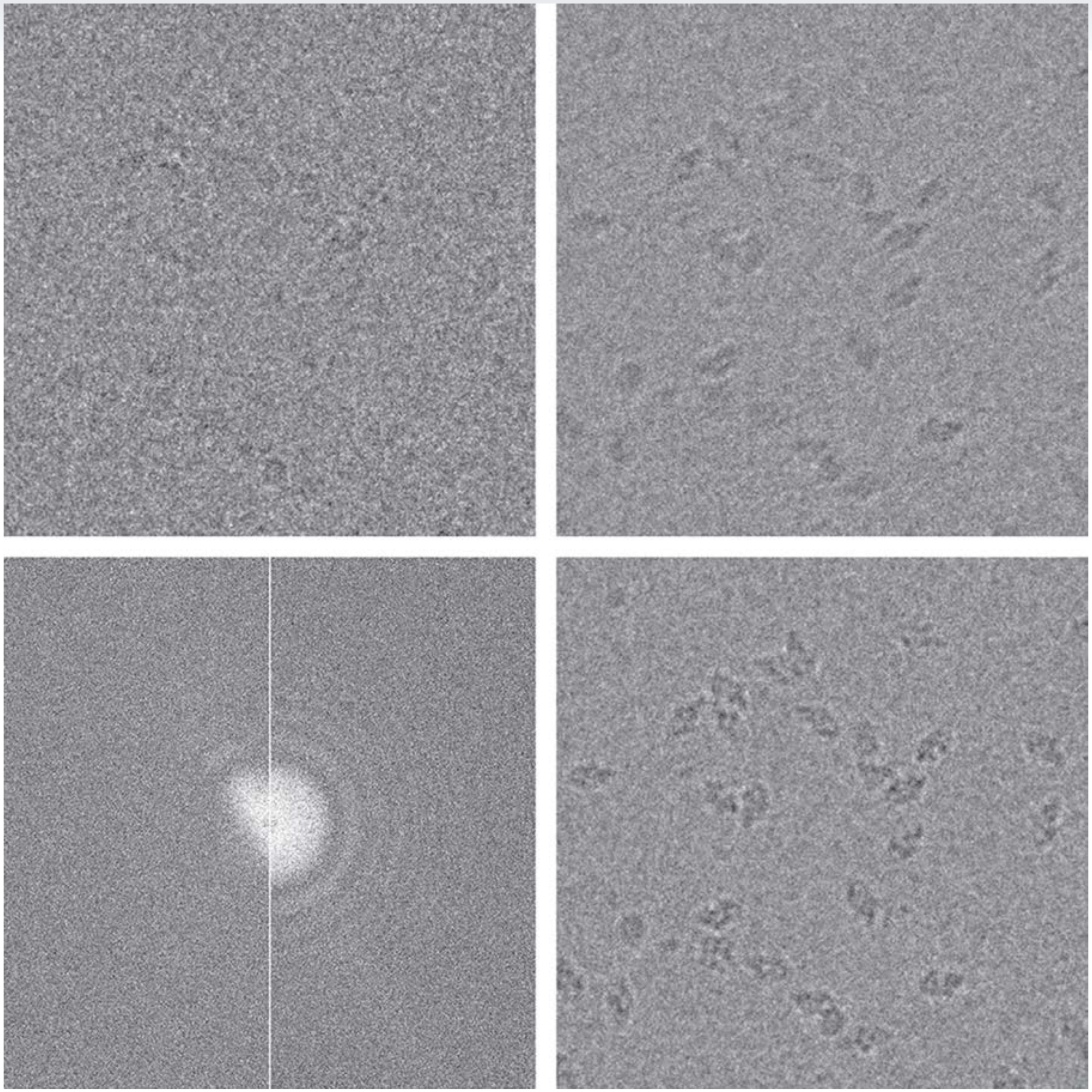


Bai *et. al*, 2015, TBS

Movies instead of snapshots

Single Frame

Summed frames
(No alignment)



Power Spectra
Unaligned /Aligned

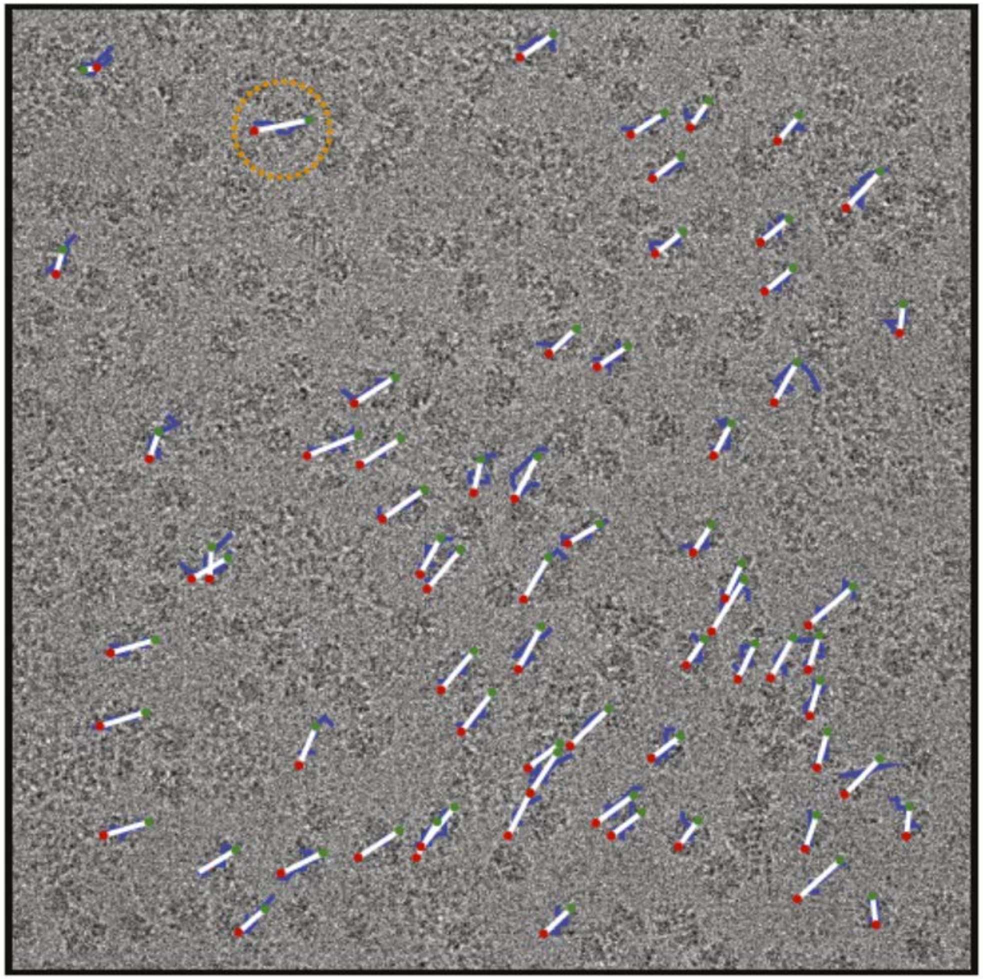
Summed frames
(With alignment)

McMullan, 2016

Individual particle tracking

Particles can move in the ice

- Electrostatic attraction
- Release of stress in the ice



Scheres, 2014, Elife

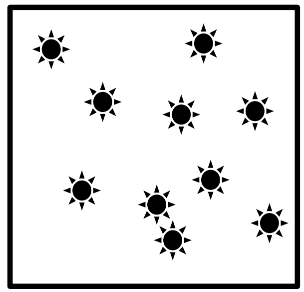
Radiation Damage

- Biological samples are radiation sensitive
- Bonds are broken and free radicals released
- Imaging performed using “Low Dose” methods
- Each micrograph receives a limited amount of electrons to prevent structure deformation
- Typically we use 40-60 electrons/Å^2 per micrograph

UNIVERSITY OF LEICESTER

Using movies to deal with radiation damage

"Old days" (before 2012)
Single image
1 second exposure at 40 e-/Å^2/sec dose rate

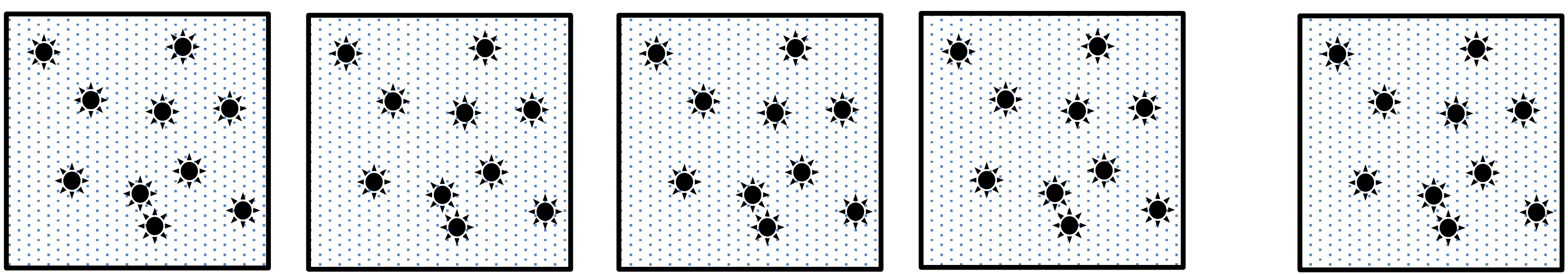


Dose per image: 40 e-/Å^2

Current date: New detectors
40 frames/sec, 1 second exposure at 40 e-/Å^2/sec dose rate

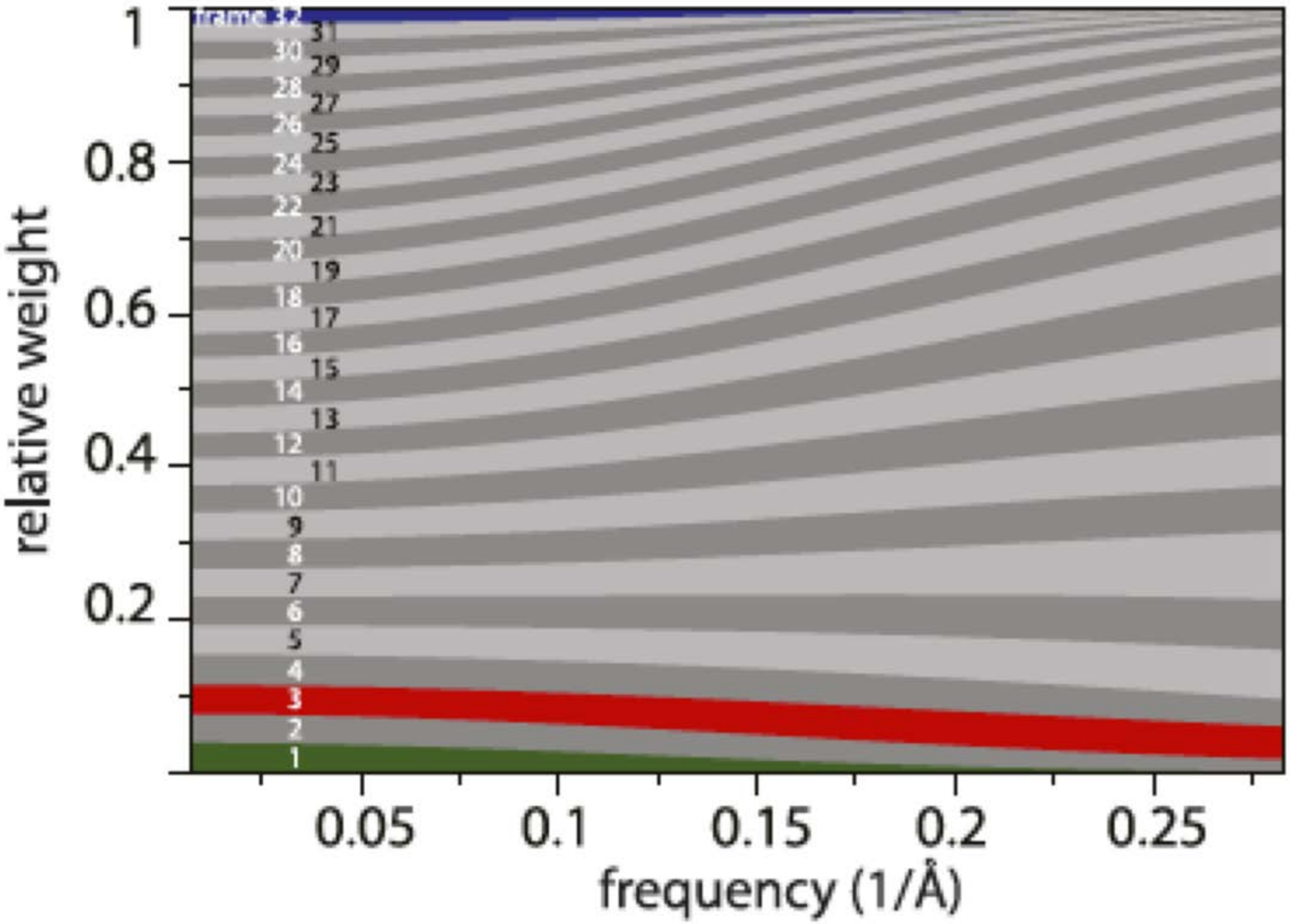
Lower radiation damage: High frequencies are signal

Higher radiation damage: High frequencies are noise



Frame	1	2	3	4	....	40
Accumulated Dose (e-/Å^2)	1	2	3	4	....	40

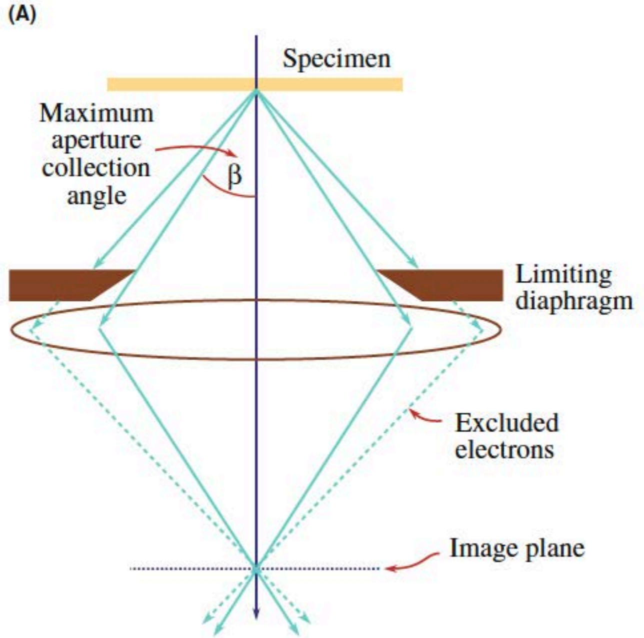
Radiation damage weighting

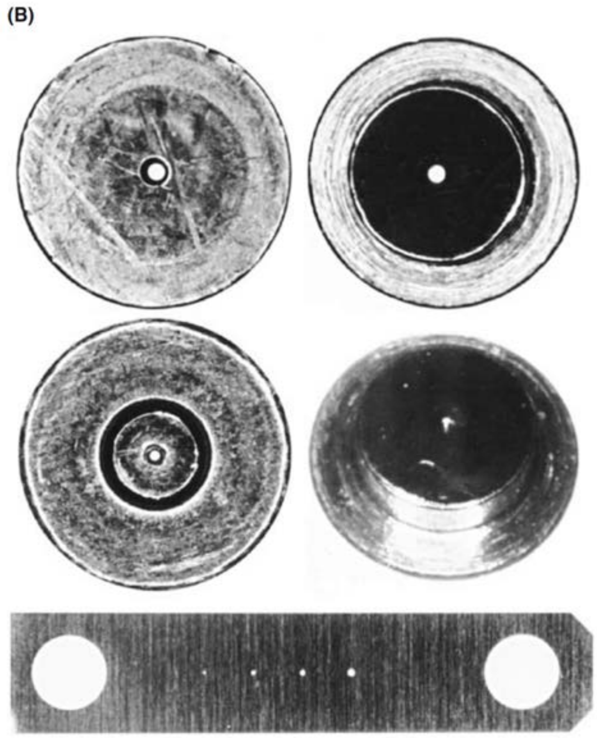


Scheres, 2014, eLife

Microscope Apertures

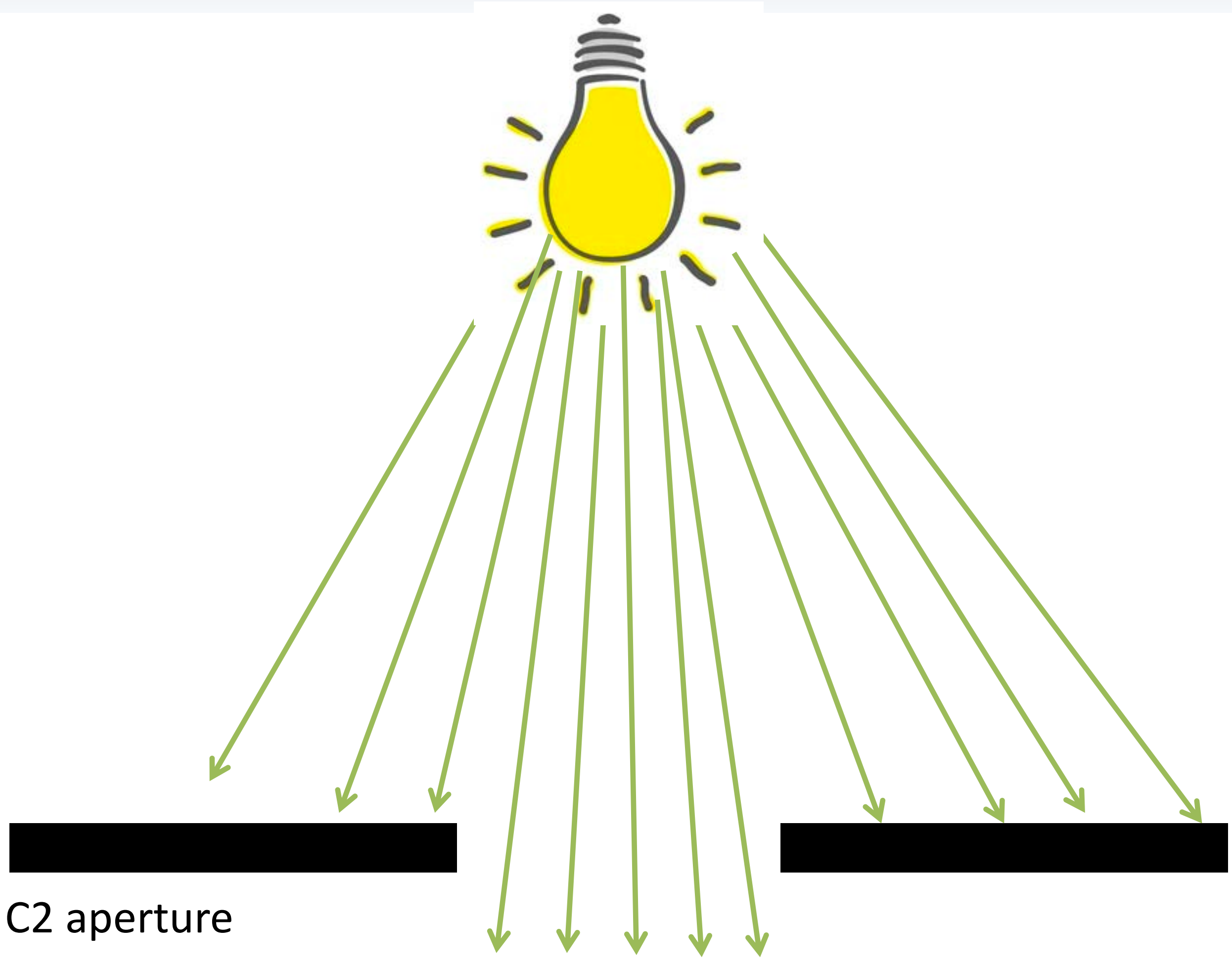
- An aperture is a small hole in a strip of metal inserted in the beam path
- Will only allow the central beam to go through





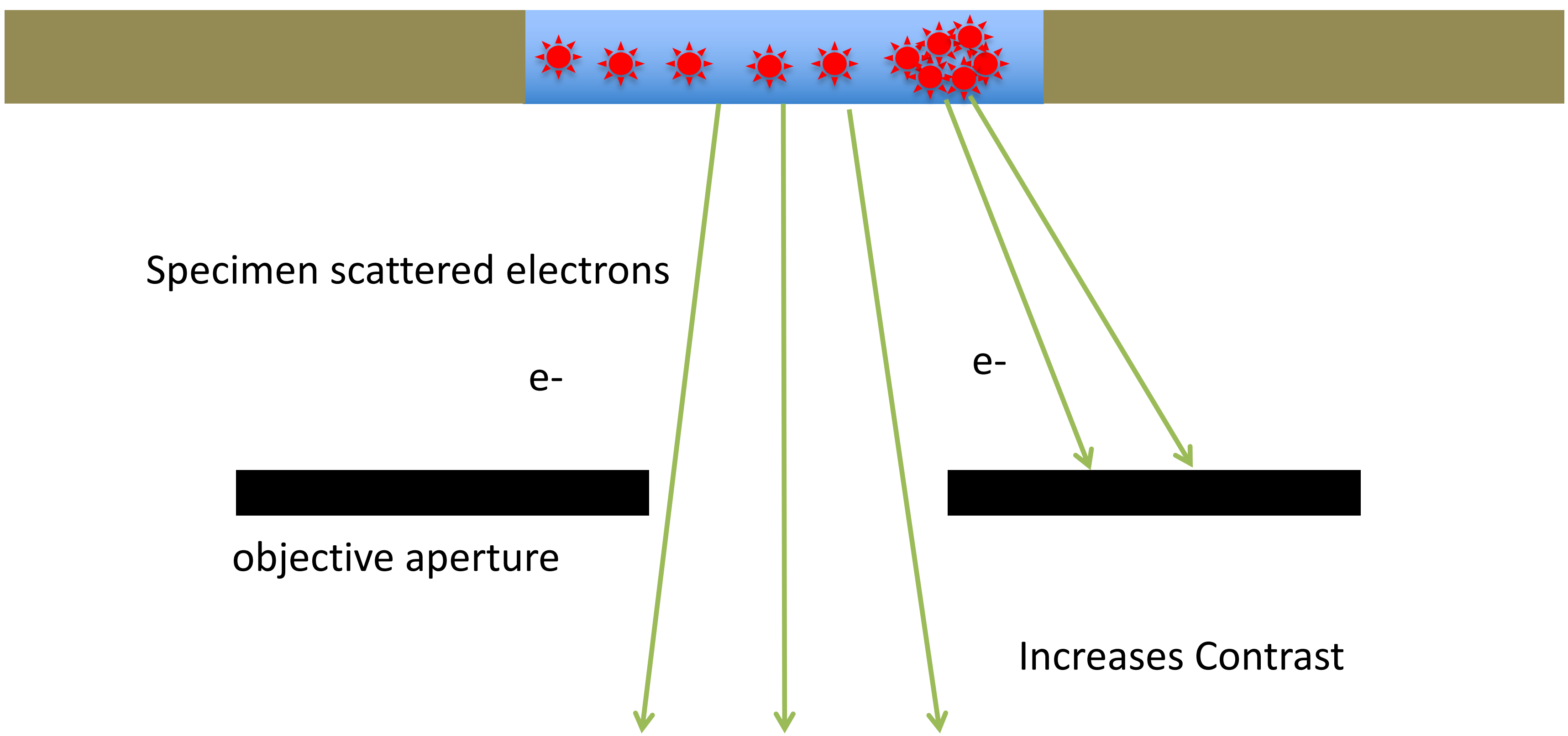
From Williams and Carter, 2009

Condenser Aperture



C2 aperture Produces a more coherent, parallel beam

Objective Aperture



Objective Aperture

Also acts as a low-pass filter removing high-resolution information

Consider resolution cut-off. e.g on Krios:

30 µm > 4 Å

70 µm > 1.8 Å

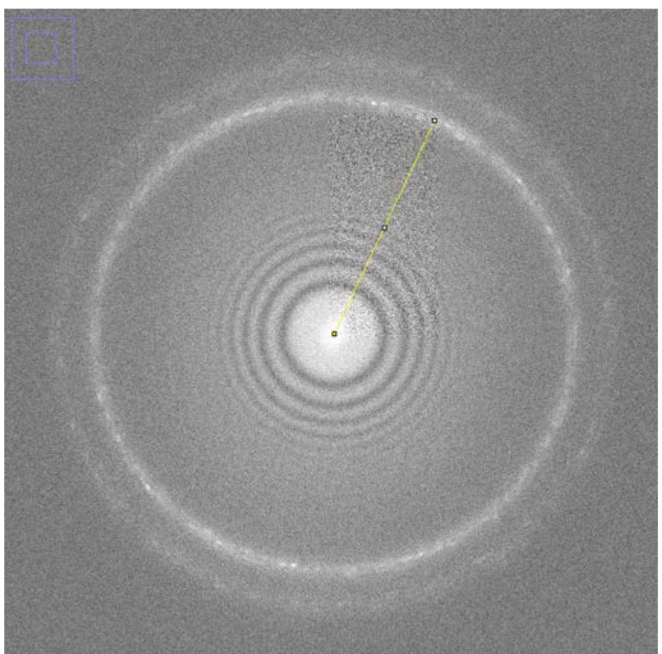
100 µm > 1.4 Å

Magnification and Pixel sizes

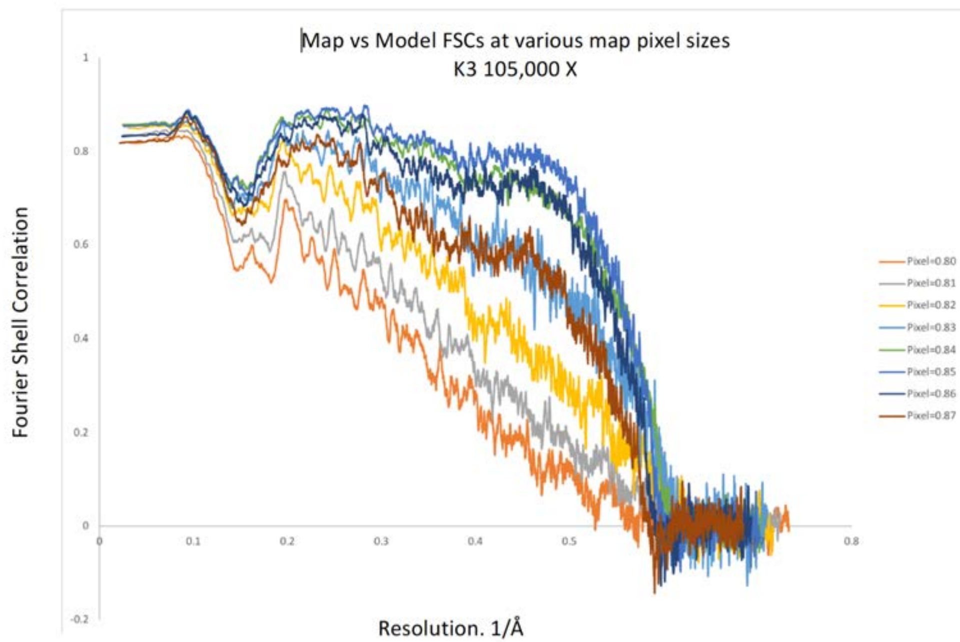
Nominal Magnification

SA 105 kx
nP EFTEM

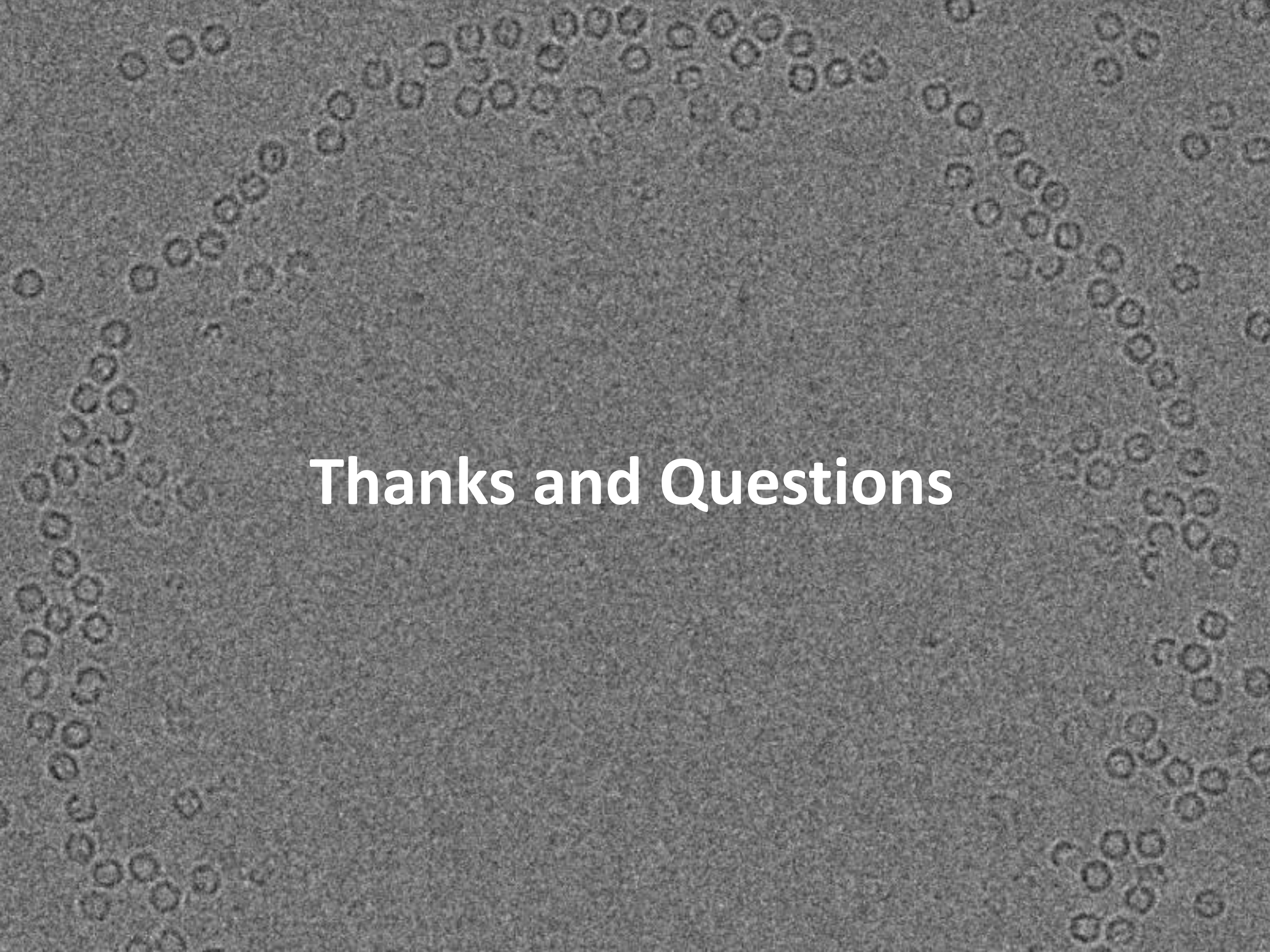
$$\text{Pixel size} = \frac{\text{Calibrated Magnification}}{\text{Physical Pixel Size}}$$



Gold diffraction: 2.35Å



Known atomic model fitting



Thanks and Questions