CRYOEM 001 :
TOOLS OF THE TRADE - MICROSCOPES AND DETECTORS

NCCAT Embedded Training – Master Class series

September 2020

NATIONAL CENTER FOR
CRYOEM ACCESS & TRAINING

New York Structural
Biology Center

SIMONS ELECTRON
MICROSCOPY CENTER

CRYOEM 001 : SINGLE PARTICLE MASTERCLASS

Introduction to cryoEM: SPA

Building a cryoEM toolkit

EM compatible samples

EM support films and grids

Sample preparation

Tools of the trade:

- microscopes and detectors

Microscope operations

Data collection strategies

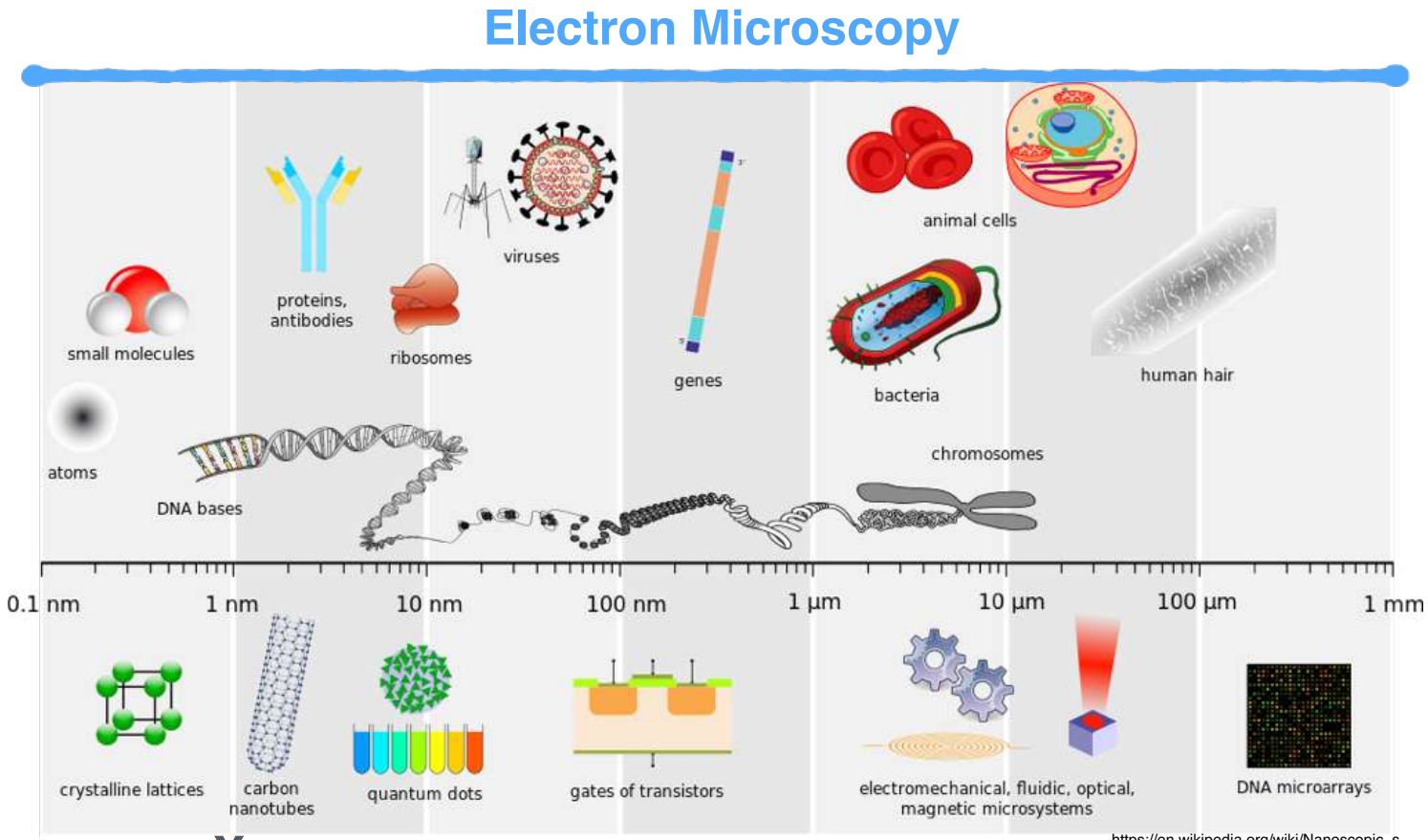
Data assessment & QC

Data processing:

- cryoEM IT infrastructure
- On-the-fly feedback
- 3D Reconstruction

Visualization and validation

CRYOEM: SCALE WITHIN BIOLOGY



https://en.wikipedia.org/wiki/Nanoscopic_scale

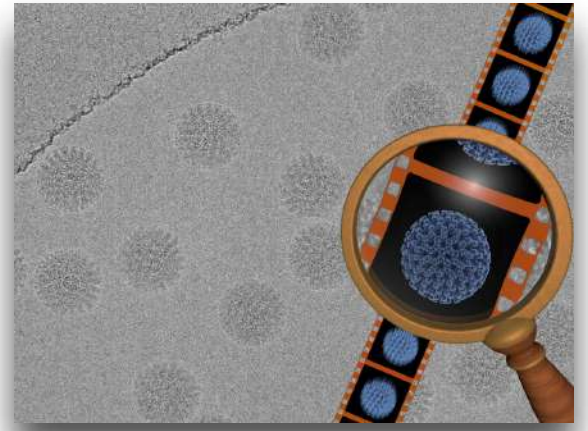
WHAT BROUGHT ABOUT THE RESOLUTION REVOLUTION

(~2012-2014)

Hardware

Microscopes

Direct Detectors



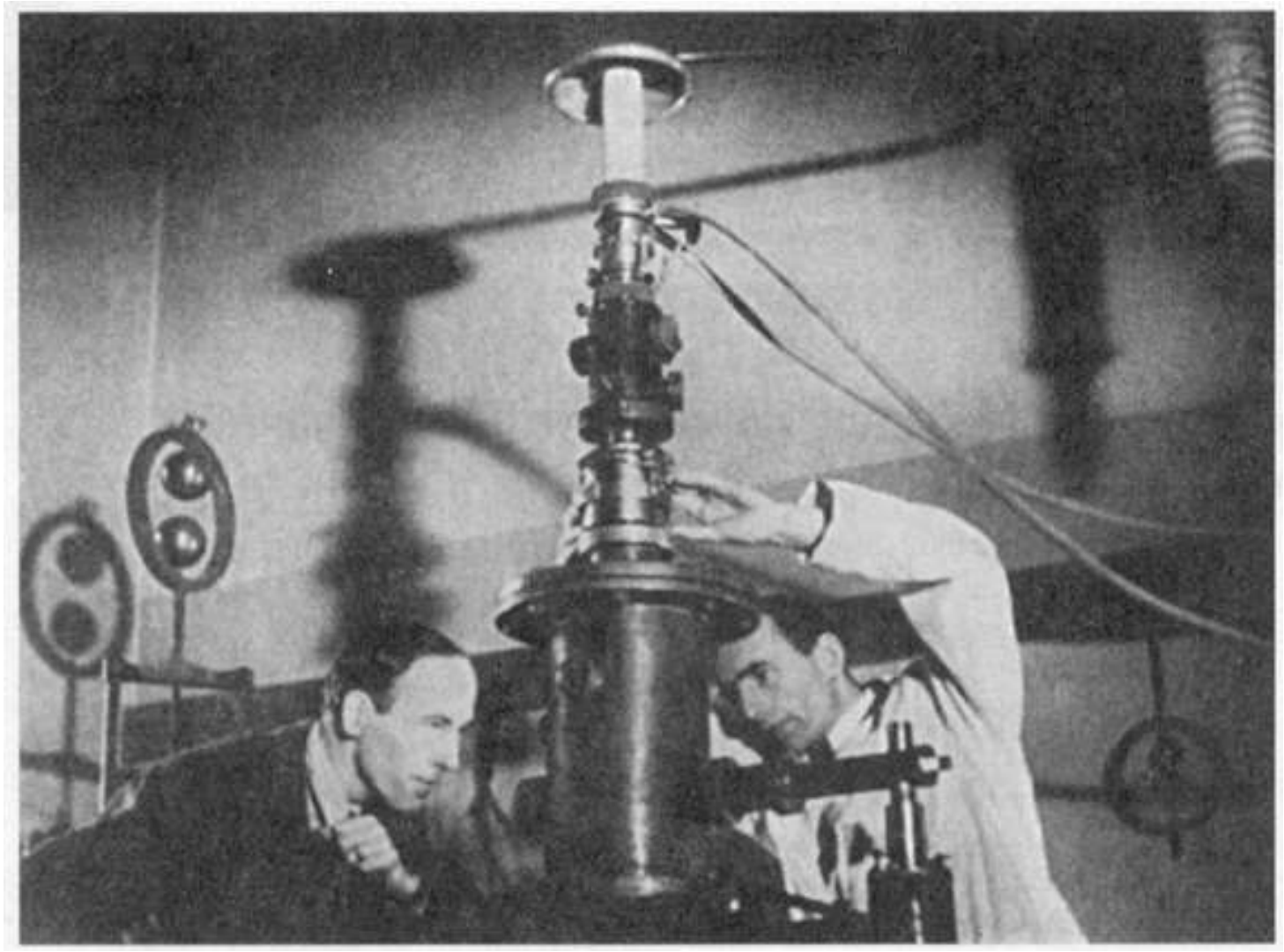
Computers

2012->2017
Cost reduced by 100x

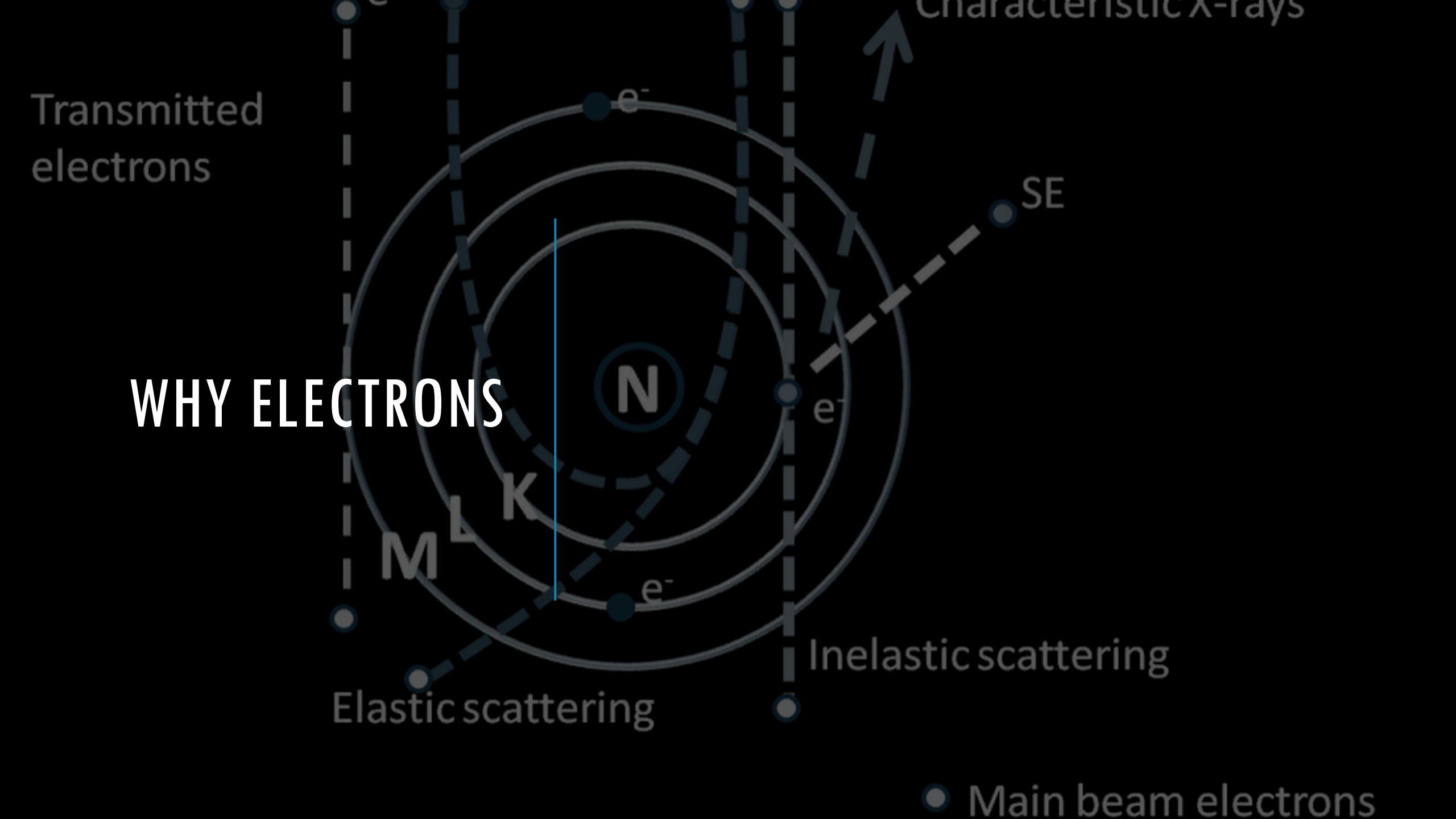
THE ELECTRON MICROSCOPE

Ruska and Knoll in Berlin in the early 1930s

-Wikipedia



WHY ELECTRONS



WHY ELECTRONS

Pros

Small wavelength

Can be focused

Cons

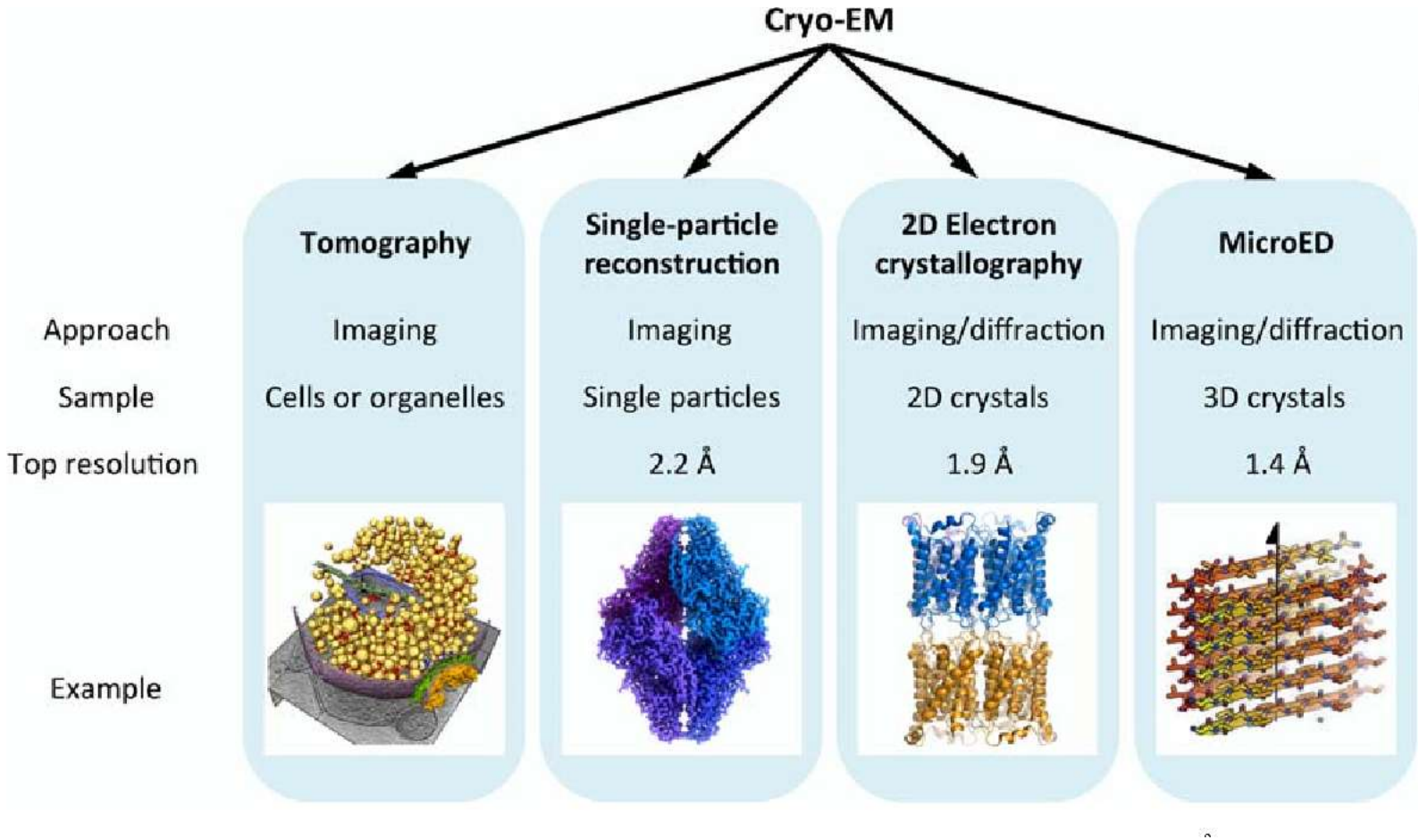
Damages sample

worse with faster electrons

Poor penetration

better with faster electrons

CRYOEM MODALITIES AND TOOLS



https://doi.org/10.1002/pro.2989

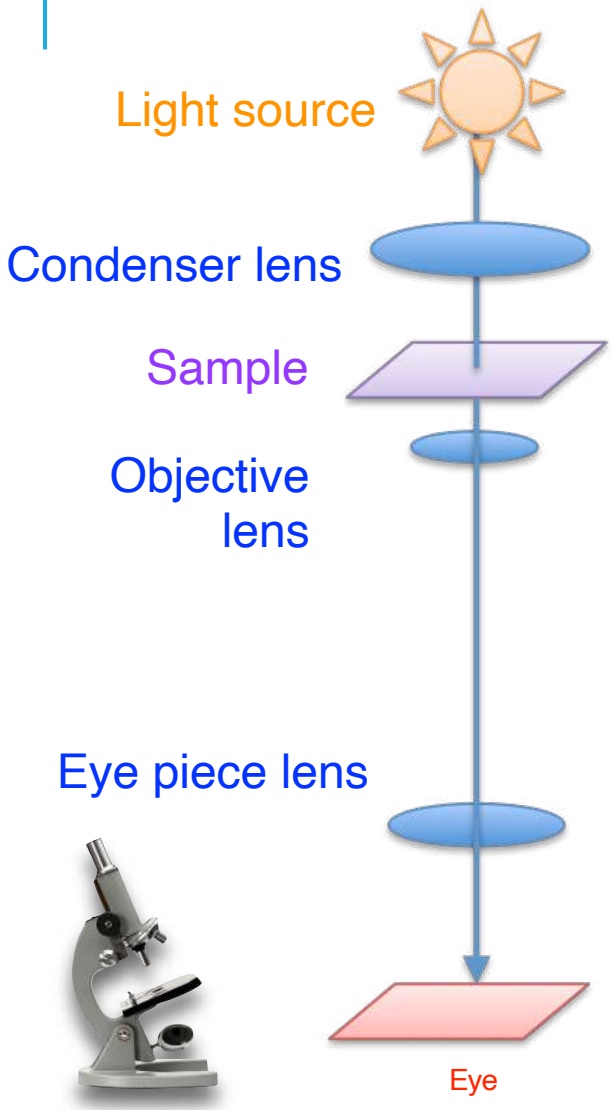
~3.4Å

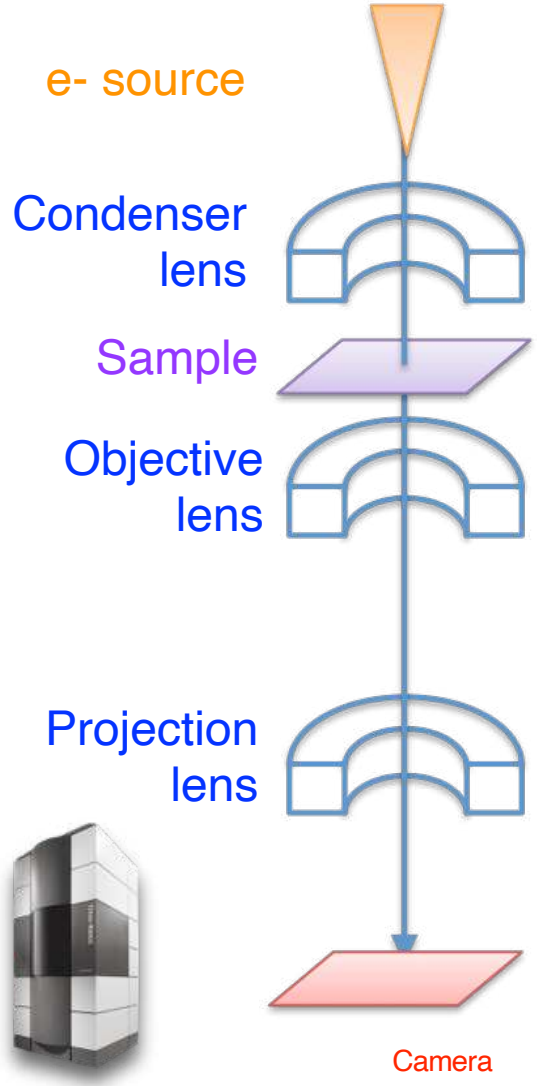
~1.2Å

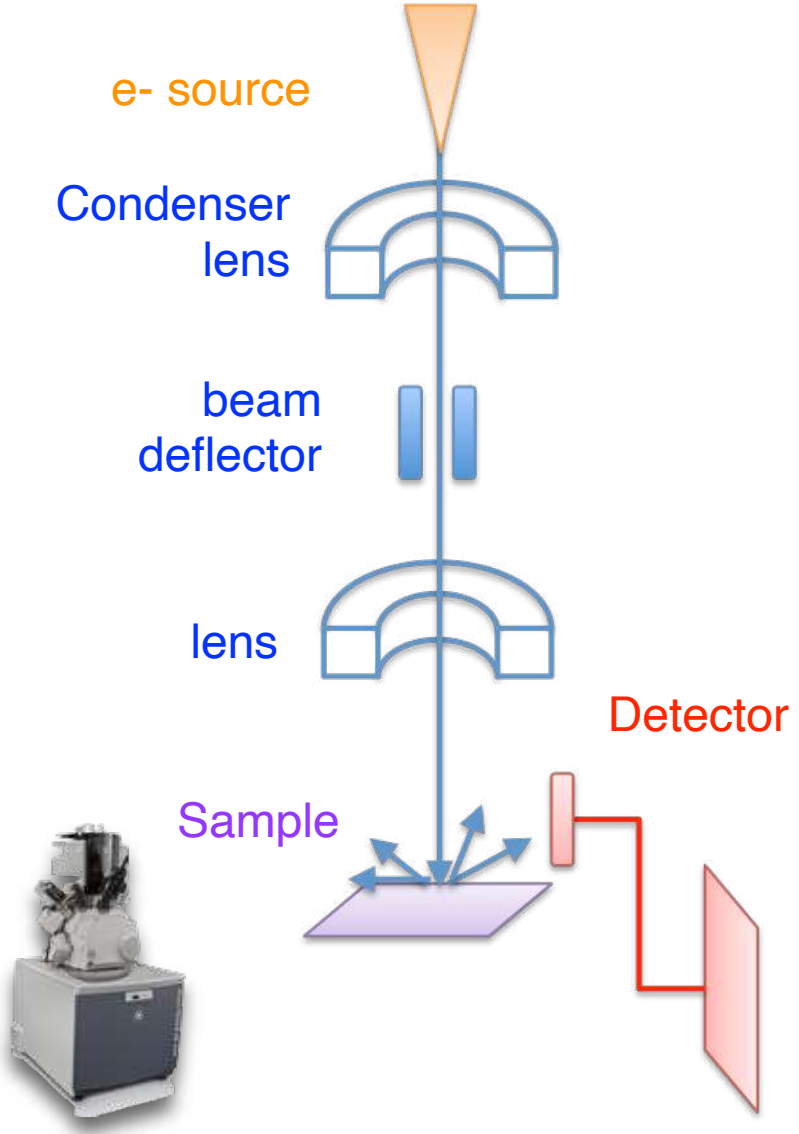
~1.9Å

~0.7Å

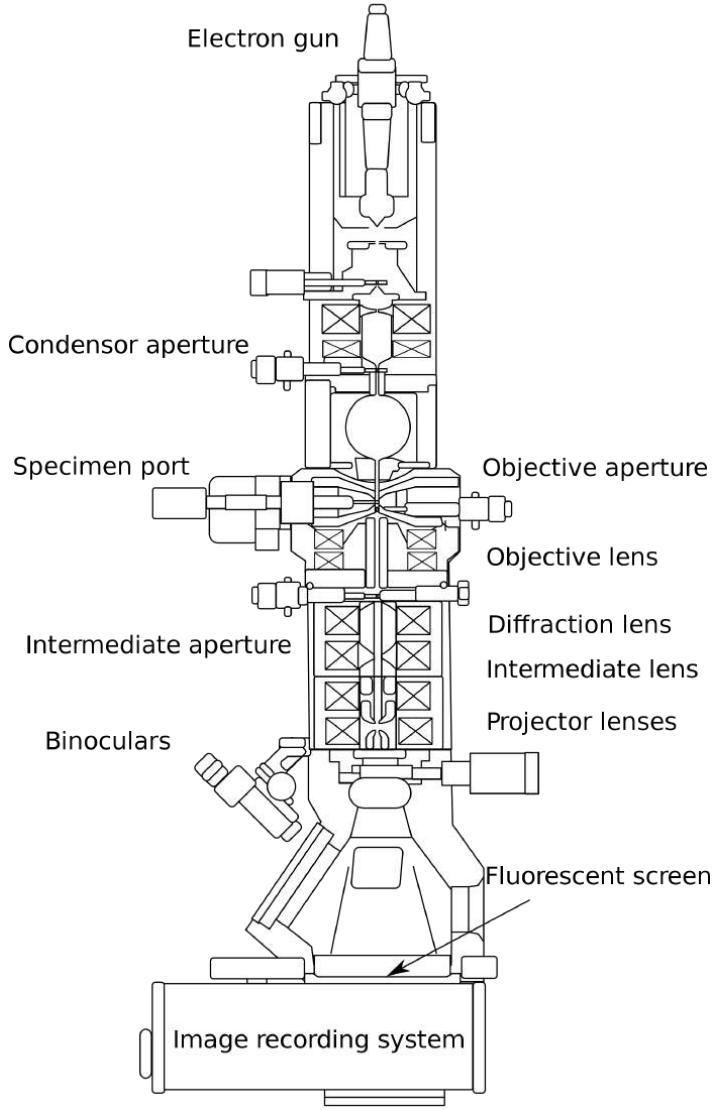
CRYOEM TOOLS







MAIN PARTS OF AN EM



Electron sources

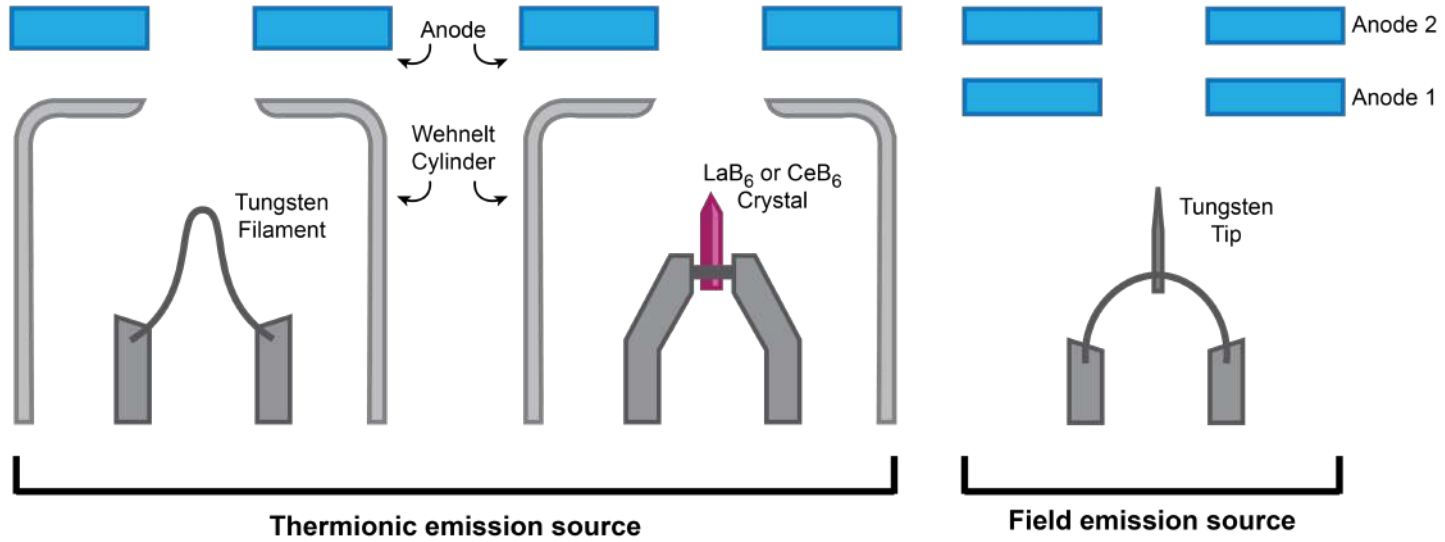
Vacuum systems

Lenses

Detectors

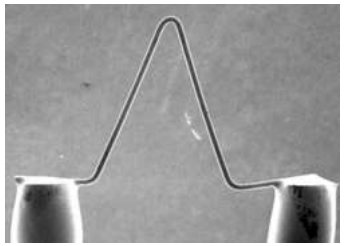
ELECTRON SOURCES

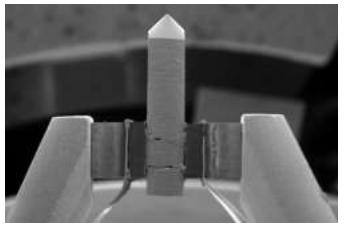
What are the 3 main kinds of electron sources?

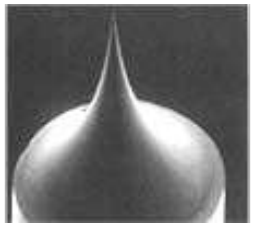


www.thermofisher.com

nanoscience.com

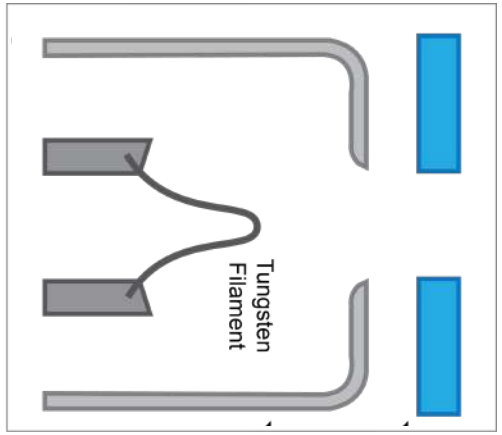


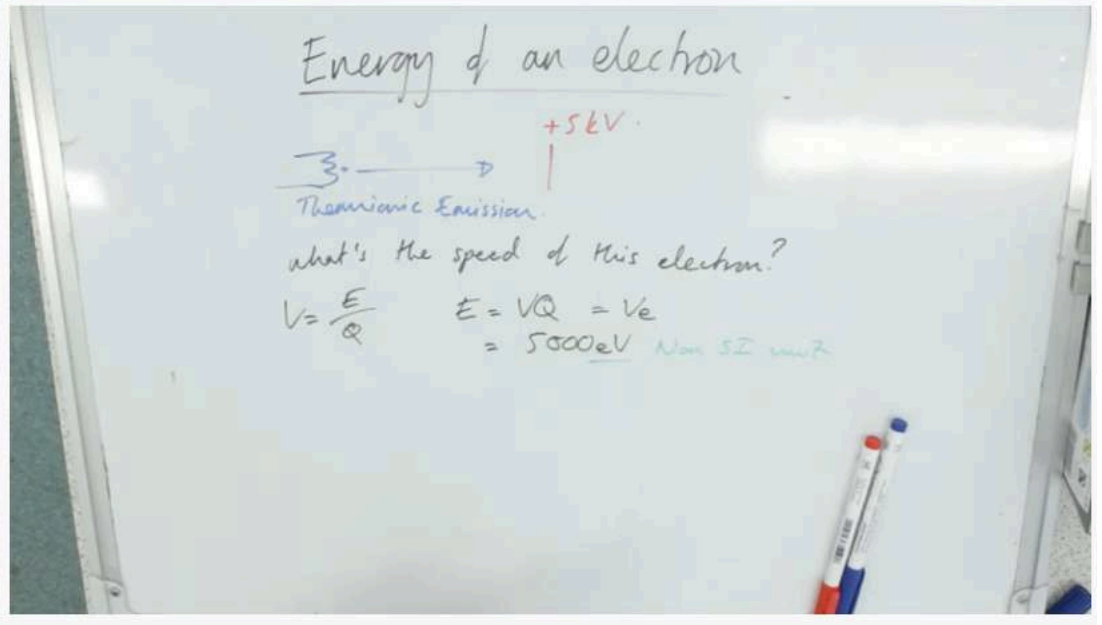




ELECTRON SOURCES

How fast are the electrons moving?

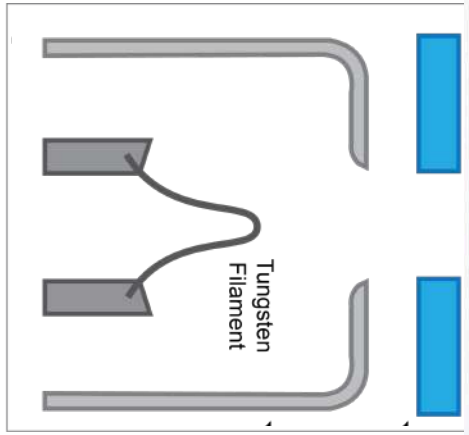


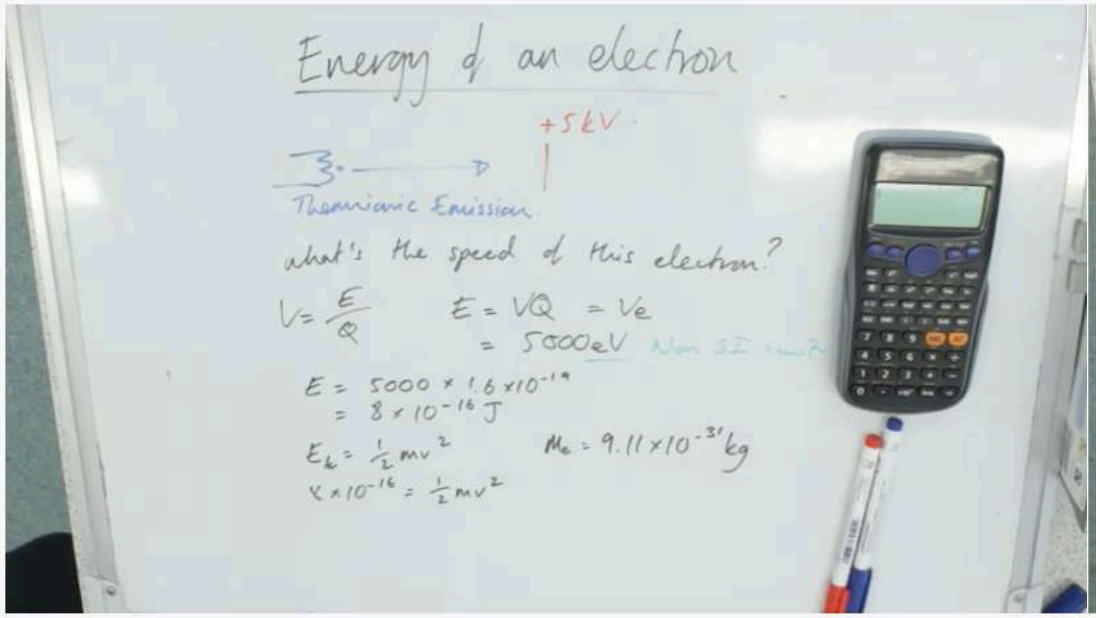


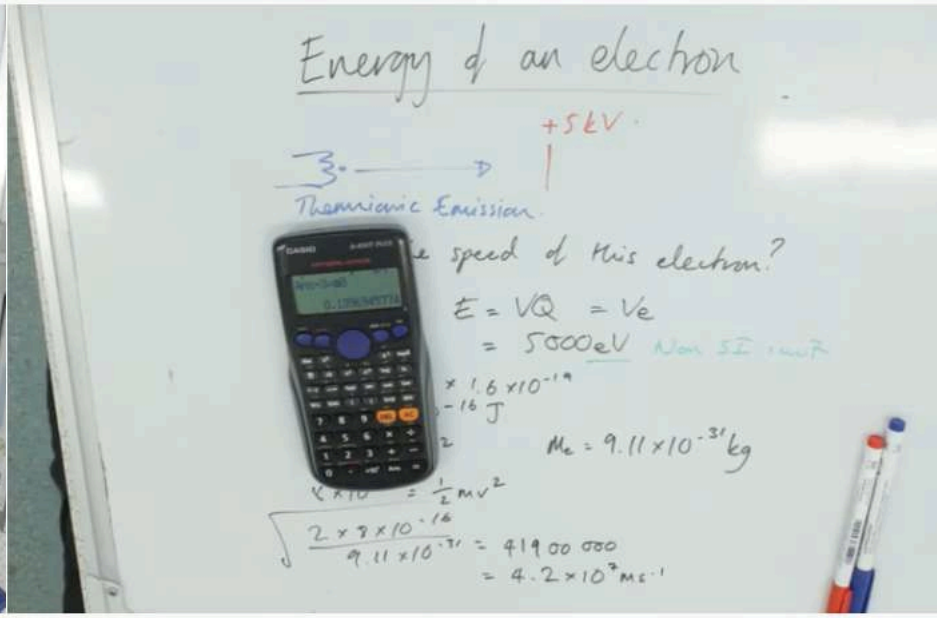
https://www.youtube.com/watch?v=tYCET6vYdYk

ELECTRON SOURCES

How fast are the electrons moving?







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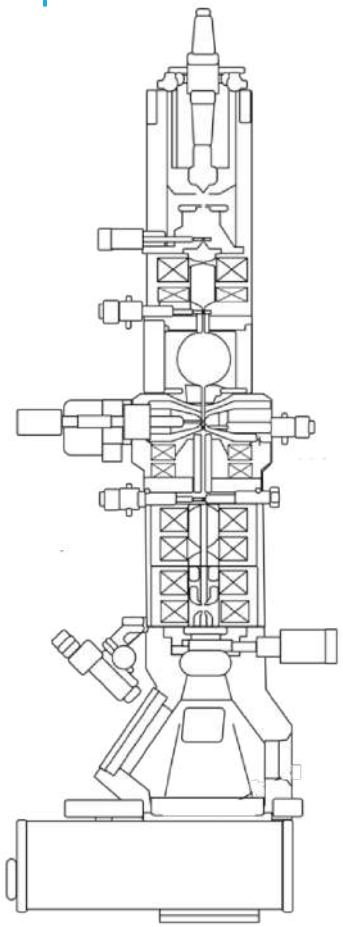
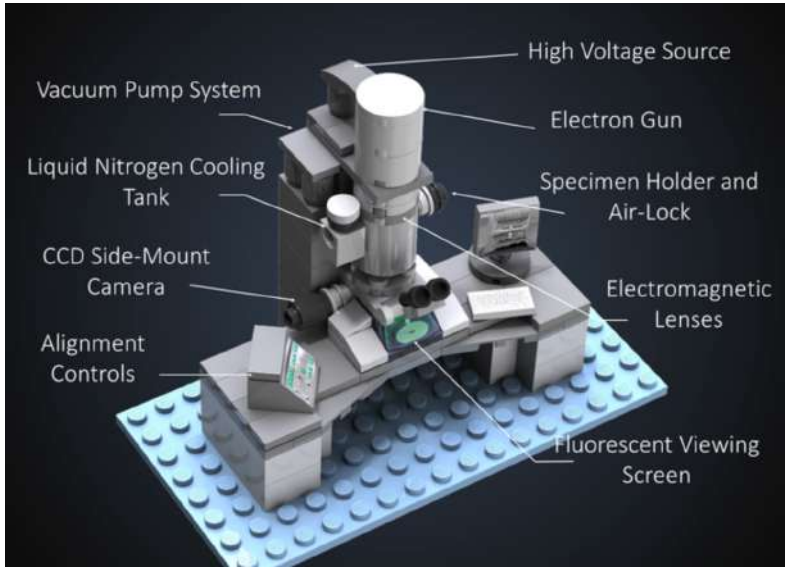
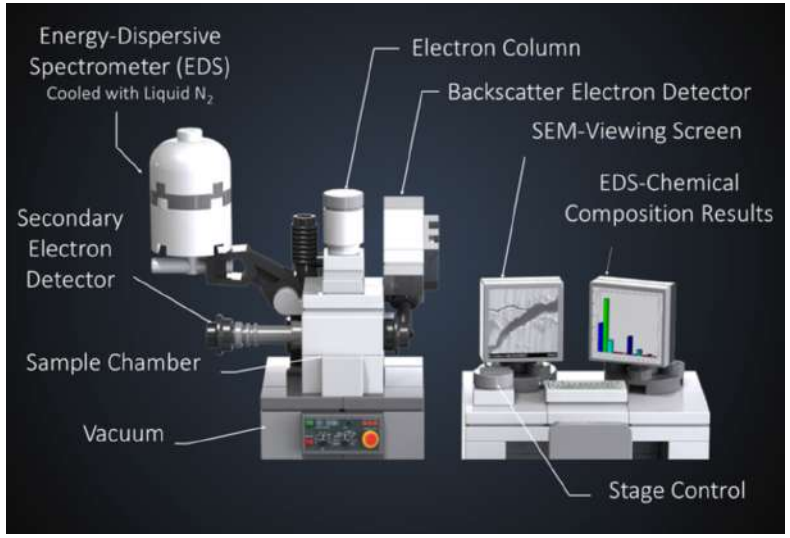
ELECTRON SOURCES & TYPES OF EMS

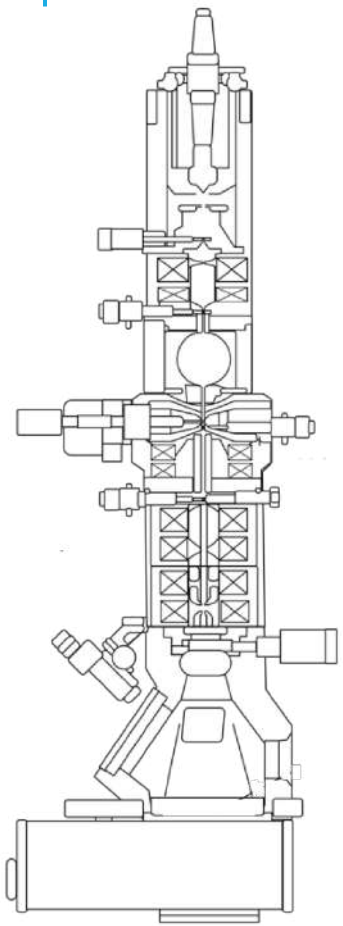




https://ideas.lego.com/projects/102281



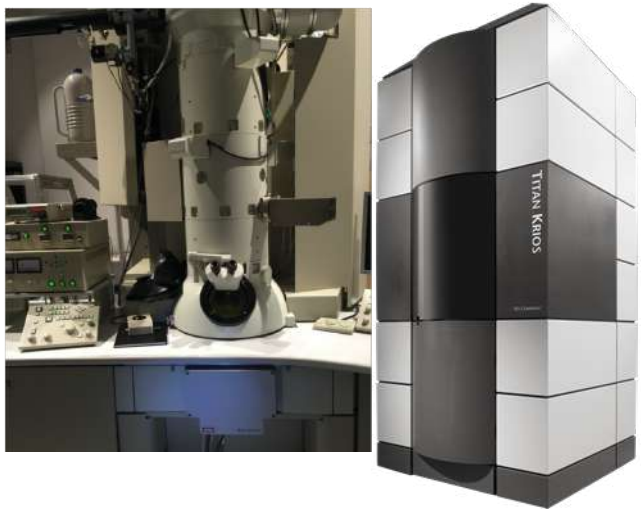
ELECTRON SOURCES & TYPES OF EMS



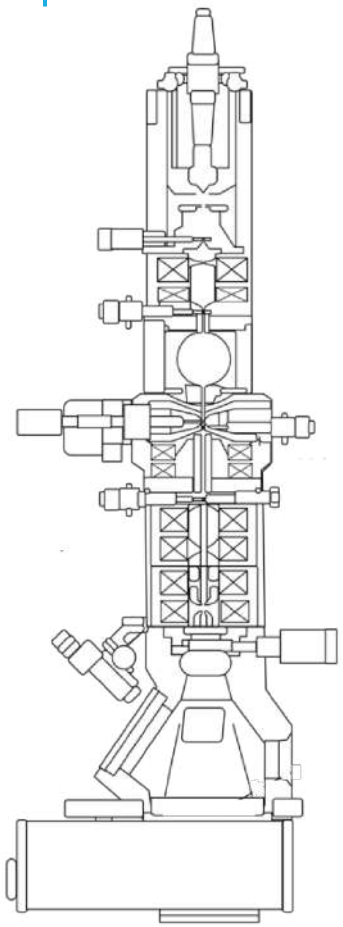
80-120 kV: JEM 1230; Tecnai T12
W or LaB6
High contrast & robust
sub-nm resolution

200 kV: JEM 2100F, Tecnai F20, Talos, Artica
FEG
2+ Å resolution (3.5-4 Å)

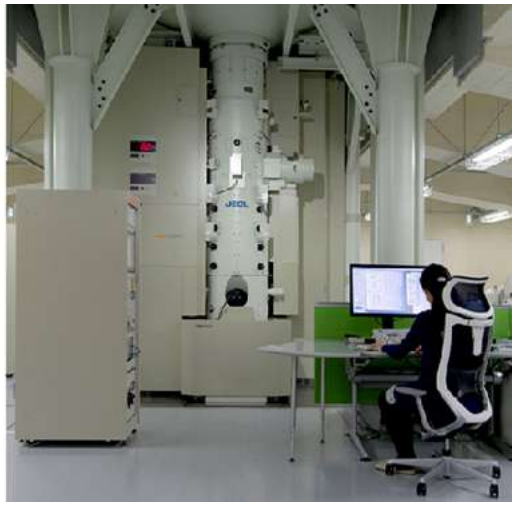
300 kV: JEM 3200FSC, cryo-ARM, Krios, Polara
FEG
Smaller effect on unwanted lens aberration
1.5-3 Å resolution



ELECTRON SOURCES & TYPES OF EMS

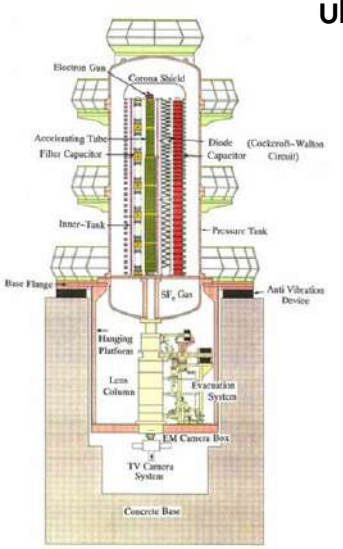


1-1.2 MV: Hitachi, JEOL
LaB6



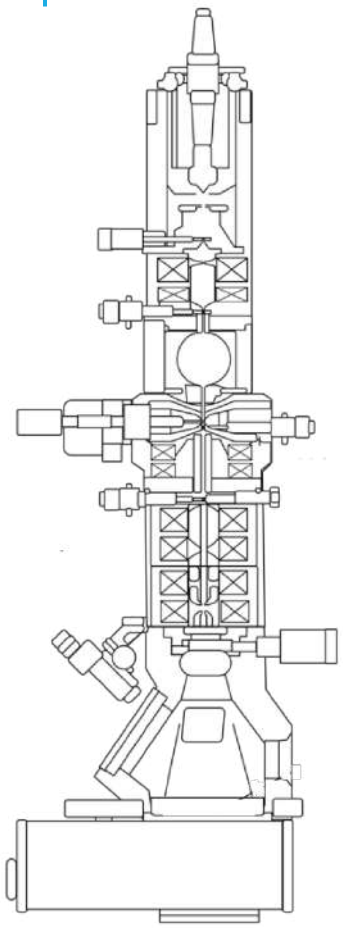
uhvem.osaka-u.ac.jp

3 MV: Hitachi H3000
LaB6



VACUUM SYSTEMS

Why do we need a vacuum?



Beam coherence - at STP mean free path ~1 cm

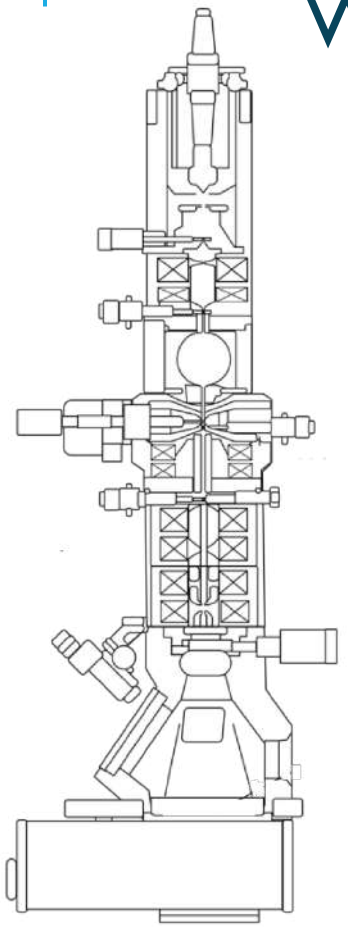
Insulation - interaction between e- and air

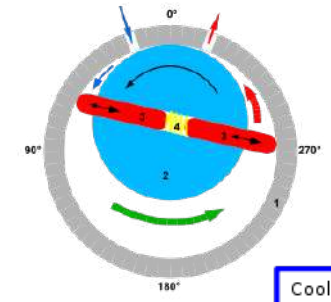
Filament - O2 will burn out source

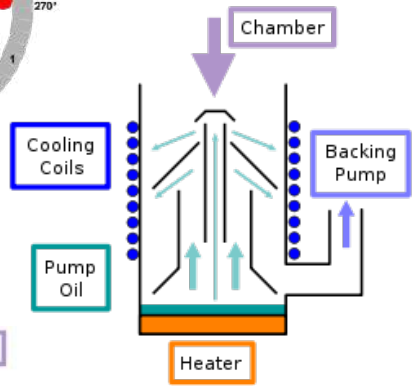
Contamination - reduce interaction gas, e-beam and sample

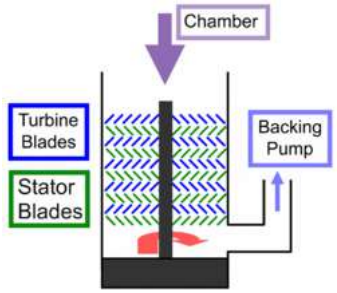
VACUUM SYSTEMS

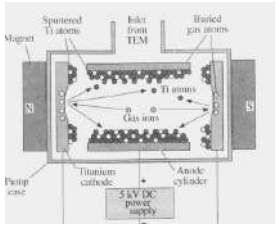
What types of pumps do we have?











wikipedia.com

1 mm Hg = 1 Torr = 10^2 Pa

1 atm = 760 Torr = 7.5×10^4 Pa

PVP / Rotary 1-10^{-3} Torr | >0.1 Pa

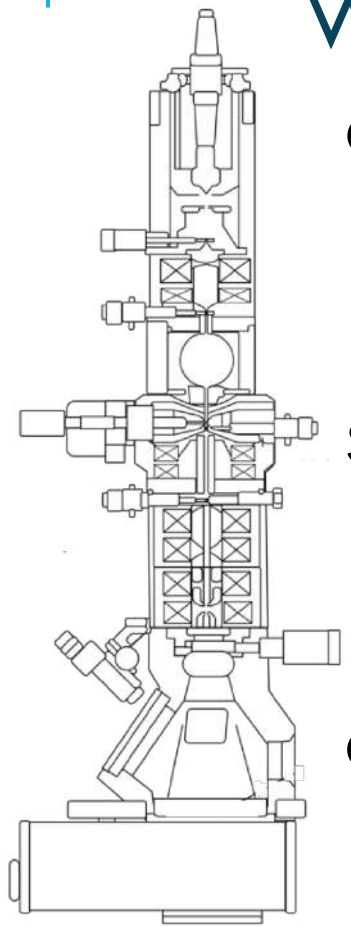
Diffusion 10^{-3}-10^{-6} Torr | 0.1-10^{-4} Pa

Turbo 10^{-6}-10^{-9} Torr | 10^{-4}–10^{-7} Pa

IGP 10^{-9}-10^{-12} Torr | 10^{-7}-10^{-9} Pa

VACUUM SYSTEMS

What types of pumps do we have?



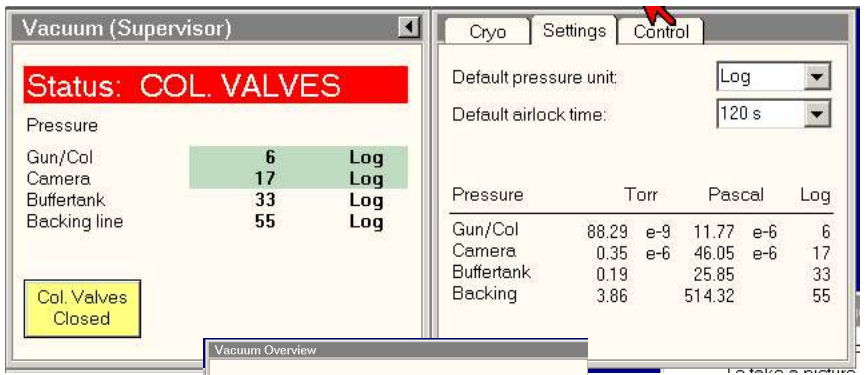
Gun 10^{-9} Torr

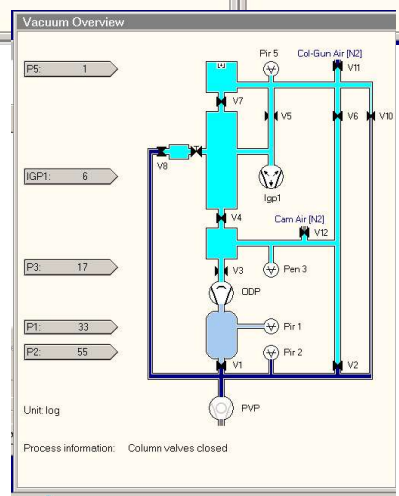
Specimen 10^{-6} -10^{-7} Torr

Chamber and Camera 10^{-5} -10^{-6} Torr

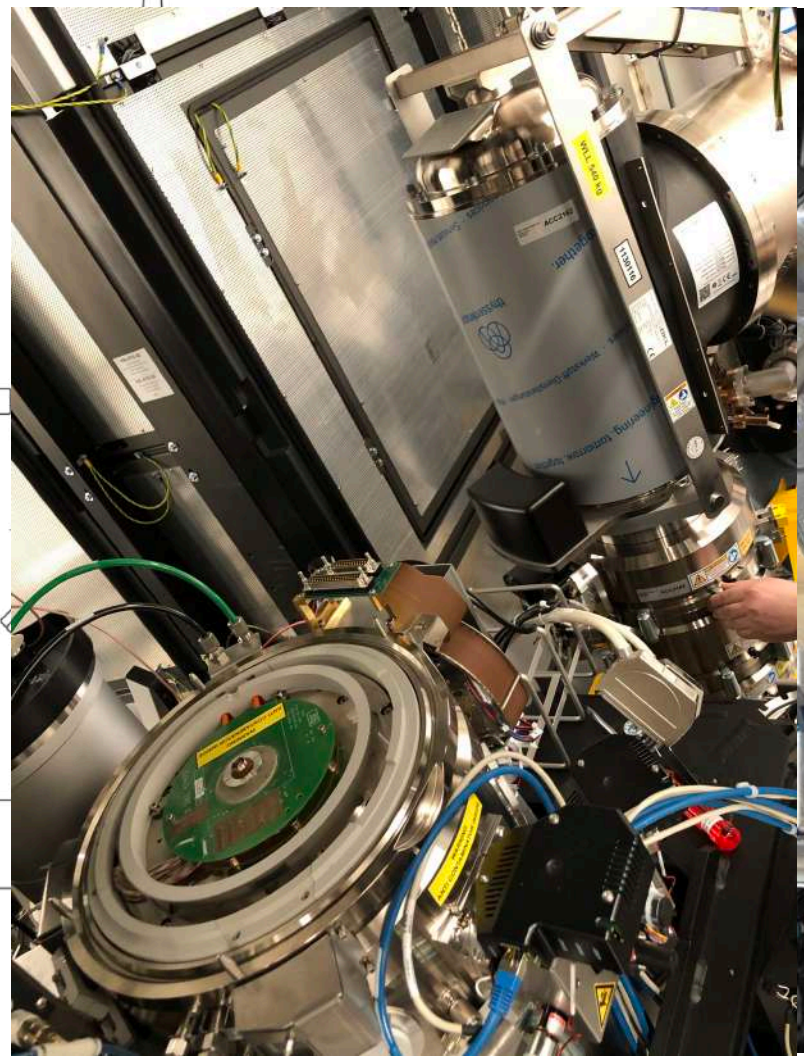
1 mm Hg = 1 Torr = 10^2 Pa

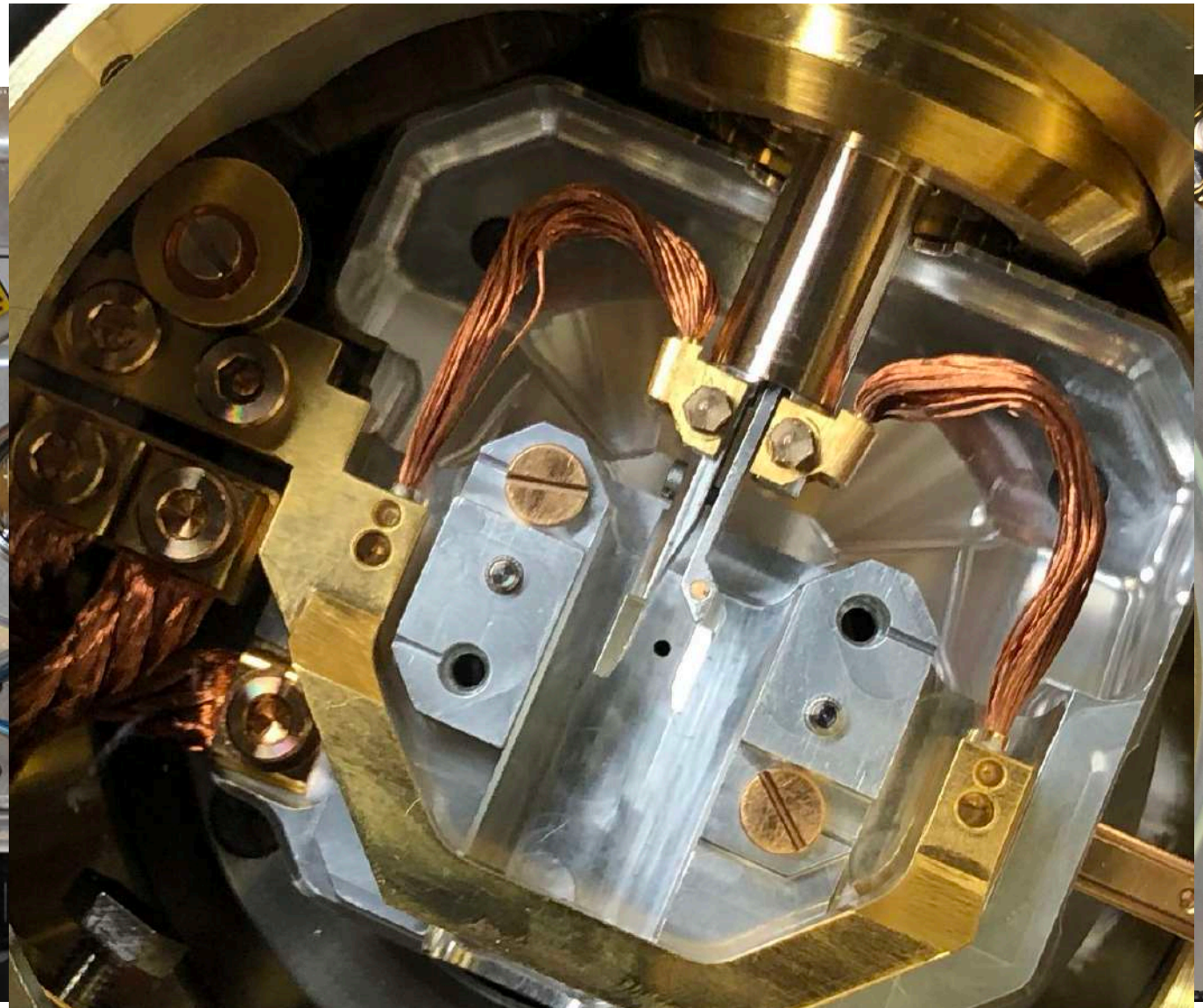
1 atm = 760 Torr = 7.5×10^4 Pa





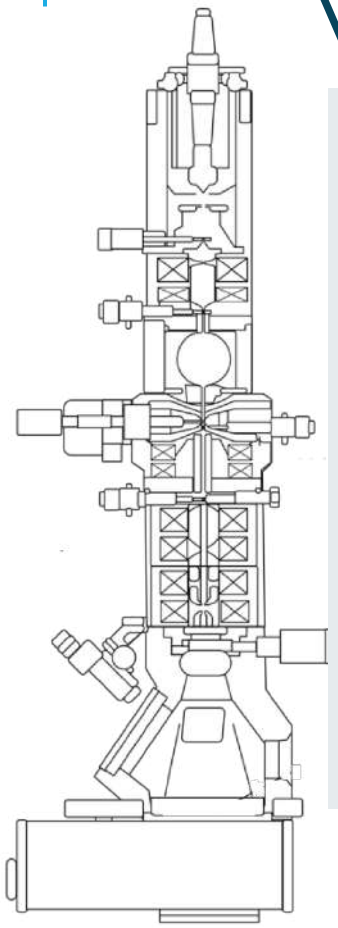
VACUUM SYSTEMS

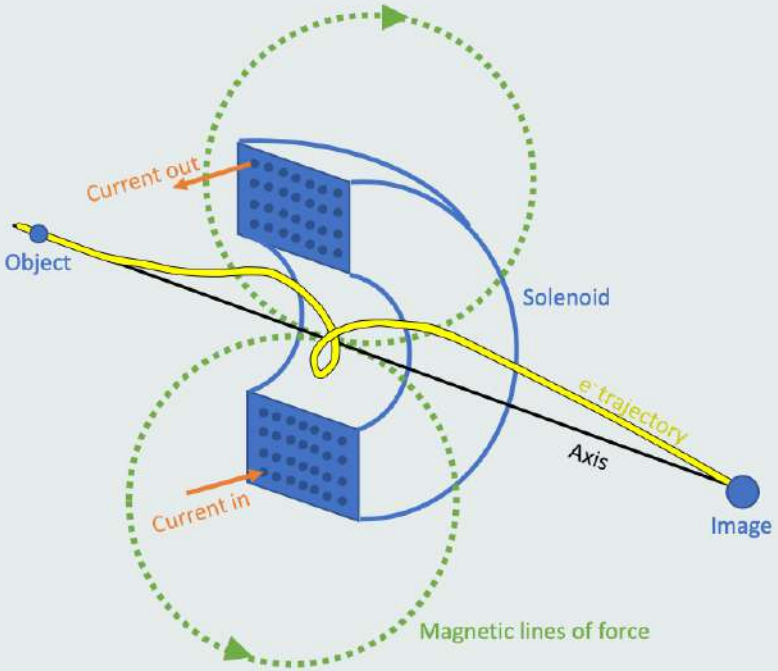




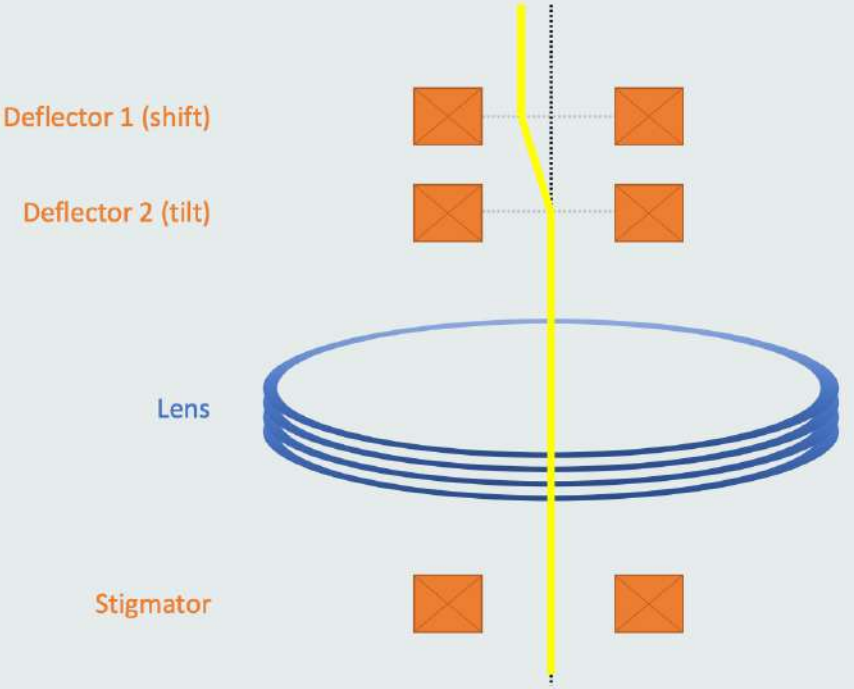
LENSES

What types of lenses do we have?





- Focus
- Magnify
- Rotate



LENSES

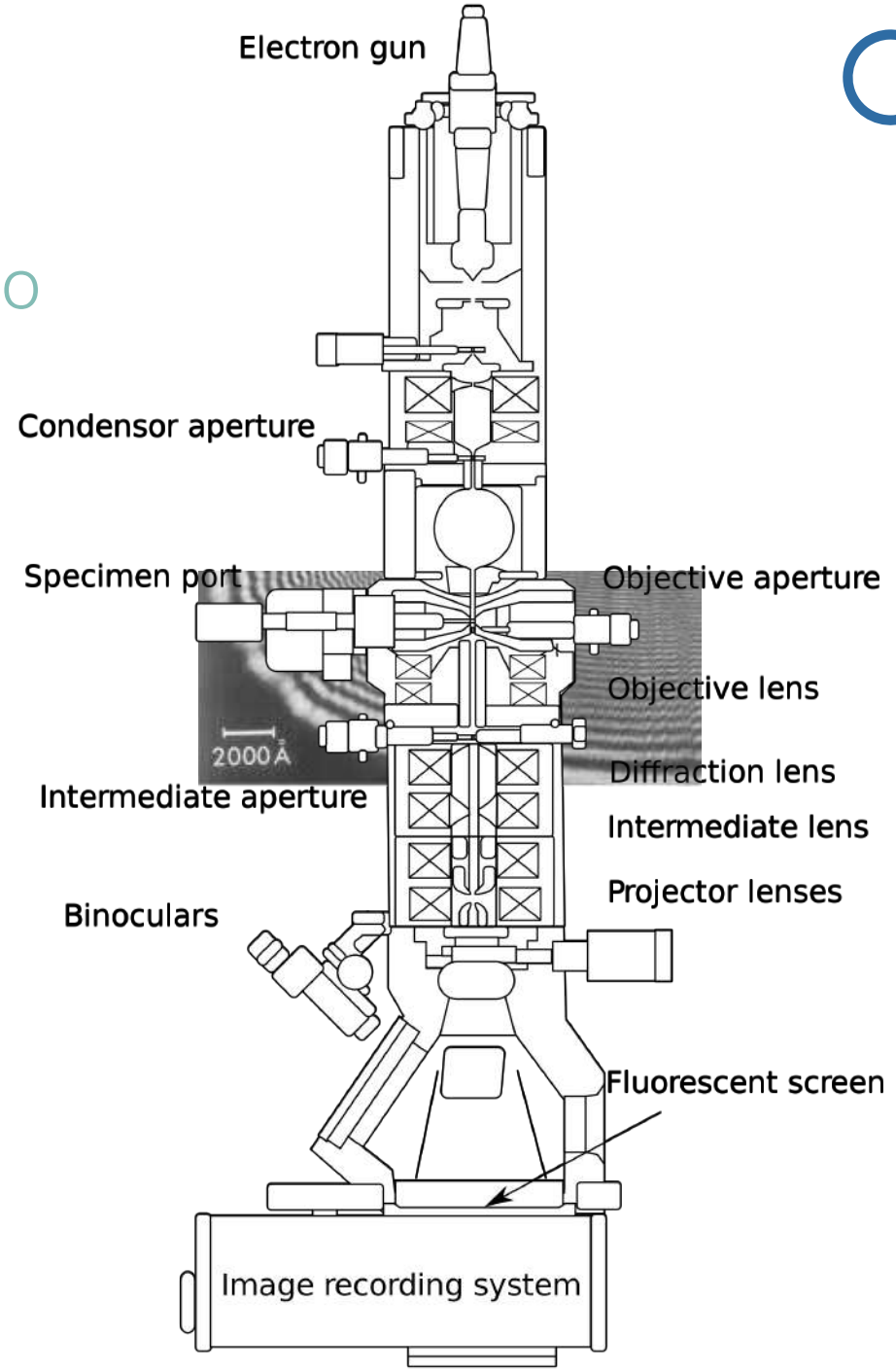
Microscope Alignments
What to do & what not to do

Do:

- Start at eucentric height and focus
- Check if it is already good before attempt
- Align from top to bottom

Not to do:

- ~~Align without a way to undo~~
- ~~Align when TEM is not stable~~ (i.e., ~~temperature~~)



DETECTORS

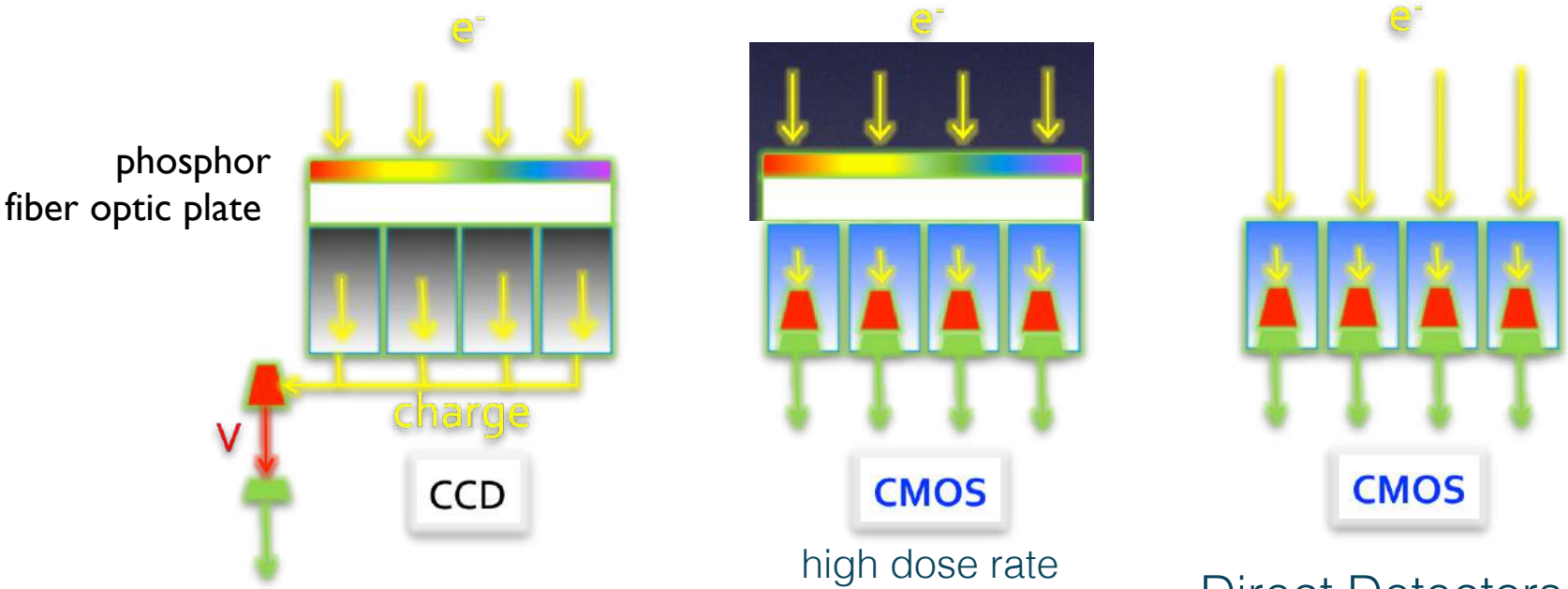
Digital Cameras for TEM

Photon converted

Direct sensing

?

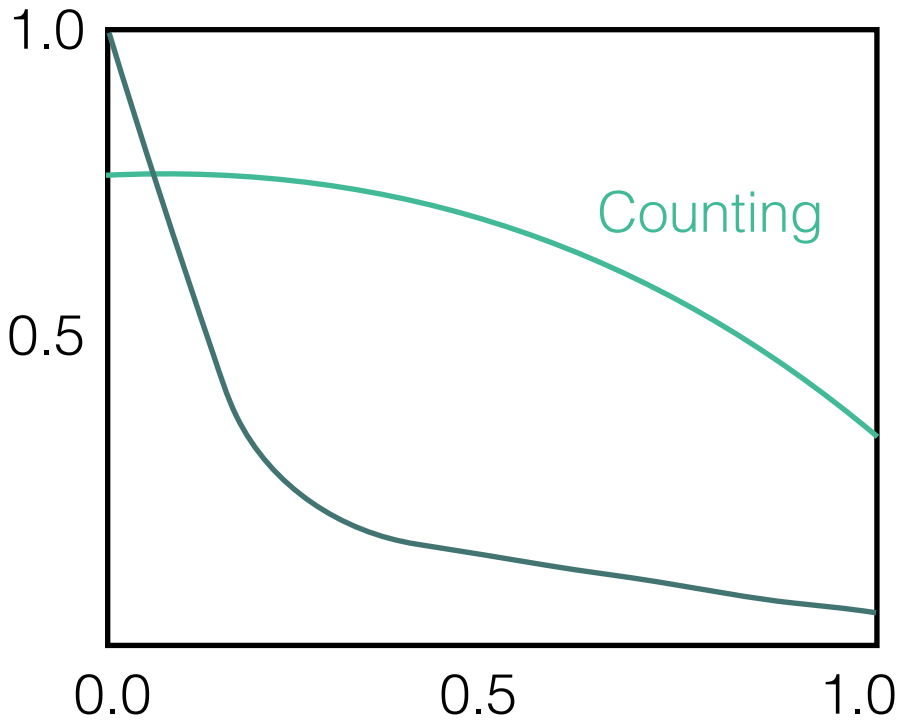
- CCD — Charge Coupled Device
- CMOS — Complementary Metal Oxide Semiconductor



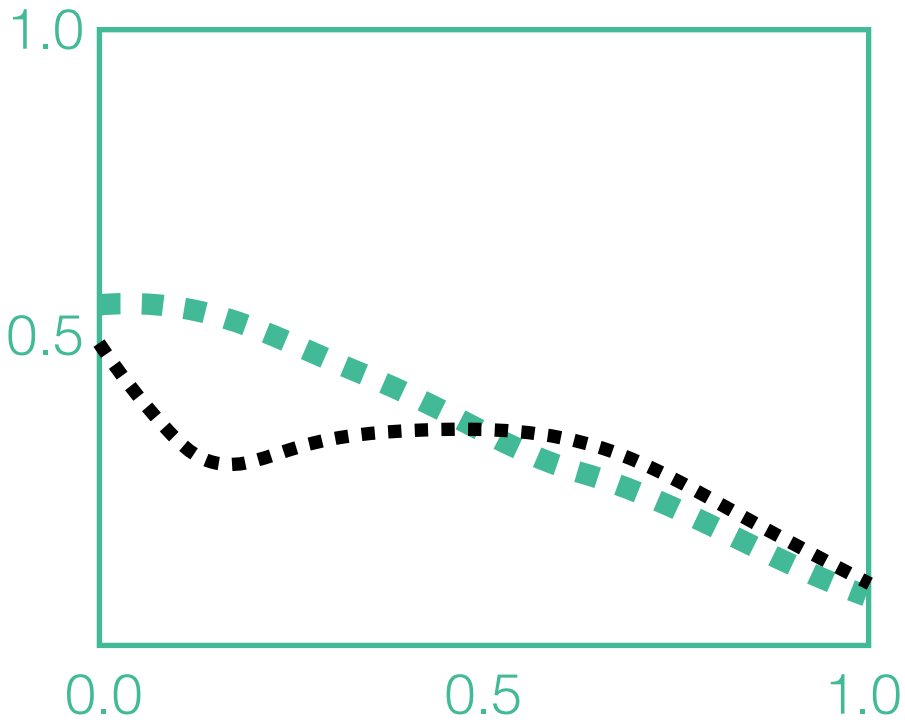
DETECTORS

Detector Performance Characterization

MTF (Modulation Transfer Transform)
contribute to signal envelope

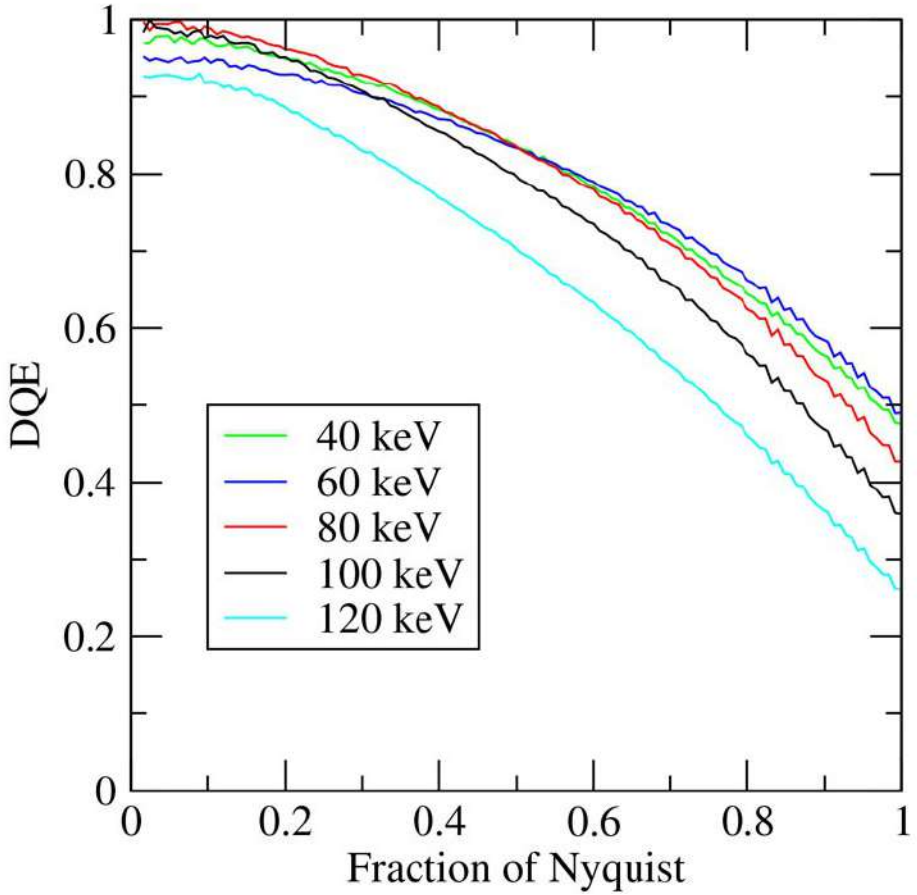


DQE (Detector Quantum Efficiency)
S/N over spatial frequency range

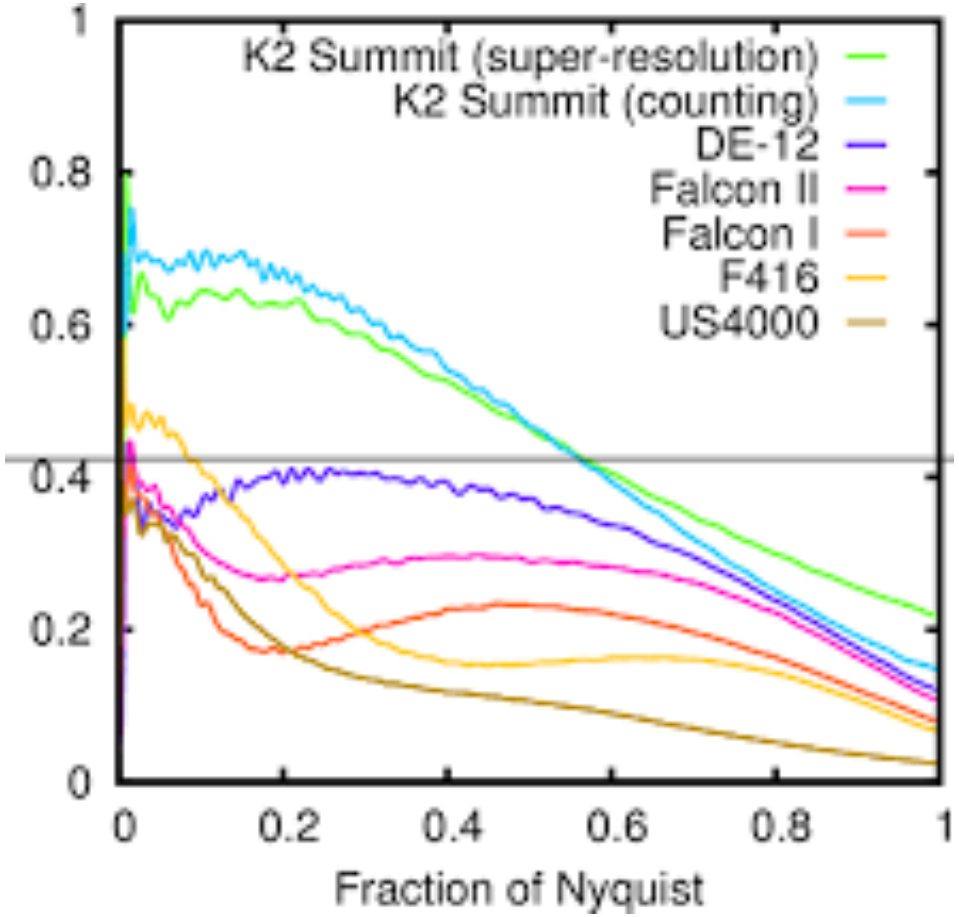


DETECTORS

Detector Performance Characterization



dectris.com



Ruskin, et al JSB

NIH P41 - National Biomedical Technology Research Resources (BTRR)


Krios1


Krios2


Krios3


Krios4


Krios5


Krios6


Krios7 2020


Tecnai F20


Tecnai12


JEOL1230

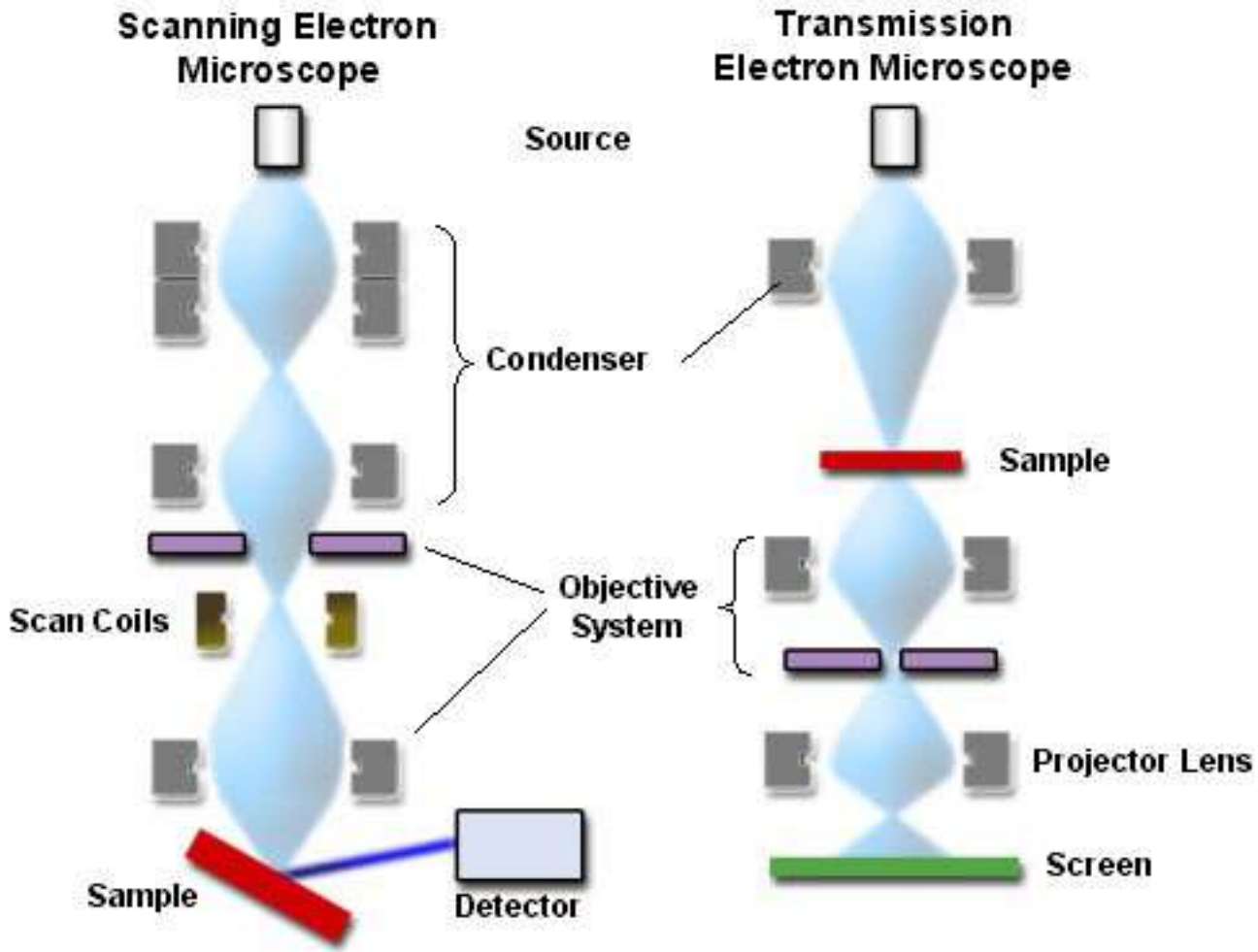

Helios650


Hitachi 7800

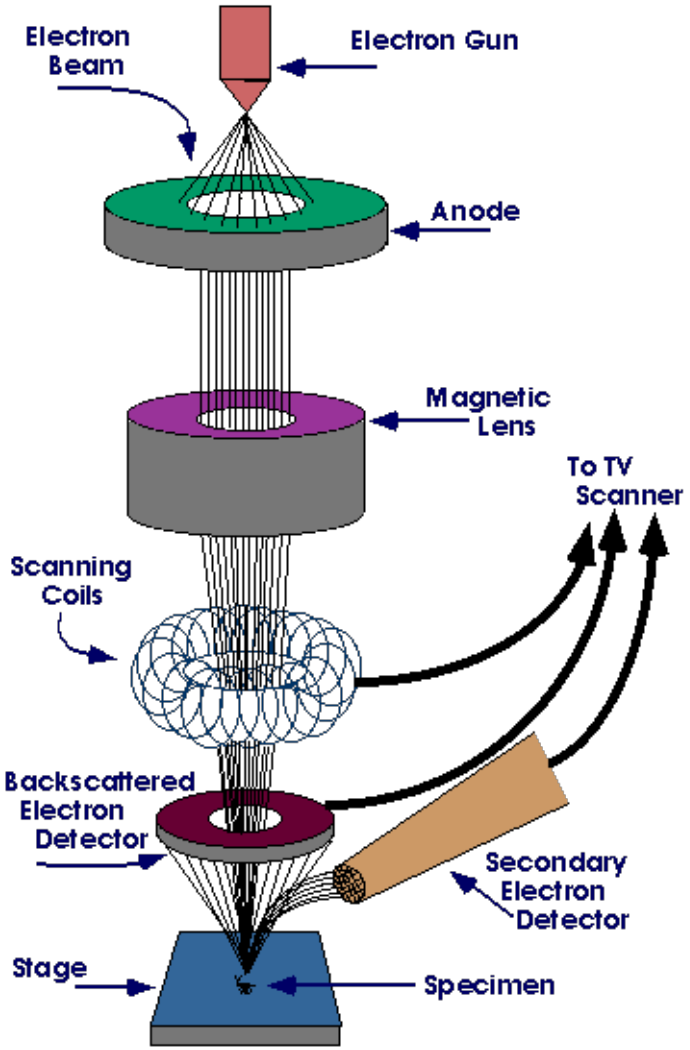

Glacios

Chameleon

ANATOMY OF AN SEM



ANATOMY OF AN SEM



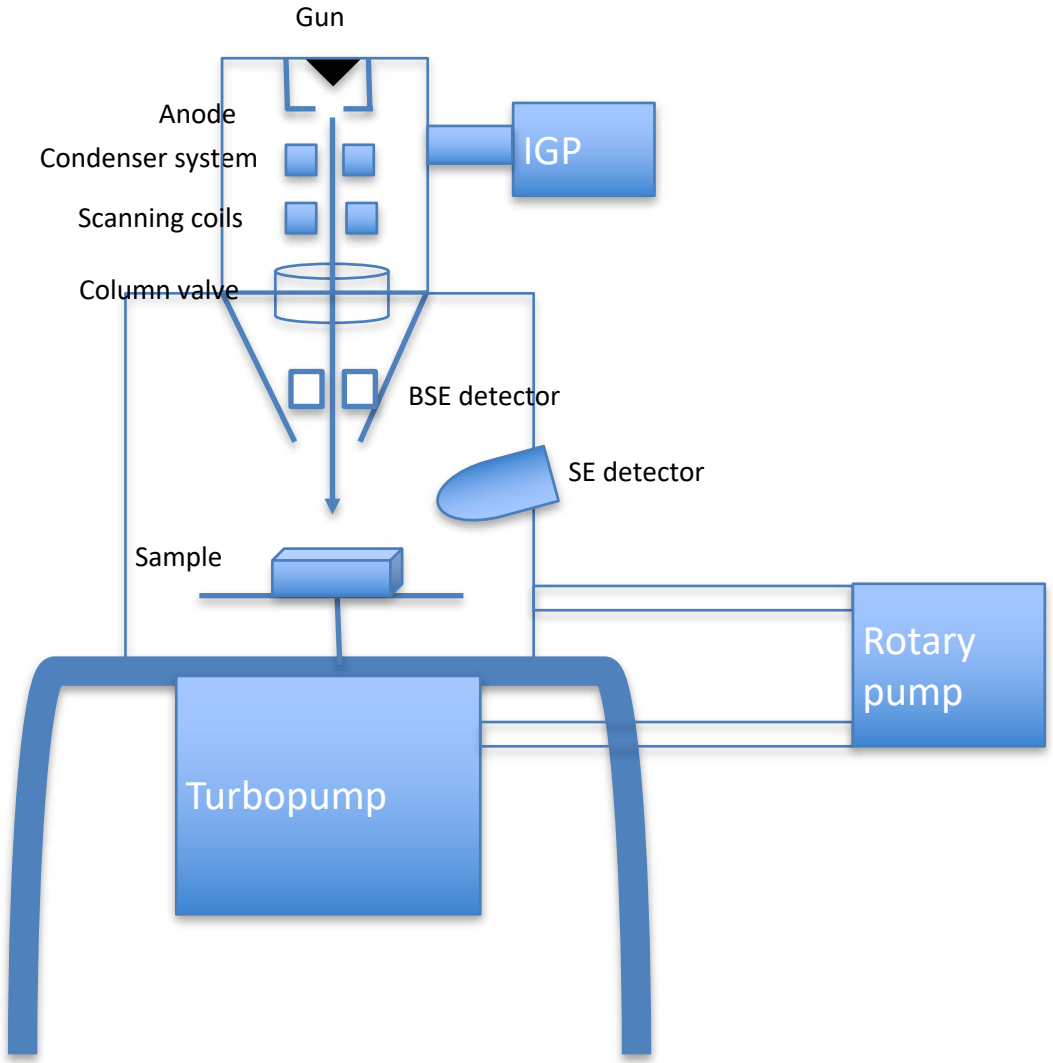
Electron gun: range from tungsten filaments in lower vacuum SEMs to FEGs which need modern high vacuum SEMs

Beam energy: 0.2 – 40 keV is focused by a condenser lens system into a spot of 0.4 – 5 nm

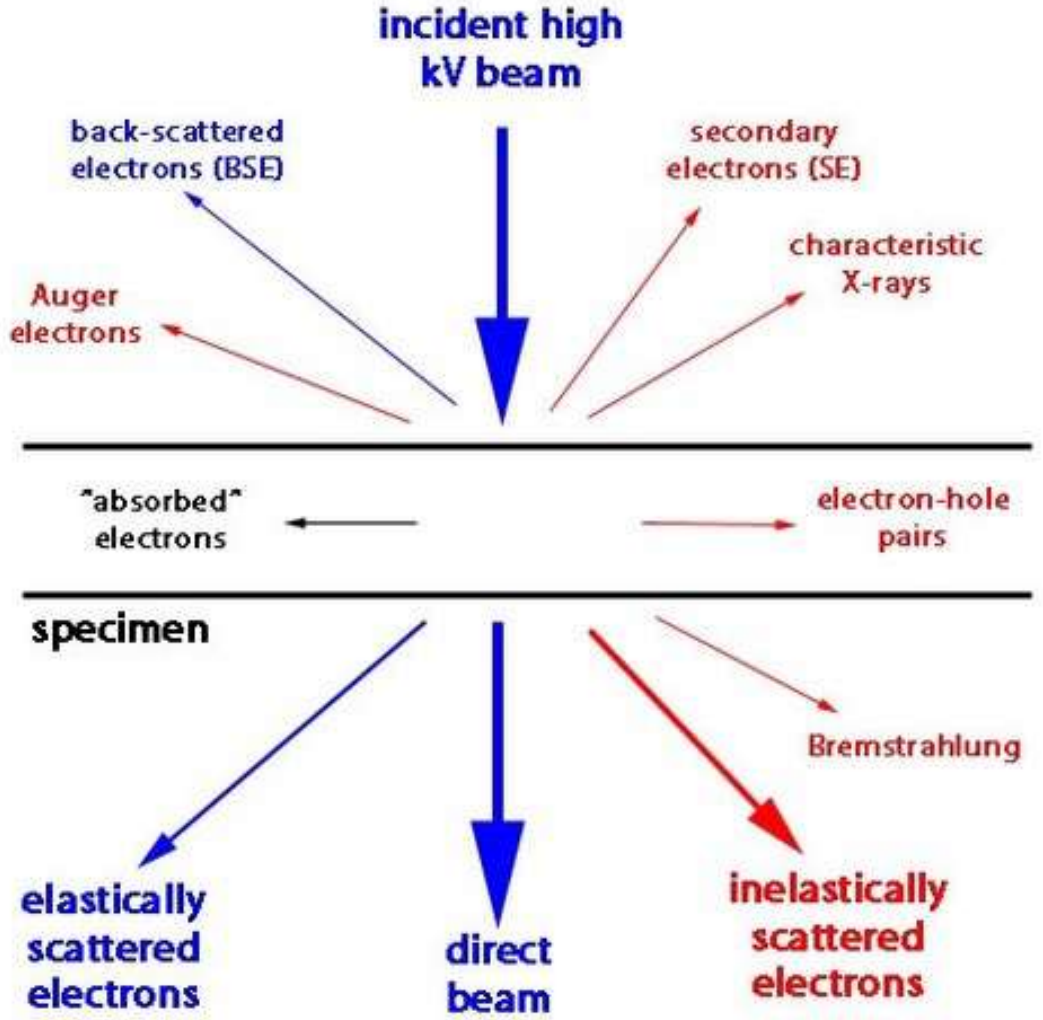
Beam is deflected by very fast scanning coils and rasters the sample surface

Typical resolution of SEM is between 1 and 20 nm where the record is 0.4 nm

ANATOMY OF AN SEM – VACUUM SYSTEMS

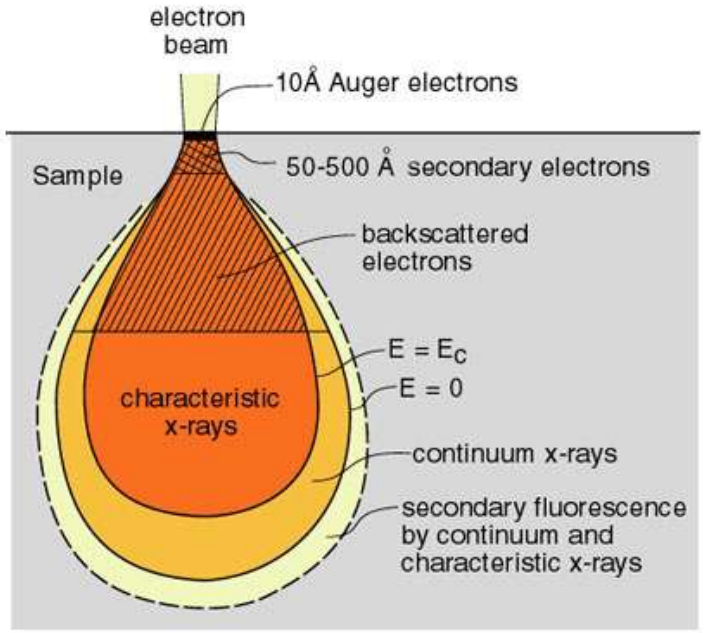


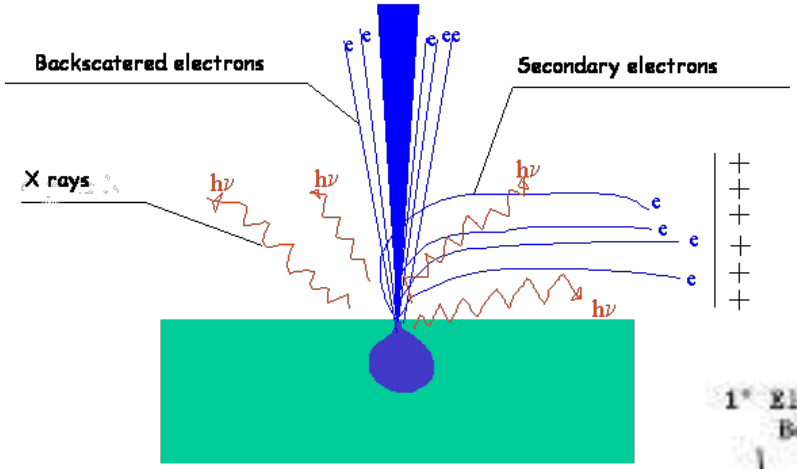
ANATOMY OF AN SEM – BEAM SAMPLE INTERACTIONS

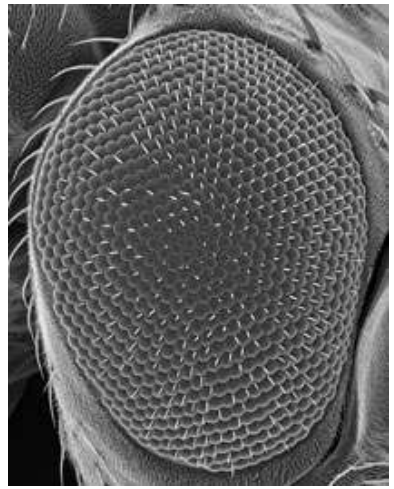


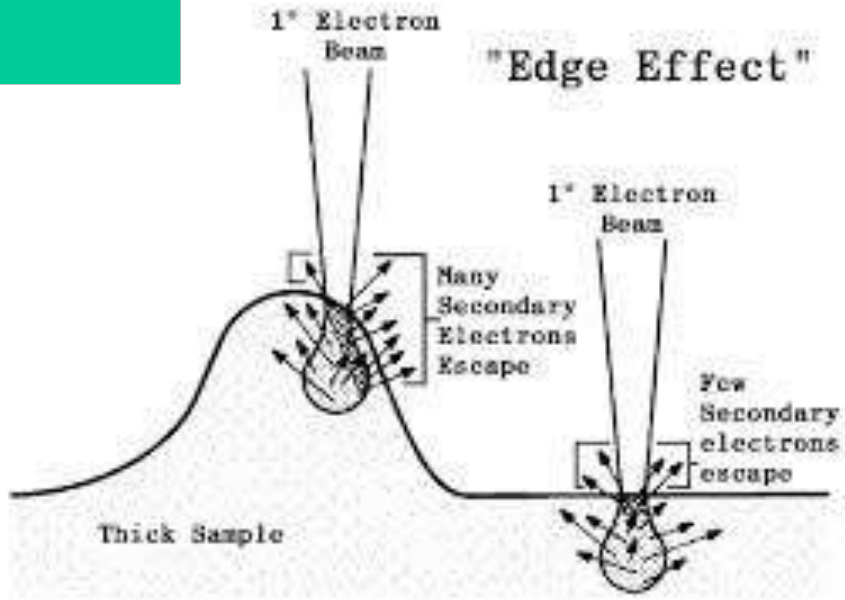
modified from Williams & Carter (1996) Fig. 1.3

ANATOMY OF AN SEM – BEAM SAMPLE INTERACTIONS &IMAGE FORMATION

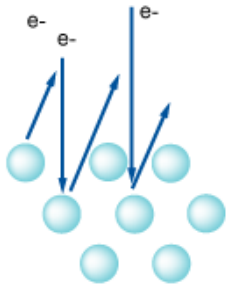




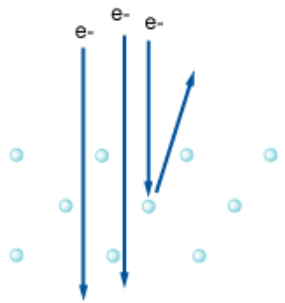




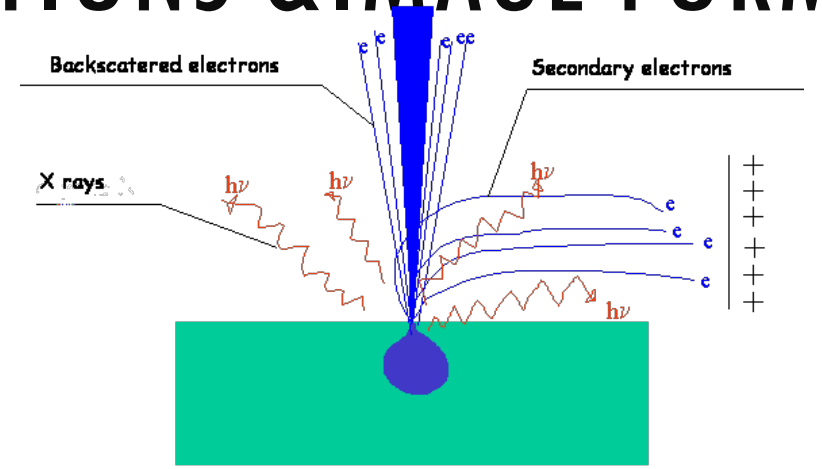
ANATOMY OF AN SEM – BEAM SAMPLE INTERACTIONS &IMAGE FORMATION

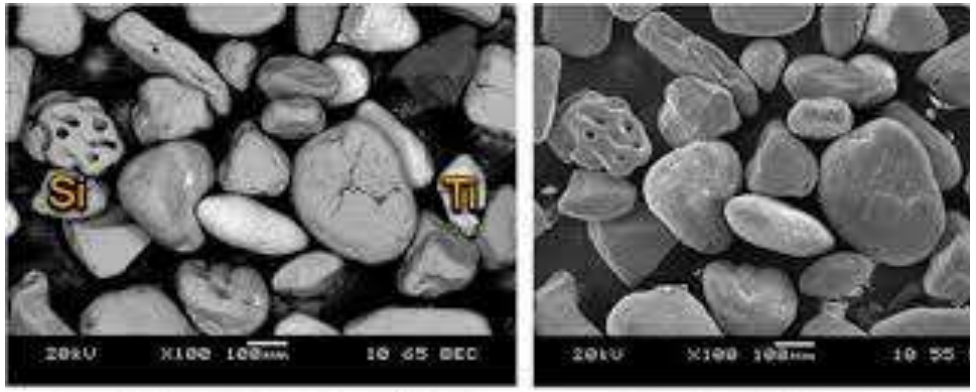


Titanium
atomic number 22



Silicon
atomic number 14





TOOLS OF THE TRADE: MICROSCOPES AND DETECTORS

Questions?

cryoEM 001 : Single Particle Masterclass

1. Building a cryoEM toolkit
2. EM compatible samples
3. EM support films and grids
4. Sample preparation
5. Tools of the trade: microscopes and detectors
6. Microscope operations
7. Data collection strategies
8. Data assessment & QC
9. Data processing:
    - cryoEM IT infrastructure
    - On-the-fly feedback
    - 3D Reconstruction
10. Visualization and validation