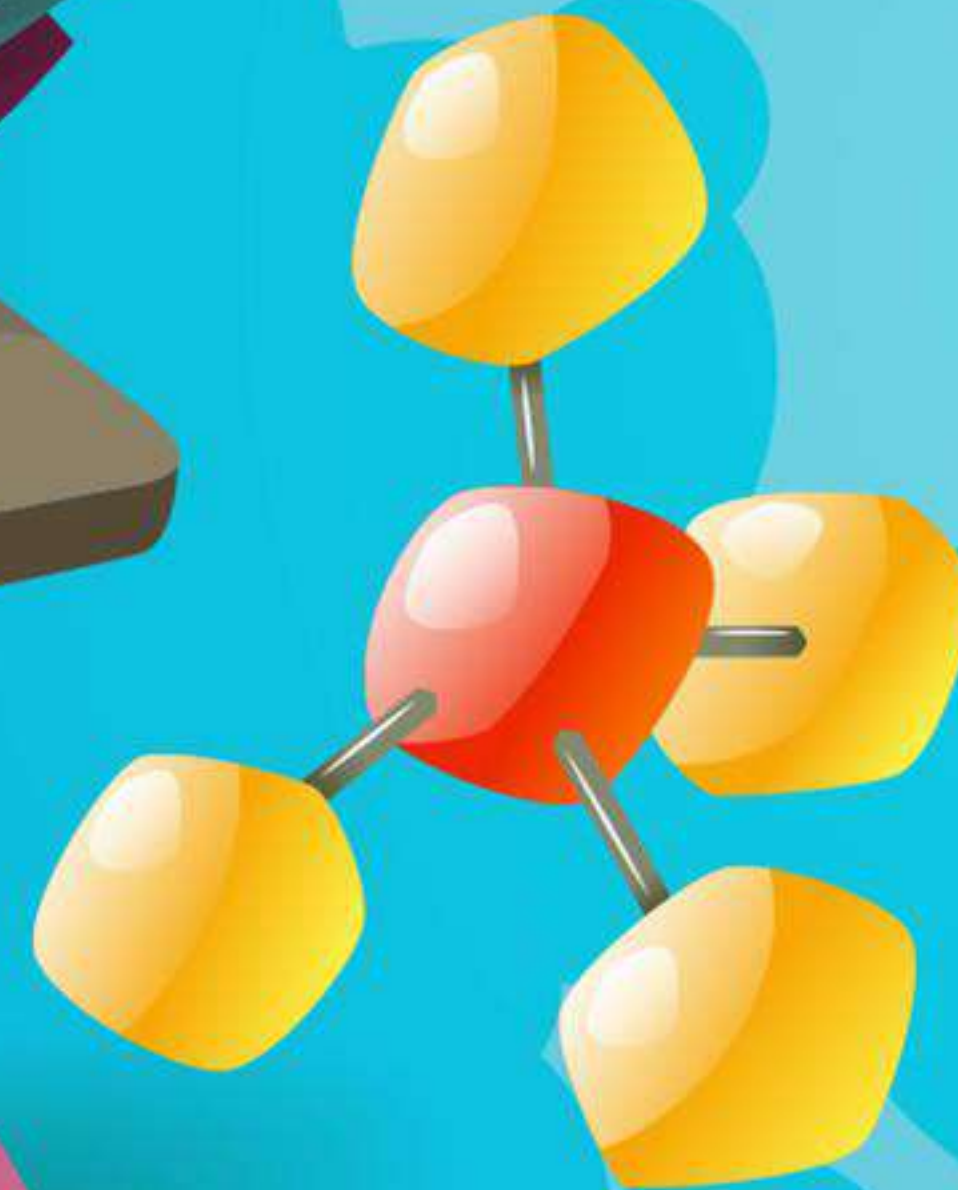
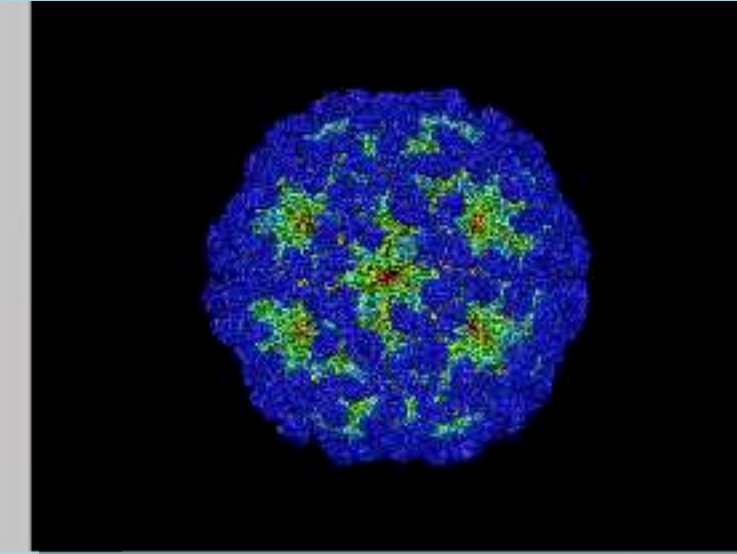
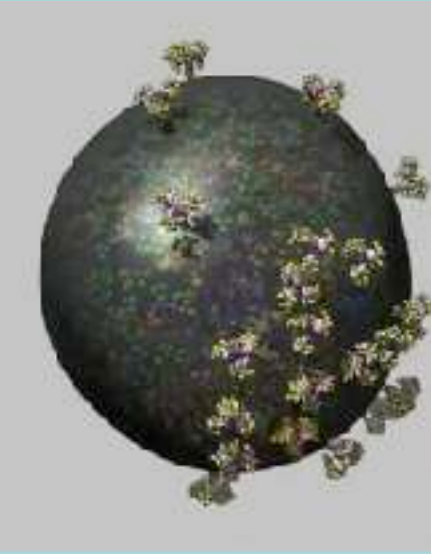
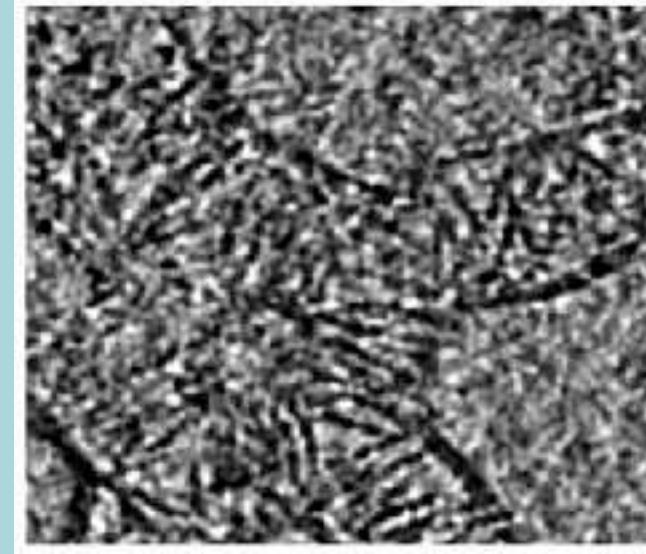


# 2022 Winter-Spring EM Course



Edward T Eng  
January 10, 2022



Simons Electron Microscopy Center

NEW YORK STRUCTURAL BIOLOGY CENTER

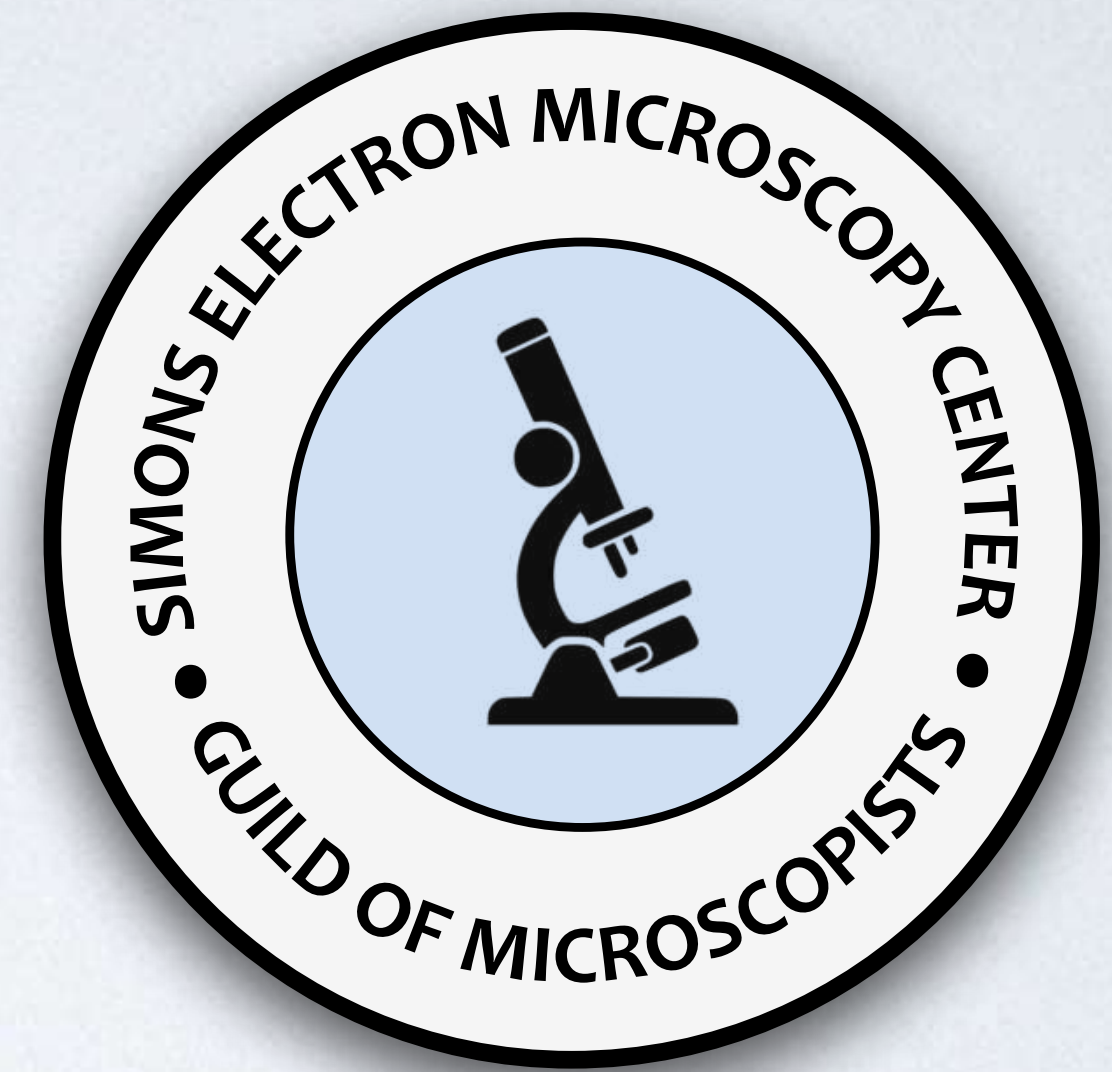




# Welcome to electron microscopy at SEMC

---

1. Welcome new students
2. Course logistics
3. Poll / Zoom Picture
4. Hybrid classroom







NMR



CoMD/  
NMR

X-ray



NYX  
@NSLS-II



Protein  
Production  
COMPPA

NIH P41 - National Biomedical Technology Research Resources (BTRR)

National Synchrotron Light Source II

BROOKHAVEN  
NATIONAL LABORATORY

19-ID

NYX



NRAMM







NMR



CoMD/  
NMR

X-ray



NYX  
@NSLS-II



Protein  
Production  
COMPPA

NIH P41 - National Biomedical Technology Research Resources (BTRR)

# 17th year of the course

photo by Swapnil Bhatkar



NRAMM





# Course logistics: main website

**[semc.nysbc.org/the-winter-spring-2022-em-course/](https://semc.nysbc.org/the-winter-spring-2022-em-course/)**



The screenshot shows the website for the SIMONS ELECTRON MICROSCOPY CENTER. The header includes the center's name and a navigation menu with links to HOME, ABOUT, USER RESOURCES, PUBLICATIONS, INSTRUMENTATION, NEWS & EVENTS, and FAQ. A search icon is also present. The main content area is titled 'Course Schedule' and lists various events and courses. On the left side, there is a sidebar with links to EVENTS, Upcoming Events, News and Past Events, Forums, and Workshops and Courses.

**SIMONS ELECTRON MICROSCOPY CENTER**

HOME ABOUT USER RESOURCES PUBLICATIONS INSTRUMENTATION NEWS & EVENTS FAQ

**EVENTS**

- Upcoming Events
- News and Past Events
- Forums
- Workshops and Courses

**Course Schedule**

Classes in SEMC seminar room (Mondays 3:30-5pm and select Wednesdays 3:30-5pm)

**EM fundamentals section**

- 1/10/22 Lecture – Introduction & Basic anatomy of the electron microscope (SEMC staff)
- 1/12/22 Practical – TEM use (SEMC staff)
- 1/17/22 *MLK Jr holiday – No class*
- 1/19/22 Practical – Sample Preparation & Support films (SEMC staff)
- 1/24/22 Lecture – New cryoEM hardware and supporting a facility (SEMC staff)
- 1/26/22 Practical – Journal club

**EM crystallography section**

- 1/31/22 Lecture – MicroED (Bill Rice – NYU)
- 2/2/22 Practical – Journal club
- 2/7/22 Lecture – Helical reconstruction (Hernando Sosa – Einstein)
- 2/9/22 Practical – Journal club

**Tomography section**

- 2/14/22 Lecture – Tomography (Wei Dai – Rutgers)
- 2/16/22 Practical – Tomography workshop – Appion/Protomo (Alex Noble – NYSBC/SEMC)
- 2/21/22 *President's day holiday – No class*
- 2/23/22 Practical – Journal club
- 2/28/22 Lecture – FIB-SEM (Bill Rice – NYU & SEMC staff)

Classes in the NYSBC seminar room (mornings)

**Single-particle section\***

- 3/14/22 : *Short course Keynote* – Intro to Single Particle

**Course  
Administrator:**

Ed Eng ([eeng@nysbc.org](mailto:eeng@nysbc.org))

**Teaching Assistants:**

Mahira Aragon,  
Eugene Chua,  
Christina Zimanyi



# Course logistics: hybrid vs flipped class

---

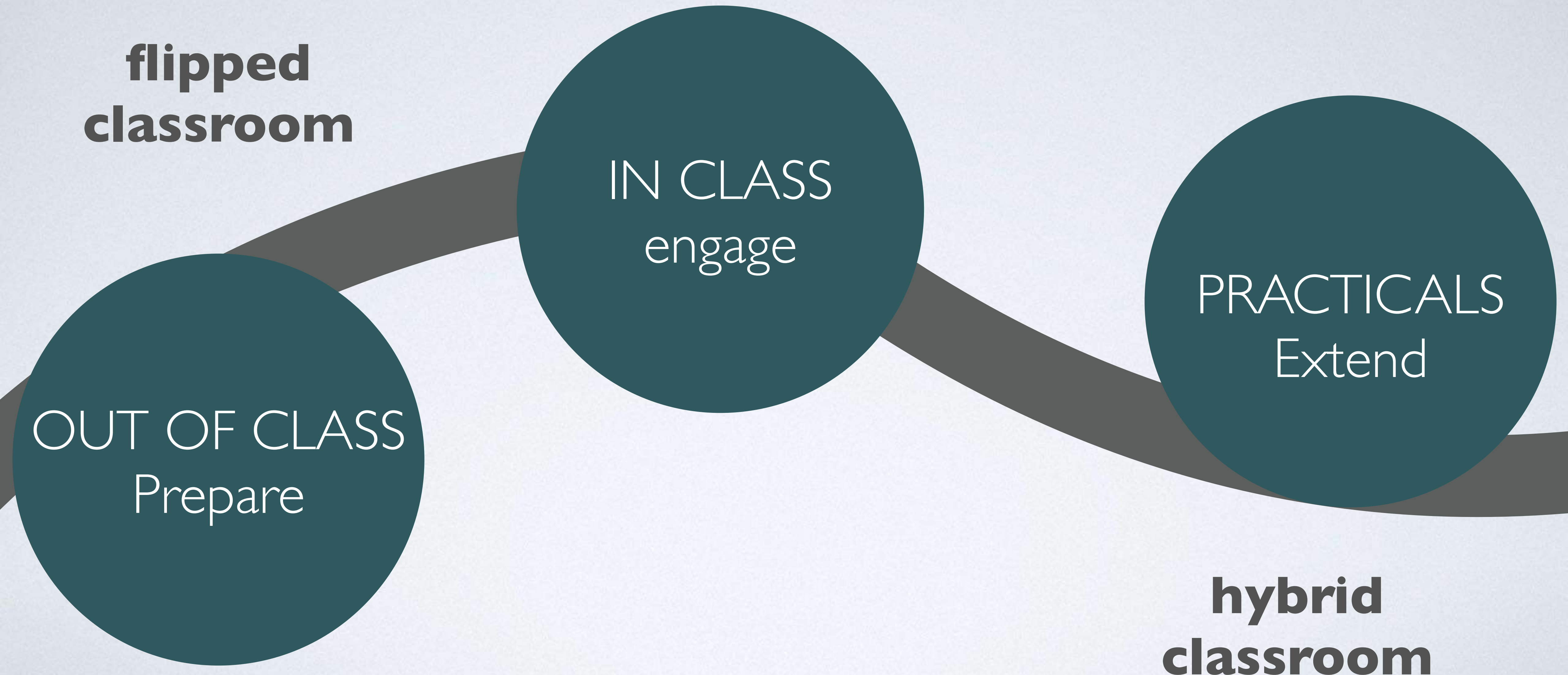
**flipped  
classroom**

IN CLASS  
engage

OUT OF CLASS  
Prepare

PRACTICALS  
Extend

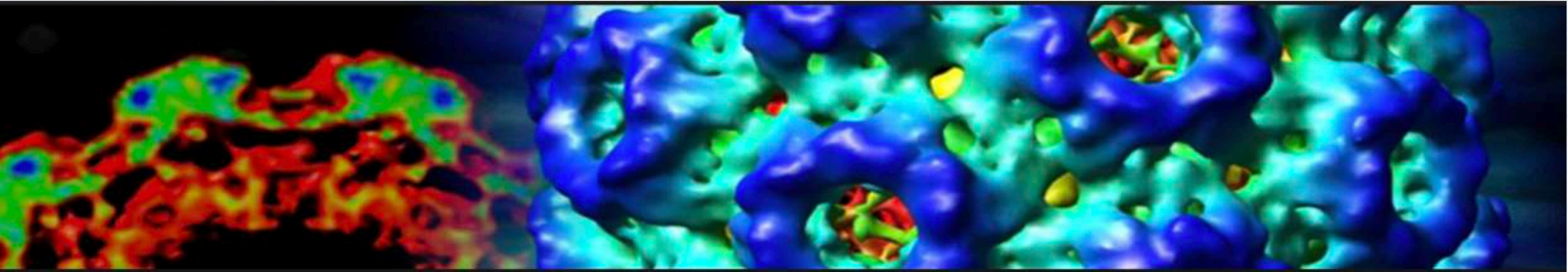
**hybrid  
classroom**






# Course logistics: resources

[youtube.com/nrammsemc](https://youtube.com/nrammsemc)






NRAMM SEMC NCCAT

803 subscribers


SUBSCRIBED



HOMEVIDEOSPLAYLISTSCOMMUNITYCHANNELSABOUT

Created playlists


SORT BY



5

SEMC Training Videos

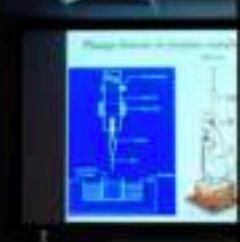
VIEW FULL PLAYLIST



16

SEMC 2021 Cryo EM Course


VIEW FULL PLAYLIST



13

NCCAT SPA Short course 2020

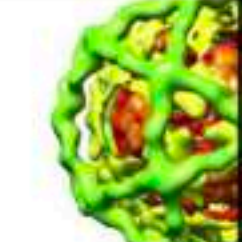
VIEW FULL PLAYLIST



3

CryoEM Facility Workshop - 2017 Control Room


VIEW FULL PLAYLIST



1

Grant Jensen Cryo-EM Lectures

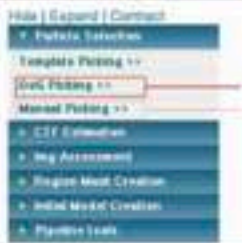
VIEW FULL PLAYLIST



48

Grant Jensen Cryo-EM Lectures


VIEW FULL PLAYLIST



2

Appion

VIEW FULL PLAYLIST



5

Data Processing

VIEW FULL PLAYLIST

[cryo-em-course.caltech.edu/videos](https://cryo-em-course.caltech.edu/videos)

Caltech

Getting Started in Cryo-EM

Welcome

Course Overview

Outline

Lecture Videos

Instructor

Links

WELCOME TO THE COURSE

Before diving into the lecture videos, start by watching the [trailer](#) and reading the course [overview](#) and [outline](#). We hope you enjoy learning about cryo-electron microscopy (cryo-EM)!

Getting Started in Cryo-EM with Professor Grant Jensen

CRYOELECTRON MICROSCOPY

LABORATORY

Established with generous support from

The Gordon and Betty Moore Foundation

and

The Agouron Institute

CryoEM 101 - Getting Started in Cryo-EM

The Jensen Lab

Email: [GettingStartedInCryoEM@gmail.com](mailto:GettingStartedInCryoEM@gmail.com)

LOG IN

[cryoem101.org](https://cryoem101.org)

CryoEM 101

HOMECHAPTERSABOUTCONTACT

Cryo EM has emerged as a powerful tool for high-resolution structure determination.

To aid the training efforts of newcomers to the field, we are creating a media-rich curriculum to augment users' own hands-on training. The training material will contain videos, animations, and interactive simulations that cover the major components of the cryo-EM workflow.

Chapter 1

Sample Purification

Chapter 2

Cryo-EM Grid Preparation

Chapter 3

Grid Screening & Evaluation

Chapter 4

Cryo-EM Data Collection

Chapter 5

Image Processing

Now available - Chapter 2: Cryo-EM grid preparation



# Course logistics: engagement & reinforcement

---

**[semc.nysbc.org/the-winter-spring-2022-em-course/](https://semc.nysbc.org/the-winter-spring-2022-em-course/)**

## **Class**

Introduction to the material



Ed



Christina

## Roundtable

Submit questions to us by  
Friday the week before.



Mahira



Eugene



# Course logistics: main topics

---

Section 1 : EM fundamentals

Section 2 : EM crystallography

Section 3 : Tomography

Section 4 : Single-particle short course\*  
March 14-18, 2022





# Course logistics

---

## **Mondays (and select Wednesdays)**

3:30-5pm - In person/Zoom meetings

### **Lecture schedule**

EM fundamentals section

Jan 10 – Introduction & Basic anatomy of the electron microscope

Jan 17 – *MLK Jr holiday*

Jan 19 – New cryoEM hardware and supporting a facility

## **Wednesdays**

Starts at 3:30 - In person/Zoom meetings

### **Recitation schedule**

Jan 12 : TEM overview

Jan 19 : Sample Preparation &  
Support films

Jan 26 : Journal club

Feb 2 : Journal club

Feb 9 : Journal club

Feb 16 : Tomography – Appion/  
Protomo workflow

Feb 23 : Journal club



# Course logistics

## Mondays (and select Wednesdays)

3:30-5pm - In person/Zoom meetings

## Wednesdays

Starts at 3:30 - In person/Zoom meetings

### Lecture schedule

EM crystallography section

Jan 31 – MicroED (Bill Rice, NYU)

Feb 2 – Helical reconstruction (Hernando Sosa, Einstein)

Tomography section

Feb 14 – Tomography (Wei Dai, Rutgers)

Feb 21 – *President's day holiday*

Feb 28 - FIB-SEM (Bill Rice, NYU)

Single particle short course

Week of March 14th



Joachim Frank (Columbia University)

Victor Chen & Wen Jian (Purdue University)

Fred Sigworth (Yale University)

Amedee des Georges & Reza Khayat (ASRC/City University of New York)

Rich Hite (Memorial Sloan Kettering Cancer Center)

Tom Walz (Rockefeller University)

Cathy Lawson (Rutgers University)

Oli Clarke (Columbia University)

Gira Bhabha & Damian Ekiert (New York University)



# Course logistics: class for credit

---

## Component

## Percentage

Recitation/Participation 50%

- *JC/HW/questions*

Practicals 10% × 3

Attendance 20%

## Wednesdays

Starts at 3:30 - SEMC conference room

### Recitation schedule

Jan 12 : TEM overview

Jan 19 : Sample Preparation &  
Support films

Jan 26 : Journal club

Feb 2 : Journal club

Feb 9 : Journal club

Feb 16 : Tomography – Appion/  
Protomo workflow

Feb 23 : Journal club





START

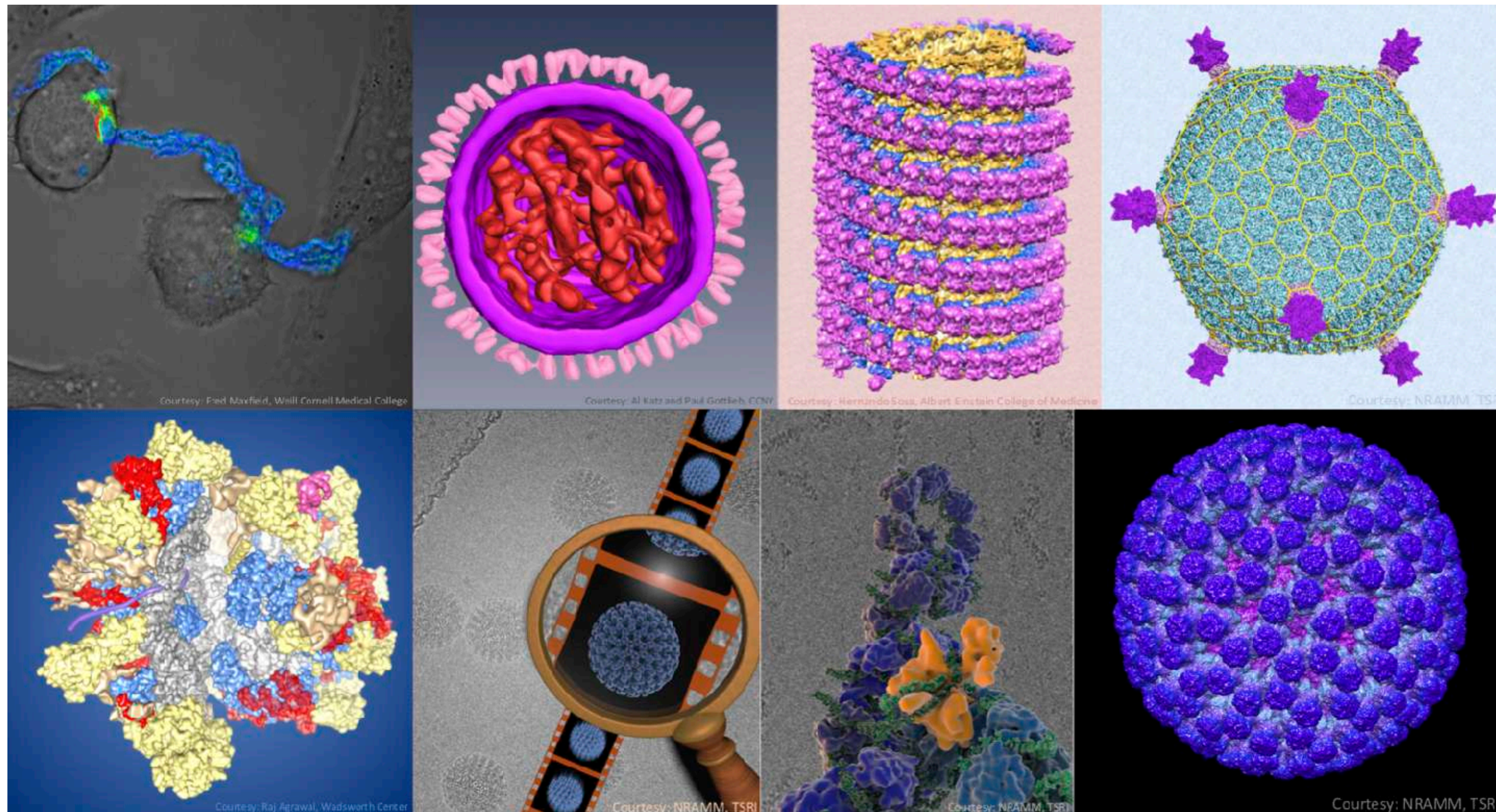


<http://etc.ch/hpD2>

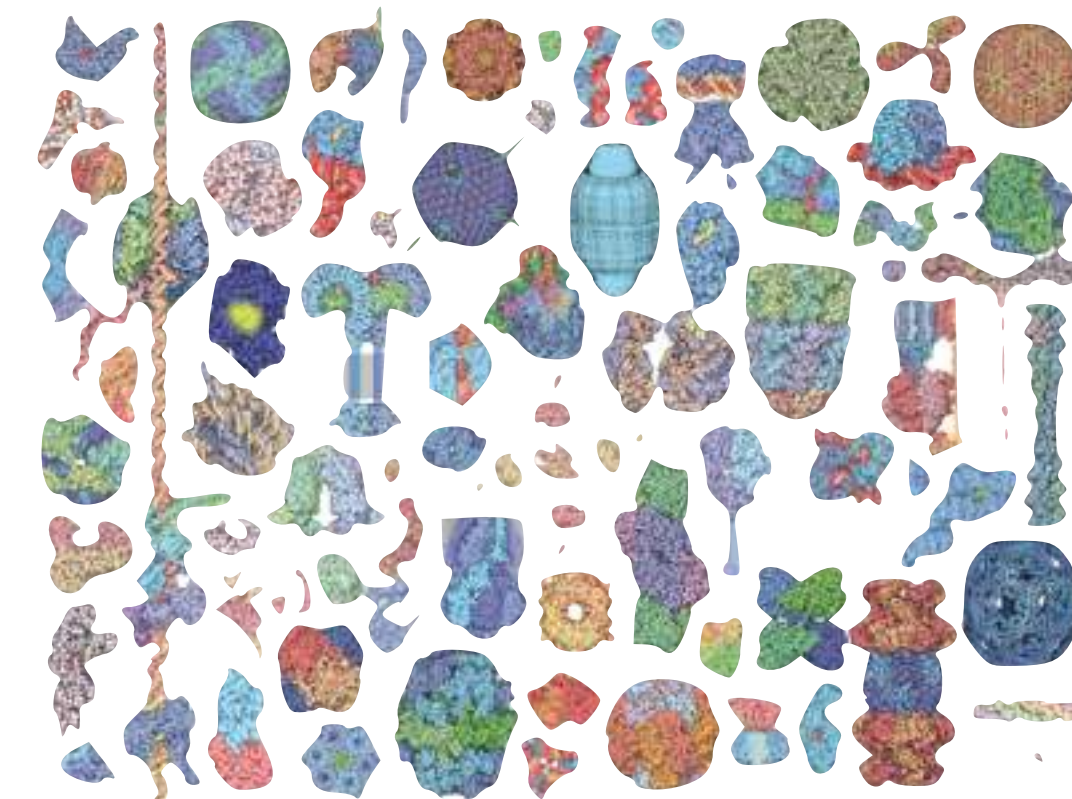
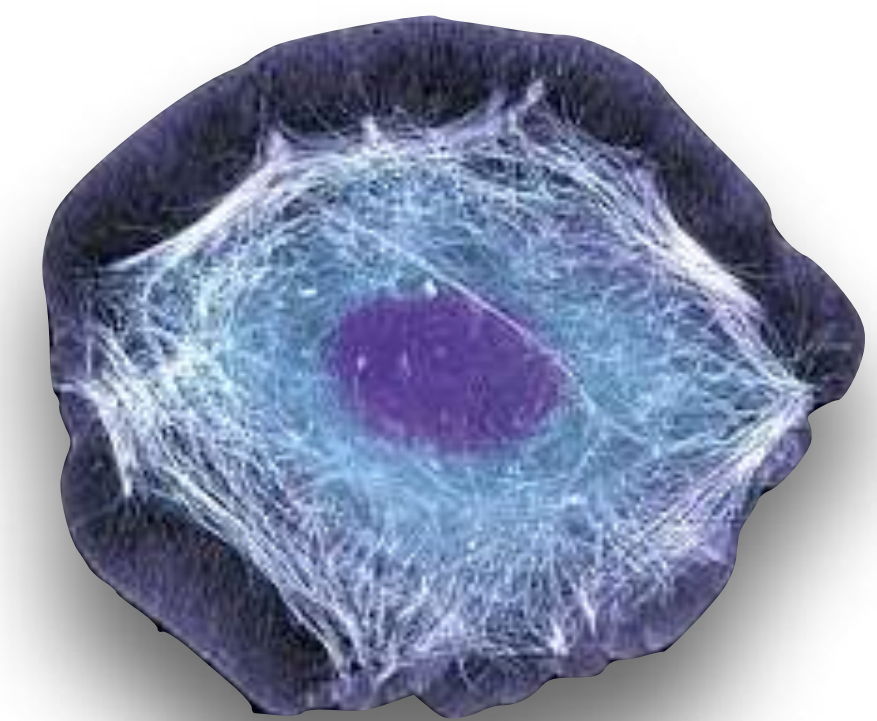




# CRYOEM: TECHNOLOGY ON THE RISE



*in situ*

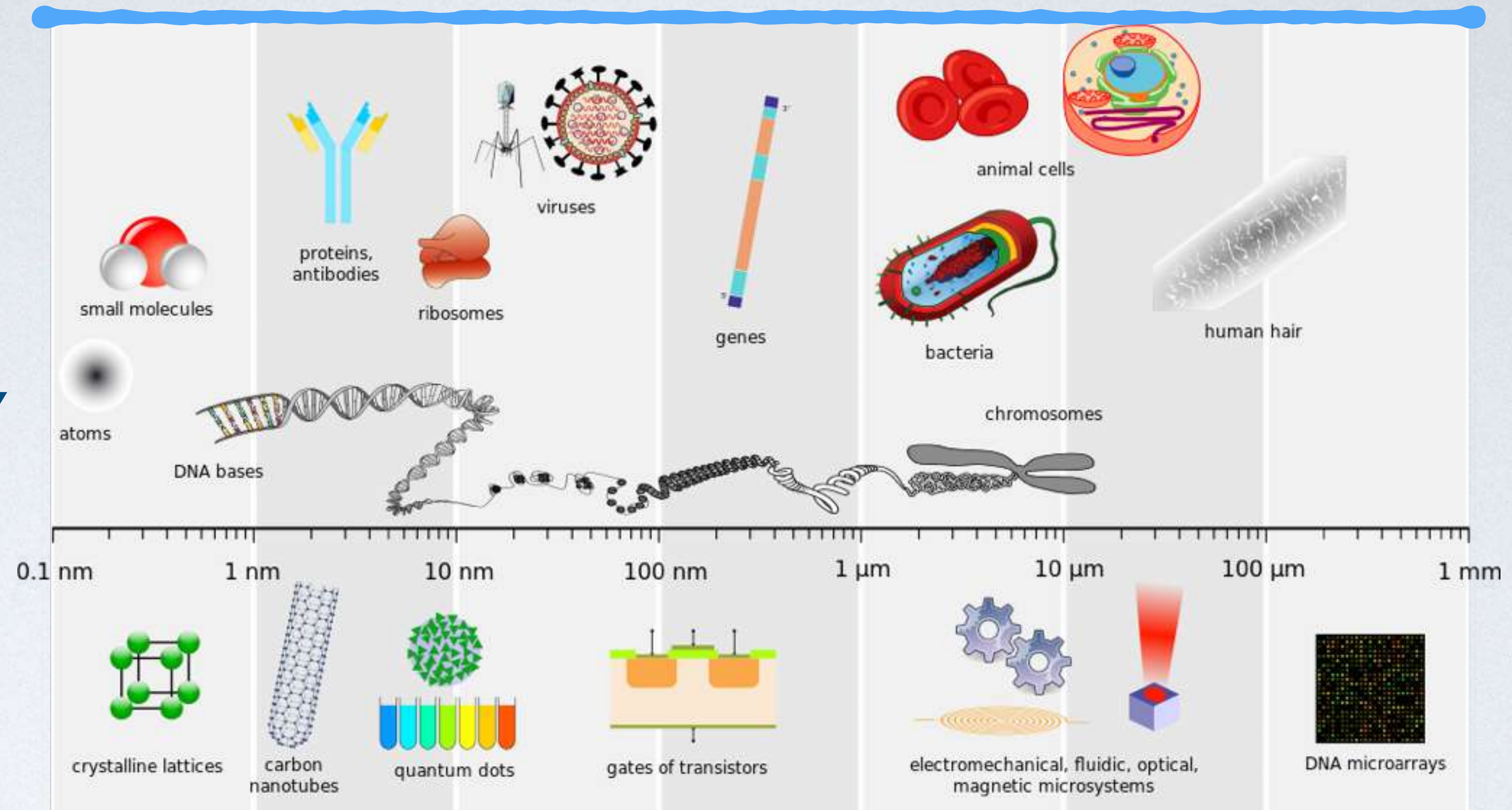


**coming soon**



# CRYOEM: SCALE WITHIN BIOLOGY

## Electron Microscopy



[https://en.wikipedia.org/wiki/Nanoscope\\_scale](https://en.wikipedia.org/wiki/Nanoscope_scale)

X-ray

NMR

AFM

Light microscopy

Naked eye



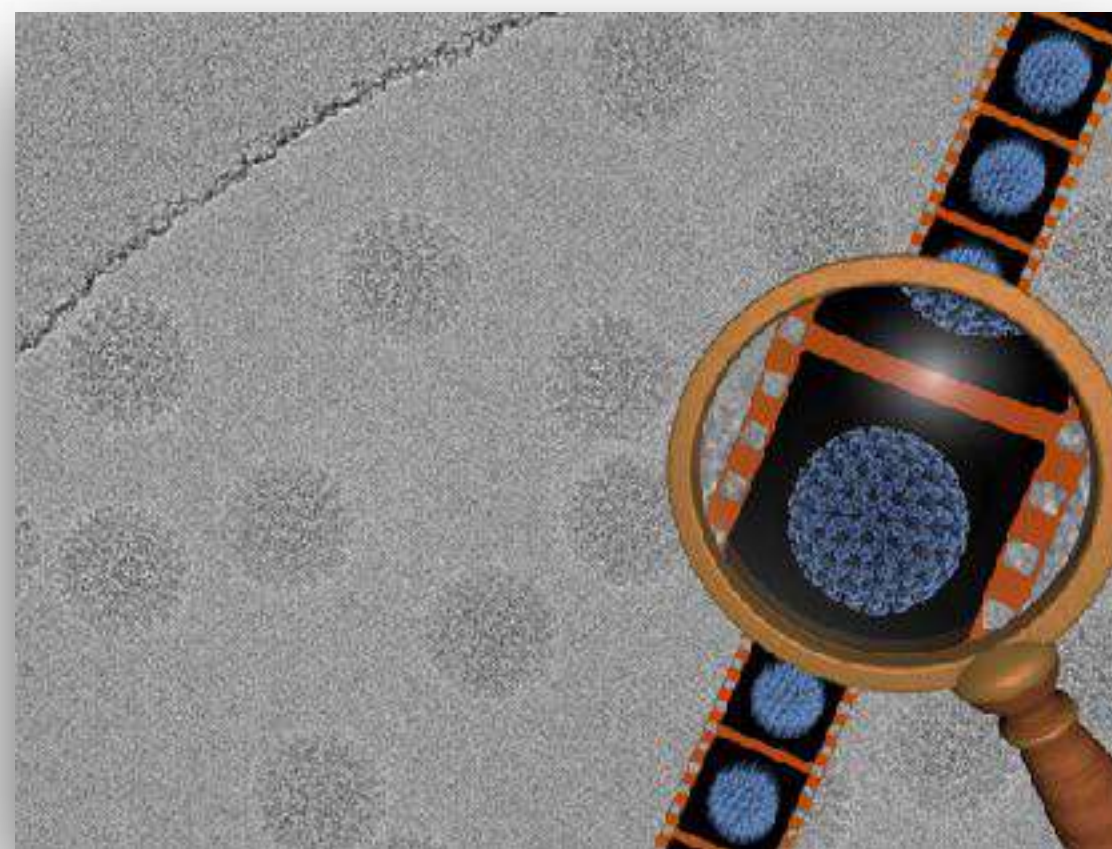
# WHAT BROUGHT ABOUT THE RESOLUTION REVOLUTION

(~2012-2014)

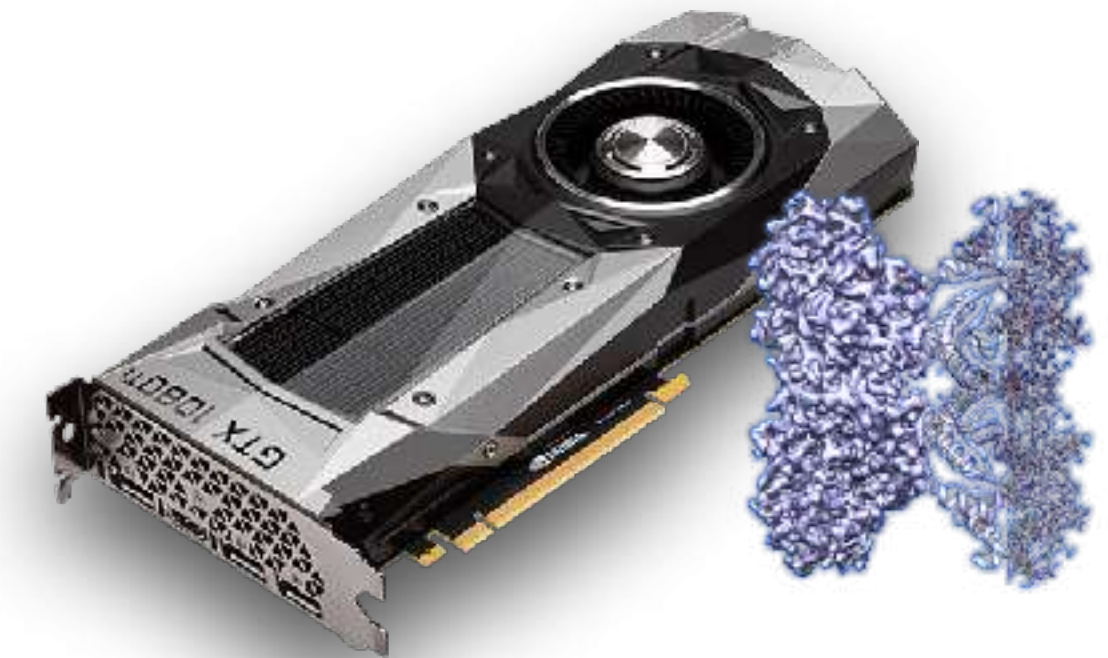
Microscopes



Direct Detectors



Computers



from 2012 to 2017  
Cost reduced by 100x



# THE ELECTRON MICROSCOPE

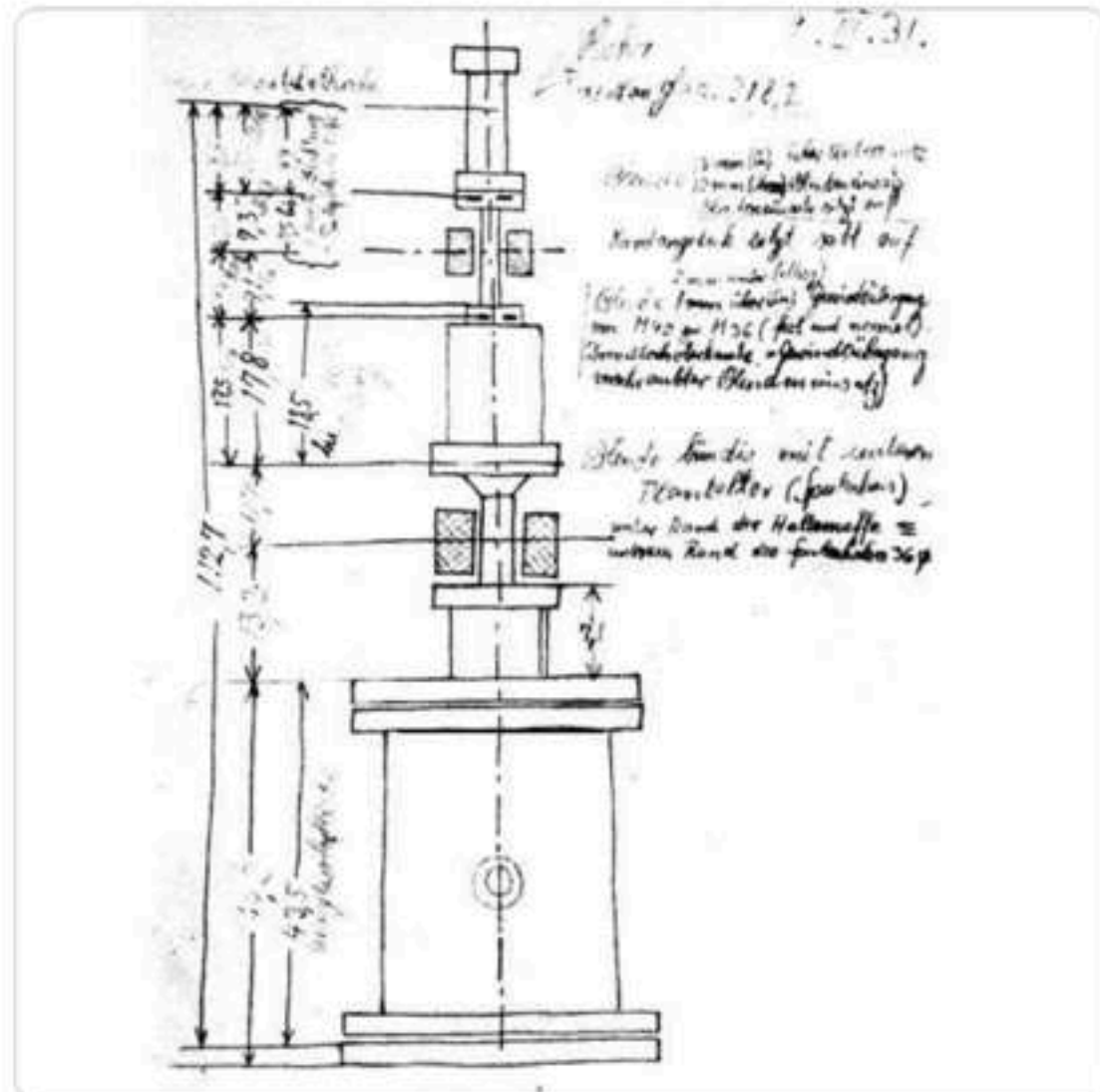
Ruska and Knoll in Berlin in the early 1930s

-Wikipedia



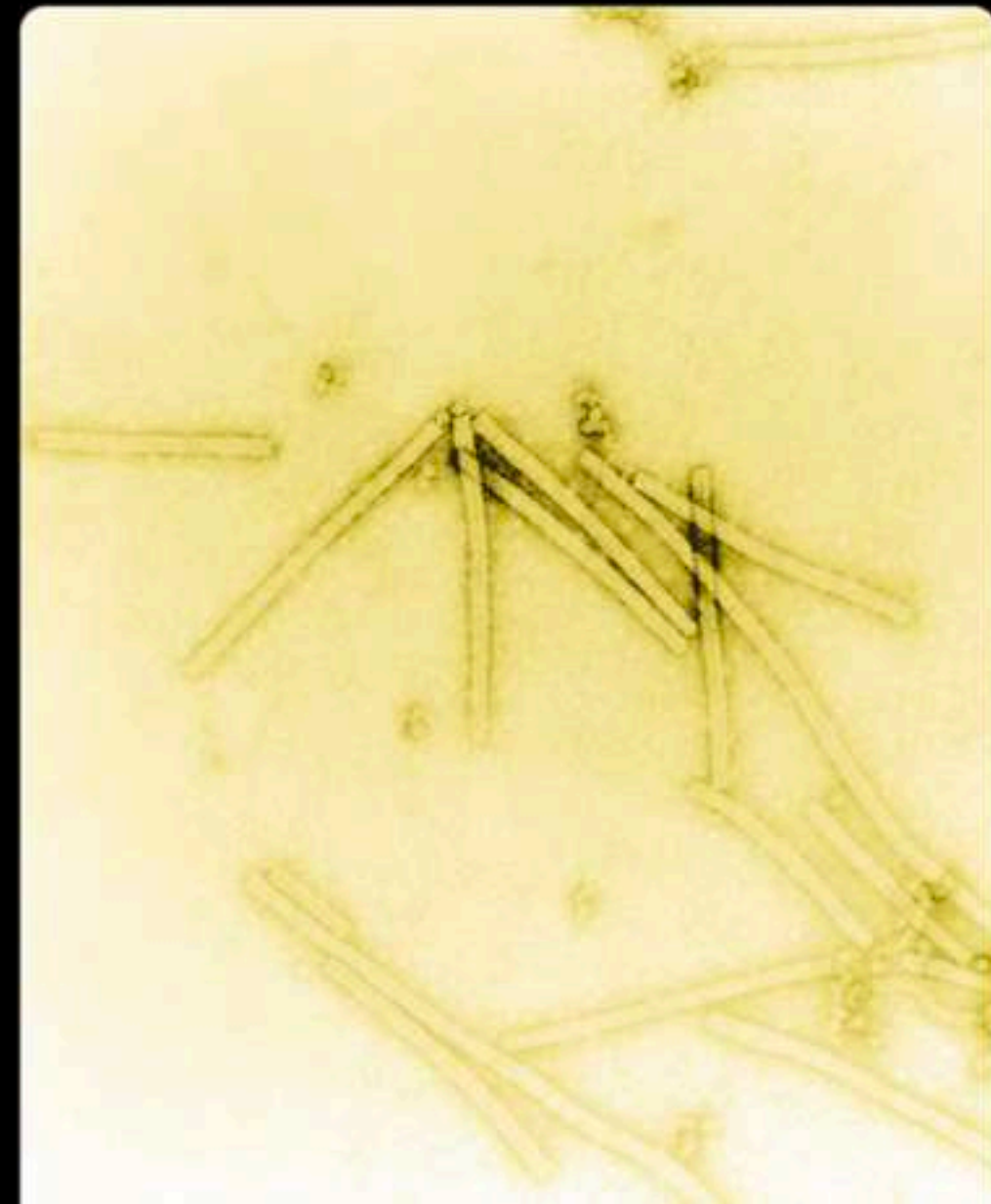
The Nobel Prize  
@NobelPrize

Take a look at a sketch by physics laureate Ernst Ruska, dated 9 March 1931, of the cathode ray tube for testing one-stage and two-stage electron-optical imaging by means of two magnetic electron lenses (electron microscope). Ruska was awarded the 1986 physics prize for his work.



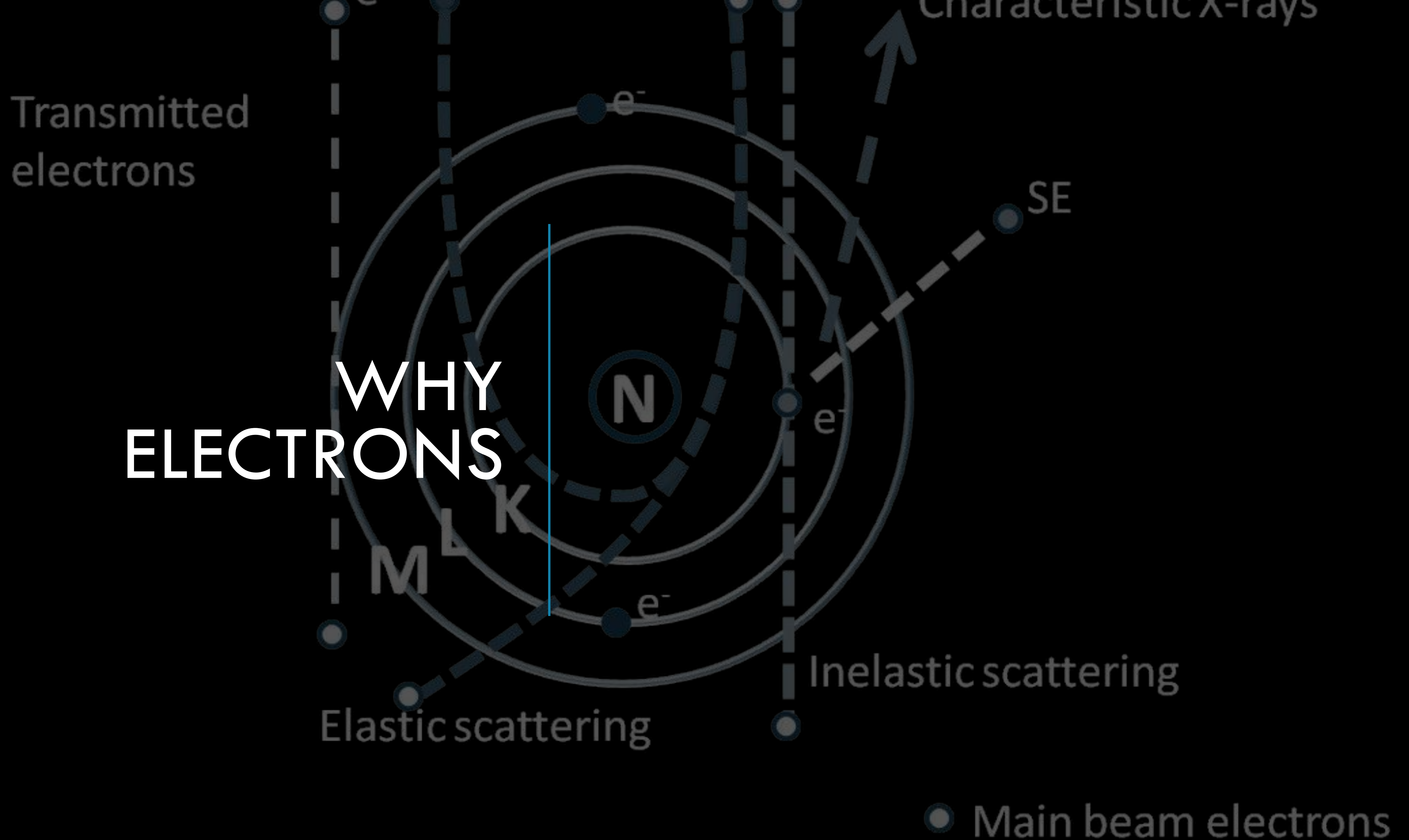
The Nobel Prize  
@NobelPrize

The electron microscope, invented by Ernst Ruska and Max Knoll in 1933, made it possible to take pictures of objects that previously could not be seen, like viruses. The tobacco mosaic virus, shown here magnified 160,000 times was the first virus captured on film.





# WHY ELECTRONS





# WHY ELECTRONS

## Pros

Small wavelength

Can be focused

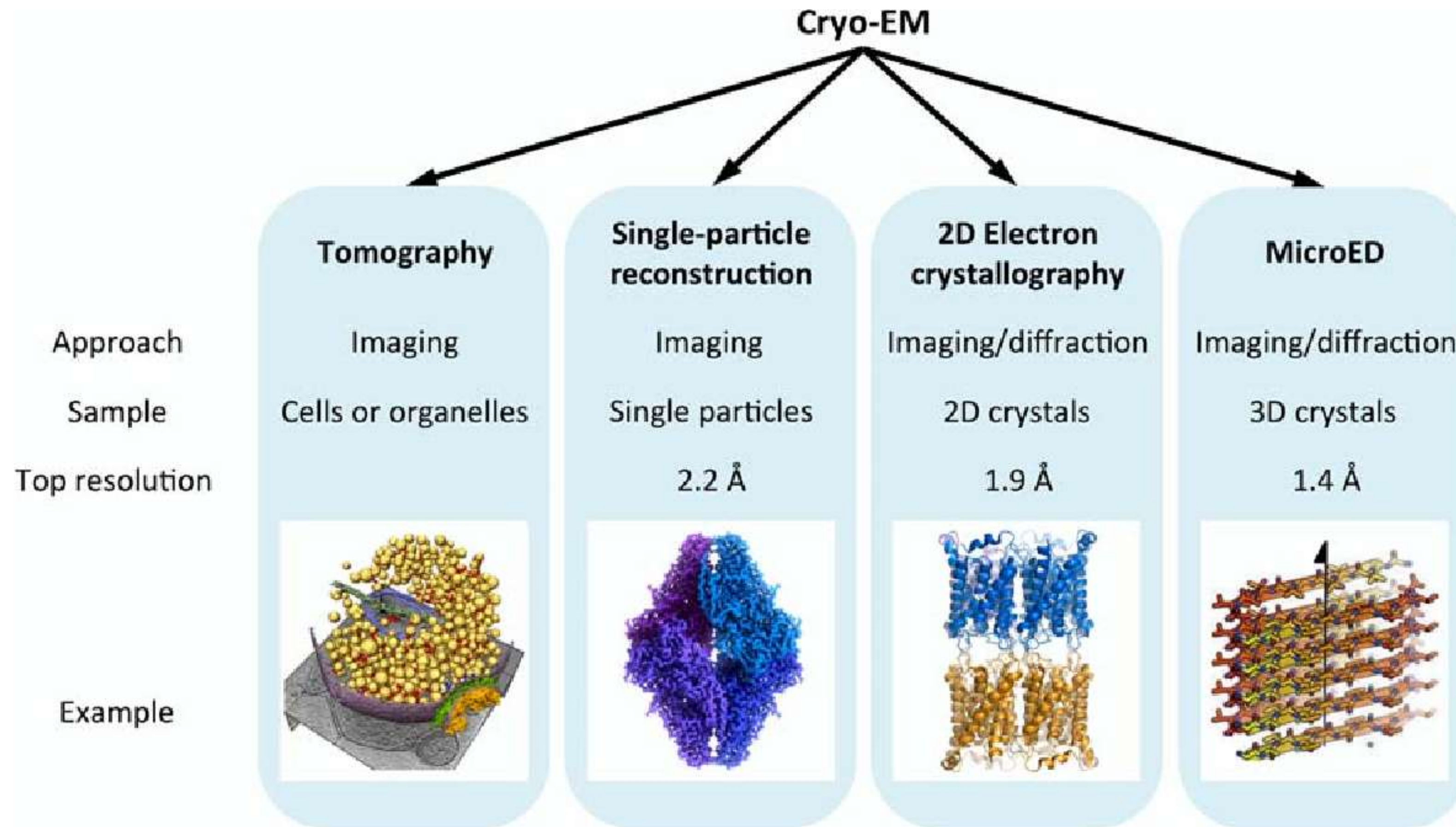
## Cons

Damages sample  
worse with faster electrons

Poor penetration  
better with faster electrons

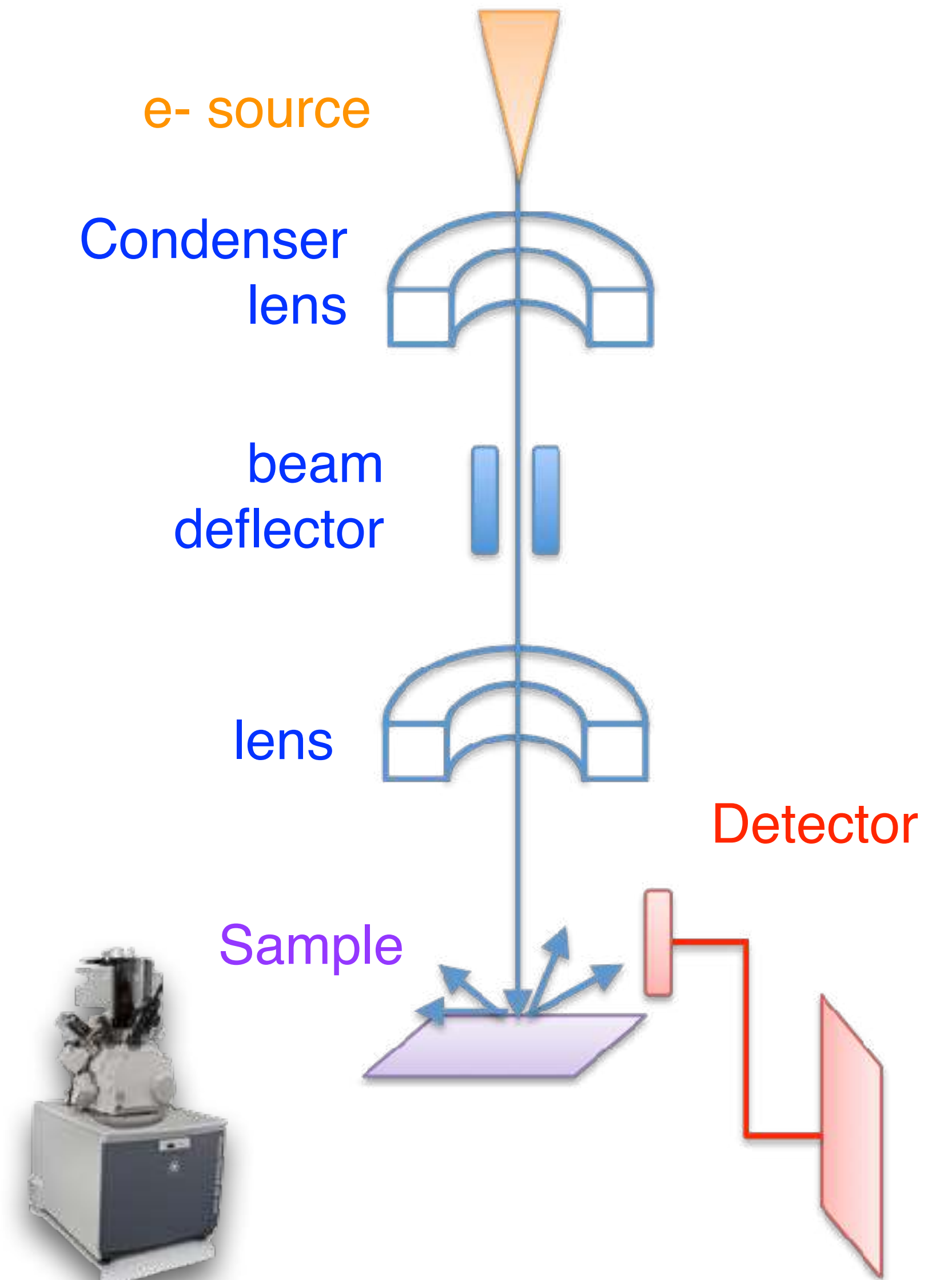
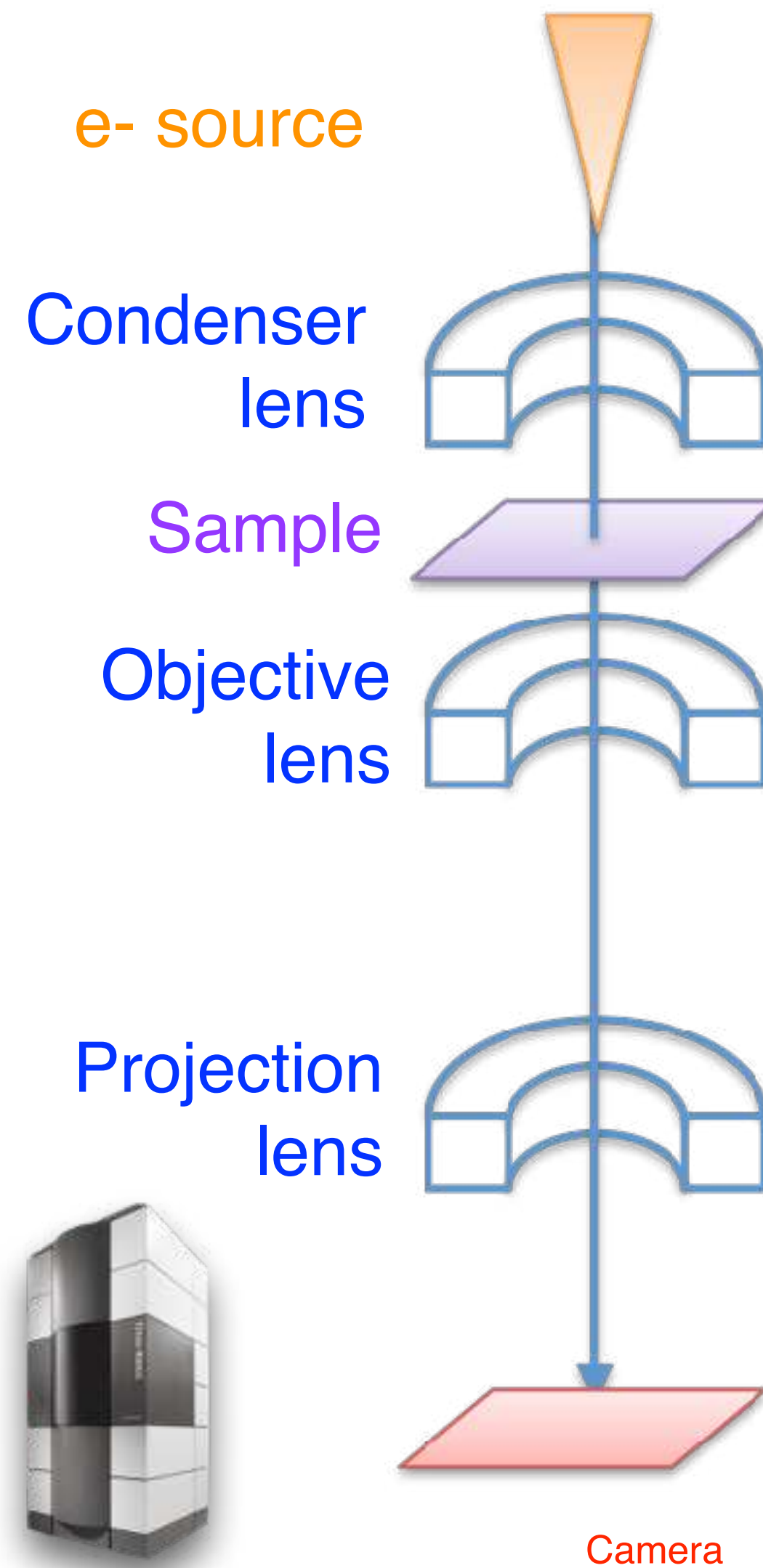
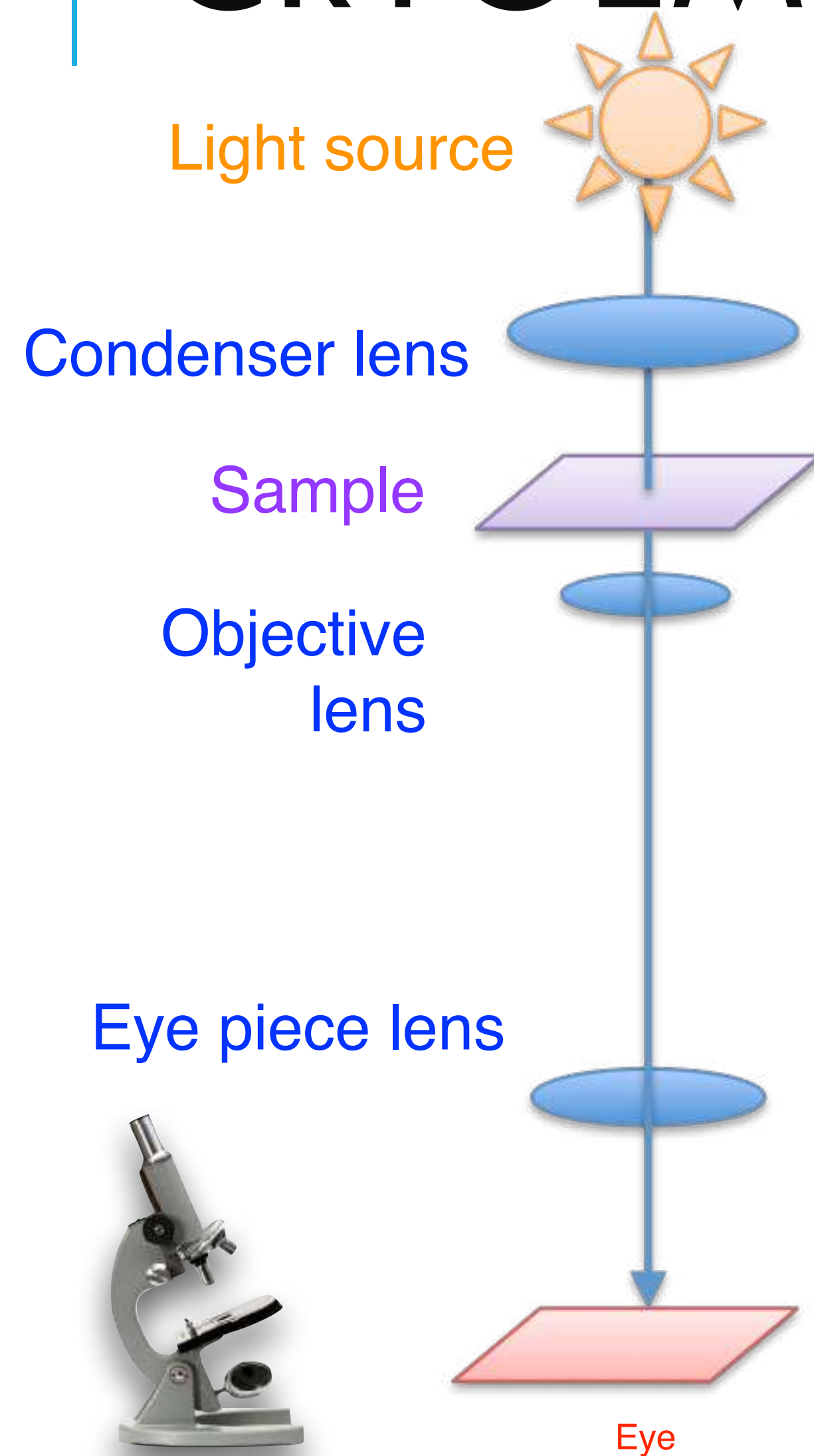


# CRYOEM MODALITIES AND TOOLS



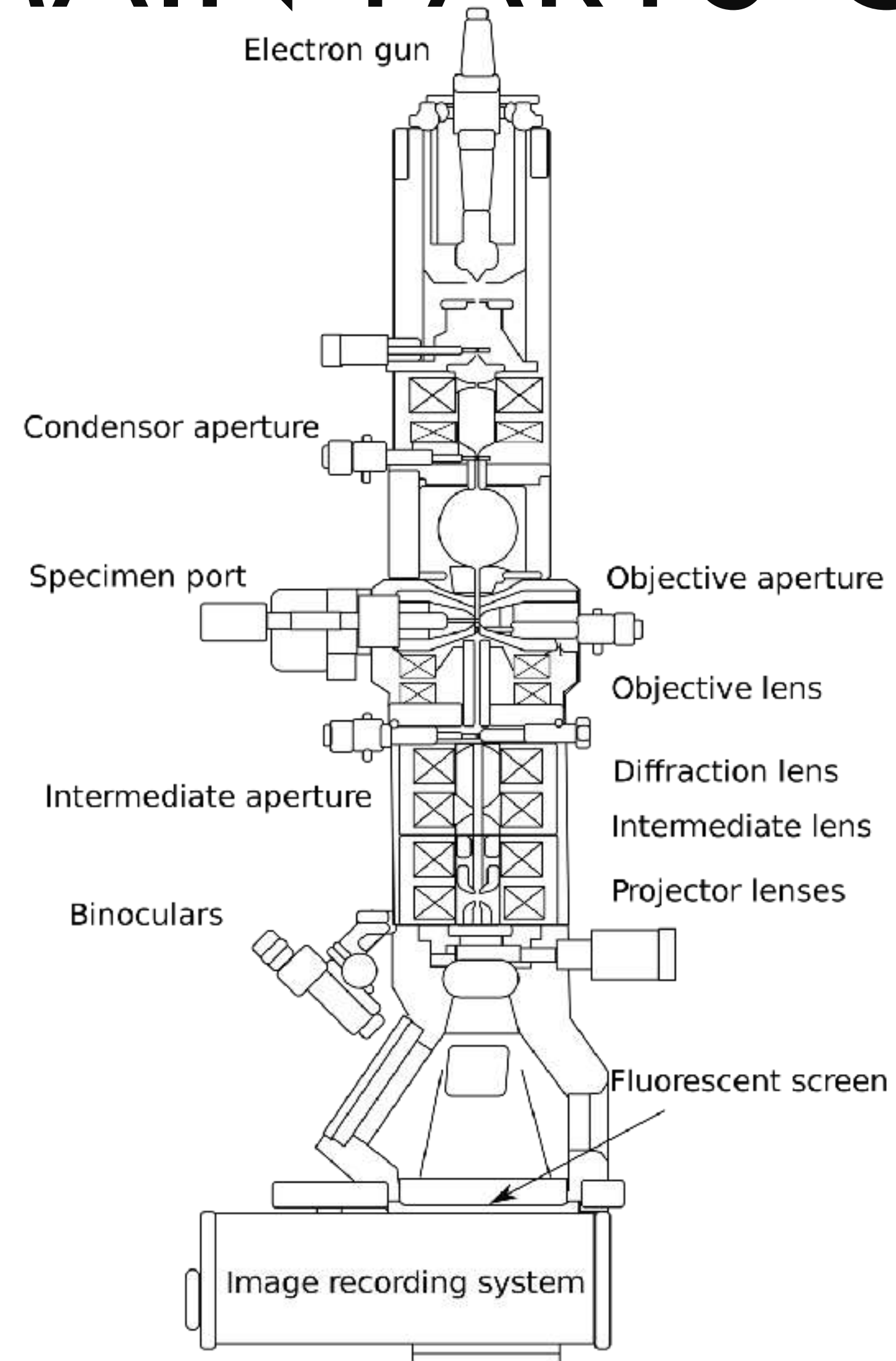


# CRYOEM TOOLS





# MAIN PARTS OF AN EM



**Electron sources**



**Vacuum systems**



**Lenses**



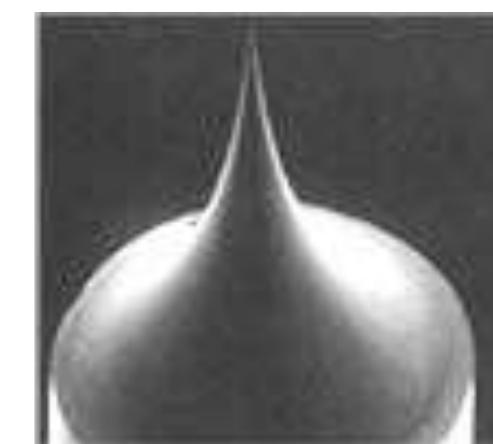
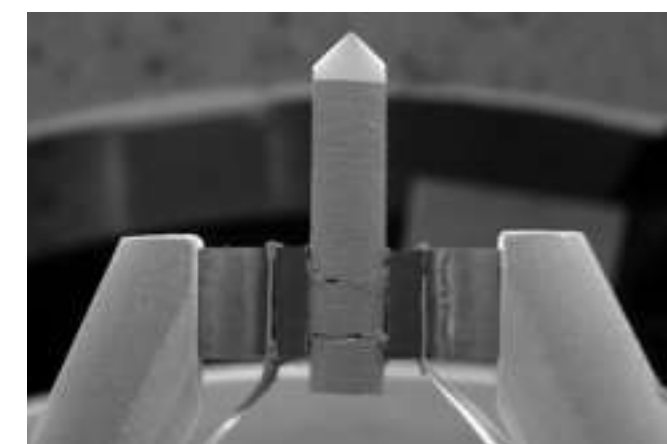
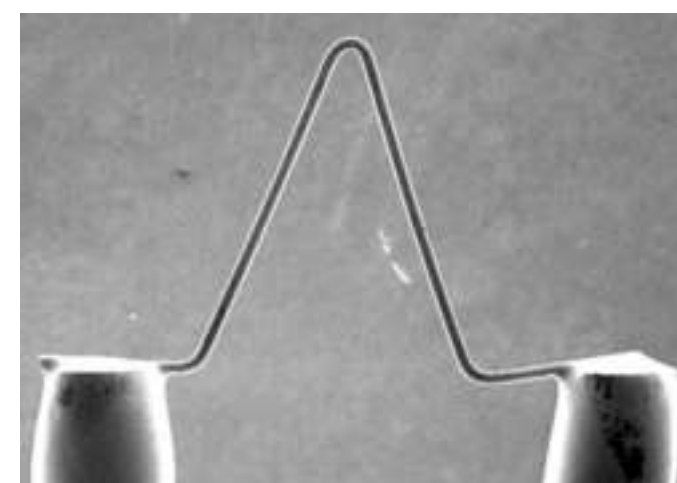
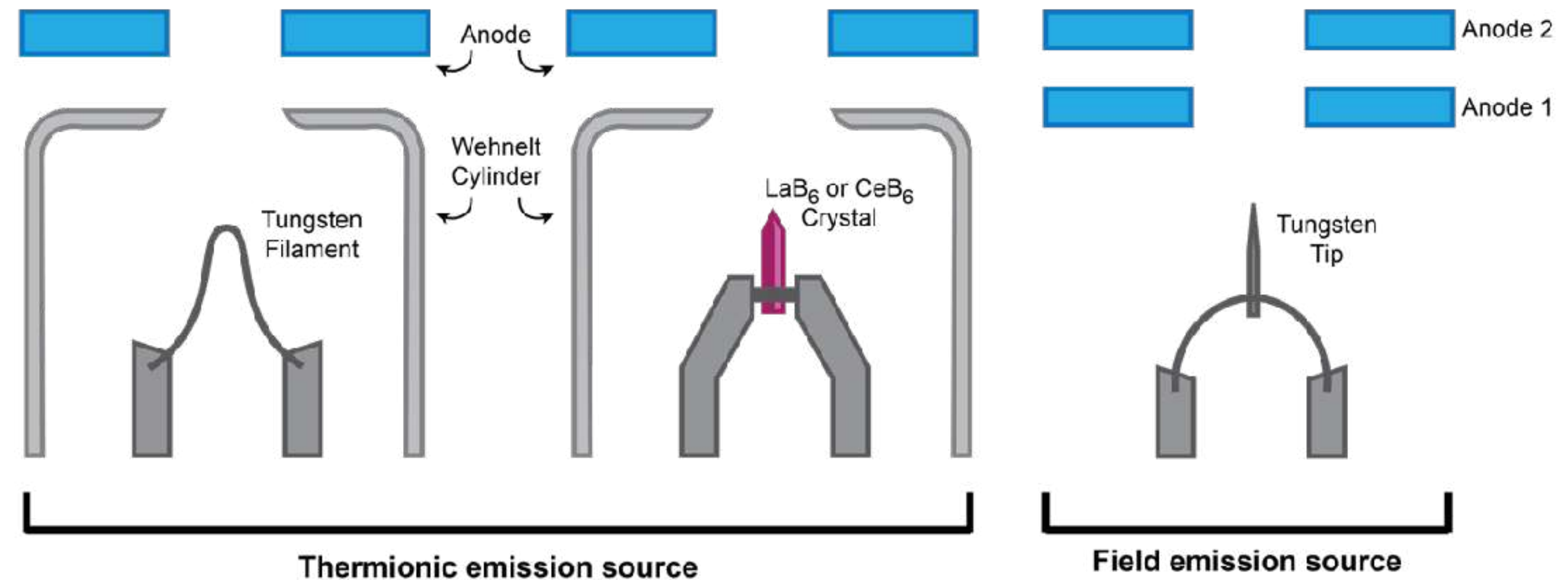
**Detectors**





# ELECTRON SOURCES

What are the 3 main kinds of electron sources?

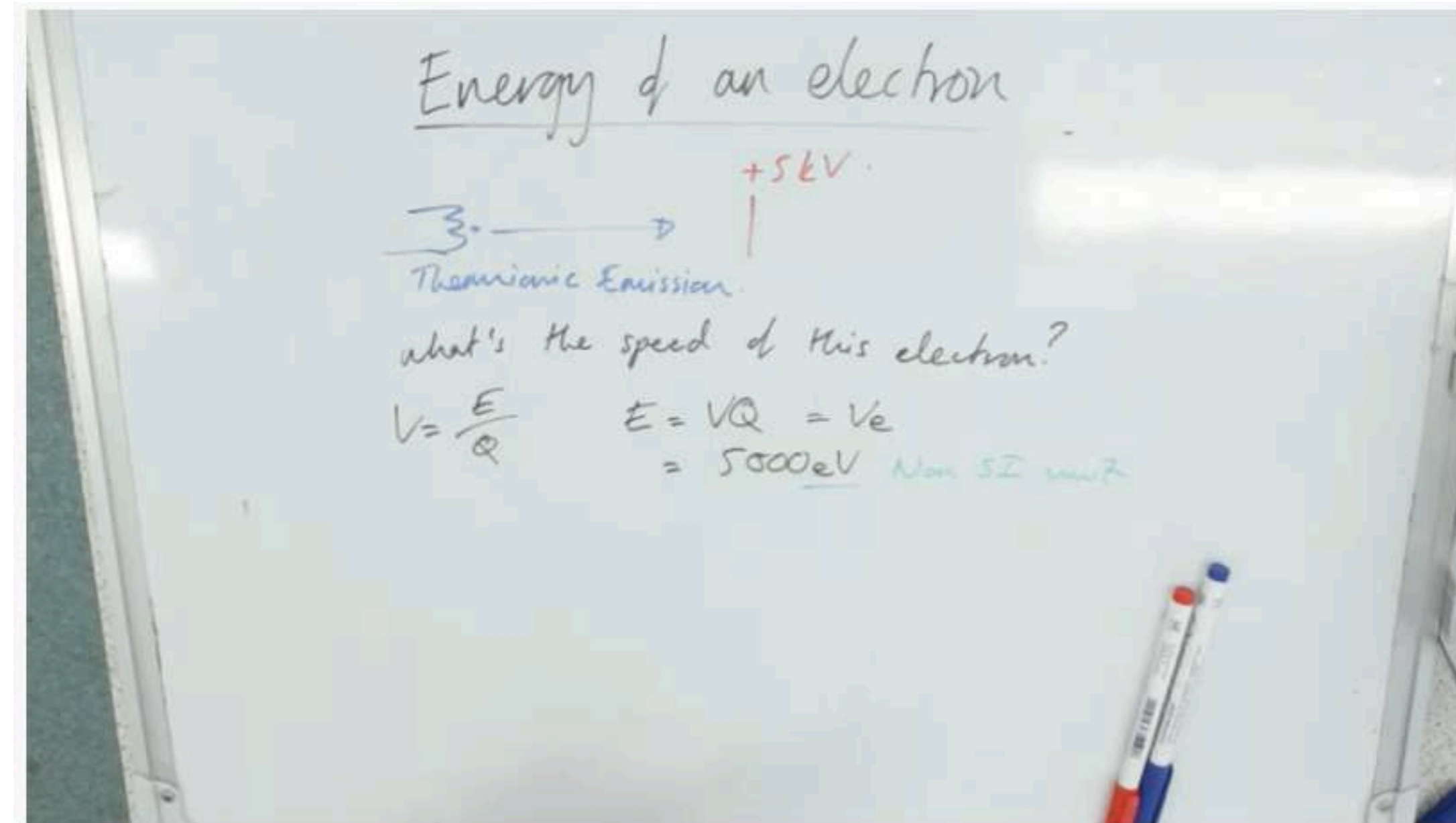
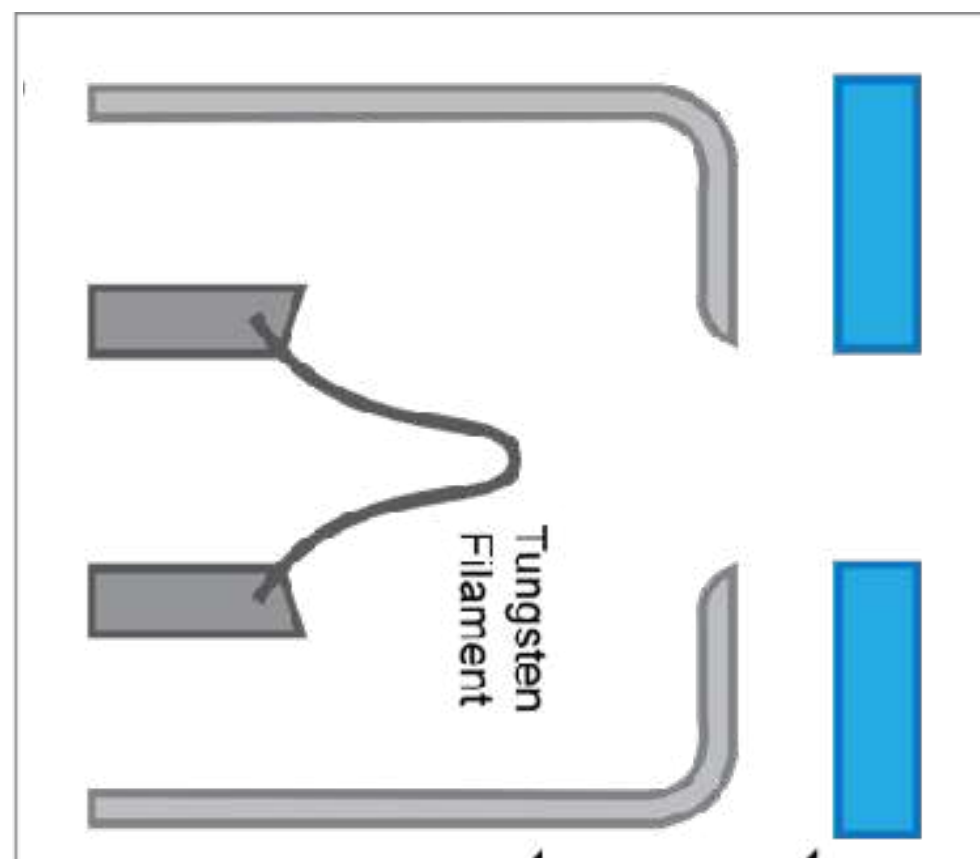






# ELECTRON SOURCES

How fast are the electrons moving?



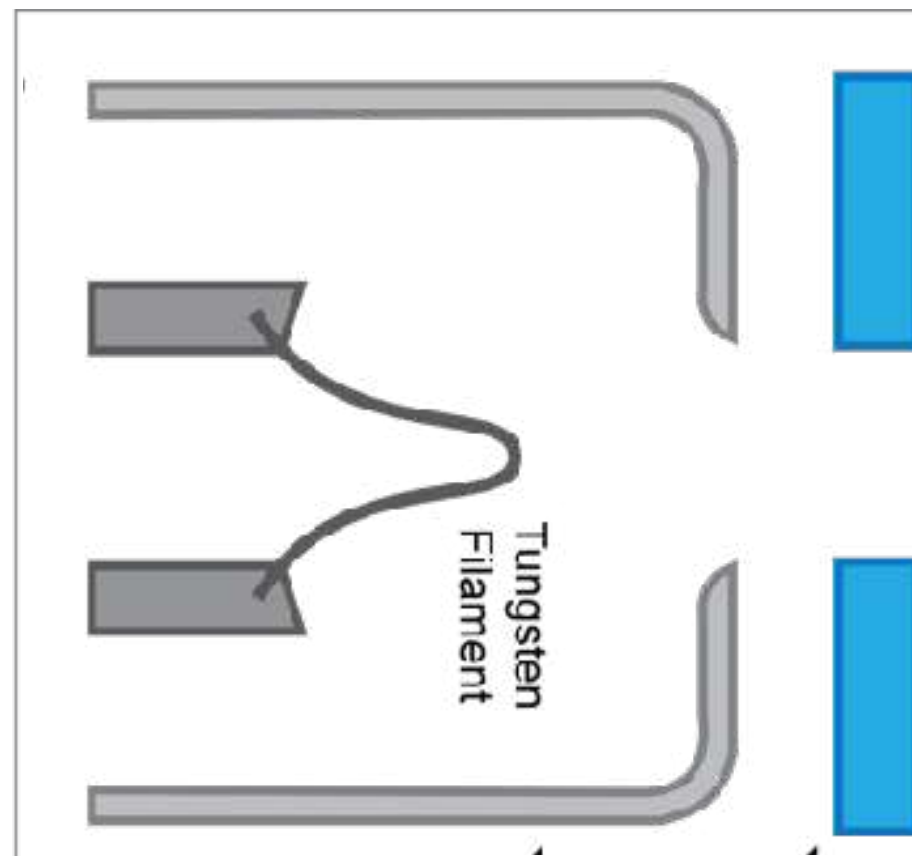
<https://www.youtube.com/watch?v=tYCET6vYdYk>





# ELECTRON SOURCES

How fast are the electrons moving?



Energy of an electron

$+5kV$

Thermionic Emission

what's the speed of this electron?

$$V = \frac{E}{Q} \quad E = VQ = Ve$$
$$= 5000eV \quad \text{Non SI unit}$$
$$E = 5000 \times 1.6 \times 10^{-19} = 8 \times 10^{-16} J$$
$$E_k = \frac{1}{2}mv^2 \quad m_e = 9.11 \times 10^{-31} kg$$
$$8 \times 10^{-16} = \frac{1}{2}mv^2$$

Energy of an electron

$+5kV$

Thermionic Emission

what's the speed of this electron?

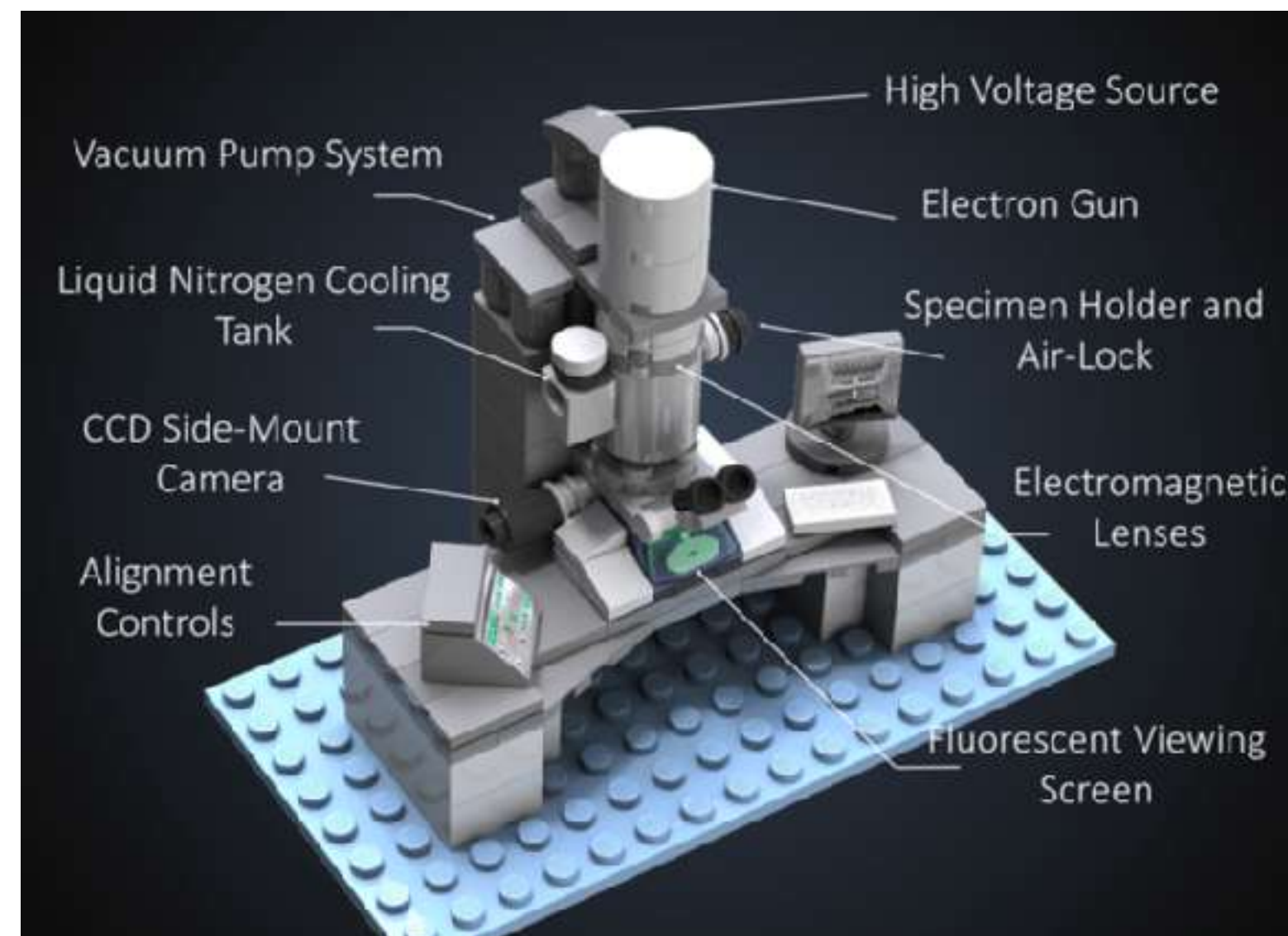
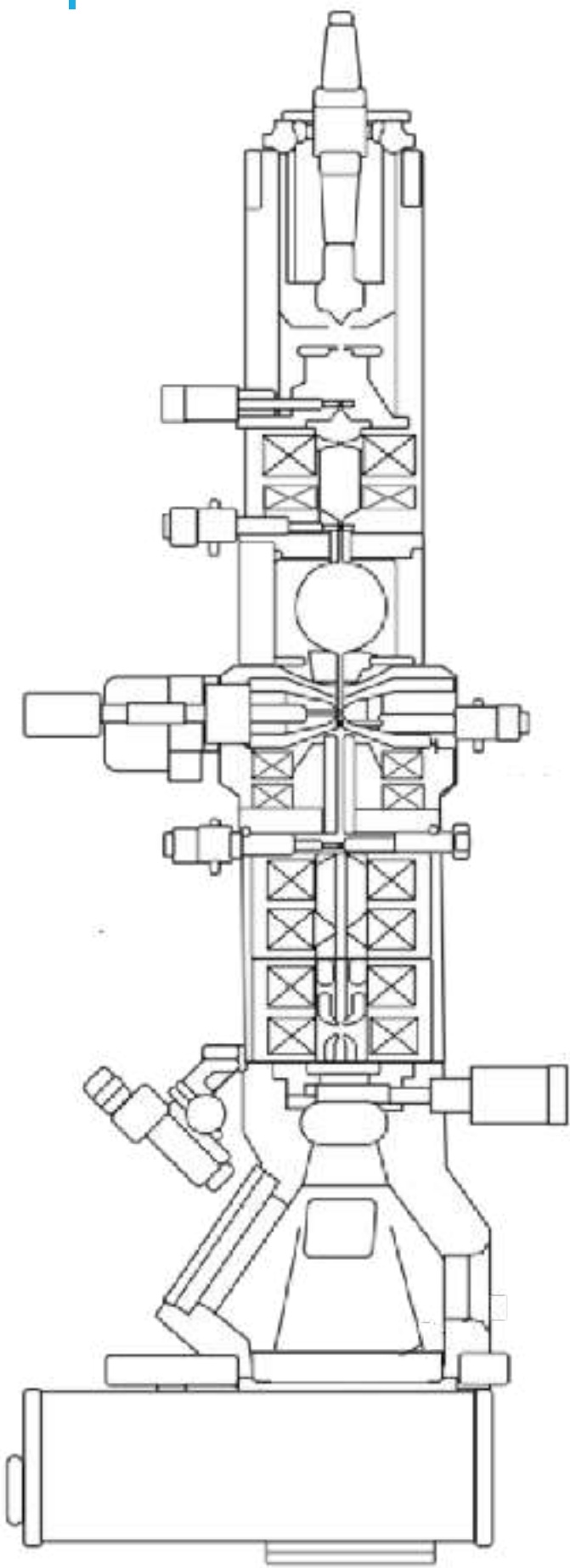
$$E = VQ = Ve$$
$$= 5000eV \quad \text{Non SI unit}$$
$$\times 1.6 \times 10^{-19} = 8 \times 10^{-16} J$$
$$8 \times 10^{-16} = \frac{1}{2}mv^2 \quad m_e = 9.11 \times 10^{-31} kg$$
$$\frac{2 \times 8 \times 10^{-16}}{9.11 \times 10^{-31}} = 41900000$$
$$= 4.2 \times 10^7 m/s$$

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

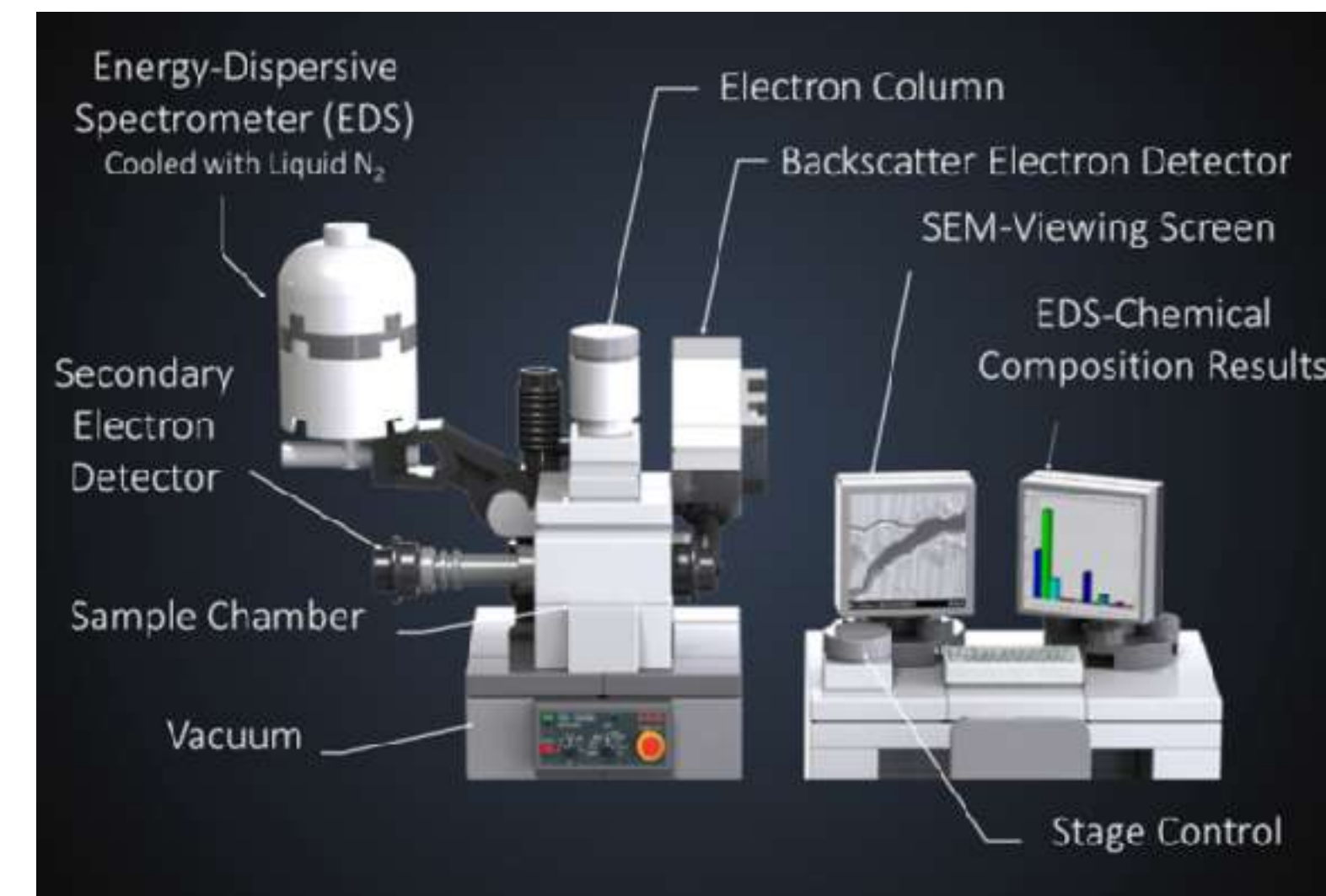




# ELECTRON SOURCES & TYPES OF EMS



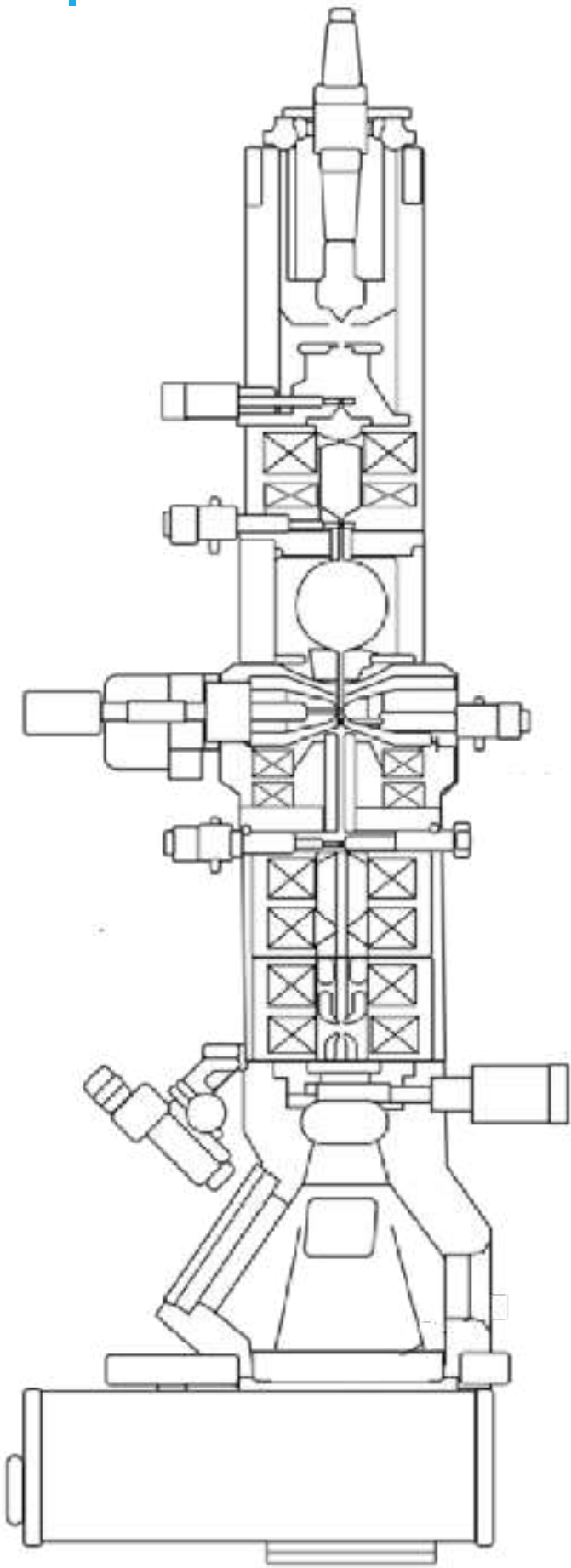
<https://ideas.lego.com/projects/102281>







# ELECTRON SOURCES & TYPES OF EMS



**80-120 kV:** JEM 1230, JEM1400; Spirit, Tecnai T12, Talos L120 W or LaB6

High contrast & robust  
sub-nm resolution



*100 kV: screening TEMs, Tundra*

*FEG*

*3-7 Å resolution*

**200 kV:** JEM 2100F, Tecnai F20, Talos, Glacios, Arctica FEG

2-5 Å resolution



**300 kV:** JEM 3200FSC, cryo-ARM, Krios, Polara FEG

Smaller effect on unwanted lens aberration  
1.5-3.5 Å resolution







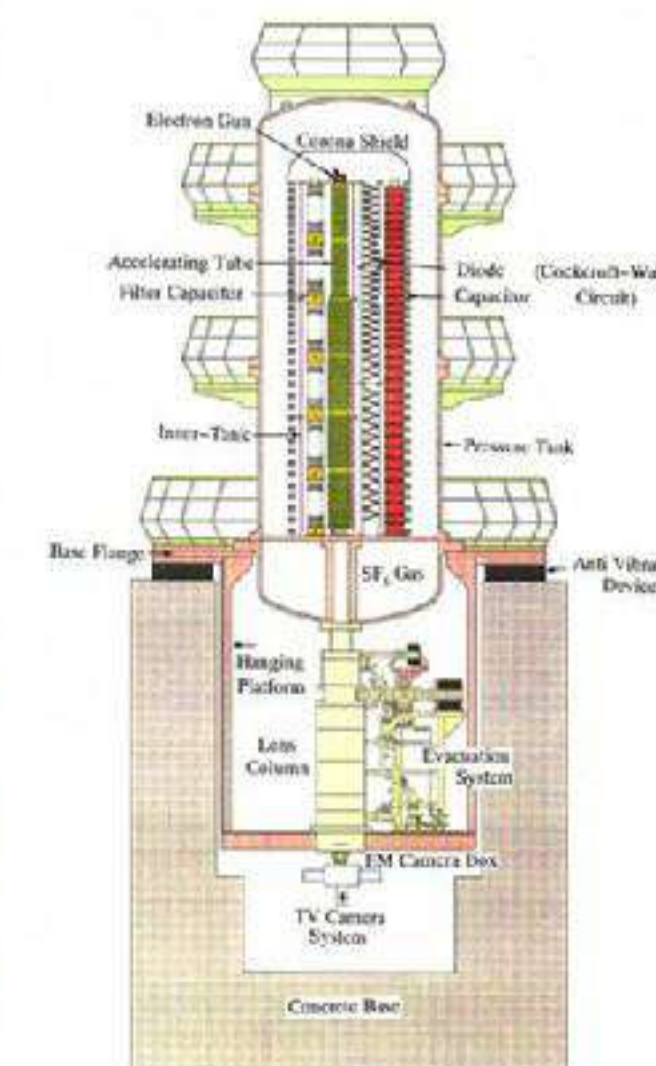
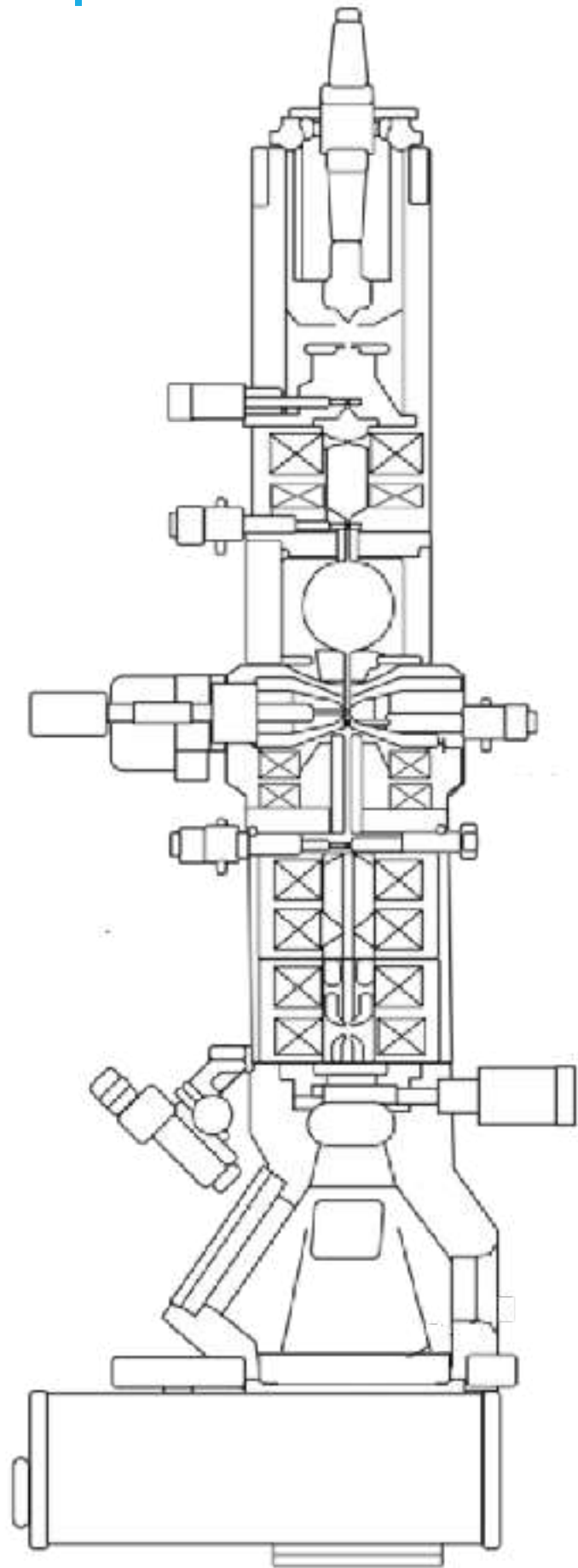
# ELECTRON SOURCES & TYPES OF EMS

**1-1.2 MV:** Hitachi, JEOL  
LaB6



[uhvem.osaka-u.ac.jp](http://uhvem.osaka-u.ac.jp)

**3 MV:** Hitachi H3000  
LaB6

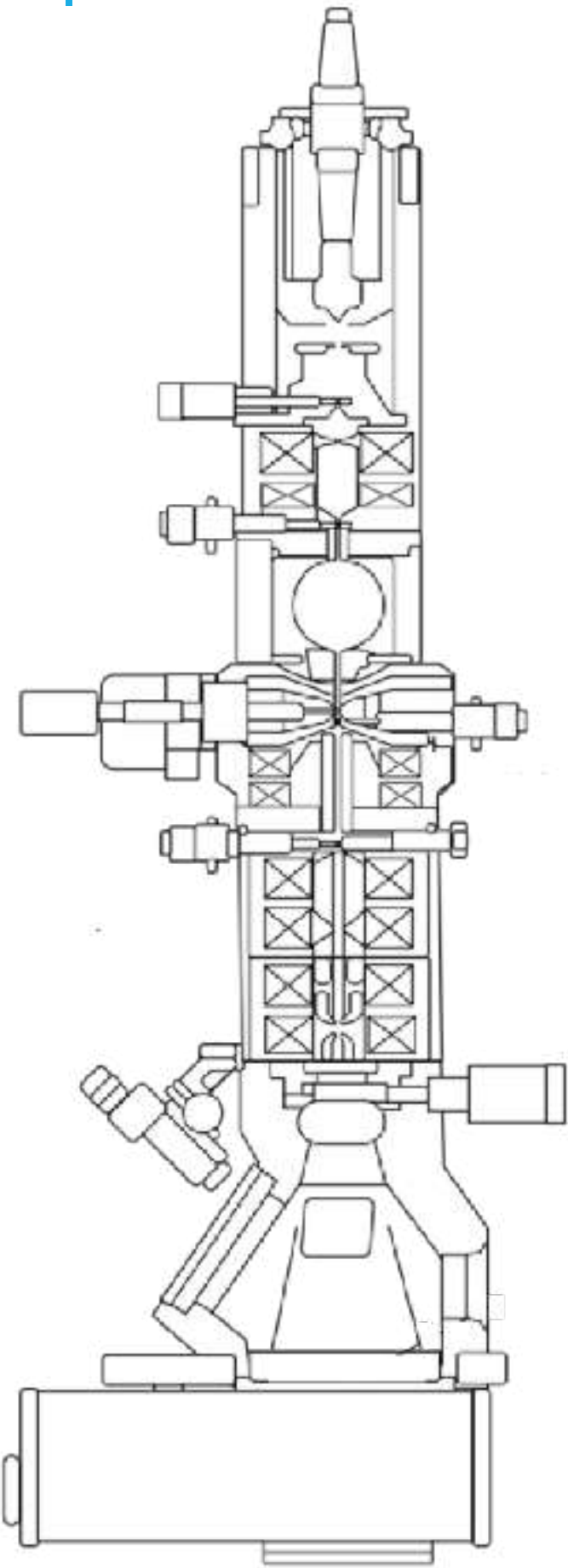






# VACUUM SYSTEMS

Why do we need a vacuum?



**Beam coherence** - at STP mean free path  $\sim 1$  cm

**Insulation** - interaction between e- and air

**Filament** - O<sub>2</sub> will burn out source

**Contamination** - reduce interaction gas, e-beam and sample





# VACUUM SYSTEMS

What types of pumps do we have?

1 mm Hg = 1 Torr =  $10^2$  Pa  
1 atm = 760 Torr =  $7.5 \times 10^4$  Pa

**PVP / Rotary**

1- $10^{-3}$  Torr |  $>0.1$  Pa

**Diffusion**

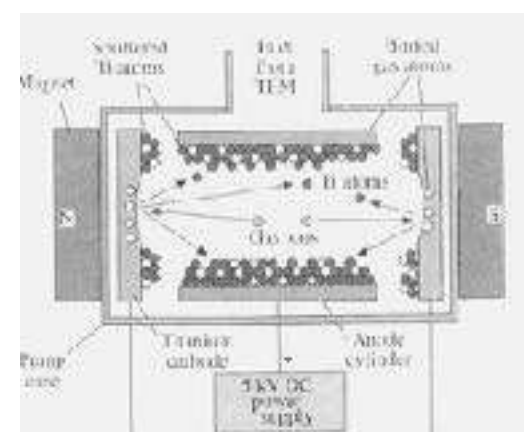
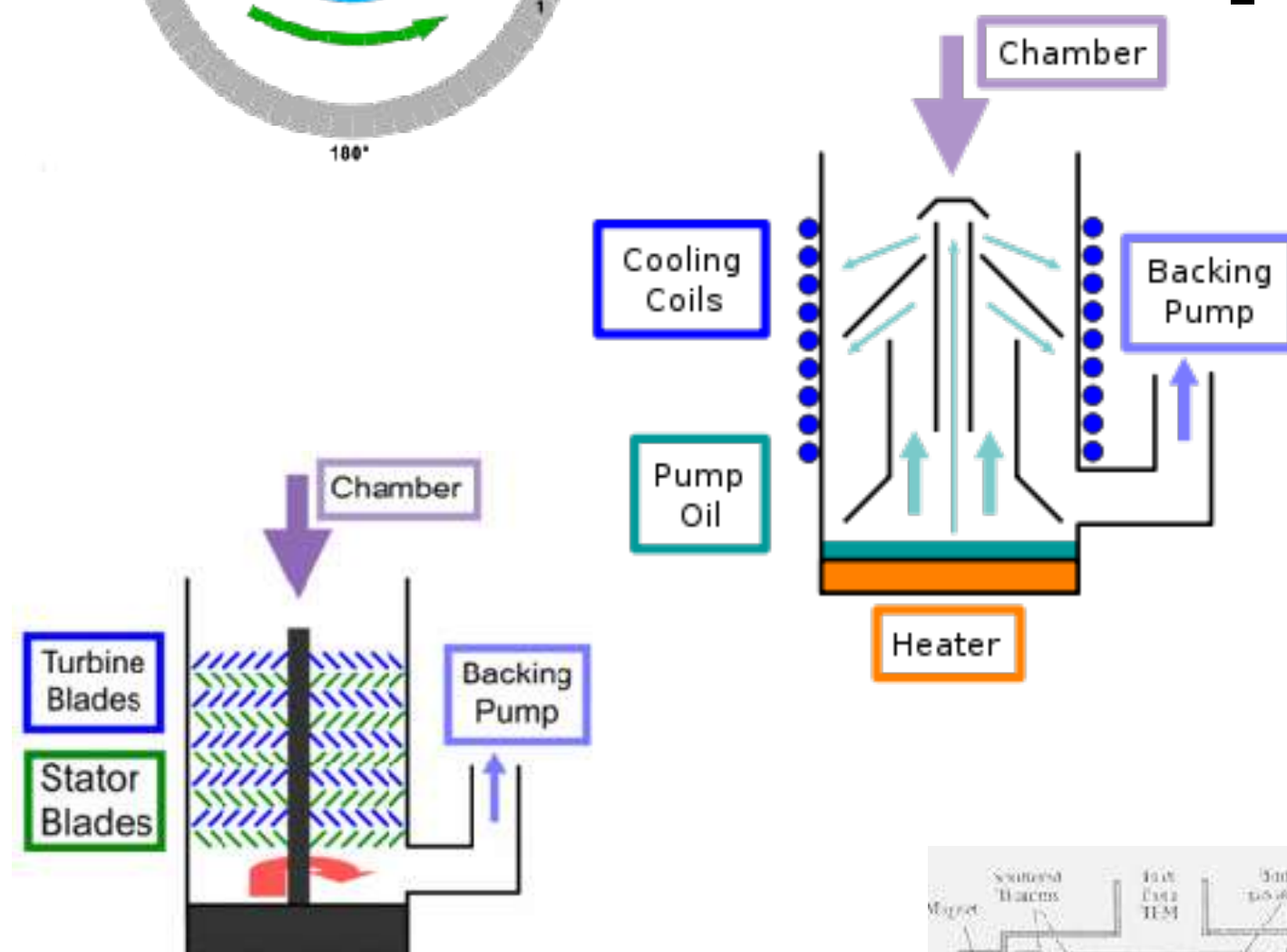
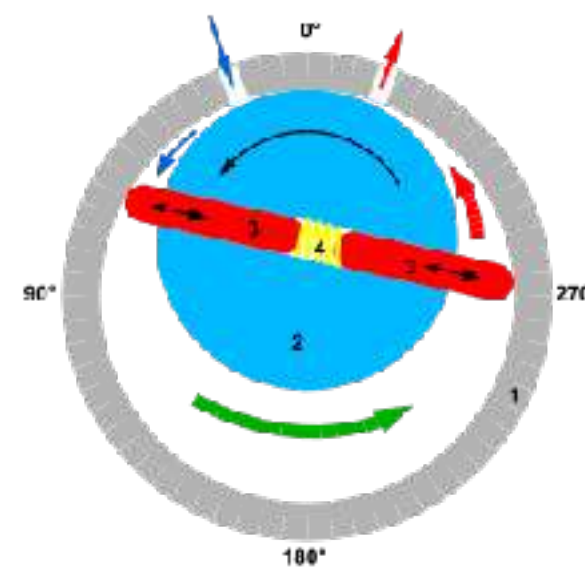
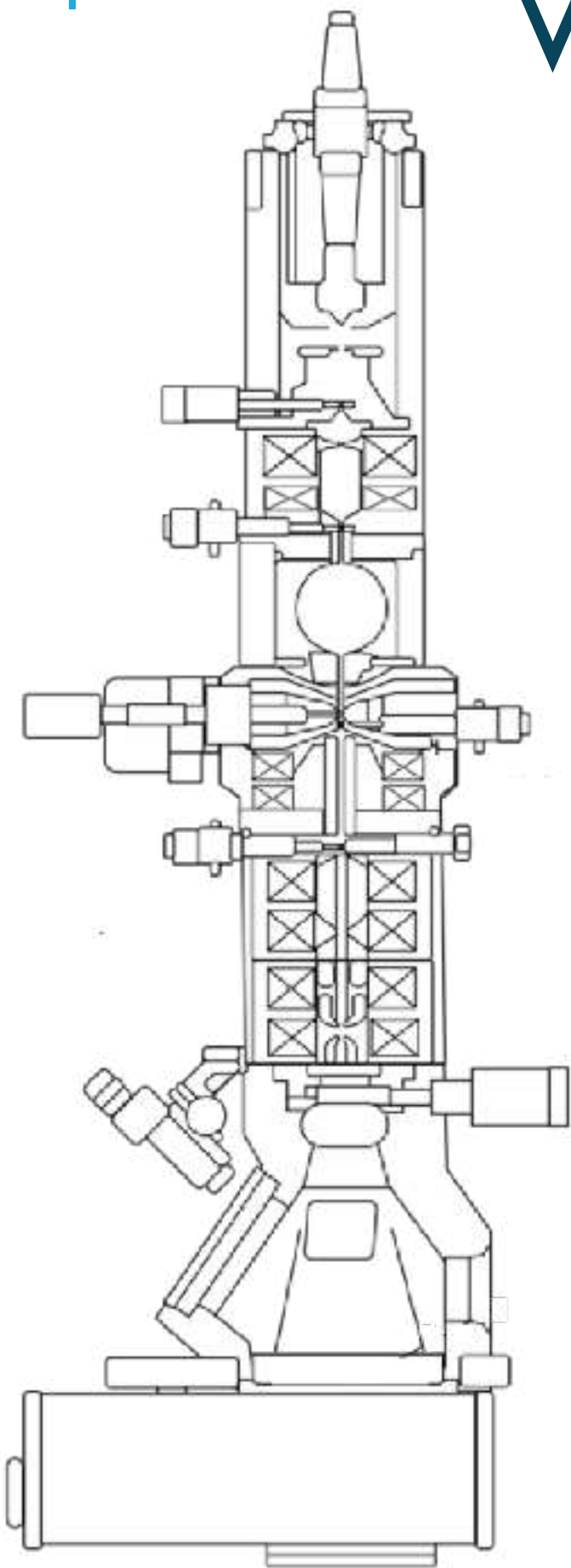
$10^{-3}$  -  $10^{-6}$  Torr |  $0.1$  -  $10^{-4}$  Pa

**Turbo**

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

**IGP**

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



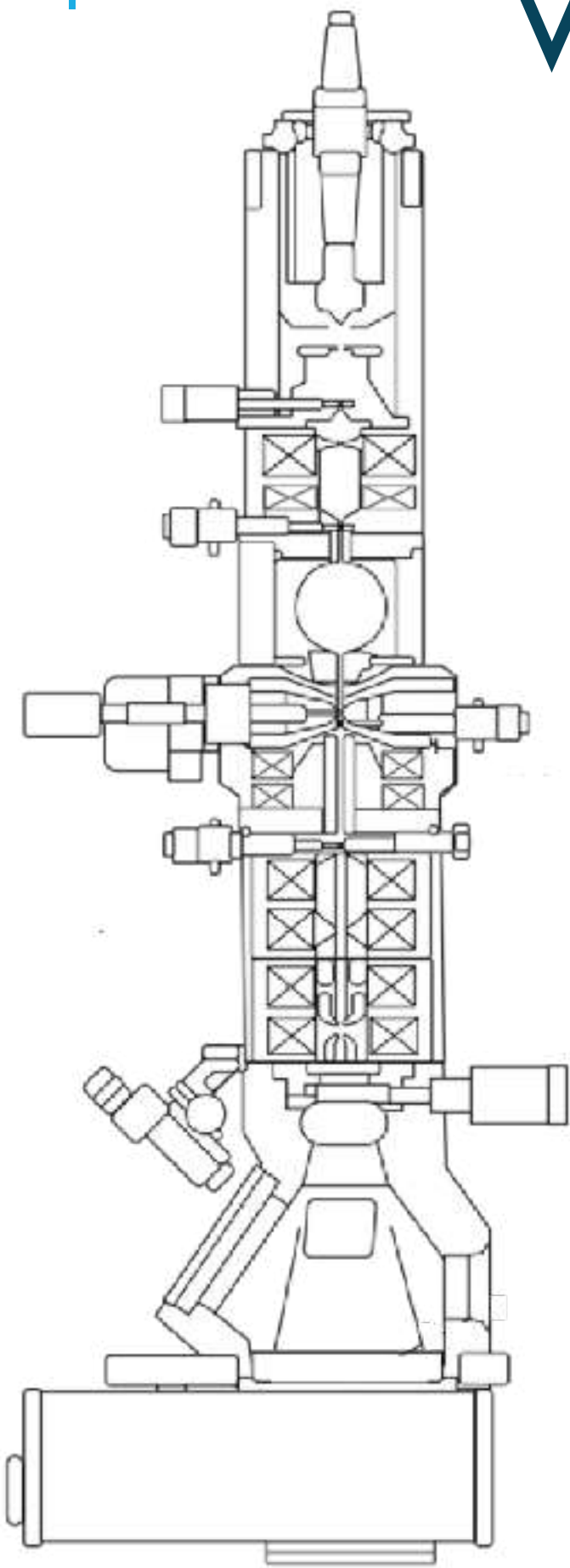
wikipedia.com





# VACUUM SYSTEMS

What types of pumps do we have?

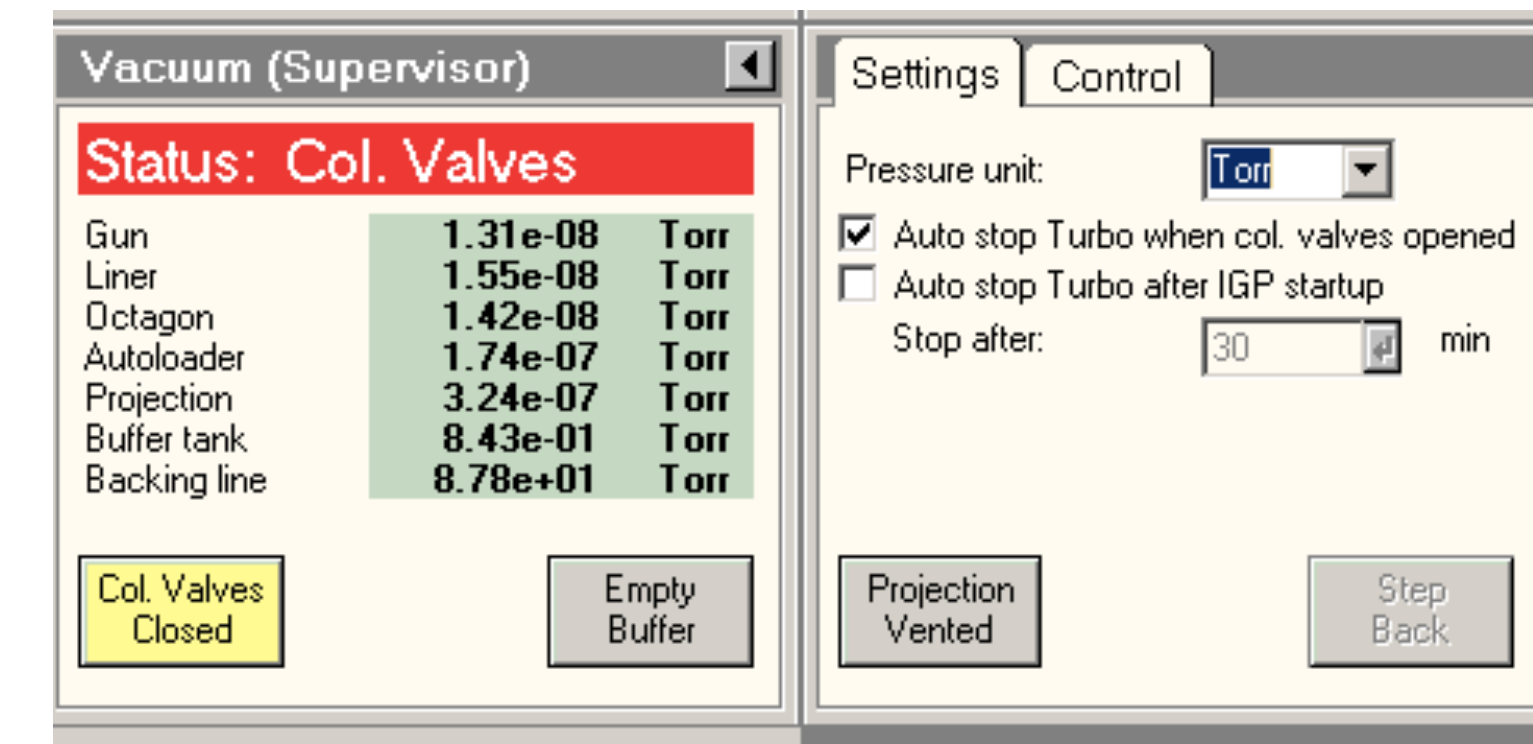
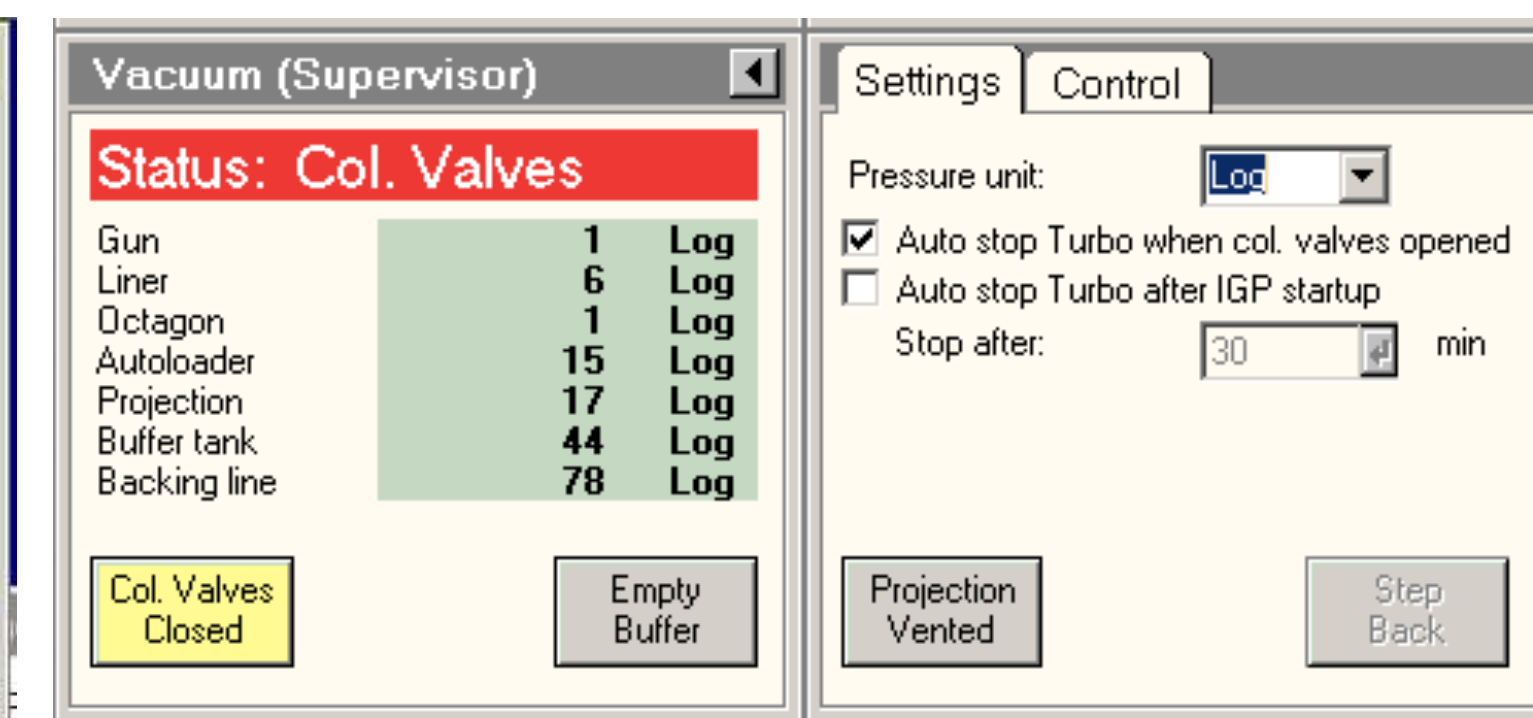
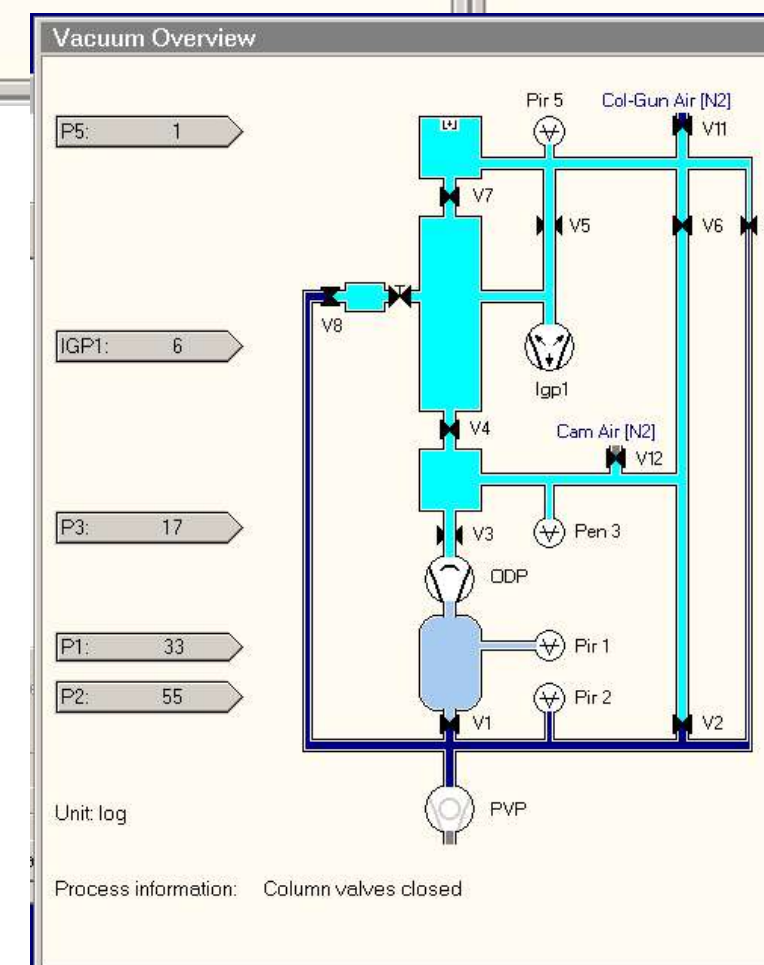
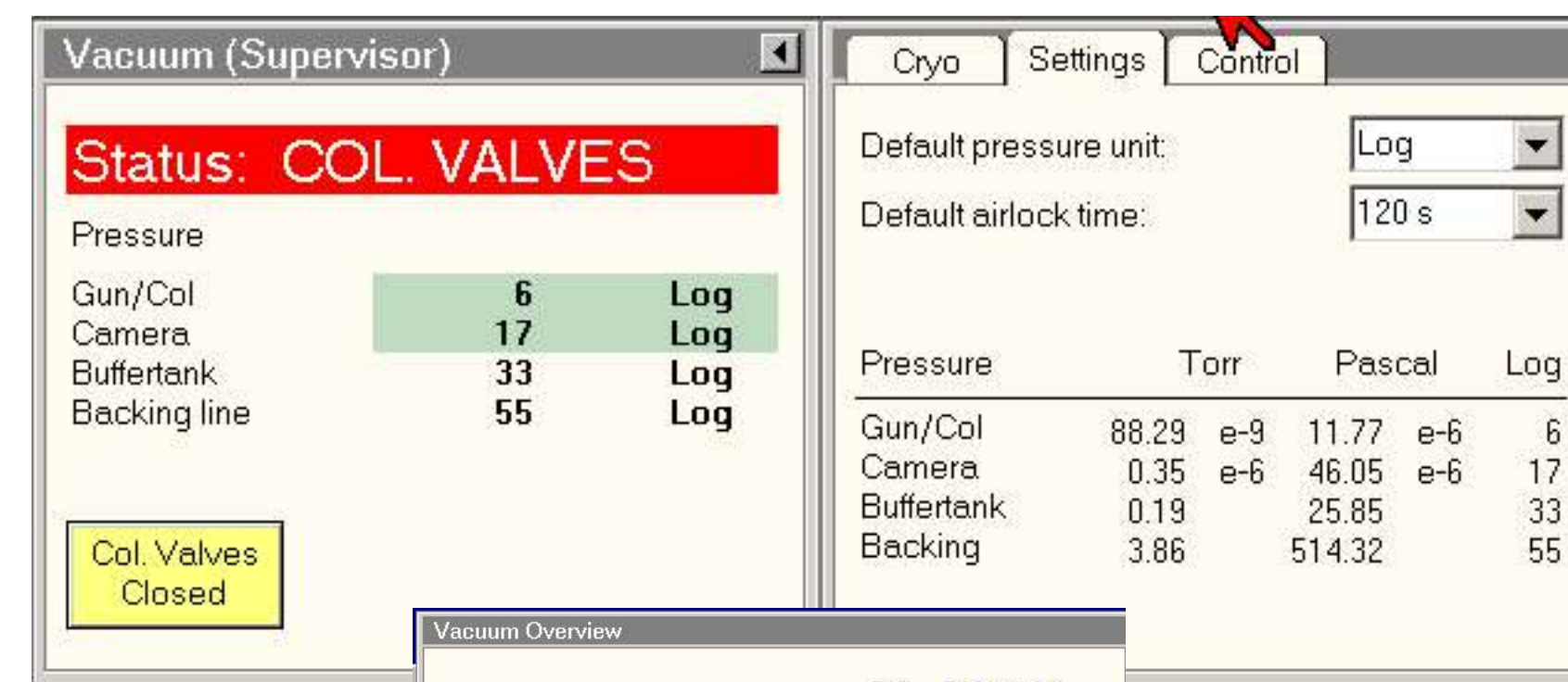


Gun  
 $10^{-9}$  Torr

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

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

$1 \text{ mm Hg} = 1 \text{ Torr} = 10^2 \text{ Pa}$   
 $1 \text{ atm} = 760 \text{ Torr} = 7.5 \times 10^4 \text{ Pa}$

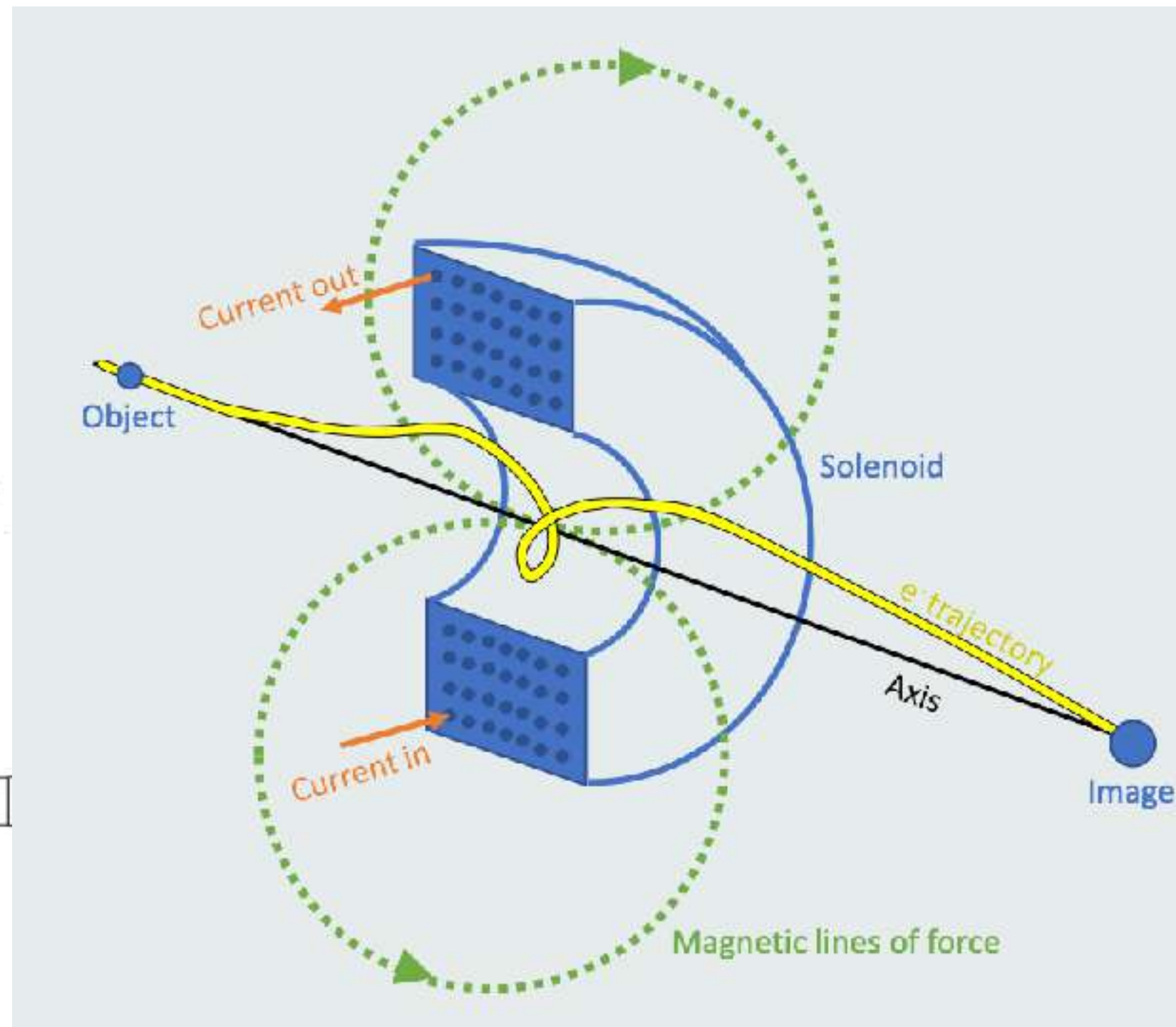
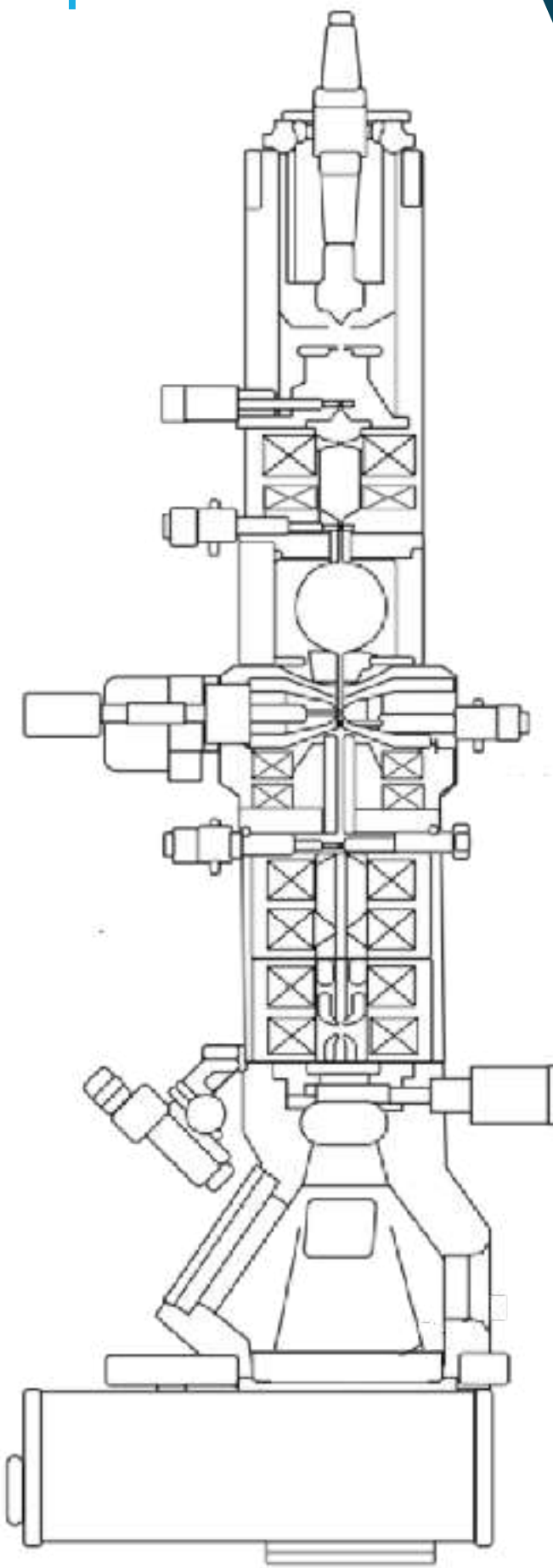






# LENSES

What types of lenses do we have?



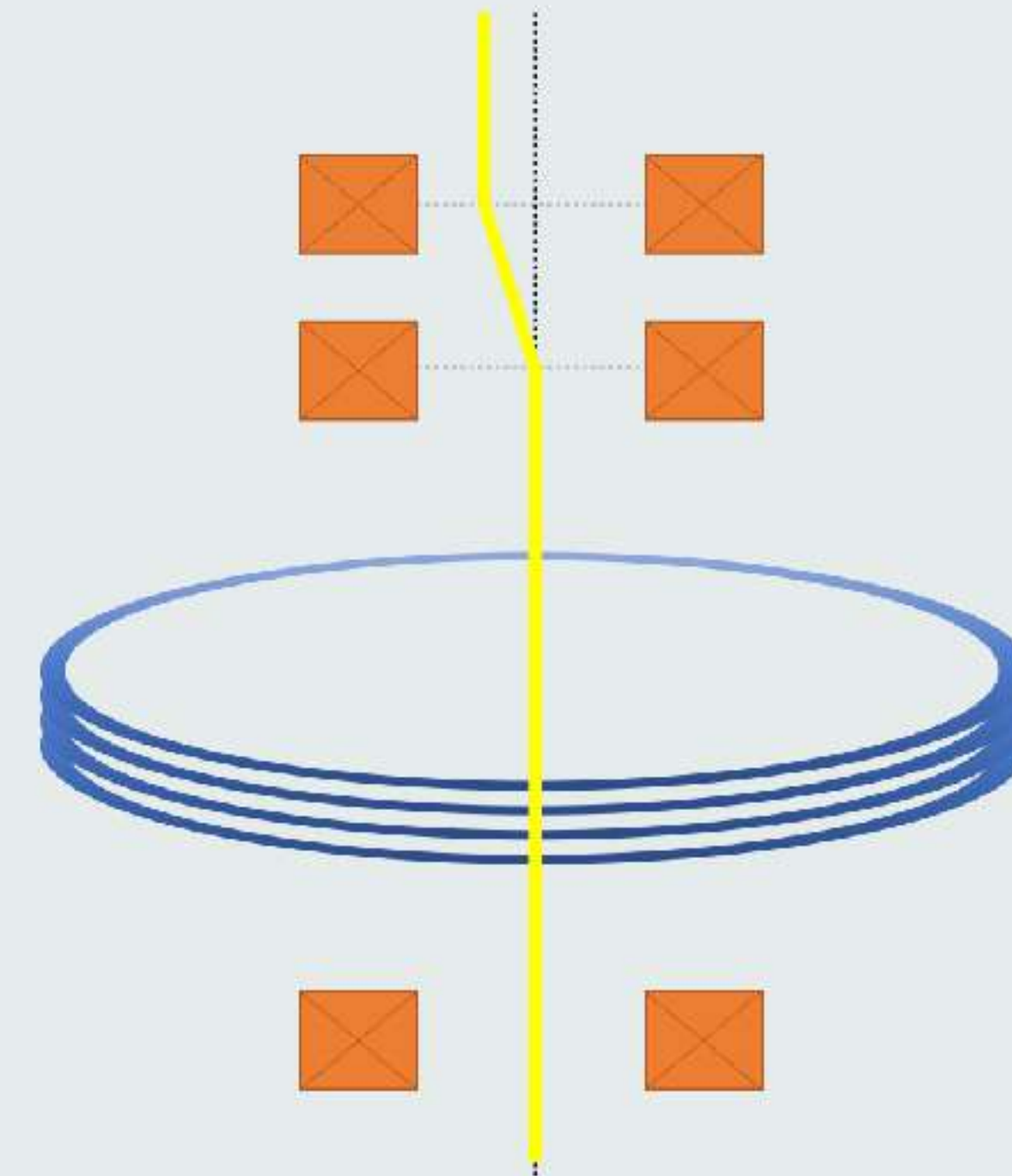
- Focus
- Magnify
- Rotate

Deflector 1 (shift)

Deflector 2 (tilt)

Lens

Stigmator





# LENSES

## Microscope Alignments

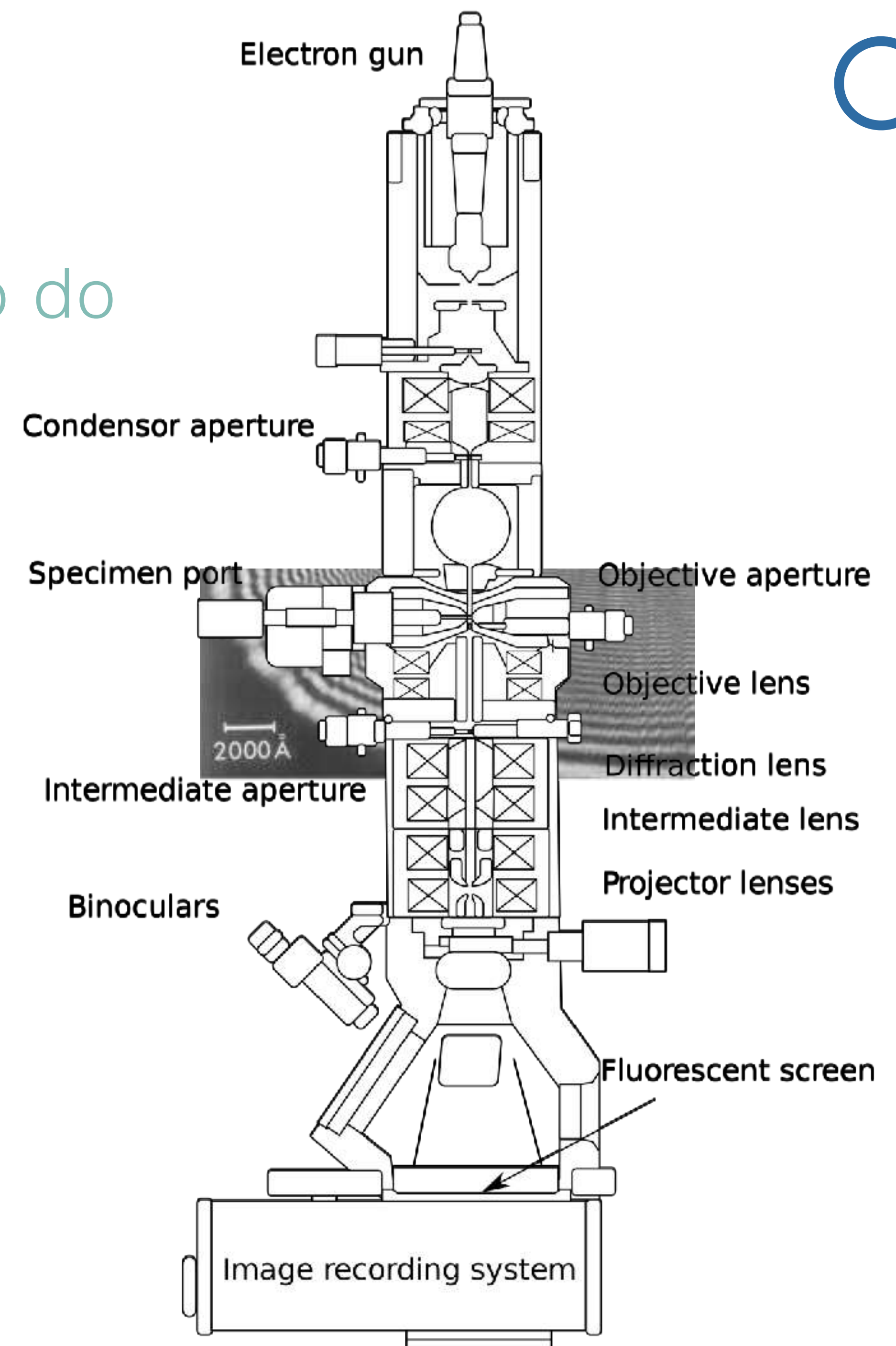
### What to do & what not to do

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)~~



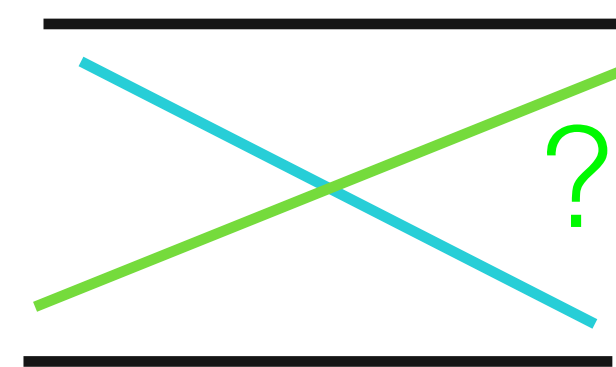




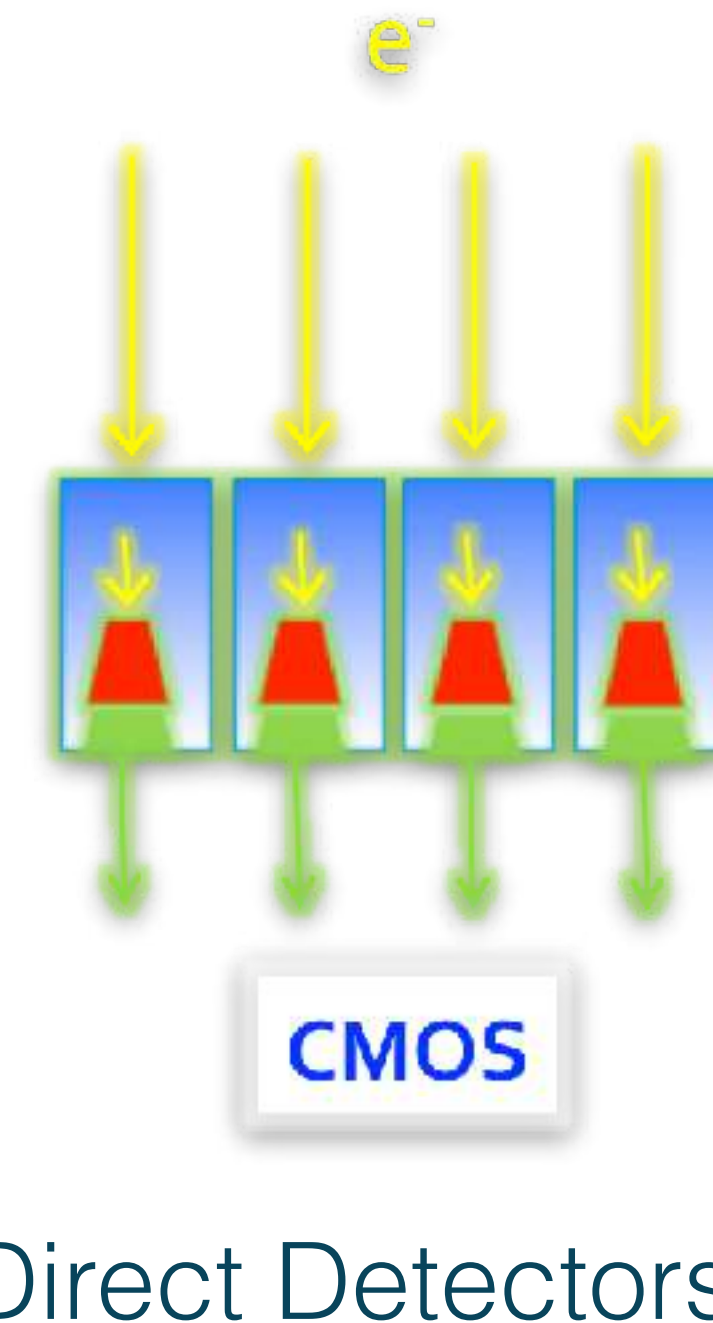
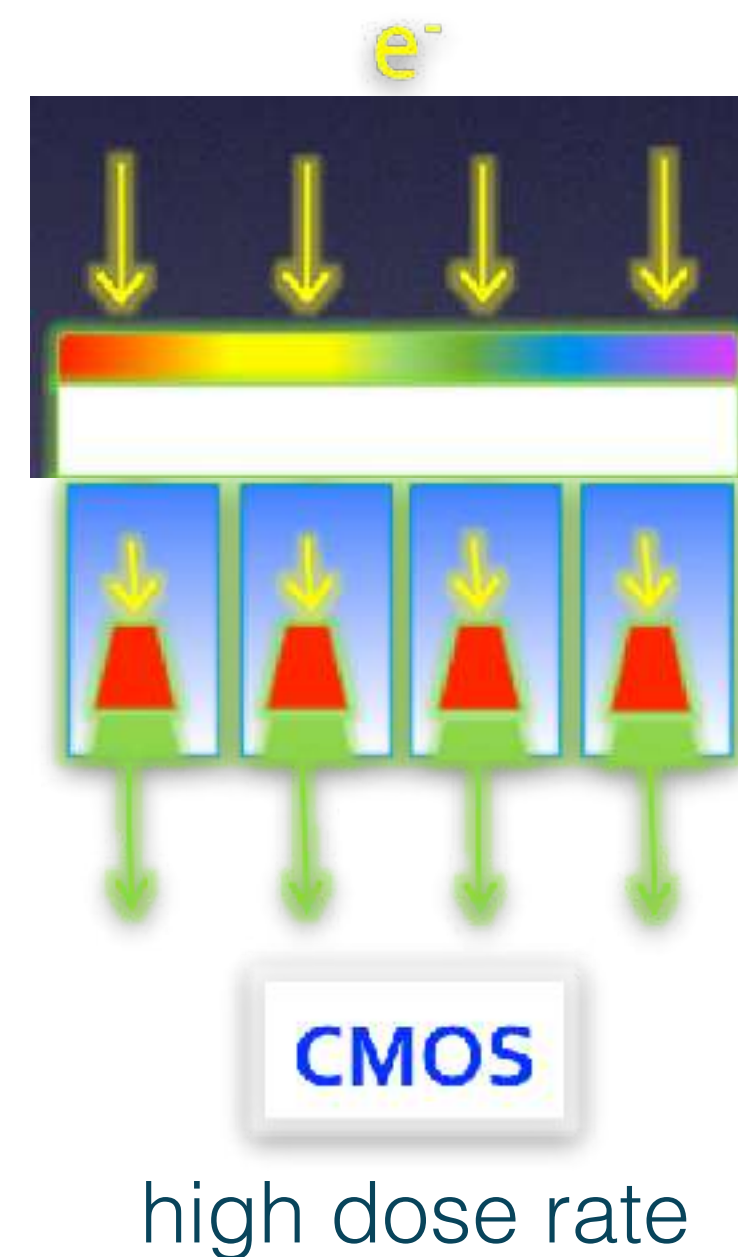
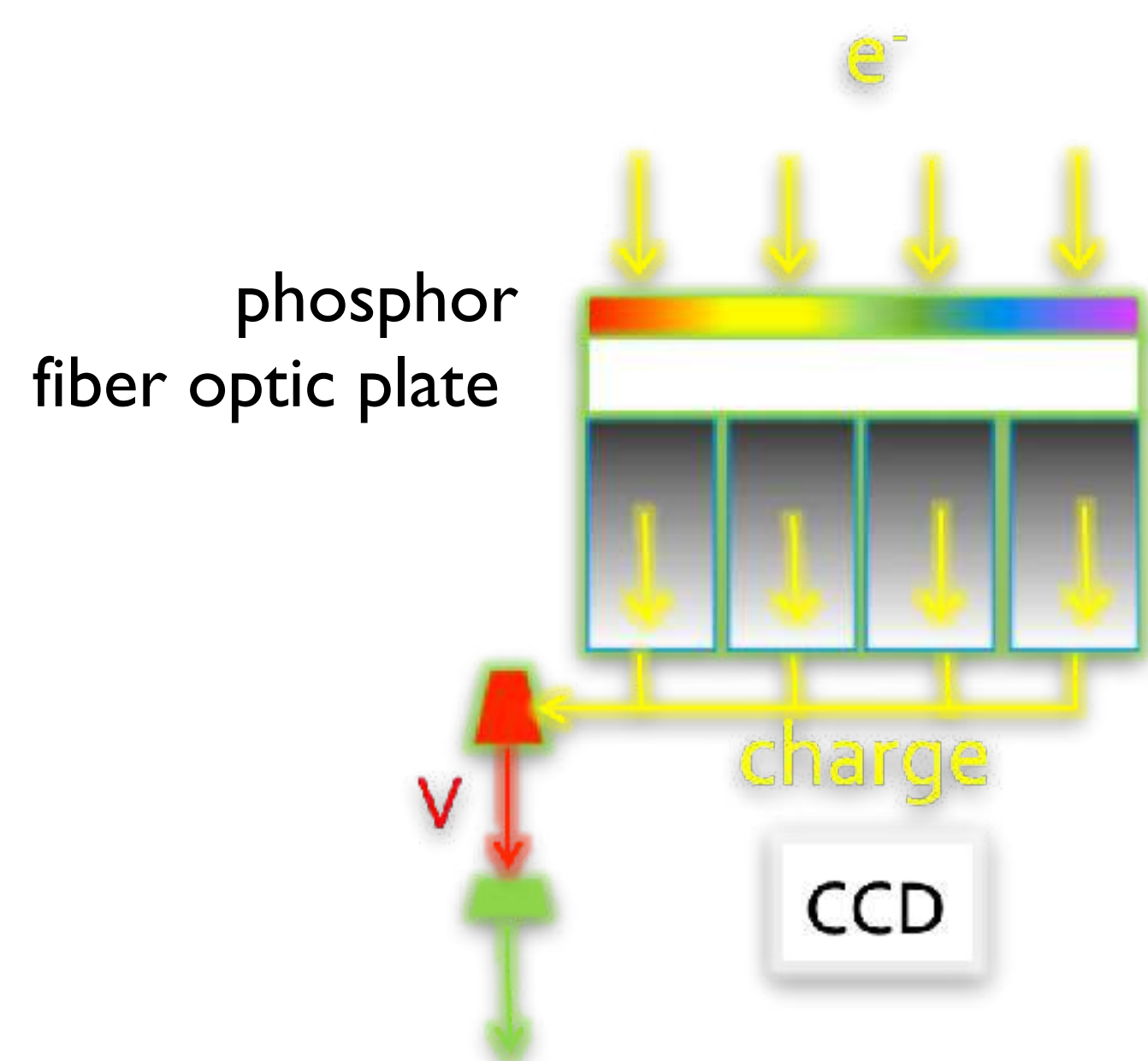
# DETECTORS Digital Cameras for TEM

Photon  
converted

Direct sensing



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



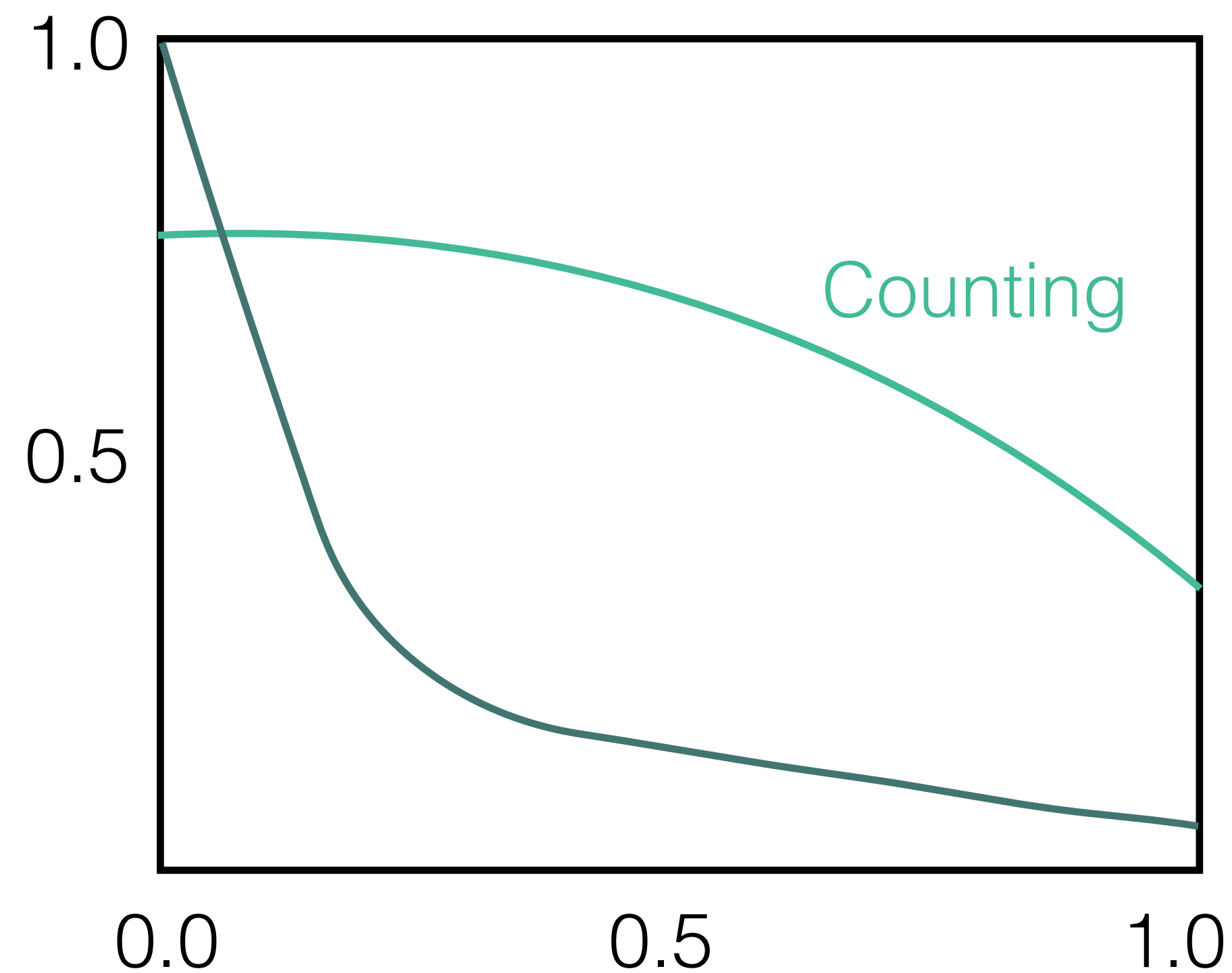




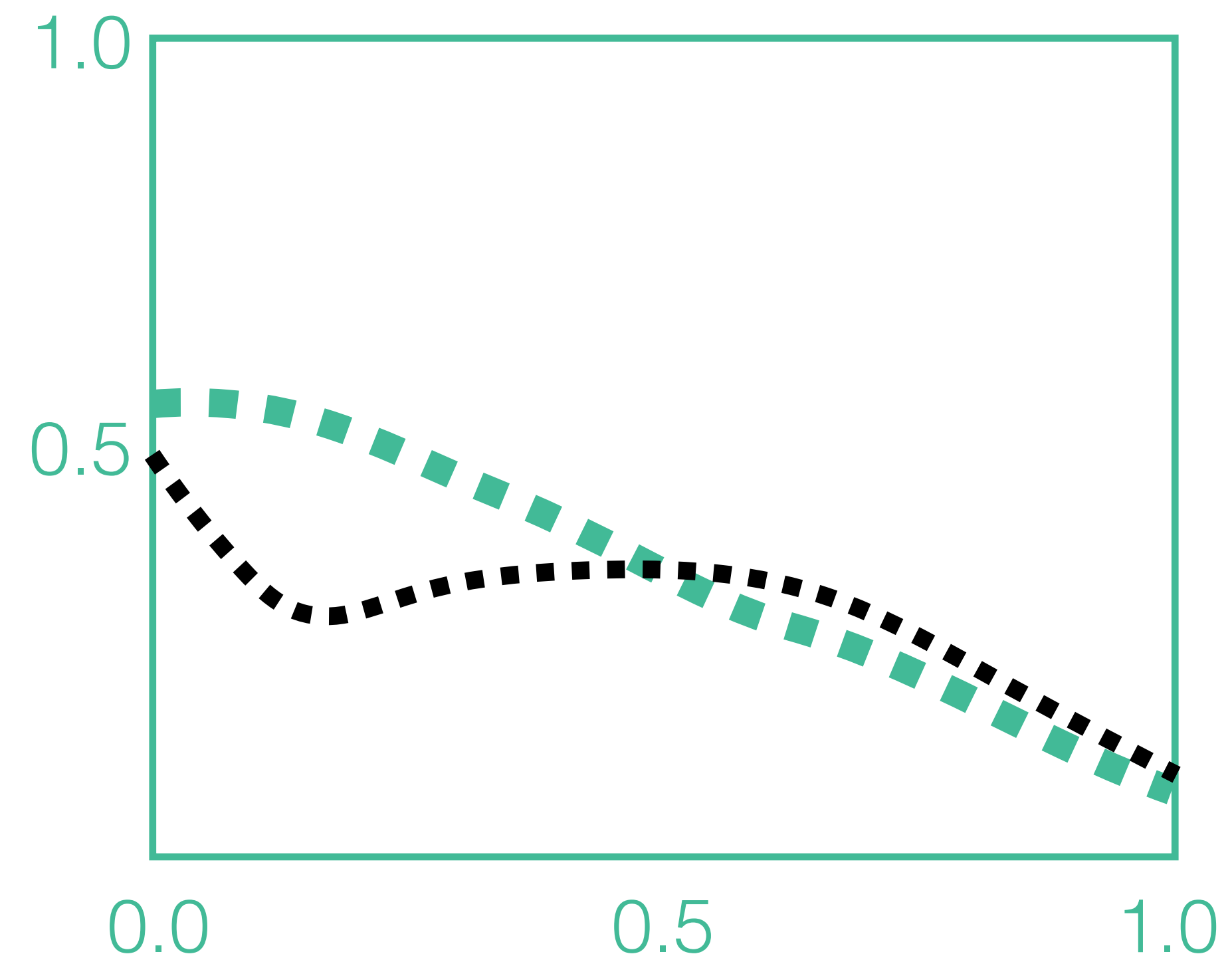
# DETECTORS

## Detector Performance Characterization

MTF (Modulation Transfer Transform)  
contribute to signal envelope



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

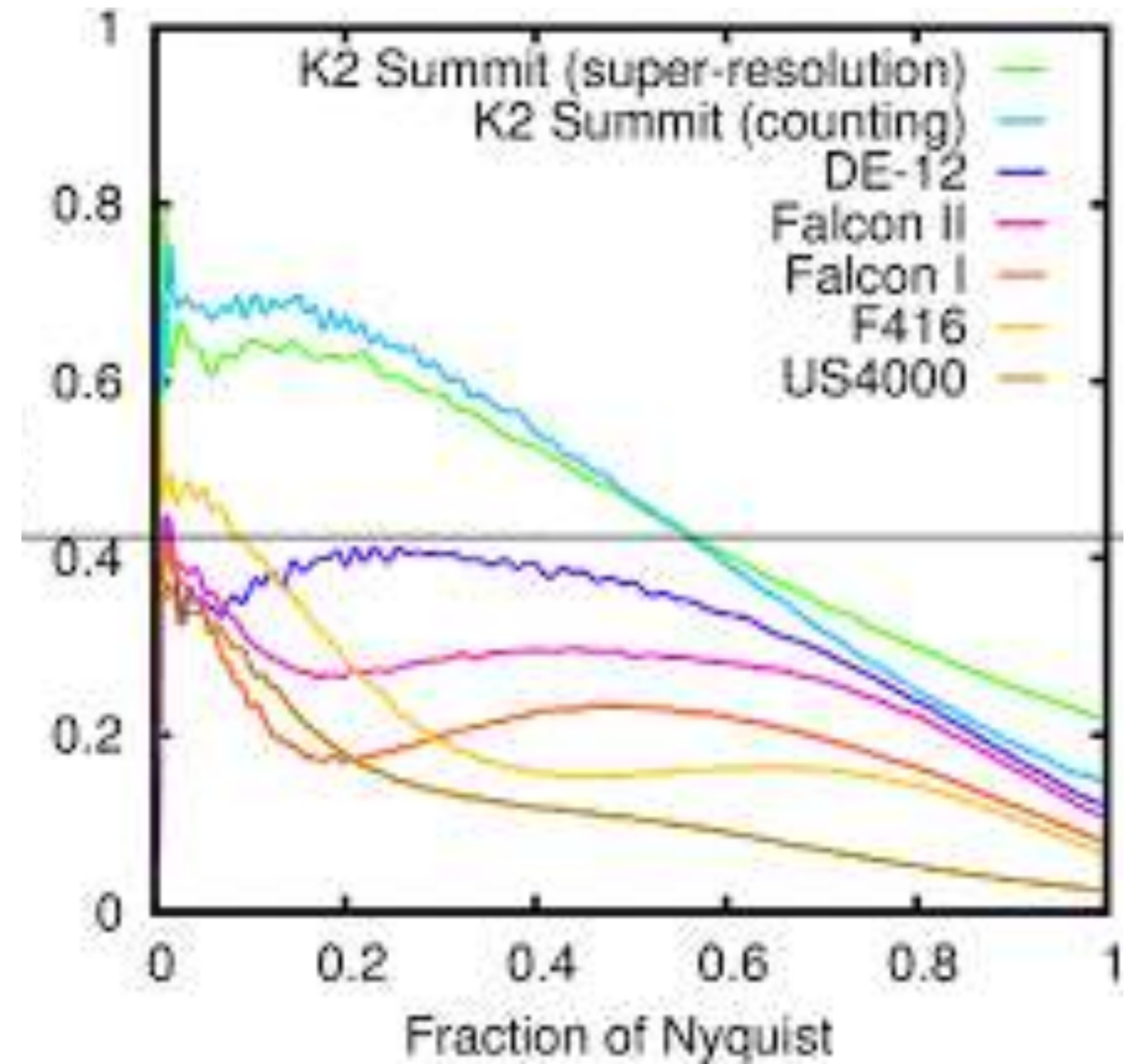
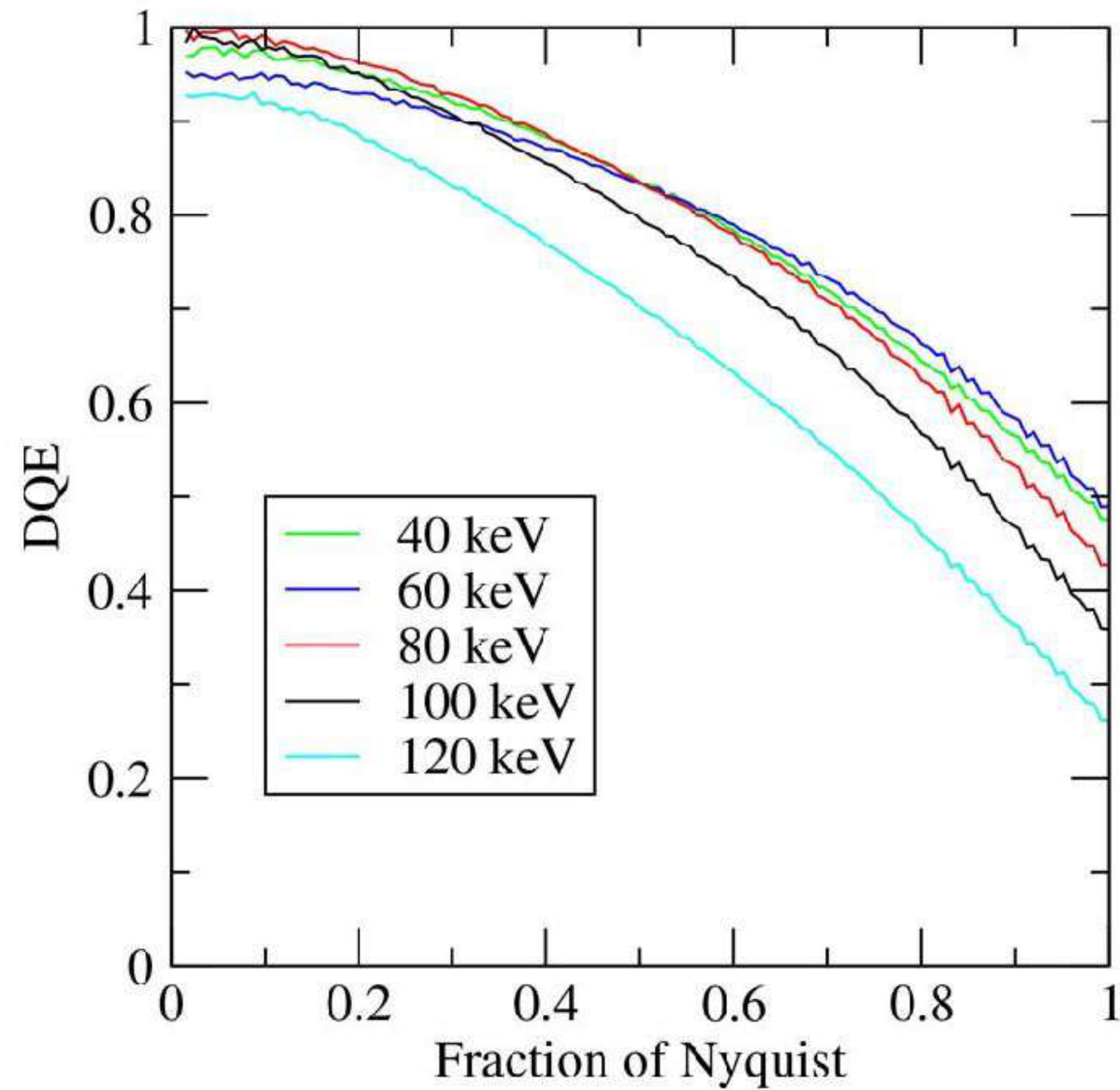






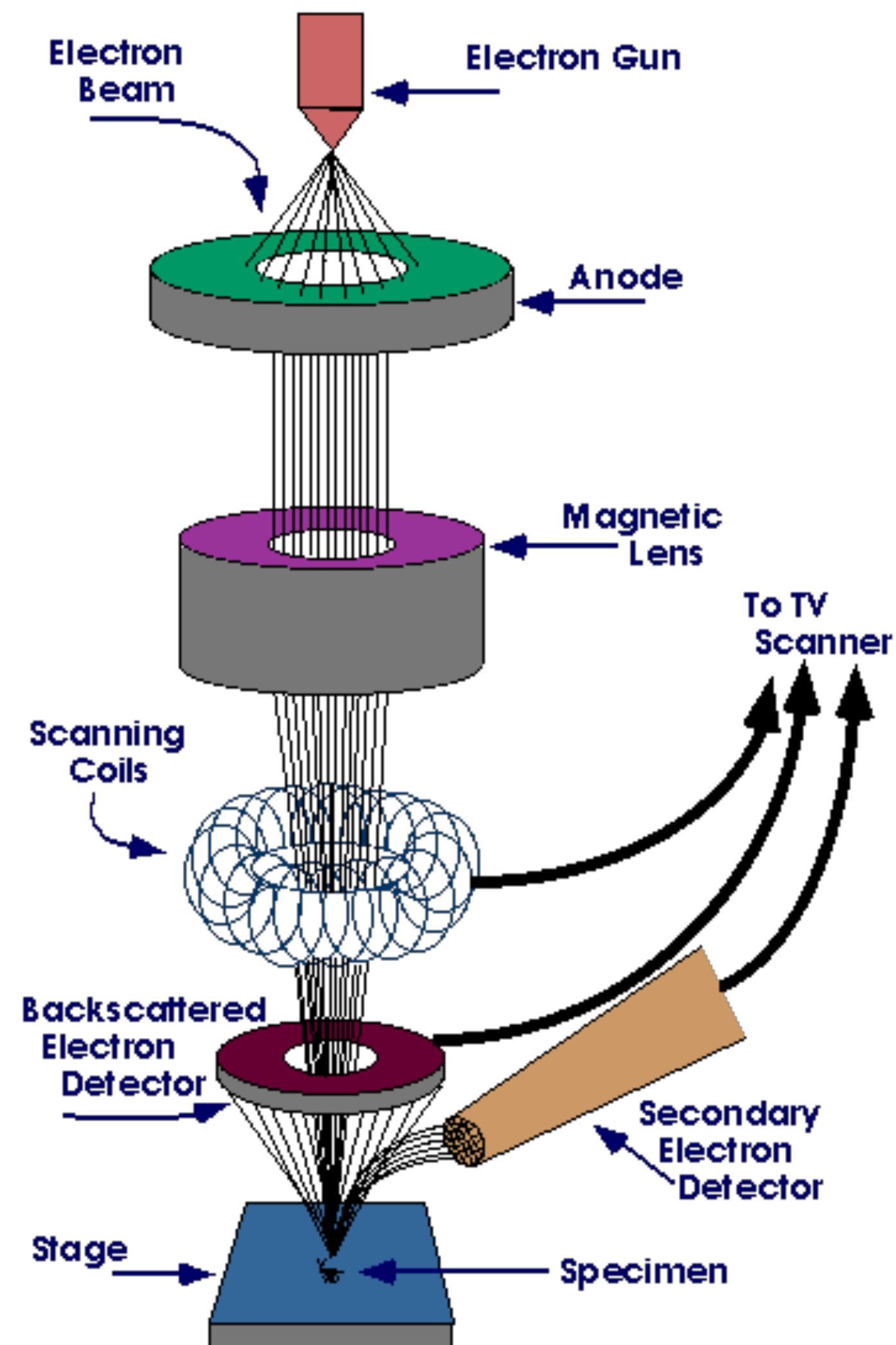
# DETECTORS

## Detector Performance Characterization





# ANATOMY OF AN SEM



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

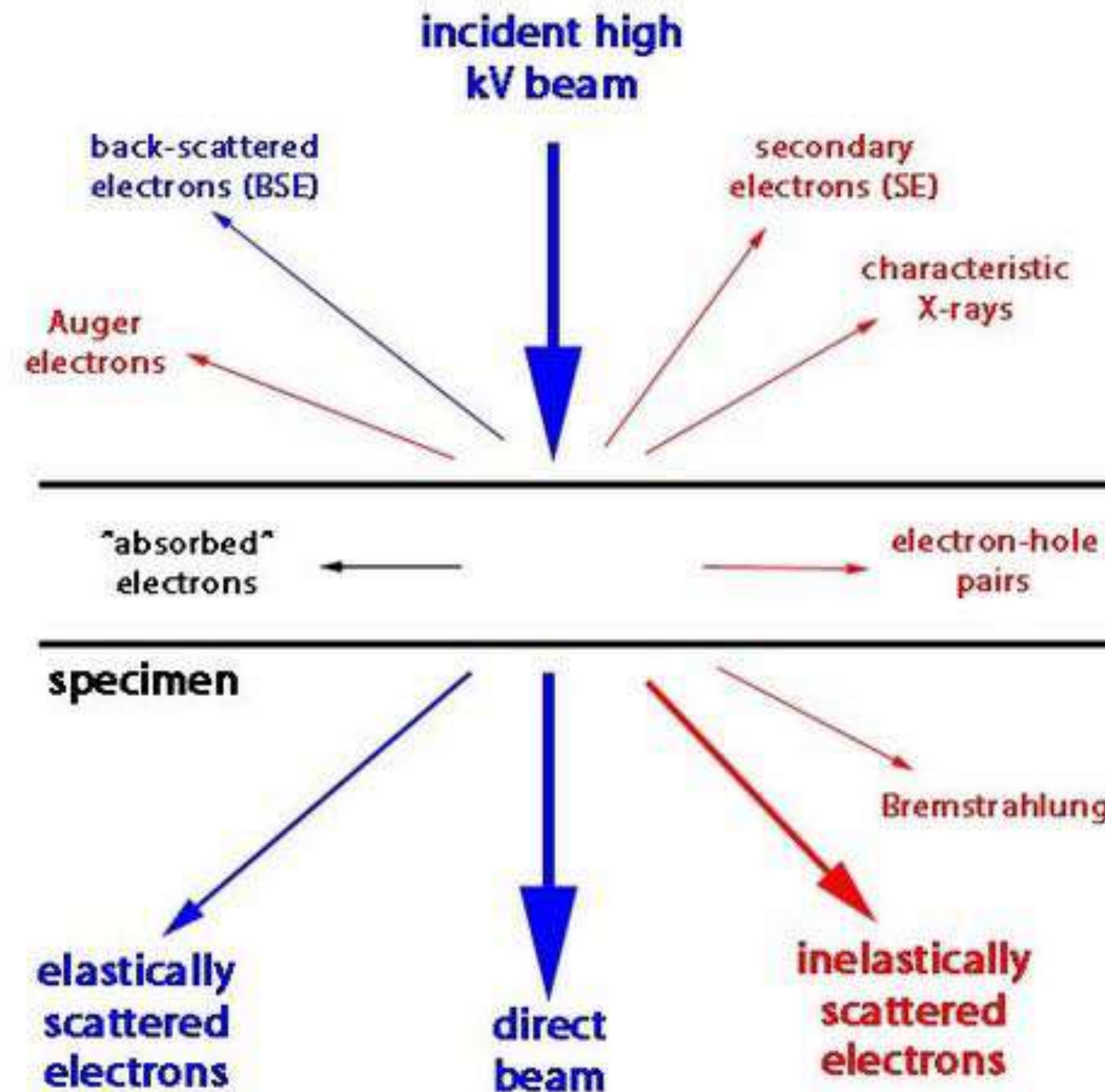
Beam energy: 0.2 – 40 keV is focused by a condenser lens system into a spot of 0.4 – 5 nm

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



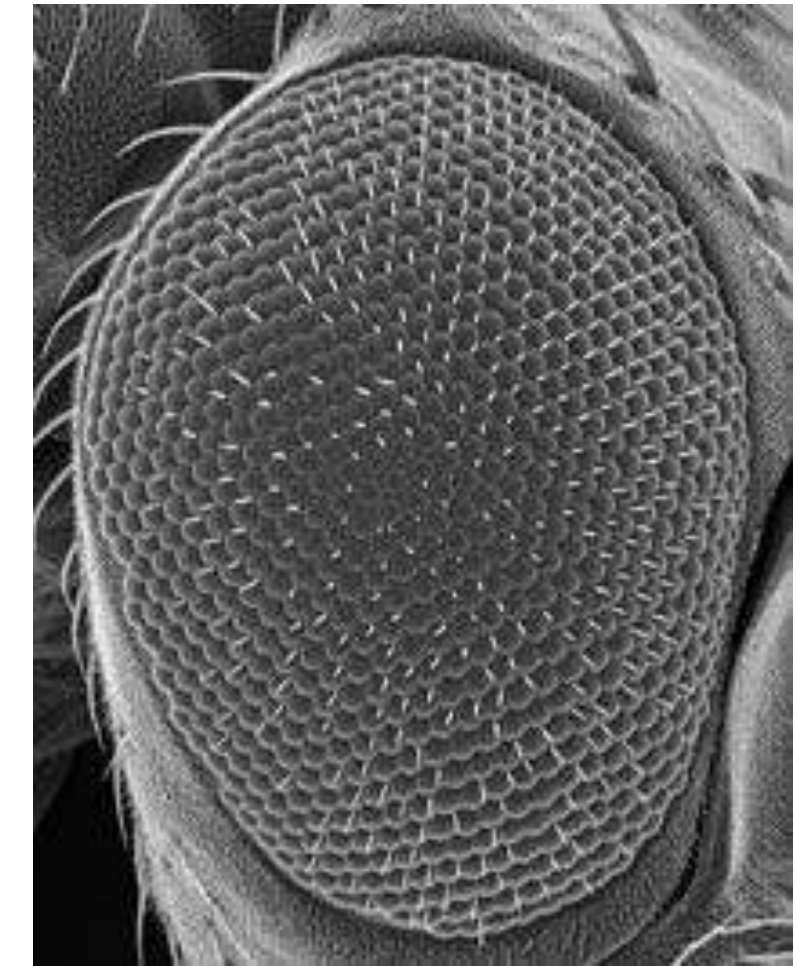
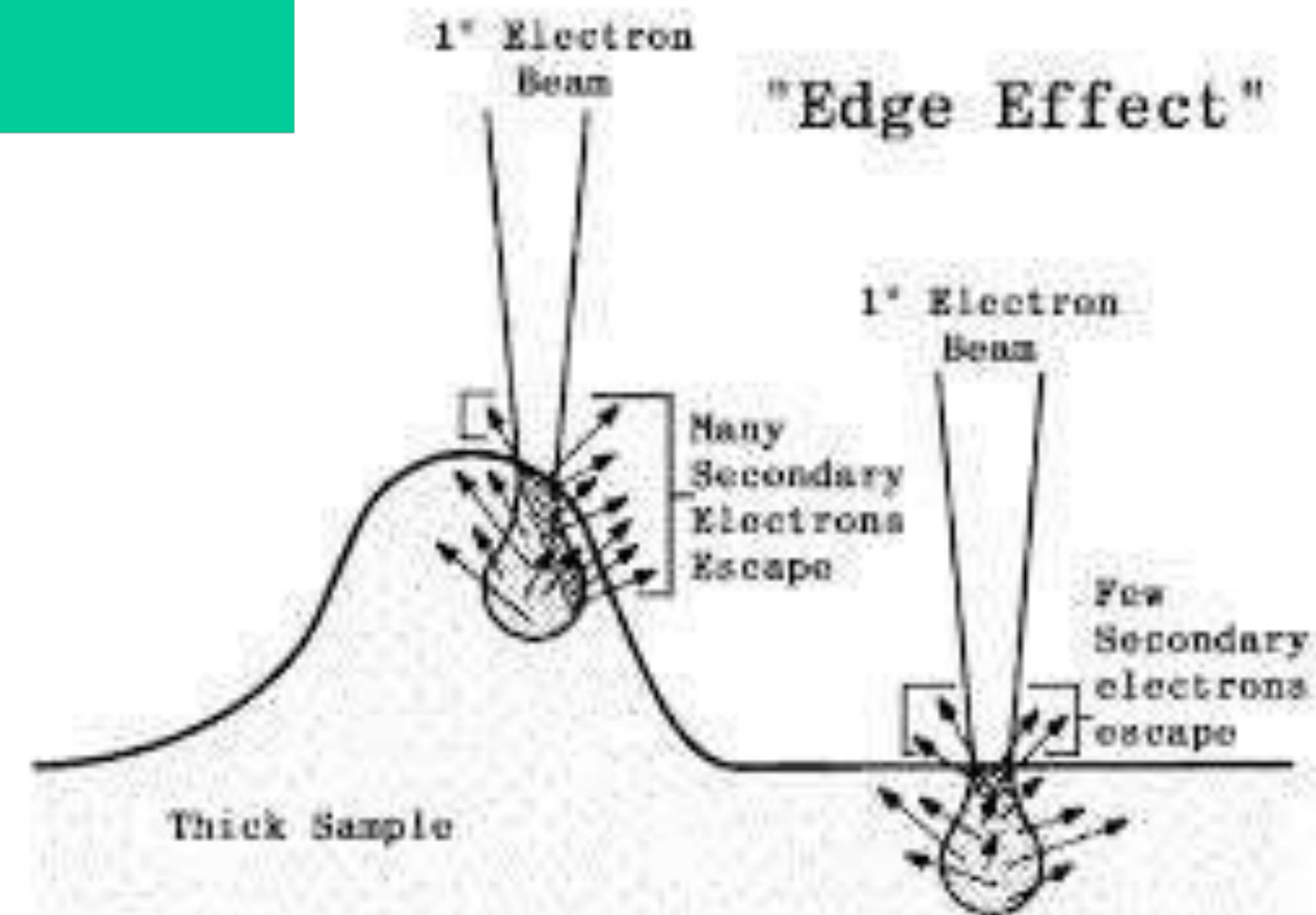
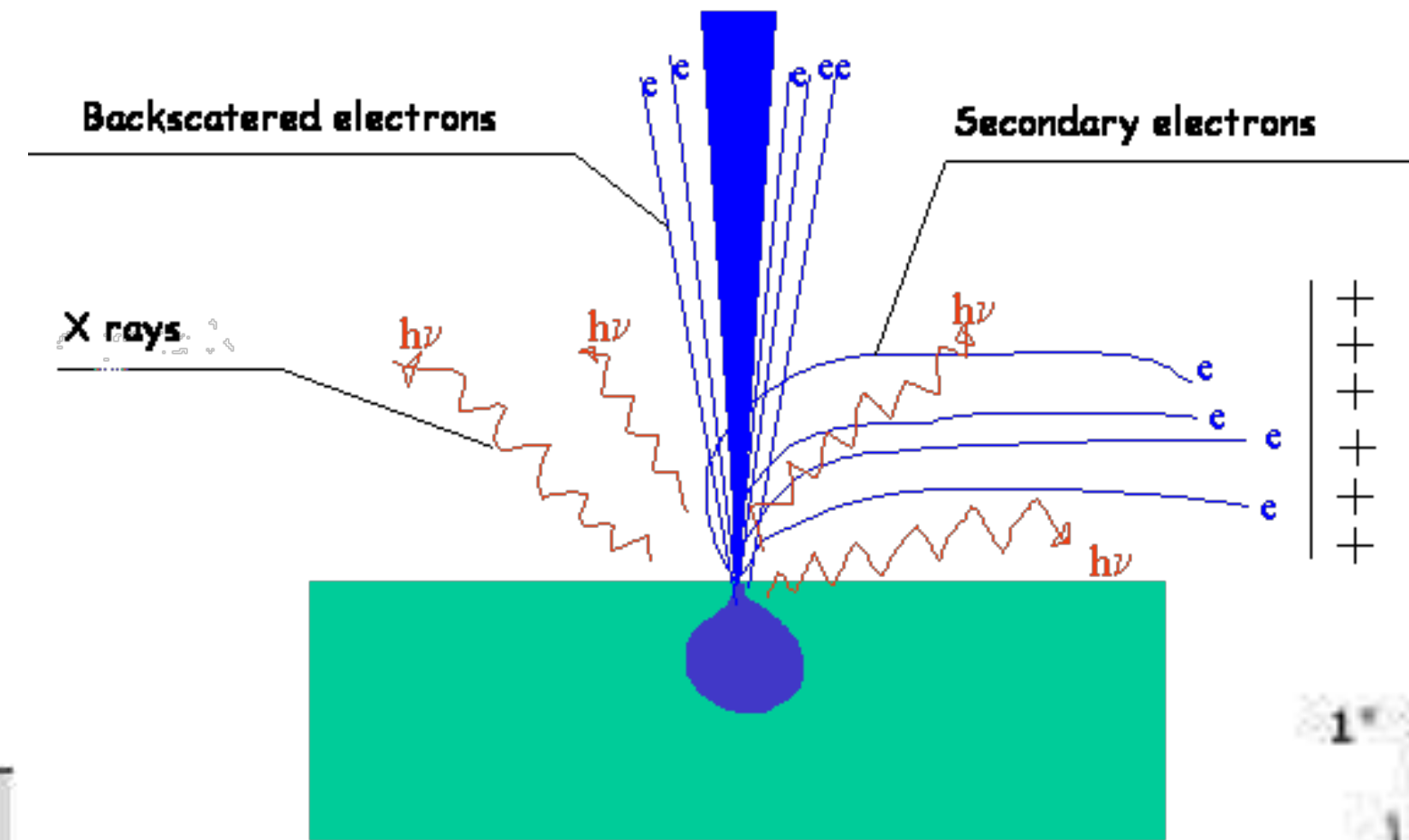
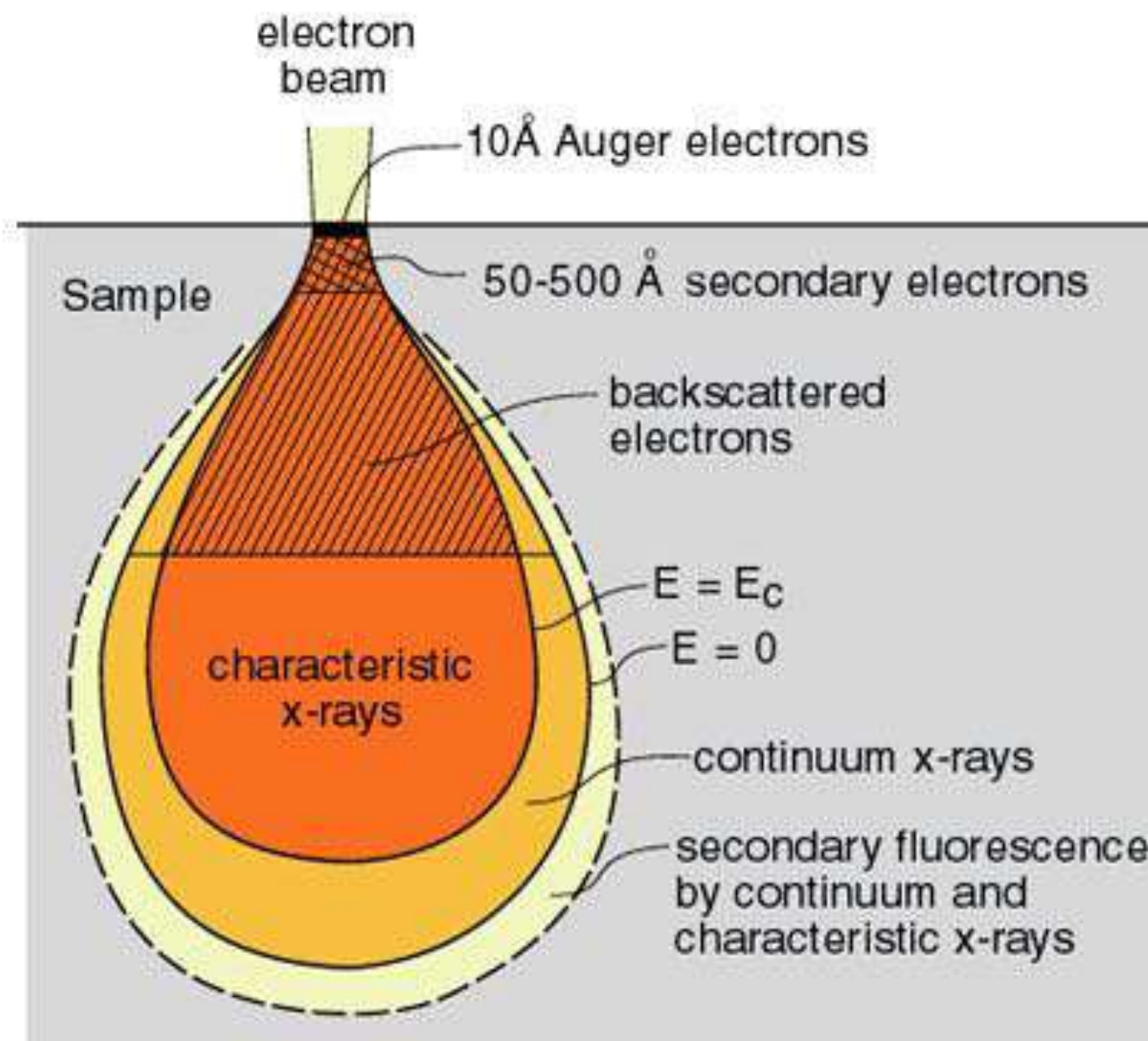
# ANATOMY OF AN SEM – BEAM SAMPLE INTERACTIONS



modified from Williams & Carter (1996) Fig. 1.3

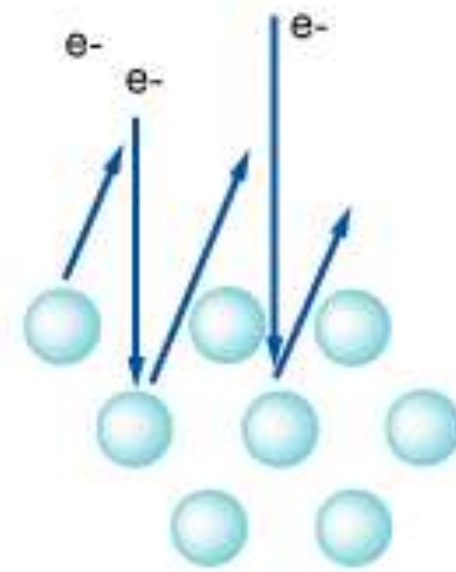


# ANATOMY OF AN SEM – BEAM SAMPLE INTERACTIONS & IMAGE FORMATION

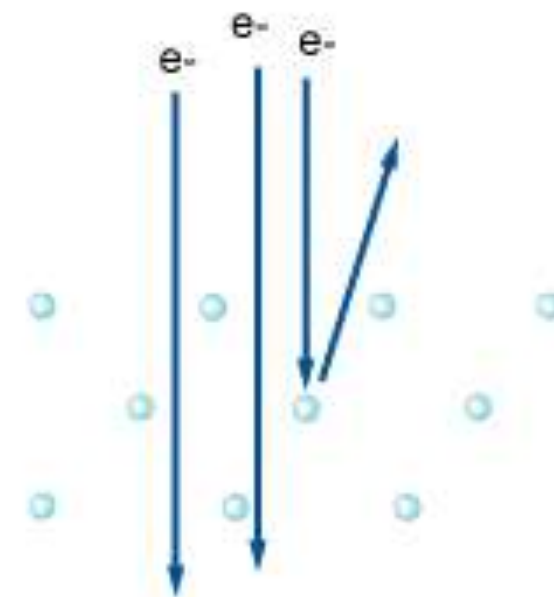




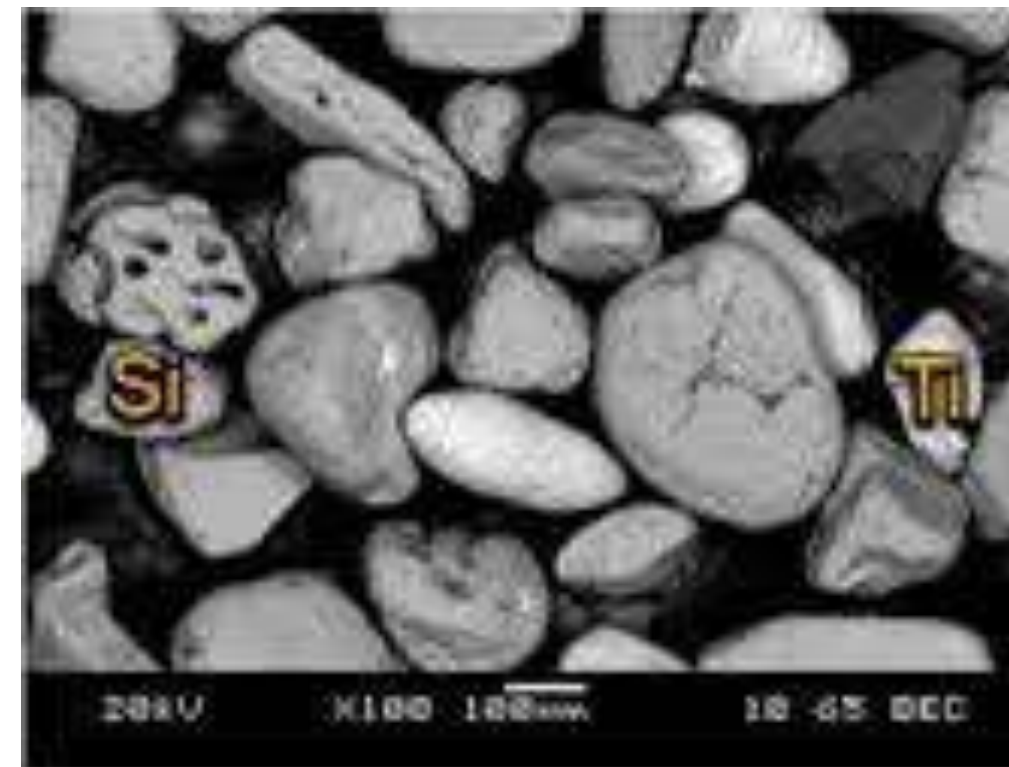
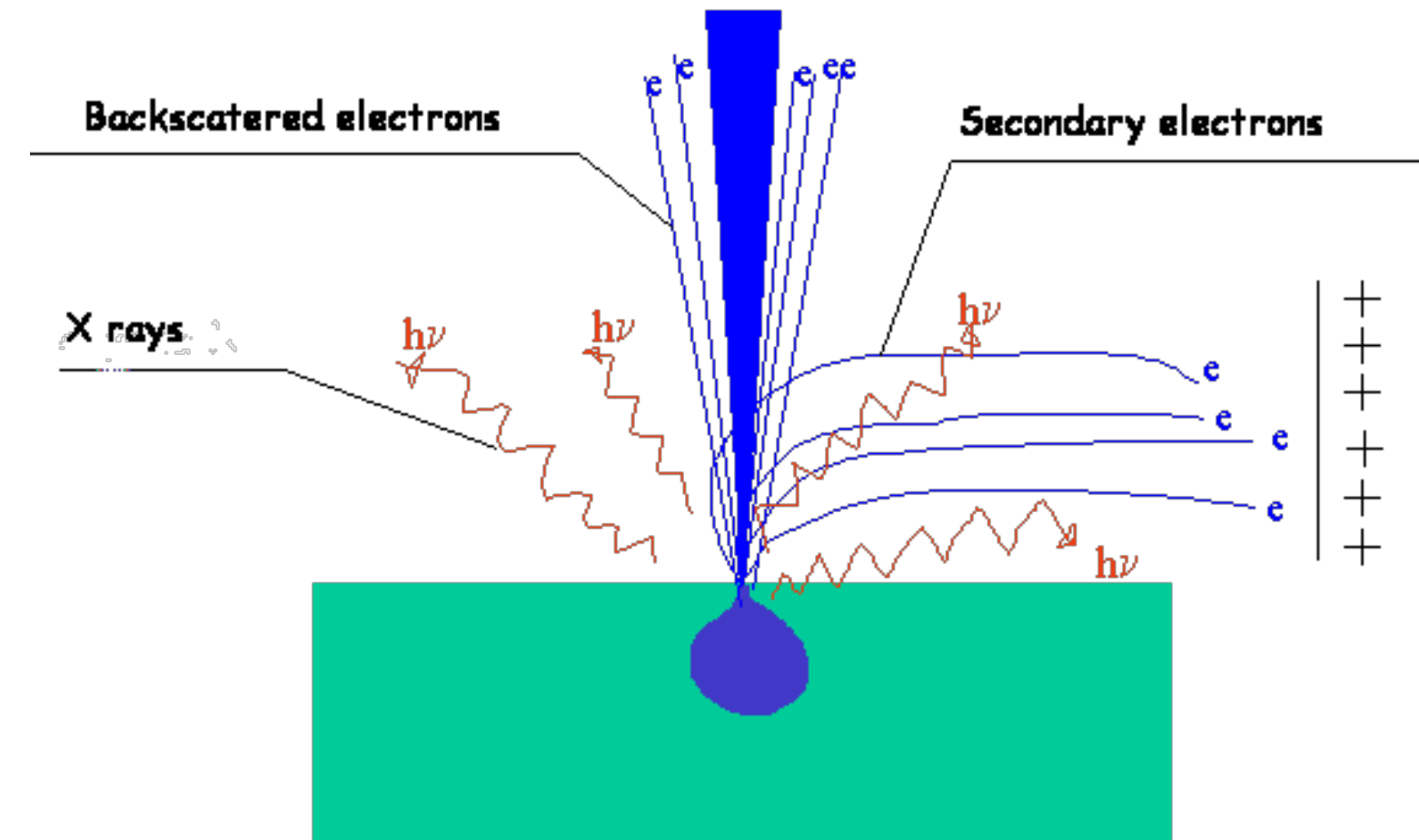
# ANATOMY OF AN SEM – BEAM SAMPLE INTERACTIONS & IMAGE FORMATION



Titanium  
atomic number 22



Silicon  
atomic number 14







# TOOLS OF THE TRADE: MICROSCOPES AND DETECTORS

Questions?