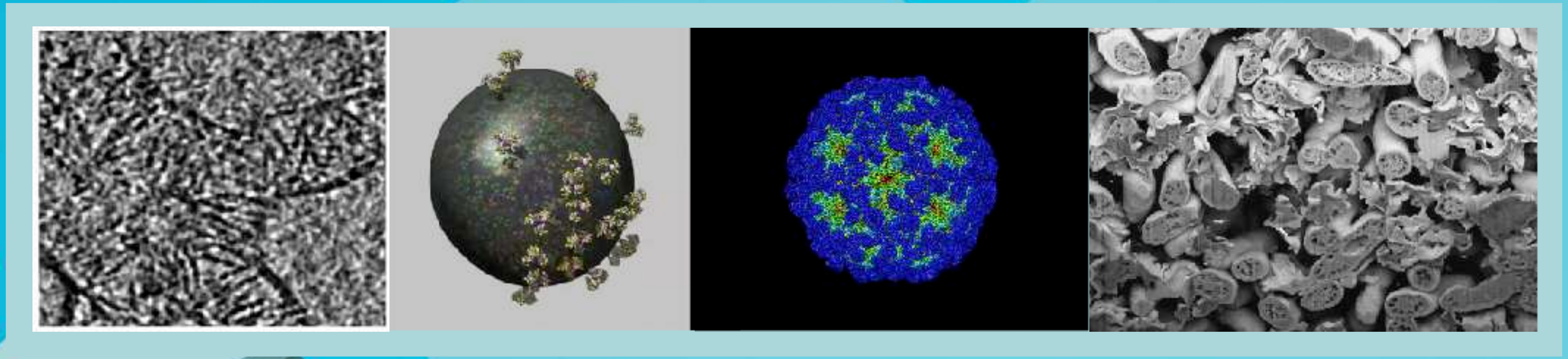
2022 Winter-Spring EM Course



Edward T Eng
January 10, 2022

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

COMPPÅ

NIH P41 - National Biomedical Technology Research Resources (BTRR)

National Synchrotron Light Source II

BROOKHAVEN NATIONAL LABORATORY

19-ID

NYX

NRAMM

NCCAT

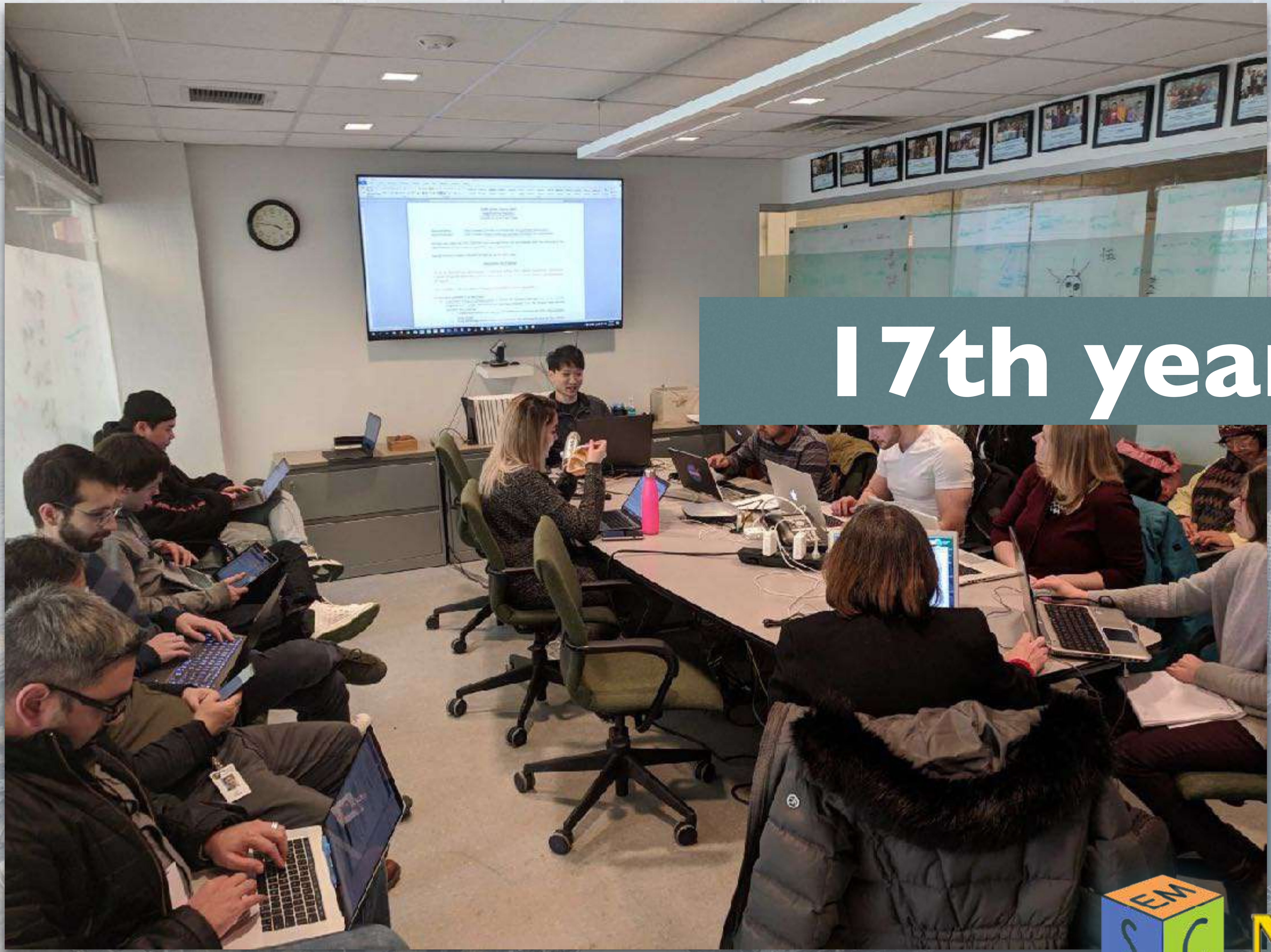
NCITU

NMR
CoMD/
NMR

X-ray
NYX
@NSLS-II

Protein
Production
COMPPÅ

NIH P41 - National Biomedical Technology Research Resources (BTRR)



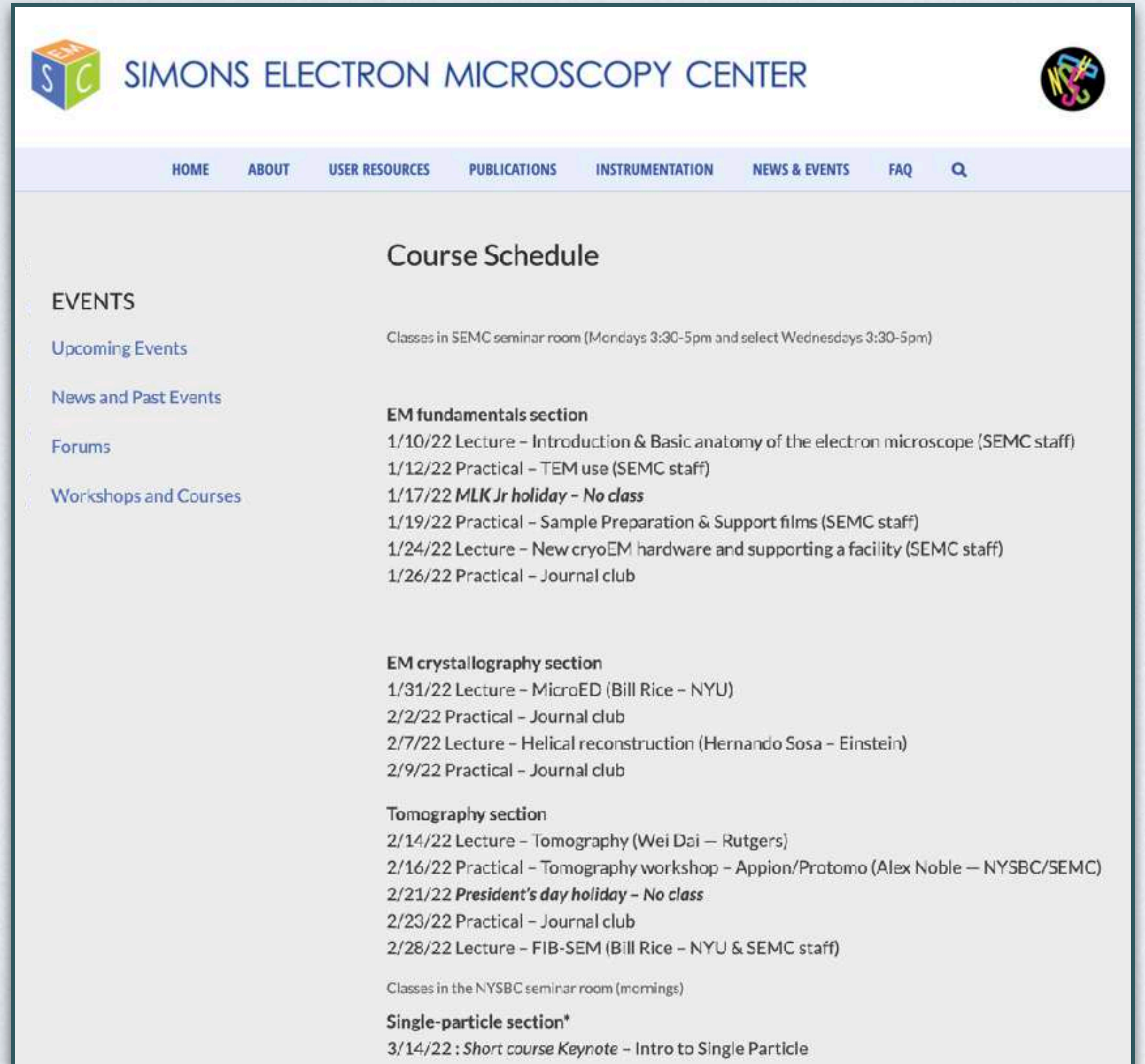
17th year of the course

photo by Swapnil Bhatkar

NRAMM NCCAT NCITU

Course logistics: main website

semc.nysbc.org/the-winter-spring-2022-em-course/


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)

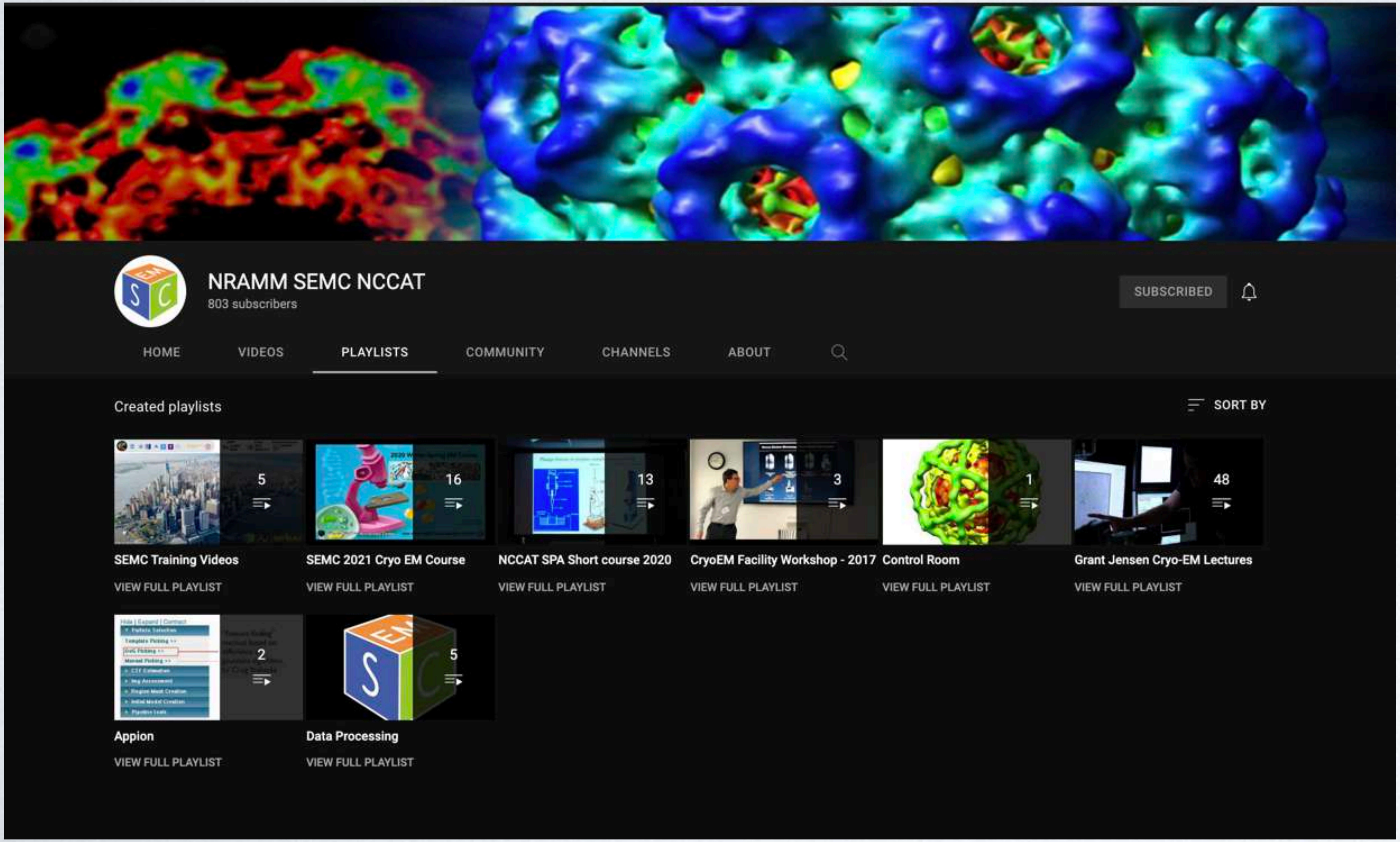
Teaching Assistants:

Mahira Aragon,
Eugene Chua,
Christina Zimanyi

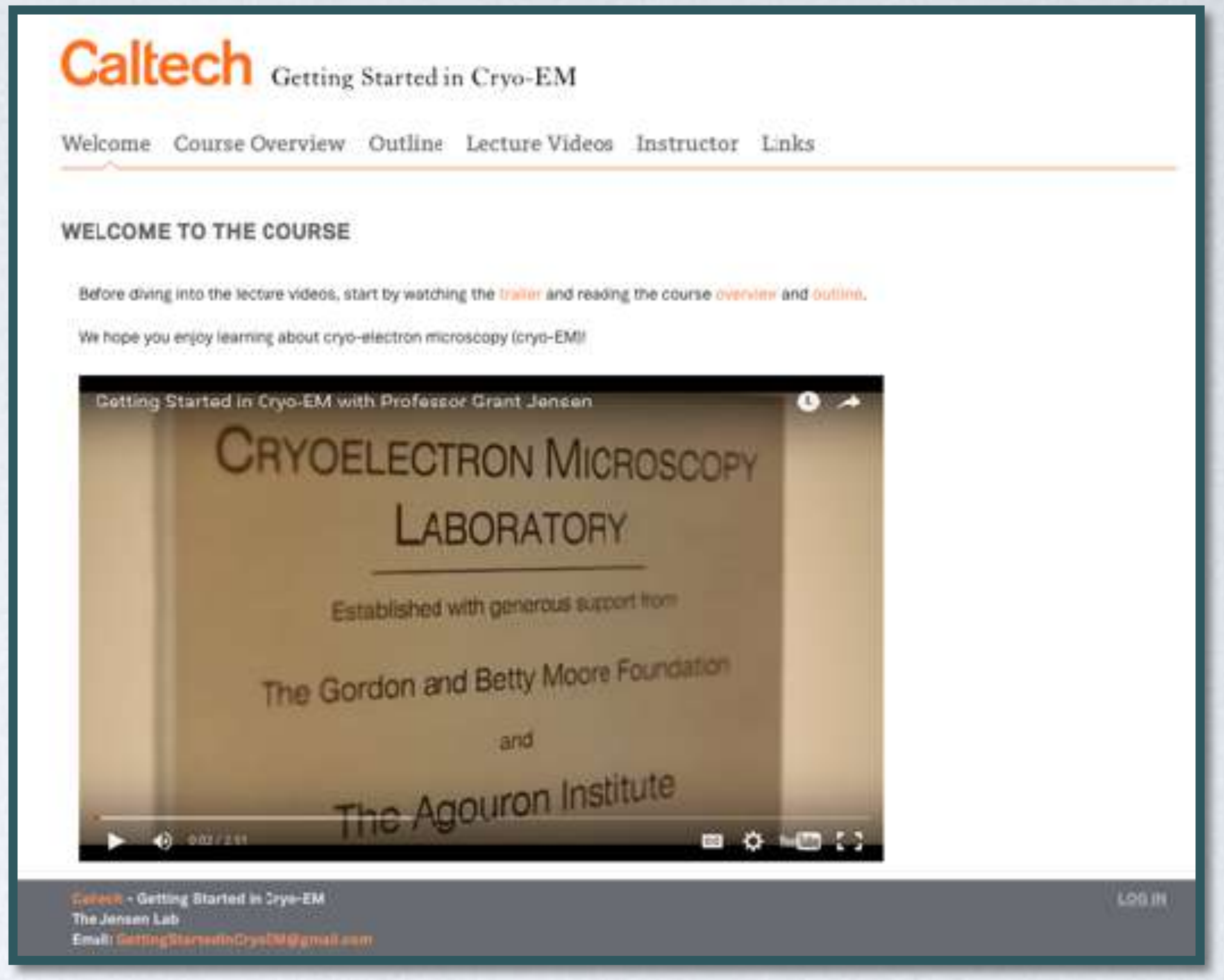
Course logistics: hybrid vs flipped class

Course logistics: resources

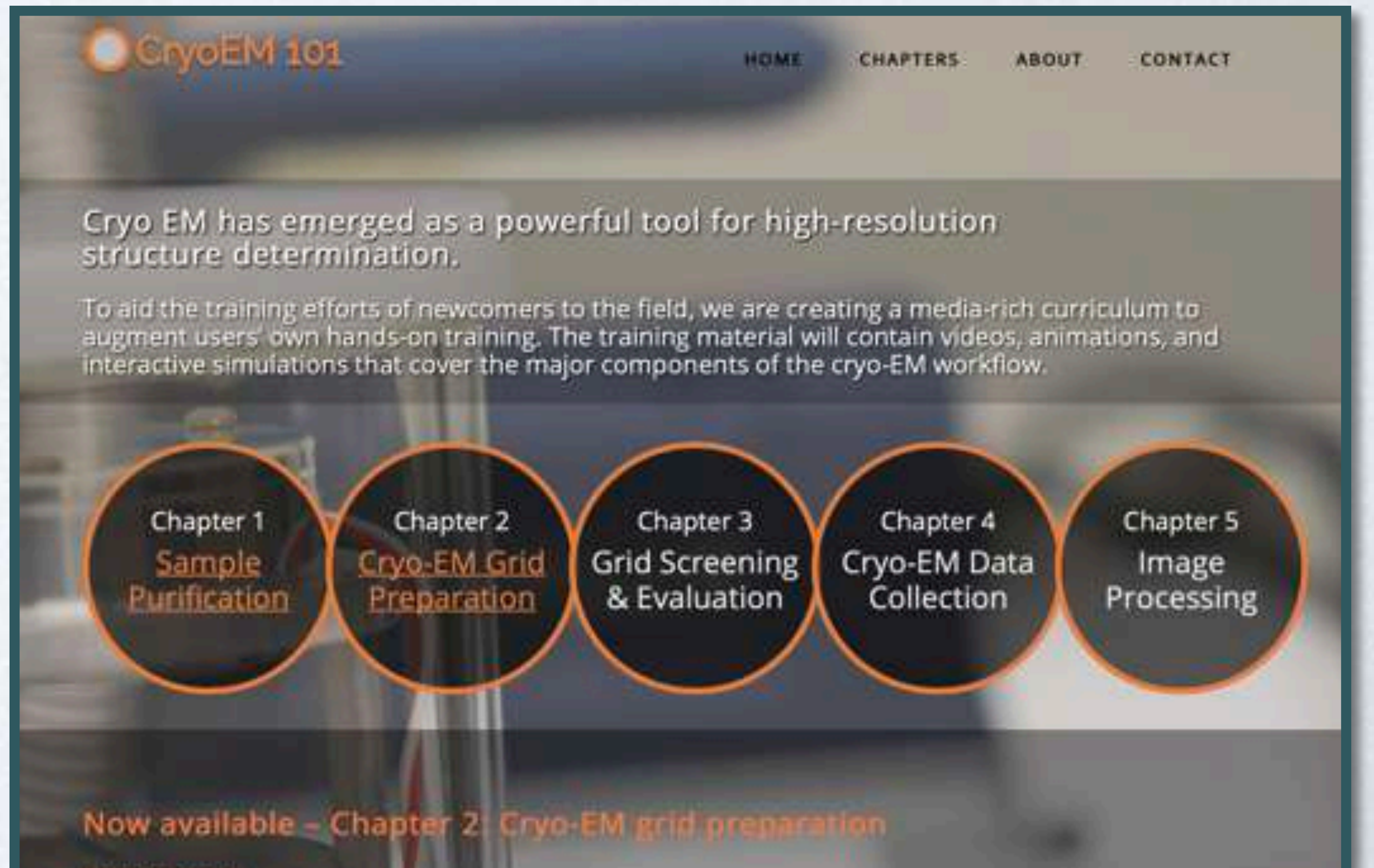
youtube.com/nrammsemc



cryo-em-course.caltech.edu/videos



cryoem101.org



Course logistics: engagement & reinforcement

semc.nysbc.org/the-winter-spring-2022-em-course/

Class

Introduction to the material

Roundtable

Submit questions to us by Friday the week before.

Ed

Christina

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

Wednesdays
Starts at 3:30 - 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

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% x 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
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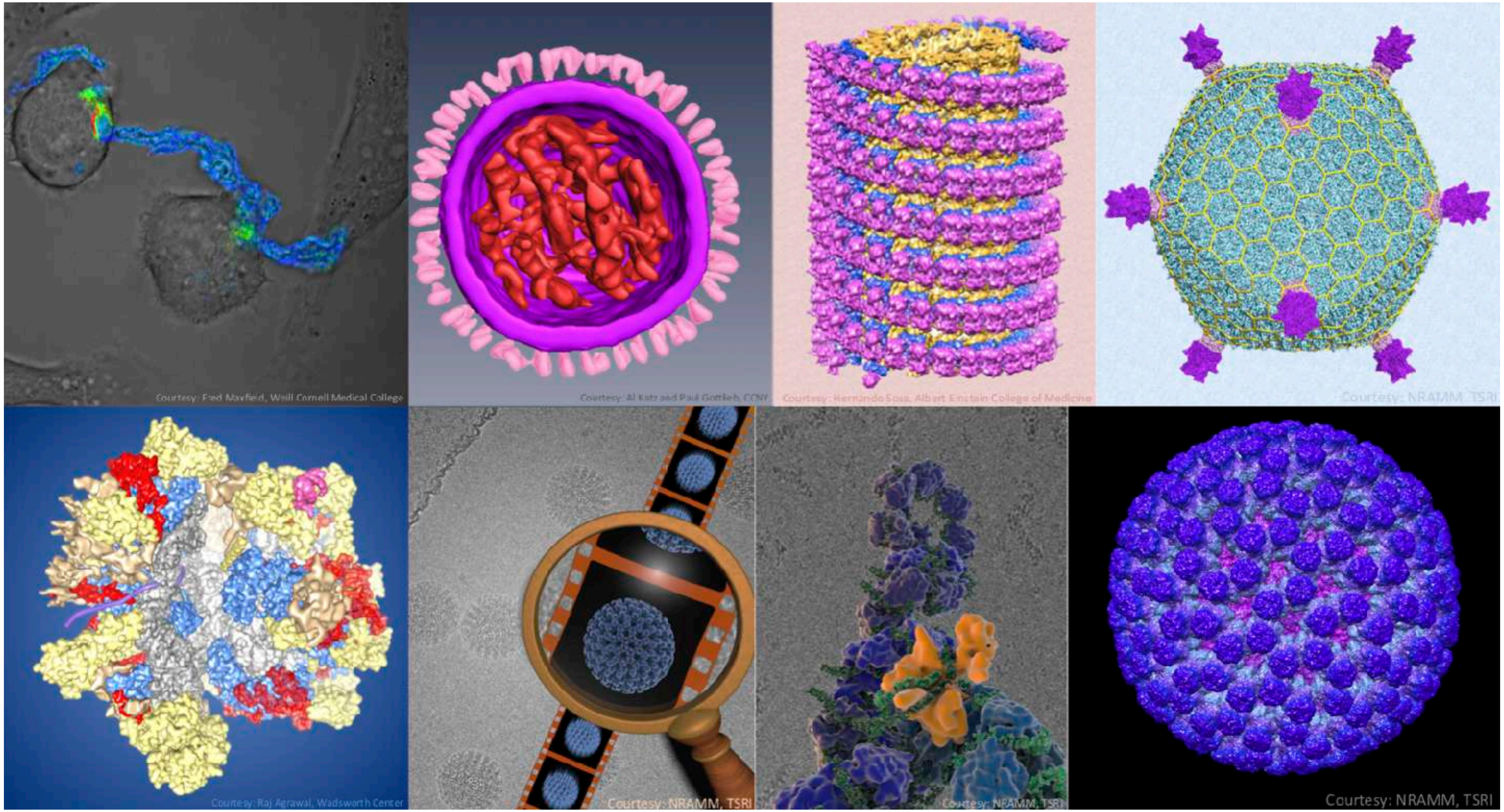
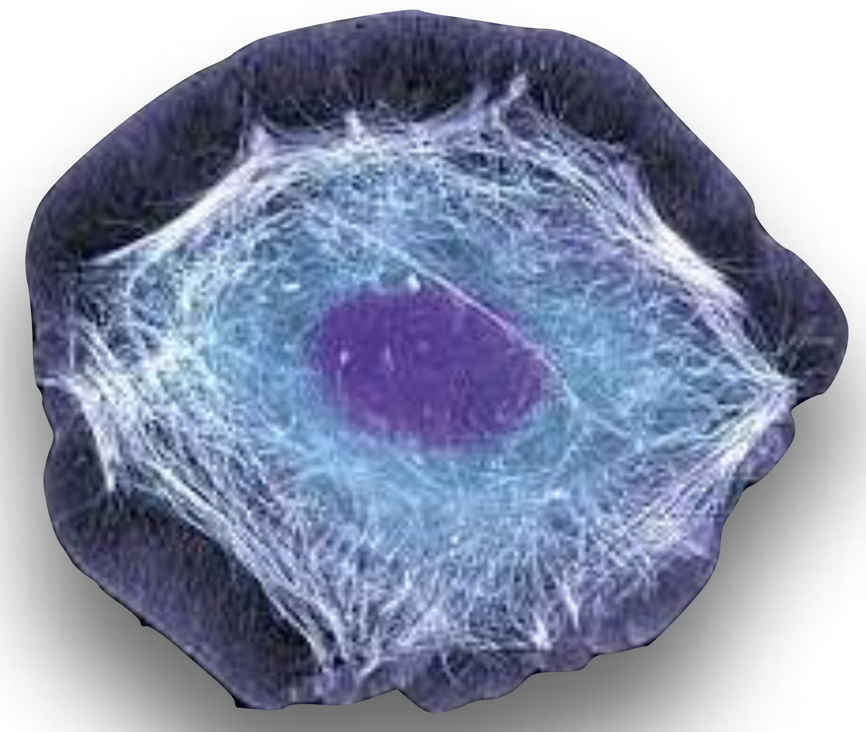
START

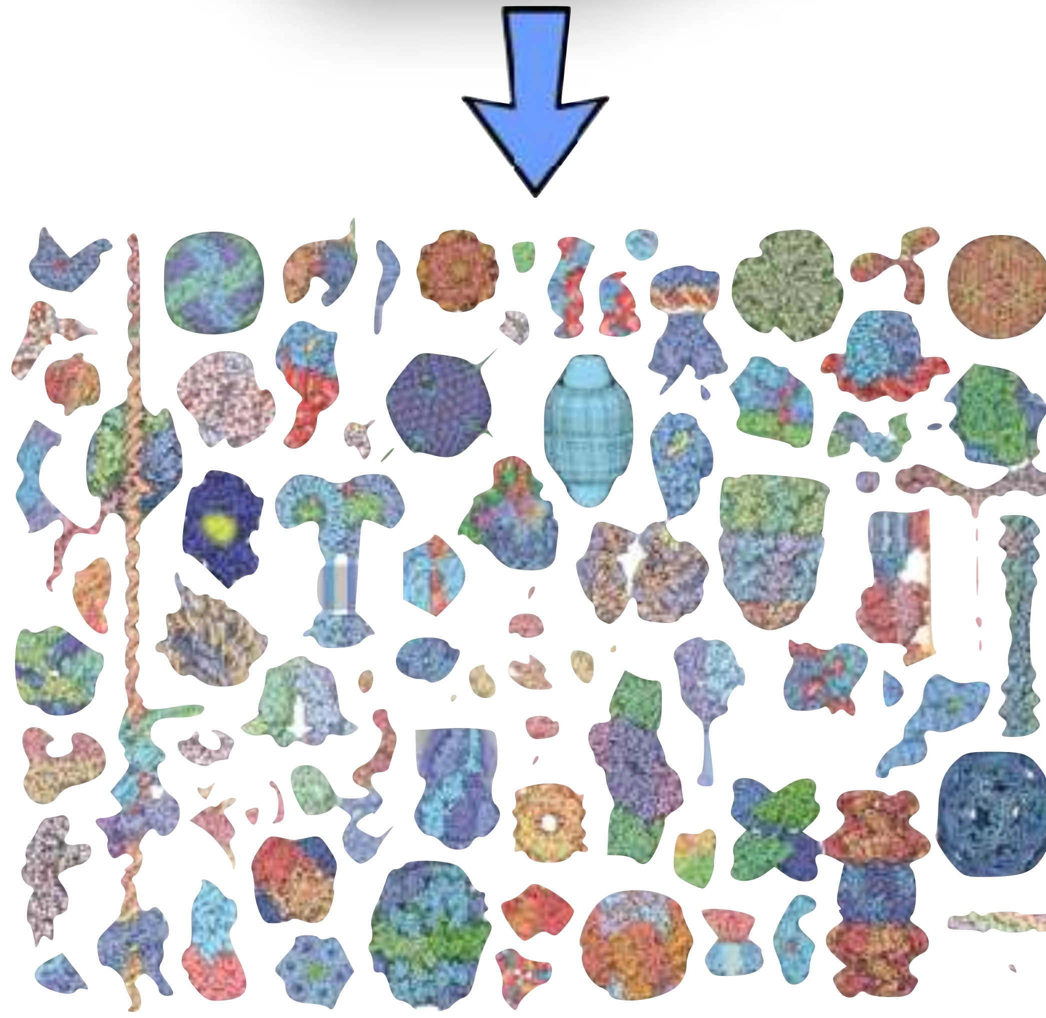
http://etc.ch/hpD2

CRYOEM: TECHNOLOGY ON THE RISE



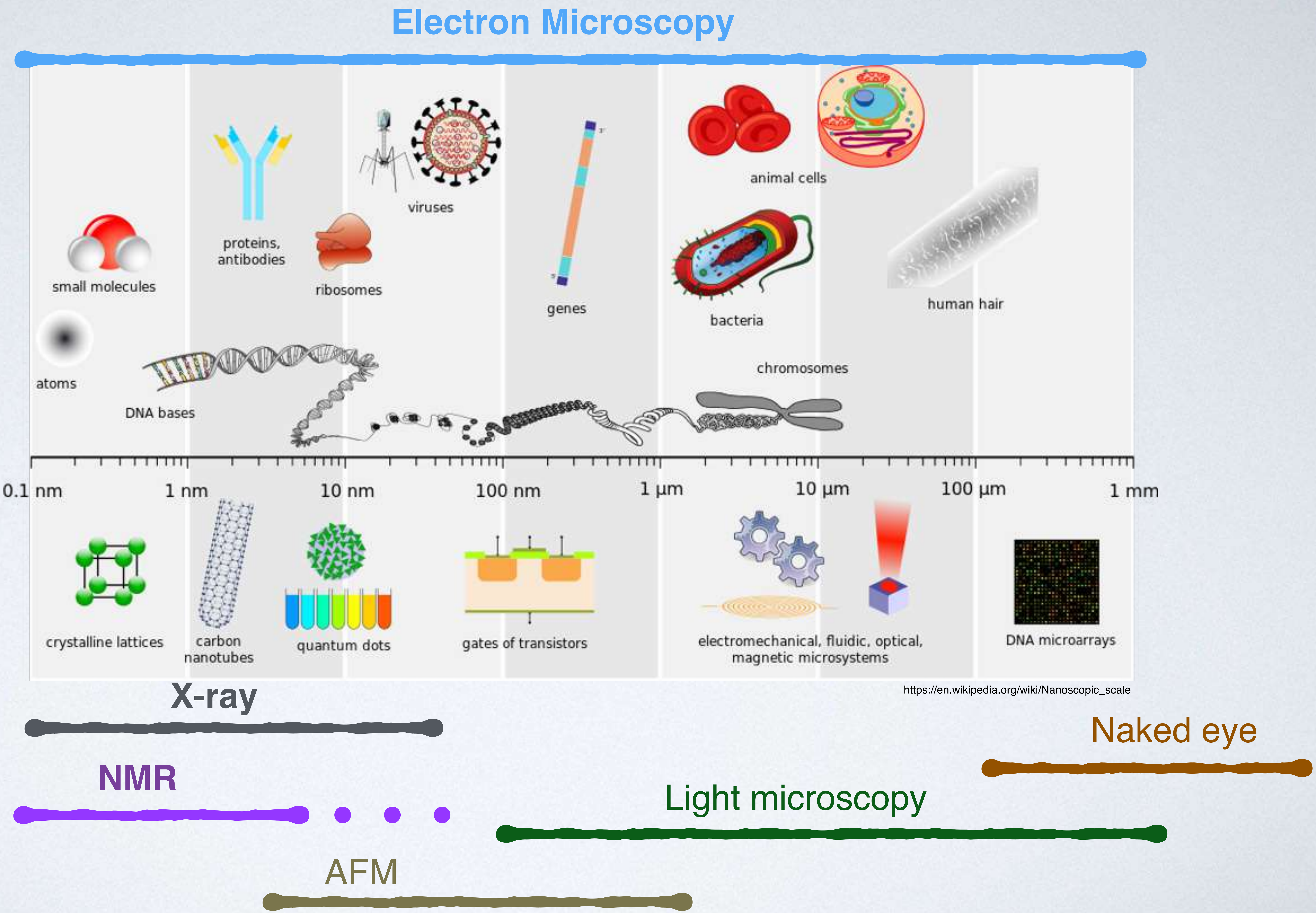
in situ





coming soon

CRYOEM: SCALE WITHIN BIOLOGY



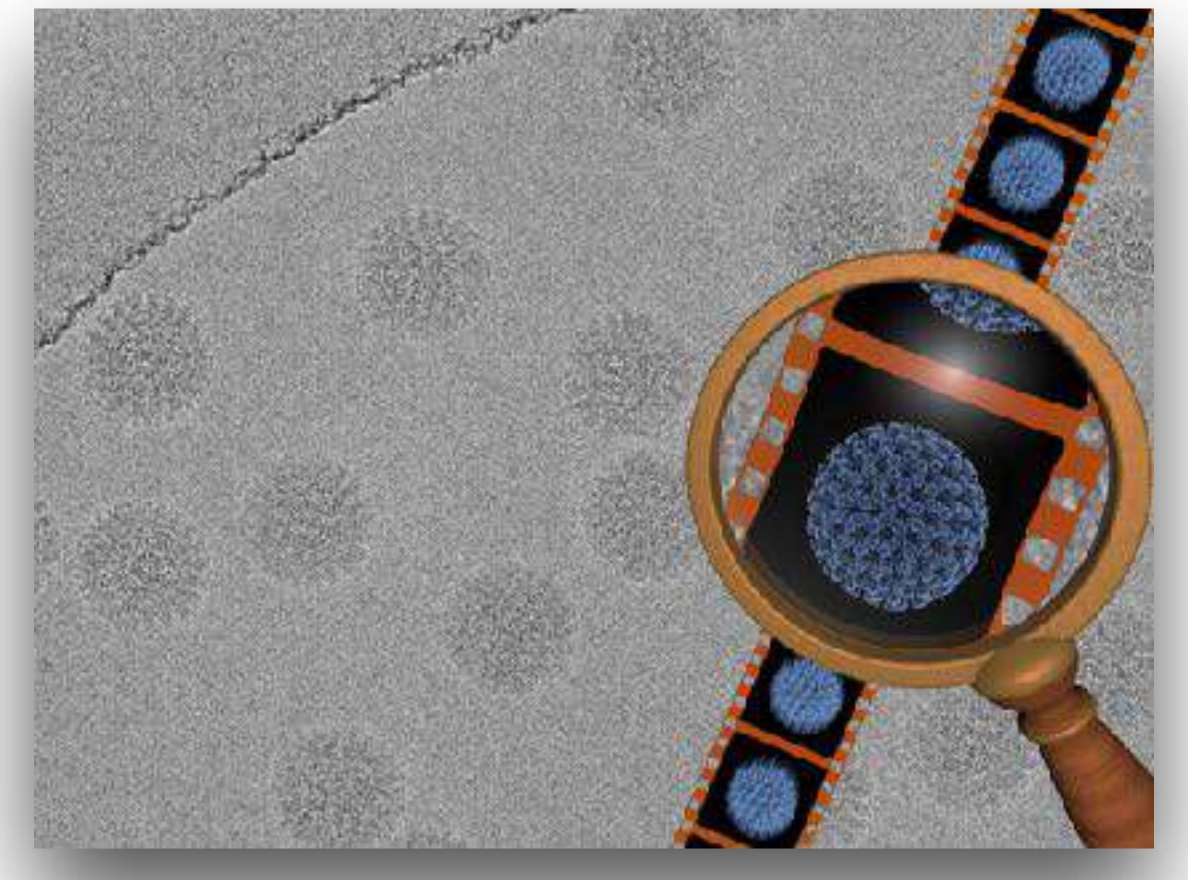
WHAT BROUGHT ABOUT THE RESOLUTION REVOLUTION

(~2012-2014)

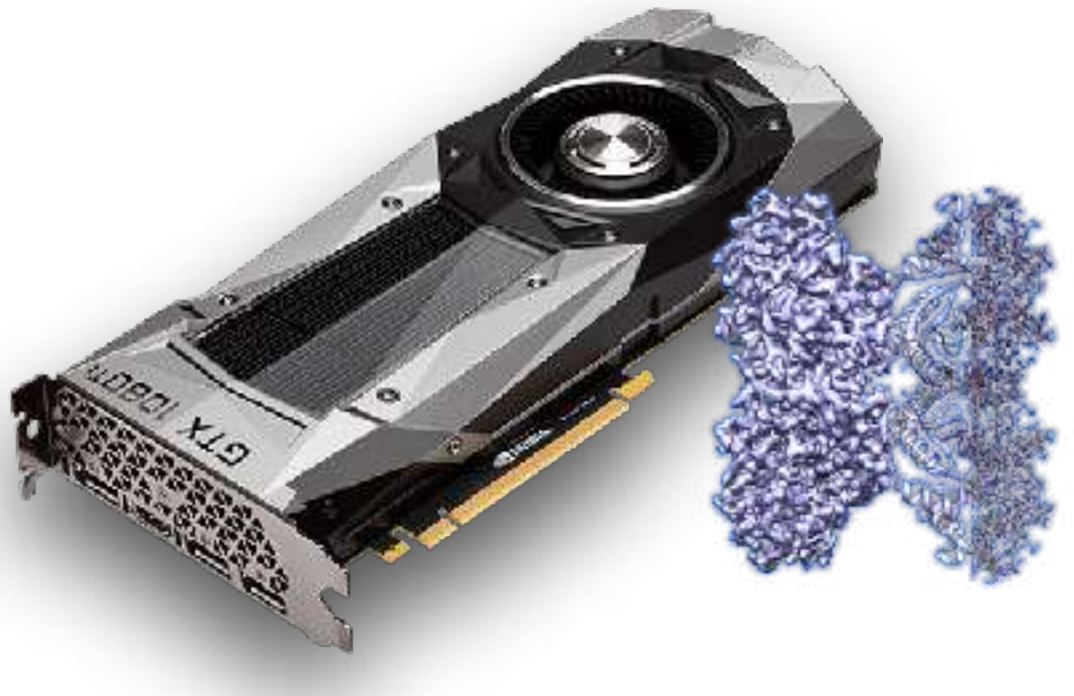
Hardware

Microscopes

Direct Detectors



Computers

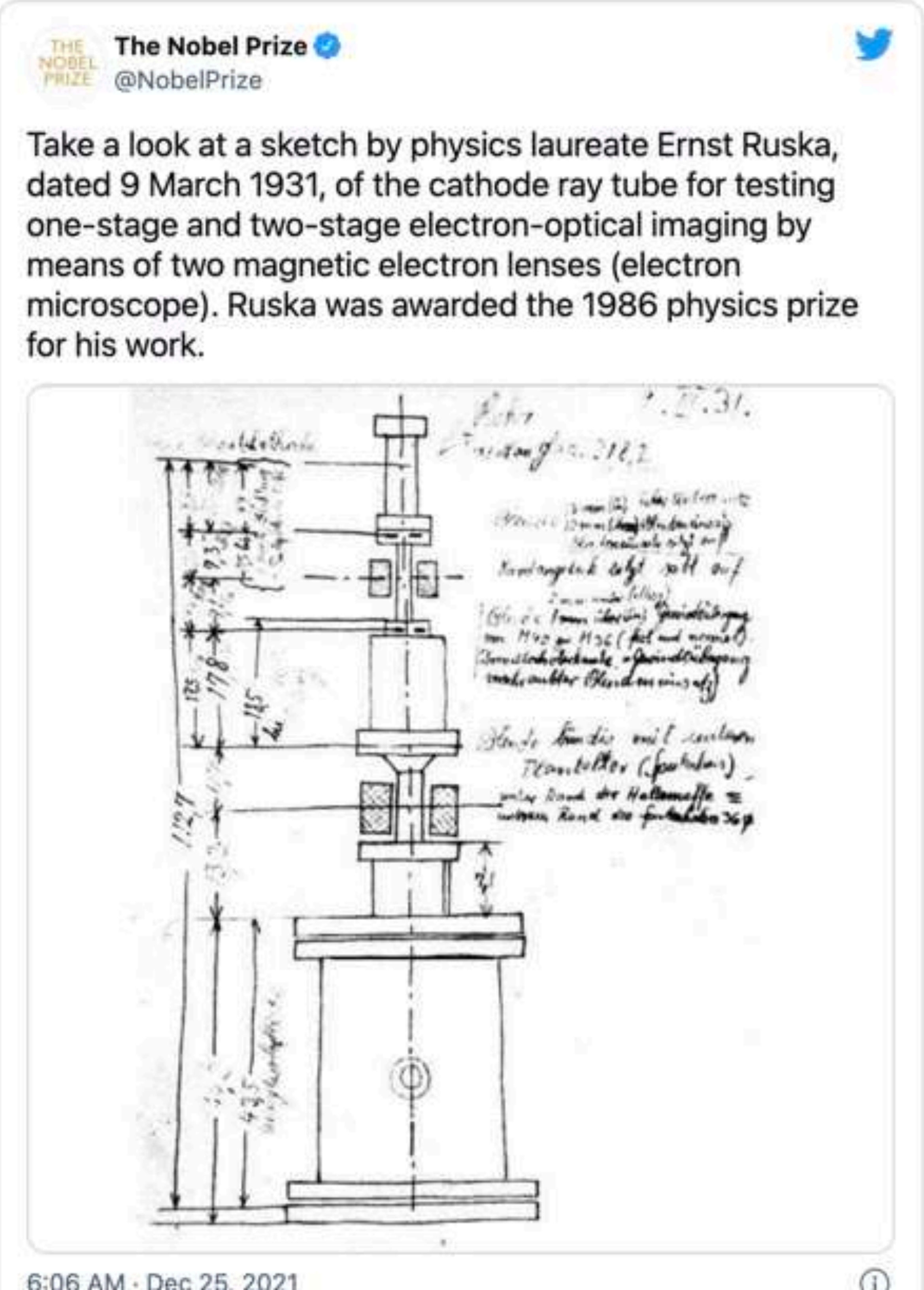


from 2012 to 2017
Cost reduced by 100x

THE ELECTRON MICROSCOPE

Ruska and Knoll in Berlin in the early 1930s

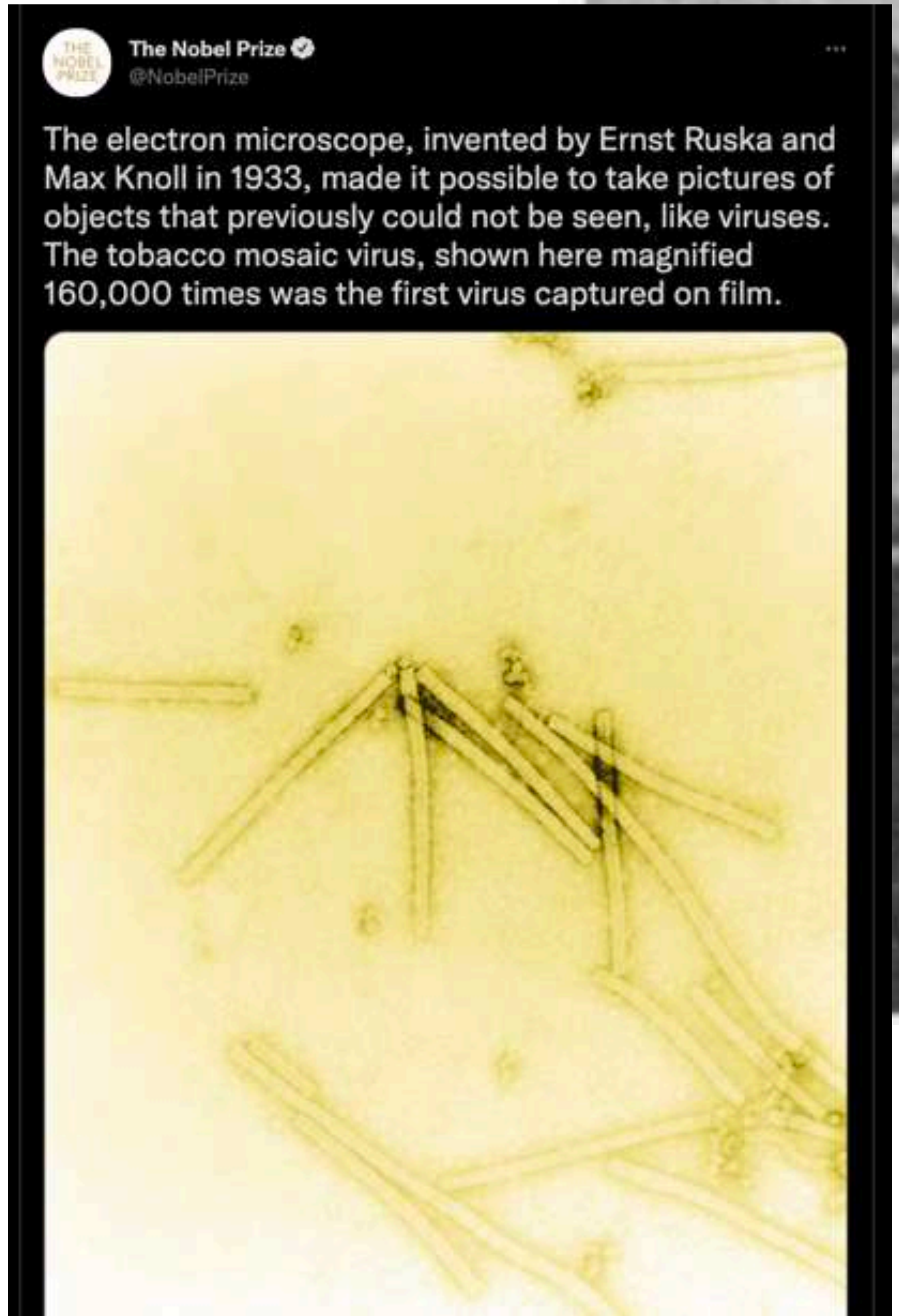
-Wikipedia



The Nobel Prize
@NobelPrize

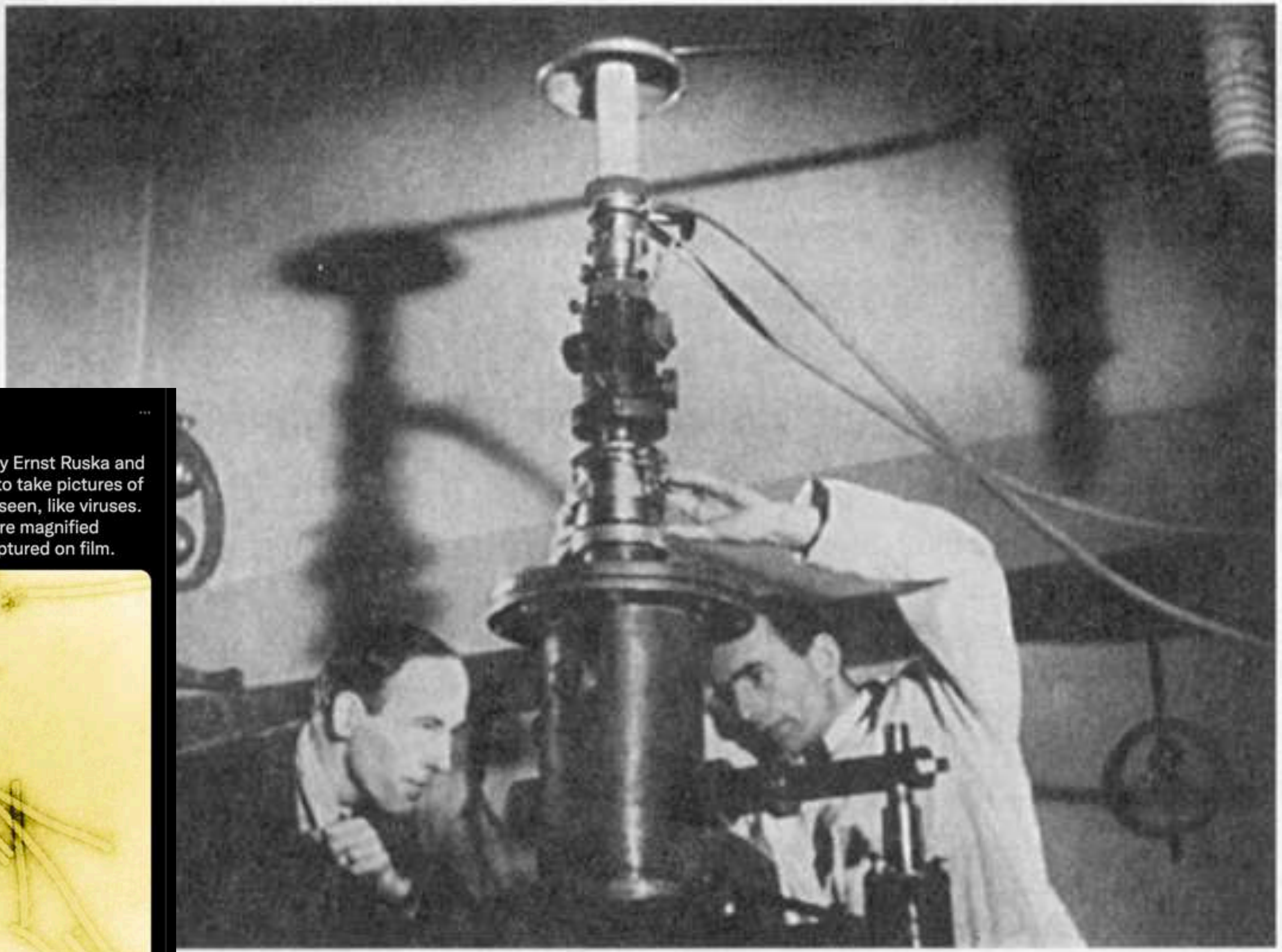
Take a look at a sketch by physics laureate Ernst Ruska, dated 9 March 1931, of the cathode ray tube for testing one-stage and two-stage electron-optical imaging by means of two magnetic electron lenses (electron microscope). Ruska was awarded the 1986 physics prize for his work.

6:06 AM · Dec 25, 2021



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

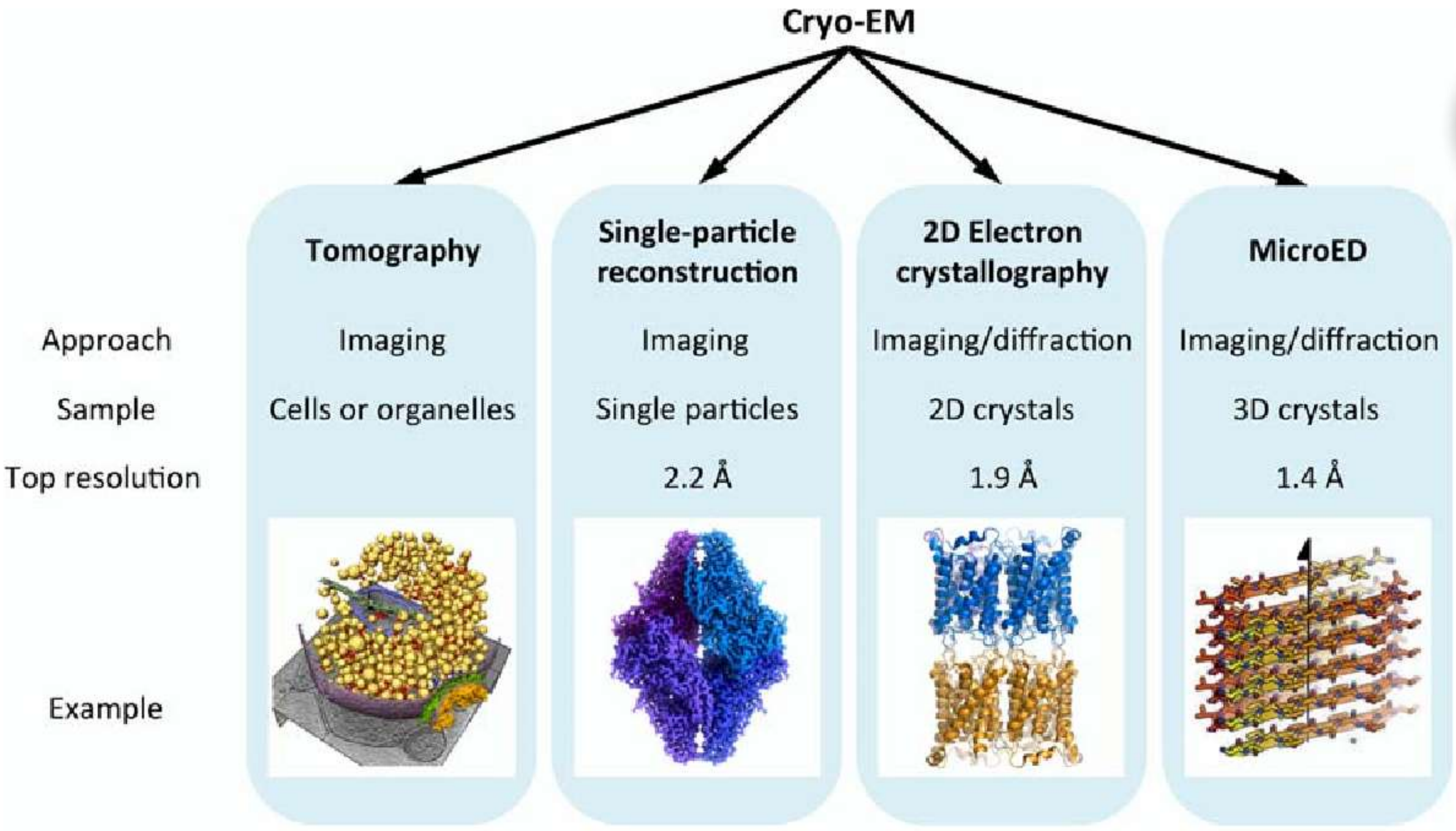
Damages sample

worse with faster electrons

Poor penetration

better with faster electrons

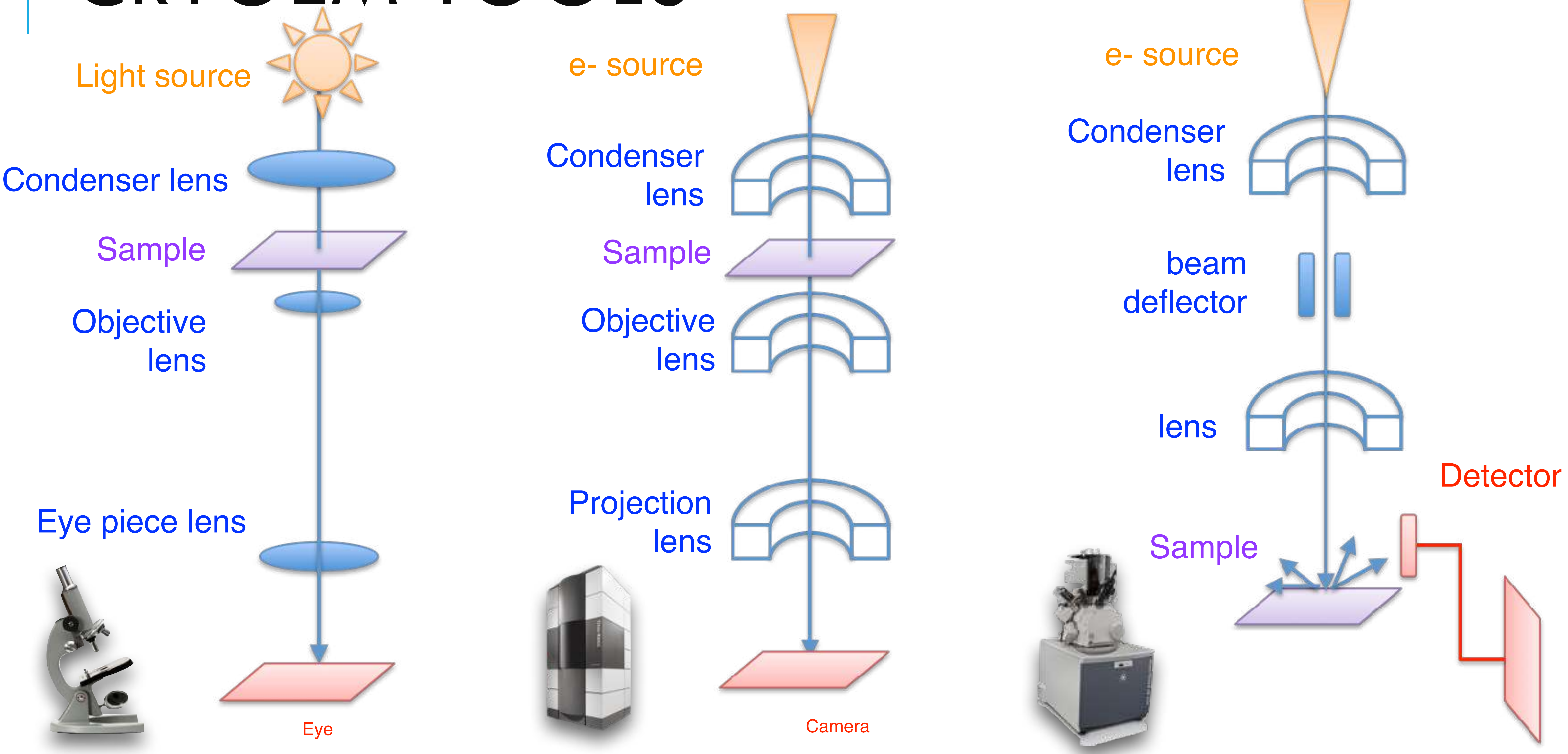
CRYOEM MODALITIES AND TOOLS



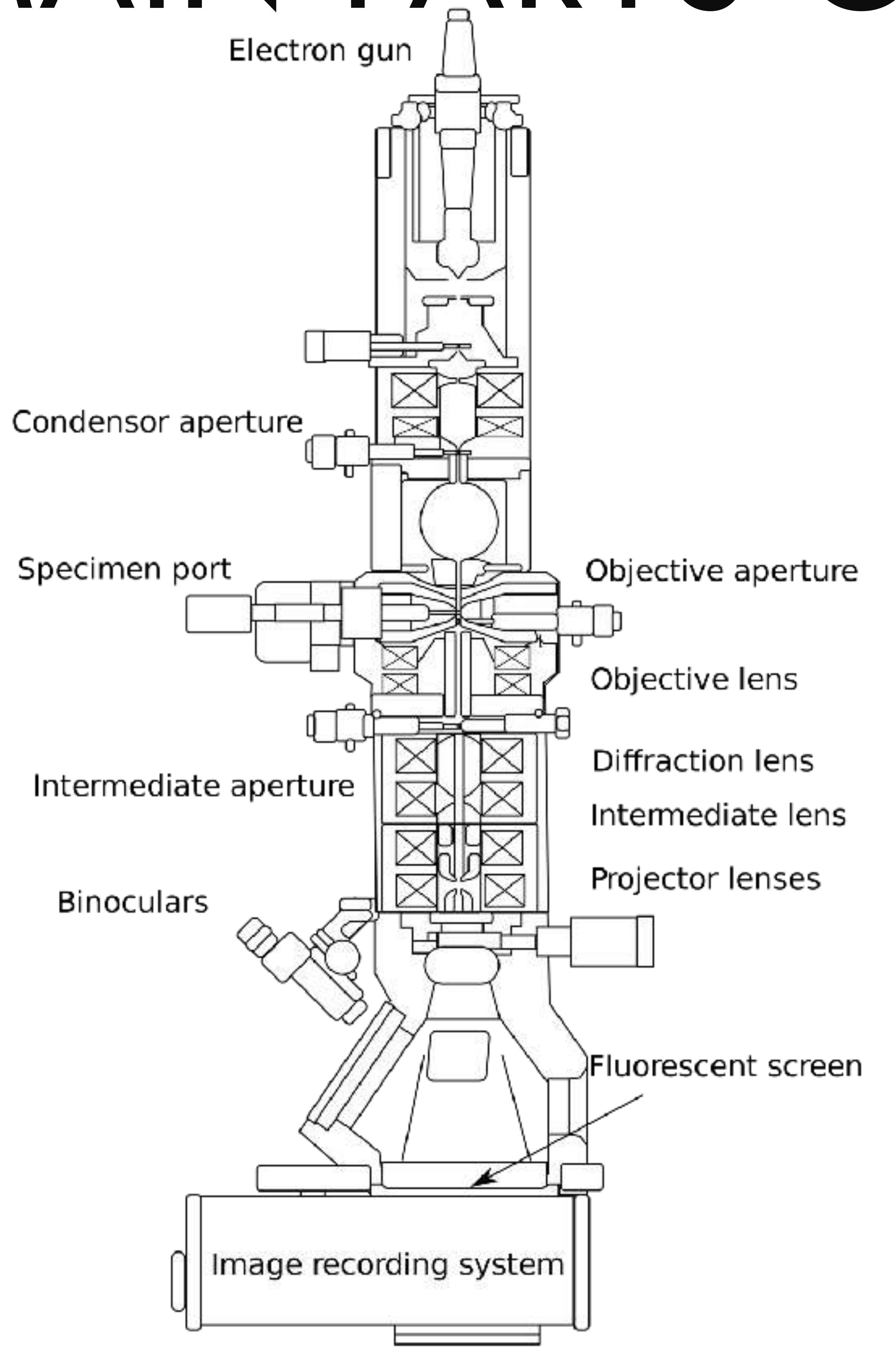
https://doi.org/10.1002/pro.2989

~3.4Å ~1.2Å ~1.9Å ~0.7Å

CRYOEM TOOLS



MAIN PARTS OF AN EM



Electron sources

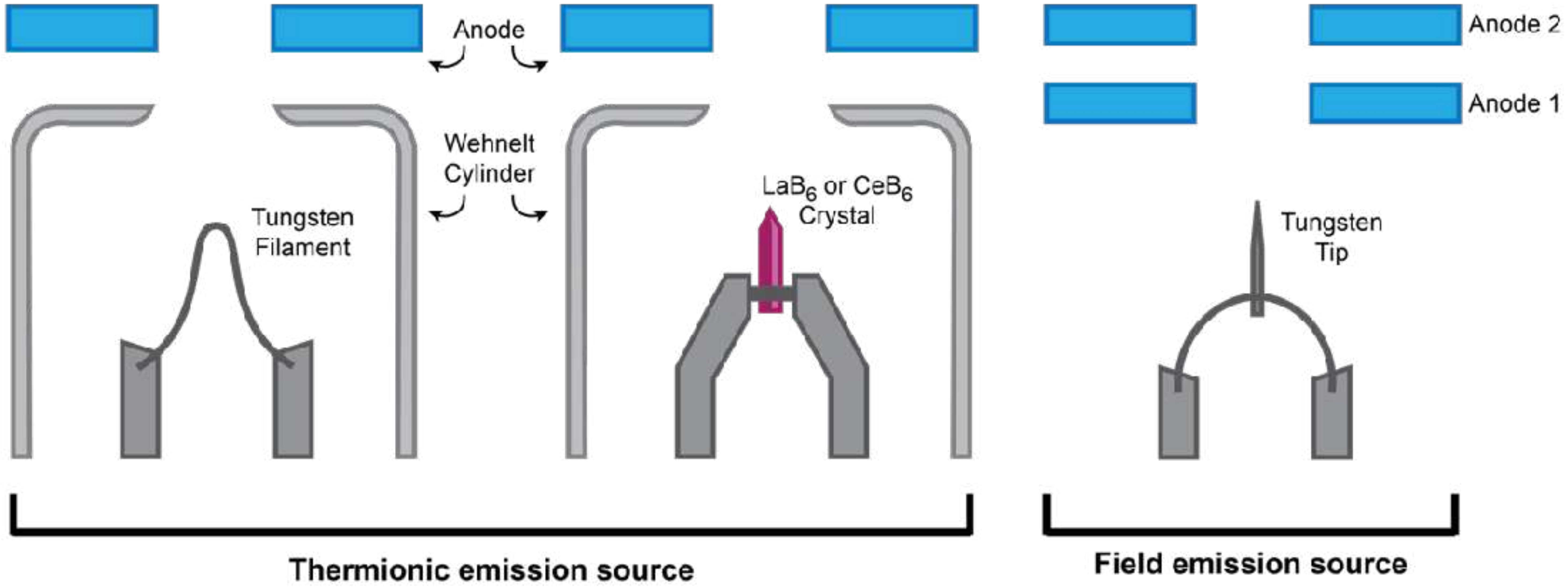
Vacuum systems

Lenses

Detectors

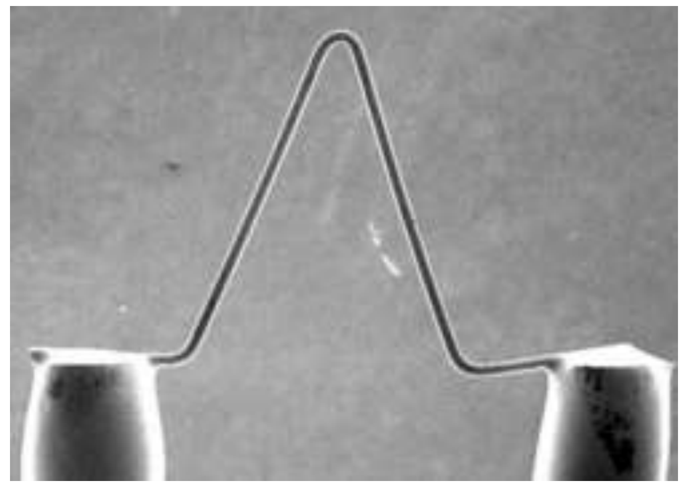
ELECTRON SOURCES

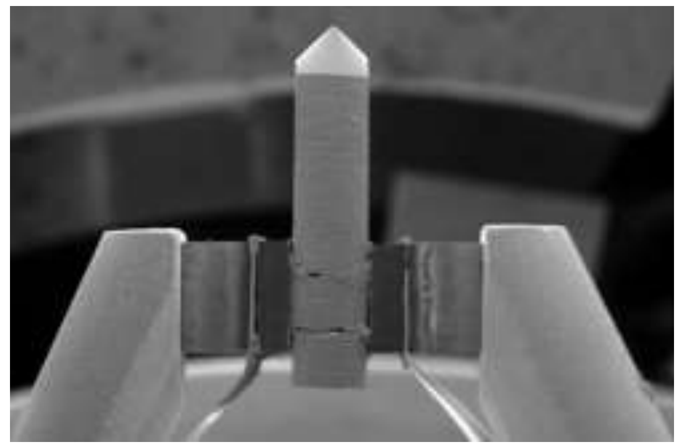
What are the 3 main kinds of electron sources?

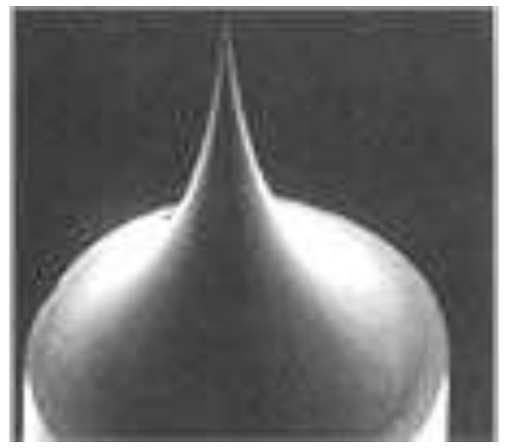


www.thermofisher.com

nanoscience.com

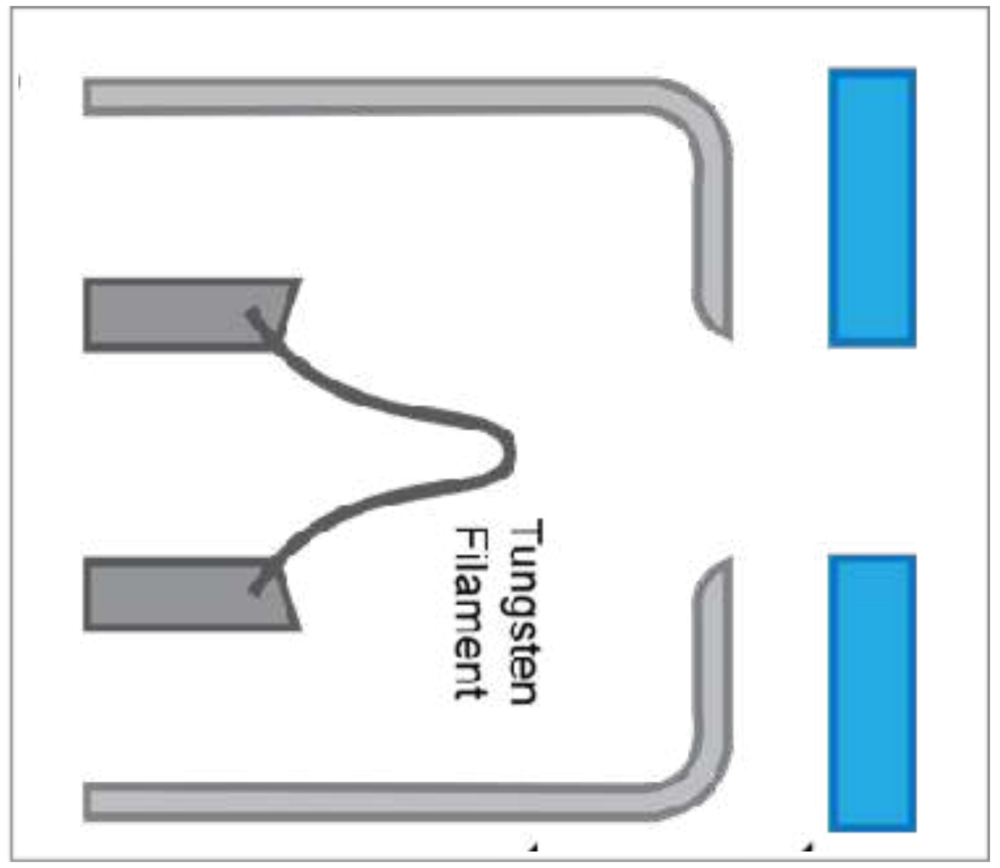


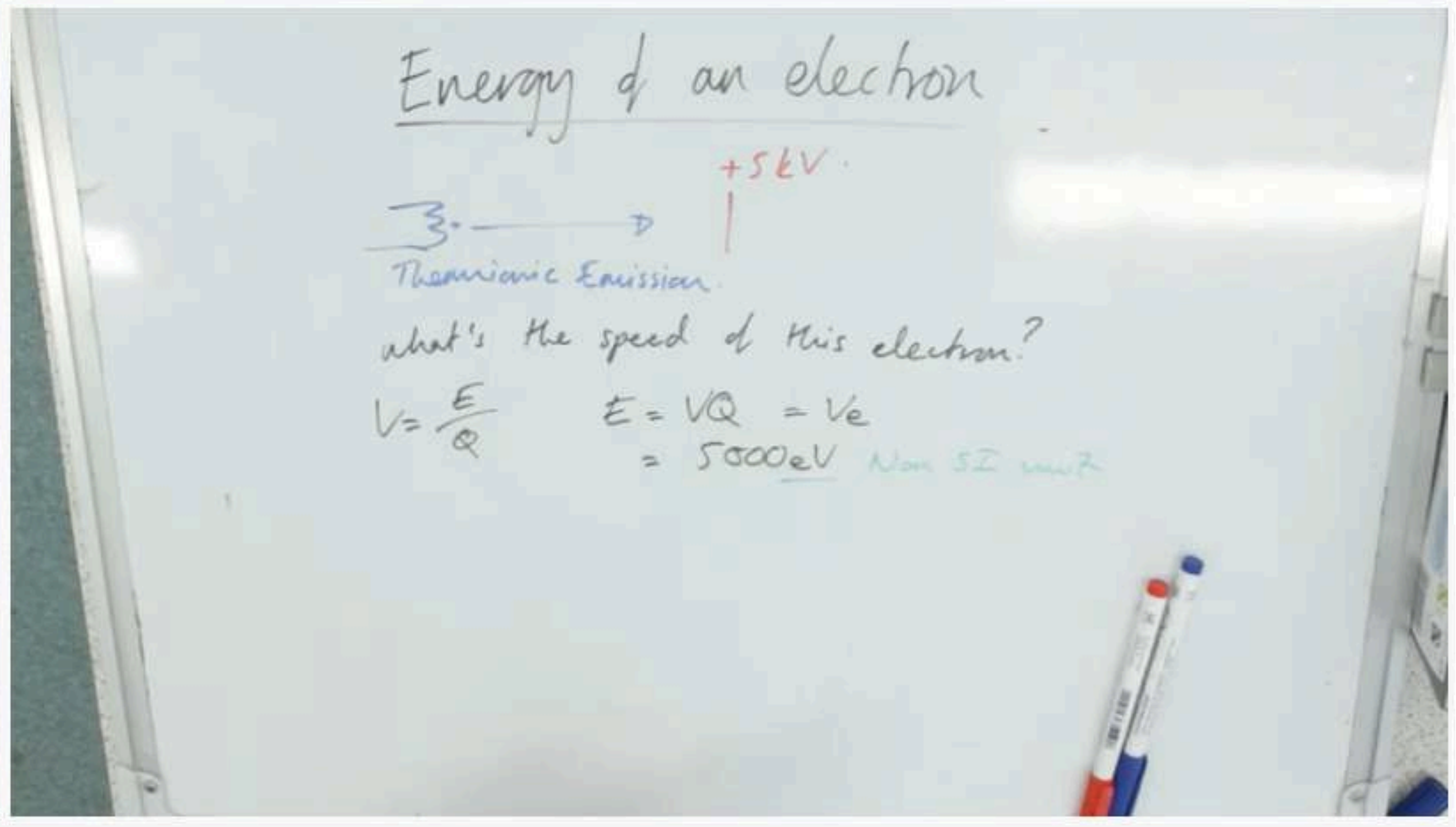




ELECTRON SOURCES

How fast are the electrons moving?

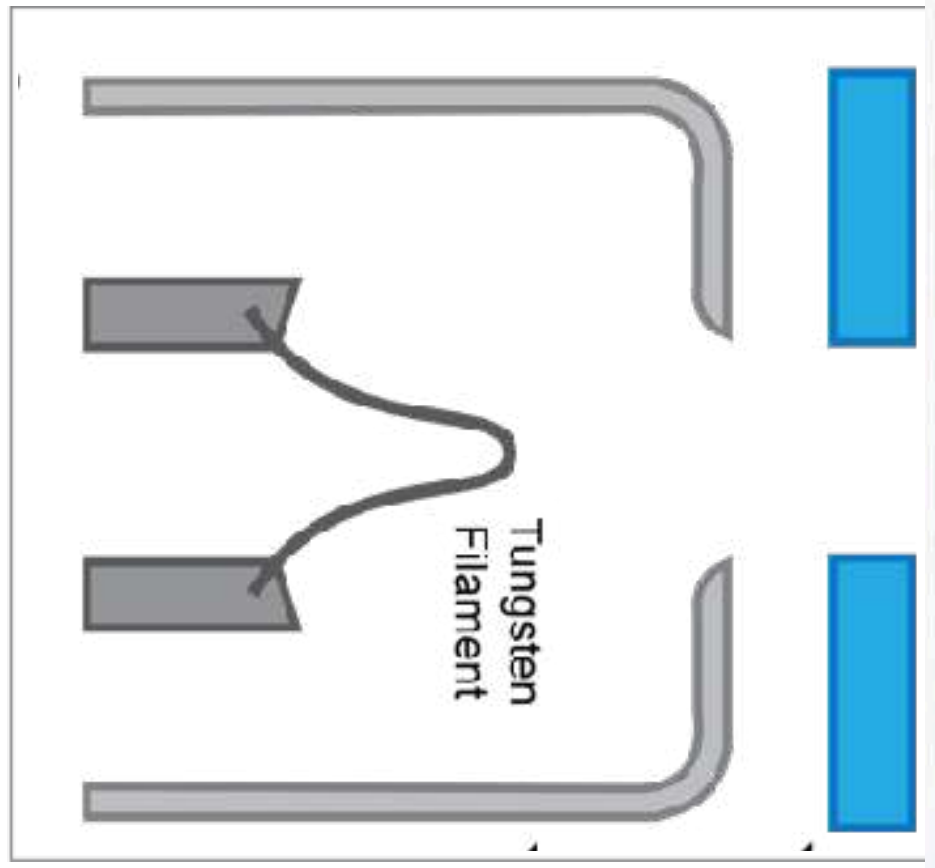


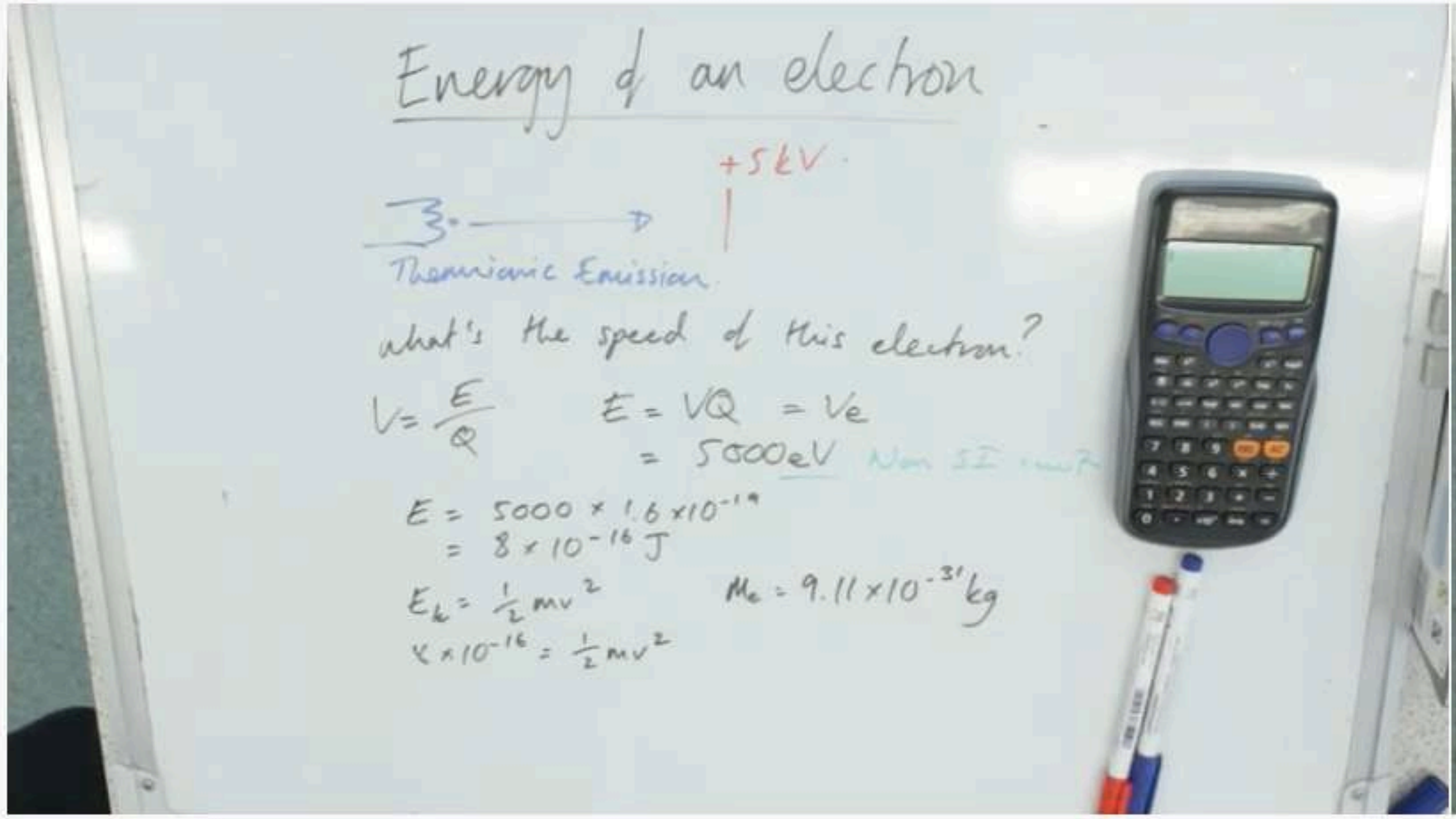


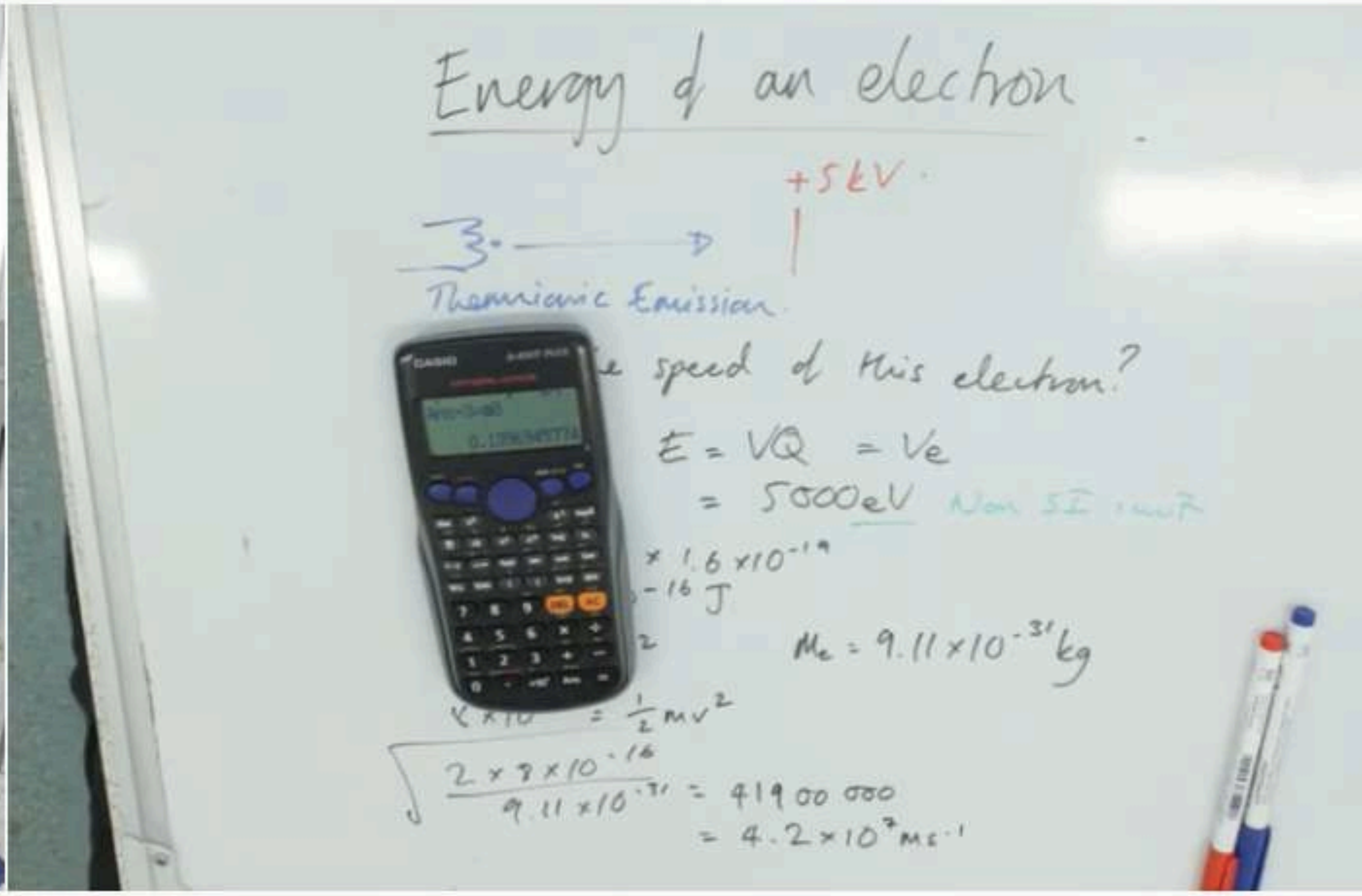
https://www.youtube.com/watch?v=tYCET6vYdYk

ELECTRON SOURCES

How fast are the electrons moving?

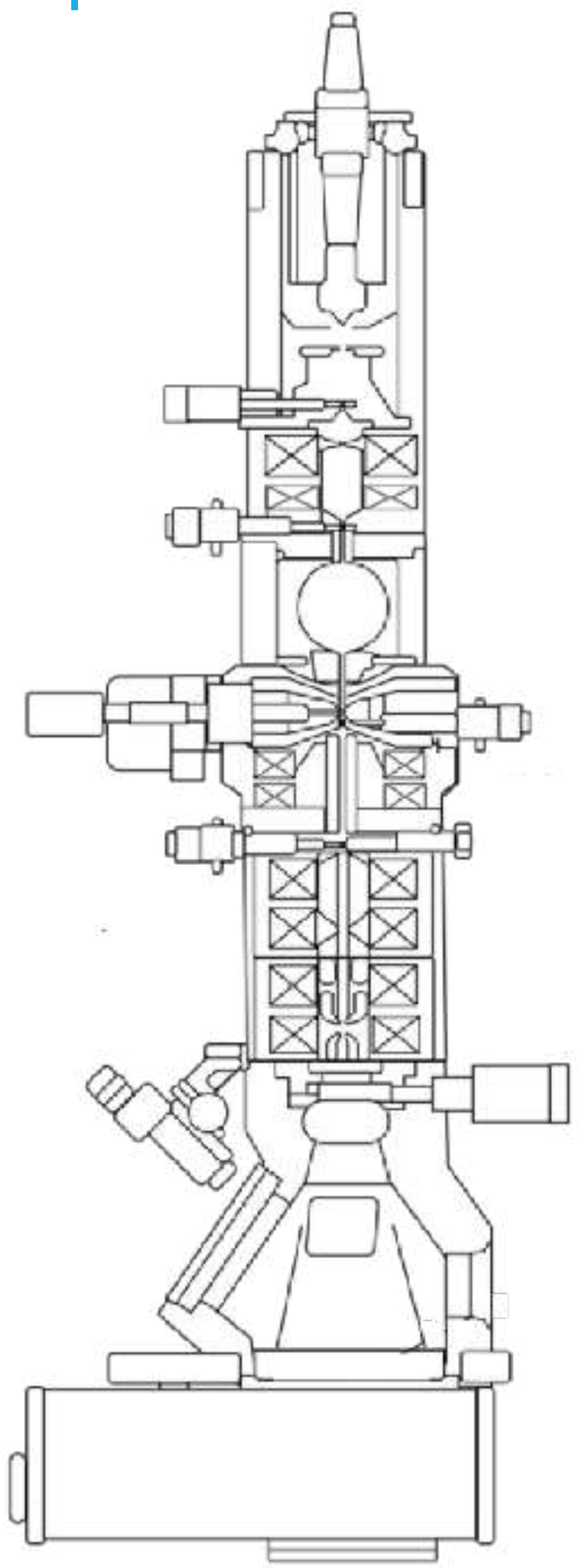


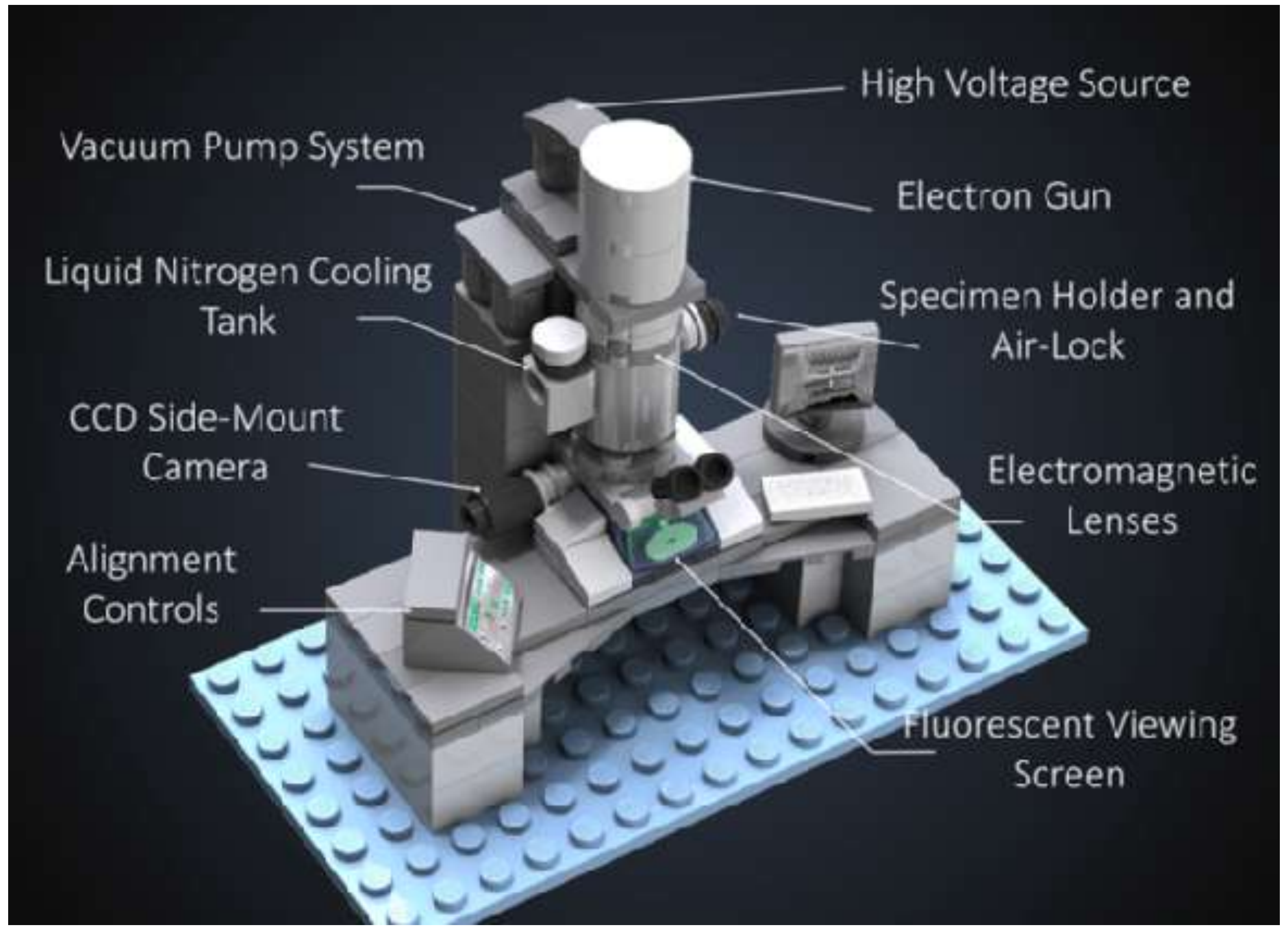




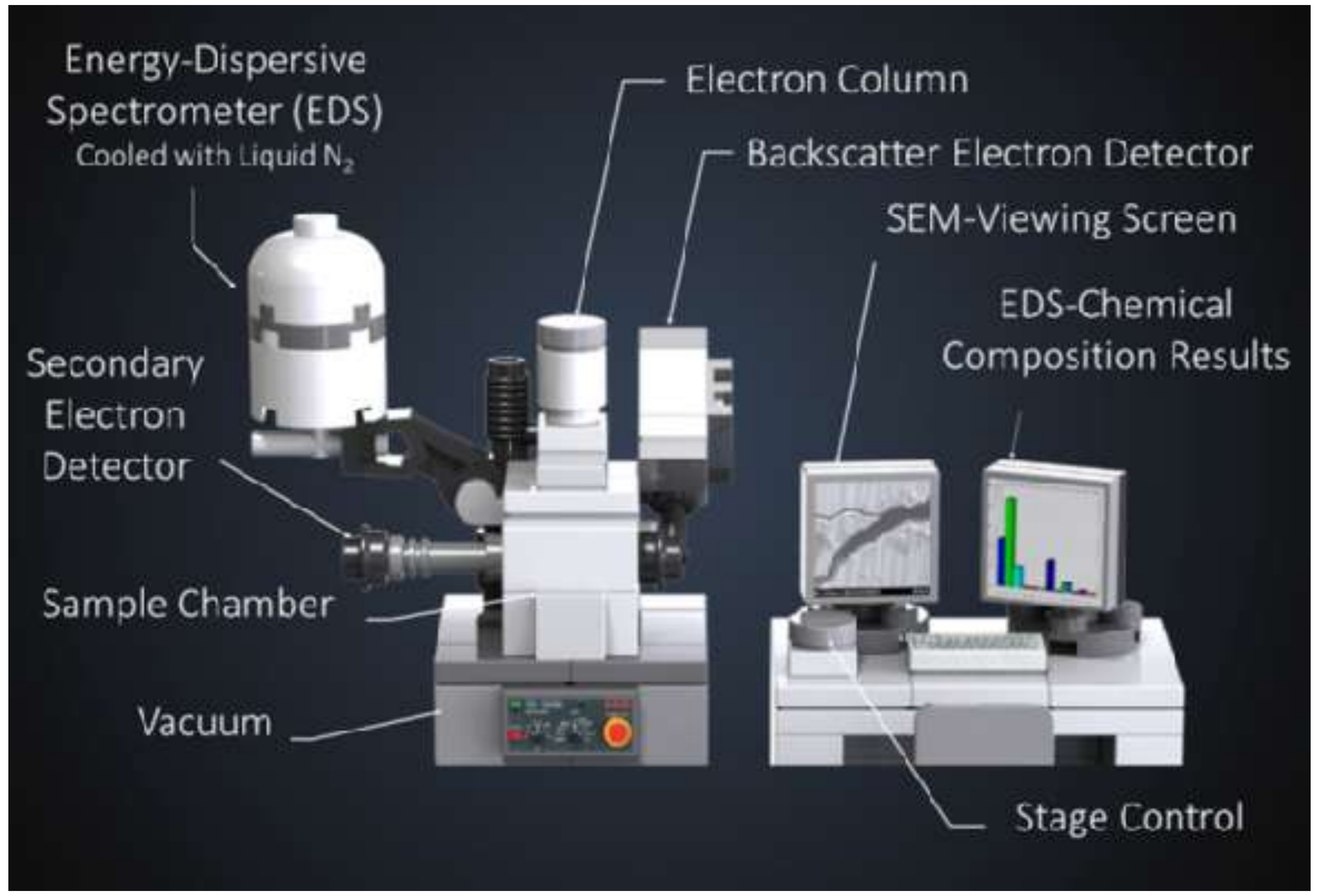
https://www.youtube.com/watch?v=tYCET6vYdYk

ELECTRON SOURCES & TYPES OF EMS

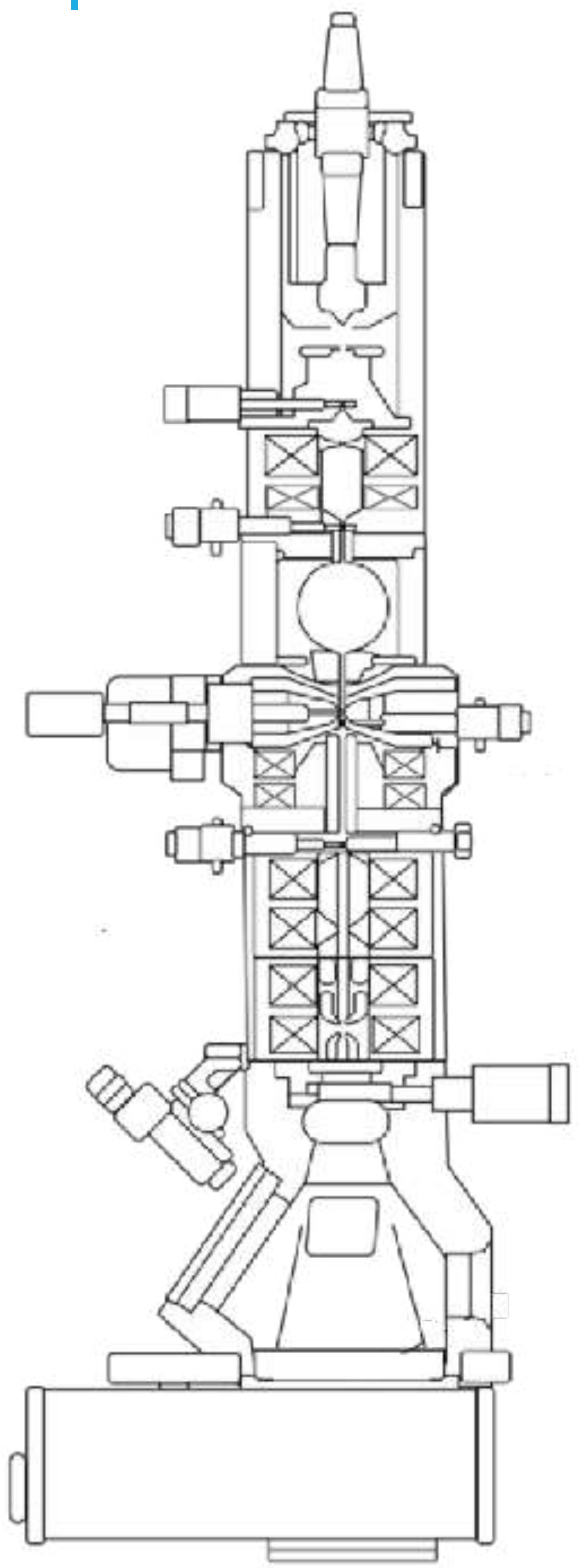




https://ideas.lego.com/projects/102281



ELECTRON SOURCES & TYPES OF EMS



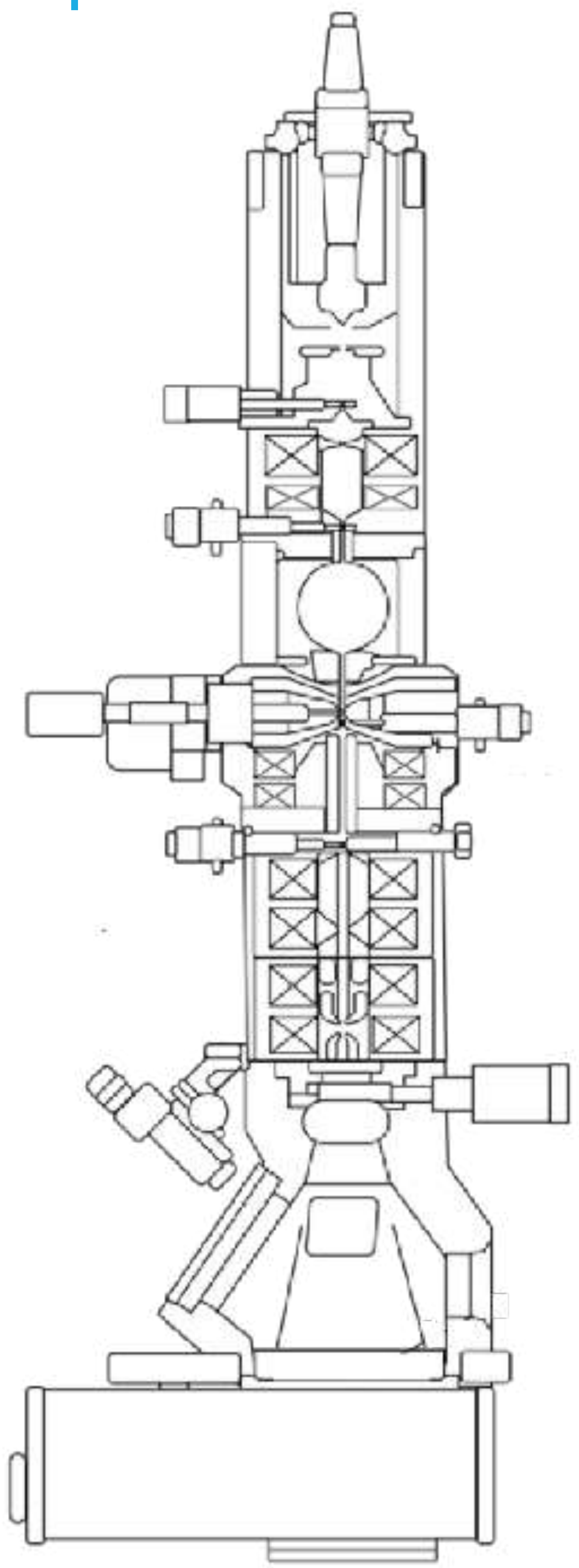
80-120 kV: JEM 1230, JEM1400; Spirit, Tecnai T12, Talos L120
W or LaB6
High contrast & robust
sub-nm resolution

100 kV: screening TEMs, Tundra
FEG
3-7Å resolution

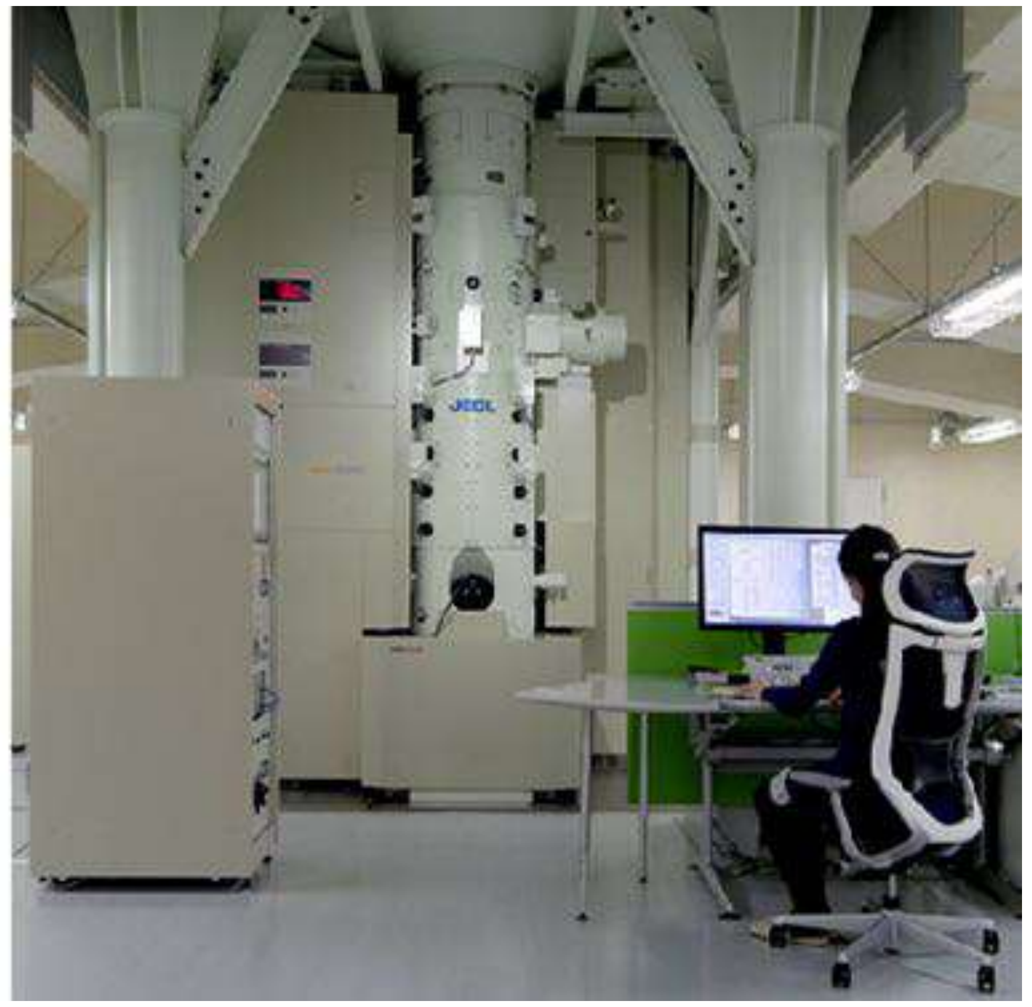
200 kV: JEM 2100F, Tecnai F20, Talos, Glacios, Arctica
FEG
2-5 Å resolution

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

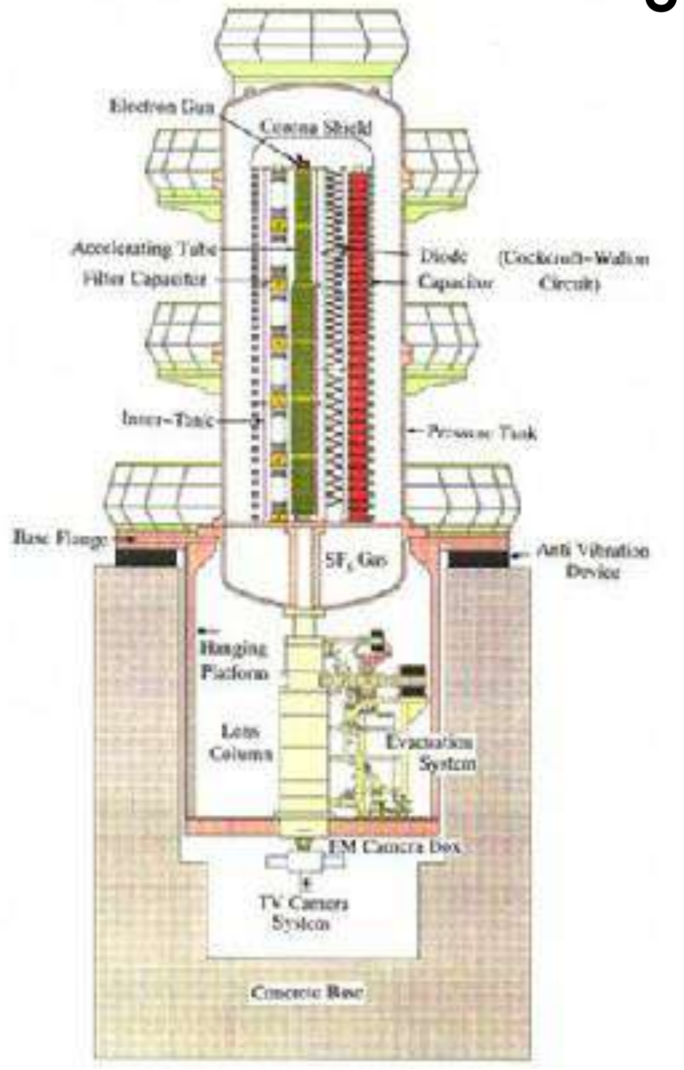


1-1.2 MV: Hitachi, JEOL
LaB6



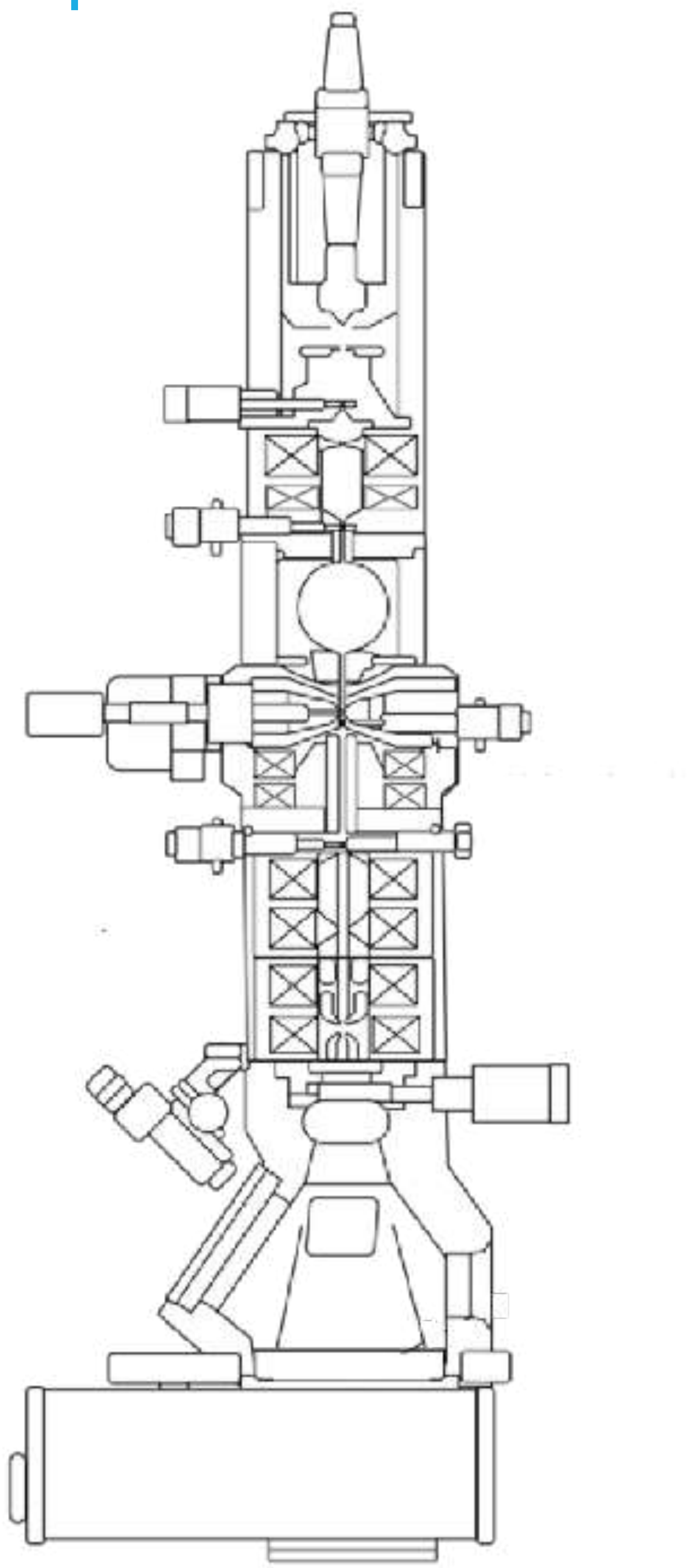
uhvem.osaka-u.ac.jp

3 MV: Hitachi H3000
LaB6



VACUUM SYSTEMS

Why do we need a vacuum?



Beam coherence - at STP mean free path ~1 cm

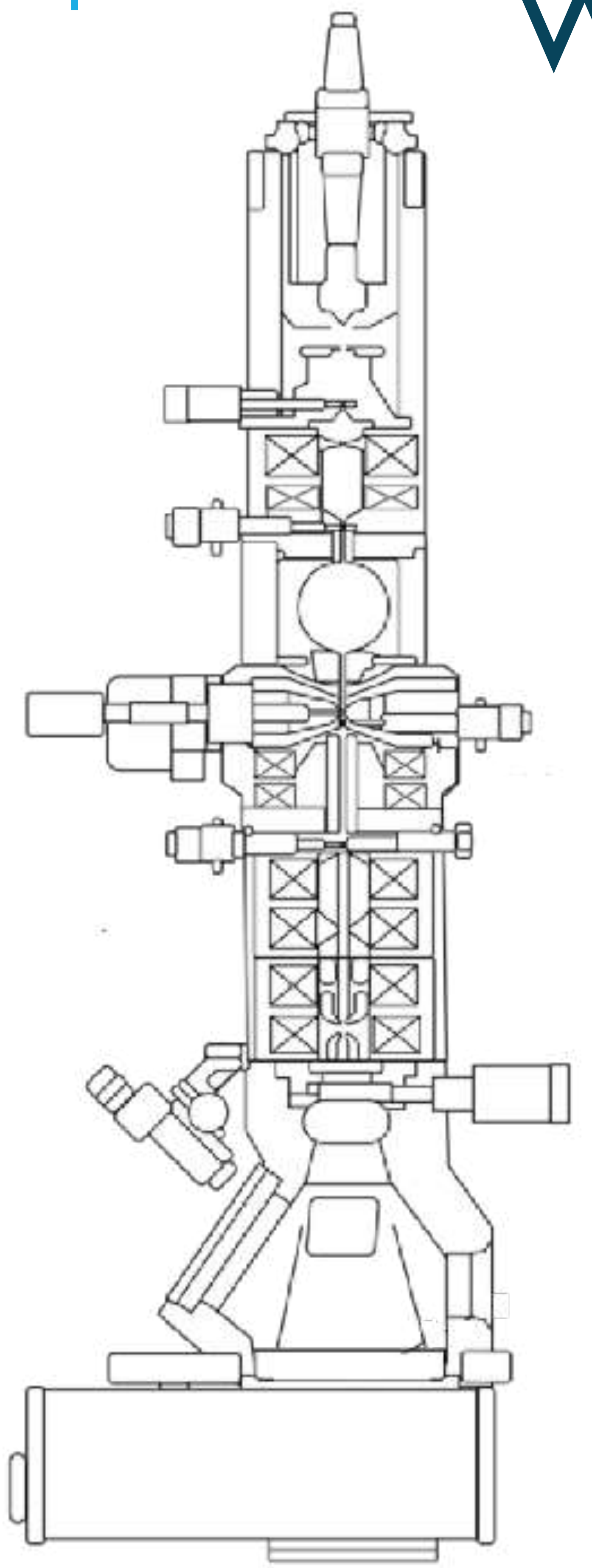
Insulation - interaction between e- and air

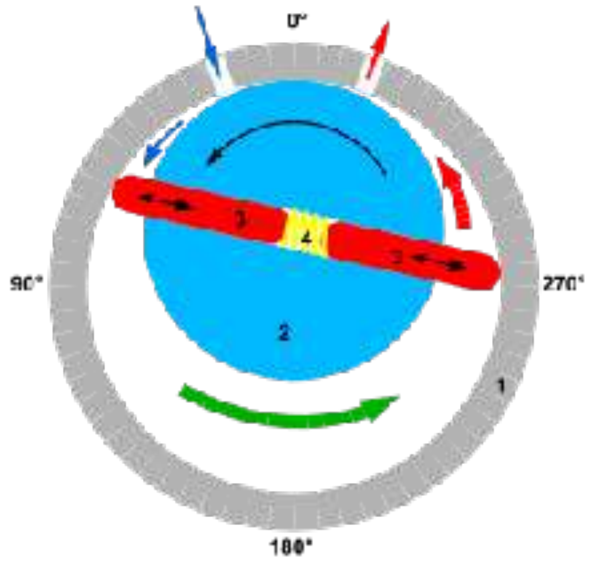
Filament - O2 will burn out source

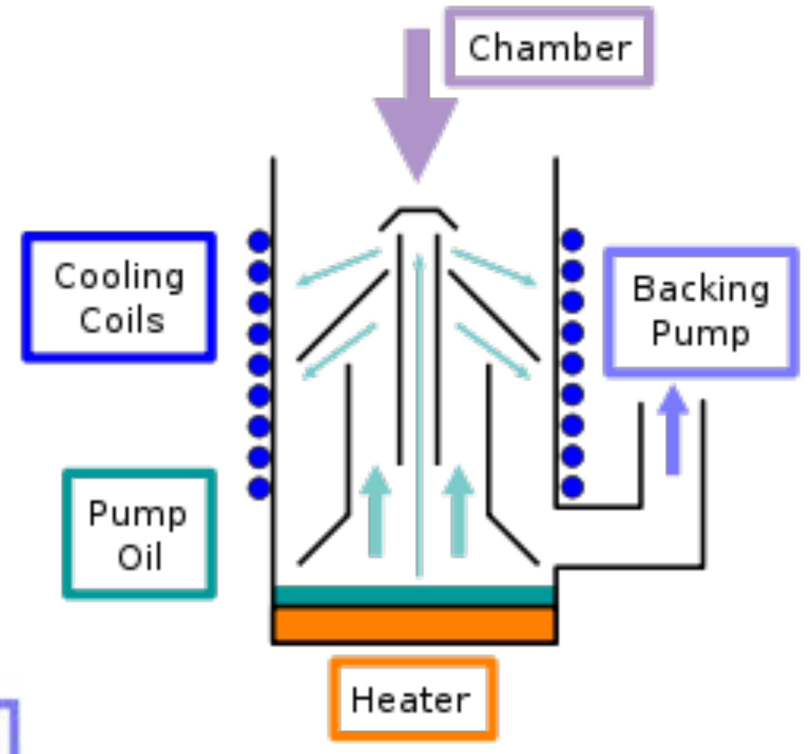
Contamination - reduce interaction gas, e-beam and sample

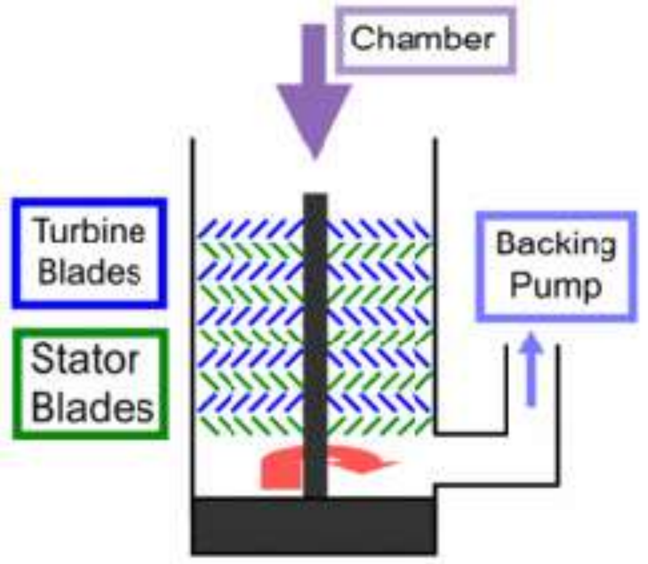
VACUUM SYSTEMS

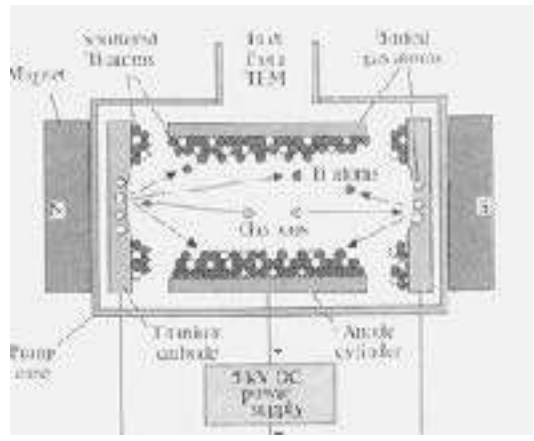
What types of pumps do we have?











wikipedia.com

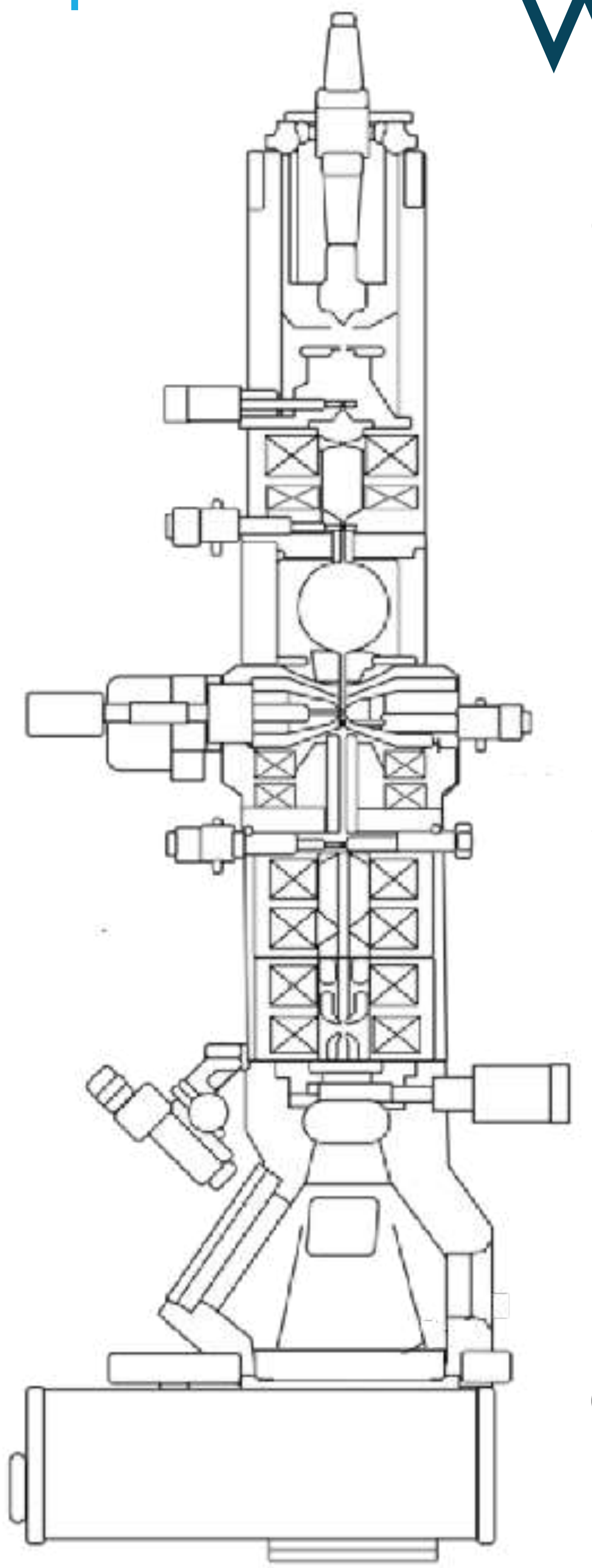
1 mm Hg = 1 Torr = 10^2 Pa

1 atm = 760 Torr = $7.5\text{x}10^4$ Pa

PVP / Rotary	$1\text{-}10^{-3}$ Torr	>0.1 Pa
Diffusion	$10^{-3}\text{-}10^{-6}$ Torr	$0.1\text{-}10^{-4}$ Pa
Turbo	$10^{-6}\text{-}10^{-9}$ Torr	$10^{-4}\text{–}10^{-7}$ Pa
IGP	$10^{-9}\text{-}10^{-12}$ Torr	$10^{-7}\text{-}10^{-9}$ Pa

VACUUM SYSTEMS

What types of pumps do we have?



Gun

10^{-9} Torr

Specimen

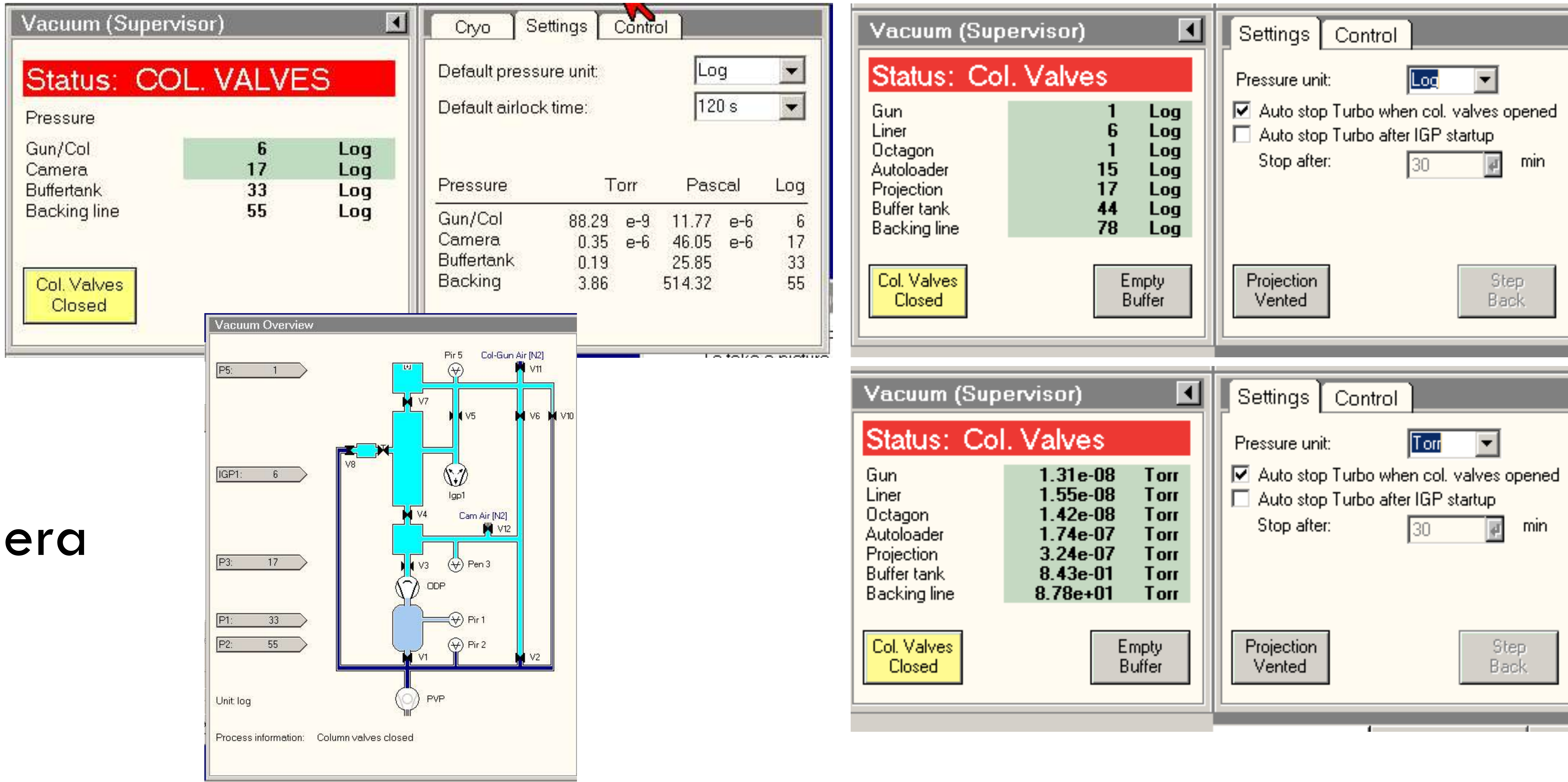
10^{-6} - 10^{-7} Torr

Chamber and Camera

10^{-5} - 10^{-6} Torr

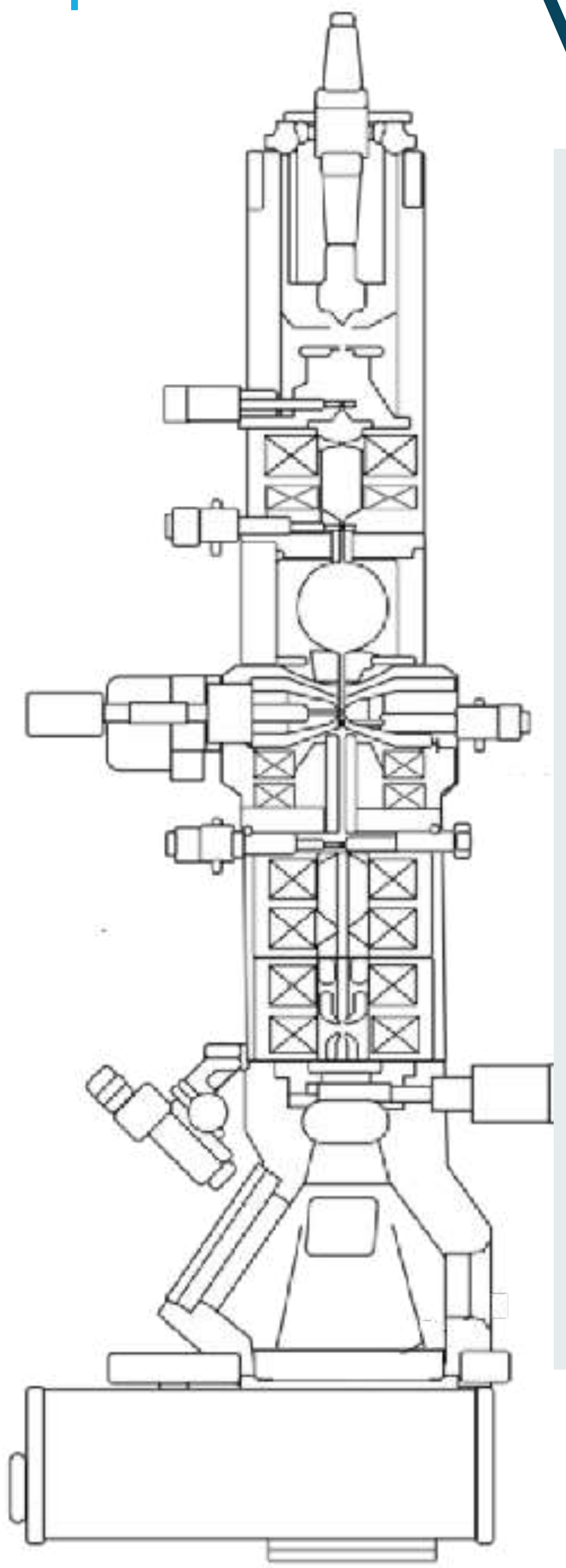
1 mm Hg = 1 Torr = 10^2 Pa

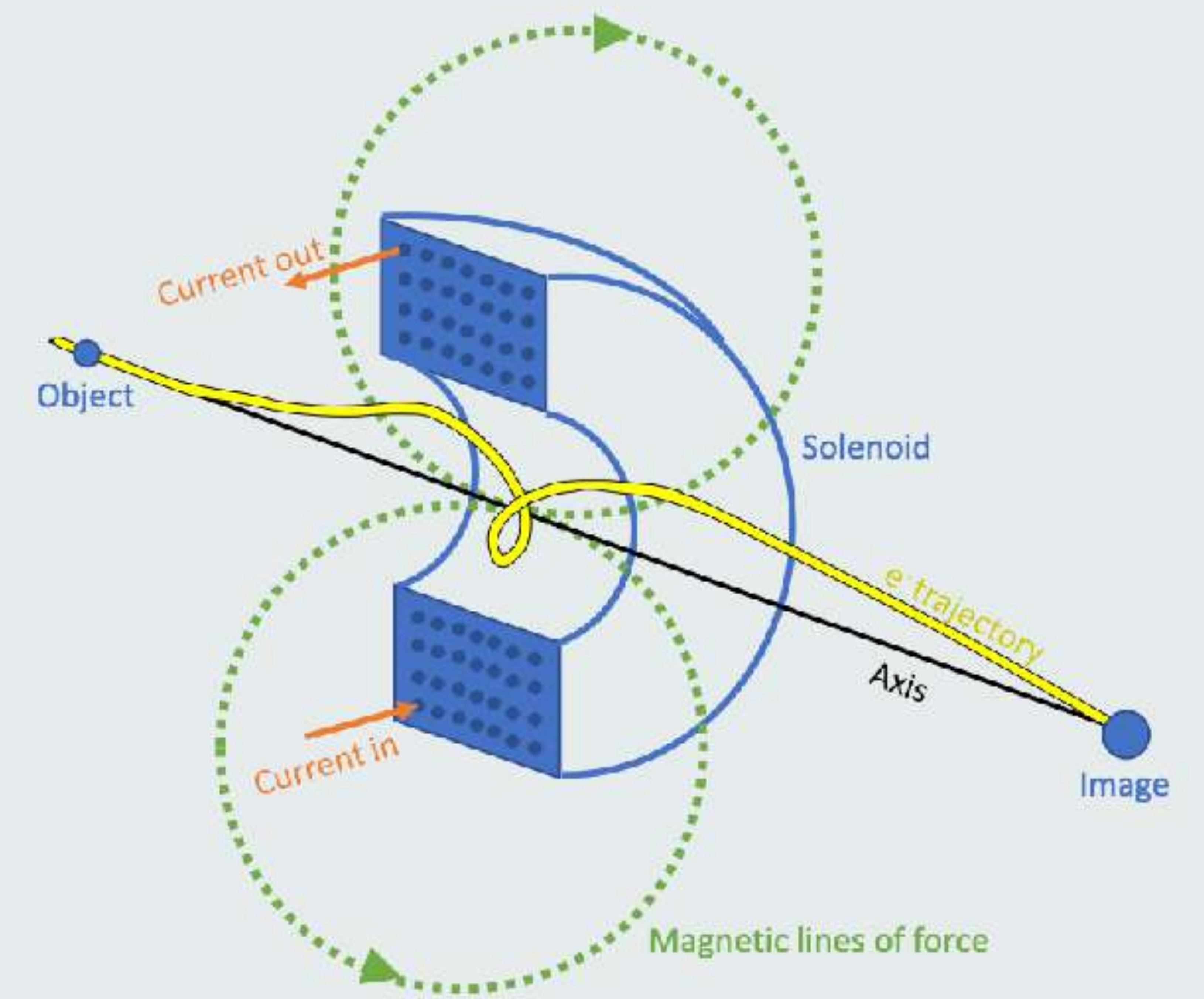
1 atm = 760 Torr = 7.5×10^4 Pa



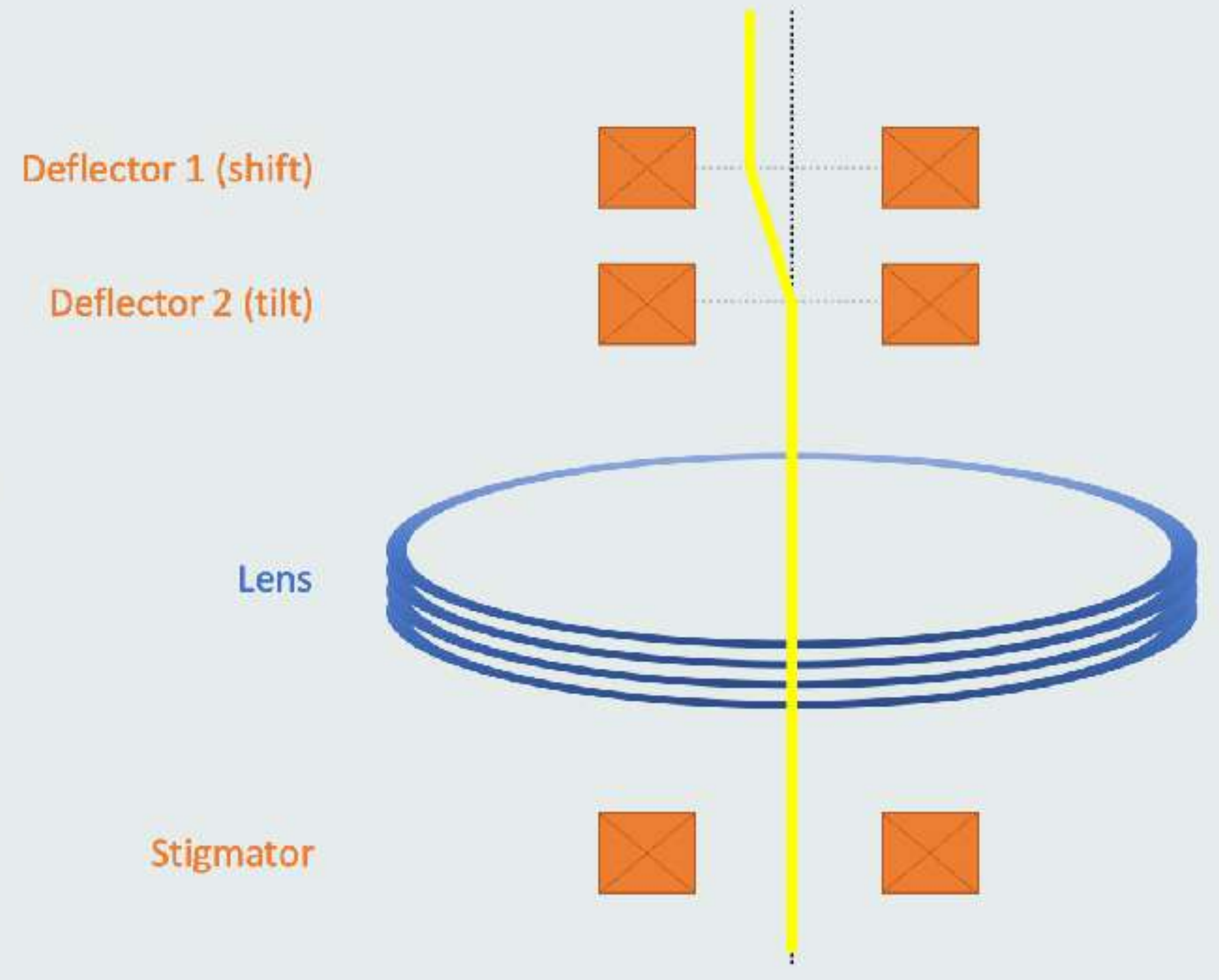
LENSES

What types of lenses do we have?





- Focus
- Magnify
- Rotate



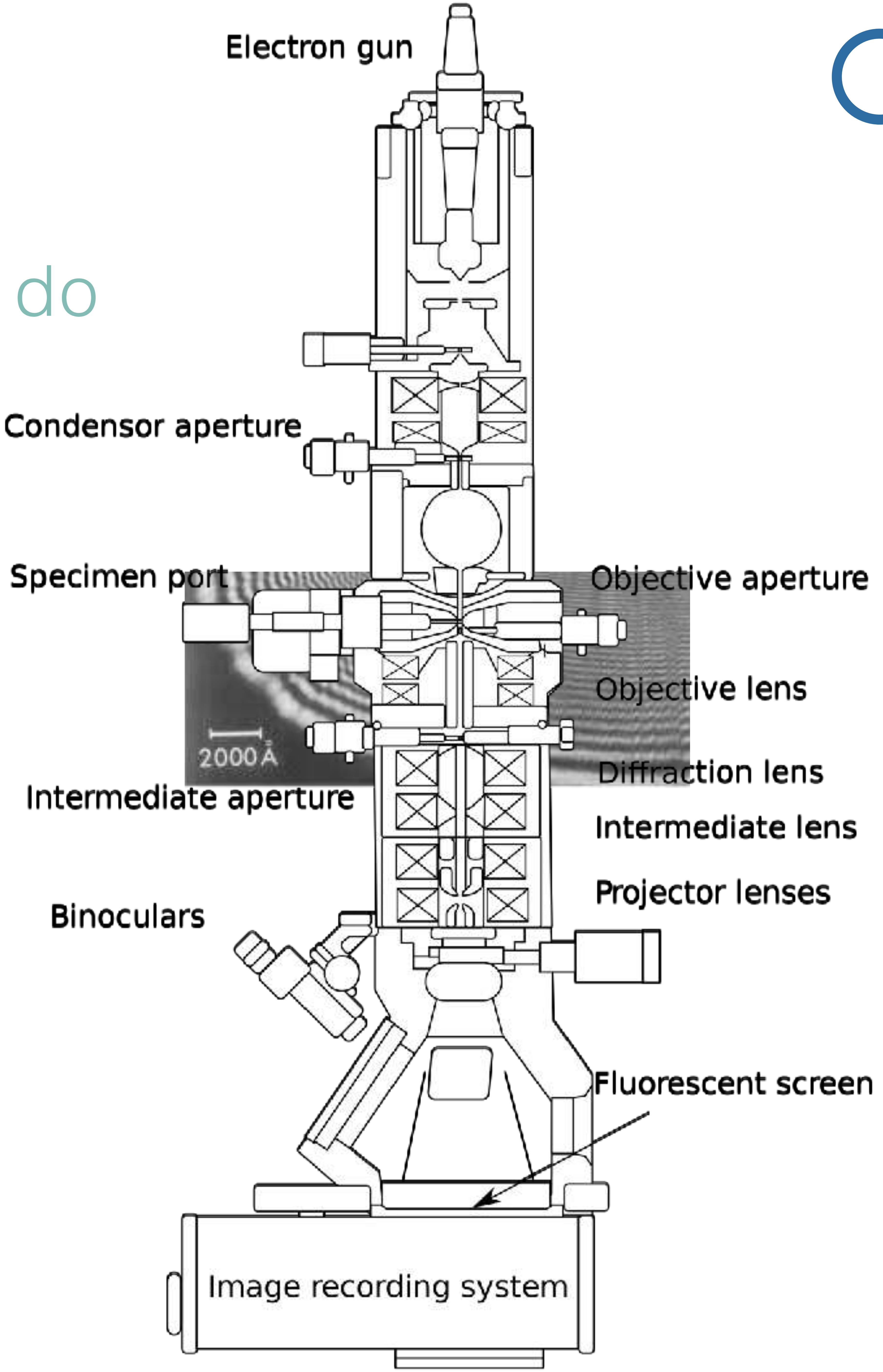
LENSES Microscope Alignments What to do & what not to do

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)~~



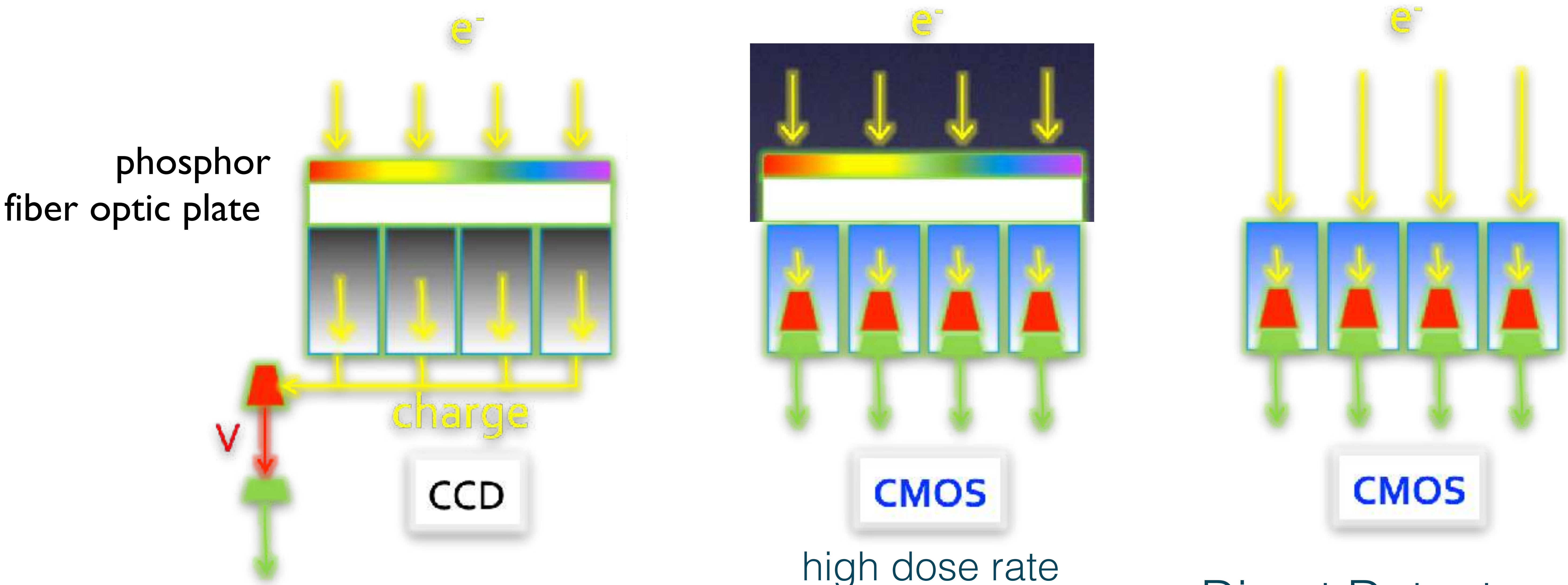
DETECTORS Digital Cameras for TEM

Photon converted

Direct sensing

?

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

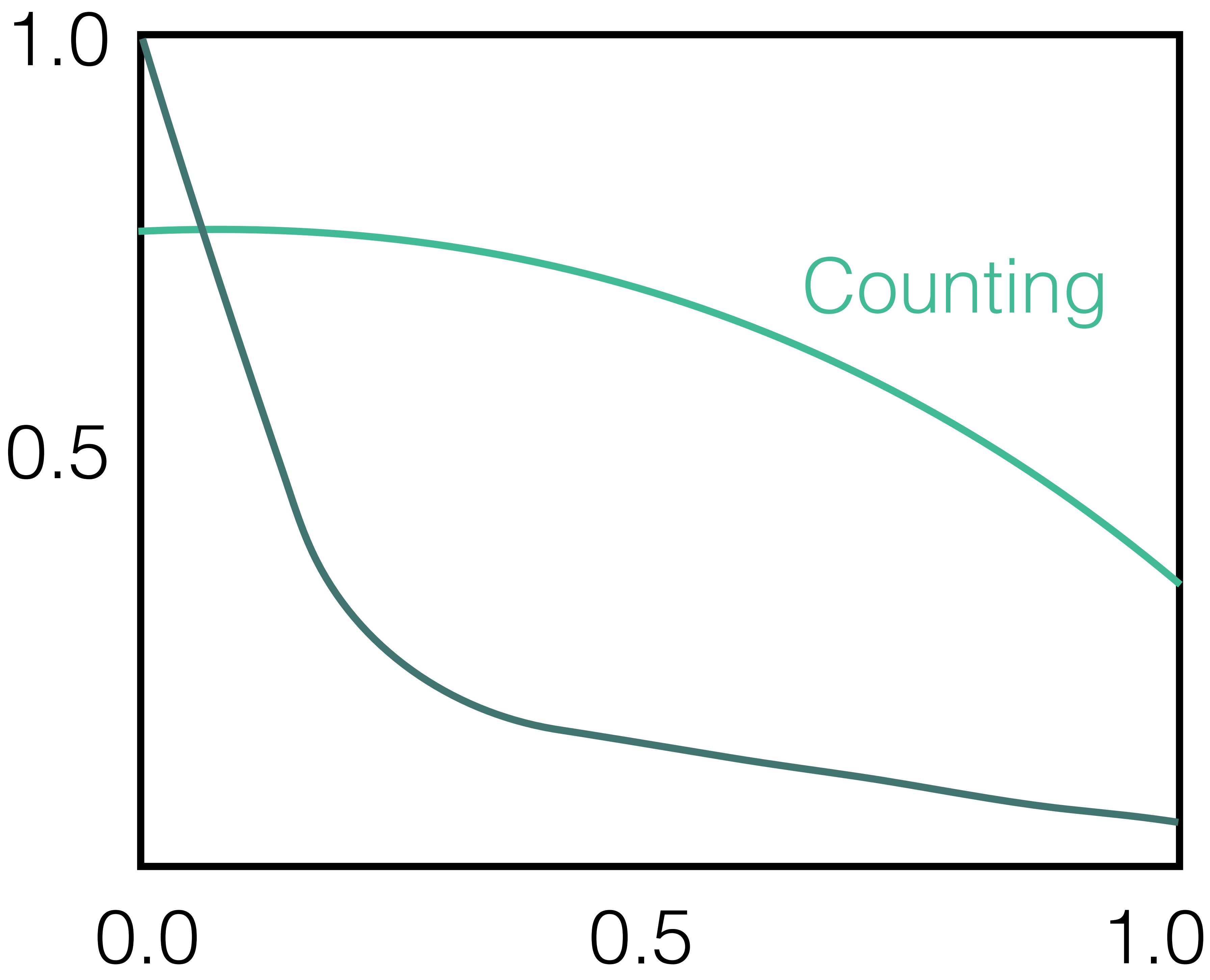


high dose rate

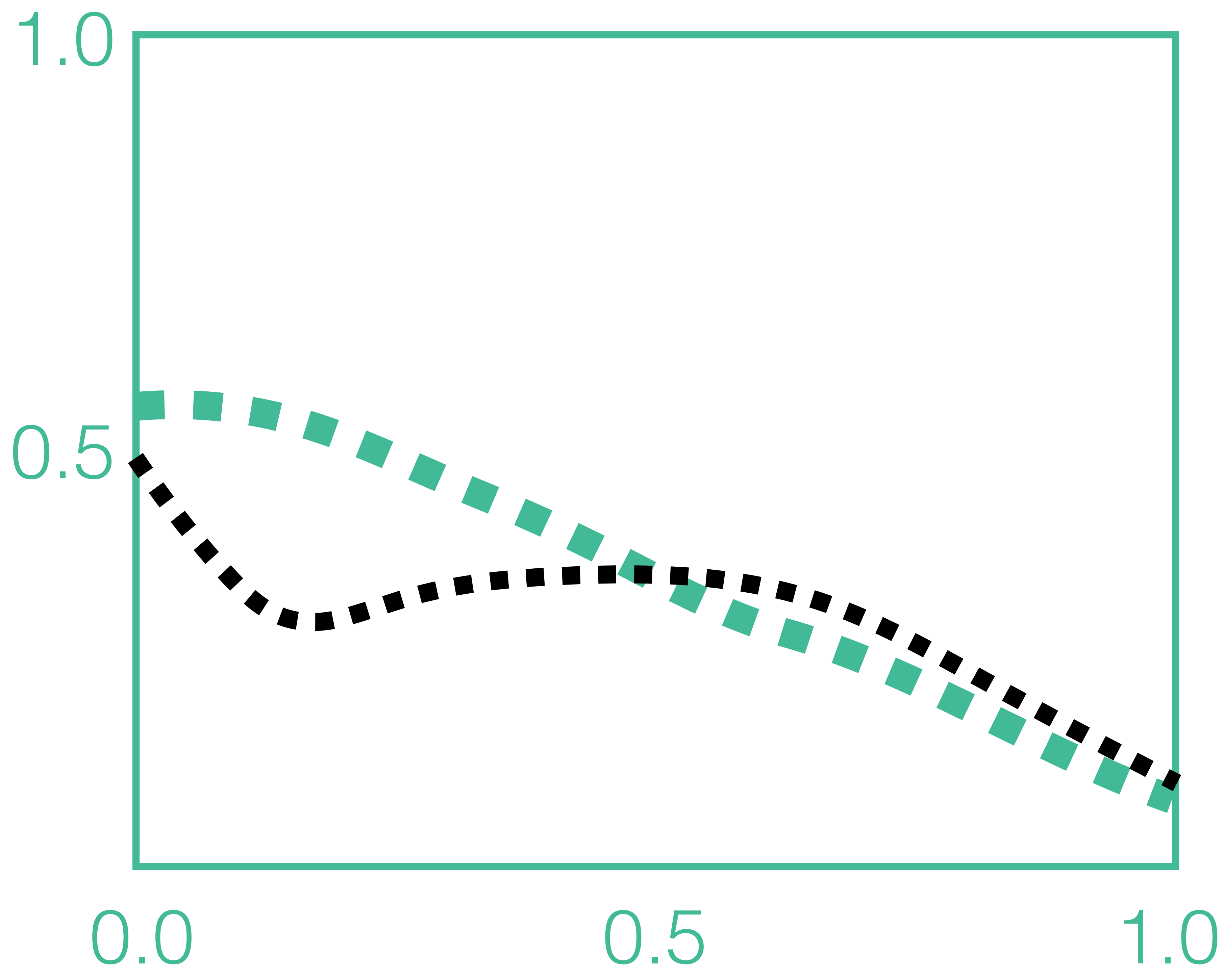
Direct Detectors

DETECTORS Detector Performance Characterization

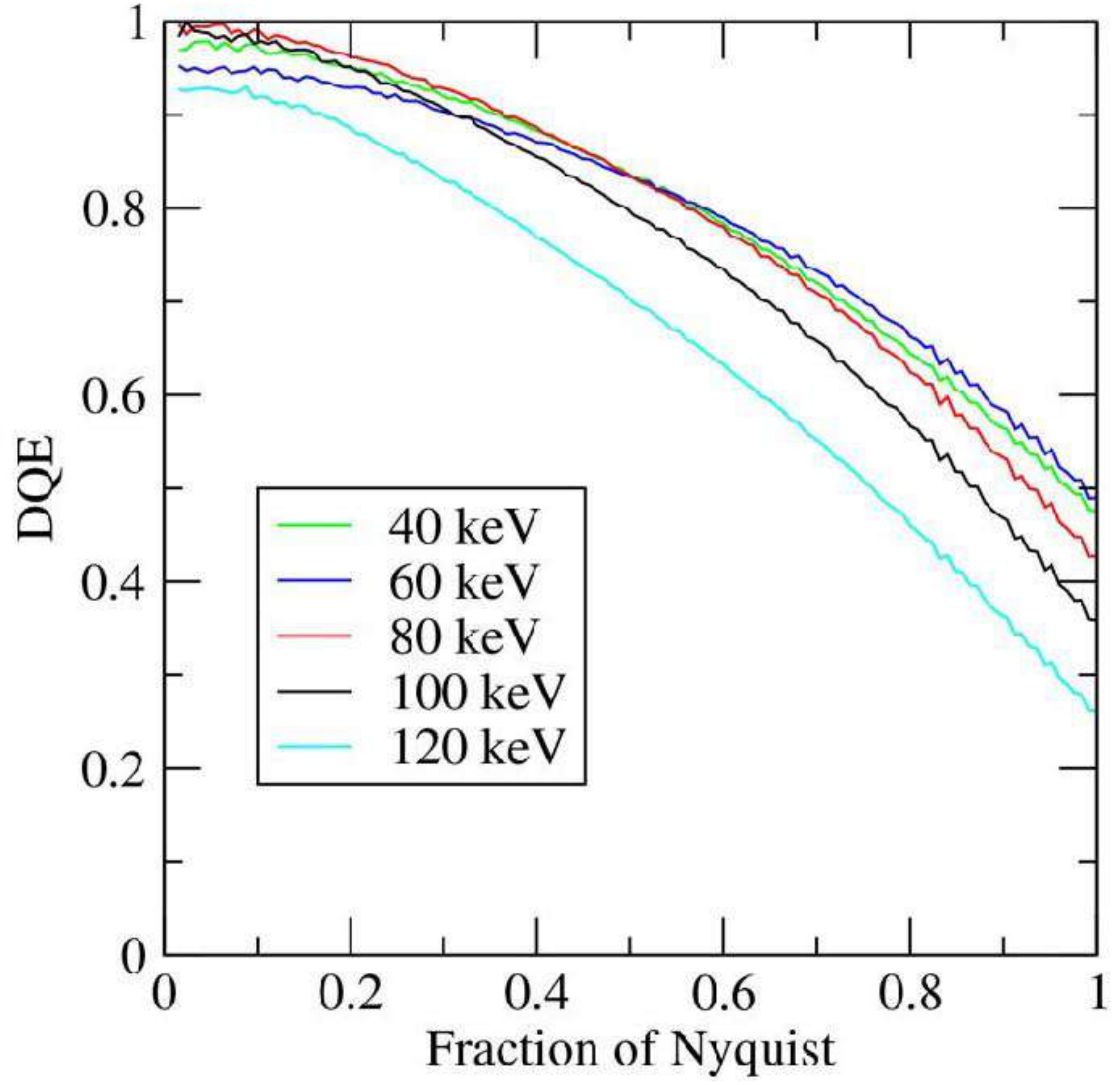
MTF (Modulation Transfer Transform)
contribute to signal envelope



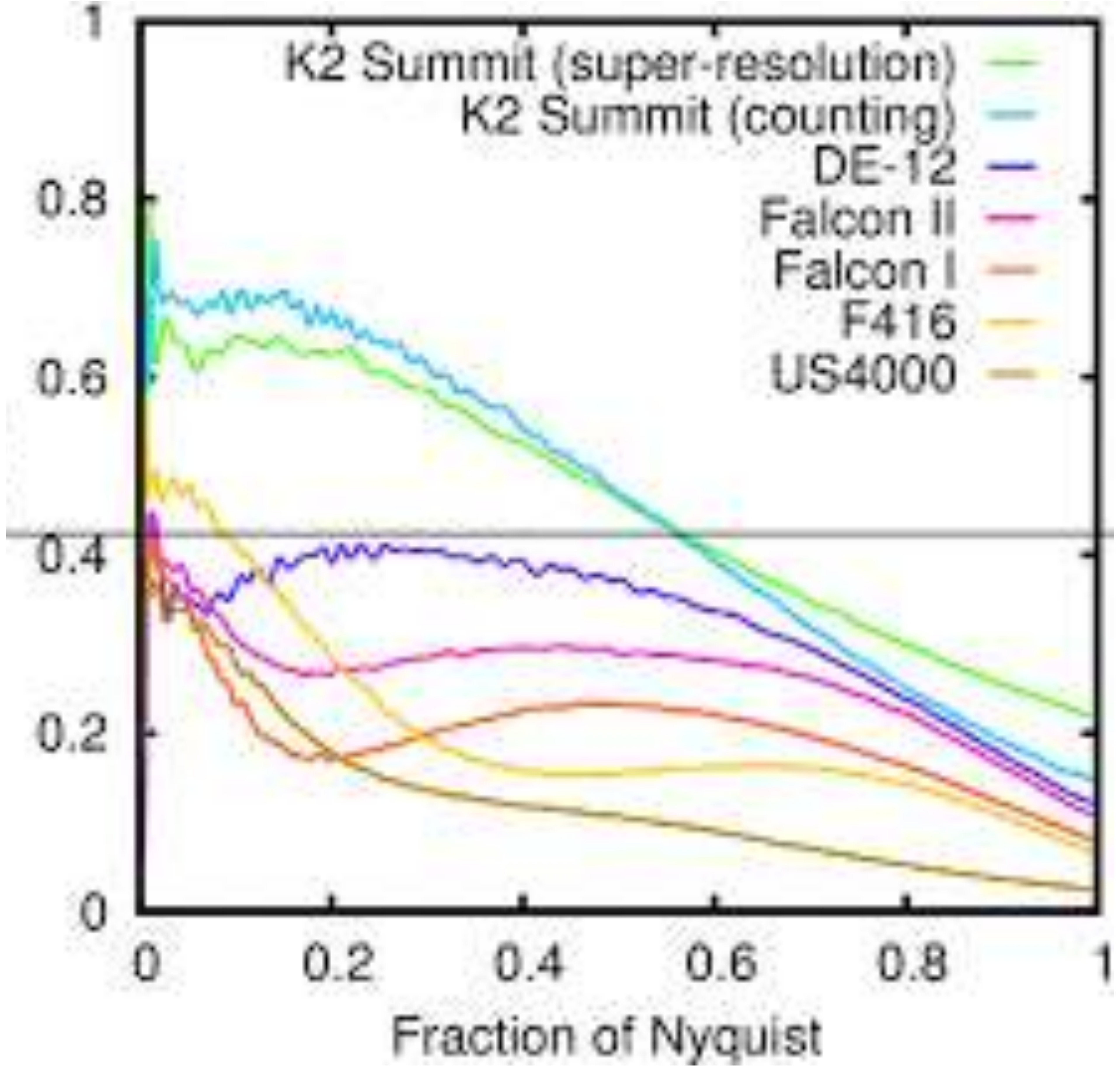
DQE (Detector Quantum Efficiency)
S/N over spatial frequency range



DETECTORS Detector Performance Characterization

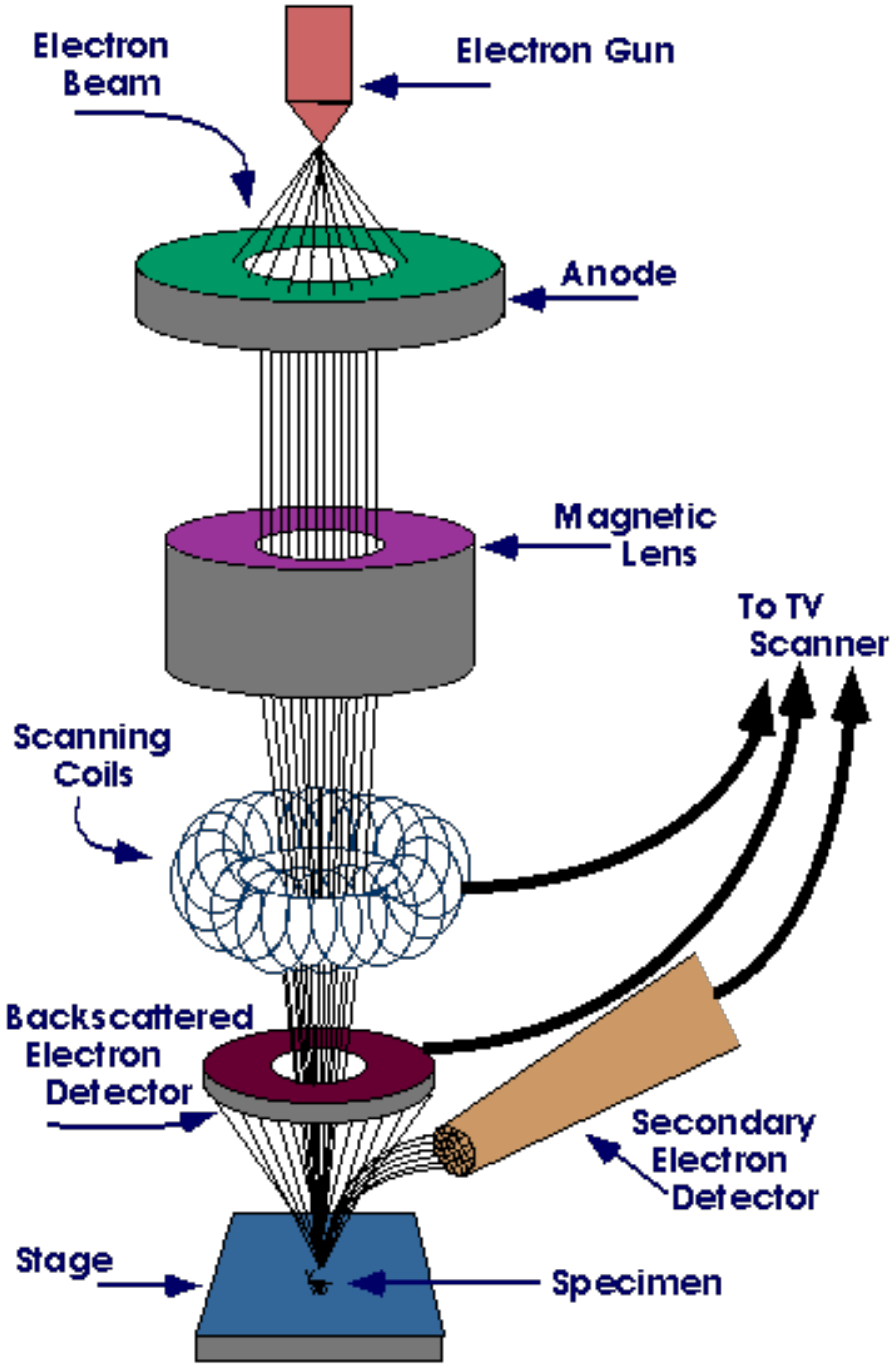


dectris.com



Ruskin, et al JSB

ANATOMY OF AN SEM



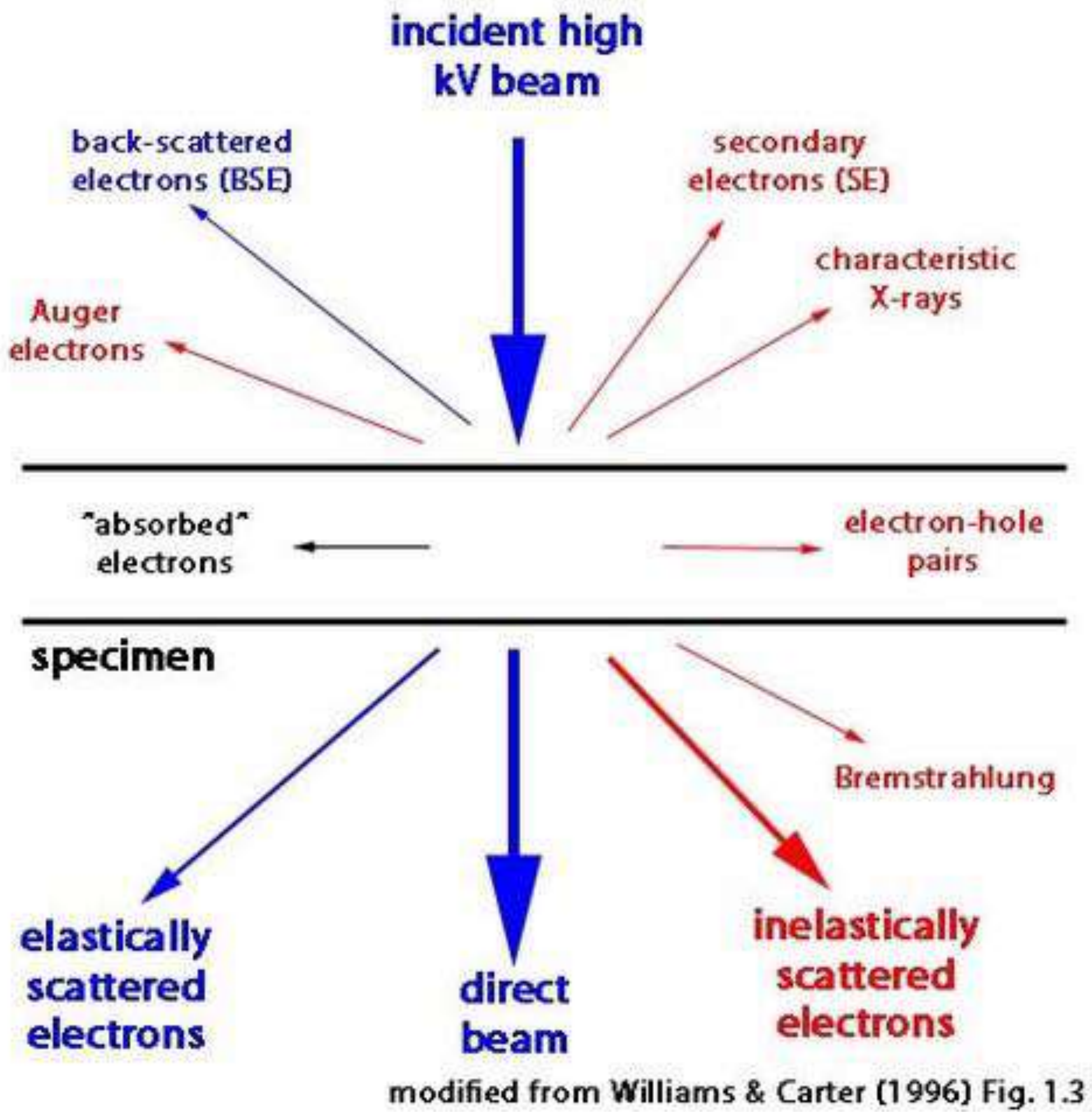
Electron gun: range from tungsten filaments in lower vacuum SEMs to FEGs which need modern high vacuum SEMs

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

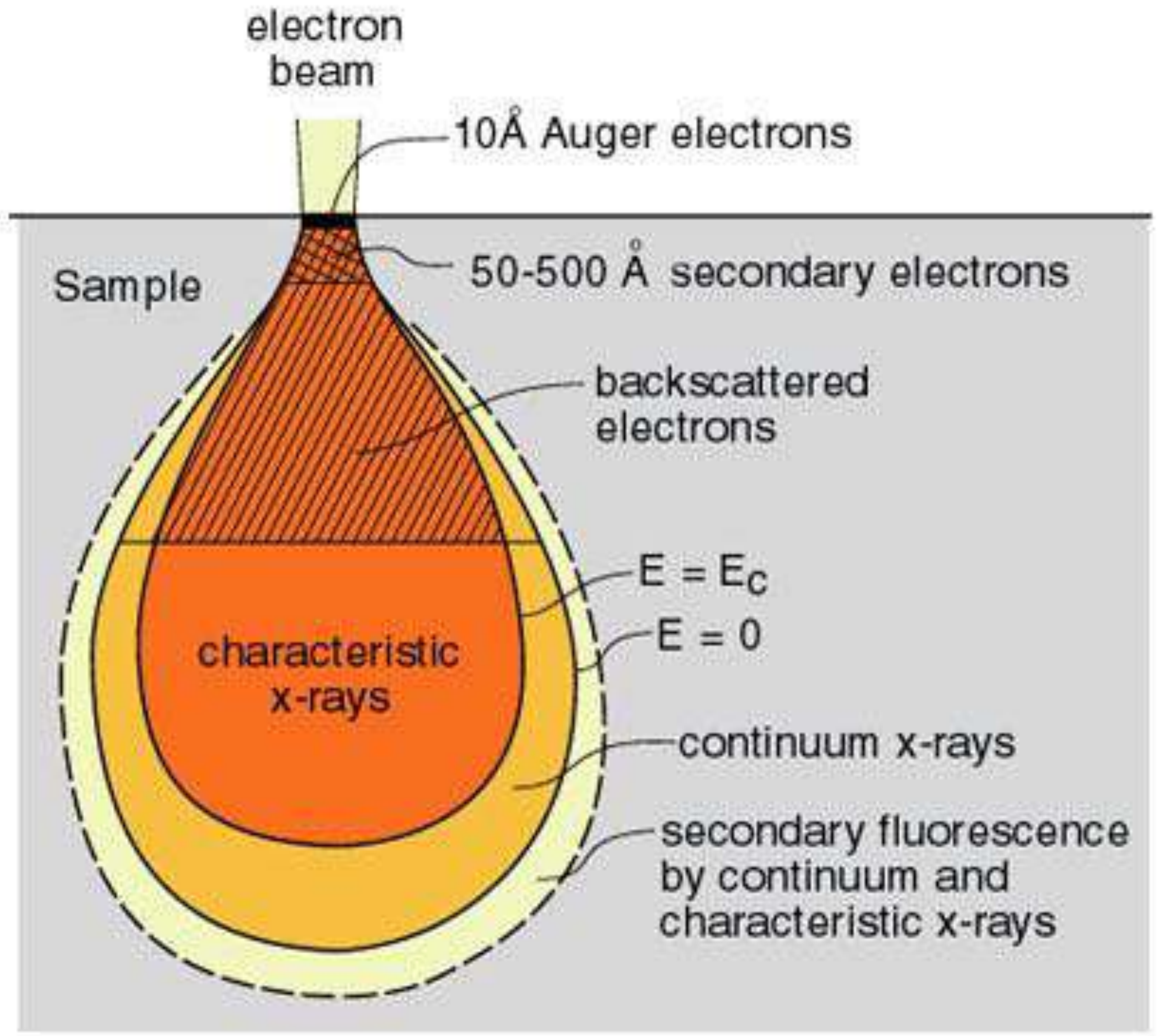
Beam is deflected by very fast scanning coils and rasters the sample surface

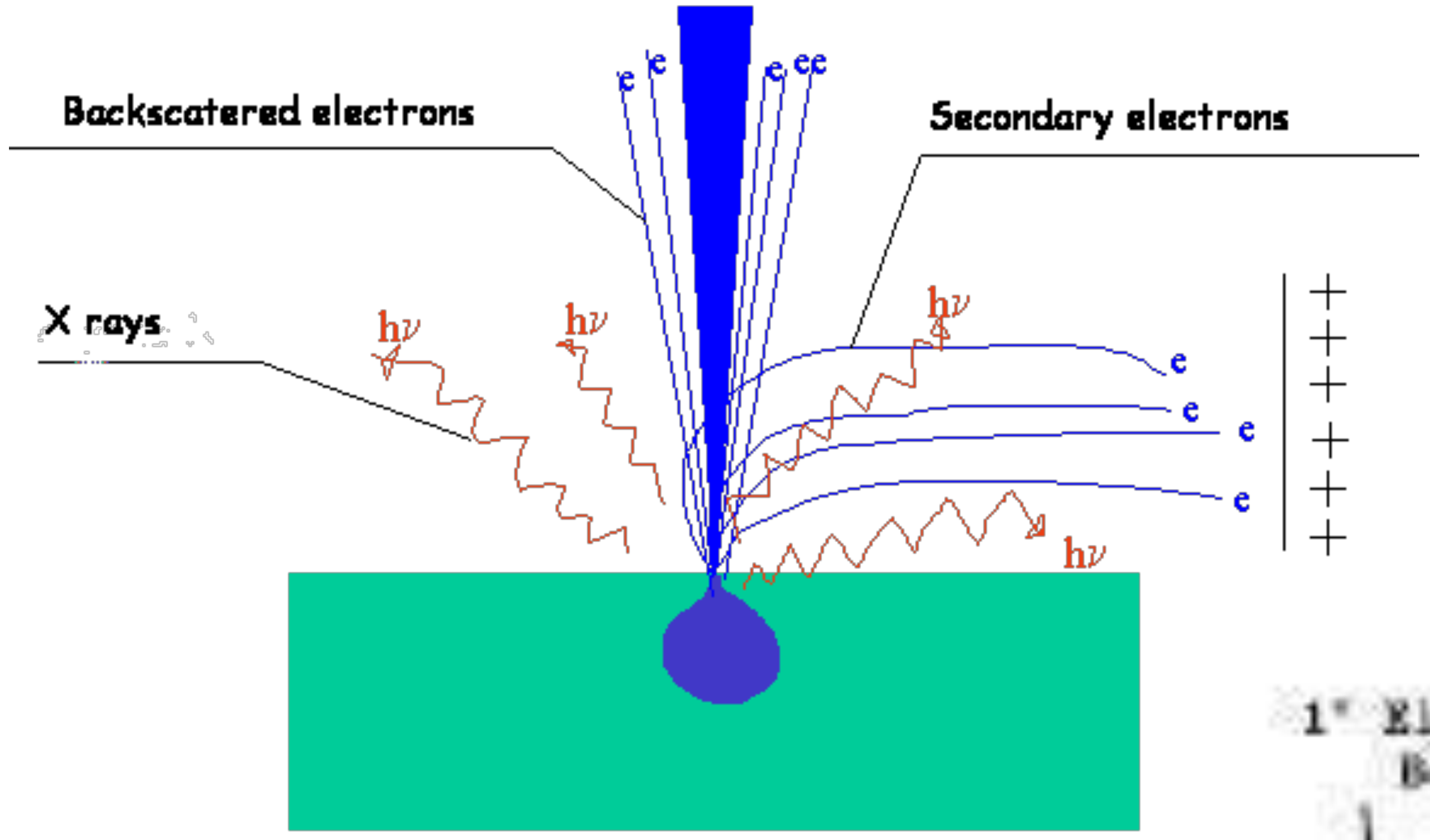
Typical resolution of SEM is between 1 and 20 nm where the record is 0.4 nm

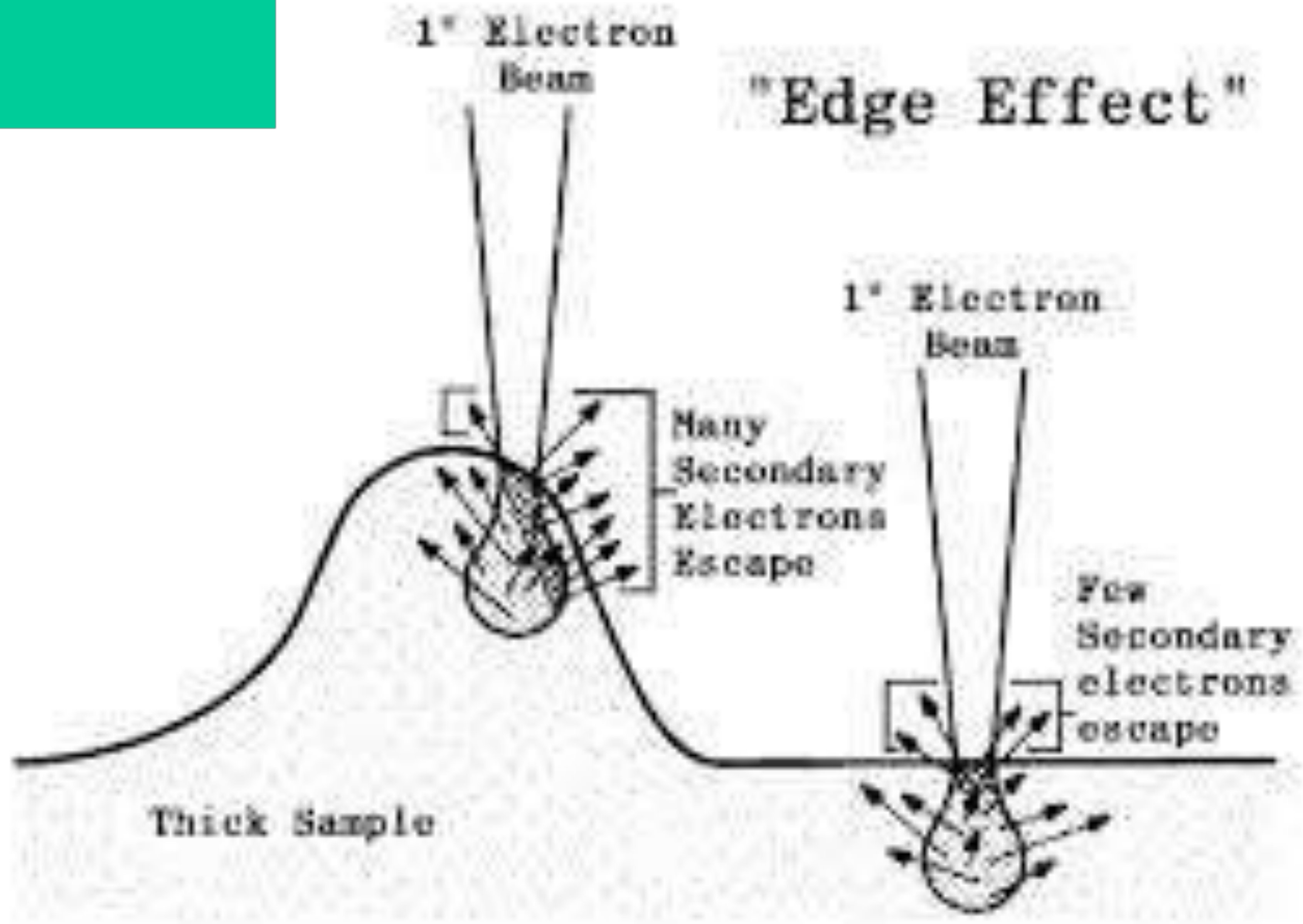
ANATOMY OF AN SEM – BEAM SAMPLE INTERACTIONS

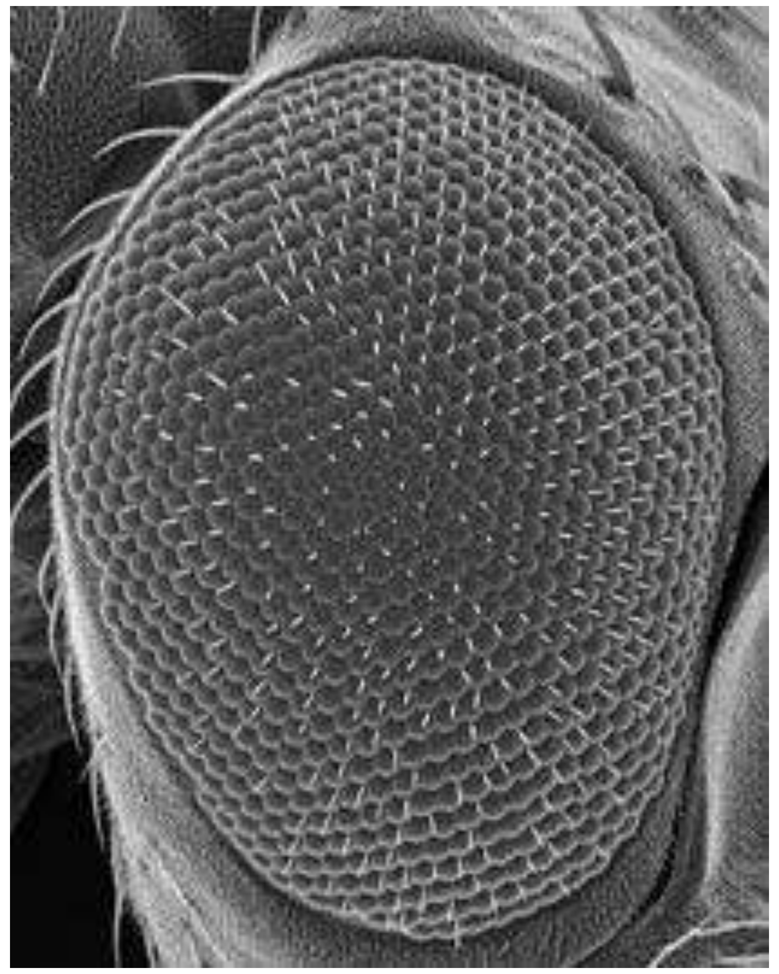


ANATOMY OF AN SEM – BEAM SAMPLE INTERACTIONS &IMAGE FORMATION

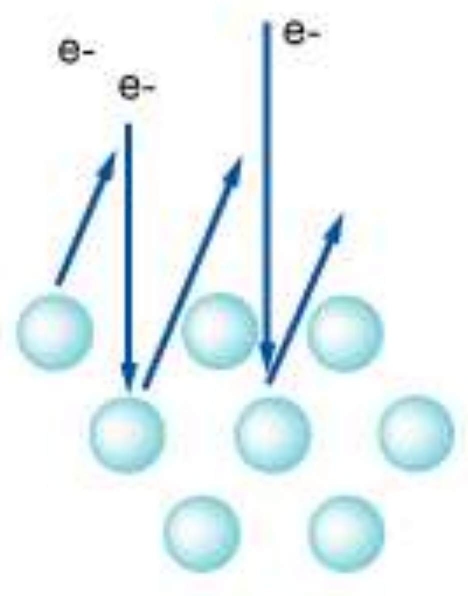




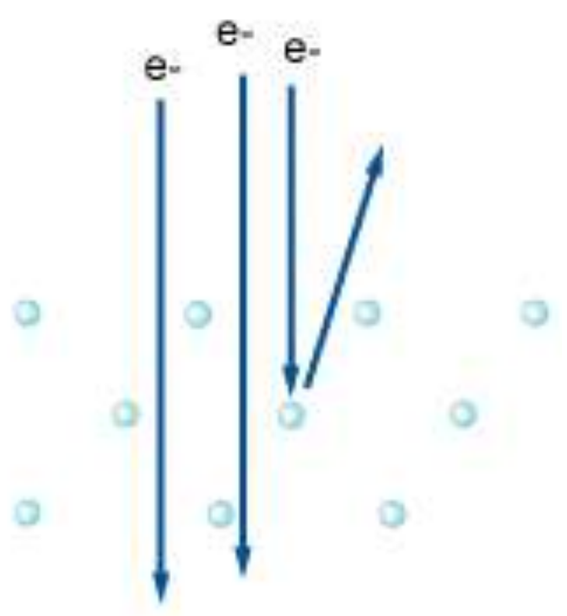




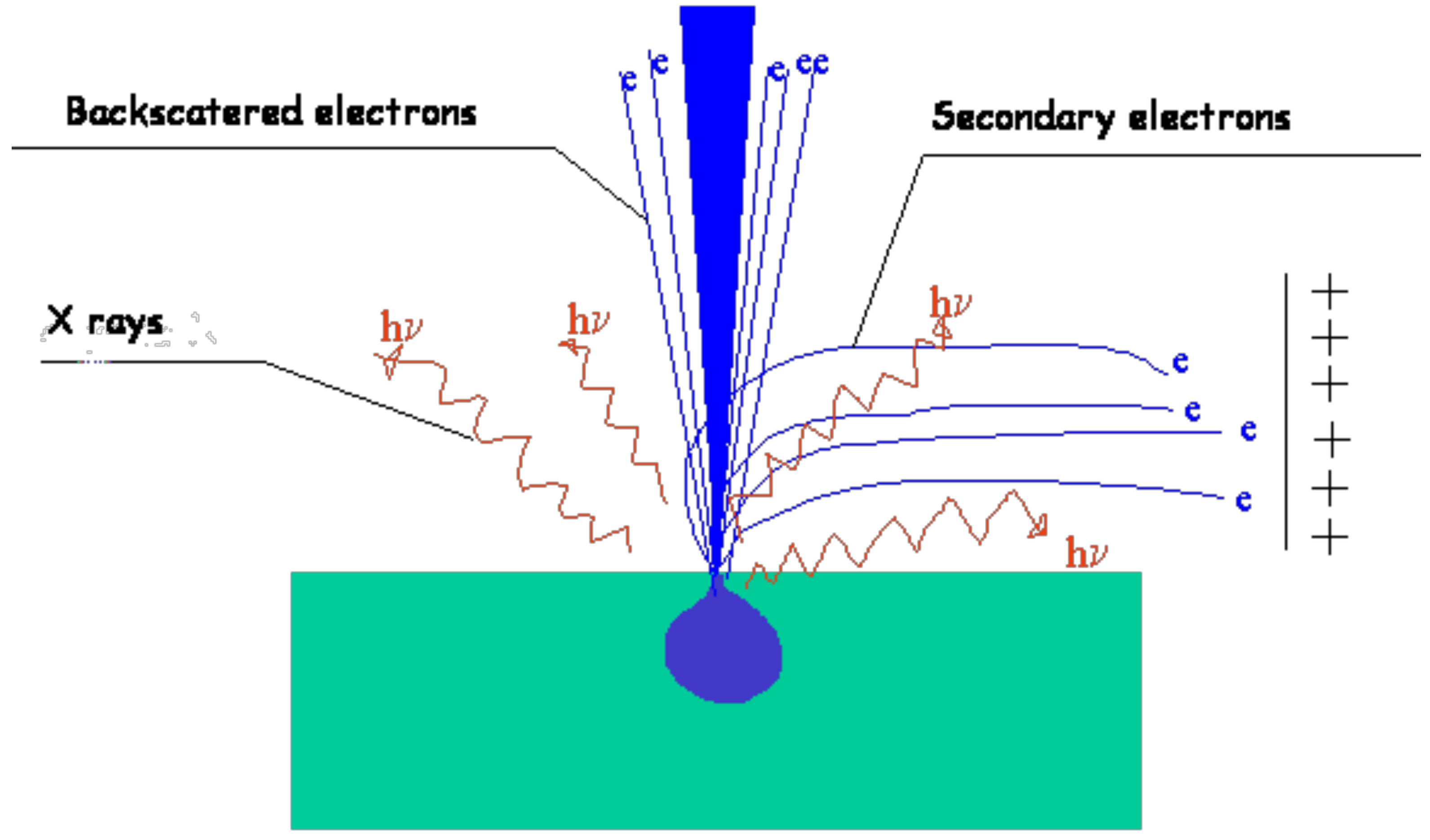
ANATOMY OF AN SEM – BEAM SAMPLE INTERACTIONS &IMAGE FORMATION

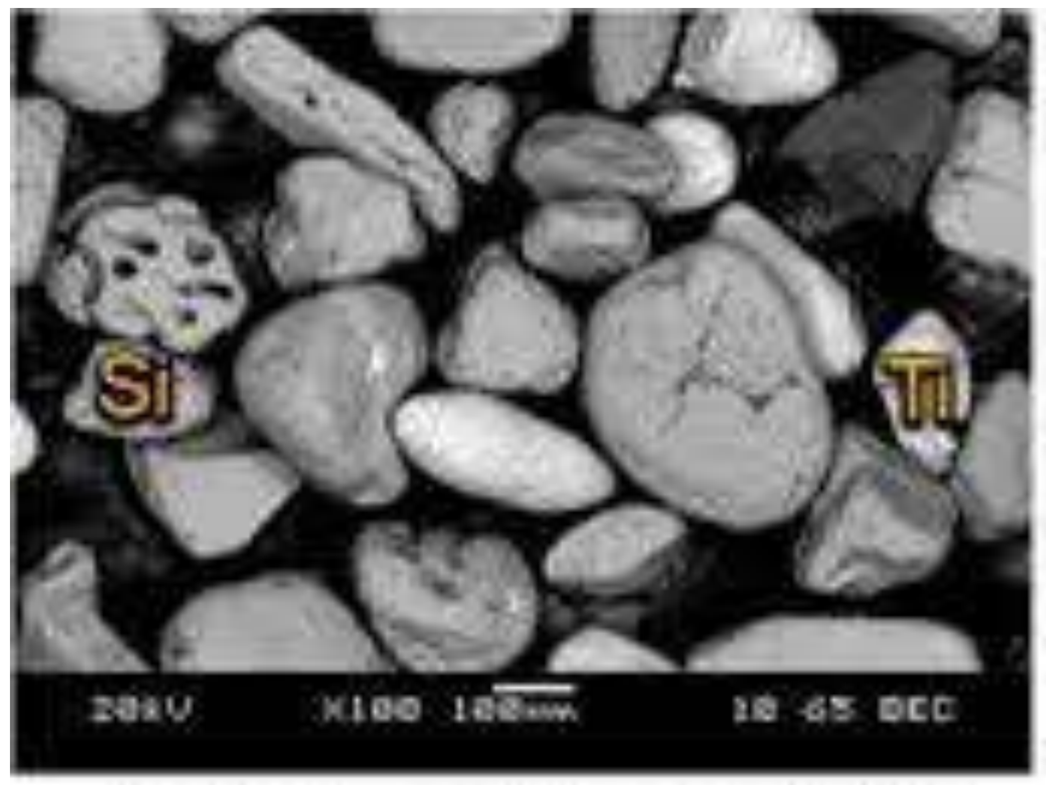


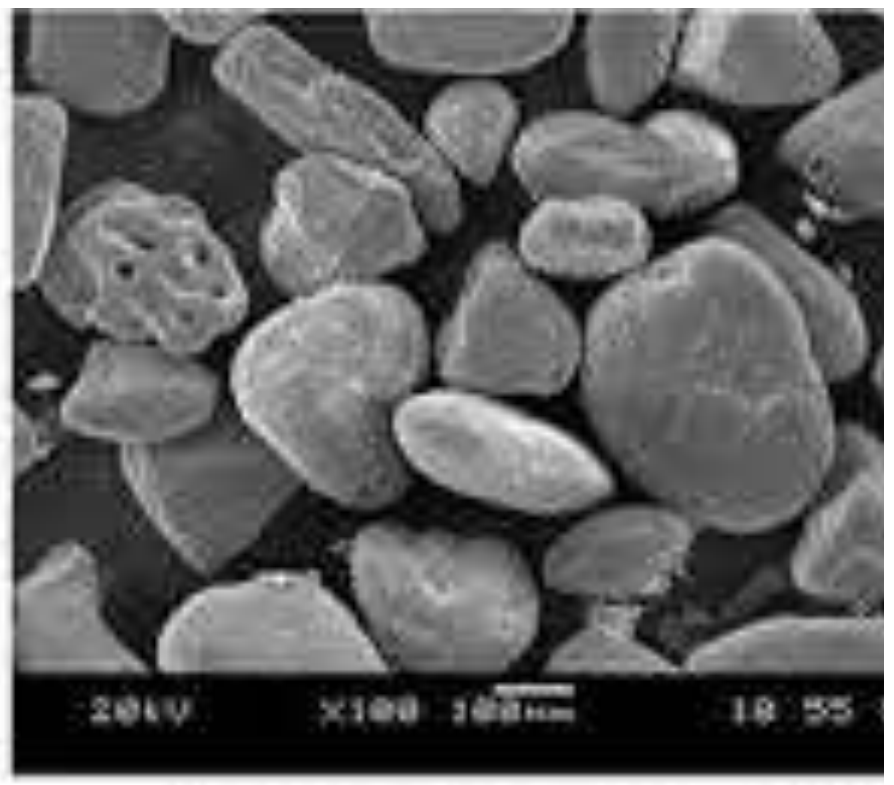
Titanium
atomic number 22



Silicon
atomic number 14







TOOLS OF THE TRADE: MICROSCOPES AND DETECTORS

Questions?