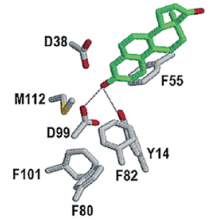
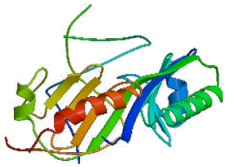
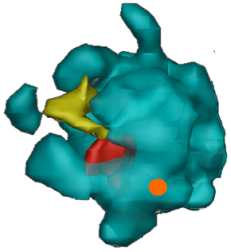
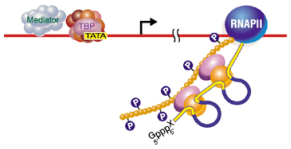
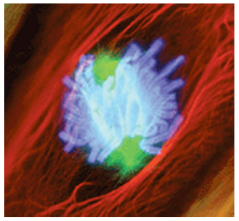
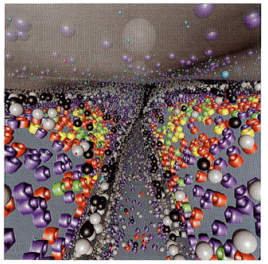
Introduction: Why electrons?

Radiations

	Advantages	Disadvantages
Visible light	Not very damaging Easily focused Eye wonderful detector	Long wavelengths (~400 nm)
X-rays	Small wavelength (Angstroms) Good penetration	Hard to focus Damage sample
Electrons	Small wavelength (pm) Can be focused	Damage sample Poor penetration
Neutrons	Low sample damage Small wavelength (pm)	Hard to produce in controlled ways Hard to focus

The structural biology continuum

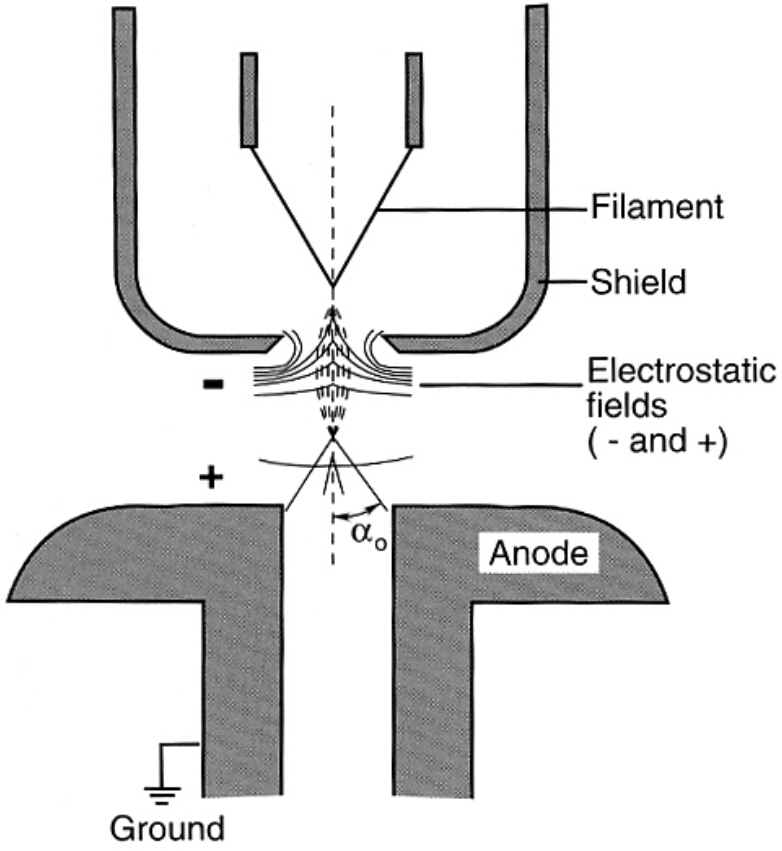
polypeptides	small proteins and domains	large proteins and complexes	multi-protein reactions	whole cells or cell sections	whole cells	whole cells and tissues
 Figure 9 from Park and Merz, JACS 125:901	 Human TBP and DNA Nikolov et al., PNAS 93:4862	 RNA Polymerase II	 Figure 3 from Orphanides and Reinberg, Cell 108:439	 *C. crescentus* cell	 Mitosis Conly Reider and Alexey Khodjakov Science 300 #5616 cover	 Synapse Stiles and Bartol, Computational Neuroscience CRC Press
molecular dynamics simulations	X-ray crystallography NMR spectroscopy	cryoEM single particle analysis or X-ray crystallography	cryoelectron tomography	cryoelectron tomography, light microscopy	fluorescence light microscopy	structurally and spatially explicit cell modeling

Introduction - Why electrons? Concept check questions:

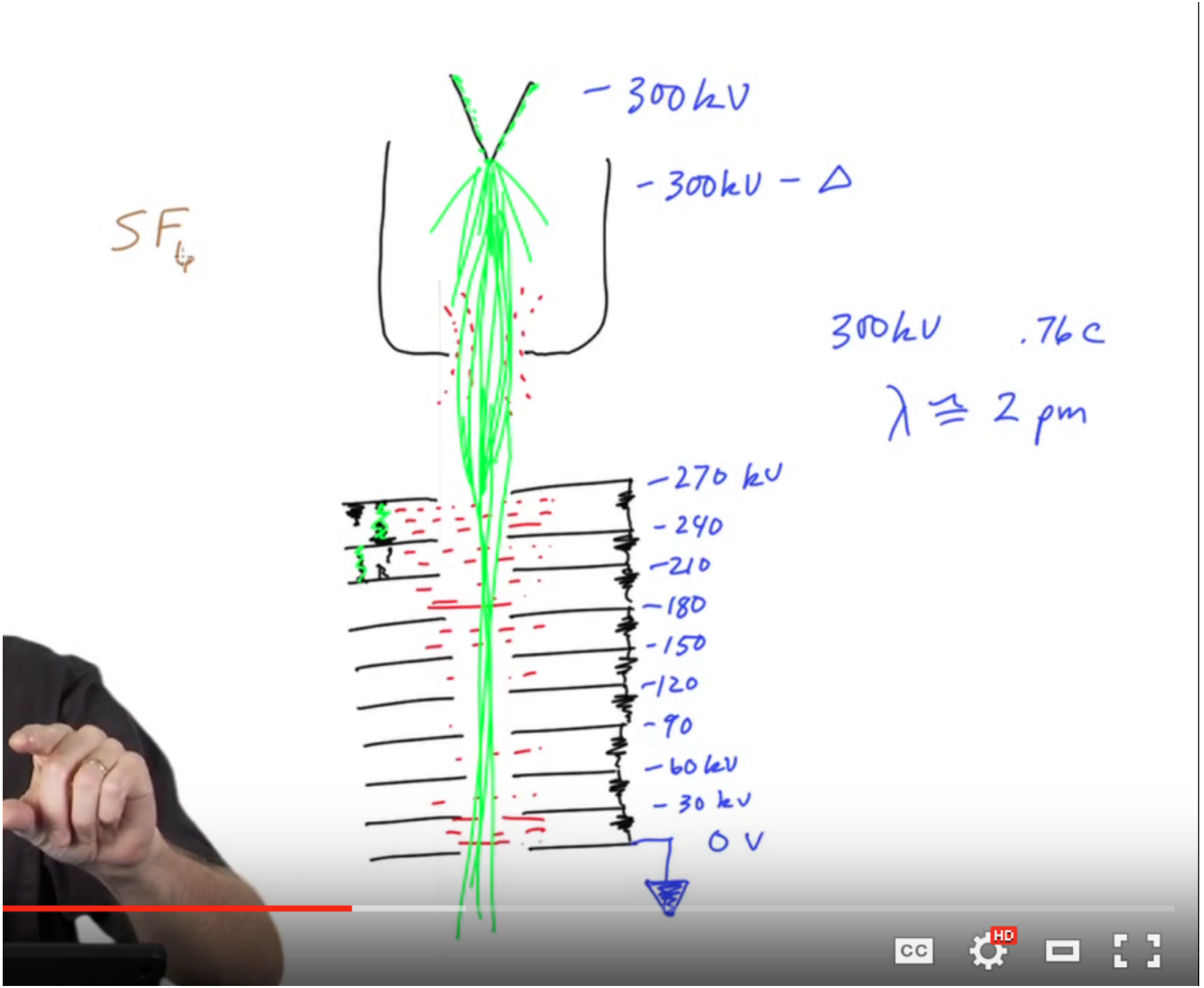
- What are the advantages and disadvantages of electrons compared to photons for microscopy? Neutrons?
- What structural biological technologies give higher resolution information than cryo-EM, and what kinds of samples and questions can they address?
- What structural biological technologies complement cryo-EM at lower resolutions, and what kinds of questions and samples do they address?

Electron Guns

Electron “guns”



- Tungsten filaments
- Lanthanum hexaboride (LaB_6) crystals
- Field emission guns

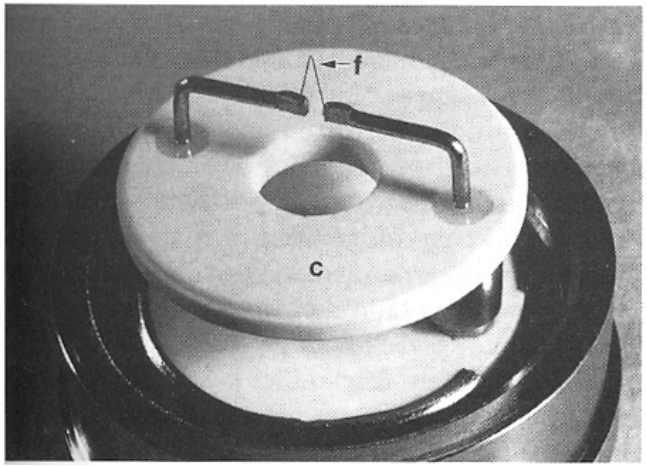

SF_6
−300kV
−300kV − Δ
300kV .76c
λ ≅ 2 pm
−270 kV
−240
−210
−180
−150
−120
−90
−60 kV
−30 kV
0 V

Two types of coherence

- ***Spatial***: do all the electrons come from the same direction?

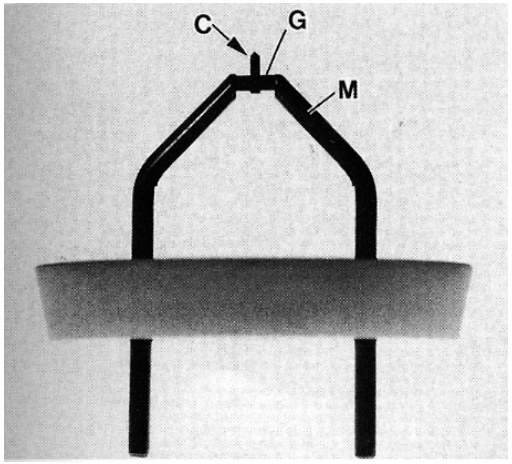
- ***Temporal***: do they all have the same speed?

1. Tungsten filaments



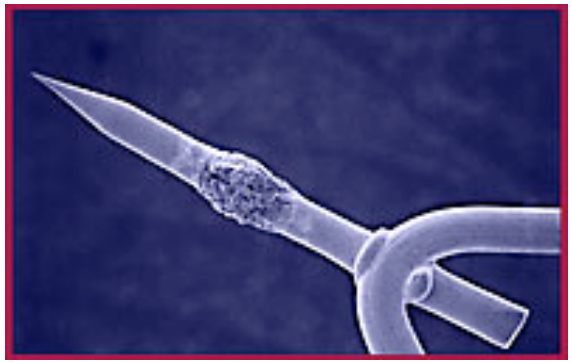
Bozzola and Russell, Fig. 6.22

2. Lanthanum hexaboride (LaB_6) crystals



Bozzola and Russell, Fig. 6.26

3. Field emission gun



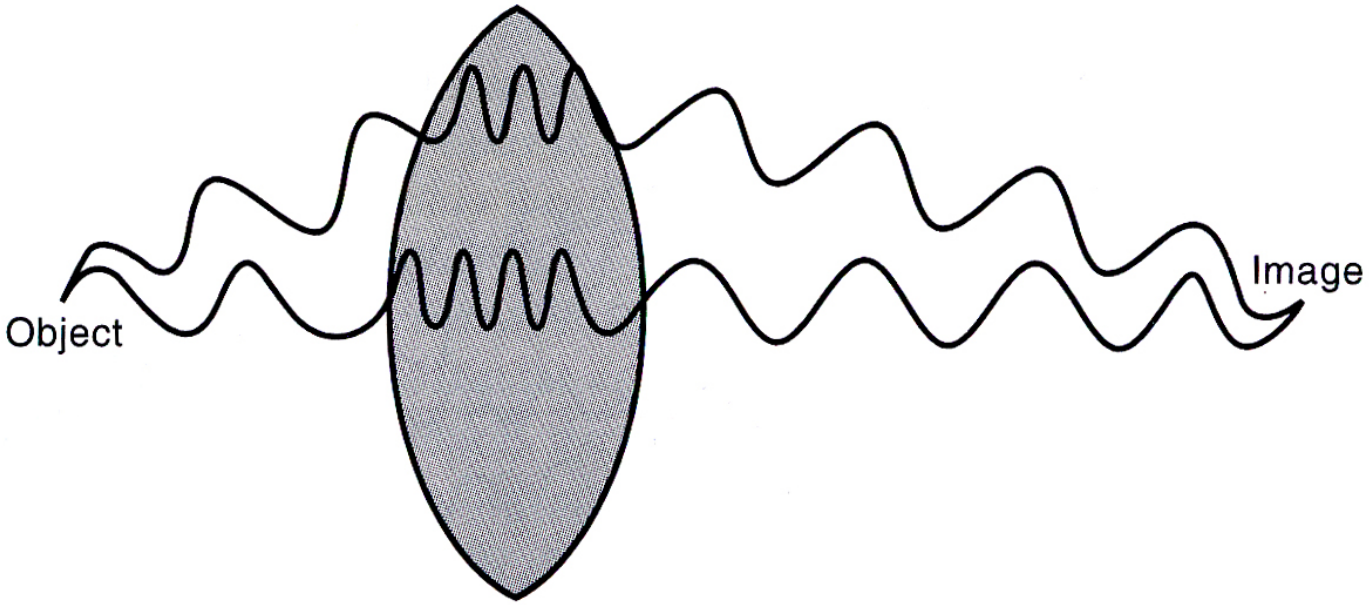
http://www.fisica.unige.it/~rocca/Didattica/Laboratorio

Electron guns
Concept check questions:

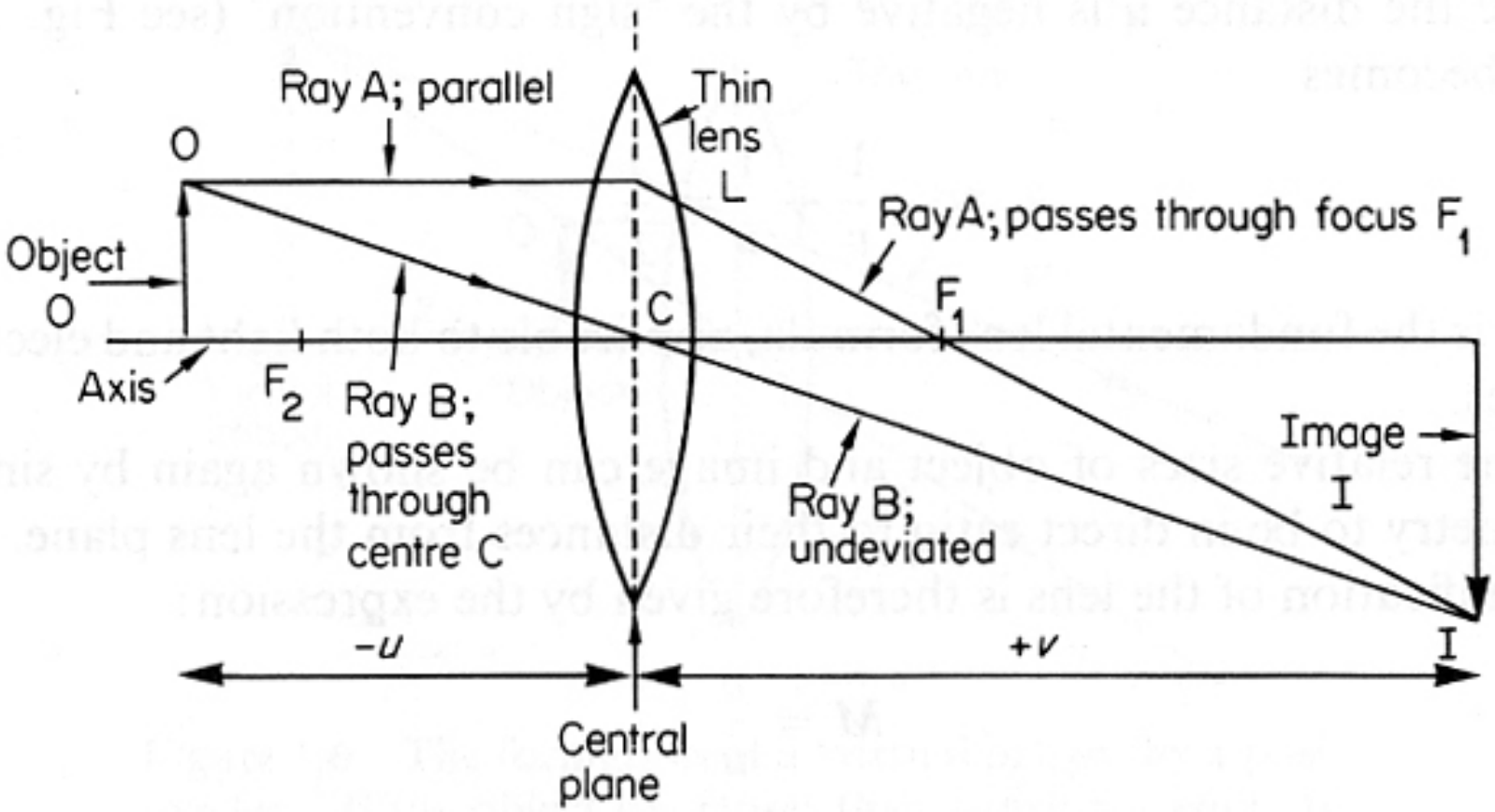
- Where do the imaging electrons in an electron microscope come from?
- What part of the gun is called the "cathode"? What should be called the "anode"?
- What is the accelerator stack a "stack" of?
- What voltages are typically used in transmission electron microscopes? What kinds of electron wavelengths does this correspond to?
- What does it mean to "condition" the gun?
- What is the difference between spatial and temporal coherence?
- What are the three main types of electron guns? What are the advantages and disadvantages of each?

Electron lenses

Lenses “focus” divergent rays



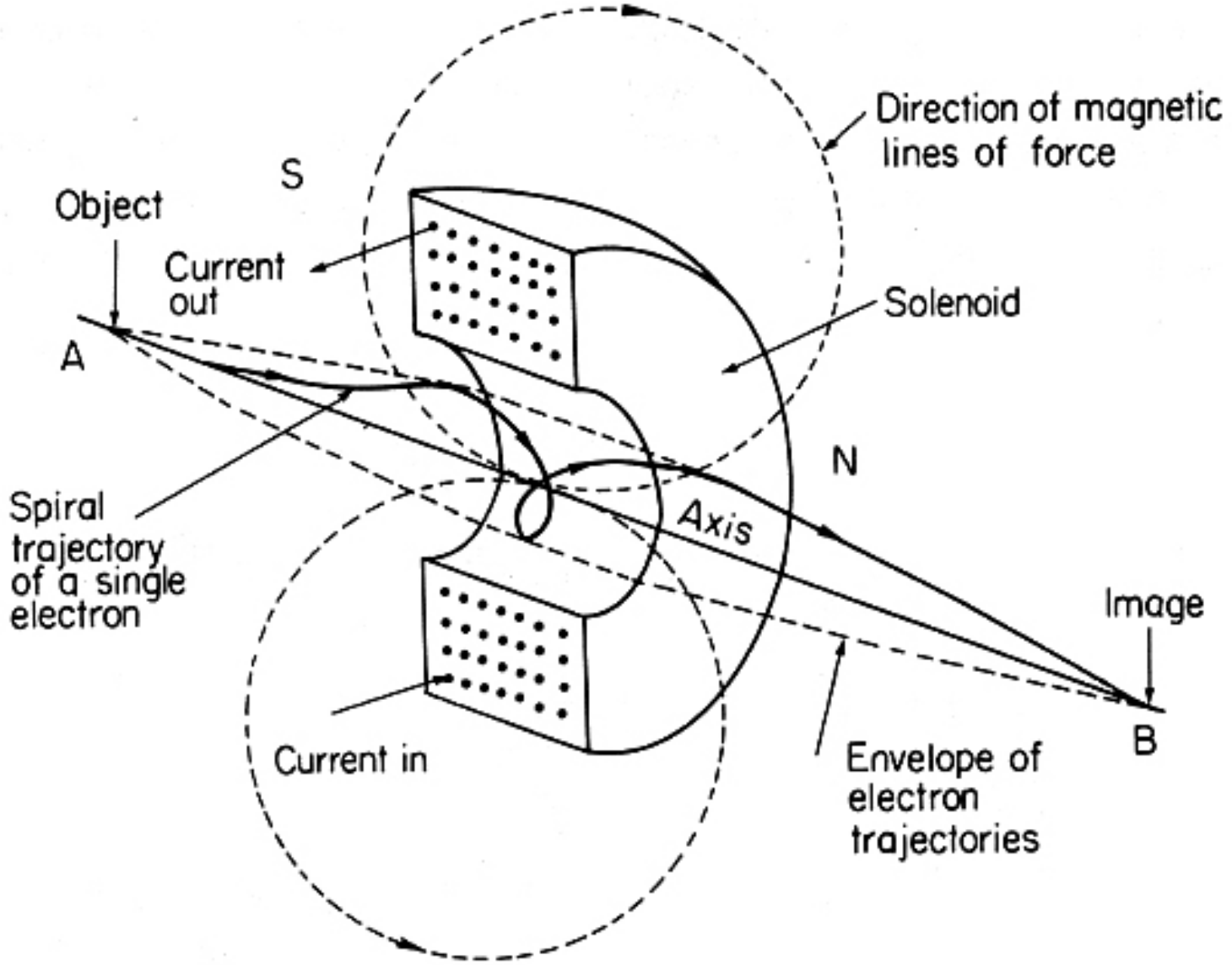
Lenses introduce the possibility of magnification



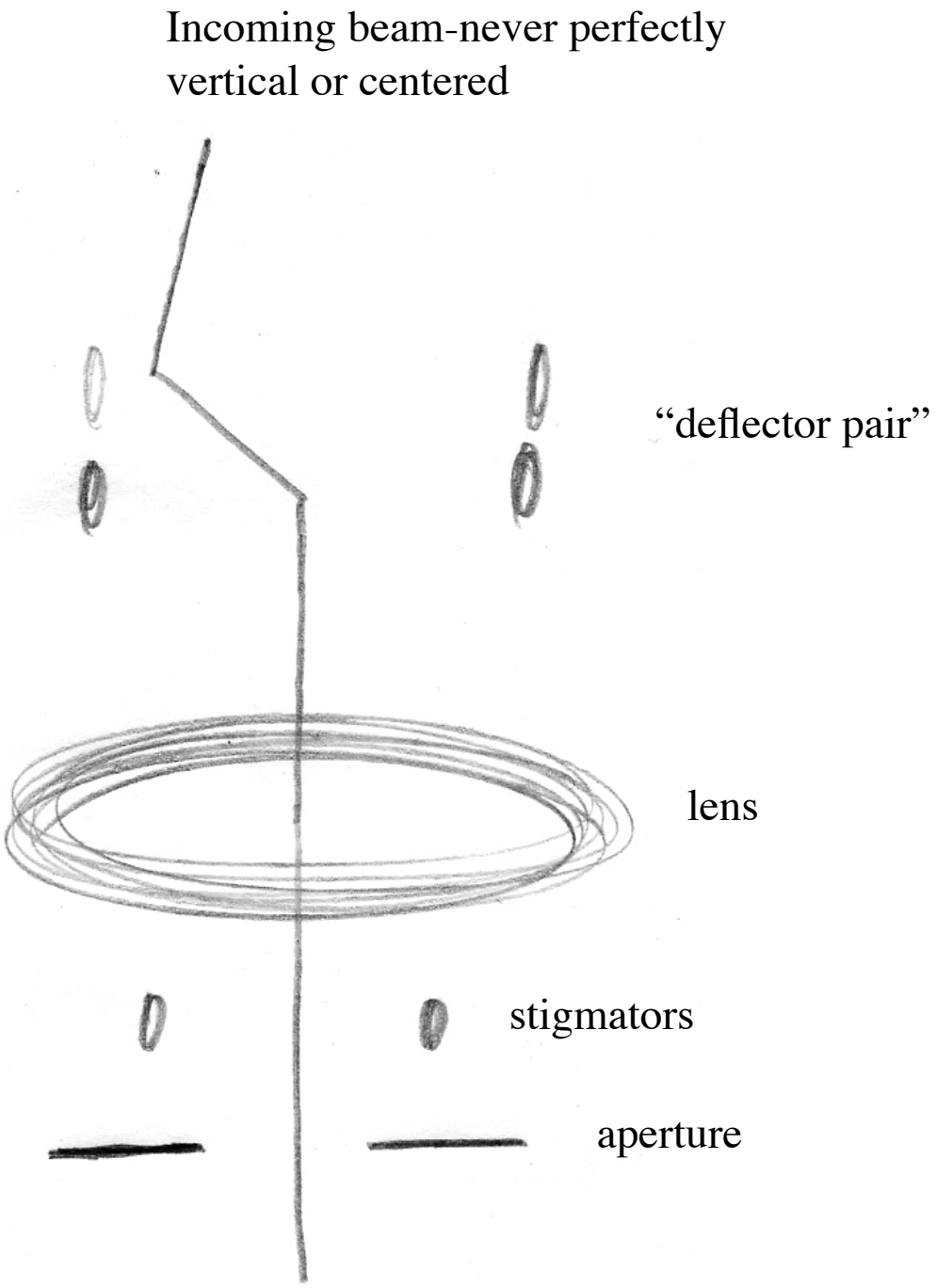
$1/f = 1/u + 1/v$

$M = v/u$

An electron lens



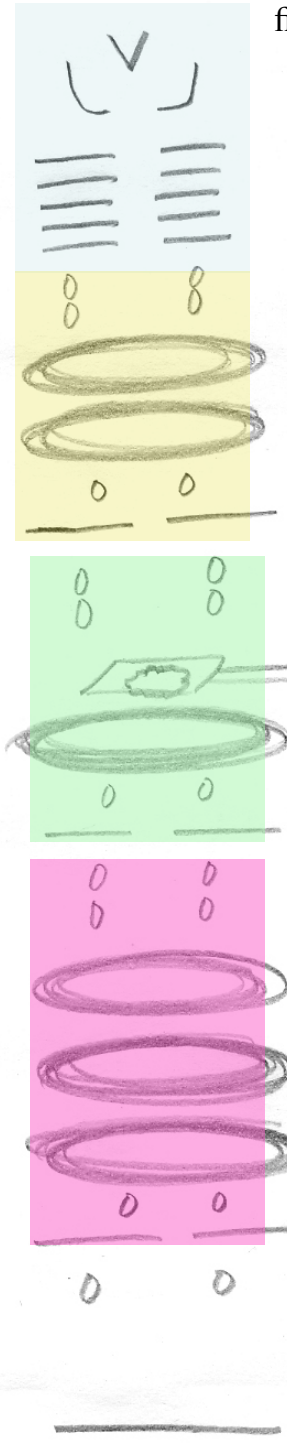
EM lens systems

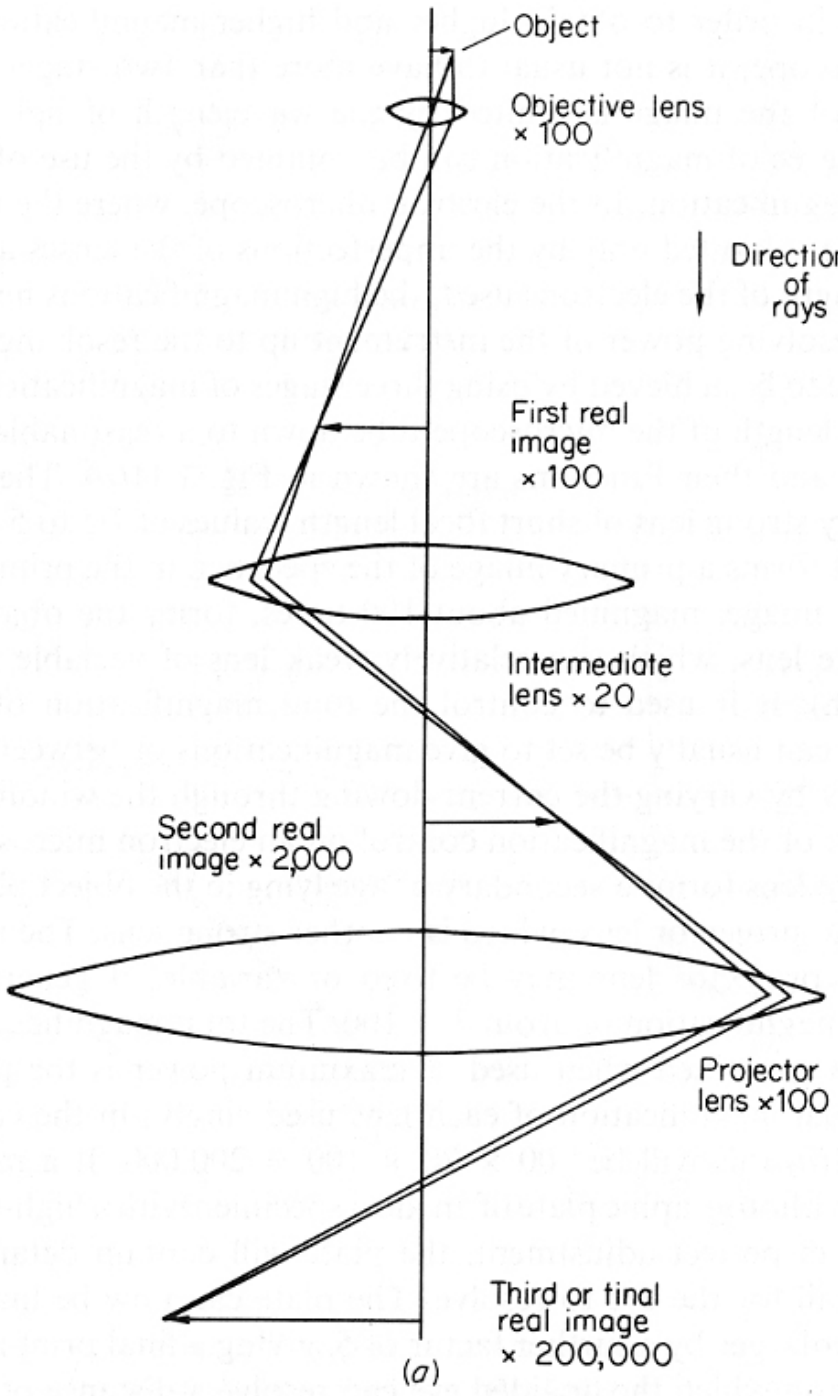


Electron lenses
Concept check questions:

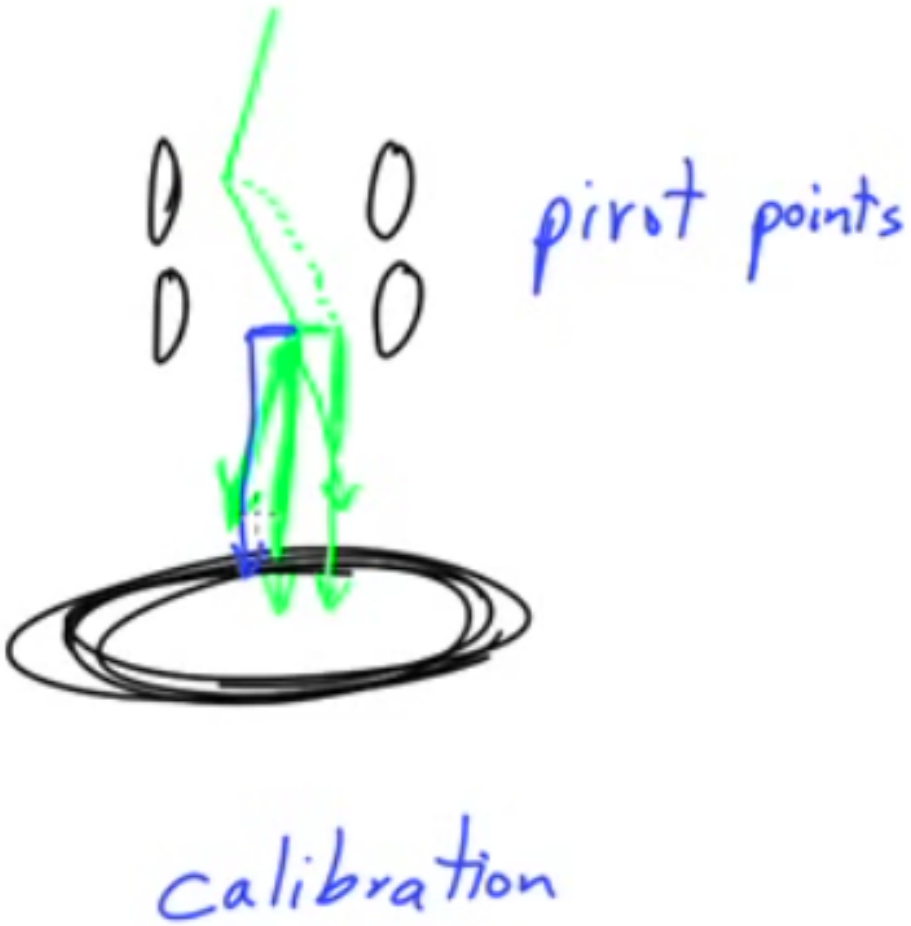
- What is the defining property of a "lens"?
- Why/how do optical lenses focus light?
- Draw a diagram that shows how a lens can be used to form a magnified image. What parameters determine the magnification?
- How do electron lenses focus electrons?
- Why do electron images rotate in an electron lens?
- What are the four main components of an electron lens system? What does each do?

Column

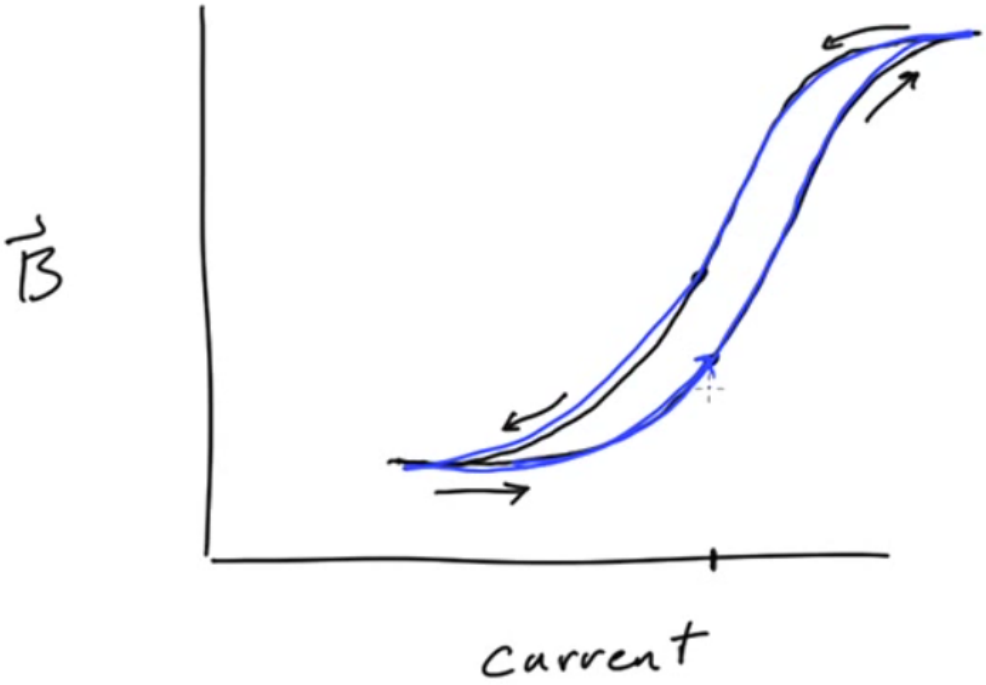

filament
Wehnelt cylinder
Gun
Accelerator stack
current (filament)
bias (emission)
voltage (high tension)
"gun" deflectors
Condenser lens system
Condenser lenses
Condenser stigmators
Condenser aperture
gun shift, tilt
spot size
intensity
condenser stigmatism
size, centering
"beam" deflectors
beam shift, tilt
Objective lens system
specimen
position, z-height, tilt
Objective lens
focus
Objective stigmator
objective stigmatism
Objective aperture
size, centering
"image" deflectors
image shift, tilt
Projector lens system
magnification
"intermediate" lenses
"diffraction" stigmator
"selected area" aperture
"projector deflectors"
divert to TV
Viewing screen
down, up for CCD



Compound microscope
Conjugate planes


pivot points
calibration

hysteresis



32k x

19k x

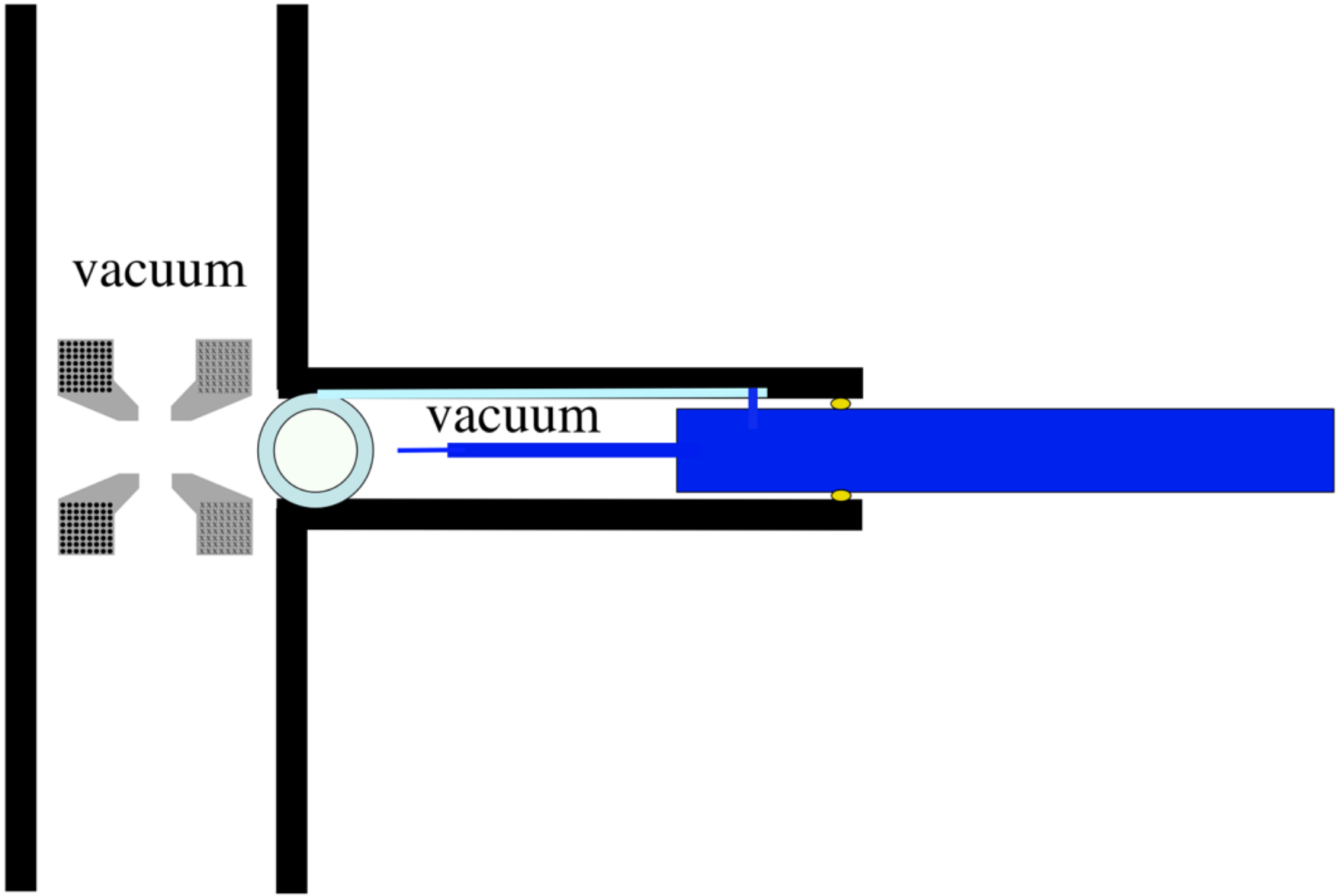
5k x

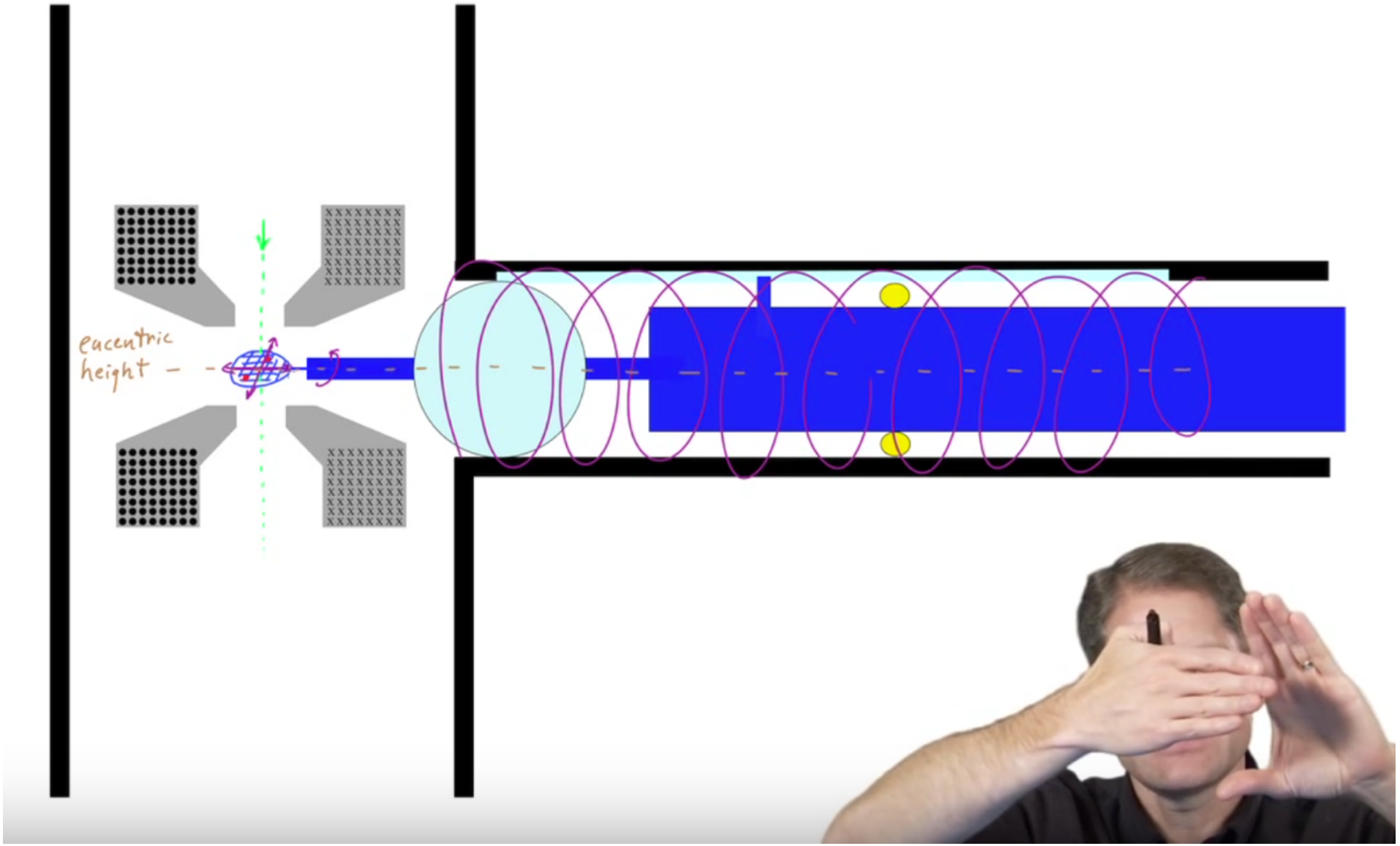
normalize

The column
Concept check questions:

- What are the three main lens systems in an electron microscope called?
- What is meant by a "conjugate plane"?
- What are the special names given to the three independent sets of deflectors?
- What current is controlled by the "filament" knob? "emission"? "spot size"? "intensity"? "focus"? "magnification"?
- What is controlled by the "high tension" knob?
- What is a "crossover"?
- Which knob controls whether the microscope is in "LM," "M," or "SA" mode? What currents change?
- What are "pivot points"?
- What does it mean to "align" the microscope?
- What is "hysteresis"?
- What does the "normalize" button do?

Sample chamber


vacuum
vacuum

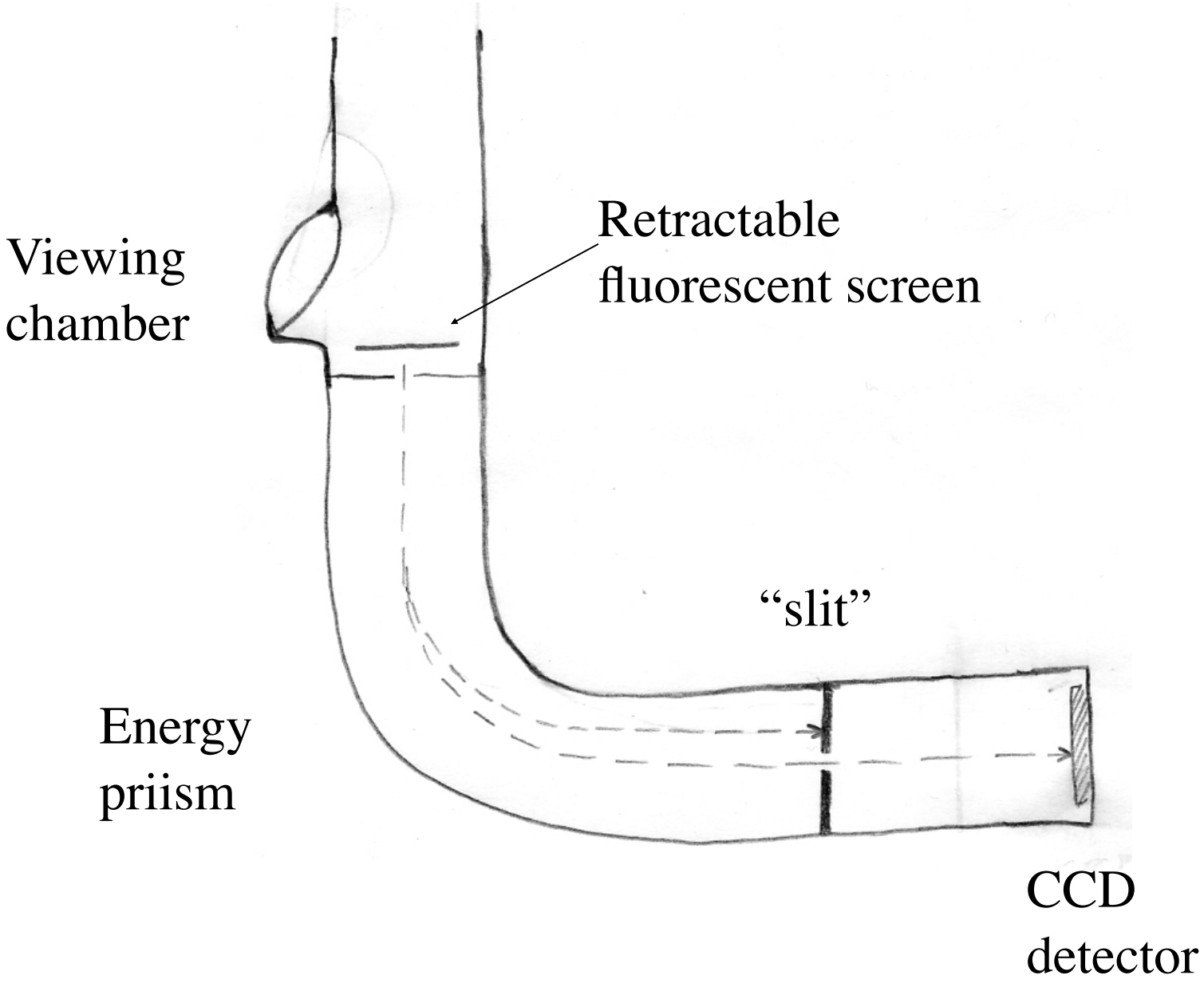

eucentric
height

The sample chamber
Concept check questions:

- In what directions/ways can the sample be moved while in the microscope?
- What is an "air-lock", and how it is relevant to the sample chamber?
- Where does the sample rest with respect to the objective lens?
- What is the "pole piece gap"?
- What is a "cryo-box"?
- What is "eucentric height"? Is it different for every grid?

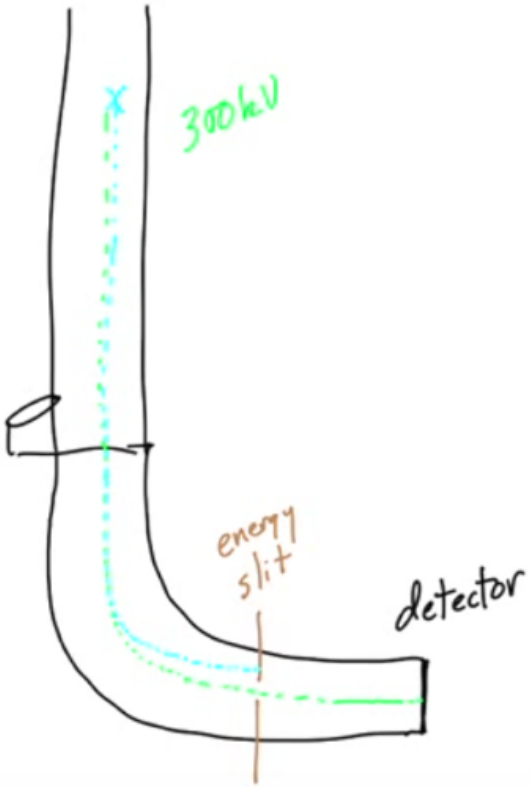
Energy filters

Energy filters



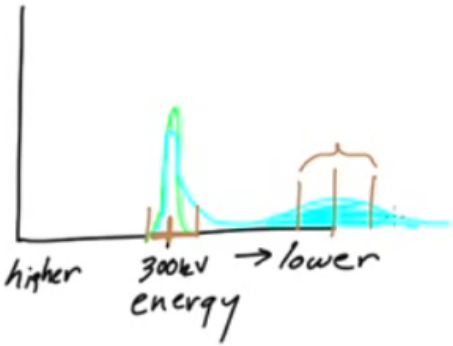
post-column filters

in-column Ω filters



slit position

slit width 20eV



Energy filters
Concept check questions:

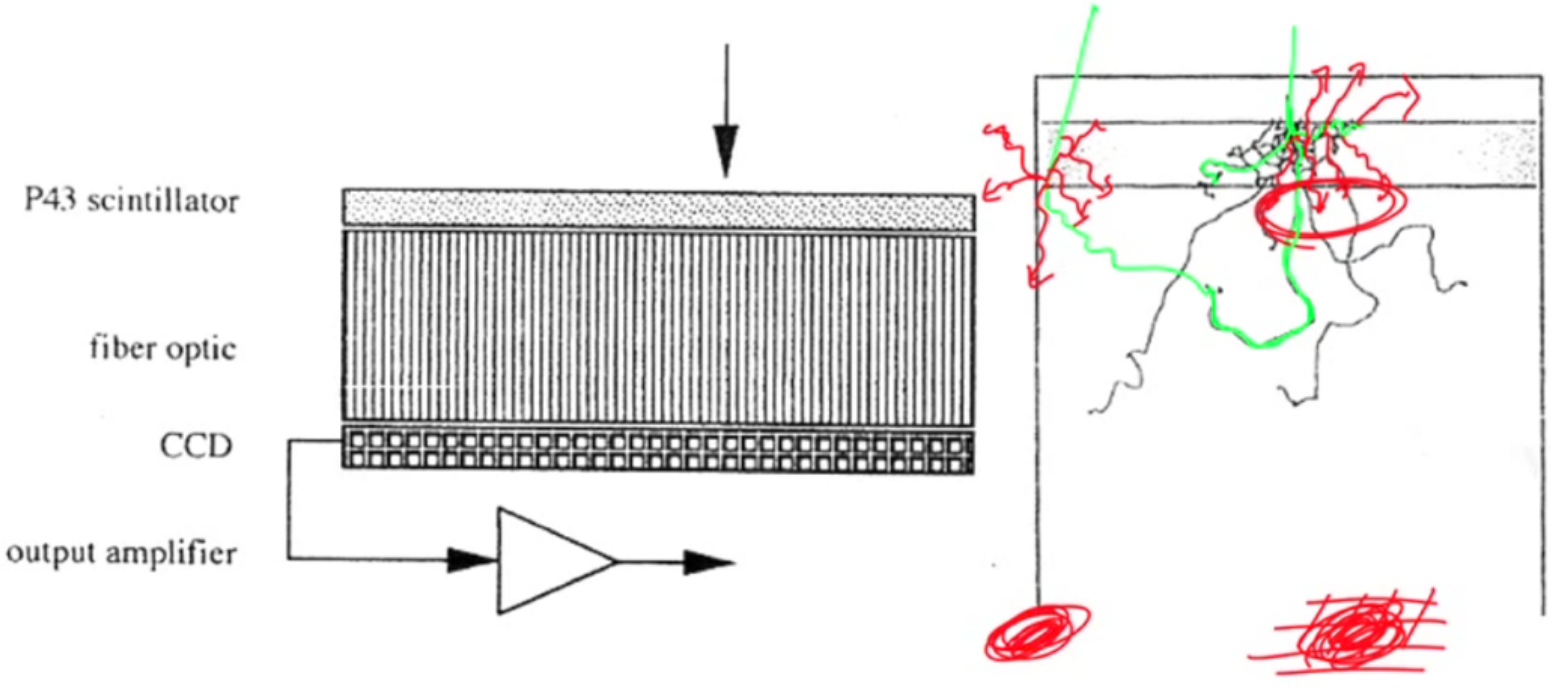
- Why are EM energy filters used?
- How are "post-column" filters different from "in-column" filters?
- What is a typical slit width for cryo-EM?
- What is a "zero-loss" peak?
- How could an energy filter allow you to image where a particular element was in the sample?

Electron detectors

Electron detectors

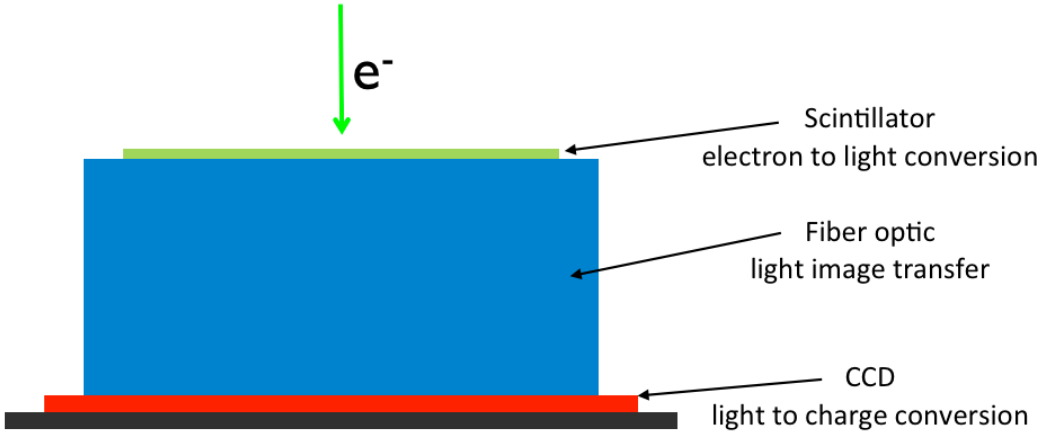
- Photographic film
- TVs
- CCDs
- Fluorescent screens
- “Direct” detectors

CCD detectors

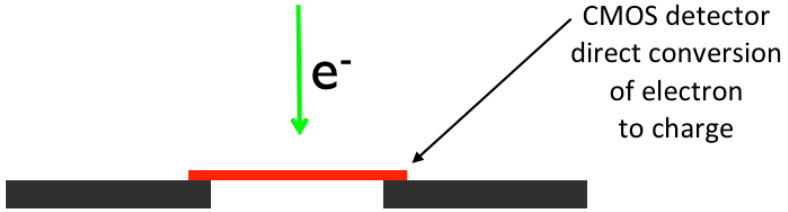


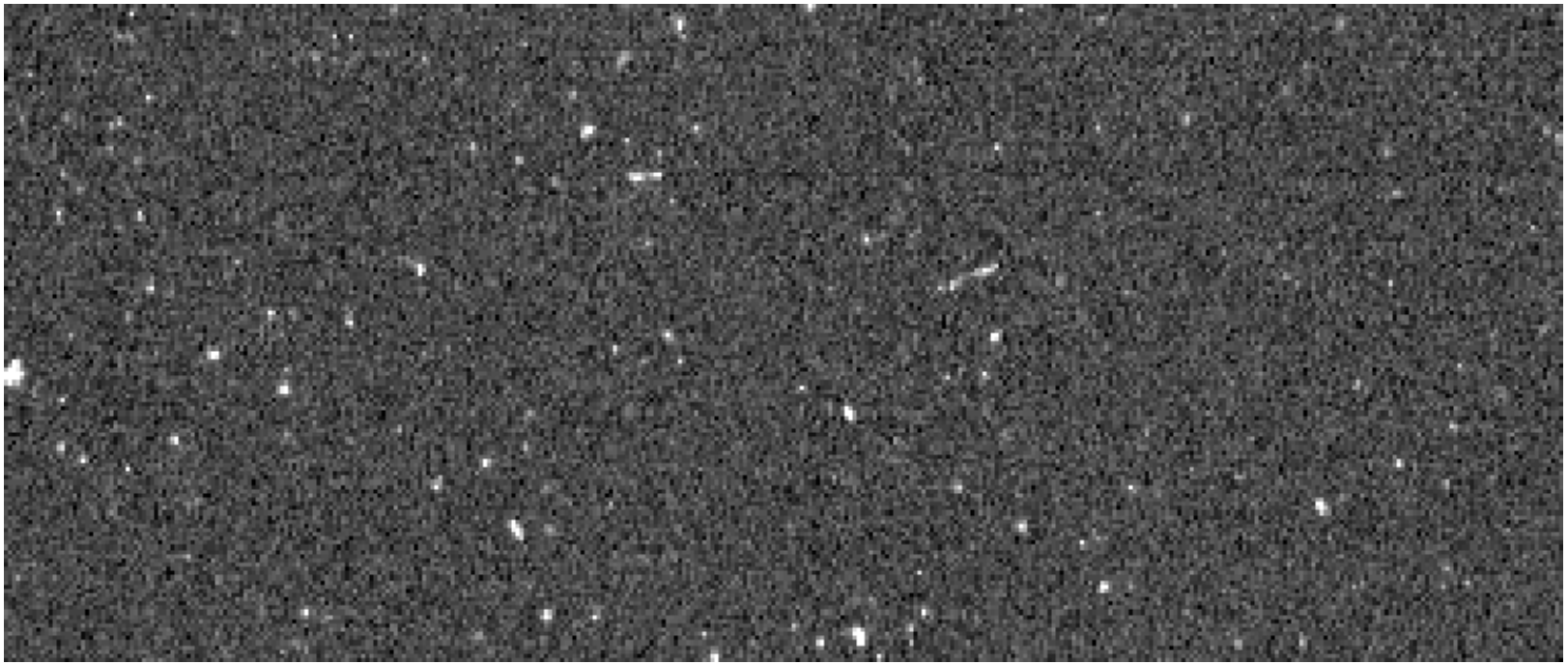
Downing et al., Ultramicroscopy 75:215 (1999)

CCD

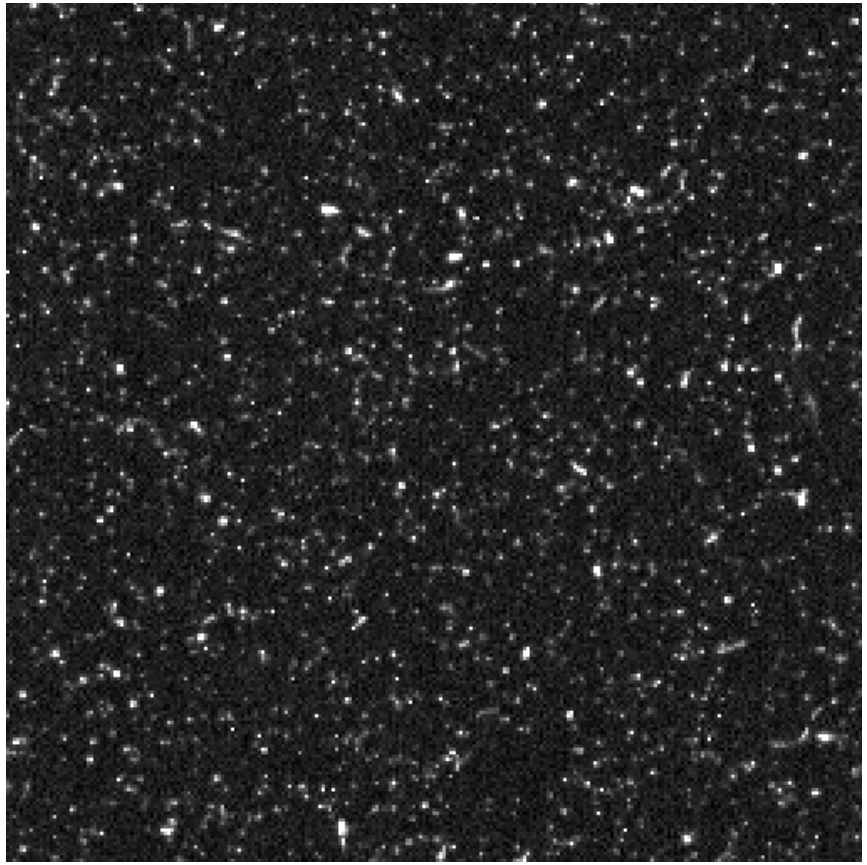


Direct Detector

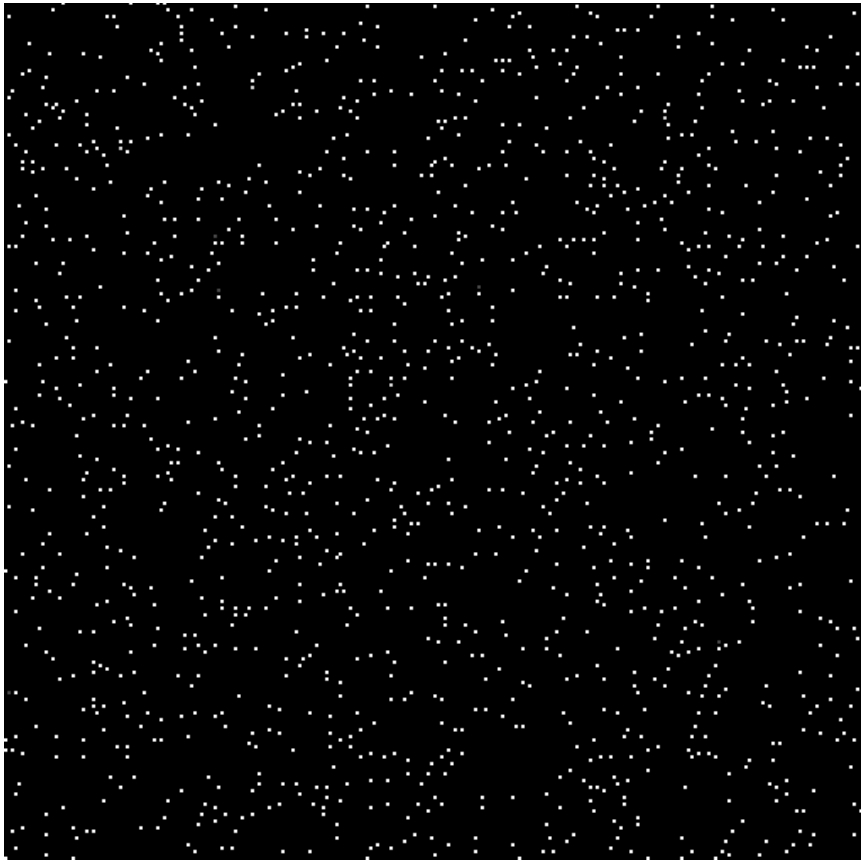




Courtesy gatan



Single 2.5 ms frame using conventional CCD-style charge read-out



Same frame after counting

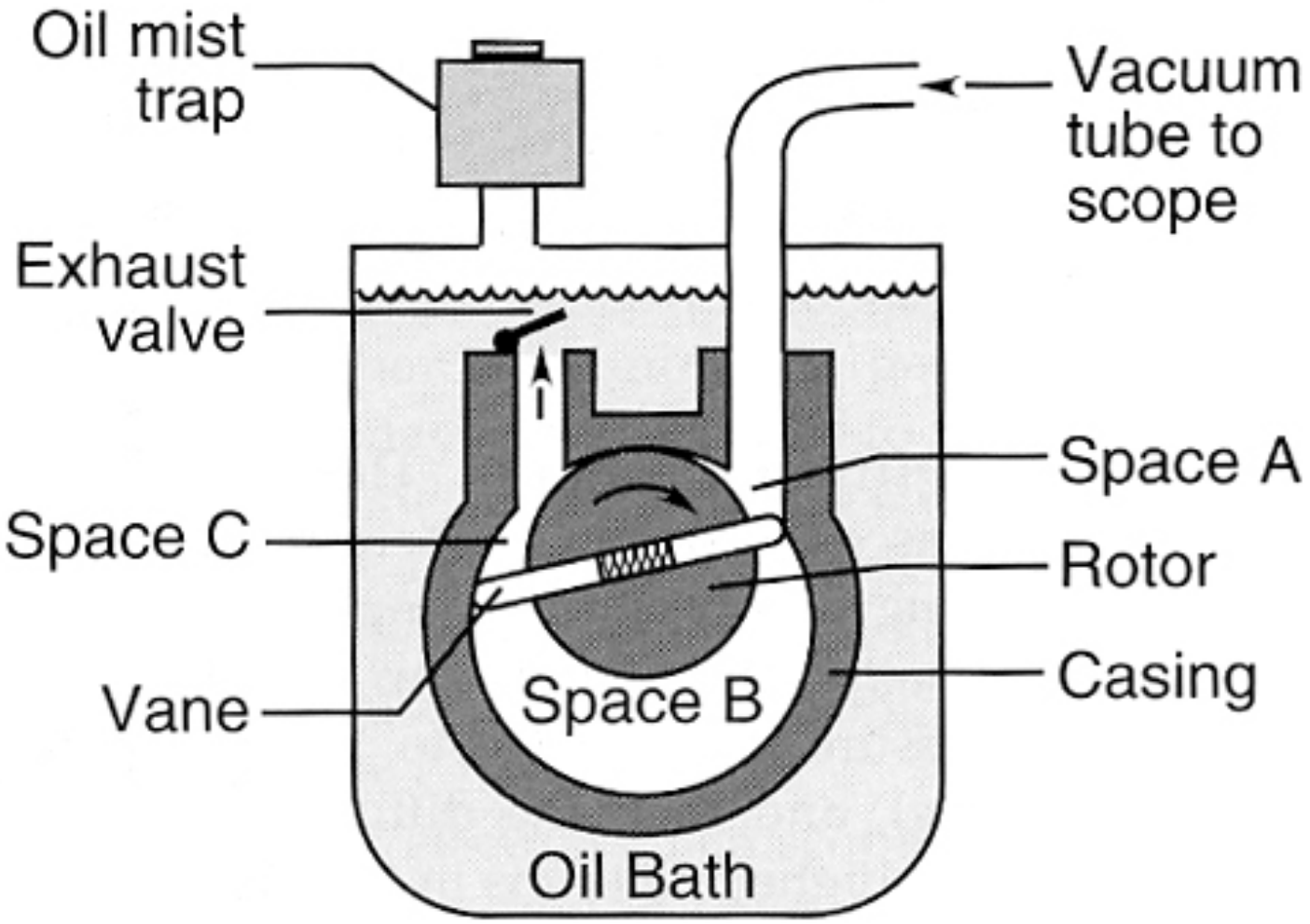
Counting removes the variability from scattering, rejects the electronic read-noise, and restores the DQE.

Electron detectors
Concept check questions:

- Name five different types of electron detectors.
- What is a "CCD"? How do they work?
- What are the advantages and disadvantages of film versus CCDs?
- What is meant by "direct" detector?
- What new capabilities do direct detectors provide?

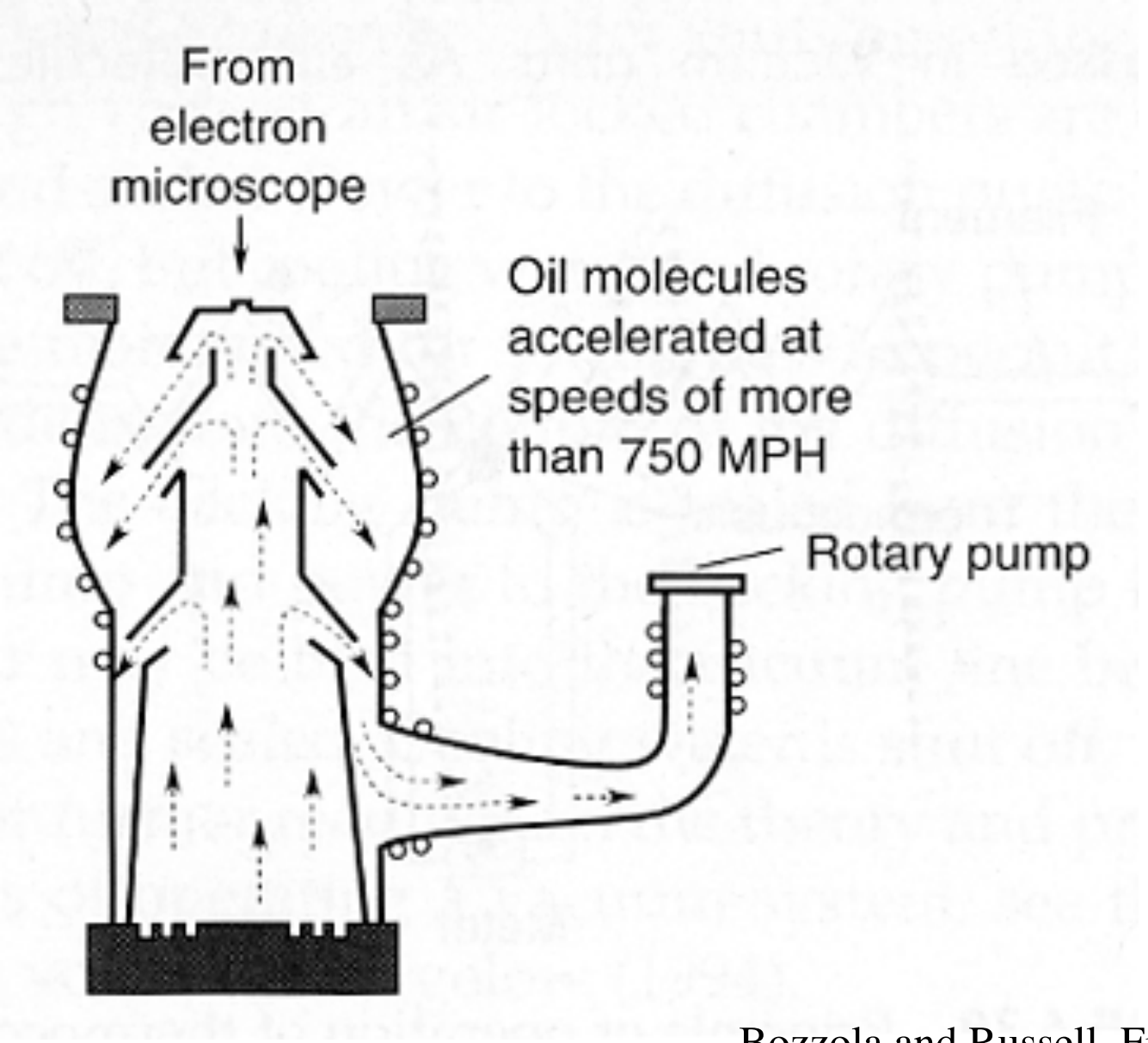
Vacuum systems

“mechanical” (rotary) pump



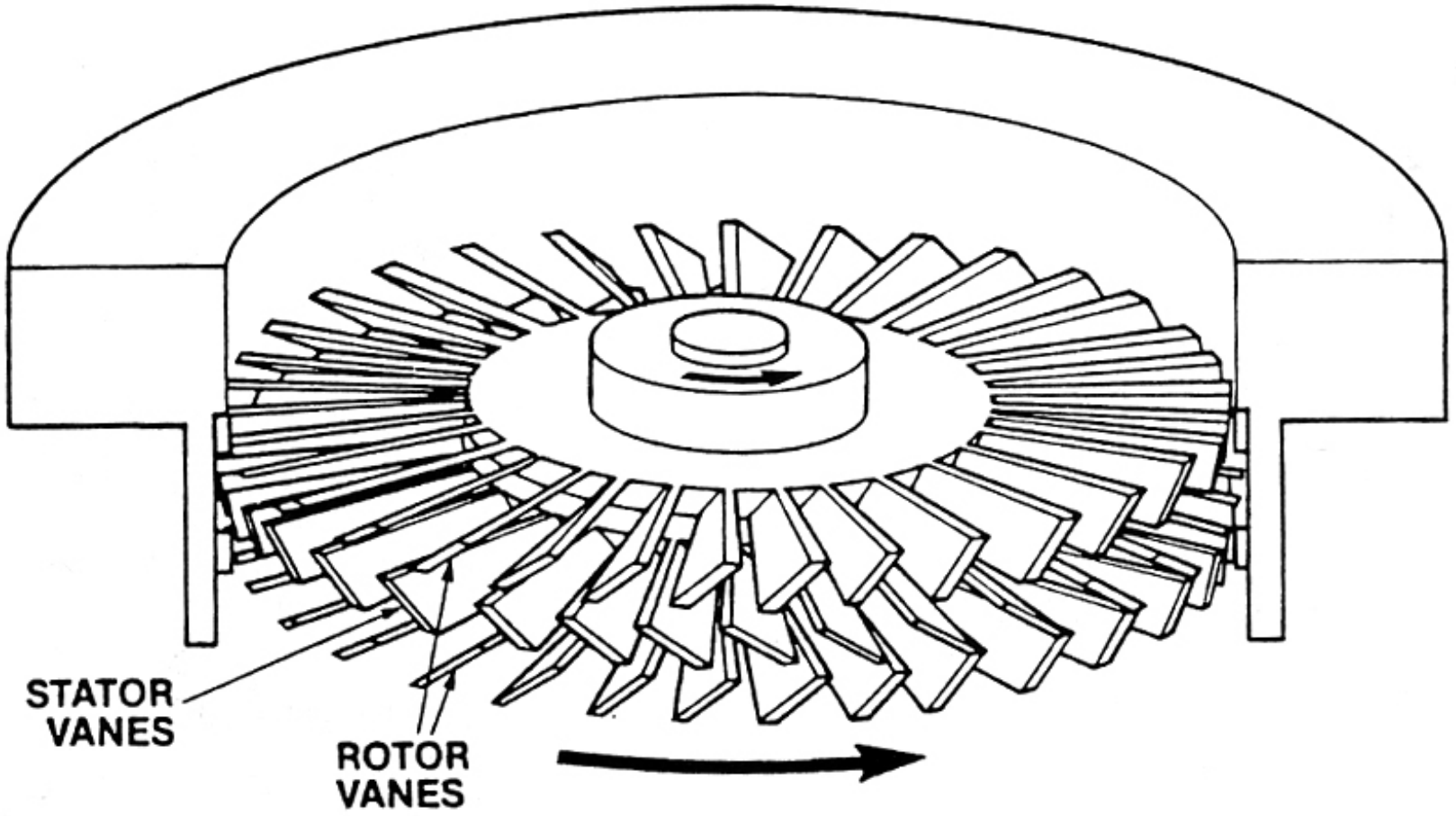
Bozzola and Russell, Fig. 6.37A

Oil-diffusion pump



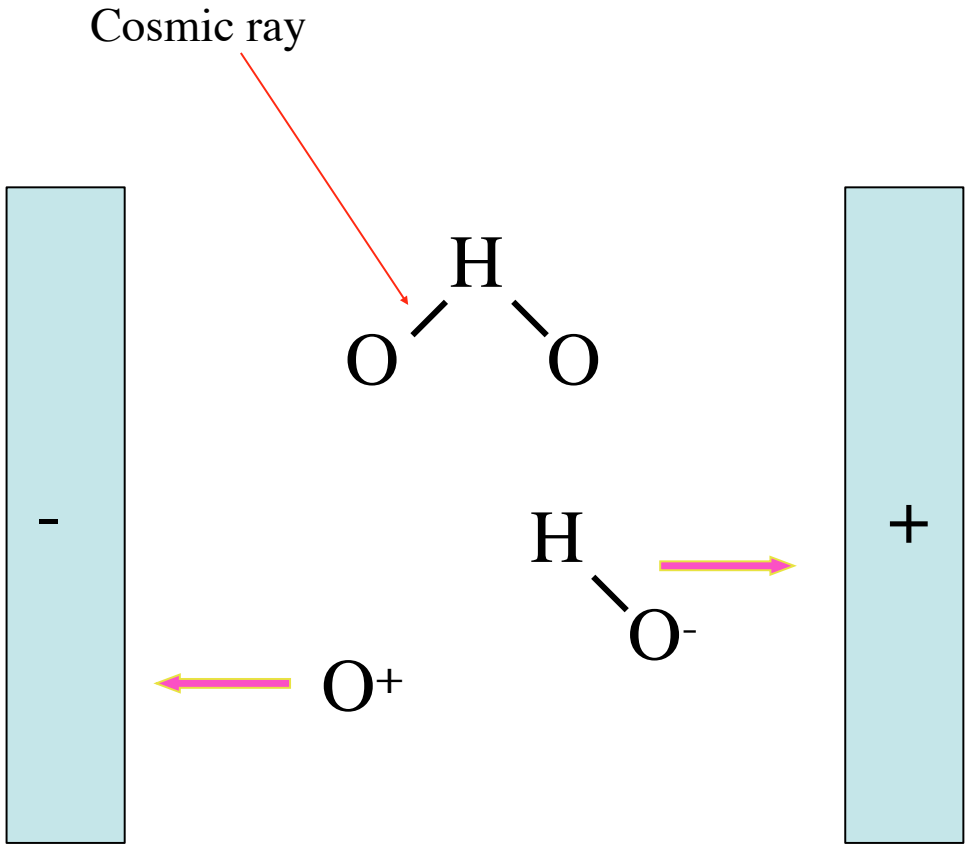
Bozzola and Russell, Fig. 6.38C

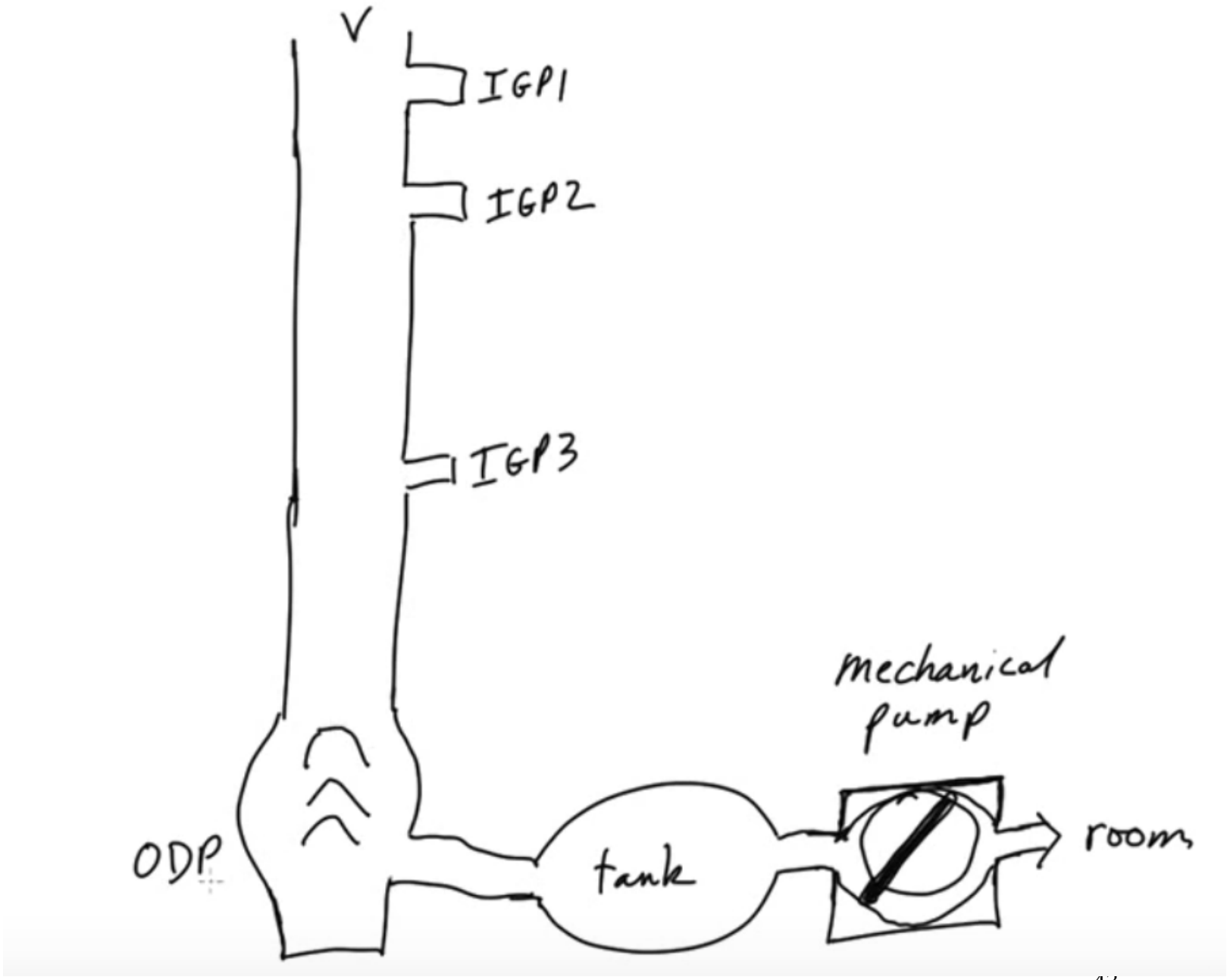
Turbo-molecular pump



Bozzola and Russell, Fig. 6.42

“Ion getter” pump

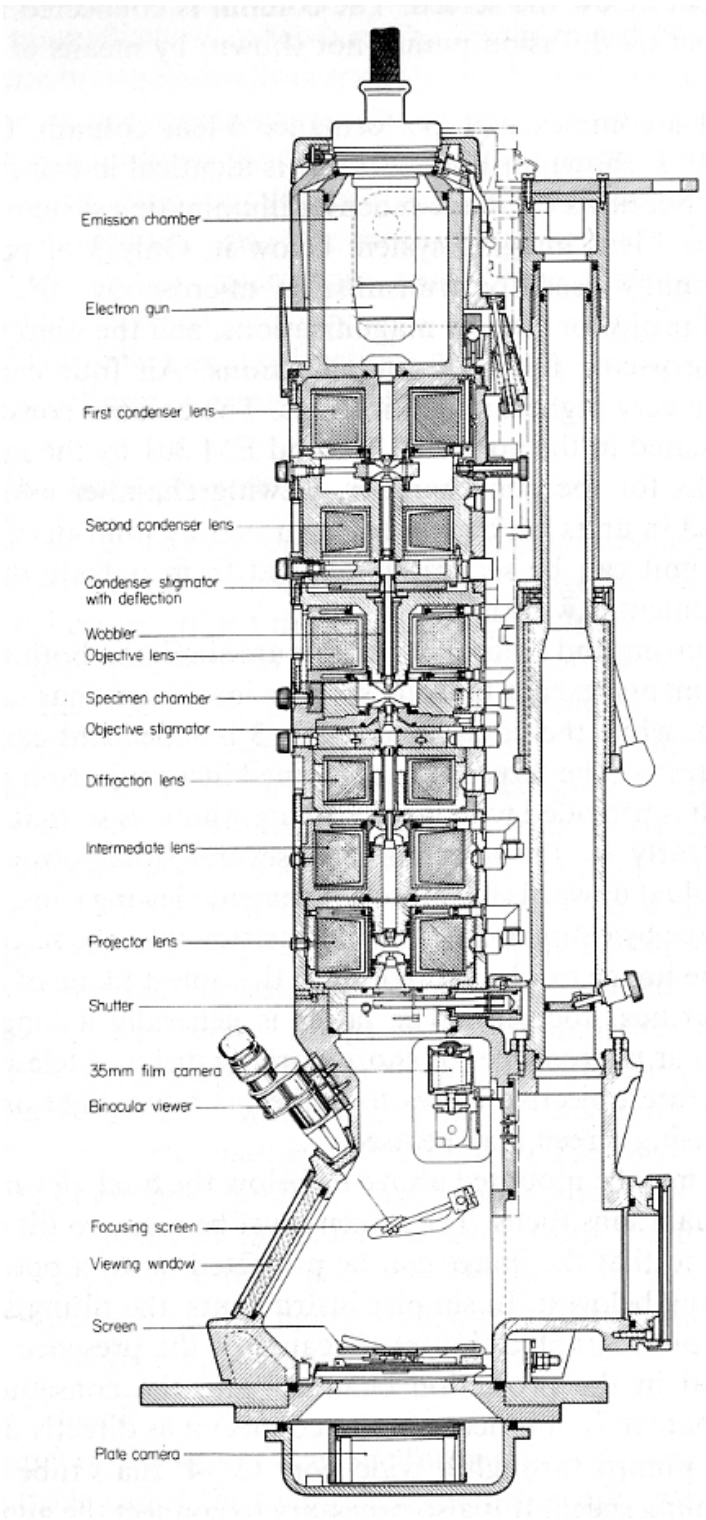



V
IGP1
IGP2
IGP3
mechanical pump
ODP
tank
room

Vacuum systems
Concept check questions:

- Name four different types of vacuum pumps. How does each work?
- Why are so many different types of pumps needed?
- What is a “backing” pump?

Summary, safety



lead shielding
SF_6
water
high voltage
liquid N_2
climbing
freezing burns

Summary, safety
Concept check questions:

- What is the purpose of the heavy lead shielding covering electron microscopes?
- Why do electron microscopes need chilled water?
- Name three lethal and at least one more non-lethal hazards associated with electron cryo-microscopes.