# Lecture Slides

Jan 17, 2013

# *P-Flasher* Question

Which of the following is designed to act as an electrostatic lens in the TEM?

A. Objective aperture

B. Objective lens

C. Specimen stage

D. Wehnelt shield

E. Second condenser lens

# *P-Flasher* Question

In the TEM, the specimen is situated very close to the front focal plane of the objective lens on the side closer to the center of the lens field.

A. True

B. False

# *P-Flasher* Question

The final image formed on the viewing screen or photographic emulsion is a virtual one and only becomes a real one when we view the screen or micrograph with our eyes.

A. True

B. False

CHM 165,265 / BIMM 162 / BGGN 262 Spring 2013

# Announcements for Jan 17, 2013

Reading assignment for Tuesday: Lecture notes pp.81-103

'Virtual homework': always check web site for new updates

Recitation session: Tomorrow at 5-6:00 pm in York 4080A

TEM facility tour: Jan 28,29 (check web site)

Reminders:

- Keep your *p-Flasher* sheets readily available during class
- Powerpoint lectures posted on Web site will include additional ('hidden') slides not shown during class

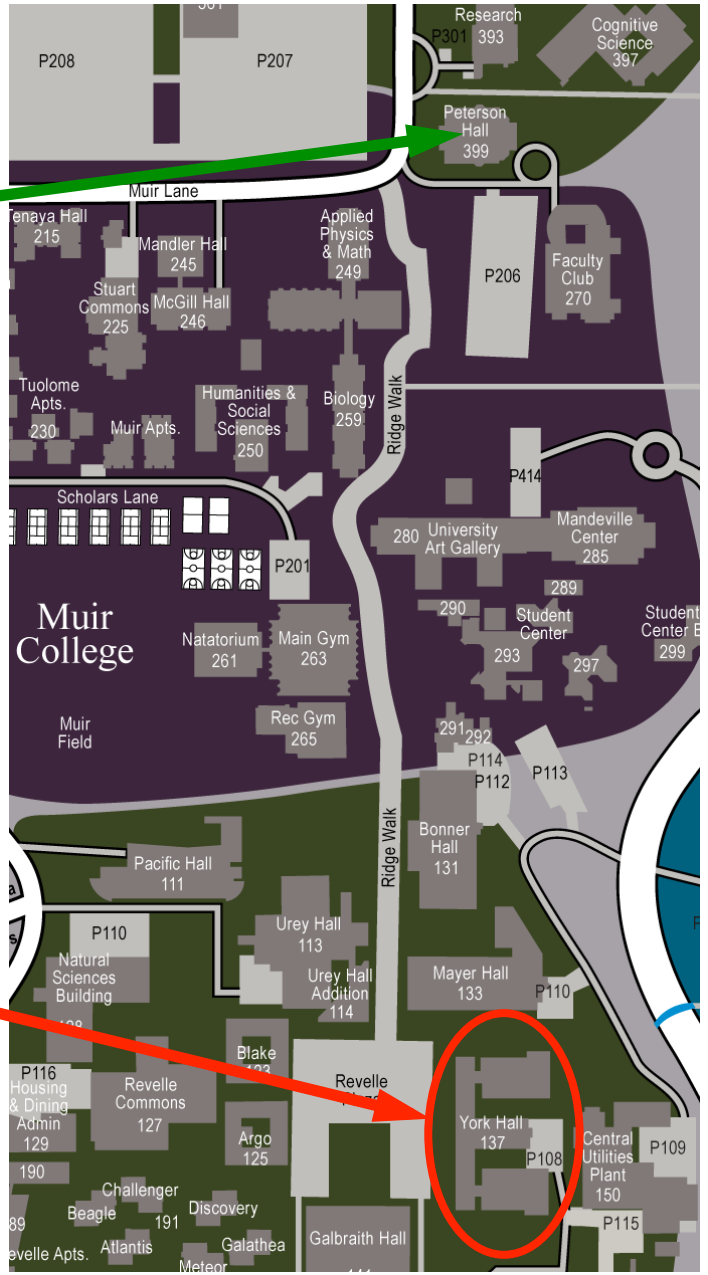
CHM 165,265 / BIMM 162 / BGGN 262 Winter 2013

**Peterson Hall, Room 103; Muir Campus**

Recitation session
***TOMORROW***, Jan 18, 2013
5:00 – 6:00 PM

Will include a laser diffraction demo, showing the relationship between simple objects and their diffraction patterns

**York Hall 4080-A Revelle Campus**



CHM 165,265 / BIMM 162 / BGGN 262 Winter 2013

# 3D Electron Microscopy of Macromolecules

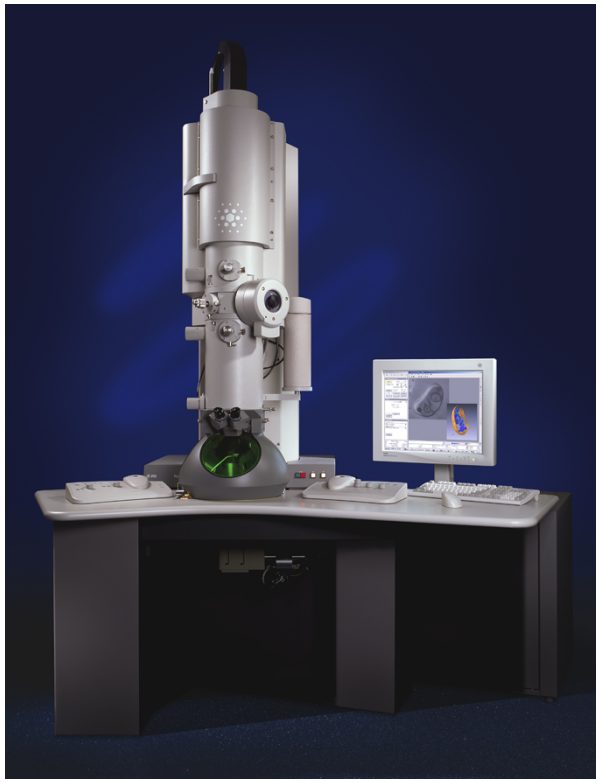
## TEM Facility Tour

Where: 1510 Bonner Hall basement

When: Mon Jan 28 and Tue Jan 29th

Check class web site for details on dates, times, and directions to facility

**Attendance is optional but 5 pts extra credit towards final grade will be awarded**



FEI Technai Sphera
(200keV; $LaB_6$; $LN_2$)

To reserve and guarantee a time slot, email **nholson@ucsd.edu**
**First come, first served.**

# Class Web Page: Jan 16, 2013

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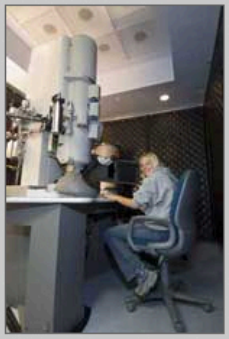
Dr. Timothy S. Baker

Intro | Members | Courses | Images | Publications | Software | Documentation and Procedures | Microscope Access | Microscope Facilities

**CHEM 165,265 / BIMM 162 / BGGN 262 - 3D Electron Microscopy of Macromolecules**
**Winter Quarter 2013**



**Class Tour of the Electron Microscopy Facility**
**Bonner Hall 1501**

Sign-up times:
Monday, January 28 -- 1 pm to 2 pm
Monday, January 28 -- 2 pm to 3 pm
Monday, January 28 -- 3 pm to 4 pm
Tuesday, January 29 -- 10 am to 11 am
Tuesday, January 29 -- 11 am to noon
Tuesday, January 29 -- 1 pm to 2 pm
Tuesday, January 29 -- 2 pm to 3 pm
Tuesday, January 29 -- 3 pm to 4 pm

To reserve a demo session write: Norm Olson
(Only four participants allowed per session - first come-first served)
(Keep an eye on this page. Closed sessions will be in ~~red strikeout~~ text)

Directions to the EM Facility

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Syllabus (PDF)

Book list (PDF)

Reference list (PDF)

The Bottom Line (PDF) -- Key concepts from daily lectures through January 15, 2013

Virtual Homework - Practice Questions for Section I (PDF) Updated January 15, 2013 (Password protected)

Lecture Notes

- Sec. IA. Principles of the transmission electron microscope (9.2 MB)
- Sec. IB. Design of the transmission electron microscope (3.5 MB)
- Sec. IC. Contrast and image formation (692 KB)

Powerpoint® presentations from lecture (PDFs)

- Introduction to the course January 8, 2013 (48 MB)
- Lecture #1 January 8, 2013 (15.5 MB)
- Lecture #2 January 10, 2013 (4.3 MB)
- Lecture #3 January 15, 2013 (20.8 MB)

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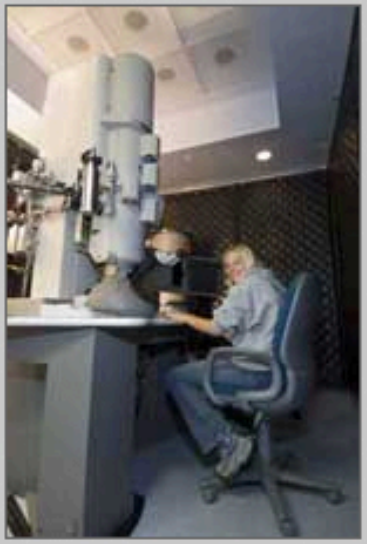

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Directions to the EM Facility

# I.B DESIGN OF THE TEM

## KEY CONCEPTS FROM LECTURE #3

- **Thermionic emission** creates a source of electrons

- Electrons passing through an **electric or a magnetic field** are bent or **refracted**

- **Focal length** of electromagnetic lens determined by **field strength** and by **electron speed**

$$f = \frac{KV_r}{(N \cdot I)^2}$$

- **Wehnelt shield** (gun cap) controls beam shape and emission in the $e^-$ gun

- **Gun-crossover** **considered** the actual source of electrons for the TEM

- **Double condenser lens system** focuses / concentrates the electron beam onto the specimen (gives optimal illumination for viewing and recording images)

- Smaller **condenser apertures** increase coherence of the electron beam and hence, phase-contrast effects in electron images

# I.B DESIGN OF THE TEM

## MORE KEY CONCEPTS FROM LECTURE #3

- Electromagnetic lenses are **'crummy' :** they are reason why resolving power is **much worse** than estimated according to the simple 1/2 λ criteria

- **Spherical aberration** (of objective lens): main culprit and the principal factor limiting resolving power in the TEM

- **Chromatic aberration**: lens focal length varies with electron/photon wavelength; images with CA are blurred (superposition of a series of images with different focal planes, rotations, and magnifications)

- **Lens asymmetry:** no real lens can be manufactured that is perfectly axially symmetric. Result: focal length varies with direction. Condenser and objective lens astigmatism are controlled (i.e minimized) by the microscopist.

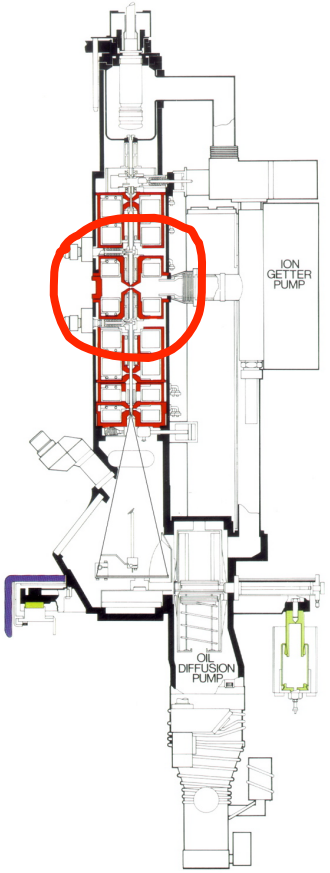
- **Objective lens: most critical** lens of TEM; performs 1st stage of imaging and determines instrument resolving power and image contrast

- **Objective aperture:** at **back focal plane** of the objective lens; intercepts electrons scattered by the specimen through large angles

# I.B DESIGN OF THE TEM

## The TEM Top to Bottom:

- Electron gun
- Condenser lens(es)
- Lens aberrations
- Objective lens and specimen stage
- Projector lenses
- Camera and viewing system
- Vacuum system
- Electrical system

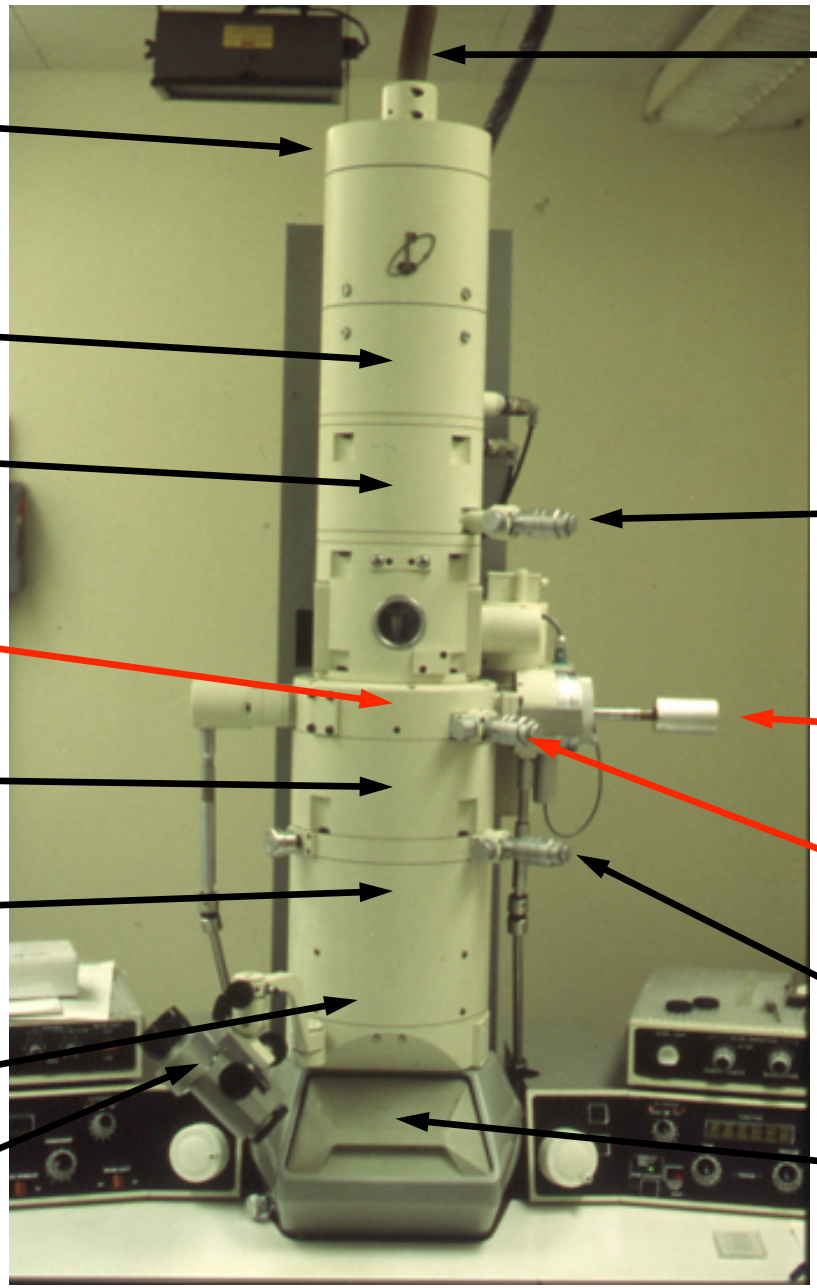


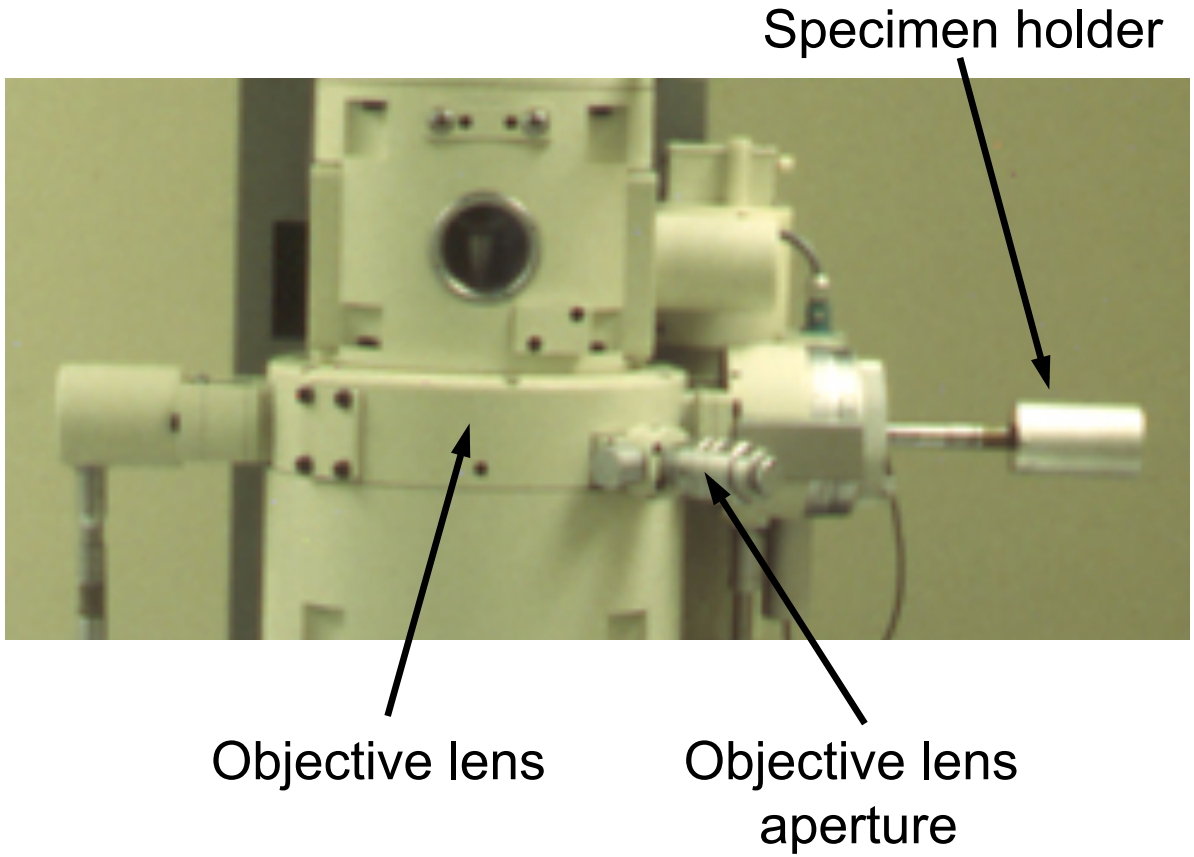
# § I: The Microscope

## I.B Design of the TEM

## I.B.4 Objective Lens and Specimen Stage

(pp.54-62 of lecture notes)


Electron gun
Condenser lens 1
Condenser lens 2
Objective lens
Intermediate lens 1
Intermediate lens 2
Projector lens
Binoculars
High tension cable
Cond. 2 aperture
Specimen holder
Obj. lens aperture
Intermediate lens aperture
Viewing port


Specimen holder
Objective lens
Objective lens aperture

I.B.4 Objective Lens and Specimen Stage

# Bottom Line:

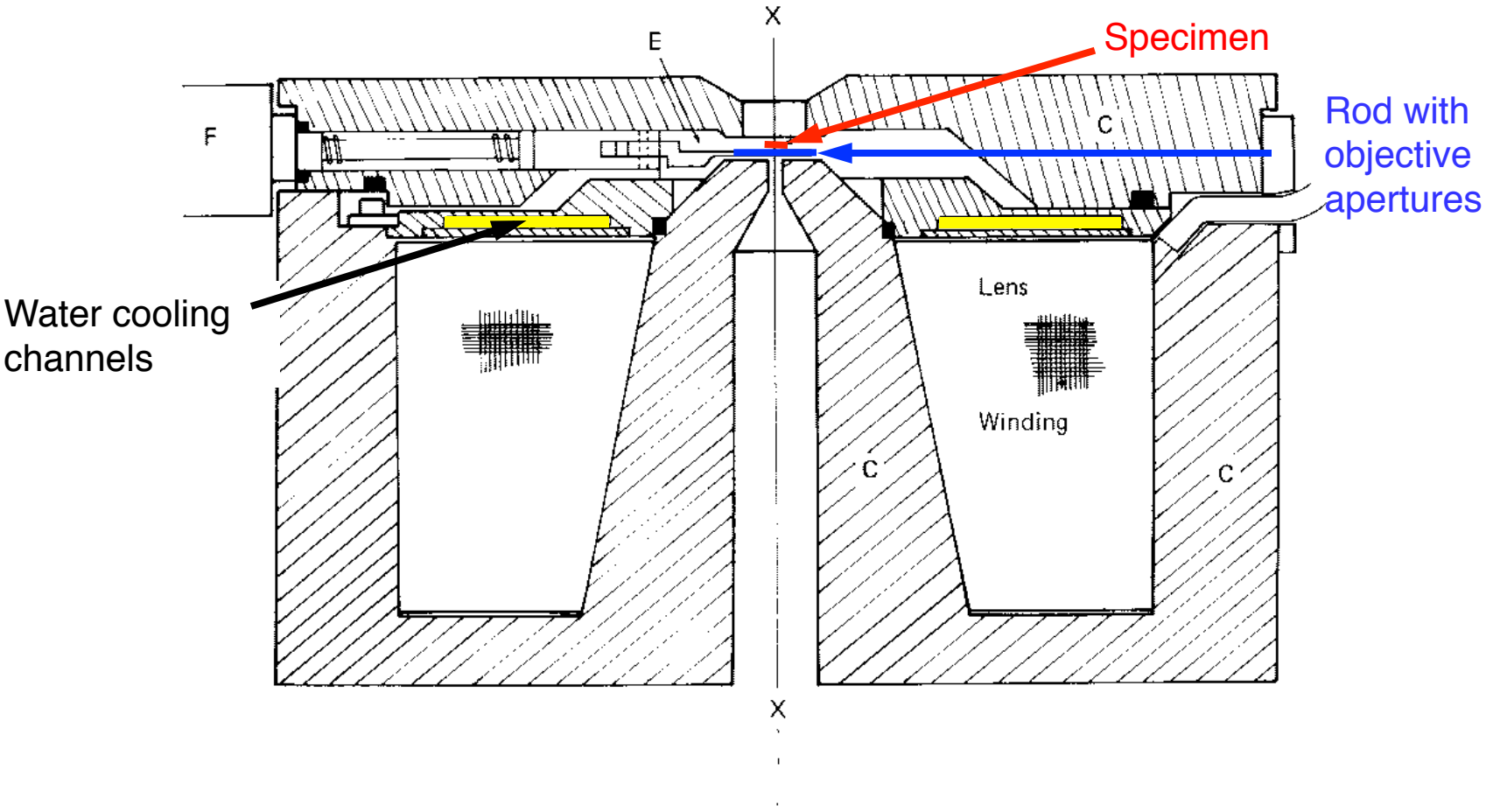
**Objective** lens is the **most critical** lens in the TEM

- Performs **first stage of imaging**

- Determines instrument **resolving power** and **image contrast**

# I.B.4 Objective Lens and Specimen Stage

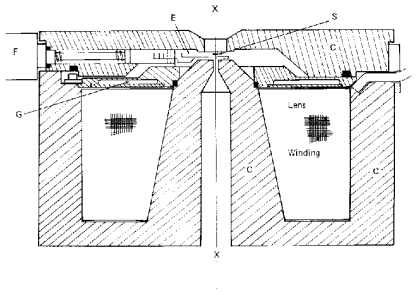
## I.B.4.b Objective Lens Construction




From Agar, Fig. 2.11, p.51

# I.B.4 Objective Lens and Specimen Stage

## I.B.4.b Objective Lens Construction




### Main Requirements

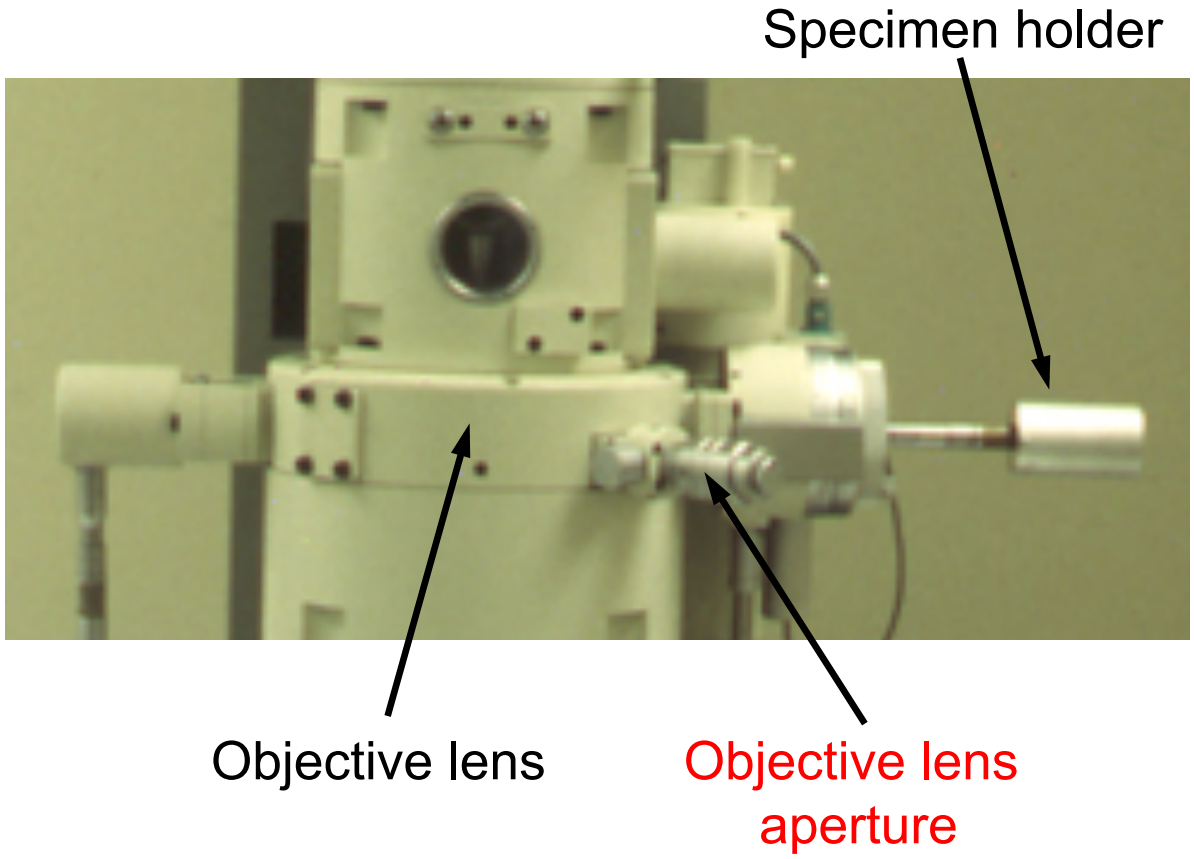
- **Specimen** situated **close to** and **before** the **front focal plane** of the objective lens

- Specimen sits ***inside*** the **lens field** (necessary to obtain short focal length)

- **Space is very cramped** (need adequate clearance for inserting several items):
  - Specimen
  - Aperture
  - Anticontaminator
  - Stigmators to correct for asymmetries in the lens field

# I.B.4 Objective Lens and Specimen Stage

## I.B.4.e Objective Aperture (OA)



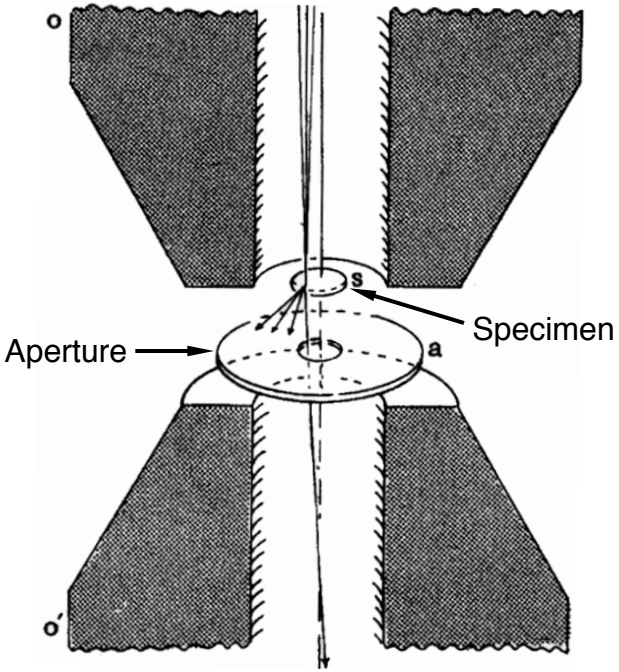
# I.B.4 Objective Lens and Specimen Stage

## I.B.4.e Objective Aperture (OA)

**FUNCTION:** Intercepts electrons **scattered** by the specimen through **large angles**

**POSITION:** Right at the ***back*** **focal plane** of the objective lens

Schematic of lengthwise section through objective lens pole pieces




From Wischnitzer 2nd ed., Fig. 51, p.60

OA does not restrict field of view

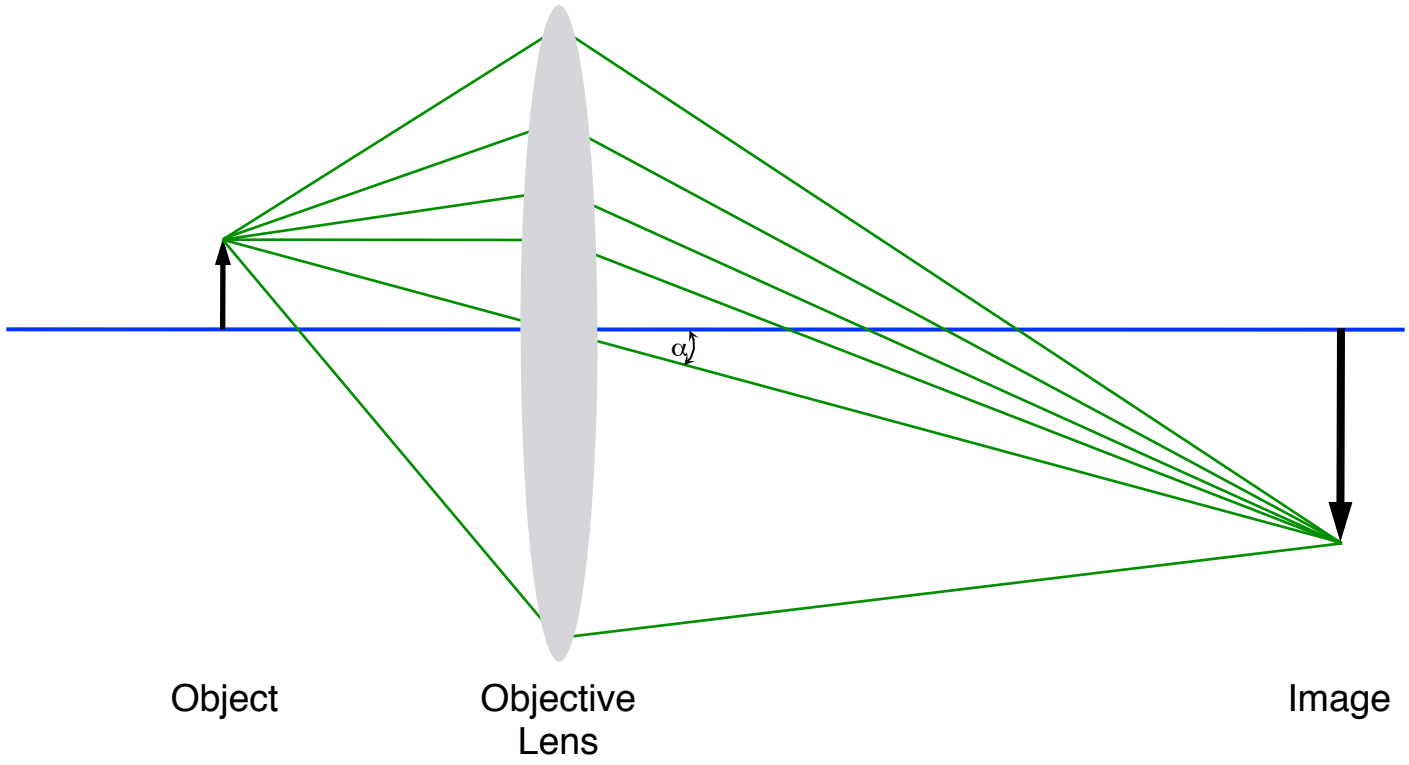
# I.B.4 Objective Lens and Specimen Stage

## I.B.4.e Objective Aperture (OA)

**FUNCTION:** Intercepts electrons **scattered** by the specimen through **large angles**

**POSITION:** Right at the ***back*** **focal plane** of the objective lens

Here, the OA screens out widely scattered electrons from being imaged



# I.B.4 Objective Lens and Specimen Stage

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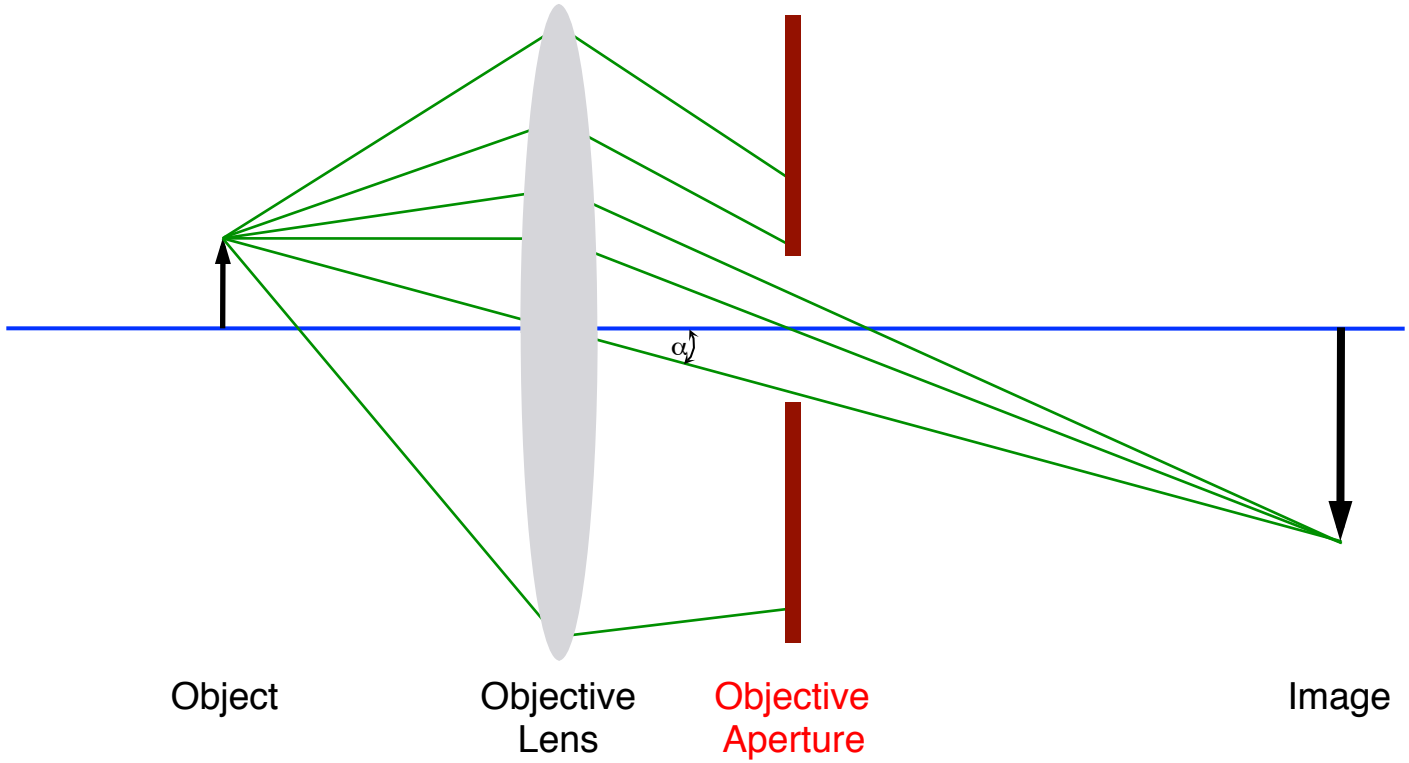


# I.B.4 Objective Lens and Specimen Stage

## I.B.4.e Objective Aperture (OA)

### *Miscellaneous Factoids:*

- Hole diameter generally **~ 25-75 μm**

- Must be **perfectly circular** and clean

  A 'dirty' aperture will distort the imaging field

  Will act like a weak electrostatic lens and cause image astigmatism

# I.B.4 Objective Lens and Specimen Stage

## I.B.4.e Objective Aperture (OA)

### *Miscellaneous Factoids:*

- Hole diameter generally **~ 25-75 μm**

- Must be **perfectly circular** and clean (or imaging field will be distorted)

  Contaminated aperture can act like a weak electrostatic lens and cause image astigmatism

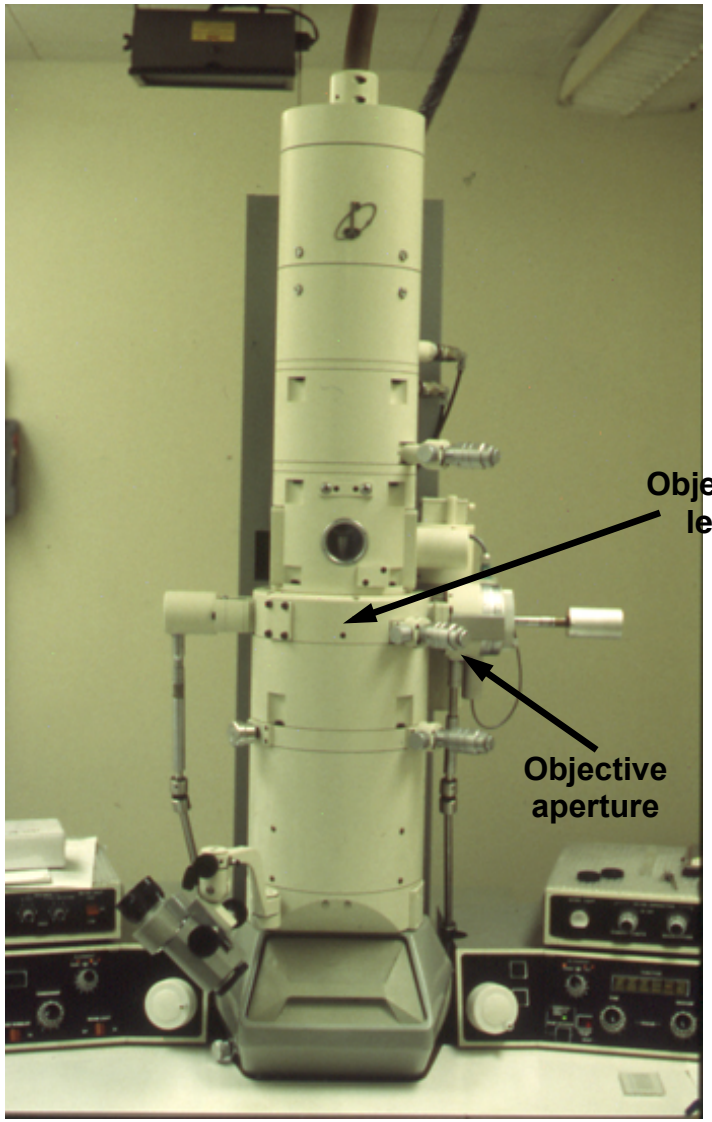
- **Ultrathin, self-cleaning** metal apertures are best

- **Smallest** apertures give **best image contrast** but exhibit more serious contamination effects

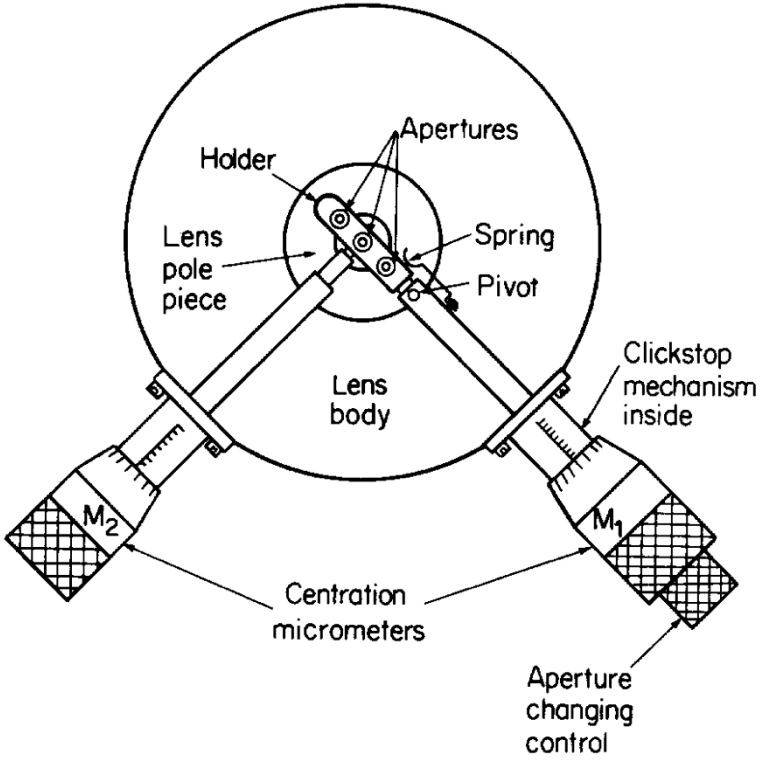
Slide not shown in class lecture

# I.B.4 Objective Lens and Specimen Stage

## I.B.4.e Objective Aperture (OA)




**Most TEMs have an objective aperture holder with space for 3 apertures**




From Meek, 1st ed., Fig. 5.2, p.95

# I.B.4 Objective Lens and Specimen Stage

## I.B.4.f Specimen Stage

## Desirable Properties of a Specimen Stage

- Specimen exchange should be **simple and rapid**

- Must have a specimen **airlock**

- Should sit in a plane that is well defined with respect to its position along the axis of the optical system

- Provide **minimum mechanical backlash, drift,** and **vibrations**, and minimal thermal motions and other movements

See p.59 of lecture notes for additional properties

# I.B.4 Objective Lens and Specimen Stage

## I.B.4.f Specimen Stage

**How do I get the sample into the TEM?**

**Microscopes come in only two "flavors":**

**Side Entry** ← **Most common**

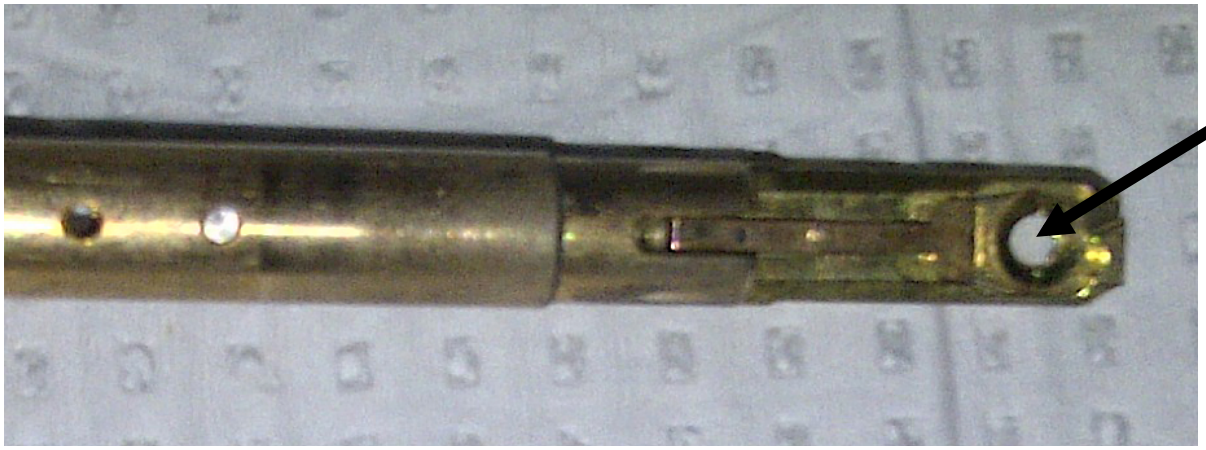
**Top Entry**

# I.B.4 Objective Lens and Specimen Stage

## I.B.4.f Specimen Stage

**How do I get the sample into the TEM?**

## Side Entry



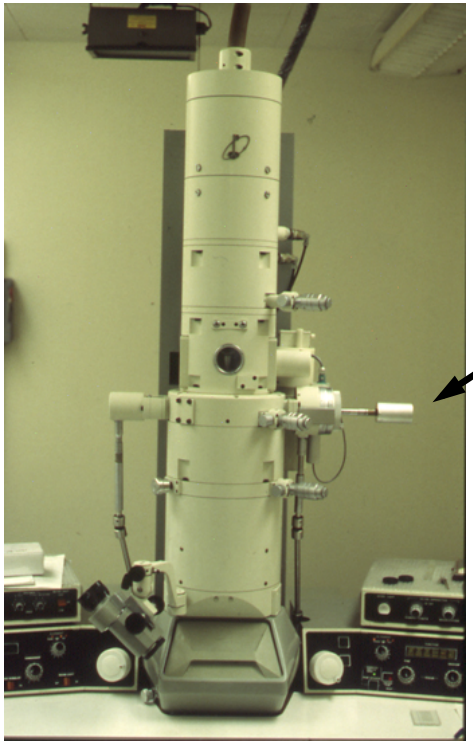

Photos courtesy of N. Olson

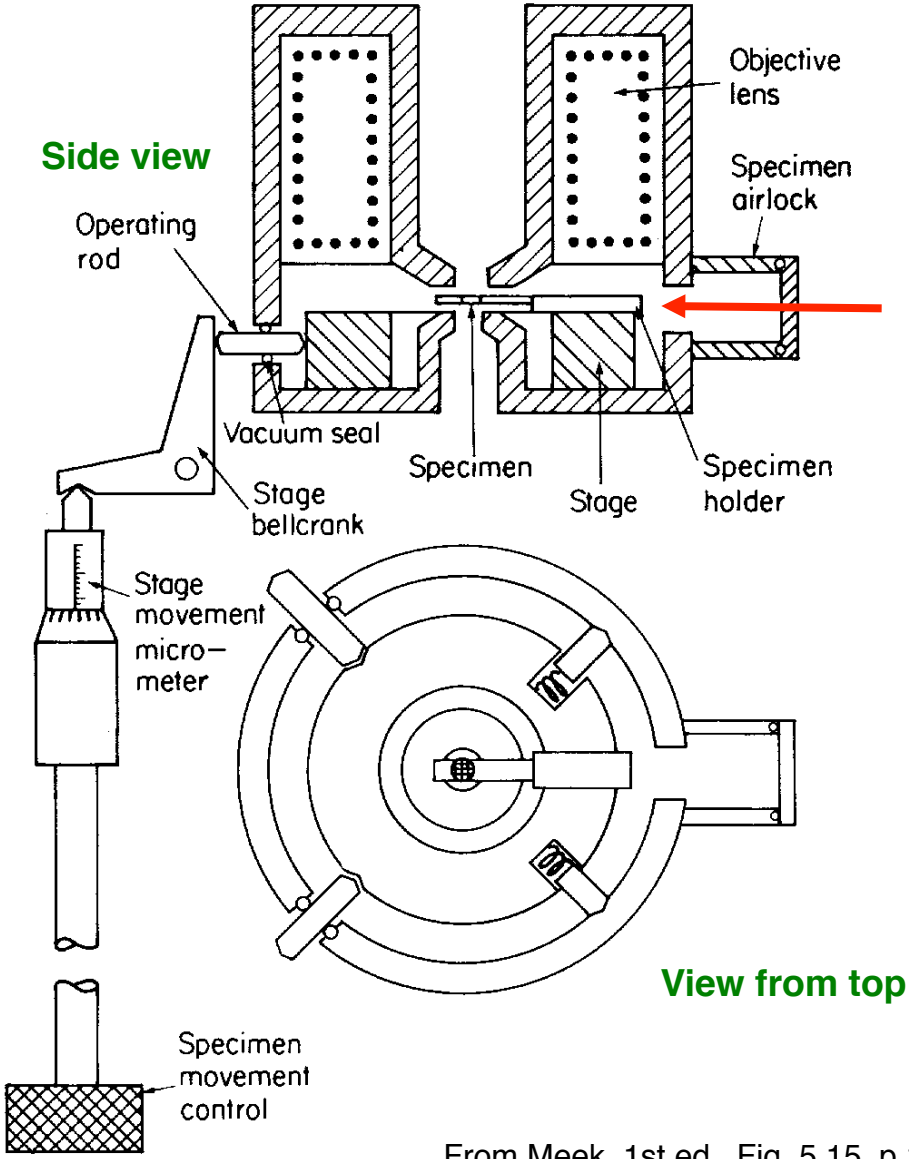
# I.B.4 Objective Lens and Specimen Stage

## I.B.4.f Specimen Stage

### Side Entry Stage

Specimen mounted at end of a long rod, inserted into center of objective lens



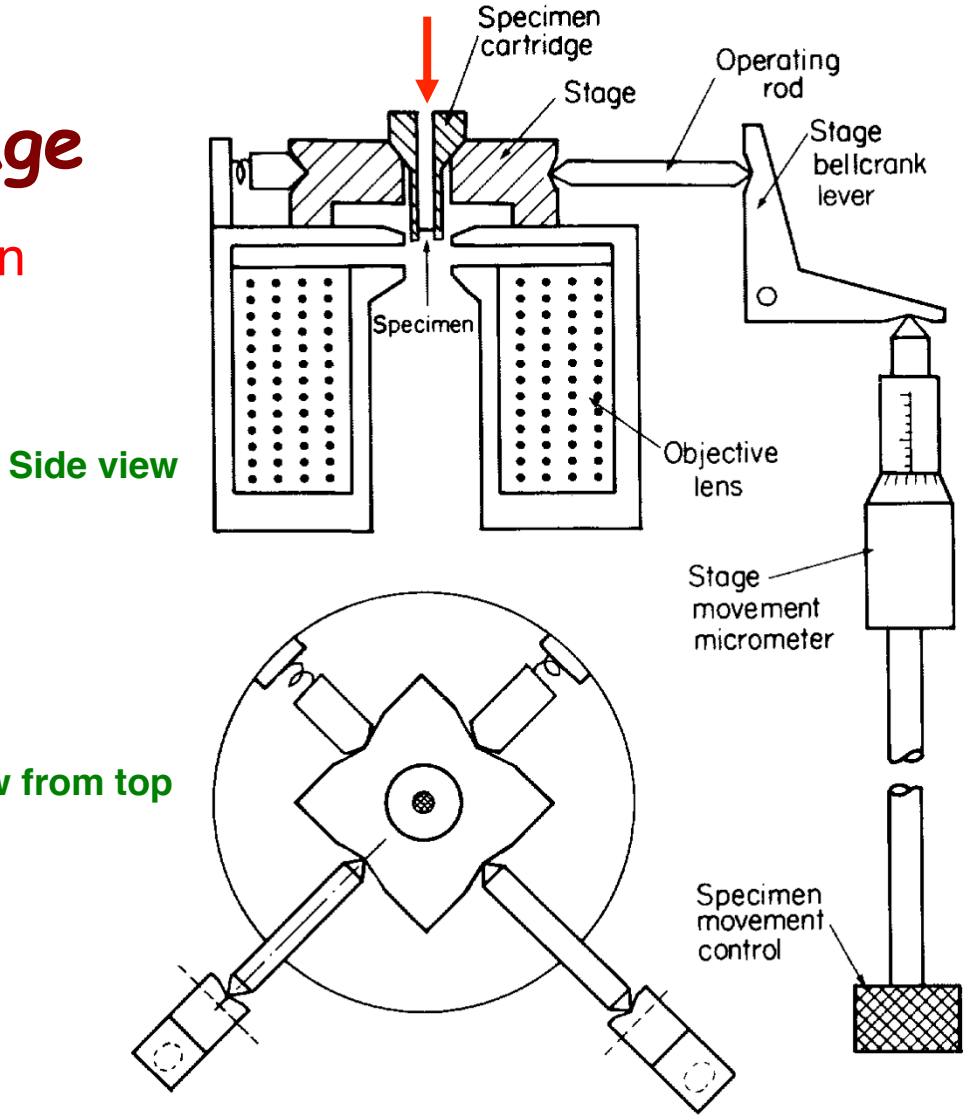

From Meek, 1st ed., Fig. 5.15, p.115

# I.B.4 Objective Lens and Specimen Stage

## I.B.4.f Specimen Stage

### Top Entry Stage

Specimen mounted in drop-in cartridge




From Meek, 1st ed., Fig. 5.14, p.114

# I.B.4 Objective Lens and Specimen Stage

## I.B.4.g Special Stages

**One for nearly every need!!!**

- Tilt stage
- Multiple specimen stage
- Furnace heating stage
- Grid heater stage
- Cold stage
- Straining stage
- Gas reaction stage
- Hydration or ‘wet’ stage
- *Many, many more….*

See notes (p.61) for some details

# I.B.4 Objective Lens and Specimen Stage

## I.B.4.g Special Stages

**One for nearly every need!!!**

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- Multiple specimen stage
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- **Cold stage**
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# I.B DESIGN OF THE TEM

## The TEM Top to Bottom:

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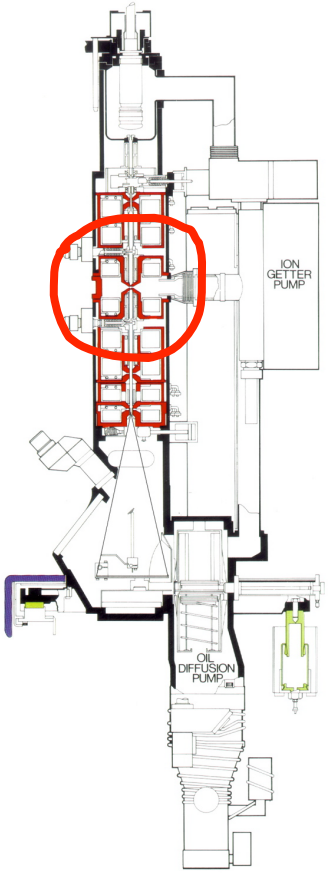

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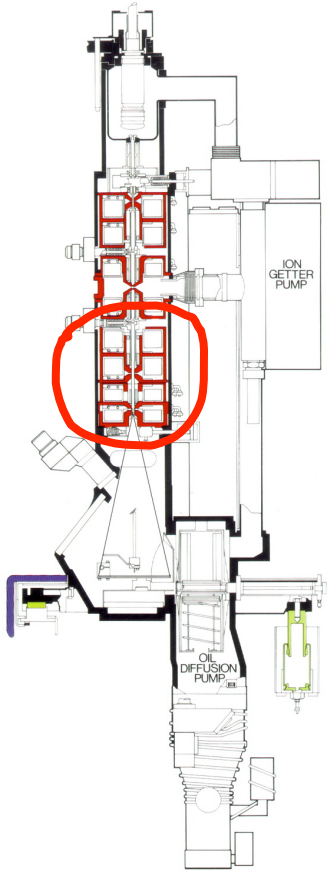
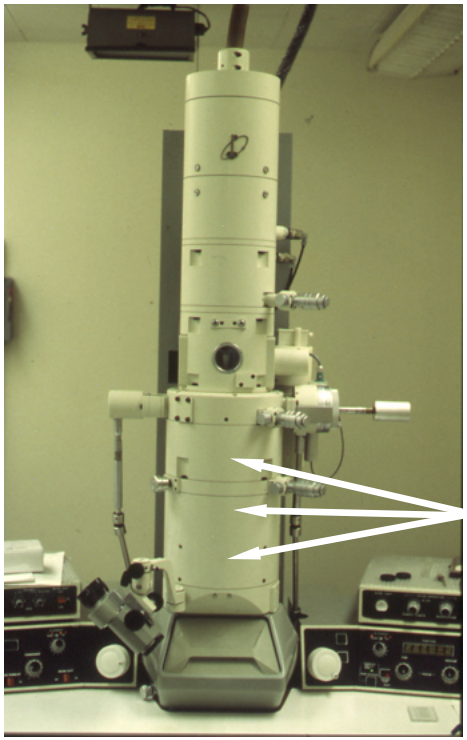


# § I: The Microscope

I.B Design of the TEM

I.B.5 Projector Lens(es)

(pp.62-64 of lecture notes)




# I.B.5 Projector Lenses

## I.B.5.a Description

## Projector Lens Systems

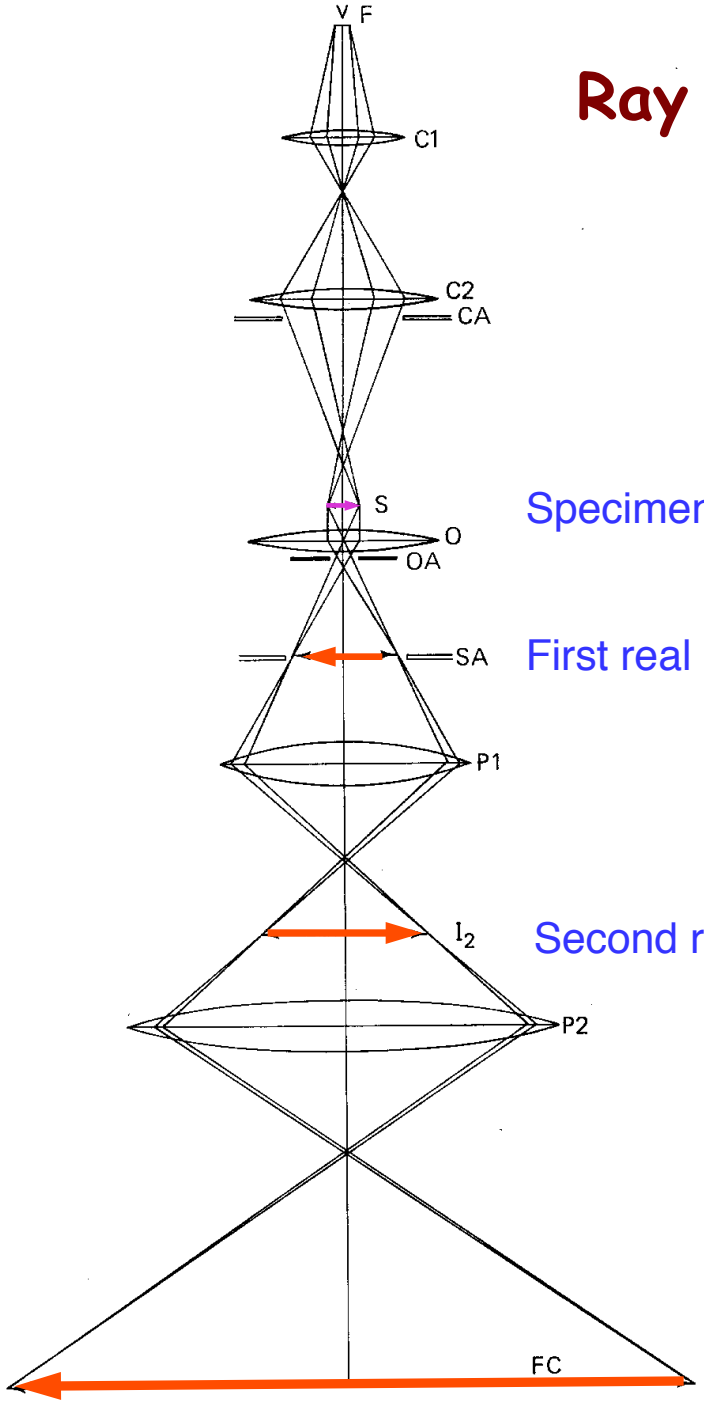
Produce and control magnification of final image

3-4 lenses (diffraction, intermediate, plus one or two projector lenses) give a wide magnification range (~1000X up to >500,000X)

**Intermediate image** produced by objective lens serves as **object** of 1st 'projector' lens, and so forth.

# Ray diagram for a two projector TEM

(Shown during lecture #2)




Specimen

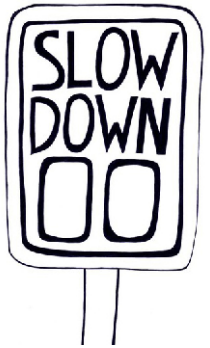
First real image

Second real image

Final real image on viewing screen

From Agar, Fig. 1.30, p. 35

I.B.5 Projector Lenses



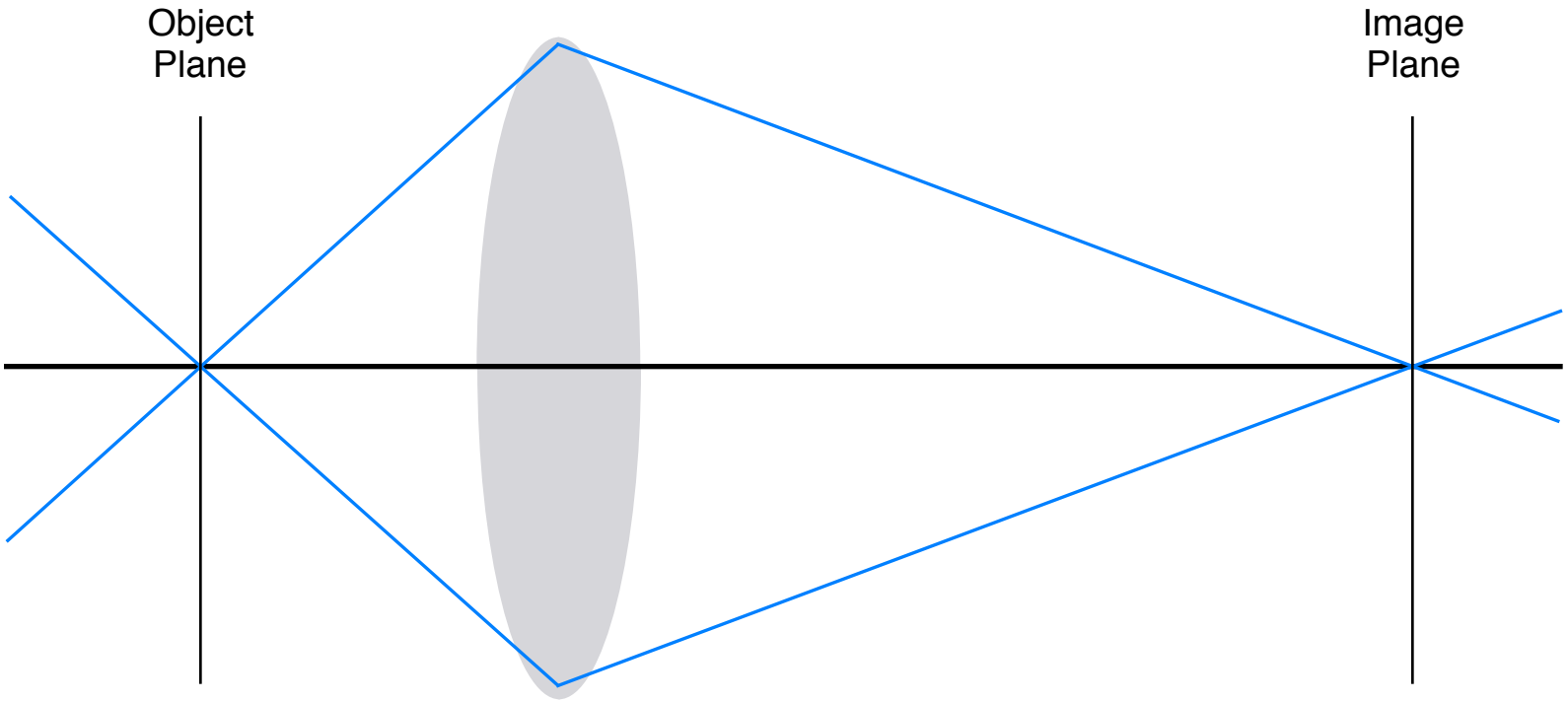

**KEY CONCEPTS**

# Depth of Field
# Depth of Focus

# I.B.5 Projector Lenses

## I.B.5.c Depth of Field and Depth of Focus

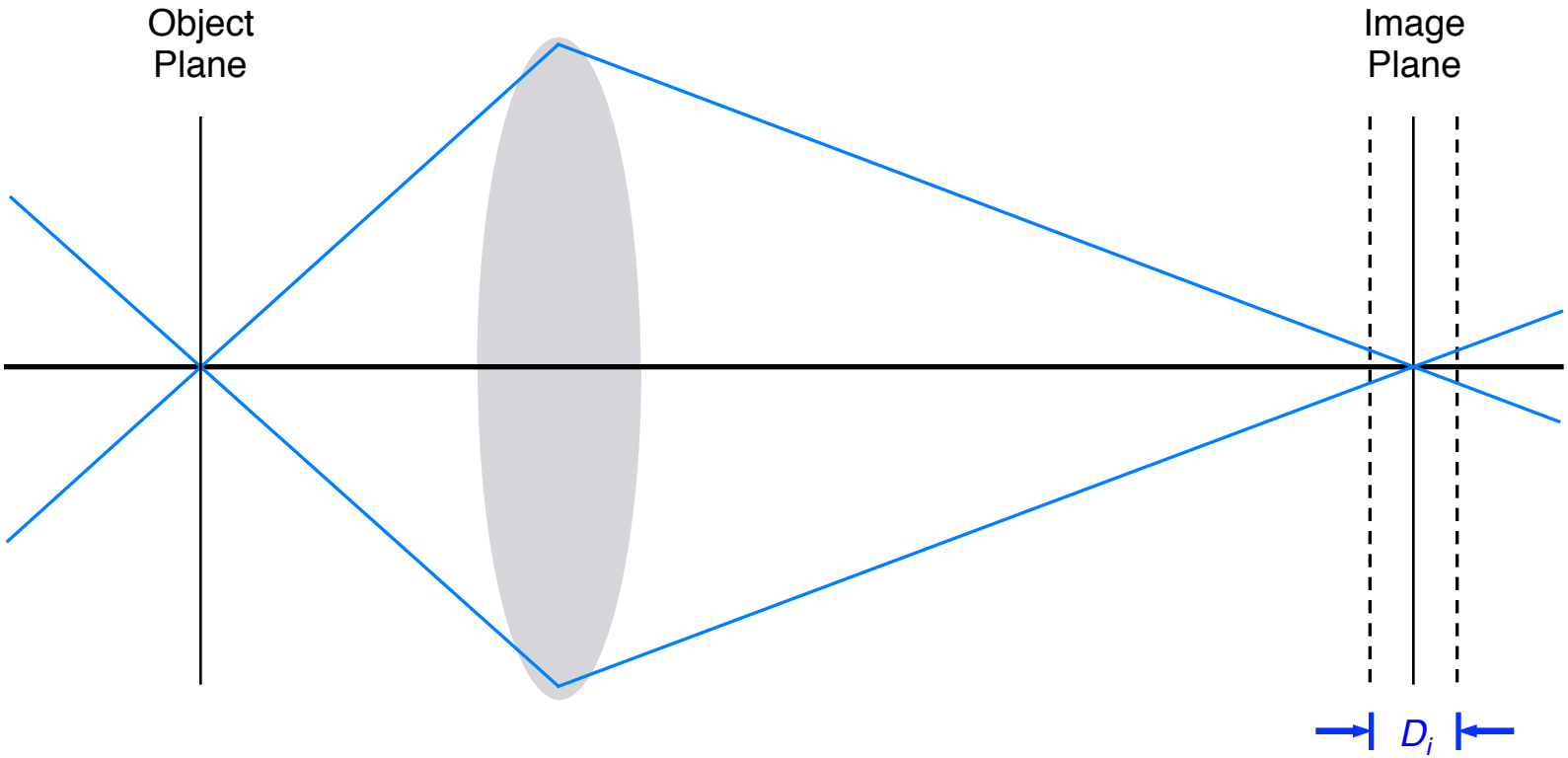
**Recall:** A **real** lens images each object point as an **Airy disc**, the radius of which = lens resolving power



# I.B.5 Projector Lenses

## I.B.5.c Depth of Field and Depth of Focus

There is a finite distance along the optic axis, $D_i$, where the **image** appears essentially unchanged. This is distance is called **depth of focus.**



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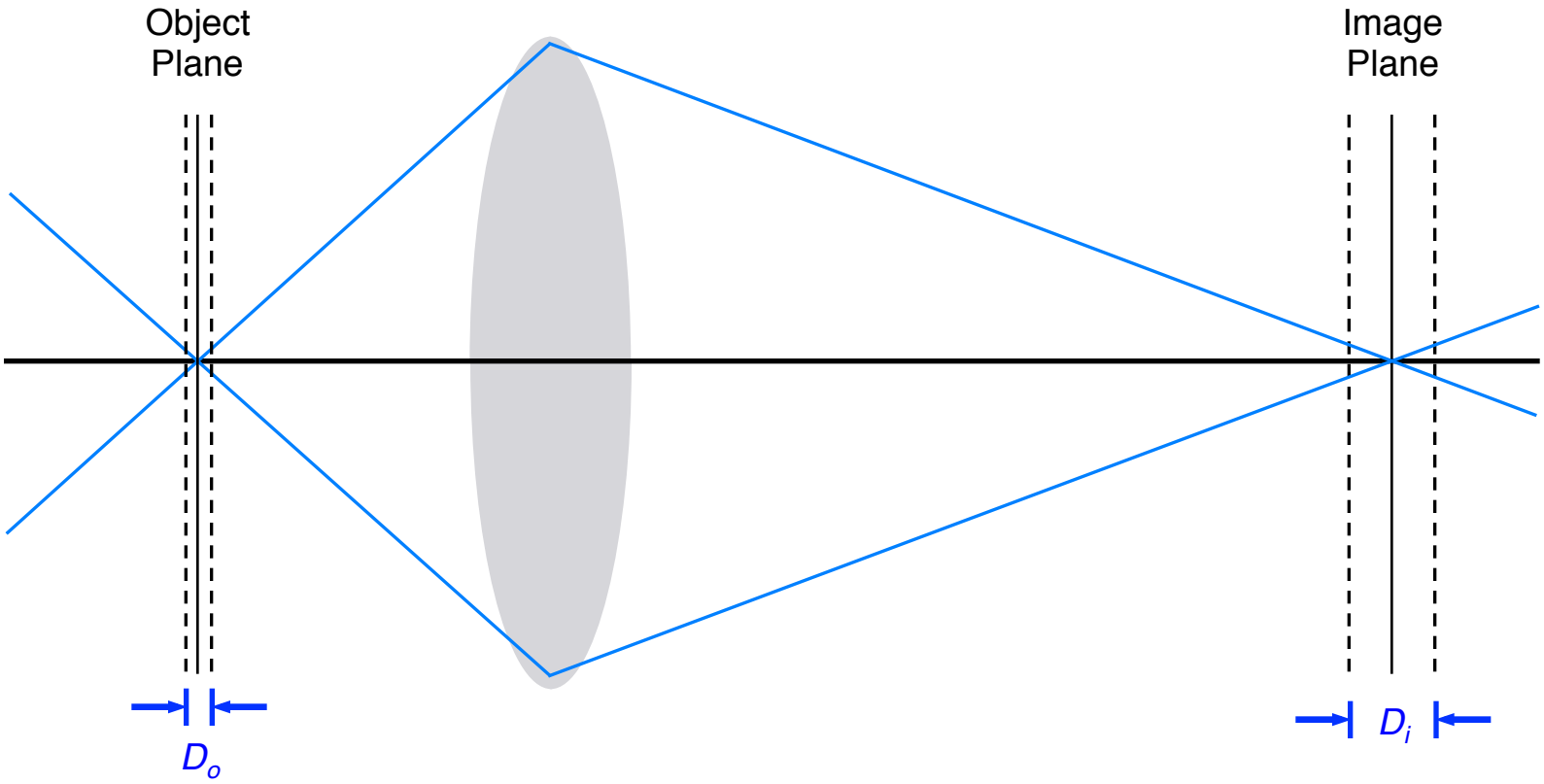
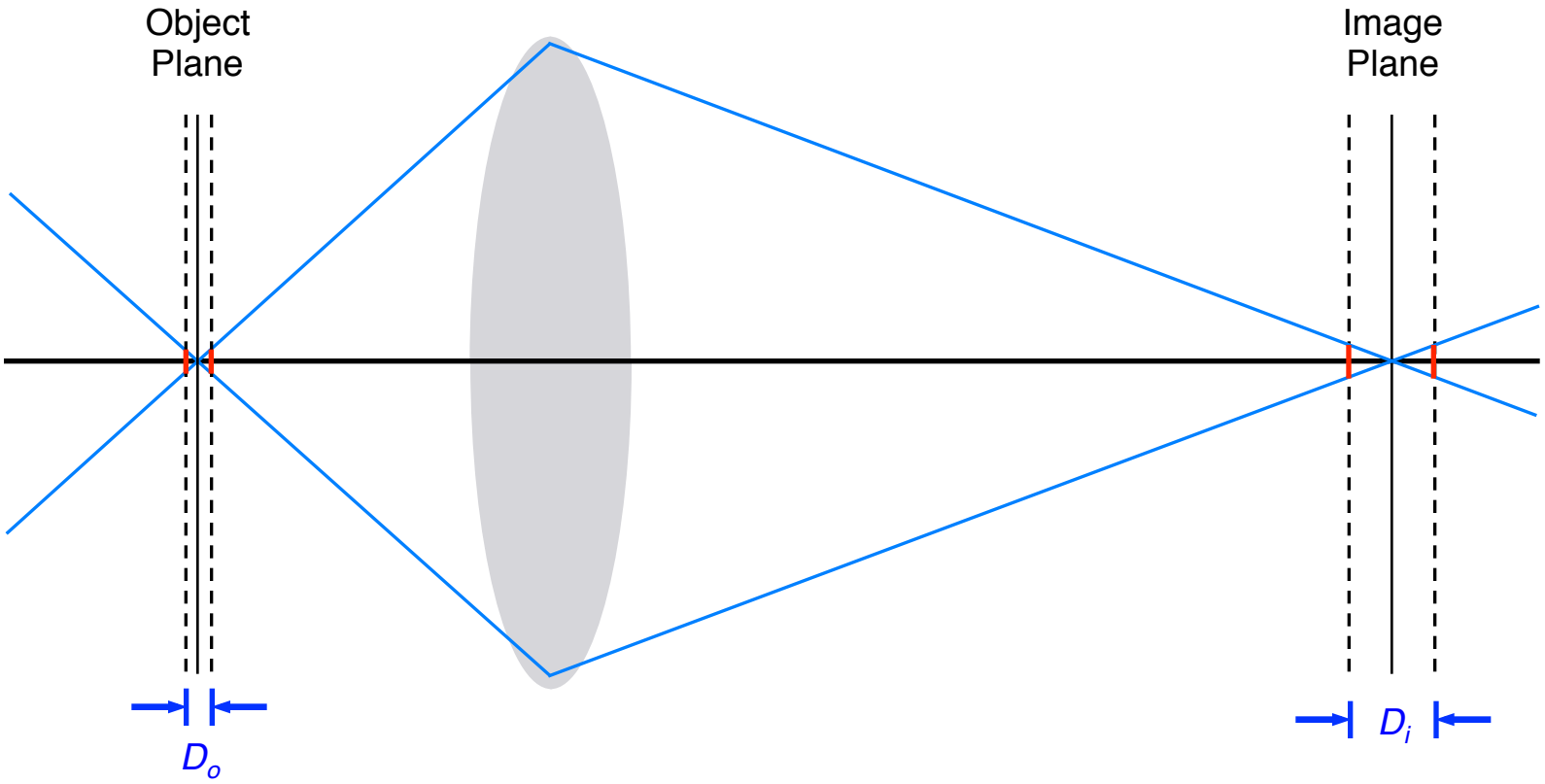



An analogous distance, $D_o$, exists along the optic axis on the **object side** over which the object **could** be moved and still give a maximally sharp image (at position of "exact" image plane). This distance is called the **depth of field.**

# I.B.5 Projector Lenses

## I.B.5.c Depth of Field and Depth of Focus

*Decreasing* the aperture of the lens *increases* **both** $D_o$ and $D_i$.



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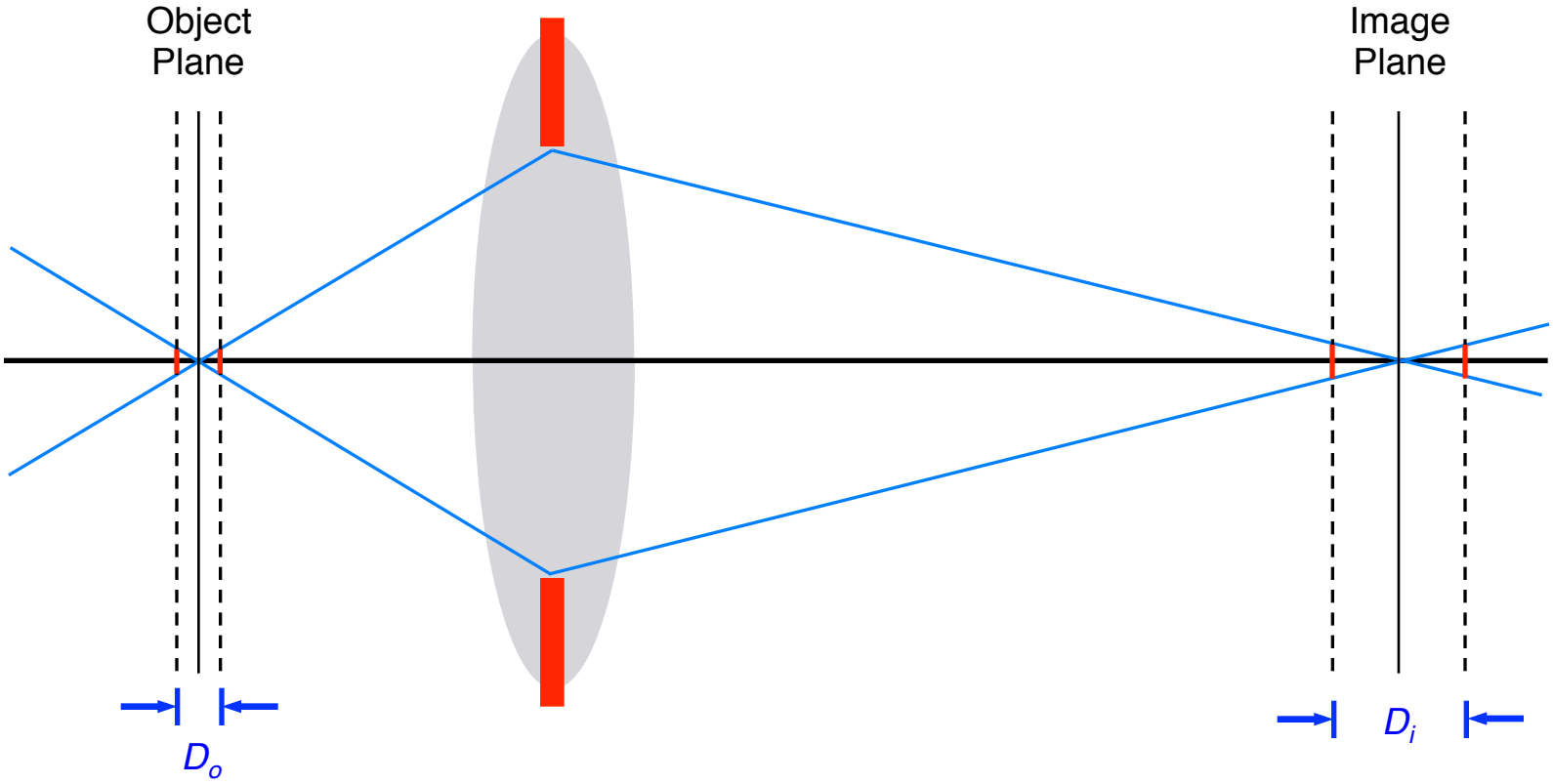
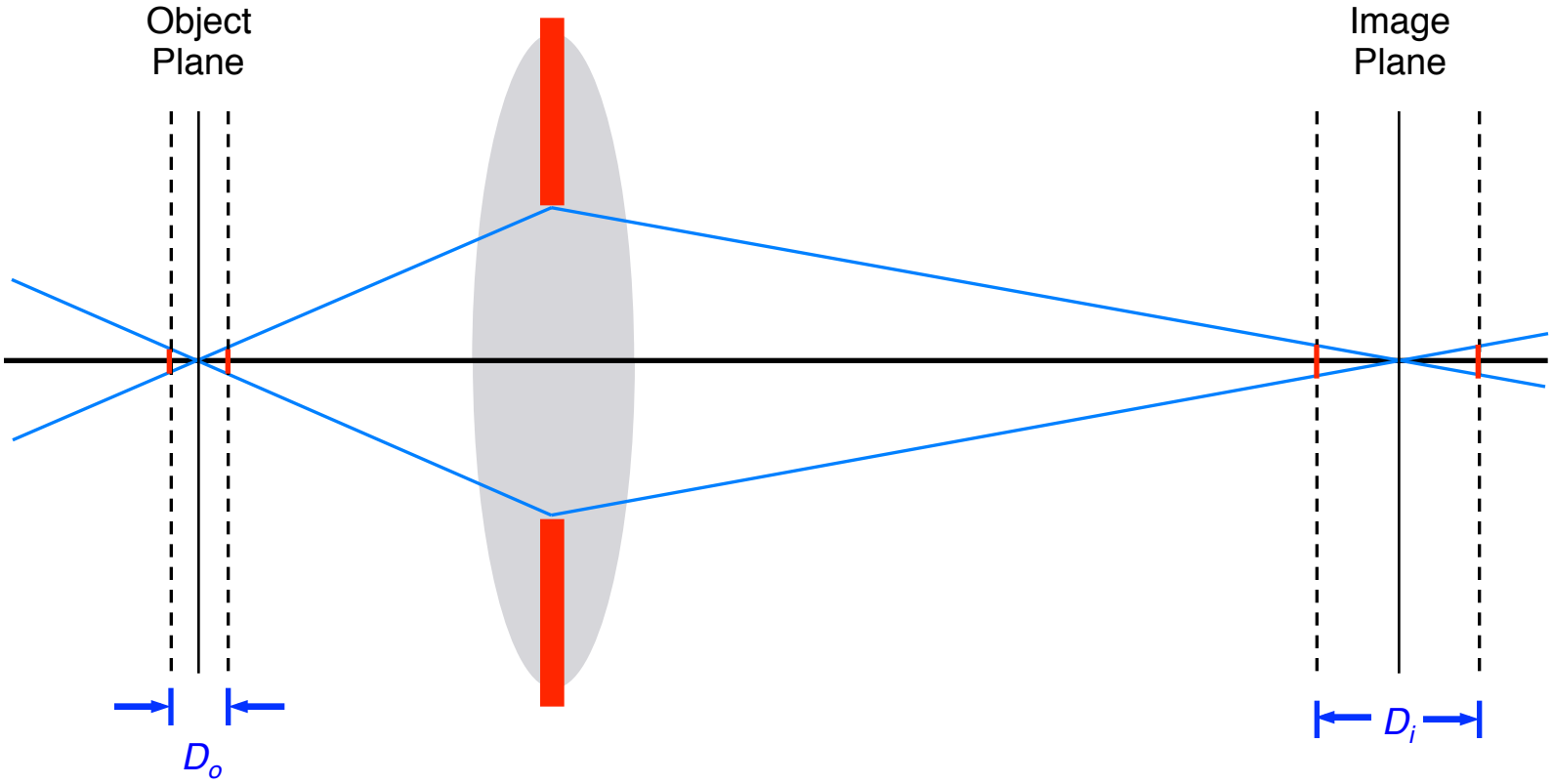


# I.B.5 Projector Lenses

## I.B.5.c Depth of Field and Depth of Focus

*Decreasing* the aperture of the lens *increases* **both** $D_o$ and $D_i$.



# I.B.5 Projector Lenses

## I.B.5.c Depth of Field and Depth of Focus

### Depth of Field (Object/Specimen Plane)

$$D_o = \frac{2d}{\tan \alpha_o}$$

$d$ = minimum object spacing one hopes to resolve

$\alpha_o$ = semi-angular aperture of **objective lens**

**EXAMPLE:** For $d$ = 1.0 nm and $\alpha_o$ = 5 x $10^{-3}$ radians, $\boldsymbol{D_o}$ **= 400 nm**

(thicker than all macromolecules and most TEM specimens)

**CONSEQUENCE:** The **entire** 3D contents of a **thin** specimen appear **equally sharp** in 2D electron images

# I.B.5 Projector Lenses

## I.B.5.c Depth of Field and Depth of Focus

### Depth of Focus (Image Plane)

$$D_i = \frac{M^2 2d}{\tan \alpha_O} = D_o M^2$$

$M$ = **total** magnification of the compound magnifying system

$d$ = minimum object spacing one hopes or expects to resolve

$\alpha_o$ = semi-angular aperture of **objective lens**

**EXAMPLE:** If $M = 50{,}000X$, $d = 1.0$ nm, and $\alpha_o = 5 \times 10^{-3}$ radians, then $D_i$ **= 1000 meters!!!**

**OK, so what the heck does this mean?**

# I.B.5 Projector Lenses

## I.B.5.c Depth of Field and Depth of Focus

## Practical Consequences

- Fluorescent screen, photographic film or CCD/DDD camera can be placed **ANYWHERE** on the optic axis **beneath** the projector lens and the final image will **look** essentially the same (but the **magnification DOES change**)

- Large depth of field or focus does **NOT** eliminate the requirement for **VERY CAREFUL FOCUSING** of the image (by adjusting the objective lens strength)

**Ugh…..just when you were beginning to think something might actually make some sense !!!**

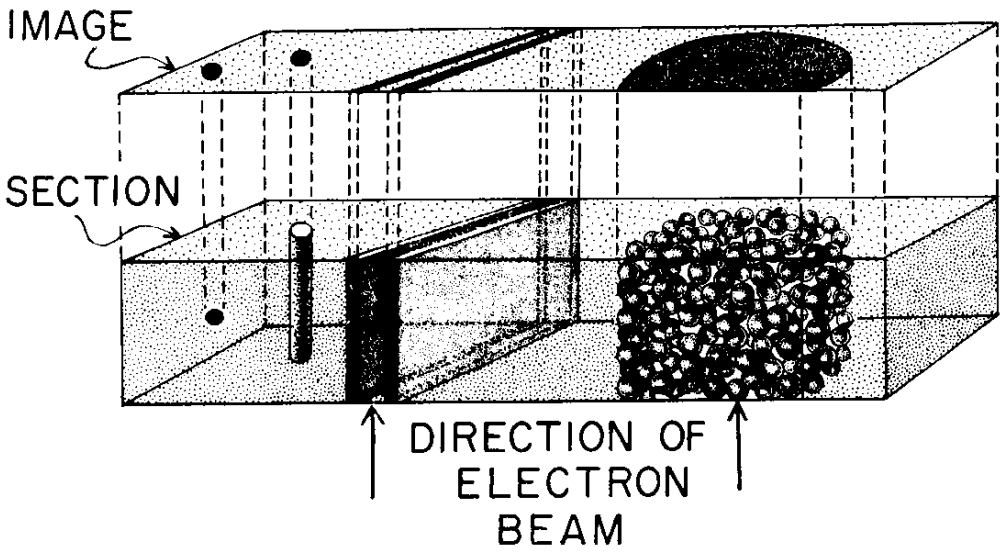
# I.B.5 Projector Lenses

## I.B.5.c Depth of Field and Depth of Focus

## Consequence of large relative depth of field in the TEM

Images are (to a first approximation) **PROJECTIONS** of the **entire contents** of a specimen.

Each portion of the 2D image represents the projected contributions from all points in the 3D specimen in the direction of the electron beam.



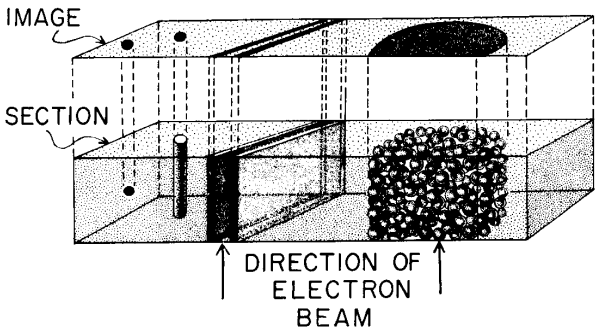

From Sjostrand, Fig. IV-23, p. 123

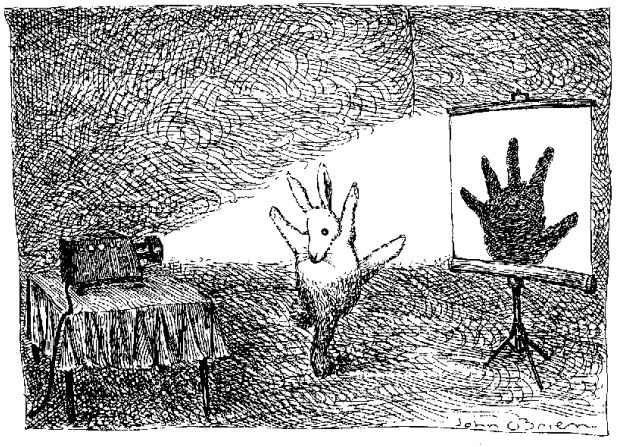
# I.B.5 Projector Lenses

## I.B.5.c Depth of Field and Depth of Focus

## IMPORTANT DISTINCTION:

Projection images are ***NOT*** shadow-graphs, where light is **not** transmitted **through** the object.



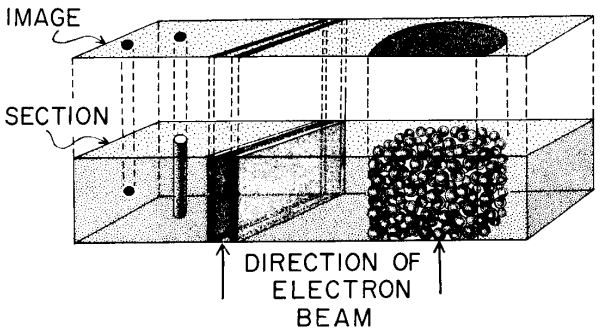
From Sjostrand, Fig. IV-23, p. 123

# I.B.5 Projector Lenses

## I.B.5.c Depth of Field and Depth of Focus

## IMPORTANT DISTINCTION:

Projection images are ***NOT*** shadow-graphs, where light is **not** transmitted **through** the object, but are **similar to X-ray photos**.





From Sjostrand, Fig. IV-23, p. 123

www.worth1000.com

# I.B.5 Projector Lenses

## I.B.5.c Depth of Field and Depth of Focus

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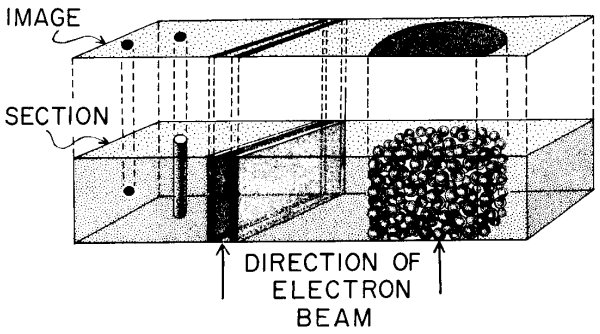
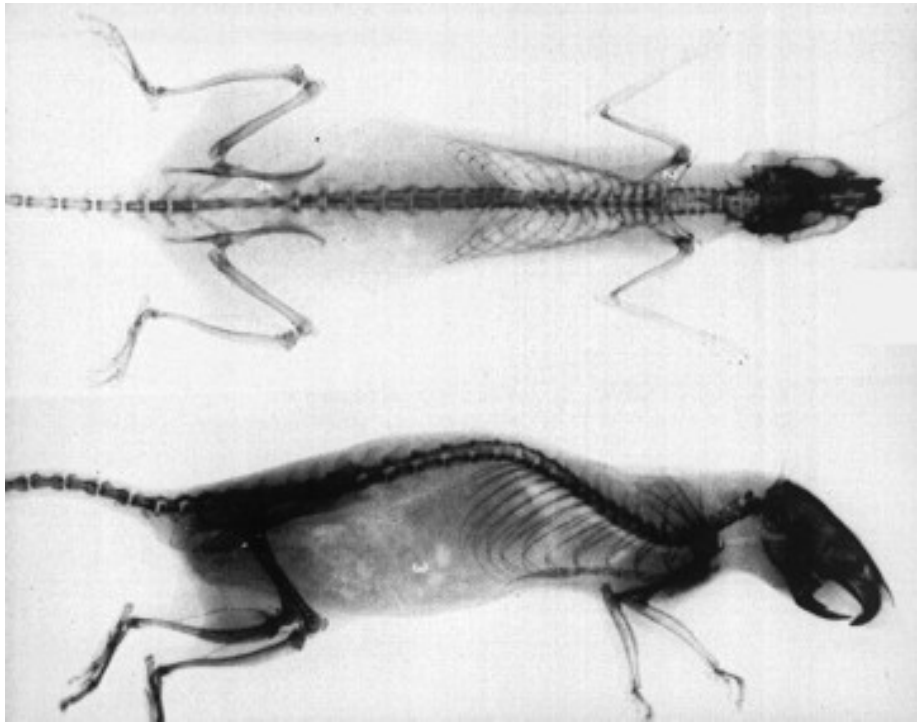


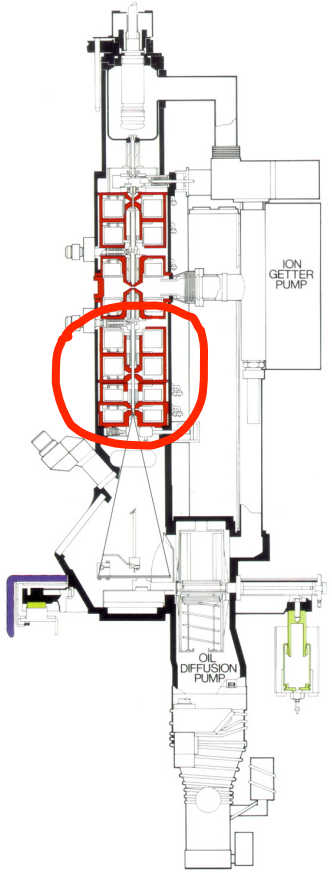
From Sjostrand, Fig. IV-23, p. 123

www.tegascience.co.jp/products/stn/applications/xray_20RatSkeleton.jpg

# I.B DESIGN OF THE TEM

## The TEM Top to Bottom:

- Electron gun
- Condenser lens(es)
- Lens aberrations
- Objective lens and specimen stage
- Projector lenses
- Camera and viewing system
- Vacuum system
- Electrical system



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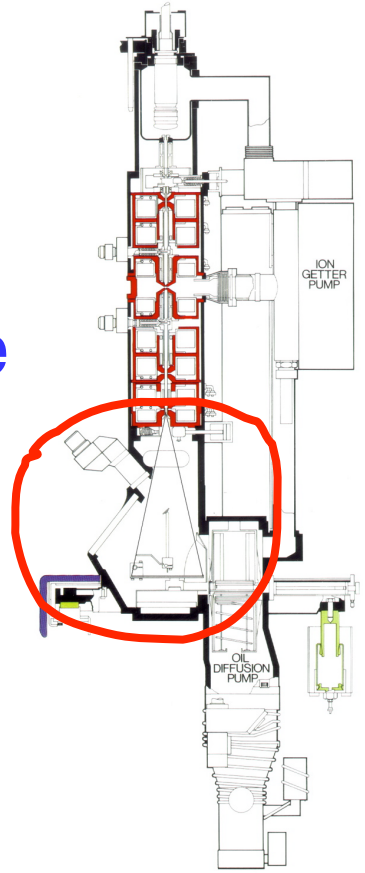

# § I: The Microscope

## I.B Design of the TEM

## I.B.6 Camera and Viewing System

(pp.65-66 of lecture notes)

# I.B DESIGN OF THE TEM

## I.B.6 Camera and Viewing System

### I.B.6.a Viewing the Image

- Electron optical image is projected onto a fluorescent screen (coated with zinc sulfide crystals)

- Kinetic energy of electrons in the image is transformed into light energy through fluorescence

- Resolution of image seen on **SCREEN** determined by size of ZnS crystals (~50-75 μm)

# I.B.6 Camera and Viewing System

## I.B.6.b Recording the Image

- Photographic recording must be done at a magnification **sufficient** to capture resolution present in the **electron** image

- Electron images typically recorded on CCD camera or photographic emulsion (DDD cameras may soon replace photographic emulsions)

- Resolution of CCD: depends on pixel size, which is typically 15 μm (*i.e.* superior to the fluorescent screen)

- Resolution of photographic emulsions: depends on size of silver halide crystals (~1-5 μm, hence >10 times better than the fluorescent screen)

**NOTE: More details about recording electron images later (§ I.E10-12).**

# I.B DESIGN OF THE TEM

## The TEM Top to Bottom:

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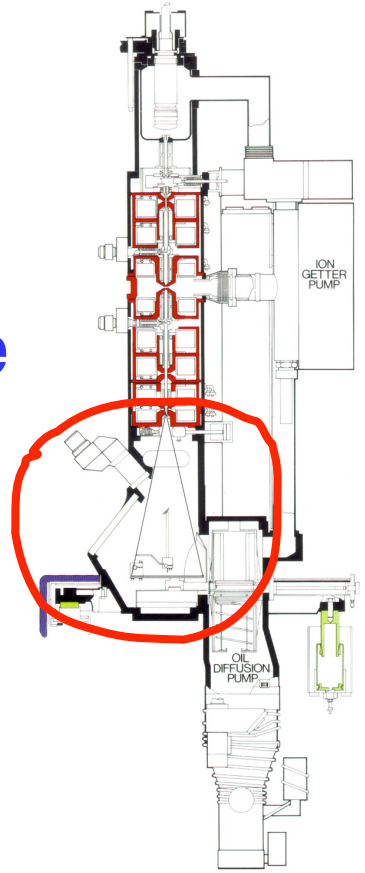

# I.B DESIGN OF THE TEM

## The TEM Top to Bottom:

- Electron gun
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- Lens aberrations
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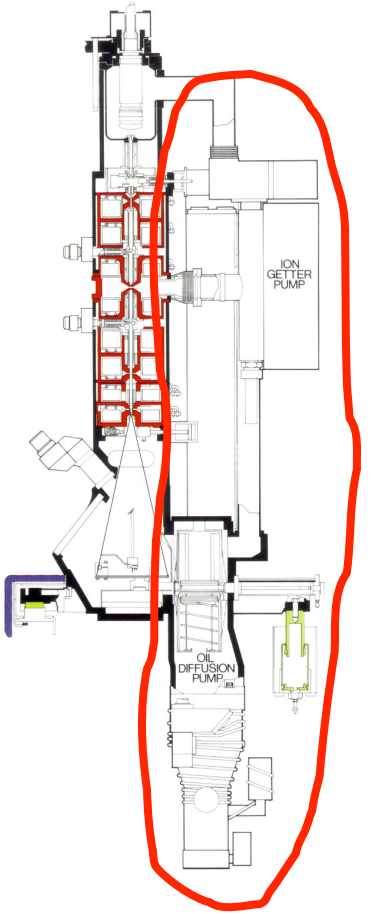
- Vacuum system (notes pp.66-71)
- Electrical system



# § I: The Microscope

## I.B Design of the TEM

## I.B.7 TEM Vacuum System

(pp.66-71 of lecture notes)

# I.B DESIGN OF THE TEM

## I.B.7 TEM Vacuum Systems

**Bottom Line: Electron beam needs a 'free path'**

- **Mean free path** of a moving electron in air (760 Torr) is **< 100 nm** (*i.e.* < 0.1 μm) **!!!**

- High vacuum essential to allow electron beam to pass through entire TEM column (> 1 meter)

- TEMs use **$10^{-6}$ Torr** or lower (MFP is **10s of meters**)

- TEMs generate high vacuum by using **two or more** different type **pumps in tandem**

# I.B.7 TEM Vacuum Systems

## I.B.7.b Types of Pumps

## Bottom Line: A Pump for Every Need

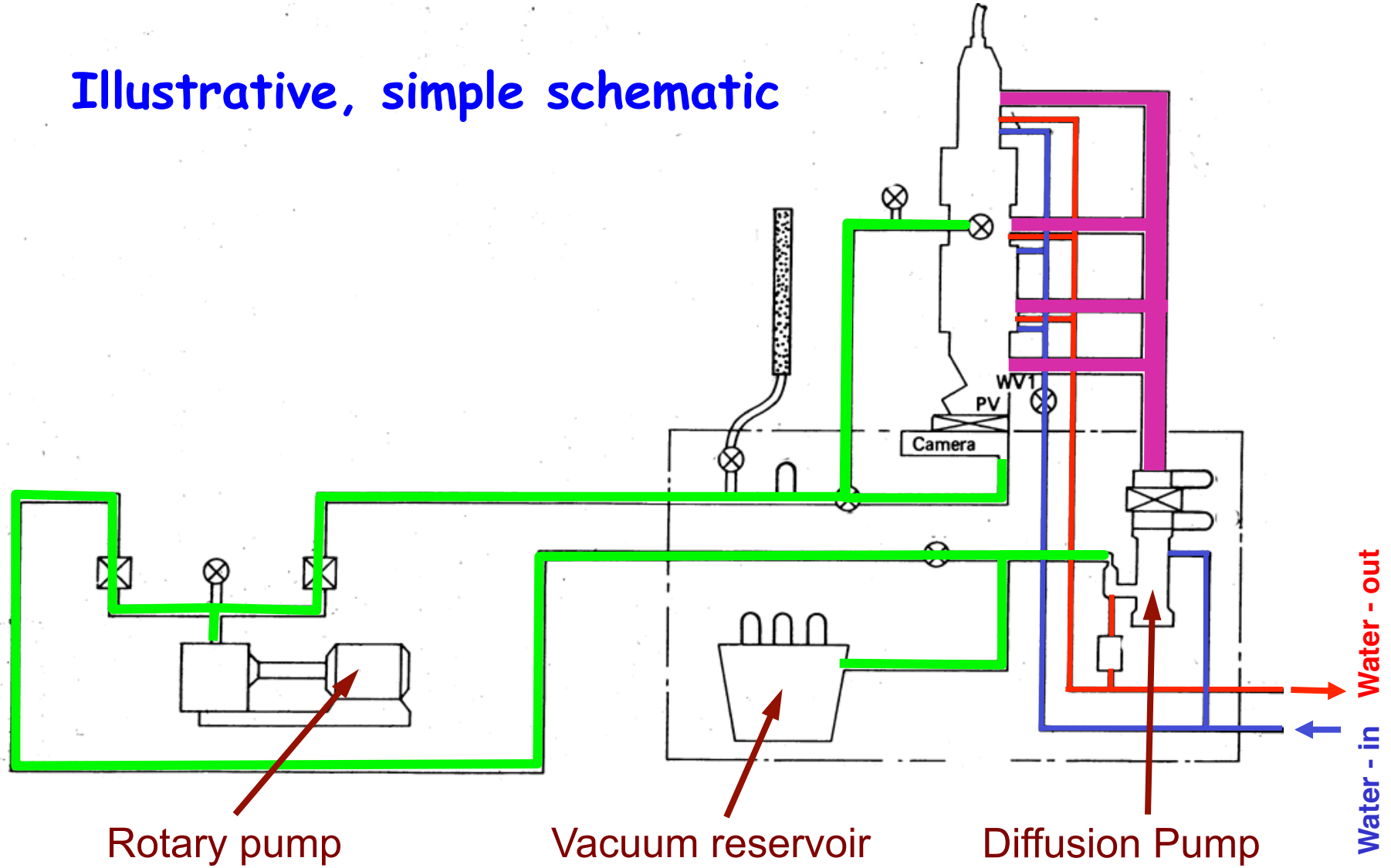
- Rotary Vane ("Roughing") $10^{-3}$ to $10^{-4}$ Torr
- Diffusion $10^{-5}$ to $10^{-7}$ Torr
- Ion $10^{-7}$ to $10^{-9}$ Torr
- Turbomolecular $10^{-7}$ to $10^{-10}$ Torr
- Cryo $10^{-8}$ to $10^{-11}$ Torr

**NOTE: 1 atmosphere (1 atm) = 760 Torr**

See lecture notes pp.66-69 for brief descriptions of each pump

# I.B.7 TEM Vacuum Systems

**Illustrative, simple schematic**




From Agar, Fig. 2.32, p.76

# I.B.7 TEM Vacuum Systems

## I.B.7.b Measuring Vacuum (Gauges)

**Quality** of the vacuum obtained by each type of pump is monitored by different type gauges:

- Thermocouple gauge
- Pirani gauge
- Ion gauge
- Penning gauge

See lecture notes pp.69-70 for brief descriptions

I.B.7 TEM Vacuum Systems

# Problems Caused by Poor Vacuum

**Specimen contamination**

**Specimen etching**

**Decreased filament life**

I.B.7 TEM Vacuum Systems

# Problems Caused by Poor Vacuum

**Specimen contamination:**

Hydrocarbon residues from oil, grease, etc., when hit by the $e^-$ beam, decompose into H and C. C atoms crosslink to the specimen, obscure details, and reduce resolution.

**Specimen etching:**

Residual $H_2O$ in or near the specimen is ionized by the $e^-$ beam, producing highly reactive $OH^-$ ions. These attack carbon in the specimen, producing volatile CO. The specimen essentially 'burns' up in the beam.

**Decreased filament life:**

Filament quickly etches in a poor vacuum

I.B.7 TEM Vacuum Systems

# Problems Caused by Poor Vacuum

**Specimen contamination:**

**Specimen etching:**

Reduce or eliminate these two problems with an **anti-contaminator**

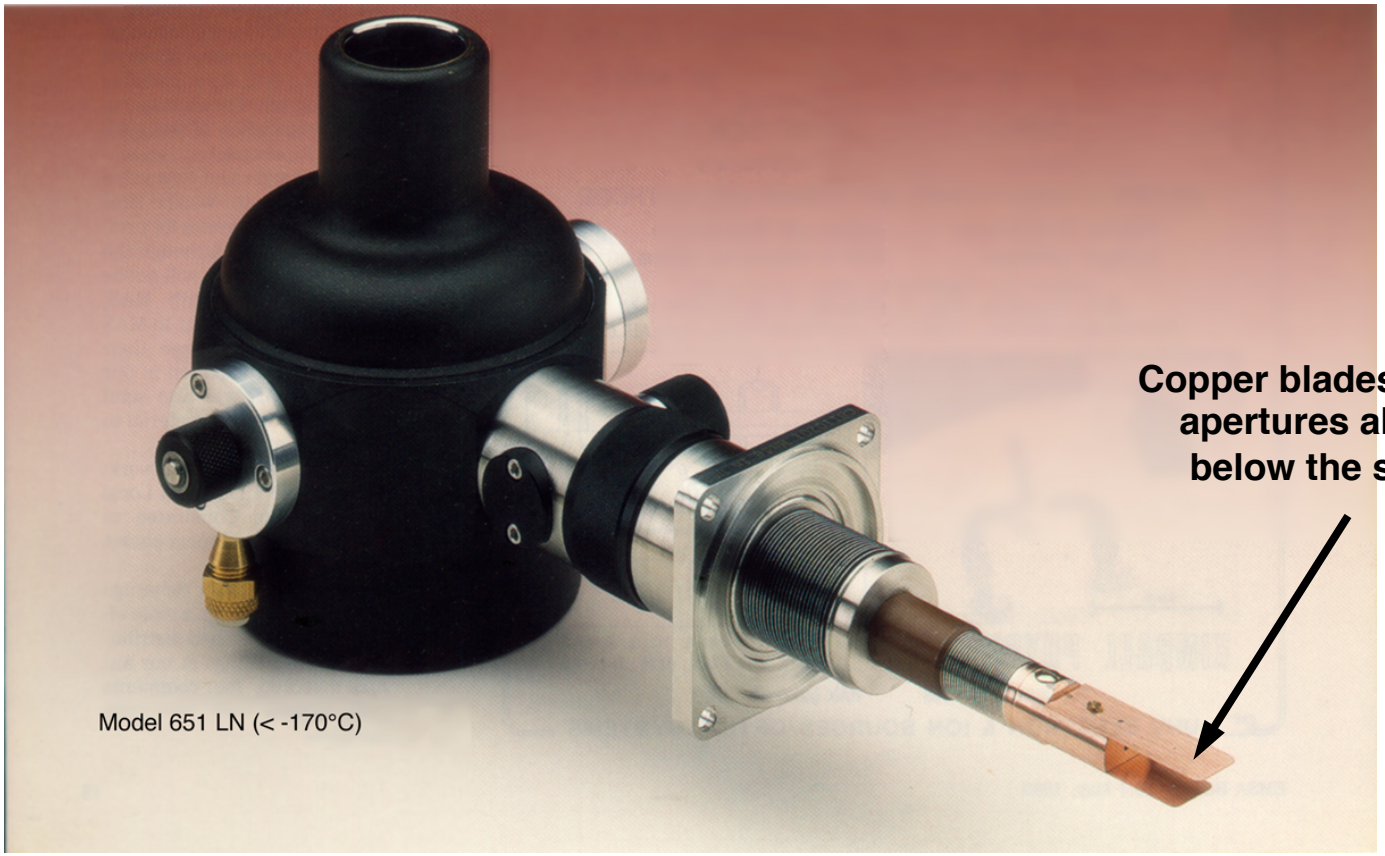
Device that **traps contaminants** before they reach the specimen

# I.B.7 TEM Vacuum Systems

## Anticontaminator

A **cooled surface** placed close to the specimen traps residual gases in the column, preventing them from interacting unfavorably with the specimen.

Most anticontaminators are cooled with **liquid $N_2$**.

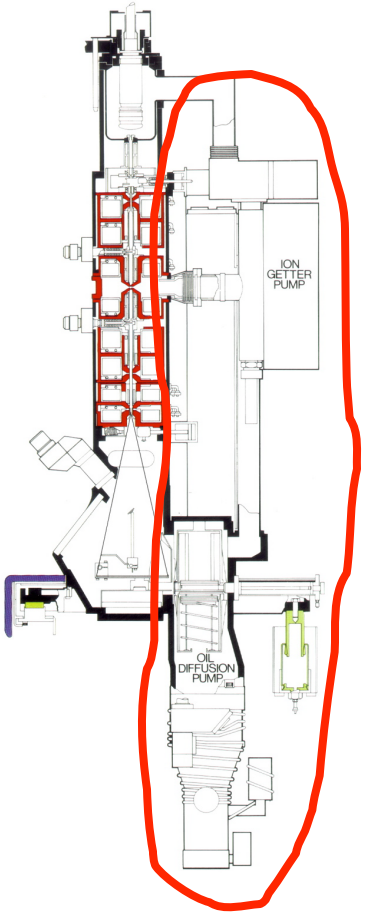



From www.gatan.com/holders/index.html

# I.B DESIGN OF THE TEM

## The TEM Top to Bottom:

- Electron gun
- Condenser lens(es)
- Lens aberrations
- Objective lens and specimen stage
- Projector lenses
- Camera and viewing system
- Vacuum system (notes pp.66-71)
- Electrical system



# I.B DESIGN OF THE TEM

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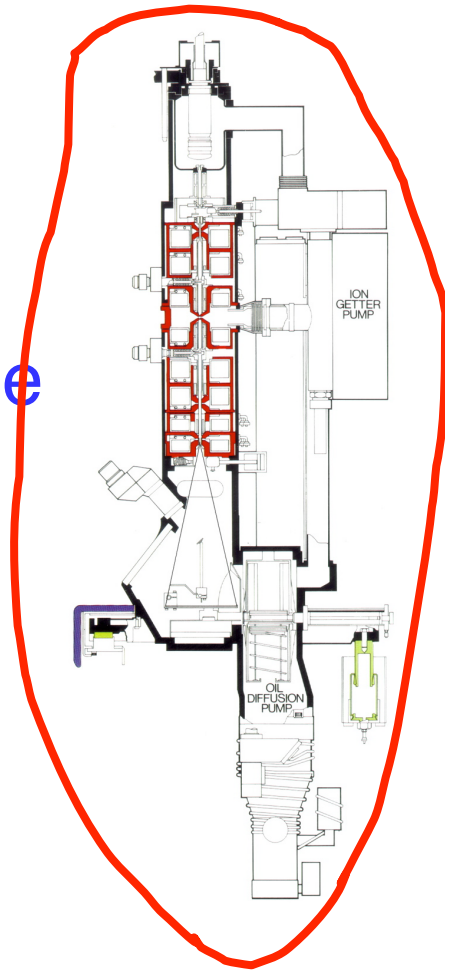
- Vacuum system
- Electrical system (notes pp.71-72)



# § I: The Microscope

I.B Design of the TEM

I.B.8 TEM Electrical System

(pp.71-72 of lecture notes)

# I.B DESIGN OF THE TEM

## I.B.8 Electrical System

### TEMs are full of electronics:

- Current to heat the filament (generate beam electrons)
- High voltage to accelerate the emitted $e^-$ beam
- Current to all lenses to generate magnetic fields to focus electrons
- Power to many circuits:

Stigmators
Beam deflectors
Camera and camera shutter
Exposure meter
Focus wobbler
Safety devices

Relay switches
Diffusion, rotary, getter pumps
Microprocessor
etc. etc. etc.

# I.B DESIGN OF THE TEM

## I.B.8 Electrical System

## Bottom Line:

**1. Current supplies** for each lens must be **very stable**

**Objective lens:** strictest stability requirement (1 part in 100,000)

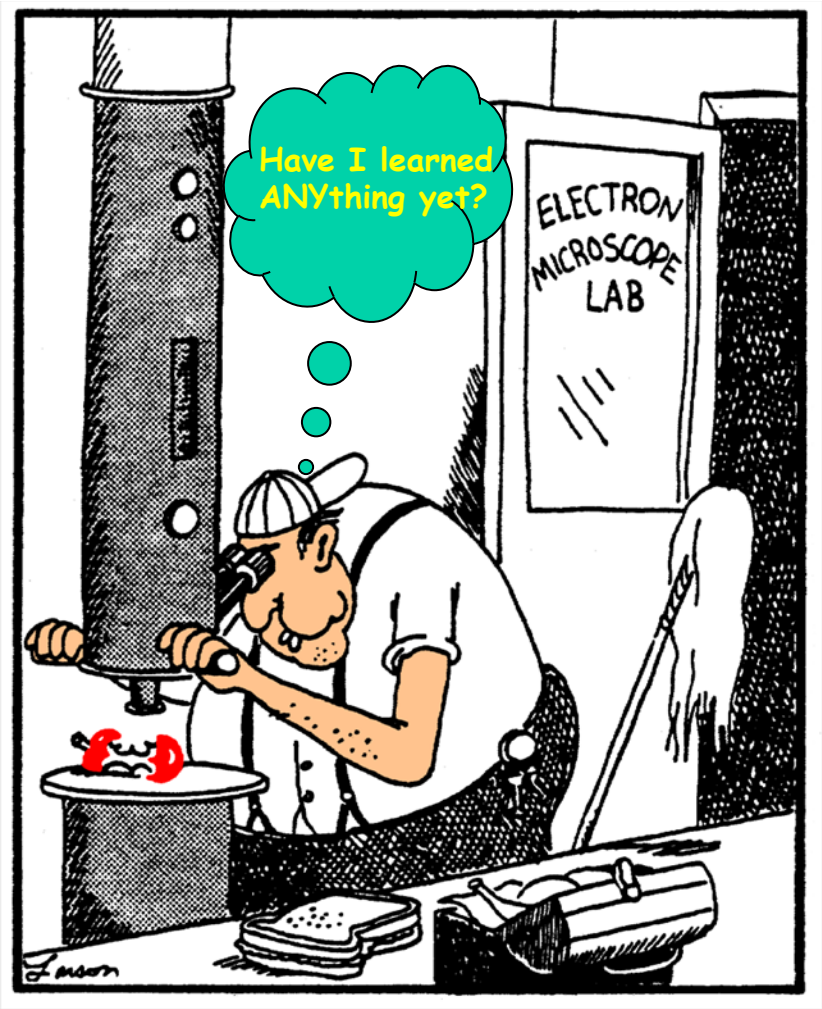
If current varies through any imaging lens, the image rotates about a point called the **current rotation center**

Any micrograph recorded ***while*** this happens will be **blurred**

**2. Voltage** should also be stable (~1 part in 50,000)

Fluctuations produce **magnification changes** (image grows or contracts radially about the **voltage center**)

Fluctuations that occur during recording of the micrograph lead to **image blurring**




# TOPICS

- Principles of TEM
  - Electrons, lenses and optics
- Design of TEM
  - Components top to bottom
- Contrast and image formation
  - Electron scattering from object
- Optimizing TEM performance
  - Alignment assures ‘best’ images
- Operation of TEM
  - “What do all these buttons do?”
- Other modes of TEM
  - Many ways to ‘observe’ specimens
- Specimen preparation for TEM
  - Getting specimen ready
- Radiation damage
  - Less is better
- 3D reconstruction
  - Specimen 3D structure from 2D images

# § I: The Microscope

I.A Principles of TEM

→ I.B Design of the TEM

→ I.C Contrast and Image Formation

(pp.73-80 of lecture notes)

# I.C CONTRAST AND IMAGE FORMATION

## KEY CONCEPTS

- Specimens are made of **atoms** (mainly empty space)

- **Electron beam** interacts with (*i.e.* scatters from) specimen atoms

- In TEM, **image contrast** arises in two ways:

  Removal of electrons by objective **aperture** (**amplitude**)

  **Interference** of electron waves (**phase**)

# I.C CONTRAST AND IMAGE FORMATION

## MORE CONCEPTS

- **Resolution** of features in electron images of **biological** specimens (or virtually any *thin* specimen) is normally limited by **contrast BUT NOT** lack of **resolving power**

- **RECALL: resolving power** of TEM: 0.2-0.3 nm (2-3 Å)

**Limited by optics, not by the specimen or electron λ**

- **Resolution limit** in TEM images of **biological** specimens:

***Generally*** 1-5 nm (10 – 50 Å)

**BUT:** You will soon learn that sometimes, with judicious specimen preparation and microscopy procedures, and with computer processing of images, higher resolutions **CAN** be achieved

# I.C CONTRAST AND IMAGE FORMATION

## IMAGE CONTRAST IN THE TEM

Determined by two factors:

1. The nature and extent of **interactions** between the electron beam and the specimen

2. How the electron image forms

# I.C CONTRAST AND IMAGE FORMATION

## IMAGE CONTRAST IN THE TEM

Two sources of contrast:

Specimen (**inherent contrast**)

Microscope (**instrumental contrast**)

***Both*** are important !!!

# I.C CONTRAST AND IMAGE FORMATION

## DEFINITION: CONTRAST

*The **relative** difference in intensity between an image point and its surroundings*

$$Percent\ contrast = 100 \times \frac{|I_i - I_b|}{I_b}$$

$I_i$ = intensity of an image point

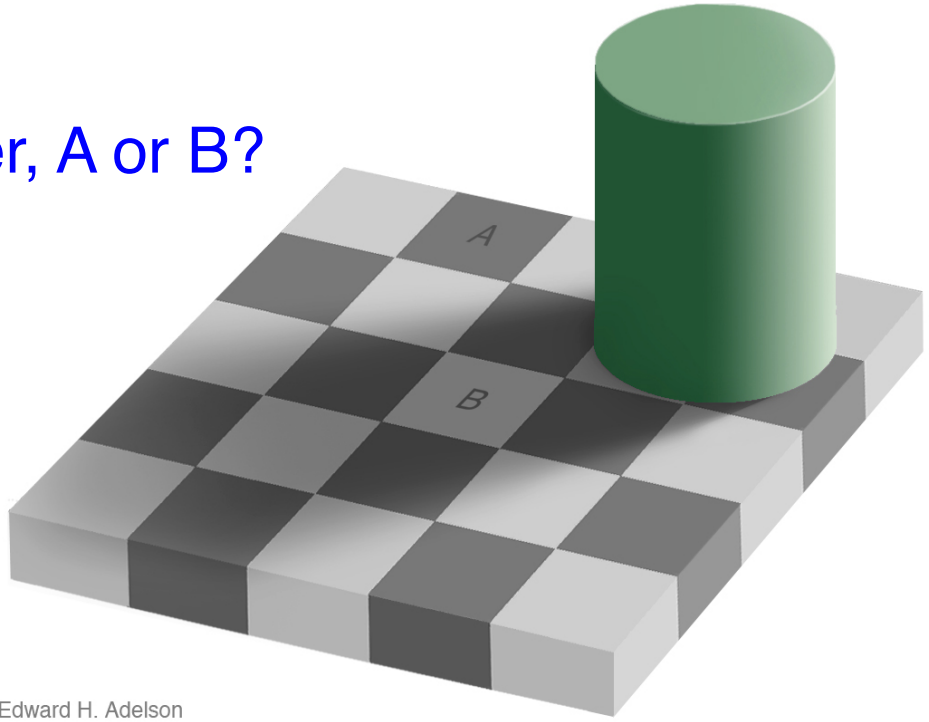
$I_b$ = intensity of the background **adjacent** to the image point

# I.C CONTRAST AND IMAGE FORMATION

$$Percent\ contrast = 100 \times \frac{|I_i - I_b|}{I_b}$$

*The **relative** difference in intensity between an image point and its surroundings*

Which is darker, A or B?




Edward H. Adelson

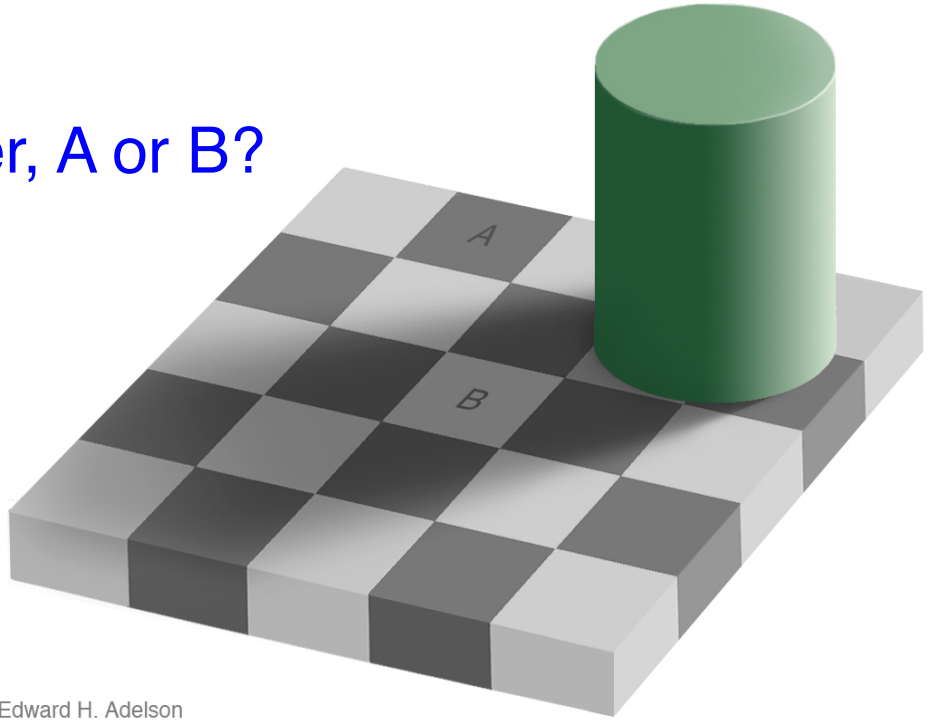
# I.C CONTRAST AND IMAGE FORMATION

$$Percent\ contrast = 100 \times \frac{|I_i - I_b|}{I_b}$$

*The **relative** difference in intensity between an image point and its surroundings*

Which is darker, A or B?

Neither one!
(of course)



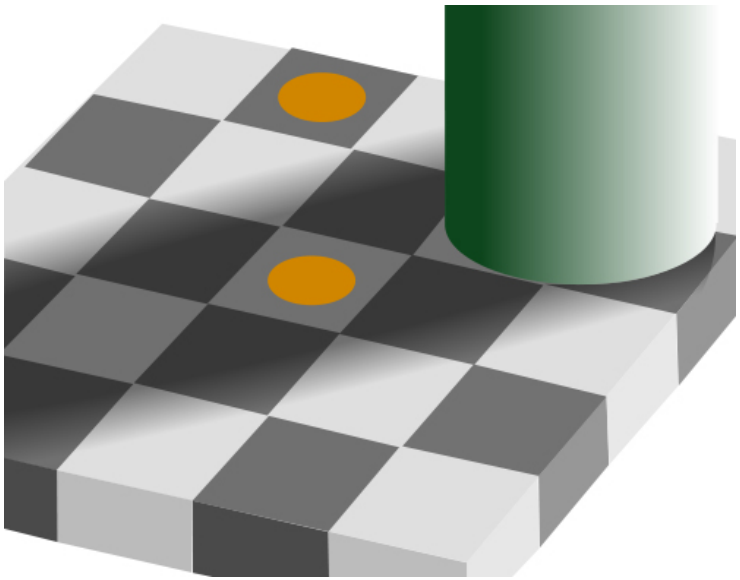

Edward H. Adelson

# I.C CONTRAST AND IMAGE FORMATION

$$Percent\ contrast = 100 \times \frac{|I_i - I_b|}{I_b}$$

*The **relative** difference in intensity between an image point and its surroundings*

What about the orange disks?



Slide not shown in class lecture

# I.C CONTRAST AND IMAGE FORMATION

## Contrast in LM vs. TEM

**LM** - differential **absorption** of photons
- depends mainly on staining

**EM** - differential **scattering** of electrons
- negligible absorption of electrons for "thin" specimens (*i.e.* <100-200 nm)

# § I: The Microscope

## I.C Contrast and Image Formation

### I.C.1 Electron Scattering

(pp.73-75 of lecture notes)

# I.C CONTRAST AND IMAGE FORMATION

## I.C.1 Electron Scattering

### *A few important facts:*

- **Amount** of $e^-$ scattering from a finite region of a specimen depends on the **density AND overall thickness** of the specimen in the direction of the beam

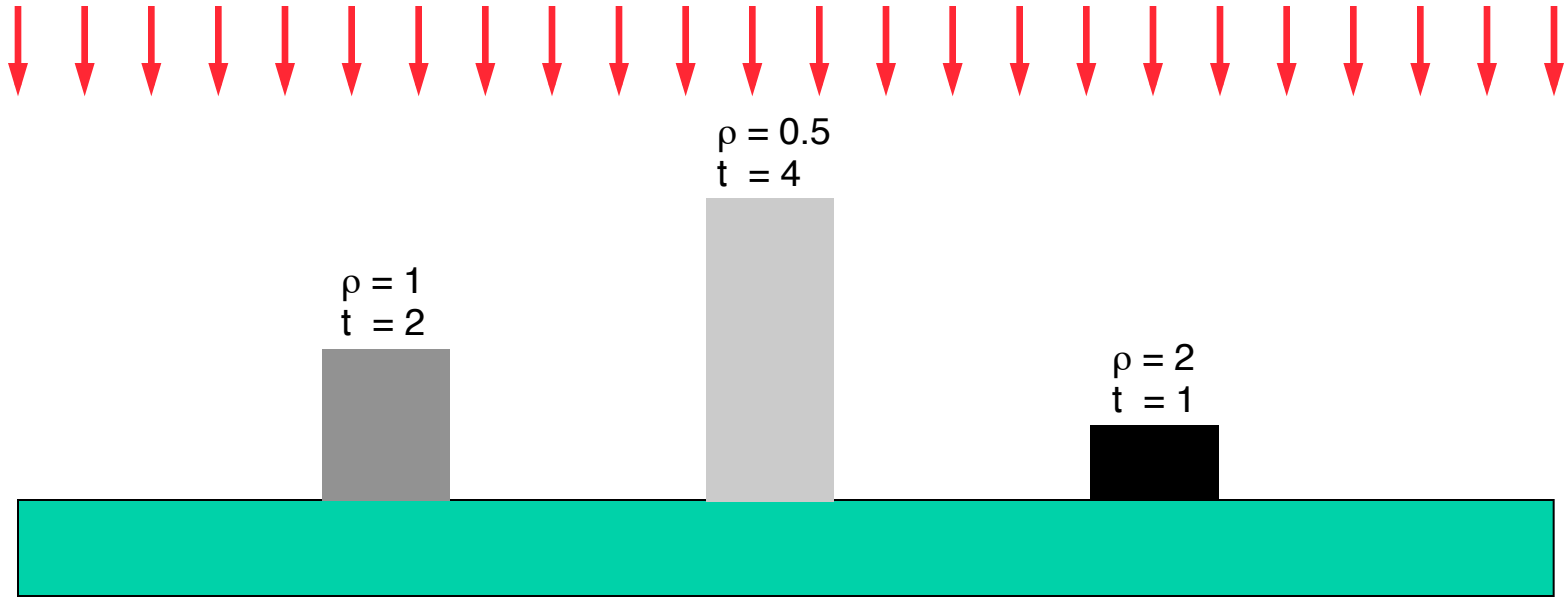
- Scattering **probability** increases as **mass thickness** increases

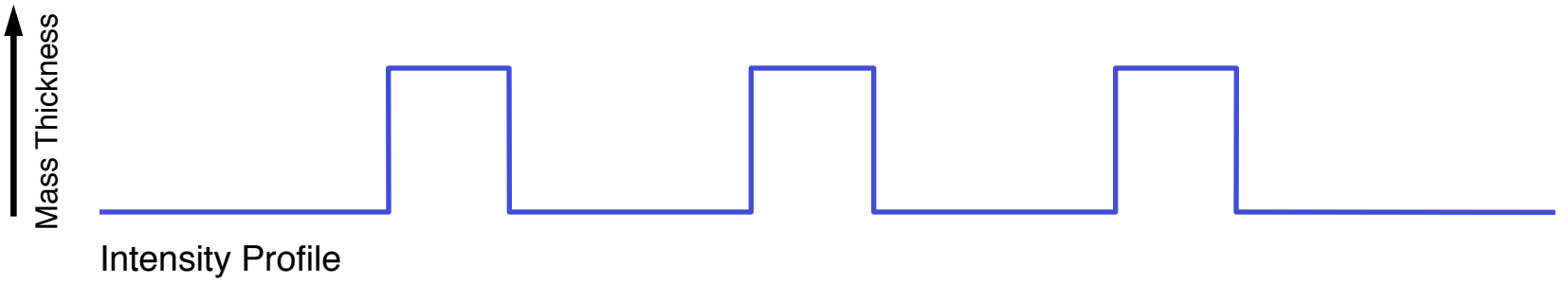
- To a ***first approximation***, scattering is **independent** of chemical composition and other specimen properties

# I.C CONTRAST AND IMAGE FORMATION

## I.C.1 Electron Scattering

Mass thickness = density x thickness

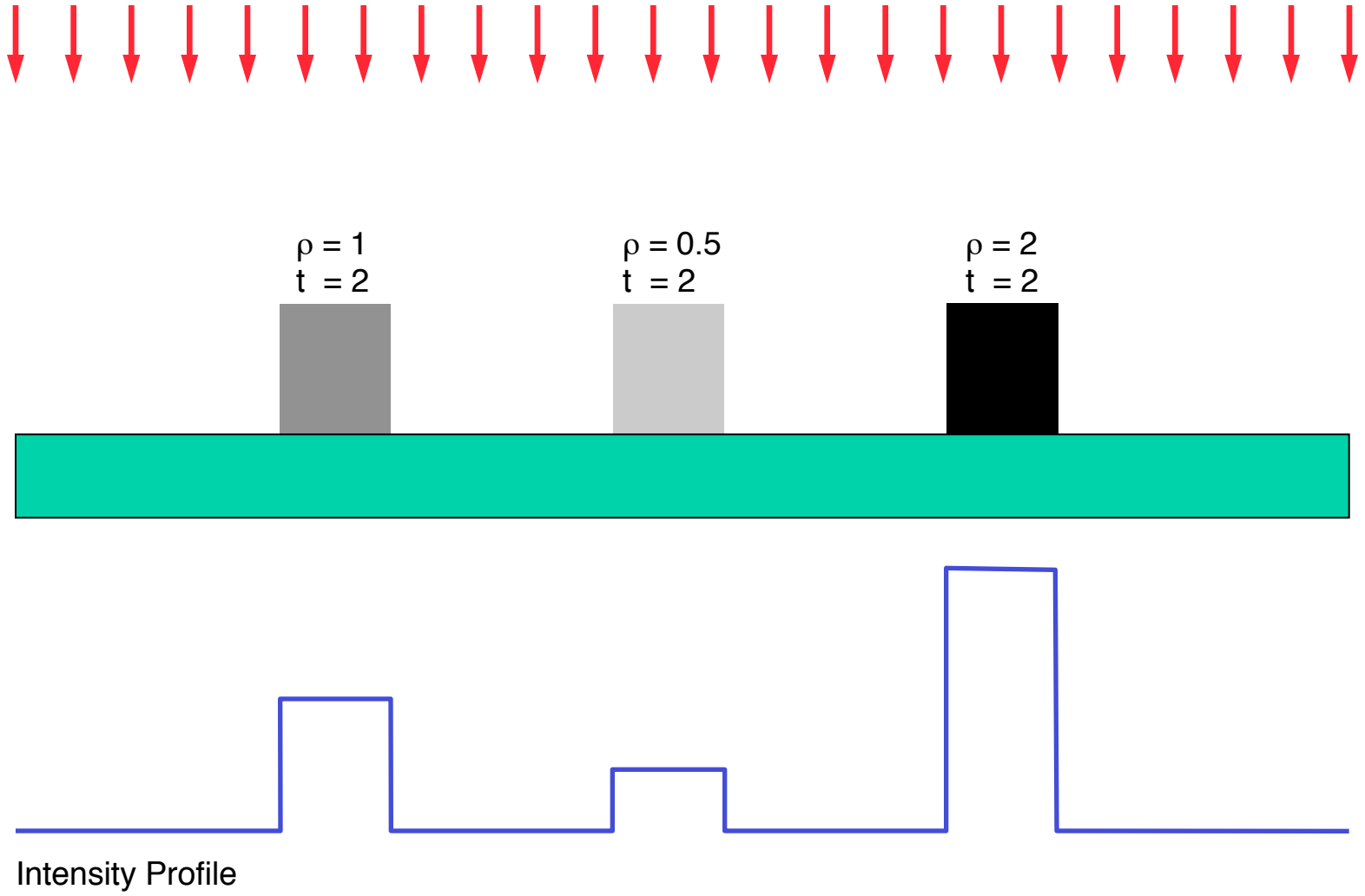


# I.C CONTRAST AND IMAGE FORMATION

## I.C.1 Electron Scattering

Mass thickness = density x thickness



# I.C CONTRAST AND IMAGE FORMATION

## I.C.1 Electron Scattering

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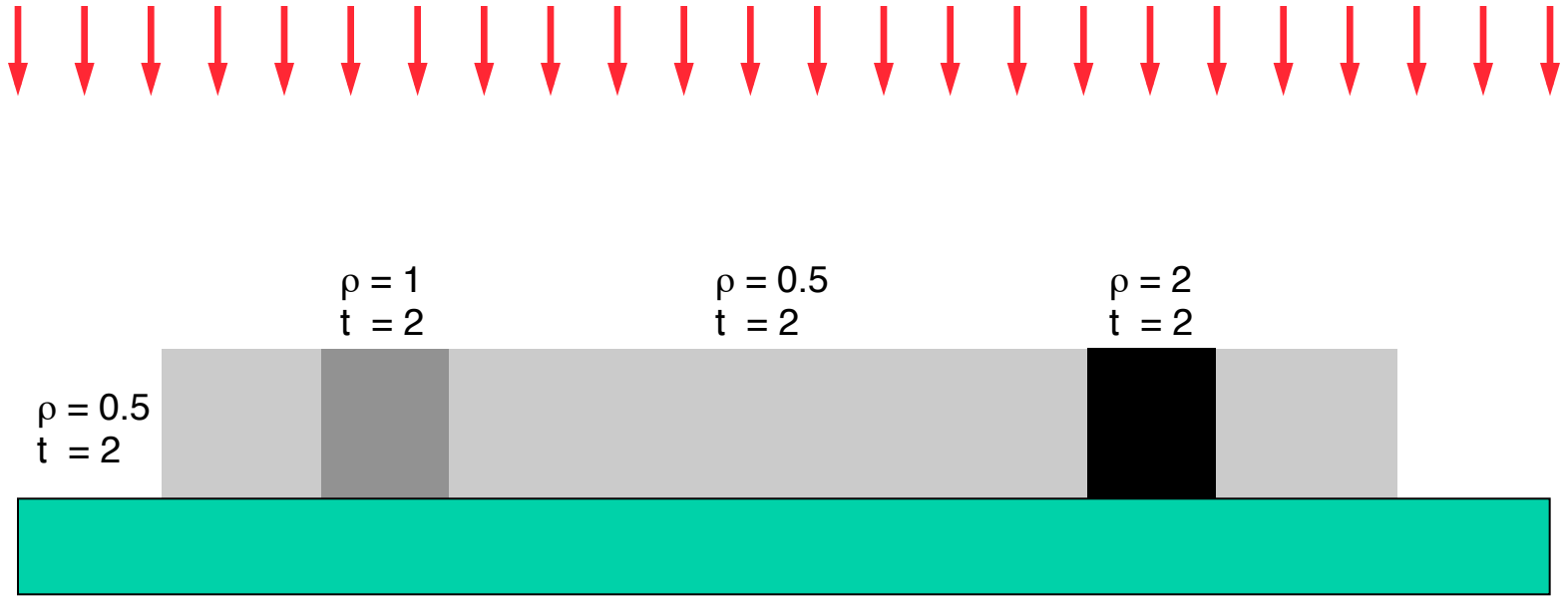
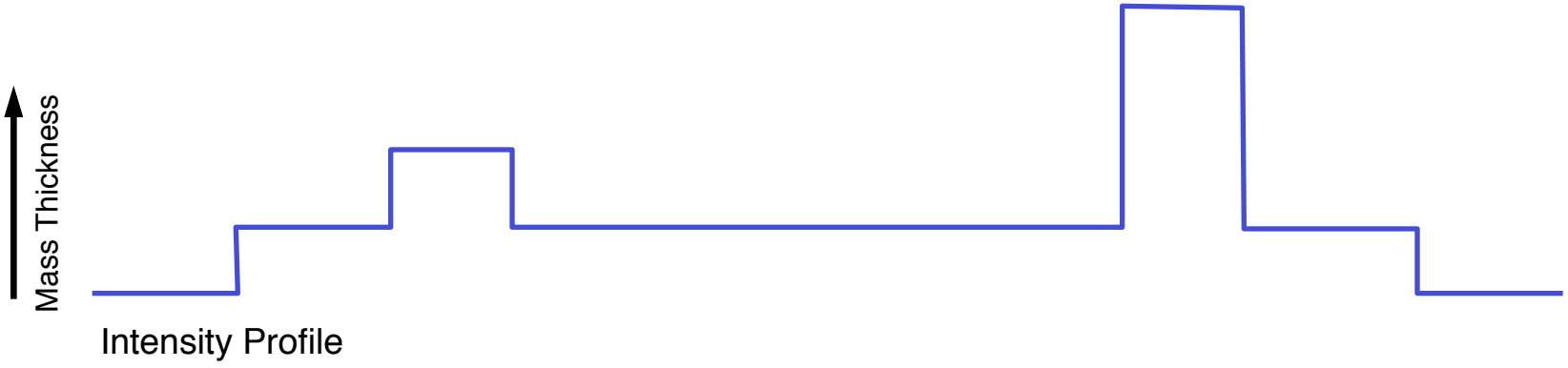
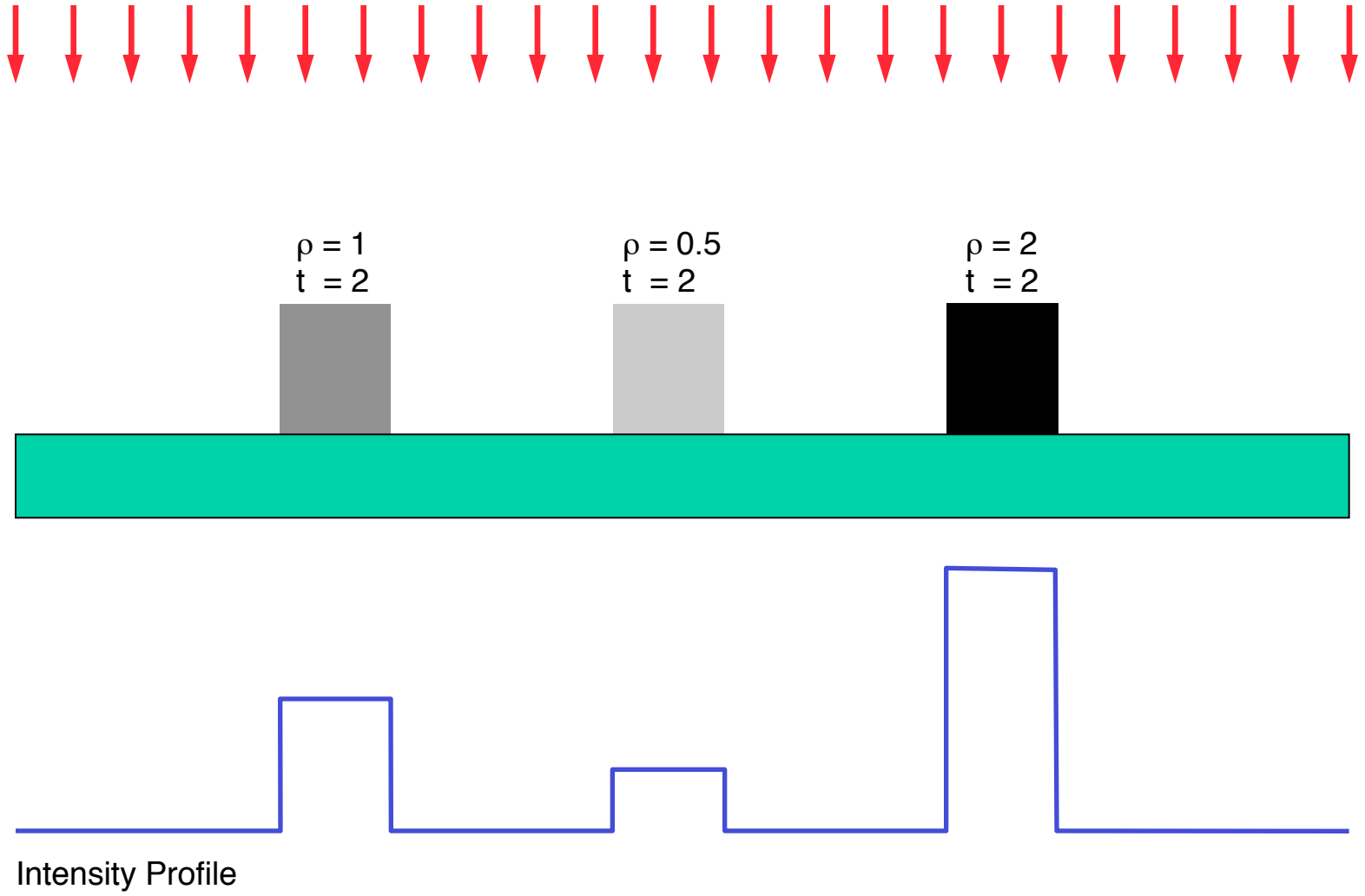


# I.C CONTRAST AND IMAGE FORMATION

## I.C.1 Electron Scattering

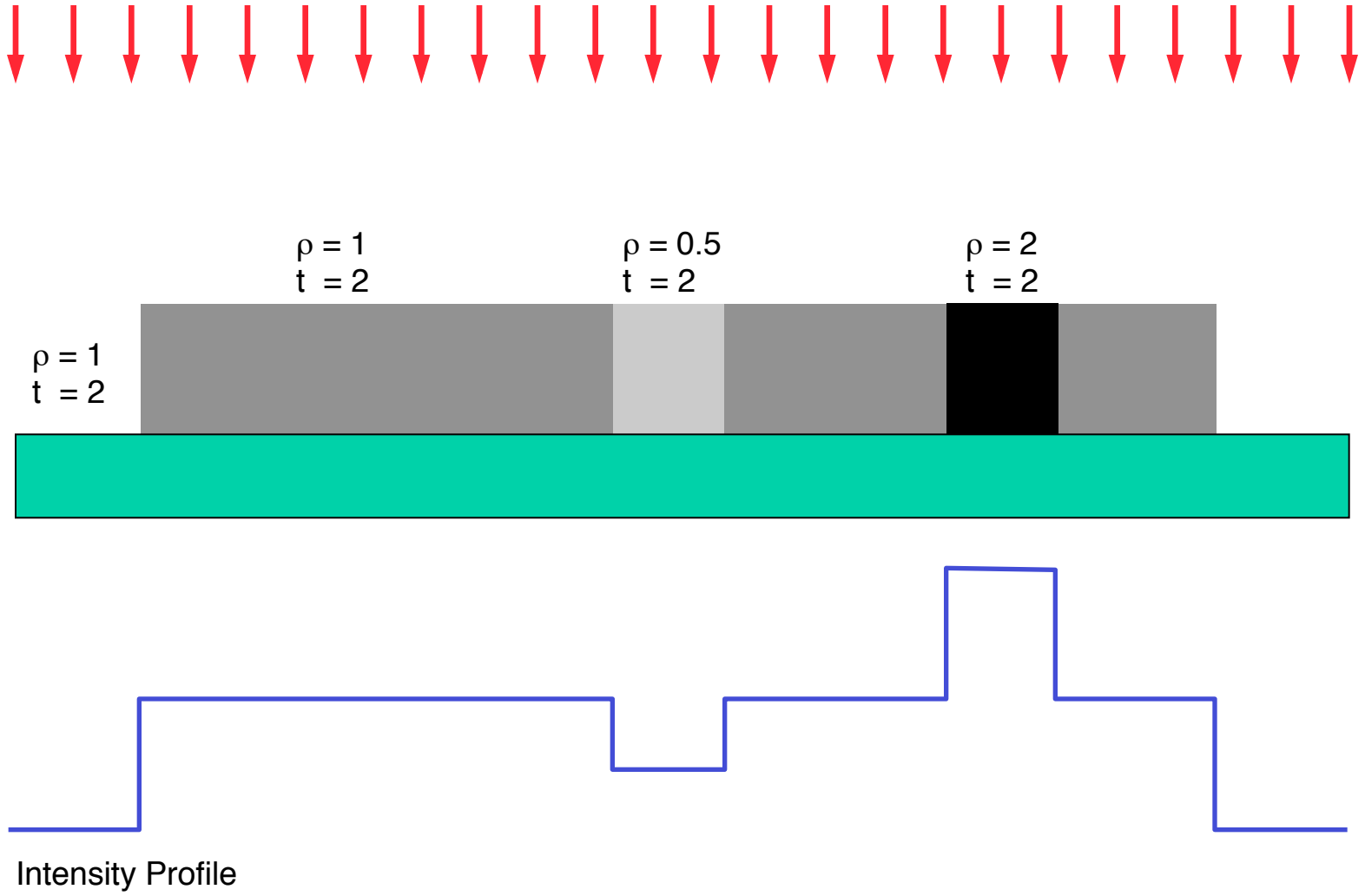
Mass thickness = density x thickness



# I.C CONTRAST AND IMAGE FORMATION

## I.C.1 Electron Scattering

Mass thickness = density x thickness



# I.C CONTRAST AND IMAGE FORMATION

## I.C.1 Electron Scattering

### A few more relevant facts:

**Biological** specimens have **low inherent contrast**
(mainly composed of light atoms: **H, C, O, N**)

**Weak contrast** is a **limiting problem** in imaging biological specimens (or **any** thin specimen)

### How is inherent contrast increased?

Add materials of **high atomic number** to the specimen
(basis of most "conventional" biological specimen preparation procedures)

# I.C CONTRAST AND IMAGE FORMATION

## I.C.1 Electron Scattering

## "Physics" of Electron Scatter

Consider electron beam as it passes ***through*** a specimen

**Paths** of **beam** electrons affected **primarily** by **electrostatic interactions** with the specimen atom components:

- **Nuclei** (protons + neutrons)
- **Electrons**

(in the electron shells or 'cloud' that surround the atomic nuclei)

# I.C CONTRAST AND IMAGE FORMATION

## I.C.1 Electron Scattering

### "Physics" of Electron Scatter

**Recall:** Matter is primarily **empty space**

- **Beam** electrons must pass **very close** to an atomic **nucleus or electron** to be **scattered**

- Beam electrons passing **beyond** the range of the electrostatic fields produced by atomic nuclei and electrons are **not scattered**

- **Rare** for beam electrons to **collide directly** with atomic nuclei or electrons

# I.C CONTRAST AND IMAGE FORMATION

## I.C.1 Electron Scattering

### "Physics" of Electron Scatter

Primary types of electron/specimen scatter:

**Elastic**

**Inelastic**

**None**

# I.C CONTRAST AND IMAGE FORMATION

## I.C.1 Electron Scattering

## "Physics" of Electron Scatter

Primary types of electron/specimen scatter:

**Elastic** No energy loss (no change in velocity or $\lambda$)

**Inelastic** Some energy loss (~10-20 eV per event)

**None** No energy loss

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## "Physics" of Electron Scatter

Primary types of electron/specimen scatter:

**Elastic** No energy loss (no change in velocity or λ)

**Inelastic** Some energy loss (~10-20 eV per event)

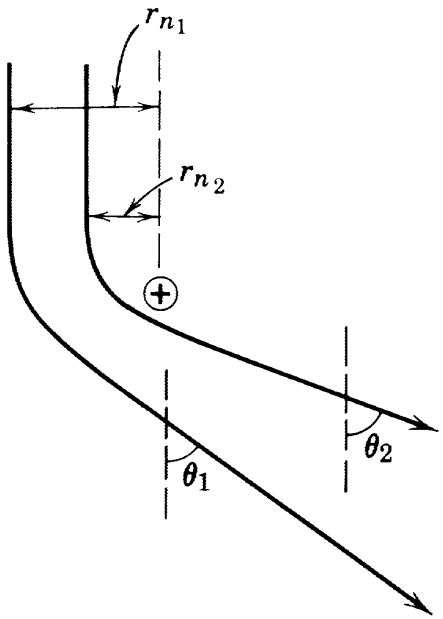
- Main cause of **radiation damage** in biological specimens
- Velocity of **imaging** electron **decreases** (λ increases)

**None** No energy loss

- Significant fraction of beam electrons are **unscattered** after passing through a **"thin" specimen**

# I.C.1 Electron Scattering

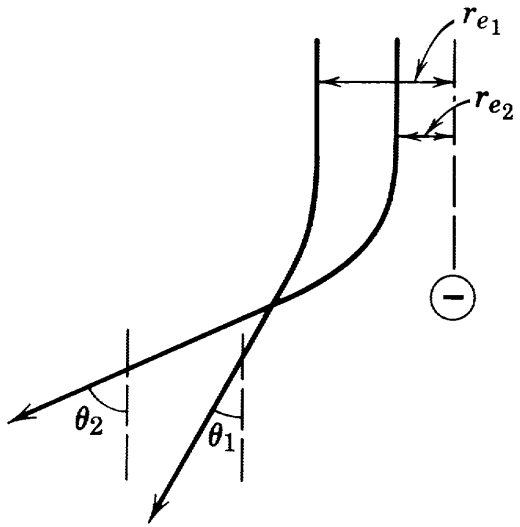
## I.C.1.a Elastic Scattering




Beam electron trajectories near specimen atom **nucleus**

From Slayter, Fig. 19-1, p. 423

## I.C.1.b Inelastic Scattering

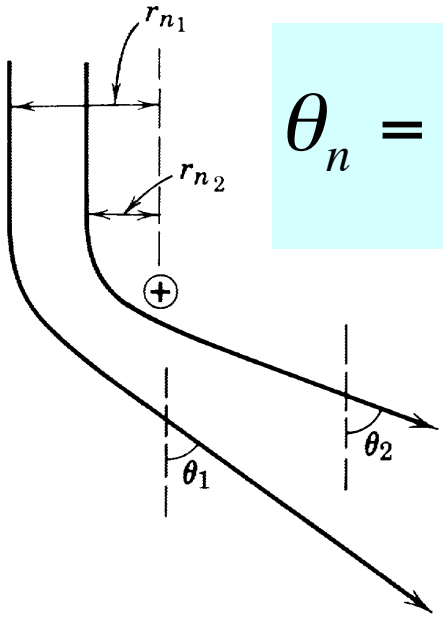



Beam electron trajectories near specimen atom **'stationary' electron**

From Slayter, Fig. 19-2, p. 423

# I.C.1 Electron Scattering

## I.C.1.a Elastic Scattering



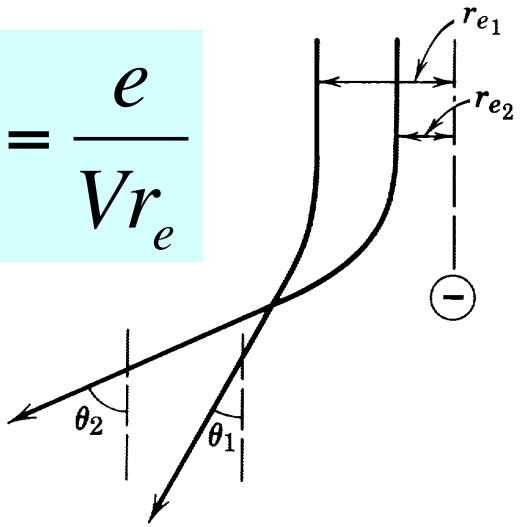

$$\theta_n = \frac{Ze}{Vr_n}$$

Beam electron trajectories near specimen atom **nucleus**

$Z$ = atomic number of specimen atom
$e$ = charge of an electron
$V$ = accelerating voltage of illumination beam
$r_n$ = distance of beam electron from specimen **atom nucleus**

From Slayter, Fig. 19-1, p. 423

## I.C.1.b Inelastic Scattering




$$\theta_e = \frac{e}{Vr_e}$$

Beam electron trajectories near specimen atom **'stationary' electron**

$r_e$ = distance of beam electron from specimen **atom electron**

From Slayter, Fig. 19-2, p. 423

Slide not shown in class lecture

# I.C.1 Electron Scattering

## I.C.1.b Inelastic Scattering

## More facts about inelastic scattering...

- Typically just **one scattering event** as electron passes through a specimen **'thinner'** than ~100 nm (= 1000 Å)

- Each time a beam electron is inelastically scattered, it looses ~10-20 eV of energy (*i.e.* shift to **longer** wavelength and **lower** velocity)

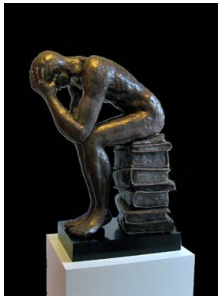
- 10-20 eV of energy loss *corresponds* to fluctuations in accelerating voltage of the order **one part in $10^4$-$10^5$**

- Hence, change in $\lambda$ produced by a **SINGLE** inelastic event is relatively insignificant, but does contribute to **chromatic aberration**

- **Multiple scattering** occurs in **thick** specimens and can be a serious source of chromatic aberration and loss of resolution

Slide not shown in class lecture

# I.C CONTRAST AND IMAGE FORMATION



## I.C.2 Amplitude/Phase Contrast

**...and now (of course) the plot thickens...**

**Contrast** in electron images arises from **scattering AND interference** effects

**SCATTERING (AMPLITUDE or APERTURE) CONTRAST:**

Produced by **loss of amplitude** (*i.e.* electrons) from the imaging beam (**PARTICLE nature** of electrons)

**INTERFERENCE (PHASE) CONTRAST:**

Originates from **shifts in relative phases** of portions of the imaging beam that combine and contribute to the image (**WAVE nature** of electrons)

# I.C CONTRAST AND IMAGE FORMATION

## SCATTERING (APERTURE) CONTRAST

Primary source of electron contrast for most biological specimens prepared for TEM using **"conventional"** methods (*i.e.* those stained or shadowed with heavy atoms to **increase mass thickness**)

## INTERFERENCE (PHASE) CONTRAST

Arises primarily from two factors:

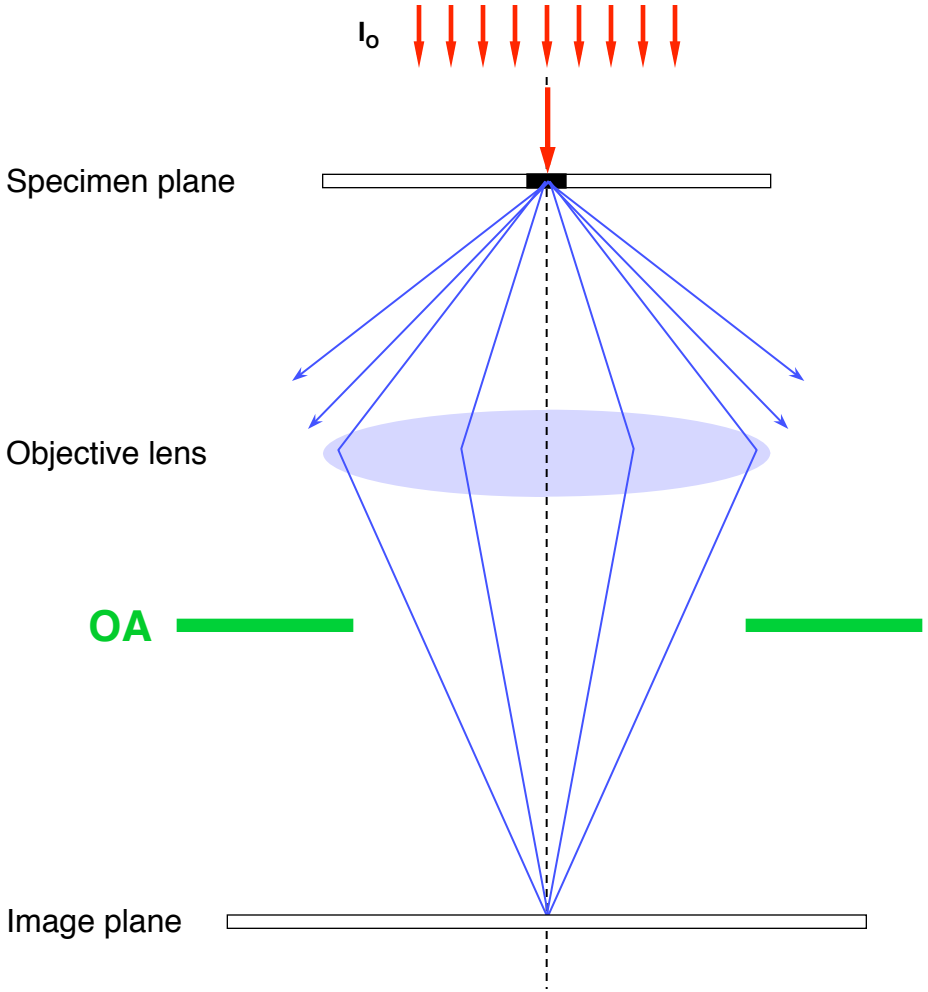
**Defocusing** the **objective lens**

**Spherical aberration** in the objective lens

Importance of interference contrast increases as the **limit of resolution** in the TEM is approached, and for **small / thin** structures

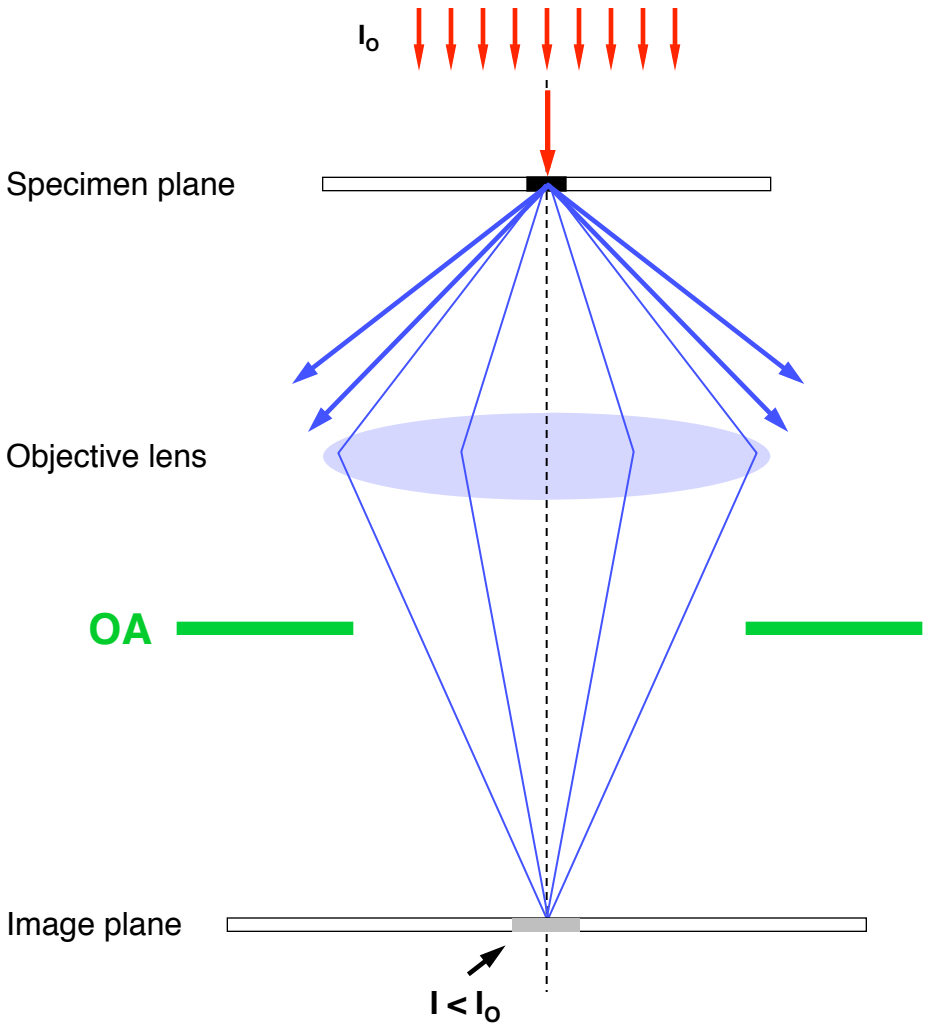
**Dominant source of contrast** for very small or thin objects of low atomic number

# I.C.2.a Scattering (Aperture) Contrast




**Electron 'opaque'** object points produce **appreciable scattering** of beam electrons through relatively **large angles**

# I.C.2.a Scattering (Aperture) Contrast

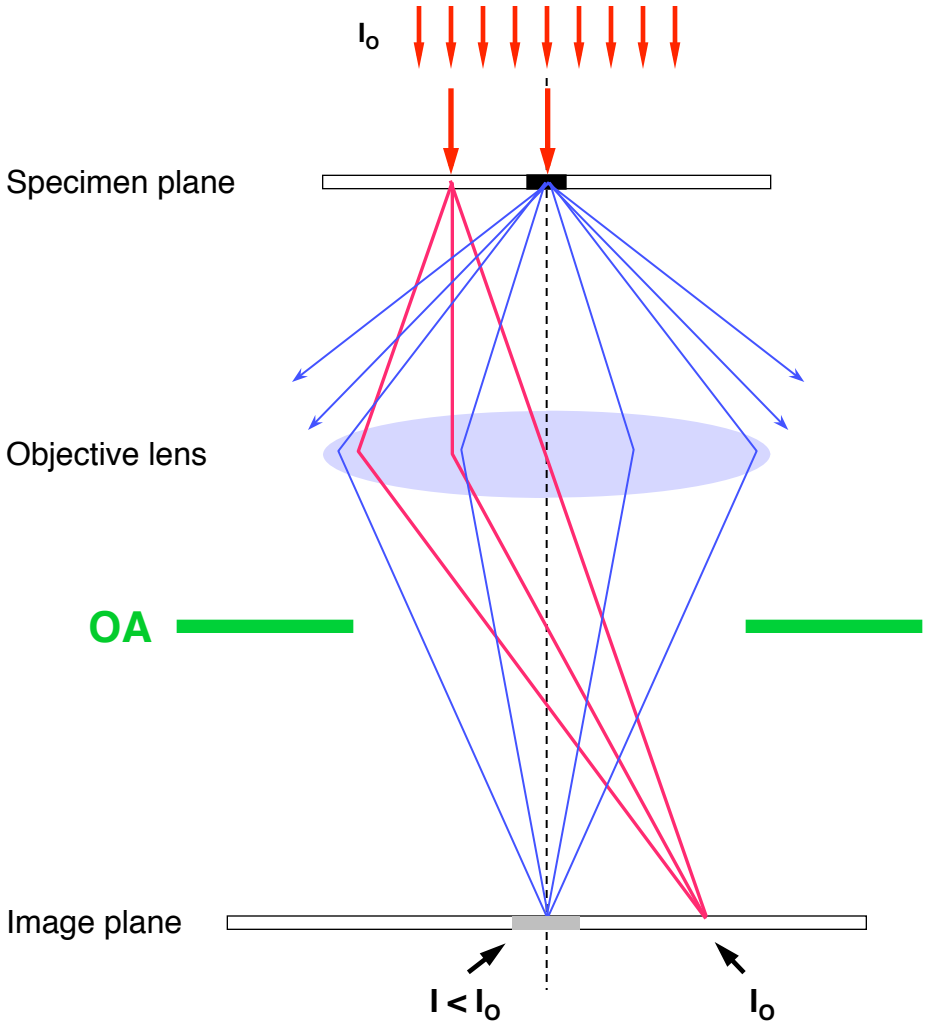



**Electron 'opaque'** object points produce **appreciable scattering** of beam electrons through relatively **large angles**

These $e^-$ are **excluded from** (*i.e.* fall outside) **the lens aperture** and don't reach the image plane

**Intensity** in these image points is correspondingly **low** **($I < I_o$)**

# I.C.2.a Scattering (Aperture) Contrast



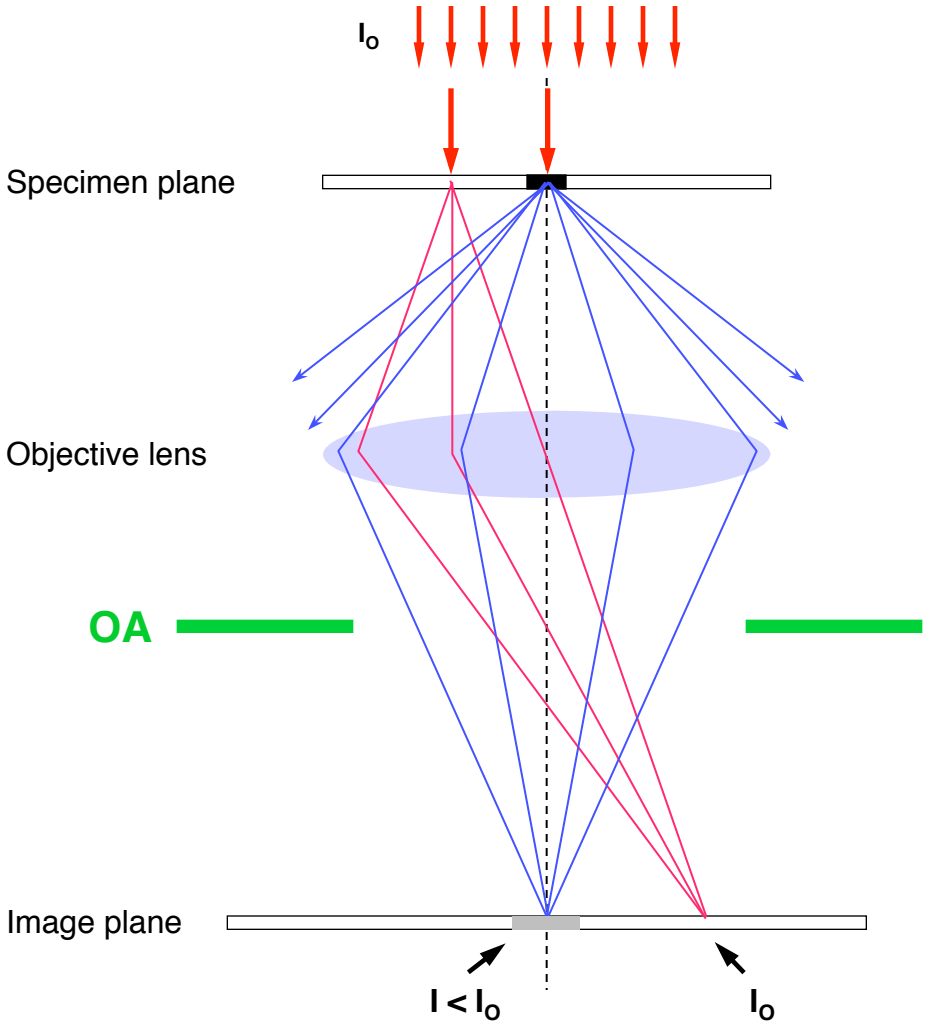

## Conversely:

**Electron 'transparent'** regions in the object (lower average atomic number and/or mass thickness) produce **little scattering outside the lens aperture**

These $e^-$ mostly fall **inside the lens aperture** and nearly all reach the image plane

**Intensity** in these image points is correspondingly **high** ($I \cong I_o$)

# I.C.2.a Scattering (Aperture) Contrast




**Aperture contrast controlled to some extent by:**

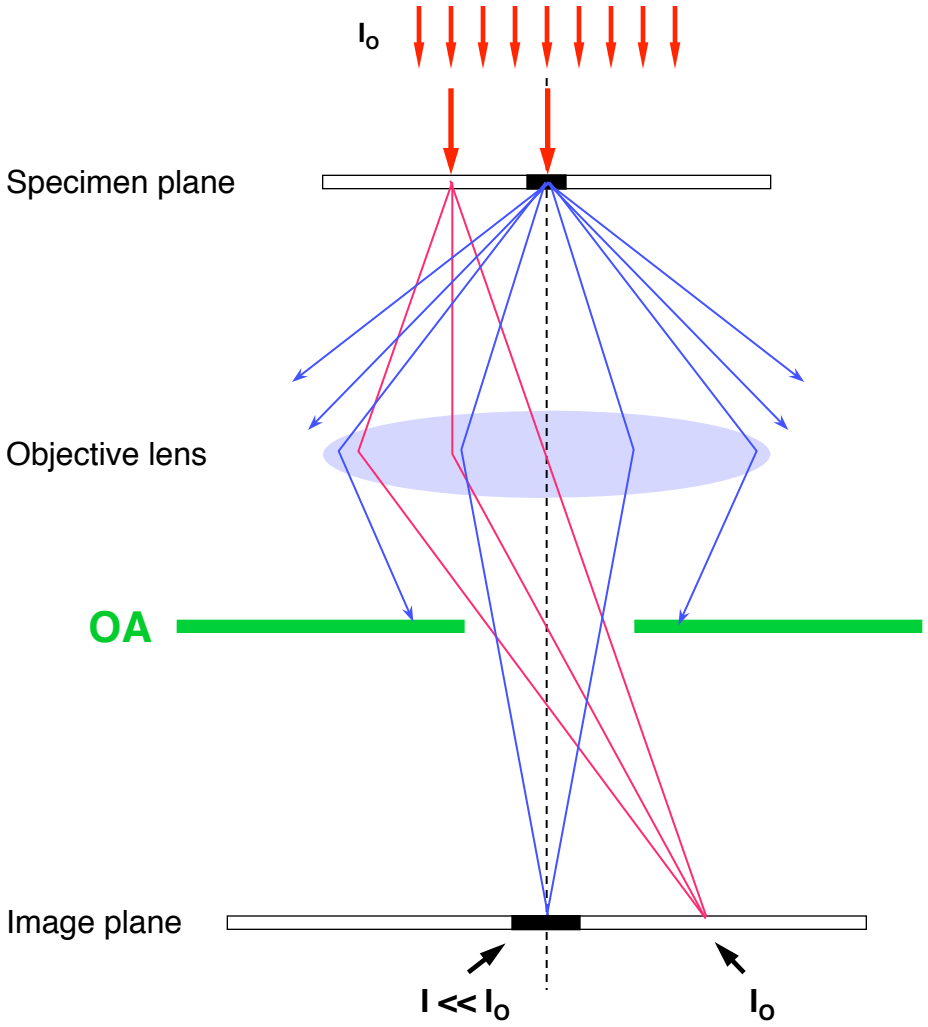
- Choice of accelerating voltage
  (contrast ↑ as V ↓)

$$\theta_n = \frac{Ze}{Vr_n}$$

$$\theta_e = \frac{e}{Vr_e}$$

- Size of objective aperture
  (contrast ↑ as aperture size ↓)

# I.C.2.a Scattering (Aperture) Contrast

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  (contrast ↑ as aperture size ↓)

## Warning:

- Lower V leads to **higher chromatic aberration** and **loss of resolution** (esp. for thicker specimens)