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

# Lecture Slides

Jan 15, 2013

## Fill In Blank (XXX)

According to Abbe's simple criterion for estimating the resolving power of an optical instrument, the smallest object spacings (or details) one can expect to resolve with the instrument are about XXX the wavelength of the radiation used to form images.

A. one tenth the size of

- B. one half the size of
- C. the same size as
- D. two times the size of
- E. ten times the size of

## Multiple Choice Question

Which dimension in the following list is largest?

A. 0.0191  $\mu\text{m}$ 

B. 19.0 nm

C. 190 Å

D. 1.91 nm

E. 1.92 x 10<sup>-10</sup> m

## True / False

The angular aperture typically used in electron optics is much smaller than what is used in light optics.

A. True B. False

### CHM 165,265 / BIMM 162 / BGGN 262 Spring 2013 Announcements for Jan 15, 2013

Reading assignment for Thursday: Lecture notes pp.65-72 'Virtual homework': always check web site for new updates Recitation session: Friday Jan 18; York 4080A; 5-6:00 pm 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 3D Electron Microscopy of Macromolecules

#### **TEM Facility Tour**

Where: 1510 Bonner Hall basement

When: Aiming for Jan 28, 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<sub>6</sub>; LN<sub>2</sub>)

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

#### Class Web Page: Jan 14, 2013

₹UCSD				Dr. Timothy S. Baker				
Intro	UNIVERSIT Members	Y OF CALL Courses	FORNIA, S Images	AN DIEGO Publications	Software	Documentation and Procedures	Microscope Access	Microscope Facilities
CHEM 165,265 / BIMM 162 / BGGN 262 - 3D Electron Microscopy of Macromolecules Winter Quarter 2013								
Syllabus (PDF)								
Book list (PDF)								
Reference list (PDF)								
The Bottom Line (PDF) Key concepts from daily lectures through January 10, 2013								
Virtual I	Homework -	Practice Que	estions for	Section I (PDF)	Updated Janua	ry 10, 2013 (Password p	protected)	
Lecture	Notes							
• 5	Sec. IA. Princ Sec. IB. Desig	iples of the tran	transmission	on electron micros	OSCODE (9.2 MB) CODE (3.5 MB)			

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)

Home Contact Site Map Search Calendar

#### I.A PRINCIPLES OF TRANSMISSION EM KEY CONCEPTS FROM LECTURE #2

- Coherence: defines variance in  $\lambda$  and phase of component waves
- Instrument resolving power: limited by  $\lambda$  of radiation; at best = 1/2  $\lambda$
- Image resolution: always ≤ resolving power of instrument
- Electron waves: potential to resolve finer object details than can photons
- Rayleigh Criterion: gives more realistic estimate of instrument resolving power for TEM (compared to Abbe's simple 1/2 wavelength rule) primarily because it accounts for effects of lens aperture.
- Geometrical (Ideal) Optics: Ray paths through lenses and apertures
- Physical (Real) Optics: Accounts for diffraction and interference effects

#### I.A PRINCIPLES OF TRANSMISSION EM MORE KEY CONCEPTS FROM LECTURE #2

- Ideal vs. Real Lenses
- Ray Diagrams
- Converging / Diverging Lenses; Real / Virtual Images
- Thin Lens Equation:
- Magnification:

$$\frac{1}{f} = \binom{1}{o} + \binom{1}{i}$$
$$M = \begin{vmatrix} i \\ 0 \end{vmatrix}$$

- Lens Aperture: determines amount of radiation arriving from object that can be focused to form an image
- High Magnification Imaging: generally requires 3-4 lenses

I.A.4 Optics (Lens Theory) I.A.4.h Magnification  $M = \begin{vmatrix} i \\ 0 \end{vmatrix}$ 

#### For a converging lens:

- If object is > 2f in front of the lens, the image formed is real, inverted, and smaller than the object (M < 1)</li>
- If object is exactly 2f in front of the lens, image is real, inverted, and the same size as the object (M = 1)
- If object is between f and 2f, image is real, inverted, and larger than the object (M > 1)
- If object is < f, image is virtual, erect, and larger than the object (M > 1)





200 Å



25 Å

200 Å





200 Å



5 Å

200 Å





#### Thermus thermophilus 70S ribosome



#### Thermus thermophilus 70S ribosome



#### Thermus thermophilus 70S ribosome

## § I: The Microscope

I.A Principles of TEM

I.A.5 Electron Optics / Electron Lenses

(pp.25-37 of lecture notes)

# I.A.5 Electron Optics / Electron Lenses NEW CONCEPTS

- Thermionic emission creates a source of beam electrons
- Charged objects produce an electric field
- Path of an electron passing through an electric field or a magnetic field is bent or refracted
- Focal length of electromagnetic lens determined by field strength and electron speed

## Thermionic Emission

Process by which **thermal energy** is supplied to loosely bound e<sup>-</sup> in a metal to form a source of 'free' e<sup>-</sup>

Simplest form of an electron gun filament is a thin tungsten wire

Wire is heated by passing an electric current through it



Electron gun tungsten filament (cathode)

From Agar, Fig. 2.5, p.45

## Take home message of next several slides:



These refract (bend) moving electrons and therefore allow them to be focused into electron images

FACT: Electrons accelerate in an applied electric field



FACT: Electrons accelerate in an applied electric field



A strong electrostatic field applied in a vacuum between a wire [cathode], at negative potential, and an anode, at positive potential, causes e<sup>-</sup> to accelerate away from the wire towards the anode



Speed of the moving e- depends on strength of the electrostatic field (voltage) between the cathode and anode.

$$v = \sqrt{\frac{2eV}{m}}$$



Speed of the moving e- depends on strength of the electrostatic field (voltage) between the cathode and anode.

Number of e<sup>-</sup> that leave the wire depends on the temperature to which the wire is heated, which depends on the filament current

I.A.5 Electron Optics / Electron Lenses I.A.5.b Electric Field / Equipotentials

Path of an electron between two parallel plates of opposite charge



From Sjostrand, Fig. II.22, p.33

# Electric Field / Equipotentials

- Electrons traveling in a direction parallel to the plates are affected by the force directed **perpendicular** to the plates
- Electrons are attracted toward the positive plate
- Path changes in a series of gradual steps at the equipotential surfaces

**RESULT:** fundamentally same as given by Snell's Law of refraction (light optics). **Curved equipotential surfaces** exhibit the **properties of a lens.** 

I.A.5 Electron Optics / Electron Lenses I.A.5.e Magnetic Fields and Magnetic Lenses

The path of a moving electron is bent when the electron encounters an <u>electric</u> field.

What happens if a moving electron encounters a <u>magnetic</u> field?

I.A.5 Electron Optics / Electron Lenses

I.A.5.e Magnetic Fields and Magnetic Lenses

Magnetic field: An electric current passing through a conductor gives rise to a magnetic field





Flux density depends on the properties of the material surrounding the conductor

**Iron** induces a higher flux density than air or a vacuum

I.A.5 Electron Optics / Electron Lenses

I.A.5.e Magnetic Fields and Magnetic Lenses Magnetic field induced by current passing through a solenoid

s N

If conductor is in the form of a **circular loop**, the lines of force form circles around the loop

Flux density is greatest at the center of the loop

From Sjostrand, Fig. II.28, p.37

I.A.5 Electron Optics / Electron Lenses I.A.5.e Magnetic Fields and Magnetic Lenses Flux density even higher if solenoid has an iron core



From Sjostrand, Fig. II.31, p.40

I.A.5 Electron Optics / Electron Lenses I.A.5.e Magnetic Fields and Magnetic Lenses *However*...

Use of iron leads to a problem: lens hysteresis

Lens strength depends to some extent on the magnetic history of the lens

Read about lens hysteresis and lens normalization (p.32, lecture notes)



From Wischnitzer, 2nd ed., Fig. 35, p.33
## I.A.5 Electron Optics / Electron Lenses I.A.5.f The Electromagnetic Lens Forces Acting on a Current in a Magnetic field



From Sjostrand, Fig. II.35-36, p.43

## I.A.5 Electron Optics / Electron Lenses I.A.5.f The Electromagnetic Lens Path of Electron Through an Electromagnetic Lens



Electron starting at point A on axis and **at an angle to it** follows a **spiral path**, returning to the axis at point B

BOTTOM LINE: Action is *similar* to a **converging** glass lens

## I.A.5 Electron Optics / Electron Lenses I.A.5.f The Electromagnetic Lens Path of Electron Through an Electromagnetic Lens





Spiral path of single electron through the electromagnetic lens. **Note:** this is a <u>highly exaggerated</u> schematic diagram, since the path does not include multiple, full rotations.

Schematic diagram of the paths of many different electrons through the electromagnetic lens, scattered from a single point in the specimen.



From Agar, Fig. 1.4, p.5

I.A.5 Electron Optics / Electron Lenses I.A.5.f The Electromagnetic Lens Properties of a Magnetic Lens

- Any **axially-symmetric** magnetic field has the properties of an ideal lens
- All formulas for the ideal lens may be applied
- Magnetic lenses are **always convergent** (*i.e.* positive)

I.A.5 Electron Optics / Electron Lenses I.A.5.f The Electromagnetic Lens Magnetic Lens Focal Length (f)

Determined by two factors:

Field strength in the lens gap AND

Speed of the e<sup>-</sup> (depends on accelerating voltage)

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

- f =focal length of the lens
- K = a constant
- $V_r$  = accelerating voltage (relativistically corrected)
- N = # of turns in the excitation coils
- I = current (in amps)

I.A.5 Electron Optics / Electron Lenses I.A.5.f The Electromagnetic Lens Magnetic Lens Focal Length (f)

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

#### How does one focus an electron image in the TEM?

Varying the current in the OBJECTIVE lens of the TEM

- Changes magnetic field strength and alters lens focal length

I.A.5 Electron Optics / Electron Lenses I.A.5.f The Electromagnetic Lens Magnetic Lens Focal Length (f)

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

#### How does one focus an electron image in the TEM?

Varying the current in the OBJECTIVE lens of the TEM

- Changes magnetic field strength and alters lens focal length
- If TEM voltage is *increased* (e<sup>-</sup> velocity increases), lens current must be increased to keep the focal length constant

## I.A.5.f The Electromagnetic Lens Objective Lens Design



- Typical construction:

**Strong field** of **short axial extent** (f = 1.5-3 mm) needed to form images at **high magnification** 

- Specimen sits inside the magnetic field of the objective lens

From Agar, Fig. 2.11, p.51

# A few take home messages:

- "Refractive index" changes gradually in electromagnetic lenses
- Electrons travel spiral paths through electromagnetic lenses
- Magnetic lens focal length determined by field strength of the lens <u>AND</u> by speed of electrons in the imaging beam

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

# § I: The Microscope

I.A Principles of TEMI.B Design of the TEM

(pp.39-72 of lecture notes)



#### 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



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



## I.B DESIGN OF THE TEM KEY CONCEPTS

- Electron gun: Produces beam of electrons
- Condenser lens(es): Focuses e<sup>-</sup> beam onto specimen
- Lens aberrations: Electromagnetic lenses stink!
- Objective lens and specimen stage: Objective is most important lens
- Projector lenses: Magnify image formed by objective
- Camera and viewing system: View and record electron image
- Vacuum system: Enables beam to travel length of TEM
- Electrical system: Needed for virtually every part of the TEM







# § I: The Microscope

I.B Design of the TEM

(pp.39-72 of lecture notes)

# § I: The Microscope

I.B Design of the TEM

I.B.1 Electron Gun

(pp.39-42 of lecture notes)

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





I.B.1 The Electron Gun

**KEY CONCEPT** 

Gun creates source of high voltage electrons

#### I.B.1 The Electron Gun

#### I.B.1.a Gun Design

Gun in most TEMs consists of a tungsten wire (filament-cathode), bent into a hairpin ("V") shape and surrounded by a shield (gun cap, wehnelt cylinder) with a circular aperture (1-3 mm diameter) centered just below the filament tip



Electron gun tungsten filament (cathode)



Wehnelt cylinder

From Crang and Ward

From Agar, Fig. 2.5, p.45

I.A.5 Electron Optics / Electron Lenses I.A.5.a Electron Emission

## **TEM Filaments**



Tungsten filament

From Agar, Fig. 2.5, p.45

\$3



LaB<sub>6</sub> filament

From Bozzola, 1<sup>st</sup> Ed., Fig. 6-26, p.155

\$1000



Tungsten, cold field-emitting tip From Watt, Fig. A4.8, p.444

\$18,000

#### I.B.1 The Electron Gun

#### I.B.1.a Gun Design

**Flectrons accelerated across** large potential difference (~100,000 volts) between cathode and anode

Wehnelt shield controls beam shape and emission

Anode controls acceleration



From Sjostrand, Fig. III.6, p.74

- Electron gun
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- Electron gun
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# § I: The Microscope

I.B Design of the TEM

I.B.2 Condenser Lens(es)

(pp.42-46 of lecture notes)

I.B.2 Condenser Lens(es)

## **Bottom Line:**

Condenser lens system **focuses / concentrates** the electron beam onto the specimen to give optimal **illumination** for viewing and recording the image



From Agar, Fig. 1.16, p.22

#### I.B.2 Condenser Lens(es) I.B.2.b Double Condenser System



#### **ADVANTAGES (lots of them!):**

- More flexible control of illumination
- Wider range of intensities
- Reduces area of object irradiated
- Reduces specimen contamination
- Improves image contrast (smaller e<sup>-</sup> source produces a higher coherence beam)
- Higher efficiency of double condenser ==> gun brightness can be reduced (increases filament life)

From Agar, Fig. 1.16, p.22

#### I.B.2 Condenser Lens(es) I.B.2.c Condenser Apertures

- CA1 is often a fixed aperture
- CA2 is generally adjustable with centering controls (aperture holder allows rapid exchange of 3 different size apertures).





From Agar, Fig. 1.16, p.22

#### I.B.2 Condenser Lens(es) I.B.2.c Condenser Apertures

- CA1 is often a fixed aperture
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From Agar, Fig. 1.16, p.22

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

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





- Electron gun
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# § I: The Microscope

I.B Design of the TEM

**I.B.3 Lens Aberrations** 

(pp.47-54 of lecture notes)

**I.B.3 Lens Aberrations** 

#### Lots and lots of them:

- Spherical aberration
- Distortion
- Chromatic aberration
- Lens asymmetry
- Lens current fluctuations
- Curvature of field
- Coma and anisotropic coma
- Space charge distortion



I.B.3 Lens Aberrations

### Lots and lots of them:

- Spherical aberration
- Distortion
- Chromatic aberration
- Lens asymmetry
- Lens current fluctuations
- Curvature of field
- Coma and anisotropic coma
- Space charge distortion
# **Bottom Line:**

## Electromagnetic lenses are 'crummy'

They are the reason why resolving power in TEM is **much** worse than estimated by the simple Abbe  $1/2 \lambda$  criterion

To reduce aberrations, the **semi-angular aperture** ( $\alpha$ ) of the **objective** lens is made **VERY small** (recall Abbe's equation?)

$$d = \frac{0.612\lambda}{n \cdot \sin \alpha}$$

I.B.3 Lens Aberrations I.B.3.b Spherical Aberration Bottom Line:

### Spherical aberration is main culprit

Spherical aberration in TEM electromagnetic lenses is the **principal factor** that **limits TEM resolving power** 

#### I.B.3.b Spherical Aberration

**Recall:** For an **IDEAL** lens, all rays entering lens parallel to the optic axis are focused behind the lens on the axis at a **single point**, the focal point



#### I.B.3.b Spherical Aberration

For a **REAL** lens (esp. electromagnetic), rays entering different parts of the lens experience different lens (or field) strength and hence are not focused at a single point on the optic axis



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I.B.3 Lens Aberrations I.B.3.b Spherical Aberration



I.B.3 Lens Aberrations I.B.3.b Spherical Aberration



Region of focus where the marginal and paraxial rays is smallest is the *disc of least confusion* with diameter of disc,  $d_{sa}$ , given by:

$$d_{sa} = \frac{C_s \alpha^3}{2}$$

 $\alpha$  = semi-angle of illumination

 $C_s$  = spherical aberration coefficient of objective lens

So, to **improve resolution** (*i.e.* get smaller  $d_{sa}$ ), all one has to do is **reduce** the lens aperture (*i.e.* reduce  $\alpha$ ).....all the way to zero???

I.B.3.b Spherical Aberration



## Life should be so simple...

SA can be minimized by 'stopping down' the lens with an aperture, but only so far because this **worsens** diffraction-limited resolution

HENCE: Decreasing  $\alpha$  to reduce spherical aberration must be <u>balanced</u> against loss of resolution caused by diffraction effects



Resolution limit due to spherical aberration

 $0.612\lambda$  $n \cdot \sin \alpha$ 

Resolution limit due to diffraction (Abbe equation)

See Tables (p.48, lecture notes) listing values of  $\alpha$  (for different  $C_S$  values) that give 'optimum' resolution.

I.B.3.b Spherical Aberration



## More thoughts about spherical aberration... Glass optics:

Effects of SA CAN be corrected by judicious combination of converging (positive) and diverging (negative) lenses [SA of one lens counteracts the SA of the other lens]

#### **Electron Optics:**

**NOT** generally possible since electromagnetic lenses only work as converging (positive) lenses

I.B.3 Lens Aberrations

- Spherical aberration
- Distortion
- Chromatic aberration
- Lens asymmetry
- Lens current fluctuations
- Curvature of field
- Coma and anisotropic coma
- Space charge distortion

I.B.3 Lens Aberrations

- Spherical aberration
- Distortion (lecture notes, pp.49-50)
- Chromatic aberration
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I.B.3 Lens Aberrations

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- Distortion (lecture notes, pp.49-50)
- Chromatic aberration
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- Coma and anisotropic coma
- Space charge distortion

## OK, what is chromatic aberration?

khroma: Greek for "color"

**Recall:** For an **IDEAL** lens, all **rays** from an object point will be focused by the lens at a point in the image plane



**REAL** lens: <u>Electrons</u> of different wavelength (velocity) leaving a point in object space are not brought to the same point in image space



**REAL** lens: <u>Electrons</u> of different wavelength (velocity) leaving a point in object space are not brought to the same point in image space



I.B.3.d Chromatic Aberration

#### What is the nature of an image with CA?

Images with CA are the combination (superposition) of a series of images

- A given lens has a **different** focal length for <u>each</u>  $\lambda$  electron
- For each  $\lambda$ , an 'in focus' image forms at a specific and different image plane behind the lens and at a particular magnification
- Final image formed at <u>a particular</u> image plane is a superposition of images, each at a different <u>rotation and magnification</u>, and <u>only</u> <u>one</u> of which is in focus



I.B.3.d Chromatic Aberration

#### What is the nature of an image with CA?

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- Final image formed at <u>a particular</u> image plane is a superposition of images, each at a different rotation and magnification, and <u>only</u> <u>one</u> of which is in focus
- The effects of CA in images become **progressively worse** for image points at **increasing distances from the optic axis**

#### Chromatic Change of Magnification



1000 Å tissue section imaged by a 30kV beam

Central part of the micrograph is the sharpest. Out-of-focus effect becomes increasingly noticeable at increasing distance from the optical axis of the TEM. Effect is more noticeable at low magnifications.

I.B.3.d Chromatic Aberration

#### What about photons and glass lenses?

Exactly the **opposite** occurs with glass optics

Photons of short  $\lambda$  (blue) are refracted more in glass than those of longer  $\lambda$  (red)



Net effect (image blurring) occurs with electrons and photons



#### What causes electrons to have different velocities?

- Instabilities in high tension ( $\Delta V/V < 10^{-5}$ )
- Variation in velocity of  $e^{-}$  emitted by the cathode (±3.5 parts/10<sup>6</sup>)
- Energy losses when beam electrons interact with specimen atoms

I.B.3 Lens Aberrations I.B.3.d Chromatic Aberration Does CA limit resolution?

Of course it does.....BUT....

... for TEM imaging of most specimens (**thin ones: ≤ 100 nm**), chromatic aberration is **NOT** a major limit to resolution in electron images.

... however, for thick specimens, effects of CA can be appreciable.

Limit to resolving power strictly due to CA estimated as follows (notes p.52):

$$d_{cv} = C_C \alpha_0 \frac{\Delta V}{V}$$
$$d_{ci} = 2C_C \alpha_0 \frac{\Delta I}{I}$$

I.B.3 Lens Aberrations

- Spherical aberration
- Distortion (lecture notes, pp.49-50)
- Chromatic aberration
- Lens asymmetry
- Lens current fluctuations
- Curvature of field
- Coma and anisotropic coma
- Space charge distortion

I.B.3 Lens Aberrations I.B.3.e Lens asymmetry - Astigmatism

- Impossible to produce lens pole pieces completely free from mechanical and magnetic imperfections
- Irregularities induce an asymmetry in the magnetic field (focal length varies with direction)



www.tedmontgomery.com/.../Astigmatism-grphc.jpg

# I.B.3 Lens Aberrations I.B.3.e Lens asymmetry - Astigmatism Image formation with an astigmatic lens Focal lines 0 $Z_a$ Rays normal to the plane of the paper

Lens

Lens **stronger** in plane  $\perp$  to the screen compared to plane of screen

Point object O is imaged into **two focal lines** in  $\perp$  planes, Z<sub>a</sub> apart

From Agar, Fig. 1.8, p.12

## **I.B.3 Lens Aberrations** I.B.3.e Lens asymmetry - Astigmatism

#### **Condenser Lens Astigmatism**

Uncorrected (astigmatic)

Images of focused electron beam

Corrected (stigmated)

From Agar, Fig. 4.6, p.129

## I.B.3 Lens Aberrations I.B.3.e Lens asymmetry - Astigmatism Objective Lens Astigmatism



Stigmated

Astigmatic

From Agar, Fig. 4.12, p.139

## I.B.3 Lens Aberrations I.B.3.e Lens asymmetry - Astigmatism Objective Lens Astigmatism



Stigmated

Stigmated

From Agar, Fig. 4.12, p.139

## 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.4 Objective Lens and Specimen Stage

(pp.54-62 of lecture notes)



# 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



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

Specimen holder



## 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



OA does not restrict field of view

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

# 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



# 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

