Lecture Slides

Jan 17, 2013
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
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
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
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
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
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

To reserve and guarantee a time slot, email nholson@ucsd.edu
First come, first served.
Class Web Page: Jan 16, 2013

CHEM 165,265 / BIMM 162 / BGHN 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-strikethrough text)

Directions to the EM Facility

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 1 (PDF) Updated January 15, 2013 (Password protected)

Lecture Notes

- Sec. 1A. Principles of the transmission electron microscope (822 KB)
- Sec. 1B. Design of the transmission electron microscope (251 KB)
- Sec. 1C. 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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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 $1/2 \lambda$ 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)
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

![Diagram showing water cooling channels, a rod with objective apertures, and the specimen path.](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

OA does not restrict field of view

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

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

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

**Miscellaneous Factoids:**

- Hole diameter generally $\sim 25-75 \ \mu m$

- 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
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

View from top

Side view

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!!!

- **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 DESIGN OF THE TEM

The TEM Top to Bottom:
- Electron gun
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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
I.B.5 Projector Lenses

KEY CONCEPTS

Depth of Field
Depth of Focus
Recall: A *real* lens images each object point as an *Airy disc*, the radius of which = lens resolving power.
There is a finite distance along the optic axis, $D_i$, where the image appears essentially unchanged. This distance is called depth of focus.
There is a finite distance along the optic axis, $D_i$, where the image appears essentially unchanged. This distance is called depth of focus.

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.
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
Decreasing the aperture of the lens increases both $D_o$ and $D_i$. 
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 \(\alpha_o = 5 \times 10^{-3}\) radians, \(D_o = 400\) 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)

\[ D_i = \frac{M^2 2d}{\tan \alpha_o} = D_o M^2 \]

\( M = \textbf{total} \) magnification of the compound magnifying system
\( d = \) minimum object spacing one hopes or expects to resolve
\( \alpha_o = \) semi-angular aperture of \textbf{objective lens}

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

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
IMPORTANT DISTINCTION:

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

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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

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I.B DESIGN OF THE TEM

The TEM Top to Bottom:
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- Vacuum system \(\text{(notes pp.66-71)}\)
- 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 $< 100 \text{ nm}$ *(i.e. $< 0.1 \text{ \mu m}$)* !!!

- 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")**  \(10^{-3} \text{ to } 10^{-4} \text{ Torr}\)
- **Diffusion**  \(10^{-5} \text{ to } 10^{-7} \text{ Torr}\)
- **Ion**  \(10^{-7} \text{ to } 10^{-9} \text{ Torr}\)
- **Turbomolecular**  \(10^{-7} \text{ to } 10^{-10} \text{ Torr}\)
- **Cryo**  \(10^{-8} \text{ to } 10^{-11} \text{ Torr}\)

**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

Rotary pump
Vacuum reservoir
Diffusion Pump

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 N₂*.
I.B DESIGN OF THE TEM

The TEM Top to Bottom:
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- Camera and viewing system
- Vacuum system  (notes pp.66-71)
- Electrical system
I.B DESIGN OF THE TEM

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- 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

Have I learned ANYthing yet?
§ 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 \( \lambda \)

- **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.
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

\[
\text{Percent contrast} = 100 \times \frac{|I_i - I_b|}{I_b}
\]

\(I_i\) = 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?
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)
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?
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.
Mass thickness = density $\times$ thickness

I.C.1 Electron Scattering

$\rho = 1 \quad t = 2$

$\rho = 0.5 \quad t = 4$

$\rho = 2 \quad t = 1$

Intensity Profile

Mass Thickness
I.C CONTRAST AND IMAGE FORMATION

I.C.1 Electron Scattering

Mass thickness = density \times thickness

Intensity Profile

Mass Thickness

\begin{align*}
\rho = 1 & \quad t = 2 \\
\rho = 0.5 & \quad t = 2 \\
\rho = 2 & \quad t = 2
\end{align*}
I.C CONTRAST AND IMAGE FORMATION

I.C.1 Electron Scattering

Mass thickness = density x thickness

ρ = 1

ρ = 0.5

ρ = 2

t = 2

t = 2

t = 2
I.C CONTRAST AND IMAGE FORMATION

I.C.1 Electron Scattering

Mass thickness = density x thickness

Mass Thickness

Intensity Profile
I.C CONTRAST AND IMAGE FORMATION

I.C.1 Electron Scattering

Mass thickness = density x thickness

\[ \rho = 1 \]
\[ t = 2 \]

\[ \rho = 0.5 \]
\[ t = 2 \]

\[ \rho = 2 \]
\[ t = 2 \]
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)
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

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

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

**None**  
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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)
  - Main cause of radiation damage in biological specimens
  - Velocity of imaging electron decreases ($\lambda$ 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

I.C.1.b Inelastic Scattering

Beam electron trajectories near specimen atom **nucleus**

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

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

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

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. 19-1, p. 423

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

...and now (of course) the plot thickens...
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 < I₀).
I.C.2.a Scattering (Aperture) Contrast

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

Conversely:

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 ↓)

\[ \theta_a = \frac{Ze}{Vr} \]

\[ \theta_c = \frac{e}{Vr} \]

- Size of objective aperture (contrast ↑ as aperture size ↓)
I.C.2.a Scattering (Aperture) Contrast

Aperture contrast controlled to some extent by:

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

\[
\theta_a = \frac{Ze}{V_r}
\]

\[
\theta_c = \frac{e}{V_r}
\]

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

Warning:

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