What's Next?

Contrast and Image Formation

- Electron Scattering

On the horizon…

Feb 3: Alignment and Performance of the TEM (J. Turek)

- Optimizing the resolving power of the TEM

Feb 5: TEM Demo (D. Sherman/M. Sherman)

- The TEM up close and personal

Feb 10: Operation of the TEM (J. Turek)

- The ABCs of TEM operation

I.C CONTRAST AND IMAGE FORMATION

KEY CONCEPTS:

- Specimens are made of **atoms**

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

- **Image contrast** arises both from electron **scattering** and **interference** (phase) effects

I.C CONTRAST AND IMAGE FORMATION

MORE KEY CONCEPTS:

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

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

- **Resolution** in TEM images: ***generally*** 1-5 nm for most **biological** specimens

I.C CONTRAST AND IMAGE FORMATION

IMAGE CONTRAST:

- Determined by nature and extent of interactions between electron beam and specimen

- Properties of specimen (**inherent contrast**) **AND** microscope (**instrumental contrast**) are both 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

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.C CONTRAST AND IMAGE FORMATION

I.C.1 Electron Scattering

Some facts:

- **Amount** of scattering at any particular specimen point depends on its **density and overall thickness**

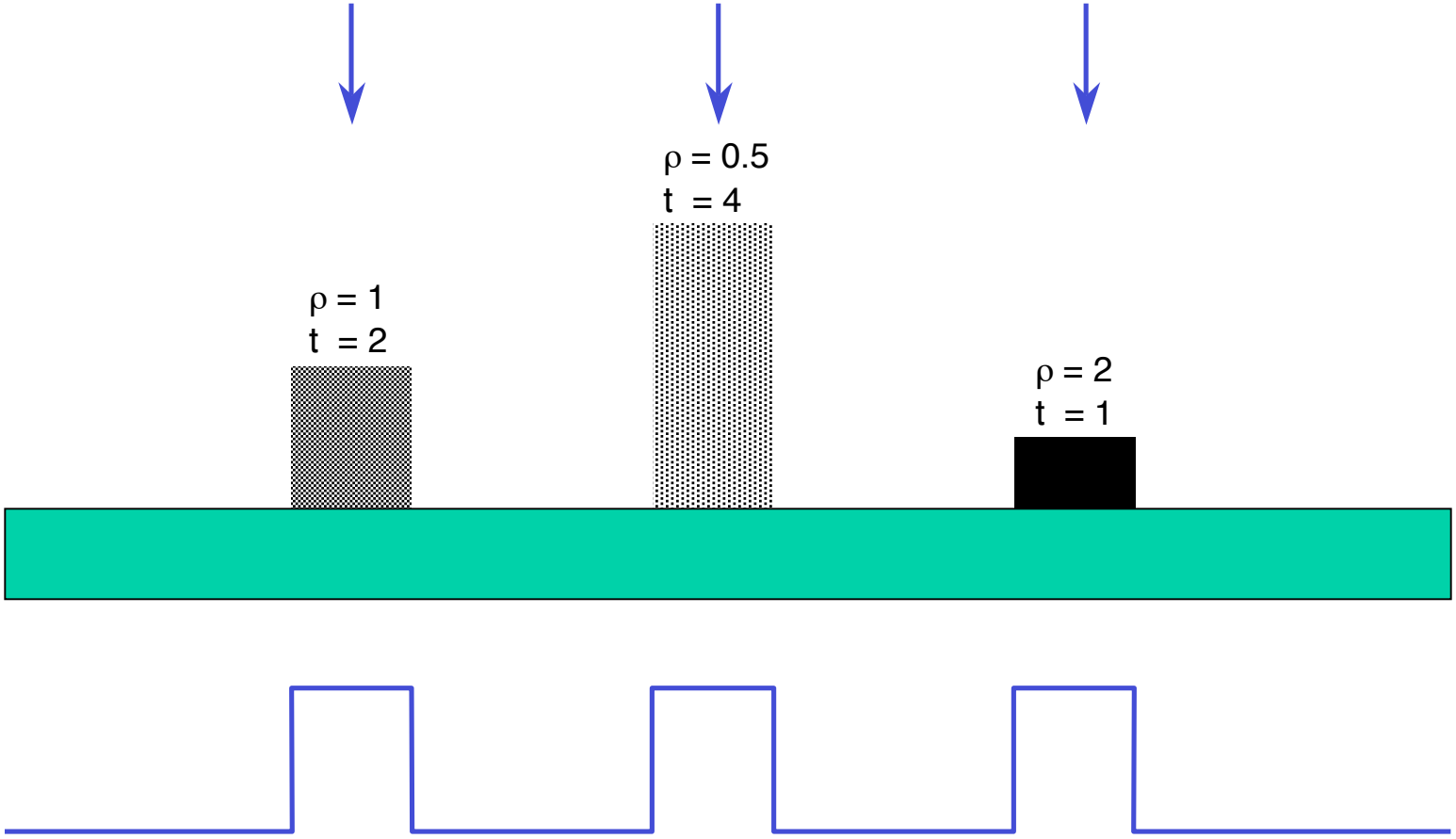
- Scattering is *relatively* **independent** of atomic number, chemical composition, or other specimen properties

- **Probability** of scattering increases with increasing **mass thickness**

I.C CONTRAST AND IMAGE FORMATION

I.C.1 Electron Scattering

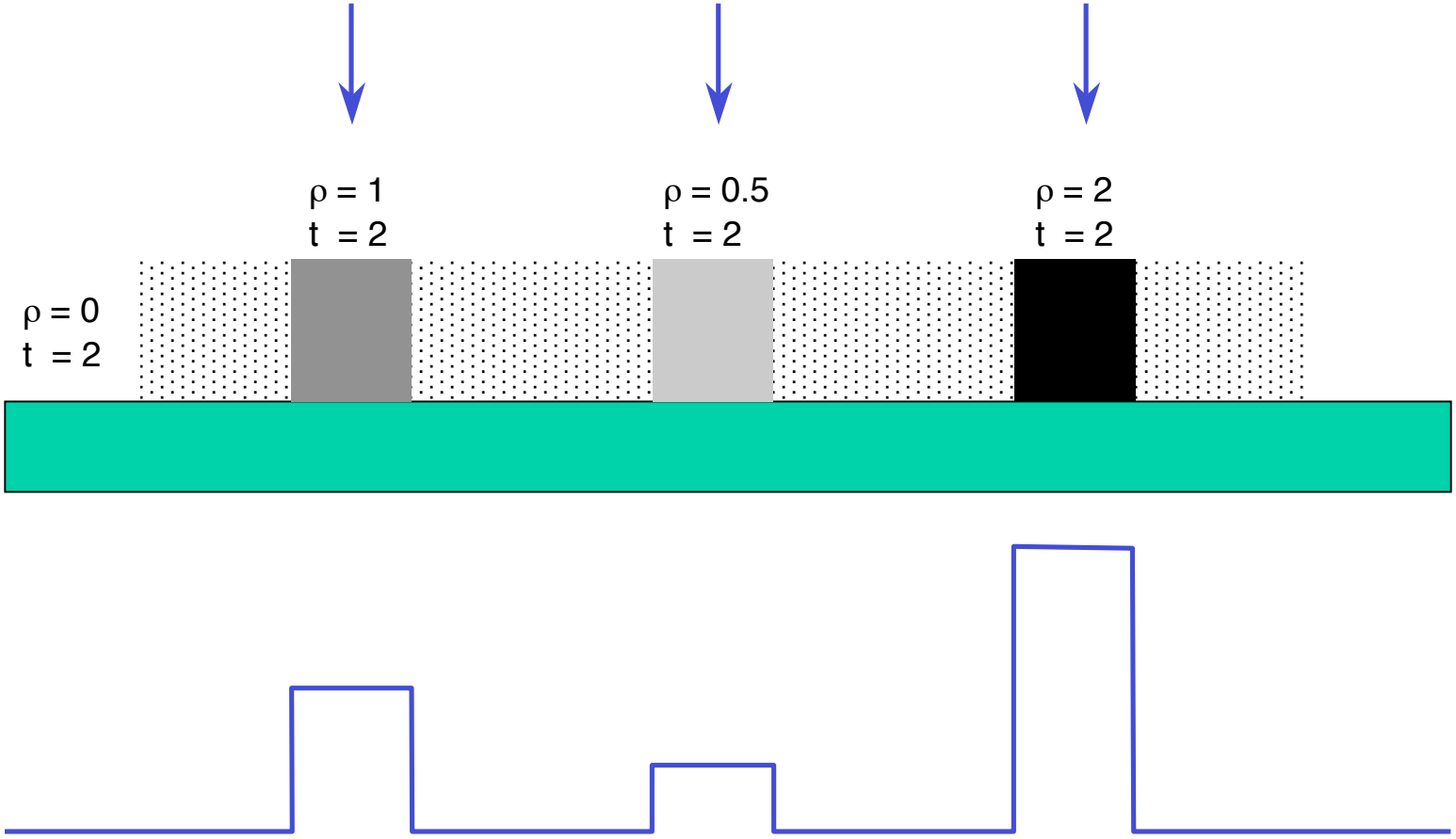
Mass thickness = density x thickness



I.C CONTRAST AND IMAGE FORMATION

I.C.1 Electron Scattering

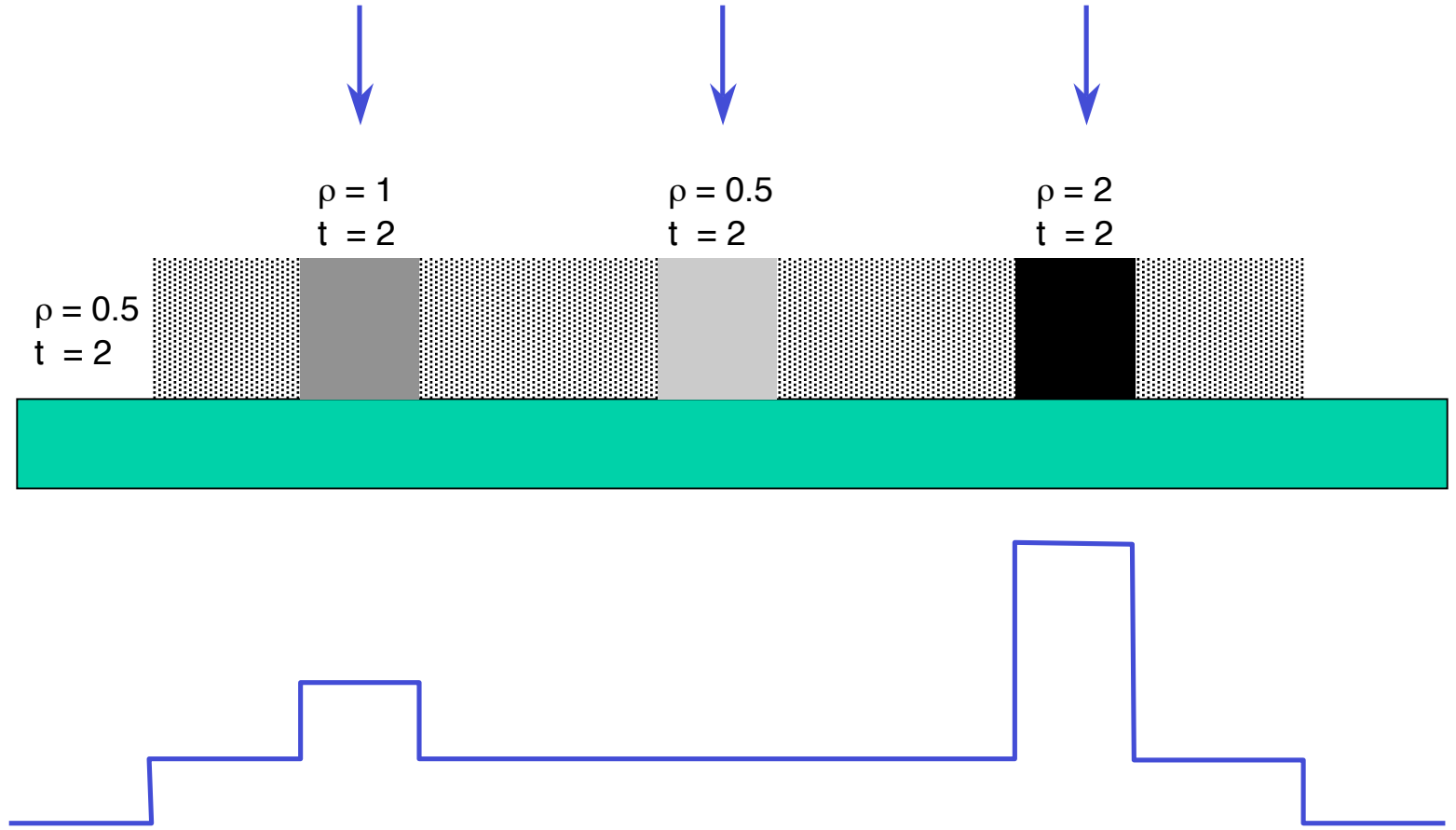
Mass thickness = density x thickness



I.C CONTRAST AND IMAGE FORMATION

I.C.1 Electron Scattering

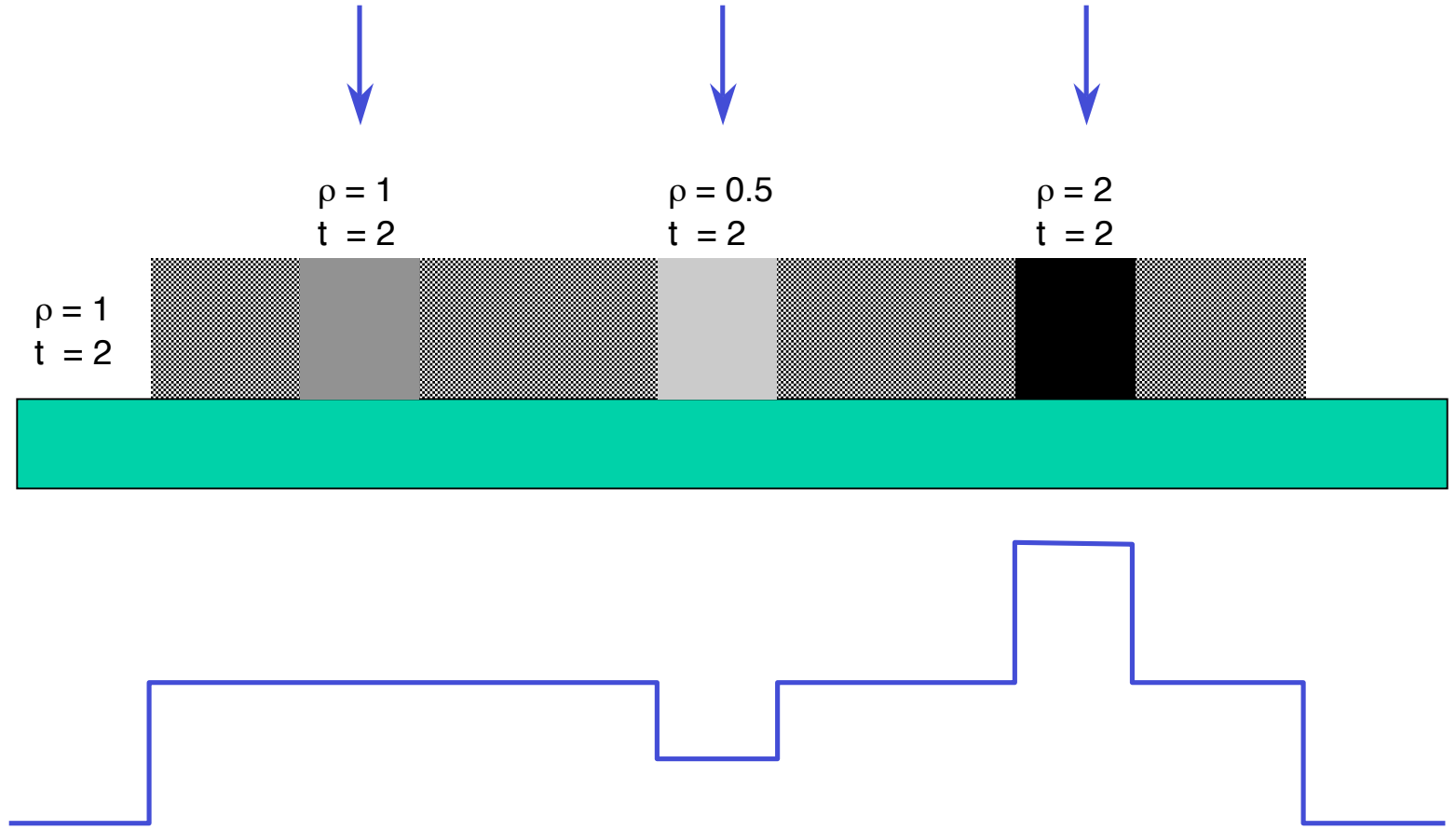
Mass thickness = density x thickness



I.C CONTRAST AND IMAGE FORMATION

I.C.1 Electron Scattering

Mass thickness = density x thickness



I.C CONTRAST AND IMAGE FORMATION

I.C.1 Electron Scattering

Mass thickness = density x thickness

- **Biological** specimens have low **Inherent contrast** because they are mainly comprised of **C, N, O, H**

- **Weak contrast** is a **limiting problem** in imaging **biological** specimens (or *very* thin specimens)

- **Inherent contrast** increased by preferential addition of materials of **high atomic number** (basis of many different specimen preparation procedures)

I.C CONTRAST AND IMAGE FORMATION

I.C.1 Electron Scattering

"Physics" of Electron Scatter

Consider an e^- beam as it passes through a specimen:

- **Path** of the beam is affected mainly by **electrostatic interactions** with the **atomic nuclei or electrons** in the electron shells surrounding the nuclei

I.C CONTRAST AND IMAGE FORMATION

I.C.1 Electron Scattering

"Physics" of Electron Scatter

Recall: Matter is mainly **empty space**

- Beam electron must pass **very close** to **atomic nucleus or electron** before it will be **deflected** (i.e. scattered)

- Electrons **rarely** scatter due to **direct collisions** with atomic nuclei and 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

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

Inelastic scatter: Some energy loss (~10-20 eV)

- Energy transferred to **atomic shell electrons**
- Main cause of **radiation damage** in biological specimens
- Velocity of imaging electrons **decreases** (λ increases)

No scatter: Beam electrons which pass **outside** the range of the electrostatic field of atomic nuclei and atomic electrons are **not** scattered

- For **"thin" specimens**, a large fraction of electrons are **unscattered**

I.C CONTRAST AND IMAGE FORMATION

I.C.1 Electron Scattering

Proportions of inelastic and elastic collisions depend on the accelerating **voltage** and the nature of the **specimen**

EXAMPLE:

50 nm thick carbon film and 50kV electrons

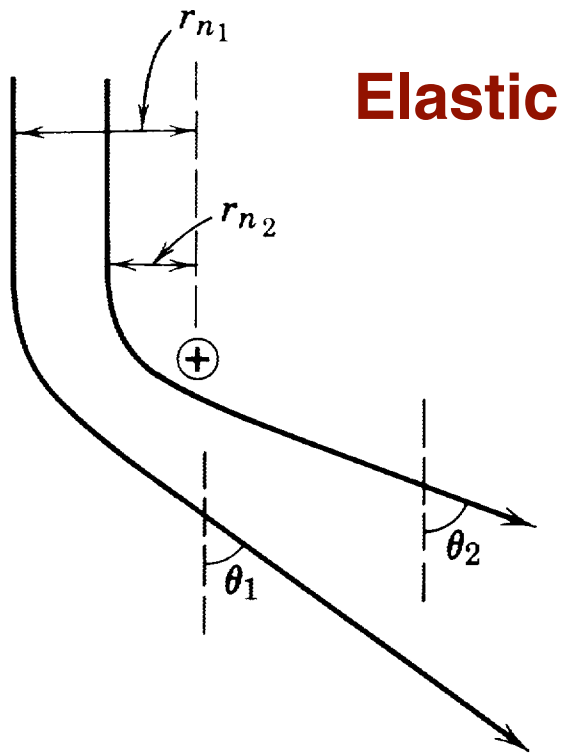
34% unscattered

11% elastically scattered

55% inelastically scattered

I.C.1 Electron Scattering

I.C.1.a Elastic Scattering



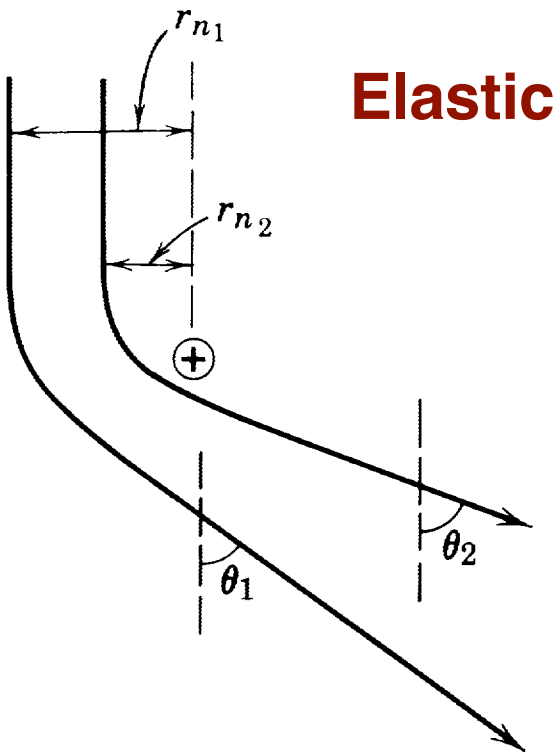
Elastic

Electron trajectories in vicinity of a **nucleus**

(From Slayter, Figs. 19-1, p. 423)

I.C.1 Electron Scattering

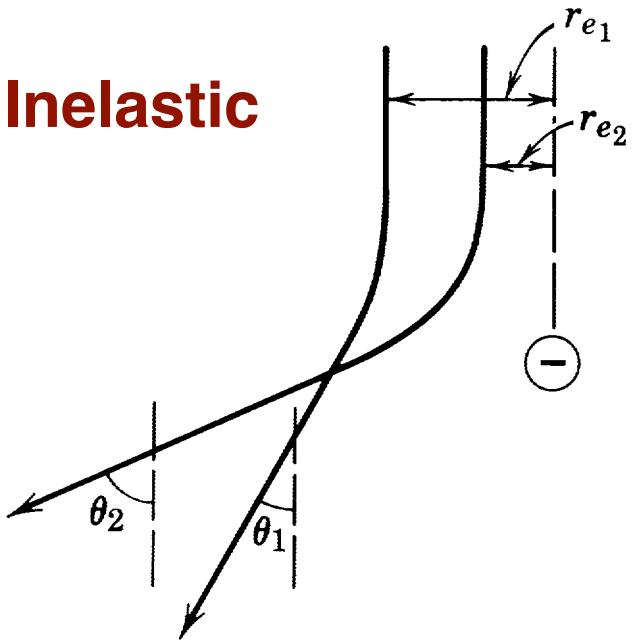
I.C.1.a Elastic Scattering



Elastic

Electron trajectories in vicinity of a **nucleus**

I.C.1.b Inelastic Scattering



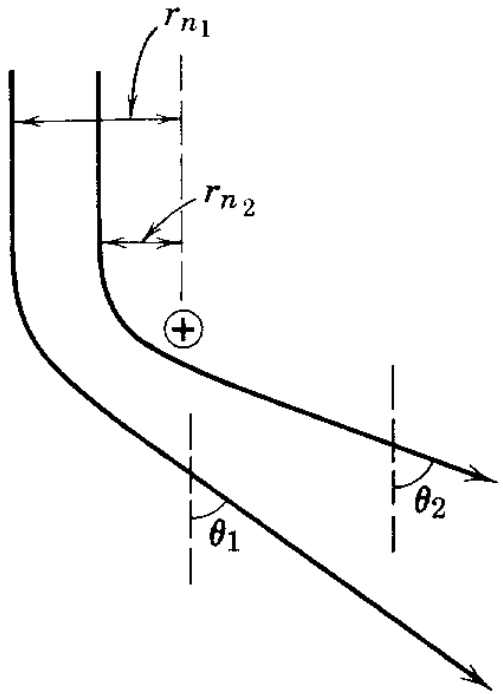
Inelastic

Electron trajectories in vicinity of a **'stationary' electron**

(From Slayter, Figs. 19-1, 19-2, p. 423)

I.C.1 Electron Scattering

I.C.1.a Elastic Scattering



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

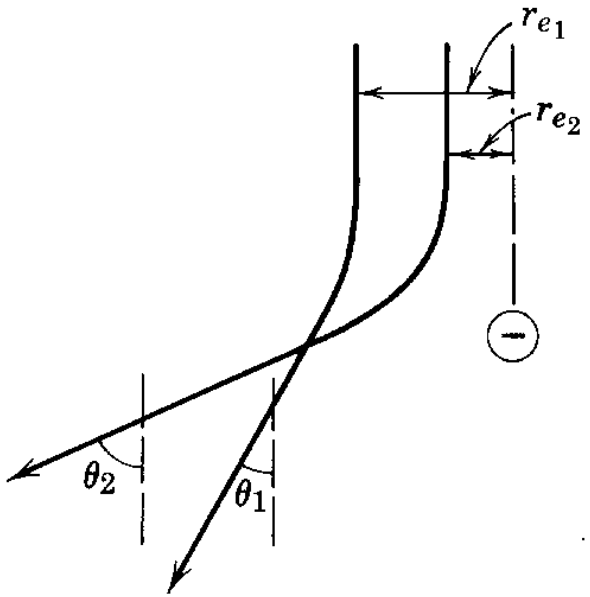
Elastic scattering of beam electrons by **electrostatic attraction** from the atomic **nucleus** leads to angular deflection, θ_n, of beam electrons

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

(From Slayter, Figs. 19-1, p. 423)

I.C.1 Electron Scattering

I.C.1.b Inelastic Scattering



$$\theta_e = \frac{e}{V r_e}$$

Inelastic scattering of beam electrons by **electrostatic repulsion** from the atomic **electrons** leads to angular deflection, θ_e, of beam electrons

e = charge of an electron
V = accelerating voltage of illumination beam
r_e = distance of beam electron from **atomic electron**

(From Slayter, Figs. 19-2, p. 423)

I.C.1 Electron Scattering

Elastic vs. Inelastic

$$\theta_n = \frac{Ze}{Vr_n} \qquad \theta_e = \frac{e}{Vr_e}$$

Nucleus has a higher scattering power than the atomic **electron** by a factor of *Z*, due to the greater concentration of charge in the nucleus

Consequence: electrons are generally scattered **elastically** to **higher angles** than those scattered inelastically (*i.e.* generally $\theta_n > \theta_e$)

I.C.1 Electron Scattering

I.C.1.b Inelastic Scattering

A few more facts...

- For **electrostatic** interactions between the **beam** electrons and the electrons surrounding the atomic nucleus, the **deflected** beam electrons undergo a **loss of energy** (*i.e.* shift to **longer wavelength** and **lower velocity**)

- Energy loss **~10-20 eV** for **'thin'** specimens (<100 nm)

- Beam electrons suffering ~10-20 eV energy loss are deflected through **very small angles** (~10^{-4} radians), thus **nearly all of them pass through the objective aperture**

- Generally only **one scattering event** as electron passes through a **'thin'** specimen

I.C.1 Electron Scattering

I.C.1.b Inelastic Scattering

And even some more facts...

- Energy loss due to inelastic collisions corresponds to fluctuations in the accelerating voltage of the order **one part in 10^4-10^5**

- Hence, **change in λ** produced by a **single** electron scattering event is **relatively insignificant**

- However, **multiple scattering**, which does occur in **thick** specimens, can be a serious source of **chromatic aberration** in TEM images

I.C CONTRAST AND IMAGE FORMATION

I.C.2 Amplitude/Phase Contrast

Contrast in electron images arises from both **"amplitude"** (**scattering**) and **"phase"** (**interference**) effects

AMPLITUDE CONTRAST:

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

PHASE CONTRAST:

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

I.C CONTRAST AND IMAGE FORMATION

I.C.2 Amplitude/Phase Contrast

Scattering vs. Interference Contrast

Terms "amplitude" and "phase" **misleading**

More appropriate: scattering (or **aperture**) and **interference** contrast

However, many EM texts and articles often use terms "amplitude" and "phase" contrast, so **BEWARE of the distinction**

I.C.2 Amplitude/Phase Contrast

Scattering vs. Interference Contrast

SCATTERING CONTRAST

- **Primary source** of **electron contrast** for **most** biological specimens prepared for TEM imaging by **"conventional"** methods (*i.e.* stained or shadowed with heavy atoms and hence of large mass thickness)

INTERFERENCE CONTRAST

- Arises from two factors:

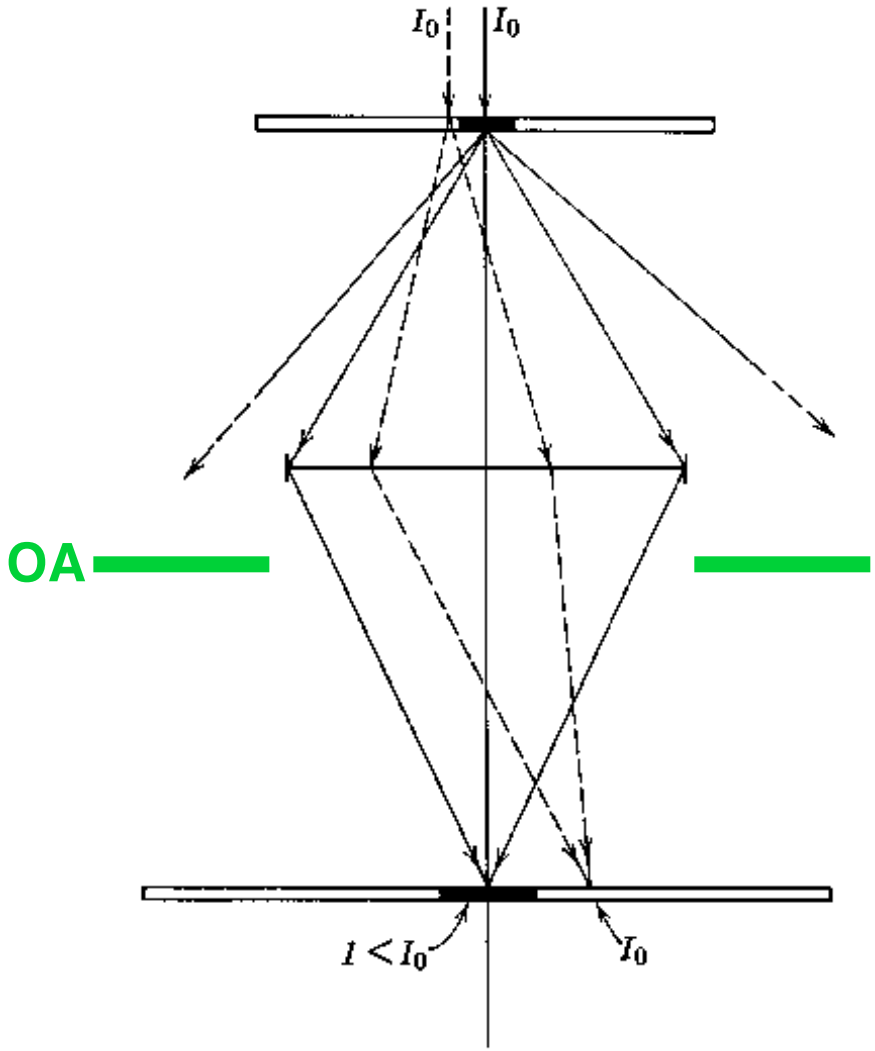
  Defocusing the objective lens

  Spherical aberration in the objective lens

- Importance increases as **limit of resolution** in TEM is approached and for **small / thin** structures
- **Dominant source of contrast** for **very small objects** of low atomic number

I.C.2 Amplitude/Phase Contrast

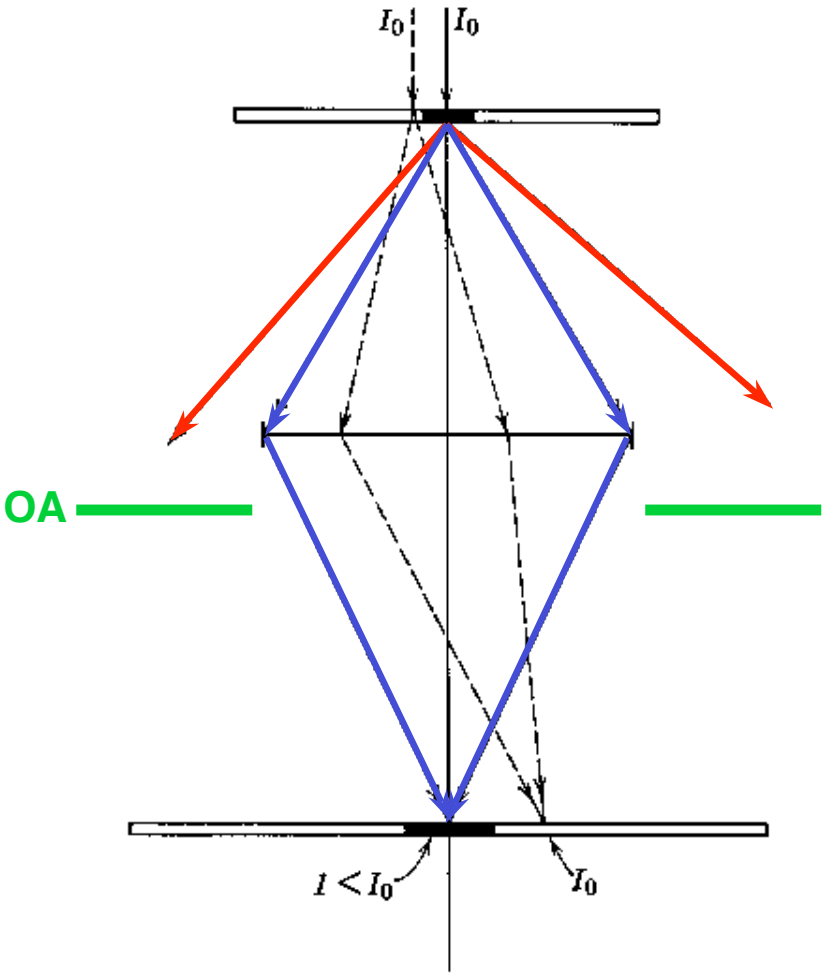
I.C.2.a Scattering (Amplitude) Contrast



(From Slayter, Fig. 19-3, p. 427)

I.C.2 Amplitude/Phase Contrast

I.C.2.a Scattering (Amplitude) Contrast



(From Slayter, Fig. 19-3, p. 427)

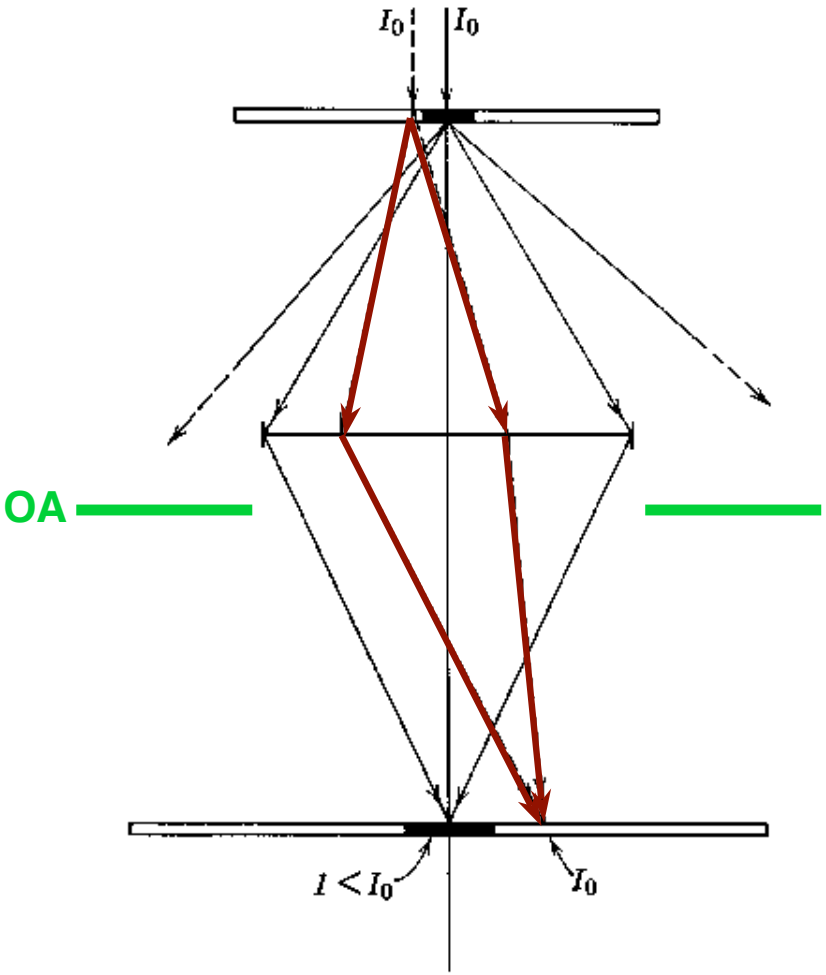
Electron opaque object points produce **appreciable scattering** of beam electrons through relatively **large angles**

These e^- are **excluded from** (*i.e.* fall outside) **the lens aperture**

Intensity in these image points is correspondingly **low**

I.C.2 Amplitude/Phase Contrast

I.C.2.a Scattering (Amplitude) Contrast



(From Slayter, Fig. 19-3, p. 427)

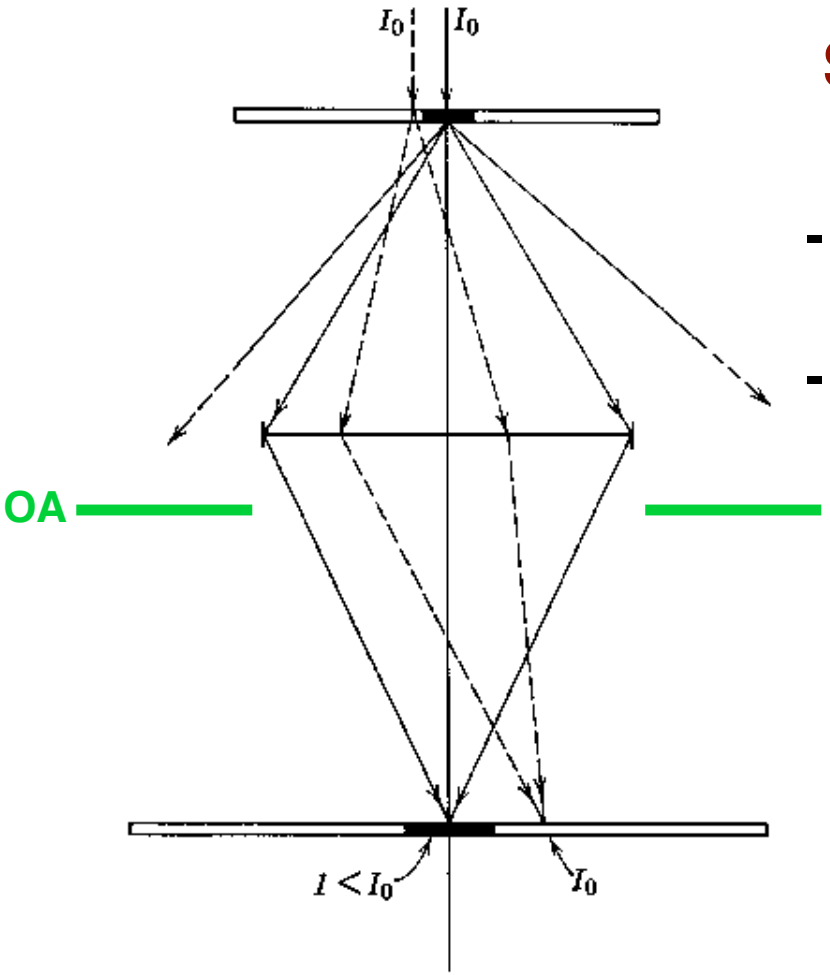
Conversely:

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

Intensity of these image points is correspondingly **high**

I.C.2 Amplitude/Phase Contrast

I.C.2.a Scattering (Amplitude) Contrast



(From Slayter, Fig. 19-3, p. 427)

Scattering contrast controlled to some extent by:

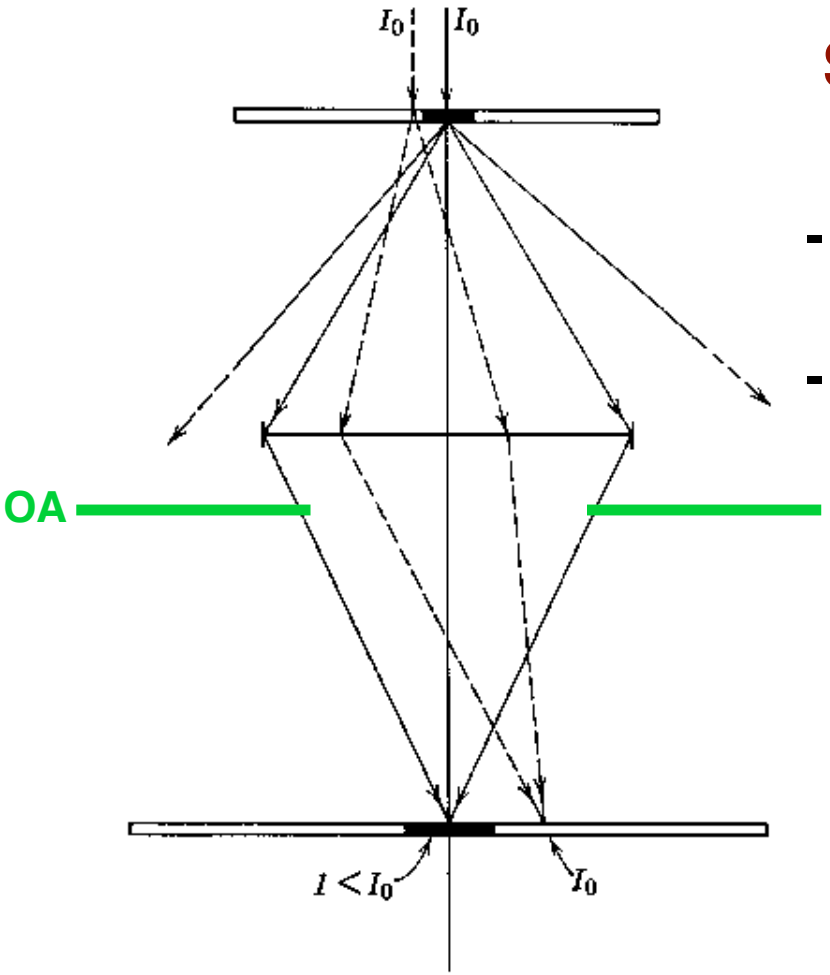
- **Choice of accelerating voltage**
  (contrast ↑ as V ↓)
- **Size of objective aperture**
  (contrast ↑ as aperture size ↓)

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

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

I.C.2 Amplitude/Phase Contrast

I.C.2.a Scattering (Amplitude) Contrast



(From Slayter, Fig. 19-3, p. 427)

Scattering contrast controlled to some extent by:

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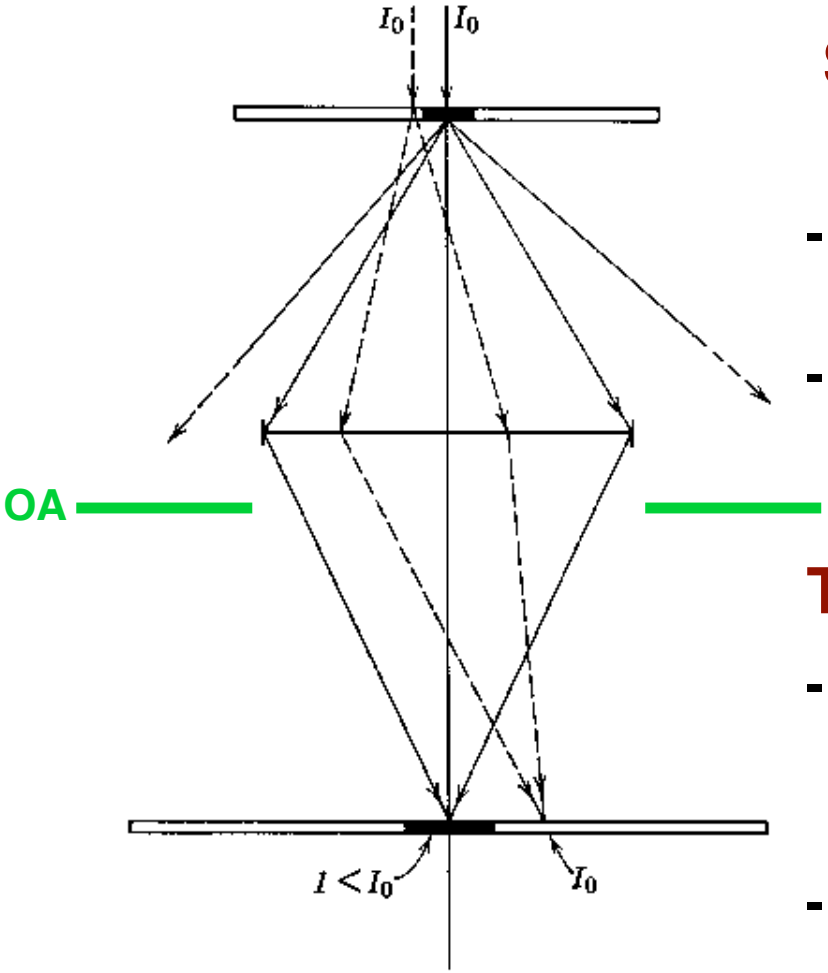
I.C.2 Amplitude/Phase Contrast

I.C.2.a Scattering (Amplitude) Contrast



(From Slayter, Fig. 19-3, p. 427)

Scattering contrast controlled to some extent by:

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

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

- **Choice of accelerating voltage** (contrast ↑ as V ↓)
- **Size of objective aperture** (contrast ↑ as aperture size ↓)

Trade offs:

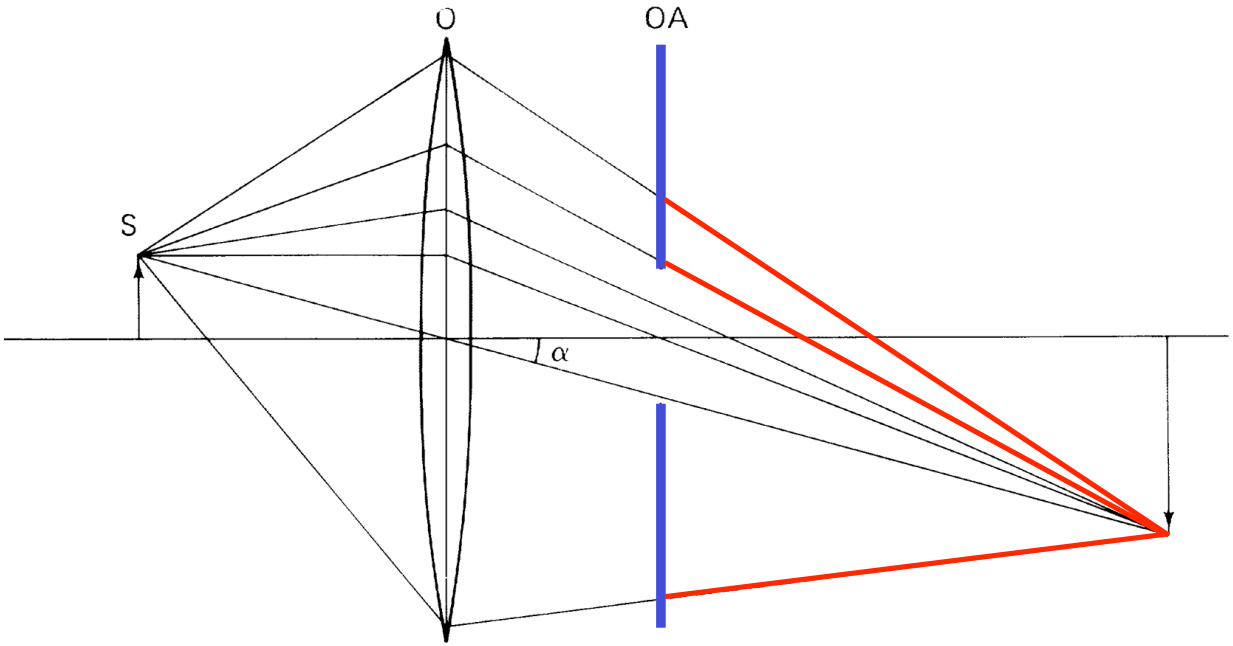
- **Higher chromatic aberration** at lower V may lead to unacceptable **loss of resolution** (unless specimen is thin)
- **Gun brightness** ↓ as V ↓

I.C.2 Amplitude/Phase Contrast

I.C.2.a Scattering (Amplitude) Contrast

Objective Aperture Affects Scattering Contrast

- Objective aperture at **back focal plane** of objective lens



From Agar 2nd ed., Fig. 1.21, p.27

I.C.2 Amplitude/Phase Contrast

I.C.2.a Scattering (Amplitude) Contrast

Objective Aperture Affects Scattering Contrast

- Objective aperture at **back focal plane** of objective lens

- As aperture size is **reduced**, more scattered electrons are stopped and “amplitude” (or aperture) **contrast improves**

- However, apertures **too small** (<20 μm) lead to loss of resolution due to **diffraction** effects (point in object leads to **larger Airy disk image**)

I.C.2 Amplitude/Phase Contrast

I.C.2.a Scattering (Amplitude) Contrast

Objective Aperture Affects Scattering Contrast

Practical problems with small objective apertures:

- More **difficult to keep aligned** on the optical axis in the back focal plane of the objective lens

- More **susceptible to the effects of contamination** (produces lens asymmetry and astigmatism, thereby reducing resolution)

I.C.2 Amplitude/Phase Contrast

I.C.2.b Interference (Phase) Contrast

Interference ("phase") contrast is generated when **scattered and unscattered** (i.e. diffracted and undiffracted) electron **waves interfere**

This produces **differences in intensity** at the electron image

Recall:

- Electrons scattered through large angles (outside lens aperture) give rise to **scattering** ("amplitude") contrast

- ***Ideally***, **all other** scattered electrons are focused by the lens at the corresponding image points, at which they arrive **in phase**

I.C.2 Amplitude/Phase Contrast

I.C.2.b Interference (Phase) Contrast

Interference contrast arises from phase differences and interference between scattered and unscattered rays in different parts of the image

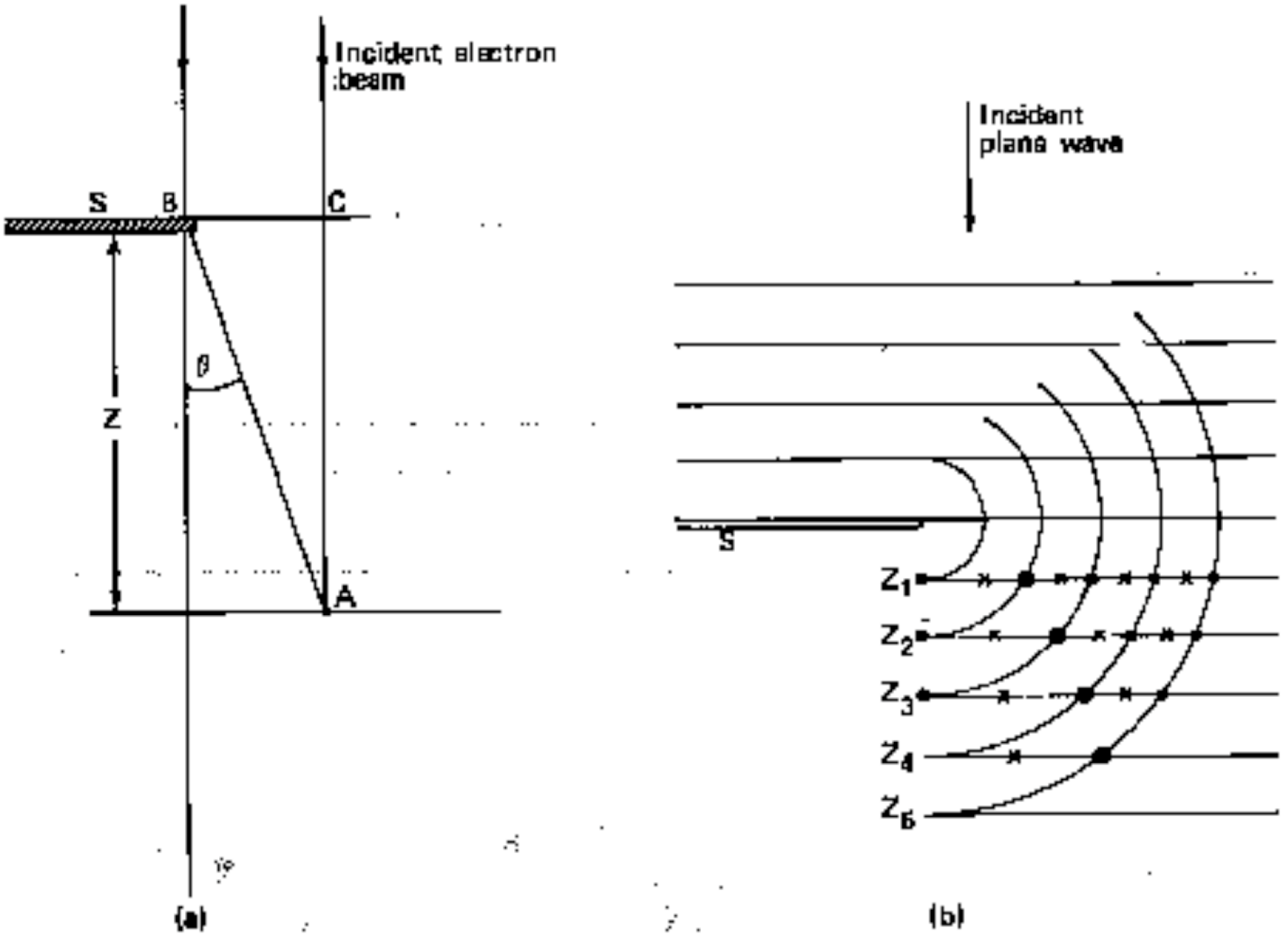
Defocusing of objective lens:

- Causes path lengths of scattered rays to change more than for the unscattered rays
- Enhances contrast due to interference effects

Contrast due to phase differences are important for **thin** objects and when working near the **resolution limit**

I.C.2 Amplitude/Phase Contrast

I.C.2.b Interference (Phase) Contrast



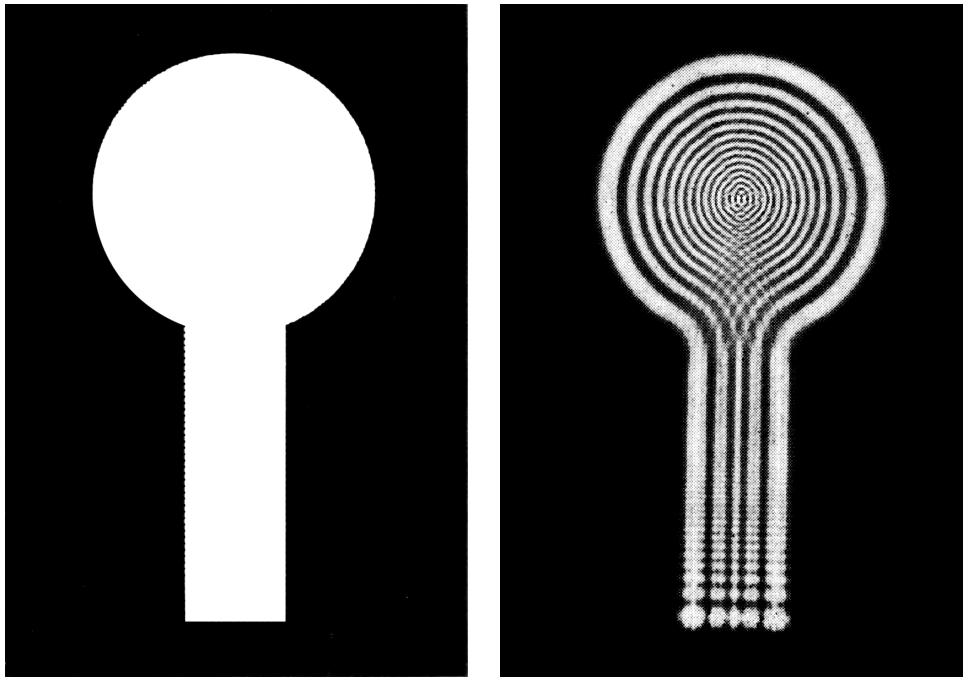
(From Agar, Fig. 3.8, p. 97)

I.C.2 Amplitude/Phase Contrast

I.C.2.b Interference (Phase) Contrast

Defocus or **phase contrast** appears as **Fresnel fringes** in the specimen

Fringes especially noticeable wherever there are **sharp changes in mass thickness**



From Young., Fig. 3-24, p.95

I.C.2 Amplitude/Phase Contrast

I.C.2.b Interference (Phase) Contrast

Fresnel fringes **disappear** at "exact" ("near" or "true") focus because phase differences are at a minimum

Practical tip:

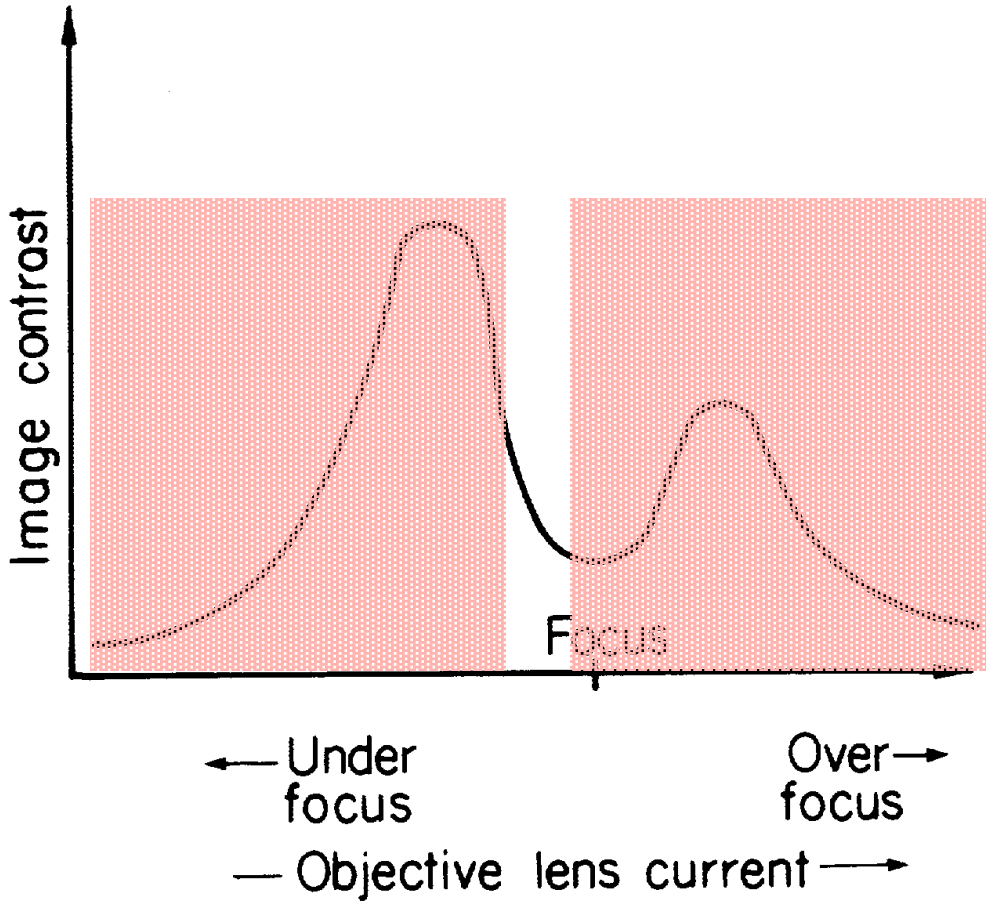
Common practice to **defocus** (underfocus) **slightly** to ↑ image interference contrast

At slight defocus, **resolution** is **not significantly reduced**

I.C.2 Amplitude/Phase Contrast

I.C.2.b Interference (Phase) Contrast

Variation of Image Contrast with Objective Lens Focus



(From Meek, 2nd ed., Fig. 5.3, p.100)

I.C.3 Phase Contrast Transfer Function

Unfortunately, electron images do **not** give a **direct or completely faithful** rendering of the specimen density distribution

- Relationship between image and specimen is described by the contrast transfer function (**CTF**) which is characteristic of:

  Particular microscope used

  Specimen

  Conditions of imaging

- Microscope CTF arises from the **objective lens focal setting** and from the **spherical aberration** present in all electromagnetic lenses

- CTF varies with the **defocus** and **accelerating voltage** according to a formula that includes both phase and amplitude contrast components

I.C.3 Phase Contrast Transfer Function

Dependence of CTF on **resolution**, **wavelength**, **defocus** and **spherical aberration** is given by:

$$CTF(\nu) = -\left\{\left(1 - F_{amp}{}^2\right)^{1/2} \bullet \sin\left(\chi(\nu)\right) + F_{amp} \bullet \cos\left(\chi(\nu)\right)\right\}$$

where

$$\chi(\nu) = \pi\lambda\nu^2\left(\Delta f - 0.5C_s\lambda^2\nu^2\right)$$

ν = spatial frequency (in $Å^{-1}$)

F_{amp} = fraction of amplitude contrast

λ = electron wavelength (in Å), where $\lambda = 12.3/\sqrt{V + 0.000000978 \cdot V^2}$

(= 0.037, 0.025, and 0.020Å for 100, 200, and 300 keV electrons, respectively)

V = voltage (in volts)

Δf = underfocus (in Å)

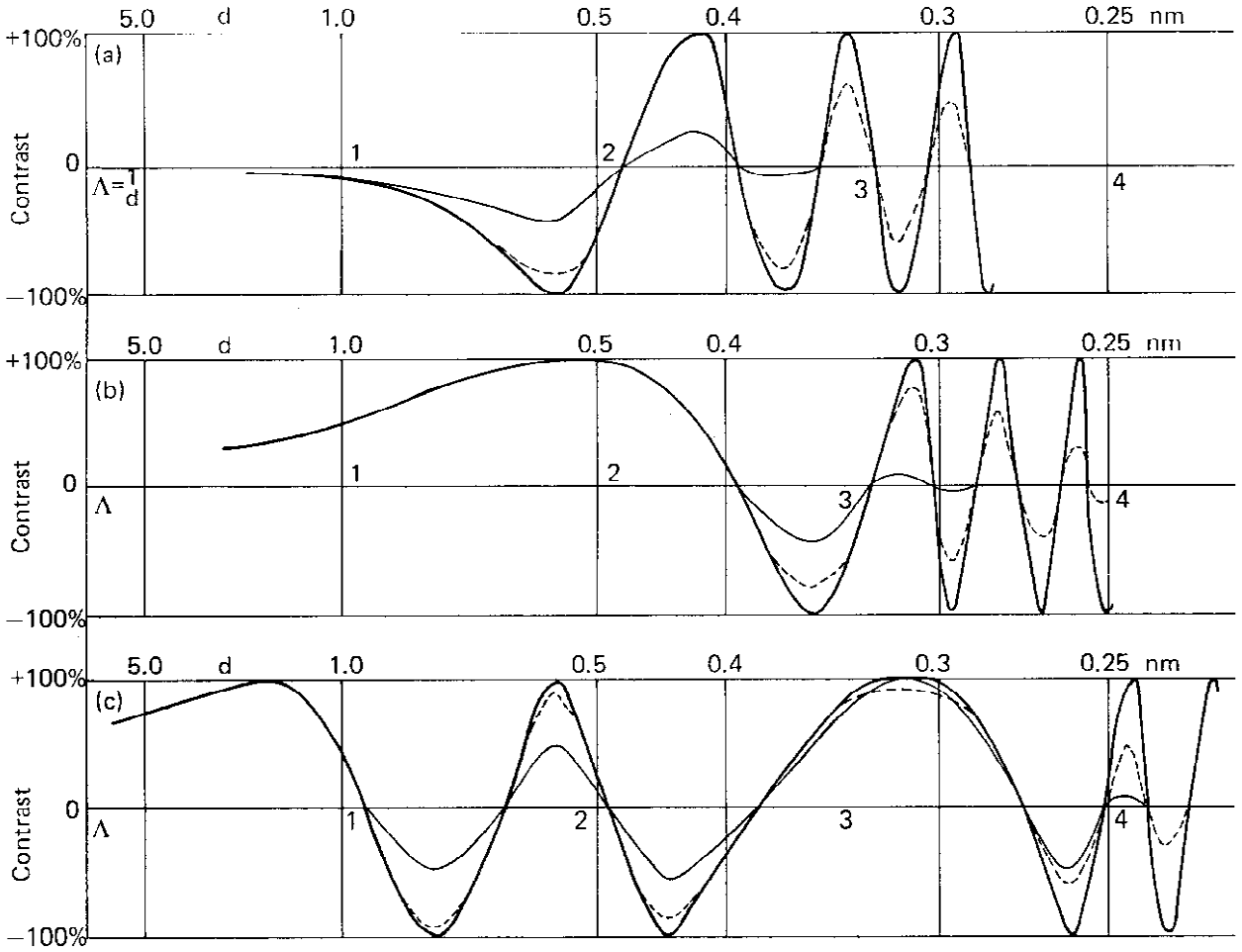
C_s = spherical aberration of objective lens of microscope (in Å)

I.C.3 Phase Contrast Transfer Function

Δf = 0 nm

Δf = -78 nm

Δf = -234 nm



Plot of phase contrast as a function of structure size. (a) Objective lens in focus. (b) Objective lens 78 nm underfocus. (c) Objective lens 234 nm underfocus

(Agar, p. 282)

I.C.3 Phase Contrast Transfer Function

FEAR NOT!!!

Though the importance of the CTF is unquestioned if one wishes to fully understand image formation in a TEM, a full discussion of the CTF is beyond the scope of an introductory course.

For **BIO595R**, you are **NOT** required to understand the CTF in any detail (and won't be quizzed on it!)

For those ***fearless*** people who enjoy the challenge, the full flavored nature of the CTF will be dissected in more detail in **BIO595W …** and you will learn how images can be manipulated to reduce CTF effects and get a more accurate view of specimen features

I.C.5 Other Methods for Enhancing Contrast

- Directly **increase specimen contrast** using various preparation procedures (*i.e.* staining, shadowing, etc.)

- Use **longer exposure time** for recording the photographic image

To be discussed in future lectures

End of Sec.I.C