I.A.1 Brief History of the Transmission Electron Microscope

DATE	NAME	EVENT
1897	J. J. Thompson	Discovers the electron
1924	Louis deBroglie	Identifies a wavelength to moving electrons
		$\lambda = h/mv$
		where λ = wavelength
		h = Planck's constant
		m = mass
		v = velocity
		(For an electron at 60kV λ = 0.005 nm)
1926	H. Busch	Magnetic or electric fields act as lenses for electrons
1929	E. Ruska	Ph. D thesis on magnetic lenses
1931	Knoll & Ruska	First electron microscope built
1931	Davisson & Calbrick	Properties of electrostatic lenses
1934	Driest & Muller	Surpass resolution of the LM
1938	von Borries & Ruska	First practical EM (Siemens) - 10 nm resolution
1940	RCA	Commercial EM with 2.4 nm resolution
1945		1.0 nm resolution

BIO595R / BMS517C - Spring 2004

Concepts, Concepts, Concepts

- TEM is not simply “knob twiddling”

- To be “good”, need to understand basic principles

- TEM relies on **many** basic principles (mostly physics)

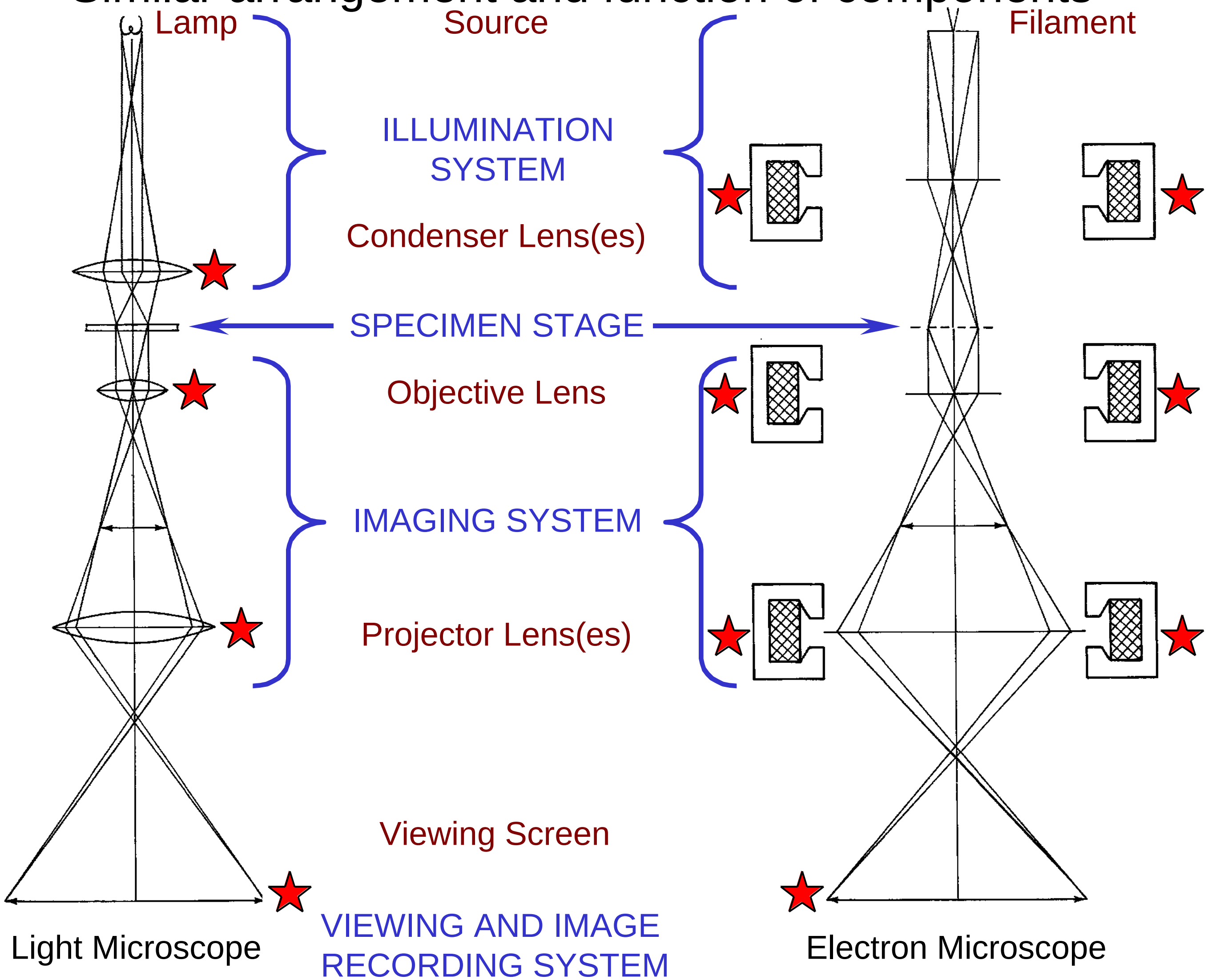
- Some concepts may not make much sense at first
  So, HANG IN THERE!

- Goal: become proficient microscopists (or judge work of others)

Concept #1: electrons and photons have much in common

I.A.2 Comparison of Light and Electron Microscope

Similar arrangement and function of components



From Agar, Fig. 1.6, p.8

I.A.2 Comparison of Light and Electron Microscope

SIMILARITIES

Similar arrangement and function of components

ILLUMINATION SYSTEM: Radiation source & condenser lens

- Source produces illumination beam
- Condenser focuses beam on specimen

SPECIMEN STAGE:

- Hold specimen between illumination & imaging systems

IMAGING SYSTEM: Objective and projector lenses

- Objective produces first (intermediate) image
- Projector(s) magnifies a portion of the intermediate image to form final image

IMAGE RECORDING SYSTEM: Photographic emulsion or CCD camera

- Converts radiation into a permanent image

I.A.2 Comparison of Light and Electron Microscope

DIFFERENCES

Light Microscope

- Optical lenses

  Glass; **fixed** focal length

- Magnification changes

  Switch objective lens or ocular (eyepiece)

- Depth of field small

  Different focal levels in specimen

- Mechanism of image formation

  Mainly **amplitude** (scattering) contrast

Electron Microscope

- Magnetic lenses

  Ferromagnetic materials & windings of copper wire

  Variable focal length (vary current.)

- Magnification changes

  Objective lens focal length 'fixed'
  Projector focal length varied

- Depth of field large

  Entire (thin) specimen is **in focus**

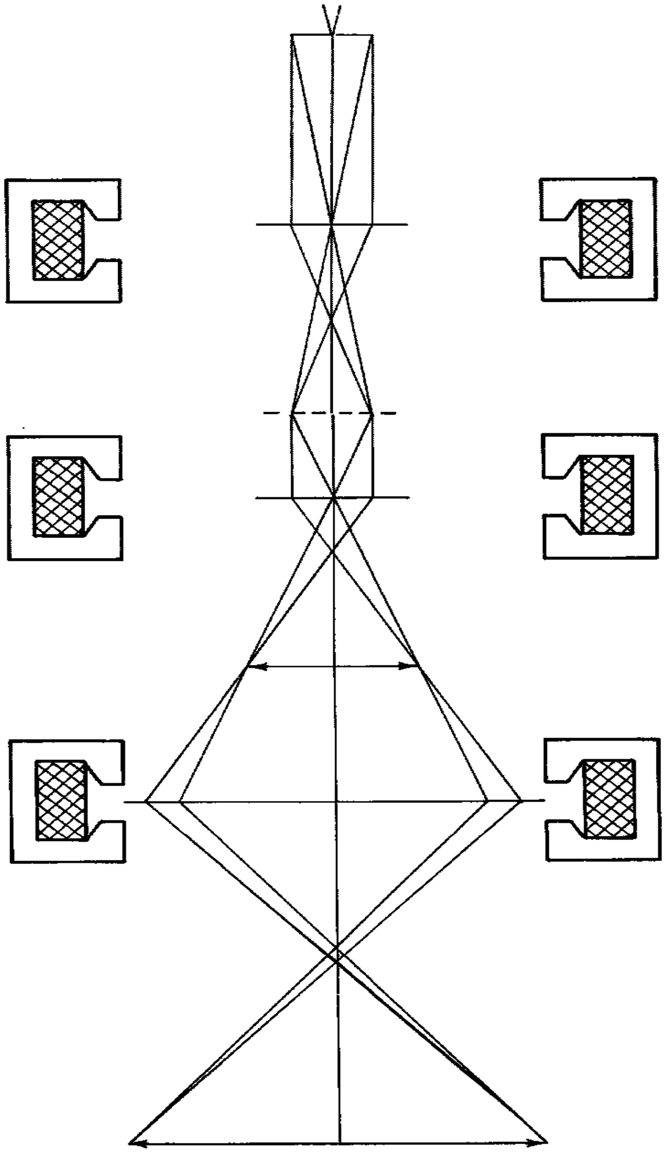
- Mechanism of image formation

  Mainly **phase** (interference) contrast

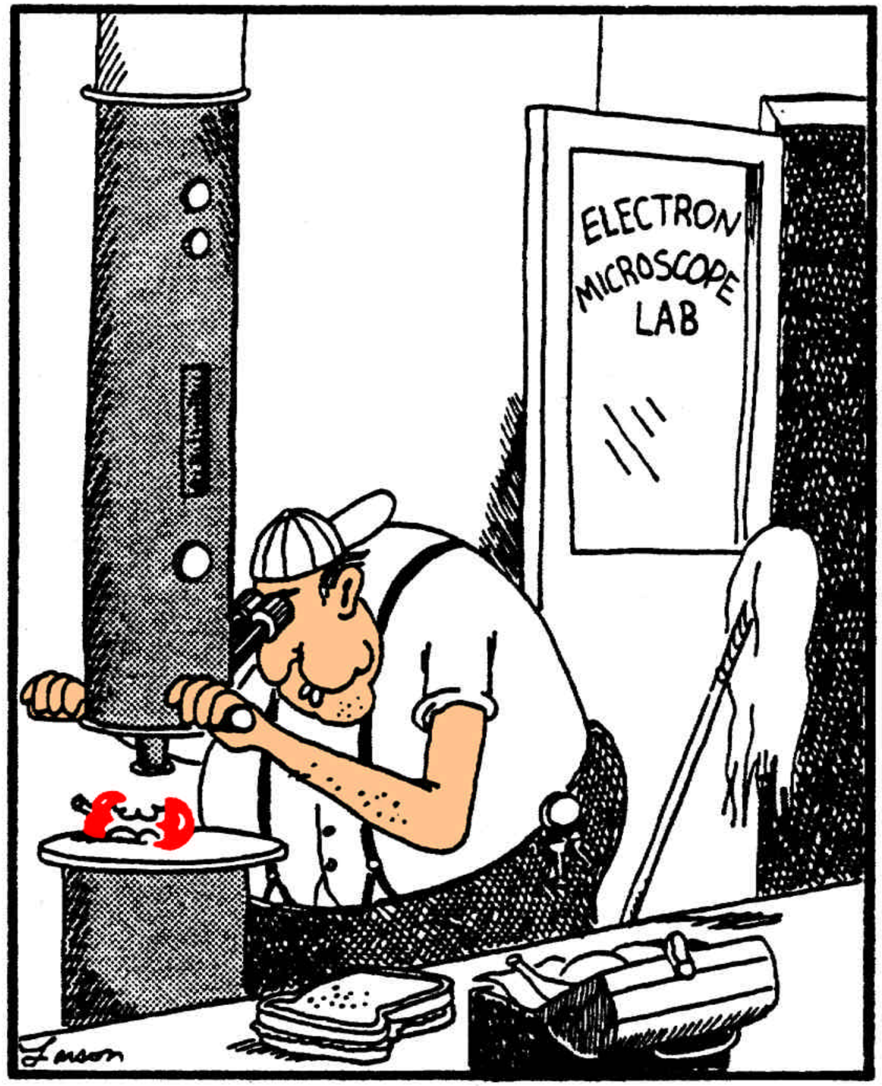
I.A.2 Comparison of Light and Electron Microscope

MORE DIFFERENCES

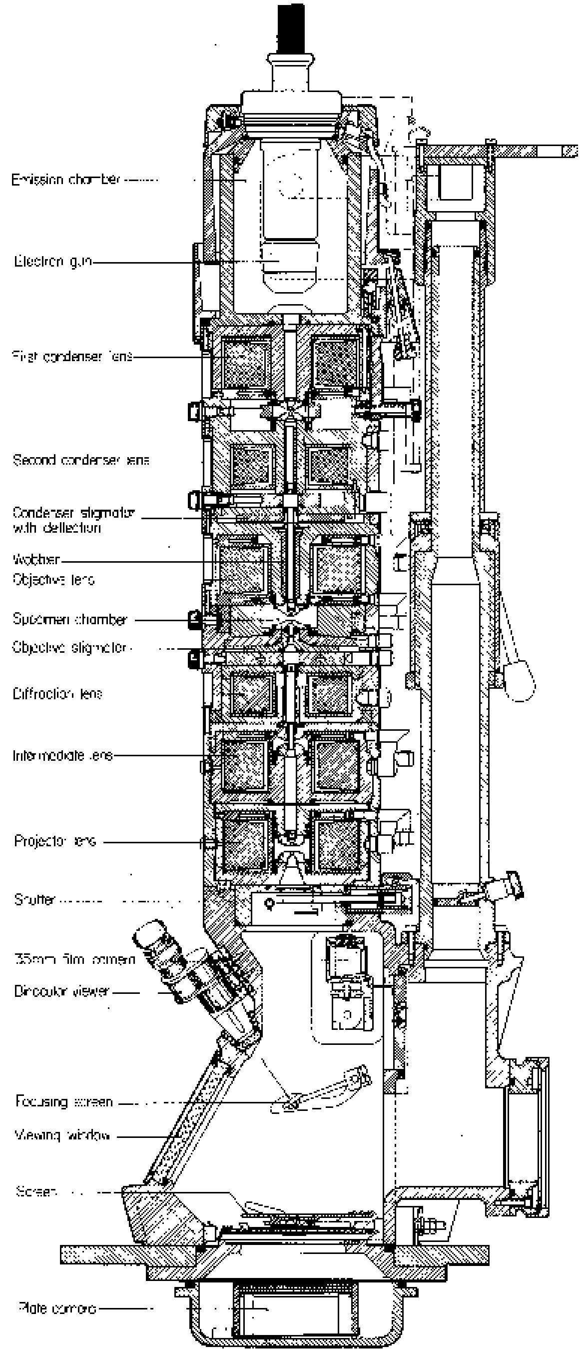
Light Microscope	Electron Microscope
- Specimen Environment -	
Nothing unusual	**High vacuum** Specimen *usually* **dehydrated** (dead!)
- Beam Effects -	
None	Biological specimens **rapidly damaged**
- Magnification/Resolution -	
~1000X or less	~ 10,000 to 100,000X or more
~0.1 µm or worse	~ 0.3 nm (0.003 µm) or better
- Orientation of Components -	
Radiation source generally at **bottom**	Radiation source at **top**
- Price Tag -	
$1000s (not confocal)	$400,000 - $2x10^6$ or more!!!

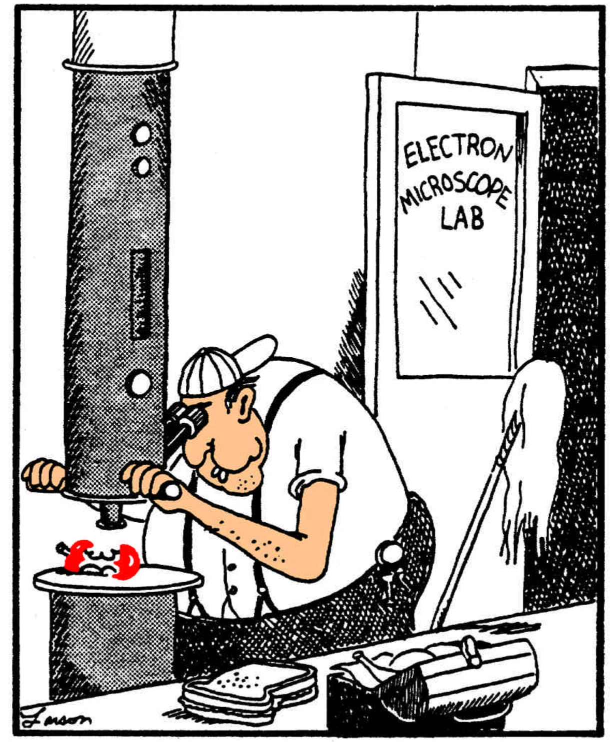


Electron Microscope



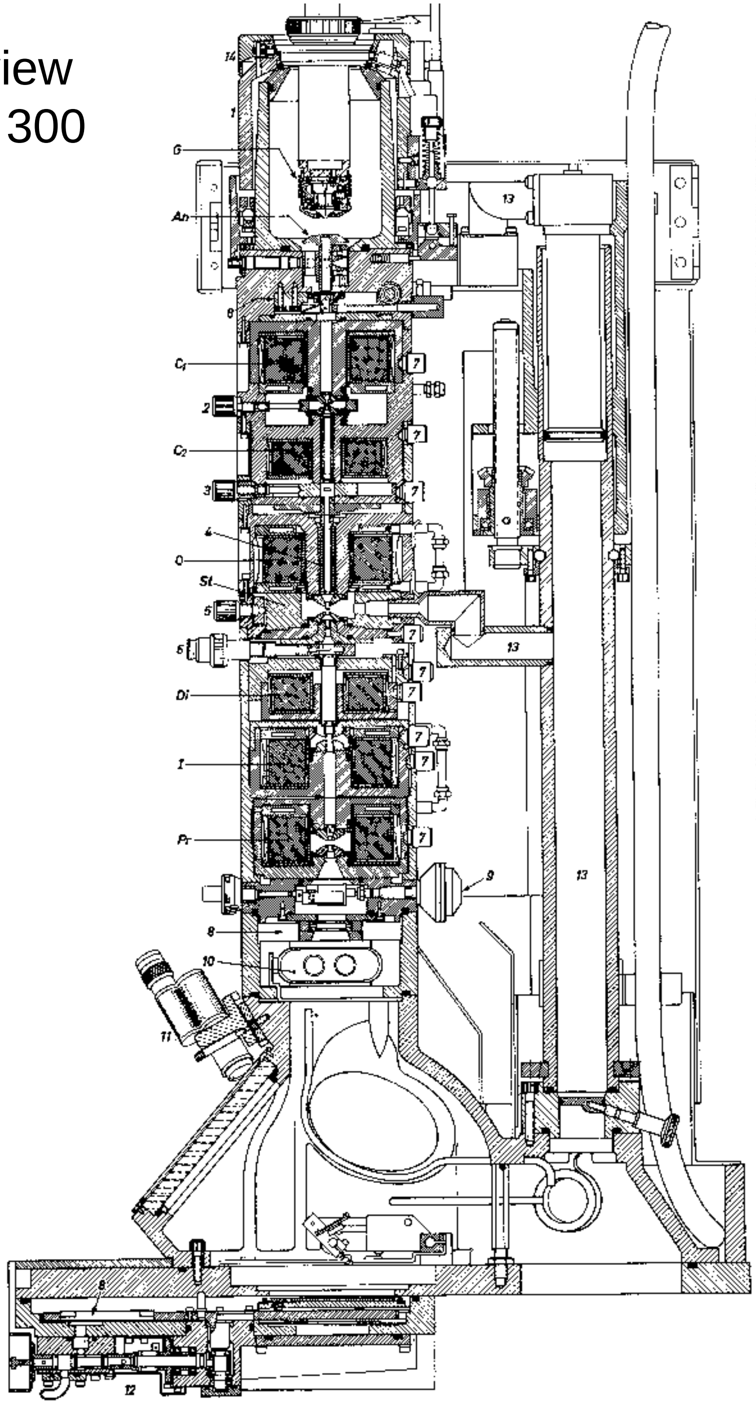
Cross-sectional view of the Philips EM 200

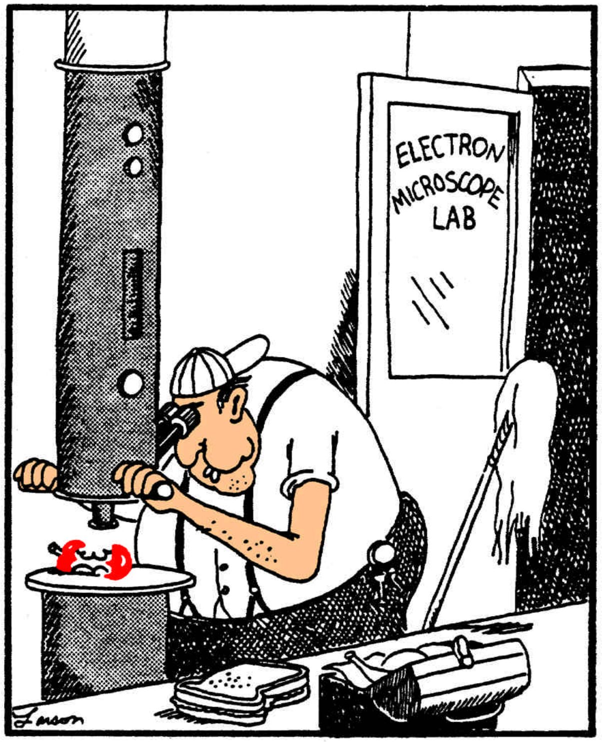




From Meek, Fig. 5.4b, p.99

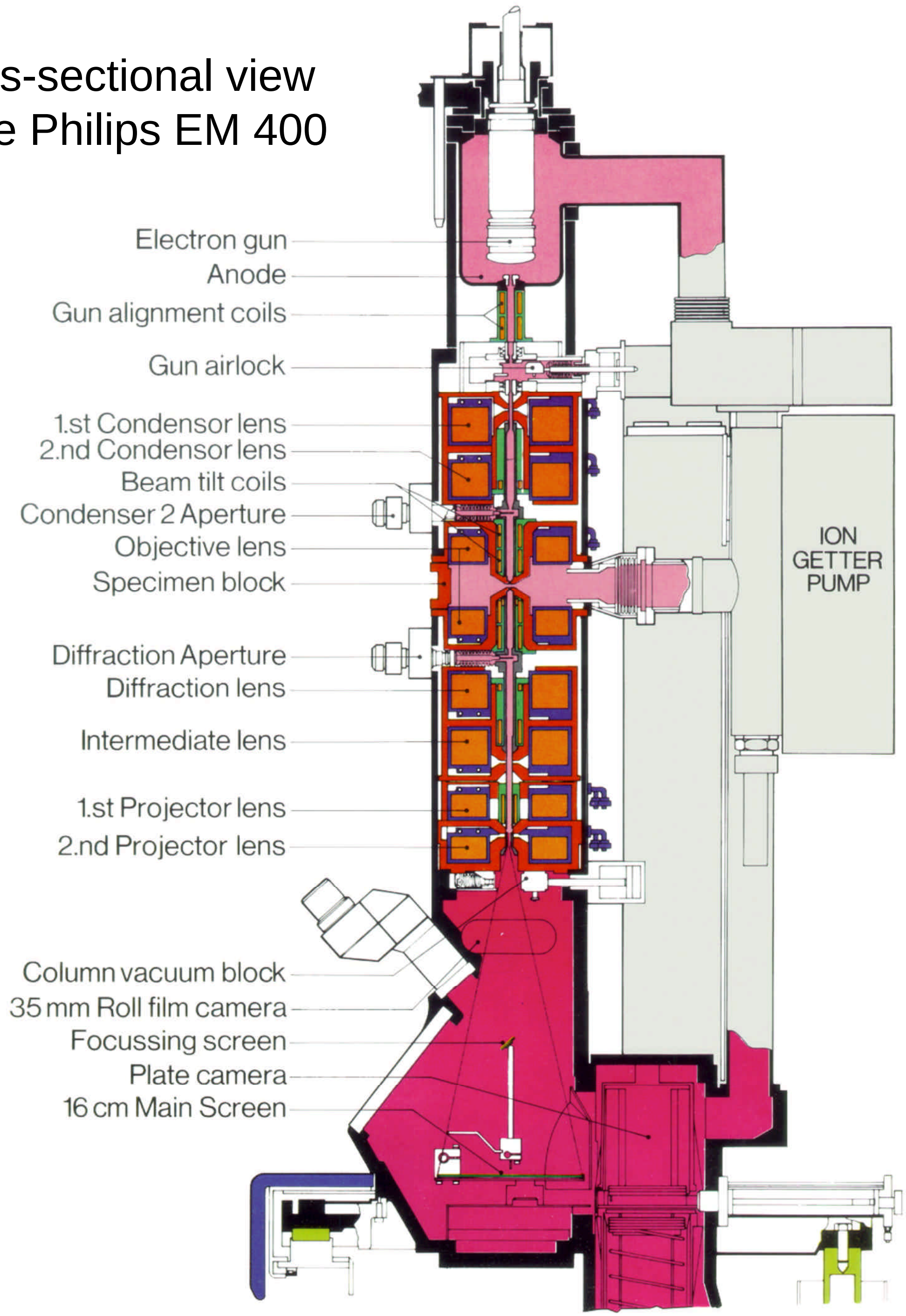
Cross-sectional view
of the Philips EM 300

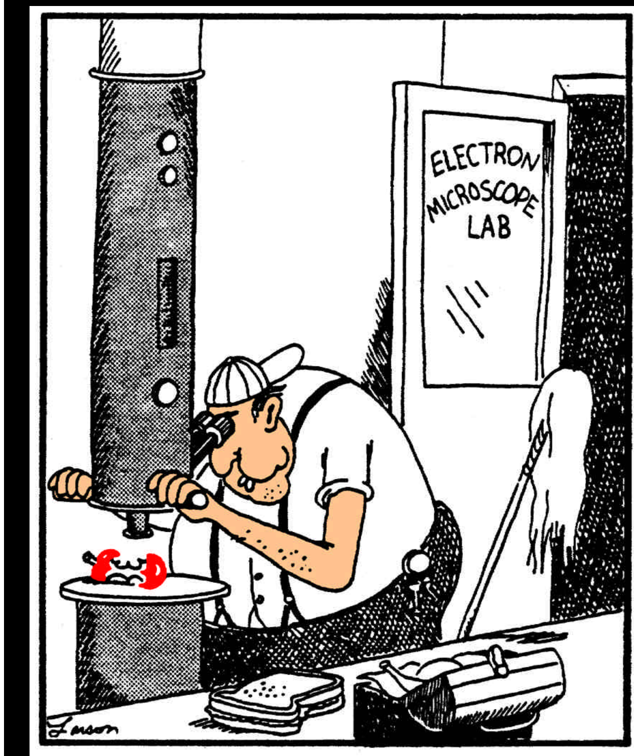


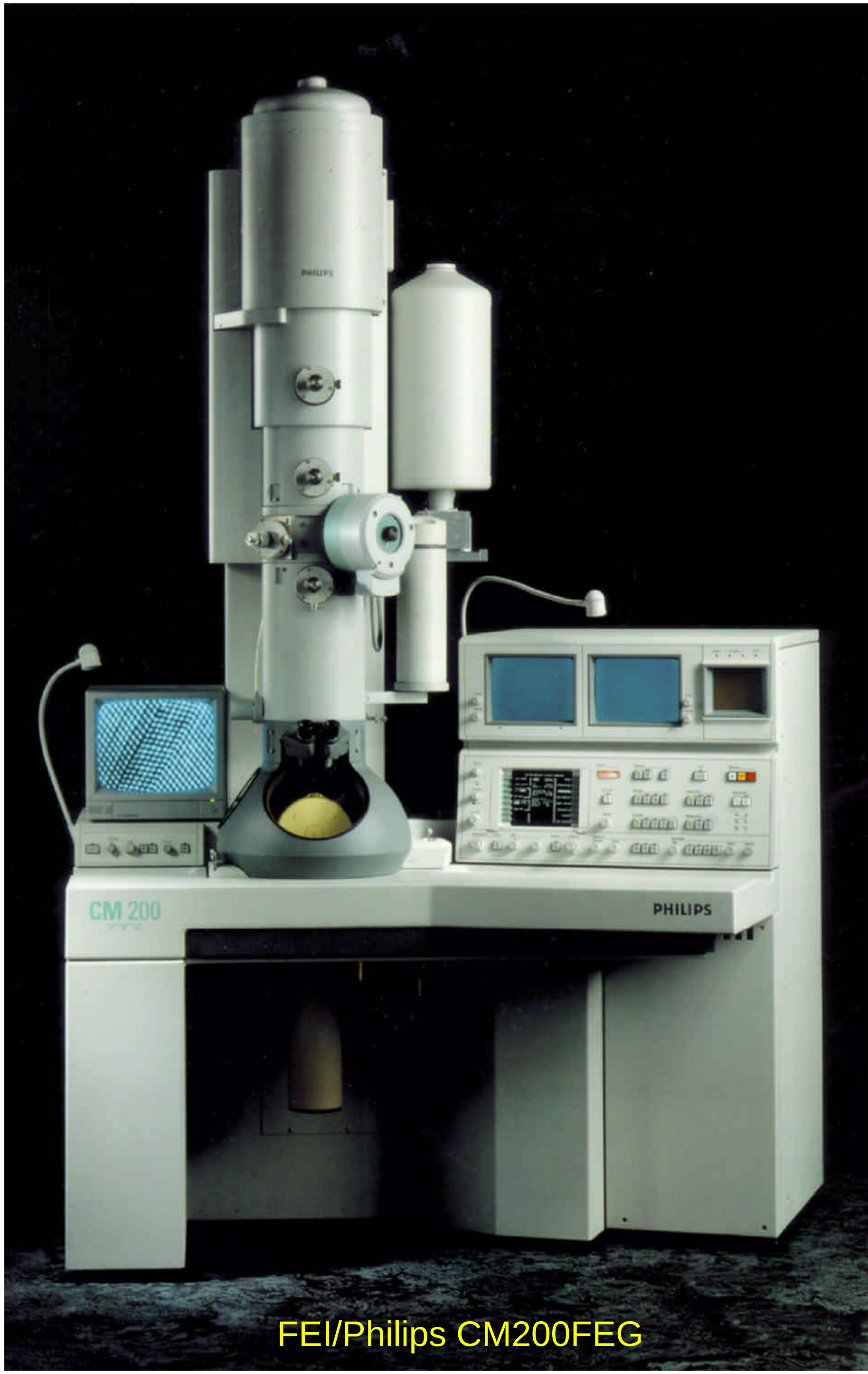


From Agar, Fig. 2.2, p.40

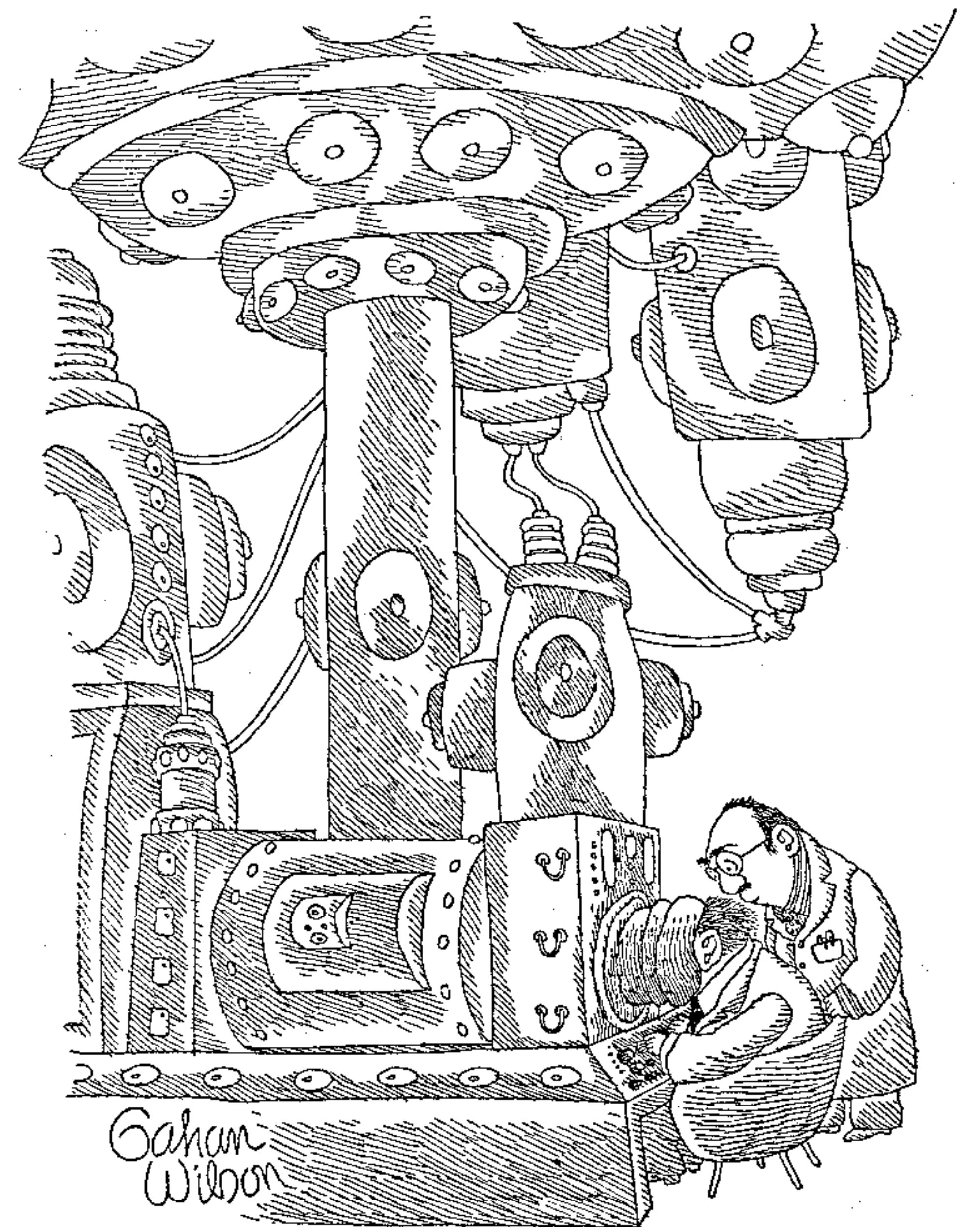
Cross-sectional view of the Philips EM 400







FEI/Philips CM200FEG



"I think I may have spotted something!"

I.A.3 Photons/Electrons

Key Concepts (lots of them!)

- Photons and electrons behave as particles **AND** waves
- **Any** moving particle has a **wavelength** associated with it
- TEM: electrons travel **very fast** (near speed of light)
- TEM: electrons have **very short** wavelengths
- **Diffraction:** path of radiation bent by ‘obstacles’
- **Interference:** combination of diffracted and undiffracted waves
- **Resolution:** ability to distinguish objects or object details
- **Instrument resolution:** limited by wavelength of radiation

I.A.3 Photons/Electrons

I.A.3.a Dual Concept of Wave and Particle

Light has both particle **and** wave properties

Dual nature explains results of various physical experiments

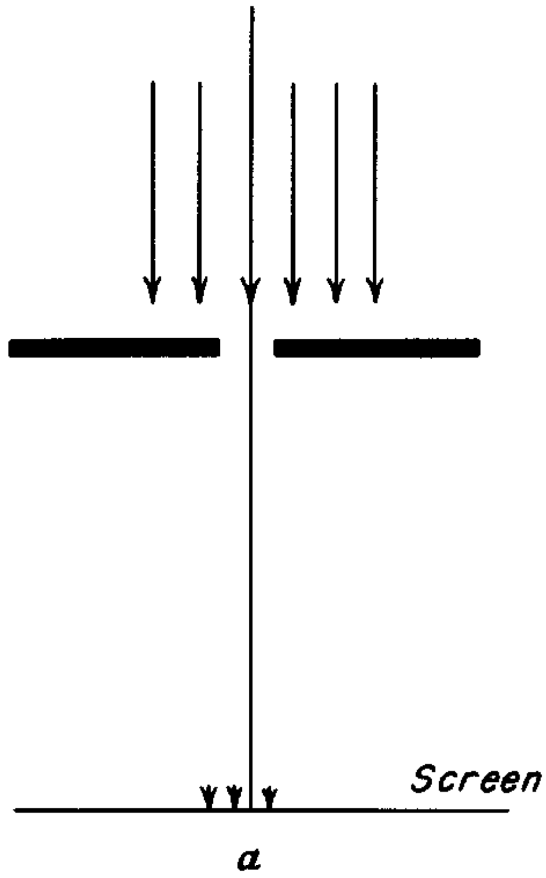
Electrons also exhibit particle **and** wave properties

Diffraction of light and electrons illustrates their wave nature

Diffraction refers to the bending of the path of radiation around ‘obstacles’

I.A.3 Photons/Electrons

I.A.3.a Dual Concept of Wave and Particle

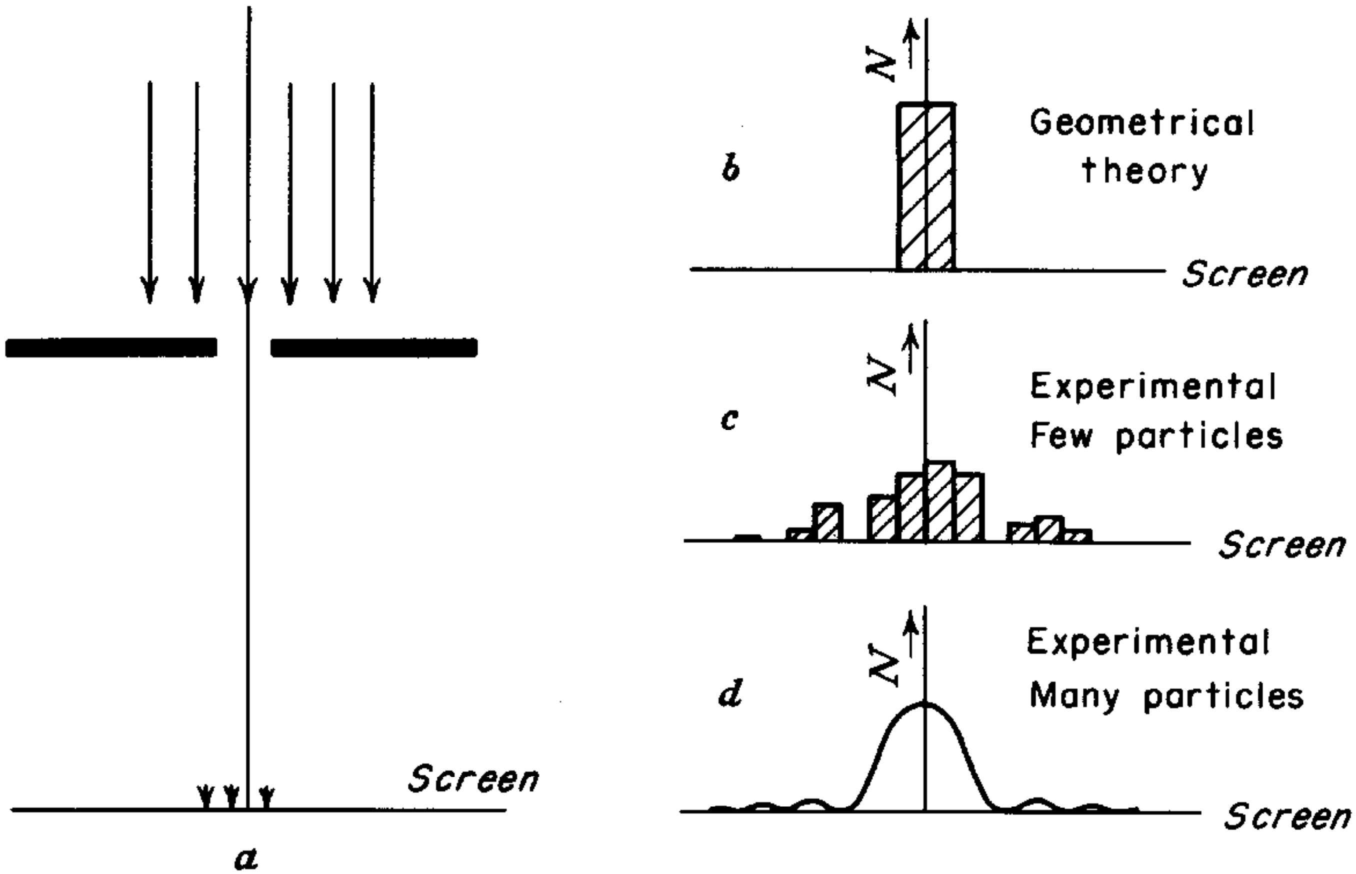


From Hall, Fig. 1.8, p.13

I.A.3 Photons/Electrons

I.A.3.a Dual Concept of Wave and Particle

Statistical Nature of Diffraction Patterns



From Hall, Fig. 1.8, p.13

I.A.3 Photons/Electrons

I.A.3.b Electron Velocity and Wavelength

DeBroglie (1924):

A particle of mass, *m*, moving at a velocity, v, has a wavelength (λ) given by:

$$\lambda = \frac{h}{mv}$$

DeBroglie wave equation

(h = Planck's constant)

Wavelength decreases as velocity increases

I.A.3 Photons/Electrons

I.A.3.b Electron Velocity and Wavelength

Electron charge = *e* (1.6 x 10^{-19} coulomb)

Electron mass = *m* (9.11 x 10^{-28} gm)

Electron passing through a potential difference of *V* volts (expressed in joules/coulomb), has a kinetic energy:

$$\frac{1}{2}mv^2 = eV$$

I.A.3 Photons/Electrons

I.A.3.b Electron Velocity and Wavelength

Kinetic energy of moving electron: $\frac{1}{2}mv^2 = eV$

Rearrange this equation to get velocity, v, of electron:

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

Velocity increases as accelerating voltage increases

I.A.3 Photons/Electrons

I.A.3.a Dual Concept of Wave and Particle

To find the relation between λ and V, substitute for v from last equation into the DeBroglie equation:

i.e. take *v* from $v = \sqrt{\frac{2eV}{m}}$ and plug into: $\lambda = \frac{h}{mv}$

to get:

$$\downarrow \lambda = \left(\frac{h}{m}\right)\frac{1}{\sqrt{\frac{2eV}{m}}} = \sqrt{\frac{h^2}{2meV}} \uparrow$$

Wavelength decreases as accelerating voltage increases

I.A.3 Photons/Electrons

I.A.3.a Dual Concept of Wave and Particle

Starting with: $$\lambda = \sqrt{\frac{h^2}{2meV}}$$

Substitute appropriate values for *h, m,* and *e*:

$$\lambda = \sqrt{\frac{150}{V}}\ 10^{-8}\,cm = \frac{1.23}{\sqrt{V}}\,nm$$

Example: if V = 60,000 volts, λ = 0.005 nm

I.A.3 Photons/Electrons

I.A.3.b Electron Velocity and Wavelength

At high voltage, electron velocity is comparable to the speed of light in a vacuum (c = 3 x 10^{10} cm/sec)

V	λ (nm)	v (x10^{-10} cm/sec)	v/c
10,000	0.0123	0.593	0.198
50,000			
100,000			
1,000,000			

I.A.3 Photons/Electrons

I.A.3.b Electron Velocity and Wavelength

At high voltage, electron velocity is comparable to the speed of light in a vacuum ($c = 3 \times 10^{10}$ cm/sec)

V	λ (nm)	v ($\times 10^{-10}$ cm/sec)	v/c
10,000	0.0123	0.593	0.198
50,000	0.0055	1.326	0.442
100,000			
1,000,000			

I.A.3 Photons/Electrons

I.A.3.b Electron Velocity and Wavelength

At high voltage, electron velocity is comparable to the speed of light in a vacuum ($c = 3 \times 10^{10}$ cm/sec)

V	λ (nm)	*v* ($\times 10^{-10}$ cm/sec)	*v*/*c*
10,000	0.0123	0.593	0.198
50,000	0.0055	1.326	0.442
100,000	0.0039	1.875	0.625
1,000,000			

I.A.3 Photons/Electrons

I.A.3.b Electron Velocity and Wavelength

At high voltage, electron velocity is comparable to the speed of light in a vacuum (c = 3 x 10^{10} cm/sec)

V	λ (nm)	*v* (x10^{-10} cm/sec)	*v/c*
10,000	0.0123	0.593	0.198
50,000	0.0055	1.326	0.442
100,000	0.0039	1.875	0.625
1,000,000	0.0012	5.930	**1.977!**

I.A.3 Photons/Electrons

I.A.3.b Electron Velocity and Wavelength

Equation breaks down when the electron velocity approaches *c*.

Relativistic correction must be made for the value of the mass:

$$m_1 = \frac{m_0}{\sqrt{1 - \frac{v^2}{c^2}}}$$

Relation between λ and ***V*** more correctly given by:

$$\lambda = \frac{1.23}{\sqrt{V + 10^{-6} V^2}}\ nm$$

I.A.3 Photons/Electrons

I.A.3.b Electron Velocity and Wavelength

With relativity effects **not** included:

V	λ (nm)	*v* (x10^{-10} cm/sec)	*v/c*
10,000	0.0123	0.593	0.198
50,000	0.0055	1.326	0.442
100,000	0.0039	1.875	0.625
1,000,000	0.0012	5.930	**1.977!**

I.A.3 Photons/Electrons

I.A.3.b Electron Velocity and Wavelength

With relativity effects included:

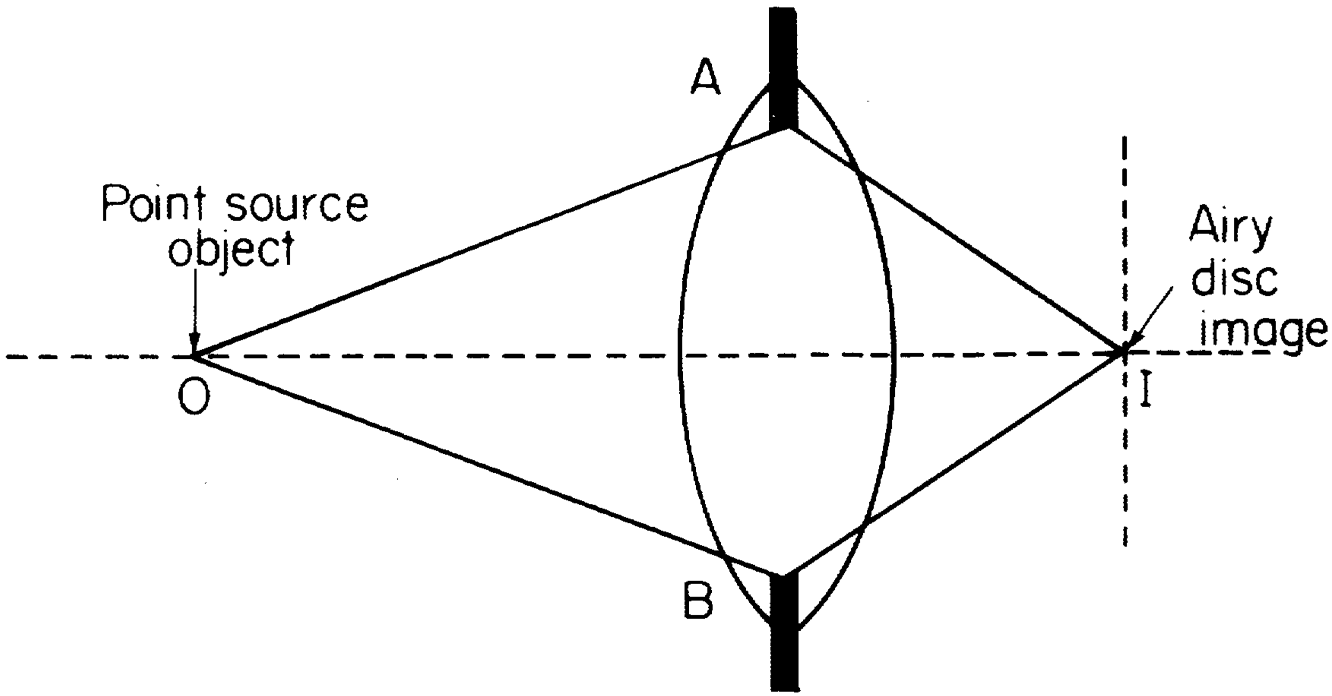
V	λ (nm)	v (x10^{-10} cm/sec)	v/c
10,000	0.0122	0.585	0.195
50,000	0.0054	1.237	0.414
100,000	0.0037	1.644	0.548
1,000,000	0.0009	2.822	**0.941**

I.A.3 Photons/Electrons

I.A.3.c Interference / Diffraction / Coherence

IDEAL LENS: takes each object point and represents it exactly as a point in the image.

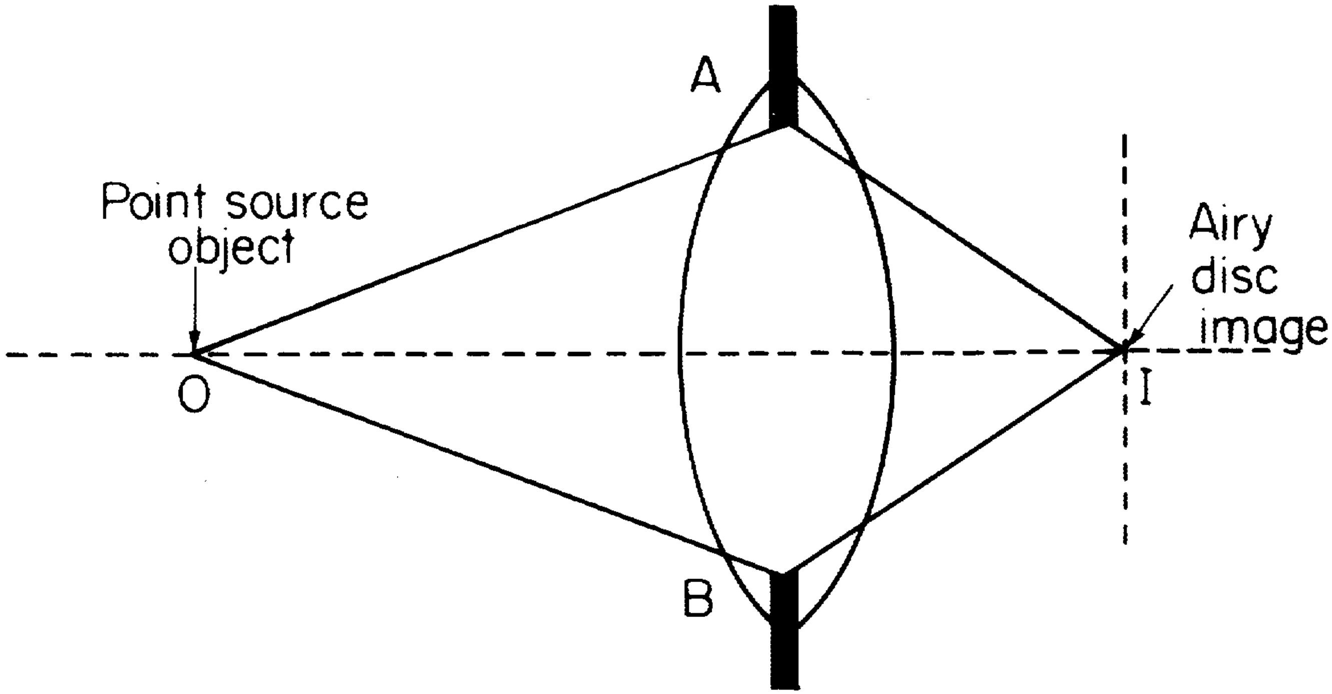
REAL LENS: takes each object point and spreads it out into a circular disk (**Airy disk**) in the image plane.

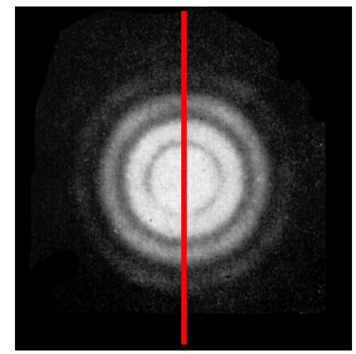


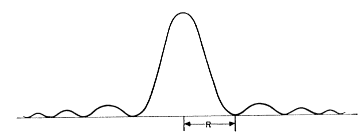
From Meek, 1st ed., Fig. 1.22, p.35

I.A.3 Photons/Electrons

I.A.3.c Interference / Diffraction / Coherence







Diameter of Airy disk depends on the lens angular aperture.

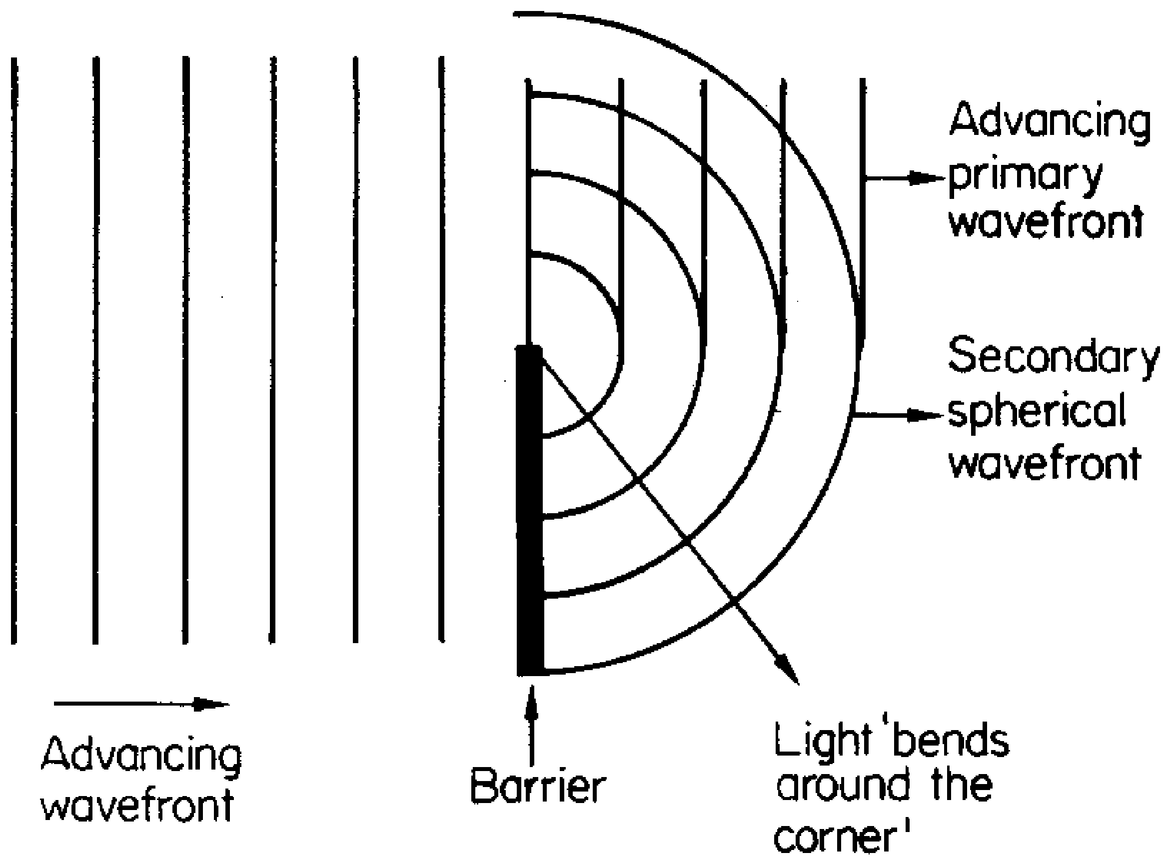
Airy disk image is caused by diffraction from the aperture.

From Meek, 1st ed., Fig. 1.22, p.35
and Sjostrand, Fig. IV.18, p.115

I.A.3 Photons/Electrons

I.A.3.c Interference / Diffraction / Coherence

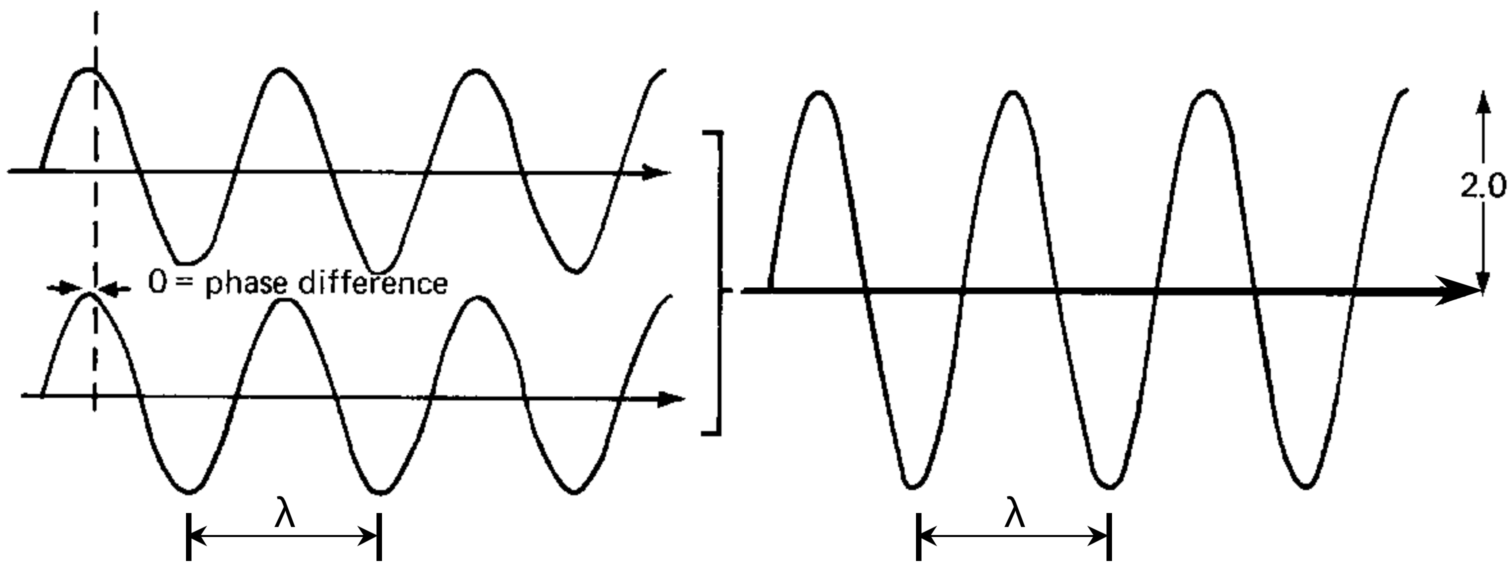
Diffraction phenomena: bending of the path of radiation passing close to an obstacle.



From Meek, 1st ed., Fig. 1.14, p.22

I.A.3 Photons/Electrons

I.A.3.c Interference / Diffraction / Coherence

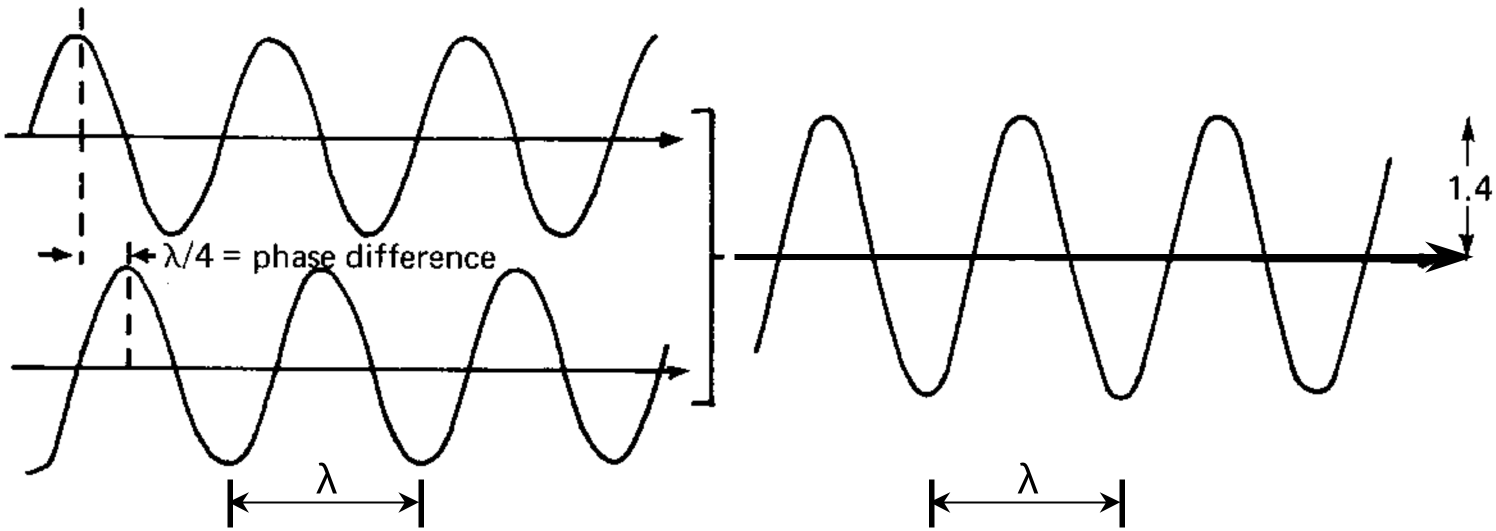


Total constructive interference
“In phase”

From Glusker and Trueblood., Fig. 5, p.19

I.A.3 Photons/Electrons

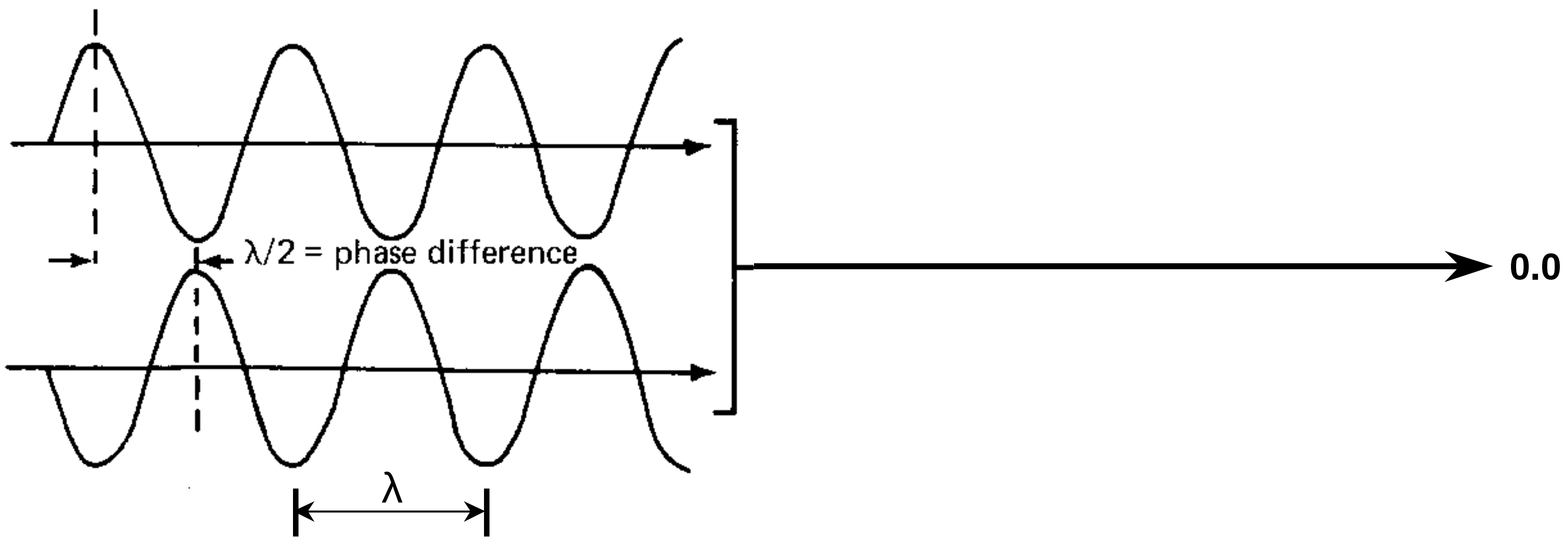
I.A.3.c Interference / Diffraction / Coherence



From Glusker and Trueblood., Fig. 5, p.19

I.A.3 Photons/Electrons

I.A.3.c Interference / Diffraction / Coherence



Total destructive interference
"Out of phase"

From Glusker and Trueblood., Fig. 5, p.19

BIO595R / BMS517C - Spring 2004

Introduction to Transmission Electron Microscopy

See you on Thursday!

I.A PRINCIPLES OF TRANSMISSION EM

Key Concepts from last class:

- LM and TEM have similar arrangement and function of components
- Photons and electrons exhibit properties of particles AND waves
- A moving particle has a wavelength associated with it
- In TEM, electrons travel *very* fast and have *very* short wavelengths
- A real lens images each object point as an Airy disk in the image plane
- Diffraction occurs when radiation encounters and is bent by ‘obstacles’
- Interference occurs when diffracted and undiffracted waves combine

I.A PRINCIPLES OF TRANSMISSION EM

Key Concepts for today:

- Coherence**:** property of a beam of radiation that defines the variance in wavelength and phase of the component waves

- Wavelength of radiation used limits the ultimate resolving power of any microscope (and hence the size of object details one can resolve)

- **Rule of thumb: Ultimate** resolving power of any instrument is equal to 1/2 the wavelength of the radiation used for imaging

- **Reality:** Image resolution is always ≤ resolving power of instrument

- The maximum magnification of an instrument is limited

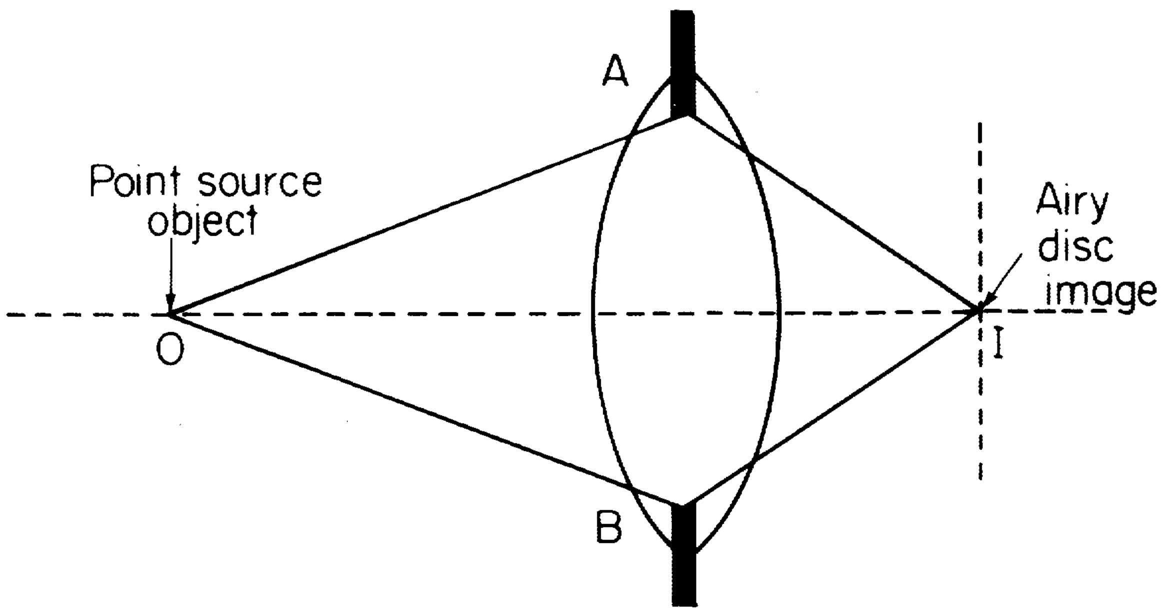
- Electrons "*beat the pants off*" photons at resolving details in objects

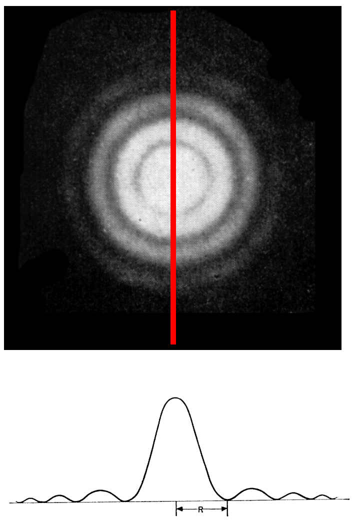
I.A.3 Photons/Electrons

I.A.3.c Interference / Diffraction / Coherence

Diffraction effects limit microscope resolving power

An image point produced by a lens is a diffraction image (Airy image) of the opening of the lens or the aperture.



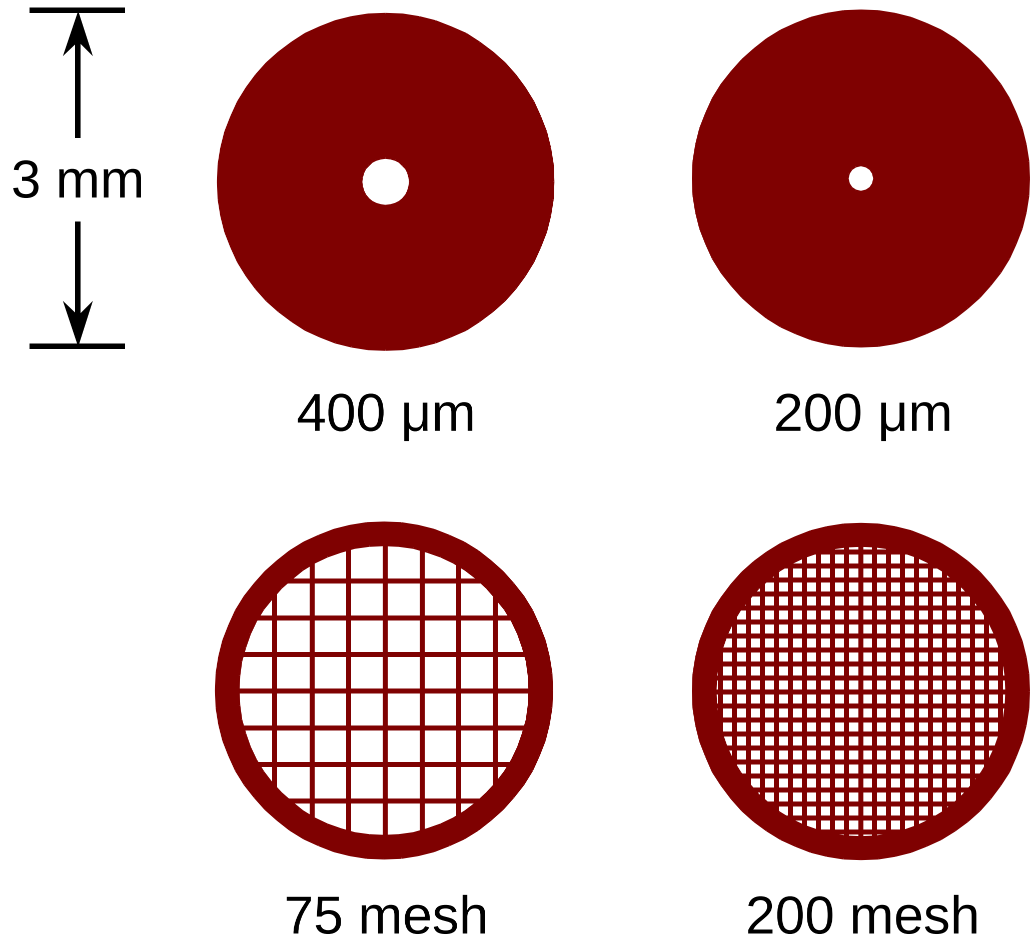


From Meek, 1st ed., Fig. 1.22, p.35
and Sjostrand, Fig. IV.18, p.115

For diffraction demo

I.A.3 Photons/Electrons

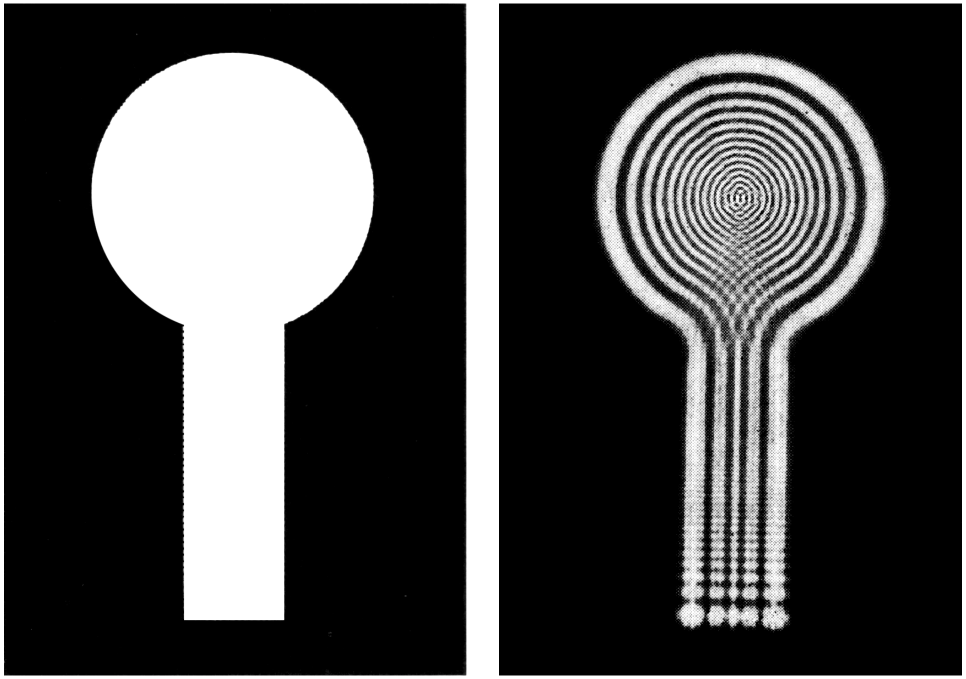
I.A.3.c Interference / Diffraction / Coherence



I.A.3 Photons/Electrons

I.A.3.c Interference / Diffraction / Coherence

Fresnel diffraction pattern (right) formed by an irregularly shaped aperture



Pattern results from interference between non-diffracted light and a wave of light diffracted at the edges

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

I.A.3.c Interference / Diffraction / Coherence

PREREQUISITE FOR INTERFERENCE:

Superposition of wave systems whose **phase difference remains constant in time**

Two beams are **coherent** if, when combined, they produce an interference pattern

Two beams are **incoherent** when they are **incapable** of producing an interference pattern (e.g. two flashlights)

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

I.A.3.d Resolution

1) Definitions

- **RESOLUTION**: ability to distinguish closely spaced points as separate points

- **RESOLUTION LIMIT**: smallest separation of points which can be recognized as distinct

- **RESOLVING POWER**: resolution achieved by a particular instrument under optimum viewing conditions

I.A.3 Photons/Electrons

I.A.3.d Resolution

2) Distinction between resolution and resolving power

Resolving power: Property of the **instrument**

May be estimated on theoretical grounds

Resolution: always ≤ resolving power

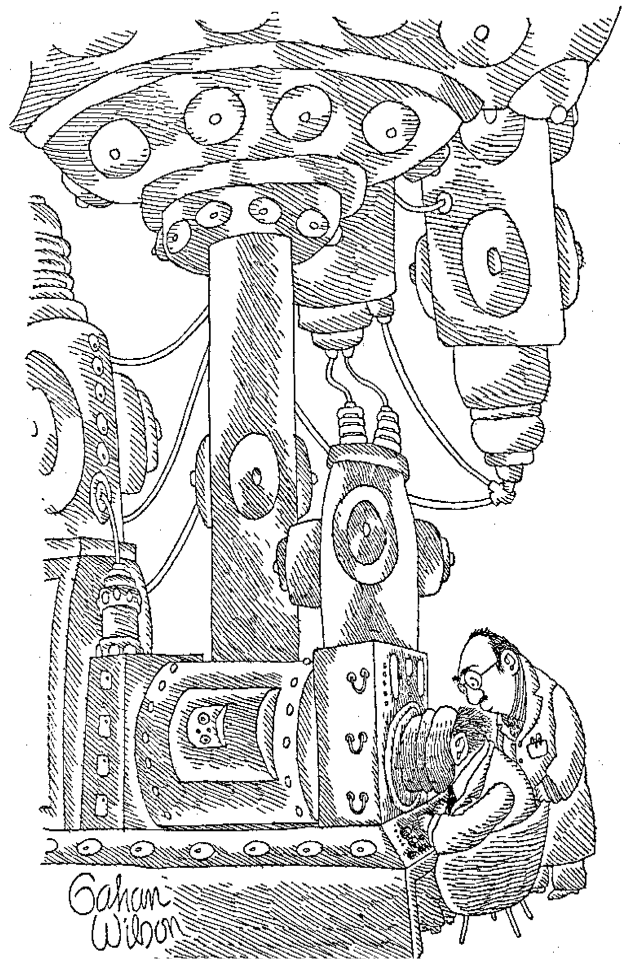
Quantity observed under any given set of experimental conditions

In the TEM (esp. with biological samples), resolution achieved is often **considerably inferior** to the theoretical instrument resolving power

I.A.3 Photons/Electrons

I.A.3.d Resolution

Microscopy: The science of seeing the very small

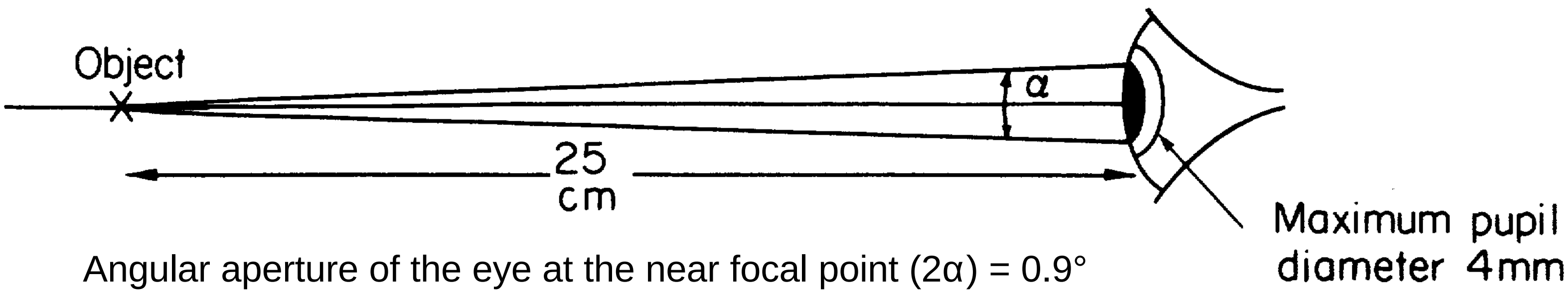


"I think I may have spotted something!"

I.A.3 Photons/Electrons

I.A.3.d Resolution

Microscopy: The science of seeing the very small



Angular aperture of the eye at the near focal point (2α) = 0.9°

Under <u>ideal</u> conditions:

Eye can focus on objects ~ 250mm away

Smallest object or detail we can resolve is about 0.07mm (70µm)

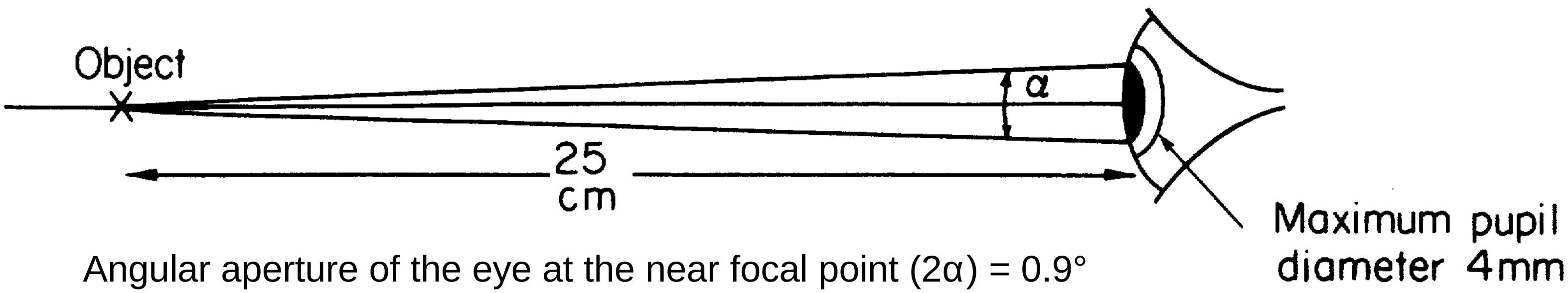
- Limit related to size of receptors in the retina
- Limit related to small angular aperture of eye
- Limit related to how close we can place object to eye

From Meek, 1st ed., Fig. 1.8, p.13

I.A.3 Photons/Electrons

I.A.3.d Resolution

Tennis Ball Analogy (an aside)



Angular aperture of the eye at the near focal point (2α) = 0.9°

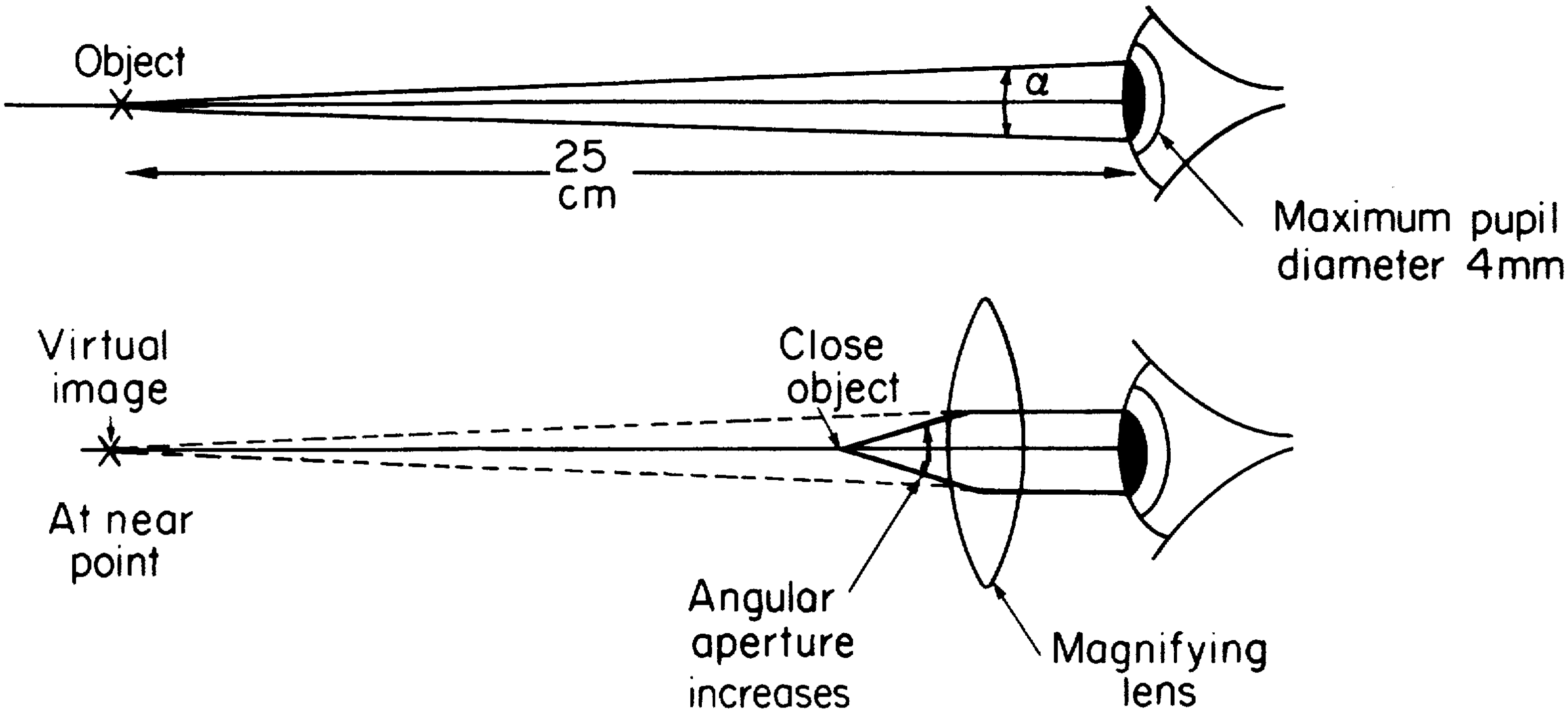
- Eye can resolve 3 cm object at 100 meters
- Hence, a tennis ball is clearly visible (resolvable) at 100 meters

But.........it's not **just** a question of resolution...

From Meek, 1st ed., Fig. 1.8, p.13

I.A.3 Photons/Electrons

I.A.3.d Resolution



A single biconvex lens (a simple microscope):

Allows us to bring objects closer to the eye

Increases the angular aperture of the eye (gather more info)

Magnifies the image falling on the retina

From Meek, 1st ed., Figs. 1.8-1.9, pp.13-14

I.A.3 Photons/Electrons

I.A.3.d Resolution

3) Abbe Simple Criteria of Resolution

- Wave nature of light poses limits on the size of details that can be resolved

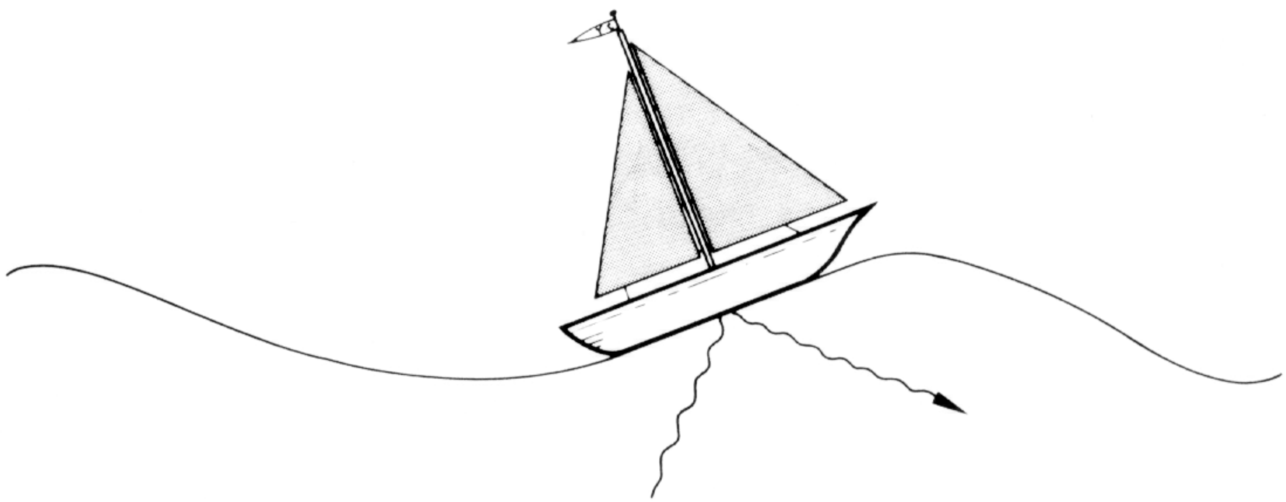
- Smallest resolvable distance is about **1/2 the wavelength** of light used

- **Abbe rule of thumb:** 1/2 the wavelength of the radiation used is the **ultimate** resolving power of **any** instrument

- Theory applies for **light or electron** waves

3) Abbe Simple Criteria of Resolution

Simple Rule: 1/2 the wavelength of the radiation used is the **ultimate** resolving power of any instrument



Interaction of waves with an obstacle

Observer who wishes to detect the boat can do so only by observing waves that have wavelengths comparable to or smaller than the length of the boat.

From Sherwood, Fig. 1.9, p.19

I.A.3 Photons/Electrons

I.A.3.d Resolution

4) Magnification Limits

Maximum magnification of an instrument is limited

$$\textbf{Maximum mag.} = \frac{\textbf{resolving power of eye}}{\textbf{resolving power of microscope}}$$

For LM:

For a microscope resolving power of ~ 0.25 µm
Maximum (useful) magnification is about 250 µm/0.25 µm = 1000X

Any higher magnification represents **empty magnification**

...meaning you get **no more** useful information but just a **magnified blur**

I.A.3 Photons/Electrons

I.A.3.d Resolution

4) Magnification Limits

According to Abbe's simple criteria:

At 60kV (λ=0.05 nm), TEM **ultimate** resolving power is ~ 0.0025 nm

==> Max useful mag of ~100 x 10^6 (= 250 μm/0.0025 nm)

In practice: Max useful mag. at 60 kV is limited to << 1 x 10^6

The good news/bad news:

LM nearly obeys the Abbe simple criteria

TEM falls *way way way* short

I.A.3 Photons/Electrons

I.A.3.d Resolution

4) Magnification Limits

The good news/bad news:

LM nearly obeys the Abbe simple criteria
TEM falls *way way way* short

"And why is this ?" you ask:

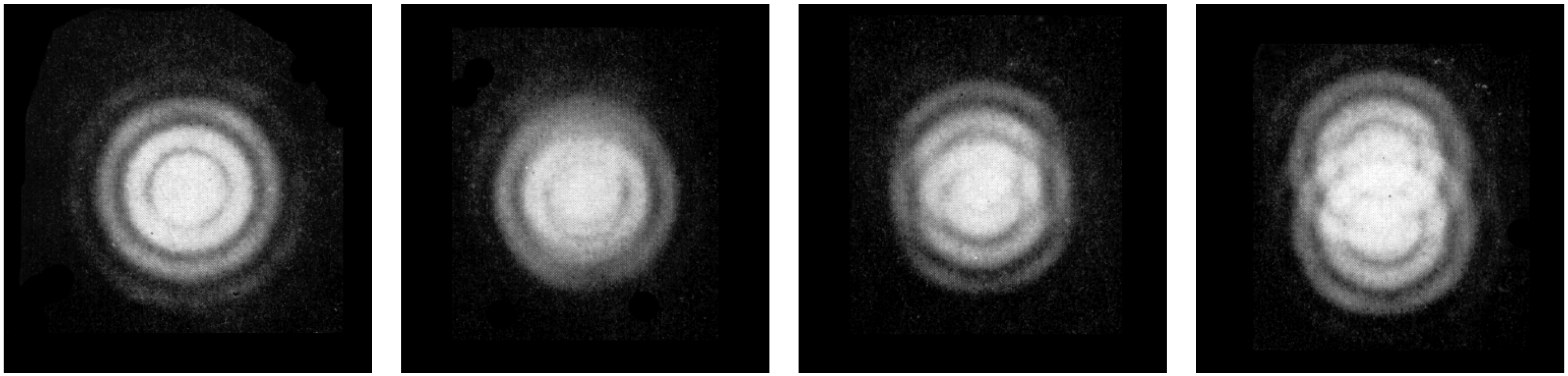
Main limiting factors in achieving the theoretical resolving power:

- nature of the imaging lenses
- nature of the image formation process

I.A.3 Photons/Electrons

I.A.3.d Resolution

5) Raleigh Criteria



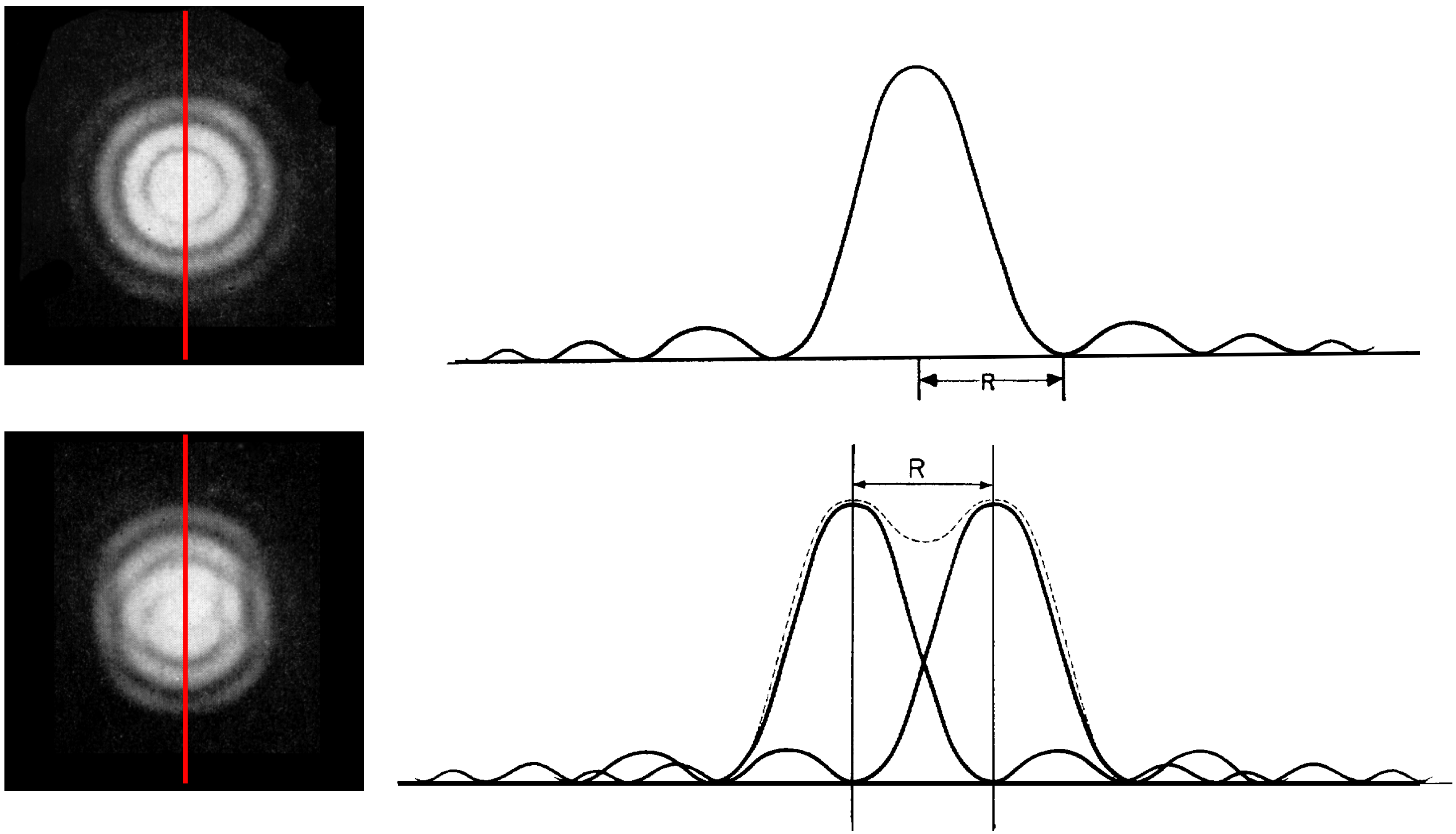
The shortest distance between 2 Airy disks at which the two disks appear partially separated corresponds to about 1/2 the width of the disks

From Sjostrand, Fig. IV.18, p.115

I.A.3 Photons/Electrons

I.A.3.d Resolution

5) Raleigh Criteria



From Sjostrand, Fig. IV.18, p.115

I.A.3 Photons/Electrons

I.A.3.d Resolution

5) Raleigh Criteria

The shortest distance between 2 Airy disks at which the two disks appear partially separated corresponds to about 1/2 the width of the disks

The distance, *d*, in object space is given by the Abbe Equation:

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

λ = wavelength of the radiation
n = refractive index of the media
α = lens semi-angular aperture

Note: *n* sinα = lens numerical aperture (N.A.)

I.A.3 Photons/Electrons

I.A.3.d Resolution

5) Raleigh Criteria

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

To maximize resolving power, λ must be decreased, *n* increased, or α increased

	n	*sin* α	λ	*d*
LM	1.5	0.87	400 nm	0.2 μm
TEM				

* λ = 400 nm for violet light

I.A.3 Photons/Electrons

I.A.3.d Resolution

5) Raleigh Criteria

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

To maximize resolving power, λ must be decreased, *n* increased, or α increased

	n	*sin* α	λ	*d*
LM	1.5	0.87	400 nm	0.2 µm
TEM	1.0			

* λ = 400 nm for violet light

I.A.3 Photons/Electrons

I.A.3.d Resolution

5) Raleigh Criteria

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

To maximize resolving power, λ must be decreased, *n* increased, or α increased

	n	*sin* α	λ	*d*
LM	1.5	0.87	400 nm	0.2 µm
TEM	1.0	0.01		

* λ = 400 nm for violet light

I.A.3 Photons/Electrons

I.A.3.d Resolution

5) Raleigh Criteria

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

To maximize resolving power, λ must be decreased, *n* increased, or α increased

	n	*sin* α	λ	*d*
LM	1.5	0.87	400 nm	0.2 µm
TEM	1.0	0.01	0.005 nm	

* λ = 400 nm for violet light; = 0.005 nm for 60kV electrons

I.A.3 Photons/Electrons

I.A.3.d Resolution

5) Raleigh Criteria

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

To maximize resolving power, λ must be decreased, *n* increased, or α increased

	n	*sin* α	λ	*d*
LM	1.5	0.87	400 nm	0.2 μm
TEM	1.0	0.01	0.005 nm	0.3 nm

* λ = 400 nm for violet light; = 0.005 nm for 60kV electrons