

2009 spring

***Microstructural Characterization
of
Materials***

03.16.2009

Eun Soo Park

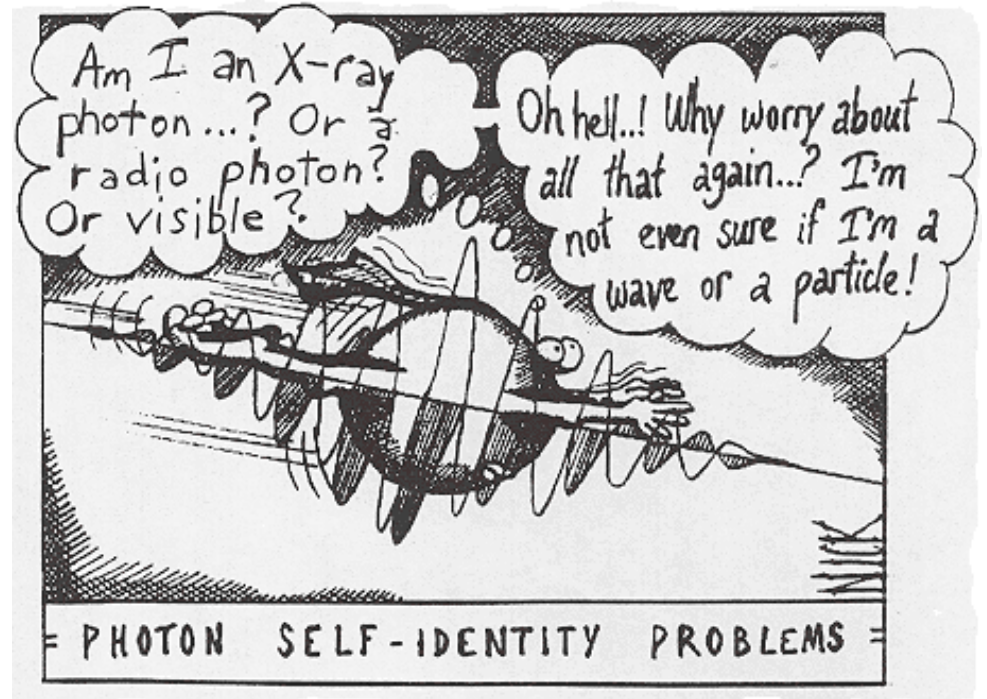
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Office hours: by an appointment ¹

So is light a wave or a particle ?



On **macroscopic scales**, we can treat **a large number of photons as a wave**.

When dealing with **subatomic phenomenon**, we are often dealing with a **single photon**, or a few. In this case, you cannot use the wave description of light. It doesn't work !

Contents for previous class

□ Light is made up of photons, but in macroscopic situations, it is often fine to treat it as a wave.

□ When looking at the microscopic world, there is only 1 thing that works... Light is made up of photons (particles of light).

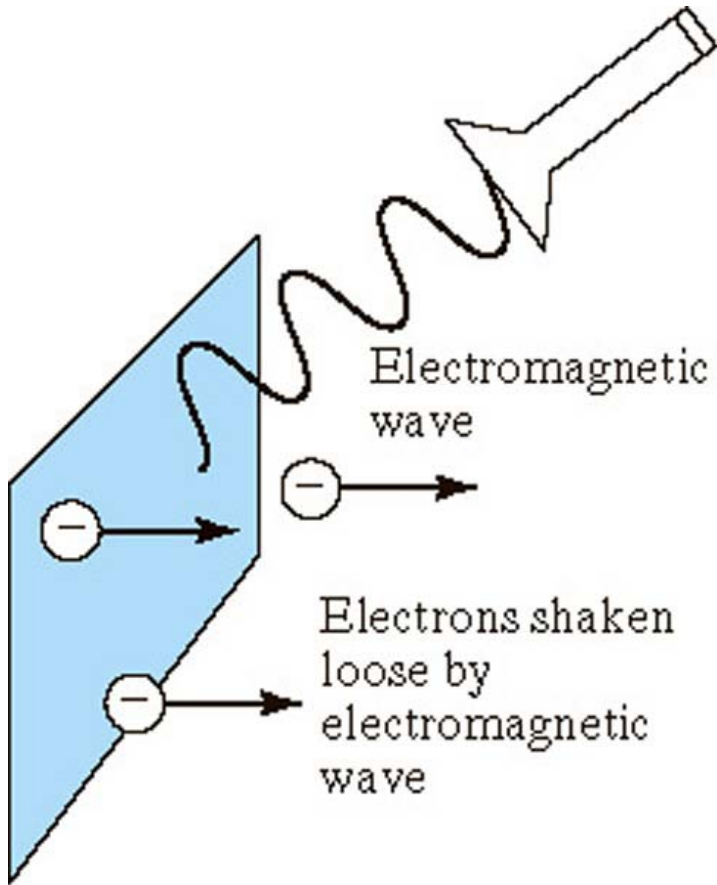
□ Photons carry both energy & momentum.

$$E = hc / \lambda \qquad p = E/c = h / \lambda$$

□ Matter also exhibits wave properties. For an object of mass m , and velocity, v , the object has a wavelength, $\lambda = h / mv$.

□ One can probe ‘see’ the fine details of matter by using high energy particles (they have a small wavelength !) → Can reveal the tiniest things !

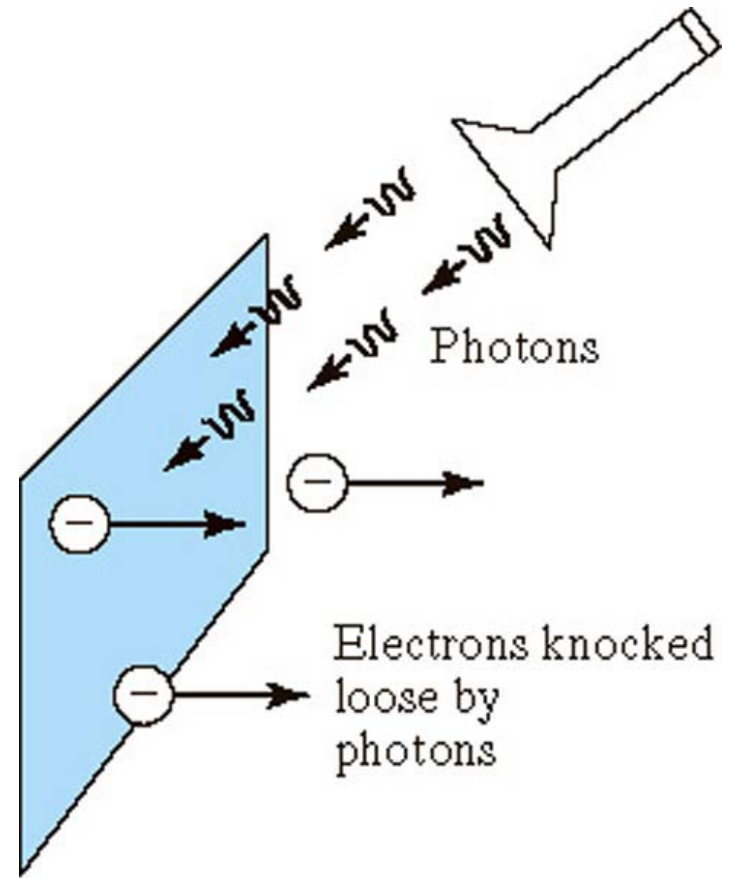
Classical Picture



Macroscopic situations

$$\text{Energy of wave} \propto (\text{Amplitude})^2$$

Quantum Picture



Microscopic situations

$$\text{Energy/photon} = hc / \lambda$$

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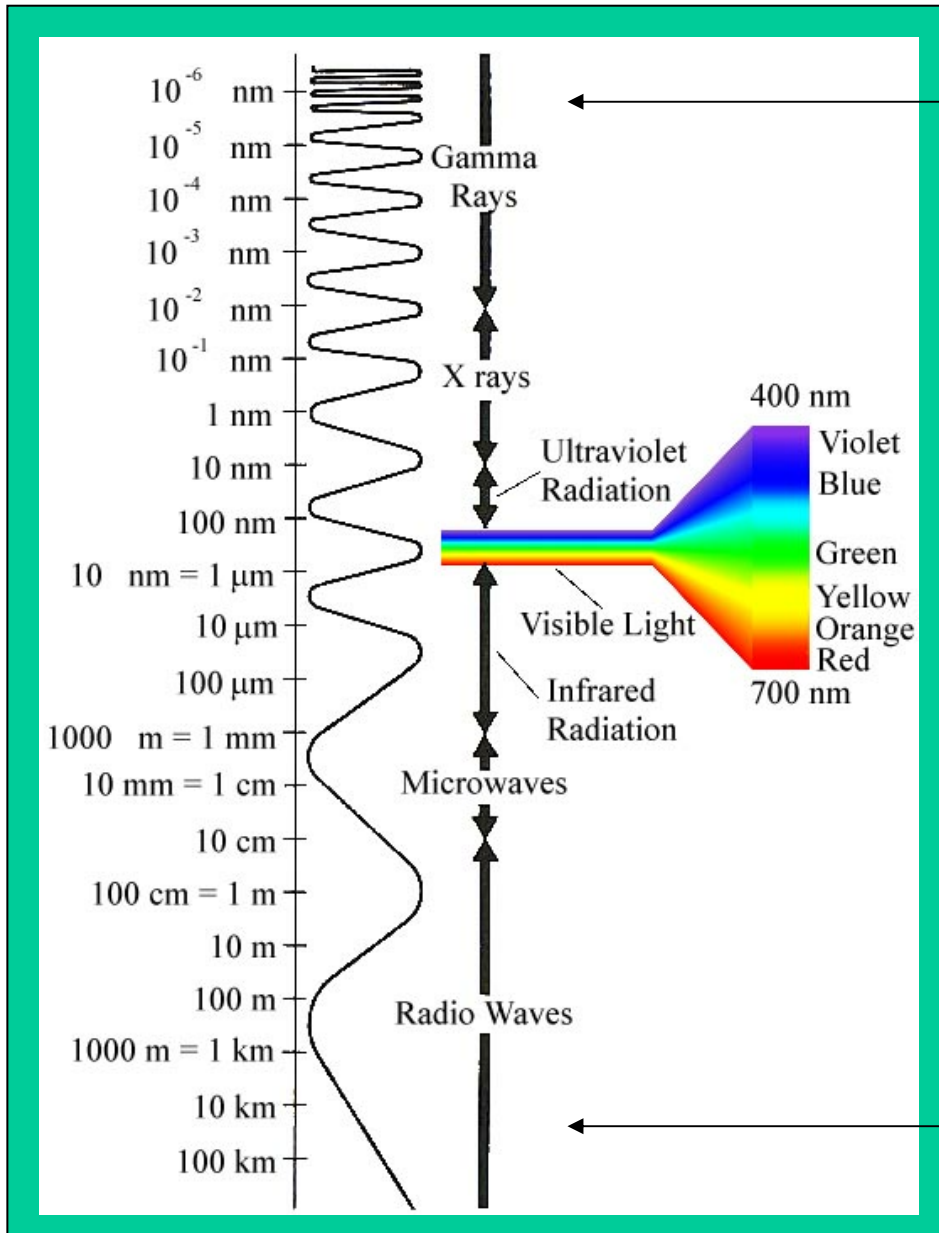
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The Electromagnetic Spectrum



Shortest wavelengths
(Most energetic photons)

$$E = h\nu = hc/\lambda$$

$$h = 6.6 \times 10^{-34} \text{ [J*sec]}$$

(Planck's constant)

Longest wavelengths
(Least energetic photons)

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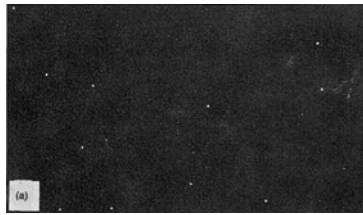
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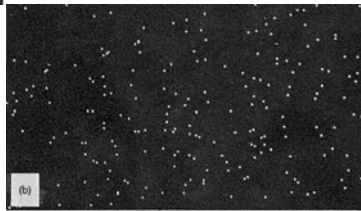
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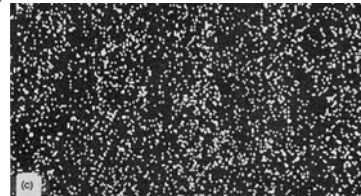
Real photographs of an electron interference pattern...



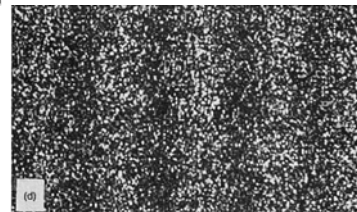
.01 [s]
10 electrons



.1 [s]
100 electrons

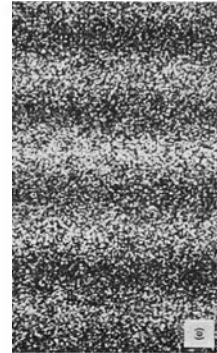
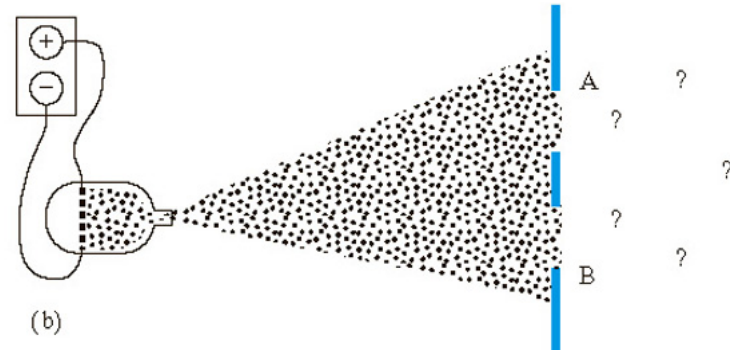


3 [s]
3000 electrons



70 [s]
70,000 electrons

Notice the clear interference fringes. Clear indication of wave phenomenon.



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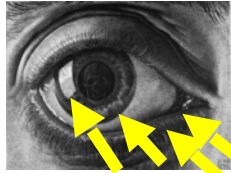
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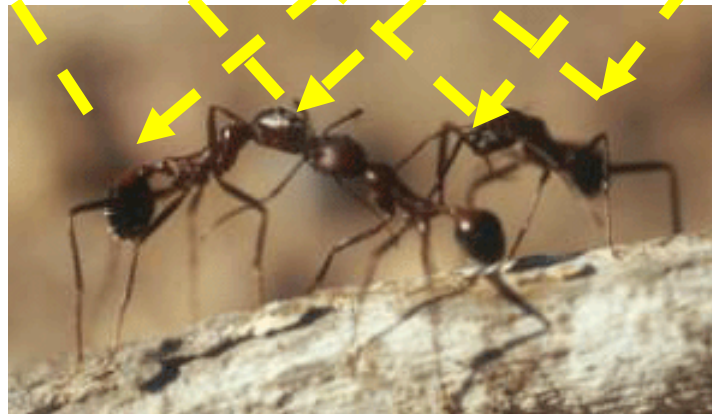
How do we see ?



Light reflects (scatters) from a surface and reaches our eye.



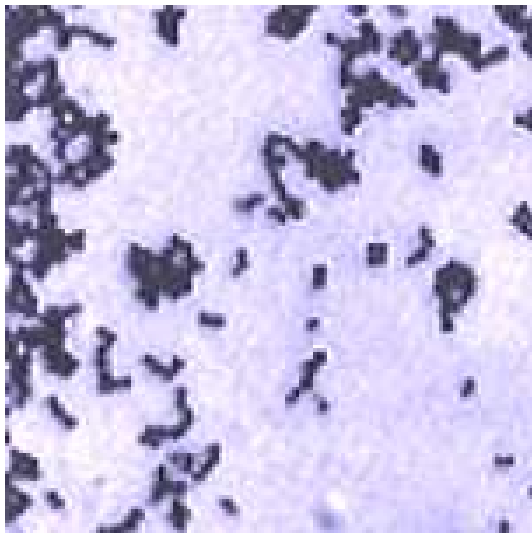
Our eye forms an image of the object.



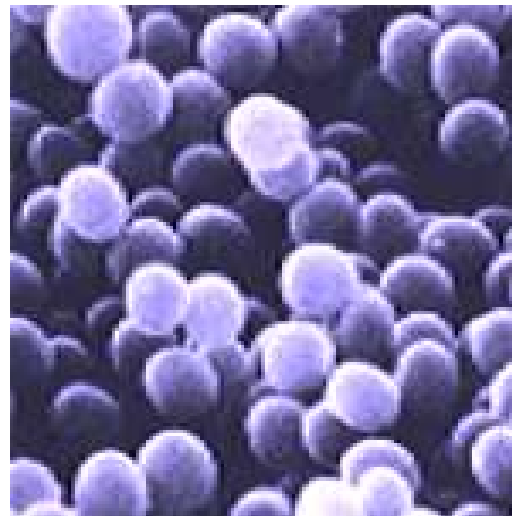
Wavelength versus Size

Even with a visible light microscope, we are limited to being able to resolve objects which are at least about 10^{-6} [m] = 1 [μm] = 1000 [nm] in size.

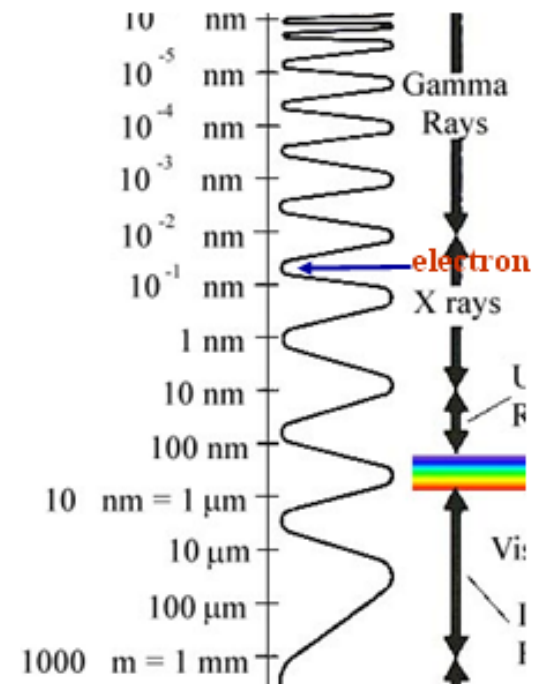
This is because visible light, with a wavelength of ~ 500 [nm] cannot resolve objects whose size is smaller than it's wavelength.



Bacteria, as viewed using visible light



Bacteria, as viewed using electrons !

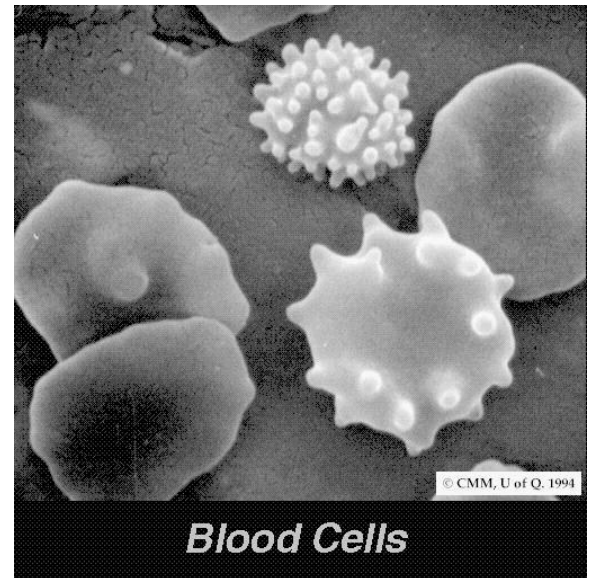


Electron Microscope

→ The electron microscope is a device which uses the wave behavior of electrons to make images which are otherwise too small for visible light!

This image was taken with a Scanning Electron Microscope (SEM).

These devices can resolve features down to about 1 [nm]. This is about 100 times better than can be done with visible light microscopes!



IMPORTANT POINT HERE:

High energy particles can be used to reveal the structure of matter !

Contents for today's class

Optical Microscope

- What is a Microscope?
- History of Optical Microscope
- Refraction and Snell's law
- Relationship between the Microscope and the human eye
- How the Optical Microscope Works?
 - Important terms



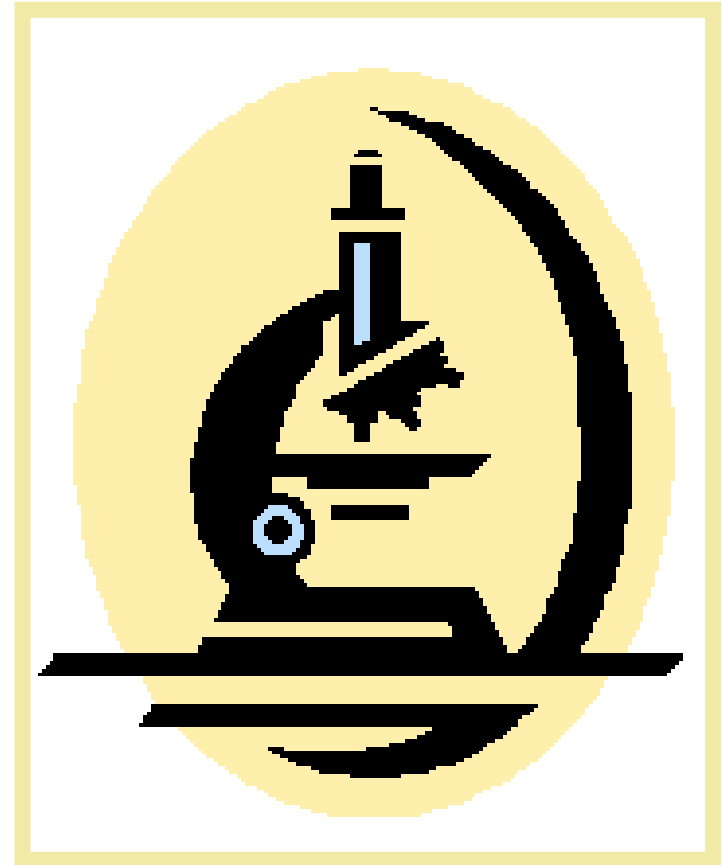
Suggested Reading

1. ASM Handbook, Volume 9, ASM International, Materials Park, OH (2004).
2. G.F. Vander Voort, Metallography Principles and Practice, ASM International, Materials Park, OH (1999).
3. D. Brandon and W.D. Kaplan, Microstructural Characterization of Materials, Wiley (1999). Newer edition is also good.
4. Y. Leng, Materials Characterization, (2008), Wiley, Hoboken, NJ.

What is a Microscope?

Microscopes are instruments designed to produce magnified visual or photographic images of small objects. The microscope must accomplish three tasks:

- produce a magnified image of the specimen
- separate the details in the image
- render the details visible to the human eye or camera

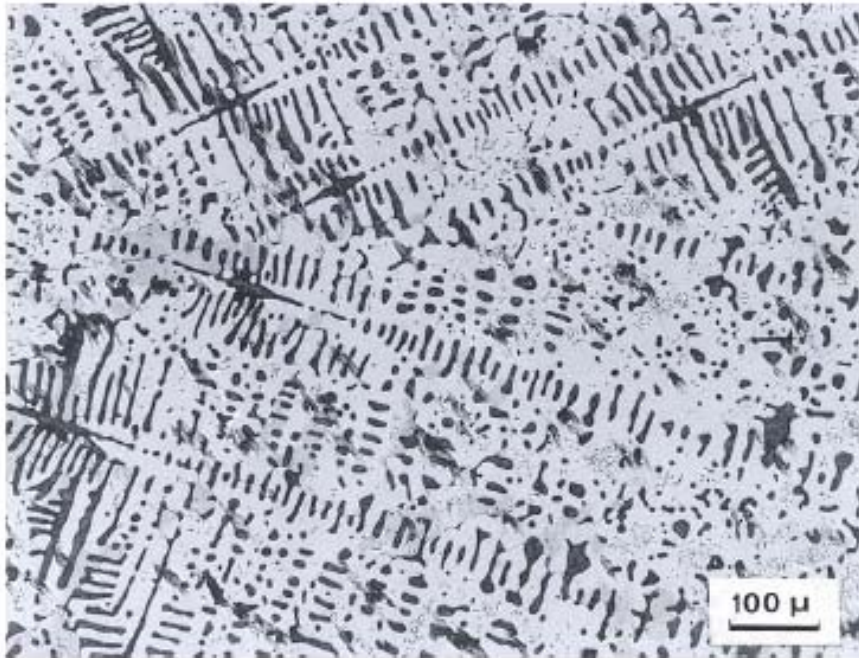


A light microscope is also known as an optical microscope.

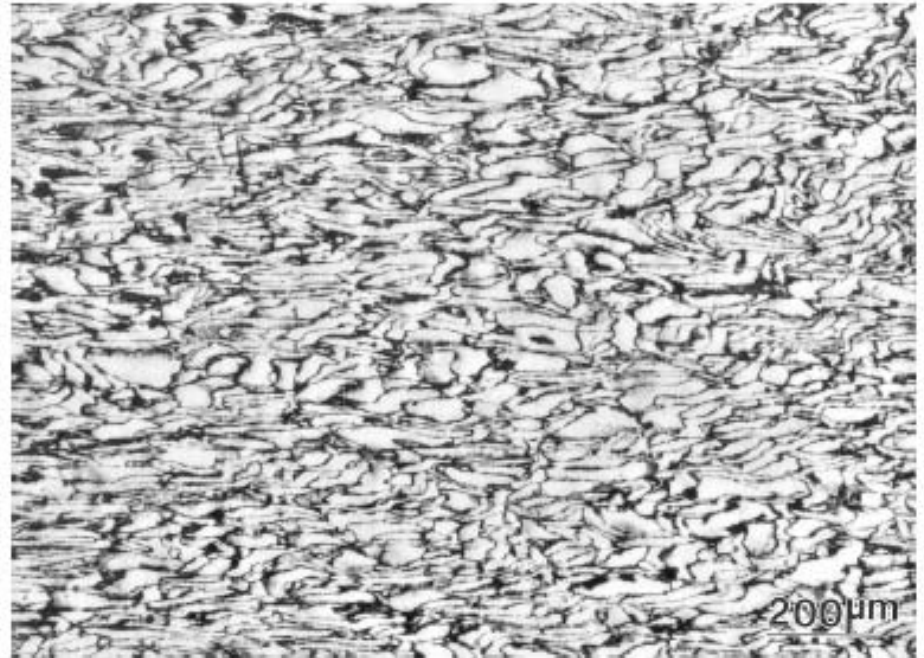
Light Optical Microscopy (LOM)

- **The primary technique used by scientists and engineers to examine the microstructures of materials.**
- **Basis for field of metallography**
- **Technique is used to examine all classes of materials**
- **Origin can be traced back to the 1600s.**

- **Basis for field of metallography**



Casting microstructure of Ni-Cr alloy



Microstructure of powder metallurgy Al alloy

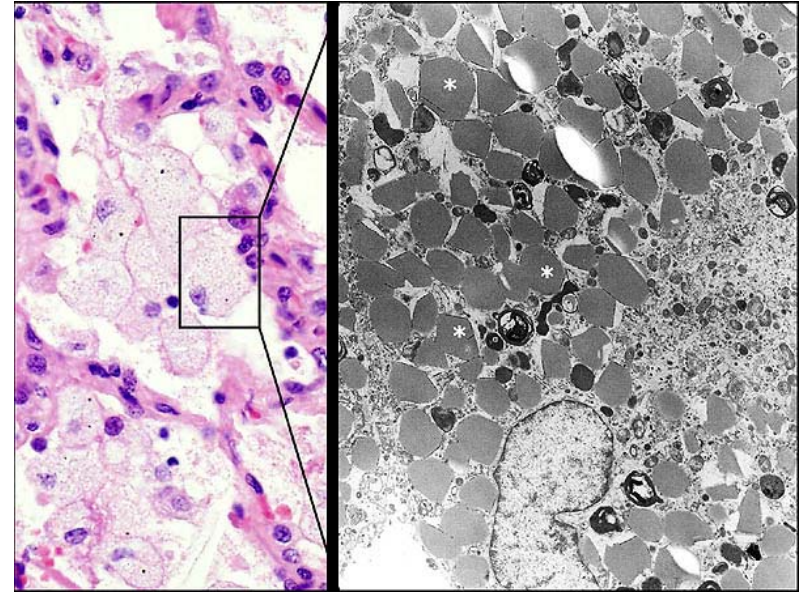
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탄소강 조직사진



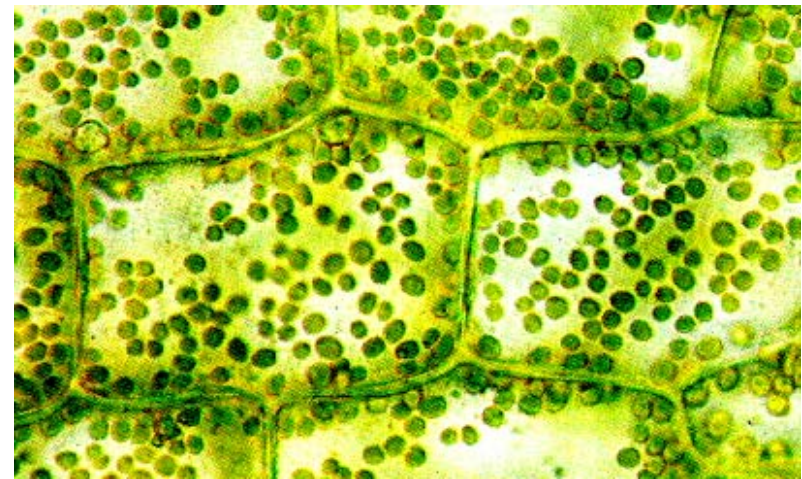
폐 세포 속의 대식세포



곤충 사진



식물성 플랑크톤



식물세포내 엽록체

Types of Optical Microscopes:

(1) Simple OM:

One lens; 25x; 10 μm resolution

(2) Stereo OM:

Two lens trains; 6-8x;

(3) Compound OM:

Objective + eyepiece + condenser lenses

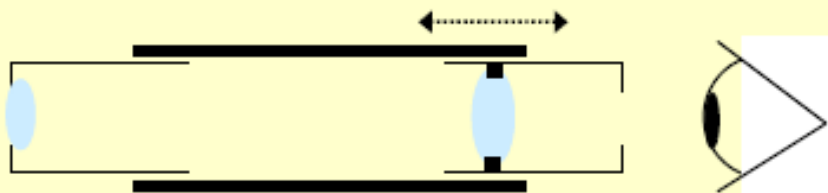
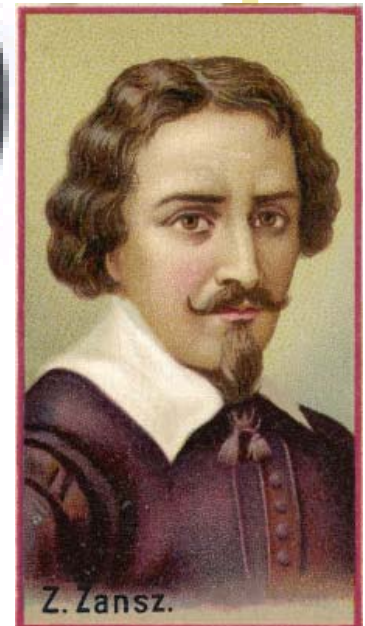
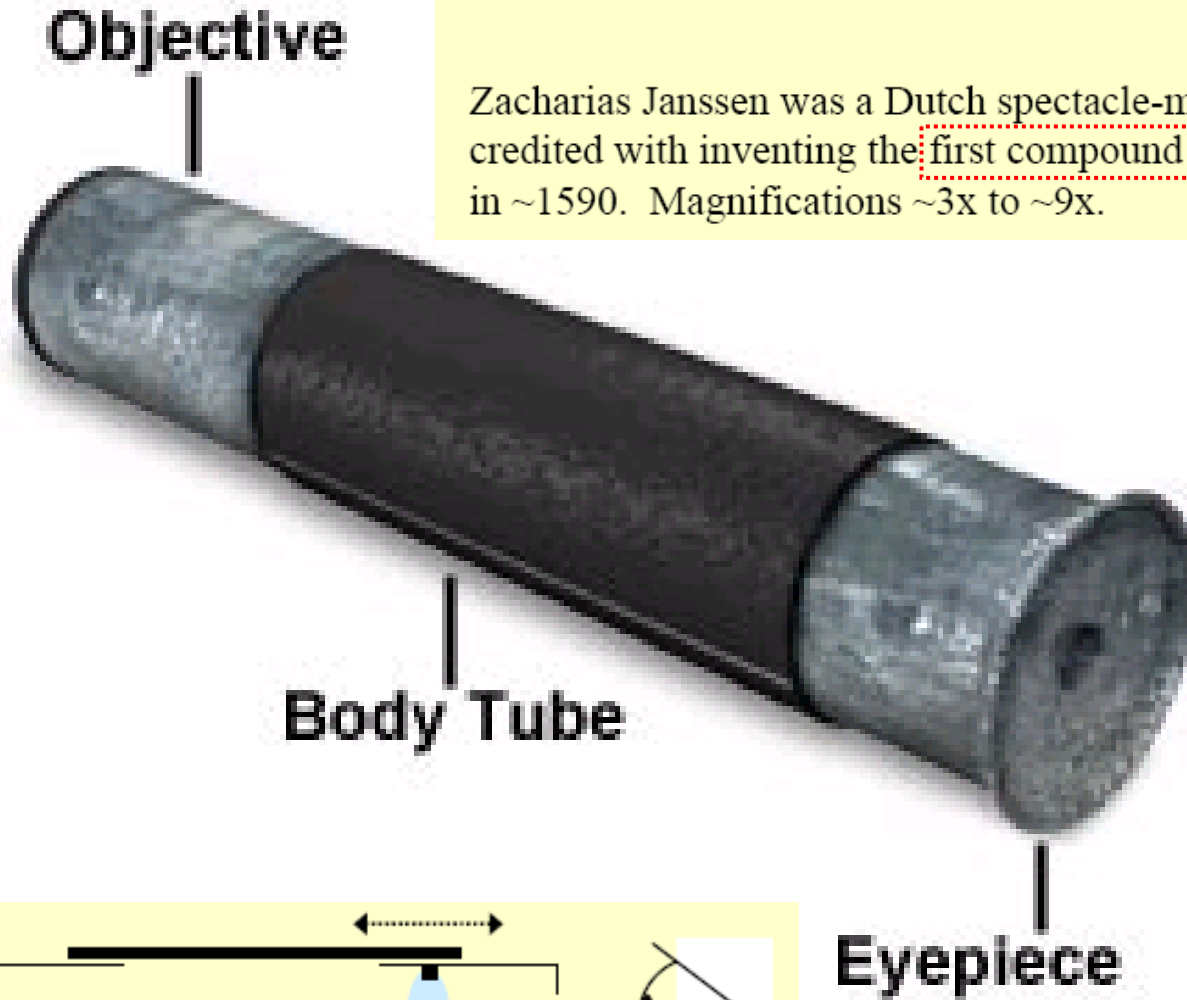
1300x; 1 μm resolution



History of Optical Microscope

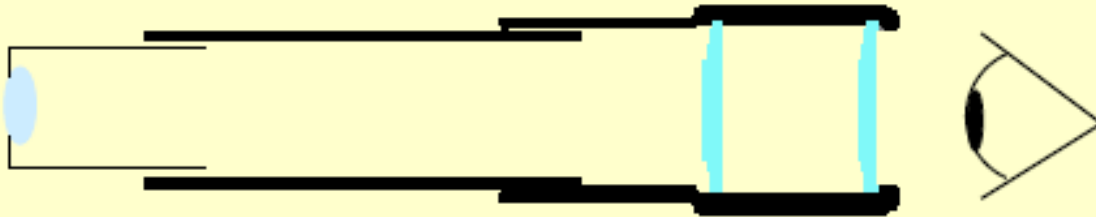
Janssen

Zacharias Janssen was a Dutch spectacle-maker credited with inventing the **first compound microscope** in ~1590. Magnifications ~3x to ~9x.



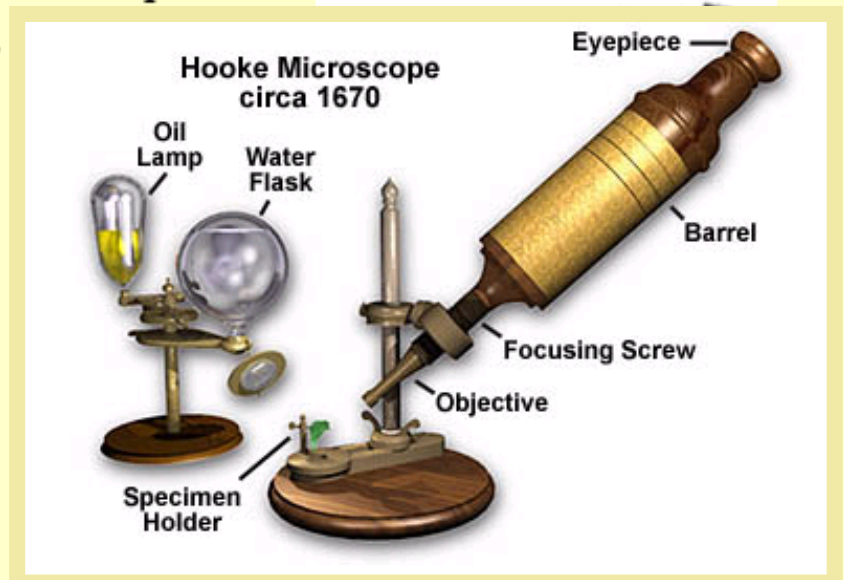
Huygens

Christiaan Huygens (1629–1695) was a Dutch mathematician, astronomer and physicist developed an improved two lens eye piece. Optical errors in two half curved lenses tend to cancel out. Can get more magnification.



Hooke

Robert Hooke improved the design of the new compound microscope, including a light source ~1655. Developed micrometer.



History of Optical Microscope

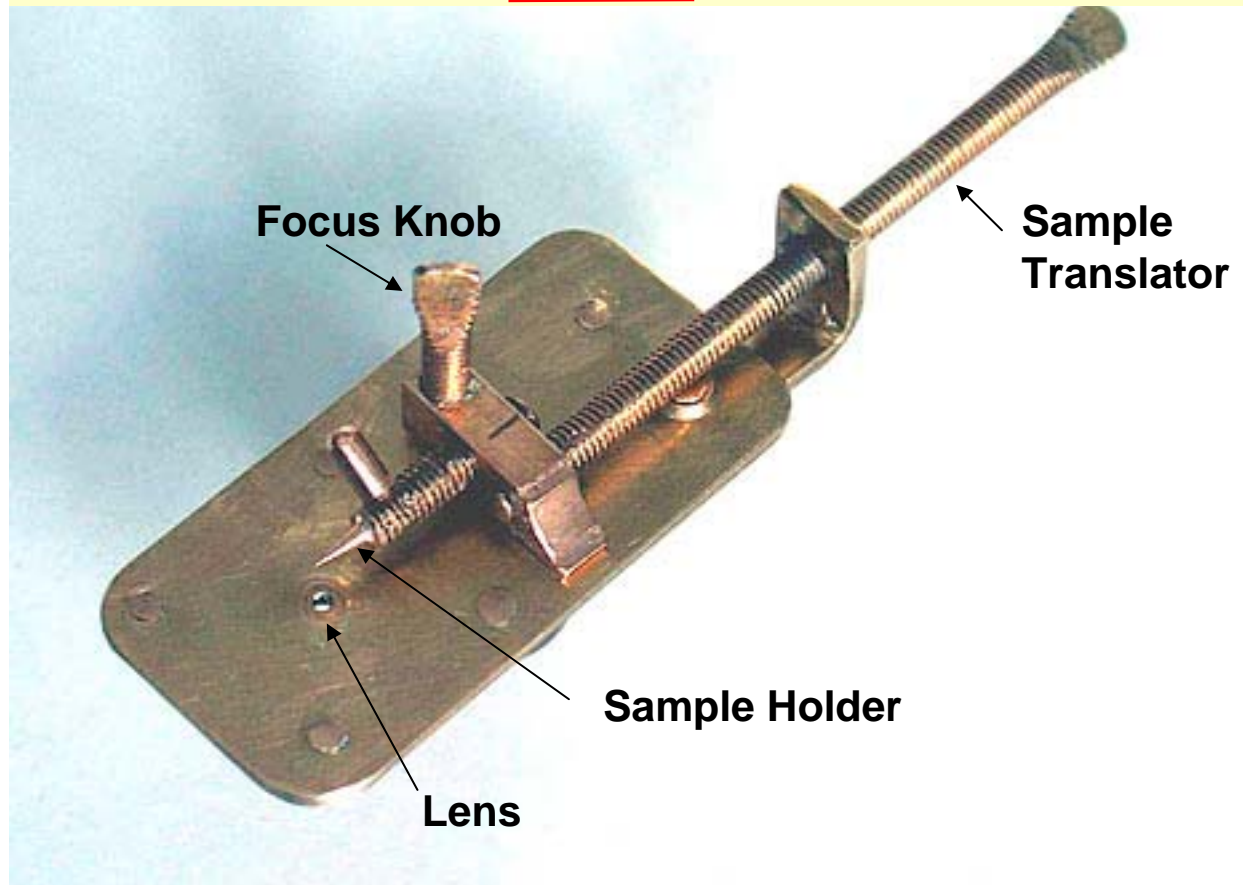
Von Leewenhoek Microscope (circa Late 1600s)

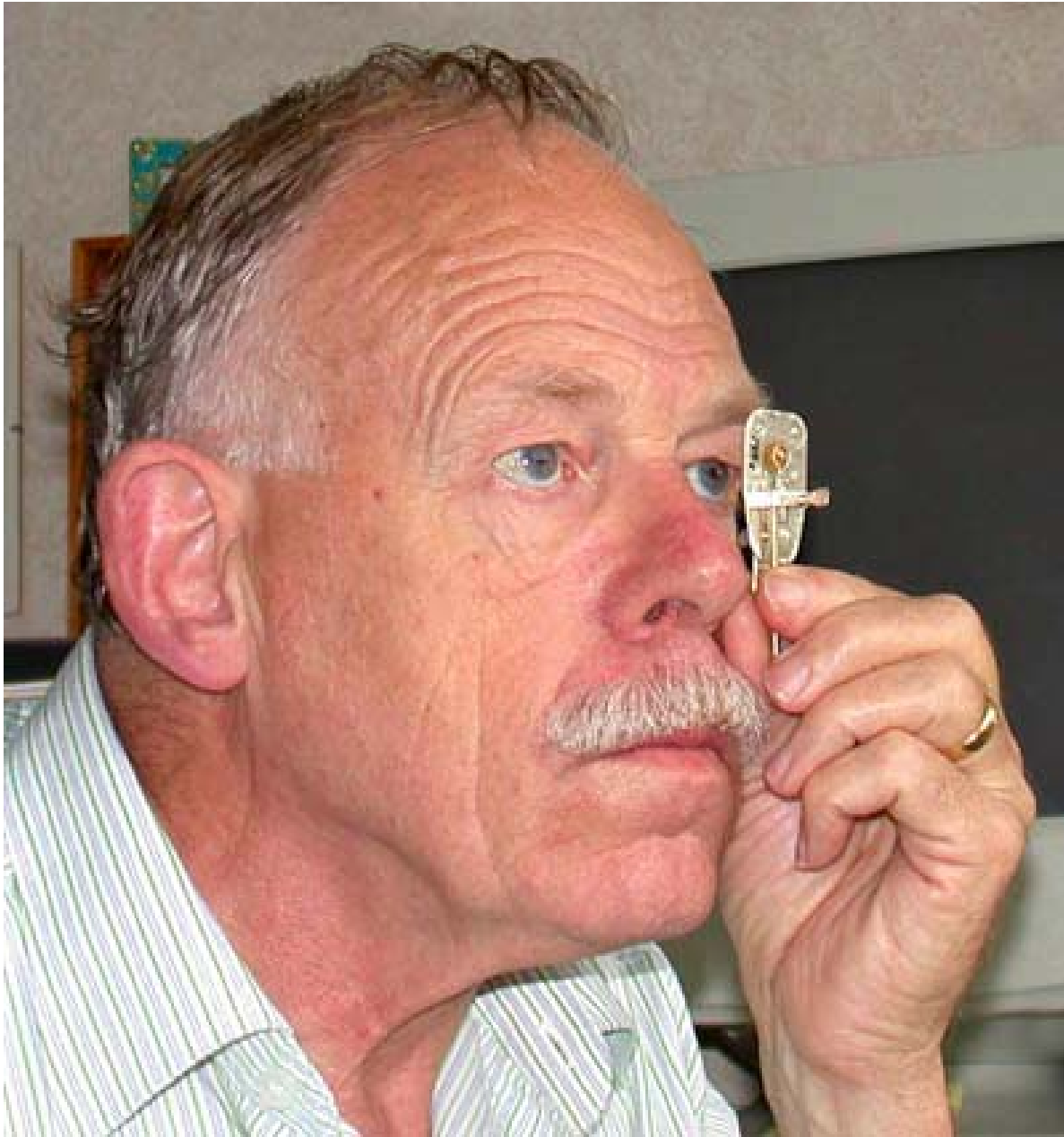


Father of Microbiology

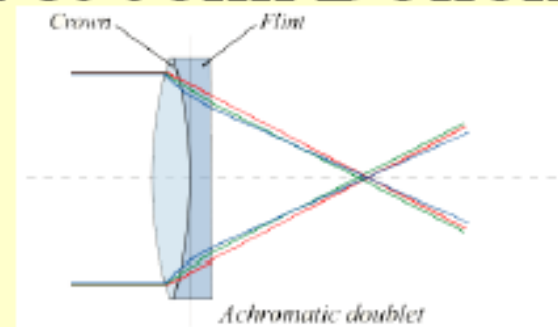
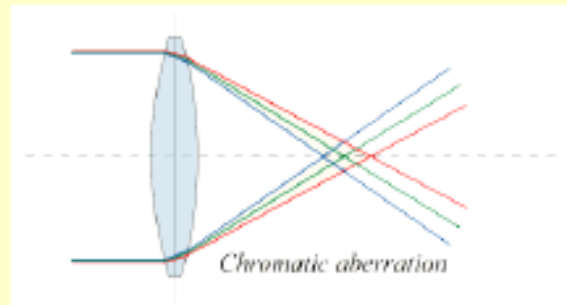


Anton van Leeuwenhoek simple microscope (~1675) used a single lens which yielded high magnifications (~70x to ~300x) and excellent resolution (~1 μm). He reported seeing many kinds of microorganisms including bacteria!





Chester Moore Hall & John Dollond

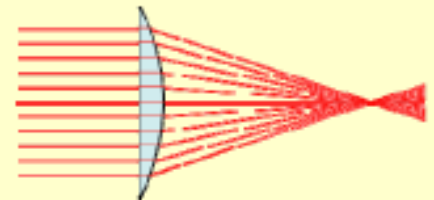


- **Achromatic refracting lens** was invented in 1733 by an English barrister named Chester Moore Hall
- Patented by John Dollond.

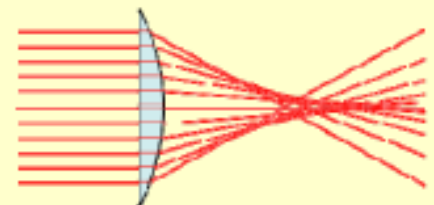


Lister

Tight focus

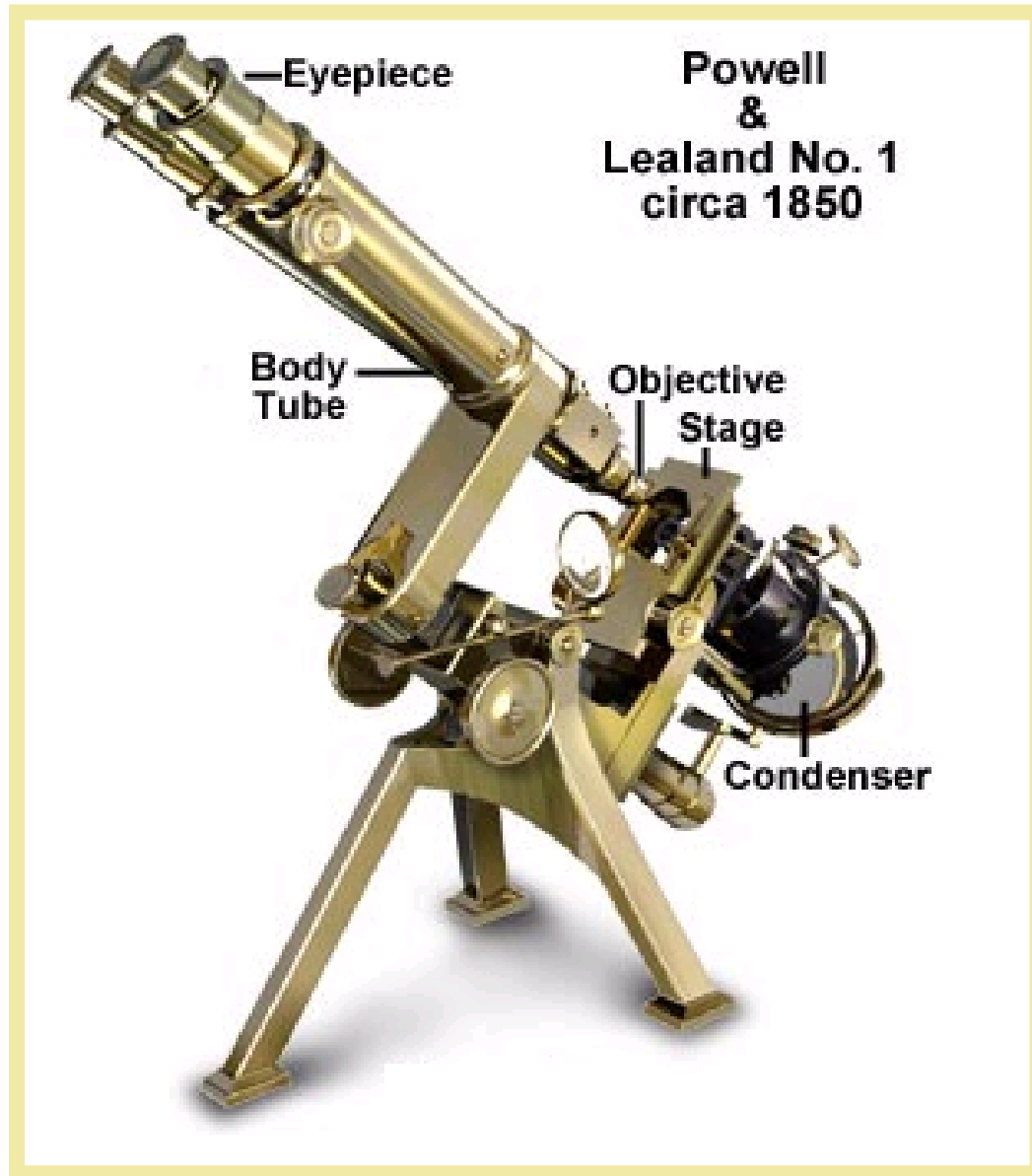


Spherical aberration



Joseph Jackson Lister (1786-1869) design and construct superior complex lenses by combining lenses of crown and flint glasses of different dispersion, but separated in order to both correct chromatic aberration and minimize spherical aberration.

History of Optical Microscope



Abbe

Ernst Abbe (1840-1905) applied mathematical principles to the design of lenses, which dramatically facilitated the manufacturing high quality optical instruments by the Carl Zeiss corporation. Abbe's realization that the performance of a microscope was limited by the diffraction of light was not well accepted for decades.



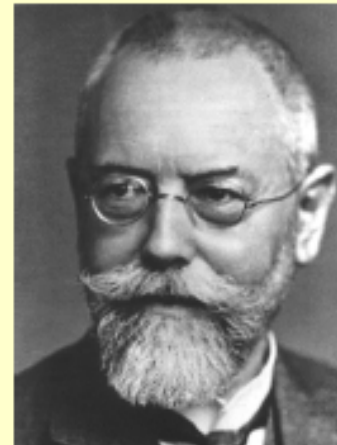
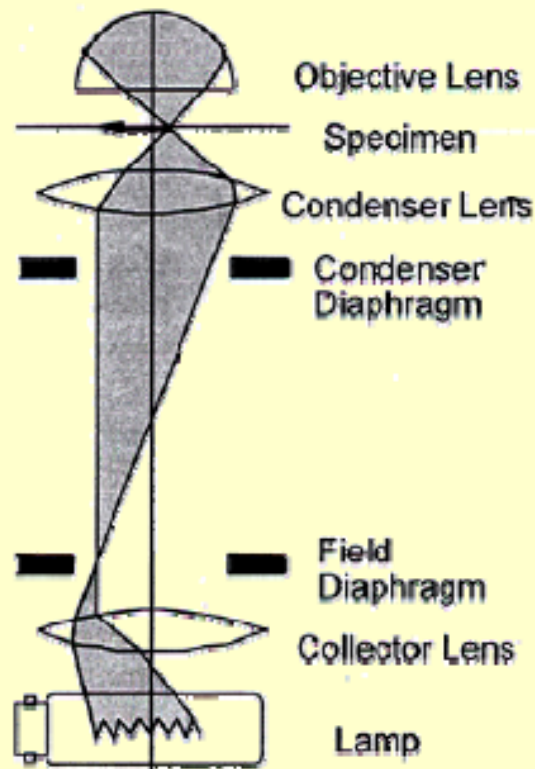
The Abbe limit formula

$$d = \frac{\lambda}{2 \sin \alpha}$$

$$E = h\nu$$


Köhler

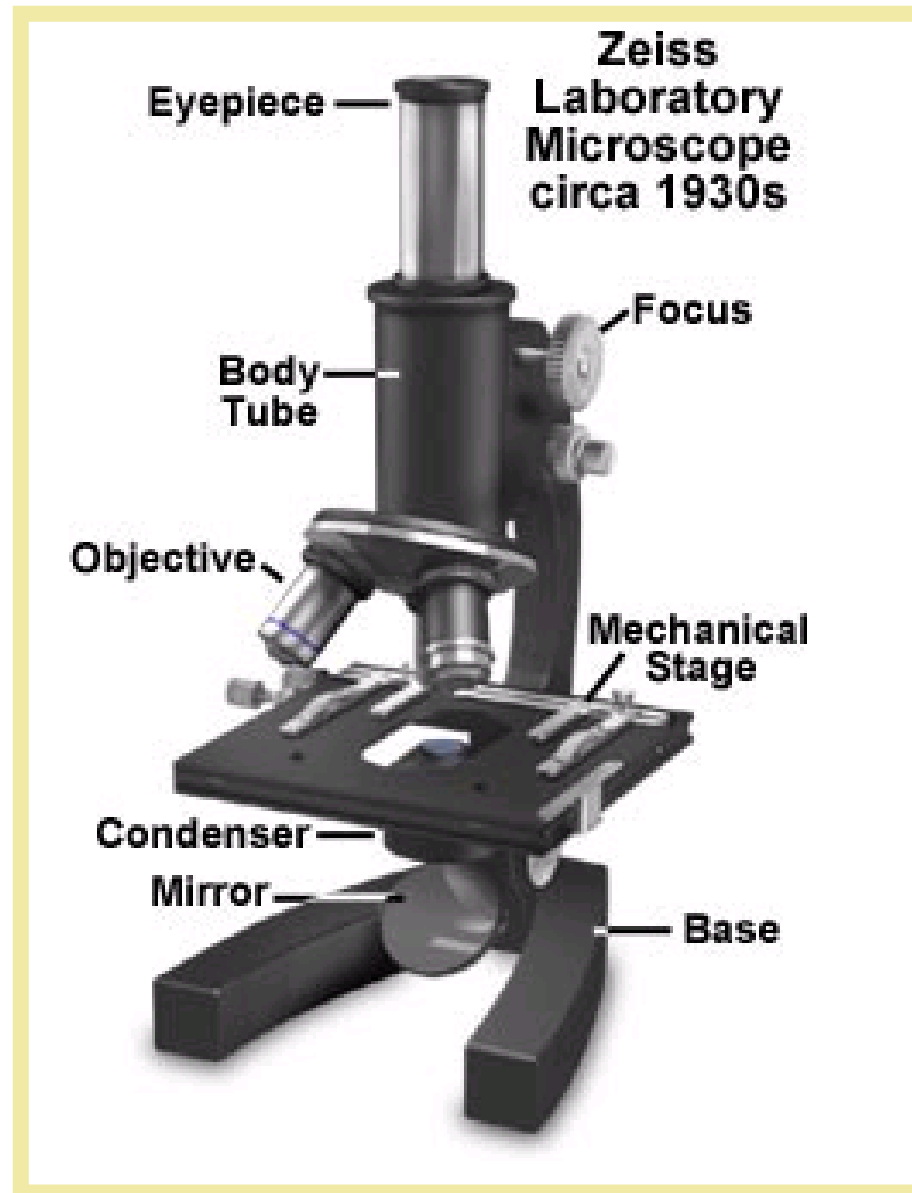
In 1893 August Köhler (1866-1948) invented a method of providing optimum illumination of a microscope specimen while working at the Zeiss Corporation. Improved resolution and evenness of light illumination made photomicrography possible.



$$E = h\nu$$

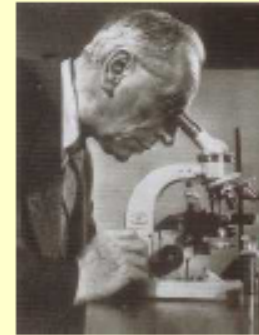
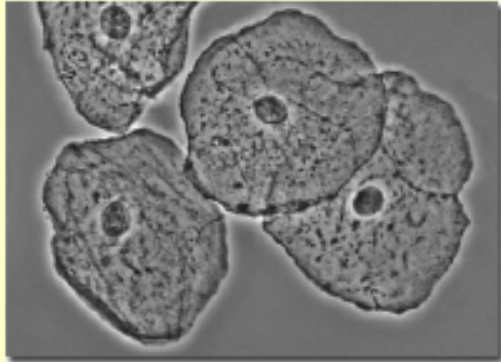


History of Optical Microscope



Zernike

Frederik Zernike (1888–1966) invented phase contrast microscopy in 1933, a way to see unstained cells. Nobel prize 1953.



Nomarski

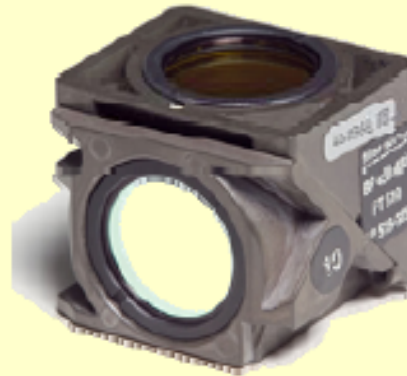
Georges Nomarski (1919-1997) developed the differential interference contrast (DIC) microscopy technique, which bears his name.



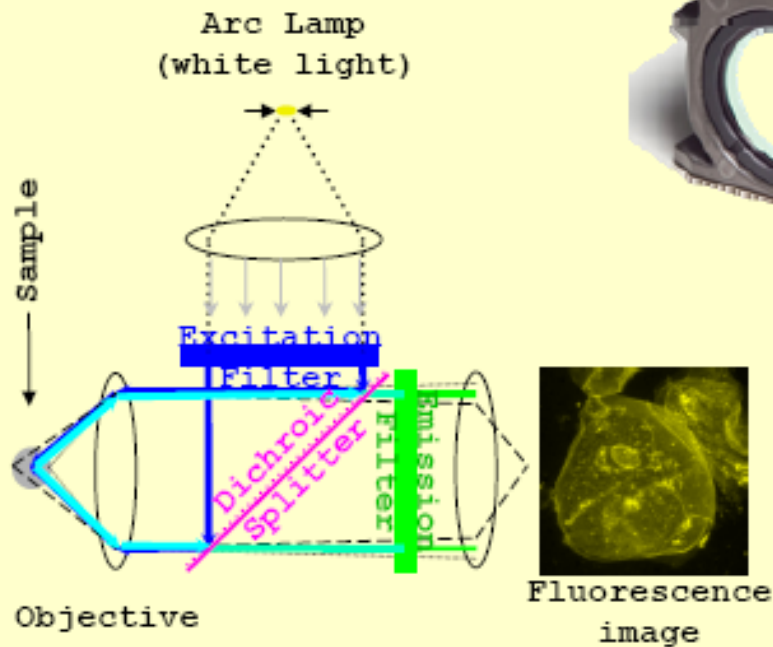
Ploem

Johas Ploem invented the epi-illumination cube used in **fluorescence microscopy**

Dichroic filter cube



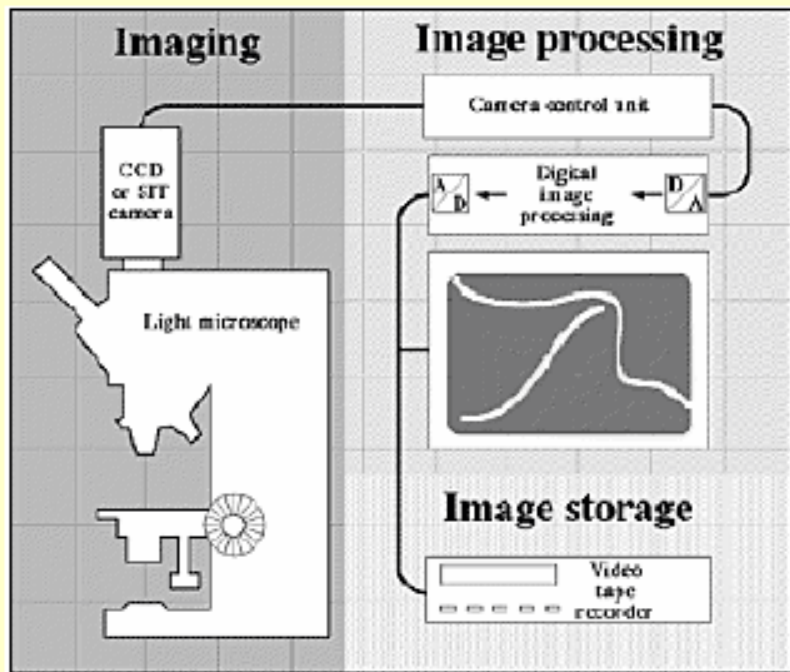
Johan Sebastian Ploem
(1927-Present)



$$E = h\nu$$


Inoue & D. Allen & N. Allen

Video enhanced microscopy – use electronic camera and computer generated contrast enhancement



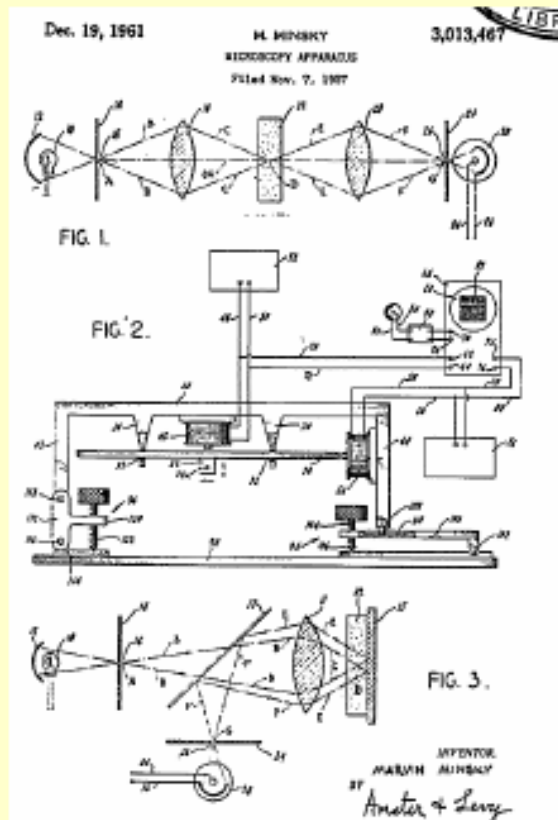
Shinya Inoue



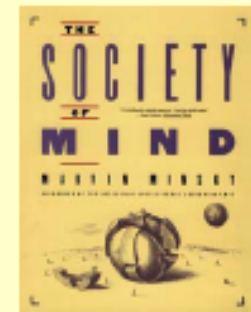
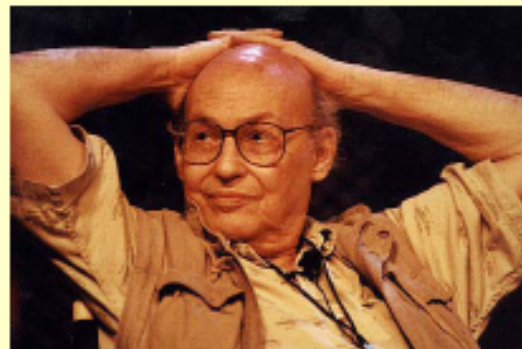
Nina Allen

Marvin Minsky

1957 Patented the **Confocal Scanning Microscope** U.S. Patent 3013467



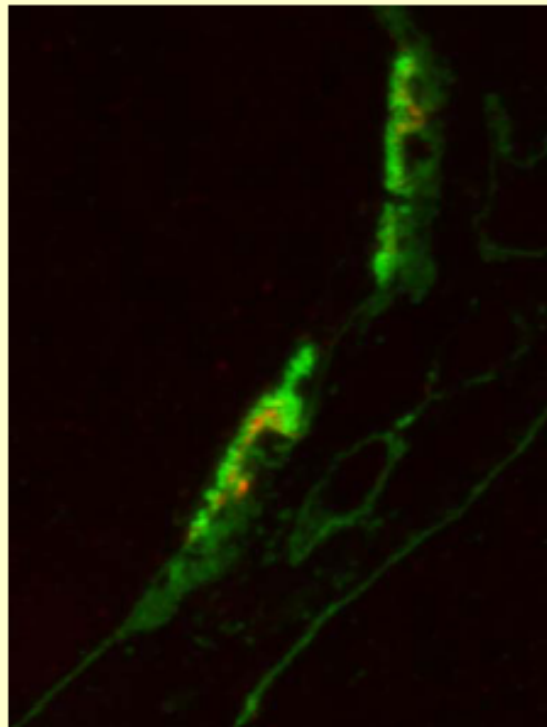
Practical Confocal microscope systems became available in the late 1980s. Yields improved contrast, resolution and optical sectioning.



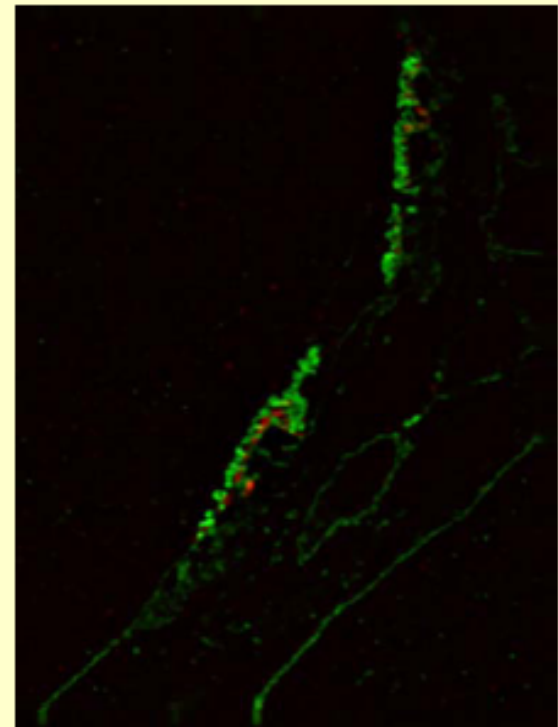
$$E = hv$$


Sedat, Amos & Agrad

1980s Digital deconvolution microscopy removes haze.



Mathematical
Transformation
→
+ time

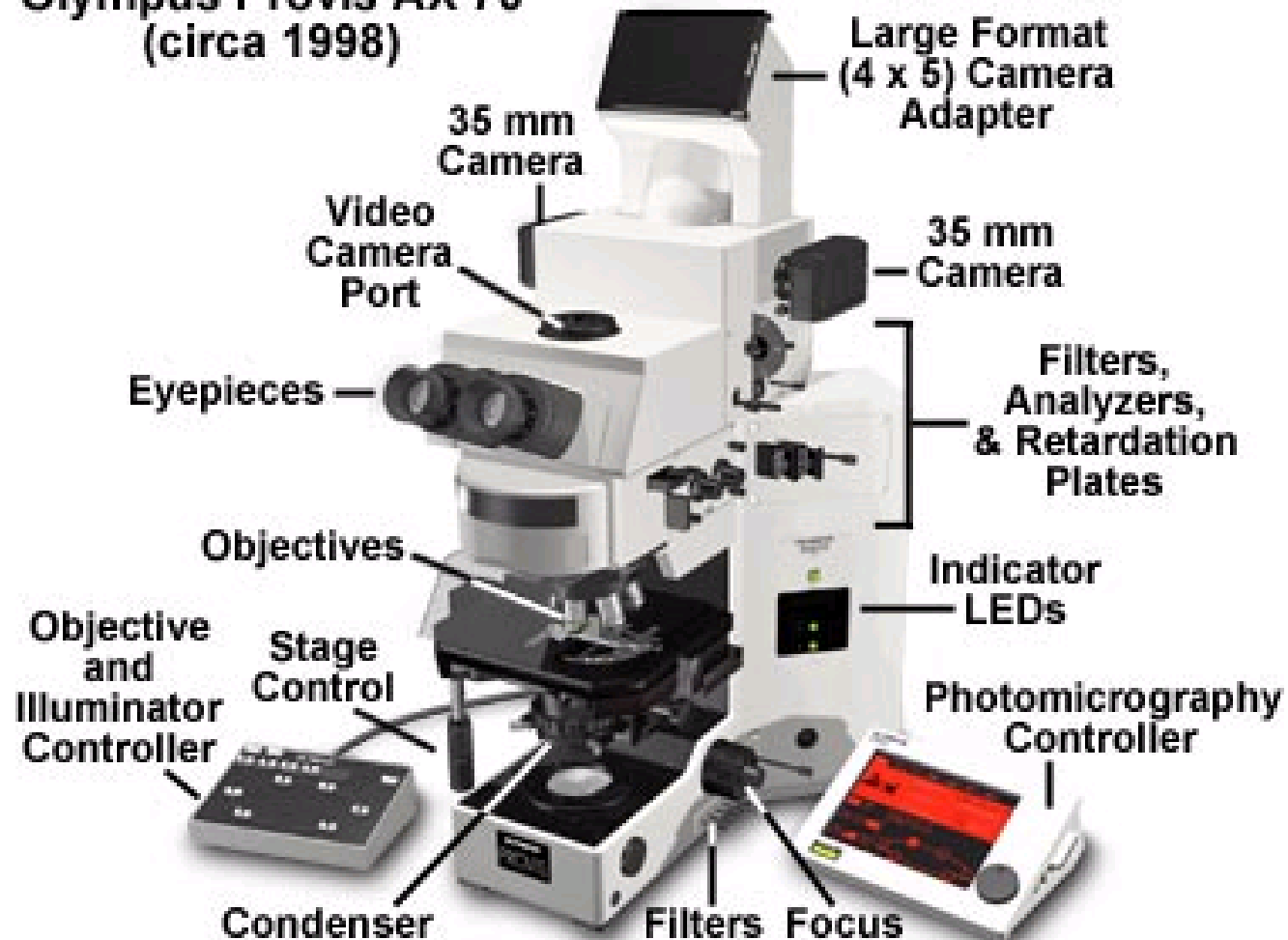


+ a powerful computer
with extensive storage

$$\underline{E = h\nu}$$


History of Optical Microscope

Olympus Provis AX 70
(circa 1998)



History Summary - Recent Evolution

Dyes – fixed, vital, indicators
Immunostaining /Antibodies
Molecular Biology
Illumination
Lasers
Electronics
Cameras – CCDs, Intensifiers, high speed
Optics – ATOF, ATOM, fibers
Computers
Algorithms & software
Techniques – Time lapse, FRAP, FRET, FLIM
Control systems – focus, x-y movement, shutters
Live cell environmental control

Better resolution
More sensitivity
Lower noise
Faster detection
Greater specificity
Easier analysis
Bigger storage
New capabilities

Increased complexity
Increased cost
More raw data

$$E = hv$$


Refraction

- Deflection of light due to changes in **density** from one substance to the next.
- Defined by refractive index, n

Waves do not have the same speed in all materials.

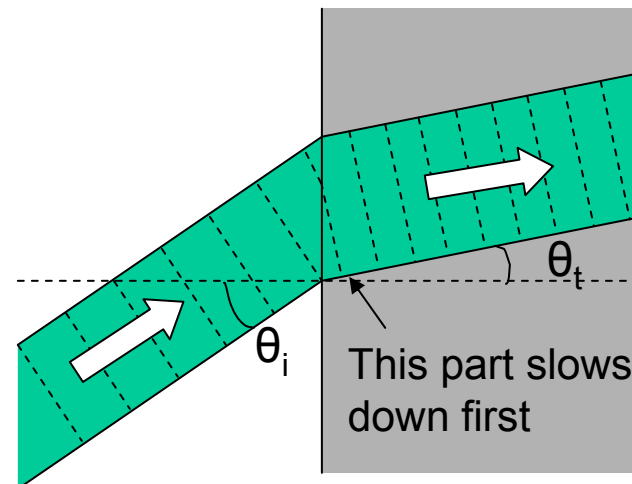
The speed of light in vacuum is

$$c = 2.998 \times 10^8 \text{ m/s.}$$

The speed of light in glass is slower, only about $v = 2.000 \times 10^8 \text{ m/s}$

The refractive index is the ratio of these two speeds

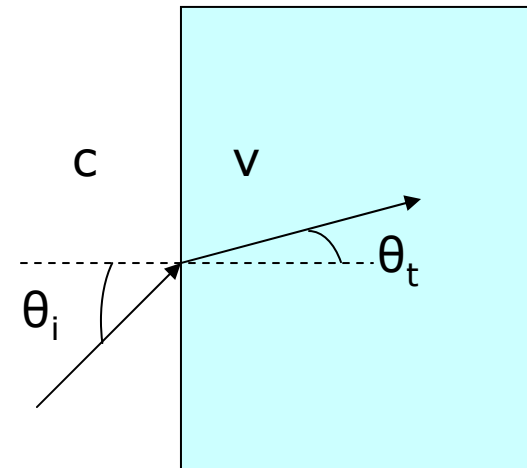
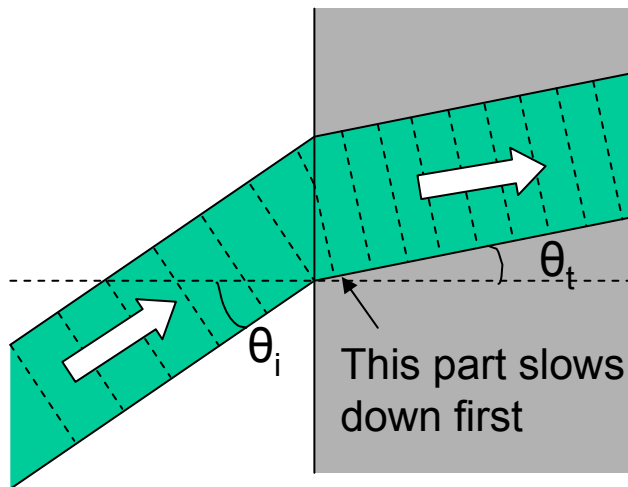
$$n = \frac{c}{v}$$



Refraction and Snell's Law

Snell's law describes refraction of sound (a change in direction as it passes through a boundary).
If the incident angle is not zero and the velocities are not equal.

$$\frac{c}{v} = \frac{\sin \theta_i}{\sin \theta_t} \approx \frac{\theta_i}{\theta_t}$$

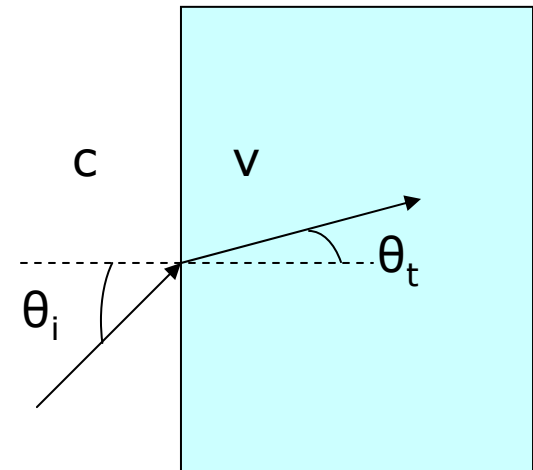


Snell's Law

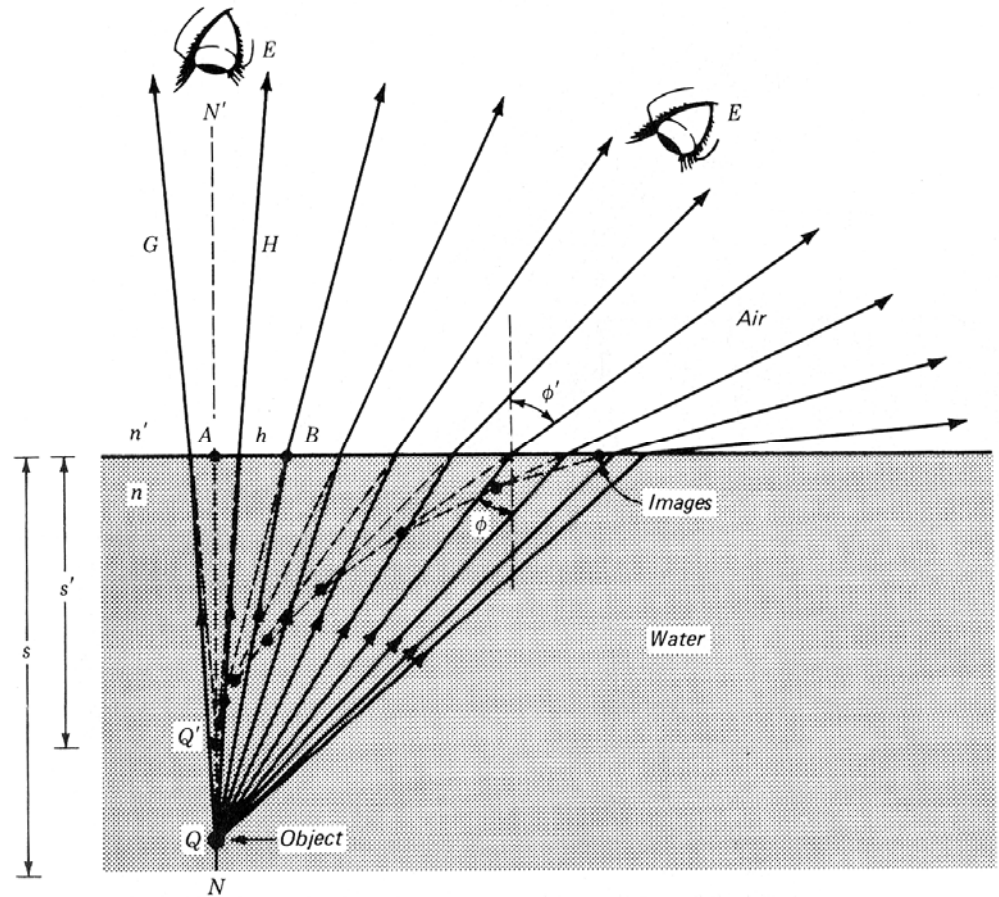
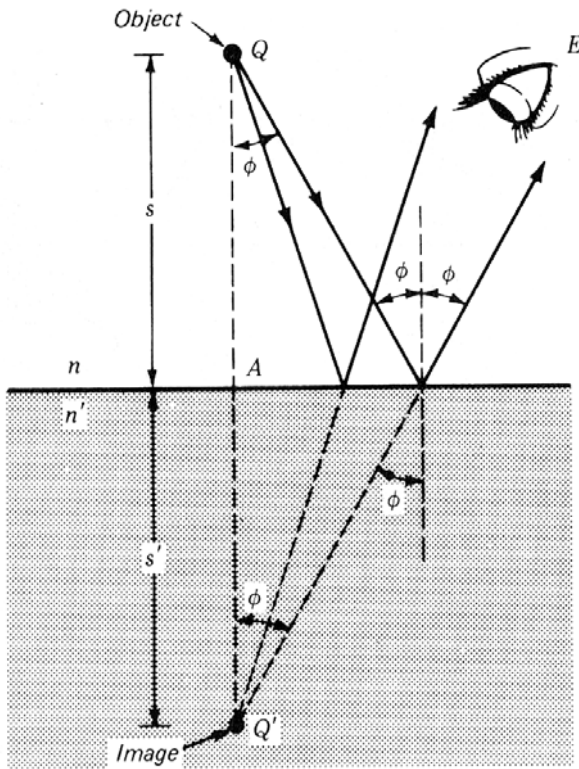
Example) A light wave traveling through a air with propagation speed 2.998×10^8 m/s passes into water with propagation speed 2.308×10^8 m/s with an incident angle of 50° .

What is the angle of transmittance?

$$\frac{c}{v} = \frac{\sin \theta_i}{\sin \theta_t} \approx \frac{\theta_i}{\theta_t}$$

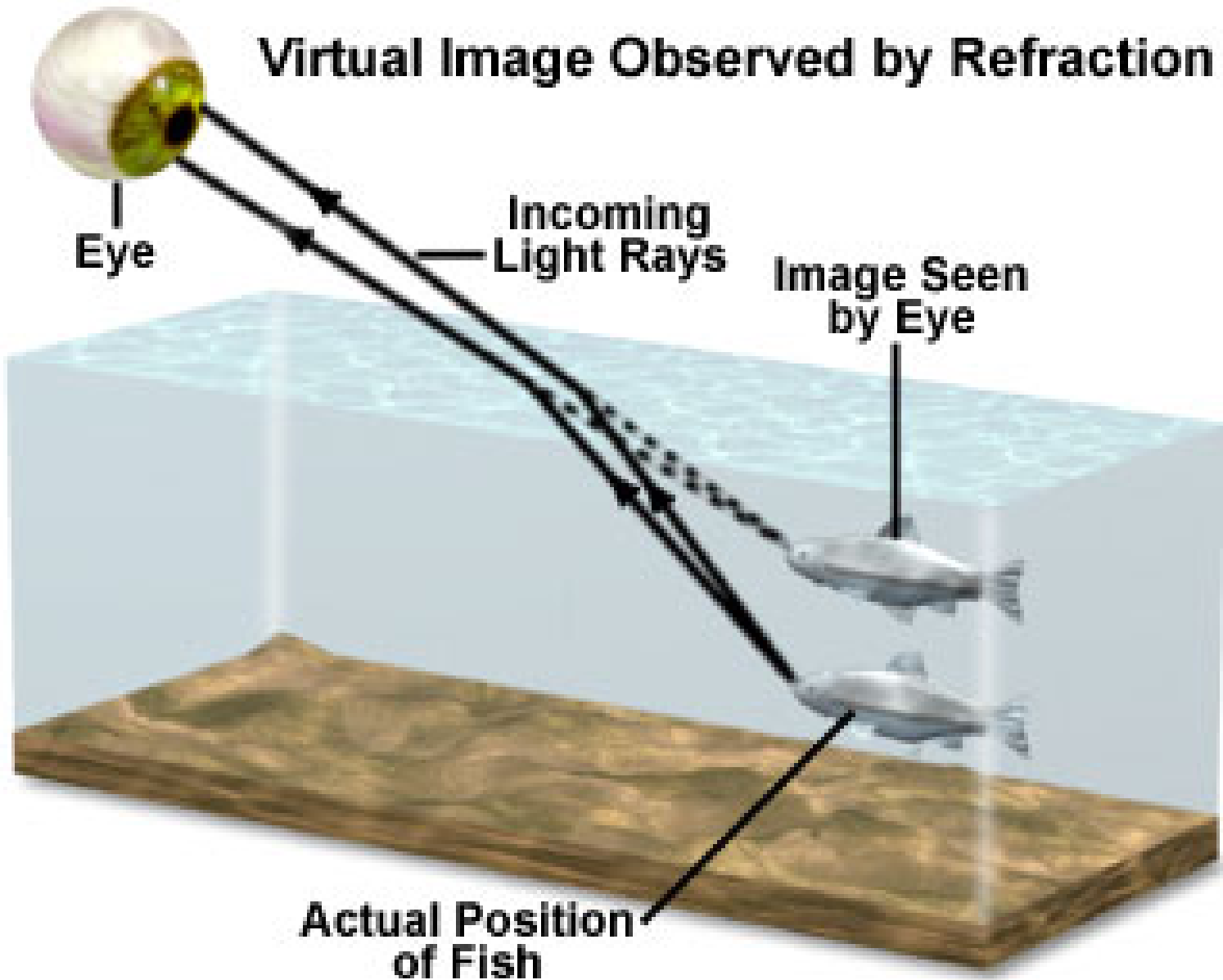


Illusion by refractive index



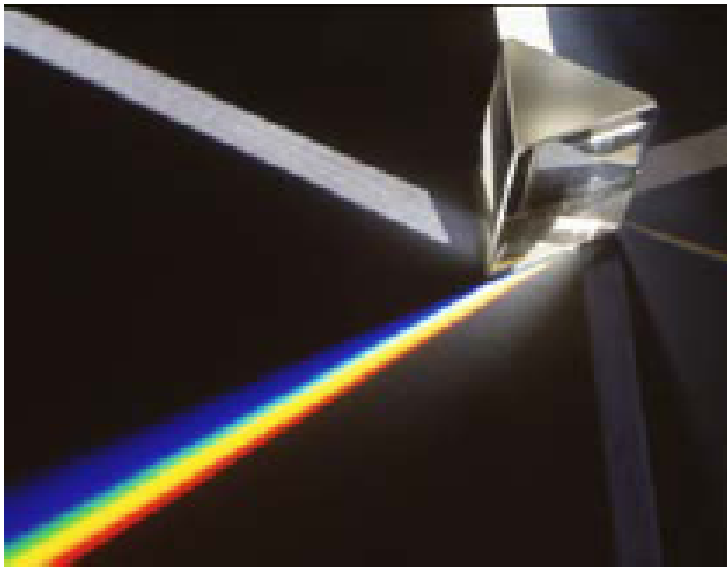


Virtual Image Observed by Refraction

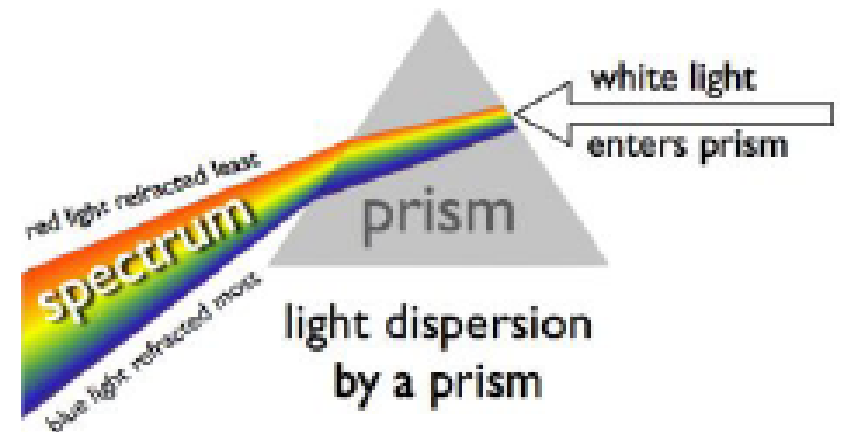


Refractive index

- Depends on wavelength (λ)
- achromatic lenses: minimize this effect



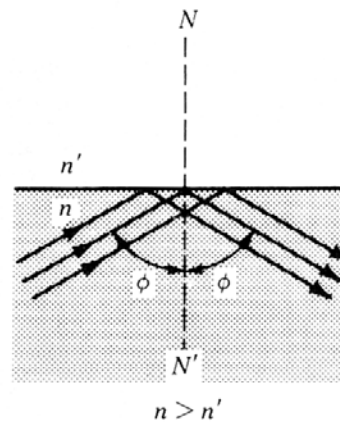
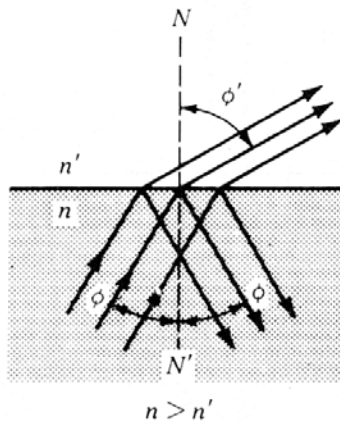
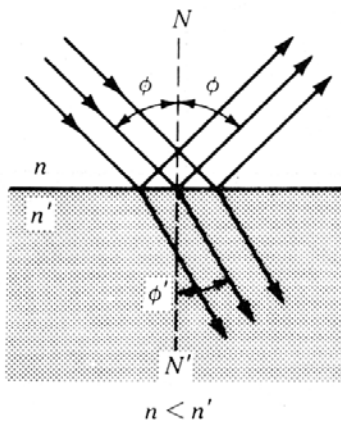
http://gallery.hd.org/_c/natural-science/prism-and-refraction-of-light-into-rainbow-AJHD.jpg.html



www.rkm.com.au/.../animation-physics-prism.html

<http://science.howstuffworks.com/question41.htm>

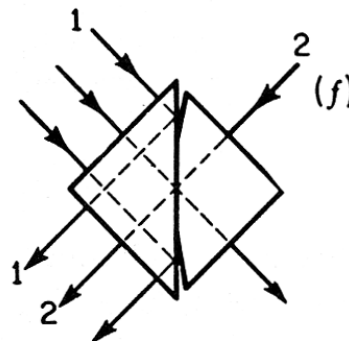
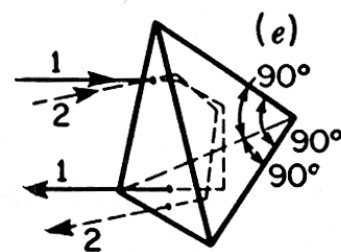
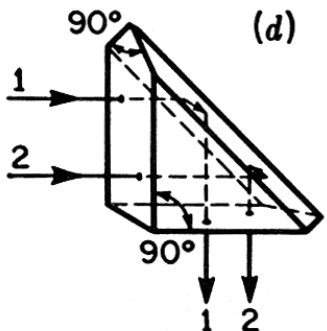
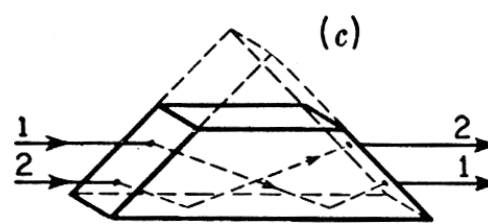
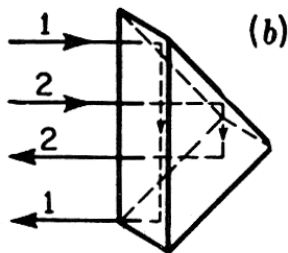
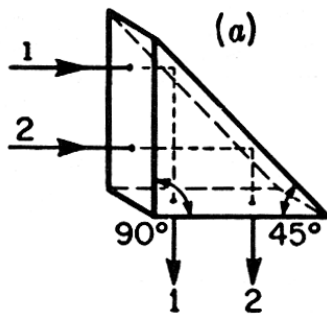
Control of light



Total reflection

Porro

Dove or inverting



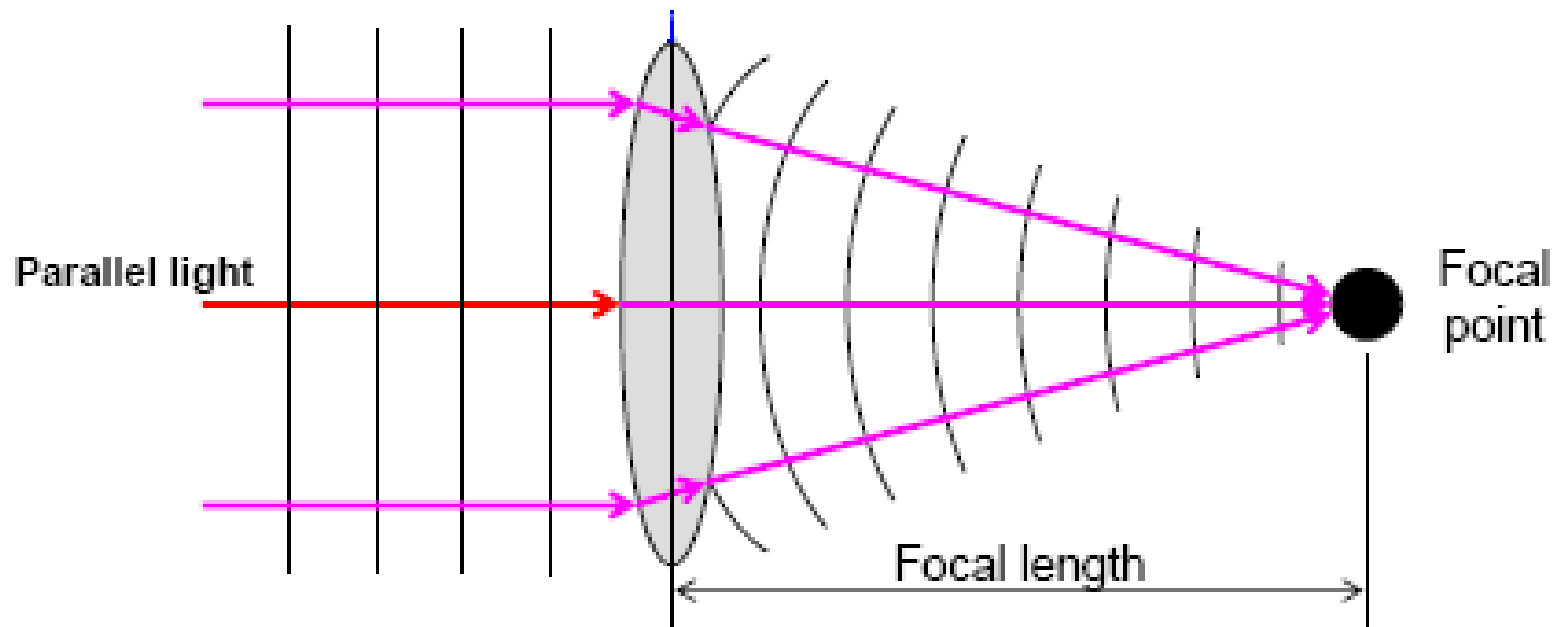
Amici or roof

Triple mirror

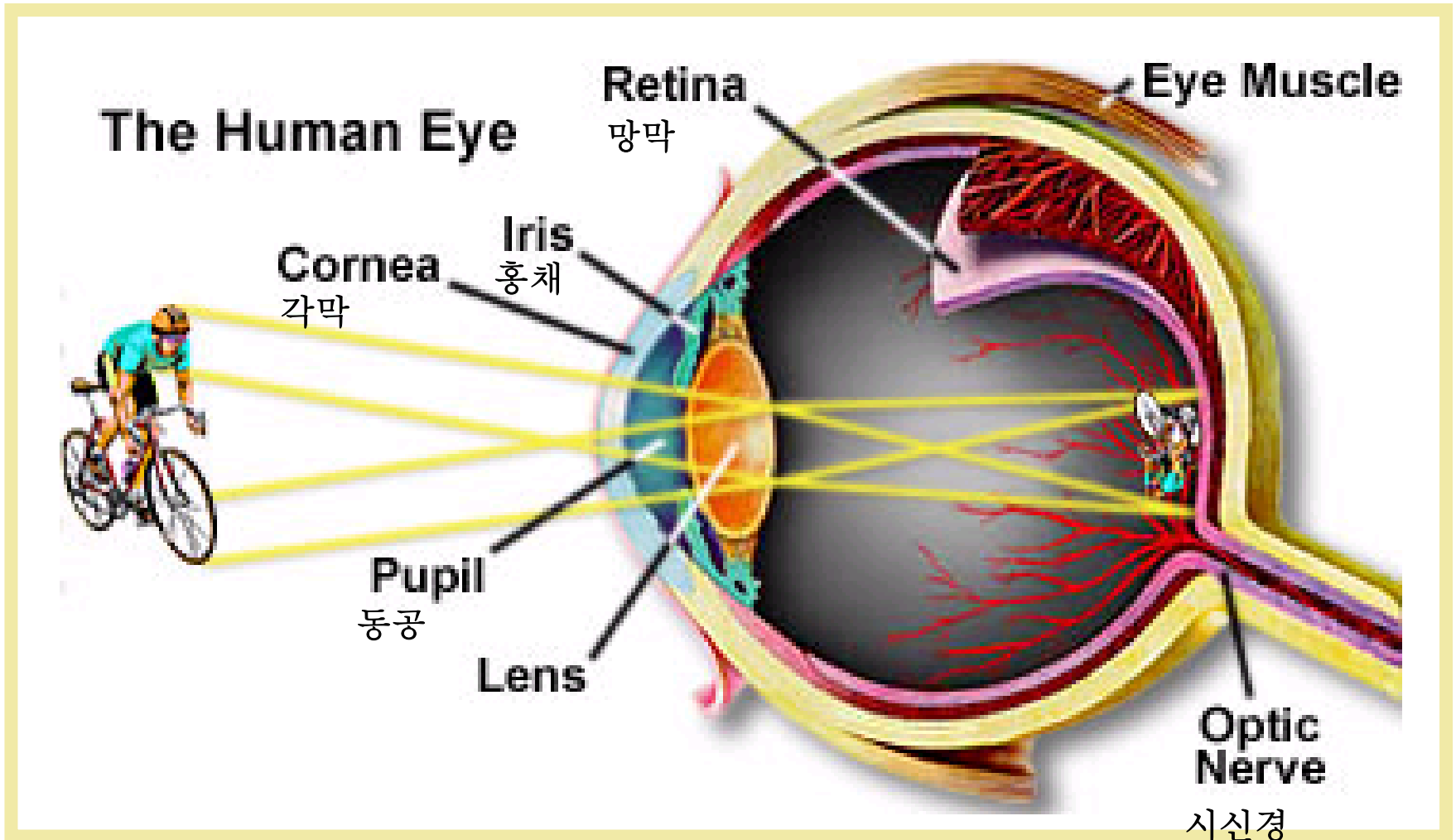
Lummer-Brodhun

Effect of Refraction in Lenses

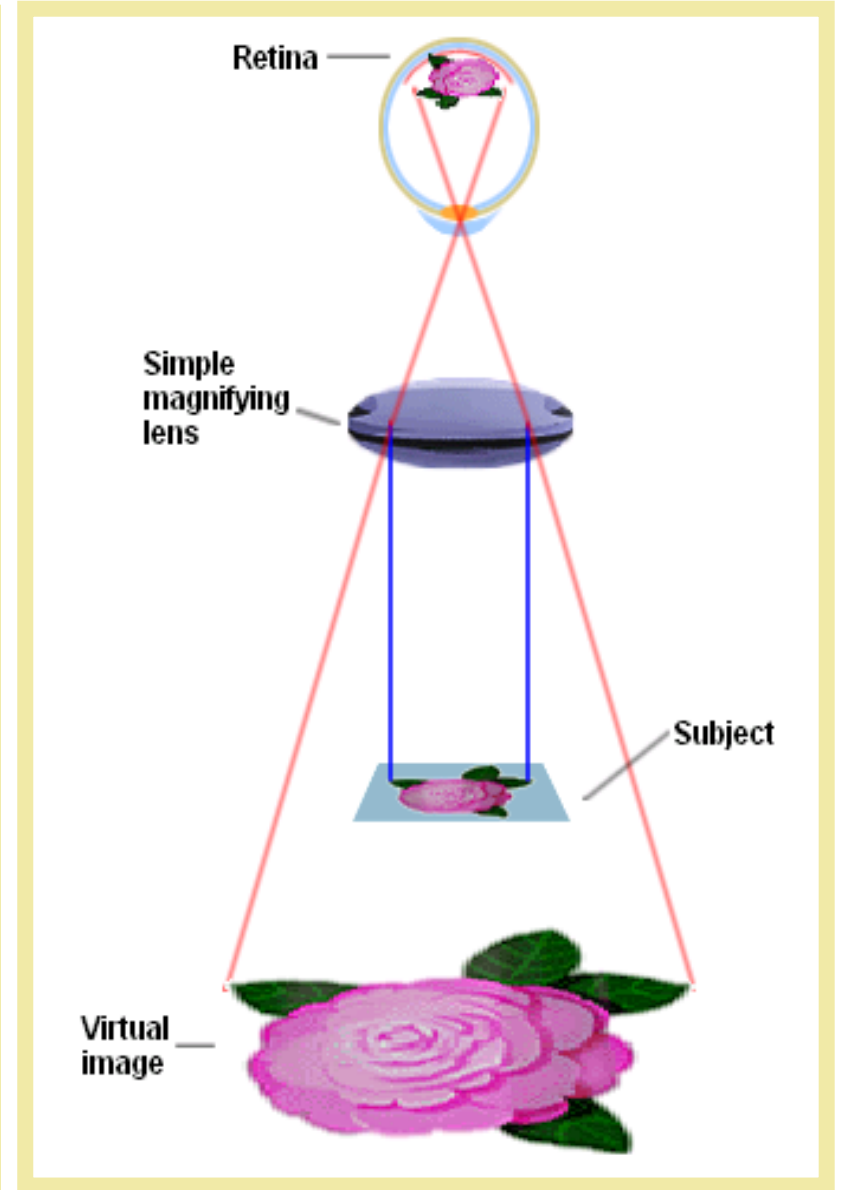
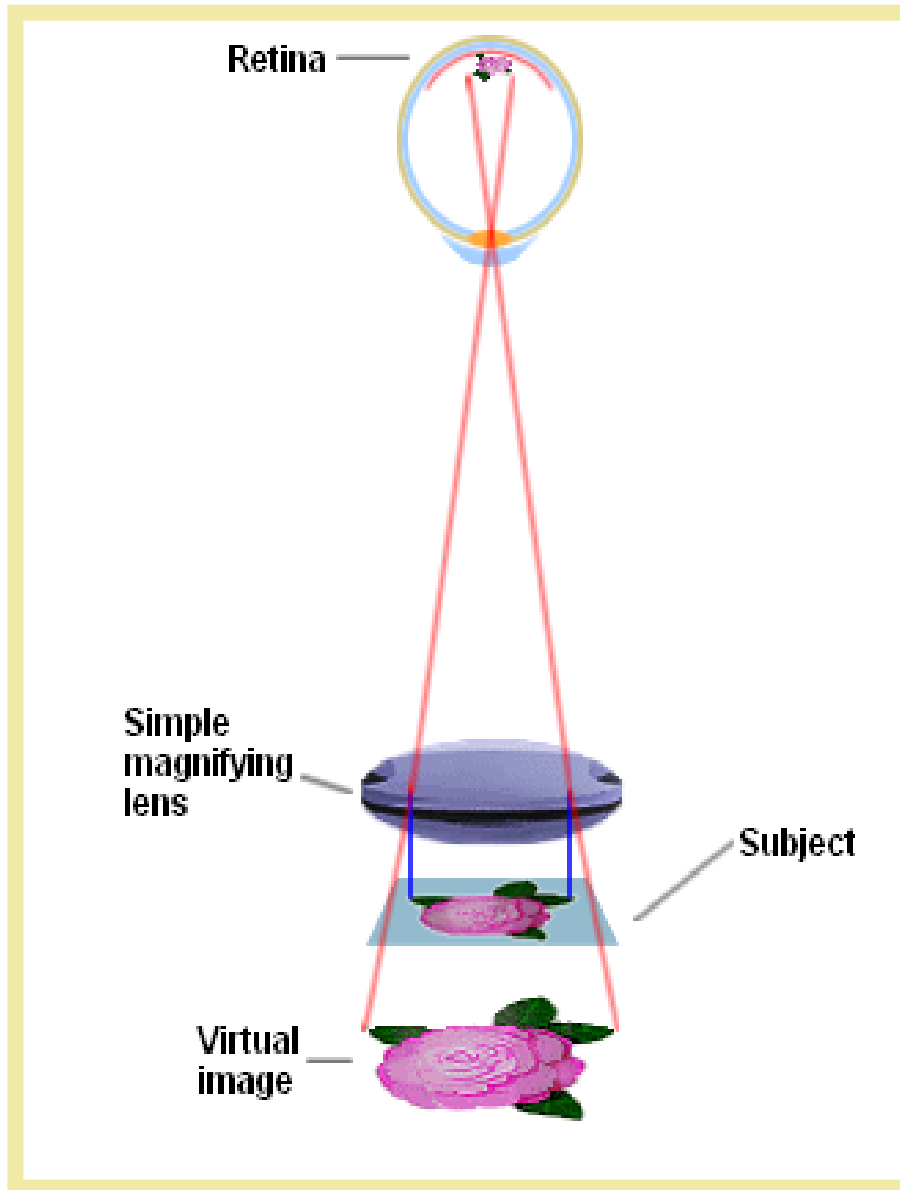
- **Because refractive angle depends on incident angle, convex lenses can be prepared which will focus light to a point at a specified distance form the lens.**



Relationship between the Microscope and the human eye



Relationship between the Microscope and the human eye

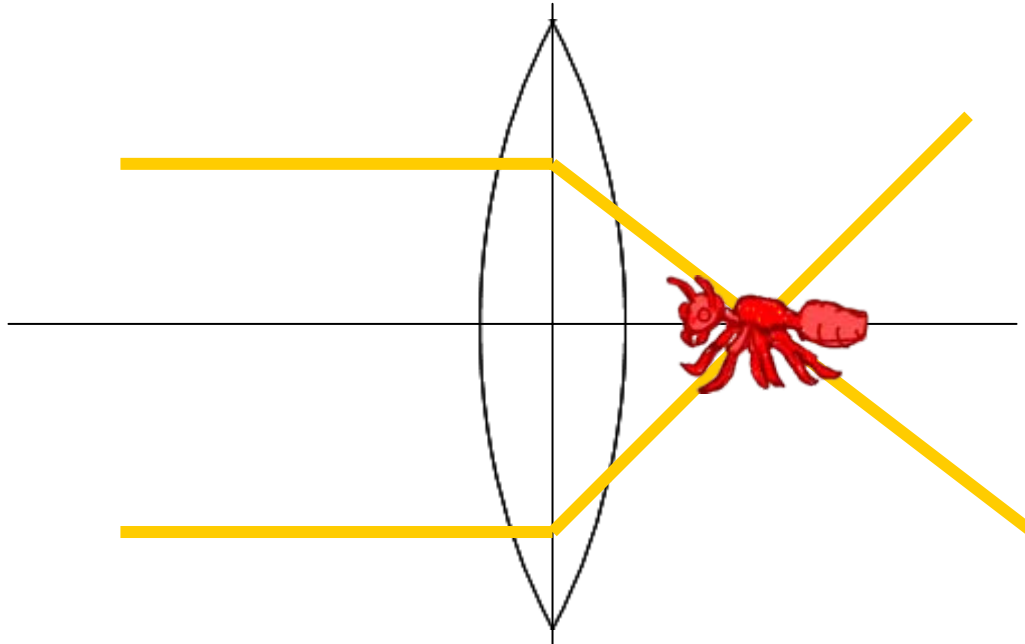


How the Microscope works?

Important Terms

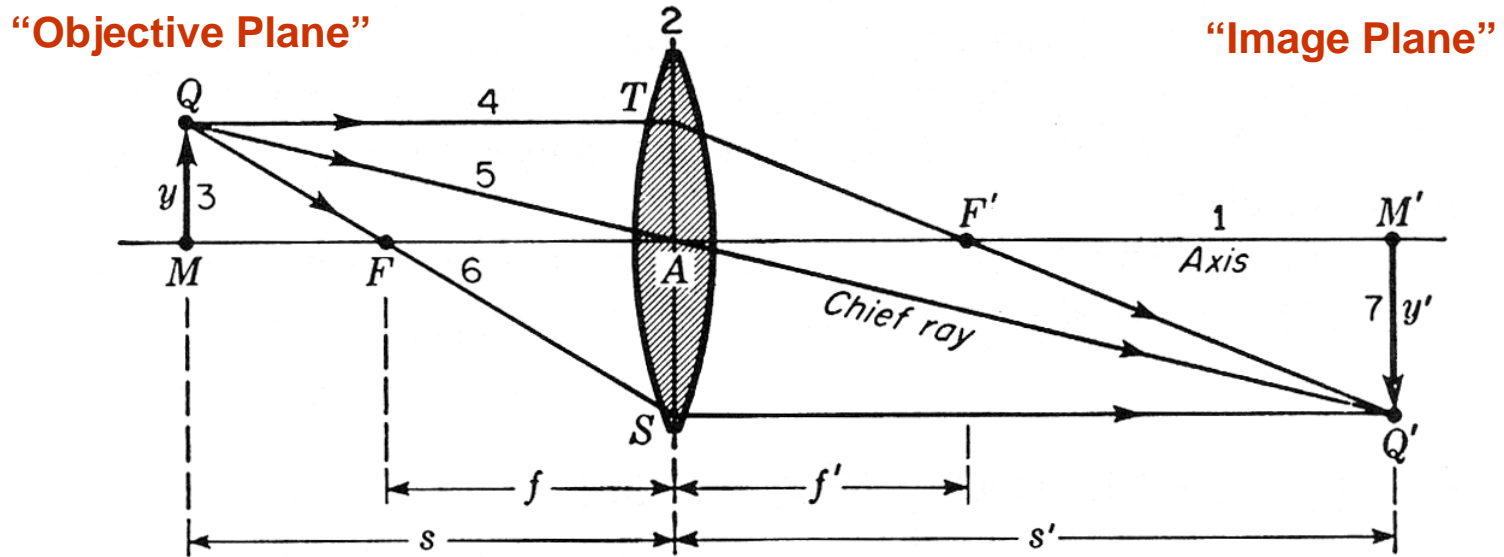
- ***Depth of field*** - vertical distance, from above to below the focal plane, that yields an acceptable image
- ***Field of view*** - area of the specimen that can be seen through the microscope with a given objective lens
- ***Focal length*** - distance required for a lens to bring the light to a focus (usually measured in microns)
- ***Focal point/focus*** - point at which the light from a lens comes together
- ***Magnification*** - product of the magnifying powers of the objective and eyepiece lenses
- ***Numerical aperture*** - measure of the light-collecting ability of the lens
- ***Resolution*** - the closest two objects can be before they're no longer detected as separate objects (usually measured in nanometers)

A Convex Lens



The focal point of a lens

Lens Equation



For focal length f at object at s gives an image at M which is magnified By a factor M , where"

- Focal Length
$$\frac{f}{f''} = \frac{n}{n''}$$

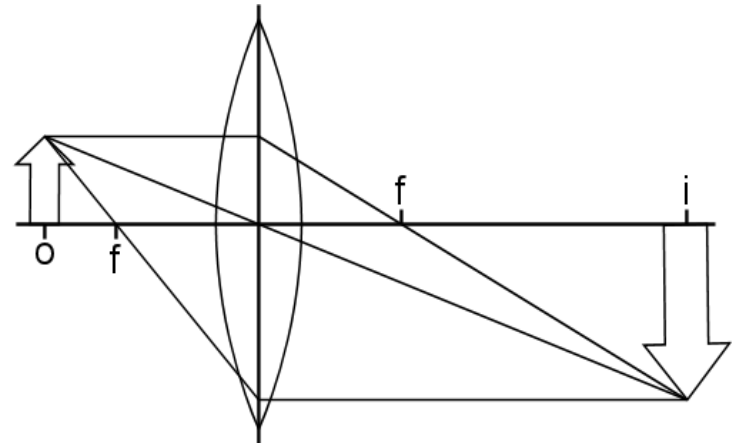
Ignores effects of lens defects, wavelength dependency of index, etc.

- Magnification
$$\frac{1}{f'} = \frac{1}{s} + \frac{1}{s'} \quad \text{and} \quad M = \frac{s'}{s}$$

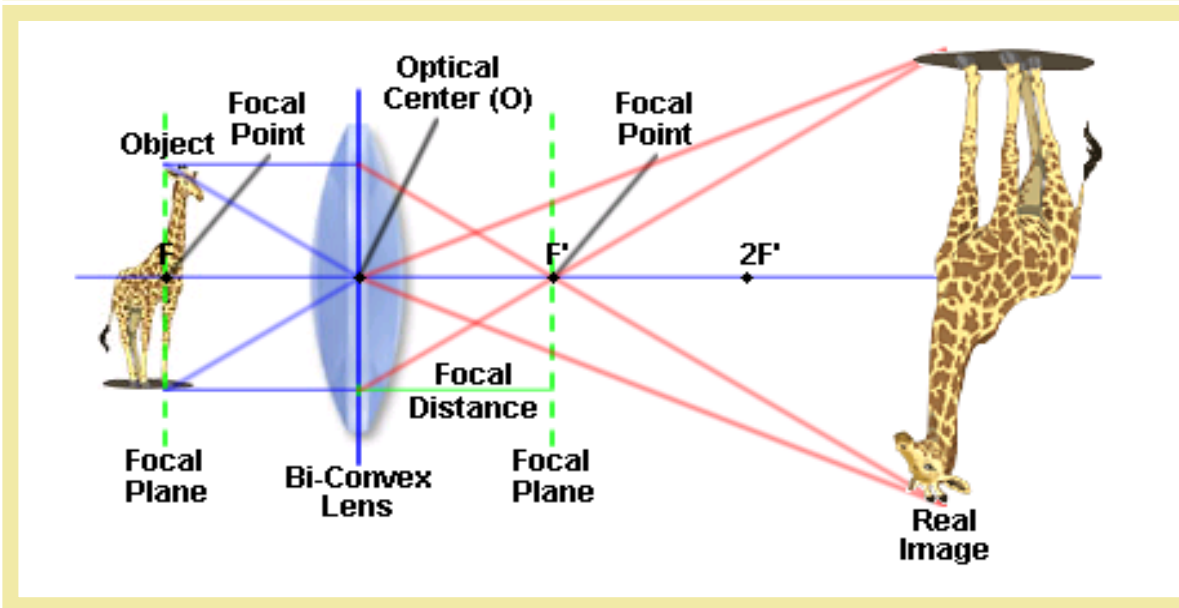
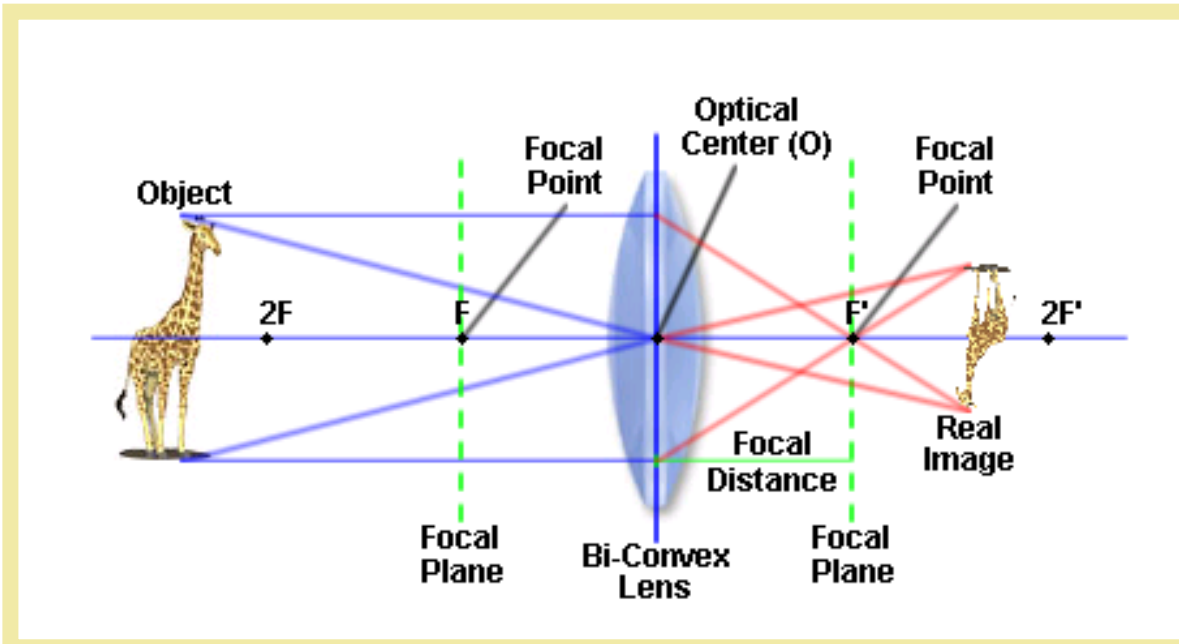
Note: asymmetric lenses can have multiple focal lengths.

Example) Thin Lens Calculations

$$\frac{1}{o} + \frac{1}{i} = \frac{1}{f} \quad M = \frac{-i}{o}$$



If a lens has a 4.5 cm focal length (f) and a specimen is 5.3 cm from it (o) where will the image appear to be and what will be the magnification?



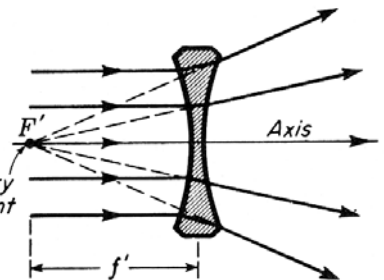
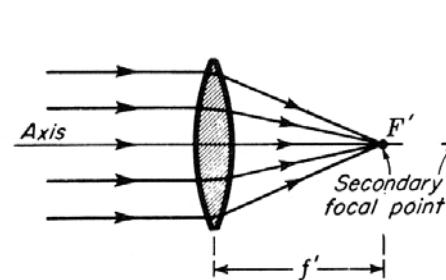
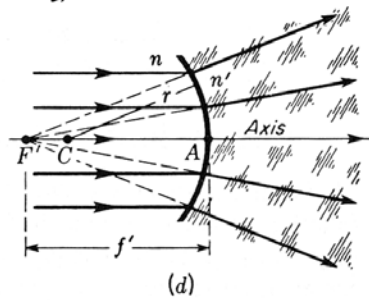
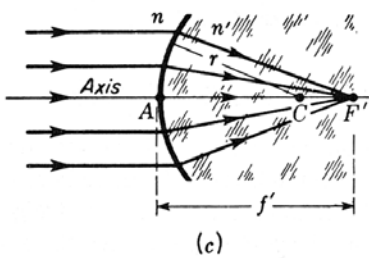
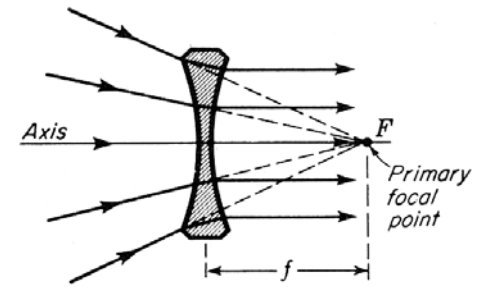
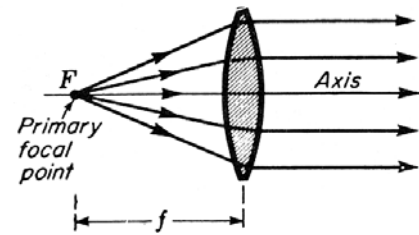
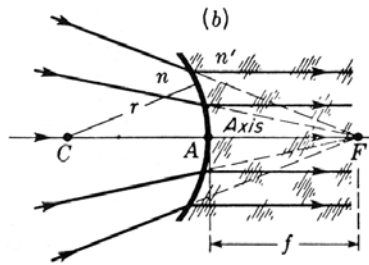
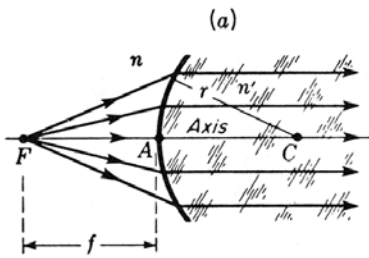
Waves

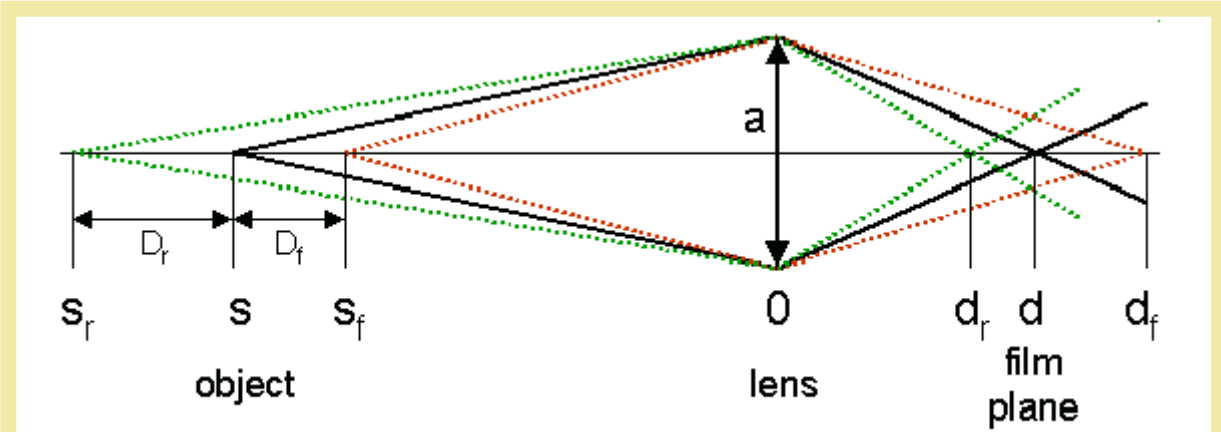
- Concept of Plane wave

- http://physica.gsnu.ac.kr/physedu/wavelight/lens_mirror/mirror.html

부제: 광선과 거울

Focusing light





Little/Shallow depth of field



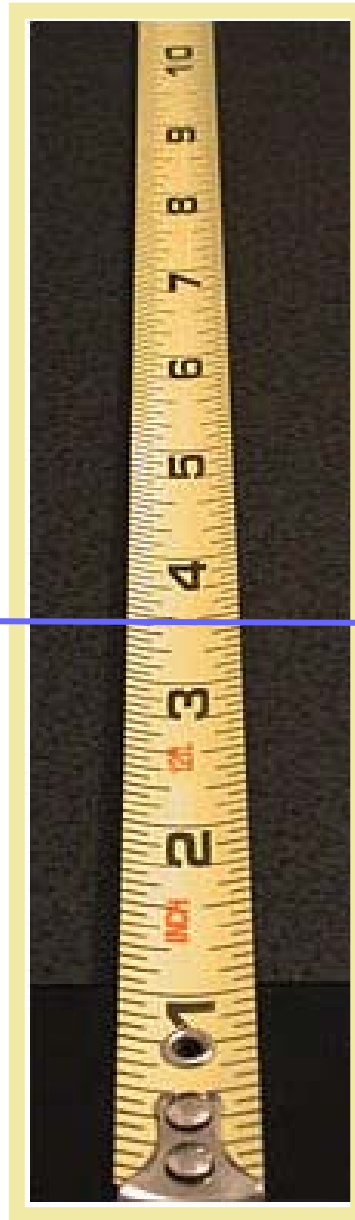
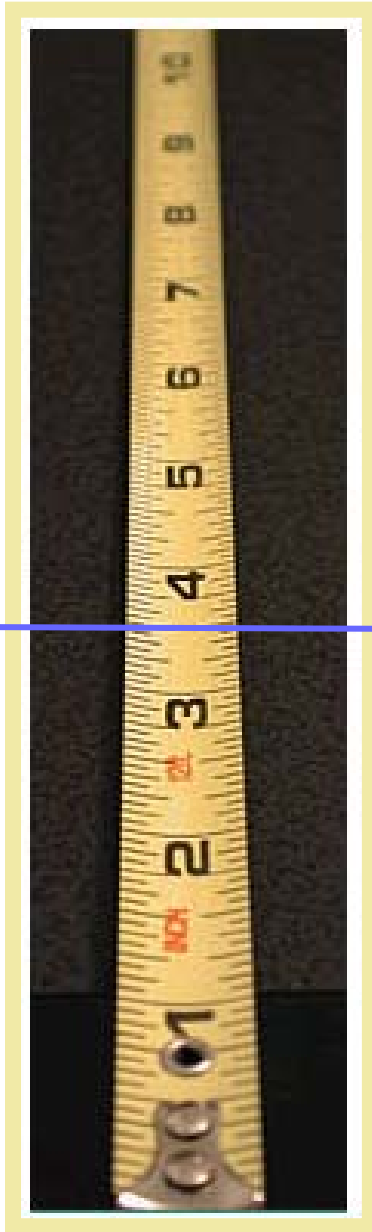
Greater/More depth of field



Little depth of field

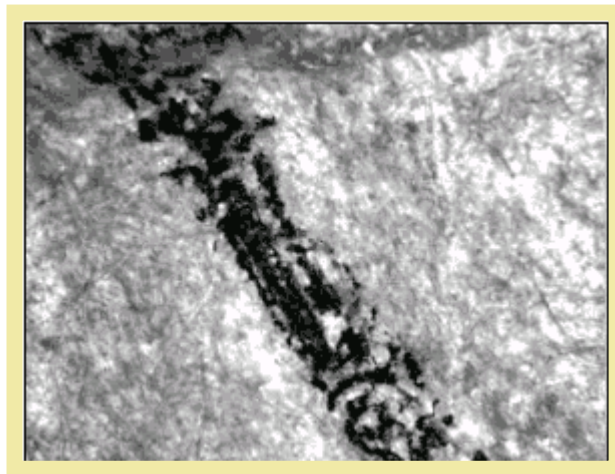
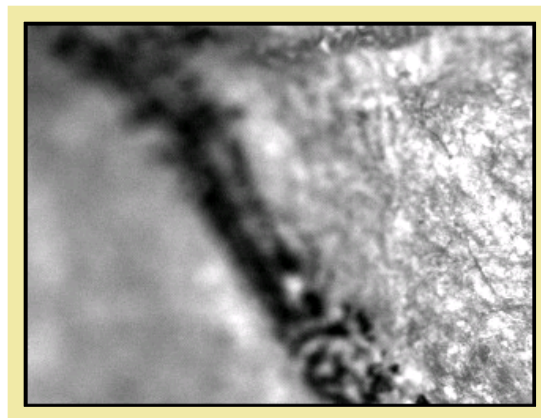
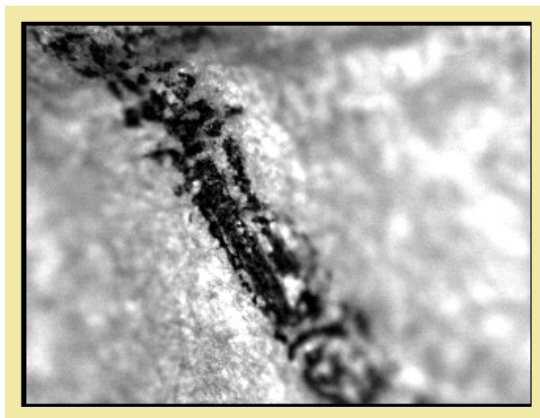
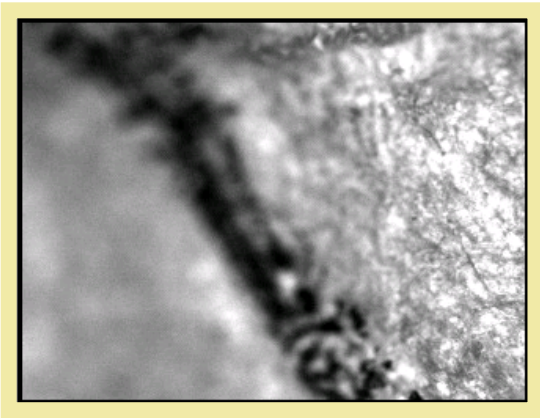


Depth of Field



Focal Plane

Depth of Field



Magnification

- Objective lenses up to 100x or 150x
- Eyepieces 10x ~ 15x
- Magnification = OL x EP
- The limit of optical magnification is about **2000x**.

Resolution is the ability to tell two points apart as separate points. If the resolving power of your lens is $2\mu\text{m}$ that means two points that are $2\mu\text{m}$ apart can be seen as separate points. If they are closer together than that, they will blend together into one point.

Optical microscopes are used daily in our lives for **example eyeglasses and a simple magnifying glass**. To increase the magnification of a microscope the number of lenses must be increased. Although sometimes the image becomes unclear that's when the microscope's resolving power decreases. The resolving power is the microscope's ability to produce a clear image.

In the 1870s, a man named Ernst Abbe explained why the resolution of a microscope is limited. He said that since **the microscope uses visible light and visible light has a set range of wavelengths**. The microscope can't produce **the image of an object that is smaller than the length of the light wave**.

The value for the resolution of a light microscope has been constant at **200 nm** (2,000 angstroms).