臺北醫學大學

Biomedical Imaging
生物醫學影像學

楊自森 助理教授

牙體技術學系

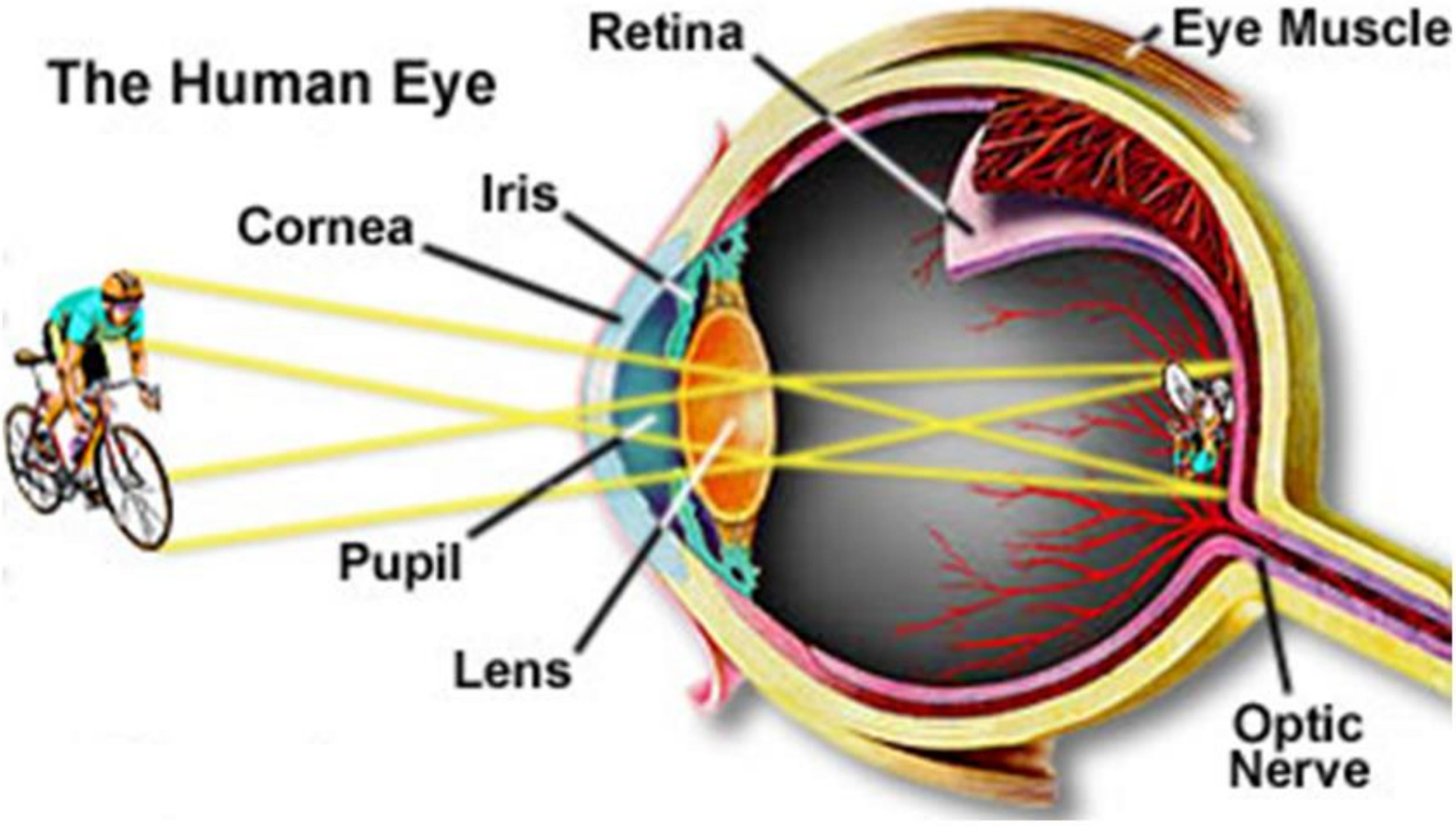
2013/02/24

tsyang@tmu.edu.tw

Course Outline

1. Course Introduction
2. **Basic Optics and Light Microscopes**
3. Fluorescence/Confocal/TIRF Microscopes
4. FRET Techniques and Photo-Spectroscopic Imaging
5. Single Molecule Detection
6. Cell Imaging
7. Atomic Force Microscopy (AFM)
8. Scanning Electron Microscope (SEM)
9. Transmission Electron Microscopy (TEM)
10. Digital Image Processing Using MATLAB

The Human Eye



http://www.olympusmicro.com/primer/anatomy/introduction.html

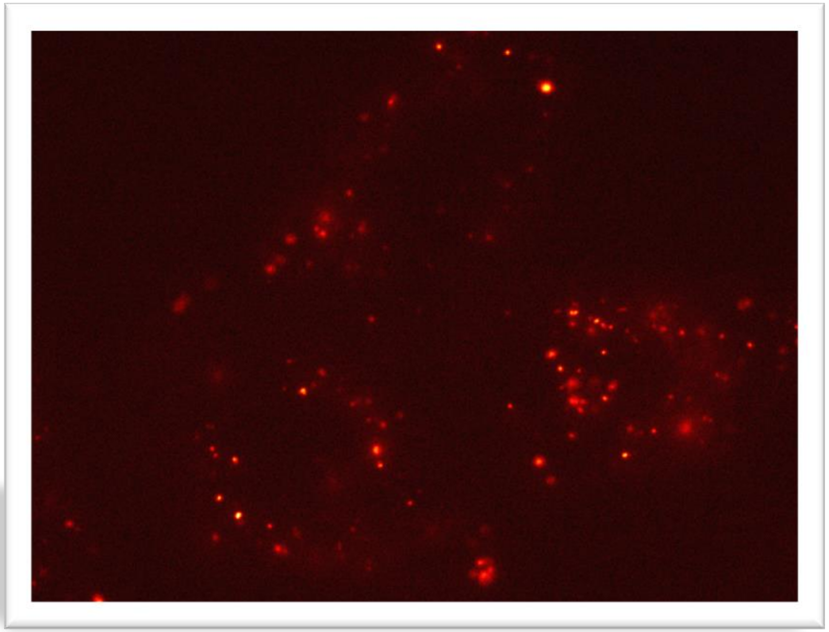
1858

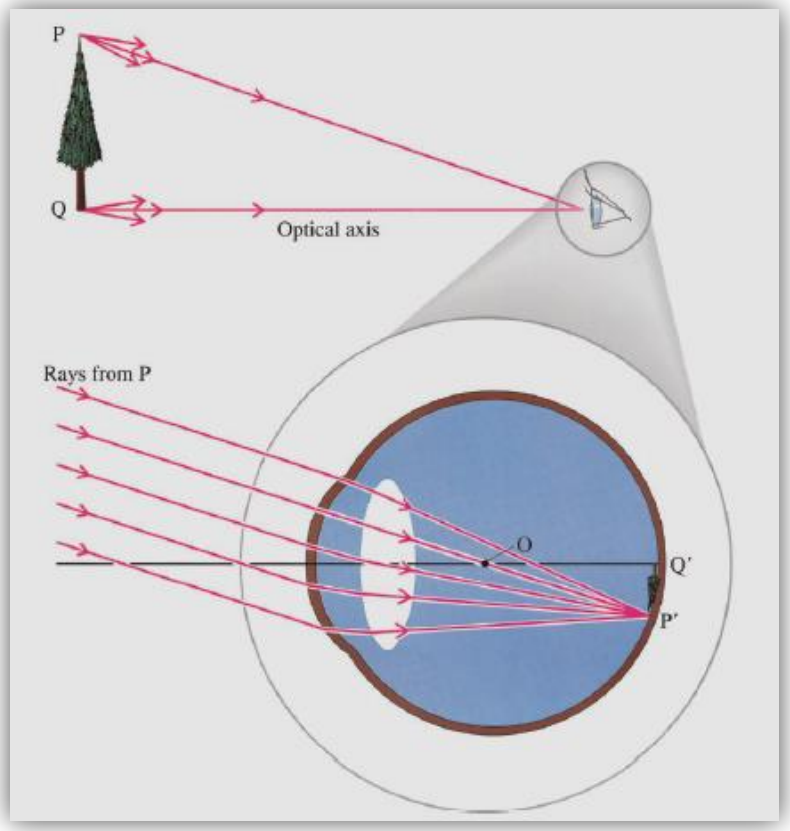
1881

1910

The Eye and Optical Instruments

The magnifying glass, the microscope, and the telescope are optical instruments, all of which have the same general purpose: to increase the size of the retina's image of an object viewed through them.





Part I

Basic Optics

Hubble Telescope—Deep Space Photo http://www.hubblesite.org

Descriptions of Light

Macroscopic View of Light

Quantum properties

Polarization

Electromagnetic Waves ⇐ **Photons**

Absorption and emission of light

Interference and diffraction

Scalar Waves (Electric Vector)

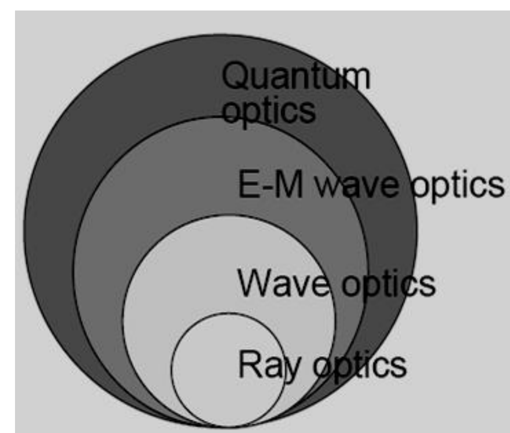
Particle optics

Laws of refraction and lens design

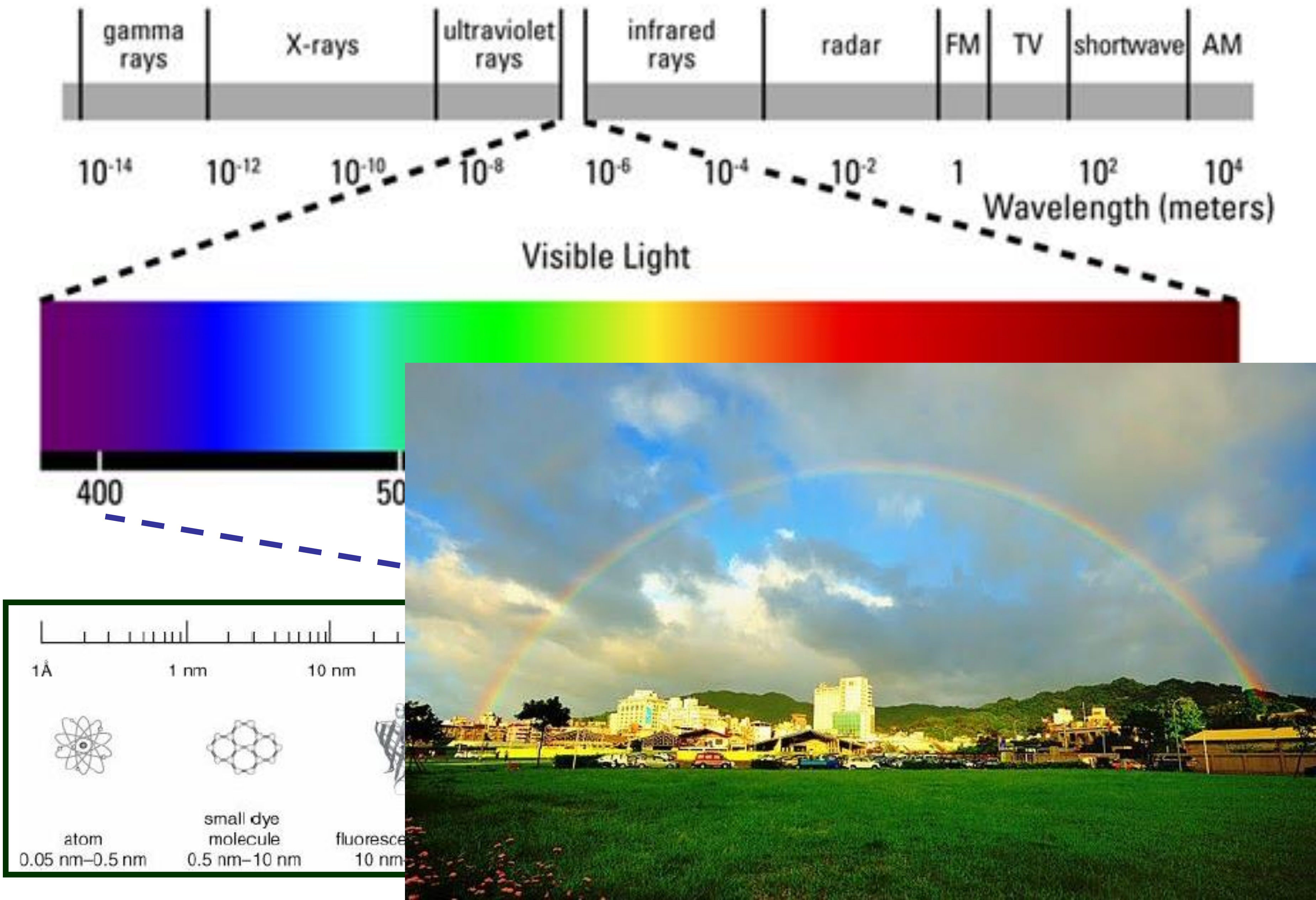
Ray/Geometrical Optics

First-order optics and thin-lens formula

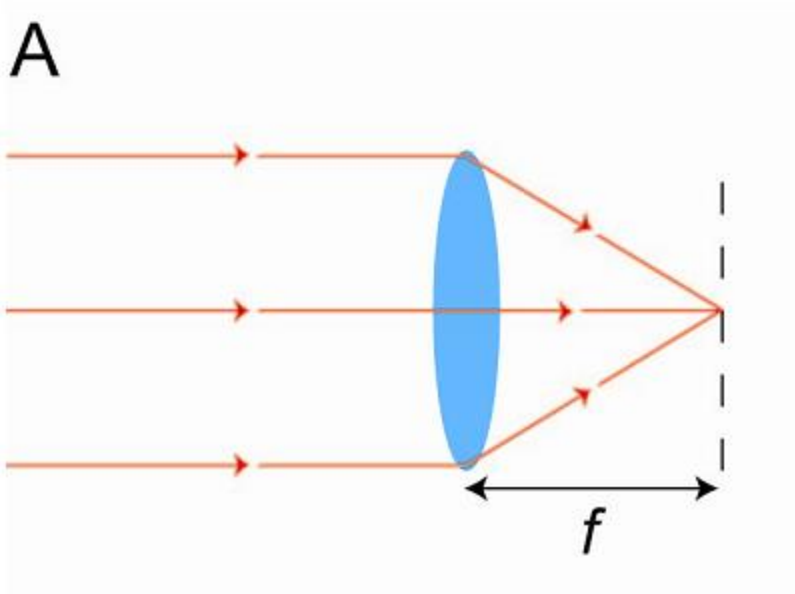
Gaussian/Paraxial Optics



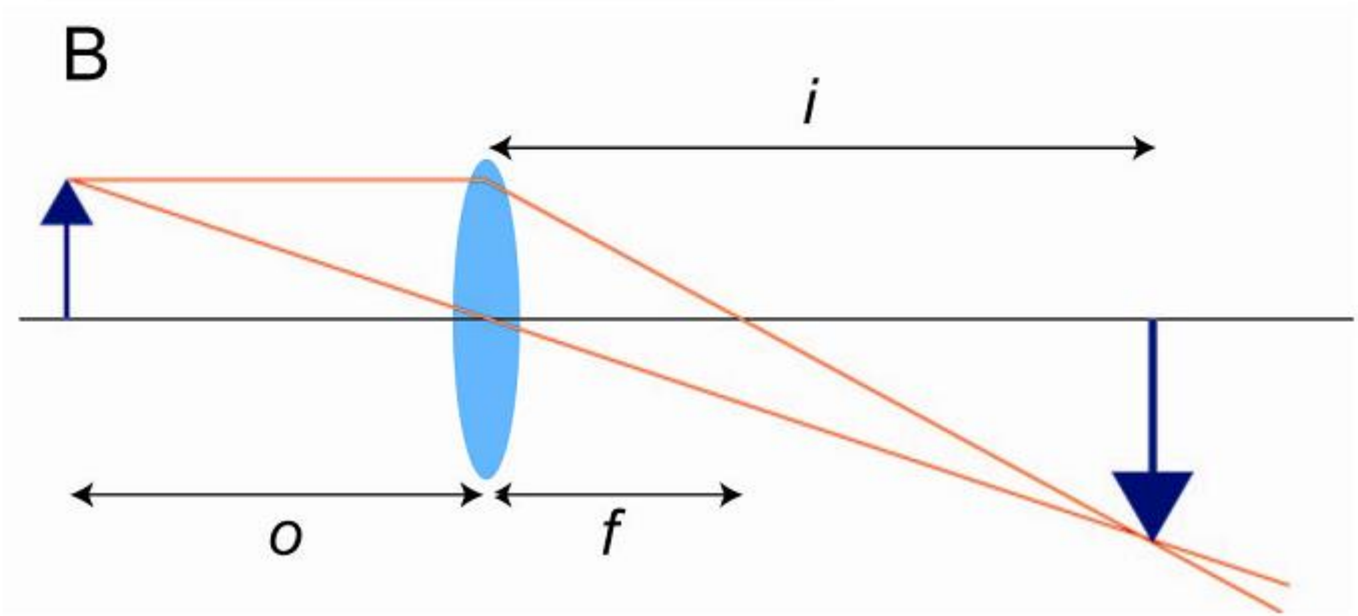
The Electromagnetic Spectrum



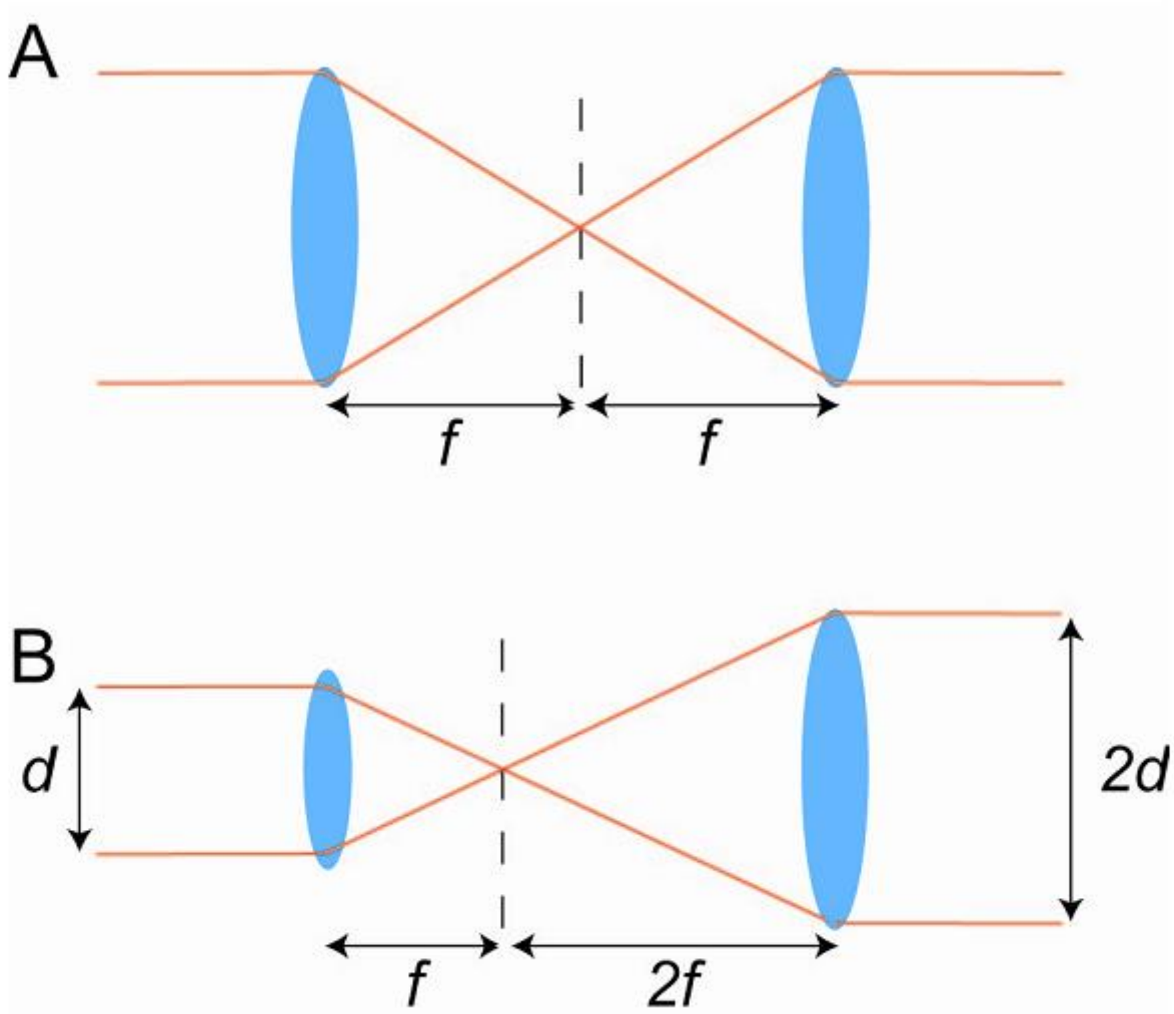
Lens Basics



$$\frac{1}{i} + \frac{1}{o} = \frac{1}{f}$$

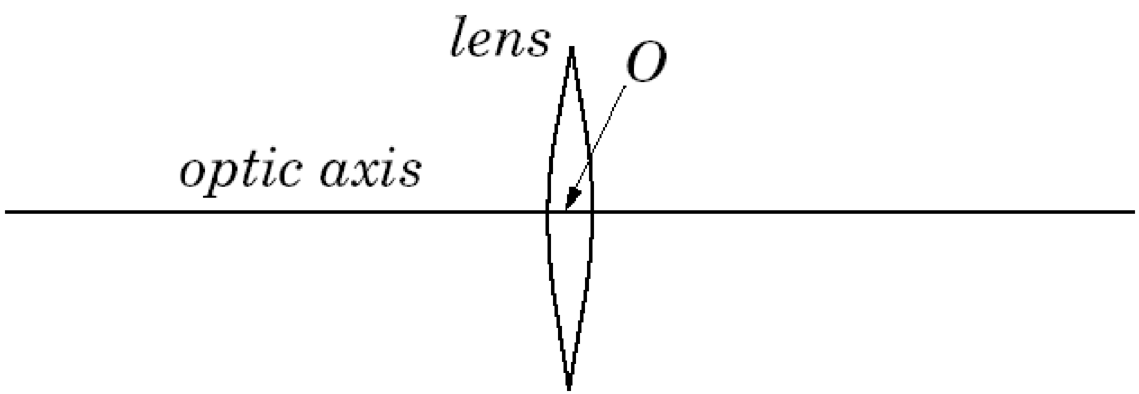


Keplerian Telescopes



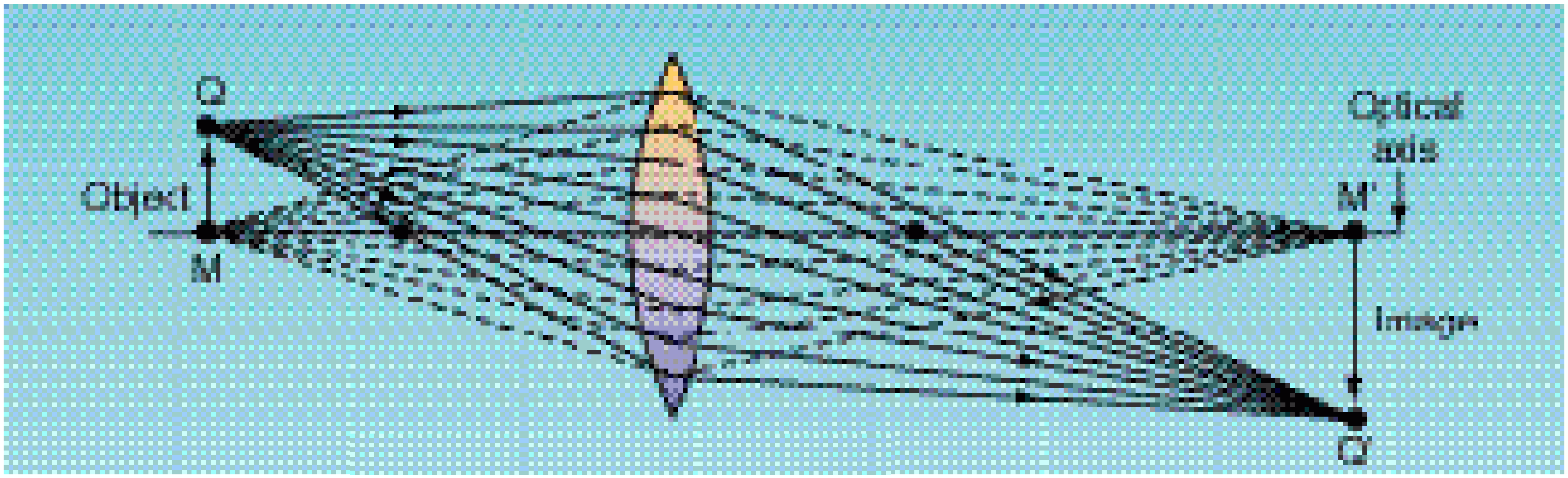
Gaussian/Paraxial optics

~ Paraxial Theory (近軸近似) ： ***thin lens***



Gaussian Optics

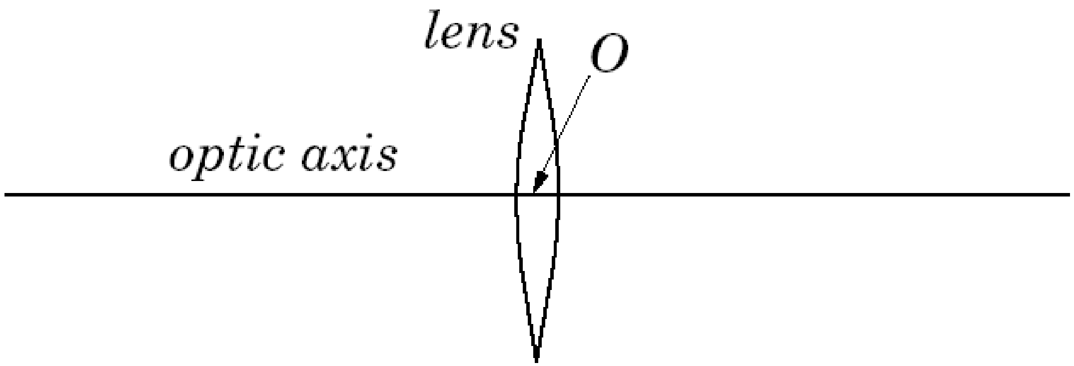
object image



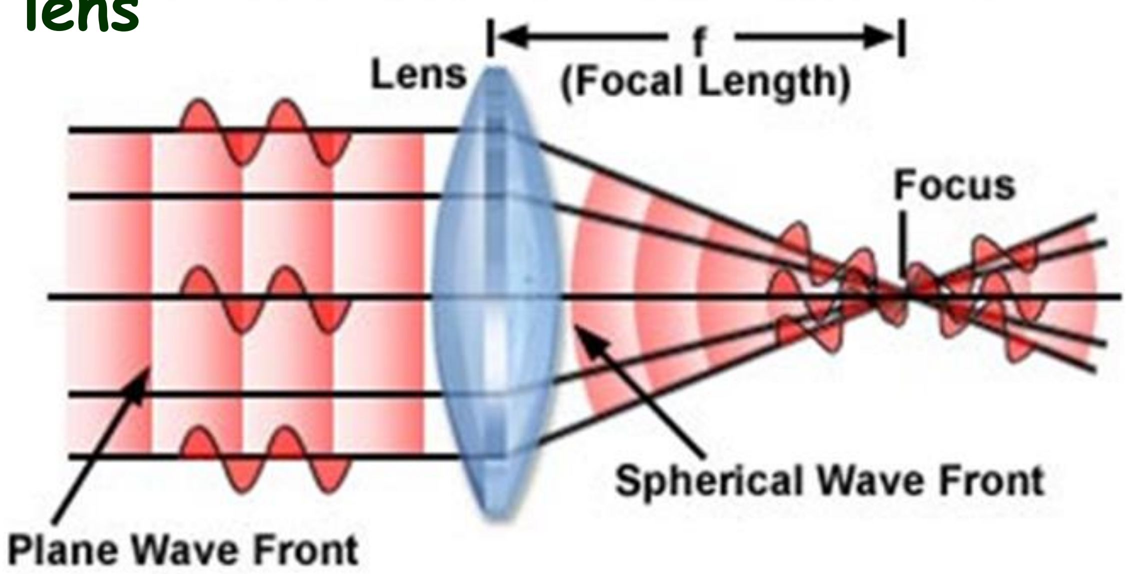
In Gaussian optics, a convex lens will focus light rays from each **point** of the object to a corresponding **point** in the image. The imaging characteristics of this spherical lens can be determined by drawing a set of rays.

Paraxial Theory (近軸近似): thin lens

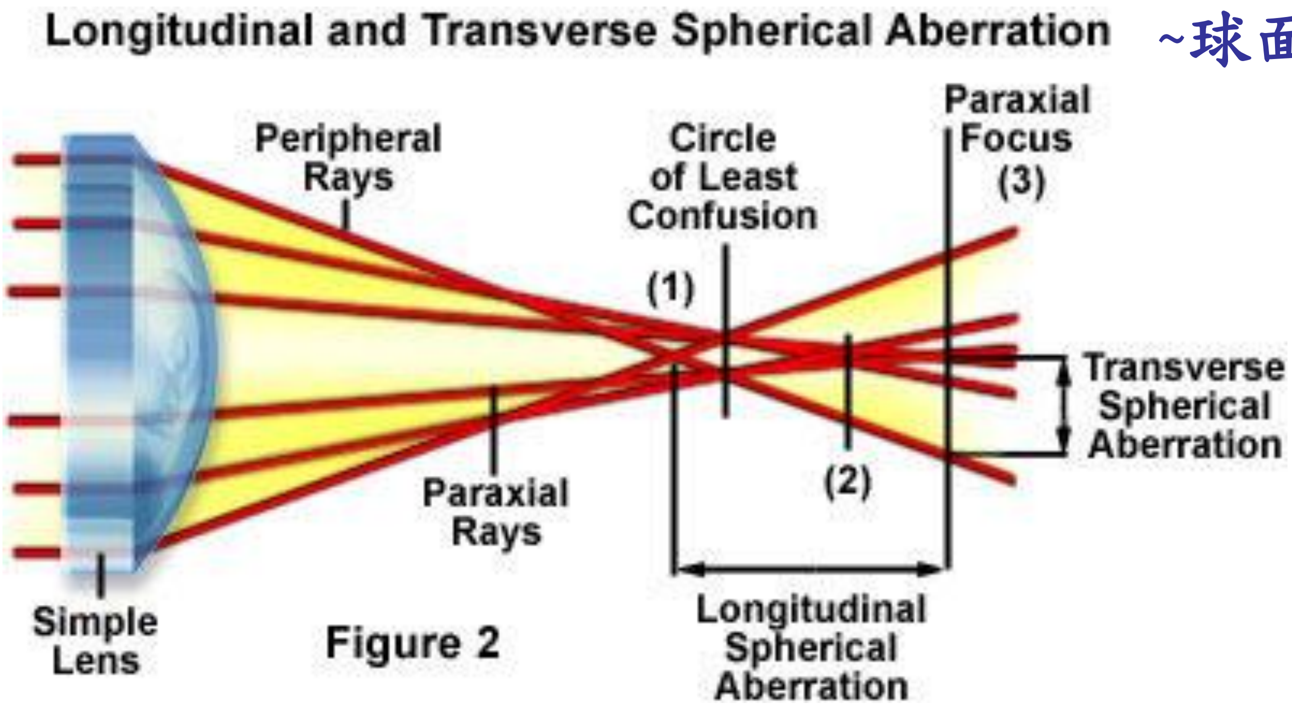
Based on Gaussian optics, assume the rays all make <u>small angle</u> with the optical axis.



A perfect lens



Spherical Aberration: Non-paraxial Optics

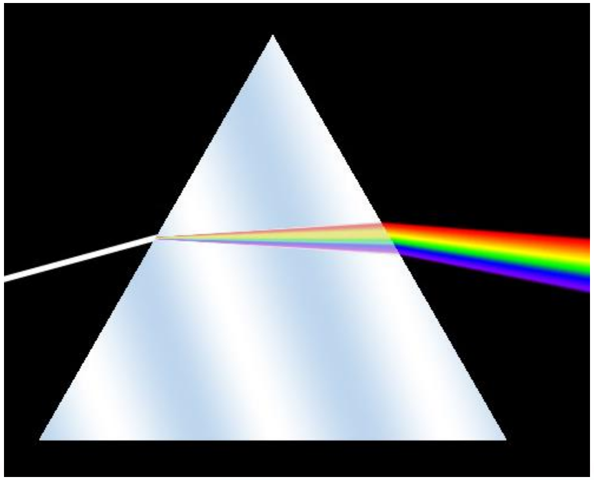


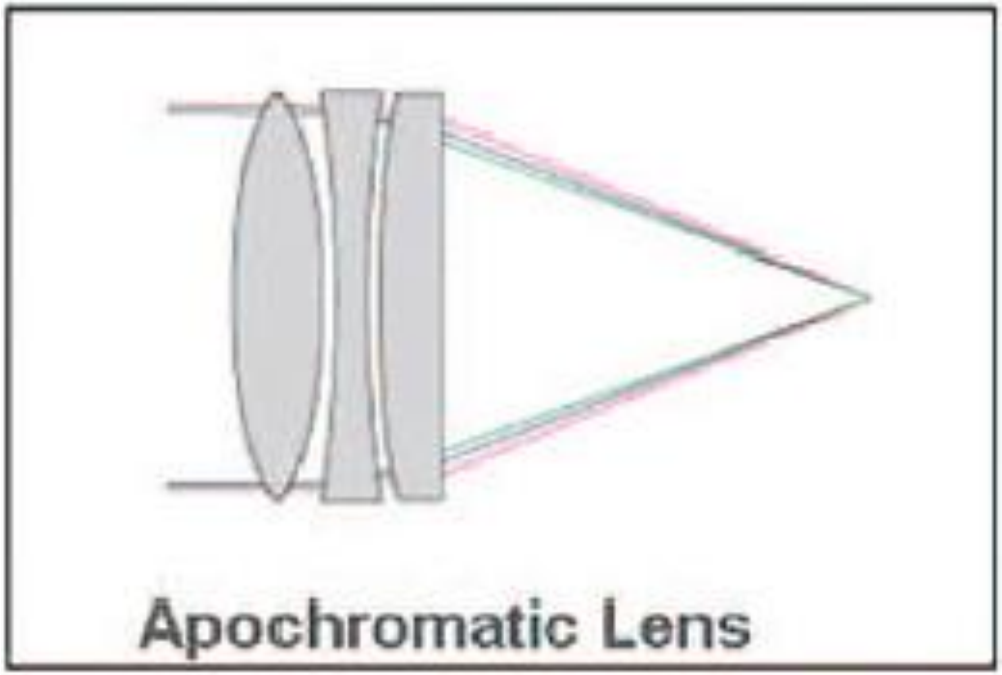
~球面像差

- These artifacts occur when light waves passing through the periphery of a lens are not brought into focus with those passing through the center as illustrated in Figure 2. Waves passing near the center of the lens are refracted only slightly, whereas waves passing near the periphery are refracted to a greater degree resulting in the production of different focal points along the optical axis. This is one of the most serious **resolution artifacts** because the image of the specimen is spread out rather than being in sharp focus.

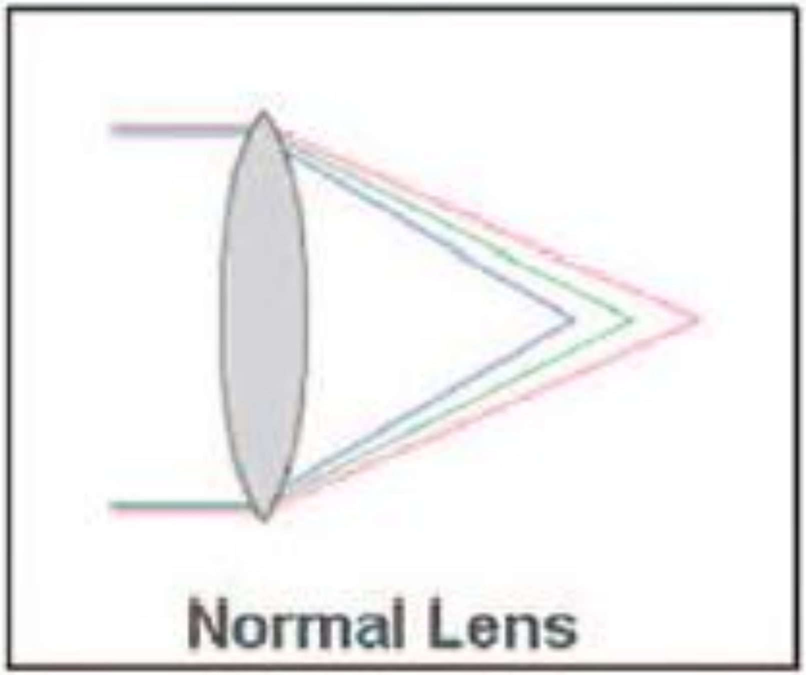
Chromatic Aberration (色差)

Apochromatic （消色差） optics design could correct the chromatic aberration of three types of colored light effectively. It ensures the reduction and saturation of image color and improves the resolution, contrast and depth of image.





Color Aberrations from Visible Lights Are Corrected



Visible Light Refraction Forms Color Aberrations

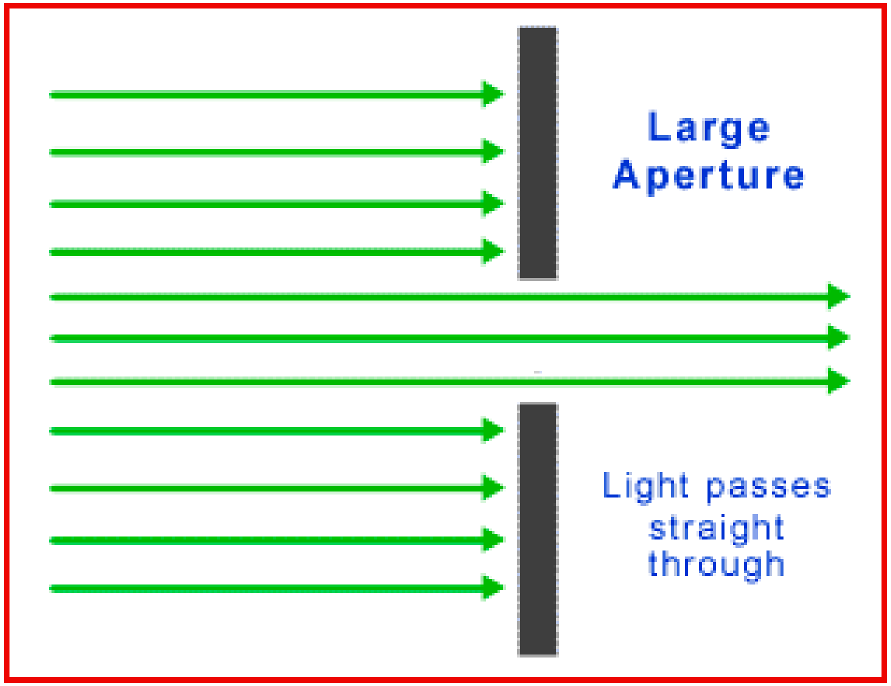
Wave Optics

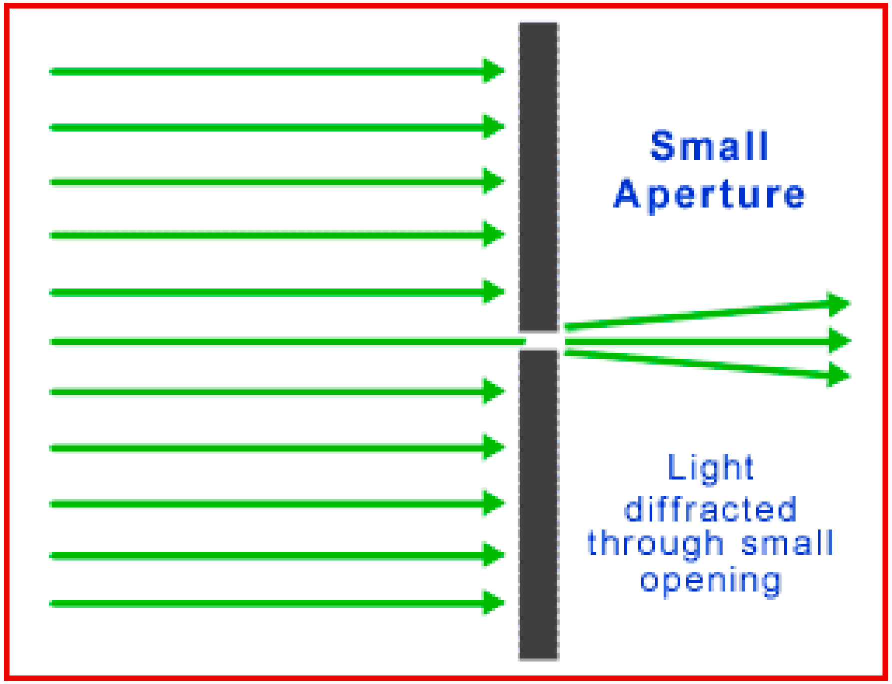
Propagation of Waves:
Interference and Differaction

Basic Concepts - Wave Optics

Ray/Geometrical Optics

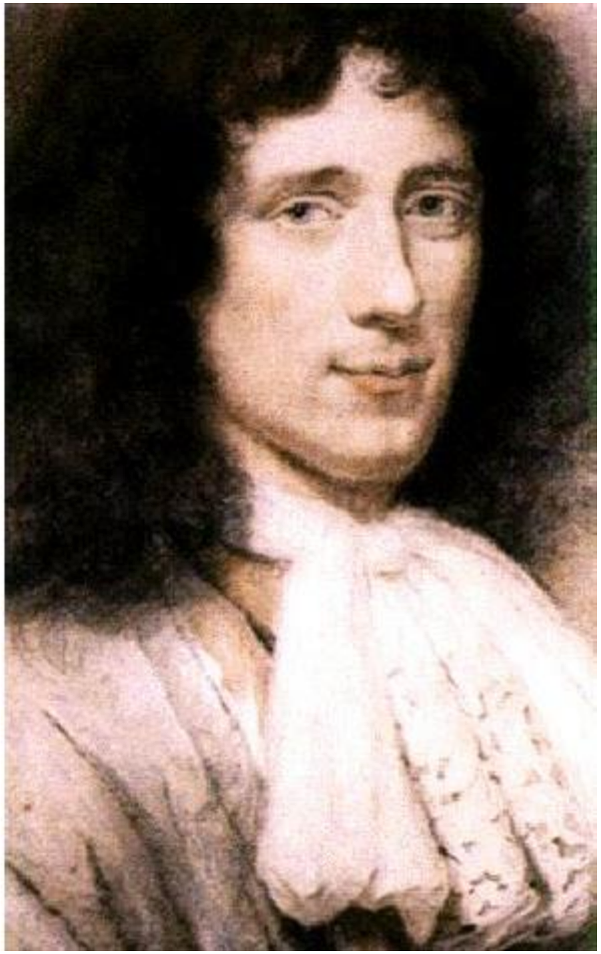
Wave Optics





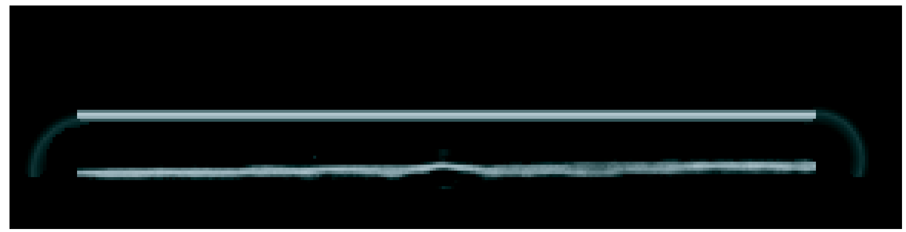
objects of sizes comparable to or smaller than a wavelength of light.

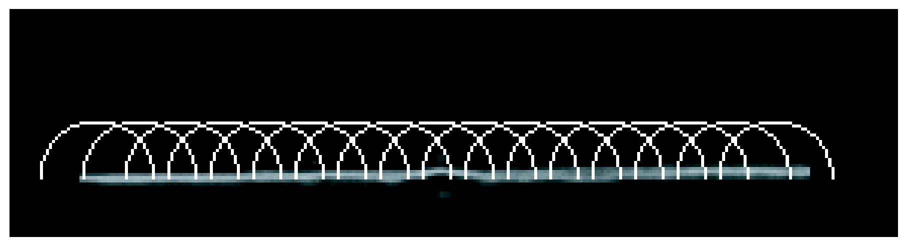
The Correspondence Principle



Christiaan Huygens (1629-1695).

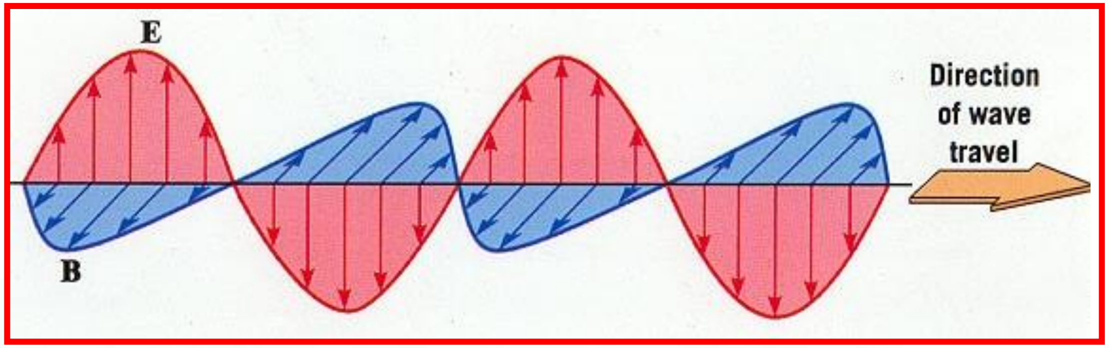
Huygens' Principle

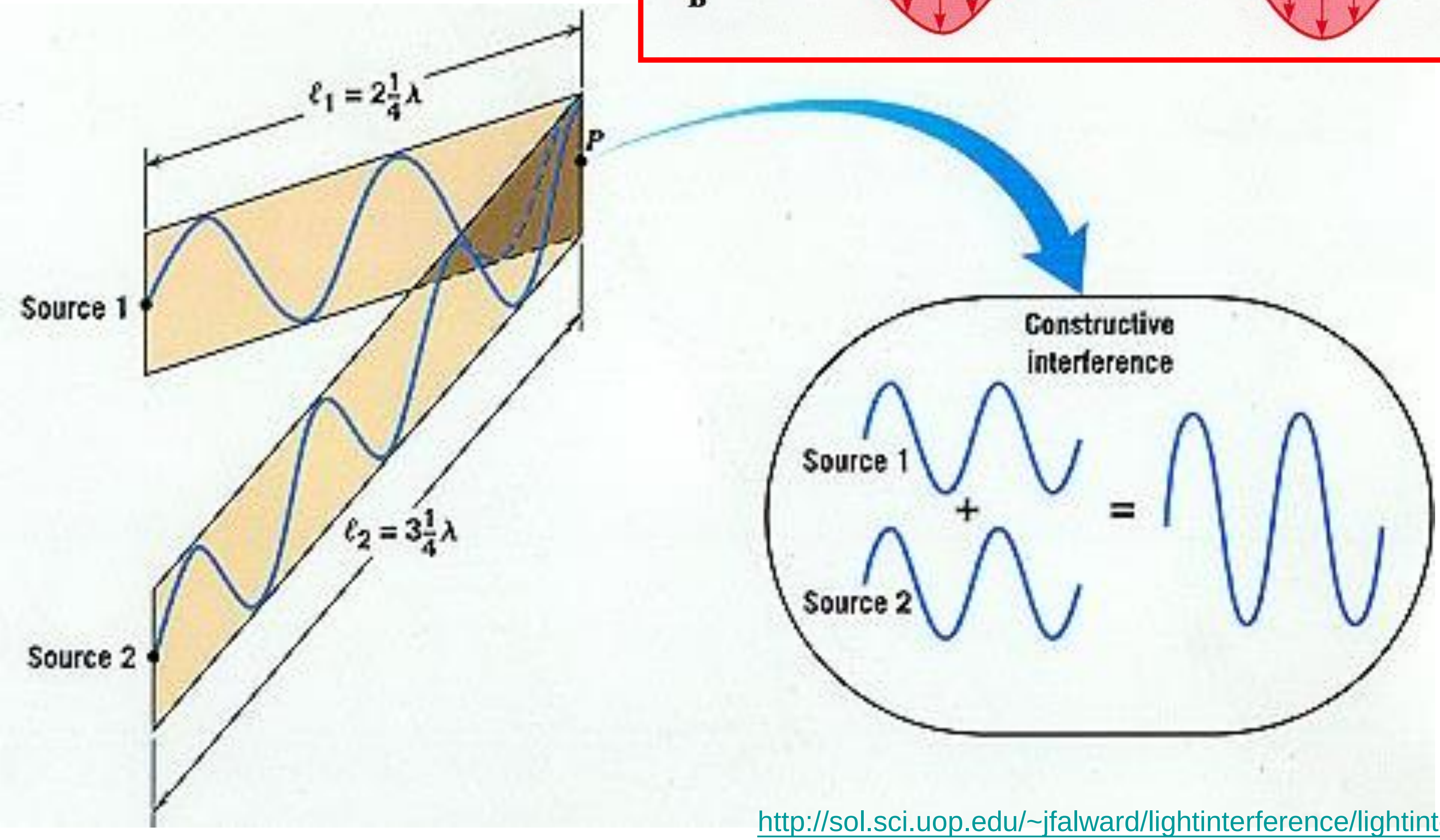




If it was by itself, each of the parts would spread out as a circular ripple. Adding up the ripples produces a new wavefront.

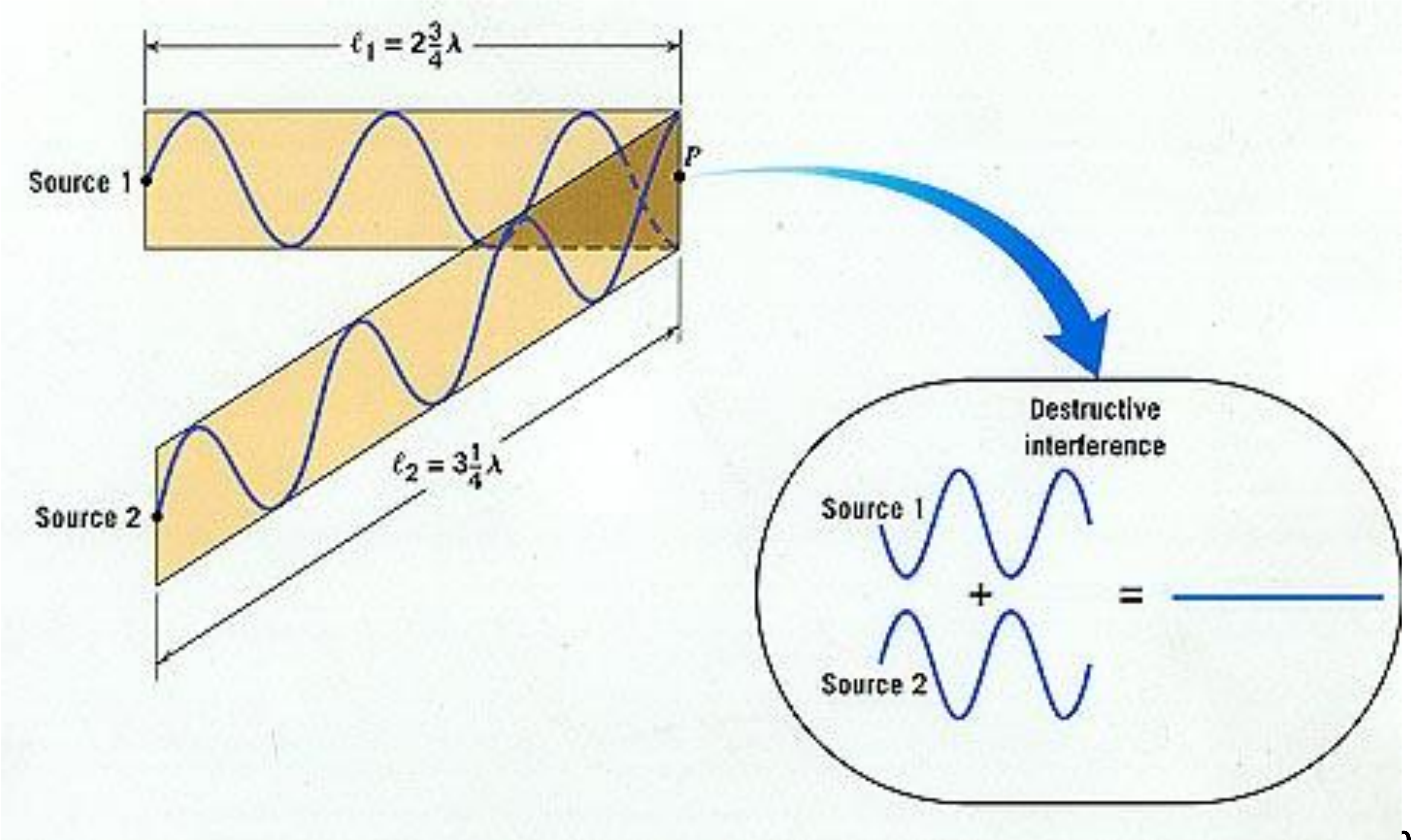
Constructive Interference



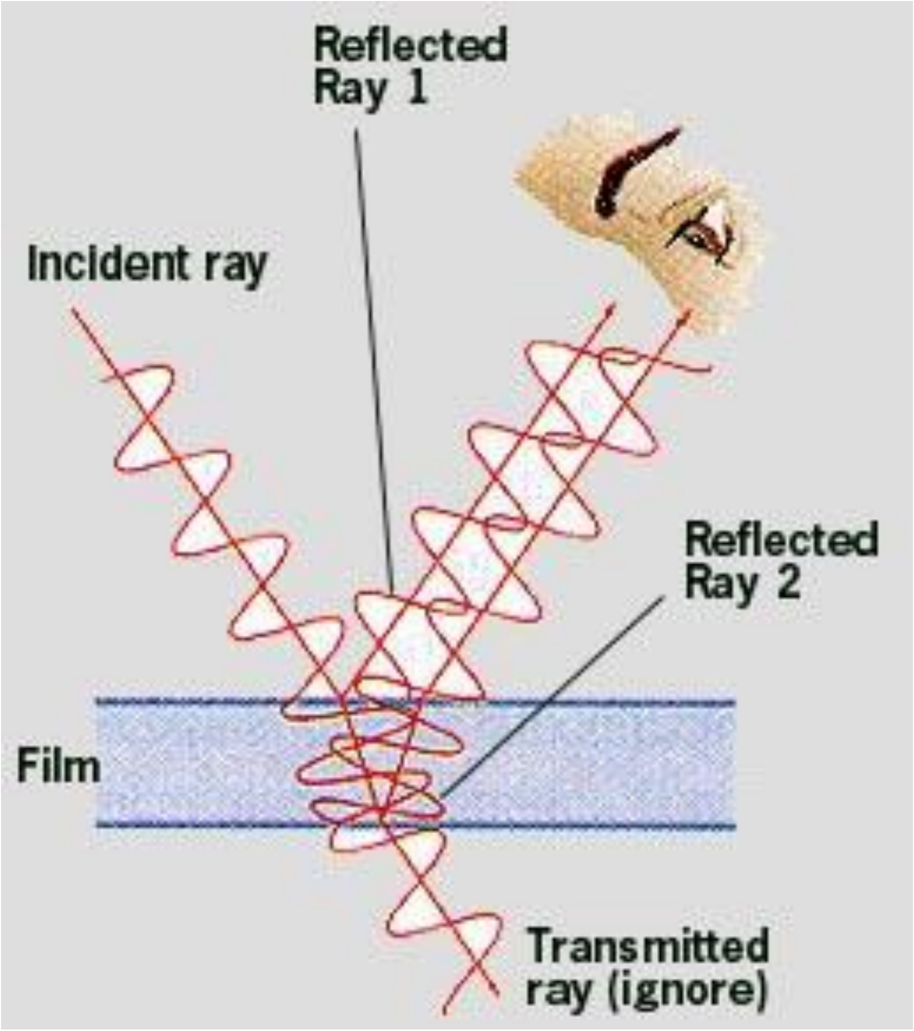


http://sol.sci.uop.edu/~jfalward/lightinterference/lightinterference.html

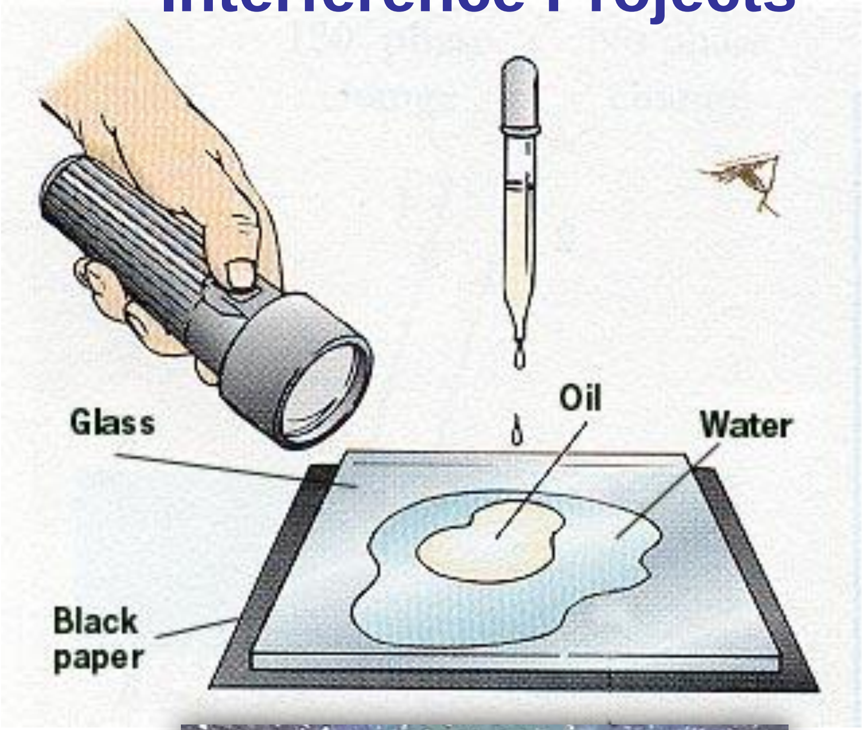
Destructive Interference



Reflection in Thin Films



Interference Projects



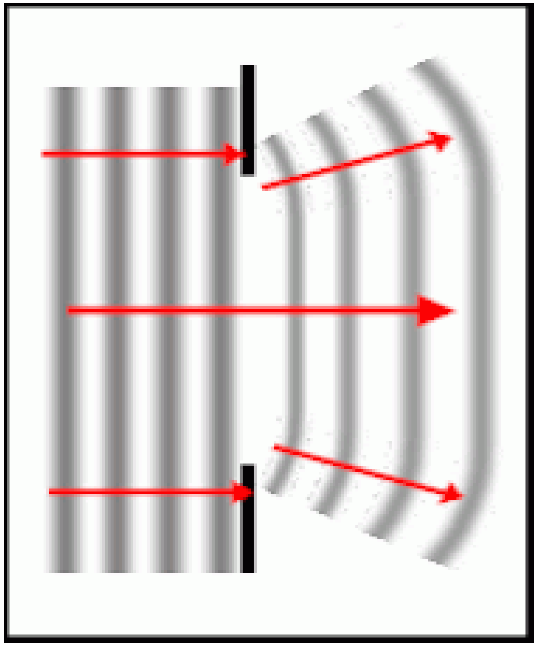
Diffraction

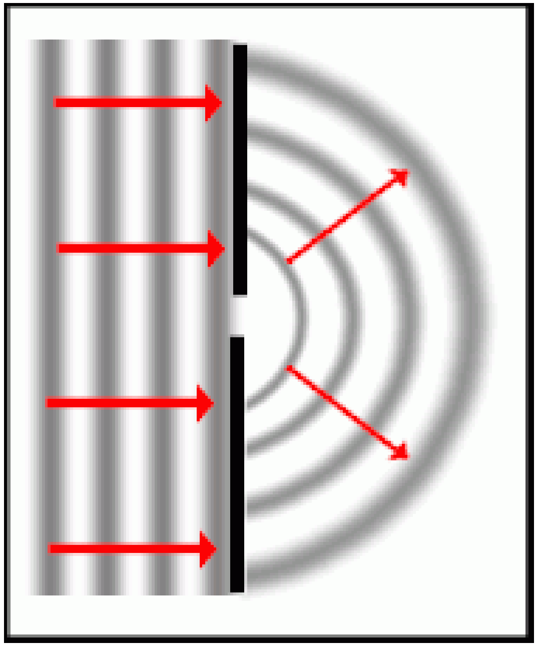
Diffraction occurs for all waves, whatever the phenomenon.

Ocean waves passing through slits in Tel Aviv, Israel

Diffraction

The bending of waves behind obstacles into the "shadow region" is known as diffraction.

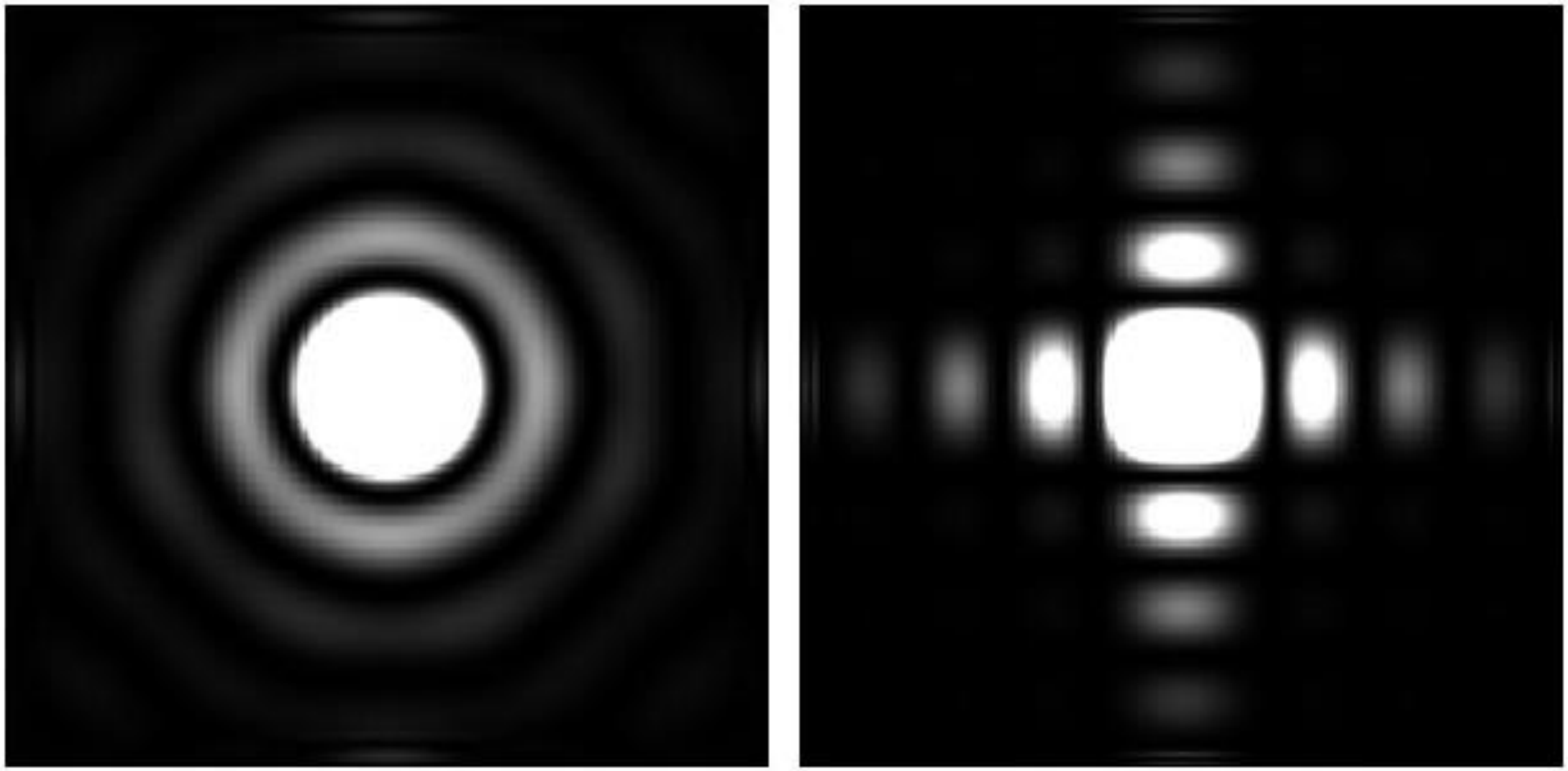




Resulting wave fronts that move out in a circular pattern.

*Lines represent peaks of waves

Diffraction

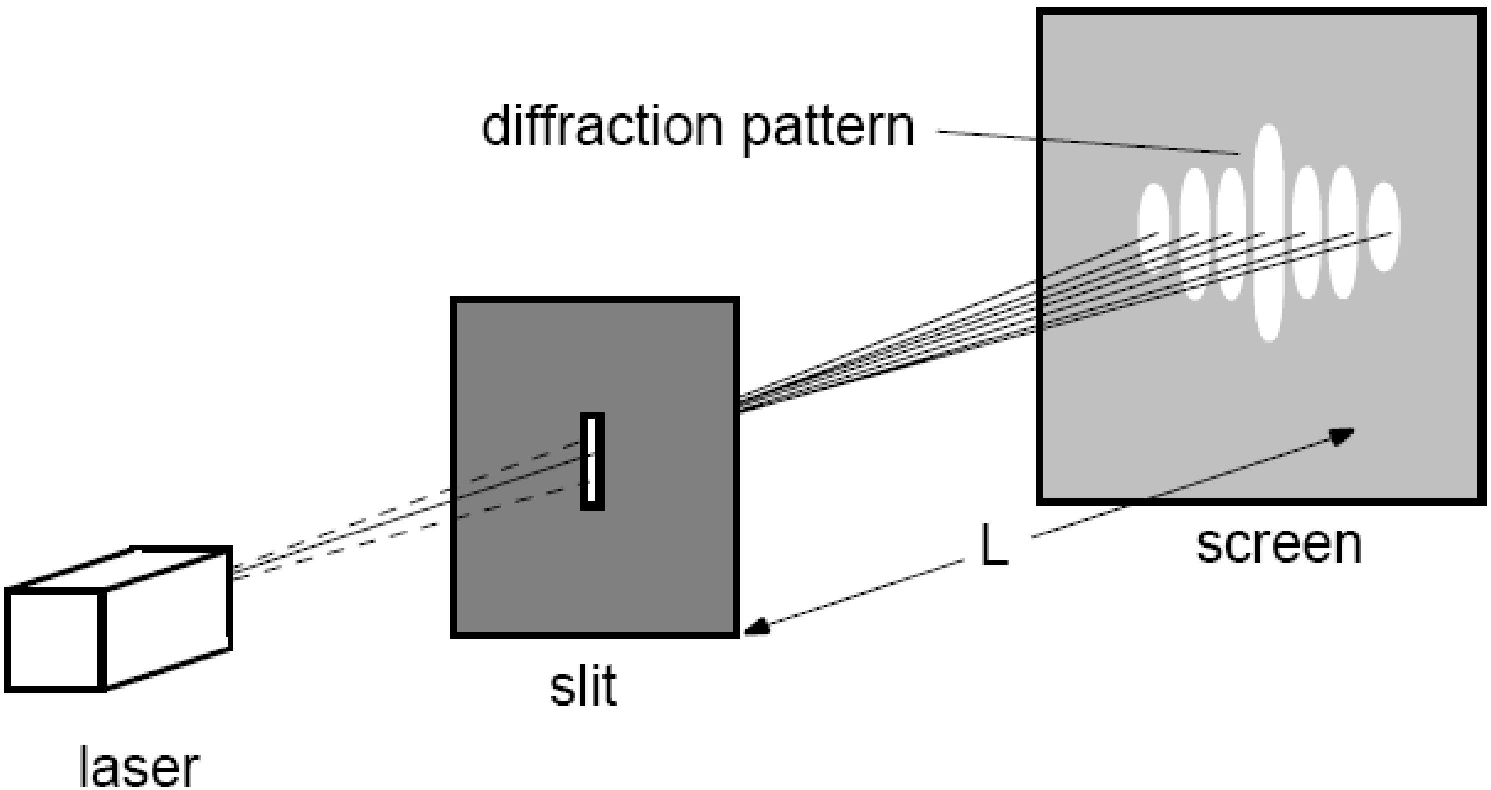


Fraunhofer Diffraction

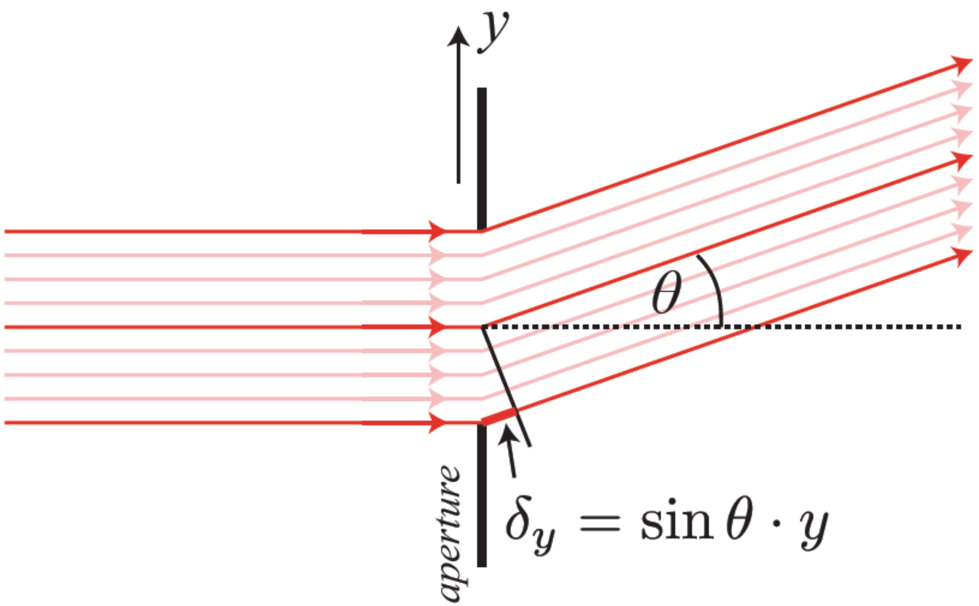
Fraunhofer diffraction: *far-field* diffraction

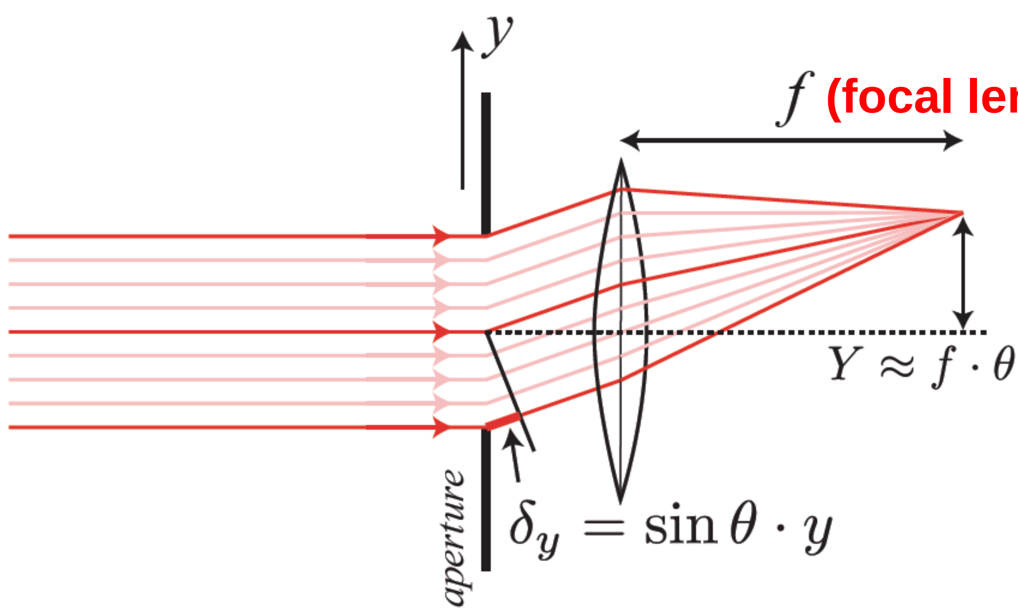
the light approaching the diffracting object is parallel and monochromatic, and the image plane is at a distance large compared to the size of the diffracting object.

***far-field* diffraction pattern**

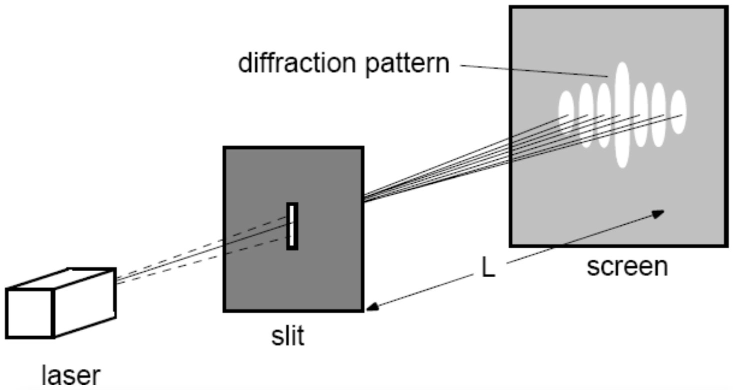


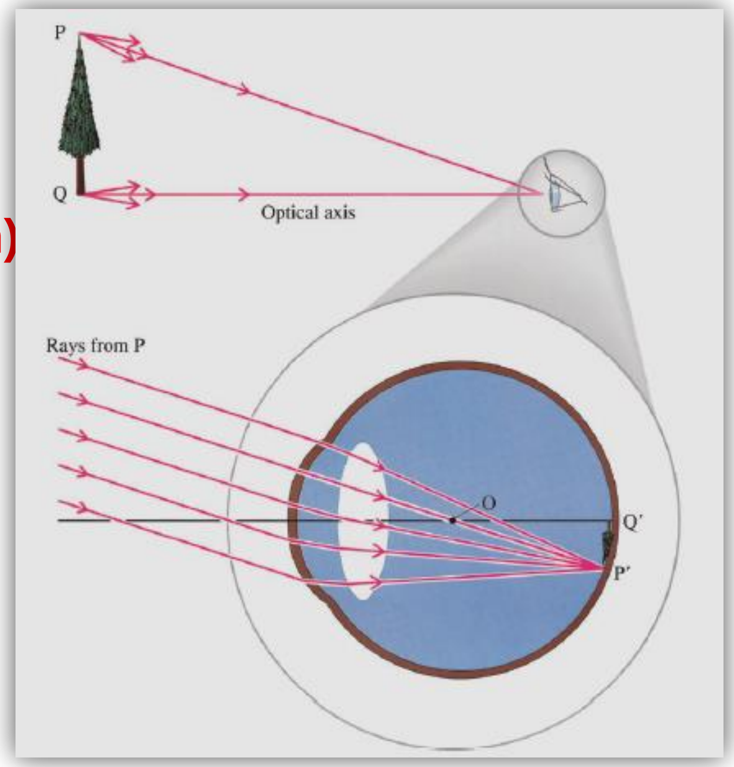
Diffraction by a single slit





***far-field* diffraction pattern**

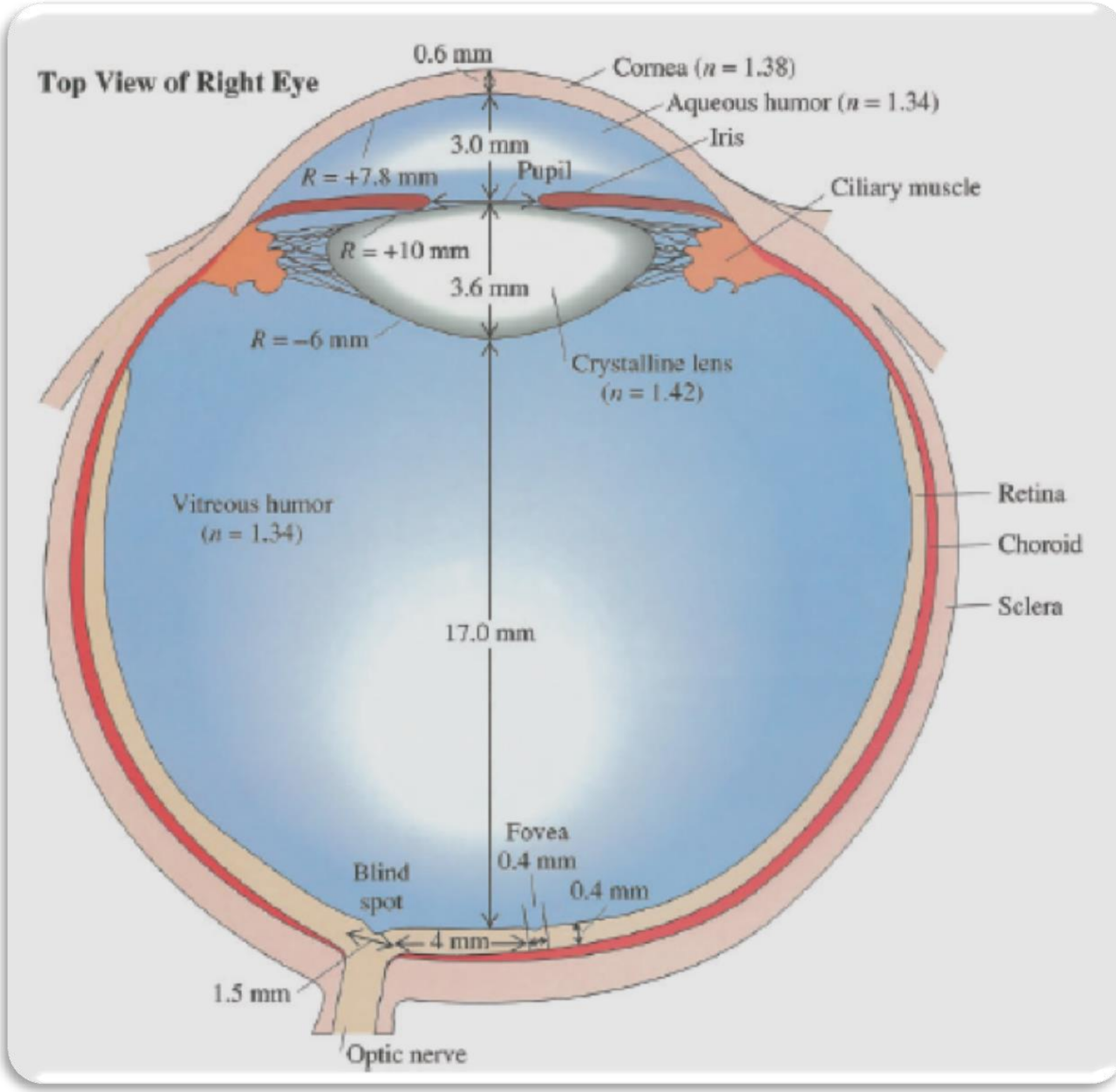




The Human Eye

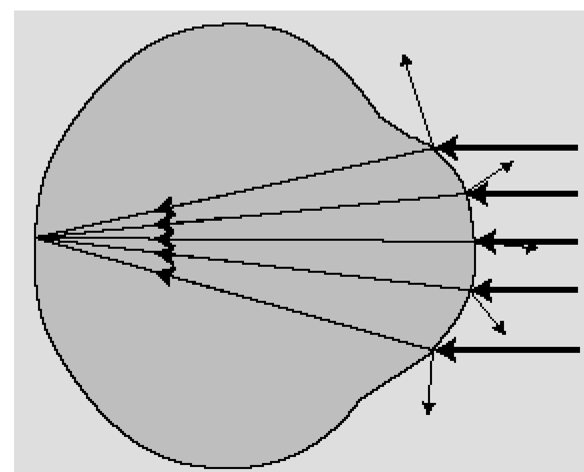
n (Refractive Index) = c/v

where **c** is the speed of light in a vacuum and **v** is the velocity of light in the material.



Eye, Aqueous humor 1.33
Eye, Cornea 1.38
Eye, Lens 1.41
Eye, Vitreous humor 1.34

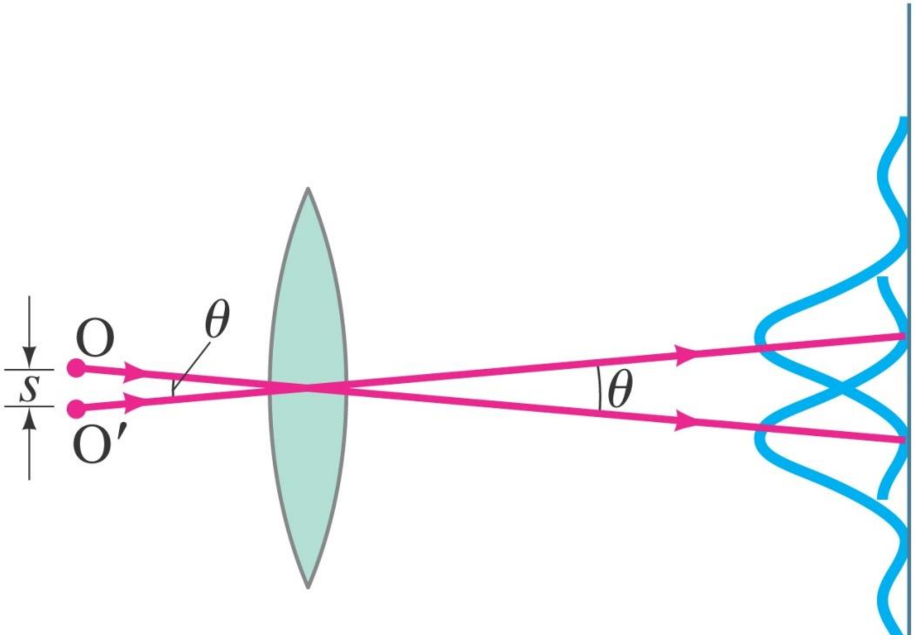
http://www.robinwood.com/Catalog/Technical/Gen3DTuts/Gen3DPages/RefractionIndexList.html



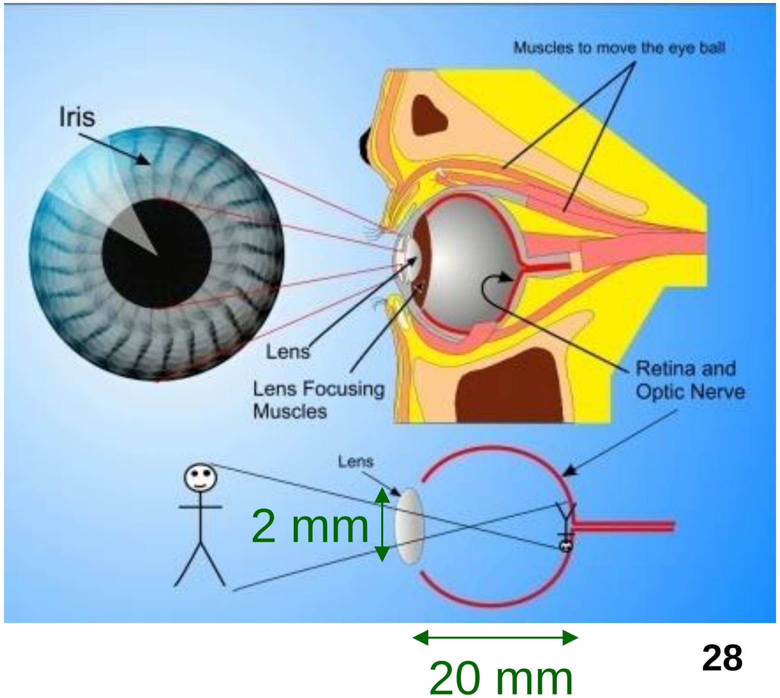
Limits of Resolution: Circular Apertures

The Resolution of the Human Eye

The **Rayleigh criterion** states that two images are just resolvable when the center of one peak is over the first minimum of the other.

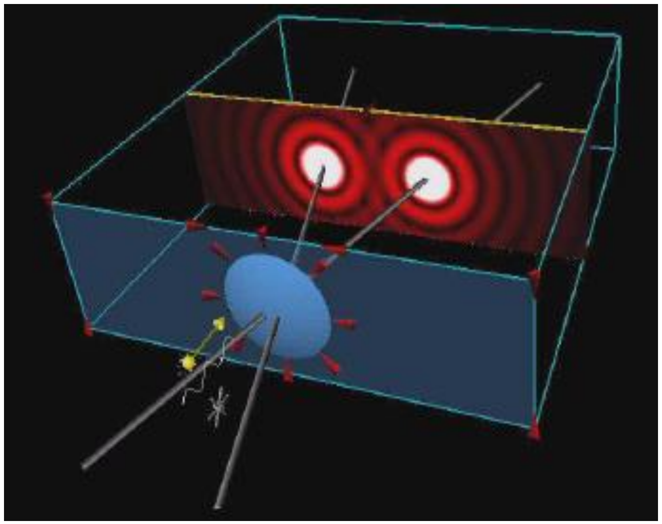


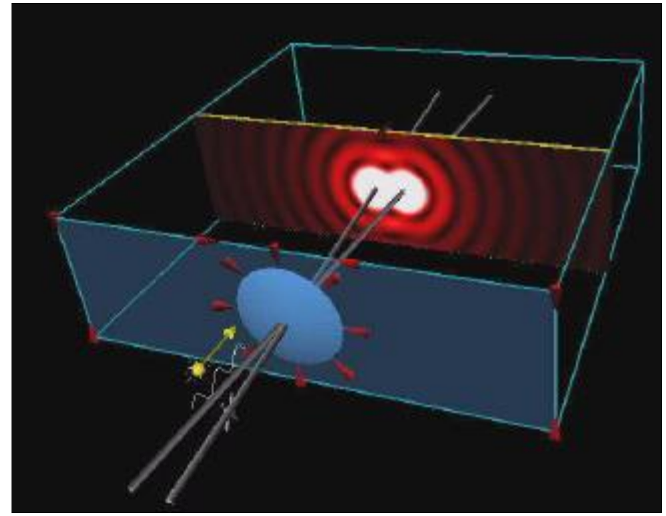
resolution=R=(1.22λf/D)

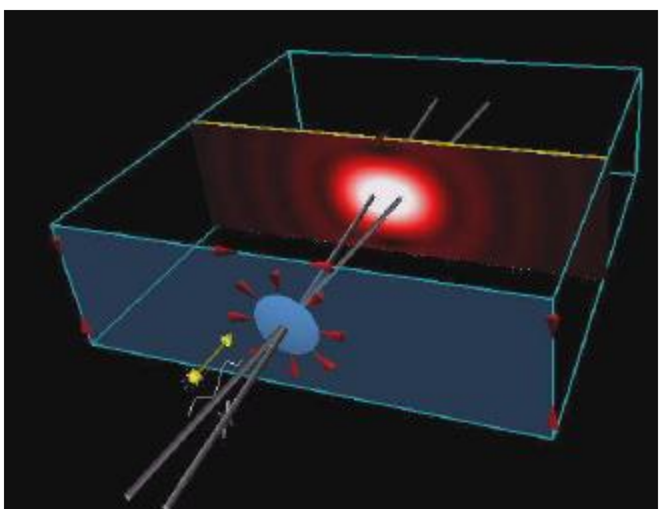


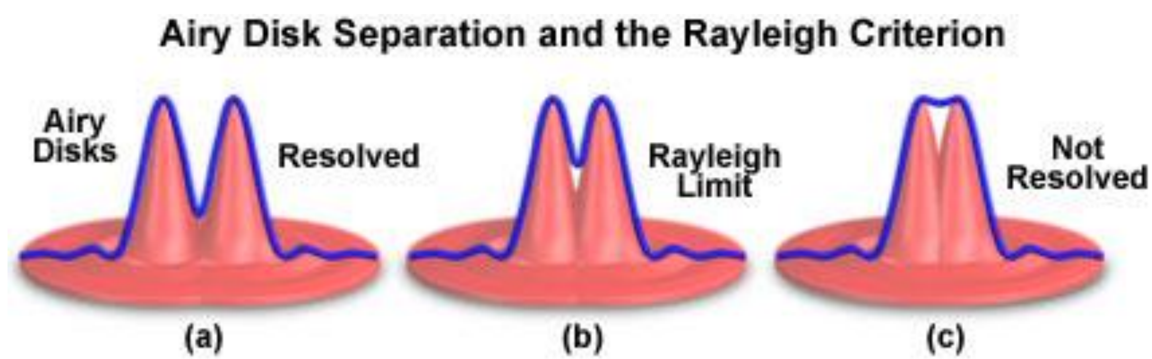
Airy Disks and Resolution

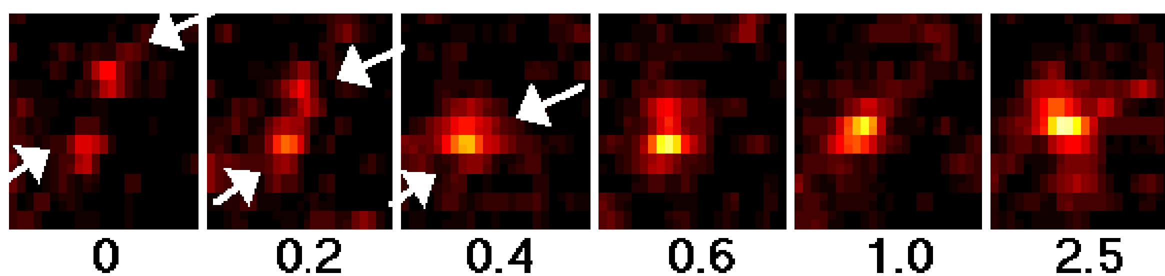
Diffraction Limited Resolution





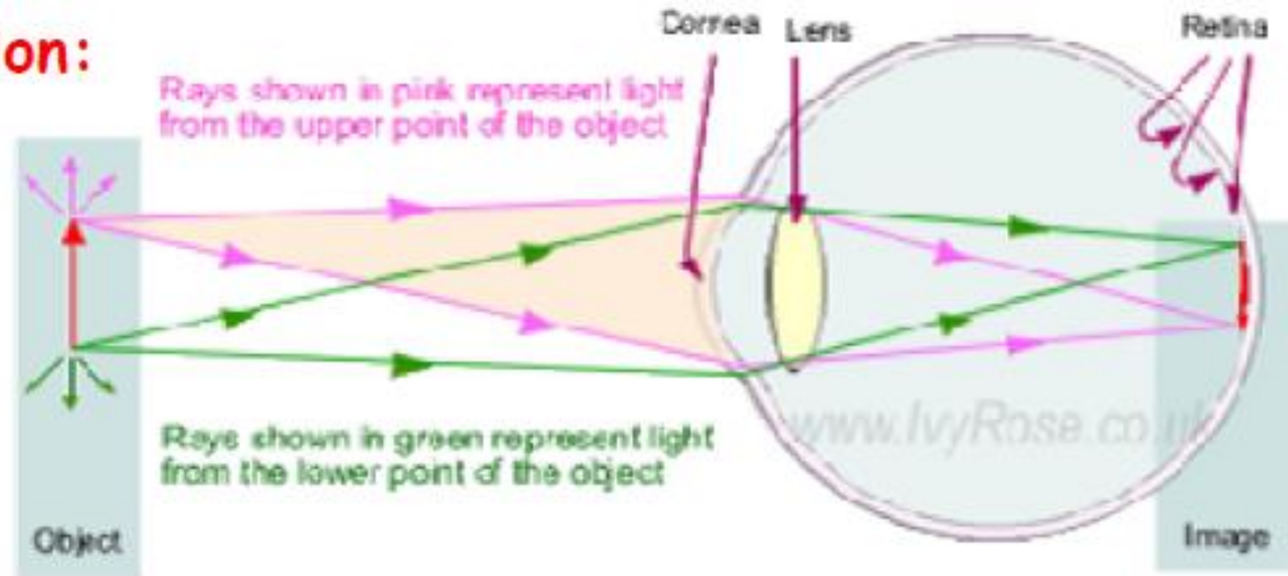




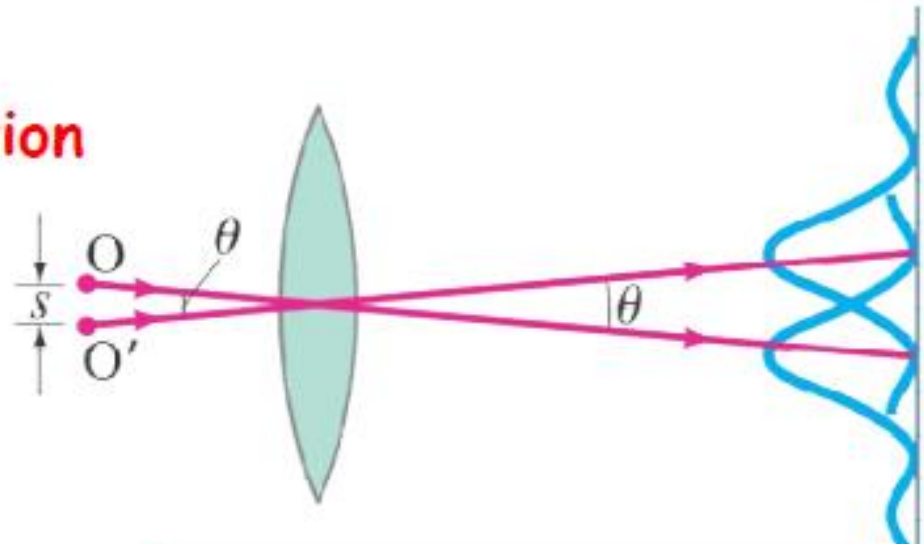


In Summary

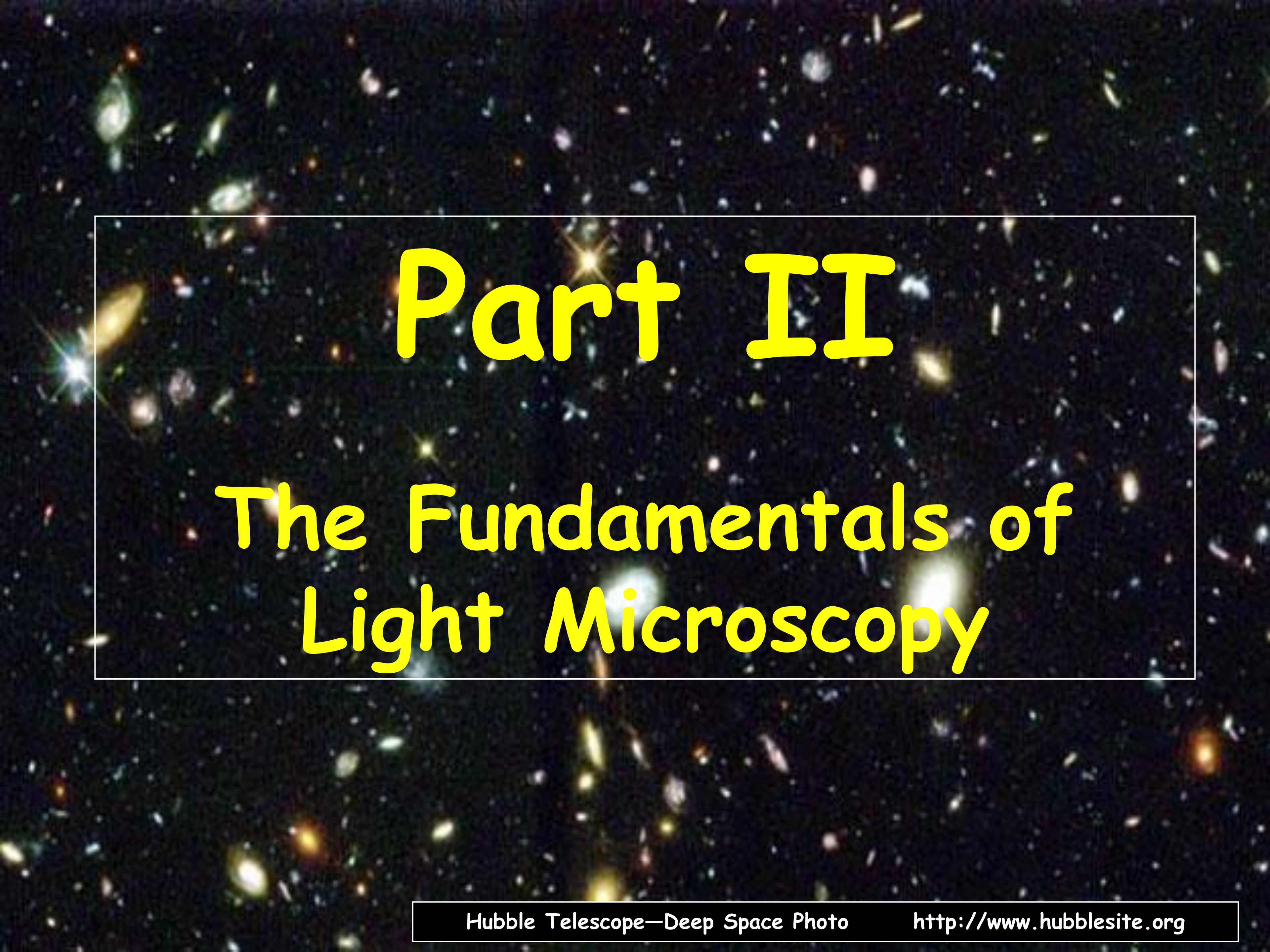
Refraction:



Diffraction:
Limits of Resolution



resolution=R=(1.22λf/D)



Part II

The Fundamentals of Light Microscopy

Hubble Telescope—Deep Space Photo http://www.hubblesite.org

Resolution and Contrast

- **Resolution**

  The resolving power of an objection

  The role of diffraction in image formation

- **Contrast**

  明視野 Bright Field

  暗視野 Dark Field

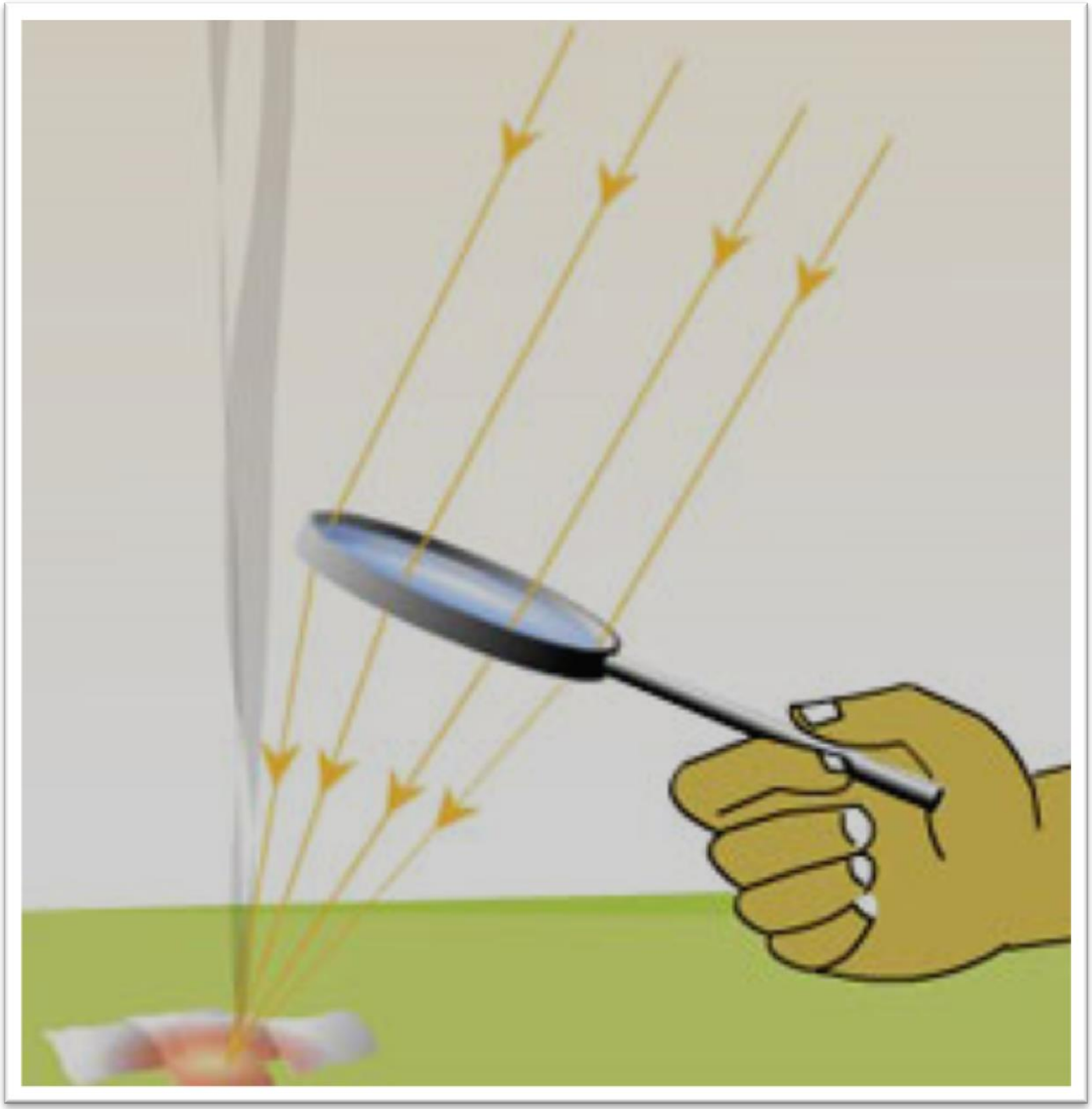
  相位差 Phase Contrast

  偏光 Polarization

  干涉相位差 Differential Interference Contrast

  螢光 Fluorescent

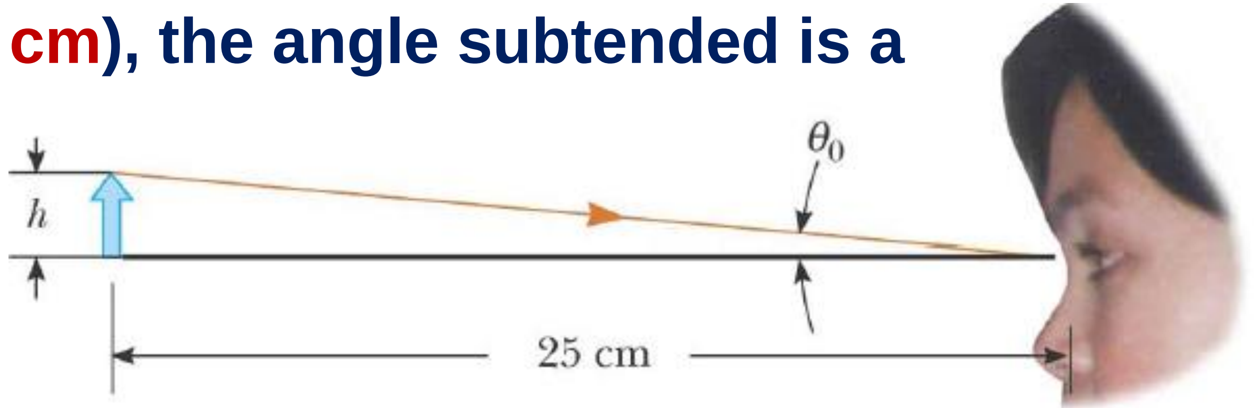
Magnifying glass lens used as a Burning lens



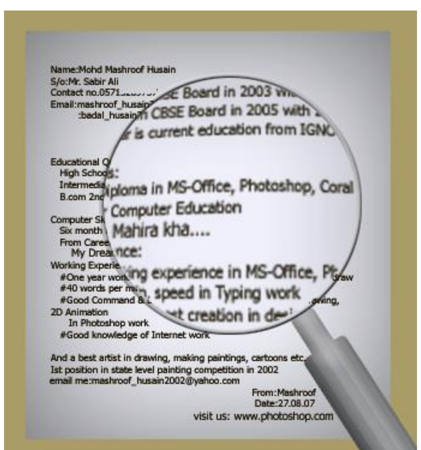
http://www.tutorvista.com/physics/magnifying-glass-lens#

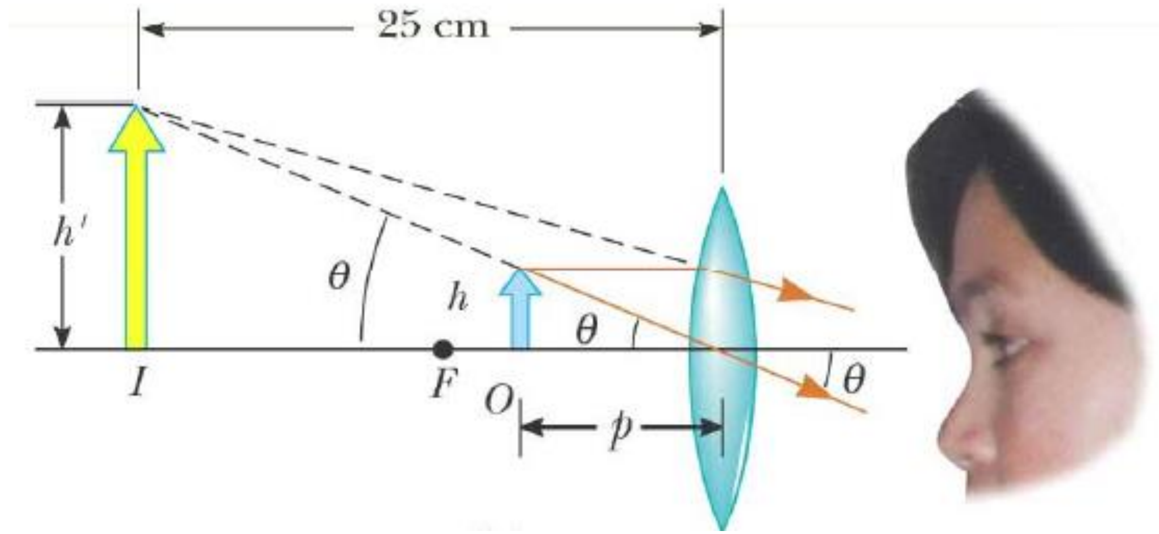
Simple Microscope: Magnifying Glass Lens

When an object is placed at the near point (the near point is about 25 cm), the angle subtended is a maximum



When the object is placed near the focal point of a converging lens, the lens forms a virtual, upright, and enlarged image

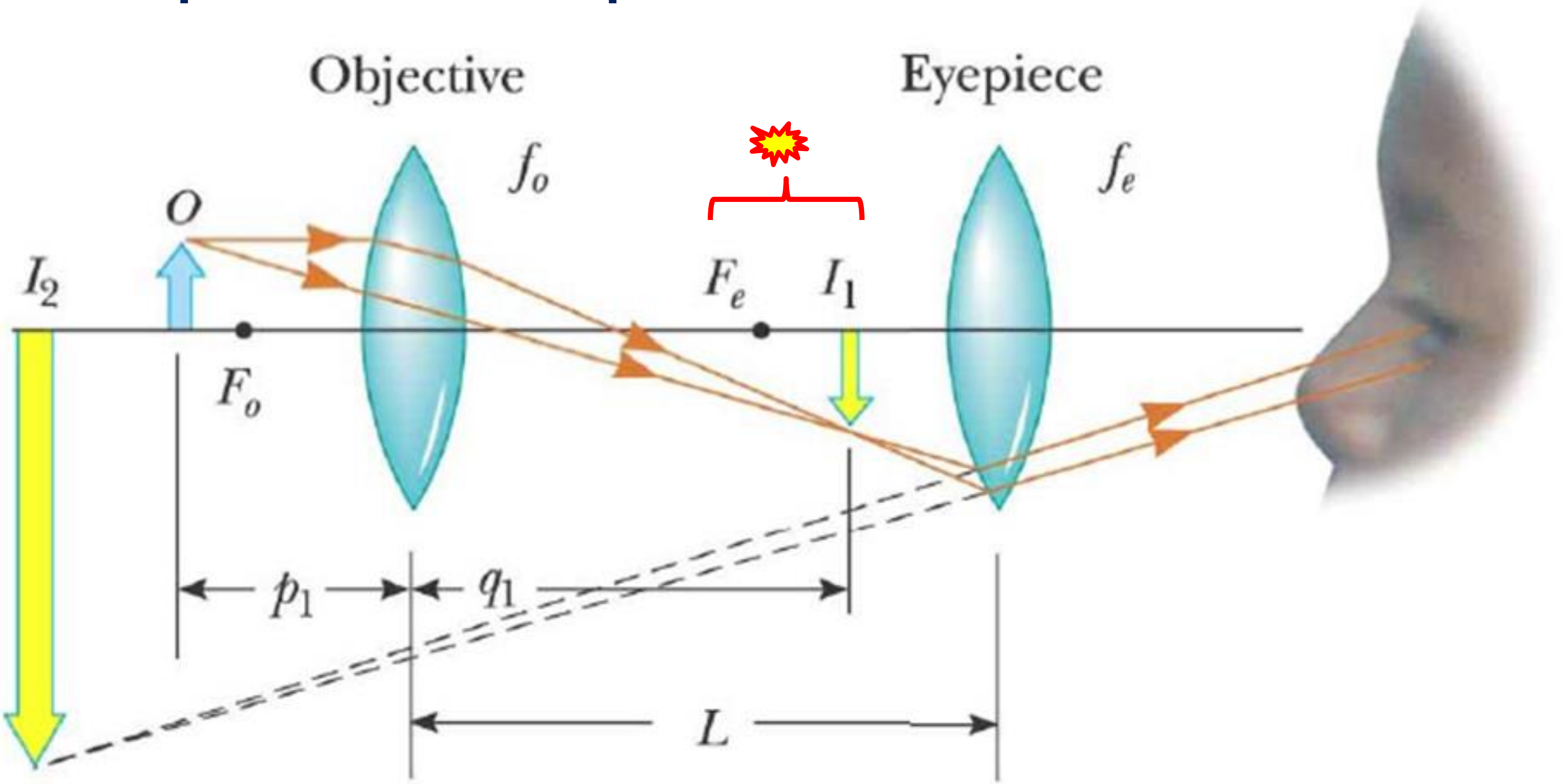




http://www.tutorvista.com/physics/magnifying-glass-lens#

Compound Microscope (複合式)

A compound microscope consists of two lenses:

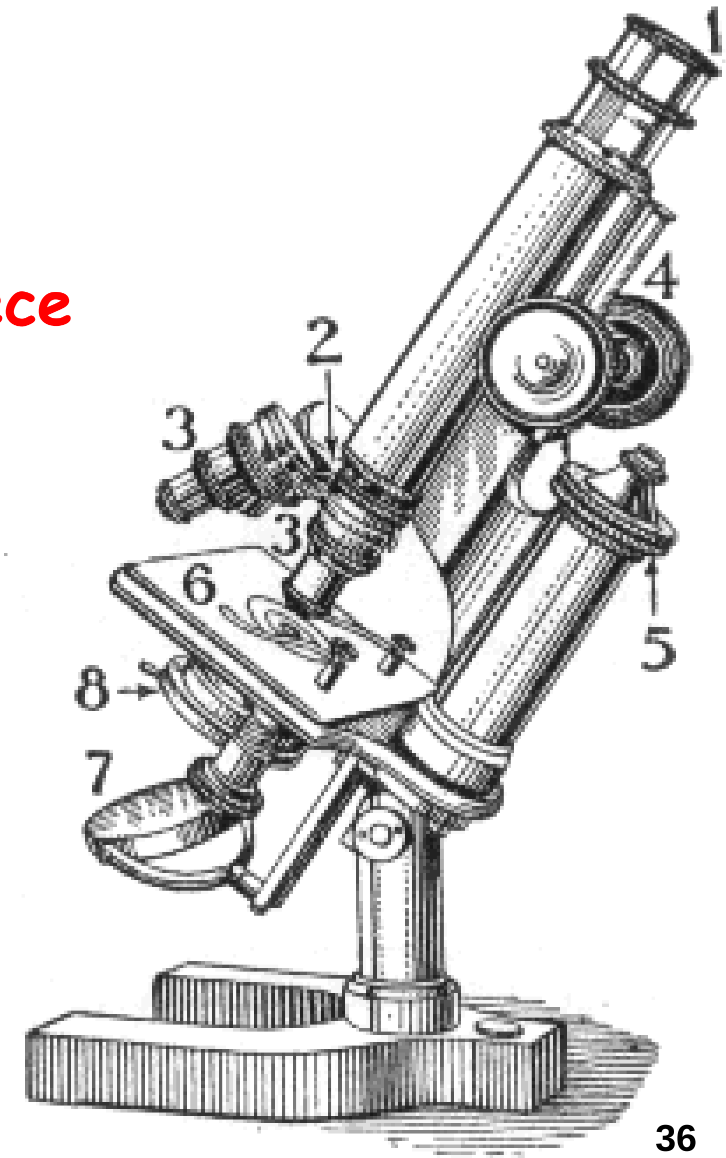


1) **The objective lens has a short focal length (< 1 cm).**
2) **The eyepiece has a focal length of a few cm.**

Compound Microscope (複合式)

Upright Microscope (正立式)

1. Ocular lens or eye-piece
2. Objective turret, or nosepiece (鼻輪/顯微鏡裝接物鏡的旋座)
3. Objective lenses
4. Coarse adjustment knob
5. Fine adjustment knob
6. Object holder or stage
7. Mirror
8. Diaphragm and condenser (集光器)



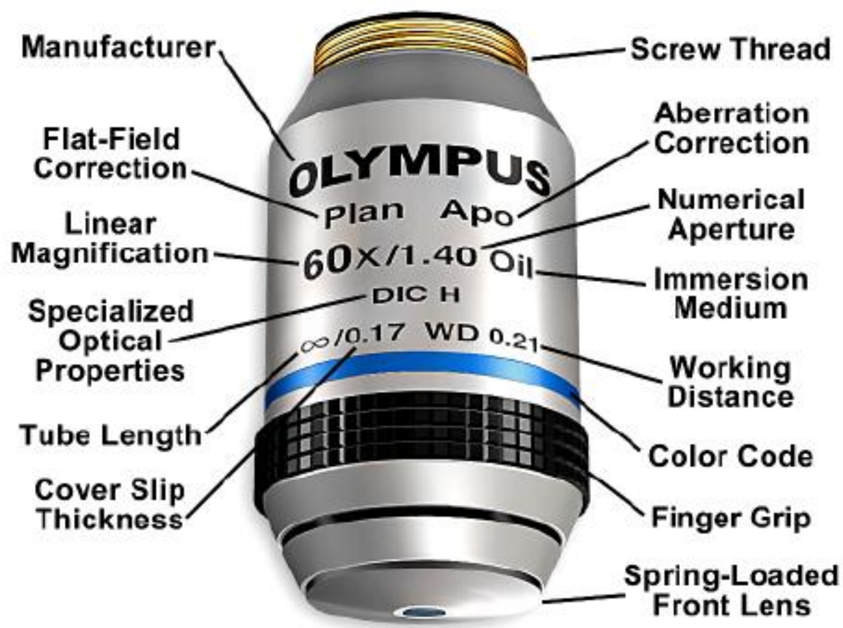
Microscope: TE-2000U, Nikon

Inverted Microscope (倒立式)

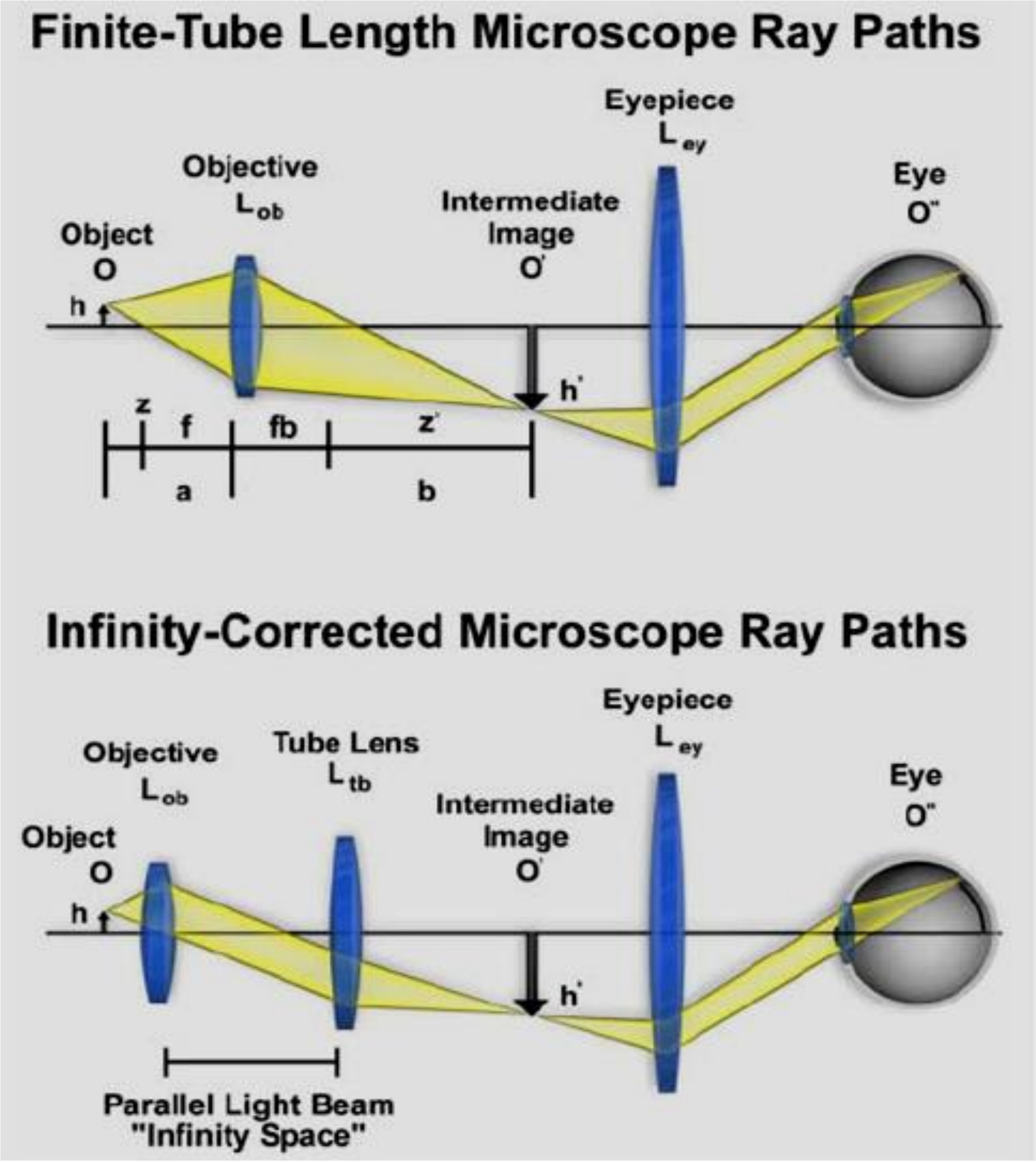
Common Objective Working Distances

Manufacturer	Correction	Magnification	Numerical Aperture	Working Distance
Nikon	PlanApo	10x	0.45	4.0 mm
Nikon	PlanFluor	20x	0.75	0.35 mm
Nikon	PlanFluor (oil)	40x	1.30	0.20 mm
Nikon	PlanApo (oil)	60x	1.40	0.21 mm
Nikon	PlanApo (oil)	100x	1.40	0.13 mm

Objective Working and Parfocal Distance

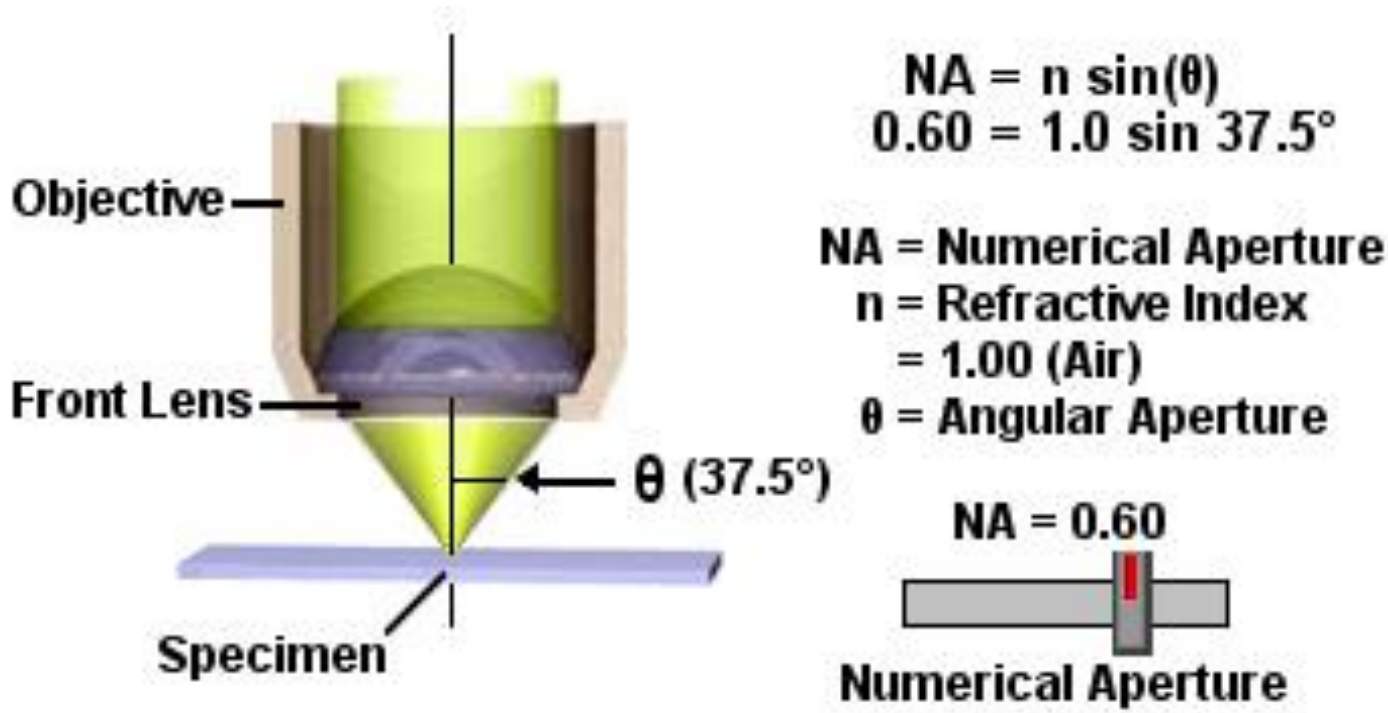


Infinity-corrected Microscope Systems



Optional stage risers create space for other attachments.

Numerical Aperture (數值孔徑)



Numerical Aperture (NA) = n • sin(θ)

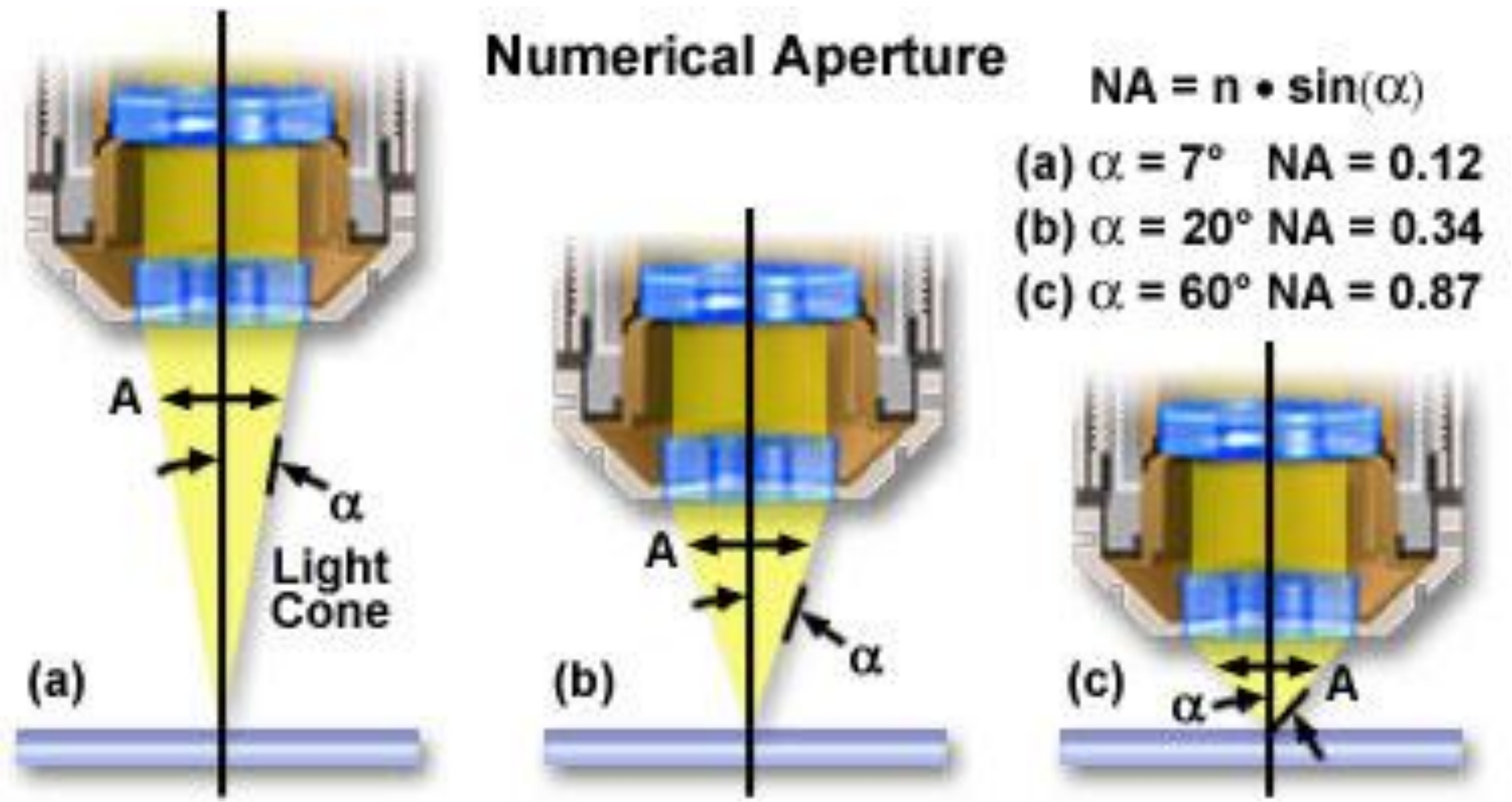
Approximate Magnification **40x**

http://www.microscopyu.com/tutorials/java/objectives/nuaperture/index.html

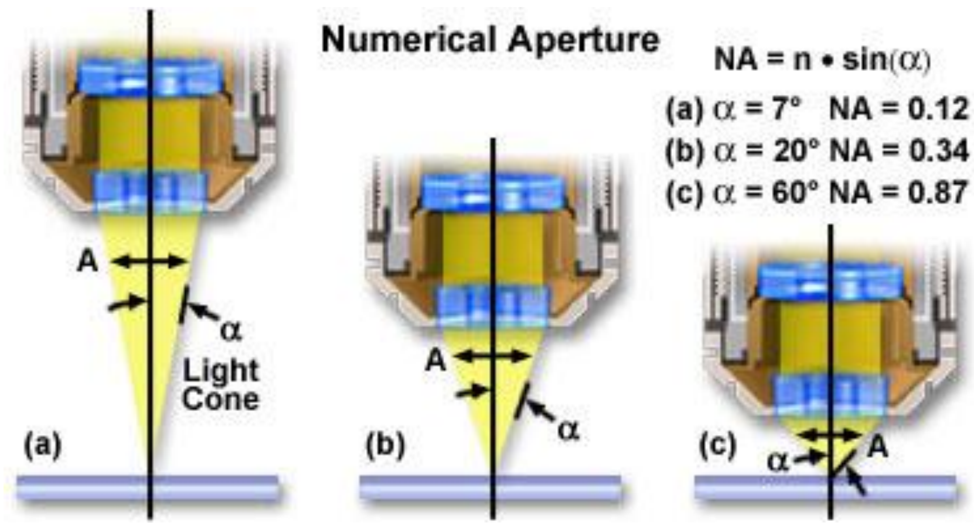
Numerical Aperture (數值孔徑)

generate a highly focused laser beam

Numerical Aperture (NA) = n • sin(α)

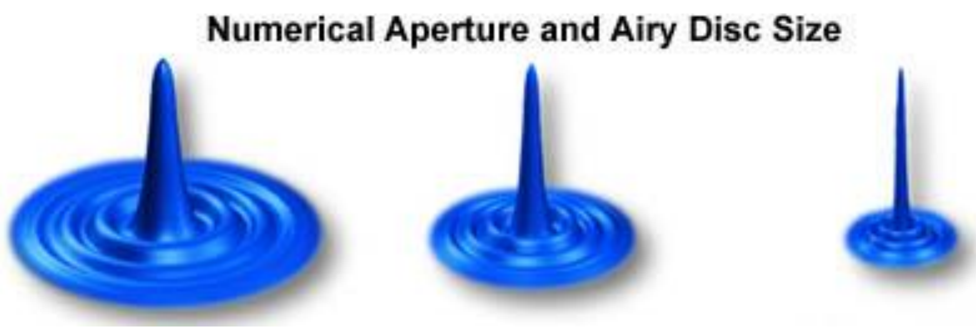


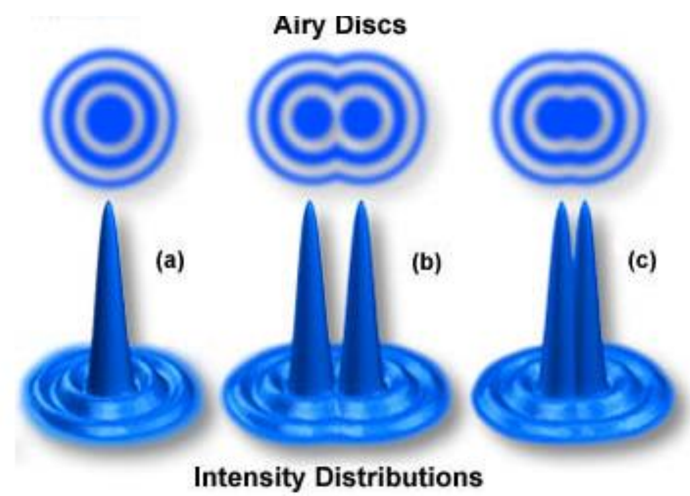
Airy Disks and Resolution



R=(1.22λf/D)
if both λ and D are the same,
∴NA↑⇒ resolution ↑

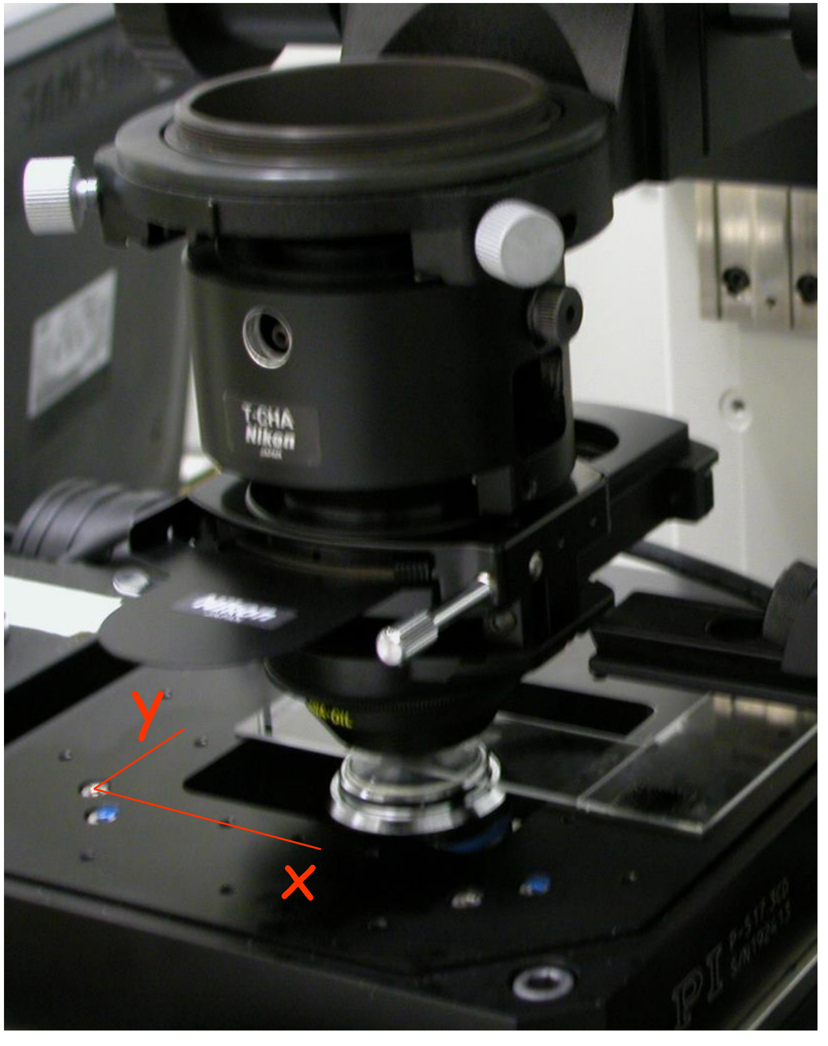
Overlapping images

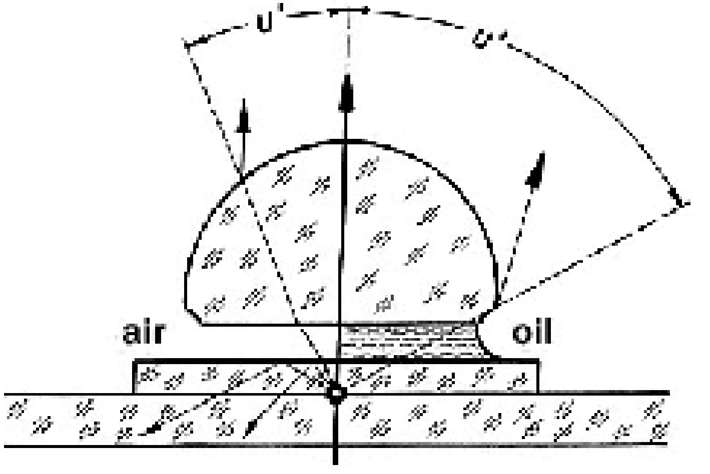




http://micro.magnet.fsu.edu/primer/anatomy/numaperture.h

High N.A Objective





Air = 1.0003

Water = 1.33

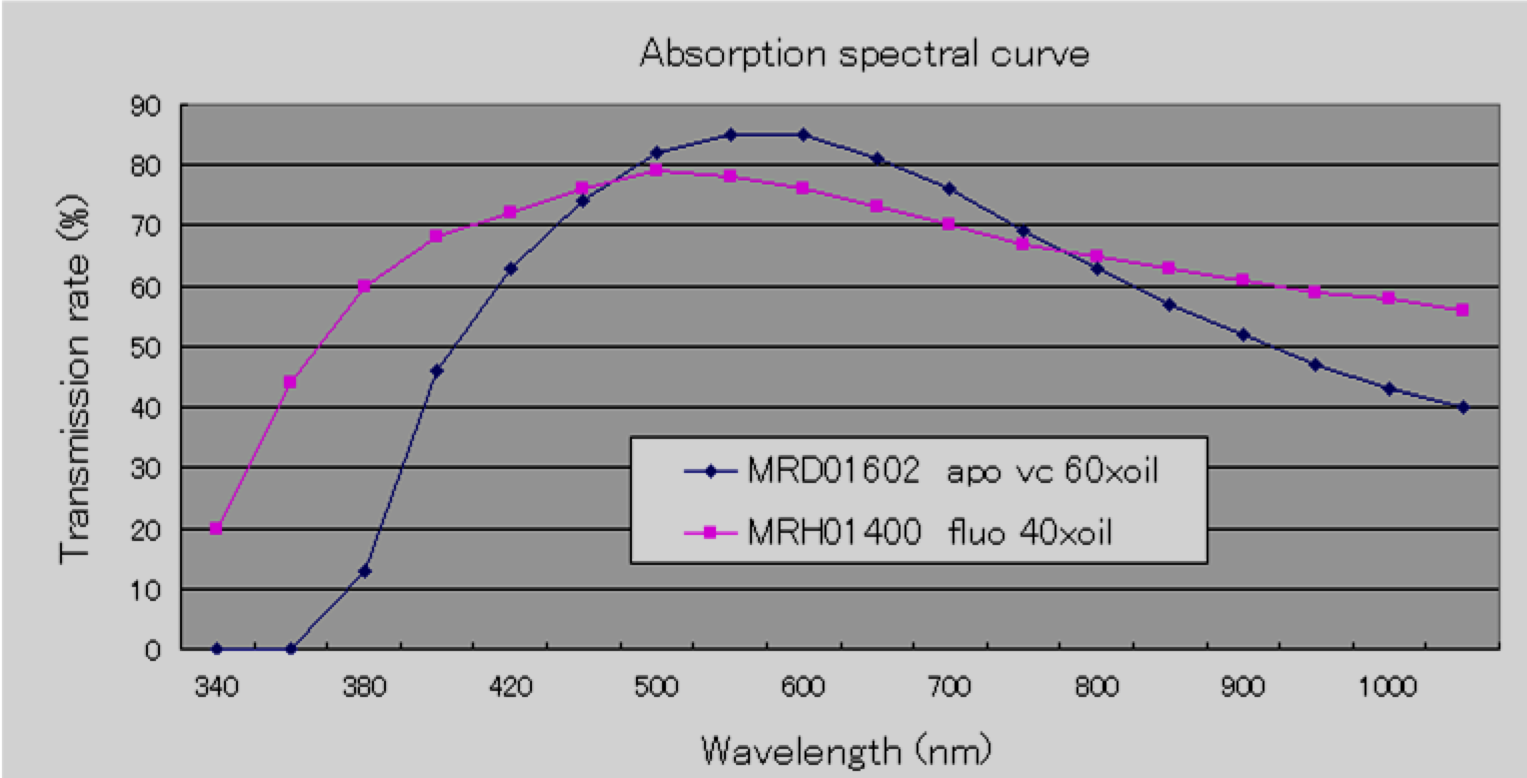
Glycerol = 1.47

Immersion Oil = 1.515

Crown Glass = 1.52

Diamond = 2.42

Cells = 1.38

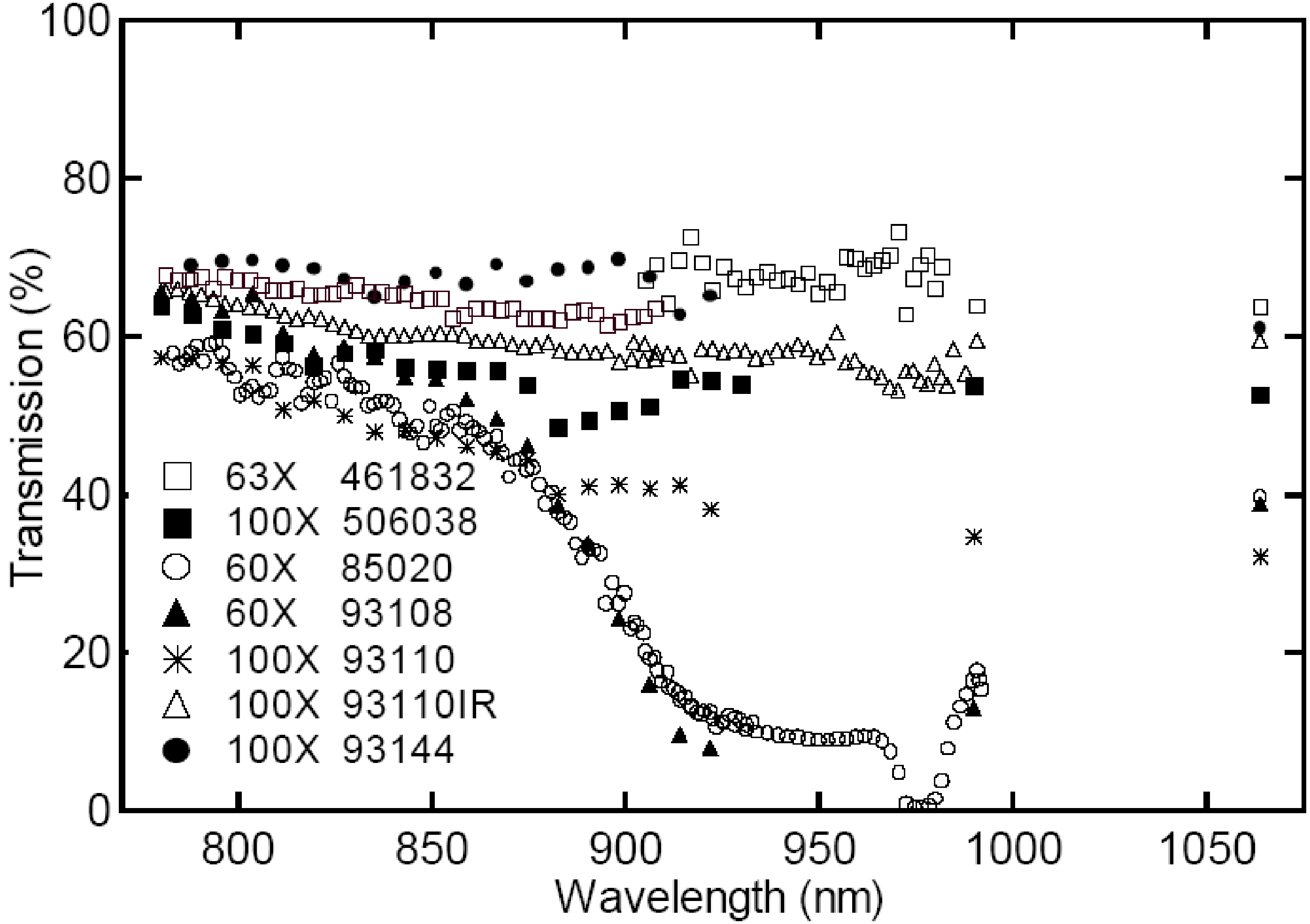


CFI Plan Apochromat VC Series

CFI Plan Apochromat VC 60X Oil, N.A. 1.40
CFI Plan Apochromat VC 60X WI, N.A. 1.20
CFI Plan Apochromat VC 100X WI, N.A. 1.40

- Chromatic aberrations have been thoroughly correct throughout the view field. Suitable for digital imaging.
- Perfect choice for multi-stained, fluorescence specimens and when using brightfield and DIC techniques.
- Axial chromatic aberration has been corrected up to the violet range (405nm), making these objectives highly effective for confocal applications.
- Excellent brightness throughout the view field.
- The 60X water-immersion type, in particular, features high spectral transmittance, even in the 360nm wavelength range.

High N.A Objective



Keir Cajal Neuman, PhD Thesis (2002)

Depth of Field and Depth of Focus

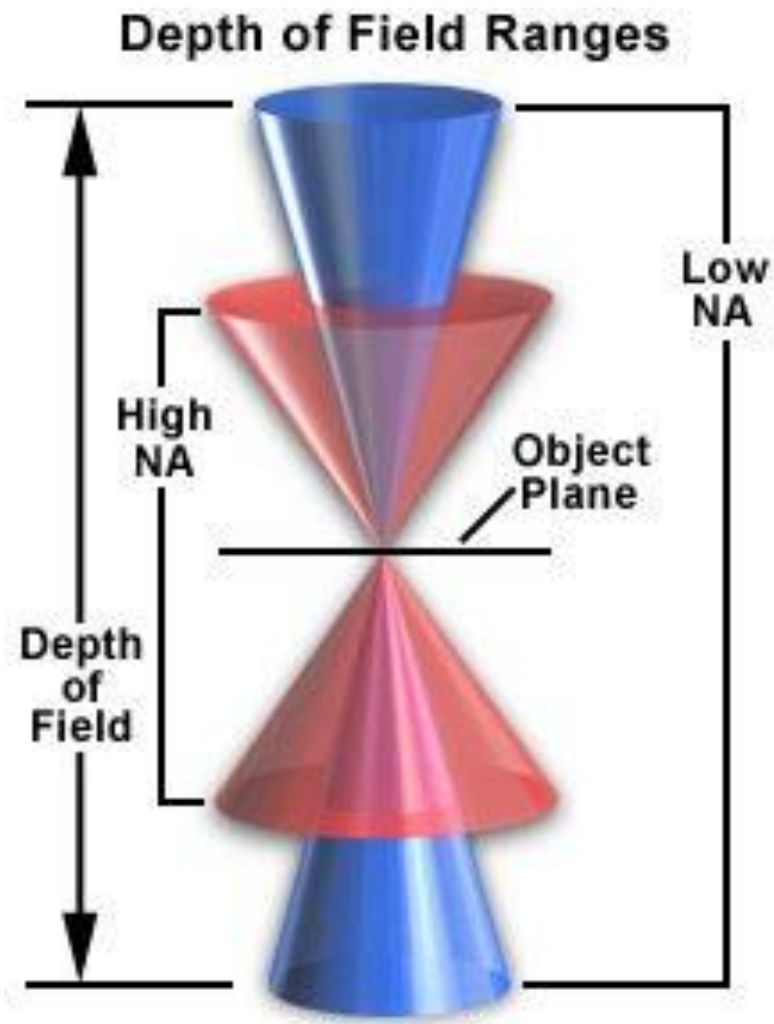
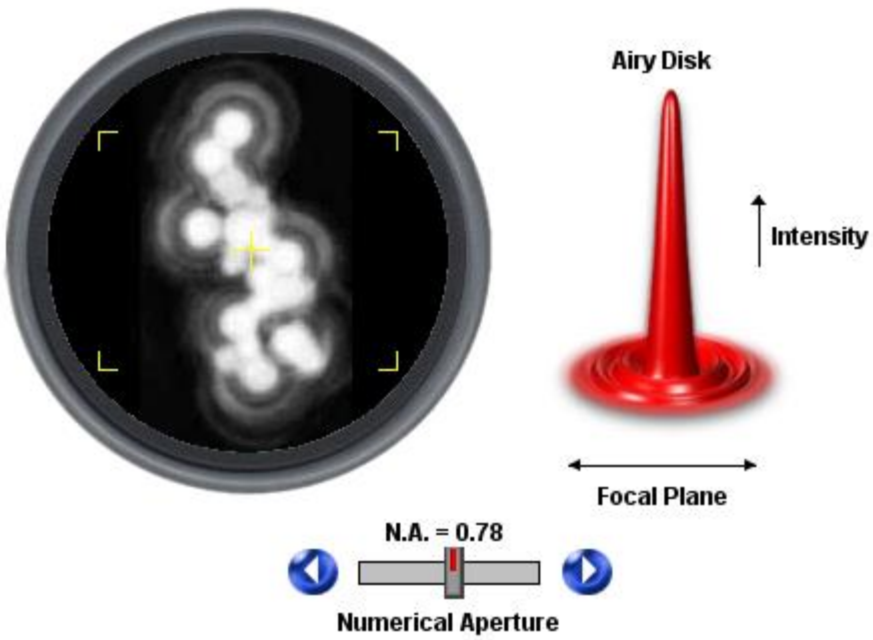


Figure 1

When considering resolution in optical microscopy, a majority of the emphasis is placed on point-to-point lateral resolution in the plane perpendicular to the optical axis (Figure 1). Another important aspect to resolution is the axial (or longitudinal) resolving power of an objective, which is measured parallel to the optical axis and is most often referred to as depth of field.

Image Formation

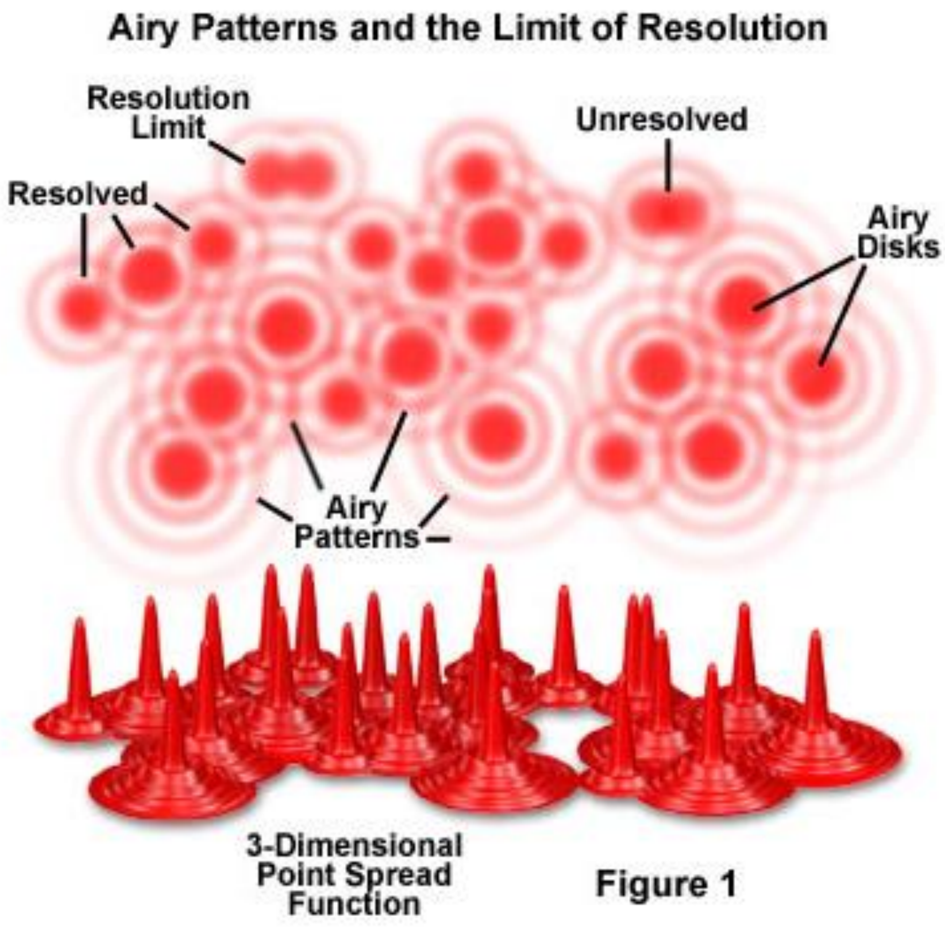
Numerical Aperture and Image Resolution



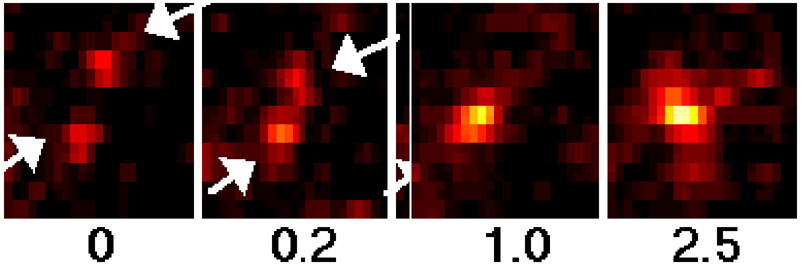
The image formed by a perfect, aberration-free objective lens at the intermediate image plane of a microscope is a diffraction pattern produced by spherical waves exiting the rear aperture and converging on the focal point. This tutorial explores the effects of objective numerical aperture on the resolution of the central bright disks present in the diffraction pattern, commonly known as Airy disks.

http://www.microscopyu.com/tutorials/java/imageformation/airyna/index.html

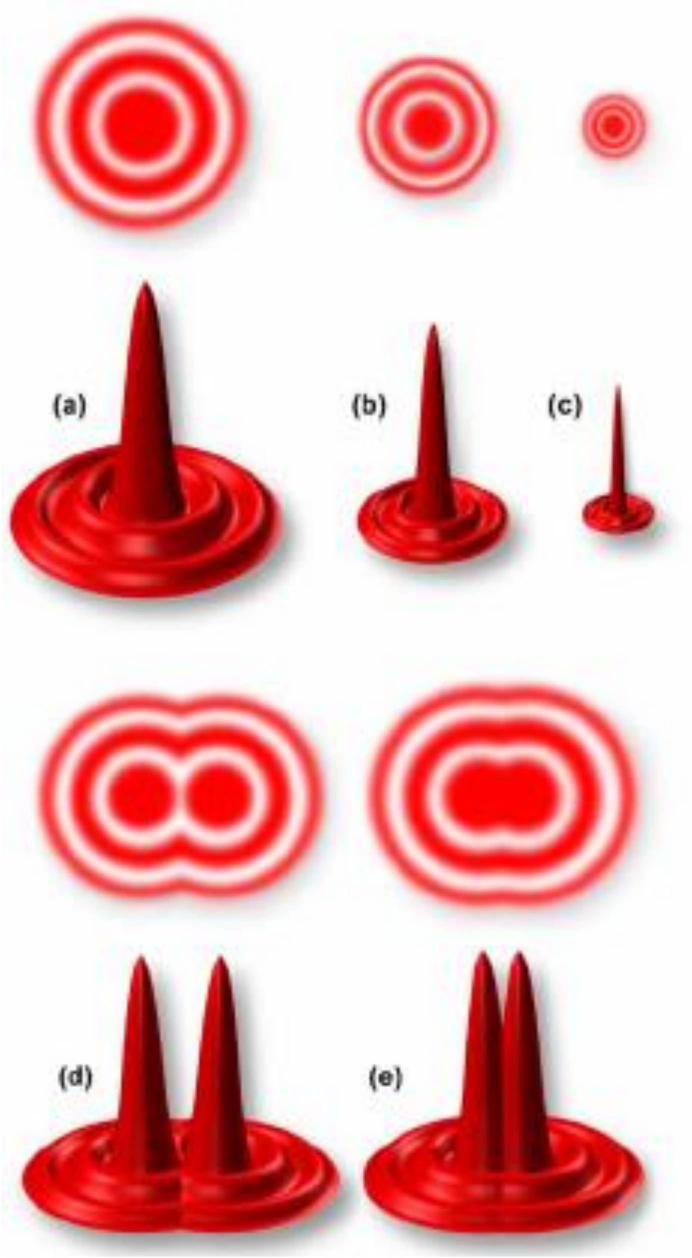
Resolution: Diffraction Limit



The resolution of an optical microscope is defined as the shortest distance between two points on a specimen that can still be distinguished by the observer or camera system as separate entities.



Resolution: Diffraction Limit



Airy disks and resolution. (a-c) Airy disk size and related intensity profile (point spread function) as related to objective numerical aperture, which decreases from (a) to (c) as numerical aperture increases. (e) Two Airy disks so close together that their central spots overlap. (d) Airy disks at the limit of resolution.

Koehler Illumination (柯氏照明)

Conjugate Planes

1) Conjugated Planes: set of planes such that an image focused on one plane is automatically focused on all other conjugate planes.

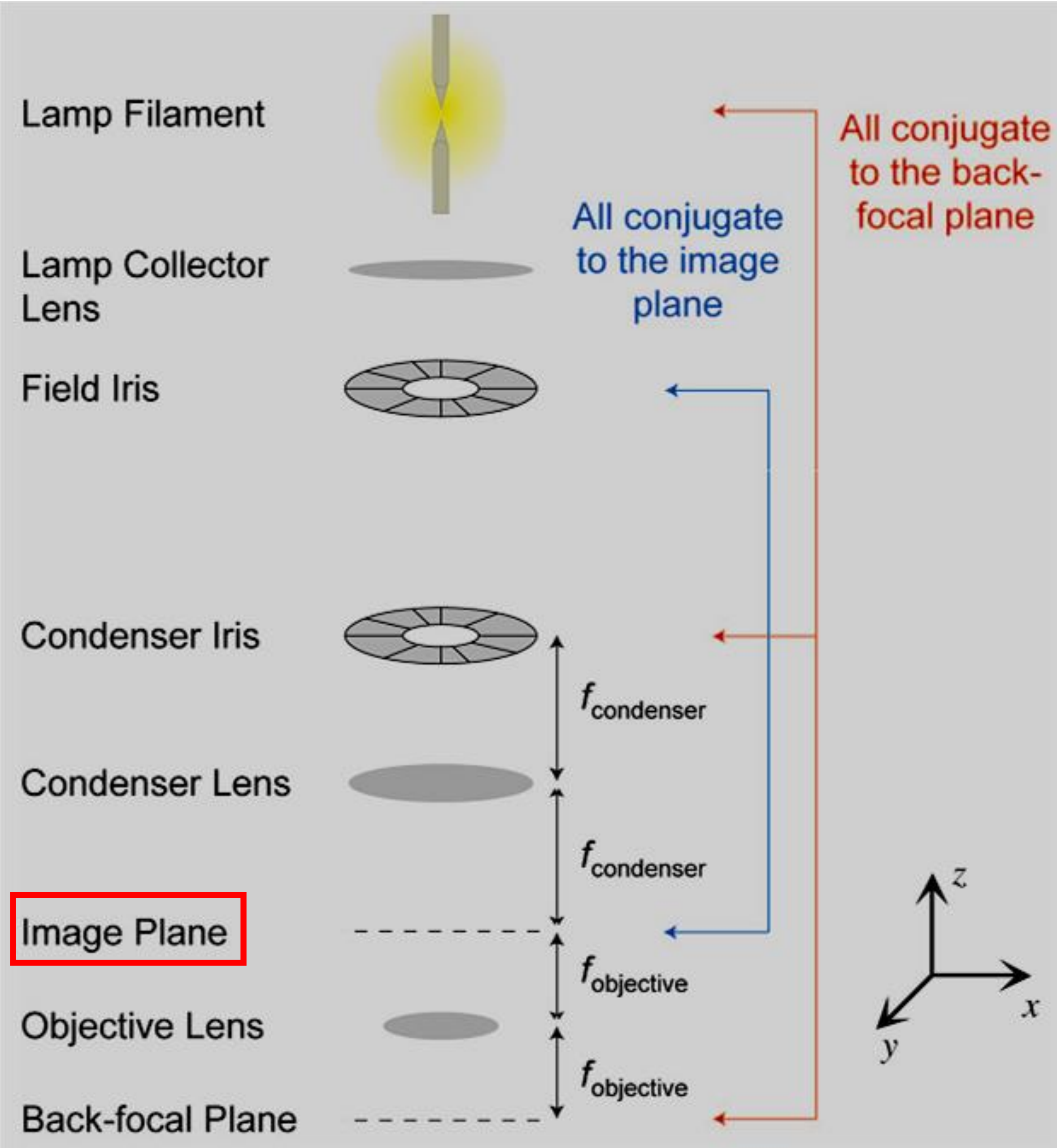
2) Light ray path produces focused images of the lamp filament at the plane of the condenser aperture, back focal plane of the specimen and at the eye point of the eyepiece.

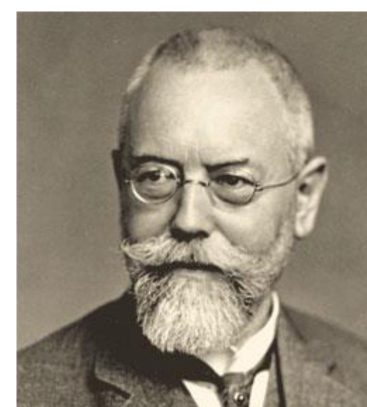
3) These planes called conjugated planes.

4) Provides an evenly illuminated field of view with a bright image, without glare (刺眼) and minimum heating of the specimen.

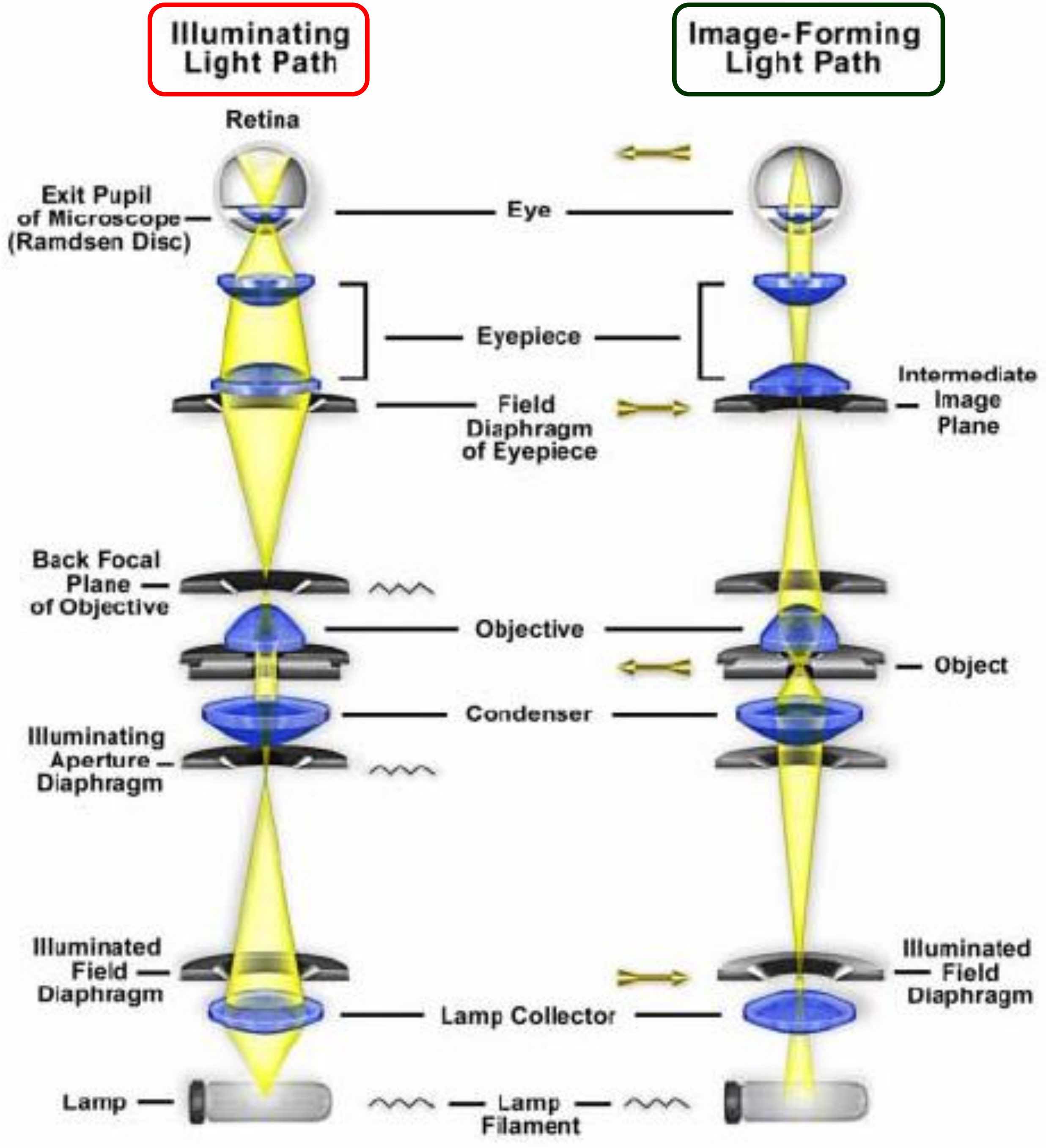
5) Very common in transmission microscopes.

Koehler Illumination (柯氏照明)





August Koehler


Illuminating Light Path
Image-Forming Light Path
Retina
Exit Pupil of Microscope (Ramdsen Disc)
Eye
Eyepiece
Field Diaphragm of Eyepiece
Intermediate Image Plane
Back Focal Plane of Objective
Objective
Object
Condenser
Illuminating Aperture Diaphragm
Illuminated Field Diaphragm
Lamp Collector
Illuminated Field Diaphragm
Lamp
Lamp Filament

Thanks For Your Attention

53