Modellistica 3D di Componenti Cellulari

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Home » Didattica

Materiale Didattico a.a. 2014/2015

- **Biologia dei Microrganismi (Prof.ssa Laura Marri)**
- **Corso di prevenzione e sicurezza nei laboratori**
- **Istituzioni di Matematiche e Fondamenti di Biostatistica (Prof.ssa Lucia Doretti)**

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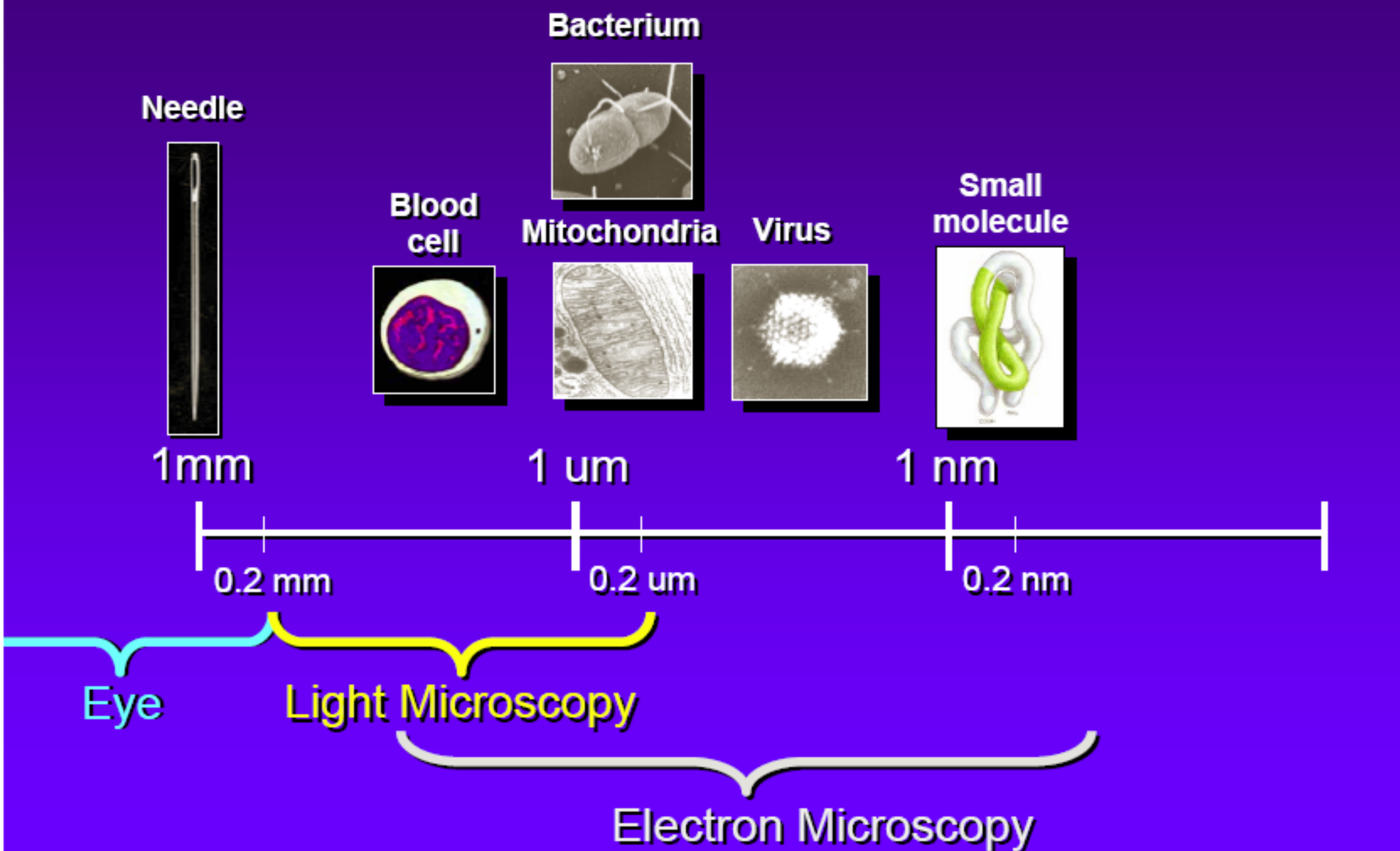
San Miniato, via Aldo Moro, 2
Via Pier Andrea Mattioli, 4
53100 Siena - Italia

Microscopy



Bacterium
Needle
Blood cell
Mitochondria
Virus
Small molecule
1mm
1 um
1 nm
0.2 mm
0.2 um
0.2 nm
Eye
Light Microscopy
Electron Microscopy

Light Microscopy

History of optical light microscopes

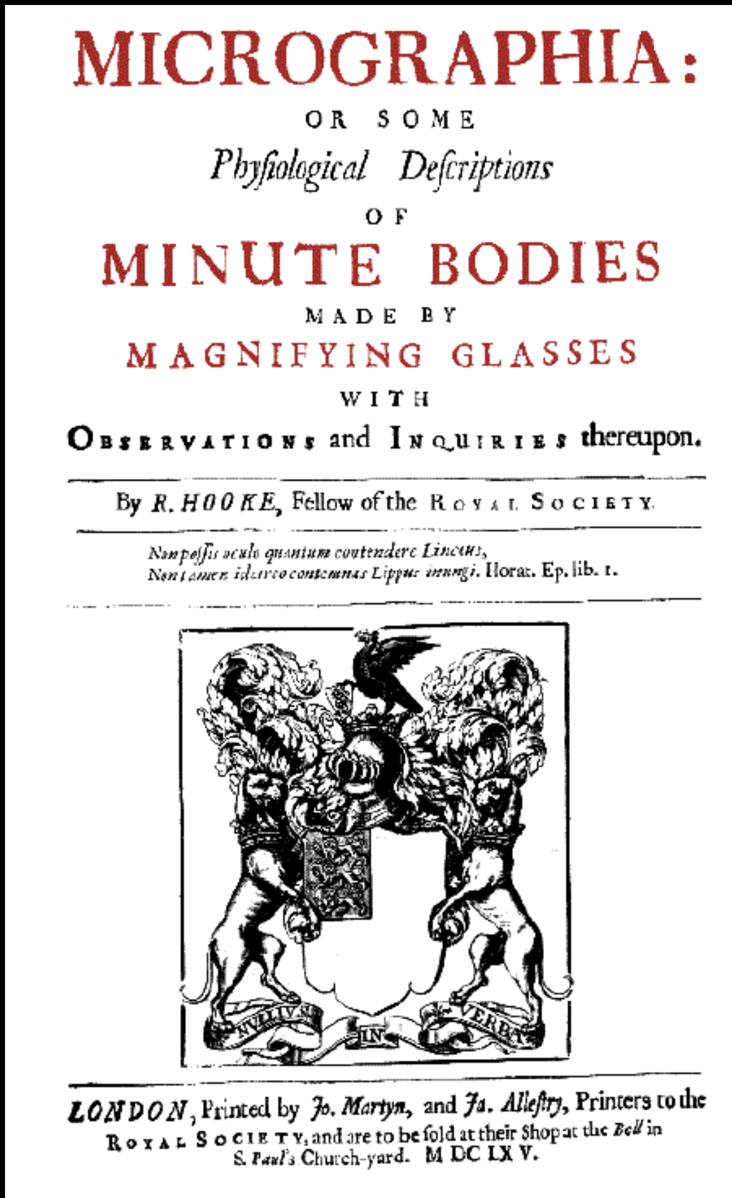
2nd Century BC - Claudius Ptolemy described a stick appearing to bend in a pool of water, and accurately recorded the angles to within half a degree.

1st Century - Romans were experimenting with glass and found objects appeared larger when viewed through this new material.

12th Century - Salvino D'Armate from Italy made the first eye glass, providing the wearer with an element of magnification to one eye.

http://www.history-of-the-microscope.org/invention-of-glass-lenses-and-the-history-of-the-light-microscope.php

Robert Hooke
(1635-1703)


MICROGRAPHIA:
OR SOME
Physiological Descriptions
OF
MINUTE BODIES
MADE BY
MAGNIFYING GLASSES
WITH
OBSERVATIONS and INQUIRIES thereupon.

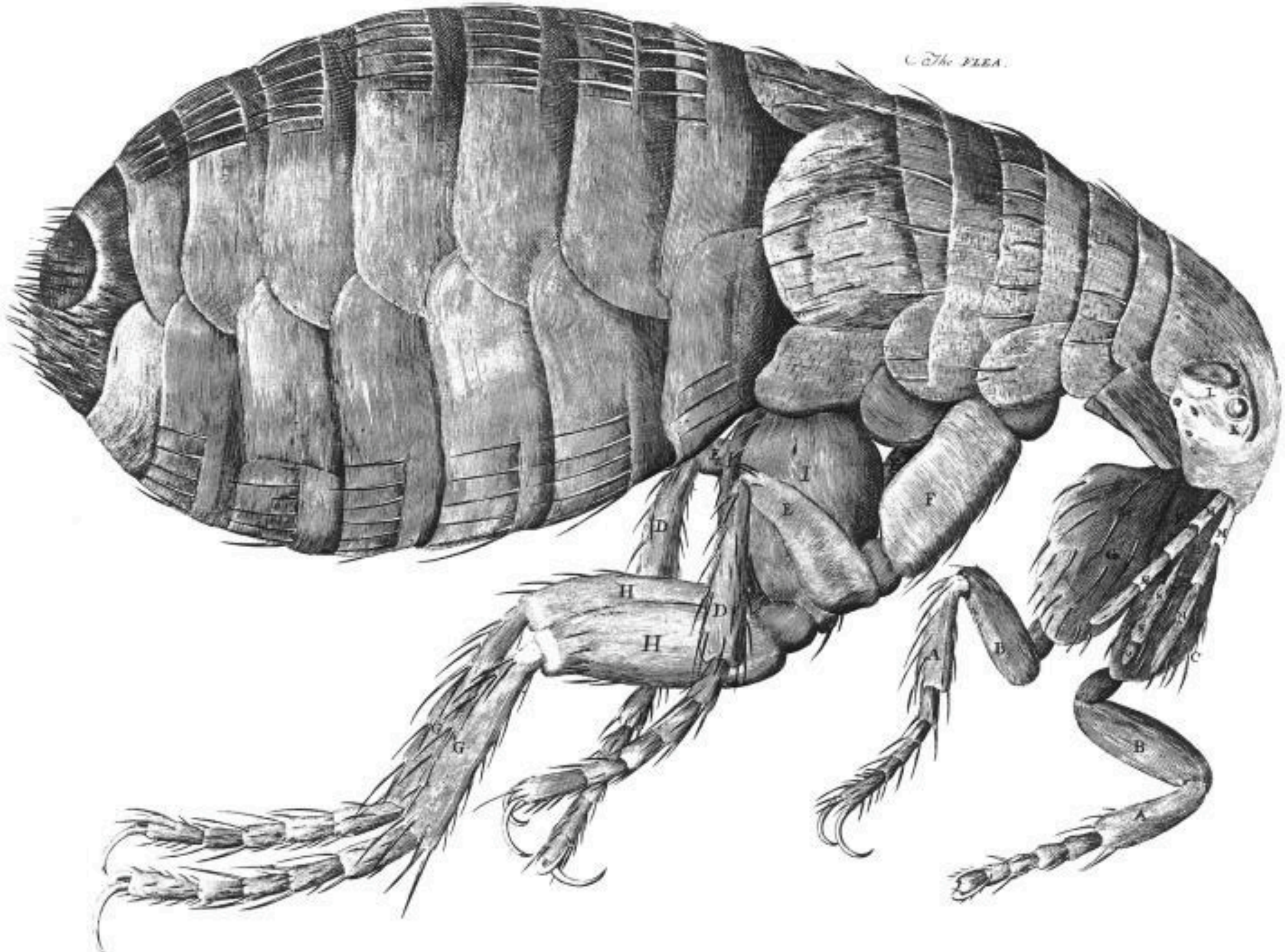
By *R. HOOKE*, Fellow of the ROYAL SOCIETY.

Non possis oculo quantum contendere Linceus,
Non tamen idcirco contemnas Lippus inungi. Horat. Ep. lib. 1.

LONDON, Printed by *Jo. Martyn*, and *Ja. Allestry*, Printers to the ROYAL SOCIETY, and are to be sold at their Shop at the *Bell* in S. *Paul's* Church-yard. M DC LX V.

1665 - Robert Hooke's book called Micrographia officially documented a wide range of observations through the microscope.

. . . I could exceedingly plainly perceive it to be all perforated and porous, much like a Honey-comb, but that the pores of it were not regular. . . . these pores, or cells, . . . were indeed the first *microscopical* pores I ever saw, and perhaps, that were ever seen, for I had not met with any Writer or Person, that had made any mention of them before this. . .


The FLEA.

1674 - Anton van Leeuwenhoek used his knowledge of grinding lenses to achieve greater magnification which he utilised to make a microscope, enabling detailed observations to be made of bacteria.

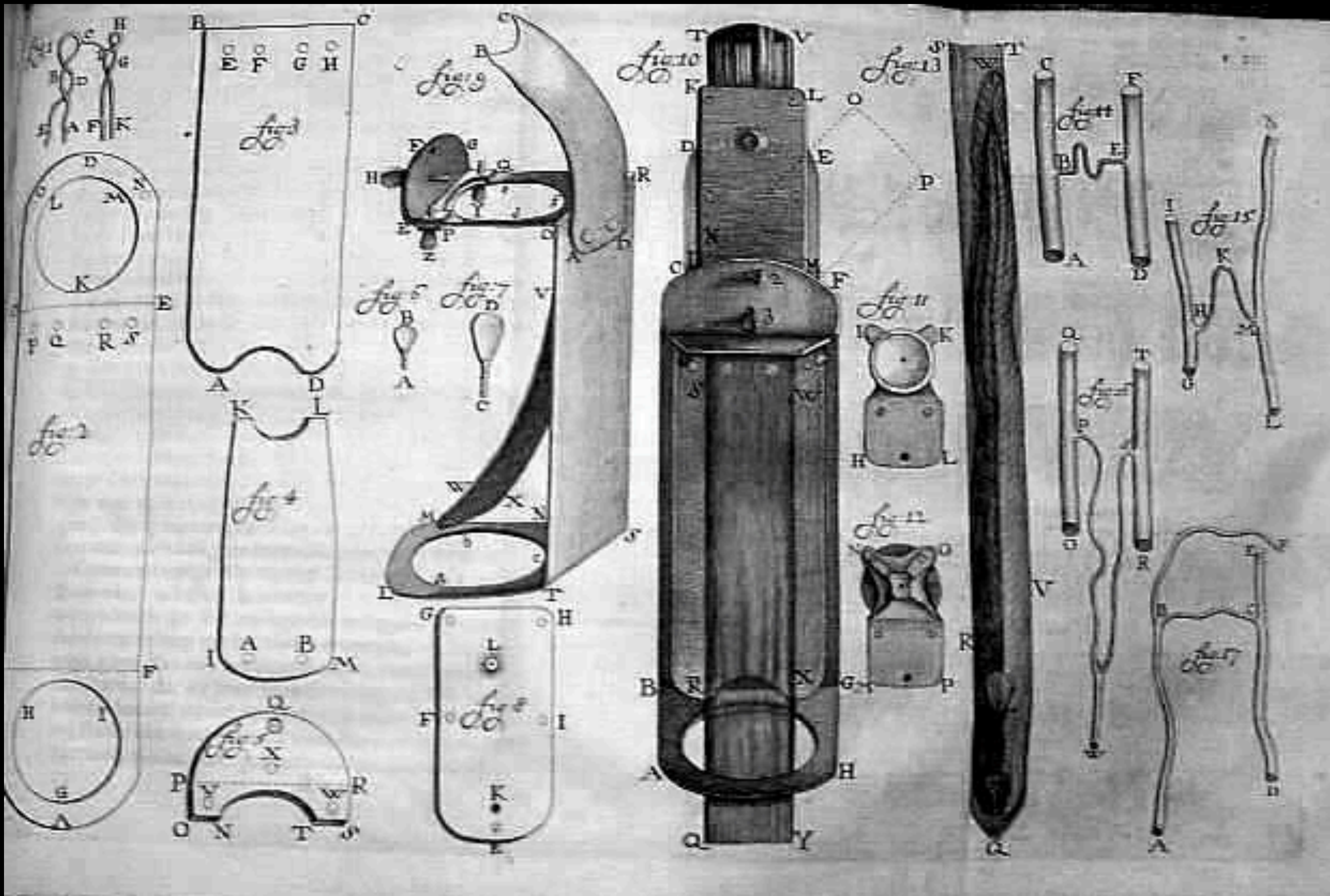
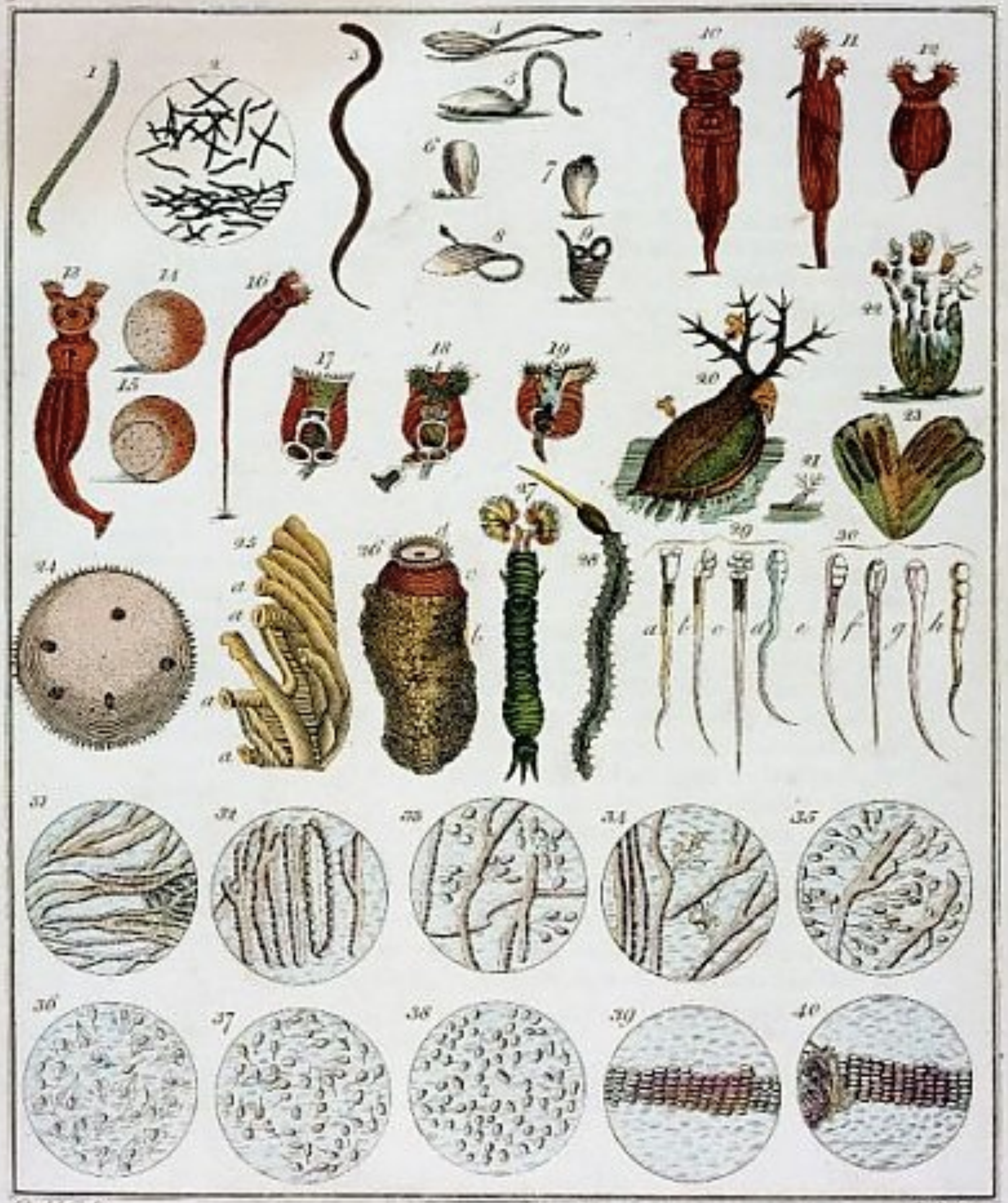


fig. 1
fig. 2
fig. 3
fig. 4
fig. 5
fig. 6
fig. 7
fig. 8
fig. 9
fig. 10
fig. 11
fig. 12
fig. 13
fig. 14
fig. 15
fig. 16
fig. 17

. . . my work, which I've done for a long time, was not pursued in order to gain the praise I now enjoy, but chiefly from a craving after knowledge, which I notice resides in me more than in most other men. And therewithal, whenever I found out anything remarkable, I have thought it my duty to put down my discovery on paper, so that all ingenious people might be informed thereof.
Antony van Leeuwenhoek. Letter of June 12, 1716

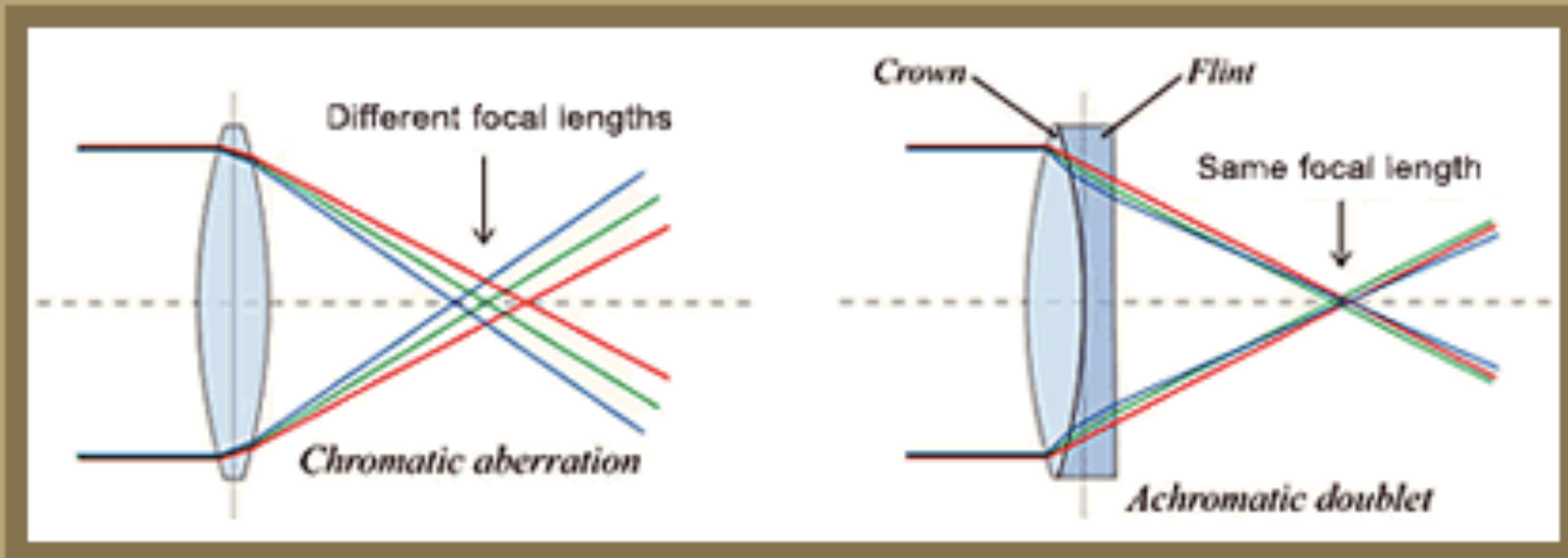


Dodd Delin. Pass Sculp.

Animalcules.

Hooke had discovered plant cells -- more precisely, what Hooke saw were the cell walls in cork tissue. In fact, it was Hooke who coined the term "cells": the boxlike cells of cork reminded him of the cells of a monastery. Hooke also reported seeing similar structures in wood and in other plants. In 1678, after Leeuwenhoek had written to the Royal Society with a report of discovering "little animals" -- bacteria and protozoa -- Hooke was asked by the Society to confirm Leeuwenhoek's findings. He successfully did so, thus paving the way for the wide acceptance of Leeuwenhoek's discoveries. Hooke noted that Leeuwenhoek's simple microscopes gave clearer images than his compound microscope, but found simple microscopes difficult to use: he called them "offensive to my eye" and complained that they "much strained and weakened the sight."

1826 - Joseph Jackson Lister created an achromatic lens to eradicating the chromatic effect caused by different wavelengths of light.



1860s - Ernst Abbe discovers the Abbe sine condition (a condition that must be fulfilled by a lens or other optical system in order for it to produce sharp images), a breakthrough in microscope design, which was until then largely based on trial and error.

1931 - Ernst Ruska starts to build the first electron microscope.

1590 - Two Dutch spectacle makers, Zacharias Jansen and his father Hans started experimenting by mounting two lenses in a tube, the first compound microscope.

1609 - Galileo Galilei develops a compound microscope with a convex and a concave lens.

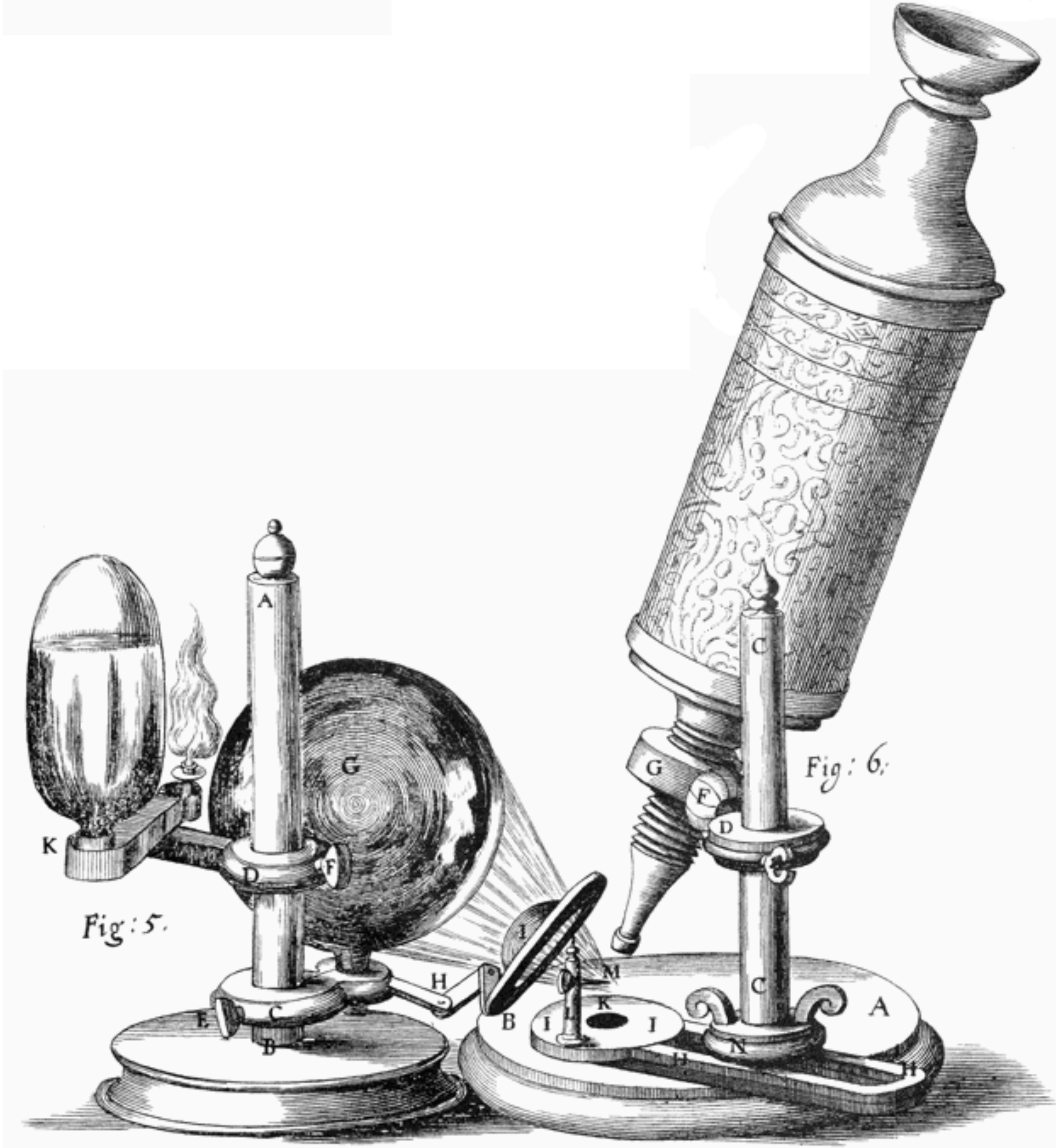


Fig: 5.
Fig: 6.

Today's light microscopes…

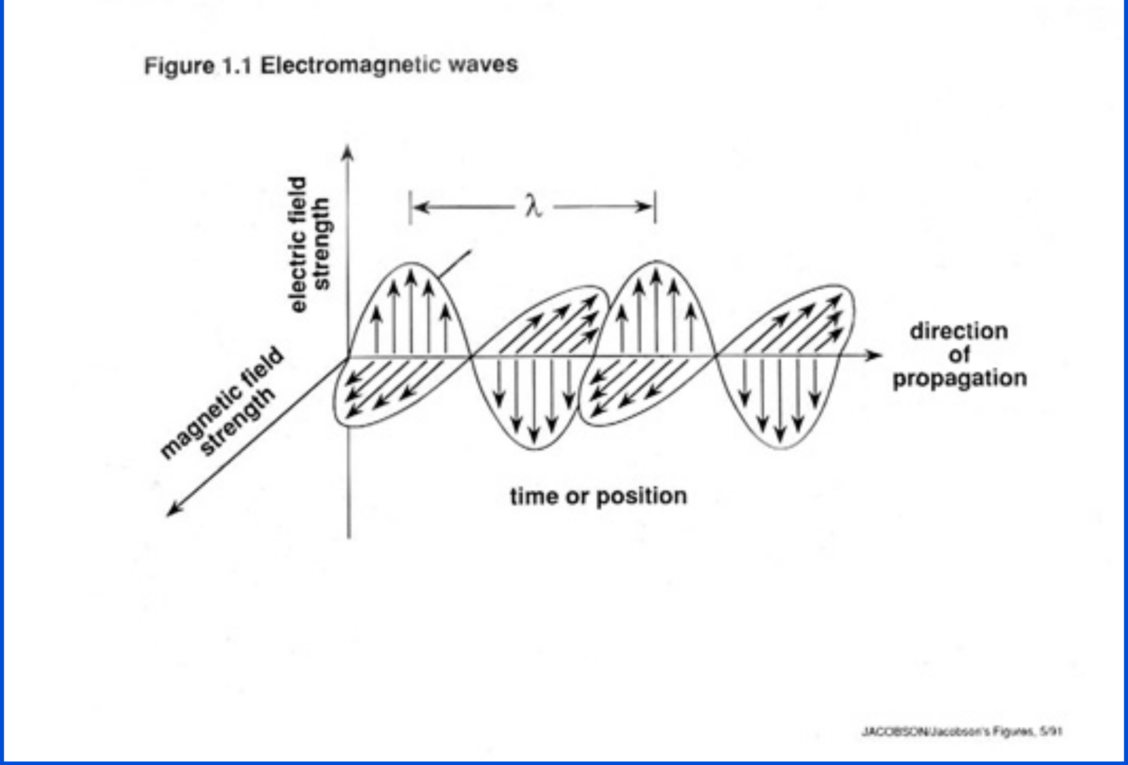
Light microscopy: topics

- Properties of light
- Compound microscope
- Resolution & contrast
- Contrast modes in light microscopy
- Koehler Illumination

General References

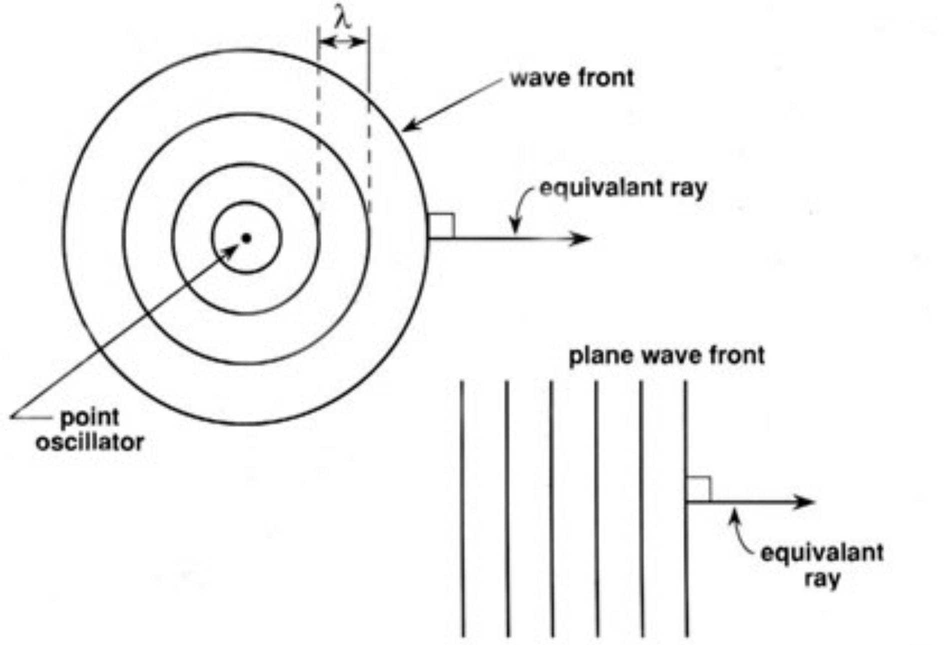
- Salmon, E. D. and J. C. Canman. 1998. Proper Alignment and Adjustment of the Light Microscope. Current Protocols in Cell Biology 4.1.1-4.1.26, John Wiley and Sons, N.Y.
- Murphy, D. 2001. Fundamentals of Light Microscopy and Electronic Imaging. Wiley-Liss, N.Y.
- Keller, H.E. 1995. Objective lenses for confocal microscopy. In "Handbook of biological confocal microsocpy", J.B.Pawley ed. , Plenum Press, N.Y.
- Microscopes: Basics & Beyond [pdf available]

Some properties of light important for the microscope



Light as electromagnetic wave with mutually perpendicular E, B components characterized by wavelength,λ, and frequency, ν, in cycles/s. Wave velocity = ν x λ. [λ=500nm--> ν=6x10^{14} cycles/s]

Figure 1.2 Spherical wavefronts emanating from a point oscillator source



Defining wavefronts and rays

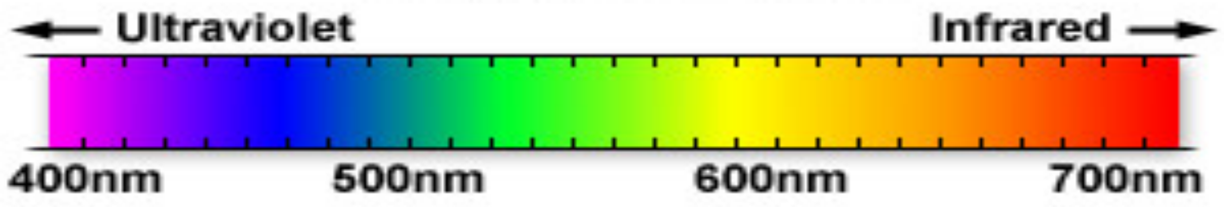
Light microscopy

Part II

Velocity of light in different media

- Index of refraction, n =c/v
  - C=speed of light in vacuum=$3x10^8$ m/s; v= velocity in media
- Light travels slower in more dense media

Index of refraction for different media at 546 nm

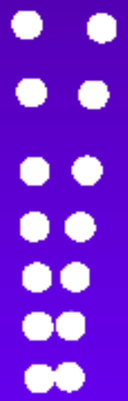


Air	1.0
Water	1.3333
Cytoplasm	1.38
Glycerol	1.46
Crown Glass	1.52
Immersion Oil	1.515
Protein	1.51-1.53
Flint Glass	1.67

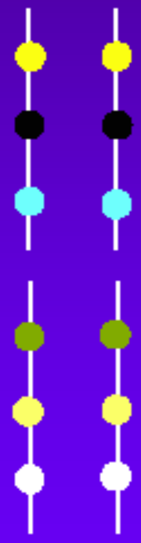
n increases with decreasing λ

Visibility

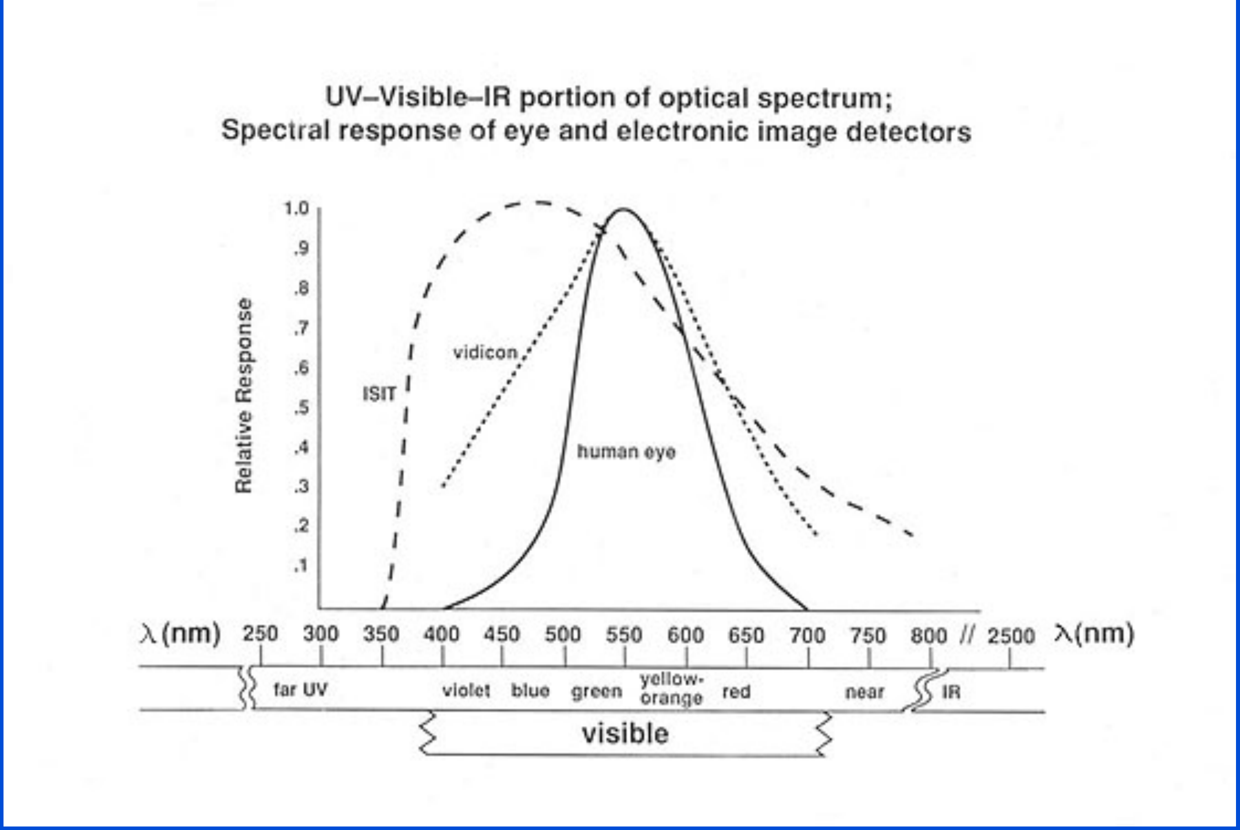
Resolution



Contrast

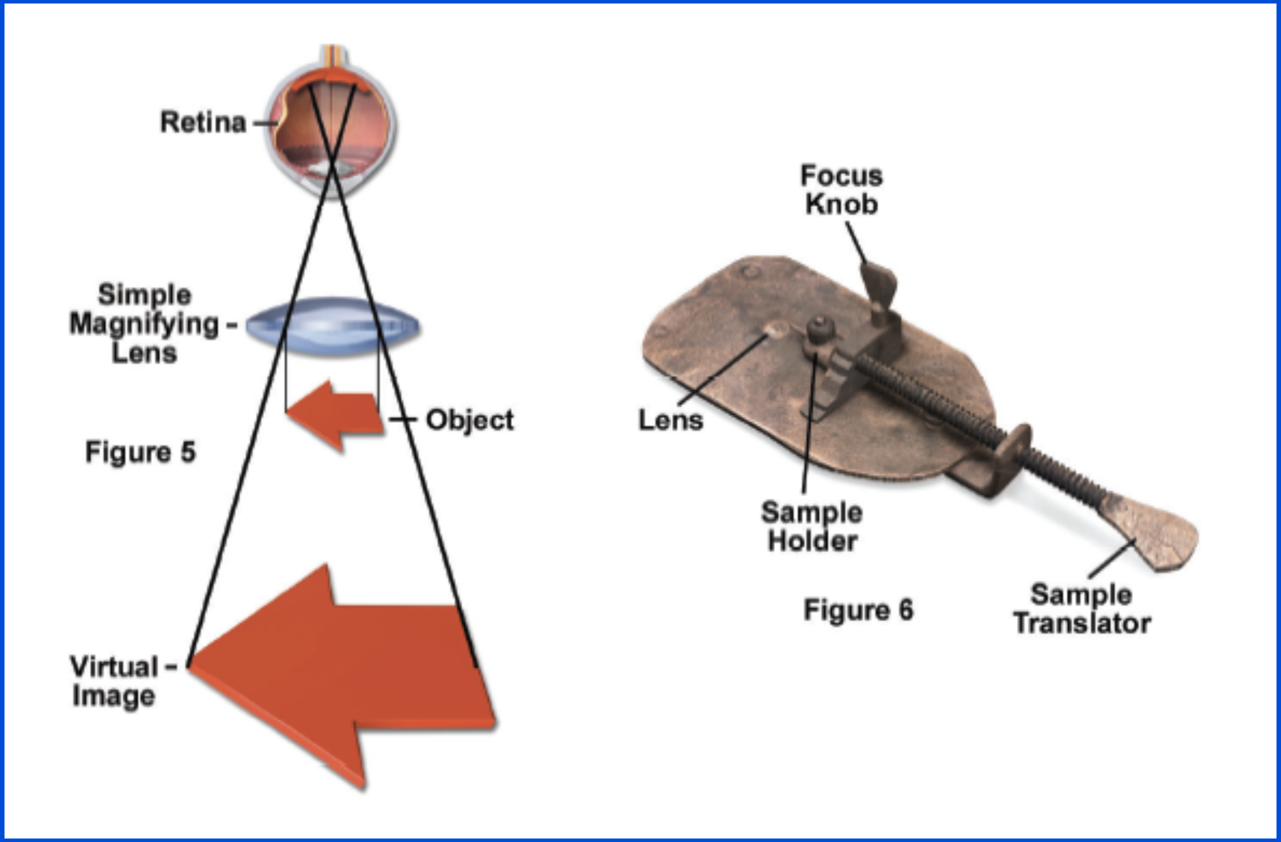


Difference in density or color



Note: electronic cameras do not have same spectral response as eyes

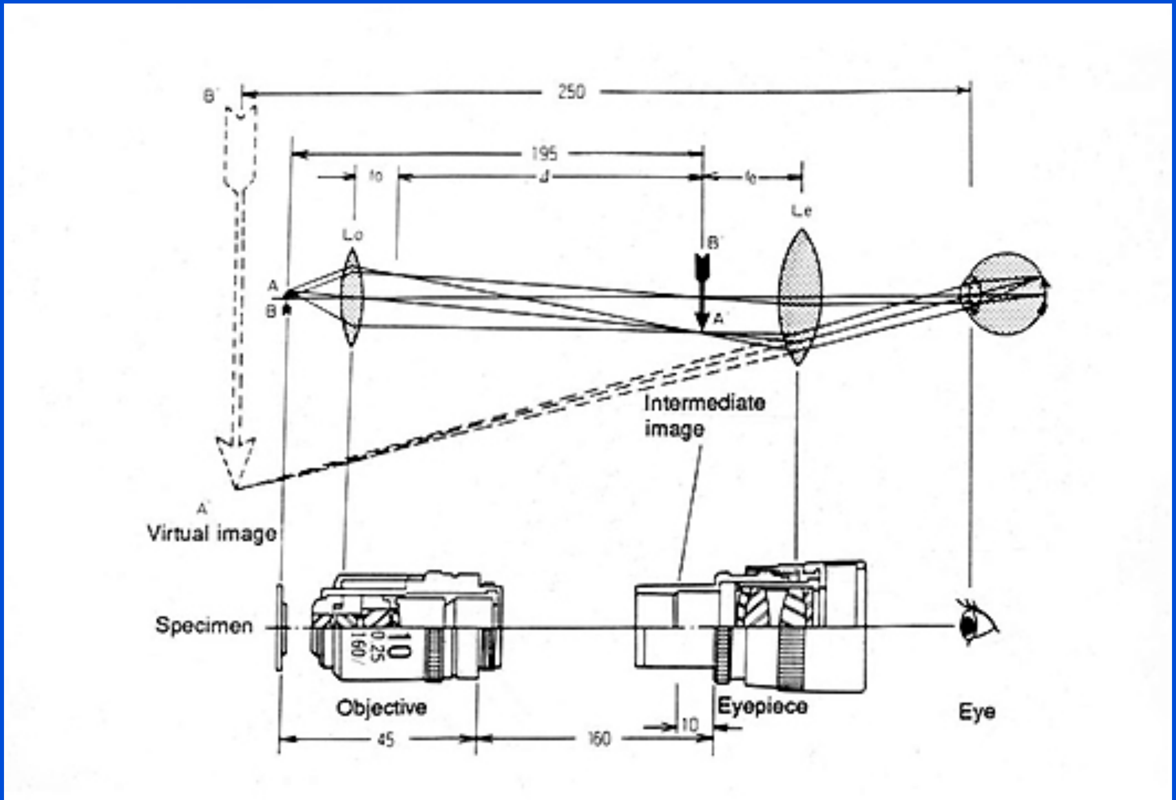
The simplest microscope: a magnifier



The compound microscope

The purpose of the microscope is to create **magnification** so that structures can be resolved by eye and to create **contrast** to make objects visible.

In the compound microscope, the objective forms a real, inverted image at the eyepiece front focal plane (the primary image plane)



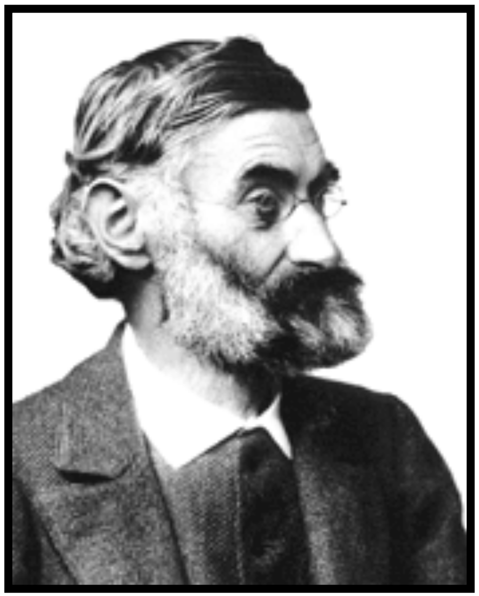
The optical tube length (OTL), typically 160mm, is the distance between the rear focal plane of the objective and the intermediate image plane

Total magnification in the compound microscope

$$M_t = M_{obj} \times M_{ep}$$

Max M_t = 1000xNA; > 1000NA, empty mag.

1860s - Ernst Abbe discovers the Abbe sine condition (a condition that must be fulfilled by a lens or other optical system in order for it to produce sharp images), a breakthrough in microscope design, which was until then largely based on trial and error.



Ernst Abbe
1840 - 1905

N.A. = n (sine α)
n = index of refraction
α = half angle of illumination

$$R.P. = \frac{0.61\ \lambda}{N.A.}$$

$$\text{R.P.} = \frac{0.61\,\lambda}{\text{N.A.}}$$

In light microscopy the N.A. of a lens and therefore resolution can be increased by a) increasing the half angle of illumination, b) increasing the refractive index of the lens by using Crown glass and c) decreasing the wavelength (λ) of illumination.

In microscopy, the numerical aperture of an optical system such as an objective lens is

$$N.A. = n (\text{sine } \alpha)$$

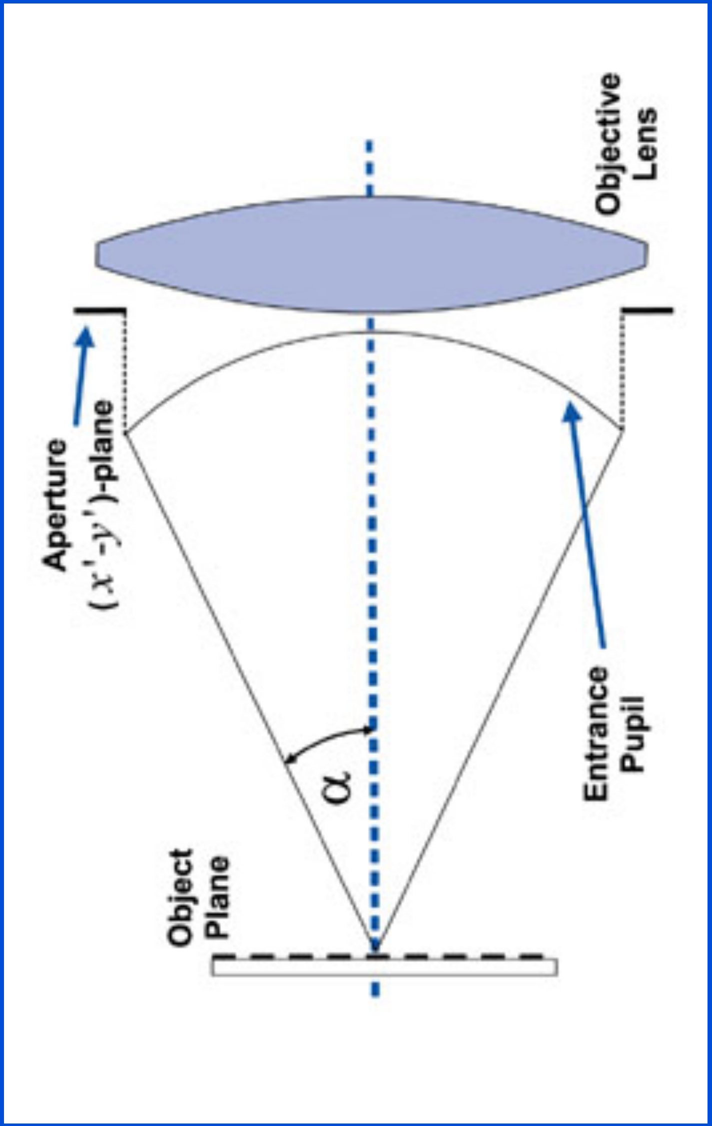
where n is the index of refraction of the medium in which the lens is working (1.00 for air, 1.33 for pure water, and typically 1.52 for immersion oil;

and **α** is the half-angle of the maximum cone of light that can enter or exit the lens.

In microscopy, NA is important because it indicates the resolving power of a lens. The size of the finest detail that can be resolved is proportional to λ/2NA, where λ is the wavelength of the light.

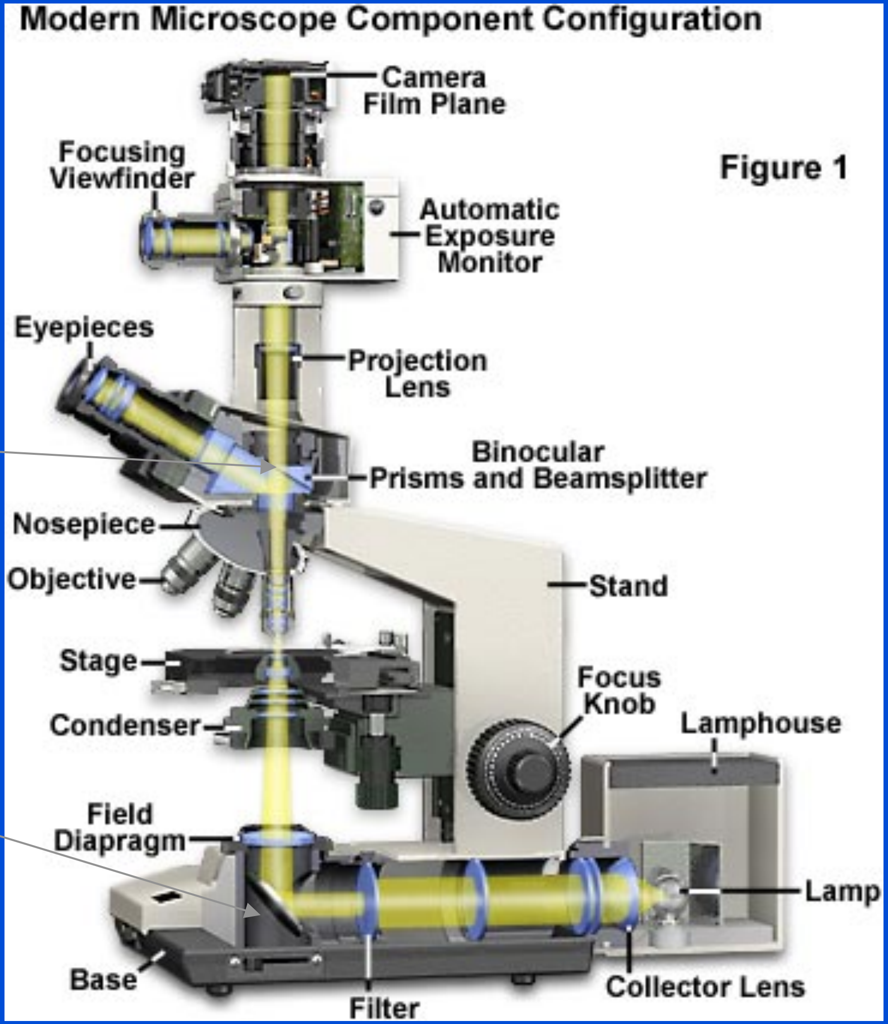
A lens with a larger numerical aperture will be able to visualize finer details than a lens with a smaller numerical aperture.

Assuming quality (diffraction limited) optics, lenses with larger numerical apertures collect more light and will generally provide a brighter image, but will provide shallower depth of field.



Modern microscope component identification

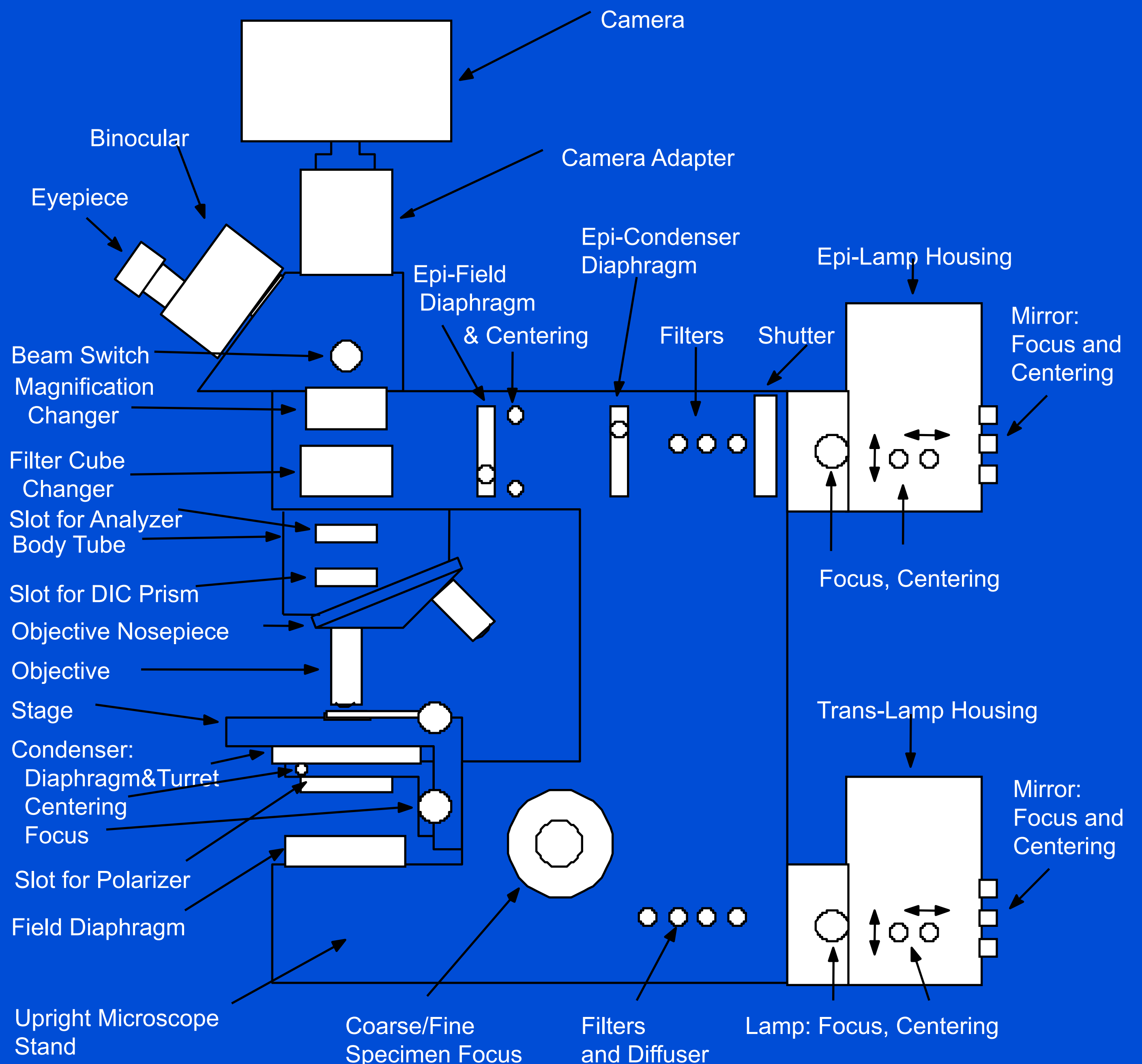
Prisms Used to Re-Direct Light In Imaging Path While Mirrors Are Used in Illumination Path



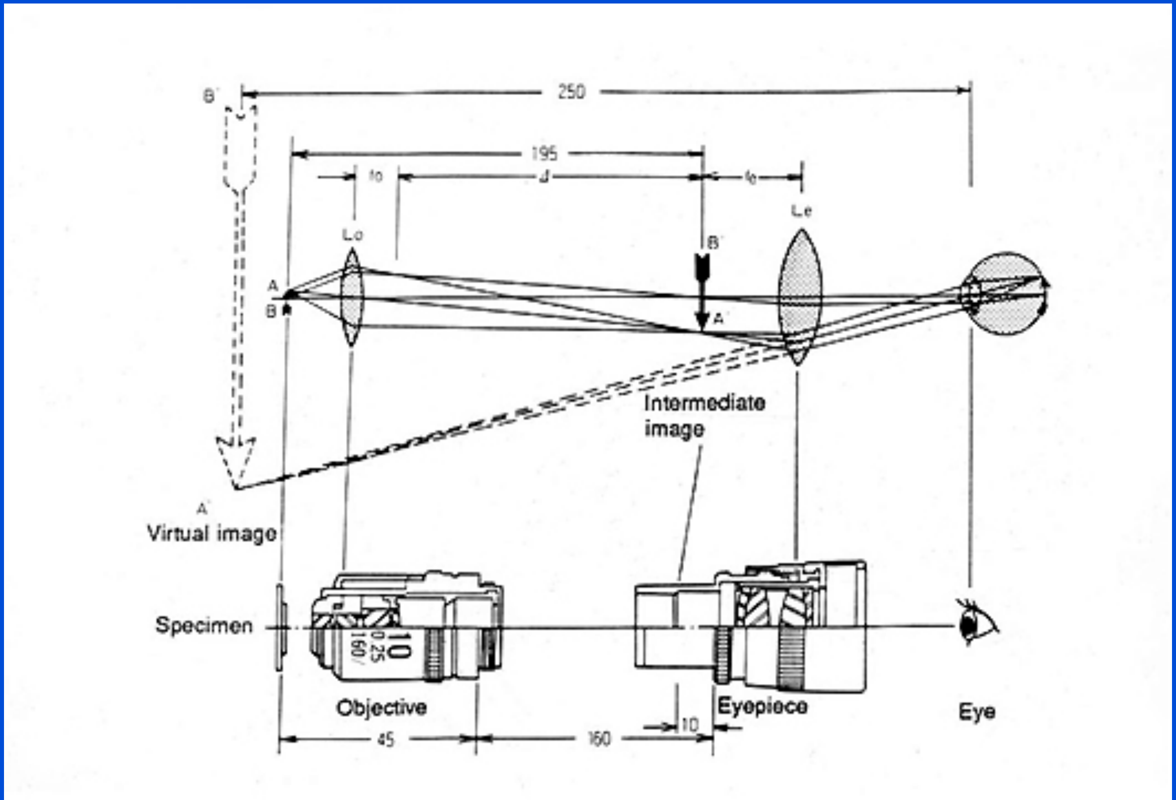
E.D.Salmon

Identify Major Components And Their Locations And Functions Within Modern Research Light Microscope (See Salmon And Canman, 2000, Current Protocols in Cell Biology, 4.1)

MICROSCOPE COMPONENTS



In the compound microscope, the objective forms a real, inverted image at the eyepiece front focal plane (the primary image plane)



The optical tube length (OTL), typically 160mm, is the distance between the rear focal plane of the objective and the intermediate image plane

A word about infinity corrected optics and its advantages.
[object is set at front focal plane of objective]

Eliminates ghost images caused by con-verging light, allows filters and polarizers to be inserted in 'infinity' space without corrections

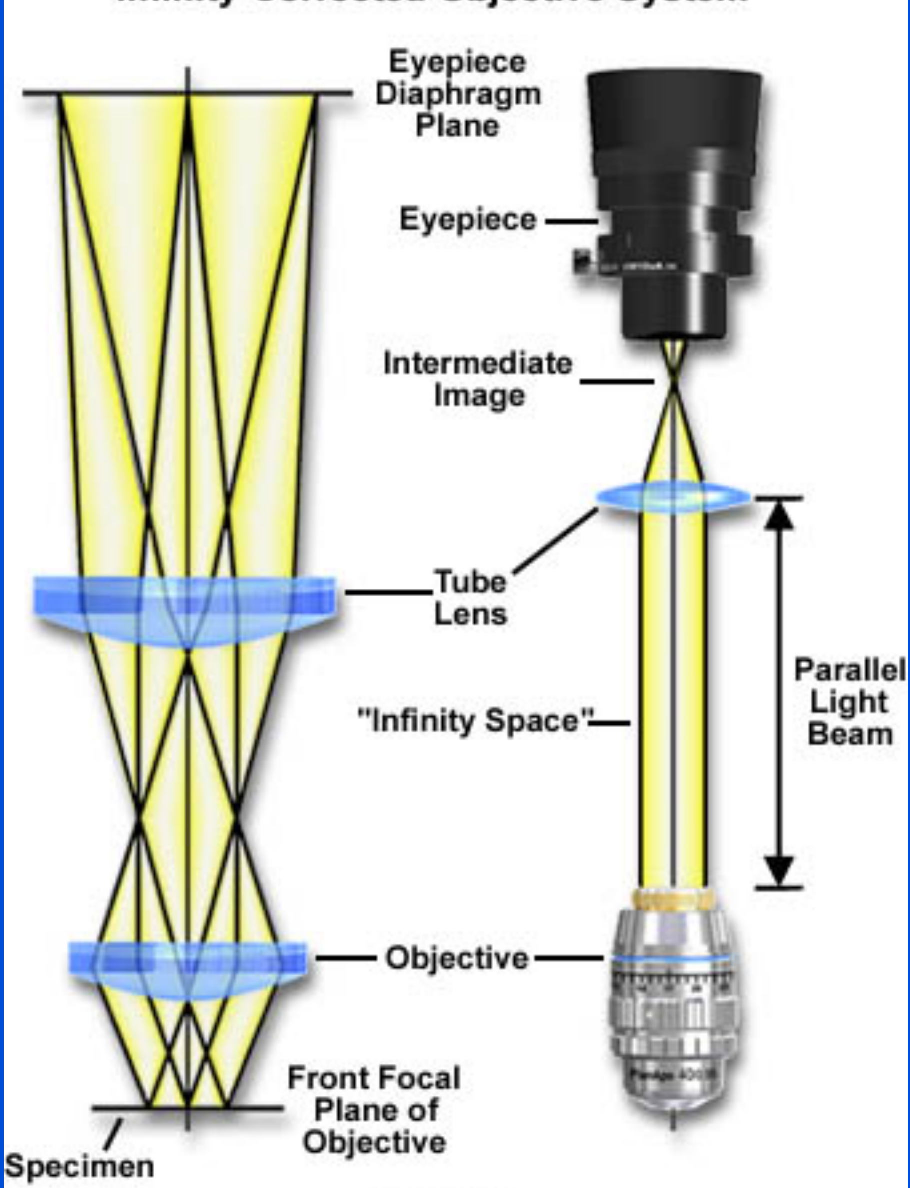
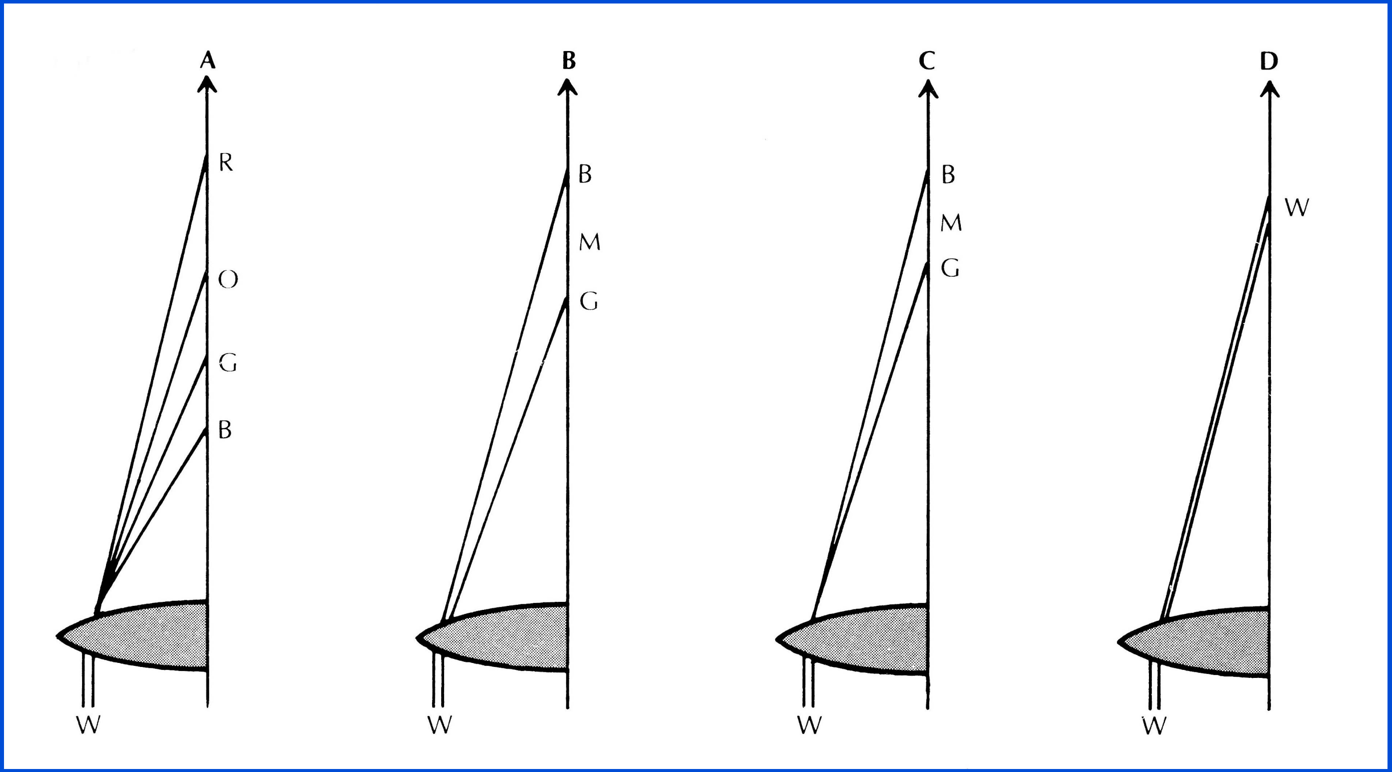


Figure 3

Key component: the objective

--> Aberrations

Chromatic aberration and its correction



Achromat
R,B corrected

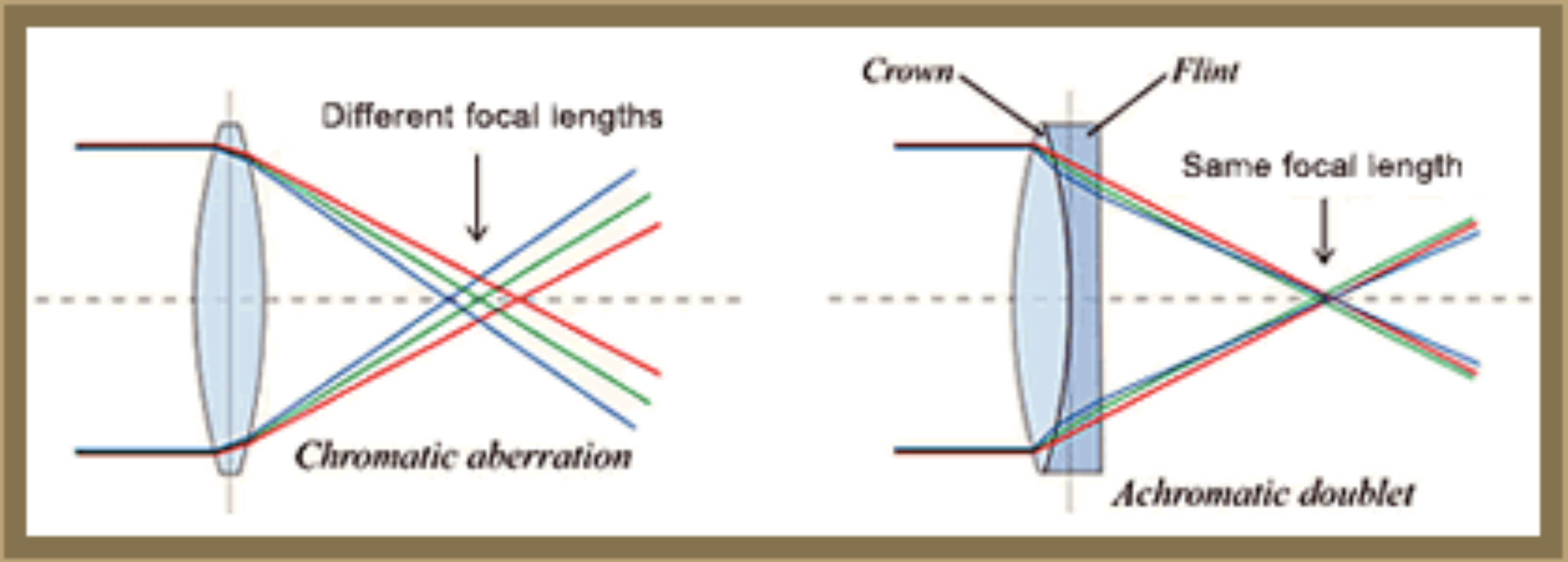
Fluorite
R,B corrected

Apochromat
R,G,B corrected

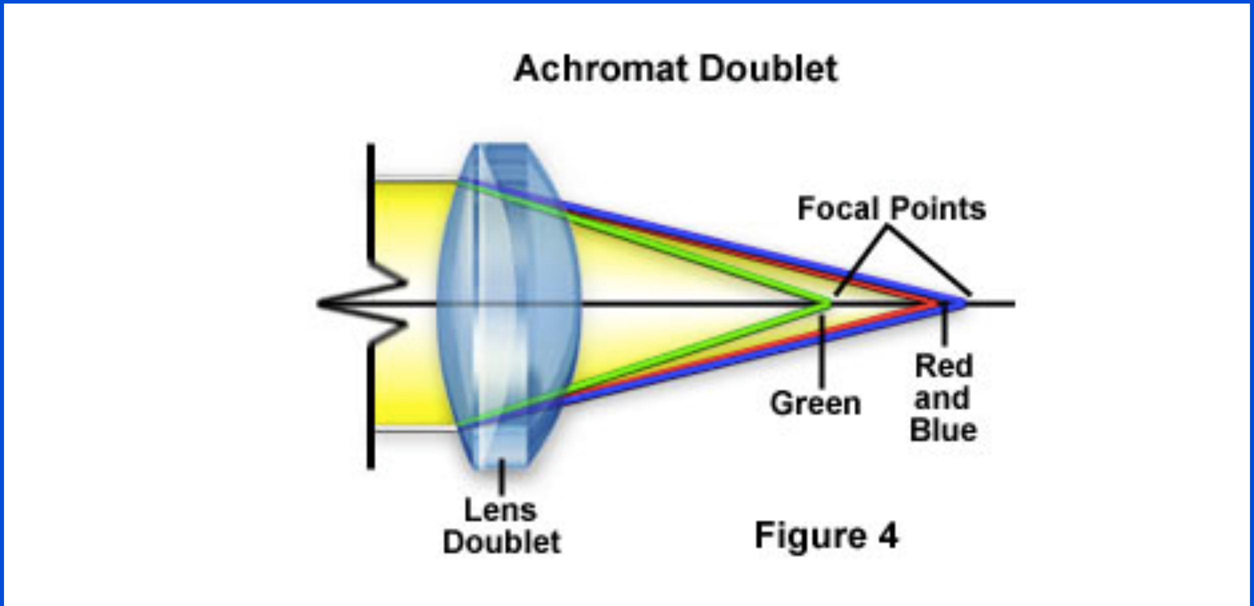
Lens designer, using various glasses & elements, tries to bring all colors to common focus

Chromatic aberration

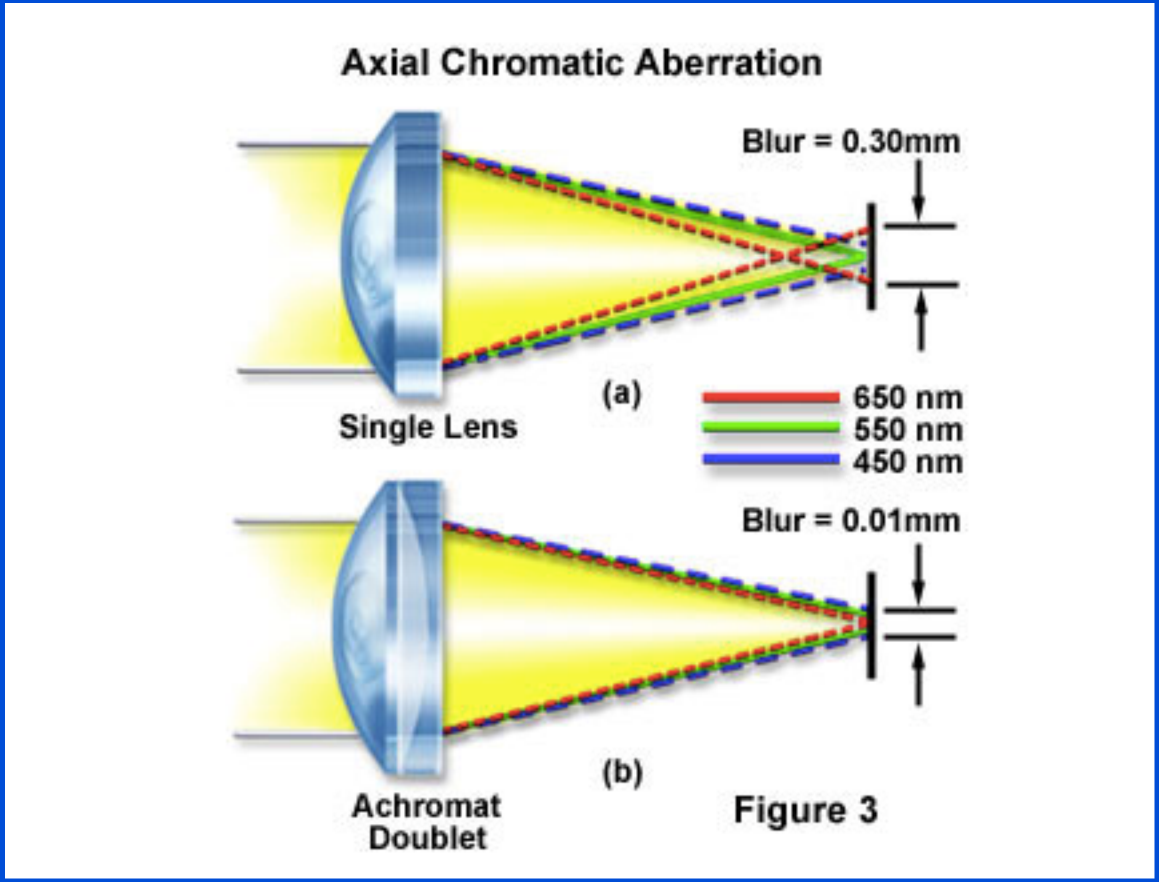
1826 - Joseph Jackson Lister created an achromatic lens to eradicating the chromatic effect caused by different wavelengths of light.

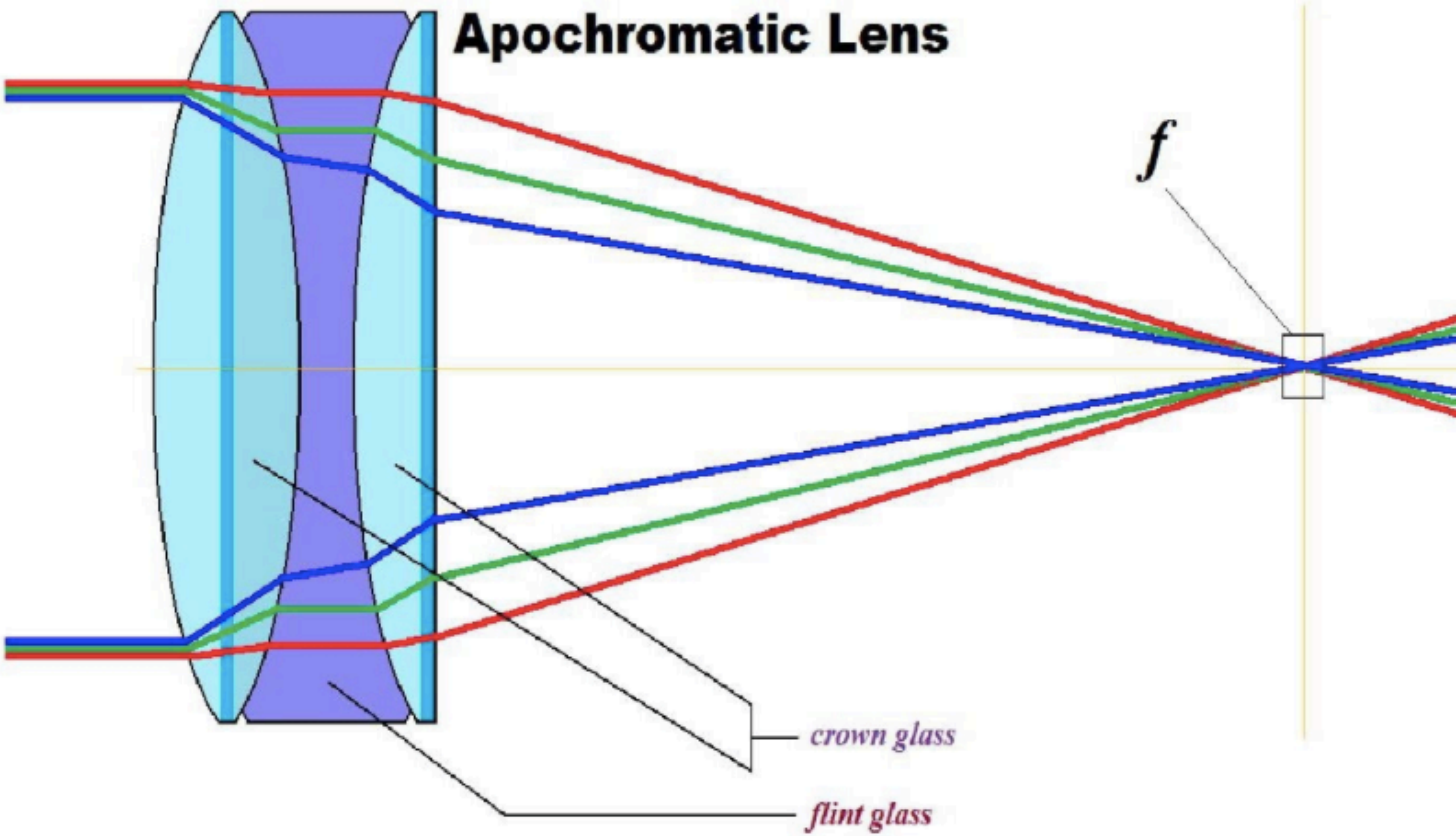


Achromat and fluorites objectives



Achromat and fluorites objectives




Apochromatic Lens
f
crown glass
flint glass

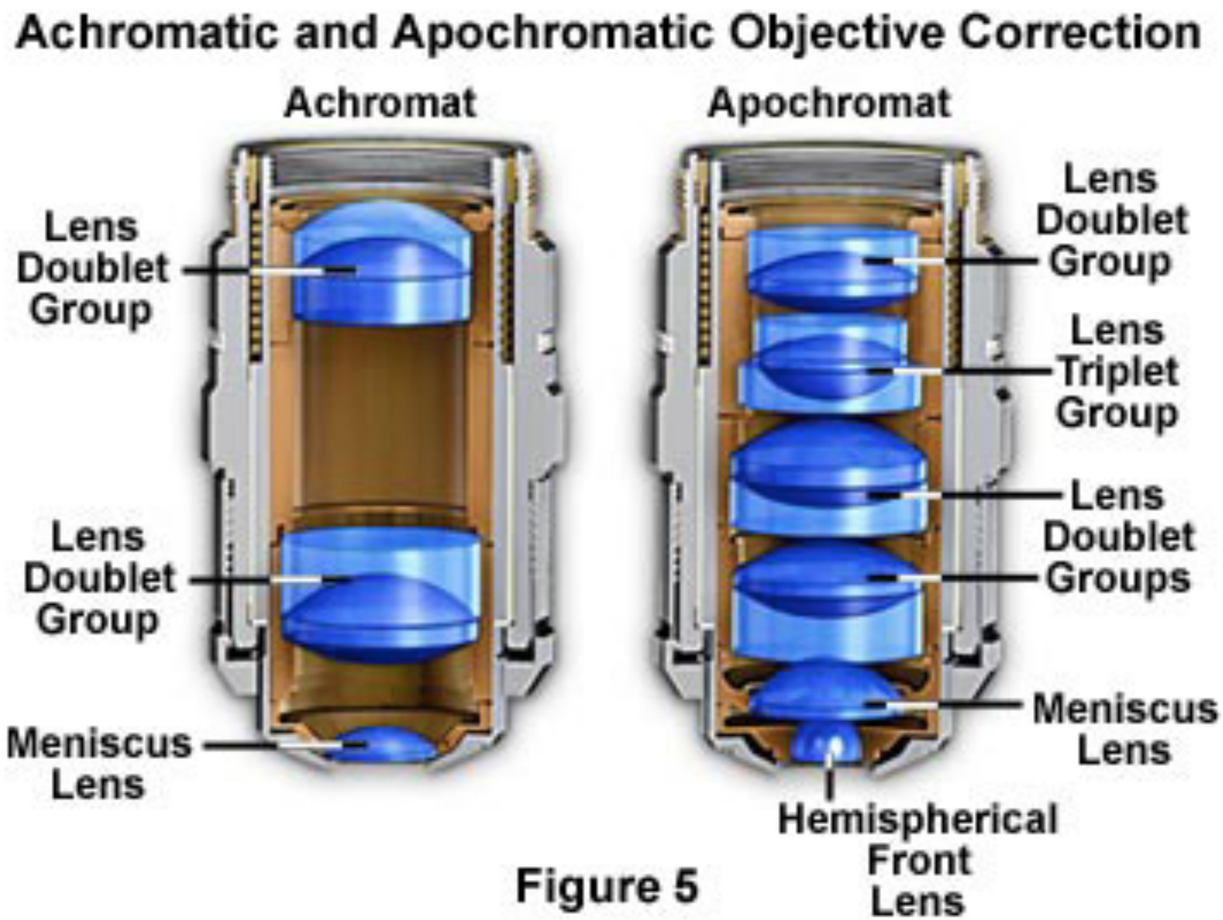
Crown & Flint glass

Crown glass

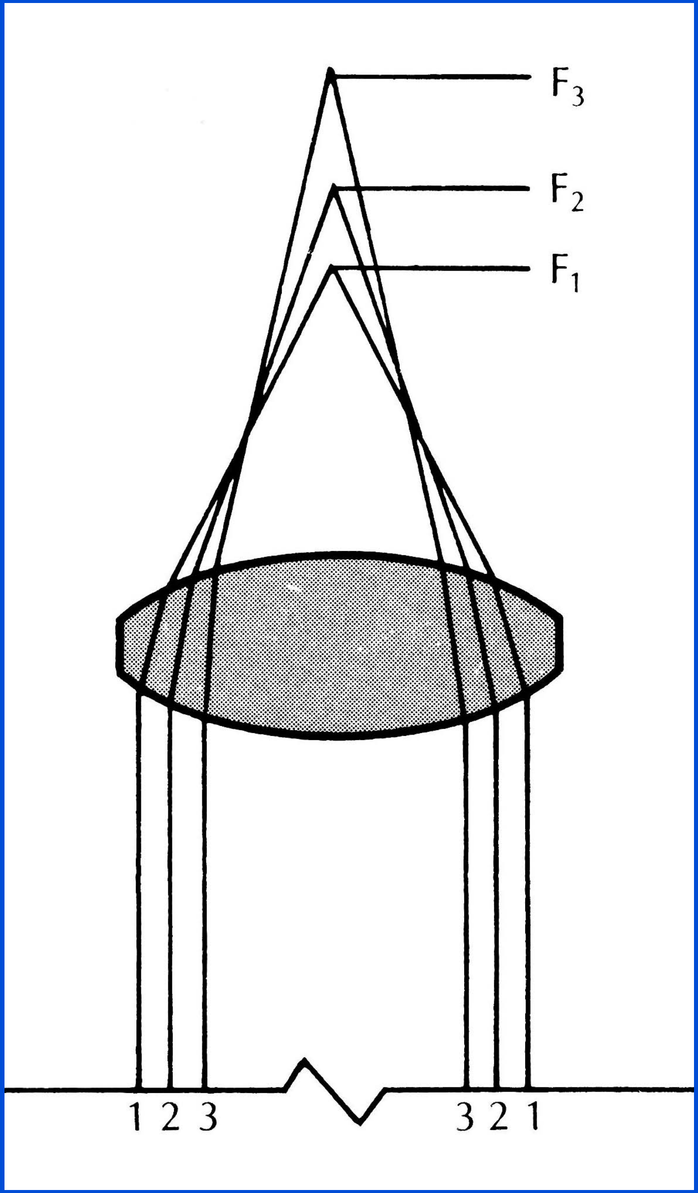
Is a type of glass used in lenses and other optical components. It has relatively low refractive index (≈1.52) and low dispersion (with Abbe numbers around 60). Crown glass is produced from alkali-lime (RCH) silicates containing approximately 10% potassium oxide and is one of the earliest low dispersion glasses.

Flint glass

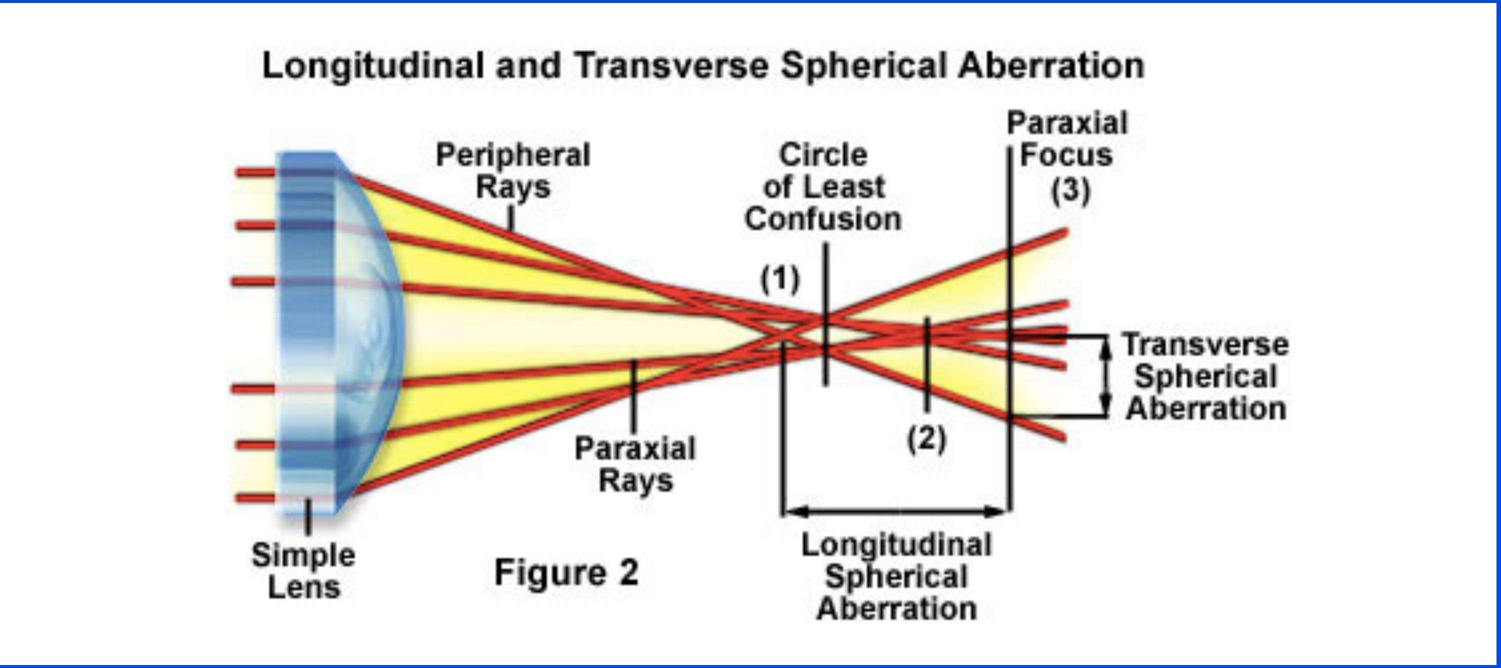
Is optic glass that has relatively high refractive index and low Abbe number (high dispersion). Flint glasses are arbitrarily defined as having an Abbe number of 50 to 55 or less. The currently known flint glasses have refractive indices ranging between 1.45 and 2.00. A concave lens of flint glass is commonly combined with a convex lens of crown glass to produce an achromatic doublet lens because of their compensating optical properties, which reduces chromatic aberration (colour defects).


Achromatic and Apochromatic Objective Correction
Achromat
Apochromat
Lens Doublet Group
Lens Doublet Group
Meniscus Lens
Lens Doublet Group
Lens Triplet Group
Lens Doublet Groups
Meniscus Lens
Hemispherical Front Lens
Figure 5

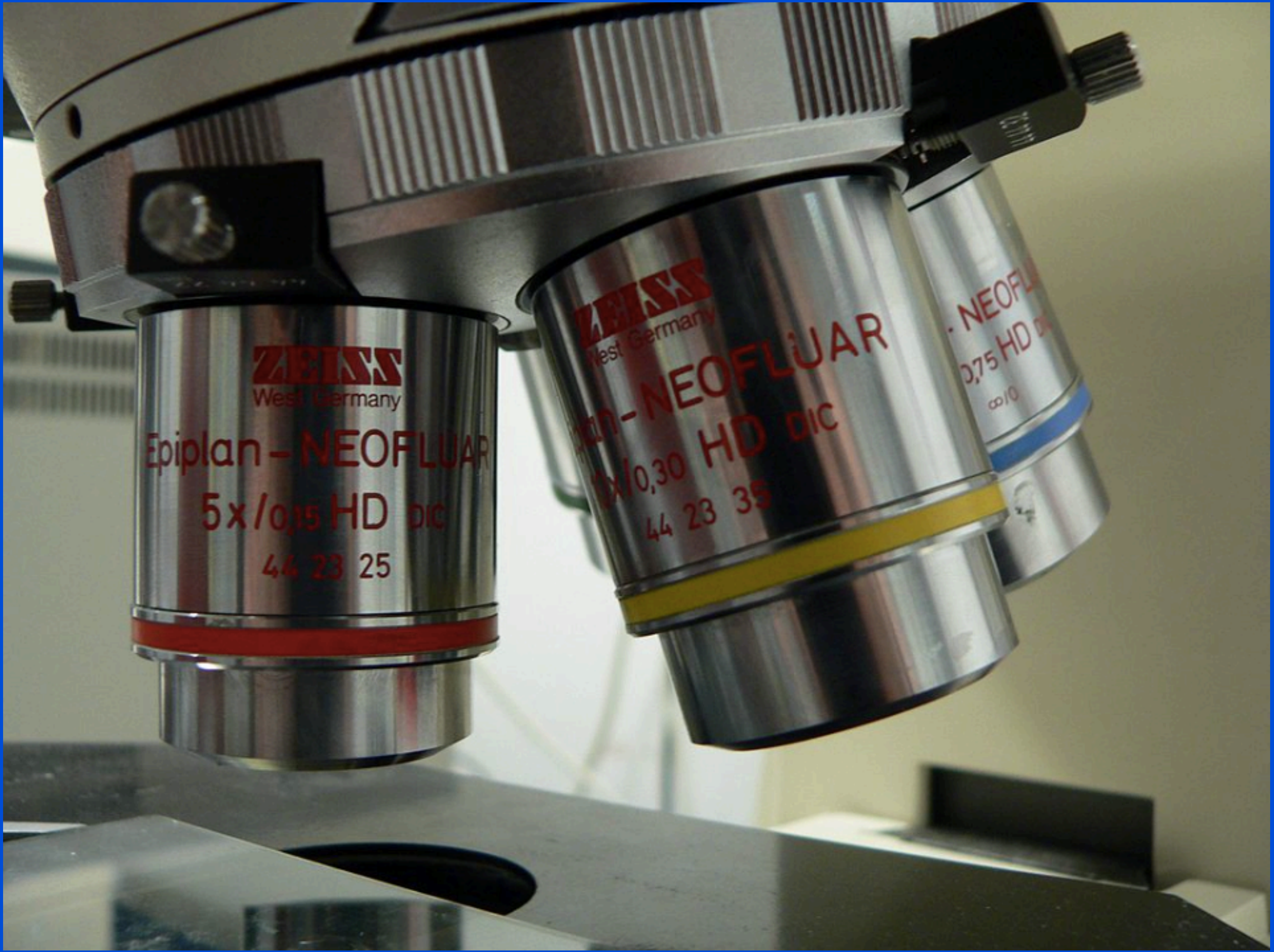
Spherical Aberration



Shperical aberration



Spherical aberrations are very important in terms of the resolution of the lens because they affect the coincident imaging of points along the optical axis and degrade the performance of the lens, which will seriously affect specimen sharpness and clarity. These lens defects can be reduced by limiting the outer edges of the lens from exposure to light using diaphragms and also by utilizing aspherical lens surfaces within the system. The highest-quality modern microscope objectives address spherical aberrations in a number of ways including special lens-grinding techniques, improved glass formulations, and better control of optical pathways.


ZEISS
West Germany
Epiplan-NEOFLUAR
5x/0,15 HD DIC
44 23 25
ZEISS
West Germany
NEOFLUAR
0,30 HD DIC
44 23 35

Objective Classes

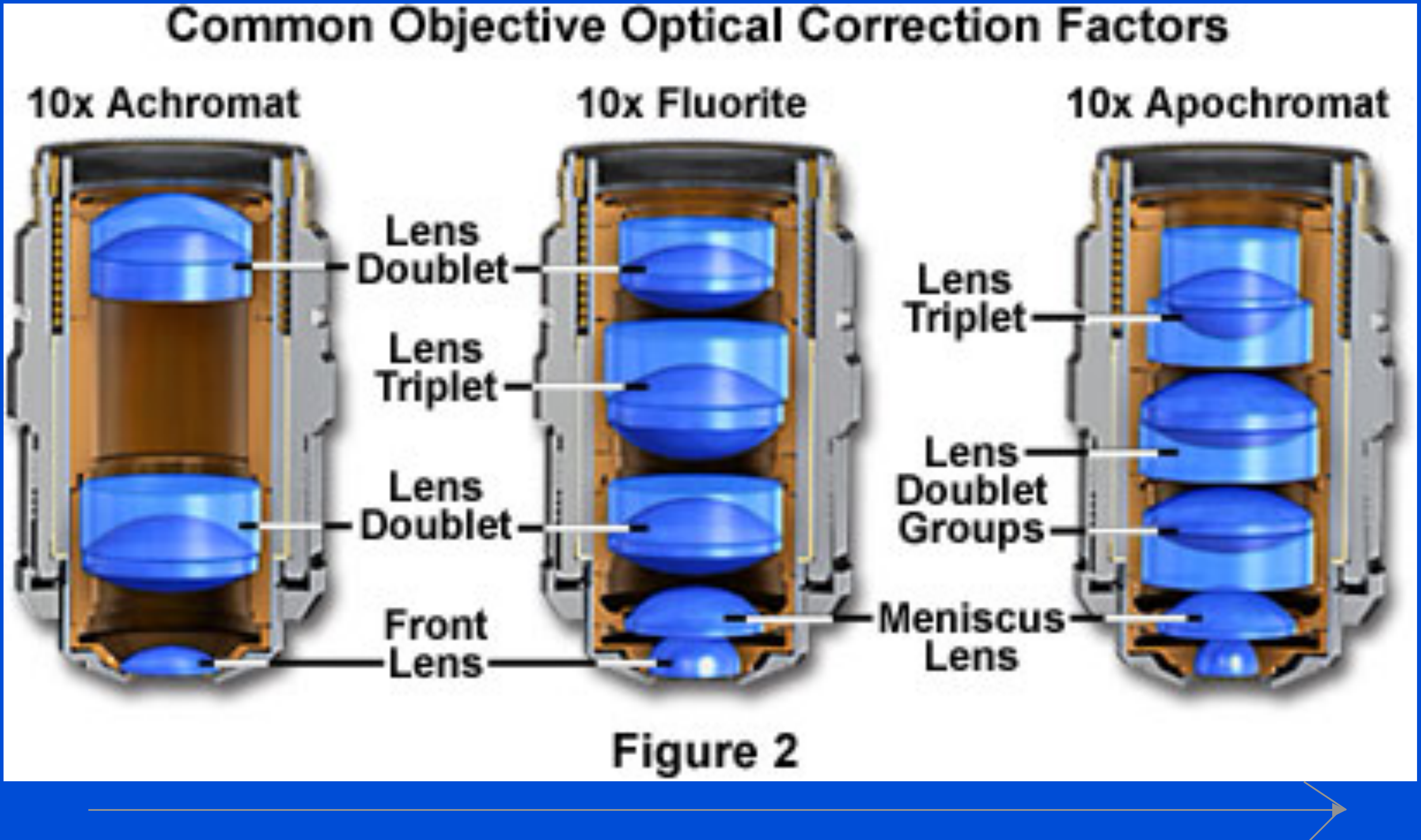
Achromats: corrected for chromatic aberration for red, blue

Fluorites: chromatically corrected for red, blue; spherically corrected for 2 colors

Apochromats: chromatically corrected for red, green & blue; spherically corrected for 2 colors

Plan-: further corrected to provide flat field

The 3 Classes of Objectives



Chromatic and Mono-Chromatic Corrections

E.D. Salmon

Multilayer anti-reflection coatings

- Highly corrected objectives may have 15 elements. Each uncoated glass-air interface can reflect 4-5%, dropping objective thruput to as low as 50%.
- Multi layer AR coatings suppress reflections increasing transmission > 99.9% as well as reducing ghosts and flare to preserve contrast.

Objective Specifications

Figure 1

E.D. Salmon