Light Microscopy

History of optical light microscopes

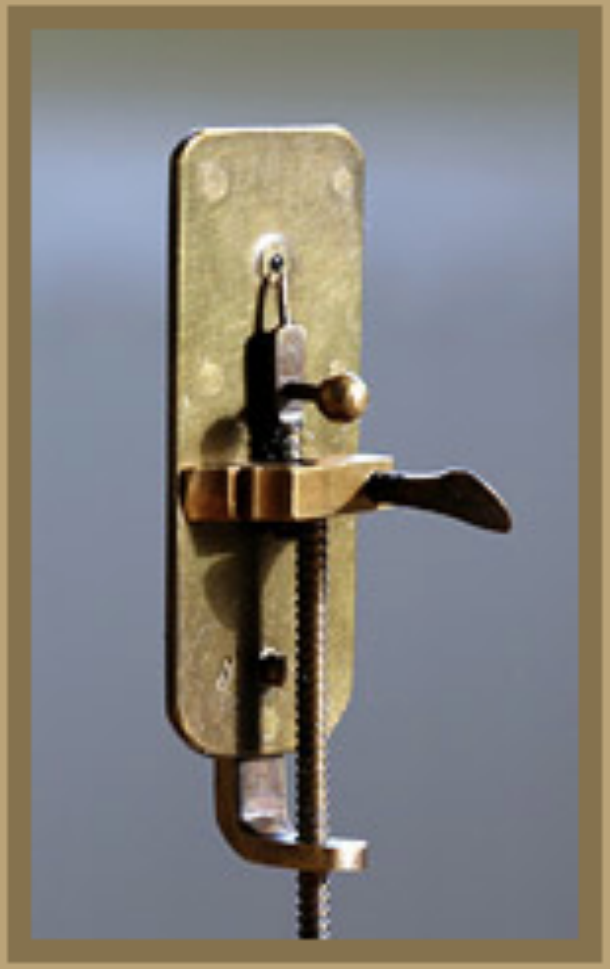
2nd Century BC - Claudius Ptolemy described a stick appearing to bend in a pool of water, and accurately recorded the angles to within half a degree.

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.

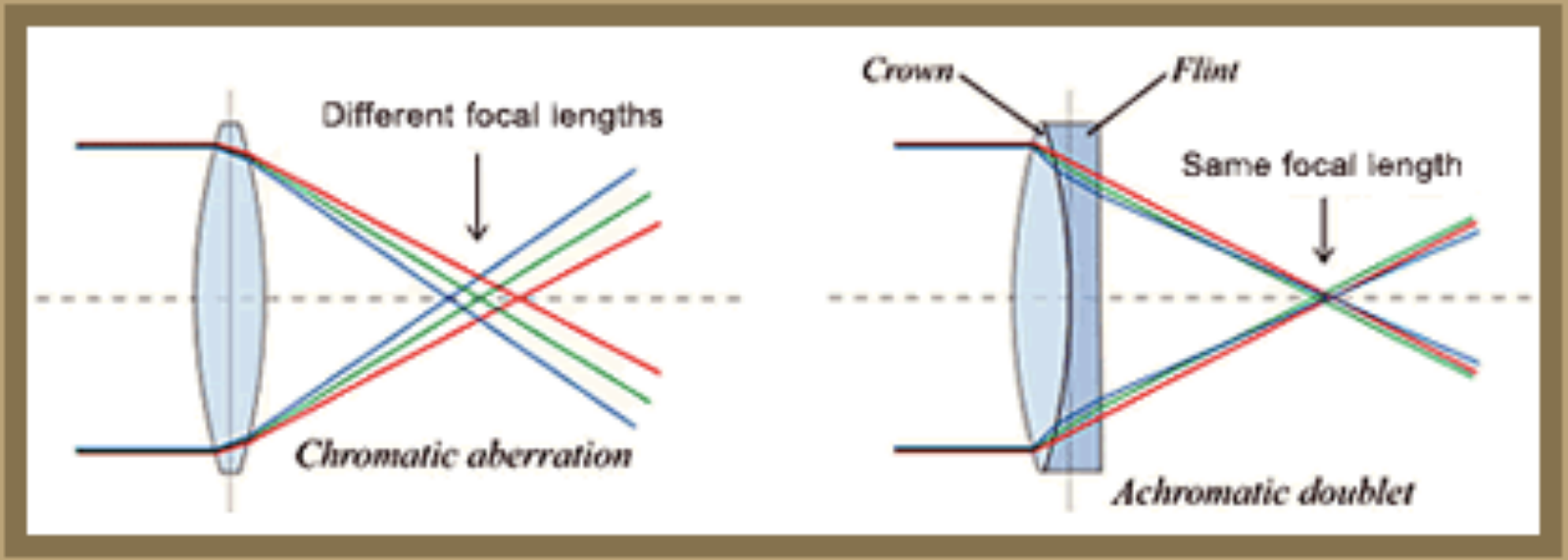
http://www.history-of-the-microscope.org/invention-of-glass-lenses-and-the-history-of-the-light-microscope.php

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



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.

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.

Today's light microscopes…

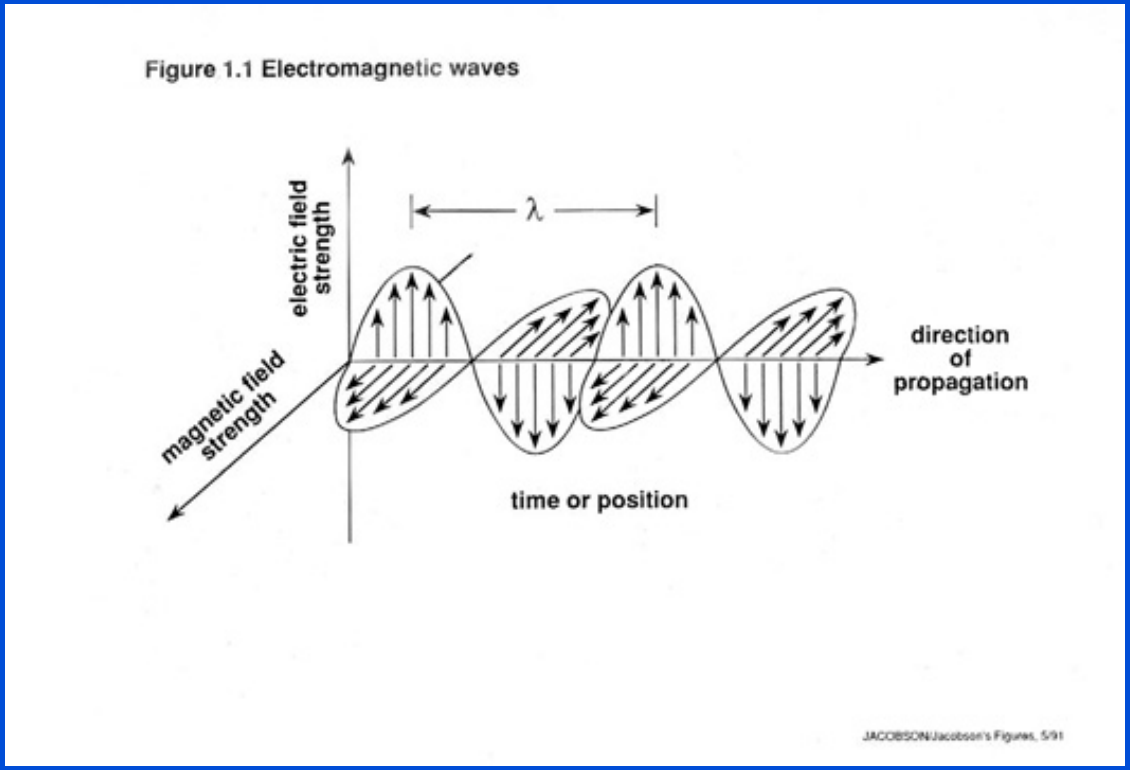
Light microscopy: topics

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

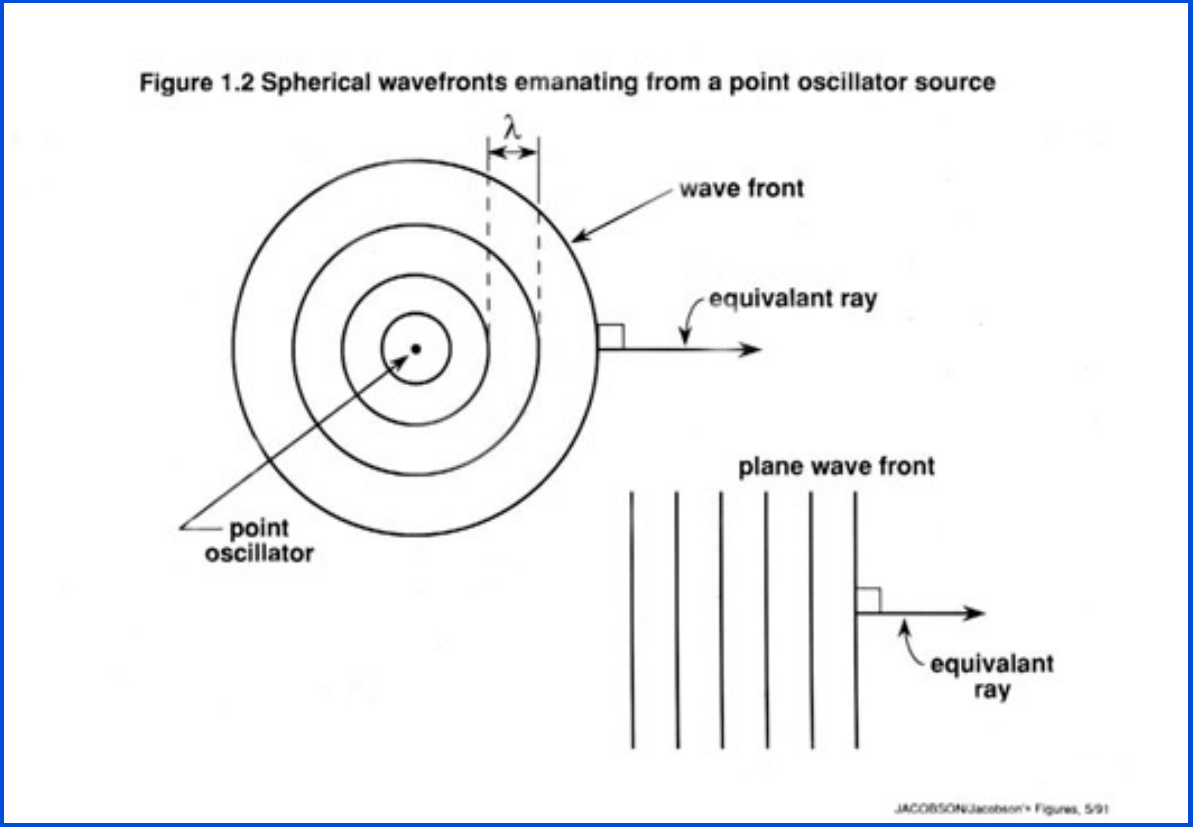
General References

- 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]

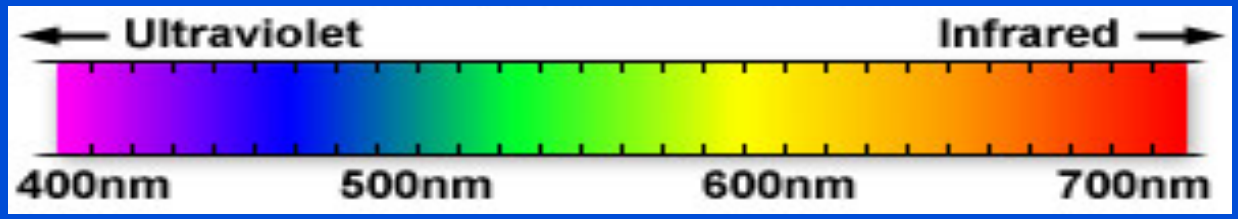


Defining wavefronts and rays

Velocity of light in different media

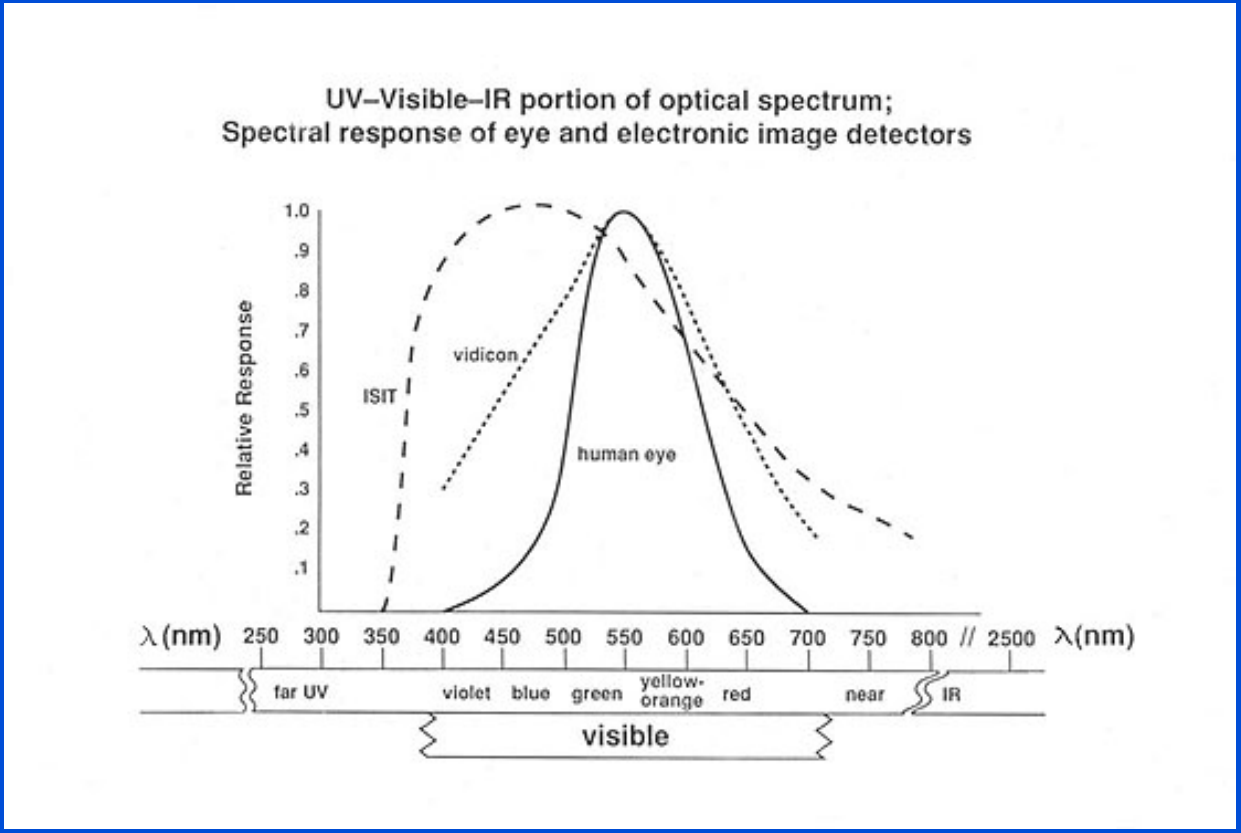
- Index of refraction, n =c/v
  - C=speed of light in vacuum=$3x10^8$ m/s; v= velocity in media
- Light travels slower in more dense media

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



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

The simplest microscope: a magnifier

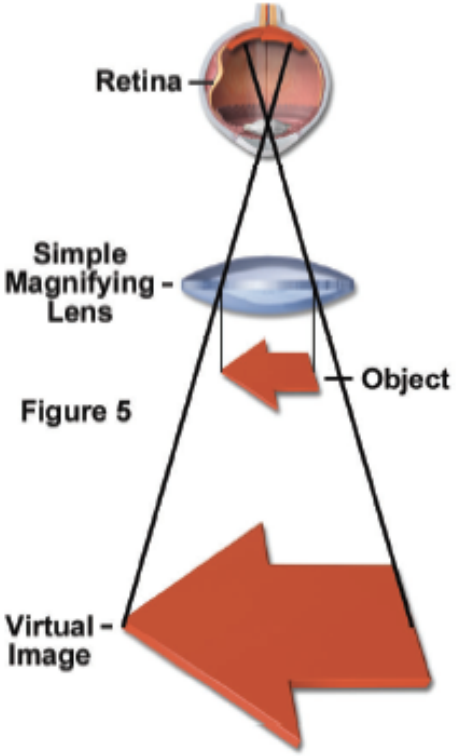


Figure 5

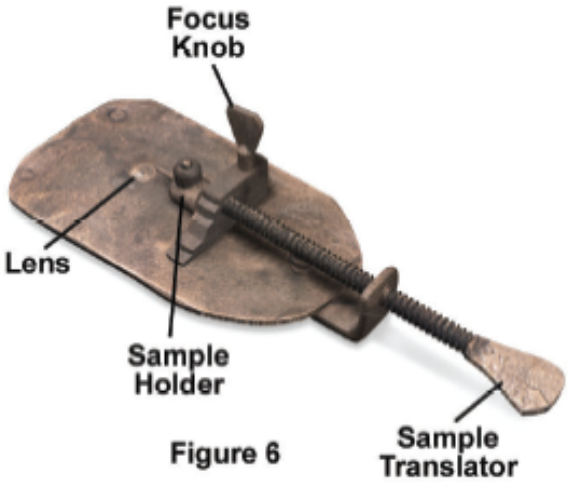
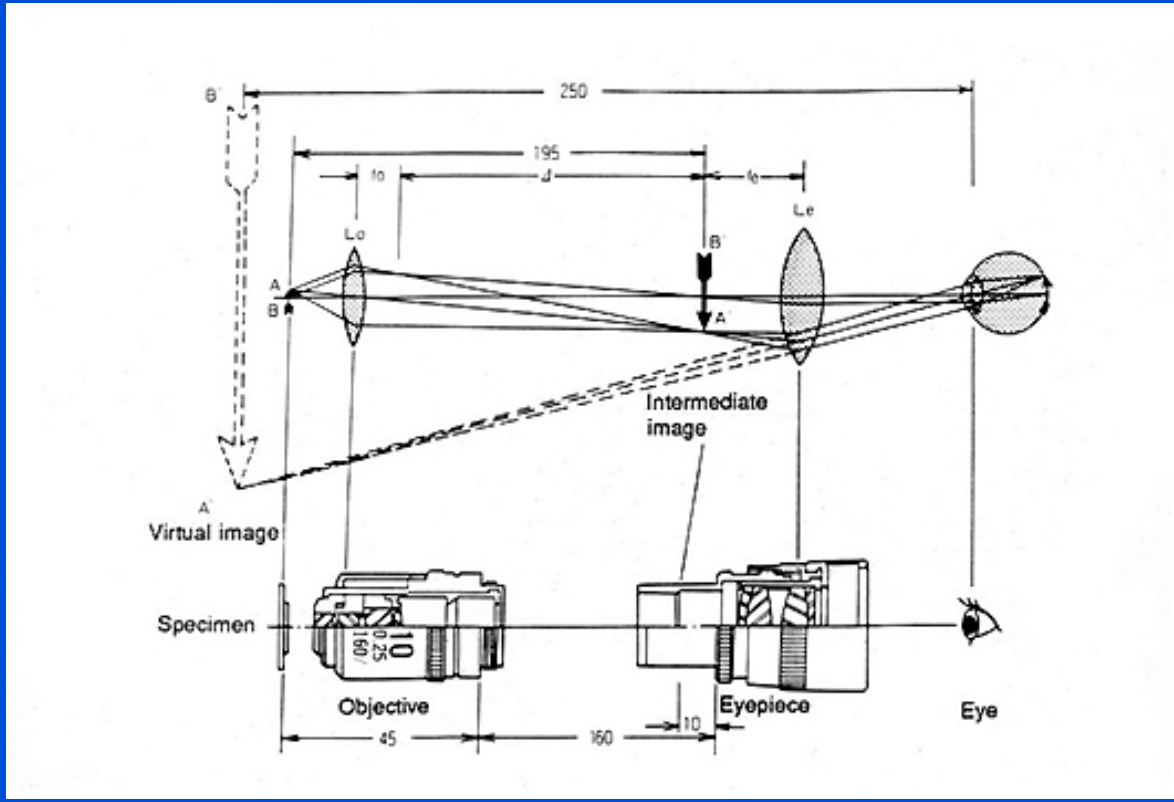


Figure 6

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

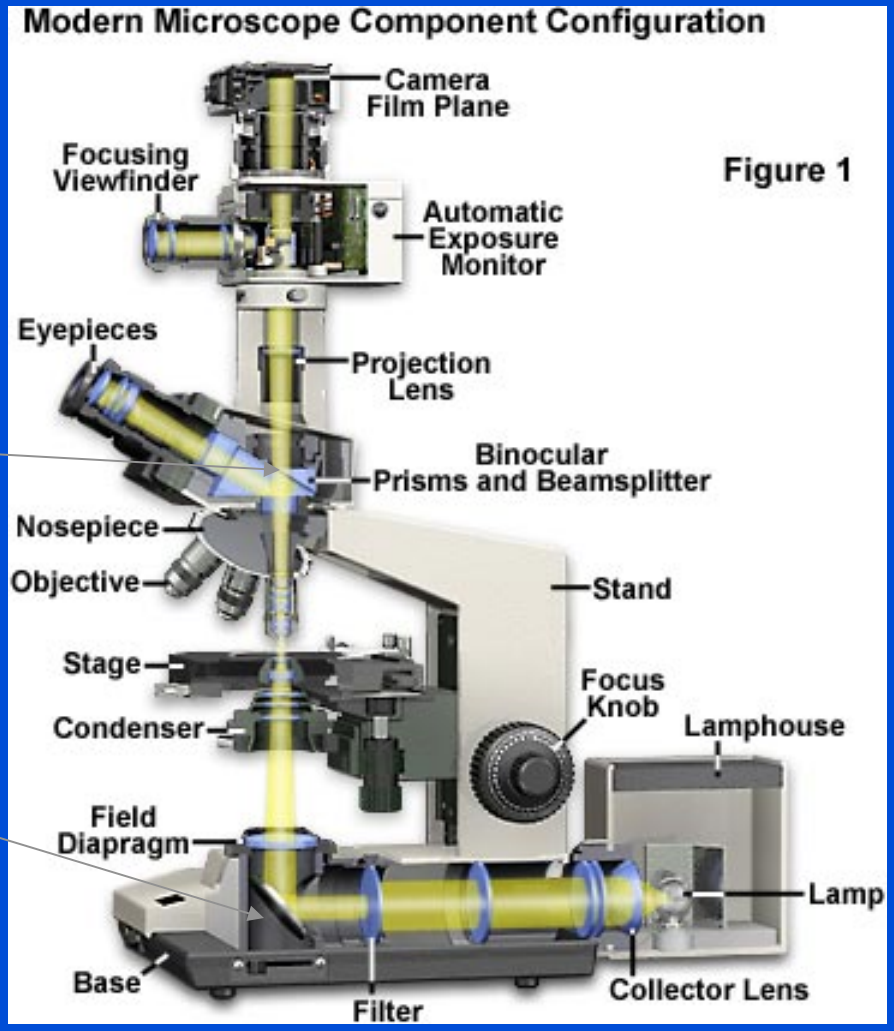
Total magnification in the compound microscope

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

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

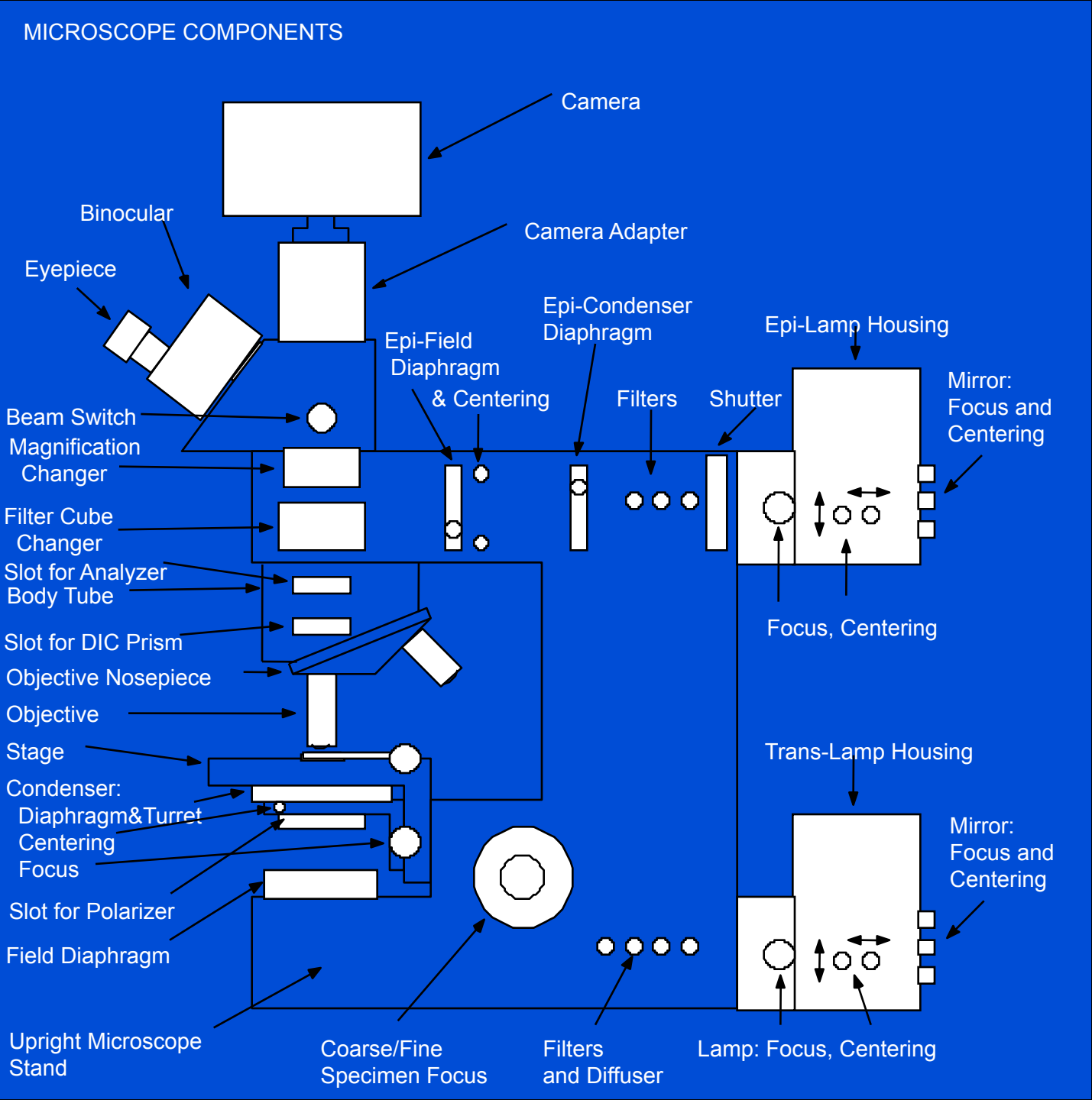
Modern microscope component identification

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)



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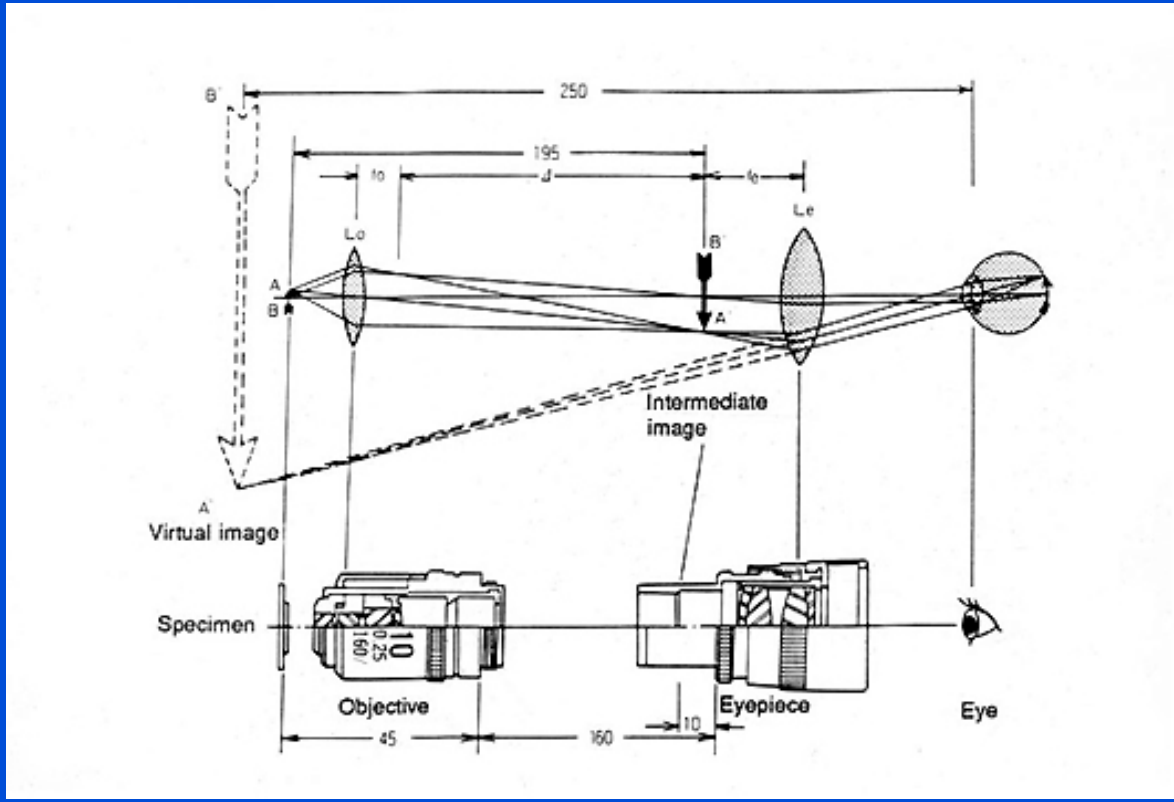

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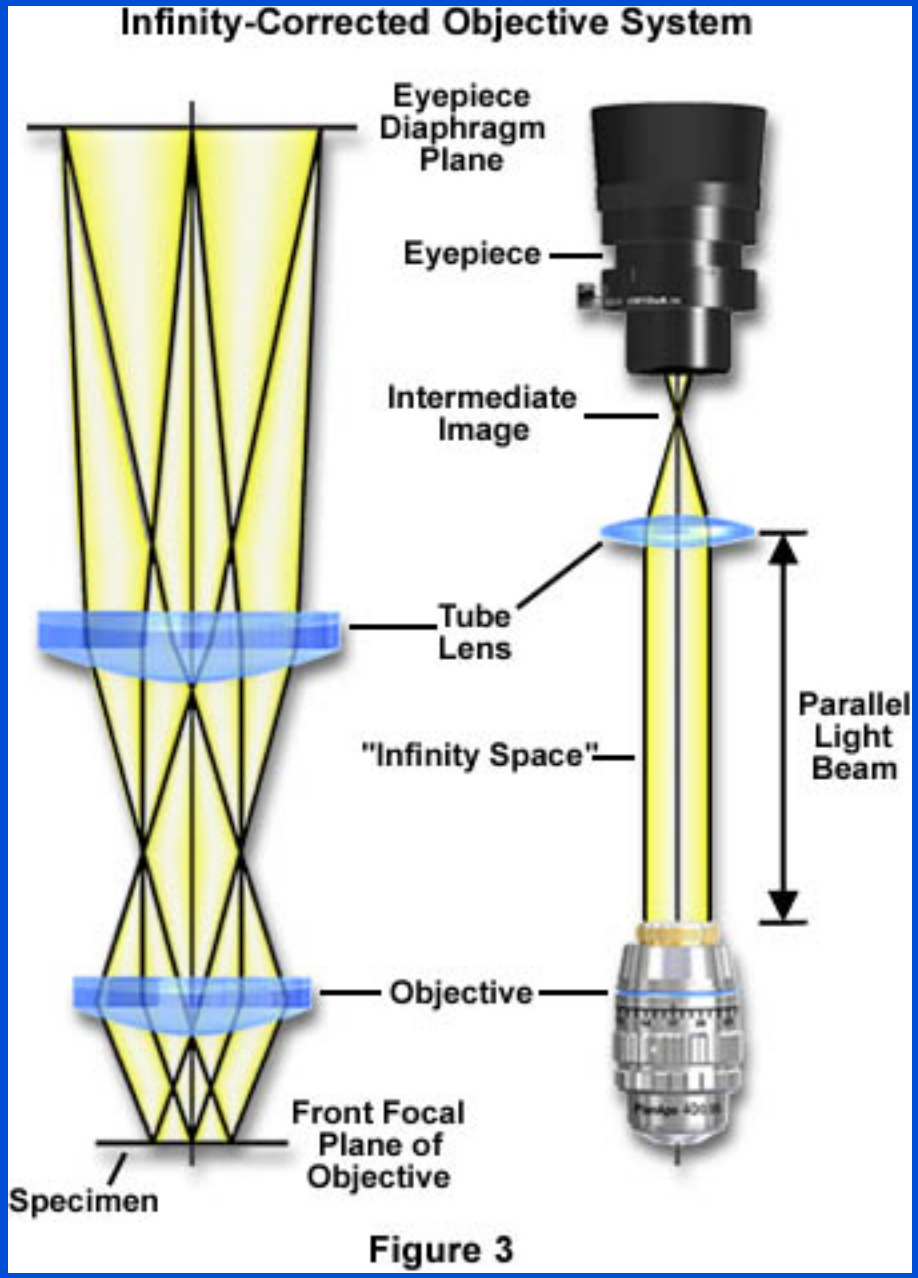
A word about infinity corrected optics and its advantages.
[object is set at front focal plane of objective]

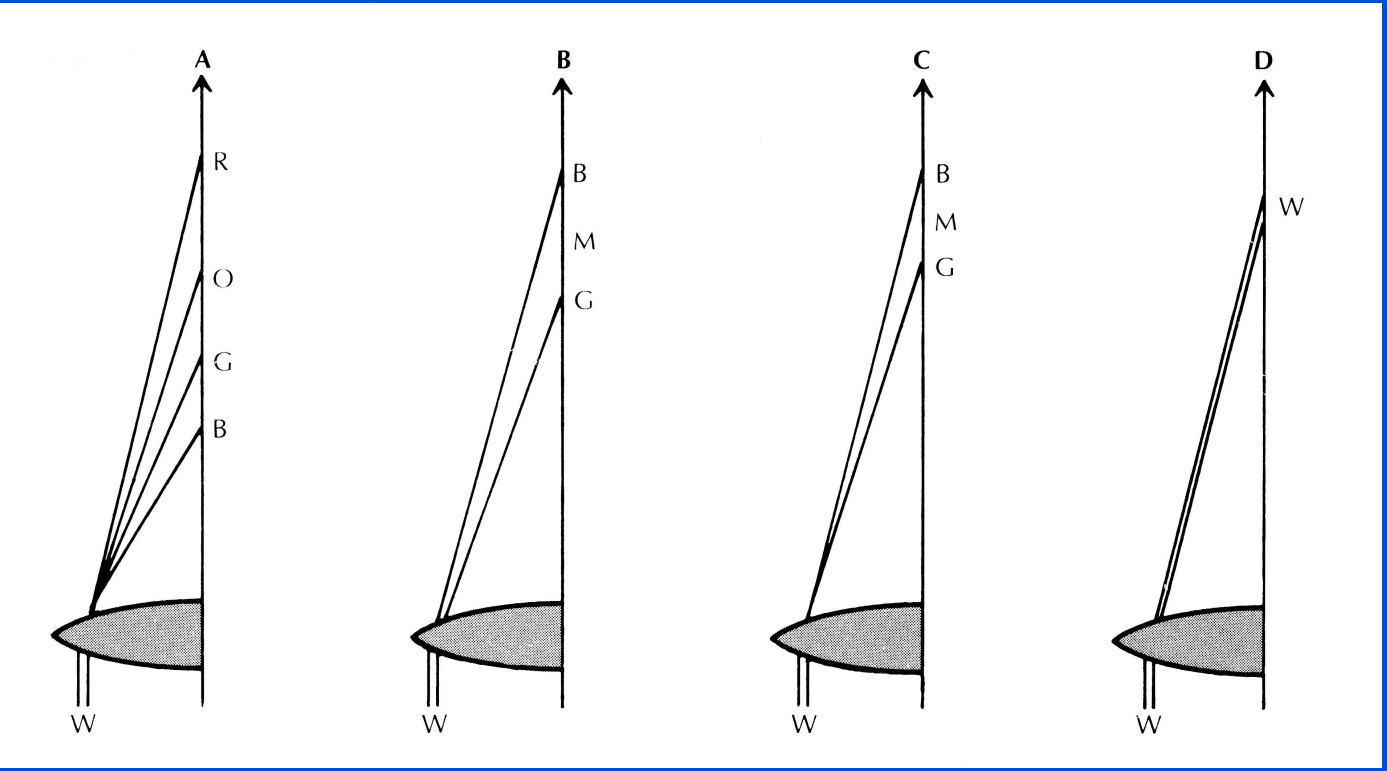
Eliminates ghost images caused by converging light, allows filters and polarizers to be inserted in 'infinity' space without corrections



Key component: the objective

--> Aberrations

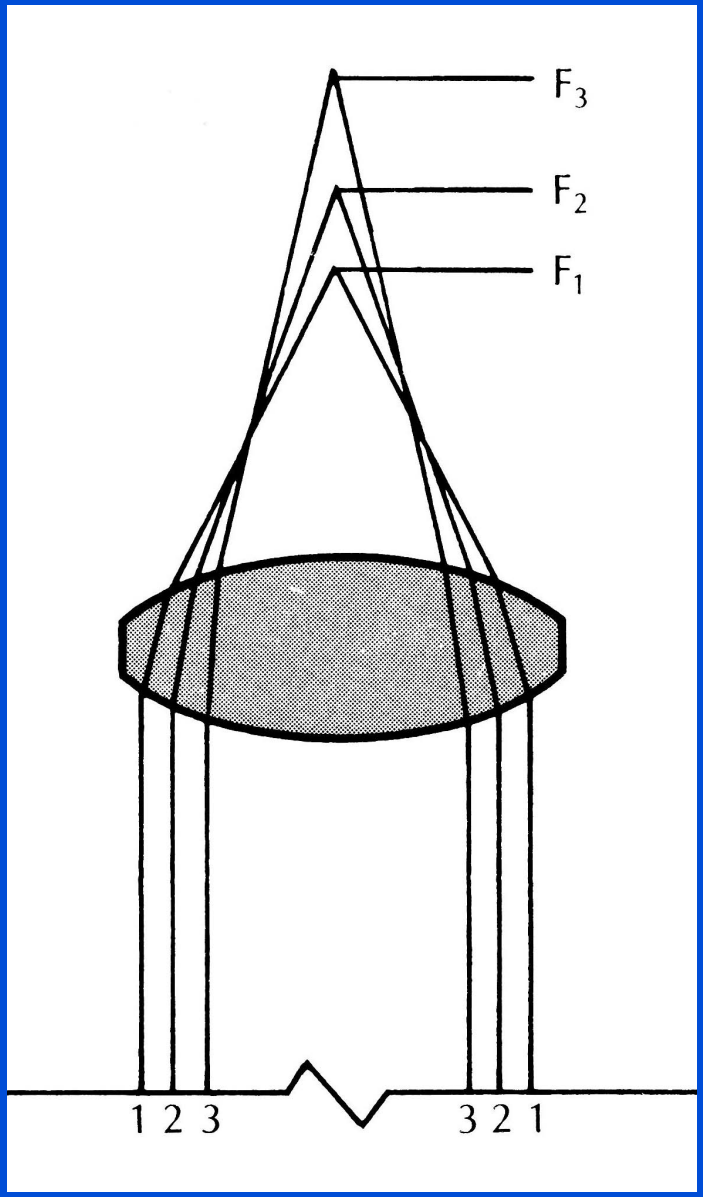
Chromatic aberration and its correction



Achromat	Fluorite	Apochromat
R,B corrected	R,B corrected	R,G,B corrected

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

Spherical Aberration



Objective Classes

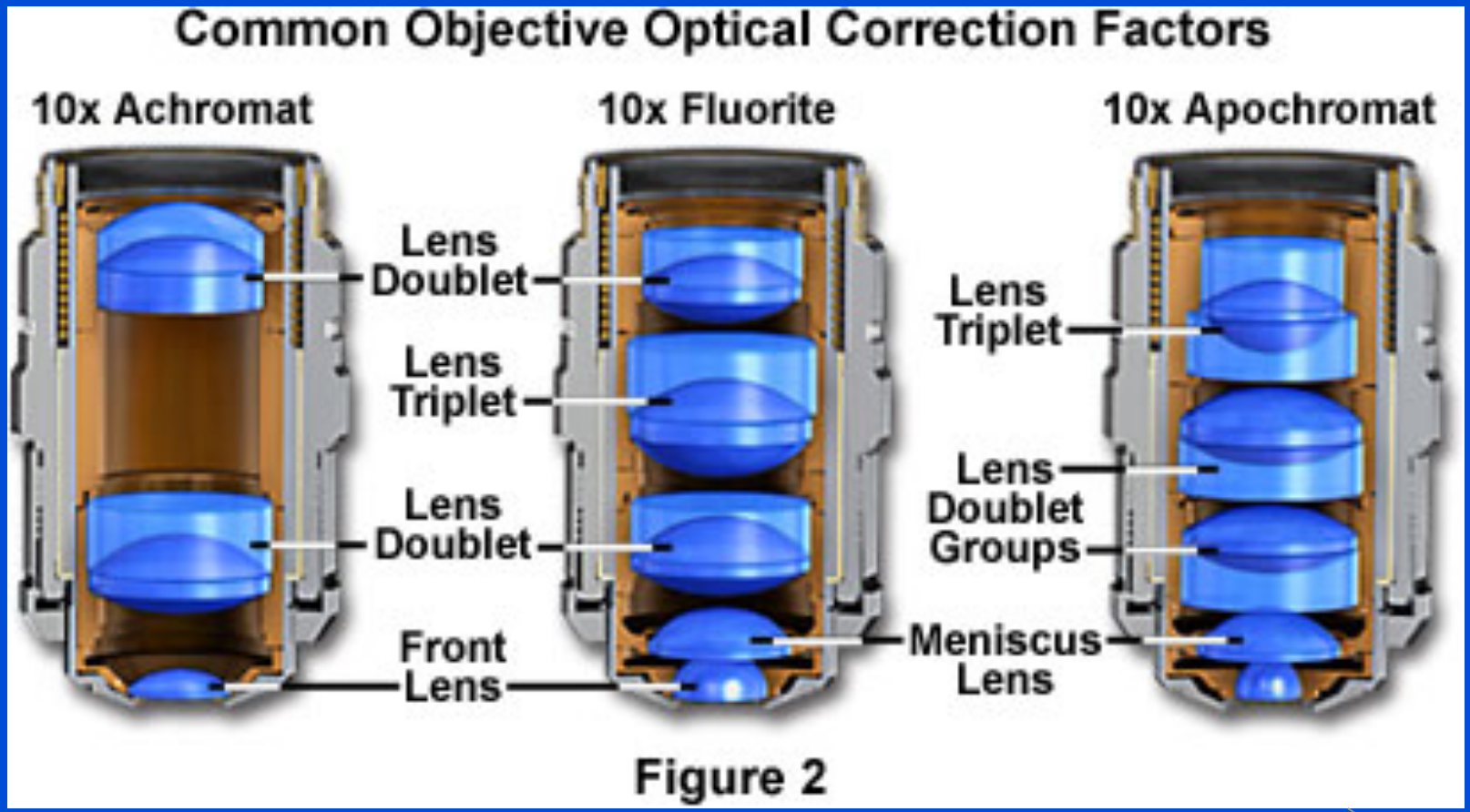
Achromats: corrected for chromatic aberration for red, blue

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