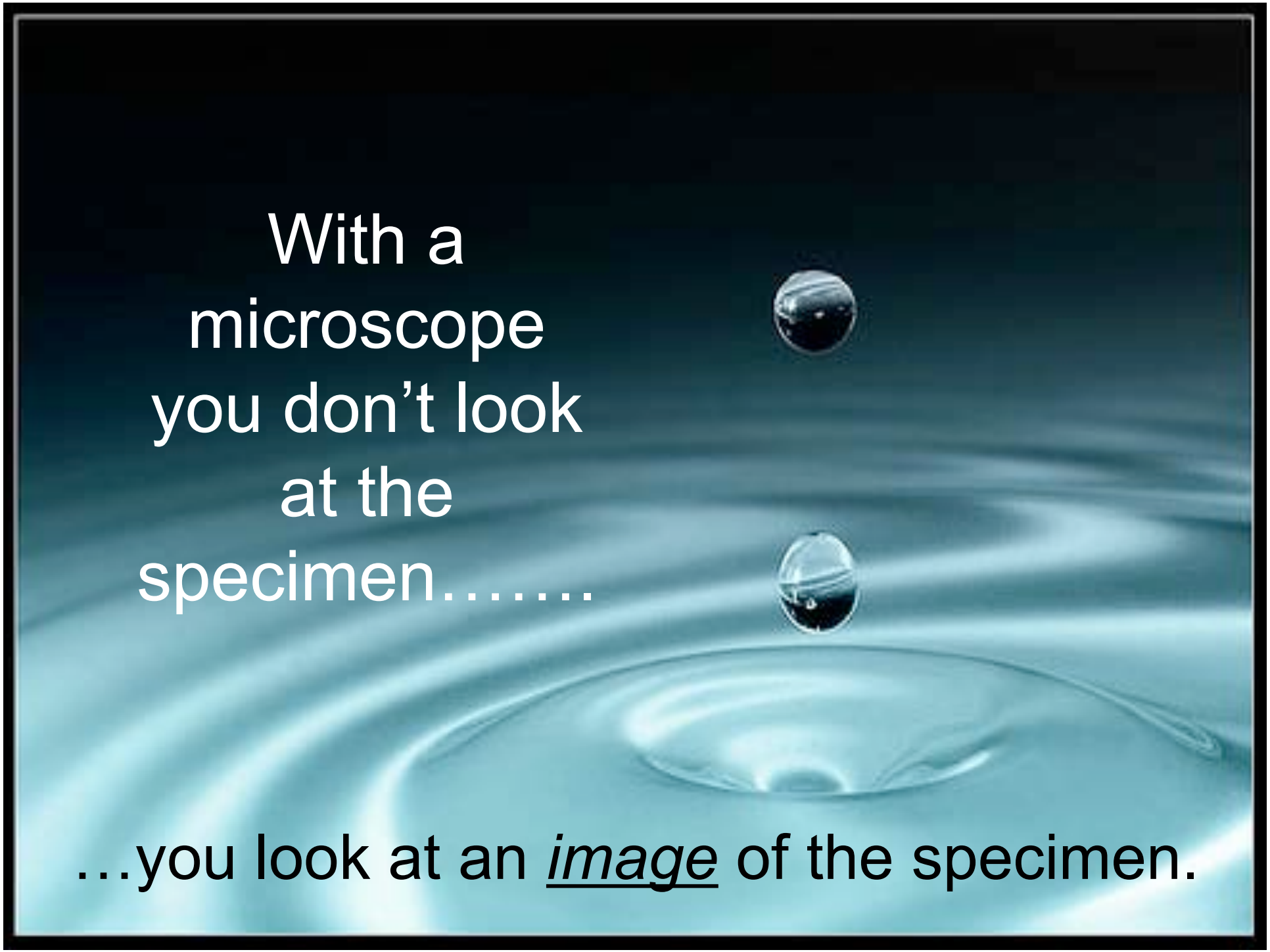
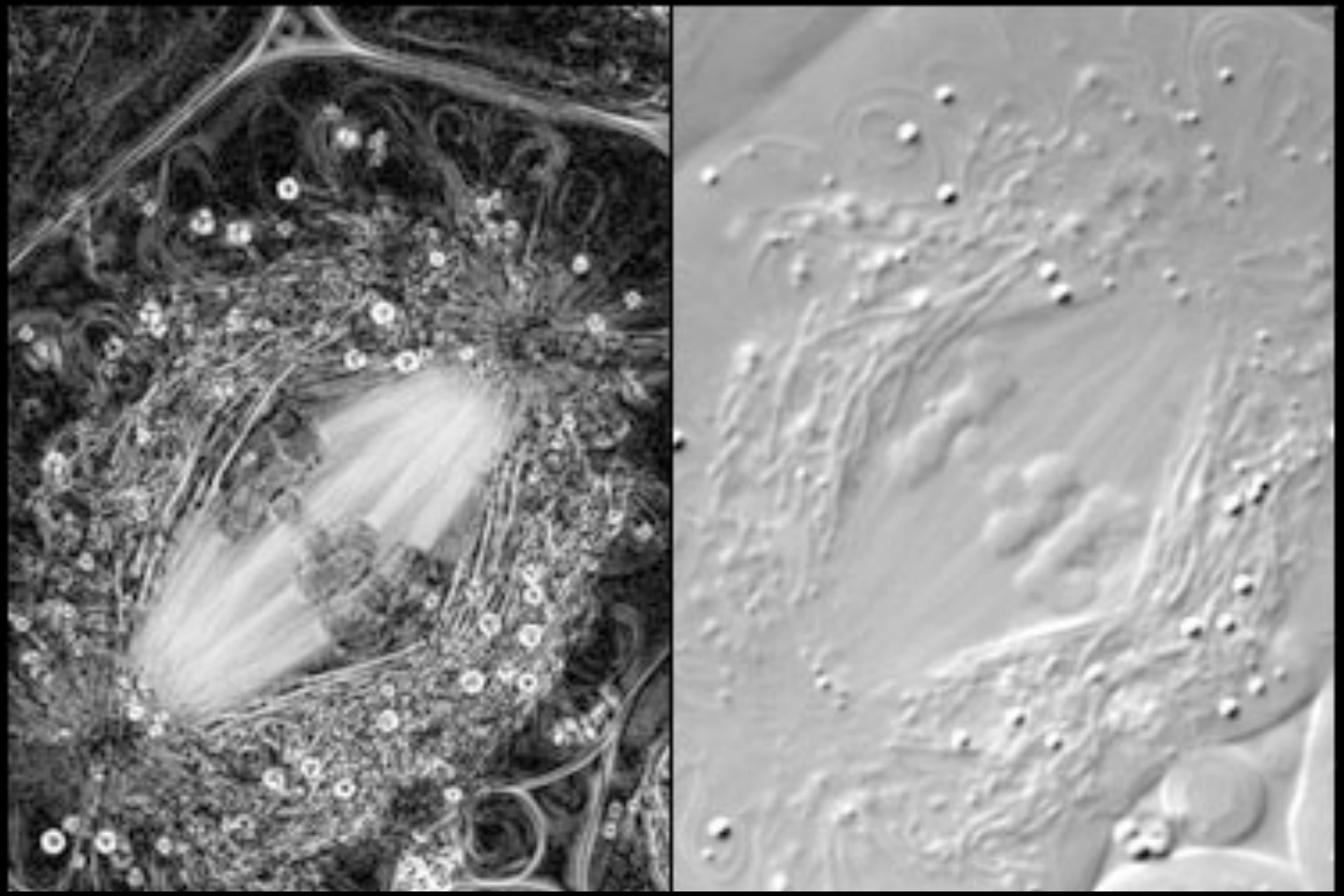
LIGHT MICROSCOPY

WHAT IS AN IMAGE???

1. A reproduction of the form of a person or object, especially a sculptured likeness.

2. An optically formed duplicate, counterpart,
or other representative reproduction of an object,
especially an optical reproduction formed by a lens or mirror.

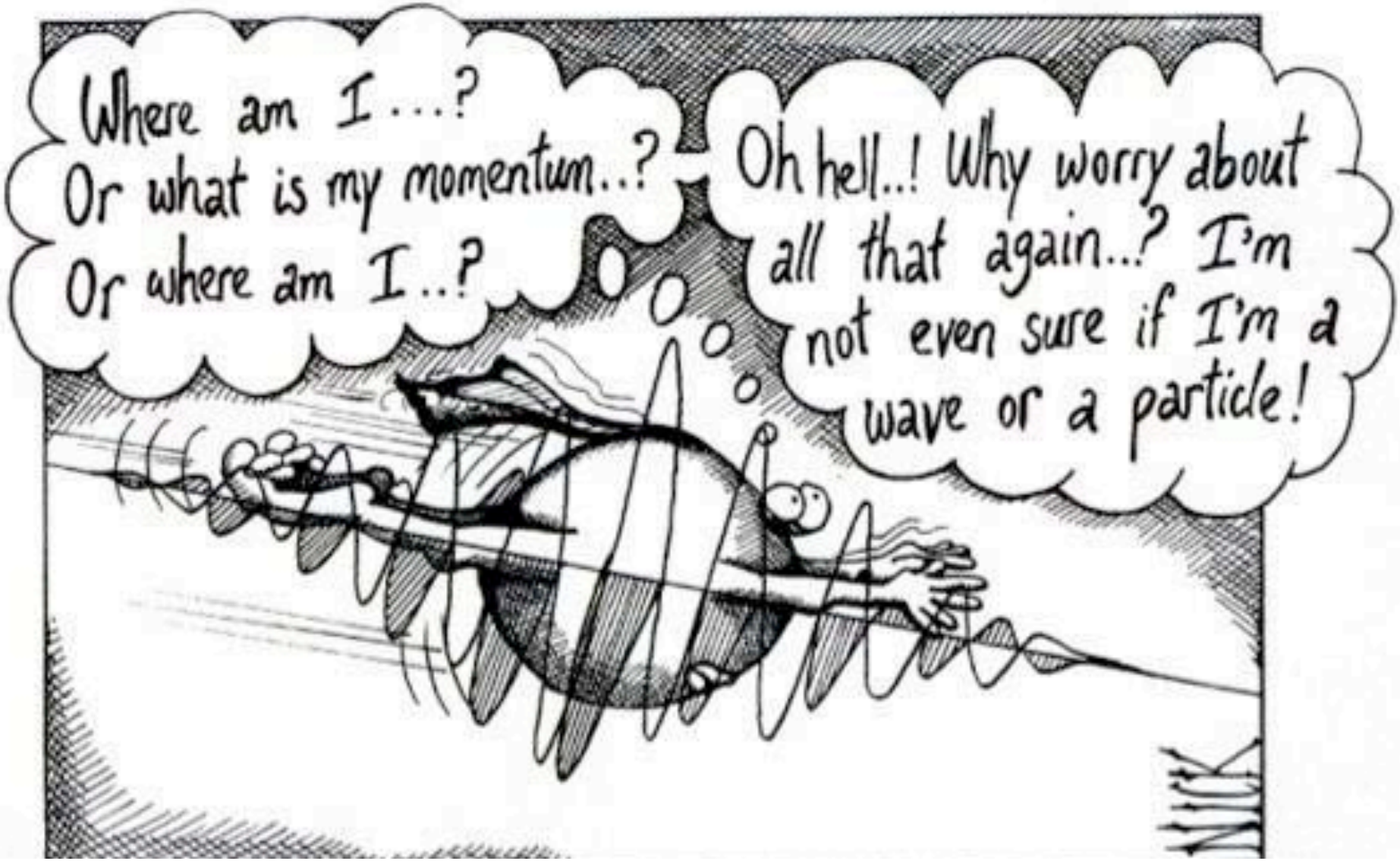

With a microscope you don't look at the specimen…….
…you look at an *image* of the specimen.

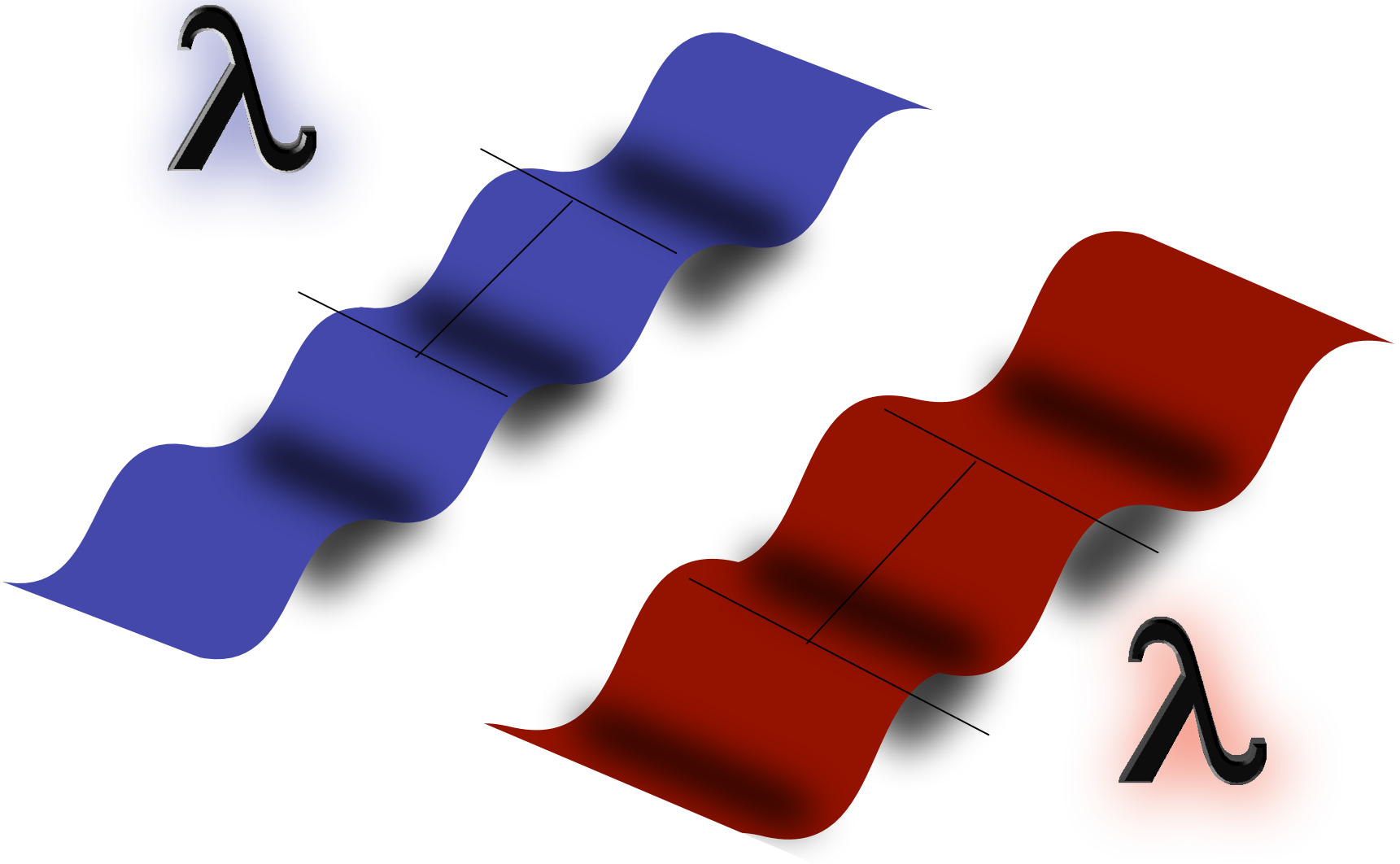


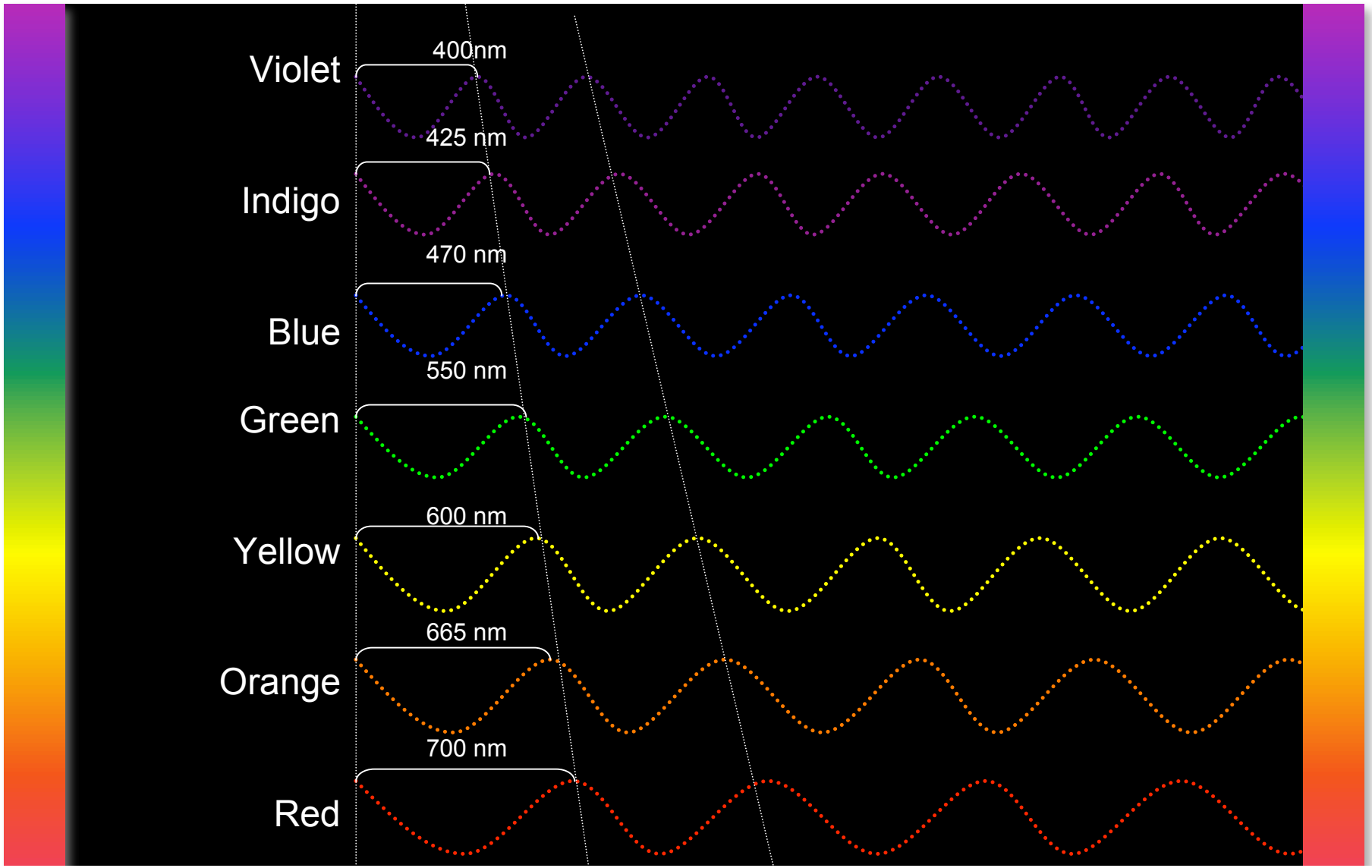
Brad Amos & Stefanie Reichelt

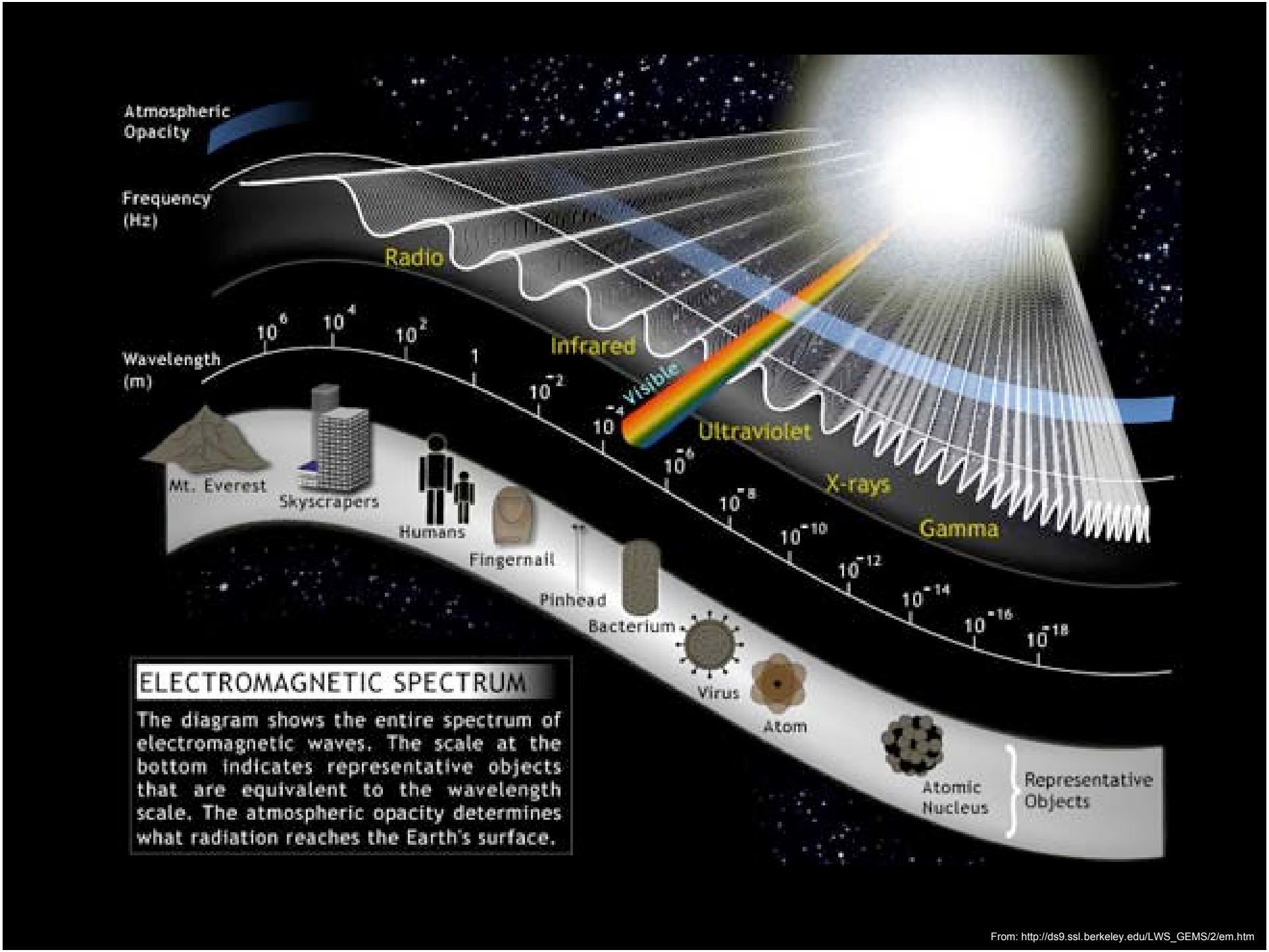

NO LIGHT……
NO IMAGE….
NO MICROSCOPY!!!

Light rays carry information!!!!!

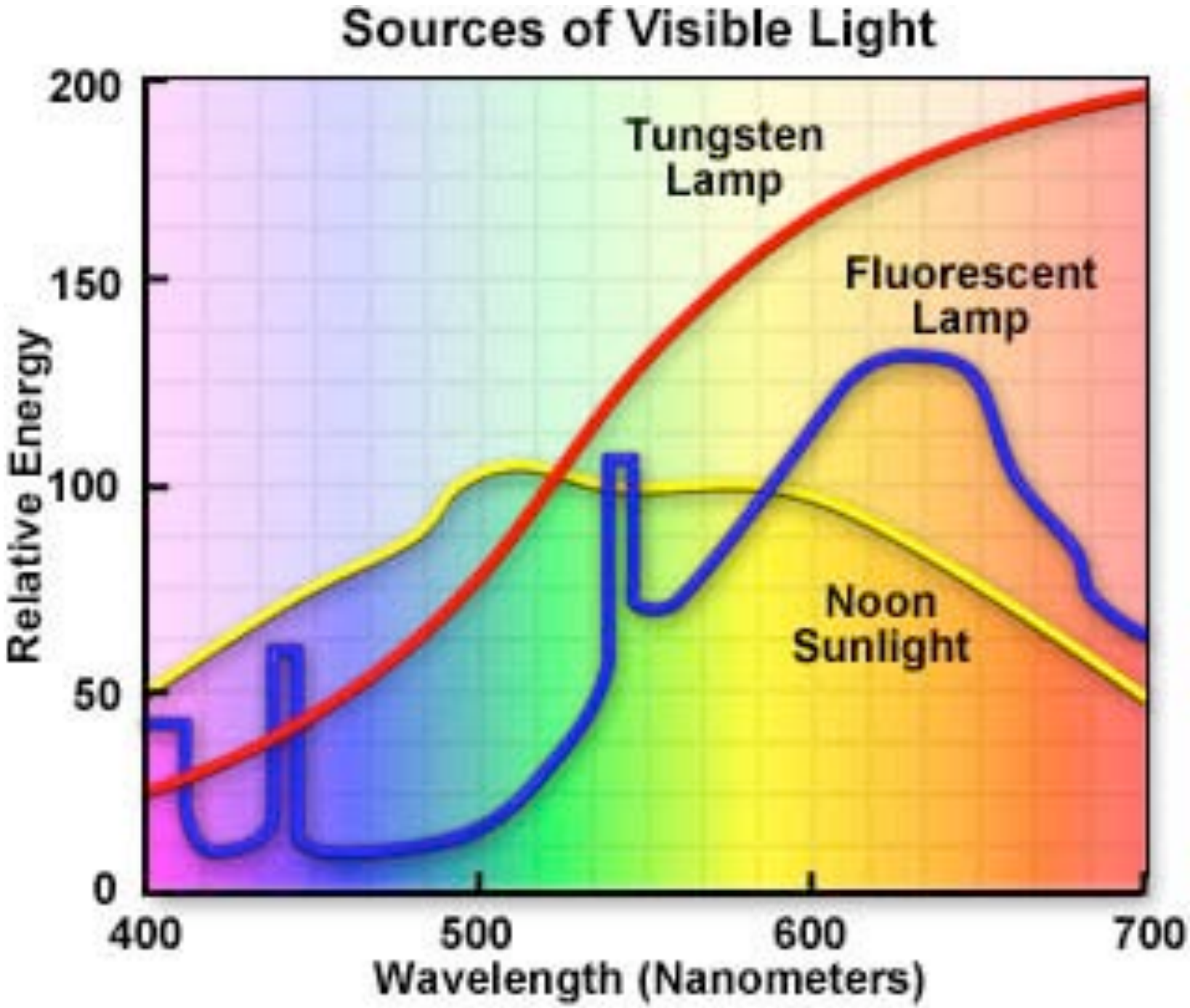

Where am I...?
Or what is my momentum..?
Or where am I..?
Oh hell..! Why worry about all that again..? I'm not even sure if I'm a wave or a particle!


λ
λ


Violet
400nm
Indigo
425 nm
Blue
470 nm
Green
550 nm
Yellow
600 nm
Orange
665 nm
Red
700 nm



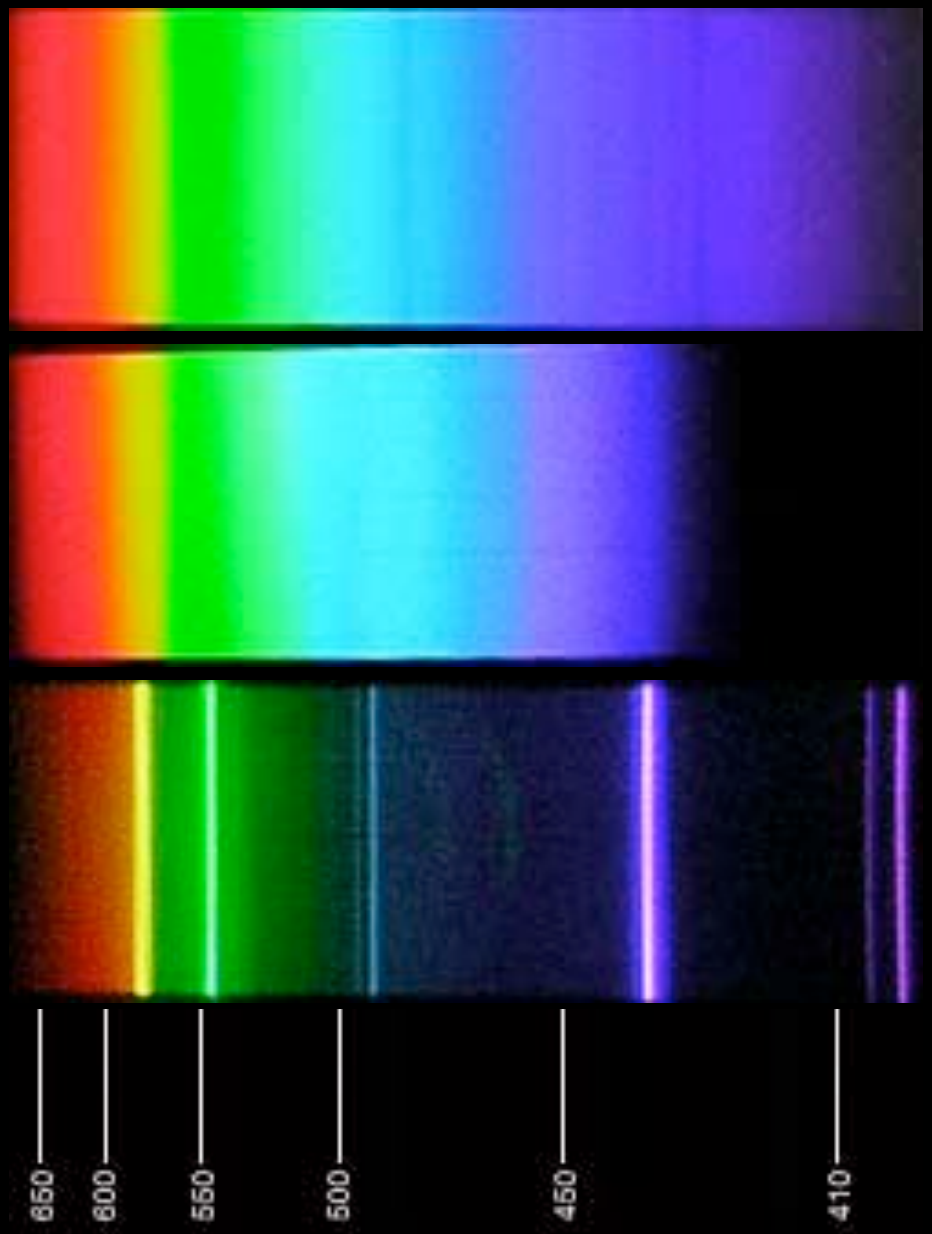
From: http://ds9.ssl.berkeley.edu/LWS_GEMS/2/em.htm


Sources of Visible Light
200
150
100
50
0
Relative Energy
Tungsten
Lamp
Fluorescent
Lamp
Noon
Sunlight
400
500
600
700
Wavelength (Nanometers)

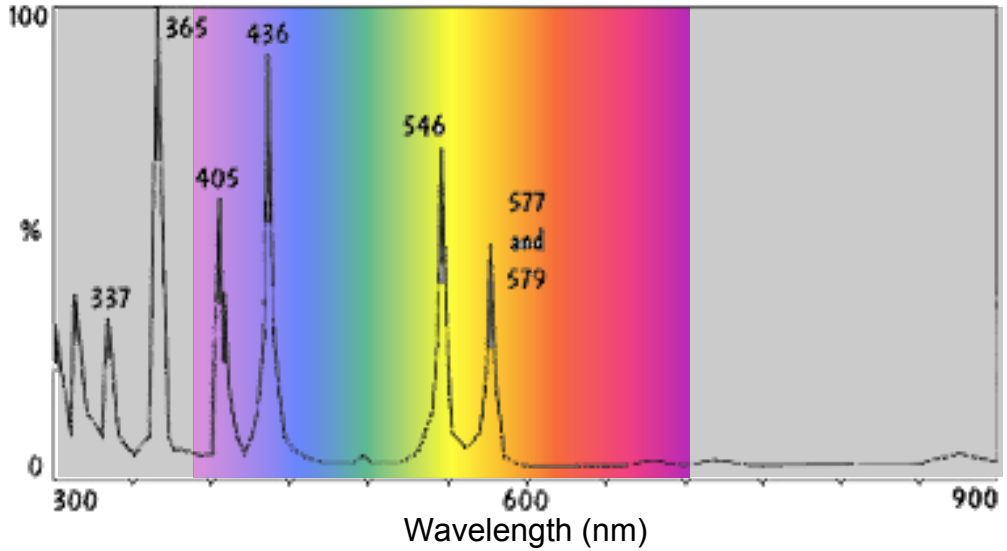
Daylight

Incandescent lamp

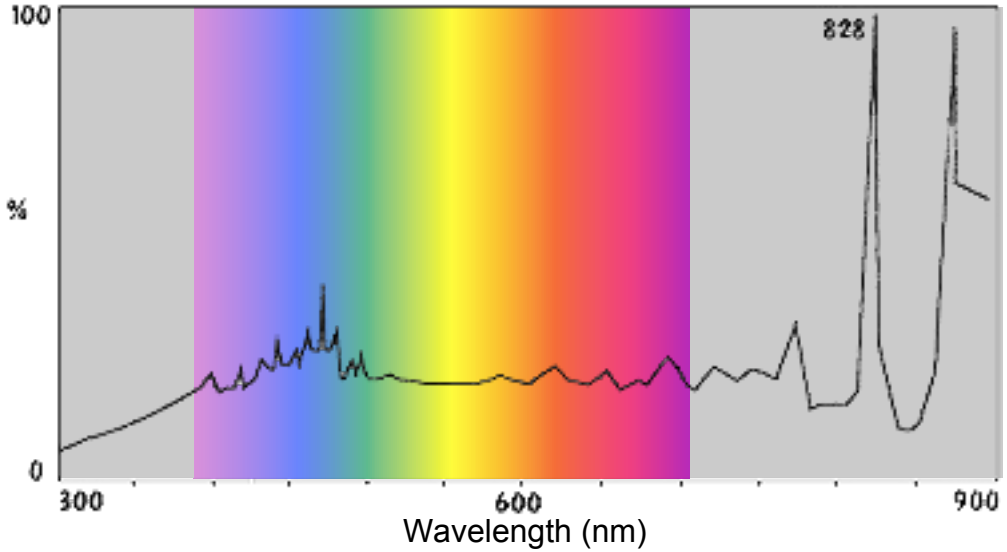
Mercury lamp



Spectrum of a Mercury Lamp

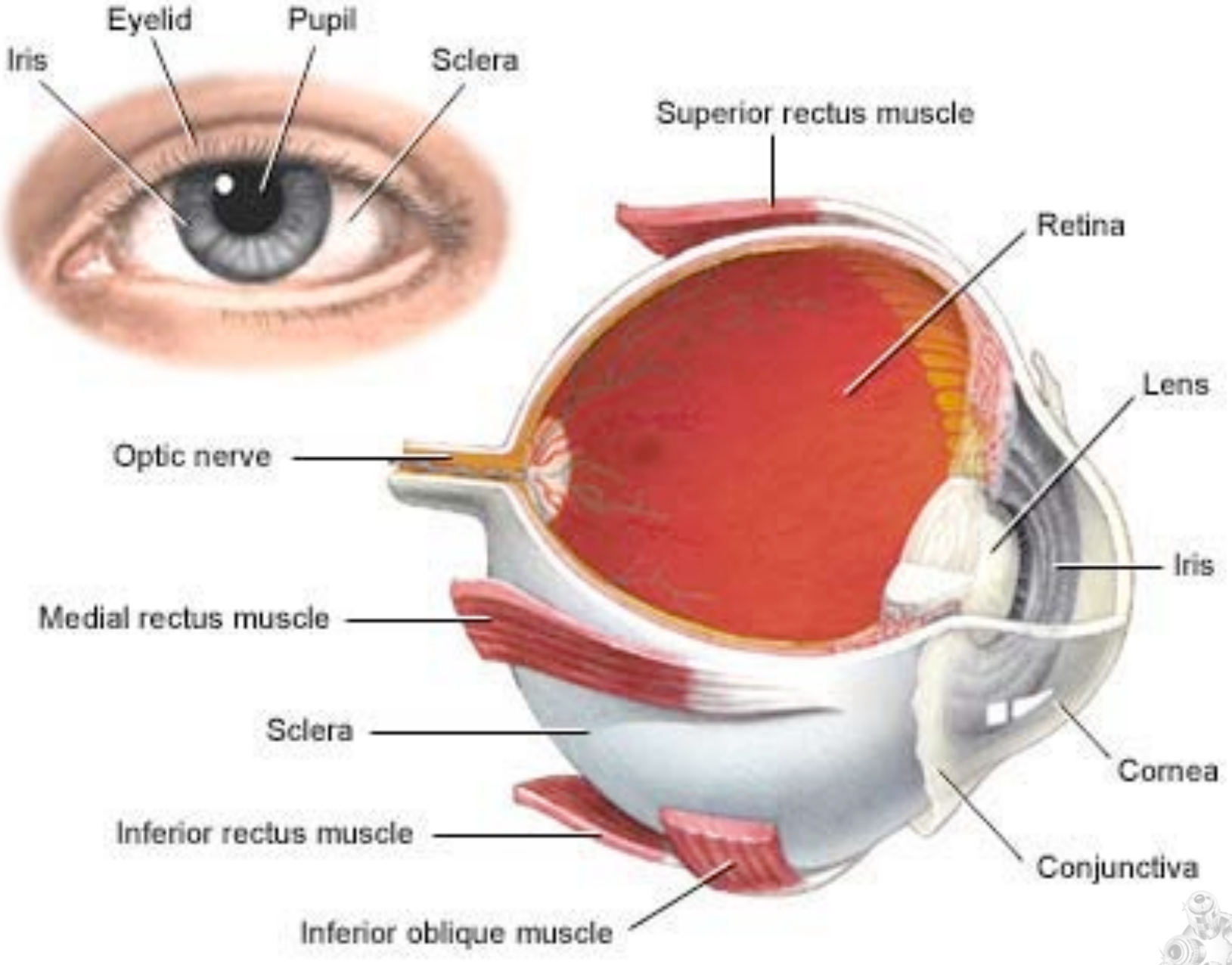


Spectrum of a Xenon Lamp



Light sources for fluorescence

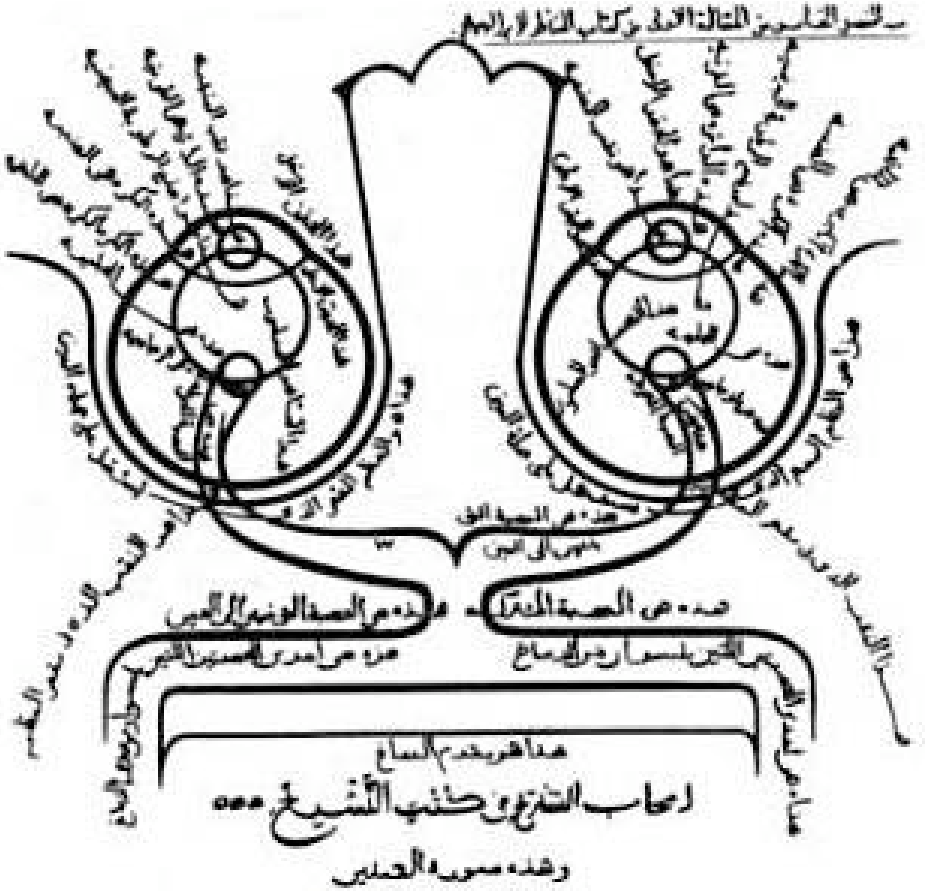
(Modified from: http://www.cairn-research.co.uk)


Eyelid
Pupil
Iris
Sclera
Superior rectus muscle
Retina
Lens
Optic nerve
Iris
Medial rectus muscle
Sclera
Cornea
Inferior rectus muscle
Conjunctiva
Inferior oblique muscle

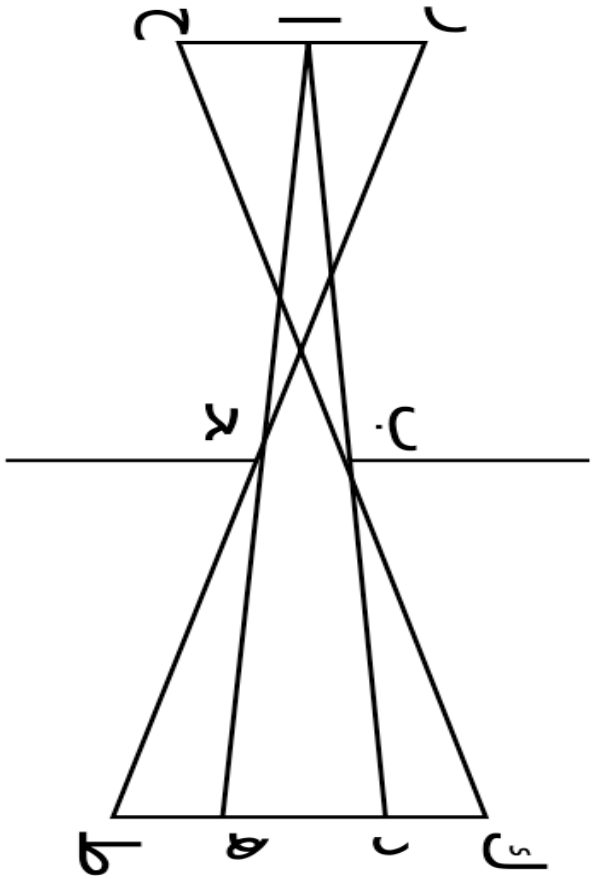
ONCE UPON A TIME……..

Alhazen
(965-1040)

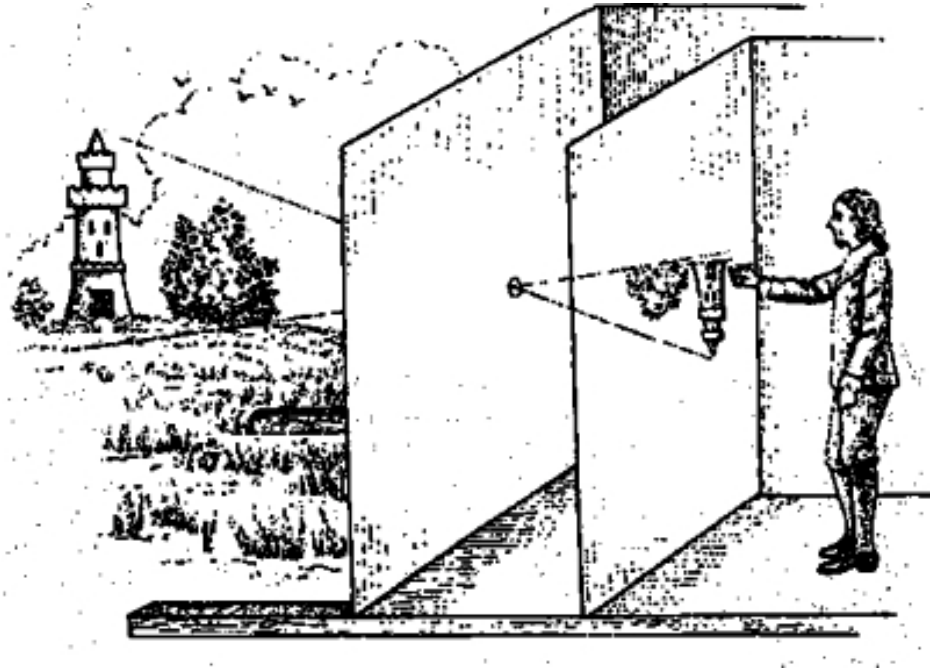
(Ibn al-Haytham)



The eye, from Al-Hazen's *Opticae Thesaurus*—AD 1038.



Alhazen was the first to show how the image is formed on the retina in the human eye, using the camera obscura as his model. However, as far as the camera obscura was concerned, he explained "Et nos non inventimus ita" we did not invent this.



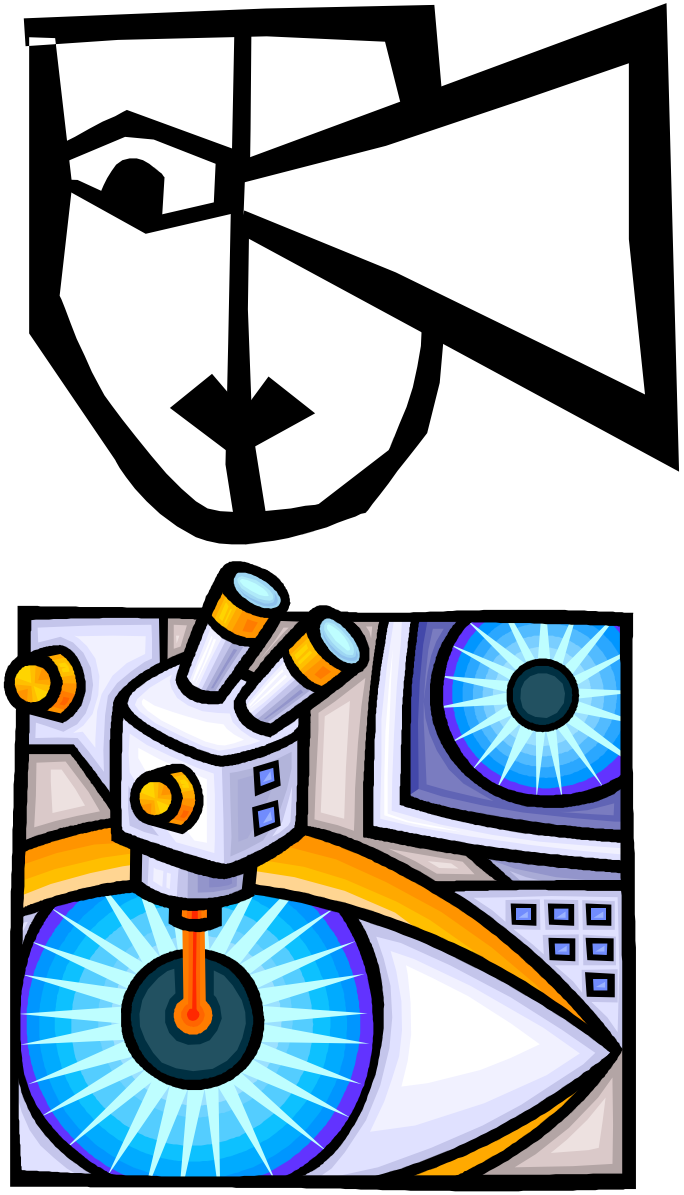
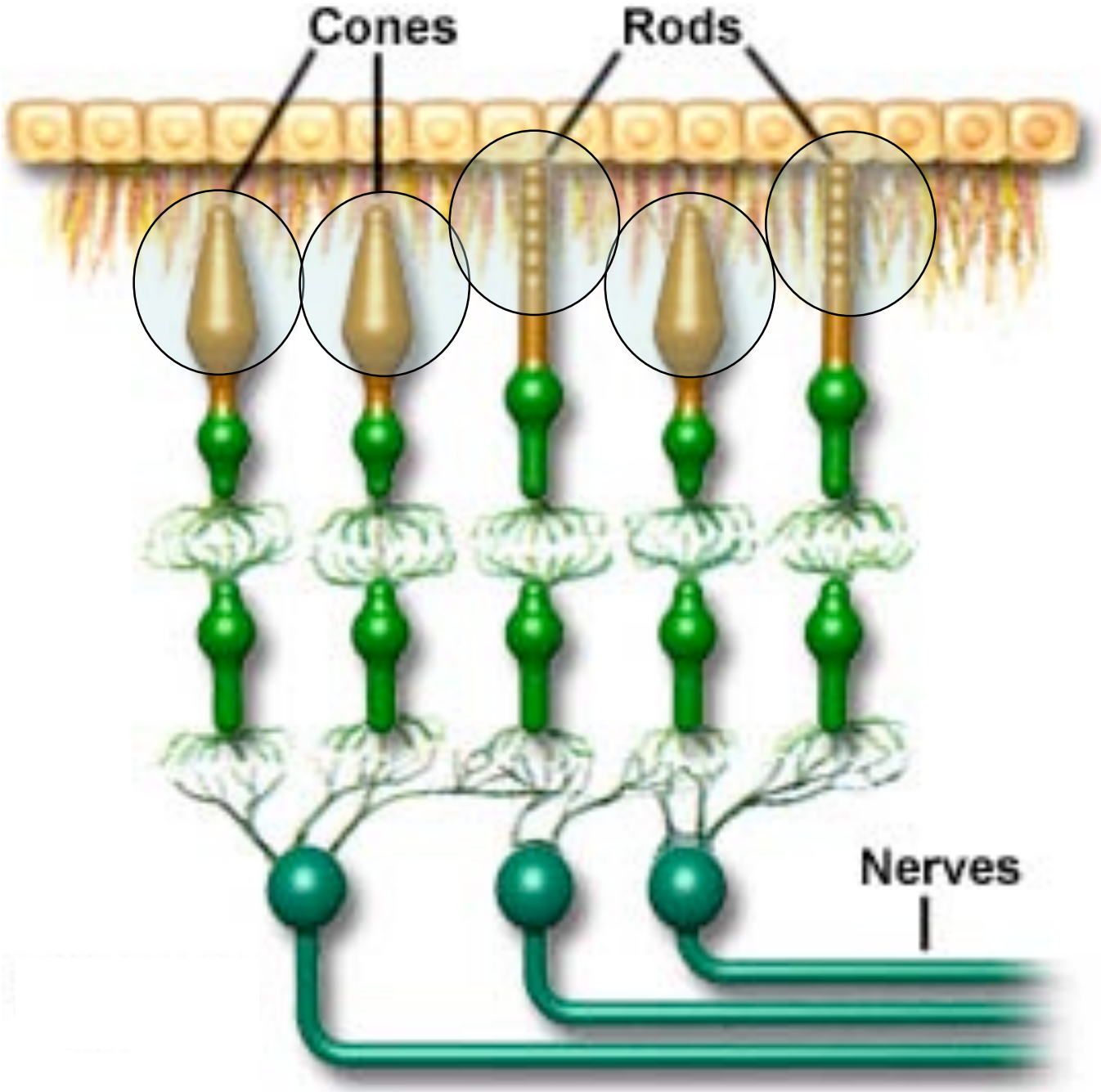


Image taken from: http://micro.magnet.fsu.edu/optics/timeline/people/alhazen.html

www.islamicspain.tv/.../Physics-and-Optics.htm


Cones
Rods
Nerves

The relative intensity of the stimuli of each type of cones determines the color!!!!

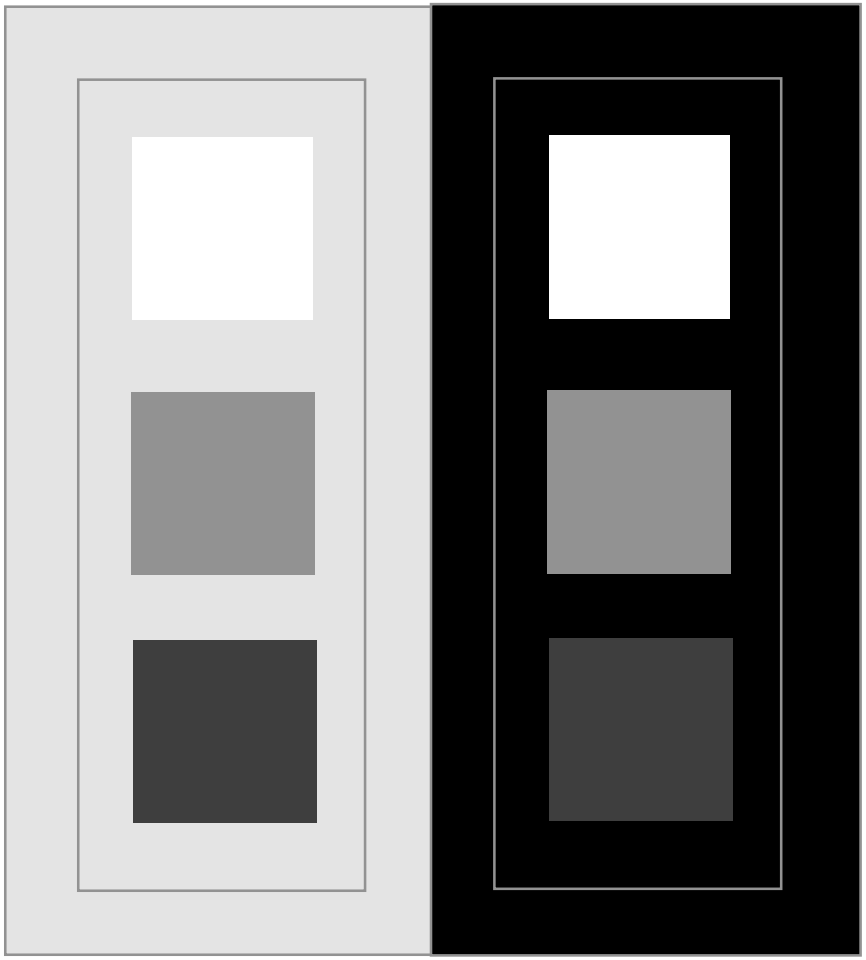
430nm light stimulate blue light sensitive cones. You see blue!

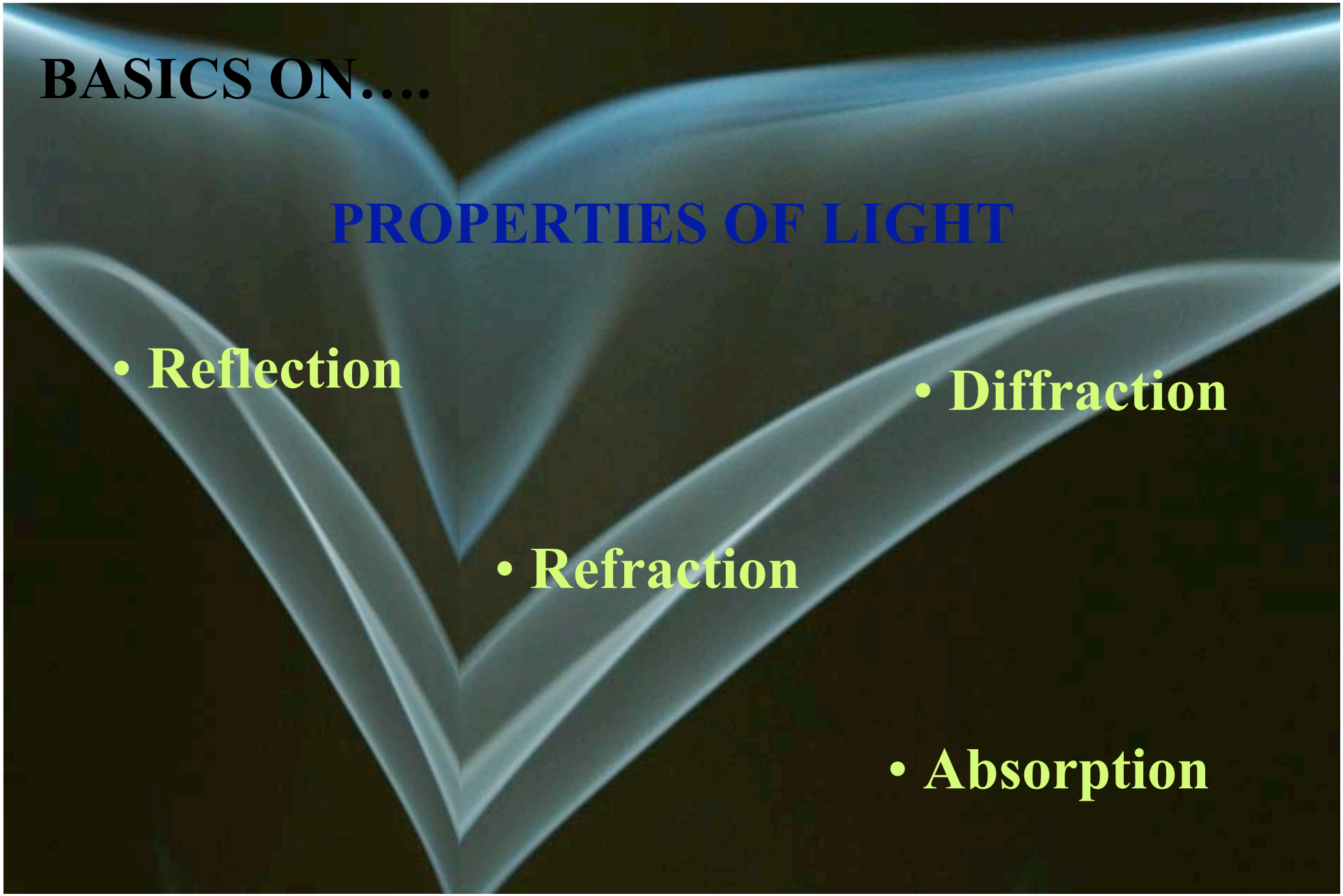
When the three types of cones are stimulated at the same time….

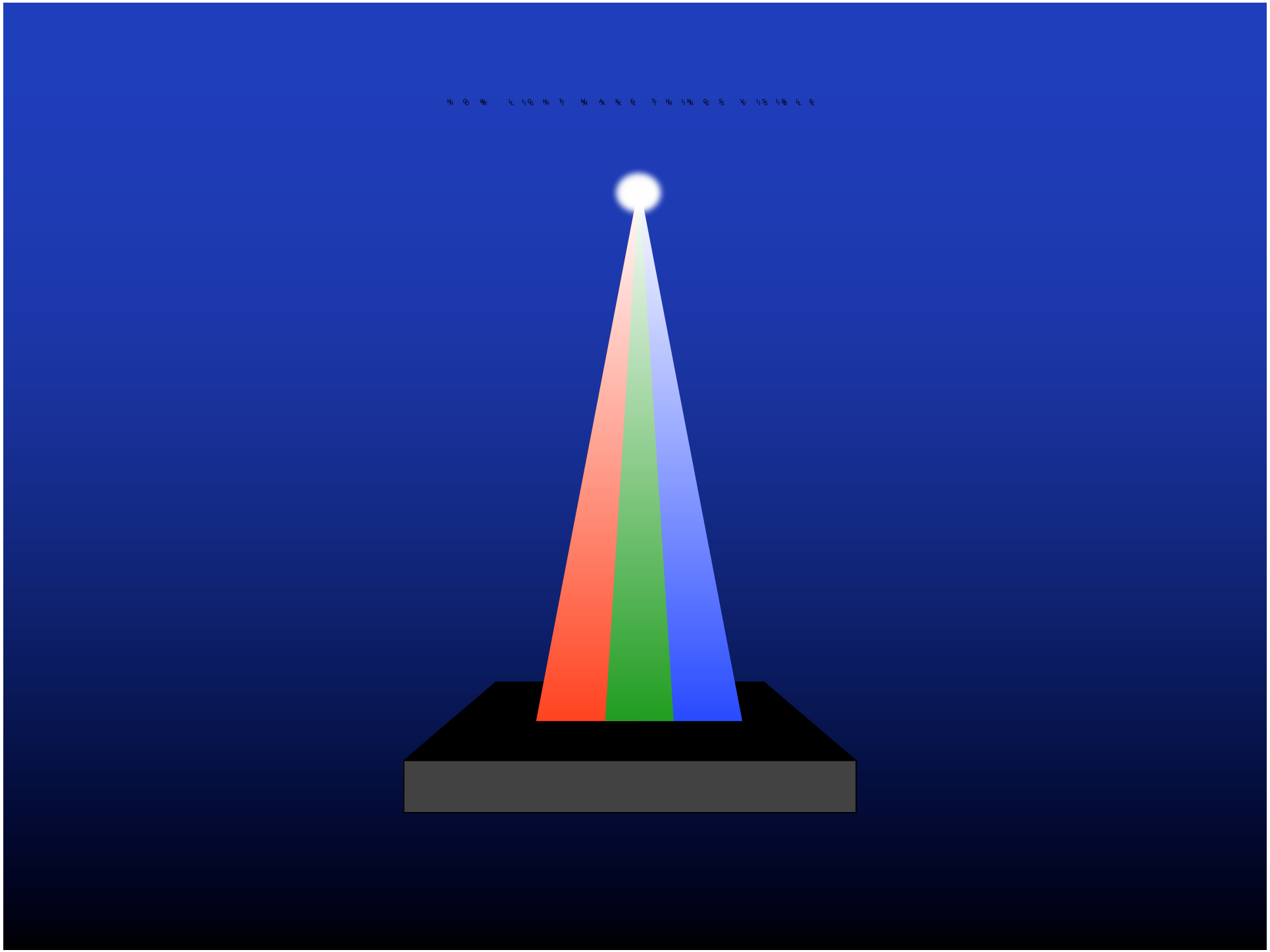
With 550nm light you see green

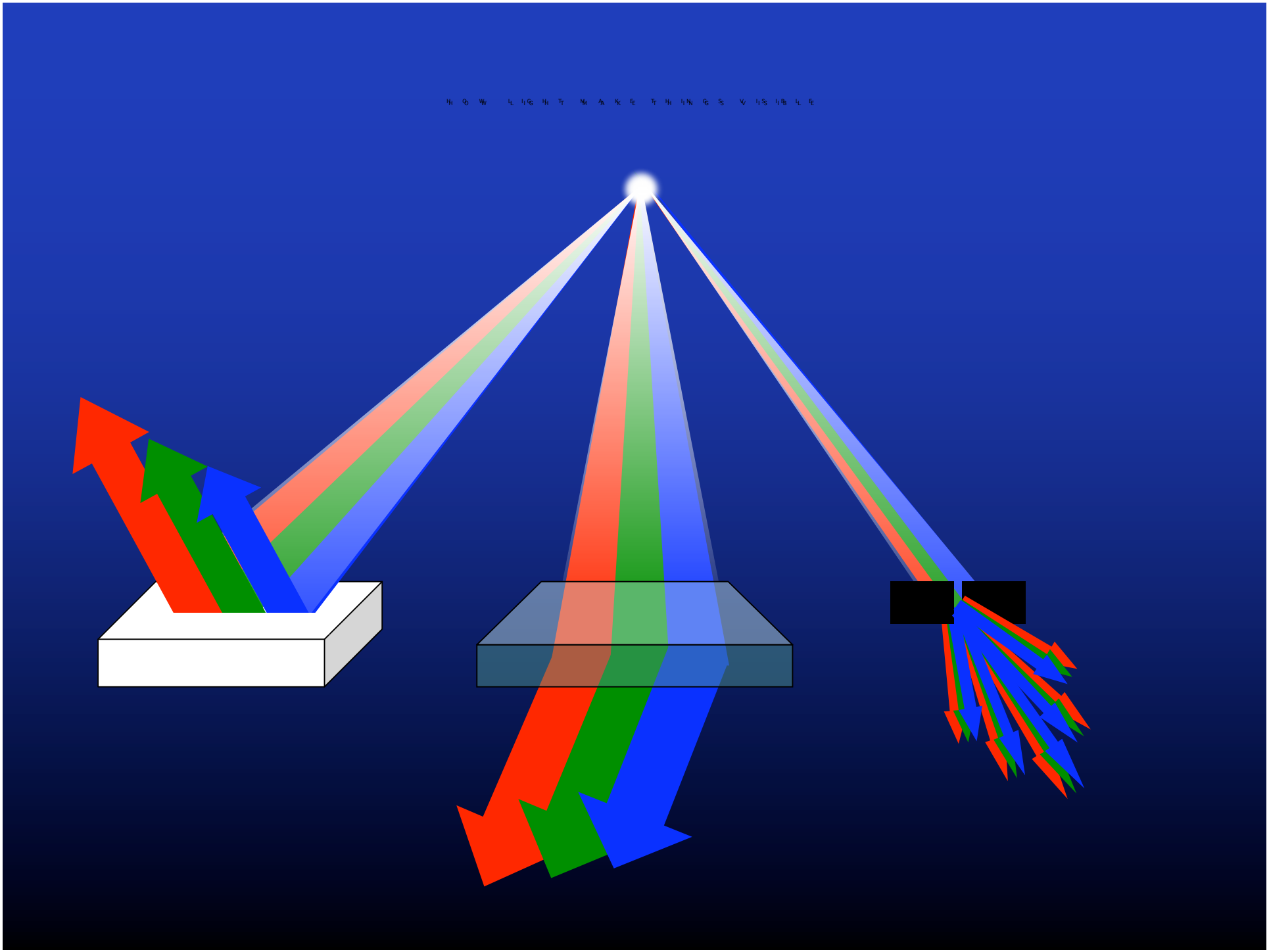
Your eyes see ACHROMATIC light… WHITE LIGHT

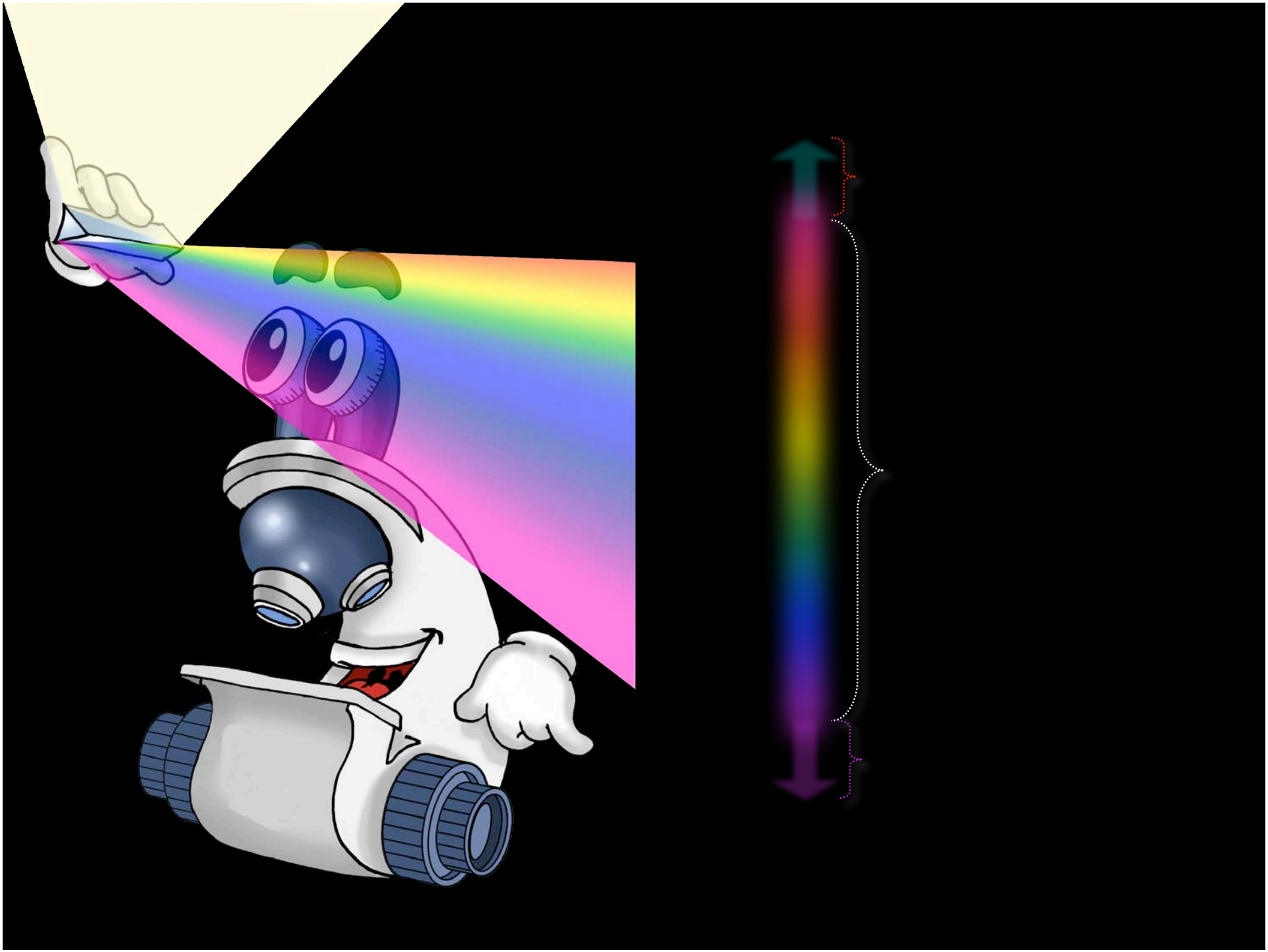
At 630nm you see red light

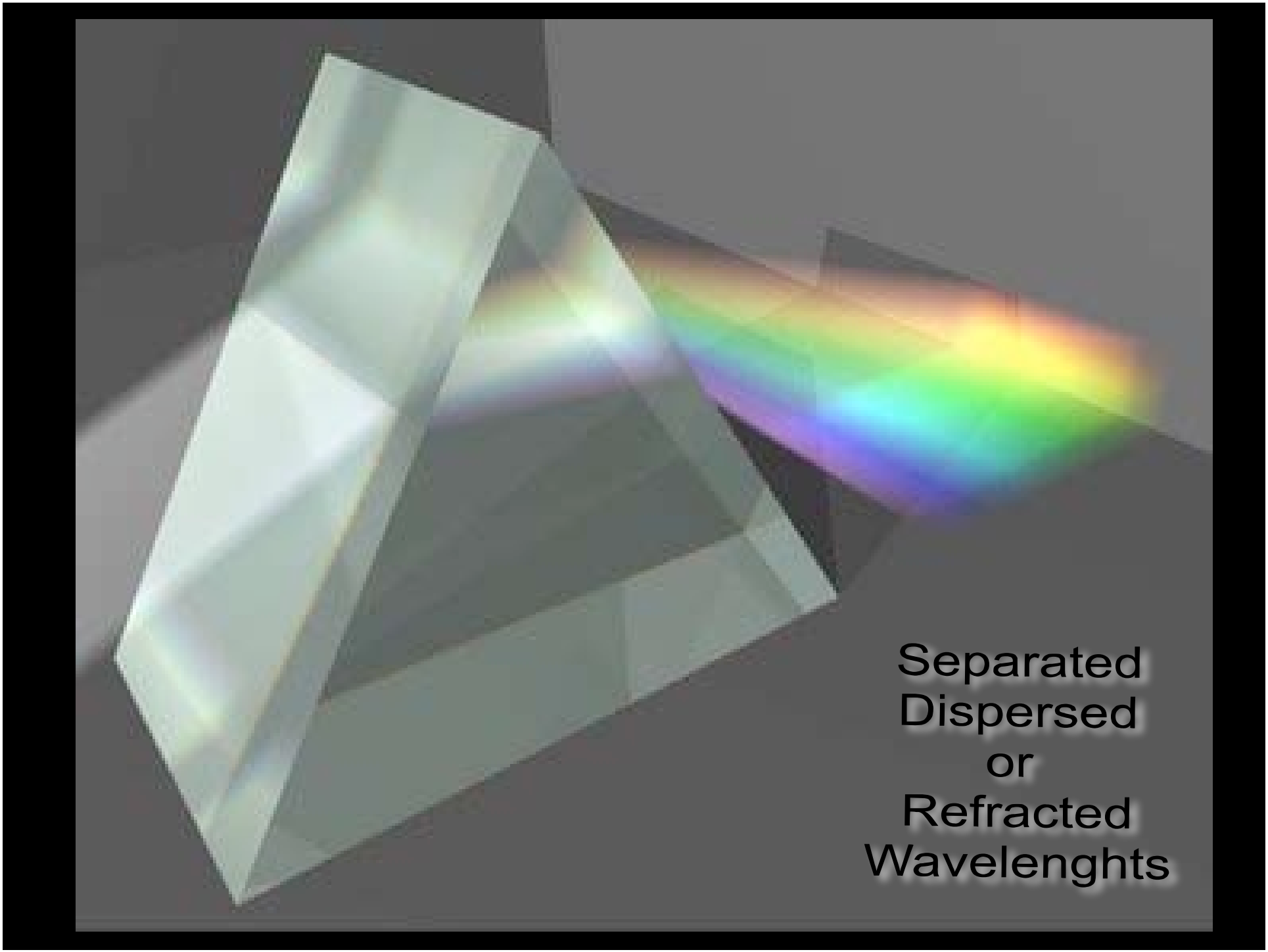


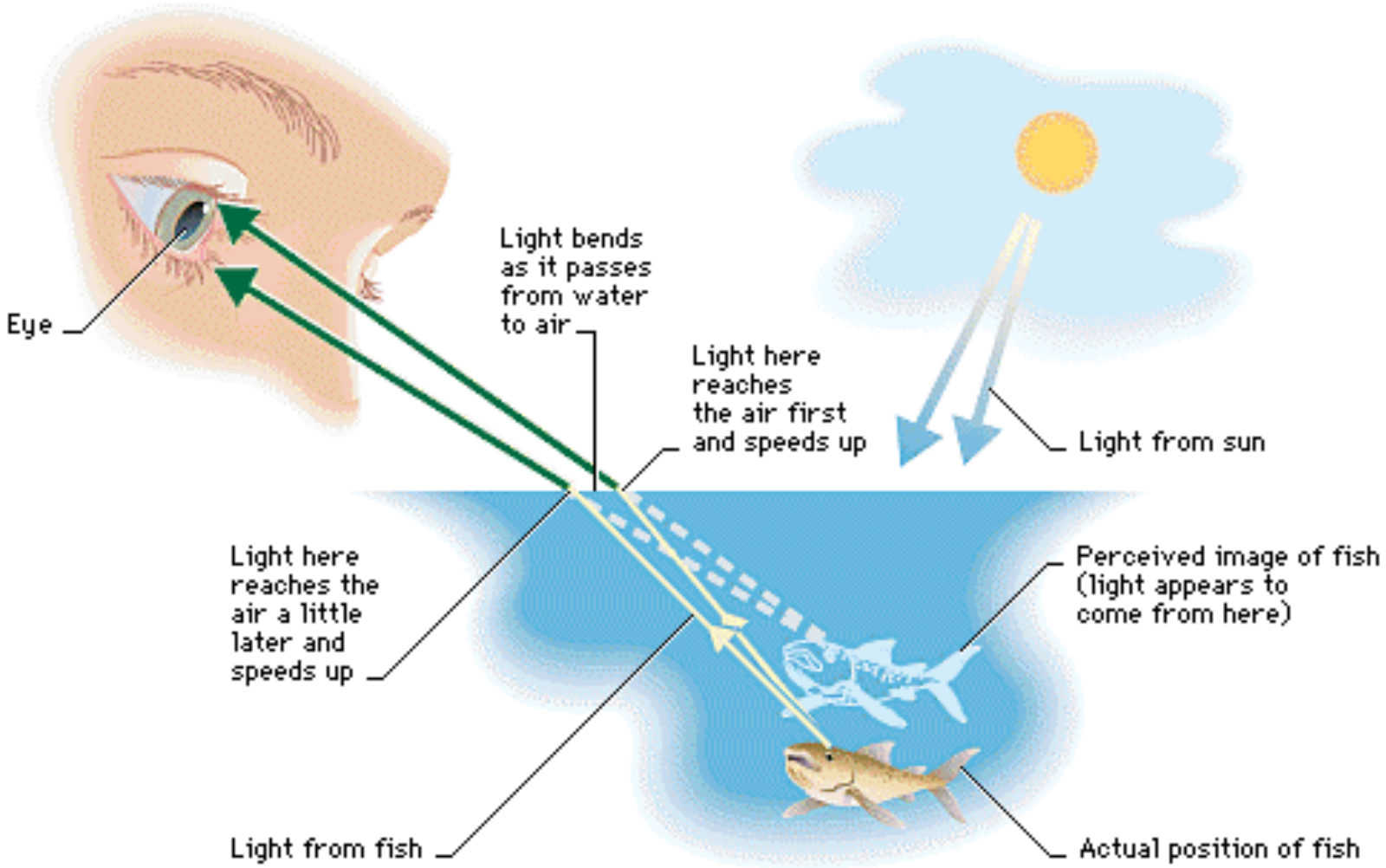

BASICS ON….
PROPERTIES OF LIGHT
• Reflection
• Diffraction
• Refraction
• Absorption

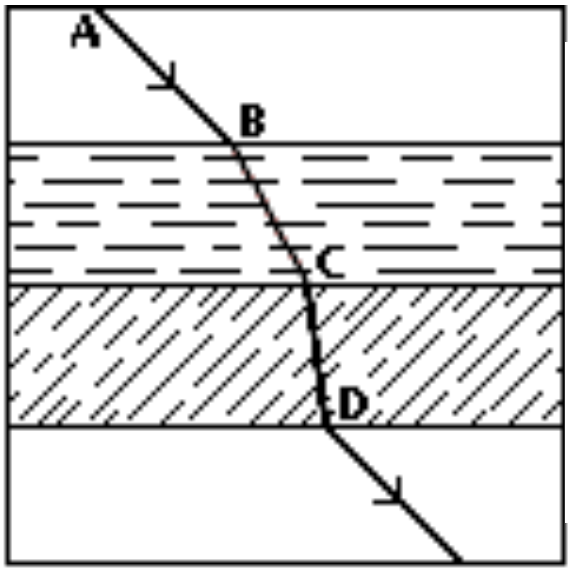




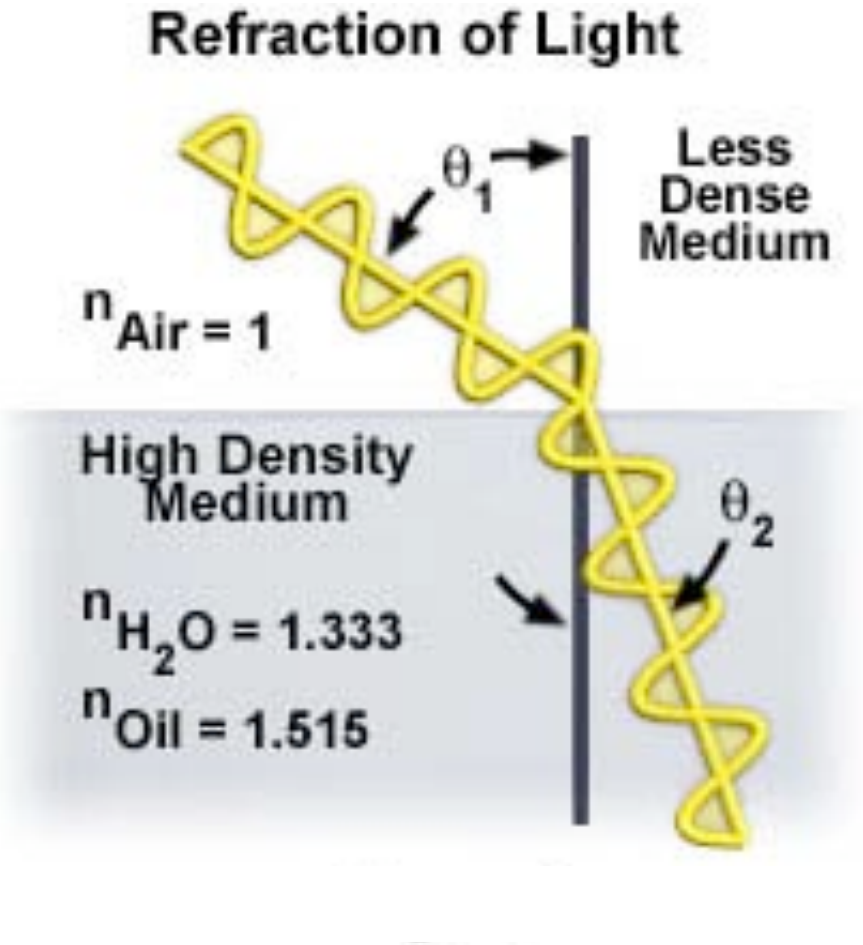


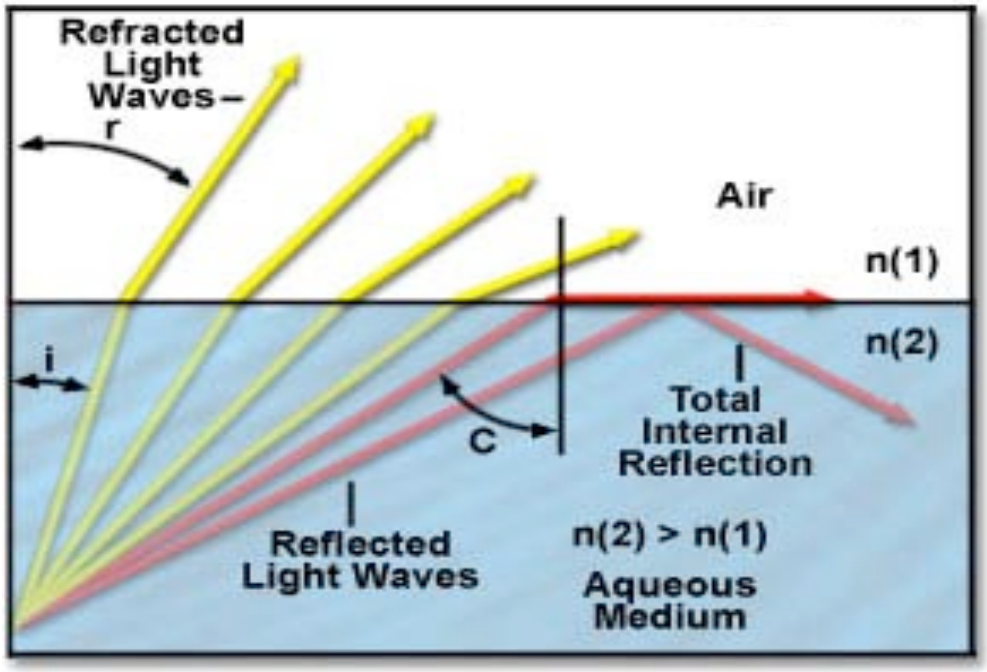

Separated
Dispersed
or
Refracted
Wavelenghts

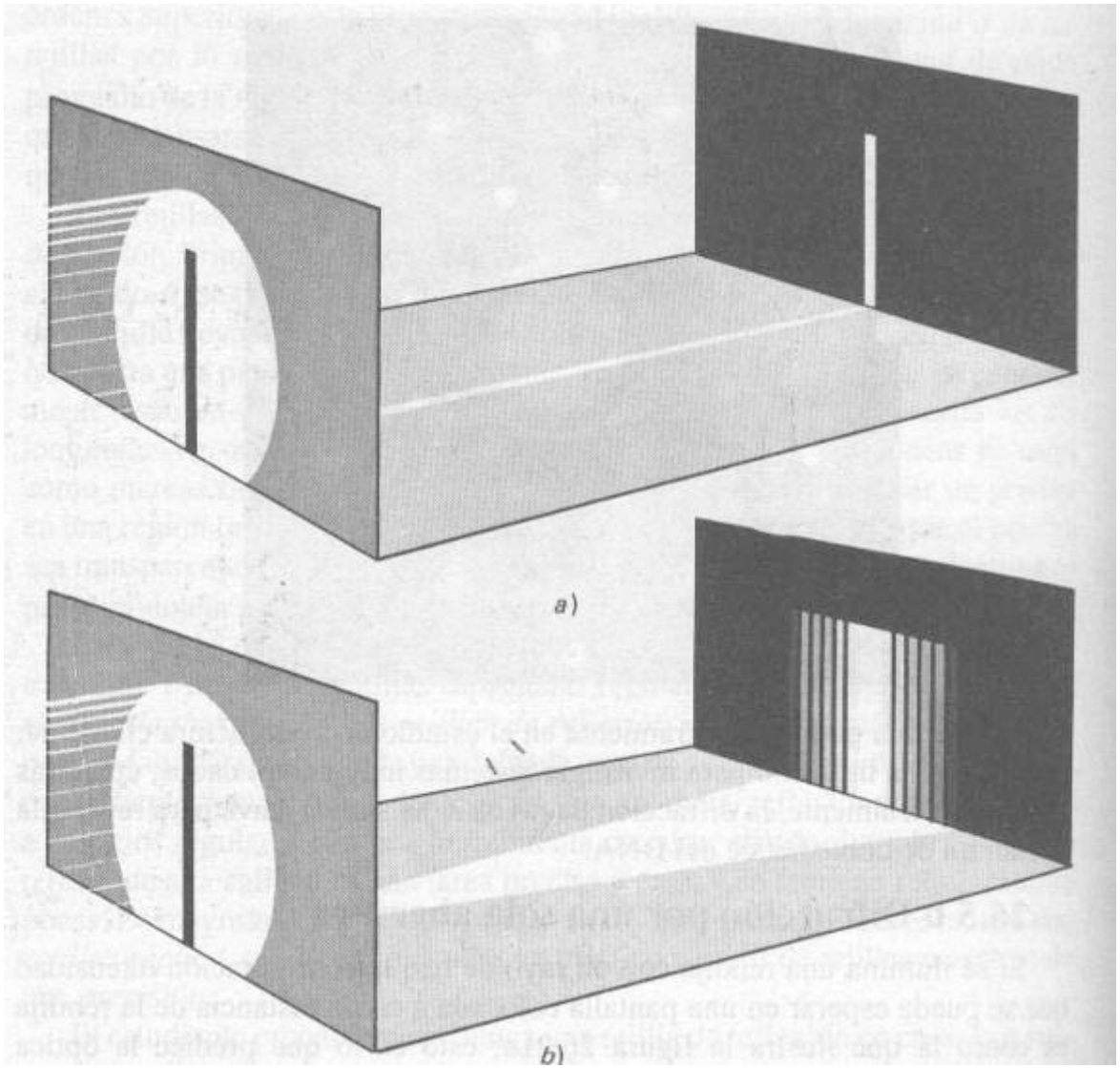

Eye
Light bends as it passes from water to air
Light here reaches the air first and speeds up
Light from sun
Light here reaches the air a little later and speeds up
Perceived image of fish (light appears to come from here)
Light from fish
Actual position of fish



Refracted rays passing through three different media




Refracted
Light
Waves –
r
Air
n(1)
n(2)
i
C
Total
Internal
Reflection
Reflected
Light Waves
n(2) > n(1)
Aqueous
Medium



DIFRACTION

INTRODUCTION TO LENSES

13th Century

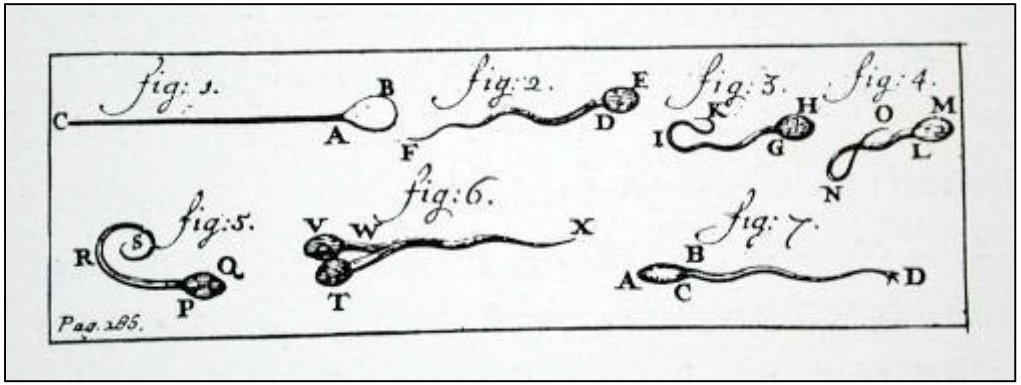
Reading stones

Early Microscope Attributed to Jansen
(about 1595)

XV Century. Leonardo Da Vinci …
The necessity to use lenses to facilitate vision and too see small things….

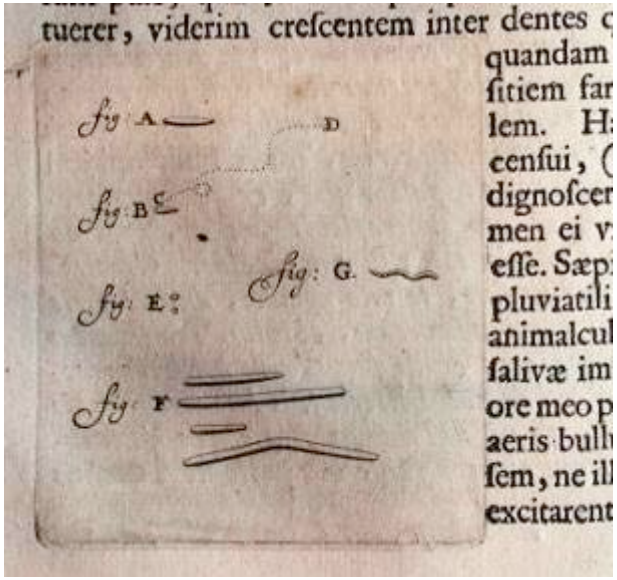
XVII Century.
Zaccharias & Hans Jansen 1590
Galileo Galilei 1609
Christiaan Huygens 1621

Images taken from: http://micro.magnet.fsu.edu/optics/timeline/people/alhazen.html
http://www.biol.unlp.edu.ar/historiabiologiacelular.htm

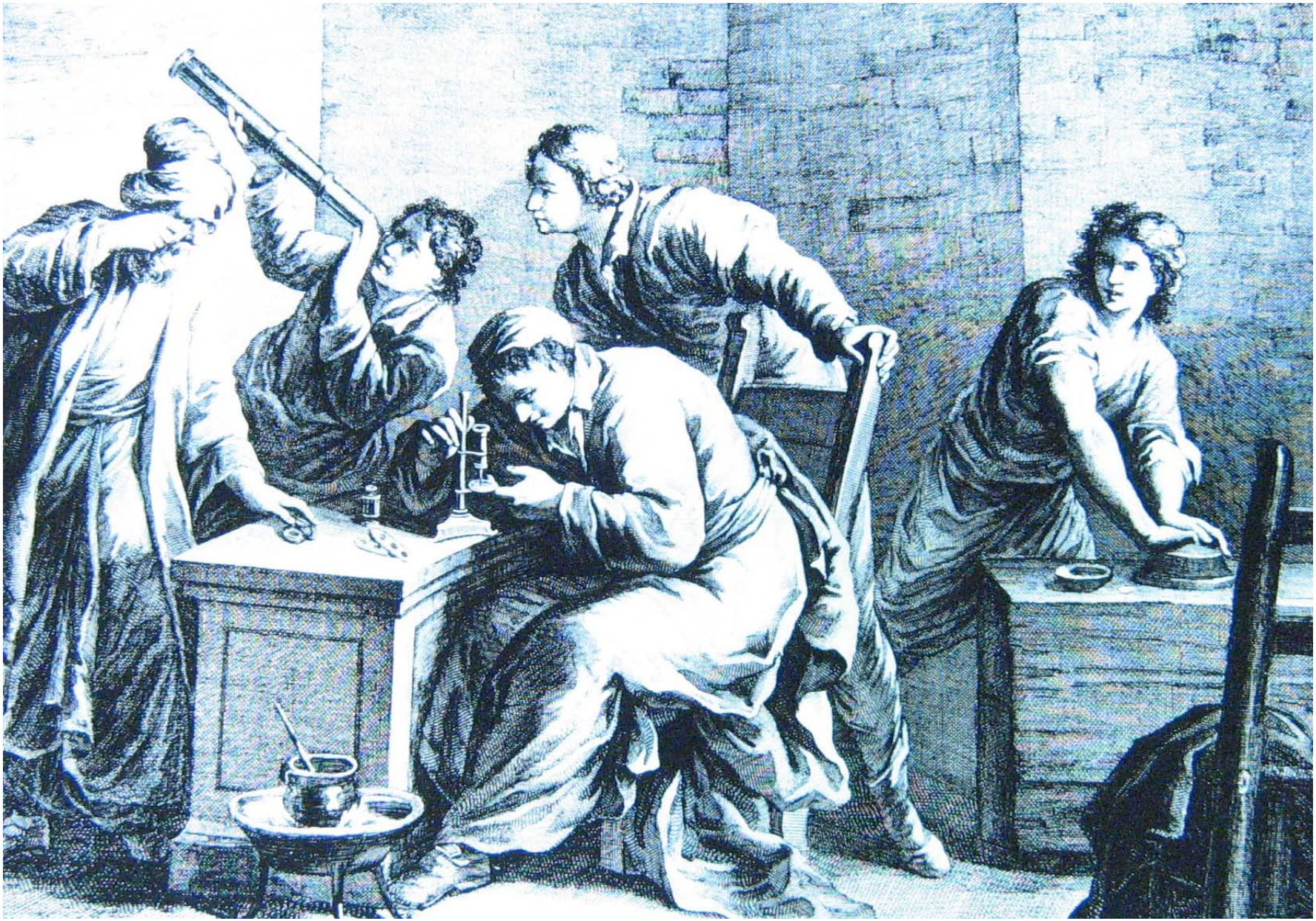


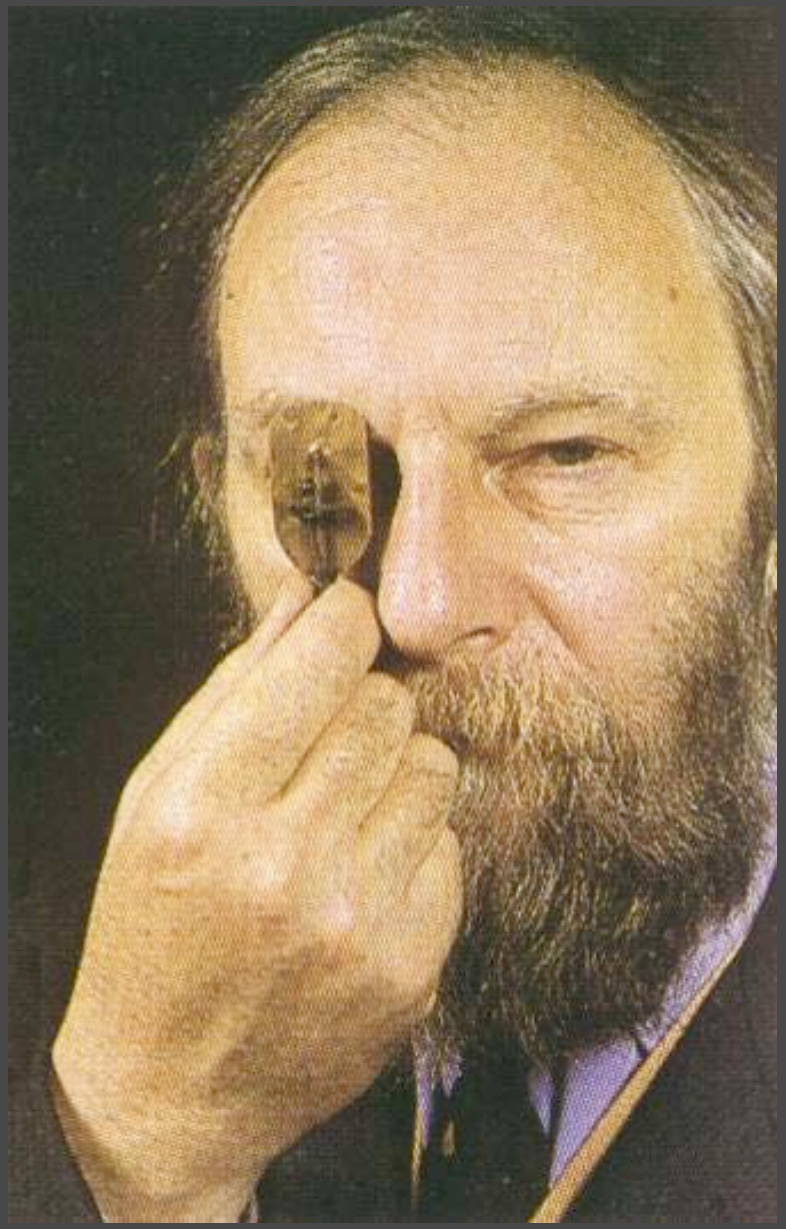
Human sperm

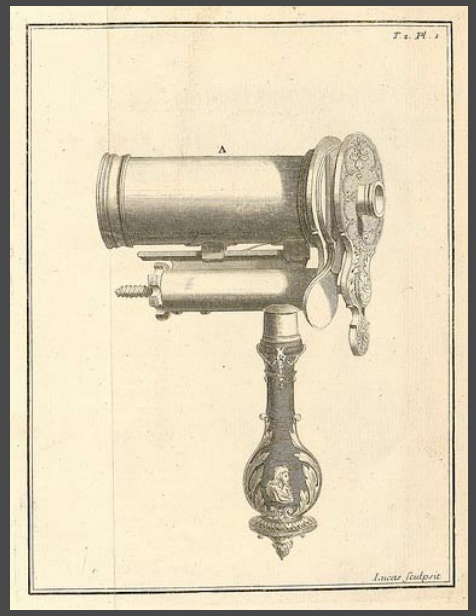
Antony van Leeuwenhoek
1632 - 1723

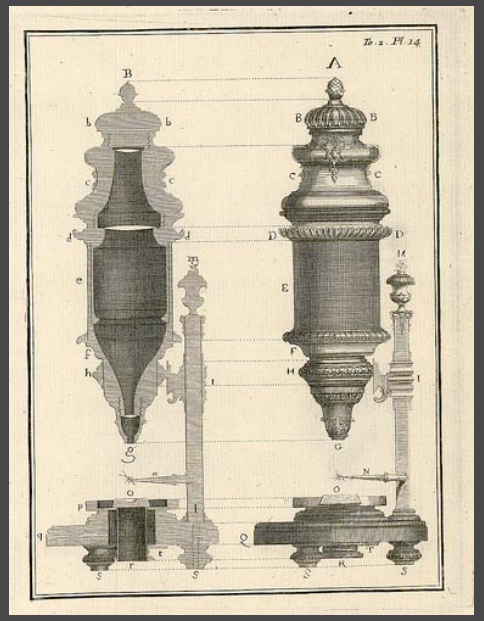


Bacteria






T. 2. Pl. 1
A
Lucas fculpsit.


To. 2. Pl. 14.
A
B


Tab. V
Abbildungen allen zu einem guten Microsco-
pen Behörigen Theilen wie selbige
in dem Futteral befindlich
Dresden in der Heckelschen Buchhandlung zu haben

MICROGRAPHIA:

OR SOME

Physiological Descriptions

OF

MINUTE BODIES

MADE BY

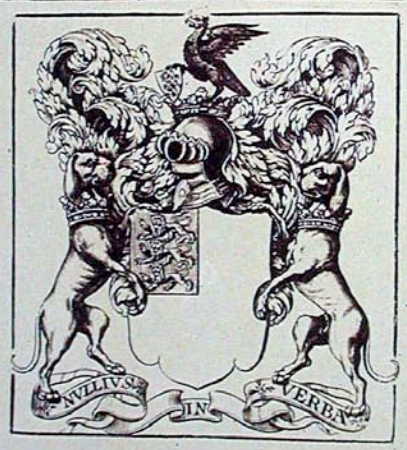
MAGNIFYING GLASSES.

WITH

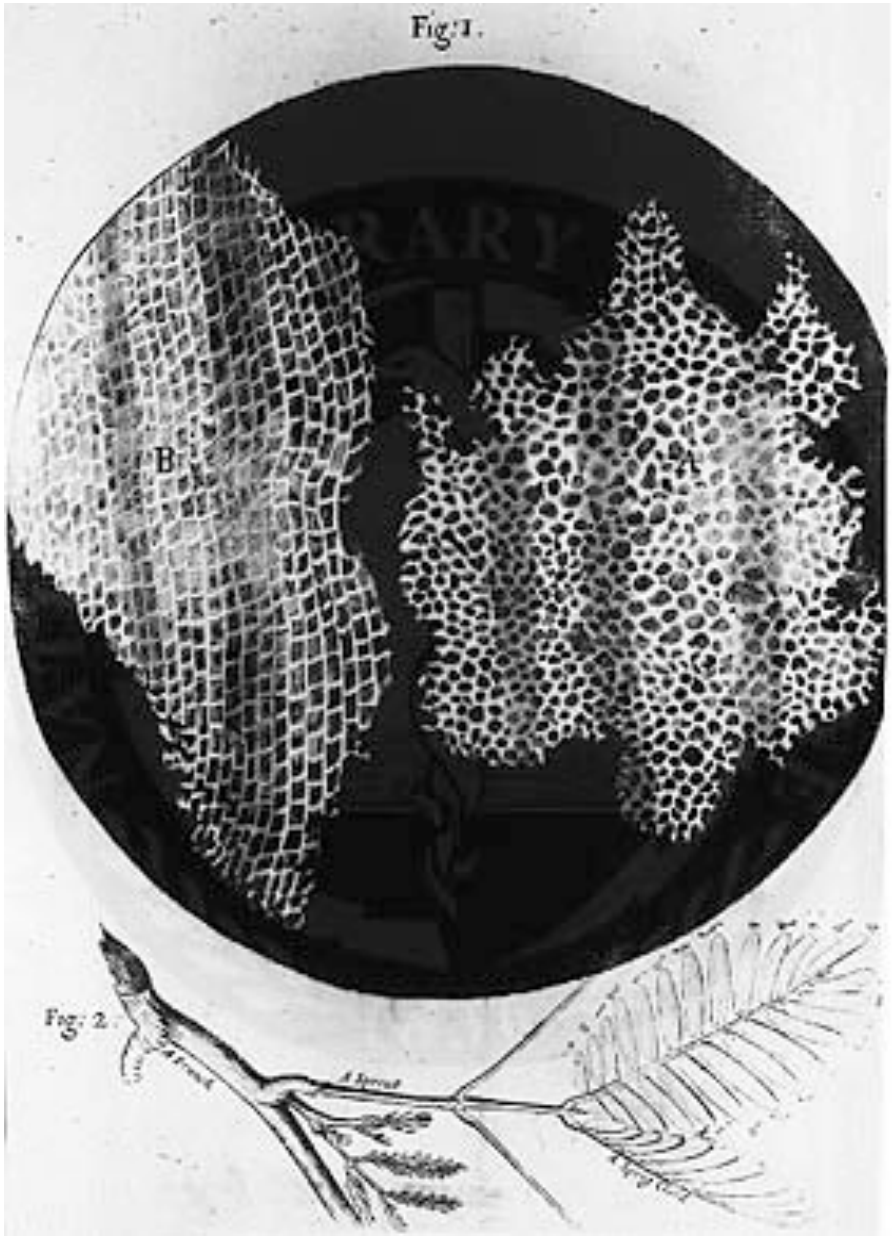
OBSERVATIONS and INQUIRIES thereupon.

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

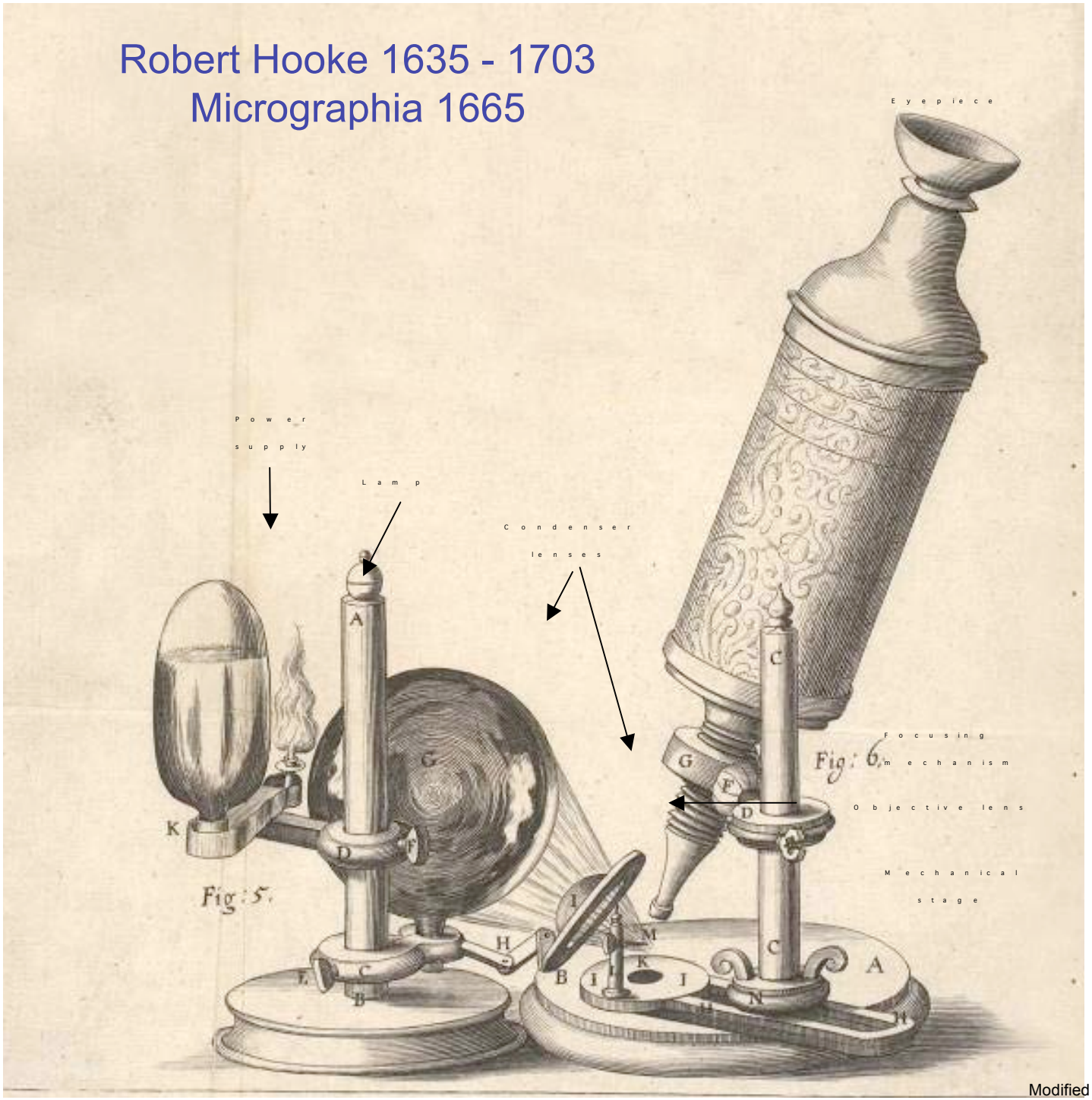
Non possis oculo quantum contendere Linceus,
Non tamen idcirco contemnas Lippus inungi. Horat. Ep. lib. 1.



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.



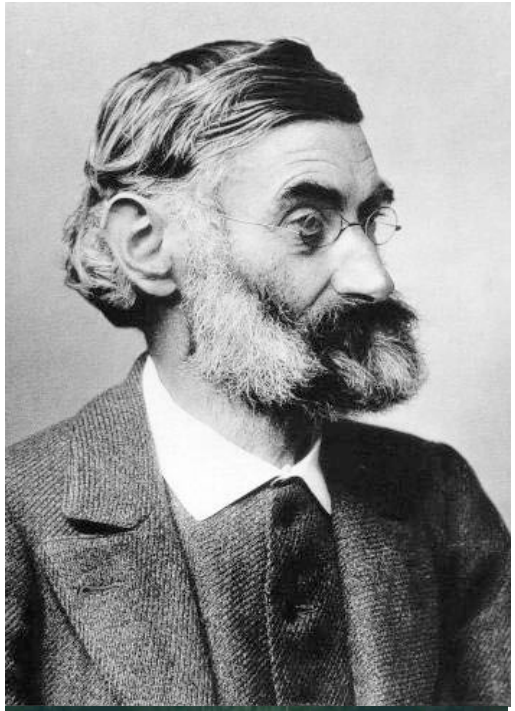
Robert Hooke 1635 - 1703
Micrographia 1665

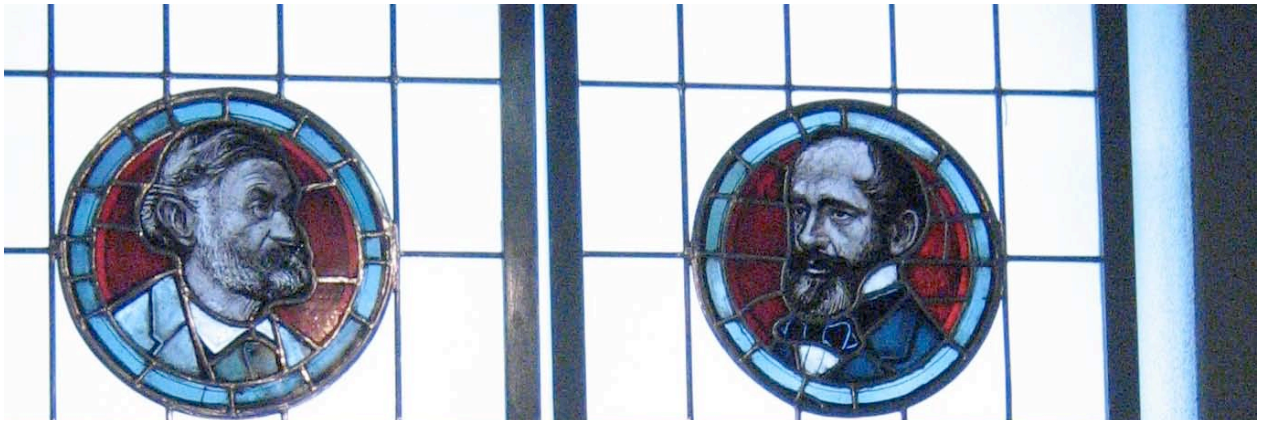


Modified from:www.its.caltech.edu

INVERTED

UPRIGHT

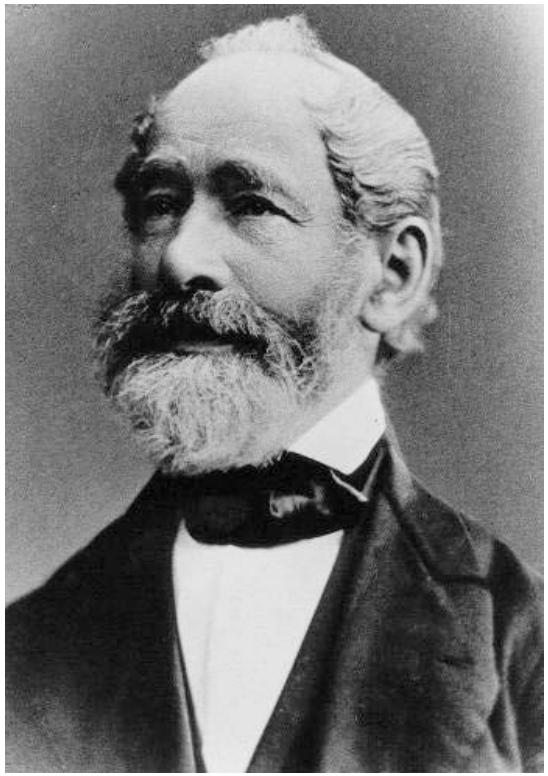


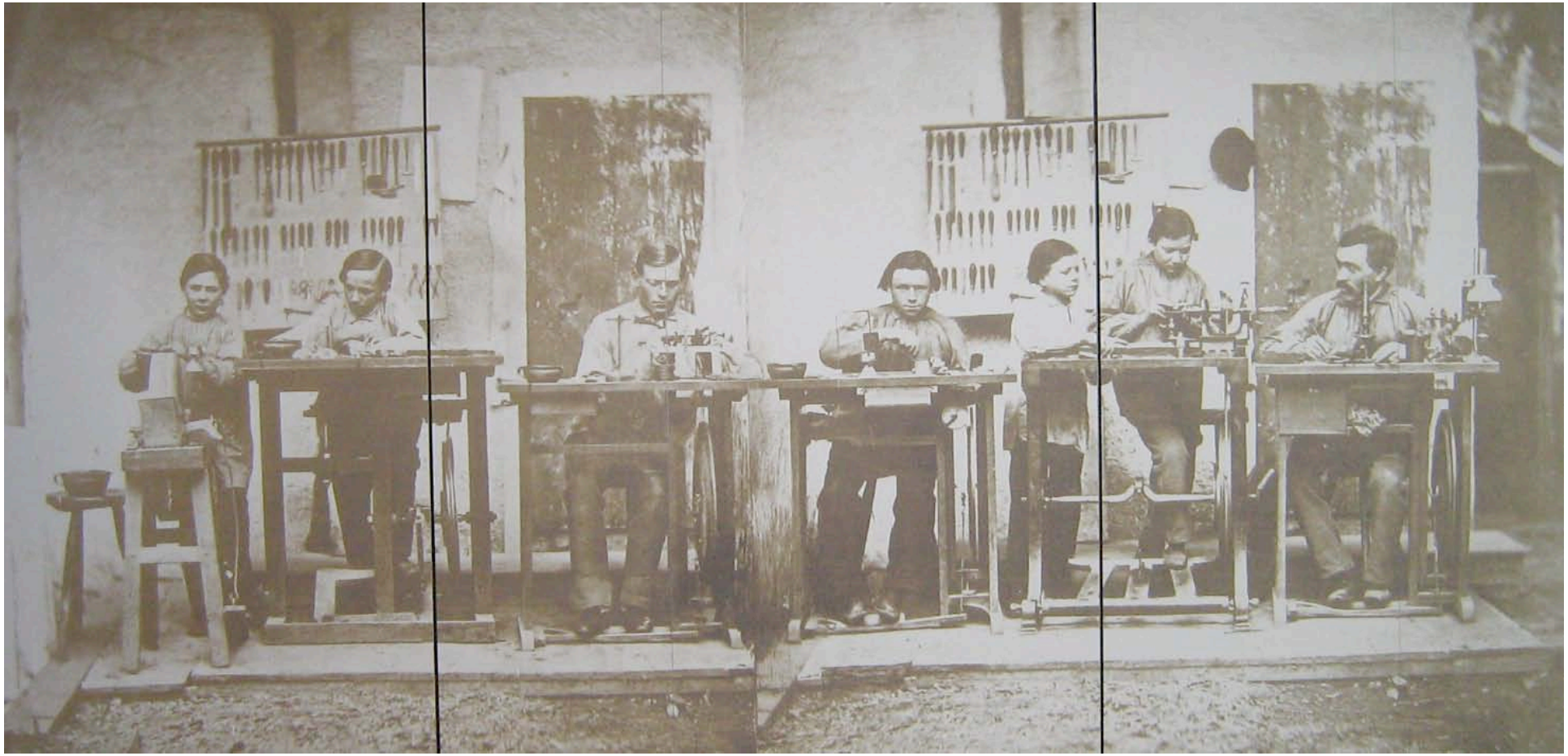


Ernst Abbe
1840 - 1905

Carl Zeiss
1816 - 1888

Otto Schott
(1851 - 1935)

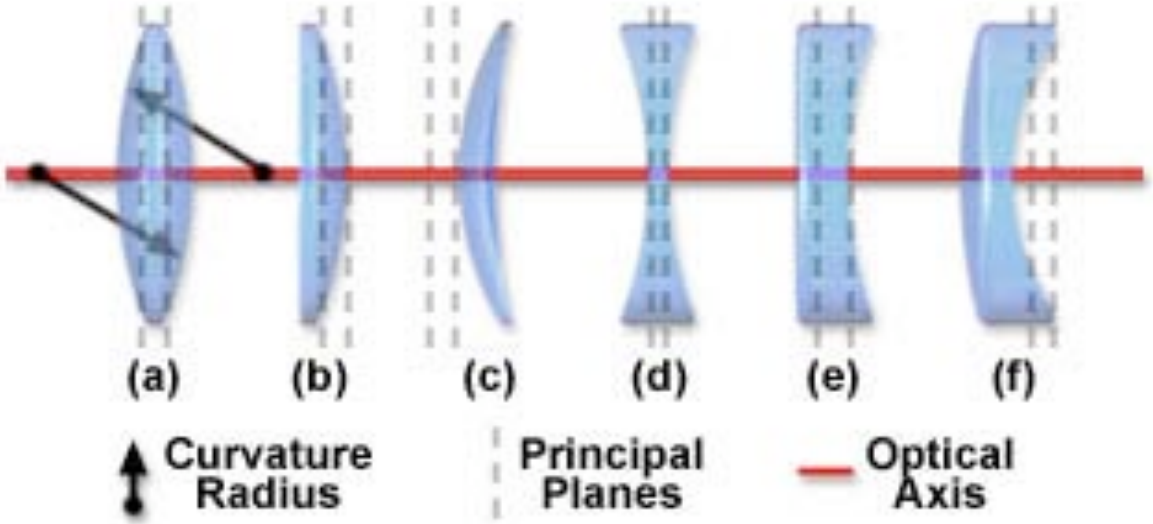




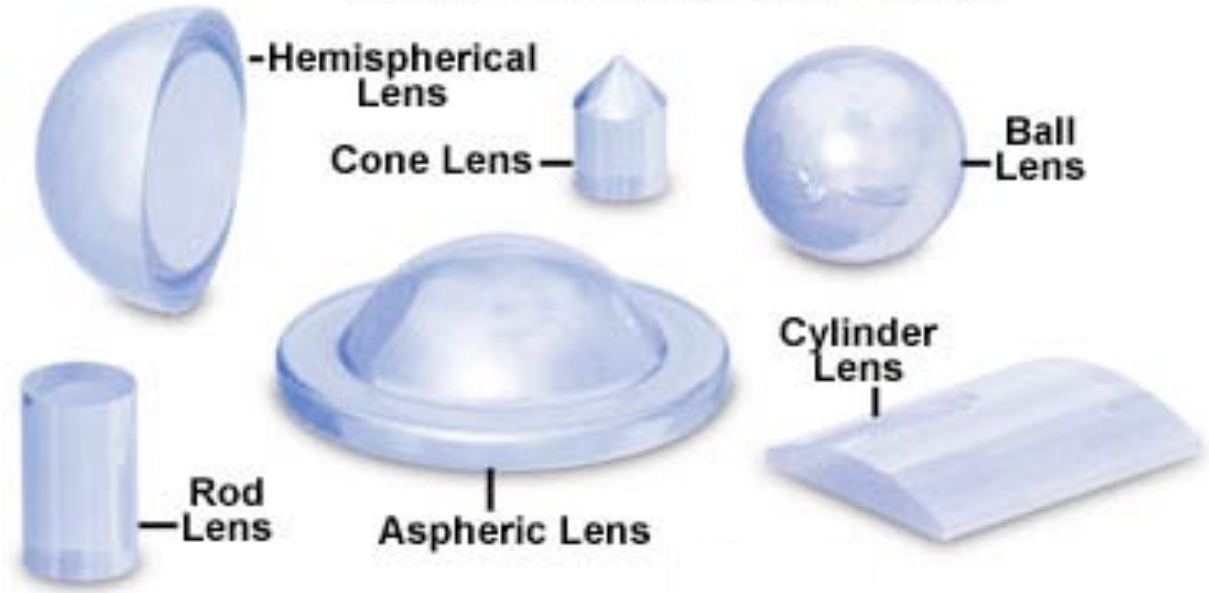
Carl Müller. Friedrich Pfaffe. Joseph Rudolph. Wilhelm Böber. Heinrich Pape. Fritz Müller. August Löber.

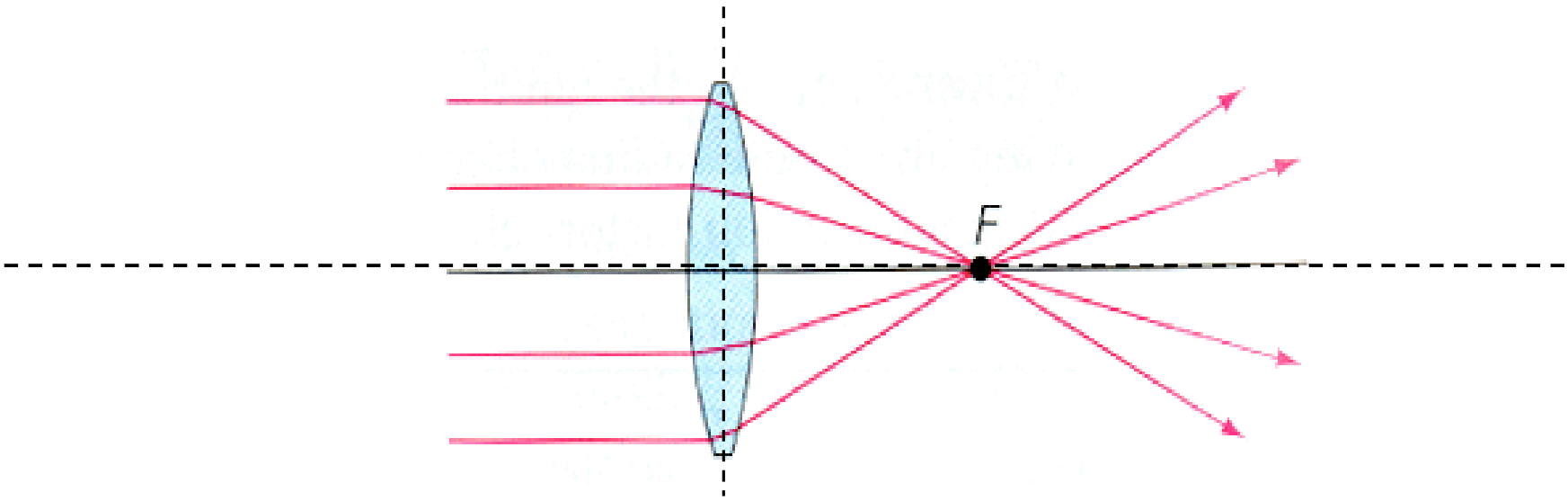
Optische Werkstatt von 1864.

Shapes and Principal Planes of Simple Lenses

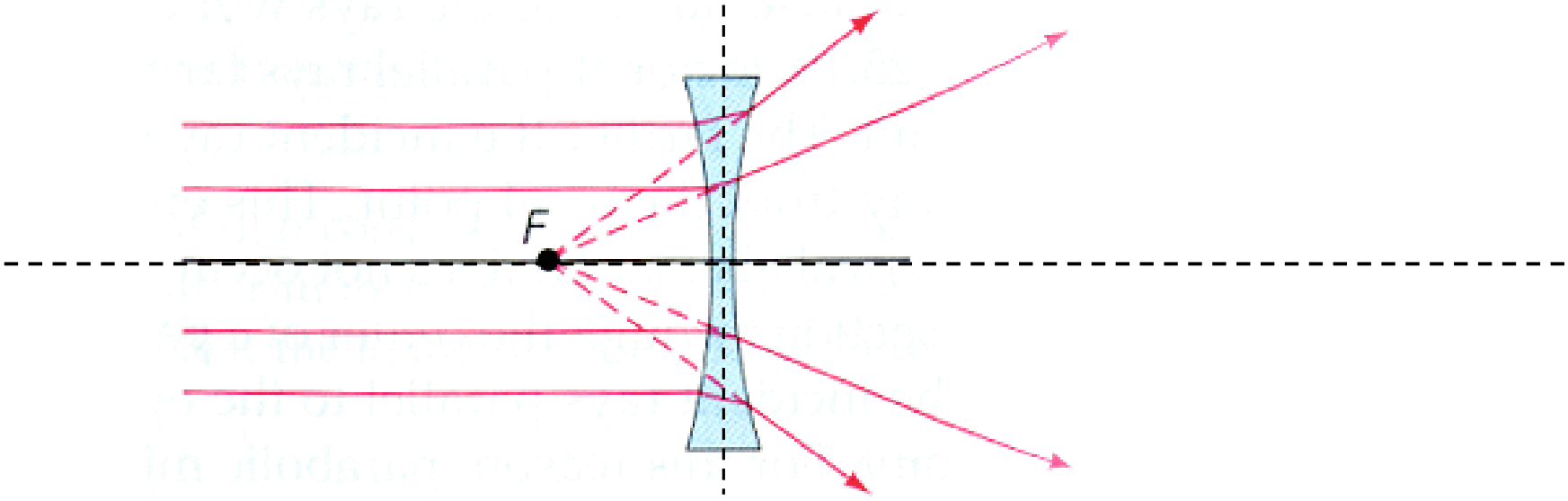


Uncommon Lens Geometries

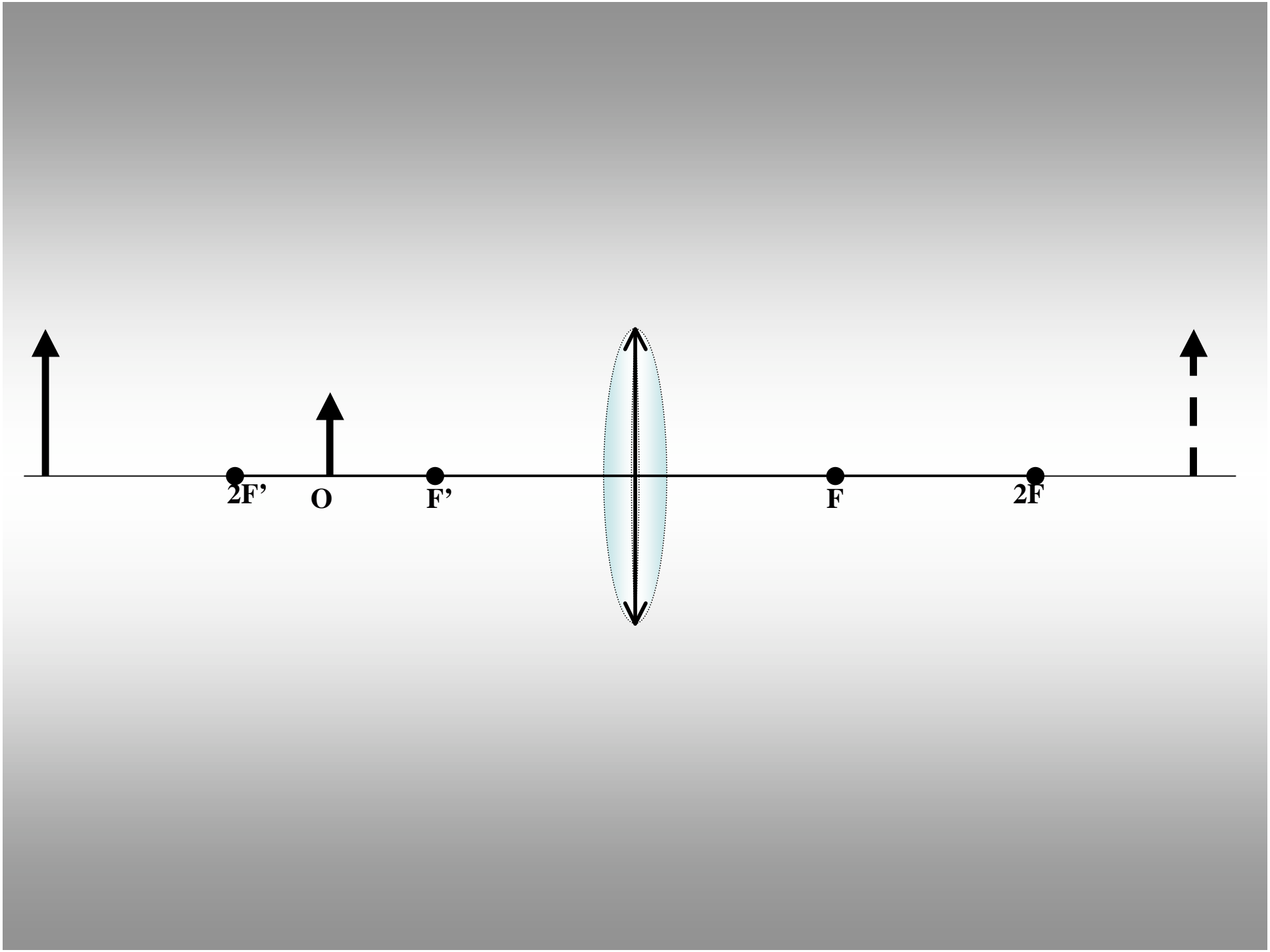


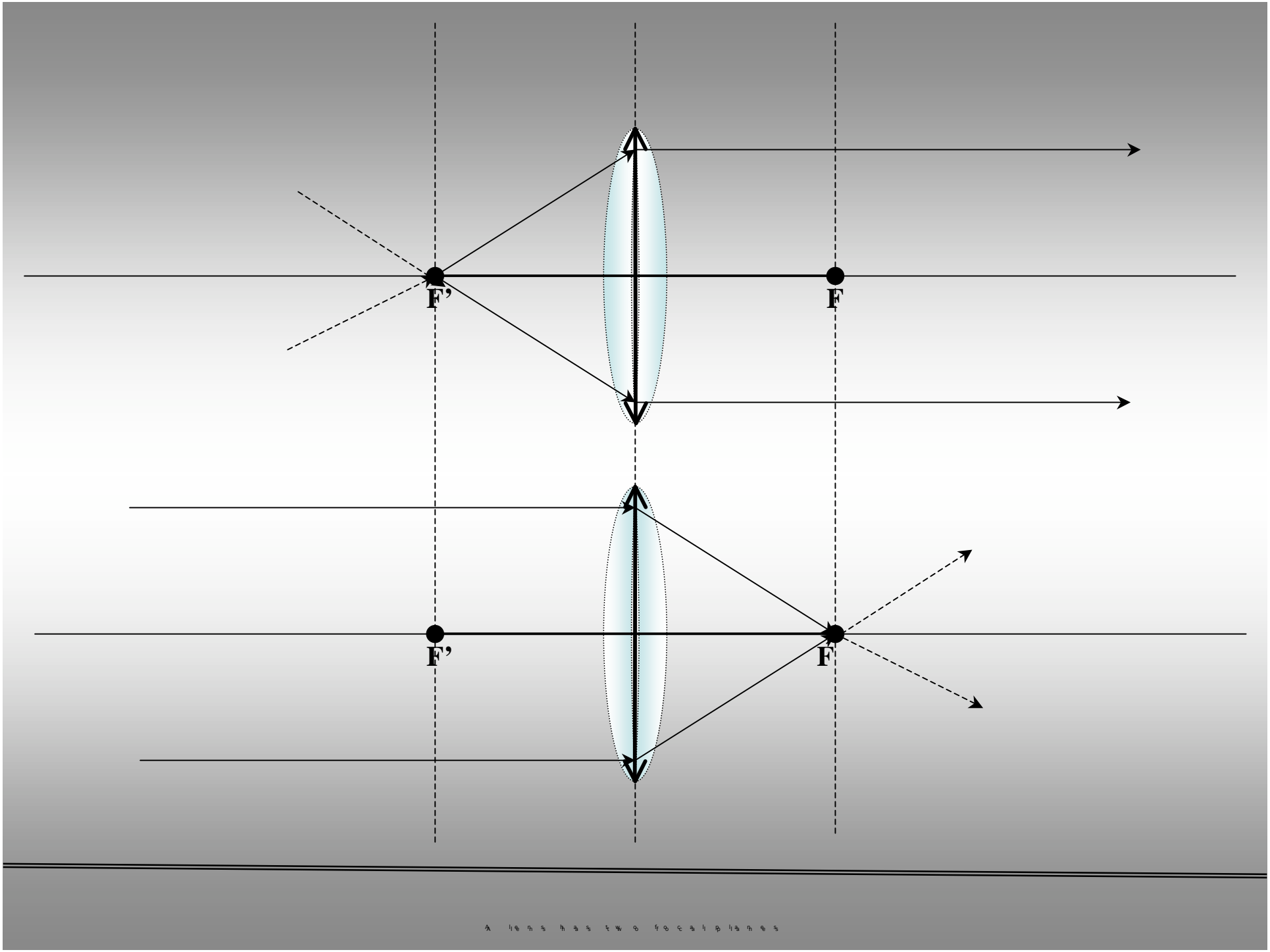


Converging lens

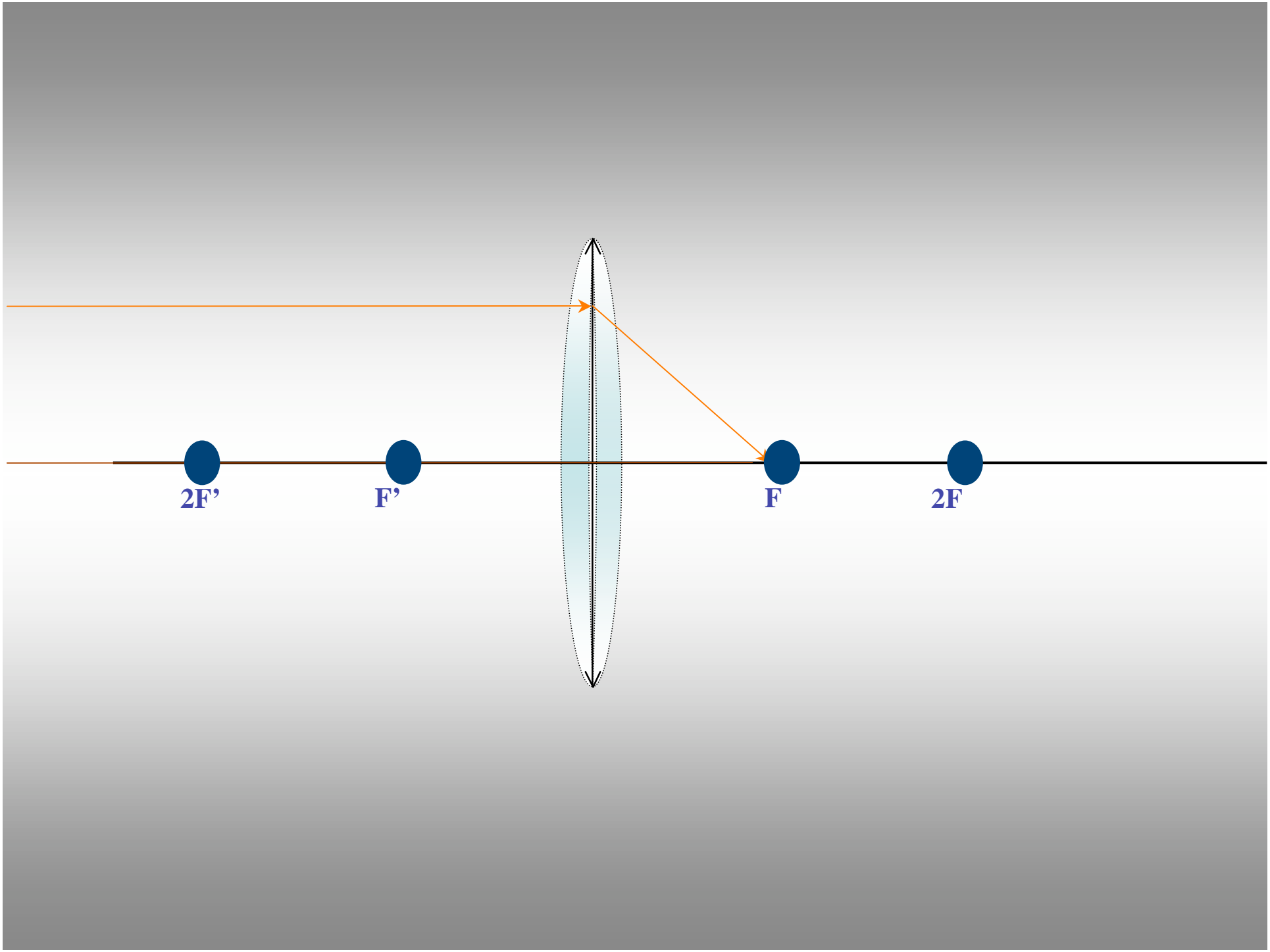


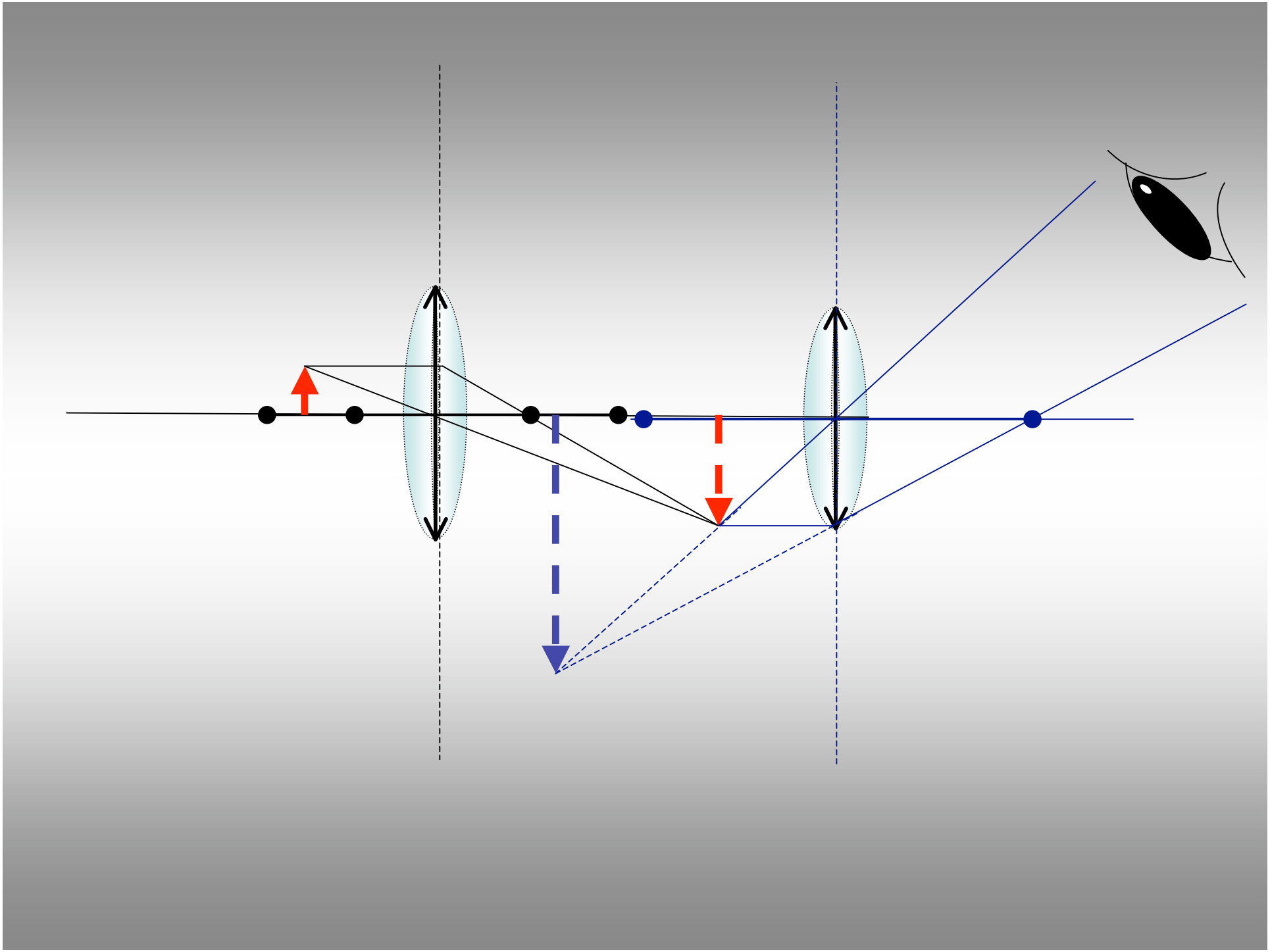
Diverging lens

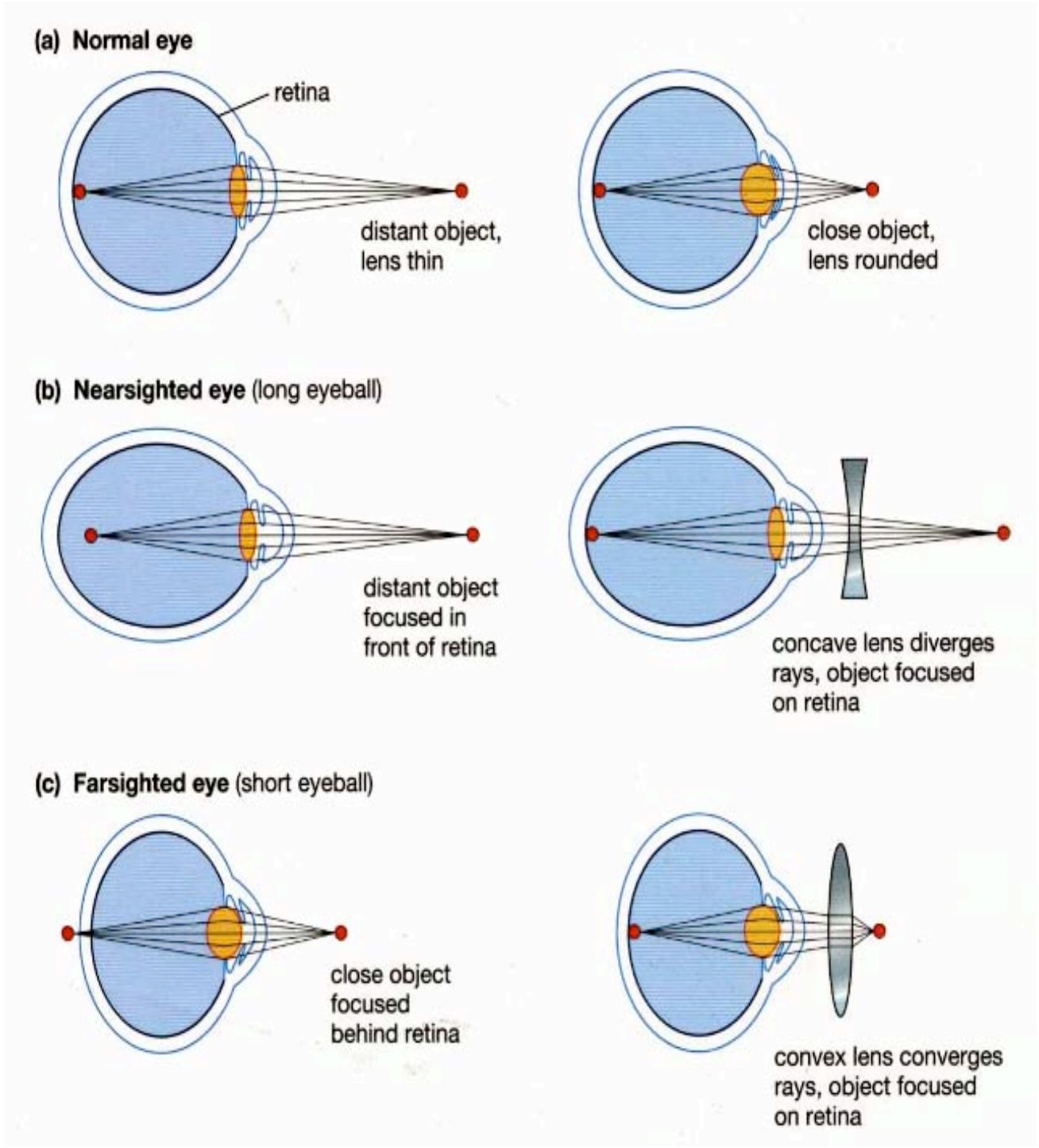

2F'
O
F'
F
2F



A lens has two focal lengths


2F’
F’
F
2F

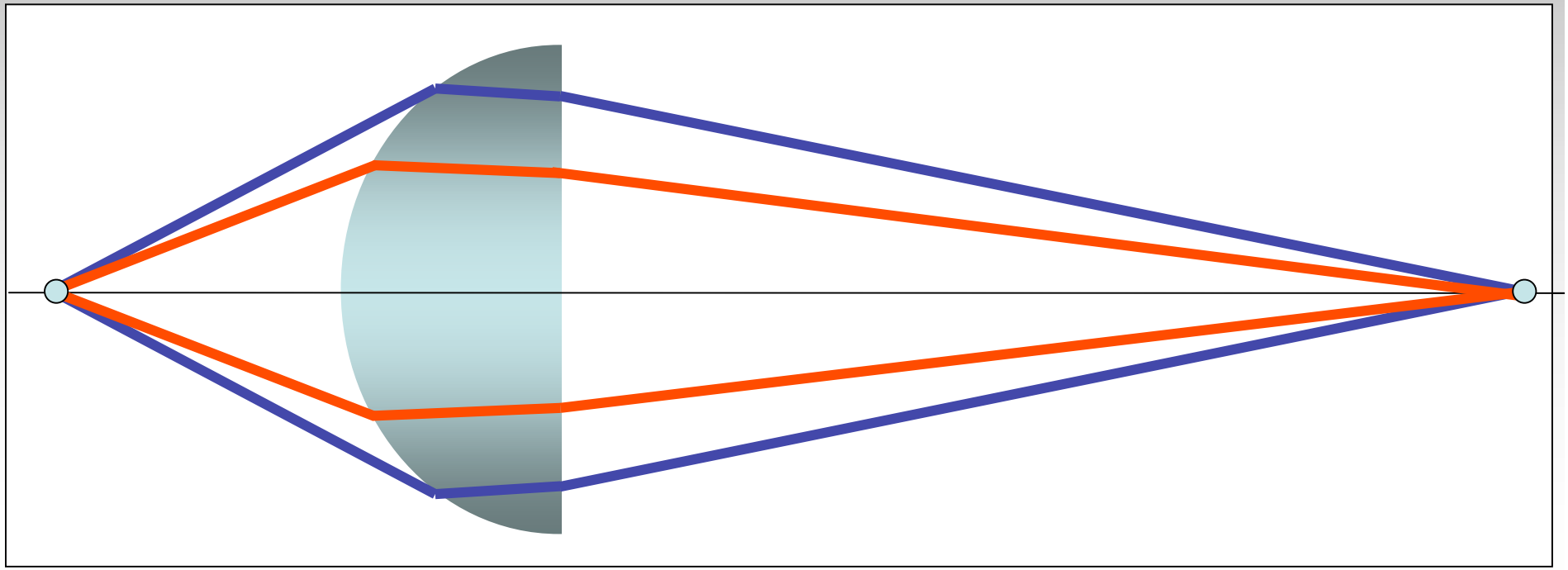



(a) Normal eye
retina
distant object, lens thin
close object, lens rounded
(b) Nearsighted eye (long eyeball)
distant object focused in front of retina
concave lens diverges rays, object focused on retina
(c) Farsighted eye (short eyeball)
close object focused behind retina
convex lens converges rays, object focused on retina

The lenses of the microscope

The important lenses of the microscope are *positive* or *converging* lenses,

- thicker in the middle than at their edges

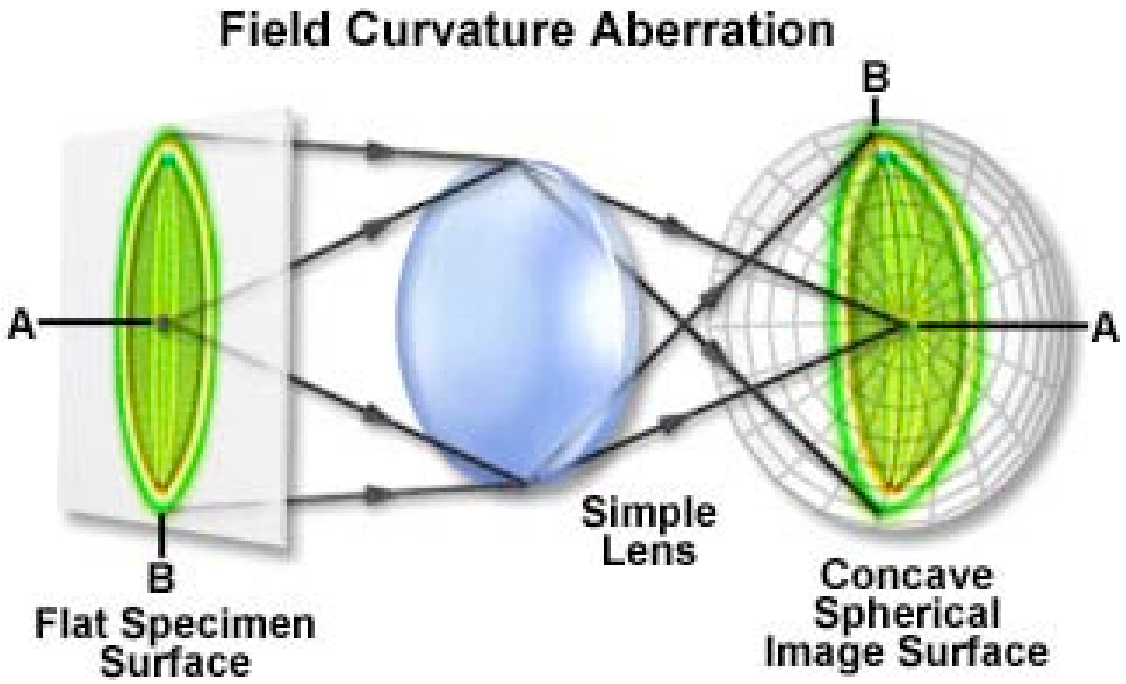


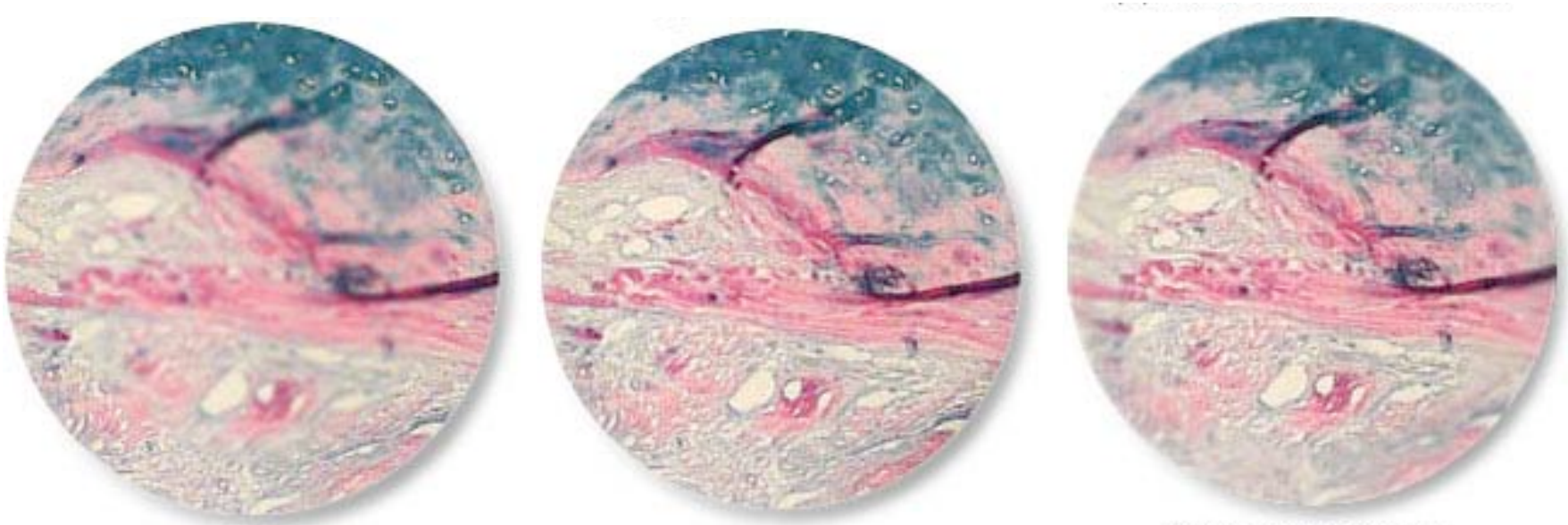
From Peter Evennett

The job of an ideal lens

- To accept as many rays as possible from each point in an object
- To reassemble all the rays from each point at corresponding points in the image…
- In such a way that the distance travelled by all the rays from each object point to its corresponding image point is the same
  - so that they all arrive ‘in phase’.

This is unfortunately not possible with a single-element lens
because of several *aberrations*
- spherical, chromatic and others

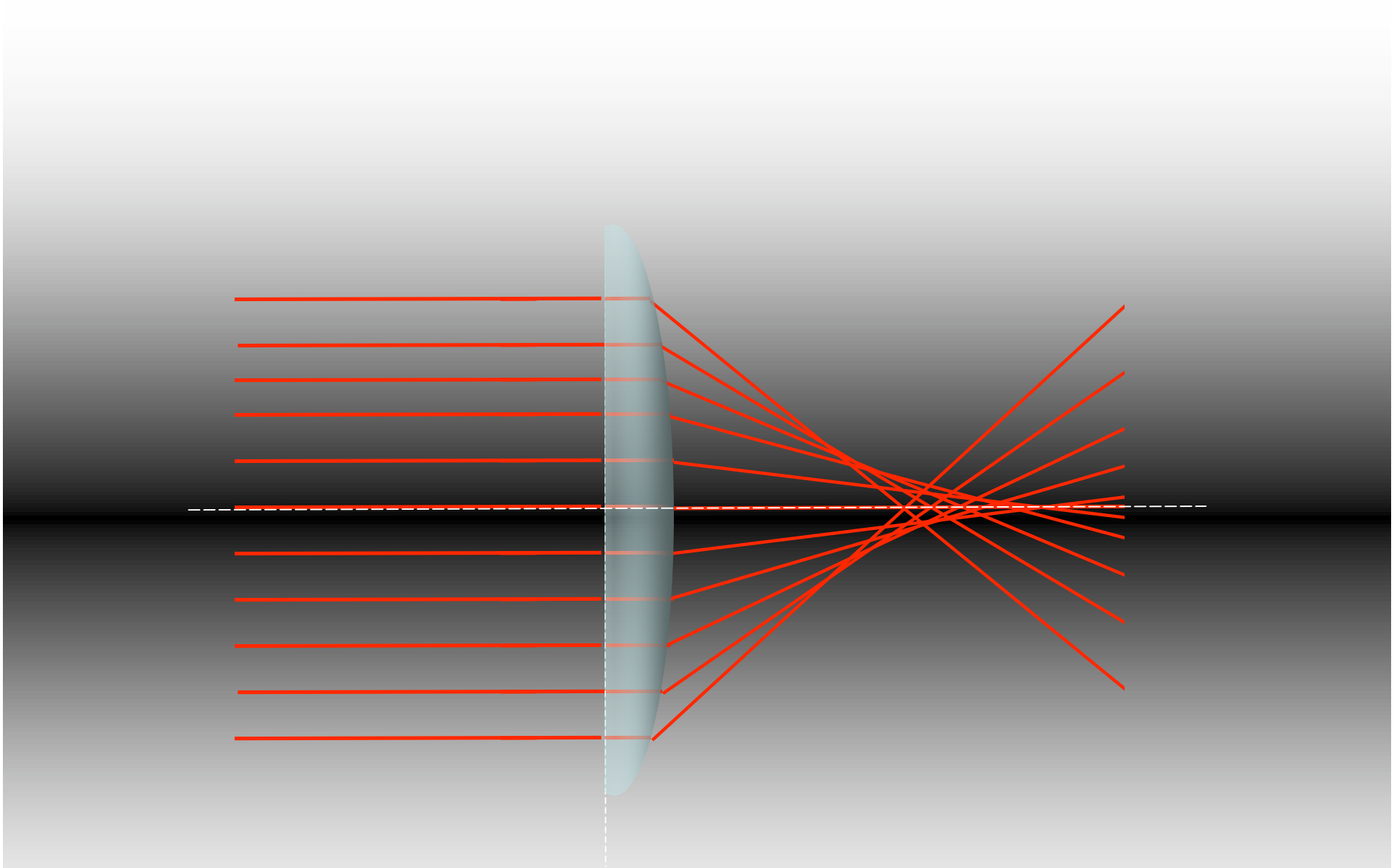




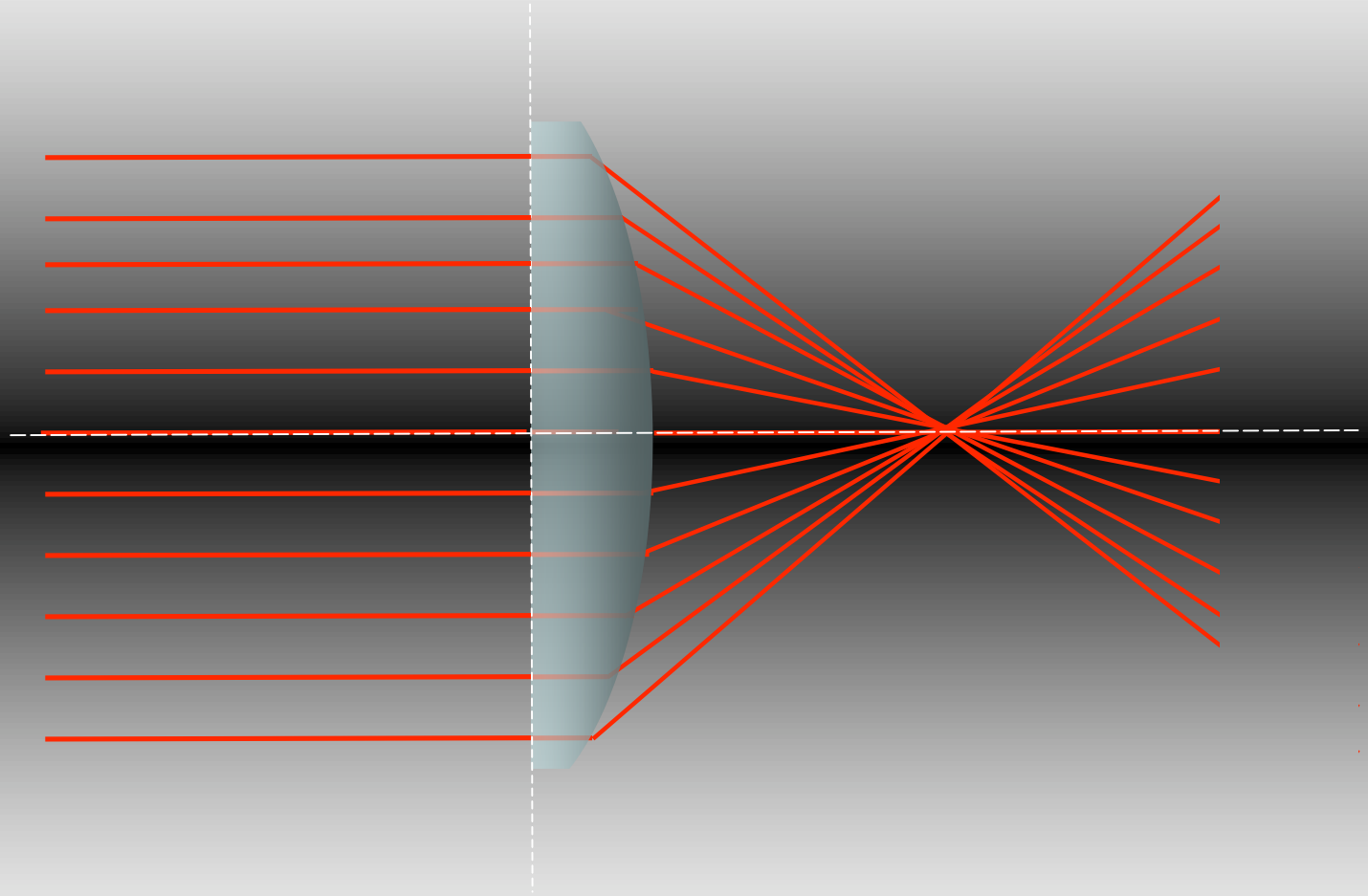
(a) Edges in Focus

(b) Entire Viewfield in Focus

(c) Center in Focus

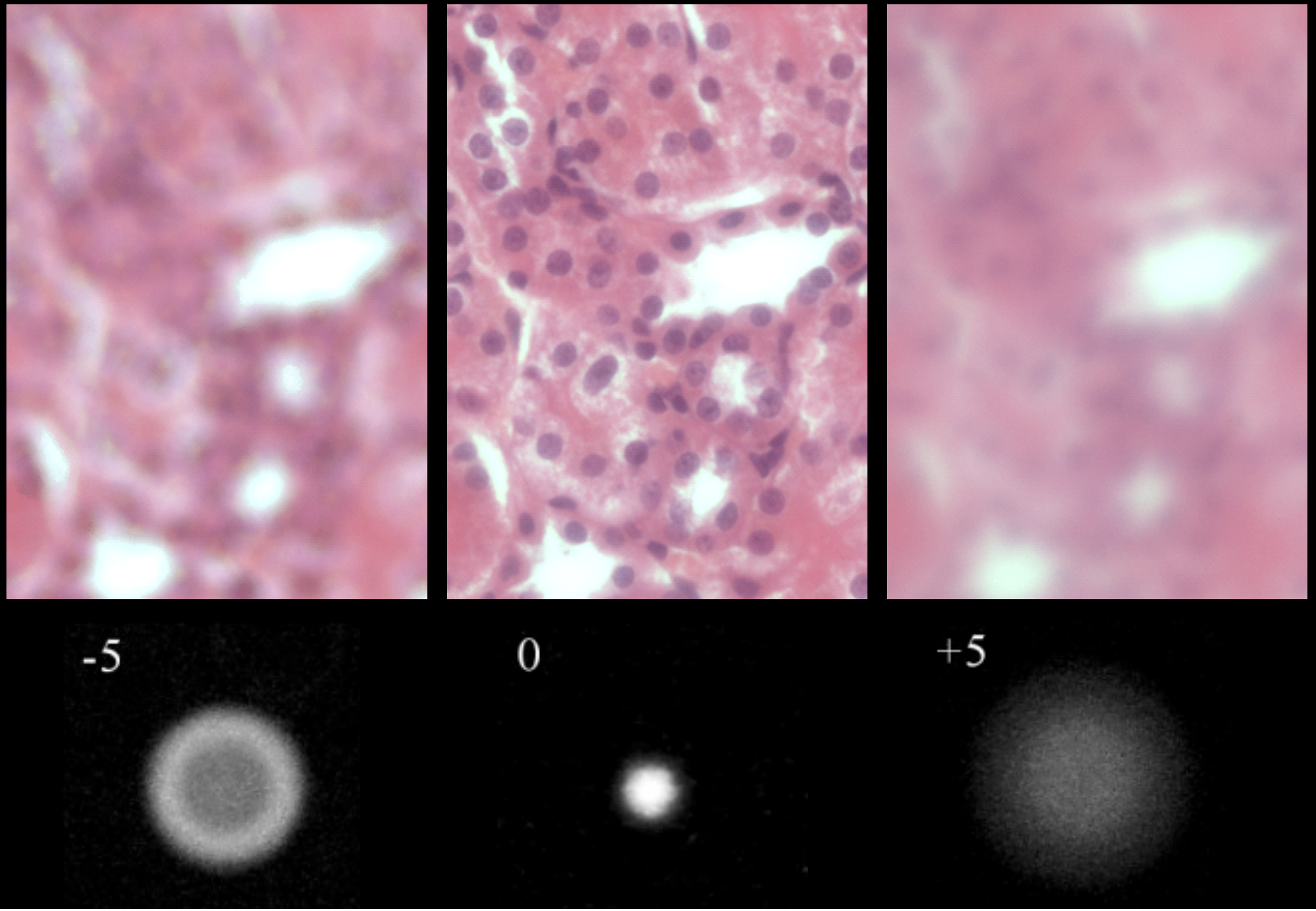


Spheric Aberration

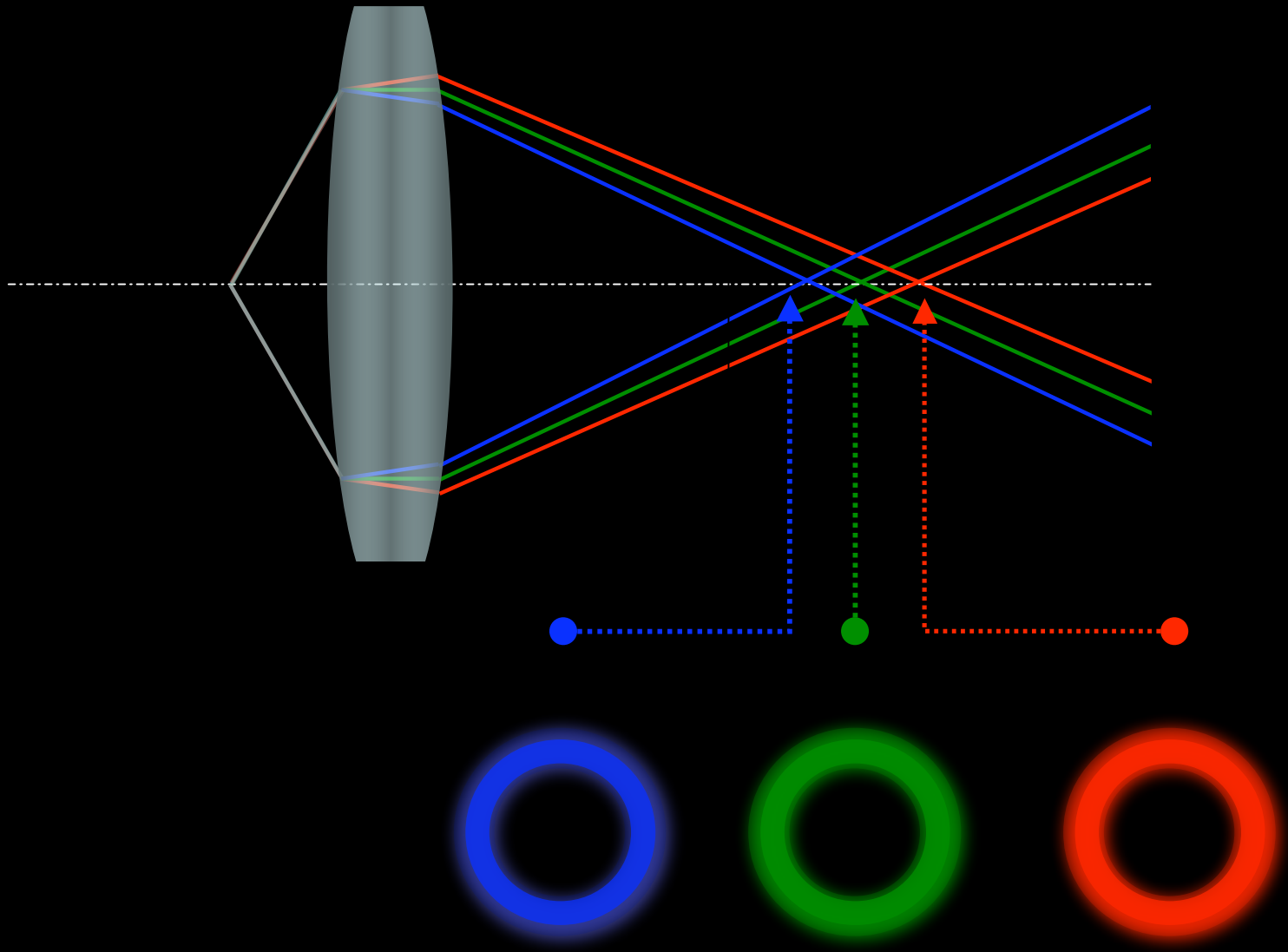


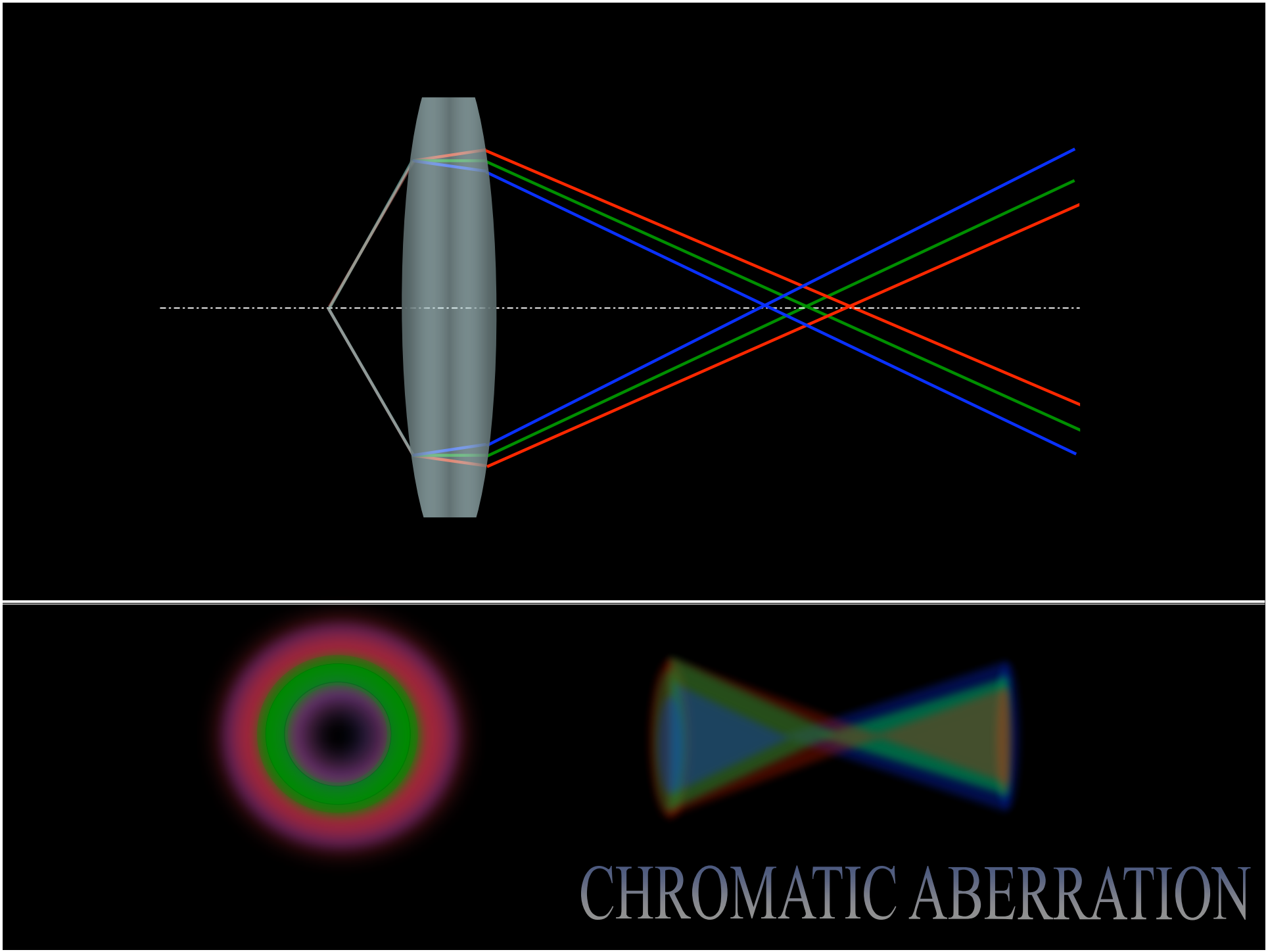
Spheric Aberration Correction

Spheric Aberration

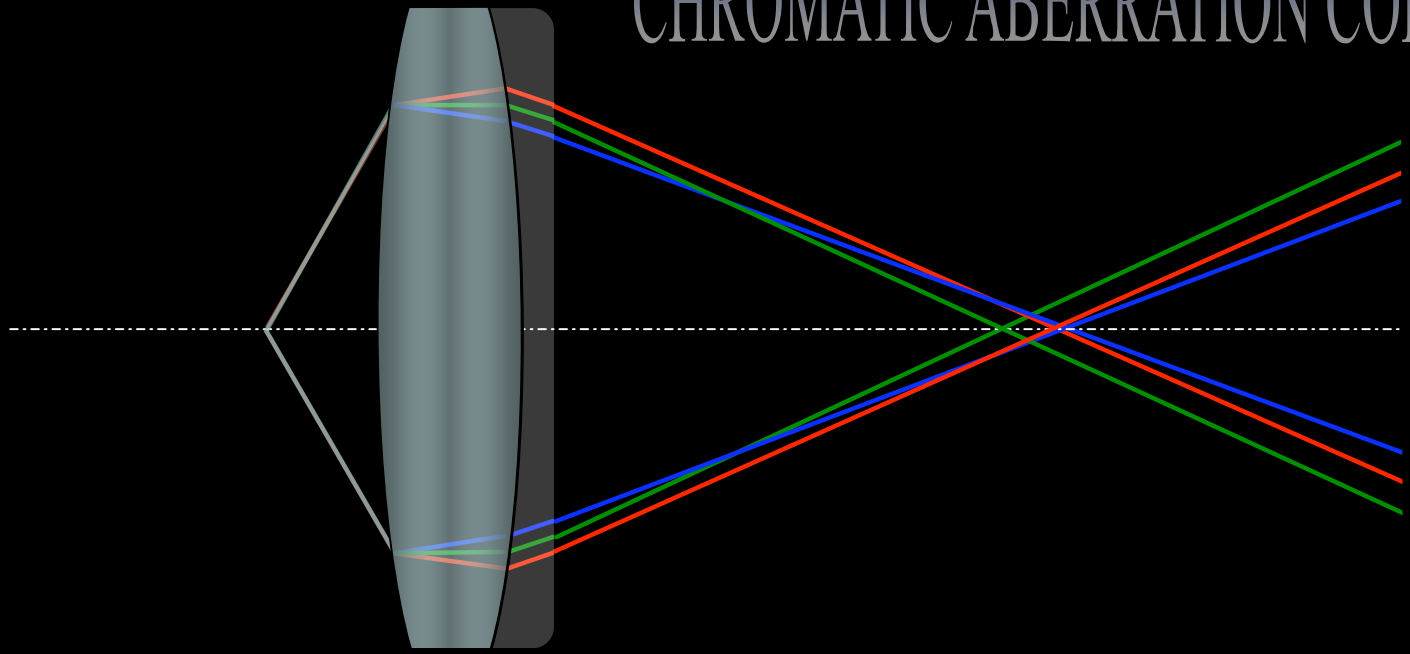


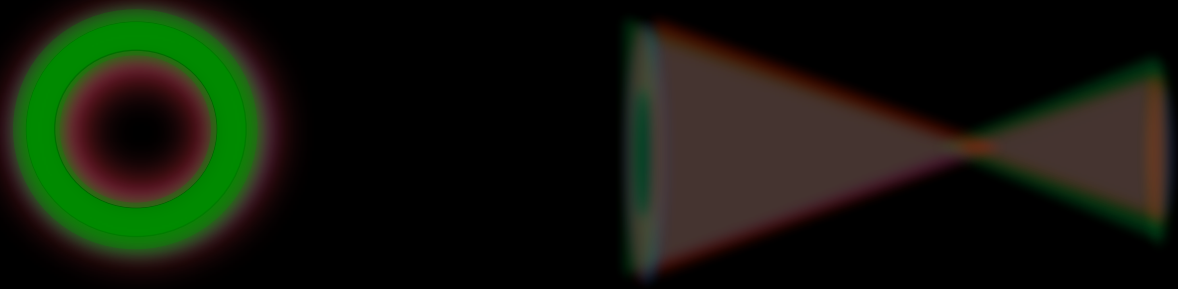
CHROMATIC ABERRATION

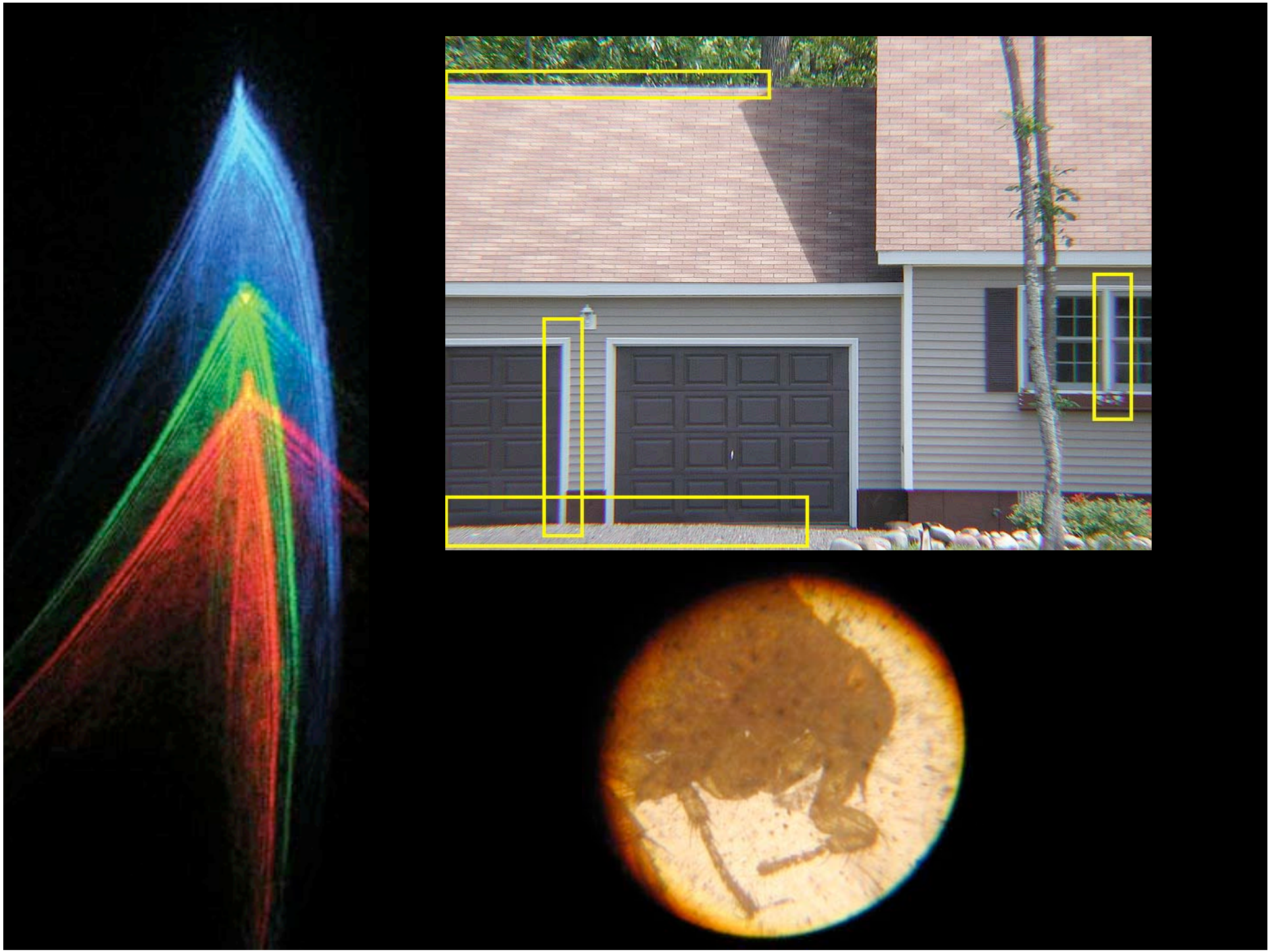


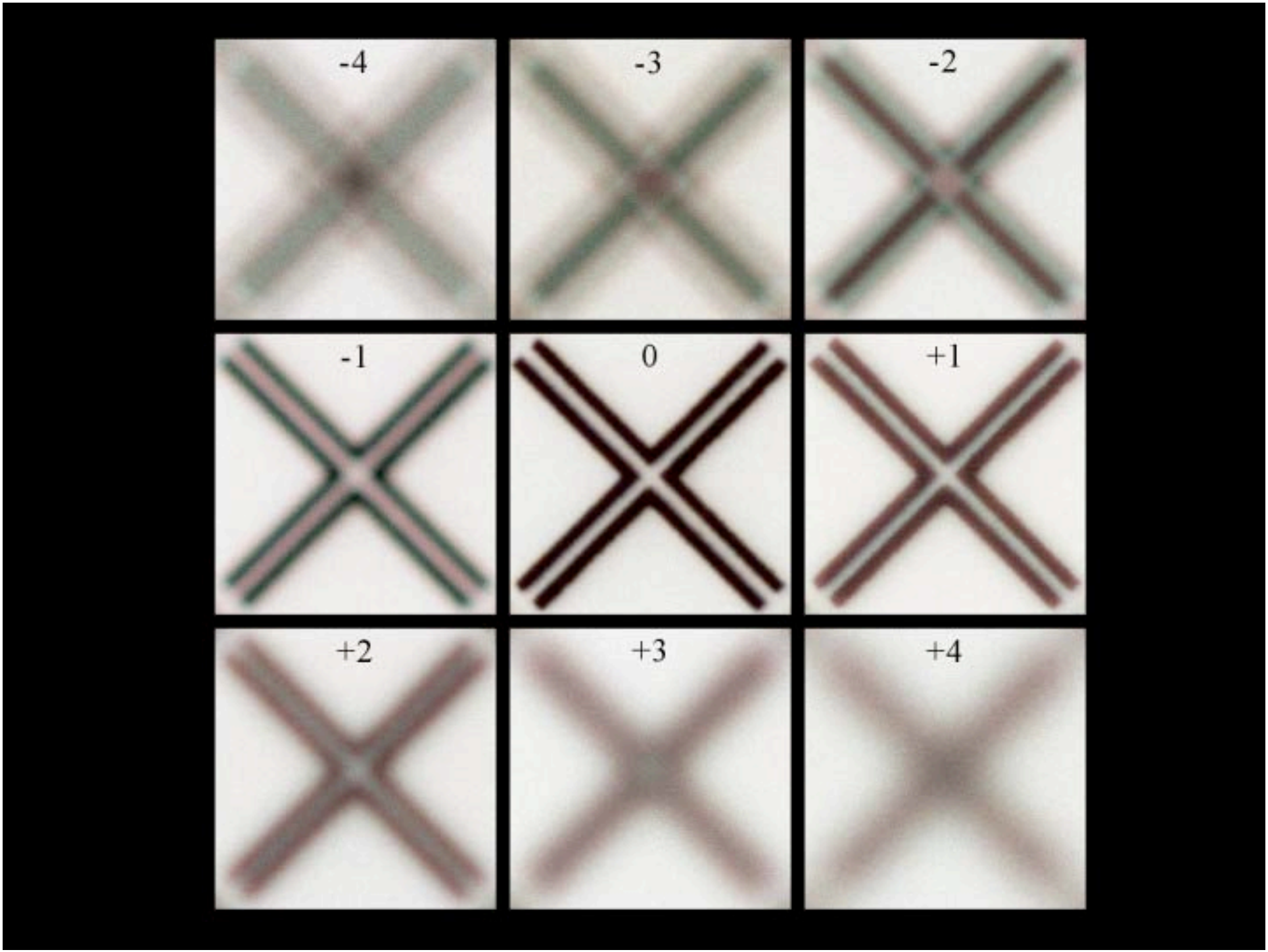

CHROMATIC ABERRATION

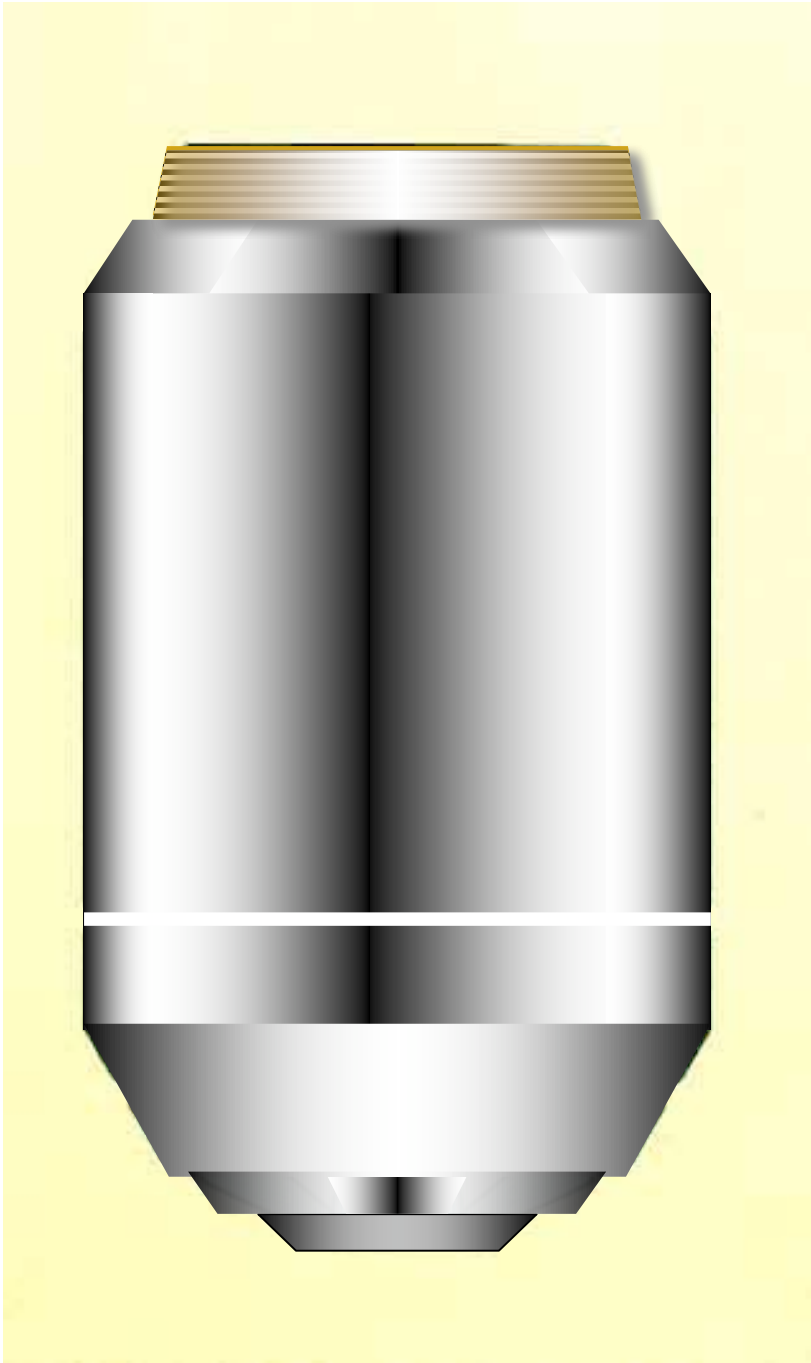
CHROMATIC ABERRATION CORRECTION







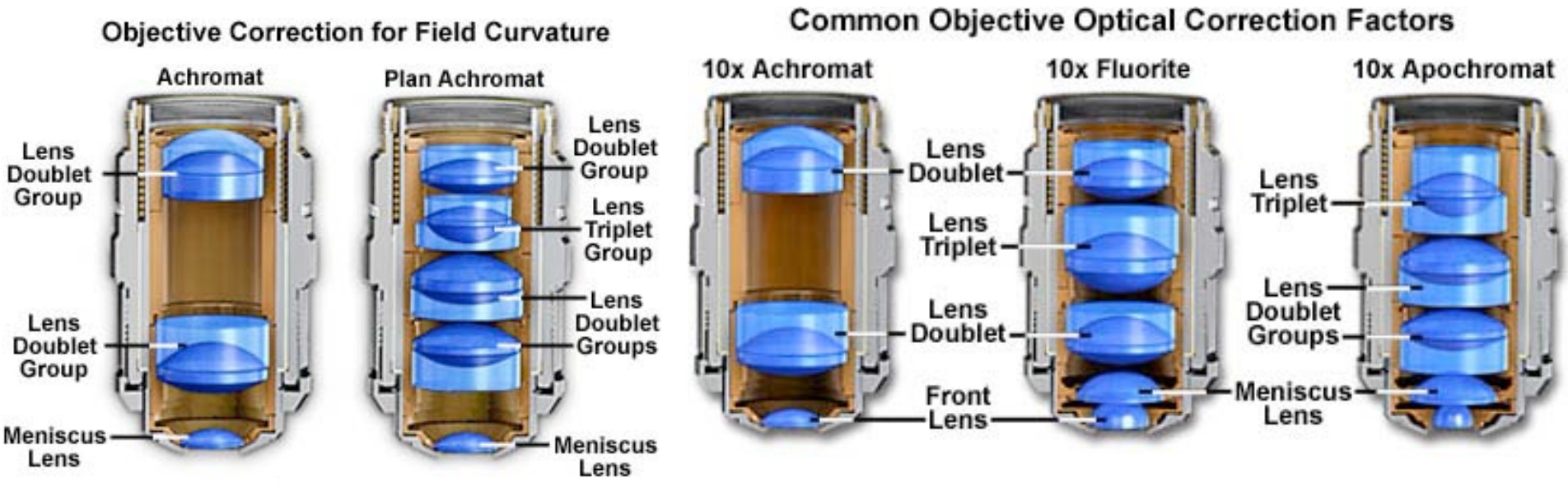

-4
-3
-2
-1
0
+1
+2
+3
+4



The objective lens

Is the microscope

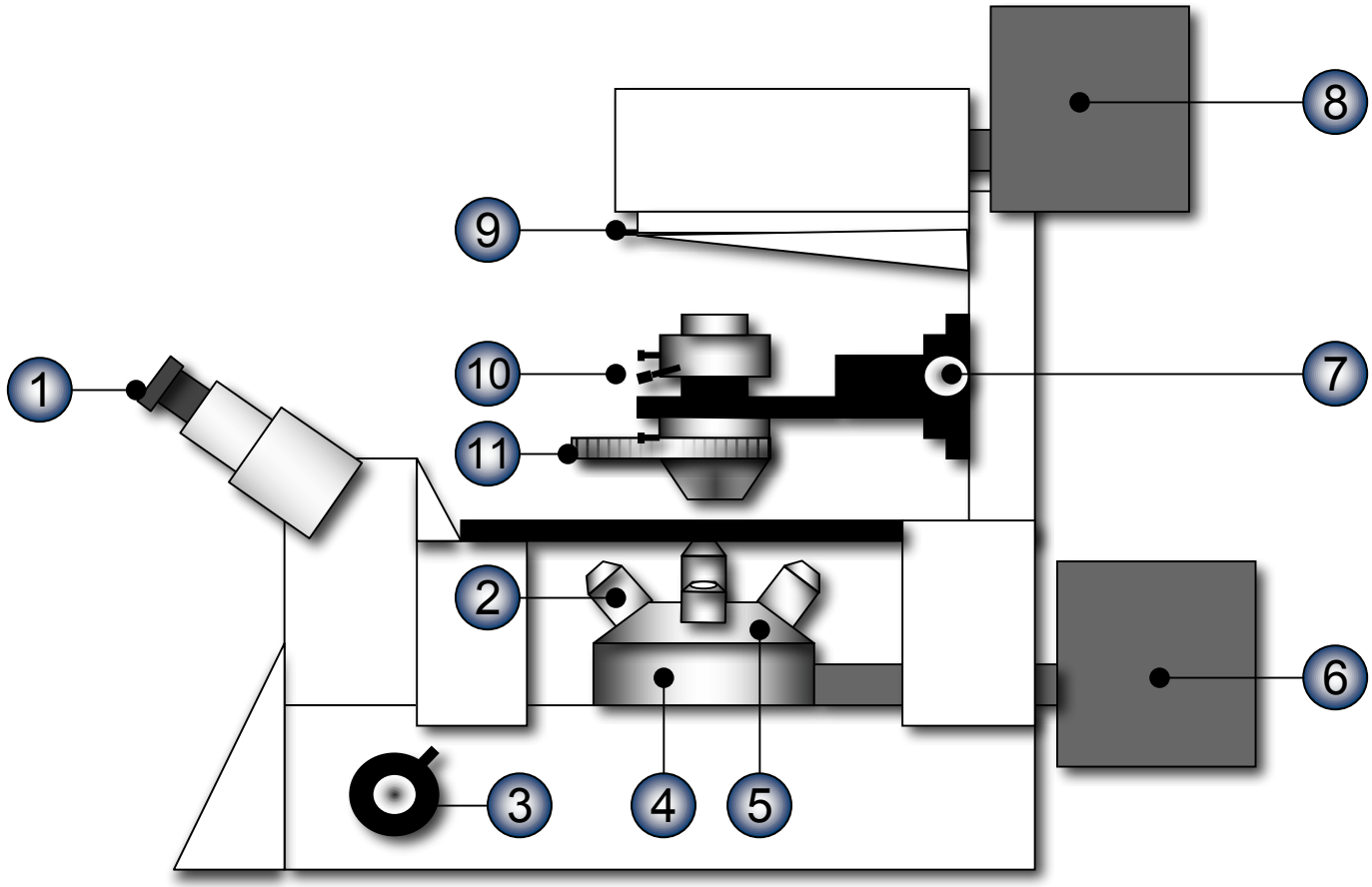
The other parts support its function
and adapt the image
to the receiving device



Objective Correction for Optical Aberration

Objective Type	Spherical Aberration	Chromatic Aberration	Field Curvature
Achromat	1 Color	2 Colors	No
Plan Achromat	1 Color	2 Colors	Yes
Fluorite	2-3 Colors	2-3 Colors	No
Plan Fluorite	3-4 Colors	2-4 Colors	Yes
Plan Apochromat	3-4 Colors	4-5 Colors	Yes

micro.magnet.fsu.edu/.../anatomy/objectives.html



1 Eyepieces

2 Objectives

3 Focusing Knob

4 Filter wheel

5 Revolver

6 Lamp house (Halogen lamp)

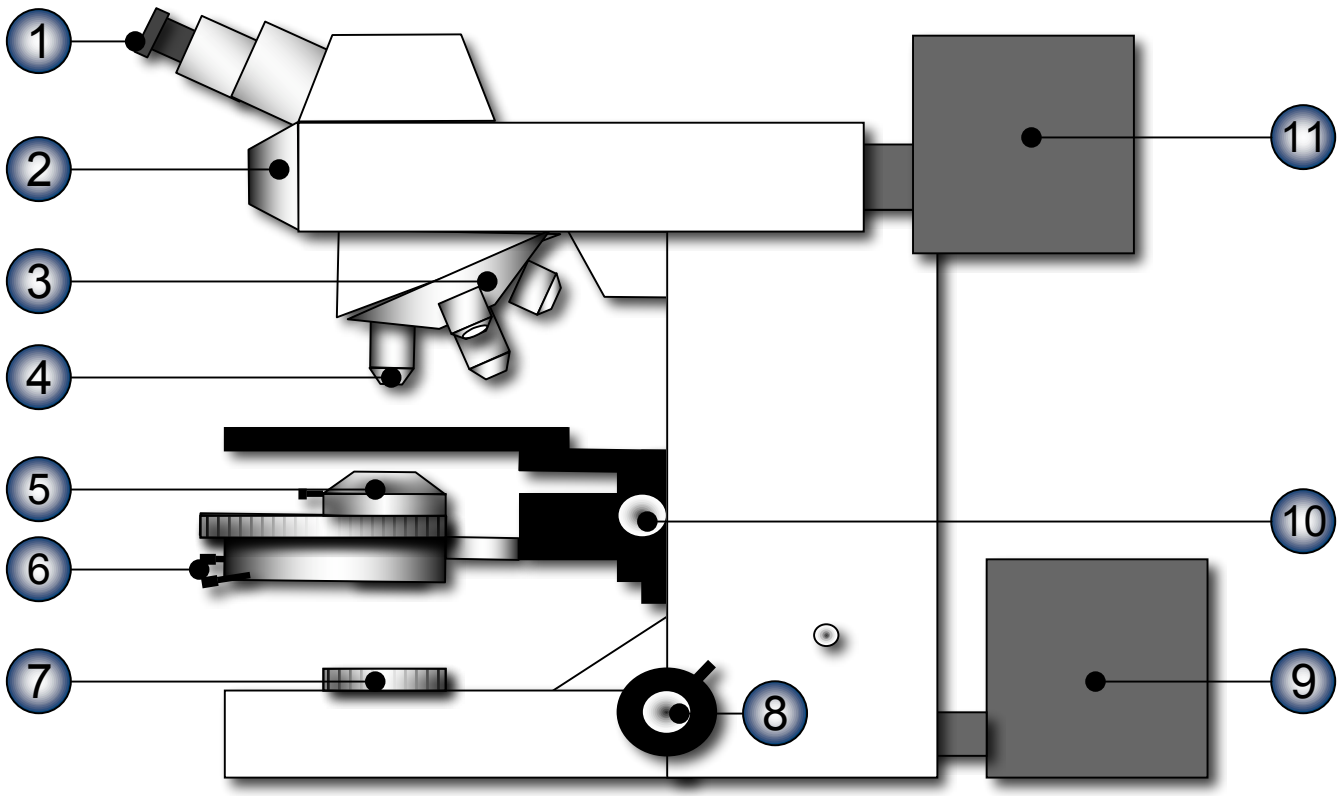
7 Condenser adjusting knob

8 Lamp house (Mercury lamp)

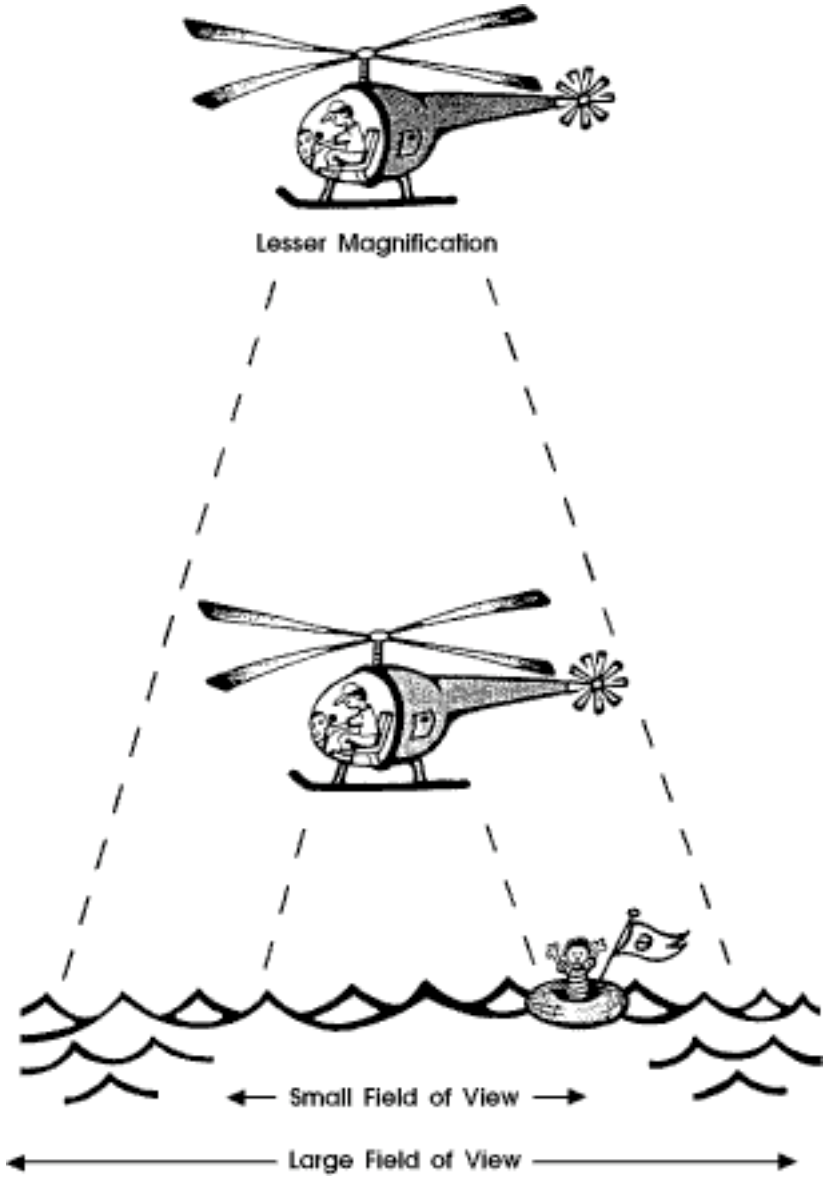
9 Field Diaphragm

10 Condenser Diaphragm

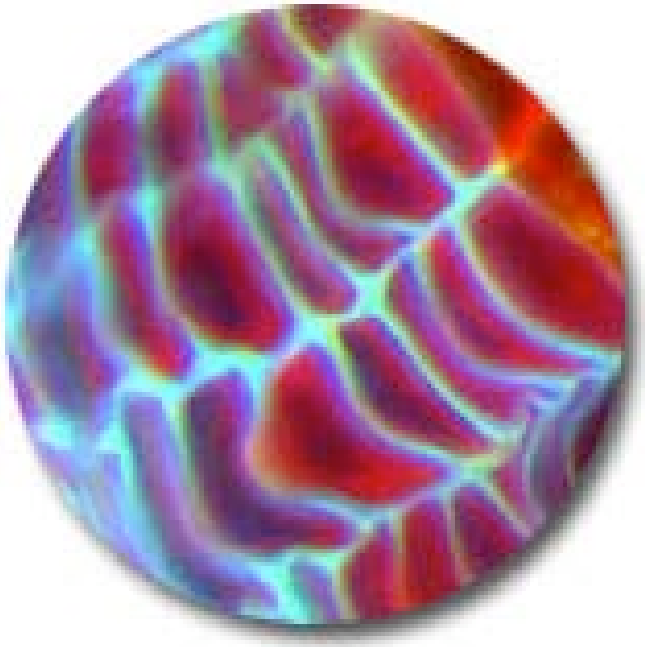
11 Condenser



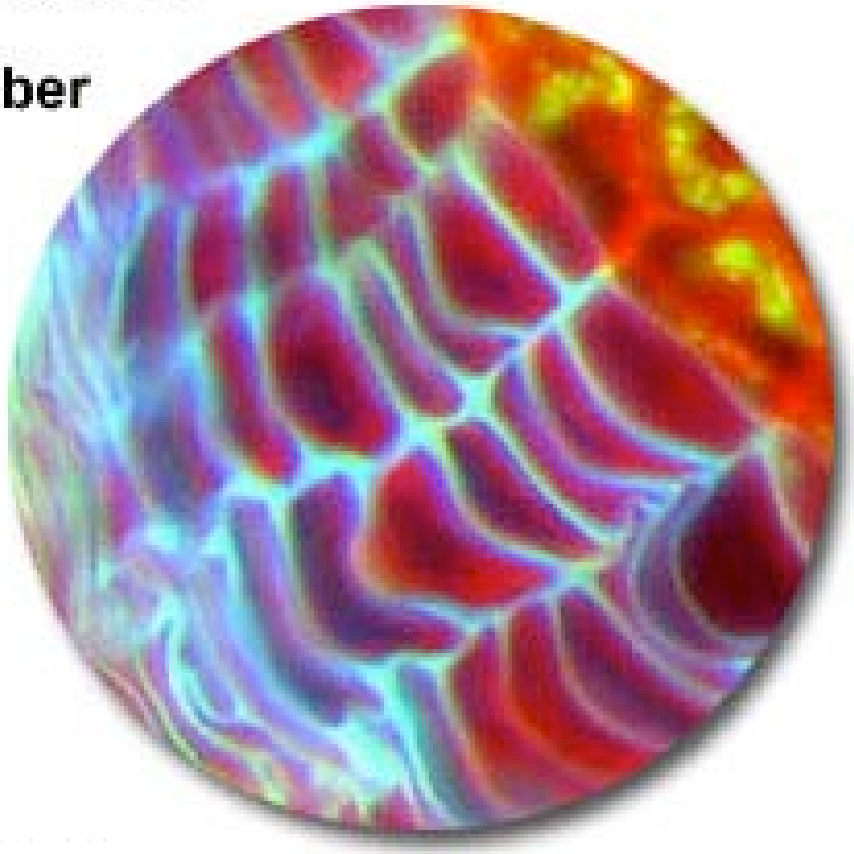
1. Eyepieces
2. Filter wheel
3. Revolver
4. Objectives
5. Condenser
6. Condenser Diaphragm
7. Field Diaphragm
8. Focusing Knob
9. Lamp house (Halogen lamp)
10. Condenser adjusting knob
11. Lamp house (Mercury lamp)


Lesser Magnification
Small Field of View
Large Field of View

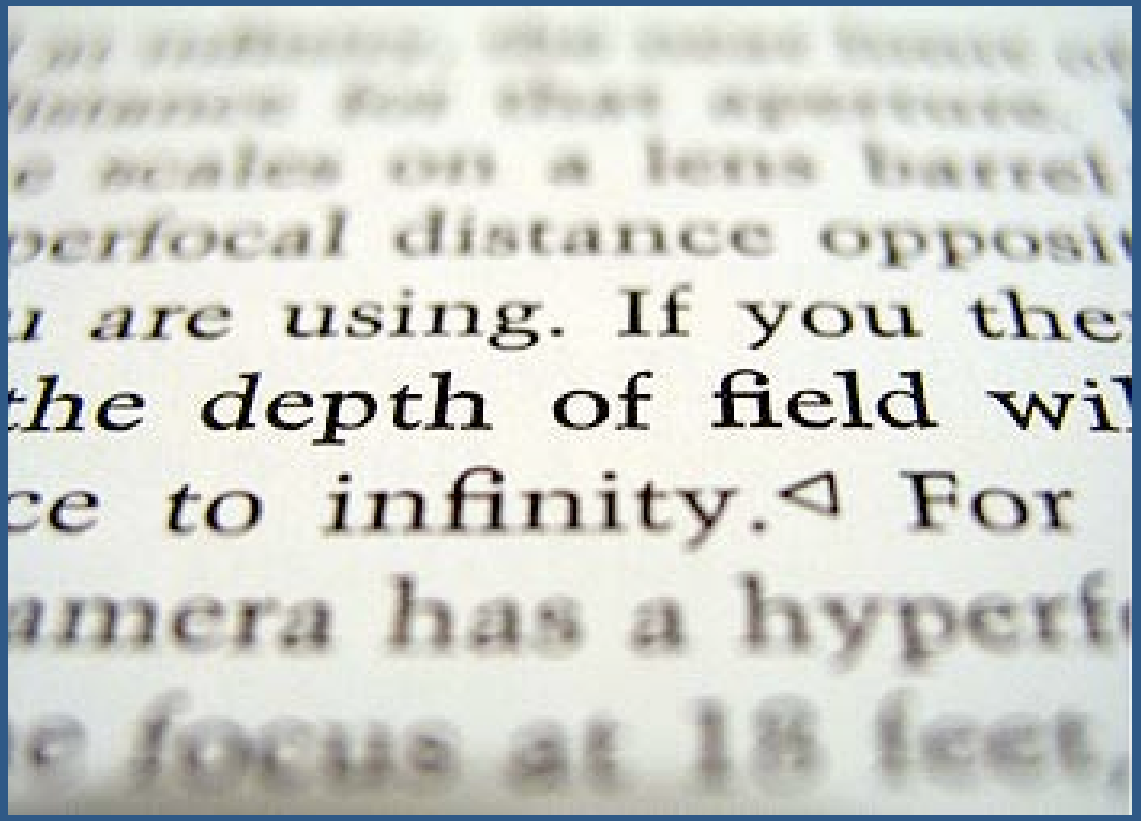
Field Size Variations with Field Number

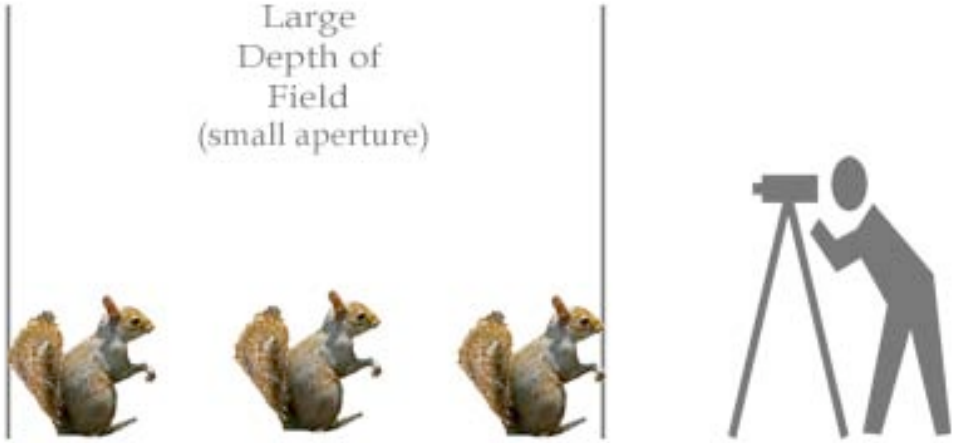


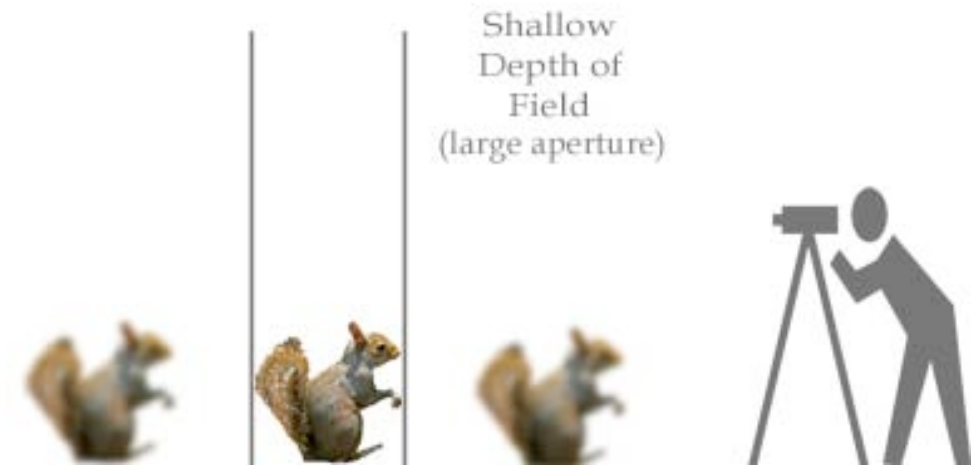
Field Number = 20

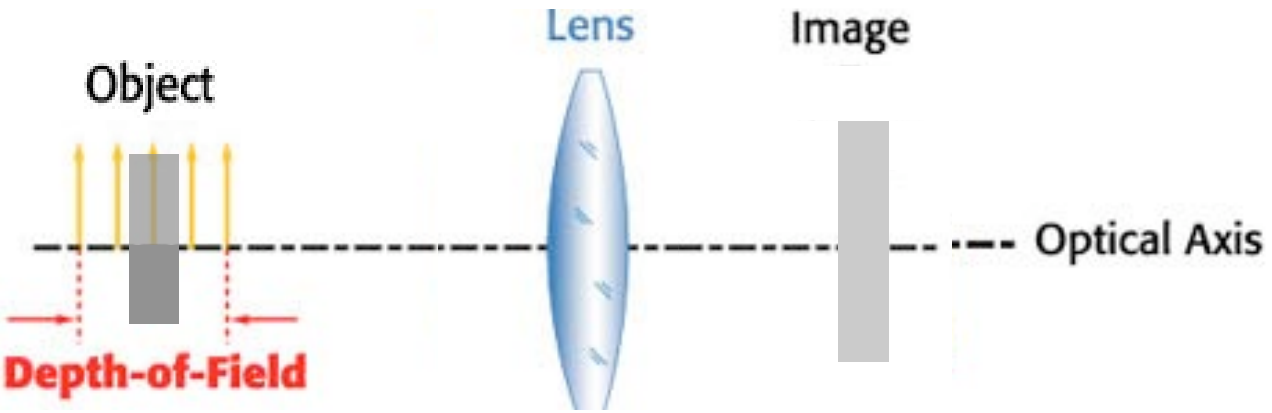


Field Number = 26


perfocal distance
are using. If you
the depth of field
ce to infinity. For
amera has a


Large
Depth of
Field
(small aperture)


Shallow
Depth of
Field
(large aperture)


Lens
Image
Object
Optical Axis
Depth-of-Field


Illumination!!

Microscope Illumination

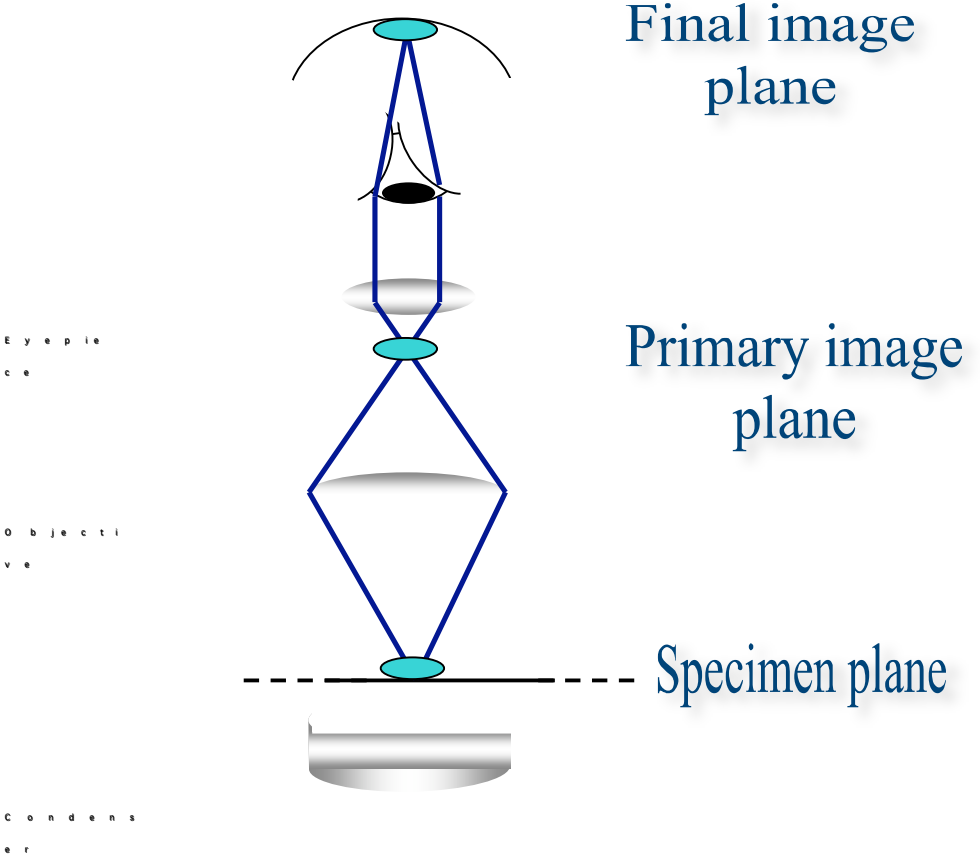
Two basic methods of illumination:

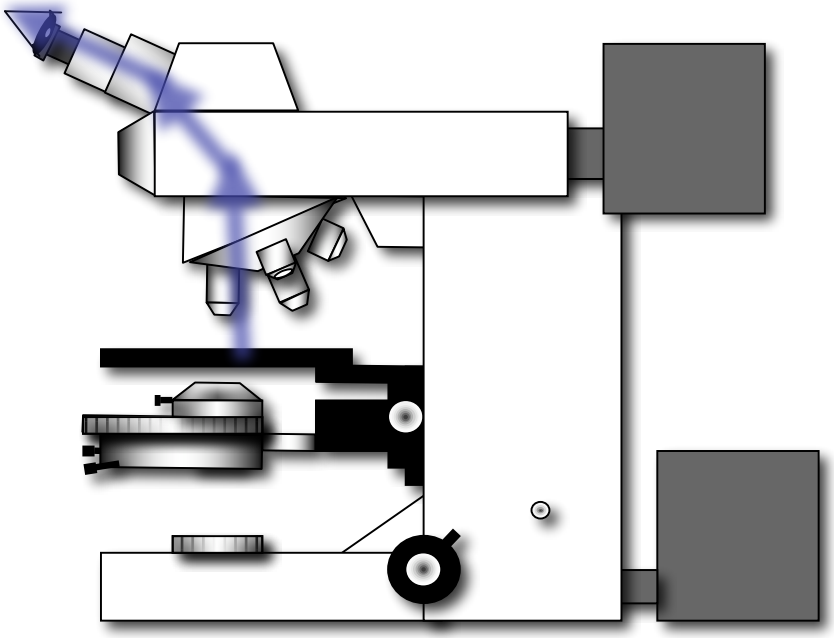
Source-focused or ‘Critical’ Illumination:

Light-source imaged on to specimen

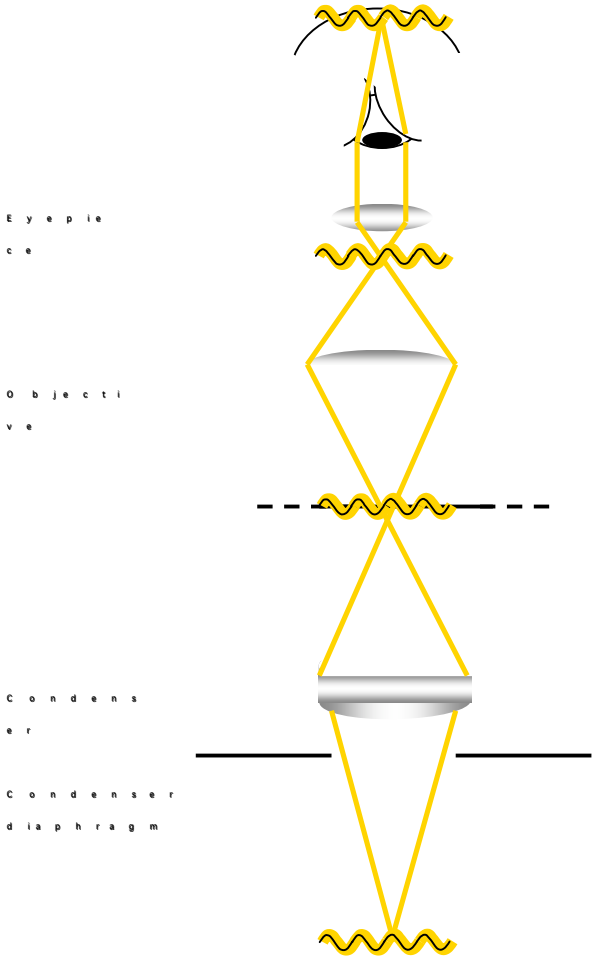
Köhler Illumination:

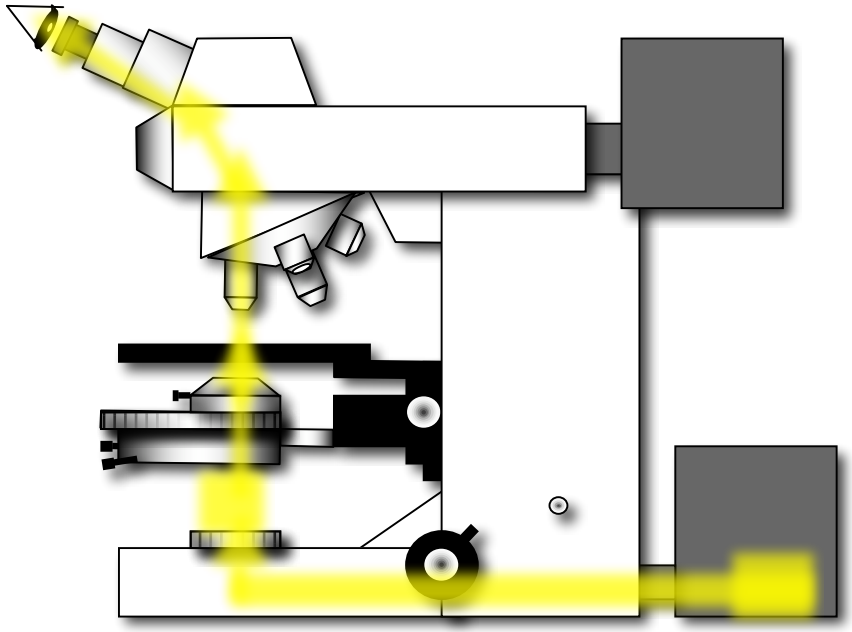
Light-source imaged in the aperture of the condenser

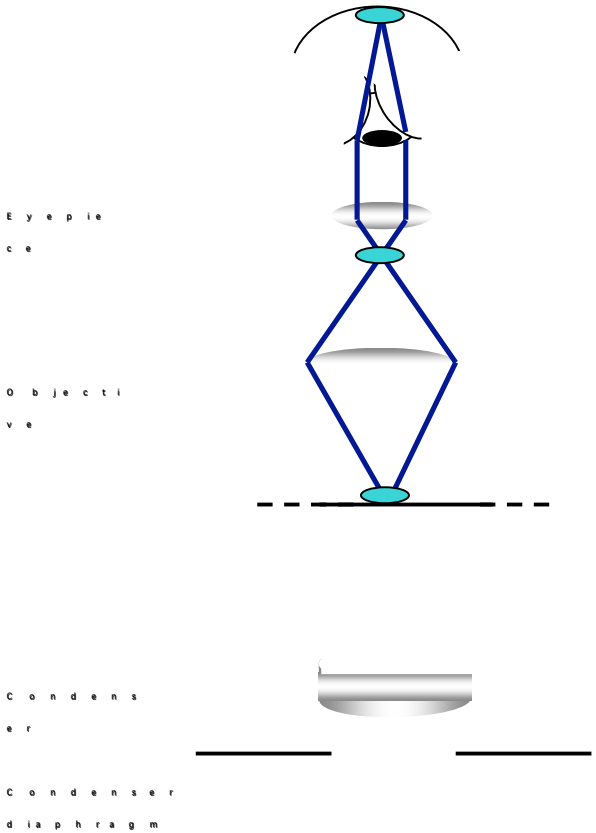


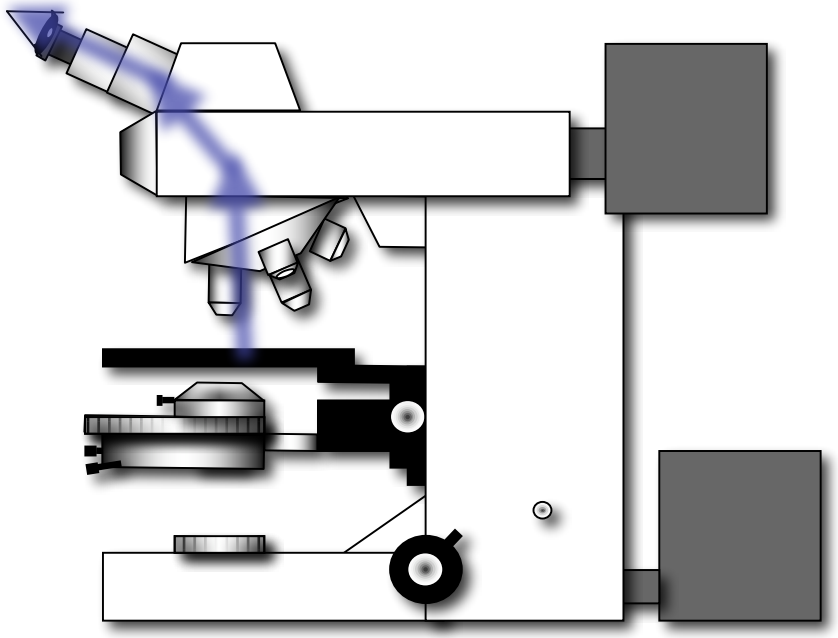


What are conjugated planes?

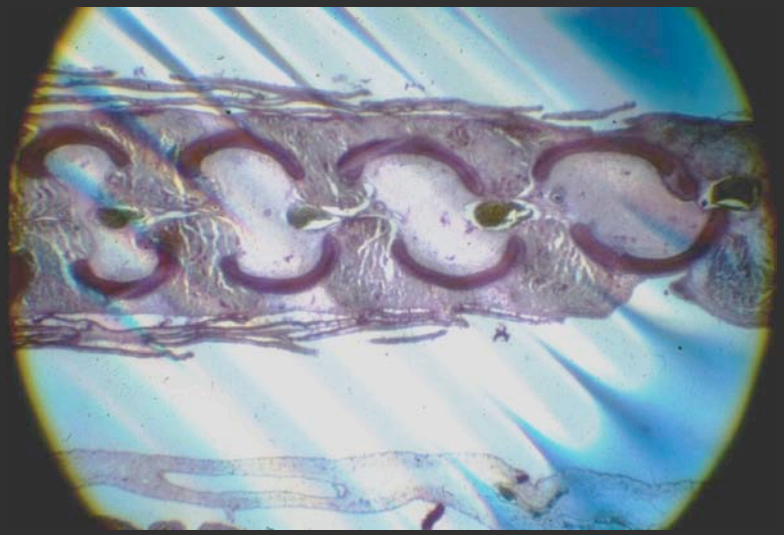

Eyepiece
Objective
Condenser
Condenser diaphragm

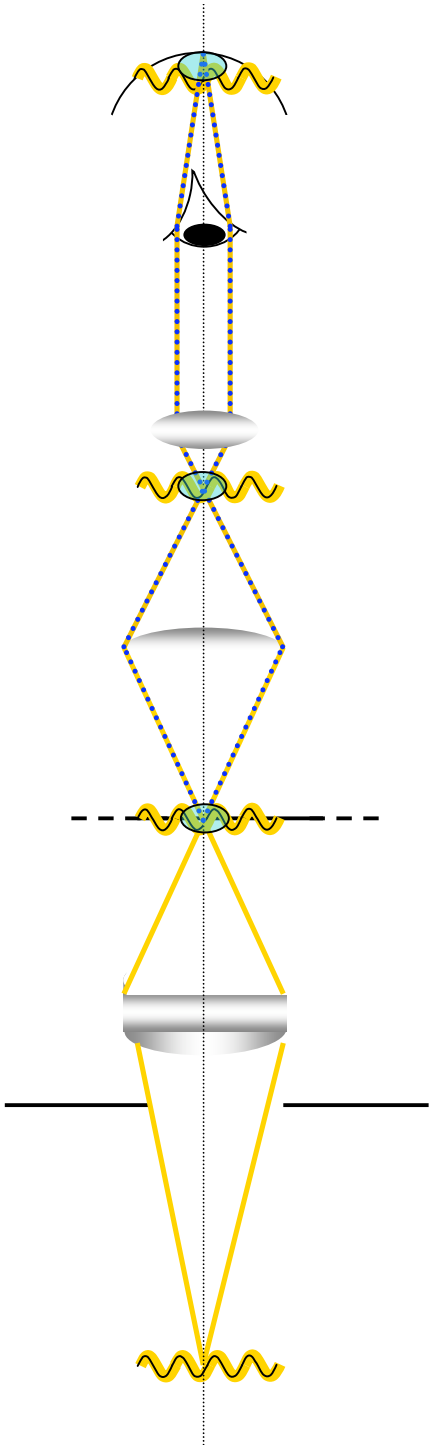


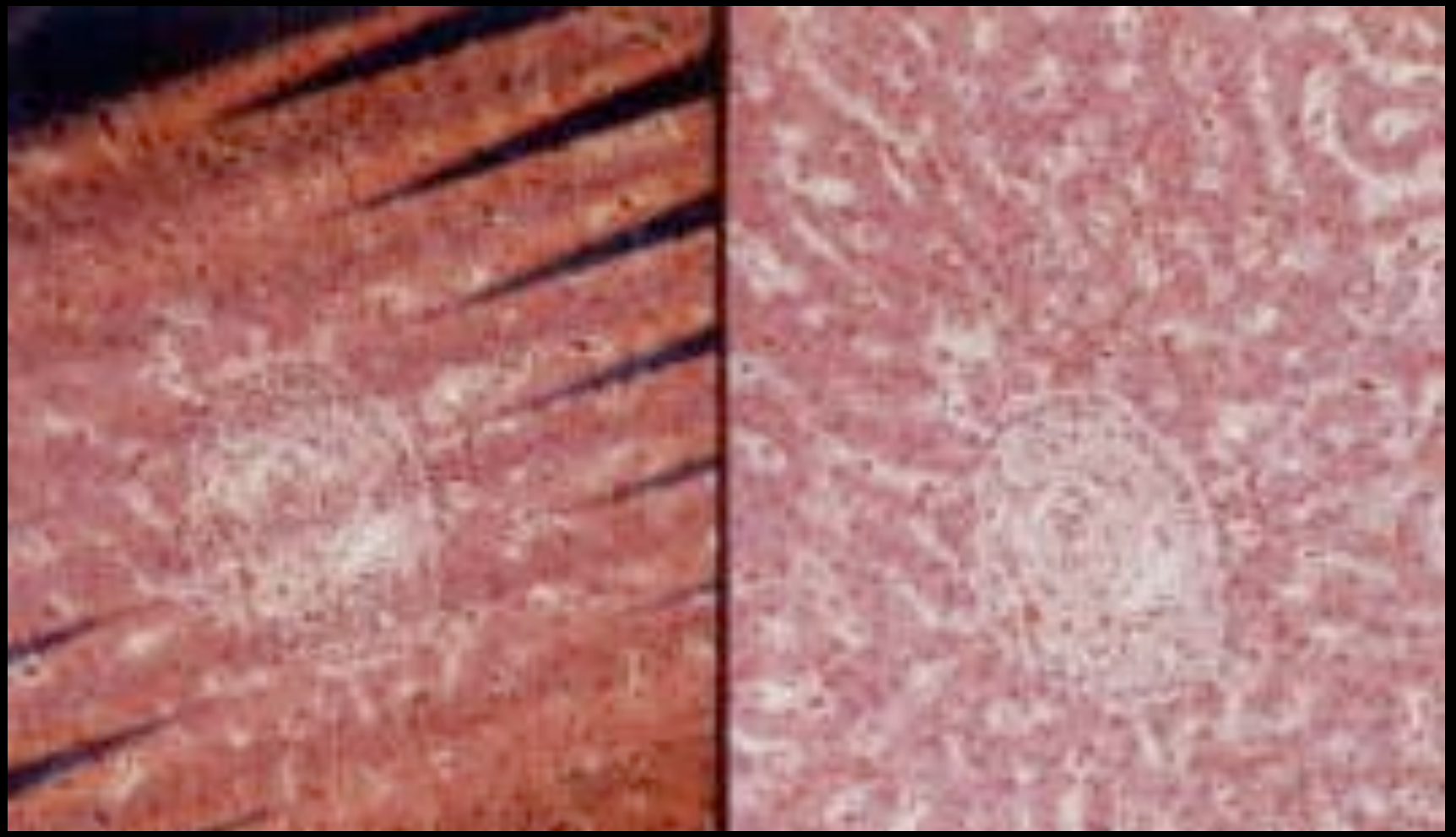

Eyepiece
Objective
Condenser
Condenser diaphragm



C R I T I C A L I L L U M I N A T I O N







August Köhler

1866 - 1948

Published

A new system of illumination for photomicrographic purposes

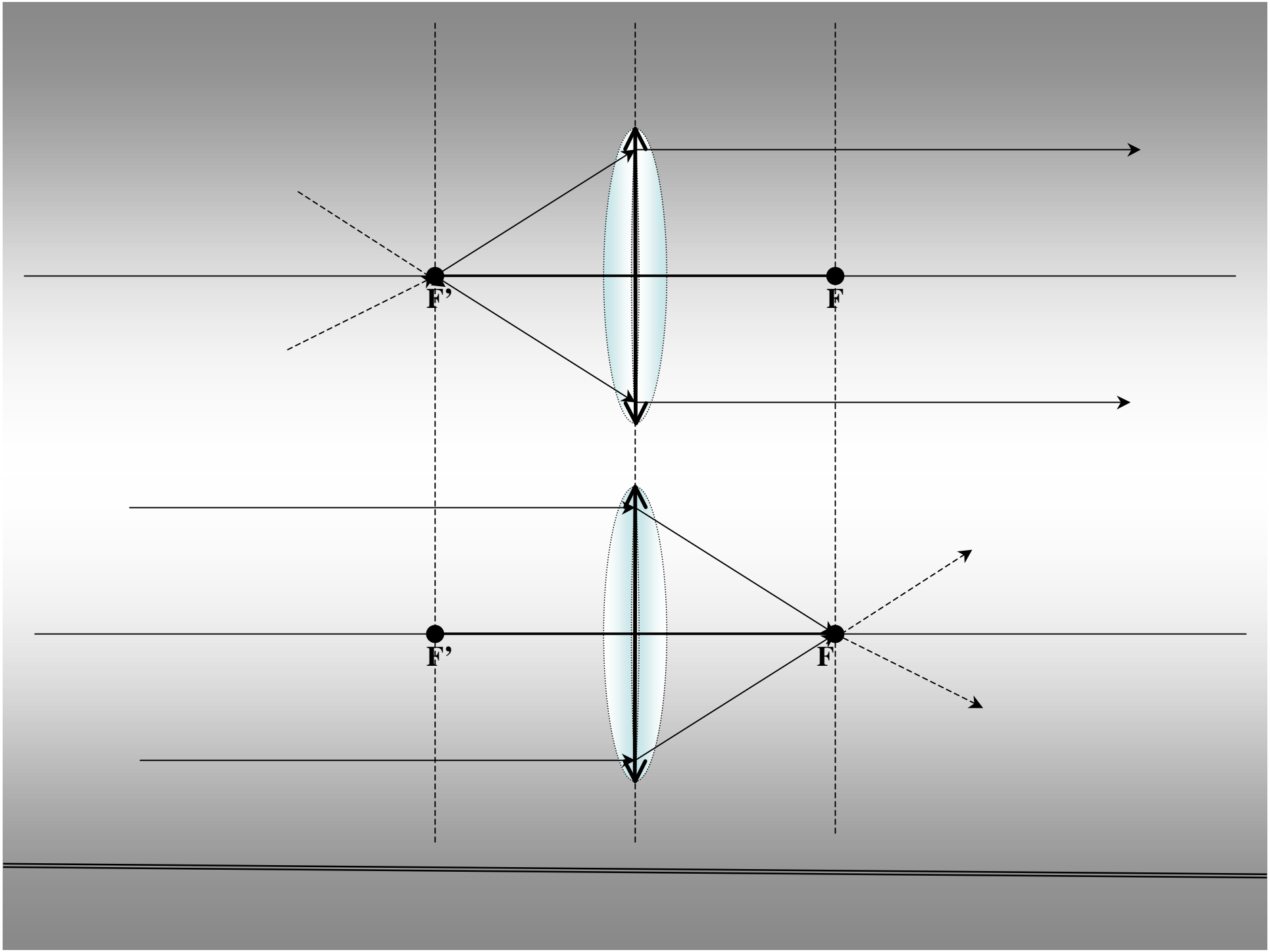
(in German) in 1893.

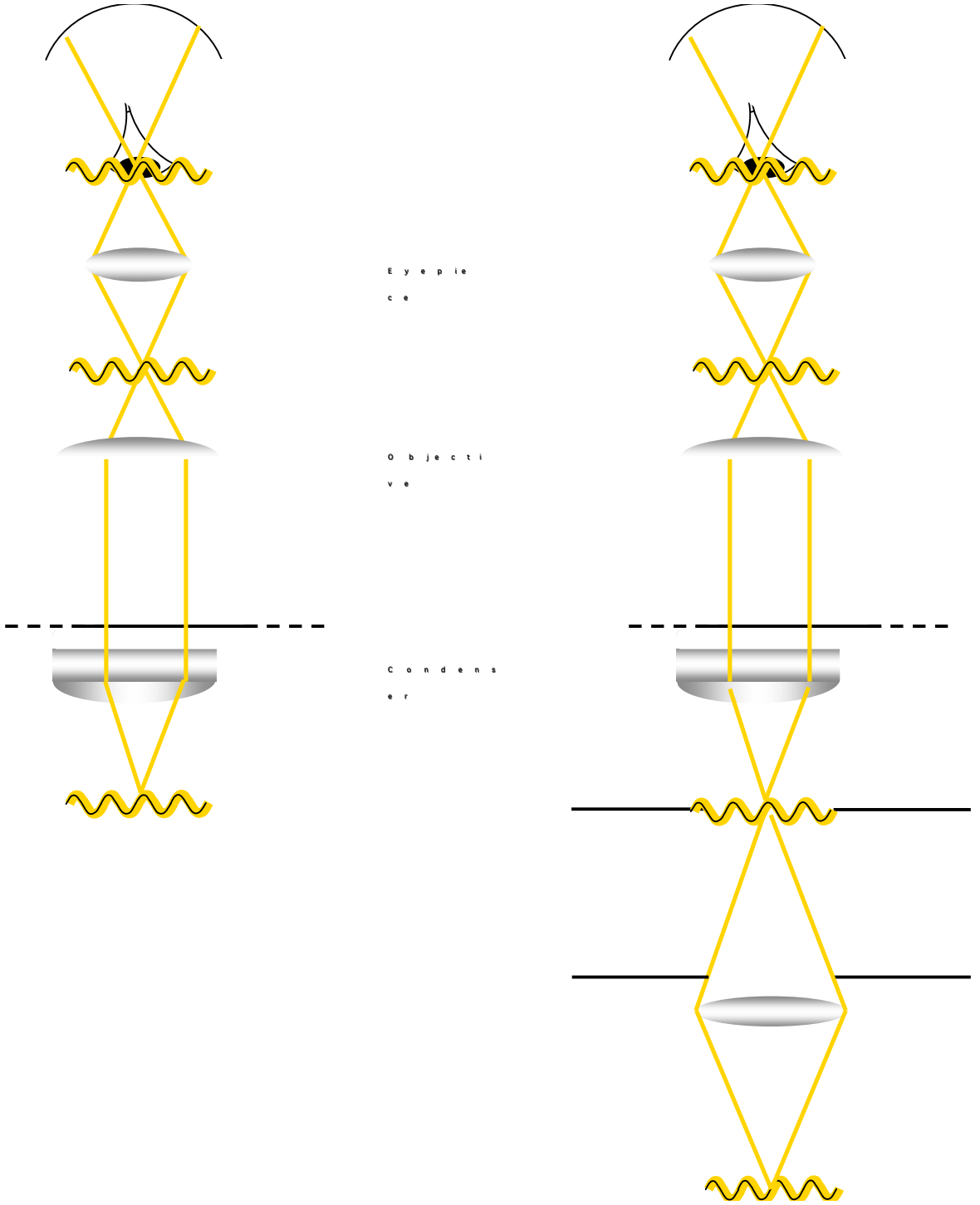
Köhler illumination

Köhler alignment

Microscope alignment

Did you.....Köhler??


F'
F
F'
F


Eyepie
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Condens
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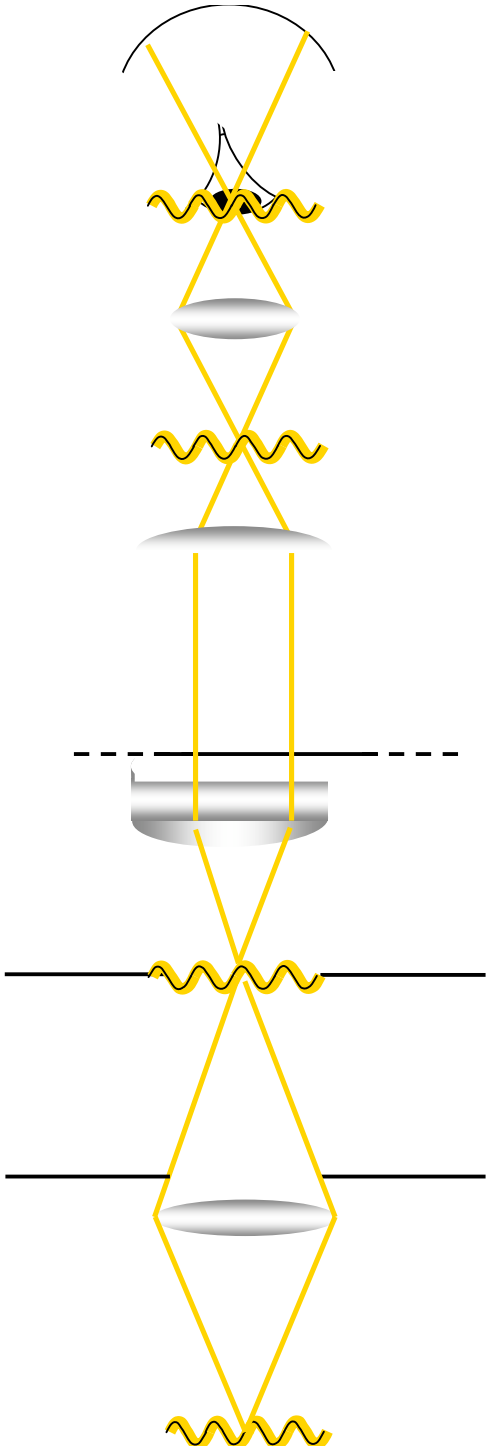
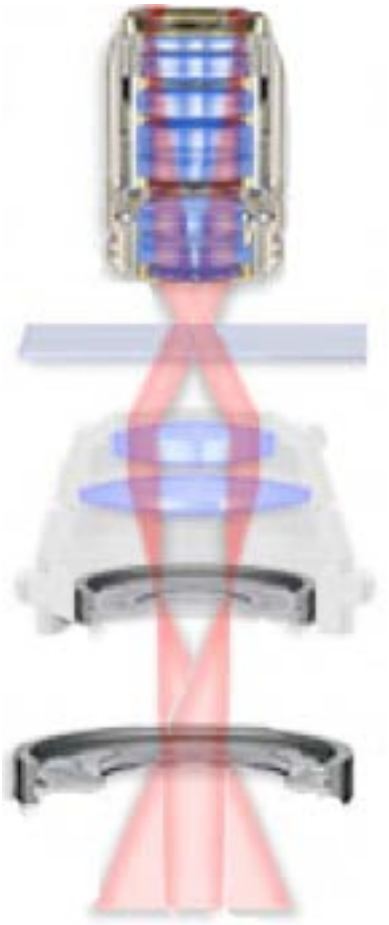
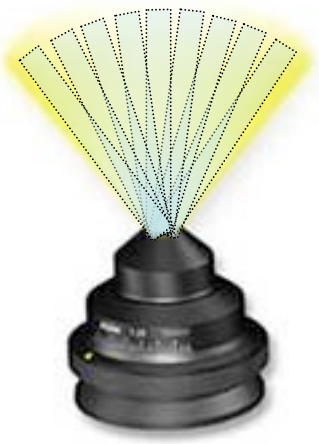
Light
source
Light
source

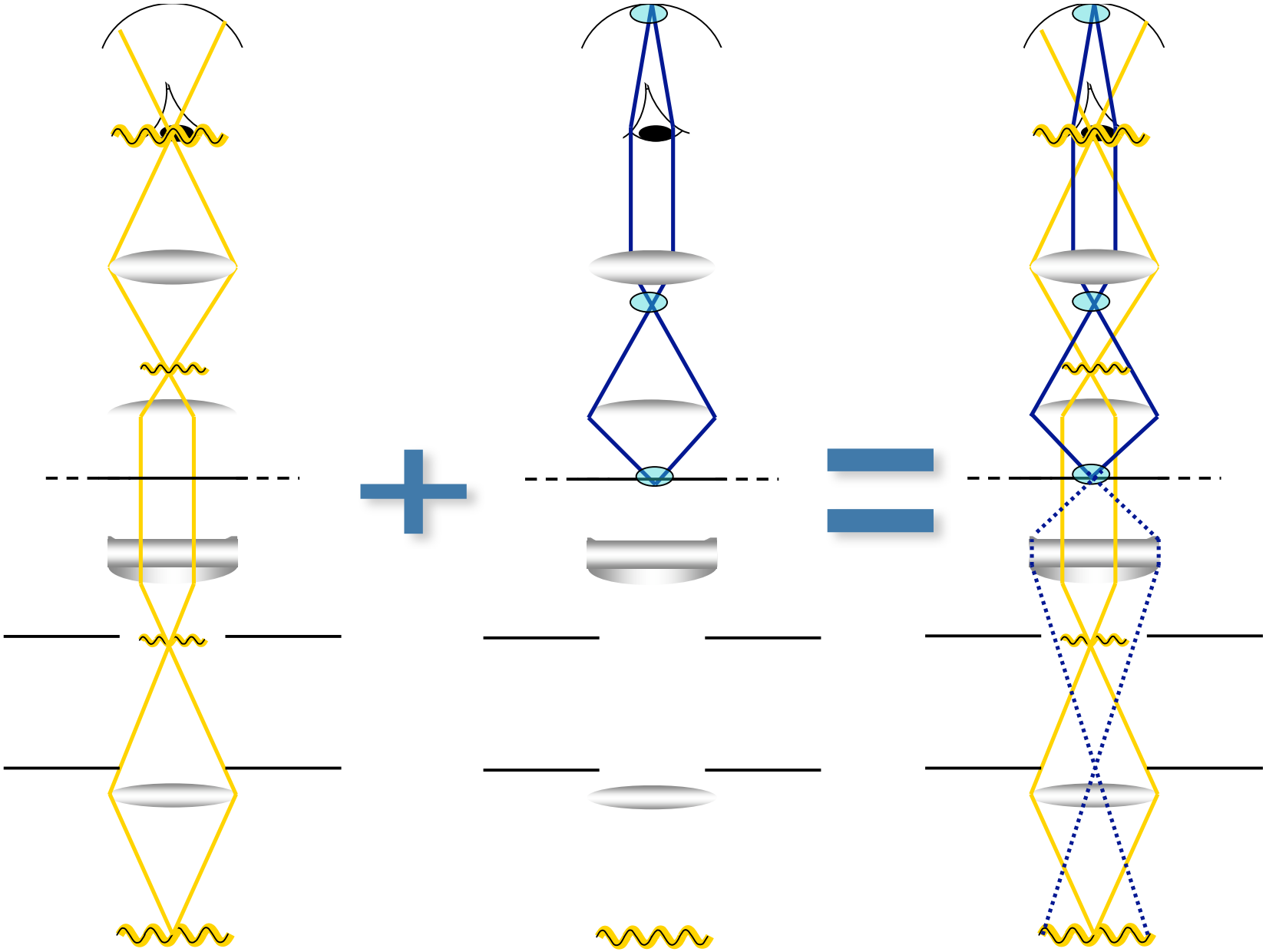


OPTIONAL (JUST IN CASE)

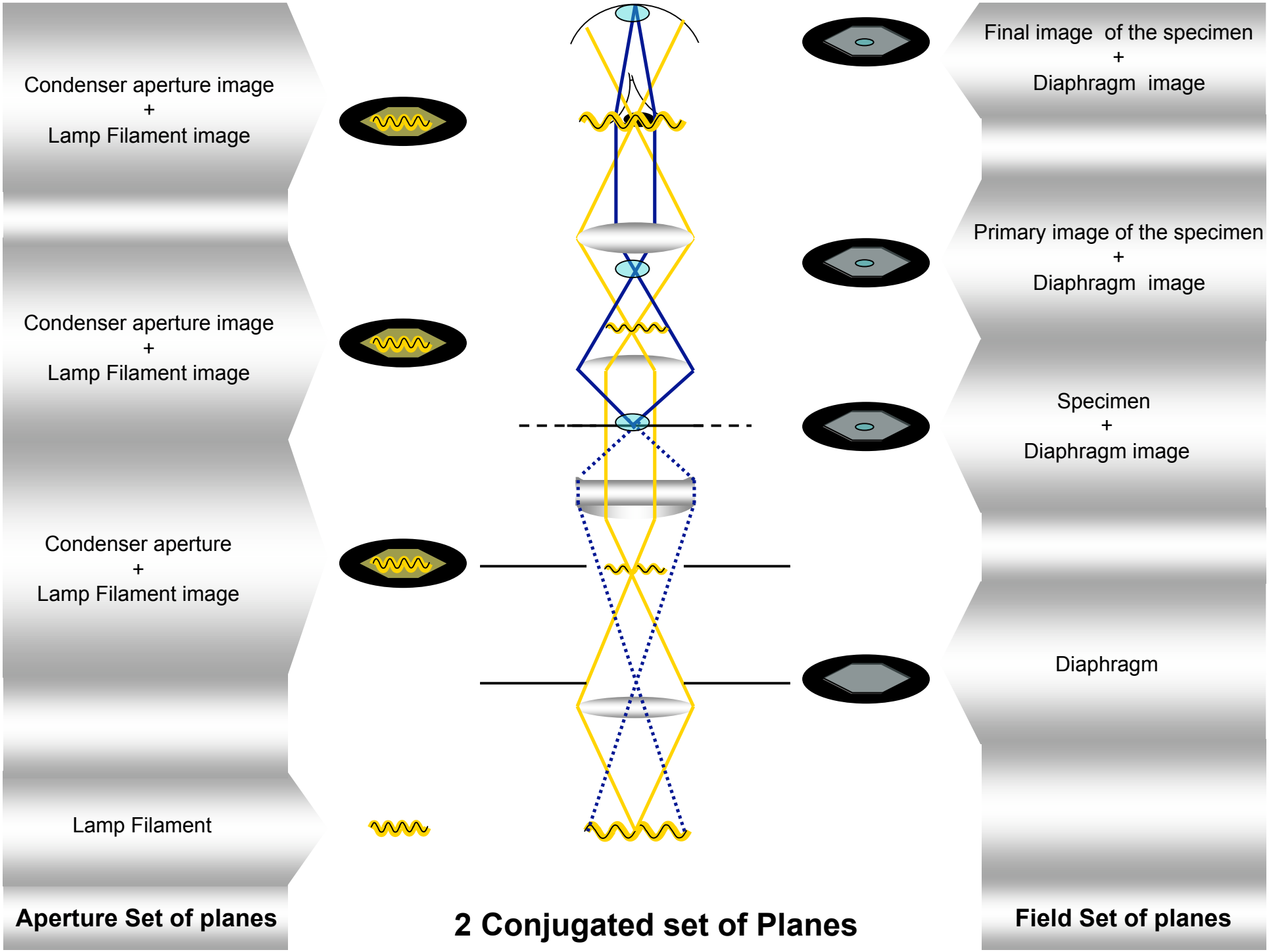
Light up the *specimen* uniformly

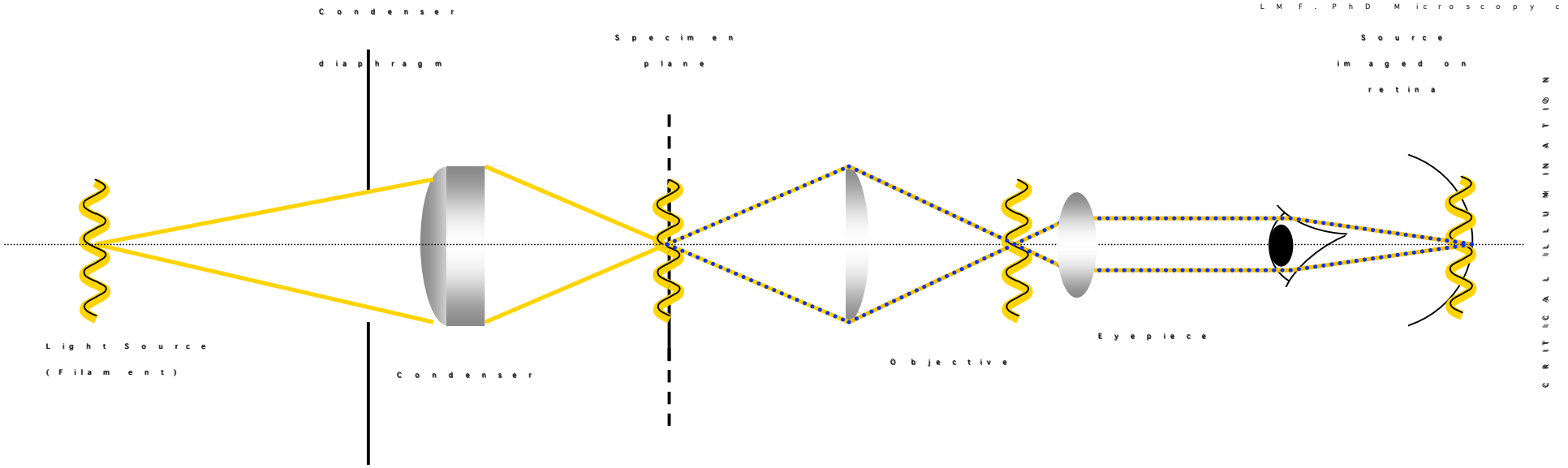


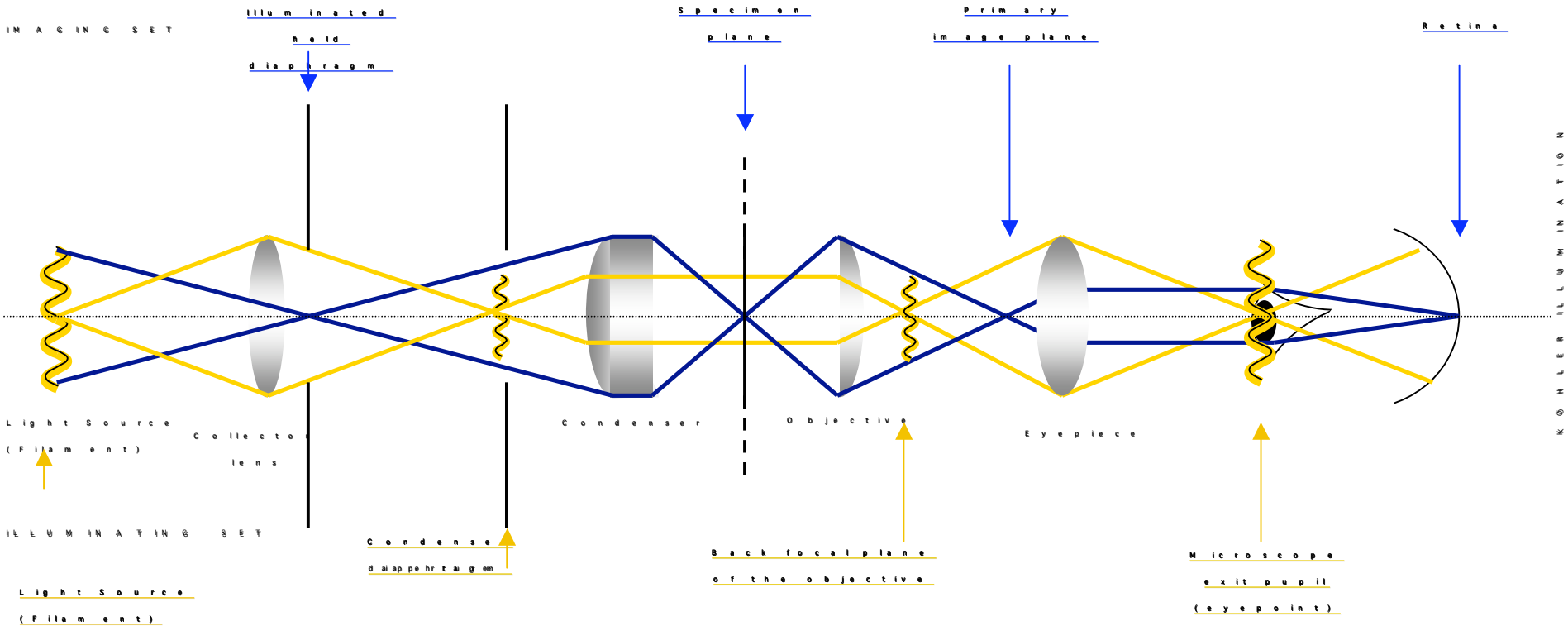




Set of “aperture” Conjugated planes + Set of “field” Conjugated planes = 2 Sets of conjugated Planes


Condenser aperture image
+
Lamp Filament image
Condenser aperture image
+
Lamp Filament image
Condenser aperture
+
Lamp Filament image
Lamp Filament
Aperture Set of planes
Final image of the specimen
+
Diaphragm image
Primary image of the specimen
+
Diaphragm image
Specimen
+
Diaphragm image
Diaphragm
Field Set of planes
2 Conjugated set of Planes


Condenser
diaphragm
Specimen
plane
Source
imaged on
retina
CRITICAL ILLUMINATION
Light Source
(Filament)
Condenser
Objective
Eyepiece

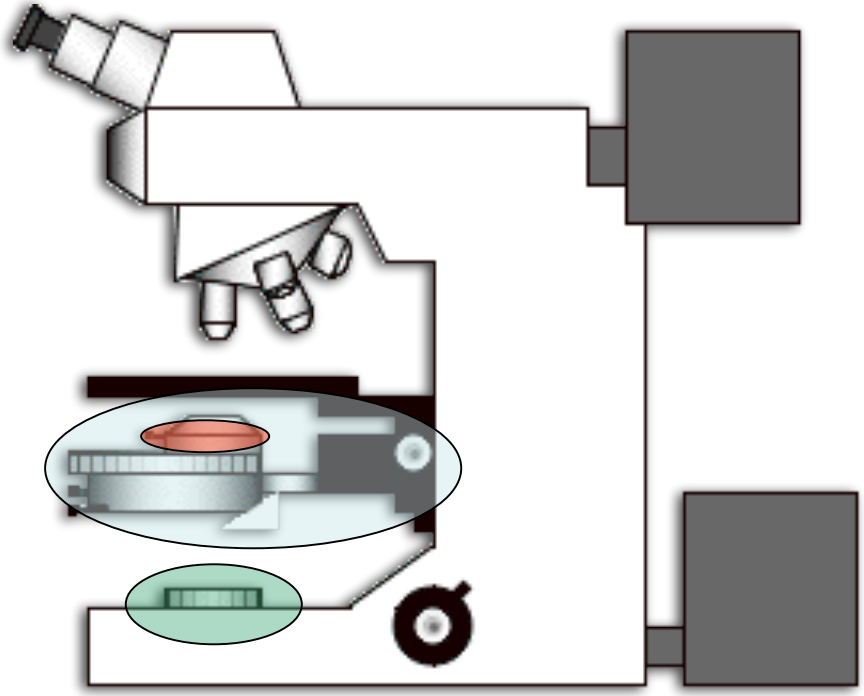

IMAGING SET
Illuminated
field
diaphragm
Specimen
plane
Primary
image plane
Retina
KÖHLER ILLUMINATION
Light Source
(Filament)
Collector
lens
ILLUMINATING SET
Condenser
Objective
Eyepiece
Condenser
diaphragm
Back focal plane
of the objective
Microscope
exit pupil
(eyepoint)
Light Source
(Filament)

What are we trying to do when illuminating a microscopical specimen??

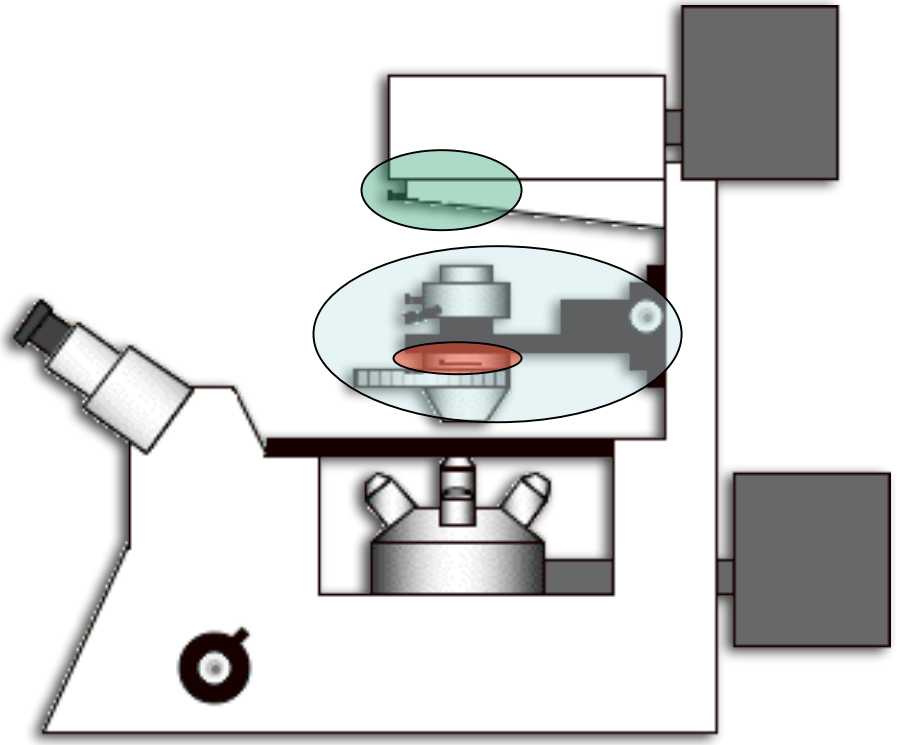
- Light up the *specimen* uniformly
  - over a controllable *area*
- Illuminate the *objective aperture* uniformly
  - over a controllable *angle*

From Peter Evennett

UPRIGHT MICROSCOPE



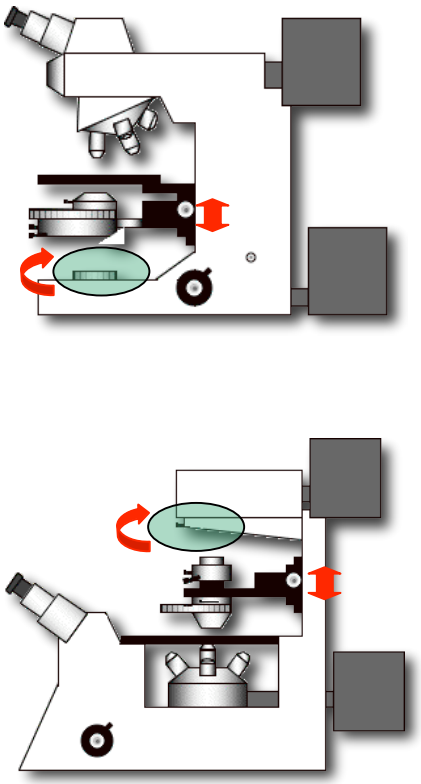
INVERTED MICROSCOPE



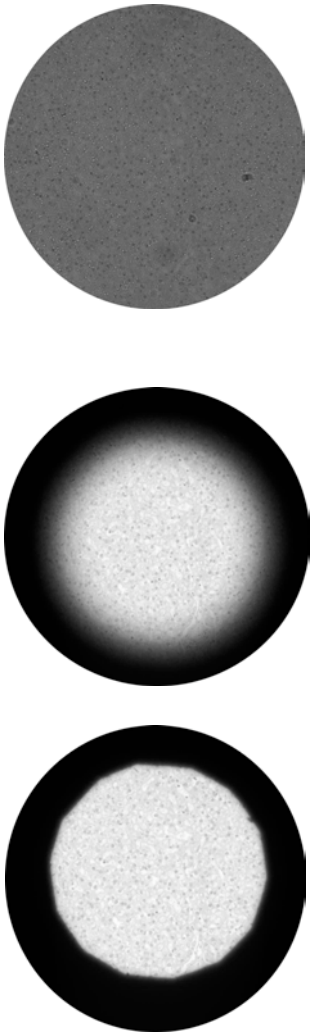
Condenser

Field Diaphragm. (FD)

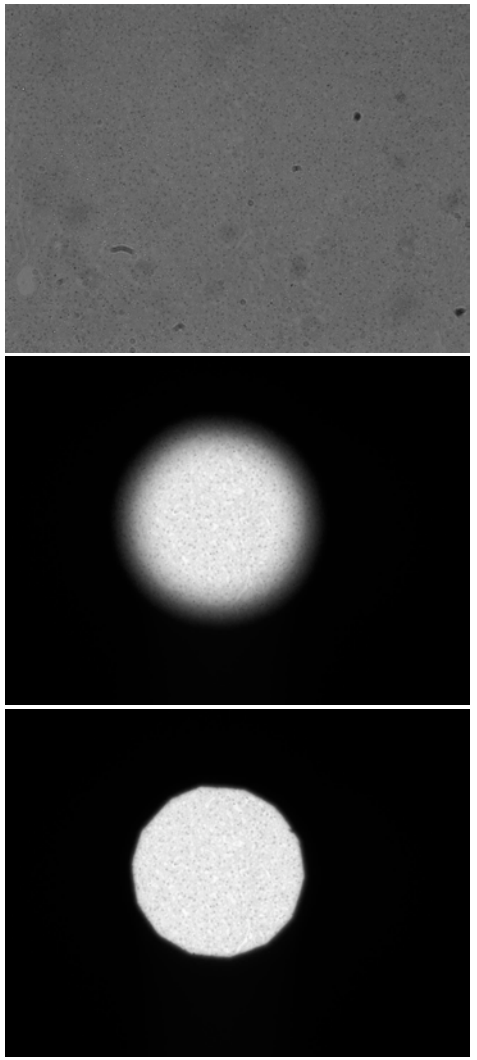
Condenser Aperture. (CD)

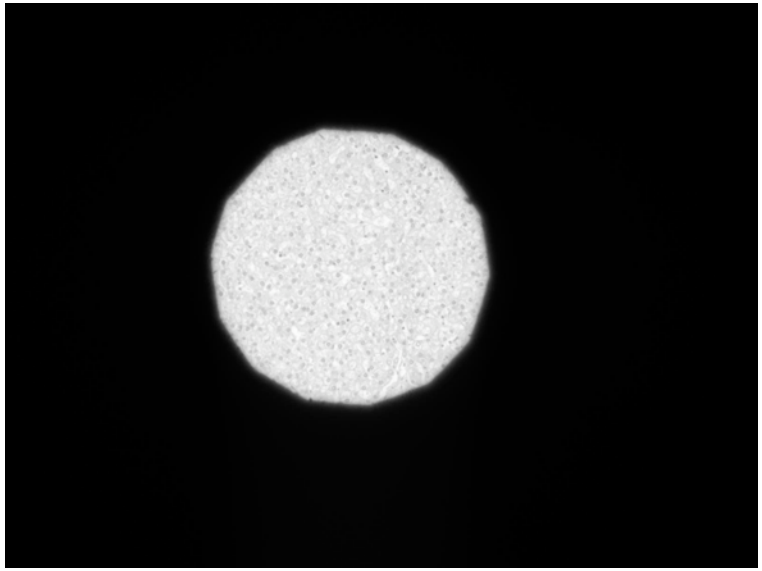


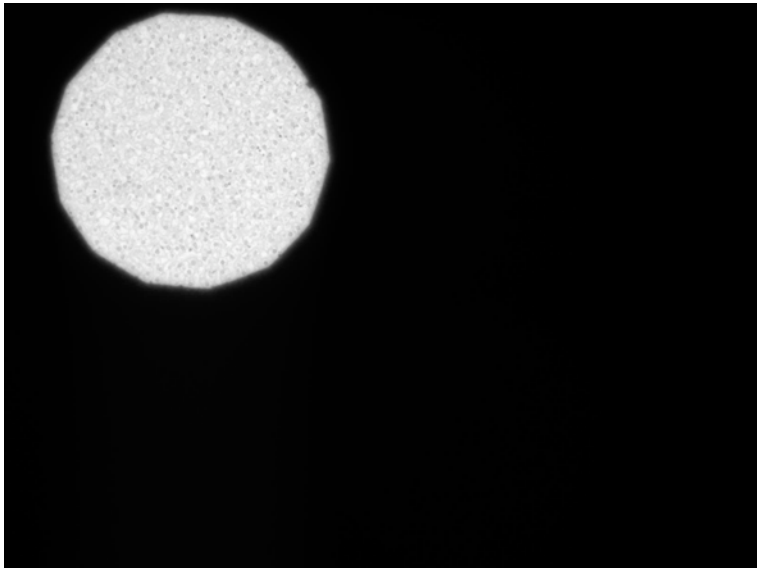
Eye

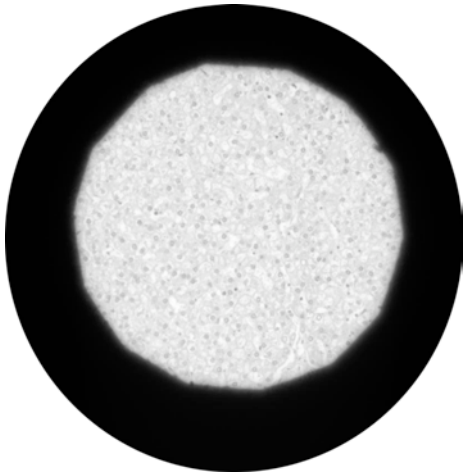


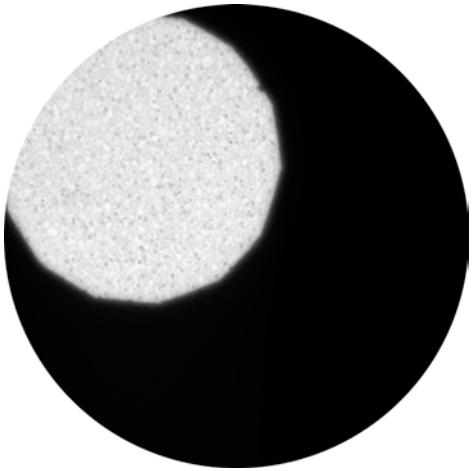
CCD

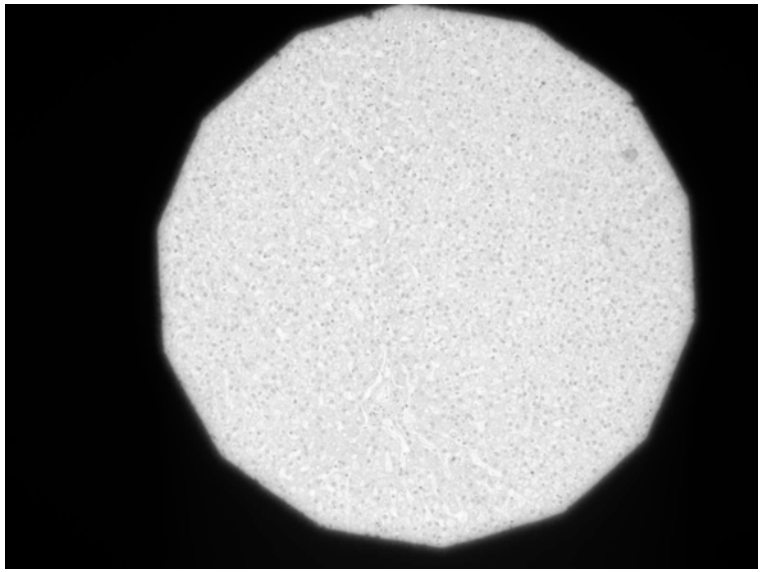


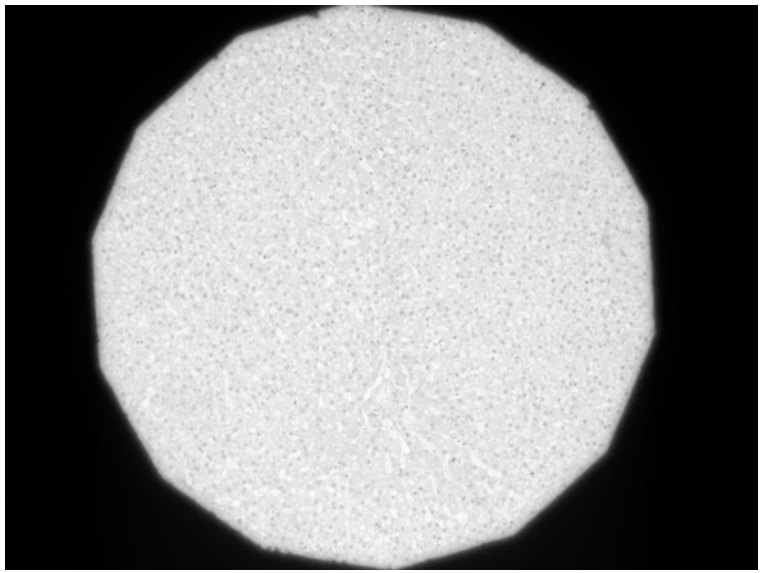


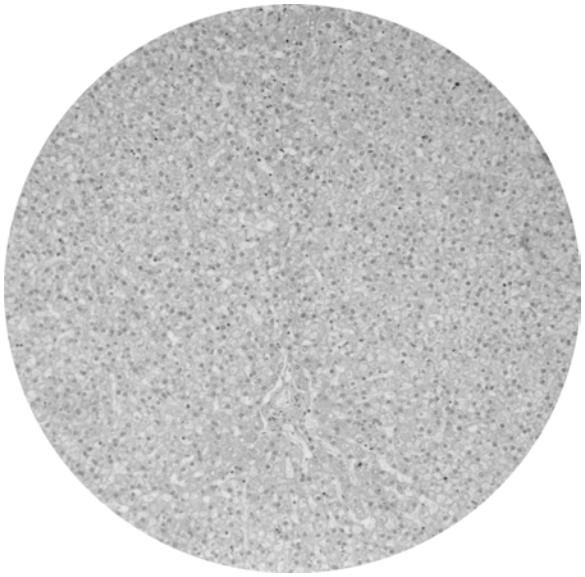


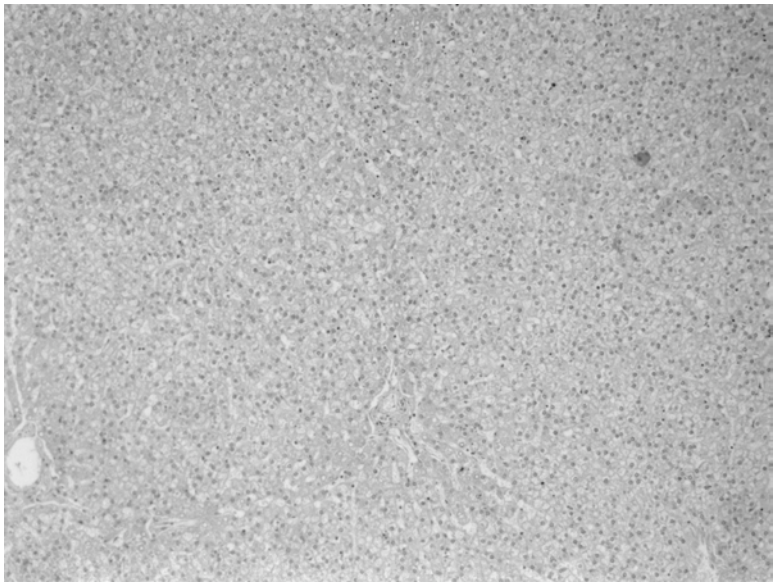






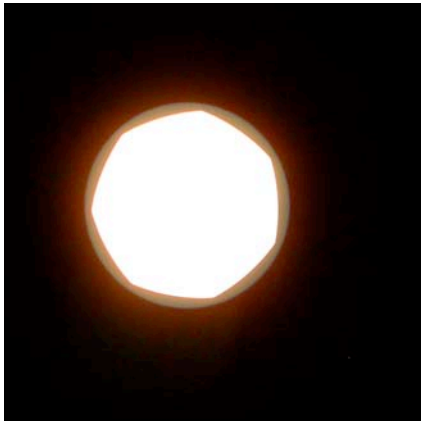




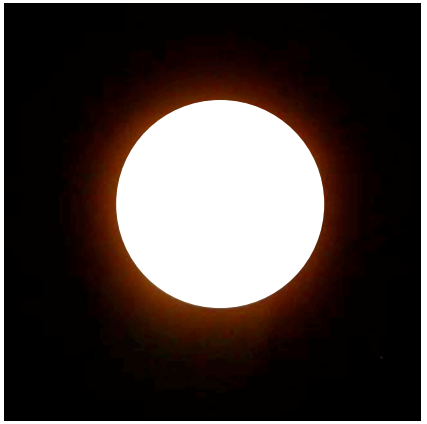


~ 6 0 %

~ 8 0 %



~ 9 5 %

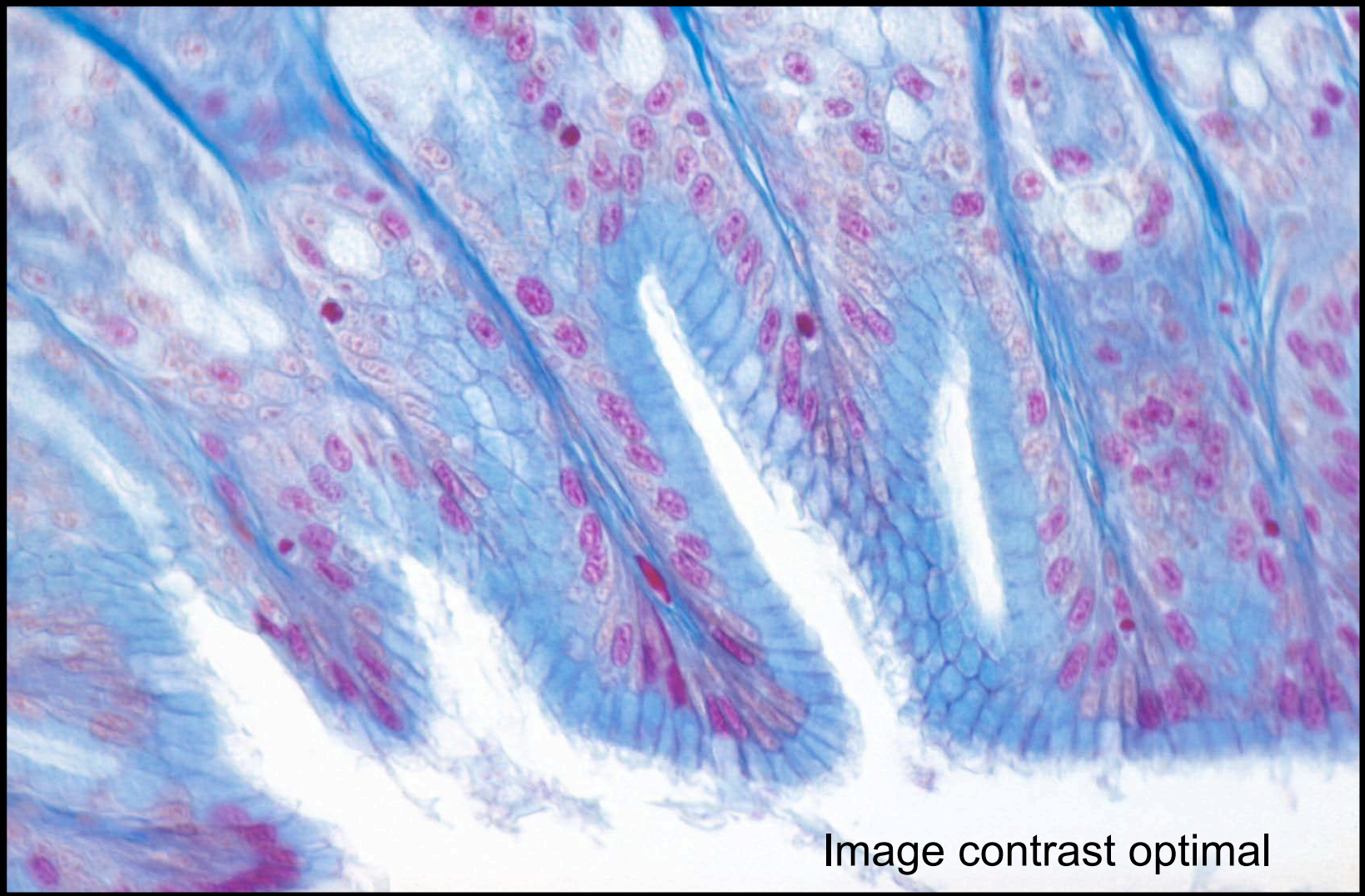


~ 1 0 0 %

Condenser Aperture too large

From Peter Evennett

Condenser Aperture correct



From Peter Evennett

Condenser Aperture too small

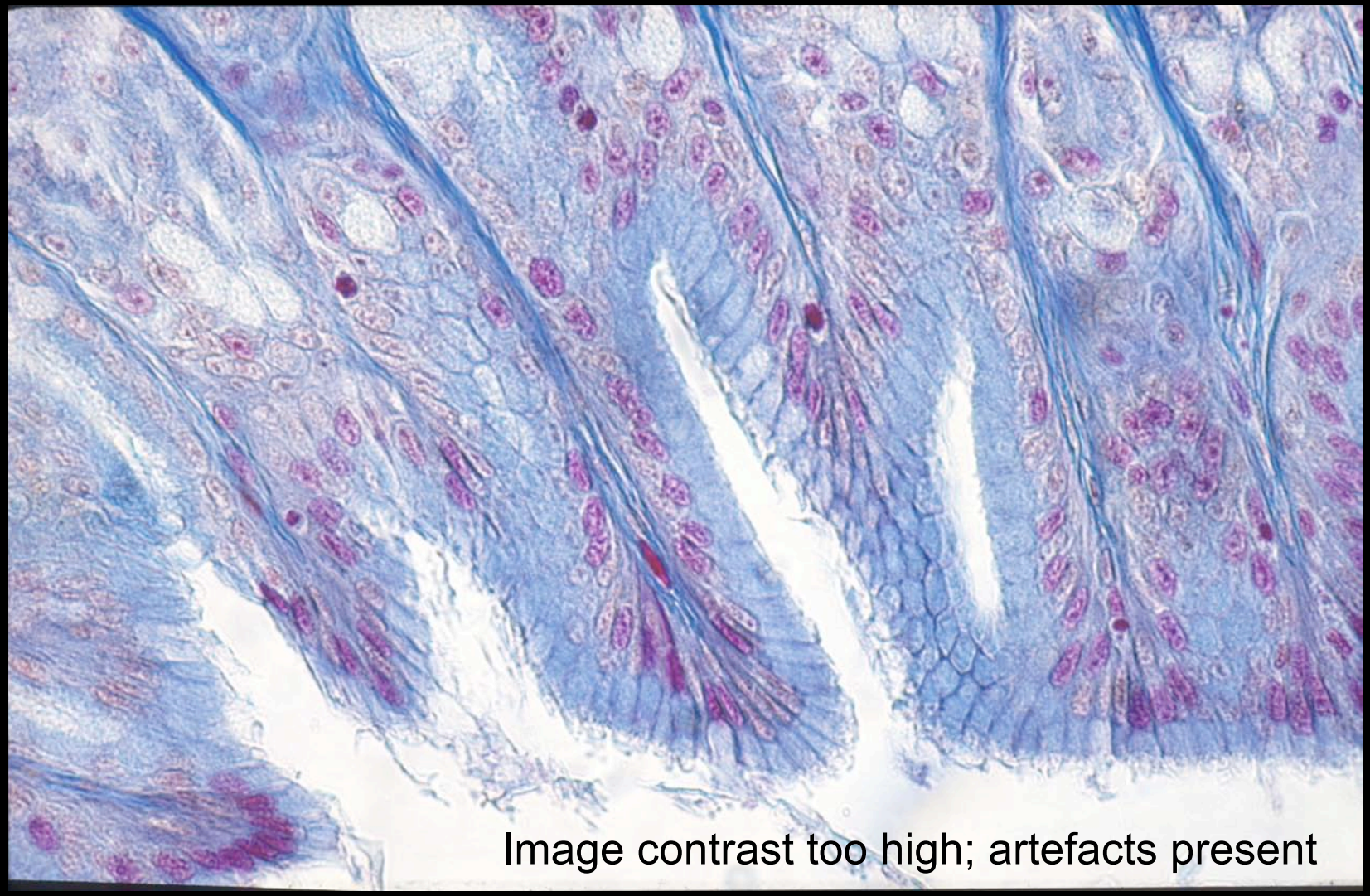
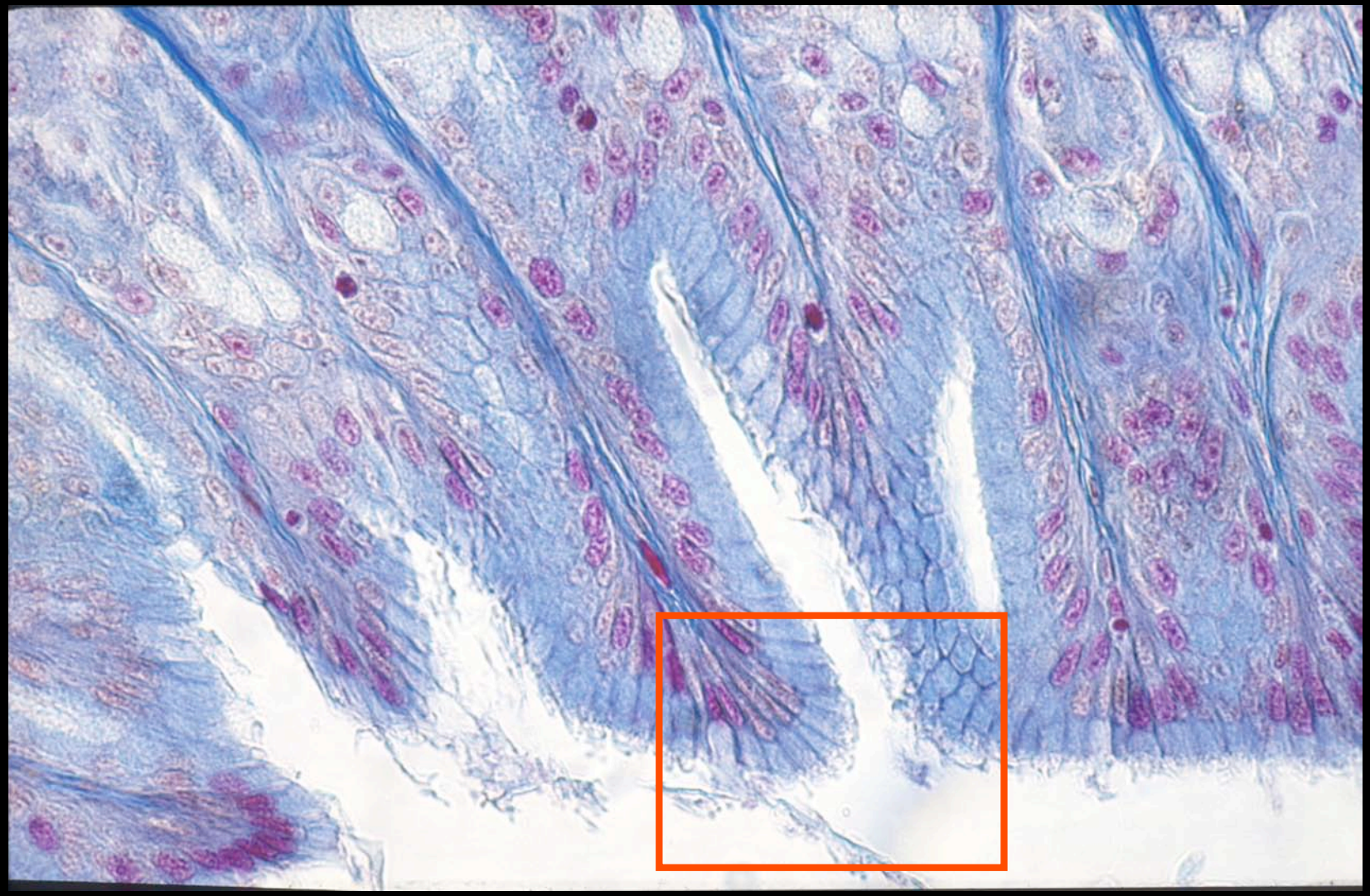


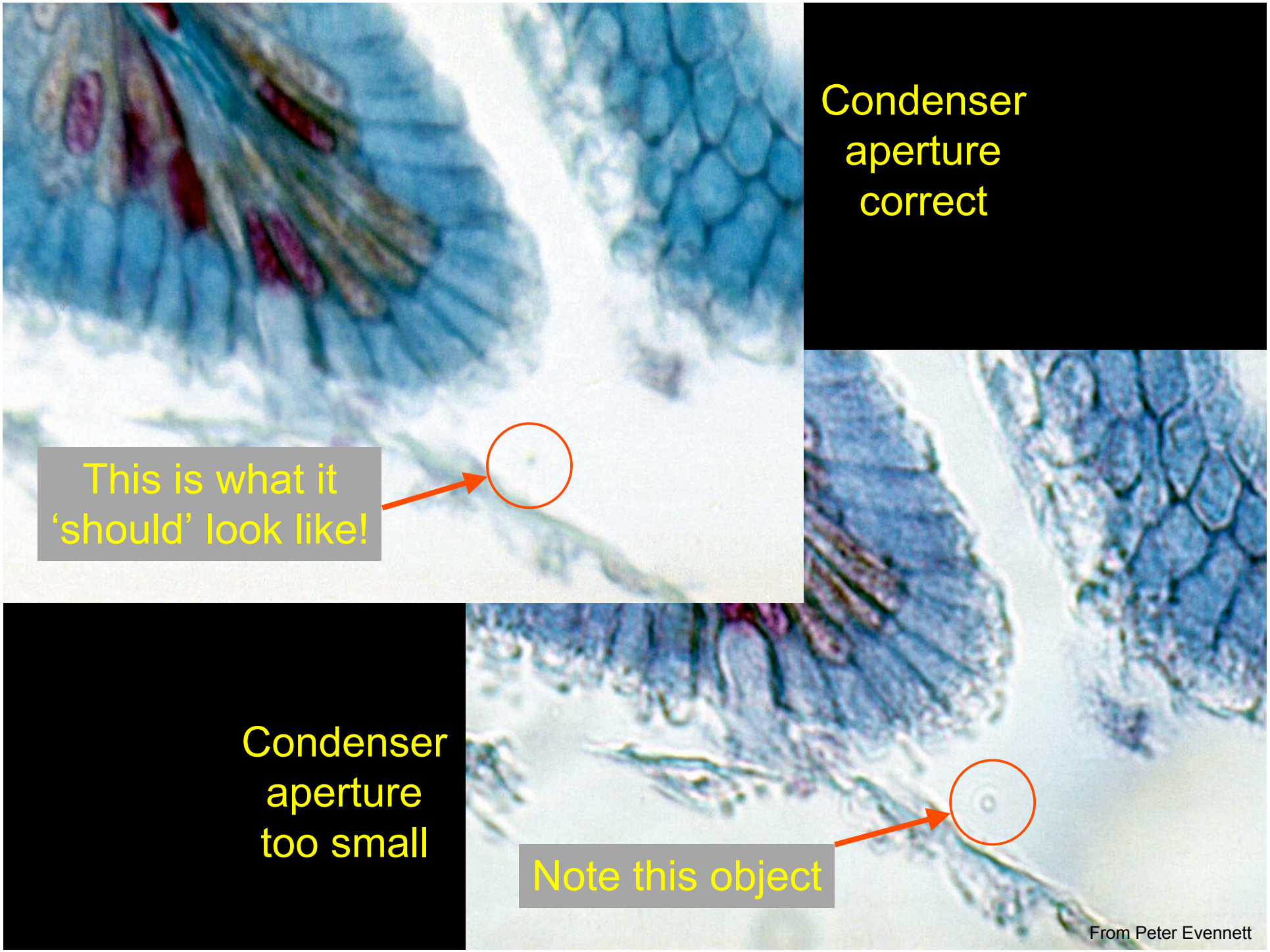
Image contrast too high; artefacts present

From Peter Evennett

Condenser Aperture too small



From Peter Evennett


Condenser aperture correct
This is what it ‘should’ look like!
Condenser aperture too small
Note this object
From Peter Evennett


From Peter Evennett

Köhler Illumination provides

Control **of Area** illuminated by the

Illuminated Field Diaphragm,

which is adjusted according to **magnification.**

Control of **Angle** of illumination by the

Illuminating Aperture Diaphragm

(the condenser aperture),

which is adjusted according to objective **aperture.**