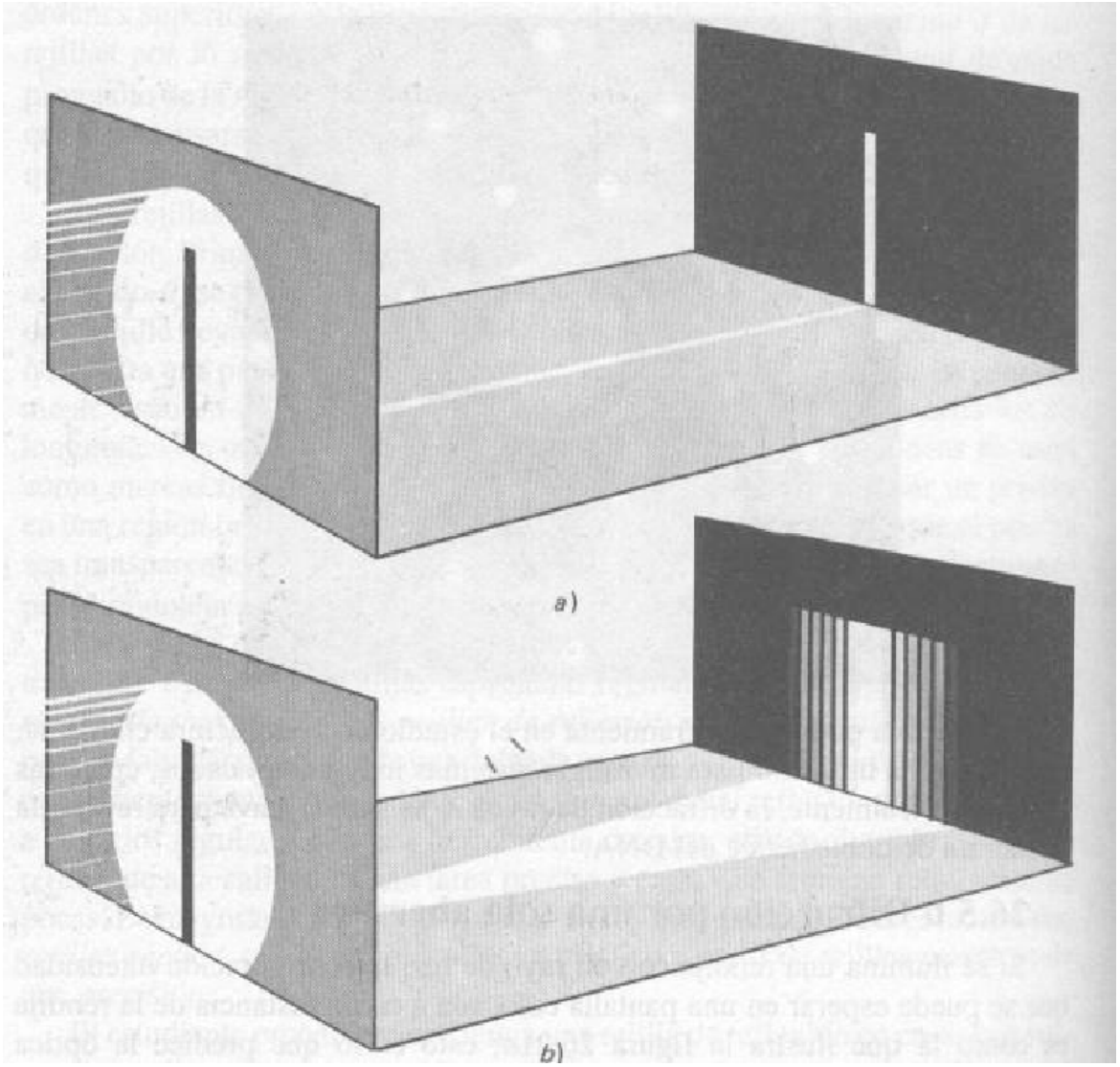
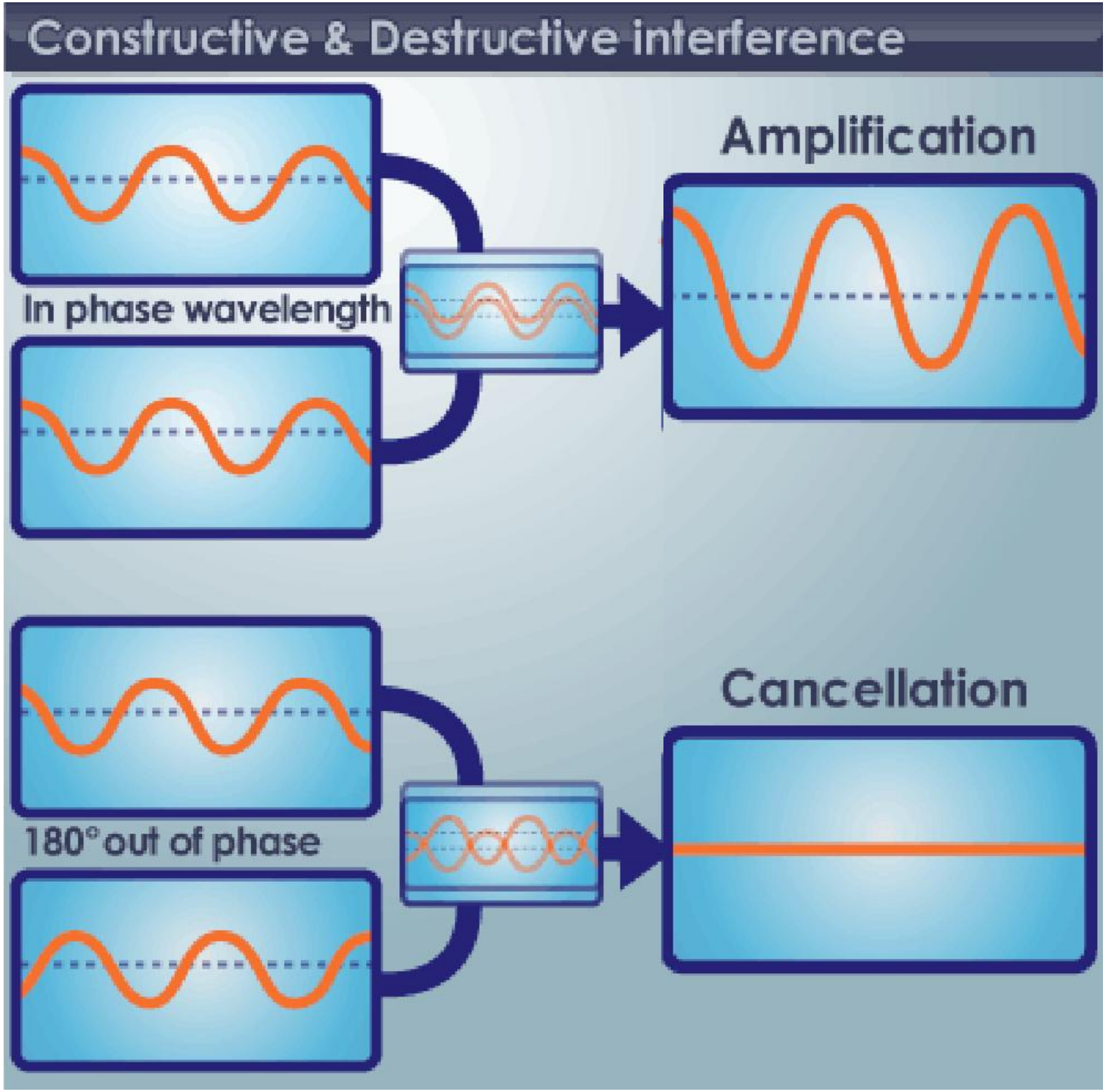
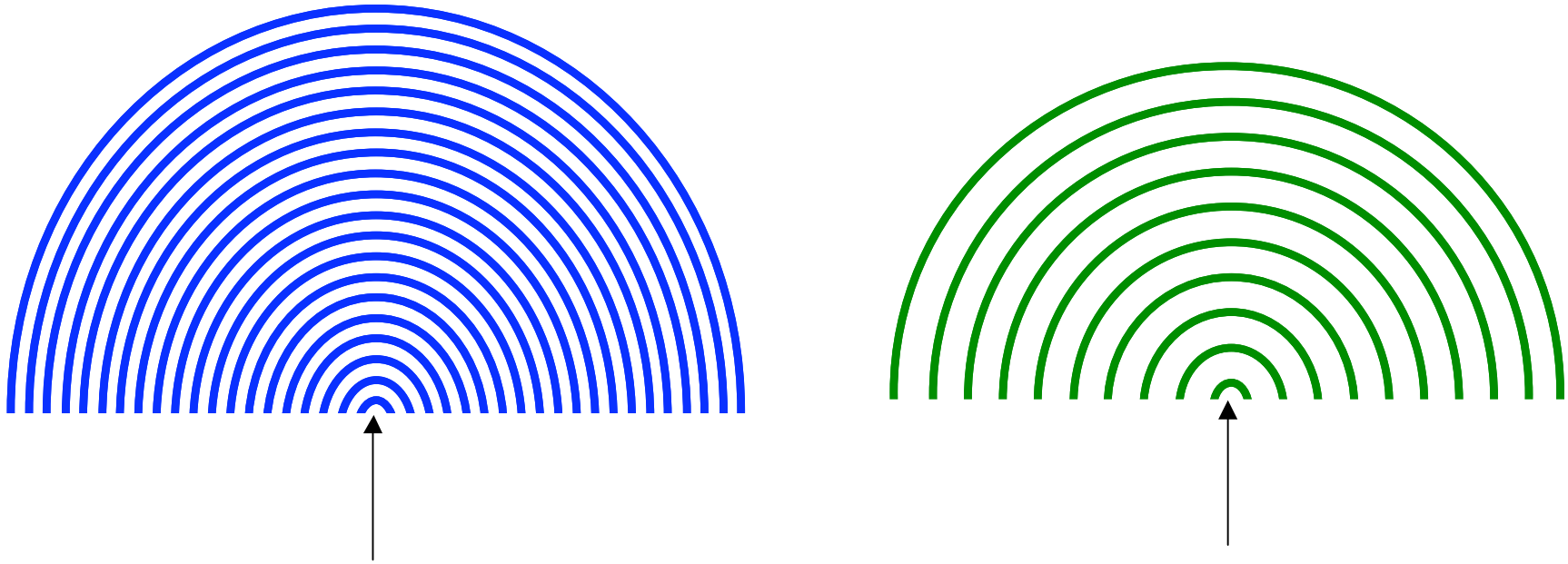
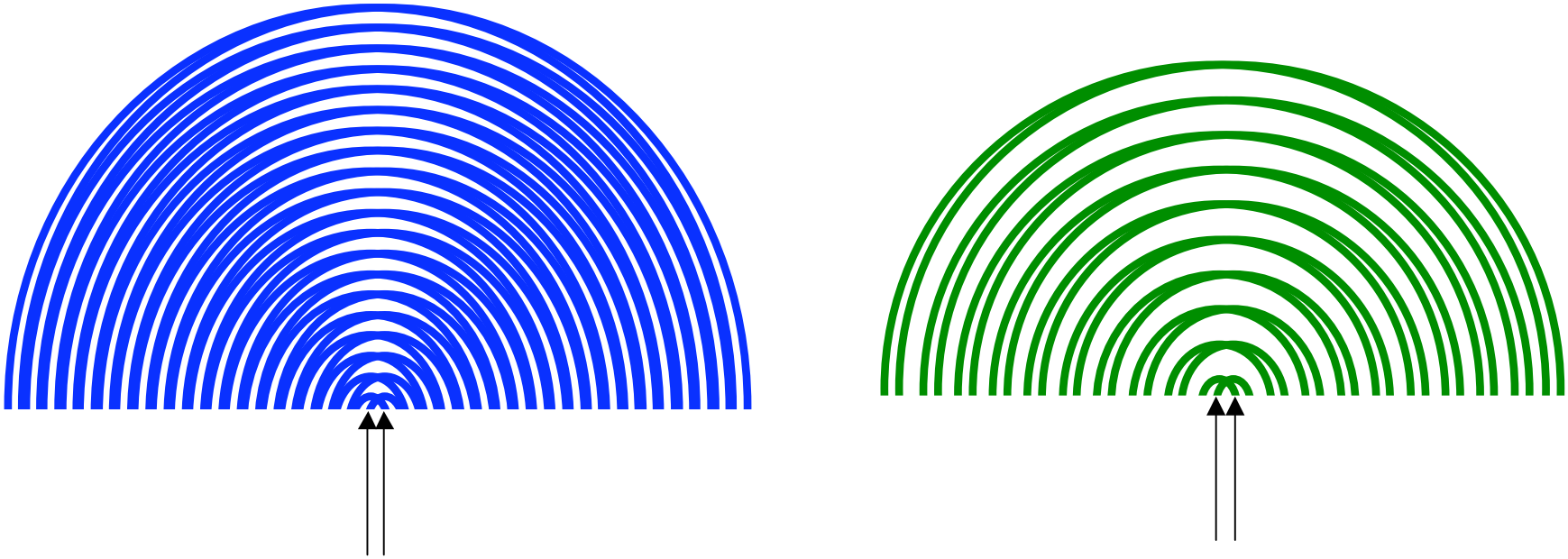
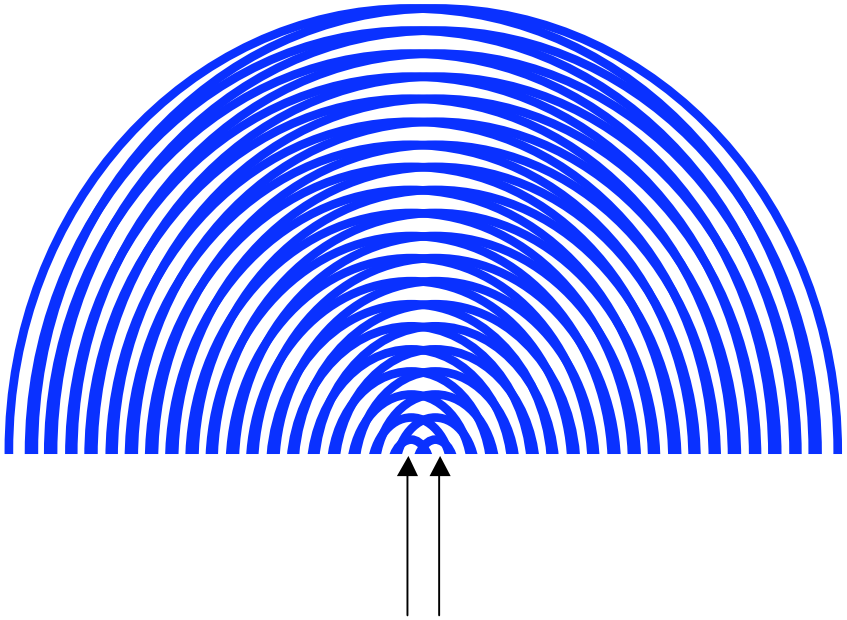


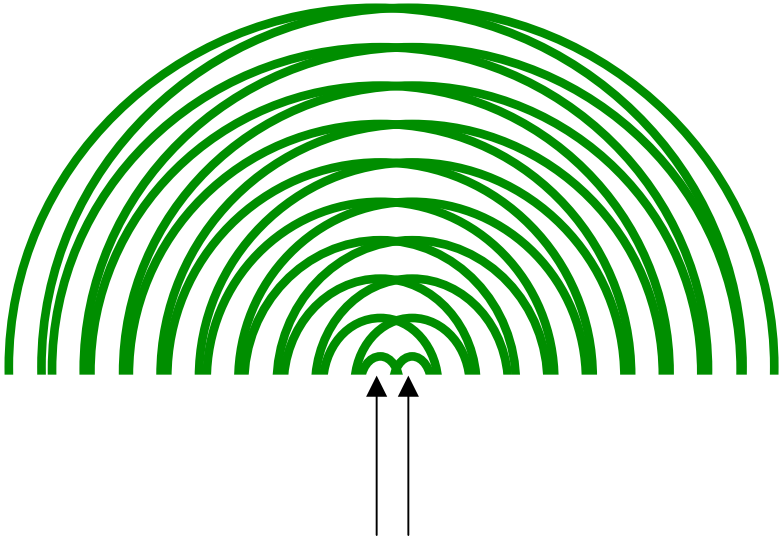
DIFRACTION

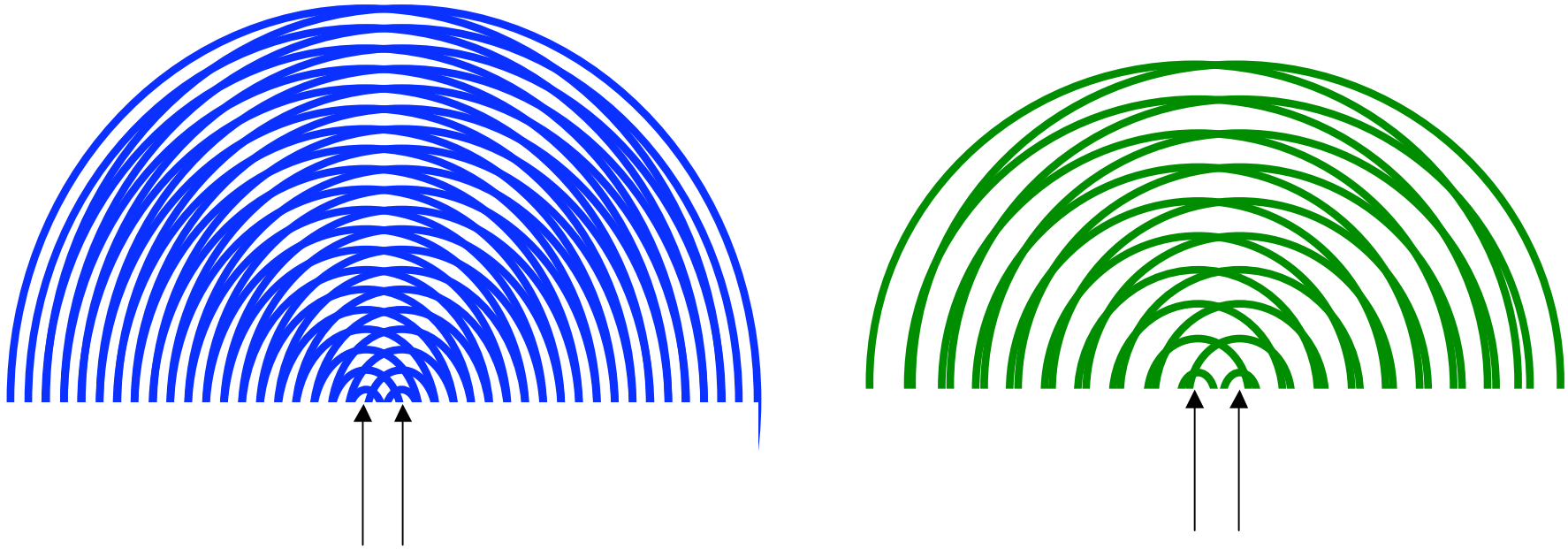

Constructive & Destructive interference
Amplification
In phase wavelength
Cancellation
180° out of phase

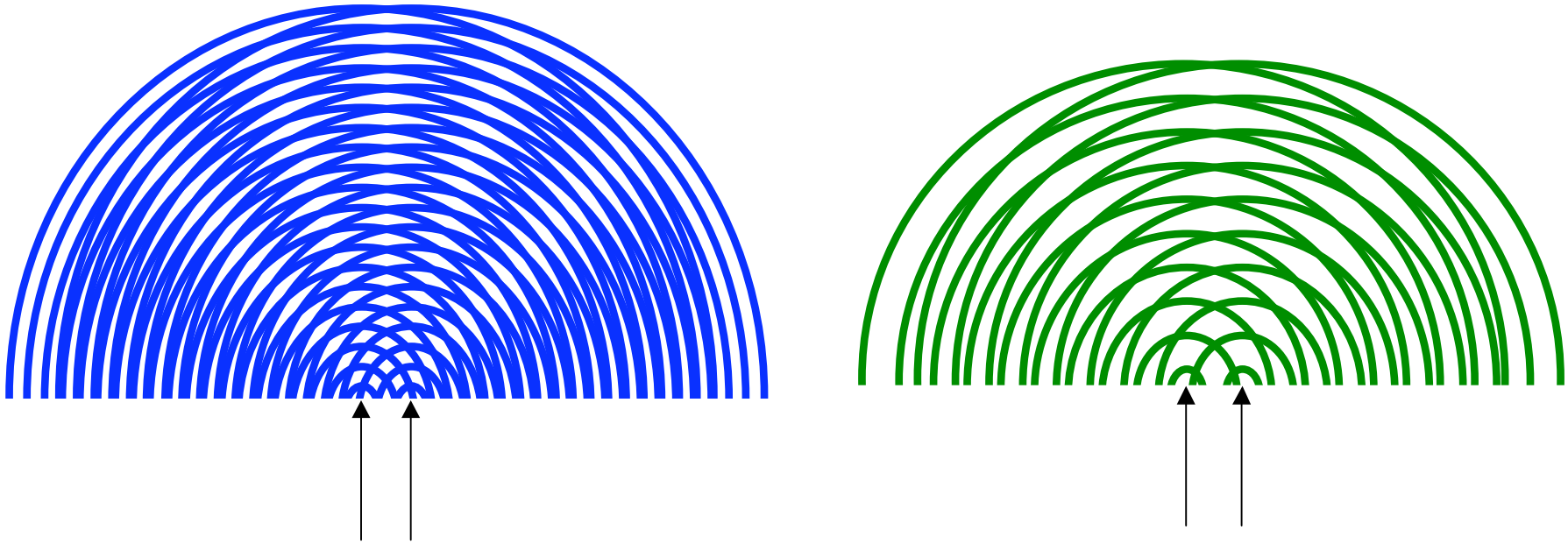


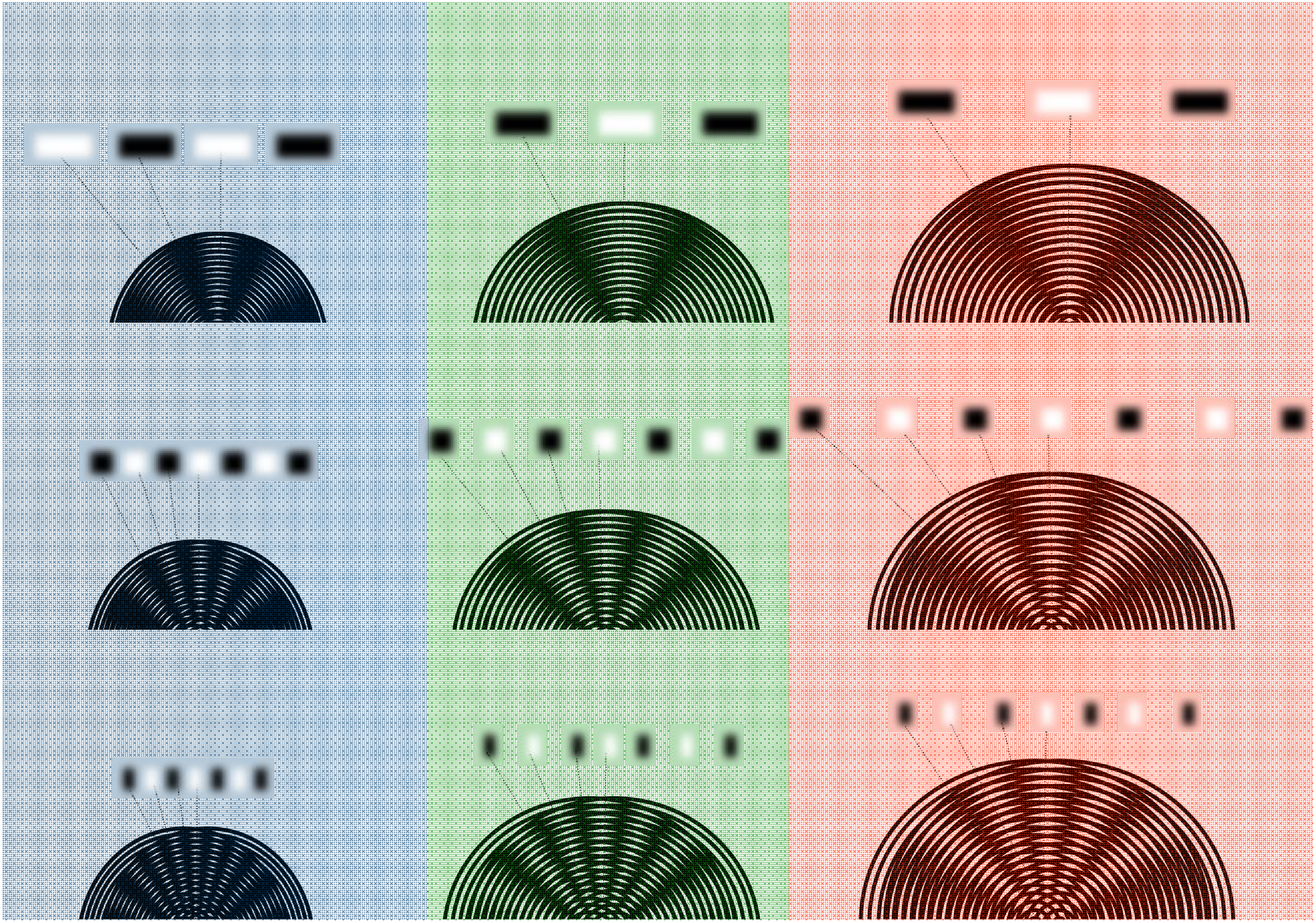




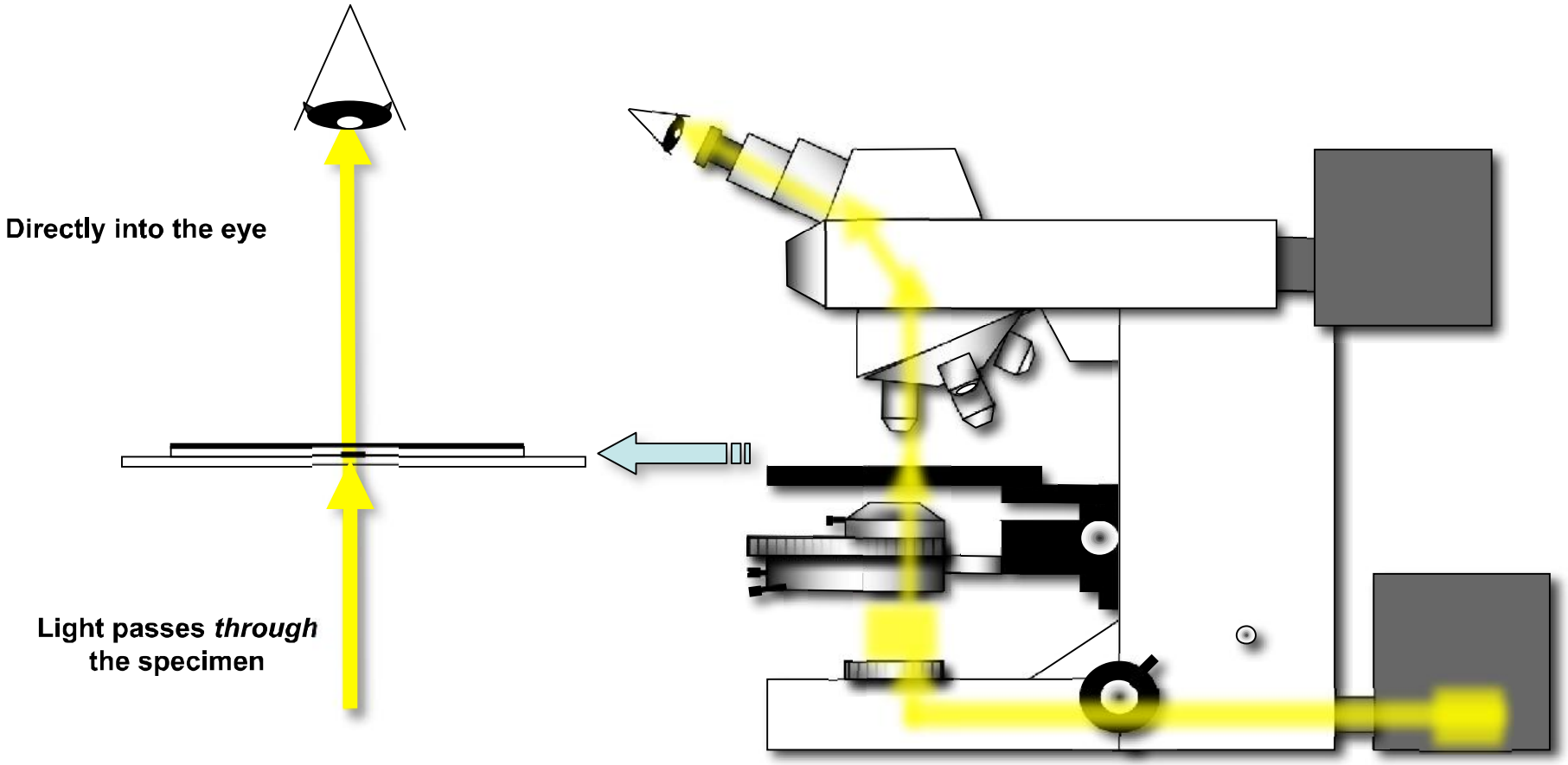




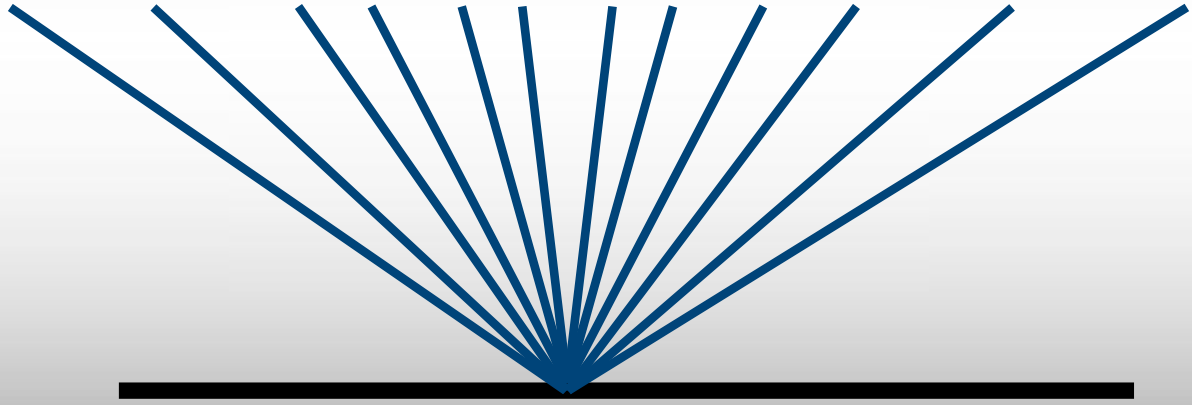




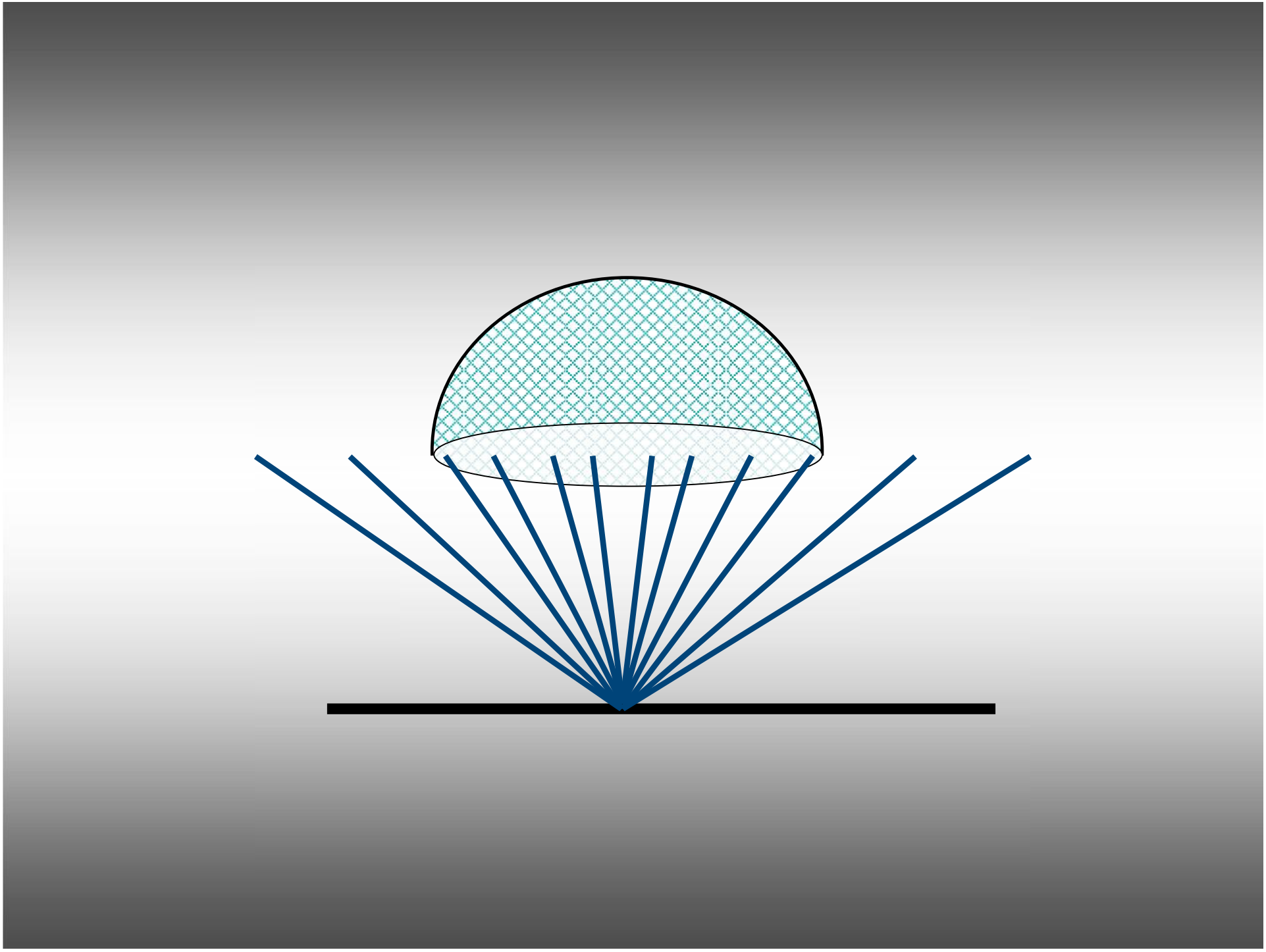
Transmitted-light. Bright-field

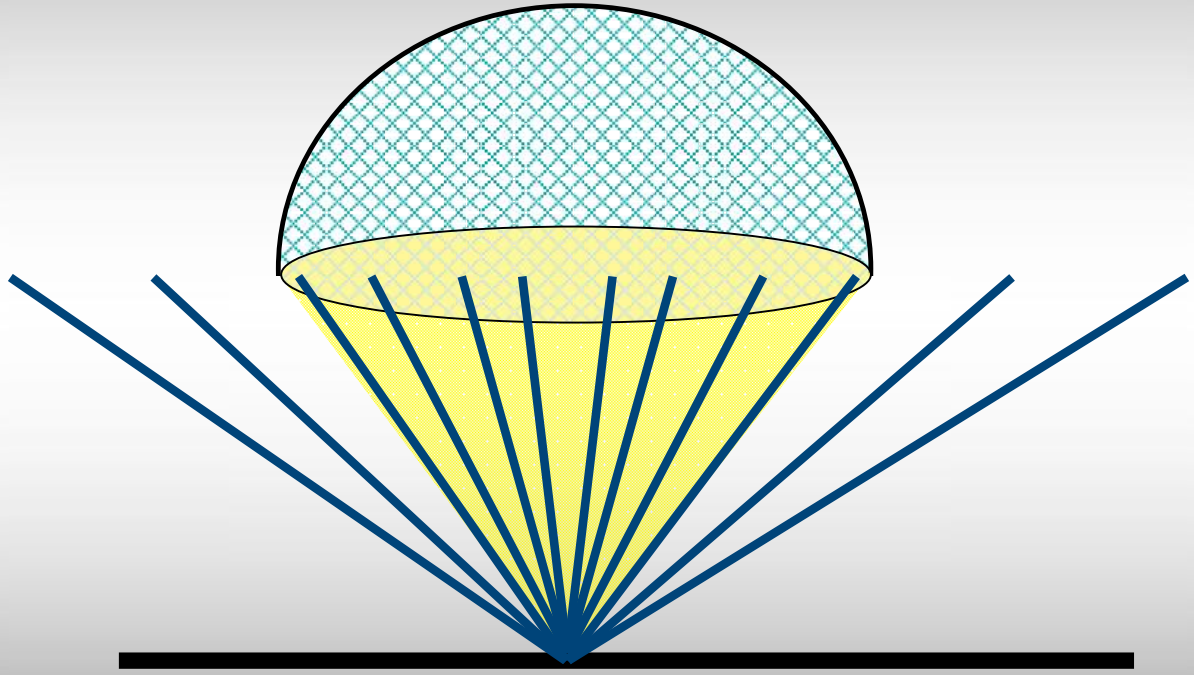


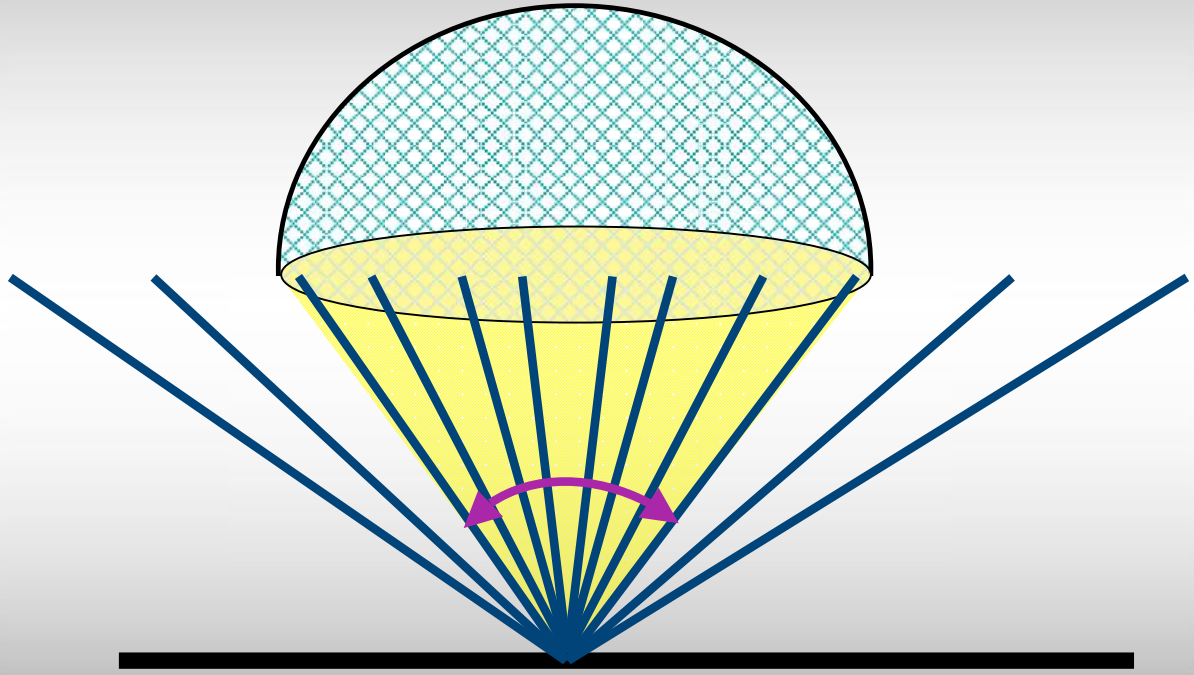
An unusual way of looking at things!



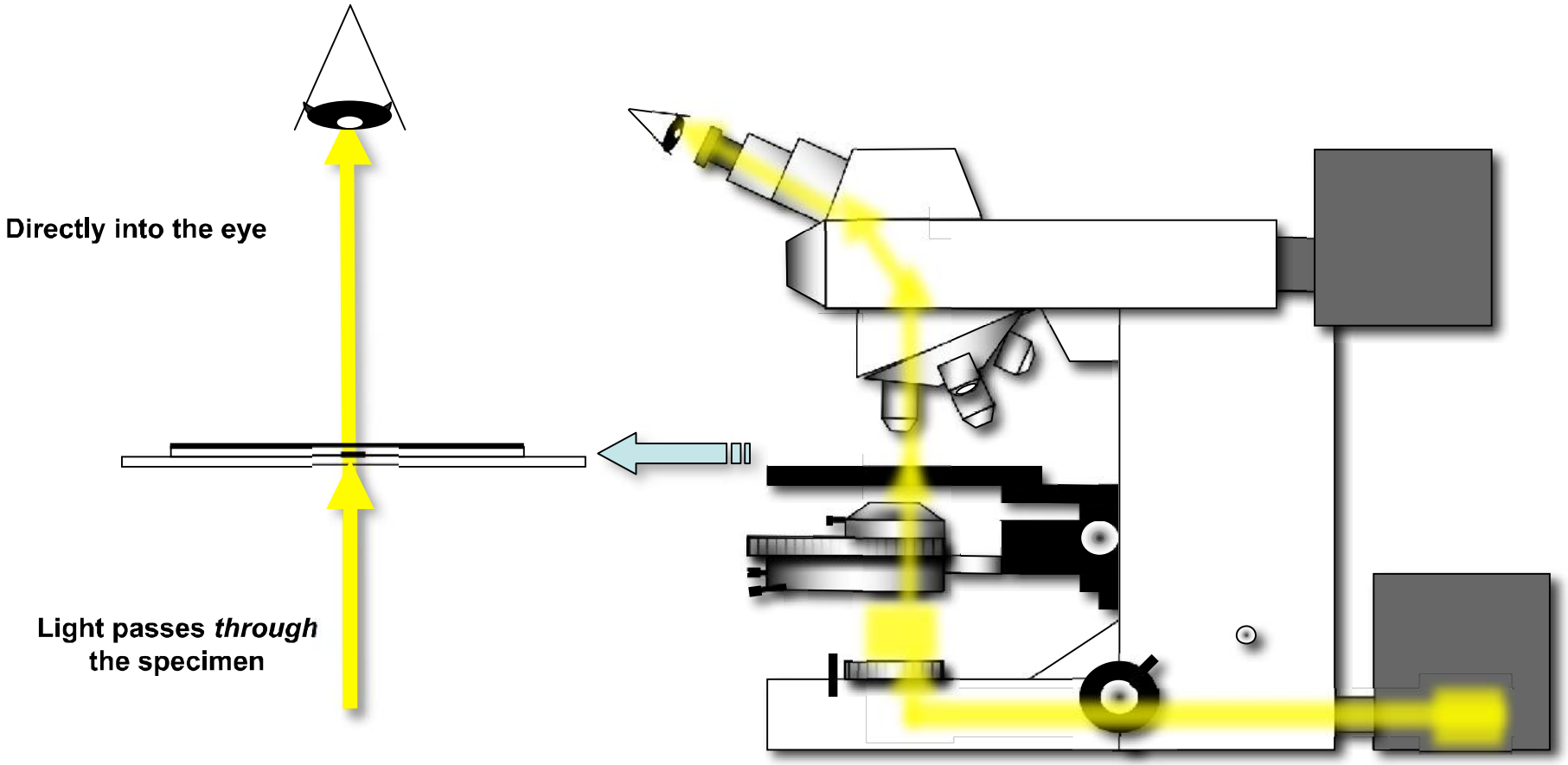
Consider that every ray leaving the object carries some information about fine detail in the object







Transmitted-light. Bright-field



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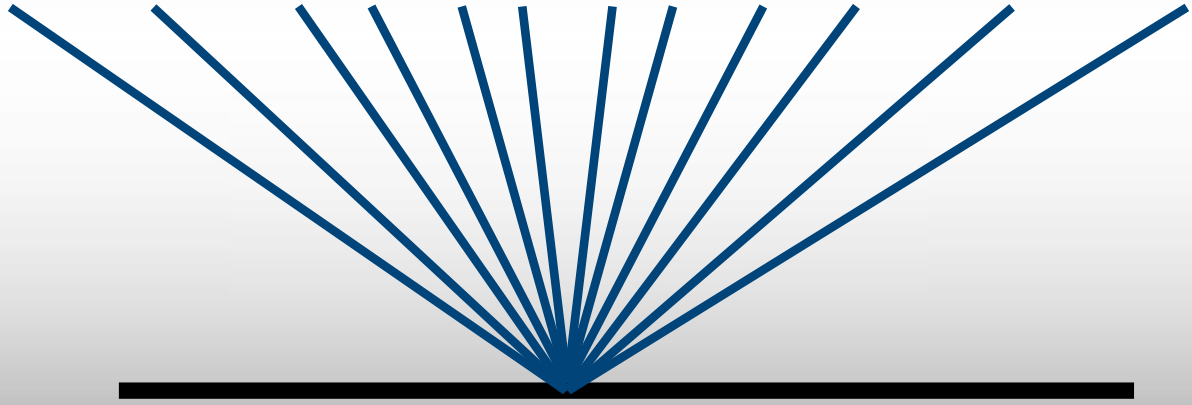
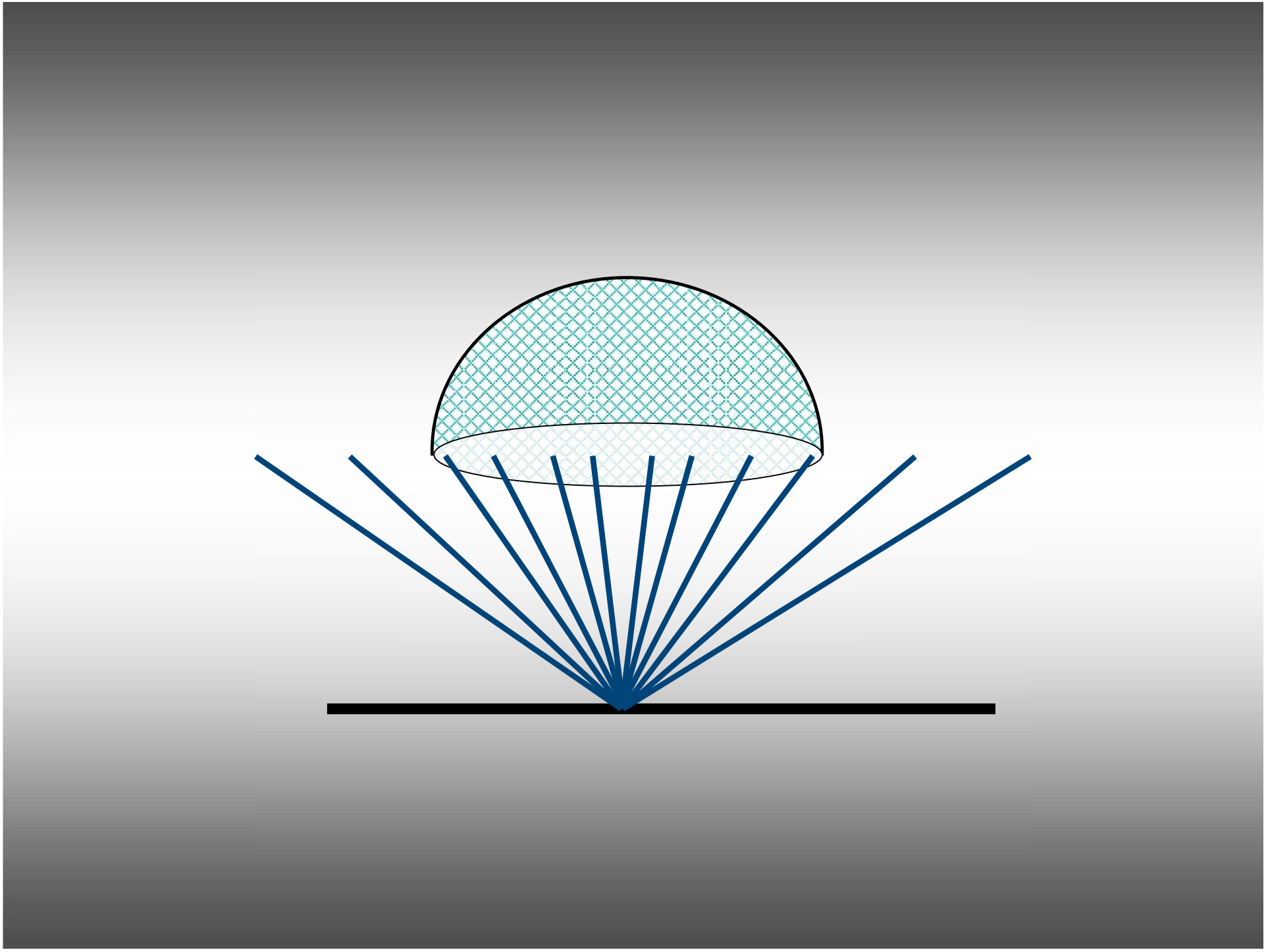
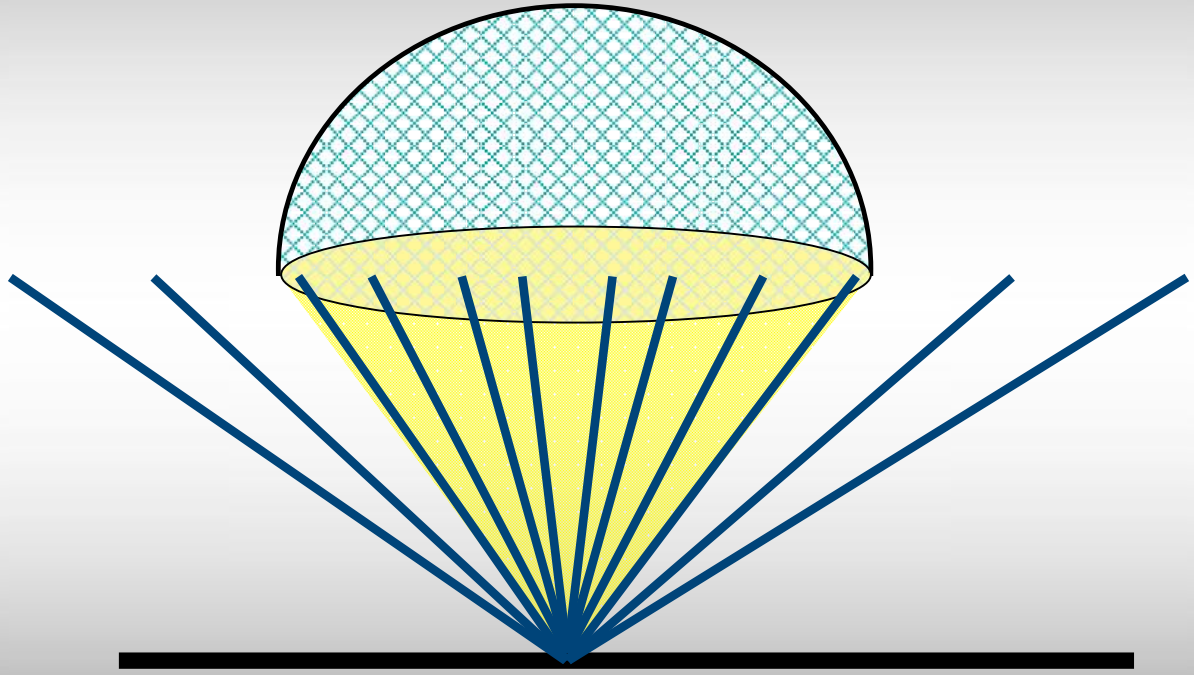
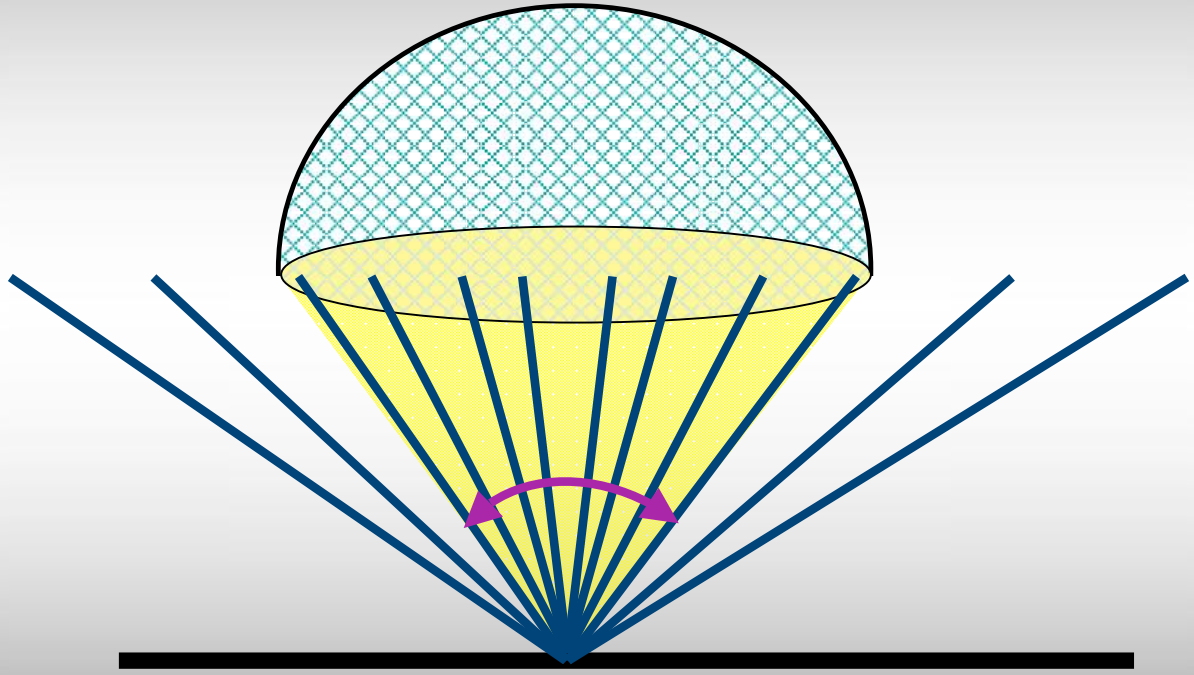


Consider that every ray leaving the object carries some information about fine detail in the object

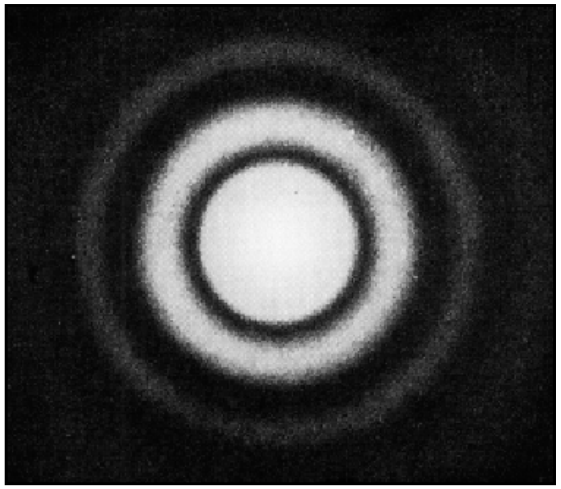







RESOLUTION
100µm

Airy and Rayleigh



Airy (1801 - 1892)

Astronomer

The image of a point source formed by a lens of finite diameter was a disk with halos around it (left) whose properties depended entirely on the size of the lens.

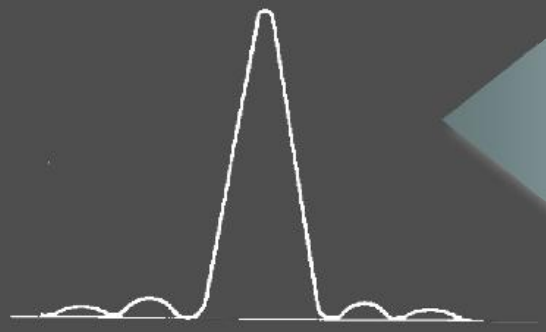
Rayleigh (1842 - 1919) explained how the wave nature of light determined how it was scattered (Rayleigh scattering). In microscopy he gave the first mathematical analysis of resolution, defining a resolution criterion based on the Airy disk and showing how it was determined by the Numerical Aperture of the objective.

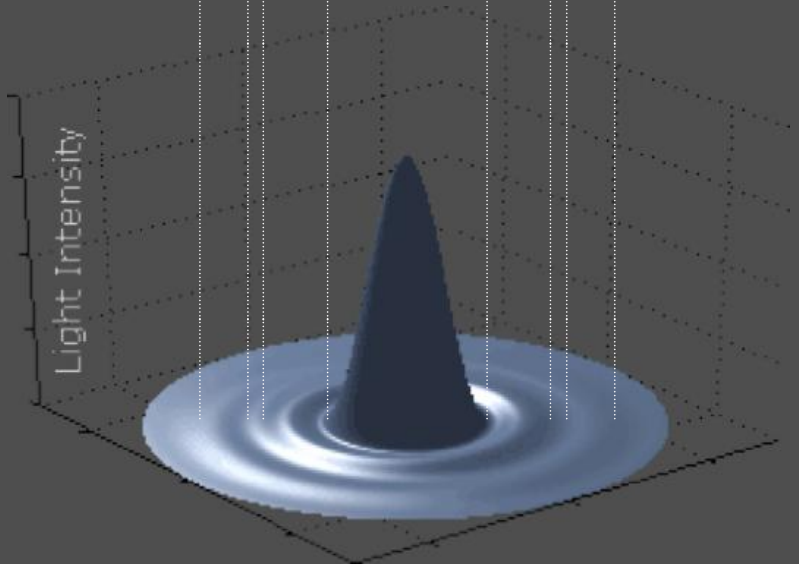
The Image of a point…is not a point

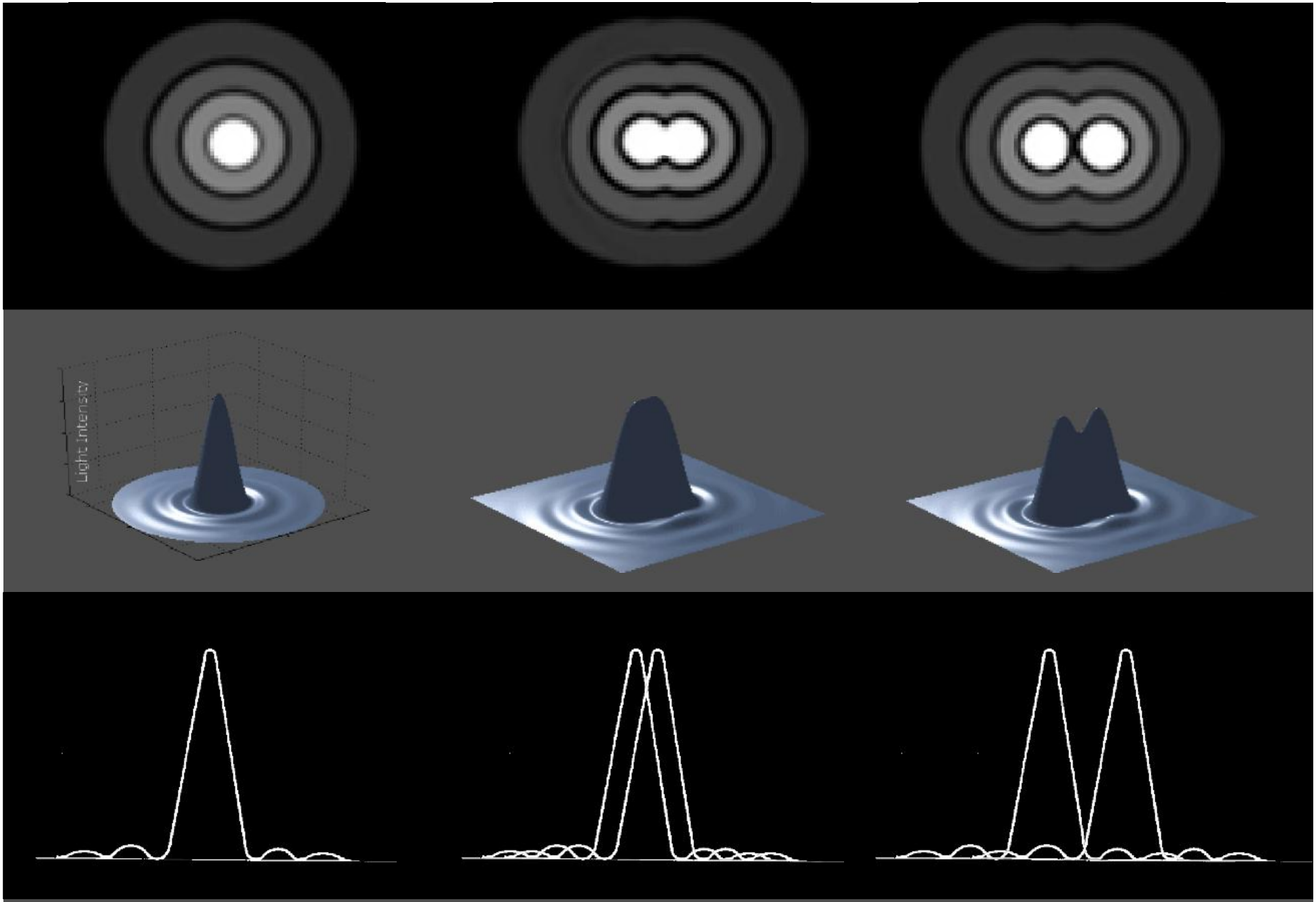
Original object

This is caused by
Diffraction

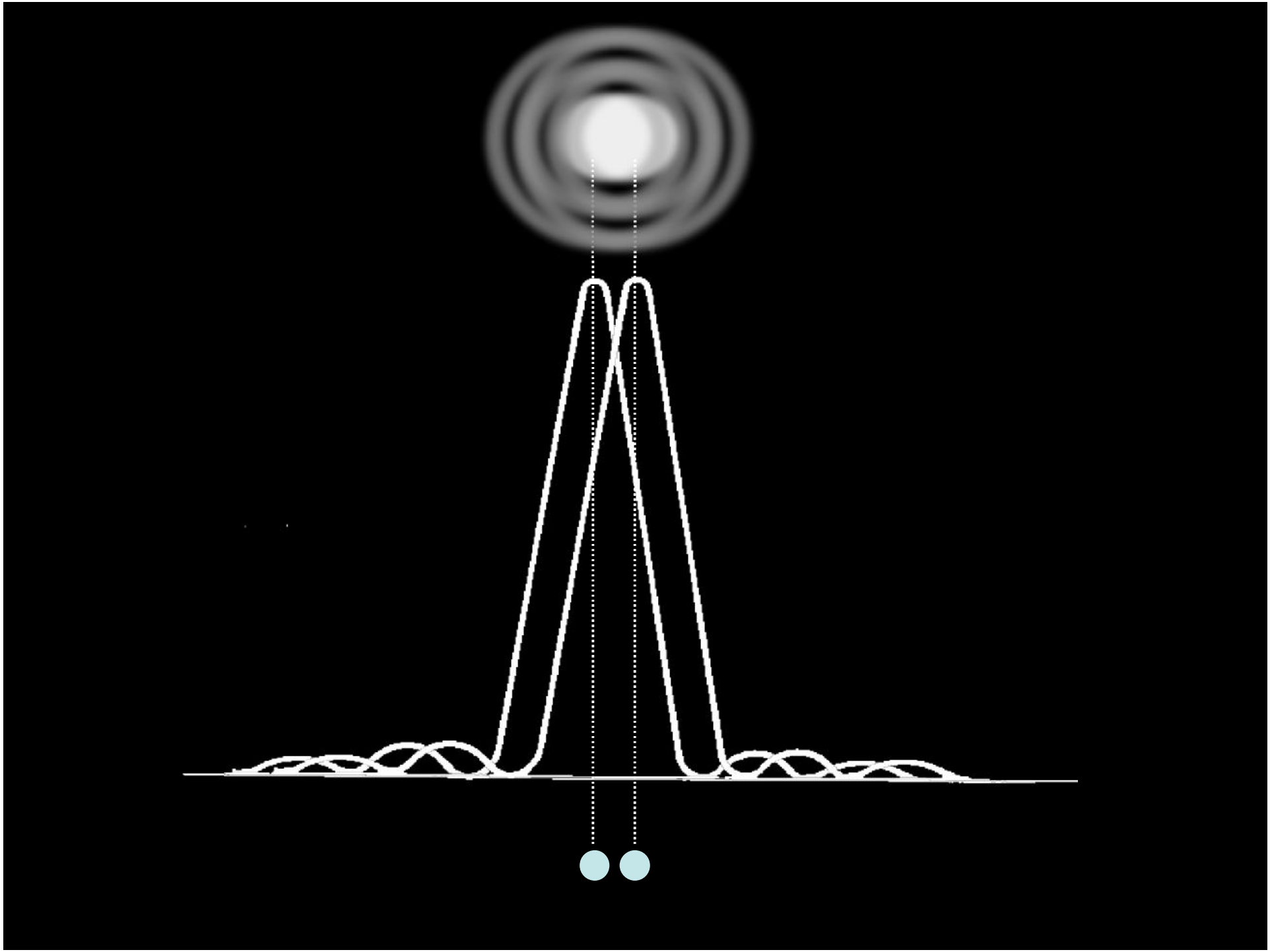
Image of a point source:
The *Airy Pattern*

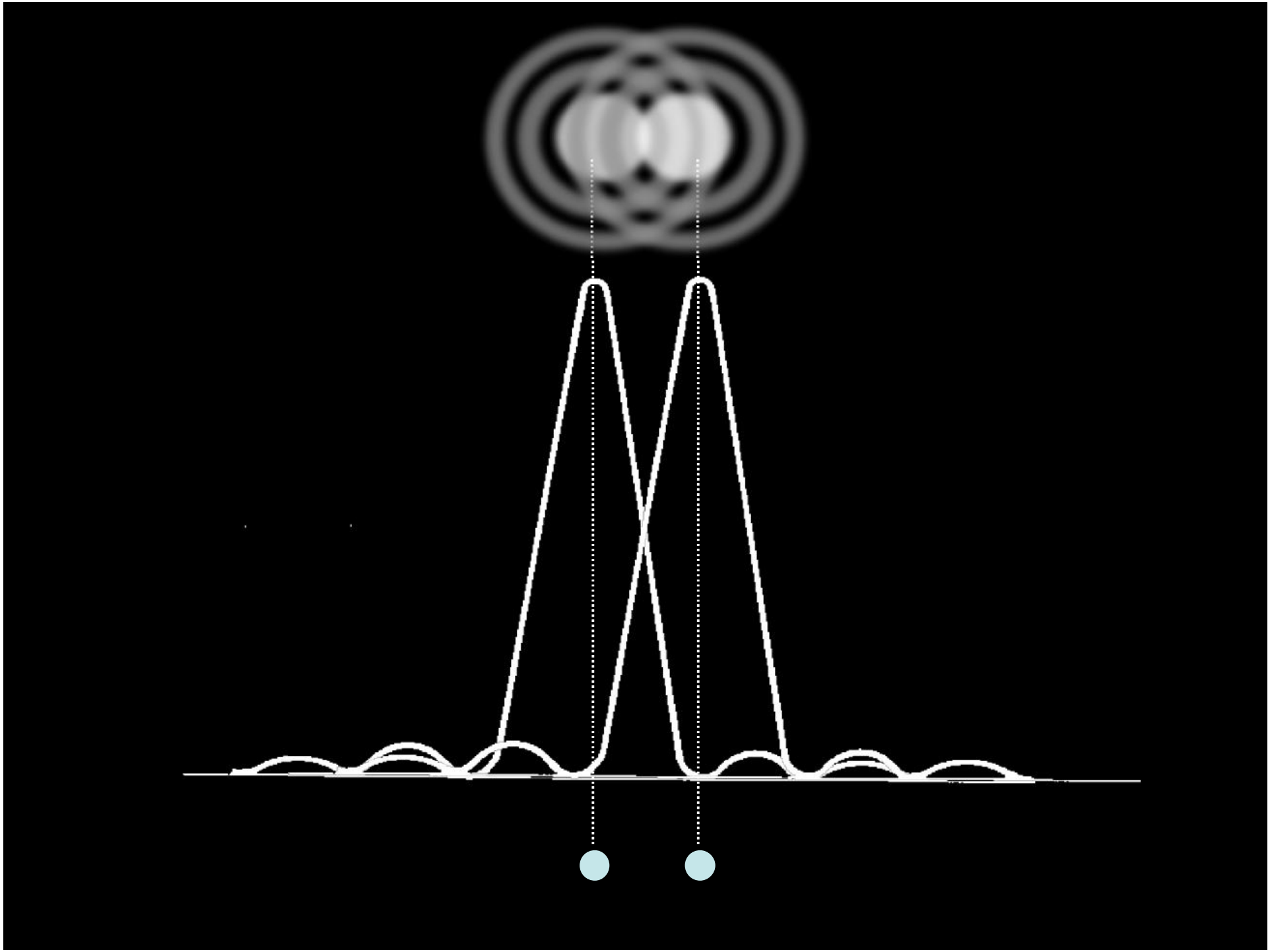


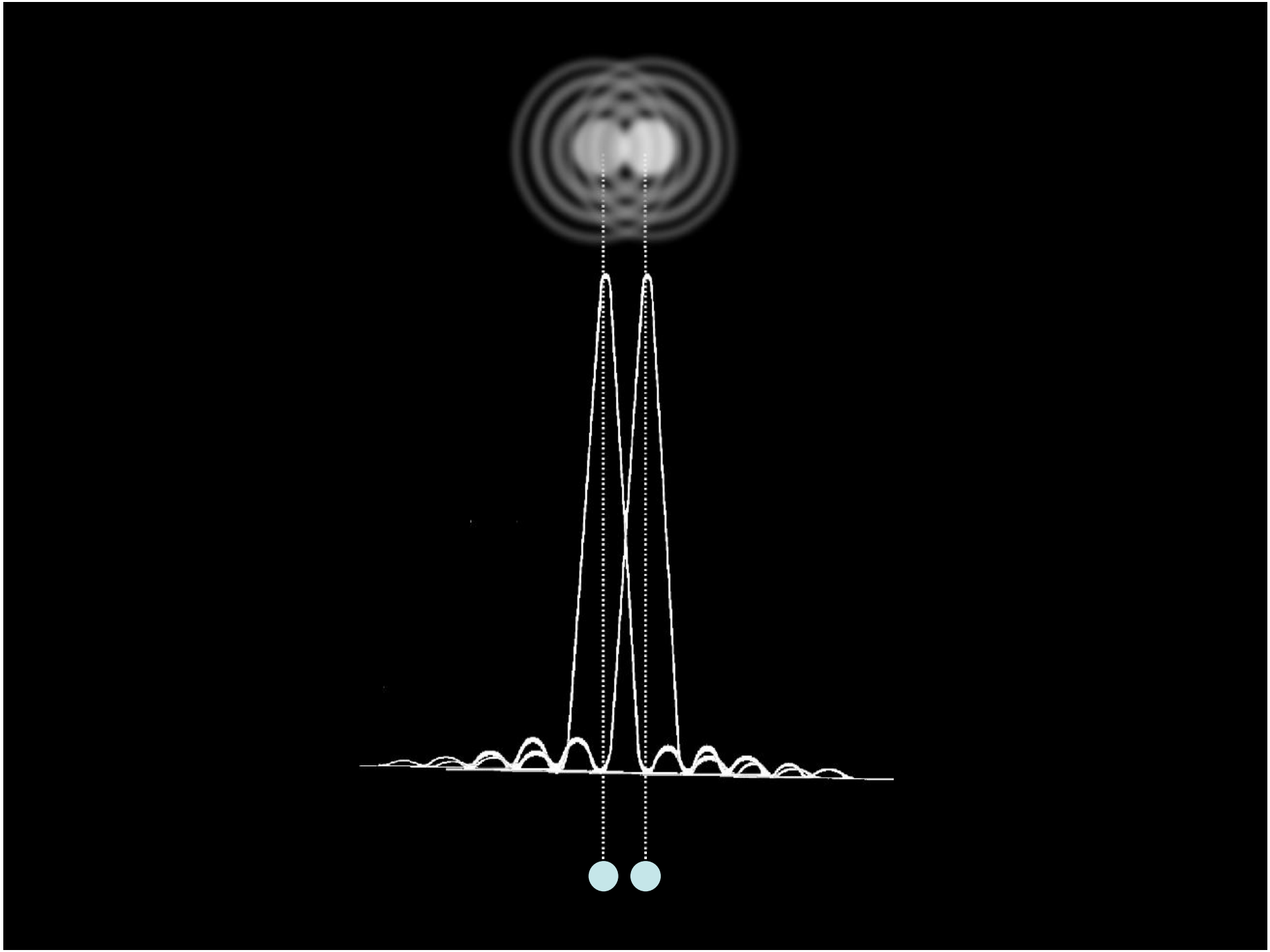




RESOLUTION is not a measure of how small an object we can see,
it is the ability to distinguish which are closer together







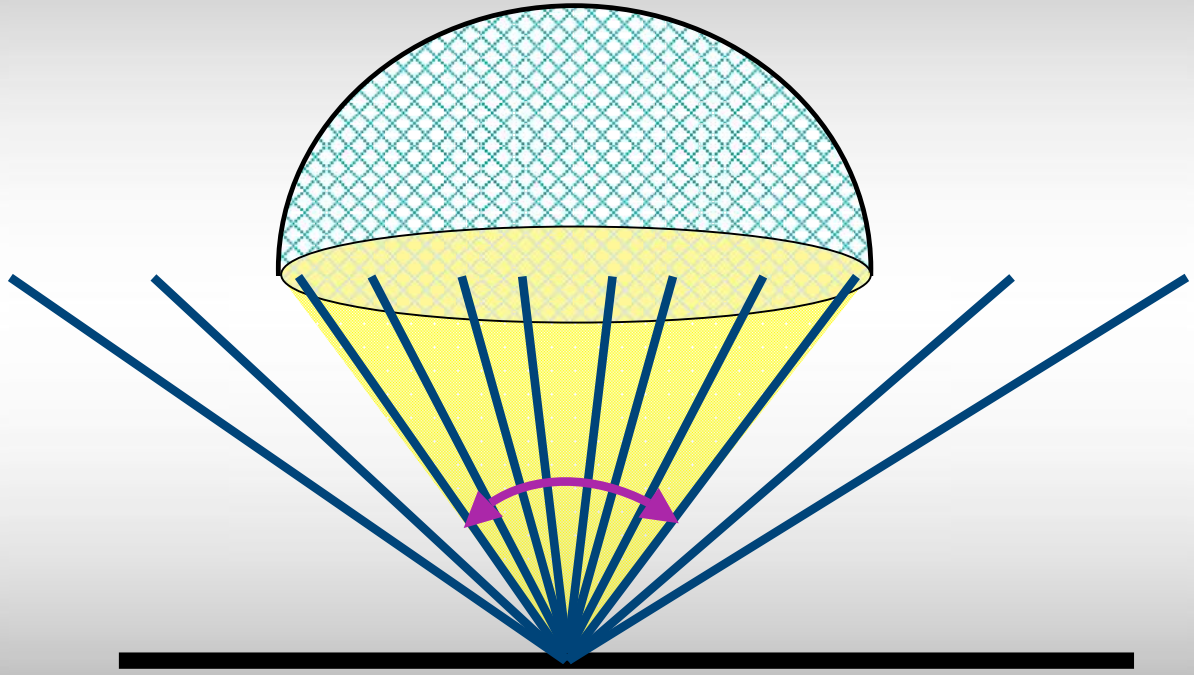
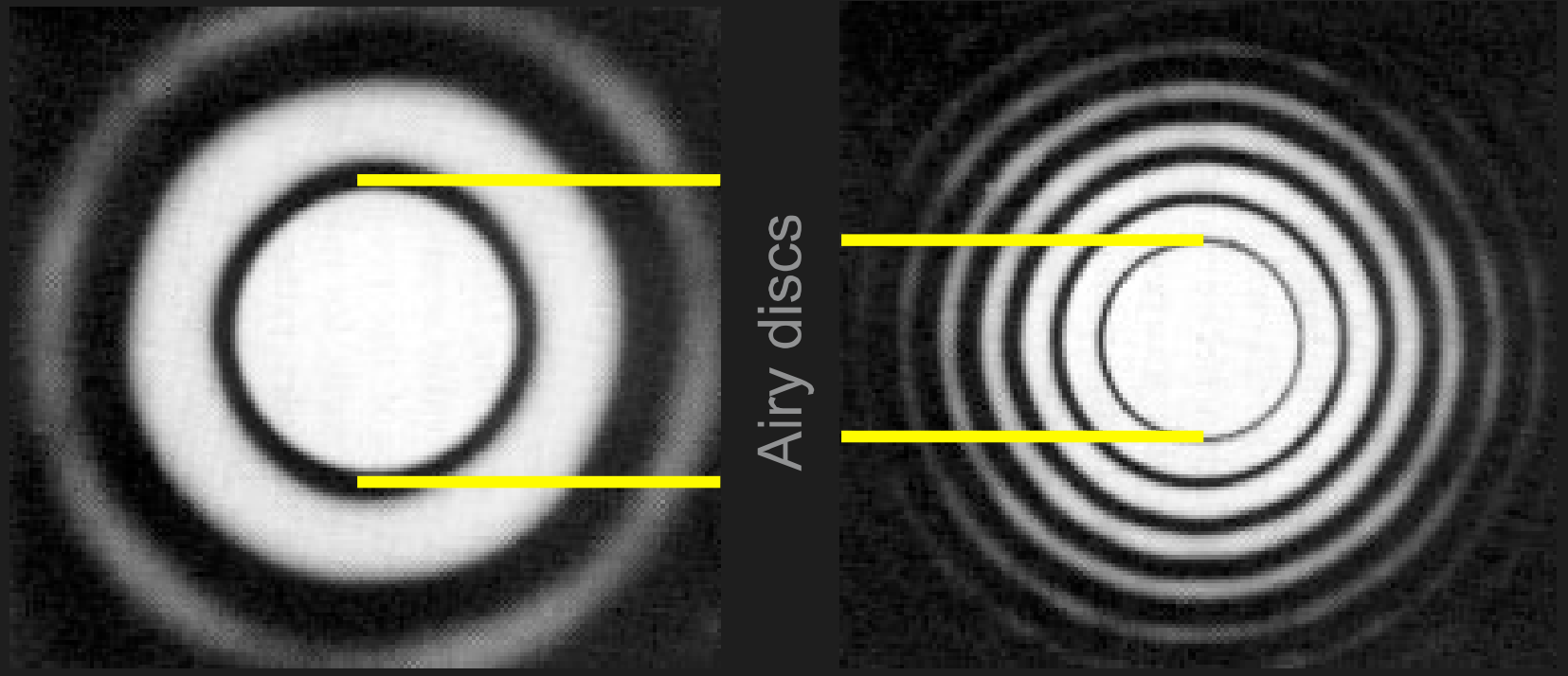


Image of a point source: the *Airy Pattern*

Small aperture

Larger aperture



The Image of a point....

Original object

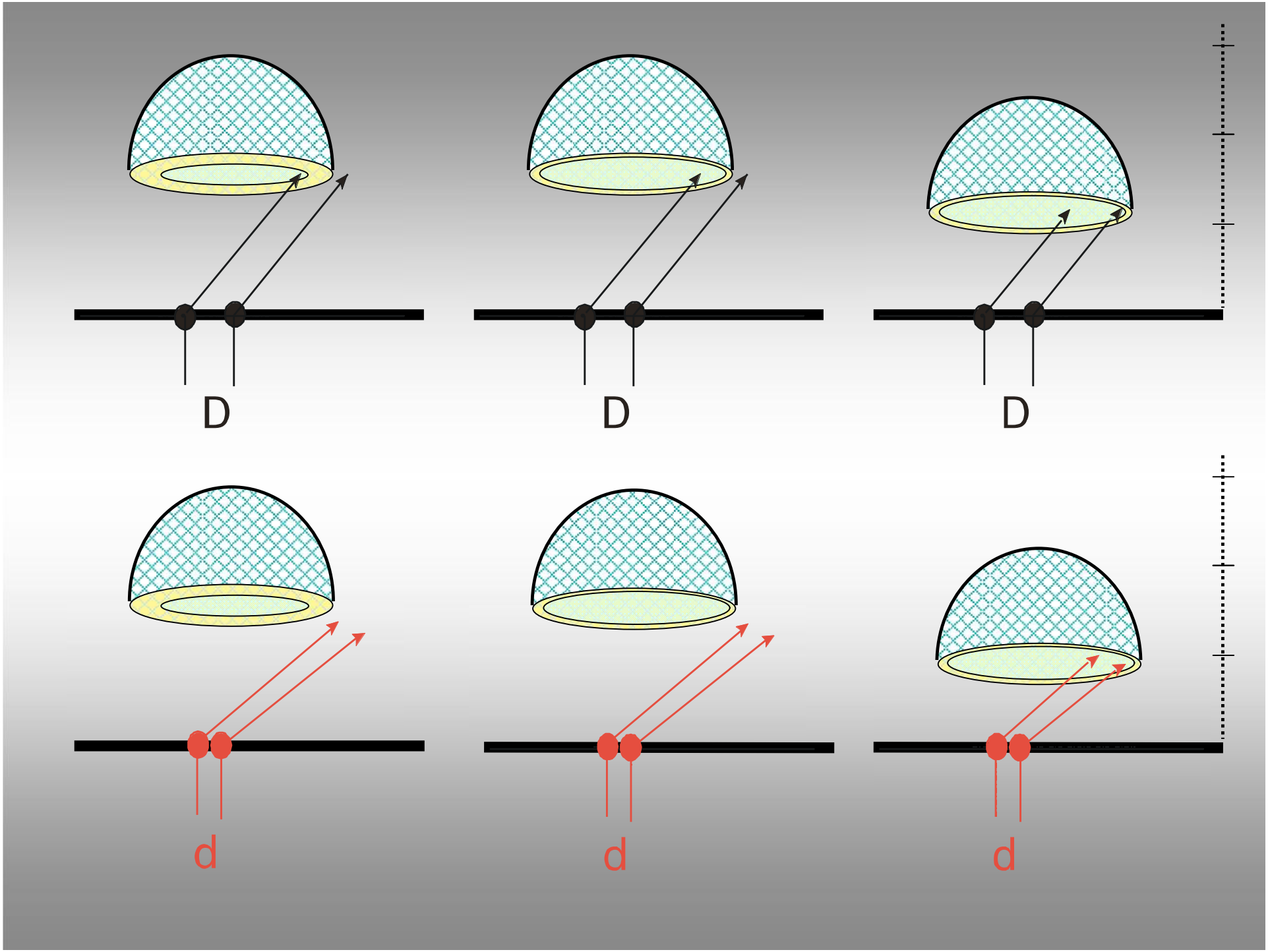
This is caused by
Diffraction

Image of a point source:
The *Airy Pattern*

Larger aperture
and
shorter wavelength

smaller disc

better resolution


D
D
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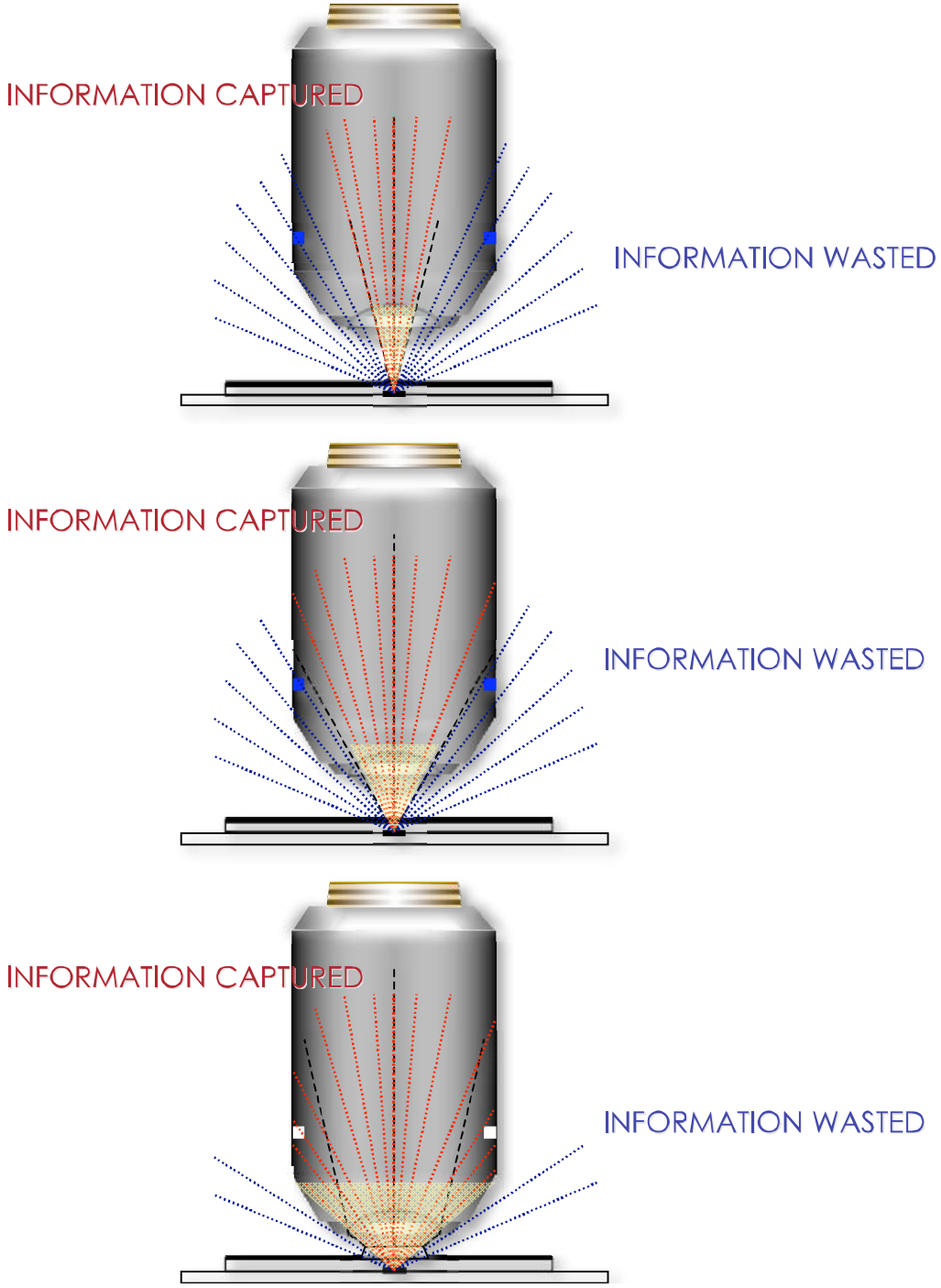
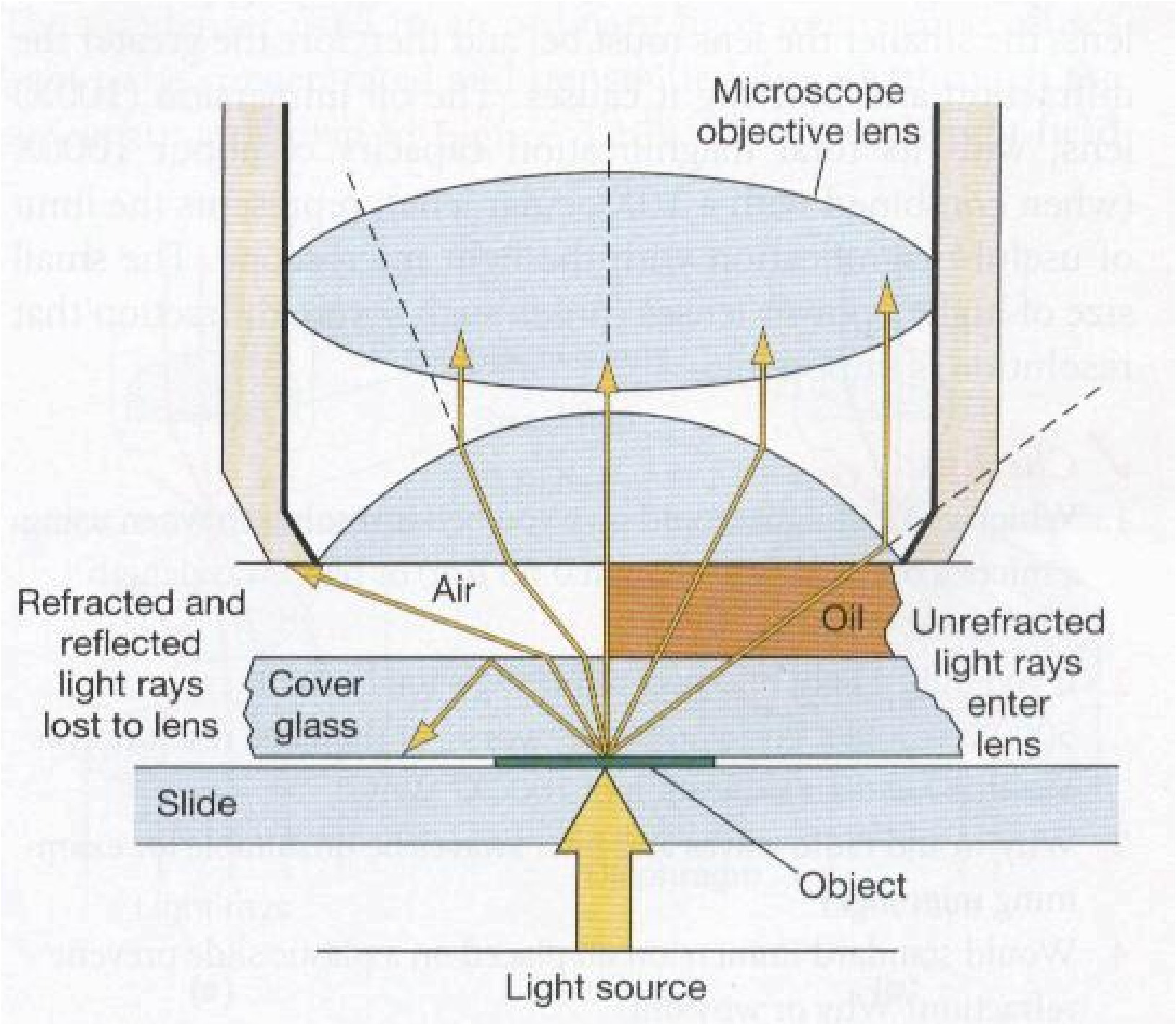
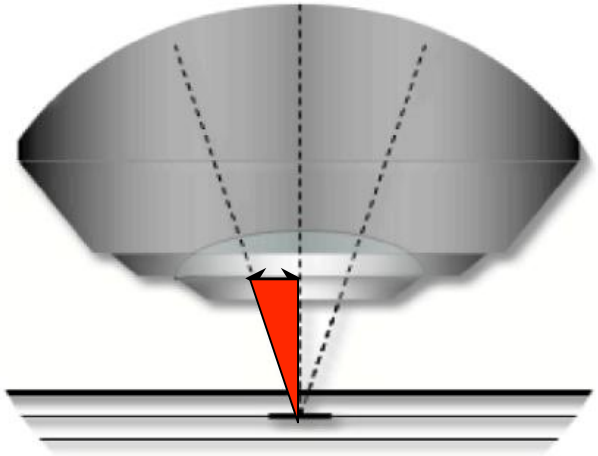
d


INFORMATION CAPTURED
INFORMATION WASTED
INFORMATION CAPTURED
INFORMATION WASTED
INFORMATION CAPTURED
INFORMATION WASTED

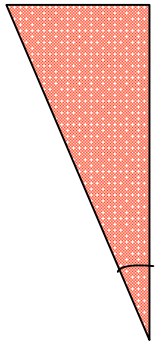

Microscope objective lens
Air
Oil
Refracted and reflected light rays lost to lens
Cover glass
Unrefracted light rays enter lens
Slide
Object
Light source

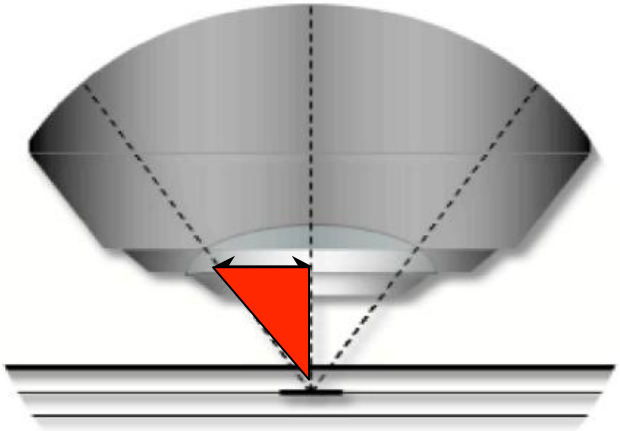


I = 1.0

32 Angle

N.A = 0.6

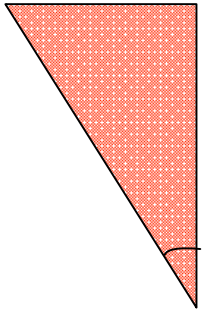


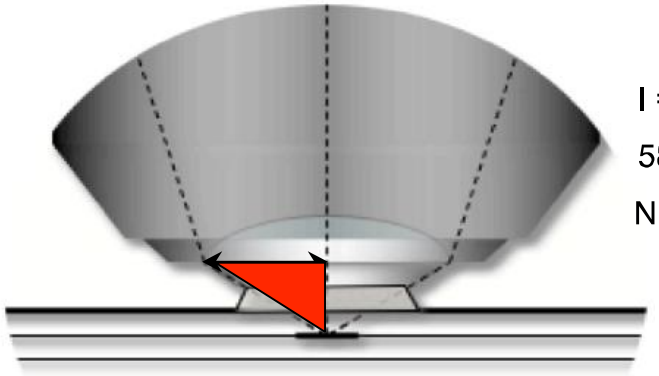


I = 1.0

48 Angle

N.A = 0.8

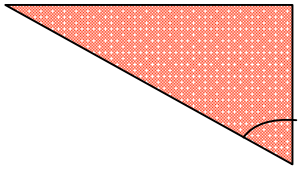


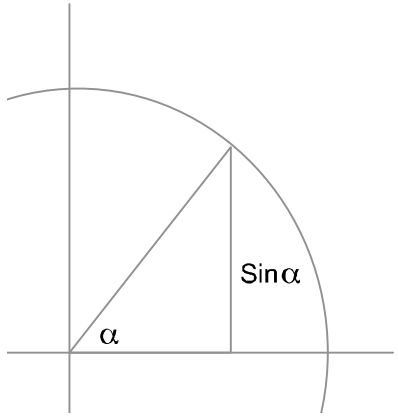


I = 1.515

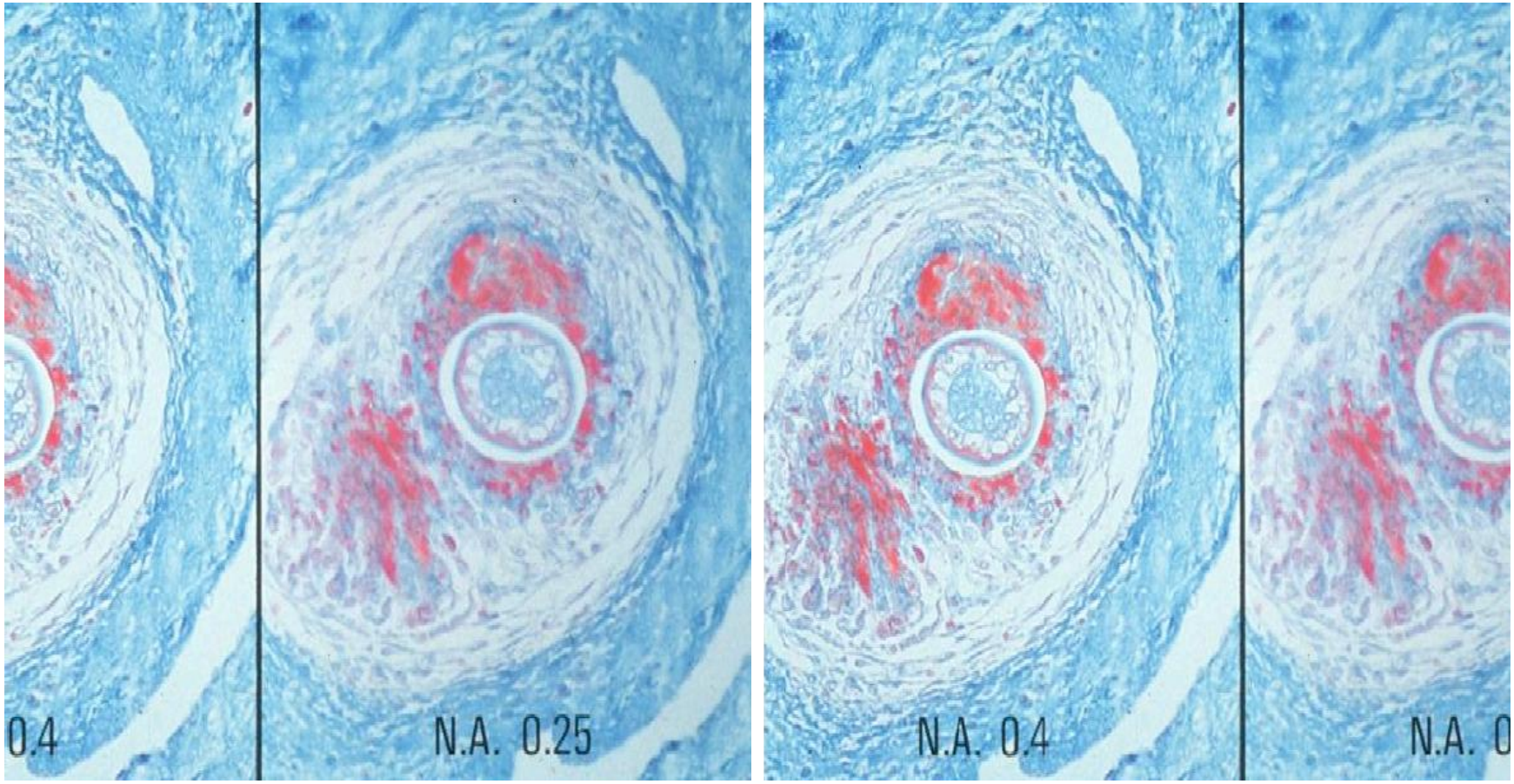
58 Angle

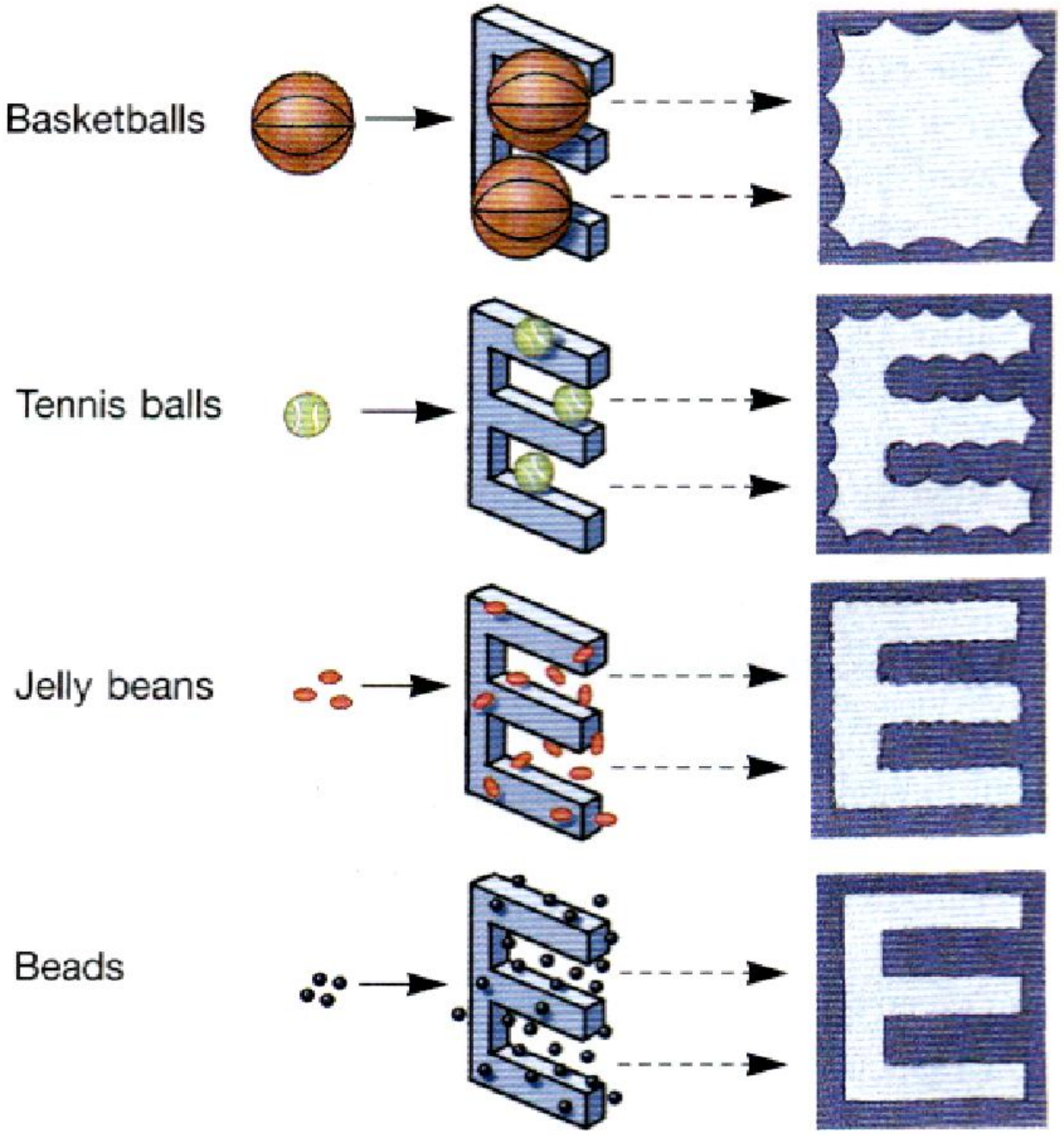
N.A = 1.3

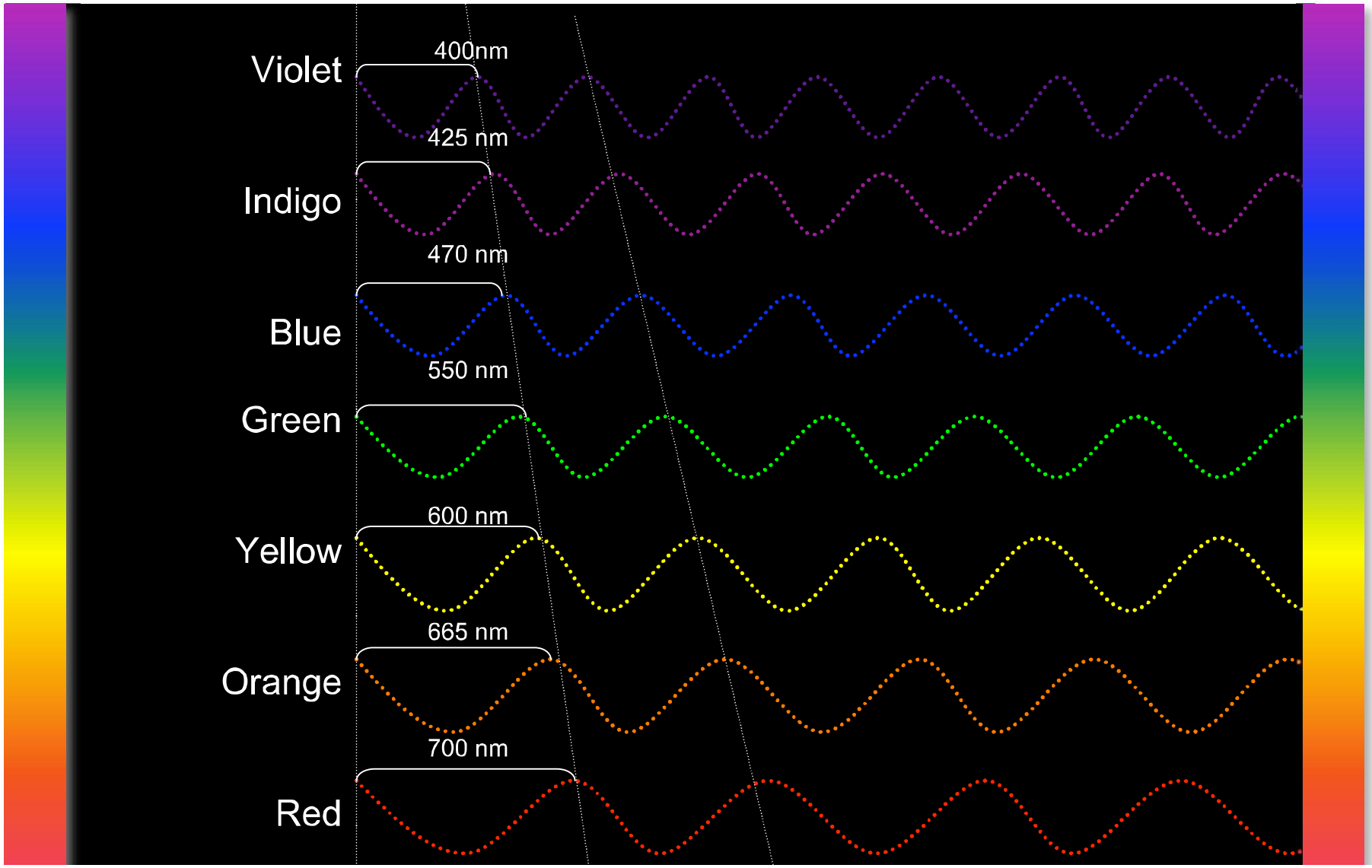




$$N.A = n.\ \mathrm{Sin}\,\alpha$$


0.4
N.A. 0.25
N.A. 0.4
N.A. 0


Basketballs
Tennis balls
Jelly beans
Beads


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

……What does it mean?

A practical example…

$$r = \lambda / 2n \sin \alpha$$

$$r = \lambda / 2NA$$

λ = 550NM (GREEN CENTRE OF THE SPECTRUM)

SIN α = 0.65 (HALF THE ANGLE 40.5o; ACCEPTANCE ANGLE 81o)

Typical value for 40X objective

$$r = \frac{550}{2 \times 0.65} = 423\text{nm}$$