

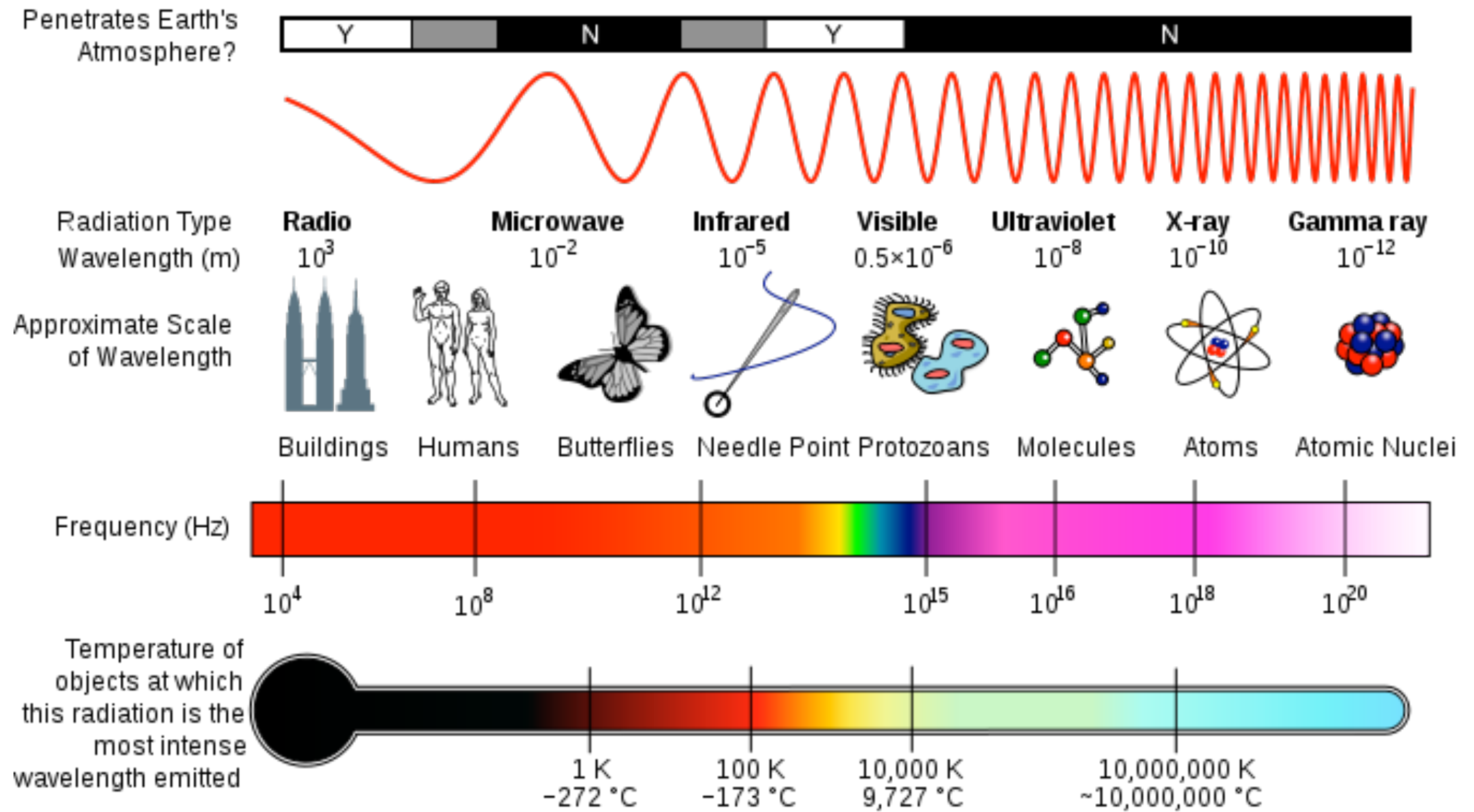


microscopy

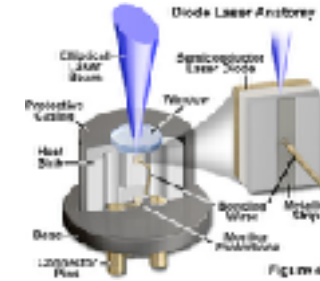
Carla Carmelo Rosa,
FCUP/INESC TEC
ccrosa@fc.up.pt

light and radiation

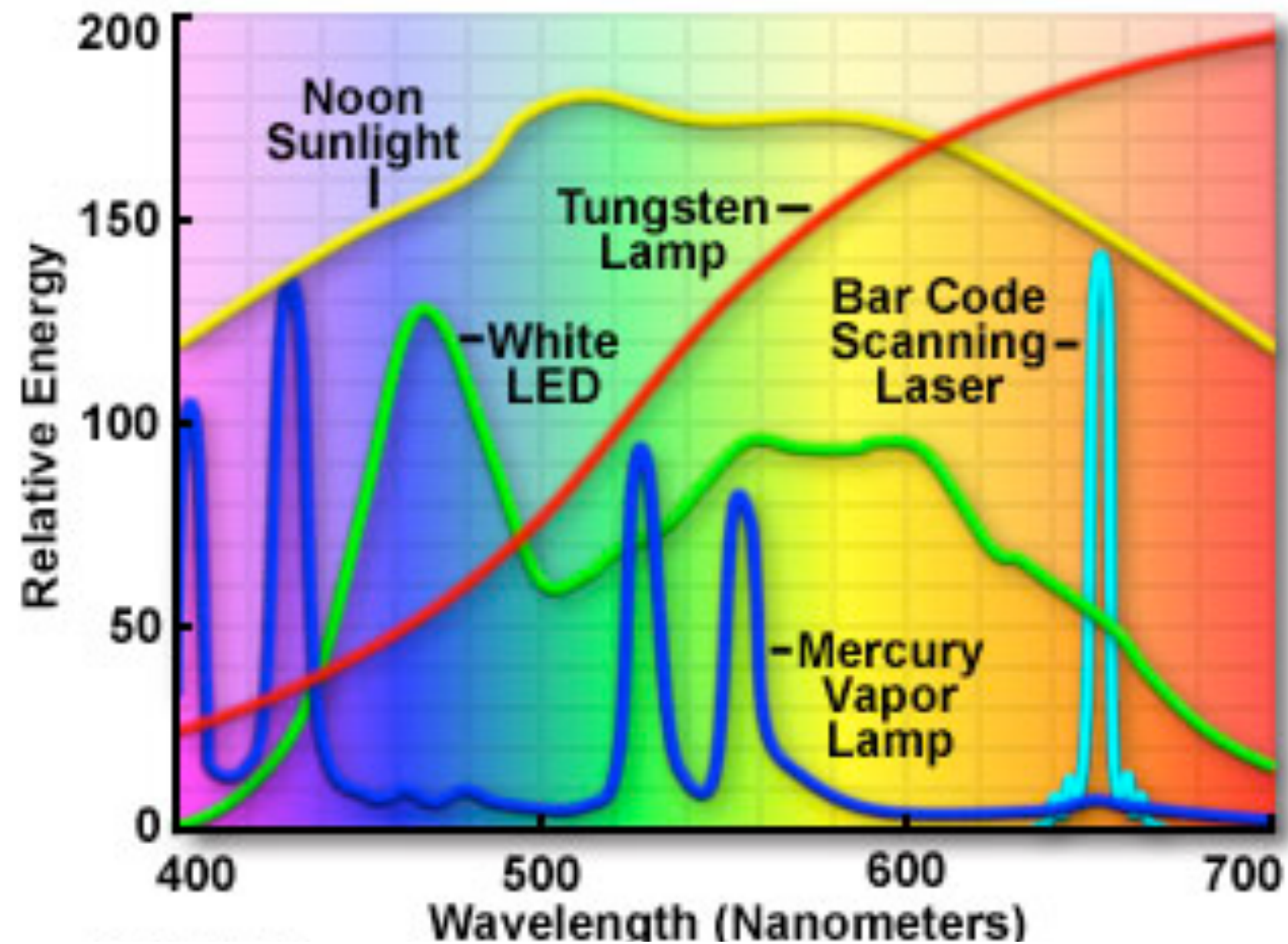
electromagnetic spectrum...



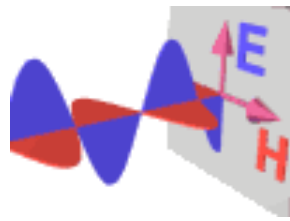
sources



Spectra From Common Sources of Visible Light

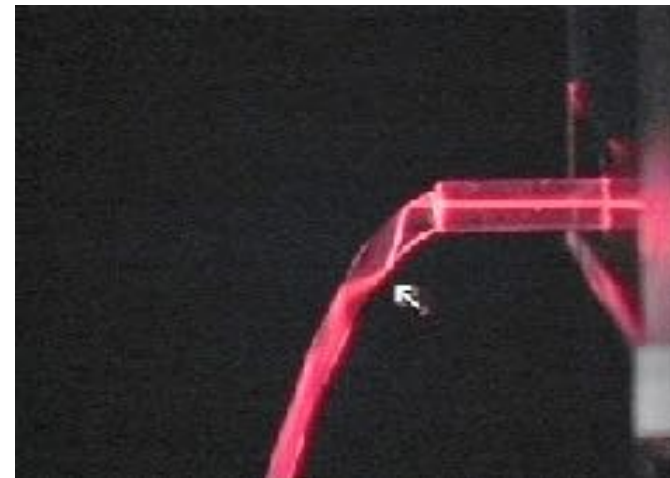
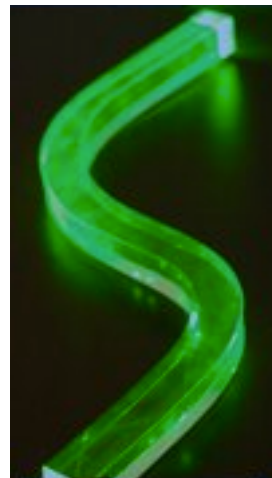


light



transfer of energy/information

- wave guides



- lenses

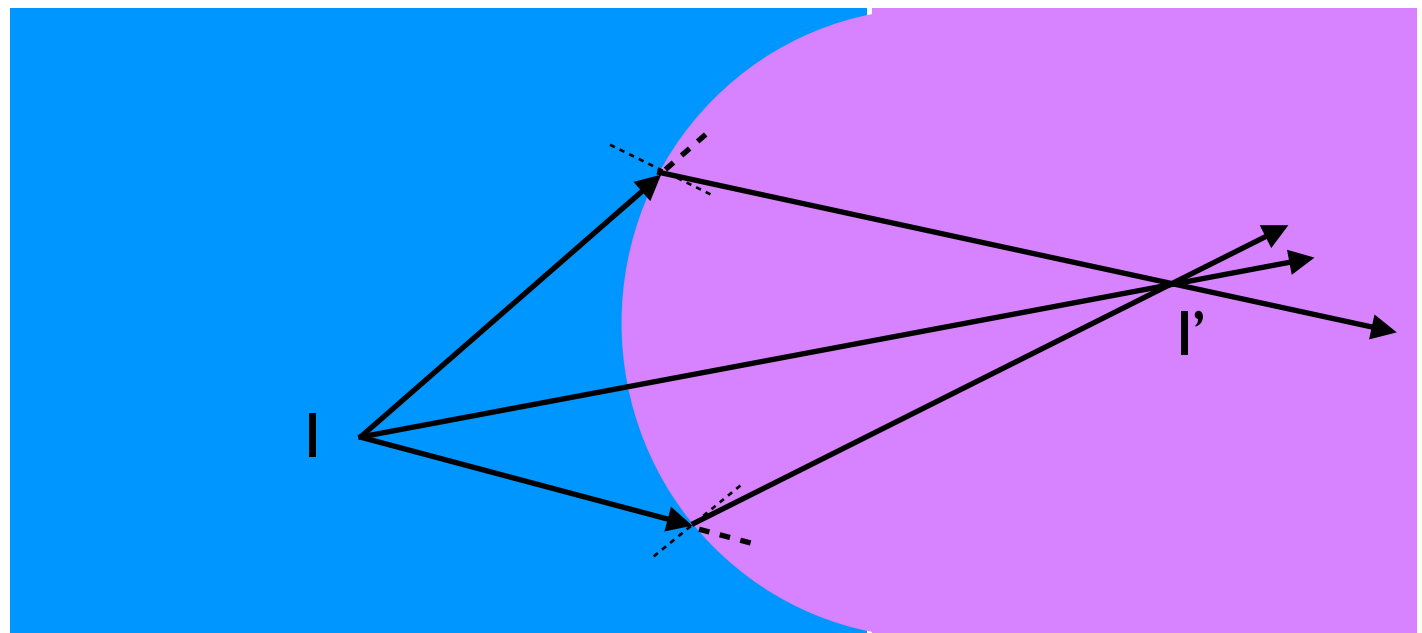
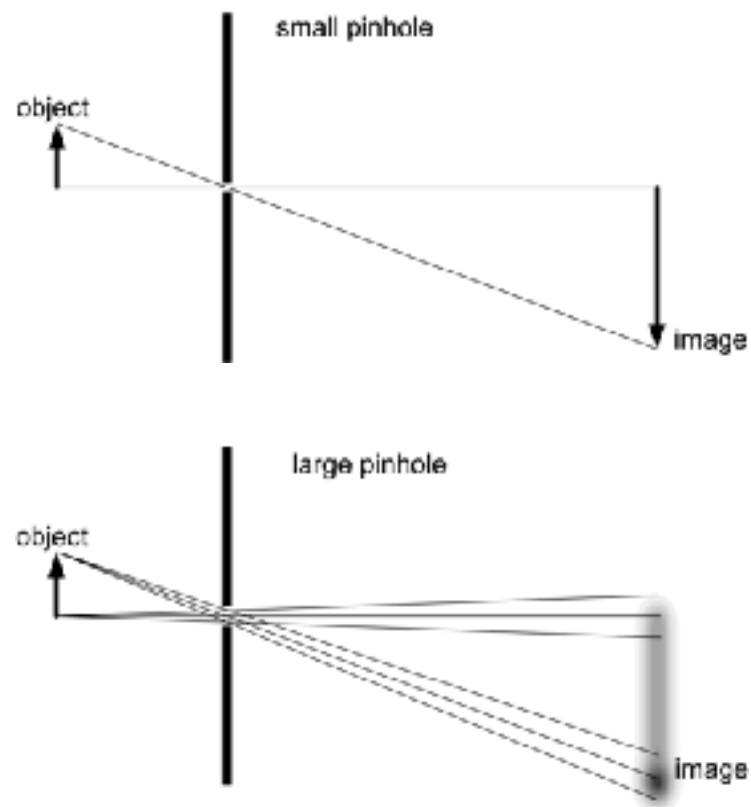


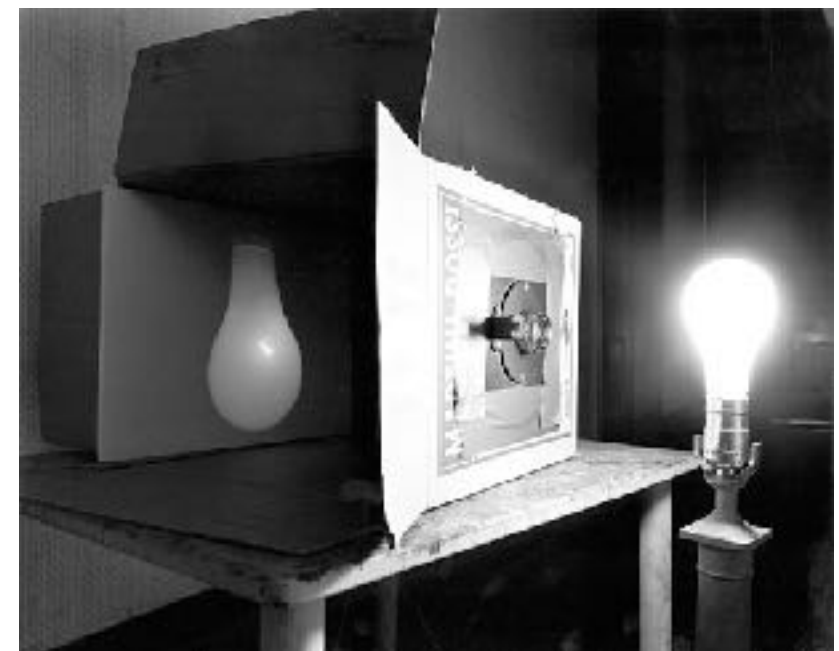
image formation

pin-hole camera

- small apertures
- lensless image formation



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image formation

lens

- refraction
- focal length f

human vision

- relaxed eye
- accommodated eye

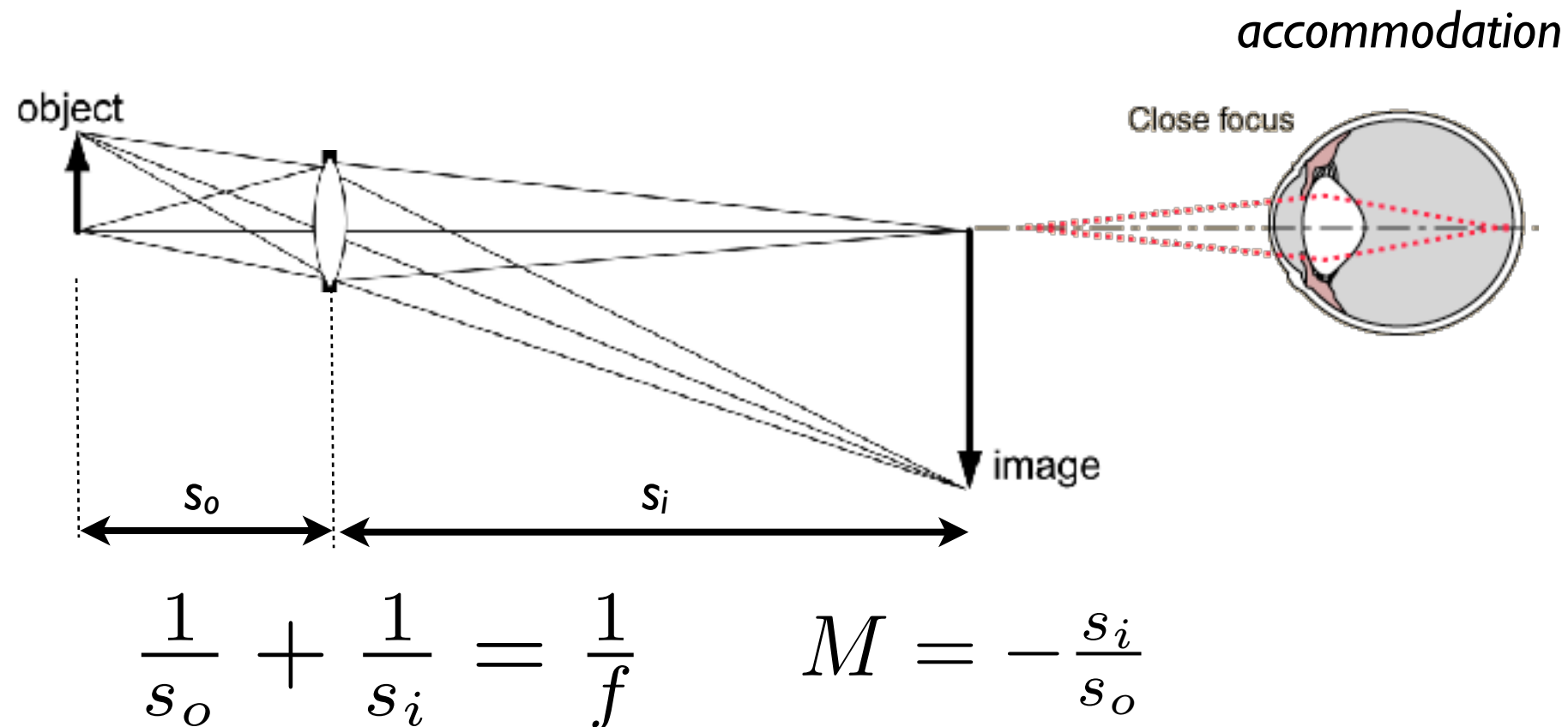


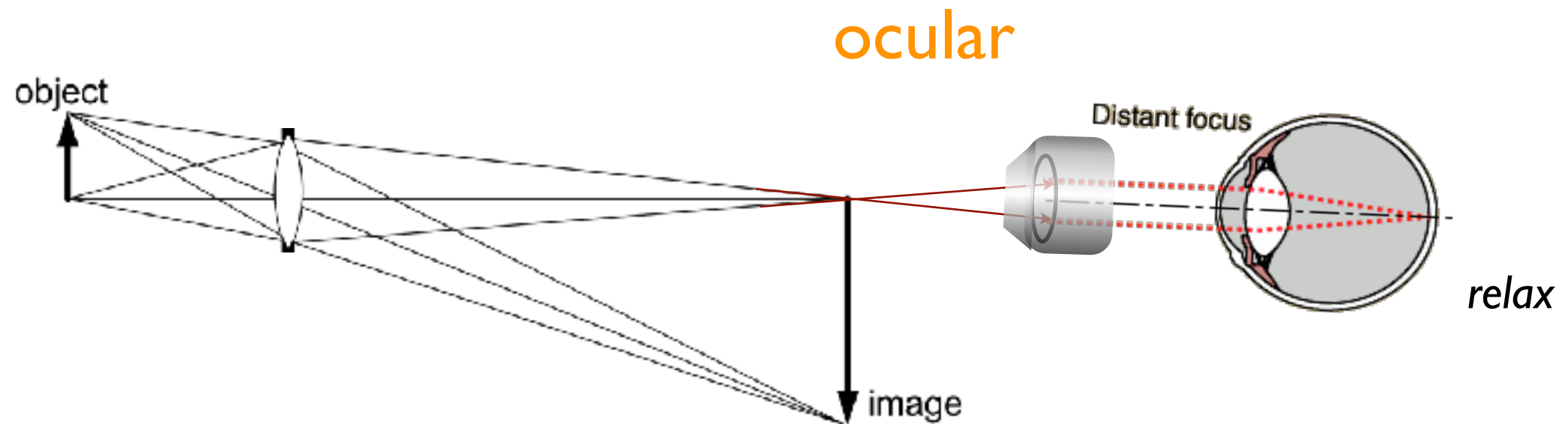
image formation

lens

- refraction
- focal length f

human vision

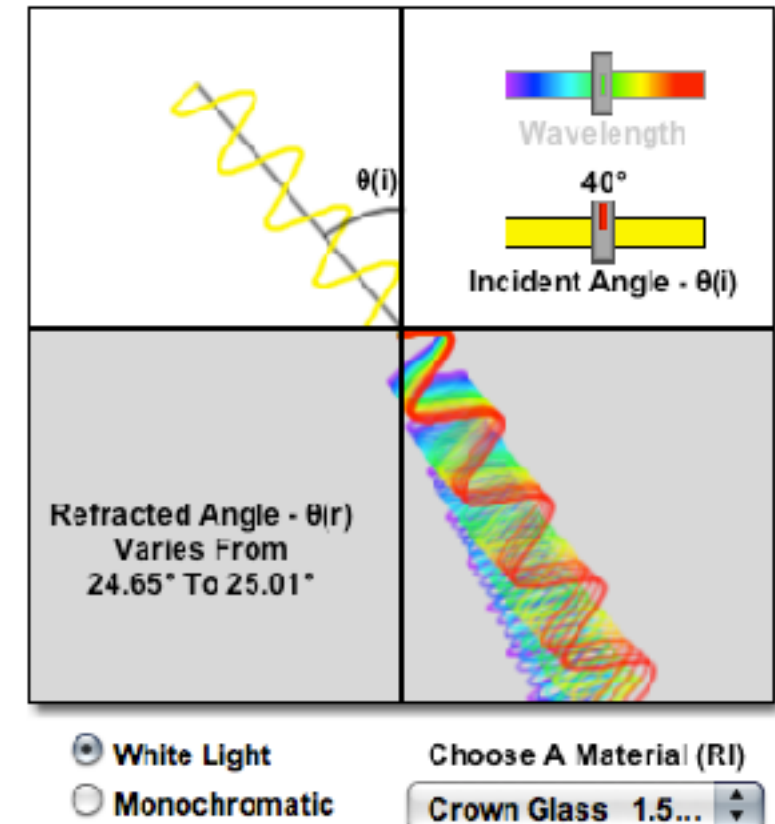
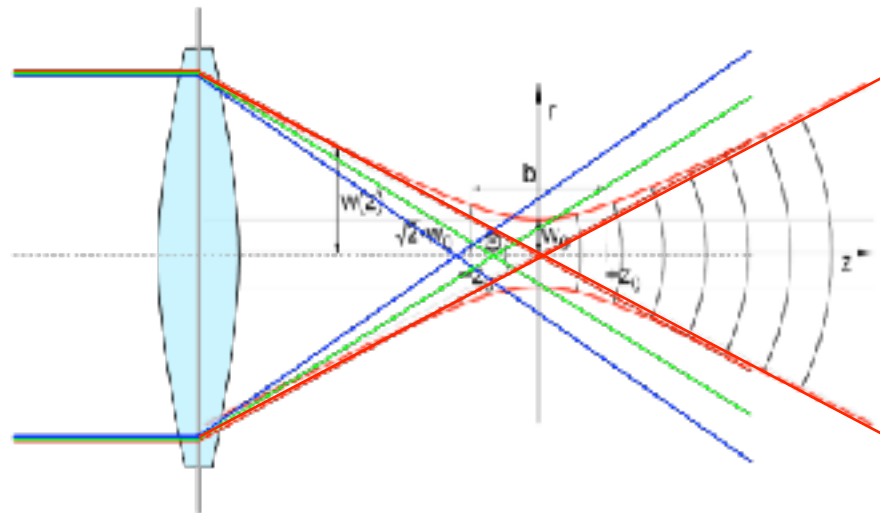
- relaxed eye
- accommodated eye



ray tracing and facts!

focal position

- chromatic aberration



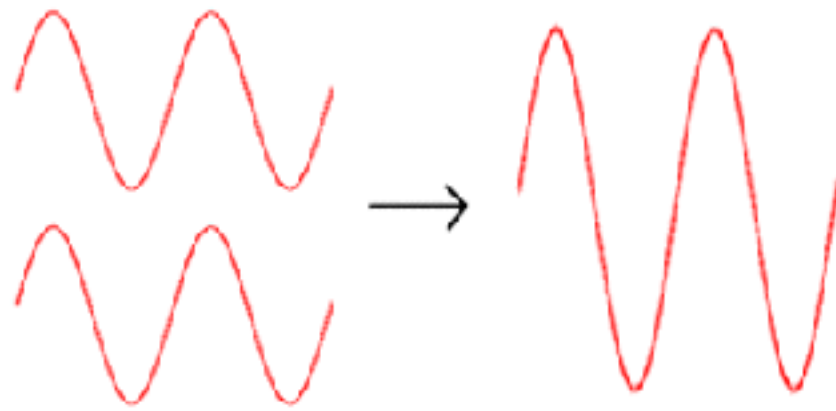
focal spot

- finite size, due to diffraction!
- depth of focus, due to diffraction

interference

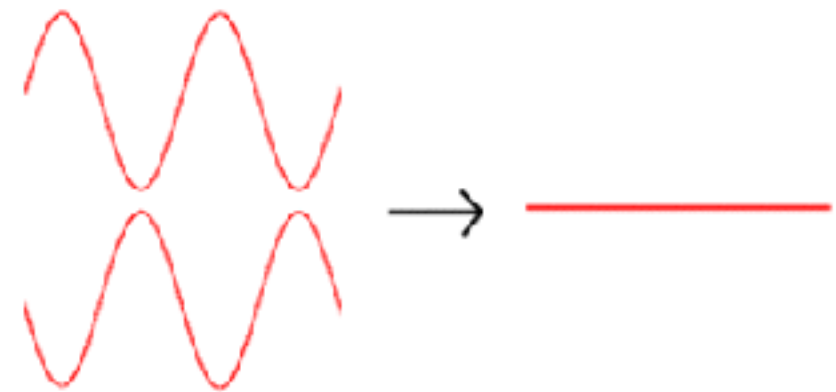
wave nature of light and matter + coherent sources (lasers)

Phase shift = 0



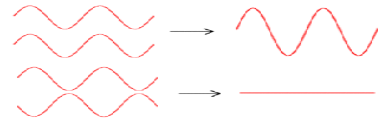
constructive
interference

Phase shift = $\frac{1}{2} \lambda$



destructive
interference

interference



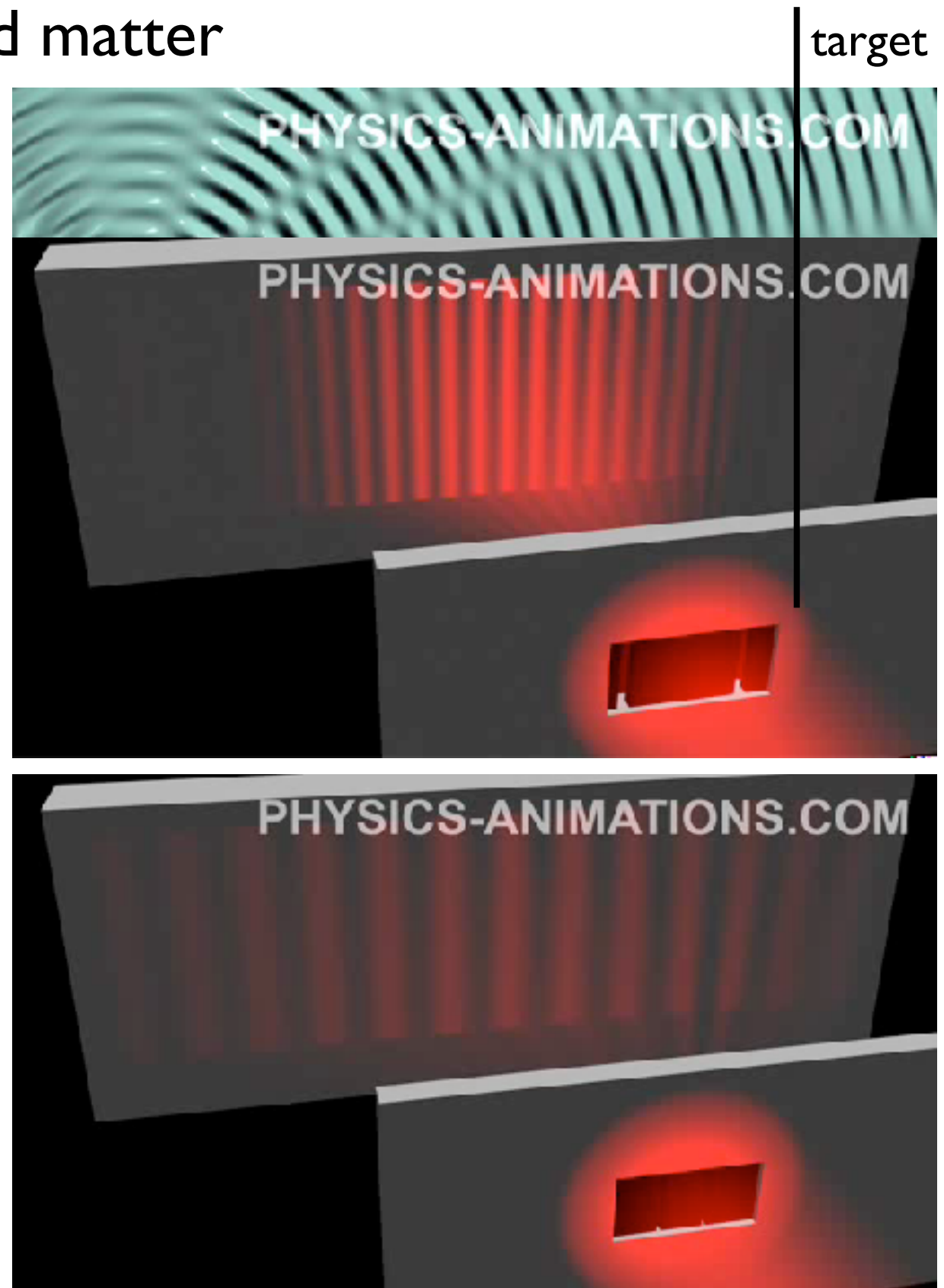
wave nature of light and matter

multiple slits

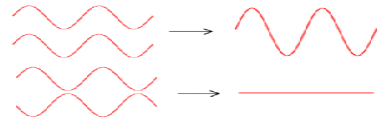
- separation d
- slit width

interference

- when $\lambda \sim d$



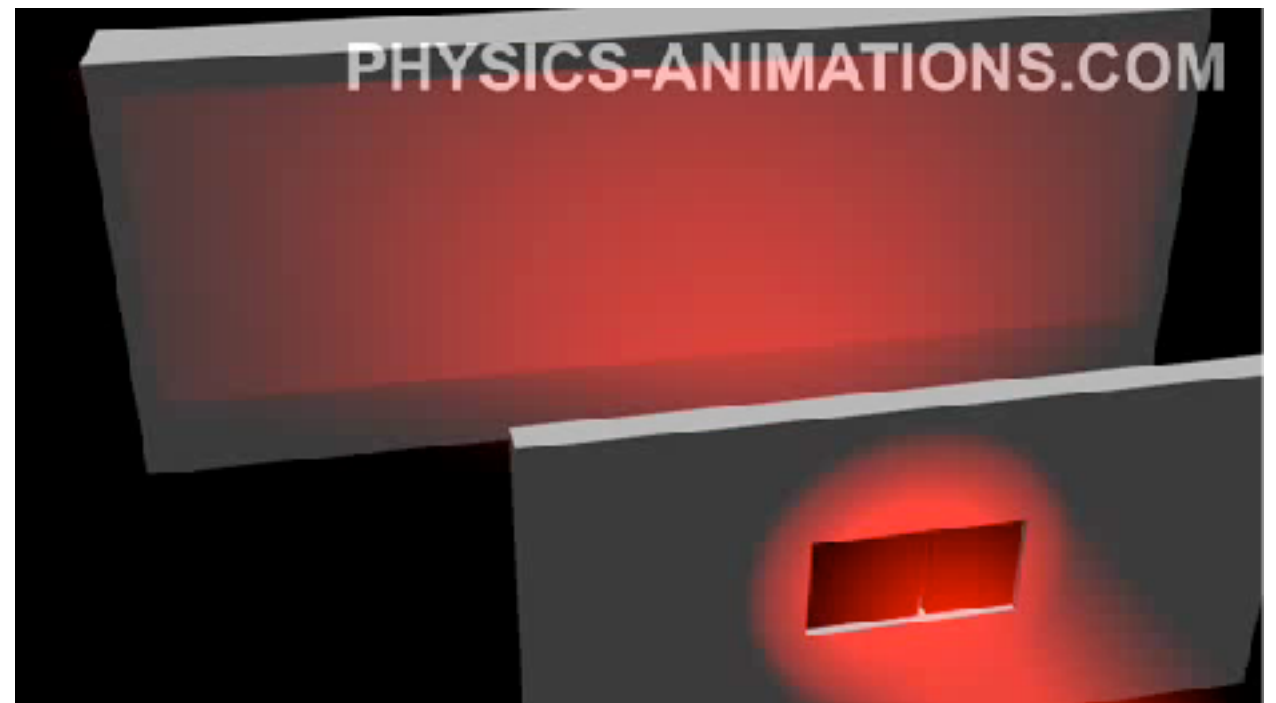
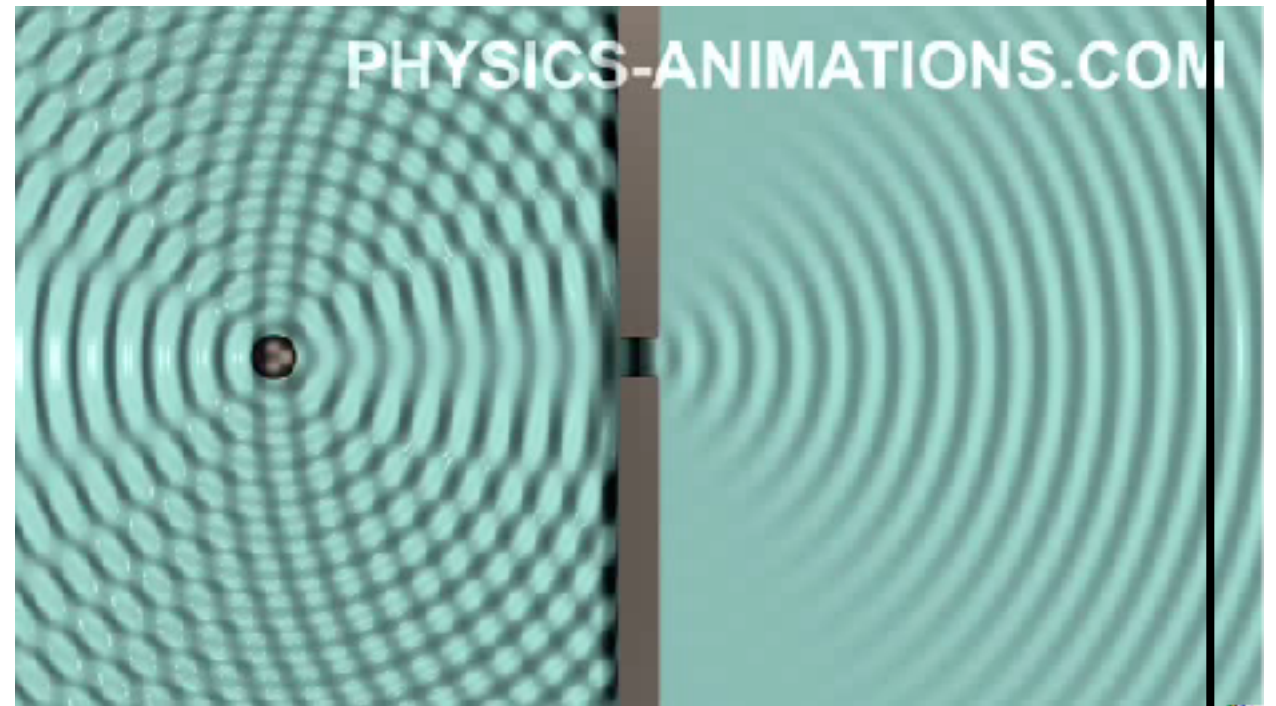
diffraction



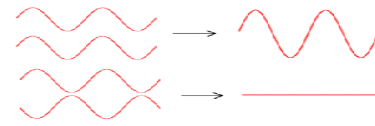
geometry

- small objects
or
small apertures
- $\lambda \sim d$

target



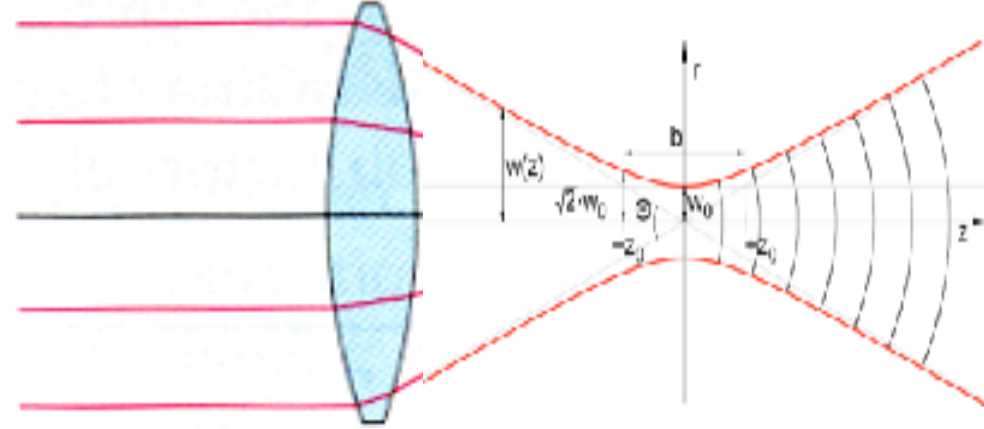
diffraction manifestations



lens focal spot

- diffraction limited

Airy disk



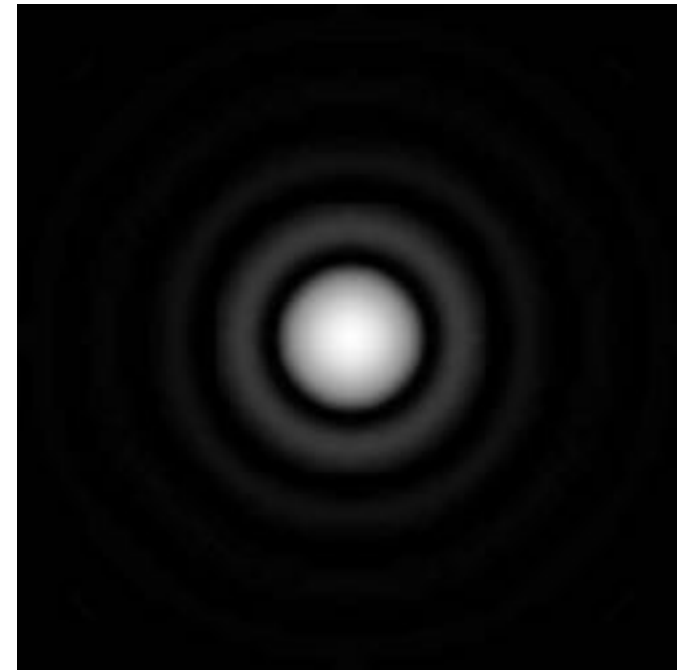
intensity at focal point

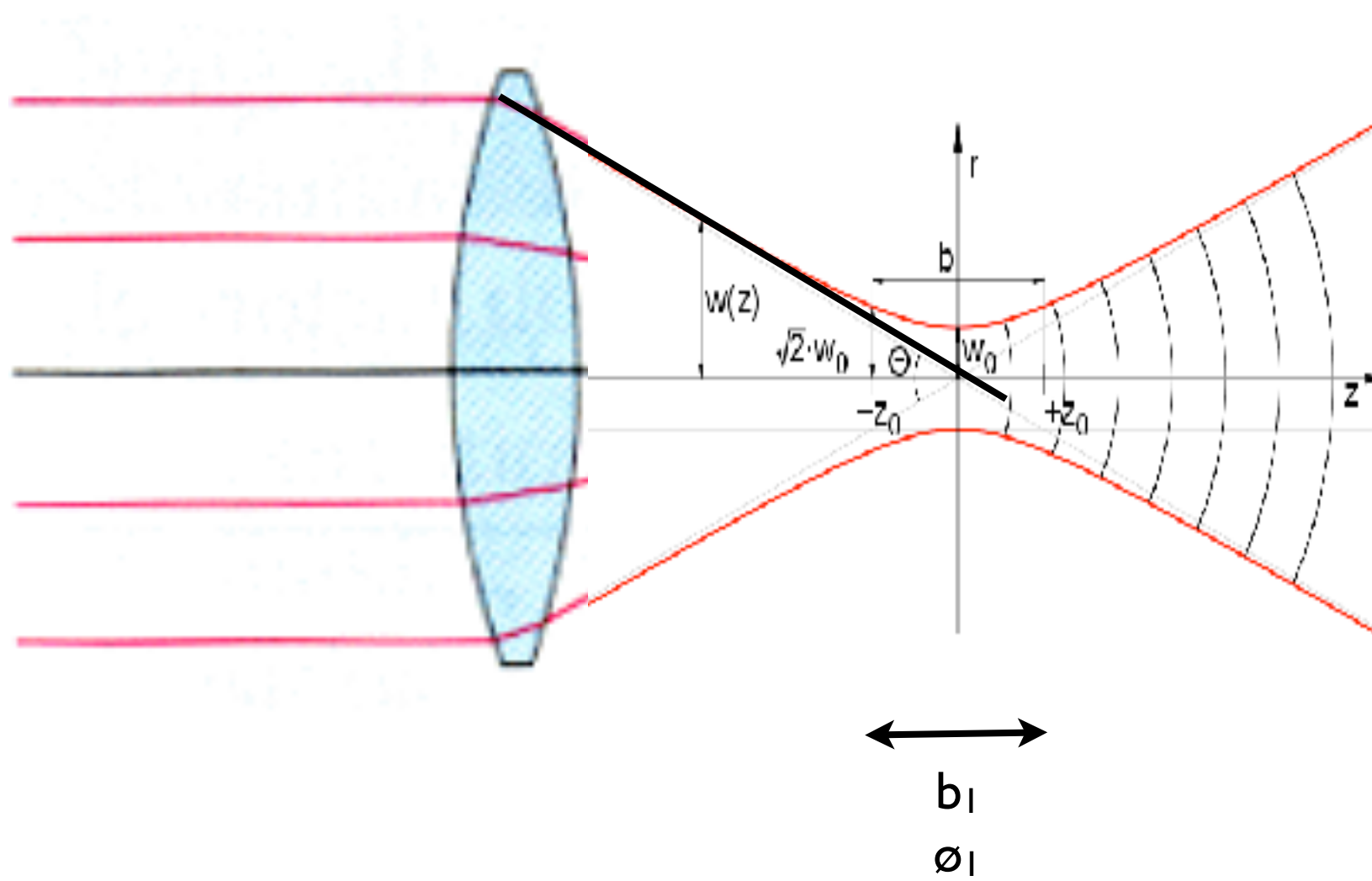
- 1st minimum location

$$r_{\text{Airy}} = 1.22 \frac{\lambda}{NA}$$

numerical aperture

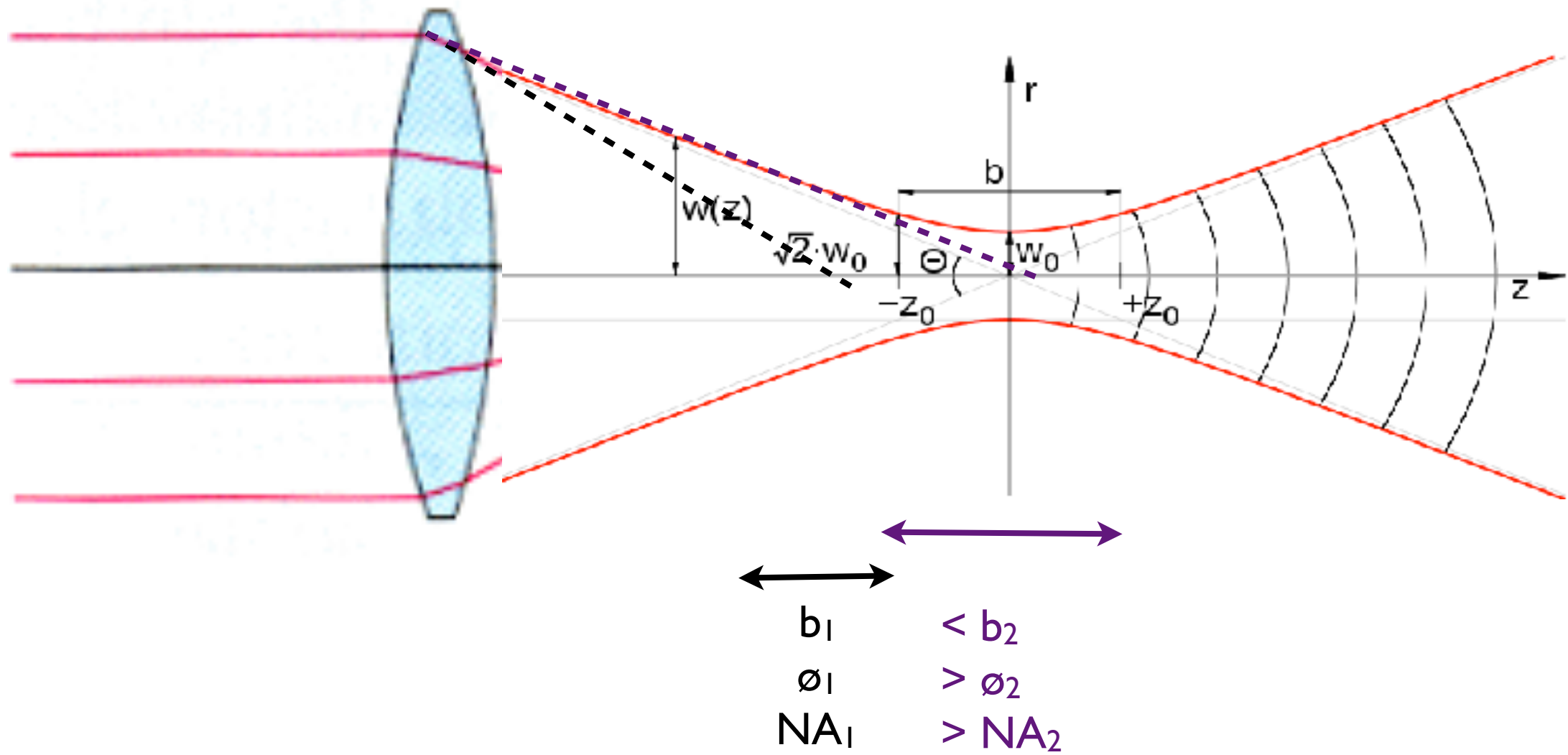
$$NA = n \sin \theta$$





$$r_{\text{Airy}} = 1.22 \frac{\lambda}{NA}$$

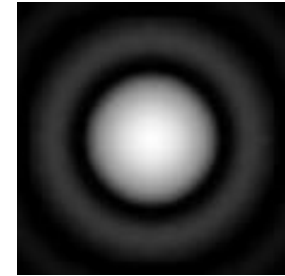
$$NA = n \sin \theta$$



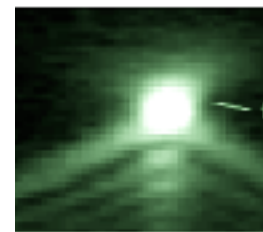
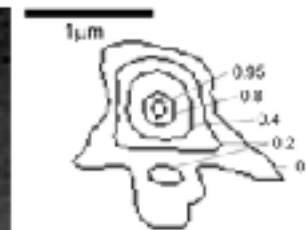
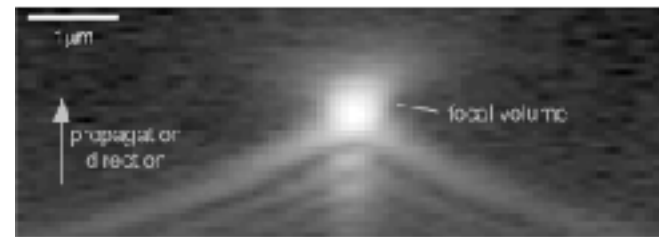
important notes regarding imaging systems

point spread function (PSF)

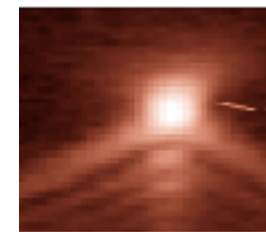
- ideal: Airy disc



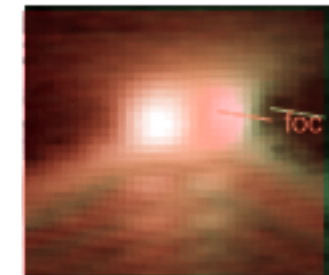
- the real case
... an example



+

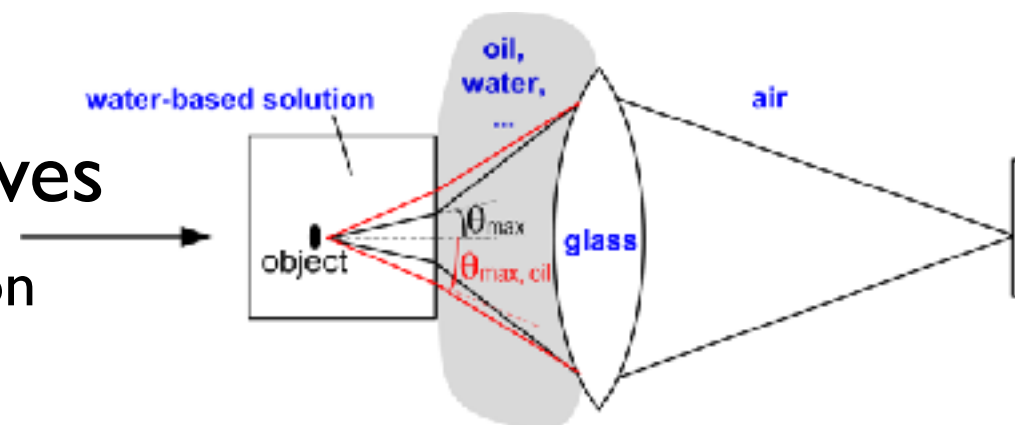


=



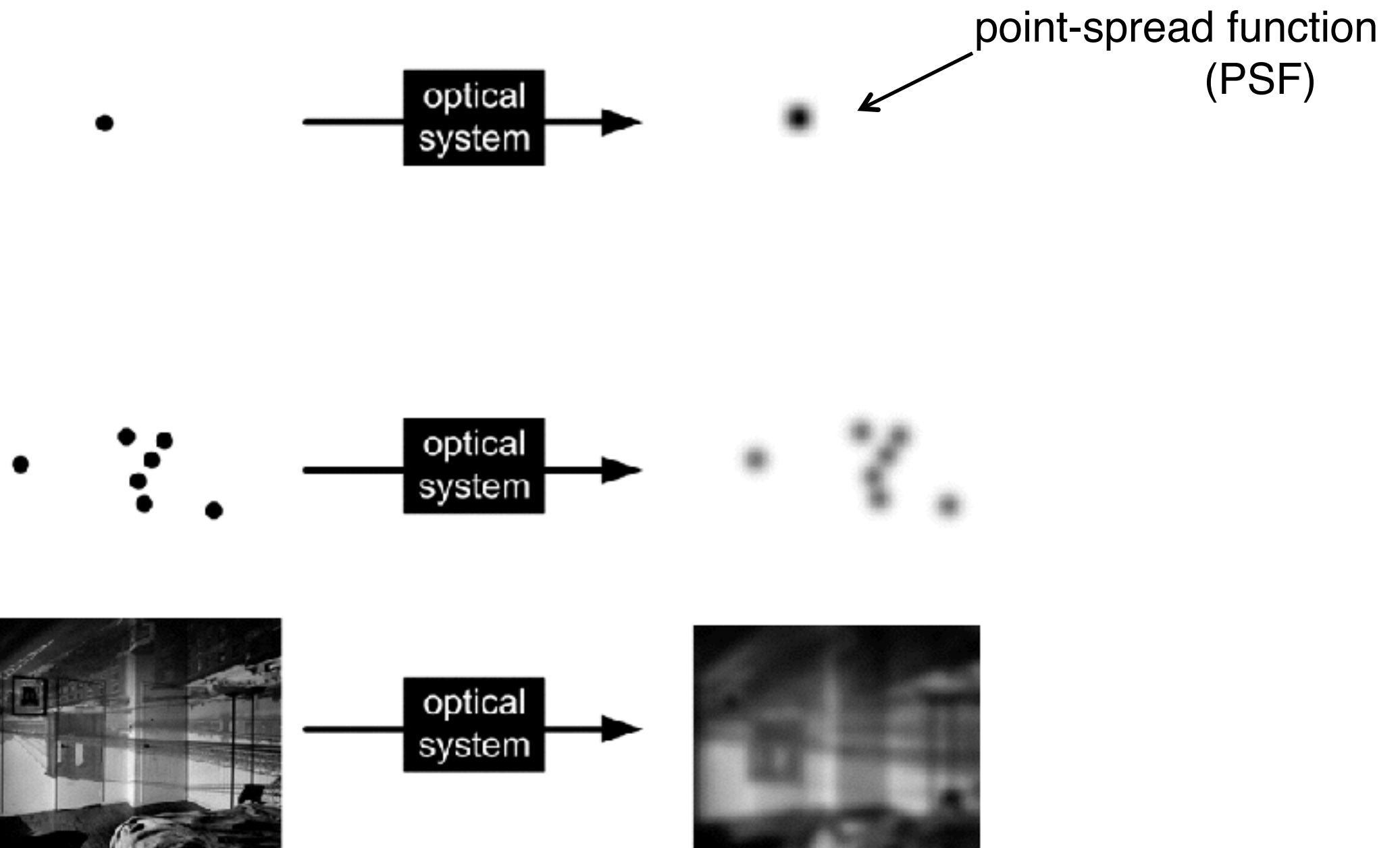
numerical aperture

- immersion oil objectives
 - improve lateral resolution



object space

image space



$$\text{Object} \otimes \text{PSF} = \text{Image}$$

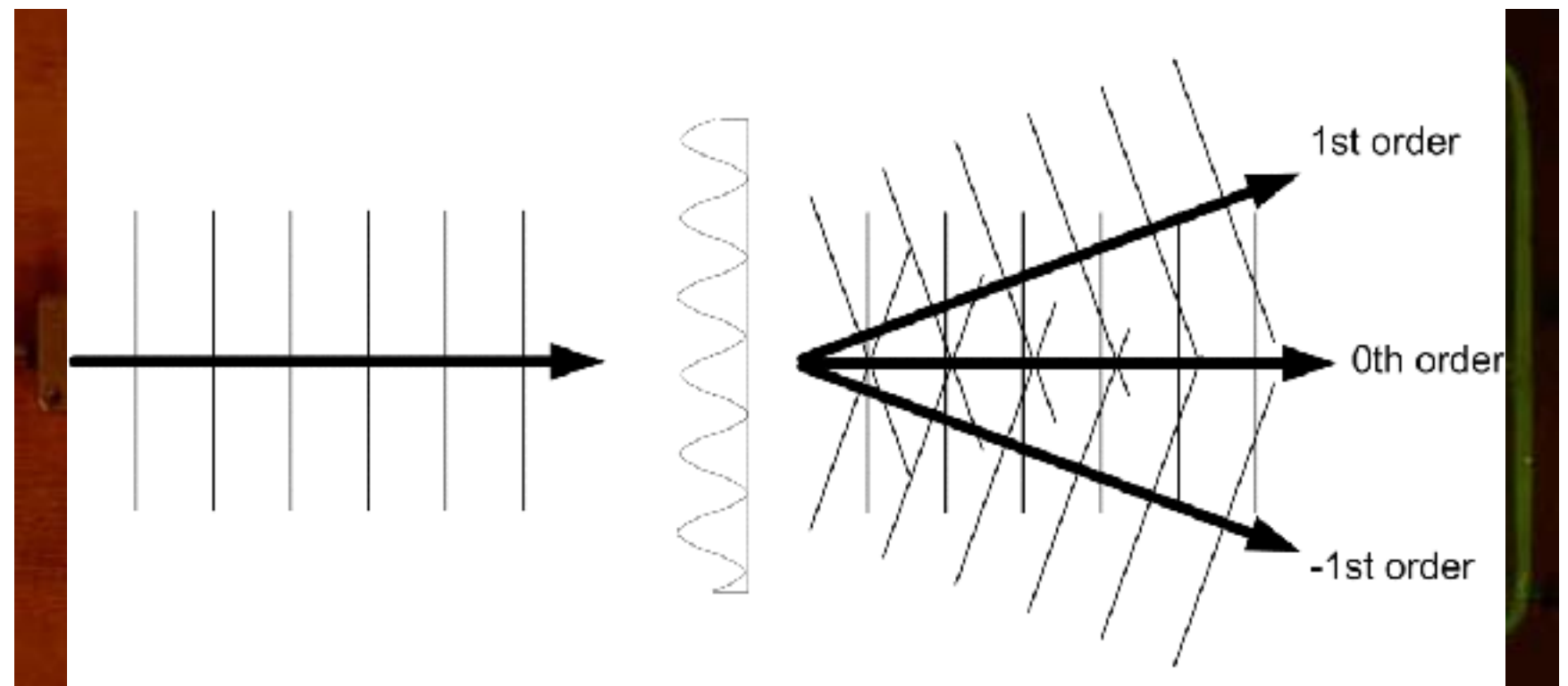
convolution

imaging thin samples

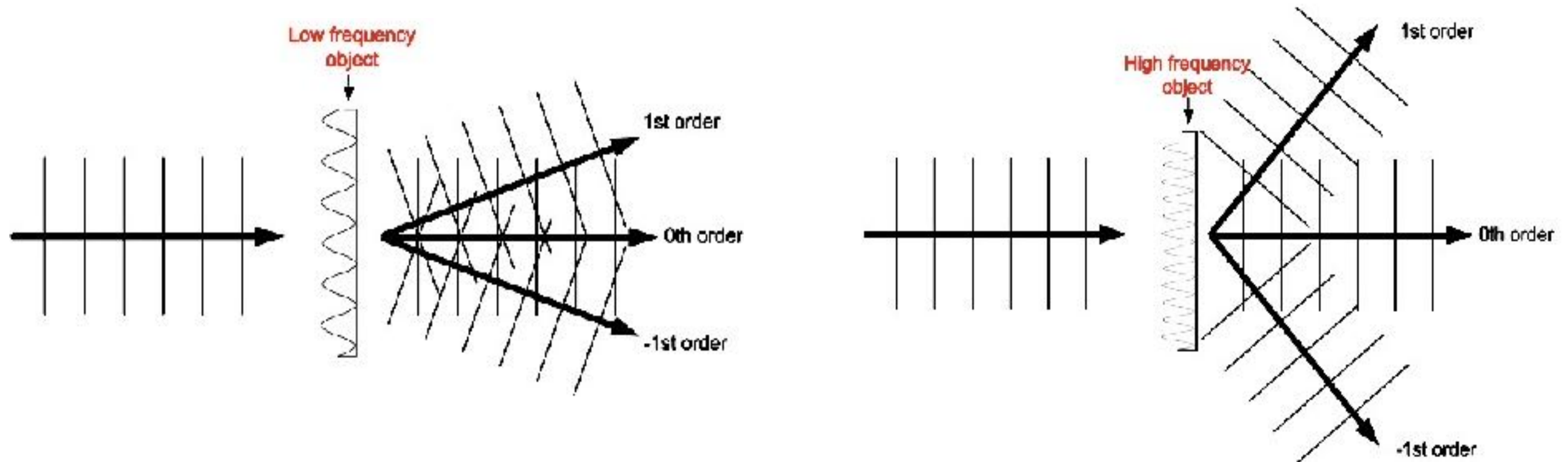
light crosses the object

- microstructures, $\lambda \sim d$
- diffraction/interference occurs!

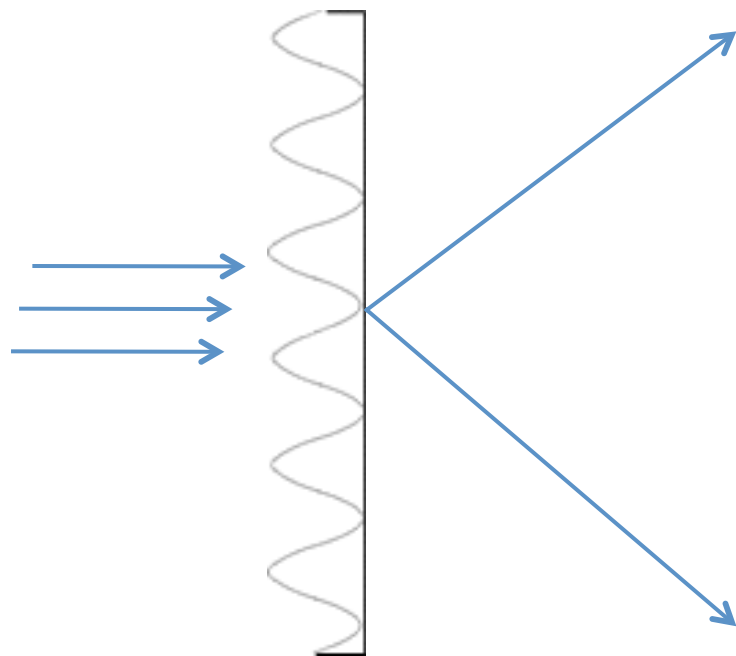
objects composed by a single
spatial frequency



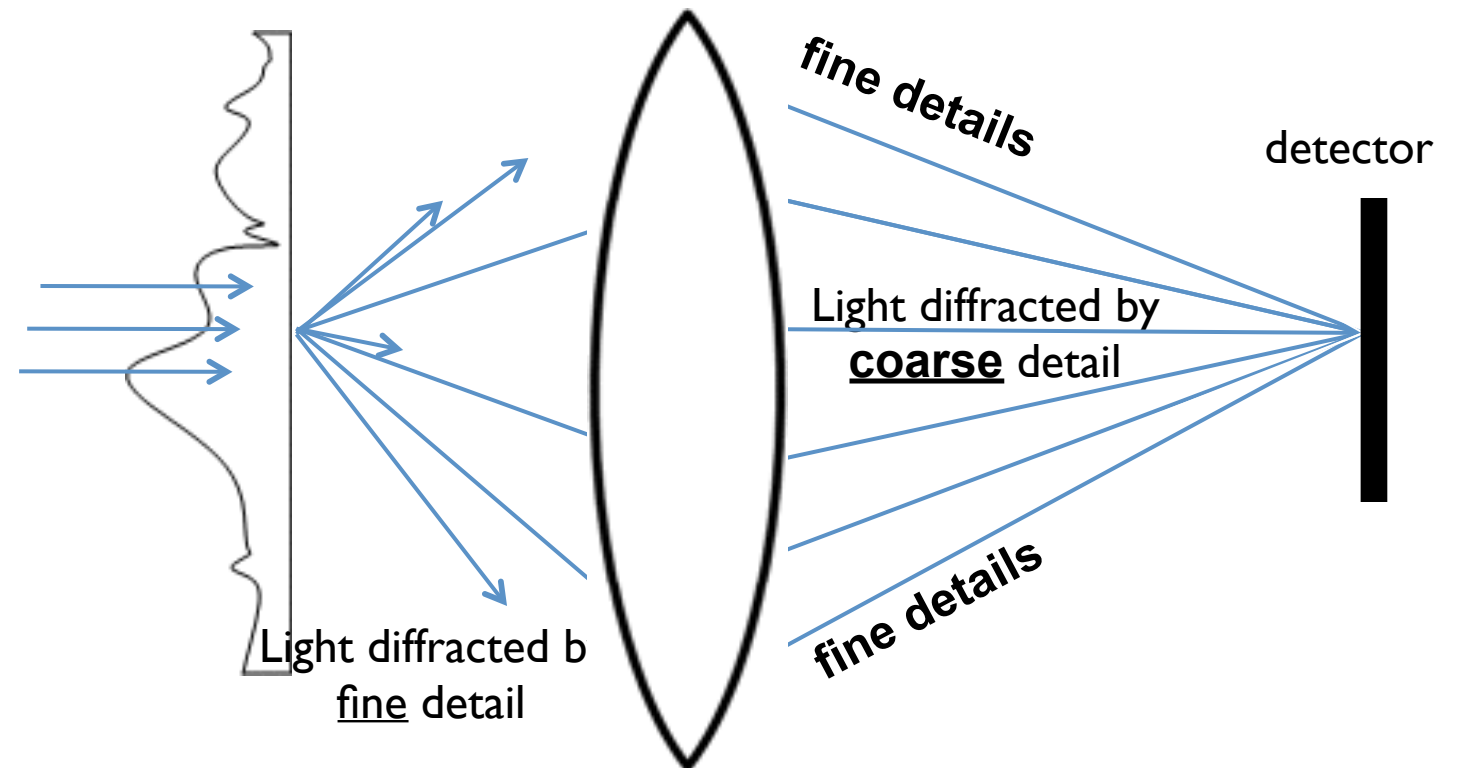
imaging *thin* samples



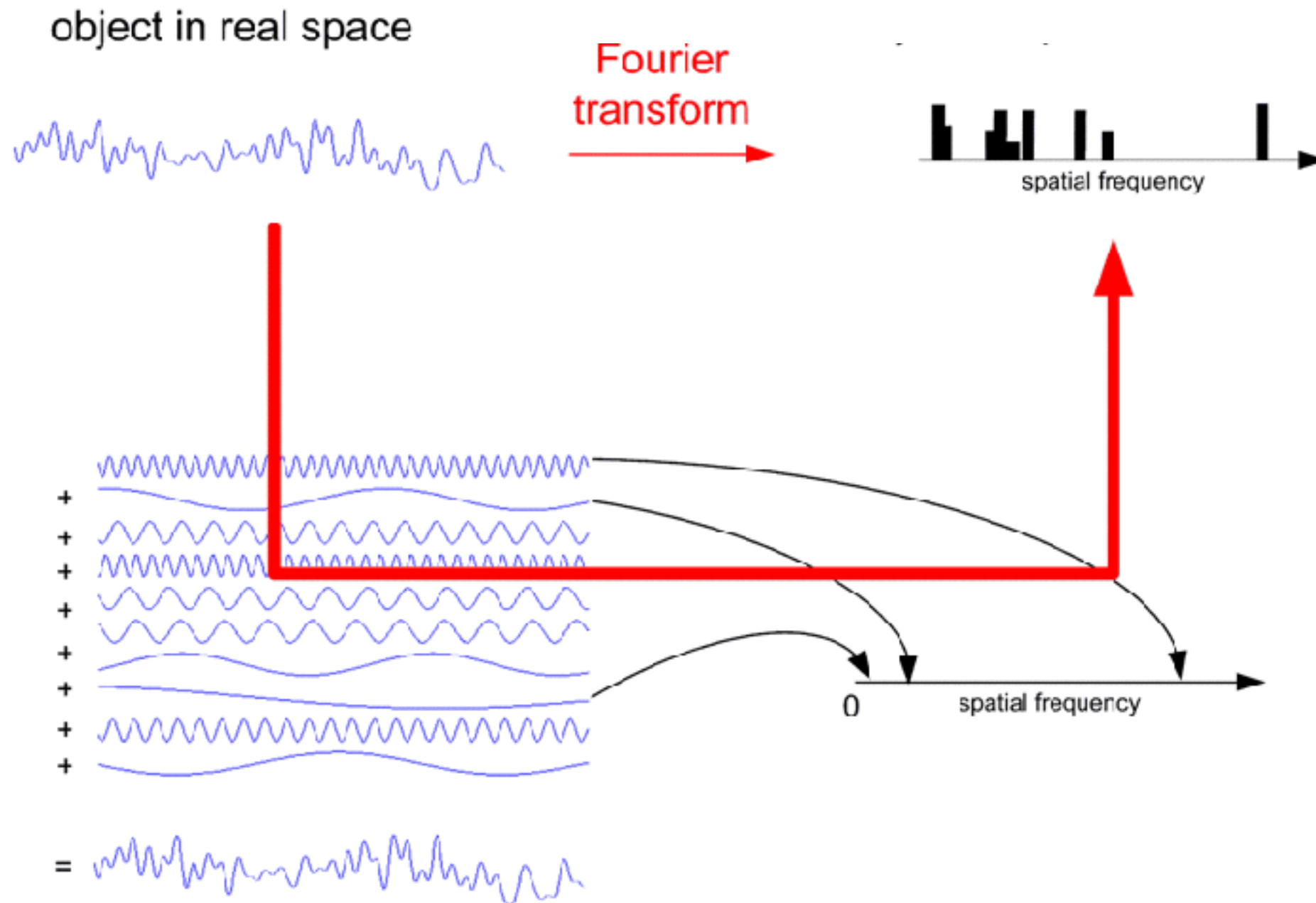
Model object



Real object



objects in the Fourier (spectral) space



Each Fourier component
(sinusoidal object)
diffracts its own beam

objects in the Fourier (spectral) space

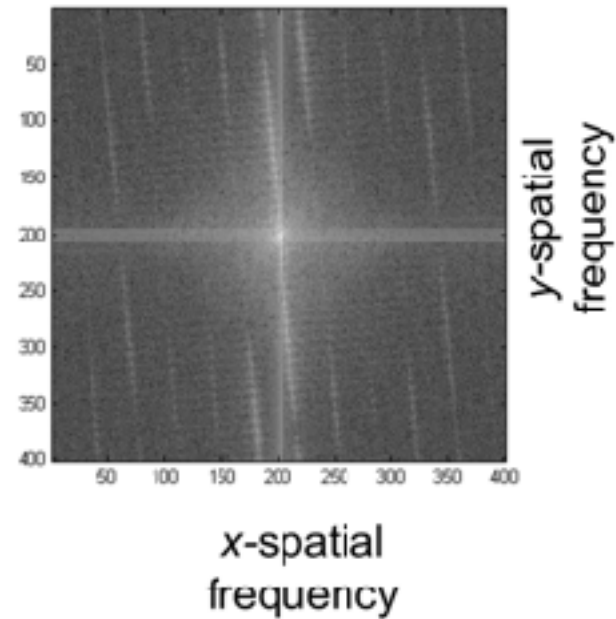
Object in real space



Fourier
transform



Object in spectral space



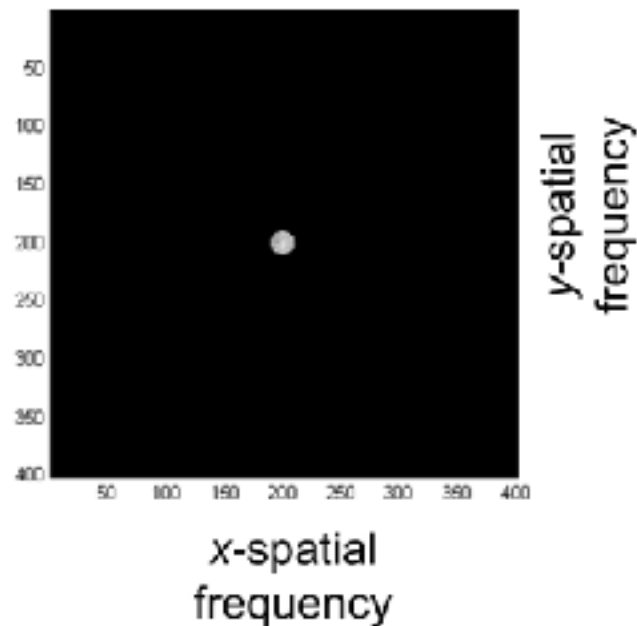
Inverse
Fourier
transform



Object in real space



removing high
frequency components



Inverse
Fourier
transform

