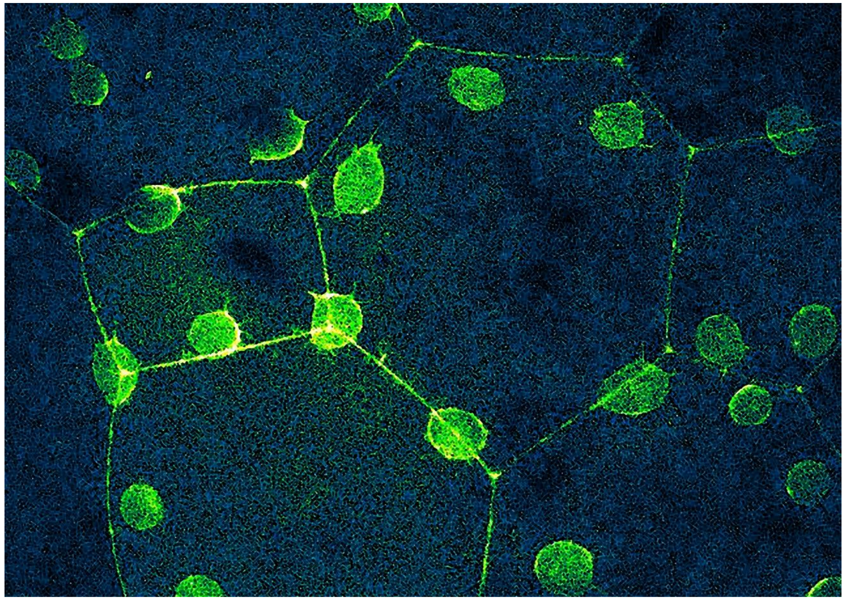
EMBO Practical Course

Optics, Forces & Microscopy 2026



Principles of Optics I

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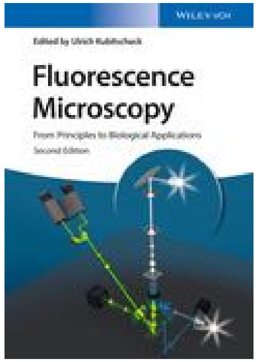
Contents

1. Additional Information

Good Collection of Online Learning Tools

http://micro.magnet.fsu.edu/primer/

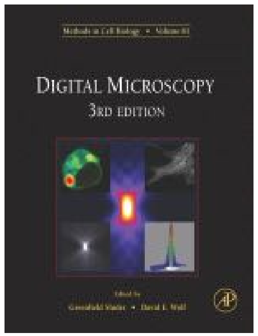
https://zeiss-campus.magnet.fsu.edu/



Books

Fluorescence Microscopy, 2017, 2nd edition, ed. U. Kubitscheck, Wiley-VCH

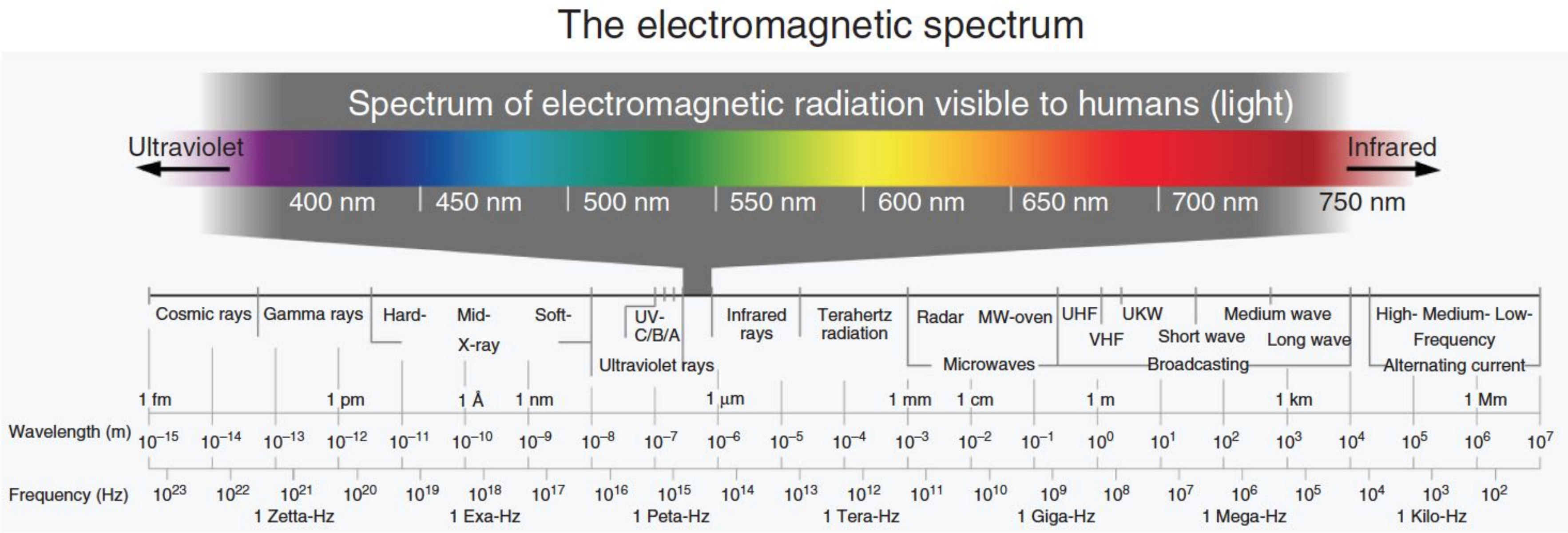
Digital Microscopy, Meth Cell Biology, 2007 ed. G. Sluder and D.E. Wolf



2. Basics:

- waves
- diffraction
- lenses
- aberrations

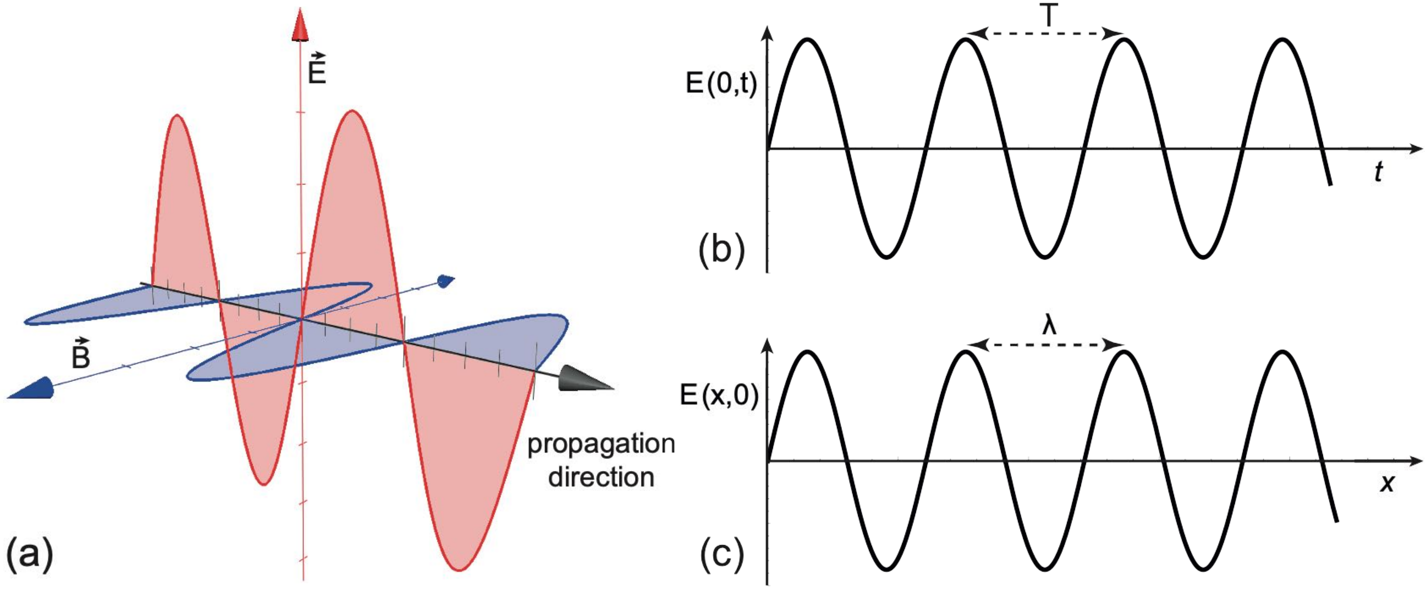
The electromagnetic spectrum



Different types of radiation are essentially electromagnetic waves with oscillation frequencies or vacuum wavelengths ranging over many orders of magnitude.

English version of a graphic by Horst Frank (https://de.wikipedia.org/wiki/Elektromagnetisches_Spektrum, https://en.wikipedia.org/wiki/GNU_Free_Documentation_License).

Electromagnetic waves



Sketch of a linearly polarized electromagnetic wave
(a) Wave with electric and magnetic field components, E and B
(b) Temporal oscillation at a fixed place in space.
(c) Still image of the wave.

$$\lambda_{vac} \nu = c_{vac}$$

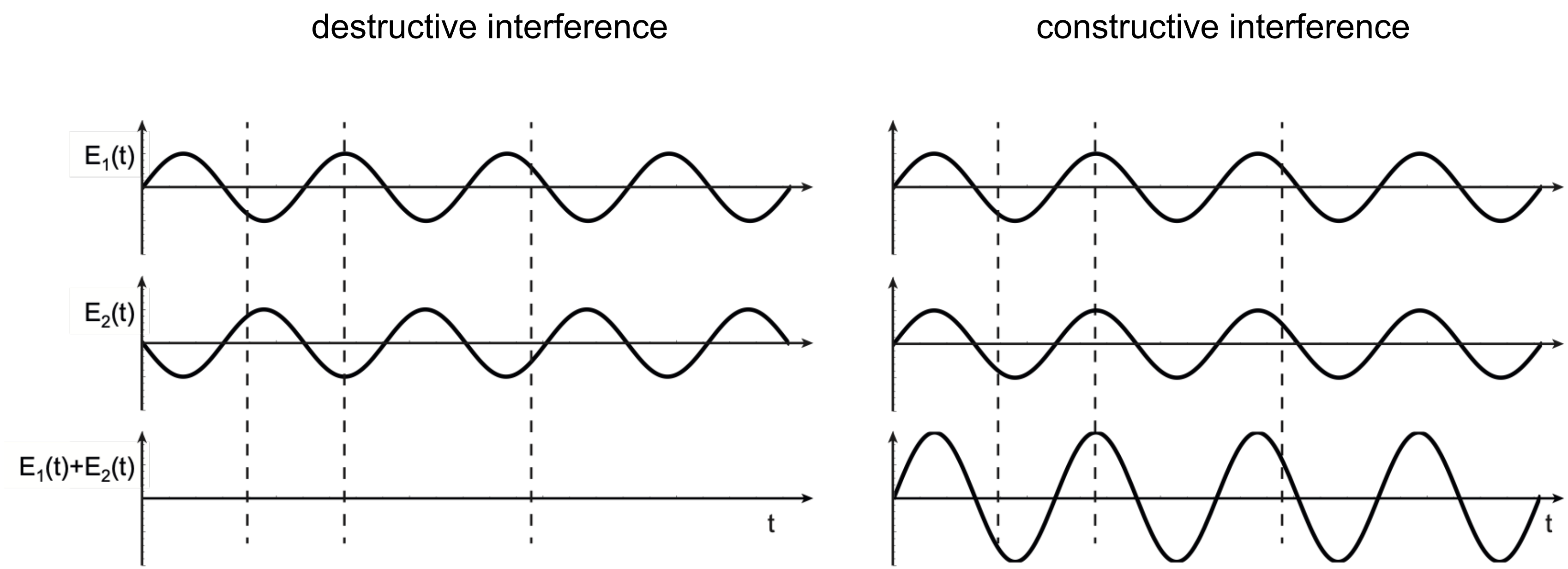
$$c_{vac} = 299.792.458 \ m/s$$
$$= 299.792,458 \ km/s$$

In media with refractive index n:

$$\frac{\lambda_{vac}}{n} \nu = \frac{c_{vac}}{n}$$

From “Fluorescence Microscopy: From Principles to Biological Applications“, edited by U. Kubitscheck. Wiley-VCH, Weinheim, 2nd edition

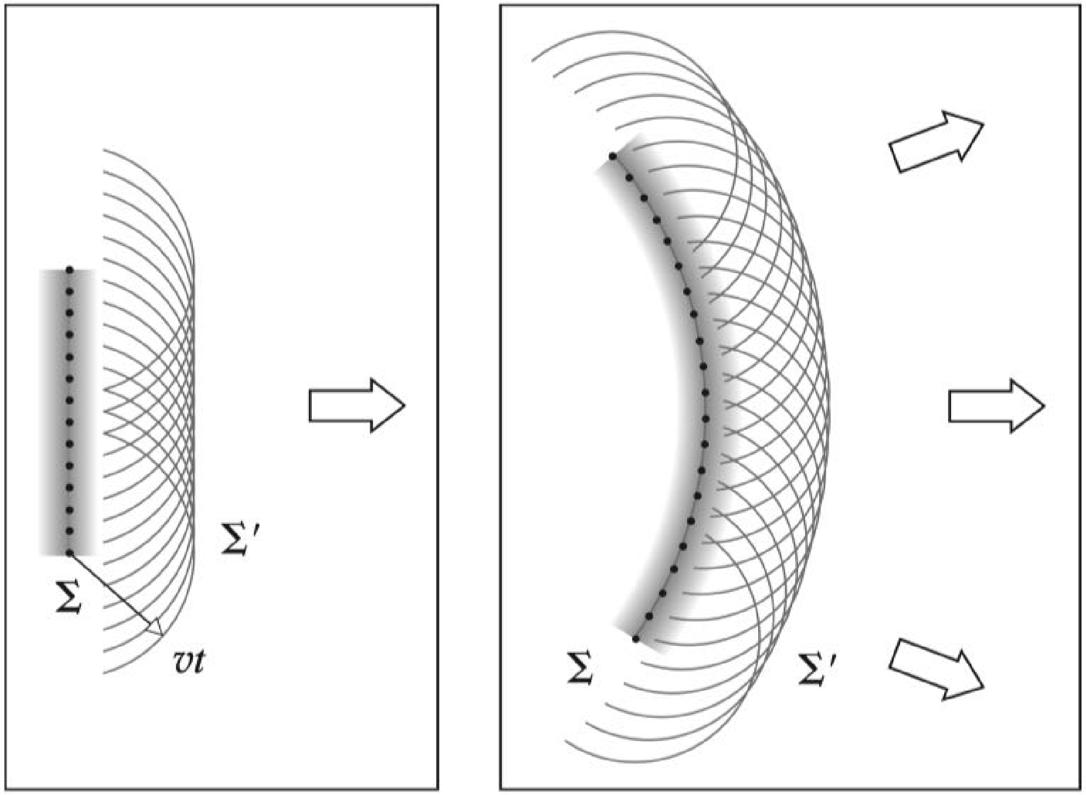
Interference of waves

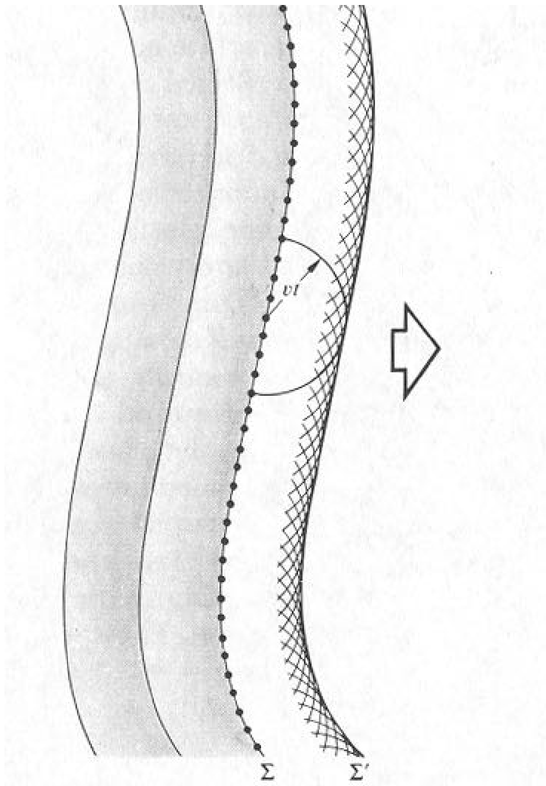


From “Fluorescence Microscopy: From Principles to Biological Applications“

The concept of Huygen´s elementary waves

„Each point of the wave front can be understood as the origin of a new elementary wave that propagates with the speed and frequency of the original wave“

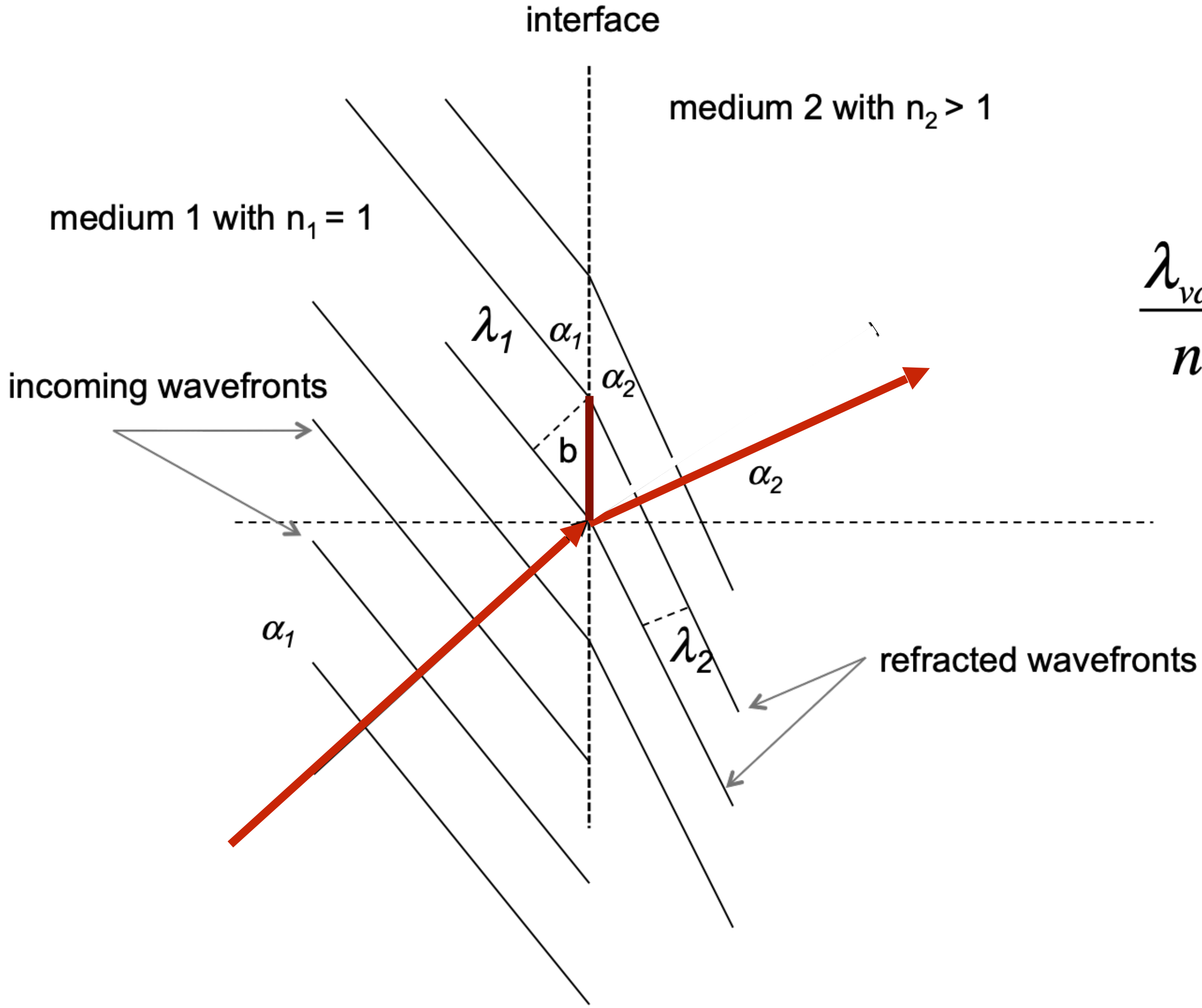




From “Optics“, Eugene Hecht, 2016

From “Optics“, Eugene Hecht, 2009

Refraction: Snell´s law



$$\frac{\lambda_{vac}}{n}\nu = \frac{c_{vac}}{n}$$

The phases of the electric field along the interface between the two materials must be identical.
The wavelengths inside the materials are given by λ_{vacuum}/n_1 and λ_{vacuum}/n_2.

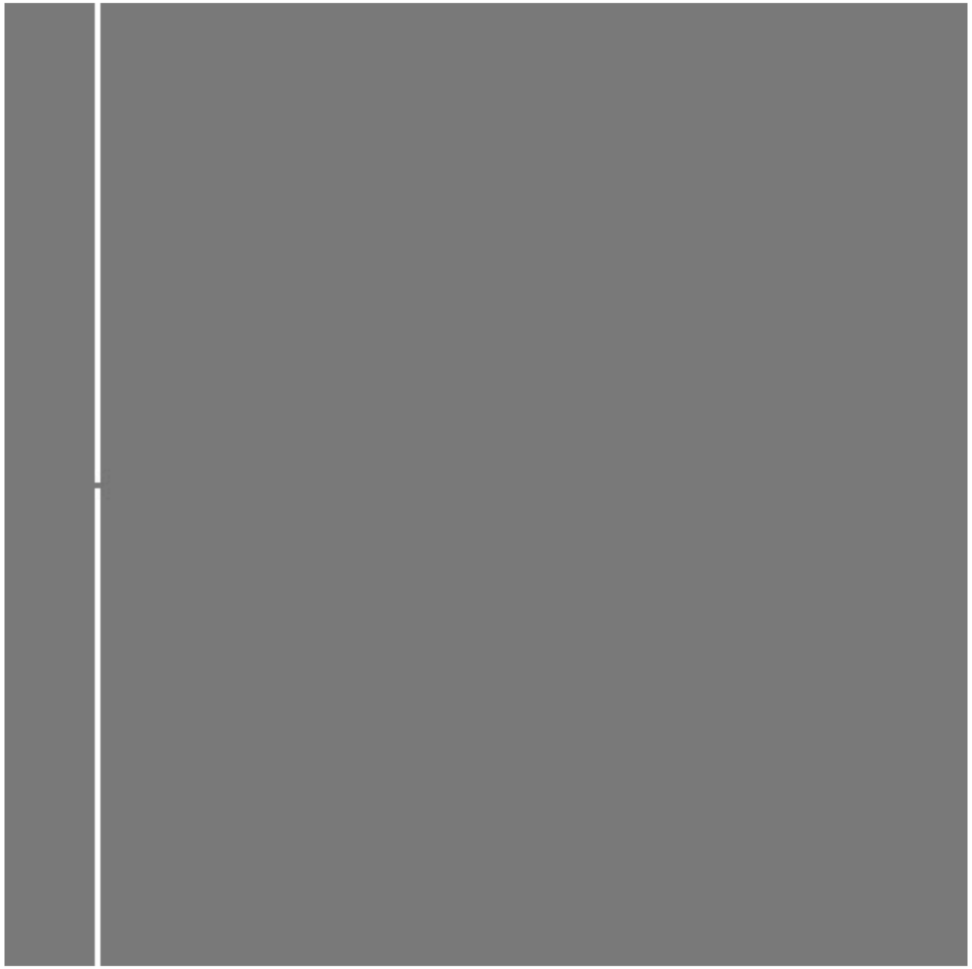
We note that $\sin \alpha_1 = \lambda_1/b$, where b denotes the distance between two wave crests at the interface,
and also that $\sin \alpha_2 = \lambda_2/b$.

Eliminating b yields $$n_1 \sin \alpha_1 = n_2 \sin \alpha_2$$

View animation:
https://www.walter-fendt.de/html5/phde/refractionhuygens_de.htm

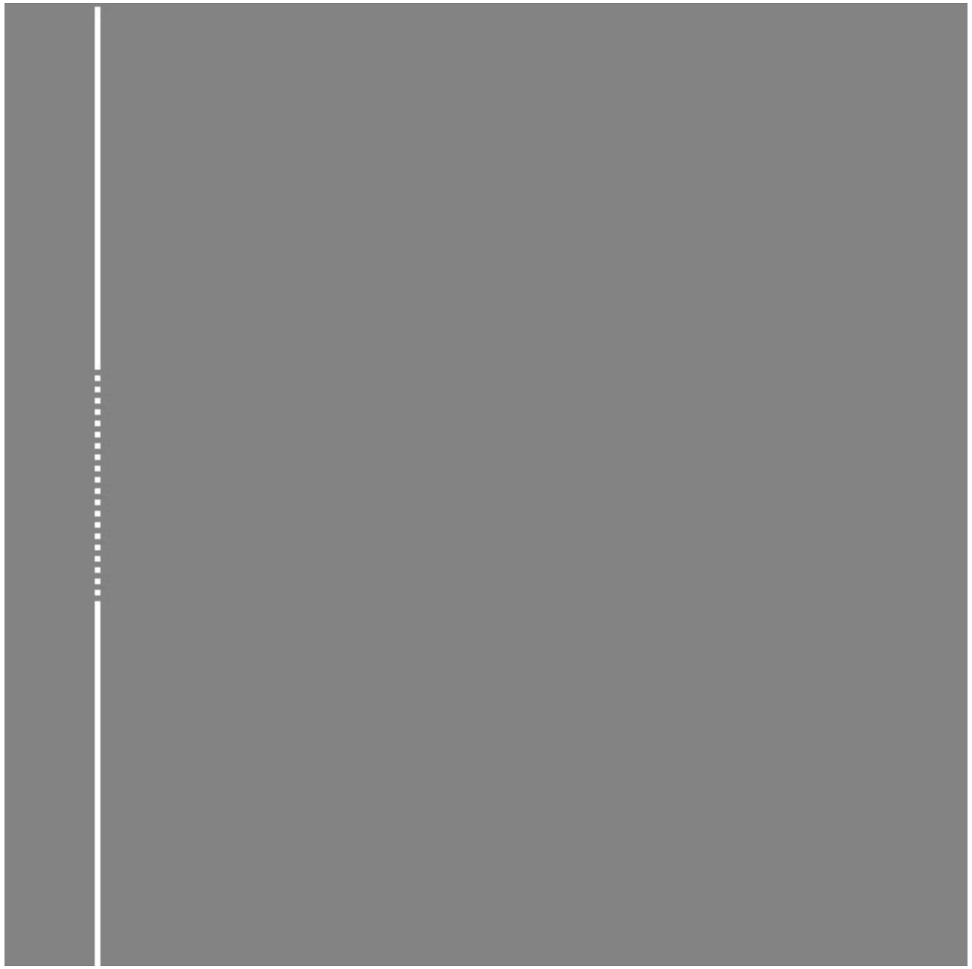
From “Fluorescence Microscopy: From Principles to Biological Applications“

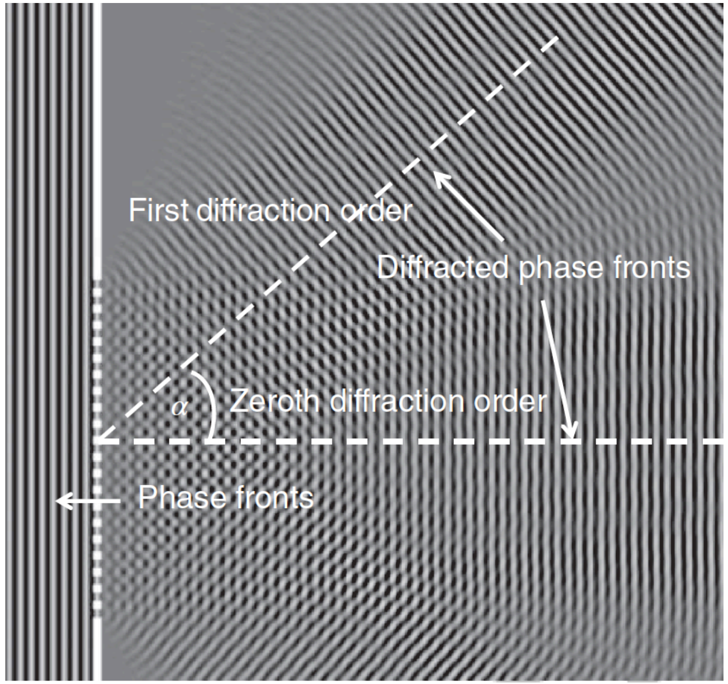
Diffraction: pinhole ➡ spherical wave



From “Fluorescence Microscopy: From Principles to Biological Applications“, online supplemental material

Diffraction: grating

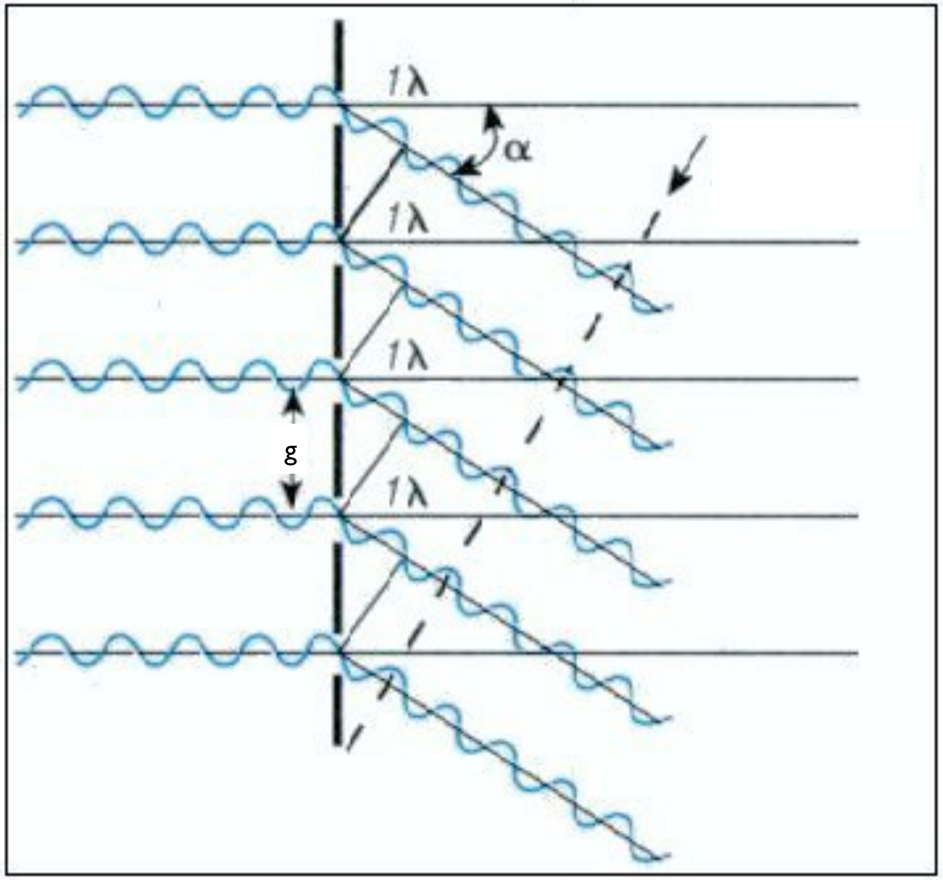




A plane wave hits perpendicularly on a grating. The directions of constructive interference, in which maxima and minima of one wave interfere constructively with the maxima and minima of the second wave are shown for the zeroth- and first-order diffraction.

From “Fluorescence Microscopy: From Principles to Biological Applications“, online supplemental material

Diffraction grating



The diffraction grating and spectrum on screen

g grating constant, λ wave length, α angle of deflection,

for main maxima we have

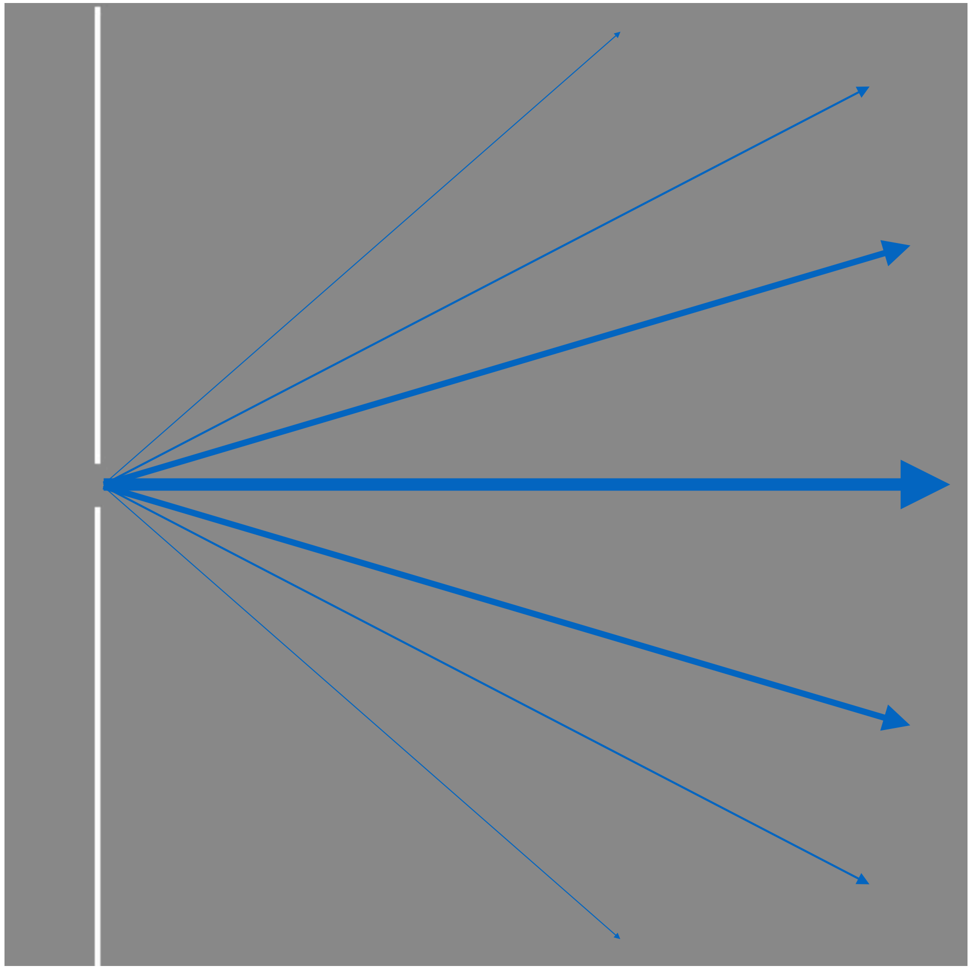
$$g \sin\alpha_n = n\lambda$$

with

g: grating constant
n=1, 2, 3, ..., order of maximum
α_n: diffraction angle of order n

Source: http://library.thinkquest.org/19662/low/eng/electron-wave-exp.html

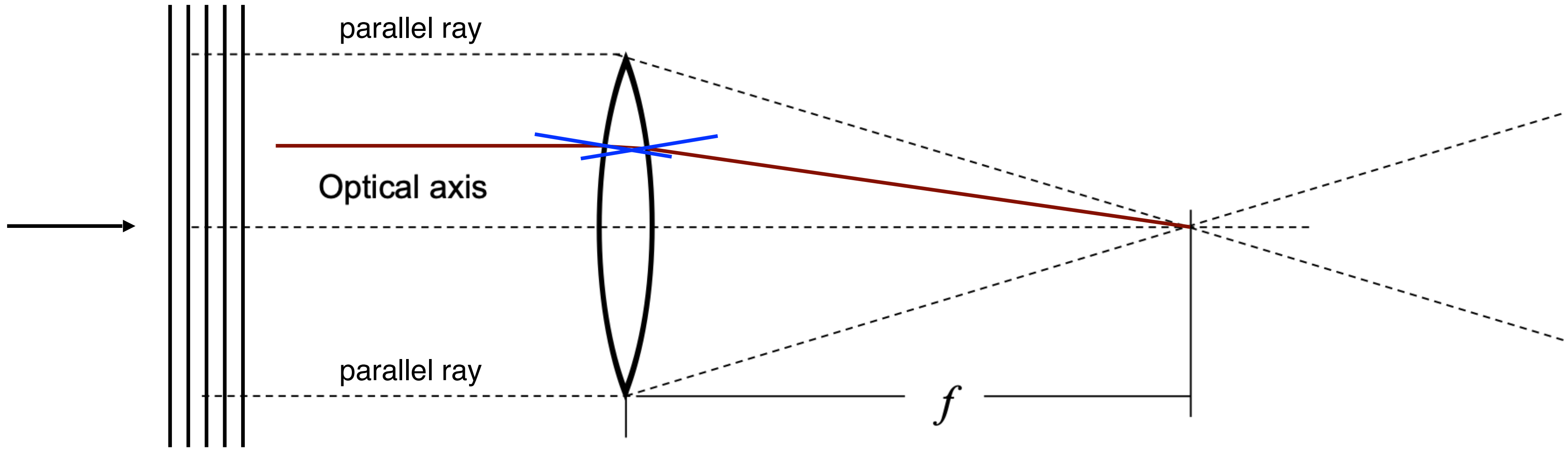
Diffraction: open pinhole



(a) A plane wave hits perpendicularly on a large pinhole.
Again we find directions of constructive and destructive interference

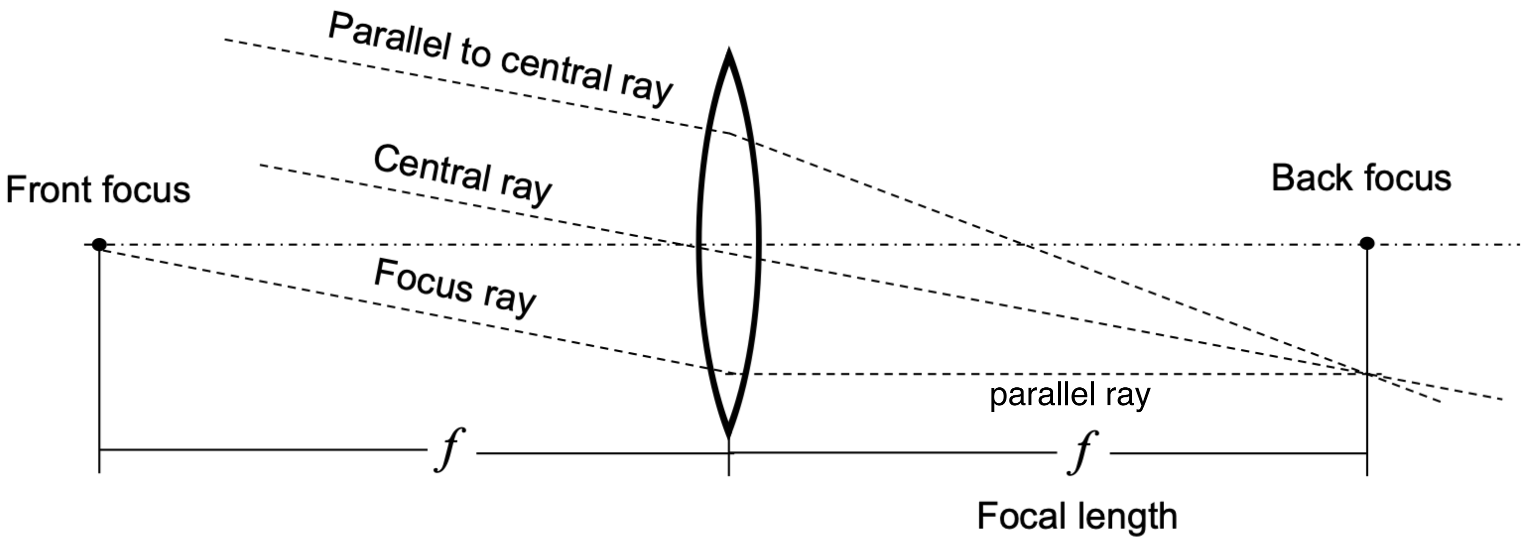
From “Fluorescence Microscopy: From Principles to Biological Applications“, edited by Ulrich Kubitscheck. Wiley-VCH, Weinheim, 2nd edition
online supplemental material

Lenses



From “Fluorescence Microscopy: From Principles to Biological Applications“

Special rays passing lenses



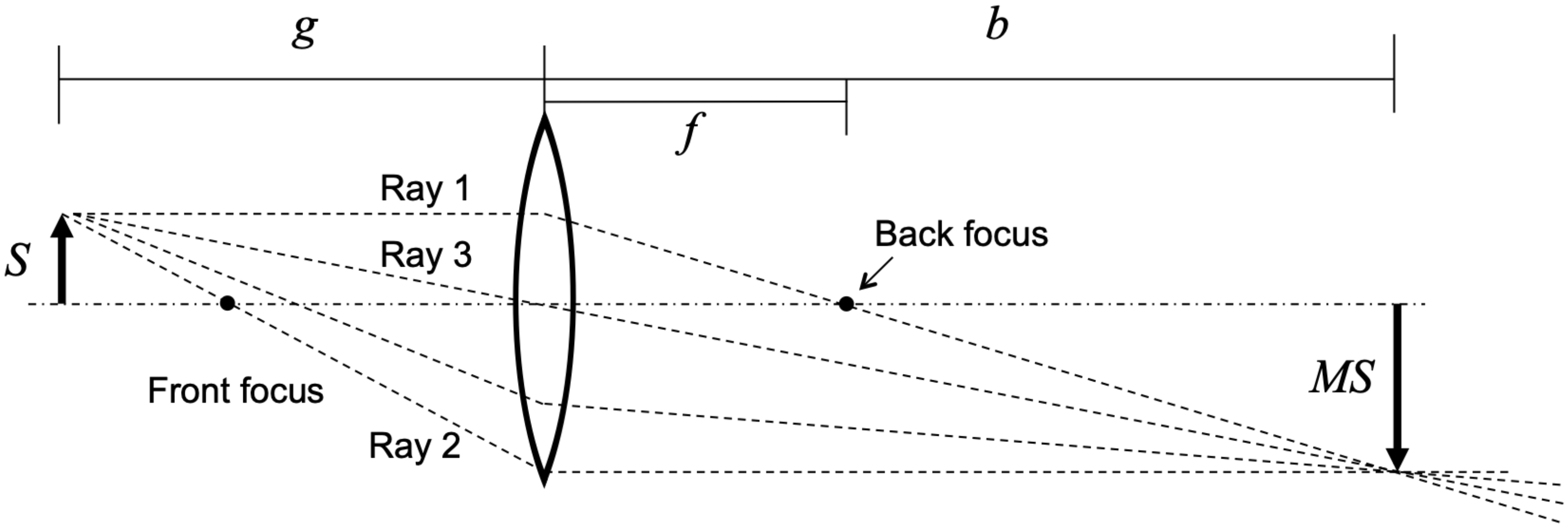
Optical reversal: retrace rays and yield identical paths

Nice Applet:

https://www.walter-fendt.de/html5/phde/imageconverginglens_de.htm

From “Fluorescence Microscopy: From Principles to Biological Applications“

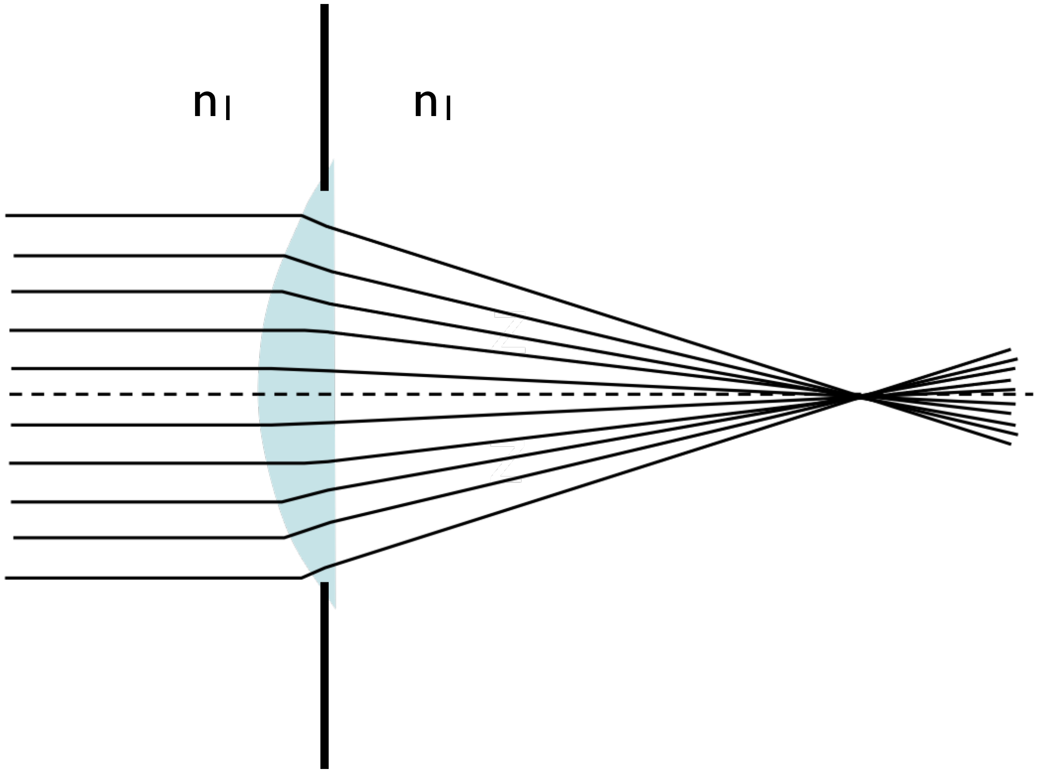
Real images

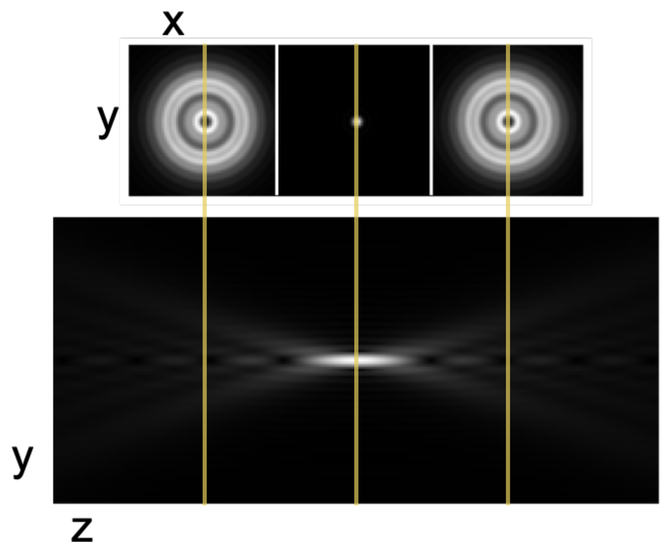


A single lens imaging an object as an example for drawing optical ray diagrams

From “Fluorescence Microscopy: From Principles to Biological Applications“

Focusing of light: ray model & wave model

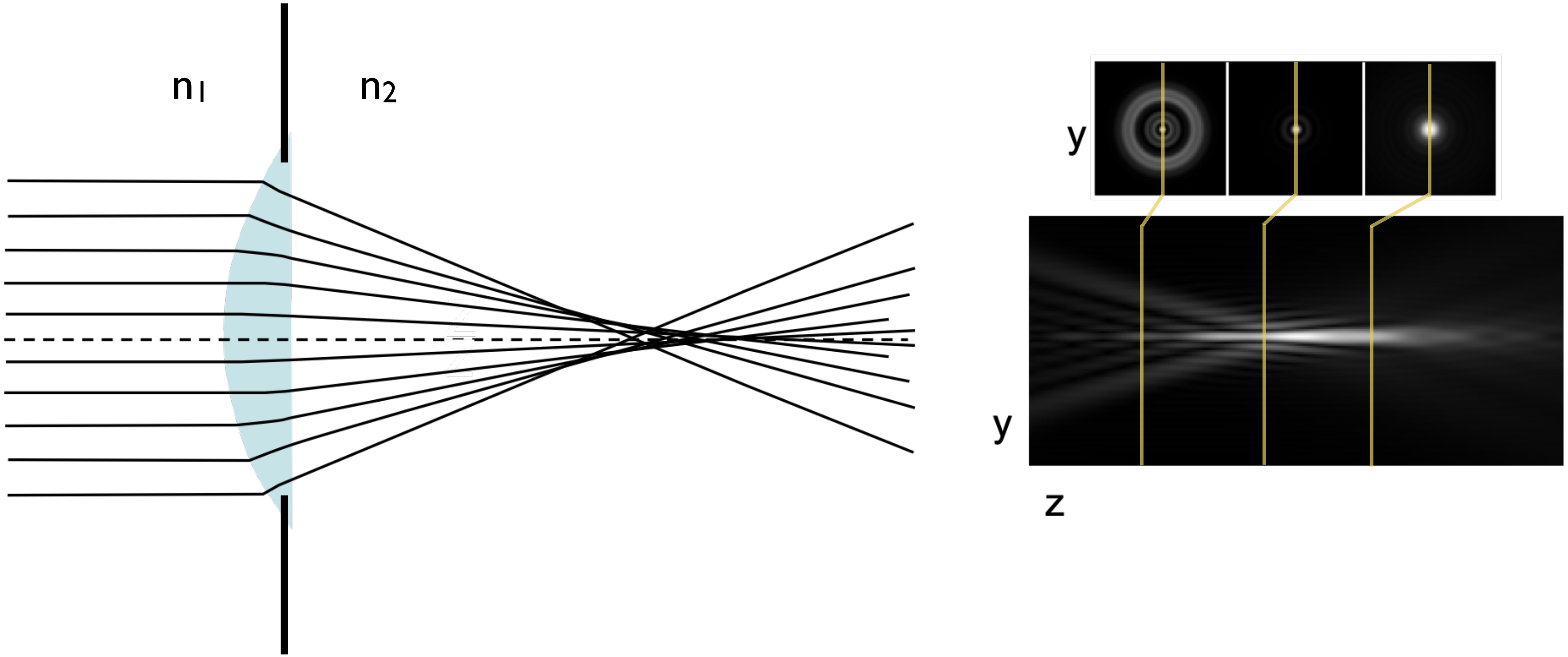




A plane wave - sketched by the parallel incoming rays - hits perpendicularly on a large pinhole.
The lens focuses the diffracted ways into its focus.
Again we find directions of constructive and destructive interference

From “Fluorescence Microscopy: From Principles to Biological Applications“, edited by Ulrich Kubitscheck. Wiley-VCH, Weinheim, 2nd edition

Focusing of light with spherical aberration



From “Fluorescence Microscopy: From Principles to Biological Applications“, edited by Ulrich Kubitscheck. Wiley-VCH, Weinheim, 2nd edition

Important aberrations in microscopy

spherical aberration

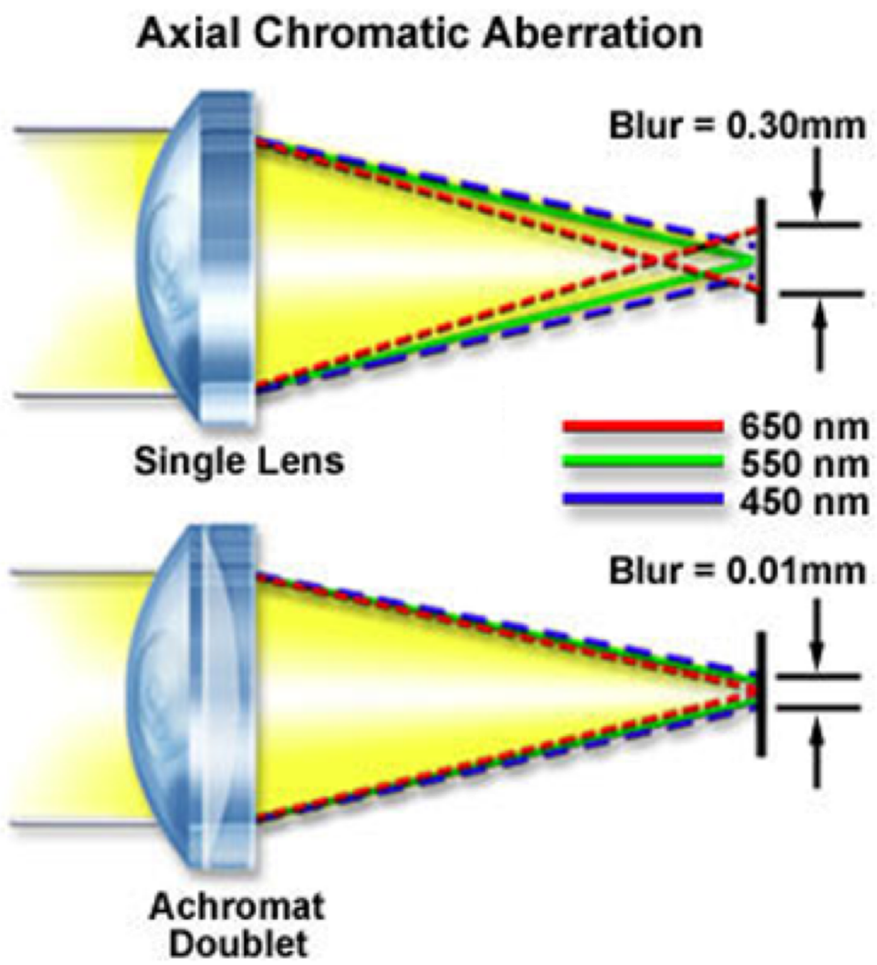
chromatic aberrations

curvature of field

coma

astigmatism

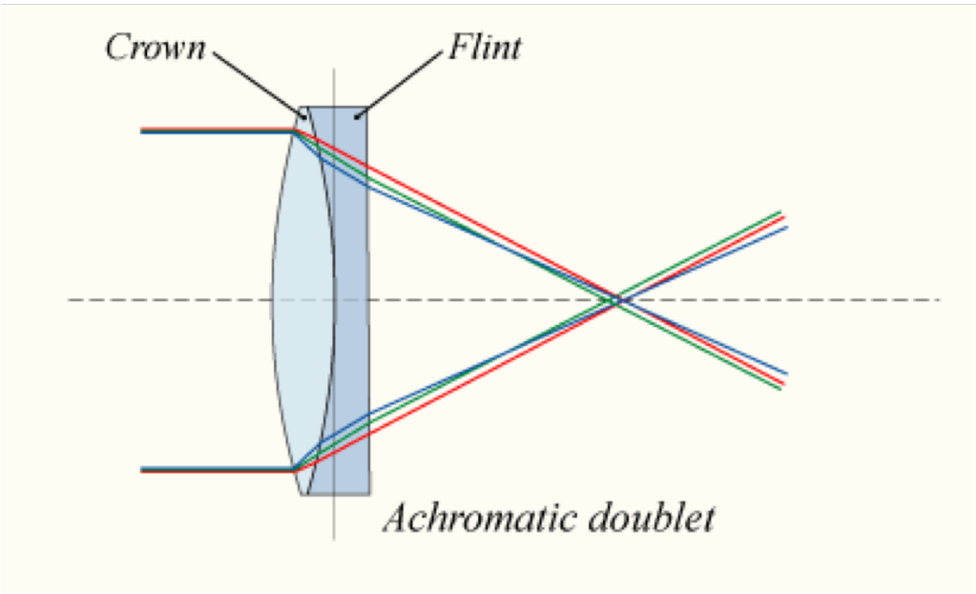
Chromatic aberrations

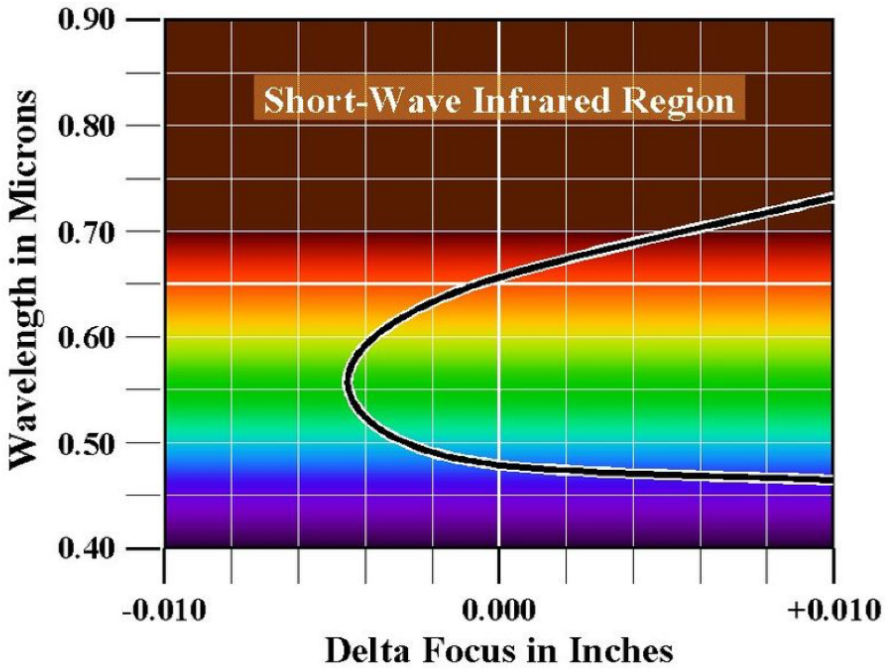


Refraction is wavelength-dependent: each color has its own focus <u>and</u> magnification

Source: microscopy primer, https://micro.magnet.fsu.edu/primer/

Correction of chromatic aberrations

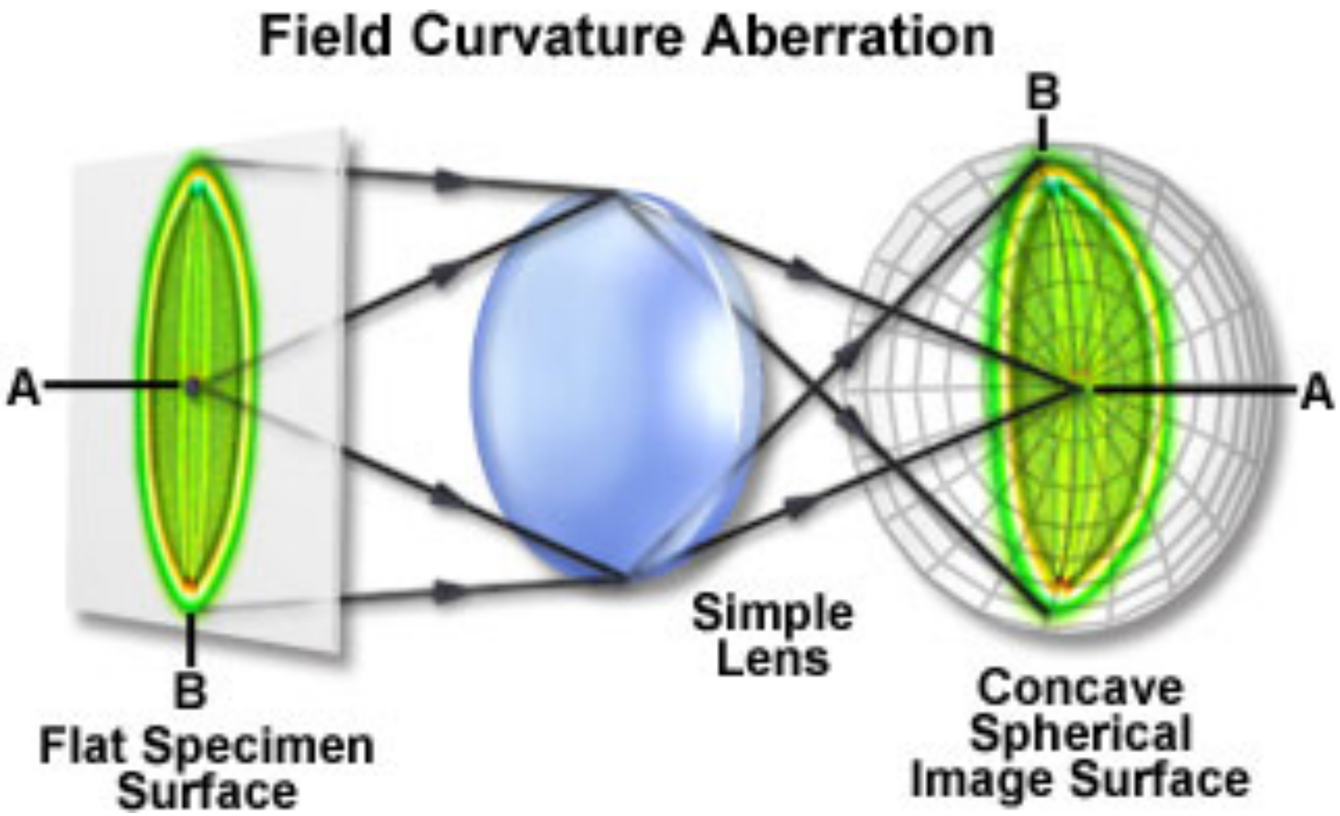




An achromatic doublet brings two wavelengths to a common focus, leaving ultraviolet and infrared uncorrected and out of focus

Source: microscopy primer, https://micro.magnet.fsu.edu/primer/

Curvature of field



The image is actually located on the surface of a sphere, hence the image of a flat object is curved with regard to the optical axis

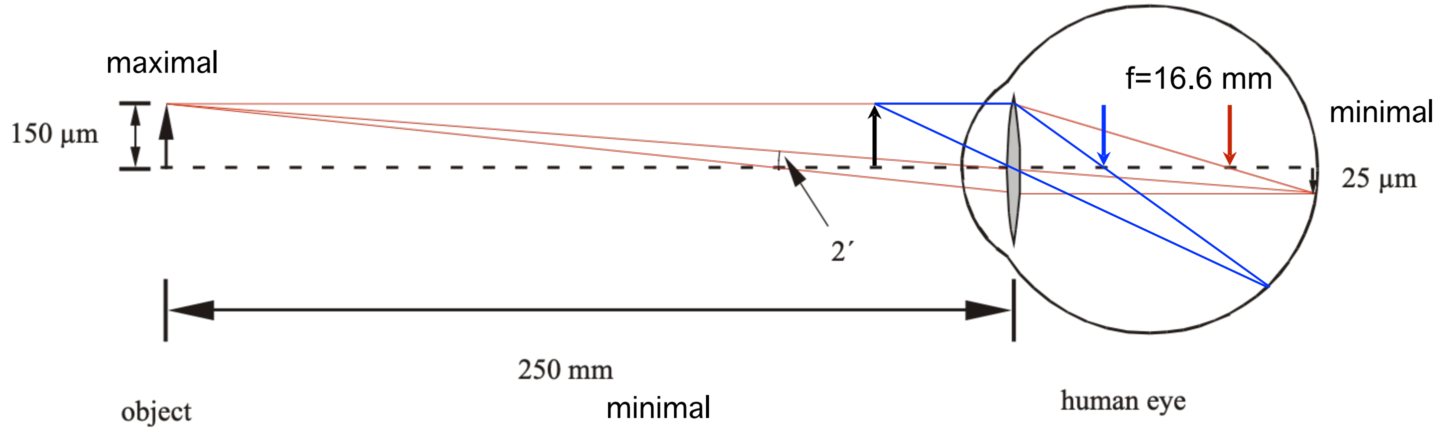
Source: microscopy primer, https://micro.magnet.fsu.edu/primer/

3. Microscope

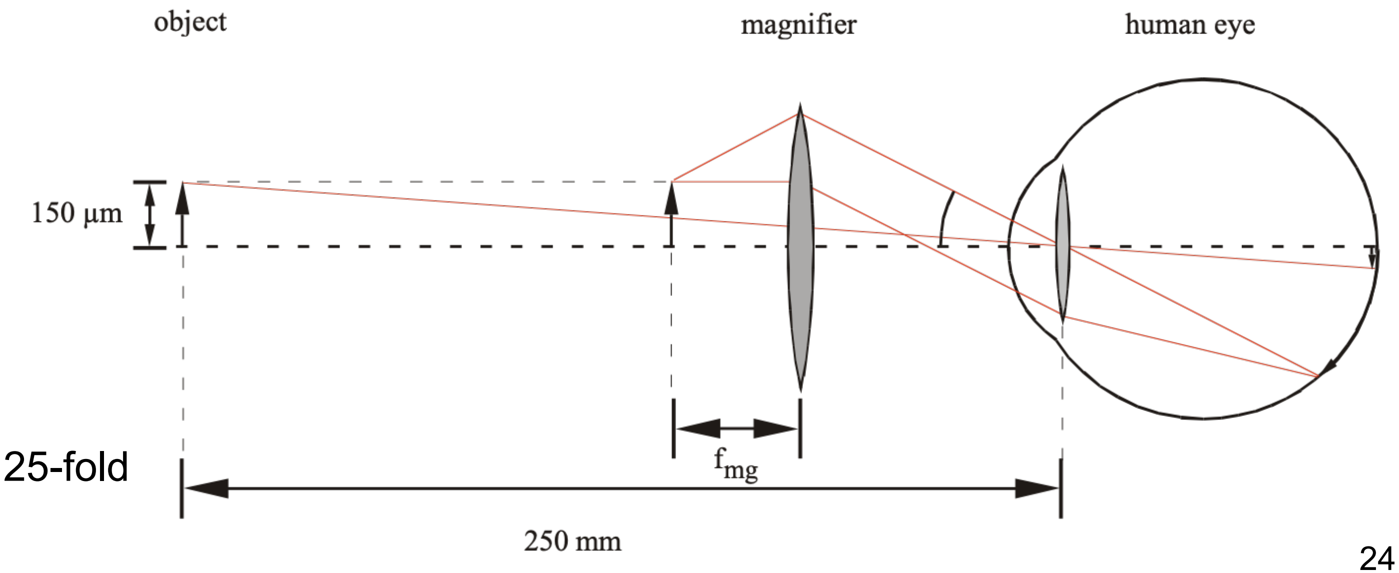
Why use a microscope?

focal length of human eye: f ≈ 16.6 mm ⇒ refractive power 1/f ≈ 60/m = 60 diopters

Imaging process of the human eye



Eye + magnifier = 1-stage microscope



maximal magnification about 25-fold

Tasks of a light microscope

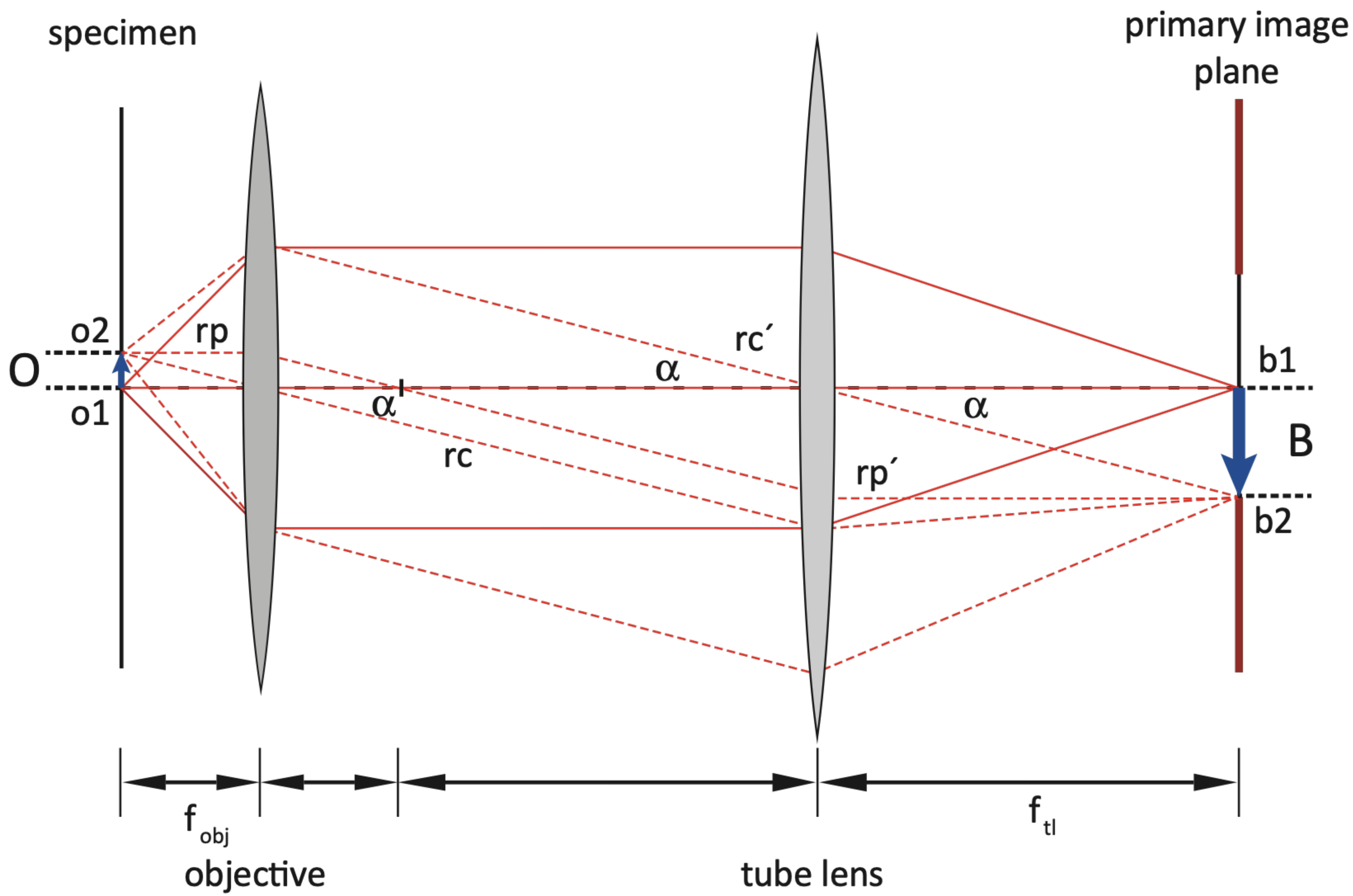
Magnification (!!!)

light detectors are sensitive for intensity,
but nor for color, neither for phase or polarisation of light

Contrast production

bright field, dark field, phase contrast, differential interference contrast, fluorescence

Imaging process using an „infinity beam path“

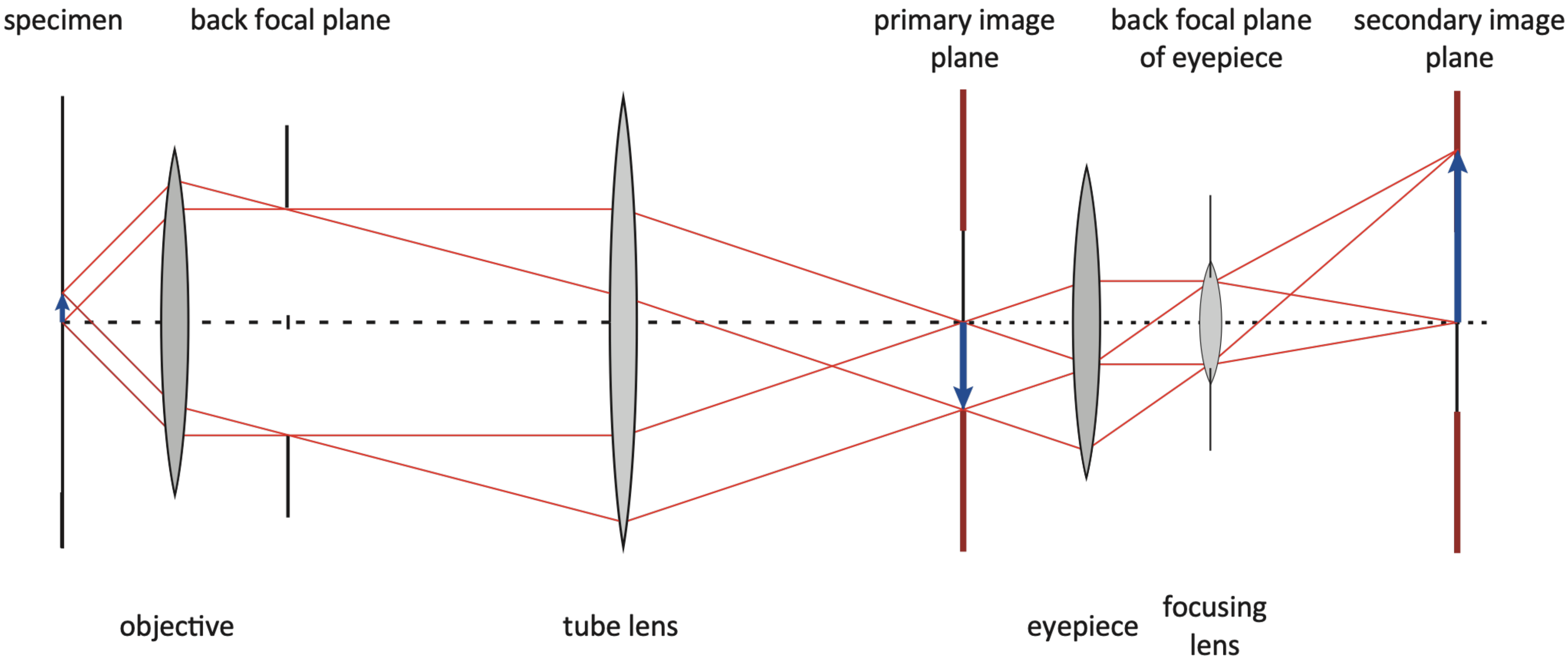


$$\tan\alpha = \frac{O}{f_{obj}} = \frac{B}{f_{TL}} \rightarrow M = \frac{B}{O} = \frac{f_{TL}}{f_{obj}}$$

From “Fluorescence Microscopy: From Principles to Biological Applications“

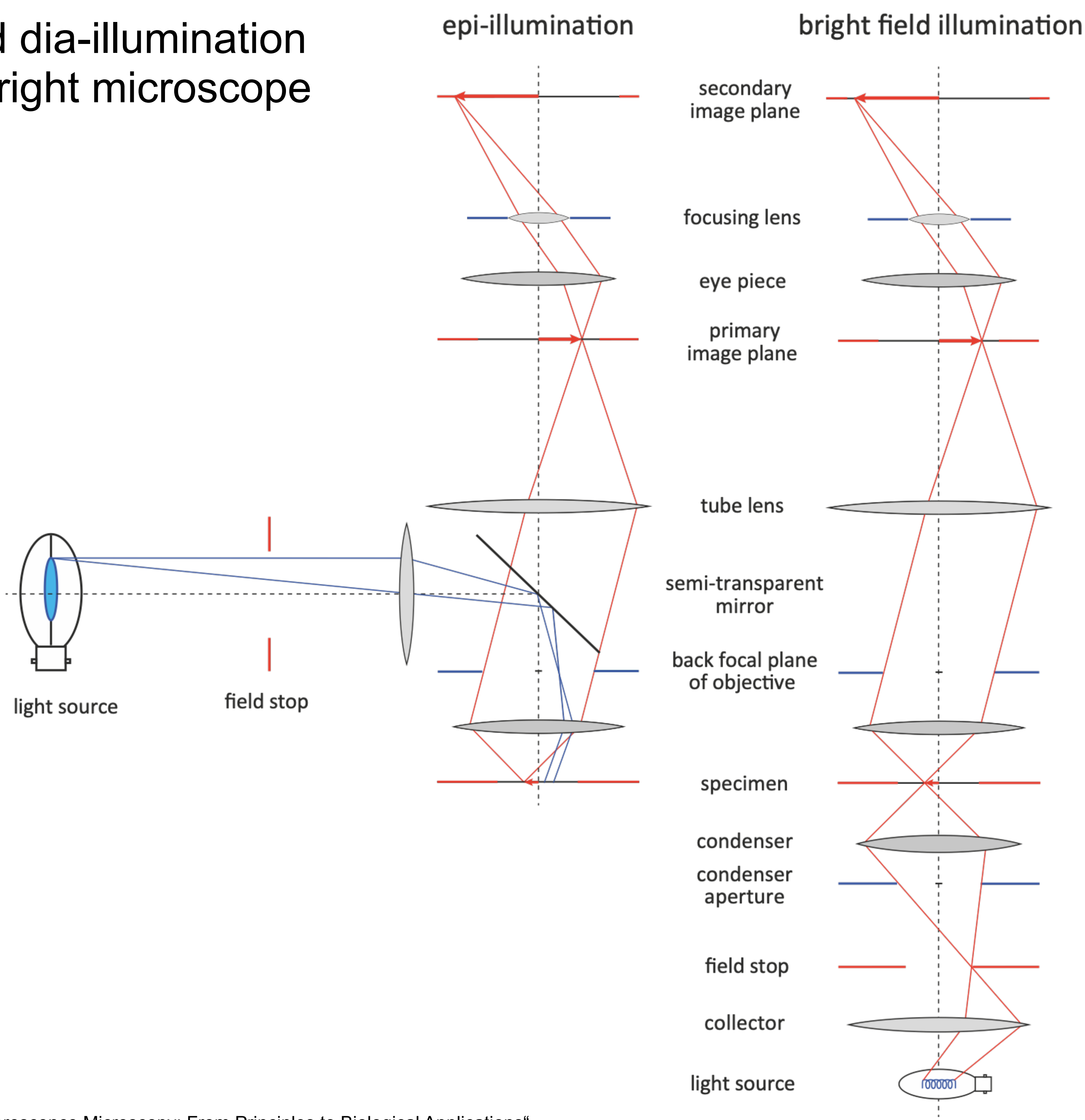
4. Two-stage microscope

Construction of a microscope
by combination of two magnification stages



From "Fluorescence Microscopy: From Principles to Biological Applications"

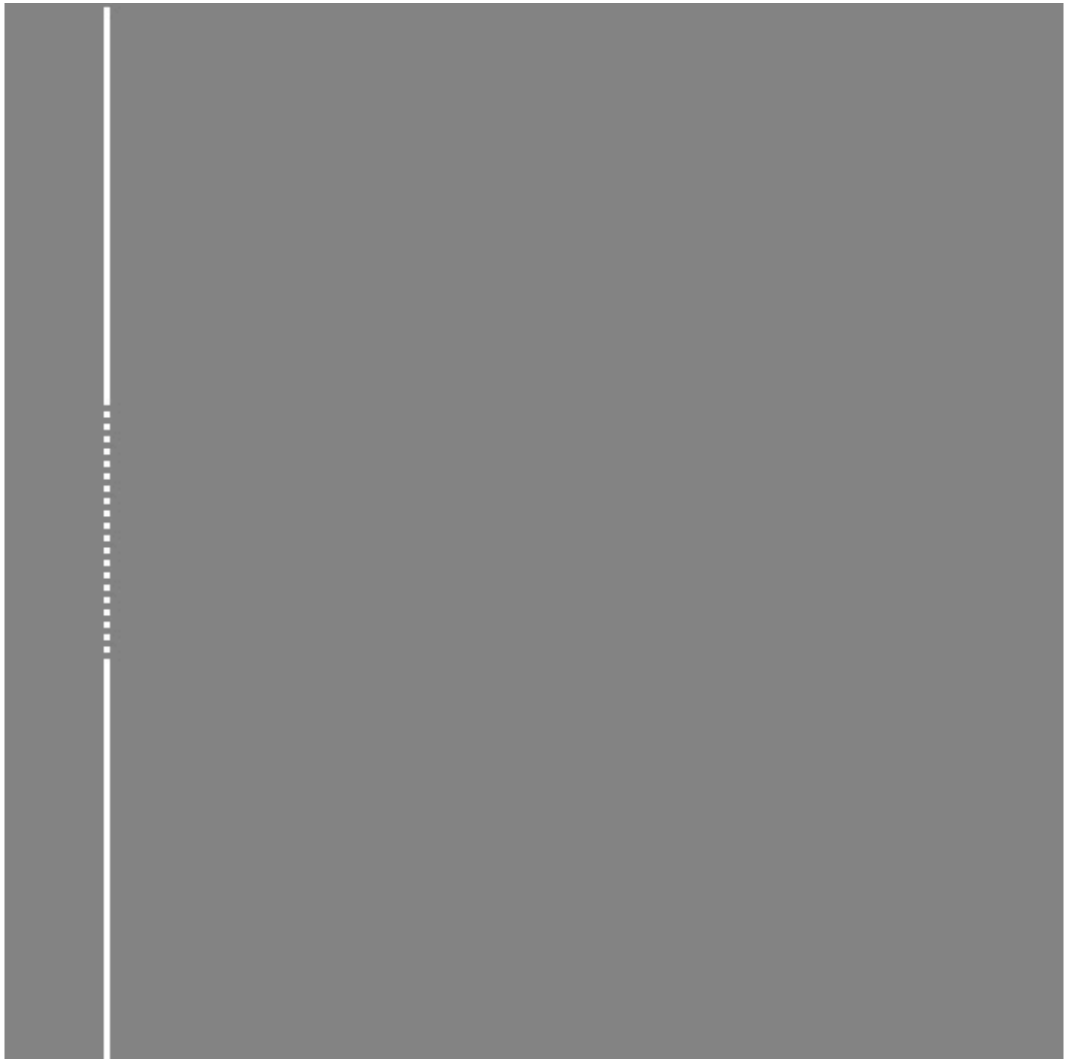
Epi- and dia-illumination in an upright microscope



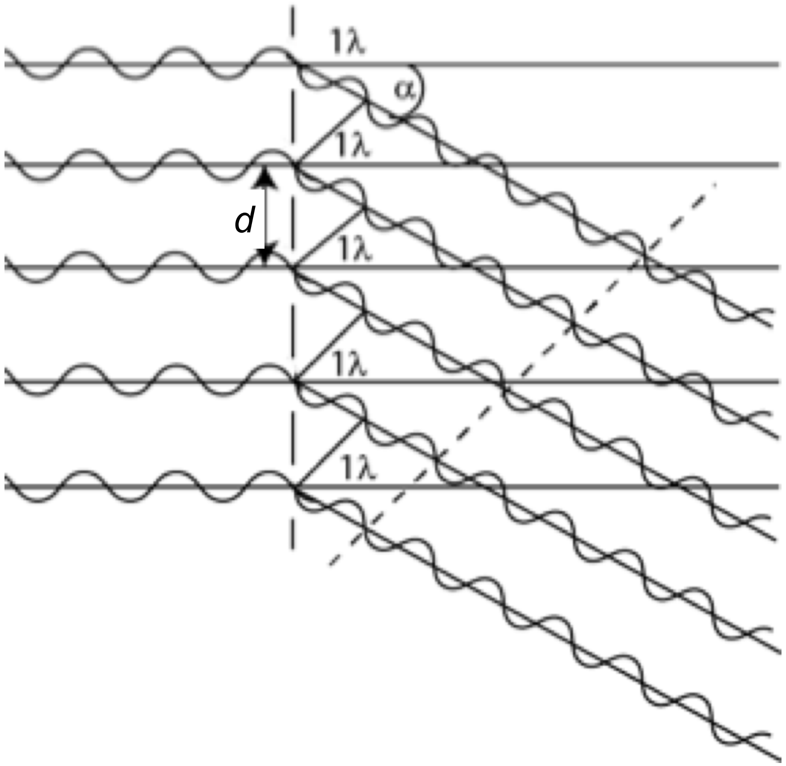
From “Fluorescence Microscopy: From Principles to Biological Applications“

5. What about resolution?
The point spread function

Diffraction at a grating I



Diffraction at a grating II



d, grid constant

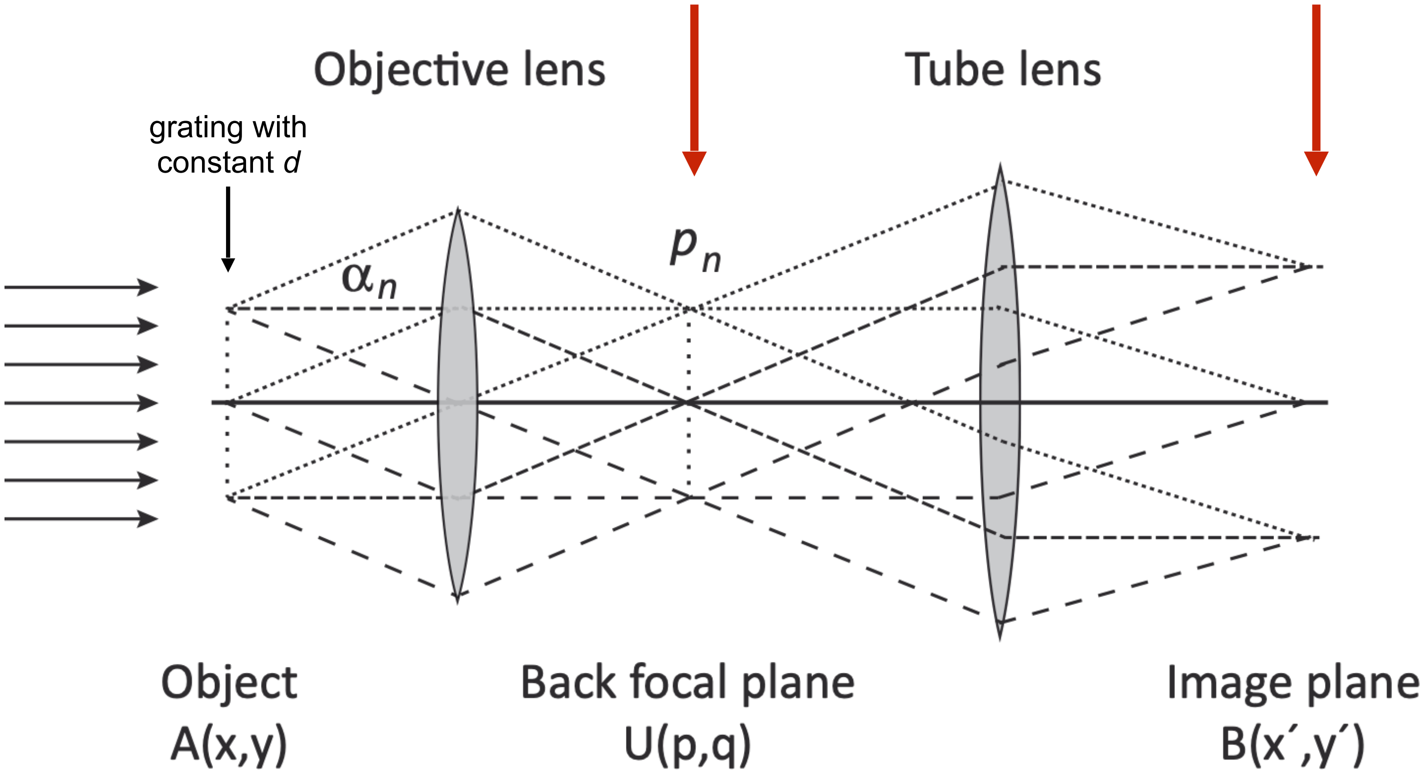
α diffraction angle

grid equation

for Fraunhofer diffraction:

$$d \sin \alpha_n = n\lambda$$

The diffraction pattern is projected into the back focal plane



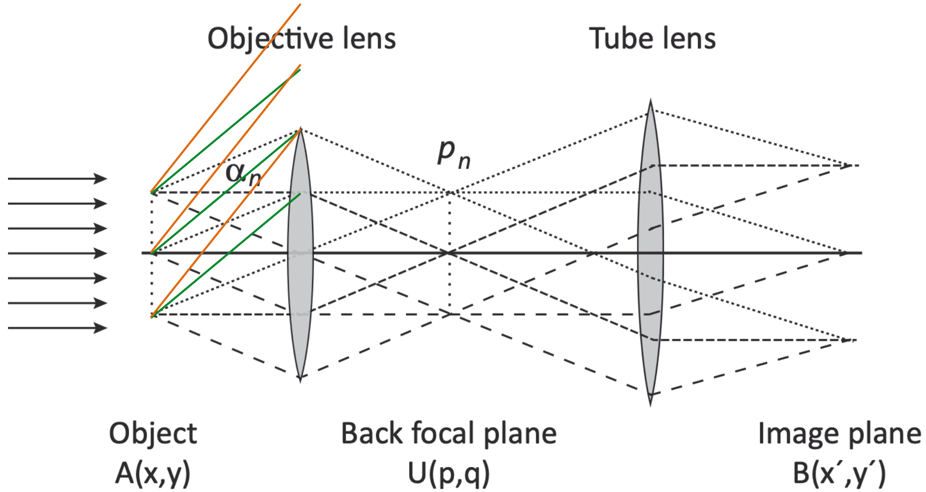
diffraction pattern p_n proportional to 1/d ….. indeed it is the *Fourier transform* of the object structure

From “Fluorescence Microscopy: From Principles to Biological Applications“

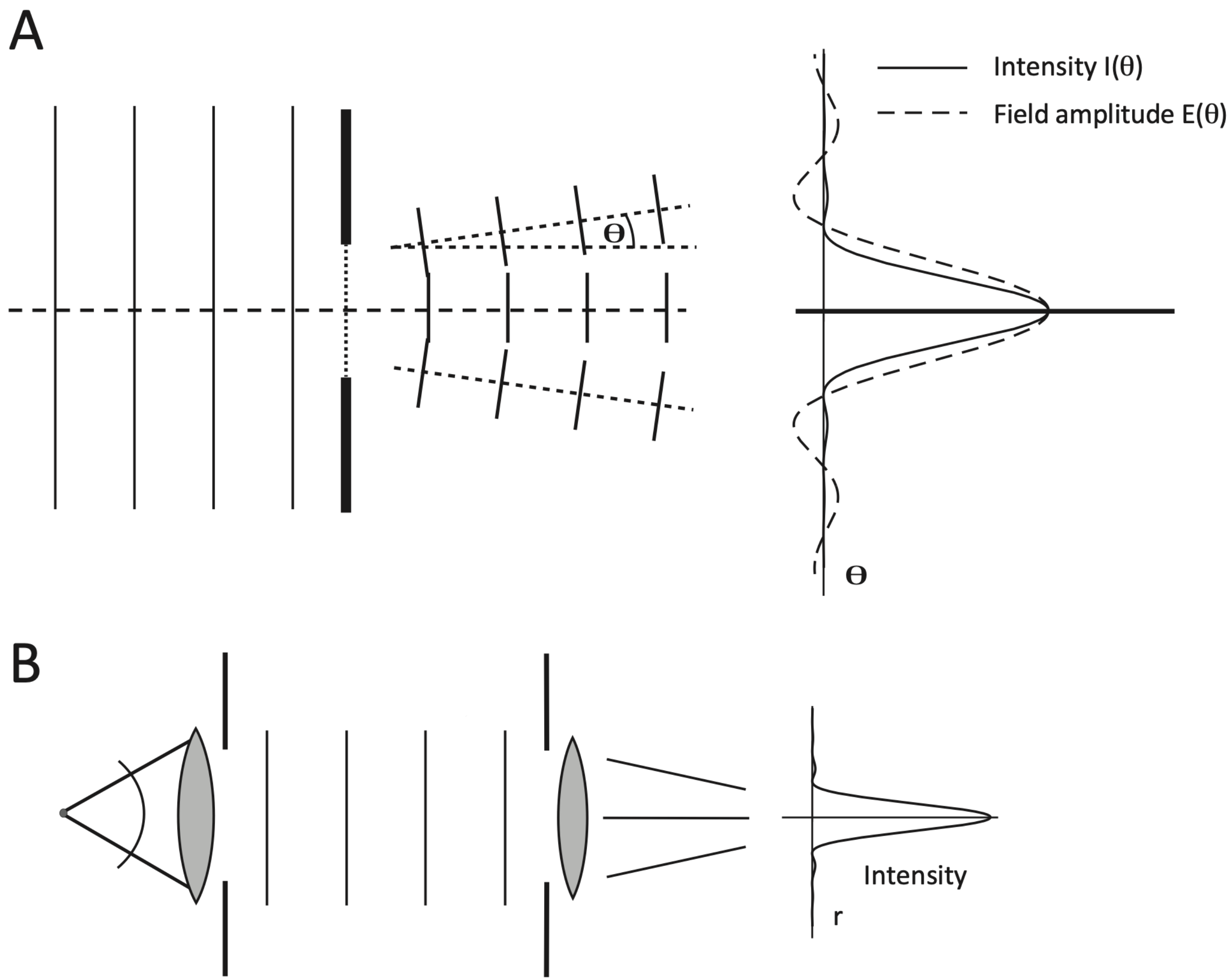
Resolution and Numerical Aperture

Grid equation $$d \sin \alpha_n = n\lambda$$

Required: at least the first diffraction maximum in the back focal plane: set n=1 … and solve for d. due to the finite lens diameter the opening angle is limited, we designate it as $\boldsymbol{\alpha_{max}}$.



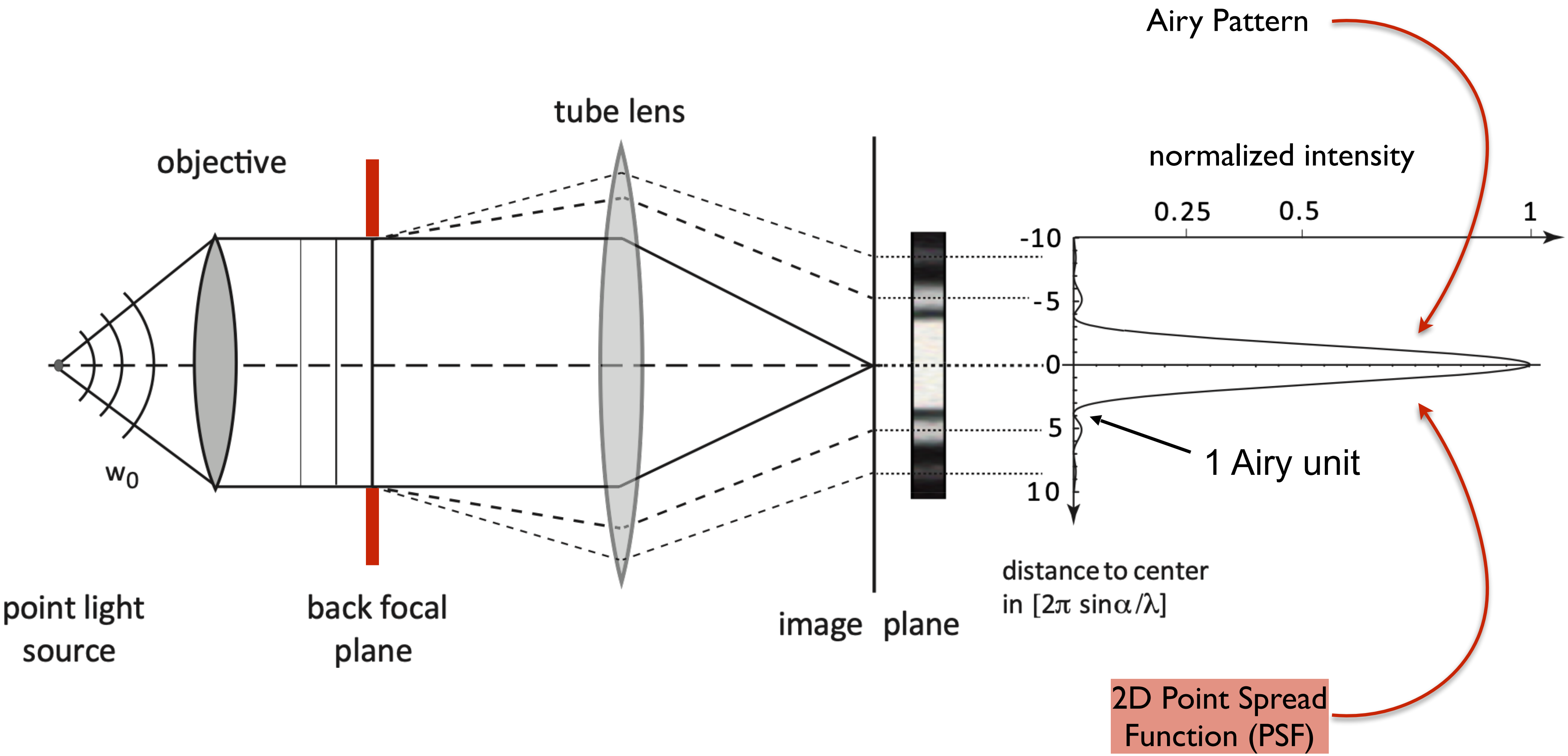
Imaging Point Objects



From “Fluorescence Microscopy: From Principles to Biological Applications“

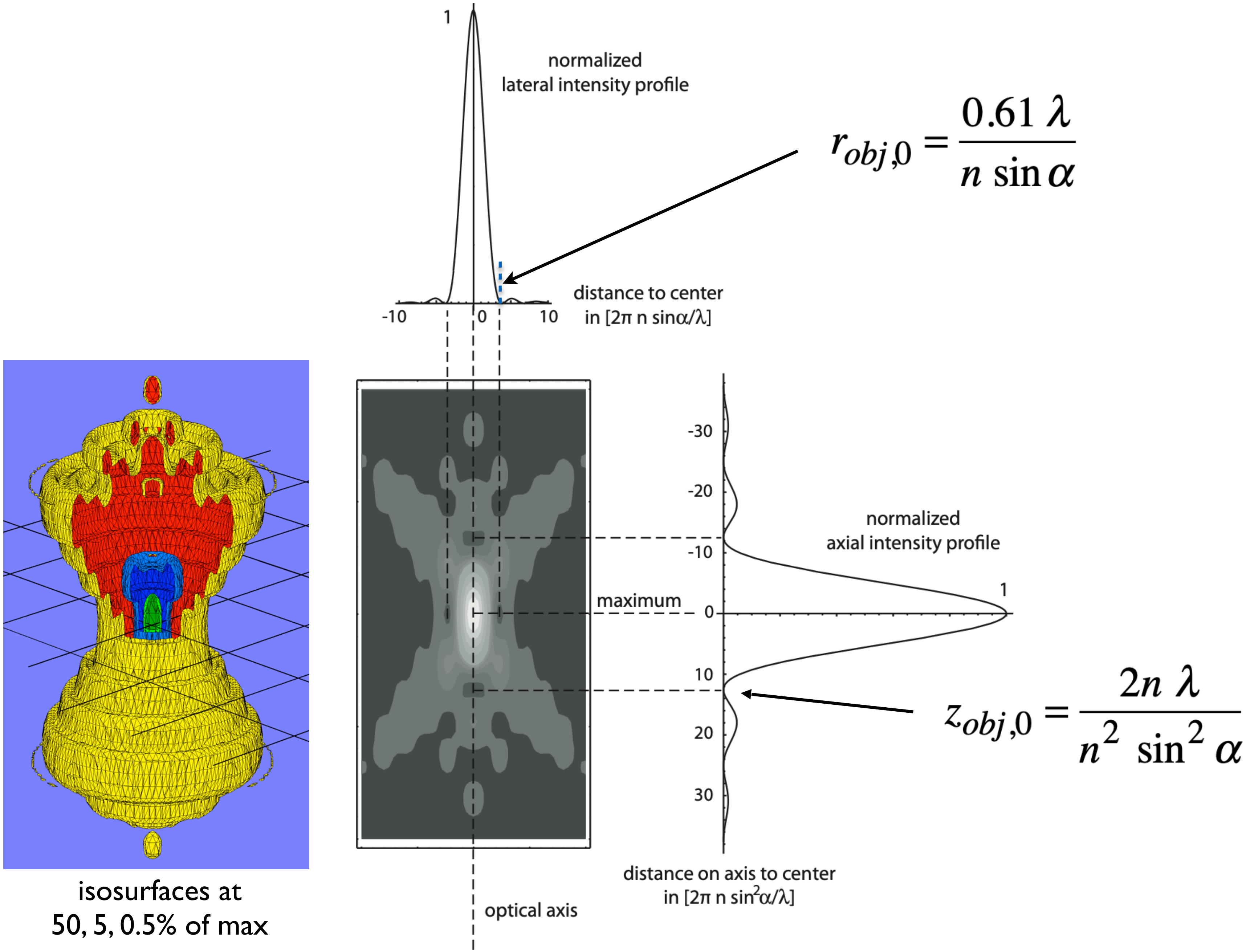
Resolution Limit, Airy Pattern and Point Spread Function

Interference of Huygens waves from the exit pupil of the objective



From “Fluorescence Microscopy: From Principles to Biological Applications“

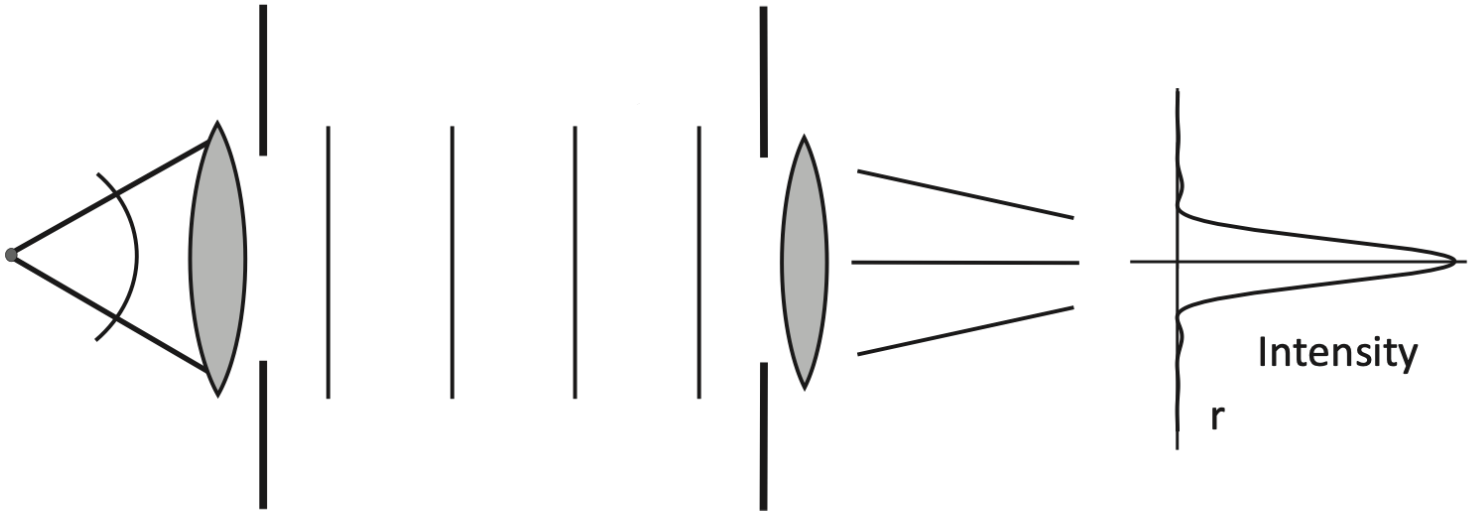
3D Point spread function (PSF)



isosurfaces at
50, 5, 0.5% of max

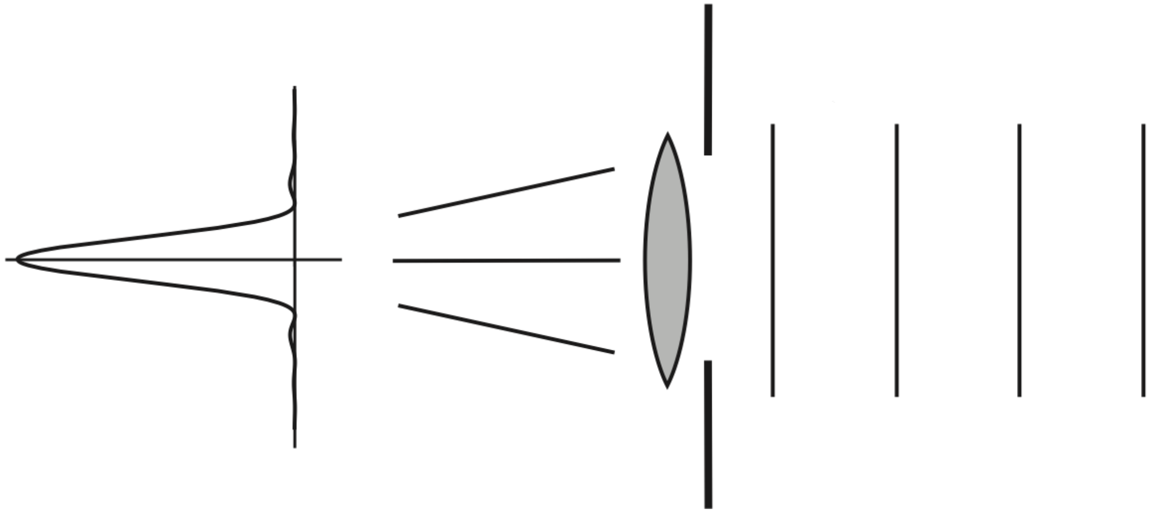
Focusing of light by a lens

Imaging of a point

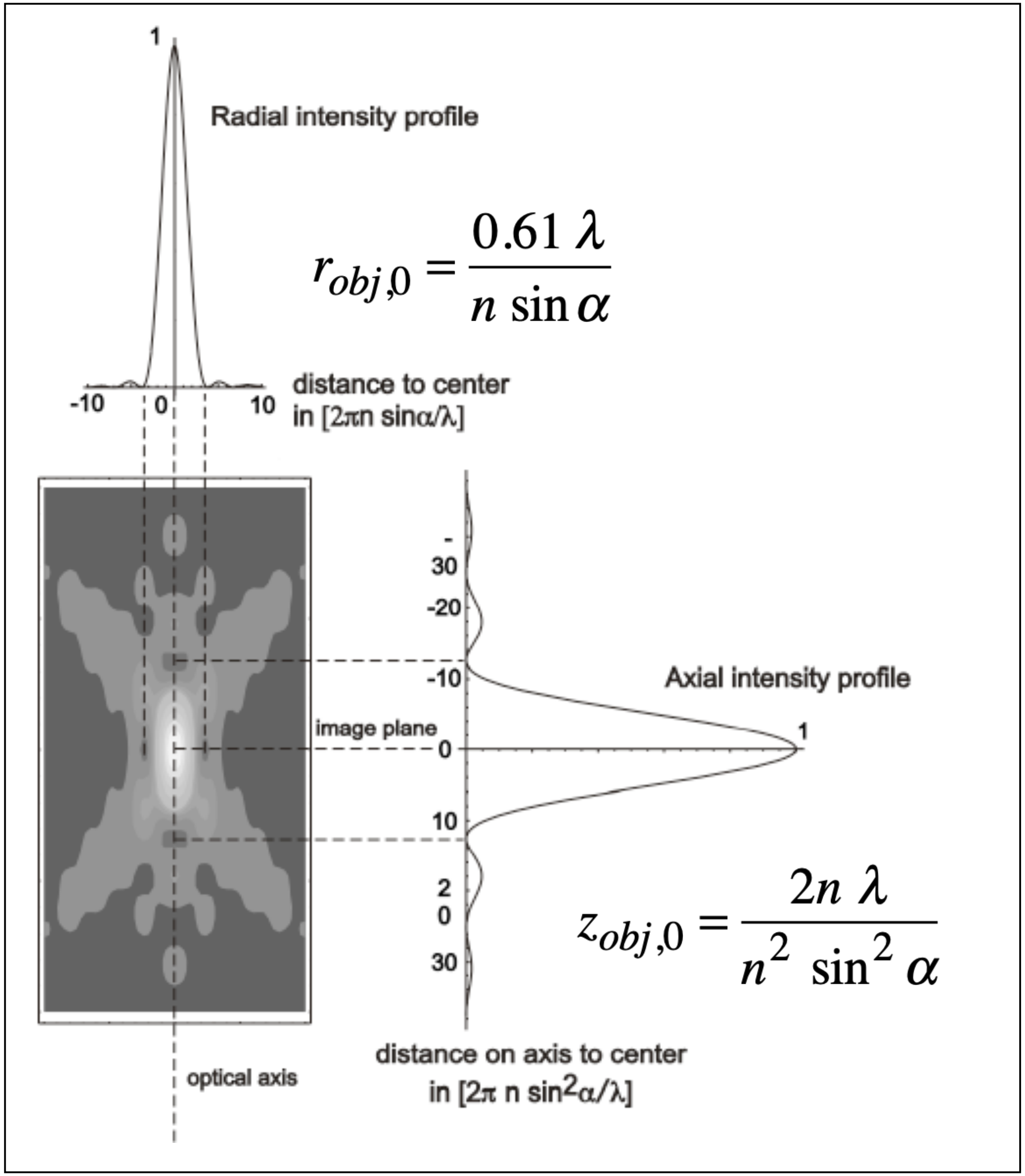


object space

Illumination of a „point“



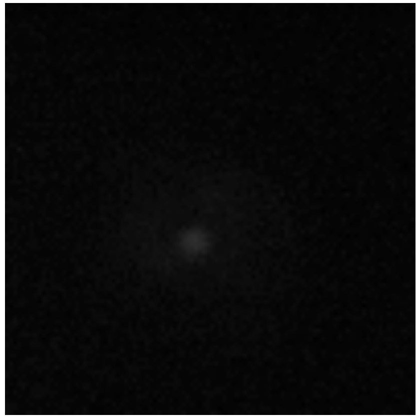
Radial and axial intensity profile of the light distribution in the focus of a lens



$$r_{obj,0} = \frac{0.61\,\lambda}{n \sin\alpha}$$

$$z_{obj,0} = \frac{2n\,\lambda}{n^2 \sin^2\alpha}$$

Bead



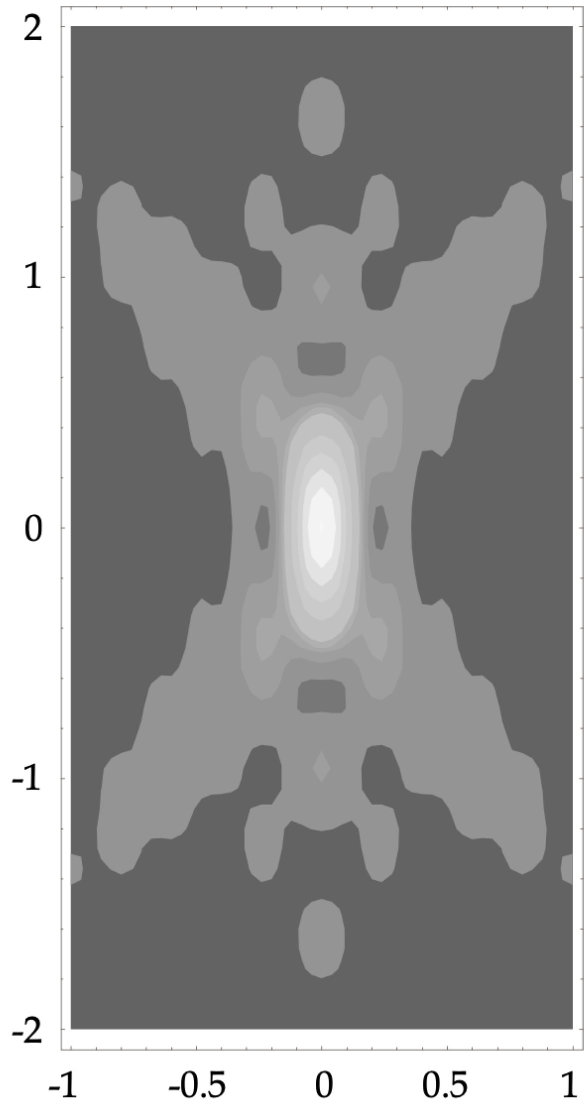
Bead
normalisiert

From “Fluorescence Microscopy: From Principles to Biological Applications“

Quantitative 3D-intensity profile in the focus of an objective lens with NA = 1.3 at 488 nm

axial distance to focus [μm]

d_z=0.88μm

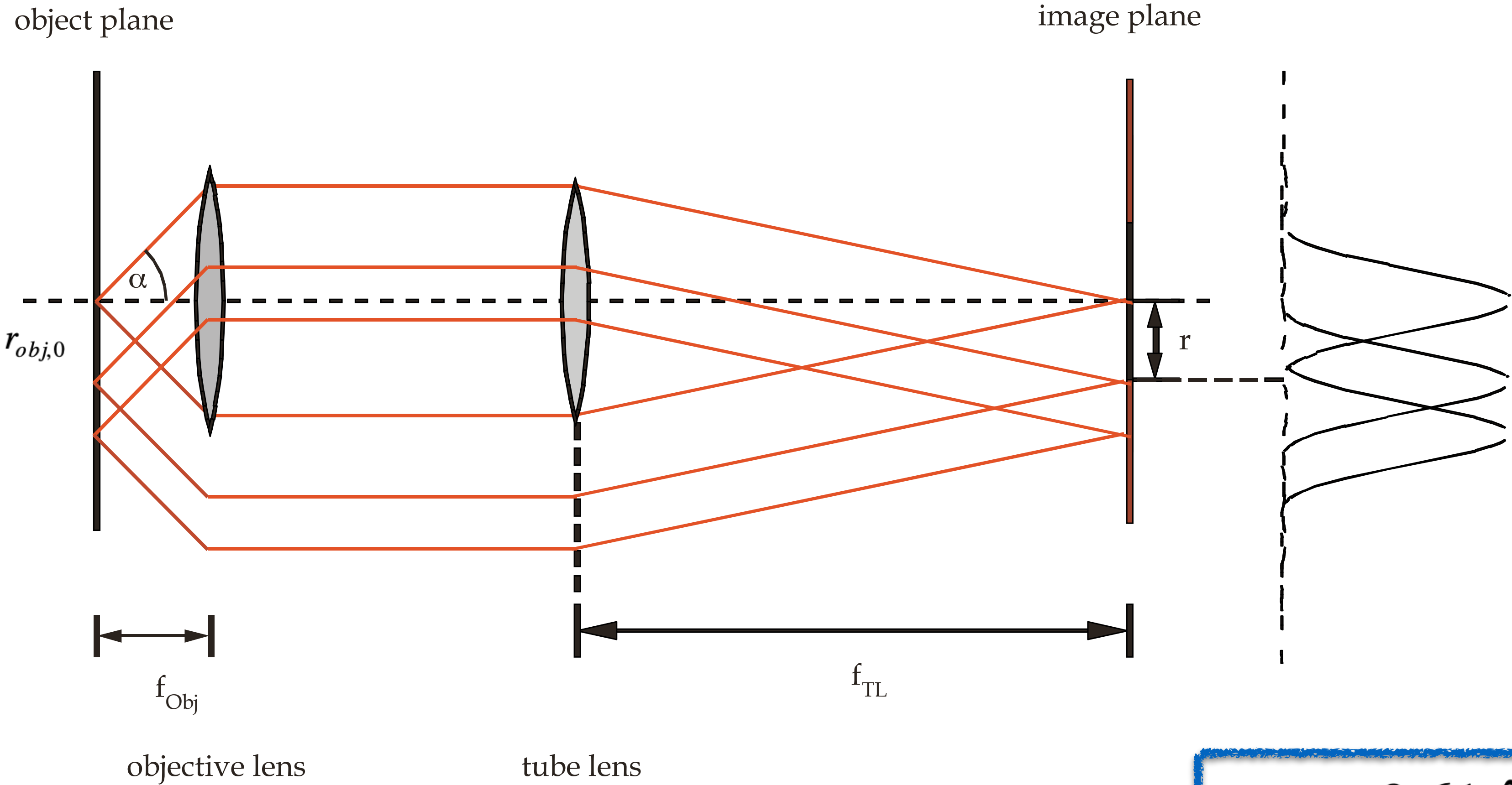


d_r=0.23μm

lateral distance to focus [μm]

3D point spread function

Resolution Limit According to Lord Raleigh

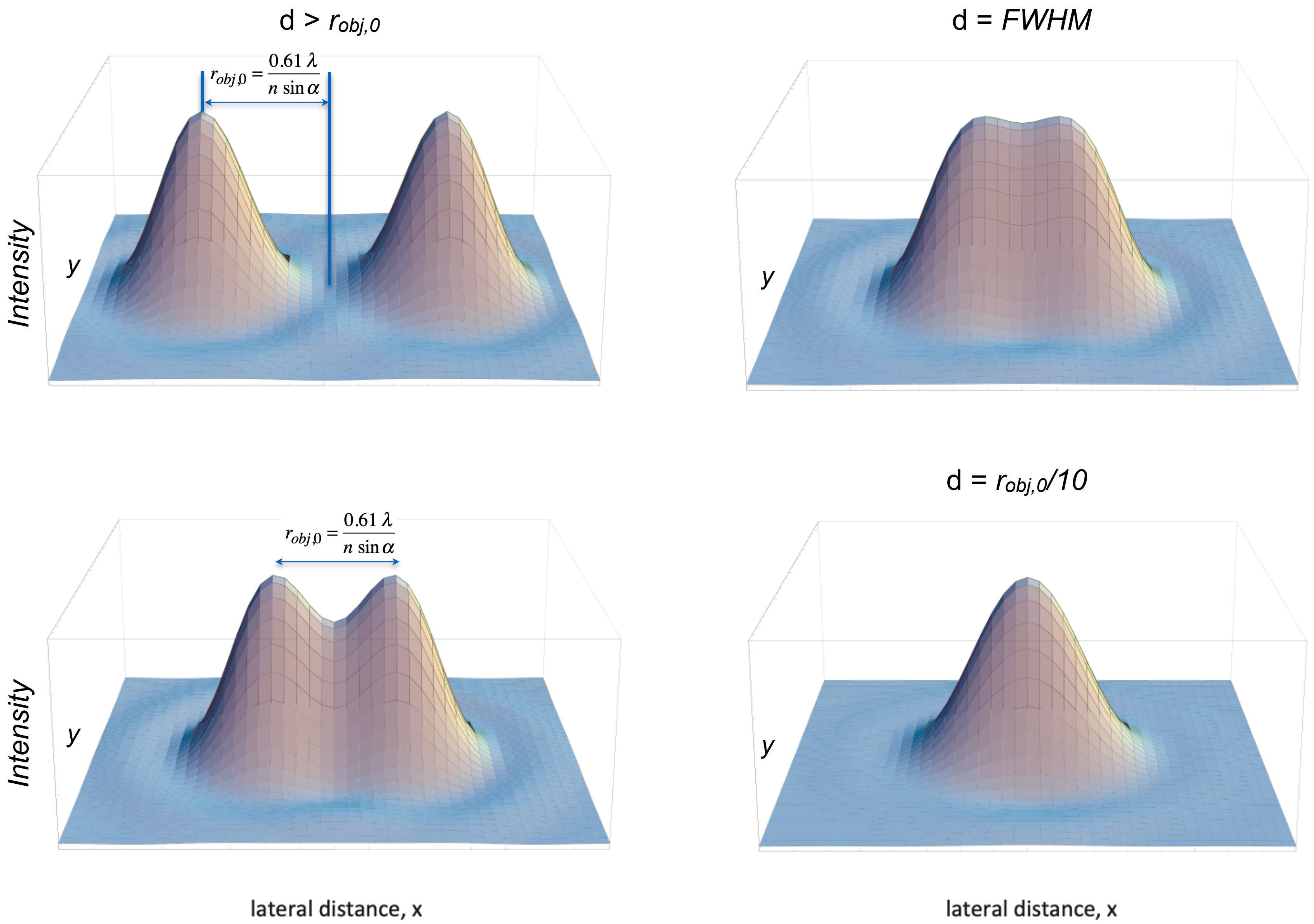


α, opening angle of the objective lens divided by 2
n, refractive index of the medium in front of the objective lens

$$r_{obj,0} = \frac{0.61\,\lambda}{n\ \sin\,\alpha}$$

$$NA_{Obj} = n\sin\alpha$$

Sum of two Point Spread Functions for incoherent point objects



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