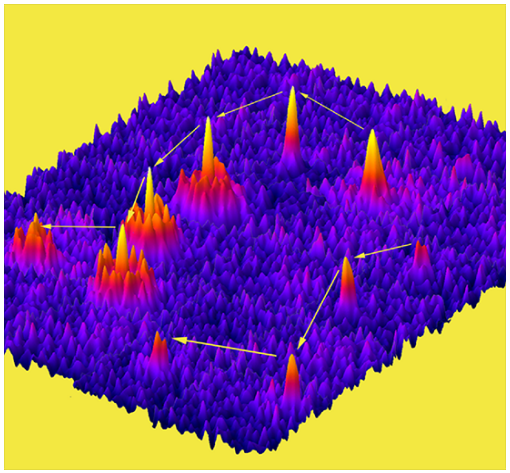
Course „Optics, Forces & Development“



Principles of Optics I

Ulrich Kubitscheck

Clausius-Institute of Physical and Theoretical Chemistry
Rheinische Friedrich-Wilhelms-Universität Bonn

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Contents

high resolution microscopy: STED, STORM
light sheet microscopy

techniques: FRET, FRAP and force measurements by light

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1. Additional Information

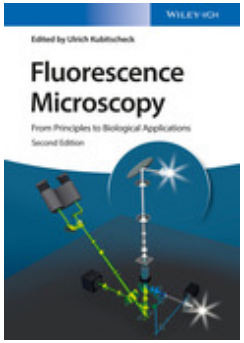
Good Collection of Online Learning Tools

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

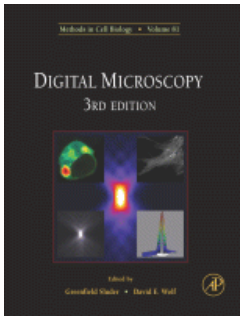
https://zeiss-campus.magnet.fsu.edu/

Books

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



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



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2. Basics: waves
diffraction
lenses
aberrations

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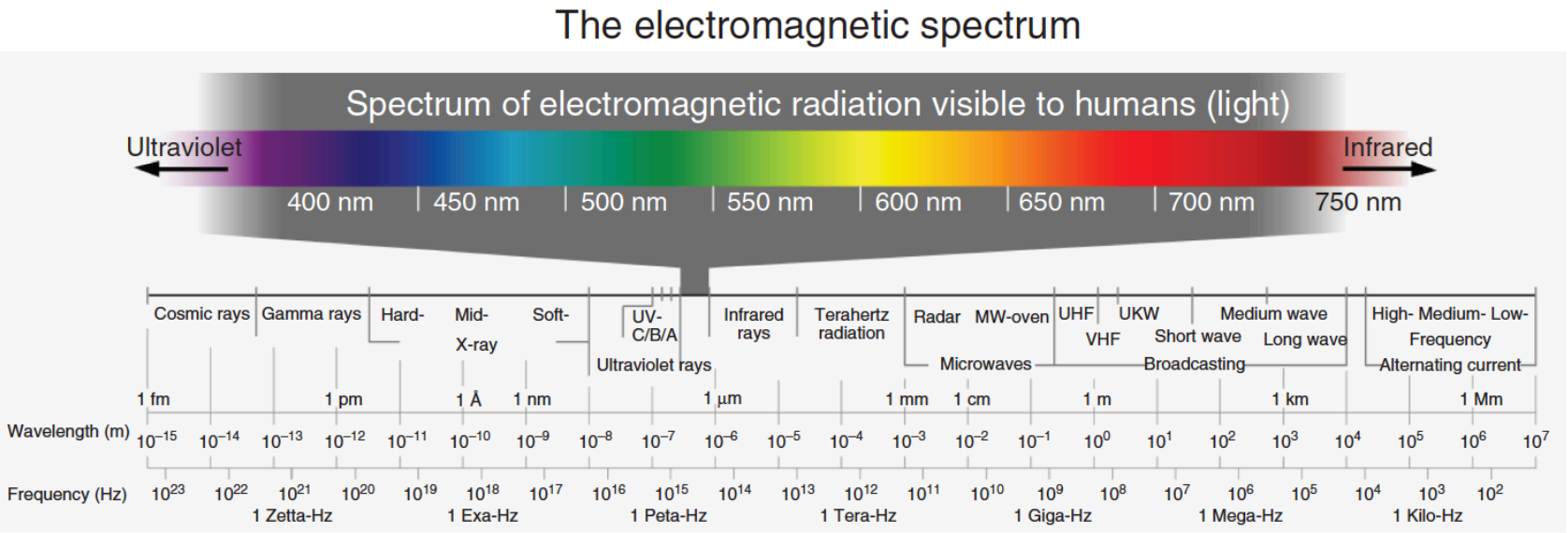
The electromagnetic spectrum



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).

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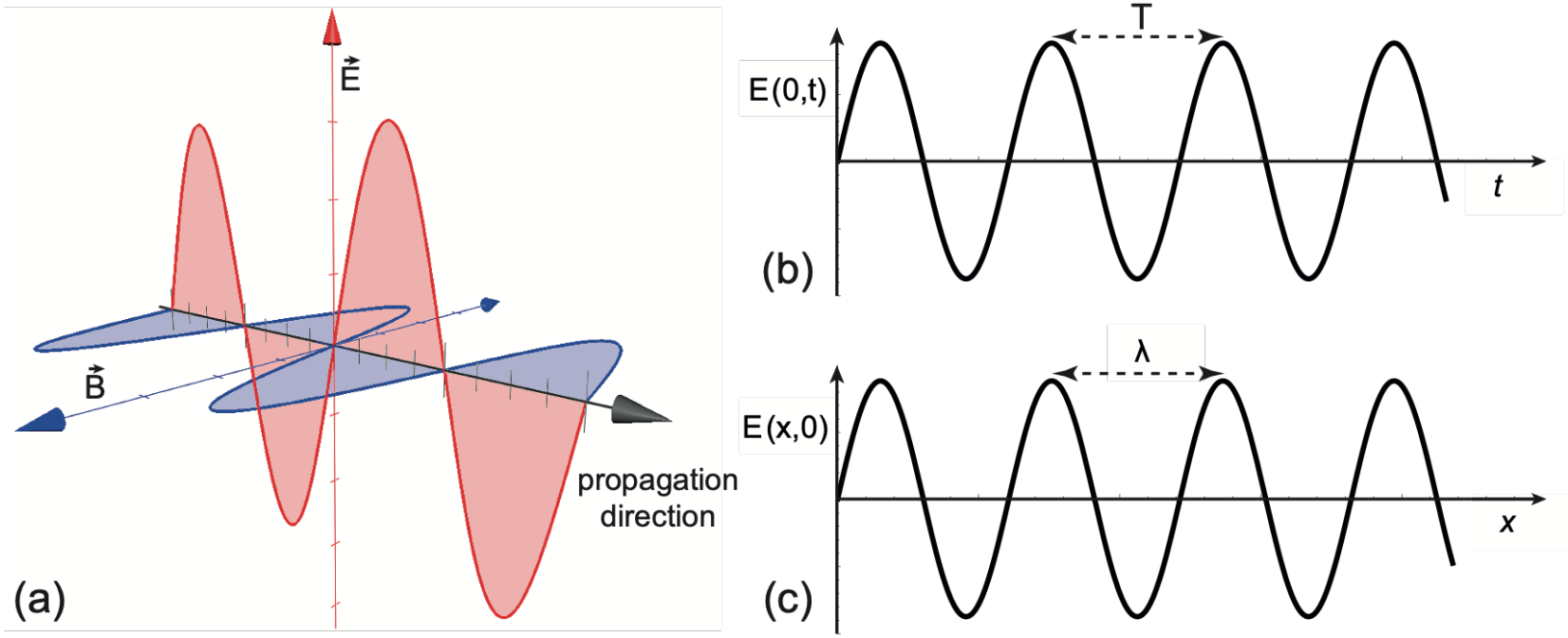
Electromagnetic waves



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.

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

$$c_{vac} = 299.792.458\ m/s$$

$$= 299.792,458\ km/s$$

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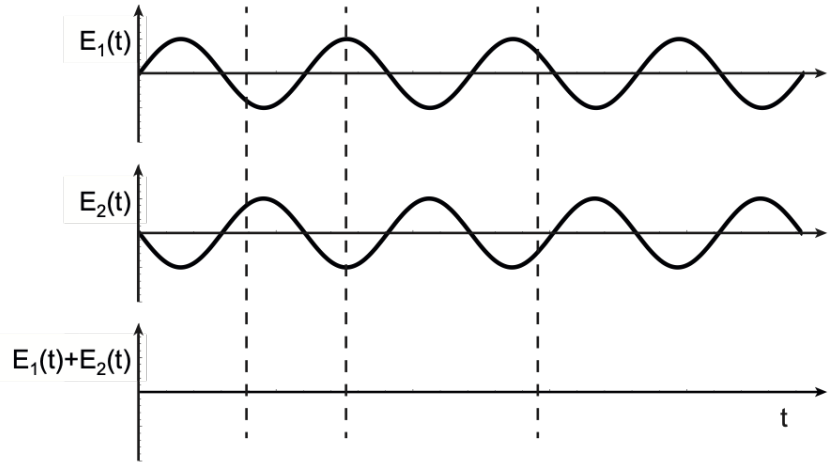
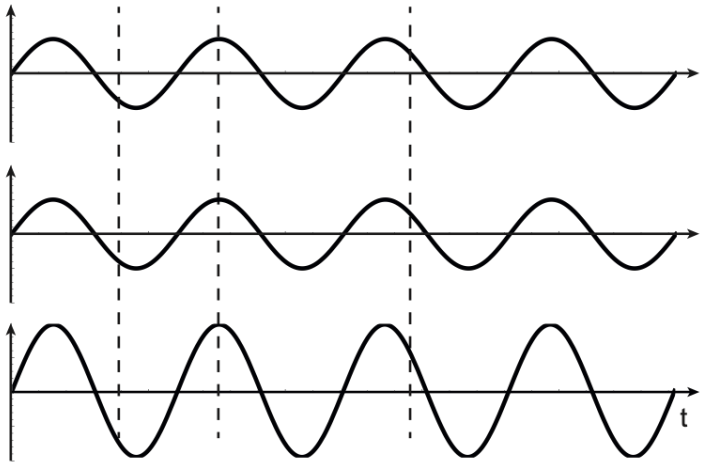
From “Fluorescence Microscopy: From Principles to Biological Applications“, edited by U. Kubitscheck. Wiley-VCH, Weinheim, 2nd edition

Interference of waves

destructive interference



constructive interference



From “Fluorescence Microscopy: From Principles to Biological Applications“

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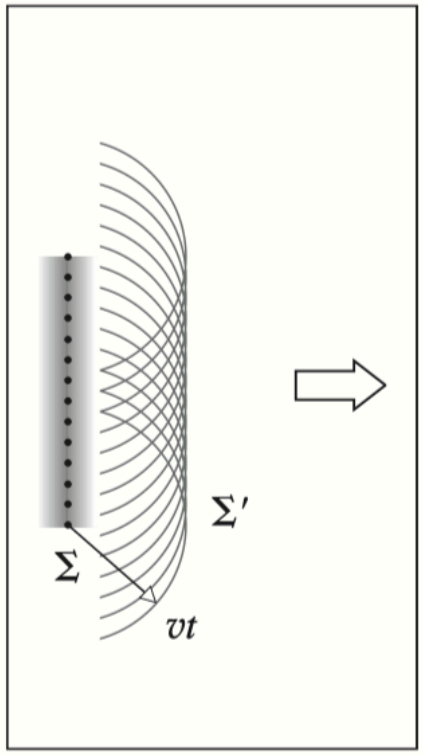
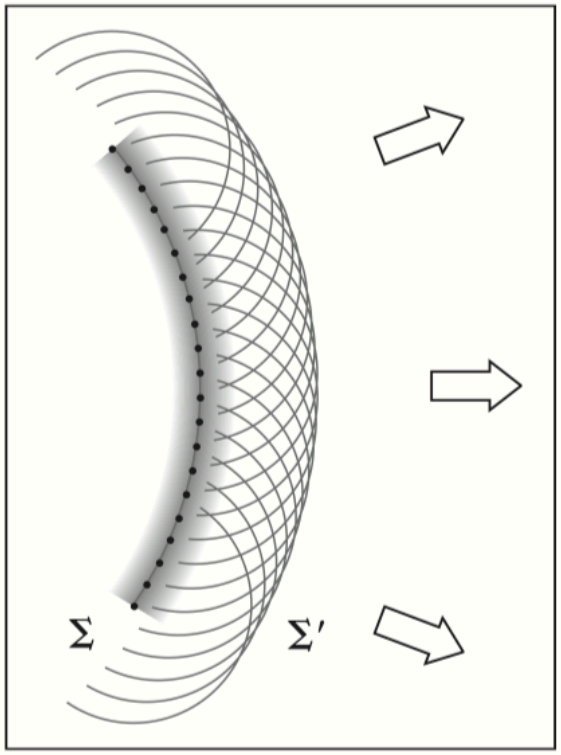
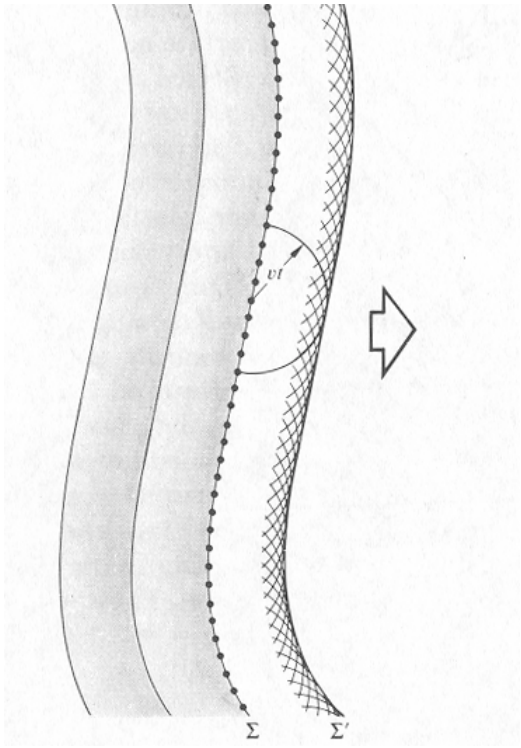
The concept of Huygen´s elementary waves

„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

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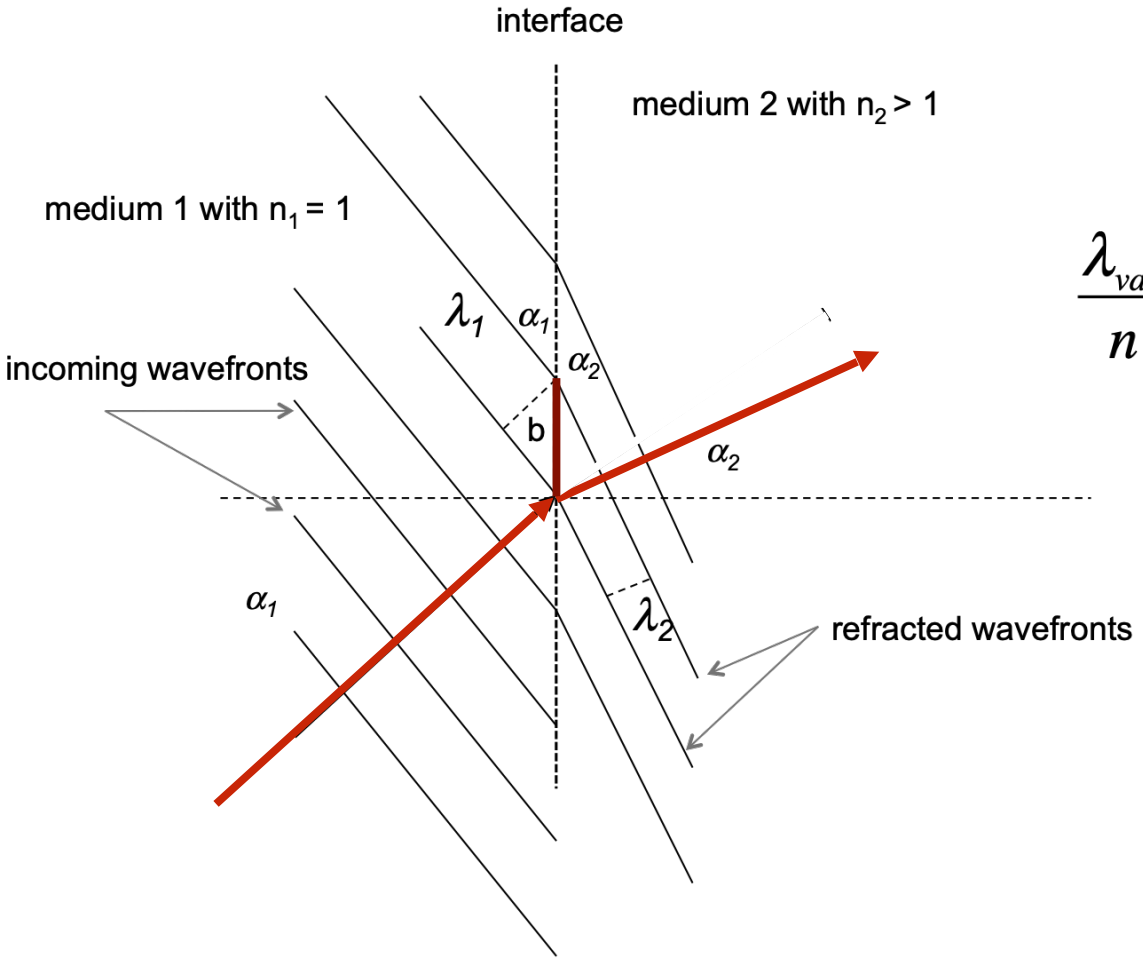
Snell´s law of refraction



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

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

$$n_1 \sin \alpha_1 = n_2 \sin \alpha_2$$

From “Fluorescence Microscopy: From Principles to Biological Applications“

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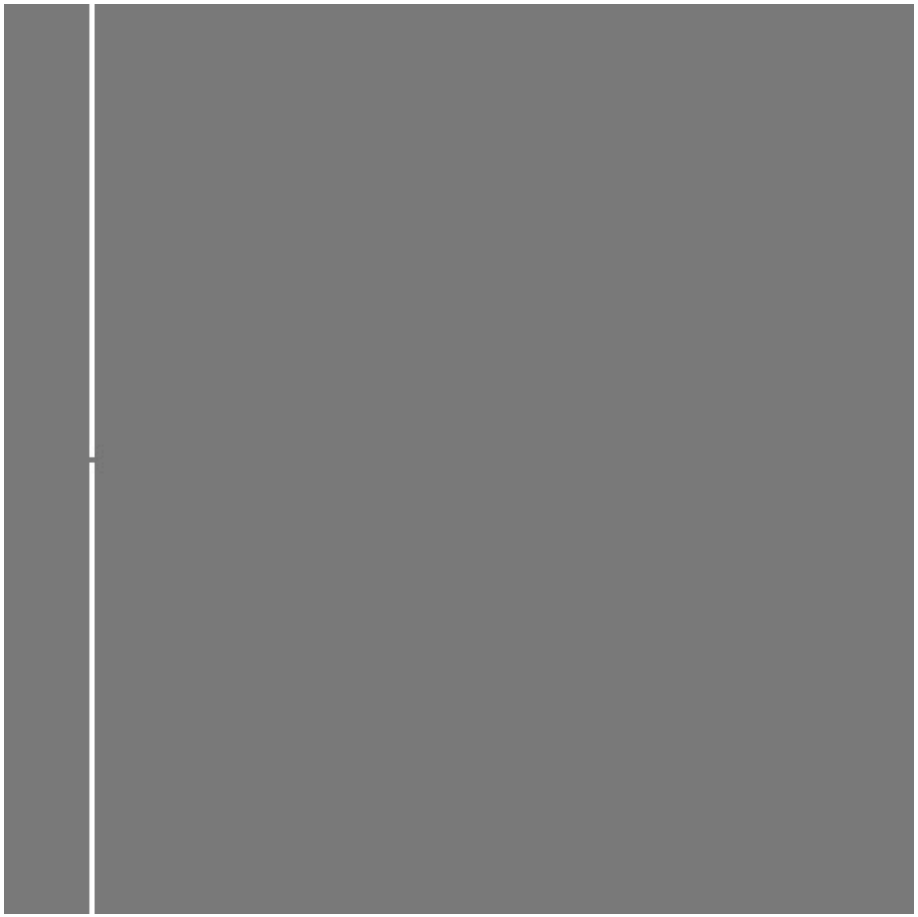
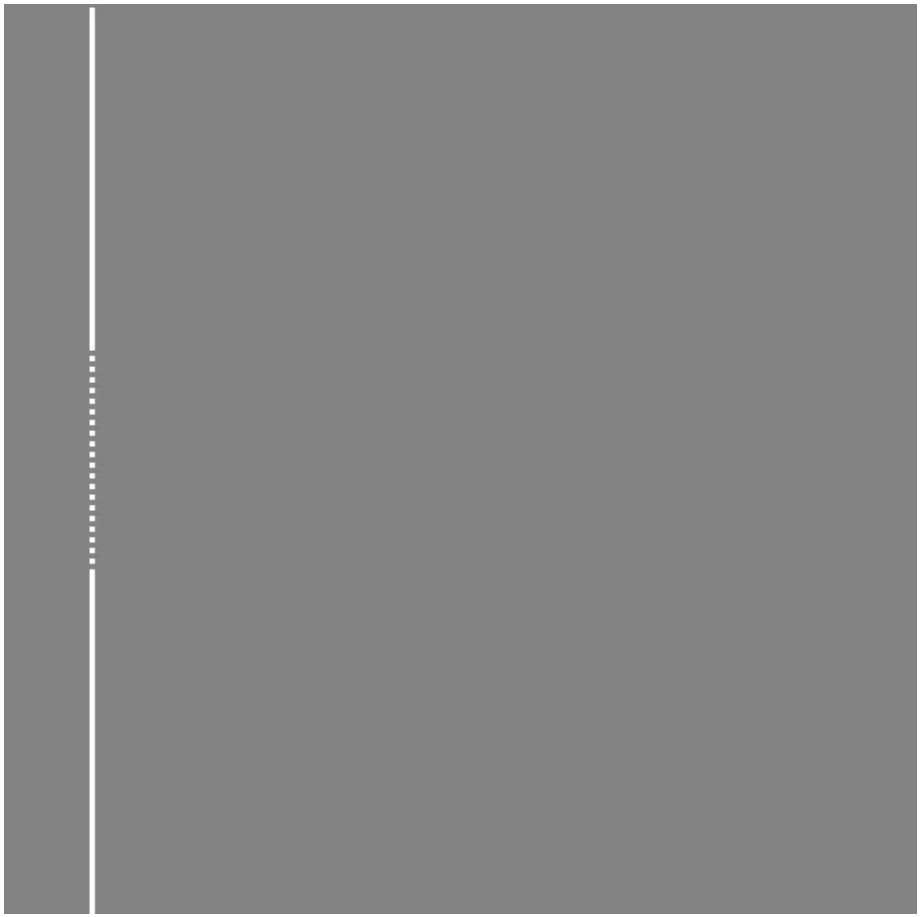
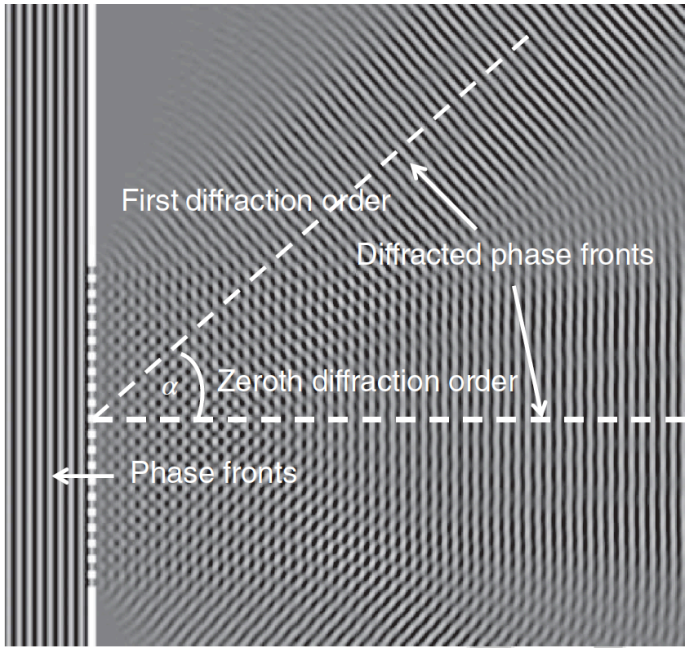
Diffraction at a pinhole ➡ spherical wave



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

Diffraction at a 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.

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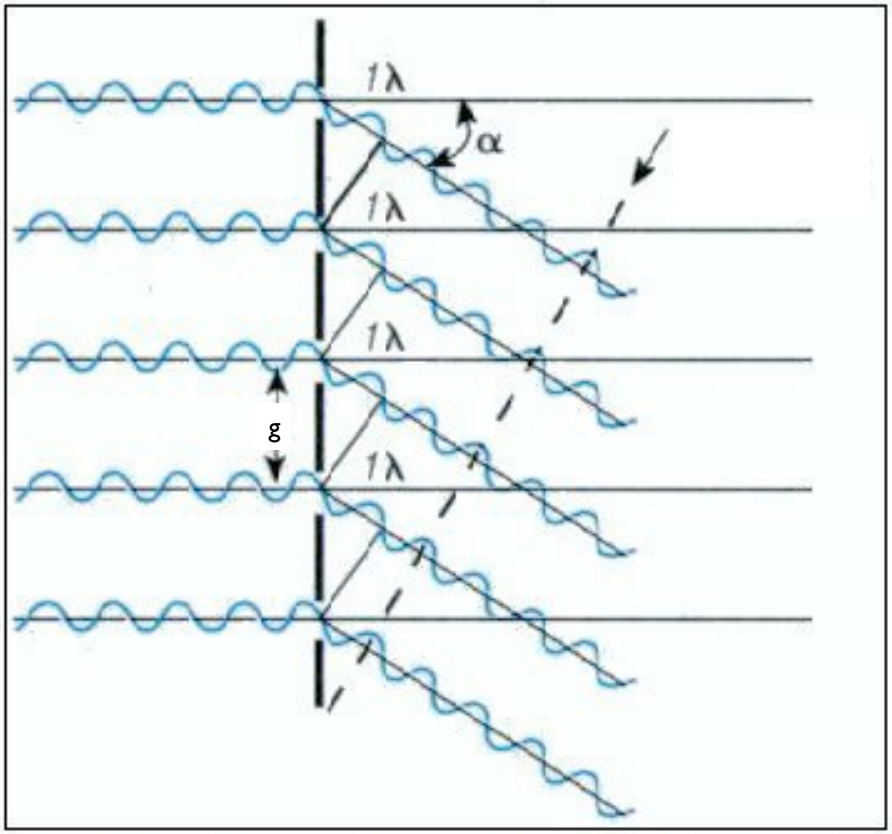
From “Fluorescence Microscopy: From Principles to Biological Applications“, online supplemental material

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

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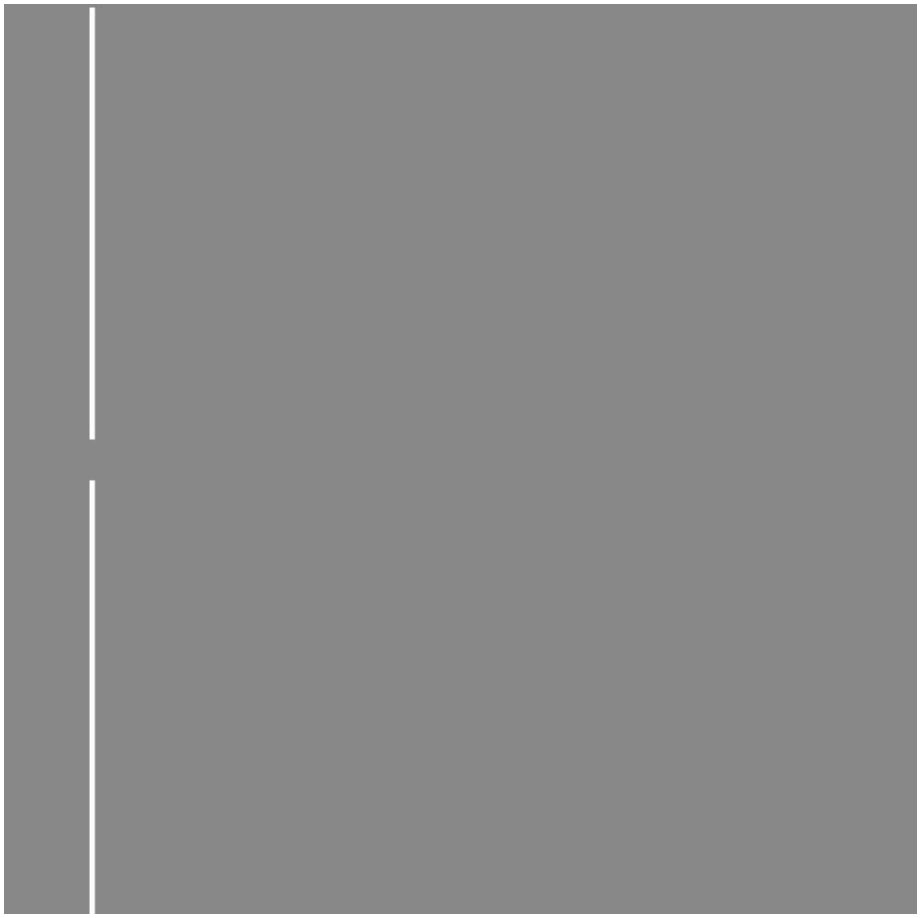
Diffraction at an open pinhole



(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

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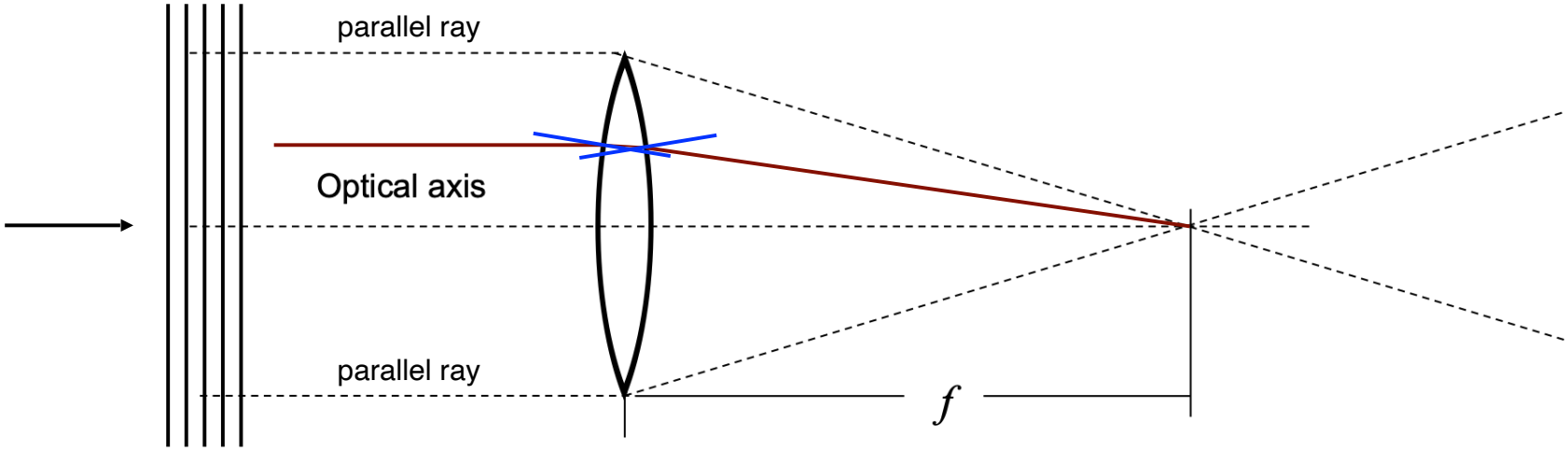
Lenses



From “Fluorescence Microscopy: From Principles to Biological Applications“

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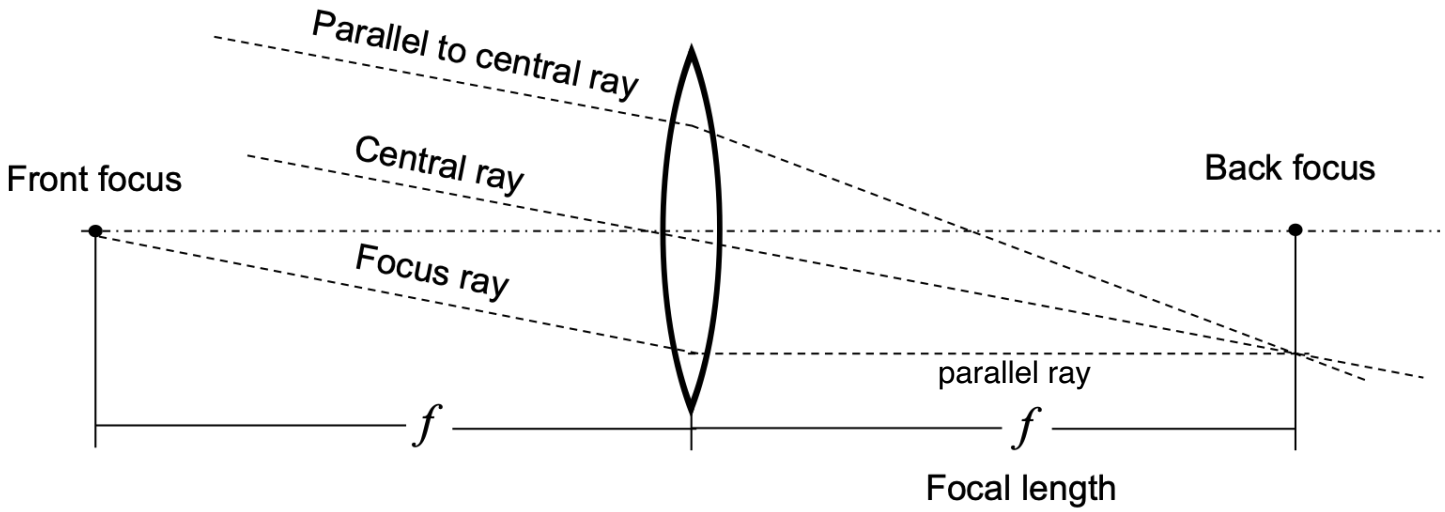
Special rays passing lenses



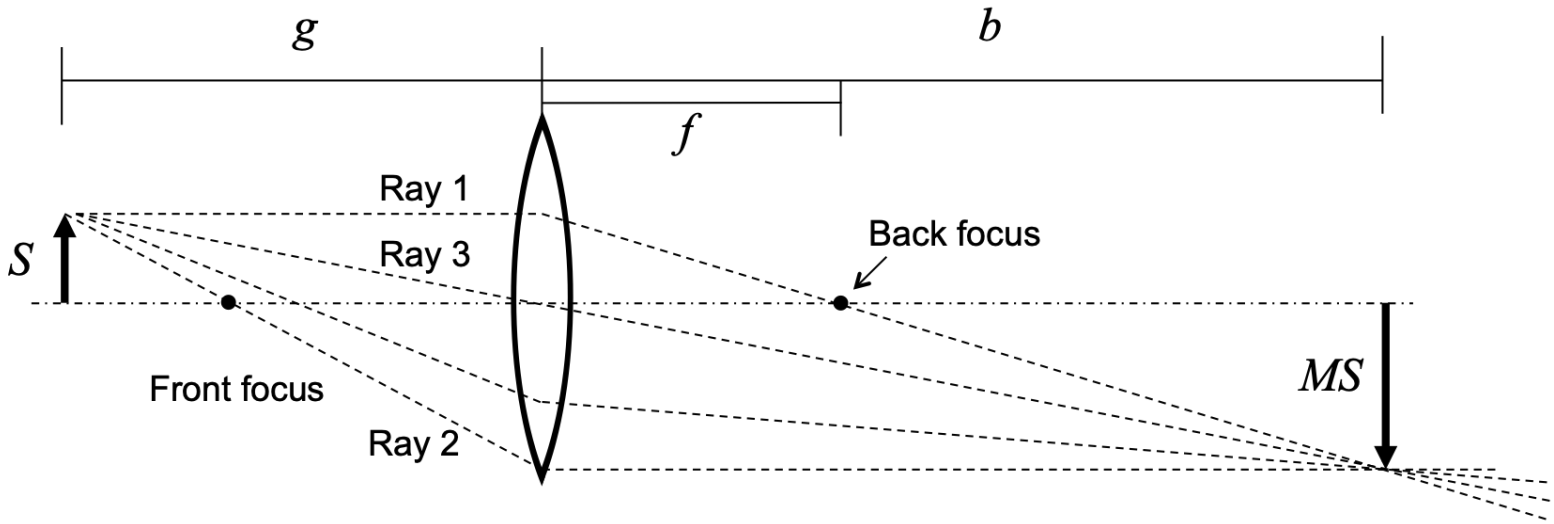
Nice Applet:

Optical reversal: retrace rays and yield identical paths

http://www.walter-fendt.de/ph14d/bildsammellinse.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“

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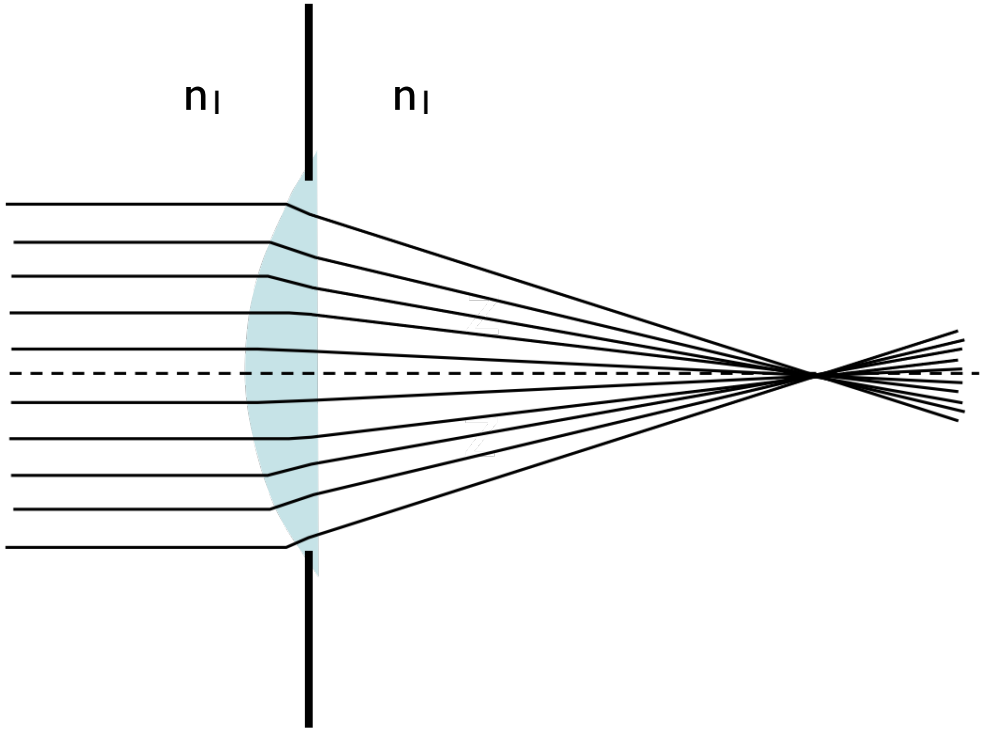
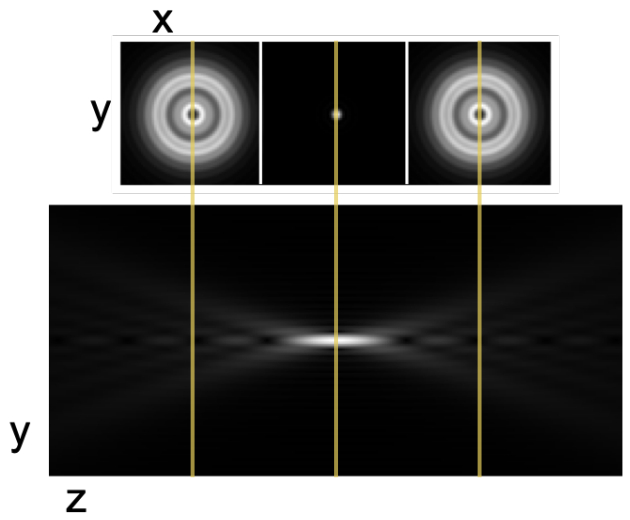
Focusing of light: ray model & wave model

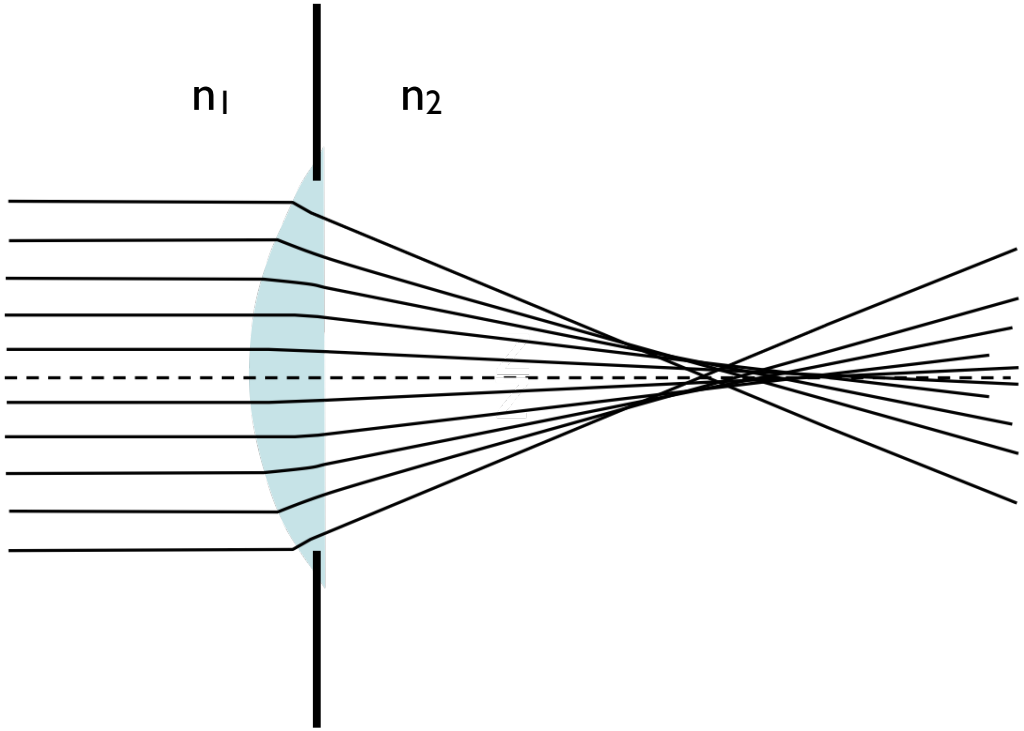


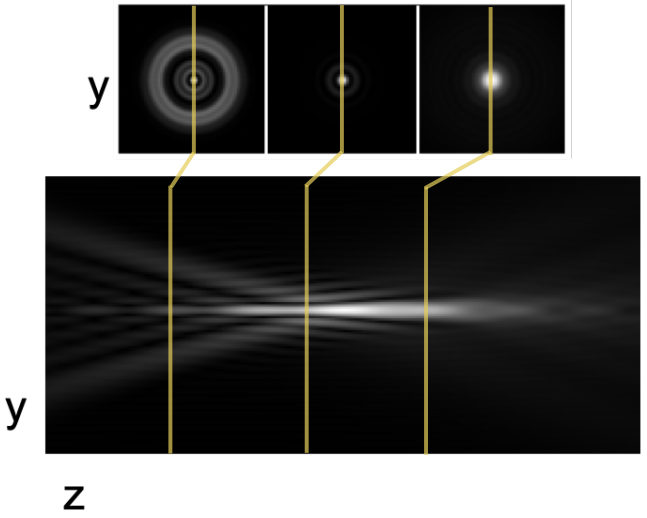


A plane wave - sketched by the parallel incoming rays - hits perpendicularly on a large pinhole.
The lens focuses the diffracted ways into its focus.
Again we find directions of constructive and destructive interference

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

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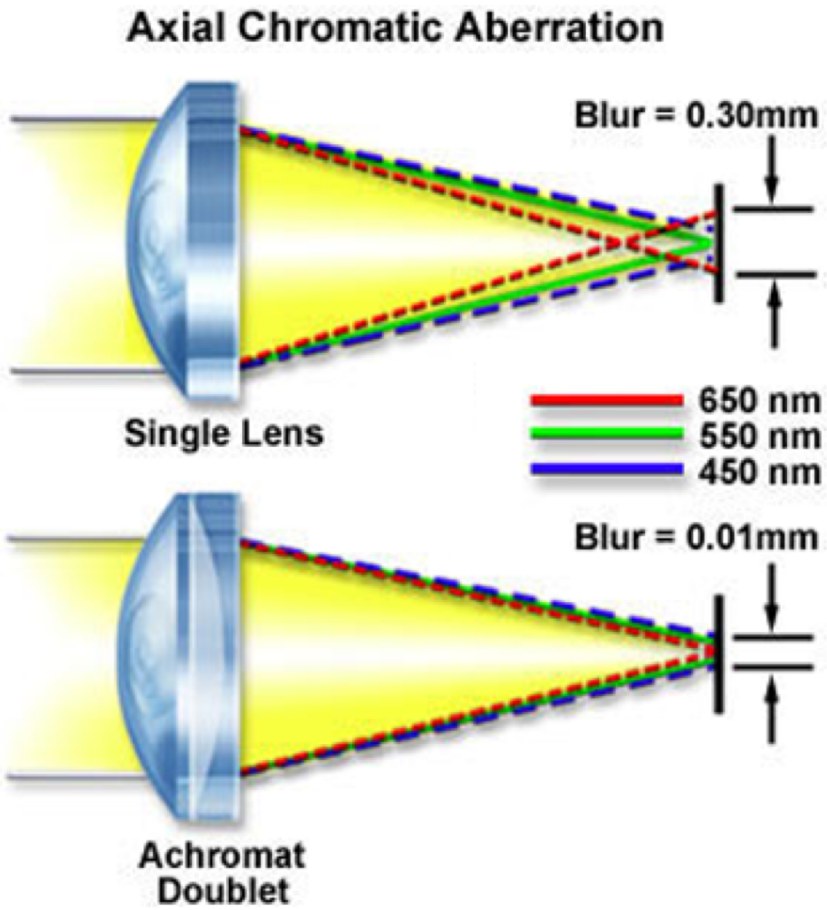
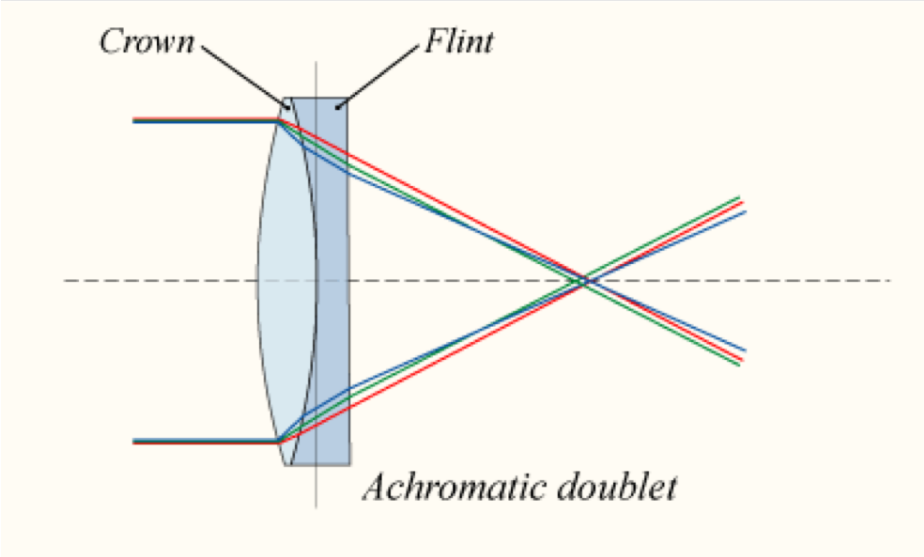
Chromatic aberrations

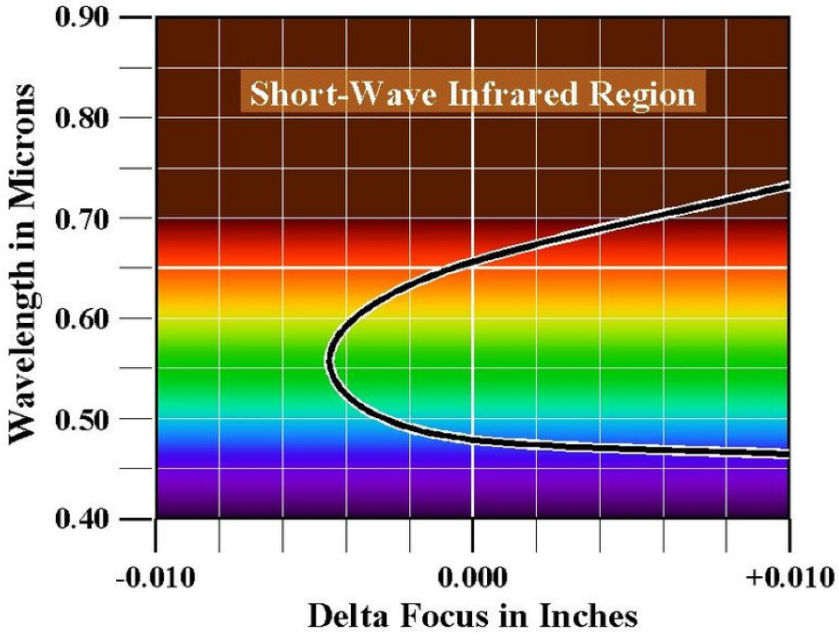


Refraction is wavelength-dependent: each color has its own focus and magnification

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

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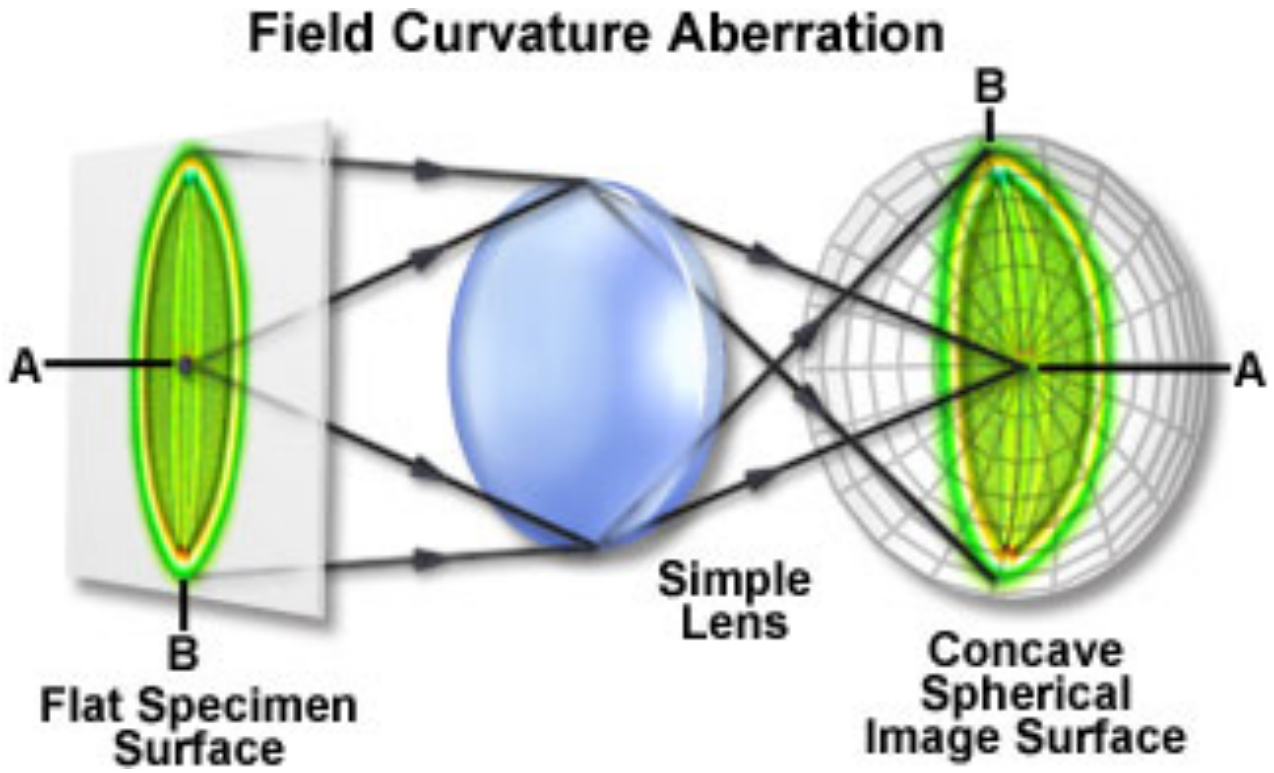
Curvature of field



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

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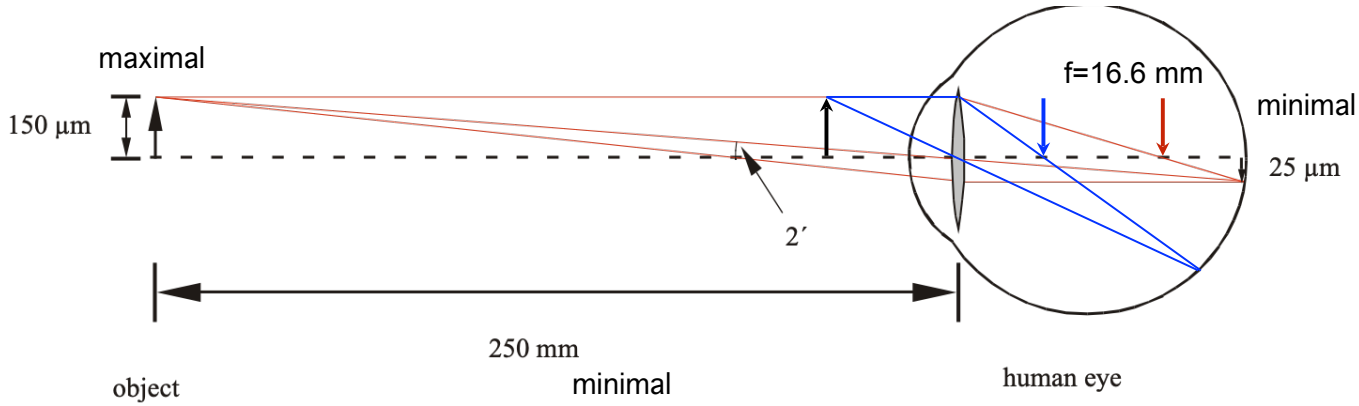
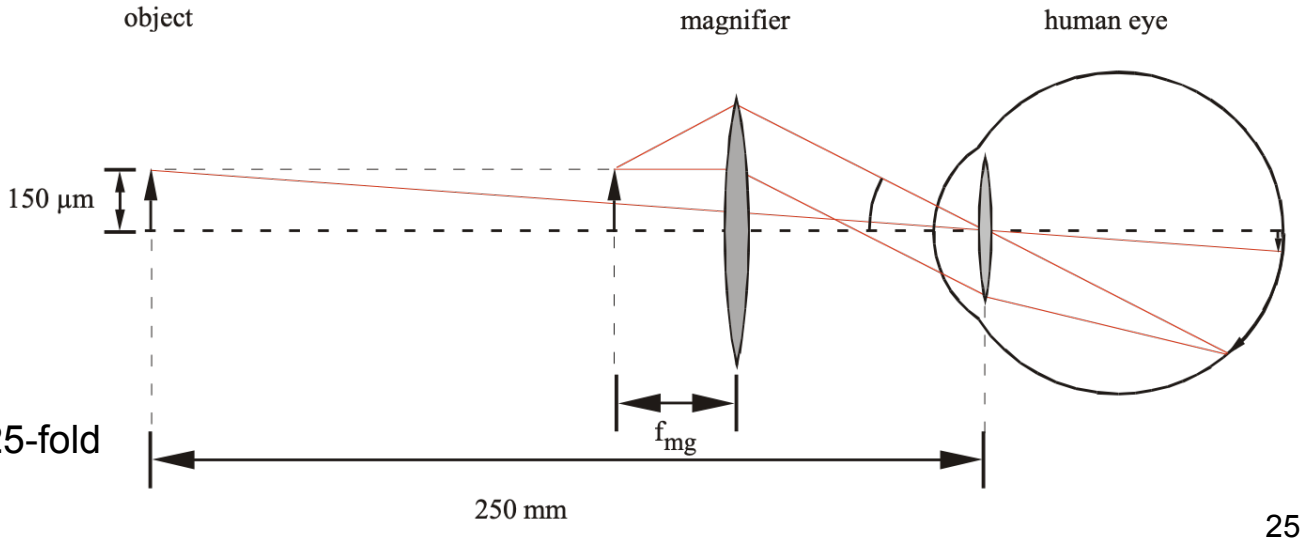
Why use a microscope?

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

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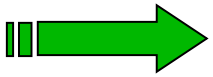
Tasks of a light microscope

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

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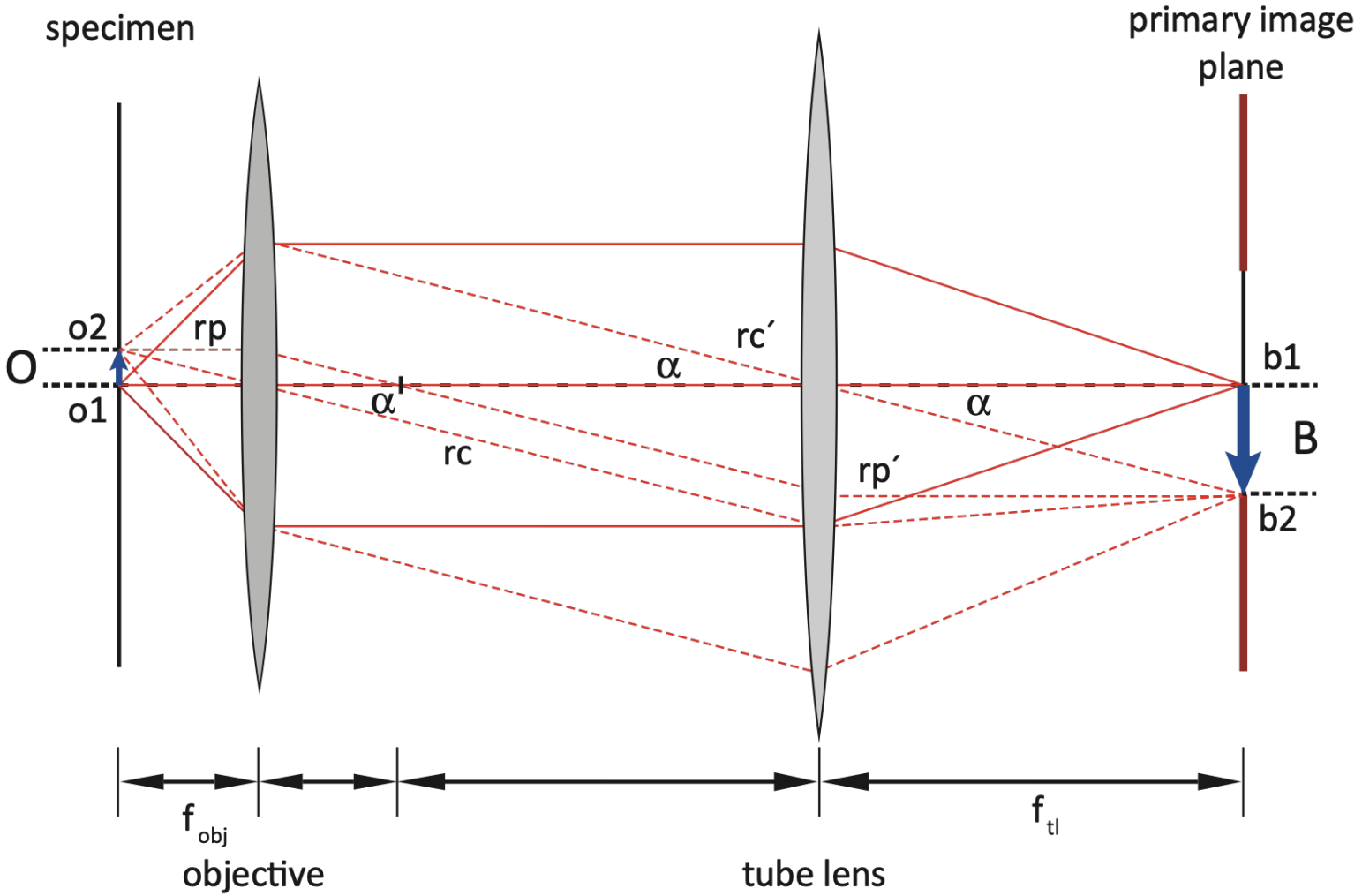
Imaging process in an „infinity beam path“



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

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

4. Two-stage microscope

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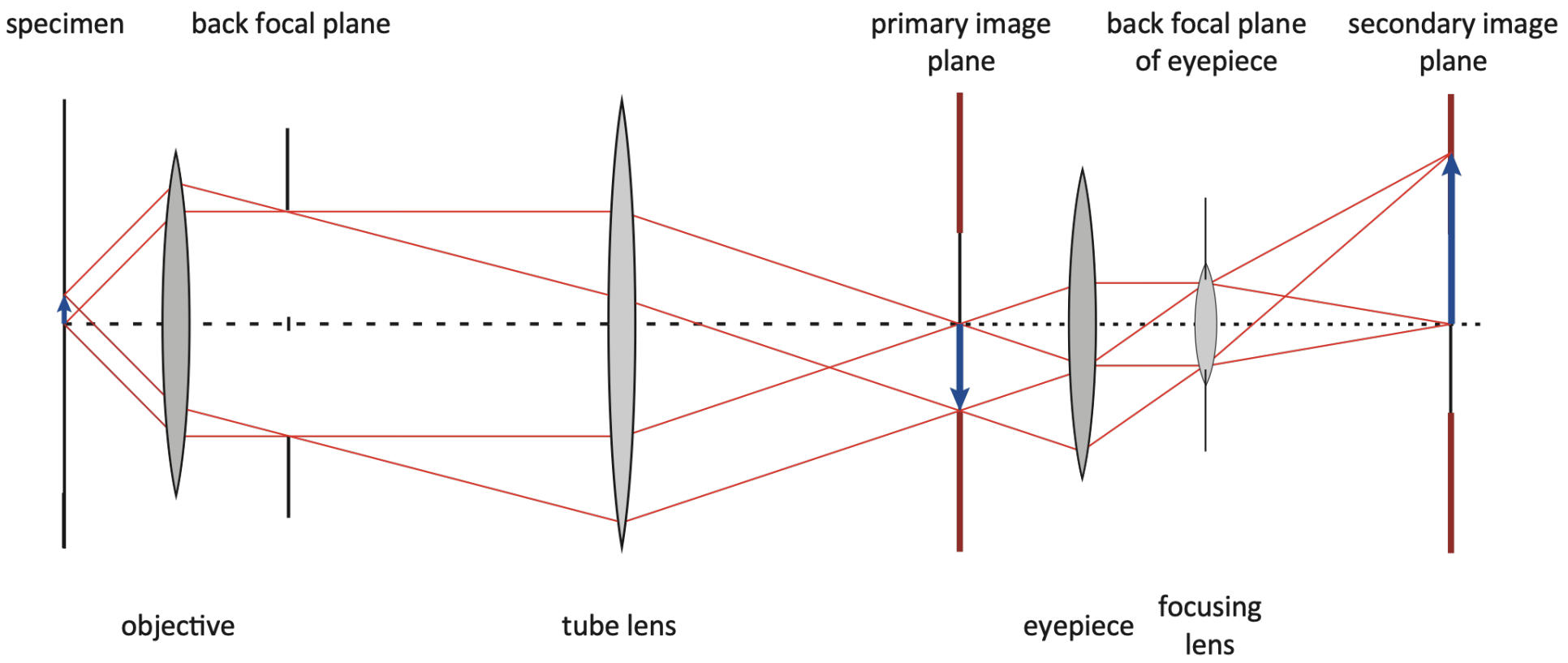
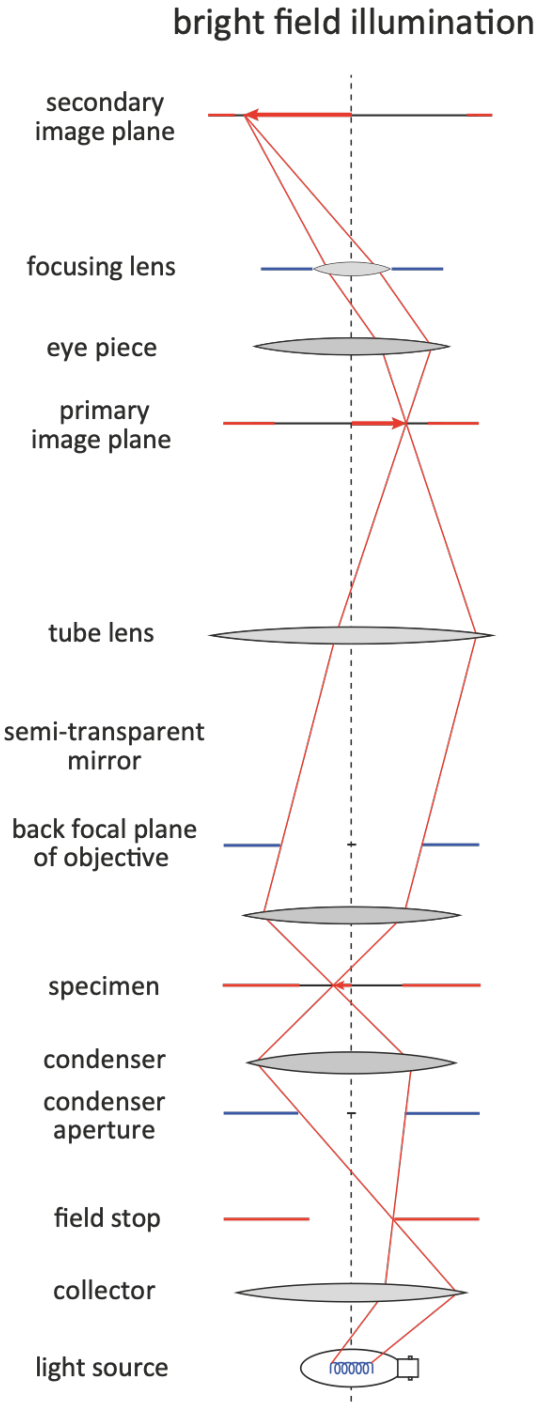
Construction of a microscope
by combination of two magnification stages



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

Epi- and dia-illumination



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

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5. What about resolution?
The point spread function

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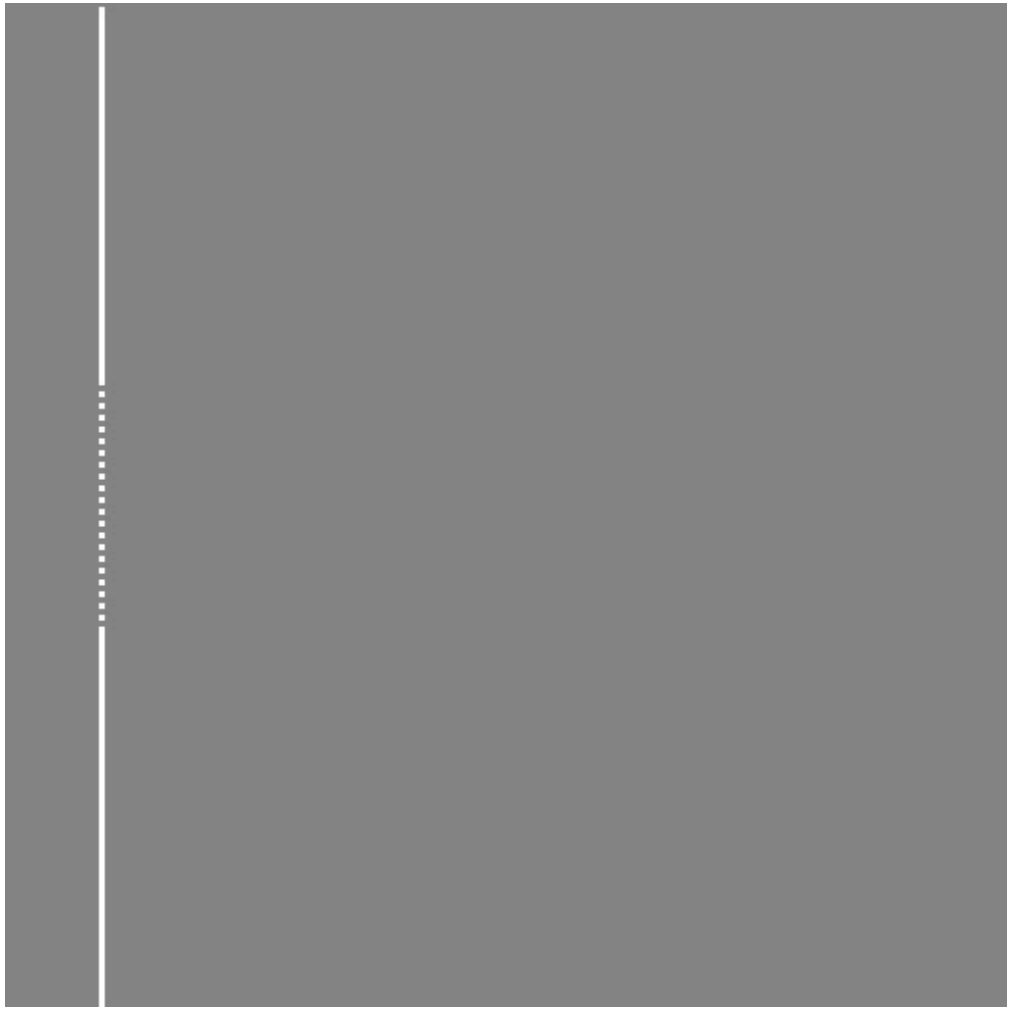
Diffraction at a grating I



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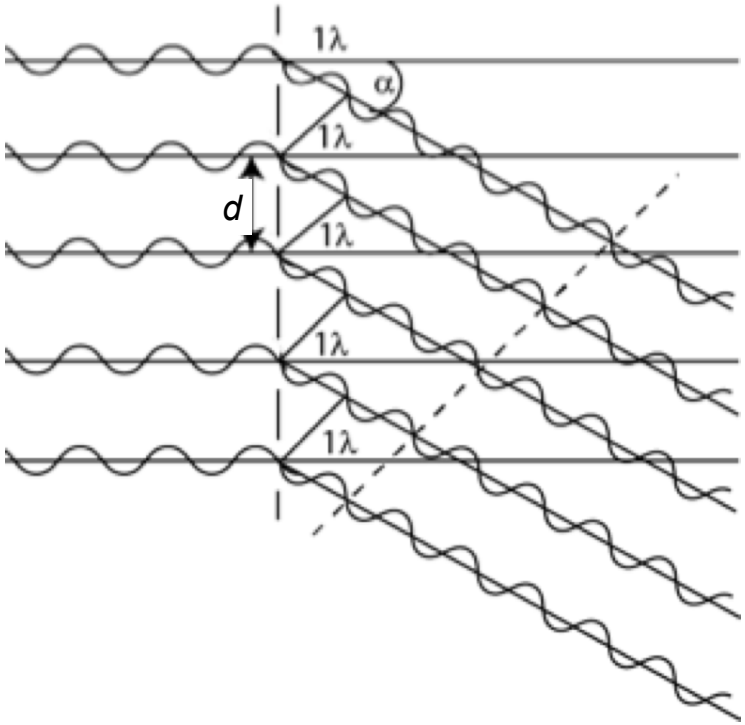
Diffraction at a grating II



d, grid constant

α diffraction angle

for Fraunhofer diffraction:

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

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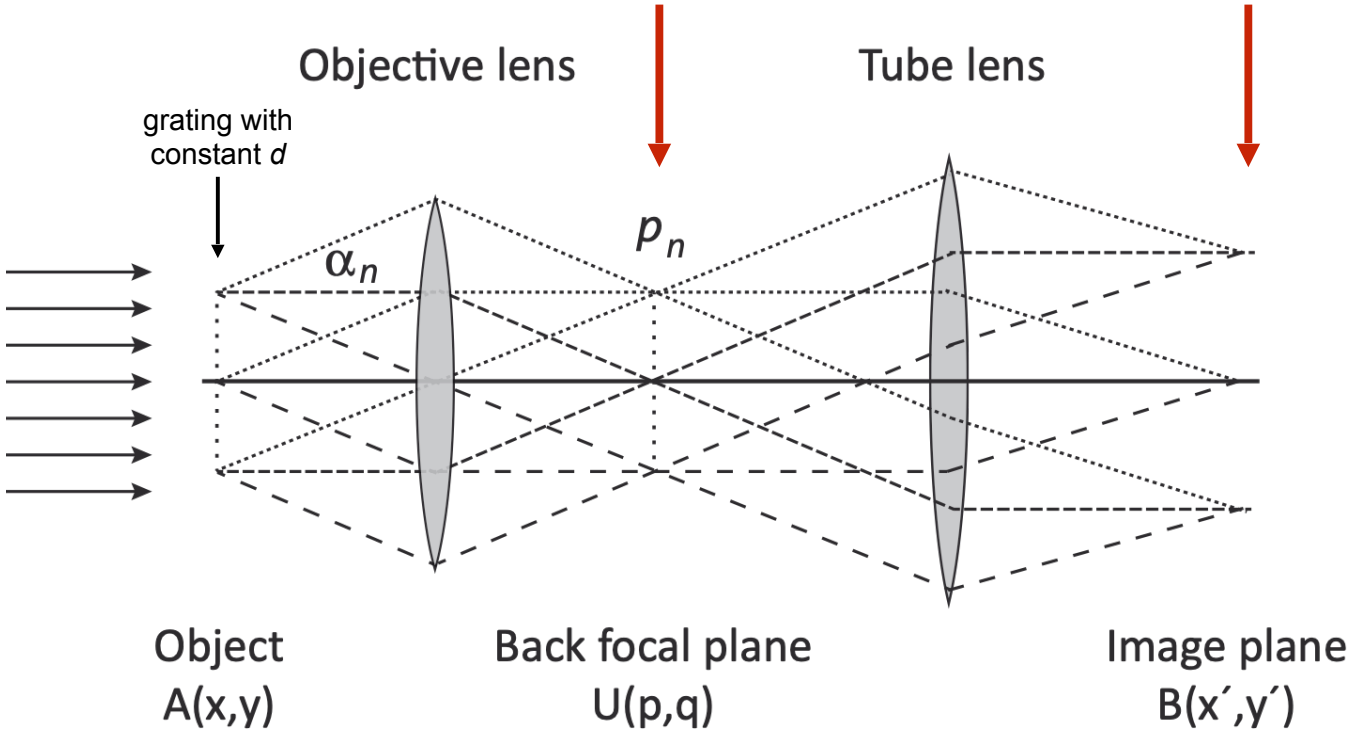
The diffraction pattern is projected into the back focal plane



von Bieren condition

$$\sin \alpha_n = \frac{p_n}{f}$$

inserting the grid equation for the sine .. thus:

$$p_n = \frac{n\lambda f}{d}$$

$$\boxed{p_n \propto 1/d}$$

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

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

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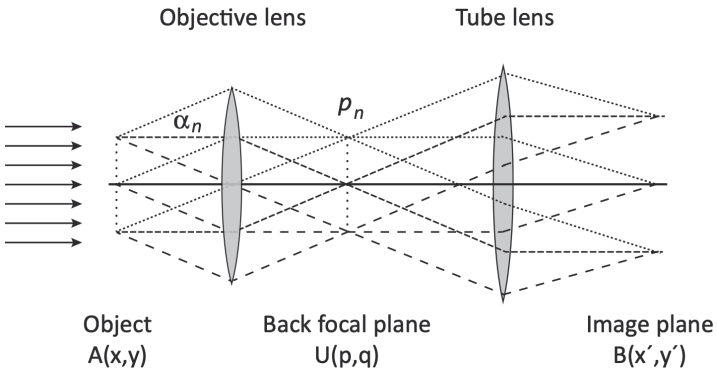
Resolution and Numerical Aperture

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

We need at least the first diffraction maximum in the back focal plane: set n=1 … and solve for d.
However, we will always have a limit of the opening angle due to the finite lens diameter.



Within a medium with refractive index *n* then replace $\lambda \rightarrow \lambda/n$

$$d = \frac{\lambda_0}{\mathrm{n} \sin \alpha_{max}}$$ is the resolution limit for regular (or grid) structures

If d is smaller, then even the first diffraction order cannot be collected and all information is lost!

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

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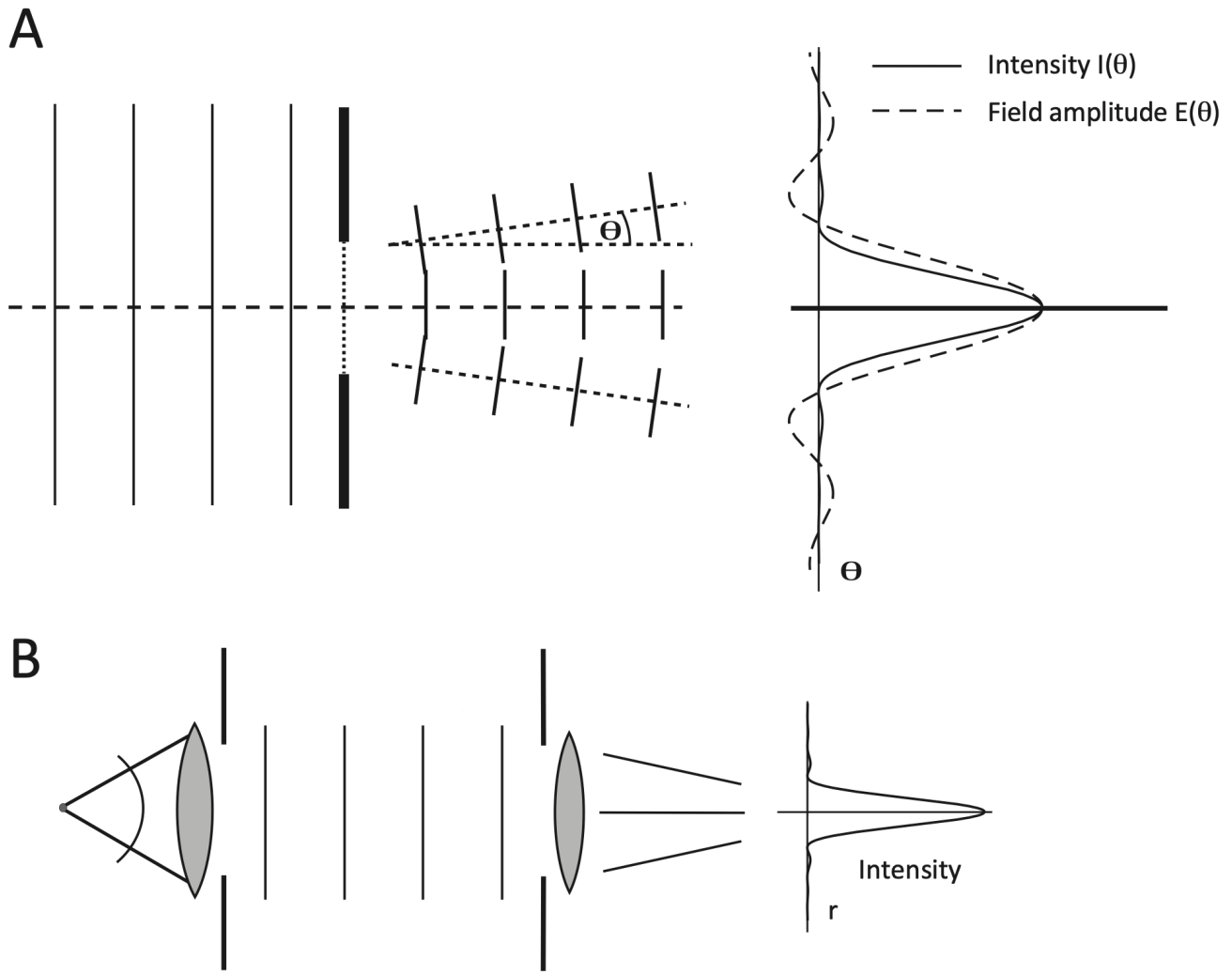
Imaging Point Objects



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

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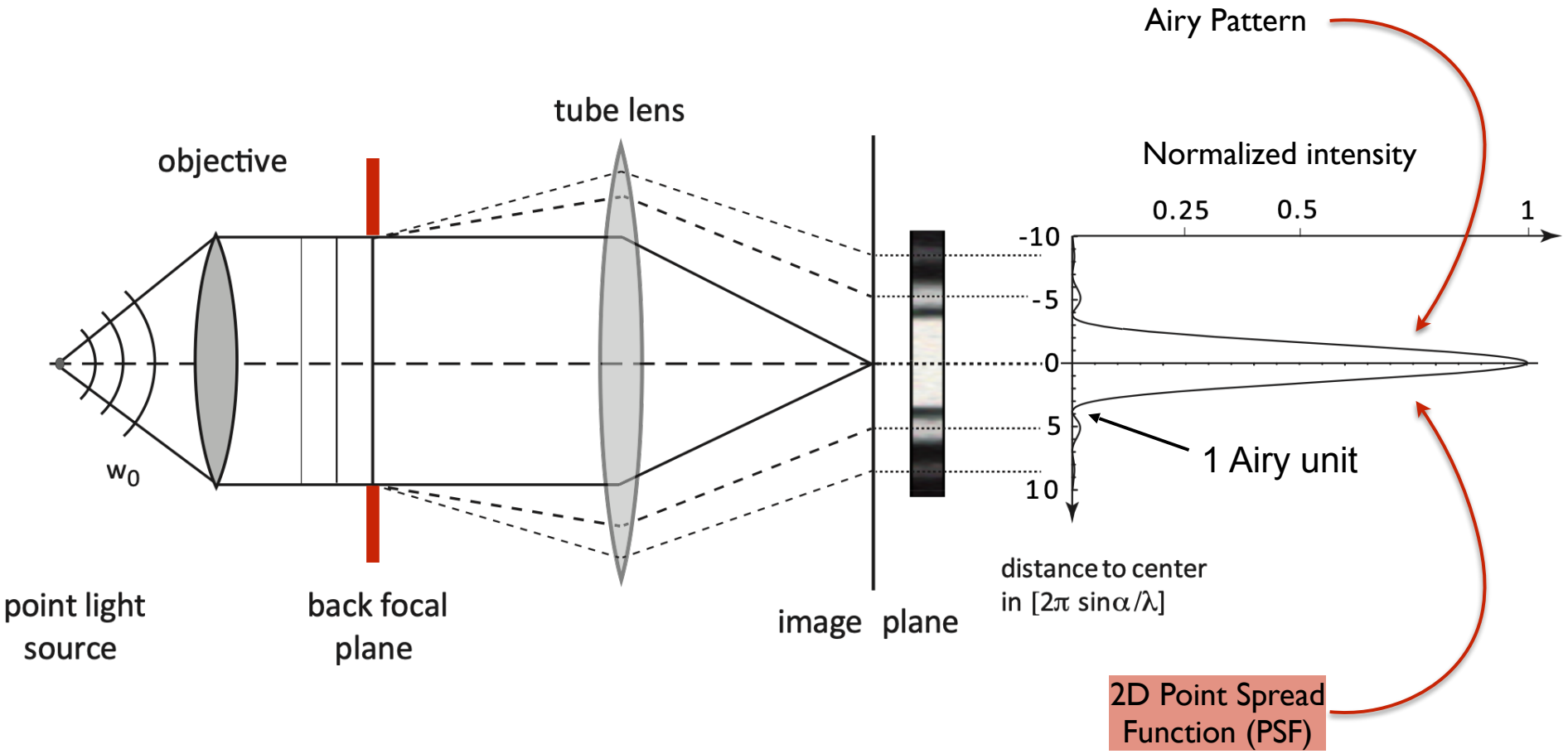
Resolution Limit, Airy Pattern and Point Spread Function

Interference of Huygens waves from the exit pupil of the objective



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

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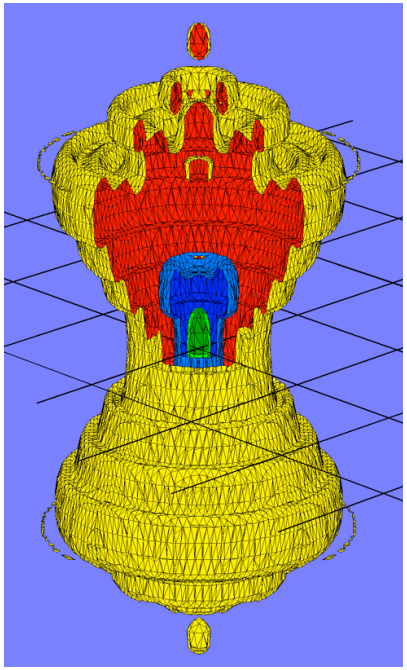
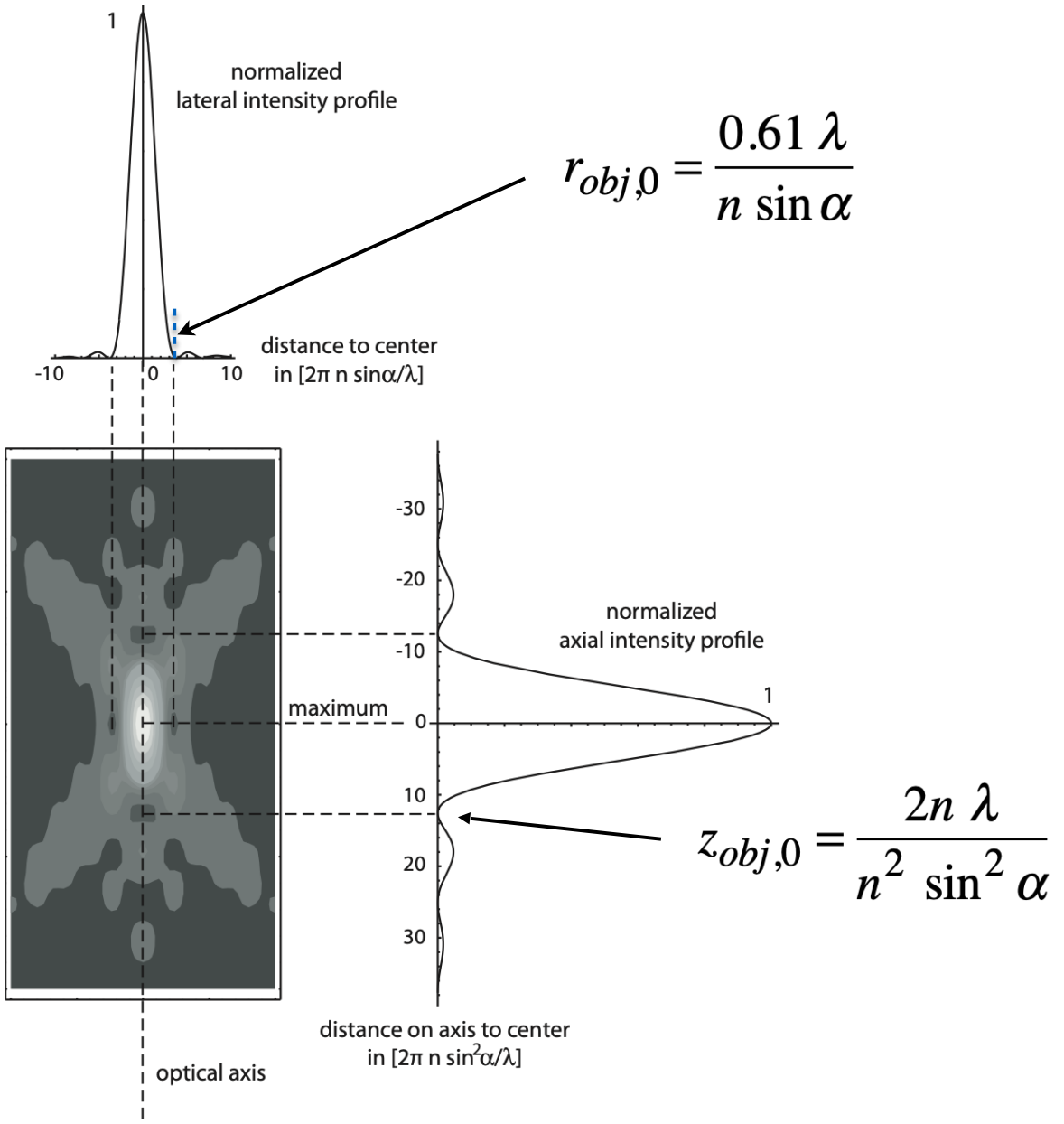
3D Point spread function (PSF)



isosurfaces at
50, 5, 0.5% of max

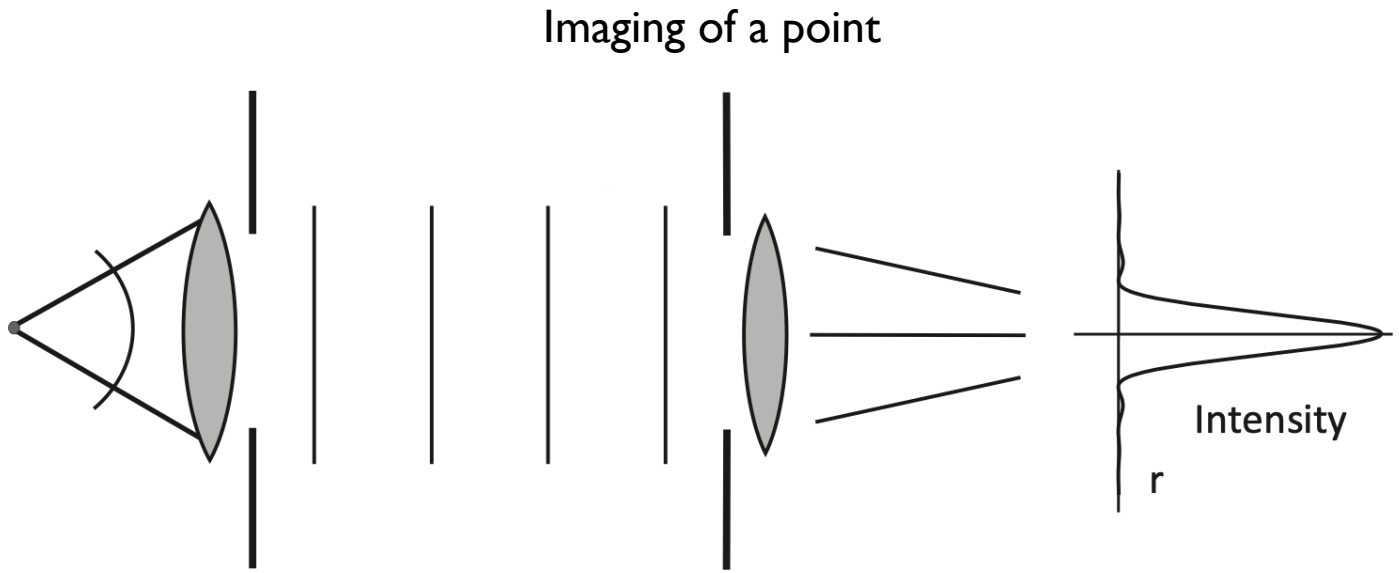


$$r_{obj,0} = \frac{0.61\,\lambda}{n\,\sin\alpha}$$

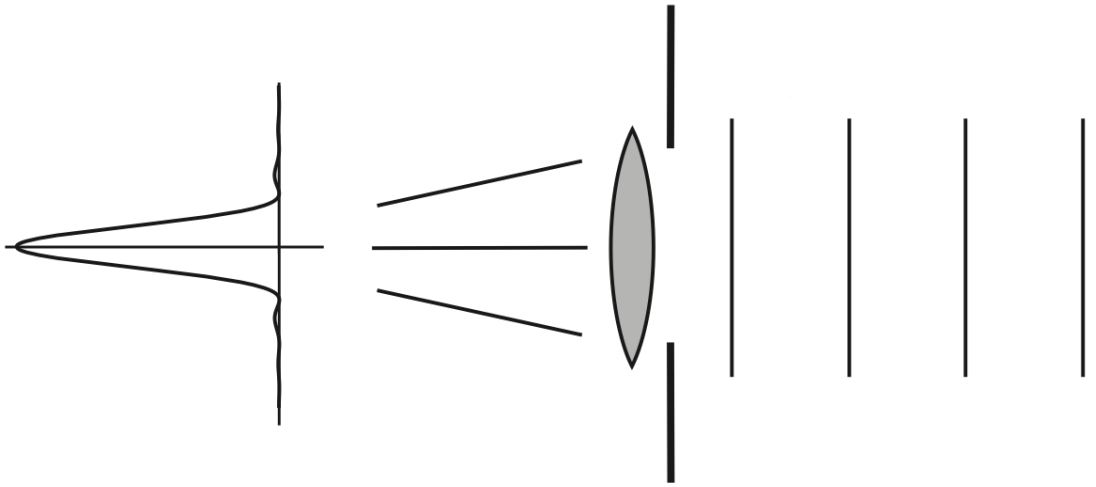
$$z_{obj,0} = \frac{2n\,\lambda}{n^2\,\sin^2\alpha}$$

38

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



object space



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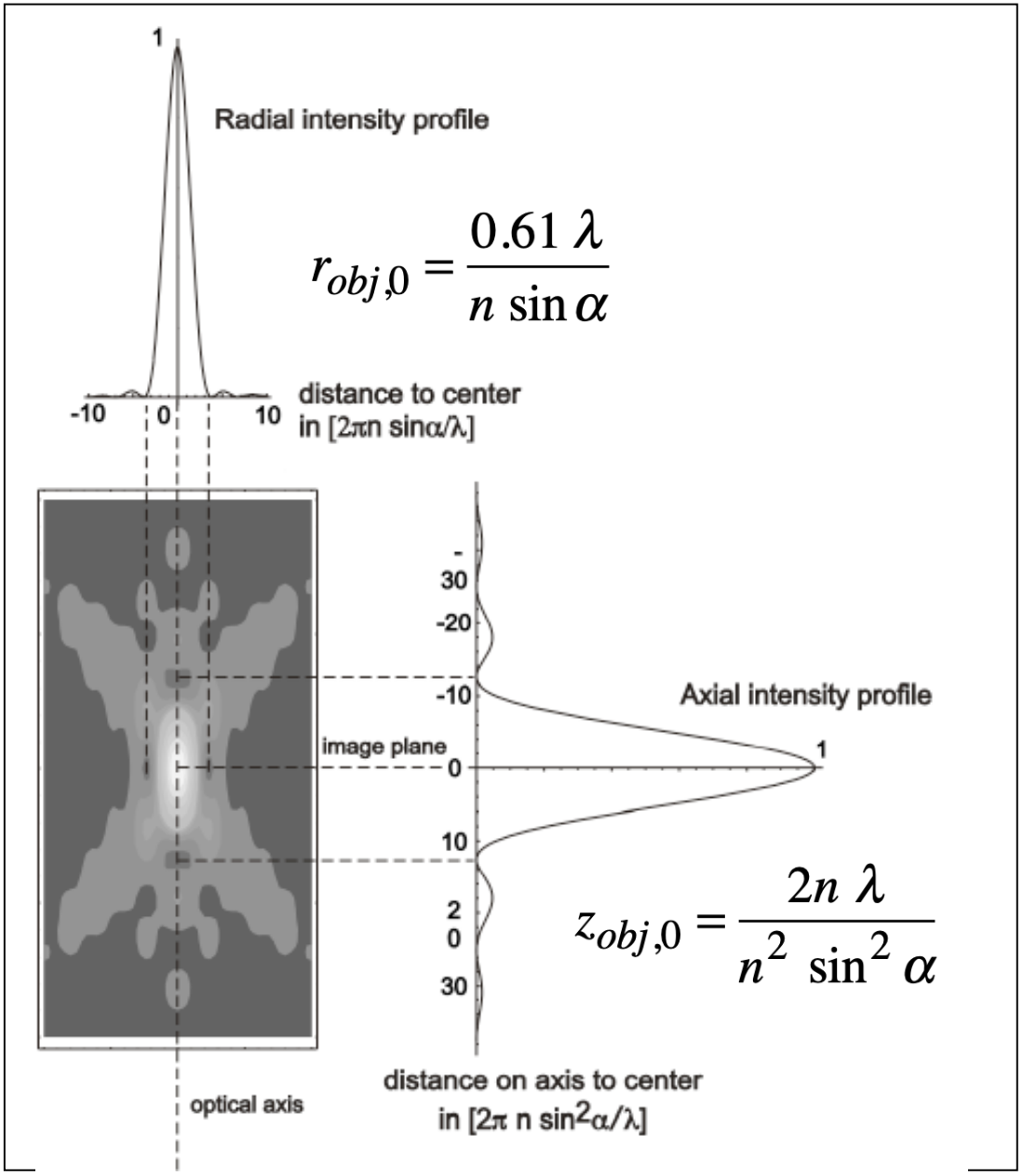
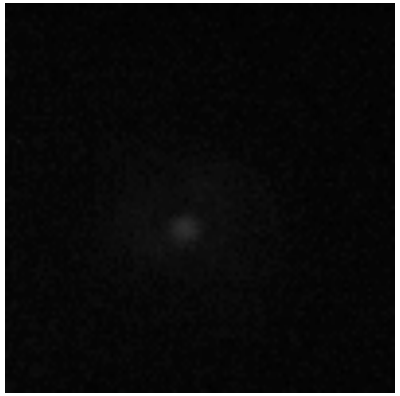
Radial and axial intensity profile of the light distribution in the focus of a lens



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

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

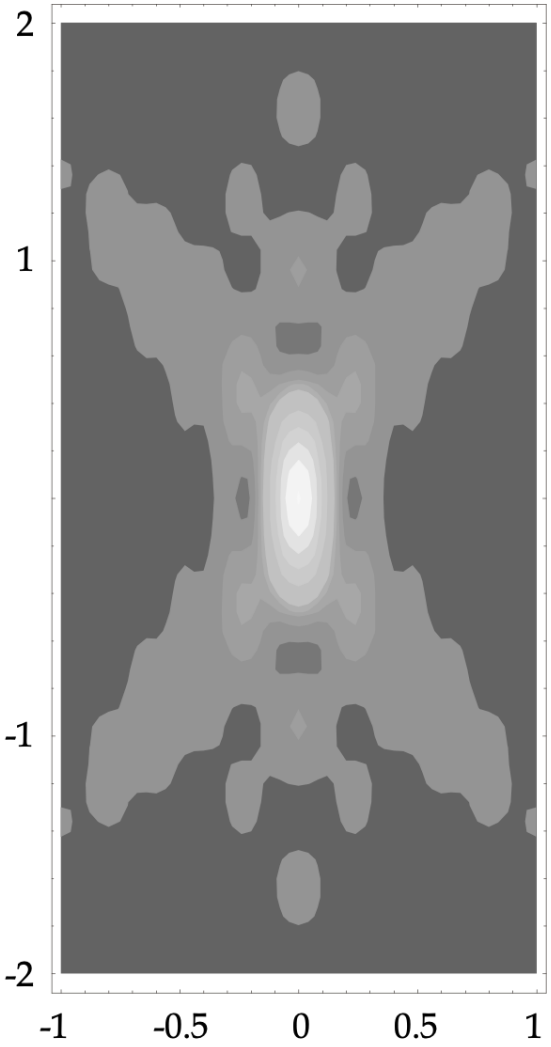
Bead



Bead
normalisiert

From “Fluorescence Microscopy: From Principles to Biological Applications“, edited by Ulrich Kubitscheck. Wiley-VCH, Weinheim, 2nd edition, Fig. 2.12

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



d_z=0.88μm

d_r=0.23μm

3D point spread function

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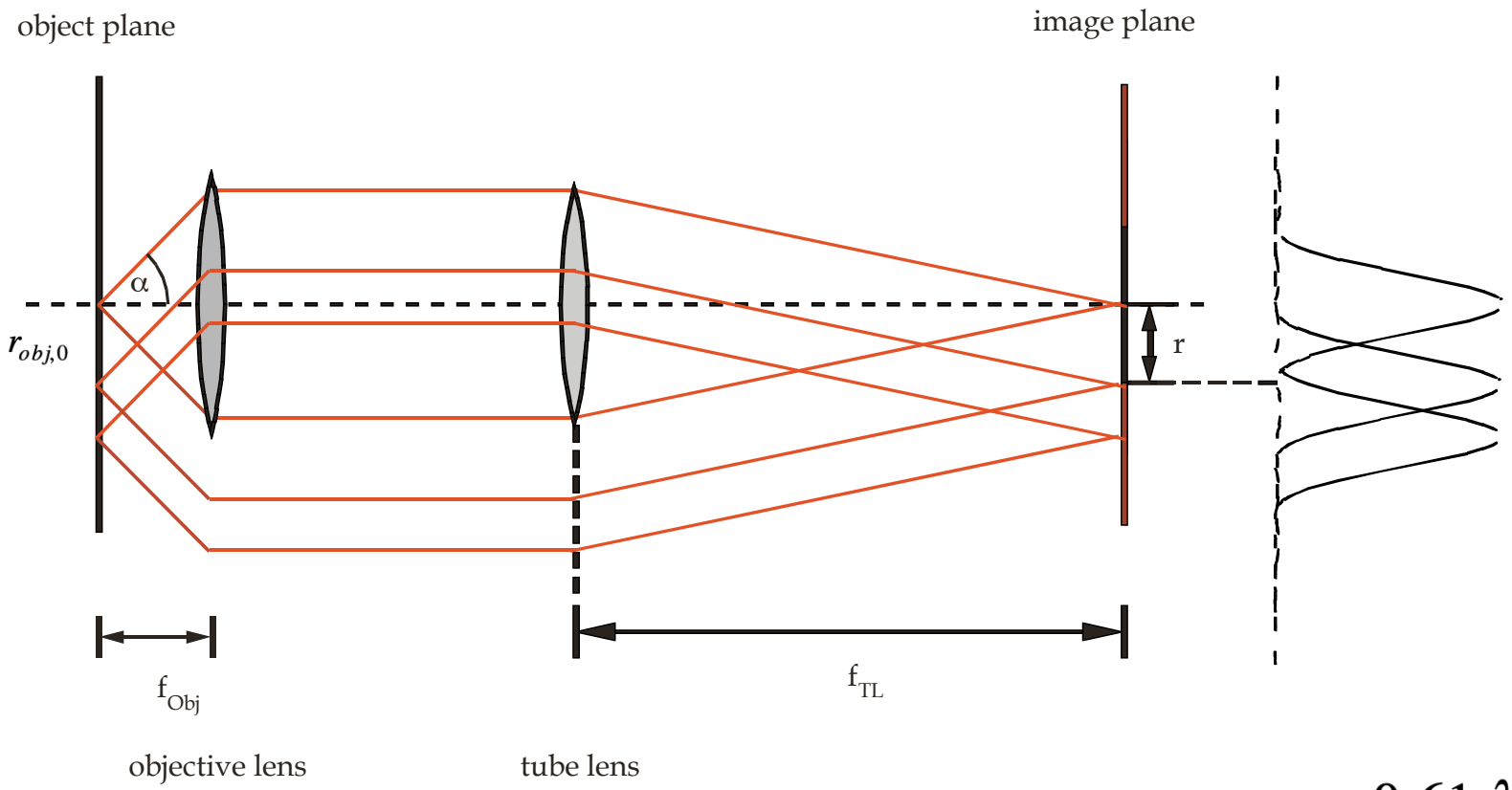
Resolution Limit According to Lord Raleigh



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

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

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

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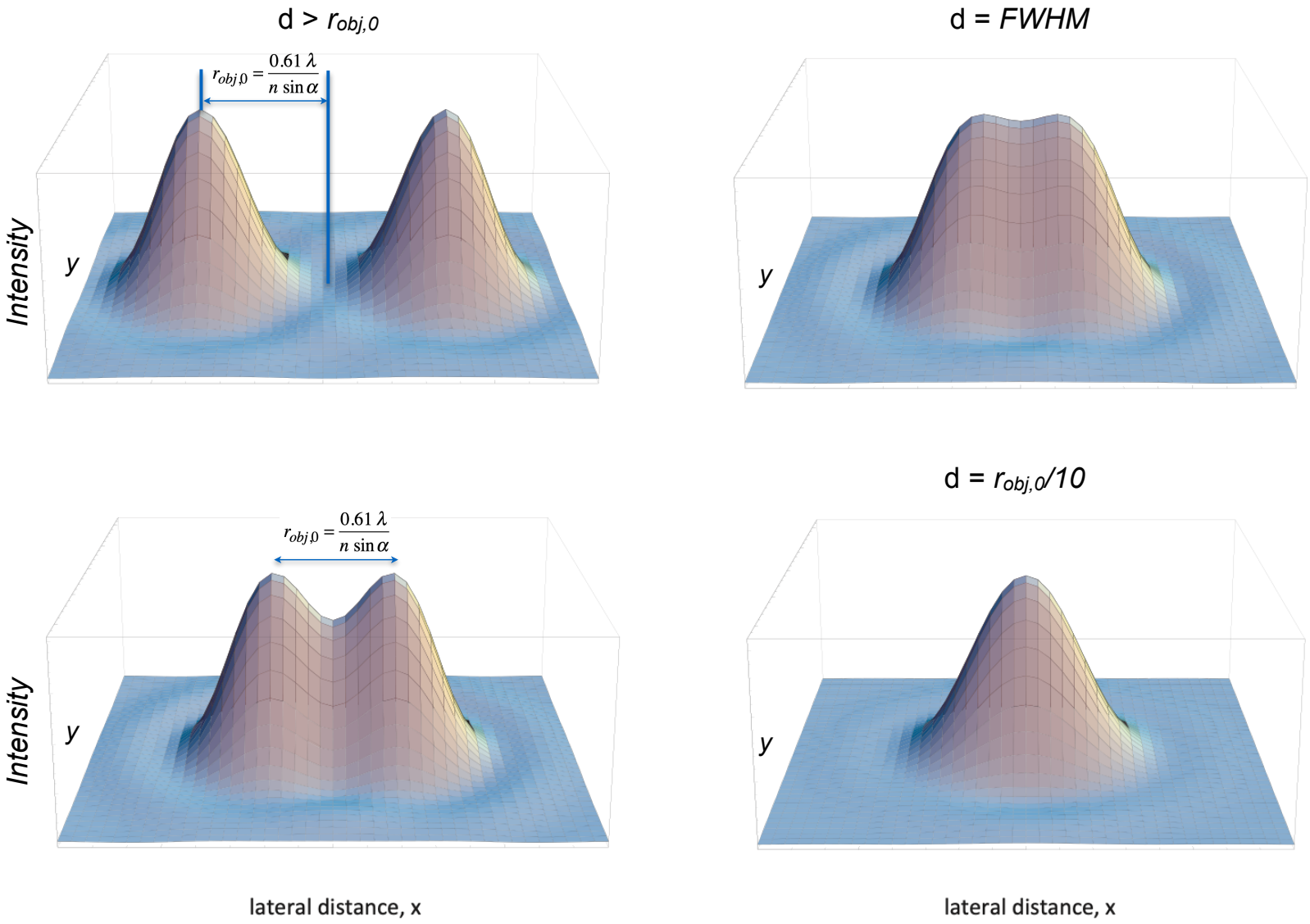
Sum of Point Spread Functions for Incoherent Point Objects



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

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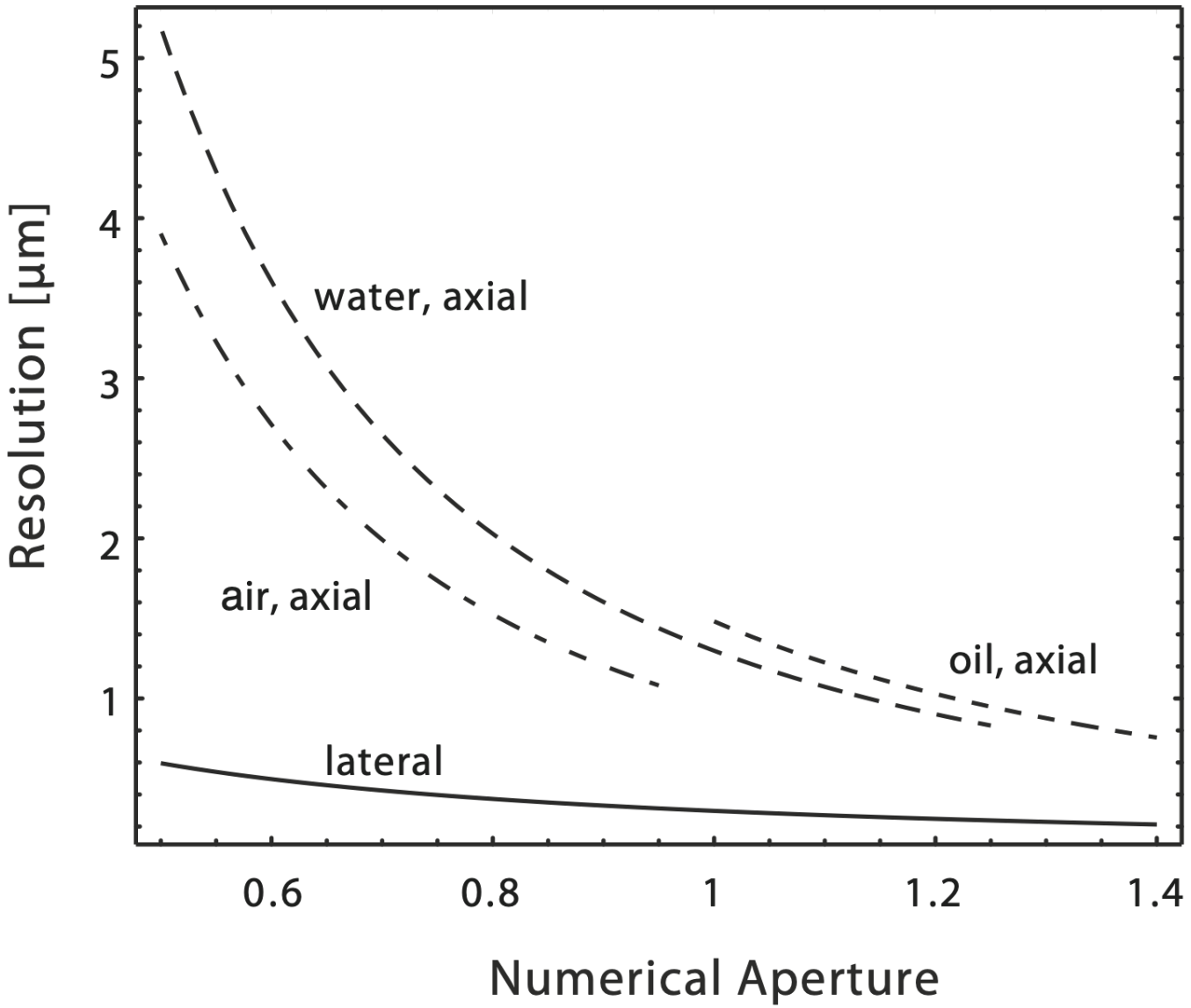
Radial and axial resolution as function of the NA



From “Fluorescence Microscopy: From Principles to Biological Applications“, edited by Ulrich Kubitscheck. Wiley-VCH, Weinheim, 2nd edition, Fig. 2.13

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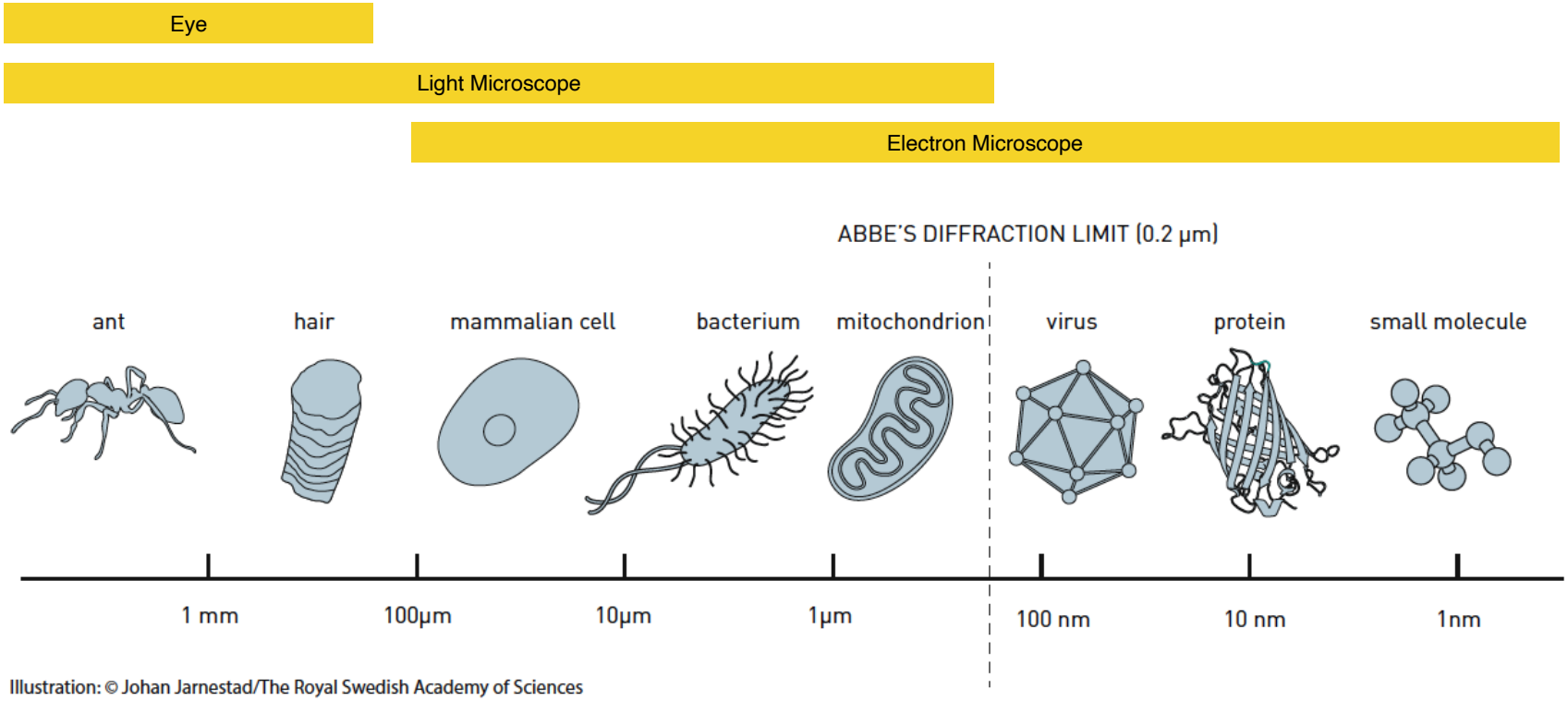
The resolution limit in the biological context



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