Part III - Microscopy

Chapter 6: Fundamentals

Resolution Limits, Modulation Transfer Function, Lens Aberrations, Comparison Optical and Electron Microscopy, 3D Imaging

Chapter 7: X-Ray and Electron Microscopy

Chapter 8: Contrast and Modulation Transfer Function

Chapter 9: Advanced Techniques

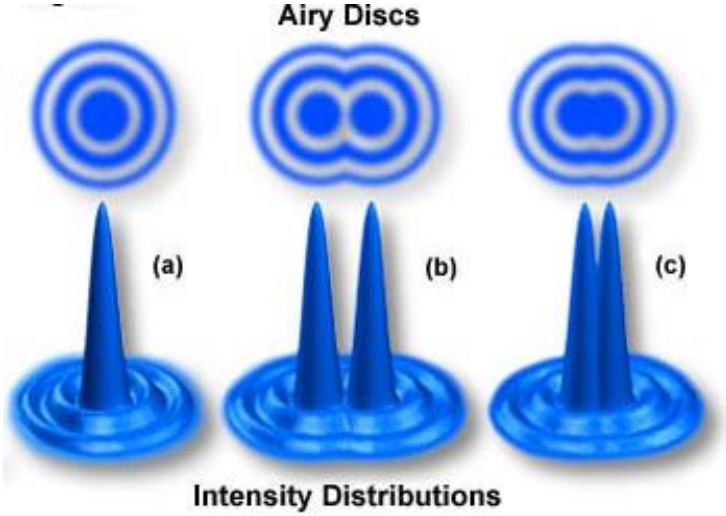
Confocal Scanning Microscopy, 3D Optical Imaging,

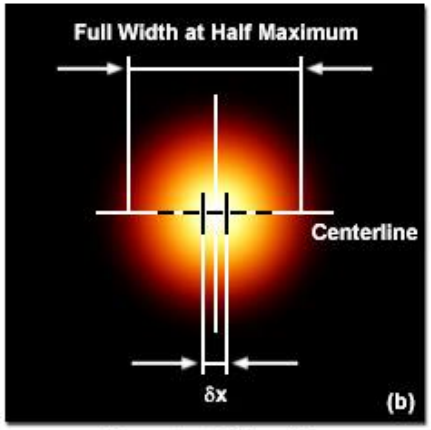
Super-resolution using PALM, STORM, STED, NSOM

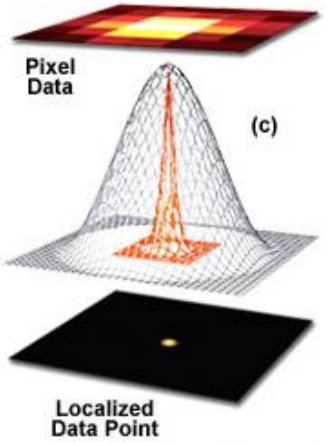
Chapter 6

Fundamentals of Optical Microscopy

Instrumentation, Resolution Limits, Aberrations,
Practical Resolution: Optical Microscopy versus TEM







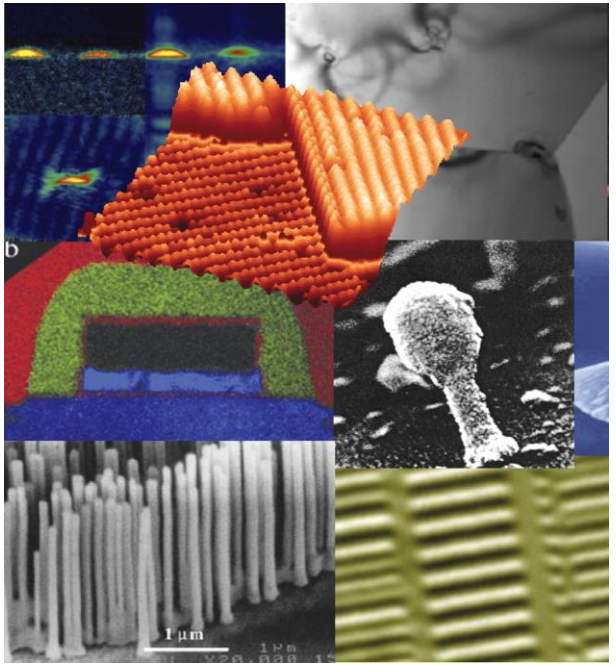
Contents - Chapter 6: Fundamentals of Optical Microscopy

6.1 Introduction

Microscopy = High resolution **2D** or **3D** real space ***spatial imaging*** , i.e., ***mapping*** of materials properties on a ***magnified scale*****.**

⇨ This yields the ***microscopic landscape*** of properties such as morphology, mass density, chemical composition, reflectivity, absorption, luminescence efficiency, or any other property, depending on which type of response signal and probe sample-interaction is recorded as imaging signal. Most common: Imaging of structure, morphology or composition of materials.

⇨ Microscopic imaging is possible in principle for all material characterization methods described in the previous chapter. However, the achievable spatial resolution can be quite different due to the different used probes.



In this chapter:

» The **principle approaches** and methods of microscopy are introduced and the **image formation processes** described,

» **Resolution limits** and conditions for best resolution are derived for optical imaging microscopy and transmission electron microscopy,

Resolution Limits for Different Probes

- **Optical microscopy (HM, PCM)**:

  Visible light: Resolution down to 200 nm (≈ λ/2).

- **Electron Microscopy:** (TEM, SEM)

  Electron beams: Small wavelengths λ << Å

  TEM: Resolution of atom rows, limited by optics,

  SEM: Resolution limited by interaction volume,

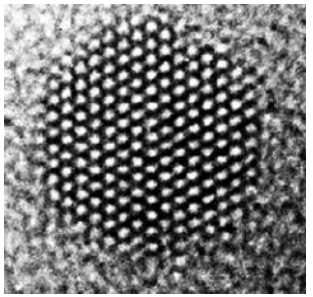
- **Field ion microscopy:** (FIM, FEM)

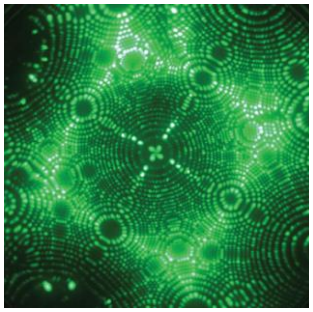
  Imaging using **electrons or ions**:
  Direct imaging of single atoms at surface steps,

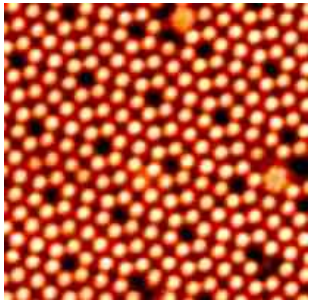
  Limited to tip-shaped samples with radius<100 nm,

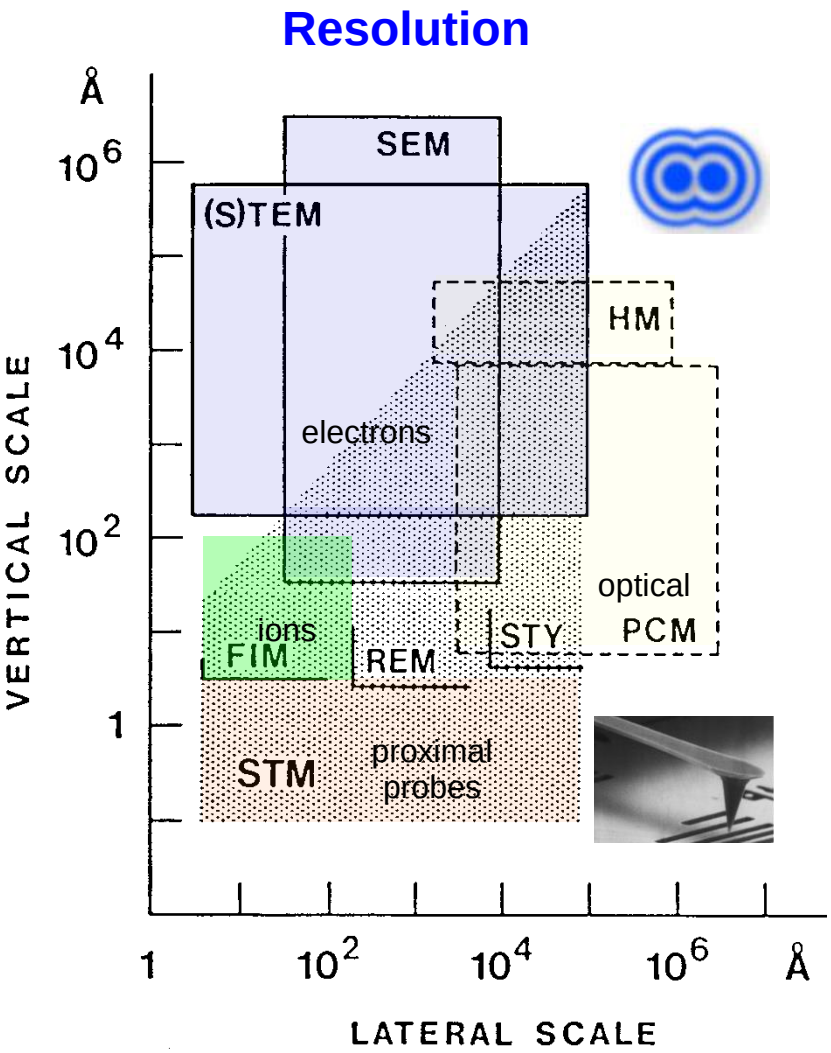
- **Scanning proximal probe microscopy**:

  Proximal probes with very small tip radius
  and localized (near-field) interaction
  Atomic resolution possible for STM & AFM.









Type of probe determines:

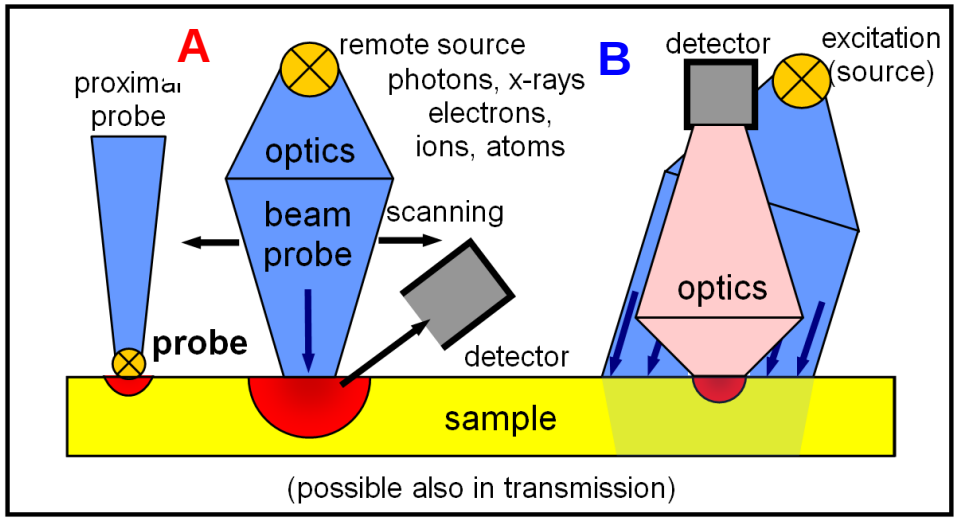
(a) Resolution, (b) probe-sample interaction (c) information type,
(d) required technical instrumentation.

6.2 How to Perform Spatial Imaging with High Resolution ?

A. *Scanning microscopy* = *Local excitation + non-local detection*

- ⇨ Excitation of a small sample volume by a tightly ***focused beam probe*** produced by ***demagnifying* optics**.
- ⇨ Global detection of the response signal from the sample by an integrating detector
- ⇨ **2D images** created by raster scanning the probe over the sample surface

Examples: Scanning electron microscopy(SEM), scanning optical (SOM), scanning probe (SPM), scanning x-ray microscopy (SXRM), …..



B. *Imaging microscopy* = *Non-local excitation + Local detection*.

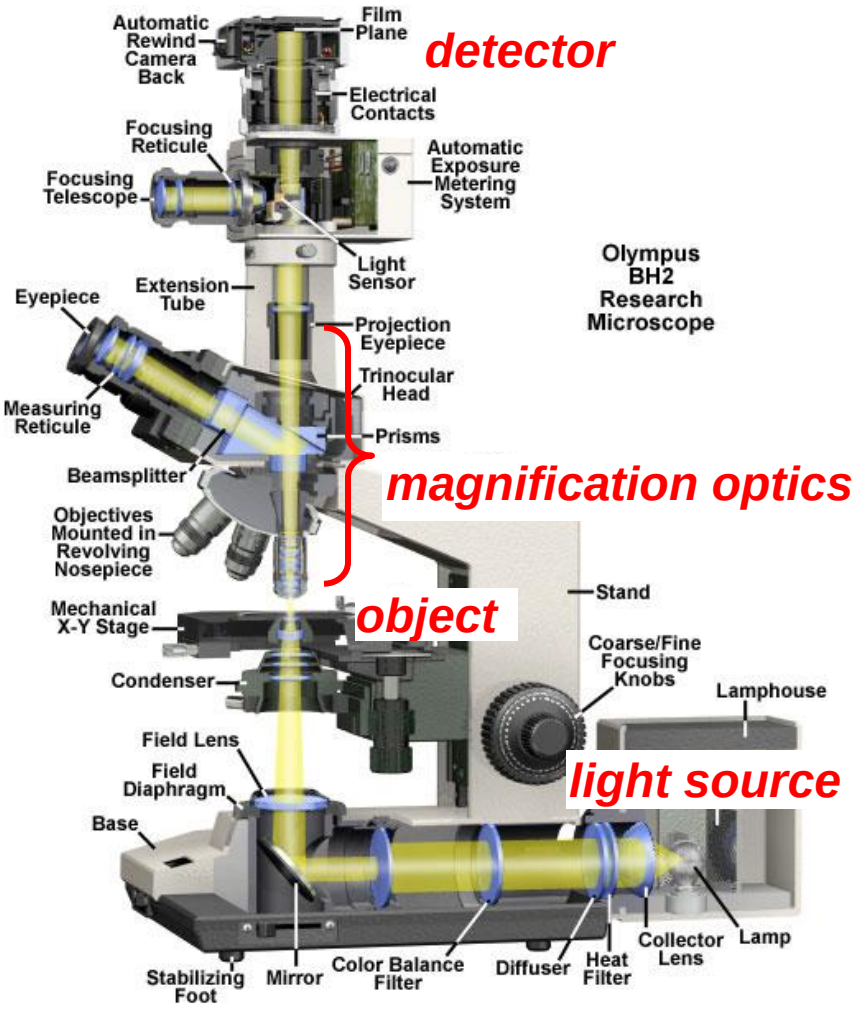
- ⇨ Excitation of a large sample volume but detection only the signal from a small volume that is projected by ***magnifying optics*** onto the detector.
- ⇨ **2D images** created usually by ***parallel*** *detection* of many individual spots using a two dimensional pixel detector such as a photoplate, human eye, CCD camera, channel plate, etc., for which one pixel corresponds to the signal from one small sample spot.

Examples: Transmission electron microscopy (TEM), optical imaging microscopy (OM) …

C. Local excitation *and* local detection: Combination of both.

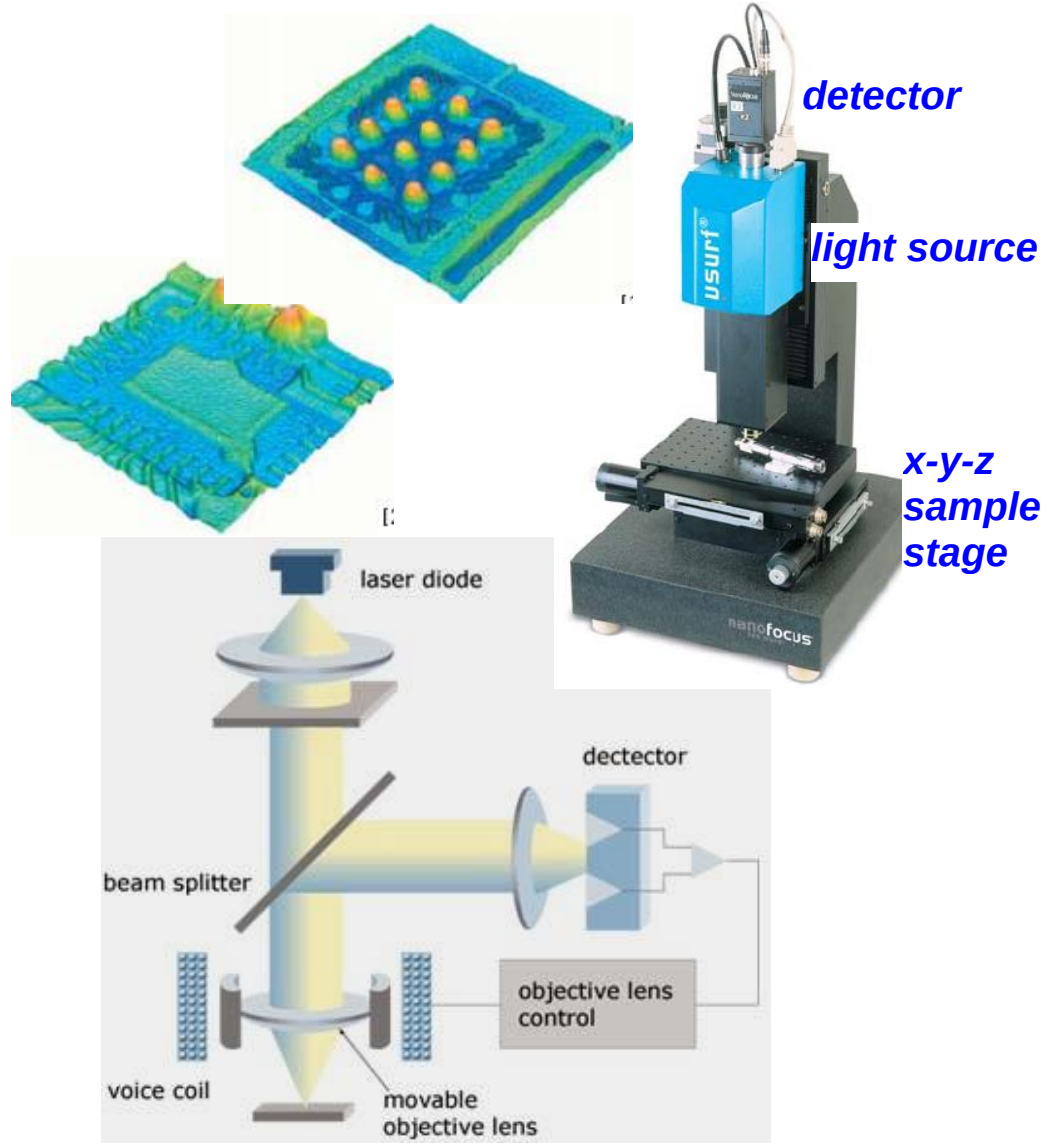
Example: Confocal scanning optical microscopy, ….

Imaging Microscopy



⇨ *Magnifying optics* required for image formation, magnification determined by focal length

Scanning Microscopy



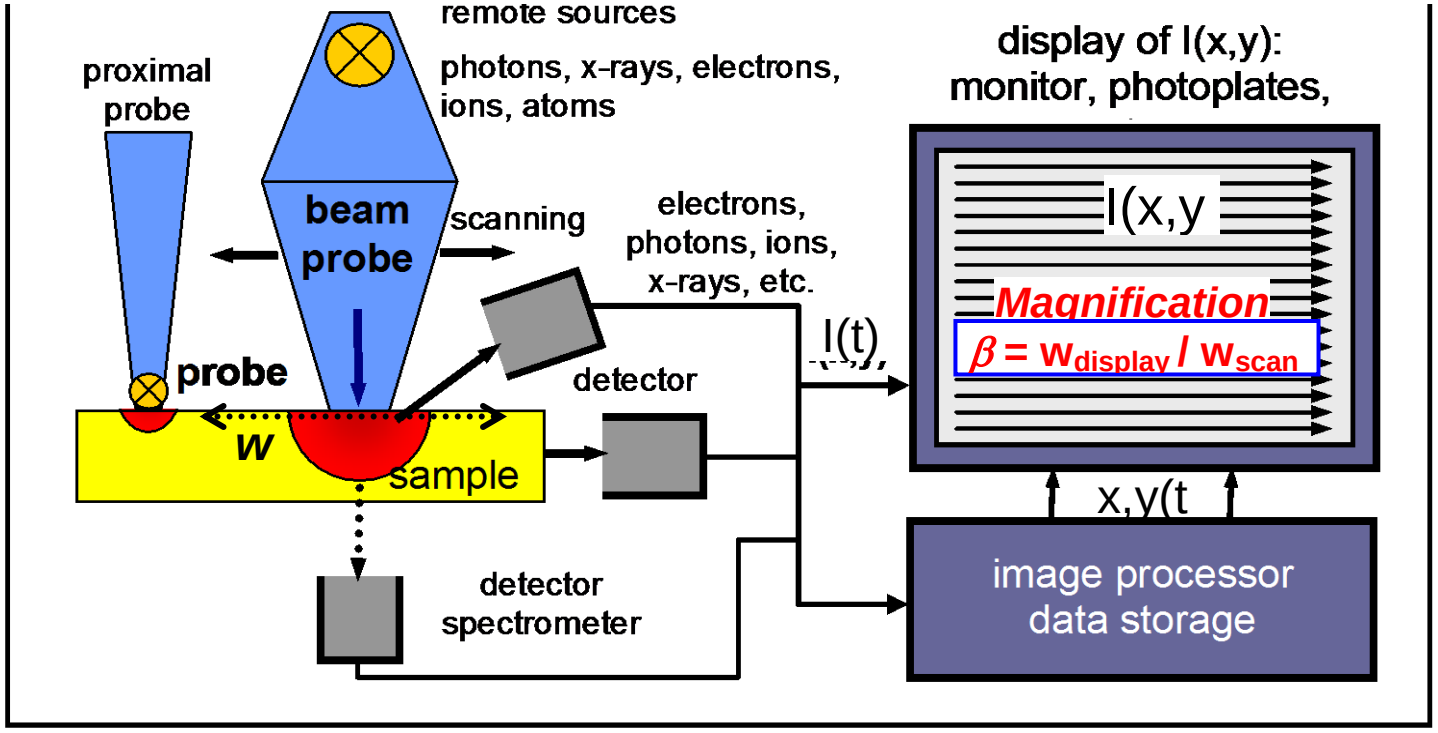
⇨ *Demagnifying* optics for probe formation, Magnification set by scan size

Note: The same type of variants exist for electron and x-ray microscopy

6.3 Scanning Microscopy

In scanning microscopy, an image is created by raster scanning a small probe that locally interacts/excites the sample and the detection of the global response signal I(t) as a function of time, where at a given time *t* the probe is at a different spot (x,y)= f(t) on the sample.

⇨ **Each image point** is recorded ***sequentially*** at a different time linked to a certain spot position !



Magnification = simply the ratio between display and scan size: $M = w_{display} / w_{scan}$

The smallest useful scan size is limited by the probe size, i.e., $w_{scan} > w_{probe}$.

Resolution is determined only by the probe size *as well as* interaction/excitation volume.

The probe size depends on the quality of the demagnifying optics and wavelength of the probe, and the interaction volume on the probe energy and interaction strength.

Characteristic Features of Scanning Microscopy

☺ *No magnifying optics* are required - only *simpler focusing optics* for probe formation, Thus, no demanding high resolution imaging optics are required.

☺ Thus, applicable to *wide range of techniques* because no imaging optics are required.

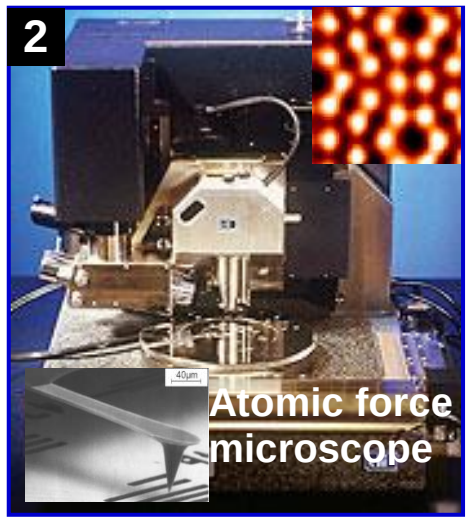
☺ Easy to combine with *spectroscopic* techniques. Thus, probing of many properties possible.

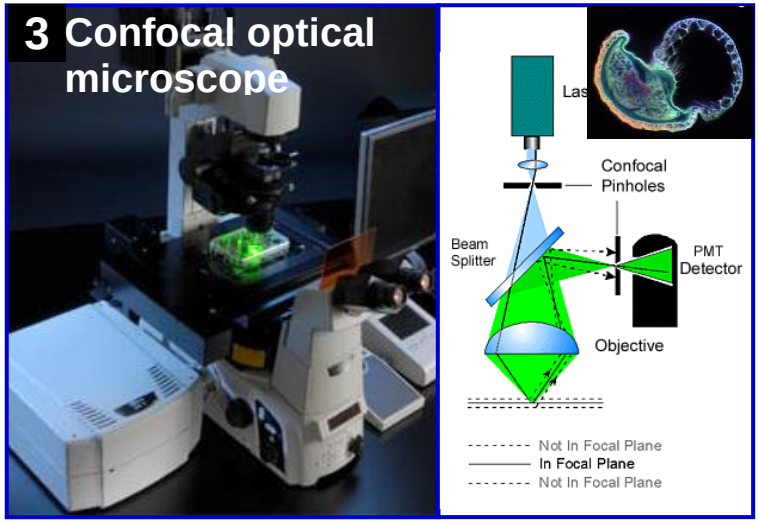
☺ *Ultimate resolution* = determined by the probe size and the interaction volume. For beam probes, the probe size is *limited by diffraction* and the *lens aberrations* of the focusing optics. The *excitation volume* depends on the probe pararmeters. In confocal fluorescenece microscopy (STED) it can be narrowed below the diffraction limit using *non-linear optical effects*.

☹ *Sequential* point-by-point recording is slow and limits the signal integration times per pixel.

😐 Overall, only a *virtual sample image* is obtained that represents measured signal versus (x,y). The image must be displayed on a monitor screen, photo plate, printer, etc. .

Variants: Scanning Electron Microscope (SEM), Scanning Tunneling (STM) and Scanning Force Microscope (AFM), Scanning Near Field (SNOM) and Scanning Confocal Optical Microscope, …

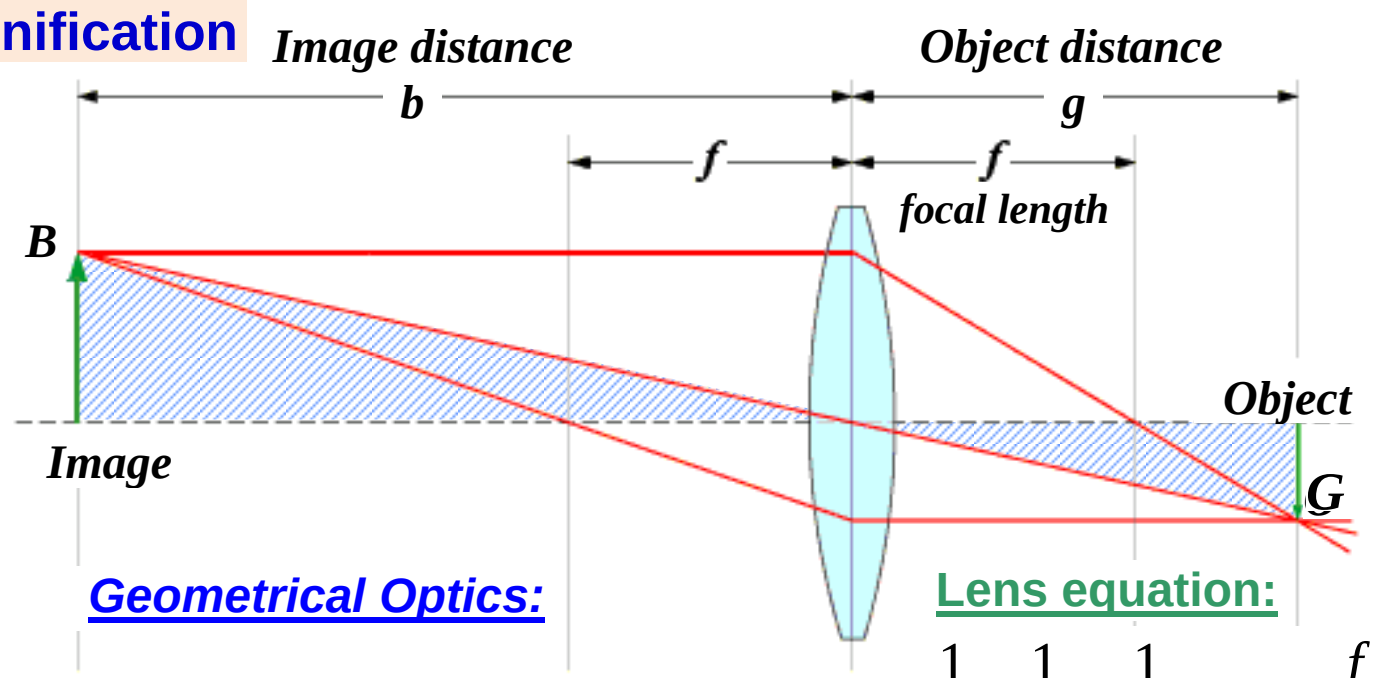




6.4 Imaging Microscopy

Local detection is achieved by creating an ***enlarged image*** of each sample point using ***magnifying optics*** of lenses or curved mirrors. The enlarged image is viewed or recorded using a 2D detector.

Magnification



Geometrical Optics:

Lens equation:

$$\frac{1}{b}+\frac{1}{g}=\frac{1}{f} \qquad b=\frac{f\cdot g}{f-g}$$

$$g \to f \Rightarrow M \to \infty$$

Magnification:

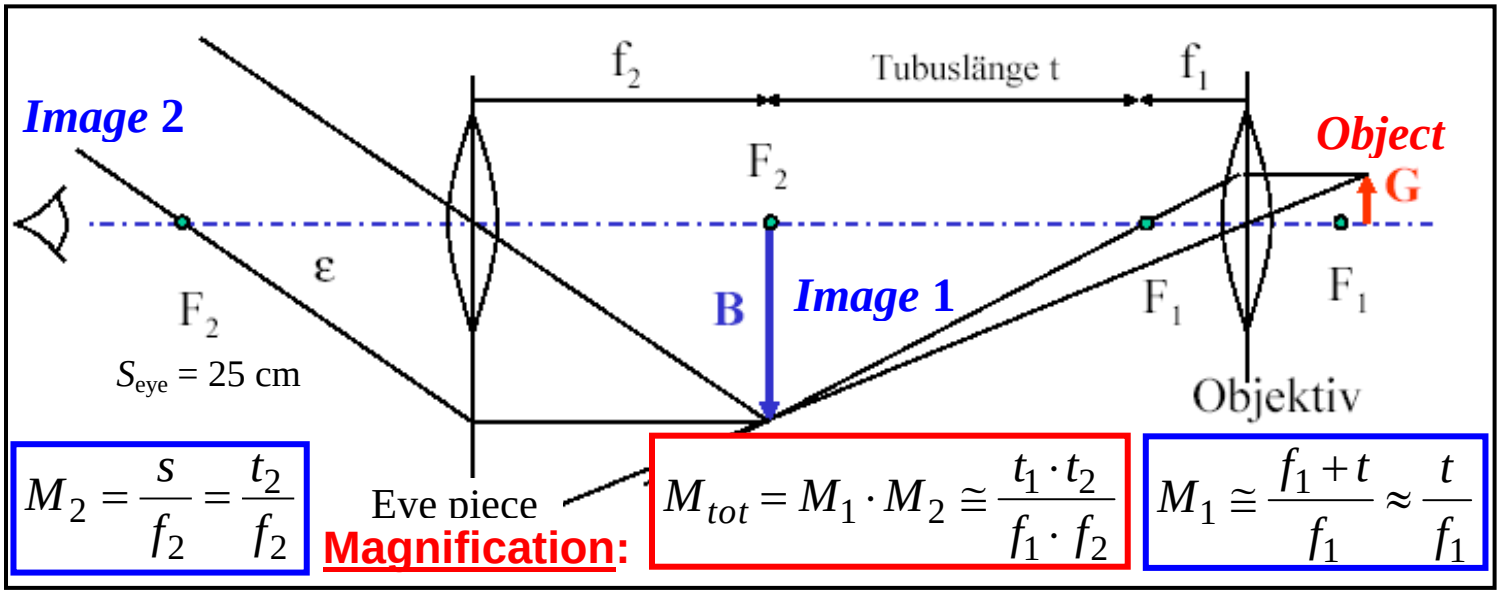
$$M=\frac{B}{G}=\frac{b}{g}=\frac{f}{g-f}$$

Magnification: $M = B / G$ is determined by the lens equation, i.e., object and image distances ***b/g***

⇨ At any time, **each point of the image** = signal collected from ***one point*** **of the sample!**

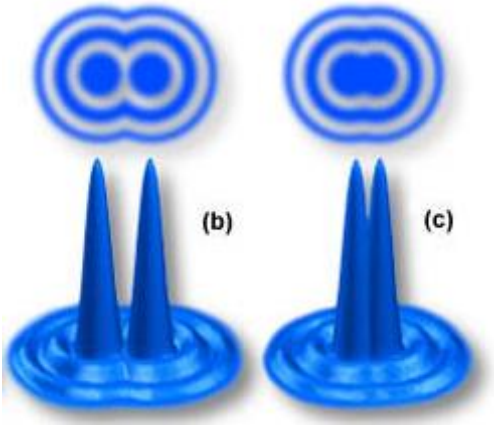
Magnification of a two lens optical microscope

Given by lens strength f_i and tube length t



⇨ The **tube length *t*** of optical microscopes is usually fixed (standardized to t =160 mm). Thus, different objective lenses with different f_i have to be used to change the magnification.

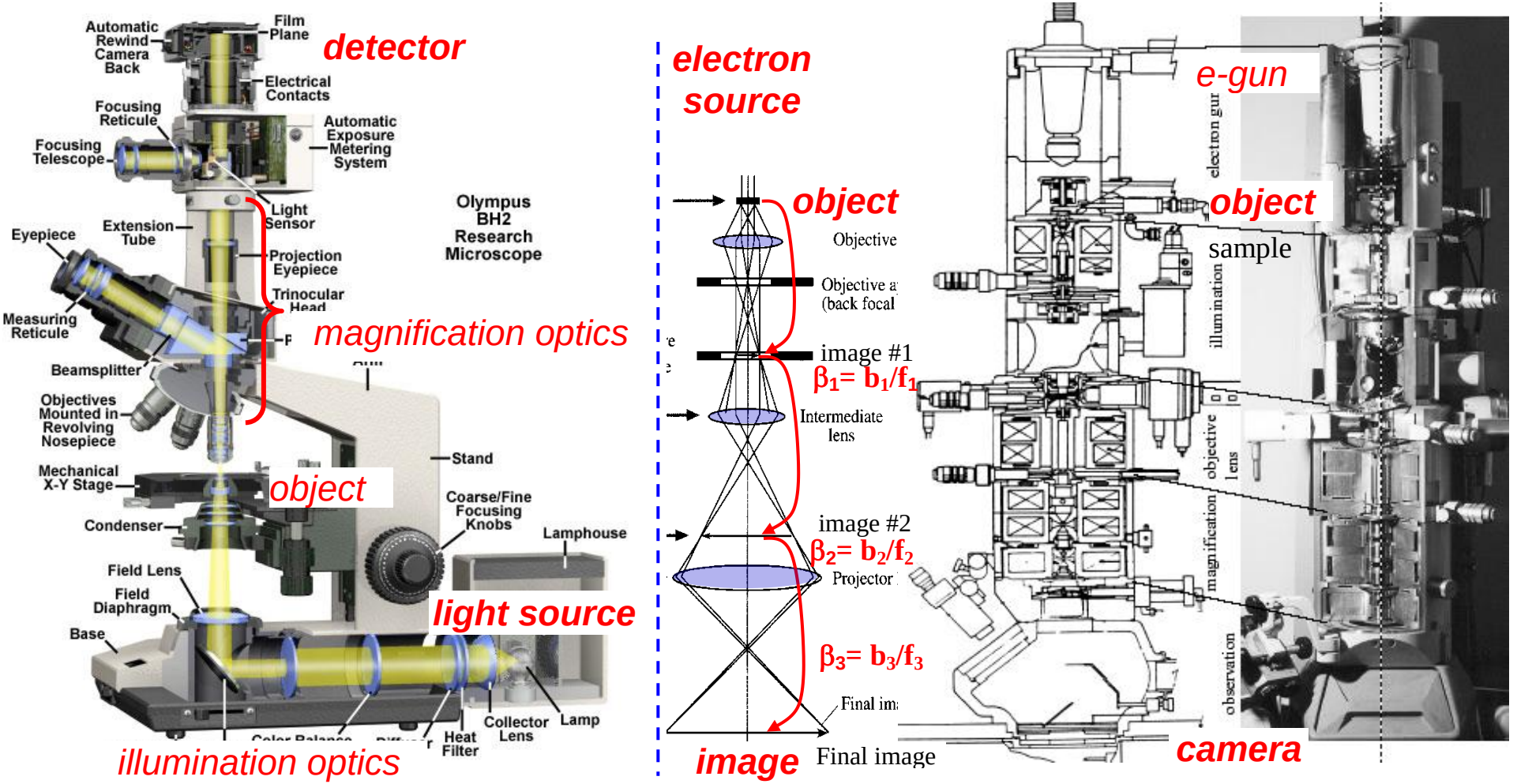
Basic features of imaging microscopy:



- ***Resolution***: Due to *diffraction* and lens imperfections, the image of each point of the object is smeared out on the detector. Thus, below a certain distance *r* , two object points can no longer be resolved. The resolution depends on the imaging wavelength λ and the quality of the optical imaging system but it is ***fundamentally limited*** to ***r > ½ λ.***
- ***Useful magnification***: Although by using strong lenses with small *f* and a large tube length *t* the *magnification* $M_{mag} \approx t / f$ can be made be arbitrarily large, due to the resolution limit the ***"useful" magnification is finite***

For the different probes very different kinds of instrumentation, i.e., beam sources, optical elements, detectors, environmental conditions, etc. is required but the basic opitical system is identical.

Example: Optical Microscope *versus* Transmission Electron Microscope



Different elements: (1) Beam sources: Light verus electrons, (2) Lenses: Glass versus magnetic/ electrostatic lenses, (3) Environment: Air versus vacuum column, (4) Photon versus electron detectors, (5) High voltage

Key features of imaging microscopy

☺ A *real* physical image of the sample is formed within the image plane.

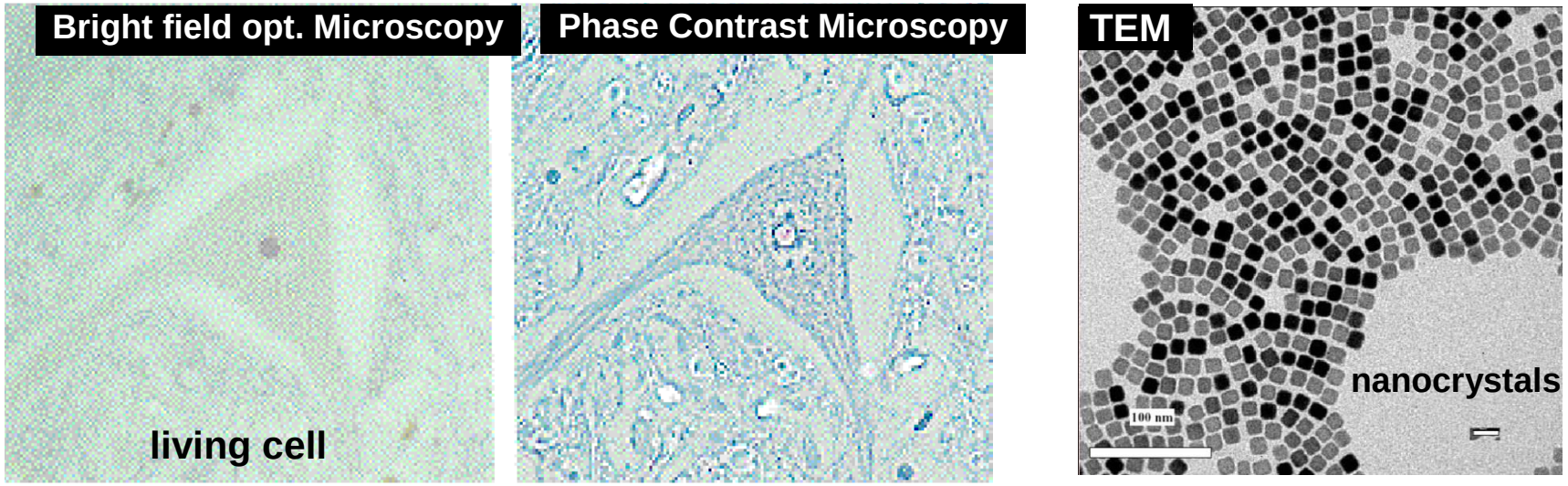
☺ Thus, all sample points are simultaneously imaged at once at the same time:
This enables *real time imaging* of the whole sample at high speeds to reveal fast dynamical changes occurring within samples as a function of time.

☺ Long integration times per point are possible that yield high signal-to-noise ratios.

☹ The *resolution* is ***fundamentally limited*** by diffraction (~wavelength) .

Variants of imaging microscopy: Light microscopy (OM) using visible/IR/UV light, transmission electron microscopy (TEM), low energy reflection electron microscopy (LEEM), photo emission electron microscopy (PEEM), field ion microscopy (FIM), etc.

Examples: Left: Optical microscopy with different contrast. Right: TEM image



Different variants of imaging microscopy are distinguished according to (i) the kind of waves used for illumination and (ii) how the contrast is formed in the image.

6.5 Resolution Limits of Imaging Microscopy

Ultimately, the lateral resolution of any microscopy system is limited by two factors:

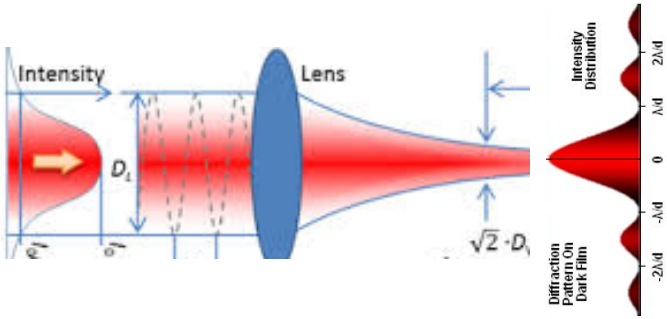
1. **Diffraction effects** due to the *finite entrance aperture* of the optical system

   = *fundamental* & ultimate resolution limit (= Abbe resolution limit,1873)

2. Image distortions/smearing caused by *lens imperfections* (aberrations)

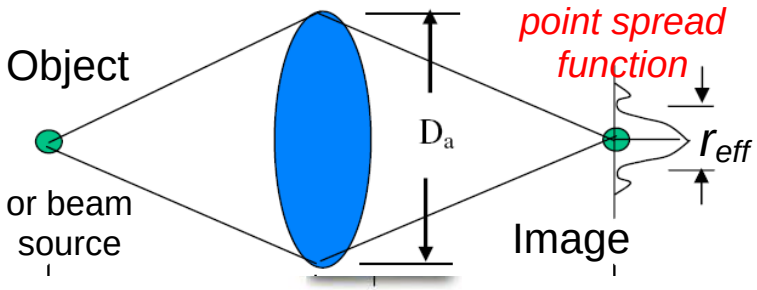
= "*practical*" resolution limit (always > diffraction limit)

⇨ Both effects limit the resolution ***independently*** of the magnification of the optical system.



For ***beam scanning microscopy***, both effects lead to a ***broadening of the probe spot size*** on the sample, with a certain minimal diameter that cannot be further reduced.

For ***imaging microscopy*** this leads to a ***broadening of the image*** of each object point, i.e., the smallest resolvable distance between to points is limited.



In sum, the total ***effective resolution*** r_{eff} is given by the square root summation:

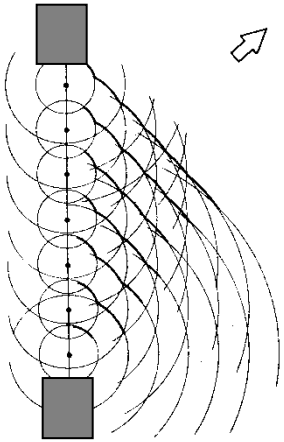
$$r_{eff} = \sqrt{r_{diff}^2 + r_{aber}^2}$$

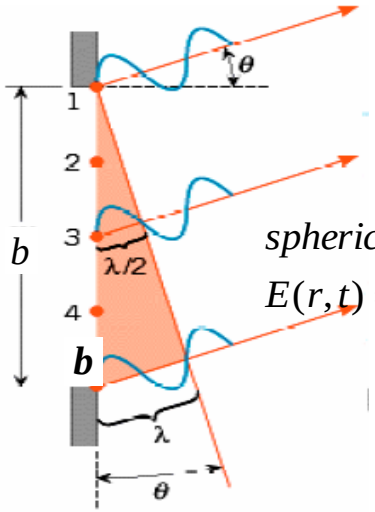
6.6 Resolution Limit due to Diffraction (see, e.g., E. Hecht: "Optics")

In any optical system, ***diffraction*** occur at all ***apertures*** confining the optical beam.

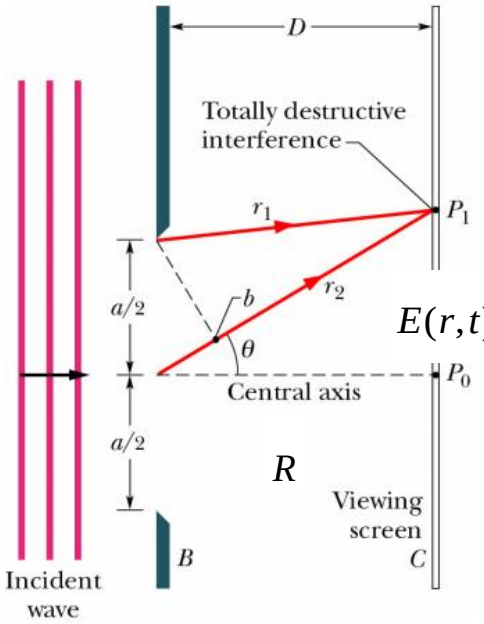
The corresponding **diffraction patterns** can be derived using the **Huygens principle**, where the electric field and thus, the intensity at a point *P* away from the aperture is calculated by ***summation over spherical waves*** emitted with wave vector k=2π/λ from every point within the aperture of area *A*.

6.6.1 Diffraction by a Single Slit with a Width *b*





spherical waves: $E(r,t) = \varepsilon / r \cdot \sin(\omega t - kr)$



$$E(r,t) = \frac{\varepsilon}{r}\sin(\omega t - kr)$$

Emitted spherical wave: $E(r,t) = \varepsilon / r \cdot \sin(\omega t - kr)$

Total field at point *P*:

$$E(R,\theta,t) = \int_A E(r,t)\,dA = \int_A (\varepsilon / r)\cdot \sin(\omega t - kr)\,dA \;\cong\; \varepsilon / R \int_{-b/2}^{+b/2} \sin(\omega t - k(R - y\sin\theta))\,dy$$

Fraunhofer far-field diffraction
parallaxial approximation (r>>w))

$$r = R - y\sin\theta + (y^2/2b)\sin^2\theta + .. \cong R - y\sin\theta \quad \text{for} \quad y << R$$

E-Field distribution:

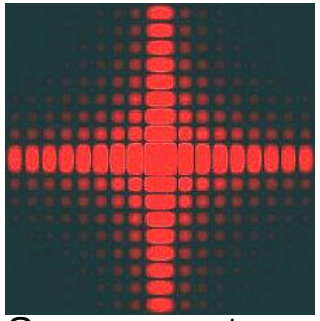
$$\Rightarrow E(R,\theta,t) = 2\frac{\varepsilon}{\mathrm{R}}\sin(\omega t)\frac{\sin(1/2\cdot kb\sin\theta)}{kb\sin\theta}$$

Resulting far-field intensity distribution $I(\theta)$ for a single slit

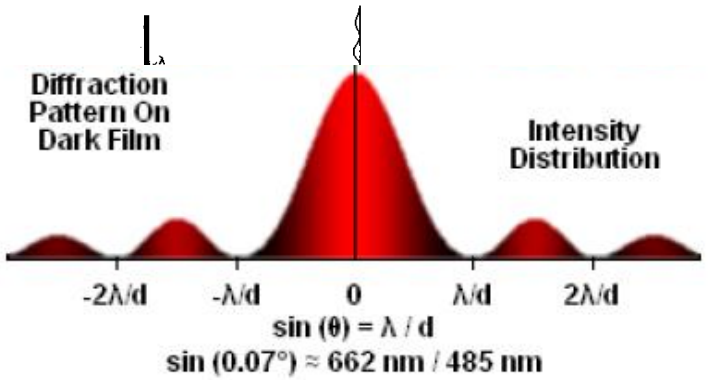
$$I(\theta) = c \cdot \varepsilon_0 \left\langle E^2(\theta,t) \right\rangle_t = c \cdot \varepsilon_0 \left\langle \sin^2(\omega t) \right\rangle_t \cdot 2E_0^2 \sin^2(\tfrac{1}{2} \cdot kb\sin\theta) \big/ (kb\sin\theta)^2$$

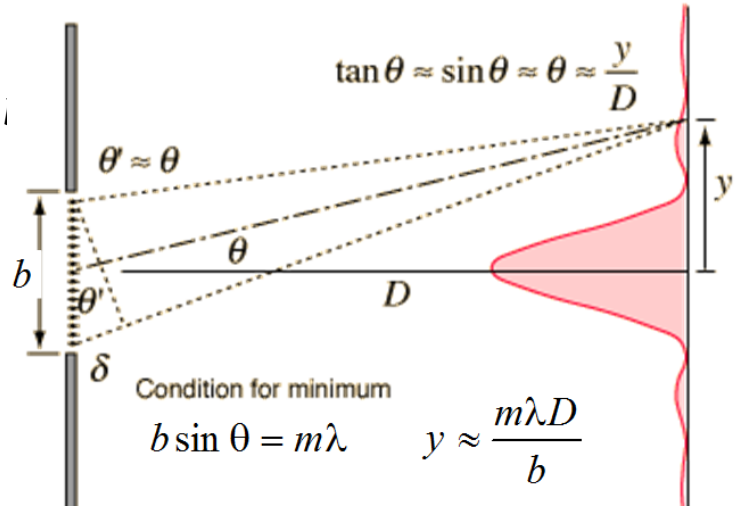
$$I(\theta) = \langle E(t)^2 \rangle = I_0 \cdot \sin^2 \beta / \beta^2$$

with $\beta = \dfrac{\pi \cdot b \cdot \sin\theta}{\lambda}$, θ = diffraction angle



Square aperture





Basic Properties:

1. **Central intensity maximum I_0** with series of side minma and maxima. $I_0 = c \cdot \varepsilon_0 \cdot E_0^2 = c \cdot \varepsilon_0 \cdot (\varepsilon / R)^2$

2. **Intensity minima** occur at: $\sin\beta = 0 \rightarrow \beta = m \cdot \pi \rightarrow \sin\theta = \lambda \cdot m / b$, $m = 1,2,3....$

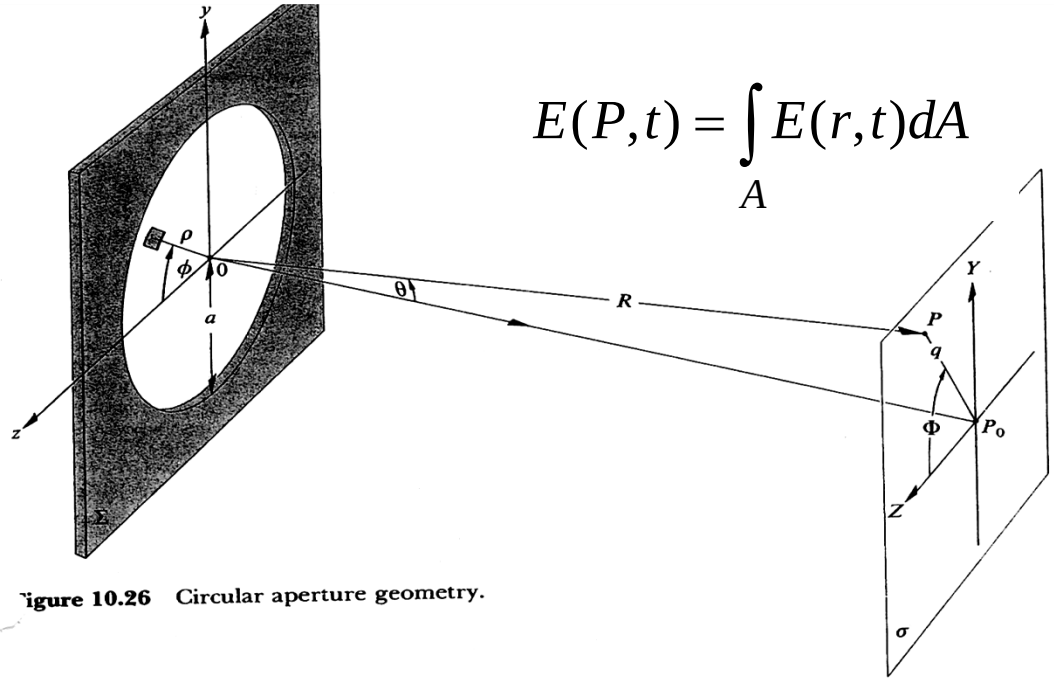
3. **Phase difference** of out-most beams for these minima: $\Delta\phi = m \cdot \lambda$ $\quad note: \lambda = \lambda_o / n$ (wavelength in medium)

4. **Broadening of the image** = Separation between the central maximum and the first side minimum: $\sin\theta_1 = \lambda / b$

5. **Stronger diffraction,** *i.e.*, larger angles and broader image **the smaller the slit width *b* .**

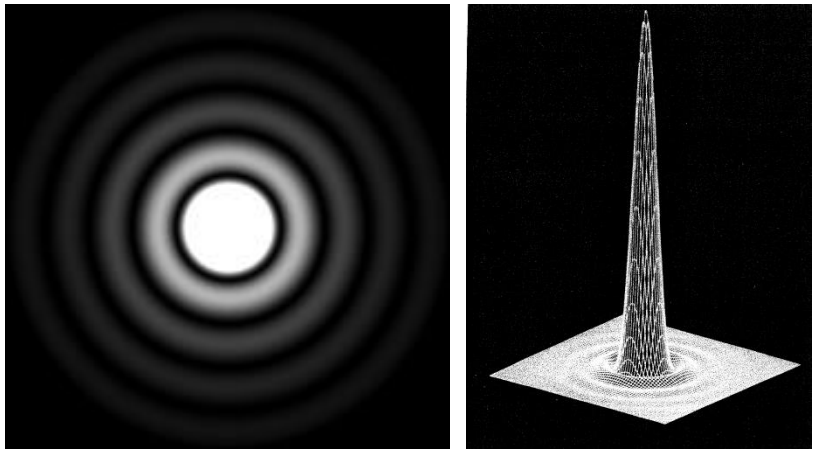
6.6.2 Diffraction by a Circular Aperture (Airy disk)

Diffraction pattern of a circular aperture (=Airy disk) with diameter D and radius R_0:
Derived in a similar way by *summation over all spherical waves* emitted from the disk area



$$E(P,t) = \int_A E(r,t)\,dA$$

Figure 10.26 Circular aperture geometry.

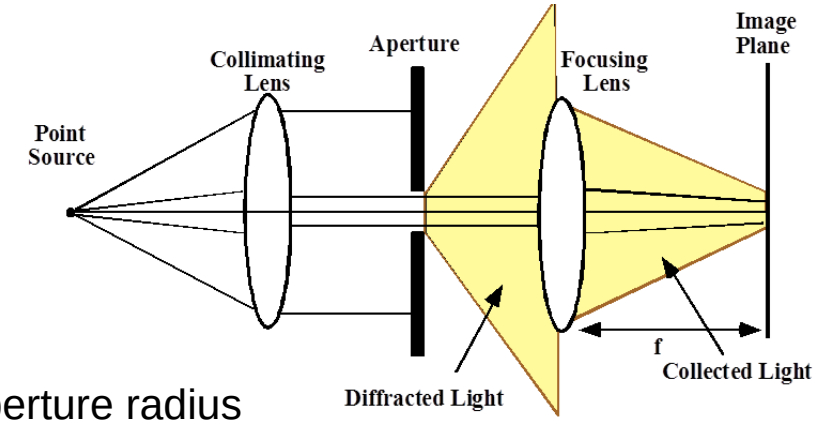


= Central intensity maximum with concentric satellite rings
"Airy intensity distribution"

The Airy intensity distribution

is described by first order Bessel function J_1

$$I(\theta) = I_0 \cdot \left[\frac{2 \cdot J_1(kR\sin\theta)}{kR\sin\theta}\right]^2$$



θ = diffraction angle, $k = 2\pi n / \lambda$ = wave vector, R = aperture radius

Properties of the Airy intensity distribution:

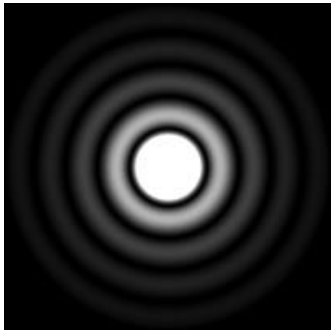
Intensity-Minima are given by zeros of the Bessel function $J_1(x) = 0$ at x = 3.83, 7.01, 10.17, …

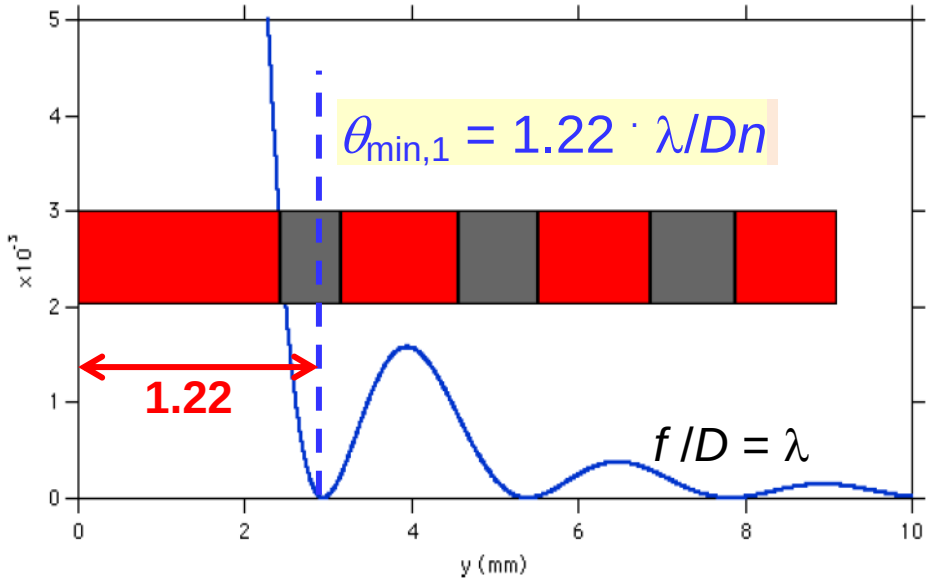
⇨ x = 3.83, 7.01, … = $\sin\theta \cdot 2\cdot\pi\cdot R/\lambda$ → $\theta_{minima}(rad) = m_i \cdot \lambda/D$ with m_i = 1.22, 2.23, 3.24 , ...

Radius of the 1st diffraction ring (Airy disk)
focused by a lens with a focal distance f onto a screen

$r_{Airy} = 1.22 \cdot \lambda \cdot f/D$

~ smallest achievable focal spot of a laser beam with diameter D and wavelength λ





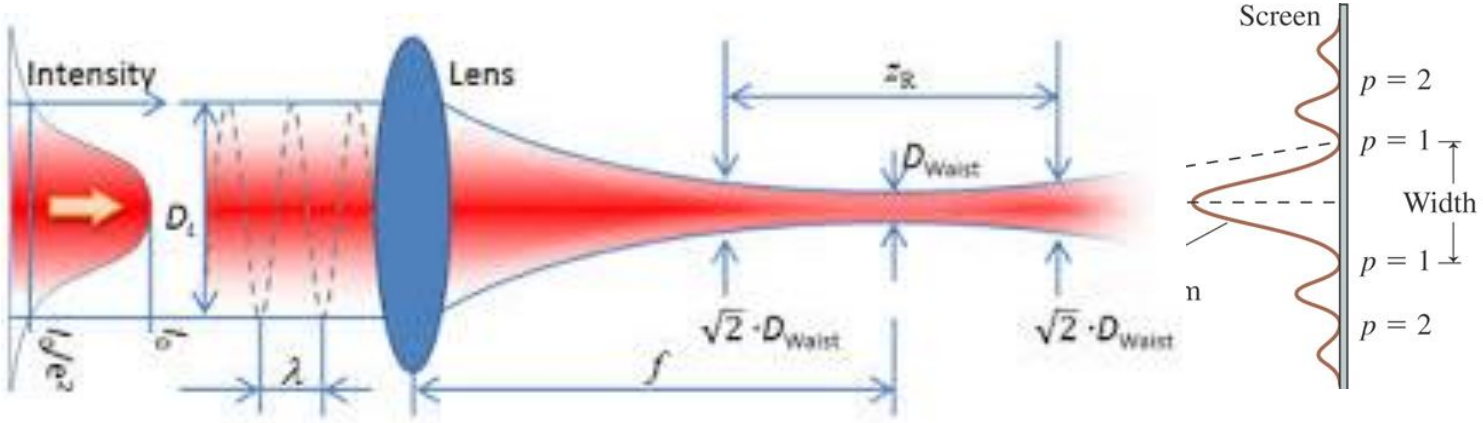
For each minimum, the path difference between outer beams is: $\Delta\phi_i = m_i \cdot \lambda$ Eq. (1)

Ring	Circular aperture m	I_{max}	I_{total}	Single slit m	I_{max}
Central maximum	0	1	1	0	1
First dark	1.220			1.000	
Second bright	1.635	0.01750	0.084	1.430	0.0472
Second dark	2.233			2.000	
Third bright	2.679	0.00416	0.033	2.459	0.0165
Third dark	3.238			3.000	
Fourth bright	3.699	0.00160	0.018	3.471	0.0083
Fourth dark	4.241			4.000	
Fifth bright	4.710	0.00078	0.011	4.477	0.0050
Fifth dark	5.243			5.000	

Note: In a medium with refractive index n, the wavelength $\lambda = \lambda_0 / n$ is shorter, accordingly: $\theta_{min} = \theta_{min,vac}/n$
i.e., *the intensity minima are more closely spaced , i.e., the diffraction broadening reduced !*

6.6.3 Application: Minimal Spot Size of a Laser Beam

The minial spot size of a quasi parallel laser beam focused onto a sample is limited by the diffraction at the lens aperture, i.e., by the finite lens diameter or finite beam diameter (=beam waist).



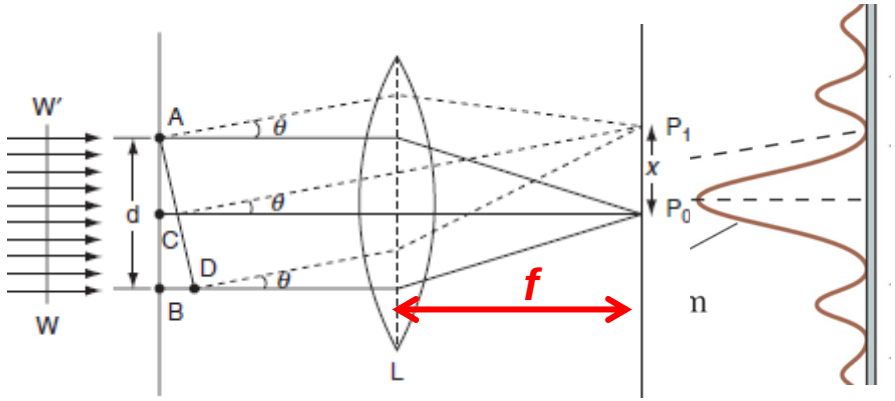
The diffraction angle of the 1st Airy disk cause by the diffraction is given by $\theta_{min,1} = 1.22 \cdot \lambda/Dn$, which is appears at a a point P_1 at a distance x away from the optical axis with $x = f \tan\theta$.

When the lens diameter D is large, θ_{min} is small and thus, $x = f \tan\theta \approx f \bullet \theta$.

As a result, the diameter of the Airy disk or beam spot size is $d_{spot} = f\ 1.22 \cdot \lambda/D$,

i.e., $\mathbf{d_{spot} = 1.22 \cdot \lambda\ f/D}$

where the parameter f/D is called "*f*" number of the lens



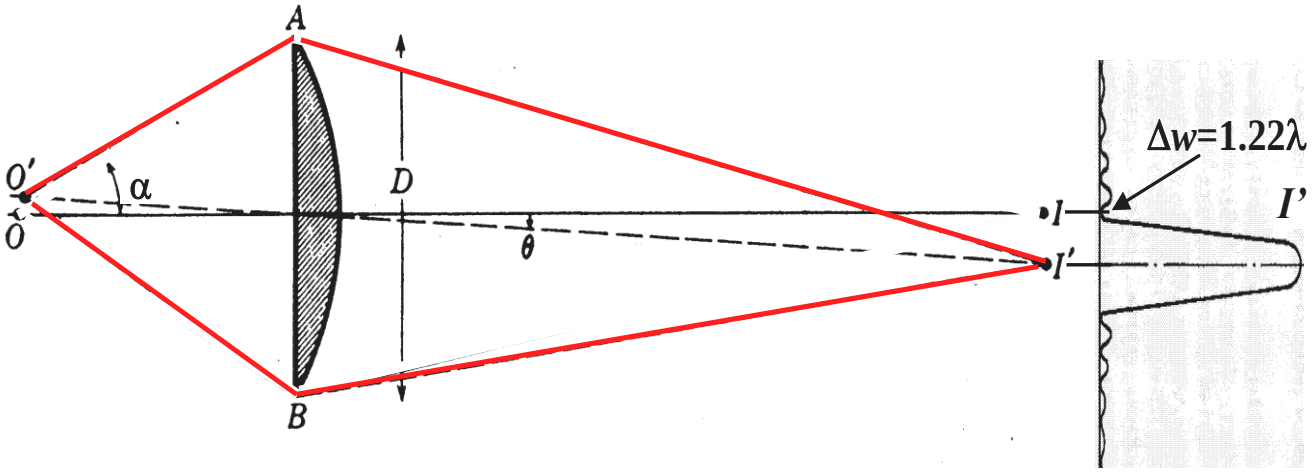
For a Gaussian beam, the laser does not fill the aperture uniformly and thus, the focal spot is slightl increased to $\mathbf{d_{spot} = 4/\pi \cdot \lambda\ f/D}$

Example: λ = 680nm, D = 5 mm, f = 5mm » d_{spot} = 1.9 µm

6.6.4 Broadening in a Magnified Image: Point Spread Function

For a microscope, the resolution is generally described by the so-called ***point spread function*** that is defined as the ***image of an ideal point source*** produced by an optical system.

Its shape characterizes the performance of a microscope system and ***includes all broadening factors*** caused by *diffraction* at the entrance aperture *as well as* by *lens aberrations*.



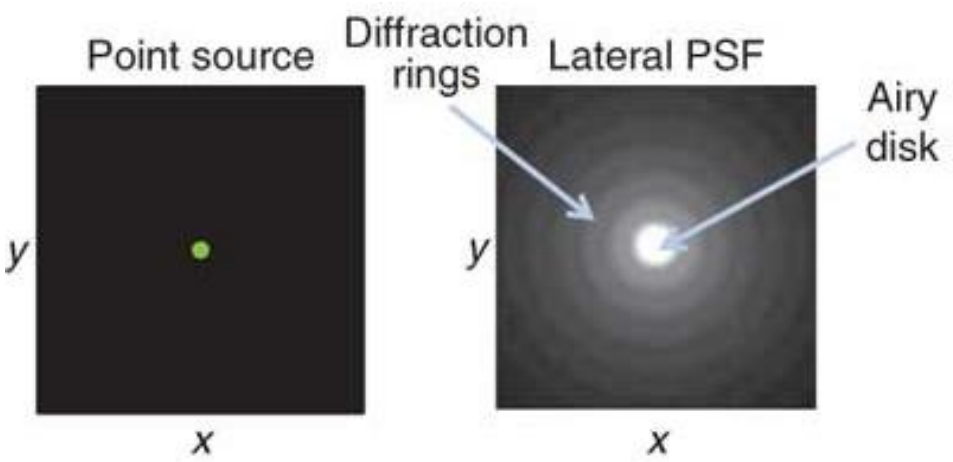
For a ***perfect* diffraction-limited optical system** without any lens aberrations, the lateral point spread function, i.e., radial intensity distribution *I*(x) of a point source is the ***Airy diffraction pattern***, which within the parallaxial approximation (small angles) is:

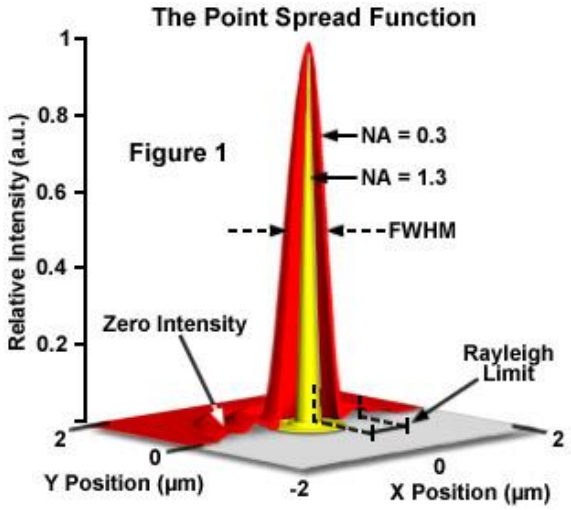
$$I_{Airy}(x) = I_0 \cdot \left[\frac{2 \cdot J_1(2\pi \cdot n \sin\alpha \cdot x / M\lambda)}{2\pi \cdot n \sin\alpha \cdot x / M\lambda} \right]^2$$

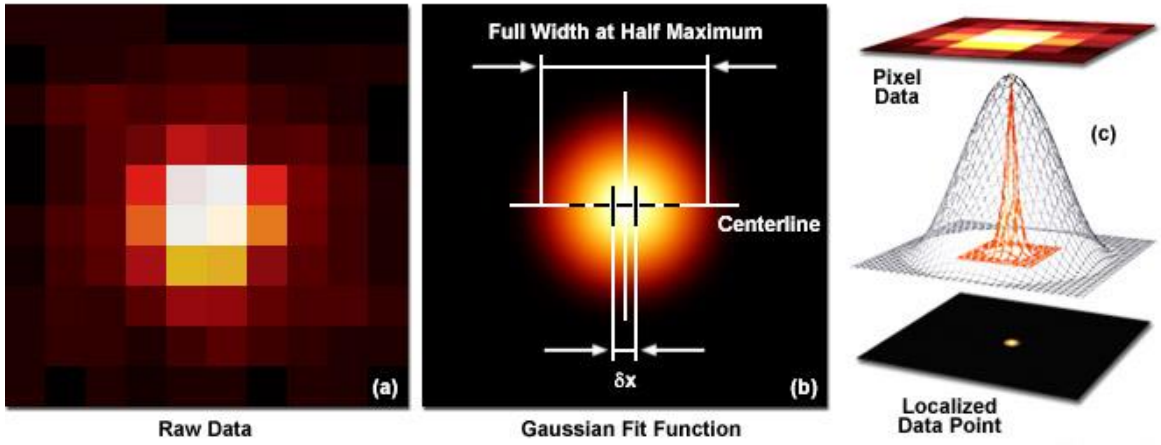
where again J_1 is the first order Bessel function, α is the aperture angle, n the refractive index of the medium between the object and lens, M the magnification and λ the wavelength of the light.

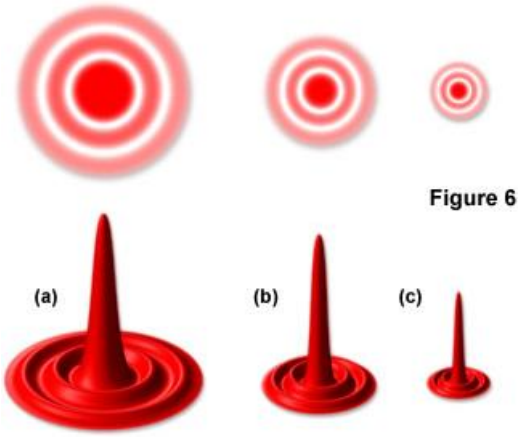
Measurement of the point spread function

The point spread function of an optical system can be directly *measured* by recording the image of a point source, for example of a single fluorescent molecule or a small nanocrystal or quantum dot.









The width of the central maximum in the image, i.e., the ***radius of the first dark ring*** determines the ultimate microscope resolution and for a perfect optical diffraction limited system is given by:

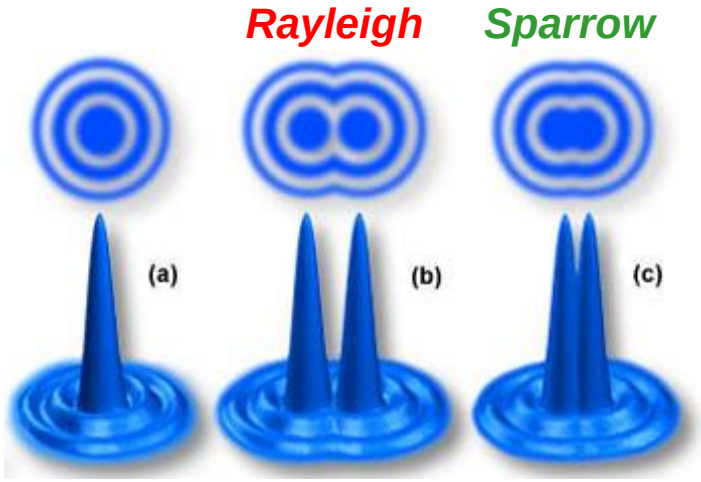
$$R_{Airy} = \frac{0.61\,\lambda}{Mn\sin\alpha}$$

6.6.4 Diffraction Limit of the Lateral Resolution (Abbe Limit)

In a microscope, the finite diameter of the objective lens acts as a finite size circular aperture that creates an Airy diffraction pattern in the image plane for every point of the object.

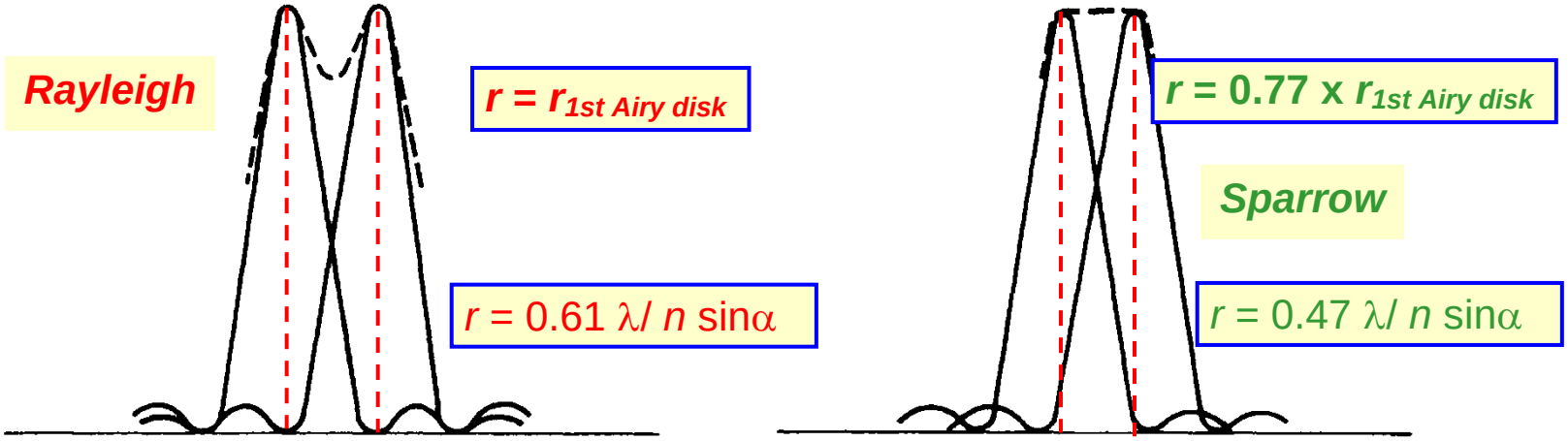
1. Resolution Criteria

When two object points are very close to each other, the point spread function (Airy patterns) of their images overlap so that eventually the two points can no longer be resolved.



(a) Rayleigh Criterion

Two point sources can be just resolved when the central ***maximum*** of the diffraction pattern of one point coincides with the ***minimum*** of the neighbouring point.



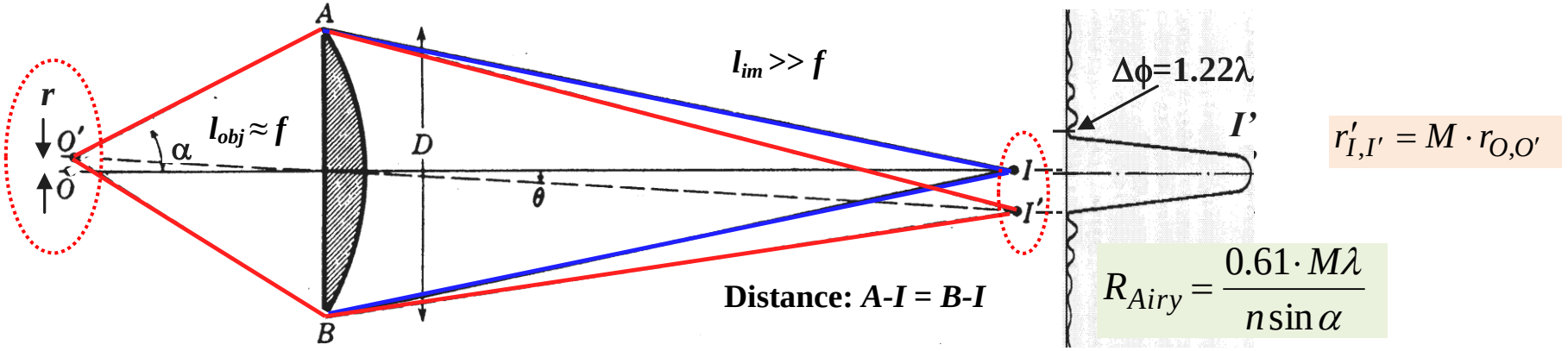
(b) Sparrow Criterion (= Ultimate resolution limit)

Two point sources can be just resolved when there starts to exists an intensity dip between the image of the points.

2. Abbe resolution limit using the Rayleigh criterion

In a **microscope**, the diameter *D* of the objective is finite and thus acts as a finite size circular aperture that creates an Airy diffraction pattern in the image plane for each point of the object.

As shown on the previous slides, for an object point *O* at the optical axis, the diffraction angle θ_{min} at which the 1st Airy intensity minimum is formed in the image is given by $\theta_{min} = 1.22 \cdot \lambda/D$ (see Figure below) where $\lambda = \lambda_{vac}/n$ is the wavelength of light in the medium between the lens and the objective with refractive index *n* that changes the wavelength in the medium.



Tracing back this position I' to the object plane, this image point corresponds to an object point at position *O'* that according to the Raleigh criterion can still be resolved.

As seen in the figure, point *O'* is separated vertically from point *O* by the distance: $r = \tan\theta_{min} \cdot l_{obj}$ where $l_{obj} \sim f$ is its distance from the objective lens.

Since for $\lambda << D$, $\tan\theta_{min} \approx \theta_{min}$ it follows that $r_{min} = \theta_{min} \cdot l_{obj} = 1.22\,\lambda/n \cdot l_{obj}/D_{len} = 0.61\lambda/n \cdot l_{obj}/R_{lens}$.

Because $l_{obj}/R = \sin\alpha$ » the ***diffraction limited resolution*** turns out to be: $r_{min} = 0.61\,\lambda / n \sin\alpha$

3. Resolution and Numerical Aperture

The **diffraction limited resolution** $r_{dif} = 0.61\ \lambda / n \sin\alpha$

increases with (i) decreasing wavelength λ, and
(ii) decreasing acceptance angle α

The denominator

NA = $n \sin\alpha$ is called ***"Numerical Aperture"***

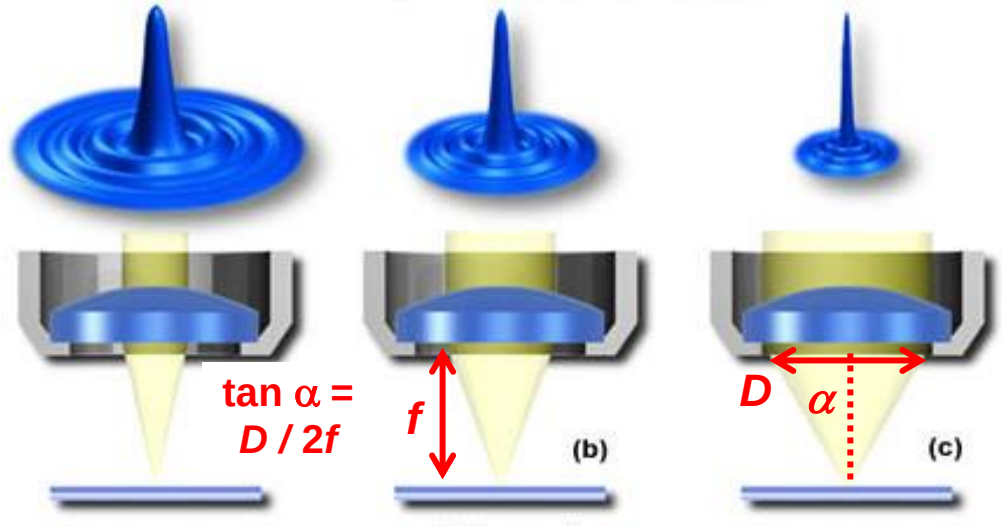
Wavelength (nm)	Resolution (nm)
360 – 450 nm (violet)	190 – 250 nm
450 – 500 nm (blue)	250 – 300 nm
500 – 570 nm (green)	300 – 350 nm
620 – 750 nm (red)	380 – 460 nm

It combines the aperture angle α of the entrance lens and the refractive index *n* of the medium between sample and lens and thus, characterizes the properties of a given optical lens system.

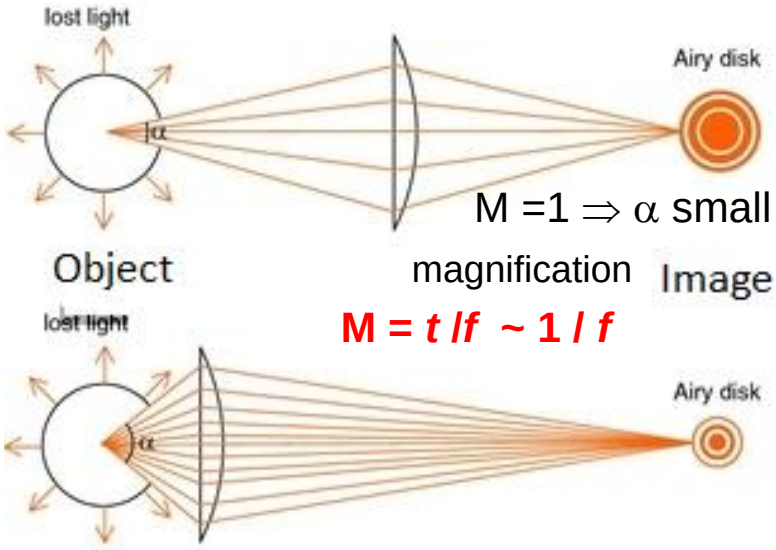
The numerical aperture NA can be increased by (a) increasing the diameter of the objective lens and/or (b) decreasing the focal length *f* of the lens as shown by the figures below:

⇨ *High NA* = small working distance WD » small *f*-number » high magnification [$M \sim t / f$]

⇨ But: For n =1, $\sin\alpha$ is limited to < 1 ⇒ NA < 1.



α = 7° NA = 0.12 α = 20° NA = 0.34 α = 60° NA = 0.87



M =1 ⇒ α small magnification

M = *t* /*f* ~ 1 / *f*

M =4 ⇒ α, NA large

6.7 Modulation Transfer Function and Resolution in Fourier Space

6.7.1 Example: Periodic Line Grating

For a $2b$ periodic line grating with line width b, the angles φ_i of the diffraction maxima are given by:

$$\sin \varphi_i = i \cdot \lambda / (2\, n\, b)$$ with i … diffraction order, n … refractive index, λ … wavelength

If the grating is imaged with an objective lens with a capture angle α, only diffracted beams with $\varphi_i < \alpha$ can contribute to the image formation.

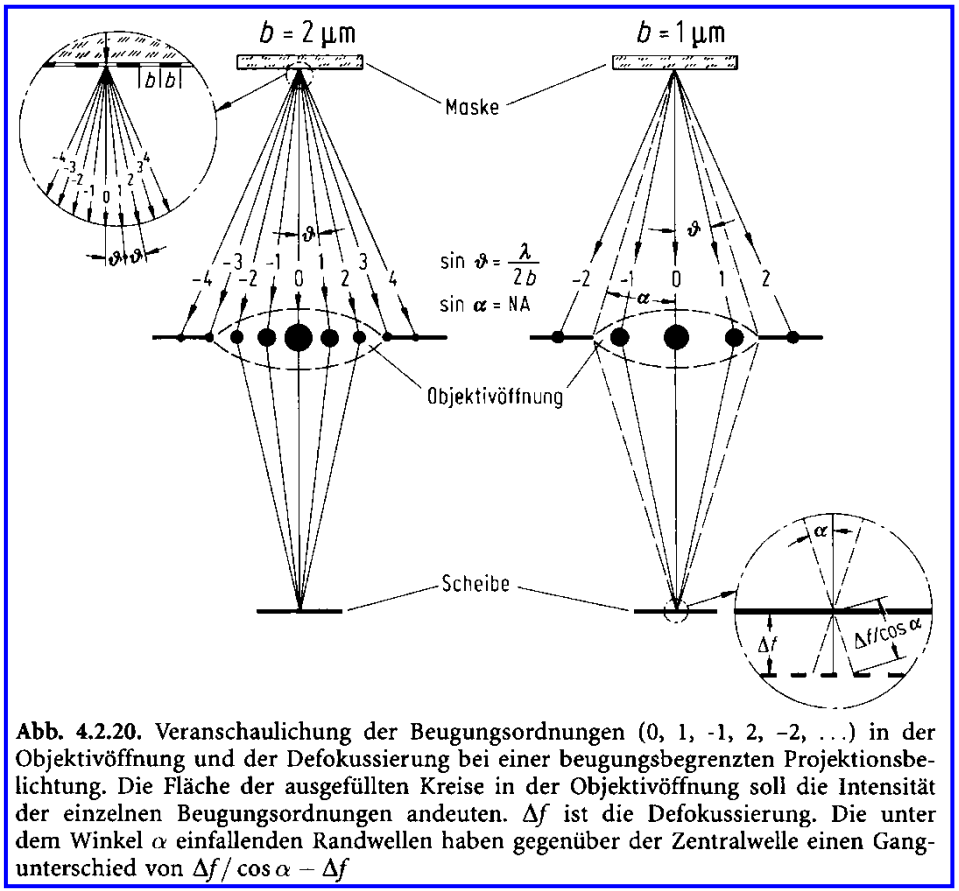


Abb. 4.2.20. Veranschaulichung der Beugungsordnungen (0, 1, -1, 2, -2, …) in der Objektivöffnung und der Defokussierung bei einer beugungsbegrenzten Projektionsbelichtung. Die Fläche der ausgefüllten Kreise in der Objektivöffnung soll die Intensität der einzelnen Beugungsordnungen andeuten. Δf ist die Defokussierung. Die unter dem Winkel α einfallenden Randwellen haben gegenüber der Zentralwelle einen Gangunterschied von $\Delta f / \cos\alpha - \Delta f$

Resolution Criterion:

The grating can only be resolved if at least the 1st order diffracted beams are still collected by the lens ($\varphi_1 \leq \alpha$).

Condition: $\sin \varphi_1 = \lambda / (2\, n\, b_{min}) =! \sin \alpha$

$\rightarrow$ $R = b_{min} = 0.5 \cdot \lambda / (n \cdot \sin \alpha)$

or $R = 0.5 \cdot \lambda / NA$

with $NA = (n \cdot \sin \alpha)$
(numerical aperture of the lens)

⇨ Thus, the objective acts as a low pass filter in diffraction space !

⇨ **Off-axis illumination** increases the resolution because more higher order diffraction maxima go through the objective.

6.7.2 Fourier Decomposition of the Sample Contrast

Any real space object with contrast $C_s(x,y)$ can be thought to be build up of a sum of sinus and cosine functions of various wavelengths λ or wave vectors $k = 2\pi/\lambda$ with amplitudes $A(k)$ or $A(\lambda)$.

$$C(x,y) = \int_{-\infty}^{\infty}\int_{-\infty}^{\infty} A(k_x,k_y)e^{-i(k_x x + k_y y)}dk_x d_y$$

(For periodic functions, the integral can be replaced by a summation:)

$$C(x) = \frac{a_0}{2} + \sum_{n=1}^{\infty} A(n)\sin(k_n x) + B(n)\cos(k_n x) \quad \text{where } k_n = \frac{2\pi n}{\lambda}$$

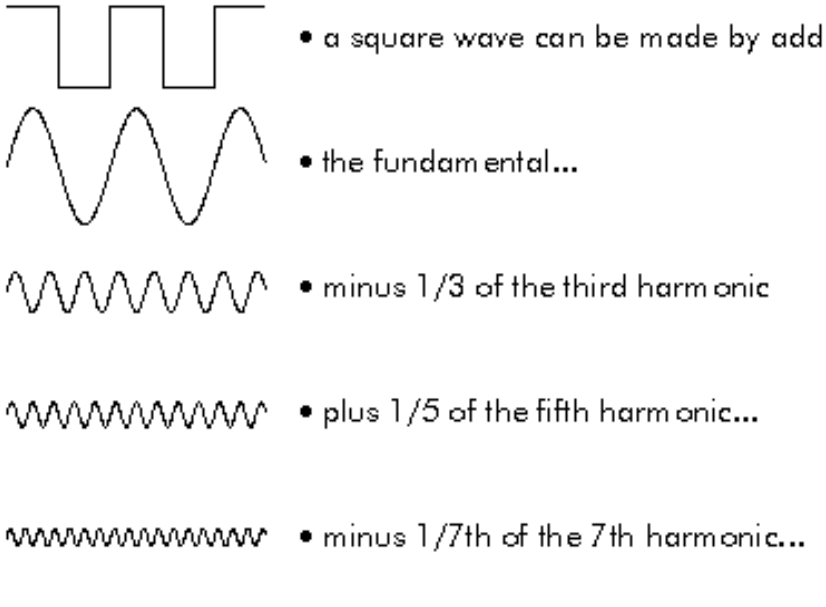
An exact 1:1 representation of $C(x)$ requires an ***infinite number* of Fourier components**.

If in an image formation process, the higher order spatial frequencies are cut off, i.e, A(k)=0 for $k > k_c$ *i.e.*, only a *finite Fourier series* contributes to the image, the sample contrast $C_s(x,y)$ is no longer exactly reproduced, i.e., $C_{image}(x,y) \neq C_{sample}(x,y)$. This is what happens in any imaging process !

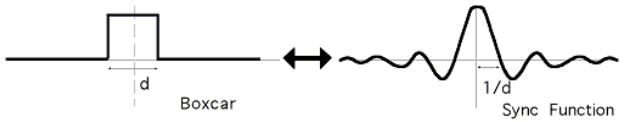
Example:

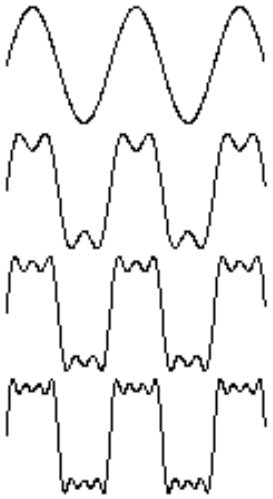
$$|A(n_{odd})| = 1/n$$

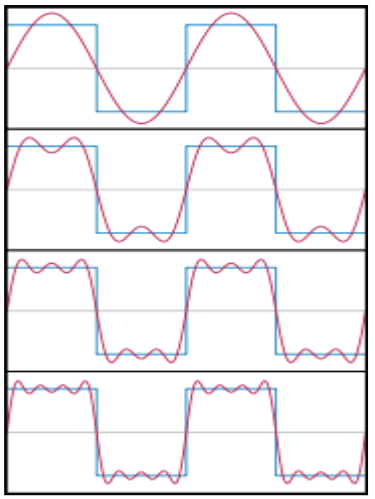
$$A(n_{even}) = 0$$



Box function:







Fourier series representation of the sample contrast

In Fourier space, the **contrast $C_s(x,y)$** of a real space object is represented by a summation of plane waves with an infinite number of spatial frequencies **$k = 2\pi/\lambda$** which are weighed by different amplitudes **$A(k_x,k_y)$** (=Fourier components), i.e.,

$$C(x,y) = \int_{-\infty}^{\infty}\int_{-\infty}^{\infty} A(k_x,k_y)e^{-i(k_x x + k_y y)}dk_x d_y$$

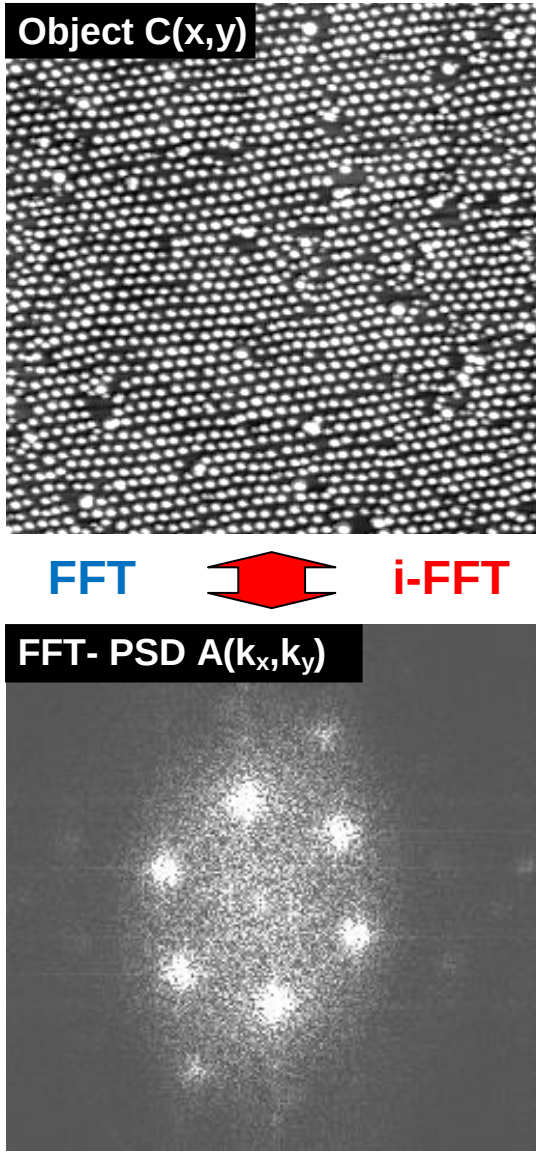
where $k_x = 2\pi/\lambda_x$ and $k_y = 2\pi/\lambda_y$ are the **wave vectors** in *x* and *y* direction inversely proportional to the corresponding **spatial wavelength $\lambda_{x,y}$**. **$A(k_x,k_y)$** is the **amplitude** of the plane wave component with wave vector (k_x,k_y). Thus: **$C(x,y) = \mathrm{FFT}(A(k_x,k_y))$**.

The **Fourier amplitudes** **$A(k_x,k_y)$** (=contribution of each frequency or wavelength to C(x,y)) can be calculated by the ***inverse*** **Fourier transformation (iFFT)** of the object contrast function **$C(x,y)$**.

Thus: **$A(k_x,k_y) = \mathrm{iFFT}(C(x,y))$**

$$A(k_x,k_y) = \frac{1}{4\pi^2}\int_{-\infty}^{\infty}\int_{-\infty}^{\infty} C(x,y)e^{+i(k_x x + k_y y)}dxdy$$

From the knowledge of the Fourier amplitudes **$A(k_x,k_y)$** the sample structure **$C(x,y)$** can be reconstructed and vice versa.



6.7.3 Modulation Transfer Function

1. Perfect optical imaging

For a **perfect imaging process**, each spatial frequency contained in the object is *perfectly* reproduced, i.e., $A_{image}(k_x,k_y) = A_{sample}(k_x,k_y)$.

This means that the sample contrast

$C(x,y) = \mathrm{FFT}(A(k_x,k_y))$ is ***exactly*** reproduced in the image without any loss of information

2. Real optical imaging

In a real ***imaging system***, different spatial frequencies are reproduced differently well, i.e.,

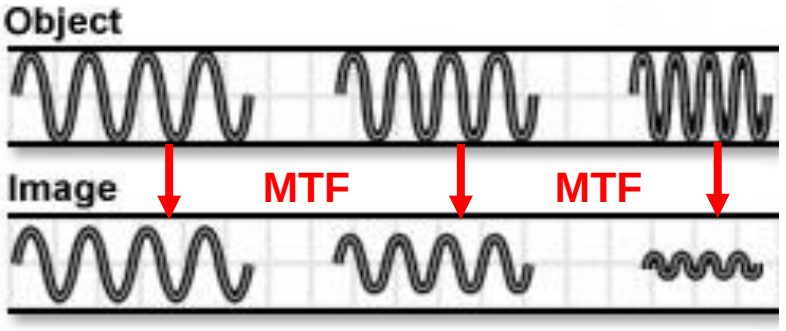
high spatial frequencies (small period structures) are generally reproduced less well than low spatial frequencies. This means that the amplitudes $A(k)$ of Fourier components with high spatial frequencies contained in the object are reduced by a certain factor in the image.

The ability of a lens system to reproduce different spatial frequencies of the object in the image is characterized by the **modulation transfer function MFT(f) or MFT(k).**

Definition of the modulation transfer function:

$$\mathrm{MTF}(k) = \frac{\text{image contrast modulation}}{\text{object contrast modulation}} = \frac{A_{im}(k)}{A_{obj}(k)}$$

at a given spatial frequency k.

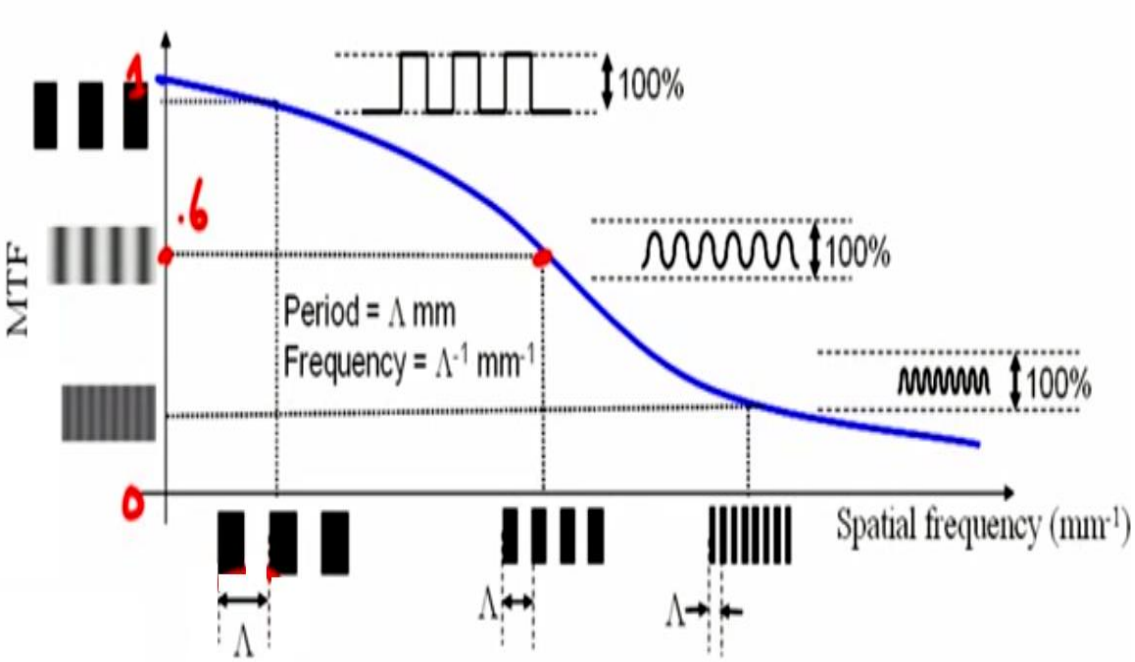


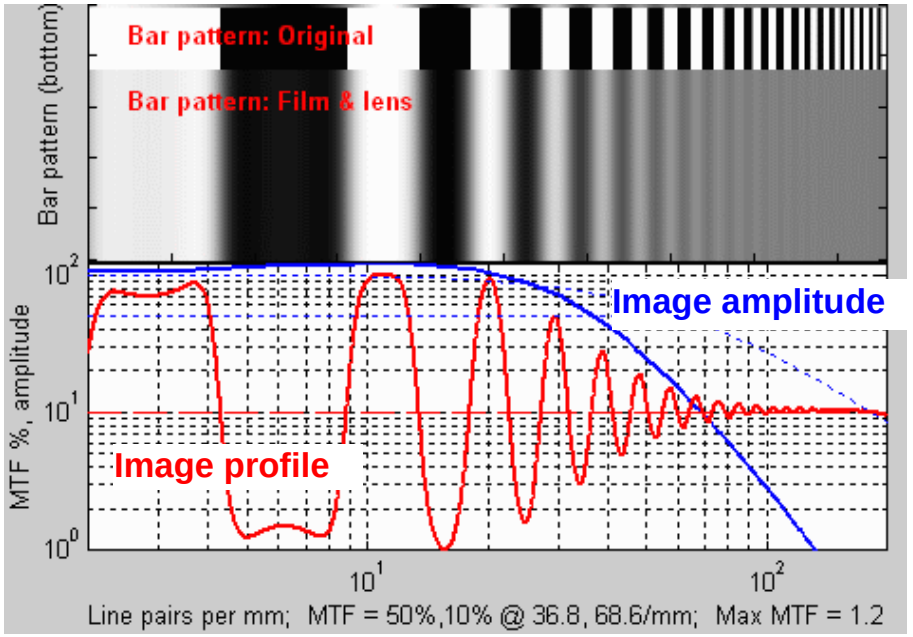
For a perfect imaging MTF = 1 at all frequencies, whereas for a real imaging system the MTF decreases with increasing wave vector, i.e., with decreasing periodicities contained in the sample.

As a result, there exists a certain **maximum *cut-off spatial frequency* k_c** above which the MTF is zero, meaning that all high frequency information is eliminated in the image formation.

⇨ **A lens system acts like a low pass Fourier filter such that the contributions of the higher spatial frequencies in the image are removed.**

Example for modulation transfer MTF(*k*) plotted versus spatial frequency $k = 1/\Lambda$

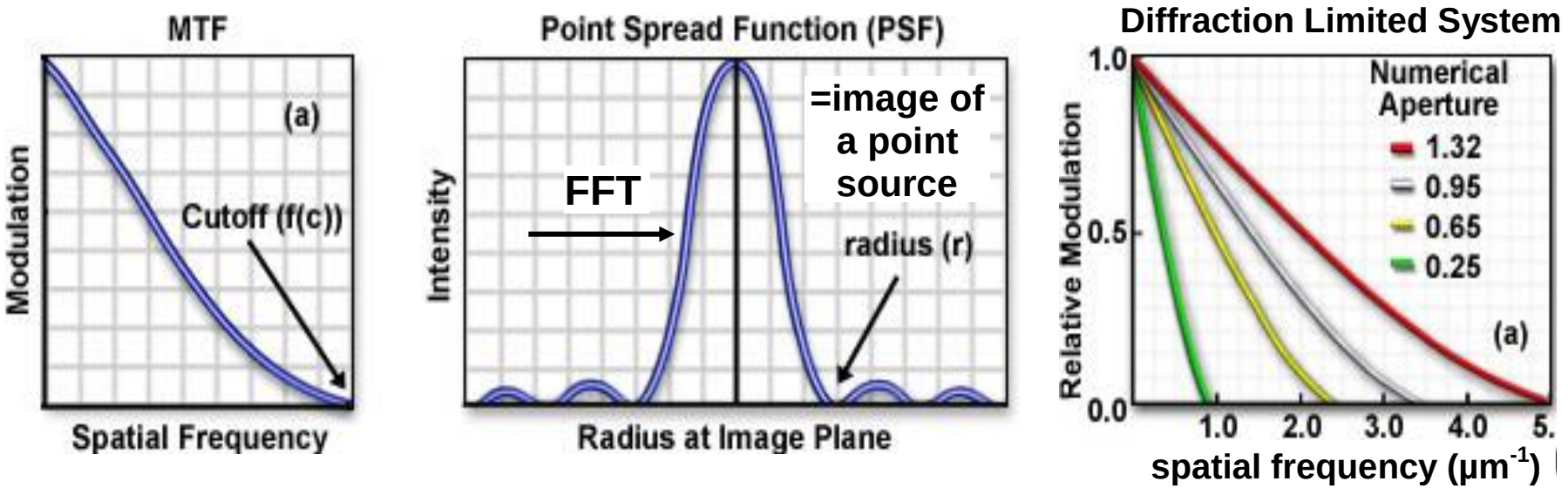




Modulation Transfer Function MTF(*k*) of a Diffraction Limited System

The modulation transfer function is the inverse FFT of the ***point spread function (=image of a point-like light source)***. For a **diffracting limited optical system** the MTF is given by:

$$MTF(k) = 2(\phi - \cos\phi \sin\phi)/\pi \quad \text{where} \quad \phi = \cos^{-1}(k\,\lambda_{illu}/2NA)$$



- At *low spatial frequencies*, MTF(k) = A_{im} / A_{obj} ~ 1 . Thus, the image contrast is equal to that of the sample. At *higher frequencies*, MTF decreases and falls to zero at the cut-off frequency k_c.
- For a diffraction limited system, the cut off frequency k_c is given by: $k_c = f_c = 2.1\ NA / \lambda$
  Thus, the highest spatial frequency is determined by the Sparrow limit.
- The corresponding radius of the first dark concentric ring surrounding the central peak of a point spread function is given by: $r = 0.61\ \lambda / NA$
- Objectives with low NA produce a wider point spread functions and thus, lower resolution.

6.7.4 Image Formation using the Modulation Transfer Function

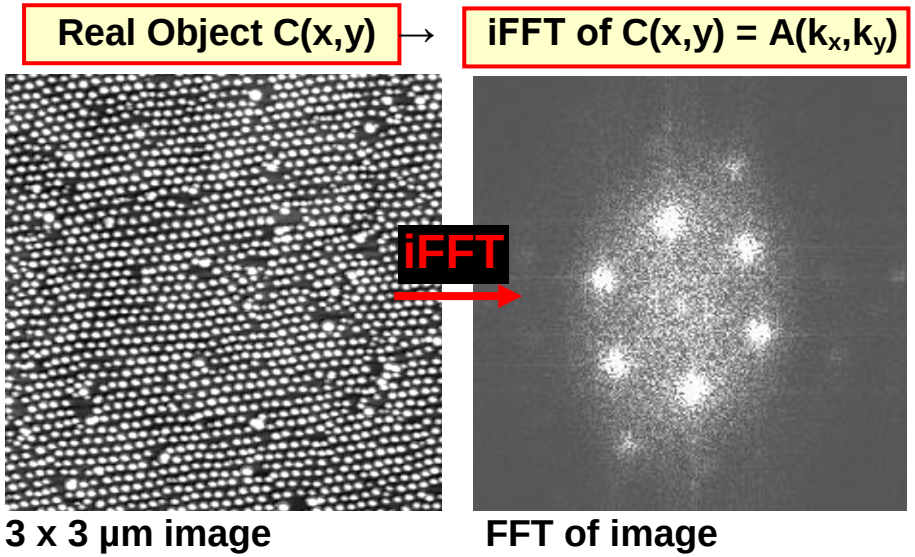
Once the modulation transfer function MTF(k) is known, the amplitude of the Fourier components of the image, i.e., $A_{image}(k_x,k_y)$ can be calculated using:

$$A_{im}(k) = \mathrm{MTF}(k) \cdot A_{obj}(k)$$

From the known $A_{image}(k_x,k_y)$, the **real space image $S_{image}(x,y)$** can be calculated by back transformation of $A_{image}(k_x,k_y)$ into real space:

$$S_{im}(x) = \mathrm{iFFT}[A_{im}(k)] = \mathrm{iFFT}[\mathrm{MTF}(k) \cdot A_{obj}(k)]$$

Example: Influence of the modulation transfer function on image formation



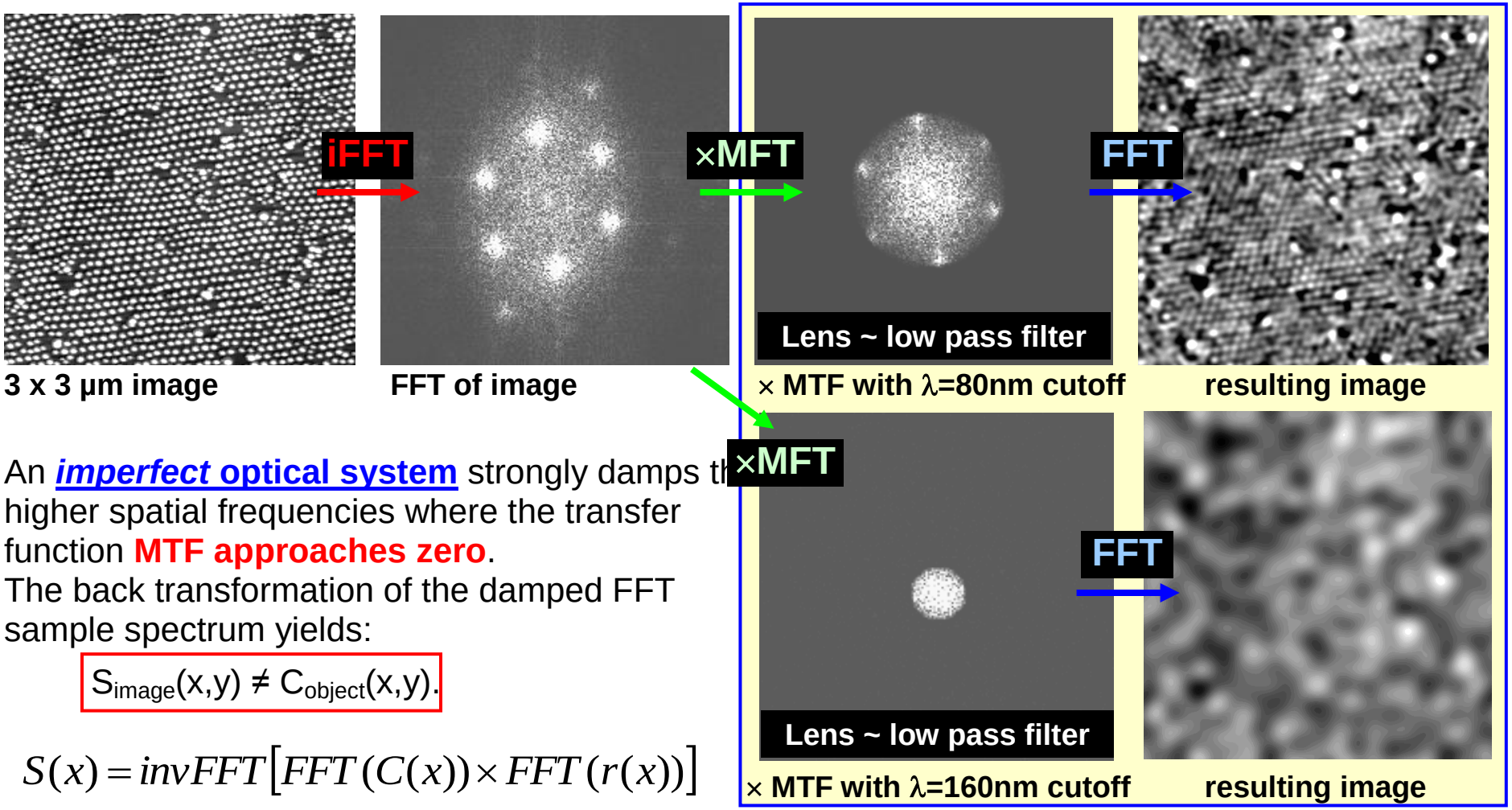
The contrast modulation of the object at a spatial frequency k is given by the corresponding **Fourier amplitude** $A(k_{x,ky})$ of the object contrast $C(x,y)$.

This amplitude is obtained by the Fourier transformation of the object contrast distribution $C(x,y)$ or $I(x,y)$ that represents the real space structure of the object

A ***perfect* optical system** with infinitely high resolution would have a modulation transfer function of **MTF = 1** (unity) **for *all* spatial frequencies**. Only then, it follows that $I_{image}(x,y) = I_{object}(x,y)$!!!

Image formation process using the modulation transfer function

Real Object C(x,y) → FFT of C(x,y) = A(k_x,k_y) → A(k_x,k_y) × MTF(k_x,k_y) → back transformation = I(x,y)



An ***imperfect* optical system** strongly damps th higher spatial frequencies where the transfer function **MTF approaches zero**.
The back transformation of the damped FFT sample spectrum yields:

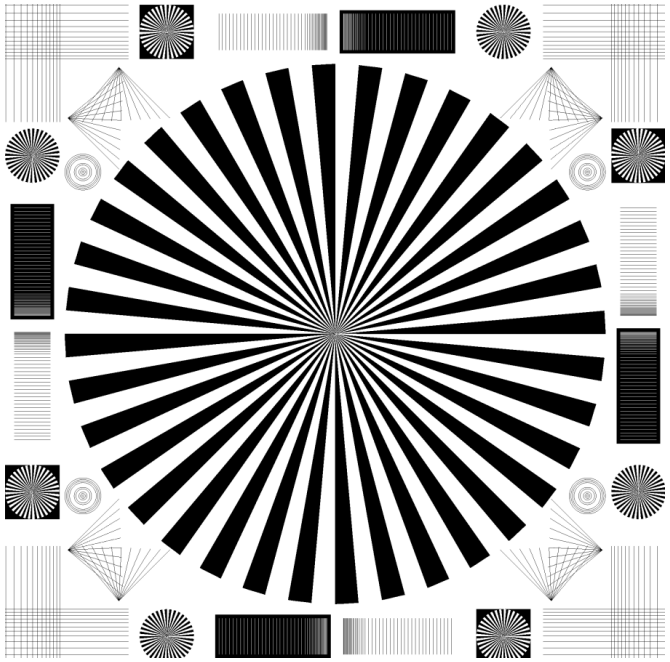
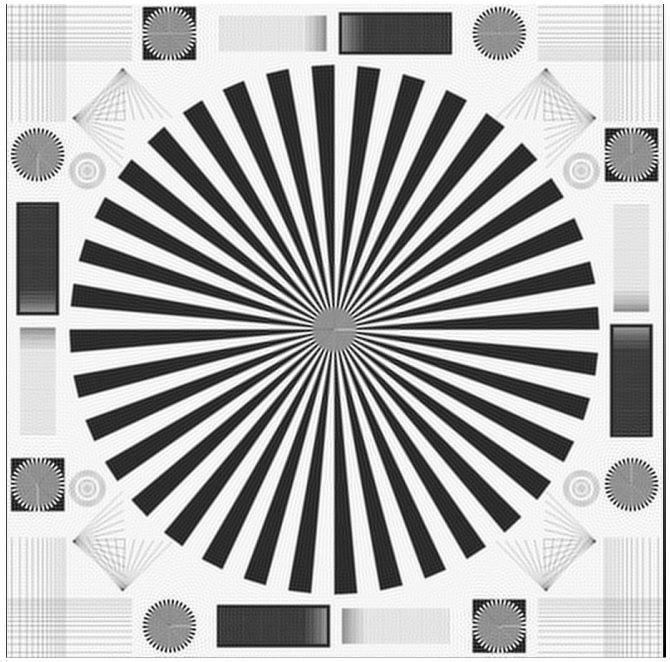
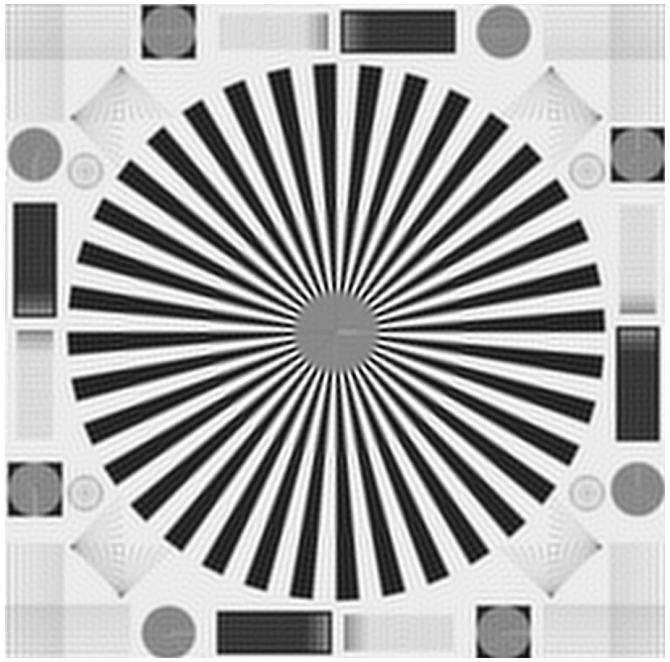
$S_{image}(x,y) \neq C_{object}(x,y)$.

$$S(x) = invFFT\left[FFT(C(x)) \times FFT(r(x))\right]$$

The **modulation transfer function includes** both image broadening effects due to **diffraction *and* Lens imperfections**. A lens system without aberrations is termed diffraction limited.

Example: Testpattern for Image Resolution

“Siemens Star”

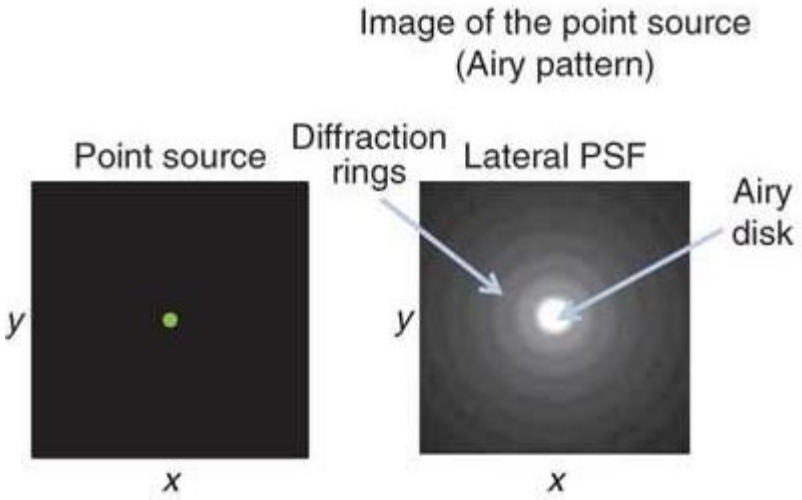
6.7.5 Experimental Determination of the Modulation Transfer Function

The **modulation transfer function** and **point spread function** of any optical system including a microscope can be ***determined*** using a **point-like light source** such as single fluorescing molecules, quantum dots or nanocrystals, or illuminated point like apertures.

Procedures:

(a) The intensity distribution of the image of the point source corresponds to the **point spread function** of the microscope.

(b) The **Fourier transform** of this intensity profile of the point source image yields the **modulation transfer function MTF(*k*)**



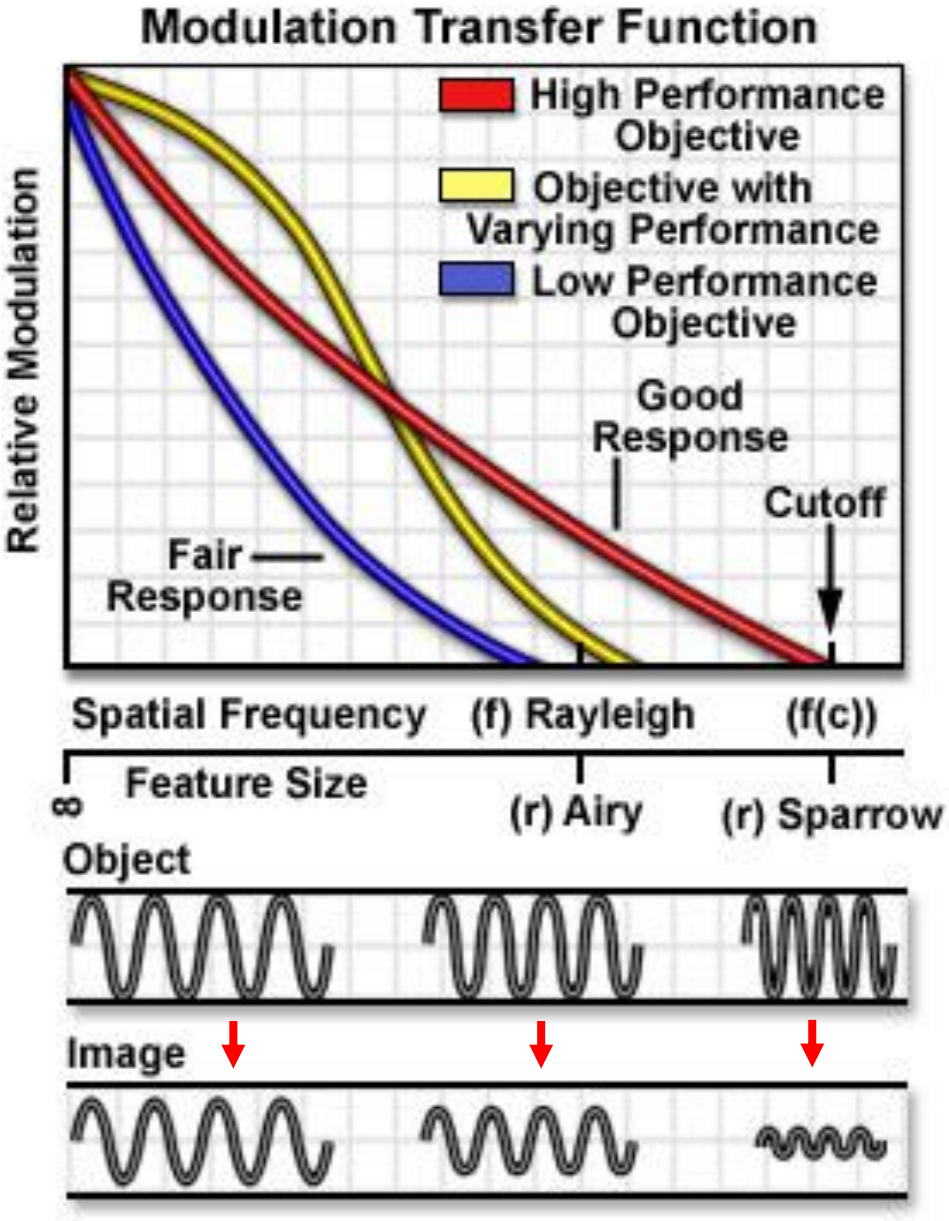
(c) The MTF can be also ***directly measured***: It is the diffraction pattern formed in the ***back focal plane*** of the objective system.

(d) The intensity profile of a sharp shadow edge can be also used to determine the MTF function, again by calculating the Fourier transform of the intensity profile in the image.

In general, for an ideal point source the **modulation transfer function** corresponds to the ***diffraction pattern*** formed in the ***back focal plane*** of the objective system, and the point spread function to the intensity distribution in the image plane.

Thus, the MTF and PSF are strictly interrelated by the Fourier transformation.

Examples for modulation transfer functions:



The modulation transfer function:

- continuously decreases with increasing spatial frequency until it reaches zero at the cut-off frequency k_c (or f_c)
- all frequencies higher than k_c are completely blocked by the optics.
- high performance objectives with large NA have a higher cut-off frequency and higher MFT values at lower frequencies.

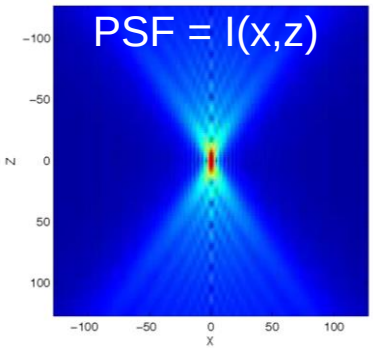
6.8 Axial Resolution and Depth of Field

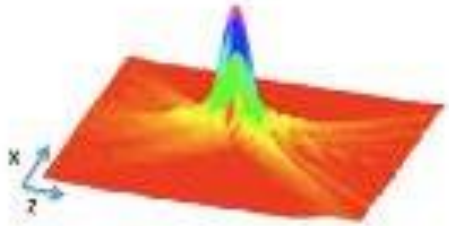
The point spread function is actually a ***3D intensity distribution*** in (x,y) as well as *z* (axial) direction.

Axial intensity distribution:

$$I_{Airy}(x=0,z) = C \cdot \frac{NA^4}{M^2}\left[\frac{\sin(\pi NA^2 \cdot z/2n*M^2\lambda)}{\pi NA^2 \cdot z/2n*M^2\lambda}\right]^2$$

Lens system



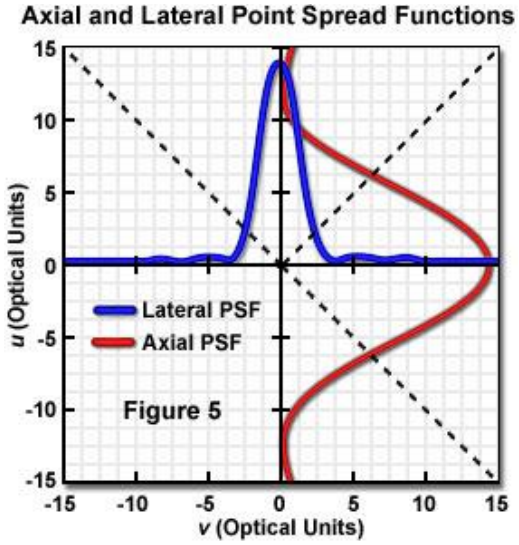


This means that the image of a point source is not only broadened in the lateral, but also *in the axial direction*, with an axial width (=1st zero of *I*(*z*)

$$\Delta z_{Airy} = 2n*\cdot M^2\lambda / NA^2$$

Thus, the ***axial resolution***, defined as minimal z-separation of two points that can be discriminated in the microscope when focusing through the object is limited to:

$$\Delta z_{res} = 2n*\lambda / NA^2$$



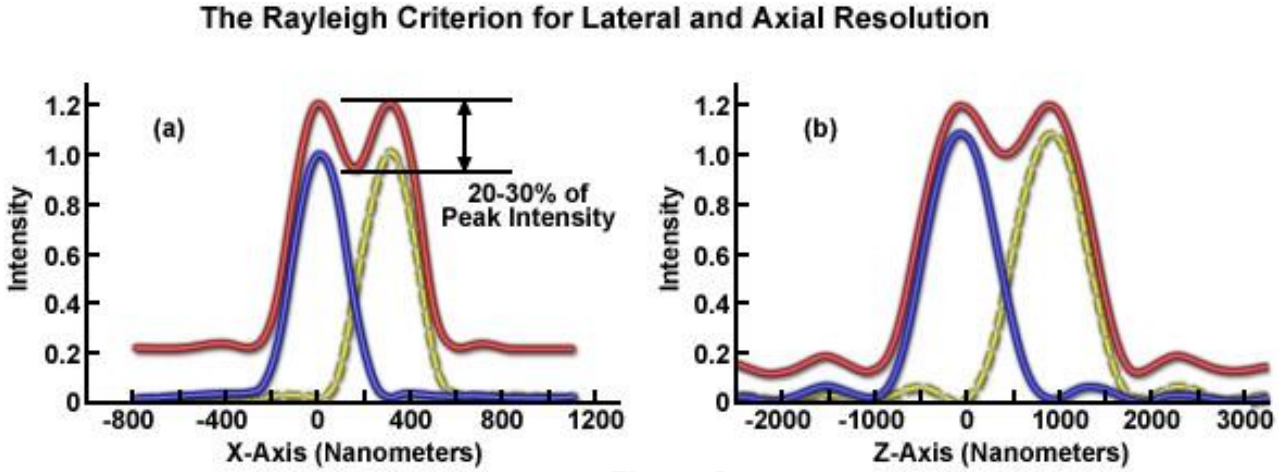
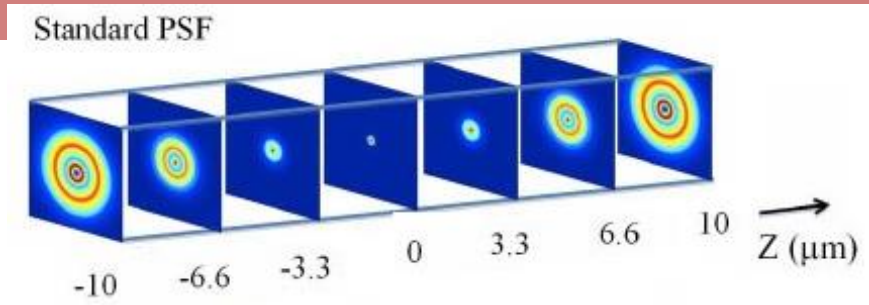


Figure 2

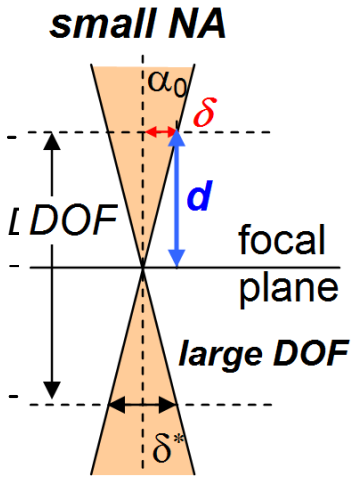
Depth-of-Field

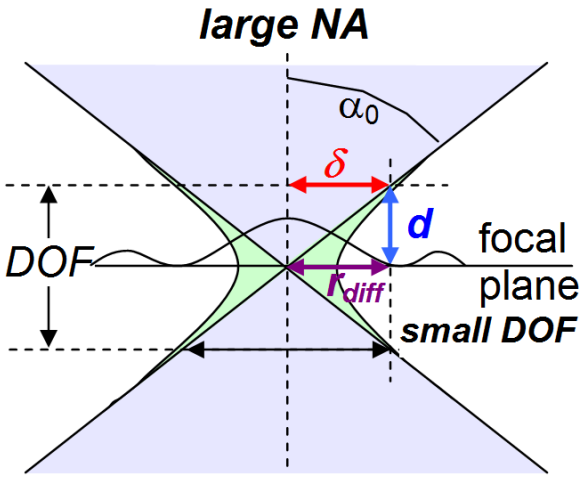


Due to the broadening of the PSF in the axial direction an object part that is offset from the ideal focal plane appears broadened in the image plane.

The **Depth-of-field (DOF)** is thus defined as the vertical distance or sample depth over which a microscopy image appears still sharp.

⇨ Like the lateral resolution, the DOF is determined by acceptance angle α_0 (*NA*) of the objective lens.





Derivation of the DOF using the following resolution criterion:

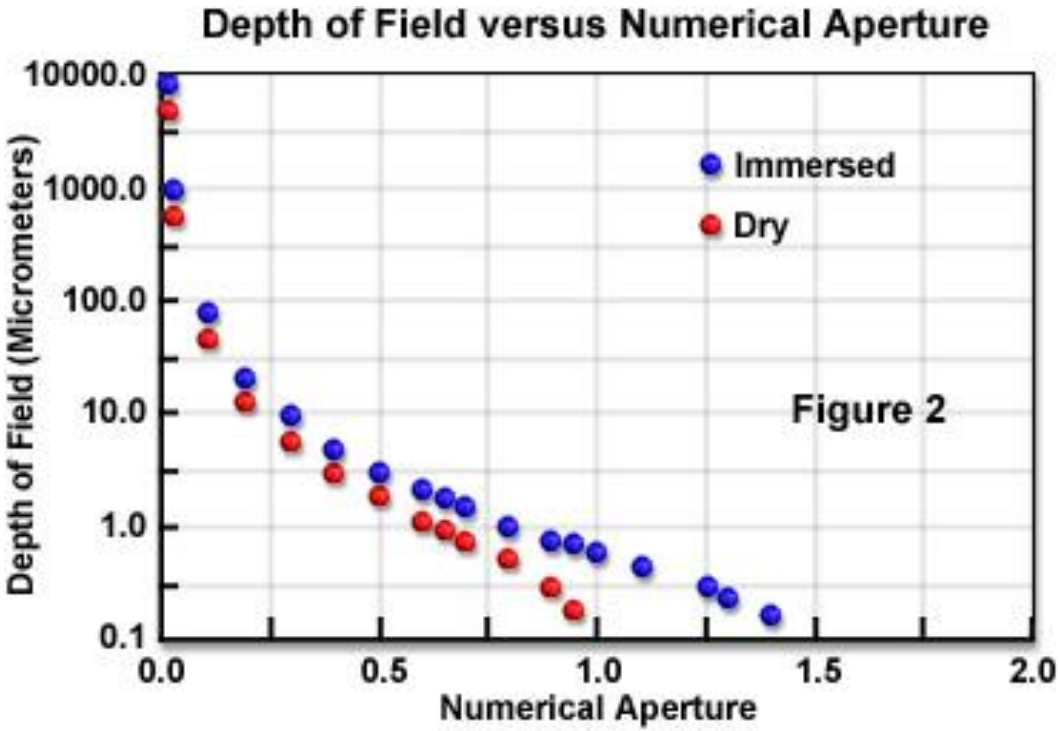
Criterion: DOF = Depth of sample across which the **broadening $\delta(z)$** due to the spreading of the light cone by α_0 is equal to the lateral resolution r_{diff} in the focal plane, i.e., $\delta(z) = r_{diff}$

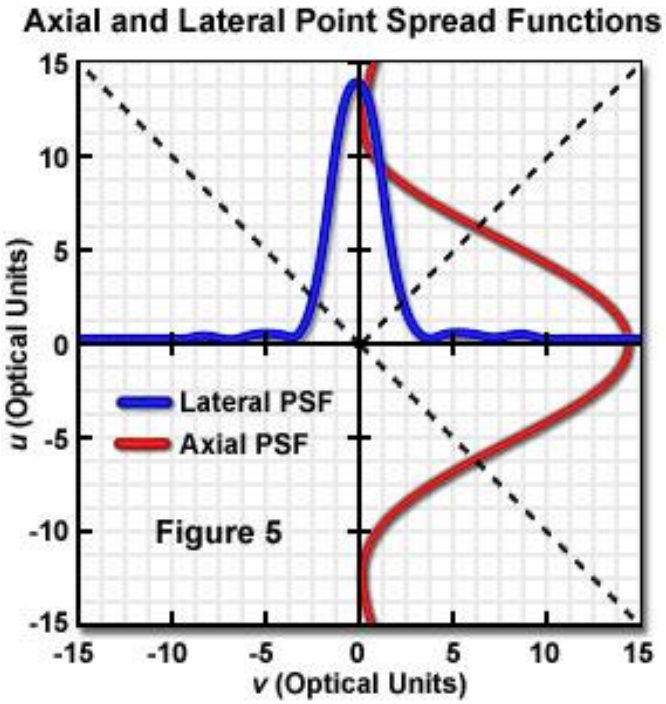
From above figure: $\delta = d \cdot \tan\alpha$ & $r_{diff} = 0.61\,\lambda / n \sin\alpha \Rightarrow d = 0.61\,\lambda / (\tan\alpha \cdot n \sin\alpha)$

$\Rightarrow$ $\mathbf{DOF = 2 \cdot d = 1.22\,\lambda \cdot (n^2 - NA^2)^{1/2} / NA^2}$ For small α: $\mathbf{DOF \approx n \cdot \lambda / NA^2}$

» Increasing the numerical aperture NA *decreases* the depth-of-field of the image !

$$DOF = 2 \cdot d = 1.22 \, \lambda \cdot (n^2 - NA^2)^{1/2} / NA^2$$ For small α: $DOF \approx n \cdot \lambda / NA^2$





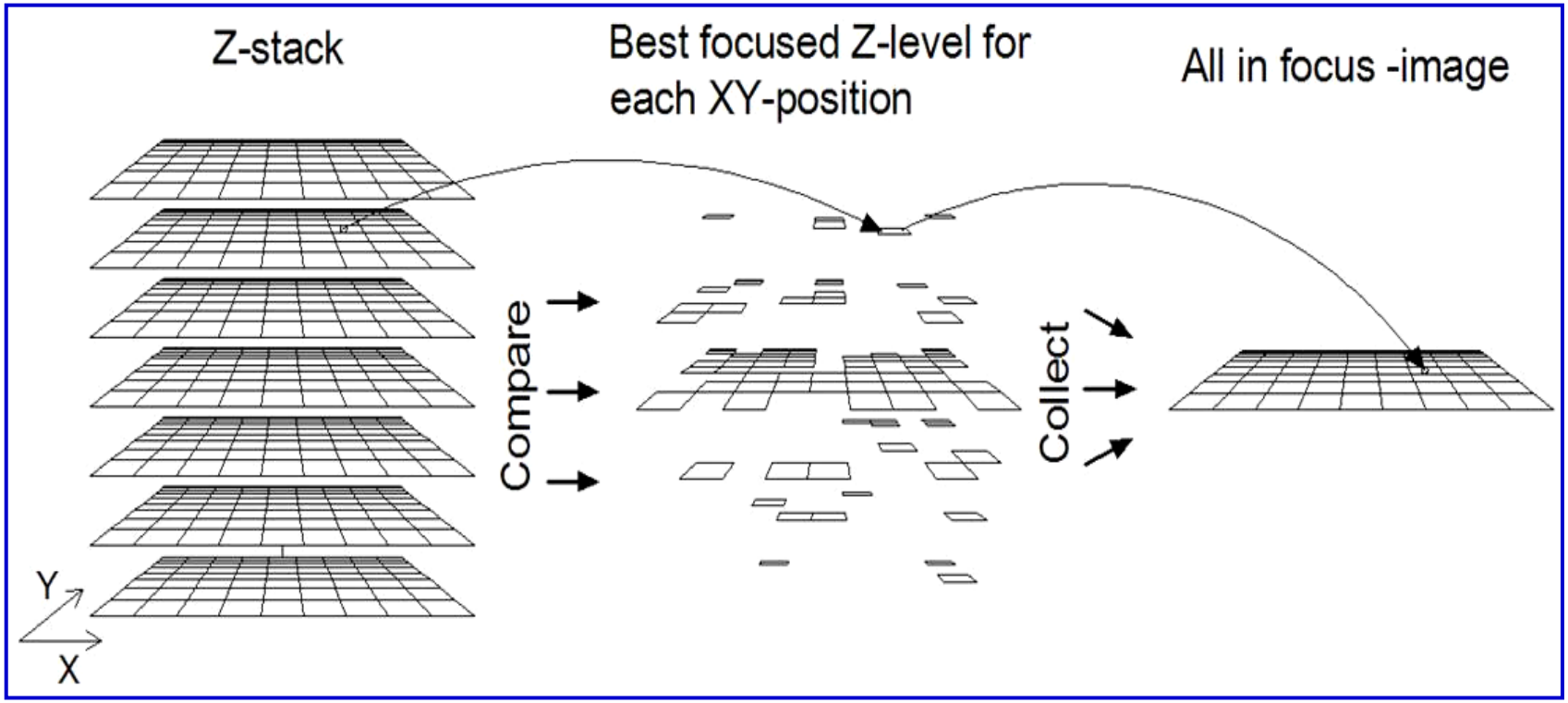
Conclusions:

- ⇨ For large sample thicknesses or large topography variations, the image is not sharp over the whole microscopy image.
- ⇨ A **high lateral resolution** and a **large DOF cannot be achieved at the same time**.
- ⇨ A **large DOF** mean a **low axial resolution** because at large DOF the microscope cannot discriminate between objects located at different z-positions. This ability is called axial resolution, which is important for 3D imaging of the objects in x,y,z direction.

6.9 Z-Series: A Method to Overcome the DOF Limitation

To overcome the limit of image sharpness due to the finite DOF, a whole series of images (**= "z-stack"**) can be recorded with incremental vertical shifts of the focal plane.

From each image, the **sharp parts** are selected by an image processing software, and a total ***virtual image*** is constructed only from the sharp regions for the whole sample series.

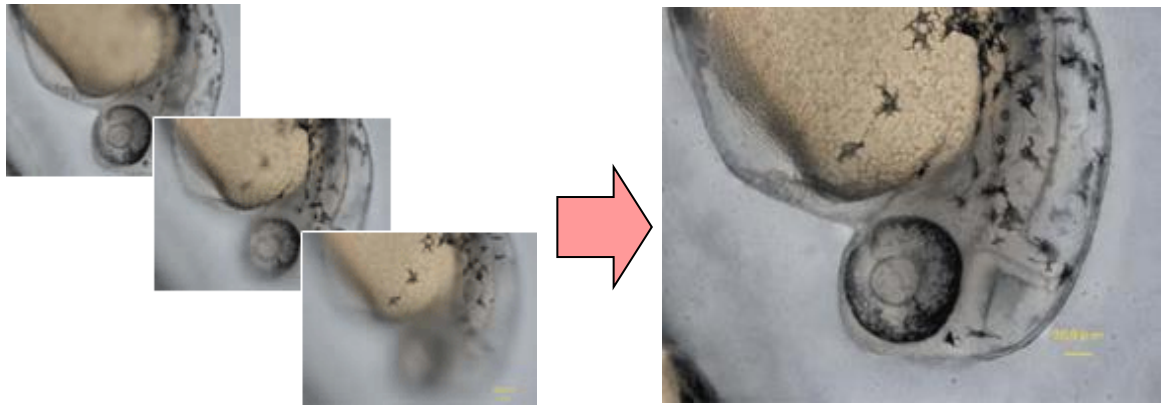


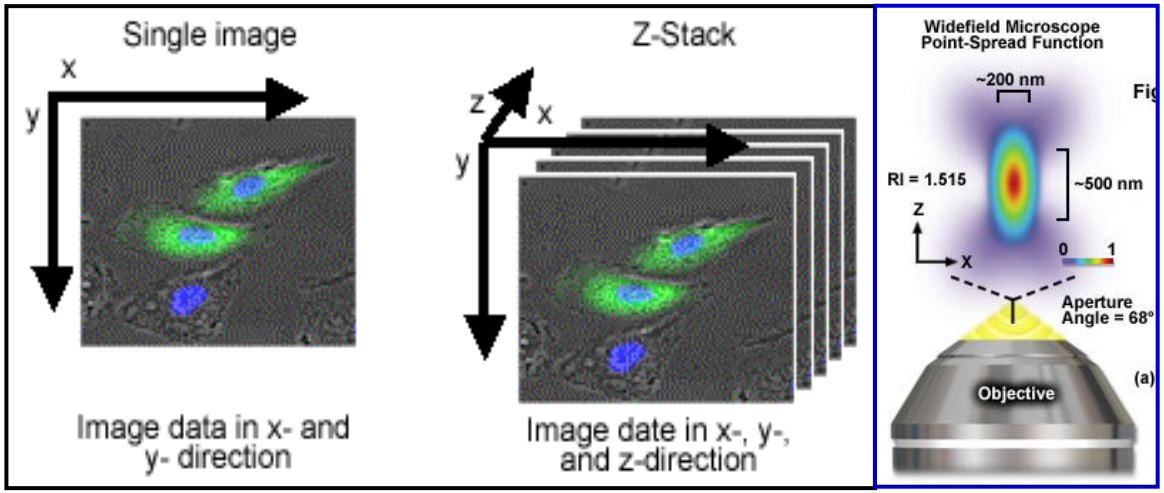
Selection criterion:
The sharpest z-element is that with the highest local contrast modulation, i.e., the element with containing the highest spatial frequencies.

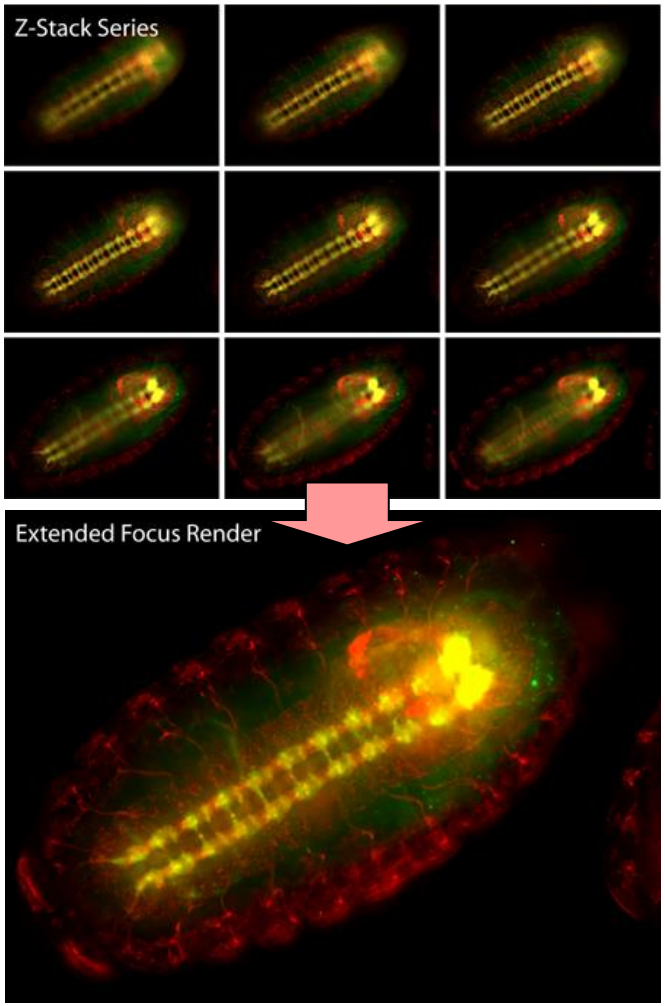
Example: Z-Series for Optical Imaging Microscopy

When a z-image series is recorded with precisely-controlled z-displacement increments Δz, the x,y coordinates of the sharp parts of the images can be correlated with their location in z-direction.
In this way, a ***complete 3D sample image*** is obtained.

Examples for z-series 3D image reconstruction





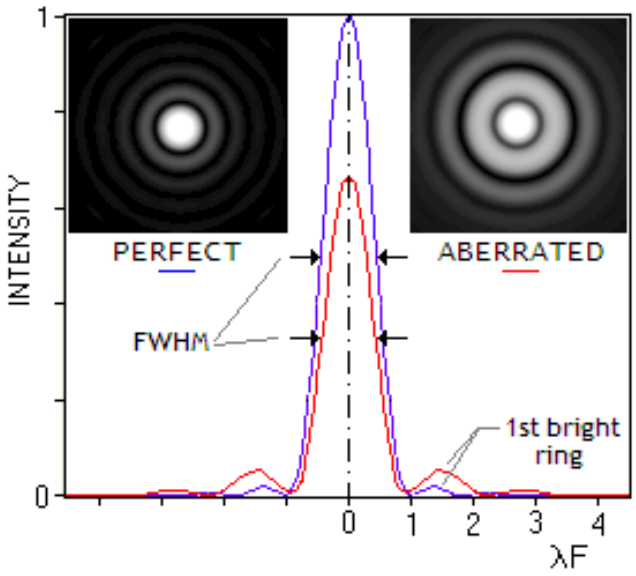


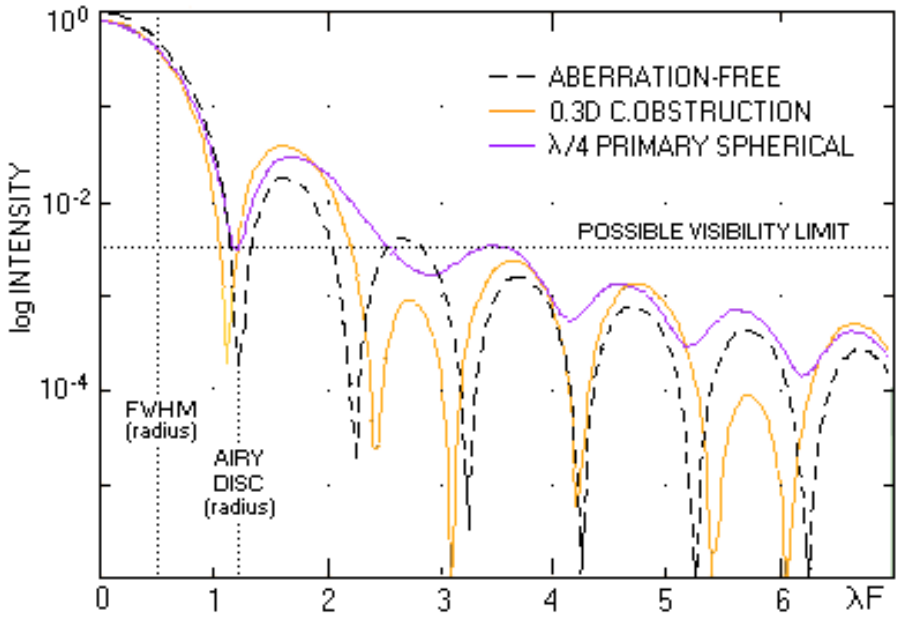
Lateral resolution: $\Delta x = 0.66\,\lambda / NA \sim \lambda/2$ **Vertical resolution:** $\Delta z = DOF \sim n \cdot \lambda / NA^2 \sim \lambda$

6.10 Resolution Limits due to Lens Aberrations

For imperfect optical systems, the PSF point spread function is ***additionally broadened***.

This is due to the fact that imperfect lenses do not focus all beams exactly on the same image spot in the image. This leads to a smearing of the image and thus, the resolution is decreased.





The resulting ***effective* resolution** is described as the ***RMS sum*** of diffraction broadening r_{diff} *and* aberration broadening r_{aber} :

$$r_{eff} = (r^2_{dif} + r^2_{aber})^{1/2}$$

The overall performance of an ojective lens is then characterized by an **effective numerical *aperture*** NA_{eff} : which is always smaller than the geometrical numerical aperture !

$$NA_{eff} = n \sin\alpha \cdot r_{dif} / r_{eff}$$

There are **three main types of lens errors** (aberrations) that degrade the spatial resolution: (1) Spherical Aberration, (2) Chromatic Aberration and (3) Astigmatism.

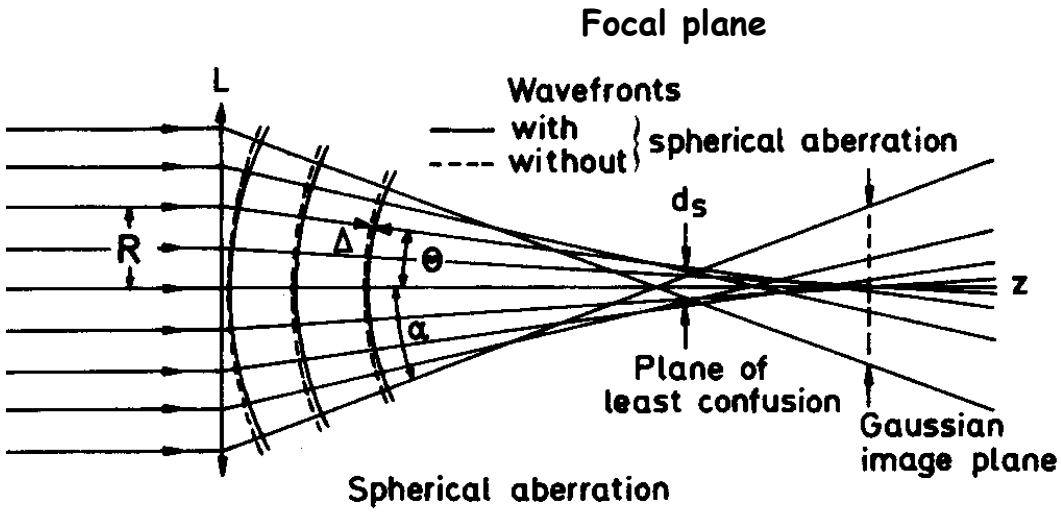
6.10.1 Spherical Aberration

Spherical aberration arises from the spherical shape of optical lenses.

A spherically shaped lens acts differently on central and far off-axis rays:

» Off-axis beams are bend more strongly towards the optical axis.

» Thus, parallel beams are not focused on the same point on the optical axis.



Result: Broadening of the spot size.

Spot radius r_{sph} in the focal plane: $r_{sph} = C_s \cdot \alpha^n$

C_s … spherical aberration constant.
α - exponent: $n \sim 3$

Radius in the plane of least confusion: $r^*_{sph} = r_{sph} / 4$

Spherical aberration decreases with *decreasing* lens curvature, i.e., *increasing* focal length.

Example: Calculation of spherical aberration for a simple *half-spherical* lens

Focal length *f* for a thin spherical lens: $\frac{1}{f} = (n-1)\left(\frac{1}{R_1} - \frac{1}{R_2}\right)$. Half-spherical lens: $\boxed{f_0 = \frac{R}{n-1}}$

(1) **Incidence angle** γ on curved lens surface: $\sin\gamma = y/R$

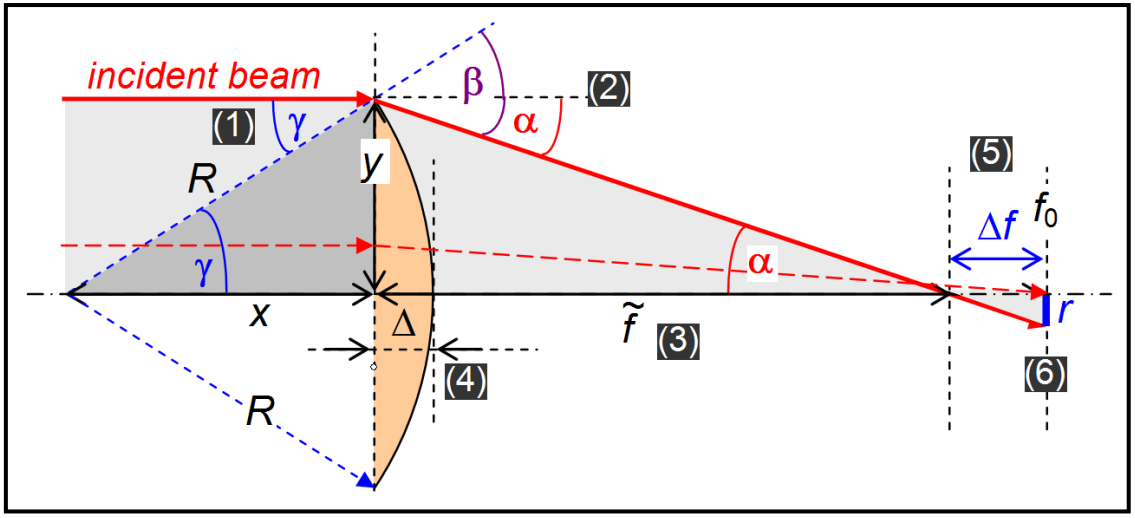
(2) **Refraction** of beam at curved surface (Snell´s law): $\sin\beta = n \cdot \sin\gamma$ » Focusing angle $\alpha = (\beta - \gamma)$

(3) **Focal distance of outer rays**: $\tilde{f}(y) = y/\tan\alpha \approx f_0\left[1 - k(y/R)^2\right]$ for $\alpha < 10°$, k = *constant*

(4) **Additional shift** Δ of origin: $\Delta(y) = R - x = R(1-\cos\gamma) \approx 1/2R \cdot (y/R)^2$ for $\alpha < 10°$

⇨ **Effective focal distance of outer rays**:

$$f(y) = \tilde{f}(y) - \Delta(y)$$

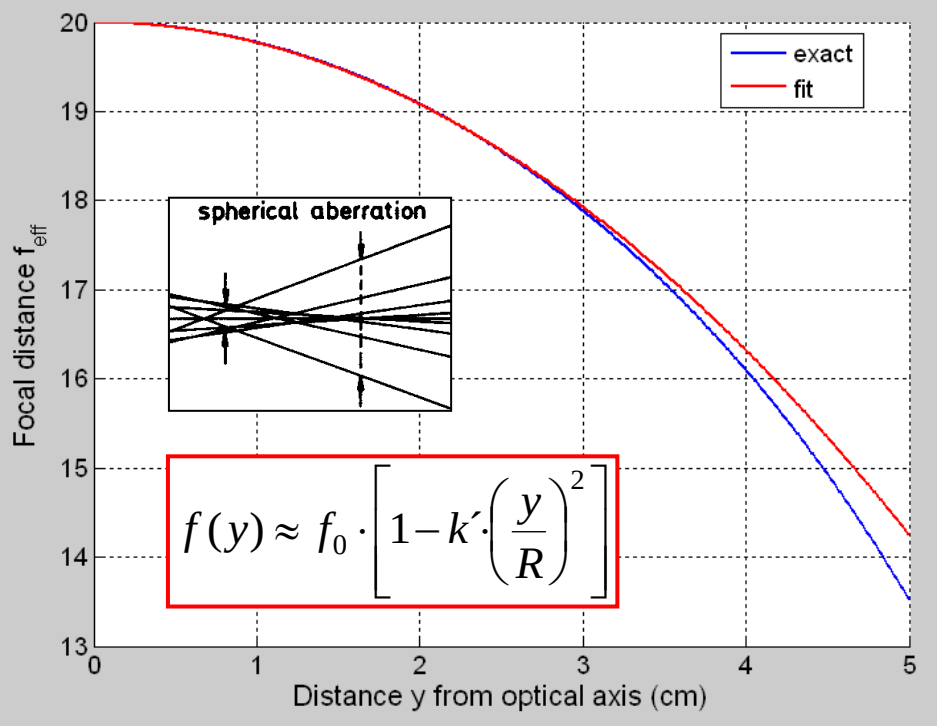


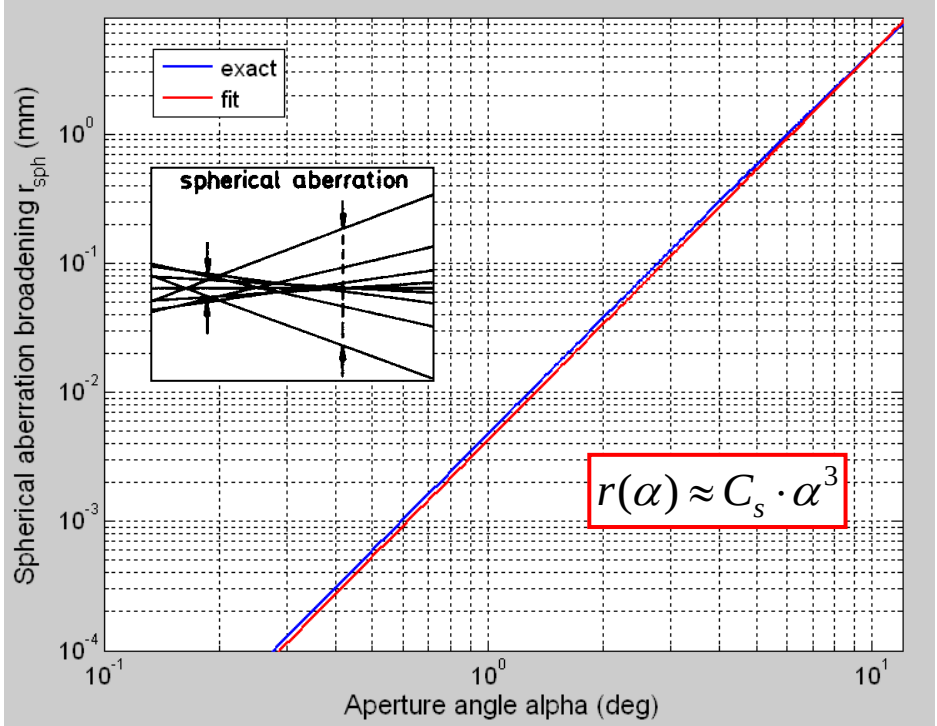
(5) **Change of focal distance**: $\Delta f(y) = f_0 - \tilde{f} - \Delta \approx f_0 \cdot k' \cdot \left(\frac{y}{R}\right)^2 = f_0(n-1)^2 \cdot k' \cdot \left(\frac{y}{f_0}\right)^2 \approx f_0(n-1)^2 \cdot k' \cdot \alpha^2$

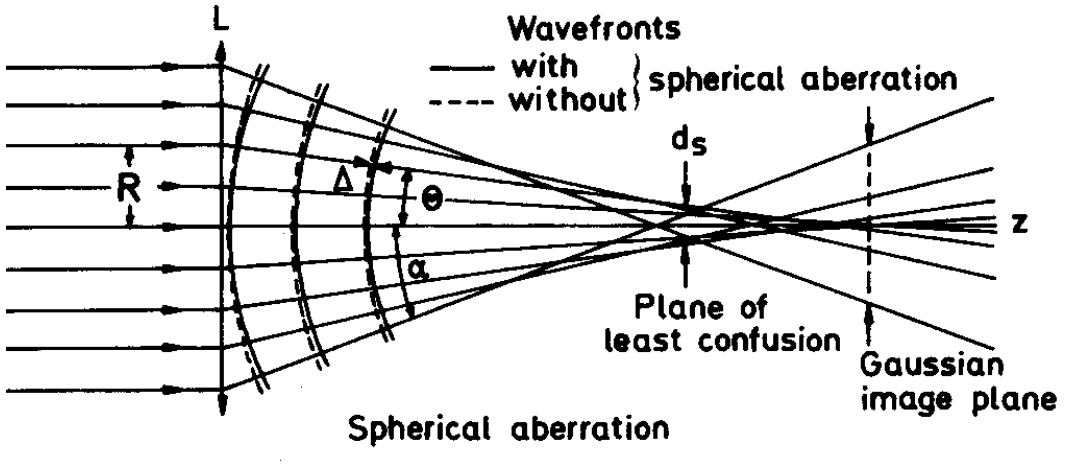
(6) **Broadening**: $r(\alpha) = \Delta f(\alpha) \cdot \tan\alpha \approx f_0(n-1)^2 \cdot k' \cdot \alpha^3$ $\boxed{r(\alpha) \approx C_s \cdot \alpha^3}$

Focal Distance and Broadening as a Function of Distance and Aperture Angle

Example: Curvature R = 10 cm, refractive index n = 1.5 , corresponding focal length f_0 = 20 cm

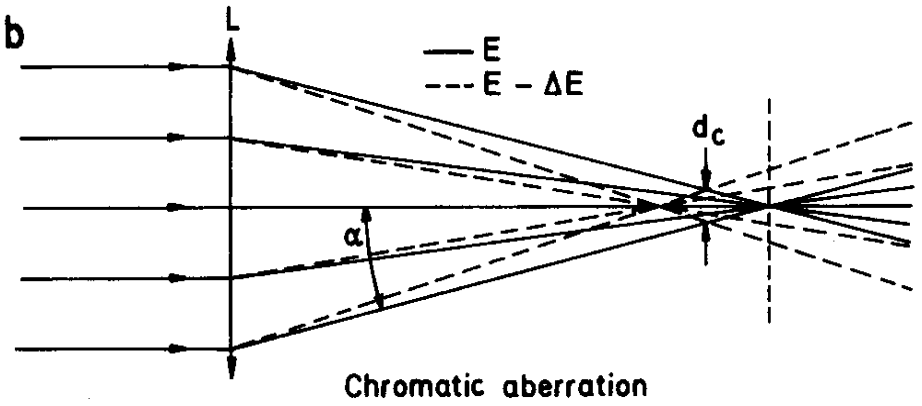






6.10.2 Chromatic Aberration

Light with different wavelengths or energies are refracted differently by the lens due to the **dispersion n(λ) of the refractive index** of the lens material.



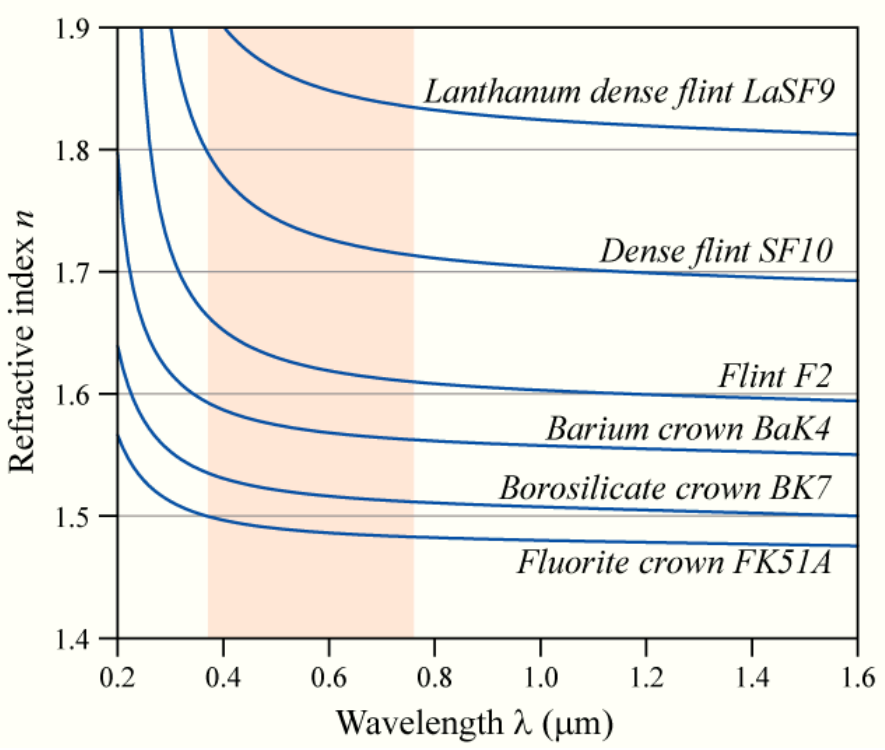
- Broadening of the spot size (spot radius r_{chr}) according to

$\Delta f \sim \Delta\lambda$:

$$r_{chr} = C_c \frac{\Delta\lambda}{\lambda} \alpha^m = C_c \frac{\Delta E}{E_0} \alpha^m$$

C_c … chromatic aberration constant: ~ dn /dλ or ~ dE /dλ or ~ df /dλ, $m \sim 1$

Example: Dispersion $n(\lambda)$ of the refractive index for typical lens



Chromatic aberration

⇨ **strongly depends on the material properties**

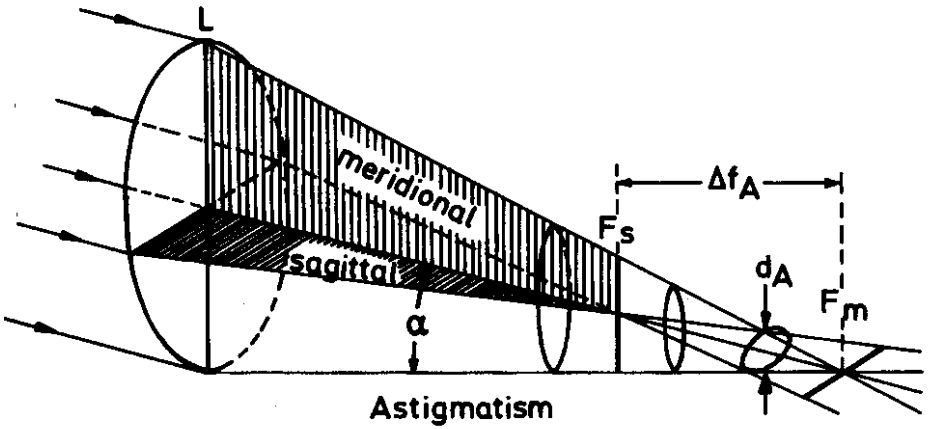
⇨ **increases with increasing wave-length spread of the used light**

6.10.3 Astigmatism

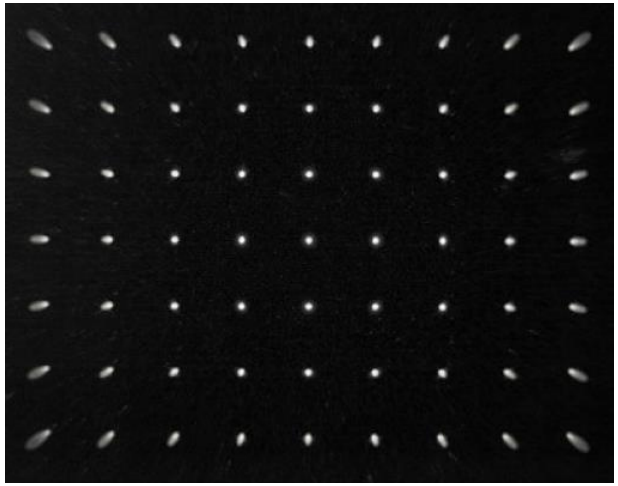
= Non-uniform action of the lens in two orthogonal directions.

Main origin:

Asymmetries of the lenses, misalignments of the optical system, electric stray fields due to charging in electron microscopy.



- Broadening of the spot size/radius r_{ast} according to $r_{ast} = \alpha \Delta f$



In all, the

⇨ **total effective resolution** is given by:

$$r_{eff} = (r^2_{dif} + r^2_{sph} + r^2_{chr} + r^2_{ast} + \ldots)^{1/2}$$

6.11 How to Maximize the Microscope Resolution ?

For *any* microscope, the **total effective resolution r_{tot}** is given by the ***sum*** *of diffraction and aberration broadening* according to:

total effective resolution:

$$r_{tot} = \sqrt{r_{dif}^2 + r_{sph}^2 + r_{chr}^2 + r_{ast}^2} = 0.61 \cdot \lambda / NA_{eff}$$

which is characterized by the *effective* numerical aperture NA_{eff}

For highest resolution ***all*** **terms must be minimized**, however:
Diffraction broadening and aberration broadening *counteract* each other !

$$r_{tot} = \sqrt{\left(\frac{0.61\lambda}{n \sin\alpha}\right)^2 + \left(C_{sph}\alpha^3\right)^2 + \left(C_{chr}\frac{\Delta\lambda}{\lambda}\alpha\right)^2 ....}$$

- *Diffraction broadening is proportional to ~1 / sin α*
- *Aberration broadening is proportional to ~ α^n*

⇨ With increasing aperture α, diffraction broadening decreases but lens aberration increase.

Consequences:

- ❖ The resolution cannot be simply increased by increasing the lens diameter: Instead a ***compromise in α*** must be made that minimizes the ***sum*** of all broadening factors.
- ❖ This means that there exists a certain ***optimum aperture angle*** α_{opt} where the *highest resolution* is achieved. This is particularly important for electron microscopy, where the lens aberrations are very large and dominate the resolution.

6.11.1 Reducing Diffraction Broadening

Numerical apertures $NA = n \cdot \sin\alpha$: Means that α as well as *n* should be maximized

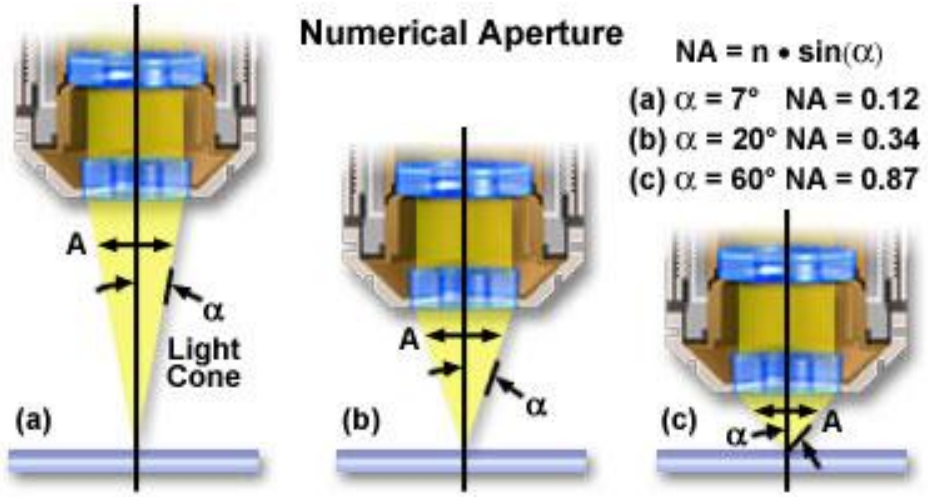
(a) Increasing the aperture angle

$\alpha = \tan(D/2f)$

requires lenses with large diameter *D* and/or smaller focal length *f*.

⇨ For microscopes with fixed tube length and with fixed objective diameter *D*, α can be increased only by **decreasing the focal length *f*** of the objective,

⇨ Reducing *f* means also reducing the working distance *w* ~ *f*



$$r_{diff} = 0.61 \cdot \lambda / n \sin\alpha$$

Consequence: Because at the same time the magnification *M* = *t*/*f*, increasing *f* simultaneously increases the magnification:

⇨ High NA (and high resolution) is obtained only at high magnification !

⇨ A high NA also gives a high brightness of the image, which is proportional to √(NA)

Resolution & Numerical Aperture for λ = 550nm (green)

Magnification	N.A.	Resolution (µm)
4x	0.10	1.375
10x	0.25	0.61
20x	0.40	0.37
40x	0.65	0.29
60x	0.75	0.29
N.A. = Numerical Aperture		

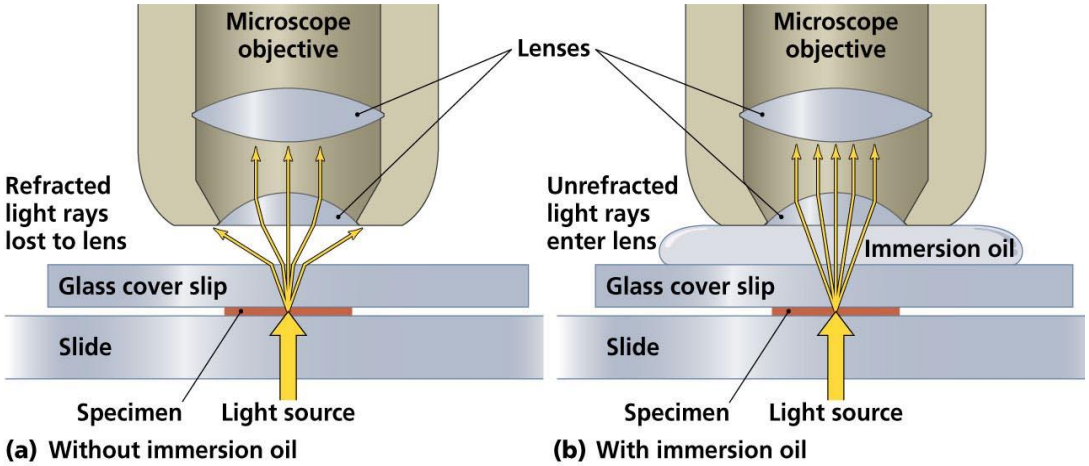
(b) Increasing the refractive index n: Immersion Microscopy

Introducing a ***liquid medium*** with refractive index $n > 1$ between the sample and objective reduces the diffraction angles and thus, increases α_{eff}.

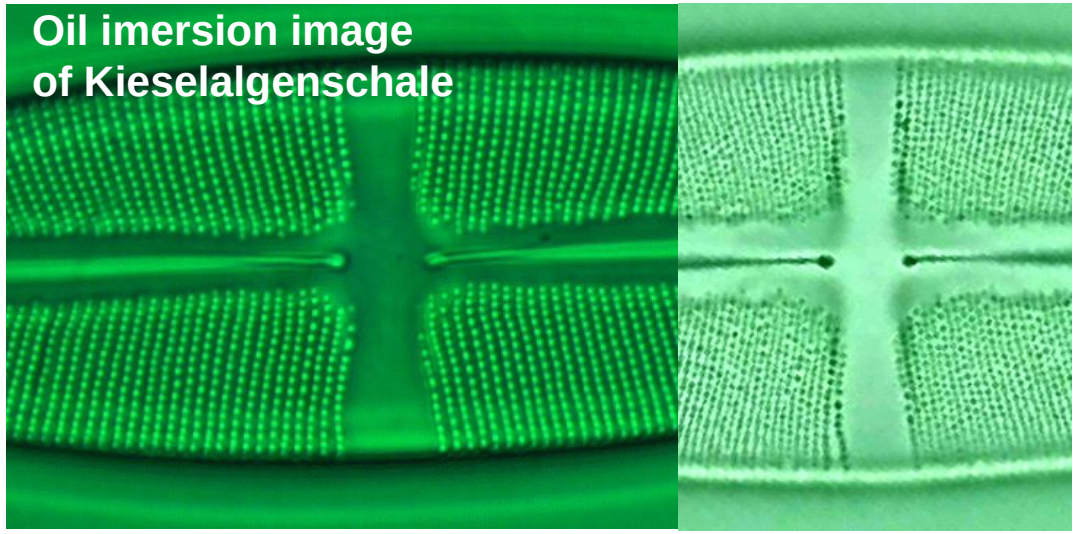
This is called ***immersion microscopy***.

Numerical apertures NA > 1 can be obtained, which reduces diffraction broadening and improves resolution

$$r_{diff} = 0.61 \cdot \lambda / n \sin\alpha$$



air: $n = 1$ **, water:** $n = 1.33$ **, glycerine:** $n = 1.47$
synthetic oils: $n = 1.51$, **Bromonaphthalene:** $n = 1.659$, **Methylene iodide:** $n = 1.74$,



6.11.2 Correction of Lens Aberrations

$$r_{eff} = \sqrt{r_{diff}^2 + r_{aber}^2} = 0.61 \cdot \lambda / NA_{eff}$$

⇨ To achieve a diffraction limited resolution, all lens errors must be corrected !

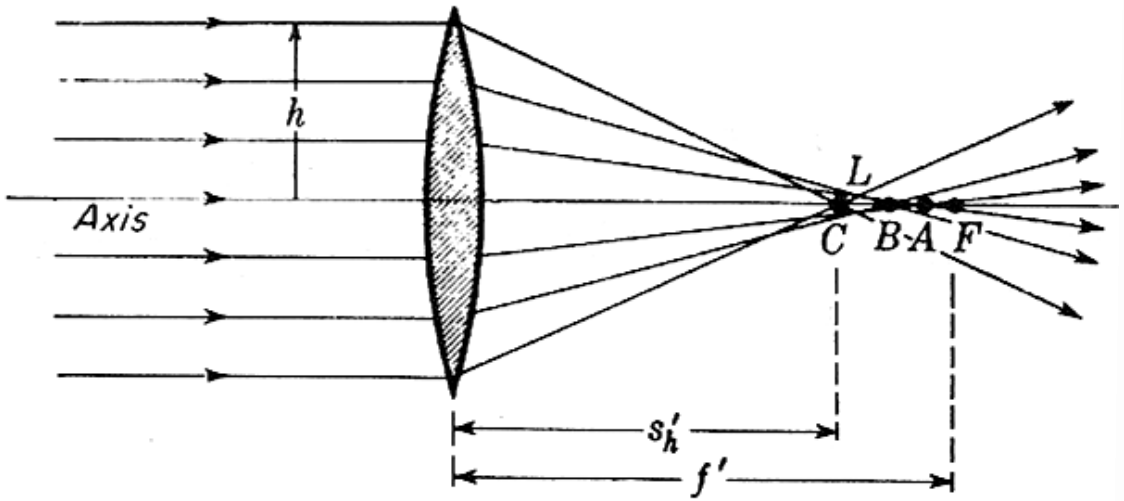
Non-perfect lens system: Performance defined by *effective numerical aperture* $NA_{eff} < n \sin \alpha$.
This value is *smaller* than $n \sin\alpha$ and accounts for additional broadenings caused by lens aberrations.

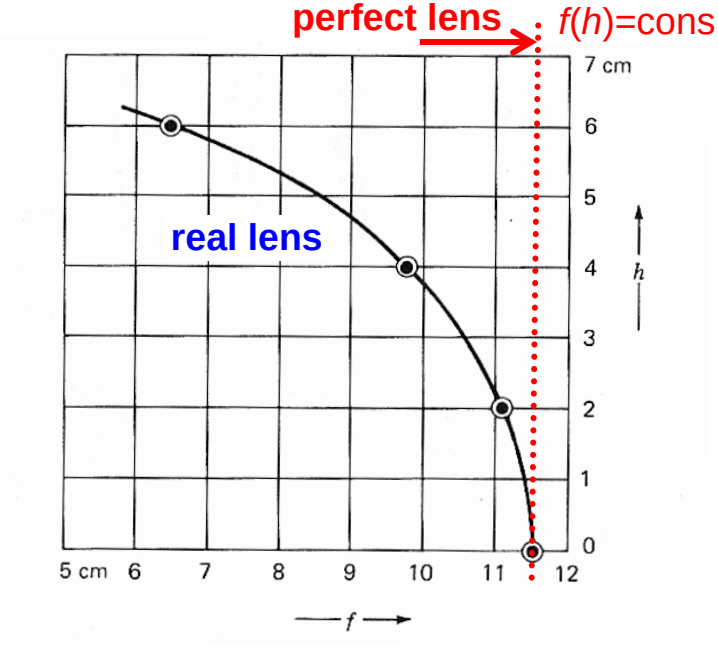
⇨ Objectives with better corrections yield higher *effective* NA_{eff} values close to $NA_{ideal} = n \sin \alpha$

⇨ Combination of several lenses in one objective allows to compensate of most lens aberrations.

(i) Spherical aberration correction: **Cause:** Spherical curvature of lenses results in stronger bending of outer rays towards the optical axis. Strong effect for "thick" lenses

Broadening: $r_{sph} \sim C_s \cdot \alpha^n$





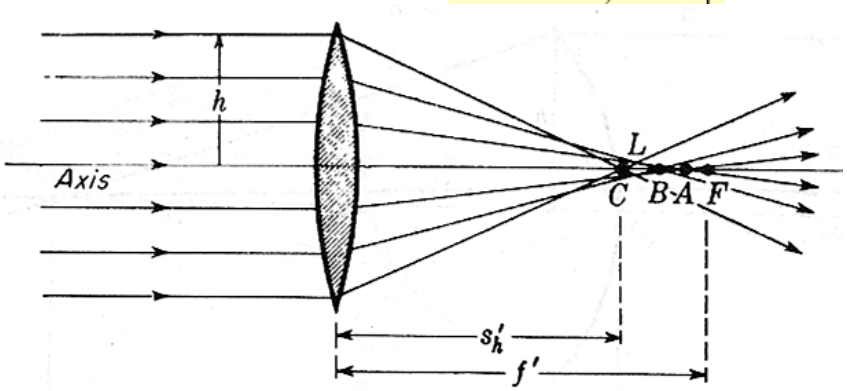
see: Jenkins & White: Fundamental of Optics

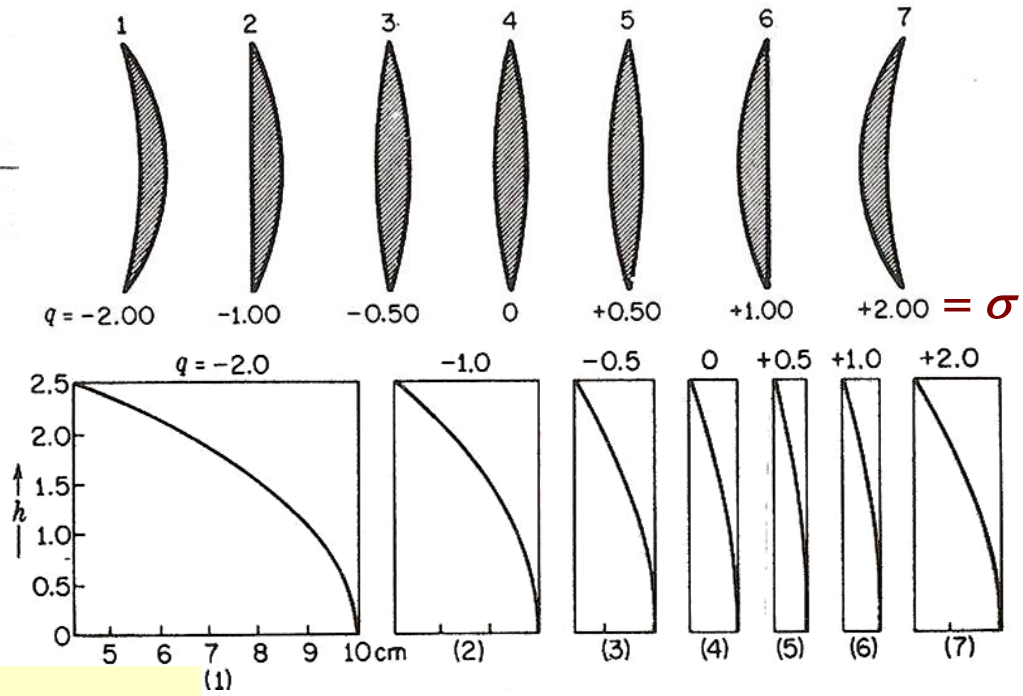
Focal point *f* versus distance *h* of ray from the optical axis

Solution #1: Use of several lenses with *smaller curvature* and smaller aberration

Solution #2: Use of *asymmetric lenses* with two different curvatures on each side to minimize spherical aberration

Lens shape factor: $\sigma = \dfrac{R_2 + R_1}{R_2 - R_1}$ determines the change of $f(h)$ ~ spherical aberration





Spherical aberration is *minimized* when:

$$\sigma = -\frac{2(n_{lens}^2 - 1)}{n_{lens} + 2} \cdot \left(\frac{s_{obj} - s_{im}}{s_{obj} + s_{im}} \right) = -\frac{2(n_{lens}^2 - 1)}{n_{lens} + 2} \cdot p$$

where s_{obj} and s'_{im} are the object and image distances, respectively.

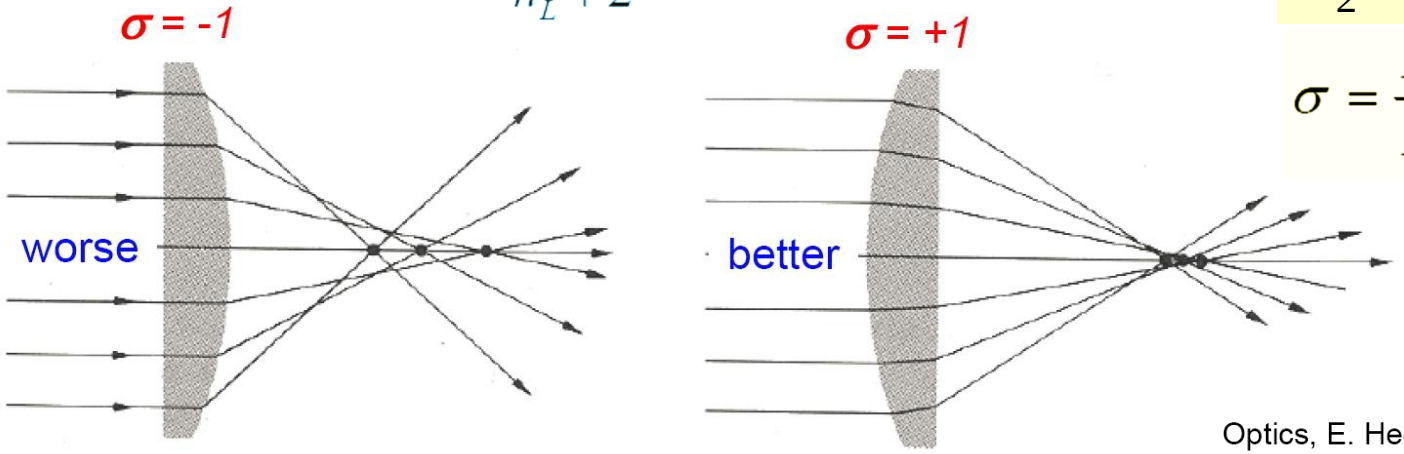
$$p = \left(\frac{s_{object} - s_{image}}{s_{object} + s_{image}} \right)$$

For an object at infinite ($s_{obj} = \infty$, $p = -1$, $n_L = 1.50$),

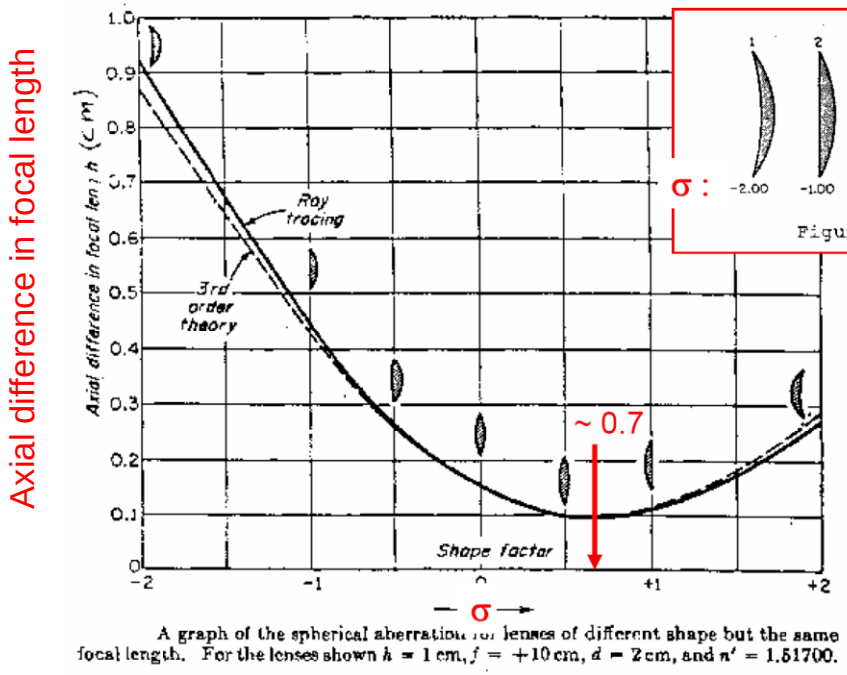
Optimum lens shape: $\sigma = -\frac{2\left(n_L^2 - 1\right)}{n_L + 2} p$ yields **$\sigma_{opt} \sim 0.7$**

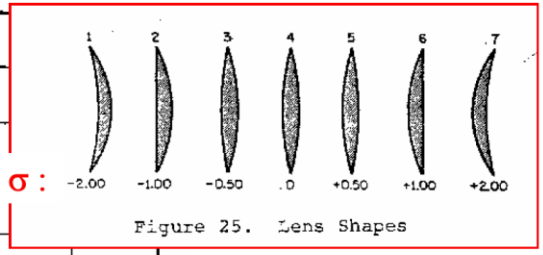
$$\frac{R_1}{R_2} = \frac{\sigma - 1}{\sigma + 1}$$

$$\sigma = \frac{R_2 + R_1}{R_2 - R_1}$$



Optics, E. Hecht, p. 222.

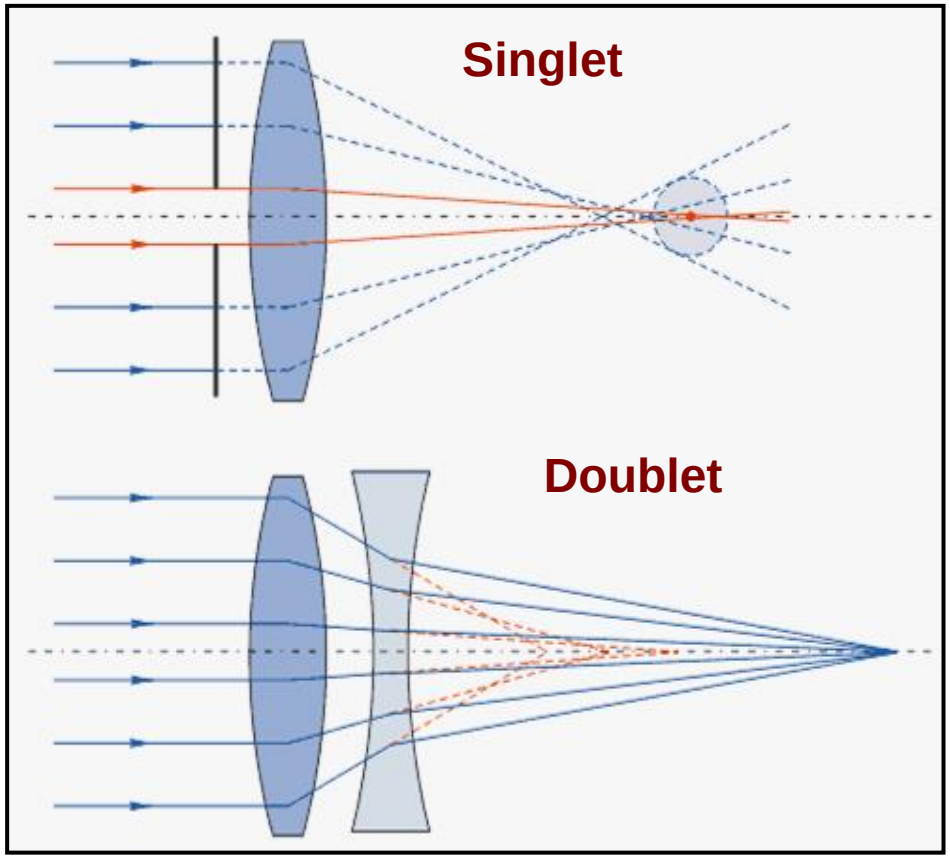


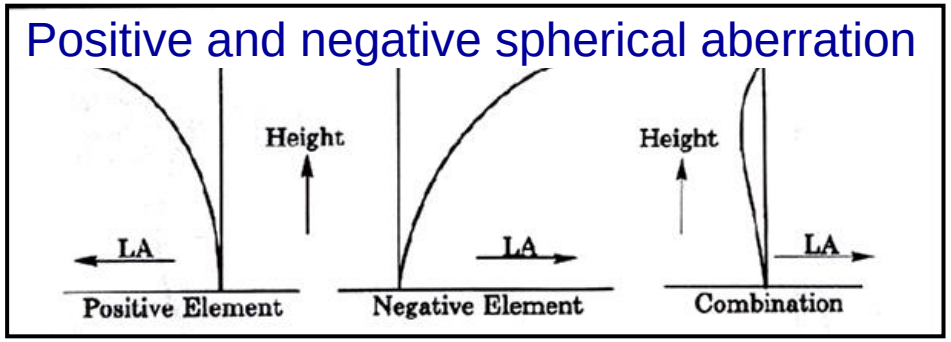


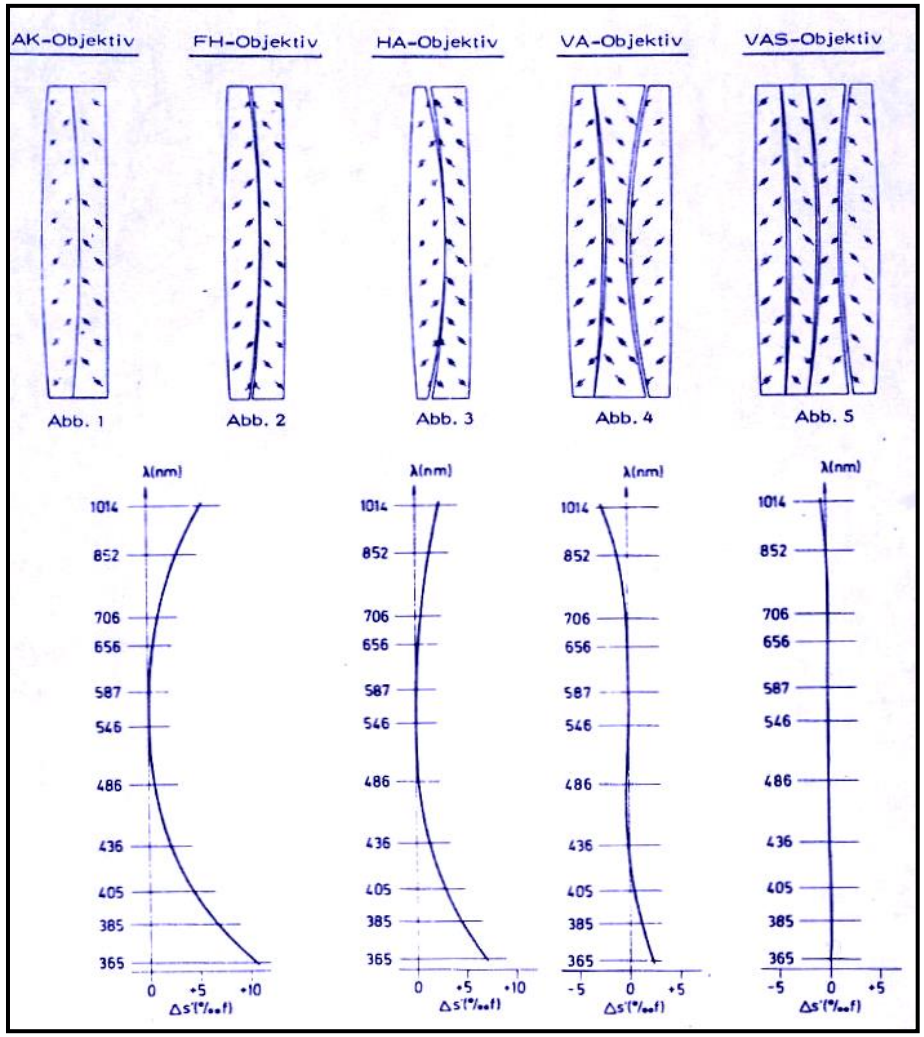
$$\sigma = \frac{R_2 + R_1}{R_2 - R_1}$$

$$p = \frac{s' - s}{s' + s}$$

Solution #3: Combination of positive (convex) and negative (concave) lenses with ***positive*** and **negative** spherical aberration constants (**dublets** and triplets)

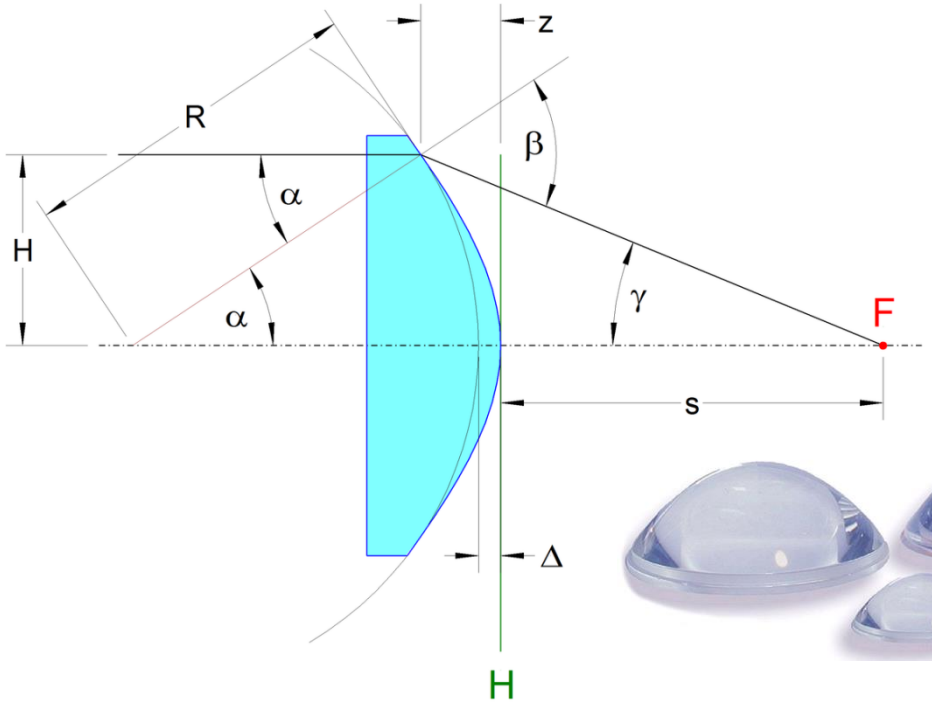


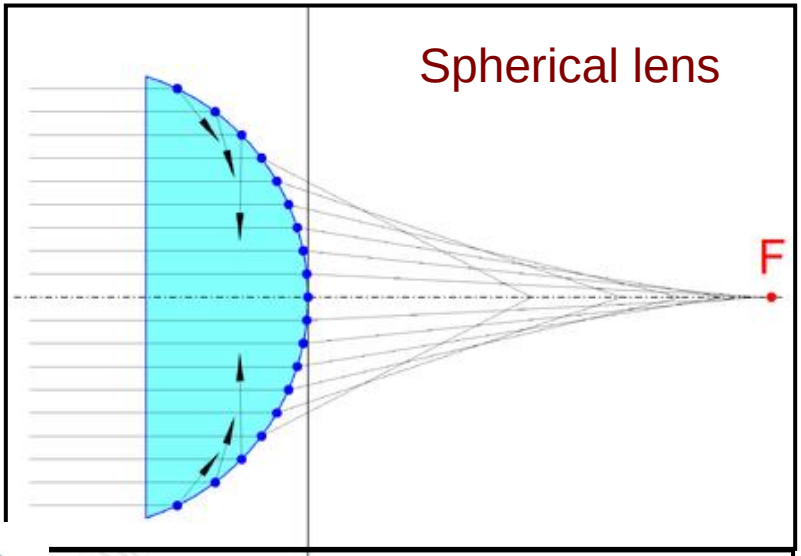


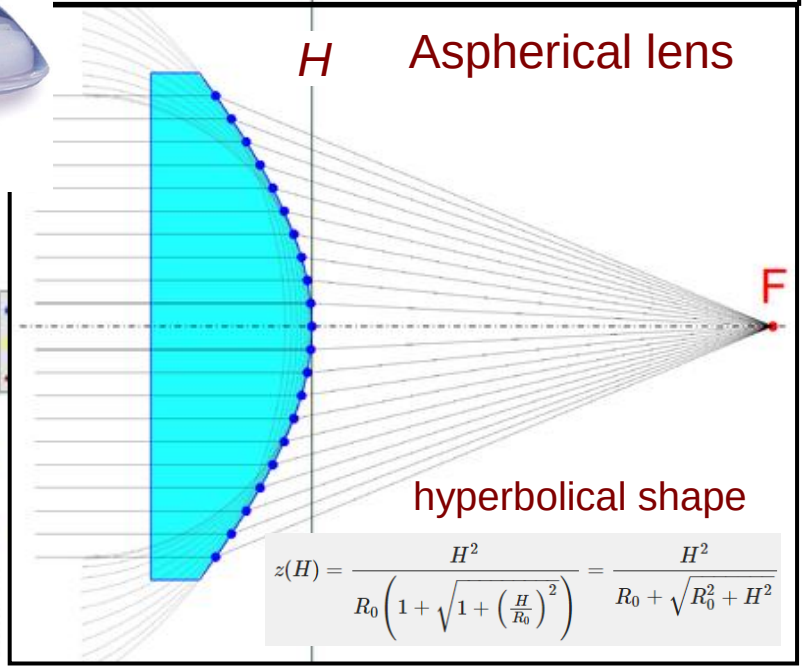


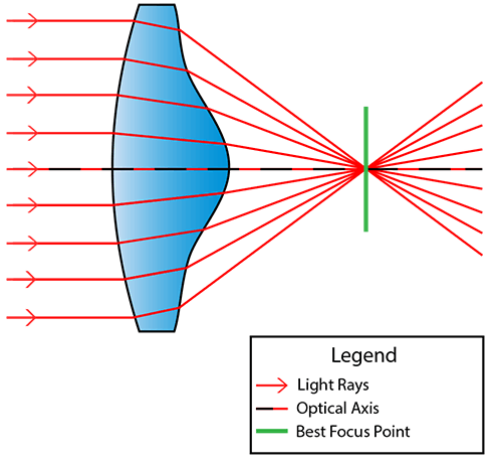
Solution #4: Use of more expensive *nonspherical lenses*

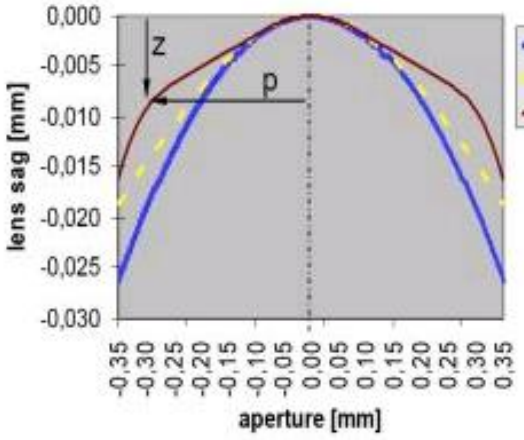
Aspheric Lenses









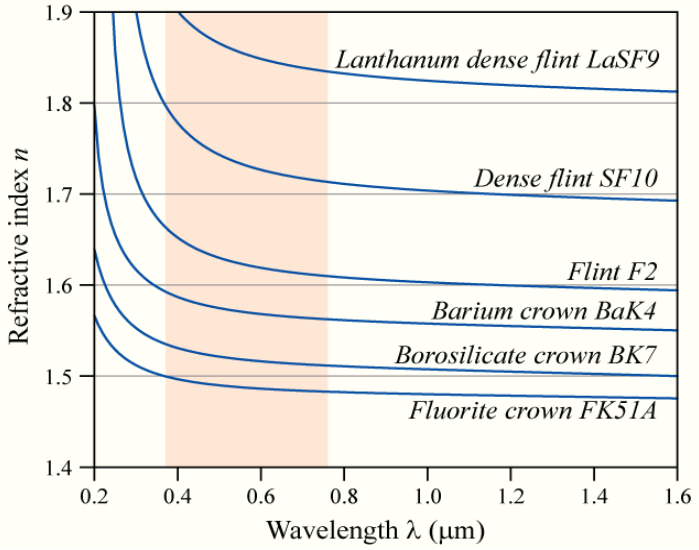


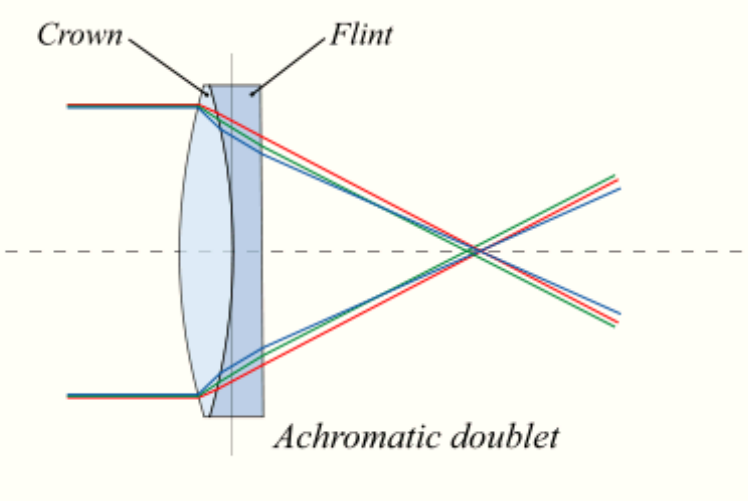
(ii) Correction of chromatic aberration

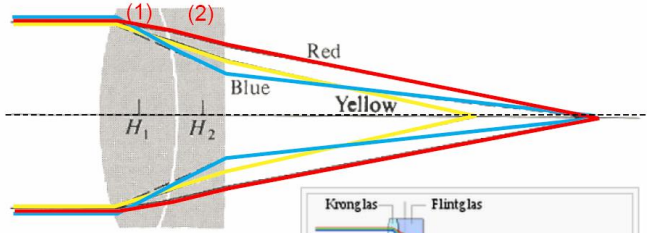
Cause:
Wavelength dependence, i.e., dispersion of the refractive index $n(\lambda)$

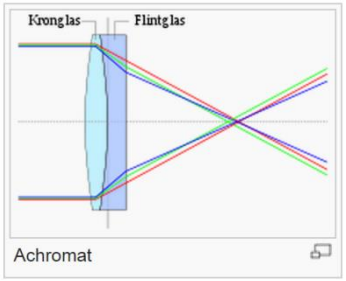
Solution:

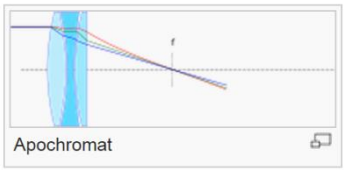
Lens ***doublets*** or ***triplets*** of convex and concave lenses consisting of glass materials with different dispersion (crown, flint glasses and fluorspar).











(iii) Correction of astigmatism

Cause: Asymmetric lenses or misaligned/unparallel optical elements

Solution: Improved lens symmetry, precise alignment.

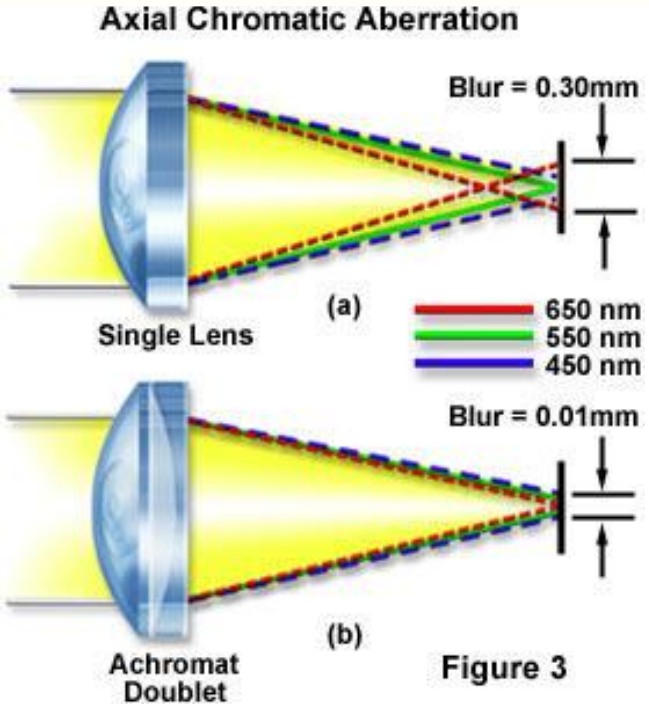


Figure 3

(iv) Curvature of field-of-view

For spherical lenses, the image is not focused on a plane but rather on a curved surface.

Cause: Curvature of lens surfaces.

Solution: Correction by additional lens elements.

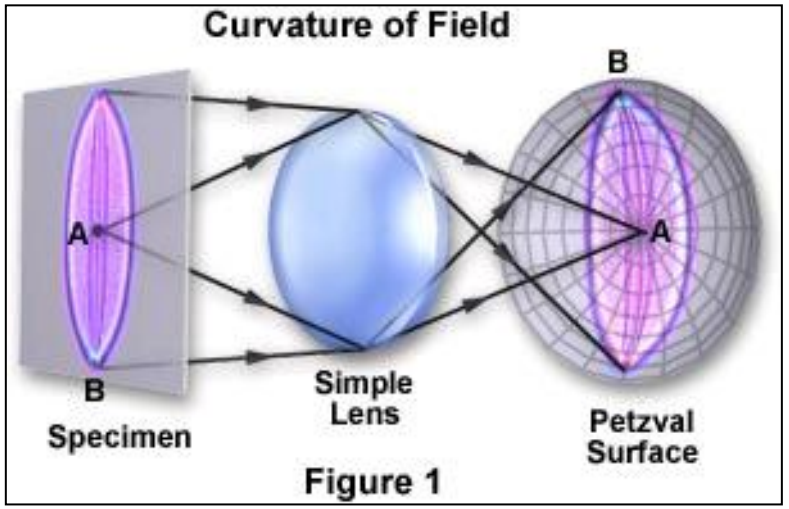


Figure 1

(v) Coma = smearing of outer part of image

Cause: Inclined beams have different focal position for outer beams through the lens.

Solution: Correction by additional lens elements.

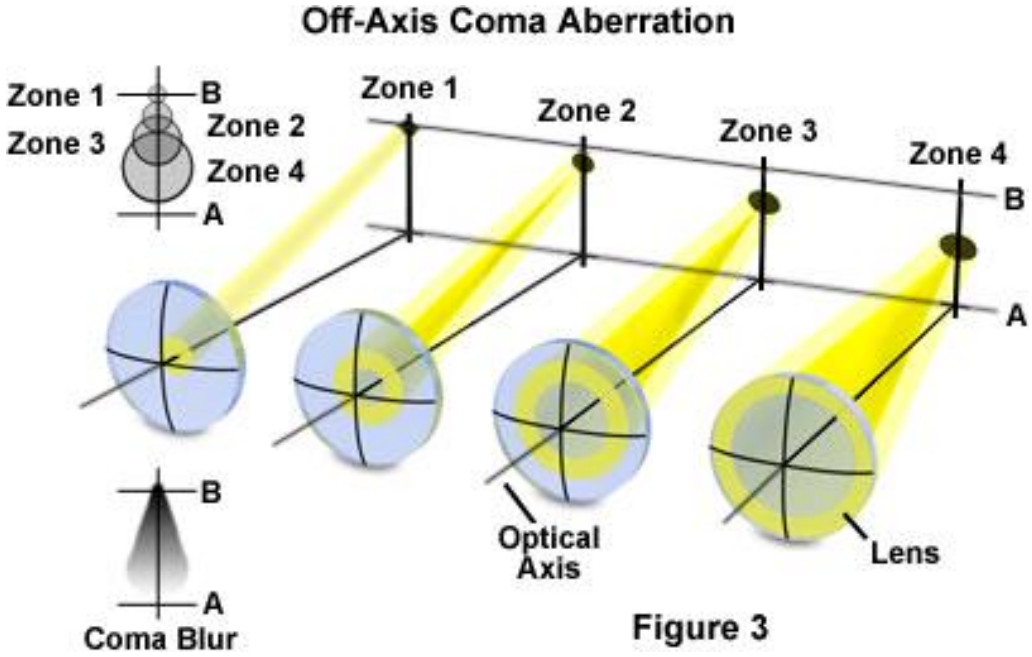
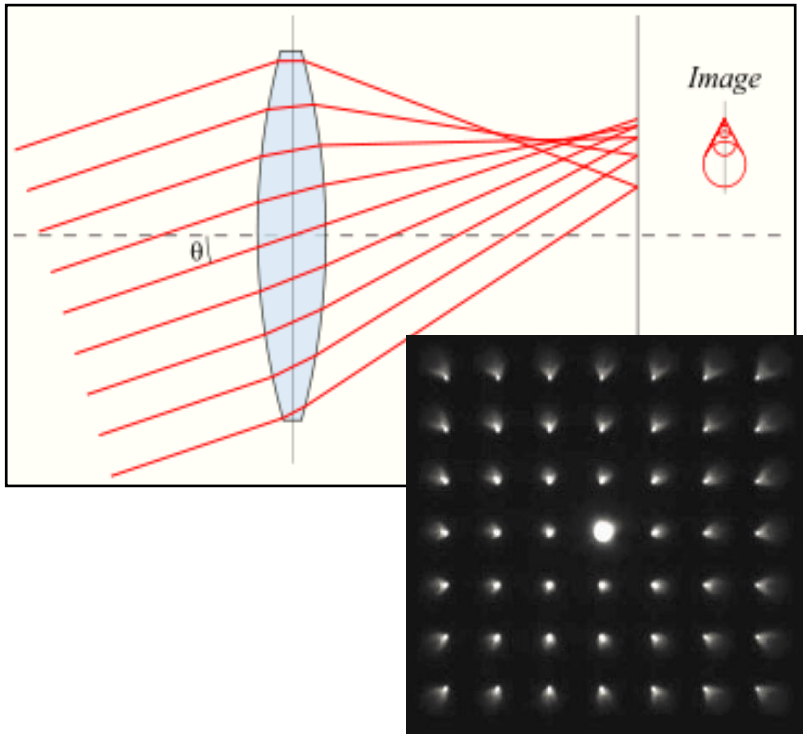


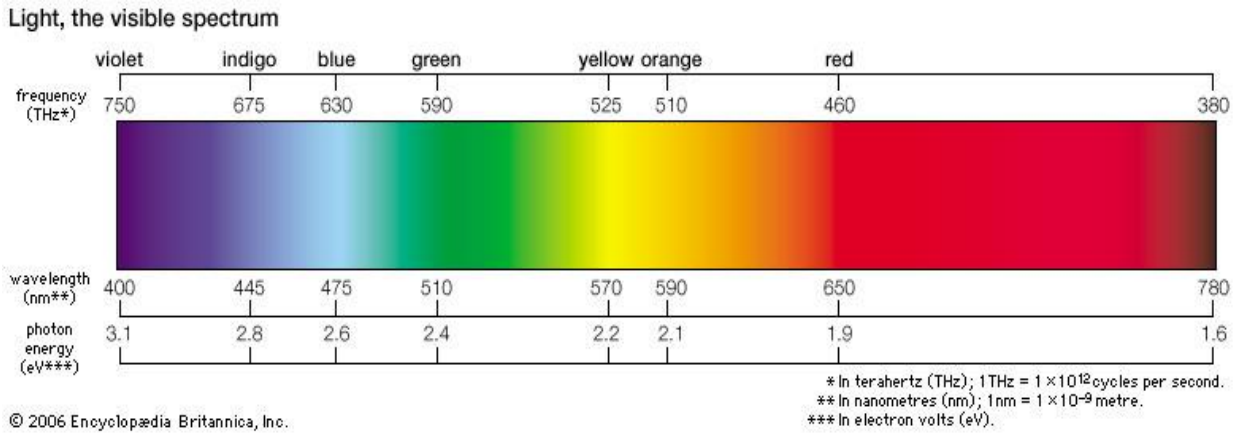
Figure 3



6.12 Application to Light Microscopy

In optical microscopy, visible (or near-infrared) light is used for imaging of the sample.

Wavelength: $\lambda[nm] = c/\upsilon = 1240/E[eV]$ **Energy:** $E[eV] = h \cdot \upsilon = 1240/\lambda[nm]$



Color	Energy	Wavelength
violet	3.2 – 2.8	380–450 nm
blue	2.8 – 2.5	450–495 nm
green	2.5 – 2.2	495–570 nm
yellow	2.2 – 2.1	570–590 nm
orange	2.1 – 1.9	590–620 nm
red	1.9 – 1.6	620–750 nm

Resolution limits: Since for VIS & NIR optical systems, effective aberration corrections exist that nearly fully eliminate aberration broadening, ***diffraction*** is the main remaining ***limitation*** for the spatial ***resolution*** of VIS & NIR microscopy.

⇨ The best resolution is about ½ λ of the illumination wavelength λ using aberration corrected objectives with high NA ~ 1

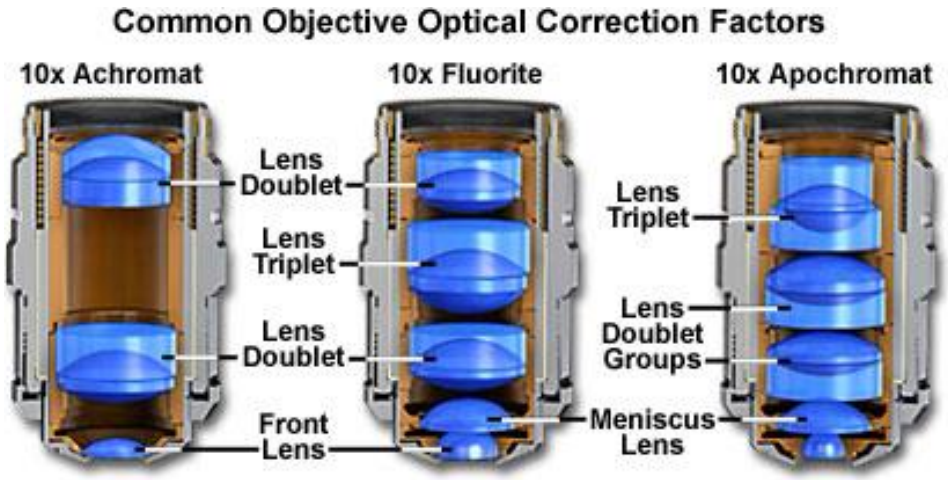
$$r_{res} = r_{diff} = 0.61\ \lambda\ /\ NA$$

⇨ Note: Recently, sub λ/2 resolution scanning fluorescence microscopy has been developed.

Wavelength (nm)	Resolution (nm)
360 – 450 nm (violet)	190 – 250 nm
450 – 500 nm (blue)	250 – 300 nm
500 – 570 nm (green)	300 – 350 nm
620 – 750 nm (red)	380 – 460 nm

Types and Specifications of Objective Lenses

Achromats: Objectives corrected for axial chromatic aberration at two wavelengths (486 nm blue and 656 nm red) and corrected for spherical aberration in the color green (546 nm; see table).



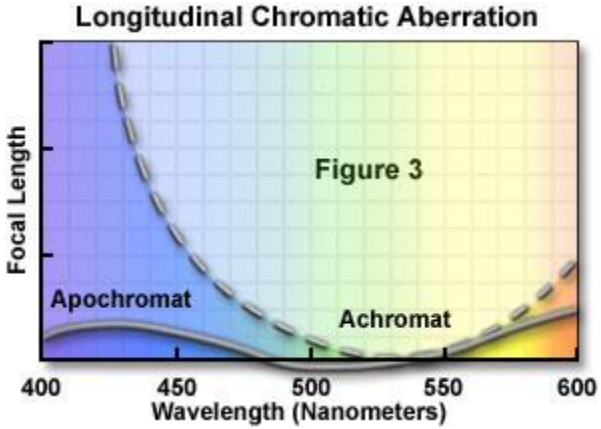
Fluorites or semic-apochromats: Additional lenses for spherical corrections at two wavelengths.

Apochromats: Best corrected objectives, correction of spherical aberrations for wide a wavelength region.

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

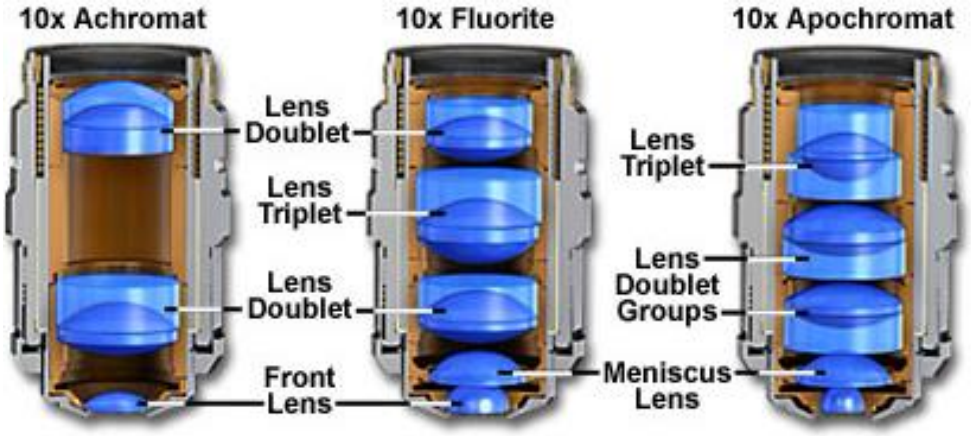
see: www.micro.magnet.fsu.edu



Plan: Lenses with field of view curvature correction are termed with additional "plan" prefix.

Specifications of Objective Lenses

- Type of Objective (Degree of correction)
- Magnification (number)
- Numerical Aperture
- Tube length (mm)
- Cover Glass correction (thickness)



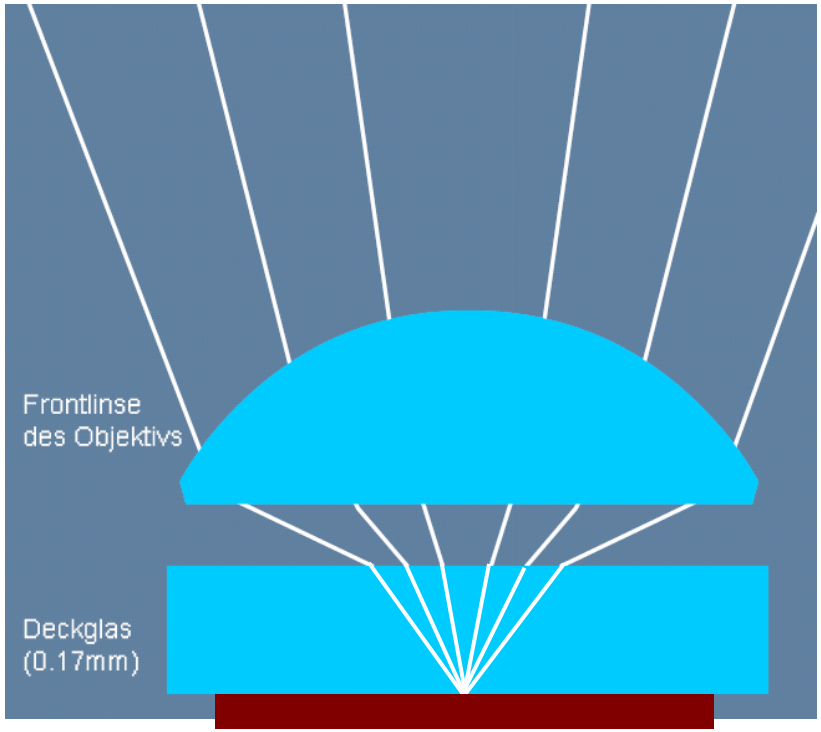
Resolution and Numerical Aperture for λ = 550nm (green)

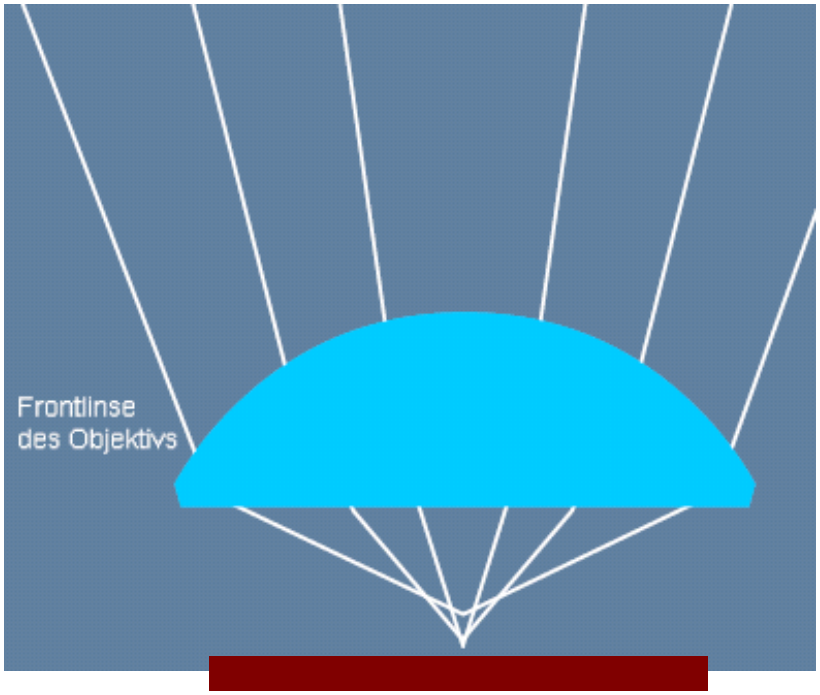
Objective Type						
	Plan Achromat		Plan Fluorite		Plan Apochromat	
Magnification	N.A.	Resolution (µm)	N.A.	Resolution (µm)	N.A.	Resolution (µm)
4x	0.10	2.75	0.13	2.12	0.20	1.375
10x	0.25	1.10	0.30	0.92	0.45	0.61
20x	0.40	0.69	0.50	0.55	0.75	0.37
40x	0.65	0.42	0.75	0.37	0.95	0.29
60x	0.75	0.37	0.85	0.32	0.95	0.29
N.A. = Numerical Aperture						

Violet: λ = 420nm (25% improvement) www.micro.magnet.fsu.edu

» Objectives with better corrections yield significantly higher resolution !

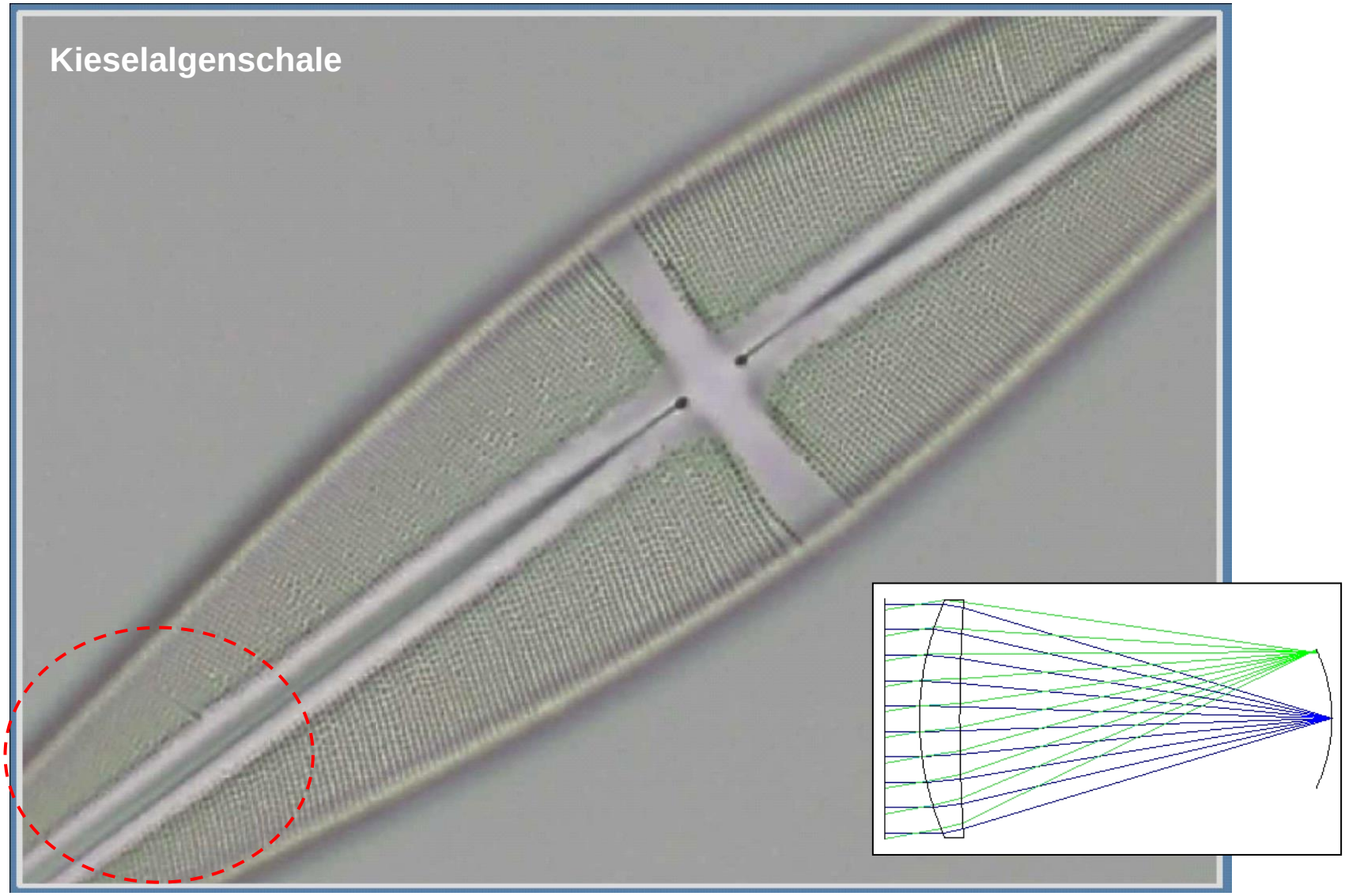
Cover Glass Correction (common for biological specimen)





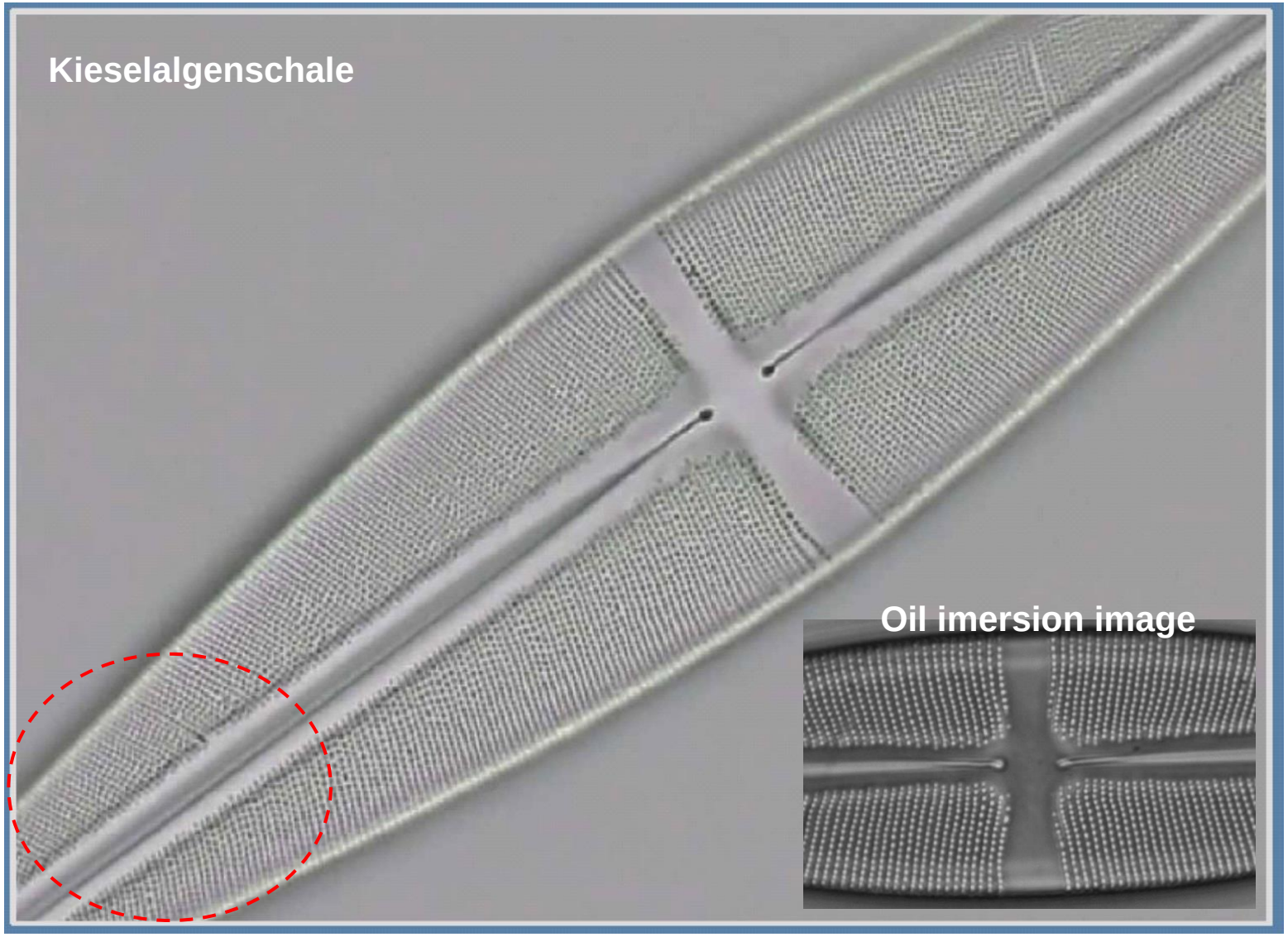
» **Distorted image without cover**

Comparison of Image Quality: Achrochromat



» **Sharp only in the center of the image due to coma and field-of-view curvature**

Planapochromat



» **Sharp over the whole image with best resolution**

6.13 Summary

- Two main approaches for microscopy exist: **Scanning and imaging microscopy**. These either consist of local excitation and non-local detector (scanning microscopy), or non-local excitation and local detection (imaging microscopy).
- In scanning microscopy, the **resolution** is limited by the spot size and interaction volume.
- In imaging microscopy, the **resolution is limited by two factors**:

  (a) Diffraction at the objective aperture, which yields: $r_{diff} = 0.61\,\lambda / n \sin\alpha$

  (b) Lens aberrations, introducing broadening $r_{sph} = C_s \cdot \alpha^n$ $n \sim 3$, $C_s \sim f$

  due to spherical & chromatic aberrations $r_{chr} = C_c \cdot dn/n \cdot \alpha^n$ $n \sim 1$, $C_c \sim f$

  Other aberration effects include coma and field of view curvature.

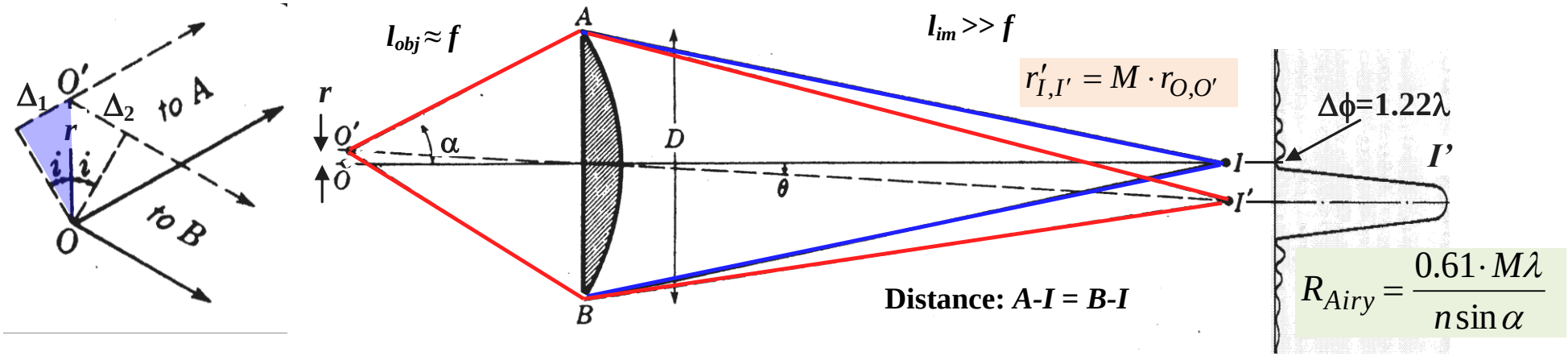
  The **total effective resolution** is thus given by: $r_{eff} = (r^2_{dif} + r^2_{aber})^{1/2}$

- Due to efficient aberrations corrections, the resolution in optical microscopy is mainly diffraction limited. The highest resolution is achieved for ***high numerical apertures*** $NA = n \sin\alpha$ and ***short wavelength*** λ imaging systems. Further increase in resolution can be achieved by immersion techniques using liquids to increase the refractive index.
- The **depth-of-focus** decreases with increasing numerical aperture: $DOF \approx n \cdot \lambda / NA^2$ By recording *z-stacks* (focus through series), 3D images can be obtained.

Alternative Derivation of the Microscope Resolution (Abbe Diffraction Limit)

Since in a microscope, the sample objects do not emit parallel beams but strongly divergent beams (see figure below), the resolution limit has to be derived differently compared to a telescope.

Derivation according to figure below: Two points O and O' in the object are separated by distance *r* and appear at the points at I and I' in the image plane. Each image point is broadened by the diffraction at the microscope aperture *D*, i.e., by the point spread Airy function.



***Rayleigh resolution condition*:** The points *O´* and *O* can be just resolved when the first side minimum of the Airy function of *O´* in the image coincides with the intensity maximum of *O*, which is assumed to be located on the optical axis, meaning that also *I* is exactly on the optical axis.

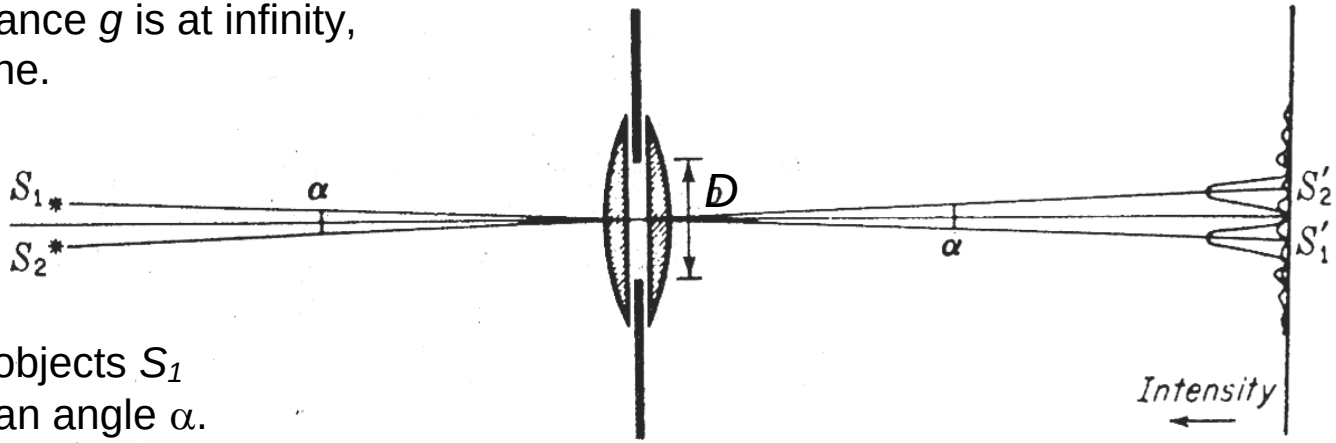
- ⇨ The 1st minimum of the Airy function of *O´* is given when the optical path difference Δw between the outmost rays is equal to: $\Delta\phi_1 = 1.22\, n\, \lambda$
- ⇨ On the other hand the path difference Δw between outer rays from O' to I is given as: $\Delta\phi_1 = \underline{O'A\,I} - \underline{O'B\,I} = \Delta_1 - \Delta_2 = 2 \cdot r \cdot \sin\alpha$

Combining yields the (Raleigh Criterion)

Diffraction Limited Resolution $r_{min} = 0.61\, \lambda / n \sin\alpha$

Angular resolution of a *telescope* for far distant objects

Telescope: Object distance *g* is at infinity, the image at the focal plane.



Resolution: Two distant objects S_1 and S_2 are viewed under an angle α.

The **circular aperture *b*** of the telescope creates an Airy diffraction pattern for each point in the image

Rayleigh Criterion: Two point objects can just be resolved when the central maximun of the **Airy diffraction pattern** of one poin coincides with the diffraction minimum of the neighboring point.

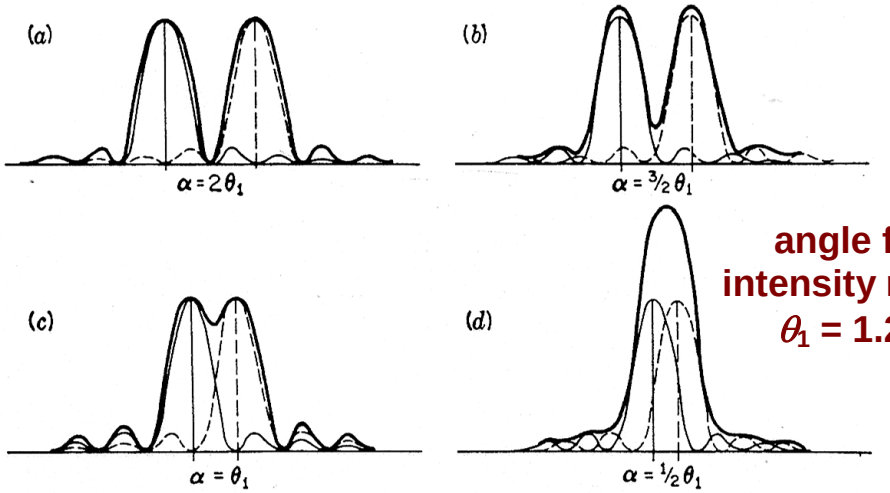
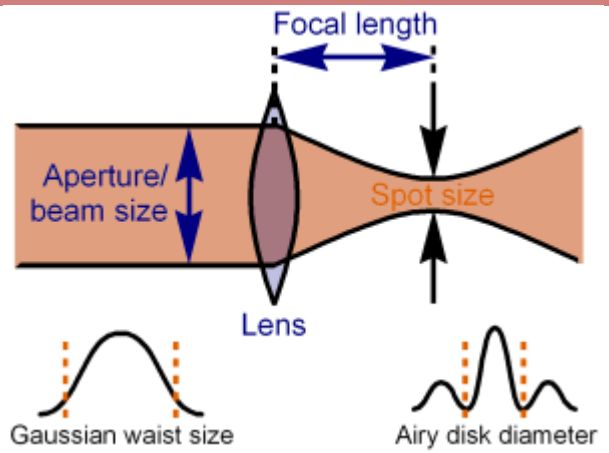


FIGURE 15I
Diffraction images of two slit sources: (*a*) and (*b*) well resolved; (*c*) just resolved; (*d*) not resolved.

angle for first intensity minimum:
$\theta_1 = 1.22\ \lambda / b$

Angle for first intensity minimum: $\theta_1 = 1.22\ \lambda / D$

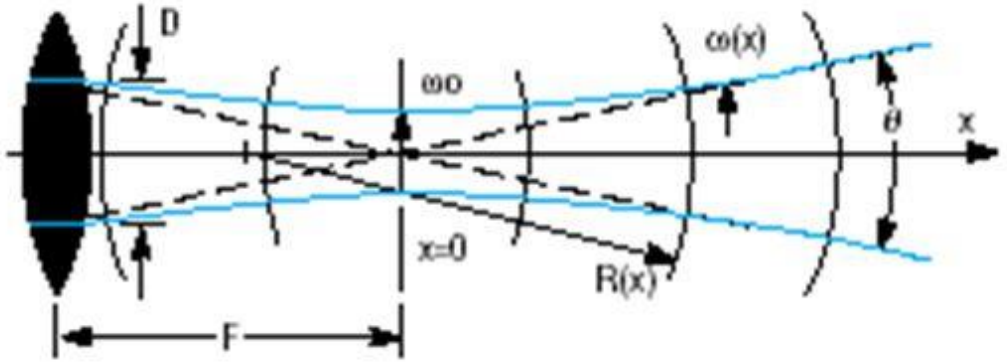
⇨ **Resolving power:** $\alpha_{min} = 1.22\ \lambda / D$ = minimum angle of resolution of a telescope with a circular aperture with diameter *D*


Focal length
Aperture/
beam size
Spot size
Lens
Gaussian waist size
Airy disk diameter

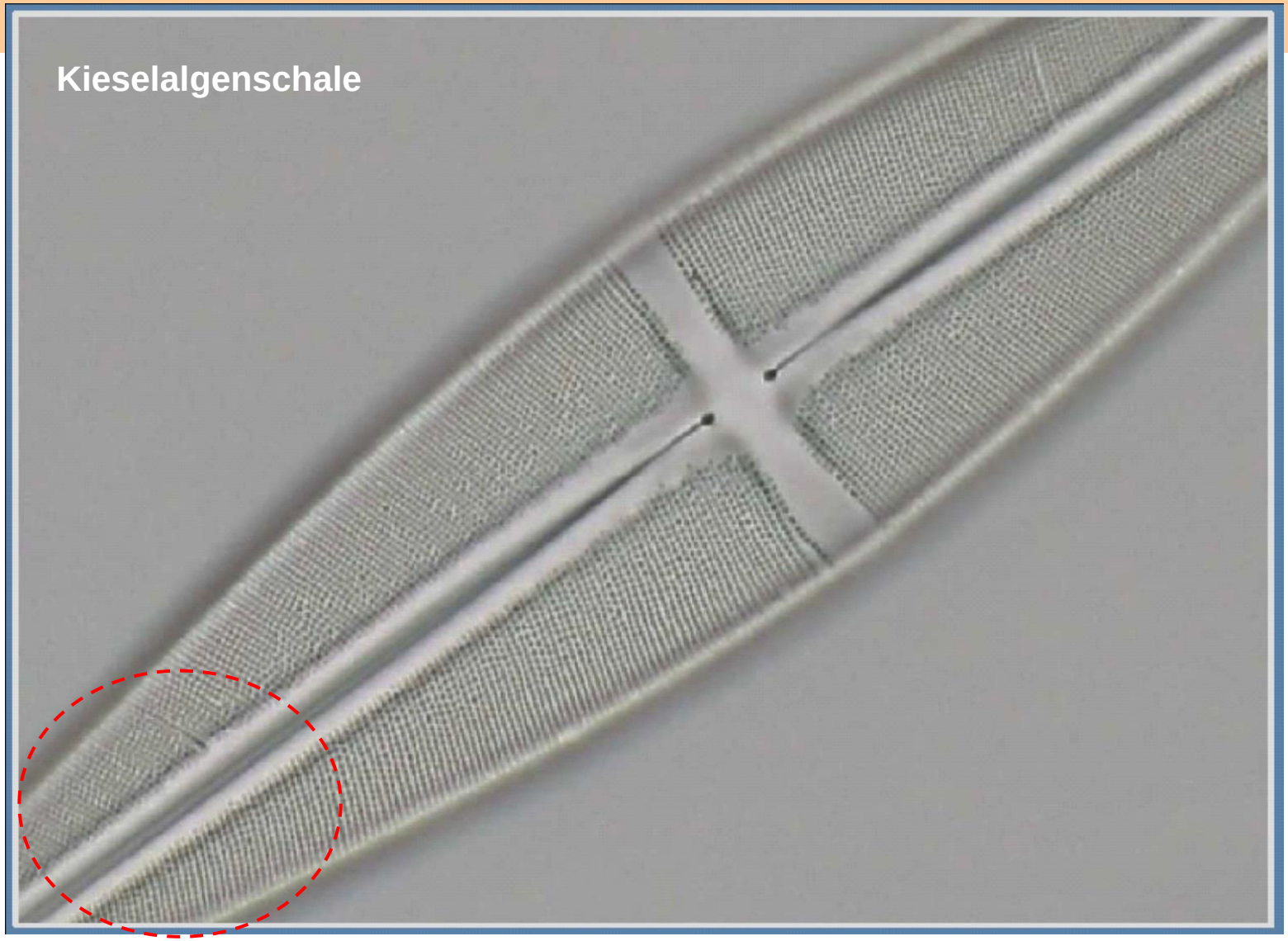
Lens resolution with laser light (Gaussian beams)

- Laser beam diameter is effective lens diameter: $D = 2w$
  - Fourier transform of Gaussian is Gaussian

	Standard lens	**Gaussian**
Aperture size	D	$2w$
Focal spot size	$1.22\, f\lambda / D$	$w_0 = (4/\pi) f\lambda / 2w = 1.27\, f\lambda / 2w$
Depth of focus	$1.22\, \lambda\, (2f / D)^2$	$z = 1.27\, \lambda\, (2f / 2w)^2$



(b) Planchromat



» **Sharp over most of the image**

Microscopic techniques are distinguished according to different criteria

(a) ***How magnification*** is achieved and the images are recorded:

» ***Optical imaging microscopy*** (such as light microscopy, TEM, PEEM, …)
Principle: Wide area (non-local) illumination / local (= spatially resolved) detection

» ***Scanning microscopy*** (such as scanning optical microscopy, SEM, SPM, …)
Principle: Local excitation / wide area (= non-local) detection

» ***Ray magnification microscopy*** (field electron & field ion microscopy, …)
Principle: Self-luminous emitting sample / spatially resolved detection.

(b) ***What kind of probes*** are used for the image recording process (see Chapter 2)
» Light, X-rays, electrons, ions, neutrons, scanning probes, …..

(c) ***What kind of contrast*** mechanism is employed:
Examples: Absorption-, phase-, mass-, density-, thickness-, diffraction contrast, etc. .
Imaging modes: Bight-field microscopy, dark field microscopy, interference contrast and phase contrast microscopy, etc. .. (see subsequent chapters)

(d) ***What is the dimensionality*** of the obtained information (2+1)D, (2+2)D, 3D ...
Plan-view imaging, cross-sectional imaging, depth profiling, 3D tomography

(e) ***What is the information depth***: Surface microscopy, transmission (bulk) microscopy, ...