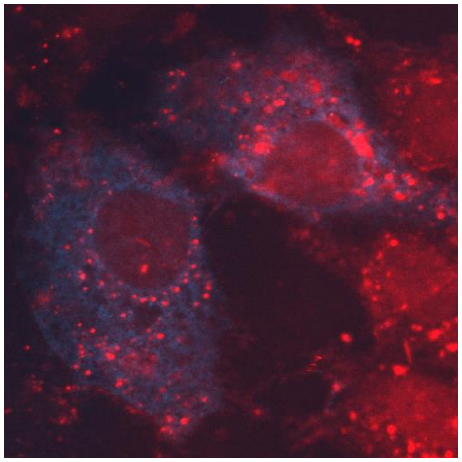
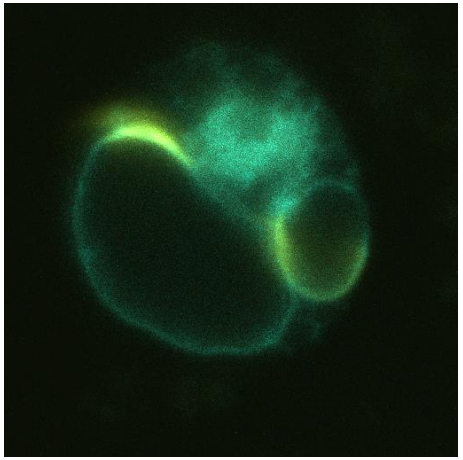
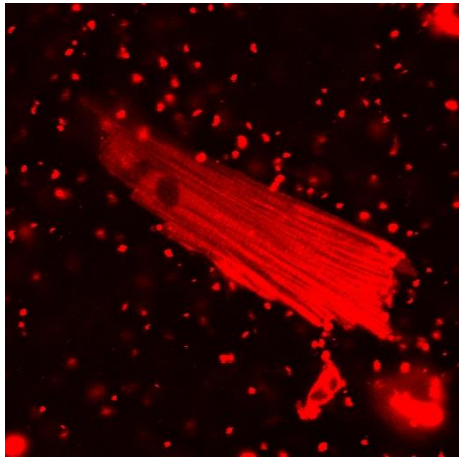
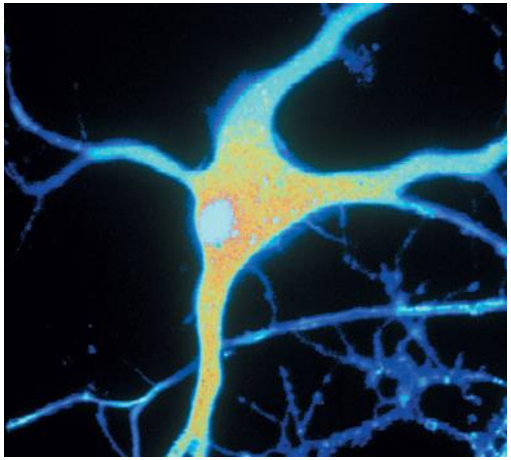
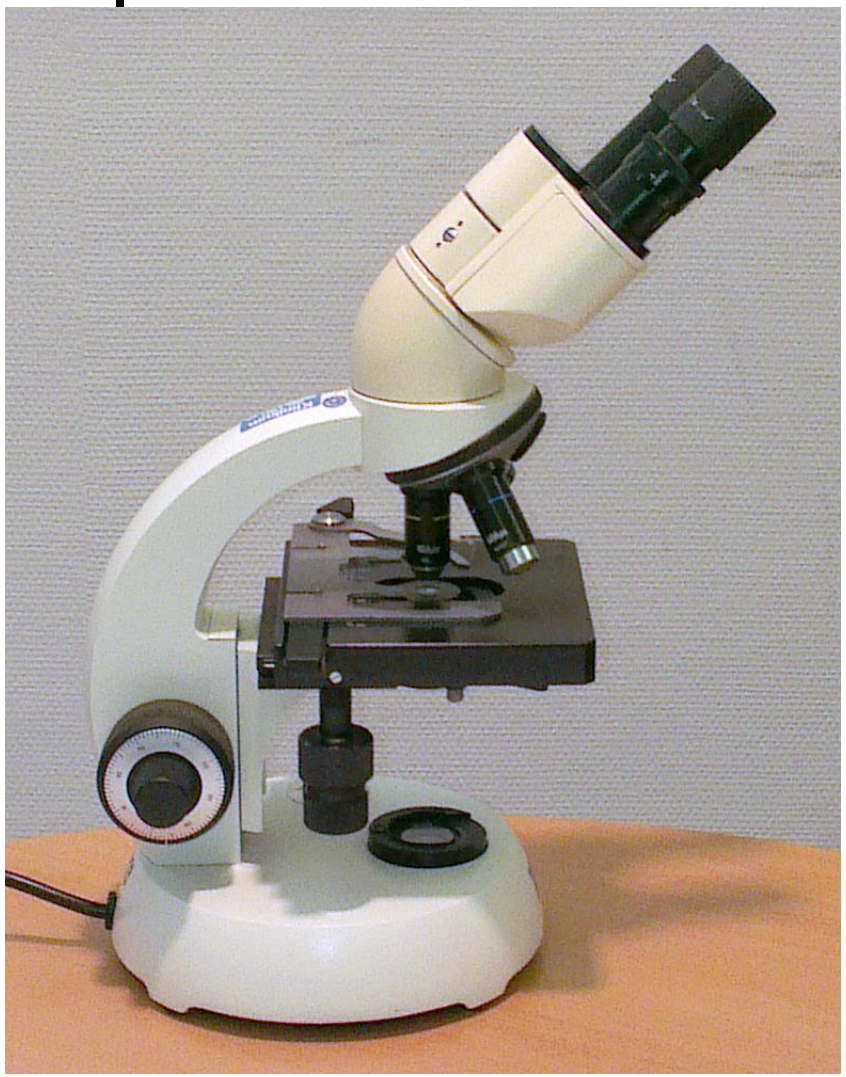
Forschungsorientierte Medizin
Mikroskopie

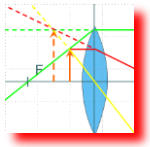
Mikroskopische Techniken

Das Programm für heute: Eine kleine Wiederholung des Histologie-Kurses ?





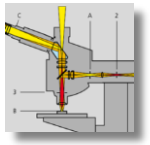
Der Plan für das heutige Seminar



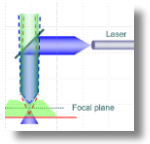
Geometrische Optik



Fluoreszenz



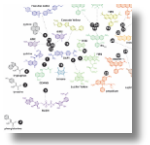
Epifluoreszenzmikroskopie



Laser Scanning Mikroskopie



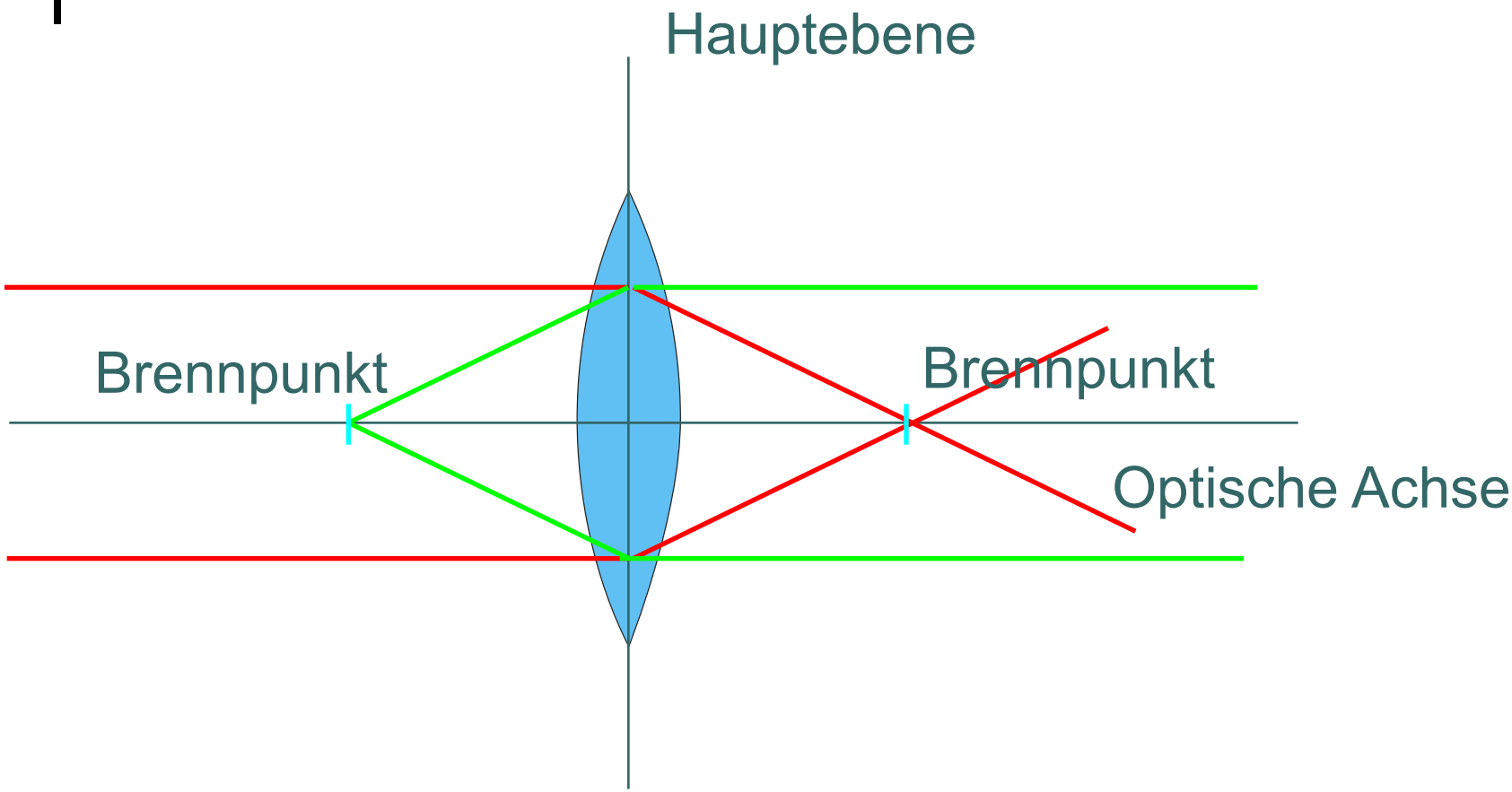
Superresolution Mikroskopie



Fluorophore

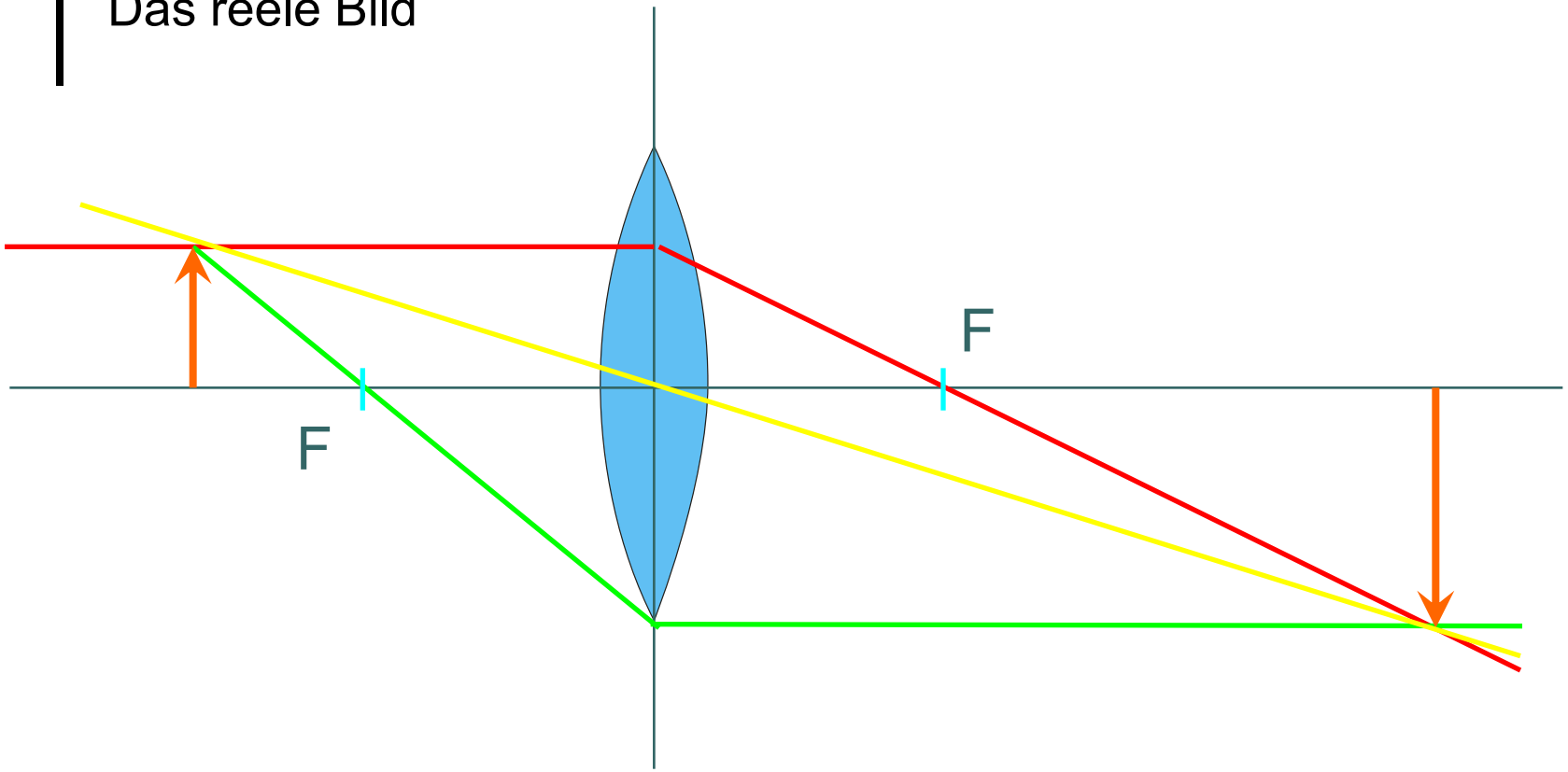
Geometrische Optik

Der Brennpunkt



Geometrische Optik

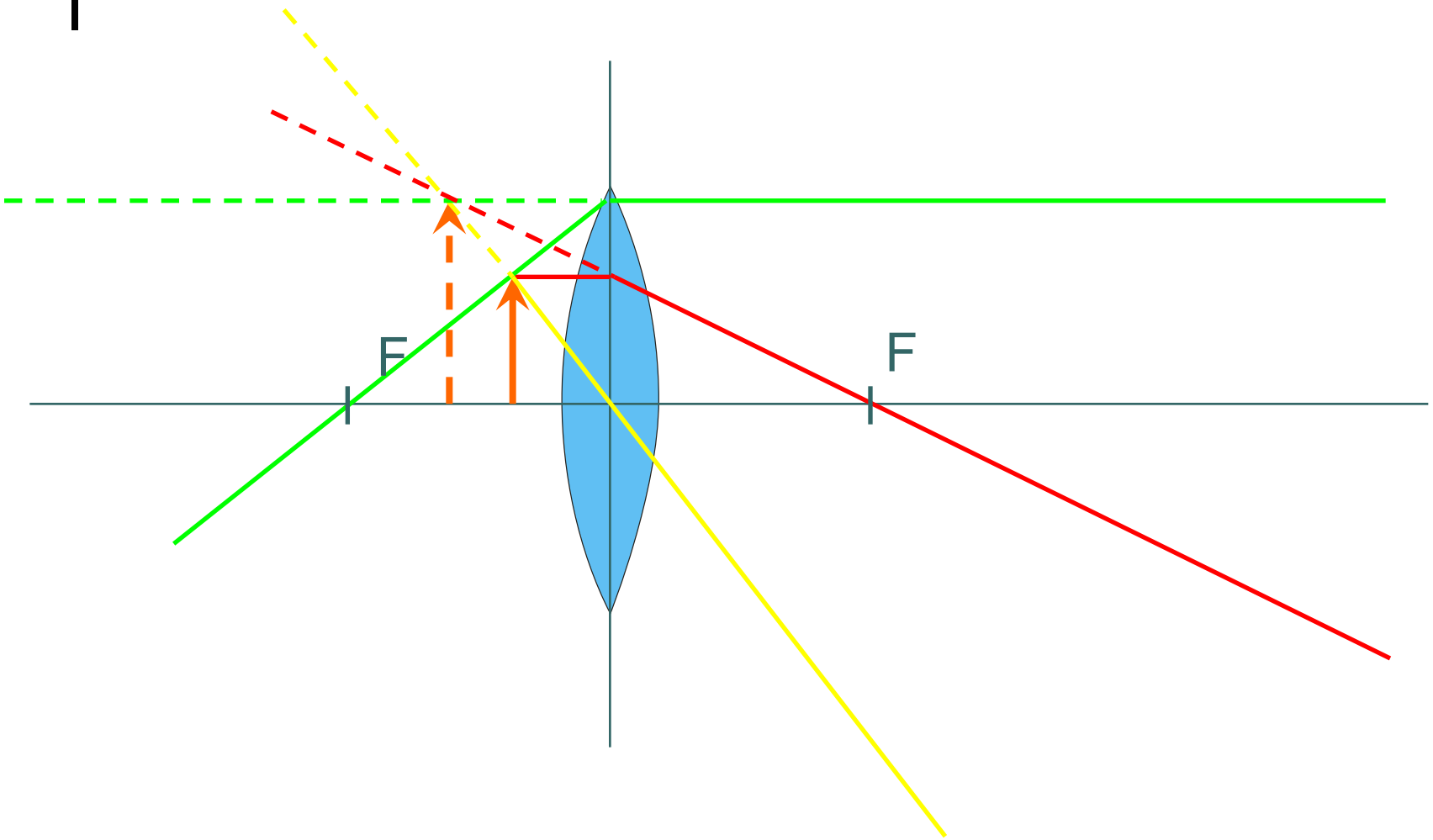
Das reele Bild



1. Ein Strahl durch den Mittelpunkt wird nicht gebrochen.
2. Ein Strahl parallel zur optischen Achse verläuft nach der Linse durch den Brennpunkt.
3. Ein Strahl aus dem Brennpunkt verläuft nach der Linse parallel zur optischen Achse.

Geometrische Optik

Das virtuelle Bild



Geschichte der Mikroskopie

Das Optische Museum in Jena







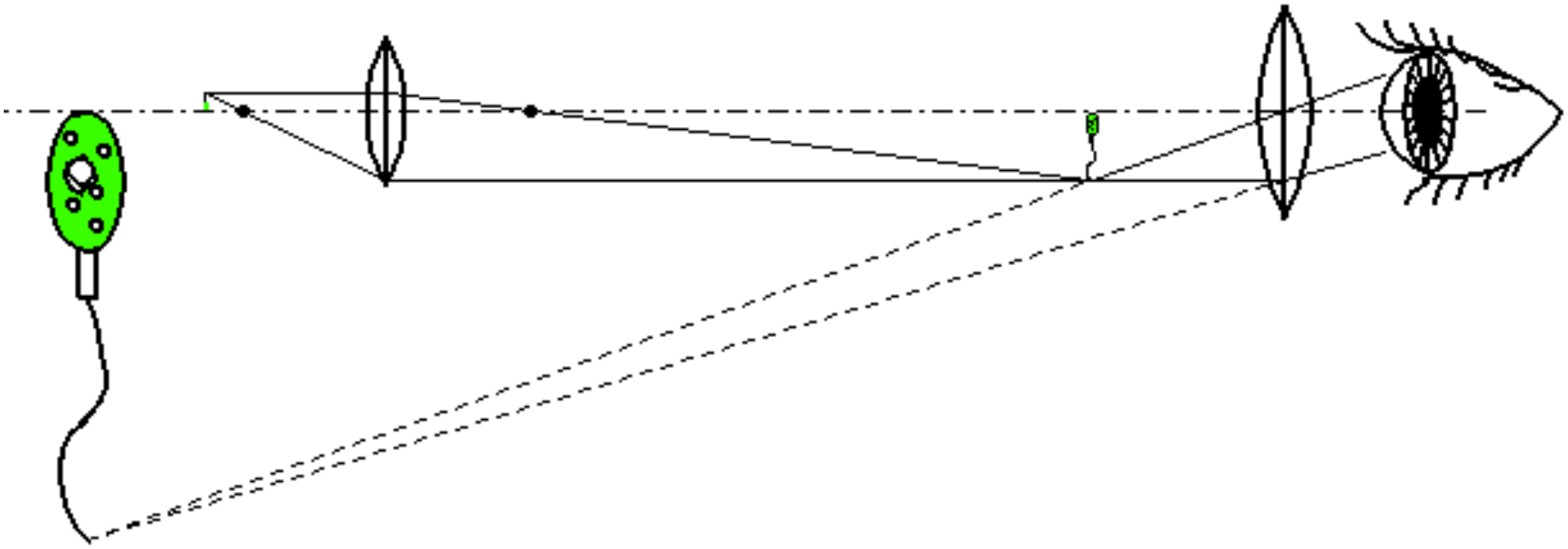






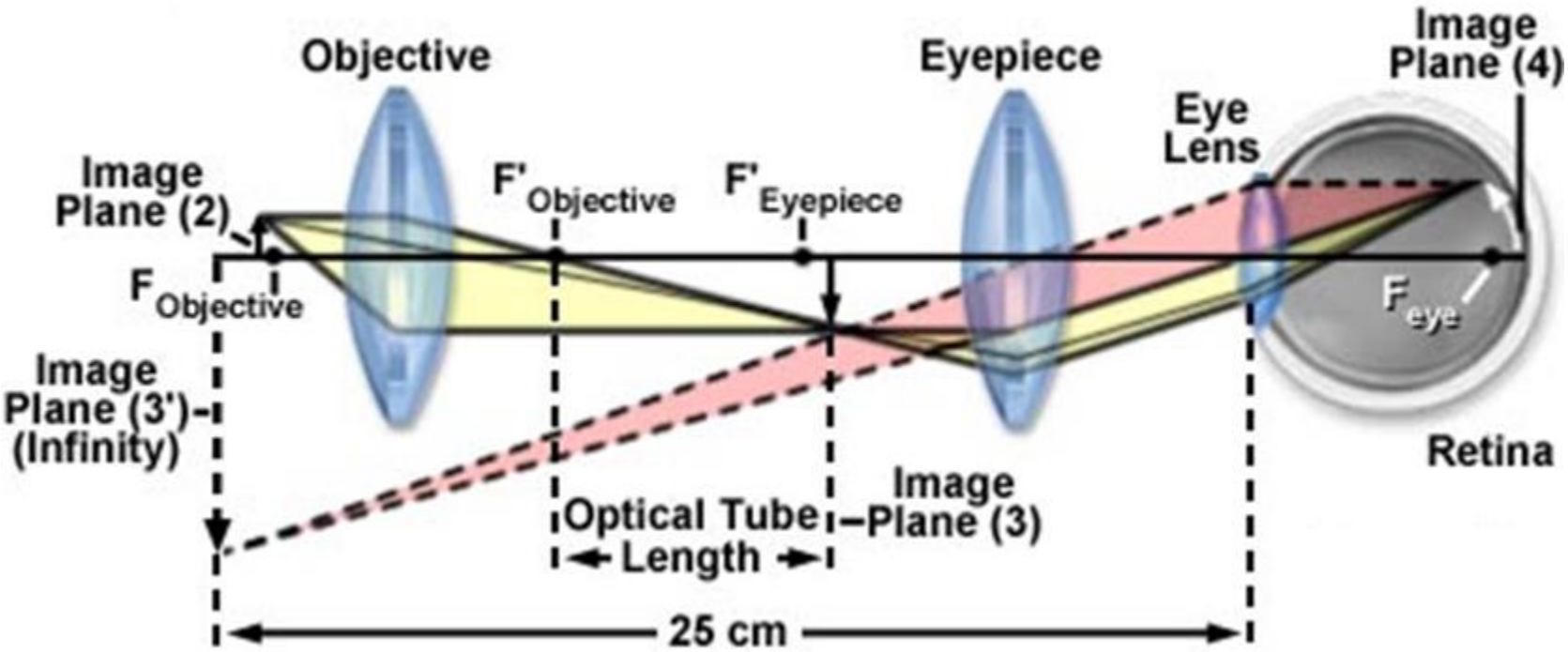
Geometrische Optik

Das zusammengesetzte Mikroskop



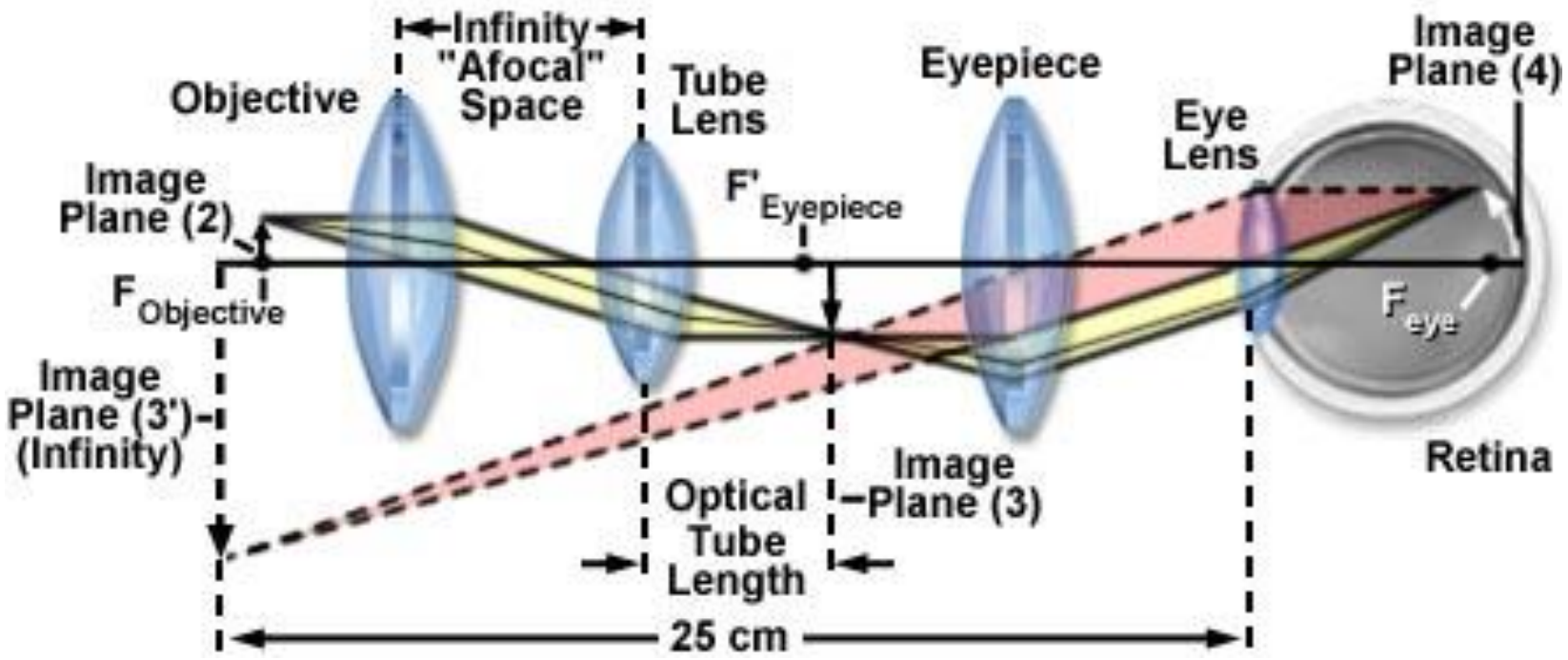
Basic microscopy

Conjugate field planes in a compound microscope



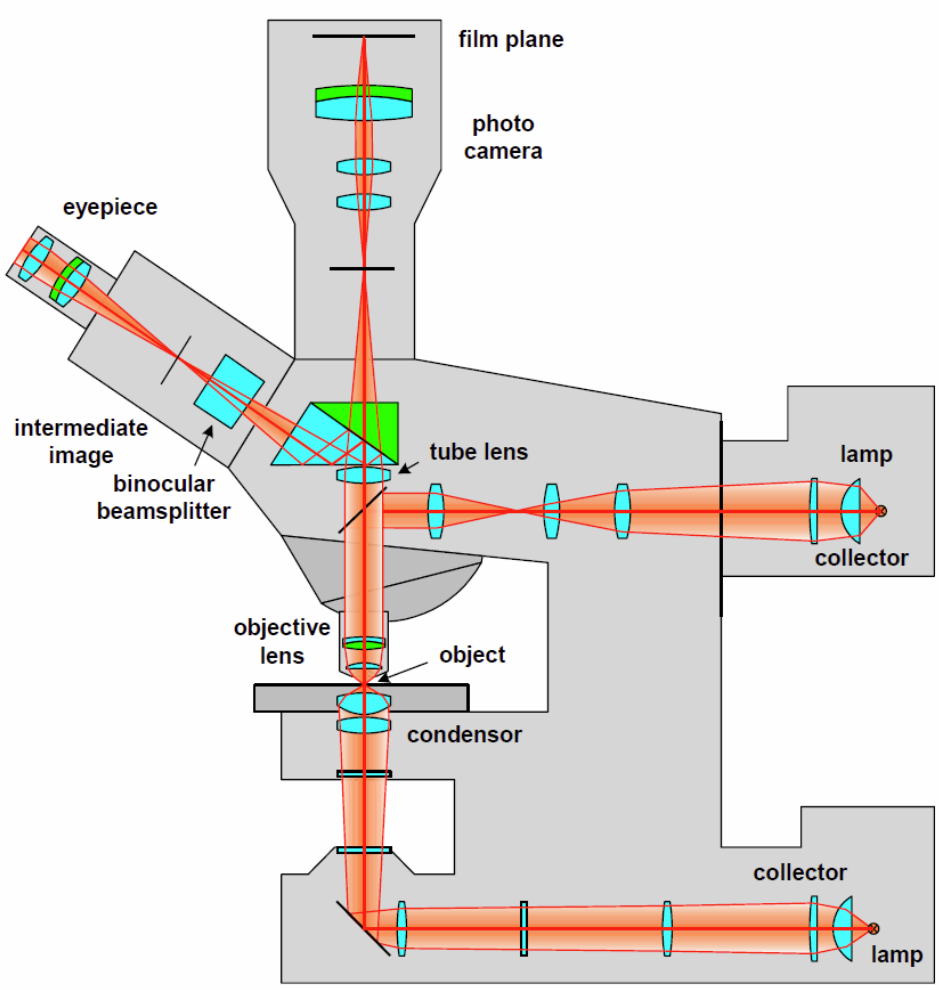
Basic microscopy

Infinity corrected microscopes



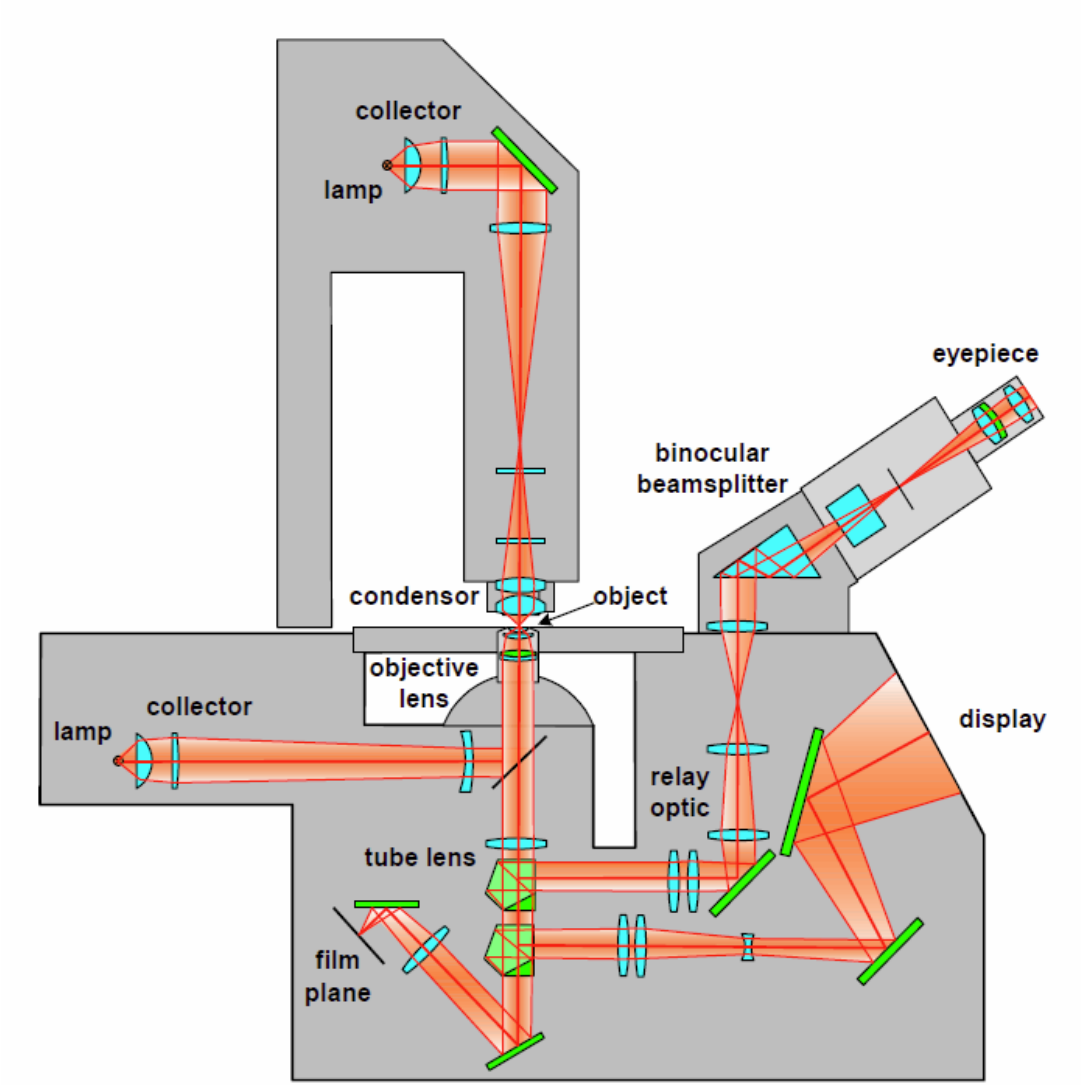
Basic microscopy

The beam path in upright microscopes



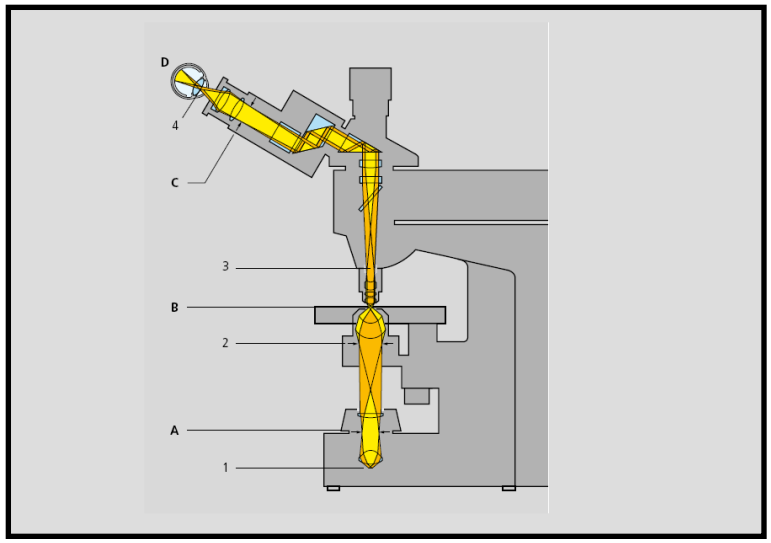
Basic microscopy

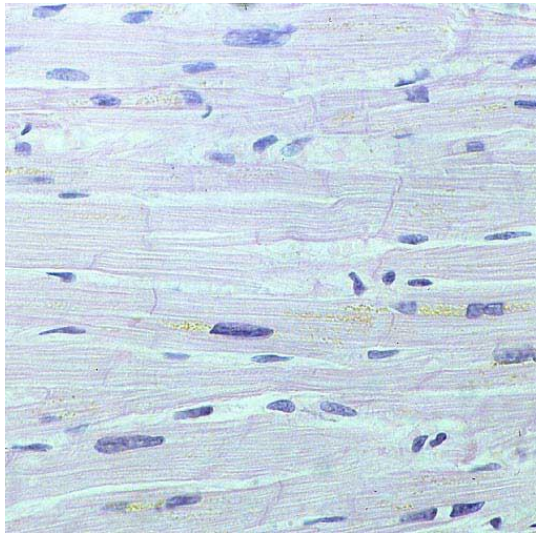
The beam path in inverted microscopes



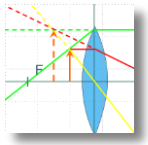
Mikroskopische Techniken

Die Transmissionsmikroskopie





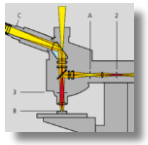
Der Plan für das heutige Seminar



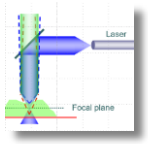
Geometrische Optik



Fluoreszenz



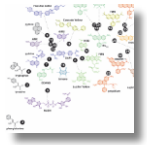
Epifluoreszenzmikroskopie



Laser Scanning Mikroskopie



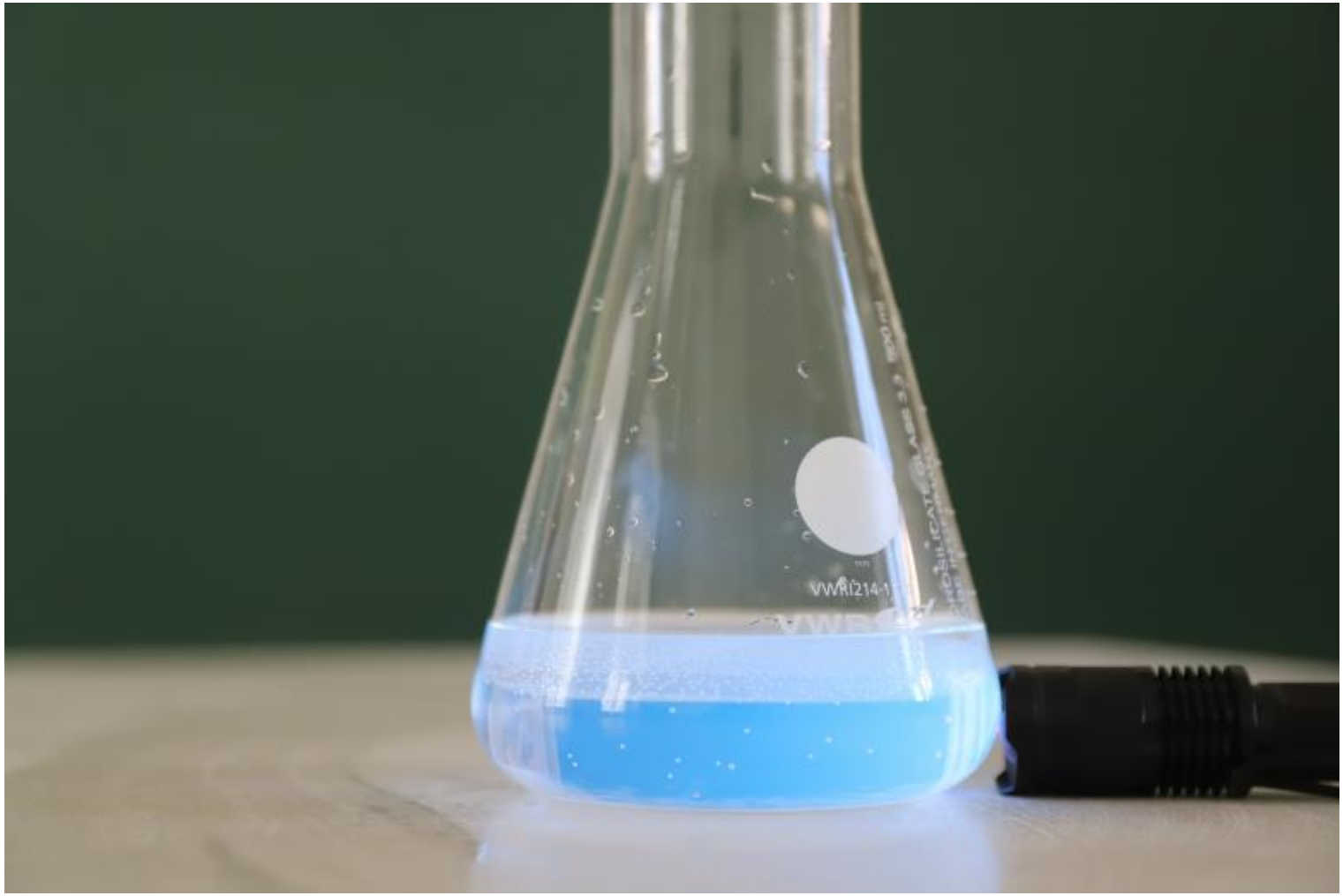
Superresolution Mikroskopie



Fluorophore

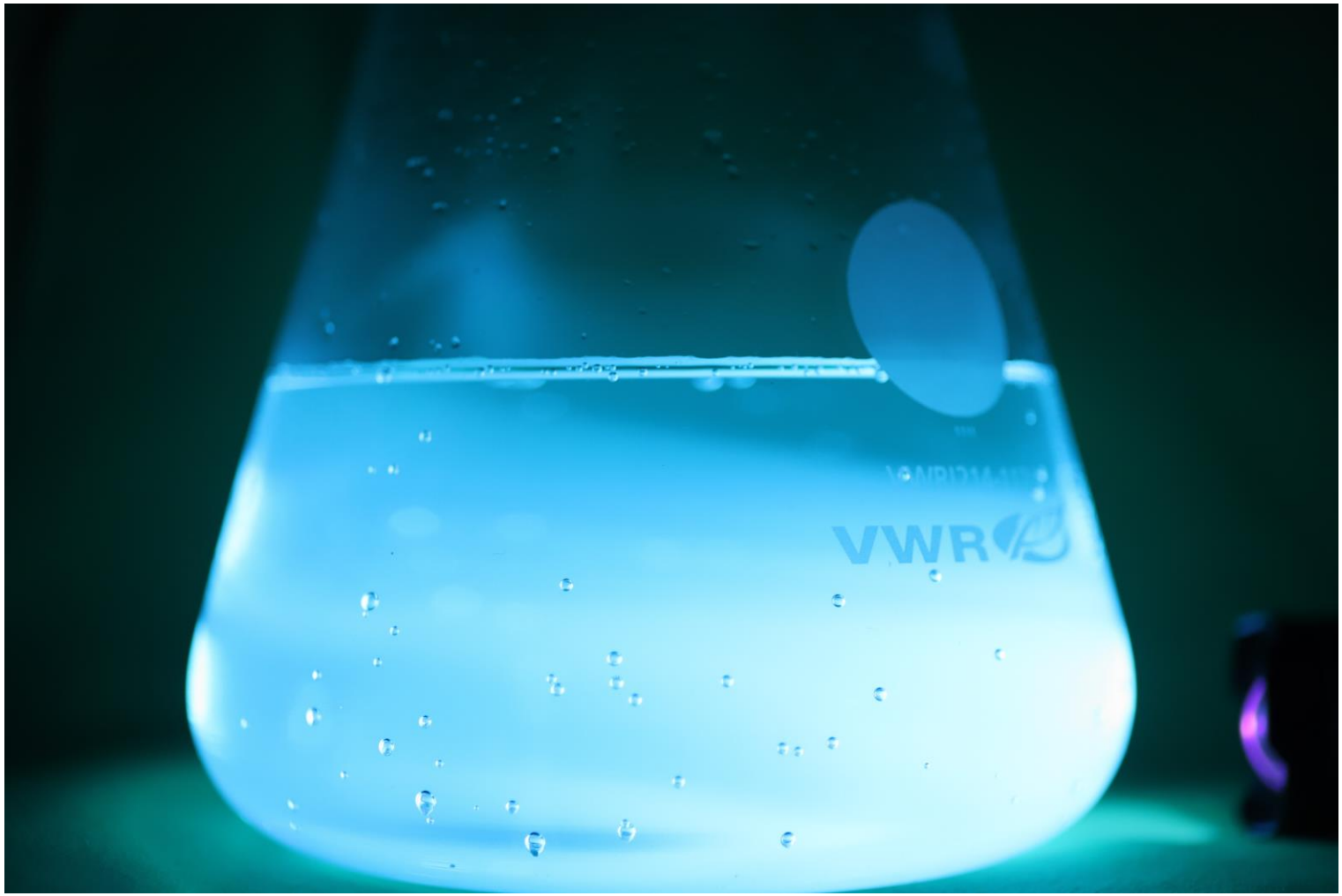
Fluoreszenz

Was ist Fluoreszenz ?



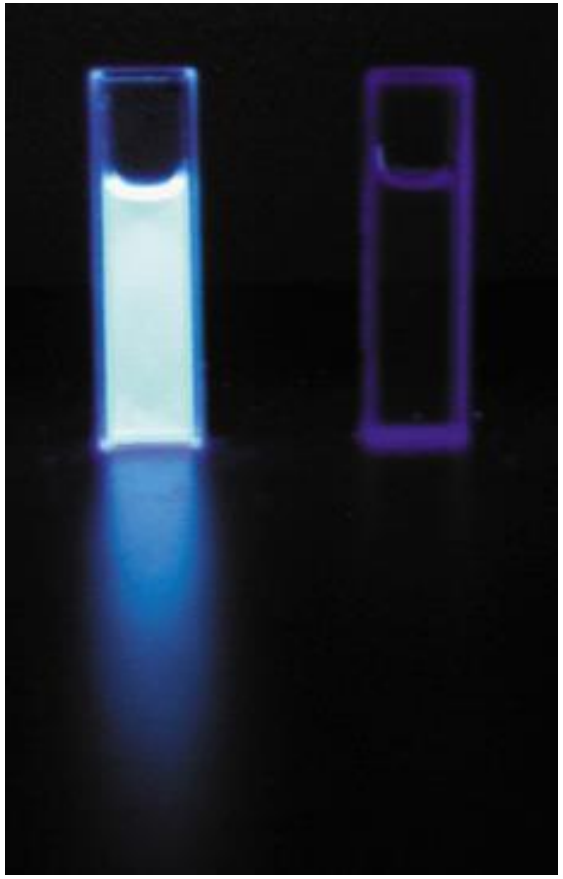
Fluoreszenz

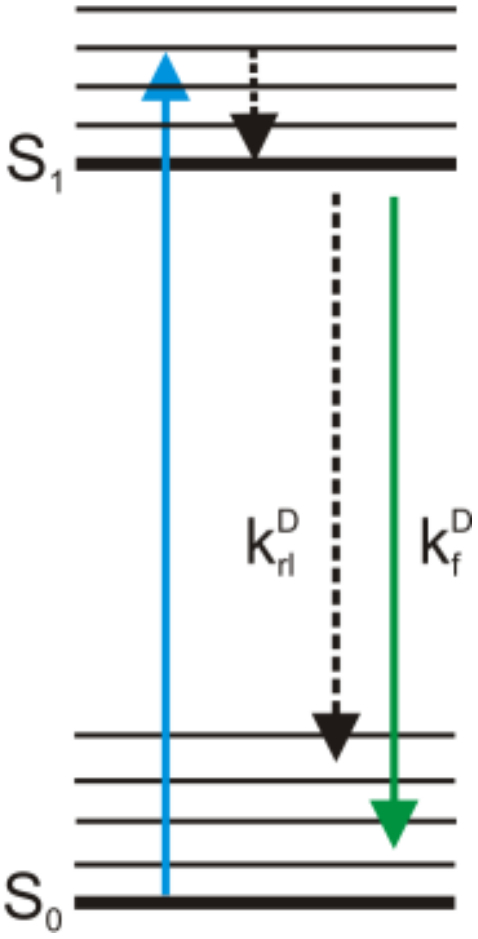
Was ist Fluoreszenz ?



Fluoreszenz

Was ist Fluoreszenz ?

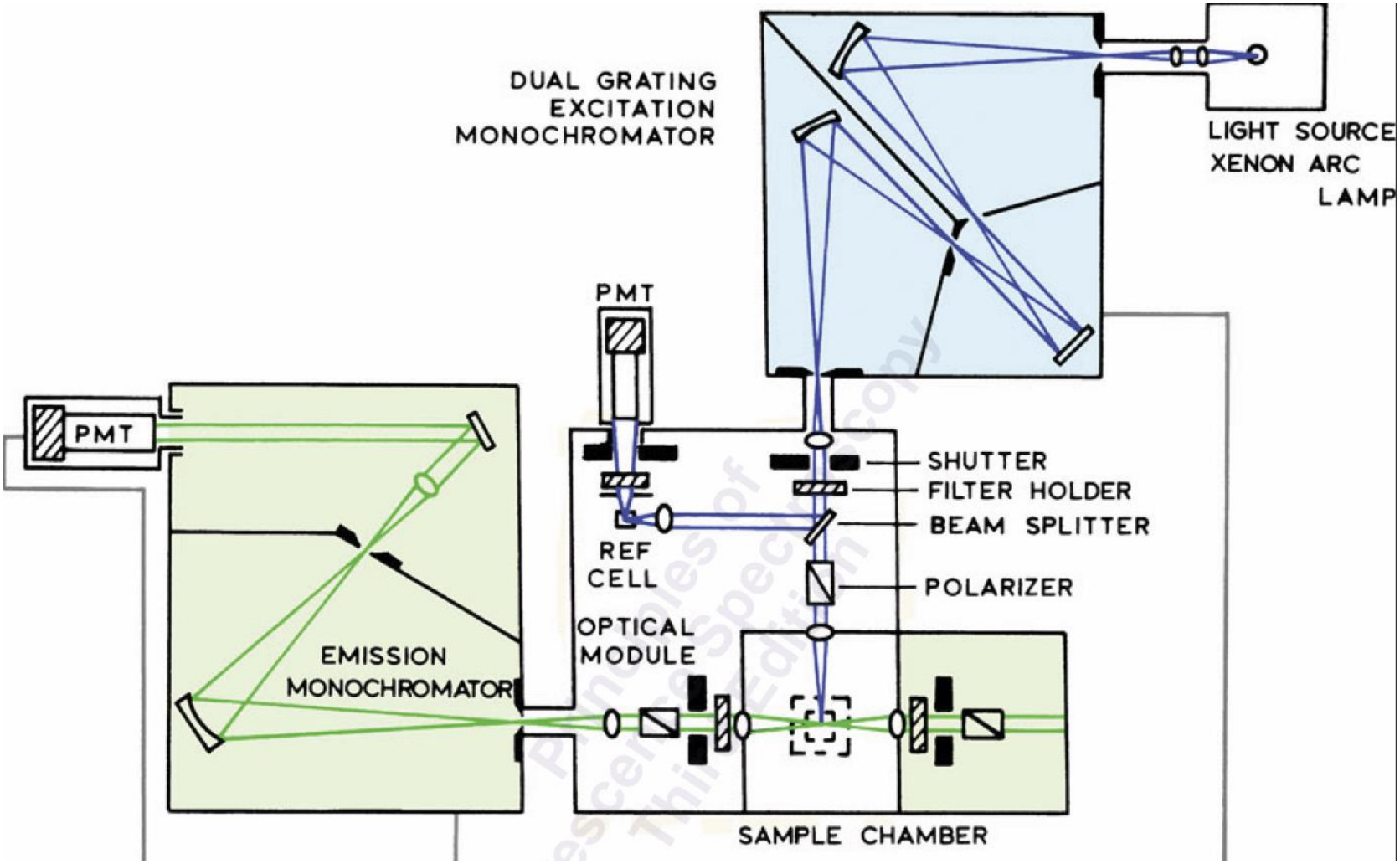




Jablonski-Schema

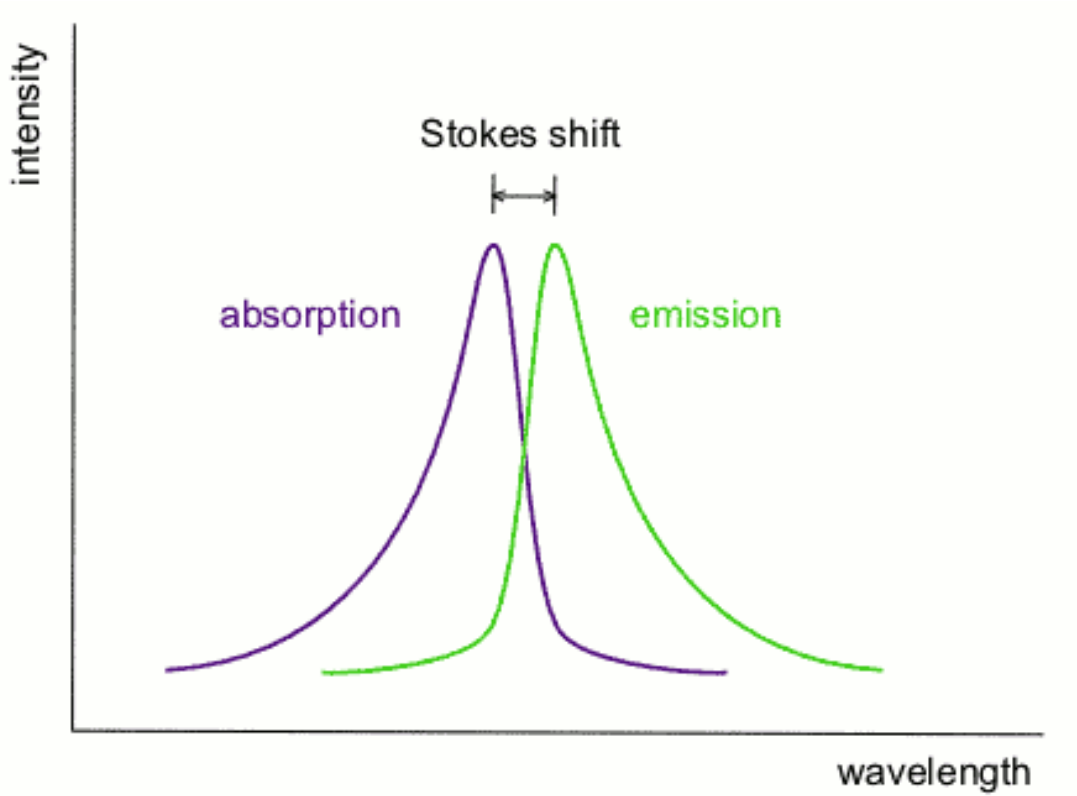
Fluoreszenzspektroskopie

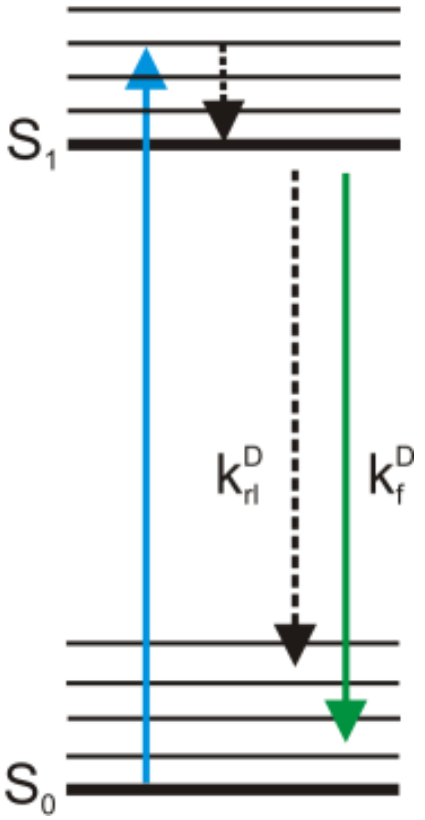
Schema eines Fluoreszenzspektrometers



Fluoreszenz

Erklärung des „Stokes Shift“

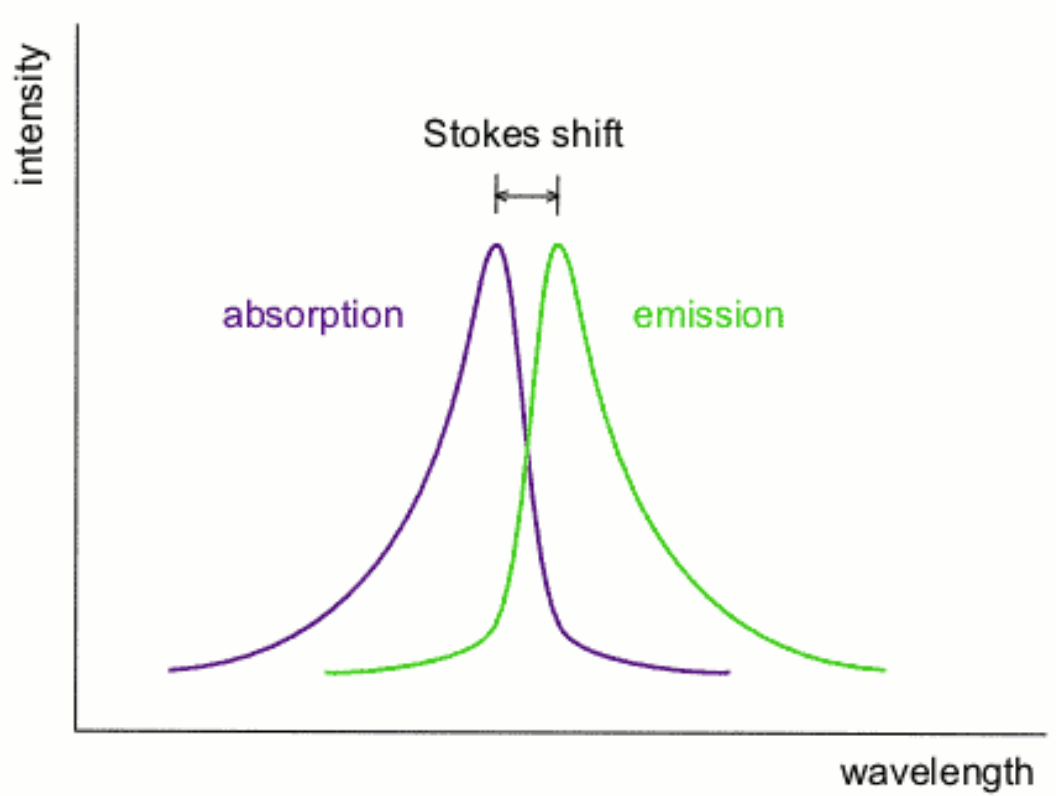


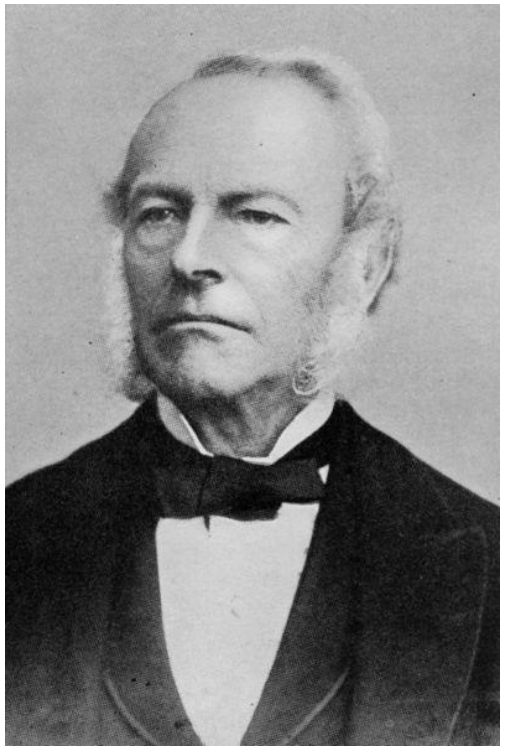


Jablonski-Scheme

Fluoreszenz

Der „Stokes Shift“

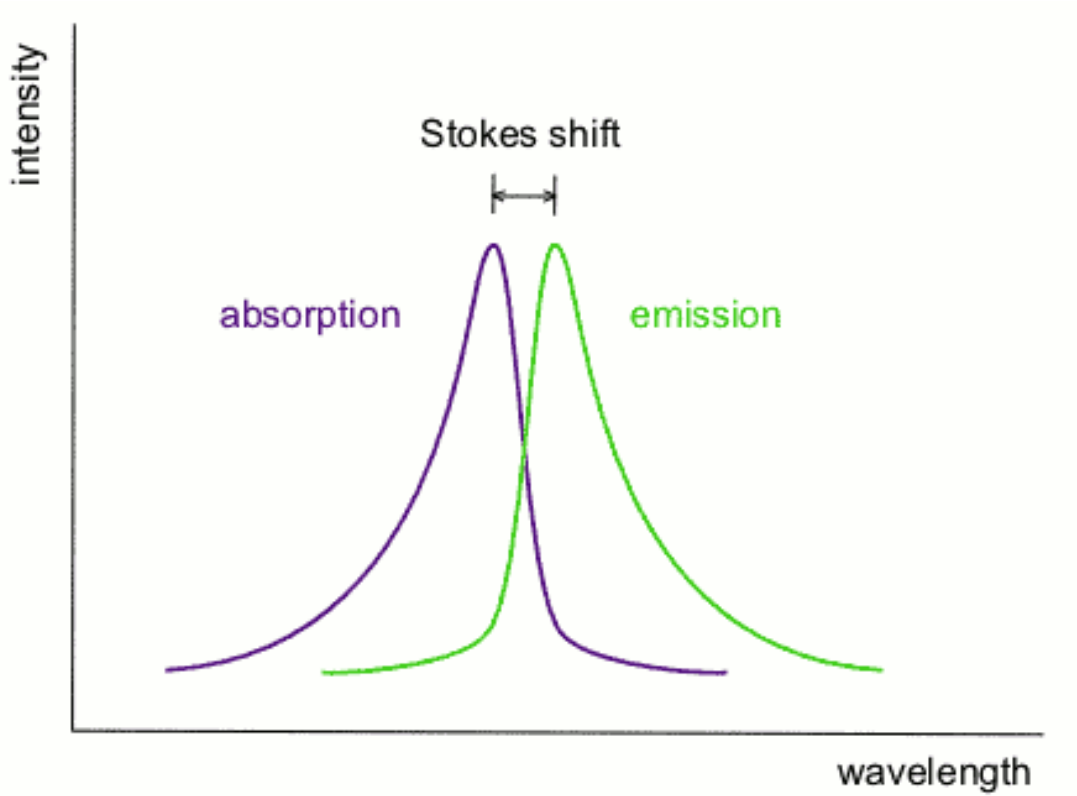


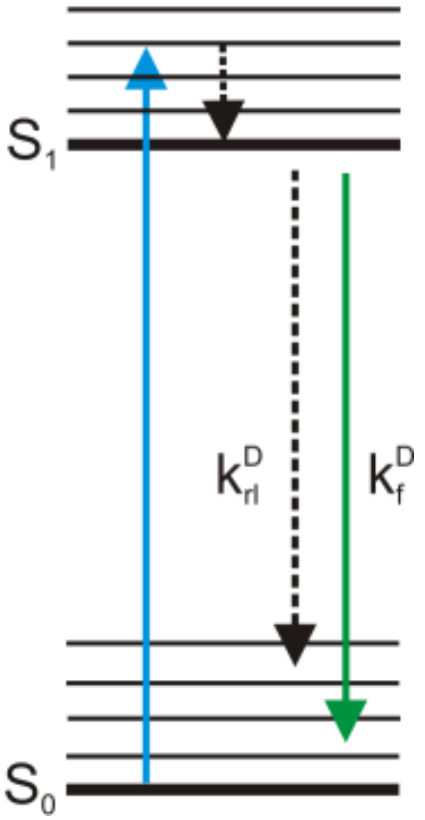


George G. Stokes

Fluoreszenz

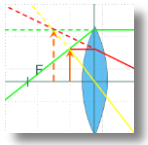
Erklärung des „Stokes Shift“





Jablonski-Scheme

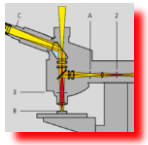
Der Plan für das heutige Seminar



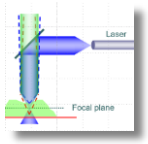
Geometrische Optik



Fluoreszenz



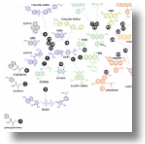
Epifluoreszenzmikroskopie



Laser Scanning Mikroskopie



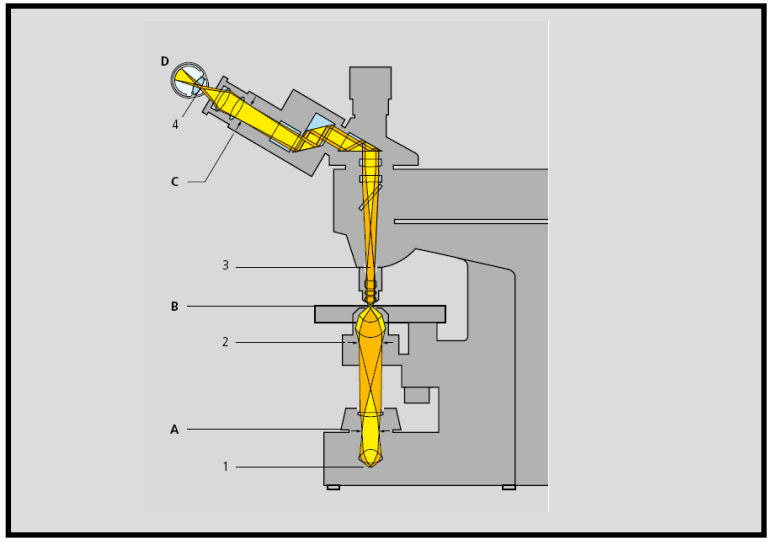
Superresolution Mikroskopie

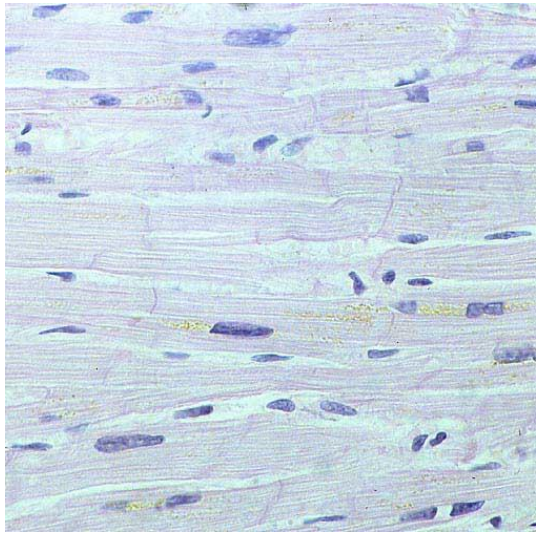


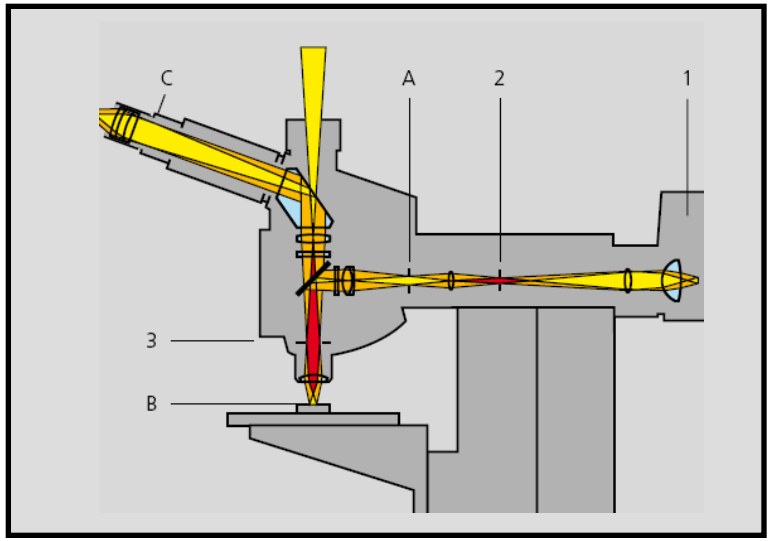
Fluorophore

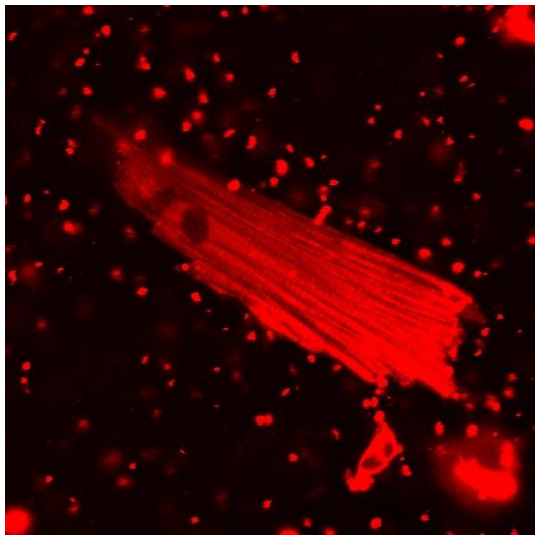
Mikroskopische Techniken

Der Strahlengang in einem Transmissions- und in einem Fluoreszenzmikroskop



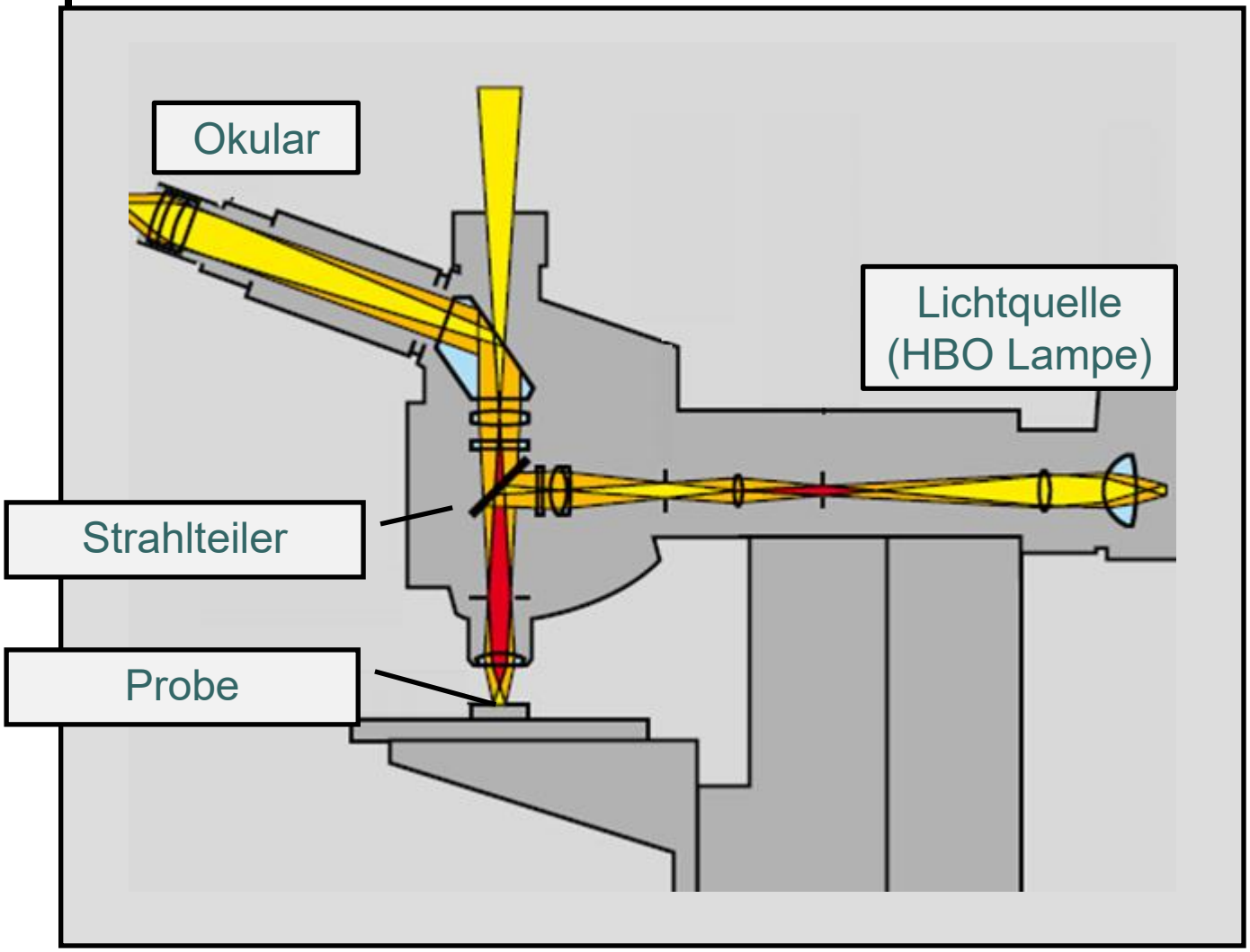






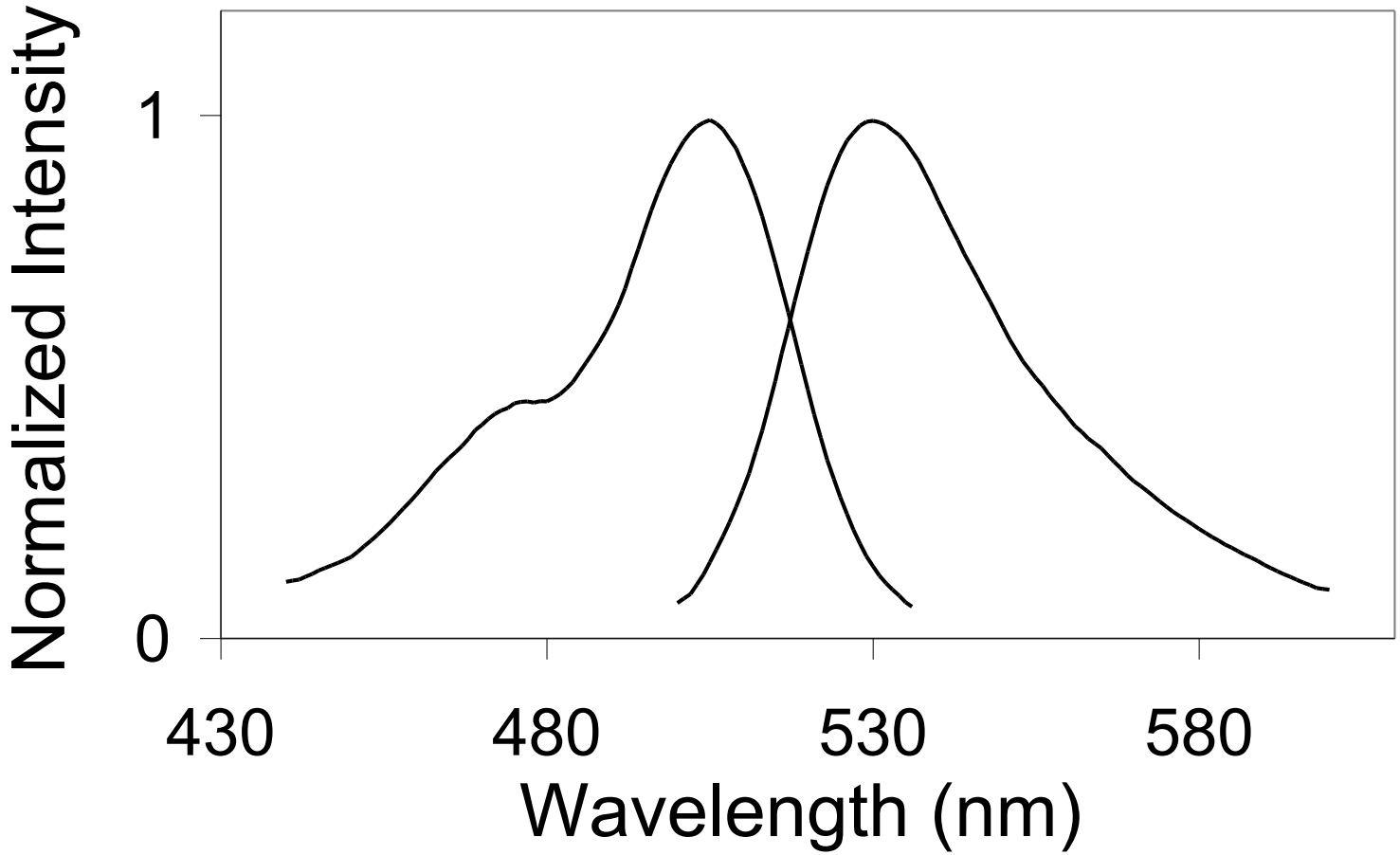
Fluoreszenzmikroskopie

Der Strahlengang im Epifluoreszenzmikroskop



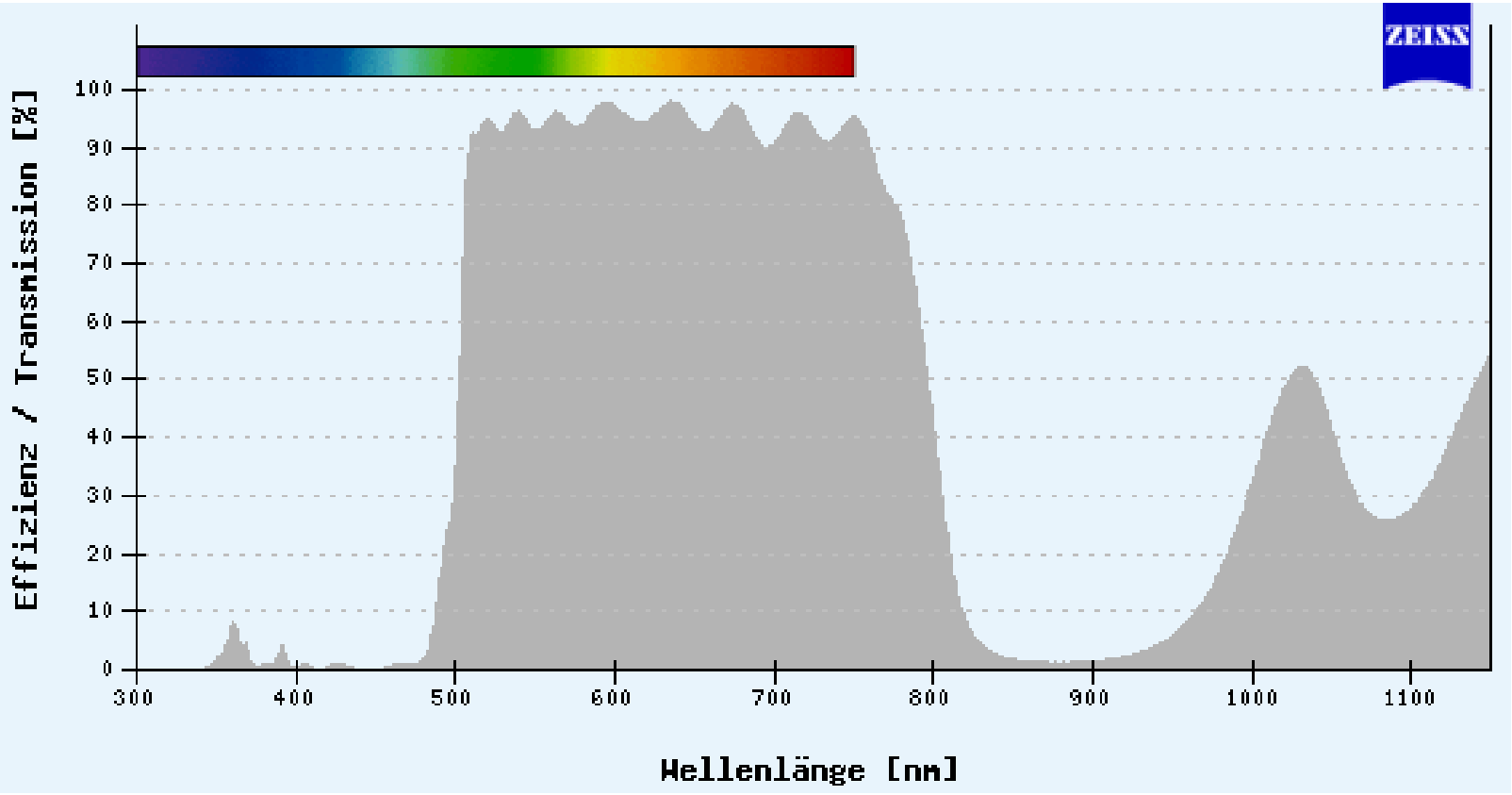
Fluoreszenzmikroskopie

Die Wahl der Filter



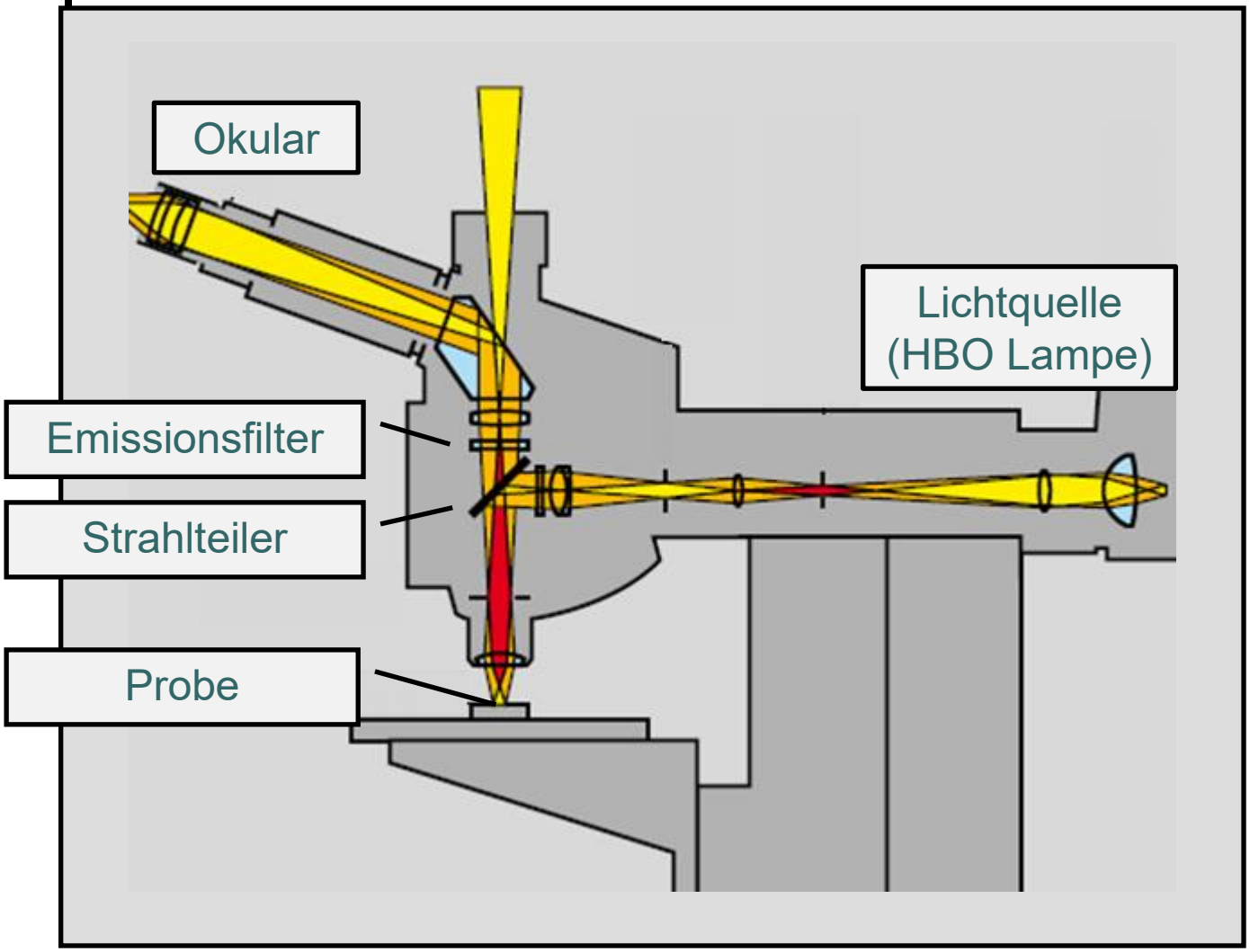
Fluoreszenzmikroskopie

Der Strahlteiler



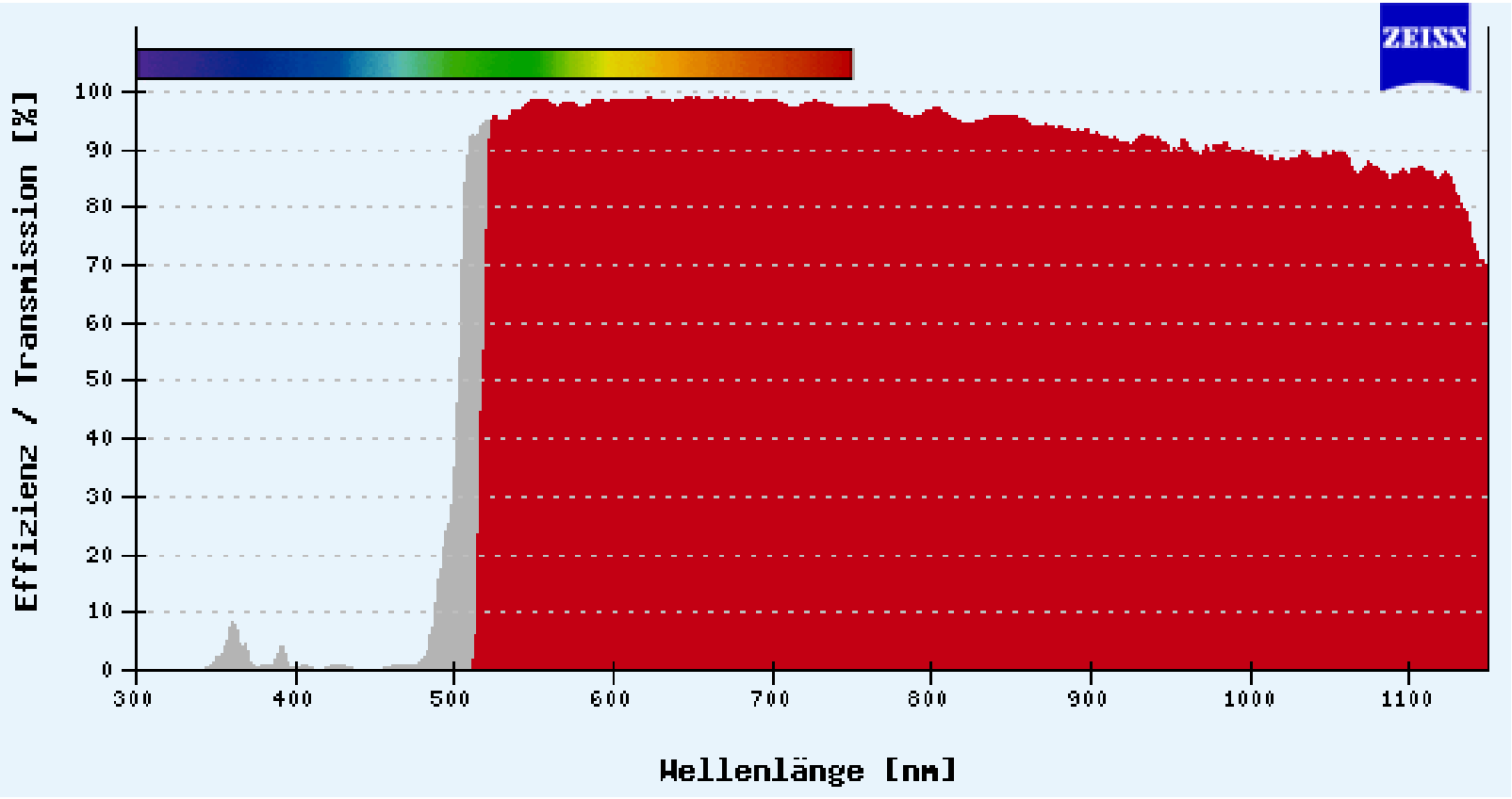
Fluoreszenzmikroskopie

Der Strahlengang im Epifluoreszenzmikroskop



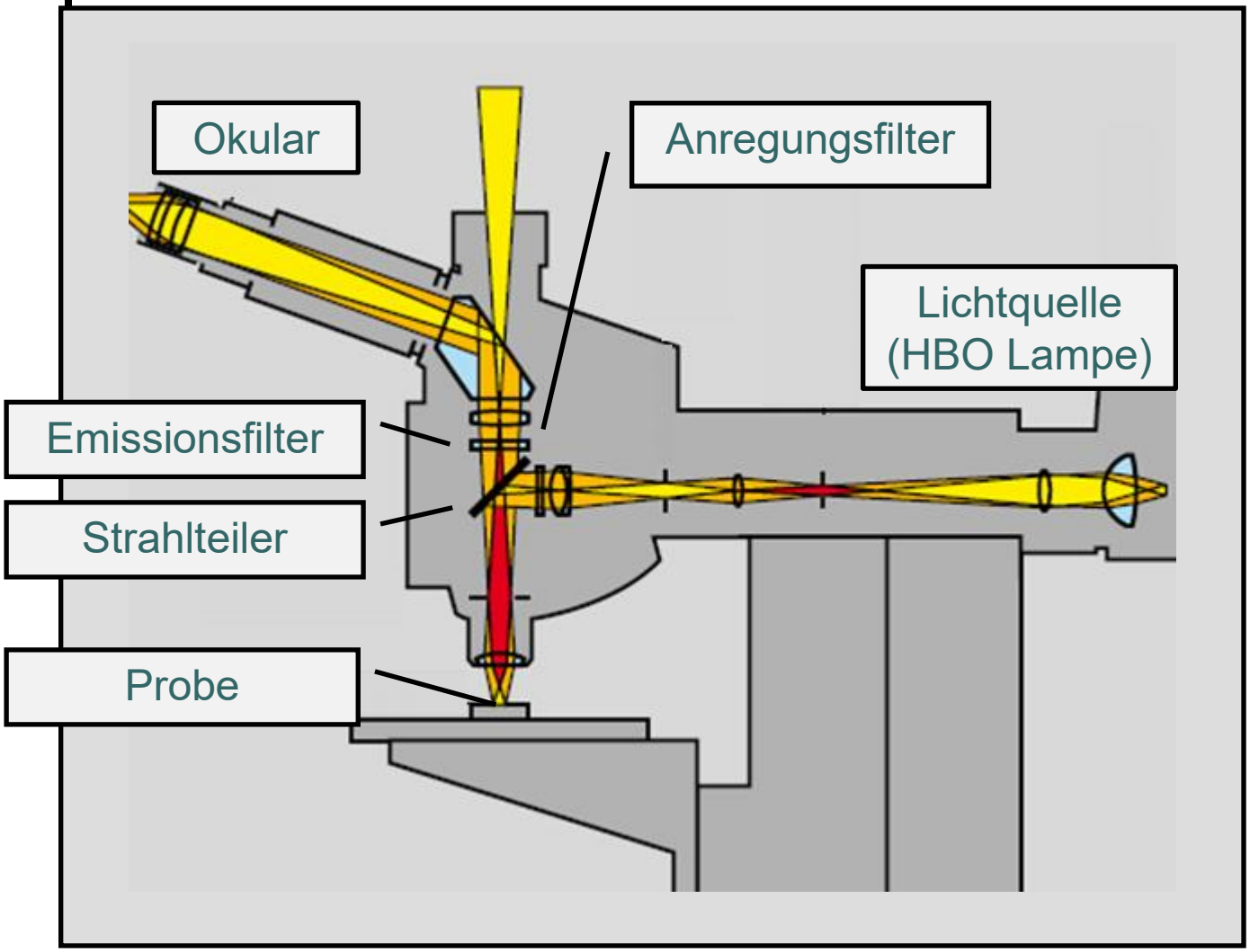
Fluoreszenzmikroskopie

Der Emissionsfilter



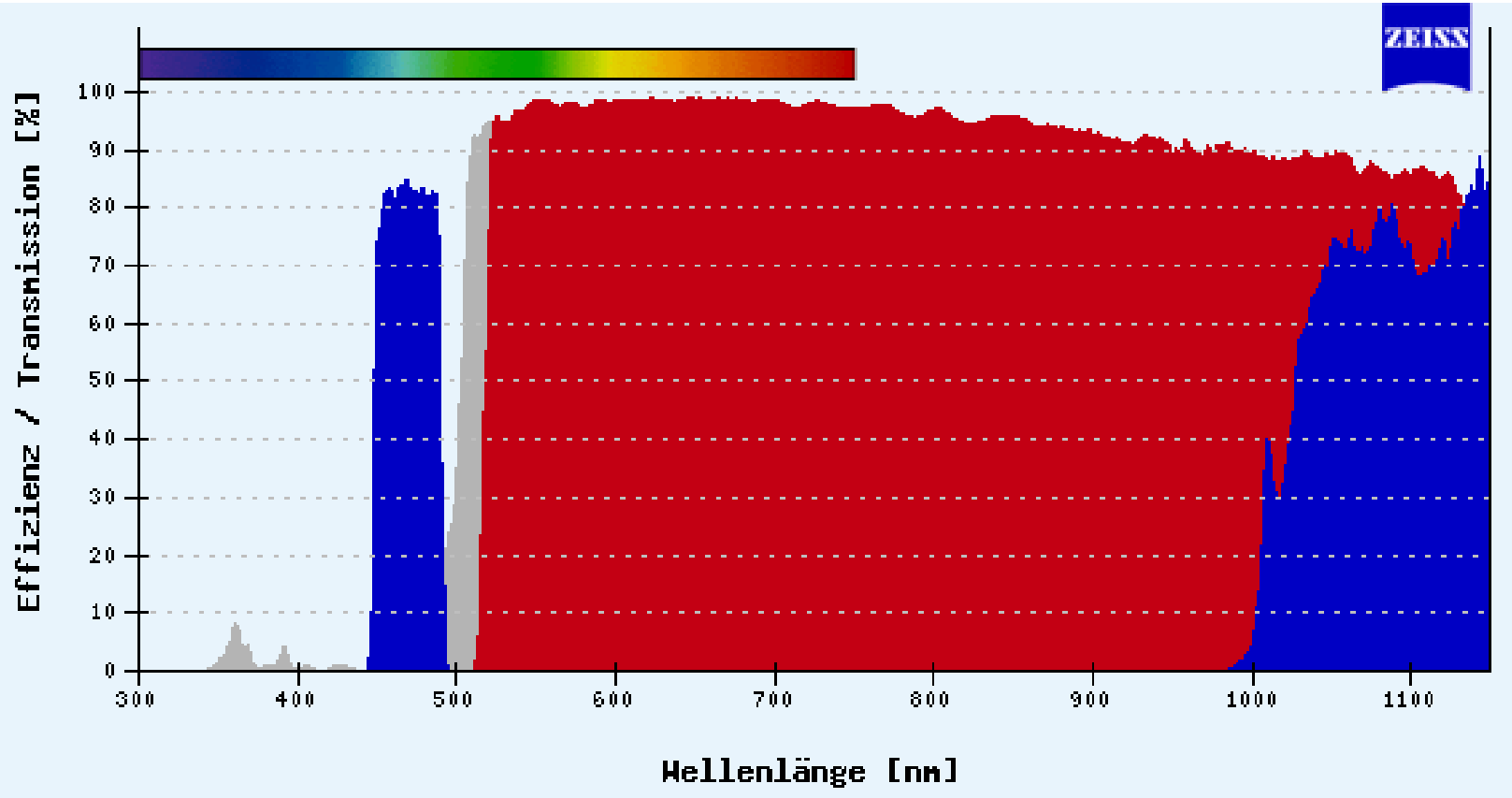
Fluoreszenzmikroskopie

Der Strahlengang im Epifluoreszenzmikroskop



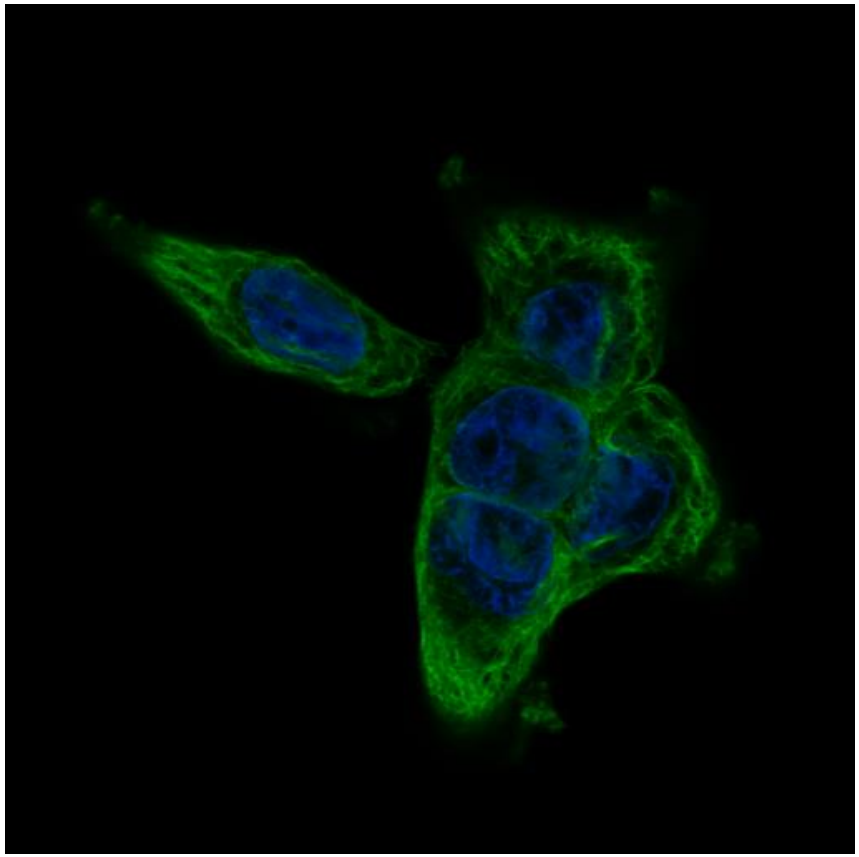
Fluoreszenzmikroskopie

Fluoreszenzfiltersatz



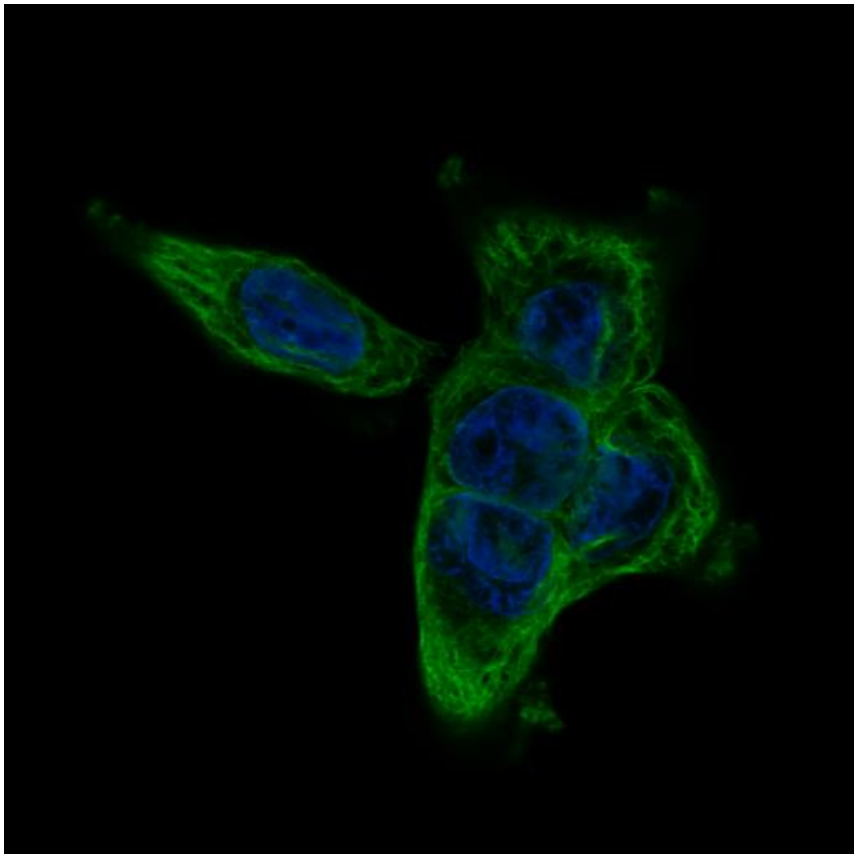
Fluoreszenzmikroskopie

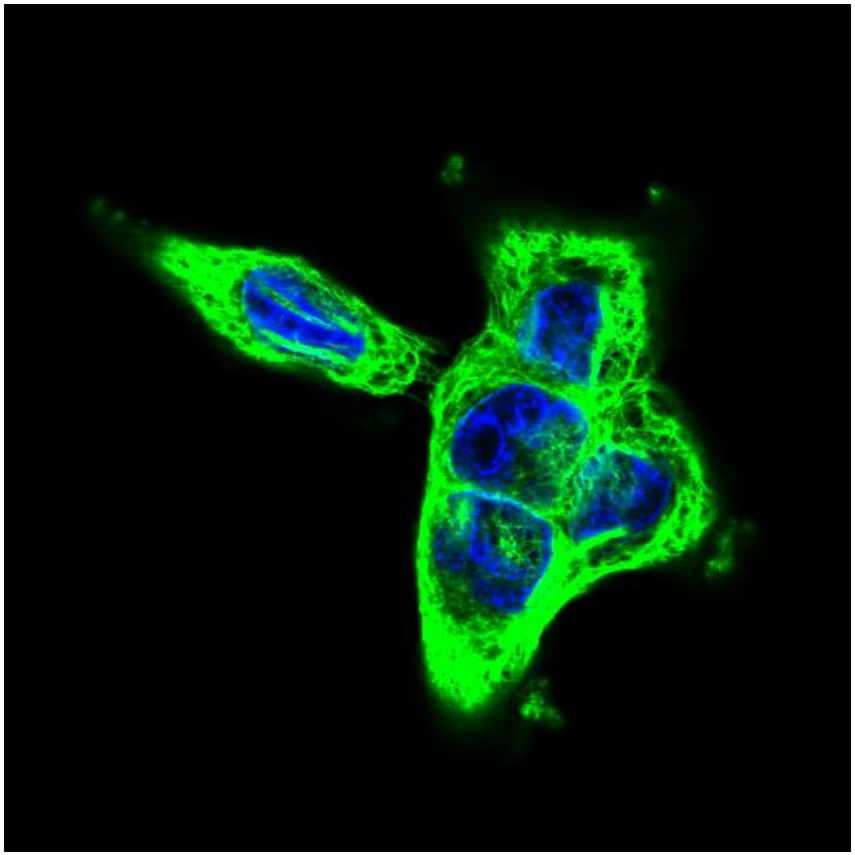
Nachteile der Epifluoreszenzmikroskopie



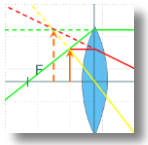
Fluoreszenzmikroskopie

Nachteile der Epifluoreszenzmikroskopie





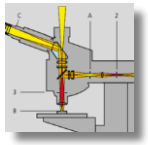
Der Plan für das heutige Seminar



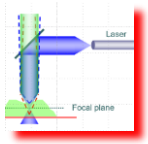
Geometrische Optik



Fluoreszenz



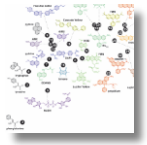
Epifluoreszenzmikroskopie



Laser Scanning Mikroskopie



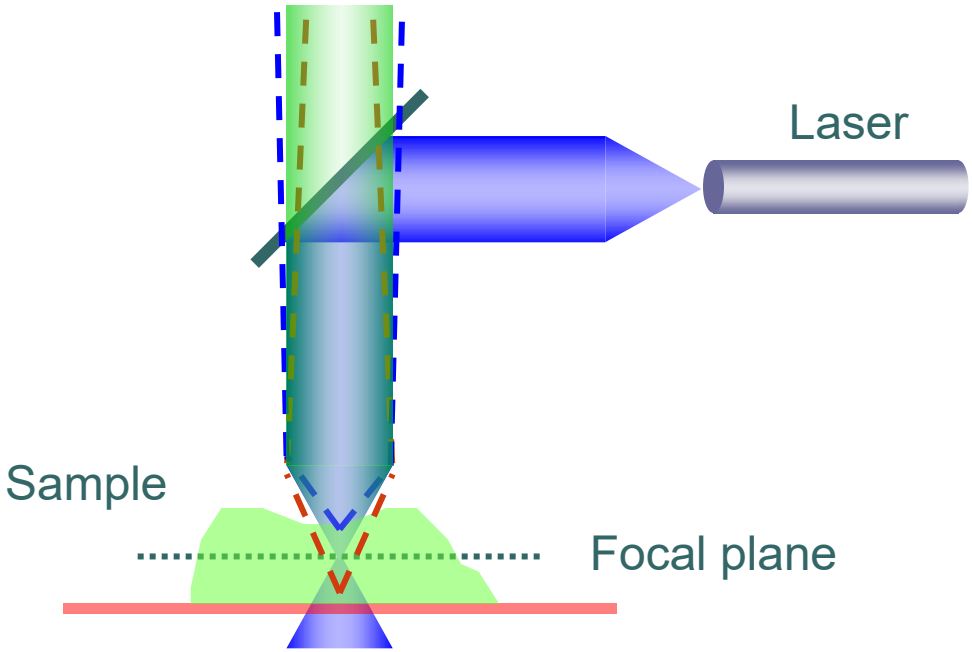
Superresolution Mikroskopie



Fluorophore

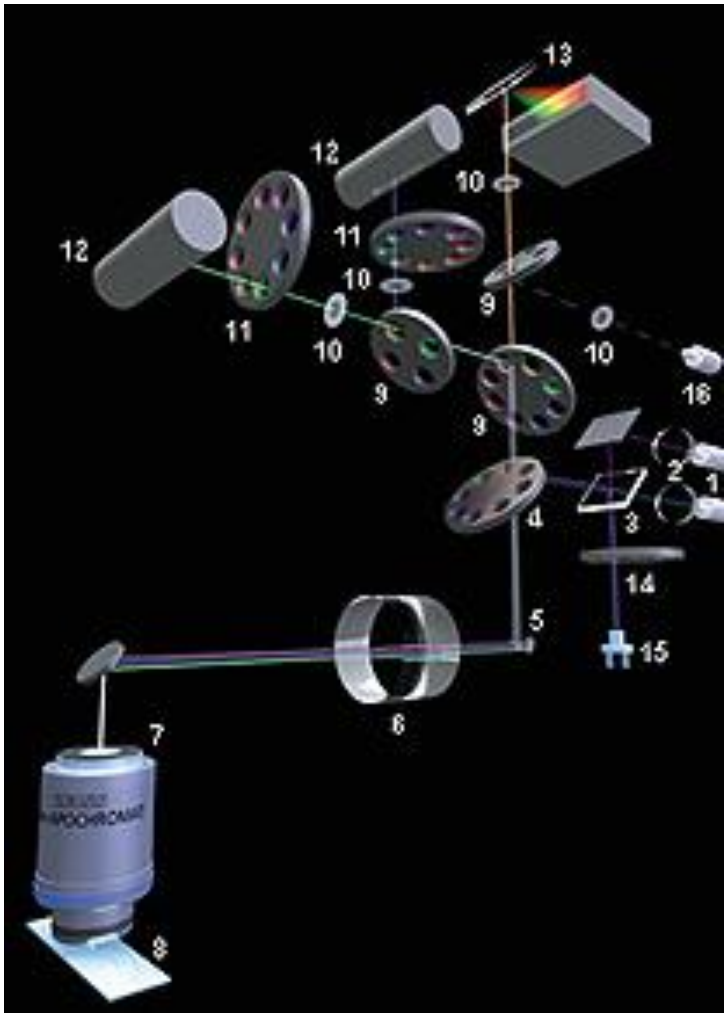
Konfokale Laser Scanning Mikroskopie

Das Prinzip der konfokalen Mikroskopie



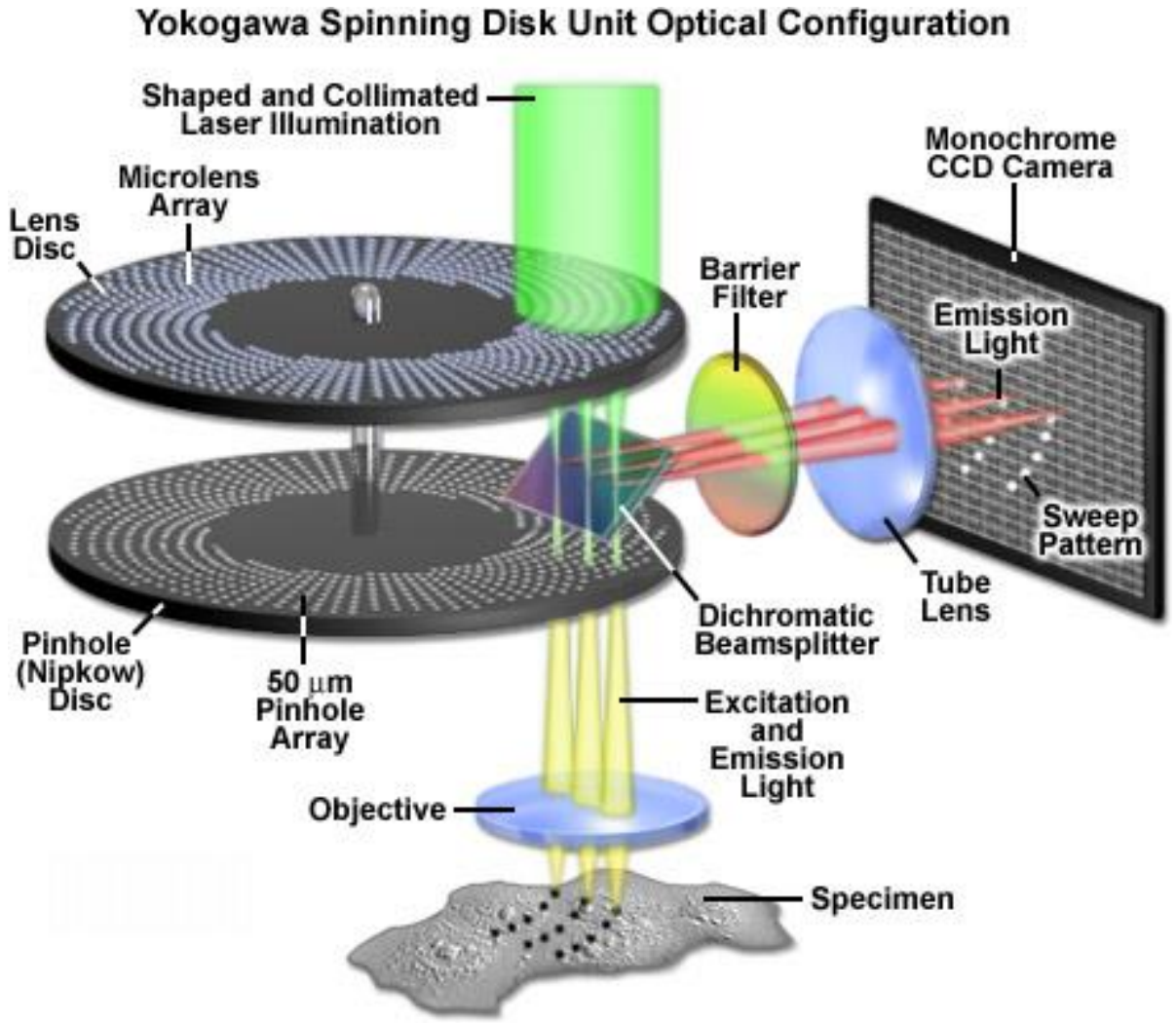
Konfokale Laser Scanning Mikroskopie

Strahlengang in konfokalen LSMs



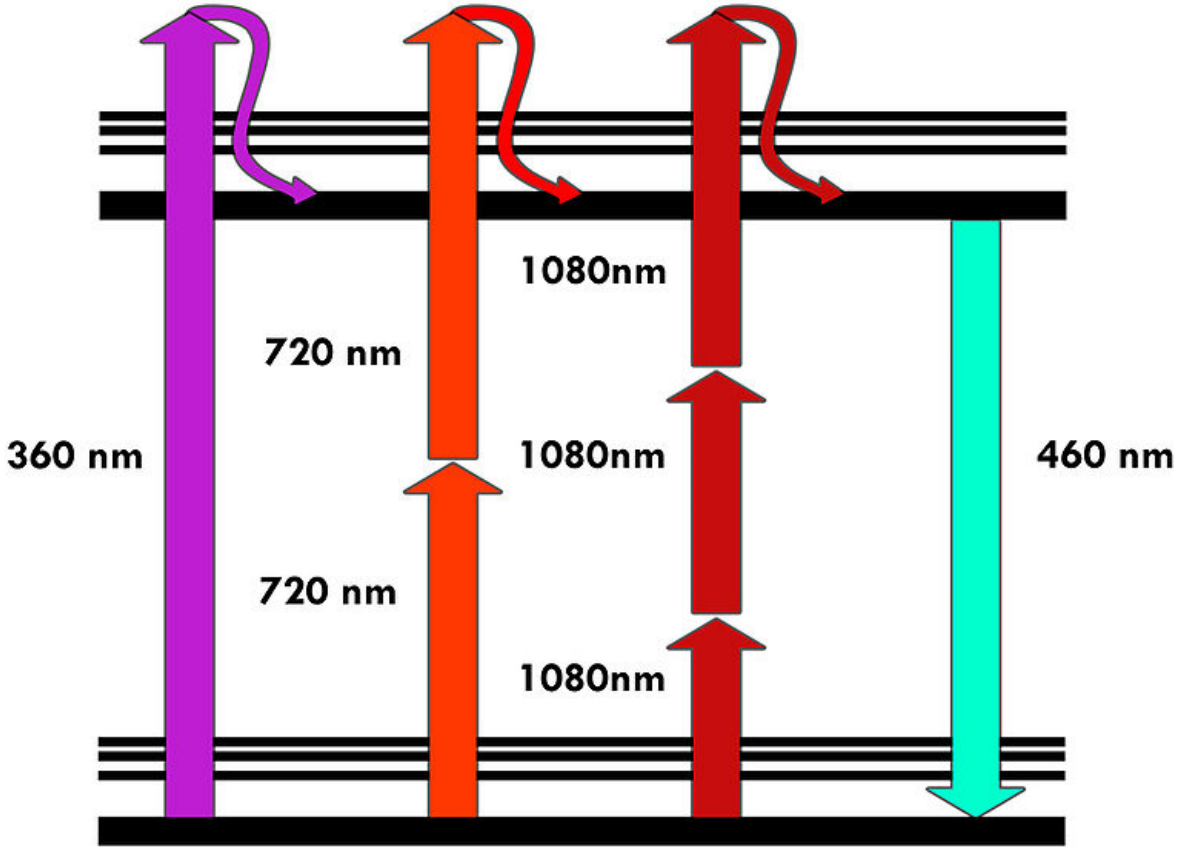
Spinning Disk Mikroskopie

Strahlengang



Multiphotonenmikroskopie

Multiphotonenanregung



Multiphotonenmikroskopie

Maria Göppert Mayer



Über Elementarakte mit zwei Quantensprüngen

Von Maria Göppert-Mayer

(Göttinger Dissertation)

(Mit 5 Figuren)

Einleitung

Der erste Teil dieser Arbeit beschäftigt sich mit dem Zusammenwirken zweier Lichtquanten in einem Elementarakt. Mit Hilfe der Diracschen Dispersionstheorie[1]) wird die Wahrscheinlichkeit eines dem Ramaneffekt analogen Prozesses, nämlich der Simultanemission zweier Lichtquanten, berechnet. Es zeigt sich, daß eine Wahrscheinlichkeit dafür besteht, daß ein angeregtes Atom seine Anregungsenergie in zwei Lichtquanten aufteilt, deren Energien in Summe die Anregungsenergie ergeben, aber sonst beliebig sind. Fällt auf das Atom Licht, dessen Frequenz kleiner ist, als die entsprechende Eigenfrequenz des Atoms, so tritt außerdem noch eine erzwungene Doppelemission hinzu, bei der das Atom seine Energie in ein Lichtquant der eingesandten und eins der Differenzfrequenz aufteilt. Kramers und Heisenberg[2]) haben die Wahrscheinlichkeit dieses letzteren Prozesses korrespondenzmäßig berechnet.

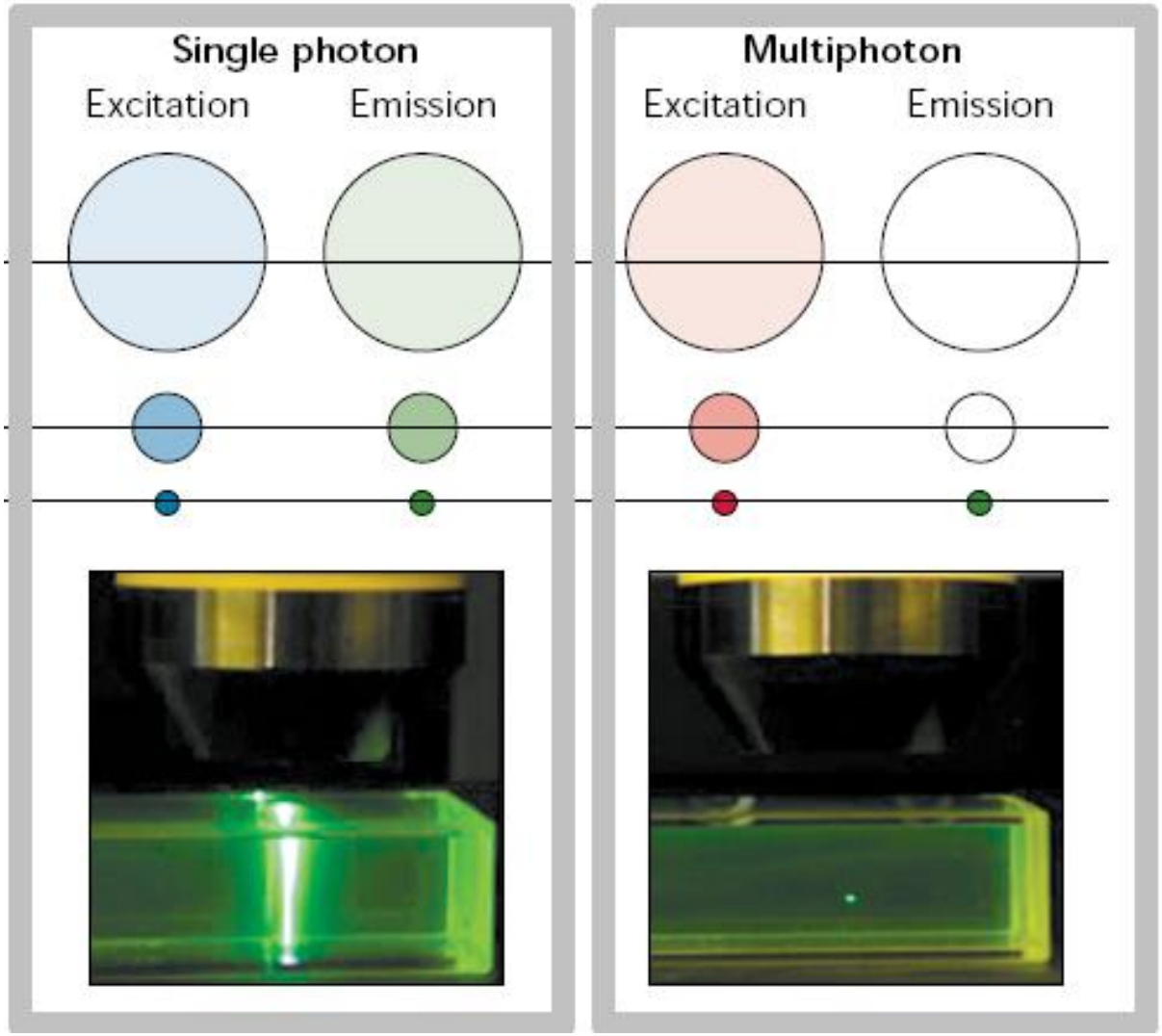
Außerdem wird die Umkehrung dieses Prozesses betrachtet, nämlich der Fall, daß zwei Lichtquanten, deren Frequenzsumme gleich der Anregungsfrequenz des Atoms ist, zusammenwirken, um das Atom anzuregen.

Ferner wird untersucht, wie sich ein Atom gegenüber stoßenden Teilchen verhalten kann, wenn es gleichzeitig die Möglichkeit hat, spontan Licht zu emittieren. Oldenberg[3]) findet experimentell eine Verbreiterung der Resonanzlinie des Quecksilbers, wenn er die angeregten Atome vielfach mit lang-

1) P. A. M. Dirac, Proc. of R. S. vol. **114**. S. 143 u. 710. 1927.
2) H. A. Kramers u. W. Heisenberg, Ztschr. f. Phys. **31**. S. 681. 1925.
3) O. Oldenberg, Ztschr. f. Phys. **51**. S. 605. 1928.

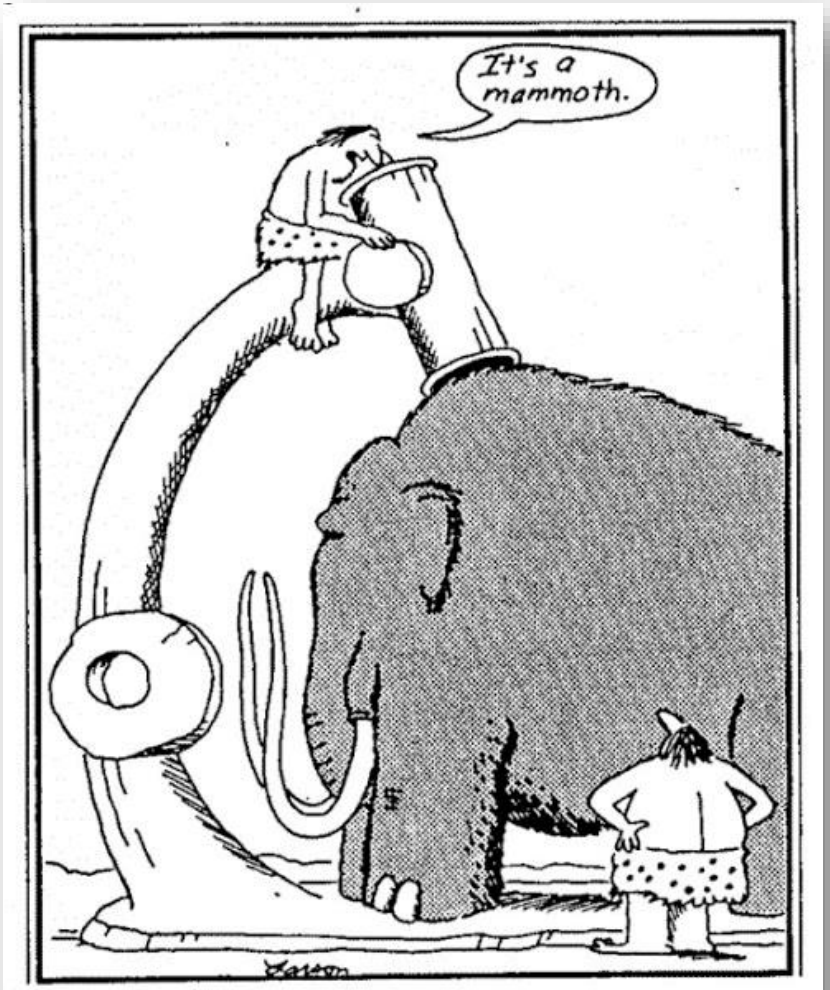
Multiphotonenmikroskopie

Multiphotonenanregung



Weitere Techniken

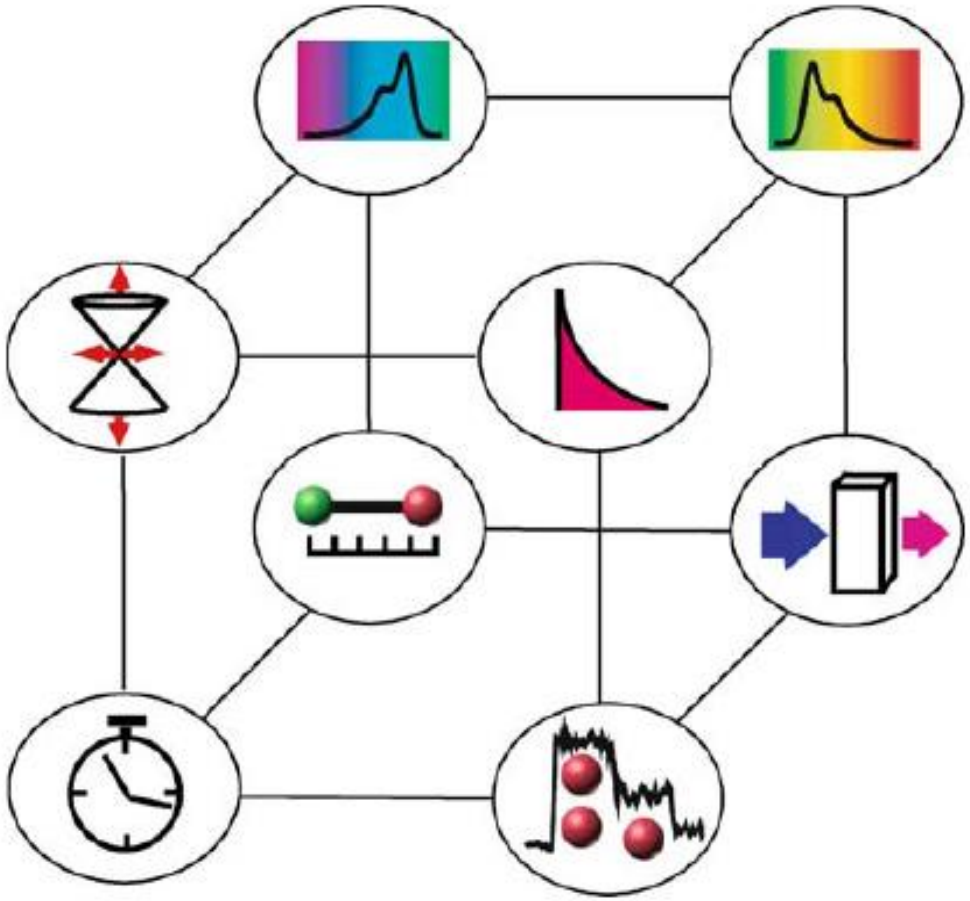
Gibt es hier noch etwas Neues zu entdecken?



Early microscope

Fluoreszenzmikroskopie

Die verschiedenen Dimensionen der Fluoreszenzmikroskopie

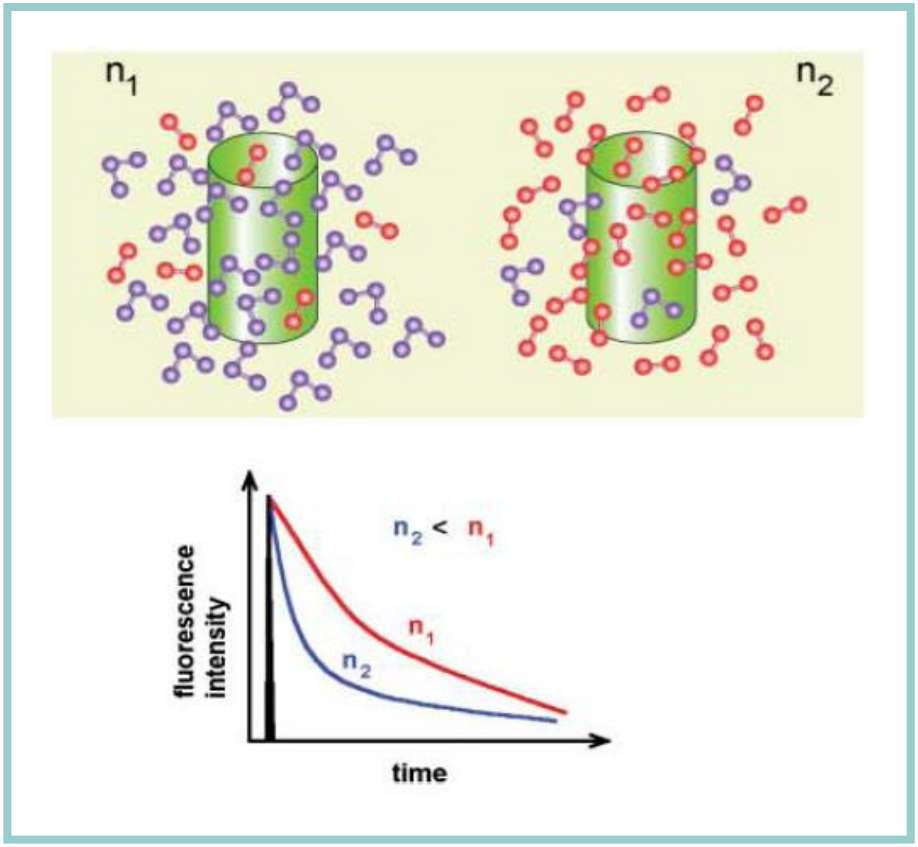


Kudryavtsev, Anal Bioanal Chem 387, 71-82 (2007)

Fluorescence lifetimes

Applications of fluorescence lifetime measurements II

Sensing of the local environment

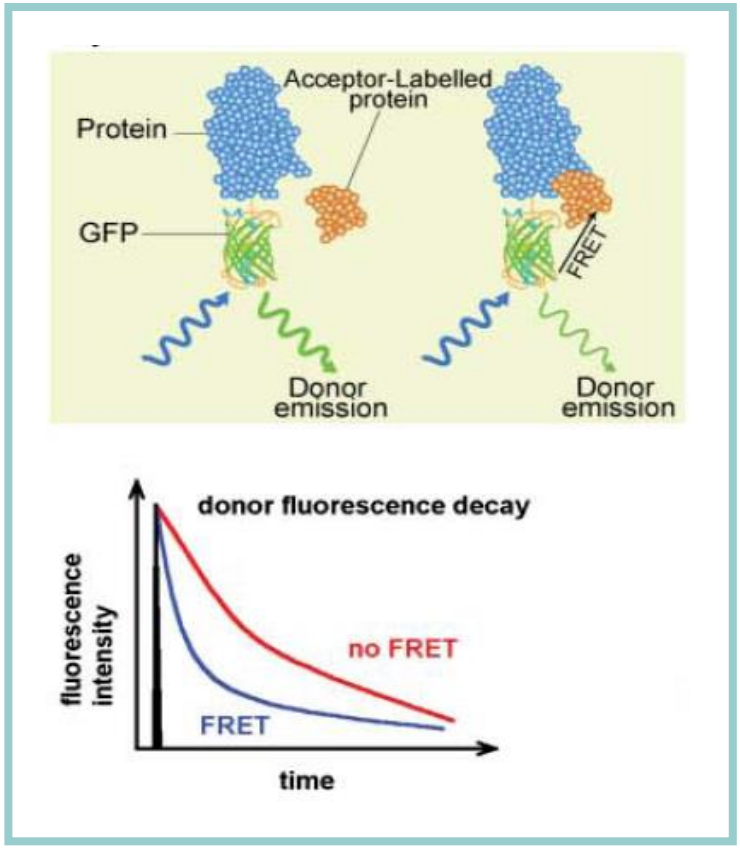


Suhling et al., Photchem. Photobiol Sci 4, 13-22 (2005)

Fluorescence lifetimes

Applications of fluorescence lifetime measurements III

Förster resonance energy transfer (FRET)

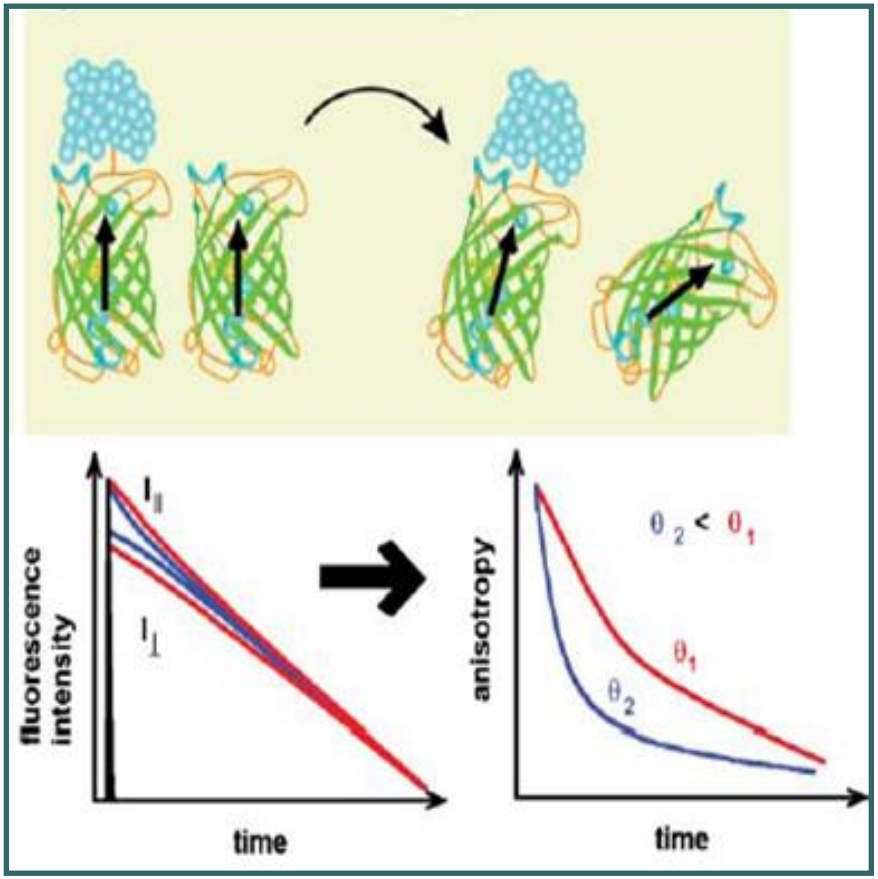


Suhling et al., Photchem. Photobiol Sci 4, 13-22 (2005)

Fluorescence lifetimes

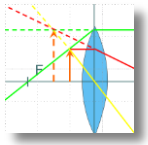
Applications of fluorescence lifetime measurements I

Rotational mobility:



Suhling et al., Photchem. Photobiol Sci 4, 13-22 (2005)

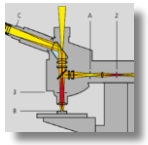
Der Plan für das heutige Seminar



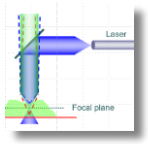
Geometrische Optik



Fluoreszenz



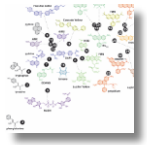
Epifluoreszenzmikroskopie



Laser Scanning Mikroskopie



Superresolution Mikroskopie



Fluorophore

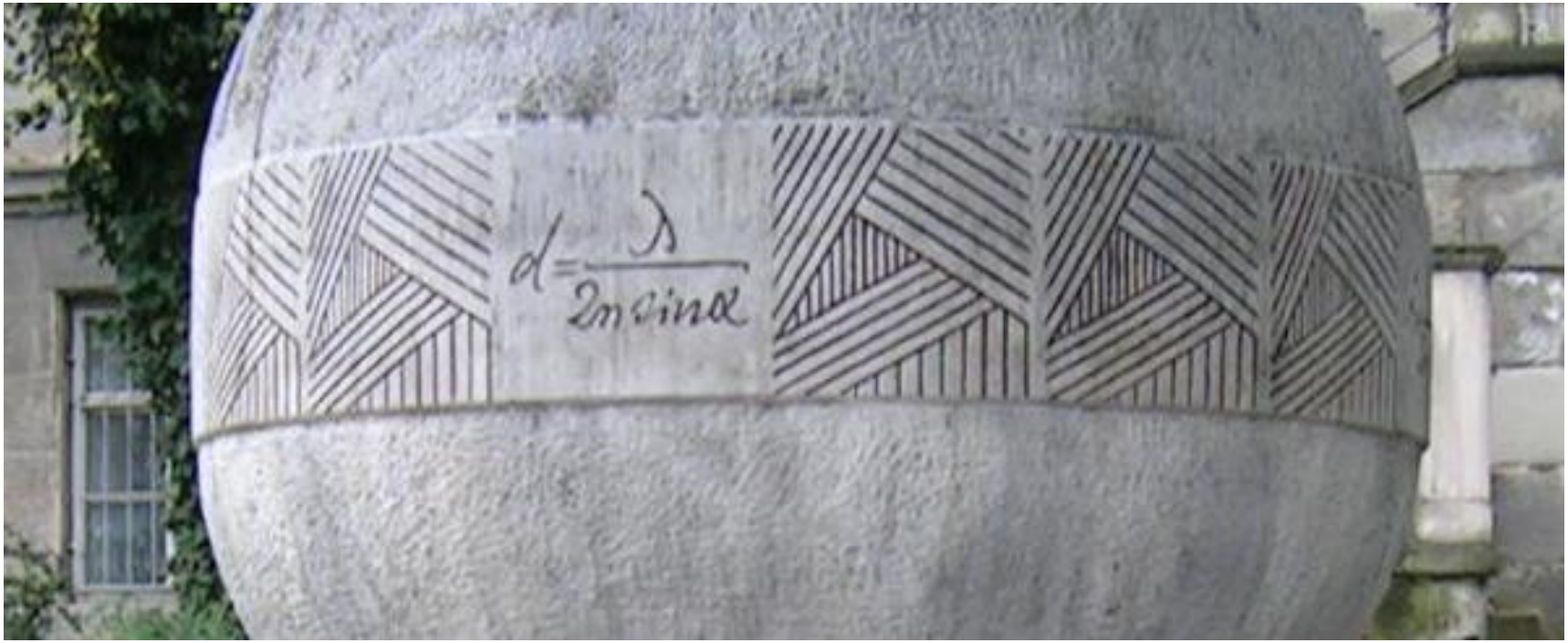
Grenzen der Lichtmikroskoie

Ein kleiner Stadtrundgang…



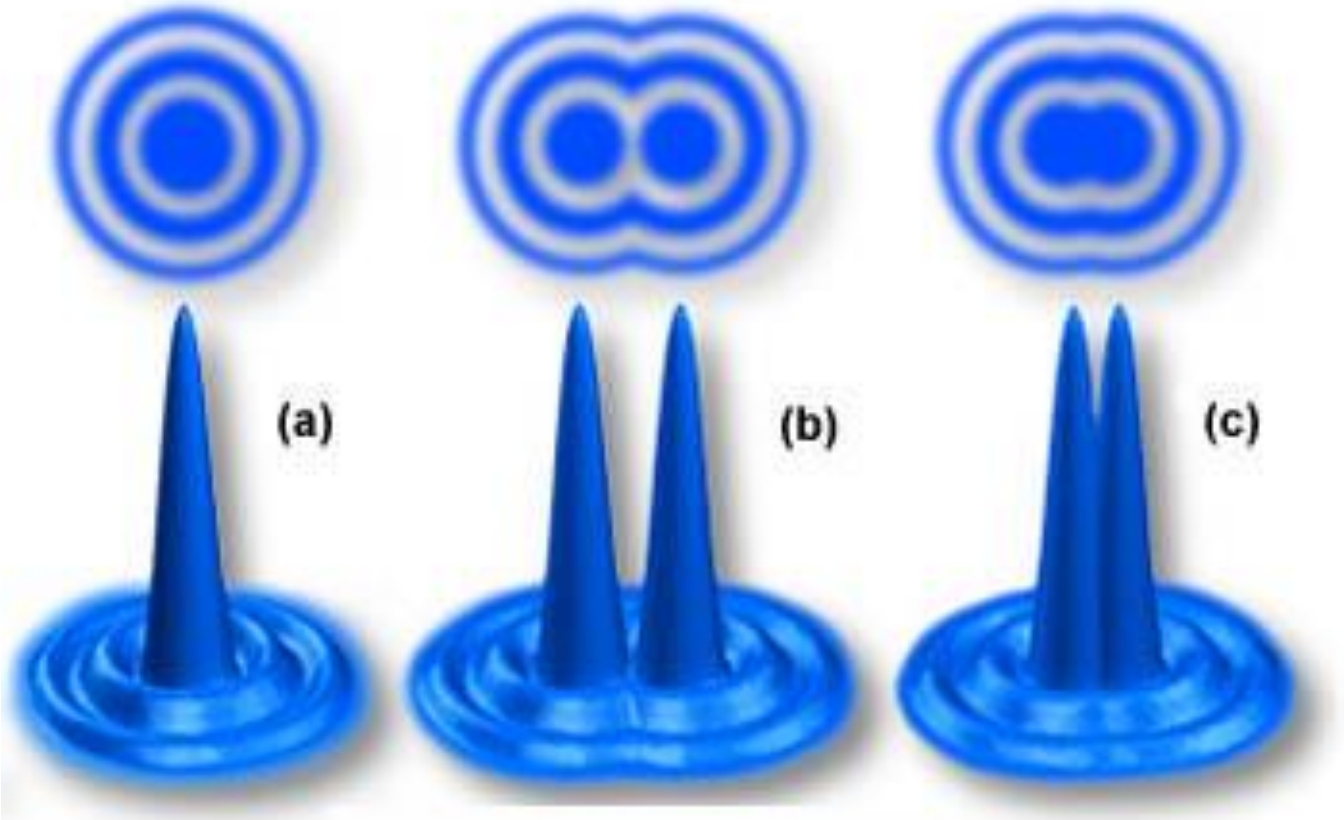
Grenzen der Lichtmikroskopie

Können wir die Grenzen umgehen?



The resolution of a microscope

Intensity distributions of two point sources



Elektronenmikroskopie

TEM
Transmission
Electron Microscopy



High resolution SEM
Scanning Electron
Microscopy



High resolution Cryo-TEM
Cryo-Transmission Electron microscopy





SEM
Scanning Electron Microscopy

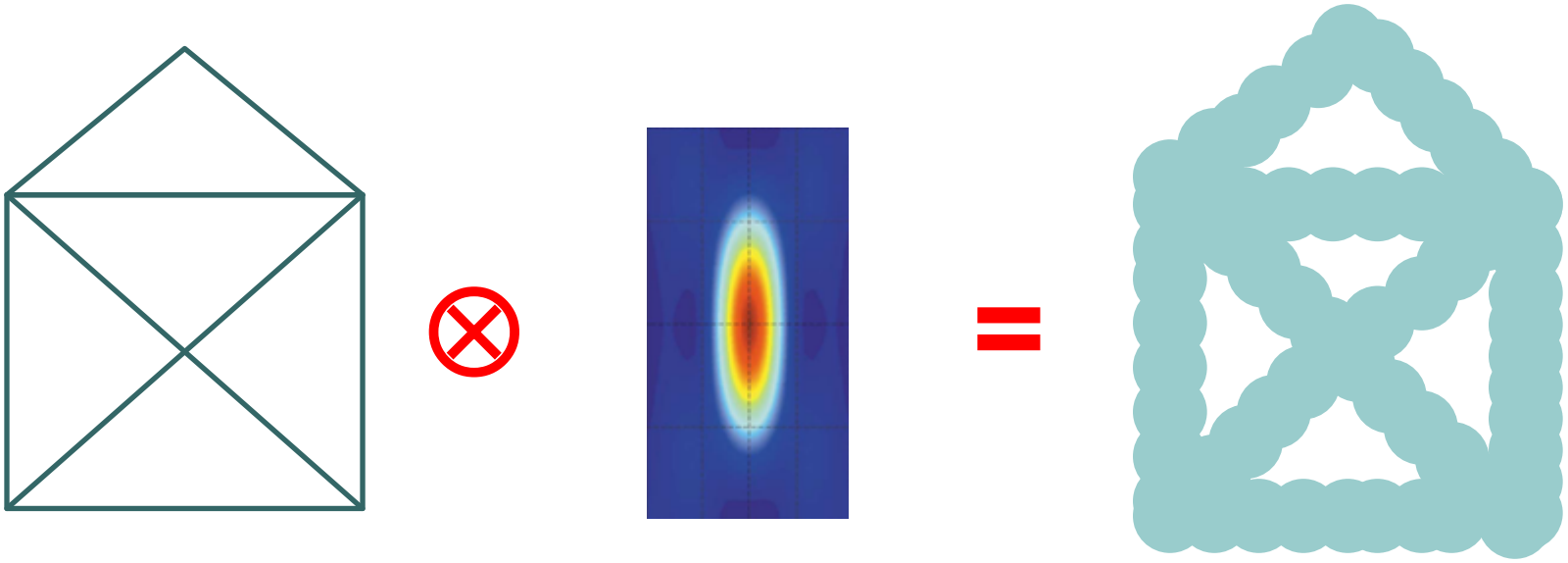
X-Ray
Spectroscopy



Freeze-fracture TEM

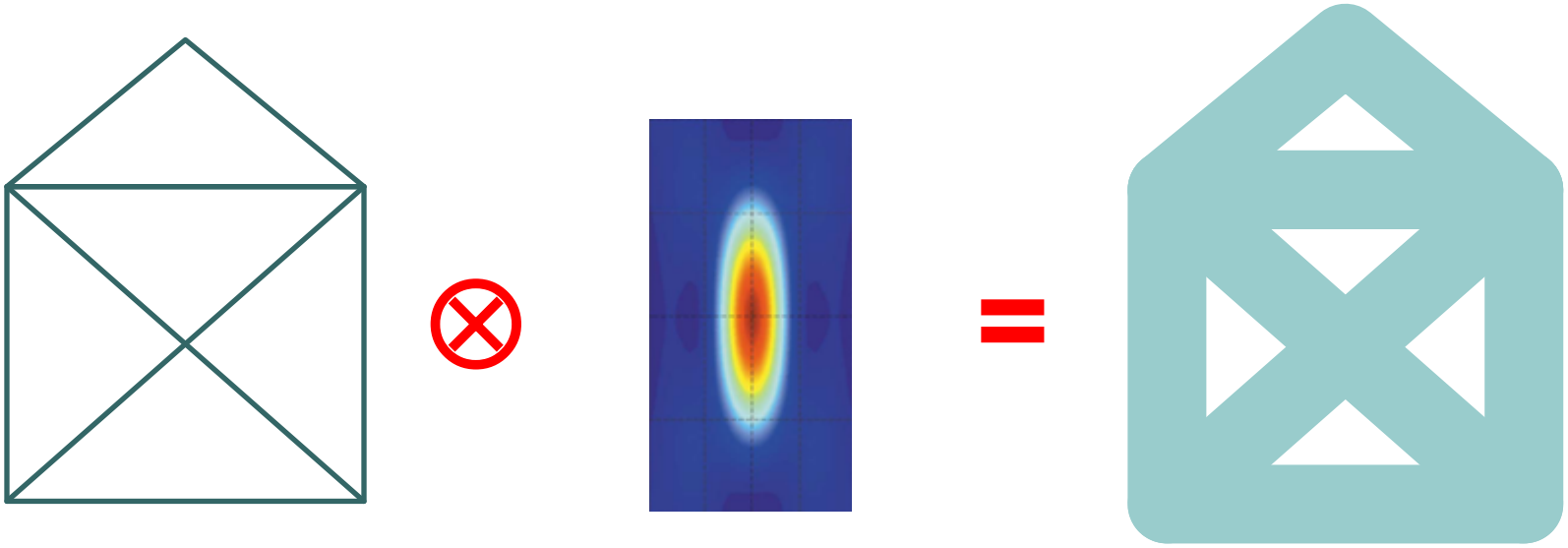
The point spread function

Object PSF Image



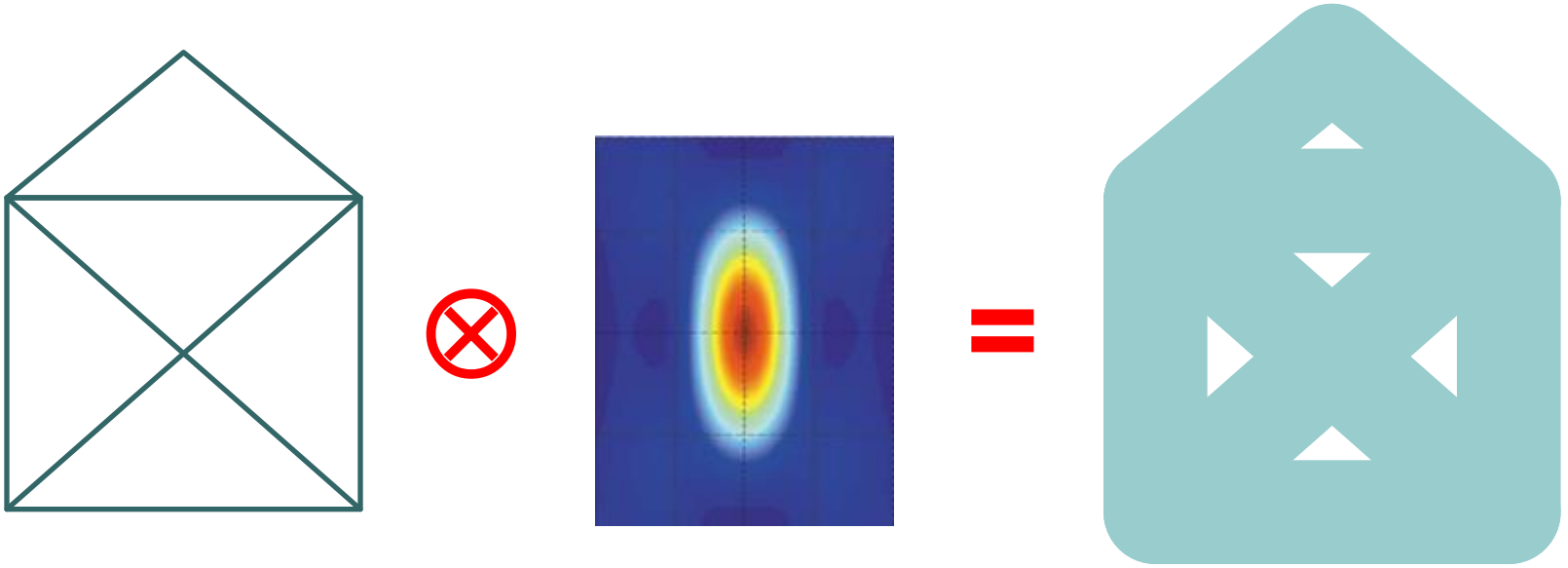
The point spread function

Object PSF Image



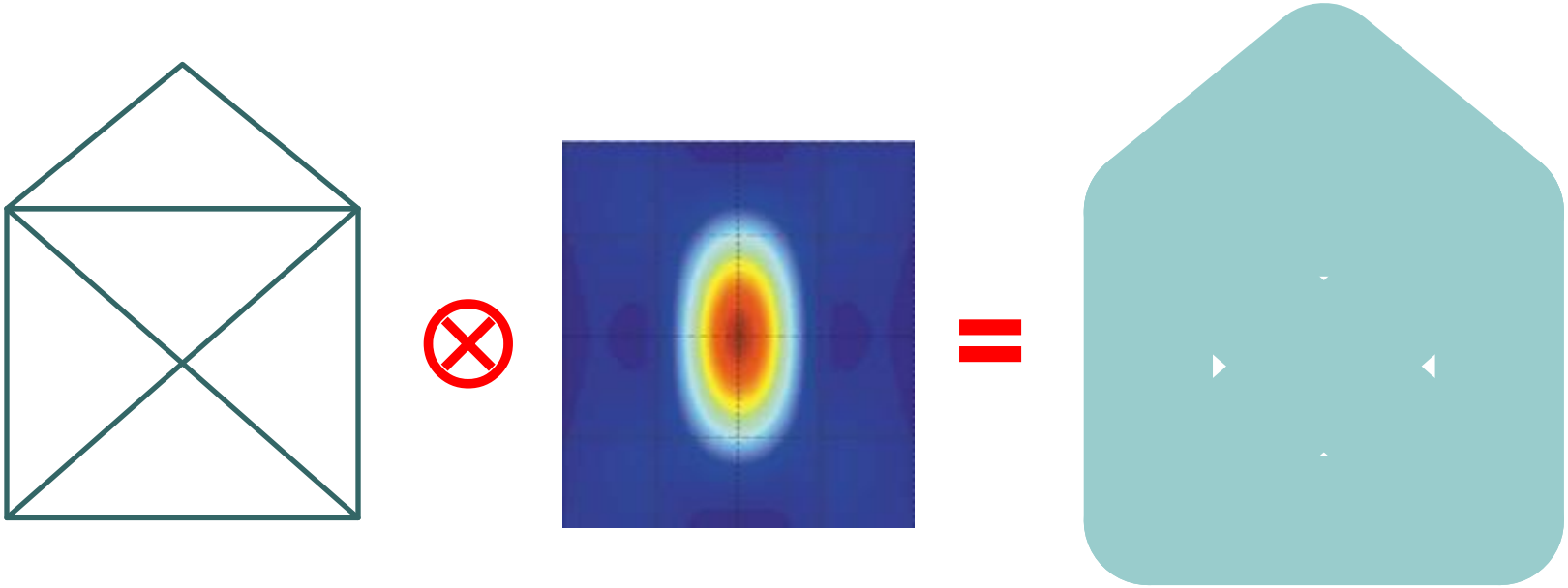
The point spread function

Object PSF Image



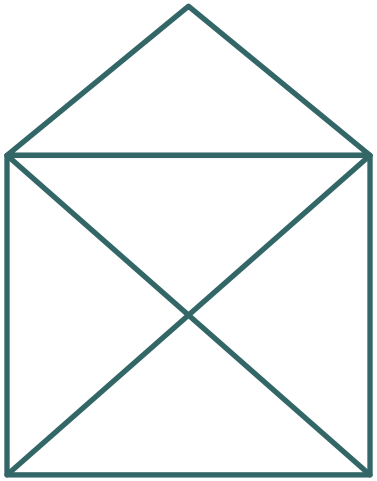
The point spread function

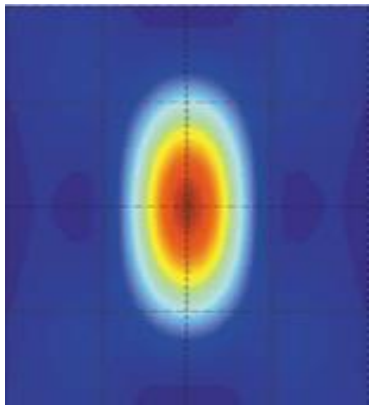
Object PSF Image



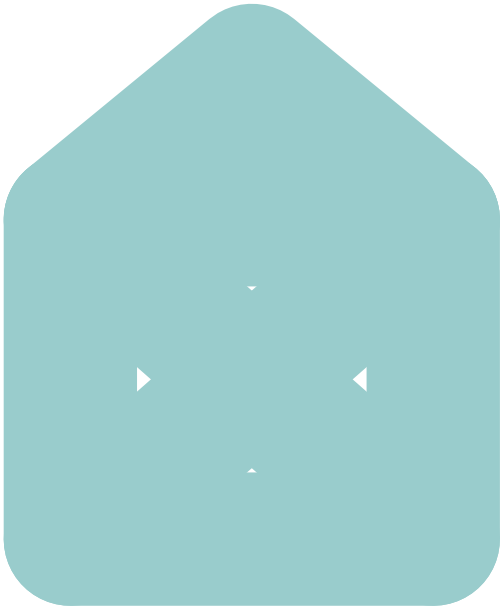
The point spread function

Object





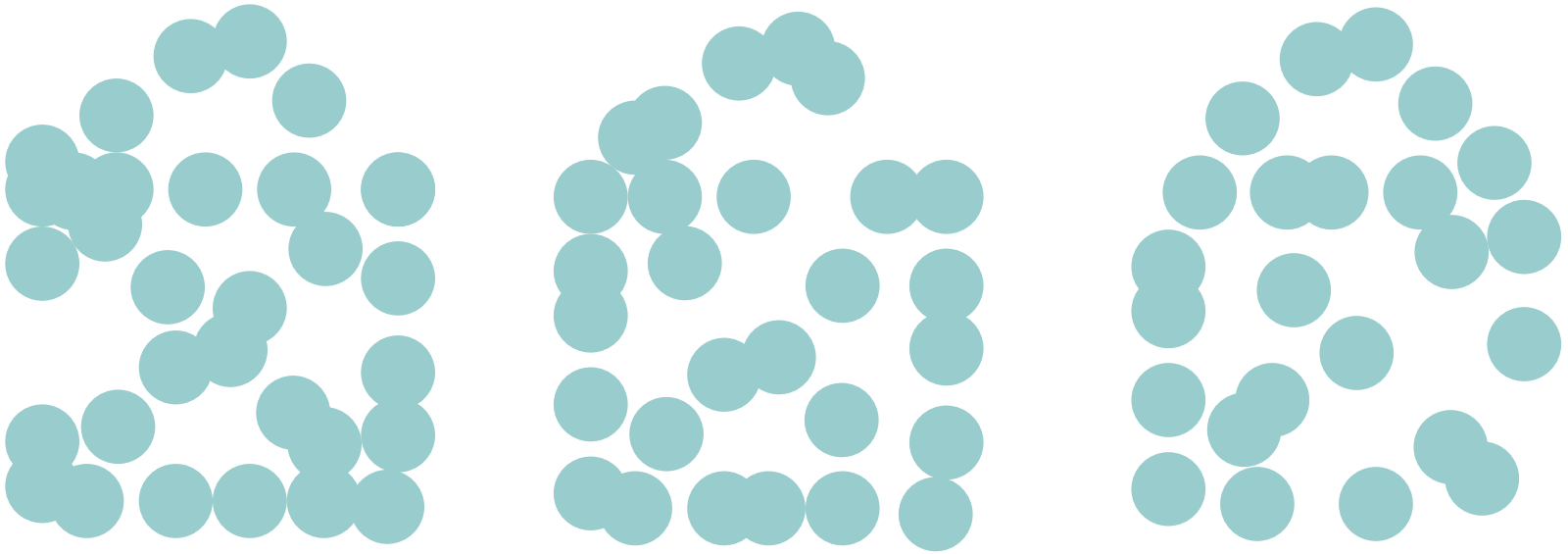
Image



spatial frequency X optical transfer function (OTF) = image frequency

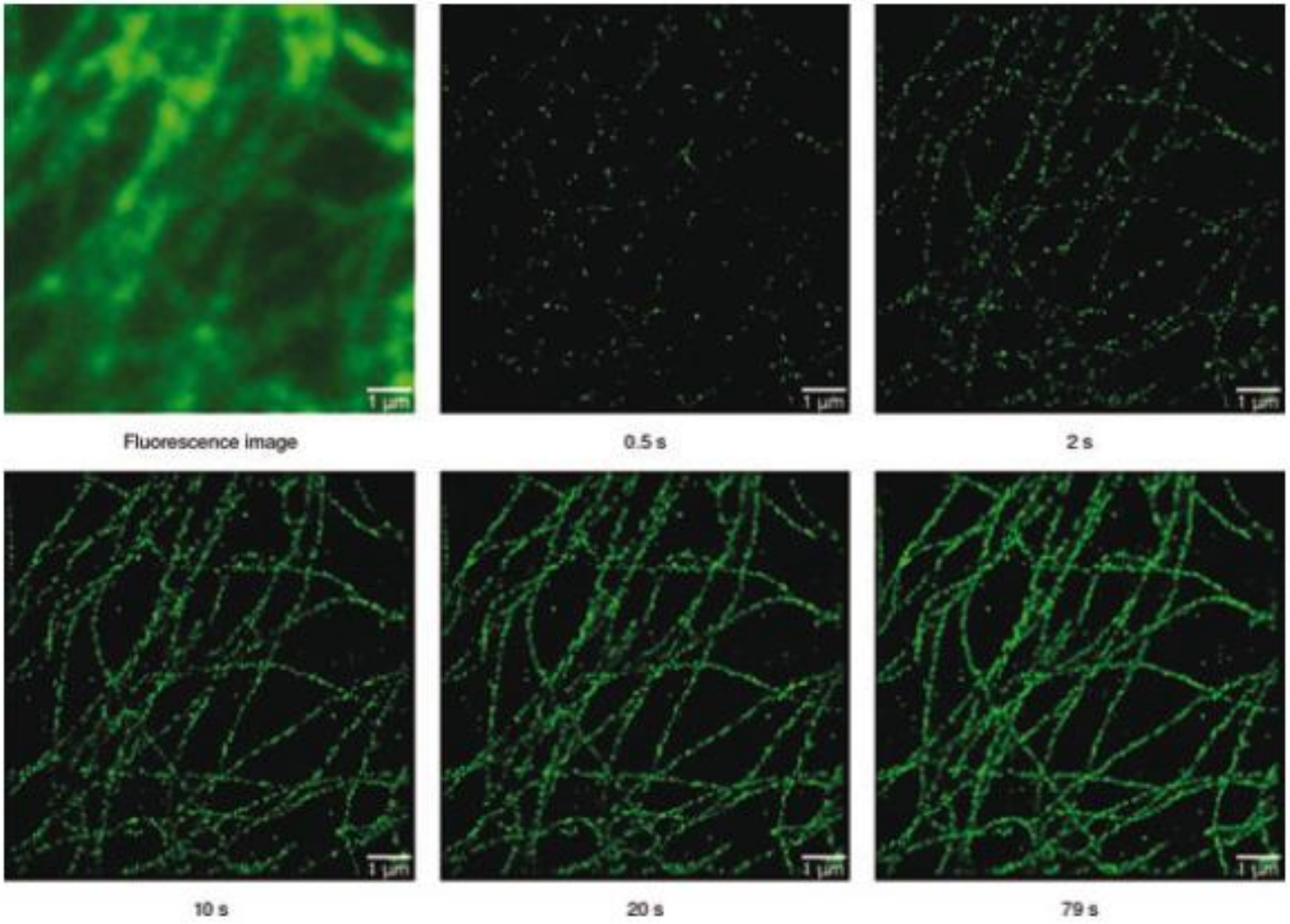
STORM

Stochastic optical reconstruction *microscopy* (*STORM*)



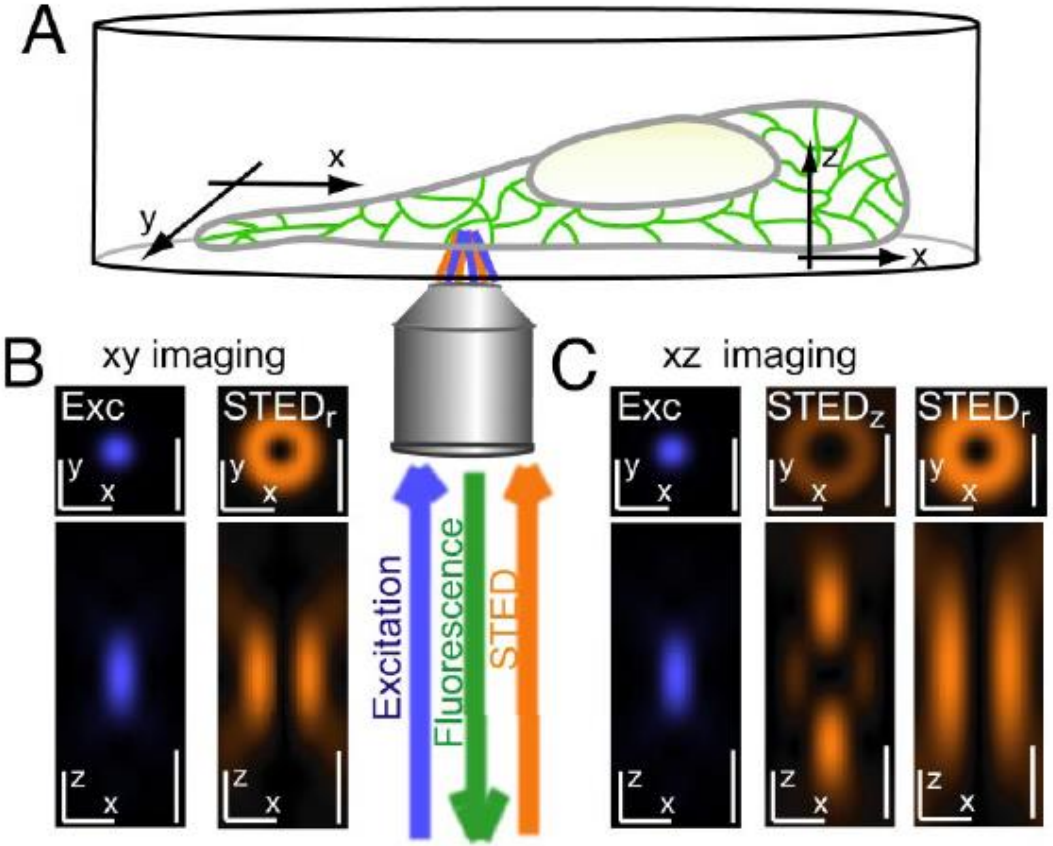
STORM

The principle



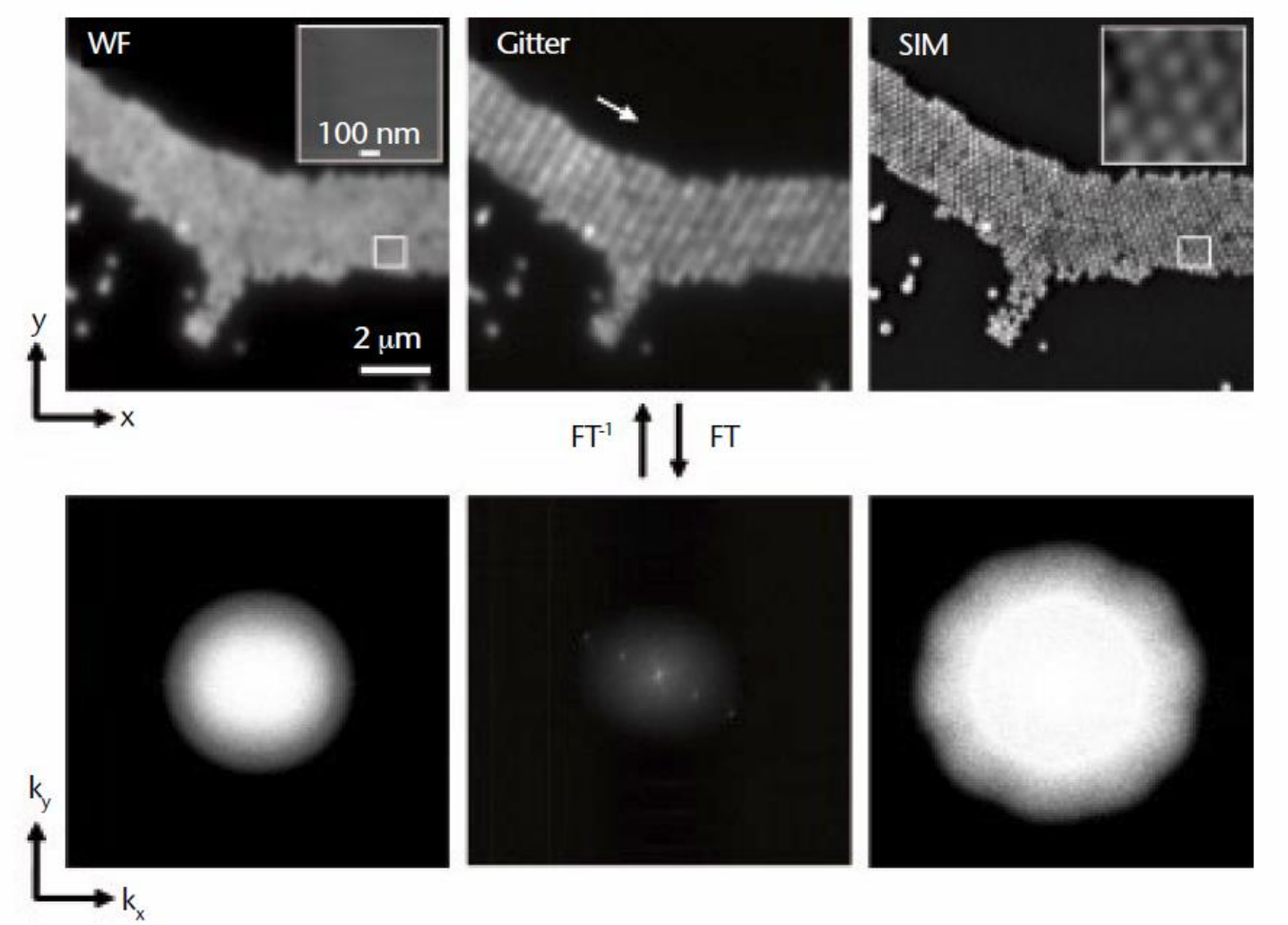
Stimulated emission depletion (STED)

How it works…



Structured illumination

The principle



Mikroskopische Techniken

Nobelpreis für Chemie 2014





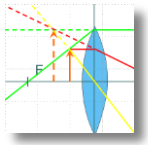






Quelle: https://www.nobelprize.org/nobel_prizes/chemistry/laureates/2014/

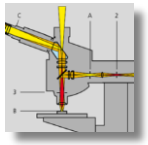
Der Plan für das heutige Seminar



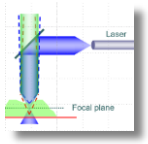
Geometrische Optik



Fluoreszenz



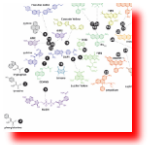
Epifluoreszenzmikroskopie



Laser Scanning Mikroskopie



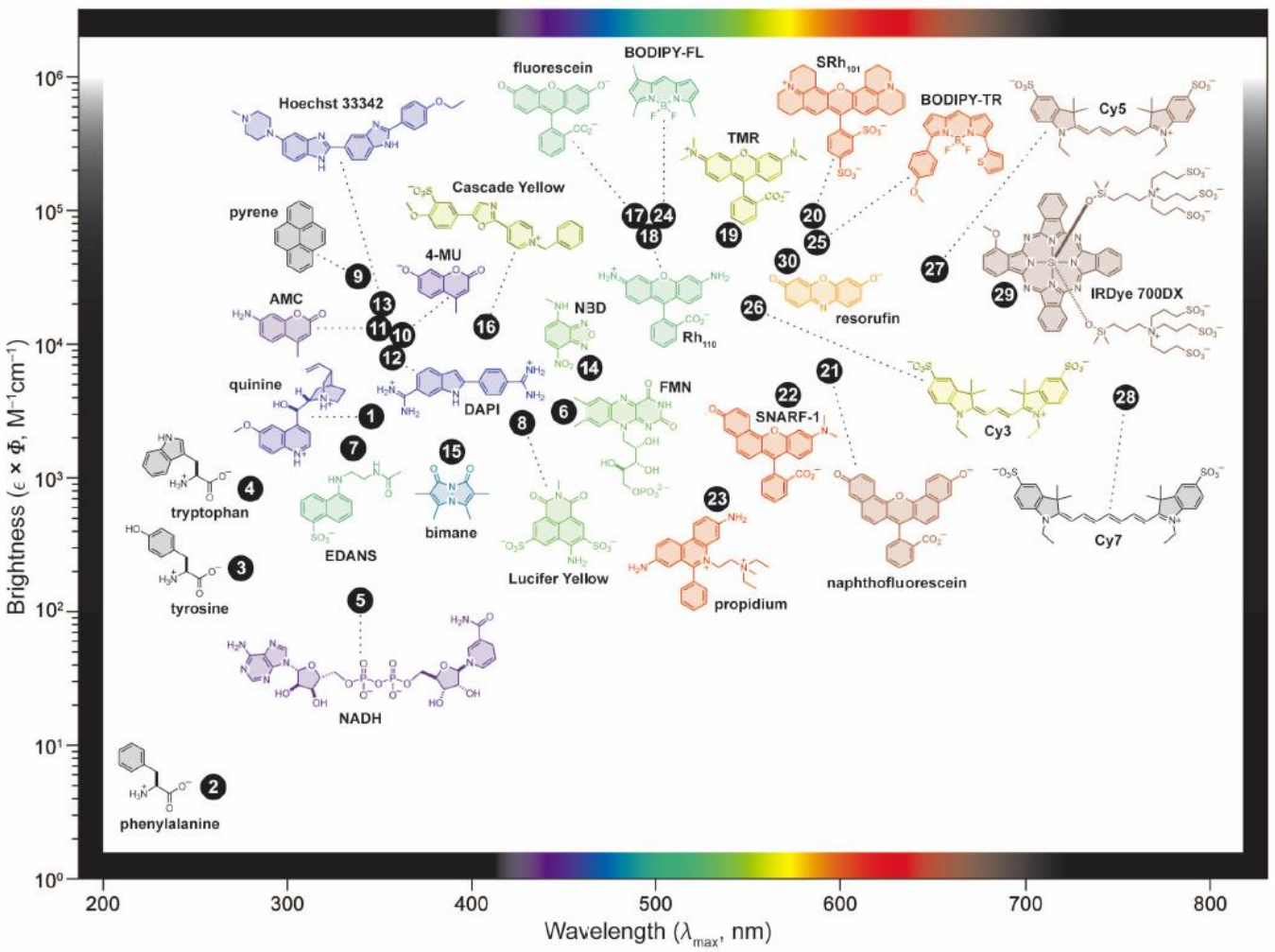
Superresolution Mikroskopie



Fluorophore

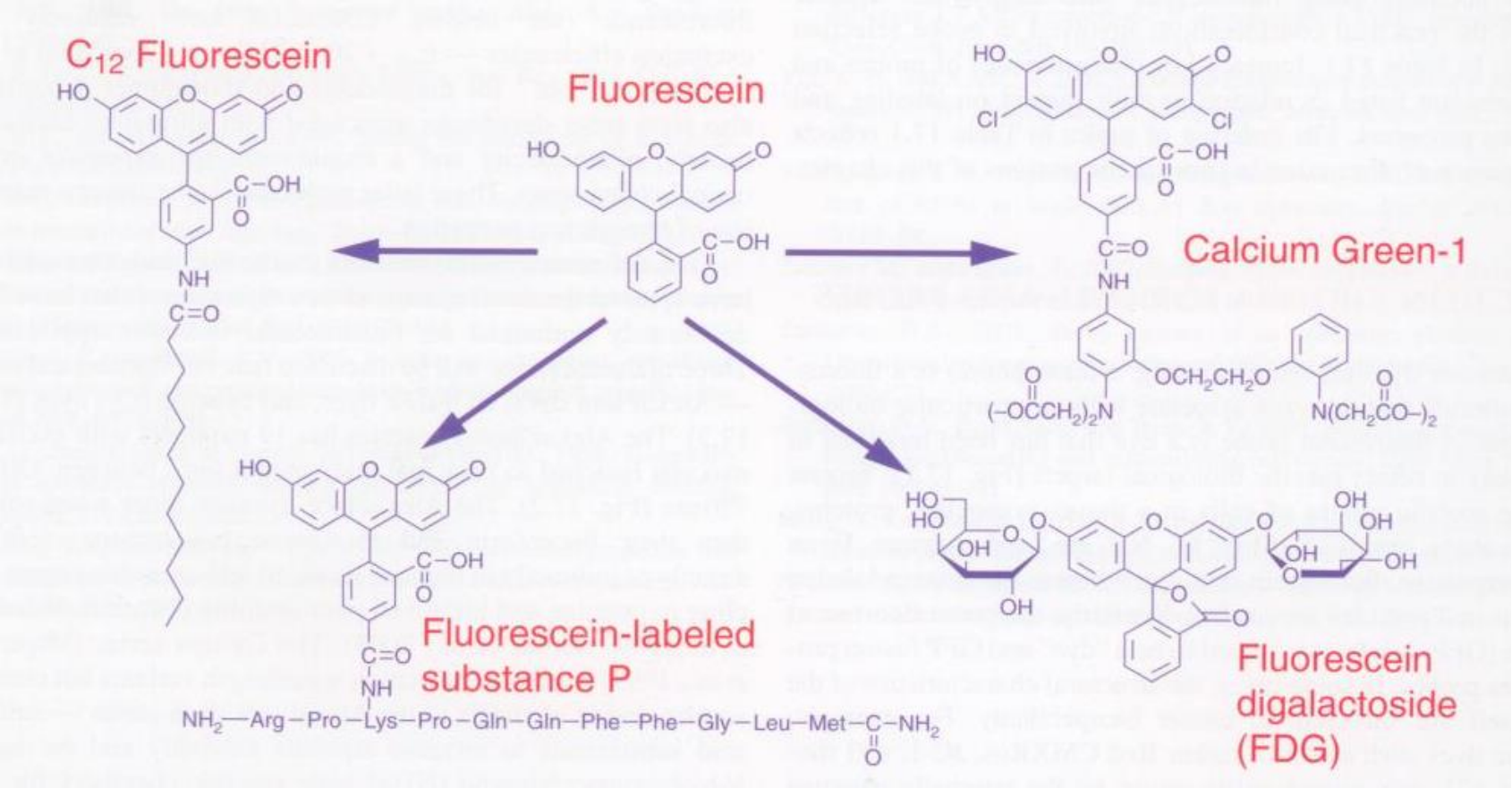
Fluorophore für die Mikroscopie

Übersicht



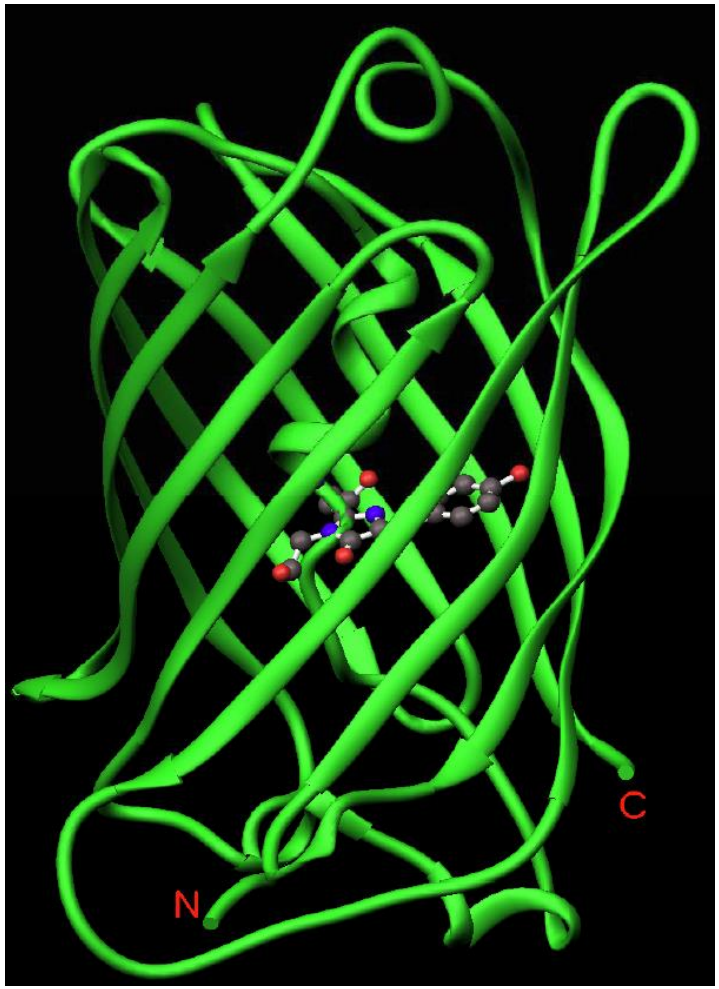
L.D. Lavis, R.T. Raines: Bright ideas for chemical biology. ACS Chemical Biology 3, 142-155 (2008)

Funktionalisierung



Green fluorescent protein (GFP)

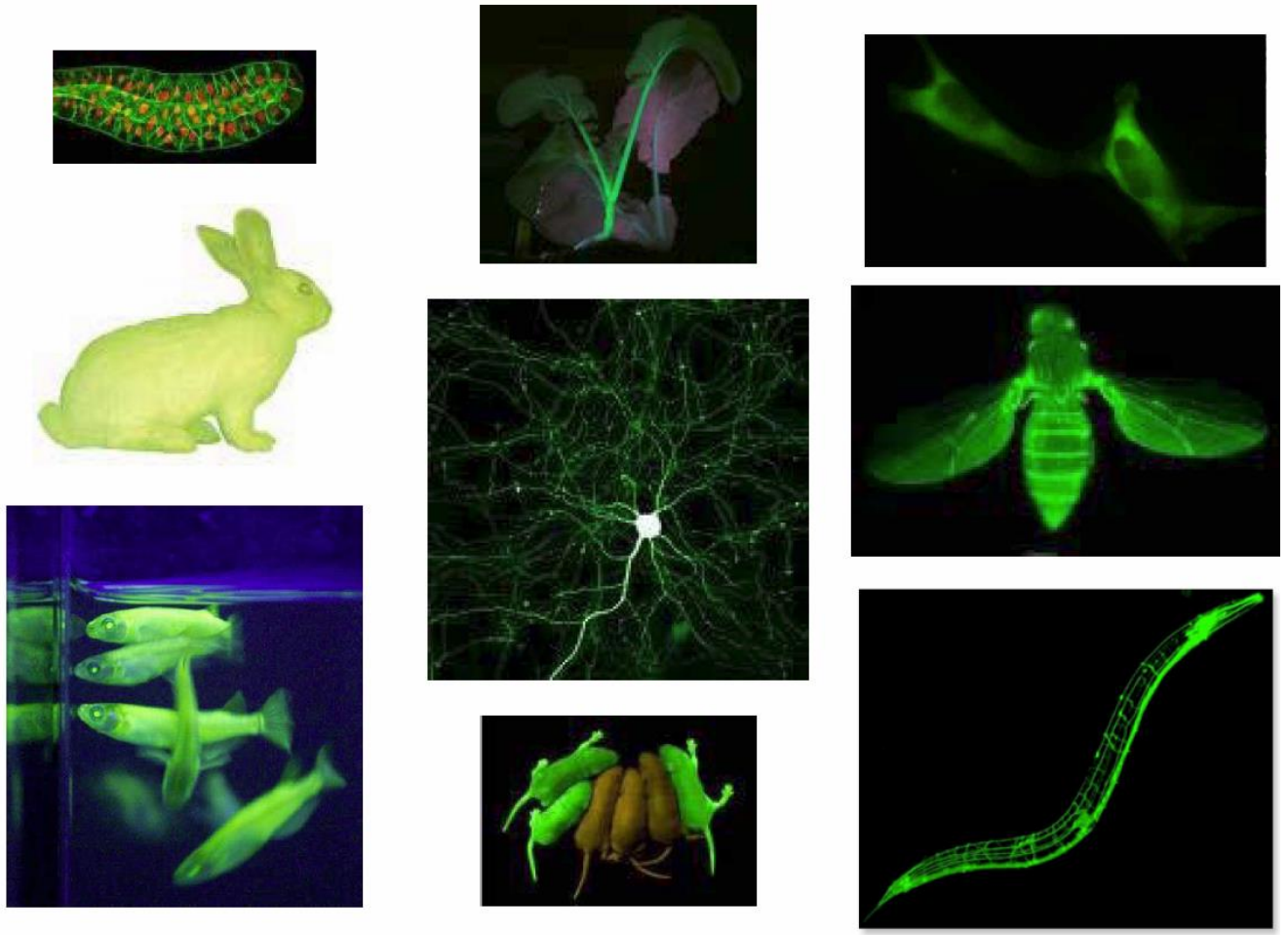
Die Kristallstruktur von S65T GFP (EGFP)



Source: Nobel lecture of Roger Y. Tsien, www.nobelprize.org

Green fluorescent protein (GFP)

Anwendungen



Source: Nobel lecture of Roger Y. Tsien, www.nobelprize.org

Green fluorescent protein (GFP)

Weitere Anwendungen?

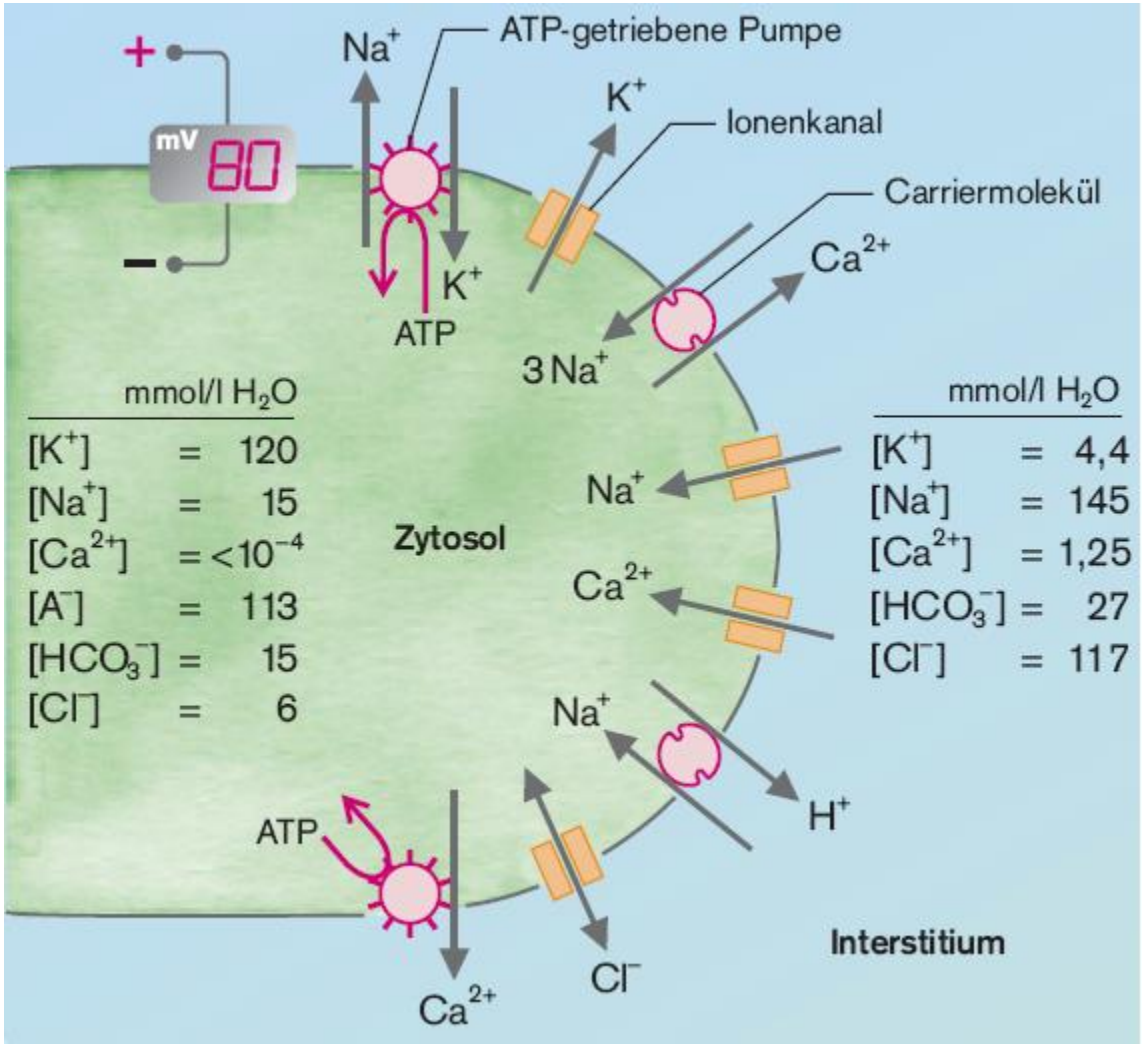






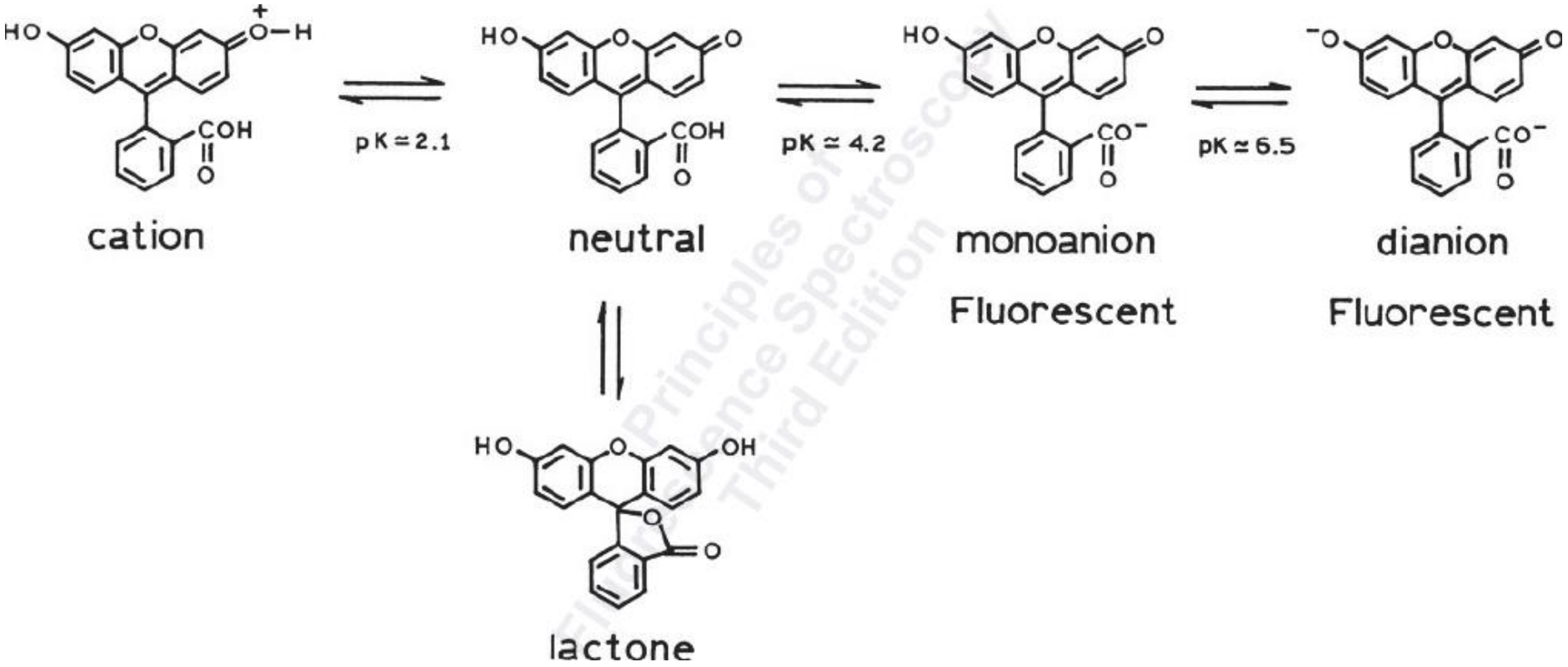
Mikroskopische Sonden

Ein Rückblick in die Physiologie



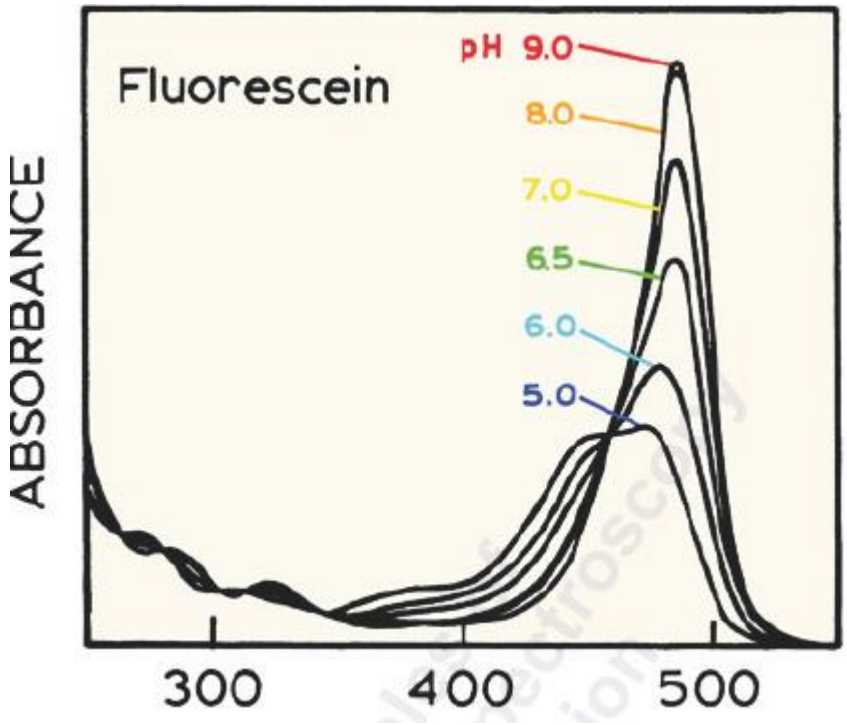
pH-Messung

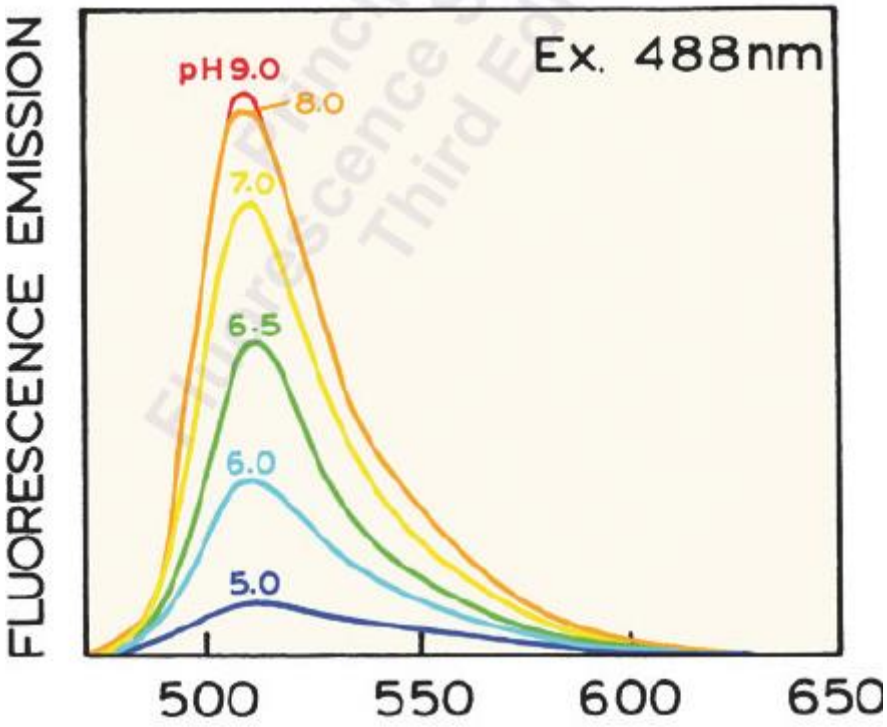
pH-dependent ionization of fluorescein



pH-Sensing

pH-dependent ionization of fluorescein

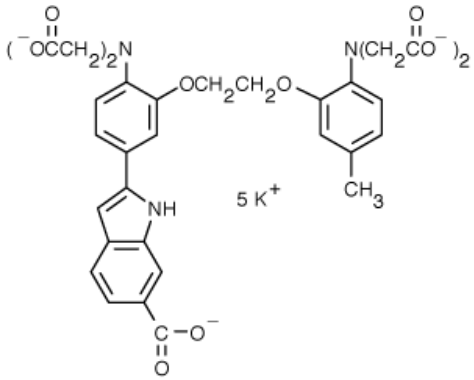




Calcium sensing

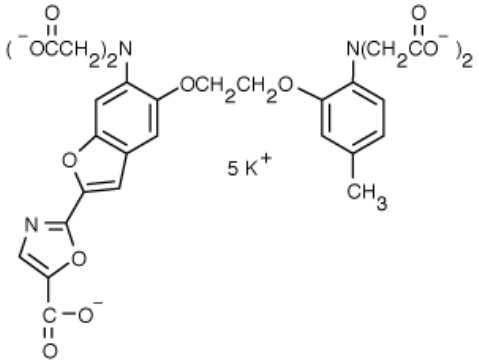
Calcium indicators

Indo-1



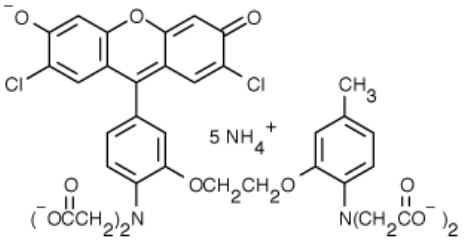
Abs = 346 nm
Em = 475 nm
Kd = 230 nM

Fura-2



Abs = 363/335 nm
Em = 512/505 nm
Kd = 145 nM

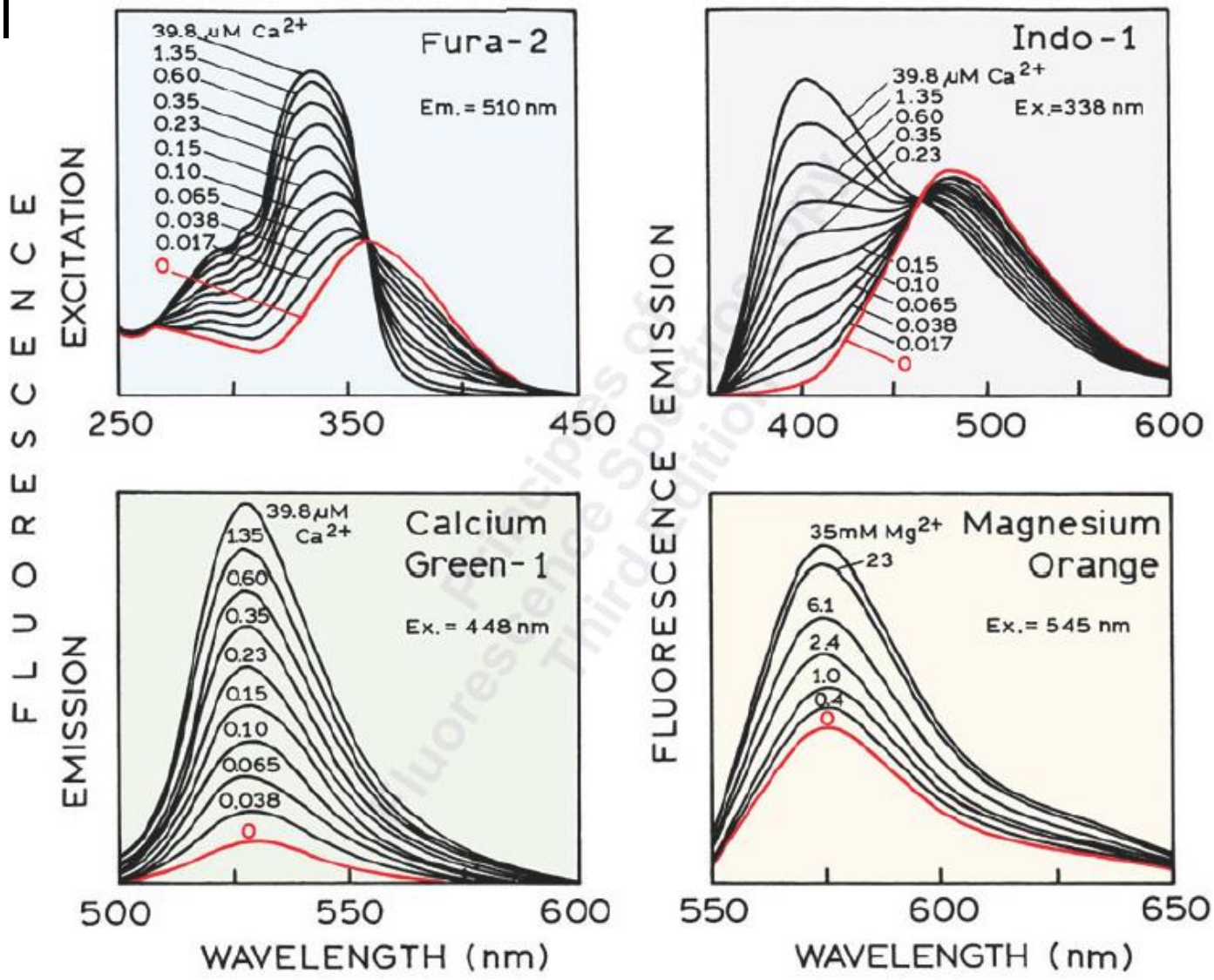
Fluo-3



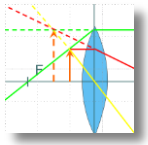
Abs = 503/505 nm
Em = 505 nm
Kd = 390 mM

Calcium sensing

Calcium indicators



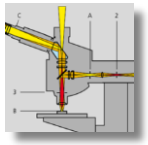
Der Plan für das heutige Seminar



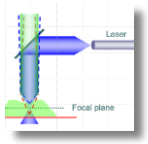
Geometrische Optik



Fluoreszenz



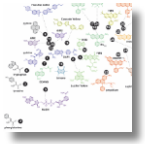
Epifluoreszenzmikroskopie



Laser Scanning Mikroskopie



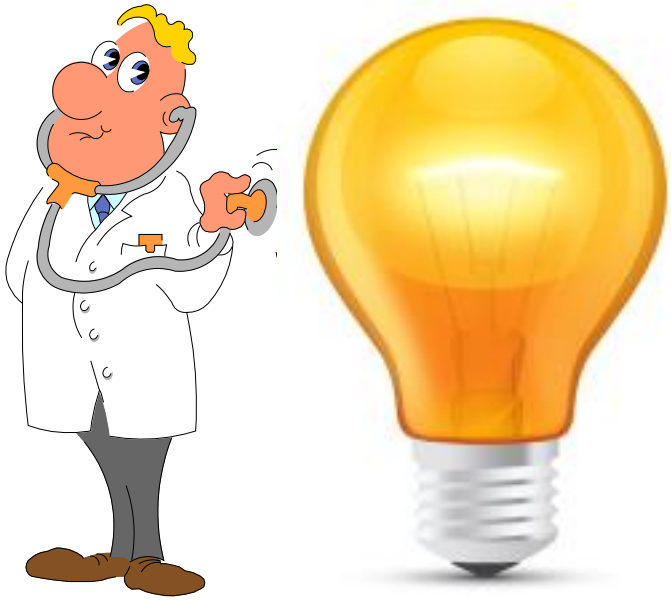
Superresolution Mikroskopie



Fluorophore

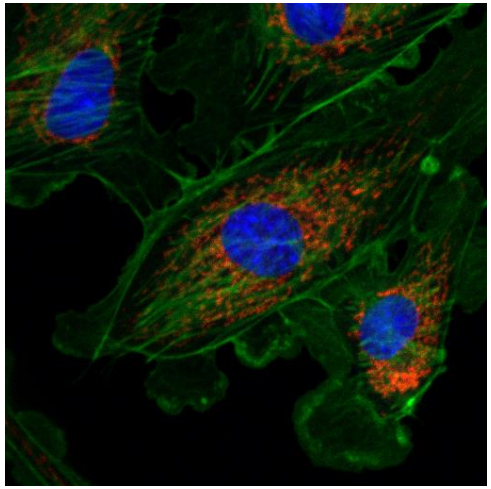
Sie wollen noch mehr über bildgebende Methoden wissen?

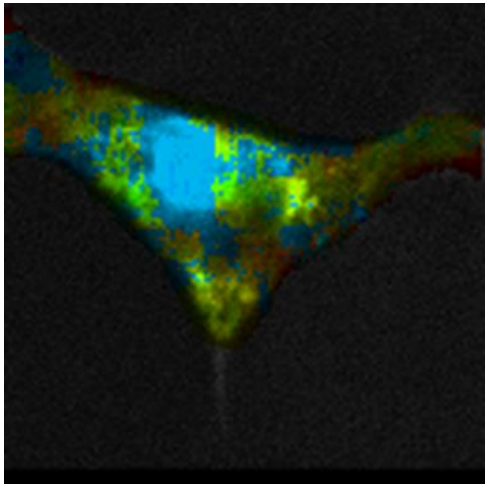
M.Sc. Medical Photonics



M.Sc. Medical Photonics

Biomedizinische Forschung







M.Sc. Medical Photonics

Medizinische Diagnostik

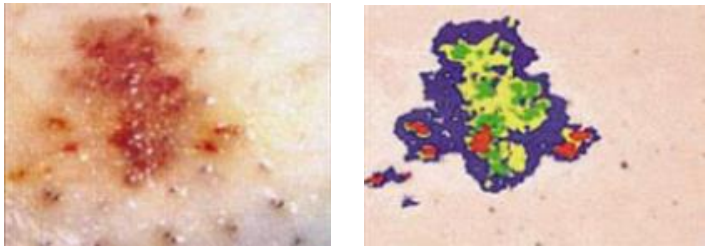




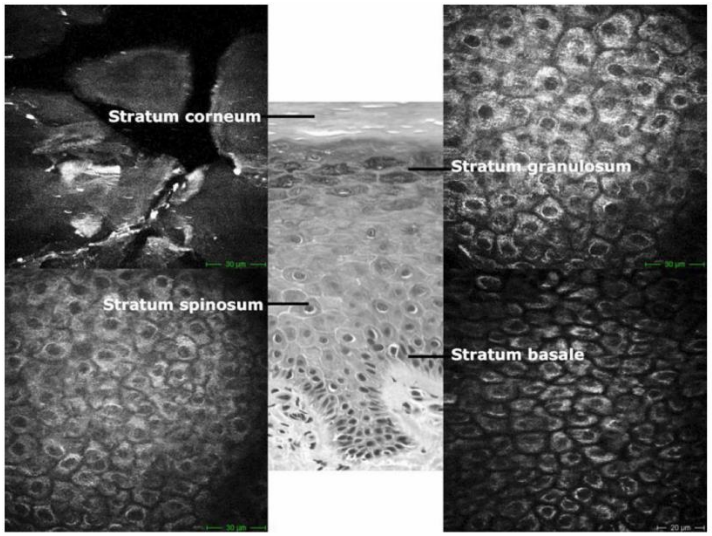
M.Sc. Medical Photonics

Medizinische Diagnostik

Dermatologie



Frakas et al. Pigment Cell Res 14, 2-8 (2002)

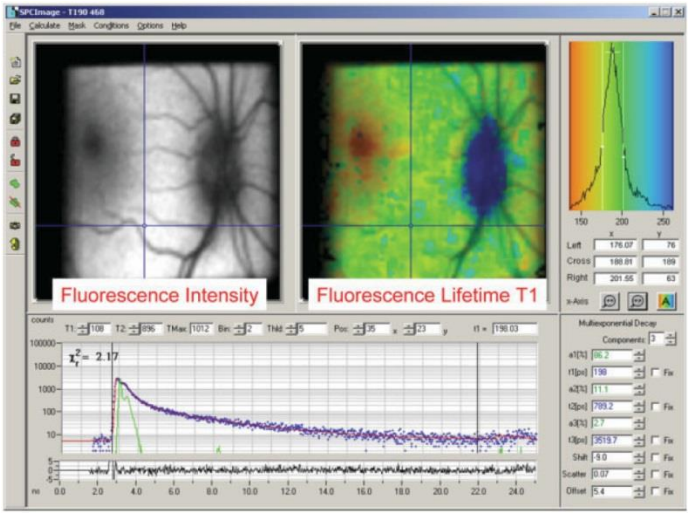


Kaatz, Hautarzt 61, 397–409 (2010)

Ophthalmologie



Canadian Neoro-Ophthalmology Group

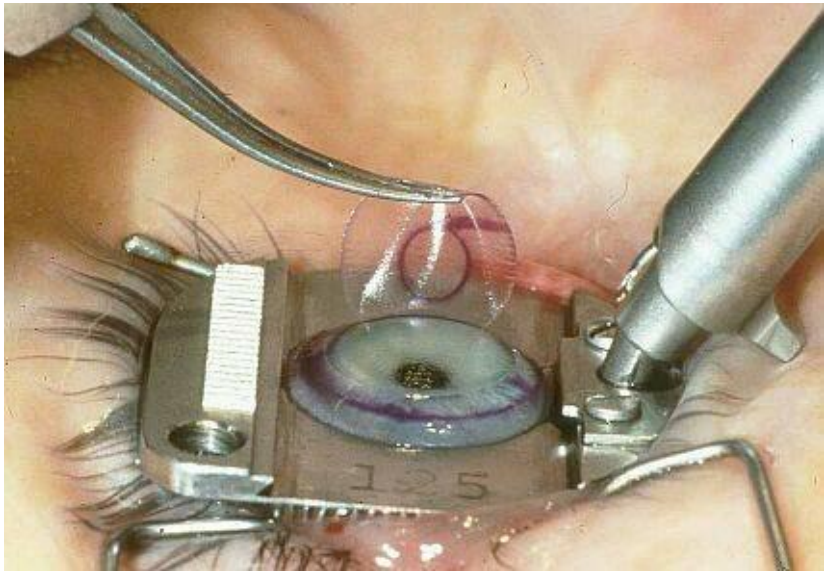


Schweitzer, Microsc Res Tech 70, 410-419 (2007)

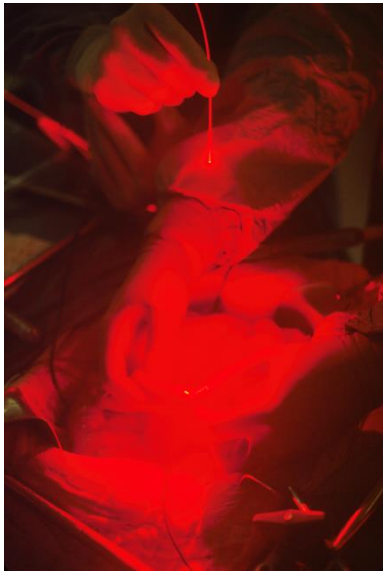
M.Sc. Medical Photonics

Therapie

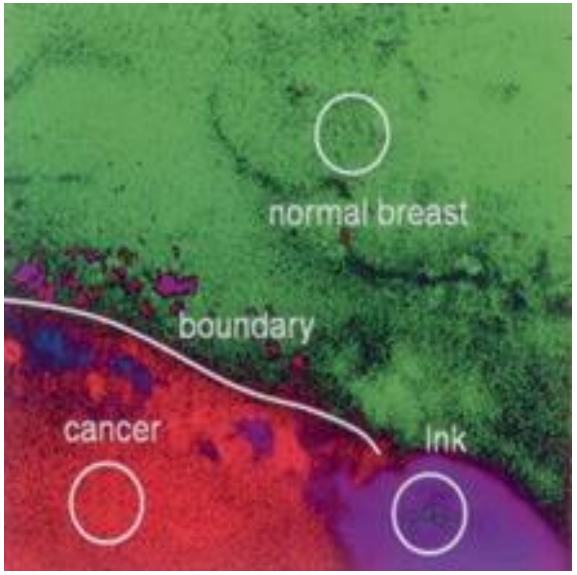
Augenheilkunde



Photodynamische Therapie

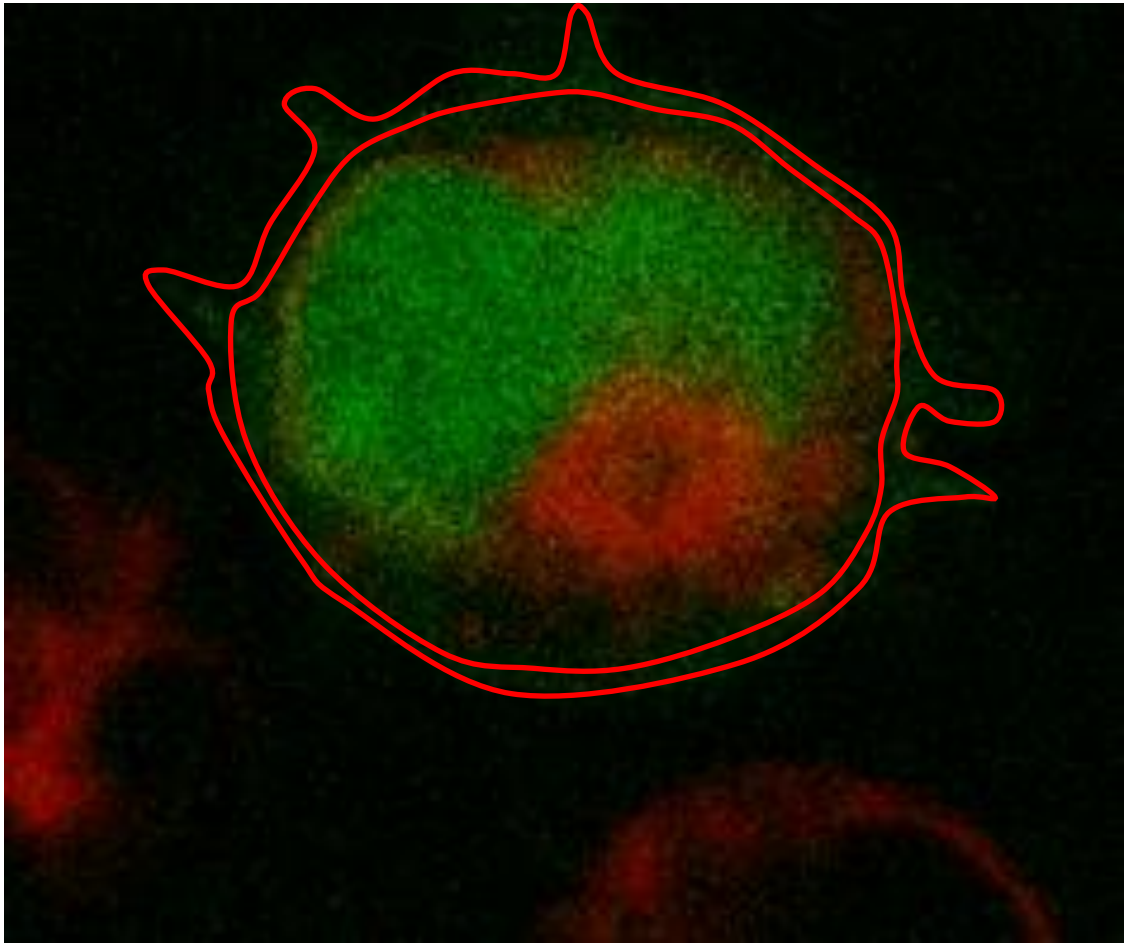


Tumorchirurgie

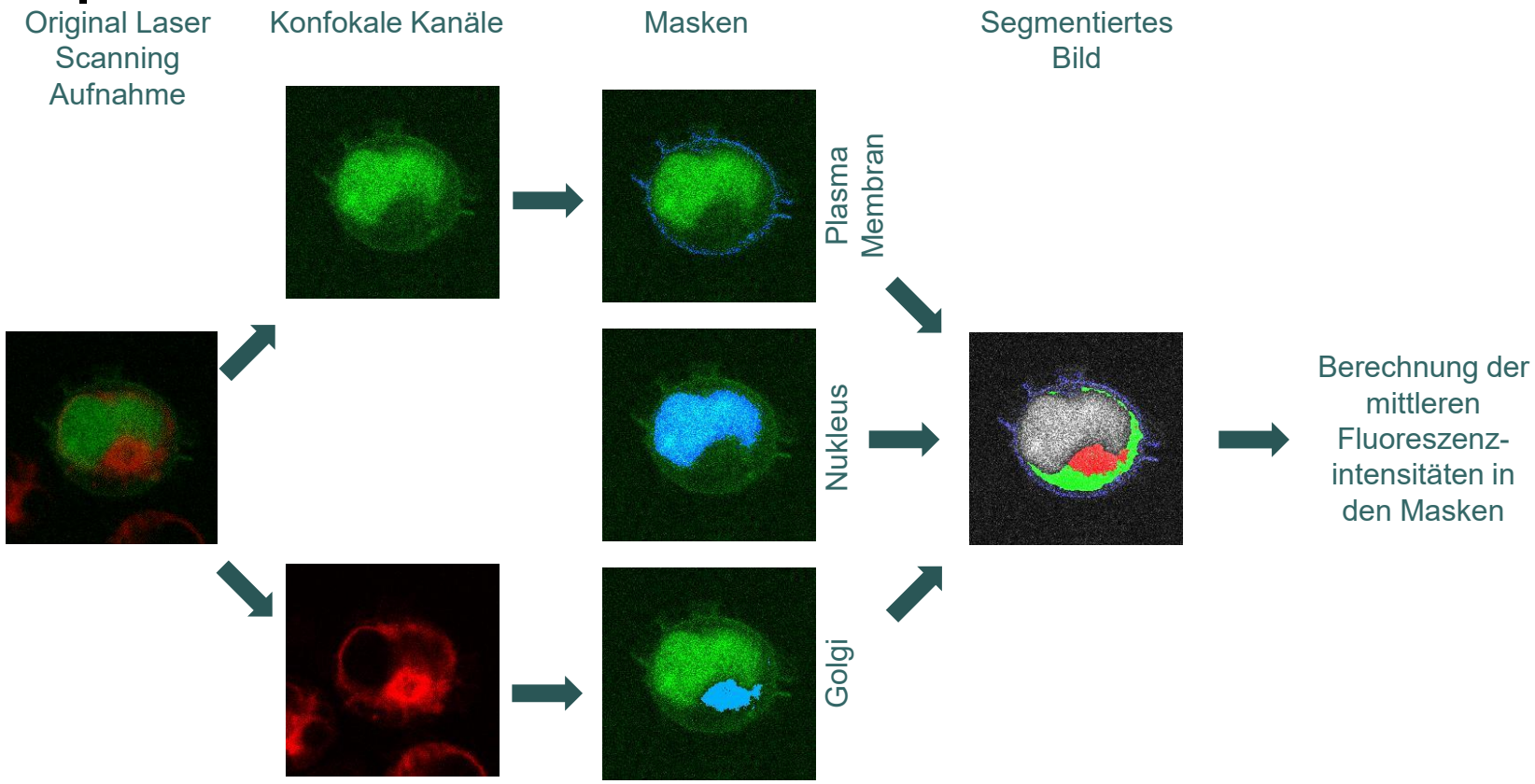


Burton et al.: Spectral optical imaging in biology and medicine.
In: Fujimoto, Farkas: Biomedical Optical Imaging, Oxford University Press, Oxford, New York 2009, S.29-72

Mikroskopische Bildverarbeitung



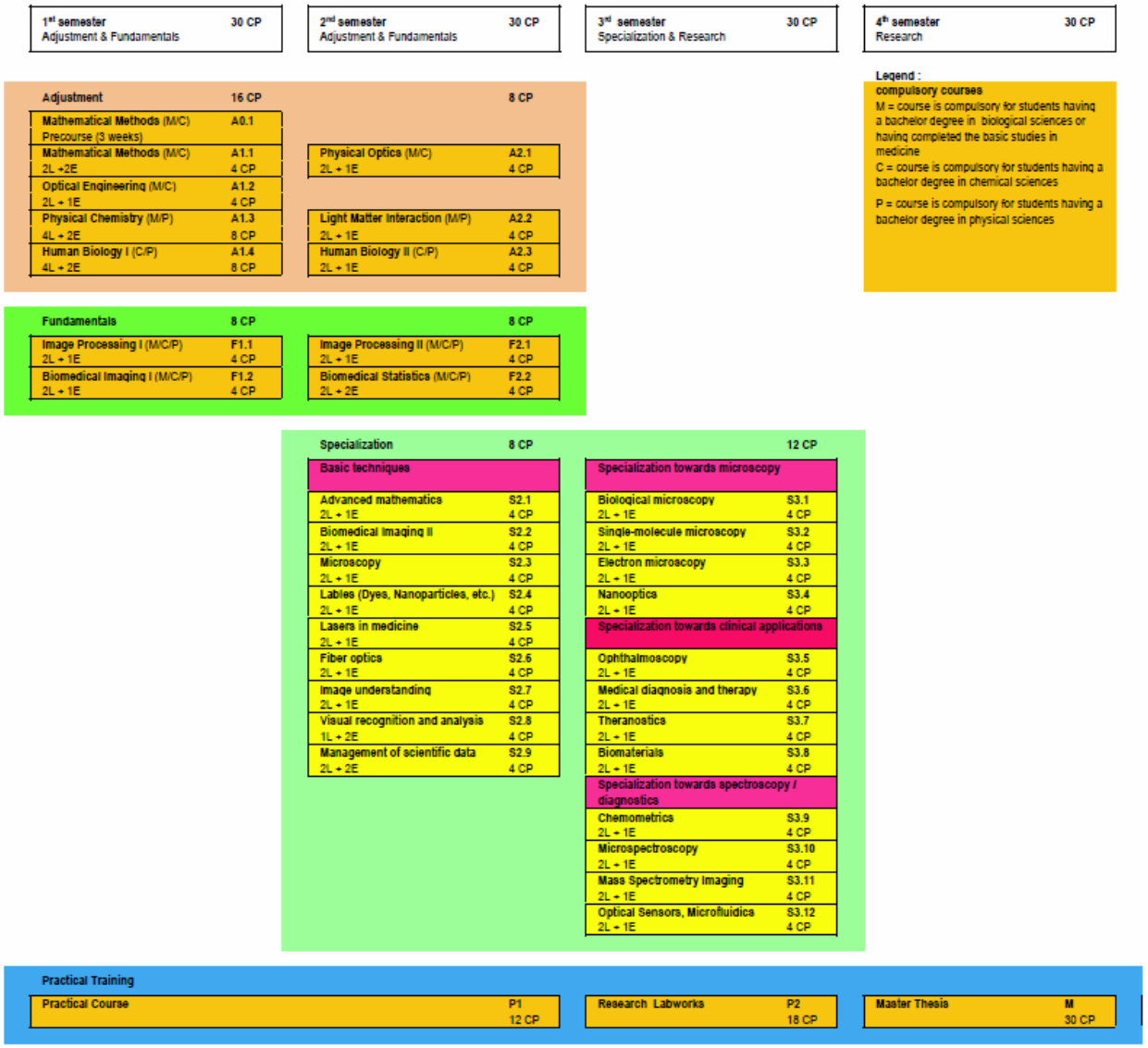
Mikroskopische Bildverarbeitung



Augsten et al., EMBO Rep. 7, 46-51 (2006).

M.Sc. Medical Photonics

Curriculum



M.Sc. Medical Photonics

Curriculum

1st semester	30 CP
Adjustment & Fundamentals	

2nd semester	30 CP
Adjustment & Fundamentals	

Adjustment	16 CP
Mathematical Methods (M/C) Precourse (3 weeks)	**A0.1**
Mathematical Methods (M/C) 2L +2E	**A1.1** 4 CP
Optical Engineering (M/C) 2L + 1E	**A1.2** 4 CP
Physical Chemistry (M/P) 4L + 2E	**A1.3** 8 CP
Human Biology I (C/P) 4L + 2E	**A1.4** 8 CP

	8 CP
Physical Optics (M/C) 2L + 1E	**A2.1** 4 CP
Light Matter Interaction (M/P) 2L + 1E	**A2.2** 4 CP
Human Biology II (C/P) 2L + 1E	**A2.3** 4 CP

Fundamentals	8 CP
Image Processing I (M/C/P) 2L + 1E	**F1.1** 4 CP
Biomedical Imaging I (M/C/P) 2L + 1E	**F1.2** 4 CP

	8 CP
Image Processing II (M/C/P) 2L + 1E	**F2.1** 4 CP
Biomedical Statistics (M/C/P) 2L + 2E	**F2.2** 4 CP

M.Sc. Medical Photonics

Curriculum

Specialization	8 CP
Basic techniques	
Advanced mathematics 2L + 1E	S2.1 4 CP
Biomedical Imaging II 2L + 1E	S2.2 4 CP
Microscopy 2L + 1E	S2.3 4 CP
Lables (Dyes, Nanoparticles, etc.) 2L + 1E	S2.4 4 CP
Lasers in medicine 2L + 1E	S2.5 4 CP
Fiber optics 2L + 1E	S2.6 4 CP
Image understanding 2L + 1E	S2.7 4 CP
Visual recognition and analysis 1L + 2E	S2.8 4 CP
Management of scientific data 2L + 2E	S2.9 4 CP

	12 CP
Specialization towards microscopy	
Biological microscopy 2L + 1E	S3.1 4 CP
Single-molecule microscopy 2L + 1E	S3.2 4 CP
Electron microscopy 2L + 1E	S3.3 4 CP
Nanooptics 2L + 1E	S3.4 4 CP
Specialization towards clinical applications	
Ophthalmoscopy 2L + 1E	S3.5 4 CP
Medical diagnosis and therapy 2L + 1E	S3.6 4 CP
Theranostics 2L + 1E	S3.7 4 CP
Biomaterials 2L + 1E	S3.8 4 CP
Specialization towards spectroscopy / diagnostics	
Chemometrics 2L + 1E	S3.9 4 CP
Microspectroscopy 2L + 1E	S3.10 4 CP
Mass Spectrometry Imaging 2L + 1E	S3.11 4 CP
Optical Sensors, Microfluidics 2L + 1E	S3.12 4 CP

M.Sc. Medical Photonics

Weitere Infos

Weitere Infos zum Studiengang auf:

www.medpho.uniklinikum-jena.de

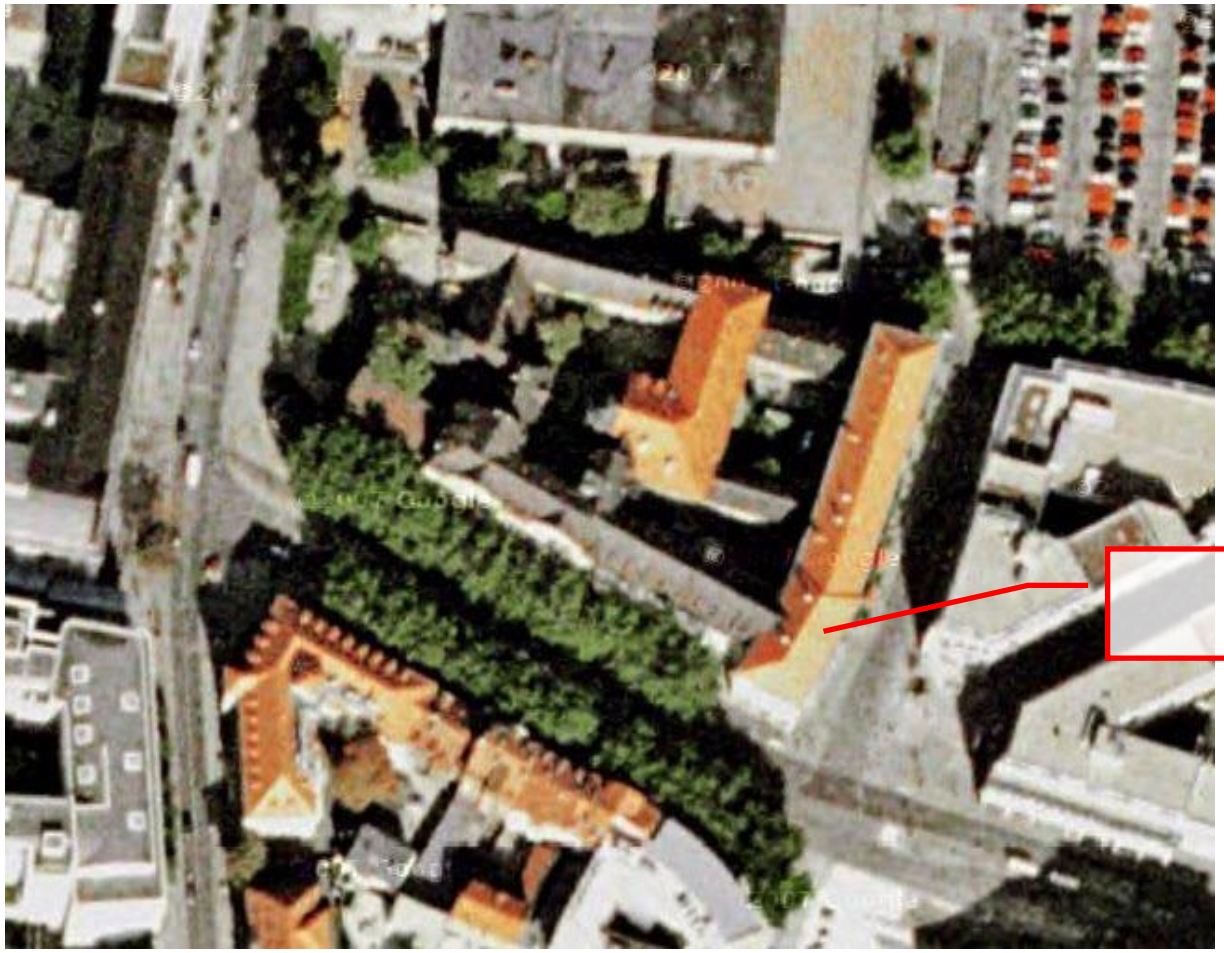
oder direkt bei:

holger.babovsky@uni-jena.de

christoph.biskup@uni-jena.de

Praktika

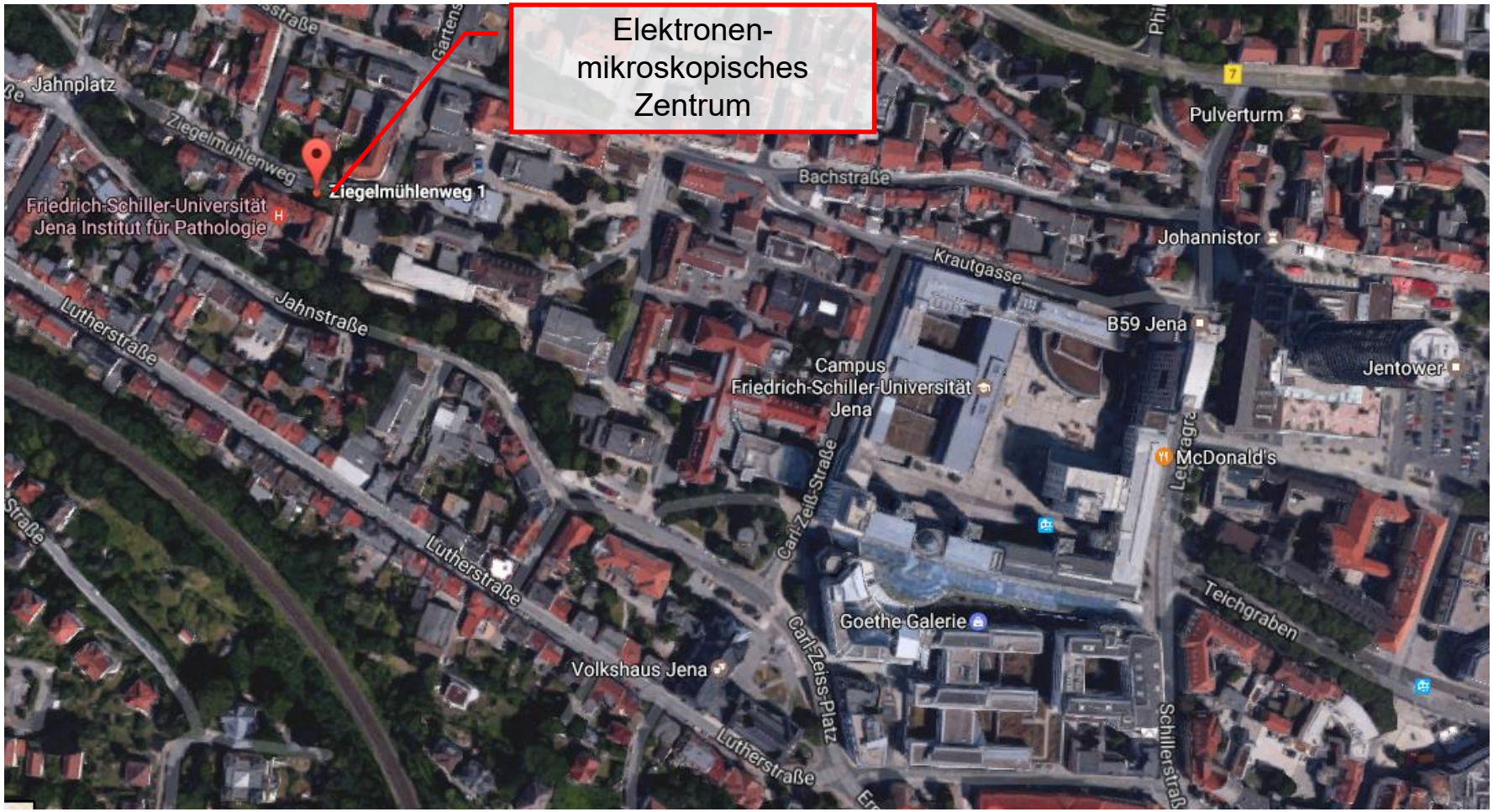
Praktika in den Medizinisch-Theoretischen Instituten



©2010 Google – Map data ©2010 Tele Atlas

Praktika

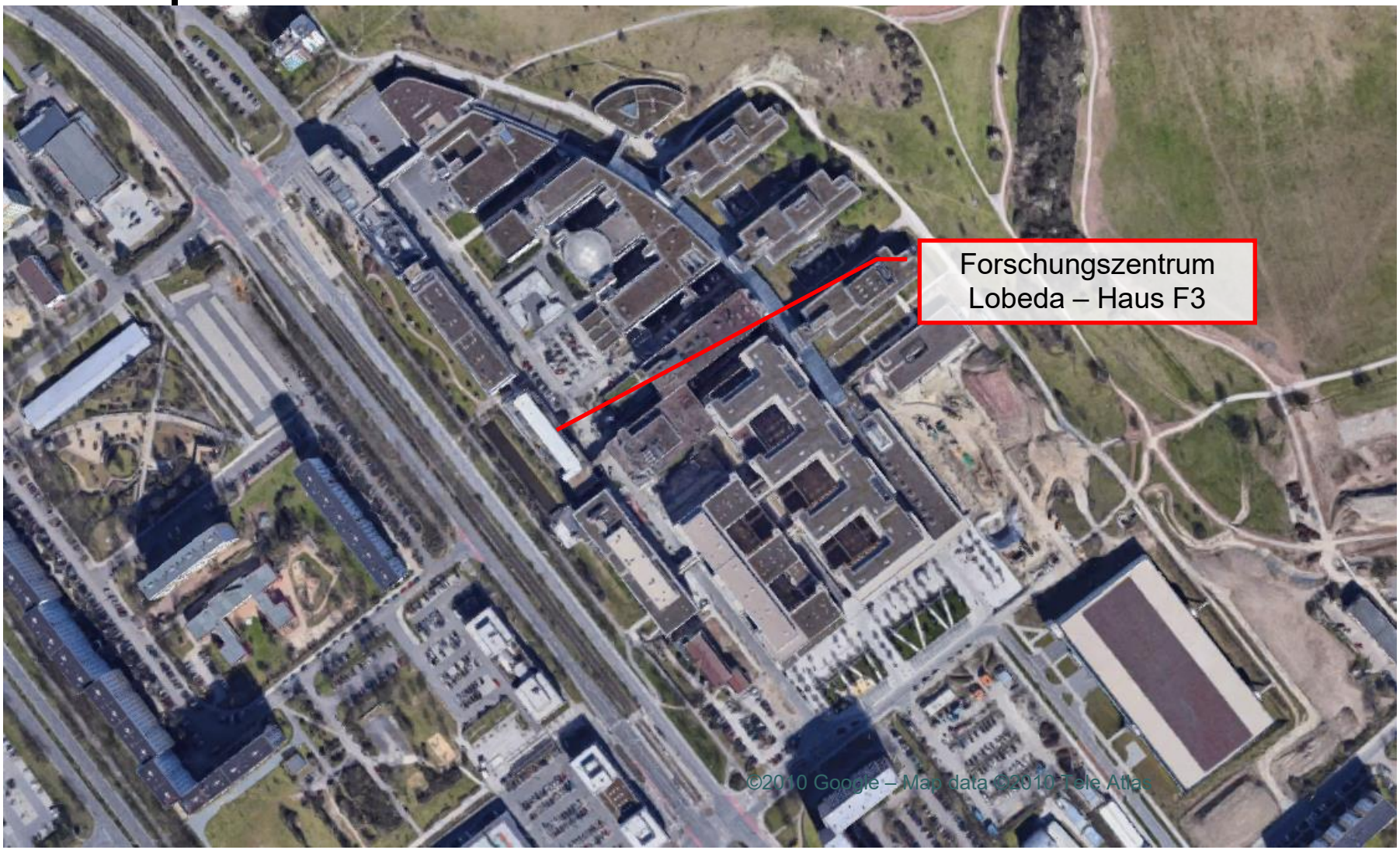
Weitere Praktika im Zentrum



©2010 Google – Map data ©2010 Tele Atlas

Praktika

Praktika in Lobeda




Have fun...!