



Einsatz von LEDs in der Fluoreszenz- mikroskopie Biologischer Proben

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What is Fluorescence?

Wikipedia



Fluorescence is a short, spontaneous emission of light that is generated during the transition process of an electronically excited state to a state with lower energy.

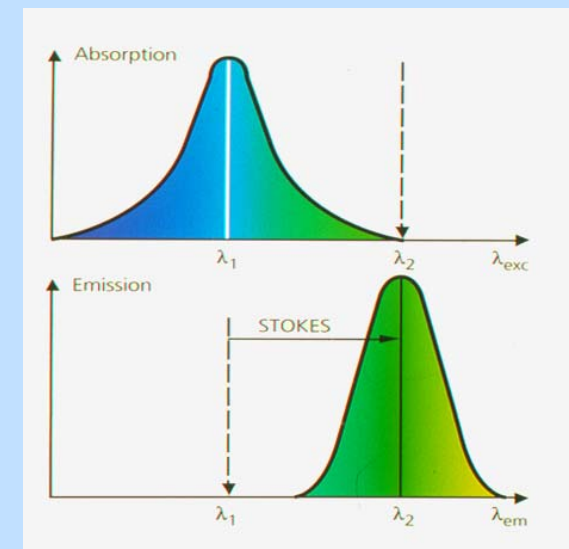
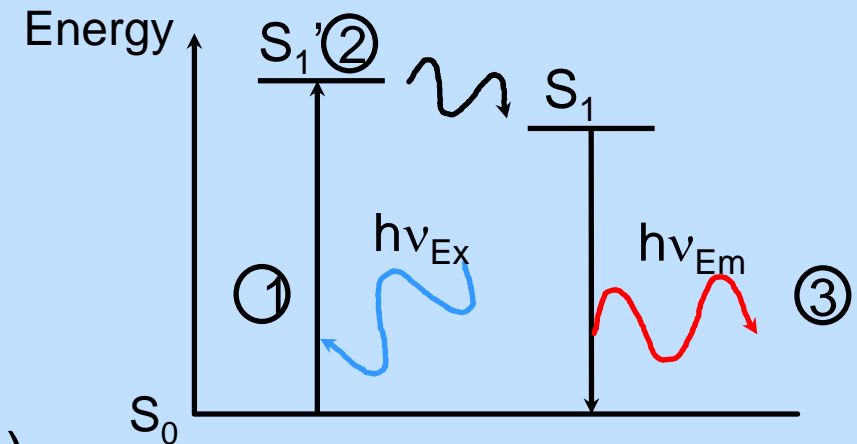
Typical Fluorophores, thus physical systems which show Fluorescence are atoms, molecules, ions and semiconductor particles. The name fluorescence is derived from the fluorescing mineral Fluorit (Fluorspar, Calcium Fluorid, CaF_2).



Excitation and Emission Process



- Starting Point: Fluorophore in a certain energy level
- Light excites a fluorophore
- Excitation: energy content of light lifts electrons to a higher level of energy
- Partially dissipation of energy (mostly heat): lower level of energy
- Emission: Return of electron to ground state after $< 10^{-6}$ s. Energy is emitted as fluorescence light of lower energy
- Stokes shift:
Excitation wavelength $<$ Emission wavelength



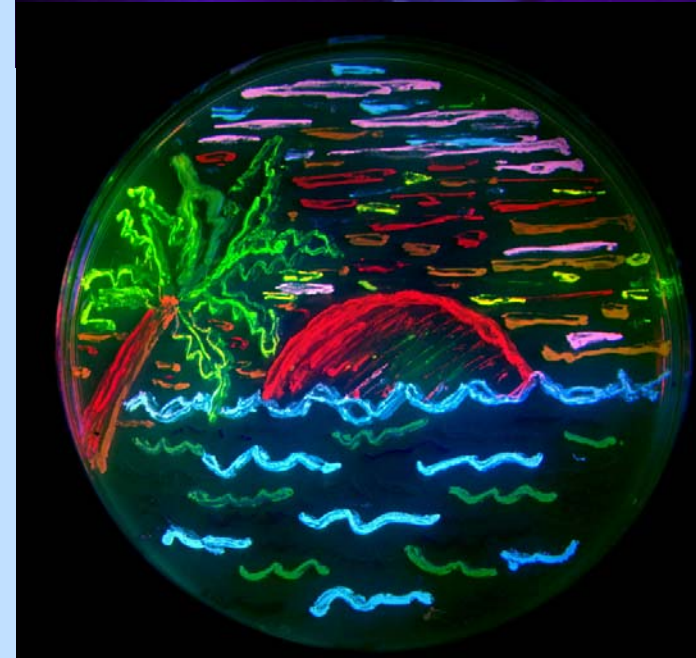
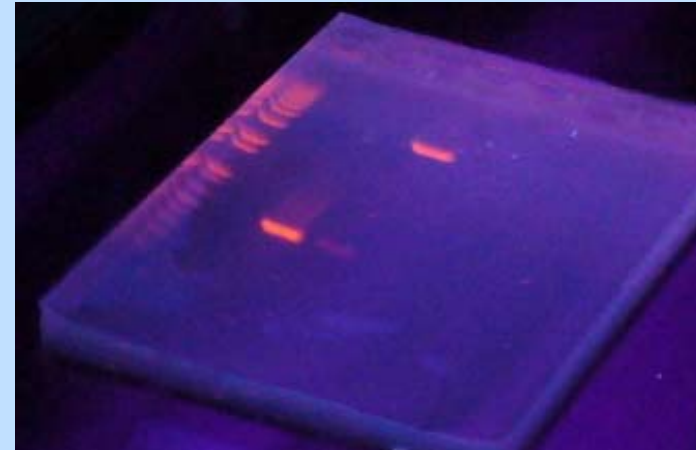
What is fluorescence in biology?



Specific detection method

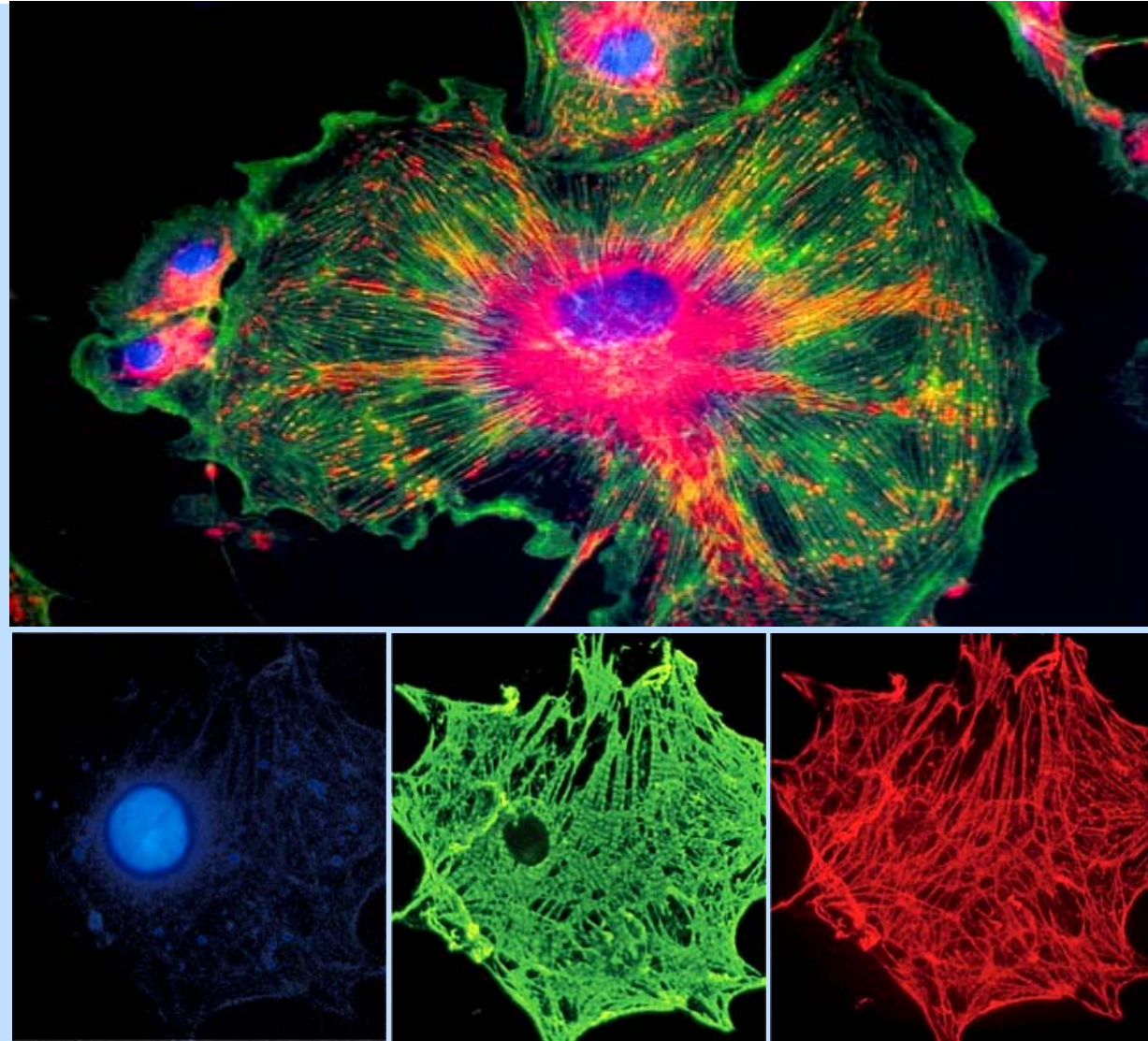
Sensitive detection method

High signal-to-background (high contrast)

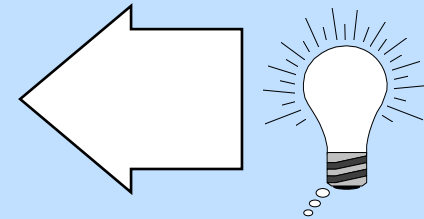
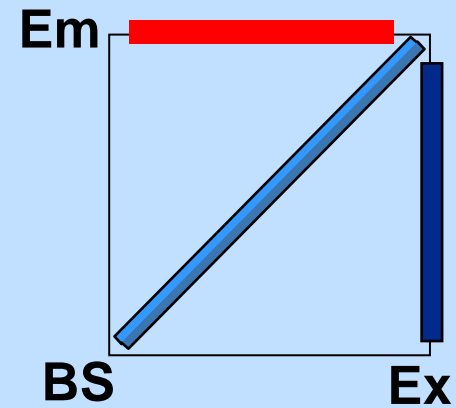


Fluorescence microscopy gives

Specific Contrast



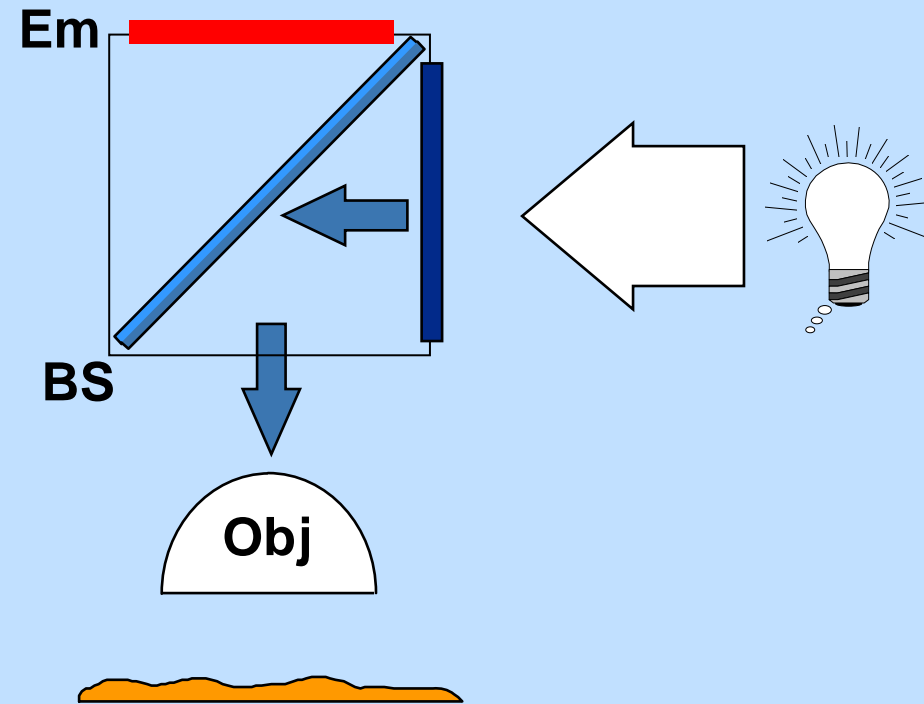
Epi-Fluorescence



- Ex Excitation Filter
- BS Beam Splitter = Dichromatic Mirror
- Em Emission Filter



Epi-Fluorescence



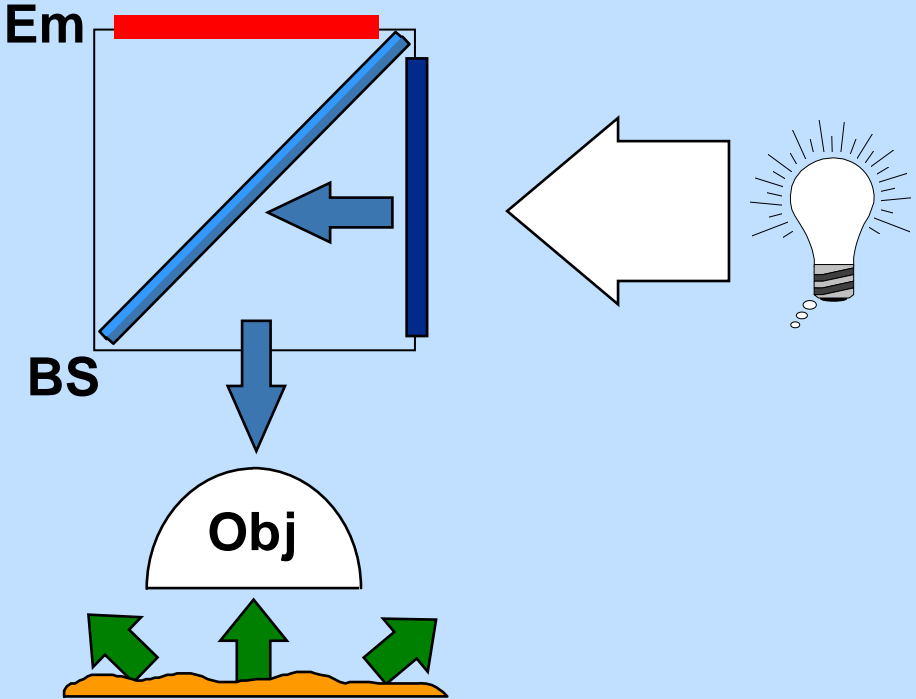
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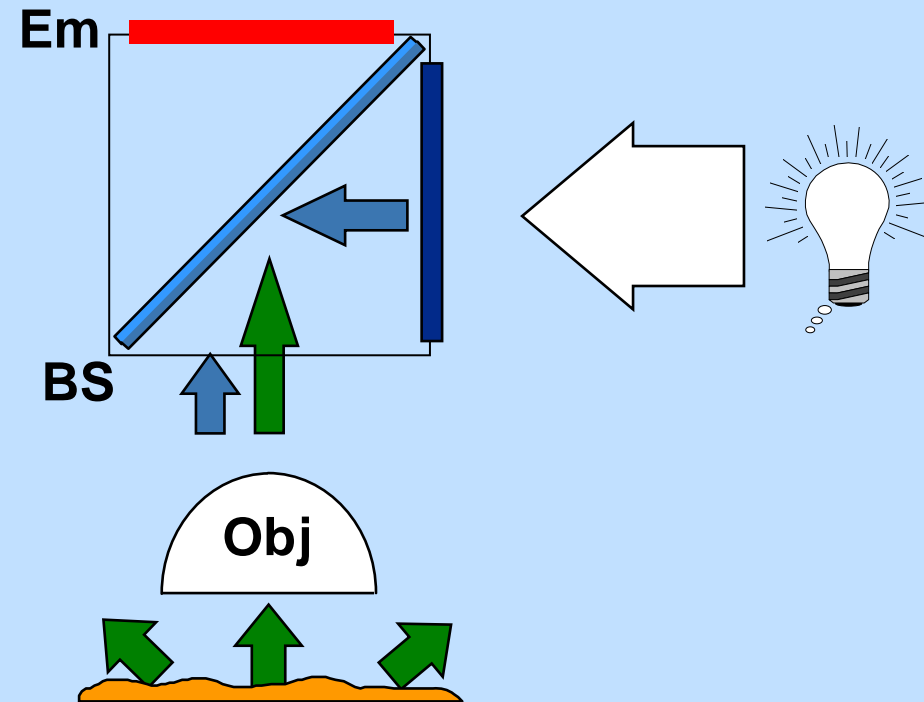
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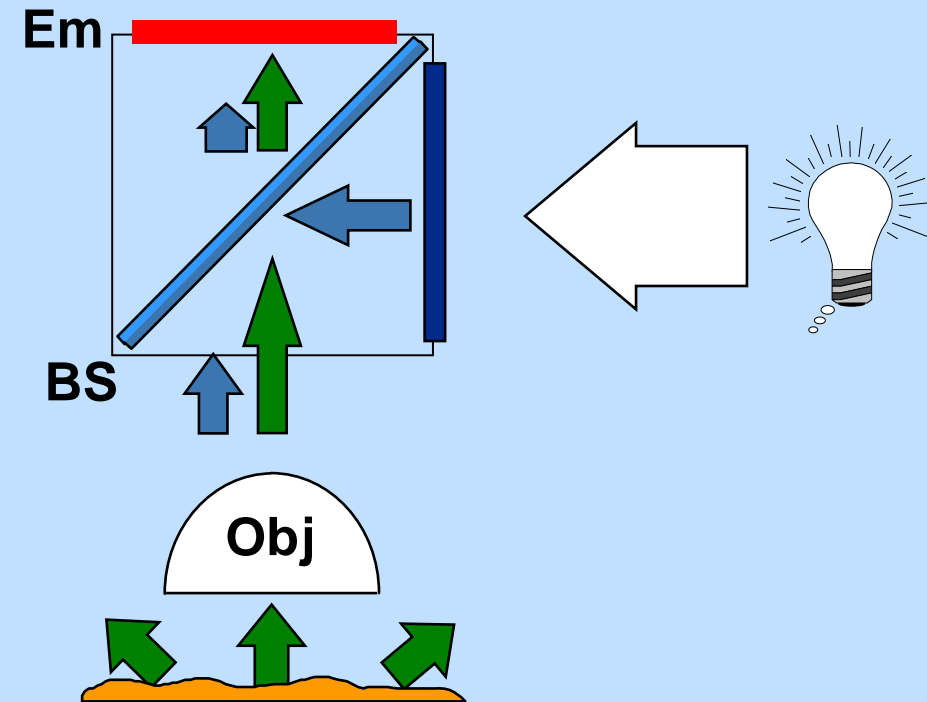
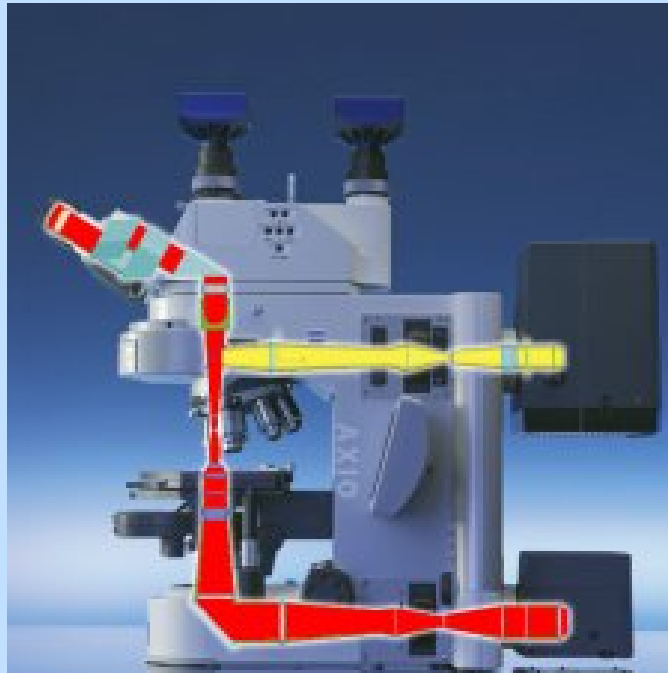
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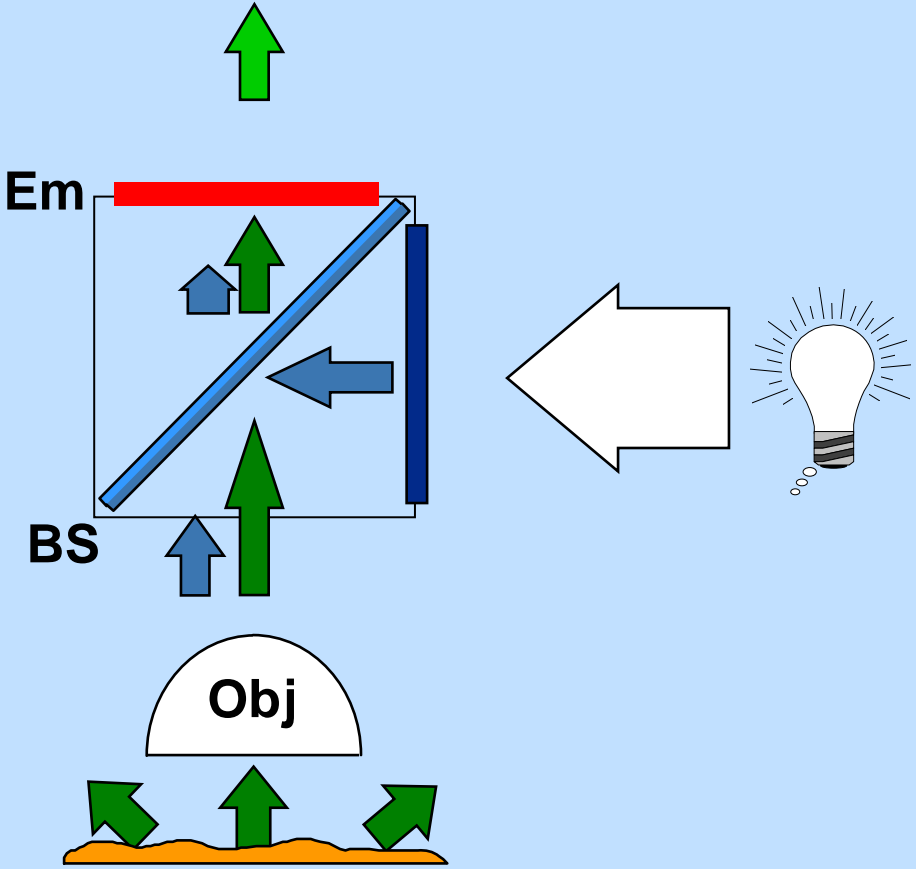
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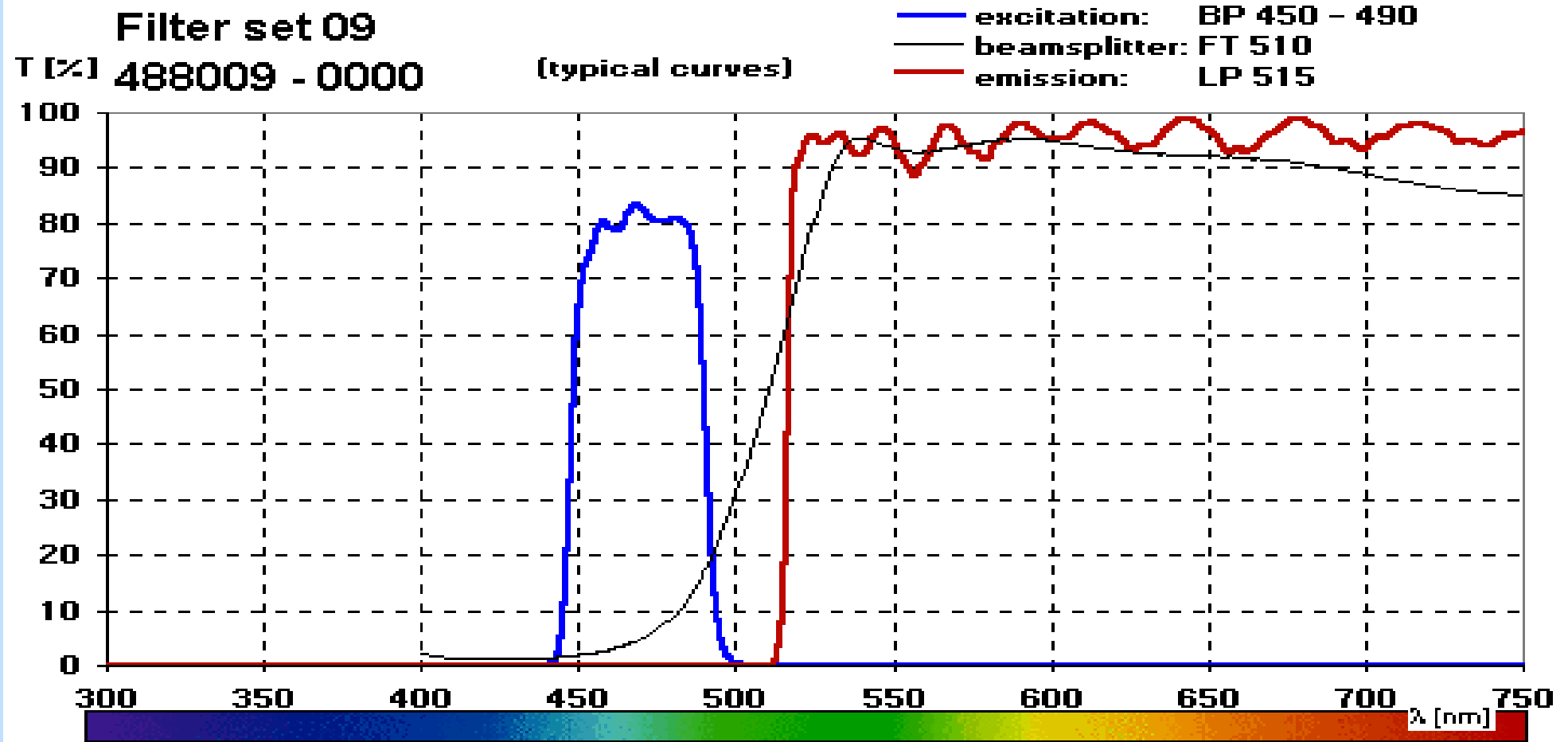


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Basic Course Light Microscopy

Fluorescence Filter Sets



A variety of fluorescence illumination systems are available on the market



HBO (50W & 100W) – Mercury arc

- Traditional work horse solution

XBO 75 – Xenon arc lamp

- Traditional light source for quantitative microscopy

HXP 120 – Metal halogenide

- External light source with fiber coupling

Sutter DG 4/5 – 175W Xenon

- External light source with fiber coupling
- Fast wavelength switching

Monochromator – 150 W Xenon

- External light source with fiber coupling
- Wavelength selection

LED light sources for fluorescence

- The future in fluorescence microscopy...



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LED light sources for fluorescence

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HBO – The classical and universal solution



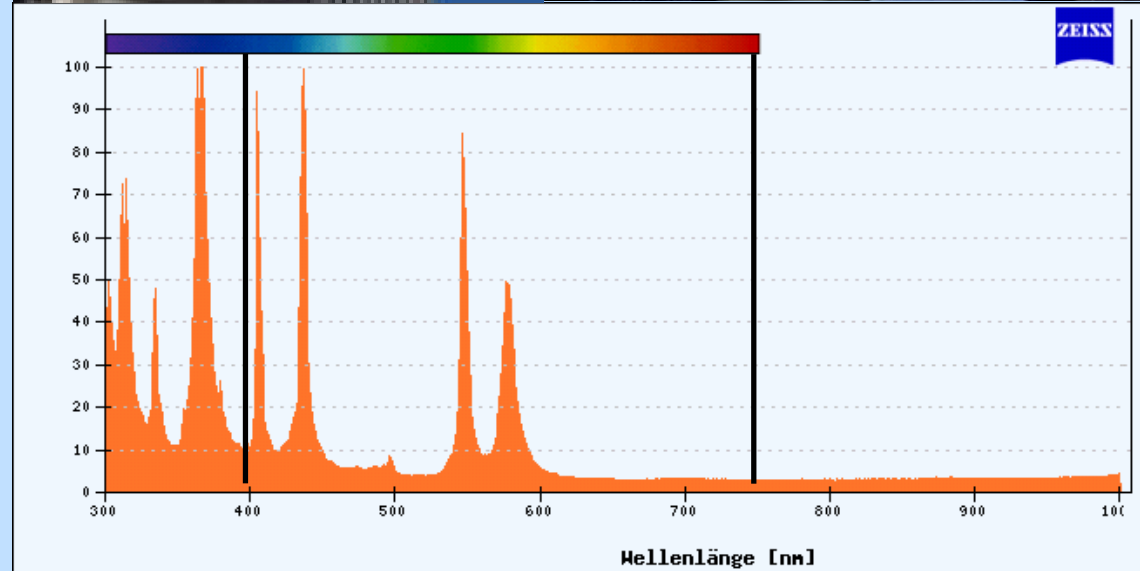
Main technical features

- 50 W or 100W Mercury light
- Peak spectrum for high intensity excitation
- Life time of 300h
- Intensity control via FluoArc or diffusor



Application

- General solution for fluorescence microscopy
- Fixed tissue
- Weak (stable) stainings



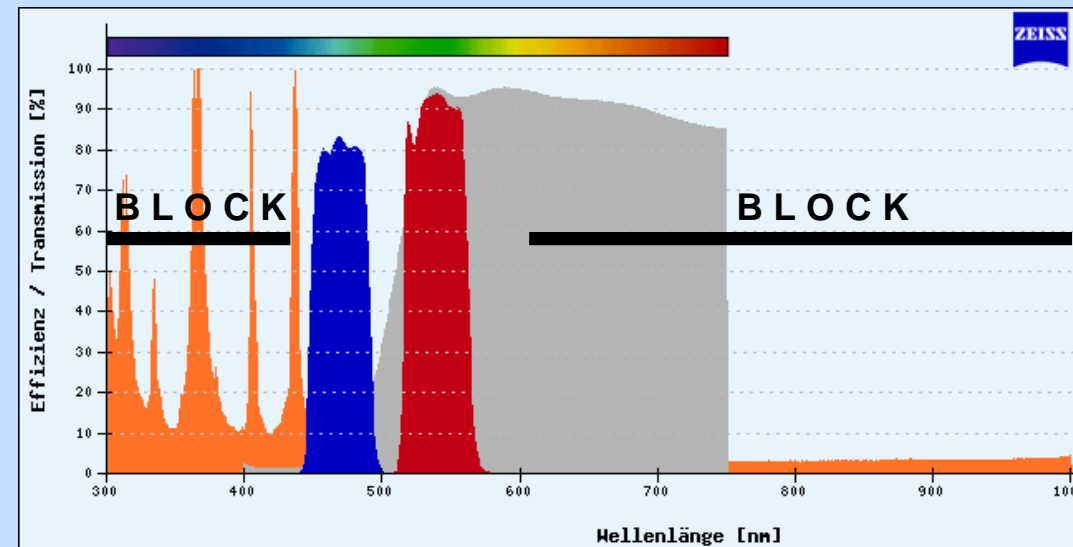
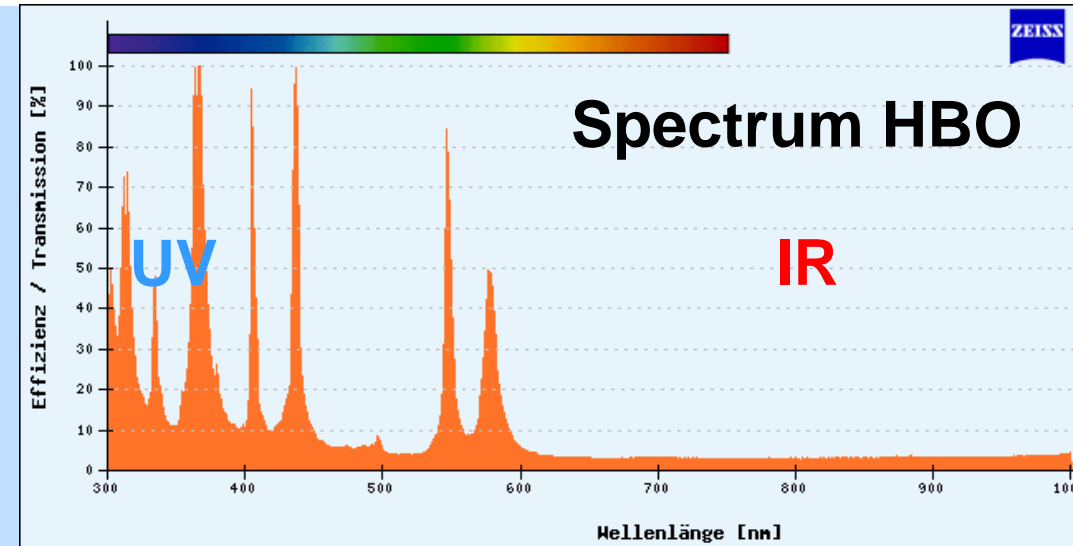
High intensity UV light has to be blocked by fluorescence filters: complex and expensive



White light: Emission of a broad band of unwanted wavelengths from **UV** to **IR**

For Live Cell Imaging:
Blocking to avoid background, artifacts, bleaching and toxic effects for living specimen

- Complex and expensive filter technology needed
- Spectral properties change over time
- Still some light leaks through



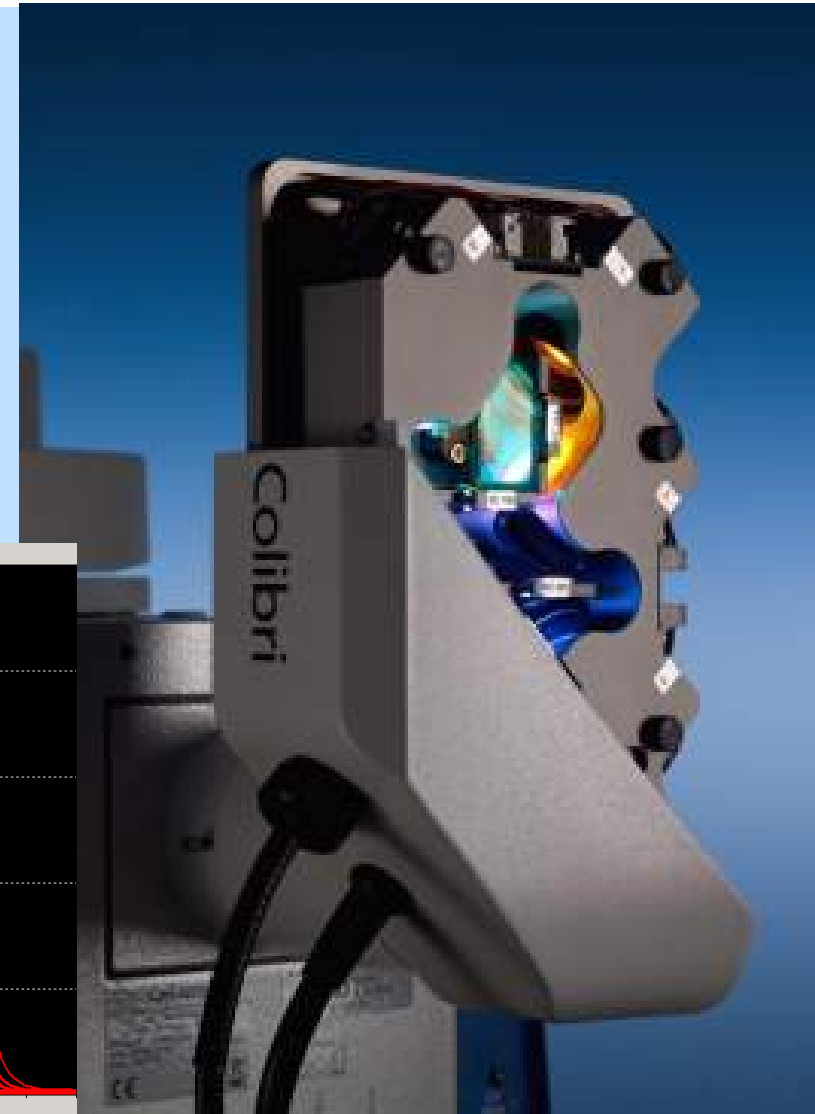
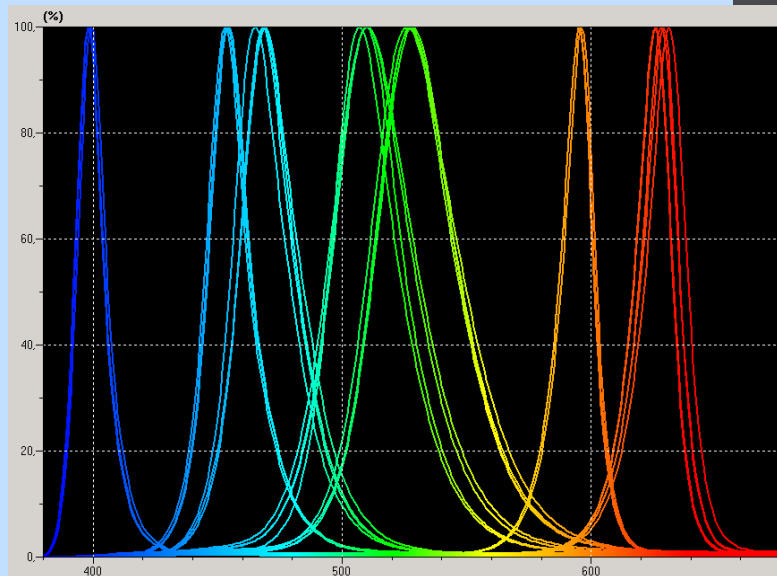
Light Emitting Diodes LED



LEDs emit a specific light band without unwanted UV or IR light which kills cells



- LED-Module 365 nm
- LED-Module 380 nm
- LED-Module 400 nm
- LED-Module 455 nm
- LED-Module 470 nm
- LED-Module 505 nm
- LED-Module 530 nm
- LED-Module 590 nm
- LED-Module 615 nm
- LED-Module 625 nm
- LED-Module White light



LED Fluorescence

LED general advantages:

- Life time of more than 10 000h
- High signal-to-background ratio
- Intensity can be controled from 0% to 100%
- Stable emission of light
- On and off switch within μs without vibration – no mechanical shutter needed
- No or very little heat generation
- LED modules are encoded and can be easily changed

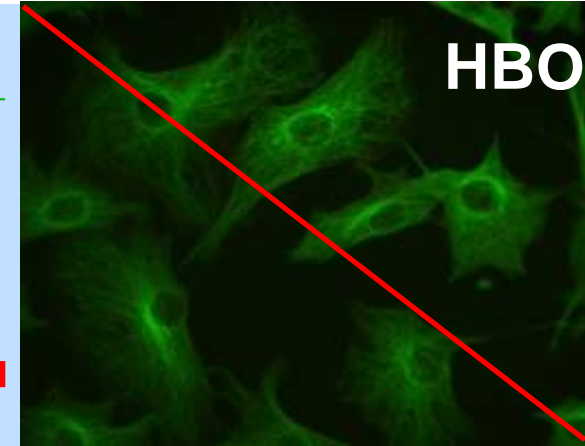
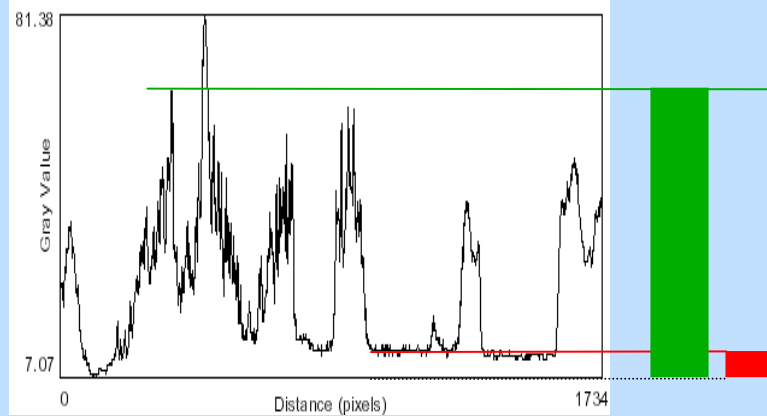


Excellent signal-to-noise for best contrast

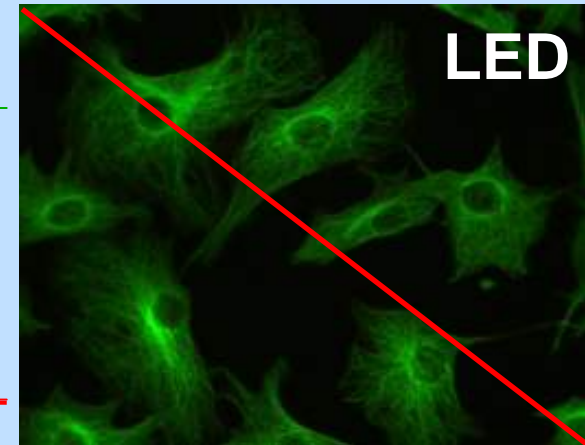
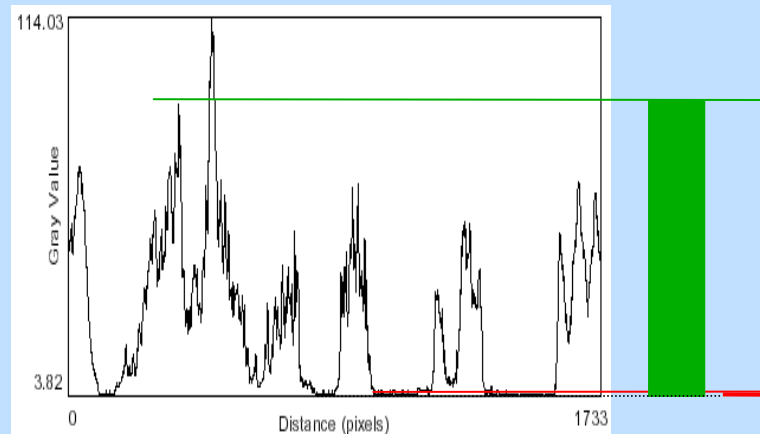


Colibri LED illumination

- Lower excitation intensity of LEDs is compensated by very low back-ground noise
- Signal to noise ratio is excellent for Live Cell Imaging with low excitation intensity



HBO



LED

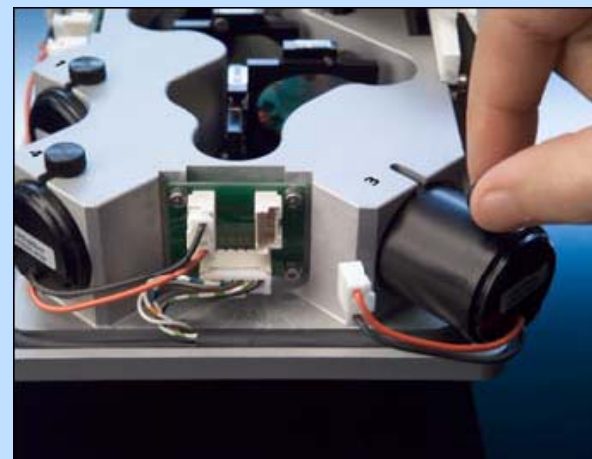


LEDs feature more advantages like „unlimited“ lifetime, fast switching, easy handling....



Up to 4 LED modules can be combined

- LEDs are encoded and can be changed easily
- Switching time below 1ms
- No mechanical interaction
- Life time of several thousand hours
- No heating



The light source can be controlled using a stand alone panel....



User panel

- Stand alone solution
- Switching on and off
- Attenuation 0 - 100%
- Switch to external light source HXP

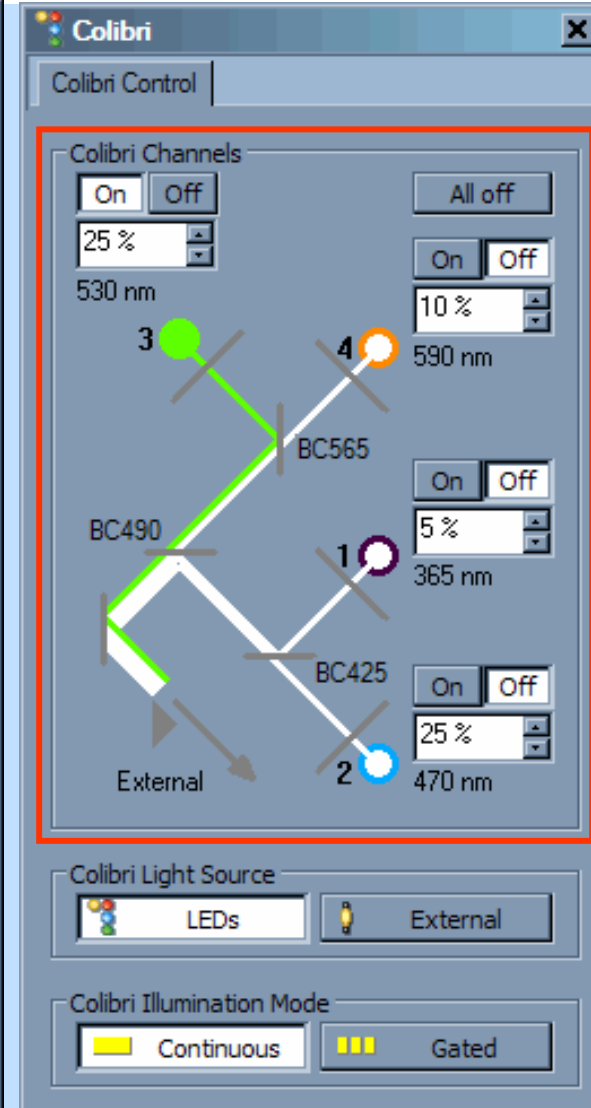
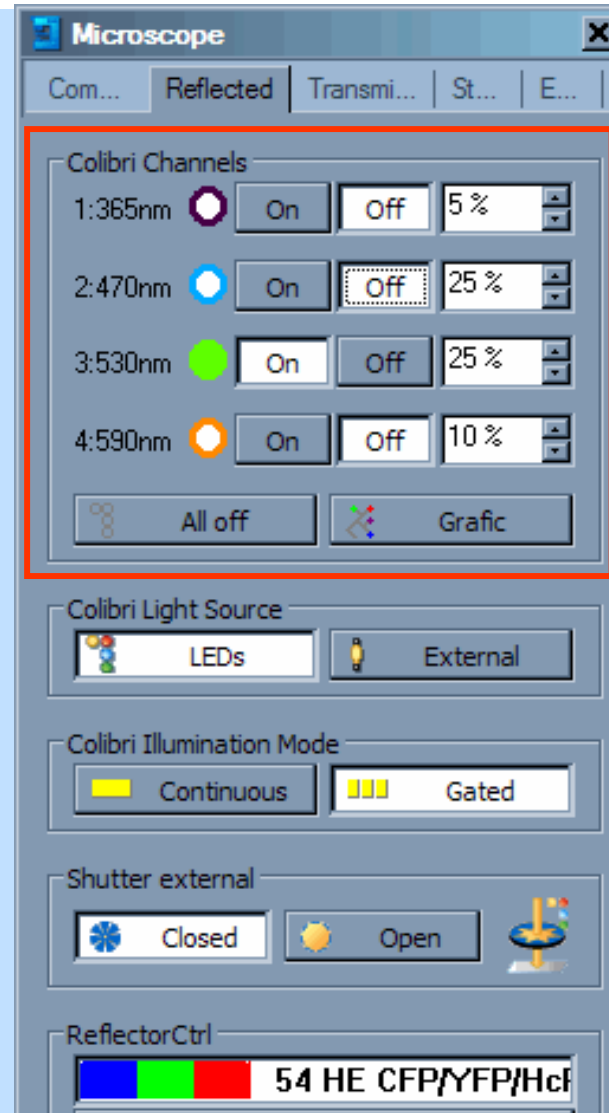


...and by software control in AxioVision for automatic image acquisition

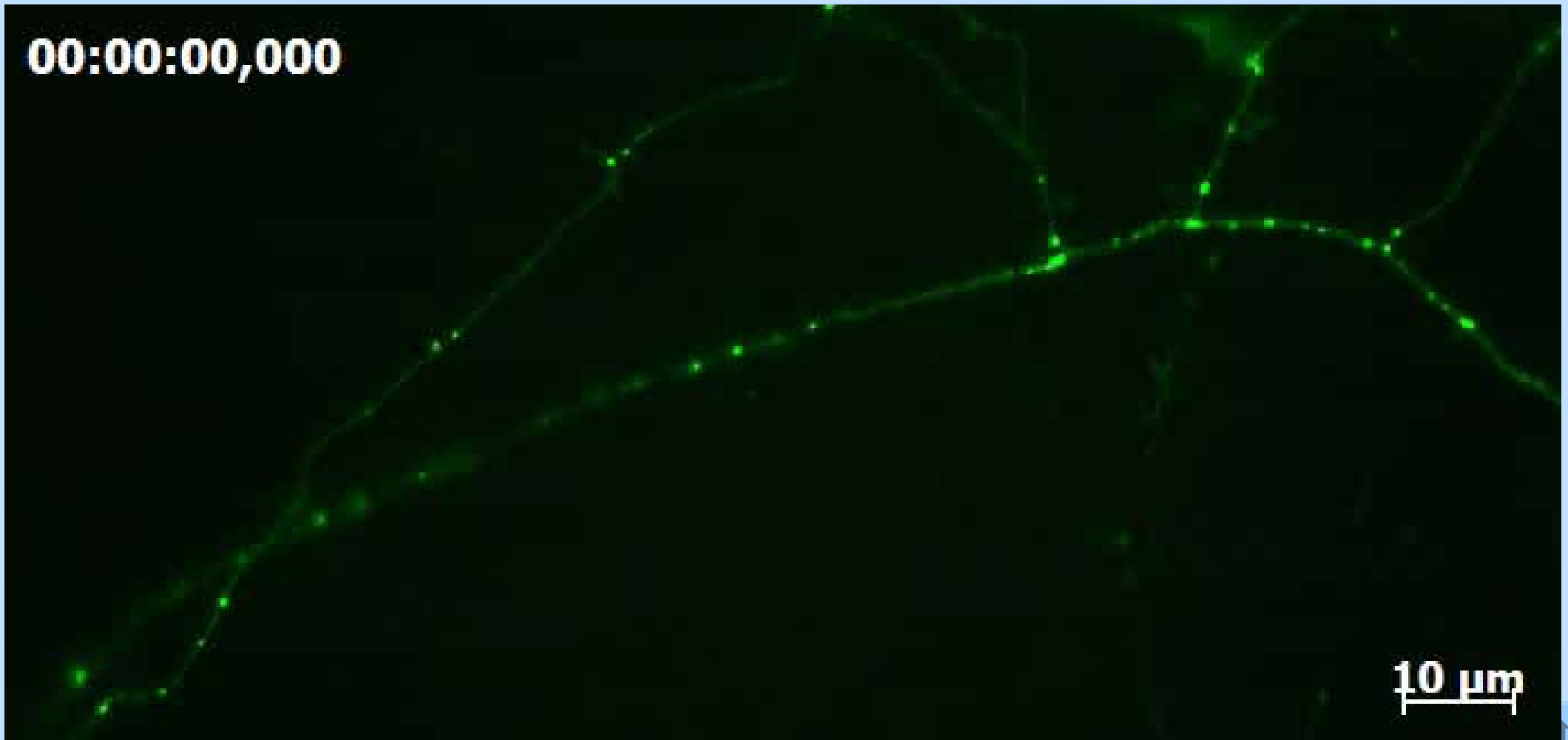


Integration in Axiovision

- Visible configuration
- On – Off functionality
- Attenuation
- Status display: beampath
- Integrated in the Cell Observer HS for High-Speed Imaging



Colibri Hypocampus Neurons GFP labeled Synaptotagmin

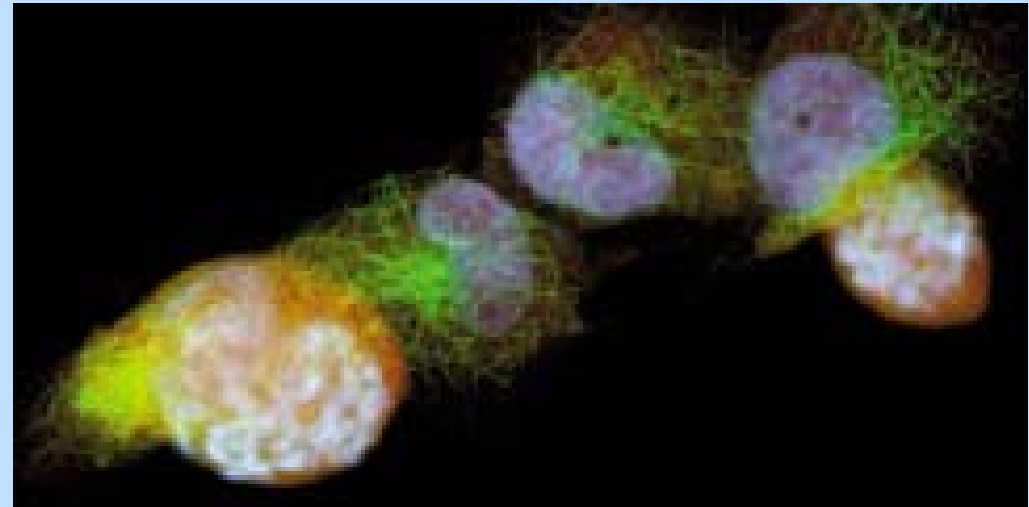
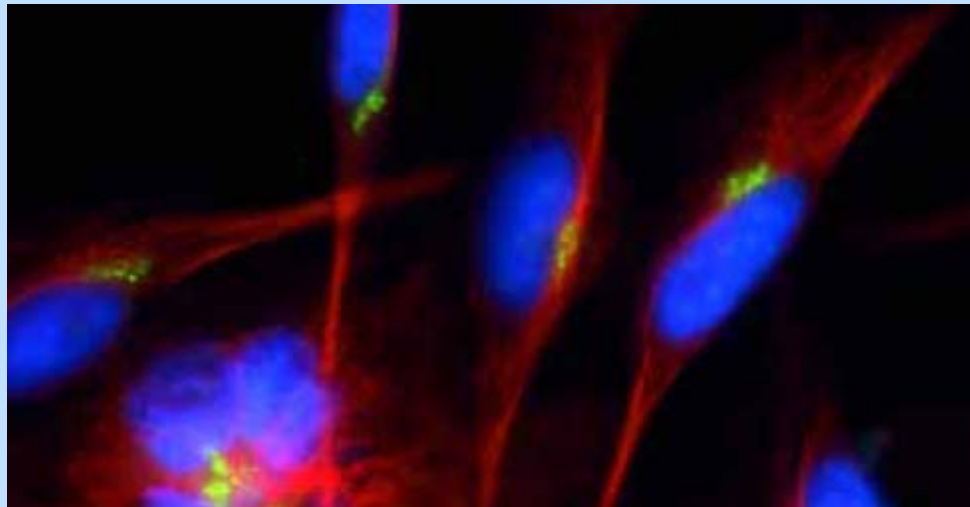


....wavelength mixing for simultaneous fluorescence observation



Possibility of simultaneous observation:

- Combination of LEDs in visible mode
- Intensity adjustment channel by channel
- Use of multibandpass filters



Fluorescence

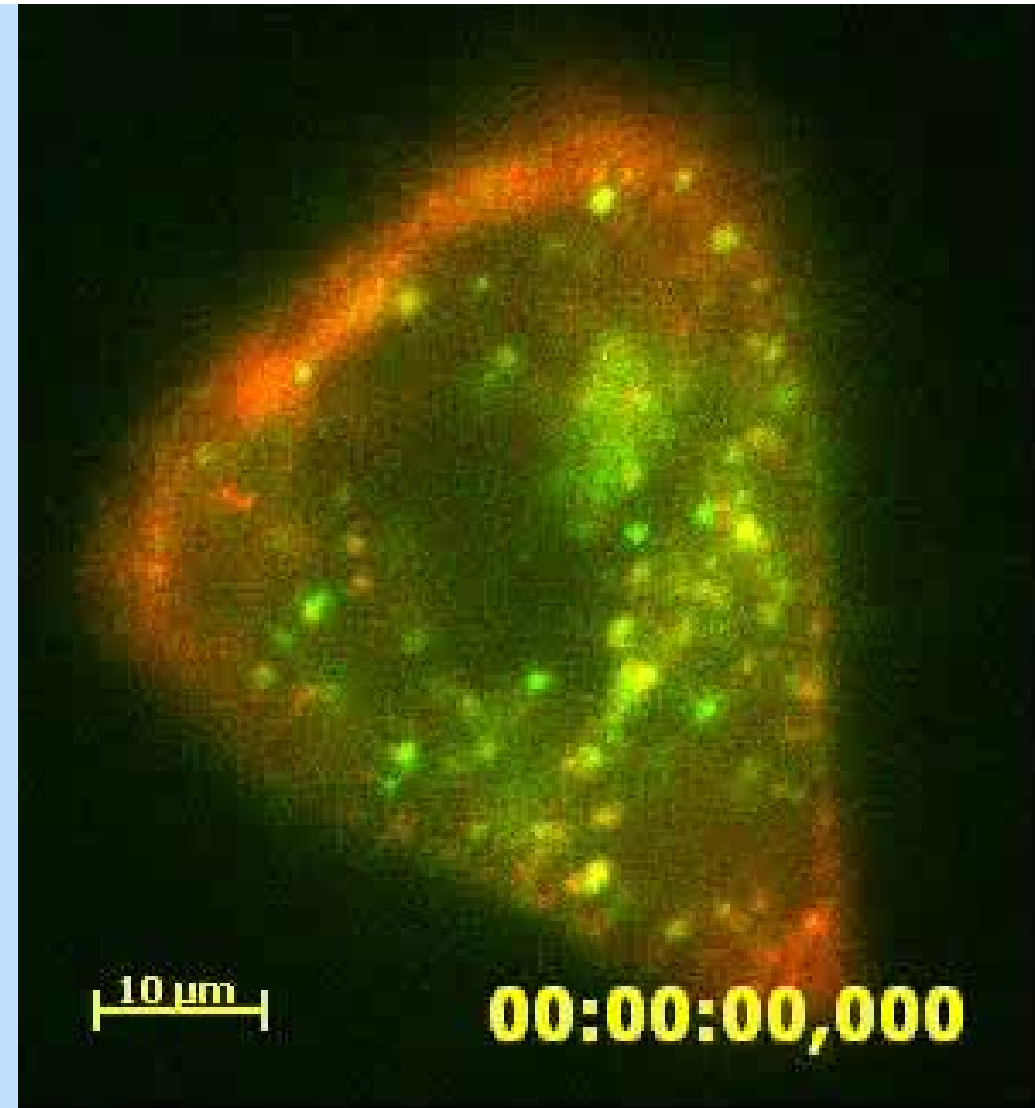


HeLa cells

Red: Cav-1-mRFP

Green: Reg-1-eGFP

Courtesy of Dr. Matthias Langhorst
University of Konstanz



Summary



- Fluorescence is a highly specific contrast method in microscopy
- Fluorescence light is emitted by excitation of a fluophore
- Microscope for (Epi) Fluorescence:
 - Fluorescence light source
 - Filter set
 - Appropriate Objective
- LED fluorescence
 - Integrated in Axio Scope
 - Add-on COLIBRI

