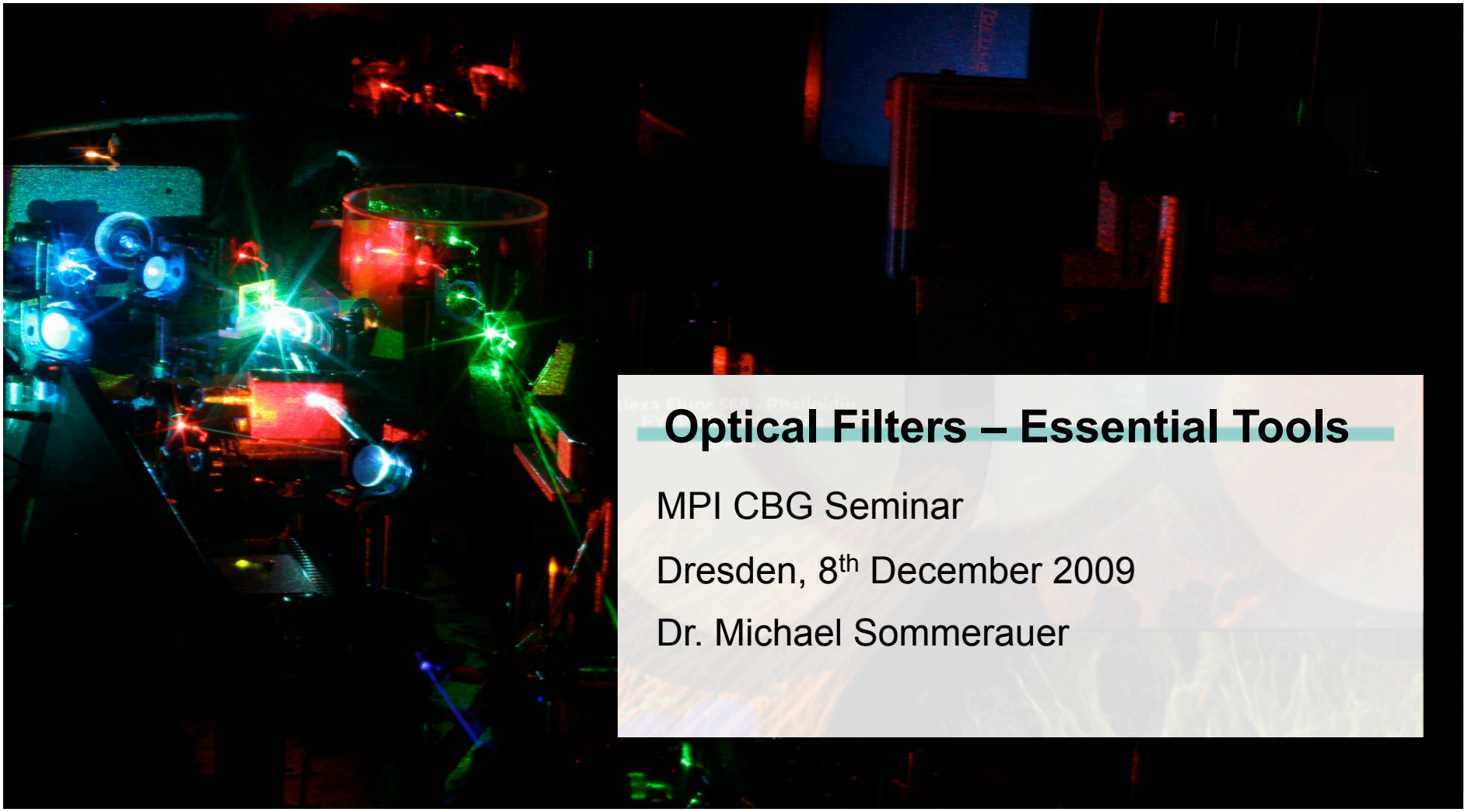


Optical Filters – Essential Tools

MPI CBG Seminar

Dresden, 8th December 2009

Dr. Michael Sommerauer

:: Company profile

AHF analysentechnik was initially founded in 1981 by Helmut Feuerbacher as an engineering company for microwave plasma spectroscopy

Accessories for ultra-trace analysis and ICP spectrometry have soon be added to the product range

Since 1992 AHF analysentechnik offers high end optical filters for any kind of fluorescence and Raman applications

In 1998 the company has been transformed into AHF analysentechnik AG.
Today AHF analysentechnik engages 14 employees

AHF ANALYSENTECHNIK

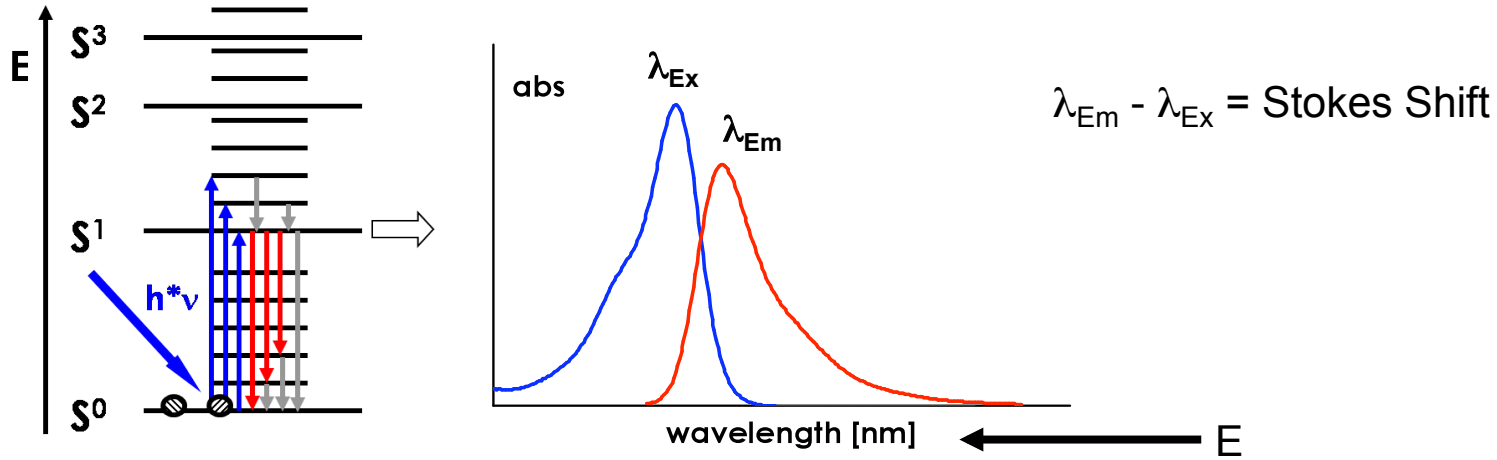
:: Content

1. Fluorescence
2. Measuring fluorescence
3. Filters
4. Fluorescence microscopy
5. Advanced microscopy techniques
6. Filter handling

:: Fluorescence

Absorption and emission

Term scheme:



$\lambda_{Em} - \lambda_{Ex}$ = Stokes Shift

absorption (10^{-15} s) of photon(s)
fluorescence emission (10^{-7} s)
relaxation without radiation (10^{-14} s)

electronic states S^n
vibronic states

:: Fluorescence

Intensity of a fluorescent dye

extinction coefficient

efficiency of absorbed energy (light)

YFP = 83 000 cm^{-1} M^{-1} at 514 nm

Q-Dot 605 = 2,8 x10^6 cm^{-1} M^{-1} at 405 nm

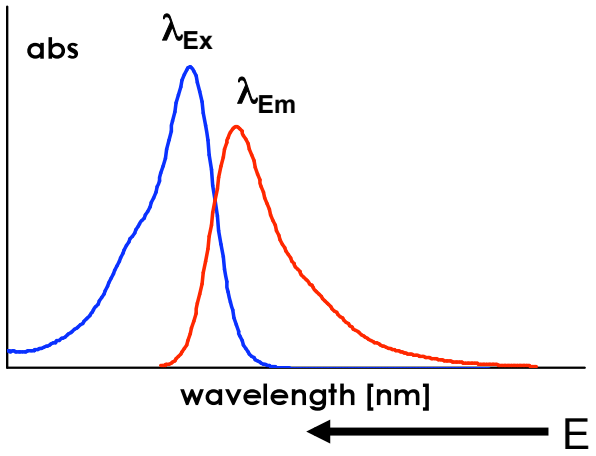
quantum yield

efficiency of released energy as fluorescence

typically 0.1 - 0.95

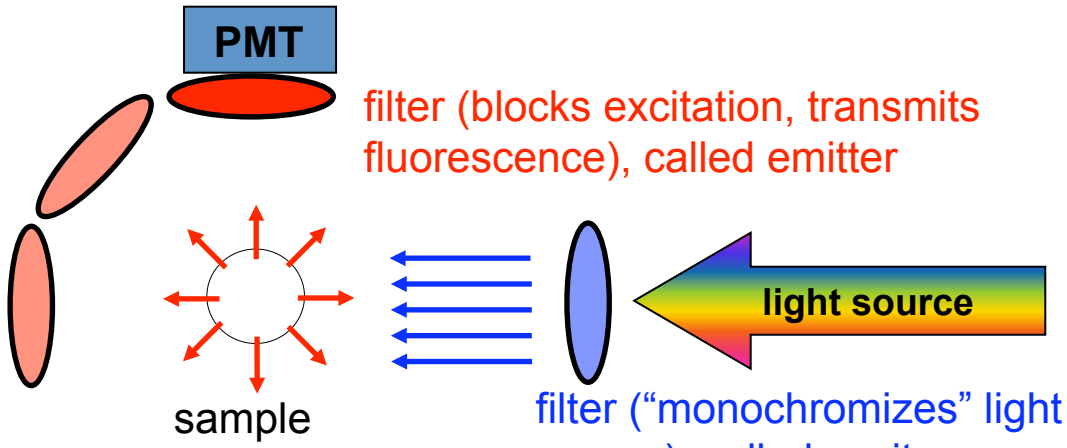
YFP = 0,64

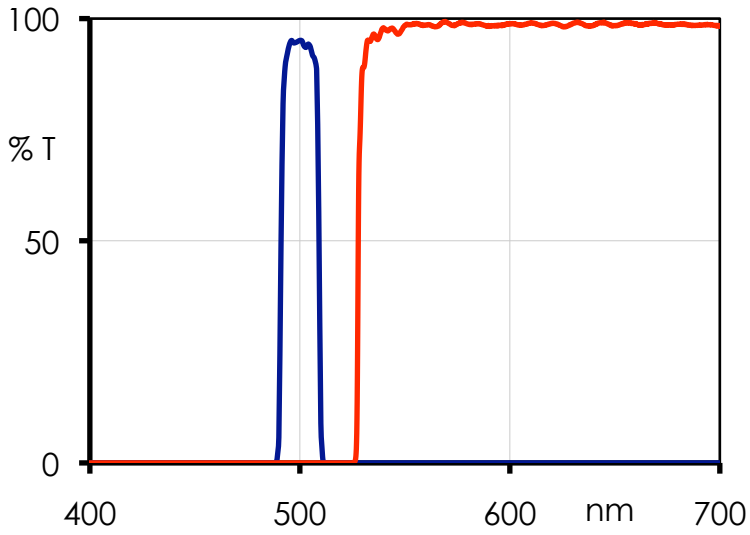
Q-Dot 605 ~ 0,6



:: Measuring fluorescence

Fluorescence spectrometers

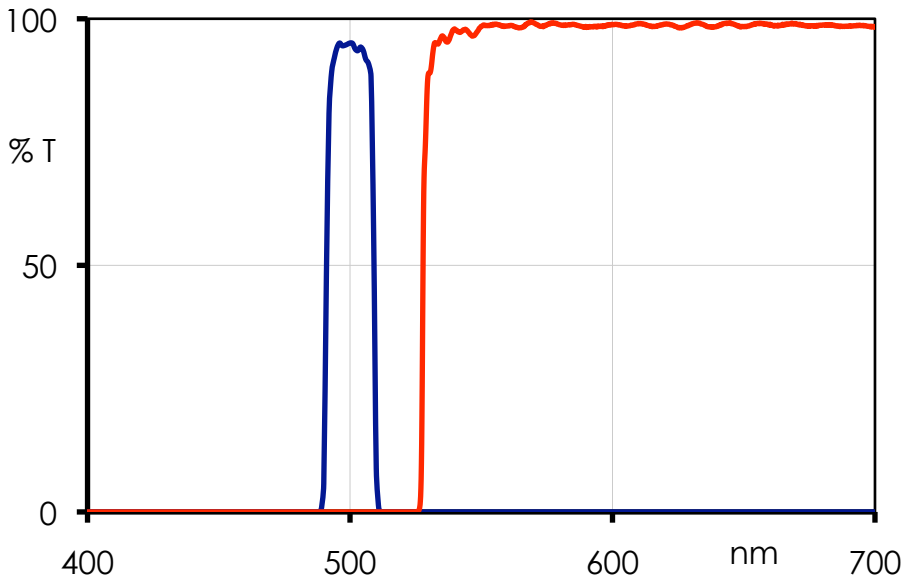




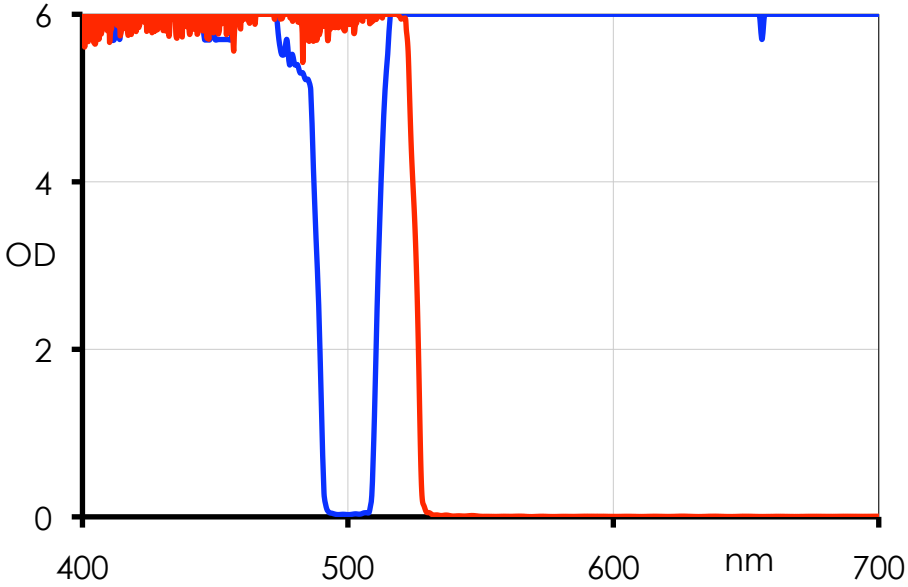
:: Measuring fluorescence

Transmission versus optical density

Intensity I: $I_{fluorescence} \sim I_{excitation} / 10.000$

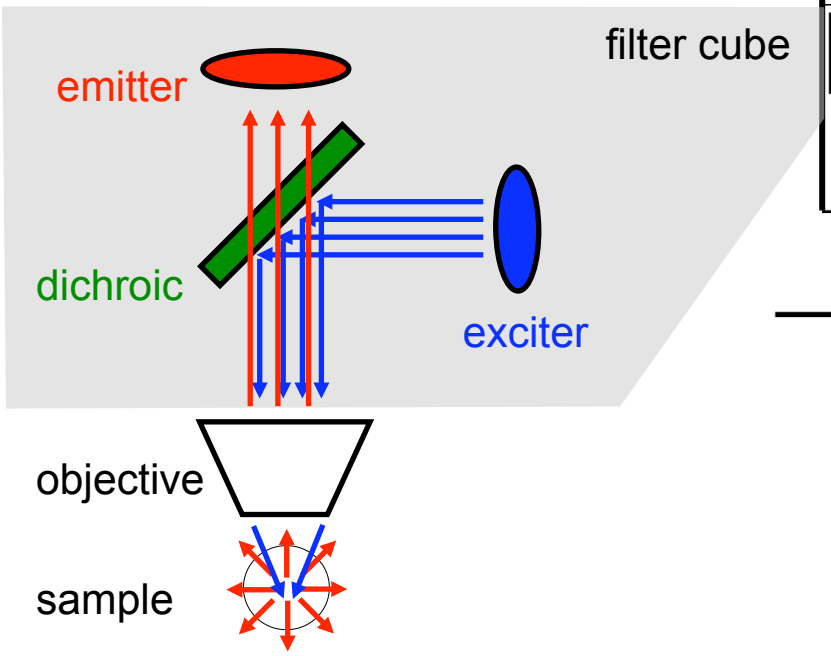


Optical density: OD = -log (%T / 100)

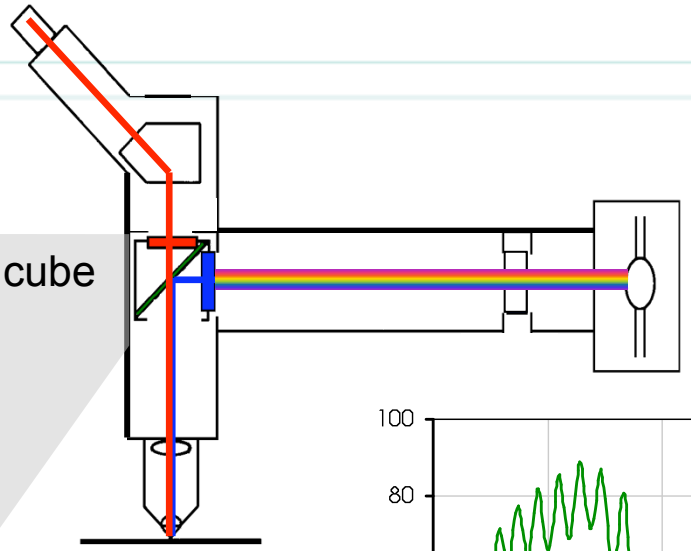


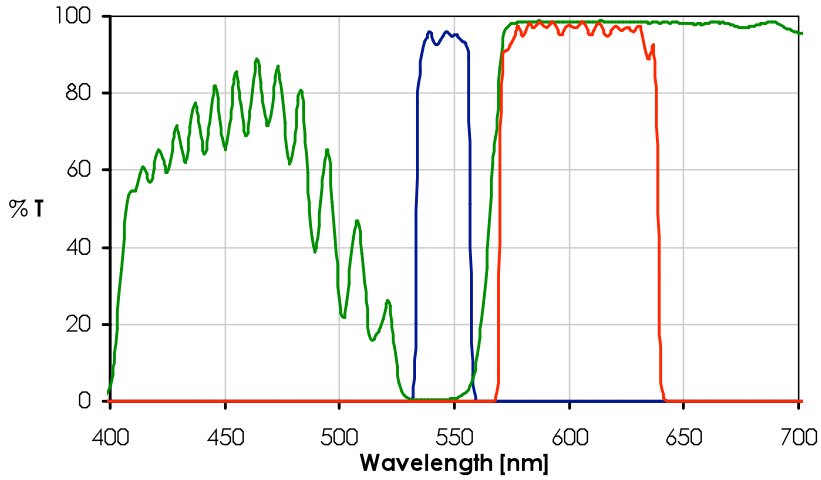
:: Measuring fluorescence

Microscopes



filter cube





:: Filters

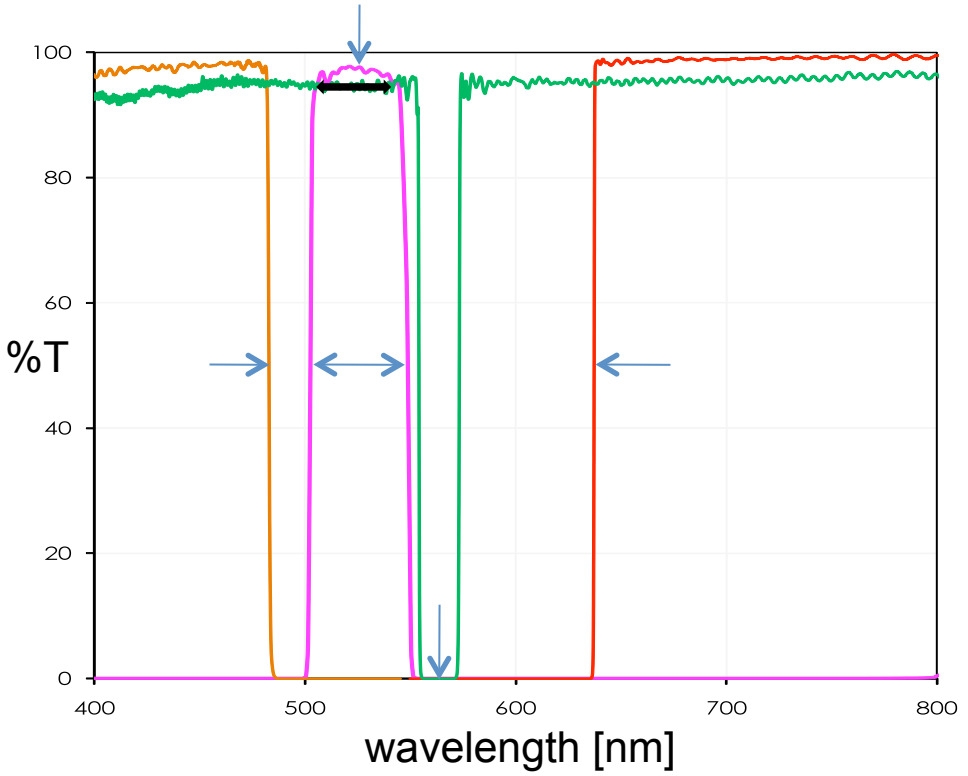
Filter types

:: Bandpass 525/50

:: Longpass 640 LP

:: Shortpass 490 SP

:: Notch 561 nm



Define:

:: CWL or notch wavelength

:: FWHM

:: GMBW*

:: Cut-on

:: Cut-off

:: Blocking range

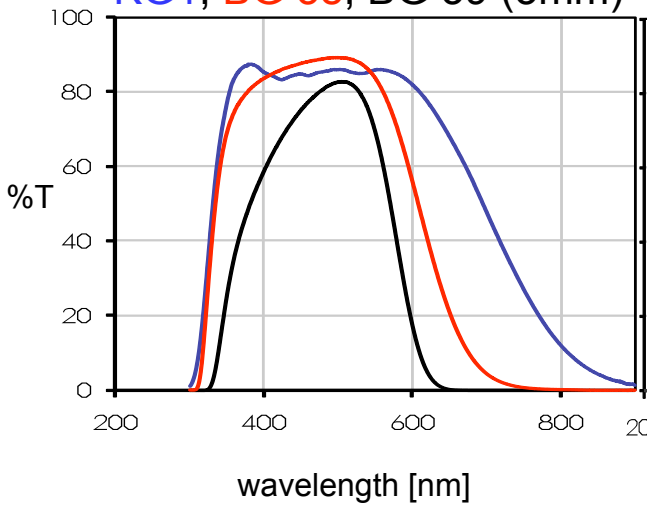
*FWHM=GMBW+ 0,01xCWL

:: Filters

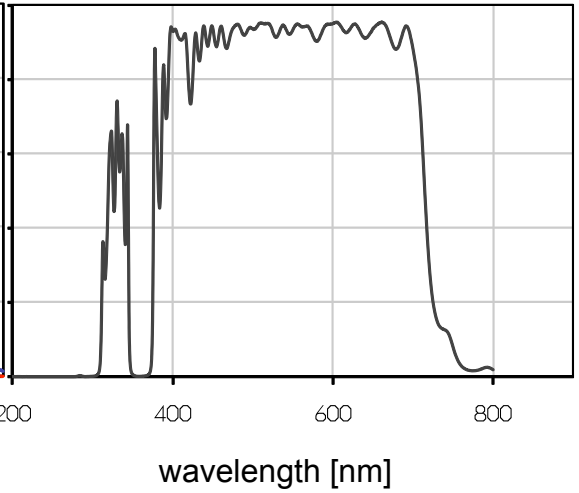
Filters for

heat protection (absorption) | heat protection (interference) | attenuation (interference)

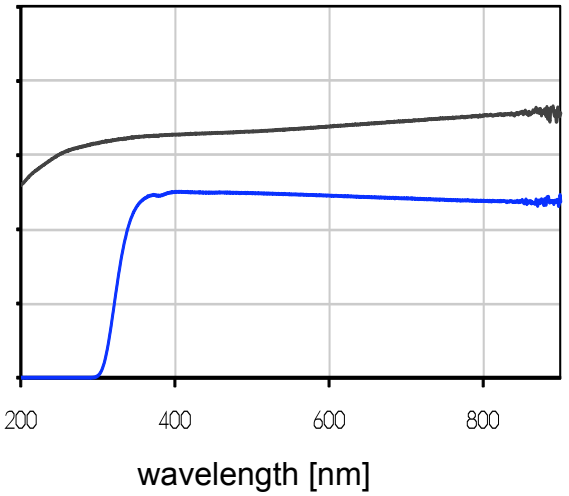
KG1; BG 38; BG 39 (3mm)



Hot Mirror



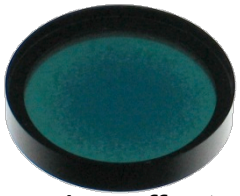
VIS-ND0,3; UV-ND0,2



:: Filters

	absorption filters	softcoated filters (D, HQ)	hardcoated filters (HC, ET)
steep cut-on/cut-off	15-30 nm	few nm	few nm
angle of incidence	insensitive	sensitive	sensitive
transmission	~90%	80-90%	>95%
burn out	no	yes	no
aging	depending	yes	no
autofluorescence	yes	no	no
flexible design (single filter)	no	yes	no

ok

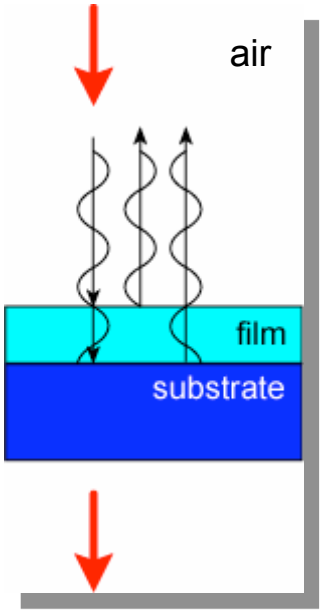


aging effects

burned

:: Filters

Thin film coating

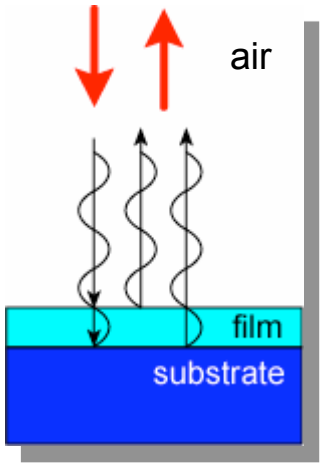


destructive interference

reflected waves add up out of phase
=> transmission

$T_{film} = \lambda/2*(2x+1)$

refraction index n: $n_{air} \neq n_{film} \neq n_{substrate}$



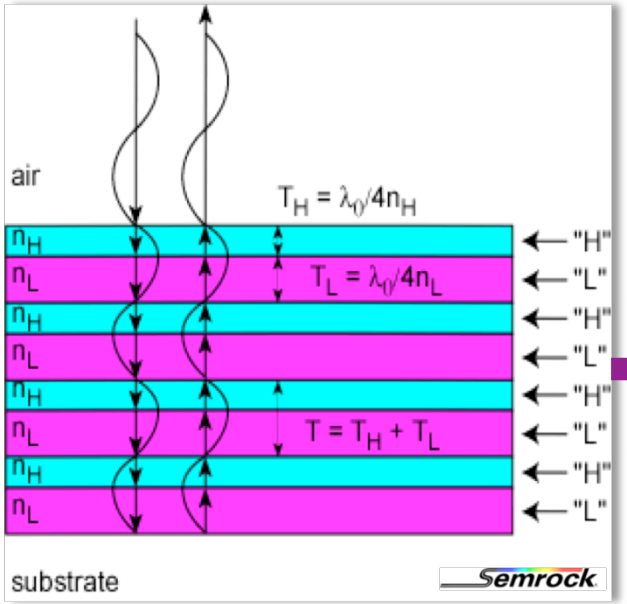
constructive interference

reflected waves add up in phase
=> reflection

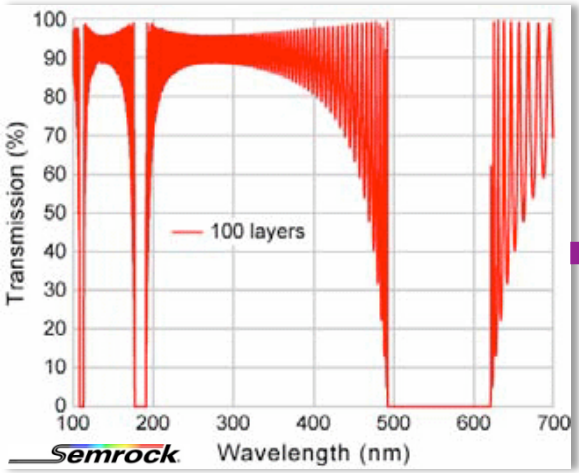
$T_{film} = \lambda/4*(2x+1)$

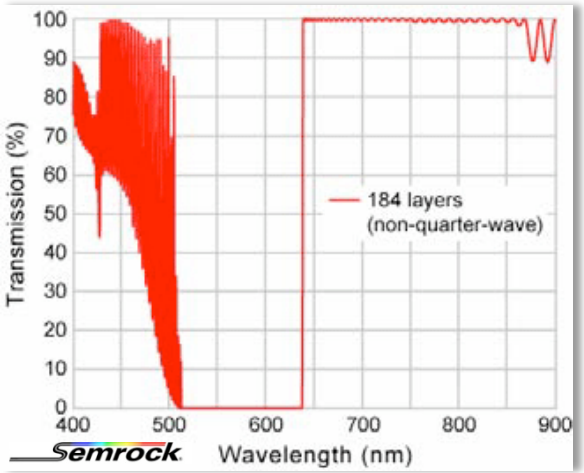
:: Filters

Thin film coating



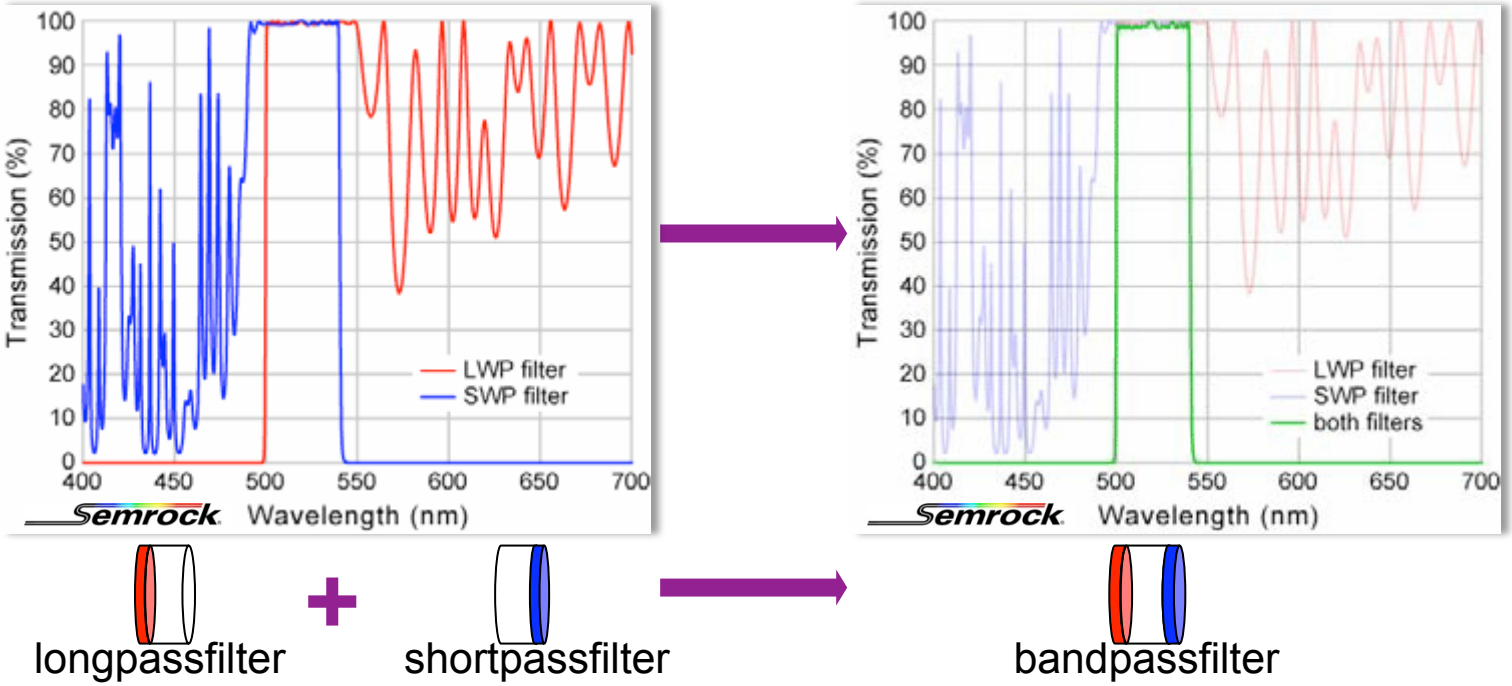
Many quaterwave layers cause strong reflection, due to constructive interference





:: Filters

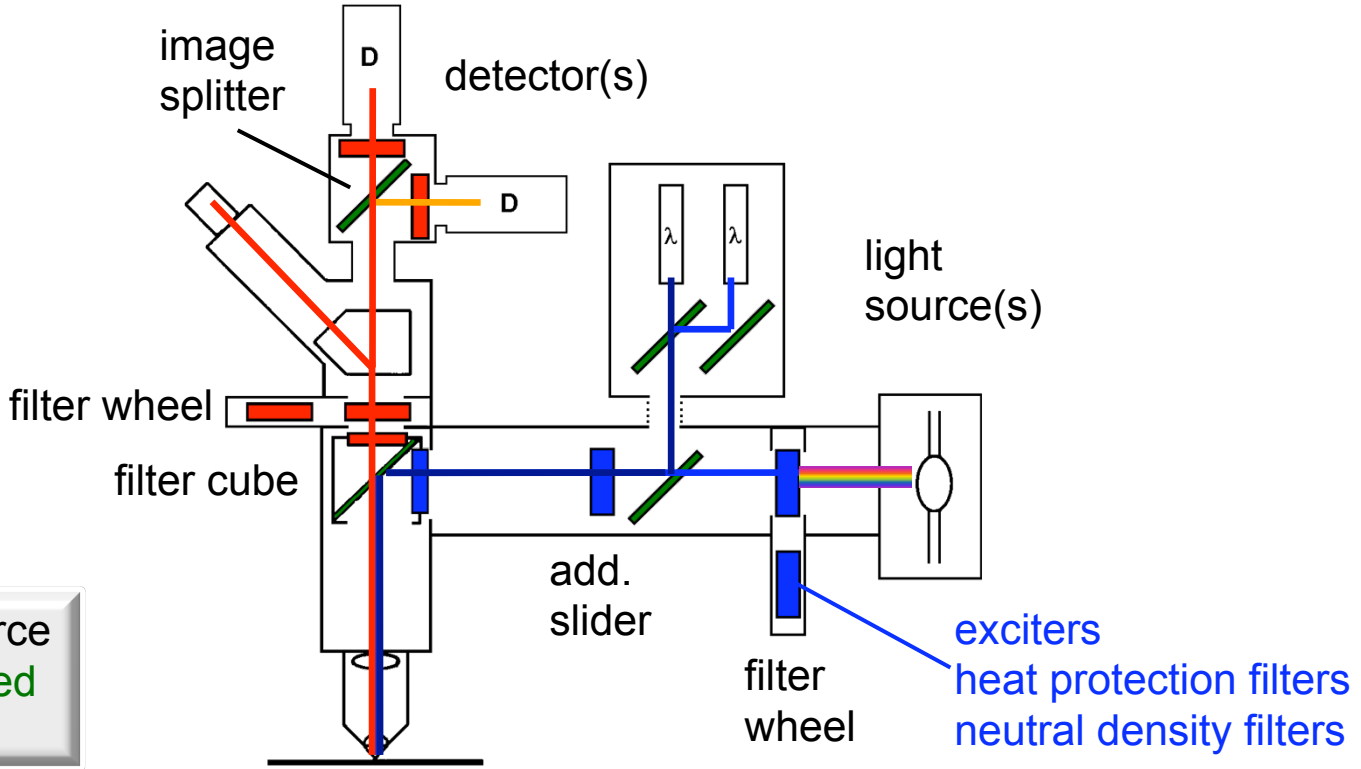
From thin film coatings to a filter



:: Fluorescence microscopy

Mounting filters into

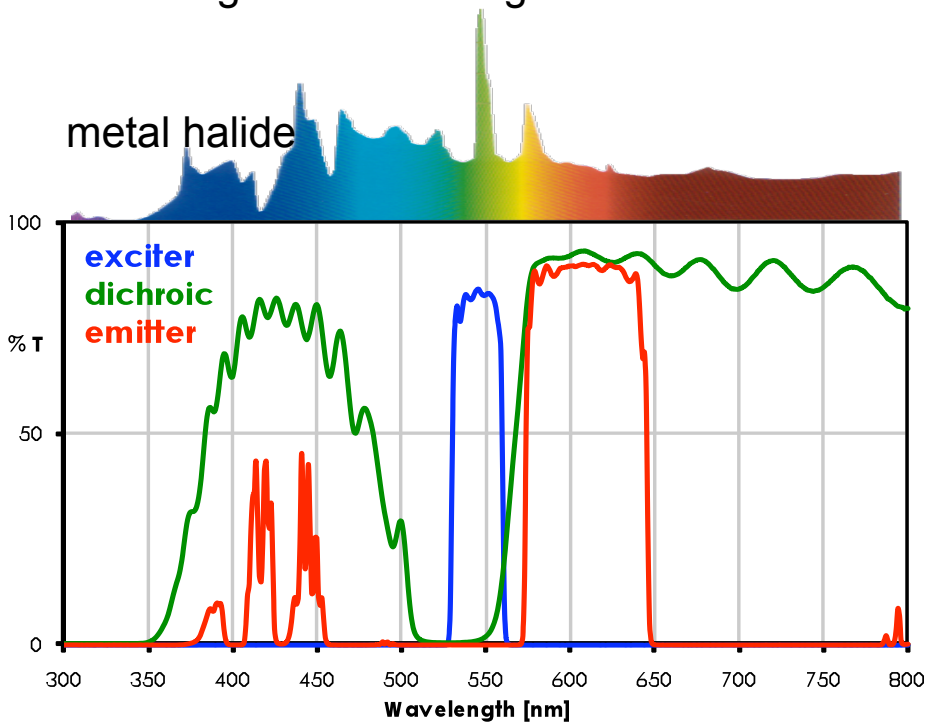
a microscope

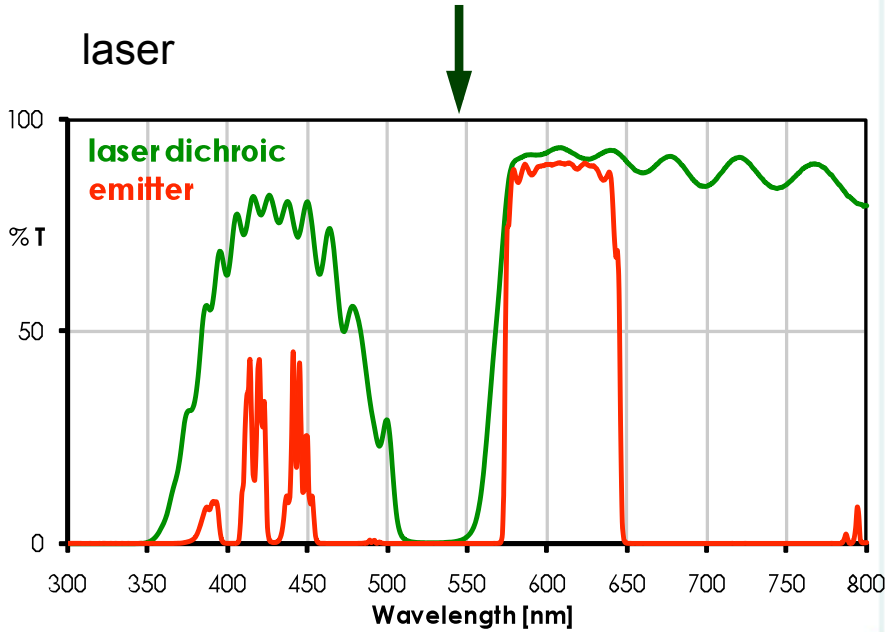


max.1 exciter / light source
beamsplitter(s) as needed
1 emitter /detector

:: Fluorescence microscopy

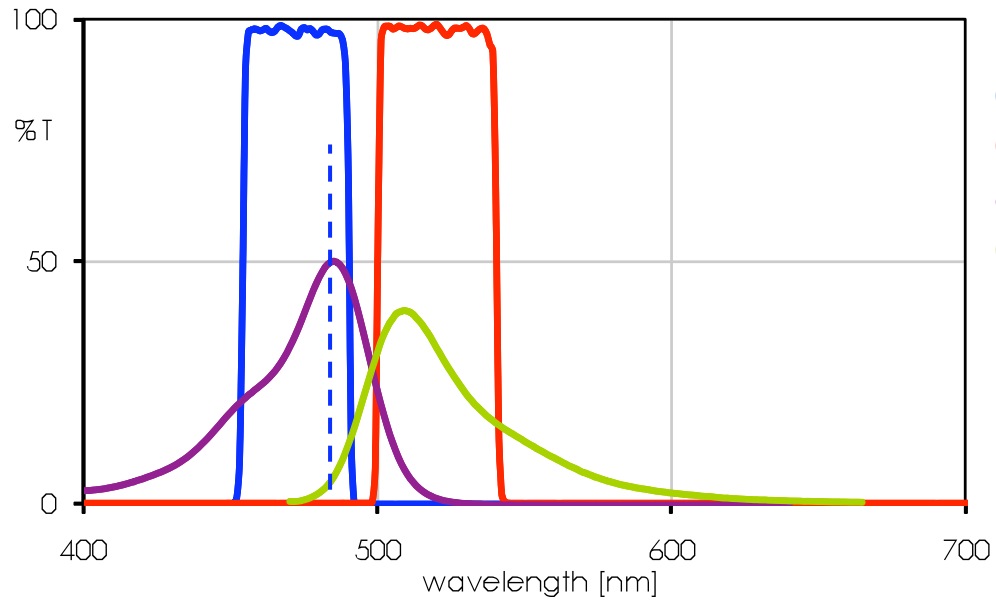
Customizing filters to the light source





:: Fluorescence microscopy

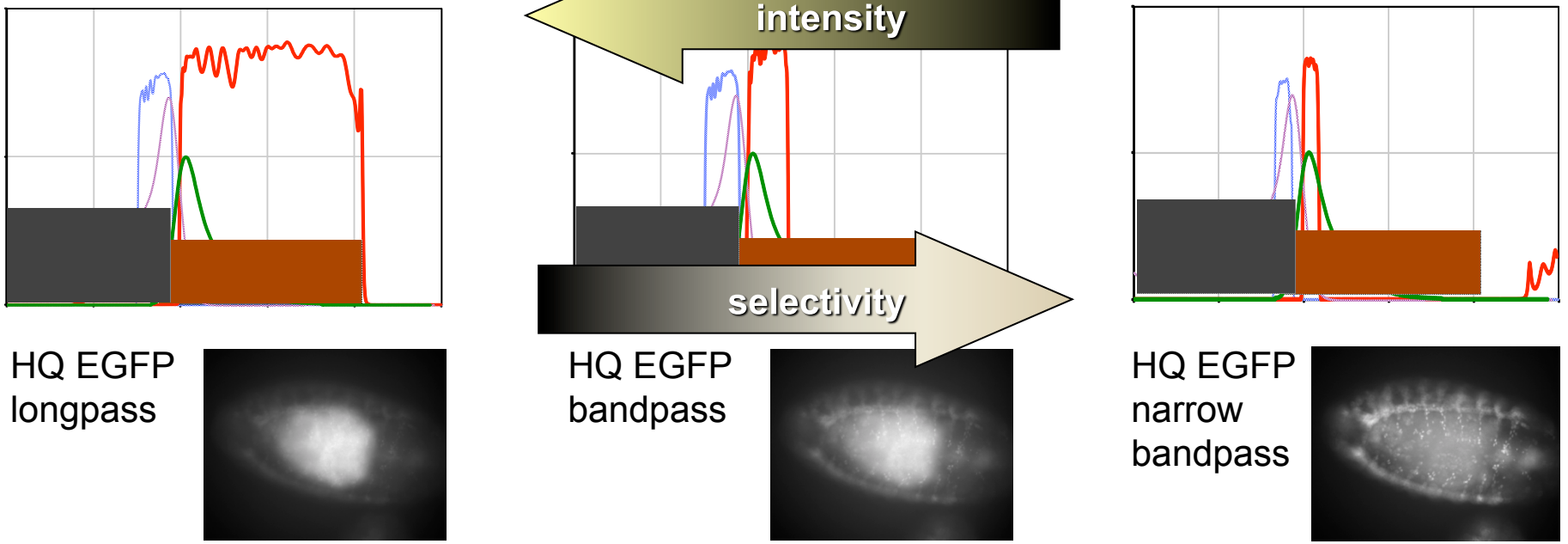
Customizing filters to the fluorescent dye



excitation filter or laser ---
emission filter
absorption EGFP
emission EGFP

:: Fluorescence microscopy

Signal / noise ratio



:: Fluorescence microscopy

Multicolor fluorescence

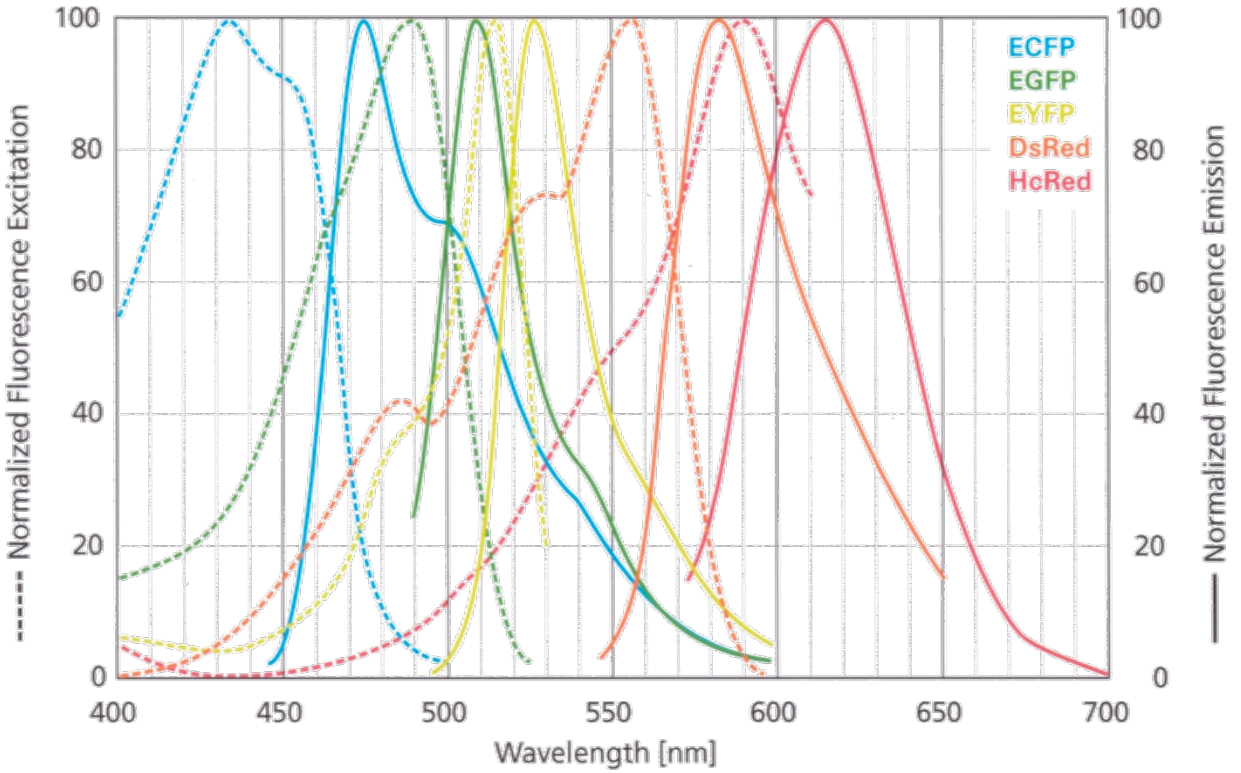
Problem:

:: Spectral overlap

Solution:

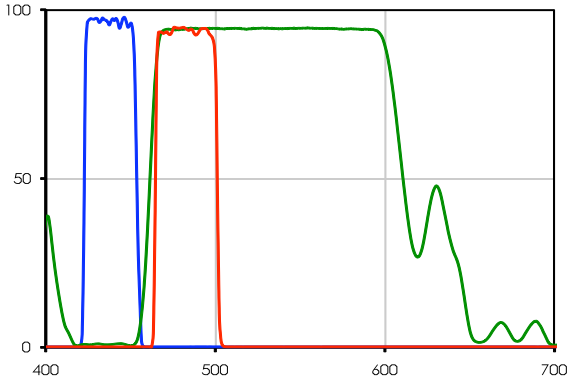
:: Selective singleband filtersets

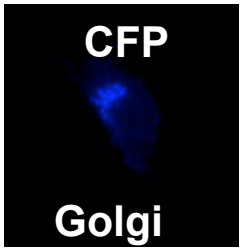
:: Multiband filtersets



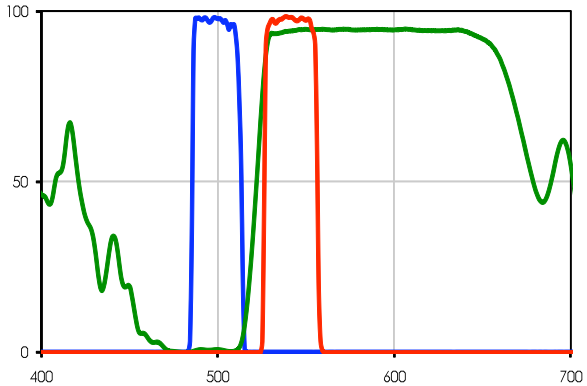
:: Fluorescence microscopy

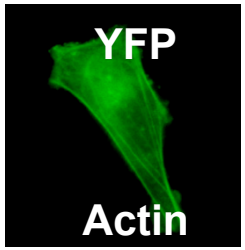
Singleband filtersets



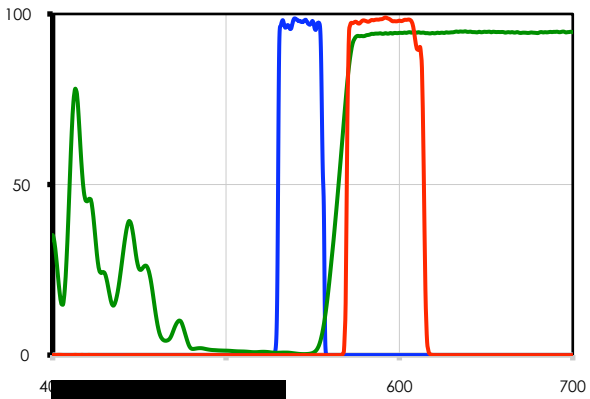


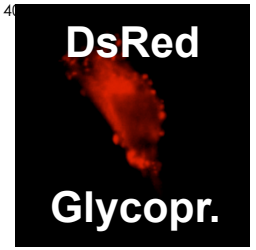
:: cuts YFP emission





:: avoids CFP excitation
:: cuts DsRed emission





:: avoids YFP excitation

Hela cells, J. Kühn, Virology University Münster

:: Fluorescence microscopy

Features of singleband filtersets

+ selective

+ perfect adaption to spectral properties

+ best s/n ratio

+ bright images

+ wide out of band blocking

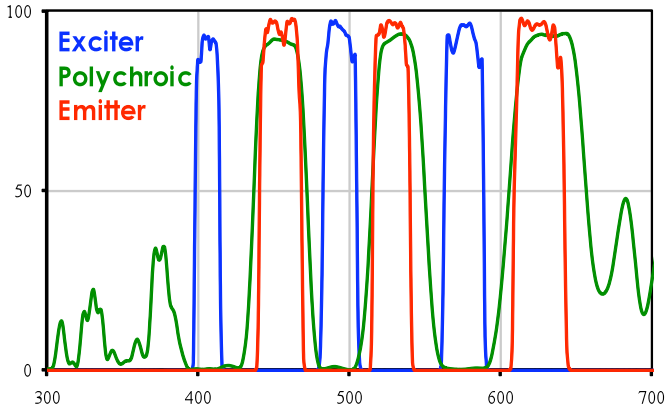
+ short irradiation time

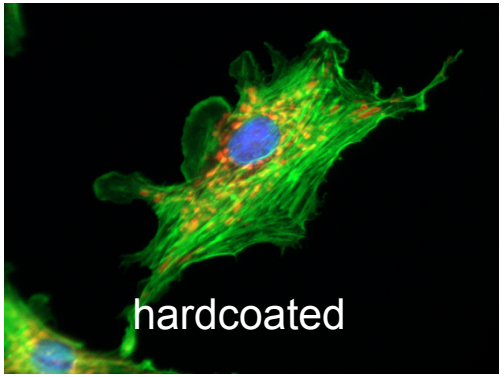
± pixelshift-free filtersets possible with/without adjustment

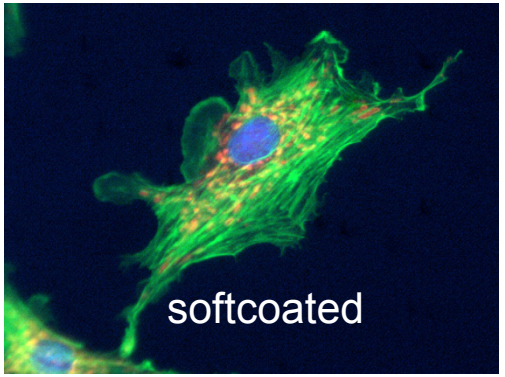
- slow switching to the next filtercube (~ 1s)

:: Fluorescence microscopy

Dual-, triple-, or multiband filtersets







:: e.g. Tripleband filterset for DAPI, FITC and Texas Red
for simultaneous imaging of all three colors

:: Fluorescence microscopy

Features of multiband filtersets

+ overview

+ no filterchange

+ real time acquisition in up to four channels

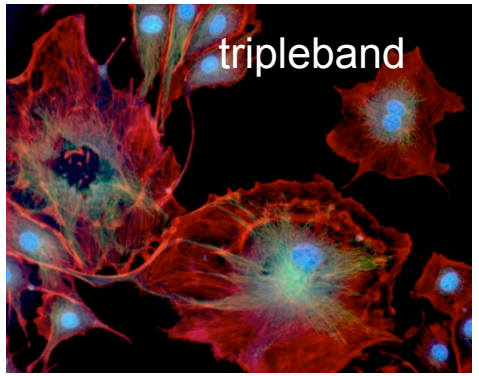
+ no pixelshift

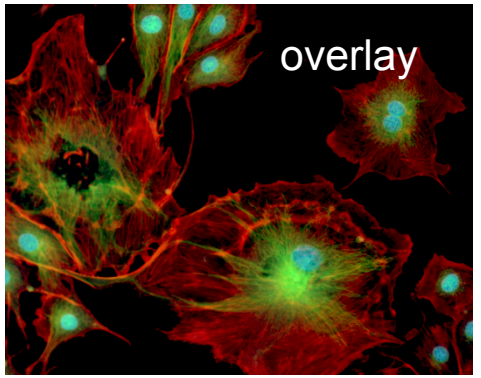
± „co-localized“ signals

± balance between the different colour channels

- loss of intensity in comparision to singleband filtersets

- bleed through due to spectral overlap possible



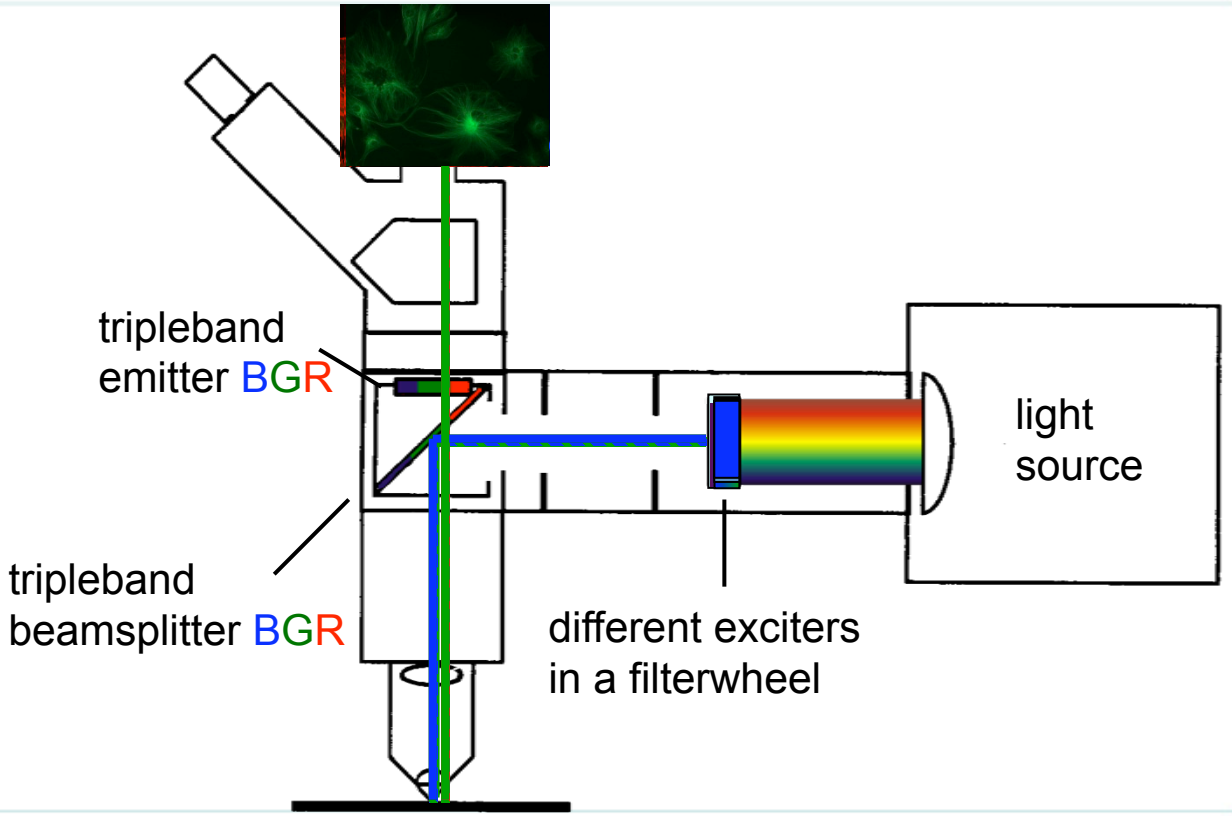


:: Fluorescence microscopy

Live cell imaging

:: Multiband filtersets with singleband exciters, the choice for time lapse experiments

:: also available with singleband exciters and singleband emitters

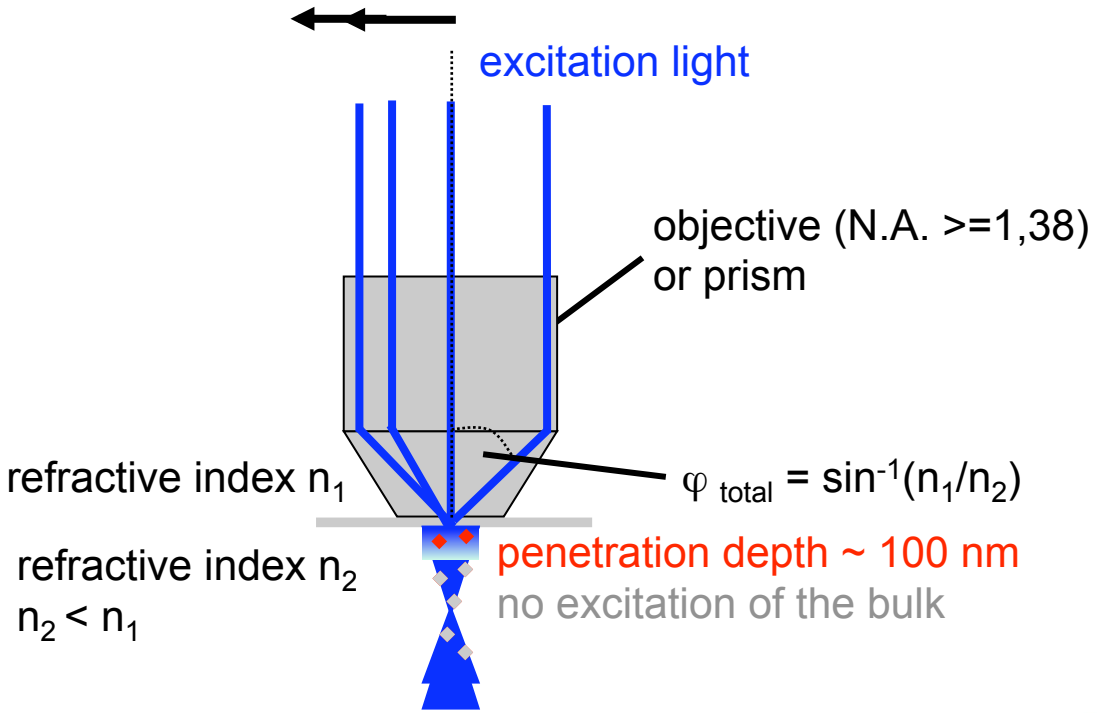


:: Advanced microscopy

Total internal reflection microscopy (TIRF)

:: laser is coupled into a microscope under total reflection conditions

:: resulting evanescent wave excites only the surface area

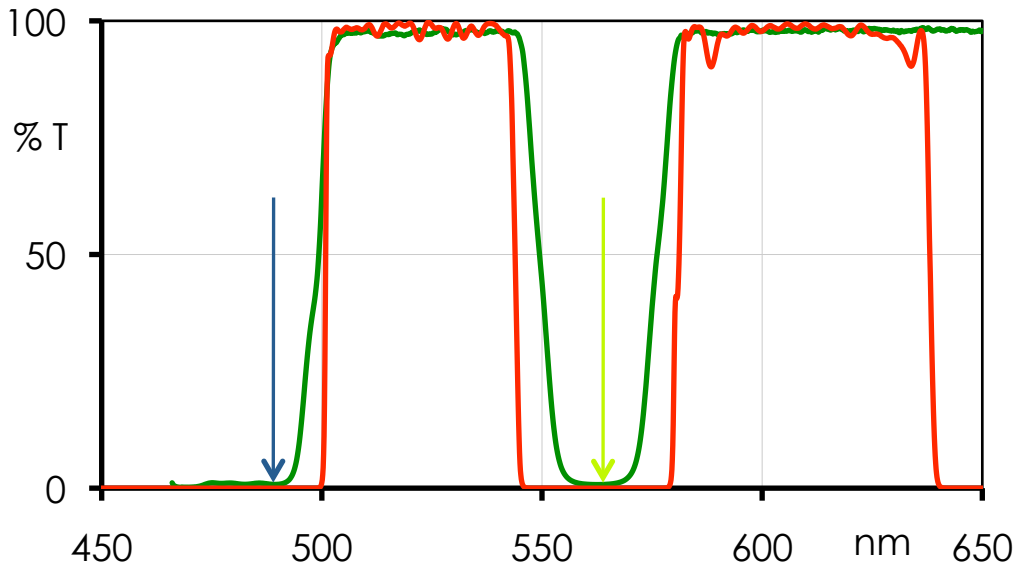


:: Advanced microscopy

Total internal reflection microscopy (TIRF)

:: filtersets for a single laser line up to four different laser lines

:: e.g. dualband GFP / mCherry lasers 473-491 nm and 559-561 nm (clean up not shown)



:: **Advanced microscopy**

Multiphoton microscopy (2P, MP)

:: nonlinear optical process

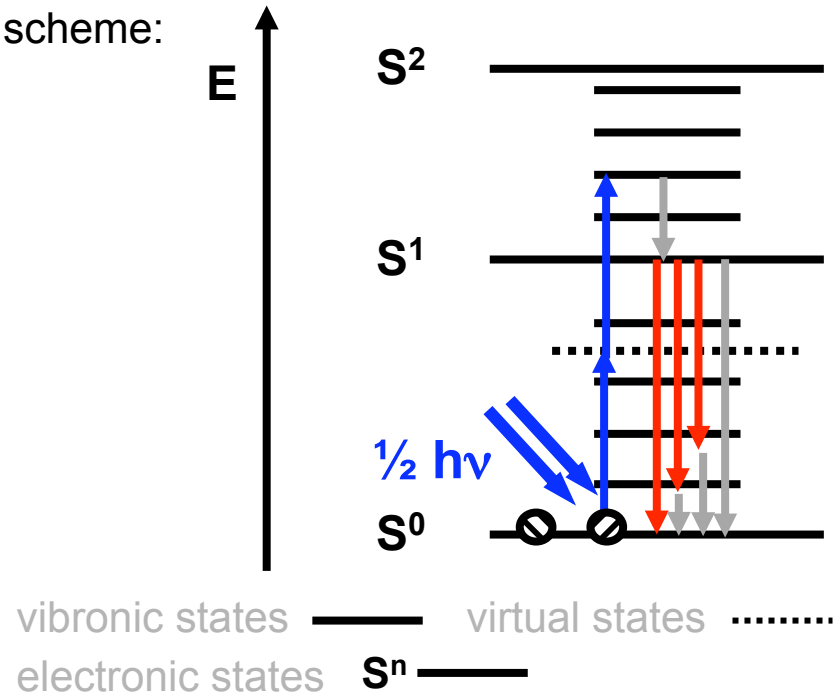
:: simultanous absoption of two photons

:: electron transition into an excited state

:: wavelength of emitted light > ½ λ_{Laser}

:: non energy conserving

Term scheme:



:: Advanced microscopy

Multiphoton microscopy (2P, MP)

Benefits:

:: less photobleaching (neglible out of focus)

:: deeper penetration into tissue

:: quasi confocal imaging

:: less scattering

:: excitation profil:

1P MP

Optical components:

:: low pulse broadening

:: deep blocked filters in the IR against the laser wavelengths

:: high near UV and VIS transmission

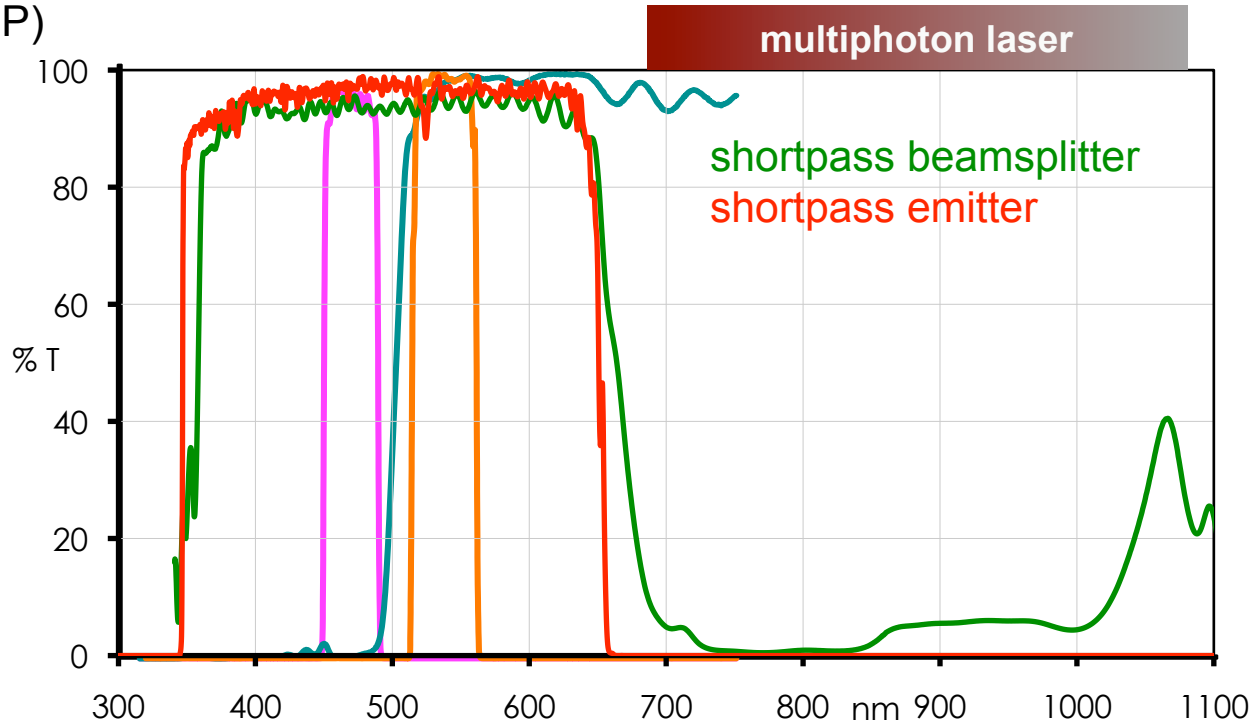
:: energy resistent coatings

:: Advanced microscopy

Multiphoton microscopy (2P, MP)

:: Epifluorescence setup

:: with emission splitting

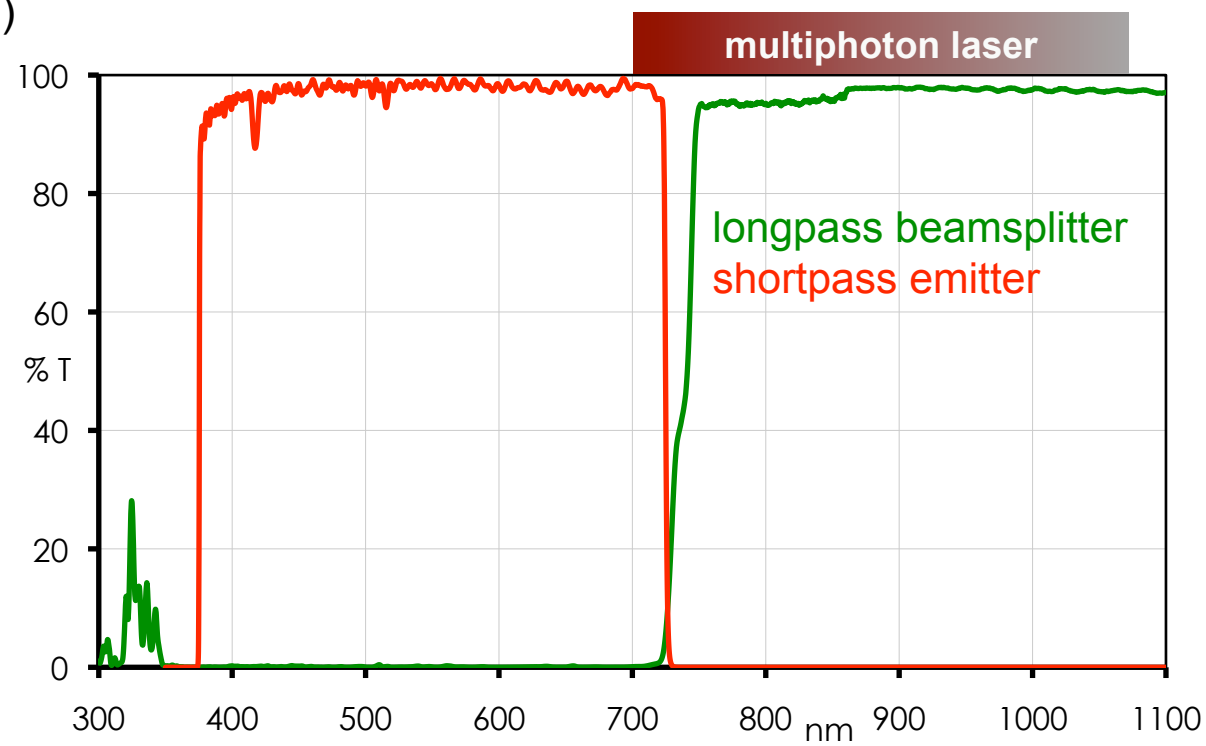


:: Advanced microscopy

Multiphoton microscopy (2P, MP)

:: sideport setup

:: excitation through transmitted light axis and detection via sideport



AHF ANALYSENTECHNIK

:: Advanced microscopy

Second harmonic generation (SHG)

:: nonlinear coherent scattering

:: energy conserving

:: no absorption of energy

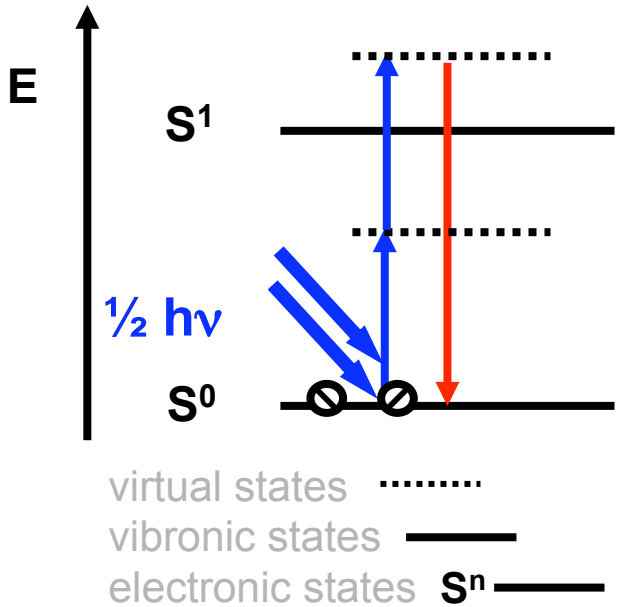
:: intrinsic property of non-centrosymmetric molecules

:: signal depends only on the laser: $\lambda_{SHG} = ½ \lambda_{Laser}$

:: peak width: $\Delta\lambda_{SHG} = \Delta\lambda_{Laser} / \sqrt{2}$

:: mostly used for collagen (incident laser ~800 nm)

Term scheme:



:: **Advanced microscopy**

Second harmonic generation (SHG)

Benefits:

:: can be combined with 2P-fluorescence

:: no staining neccessary

Optical components:

:: low pulse broadening

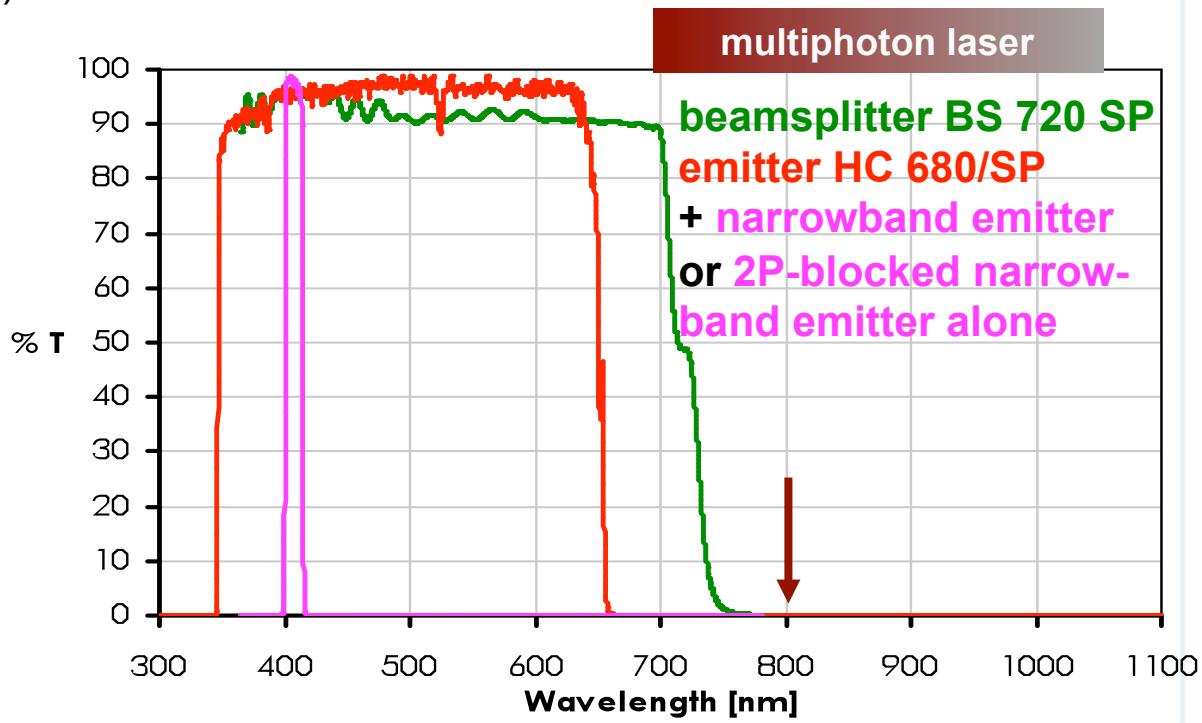
:: deep blocked filters in the IR at the laser wavelength

:: narrowband filters for blocking any 2P fluorescence

:: energy resistent coatings

Second harmonic generation (SHG)

:: Narrowband emitter separates SHG signals from 2P-fluorescence



Raman

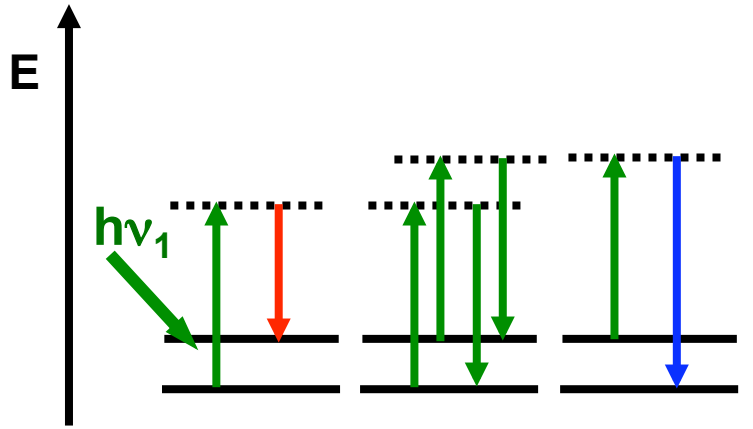
:: nonlinear optical process

:: molecules have to be polarizable

:: intensity of Raman signals are proportional to the polarizability

:: can be described as elastic (Rayleigh) and inelastic (Stokes and Antistokes) scattering of light

Term scheme:



virtual states ·····

vibronic states ——

Rayleigh, Stokes, Antistokes scattering

:: Advanced microscopy

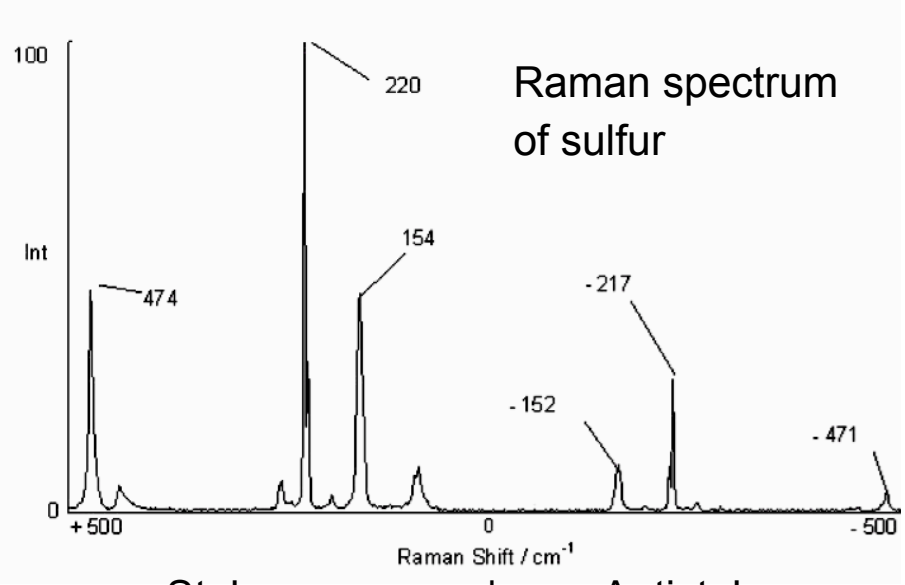
Raman

Benefits:

:: no staining neccessary

:: molecule specific

:: molecules can be characterized by their Raman spectrum



Raman spectrum of sulfur

Disadvantage

:: fluorescence might cover Stokes signals

:: low intensity (two magnitudes lower than fluorescence)

:: Advanced microscopy

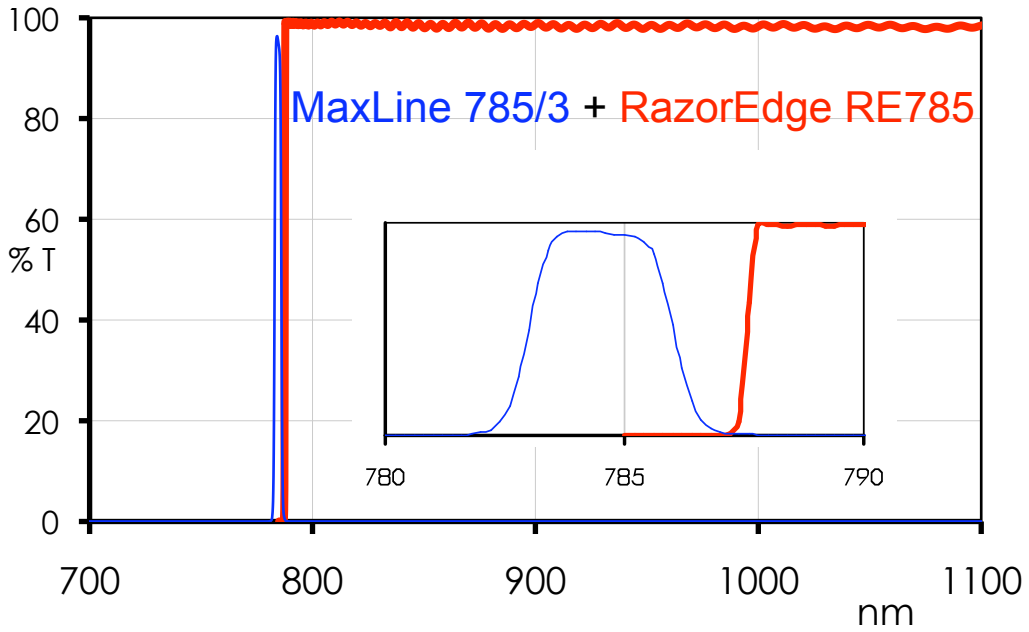
Raman

Optical components:

:: very steep filters and beamsplitters
neccessary due to small spectral shift

:: deep blocking at the pump laser line

:: notch, longpass or shortpass filters



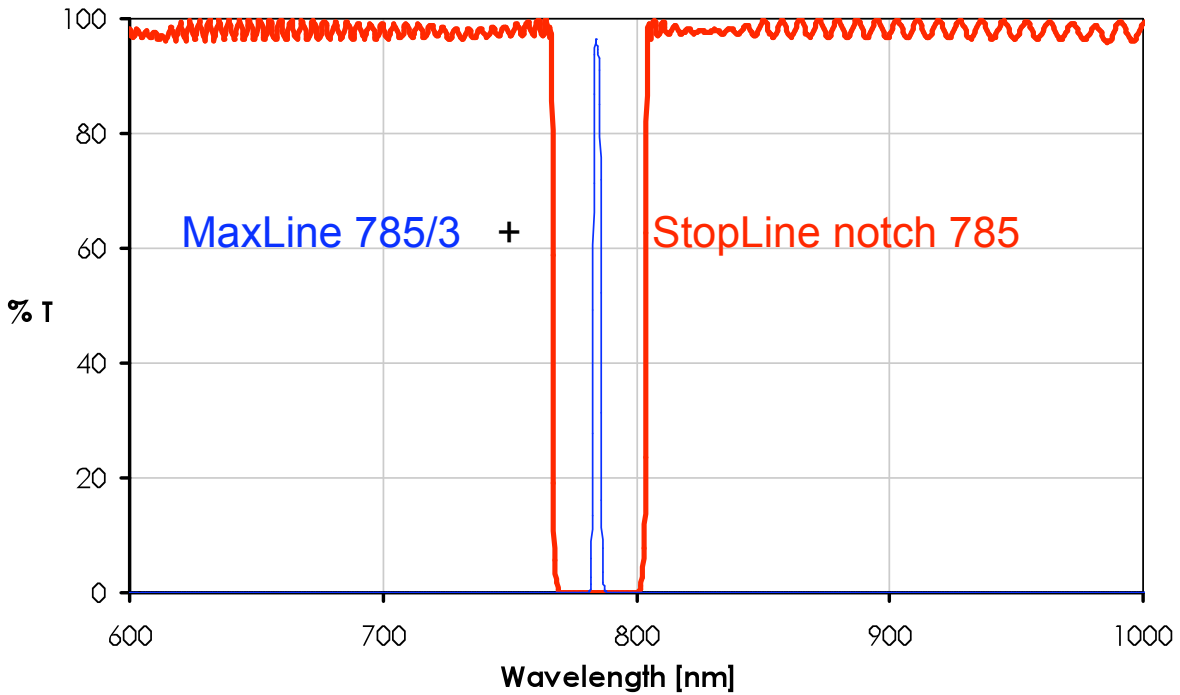
:: Advanced microscopy

Raman

:: angle tuning possible

$\lambda(\alpha) = \lambda_0 \sqrt{(1-(\sin(\alpha)/n_{eff}))^2}$

>= OD 6 under α = 14 deg.

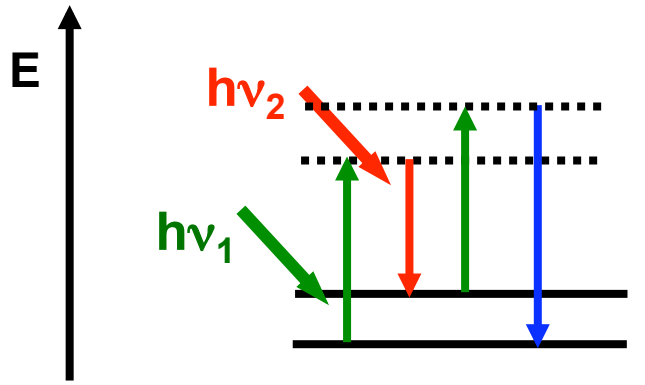


Coherent Antistokes Raman Spectroscopy (CARS)

:: nonlinear optical process

:: can be described as driven harmonic oscillator

Term scheme:



$h\nu_1$ pump laser
$h\nu_2$ Stokes laser
Antistokes scattering

virtual states
vibronic states

:: Advanced microscopy

Coherent Antistokes Raman Spectroscopy (CARS)

Benefits:

:: molecule specific imaging

:: no staining neccessary

:: factor 10^5 more intense compared to Raman

:: fluorescence does not matter

Optical components:

:: very steep filters and beamsplitters neccessary due to small spectral shift

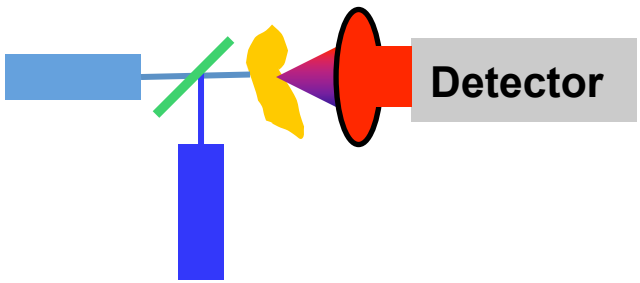
:: deep blocking at both laser lines

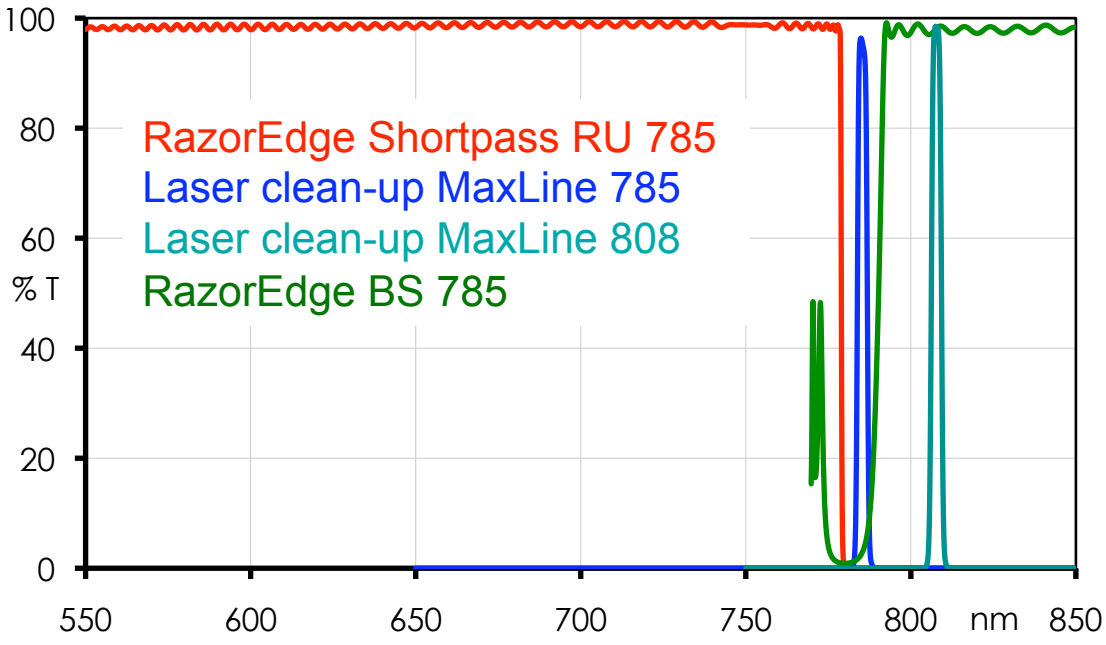
:: shortpass filters as detection filters

:: **Advanced microscopy**

Coherent Antistokes Raman Spectroscopy (CARS)

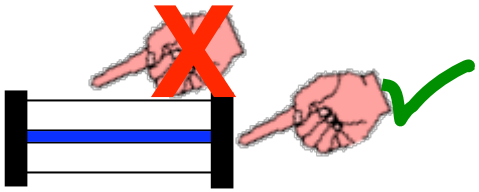
:: setup for 785 nm pump laser
and 808 nm Stokes laser



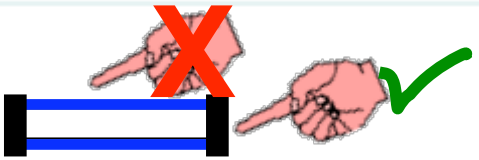


:: Filter handling

Cleaning



softcoated filter
(always with filter ring)



hardcoated filter or
beamsplitter
(with or without filter ring)

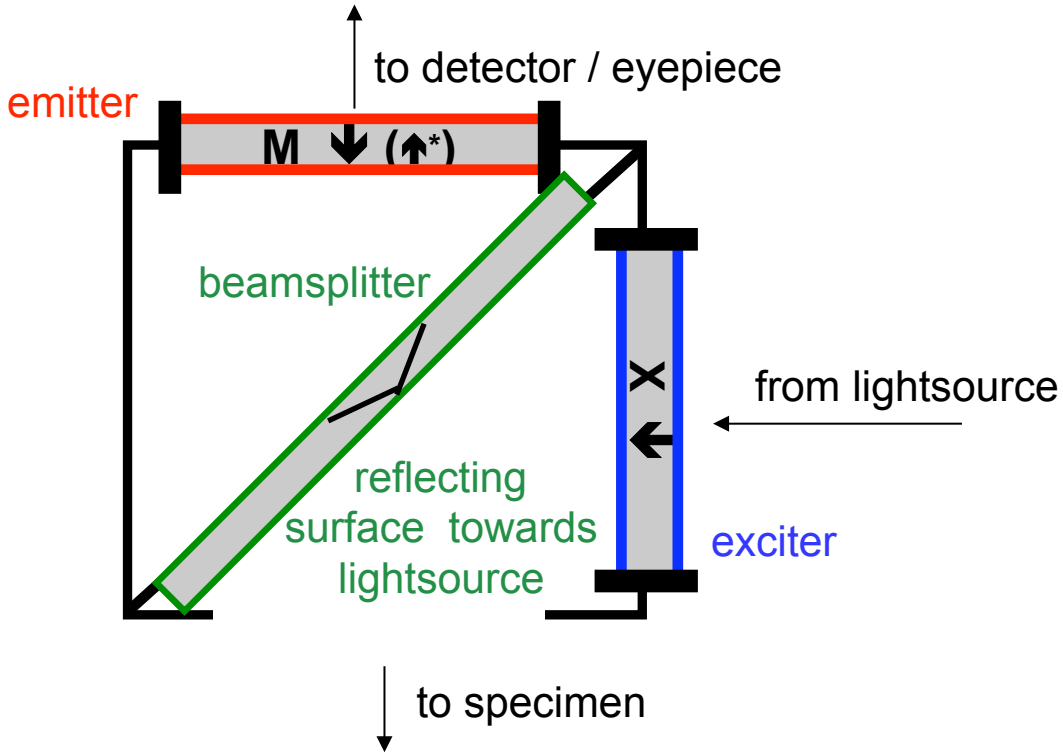
Cleaning procedure

- with filtered pressurized air (oil free!) or bulb puffer
- wipe gently with a lint-free towel and ethanol, methanol or propanol. Use new surface of towel for each wipe

Never use aceton, THF, hexane, ... and other solvents

:: Filter handling

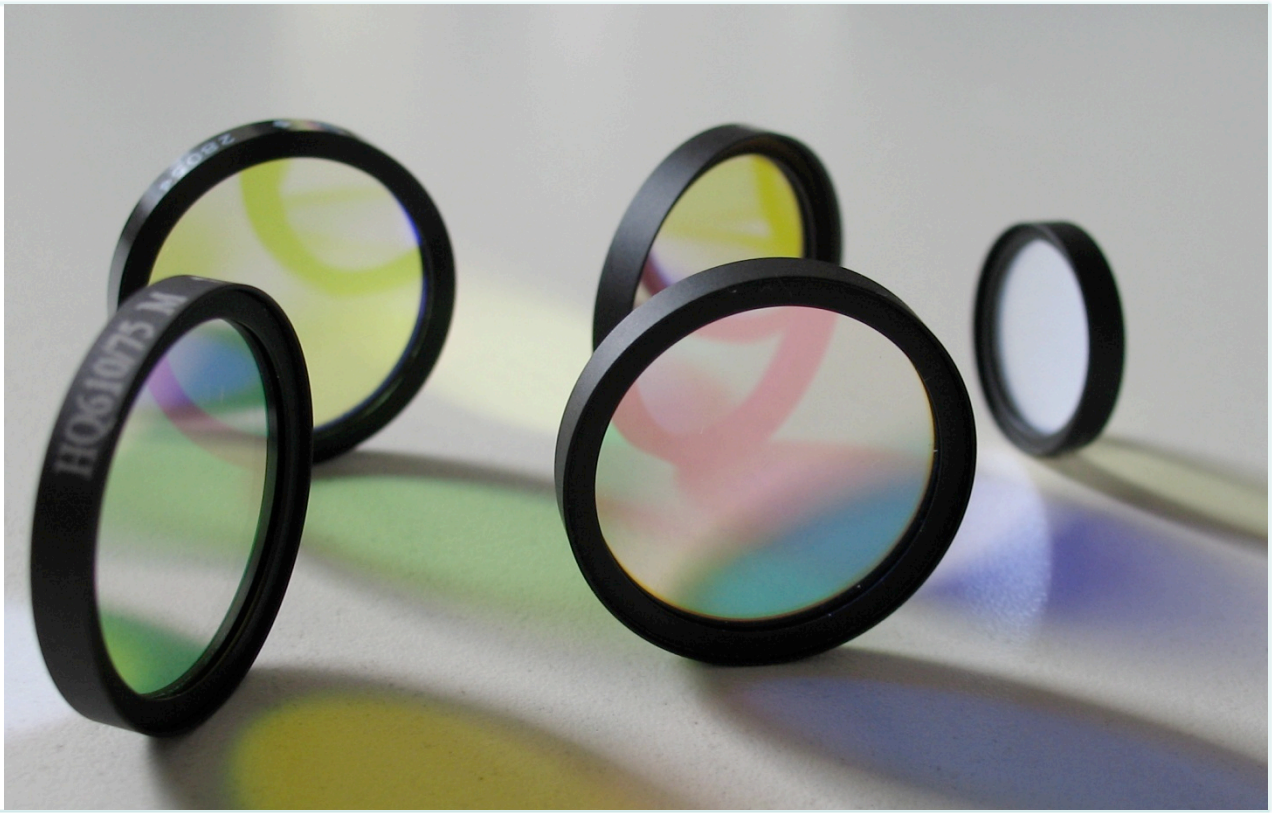
Mounting



* orientation of
BrightLine HC filters

:: Our experience – your profit

:: Thank you very much



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