

Lecture #6

Introduction to optics

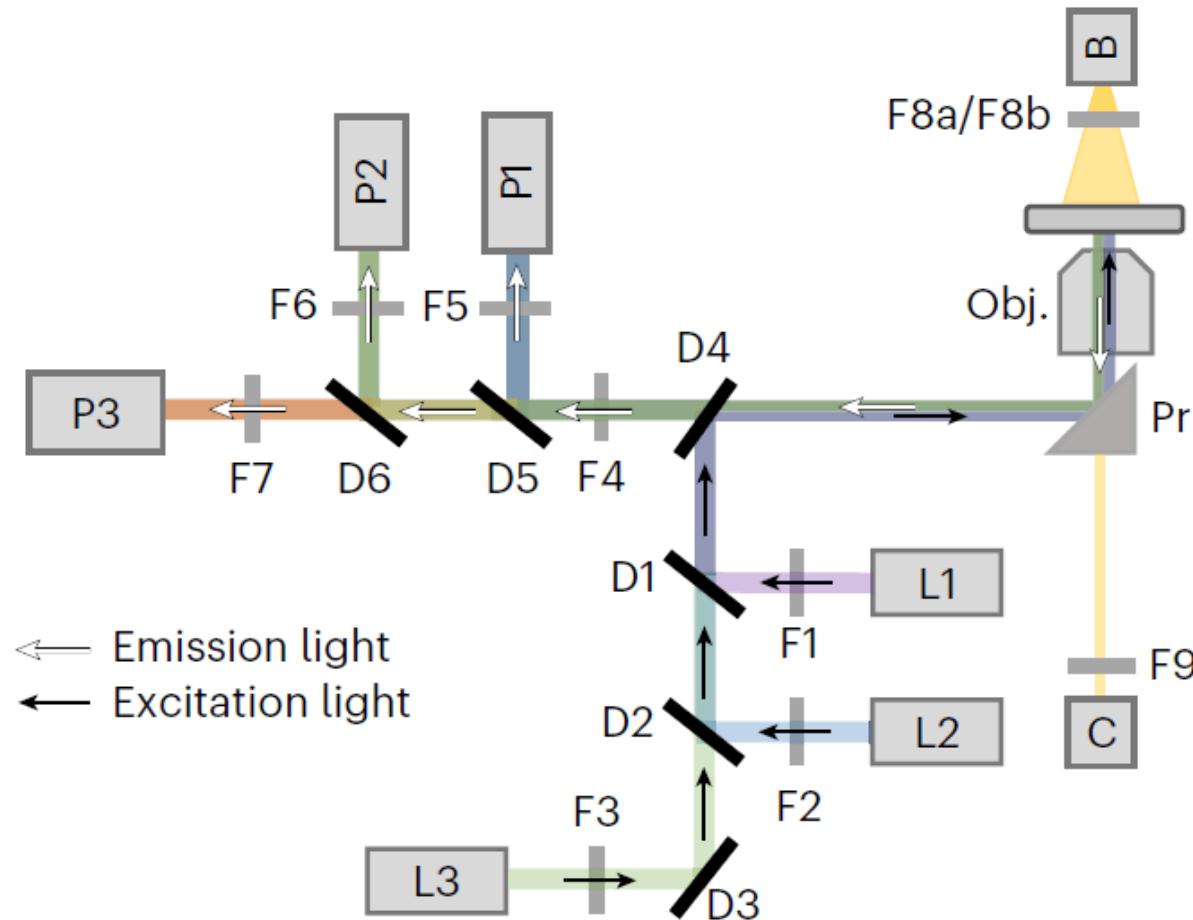
Aims:

- Understand the basic laws and concepts of geometrical optics
- Know the working principle of all optical components inside a microfluidics workstation
- Being able to design a setup and ray diagram of YOUR workstation

Lectures (CO 121)	Date & Topic	Details	Practical (location as color coded on next slide)
1	13.09 General Intro	Get to know teachers, TAs, students and aims of the course	17.09 Measure temperature using thermistor (using M&A explorer) TL
2	20.09 Lecture LabVIEW TL Group formation (A-F, 3 students, each)	Some first basic steps in LabVIEW programming	24.09 Brief intro into LabVIEW thermistor program (input and output) TL
3	27.09 Case study FACS, similarities and differences to droplet microfluidics Selection of case study topics	1.) Property to measure? 2.) Device? 3.) Working principle? 4.) Alternatives?	01.10 Preparation of bioinstrument case study
4	04.10 No course, preparation for case study		08.10 No course
5	11.10 Groups A-B presenting case study		15.10 Tour through LBMM workstation labs, intro into Nature Protocols (Groups A-B)
6	18.10 Lecture optics Homework: Students to prepare one laser/PMT blueprint FP	Mirrors, filters, microscope setup, lenses, etc.	22.10 Holidays
	25.10 Holidays, submit your blueprint by email		29.10 .10 Build workstation optics 1
7	01.11 Lecture electronics	FPGA, PMTs, amplifier, function generator	05.11 Build workstation 1 optics 2
8	08.11 Intro into enzyme concentration measurement experiment (kinetics, etc.) + task FP	Enzymes, kinetics, practical task	12.11 Build workstation electronics
9	15.11 Intro to droplet analysis software (LabVIEW) TL	Software similar to Thermistor program, pdf on installation	19.11 Build workstation software: Add output LED (mimicking sorting trigger) into analysis software
10	22.11 Fundamentals of microfluidics and microfluidic chips	Flow at the microscale, microfluidic chips (manufacturing), droplet microfluidic modules	26.11 Run microfluidic experiments, e.g. determine concentration of MMP in droplets
11	29.11 Prepare presentation		3.12 Sorting Demo on LBMM workstation1 (Groups A-B)
12	06.12 Prepare presentation		10.12
13	13.12 Groups B-A presenting results 13.12 Submit report (all!)		17.12 – TUESDAY! - Individual Q & A sessions (10min, Groups A-B)

Green shading: Single seminar/practical with all 18 students
Red shading: Individual seminar/practical with only 6 students required (= 3 sequential 90min slots, 4.5h in total)

What optical modules do we have in a microfluidic workstation?



L = lasers
F = optical filters
D = dichroic mirrors
Pr = prism
Obj. = objective
B = brightfield lamp
P = photomultiplier tube
C = camera

Which laws and concepts of geometrical optics are relevant for us?

Laws and concepts

Law of reflection

Refraction

Interference

Optical components used here

Lasers & other light sources

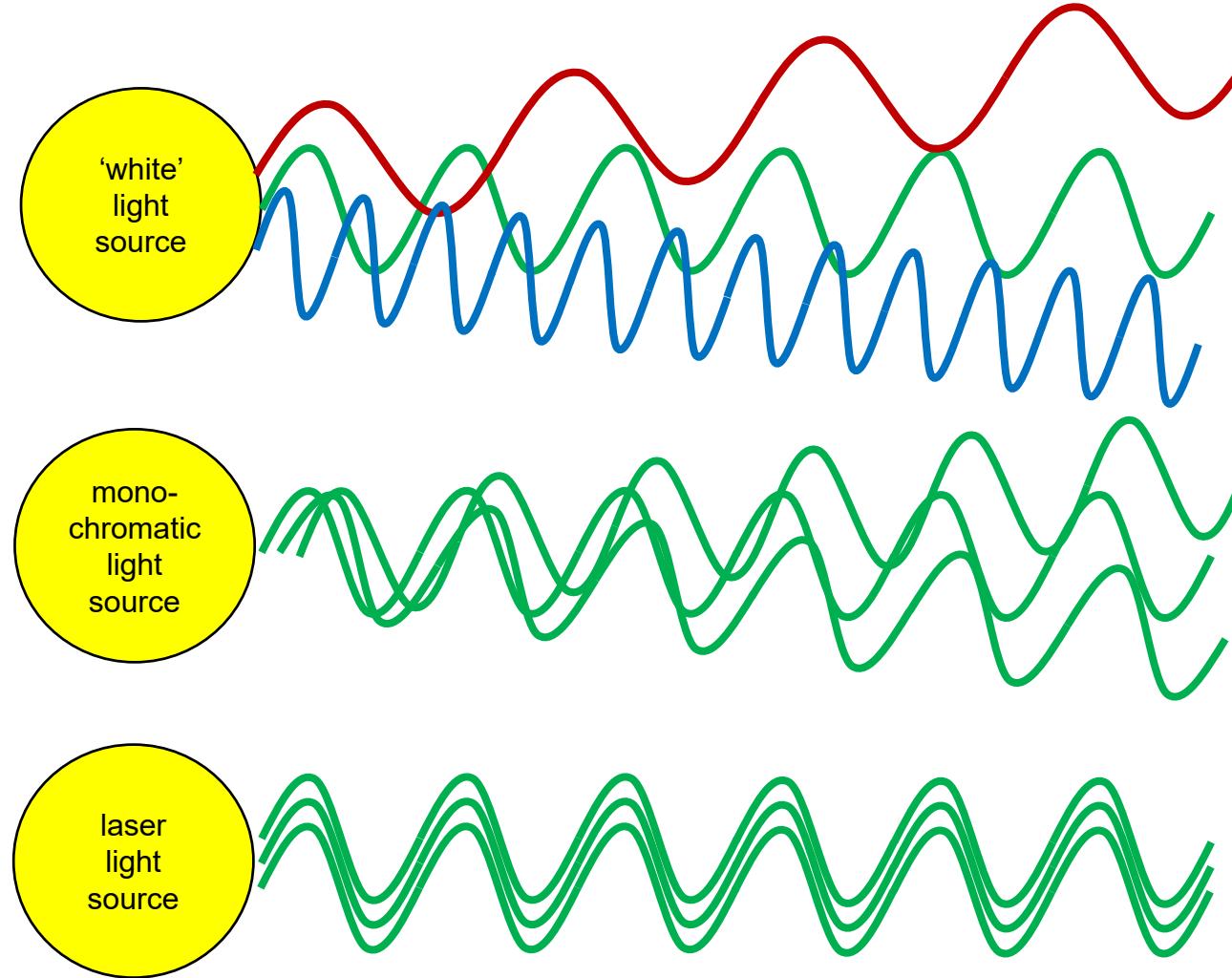
Filters

Mirrors

Prisms

Lenses

Different types of light sources



adapted from:
<https://www.quora.com/How-is-light-from-a-laser-different-from-ordinary-light>

Laser (light amplification by stimulated emission of radiation):
Monochromatic, with all waves in phase and emitted into a single direction!

Lasers used in the practical course



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[Home](#) > [420-500nm](#) > [FN Series 473nm Laser 100-1000mW](#)



FN Series 473nm Laser 100-1000mW

★★★★½ — 4

\$1800.00



Model Selection

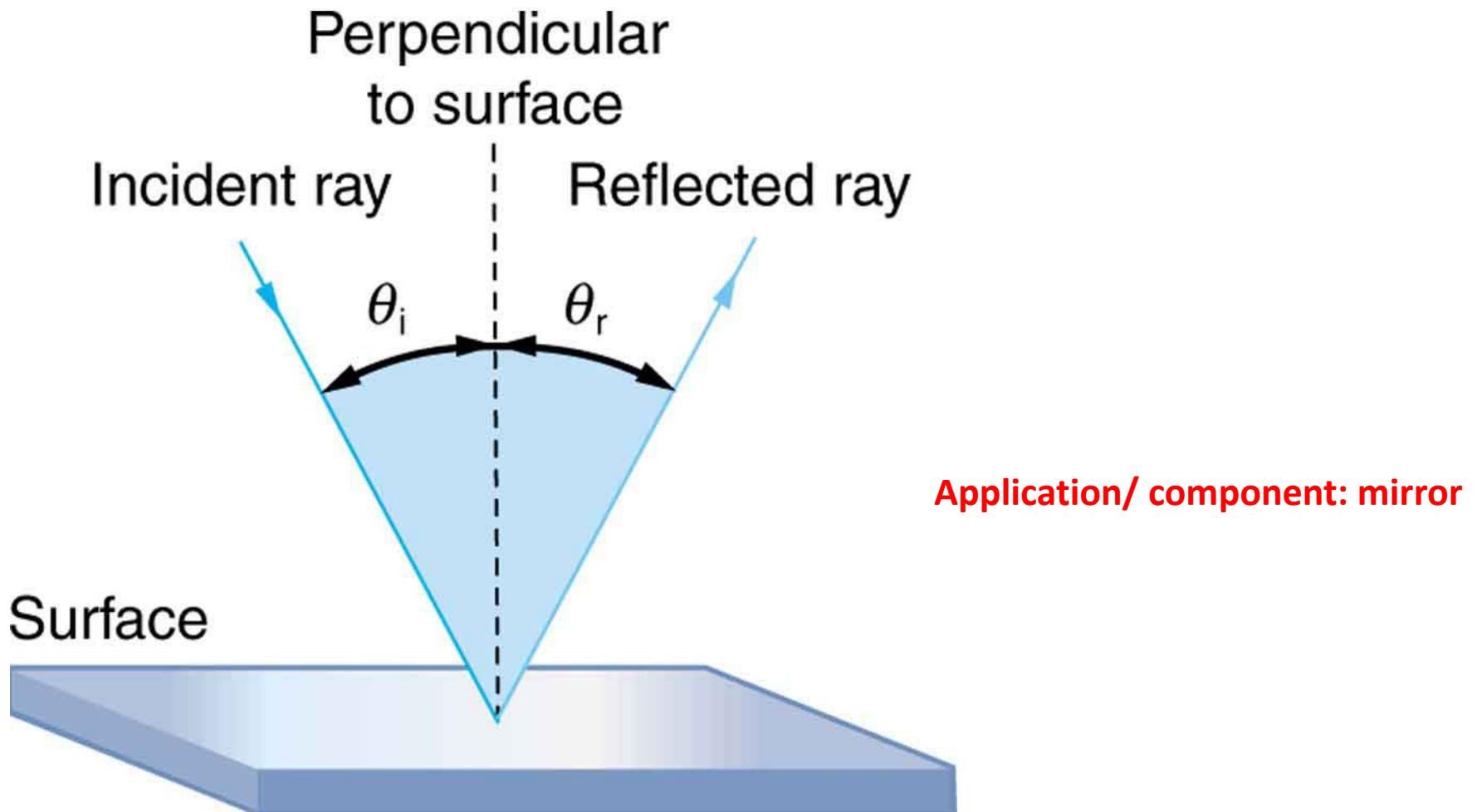
473nm 100mW

Qty:

[Add to cart](#)

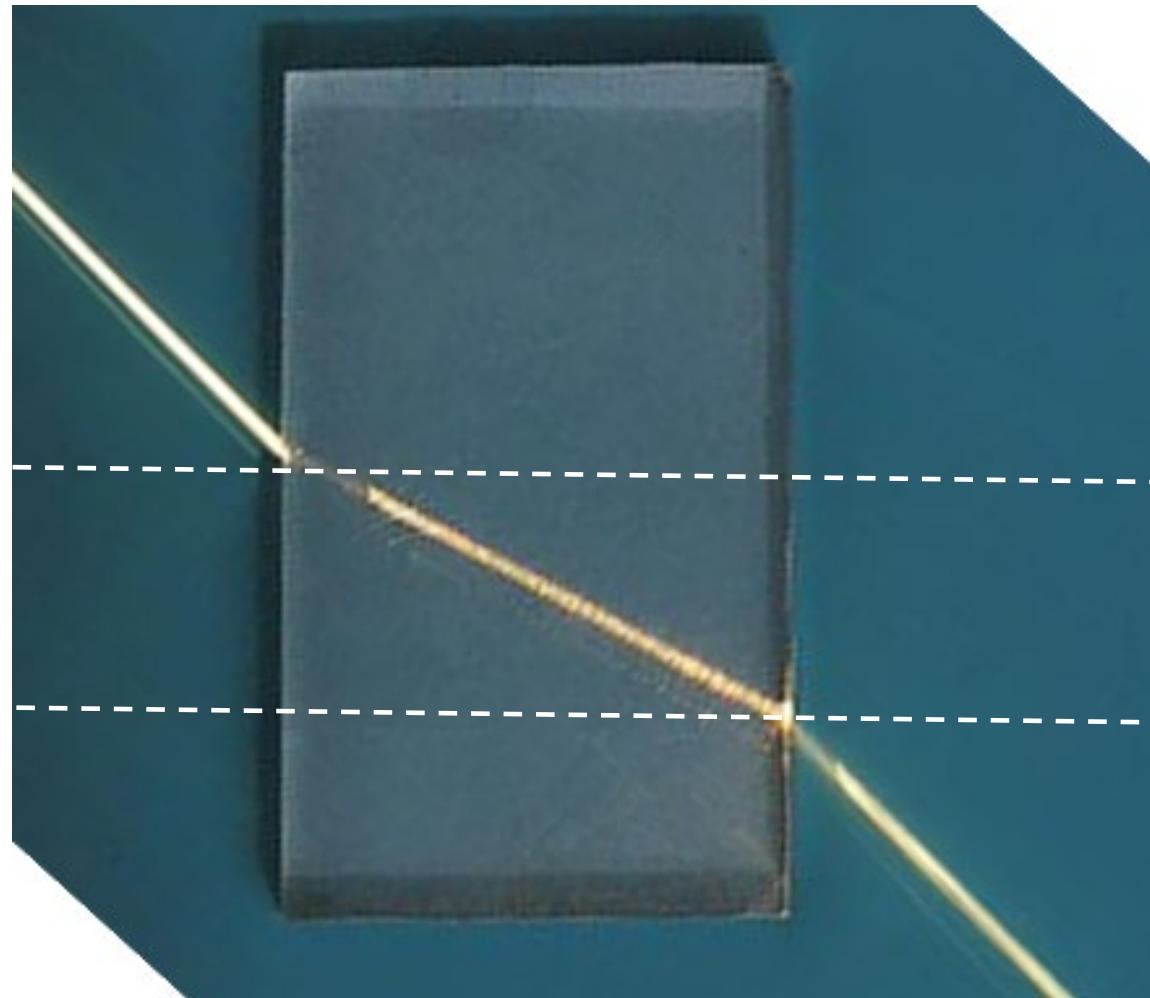
Visible light = 380 nm - 780 nm wavelength

Law of reflection



The law of reflection states that the angle of reflection equals the angle of incidence— $\theta_r = \theta_i$. The angles are measured relative to the perpendicular to the surface at the point where the ray strikes the surface

What is happening here?



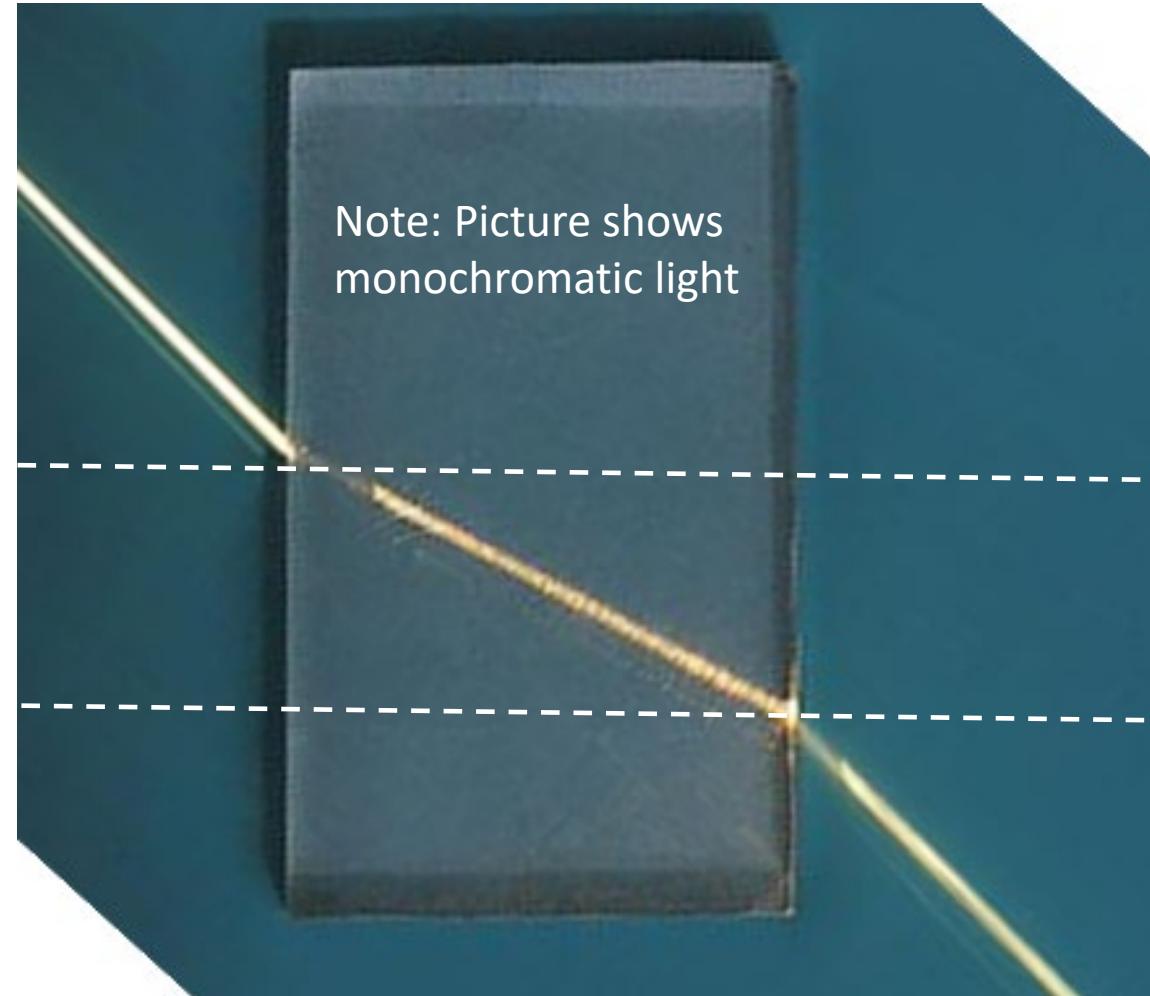
Picture taken from Wikipedia and modified

Law of refraction

Snell's law: $n_1 \sin \vartheta_1 = n_2 \sin \vartheta_2$,

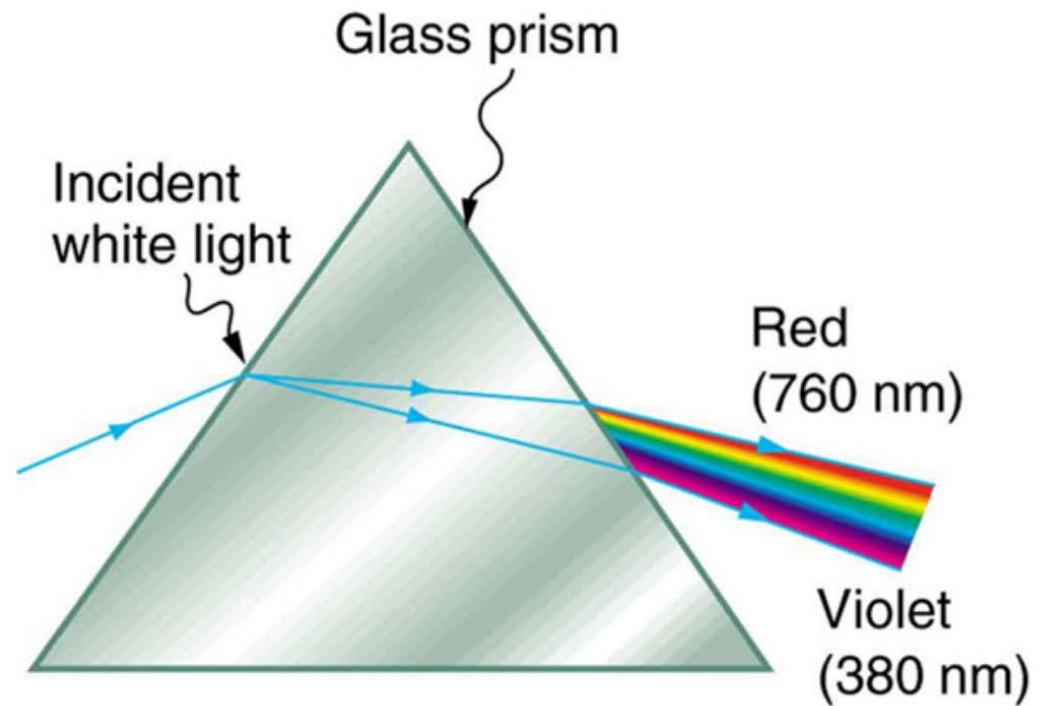
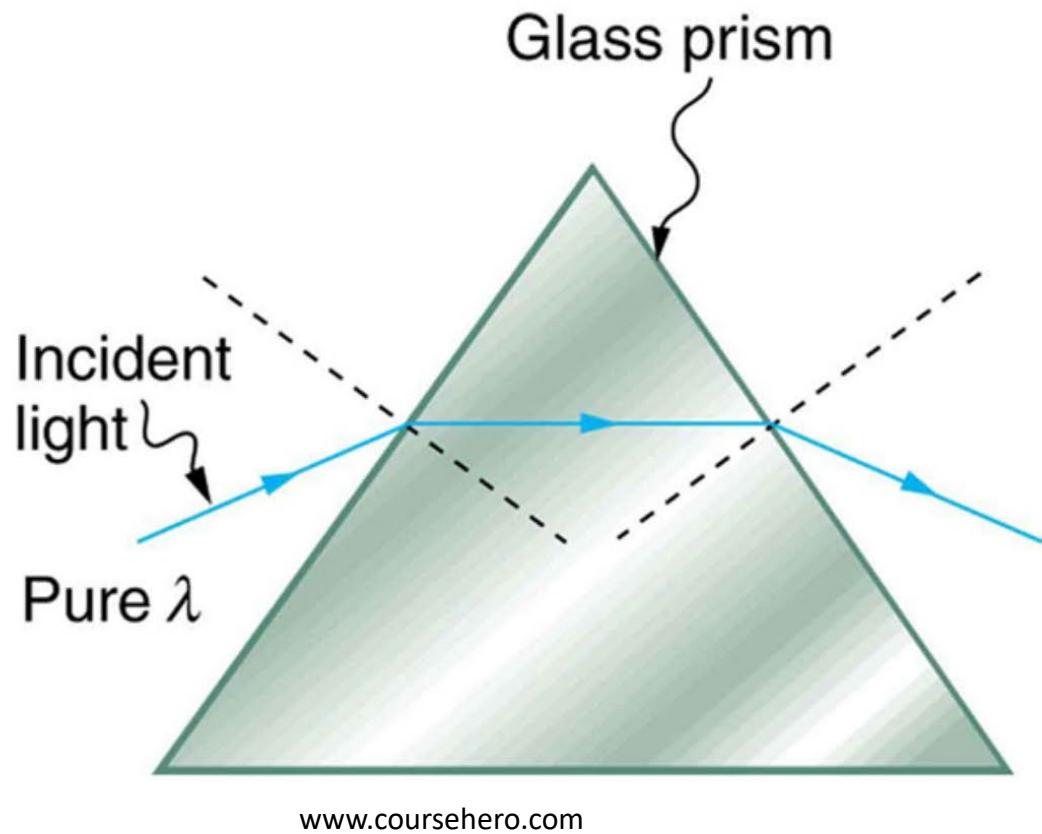
where ϑ_1 and ϑ_2 are the angle of incidence and angle of refraction, respectively, of a ray crossing the interface between two media with refractive indices n_1 and n_2 .

Refractive indices
(determined by density):
Vacuum or air = 1
Water = 1.33
Glass = 1.5
Diamond = 2.42



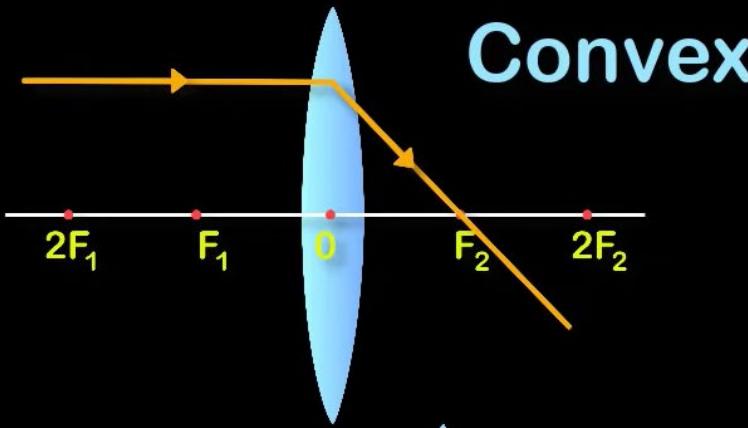
Picture taken from Wikipedia and modified

What is happening with light of different wavelengths?

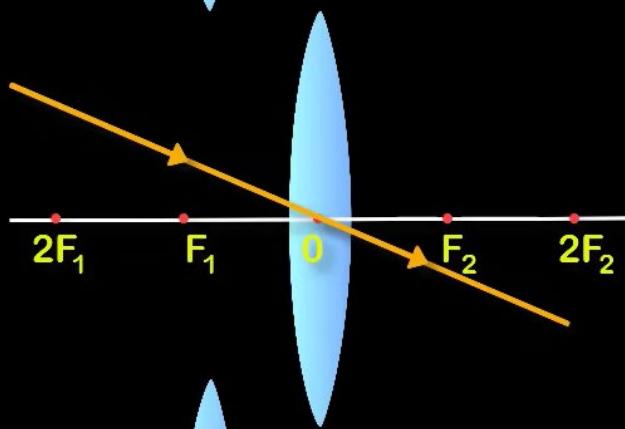


Prism – light changes its speed when passing through media with different densities, in a diamond it slows down 2.42-fold, equaling a refractive index of 2.42. However, the refractive index is slightly different for the different wavelengths of light, for example blue light (short wavelength, high frequency) is slowed down more than red light (high wavelength, short frequency), meaning that blue light also gets bent more than red light => spectral separation of light. Rays coming in at 90 degree to the surface (normal plane) are not bent.

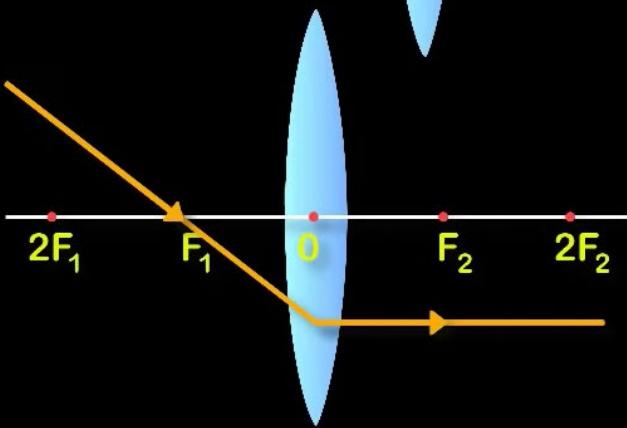
Lenses – basic rules



Rule 1



Rule 2

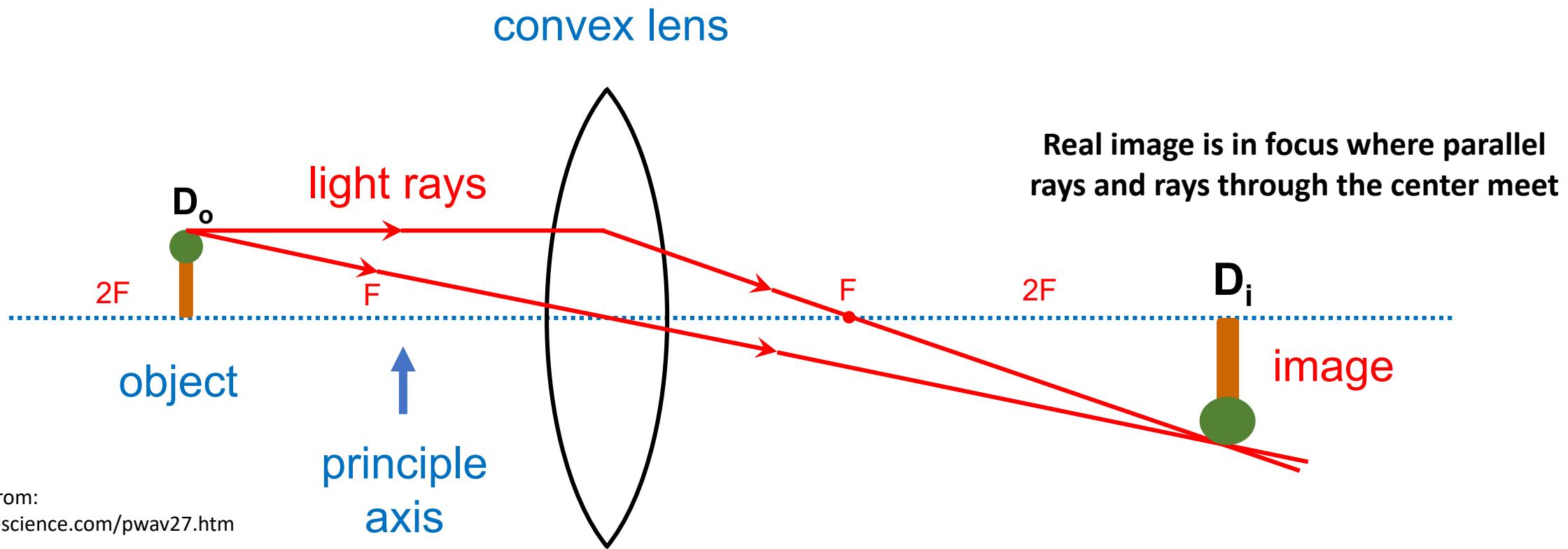


Rule 3

Rules

1. Parallel rays go through focus
2. Ray through center do not bend
3. Ray through focus goes parallel

Lenses – focus and magnification

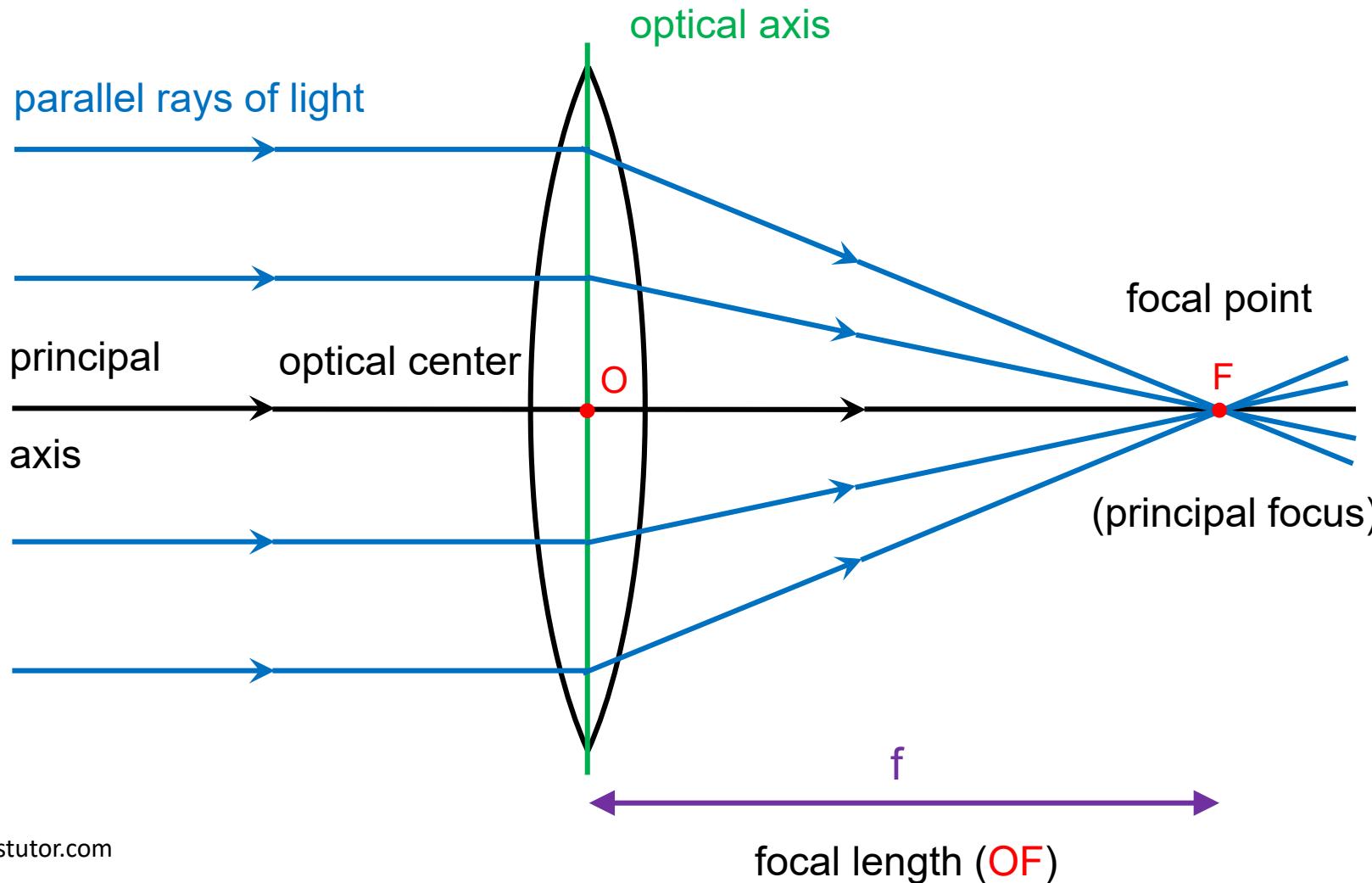


- Any object **closer than $2 \times f$** gets **magnified**.
- An object at a distance of **exactly f** is projected to **infinity**.
- An object **closer than f** results only in **virtual images on the same side** of the lens (behind the object)!

$$\text{Thin lens equation: } \frac{1}{D_i} + \frac{1}{D_o} = \frac{1}{F}$$

$$\text{Distance of the real image } D_i = \frac{F \cdot D_o}{(D_o - F)}$$

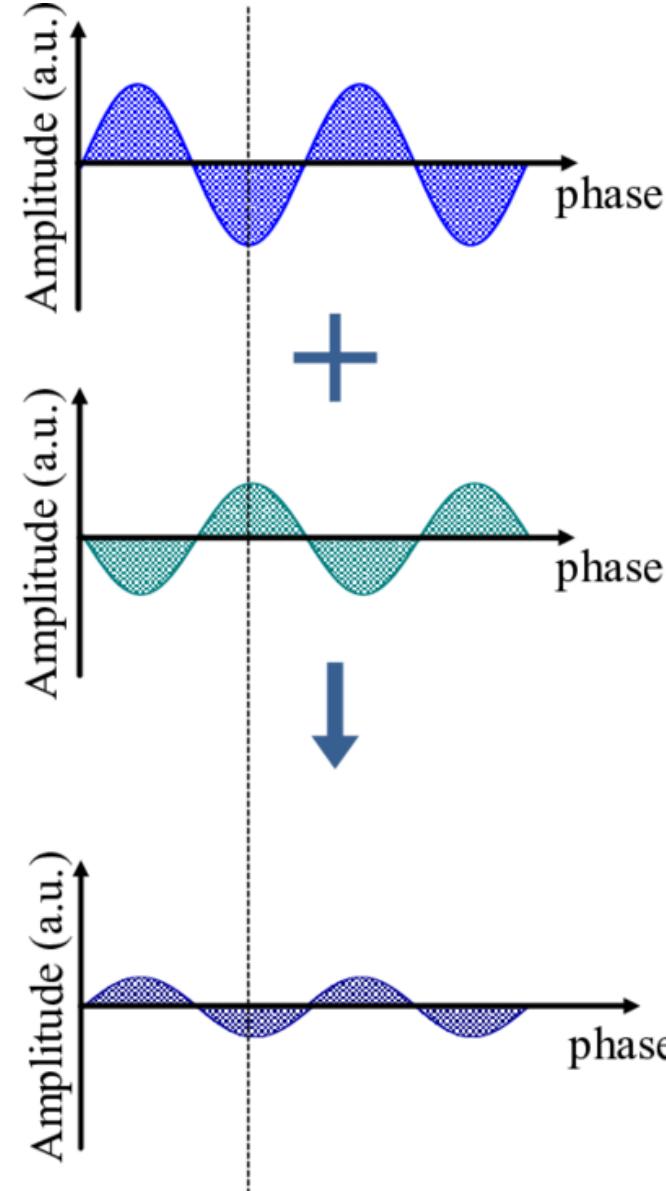
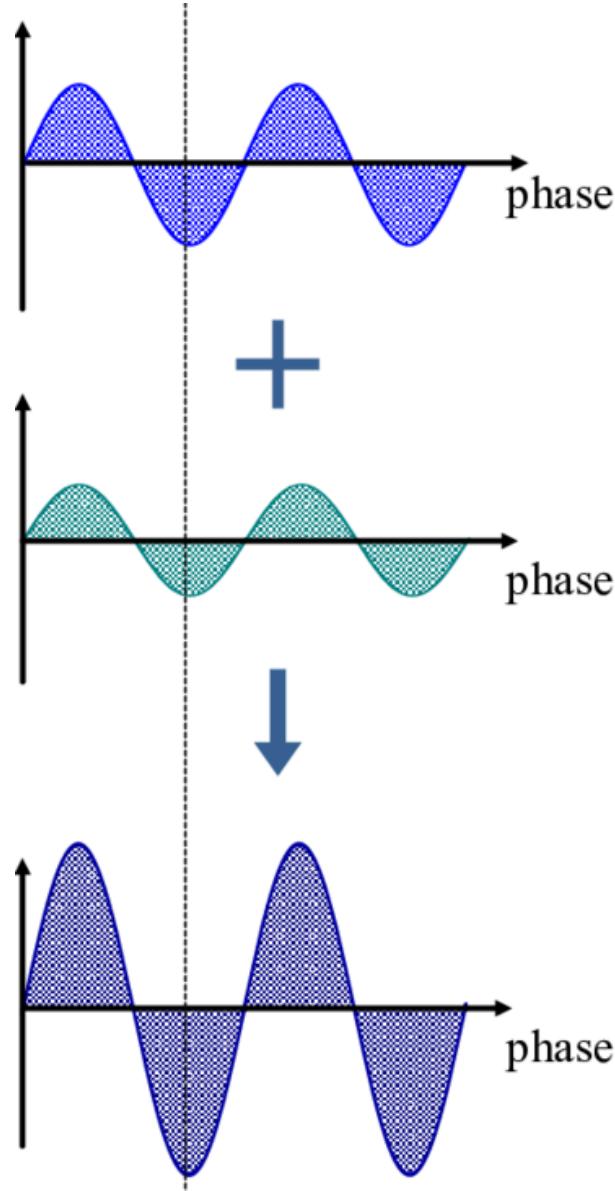
Lenses – focus and magnification



adapted from:
www.a-levelphysicstutor.com

Note you would place a PMT at F, while you would put a camera at D_i (you are only interested in maximal light intensity, not in a focused image)!

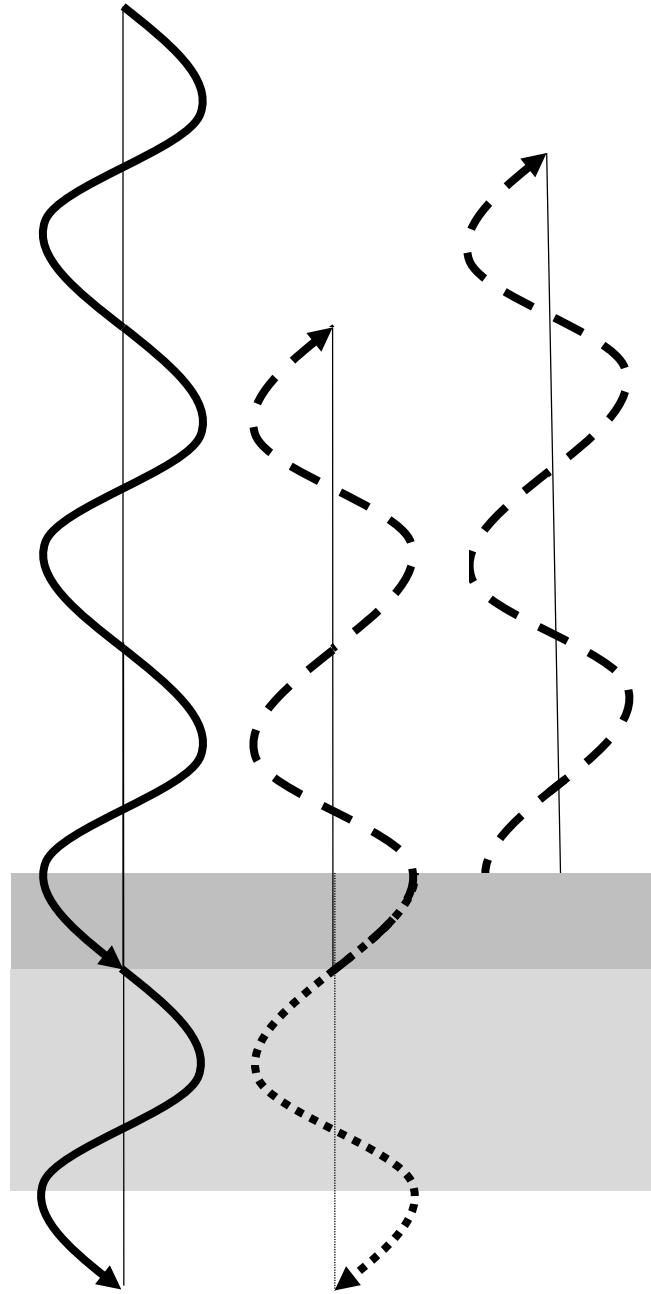
Constructive and destructive interference



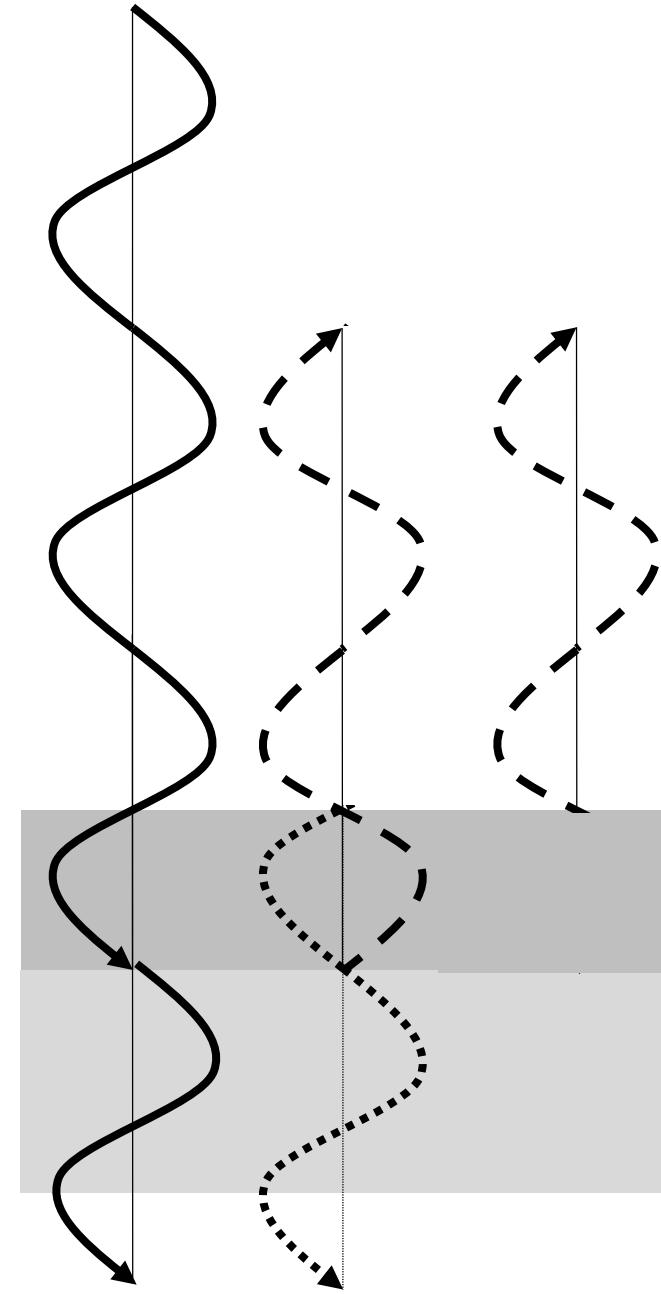
Application/
component: filters &
dichroic mirrors

Working principle of interference filters

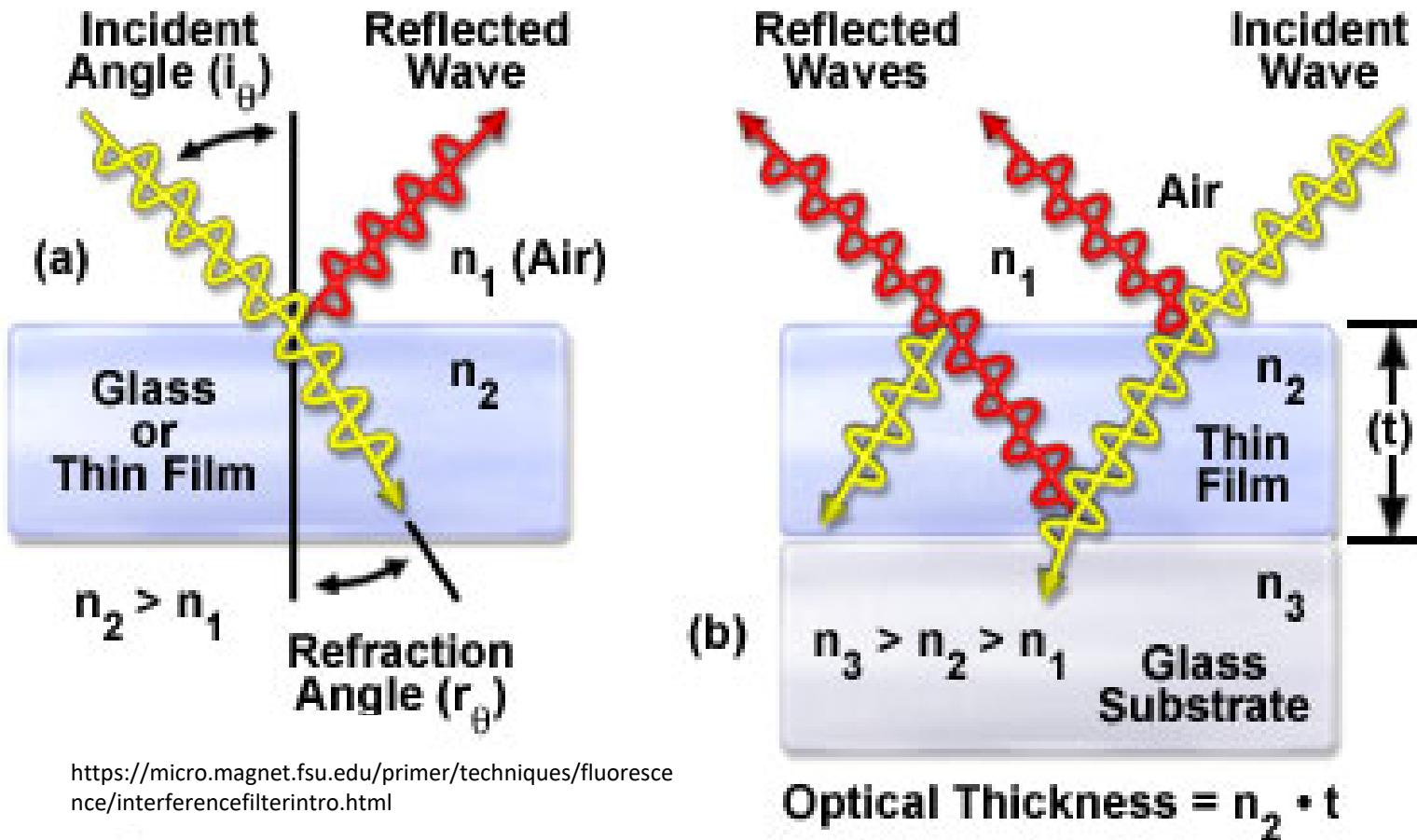
Destructive interference, layer thickness = $\lambda / 4$, phase shift = 180°



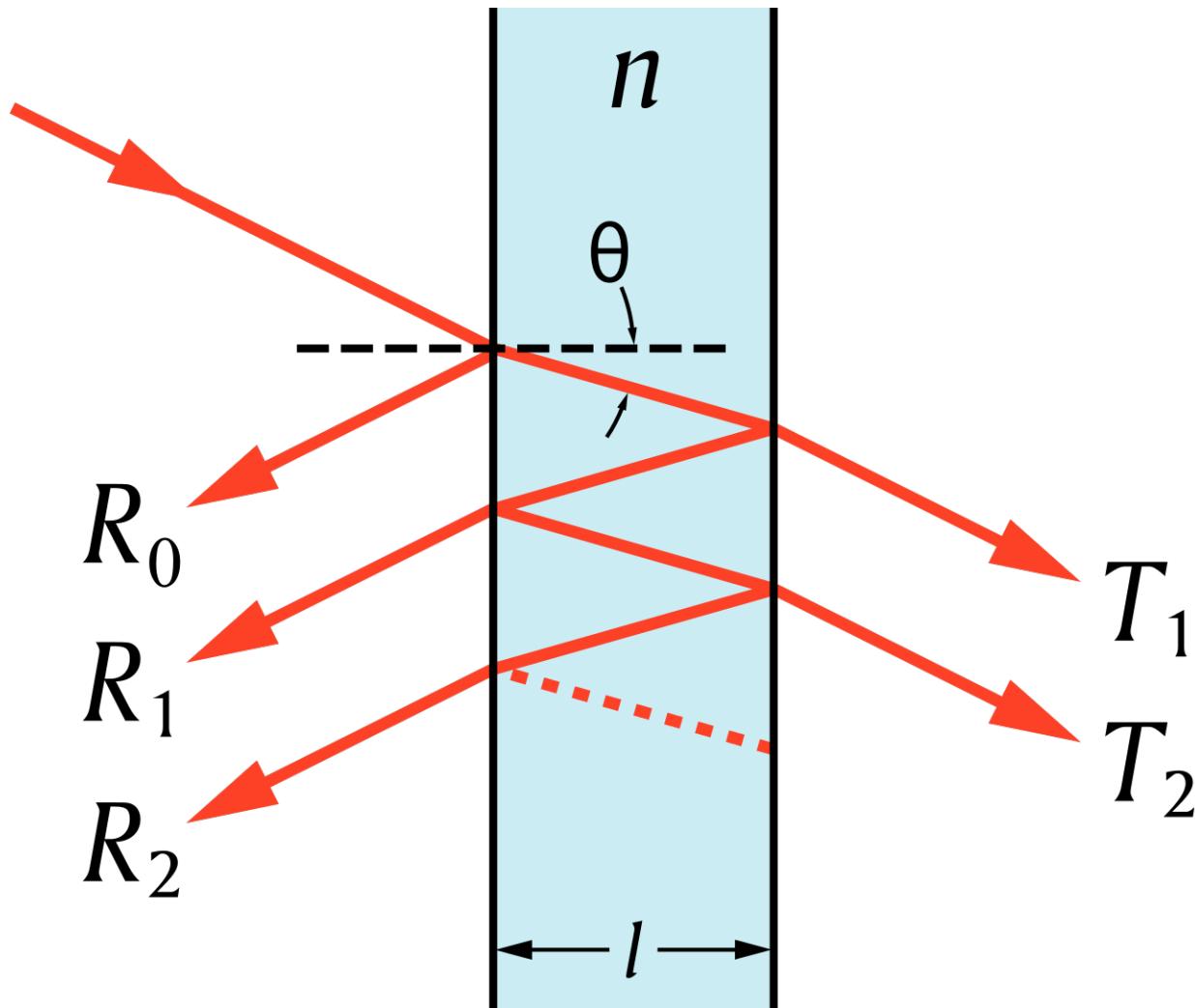
Constructive interference, layer thickness = $\lambda / 2$, phase shift = 360°



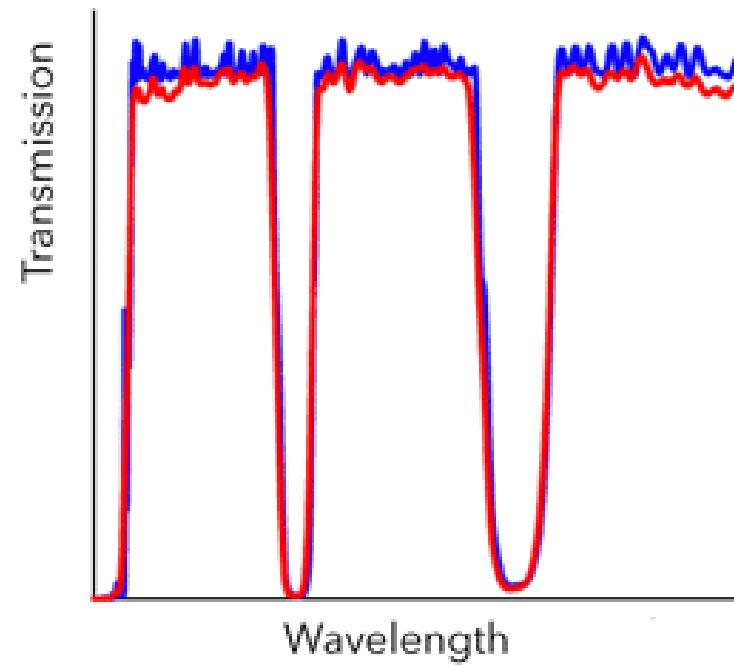
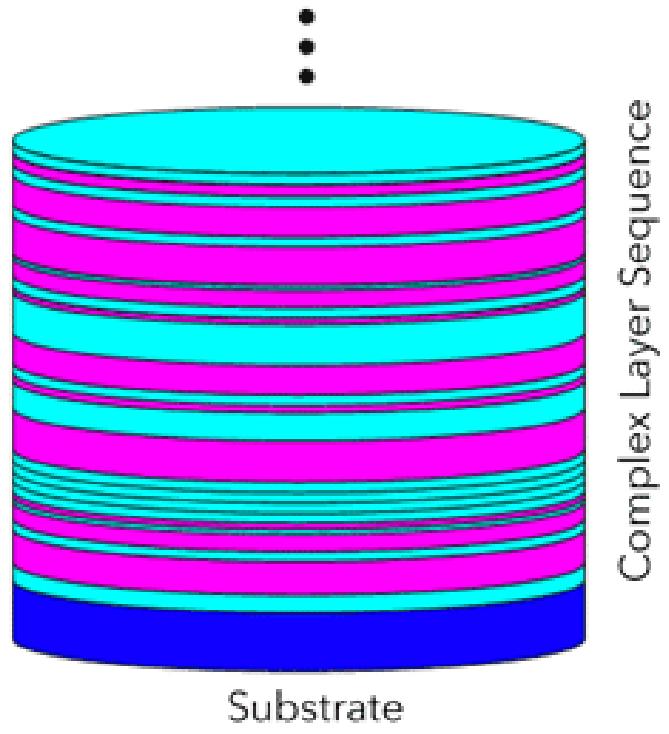
Working principle of dichroic mirrors



Working principle of dichroic mirrors



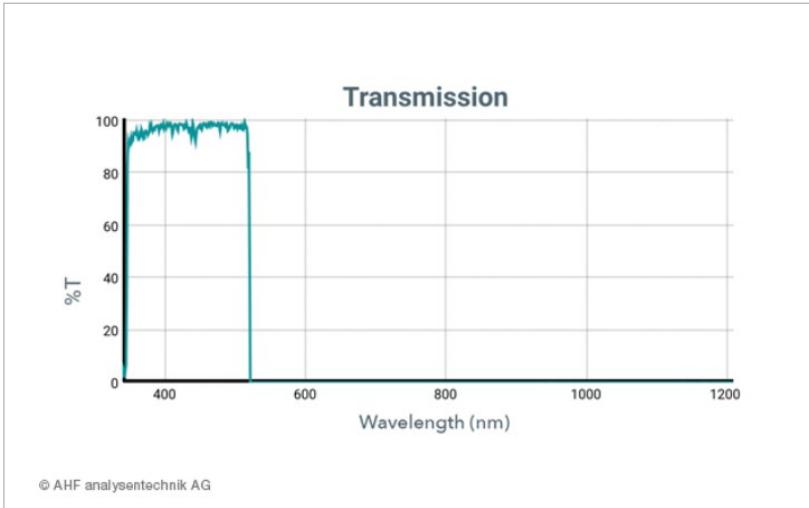
In reality it is always more than one thin layer!



Filters used in the practical course

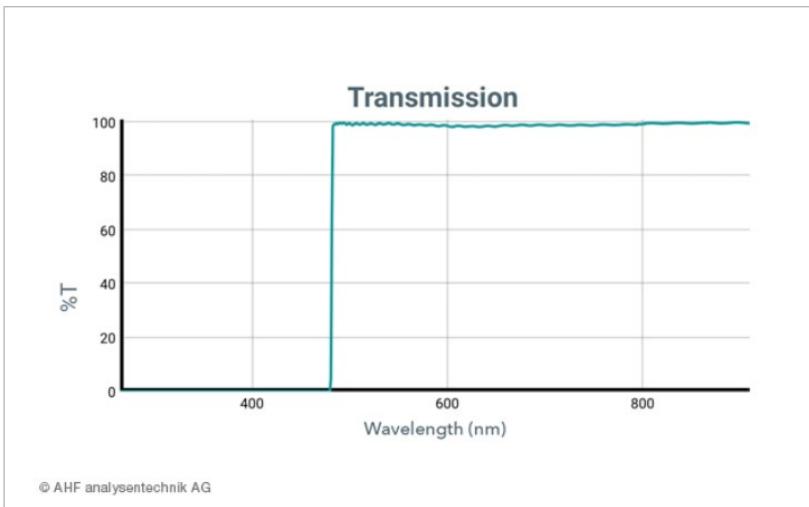
AHF Analysentechnik AG, 2023, *Optical Filters for highest performance*,
accessed 14 July 2023, '<https://www.ahf.de/en/products/spectral-analysis-photonic/optical-filters/>'

... use optical filters to control or filter light depending on its wavelength



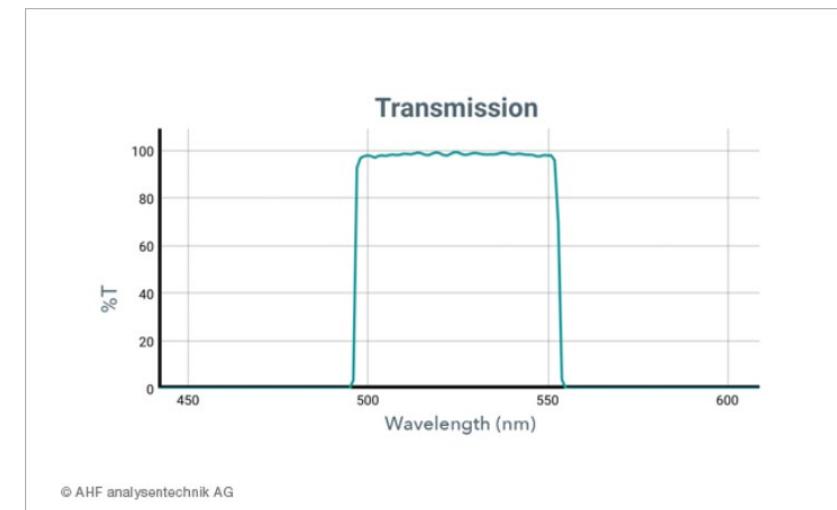
short pass filters

532 nm



long pass filters

473 nm



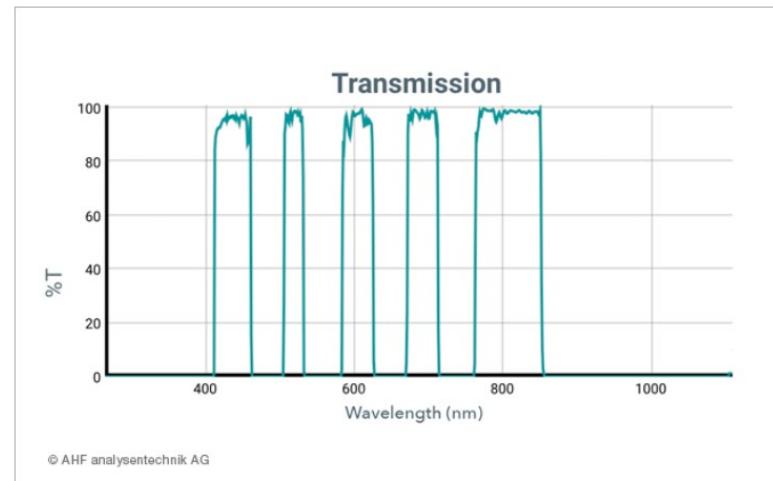
bandpass

filter

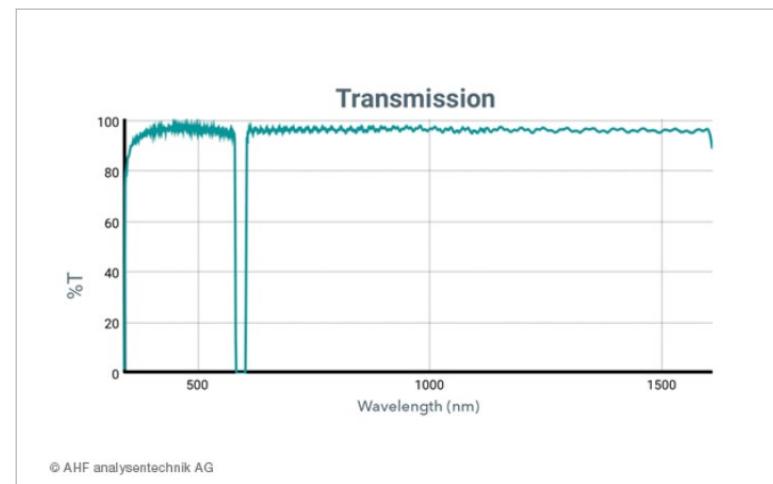
525/50 nm

Filters used in the practical course

AHF Analysentechnik AG, 2023, *Optical Filters for highest performance*,
accessed 14 July 2023, '<https://www.ahf.de/en/products/spectral-analysis-photonic/optical-filters/>'

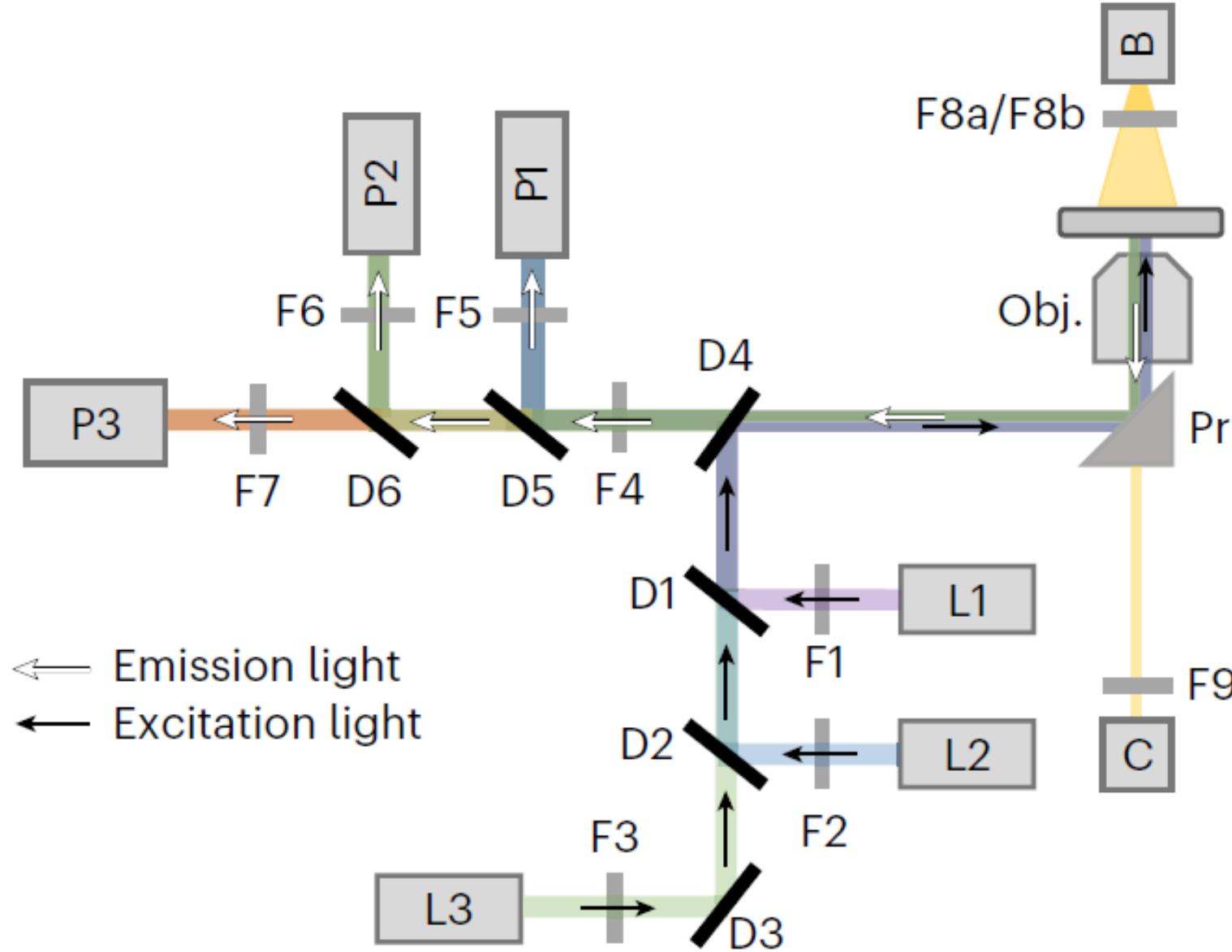


multiband filter



notch filter

A series of dichroics can be used to feed in multiple lasers or to split polychromatic light into its components



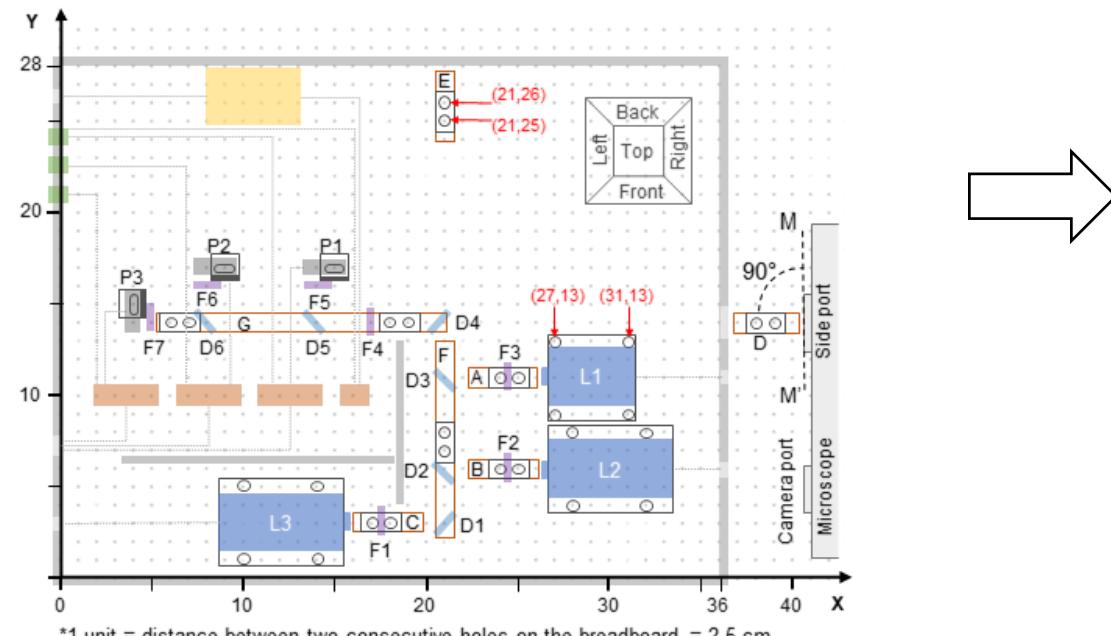
Blueprint of your instrument

Preparing a blueprint of an instrument for high throughput fluorescence analysis of microfluidic droplets – **prerequisite to pass**

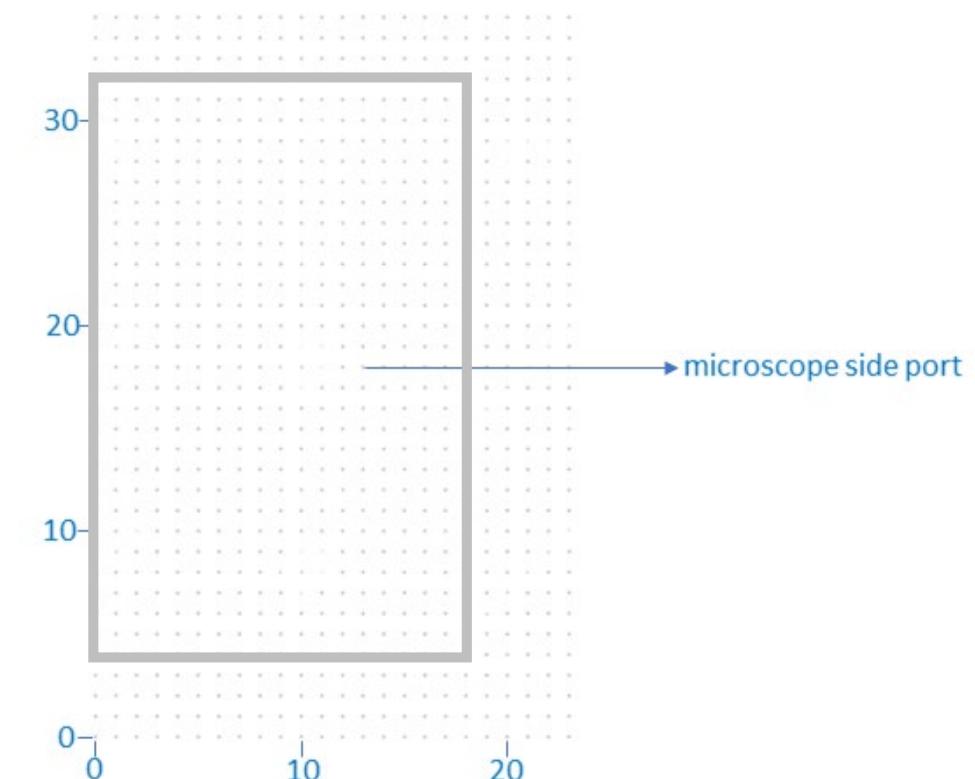
Your task: Simplification of the Panwar & Autour Nature Protocols 2023 setup, including only one laser and one PMT and only a virtual feedback rather than active sorting

a

	Breadboard
	Rails (A-G)
	Rail support blocks
	Laser (L1-L3)
	Laser support blocks
	Black box parts
	8 & 3 pin connectors
	PMT power supply
	PMT switches
	PMT on base plate (P1-P3)
	M6 screwholes
	Wirings
	Blackbox holes
	Optical filters (F1-F7)
	Dichroic mirrors (D1-D6)

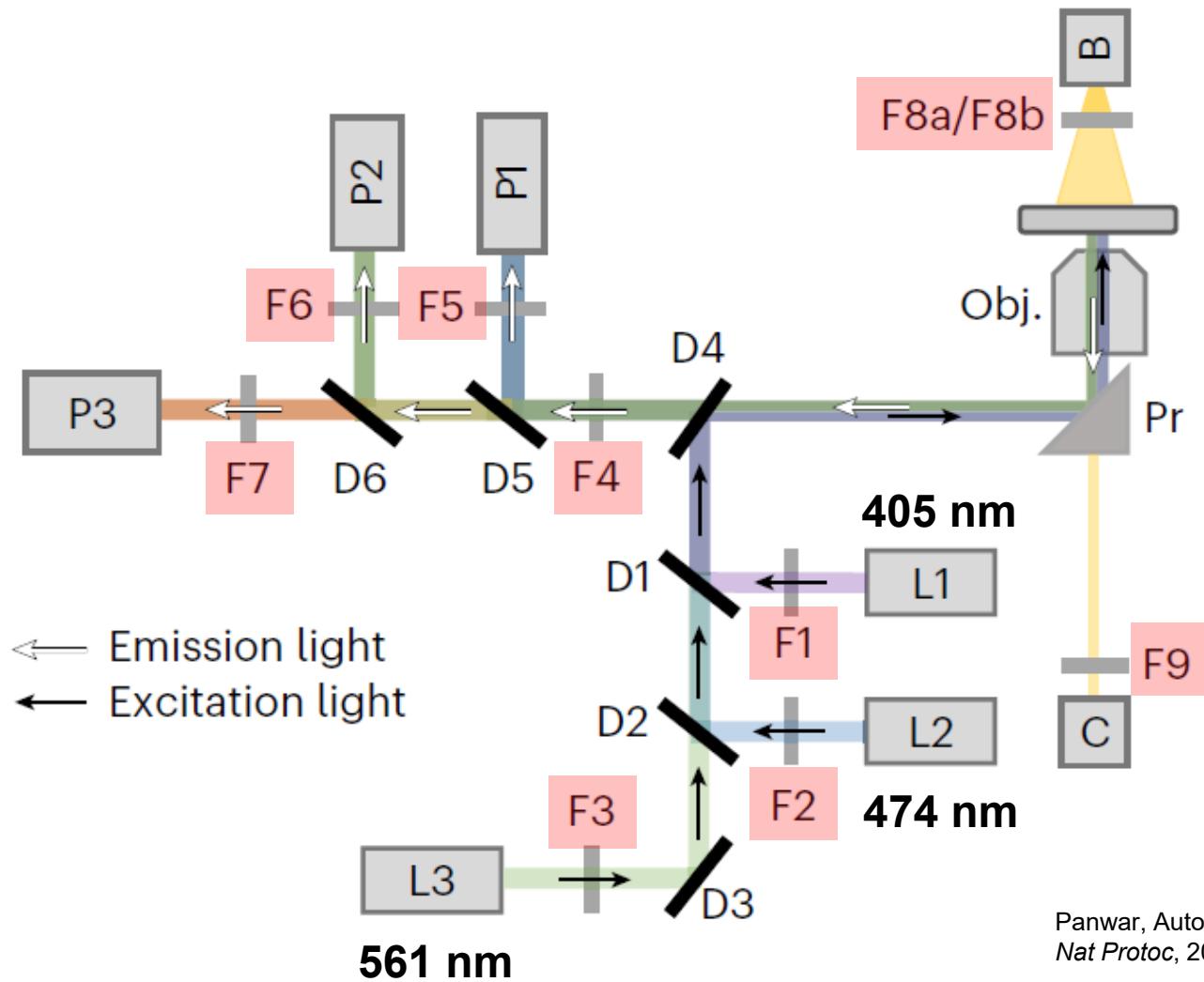


3 channel fluorescence detection



single channel fluorescence detection

What kind of tools do we need?

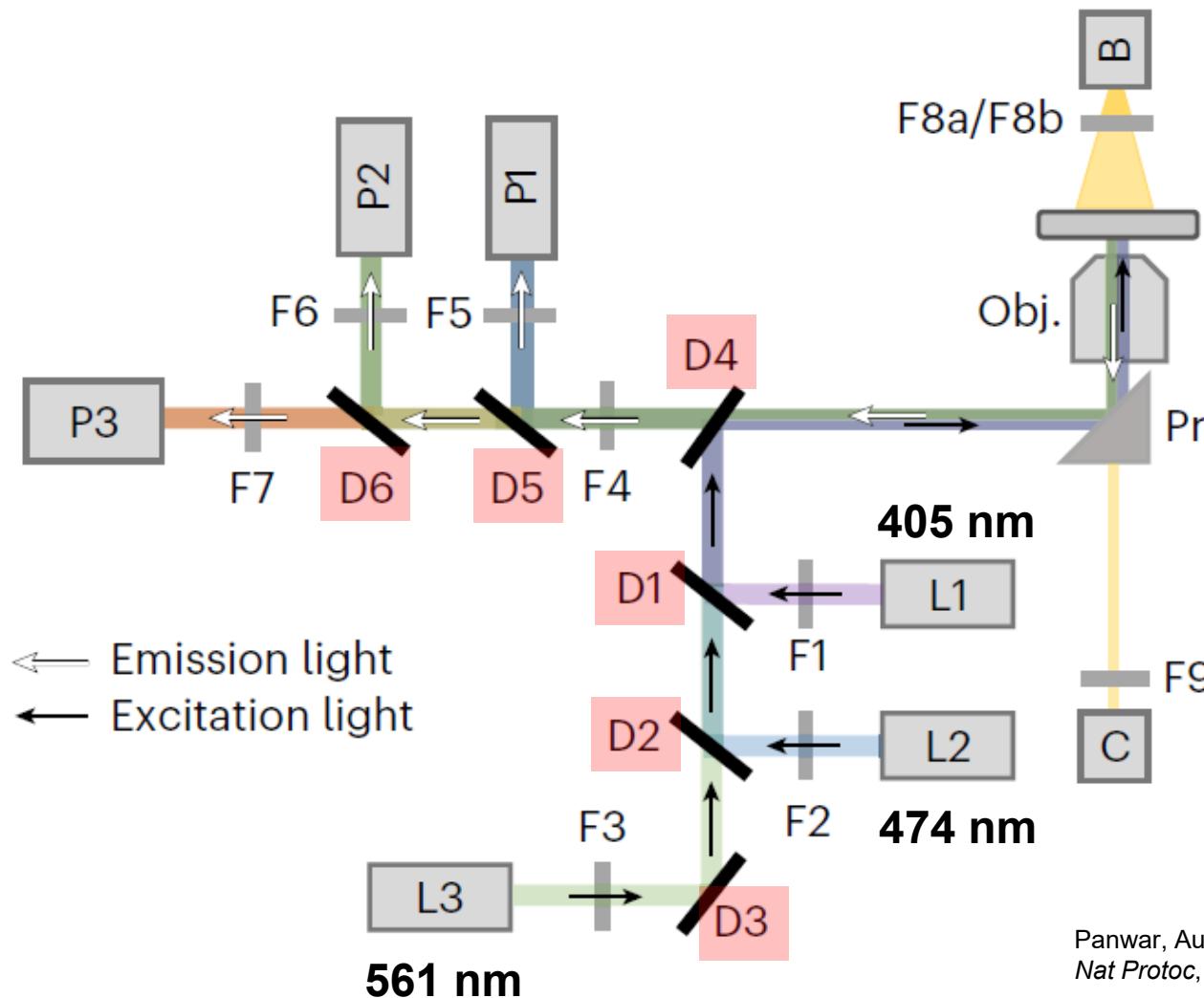


Panwar, Autour & Merten,
Nat Protoc, 2023

F1...?	405/10 nm
F2...?	488/6 nm
F3...?	563/9 nm
F4...?	405/473/561 nm
F9...?	triple band notch
F5...?	445/45 nm
F6...?	525/45 nm
F7...?	605/50 nm bandpasses
F8a...?	561 nm longpass
F8b...?	633 nm longpass

HELP: <https://www.ahf.de/en/products/spectral-analysis-photonic/optical-filters/>

What kind of tools do we need?



D1...?	409 nm
D2...?	488 nm
	beam-splitter
D3...?	full reflective mirror
D4...?	403/497/574 nm
	triple beam-splitter
D5...?	484 nm
D6...?	552 nm
	beam-splitter

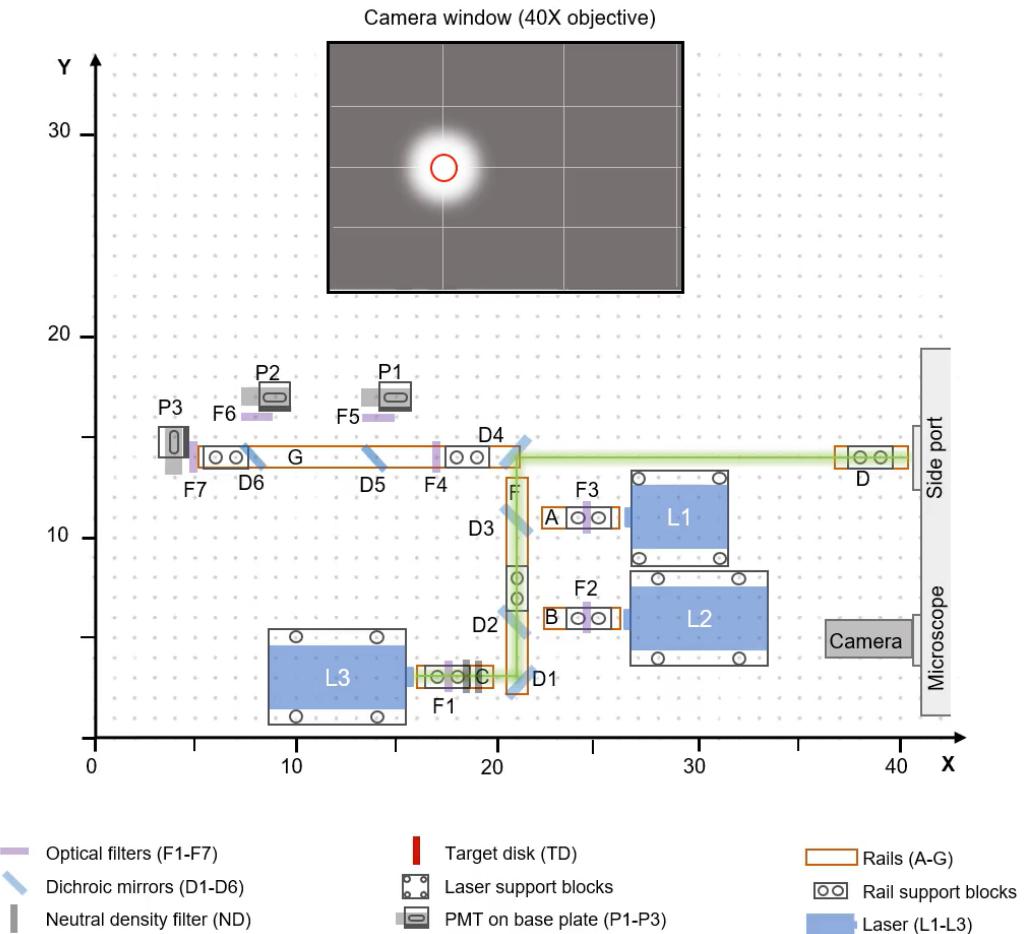
Panwar, Autour & Merten,
Nat Protoc, 2023

HELP: <https://www.ahf.de/en/products/spectral-analysis-photonic/optical-filters/>

Practical task: align all optical components

- **excite** different fluorophores (e.g., within droplets)
- **direct** the emission toward the photosensors

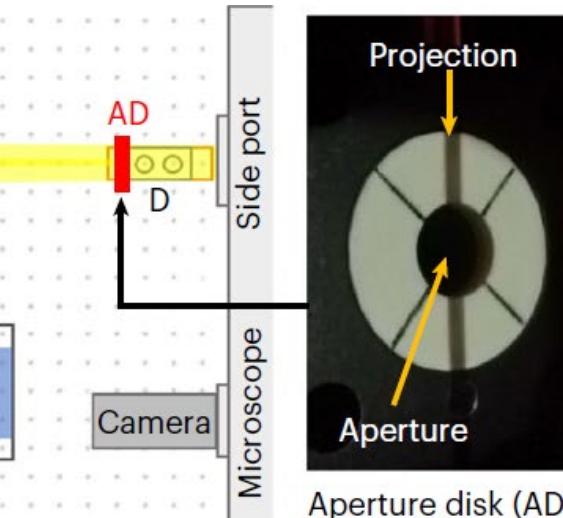
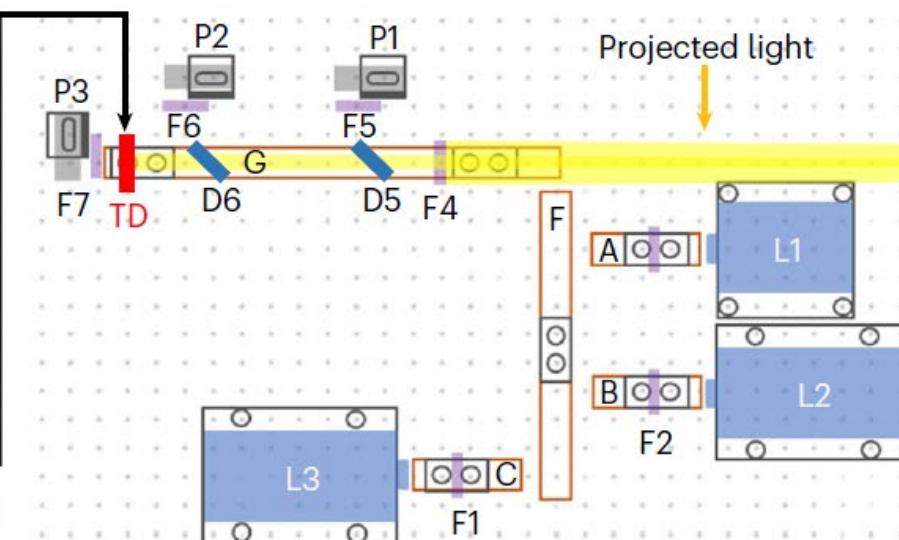
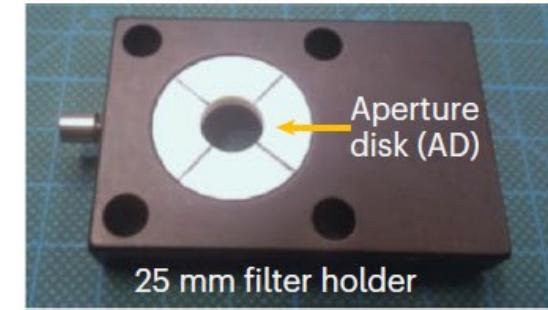
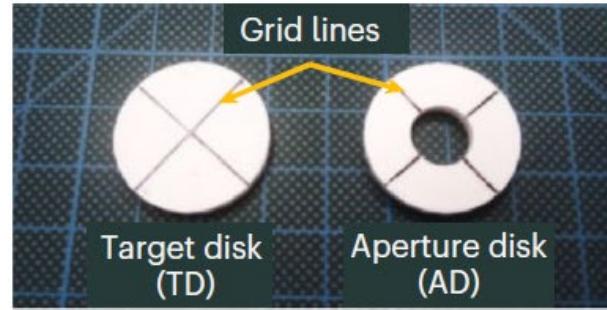
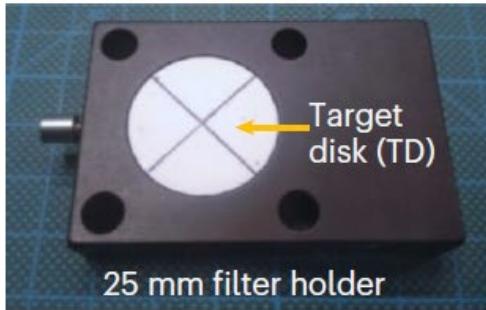
- **alignment** = directing the beam so that it follows a predetermined path



Alignment tools

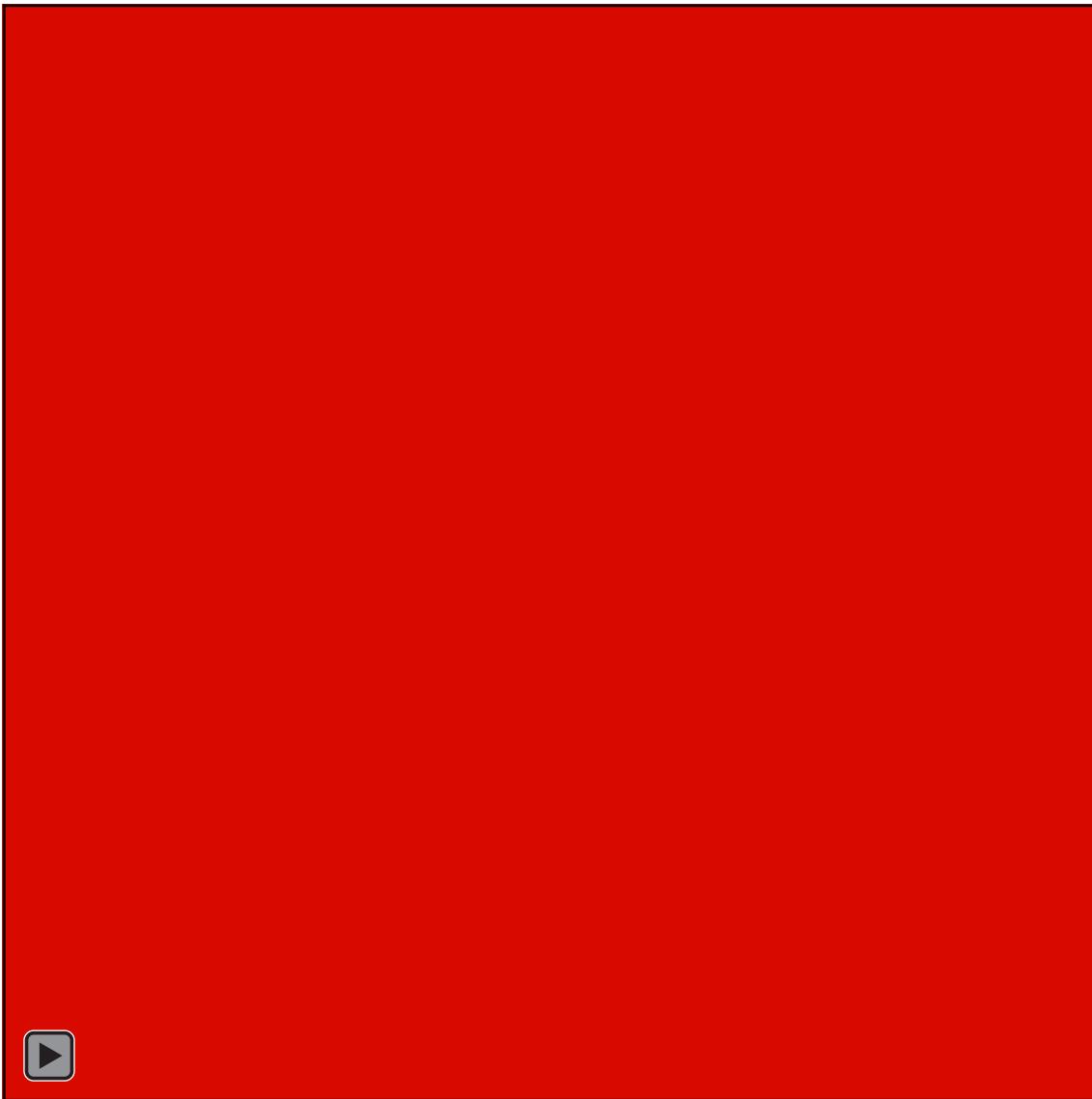
1.) emission light alignment

reduced diameter of beam



! microscope is fixed now !

Excitation light alignment (Panwar et al., 2023)

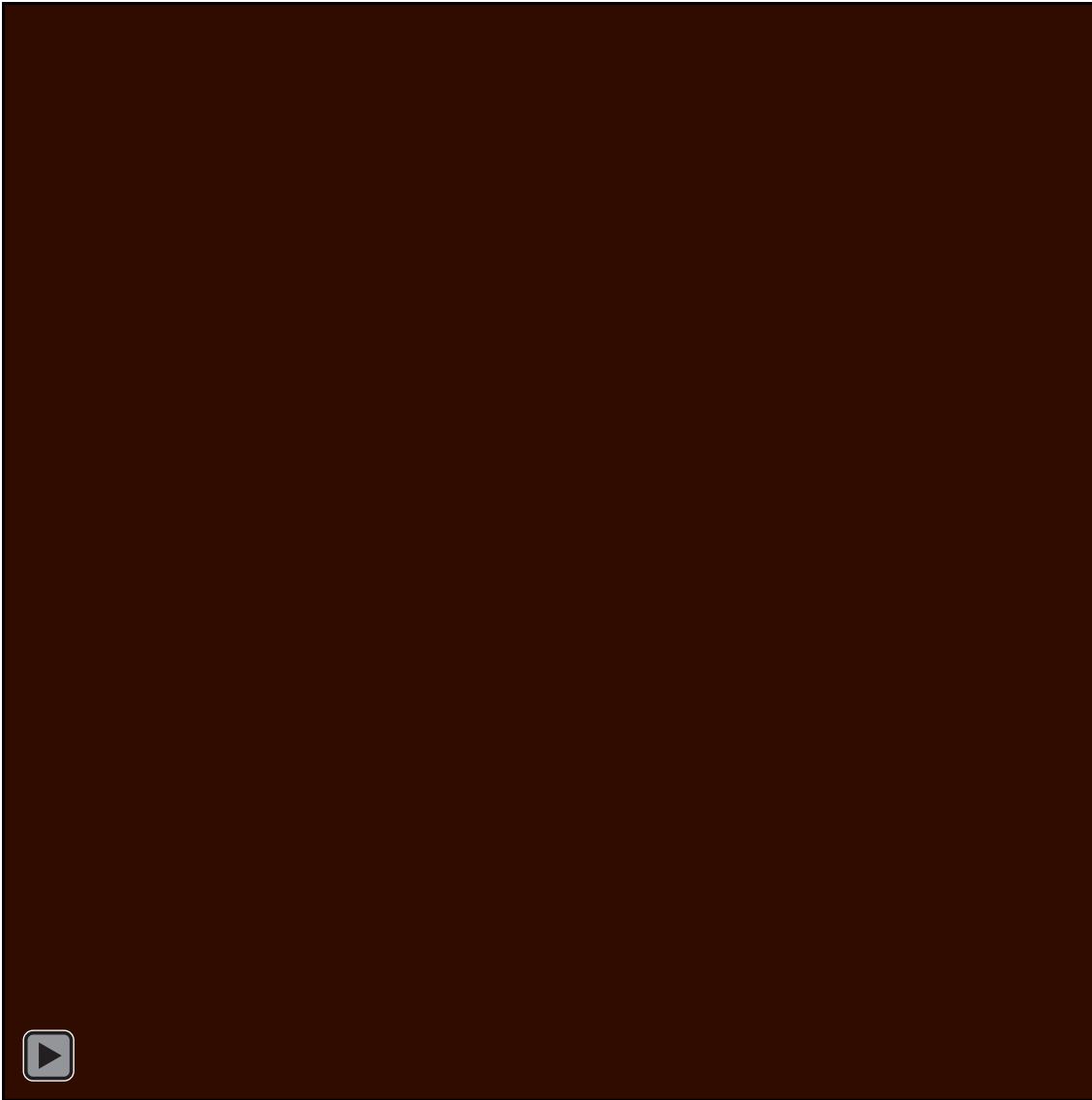


VIDEOS:

<https://www.nature.com/articles/s41596-022-00796-2#Sec75>

Most relevant: 55" and onwards

Emission light alignment (Panwar et al., 2023)

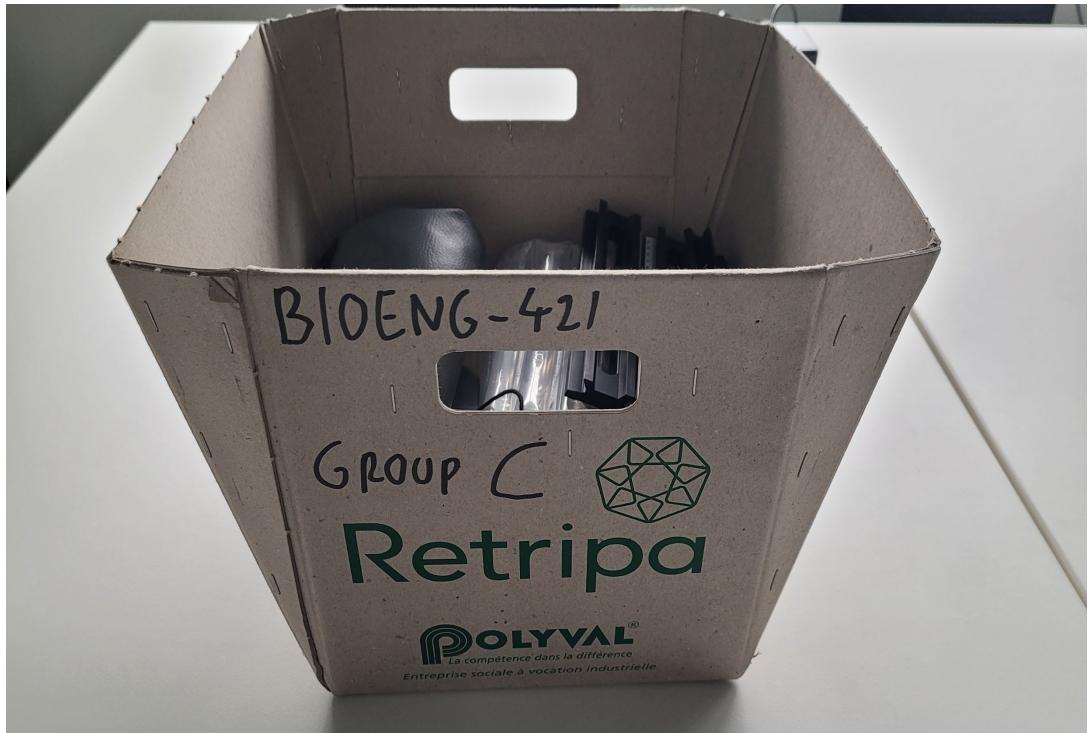


VIDEOS:

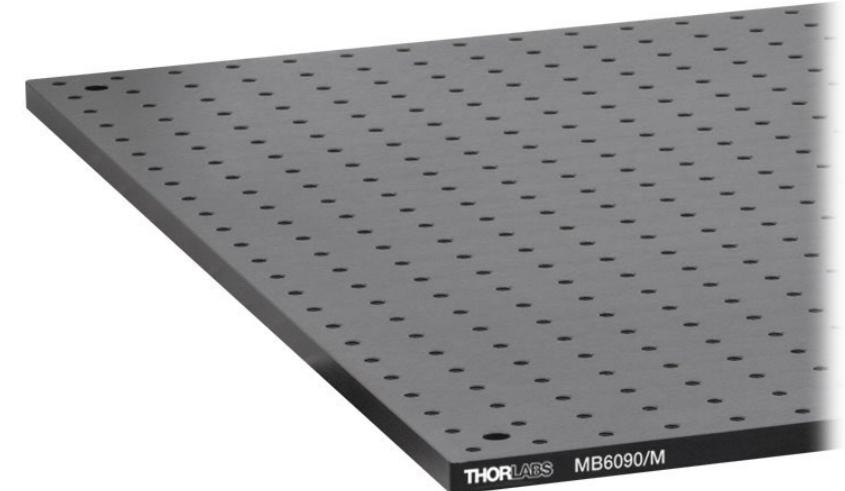
<https://www.nature.com/articles/s41596-022-00796-2#Sec75>

Most relevant: 2'40" and onwards

Your course inventory



assemble on
breadboard



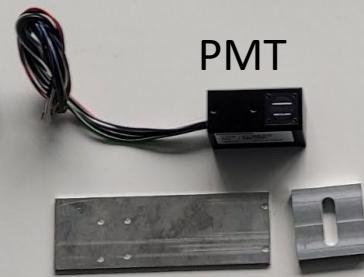
Group A: Mass Spectrometry (practical session 8.30am –10.30am, 473nm laser, microscope to the right)

Group B: NGS (practical session 8.30am –10.30am, 561nm laser, microscope to the right)

Your course inventory



Safety goggles

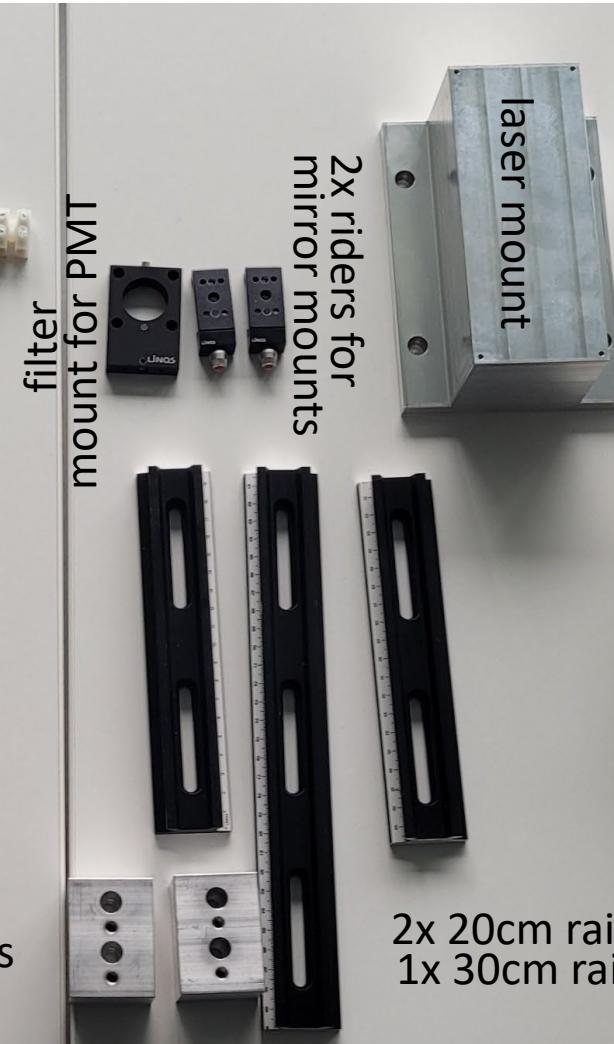


PMT mount



PMT

2x rail
mounts



2x 20cm rail
1x 30cm rail

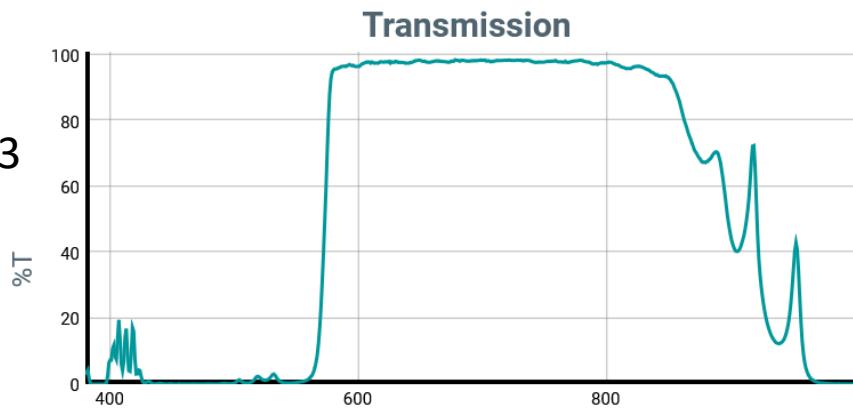


473nm OR 561nm laser

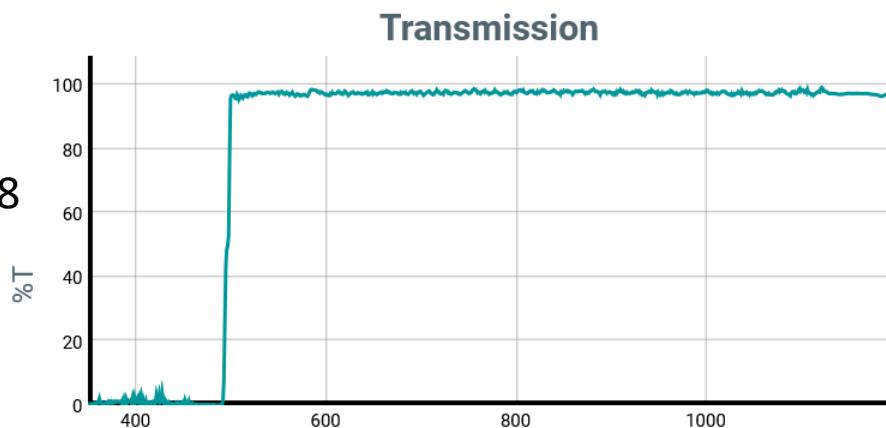
+ filters & mirrors (details on next slide)
+ screws, wires, crimps & alignment tools

Mirrors (already mounted)

Dichroic F48-553



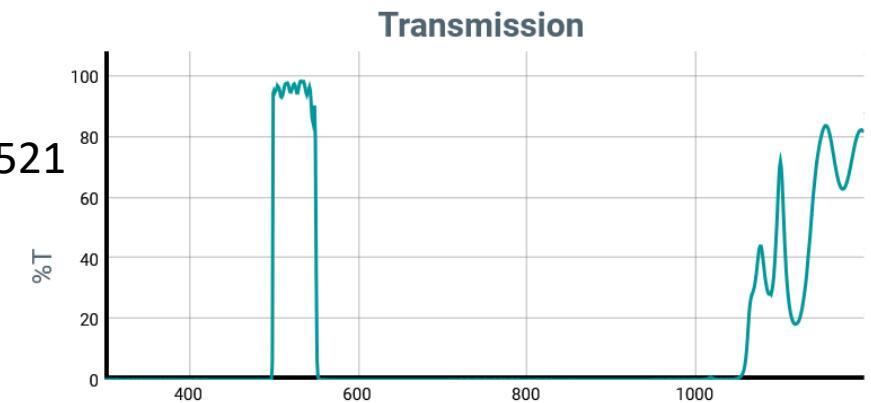
Dichroic F38-488



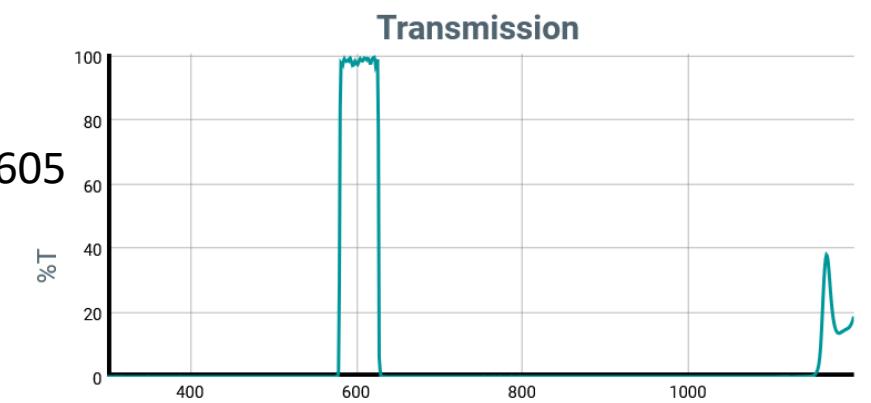
+ Full reflective mirror

Filters

Filter F37-521



Filter F49-605



BIOENG-421 students tasks for today/ this week

- **Prepare a blueprint of your instrument according to the course materials**
- **Start with the breadboard layout available on Moodle**
- **Note that you do NOT necessarily have to use all parts!**
- **Submit your blueprint at latest by Friday 25th, noon (send an email to all teachers)**
(so that it can be approved before the practical session on Tuesday)

Questions?



References and further literature



Design and construction of a microfluidics workstation for high-throughput multi-wavelength fluorescence and transmittance activated droplet analysis and sorting

Jatin Panwar^{1,2,3}, Alexis Autour^{1,2} and Christoph A. Merten^{1,2}

Single-cell analysis has revolutionized quantitative high-throughput screening, especially in the field of droplet microfluidic platforms capable of phenotypic, fluorescence-based screening and sorting are still mainly found in the screening of cell libraries for secreted factors, or even for the effects of secreted or surface-displayed factors on a specific marker. In this protocol, we provide a detailed step-by-step guide for the construction of a high-throughput droplet-based screening system for the analysis of single-cell phenotypes. The system is equipped with three lasers to excite the fluorophores in droplets and photodiodes that acquire fluorescence signals in the droplets. The droplets are sorted based on the fluorescence signal and the transmittance signal. The system is validated by sorting droplets containing fluorescent beads at 200 Hz with 99.4% accuracy. We show results from an application of our workstation in single-cell molecular biology, e.g., to analyze molecular determinants of cancer metastasis.

Introduction

Single-cell screening is an essential initial methodology for answering many biological questions, especially in genomic, transcriptomic or proteomic applications. Droplet microfluidics has emerged as a robust choice for single-cell analysis, as the droplets are well suited for screening millions of droplets at a high volume rate. ¹⁻³ At high frequencies reaching up to six million droplets per hour (ref. ⁴), these sizes and rates of formation enable single-cell encapsulation with each droplet containing a single cell and a few nanoliters of reagents and media. By changing the droplet matrix, single cells can be encapsulated with other molecularly isolated beads or mixtures of cells, or with other droplets containing different molecules. This enables the use of droplet-based high-throughput screening of these reactions. Such screening methods are commonly used in antibody discovery, drug screening, and other applications. ⁵⁻⁷ They can also be used to study the effects on enzymatic drug targets or bind to a second, co-encapsulated target (ref. ⁸). These methods can also be helpful in detecting cellular heterogeneity for example by screening single cells with different genotypes or different levels of expression of a specific gene. ⁹ This can be used for the selection of enzyme variants with 10-50 times higher catalytic activity ¹⁰ and of aptamers with improved binding properties. ¹¹ These methods can also be used to detect and sort rare microbes inside a population ¹² and to discover new promiscuous enzymes ¹³. Overall, droplet-microfluidics-based single-cell screening gains remarkable momentum in the last decade.

Institute of Bioprocessing, School of Engineering, Ecole Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland, European Molecular Biology Laboratory (EMBL), Heidelberg, Germany. ⁷These authors contributed equally to this work. ¹Correspondence to: J. Merten (merten@bioproc.epfl.ch)

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<https://doi.org/10.1038/s41596-022-00796-2>

Panwar, J., Autour, A. & Merten, C.A. *Design and construction of a microfluidics workstation for high-throughput multi-wavelength fluorescence and transmittance activated droplet analysis and sorting*. Nat Protoc **18**, 1090–1136 (2023)

OPTICS

FIFTH EDITION

Eugene Hecht



EAN: 9781292096964



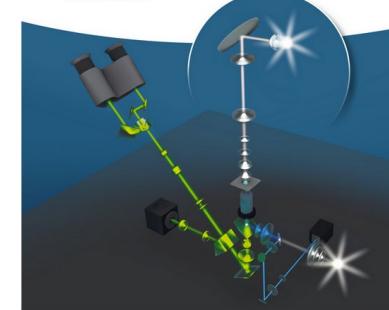
WILEY-VCH

Edited by Ulrich Kubitscheck

Fluorescence Microscopy

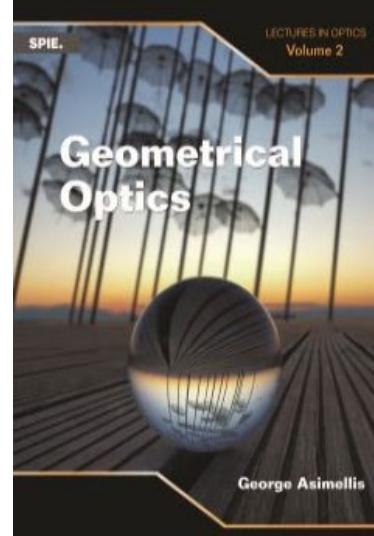
From Principles to Biological Applications

Second Edition



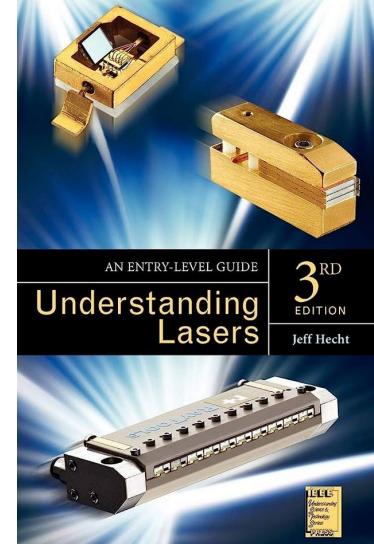
<https://doi.org/10.1002/9783527687732>

Kubitscheck, Ulrich, *Fluorescence Microscopy: From Principles to Biological Applications*, 2nd edition, Weinheim, Wiley-VCH, 2017



<https://doi.org/10.1117/3.2506310>

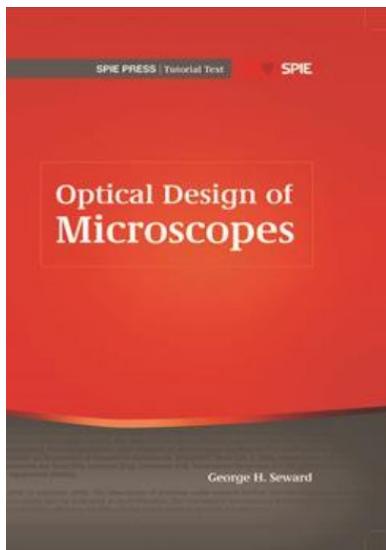
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EAN: 9781118210048

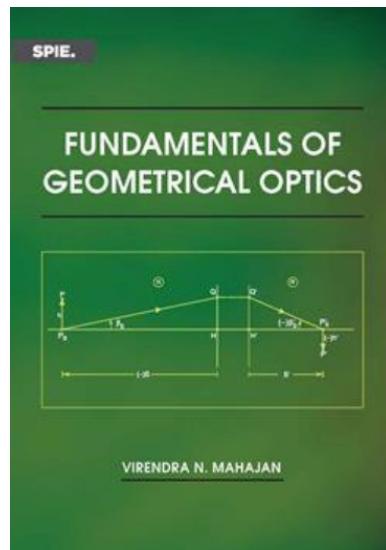
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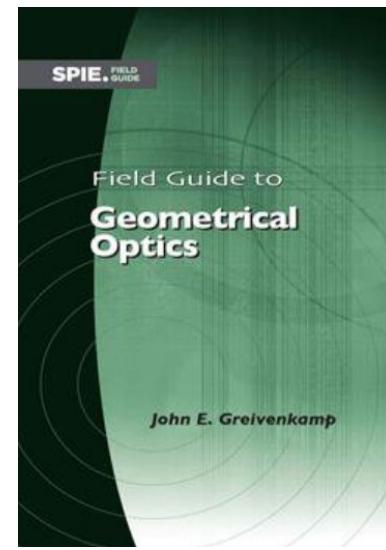
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