

Lecture #6

Introduction to optics

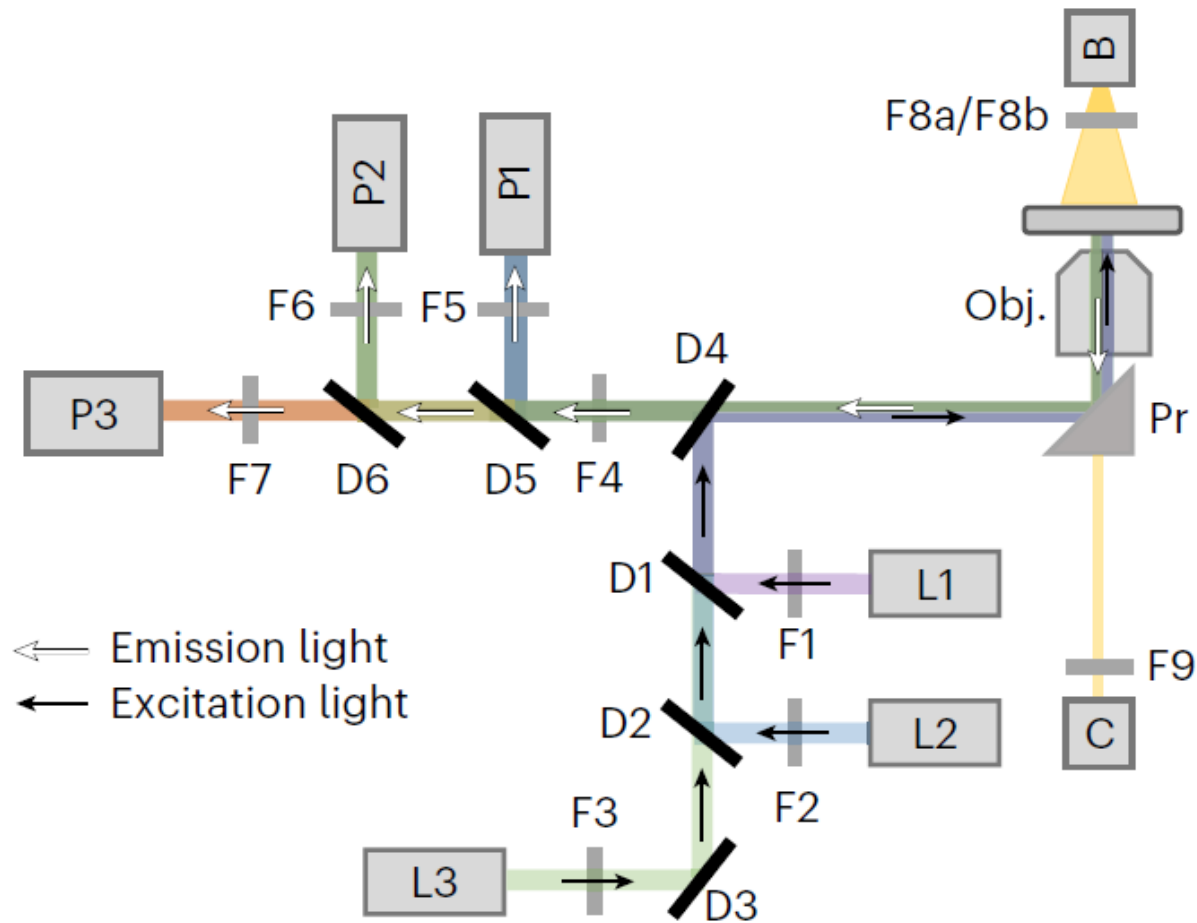
Aims:

- 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

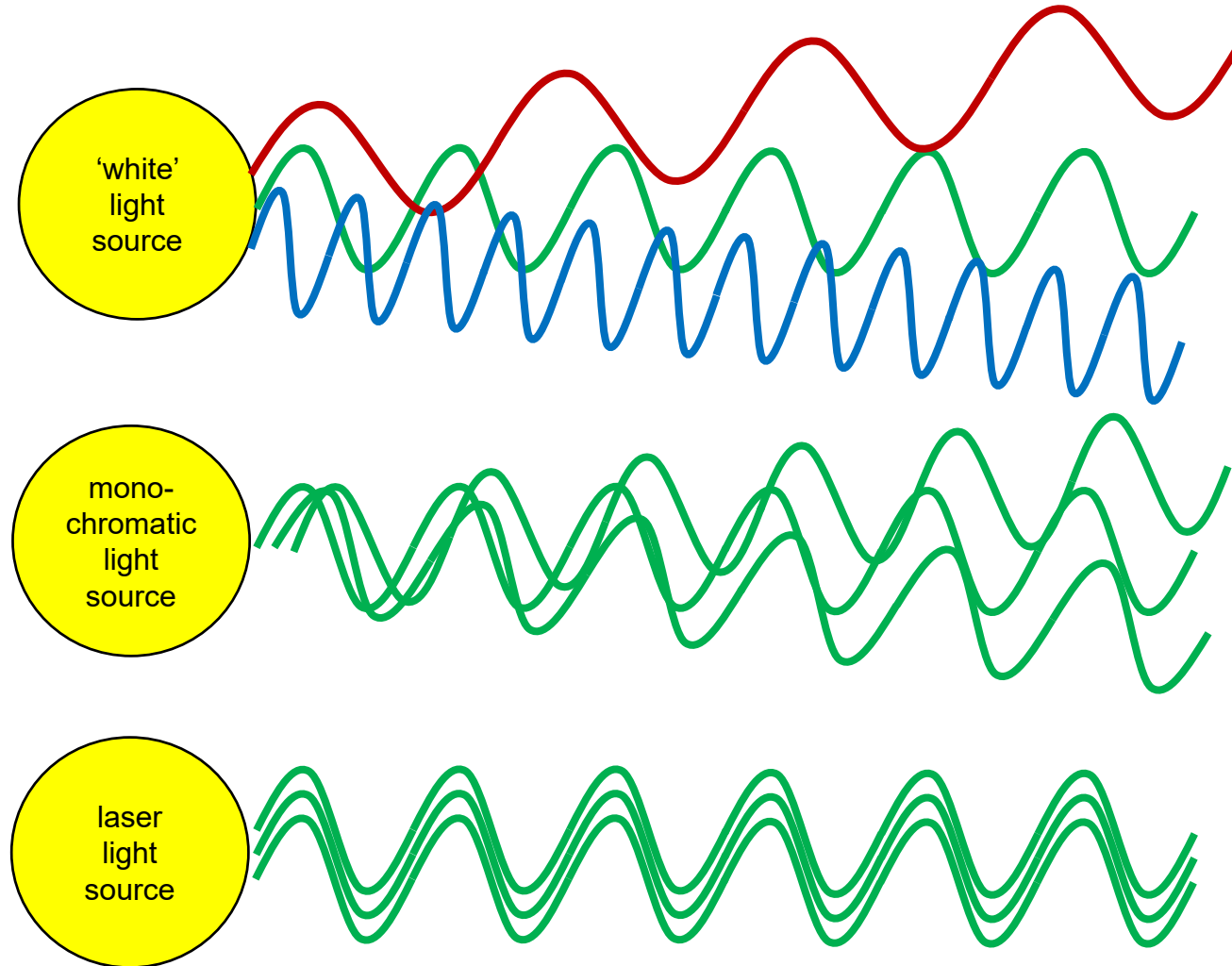
Filters

Mirrors

Prisms

Lenses



Different types of light sources



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

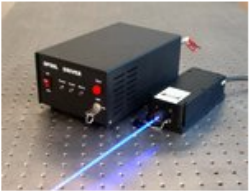
Laser (light amplification by stimulated emission of radiation):
Monochromatic, with all waves in phase and emitted into a single direction!

Lasers used in the practical course

 [Sign in](#) [Register](#)  0 items

[Home](#) [UV Lasers](#) [Blue Lasers](#) [Green Lasers](#) [Yellow Lasers](#) [Red Lasers](#) [IR Lasers](#) [Contact us](#)

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



FN Series 473nm Laser 100-1000mW

★★★★★ — 4

\$1800.00

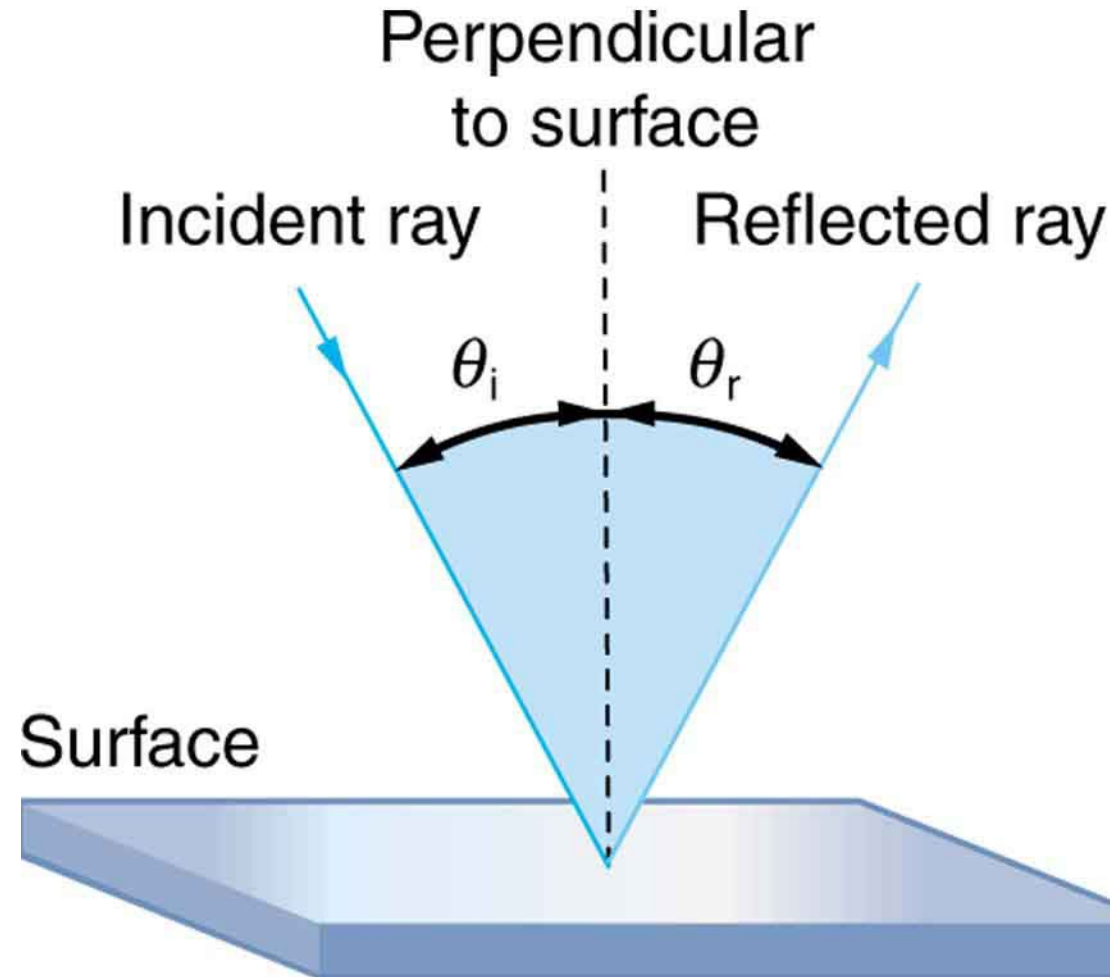
Model Selection

473nm 100mW

Qty: [Add to cart](#)

Visible light = **380 nm - 780 nm** wavelength

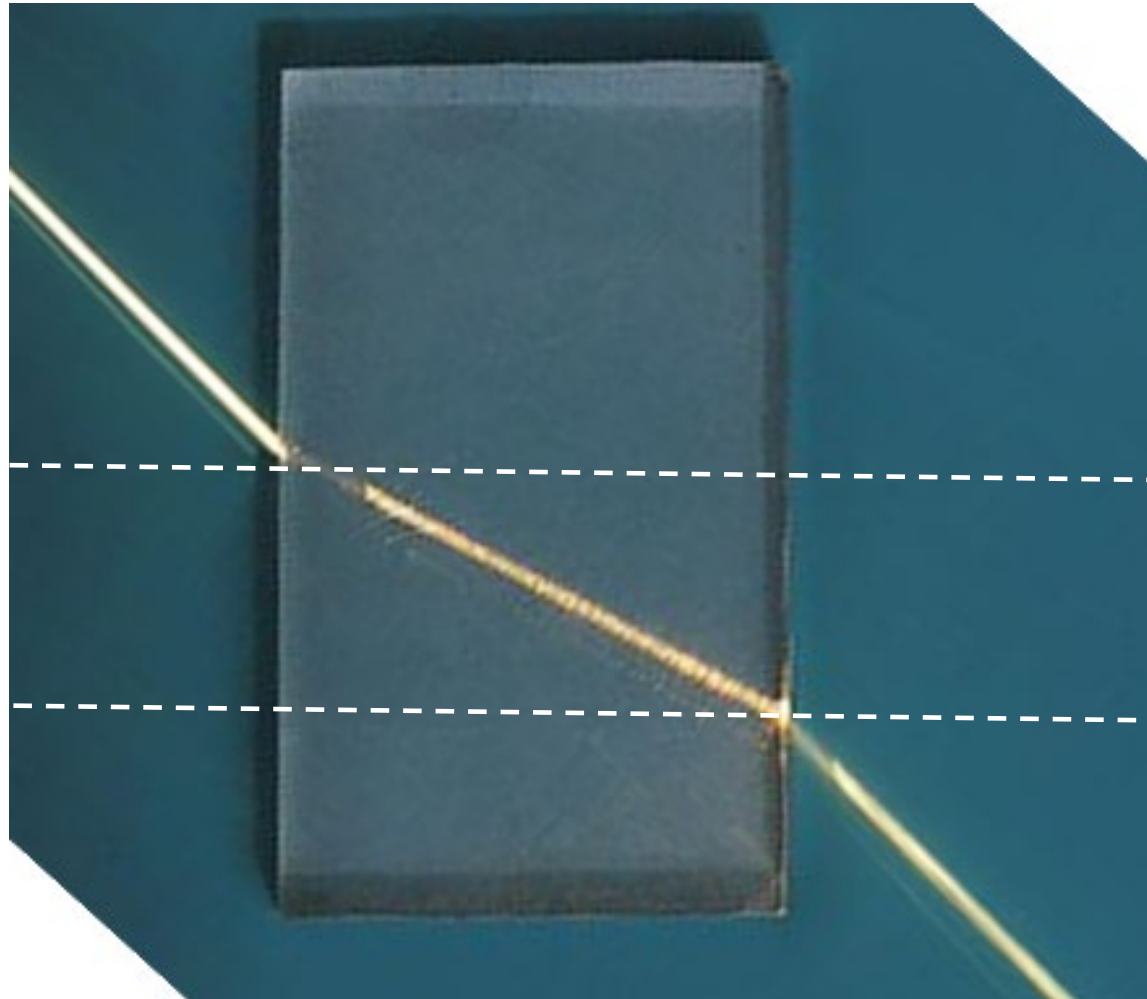
Law of reflection



Application/ component: mirror

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

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

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 .

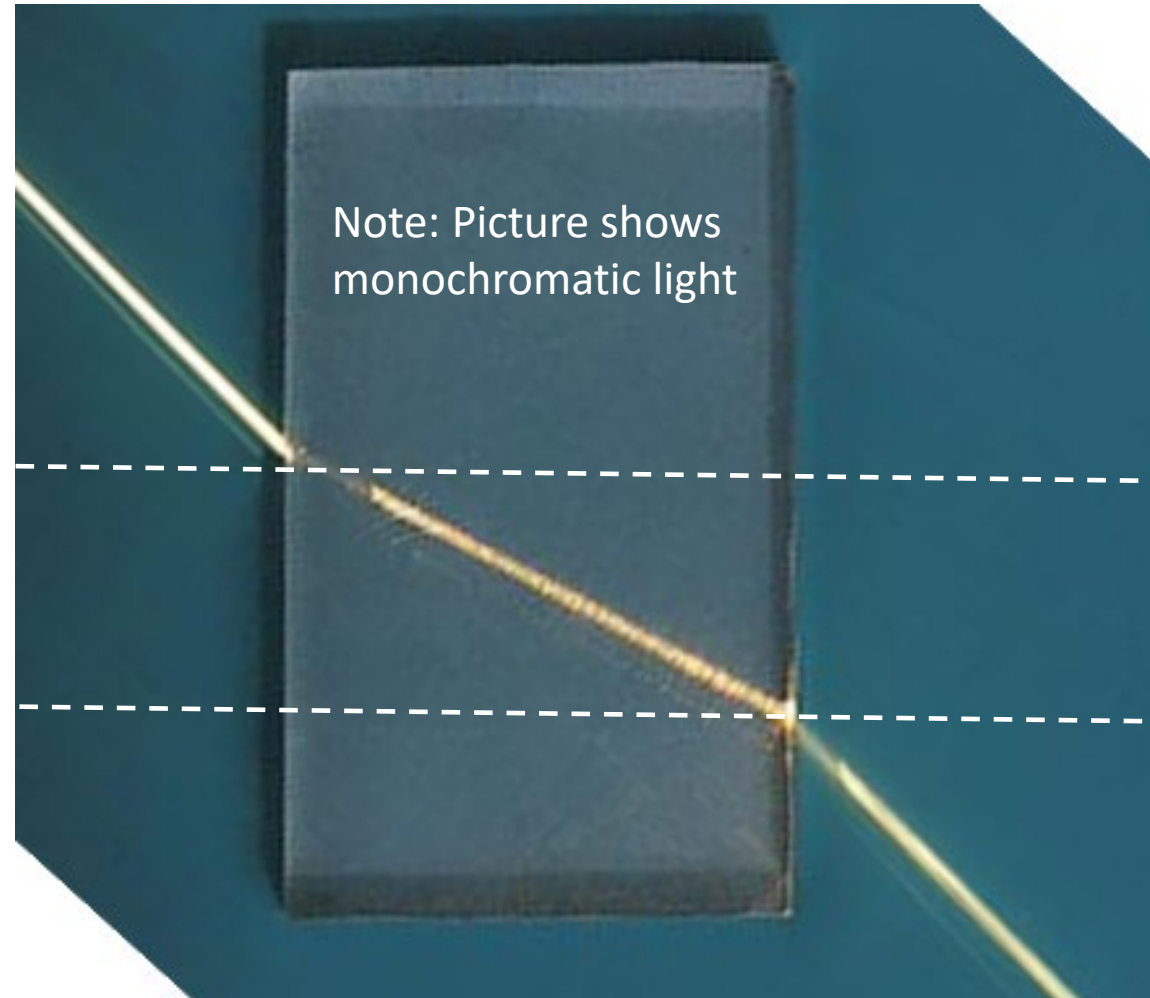
Refractive indices
(determined by density):

Vacuum or air = 1

Water = 1.33

Glass = 1.5

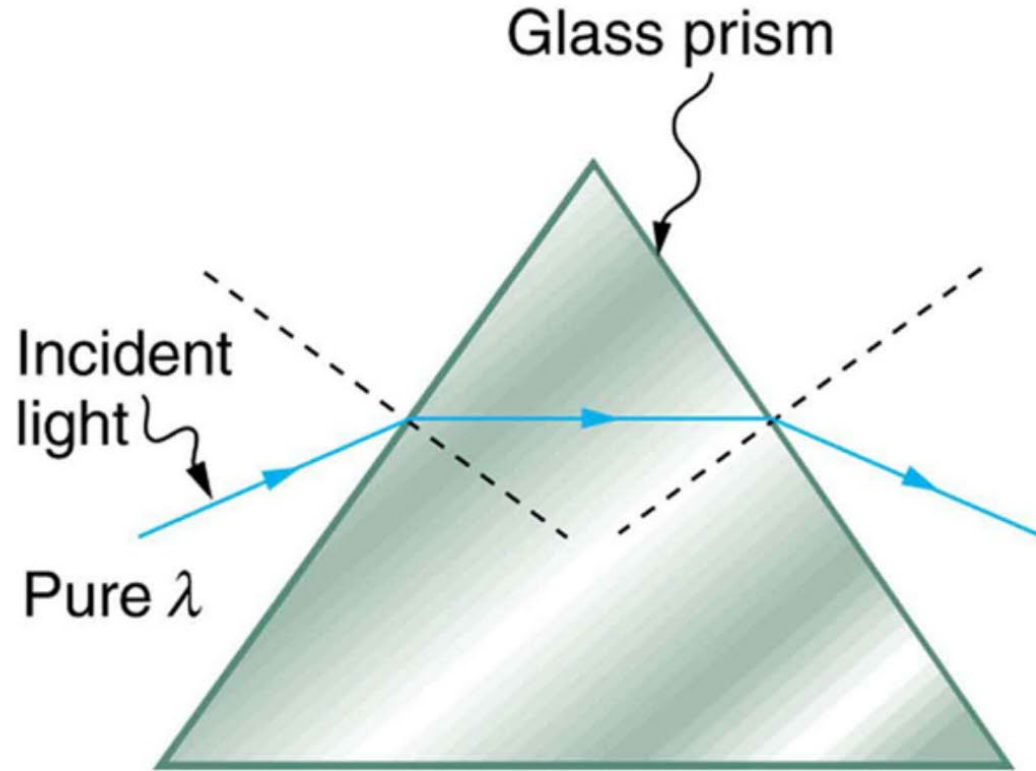
Diamond = 2.42



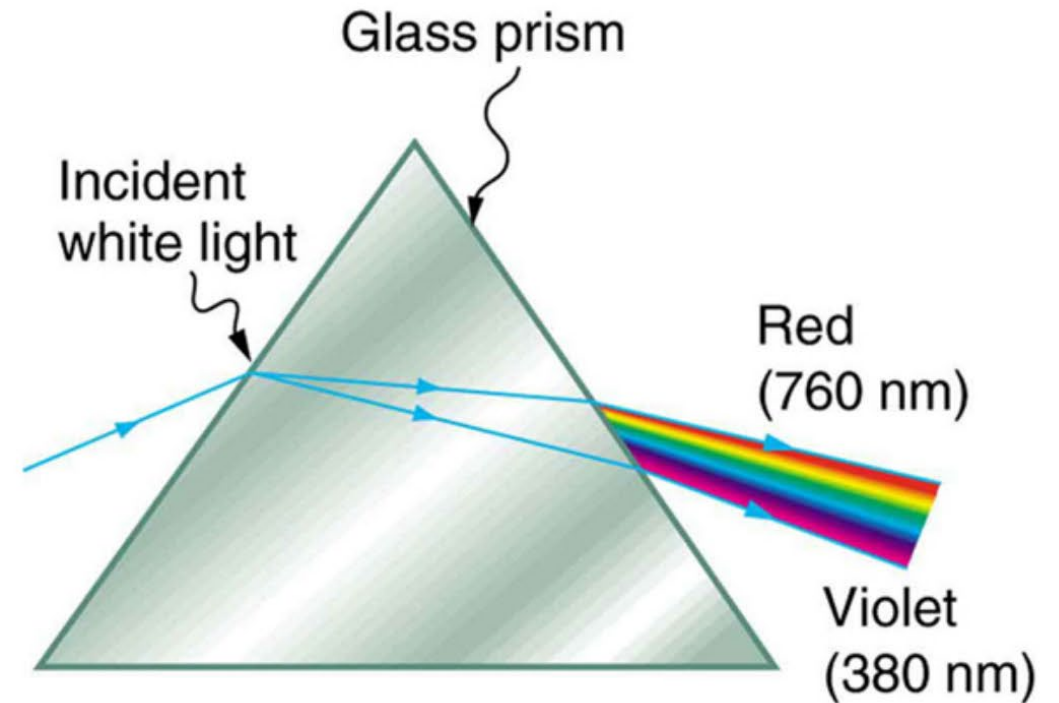
**Application/
component: prism &
lens**

Picture taken from Wikipedia and modified

What is happening with light of different wavelengths?

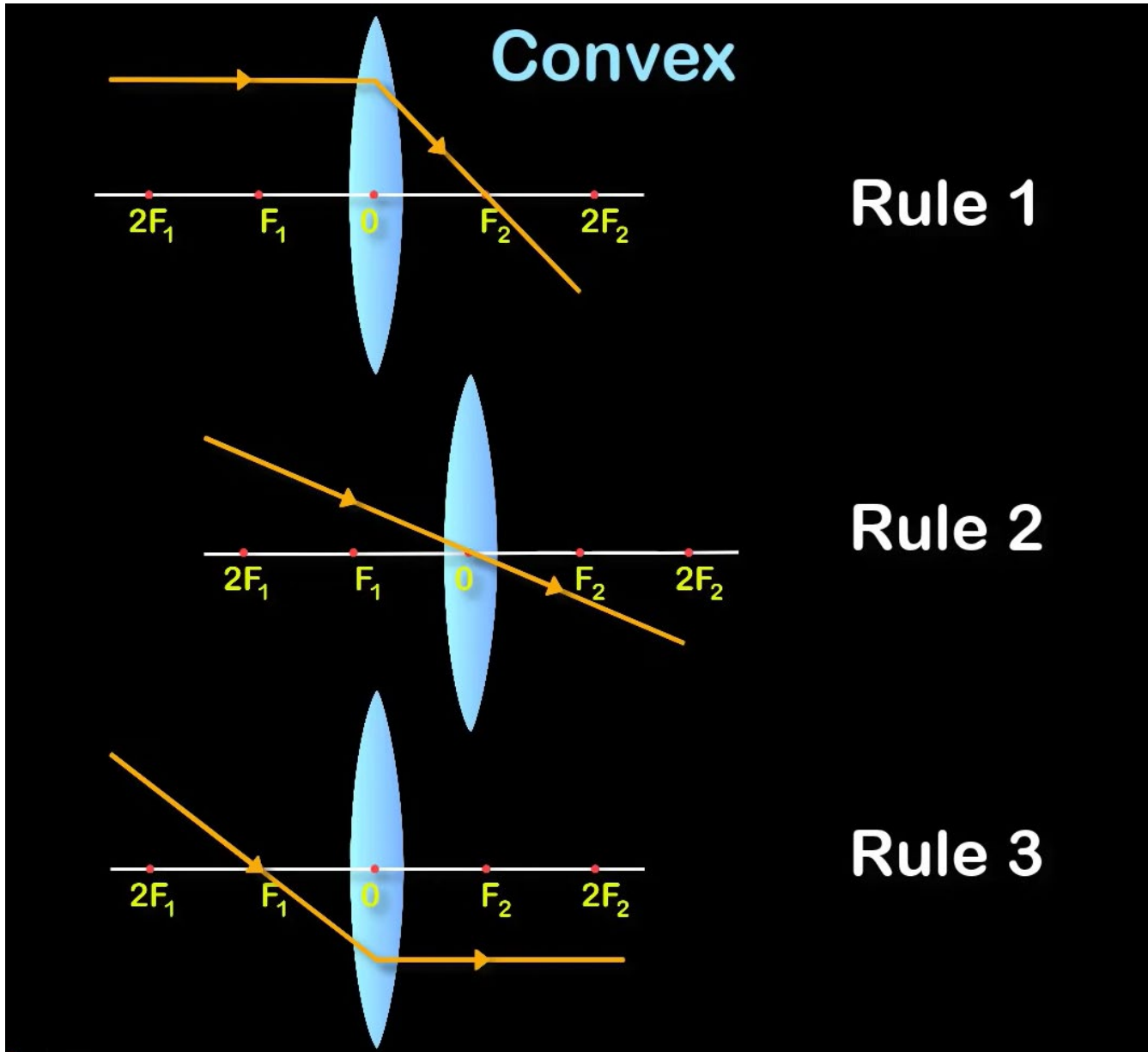


www.coursehero.com



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.

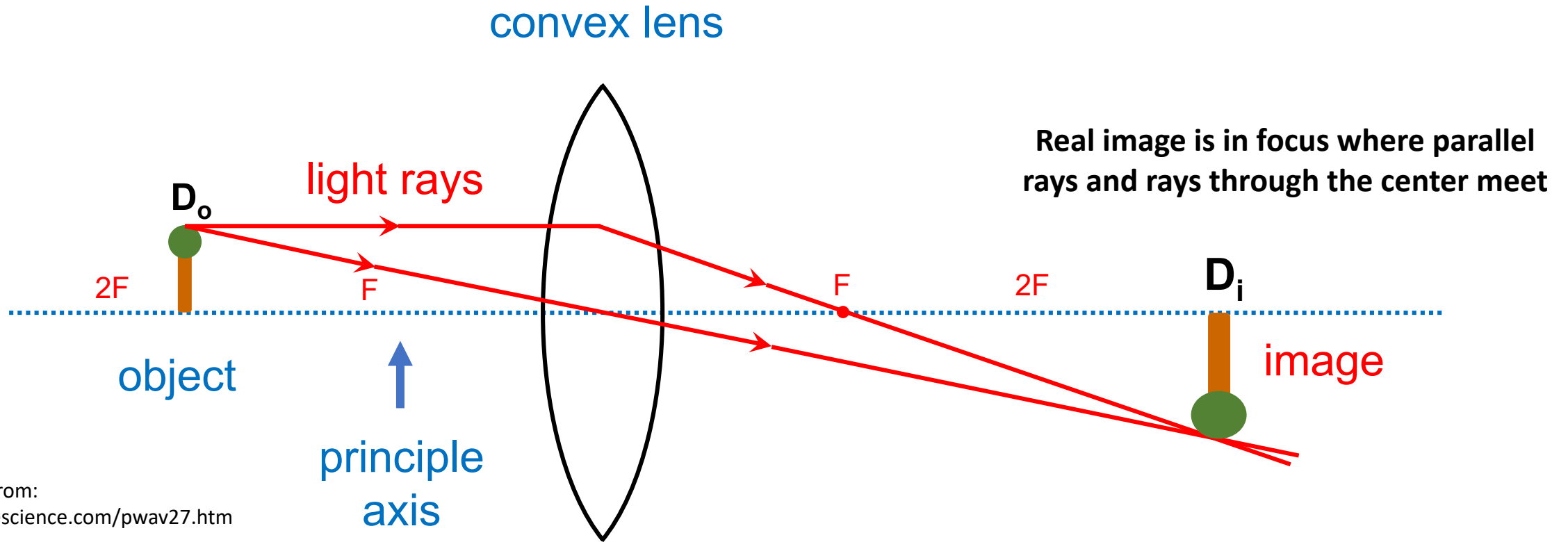
Lenses – basic rules



Rules

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

Lenses – focus and magnification



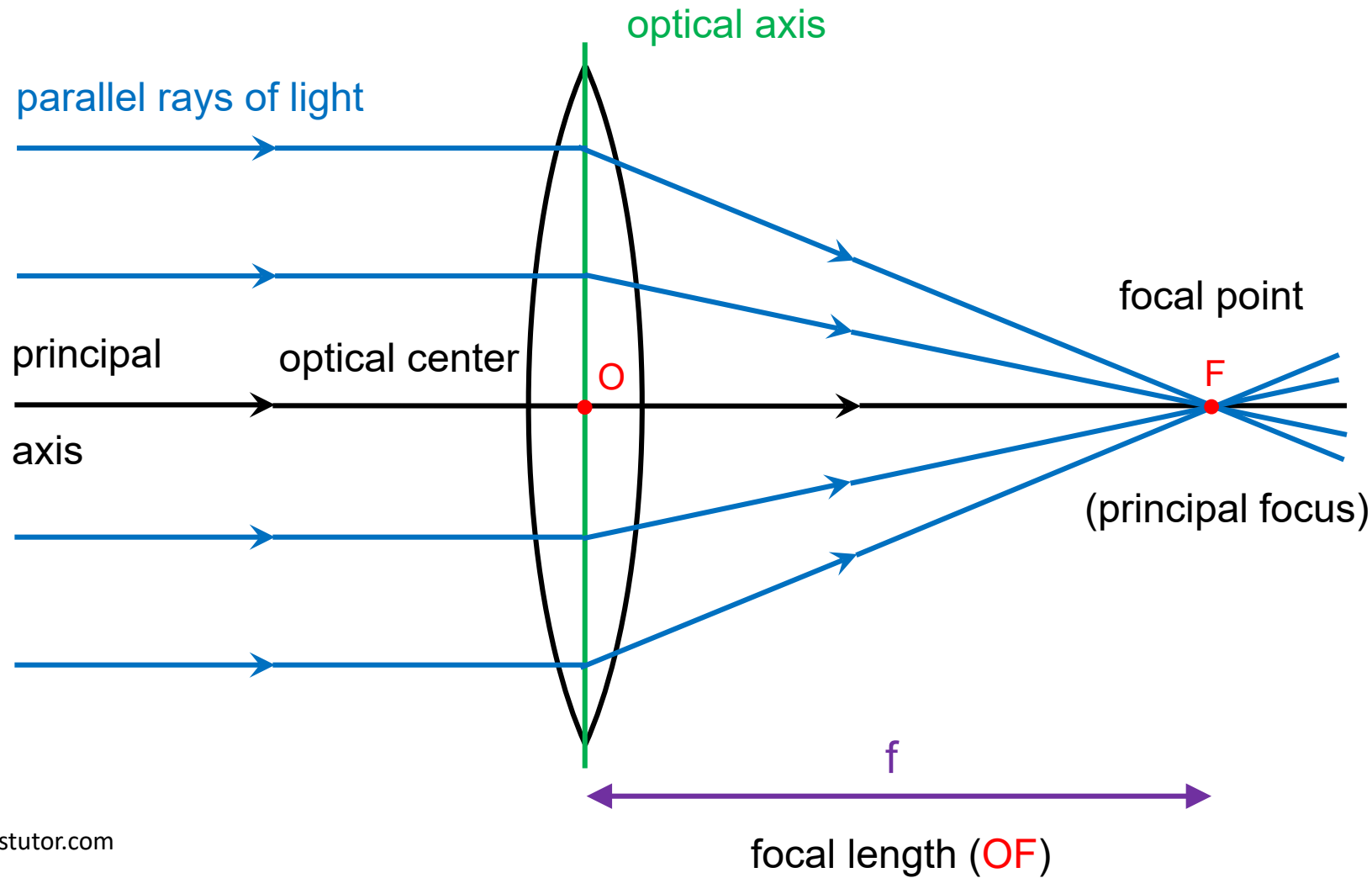
adapted from:
www.gcscience.com/pwav27.htm

- Any object **closer than 2 x 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)!

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

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

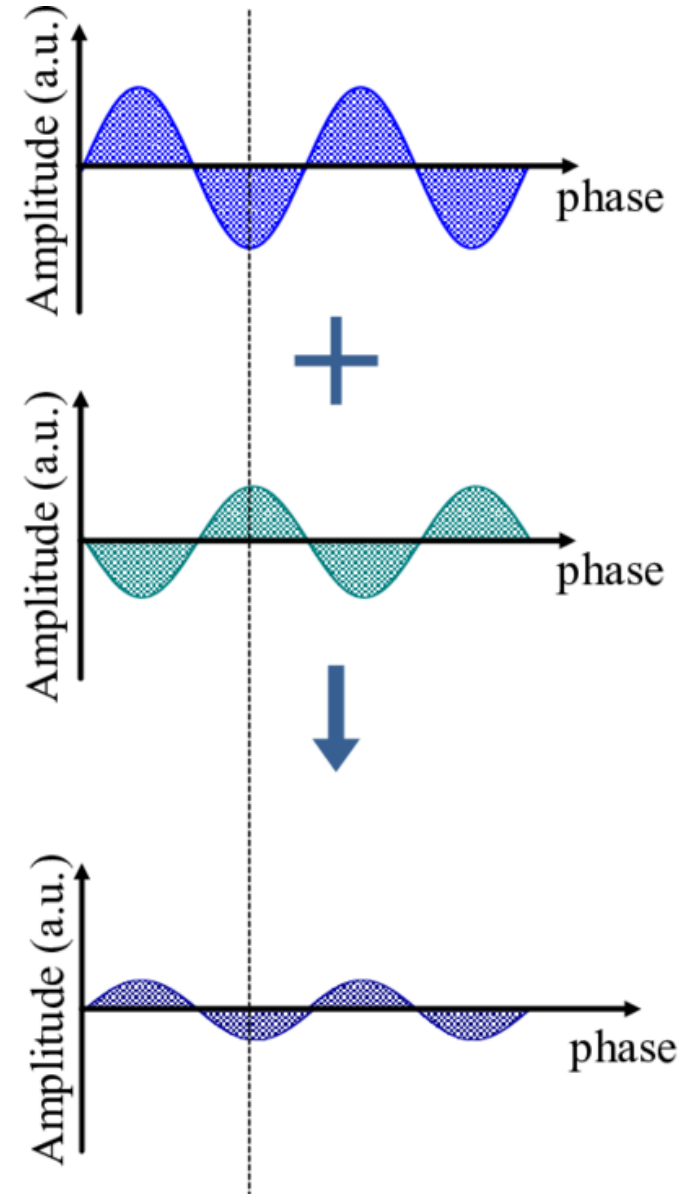
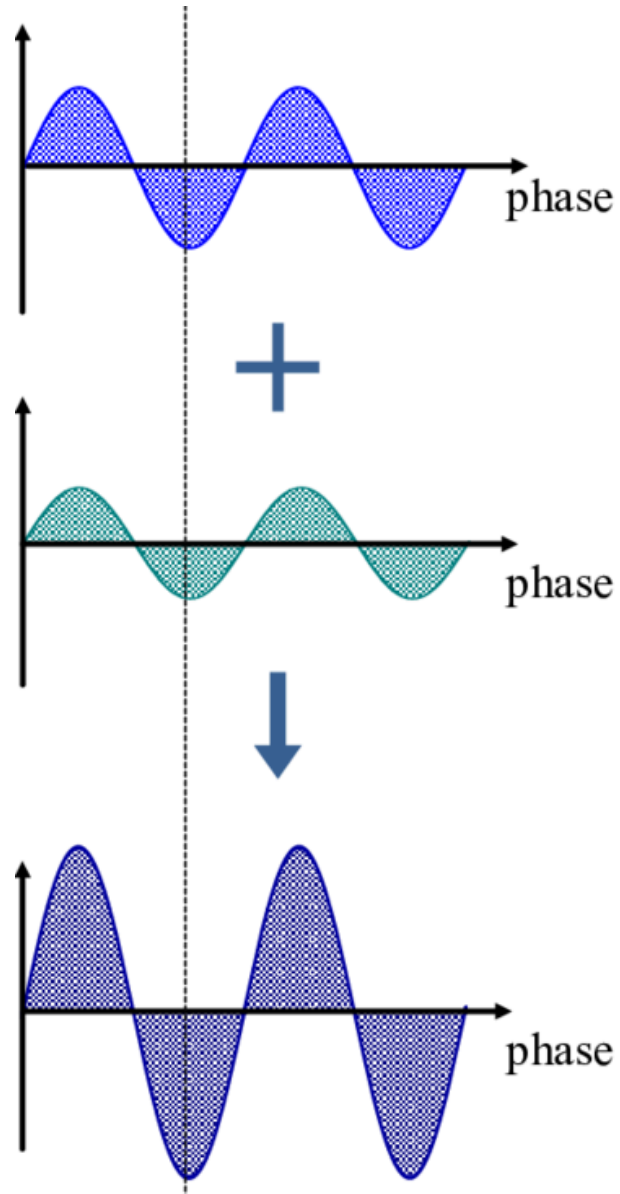
Lenses – focus and magnification



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)!

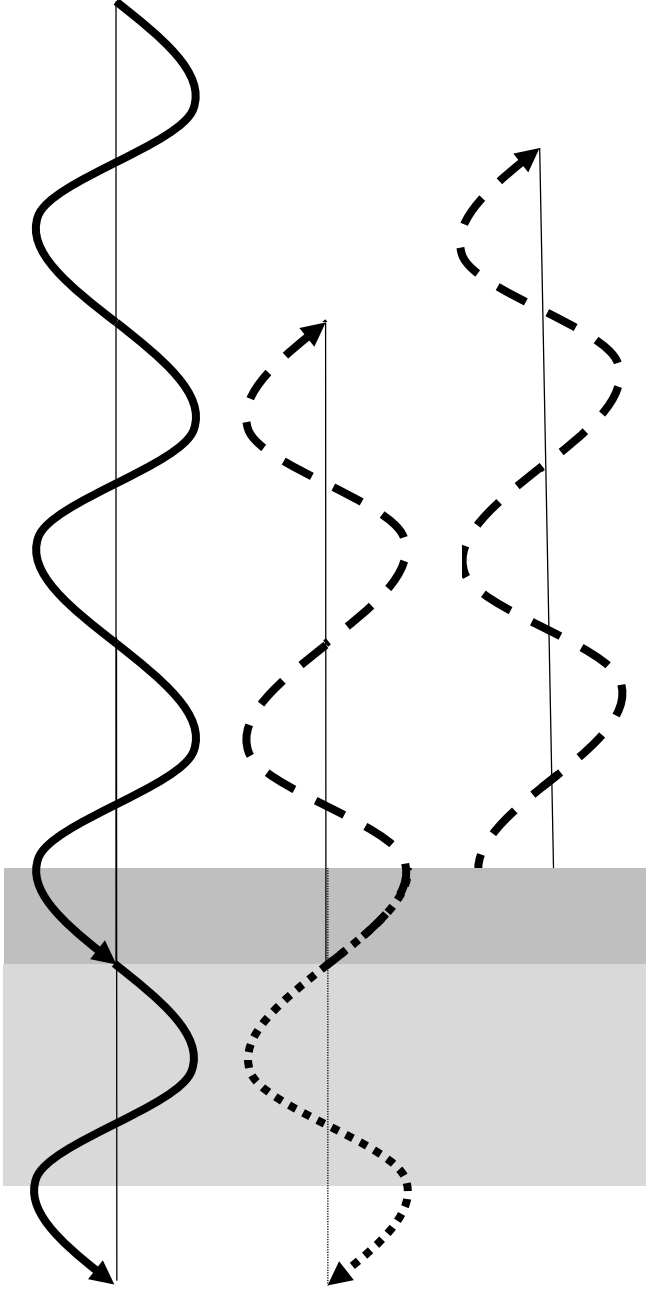
Constructive and destructive interference



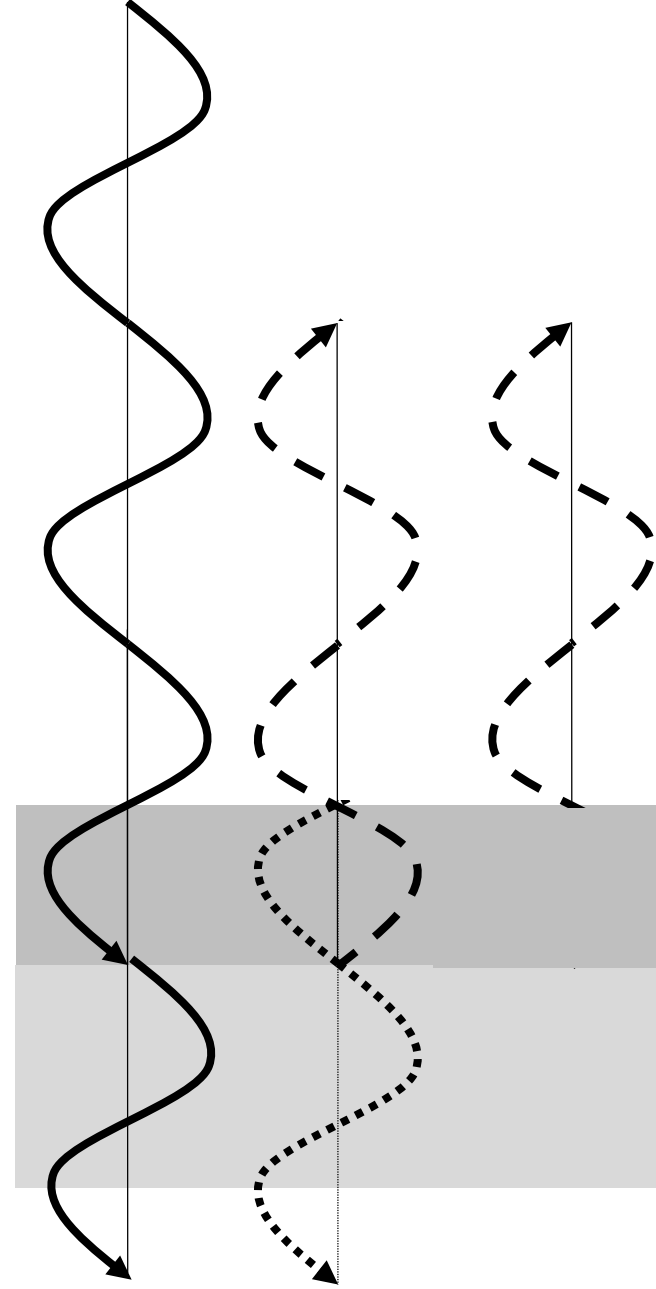
**Application/
component: filters &
dichroic mirrors**

Working principle of interference filters

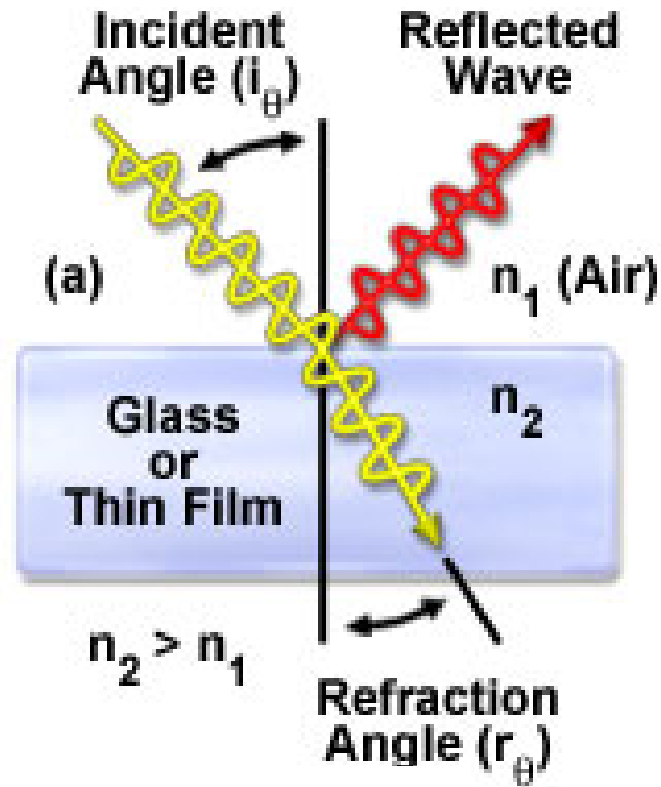
Destructive interference, layer thickness = $\lambda / 4$, phase shift = 180°



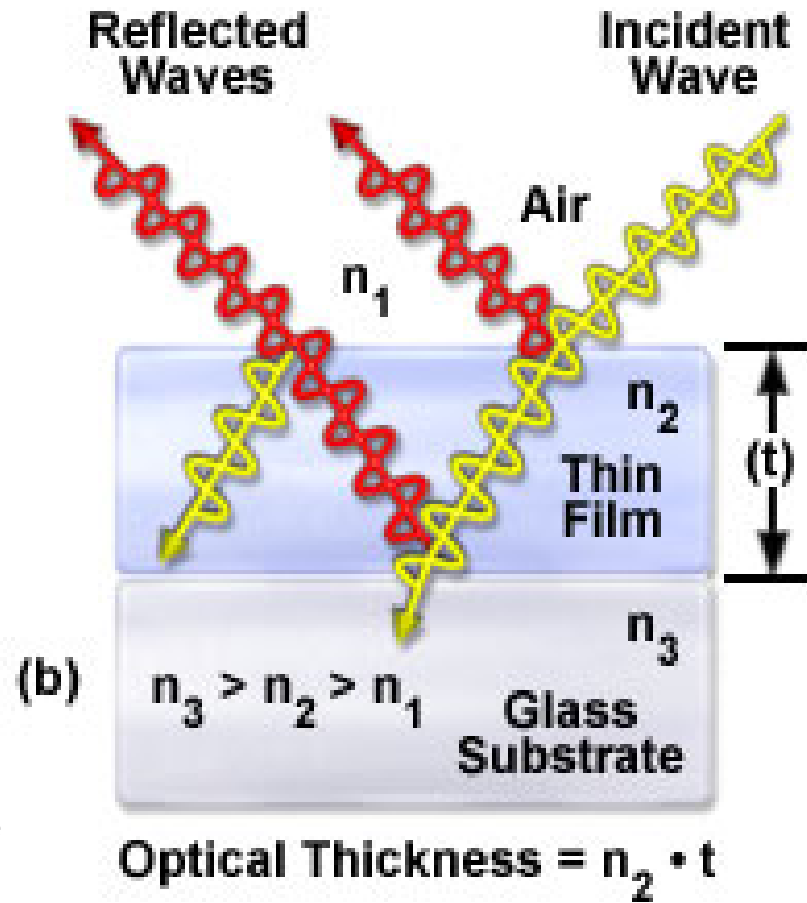
Constructive interference, layer thickness = $\lambda / 2$, phase shift = 360°



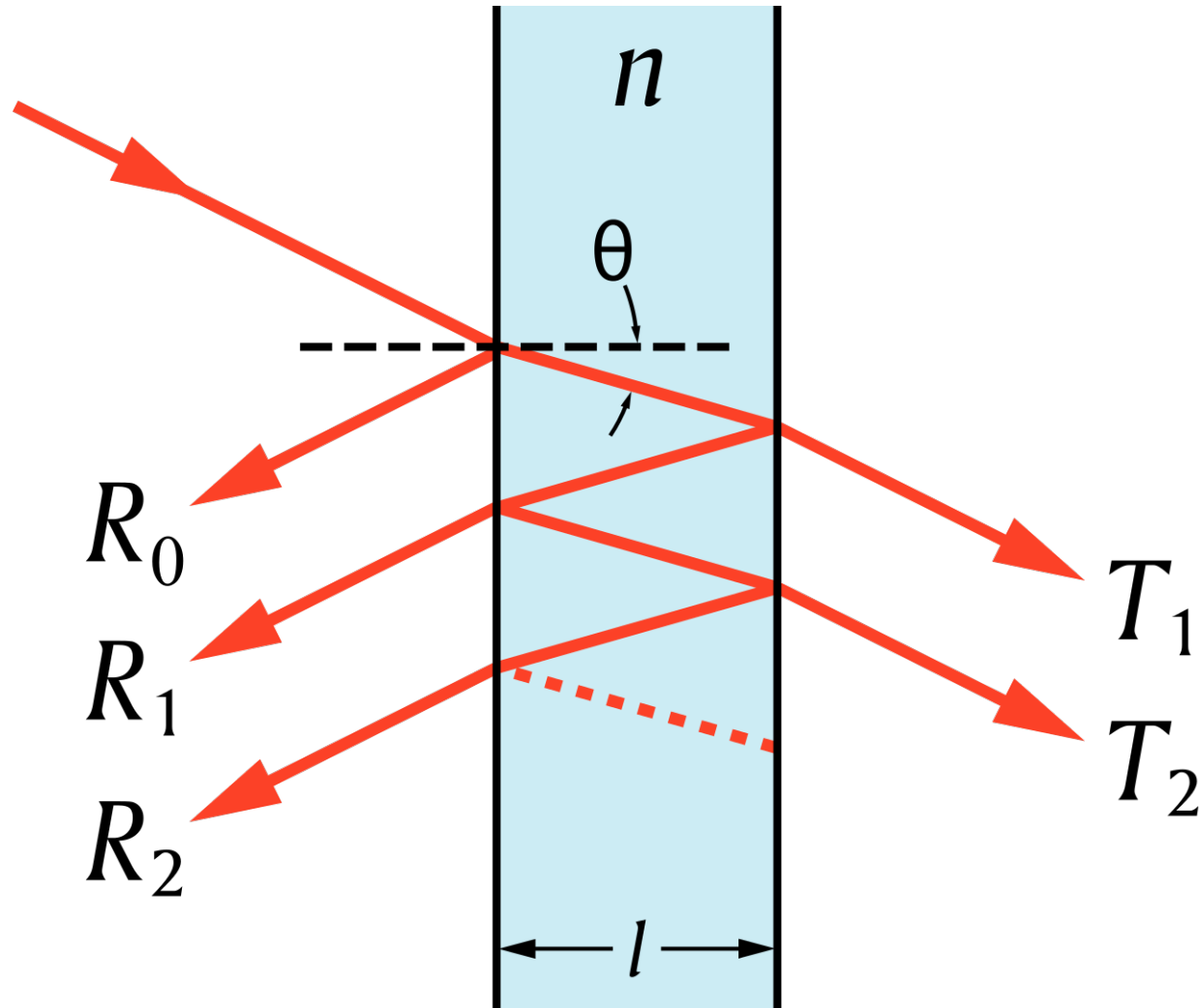
Working principle of dichroic mirrors



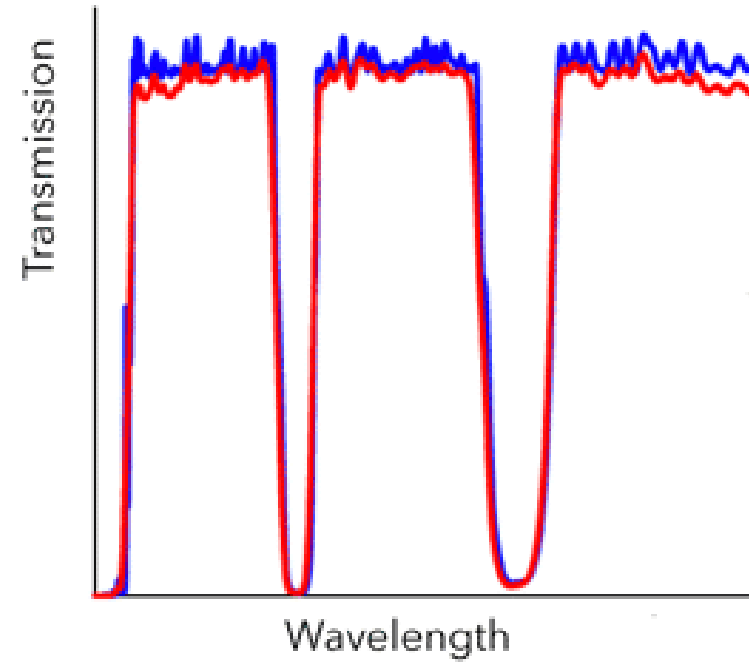
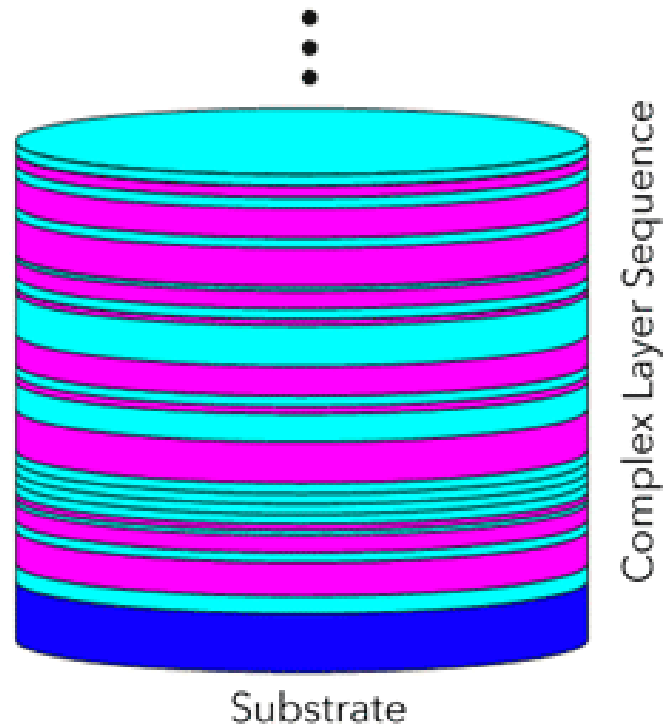
<https://micro.magnet.fsu.edu/primer/techniques/fluorescence/interferencefilterintro.html>



Working principle of dichroic mirrors



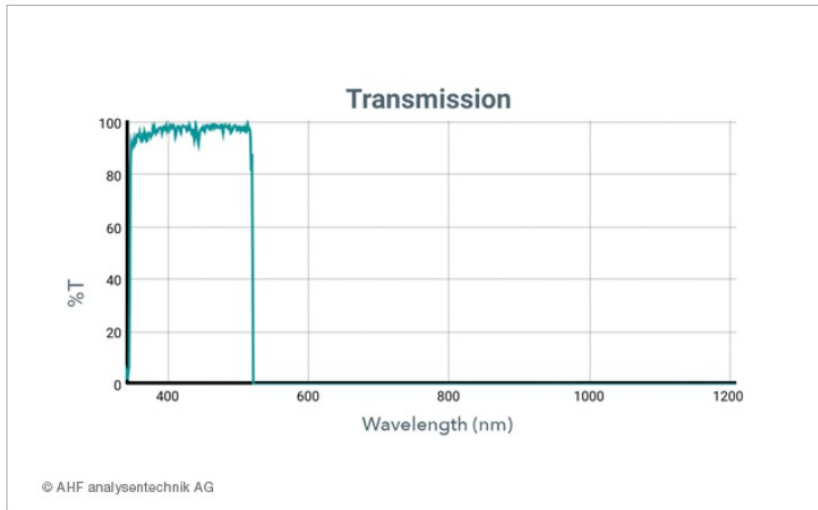
In reality it is always more than one thin layer!



Filters used in the practical course

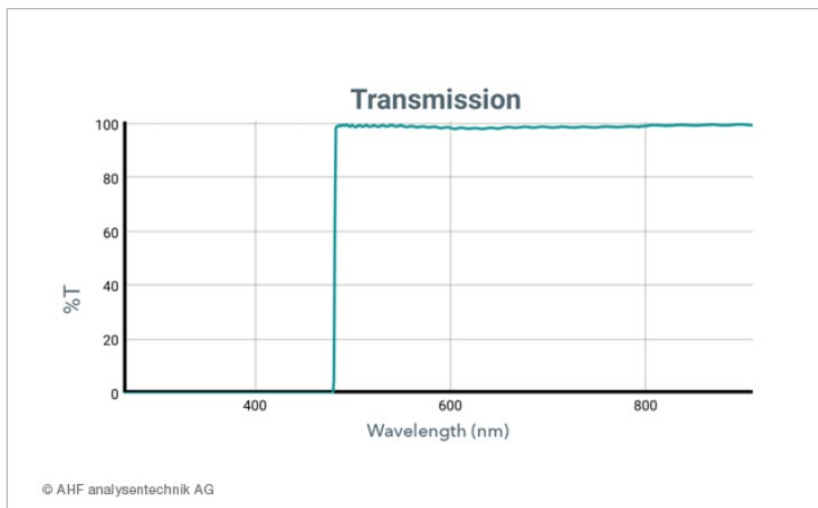
AHF Analysentechnik AG, 2023, *Optical Filters for highest performance*, accessed 14 July 2023, '<https://www.ahf.de/en/products/spectral-analysis-photonics/optical-filters/>'

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



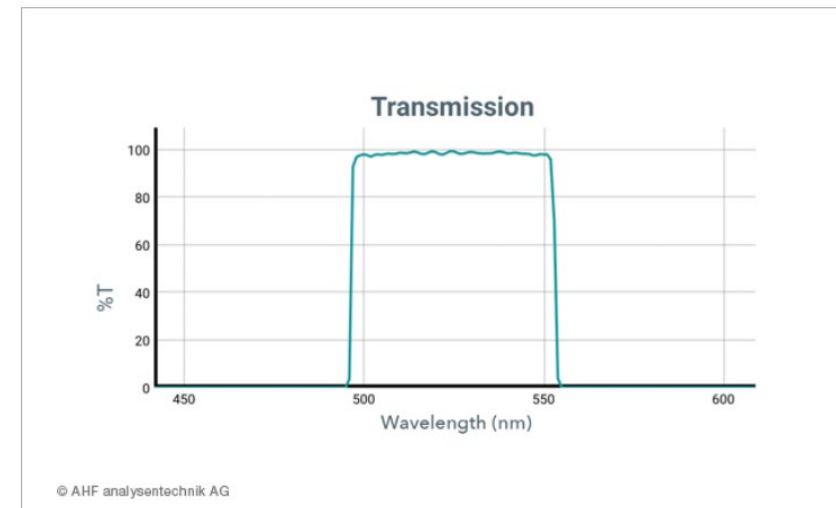
short pass filters

532 nm



long pass filters

473 nm

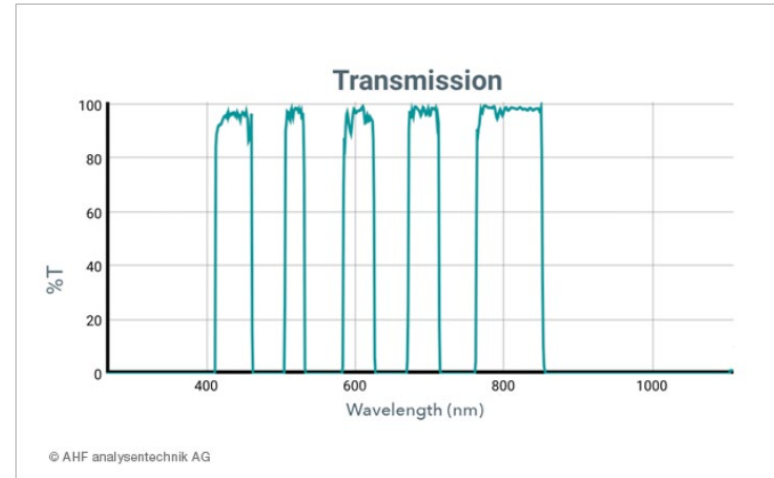


***bandpass
filter***

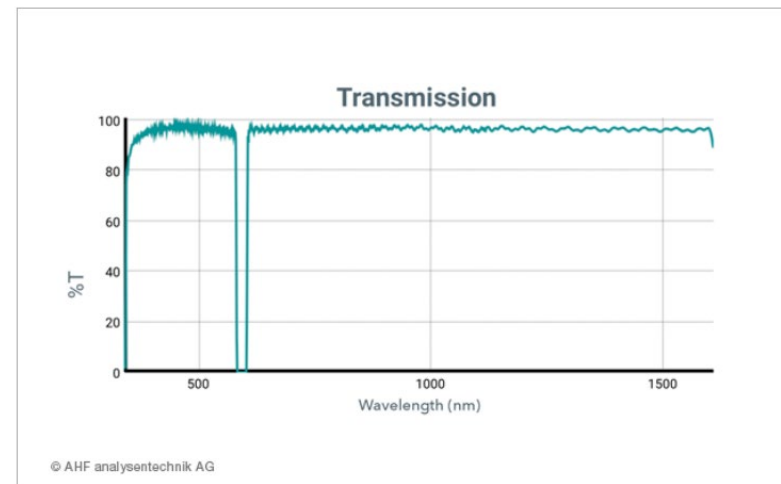
525/50 nm

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-photonics/optical-filters/>'

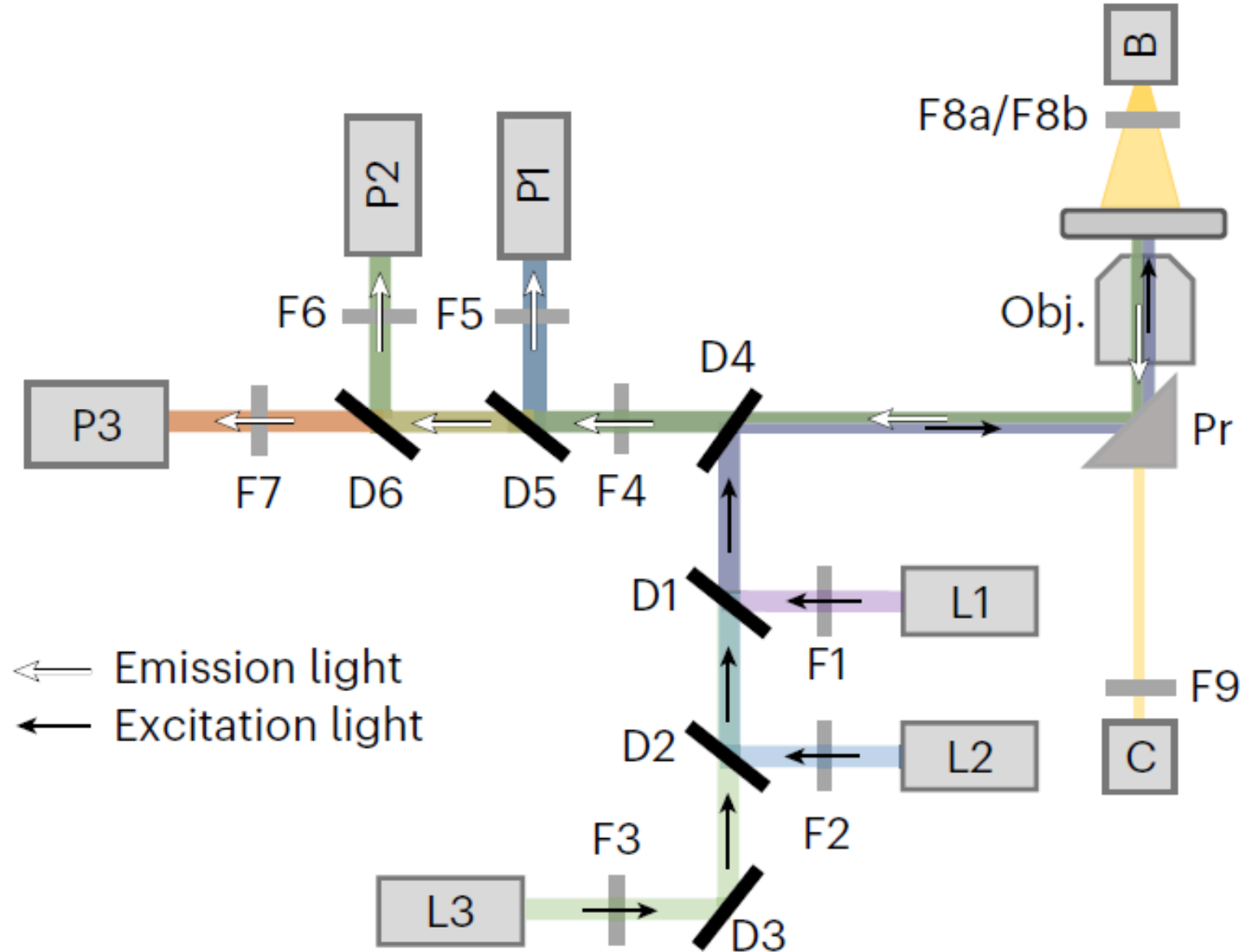


multiband filter



notch filter

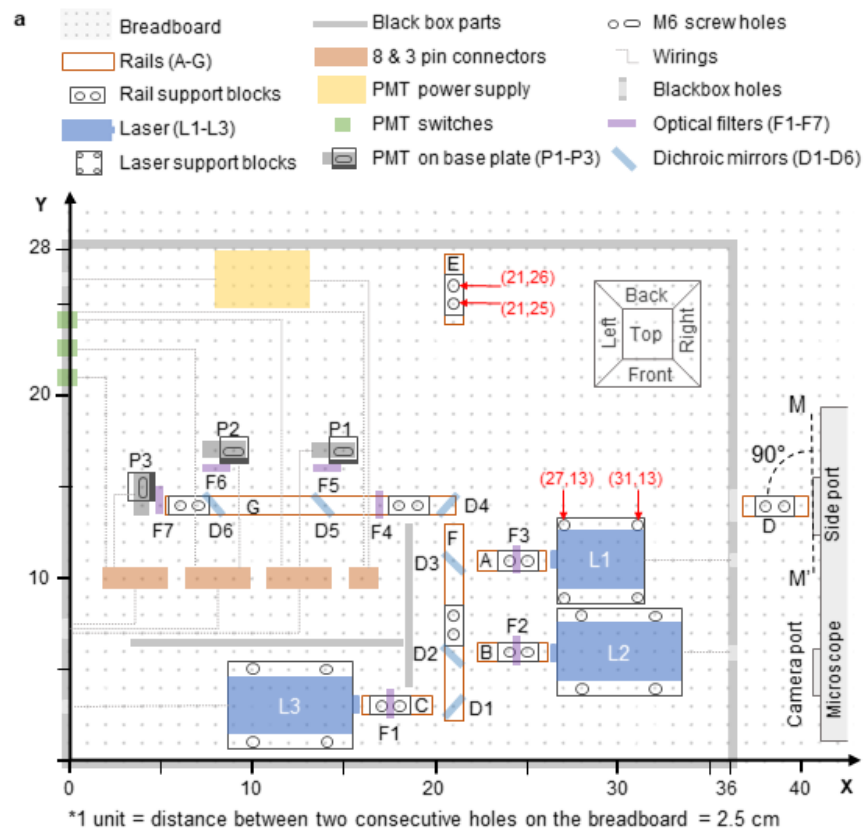
A series of dichroics can be used to feed in multiple lasers or to split polychromatic light into its components



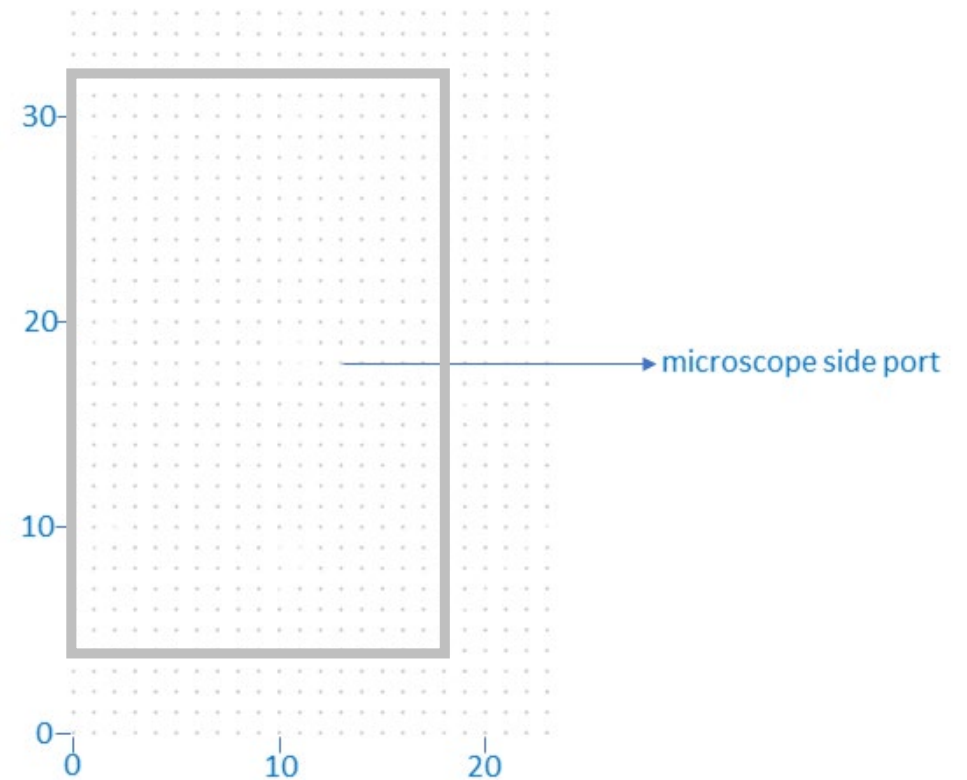
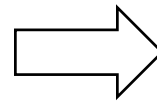
Blueprint of your instrument

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

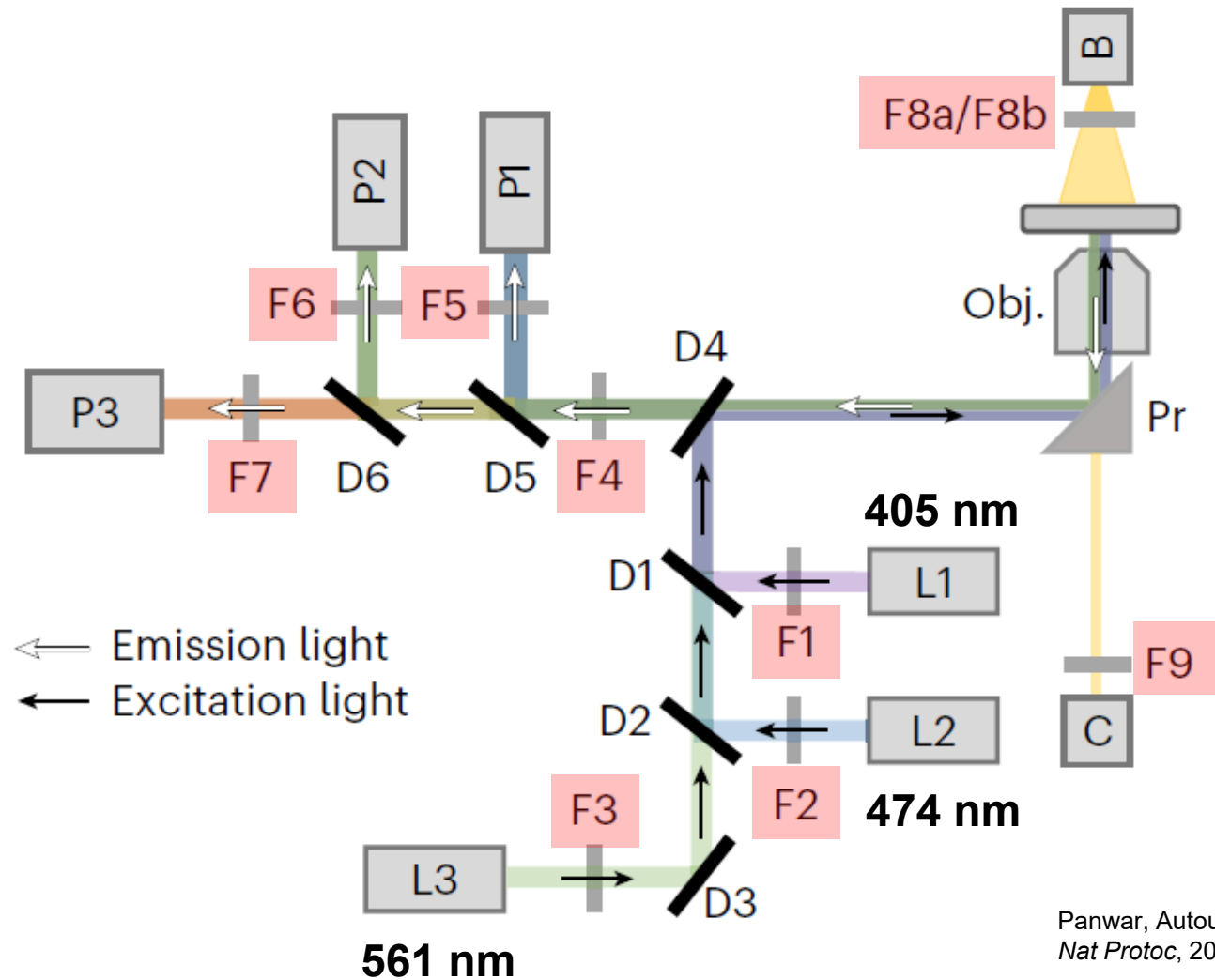


3 channel fluorescence detection



single channel fluorescence detection

What kind of tools do we need?

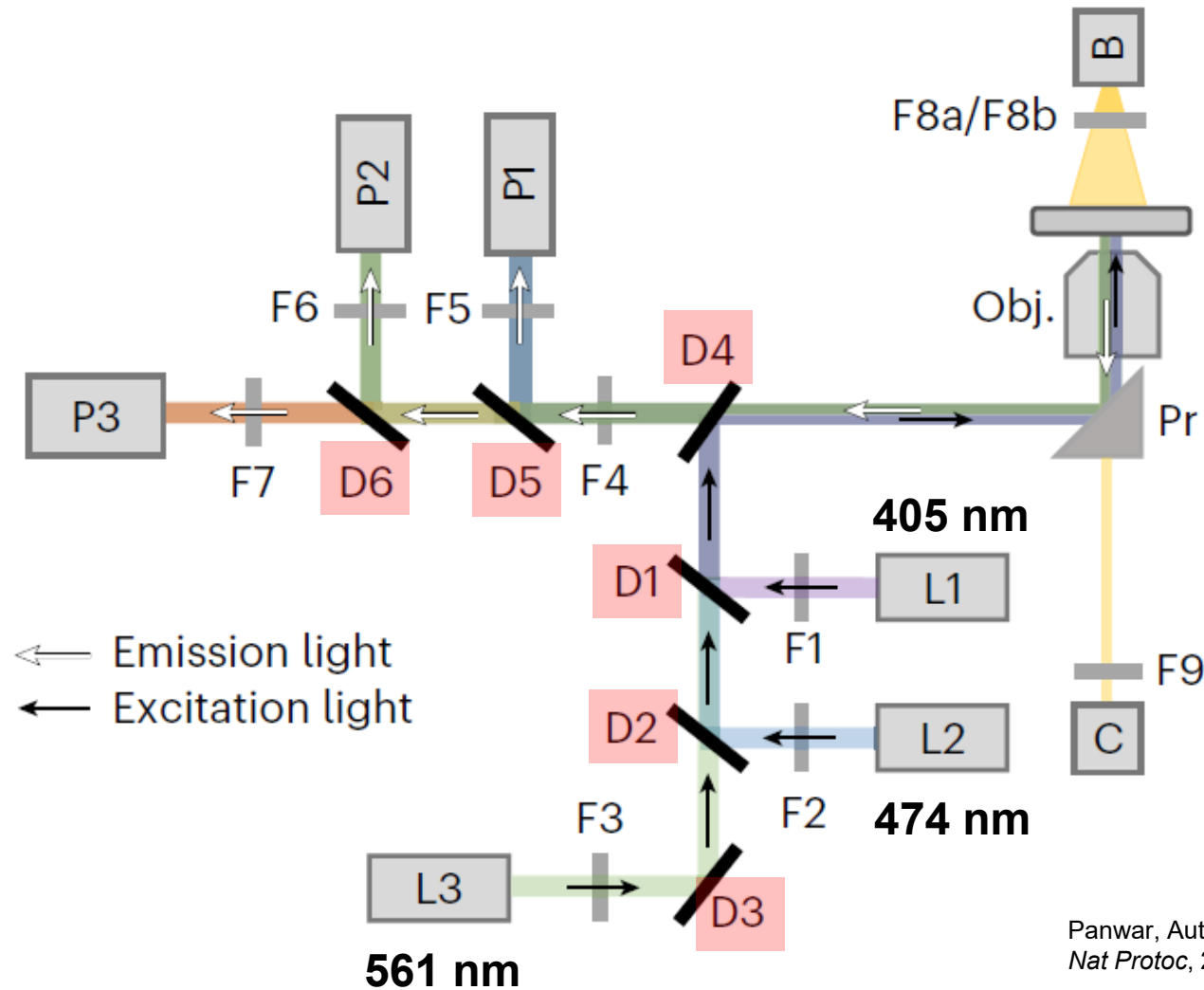


Panwar, Autour & Merten,
Nat Protoc, 2023

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?



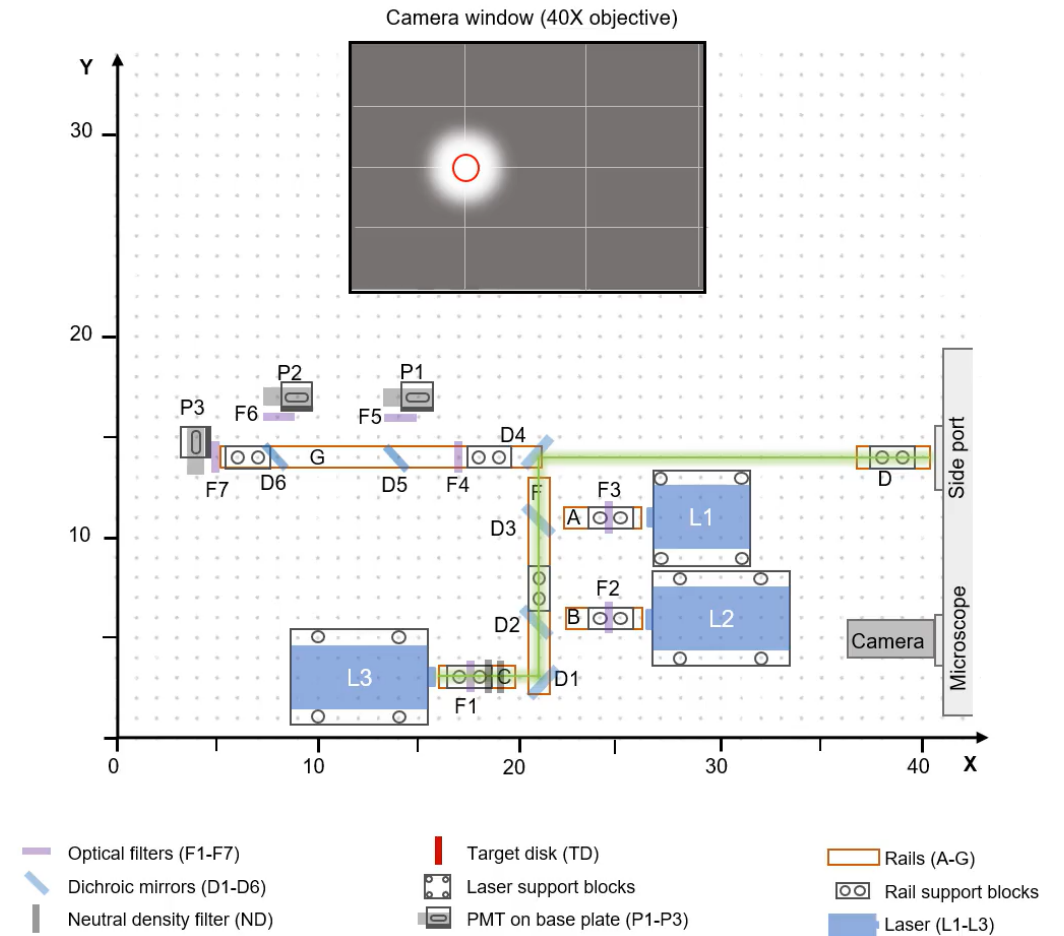
Panwar, Autour & Merten,
Nat Protoc, 2023

- 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

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

Practical task: align all optical components

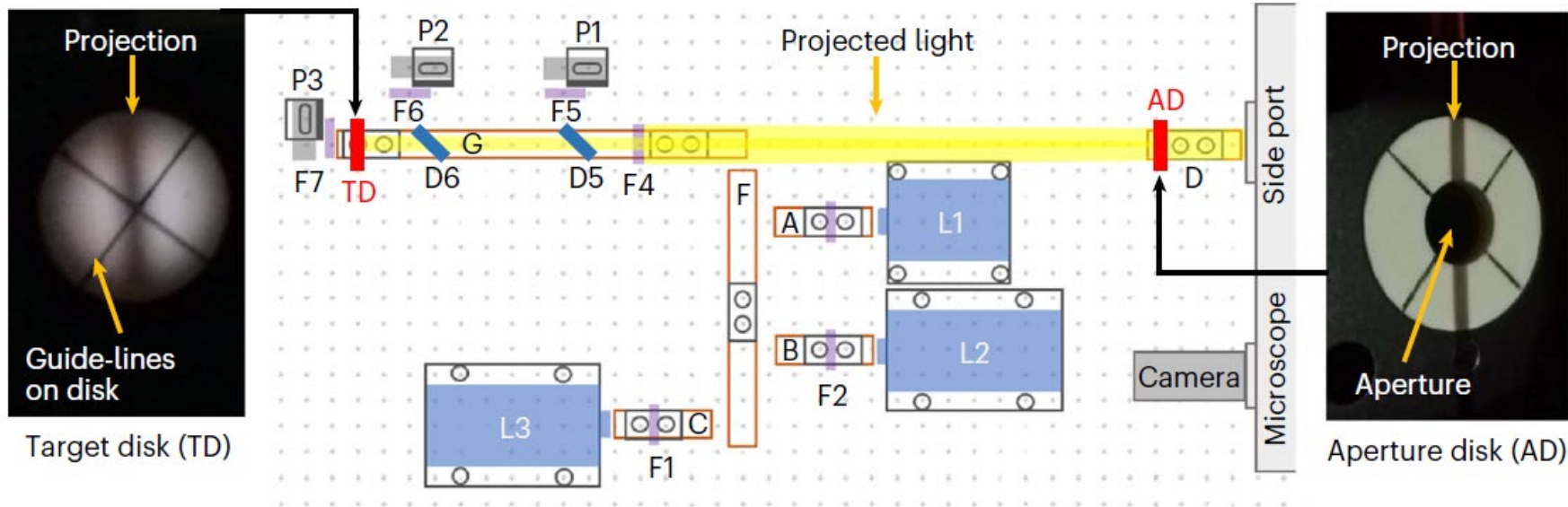
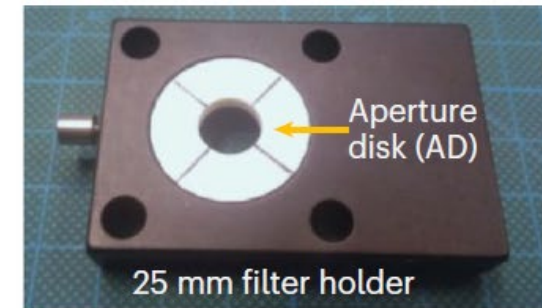
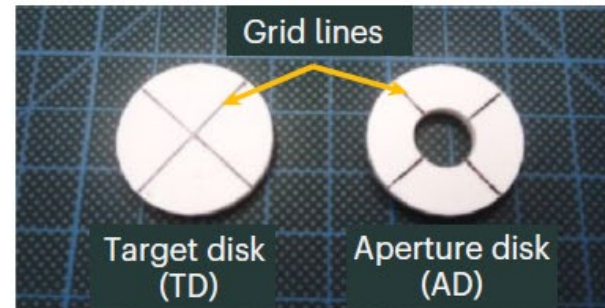
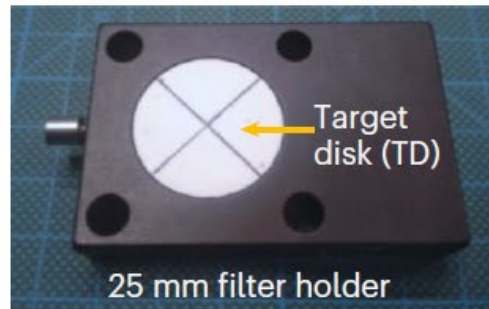
- **excite** different fluorophores (e.g., within droplets)
 - **direct** the emission toward the photosensors
-
- **alignment** = directing the beam so that it follows a predetermined path



Alignment tools

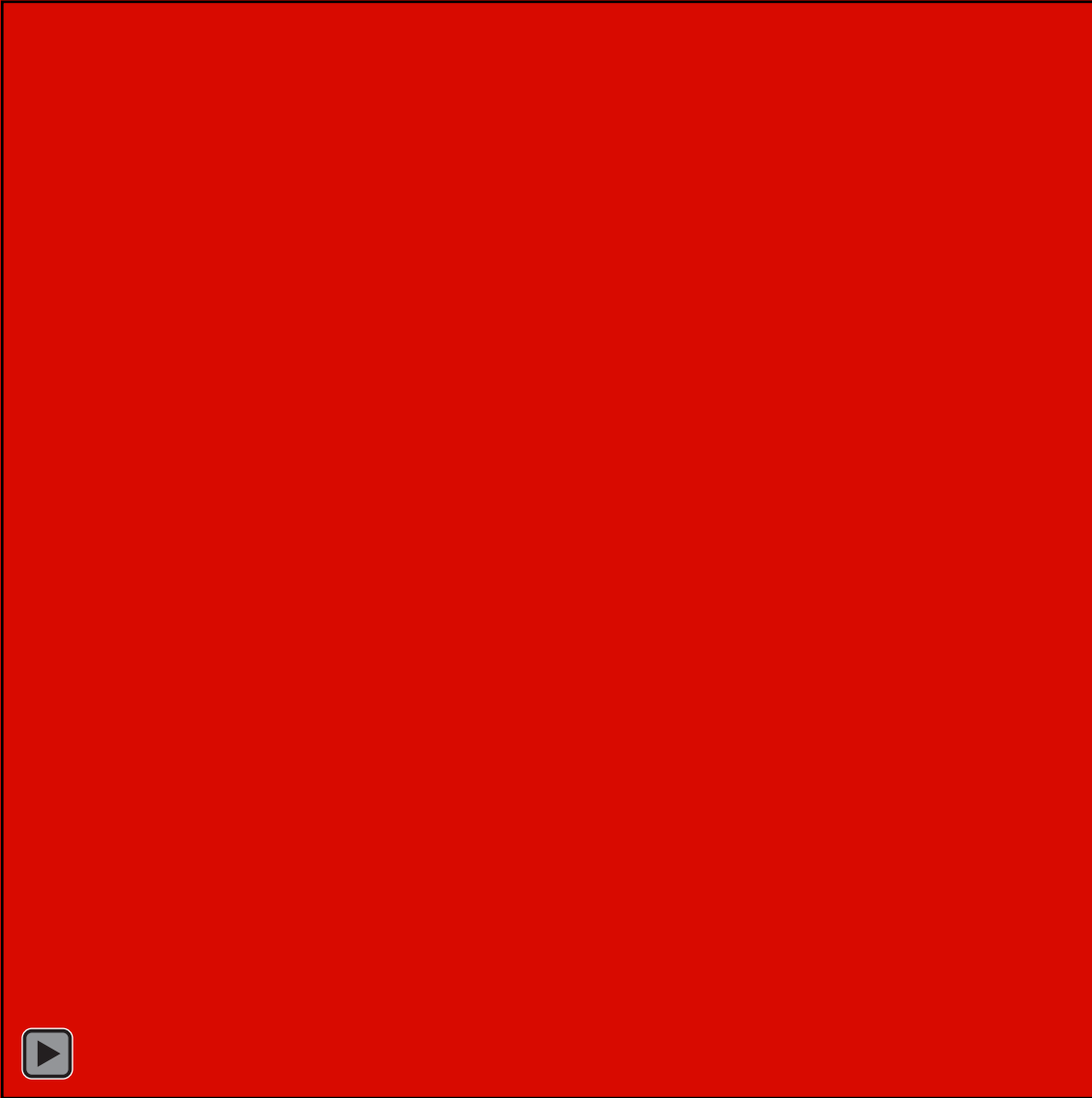
1.) emission light alignment

reduced diameter of beam



! microscope is fixed now !

Excitation light alignment (Panwar et al., 2023)

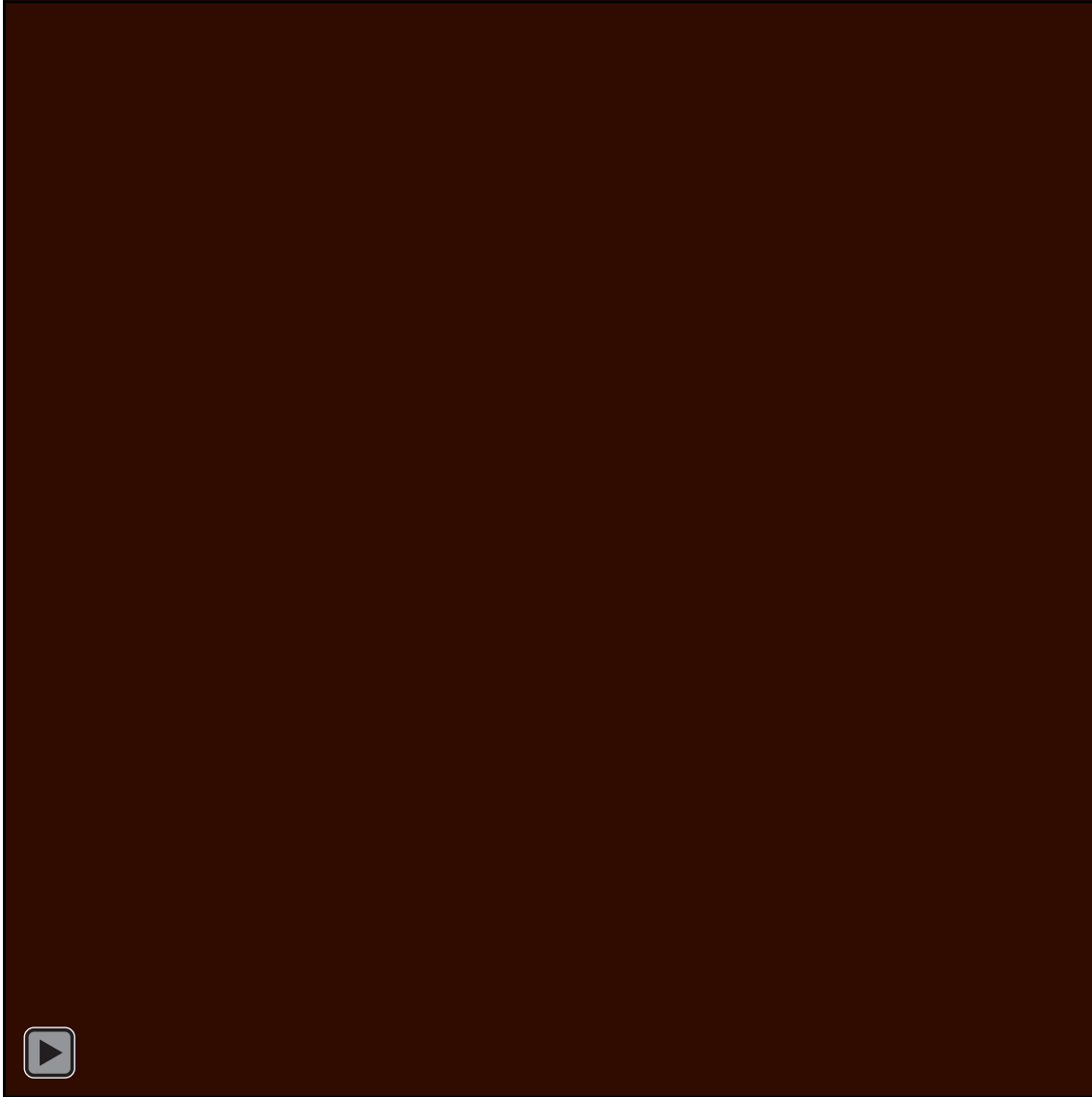


VIDEOS:

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

Most relevant: 55'' and onwards

Emission light alignment (Panwar et al., 2023)

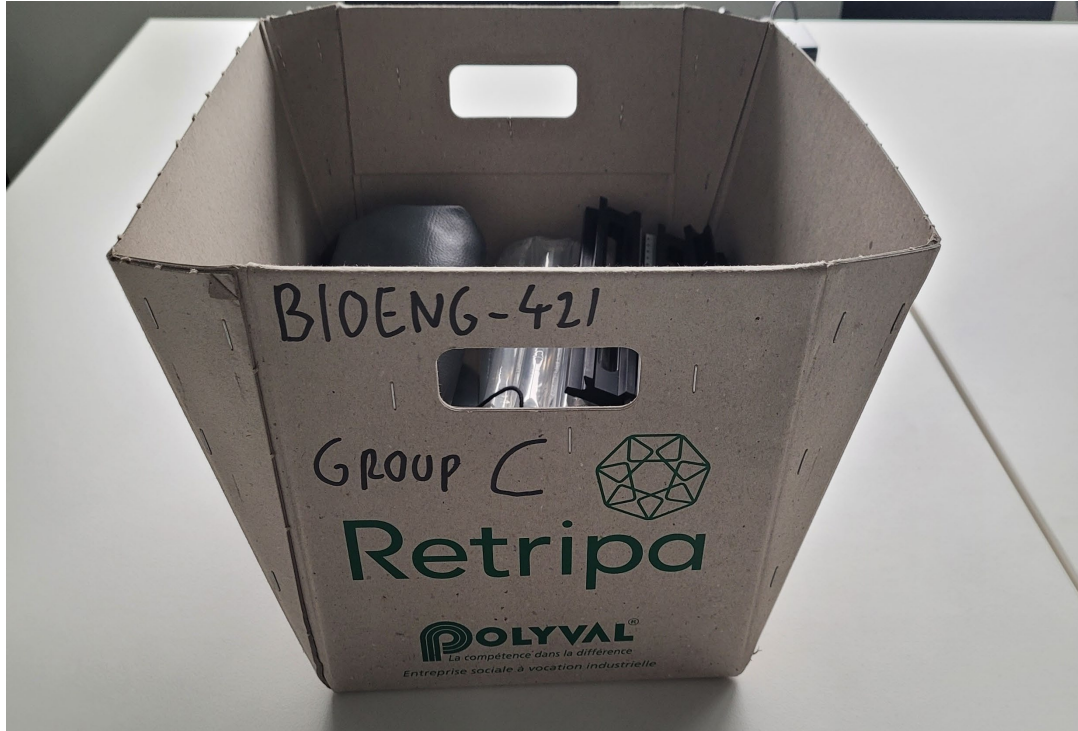


VIDEOS:

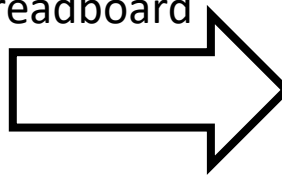
<https://www.nature.com/articles/s41596-022-00796-2#Sec75>

Most relevant: 2'40'' and onwards

Your course inventory



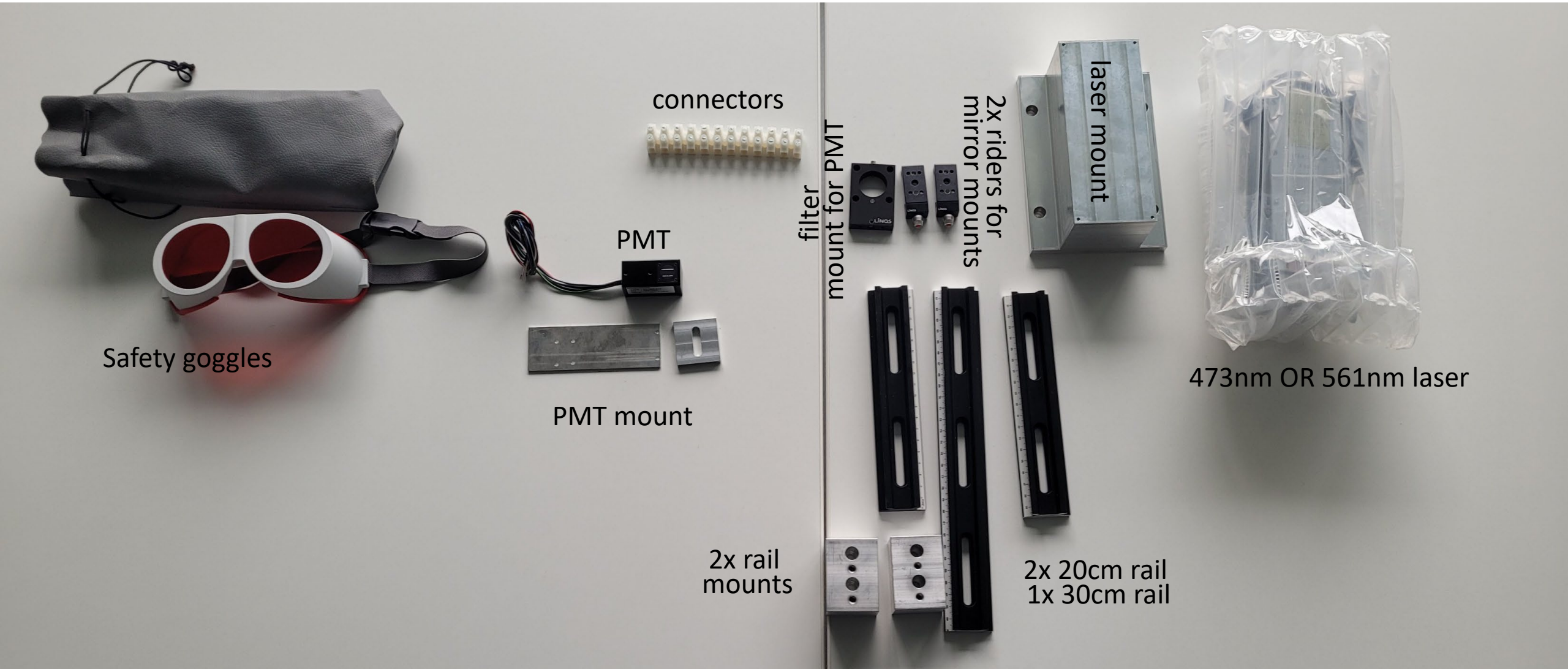
assemble on
breadboard



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**)

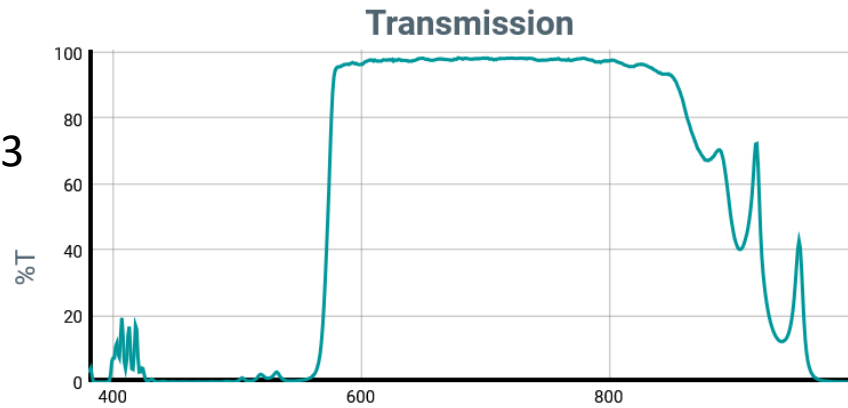
Your course inventory



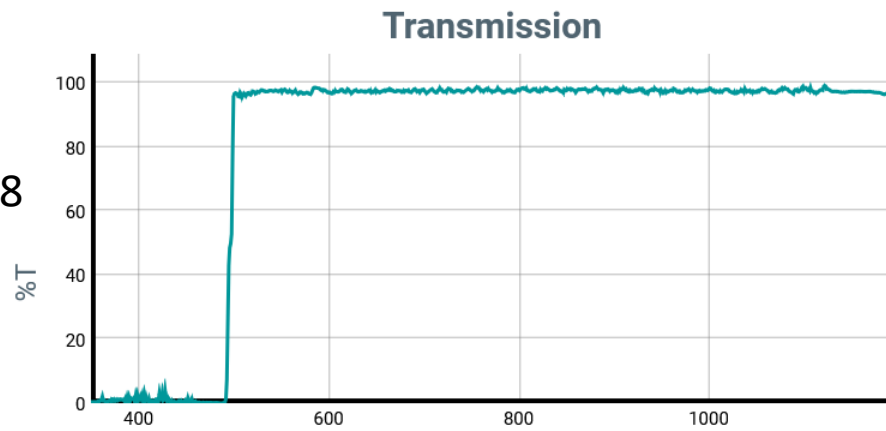
- + filters & mirrors (details on next slide)
- + screws, wires, crimps & alignment tools

Mirrors (already mounted)

Dichroic F48-553

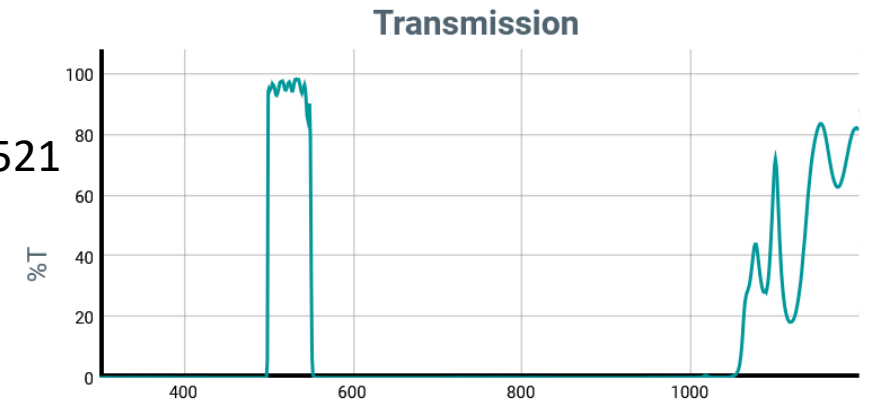


Dichroic F38-488

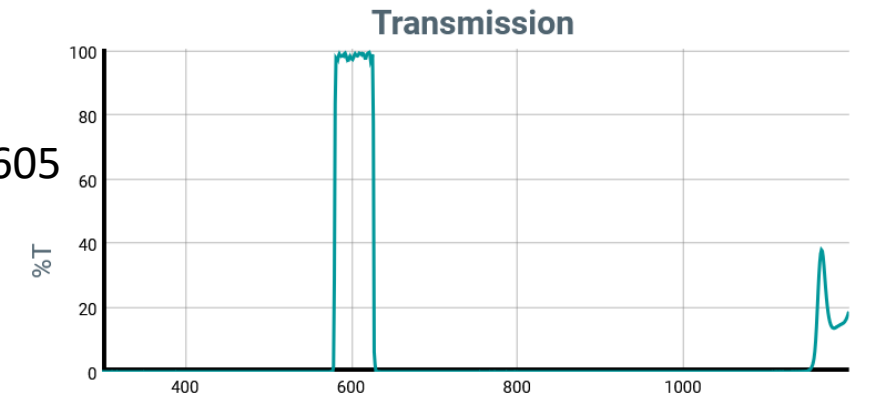


Filters

Filter F37-521



Filter F49-605



+ Full reflective mirror

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^{1,2,3} and Christoph A. Merten^{1,2,3}

Droplet microfluidics has revolutionized quantitative high-throughput bioassays and screening, especially in the field of single-cell analysis where application includes cell characterization, antibody discovery and directed evolution. However, droplet microfluidic platform capable of absorbance, fluorescence-based analysis and sorting are still mostly found in specialized labs, because their setup is complex. Complementary to commercial FACS, microfluidic droplet sorters allow the screening of cell libraries for selected factors, or even for the effects of selected or surface-related factors on a second cell type. Furthermore, they also enable PCR-activated droplet sorting for the isolation of genetic material harboring specific markers. In this protocol, we provide a detailed step-by-step guide for the construction of a high-throughput droplet analyzer and sorter, which can be accomplished in 40 working hours by nonexperts. The resulting instrument is equipped with three lasers to excite the fluorophores in droplets and photometers that acquire fluorescence signals in the blue (425–465 nm), green (505–545 nm) and red (630–650 nm) spectrum. This instrument also allows transmittance-activated droplet sorting by analyzing the light intensity transmitted through the droplets. The setup is validated by sorting droplets containing fluorescent beads at 200 Hz with 99.4% accuracy. We show results from an experiment where droplets hosting single cells were sorted on the basis of measured matrix metalloproteinase activity as 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 methodological step for assessing many biological questions especially in genomic, transcriptomic or proteomic applications. Droplet microfluidics has emerged as reliable choice for single-cell assays^{1,2}, by allowing the generation of monodispersed droplets of small volumes (10⁻¹⁰ to 10⁻¹⁶ liter lvol.) at high frequencies, reaching up to a few million droplets per hour (rd.)³. These sizes and rates of formation enable single-cell encapsulation with each droplet being its own compartment (comparable to a miniaturized reaction plate) with, by changing the droplet matrix, single cells can be encapsulated with other molecules/beads or even a second cell type (for probing interactions) making each droplet a microreactor with allowing high-throughput screening of these reactions. Such screening methods are commonly used in antibody or drug discovery experiments to test cells screening antibodies⁴. But other low dilution effects on enzymatic drug targets⁵ or bind to a second, co-encapsulated target cell^{6,7}. These methods can also be helpful in deciphering cellular biophysics for example by screening single cells with distinct cytosolic secretion levels⁸. In the field of directed evolution, droplet sorting has enabled the selection of enzyme variants with 10 to 1000-fold catalytic activity⁹ and of aptamers with customized properties for imaging cellular RNA^{10,11}. Other applications include microfluidic studies to detect and sort rare microfluidic populations^{12,13} and to discover new protein-protein interactions¹⁴. Overall, droplet microfluidics-based single-cell screening gains remarkable momentum in the life science domain.

¹ Institute of Biomechanics, School of Engineering, Ecole Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland; ² European Molecular Biology Laboratory (EMBL), Heidelberg, Germany; ³ These authors contributed equally: Jatin Panwar, Alexis Autour. *E-mail: christoph.merten@epfl.ch

DOI: 10.1038/nprot.2023.00796-2

ARTICLE IN PRESS



OPTICS

FIFTH EDITION

Eugene Hecht



EAN: 9781292096964

Hecht, Eugene, *Optics*, Global Edition, 5th edition, Harlow, Pearson, 2016

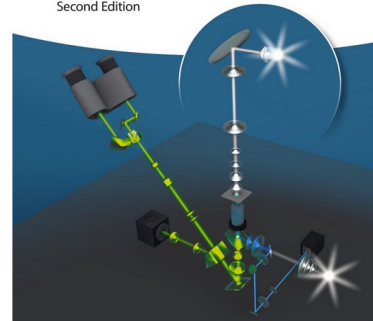


Edited by Ulrich Kubitscheck

Fluorescence Microscopy

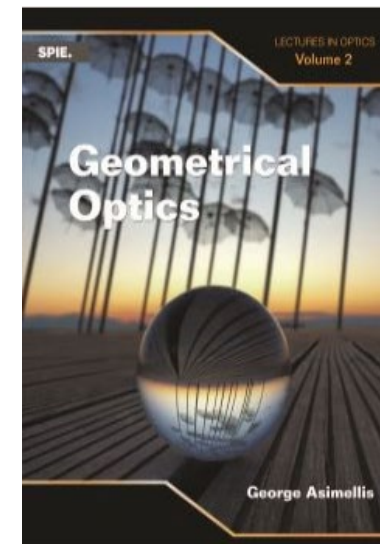
From Principles to Biological Applications

Second Edition



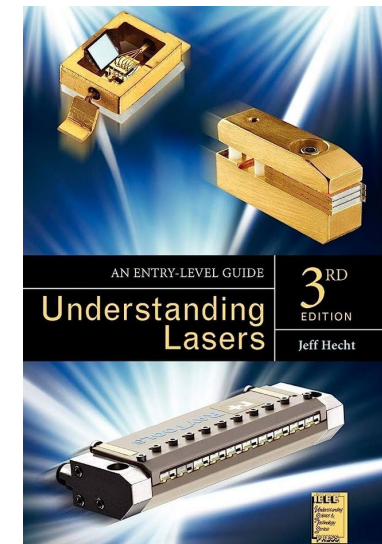
<https://doi.org/10.1002/9783527687732>

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



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

Asimellis, George, *Geometric Optics*, in: *Lectures in Optics Volume 2*, Bellingham, SPIE, 2020

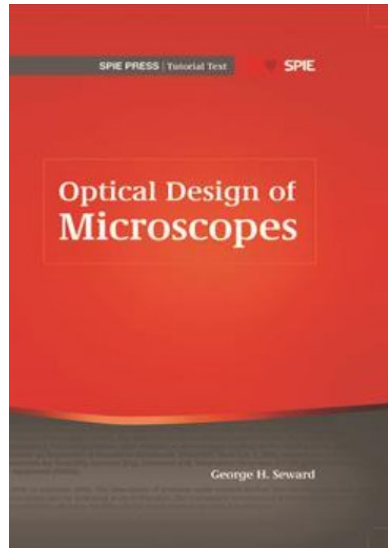


EAN: 9781118210048

Hecht, Jeff, *Understanding Lasers: An Entry-Level guide*, 3rd edition, Piscataway, IEEE, 2008

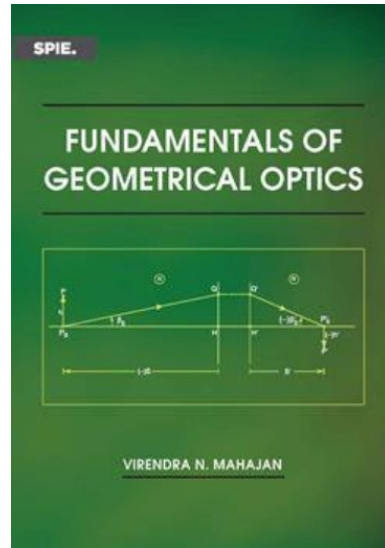
Panwar, J., Autour, A. & Merten, C.A. *Design and construction of a microfluidics workstation for high-throughput multi-wavelength fluorescence and transmittance activated droplet analysis and sorting*. Nat Protoc **18**, 1090–1136 (2023)

References and further literature



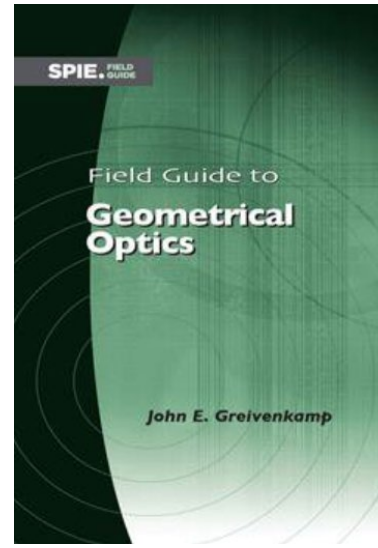
<https://doi.org/10.1117/3.855480>

Seward, George H.,
*Optical Design of
Microscopes*, Bellingham,
SPIE, 2010



<https://doi.org/10.1117/3.1002529>

Mahajan, Virendra N.,
*Fundamentals of
Geometrical Optics*,
Bellingham, SPIE, 2014



<https://doi.org/10.1117/3.547461>

Greivenkamp, John E.,
Geometrical Optics,
Bellingham, SPIE, 2003



Dragon Lasers, 2023, *FN Series 473 nm 100-1000mW*,
accessed 17 July 2023, '<https://www.dragonlasers.com/fn-series-473nm-diode-pumped-solid-state-laser-system-100mw-to-1000mw.html>'



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