Lecture #6

Introduction to optics

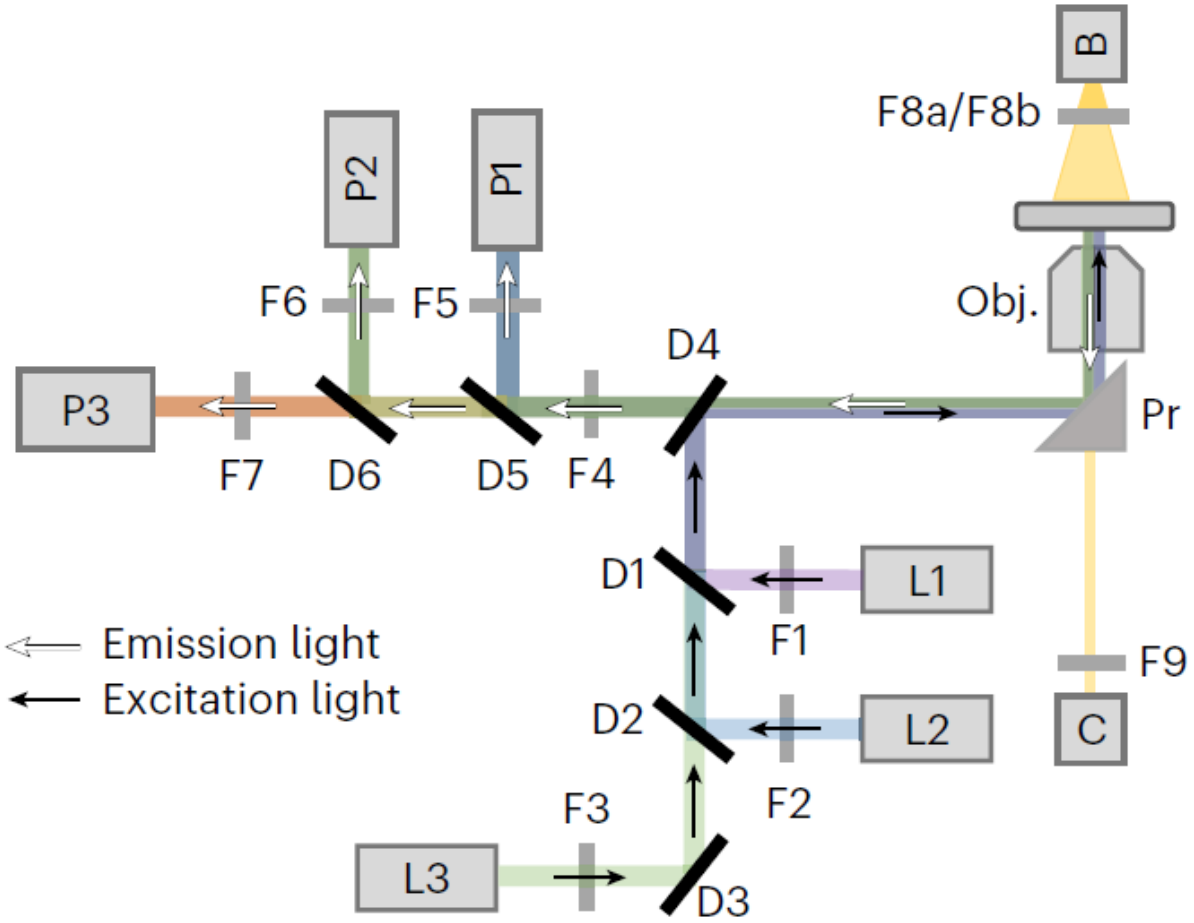
Aims:

- 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

Panwar, Autour & Merten,
Nat Protoc, 2023

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

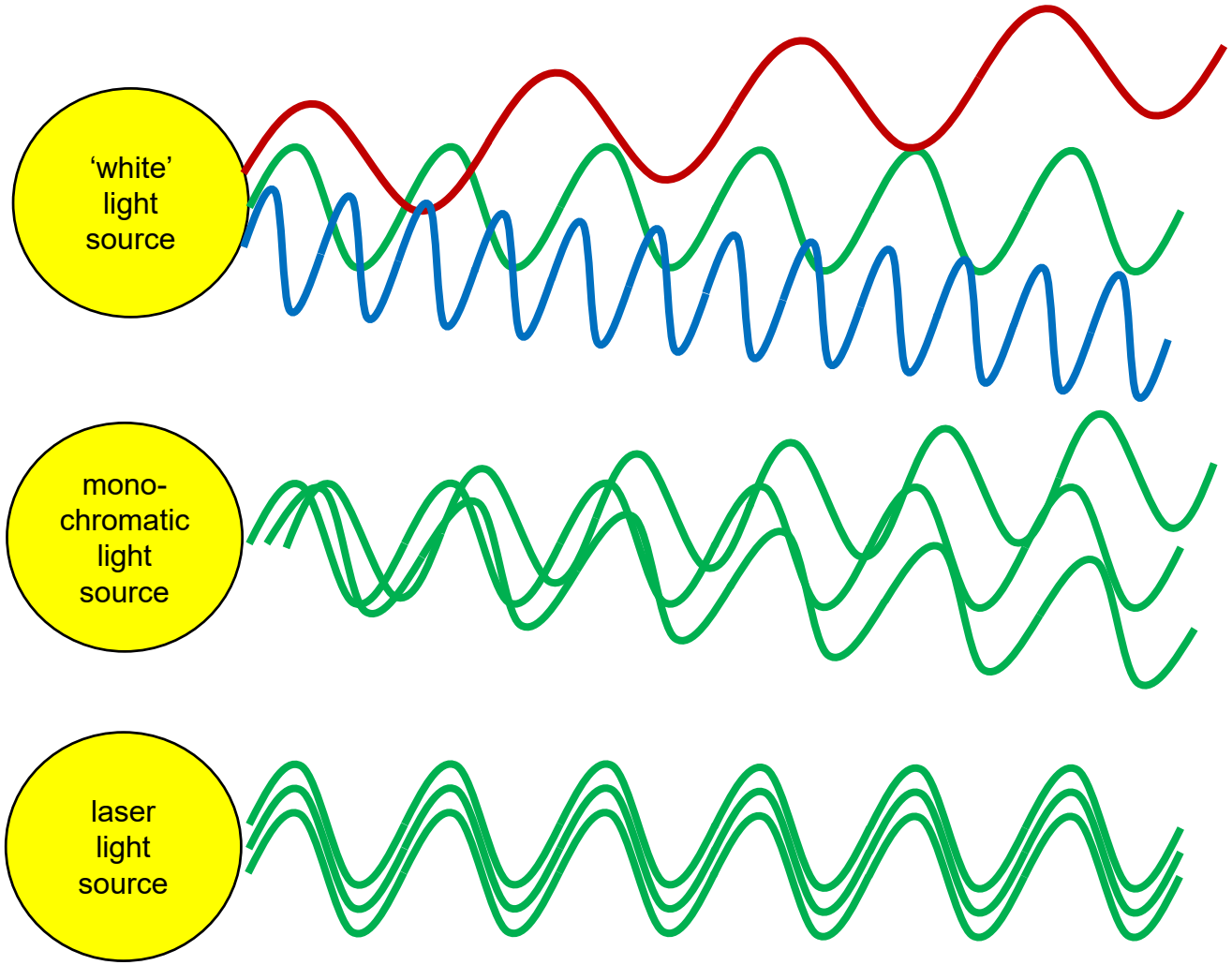
Filters

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

DRAGON LASERS

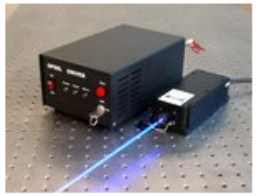
Sign in Register

Search items...

0 items

Home | UV Lasers | Blue Lasers | Green Lasers | Yellow Lasers | Red Lasers | IR Lasers | Contact us

› 420-500nm › FN Series 473nm Laser 100-1000mW



FN Series 473nm Laser 100-1000mW

★★★★★ — 4

$1800.00

Model Selection

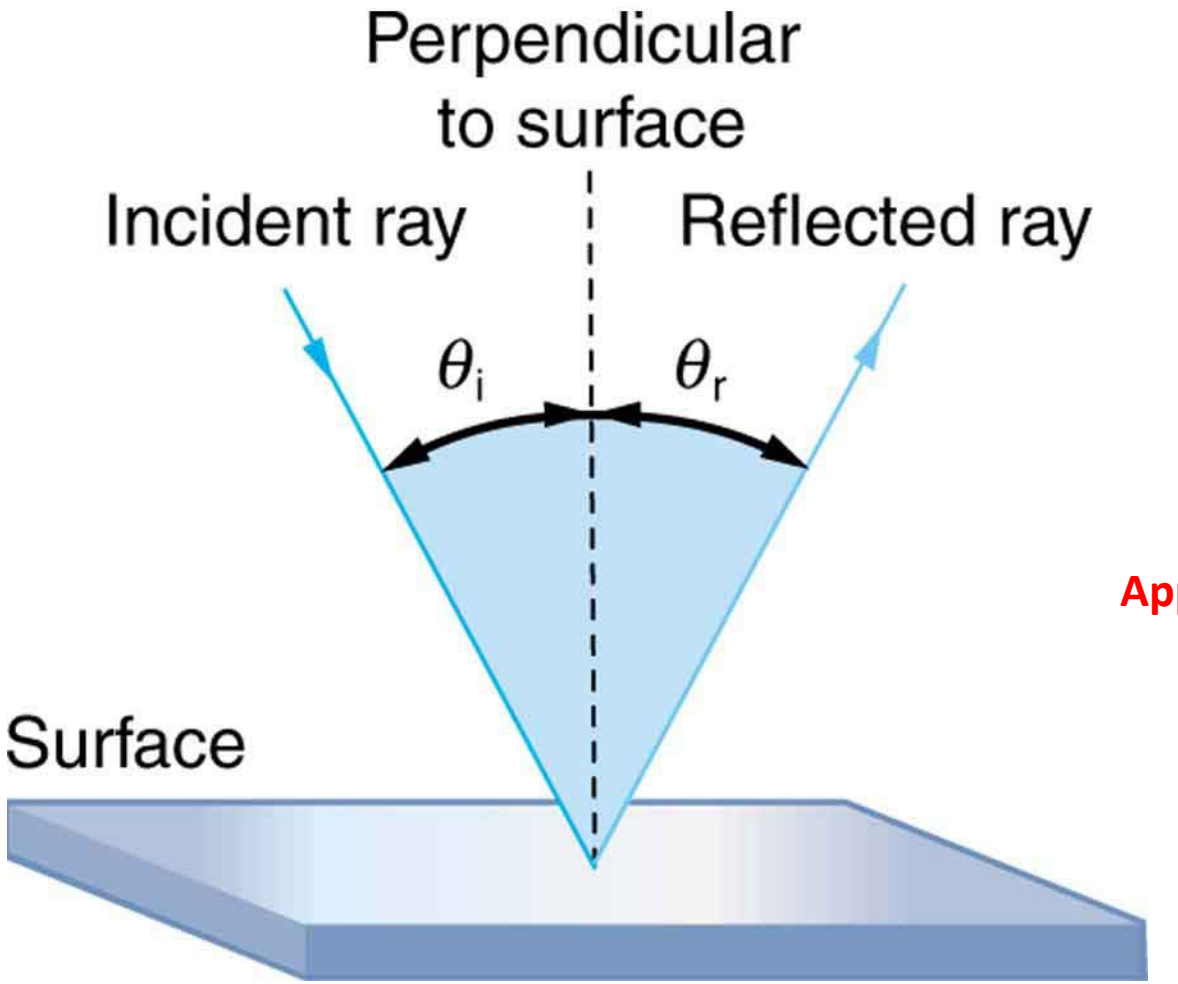
473nm 100mW

Qty: 1 Add to cart

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

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'

Law of reflection

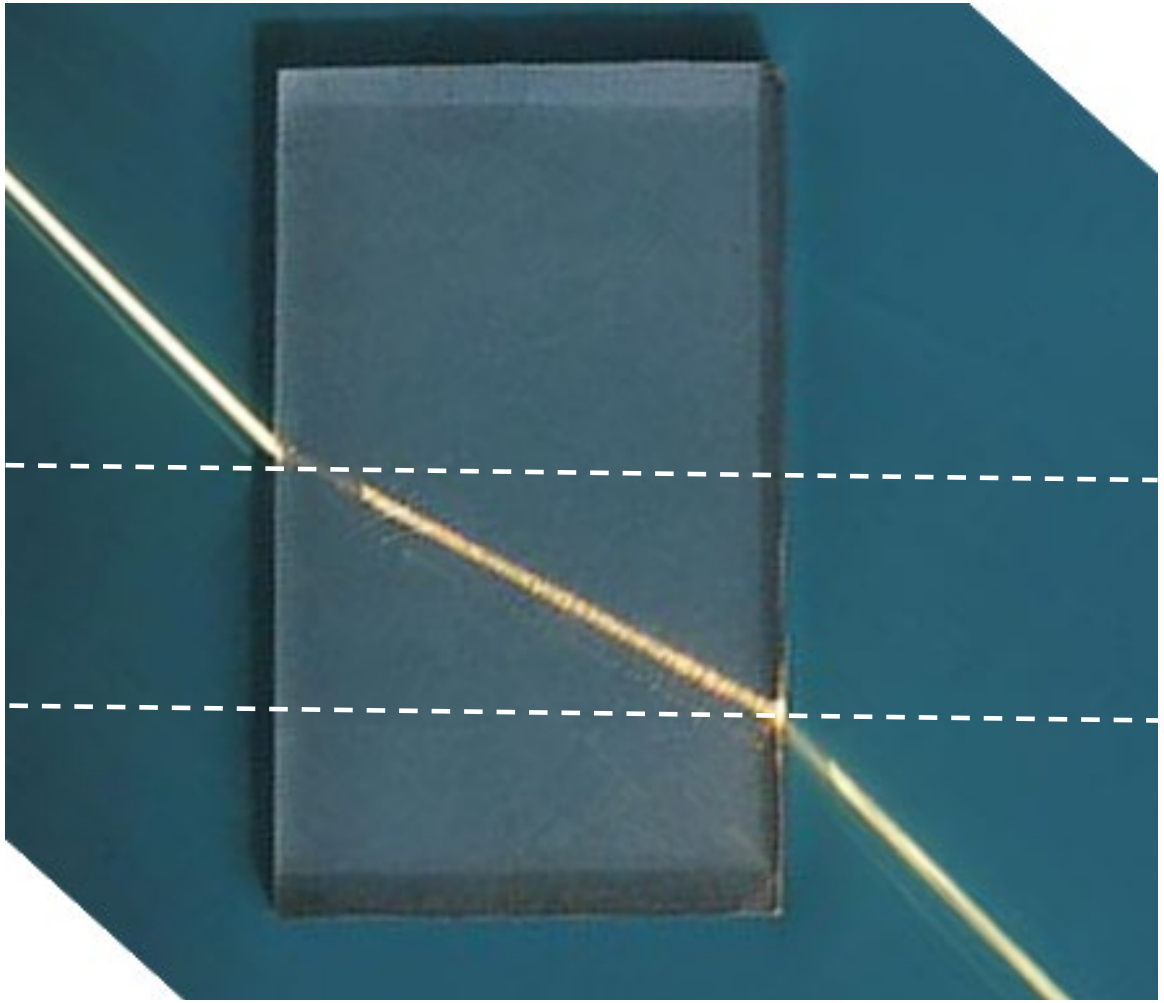


Application/ component: mirror

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

https://pressbooks.bccampus.ca: Douglas College Physics 1207

What is happening here?

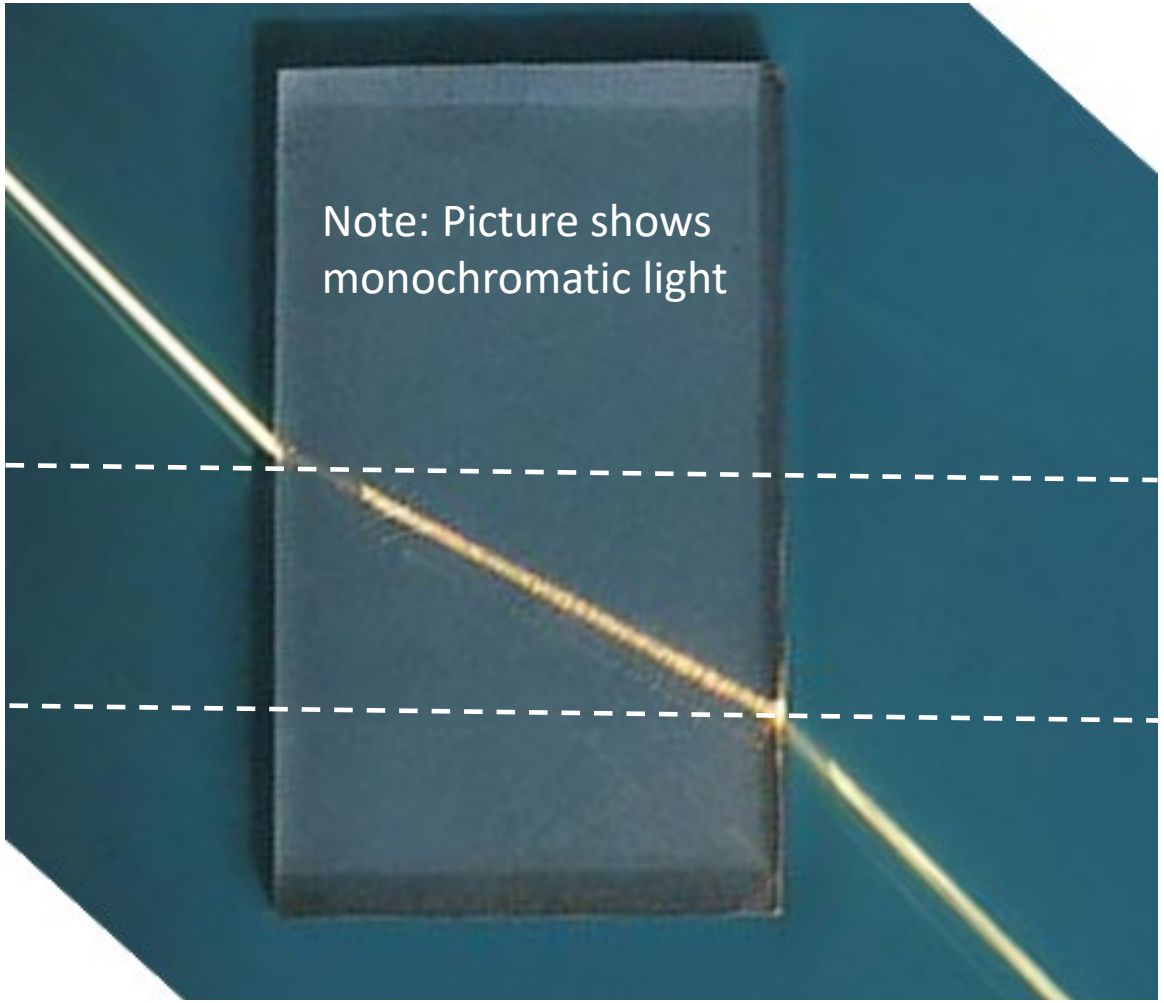


Picture taken from Wikipedia and modified

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.

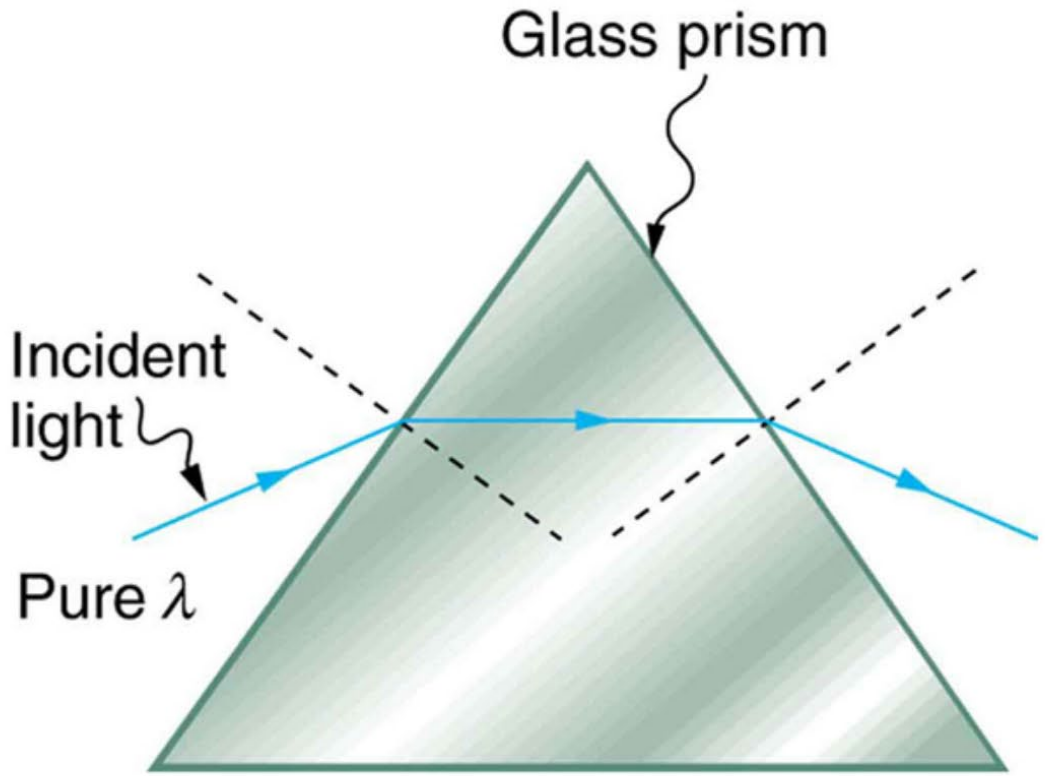


Picture taken from Wikipedia and modified

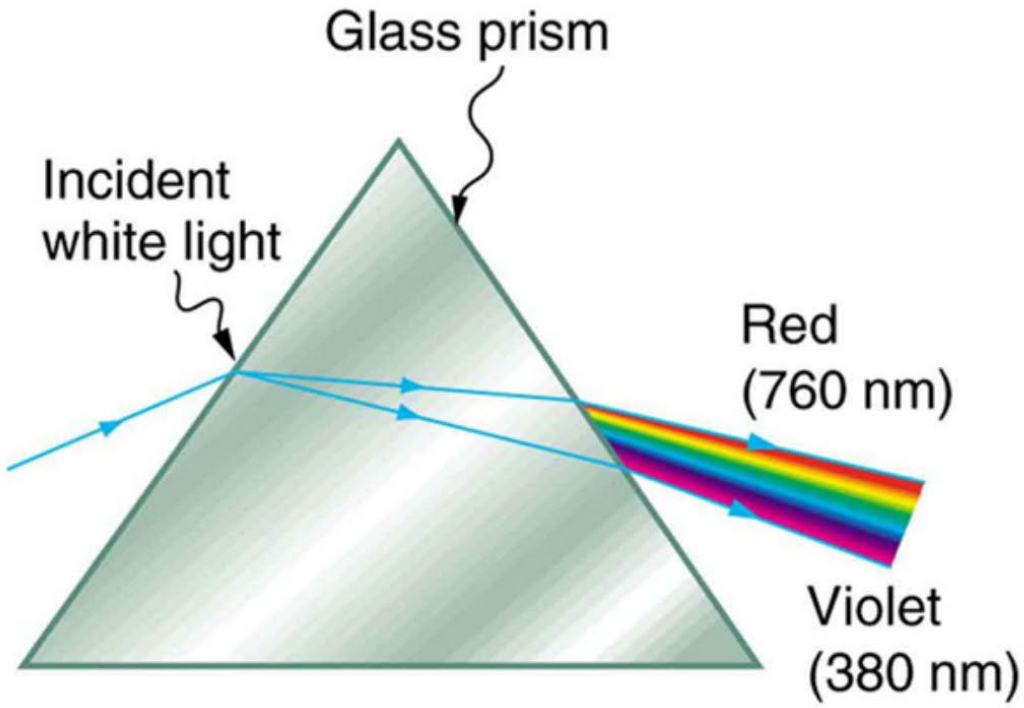
<u>Refractive indices (determined by density):</u>
Vacuum or air = 1
Water = 1.33
Glass = 1.5
Diamond = 2.42

Application/ component: prism & lens

What is happening with light of different wavelengths?

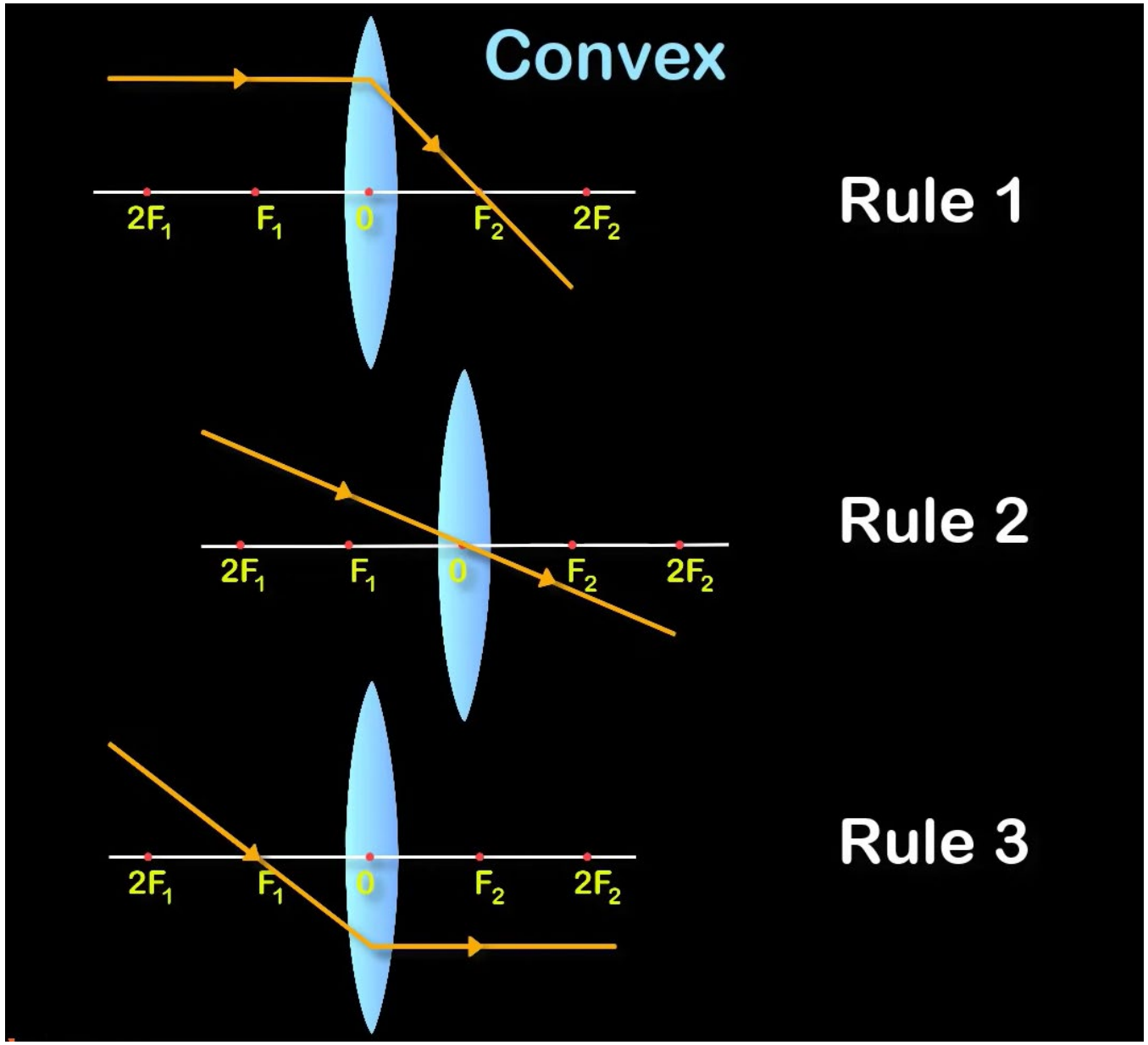


www.coursehero.com



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

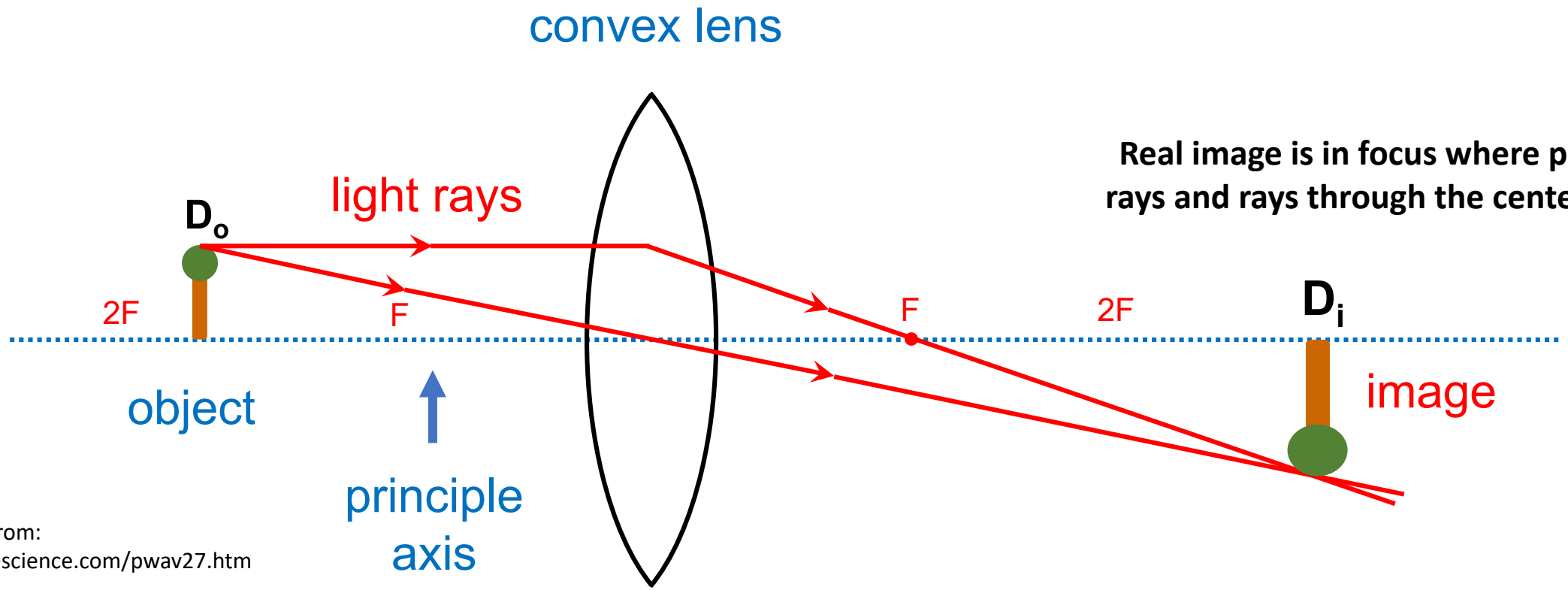
1. Parallel rays go through focus

2. Ray through center do not bend

3. Ray through focus goes parallel

www.youtube.com/watch?v=CJ6aB5ULqa0 (Manocha Academy)

Lenses – focus and magnification



Real image is in focus where parallel rays and rays through the center meet

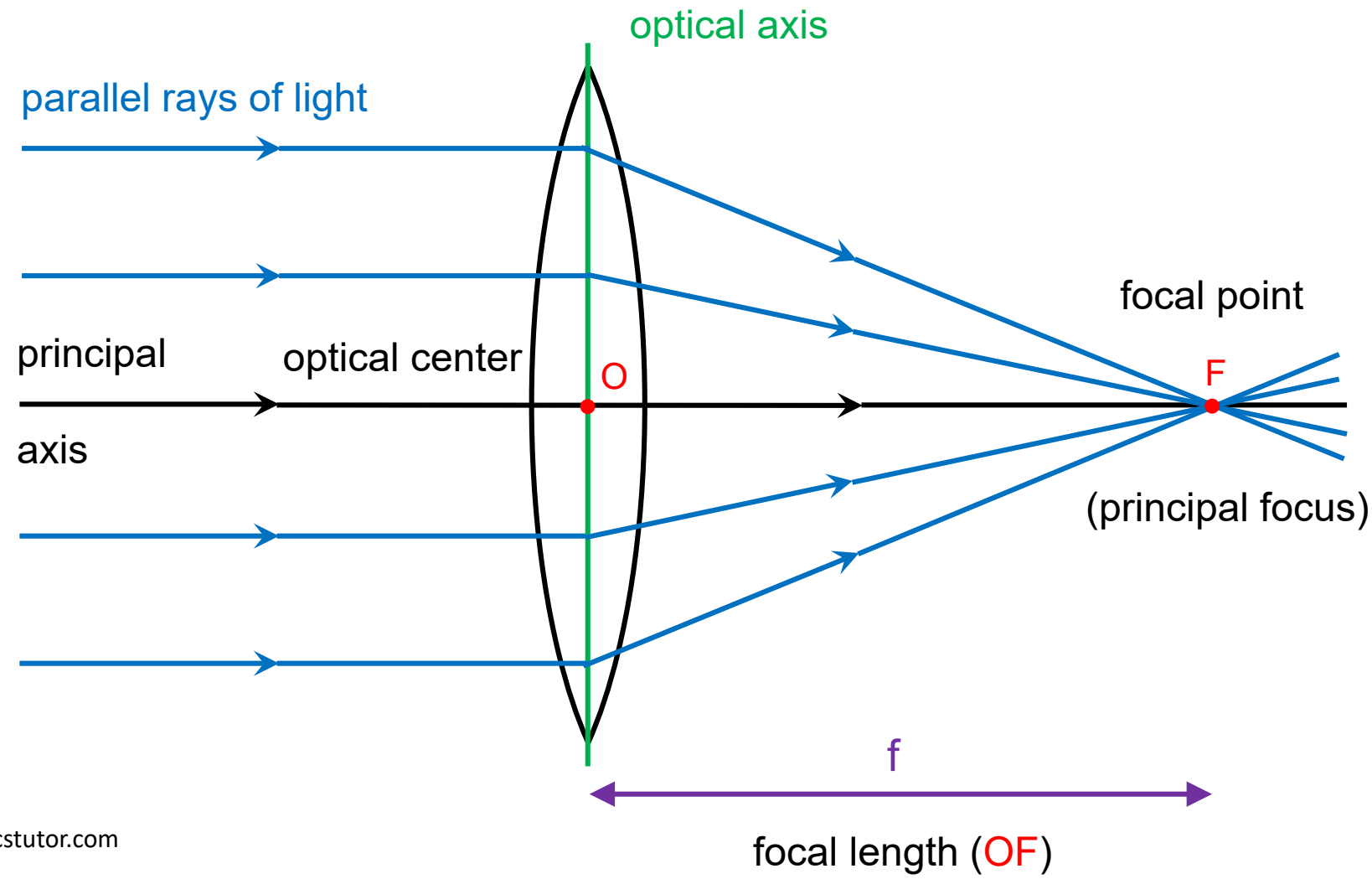
adapted from:
www.gcsescience.com/pwav27.htm

- 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: $\frac{1}{Di} + \frac{1}{Do} = \frac{1}{F}$

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

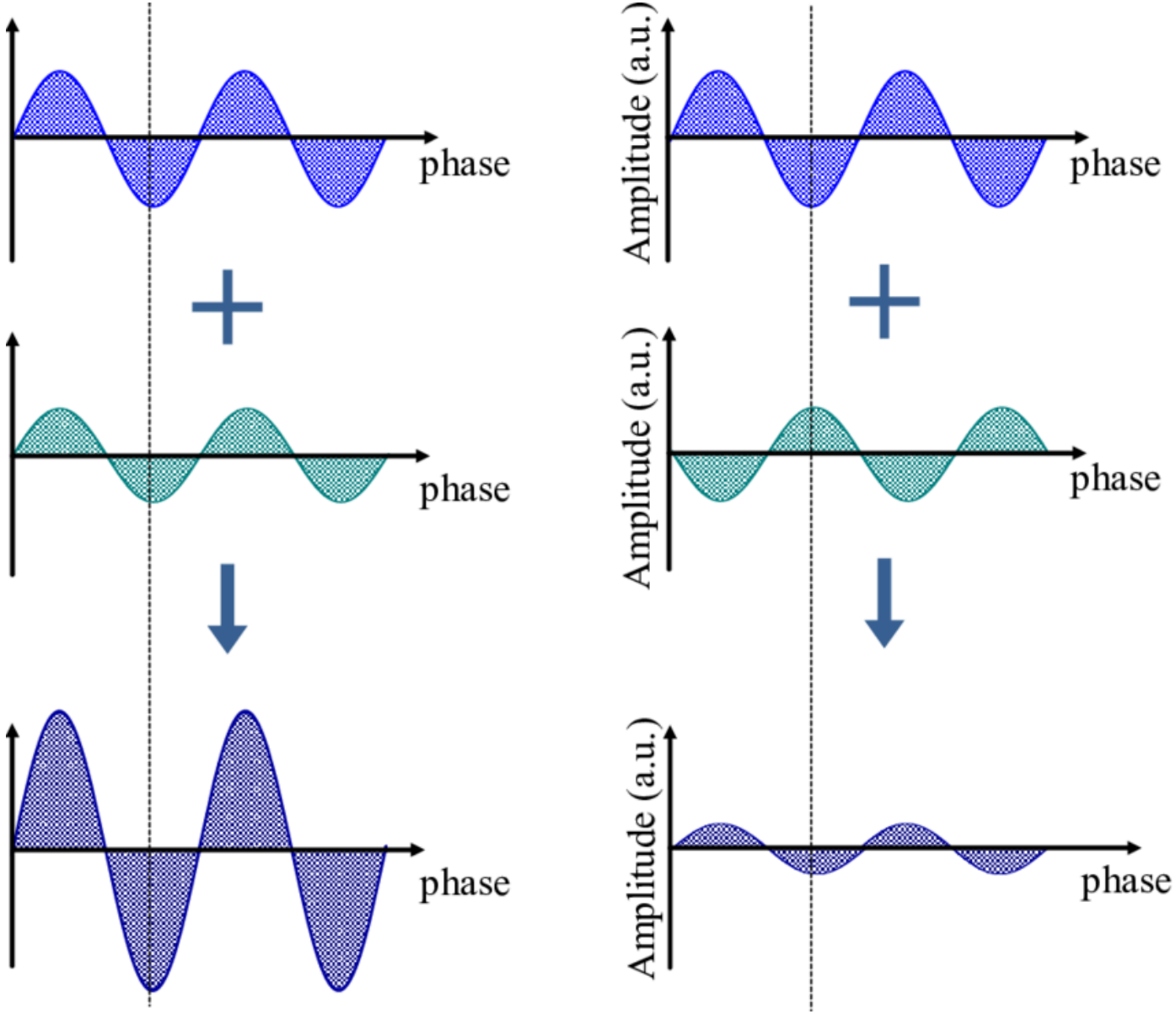
Lenses – focus and magnification



adapted from:
www.a-levelphysicstutor.com

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

Constructive and destructive interference

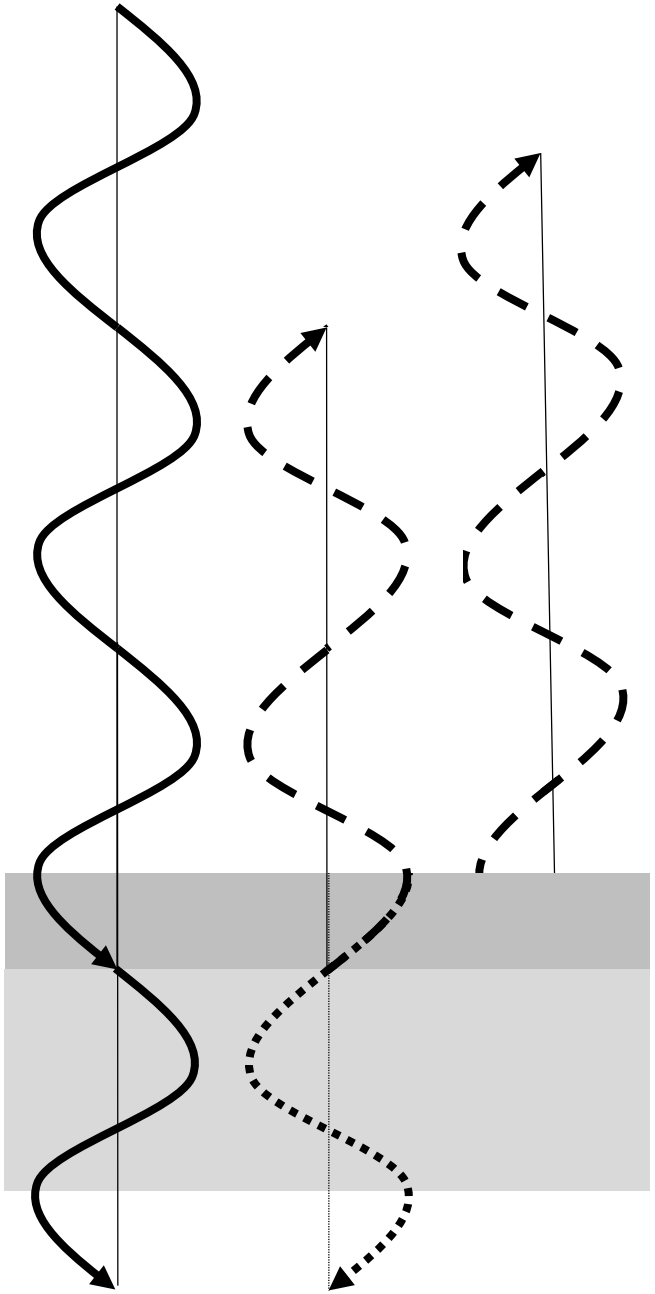


Application/ component: filters & dichroic mirrors

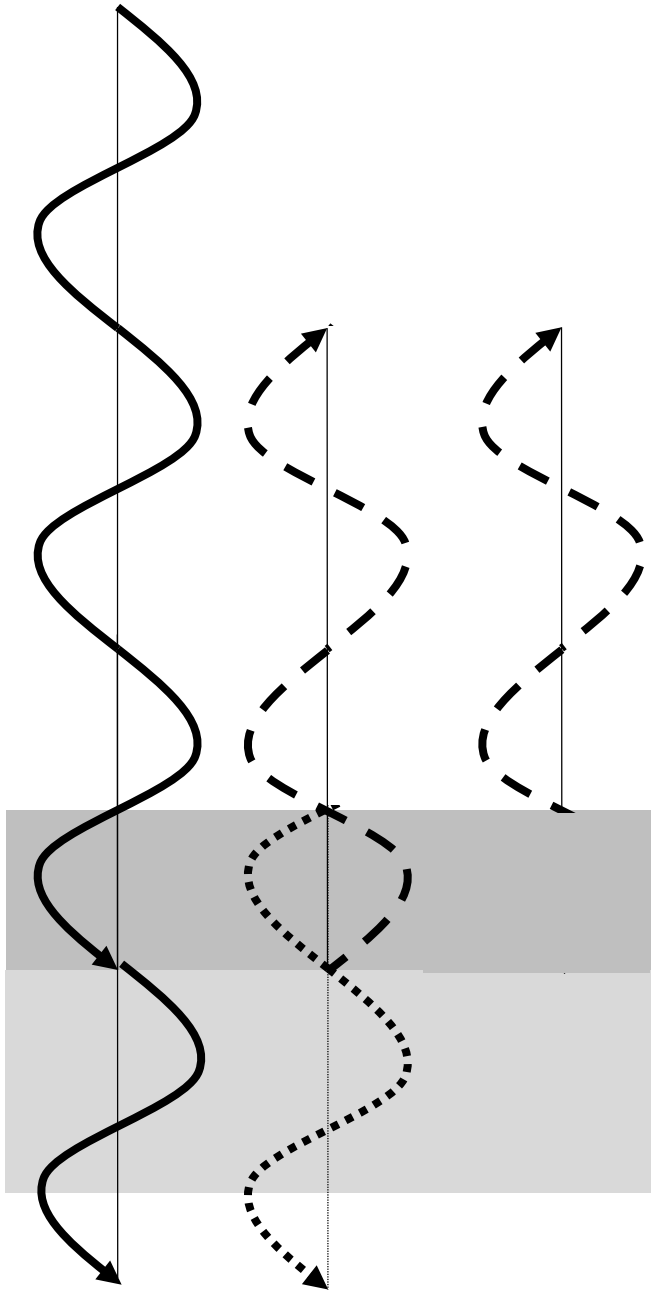
A combined theoretical and experimental analysis on performance and functionality of printed dielectric mirrors, Shuai Gao, PhD Thesis

Working principle of interference filters

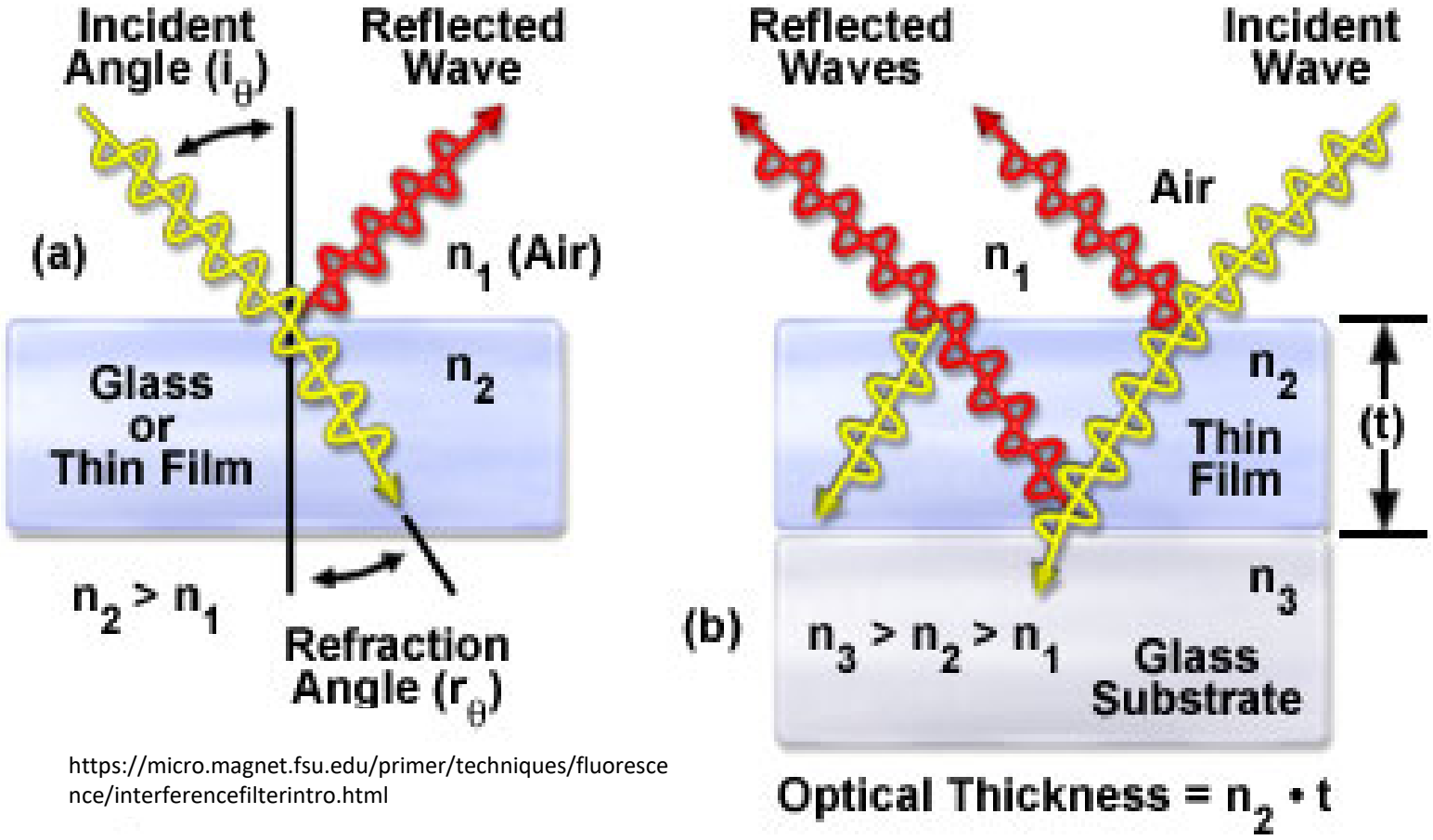
Destructive interference, layer thickness = λ /4, phase shift = 180 °



Constructive interference, layer thickness = λ /2, phase shift = 360 °



Working principle of dichroic mirrors



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

Working principle of dichroic mirrors

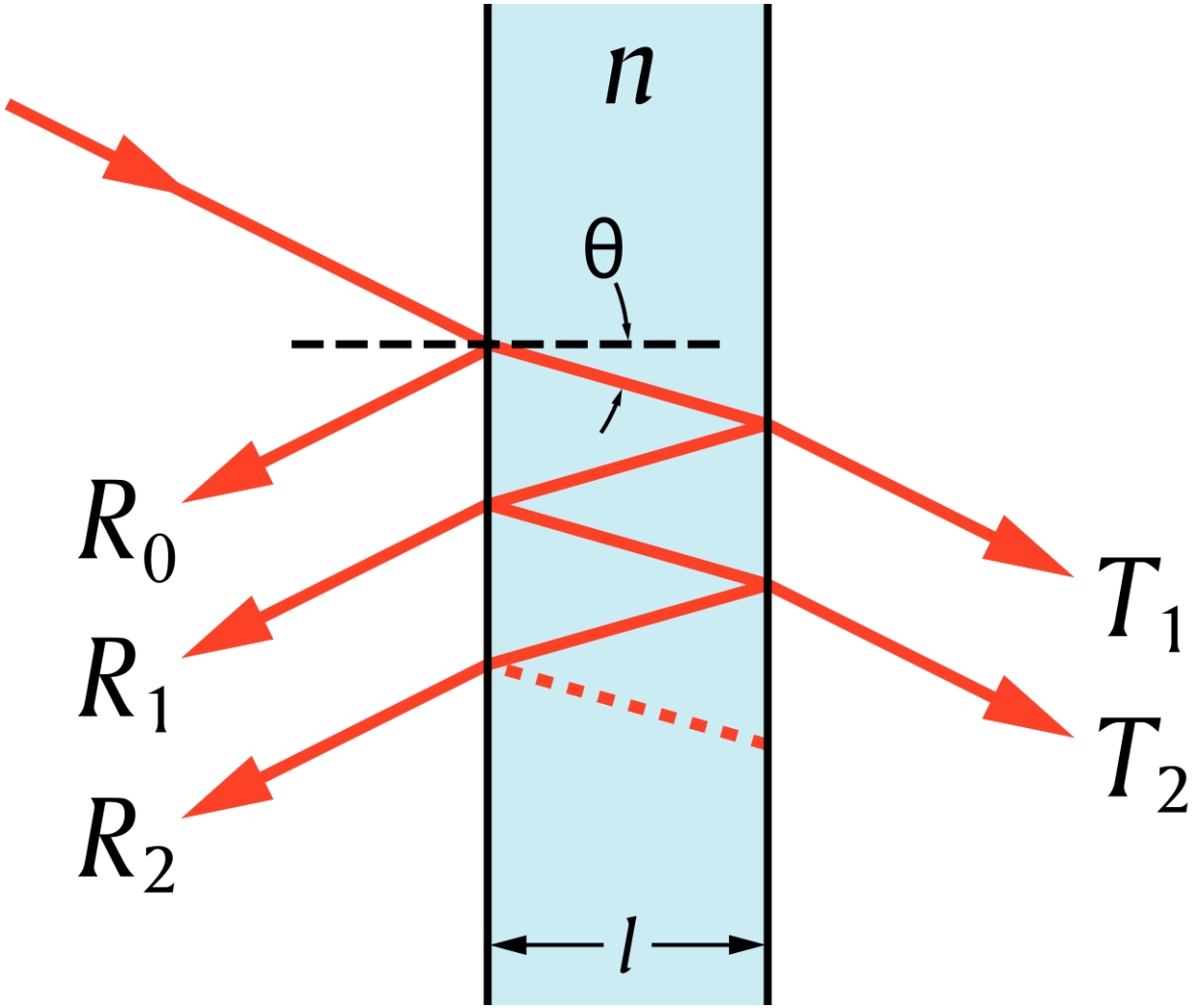
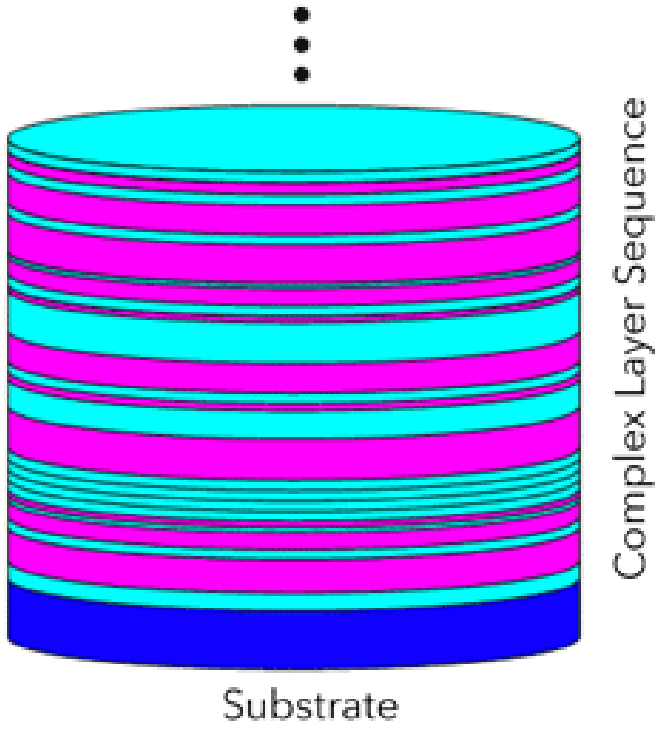
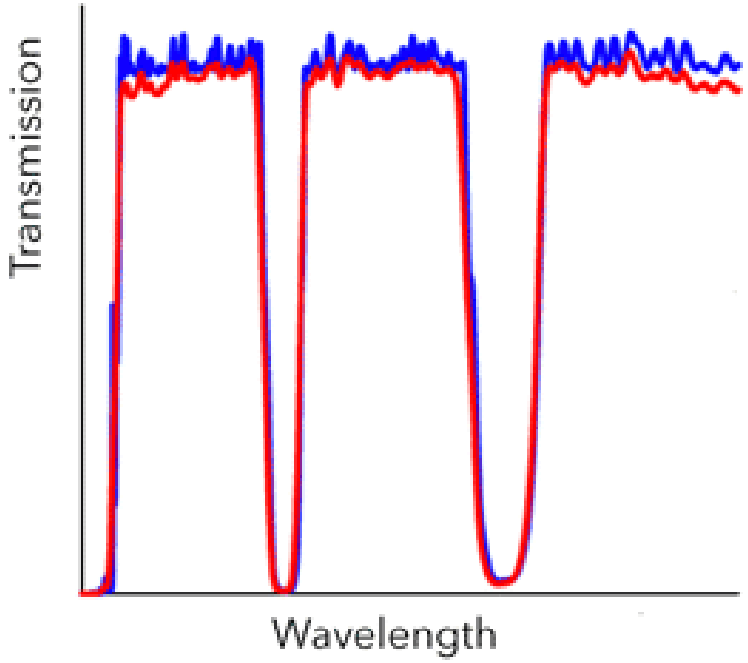


Image from wikipedia

In reality it is always more than one thin layer!

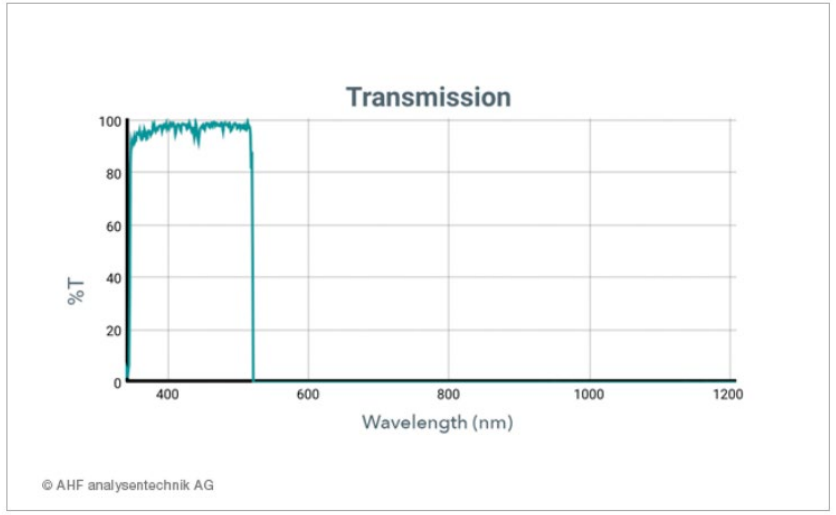




Filters used in the practical course

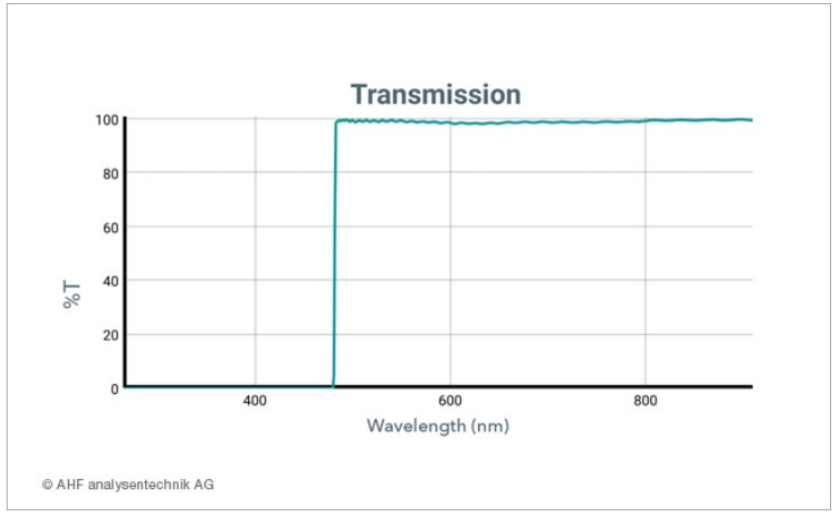
AHF Analysentechnik AG, 2023, *Optical Filters for highest performance*, accessed 14 July 2023, 'https://www.ahf.de/en/products/spectral-analysis-photonic/optical-filters/'

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



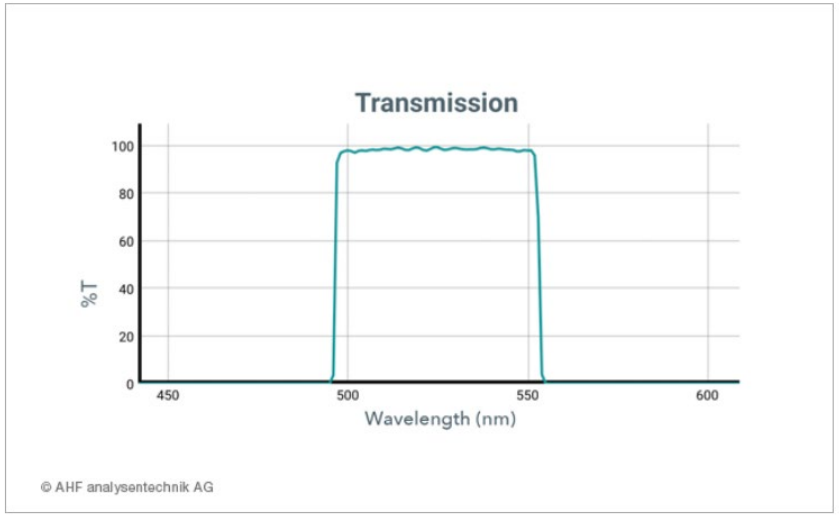
short pass filters

532 nm



long pass filters

473 nm

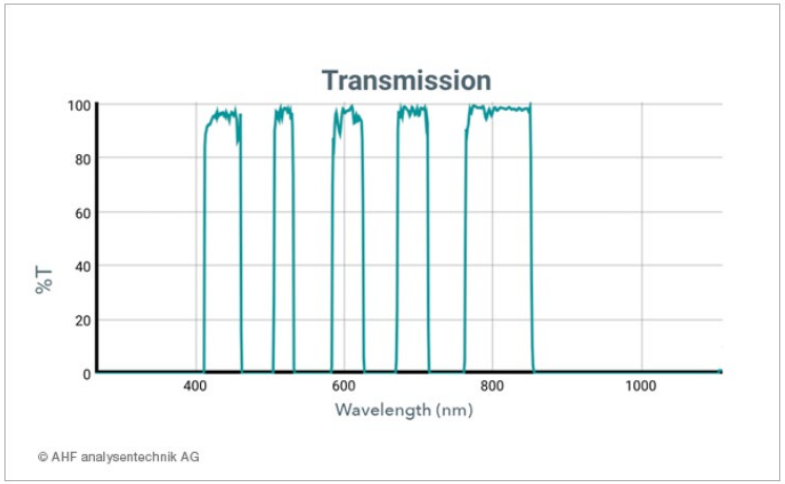


bandpass filter

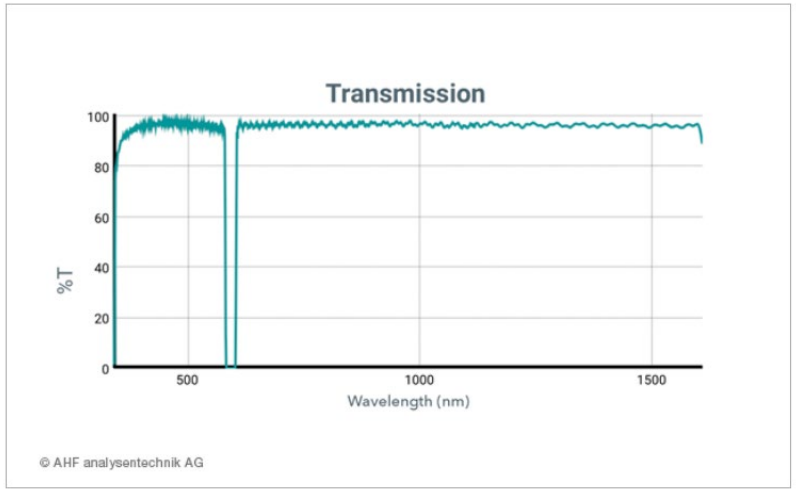
525/50 nm

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/'

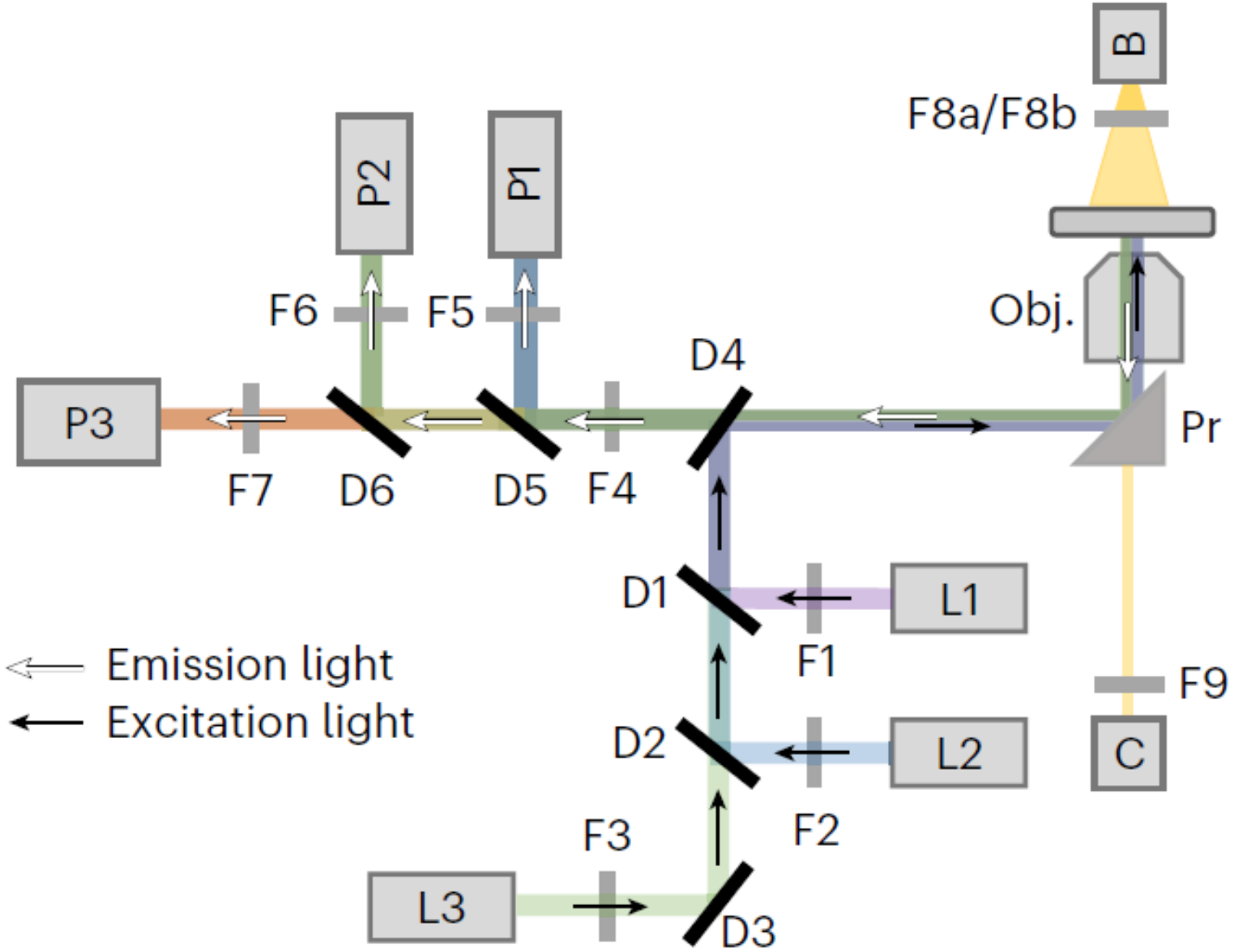


multiband filter



notch filter

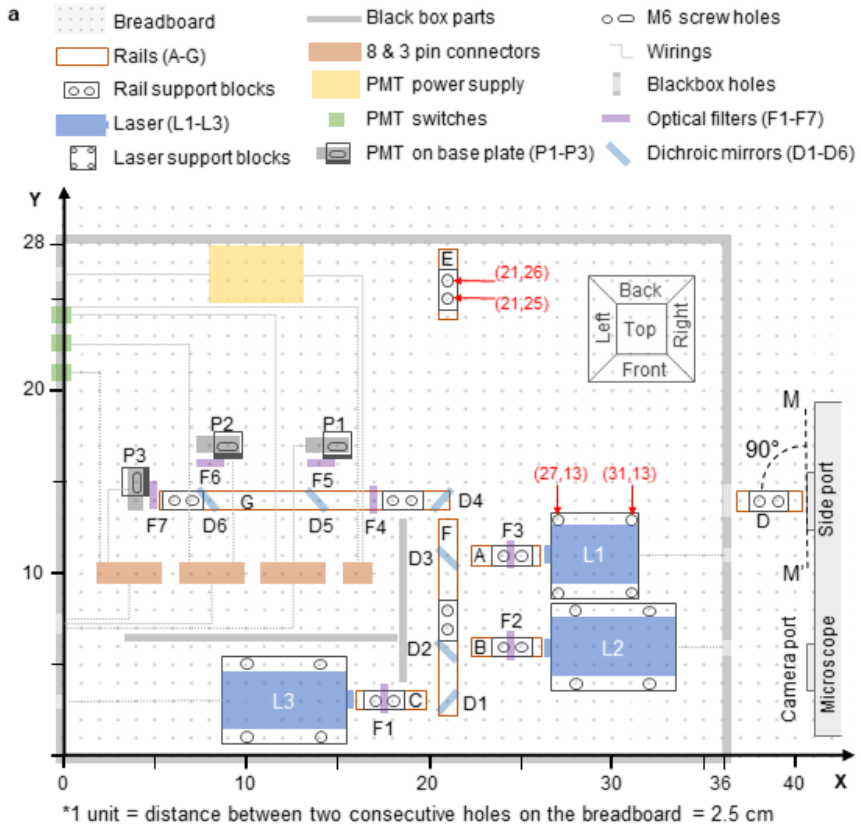
A series of dichroics can be used to feed in multiple lasers or to split polychromatic light into its components

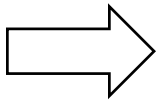


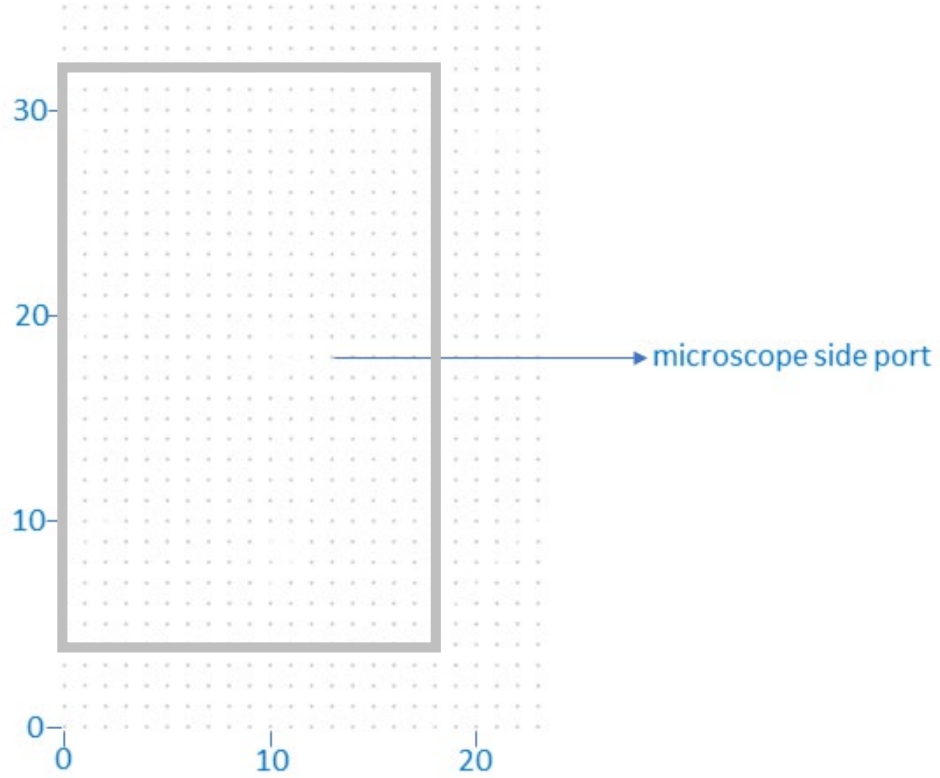
Blueprint of your instrument

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



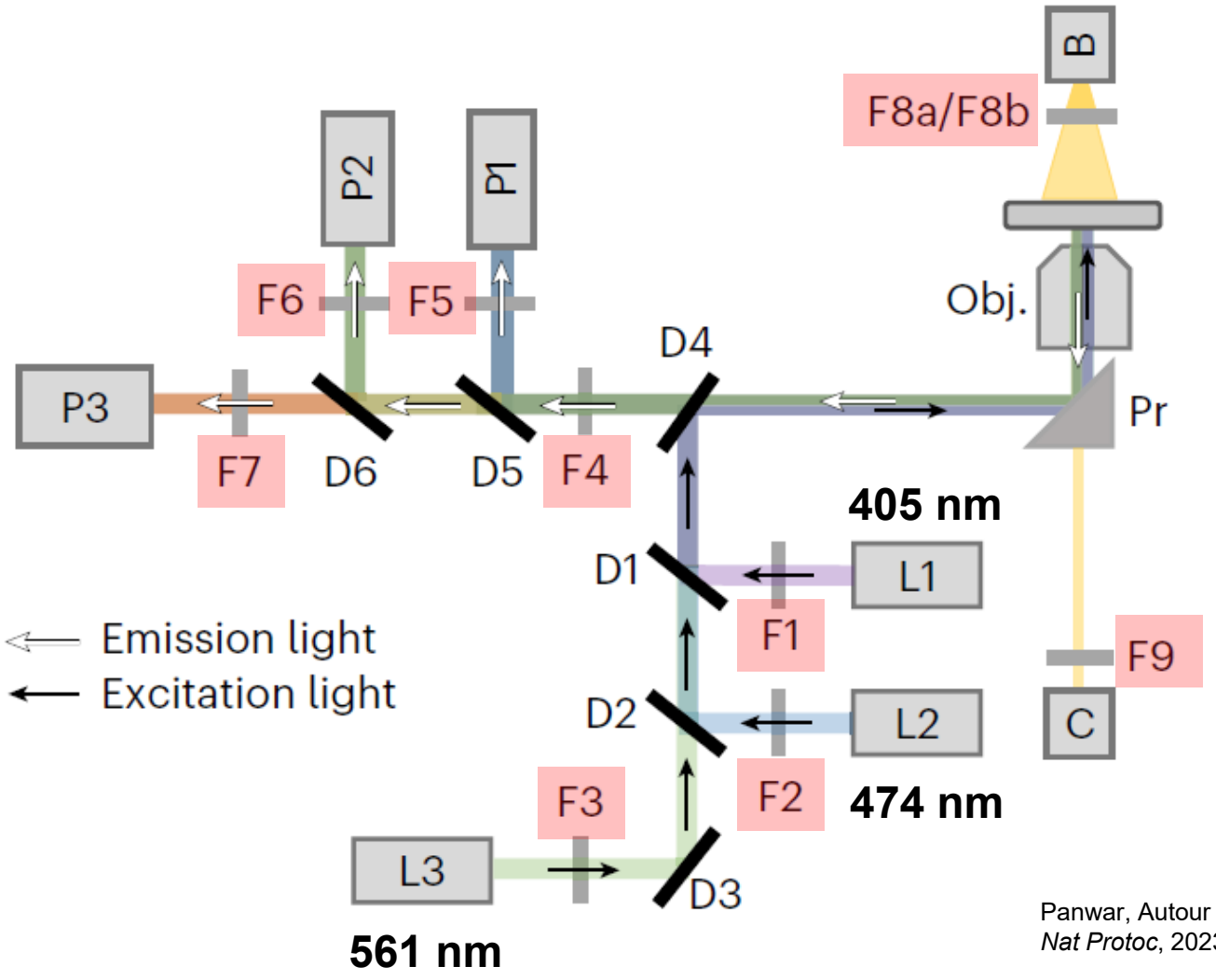




3 channel fluorescence detection

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

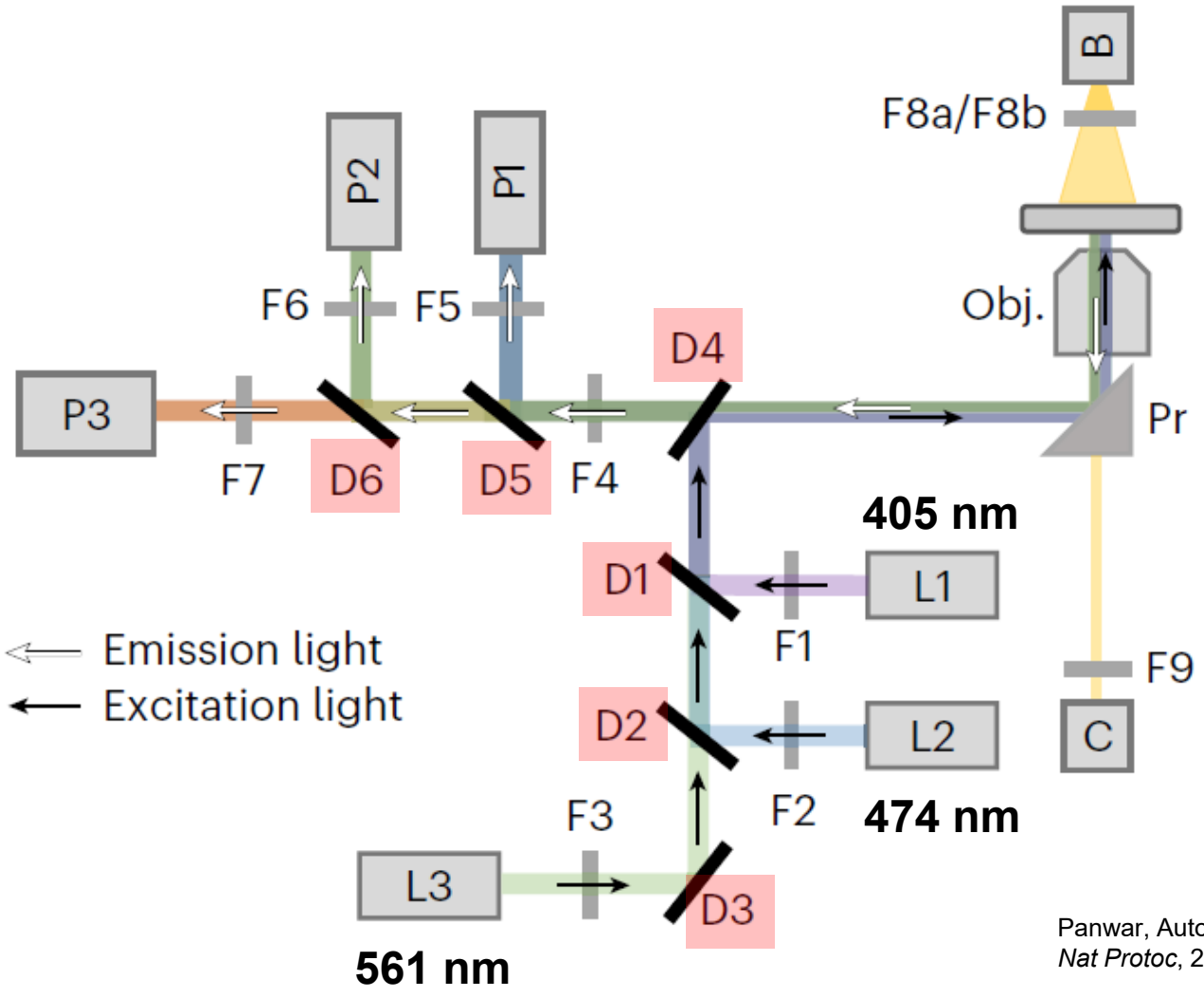
F5…? 445/45 nm
F6…? 525/45 nm
F7…? 605/50 nm
bandpasses

F8a…? 561 nm longpass
F8b…? 633 nm longpass

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

AHF ANALYSENTECHNIK

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

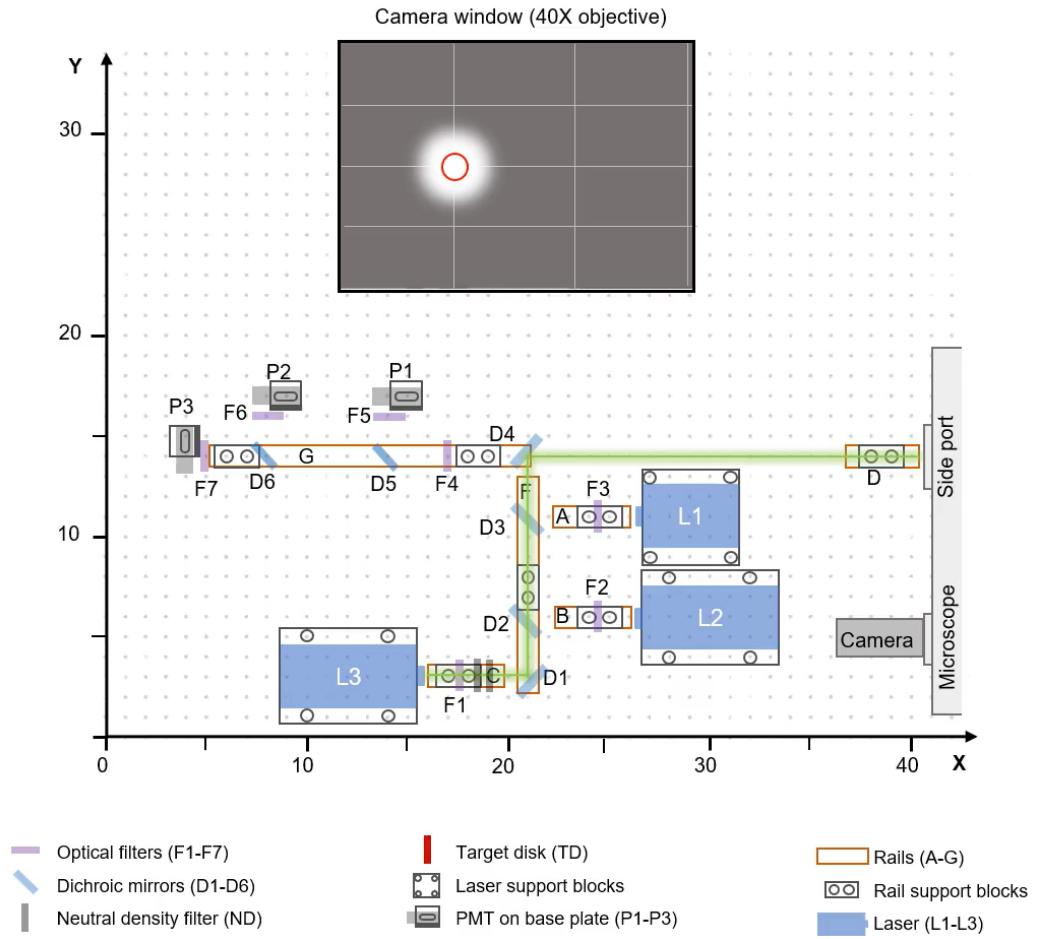
HELP: https://www.ahf.de/en/products/spectral-analysis-photonic/optical-filters/

AHF ANALYSENTECHNIK

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

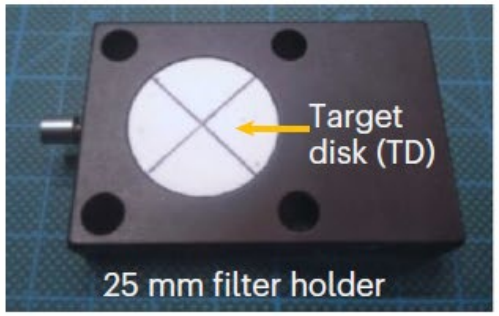


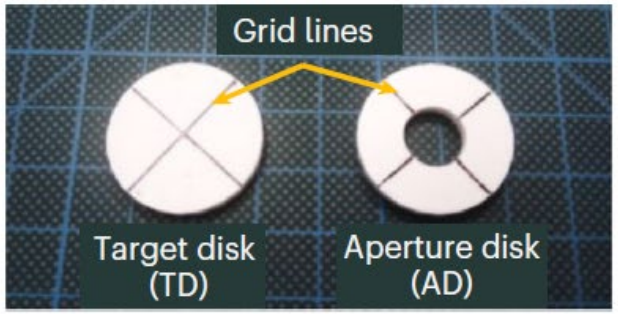
Panwar, Autour & Merten,
Nat Protoc, 2023

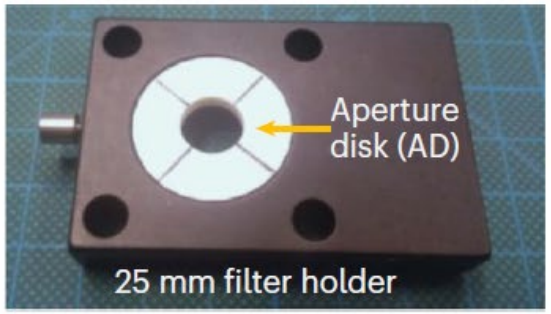
Alignment tools

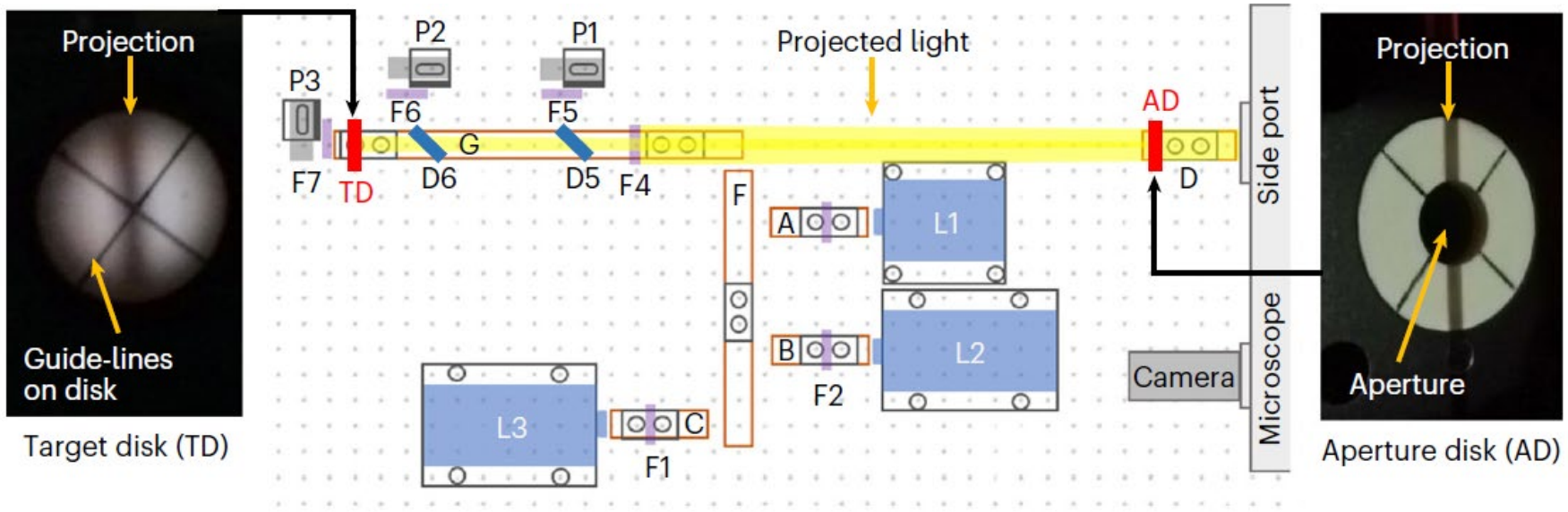
1.) emission light alignment

reduced diameter of beam





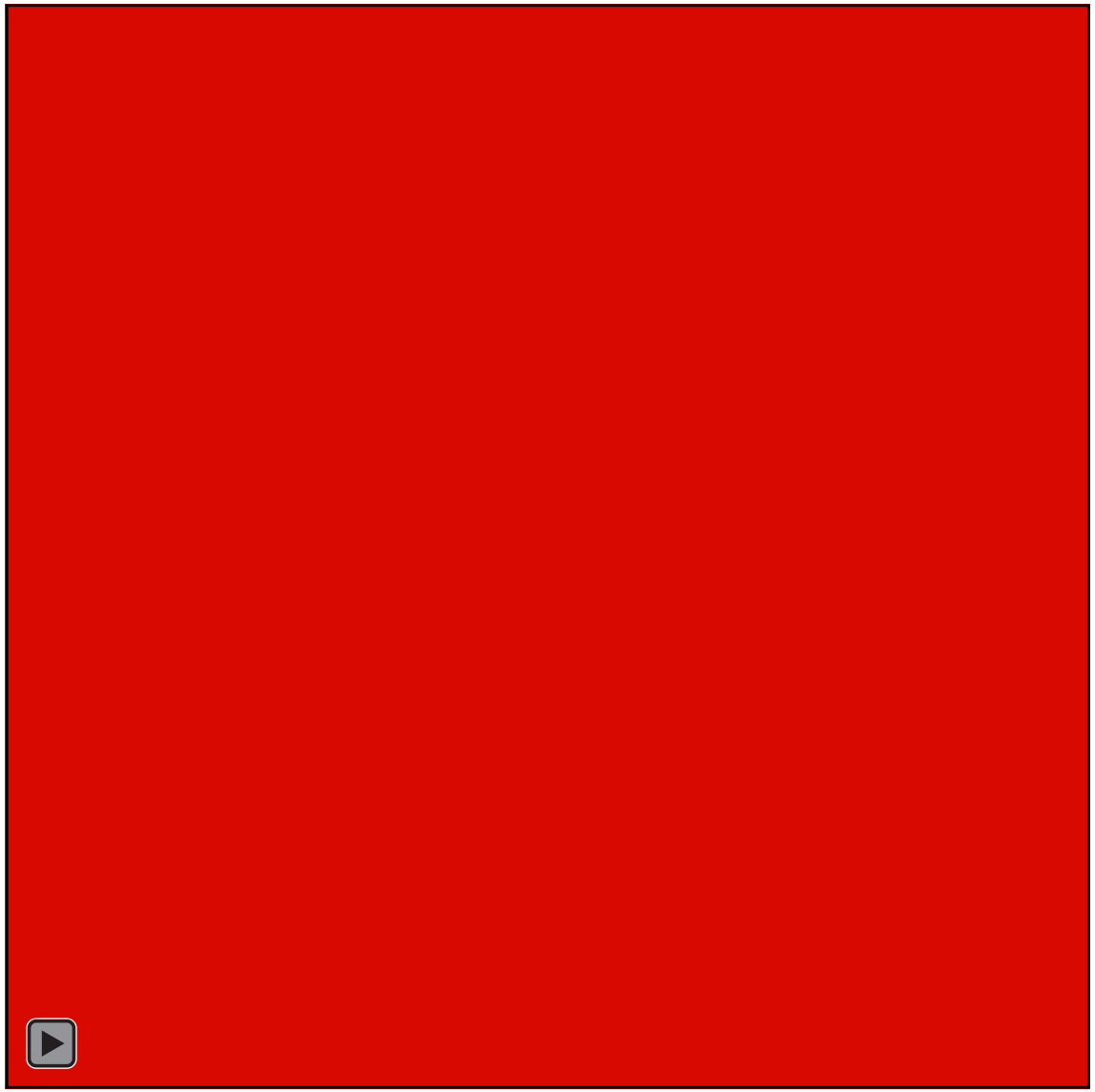




Panwar, Autour & Merten,
Nat Protoc, 2023

! microscope is fixed now !

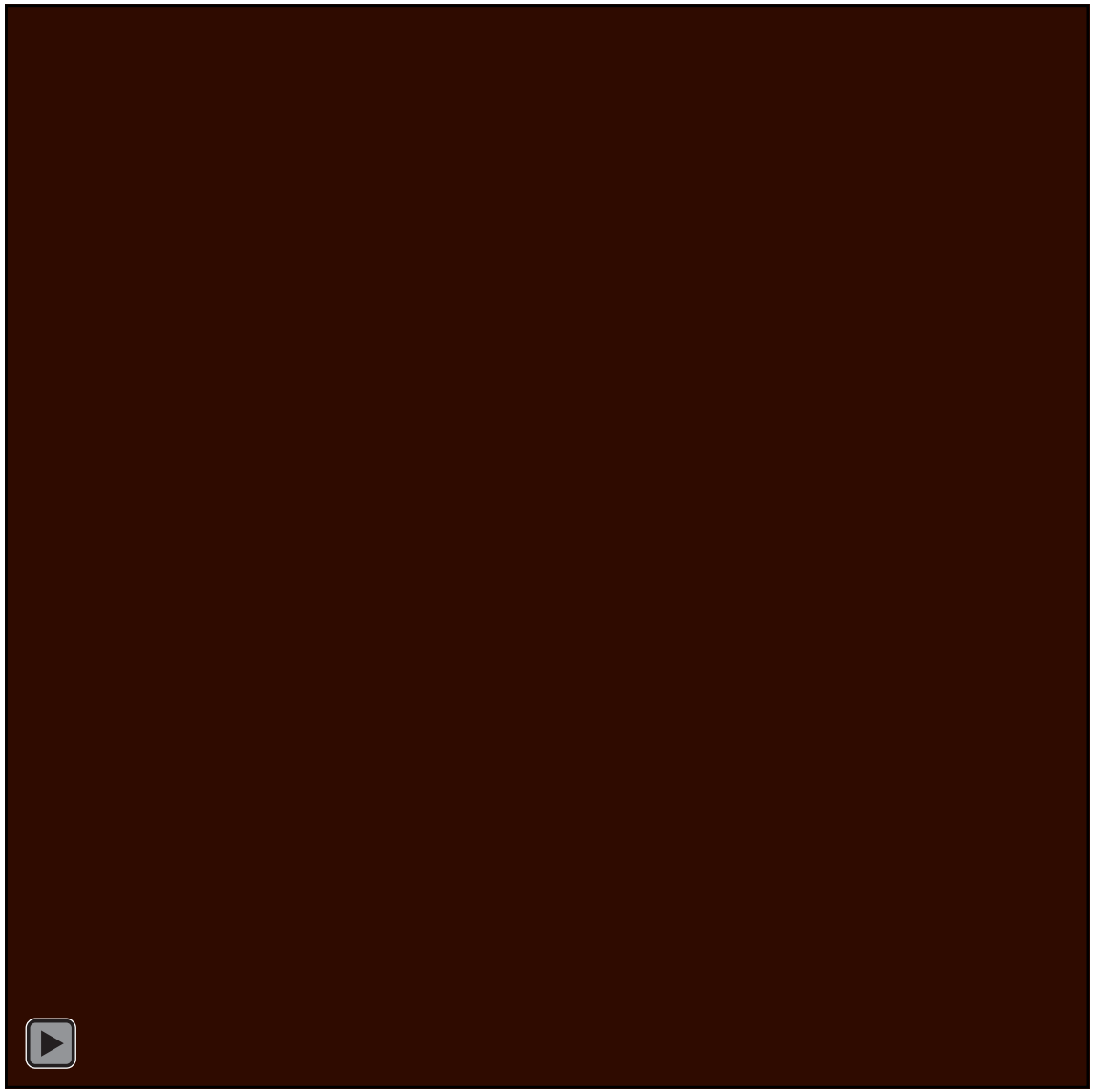
Excitation light alignment (Panwar et al., 2023)



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

Most relevant: 55’’ and onwards

Emission light alignment (Panwar et al., 2023)

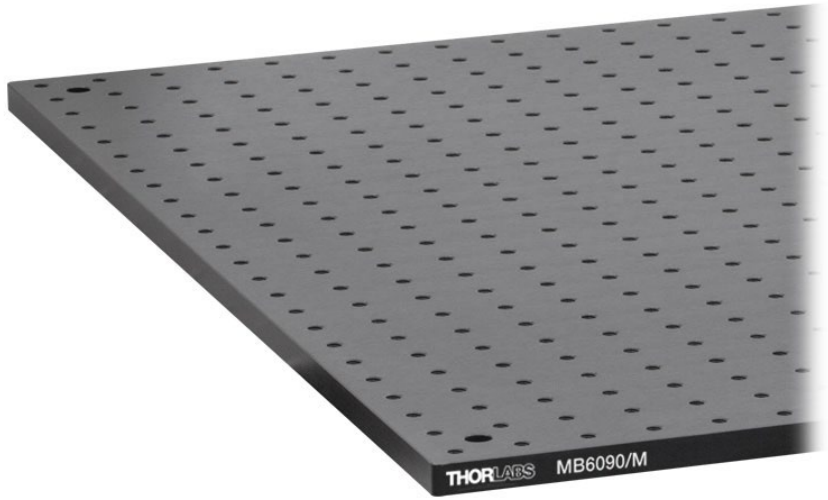


VIDEOS:
https://www.nature.com/articles/s41596-022-00796-2#Sec75

Most relevant: 2’40’’ and onwards

Your course inventory

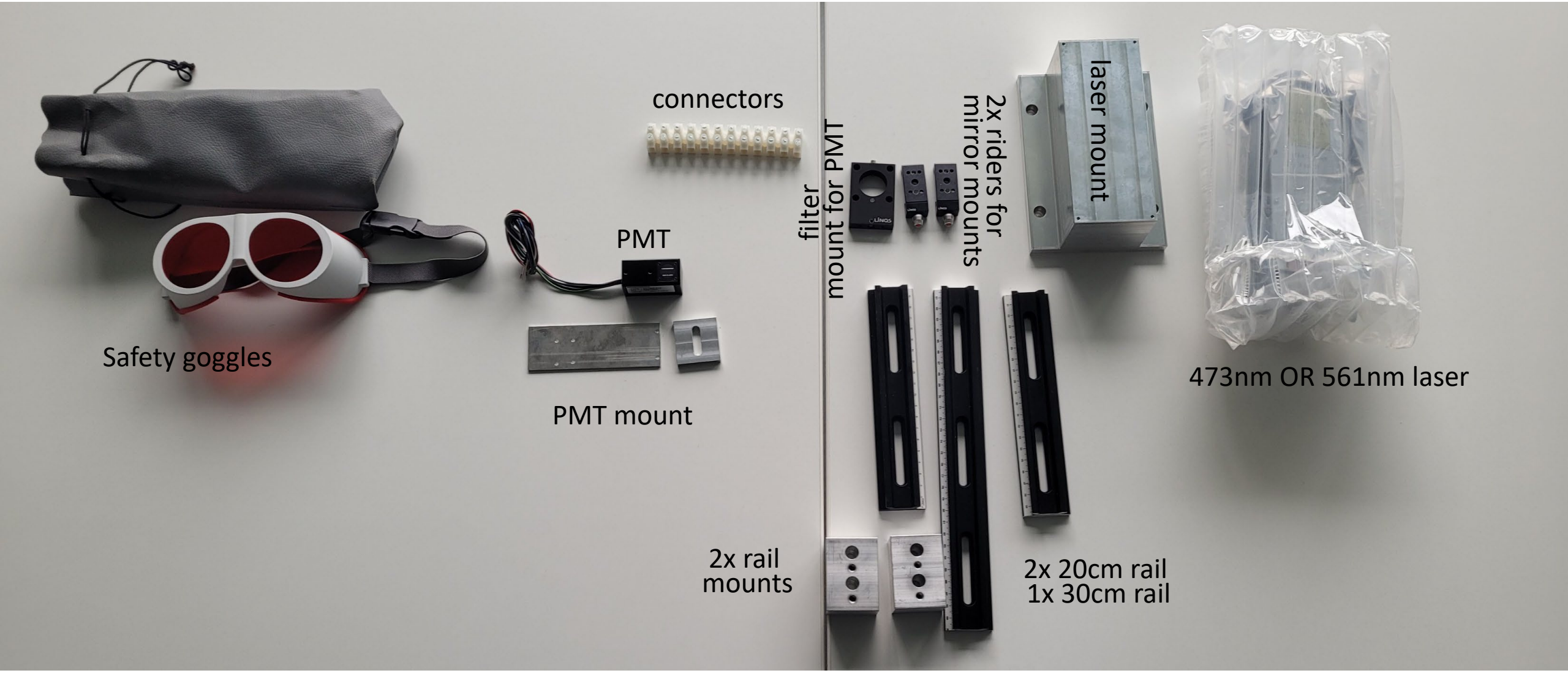
assemble on breadboard



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)

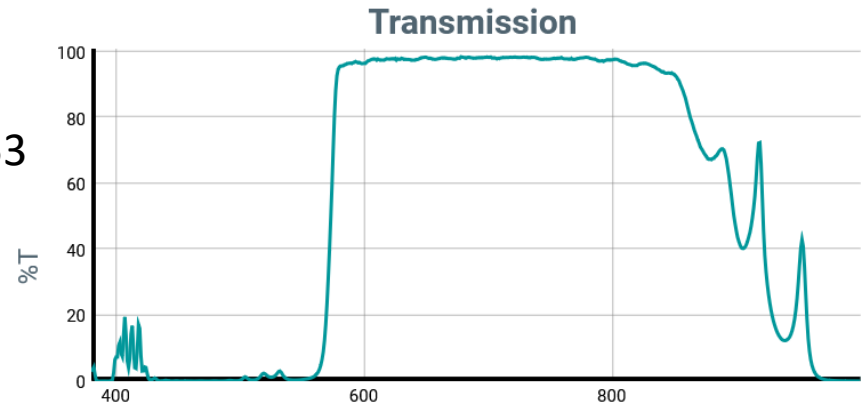
Your course inventory



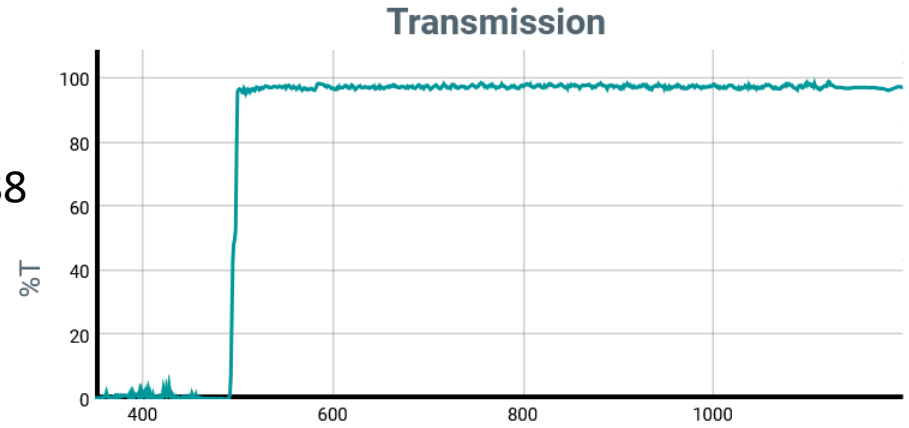
+ filters & mirrors (details on next slide)
+ screws, wires, crimps & alignment tools

Mirrors (already mounted)

Dichroic F48-553



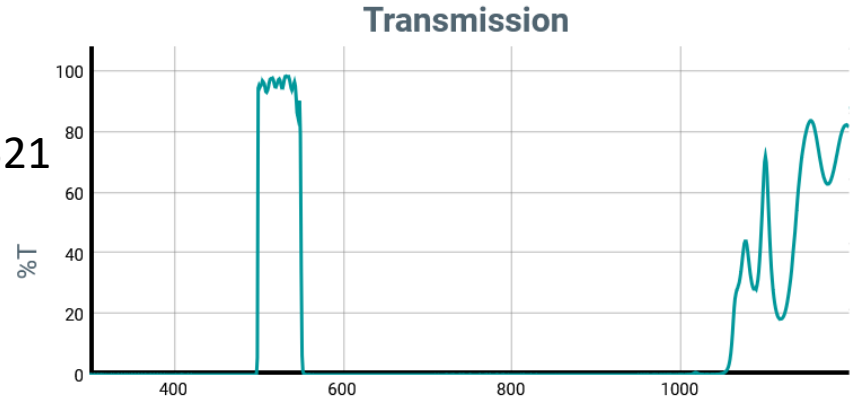
Dichroic F38-488



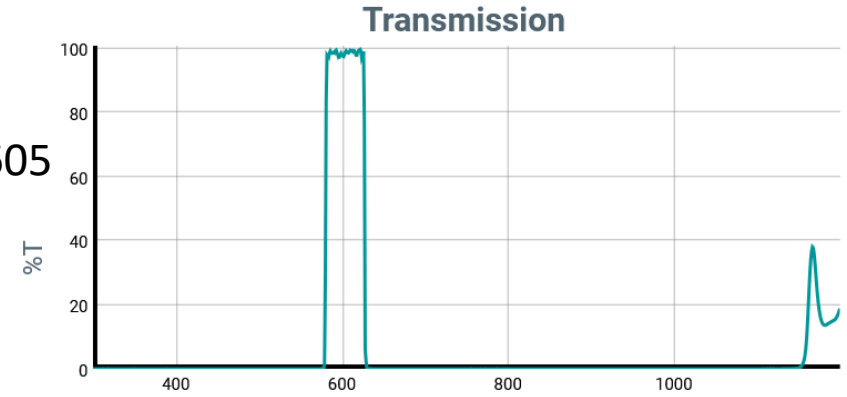
+ Full reflective mirror

Filters

Filter F37-521



Filter F49-605

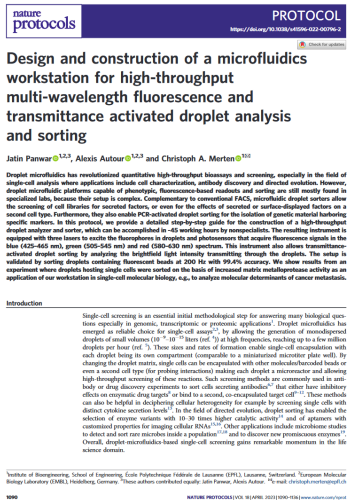


BIOENG-421 students tasks for today/ this week

- **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)

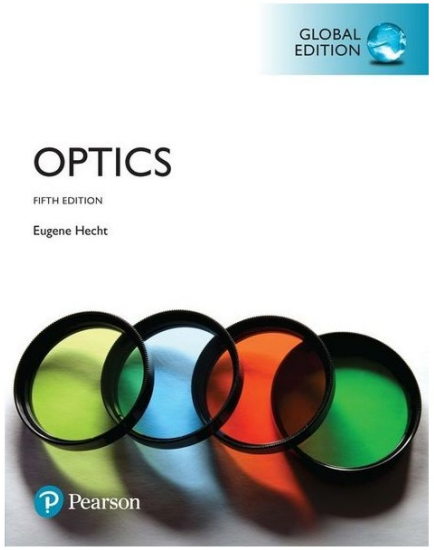
Questions?

References and further literature



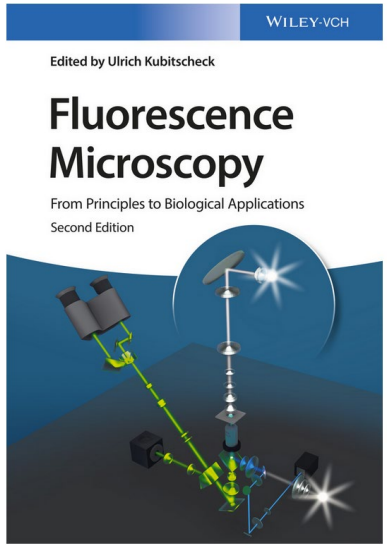
https://doi.org/10.1038/s41596-022-00796-2

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)



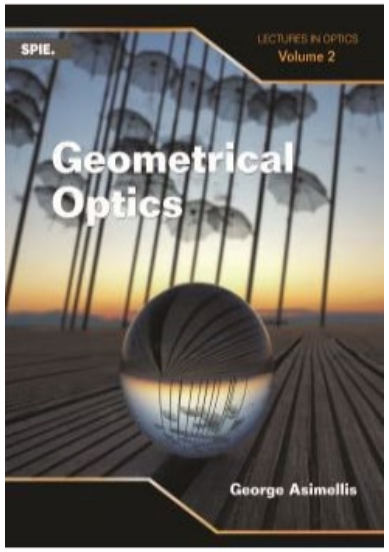
EAN: 9781292096964

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



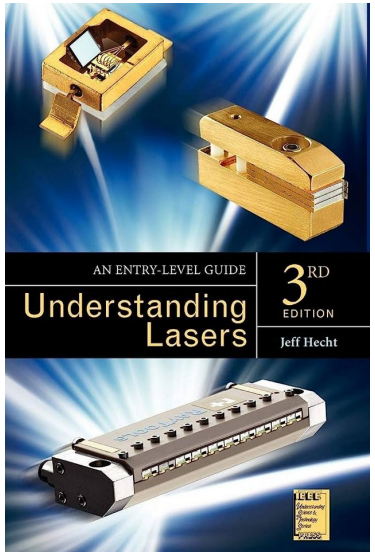
https://doi.org/10.1002/9783527687732

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



https://doi.org/10.1117/3.2506310

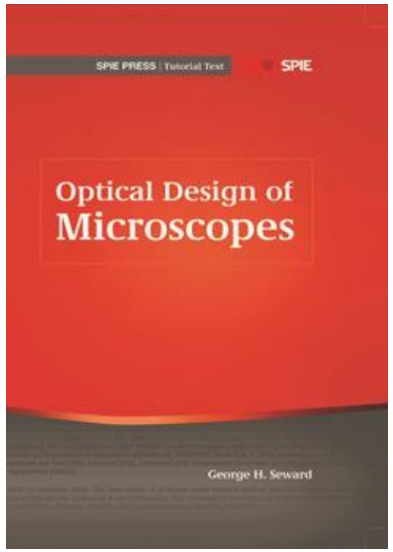
Asimellis, George, *Geometric Optics, in: Lectures in Optics Volume 2*, Bellingham, SPIE, 2020



EAN: 9781118210048

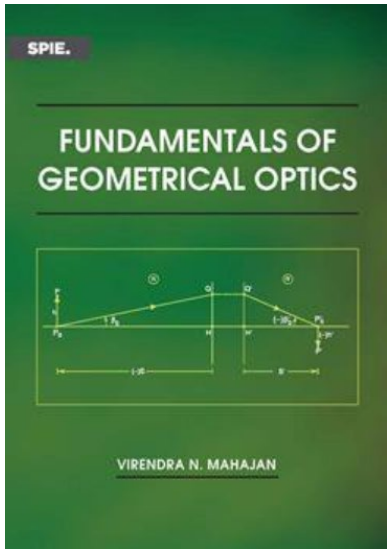
Hecht, Jeff, *Understanding Lasers: An Entry-Level guide*, 3rd edition, Piscataway, IEEE, 2008

References and further literature



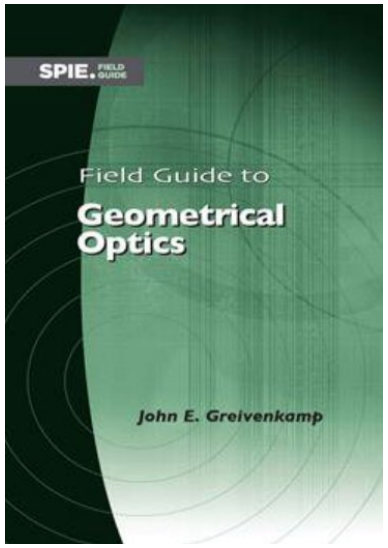
https://doi.org/10.1117/3.855480

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-photonic/optical-filters/'