

Lecture #6

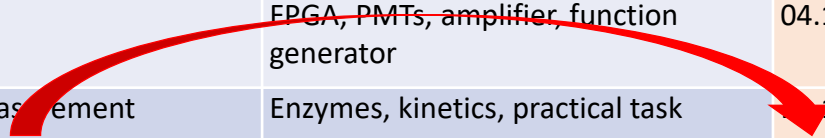
Introduction to optics

Aims:

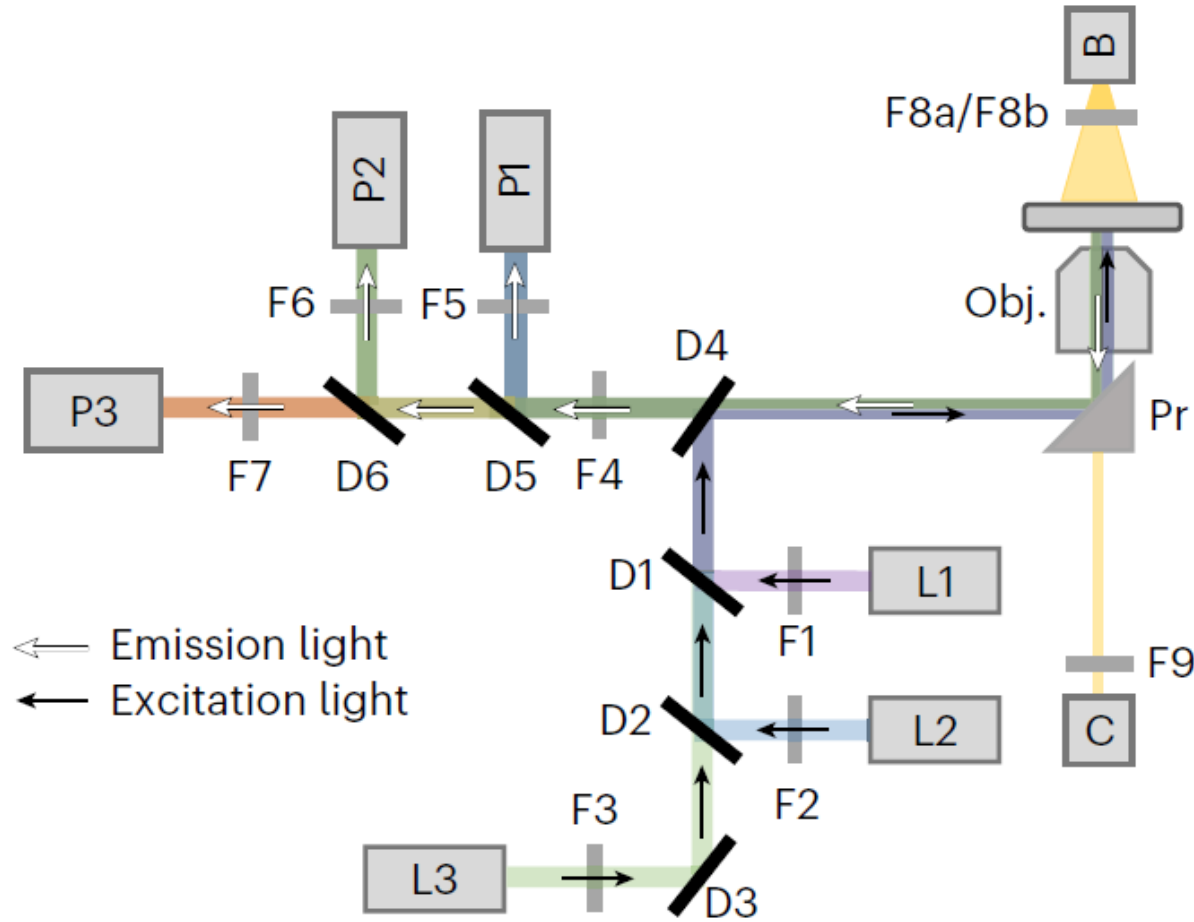
- Understand the basic laws and concepts of geometrical optics
- Know the working principle of all optical components inside a microfluidics workstation
- Being able to design a setup and ray diagram of YOUR workstation

Lectures (PHH 331)	Date & Topic	Details	Practical
1	12.09 General Intro	Get to know teachers, TAs, students and aims of the course	16.09 Measure temperature using thermistor (using M&A explorer) TL
2	19.09 Lecture LabVIEW TL Group formation (4 students, each)	Some first basic steps in LabVIEW programming	23.09 Brief intro into LabVIEW thermistor program (input and output) TL
3	26.09 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?	30.09 Preparation of bioinstrument case study
4	03.10 Preparation of bioinstrument case study		07.10 Tour through LBMM workstation labs, intro into Nature Protocols (all groups)
5	10.10 All groups presenting case study		No practical
6	17.10 Lecture optics Homework: Students to prepare one laser/PMT blueprint FP (by 21.10 23.59h)	Mirrors, filters, microscope setup, lenses, etc.	21.10 Holidays
	24.10 Holidays		28.10 Build workstation optics 1
7	31.10 Lecture electronics	FPGA, PMTs, amplifier, function generator	04.11 Build workstation 1 optics 2
8	07.11 Intro into enzyme concentration measurement experiment (kinetics, etc.) + task FP	Enzymes, kinetics, practical task	11.11 Build workstation electronics
9	14.11 Intro to droplet analysis software (LabVIEW) TL	Software similar to Thermistor program, pdf on installation	18.11 Build workstation software: Add output LED (mimicking sorting trigger) into analysis software
10	21.11 Fundamentals of microfluidics and microfluidic chips	Flow at the microscale, microfluidic chips (manufacturing), droplet microfluidic modules	25.11 Run microfluidic experiments, e.g. determine concentration of MMP in droplets
11	28.11 Prepare presentation		2.12 Sorting Demo on LBMM workstation1 (Groups A&B)
12	05.12 Groups presenting results (A-C not present)		09.12 Sorting Demo on LBMM workstation1 (Groups C&D)
13	12.12 Groups presenting results (D-F not present) 15.12 Submit report (all!)		16.12 – TUESDAY! - Individual Q & A sessions (10min, each)

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

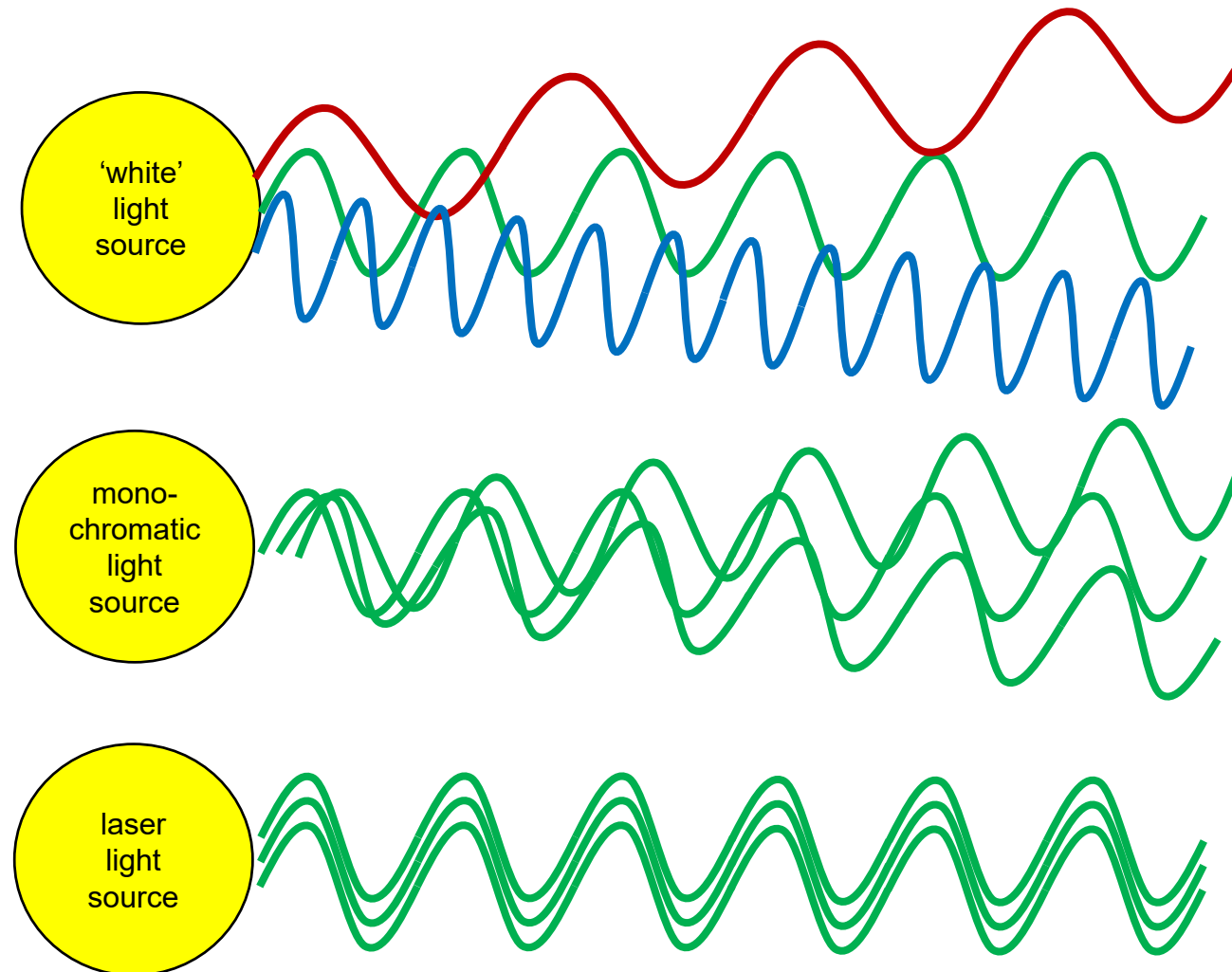
Filters

Mirrors

Prisms

Lenses

Different types of light sources



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

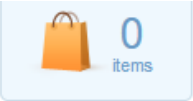
Laser (light amplification by stimulated emission of radiation):
Monochromatic, with all waves in phase and emitted into a single direction!

Lasers used in the practical course



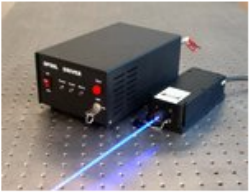
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- Blue Lasers
- Green Lasers
- Yellow Lasers
- Red Lasers
- IR Lasers
- Contact us

Home > 420-500nm > FN Series 473nm Laser 100-1000mW



FN Series 473nm Laser 100-1000mW

★★★★★ — 4

\$1800.00

Model Selection

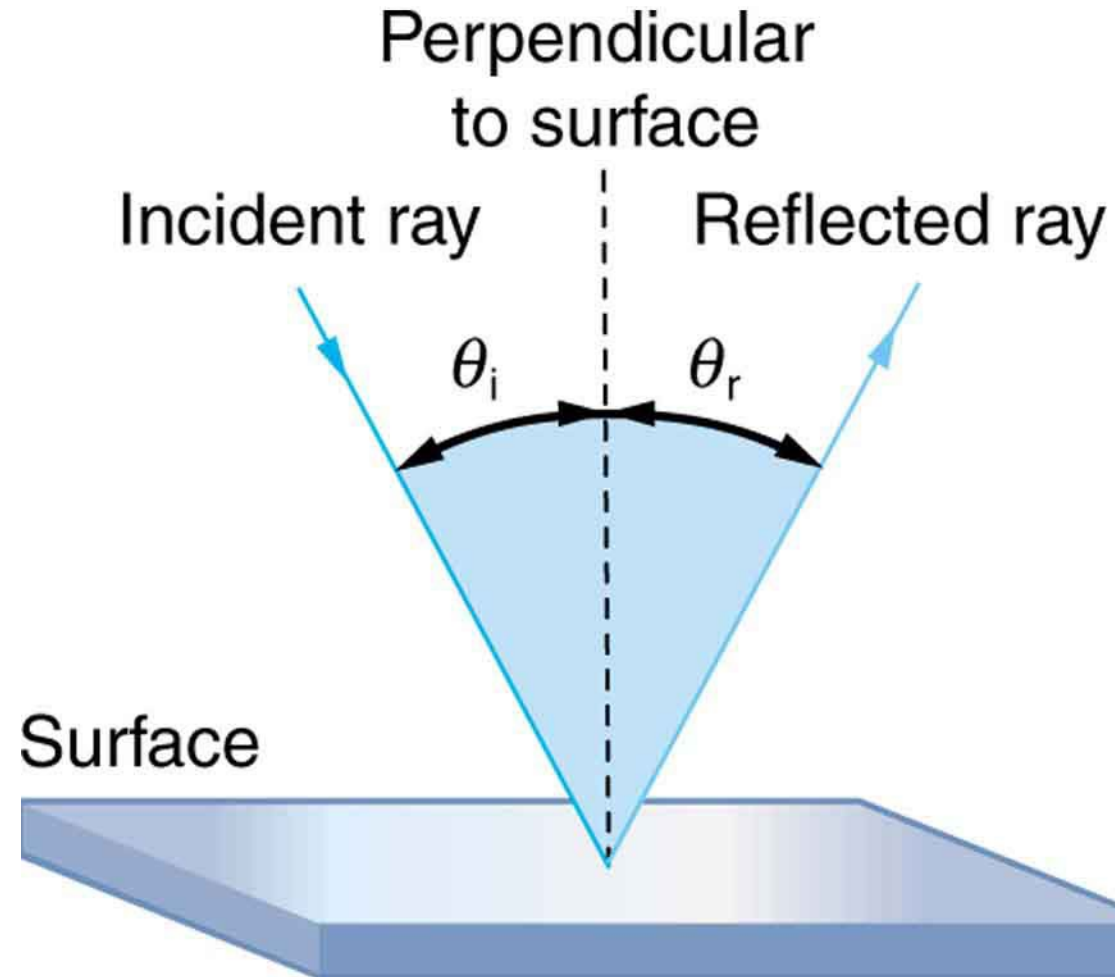
473nm 100mW

Qty:

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'

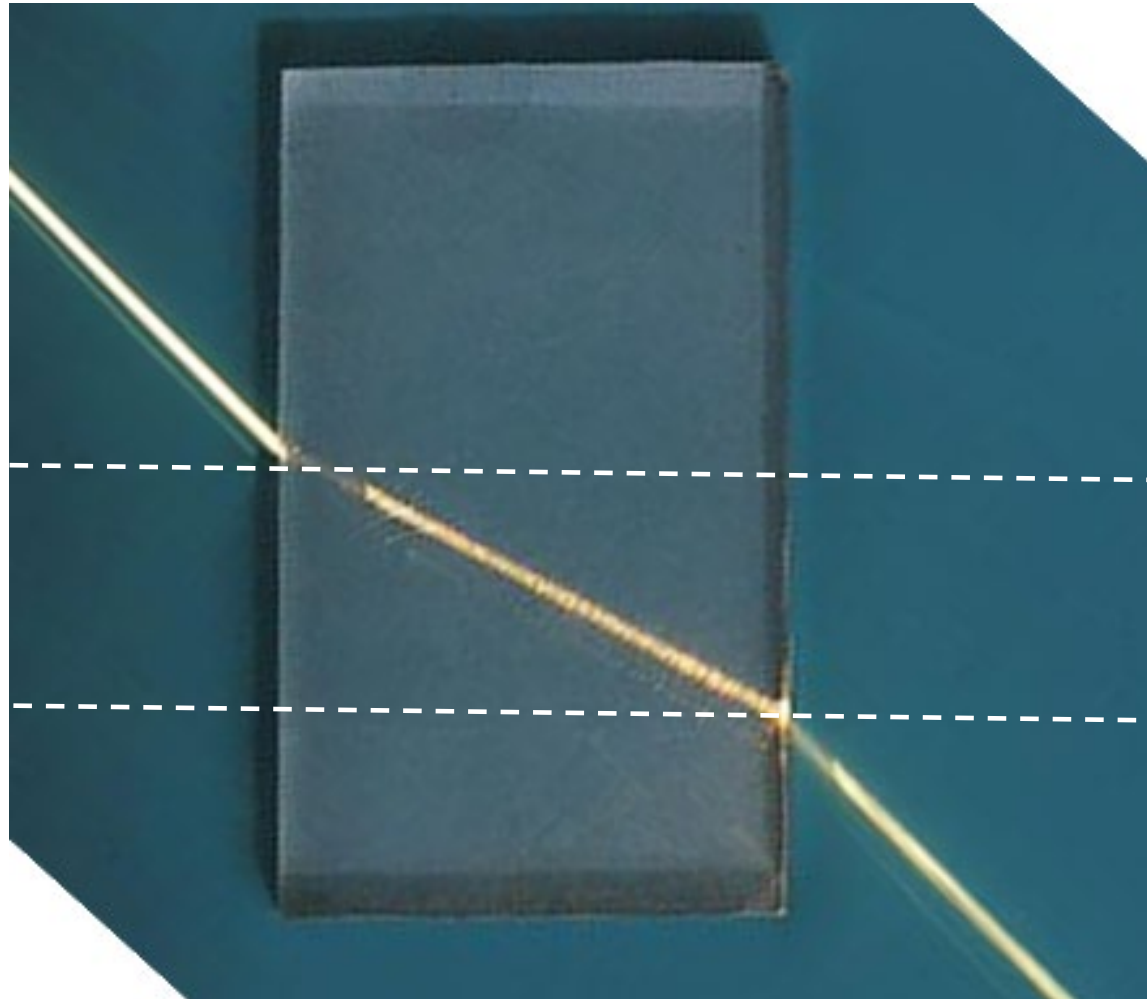
Law of reflection



Application/ component: mirror

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

What is happening here?

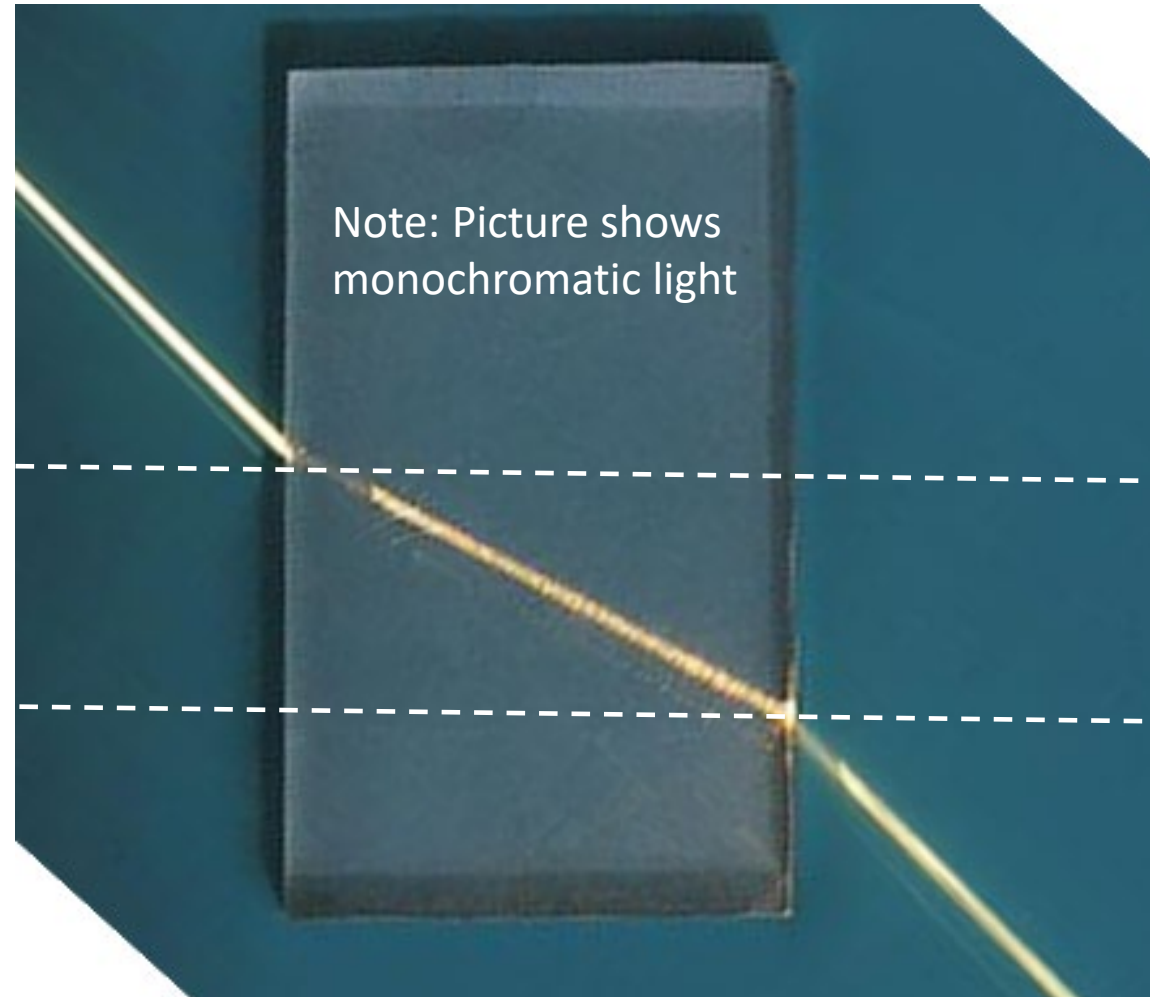


Picture taken from Wikipedia and modified

Law of refraction

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

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

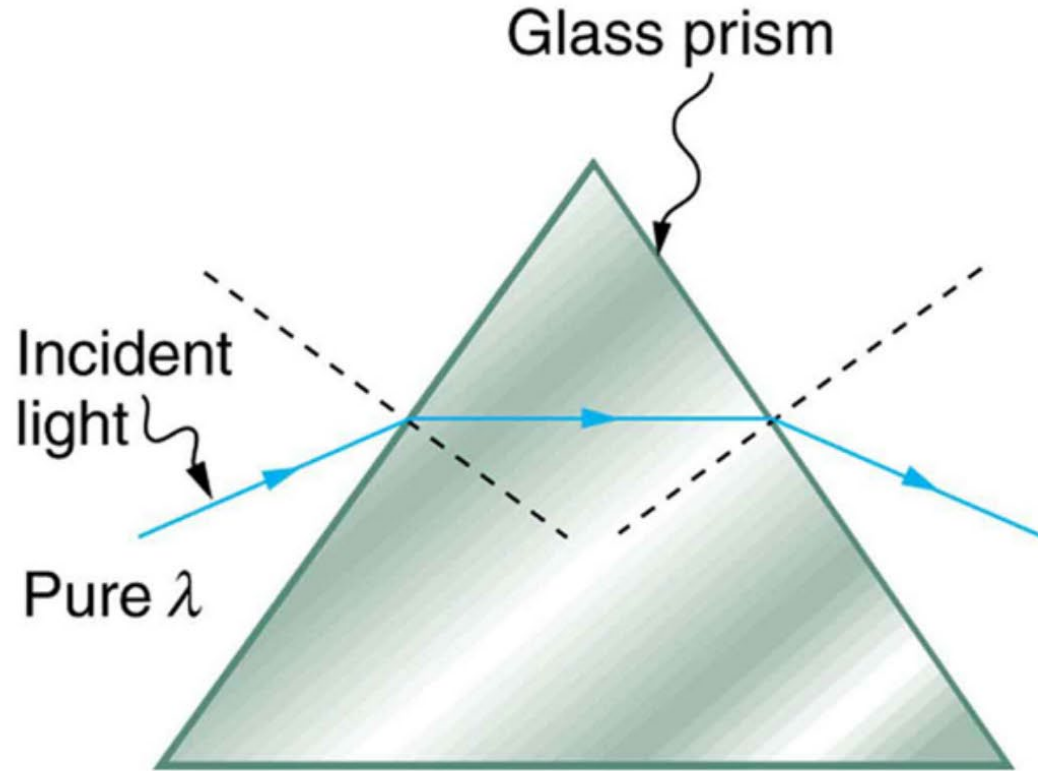
Glass = 1.5

Diamond = 2.42

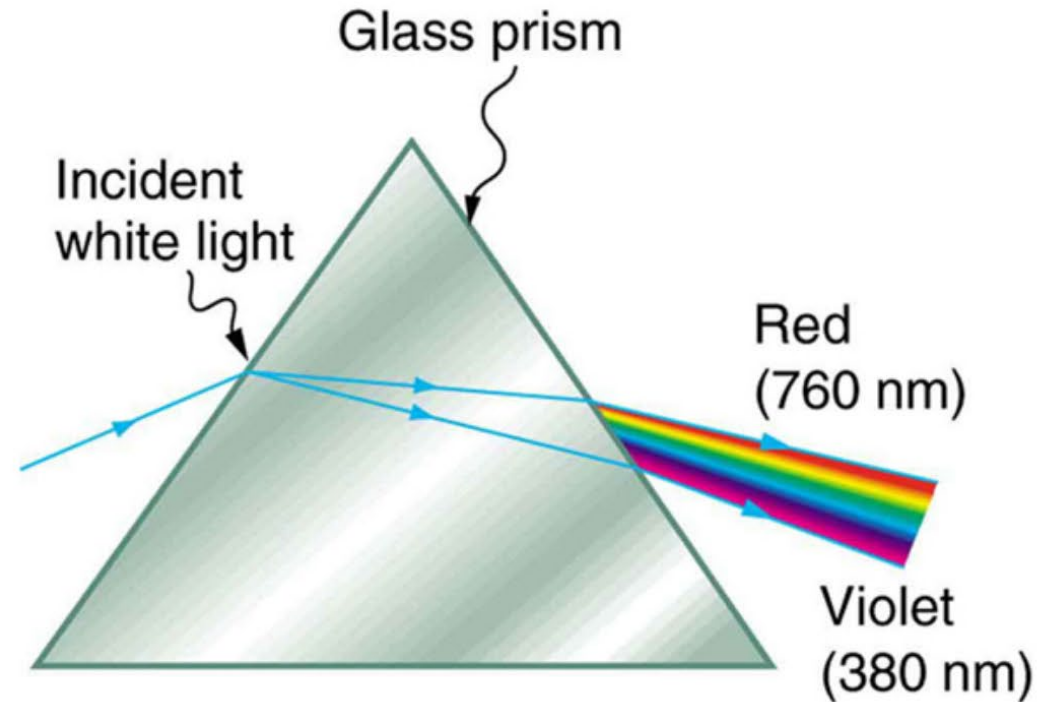
**Application/
component: prism &
lens**

Picture taken from Wikipedia and modified

What is happening with light of different wavelengths?

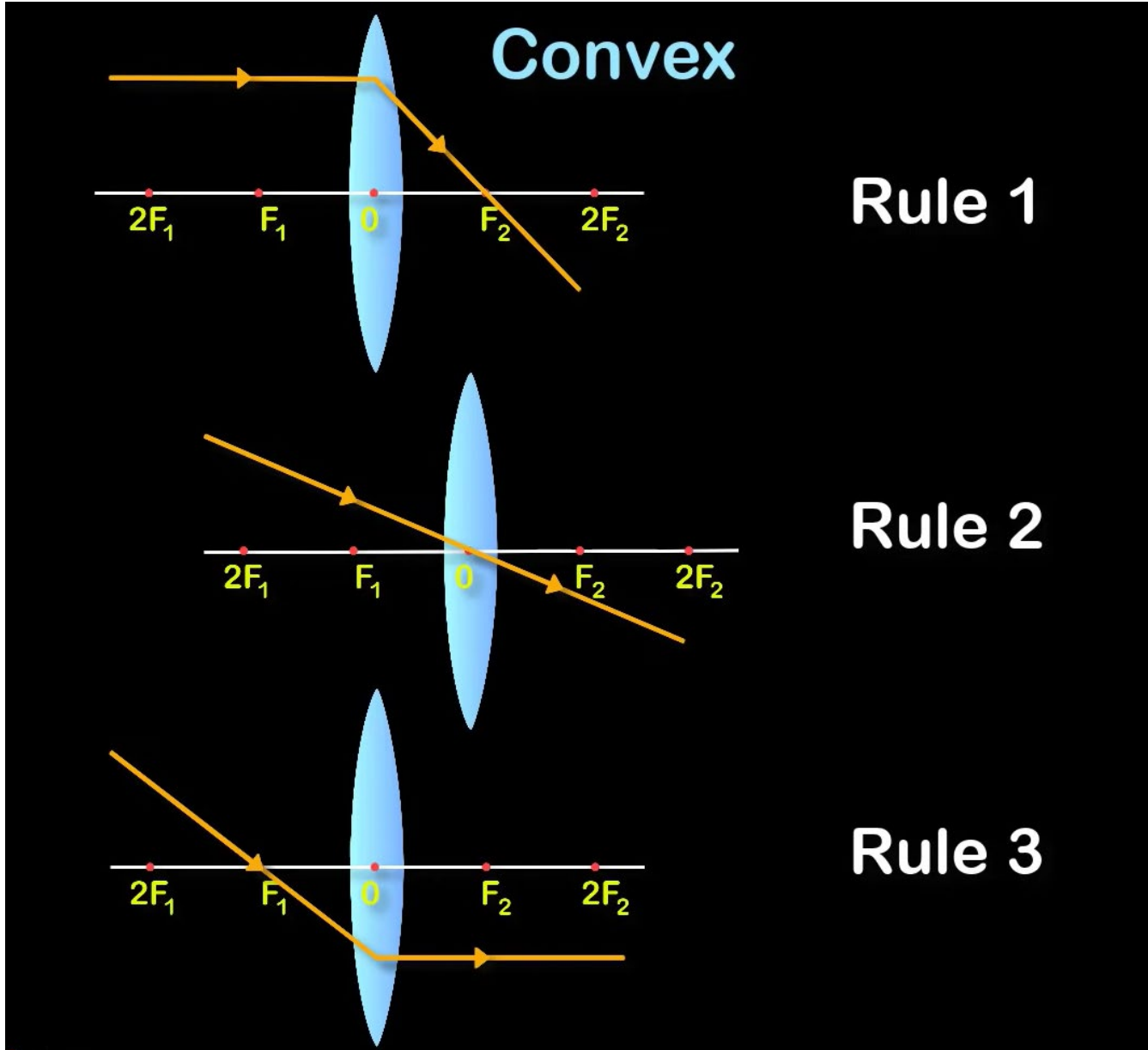


www.coursehero.com



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.

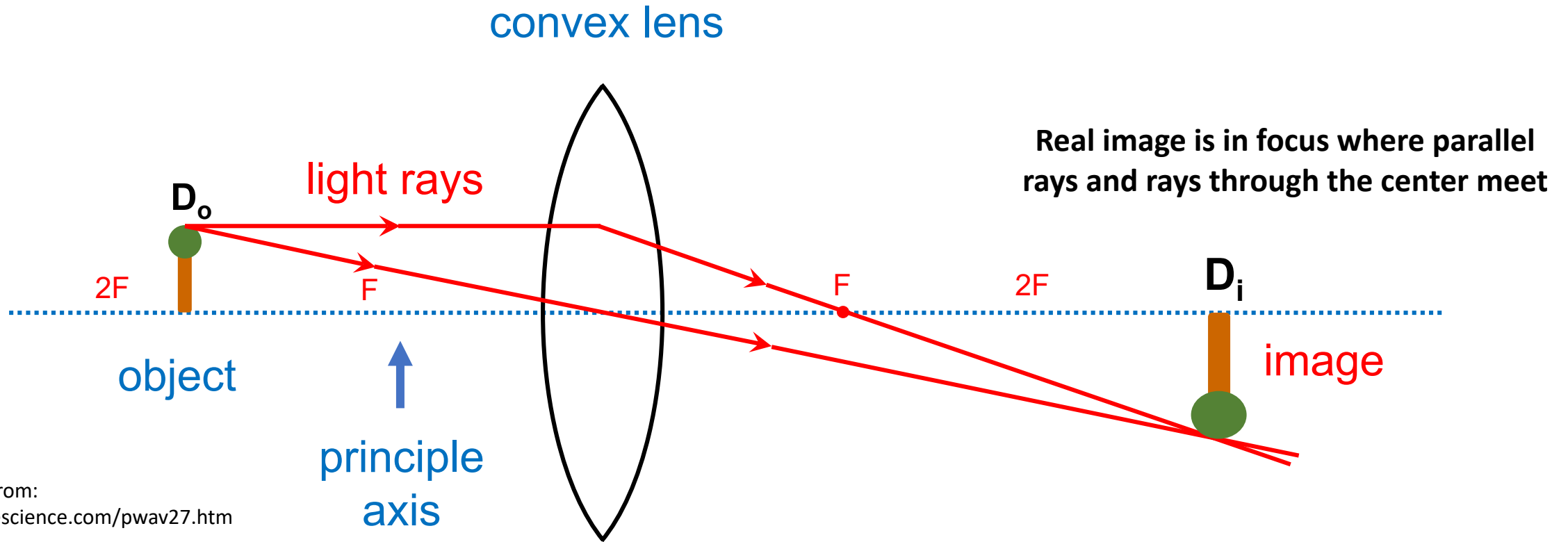
Lenses – basic rules



Rules

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

Lenses – focus and magnification



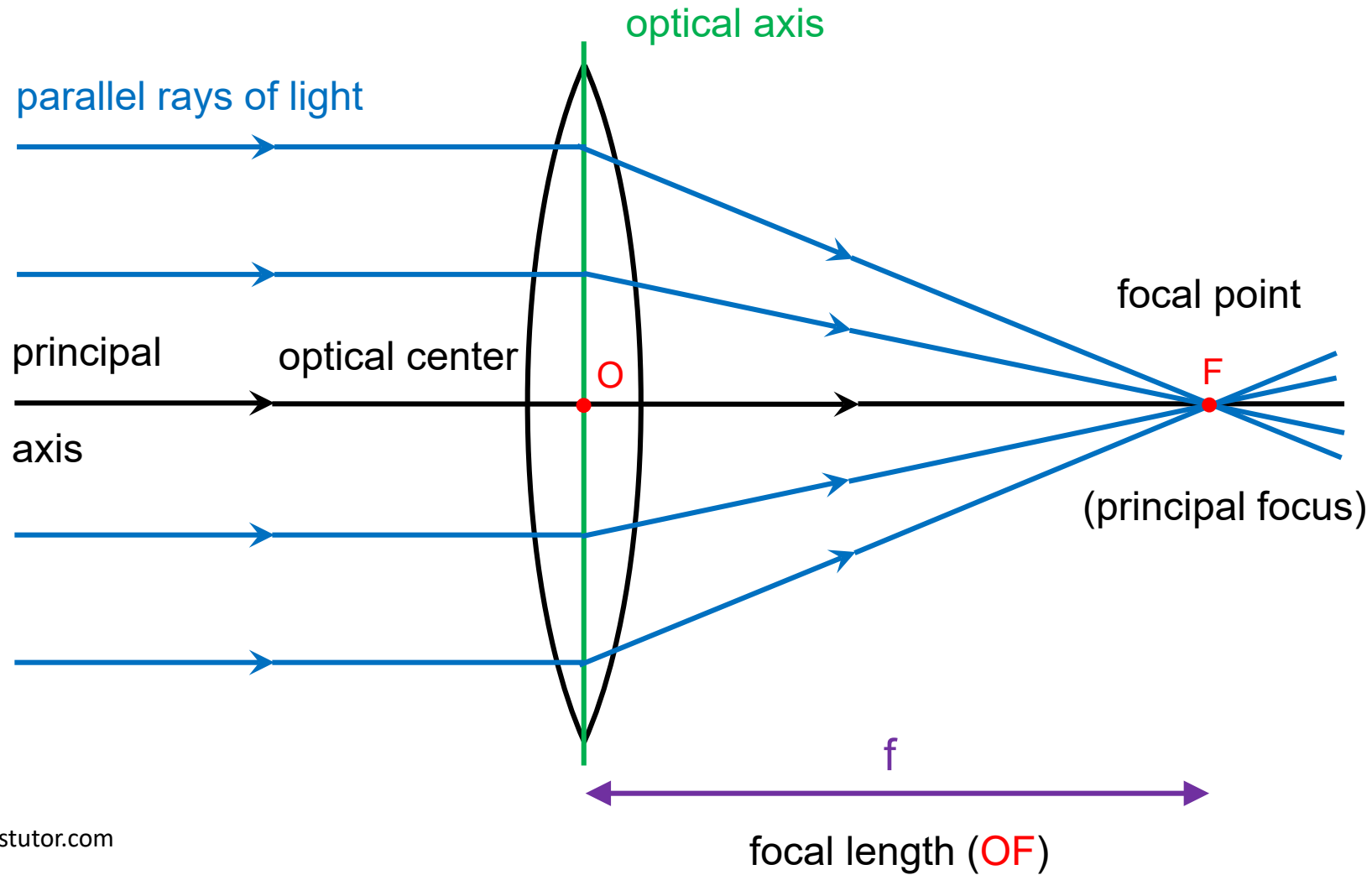
adapted from:
www.gcscience.com/pwav27.htm

- 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
$$D_i = \frac{F * D_o}{(D_o - F)}$$

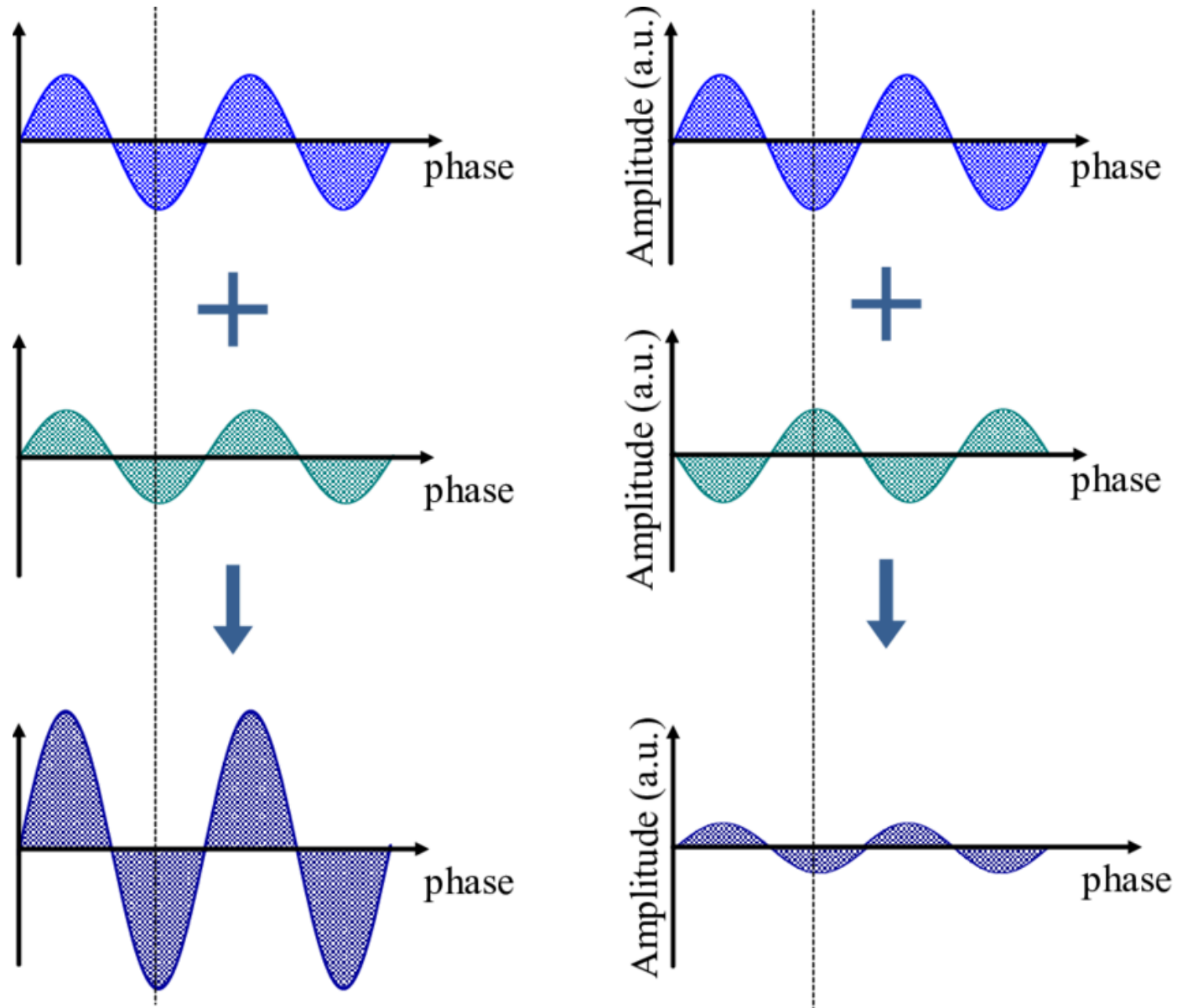
Lenses – focus and magnification



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)!

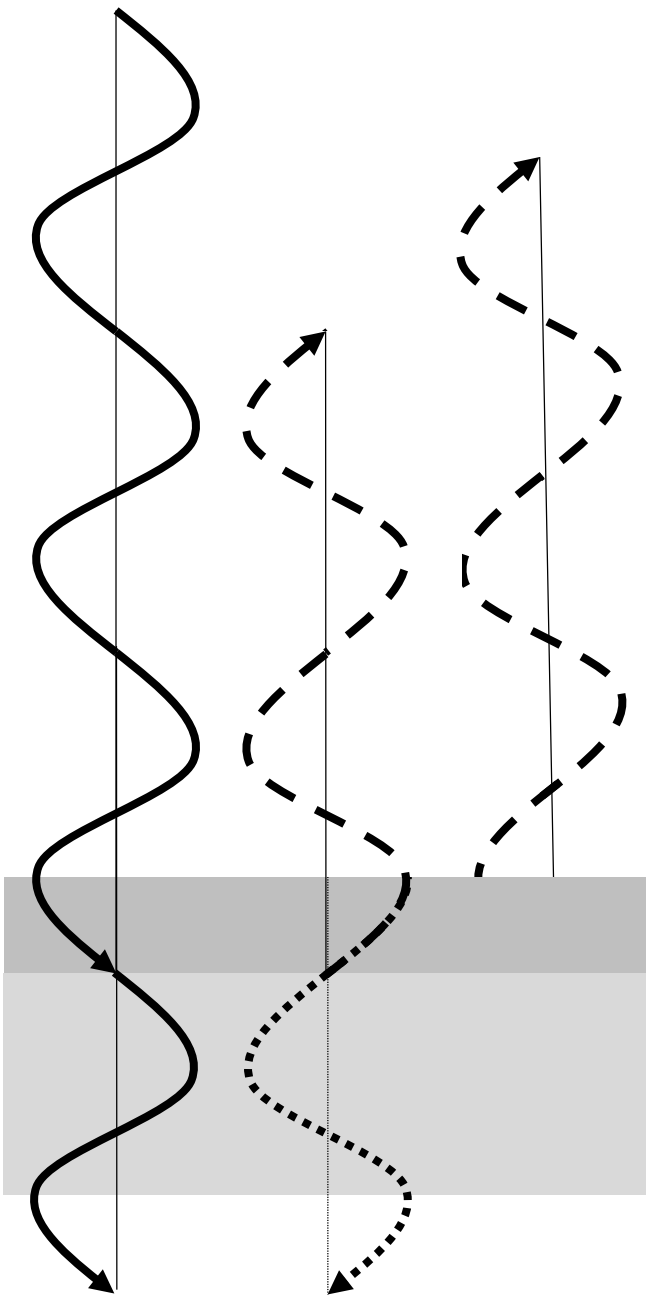
Constructive and destructive interference



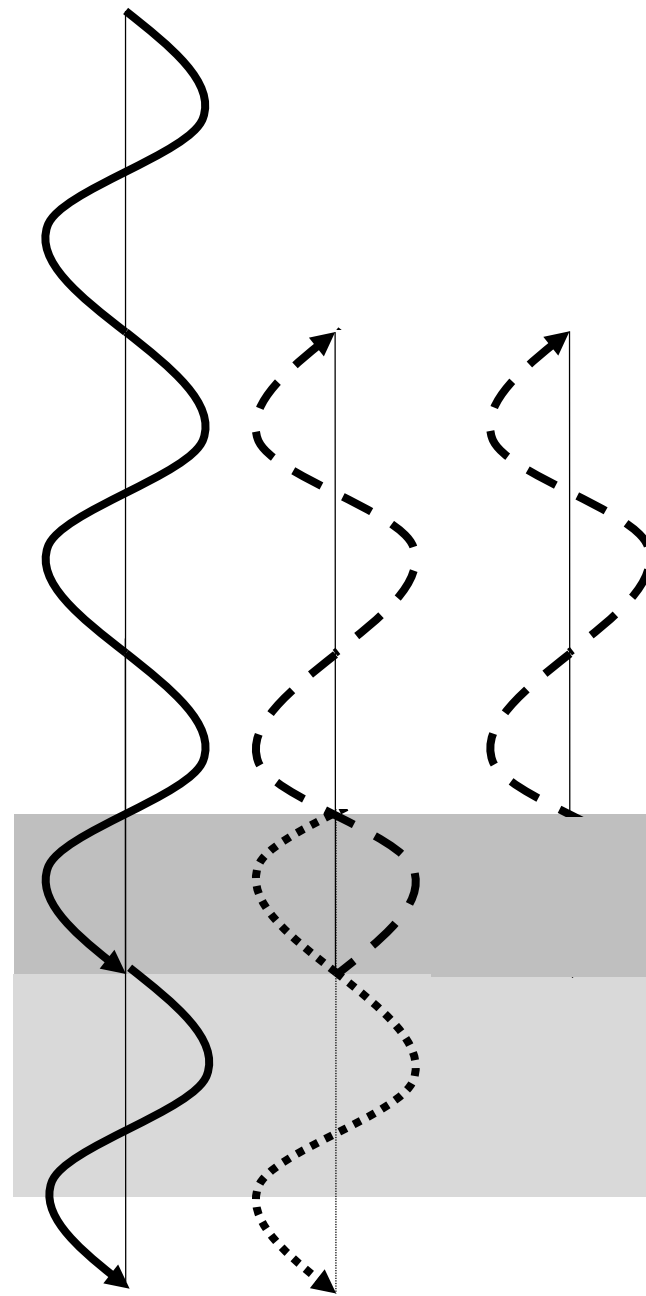
**Application/
component: filters &
dichroic mirrors**

Working principle of interference filters

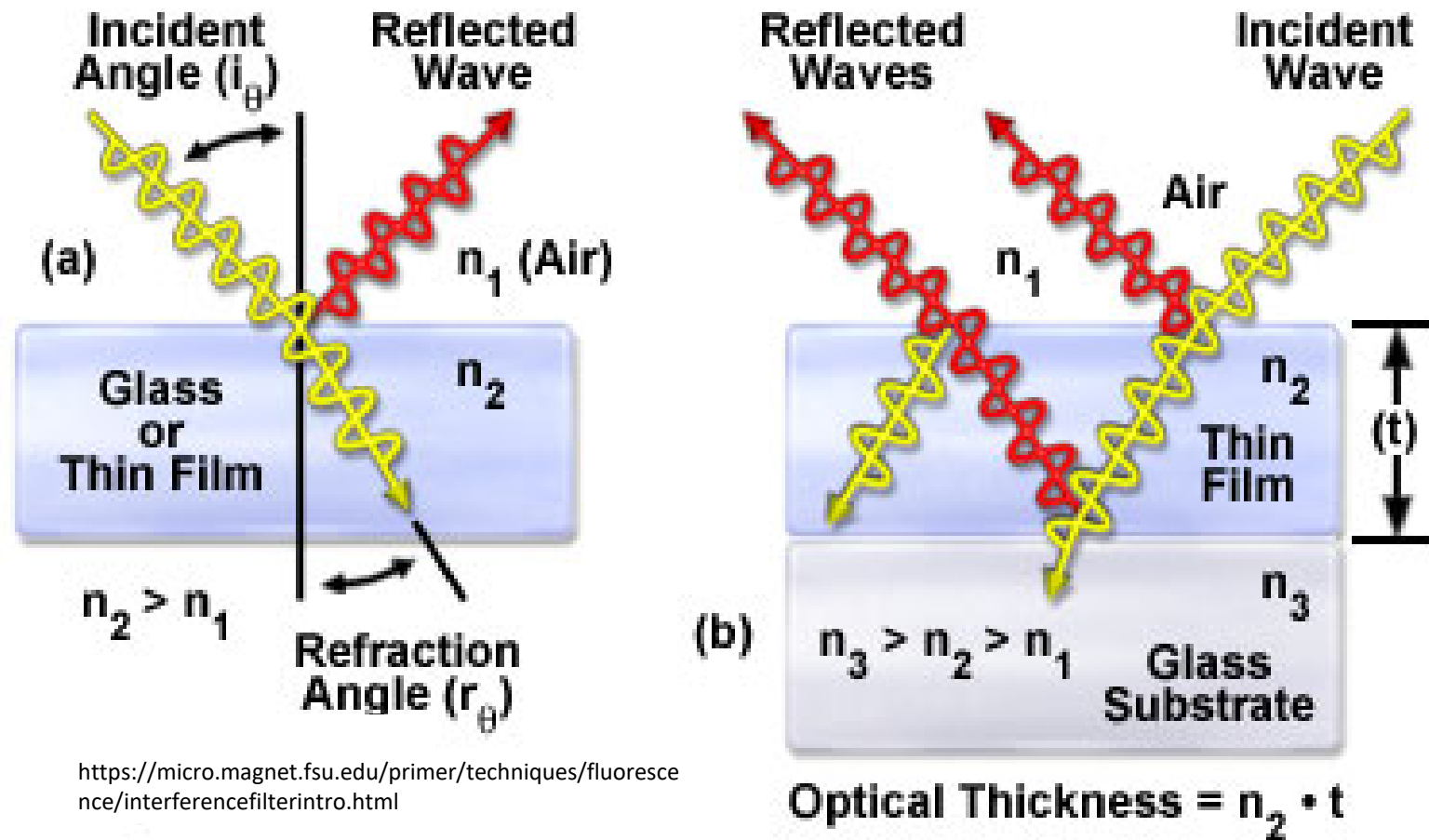
Destructive interference, layer thickness = $\lambda / 4$, phase shift = 180°



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

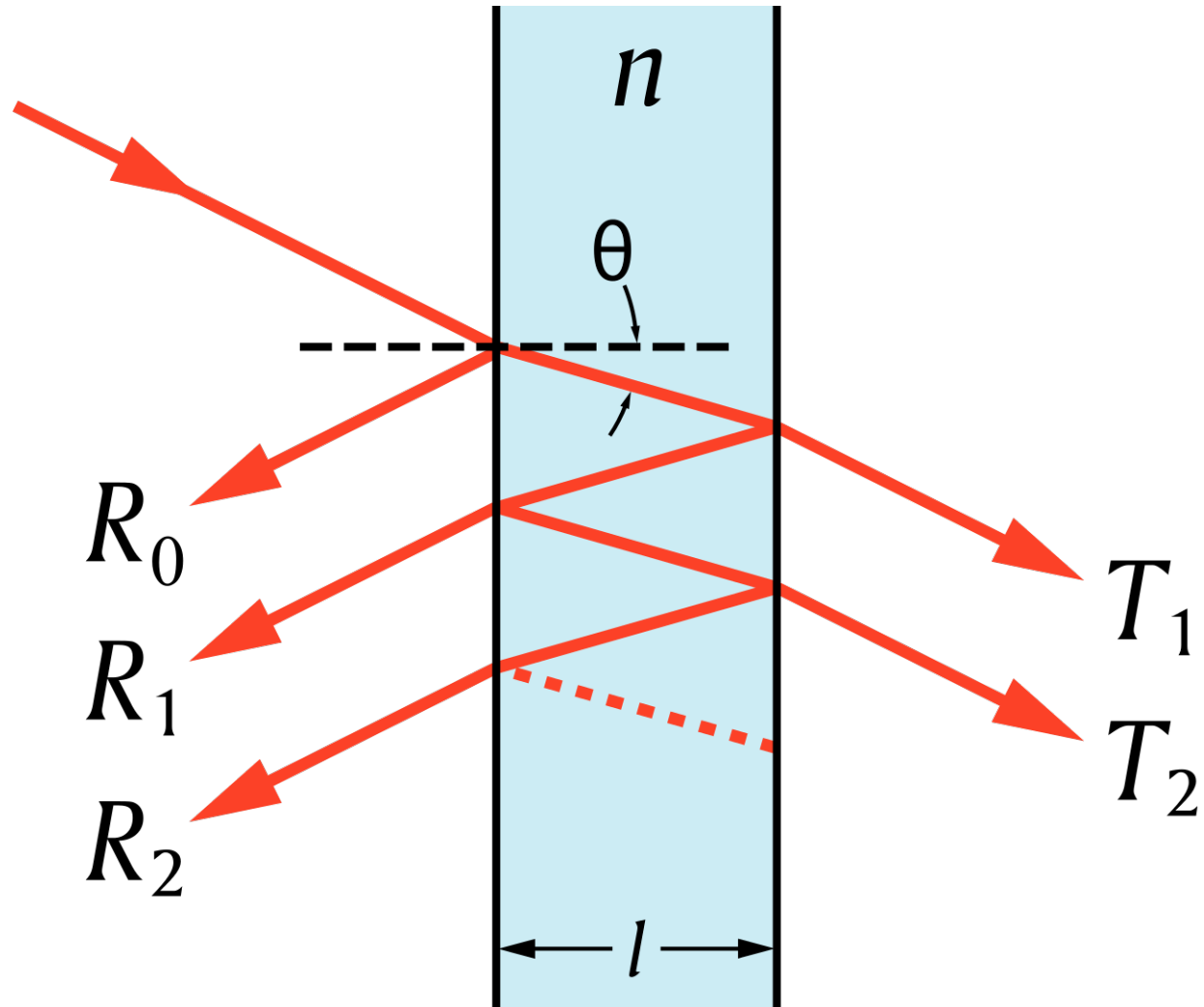


Working principle of dichroic mirrors

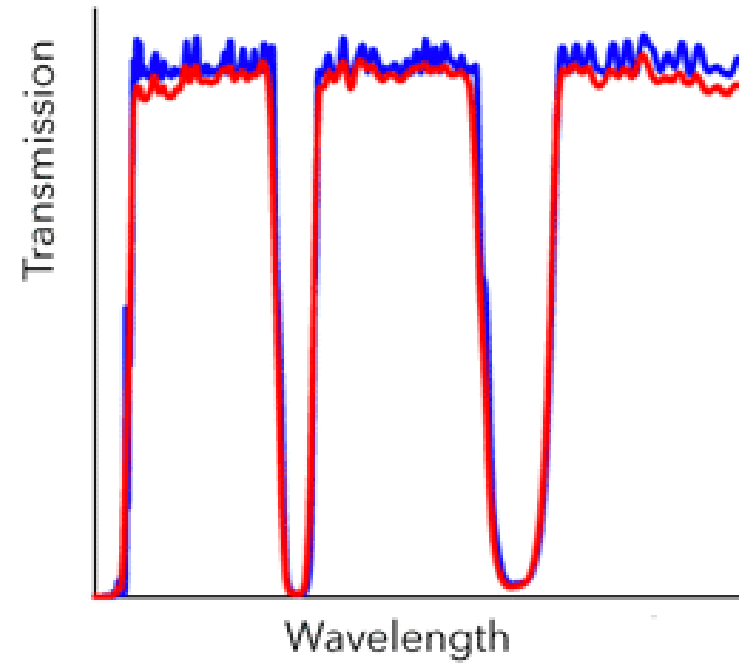
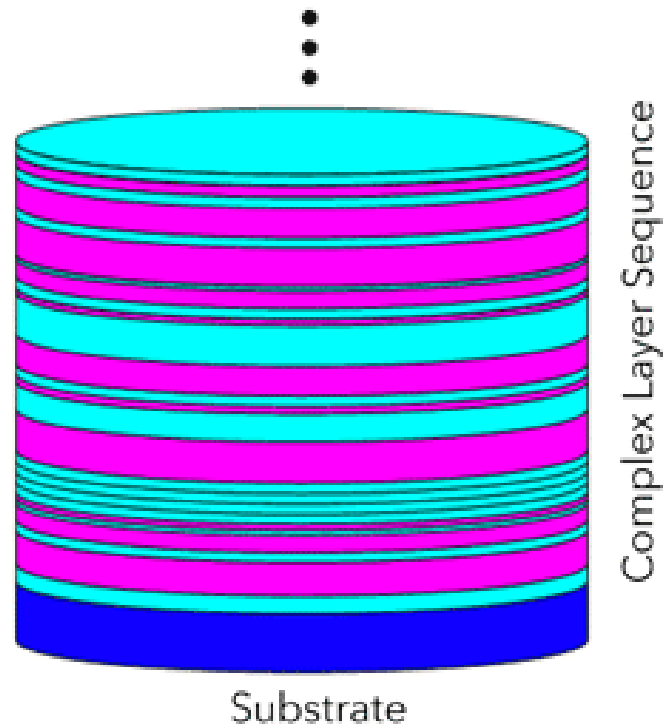


<https://micro.magnet.fsu.edu/primer/techniques/fluorescence/interferencefilterintro.html>

Working principle of dichroic mirrors



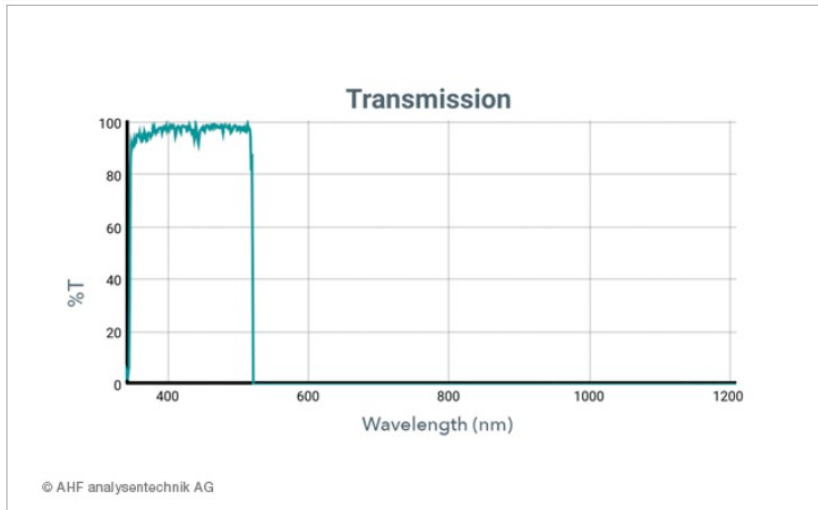
In reality it is always more than one thin layer!



© Original image: Semrock | A Unit of IDEX

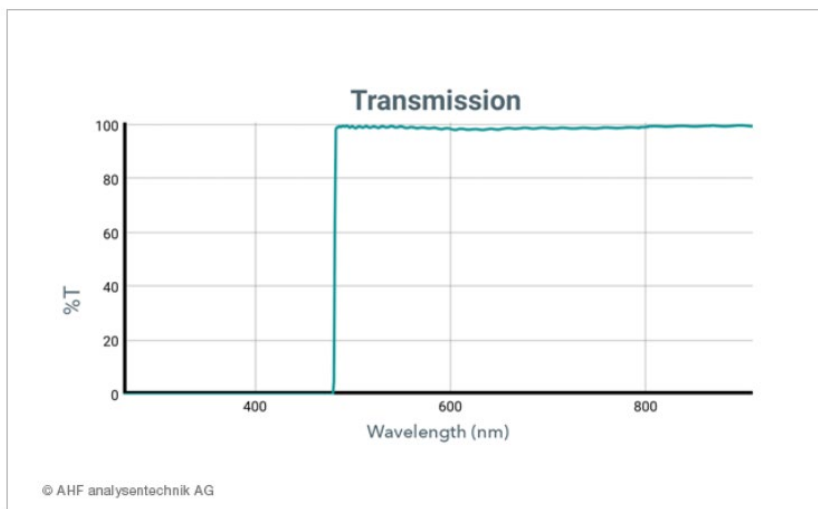
Filters used in the practical course

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



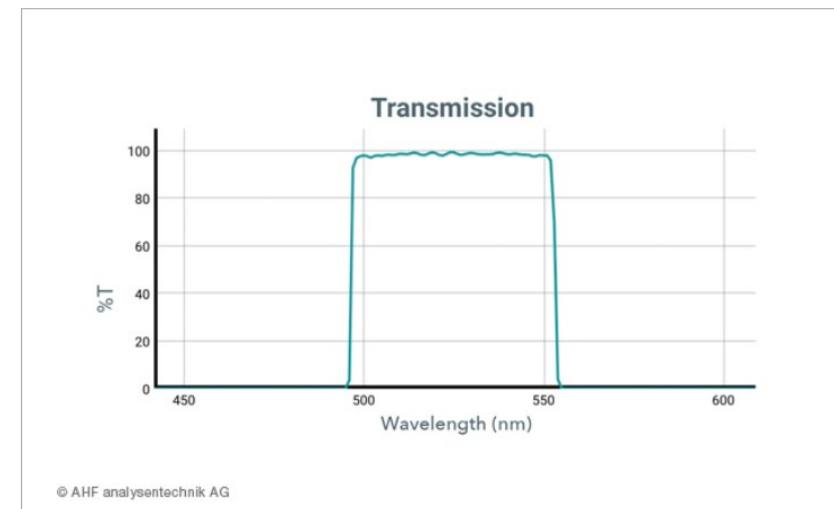
short pass filters

532 nm



long pass filters

473 nm

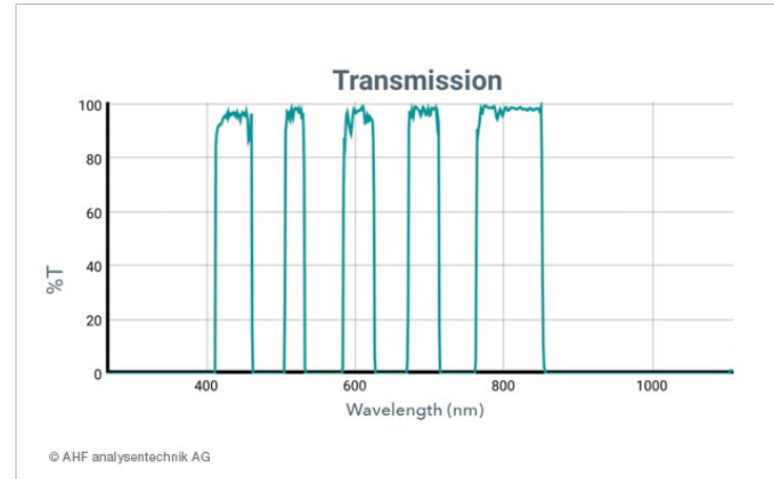


**bandpass
filter**

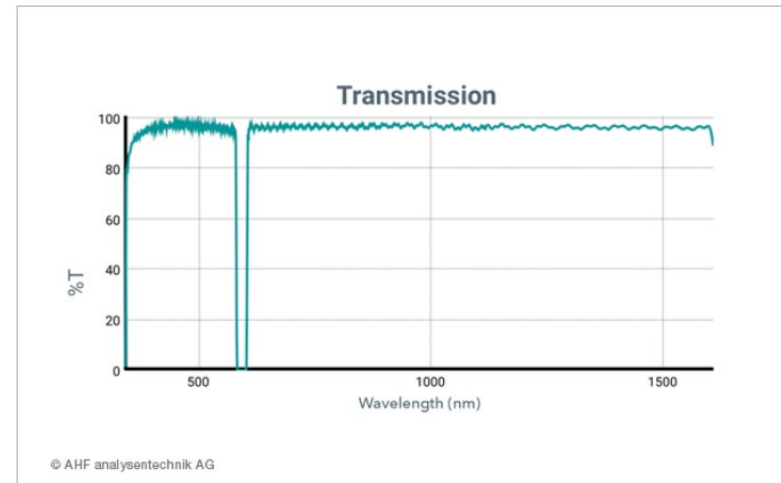
525/50 nm

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/>'

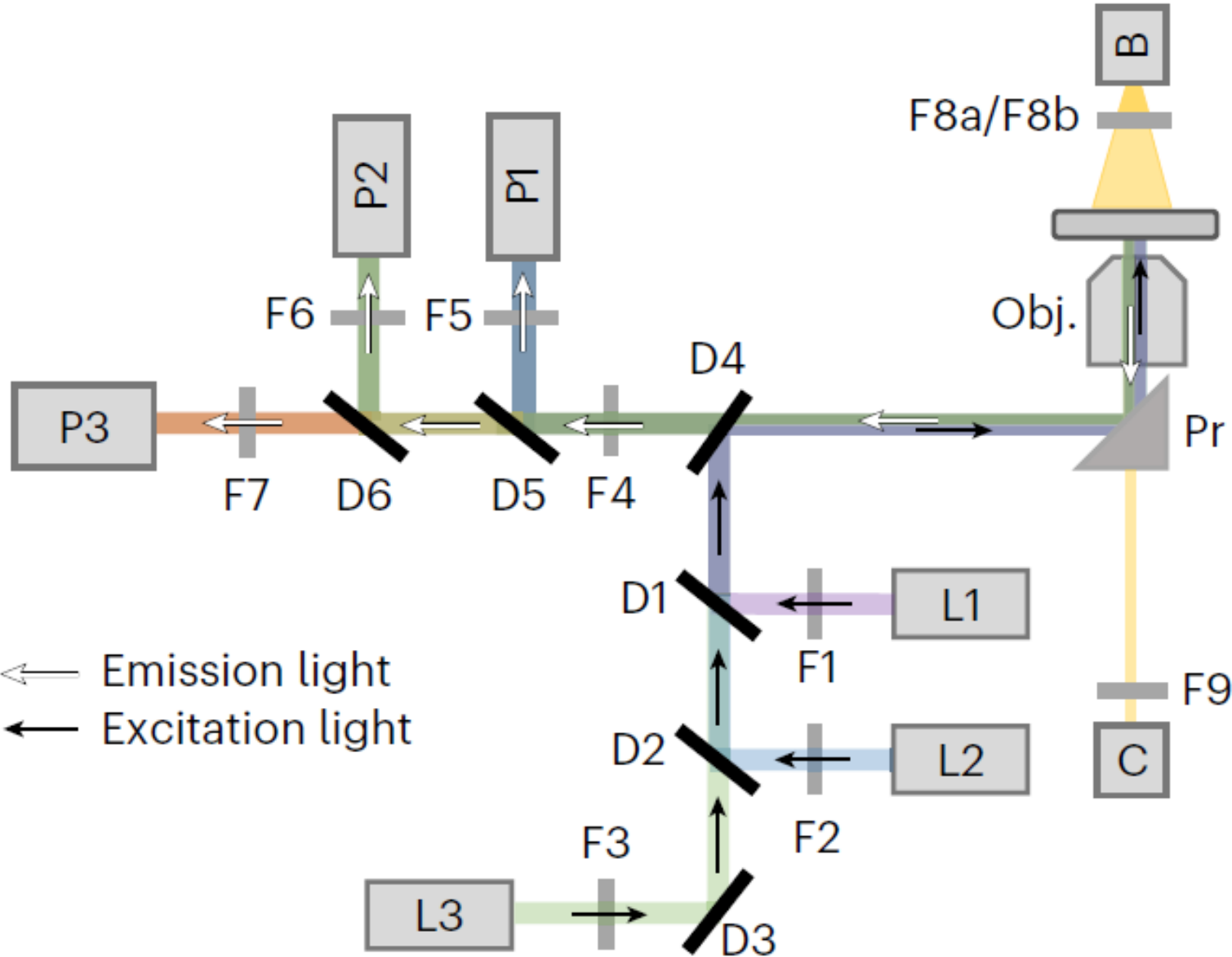


multiband filter



notch filter

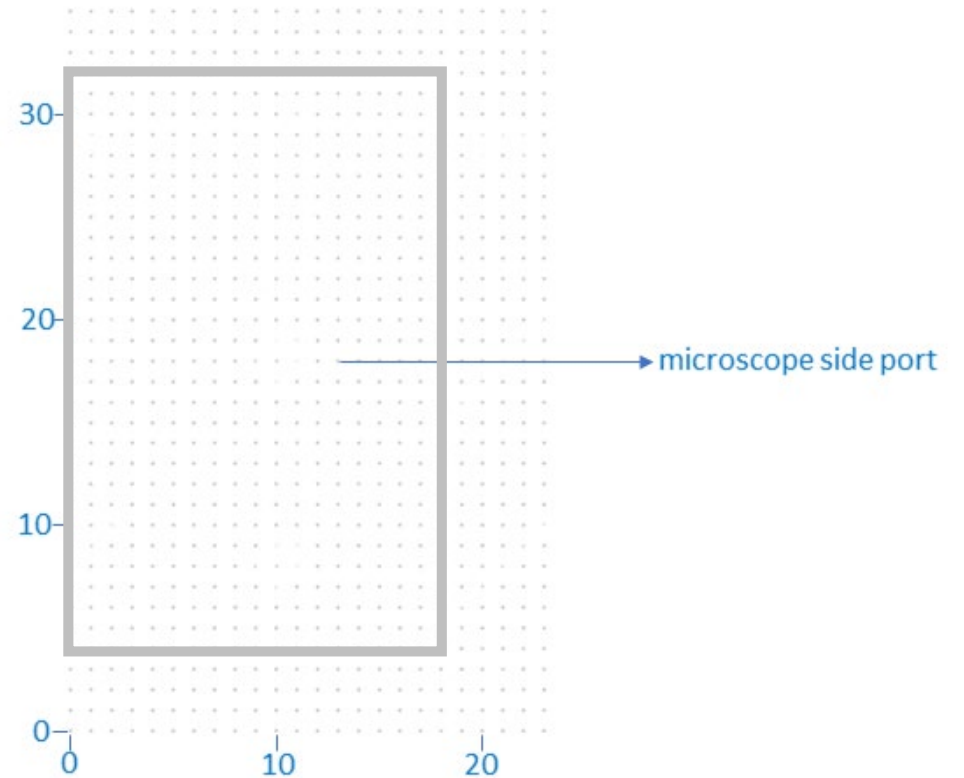
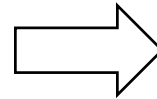
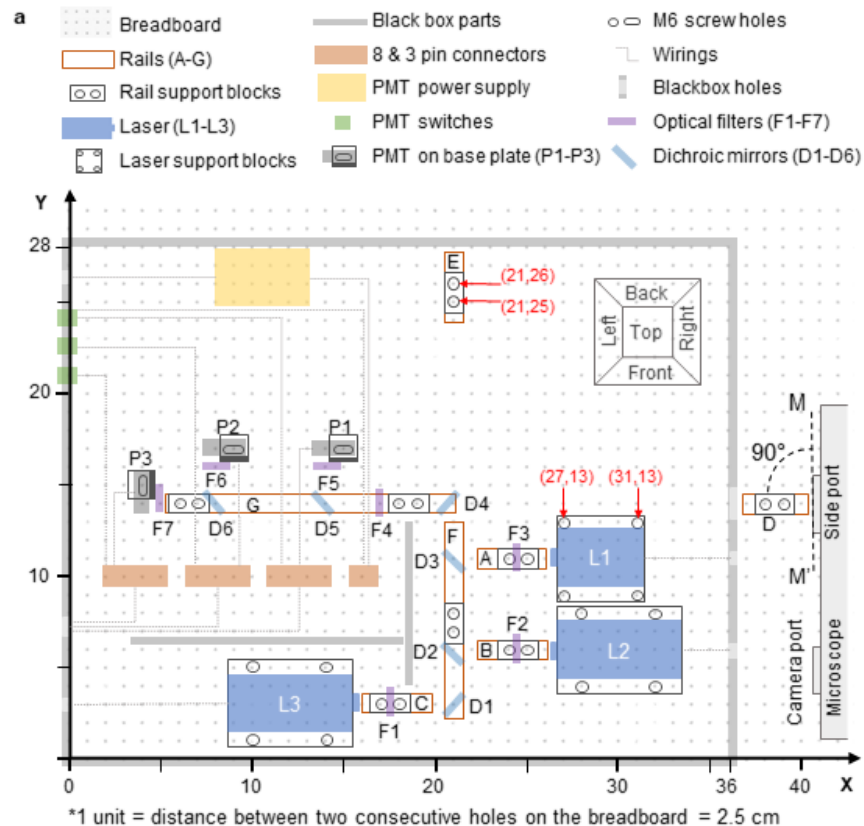
A series of dichroics can be used to feed in multiple lasers or to split polychromatic light into its components



Blueprint of your instrument

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

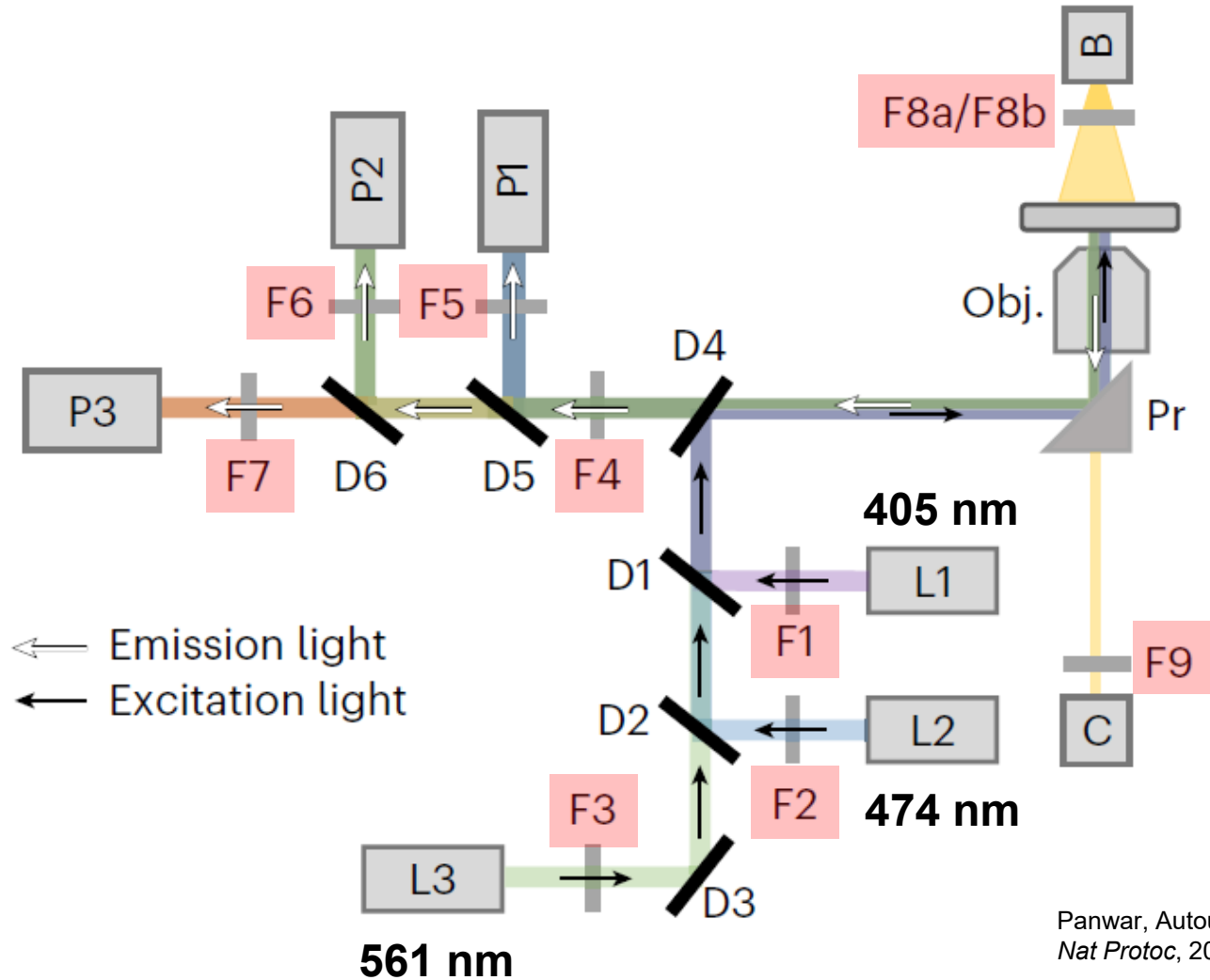
Your task: Simplification of the Panwar & Autour Nature Protocols 2023 setup, including only one laser and one PMT and only a virtual feedback rather than active sorting



3 channel fluorescence detection

single channel fluorescence detection

What kind of tools do we need?



- F1...? 405/10 nm
- F2...? 488/6 nm
- F3...? 563/9 nm

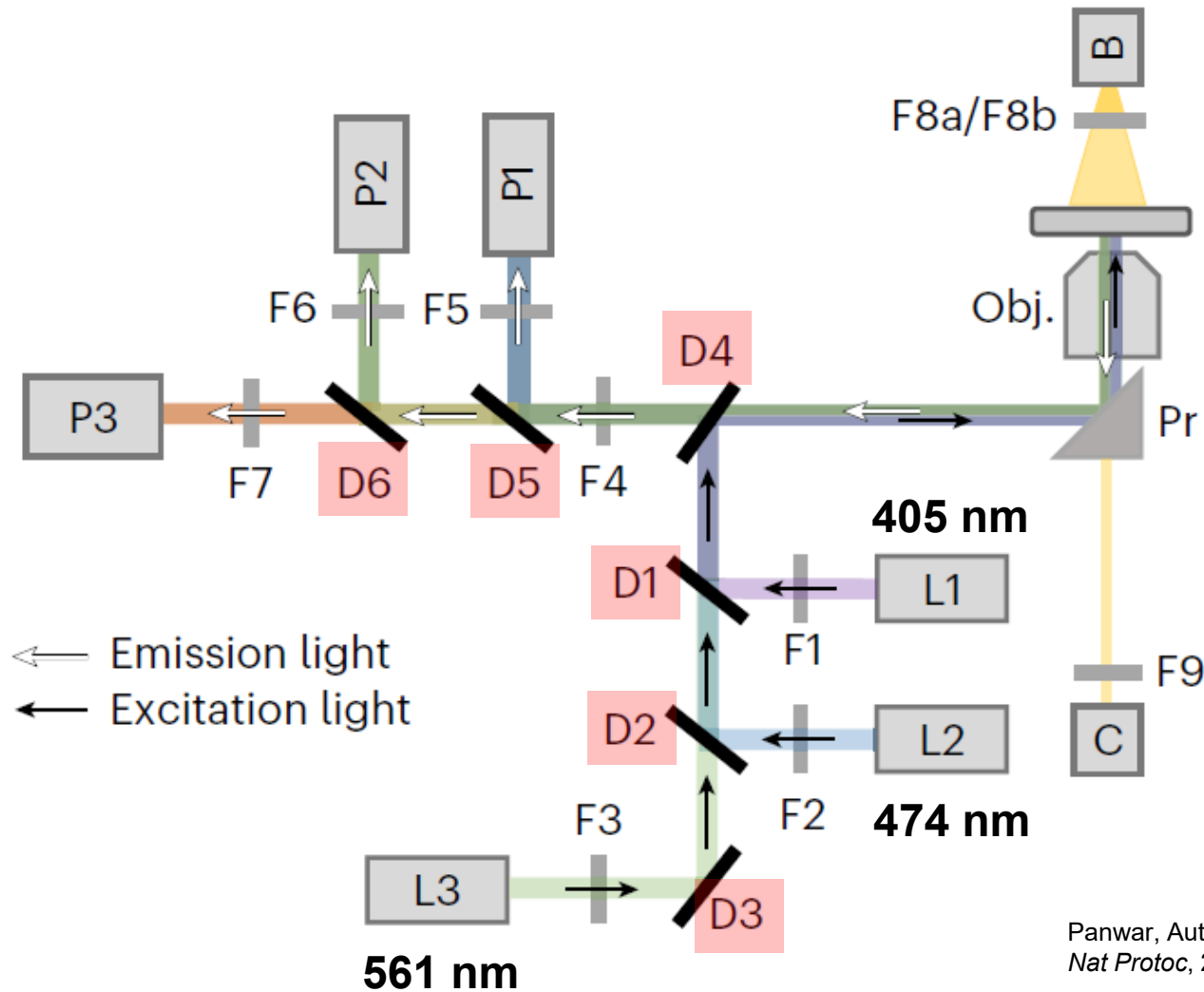
- F4...? 405/473/561 nm
- F9...? triple band notch

- 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?



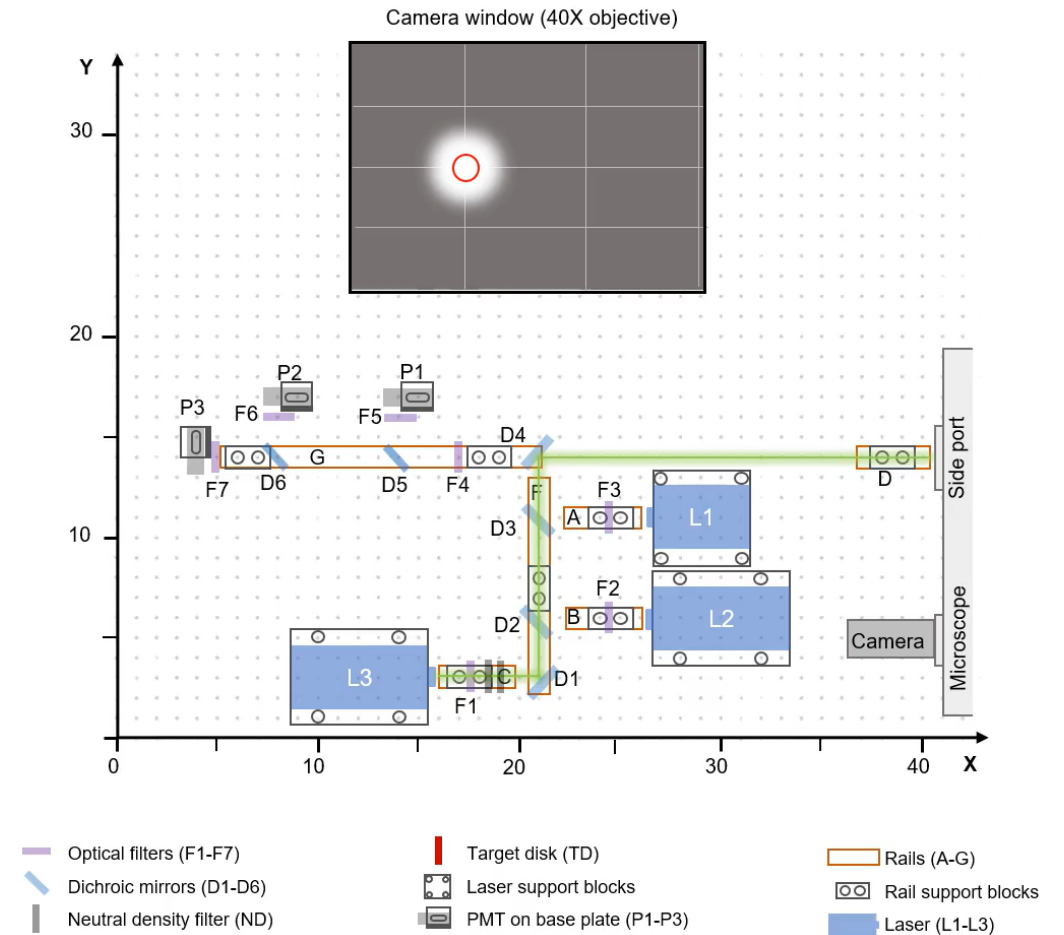
Panwar, Autour & Merten,
Nat Protoc, 2023

- 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

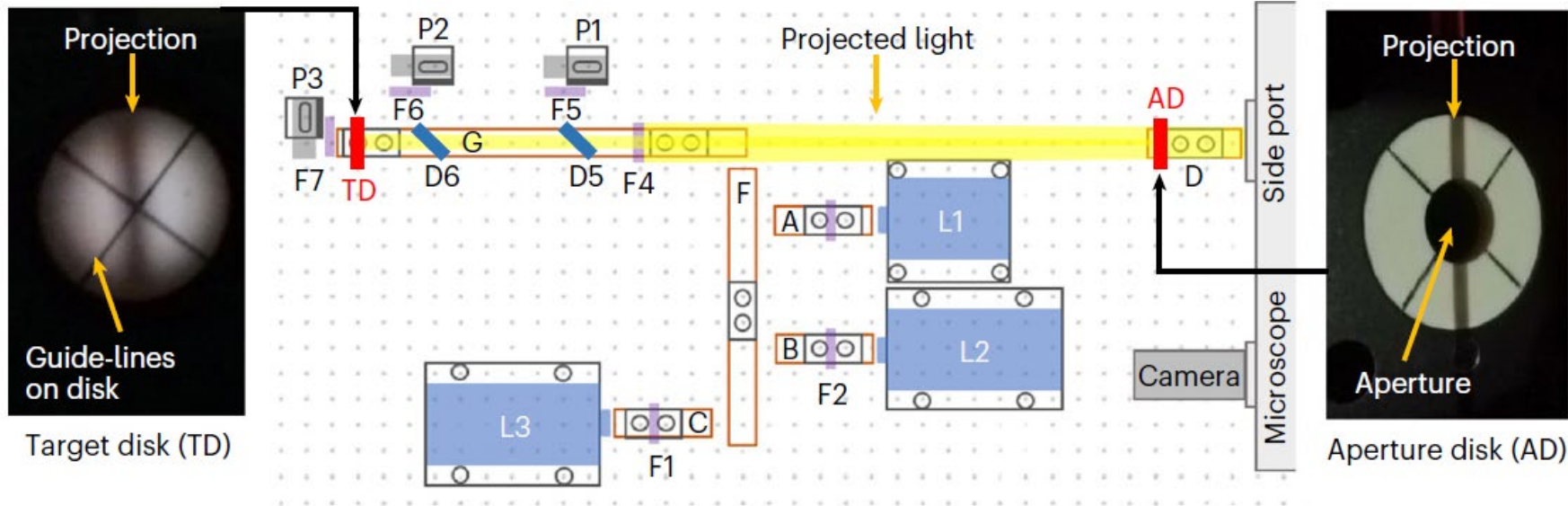
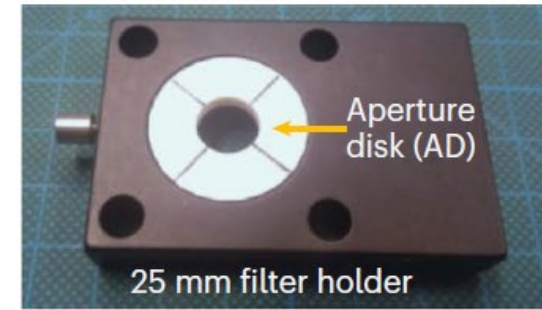
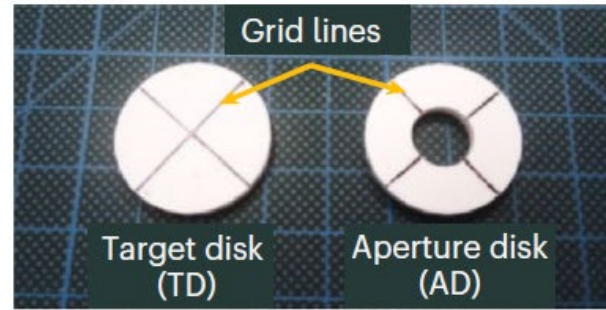
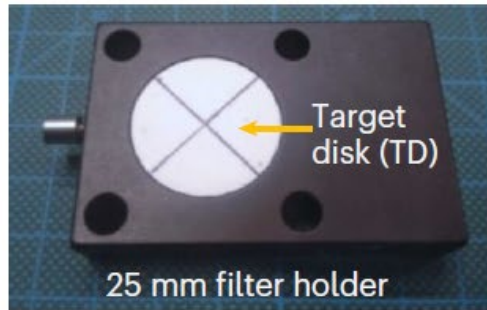
- **excite** different fluorophores (e.g., within droplets)
 - **direct** the emission toward the photosensors
-
- **alignment** = directing the beam so that it follows a predetermined path



Alignment tools

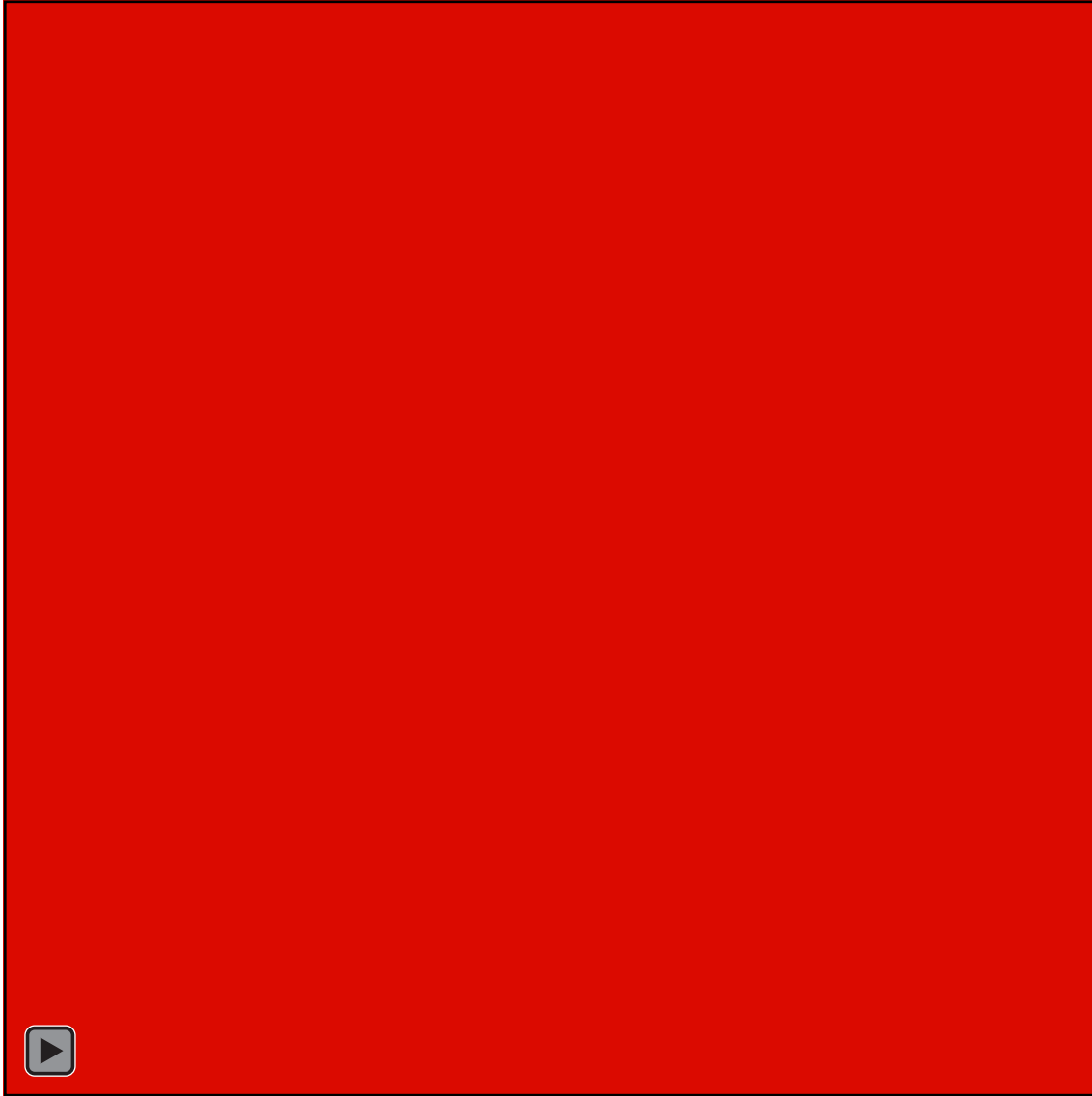
1.) emission light alignment

reduced diameter of beam



! microscope is fixed now !

Excitation light alignment (Panwar et al., 2023)

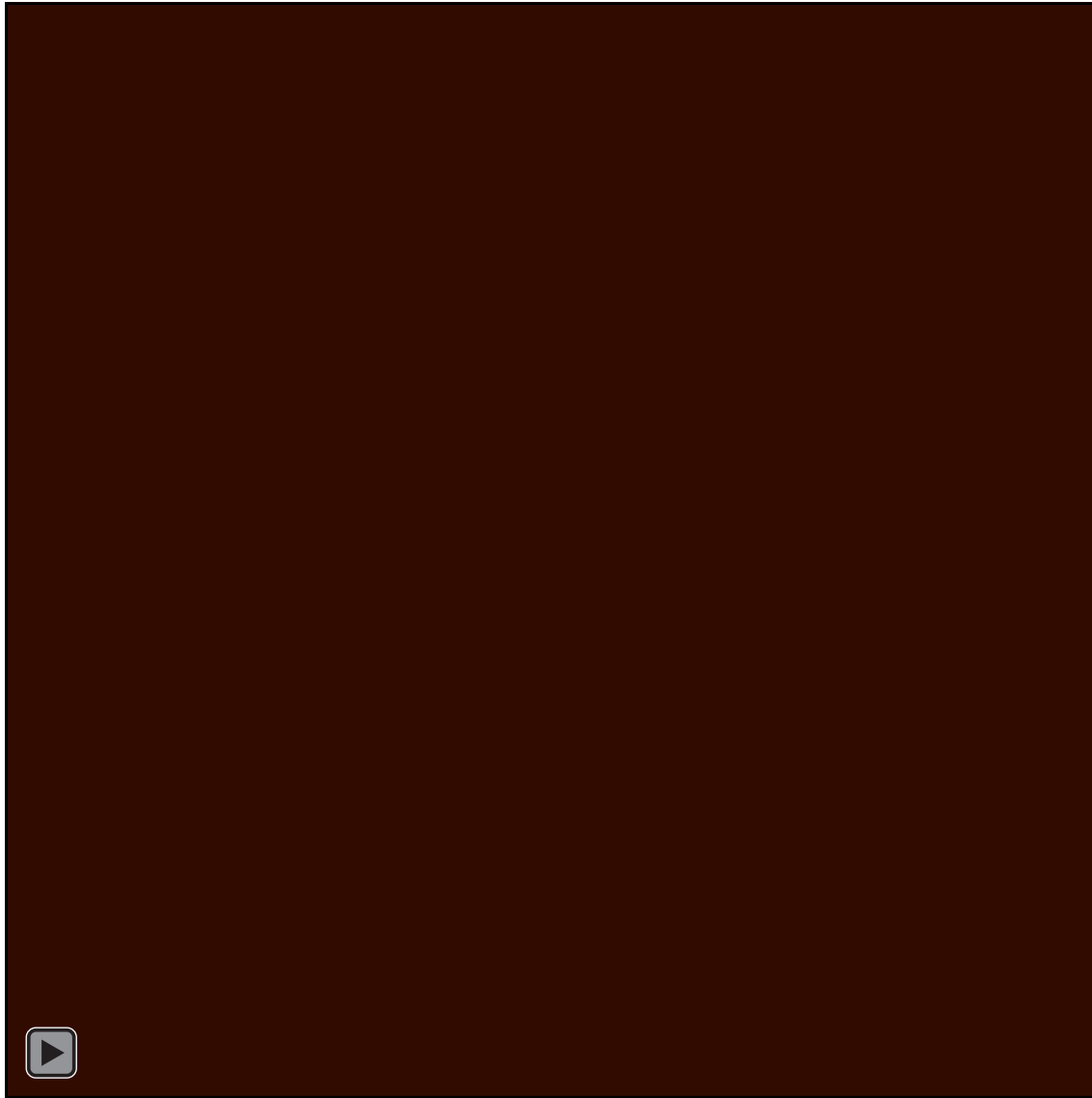


VIDEOS:

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

Most relevant: 55'' and 2'55''

Emission light alignment (Panwar et al., 2023)

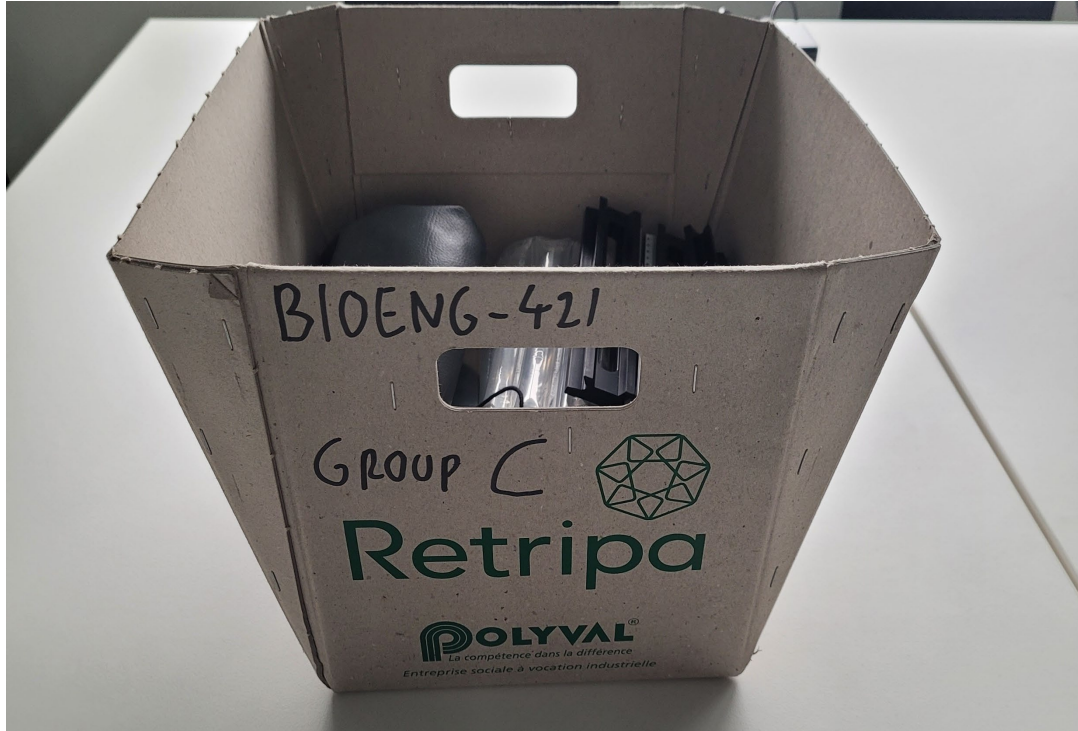


VIDEOS:

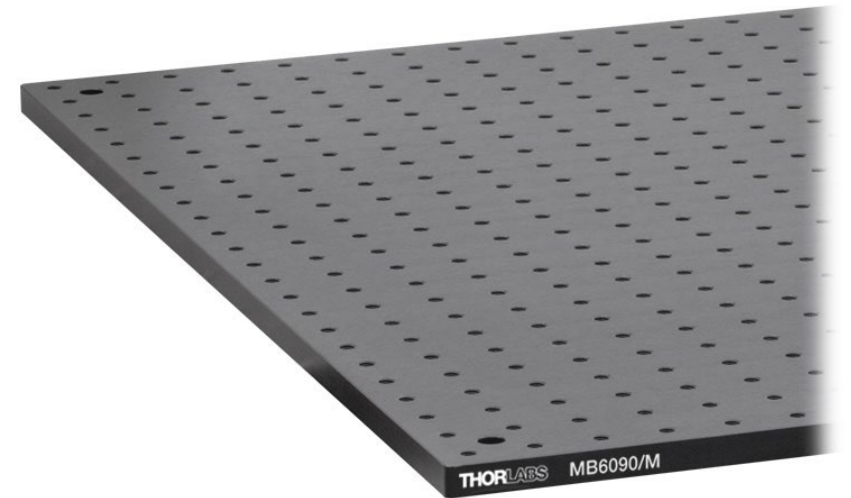
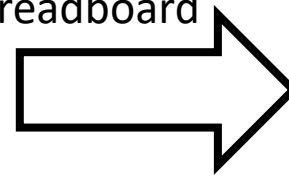
<https://www.nature.com/articles/s41596-022-00796-2#Sec75>

Most relevant: 2'40'' and onwards

Your course inventory



assemble on
breadboard



Schedule for Tuesday practical sessions:

8.15-10am: Group A (473nm) & B (561nm)

10.15 – noon: Group C (473nm) & D (561nm)

Please be on time in front of the DLL labs in the MED building!

Your course inventory



Safety goggles

connectors



PMT



PMT mount



filter
mount for PMT



2x riders for
mirror mounts



473nm OR 561nm laser



2x rail
mounts



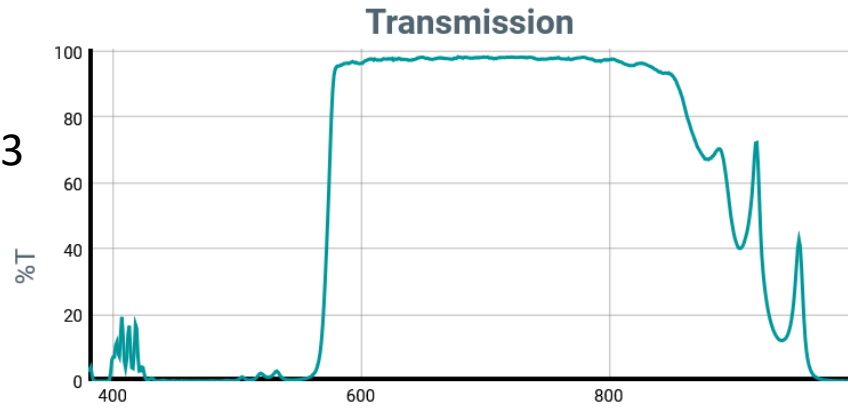
2x 20cm rail
1x 30cm rail



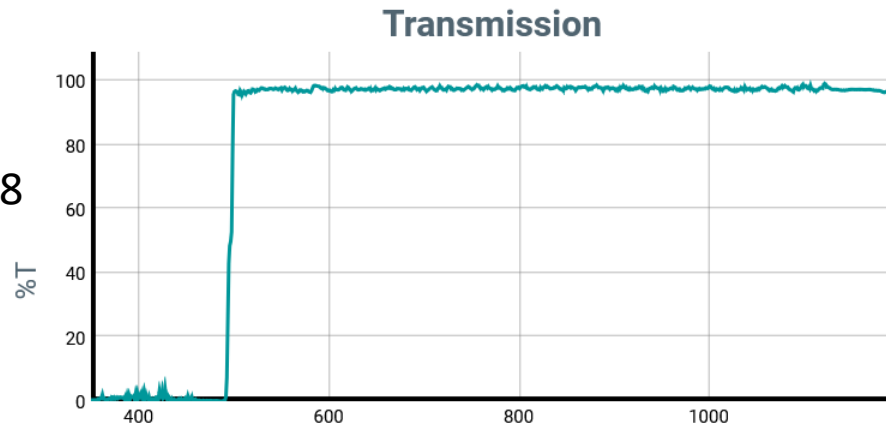
+ filters & mirrors (details on next slide)
+ screws, wires, crimps & alignment tools

Mirrors (already mounted)

Dichroic F48-553

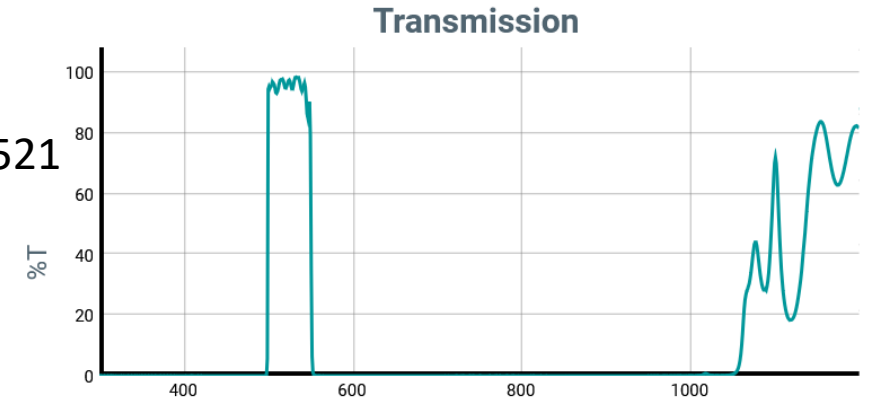


Dichroic F38-488

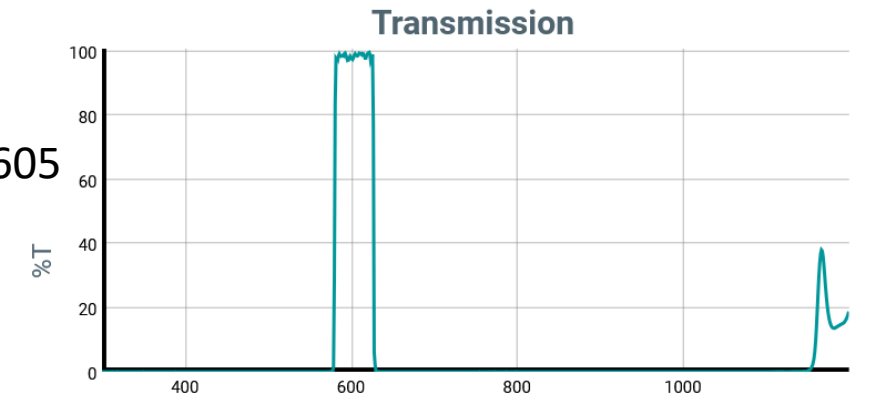


Filters

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 Tuesday 21st, 23:59h (send an email to all teachers)
(so that it can be approved before the practical session on Tuesday)

Questions?



References and further literature

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

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^{1,3}

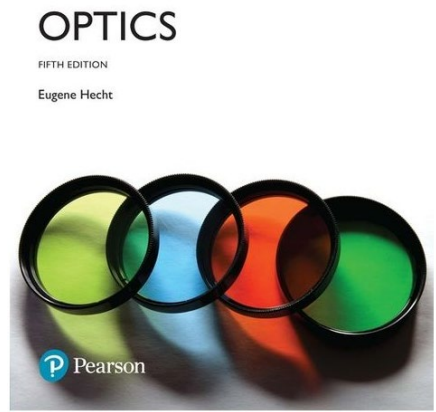
Droplet microfluidics has revolutionized quantitative high-throughput bioassays and screening, especially in the field of single-cell analysis where applications include cell characterization, antibody discovery and directed evolution. However, droplet microfluidic platforms capable of alternative, fluorescence-based analysis and sorting are still mostly found in specialized labs, because their setup is complex. Complementary to conventional FACS, microfluidic droplet sorters allow the screening of cell libraries for essential factors, or even for the effects of selected or screened 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 42 working hours by non-specialists. 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 lightfield 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 bearing 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 monodisperged droplets of small volumes (10⁻¹⁰ to 10⁻¹⁶ liters (nl)) at high frequencies, reaching up to a few million droplets per hour (ref. 3). These sizes and rates of formation enable single-cell encapsulation with each droplet being its own compartment (compatible to a microfluidic microreactor plate well). By changing the droplet matrix, single cells can be encapsulated with other molecules/beads to form a desired cell type (for protein interaction) making each droplet a microreactor and allowing high-throughput screening of these reactions. Such screening methods are commonly used in antibody or drug discovery experiments to test drug screening antibodies⁴. But other very arbitrary effects on enzymatic activity targets⁵ or bind to a second, co-encapsulated target cell^{6,7}. These methods can also be helpful in deciphering cellular signaling by changing single cells with distinct catalytic activities^{8,9}. In the field of directed evolution, droplet sorting has enabled the selection of enzyme variants with 10–100 times higher catalytic activity¹⁰ and of aptamers with distinct properties for targeting cellular RNA^{11,12}. Other applications include microbioassay studies to detect and sort rare microbe-like populations^{13,14} and to discover new protein-coding regions¹⁵. Overall, droplet microfluidics-based single-cell screening gains remarkable momentum in the life science domain.

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

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EAN: 9781292096964

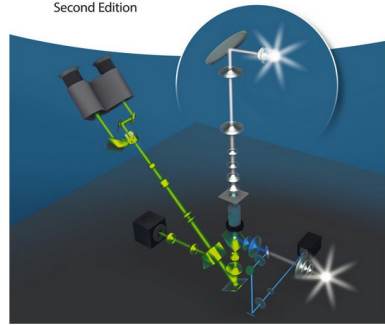
Hecht, Eugene, *Optics, Global Edition*, 5th edition, Harlow, Pearson, 2016

WILEY-VCH

Edited by Ulrich Kubitscheck

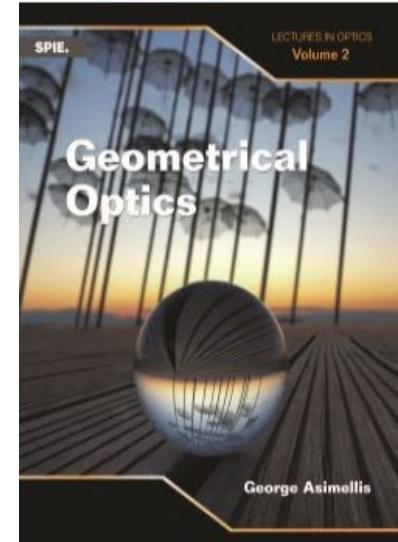
Fluorescence Microscopy

From Principles to Biological Applications
 Second Edition



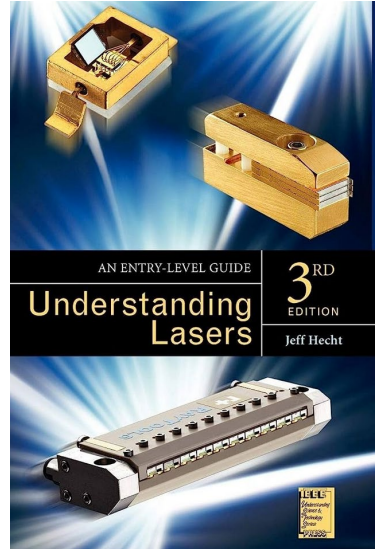
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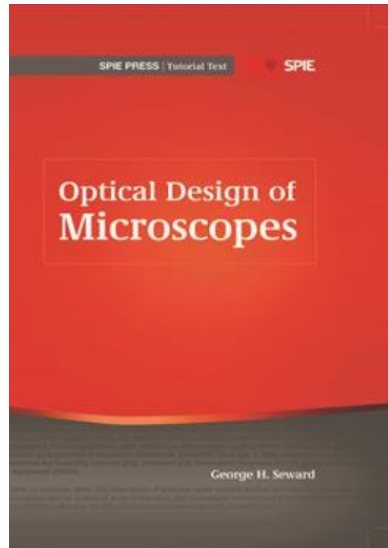
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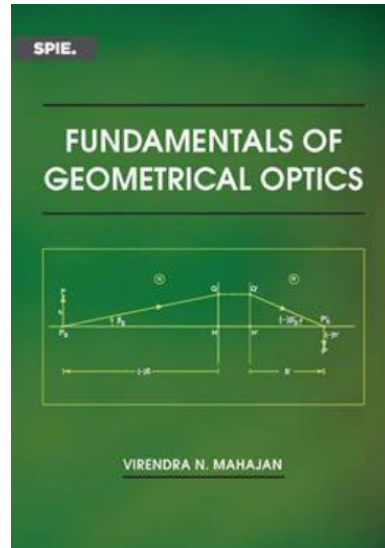
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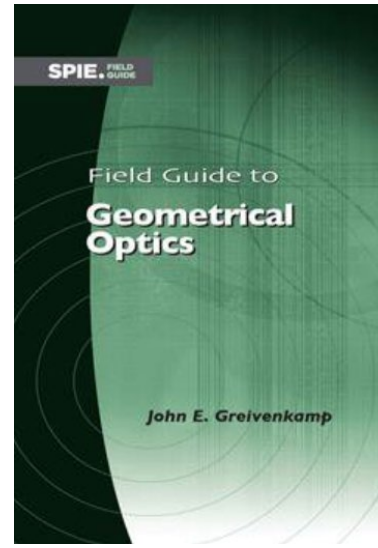
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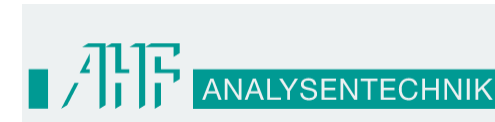


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