



Laboratory Techniques and Medical Device Approvals

Part Gauglitz: Novel Bioanalytical Approaches
Part I: Detection and Sensor Principles



Necessary basic knowledge

- Elektromagnetic radiation
- Interaction radiation/matter
 - Absorption
 - Fluorescence
 - Reflectance
- Principles of optics
- Basics in electrochemistry
- Semiconductors
- Biosensors
- Biomolecular interaction analysis
- Fundamentals in kinetics
- Data evaluation and statistics
- Some fundamental aspects of analytics
- Basic mathematics: differential equations, integration
- Lecture notes: Modul 4.5 Bachelor „Medizintechnik: Biosensoren“ des Studienganges Stuttgart/Tübingen

Point of Care

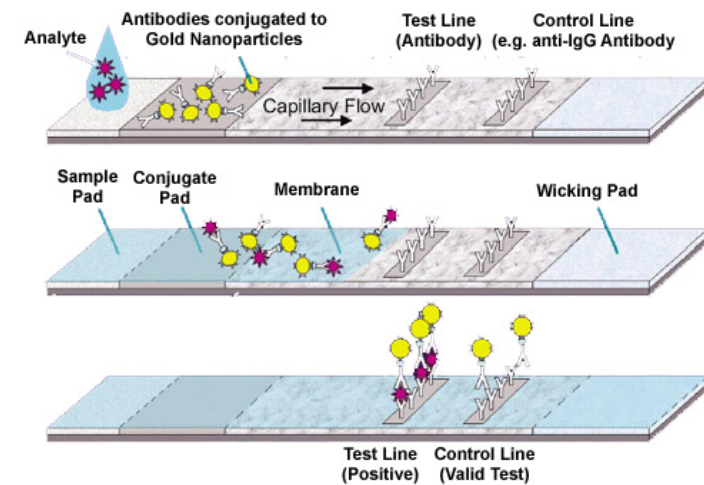


clinchem.med.uni-goettingen.de

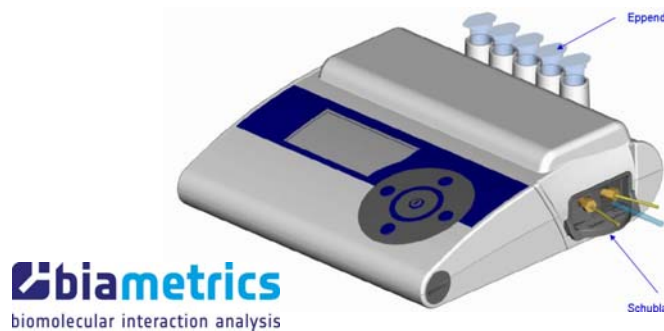


<http://www.fahrzeugbilder.de>

Lateral Flow Assay Architecture



<http://www.cytodiagnostics.com/lateral-flow-immunoassays.php>





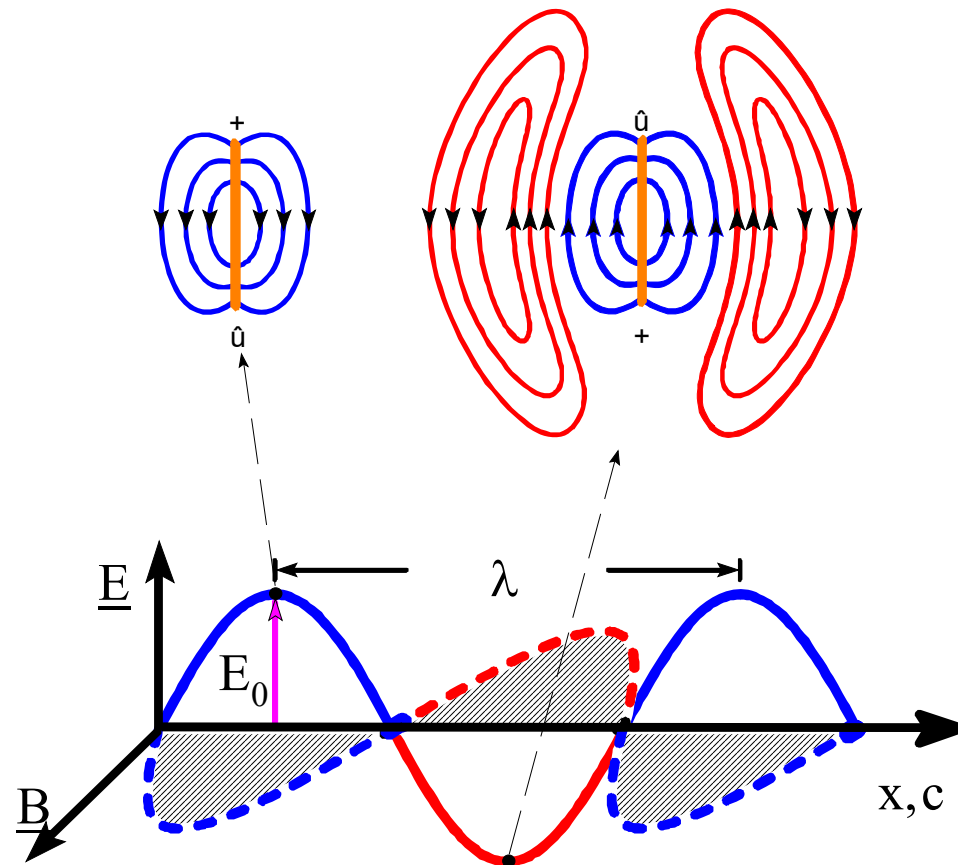
Basics in Spectroscopy

- Radiation
- Interaction radiation – matter
- Instruments



Electromagnetic radiation

- Electric and magnetic field vector are perpendicular to each other and to the direction of propagation
- At origin of radiation this dipole radiation has a phase difference of $\pi/2$ (near field)
- Beginning with a distance of $2 - 4 \lambda$ of this point both the field vectors are in phase – as long there is no interaction with matter (vacuum)
- Perpendicular to the direction of the dipole the magnetic field propagates as a ring
- deBroglie:
Dualism of *particle* und *wave*

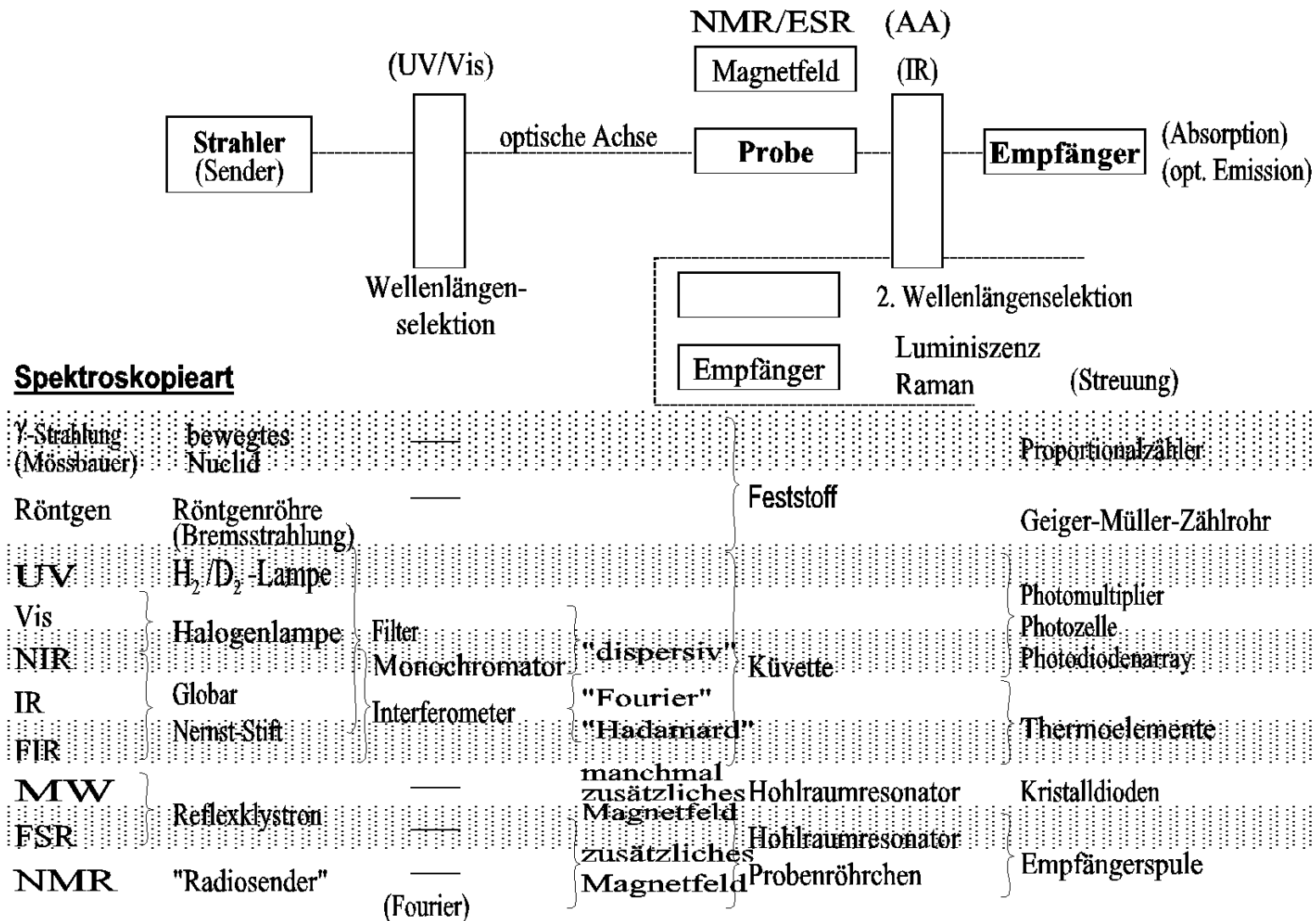




- Amplitude
 - Frequency (spectra)
 - Phase
 - State of polarisation
 - Time dependence
-
- 
- Absorption/Transmission
 - Emission (Fluorescence, Phosphorescence)
 - Scattering (Rayleigh, Raman)
 - Reflection
 - Refraction



Different spectroscopy techniques



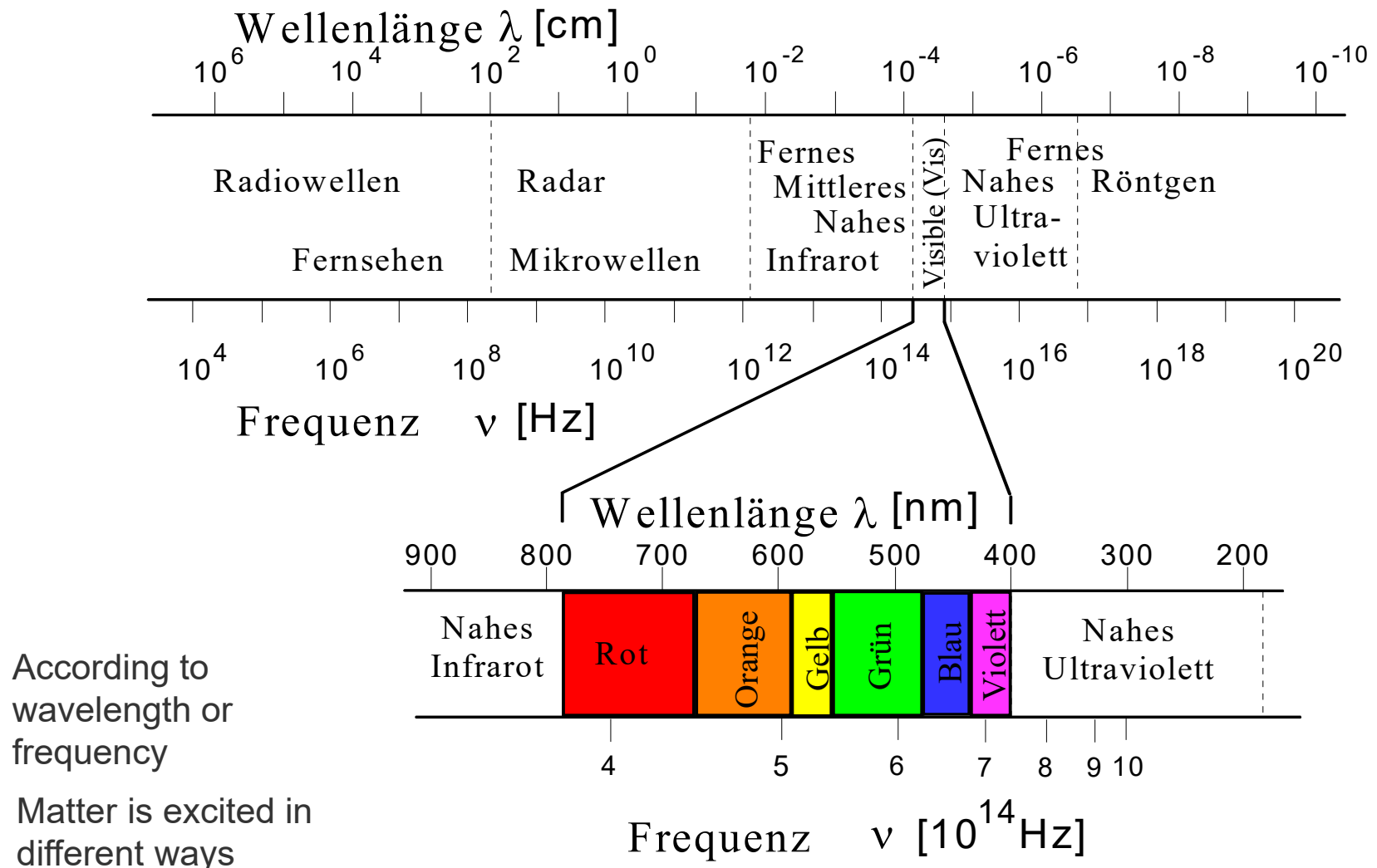


Spectral areas

Bereich	WL [nm]	Freq. [Hz]	WZ [cm ⁻¹]	Energie KJmol ⁻¹	Lichtquelle (Sender)	Dispersion	Empfänger
NMR (Kernspin)	10 ¹⁰ (10 m)	3 · 10 ⁷	10 ⁻³	1,2 · 10 ⁻⁵	Schwingkreise	FFT	Spule
ESR (Elektronenspin)	10 ⁸ (10 cm)	3 · 10 ⁹	10 ⁻¹	1,2 · 10 ⁻³	Klystron	Magnet	Diode
MW Rotation	10 ⁸	„	„	„	Klystron	Frequenzdiode	Elektronik (Resonanzkreis)
IR Atomschw	10 ⁴	3 · 10 ¹³	10 ³	12	Globar	Gitter	Thermoelement
Vis π Elektronendichte	5 · 10 ² 780 - 380	6 · 10 ¹⁴	2 · 10 ⁴	2,4 · 10 ²	Wolfram- halogen	„	PM Photodiode
UV σ-Elektron	3 · 10 ² 380 - 200	10 ¹⁵	3 · 10 ⁴	4 · 10 ²	H ₂ /D ₂ -Lampe	„	PM
Röntgen Rumpfniveaus	1	3 · 10 ¹⁷	10 ⁷	1,2 · 10 ⁵	Kathode	Braggsche Gleichung	Geiger-Zähler



Optical spectroscopy





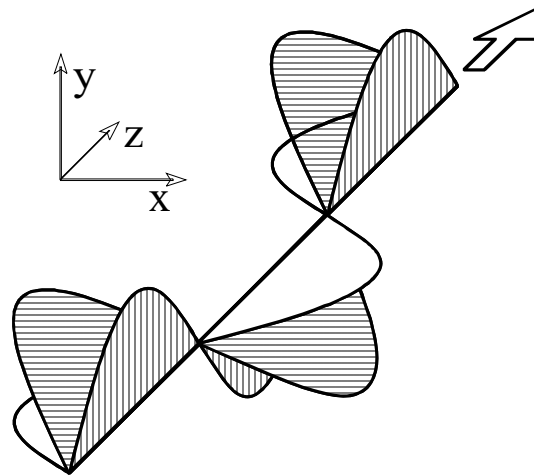
Some data

- UV
 - 200 – 380 nm
 - 200 nm 50000 cm⁻¹ 1,5 10¹⁵ Hz
 - 300 nm
 - ca. 30000 cm⁻¹
 - ca. 1 10¹⁵ Hz
 - ca. 3,72 eV
 - ca. 358 kJ/mol 85,7 kcal/mol
- Vis
 - 380 – 780 nm 5 10¹⁴ Hz
- NIR (IR-A)
 - 780 – 1400 nm 3 10¹⁴ Hz
- IR
 - 1400 – 3000 – (300000)
 - 1000000 nm



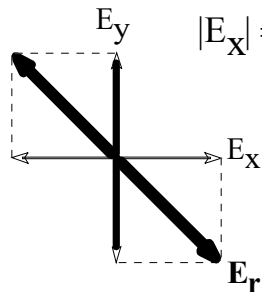
Polarisation

linear

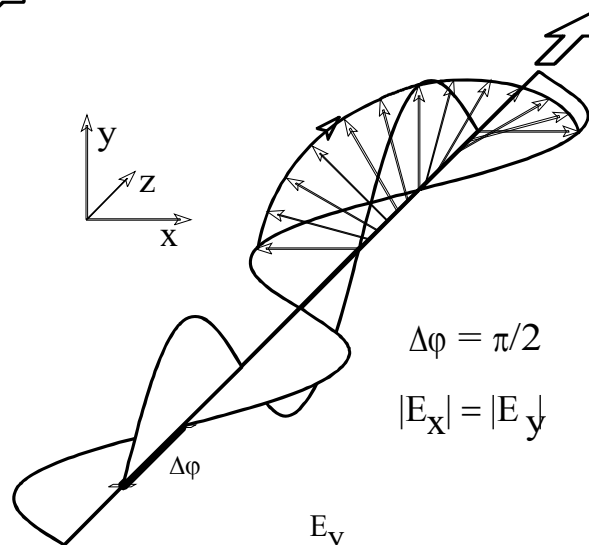


$$\Delta\phi = 0$$

$$|E_x| = |E_y|$$

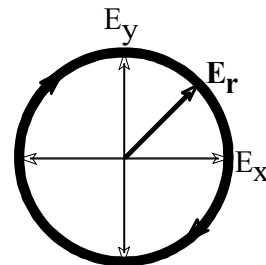


circular



$$\Delta\phi = \pi/2$$

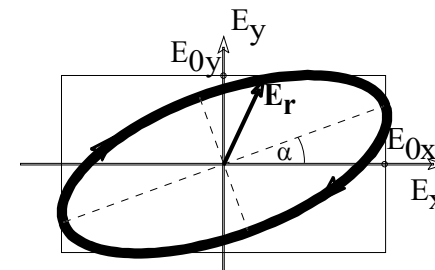
$$|E_x| = |E_y|$$



elliptical

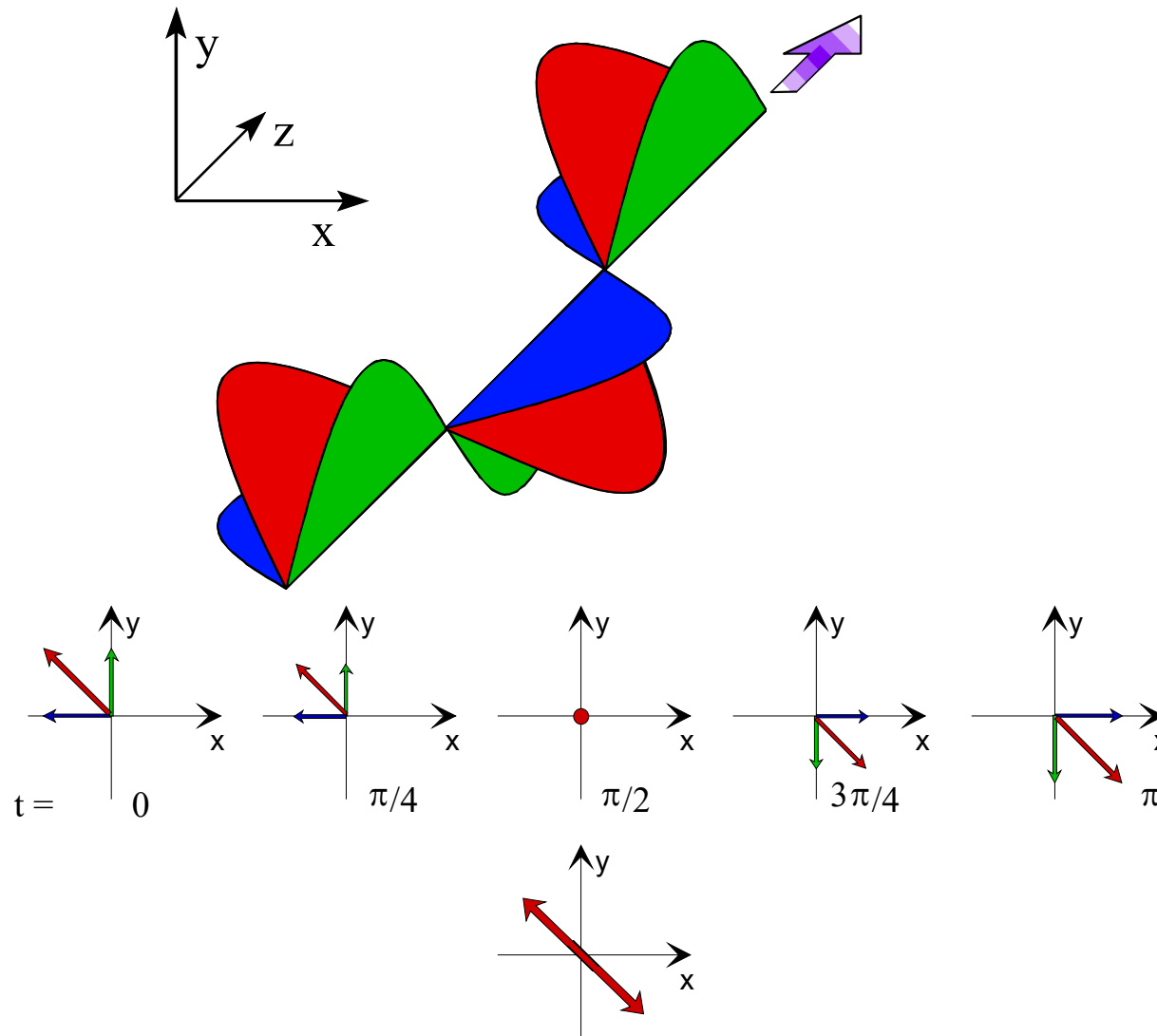
$$\Delta\phi \neq 0$$

$$|E_x| \neq |E_y|$$





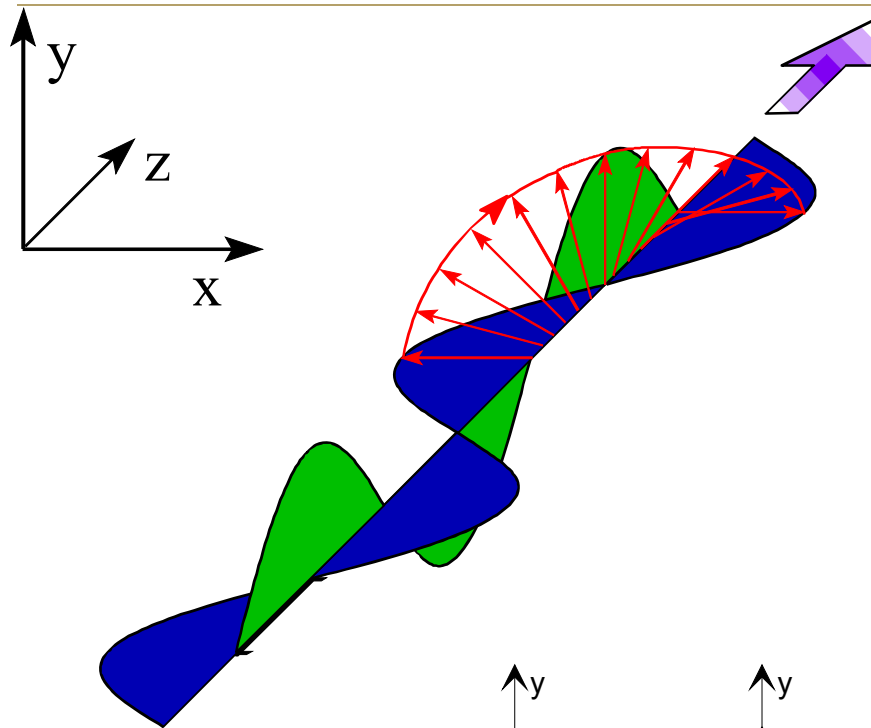
Linearly polarised radiation



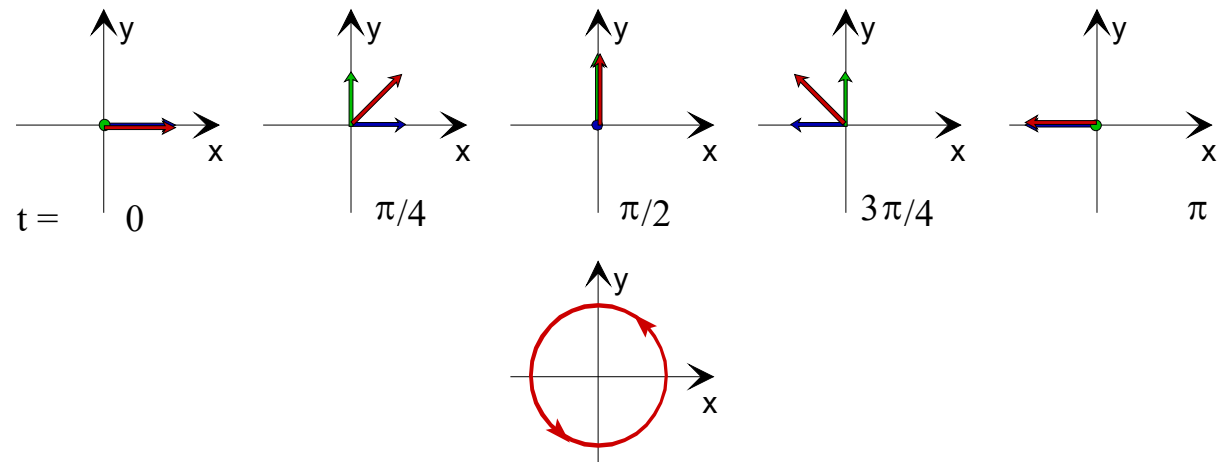
- Two linearly polarised partial beams in phase result in a new linearly polarised radiation
- If both amplitudes are the same the direction of new polarisation is on bisecting line
- Direction of polarisation varies with difference in amplitude
- ORD



circularly polarised radiation

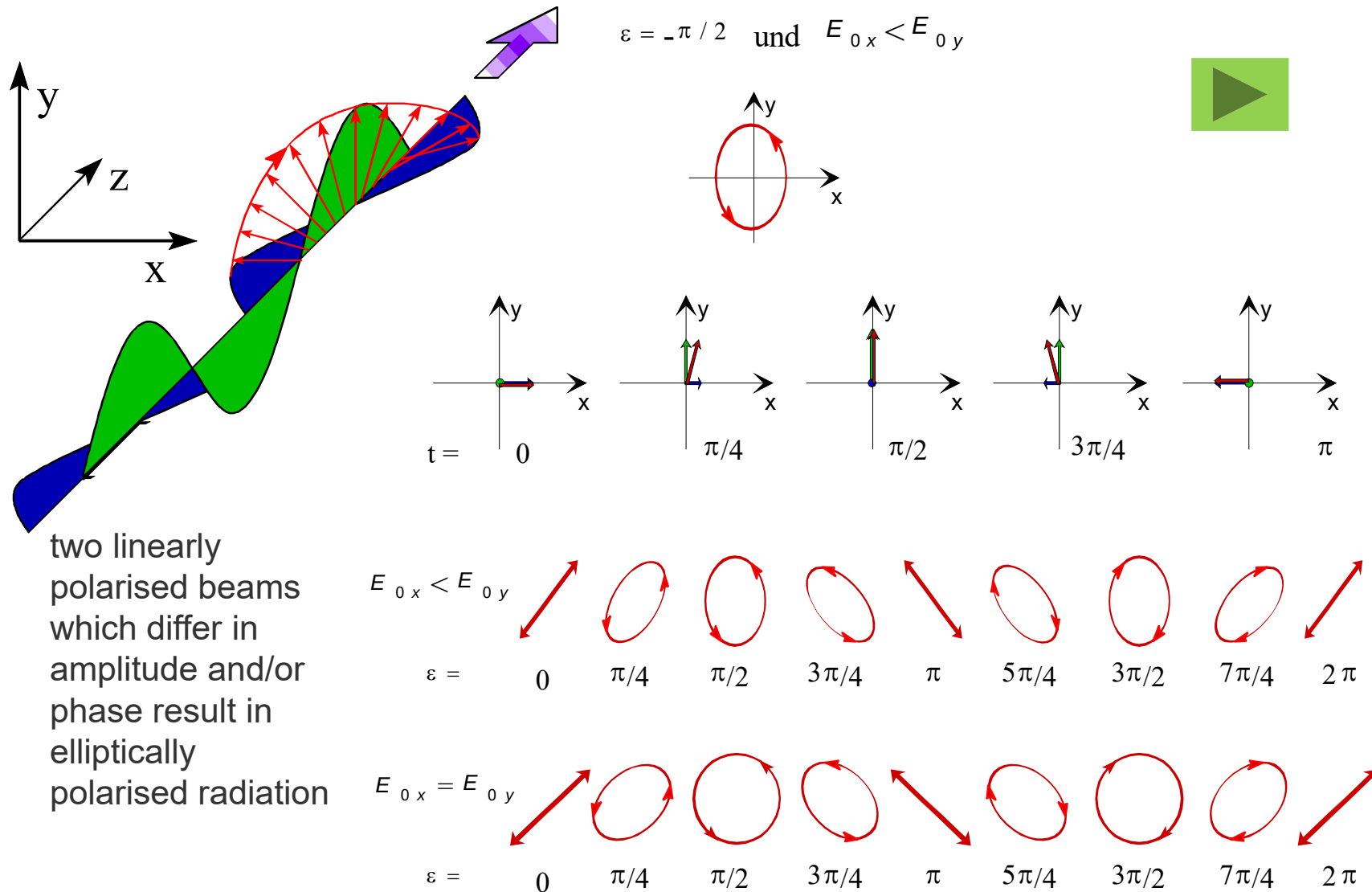


- Two linear polarised beams with equal amplitude and $\pi/2$ difference in phase result in circularly polarised radiation





Circularly and elliptically polarised radiation





Optical rotation-dispersion and radiation

specific rotation

$$[\alpha]_{\lambda} = \frac{\alpha_{\lambda}}{d \cdot c}$$

molar rotation

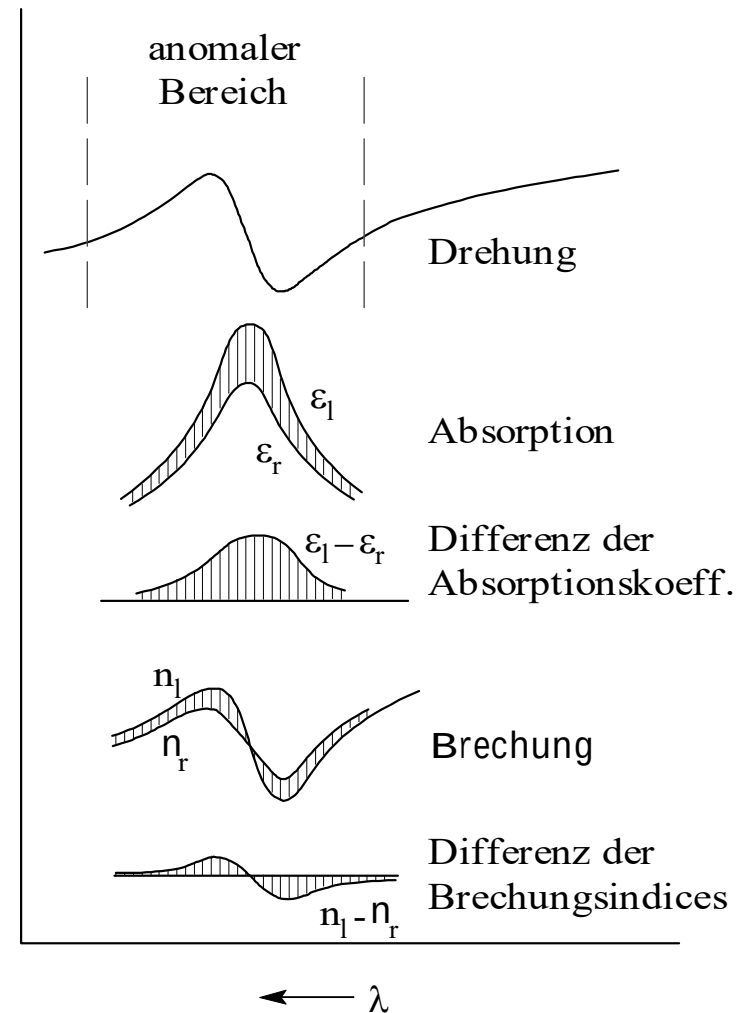
$$[\Phi]_{\lambda} = \frac{[\alpha]_{\lambda} \cdot M}{100} = \frac{100 \alpha_{\lambda}}{d \cdot c}$$

Cotton-Effect

$$\alpha = \frac{180 \cdot d}{\lambda} (n_e - n_r)$$

$$\Theta = \frac{2,303}{4} \cdot \frac{180}{\pi} (\varepsilon_e - \varepsilon_r) \cdot c \cdot d$$

Ellipsometry
Wafer control





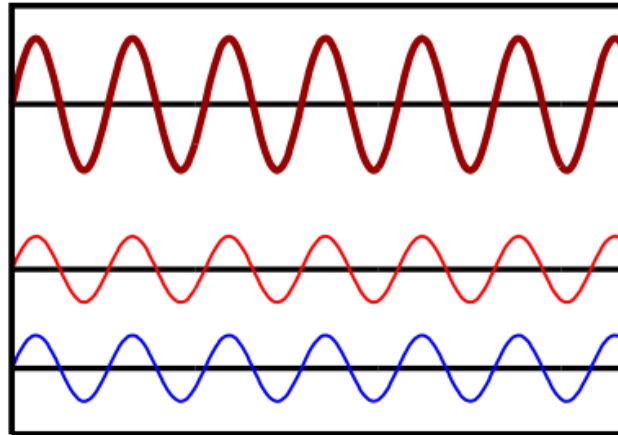
Interference

konstruktive Interferenz

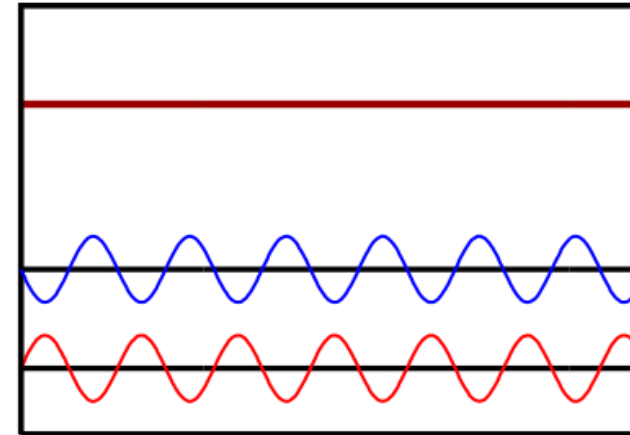
Interferenz-
muster

Welle 1

Welle 2



destruktive Interferenz



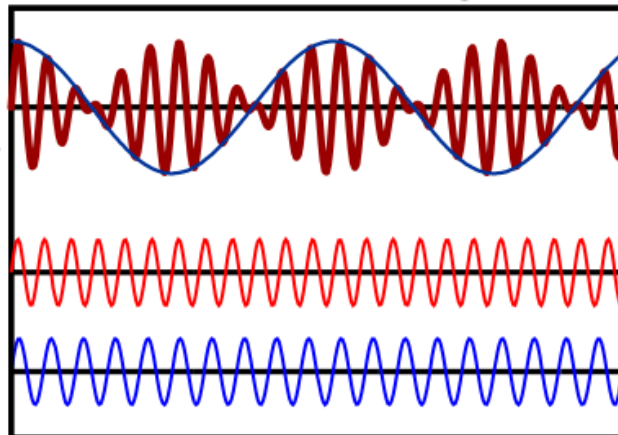
Interferenz mit Schwebung

Interferenz-
muster

Schwebungs-
frequenz

Welle 1

Welle 2



dual slit
grating
Michelson-interferometer
Mach-Zehnder
Young-Interferometer
Reflectometric interference
spectroscopy

phase and amplitude

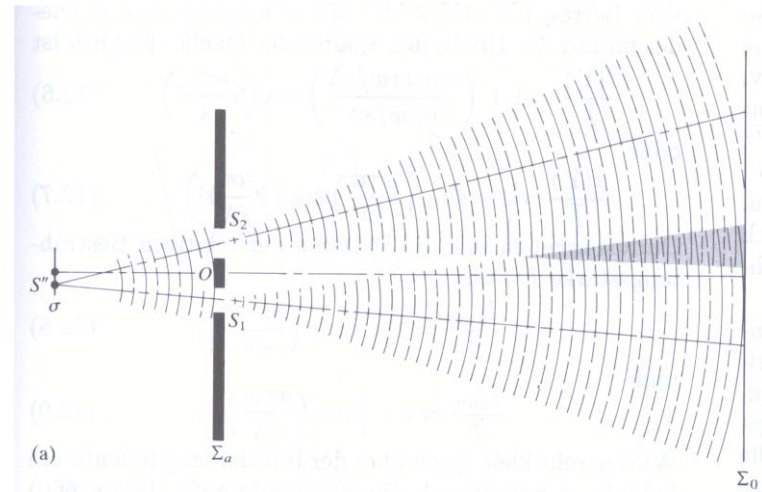
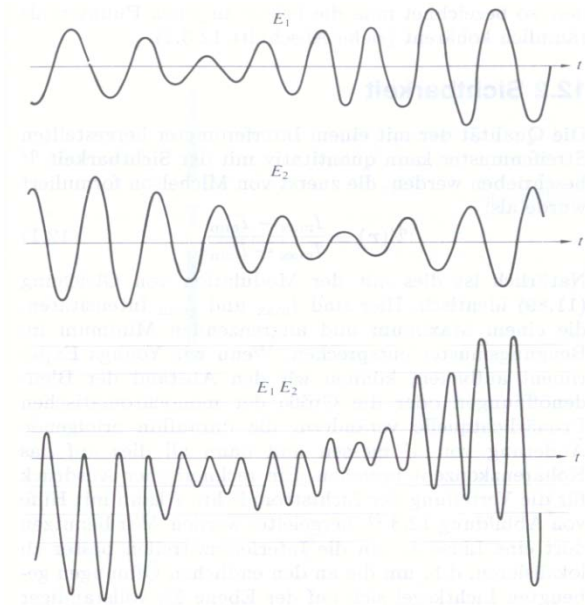


Coherence

- Coherence in time and space

Effects in time: eg. beat frequency

- two points on the same wave front (in e.g. dual slit)
- Dependent by coherence of two waves
- Non correlated result intensity zero as an average





Interaction

radiation - matter



Optical Detection

- Amplitude
- Frequency (spectra)
- Phase
- State of polarisation
- Time dependence



- Absorption/Transmission
- Emission (Fluorescence, Phosphorescence)
- Scattering (Rayleigh, Raman)
- Reflection
- Refraction

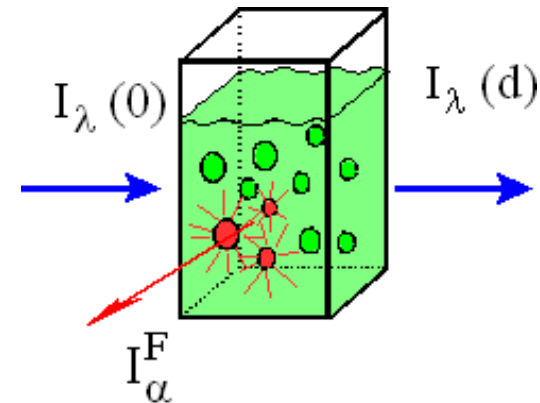
The diagram illustrates the interaction of light with a particle, showing various scattering and absorption processes. A blue wave representing incident light with wavelength λ_0 and intensity I_0 approaches a red hatched circle representing a particle. The particle's diameter is indicated as $d \ll \lambda$. The incident wave's electric field vector E is shown as a vertical double-headed arrow. The wave is partially absorbed, resulting in a transmitted wave with intensity $I_i < I_0$ and wavelength $\lambda_i = \lambda_0$. The absorption process is labeled as **Dispersion** and **Absorption**. The transmitted wave is labeled **Emission** with $\lambda_i < \lambda_0$. The scattered wave is shown as a red wave with wavelength $\lambda_i = \lambda_s$ and intensity $I_i < I_0$. The scattering process is labeled as **scattering** and **Raman**. The diagram also shows a green wave with wavelength $\lambda_i < \lambda_s$ and intensity $I_i < I_0$, labeled **Rayleigh**. The diagram includes a legend for the particle diameter: $d \ll \lambda$ (Rayleigh) and $d \sim \lambda/4$ (Mie).



Classification I: interaction principle

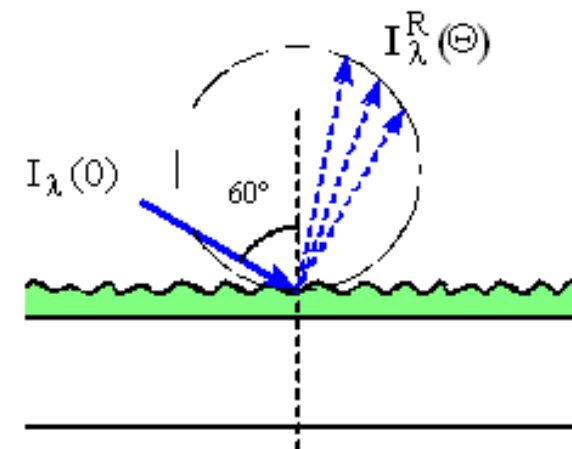
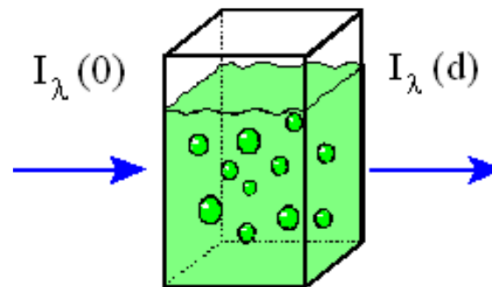
- Absorption/Transmission

$$I_{\lambda}(d) = I_{\lambda}(0) \cdot \exp[d \cdot \varepsilon_{\lambda} \cdot c]$$



- Fluorescence

$$I_{\alpha}^F(t) = I_{\lambda}(x) \cdot \varepsilon_{\lambda} \cdot c(t) \cdot f_{\alpha} \cdot \eta \cdot \Gamma$$



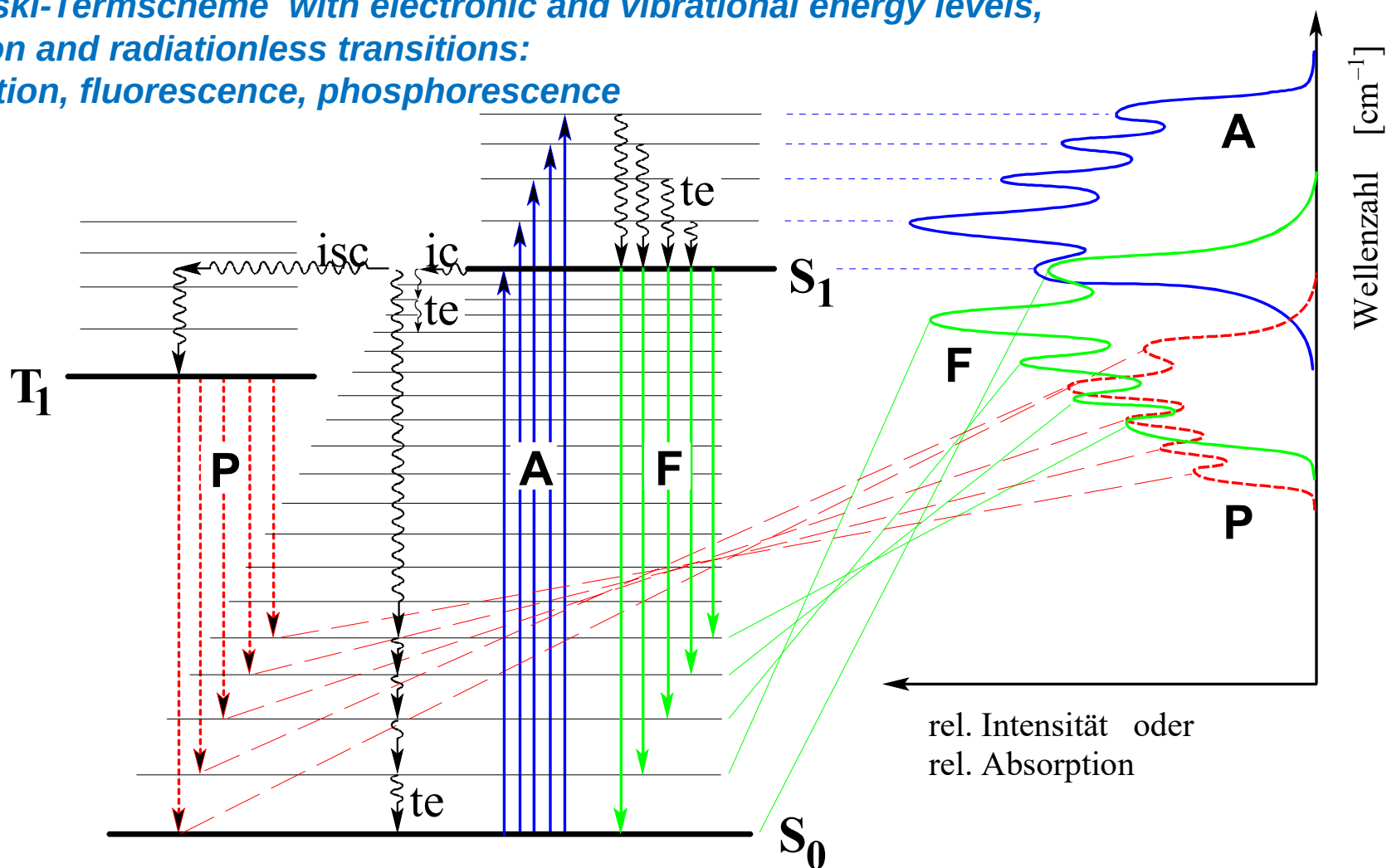
- Reflection, diffuse (remission)

Lambert cosine law



Jablonski-Termscheme

*Jablonski-Termscheme with electronic and vibrational energy levels,
radiation and radiationless transitions:
absorption, fluorescence, phosphorescence*



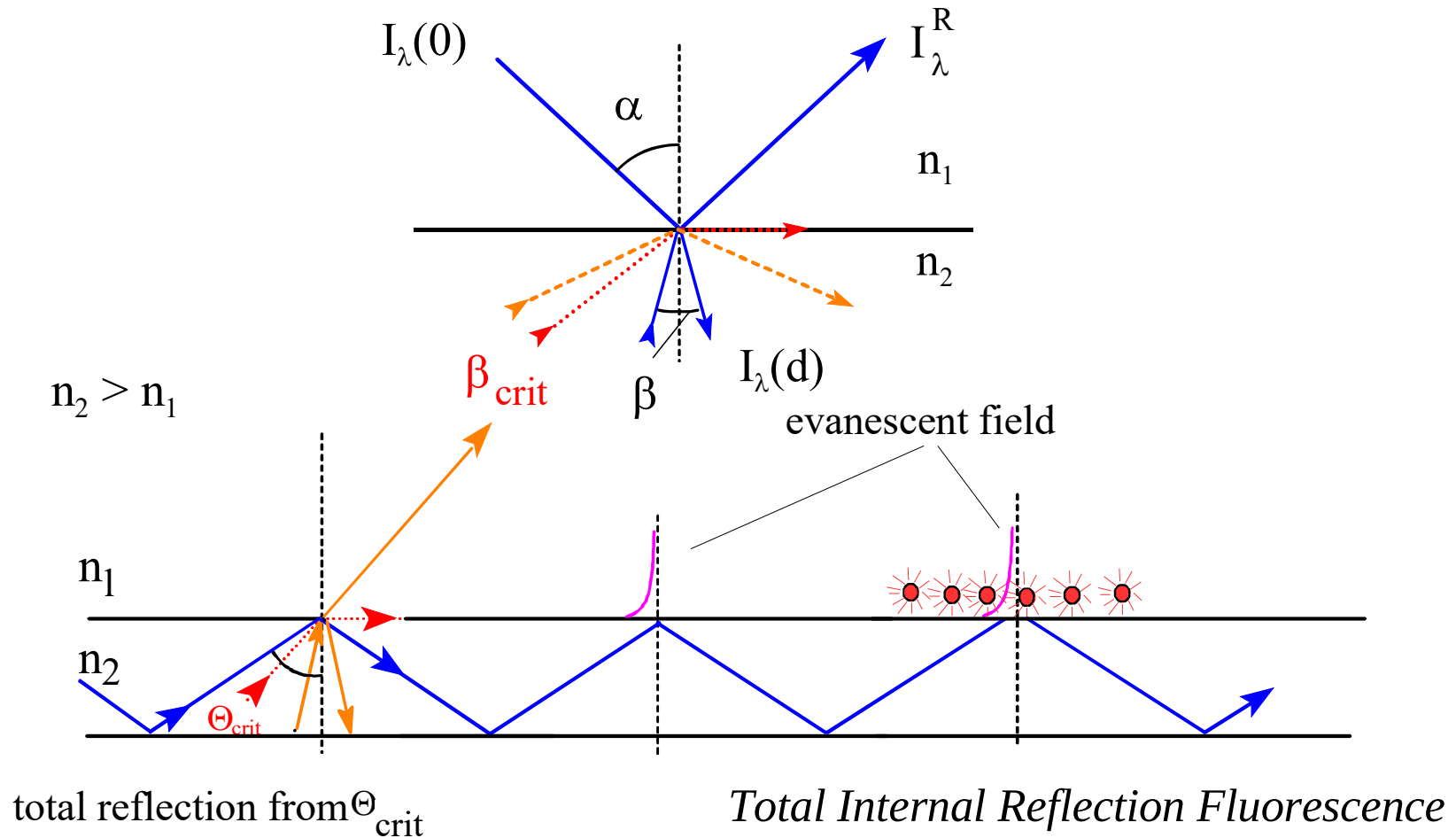


Reflectance

- Reflection regular

$$n_1 < n_2$$

$$\alpha > \beta$$

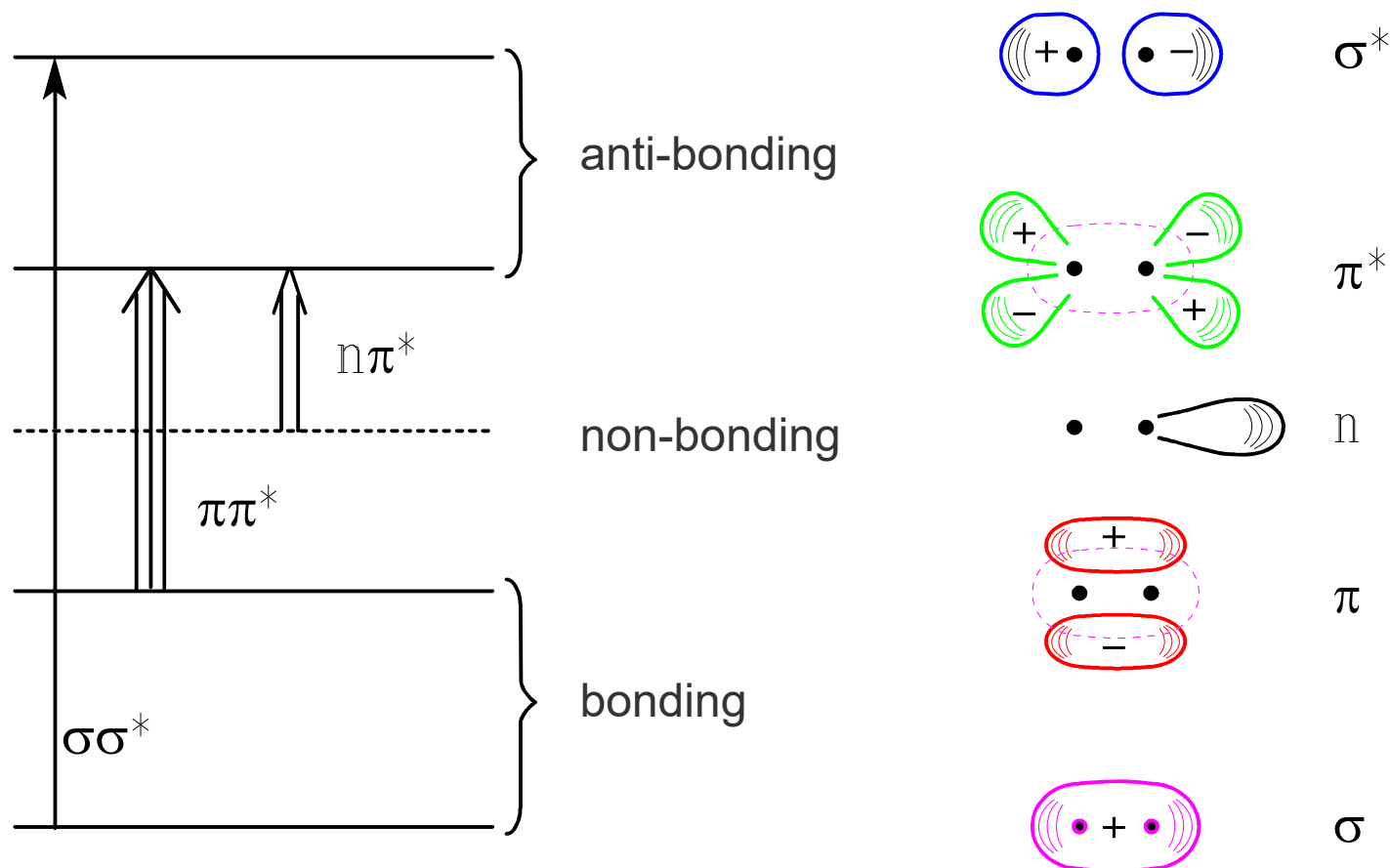




Orbitals and transitions

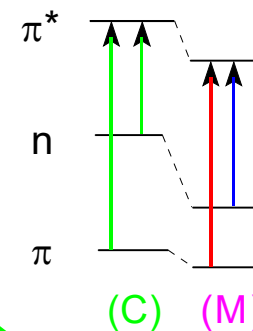
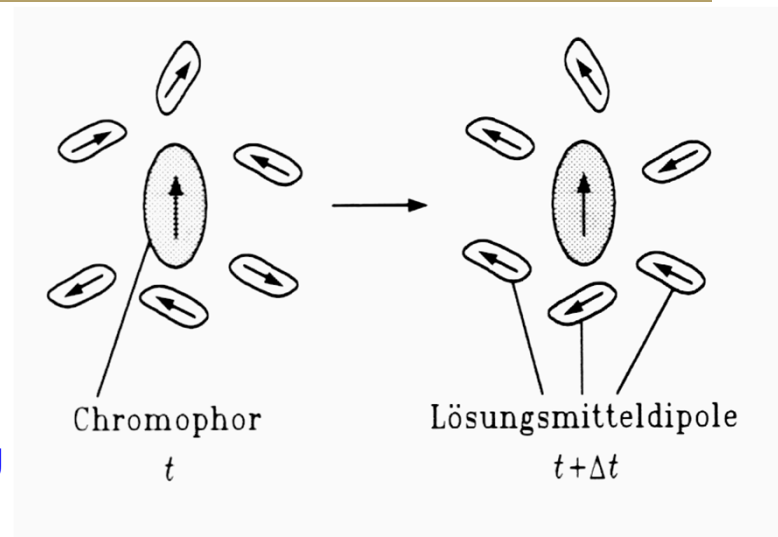
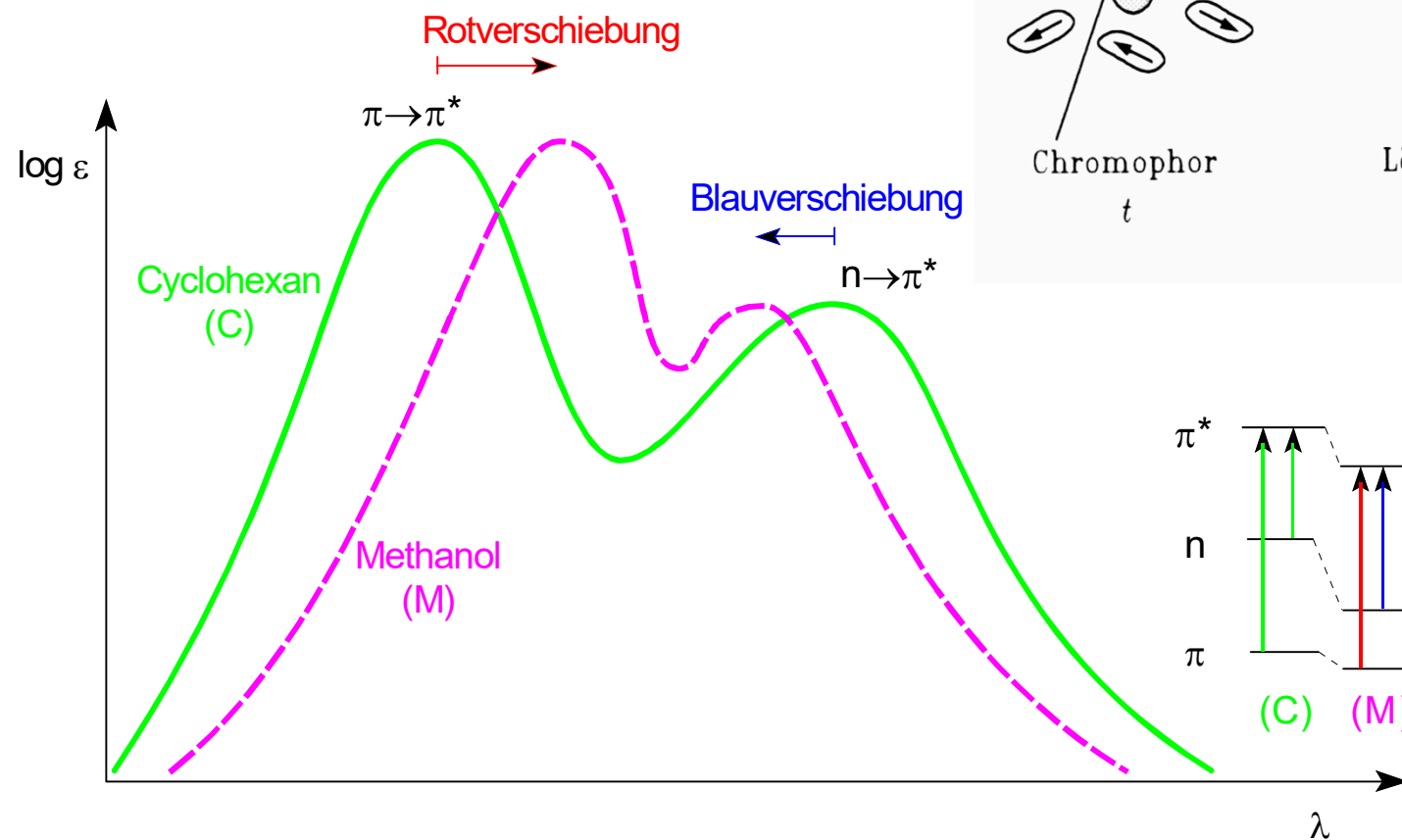
Discussion of orbitals, term symbols for single- and multi-electron states of atoms and molecules.

Possible transitions between states depending on symmetry of states and dipole momentum in transition integral





Band shift in dependence on solvent



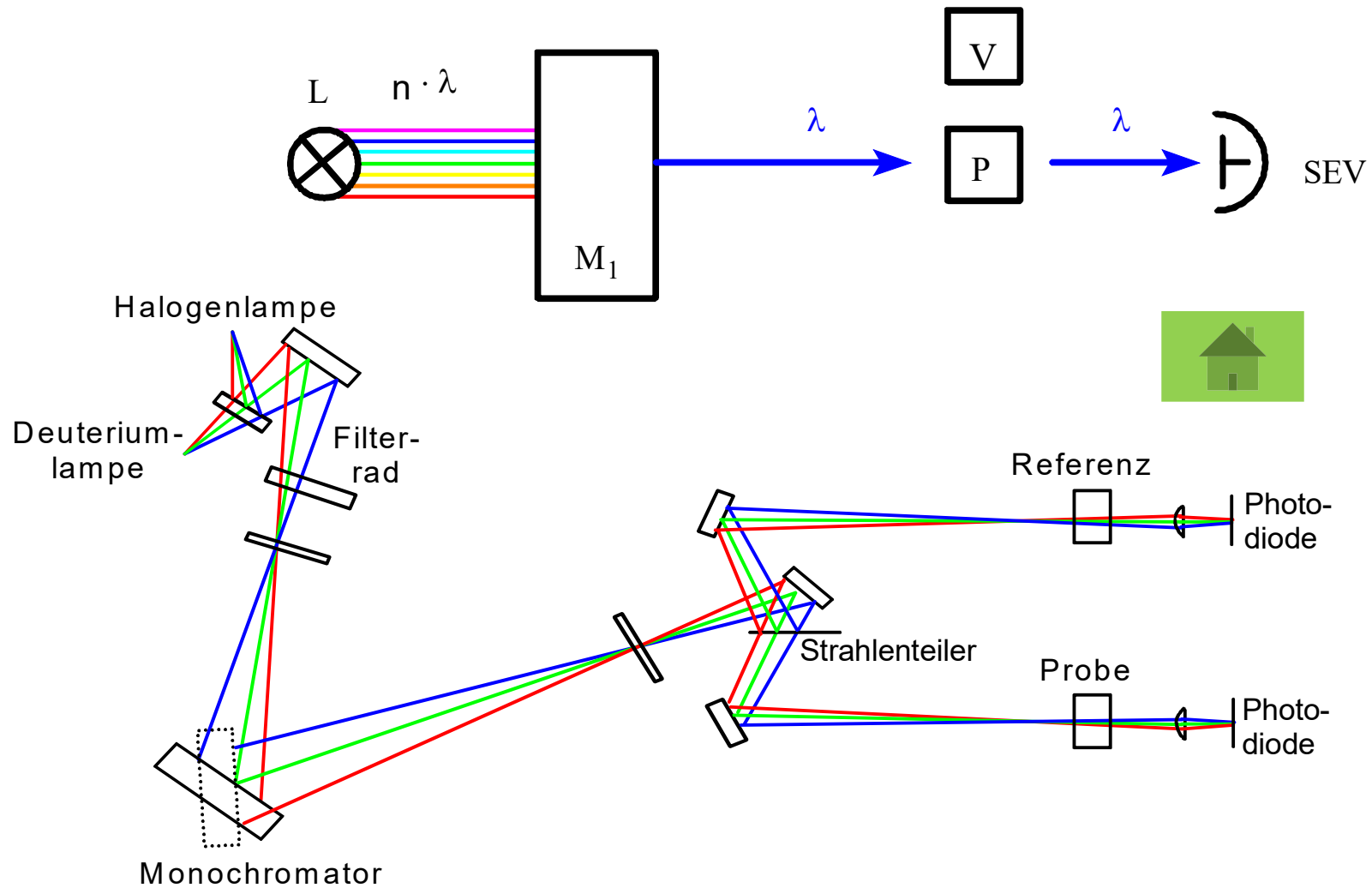


Instrumentation



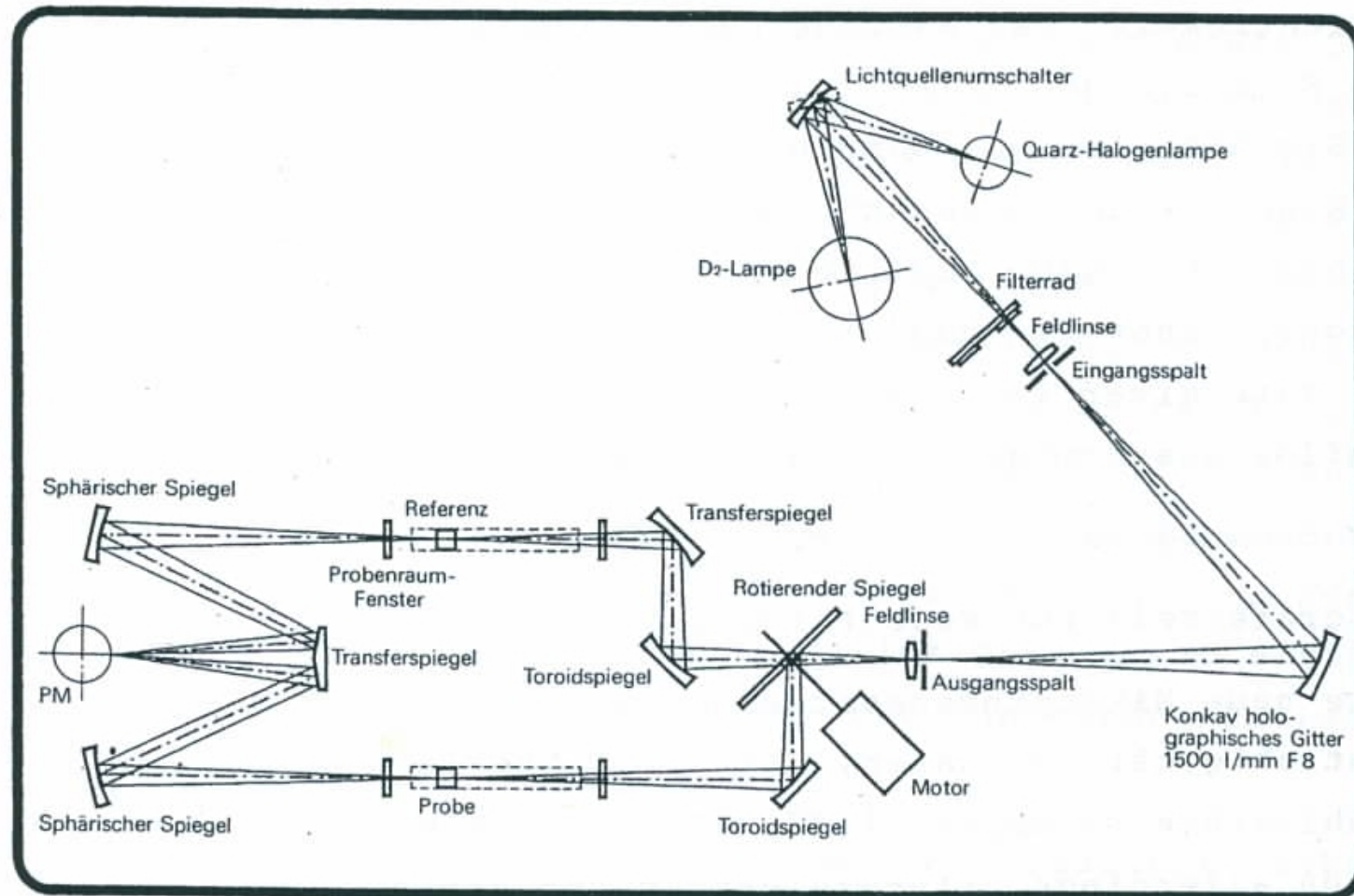
Absorption

Absorption



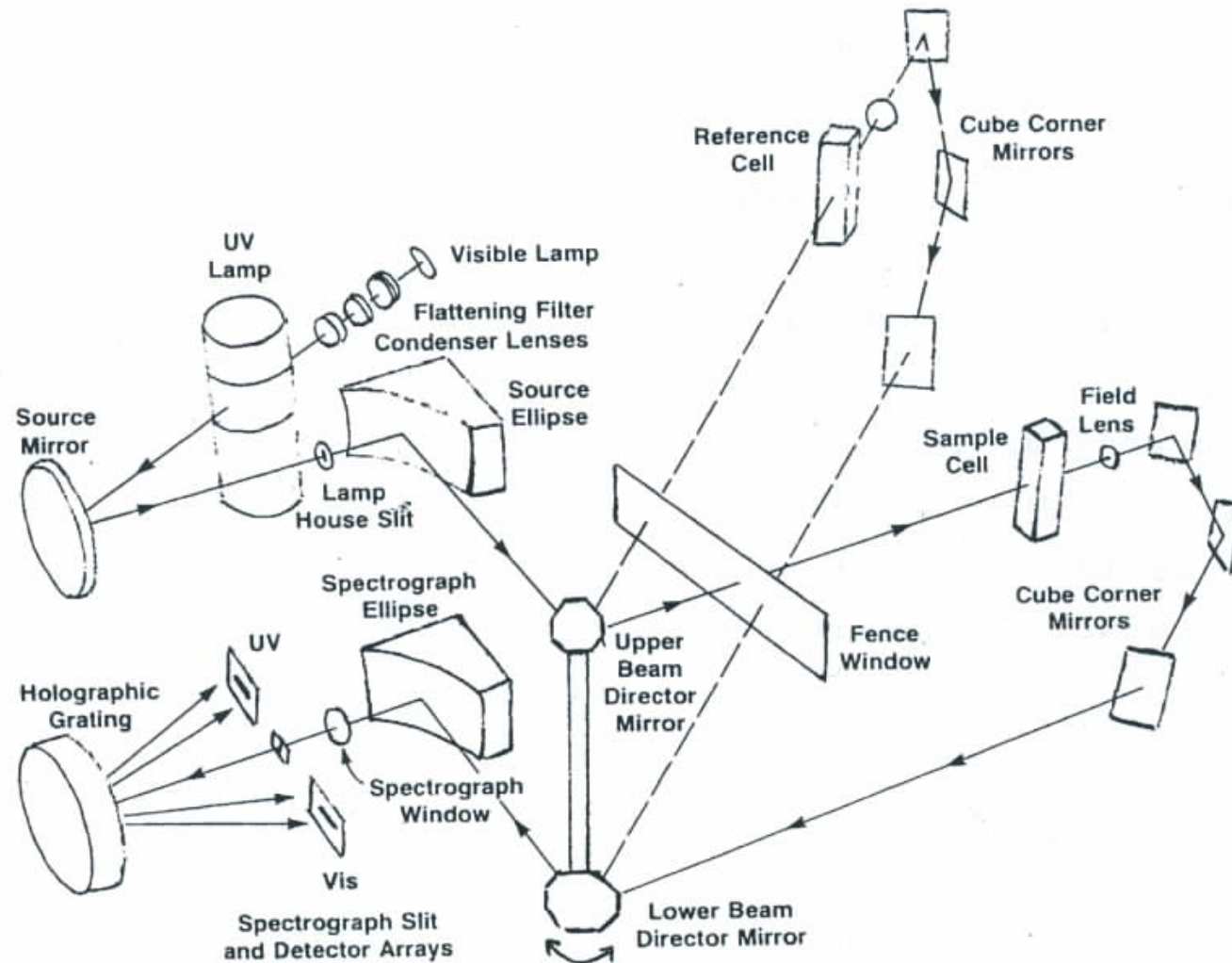


UVIKON 810/820





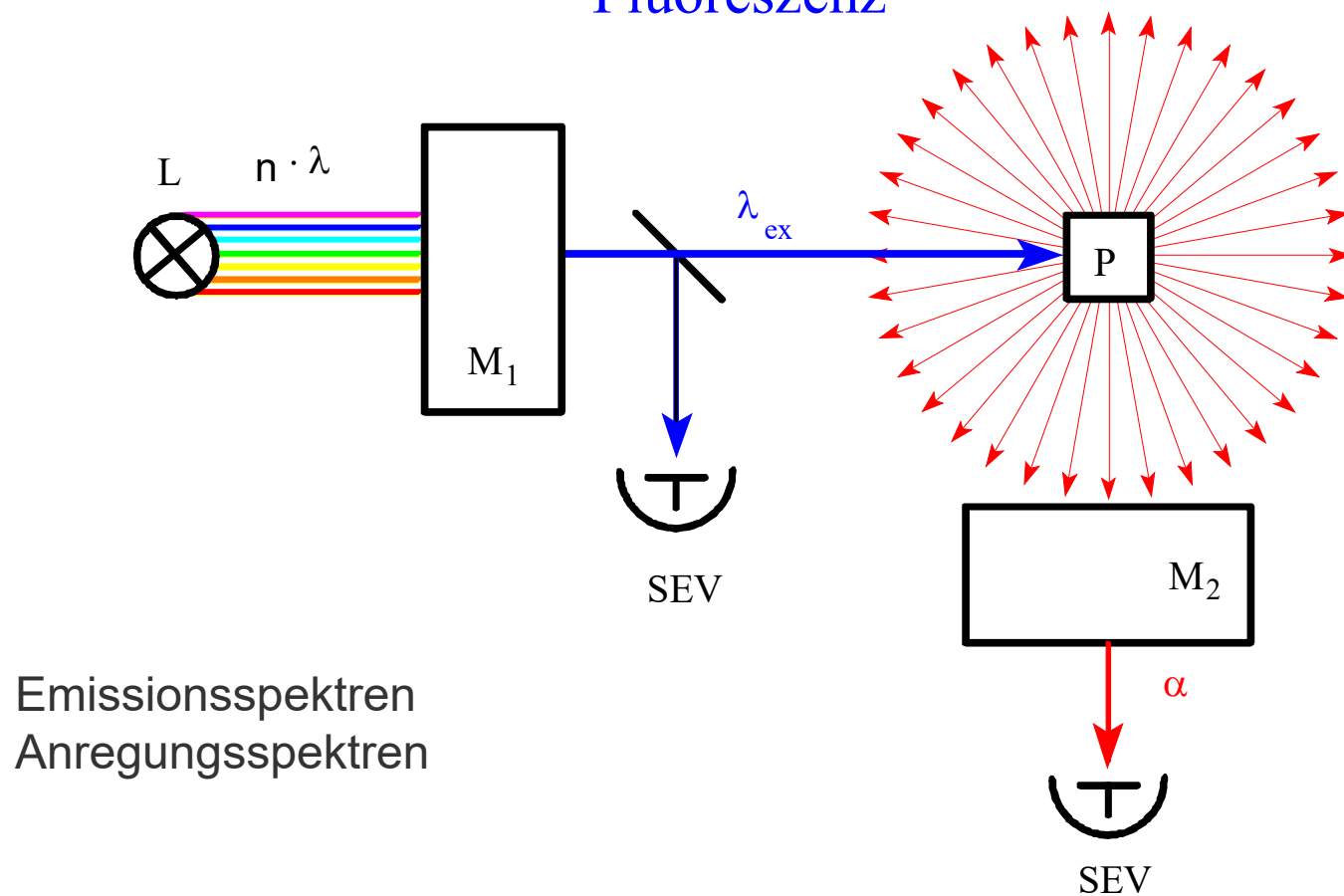
Diodenarray HP 8450A





Fluorescence

Fluoreszenz

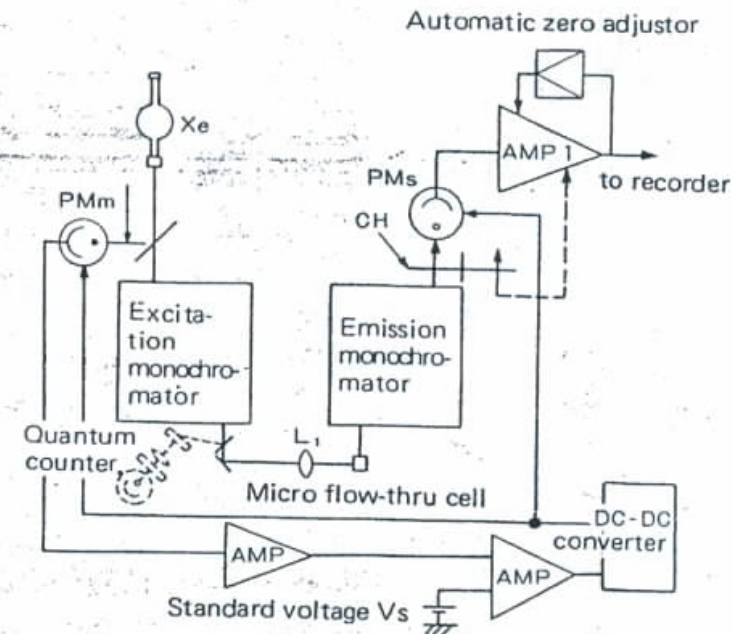
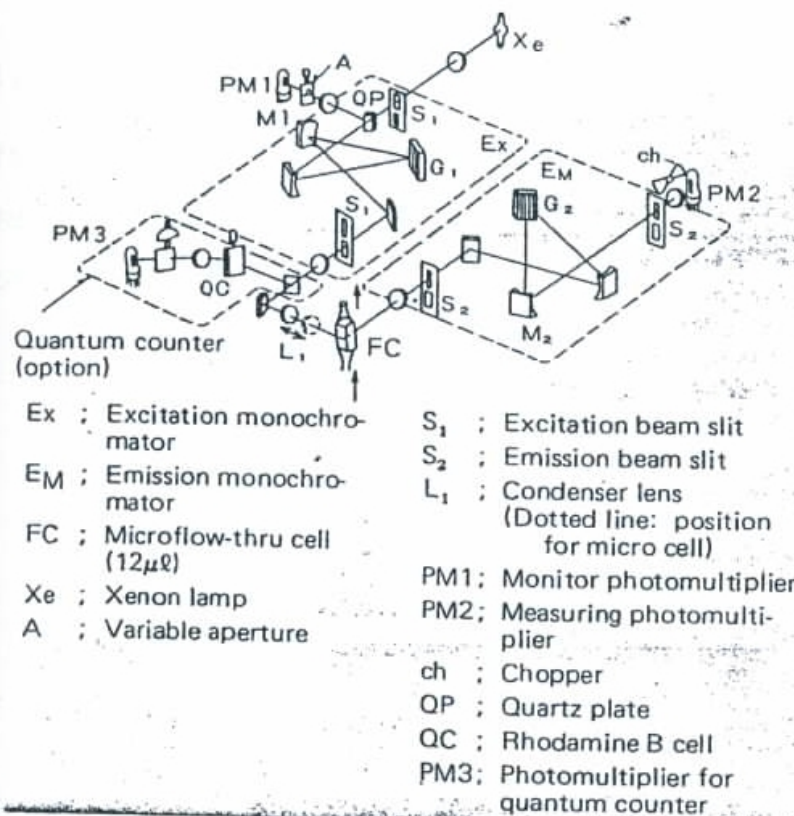




Fluorescence spectrometer

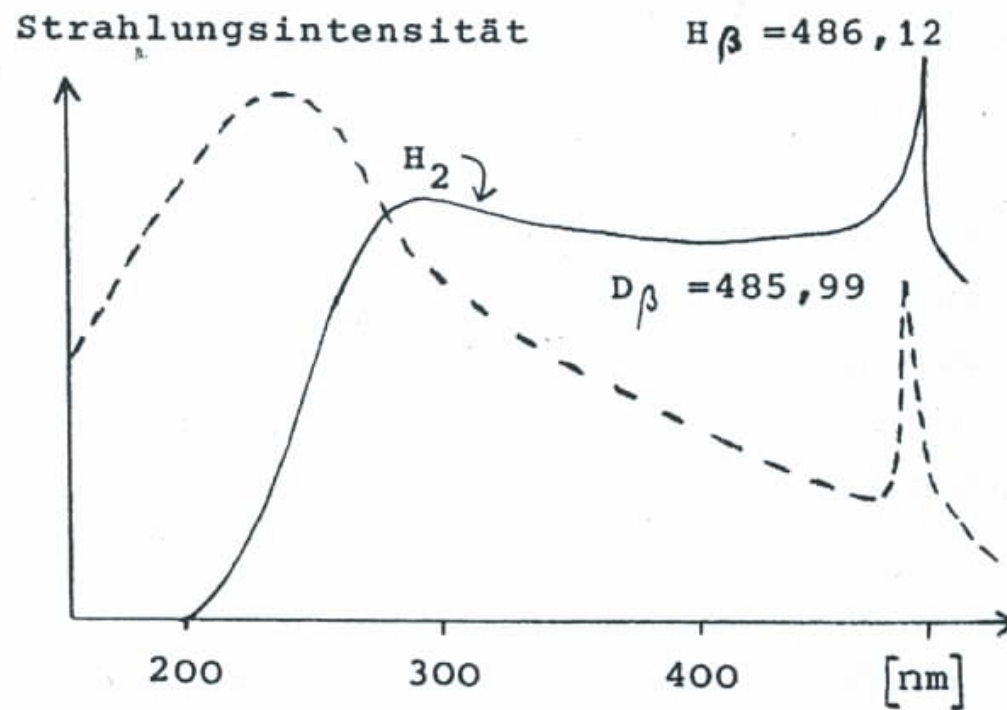
Optischer Aufbau

Schaltbild

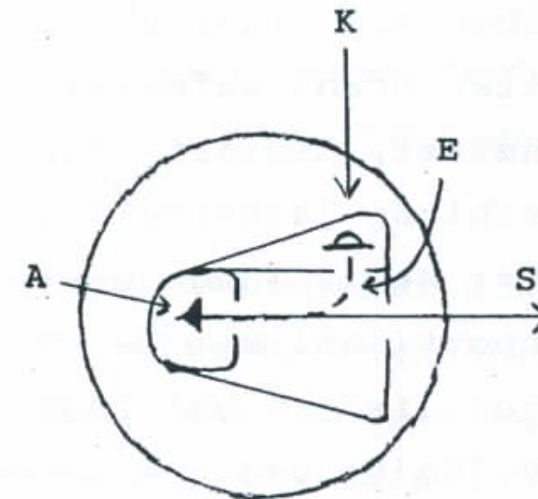




Hydrogen-/deuterium source



(a)

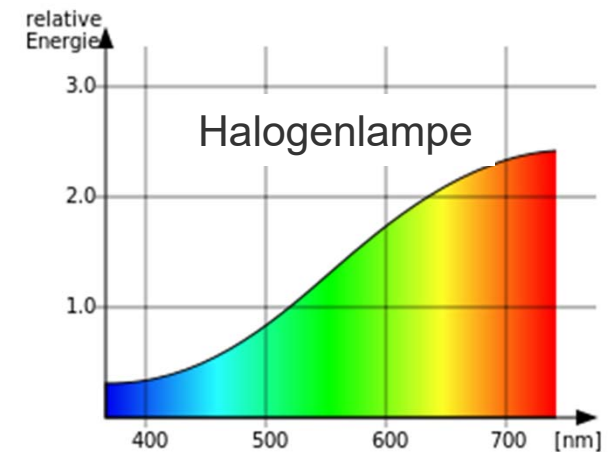
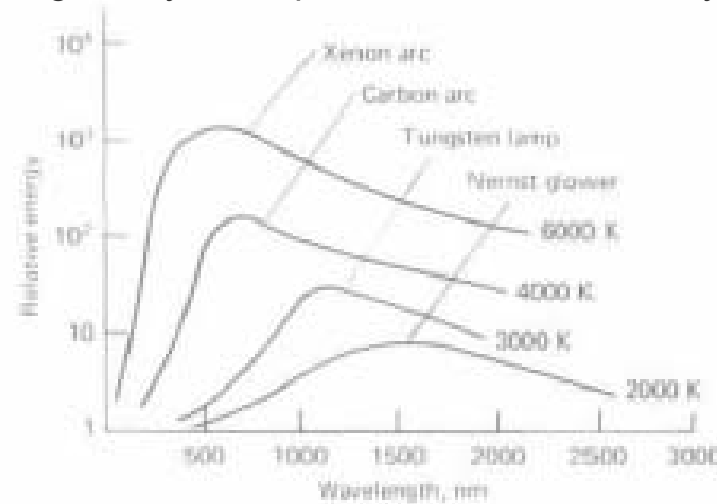


(b)



Tungsten and halogen sources

Skoog, Leary, Principles of Instrumental Analysis, Saunders College Publishing

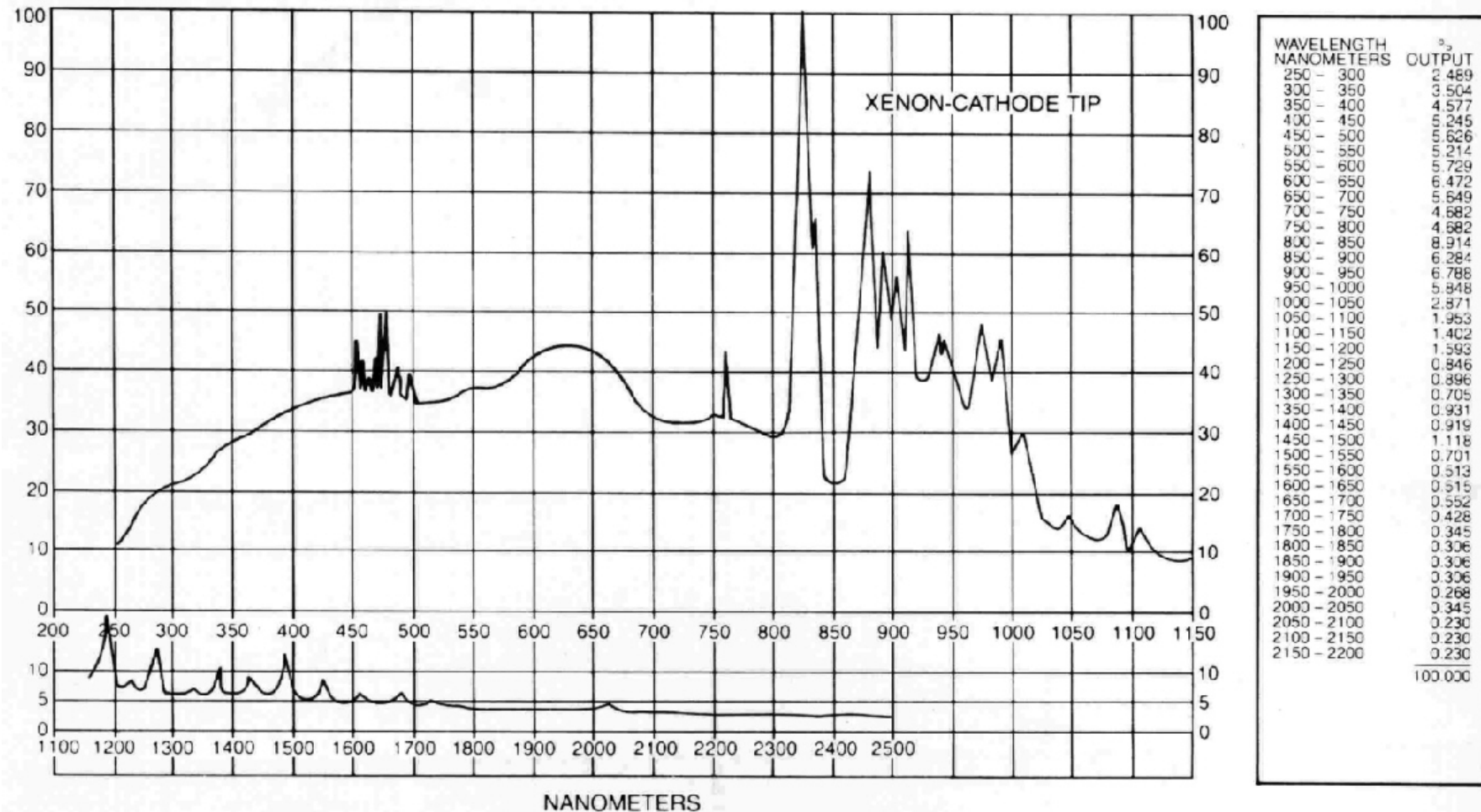


Wavelength, nm	100	200	400	700	1000	2000	4000	7000	10,000	20,000	40,000
Spectral region	VAC UV	UV	VISIBLE	NEAR IR				IR			FAR IR
(a) Sources											
Continuous	Argon lamp	Xenon lamp	H ₂ or D ₂ lamp	Tungsten lamp	Nernst glower (ZrO ₂ + Y ₂ O ₃)	Nichrome wire (Ni + Cr)	Globar (SiC)				
Line			Hollow cathode lamps								



Xenon source

Spectral emission for Xenon Compact Arc Lamps



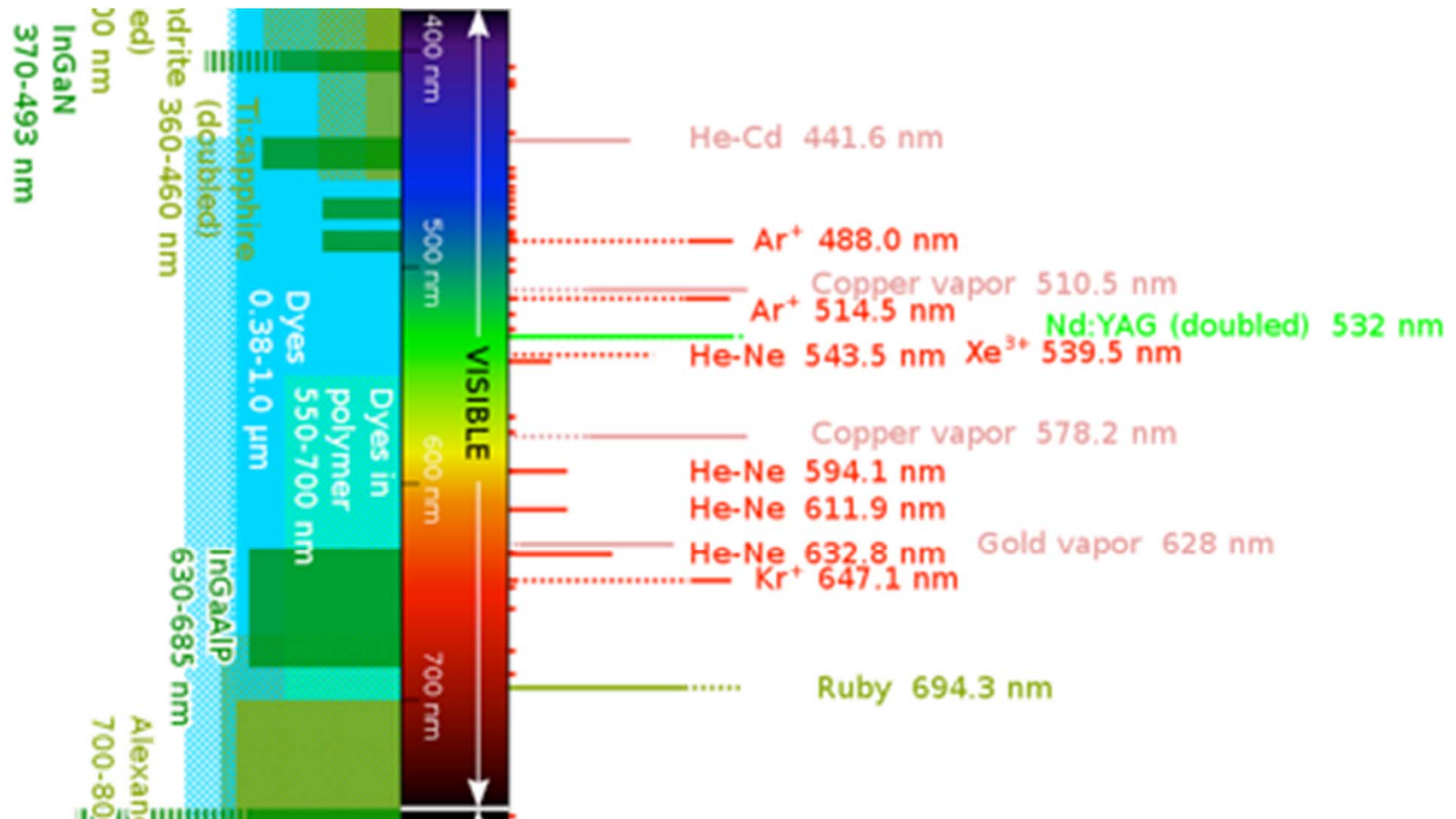
<http://de.wikipedia.org/wiki/Xenonlicht>

<http://www.muller-elektronik-optik.de/s4.pdf>



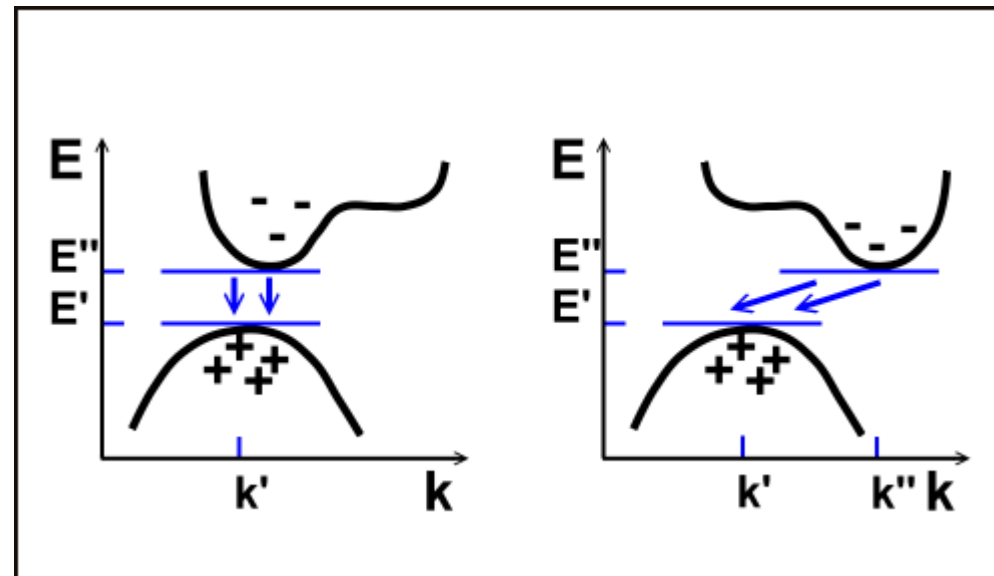
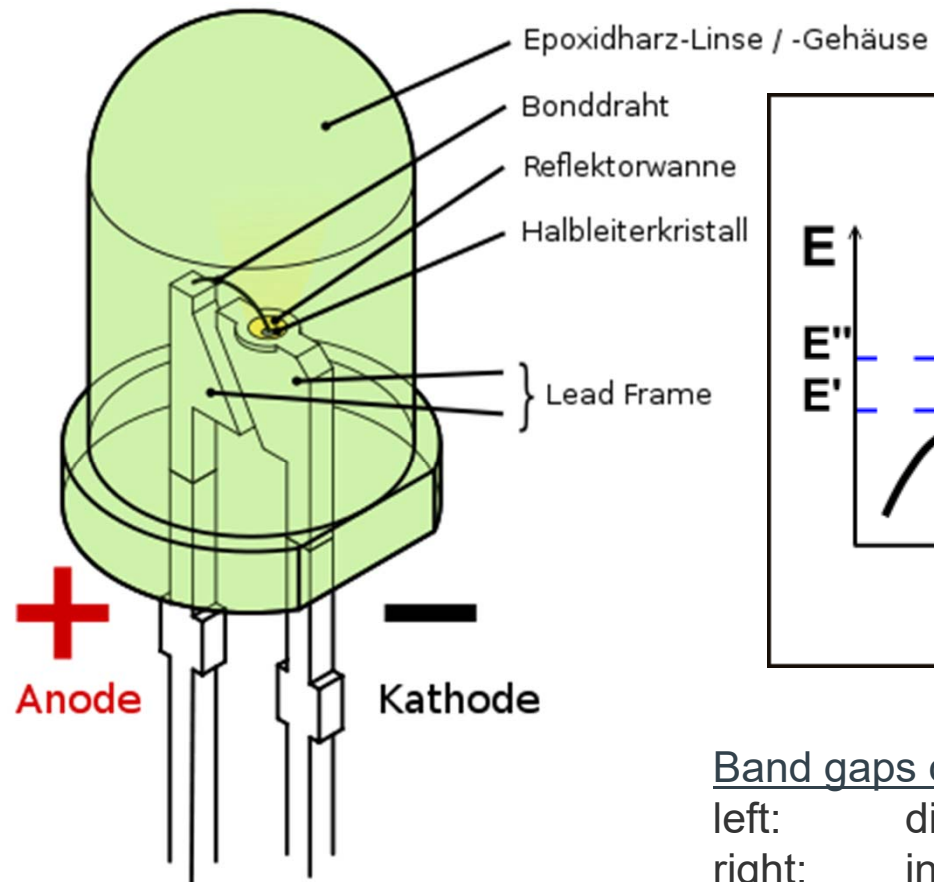
Laser in the visible

commercial lasers





LED: light emitting diodes



Band gaps of two semi conductors

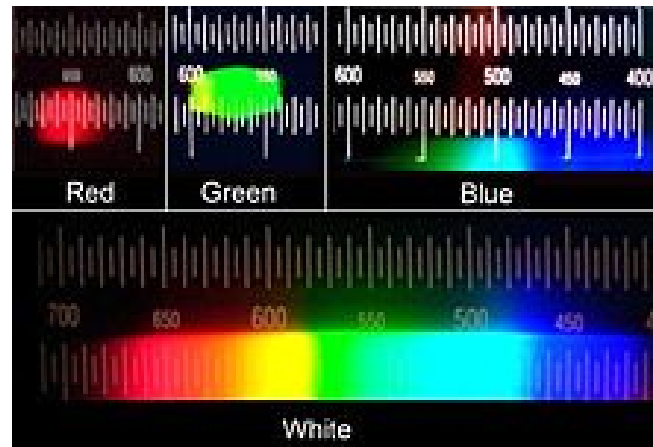
left: direct (z. B. Galliumarsenid)

right: indirect semi conductor (z. B. Silicium)

<http://de.wikipedia.org/wiki/Leuchtdiode>



LEDs colours



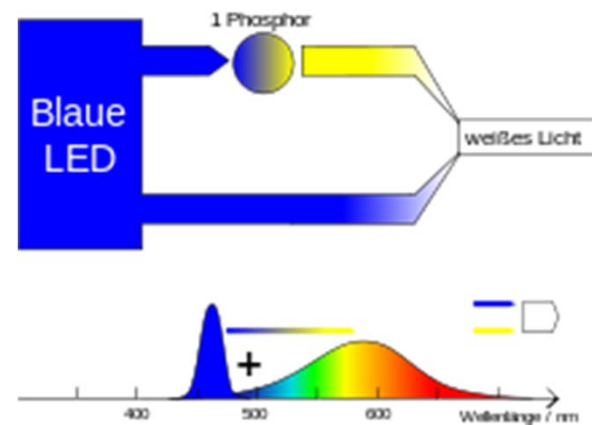
Spectrum of a red, green, blue and white LED



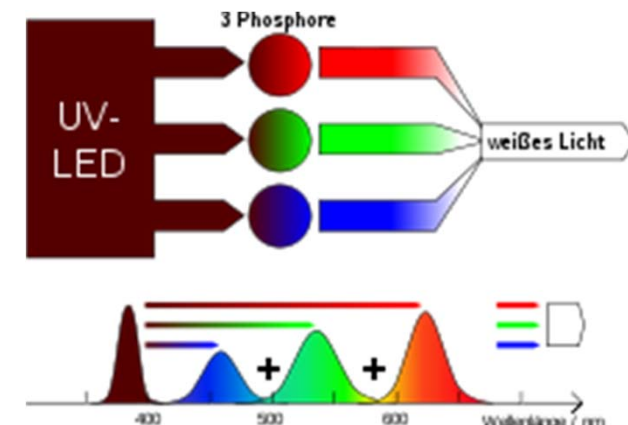
Blue LED (InGaN)



RGB-SMD-LED



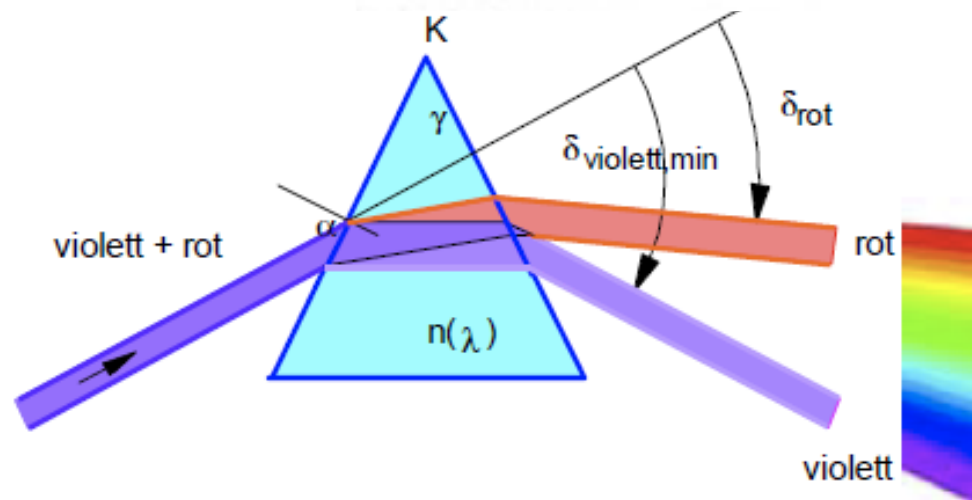
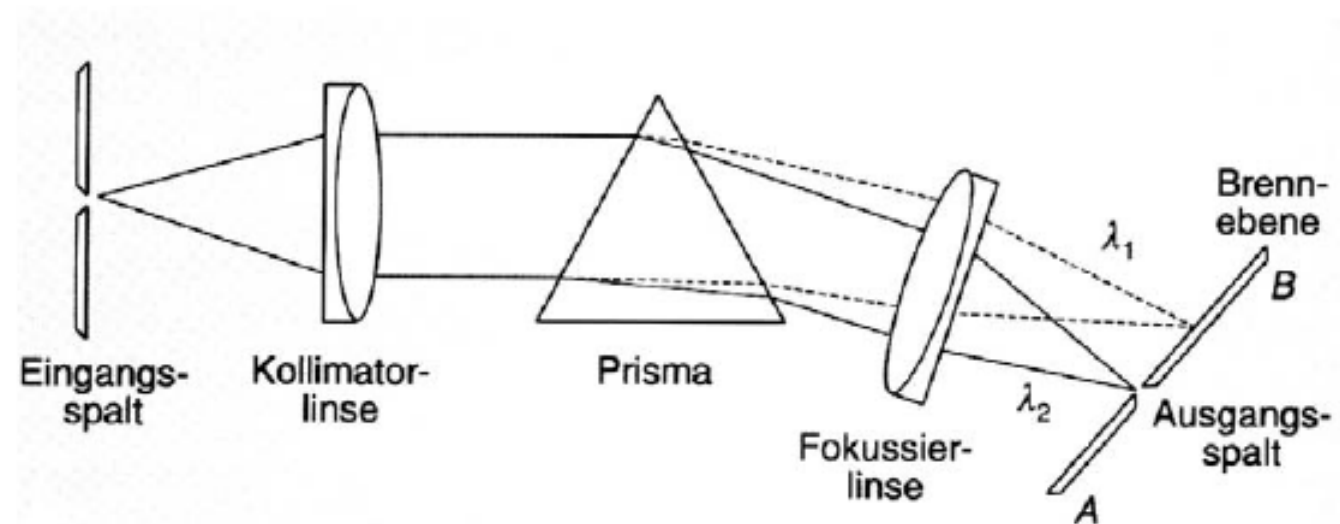
White light by a blue LED and a broad band fluorescent dye



White light by an ultra violet LED and red, green and blue fluorescent dyes



Prism monochromator



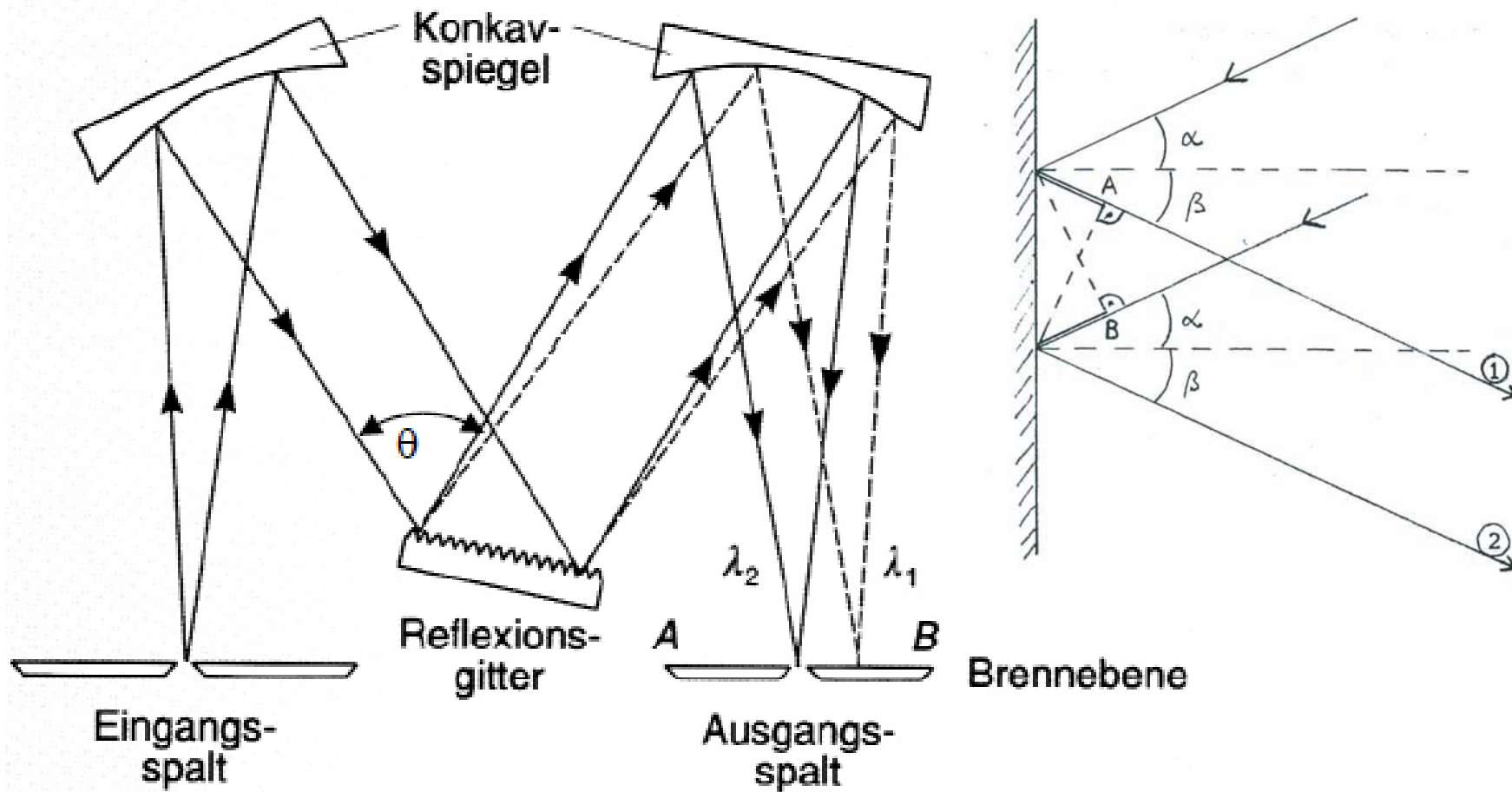
$$n_0 \sin \alpha_0 = n_1 \sin \alpha_1$$

$$\frac{n_1}{n_0} = \frac{\sin\left(\frac{\delta_{\min} + \gamma}{2}\right)}{\sin\frac{\gamma}{2}}$$

more in
UV, less
in IR
not
equivalen
t distance
-> not



Grating monochromator (Czerny-Turner)



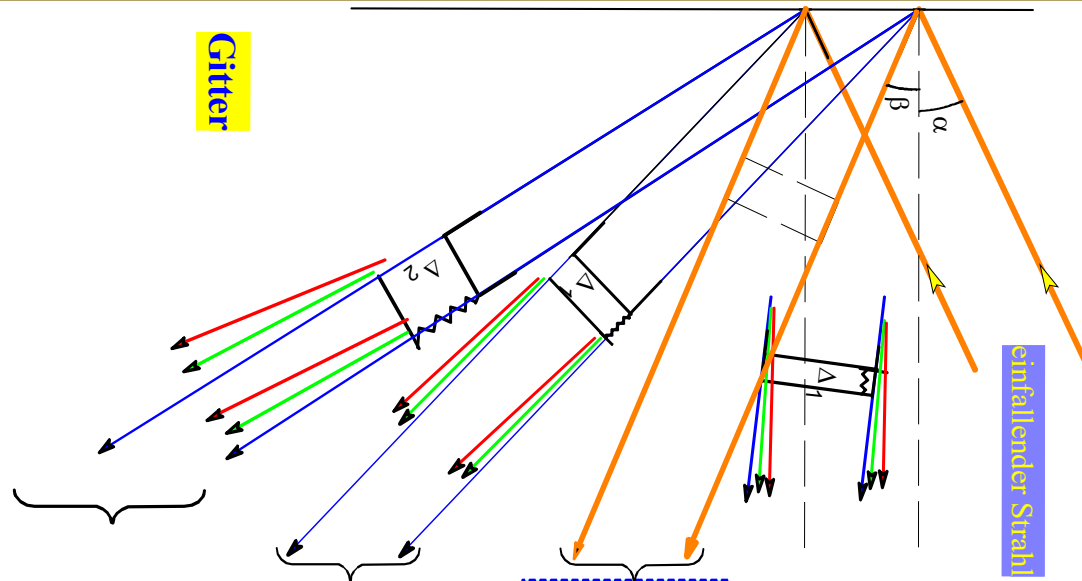
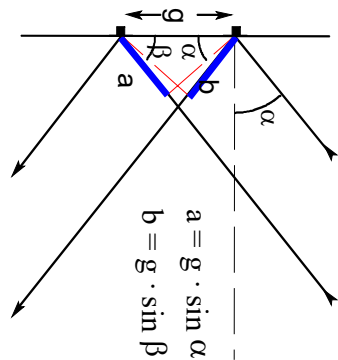
$$\sin \alpha + \sin \beta = \frac{m}{d} \cdot \lambda = m \cdot n \cdot \lambda$$

$$\Delta = A + B = d \cdot (\sin \alpha + \sin \beta) = m \cdot \lambda$$

action, differences
ism: the most
ference in the red
now linear
ave many orders,

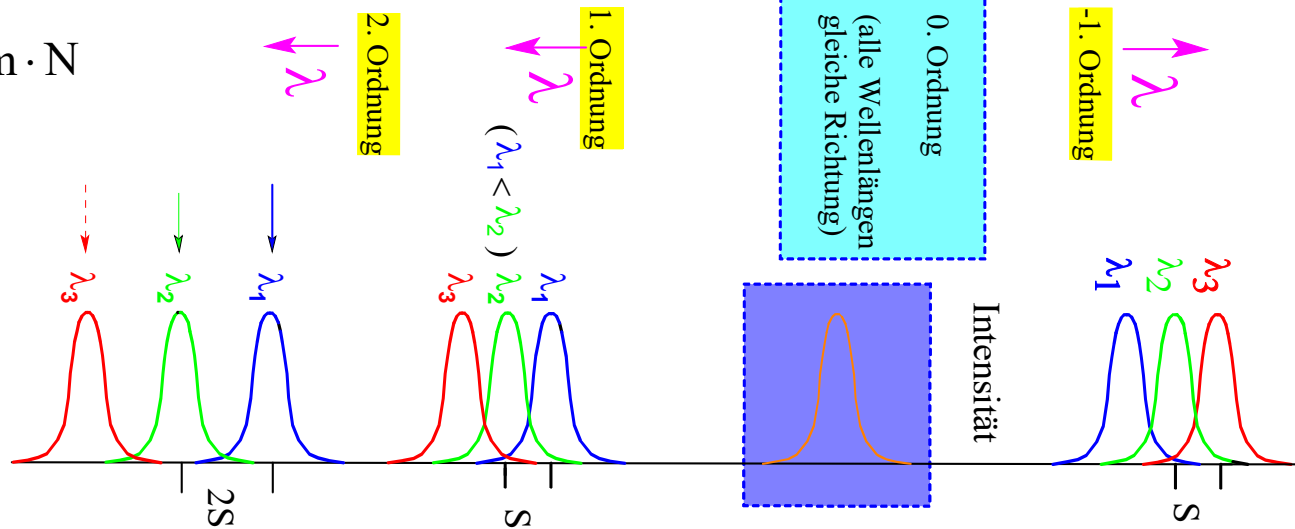


Grating



$$R = \frac{\lambda}{\Delta\lambda} = \frac{m \cdot B}{d} = m \cdot N$$

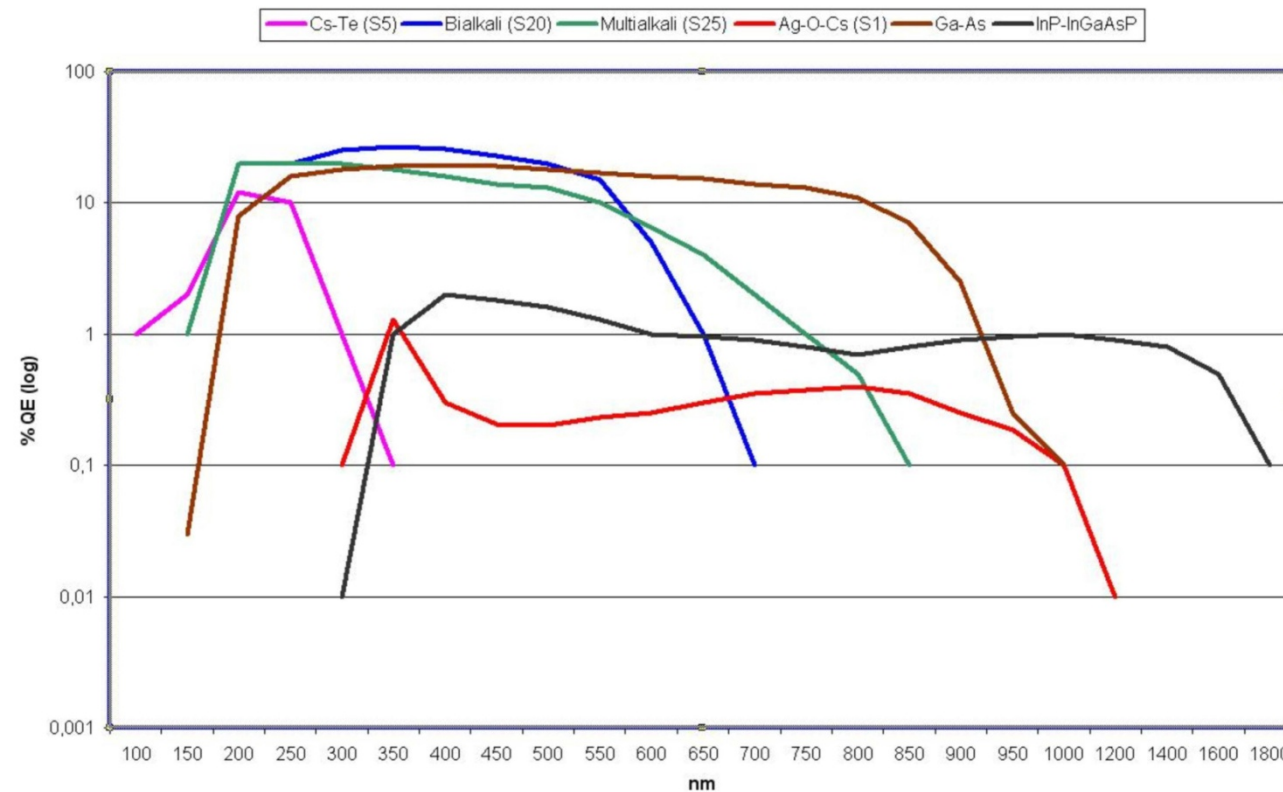
Auflösung





Photomultiplier PMT

Typical PMT QE curves

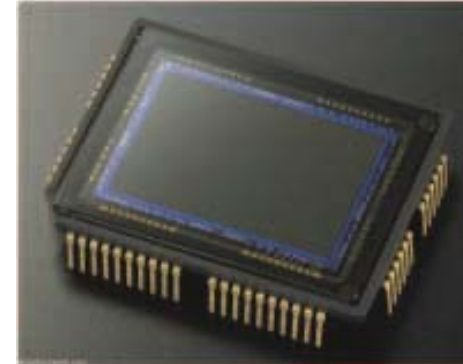


cool down photomultiplier to reduce noise



CCD und CMOS

CCD -> charge coupled device *conventional*
CMOS -> complementary metal oxide
semiconductor *modern*

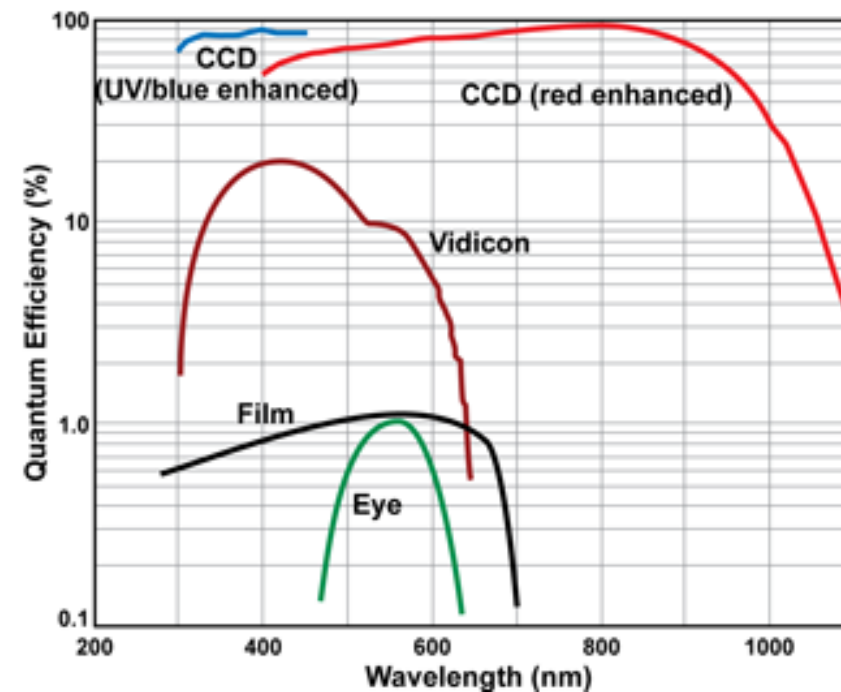


Diode array produces voltage:

- linearly proportional to intensity of incident light
- not linearly proportional of wavelength

Multi-layer system of silicium:

- Silicium is sensitive to light
- Layer structure allows laterally varying functionality





Basics in Electrochemistry



Electrode potentials single electrode potentials

- Phase equilibrium
- **Galvani-voltage**
- Half cell



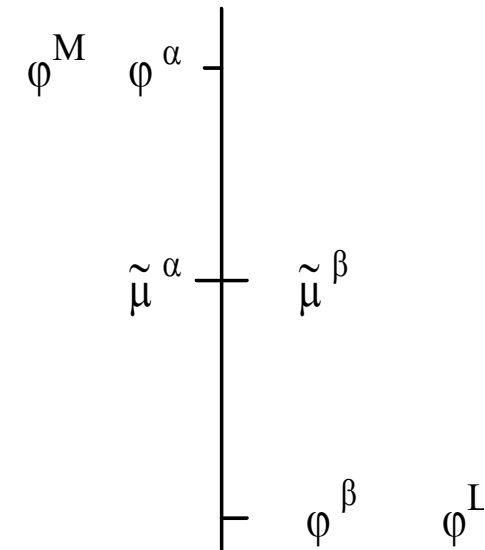
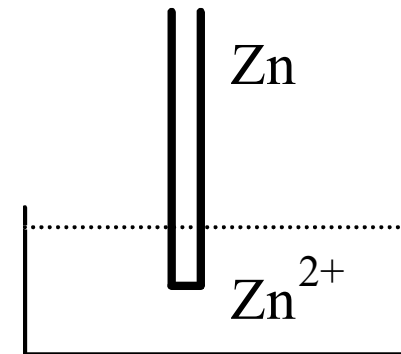
$$\tilde{\mu}_i^\alpha = \tilde{\mu}_i^\beta$$

$$\Delta\varphi_0 = \varphi^M - \varphi^L$$

Galvani voltage between the
two interfaces (e.g. liquid,
metal)

$$\mu_i^\beta + z_i F \varphi^\beta = \mu_i^\alpha + z_i F \varphi^\alpha$$

dependent on solution and metal of electrode





Electrochemical Potential

- Chemical Potential

$$\mu_i = \mu_i^0 + RT \ln a_i$$

- Additional potential, called **Galvani-Potential** φ (also named internal potential)
- Results in **electrochemical potential**

$$\tilde{\mu}_i^\alpha = \mu_i^\alpha + z_i \cdot F \cdot \varphi^\alpha$$

↑ Number of charges
(Ions, electrons)

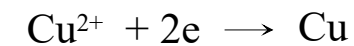
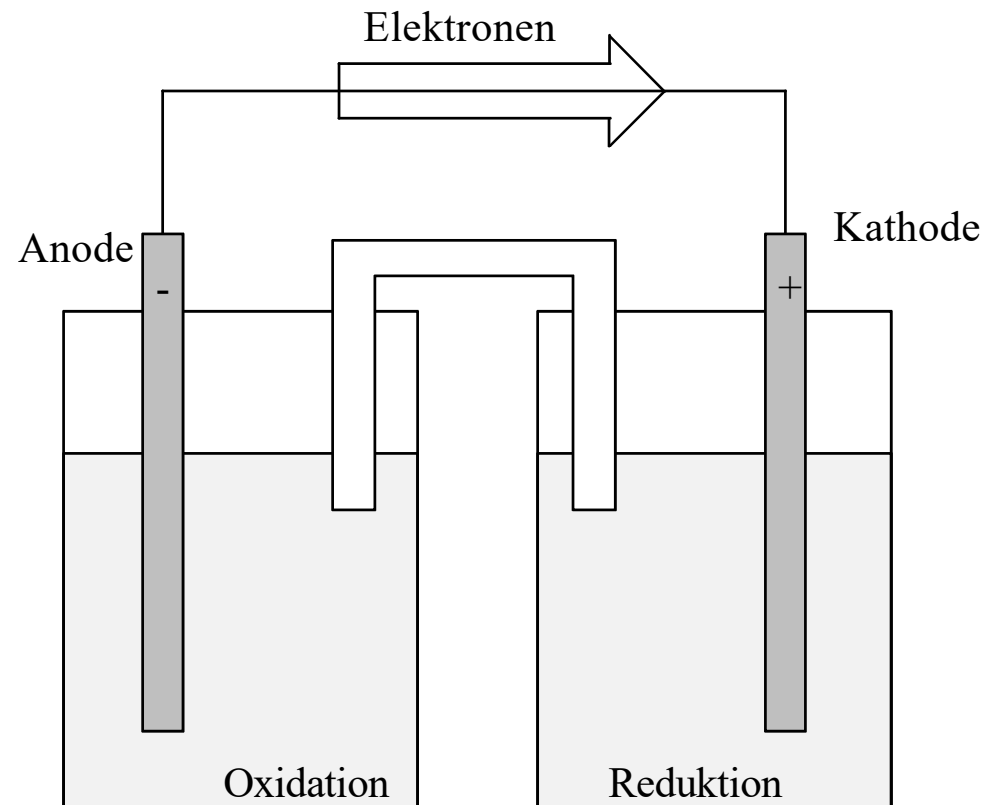
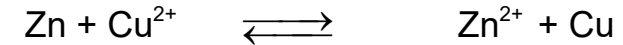
$$\tilde{\mu}_i^\alpha = \mu_i^{\alpha 0} + RT \ln a_i^\alpha + z_i F \varphi^\alpha$$



Daniell-Element

- According to Stockholm convention:
- In case reaction runs to the right side, then E_o positive

usually hydrogen reference electrode,
two properties: hydrogen of 1 bar pressure on
electrode, within an acidic solution of $\text{pH}=1 \rightarrow$
 $\ln(1)=0$





Standard-Reduction-Potential-table (Stockholm-Convention)

Kathode

- Reducing
- Right
- More positive

take more positive - less positive
e.g. Zn and Cu: $0.34 - (-0.76) = 1.1$

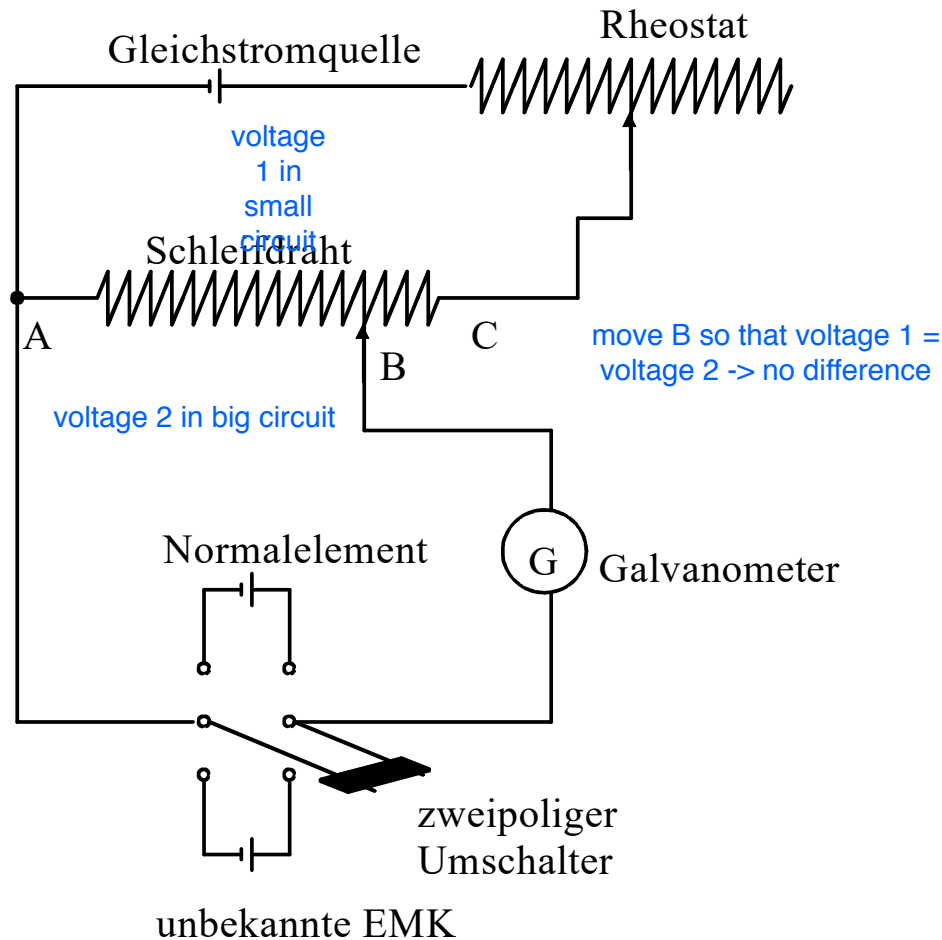
Reduktions-Halbreaktion			E°/V
Oxidierendes Agens		Reduzierendes Agens	
(stark oxidierend)			
F ₂	+ 2 e ⁻	→ 2 F ⁻	+ 2,87
S ₂ O ₈ ²⁻	+ 2 e ⁻	→ 2 SO ₄ ²⁻	+ 2,05
Au ⁺	+ e ⁻	→ Au	+ 1,69
Pb ⁴⁺	+ 2 e ⁻	→ Pb ²⁺	+ 1,67
Ce ⁴⁺	+ e ⁻	→ Ce ³⁺	+ 1,61
MnO ₄ ⁻ + 8H ⁺	+ 5 e ⁻	→ Mn ²⁺ + 4H ₂ O	+ 1,51
Cl ₂	+ 2 e ⁻	→ 2 Cl ⁻	+ 1,30
Cr ₂ O ₇ ²⁻ + 14H ⁺	+ 6 e ⁻	→ 2 Cr ³⁺ + 7H ₂ O	+ 1,33
O ₂ + 4H ⁺	+ 4 e ⁻	→ 2 H ₂ O	+ 1,23
Br ₂	+ 2 e ⁻	→ 2 Br ⁻	+ 0,81 bei pH = 7 + 1,09
Ag ⁺	+ e ⁻	→ Ag	+ 0,80
Hg ₂ ²⁺	+ 2 e ⁻	→ 2 Hg	+ 0,79
Fe ³⁺	+ e ⁻	→ Fe ²⁺	+ 0,77
I ₂	+ 2 e ⁻	→ 2 I ⁻	+ 0,54
O ₂ + 2H ₂ O	+ 4 e ⁻	→ 4 OH ⁻	+ 0,40
Cu ²⁺	+ 2 e ⁻	→ Cu	+ 0,81 bei pH = 7 + 0,34
AgCl	+ e ⁻	→ Ag + Cl ⁻	+ 0,22
2H ⁺	+ 2 e ⁻	→ H ₂	0, nach Definition
Fe ³⁺	+ 3 e ⁻	→ Fe	- 0,04
O ₂ + H ₂ O	+ 2 e ⁻	→ HO ₂ ⁻ + OH ⁻	- 0,08
Pb ²⁺	+ 2 e ⁻	→ Pb	- 0,13
Sn ²⁺	+ 2 e ⁻	→ Sn	- 0,14
Fe ²⁺	+ 2 e ⁻	→ Fe	- 0,44
Zn ²⁺	+ 2 e ⁻	→ Zn	- 0,76
2 H ₂ O	+ 2 e ⁻	→ H ₂ + 2 OH ⁻	- 0,83
Al ³⁺	+ 3 e ⁻	→ Al	- 0,42 bei pH = 7 - 1,66
Mg ²⁺	+ 2 e ⁻	→ Mg	- 2,36
Na ⁺	+ e ⁻	→ Na	- 2,71
Ca ²⁺	+ 2 e ⁻	→ Ca	- 2,87
K ⁺	+ e ⁻	→ K	- 2,93
Li ⁺	+ e ⁻	→ Li	- 3,05
		(stark reduzierend)	

compared to
hydrogen
reference
electrode



EMK measurement

- Poggendorf compensation
- Digital voltmeter (extremely high internal resistance $10^{14} \Omega$)





Sensors



- Temperature
 - Caused by heat of reaction
- Pressure
 - Acting on joints or implants
- Evoked potentials
 - Electric spikes at neurons
- Biomolecular interactions
 - Biofilms
 - Drug screening
 - Dignostics

Sensors

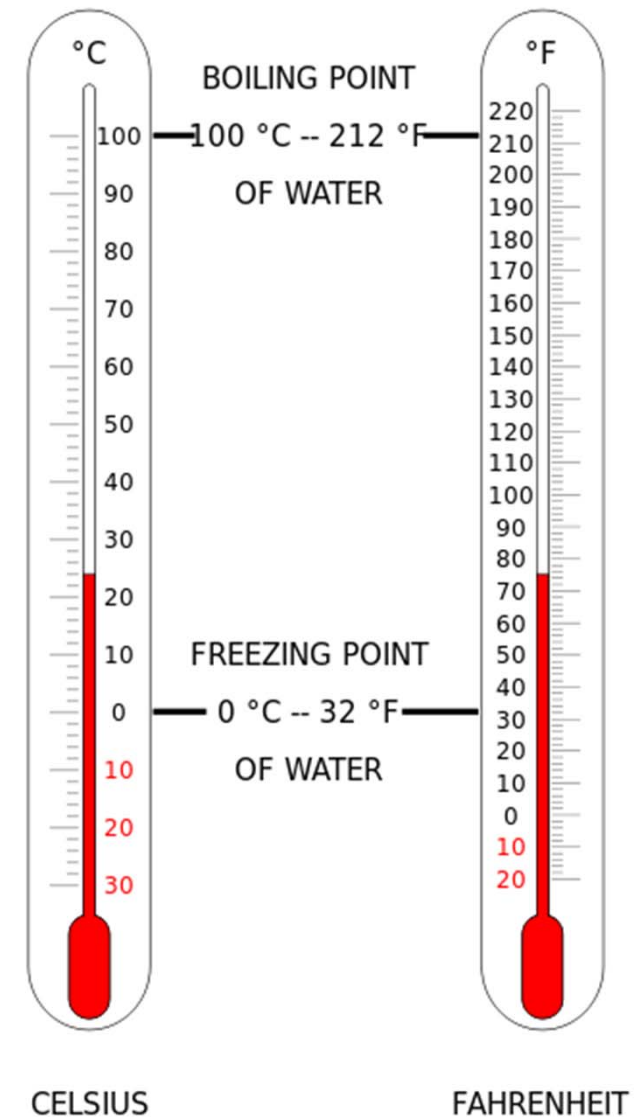


What is a *SENSOR*?

„A *sensor* is a device that *receives* and *responds* to a signal or *stimulus*“

It can be:

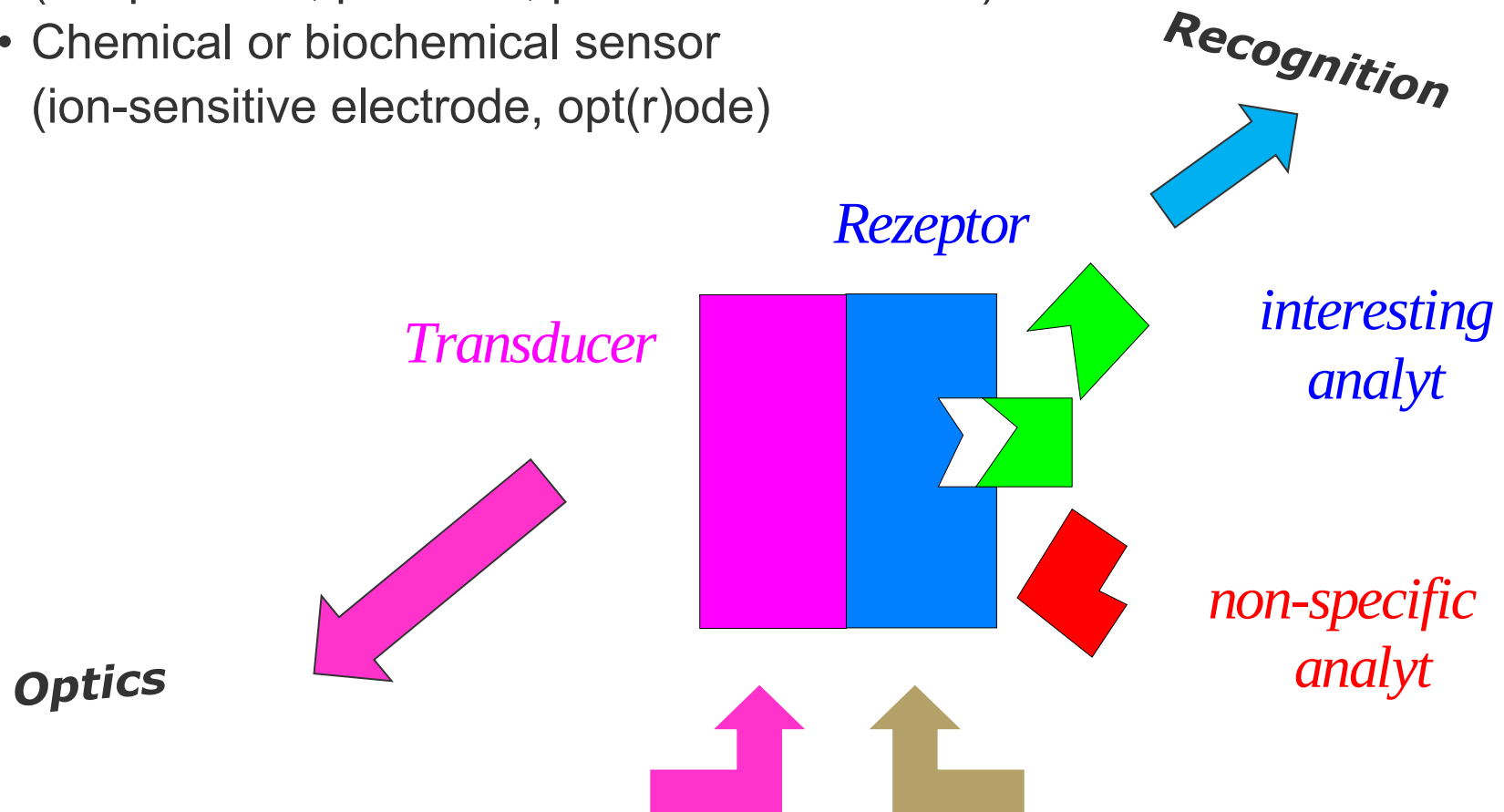
- A Thermometer
 - A thermometer has two important elements:
 - the temperature sensor
 - some means of converting this physical change into a numerical value





Sensor components

- Physical sensors
(temperature, pressure, photoelectric barrier)
- Chemical or biochemical sensor
(ion-sensitive electrode, opt(r)ode)





Sensors in Nature

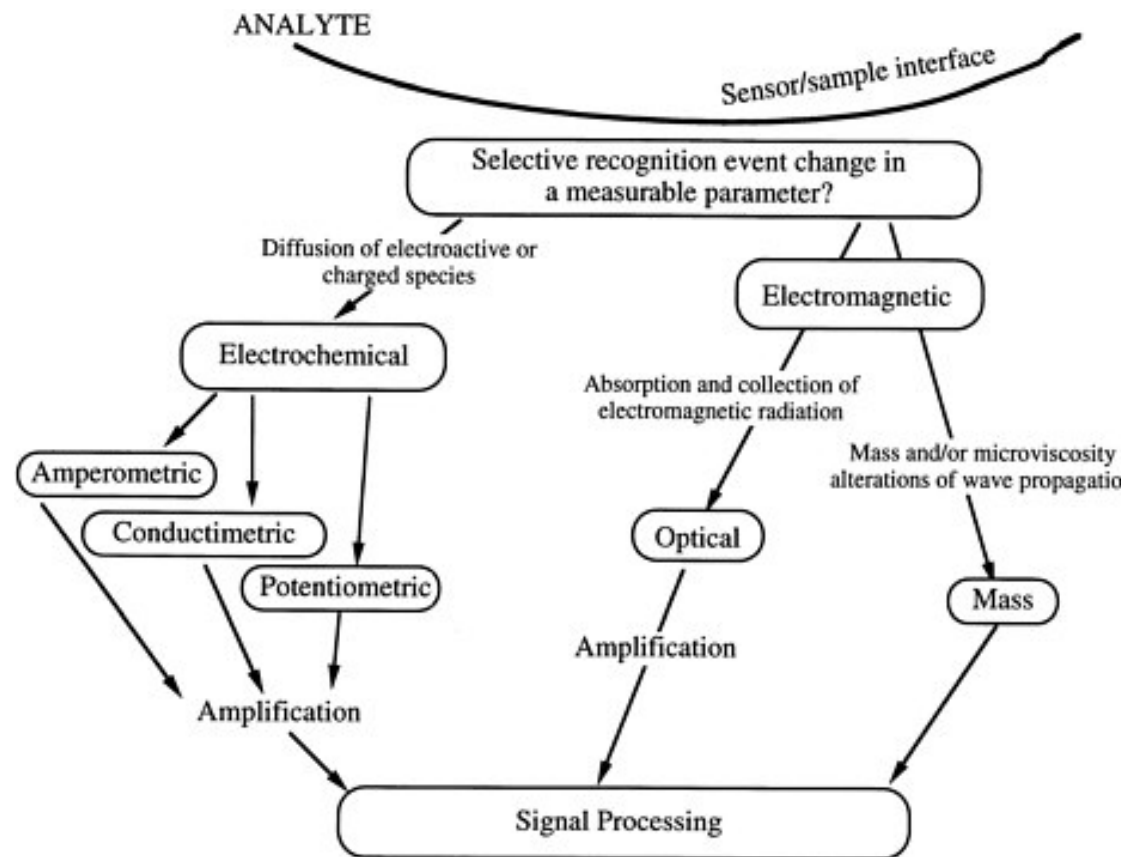
All living organisms contain **biological sensors** with functions similar to those of the mechanical devices described. Most are **specialized cells** that are sensitive to:

- Physical aspects of the external environment: Light, motion, temperature, magnetic fields, gravity, humidity, moisture, vibration, pressure, electrical fields, sound, ...
- Physical aspects of the internal environment, such as stretch, motion of the organism, and position of appendages
- **Environmental molecules**, including toxins, nutrients, and pheromones
- Internal metabolic milieu, such as glucose level, oxygen level, or osmolality
- Internal signal molecules, such as hormones, neurotransmitters, and cytokines



Transducer

- Depending on transducer the sensors can be divided into several groups:
 - Optical sensors
 - Electrochemical sensors
 - Mass sensitive sensors
 - Calorimetric sensors





Sensor Classes

- Electrochemical
 - Potentiometric
 - ISFET
 - ZrO₂
 - Amperometric
 - Conductometric
 - Chemiresistor
 - SMO_x
 - Cond-Polymer
 - Nanotube
- Optical
 - Absorption
 - IR, UV, vis
 - Emission
 - Fluorescence
- Mechanical
 - Piezoelectric
 - Resonant
- Thermal
 - TCD
 - Catalytic
 - ThermoEL
 - Pyroelectric



General requirements

- Selectivity
- Accuracy
- Repeatability
- Reproducibility
- Linear scale
- Respond quickly
- Reversibility



Ruggedness / Reliability

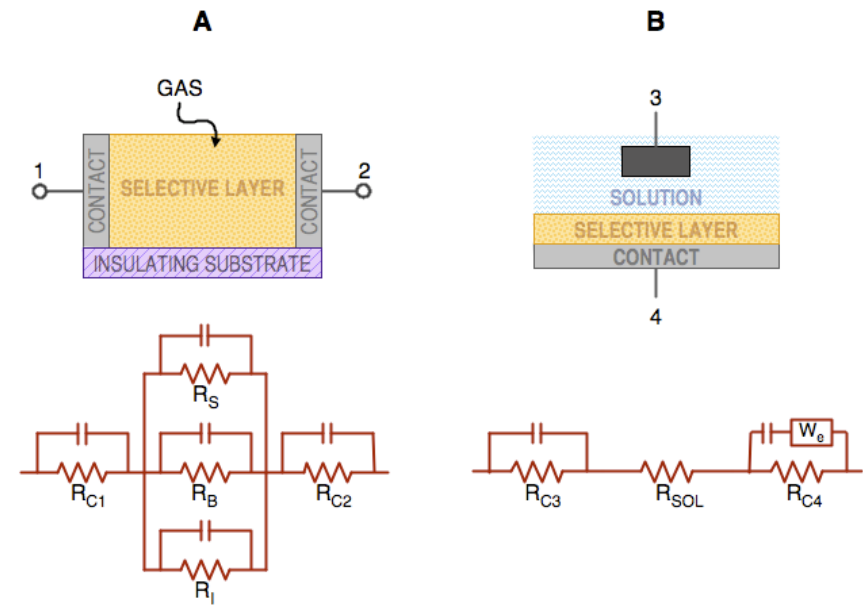
either reversible and non selective or selective but no reversible

- Small
- Cheap



Historic approach

- The idea of using semiconductors as gas sensitive devices leads back to **1953** when **Brattain and Bardeen** first reported gas sensitive effects on Germanium
- Later (1954), **Heiland** found gas sensing effect on metal oxides
- Taguchi finally brought semiconductor sensors based on metal oxides to an industrial product



Two types of conductometric sensors with their corresponding equivalent electrical circuit diagrams: (A) chemiresistor configuration typical for gas sensing; (B) conductometric biosensor.

Brattain, W. H., Bardeen, J. Bell Syst. Tech. J., 32 (1953) 1.

G. Heiland, Zum Einfluss von Wasserstoff auf die elektrische Leitfähigkeit von ZnO-Kristallen, Zeit. Phys. 138 (1954) 459–464.

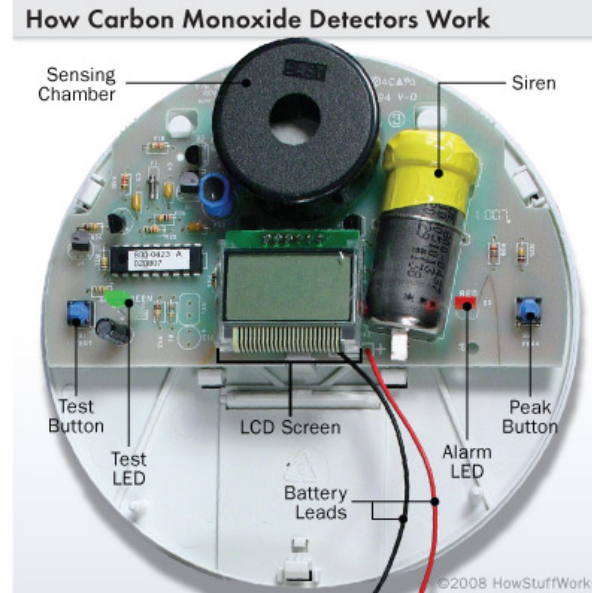
N. Taguchi, U.S. Patent 3,631,436 (1971).

Janata, J. - Principles of Chemical Sensors, Springer, 2009.



Applications

- used in gas leakage detection (propane, butane)
- ambient air quality monitoring
- in traffic (CO, NO_x)
- toxic gas detection
- smoke gas monitoring in houses and buildings





Working Principle

- Similar to solid electrolytes, but as the name states the electrolyte is liquid

Derived from the Nernst equation:

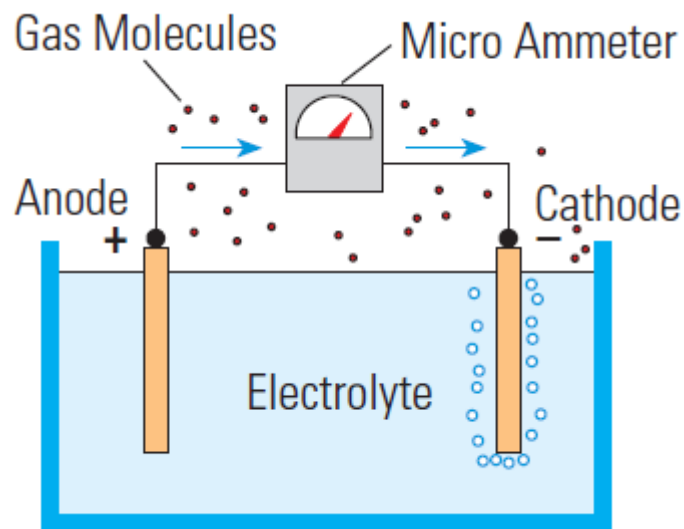
$$E = E_0 + \frac{RT}{z_e F} \ln \frac{a_{ox}}{a_{red}}$$

The emf can be calculated as:

$$emf = E_{cathode} - E_{anode}$$

To obtain:

$$emf = \frac{RT}{z_e F} \ln \frac{p(O_2)^1}{p(O_2)^2}$$



E: potential difference

E_0 : standard potential

R: gas constant

a: activity or ox./red. Spec

T: temperature

F: Faraday constant

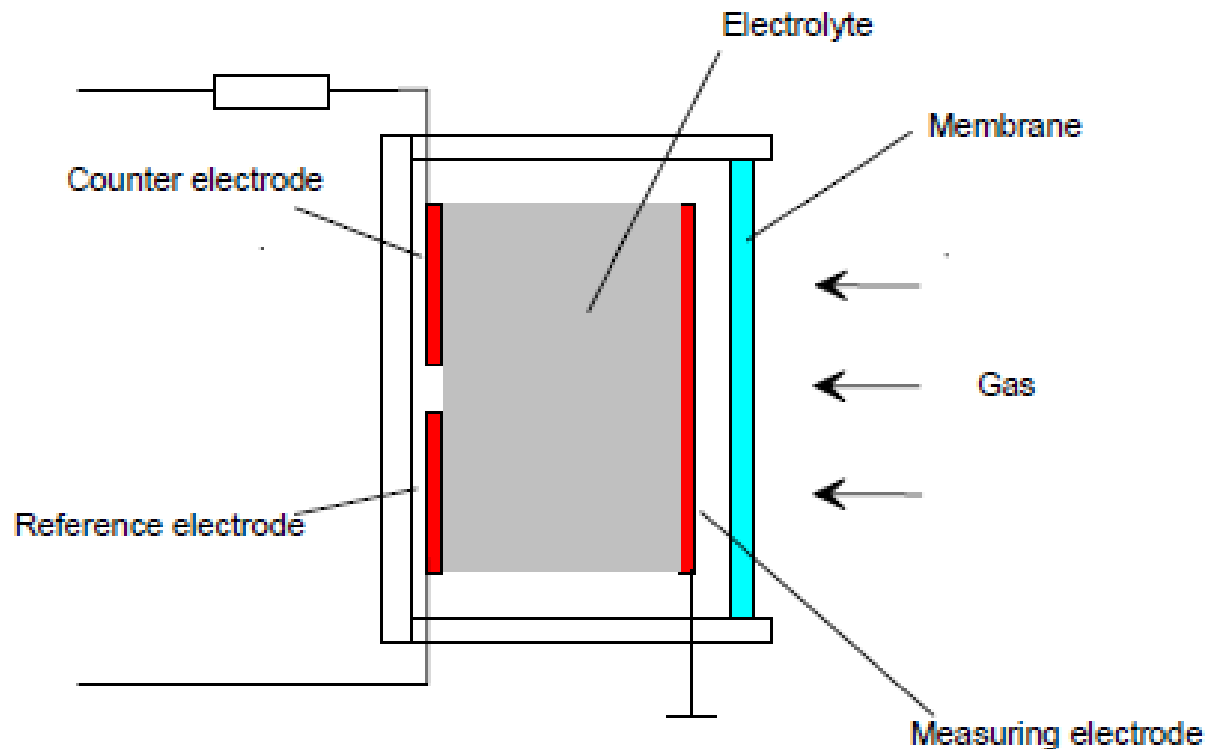
z_e : amount of e- per ion/atom

$p(O_2)$: oxygen pressure

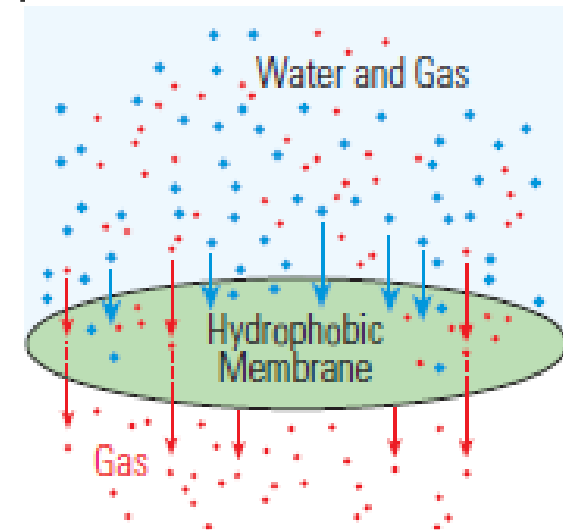


Amperometric Gas Sensors (AGS)

Schematic cell



Hydrophobic membrane



- Faraday law:
- Fick's law

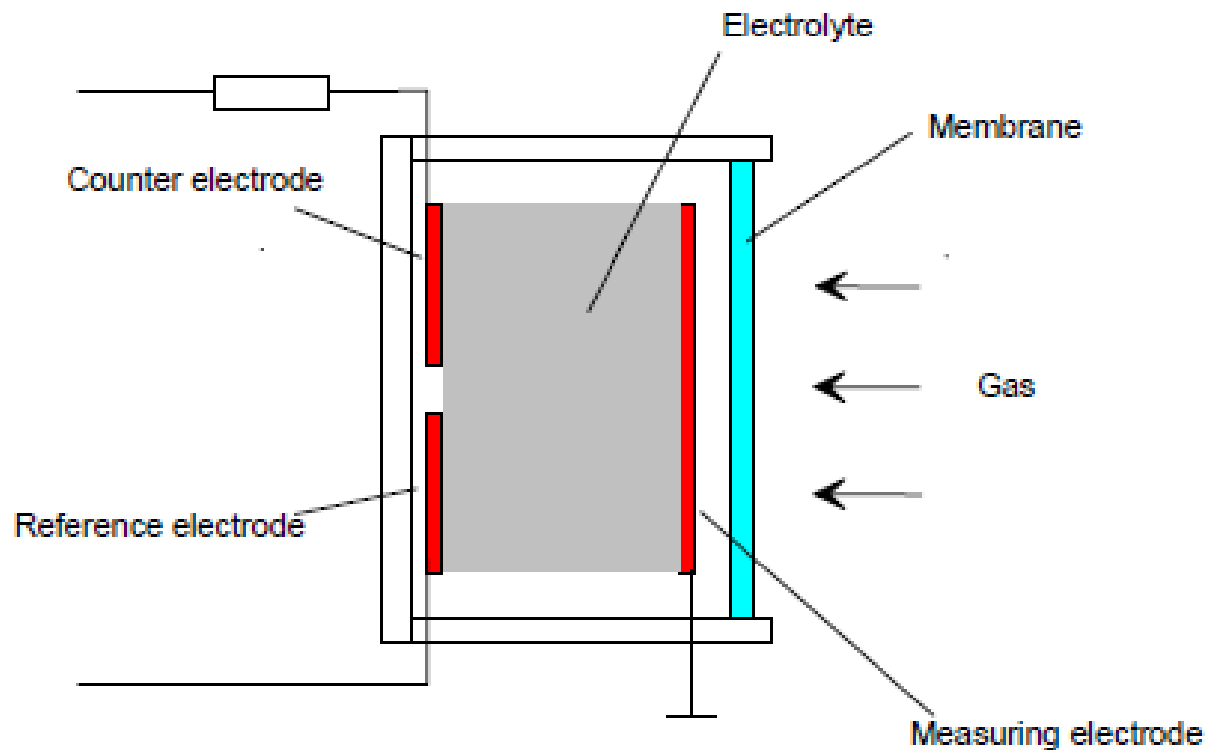
- Diffusion and mass transfer

Chou, J. - Hazardous Gas Monitors: A Practical Guide to Selection, Operation, and Applications, McGraw-Hill Professional, 1999.



Potentiometric sensors

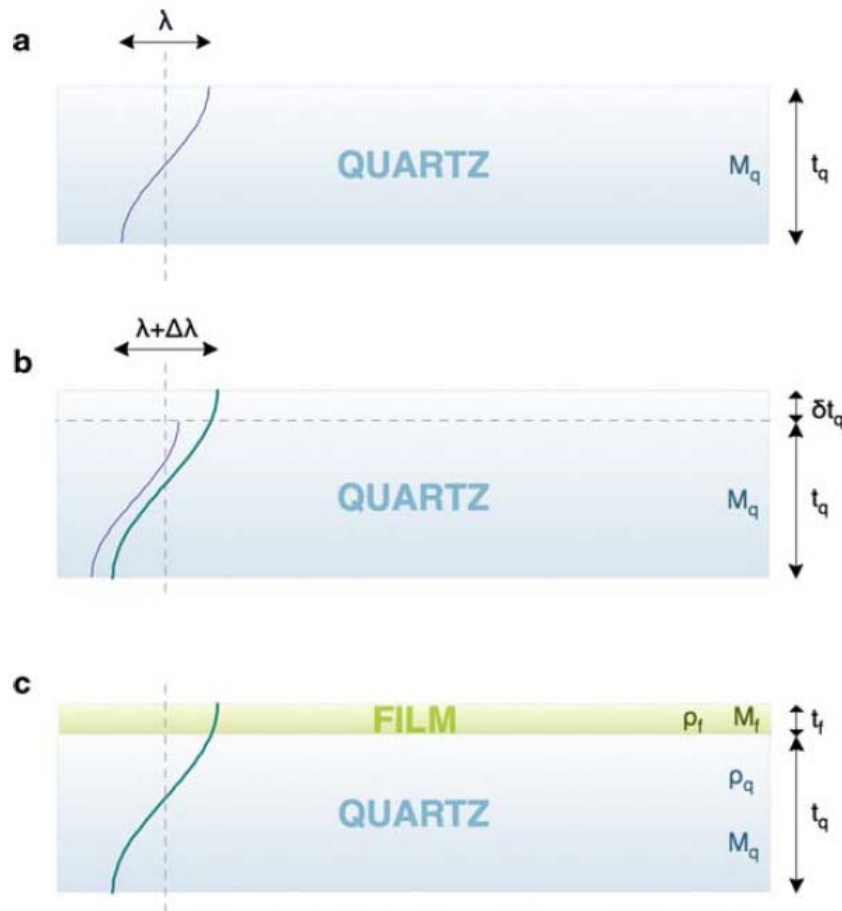
- Measured is the electrical potential of an electrode when no current is flowing
- signal is measured as the potential difference between working and reference electrode





Thickness-Shear-Mode (TSM) Resonators

ab hier nicht behandelt!



Shear mode vibrations in a thickness-shear-mode (TSM) device. Incremental increase $\Delta\lambda$ is due to increased thickness

Janata, J. - Principles of Chemical Sensors, Springer, 2009.



Acoustic wave sensors

Based on piezoelectric effect (electrical signal converted into an acoustic wave)

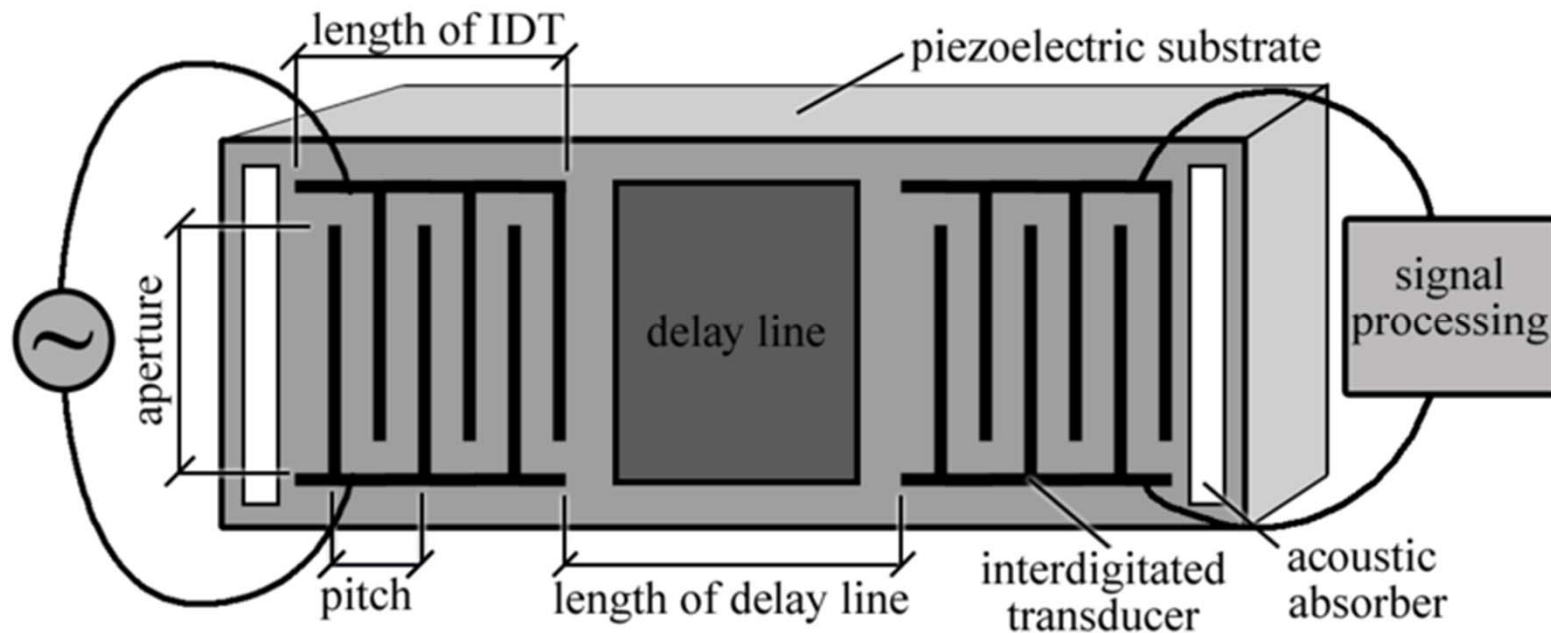
Sinusoidal electrical input

- Interdigitated transducer

⇒ alternating polarity between the fingers of an interdigitated transducer

⇒ mechanical wave (surface acoustic wave)

$$\Delta f = (k_1 + k_2)f_0^2 h q'$$





Laboratory Techniques and Medical Device Approvals I

Gauglitz Part I: Detection and Sensor Principles

End