EE 6301

Smart Biosensors and Systems for Healthcare Technology

EE6301 Week 2- Optical Biosensor (1)

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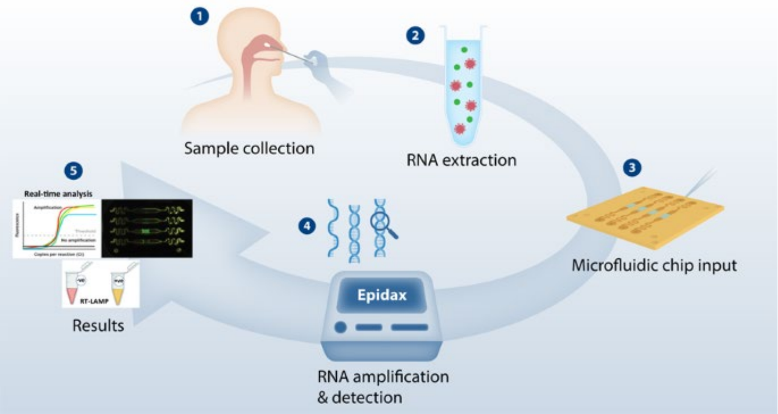
CONTENT

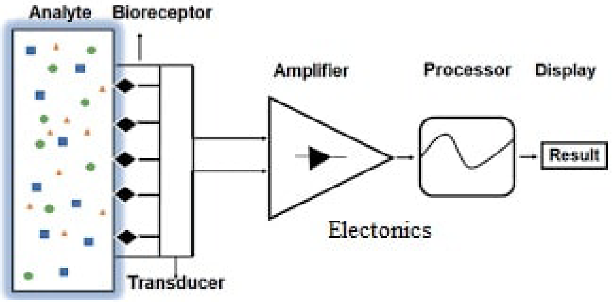
PART ONE

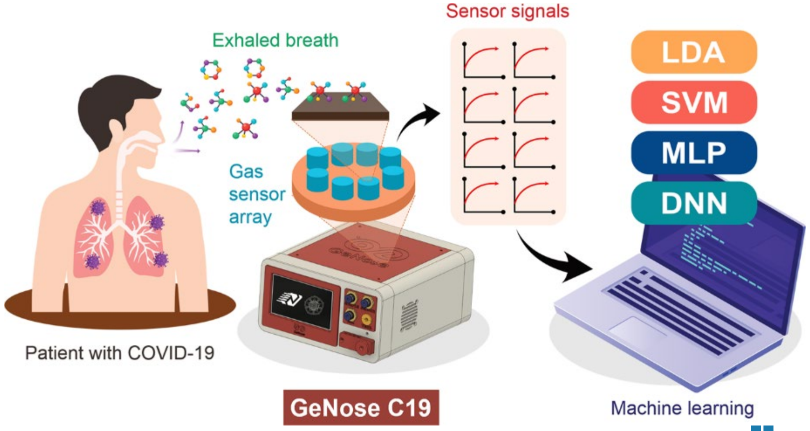
Background of Sensors

Biological Sensors

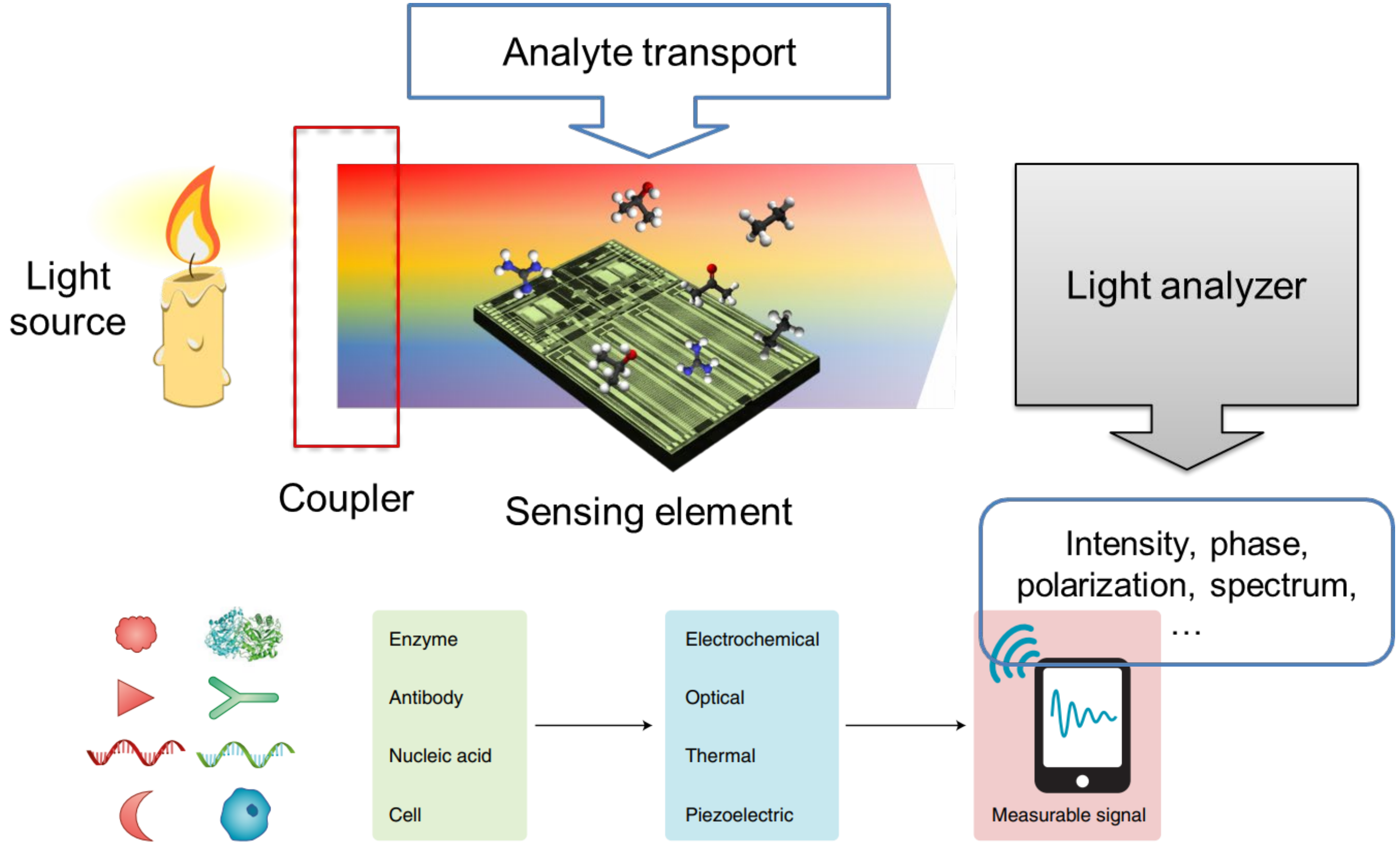
- Biosensor is **a device that consists of two main parts: bioreceptor + transducer**.
- Bioreceptor is a biological component that recognizes the target analyte.
- Transducer is a physicochemical detector component that converts the recognition event into a measurable signal.



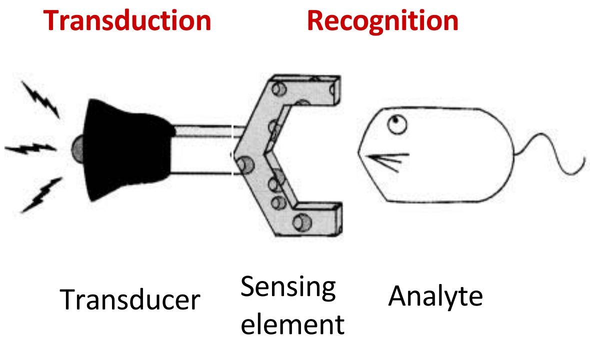


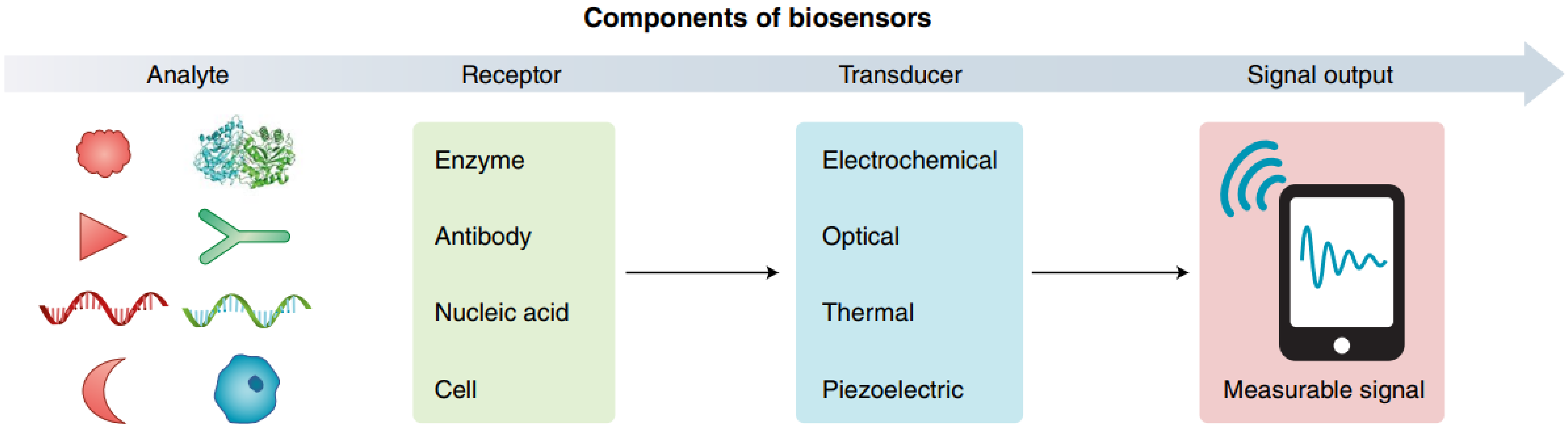


Structure of Biosensor

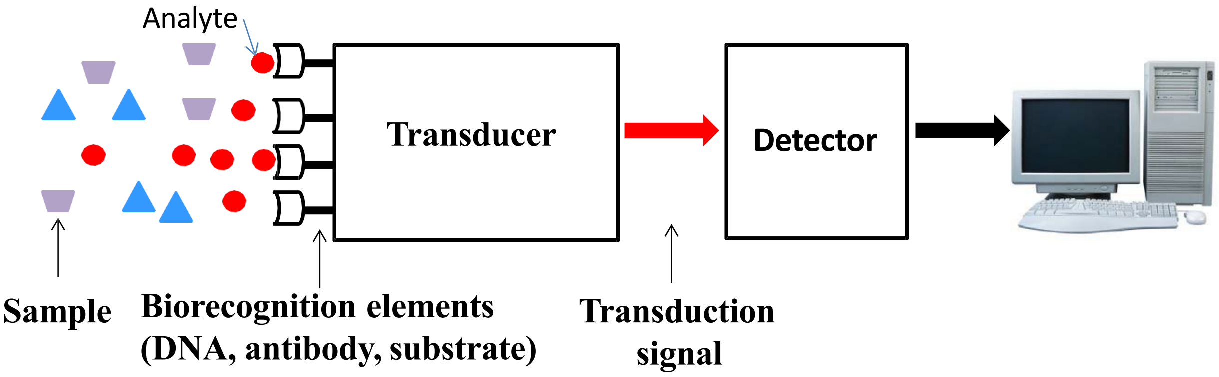


Structure of Biosensor





Key Parameters of Biosensors



A biosensor answers two fundamental questions:

1. What is the analyte being measured (**specificity**)
2. The quantity (or concentration) of the analyte (**sensitivity**)

Detection limit/
Limit of detection (LOD)

Example 1

Find the **concentration** detection limit ("limit of detection", LOD):

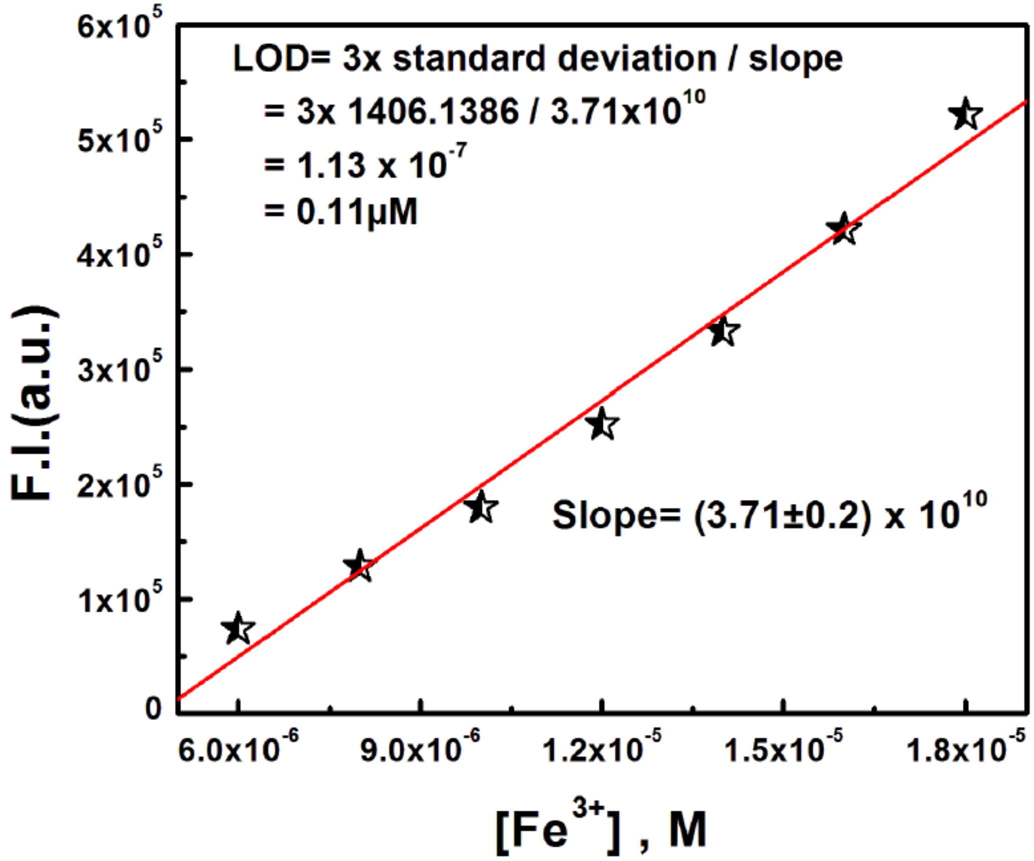
$$LOD = \frac{3s}{m}$$

where s is the standard deviation of the absorbance from the replicate low concentration measurements, and m is the slope of the calibration curve.

Given a slope of 1.54 x 10^3 au/M (au = absorbance units), the LOD is:

$$LOD = \frac{3s}{m} = \frac{(3)(0.0010\ au)}{1.54 \times 10^3\ \frac{au}{M}} = 1.9 \times 10^{-6}\ M = 2 \times 10^{-6}\ M$$

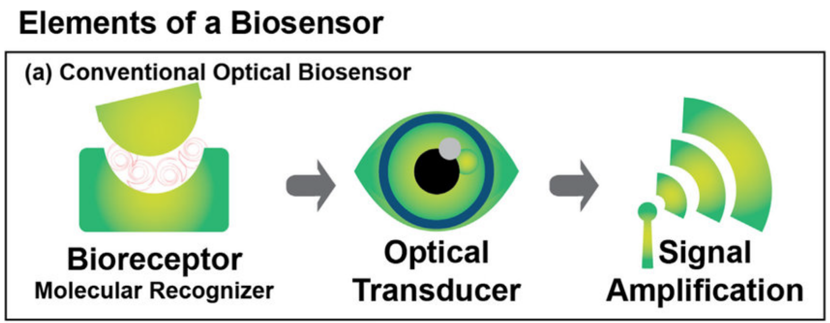
Example 2

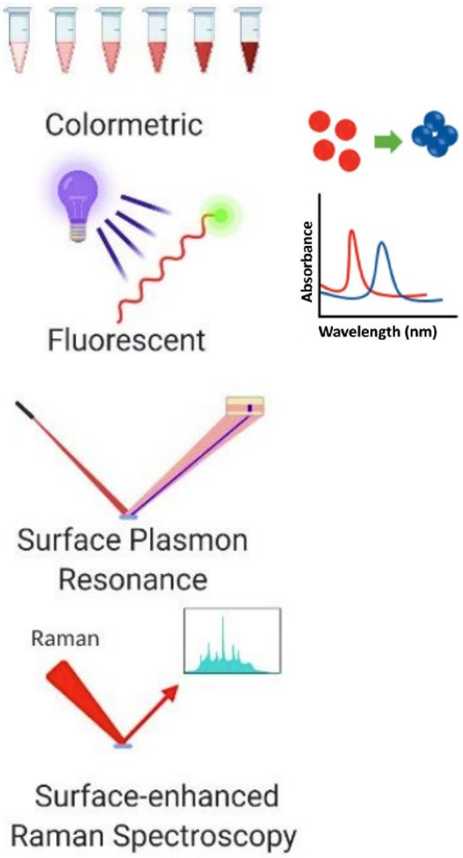


LOD= 3x standard deviation / slope
= 3x 1406.1386 / 3.71x10^10
= 1.13 x 10^-7
= 0.11μM
Slope= (3.71±0.2) x 10^10
F.I.(a.u.)
[Fe^3+] , M
6x10^5
5x10^5
4x10^5
3x10^5
2x10^5
1x10^5
0
6.0x10^-6
9.0x10^-6
1.2x10^-5
1.5x10^-5
1.8x10^-5

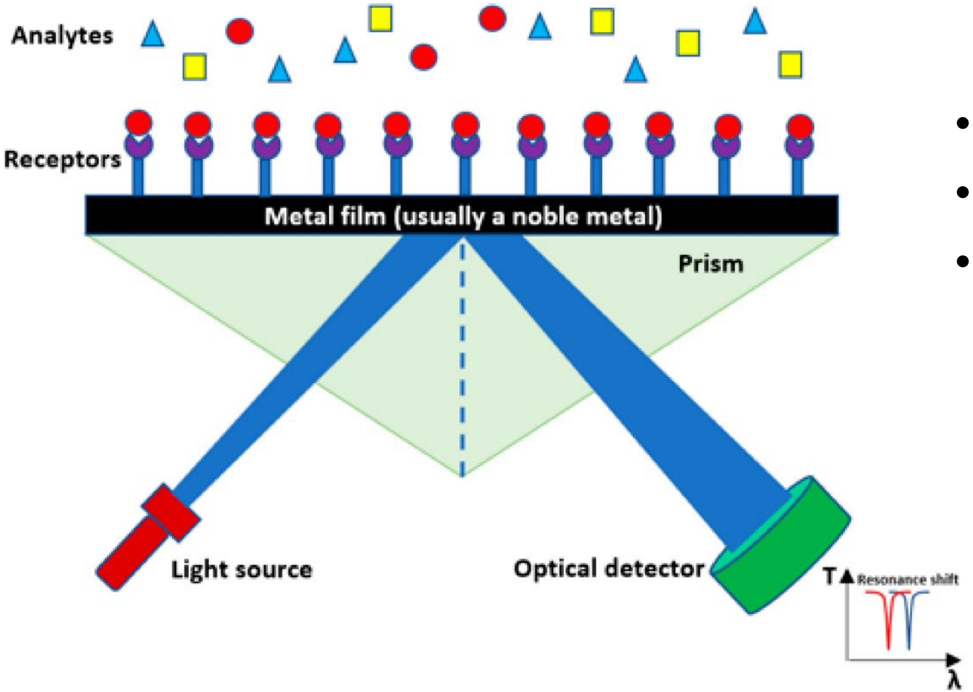
Optical biosensor

- Most common type of biosensor
- Light property changes after reactions, such as color, intensity, wavelengths, absorption, reflections.
- Compact analytical device containing a biorecognition sensing element integrated with an optical transducer system





Optical biosensor



- Light source
- Sensing interface (transducer)
- Optical detector

2

PART TWO

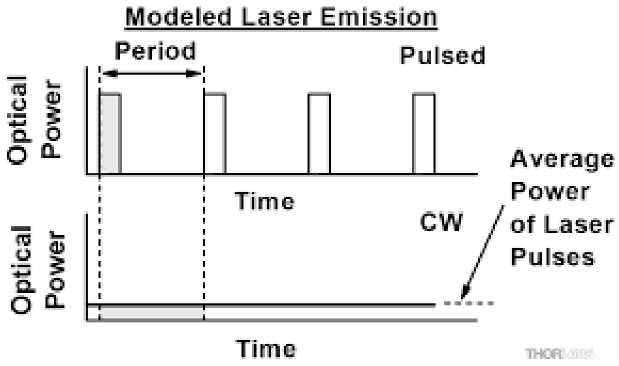
Light Source

Light sources

Lasers
- Solid state lasers
- Gas lasers
- Dye lasers

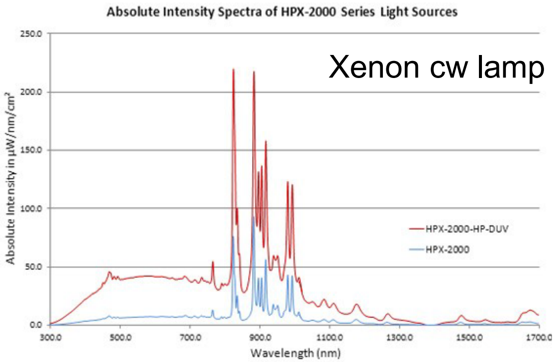
- CW (continuous wave) laser
  - Fixed
  - Tunable
- Pulse laser
  - Fixed
  - Tunable

LED (light emitting diode)

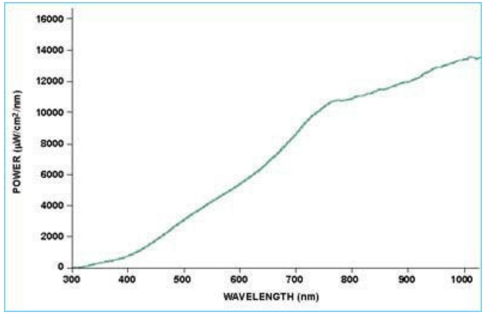


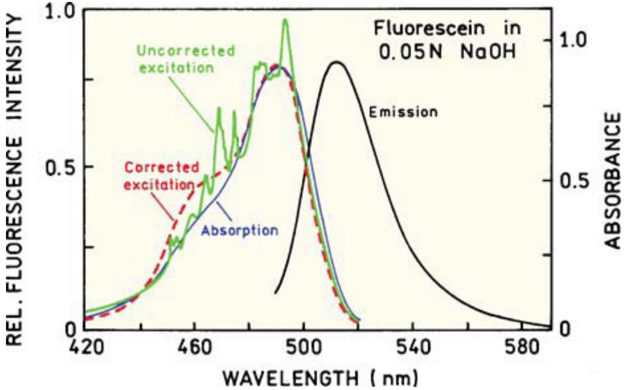
Various lamps
- Xenon lamp
- High pressure Hg lamp
- Low pressure Hg lamp
- Hg-Xe (Mercury Xenon) lamp
- Tungsten halogen lamp
- Deuterium

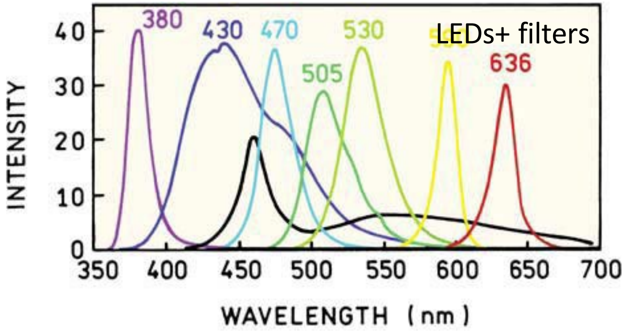
Light sources

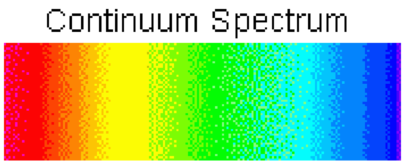


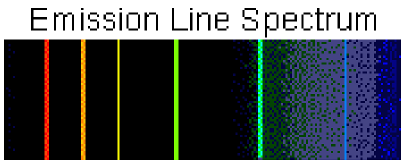
Spectral range: 185-2000 nm
Bulb power:35 W/75W
Bulb lifetime: 1000 hours





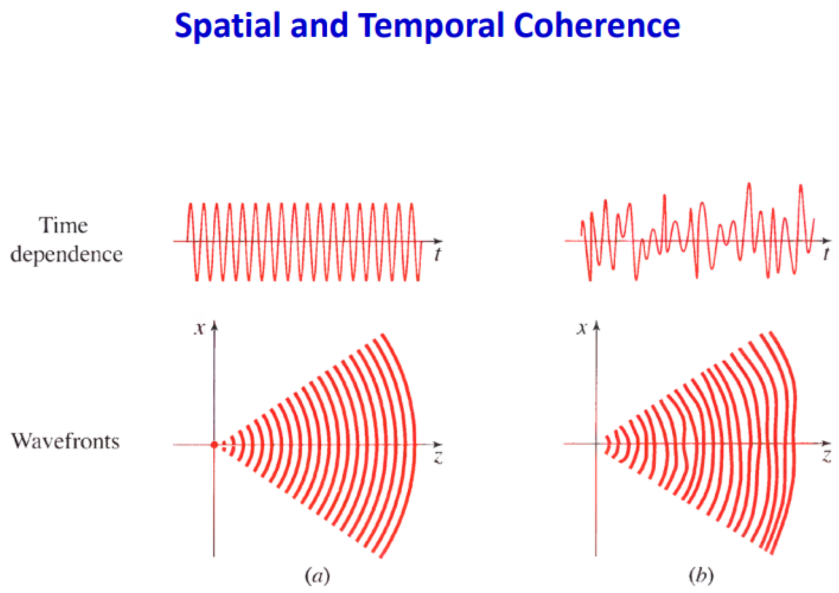


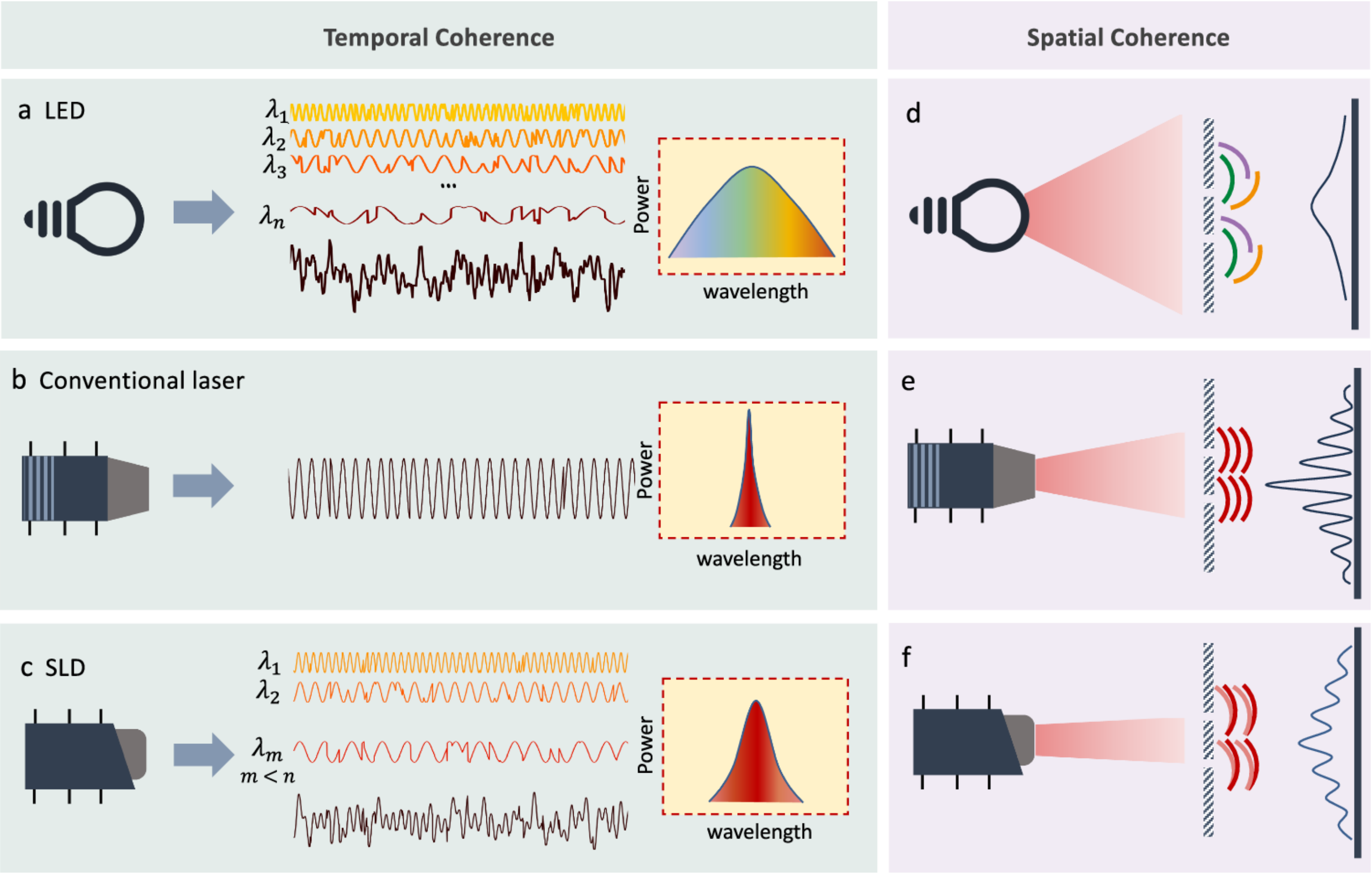




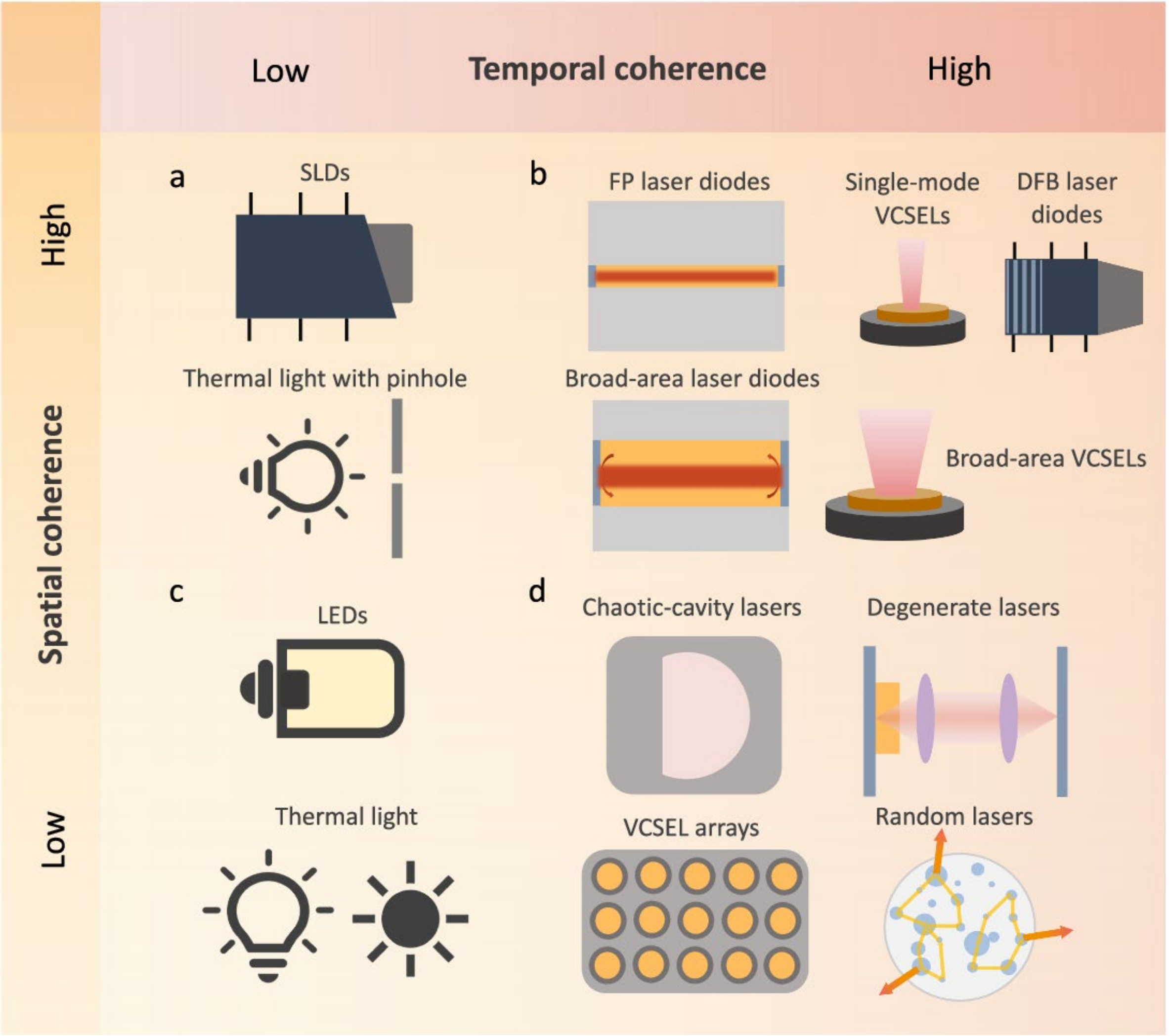
General requirements on sources

- **Spatial coherence**
  - Ideally single transverse mode
- Low noise
  - Relative intensity noise (RIN)
  - Frequency / phase noise
- Adequate power output
  - Signal-to-noise ratio (SNR)
  - Avoid nonlinearity





Temporal Coherence
Spatial Coherence
a LED
λ_1
λ_2
λ_3
...
λ_n
Power
wavelength
b Conventional laser
Power
wavelength
c SLD
λ_1
λ_2
λ_m
$m < n$
Power
wavelength
d
e
f


Low
Temporal coherence
High
High
Spatial coherence
Low
a
SLDs
Thermal light with pinhole
b
FP laser diodes
Single-mode VCSELs
DFB laser diodes
Broad-area laser diodes
Broad-area VCSELs
c
LEDs
Thermal light
d
Chaotic-cavity lasers
Degenerate lasers
VCSEL arrays
Random lasers

General requirements on sources

- Spatial coherence
  - Ideally single transverse mode
- **Low noise**
  - Relative intensity noise (RIN)
  - Frequency / phase noise
- Adequate power output
  - Signal-to-noise ratio (SNR)
  - Avoid nonlinearity

Sources of laser noise

- Quantum noise
  - Spontaneous emission
- Technical noise
  - Electrical current or optical pump fluctuations
  - Temperature changes
  - Mechanical vibrations

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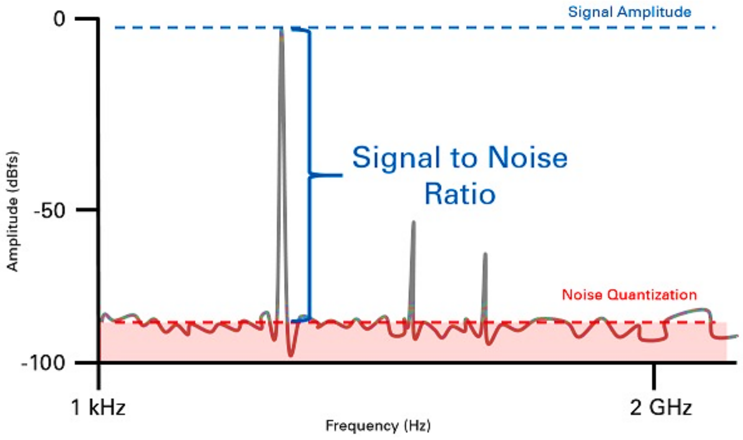
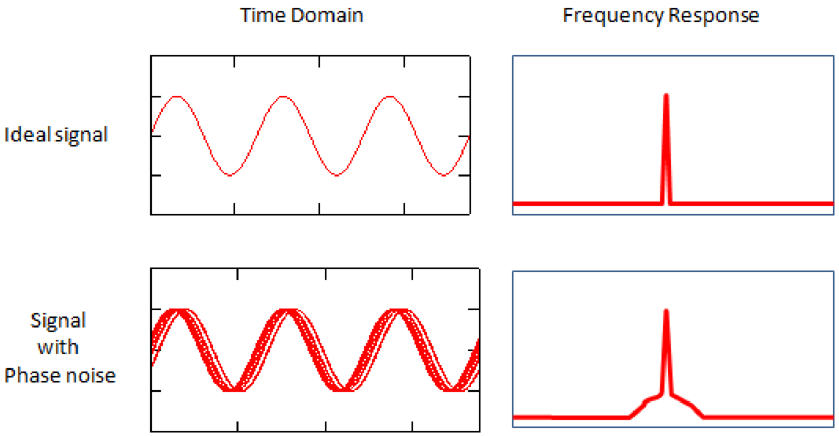
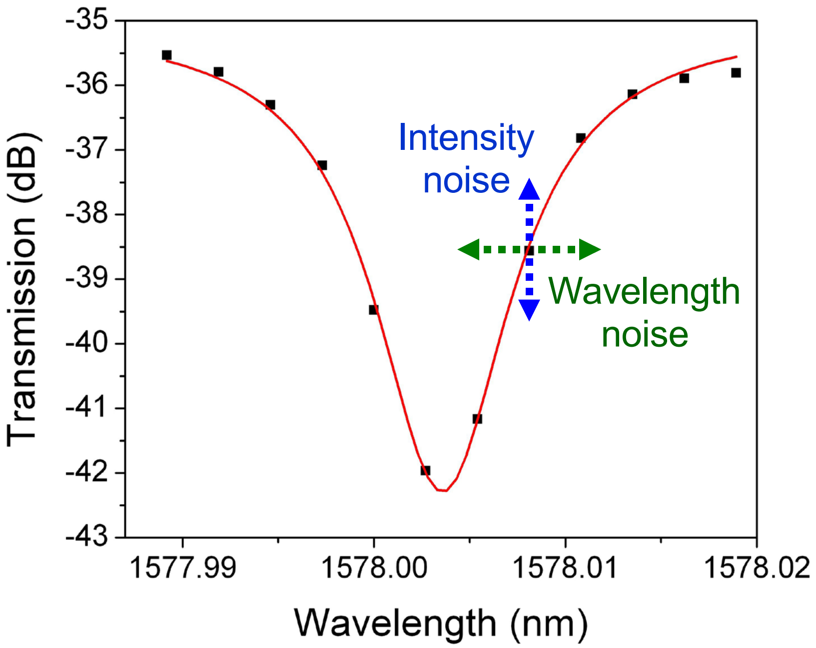
Noises

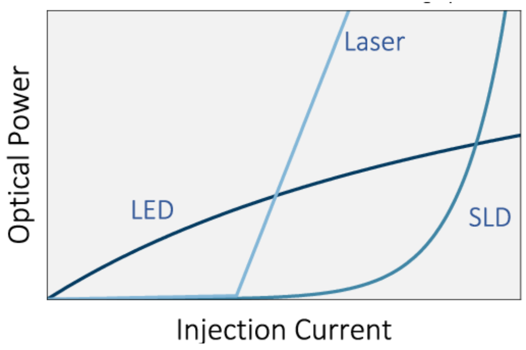

Signal Amplitude
Signal to Noise Ratio
Noise Quantization
Amplitude (dBfs)
0
-50
-100
1 kHz
2 GHz
Frequency (Hz)

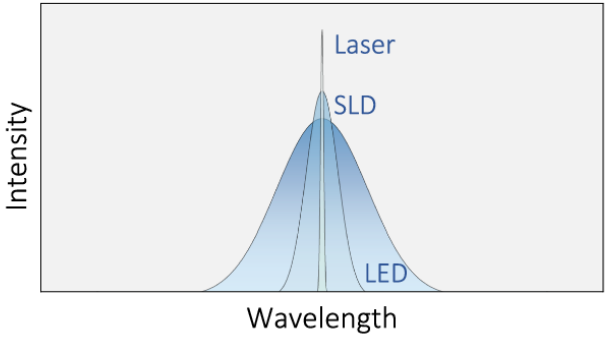

Time Domain
Frequency Response
Ideal signal
Signal with Phase noise

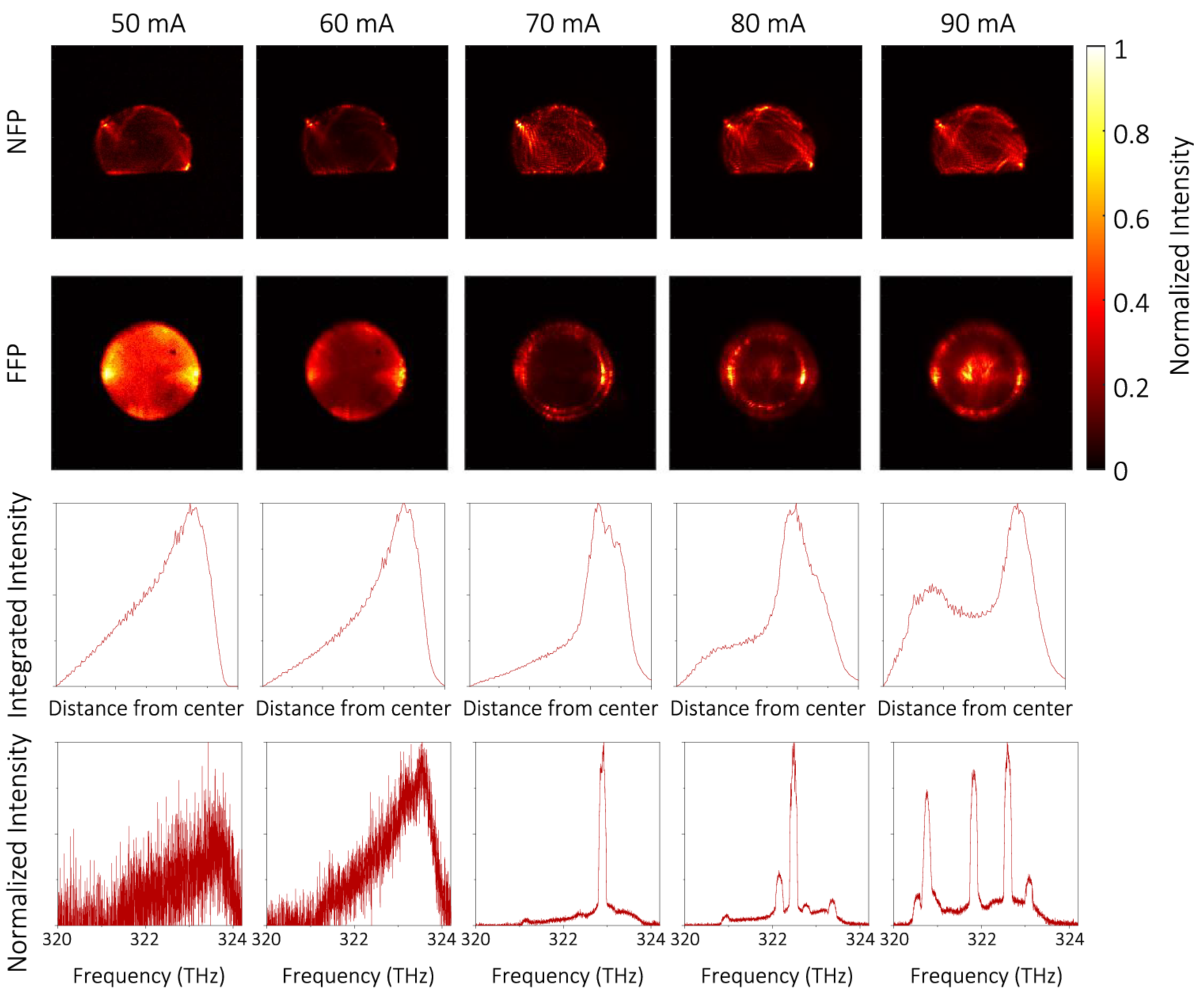

-35
-36
-37
-38
-39
-40
-41
-42
-43
Transmission (dB)
1577.99
1578.00
1578.01
1578.02
Wavelength (nm)
Intensity noise
Wavelength noise

General requirements on sources

- Spatial coherence
  - Ideally single transverse mode
- Low noise
  - Relative intensity noise (RIN)
  - Frequency / phase noise
- **Adequate power output**
  - Signal-to-noise ratio (SNR)
  - Avoid nonlinearity





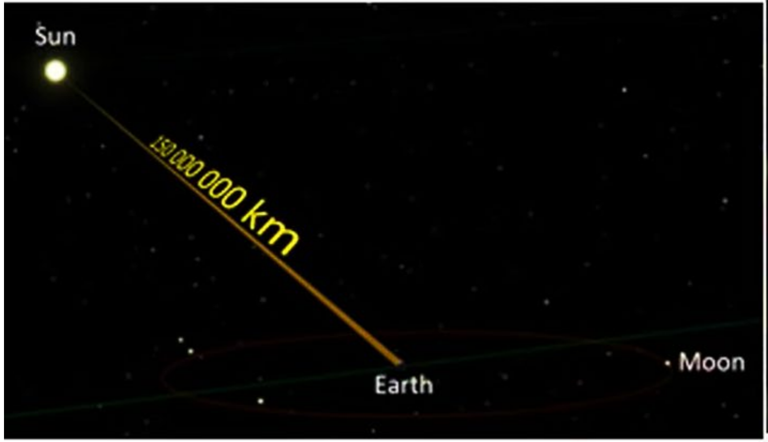


50 mA
60 mA
70 mA
80 mA
90 mA
NFP
FFP
Normalized Intensity
1
0.8
0.6
0.4
0.2
0
Integrated Intensity
Distance from center
Distance from center
Distance from center
Distance from center
Distance from center
Normalized Intensity
320
322
324
Frequency (THz)
Frequency (THz)
Frequency (THz)
Frequency (THz)
Frequency (THz)

Basic Property of Light

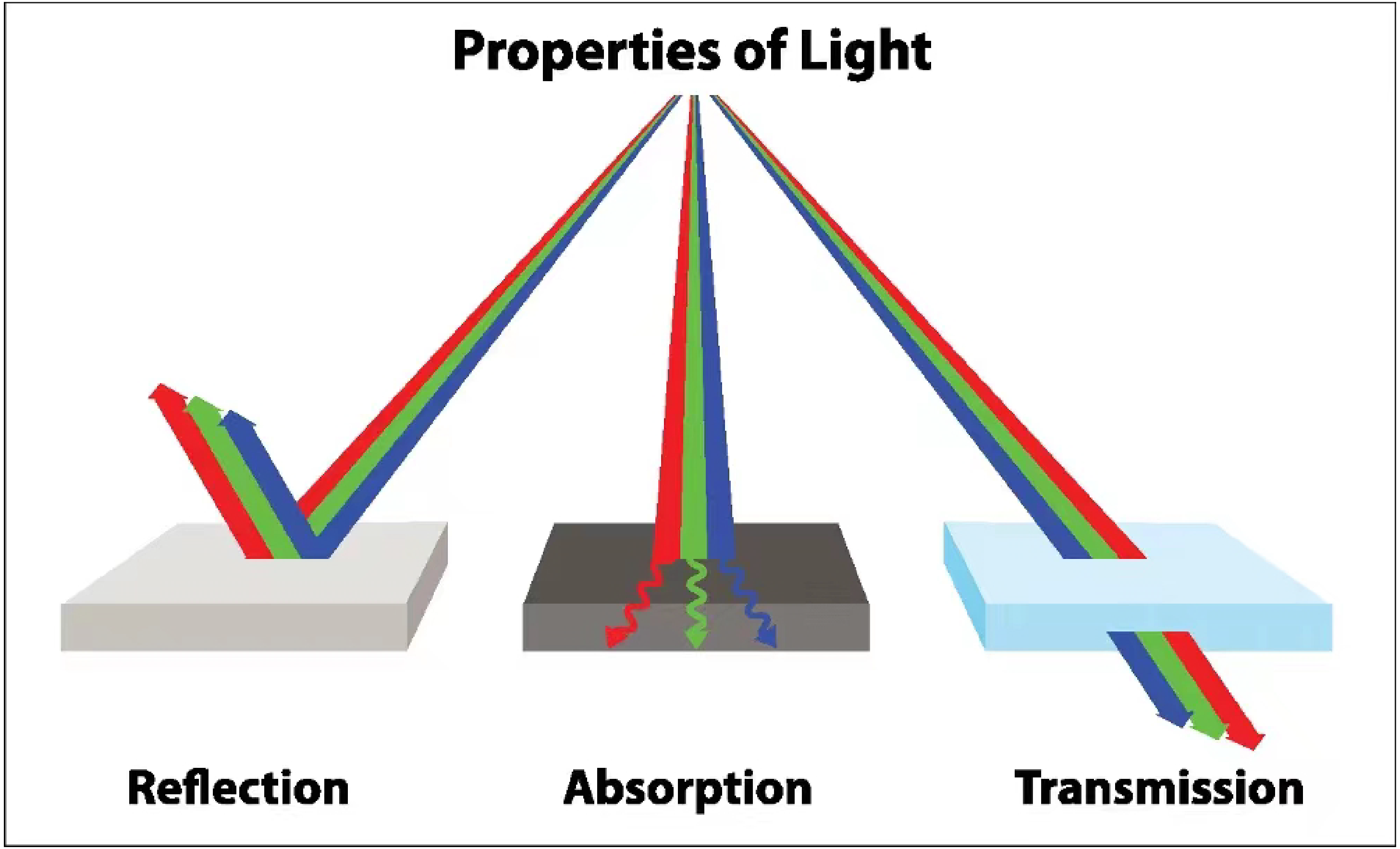
I. Light travels extremely fast

- $c = 3.0 \times 10^8$ m/s (300 000 000 m/s)
  - 300 Earth diameters per second

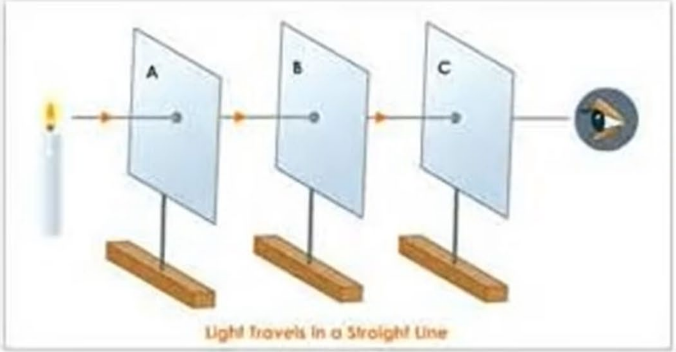


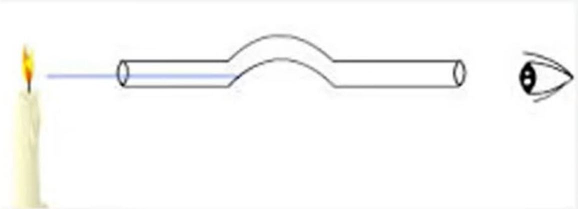
300 000 000 m/s 12.4 m/s

Basic Property of Light

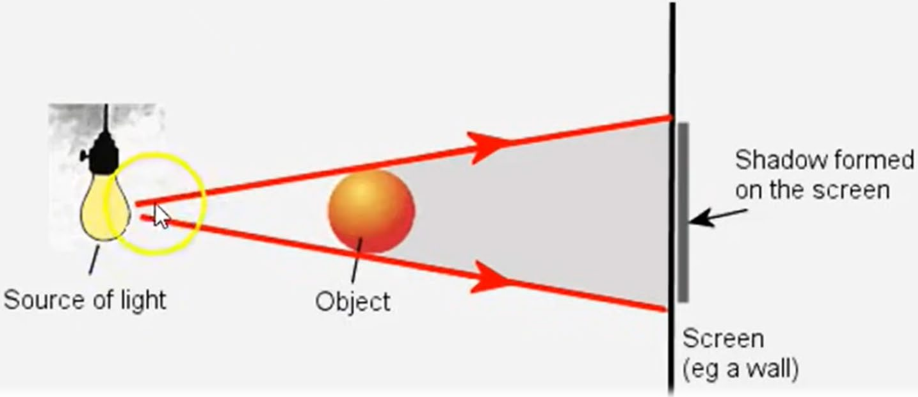


Light as Rays- The Line Model



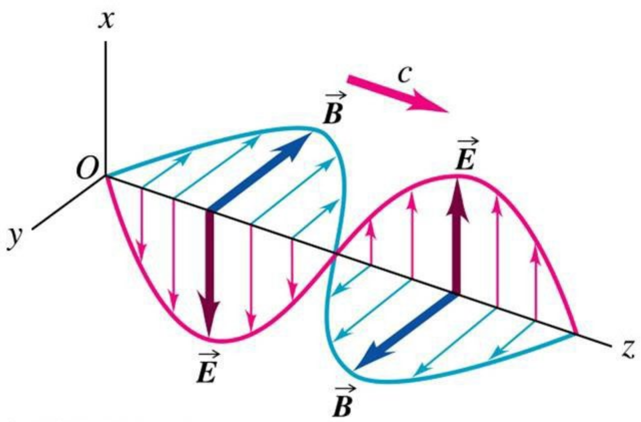


- Since light travels in straight lines **shadows** are made



Light as Waves- The EM Wave Model

- Electromagnetic wave: $E(z,t) = E_0\cos(\omega t - kz)$
  - Speed of light = $c = 3 \times 10^8$ m/s in a vacuum
  - Frequency = ν
  - Angular frequency = $\omega = 2\pi\nu$
  - Wavelength = $\lambda = c/\nu$
  - Wave vector = $k = 2\pi/\lambda$
    - Characterizes the phase
    - Vector in the direction of propagation



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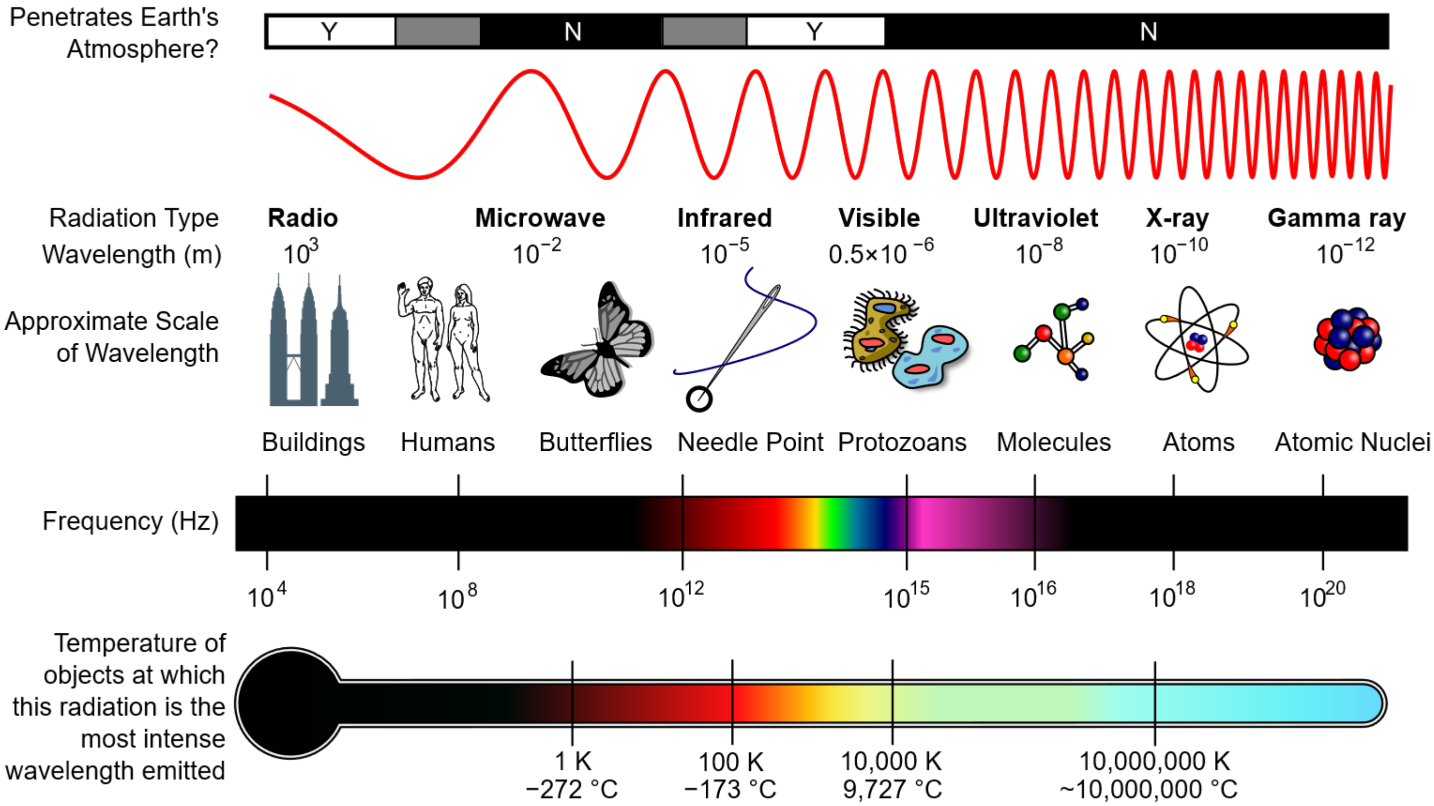
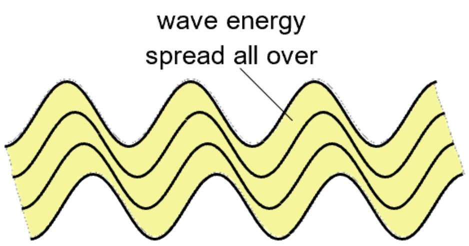
Electromagnetic Wave Spectra

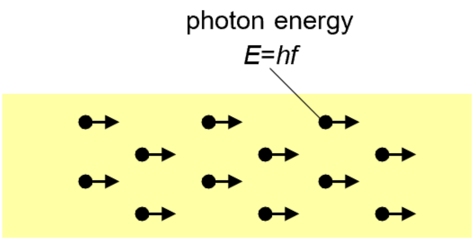


Light as Particles – The Particle Model

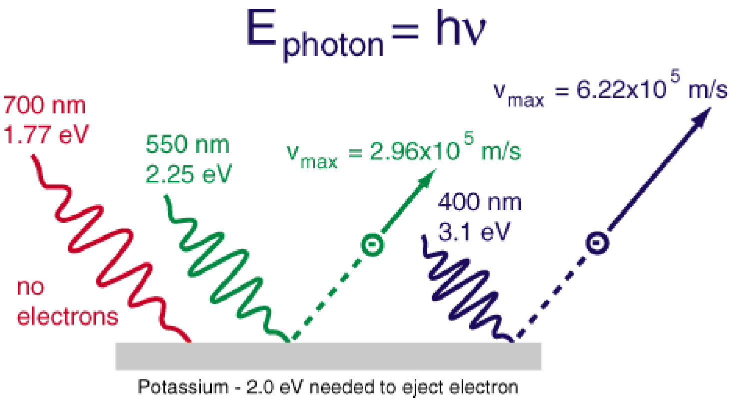
- Energy is quantized (discrete): not continuously variable
- Energy is delivered in "packets" of light - photons
- Energy of one photon $E = h\nu$
  - Planck's constant = h = 6.63×10^{-34} Joule second
- Total energy $E = Nh\nu$
- Photon momentum = $p = h/\lambda$
  - Light can deliver pressure to objects



wave model



photon model



Photoelectric effect

How to control light on a chip?

MANIPULATING LIGHT ON CHIPS

Complexity

Overall Performance

Reliability

Ergonomy

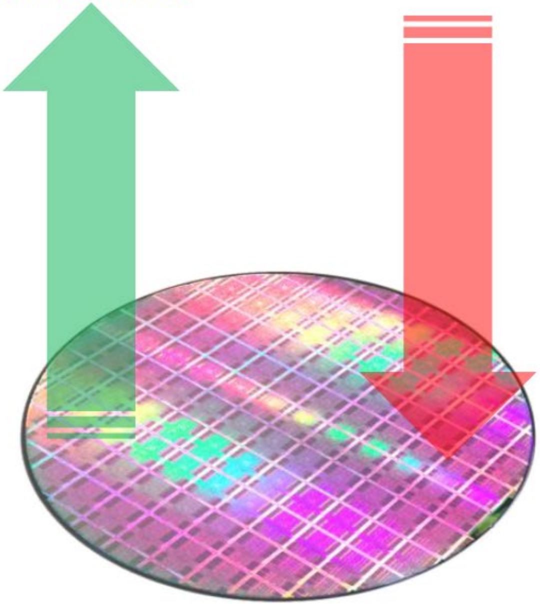
goes up

Power consumption

Ecological Footprint

Cost

goes down

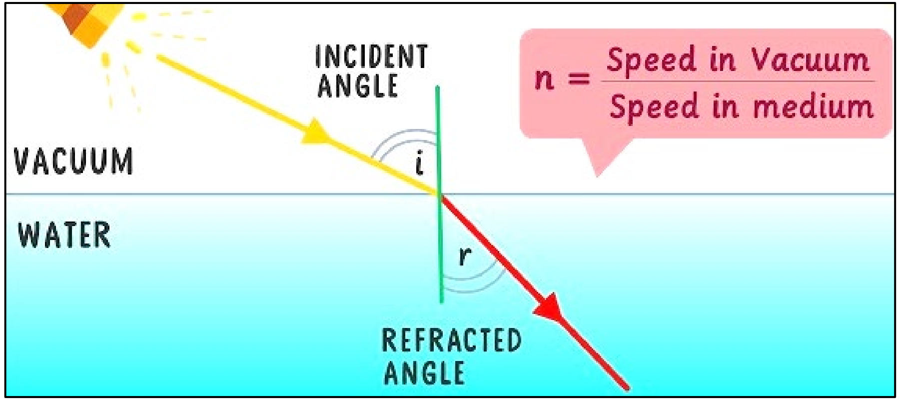


The benefits of scale

Refractive Index

Refractive index of a medium, n

Refractive index characterizes the interaction between the light (photon) and the medium.
In normal cases, it should be larger than 1



Medium	Refractive Index
Vacuum	1
Helium	1.000036
Water (typical)	1.30
Sugar Solution (30%)	1.38
Glass (typical)	1.5
Diamond	2.4

Density of medium

Low Density

High Density

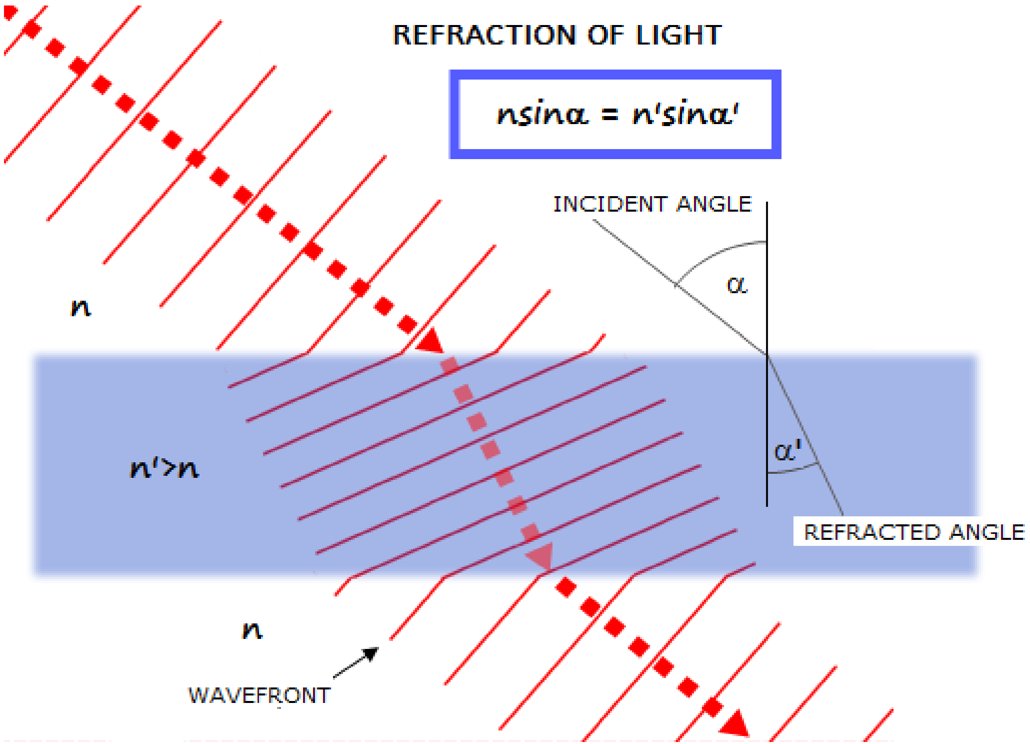
$$\frac{\sin\theta_2}{\sin\theta_1} = \frac{n_1}{n_2}$$

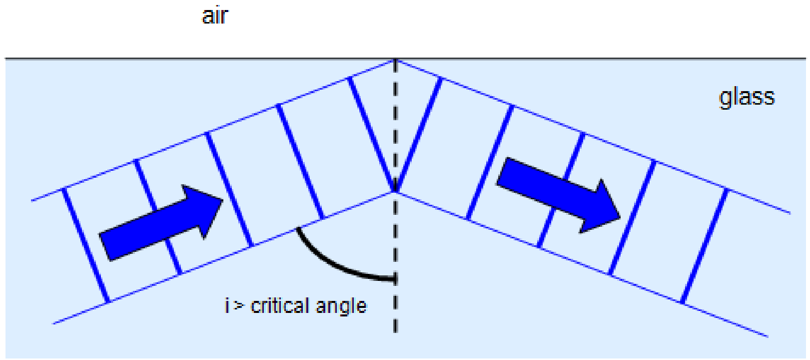
$$\boxed{n_1 \sin\theta_1 = n_2 \sin\theta_2}$$

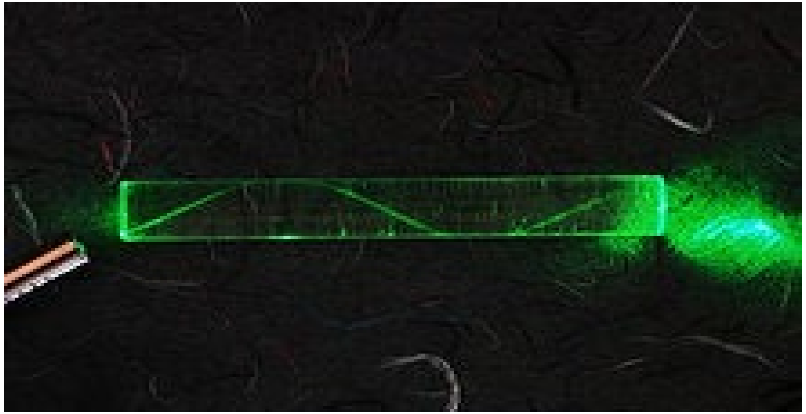
Snell's Law of Refraction.

Total Internal Reflection

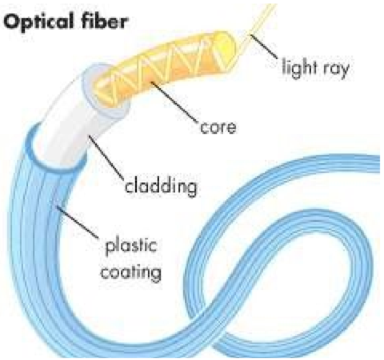
Manipulation of photons using refractive index

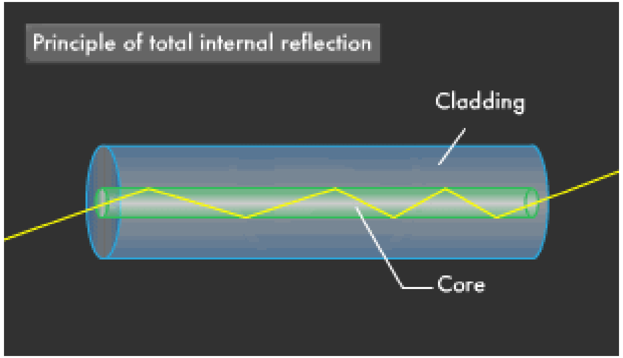


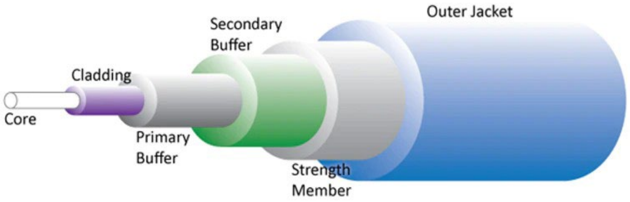


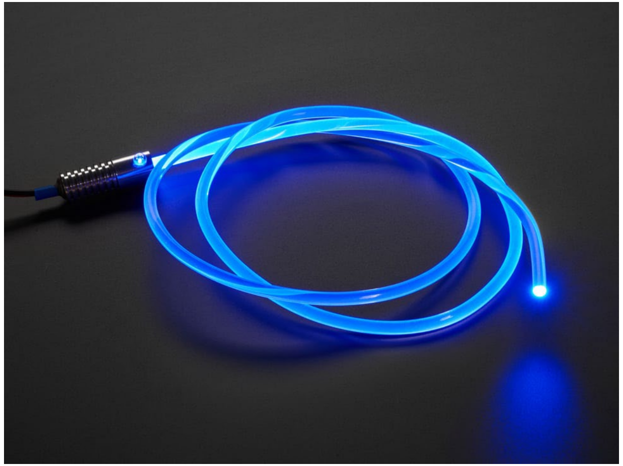


Manipulation of photons in a fiber



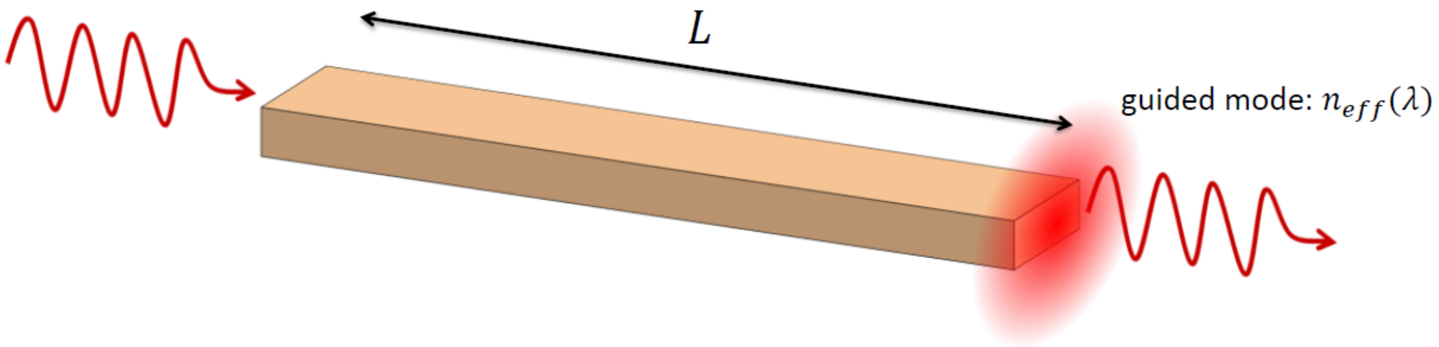






Waveguide

WAVEGUIDES



Propagate light from the input to the output

- wavefronts propagate with velocity $v_{ph}(\lambda) = \frac{c}{n_{eff}(\lambda)}$

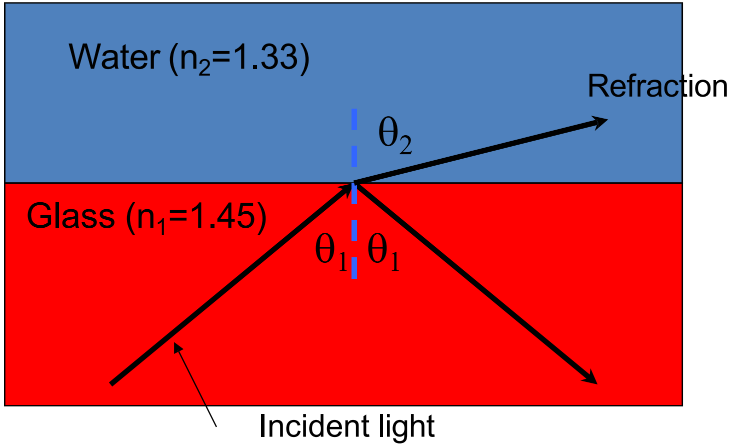
  ($n_{eff}(\lambda)$= effective refractive index)
- Dispersion: $n_{eff}(\lambda)$ is wavelength dependent
- Group velocity: time delay of a wave packet: $v_g(\lambda) = \frac{c}{n_g(\lambda)}$

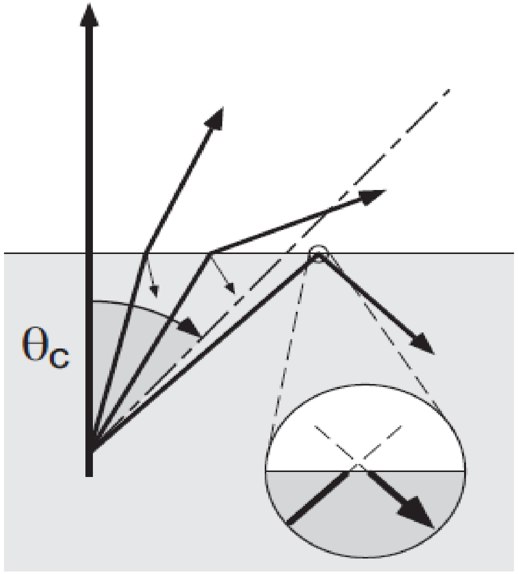
Light at Interface- Total internal Reflection

Snell's law: $n_1 \sin(\theta_1) = n_2 \sin(\theta_2)$

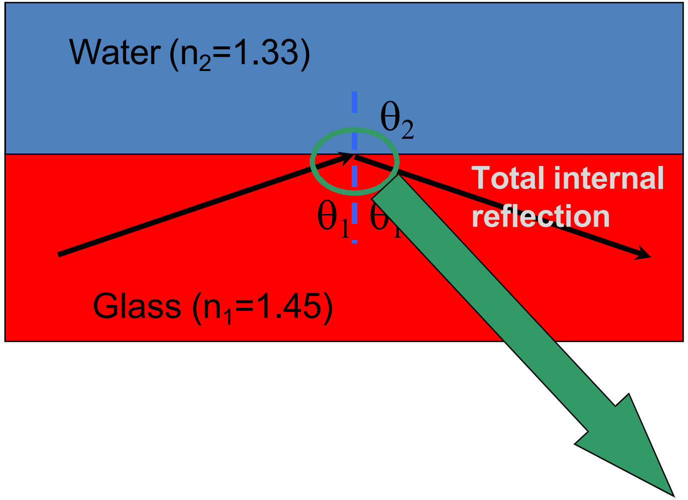
Definition of Critical Angle $\theta_c = \sin^{-1}(n_2/n_1)$

Snell's law does not work when $\theta_1 > \theta_c$

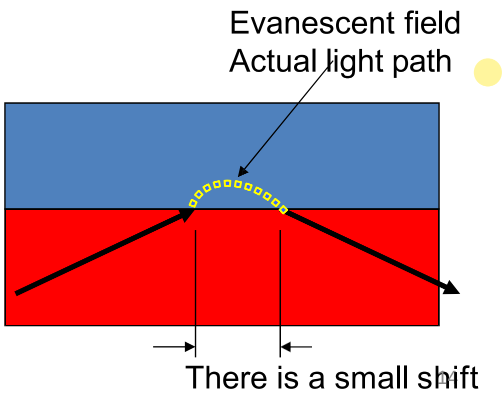




Light at Interface- Evanescent Field



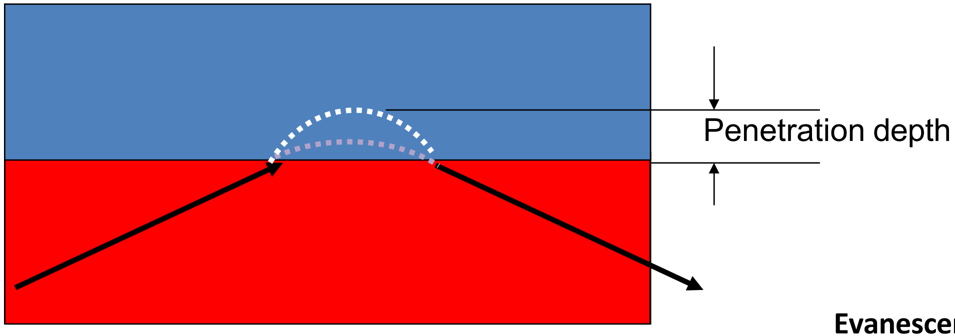
Question: Where does the light get reflected when it undergoes total internal reflection?

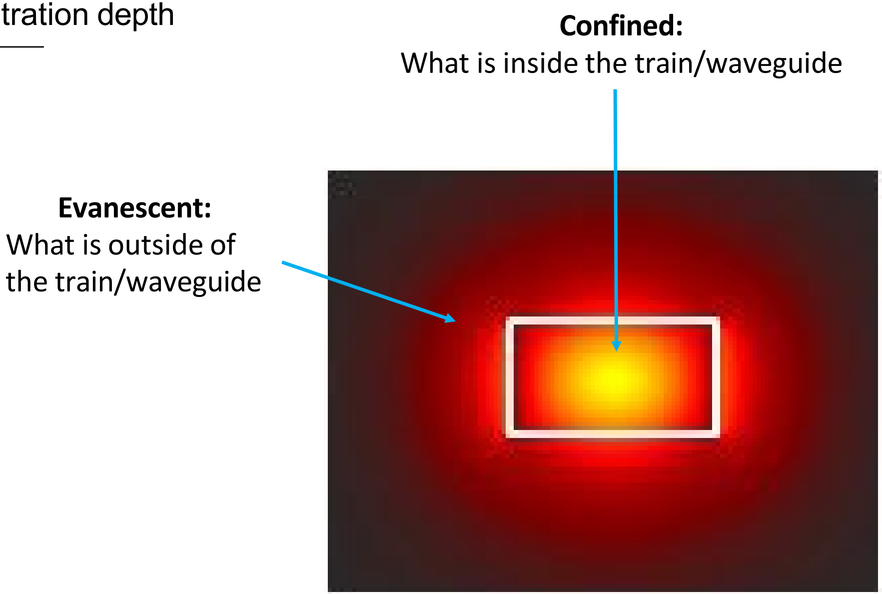


1. Light penetrates into "thinner" medium
2. Goos-Haenchen shift

Many waveguide/optical fiber based biosensors rely on evanescent field !!!

Penetration depth

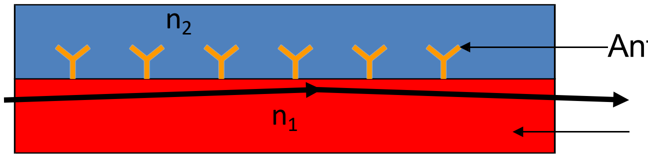




Penetration depth depends on ???

1. Wavelength
2. Refractive index contrast of two media
3. Incident angle

Penetration depth



In waveguide-based biosensors, the light incident angle is nearly 90 degrees

λ_0 = wavelength in vacuum

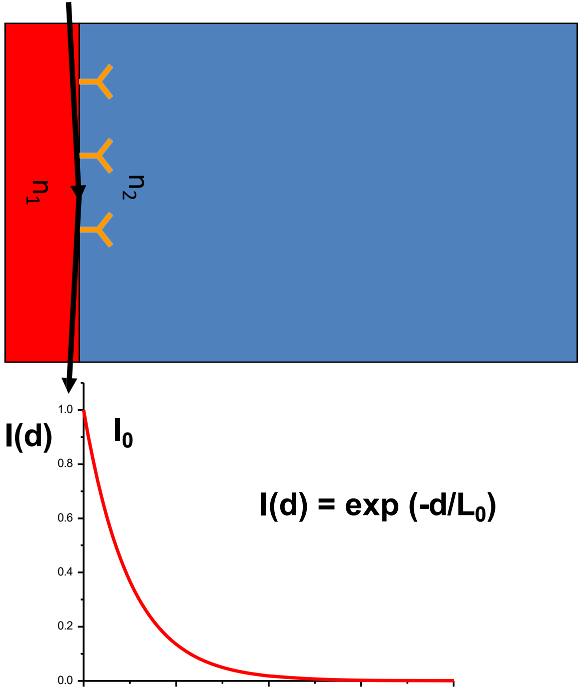
Penetration depth d (assuming incident angle = 90) =

$$\frac{\lambda_0}{4\pi\sqrt{n_1^2 \sin^2\theta_1 - n_2^2}}$$

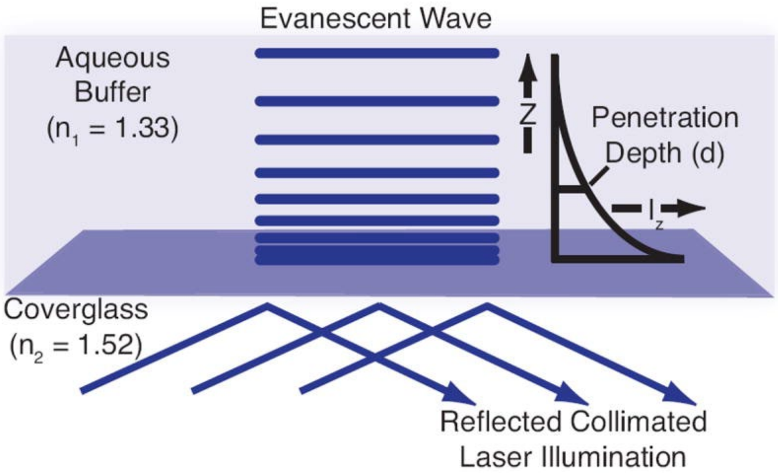
n_1 = 1.45

Calculate the penetration depth of 488 nm and 780 nm light when the substrate is water and air respectively

Evanescent Wave Field



Light intensity is attenuated exponentially in the less dense medium



Example 3

A plane wave undergoes total internal reflection at the interface between glass (n=1.6) and air (n = 1). Suppose that the angle of incidence is 45º. What is the penetration depth of the evanescent wave at the point of total reflection?

Penetration depth of evanescent wave at the point of total reflection.

Given.

$n_2 = 1.6$

$n_1 = 1$

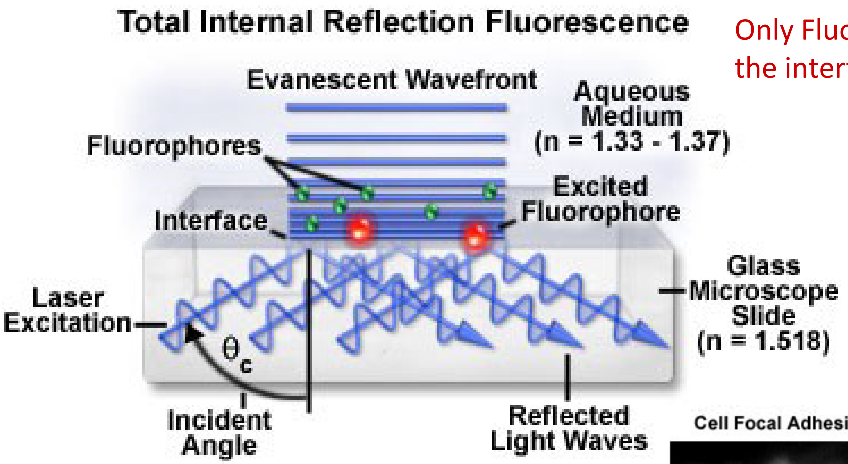
$\theta = 45^\circ$.

$$d = \frac{\lambda_0}{4\pi\sqrt{n_2^2 \sin^2\theta - n_1^2}}$$

$$= \frac{\lambda_0}{4\pi\sqrt{1.6^2 \sin^2 45 - 1^2}}$$

$$= \underline{\underline{0.15\,\lambda_0}}.$$

Total Internal Reflection Fluorescence Microscopy



Only Fluorophores within ~100 nm of the interface are illuminated

evanescent wave illumination: sharper image

Conventional widefield fluorescence microscope image: blurred

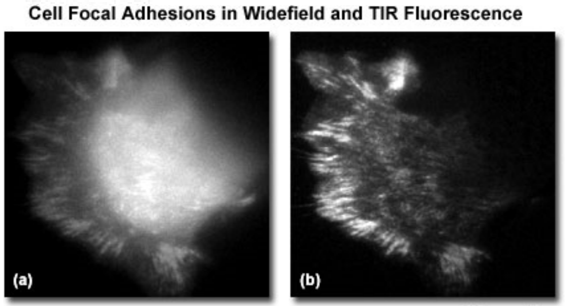
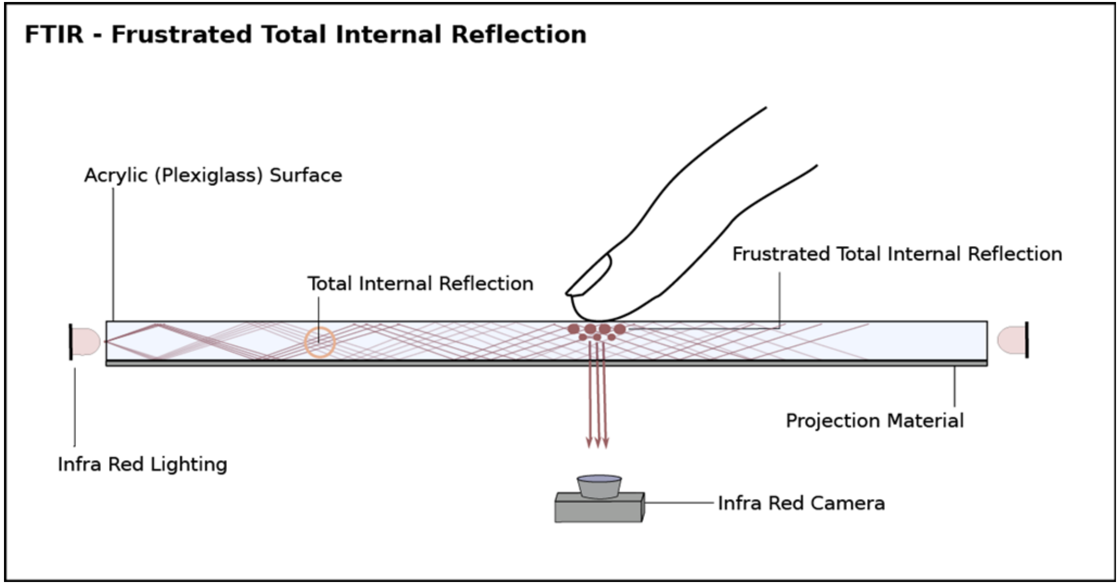


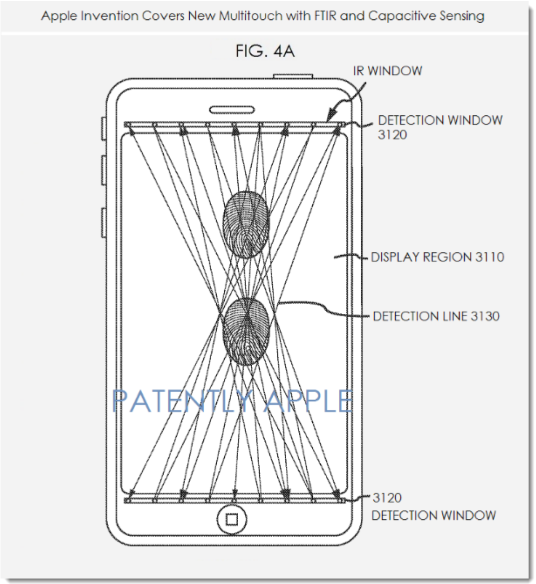
Figure 5

http://www.microscopyu.com/articles/fluorescence/tirf/tirfintro.html

Frustrated Total Internal Reflection

- Another example: touch screen





When a finger touches the acrylic surface, the infrared light is “frustrated” causing the light to escape internal reflection and scatter downwards where it is seen by an infrared camera (modified webcam).

Example 4

A light ray is moving inside a glass cube which has a refractive index of 1.58 and strikes the cube surface from the inside. If the cube is placed in air, with what minimum angle with the normal does the light ray have must incident on the glass air interface so that the light does not enter the air.

The expression for Snell's law of refraction is given by

$n_{\text{glass}} \sin\theta_a = n_{\text{air}} \sin\theta_b$

The Snell's law of refraction in terms of critical angle is,

$n_{\text{glass}} \sin\theta_{\text{crit}} = n_{\text{air}} \sin\theta_b$

Here, θ_{crit} is the critical angle of incidence for the total internal refraction.

At critical angle of incidence, the light ray does not refract into the air medium and the angle of refraction is 90° with the normal.

Substitute 90° for θ_b in $n_{\text{glass}} \sin\theta_{\text{crit}} = n_{\text{air}} \sin\theta_b$.

$n_{\text{glass}} \sin\theta_{\text{crit}} = n_{\text{air}} \sin 90^\circ$

$n_{\text{glass}} \sin\theta_{\text{crit}} = n_{\text{air}}(1)$

$n_{\text{glass}} \sin\theta_{\text{crit}} = n_{\text{air}}$

Rearrange the above equation for θ_{crit}.

$$\sin\theta_{\text{crit}} = \frac{n_{\text{air}}}{n_{\text{glass}}}$$

$$\theta_{\text{crit}} = \sin^{-1}\left(\frac{n_{\text{air}}}{n_{\text{glass}}}\right)$$

The expression for critical angle at the glass-air interface is,

$$\theta_{\text{crit}} = \sin^{-1}\left(\frac{n_{\text{air}}}{n_{\text{glass}}}\right)$$

Substitute 1.00 for n_{air} , and 1.58 for n_{glass} in the above equation.

$$\theta_{\text{crit}} = \sin^{-1}\left(\frac{1.00}{1.58}\right)$$

$$= 39.3\ ^\circ$$

Example 5

A total internal reflection fluorescence microscope uses a laser beam that undergoes total internal reflection within a glass microscope slide. The laser travels through a glass microscope slide and hits an interface with water. The index of refraction of water is 1.33 and the index of the glass is 1.52. What is the critical angle at which total internal reflection occurs within the microscope slide?

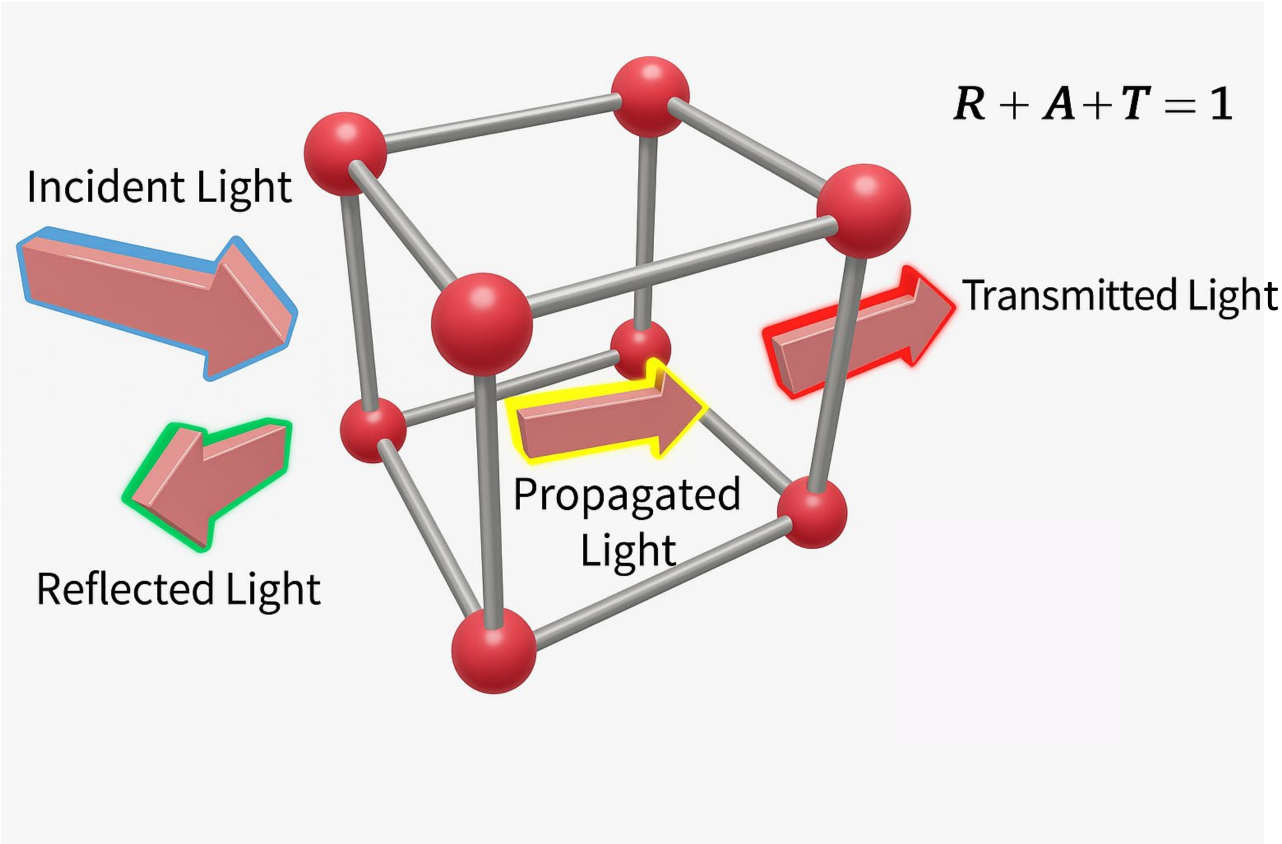
Ans: 61.045 deg

3

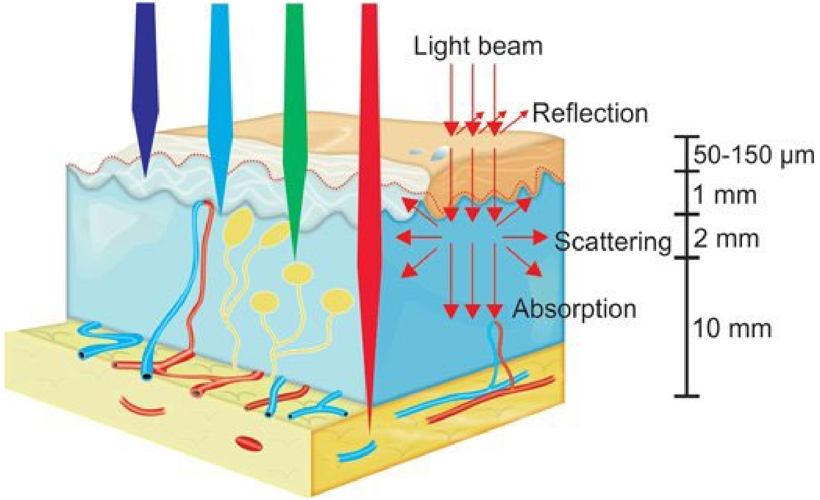
PART THREE

Sensing Interface

Light-matter Interactions



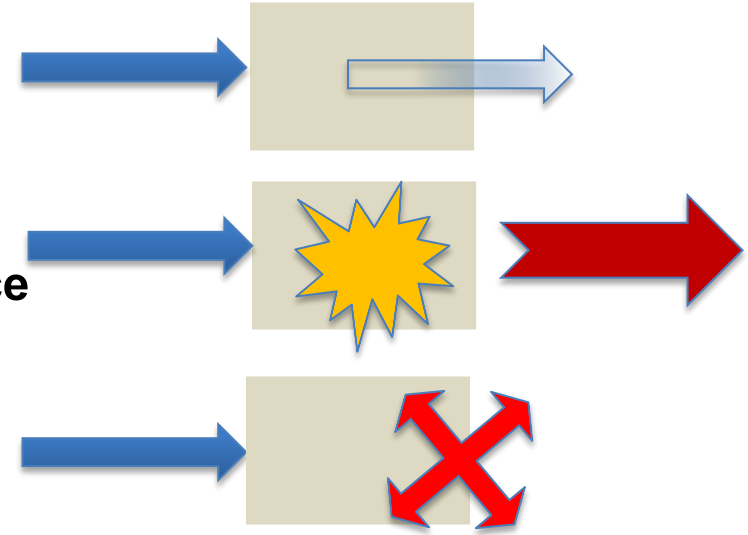
Light Interactions with Biological Materials



Absorption

Absorption+ Luminescence

Scattering

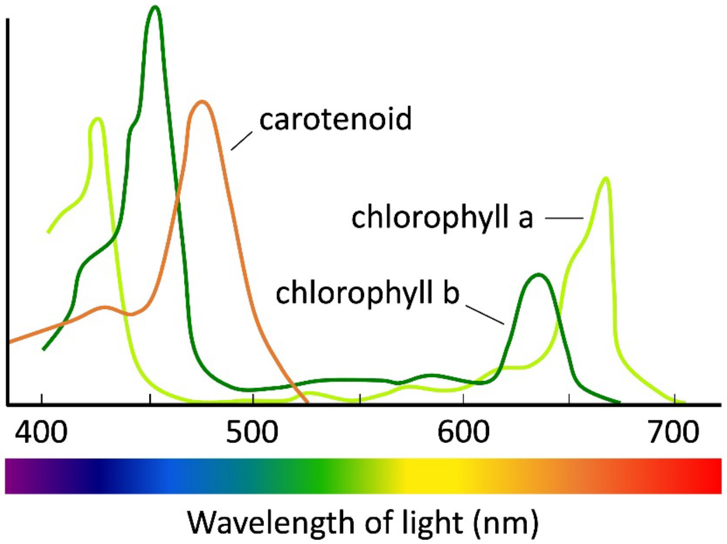


Spectroscopic Methods

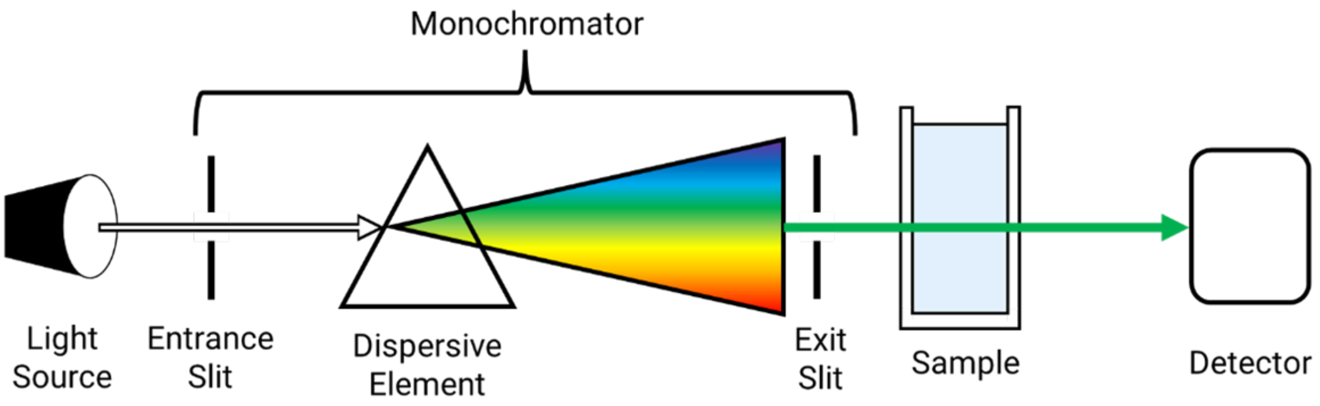
Measure wavelength dependent light intensity before/ after interaction of light with sample

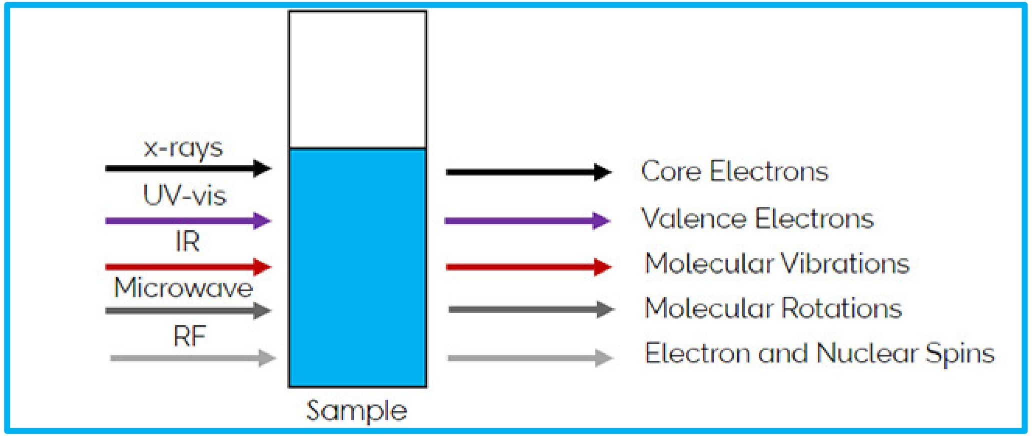
Can be used to identify types of molecules and quantitatively measure amount (concentration)

Many different techniques: e.g. absorption, fluorescence, Raman scattering, Emissions



Spectrometry



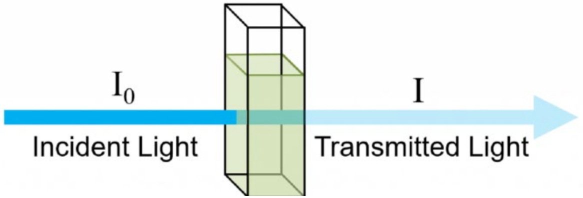


Absorption spectroscopy

- **Absorption occurs when the photon frequency matches the frequency associated with the molecule energy transition**

Beer-Lambert's law

A relationship between the attenuation of light through a substance and the properties of that substance.



$T = I/I_0$

I_0: original light intensity

I: light intensity after sample

Absorbance $= -\log_{10}(I/I_0) = \alpha L = \varepsilon c L$

$A = \varepsilon c L$

$$A = \varepsilon c l$$

A	Absorbance	
ε	Molar absorption coefficient	$M^{-1}cm^{-1}$
c	Molar concentration	M
l	optical path length	cm

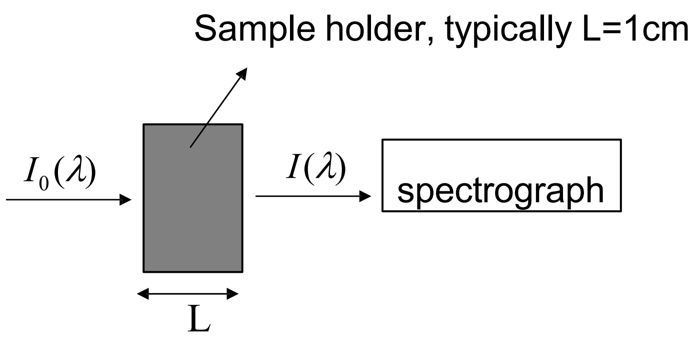
https://www.edinst.com/resource/the-beer-lambert-law/

Absorption spectroscopy

For molecules dissolved in solution

$$I(\lambda) = I_0(\lambda) \cdot e^{-\varepsilon'(\lambda)CL}$$

The wavelength dependent absorption of the molecule, $\varepsilon(\lambda)$, can be measured



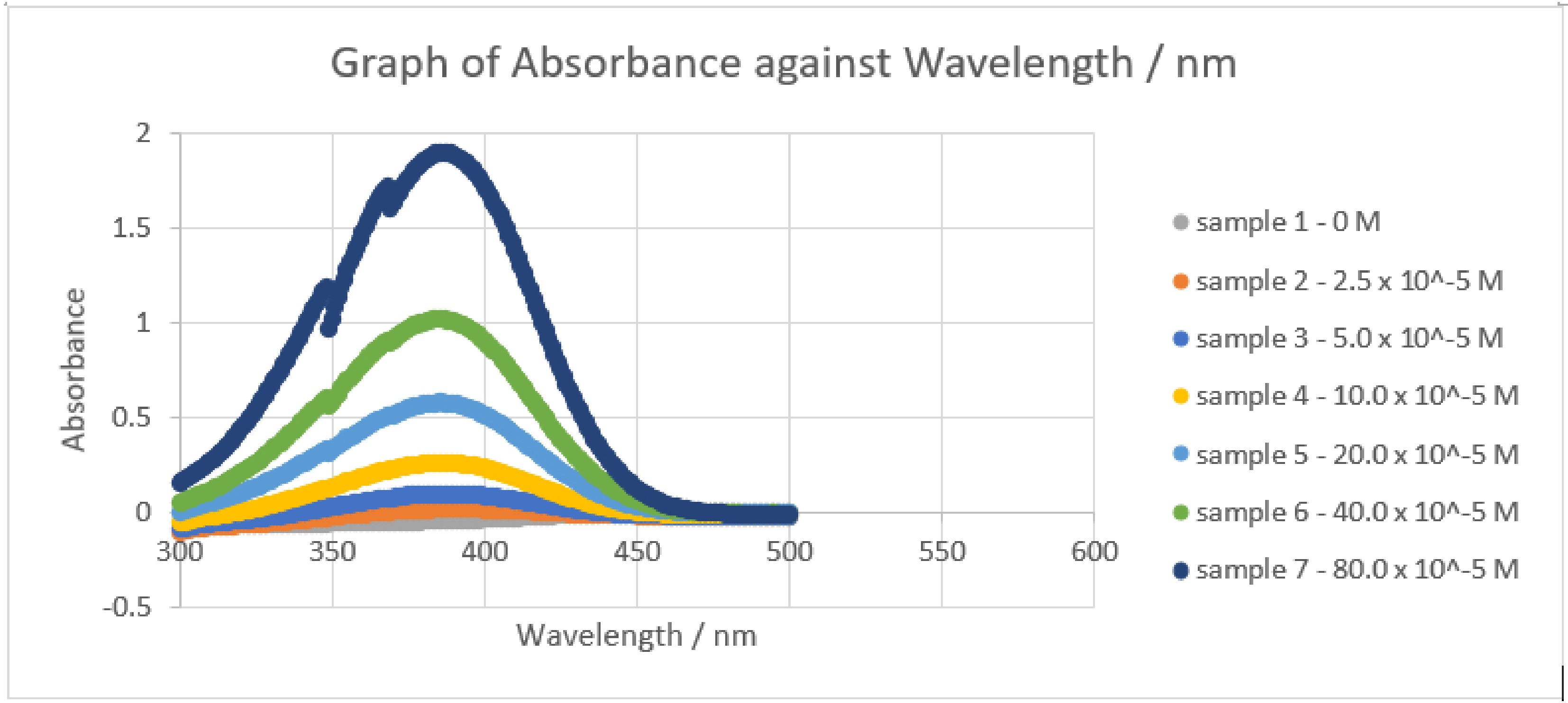
The absorbance (also called optical density) is defined as

$$A(\lambda) = -\log\left(\frac{I(\lambda)}{I_0(\lambda)}\right) = \varepsilon(\lambda)CL \qquad \varepsilon = \varepsilon'/2.303 \quad (M^{-1}cm^{-1})$$

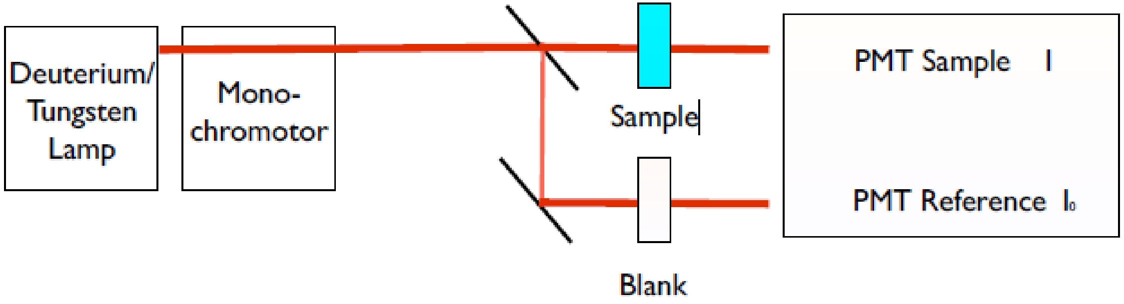
where $\varepsilon(\lambda)$ is the molar extinction coefficient ($M^{-1}cm^{-1}$) of the compound

The molar extinction coefficient is a function of wavelength and indicates the electronic energy levels of the molecule

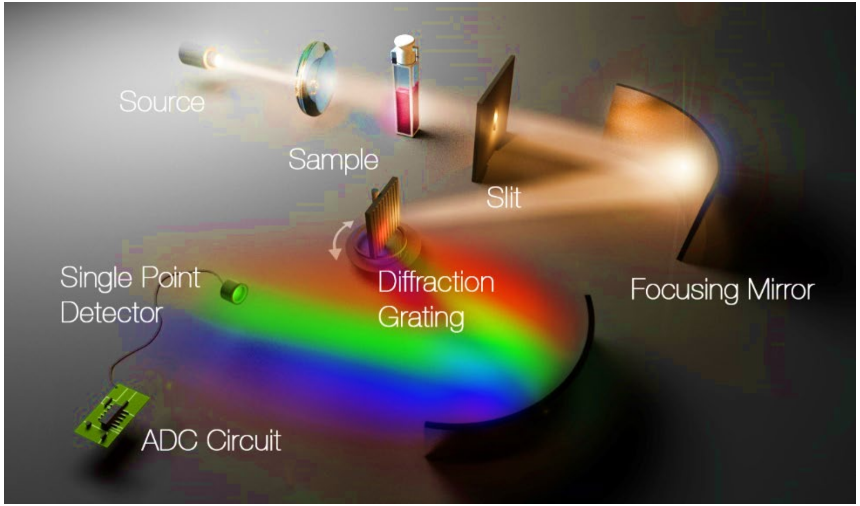
Absorption Measurement



Absorption Reading



Buffer doesn't contribute to absorption



UV-3600 UV-Vis-NIR Spectrophotometer
Double Beam, Three Detector System

http://www.ssi.shimadzu.com/products/product.cfm?product=uv3600

Example 6

Given that a concentration of $15\,\mathrm{M}$ is present for a solution of Tryptophan. This sample solution is placed in a $2.00\,\mathrm{cm}$ cuvette and the value of absorbance is identified to be 0.35 when radiations of $300\,\mathrm{nm}$ is passing through it.

A) Calculate the molar absorptivity coefficient.

B) Calculate the absorbance if the original solution's path length changes to $45\,\mathrm{mm}$.

By substituting the given absorbance in Beer's law, the molar absorptivity is calculated as follows:

$$\mathrm{A} = \varepsilon \mathrm{cl}$$

$$\varepsilon = \frac{\mathrm{A}}{\mathrm{c} \times \mathrm{l}}$$

$$\varepsilon = \frac{0.35}{15\ \mathrm{mol.L^{-1}} \times 2.00\,\mathrm{cm}}$$

$$\varepsilon = 1.2 \times 10^{-2}\,\mathrm{mol^{-1}\,L\,cm^{-1}}$$

In-order to calculate the new absorbance, first the given path length has to be changed from mm to cm.

$$1\,\mathrm{mm} = 0.1\,\mathrm{cm}$$

$$45\,\mathrm{mm} = 4.5\,\mathrm{cm}$$

By substituting the new path length in Beer's law, the absorbance can be calculated as follows:

$$\mathrm{A} = \varepsilon \mathrm{cl}$$

$$\mathrm{A} = 1.2 \times 10^{-2}\,\mathrm{mol}^{-1}\,\mathrm{L\,cm}^{-1} \times 15\,\mathrm{mol\,L}^{-1} \times 4.5\,\mathrm{cm}$$

Example 7

Consider a test solution with a concentration of $5.06\ \mathrm{mol/L}$. Given that only 15% of a specific light beam is transmitted through the solution and that the cuvette is $2.2\ \mathrm{cm}$ long. Calculate the value of molar absorptivity coefficient, (ε).

$$\mathrm{Absorbance} = -\log(\mathrm{Transmittance})$$

$$\mathrm{A} = -\log\left(\frac{15}{100}\right)$$

$$\mathrm{A} = -\log(0.15)$$

$$\mathrm{A} = 0.82$$

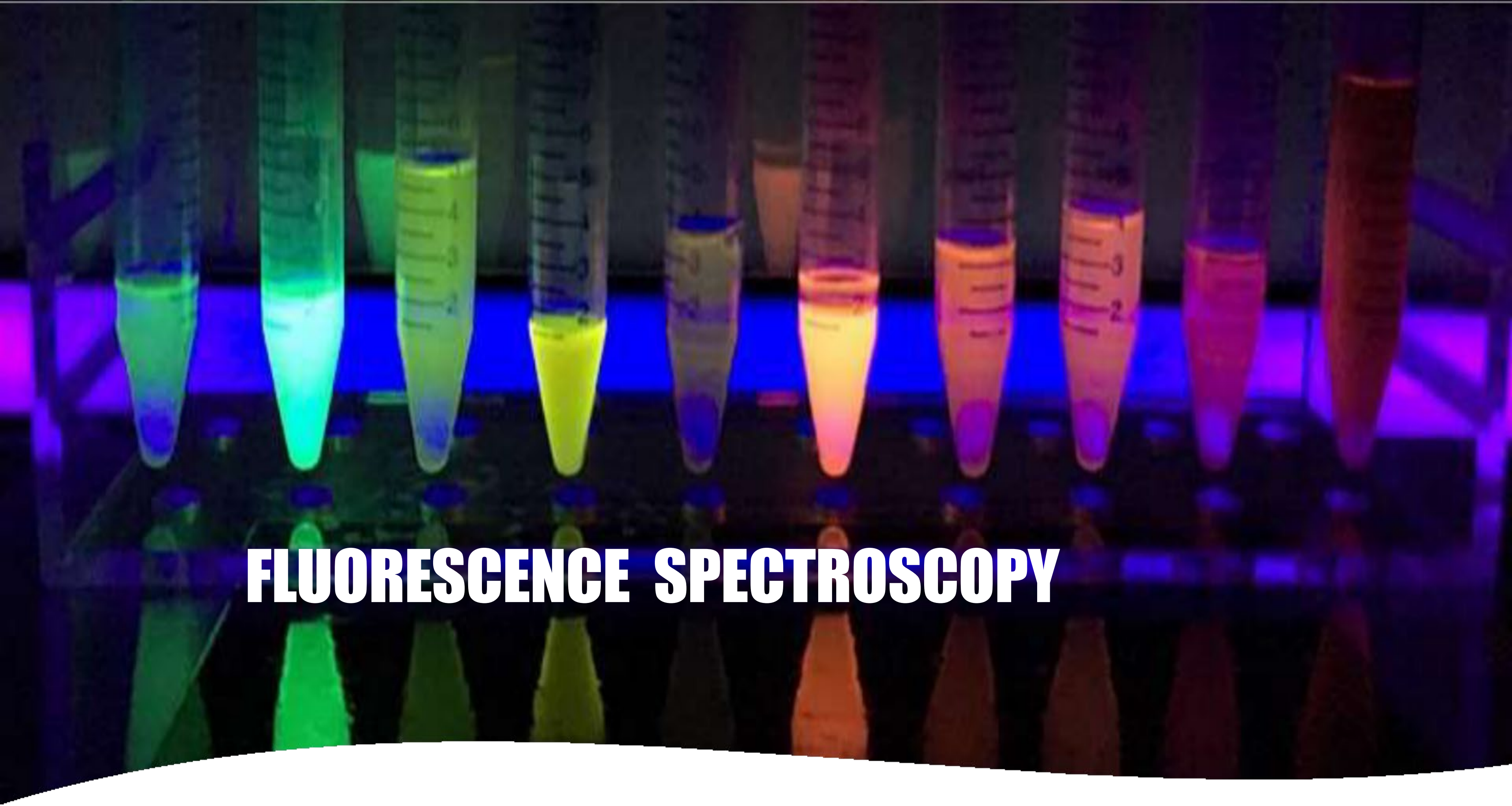
By substituting the calculated absorbance in Beer's law, the molar absorptivity is calculated as

$$\mathrm{A} = \varepsilon \mathrm{cl}$$

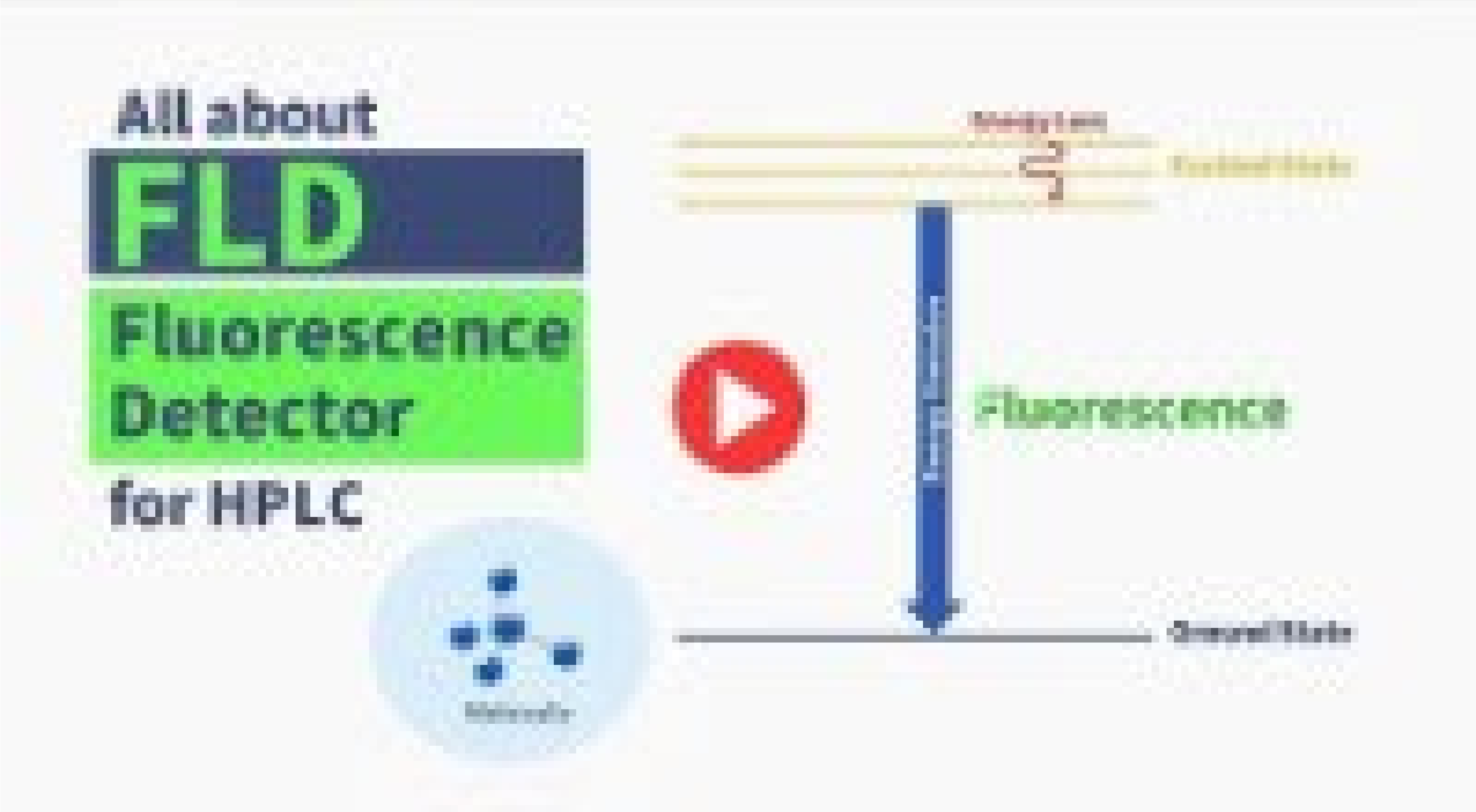
$$\varepsilon = \frac{\mathrm{A}}{\mathrm{c} \times \mathrm{l}}$$

$$\varepsilon = \frac{0.82}{5.06\,\mathrm{mol\,.L^{-1}} \times 2.2\,\mathrm{cm}}$$

$$\varepsilon = 7.4 \times 10^{-2}\ \mathrm{mol^{-1}L\,cm^{-1}}$$

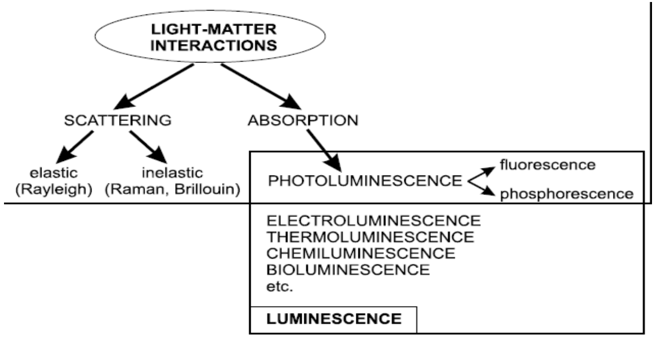


FLUORESCENCE SPECTROSCOPY

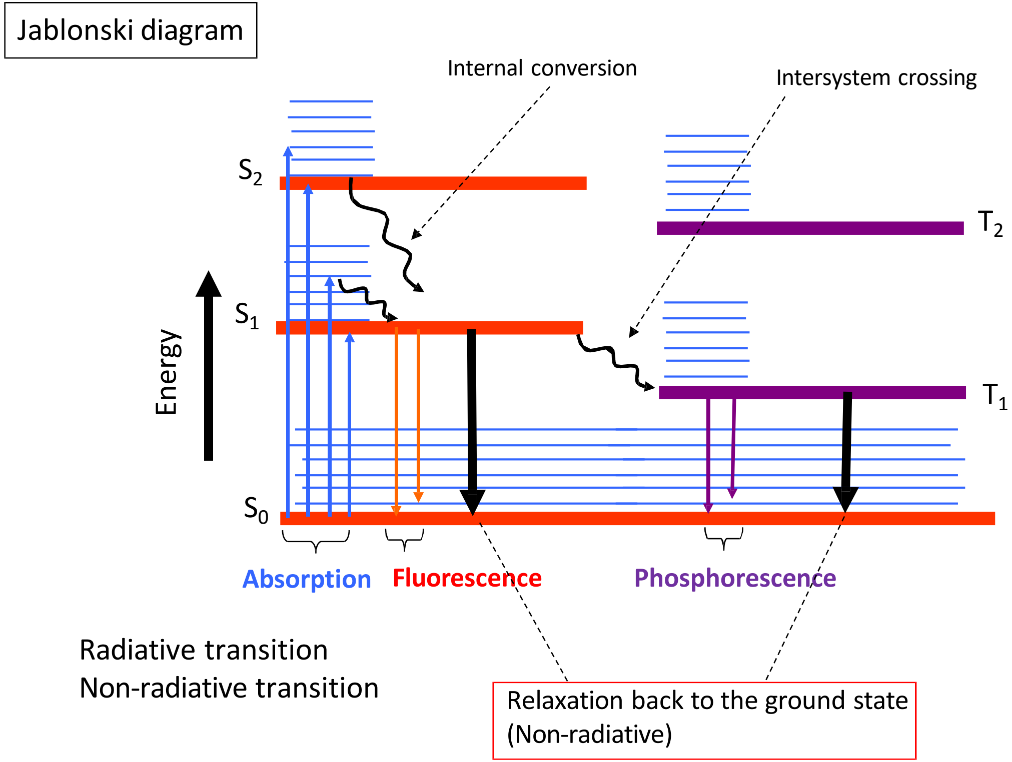

All about
FLD
Fluorescence
Detector
for HPLC
Fluorescence

Luminescence

- What is luminescence
  - It occurs when an electron returns to the electronic ground state from an excited state and loses its excess energy as a photon
  - Emission of photons from an electronically excited state
- Types of luminescence, according to mode of excitation
  - Fluorescence /phosphorescence: absorption of light
  - Chemiluminescence: chemical process (e.g. oxidation)
  - Bioluminescence: biochemical process



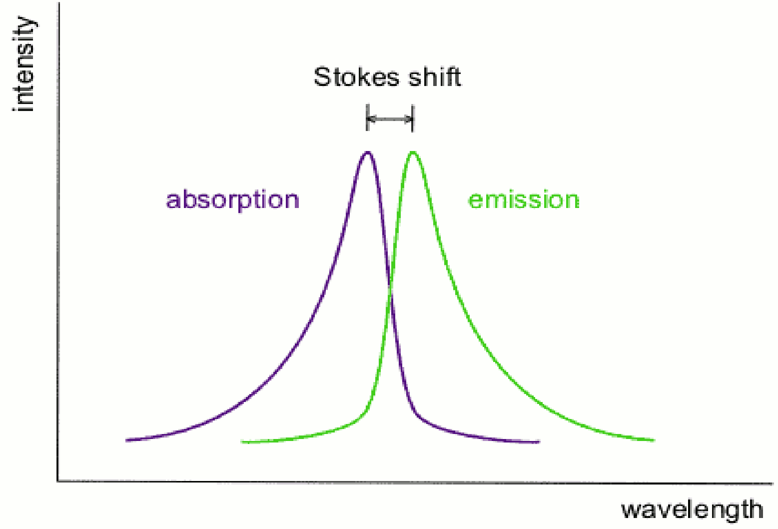
Light Absorption and Emission



Fluorescence

Energy of emitted radiation is less than that of absorbed radiation because a part of energy is lost due to vibrational or collisional processes. Hence the emitted radiation has longer wavelength (less energy) compared to Absorption

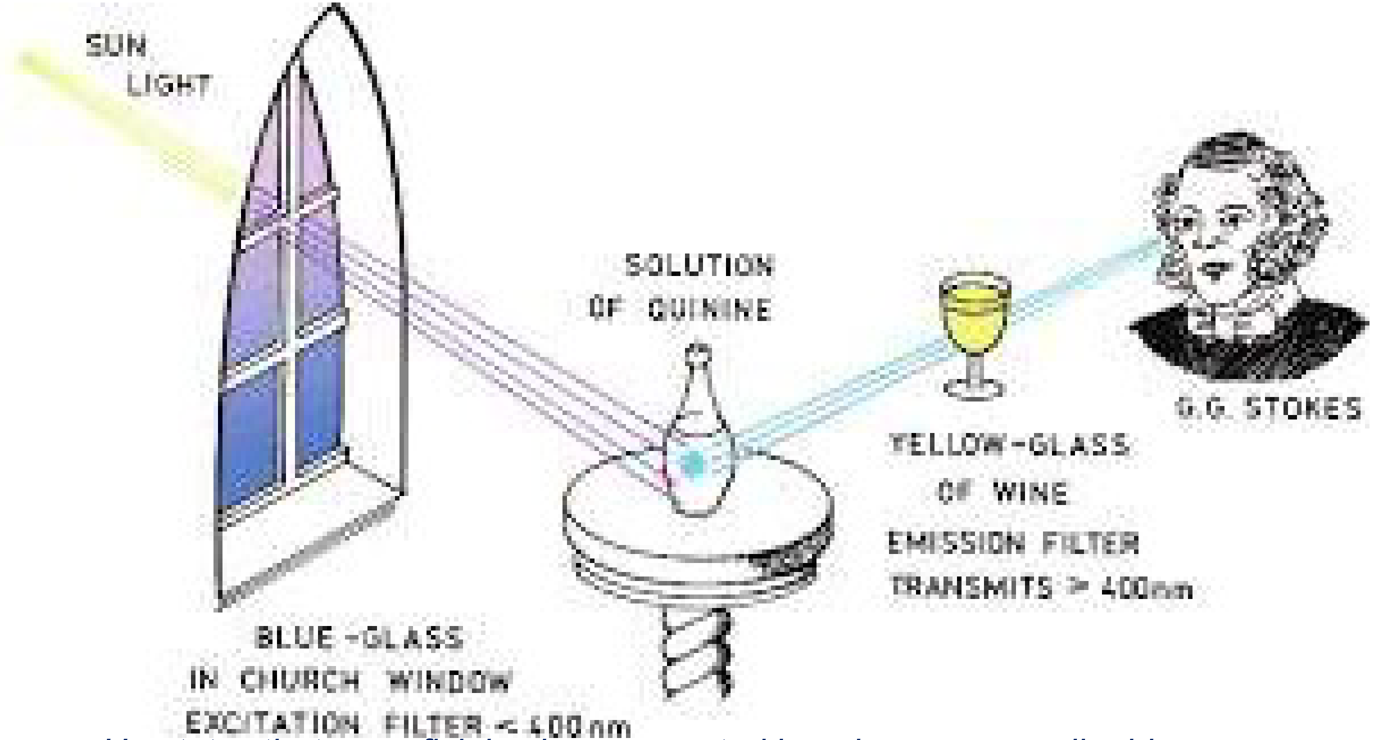
A Stokes shift is the difference between the absorption and emission peaks of a molecule.



Emission of Photons

- ✓ $S_1 \rightarrow S_0$ **radiative transition**
- ✓ **Timescale of 10^{-10} to 10^{-7} s**
- ✓ **Rapid Vibrational Relaxation And Internal Conversion**

FIRST OBSERVED FROM QUININE BY SIR J.W.HERSHEL IN



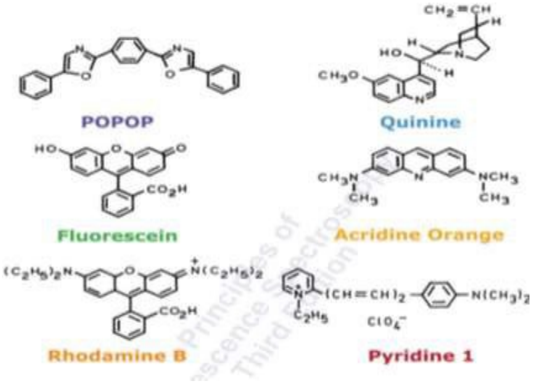
He states that superficial colour presented by a homogenous liquid internally colourless

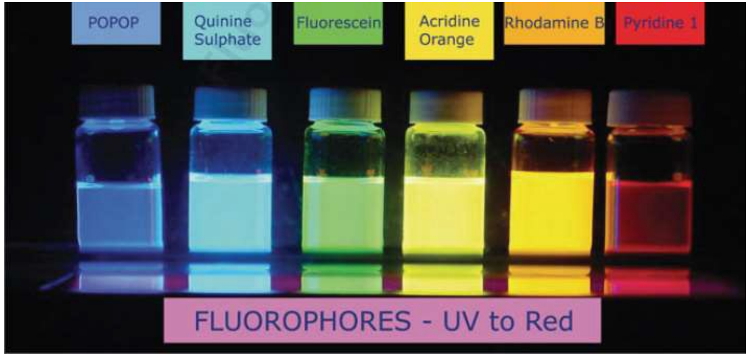
Adapted from HORIBA JobinYvon Inc., Leading the 21 st Century in Time-Resolved Fluorescence Instrumentation Dr. Adam M. Gilmore Applications Scientist Fluorescence slide share

The term fluorescence comes from the MINERAL FLUORSPAR (CALCIUM FLUORIDE)
coined by SIR GEORGE G. STOKES.

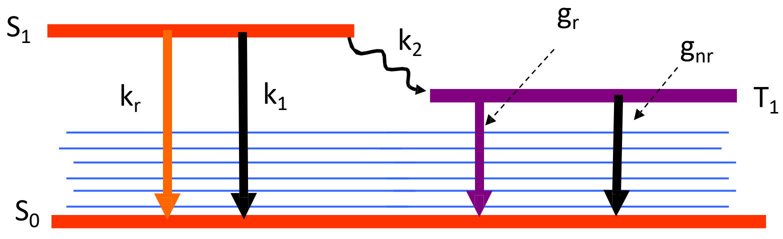
Fluorescent fluorite: Tumble-polished specimens of fluorite in normal light (top) and under short-wave ultraviolet light (bottom).

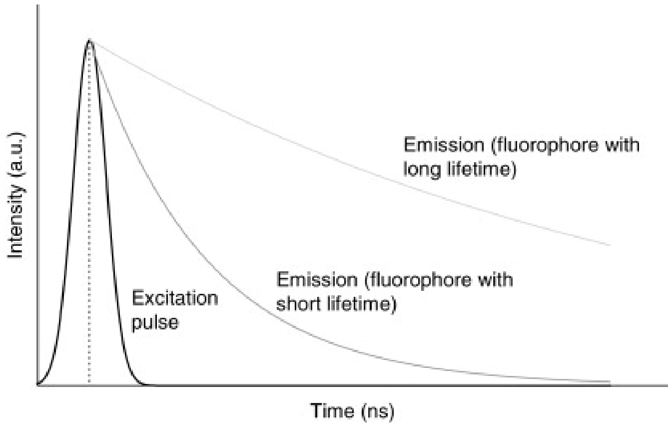
Fig Adapted Article by: [Hobart M. King](), Ph.D., RPG





Lifetime





Let's look at the population of molecules at S_1, $[S_1]$

How many channels does molecule at S_1 to leave S_1 state?

Radiative decay rate: k_r

Non-radiative decay rate: $k_{nr} = k_1 + k_2$

$$d[S_1]/dt = -(k_r + k_{nr})\,[S_1]$$

$$[S_1] = [S_1]_{initial}\, \exp(-(k_r + k_{nr})t)$$

Fluorescence lifetime (FLT) is the time a fluorophore spends in the excited state before emitting a photon and returning to the ground state. FLT can vary from picoseconds to hundreds of nanoseconds depending on the fluorophore.

Lifetime

Absorption: 10^{-15}s or fs

Vibrational Relaxation (or internal conversion): 10 ps to 100 ps

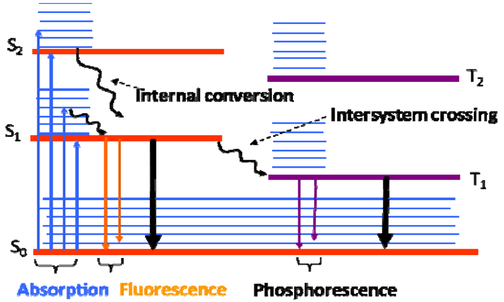
$S_1 - S_0$: 100 ps – 100 ns ← This is fluorescence

Intersystem crossing: 100 ps – 10 ns

$T_1 - S_0$: 1 μs – 1 s ← This is phosphorescence

These two processes have similar time scales.

Simplified Jablonski diagram



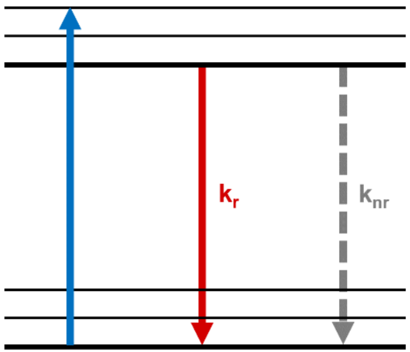
Both **fluorescence** and **phosphorescence** are radiative transitions

Quantum Yield

- The fluorescence quantum yield Φ is the fraction of excited molecules that return to the ground state S_0 with emission of fluorescence photons

$$\Phi = k_r/(k_r+k_{nr}) = k_r\tau_s$$

- Fluorescence intensity is defined as the amount of photons emitted per unit time and per unit volume of solution



Quantum Yield (Φ)

$$\Phi = \frac{N_{Photons\ Emitted}}{N_{Photons\ Absorbed}} = \frac{k_r}{k_r + \sum k_{nr}}$$

k_r = radiative rate
k_{nr} = non-radiative rate

Quantum Yield

$$\Phi = \frac{\#\text{ of photons emitted}}{\#\text{ of photons absorbed}} = 0 \text{ to } 1$$

-The most reliable method for recording Φ is the comparative method, which involves the use of well characterized standard samples with known Φ values.

-In practice, need to consider these factors:

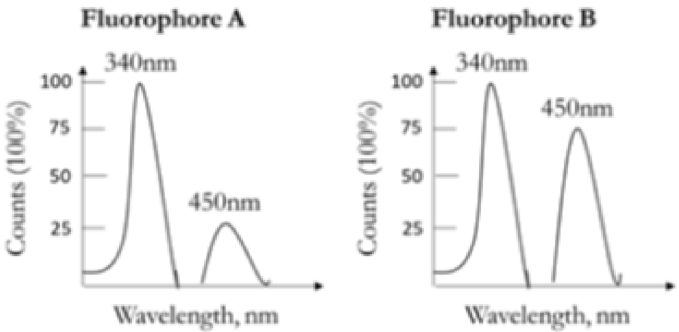
- The presence of concentration effects, *e.g.* self-quenching
- The use of different solvents for standard and test samples
- The validity in using the standard sample and its Φ value

Factors that affect QY

1) **Internal conversion (k_{ic})**
   -non radiative loss via collisions with solvent or via internal vibrations.
2) **Quenching**
   -interaction with solute molecules capable of quenching excited state
   (k_{chem}, k_{dec}, k_{ET}, k_{et})
3) **Intersystem Crossing Rate**
4) **Temperature**
   - Increasing the temperature will increase of dynamic quenching
5) **Solvent**
   - viscosity, polarity, and hydrogen bonding characteristics of the solvent
   -Increased viscosity reduces the rate of bimolecular collisions
6) **pH**
   - protonated or unprotonated form of the acid or base may be fluorescent
7) **Energy Gap Law**

Example 8

Calculate the fluorescence quantum yield of the following two fluorophores (A and B) based on the excitation and emission spectra as shown in the graphs below. Indicate which fluorophore will be brighter?



A, Fluorescence quantum yield = 25/100 = 0.25
B, Fluorescence quantum yield = 75/100 = 0.75
As the excitation count is more for B, it will be more brighter than A.

Applications

1. Environmental Monitoring,
2. Medical Diagnostics,
3. DNA Sequencing,
4. Forensics,
5. Genetic Analysis,
6. Sensing Applications.

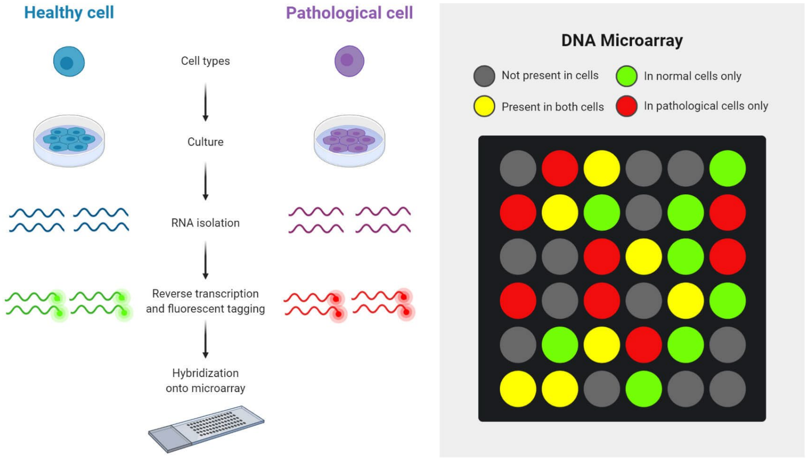
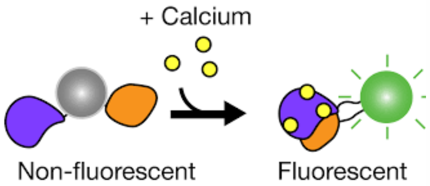
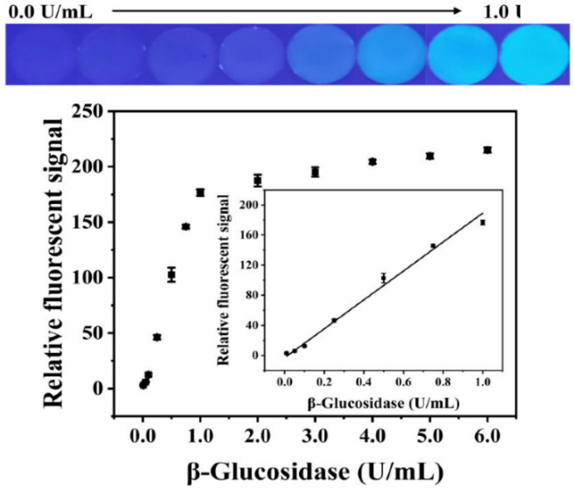
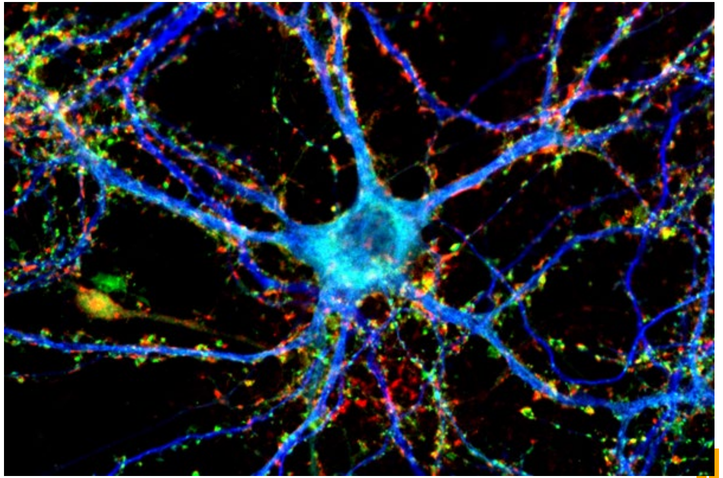


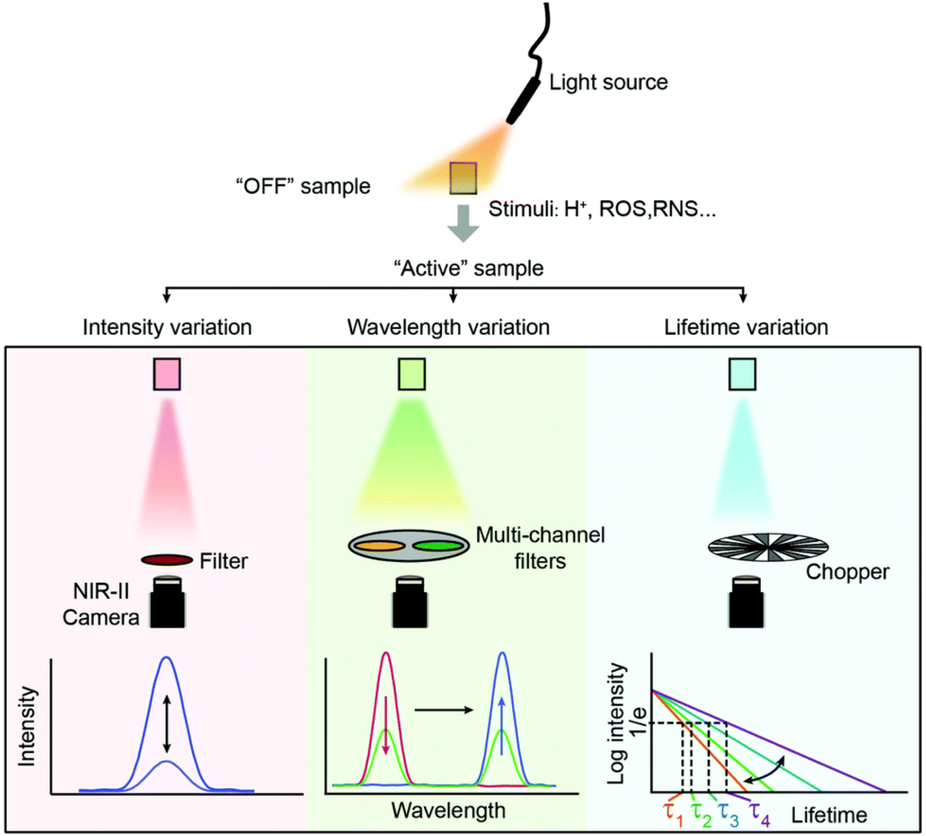
Image By Sagar Aryal, created using biorender.com

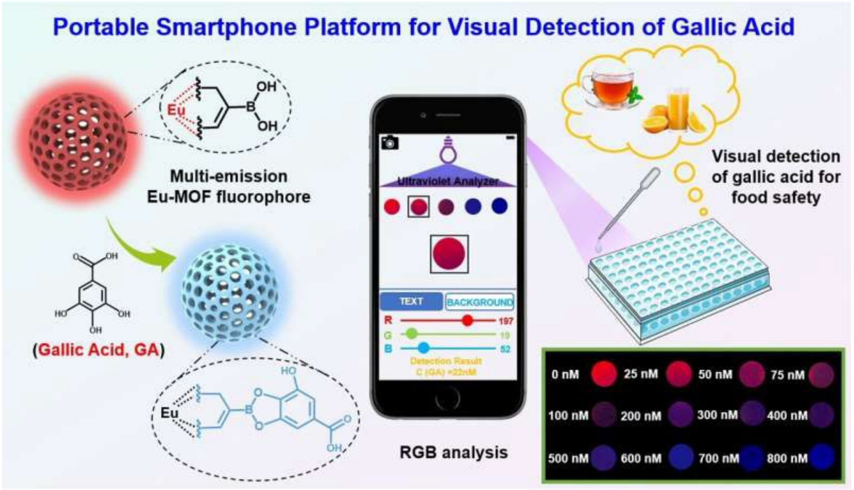




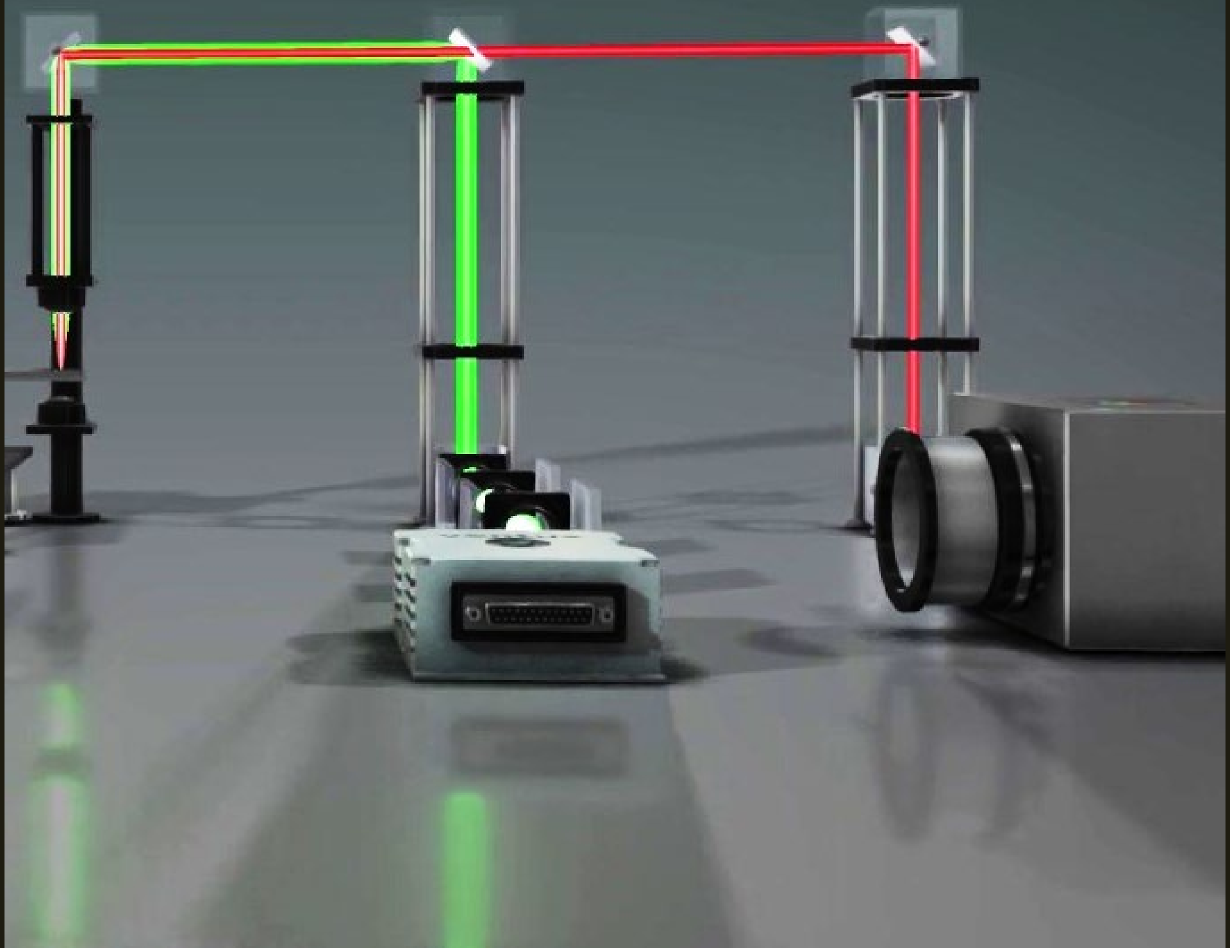


Applications



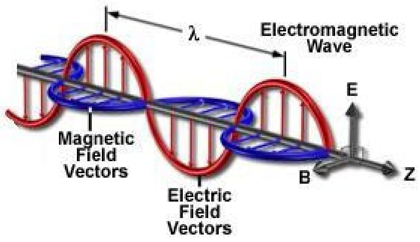


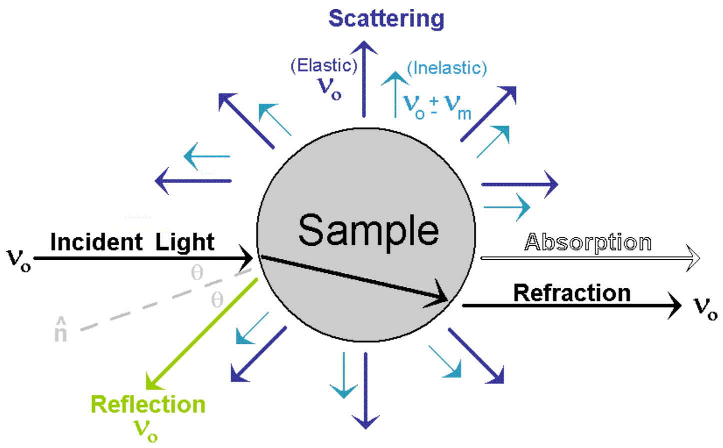
SCATTERING SPECTROSCOPY



A review of light

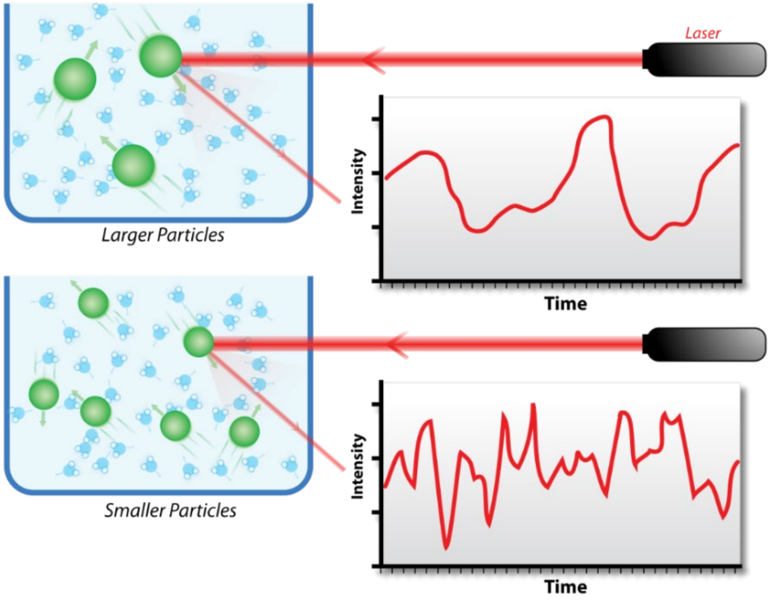
- Electromagnetic wave
  - Oscillating electric and magnetic fields
- Classical Interactions of light and matter
  - Absorption
  - Reflection
  - Refraction
  - *Scattering*
  - Elastic (Rayleigh scattering)
  - Inelastic (Stokes scattering)

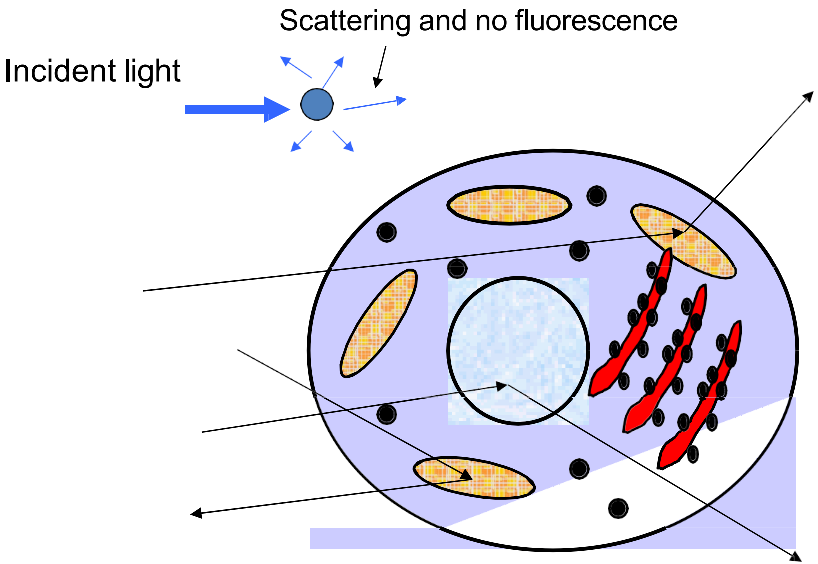




Scattering spectroscopy

Change of direction of propagation and/or energy of light by a molecular species

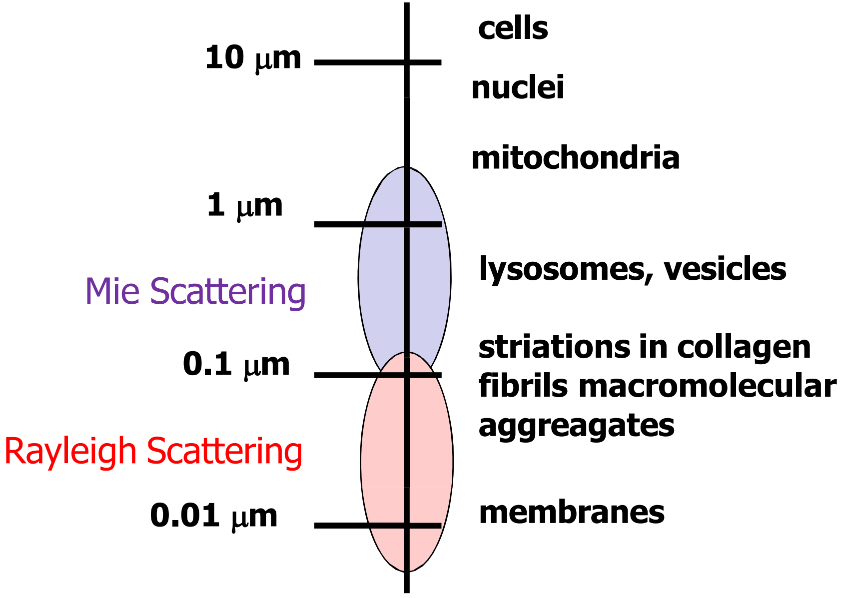




Scattering spectroscopy

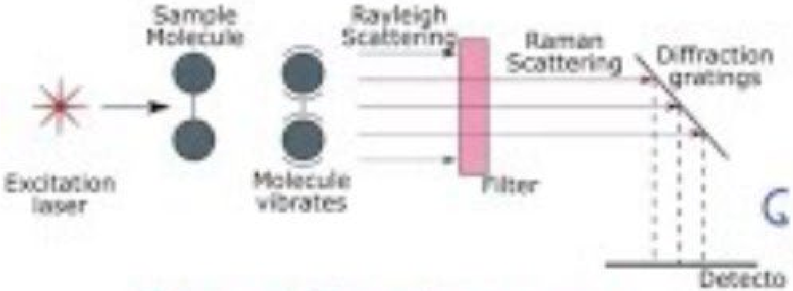
- **Elastic scattering: no energy change**
  - Frequency of the scattered wave = frequency of incident wave
  - Probe static structure of material
  - Rayleigh and Mie scattering

- **Inelastic scattering: energy change**
  - Frequency of the scattered wave ≠ frequency of incident wave
  - Internal energy levels of atoms and molecules are excited
  - Probes vibrational bonds of the molecule
  - Raman scattering (stokes↓ and anti-stokes ↑)

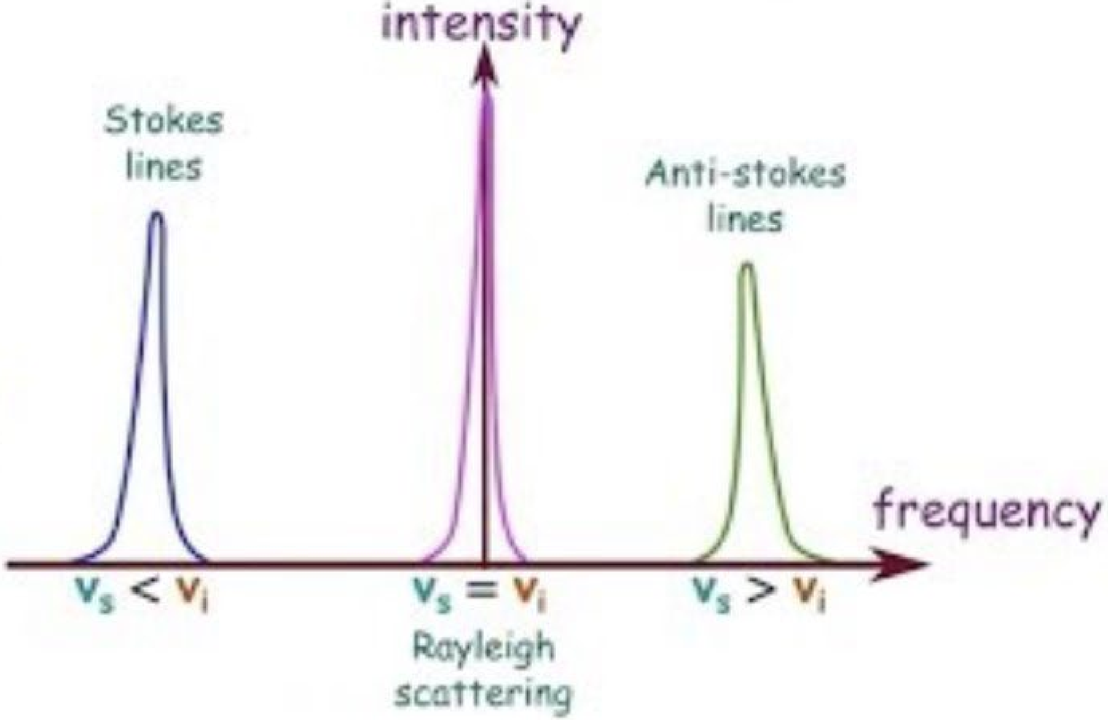


Raman Spectroscopy

Basics and Principles



Raman Spectroscopy



Origin of Raman

- When monochromatic radiation is incident on a sample then this light will interact with sample in some fashion. It may be reflected, absorbed and scattered in some manner. It is the scattering of radiation that occurs, gives information about molecular structure.
- **Raman Spectroscopy** is based on scattering of light. The sample is irradiated with a coherent source, typically a laser beam. **Three types of scattering** are obtained…….

Rayleigh scattering
(elastic scattering)

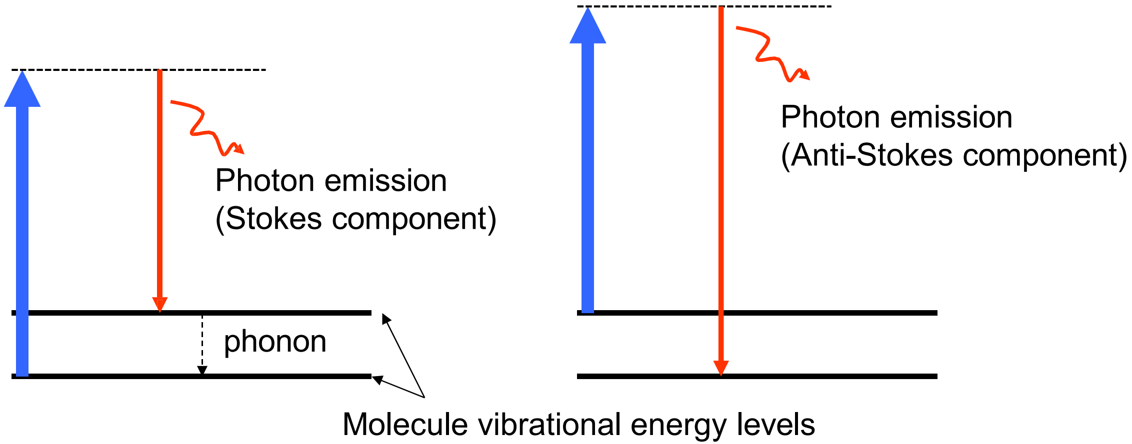
Frequency of scattered Light (v_s) is same as that of Incident light (v_i)
$(v_s = v_i)$

Stokes scattering
$(v_s < v_i)$

Anti-stoke scattering
$(v_s > v_i)$

Raman scattering/lines
(inelastic scattering)

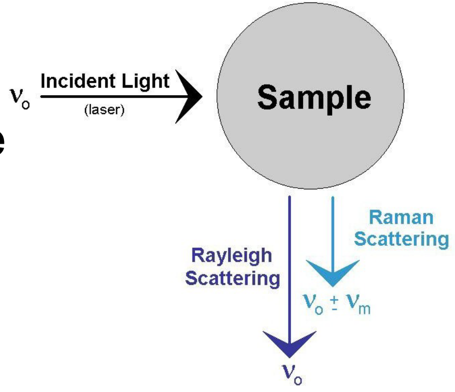
Raman scattering (inelastic)



Stokes lines are those in which the photon has *lost* energy to the molecule, $\nu_0 - \nu_t$
Anti-Stokes lines are those in which the photon has *gained* energy from the molecule, $\nu_0 + \nu_t$

Basics of Raman

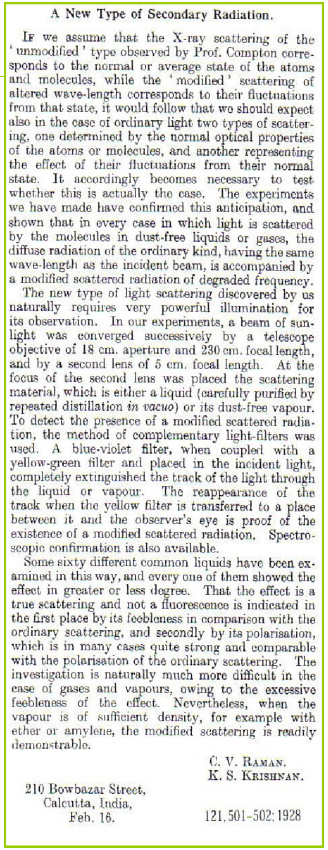
- Raman spectroscopy studies the frequency change of light due to the interaction with matter
- The energy of a vibrational mode (ν_m) depends on molecular structure and environment.
    - Atomic mass, Bond order, Molecular substituents, Molecular geometry and Hydrogen bonding all contribute
- Raman signal is 10^{-6} time weaker than incident light (ν_o)
- Photons are not absorbed
- To observe Raman scattering the molecule must be polarizable



History

- Sir. C.V. Raman discovered light scattering in 1928
- Awarded Nobel Prize in physics in 1930
- Experiment composed of light source (*sunlight*), a sample, and detector (*eye*)
- His nephew, Dr. S. Chandrasekhar, of the University of Chicago won the Nobel prize in physics in 1983

Sir. C.V. Raman



A New Type of Secondary Radiation.

If we assume that the X-ray scattering of the 'unmodified' type observed by Prof. Compton corresponds to the normal or average state of the atoms and molecules, while the 'modified' scattering of altered wave-length corresponds to their fluctuations from that state, it would follow that we should expect also in the case of ordinary light two types of scattering, one determined by the normal optical properties of the atoms or molecules, and another representing the effect of their fluctuations from their normal state. It accordingly becomes necessary to test whether this is actually the case. The experiments we have made have confirmed this anticipation, and shown that in every case in which light is scattered by the molecules in dust-free liquids or gases, the diffuse radiation of the ordinary kind, having the same wave-length as the incident beam, is accompanied by a modified scattered radiation of degraded frequency.

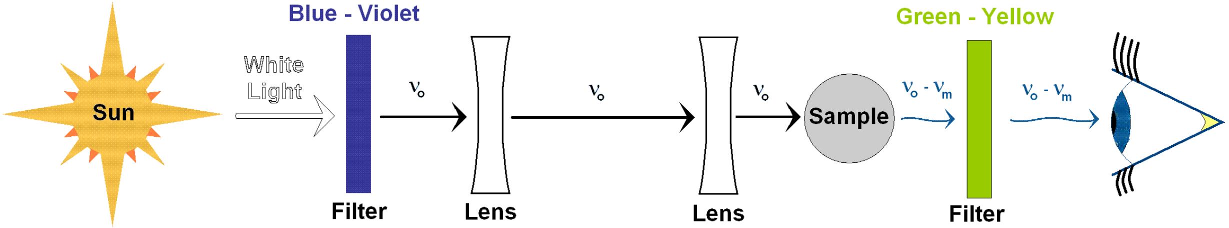
The new type of light scattering discovered by us naturally requires very powerful illumination for its observation. In our experiments, a beam of sunlight was converged successively by a telescope objective of 18 cm. aperture and 230 cm. focal length, and by a second lens of 5 cm. focal length. At the focus of the second lens was placed the scattering material, which is either a liquid (carefully purified by repeated distillation *in vacuo*) or its dust-free vapour. To detect the presence of a modified scattered radiation, the method of complementary light-filters was used. A blue-violet filter, when coupled with a yellow-green filter and placed in the incident light, completely extinguished the track of the light through the liquid or vapour. The reappearance of the track when the yellow filter is transferred to a place between it and the observer's eye is proof of the existence of a modified scattered radiation. Spectroscopic confirmation is also available.

Some sixty different common liquids have been examined in this way, and every one of them showed the effect in greater or less degree. That the effect is a true scattering and not a fluorescence is indicated in the first place by its feebleness in comparison with the ordinary scattering, and secondly by its polarisation, which is in many cases quite strong and comparable with the polarisation of the ordinary scattering. The investigation is naturally much more difficult in the case of gases and vapours, owing to the excessive feebleness of the effect. Nevertheless, when the vapour is of sufficient density, for example with ether or amylene, the modified scattering is readily demonstrable.

C. V. Raman.
K. S. Krishnan.

210 Bowbazar Street,
Calcutta, India,
Feb. 16.

121, 501–502; 1928



Raman Spectroscopy

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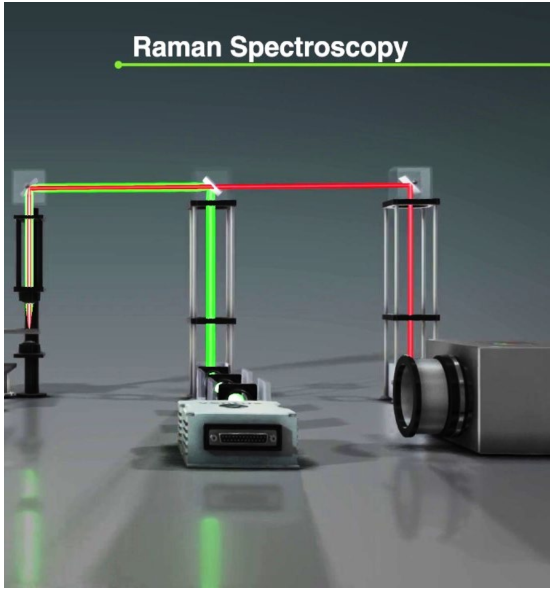
Anti-stoke scattering

$(v_s > v_i)$

Raman scattering/lines

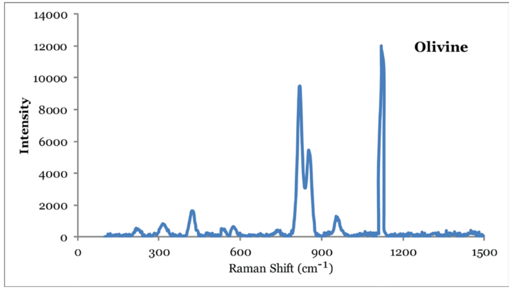
(inelastic scattering)



Raman Spectroscopy

In practice, Raman spectra are plotted as **Raman shift**. Raman shift is the difference between the peak energies and the excitation laser energy. This **allows comparison of a spectrum to other spectra even when different laser excitation energies are used**.

The x axis of a conventional Raman spectra is plotted in **wavenumber (cm^{-1}) units**, not in wavelength. For a review of wavenumbers, see 3.7 Electromagnetic Energy: Units Conversion.



The following links show examples of Raman spectra of different materials. Please **look at these examples to compare and contrast the axes of the plots.**

- InPhotonics. InPhotote Spectrometer Data. (ret. 3/17/2019) http://www.inphotonics.com/INPdata.htm National University of Ireland, Galway.
- Small Molecule/Forensics Research. (ret. 3/17/2019) http://www.nuigalway.ie/nanoscale/researchprojects/forensicsraman/

UNITS CONVERSION: WAVELENGTH TO WAVENUMBERS

In 3.6, we saw that electromagnetic energy has characteristics of both a wave and a particle (photon). The **Planck equation** relates the energy of a **photon** to its frequency:

Equation 3.7.1. $E = h\nu$

Where E = energy, h is Planck's constant ($6.62607004 \times 10^{-34}\ m^2\ kg / s$), and ν "nu" is the frequency.

3.7.3. Equation 3.7.2 relates wavelength to energy. Is wavelength proportional to or inversely proportional to energy?

Wavenumber ($\bar{\nu}$) is a common unit used for plotting spectra in the infrared region. Wavenumber is defined as

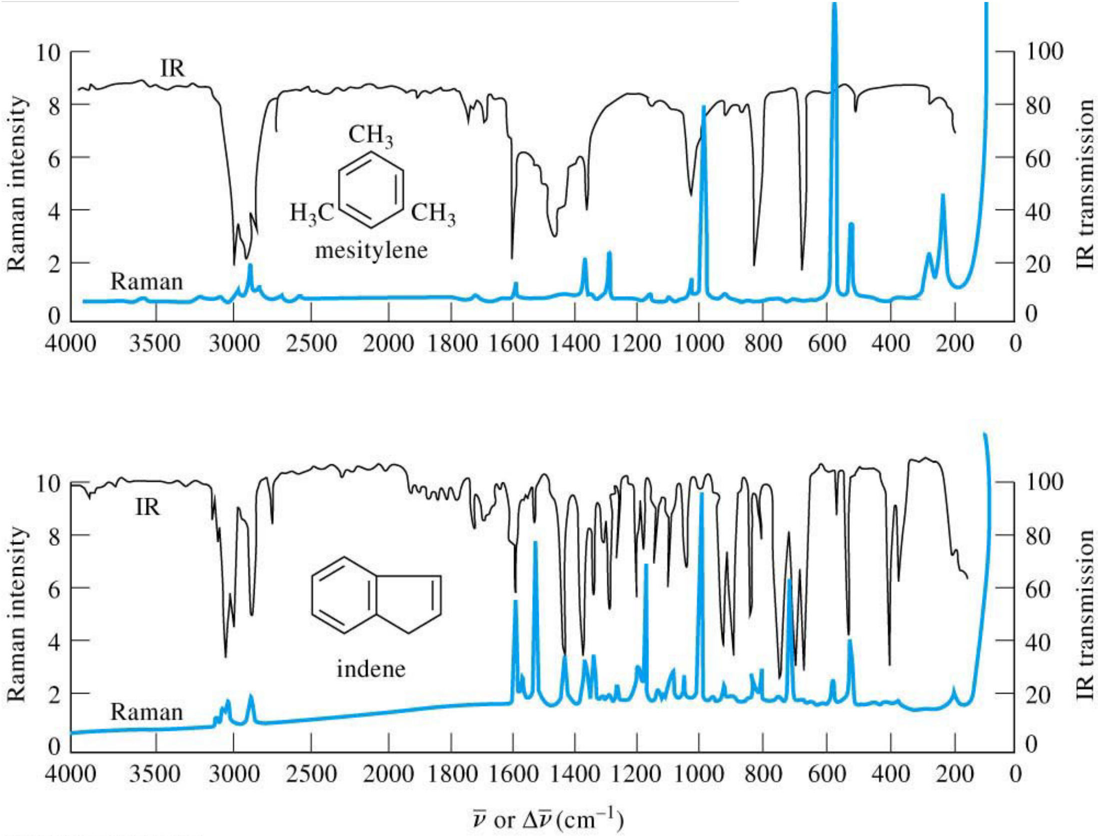
Equation 3.7.3.

$$\bar{\nu} = \frac{1}{\lambda}$$

$$\bar{\nu} = \frac{1}{\lambda} \Rightarrow unit = cm^{-1}$$

$$c = \lambda \nu$$

Infrared and Raman spectra are commonly plotted as wavenumbers in units of cm^{-1}.



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Raman is usually in the units of wavenumber (cm-1). How to calculate cm-1 to wavelength and frequency?

How to convert wavevector cm^{-1} and wavelength?

$cm^{-1} = 10^7$ /nanometers.

$$Raman\ \ shift[cm^{-1}] = \frac{10^7}{\lambda_{ex}[nm]} - \frac{10^7}{\lambda[nm]}$$

Raman Shift Calculator Tools

Do you need an easy way to calculate Raman shift for your spectrometer or experiment? Use our Raman shift / wavelength calculator tools below to convert instantly, or download our apps for Apple or Android for easy access. Enter the values you know, and the others will be automatically computed and updated (laser wavelength is required).

The Raman Shift Calculator allows you to convert a known absolute wavelength to a Raman shift in wavenumbers, or to convert a known Raman shift in wavenumbers to an absolute wavelength.

The Raman Spectral Range Calculator allows you to compute the spectral range for a Raman spectrometer in either wavelengths or shifts in wavenumber.

Raman Shift Calculator

Laser excitation wavelength, λ_{ex} (nm)

Wavelength, λ (nm)

Raman shift in wavenumbers (cm^{-1})

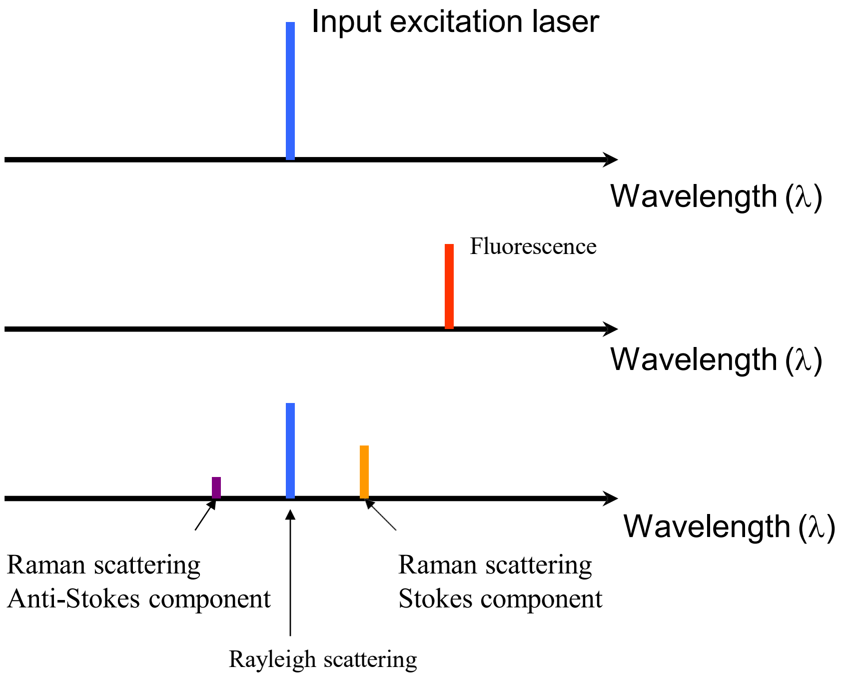
Raman Spectral Range Calculator

Laser excitation wavelength, λ_{ex} (nm)

	Wavelength (nm)	Wavenumber (cm^{-1})
Start		
End		
Range		

[Raman Shift Calculator | Online & mobile app]

Comparison



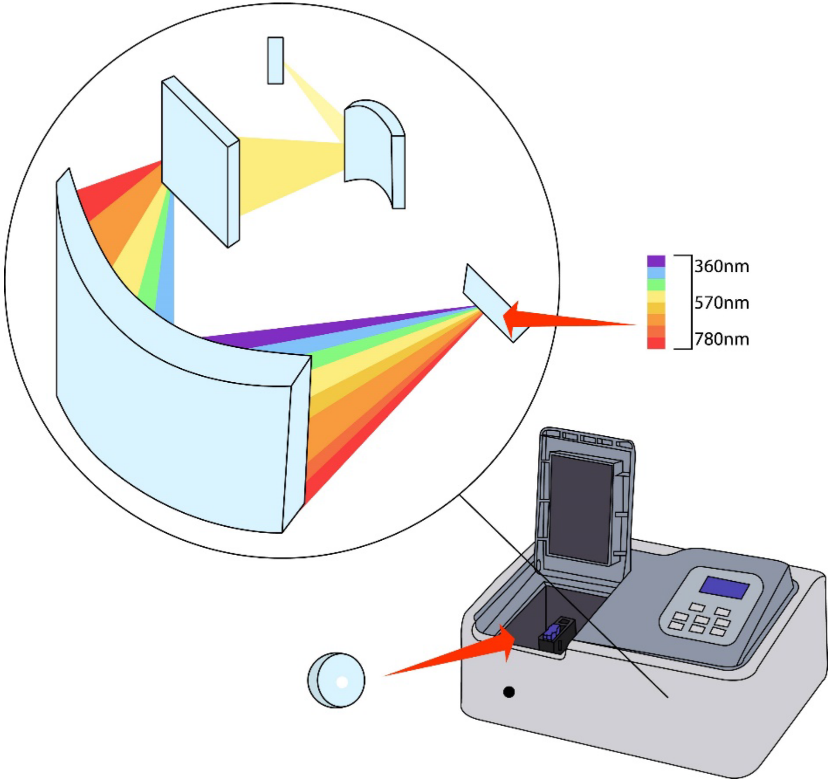
- Difference between Raman scattering and absorption/fluorescence Raman scattering is instantaneous
- Absorption/fluorescence takes time (femto-second to nano-second)
- Raman scattering involves phonon participation
- Raman scattering can occur at any wavelength, it does not need a real energy level

4

PART FOUR

Optical Detector

Spectrometer

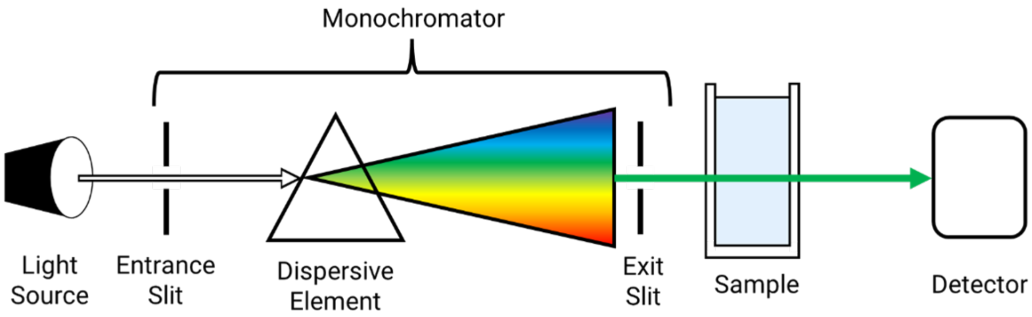


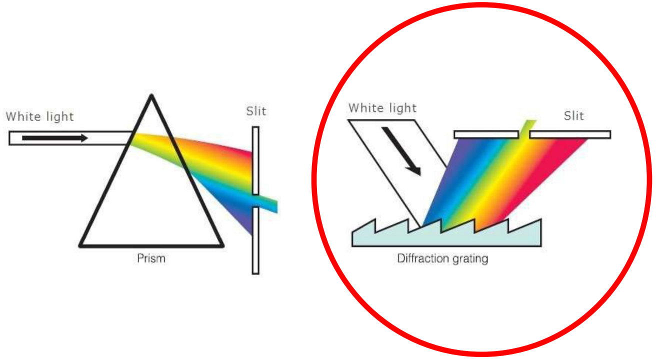
Monochromator

- A monochromator is an optical instrument which measures the light spectrum in a spectrometer.
- Monochromators are used to disperse polychromatic or white light into the various colors or wavelengths.

- This dispersion can be accomplished using prisms or diffraction gratings.

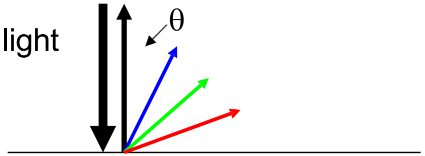
- The monochromators in most spectrofluorometers use diffraction gratings rather than prisms





Dispersion of Grating

Dispersive power is also defined as the difference in the angle of diffraction per unit change in wavelength.



Grating equation
(when the incident light is perpendicular to the grating)

$$\frac{2\pi}{\lambda} d \sin\theta = m \cdot 2\pi \quad \Rightarrow \quad \boxed{d \sin\theta = m \cdot \lambda}$$

Grating pitch

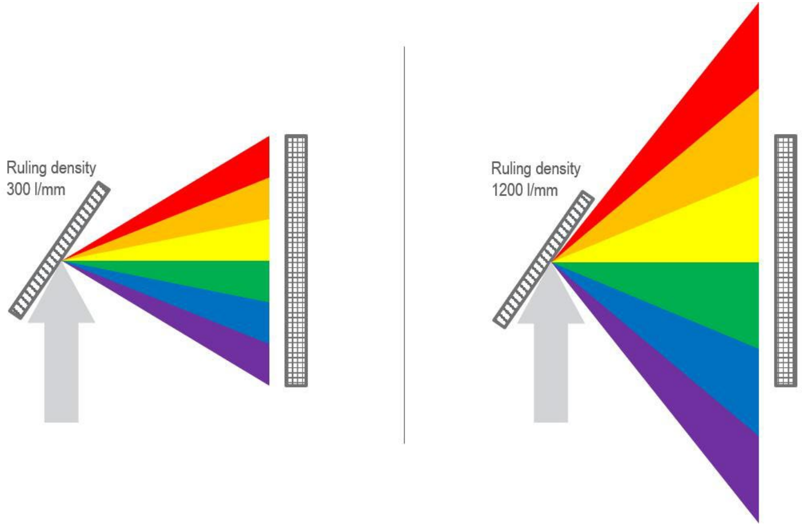
Diffraction order

m = 0: zeroth order, geometric reflection

m = 1: this is the order we usually use

Dispersion of Grating

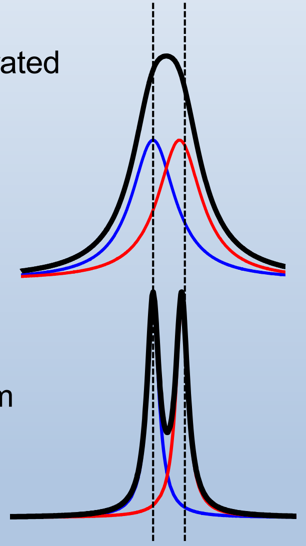
Mathematically: $$\frac{\delta\theta}{\delta\lambda}$$ ← This is Angular dispersion, as it tells how much light is separated in angle



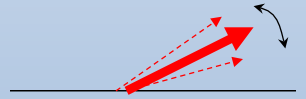
The smaller d, or the higher #groove density, the higher dispersion, higher spectral resolution

In order to full describe the grating performance, we need both “dispersion” and “resolution”

Dispersion tells you how far apart the line center is separated
Resolution tells you how narrow each line is



How to define the broadening effects of a grating on a spectroscopic line?



$\delta\Phi$: angular linewidth
Full-width at half maximum

$$\delta\Phi = \lambda/Nd\cos\theta = \lambda/W$$

N: total number of grooves
d: pitch
W: projected grating width in the diffraction direction

Resolving Power of Grating

Resolving Power or Resolution: The resolving power of a grating is a measure of its ability to spatially separate two wavelengths.

$R = mN$

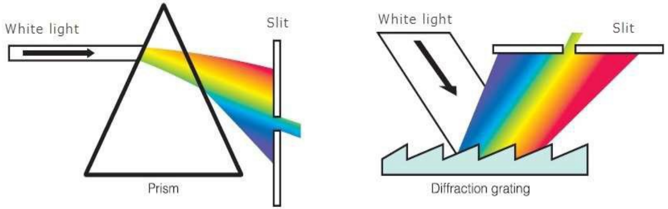
Resolving Power of a Grating

How can we increase resolving power of grating?

$$\boxed{\text{Resolving Power} = \frac{\lambda}{d\lambda} = mN}$$

Resolving power of grating is directly proportional to number of lines on grating.

Therefore, it can be increased by increasing the number of lines on the grating.



Example 9

A diffraction grating has a second-order resolving power of 1 250. (a) Find the number of illuminated lines oil the grating, (b) Calculate the smallest difference in wavelengths surrounding 525 nm that can be resolved in the Second order diffraction pattern

(a)

Rewrite the expression of the resolving power for *N* as follows:

$$N = \frac{R}{m}$$

Substitute 1250 for *R* and 2 for *m* in the above equation.

$$N = \frac{1250}{2} = 625$$

Therefore, the number of lines in grating is $\boxed{625}$.

(b)

The expression for the resolving power of the grating is,

$$R = \frac{\lambda}{\Delta\lambda}$$

Here, $\Delta\lambda$ is the smallest difference in the wavelength and λ is the wavelength in the first order diffraction.

Rewrite the above expression for $\Delta\lambda$.

$$\Delta\lambda = \frac{\lambda}{R}$$

Substitute 525 nm for λ and 1250 for *R* in the above equation.

$$\Delta\lambda = \frac{525\text{ nm}}{1250} = 0.42\text{ nm}$$

Can you detect a wavelength change or an error smaller than this value?

Therefore, the smallest difference in wavelengths is $\boxed{0.42\text{ nm}}$.

Example 10

A diffraction grating has a third-order resolving power of 1,220.
a) find the number of illuminated lines on the grating.
b) calculate the smallest difference (in nm) in wavelengths surrounding 545 nm that can be resolved in the first-order diffraction pattern

Given

$R = 1220$

$m = 3$ order

$R = mN$

$\Rightarrow N = \frac{R}{m} = \frac{1220}{3} = 406.67$

$\therefore \boxed{N \approx 407}$ (Answer)

(b) Also, we use,

$R = \frac{\lambda}{\Delta\lambda}$

where λ = wave length (given)

$\Delta\lambda$ = difference in wavelength

$\Delta\lambda = \frac{\lambda}{R} = \frac{545}{1220}$ nm

$\boxed{\Delta\lambda = 0.45\text{ nm}}$ (Answer)

Example 11

(a) A diffraction grating has a resolving power of 10^4, how close (in nm) is the closest line to 0.51223 μm that can barely be resolved?
(b) Calculate the third-order resolving order of grating that is 9.00 cm and is ruled at 165 lines/mm.

(a) A diffraction grating has a resolving power of 10^4.

$\Rightarrow$ Resolution $= \frac{\lambda}{\Delta\lambda}$

$\Rightarrow \frac{\lambda}{\Delta\lambda} = 10^4$

$\Rightarrow \Delta\lambda = \frac{\lambda}{10^4}$

$\Rightarrow \Delta\lambda = \frac{0.51223}{10^4}$ [closest line = 0.51223 μm]

$\Rightarrow \Delta\lambda = 5.1223 \times 10^{-5}$ μm

$\Rightarrow \boxed{\Delta\lambda = 0.051223 \text{ nm}}$

Ans: 0.051223 nm close is the closest line to 0.51223 μm that can barely be resolved.

17.

(b) Third-order resolving order of grating that is 9.00 cm and ruled at 165 lines/mm.

$\Rightarrow$ Resolution $= nN$

n = order, N = lines

$\boxed{9 \text{ cm} = 90 \text{ mm}}$

$\therefore$ Resolution $= 3 \times 165 \times 90$

$= 44550$

Ans: 44550 $\Rightarrow$ Resolution.

Visible light spectrophotometer

UV/Visible spectrophotometer

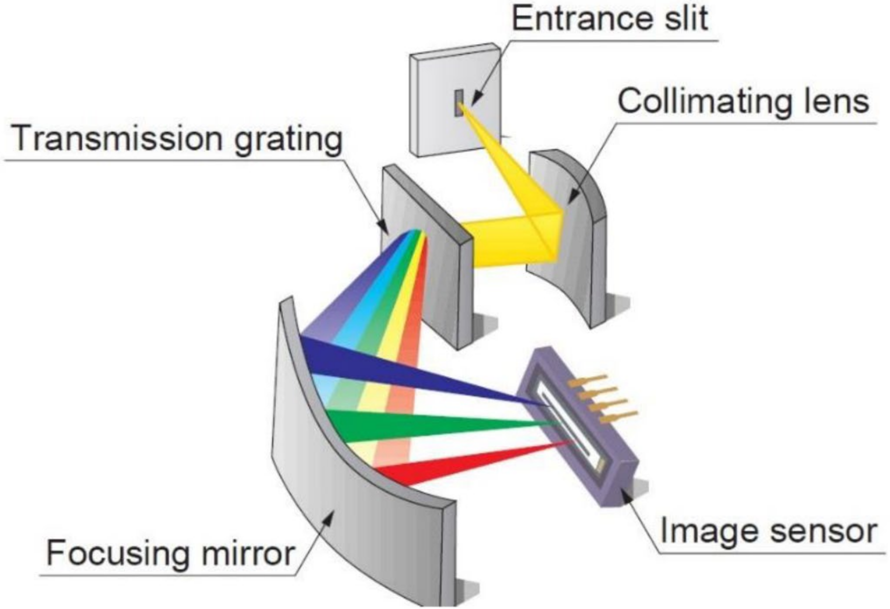
Near-infrared spectrophotometer

Nuclear Magnetic Resonance spectroscopy

Mercury spectrophotometer/analyzer

Atomic absorption

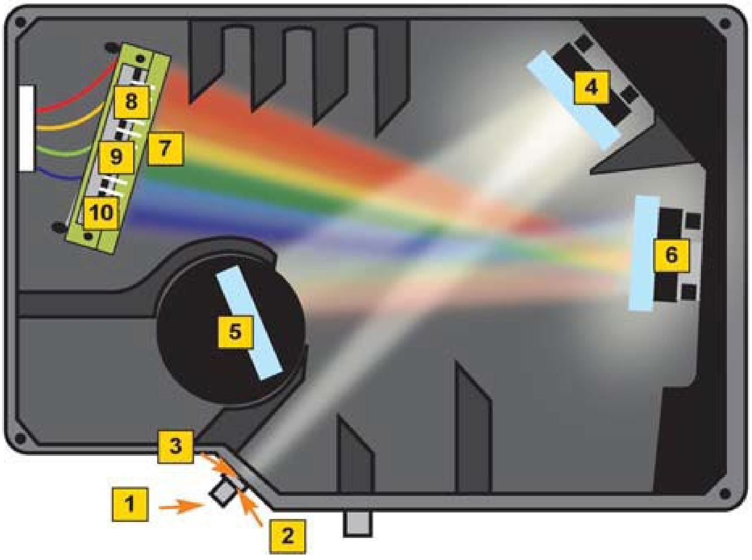
Very very important spectroscopic tool
Very expensive: >$10k - $100k
Very good performance
Big yet valuable investment in lab



Mini Spectrometer

Ocean Optics HR 4000 Spectrometer

- 1. SMA connector
- 2. Entrance slit
- 3. LP filter
- 4. Collimating mirror
- 5. grating (fixed)
- 6. focusing mirror
- 7. collection lens
- 8. CCD



https://www.youtube.com/watch?v=UTXDj1SENi8

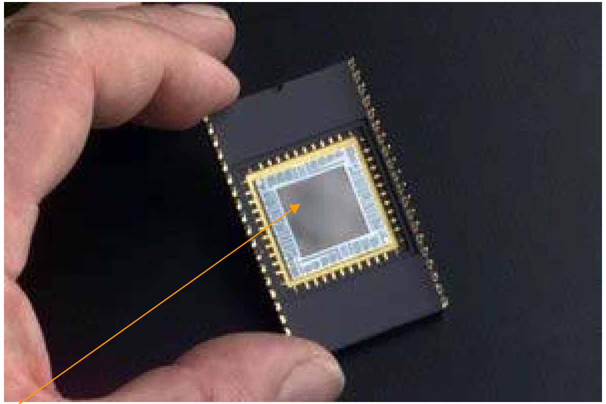
CCD

CCD: charge coupled device

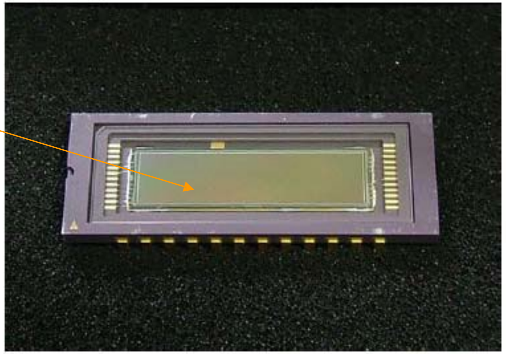
Princeton Instruments

CCD chip

A CCD camera is a solid state electrical device that is capable of converting light input into electronic signal.

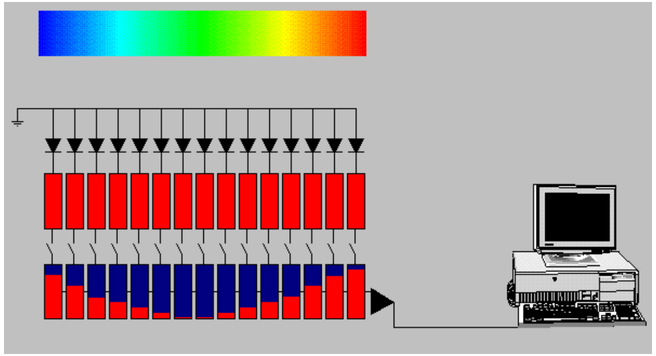


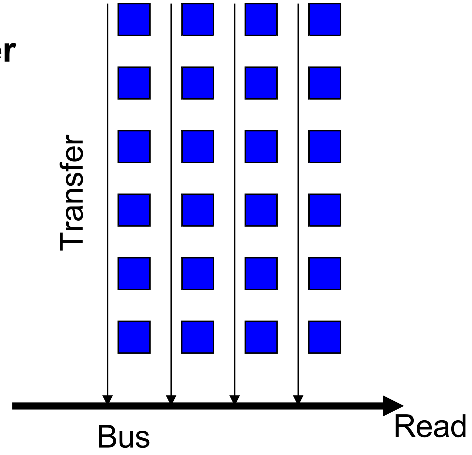
CCD chip



Think About it: How a CCD works ?

- **Charge generation**
- **Charge collection (accumulation) Charge transfer**
- **Charge measurement**





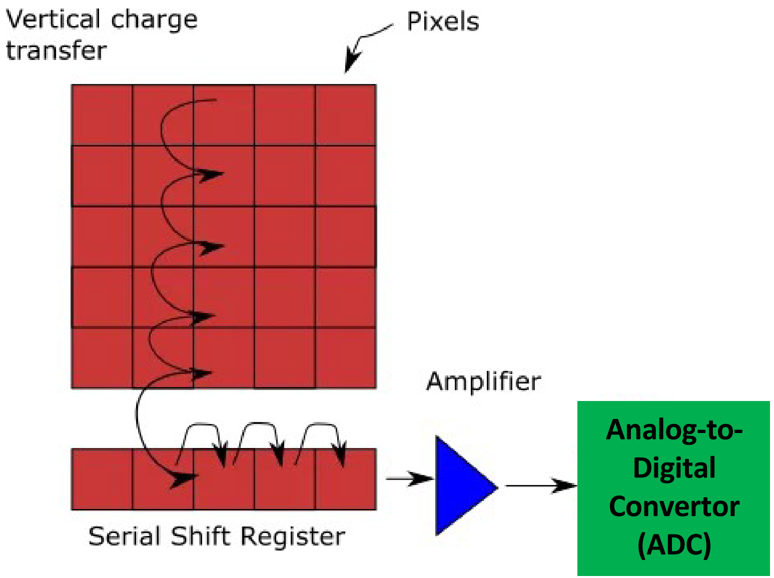
How CCD and CMOS Sensors on cameras and scanners work

https://www.youtube.com/watch?v=MAQU-smPtFY

CCD Image Sensors:
Charge Coupled Device (CCD)

CCD sensors move charges pixel to pixel, convert it to voltage through an output node

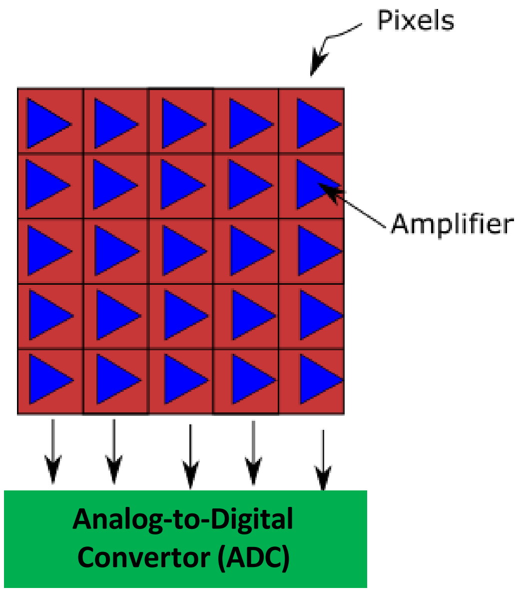
- Key Features
  - Excellent light sensitivity, all pixels devoted to light capture (100% fill factor)
  - High image quality
  - Low noise images
- Specialized applications:
  - High-end professional photography
  - High-resolution scientific imaging (e.g., astronomy, spectroscopy)
  - Medical imaging



CMOS Image Sensors:
Complementary Metal-Oxide-Semiconductor (CMOS)

CMOS pixels integrate on-chip circuitry for conversion and amplification within each pixel

- Key Features
  - Faster parallel readout and speed
  - Lower cost and power consumption
  - On-chip processing capabilities
- Applications:
  - Consumer electronics
  - Industrial embedded vision/machine vision systems
  - Automotive (in-cabin and surround view sensing)



THANKS FOR YOUR TIME