Physics of Life

PHYS-468

Spectroscopy with Light

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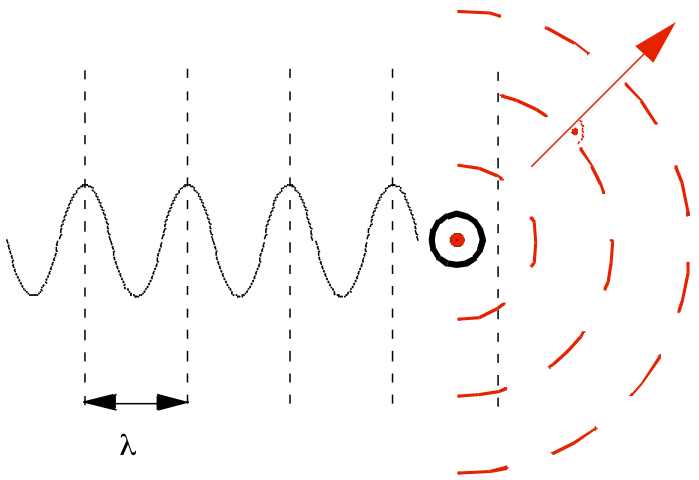
What is light?

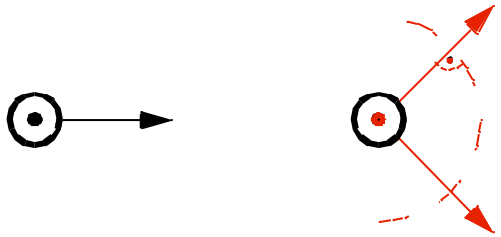
1. Electromagnetic Waves ==> Interference

2. Particles: Photons ==> Photo Effect

	Wavelength	Particle Energy
	$\lambda = \frac{c}{\nu}$	$E = h\nu$
Infrared	10 - 1 μm	0.1 - 1 eV
Visible	1 - 0.3 μm	1 - 3 eV
Ultraviolet	0.3 - 0.01 μm	3 - 500 eV
X-rays, γ	< 0.0002 μm = 2 Å	> 5000 eV

Wave-Particle Dualism





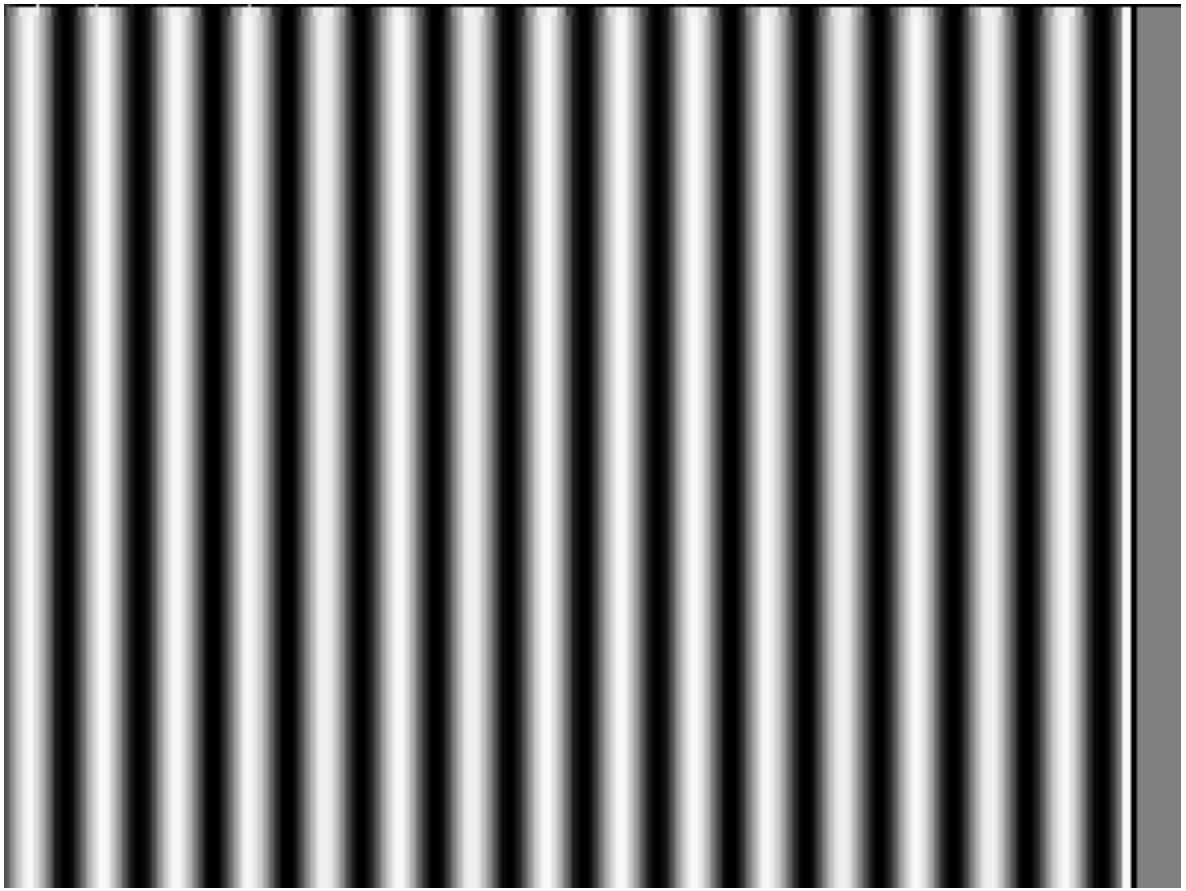
Wave			Particle	
Wavelength:	λ		Mass:	$m = m_0 \cdot \frac{1}{\sqrt{1-\left(\frac{v}{c}\right)^2}}$
Frequency:	$\nu = c/\lambda$			
Speed:	$c = \lambda\nu$		Speed:	v
Momentum:	$p = h/\lambda$		Momentum:	$p = m\,v$

Wave Front, Diffraction

Light Rays, Geometric Optics

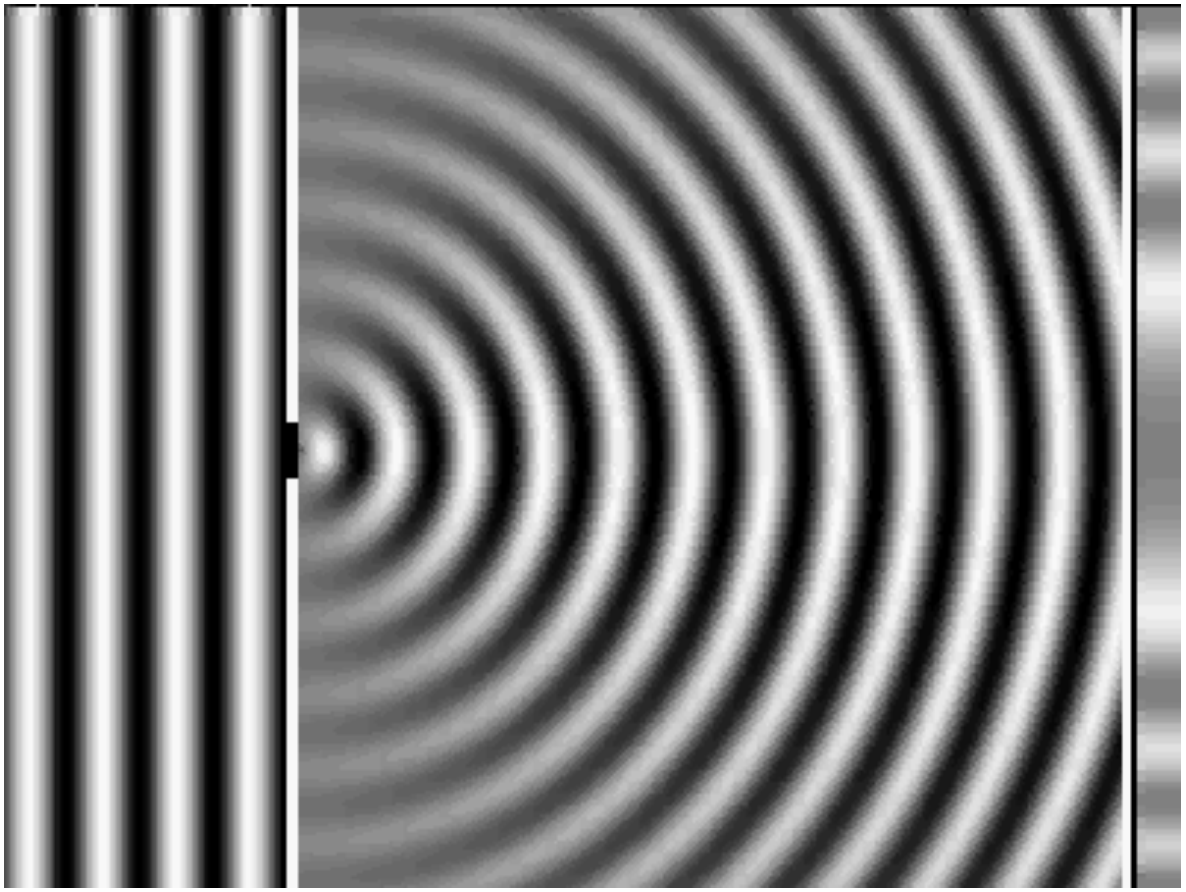
c = light speed

Light is an electro-magnetic wave.



Plane Wave

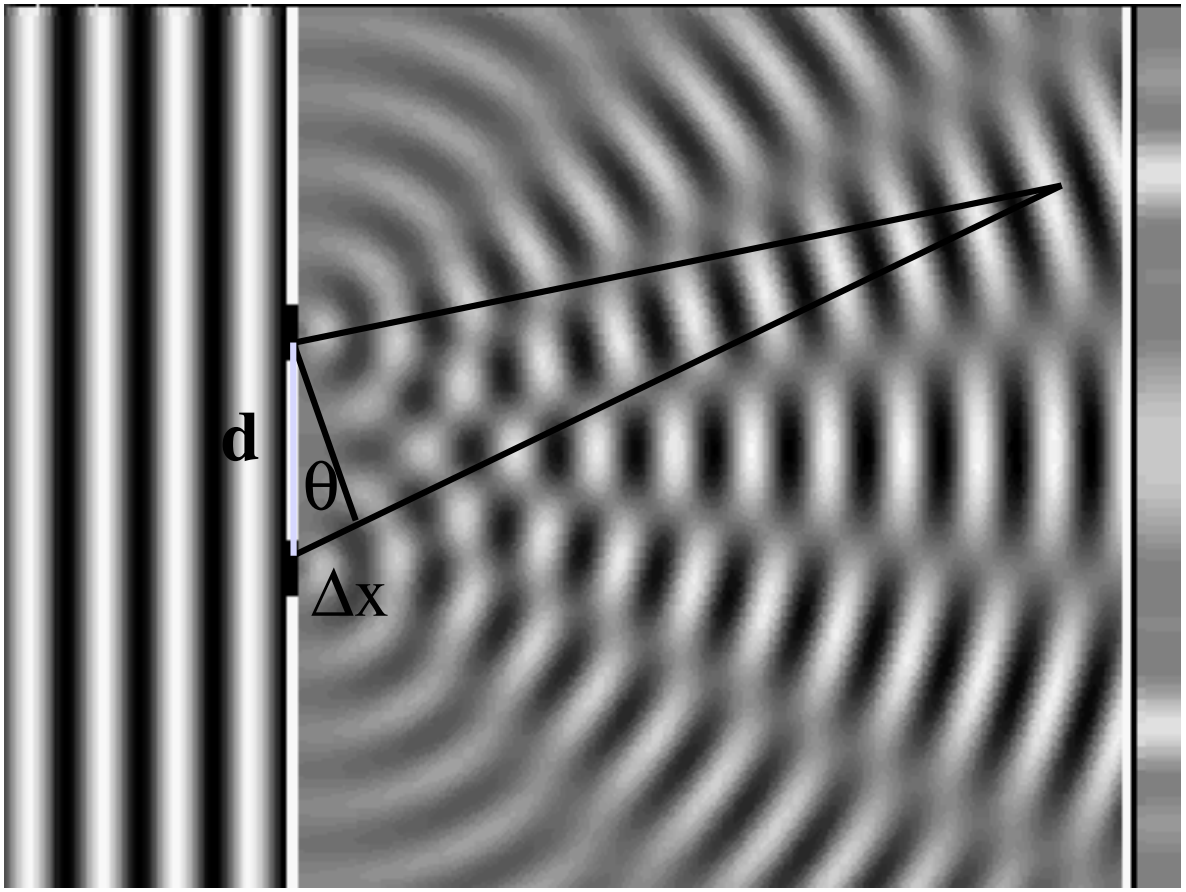
Light is an electro-magnetic wave.



Plane Wave diffracted by a slit

Light is an electro-magnetic wave.

Bragg's Equation for diffraction in a crystal: $n\lambda = 2d\sin(\theta)$



Bragg's Equation
for a double slit:

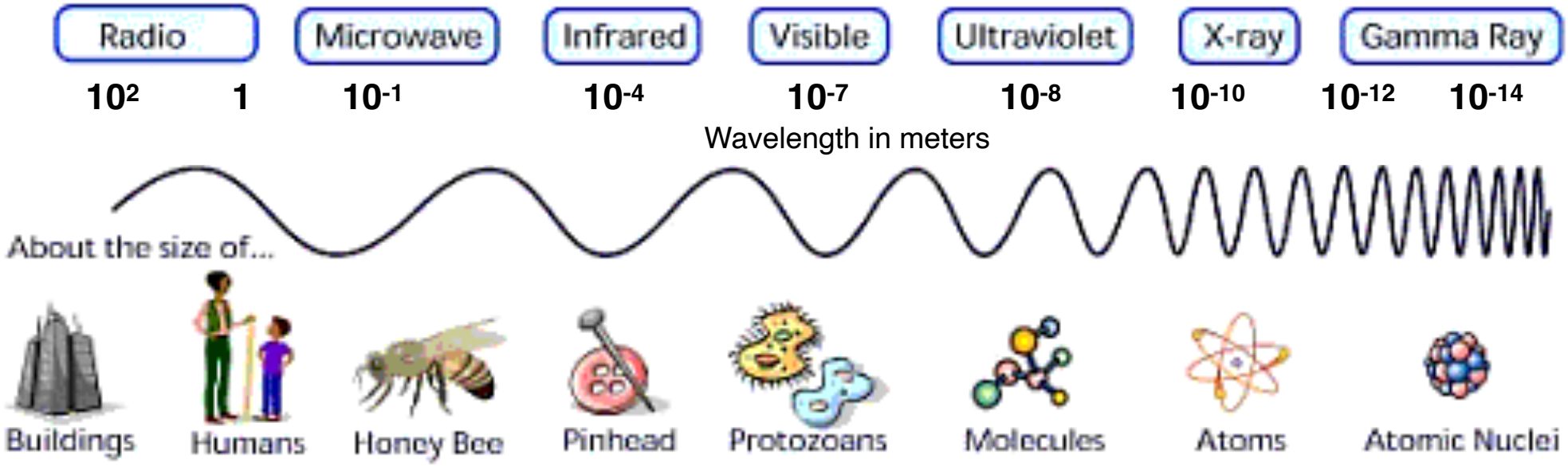
$$n\lambda = d\sin(\theta)$$

The existence of "interference" proves that light is a wave phenomenon.

Electro-Magnetic Waves

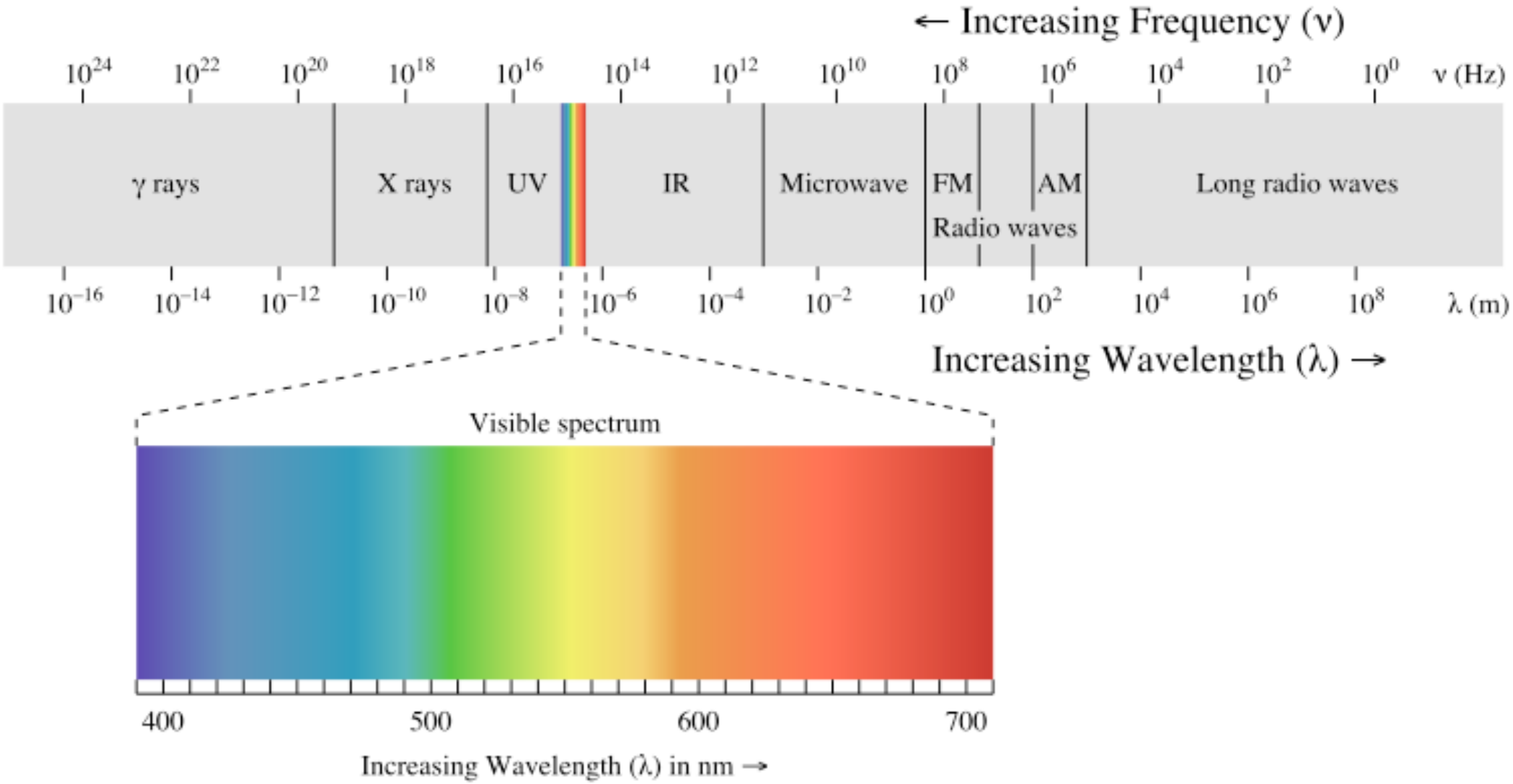
Electro-Magnetic Waves

Dualism: Wave *vs*. Particle

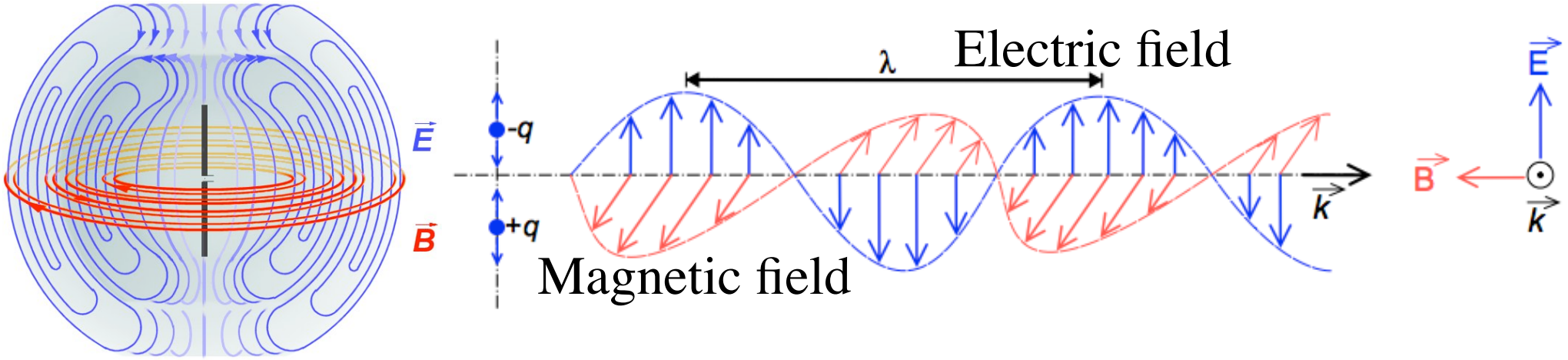


	Visible	X-Ray	Gamma
λ	500 nm	0.03 nm	0.0003 nm
ν	$6*10^{14}$ Hz	$1*10^{19}$ Hz	$1*10^{21}$ Hz
E	$3.6*10^{-19}$ J = 2.2eV	$6.4*10^{-15}$ J = 40 keV	$6.4*10^{-13}$ J = 4 MeV

Electro-Magnetic Waves



Hertz' dipole:
An antenna creates an electromagnetic wave



Maxwell equations:

Unify magnetic and electric forces.

Changes in the magnetic field B create an electric field vortex (Rotation of E):

$$\nabla \times \mathbf{E} = -\frac{\partial \mathbf{B}}{\partial t}$$

Changes in the electric field E and/or an electric current J create a rotation in the magnetic field:

$$\nabla \times \mathbf{B} = \mu_0 \mathbf{J} + \mu_0 \varepsilon_0 \frac{\partial \mathbf{E}}{\partial t}$$

Light is a particle (=> Photons)

$$E = h\nu = h\frac{c}{\lambda}$$

Experiment:

- Prepare "stopping voltage" V [Volts].
- Shine light of frequency ν=c/λ onto plate.
- Measure current I [Amperes]

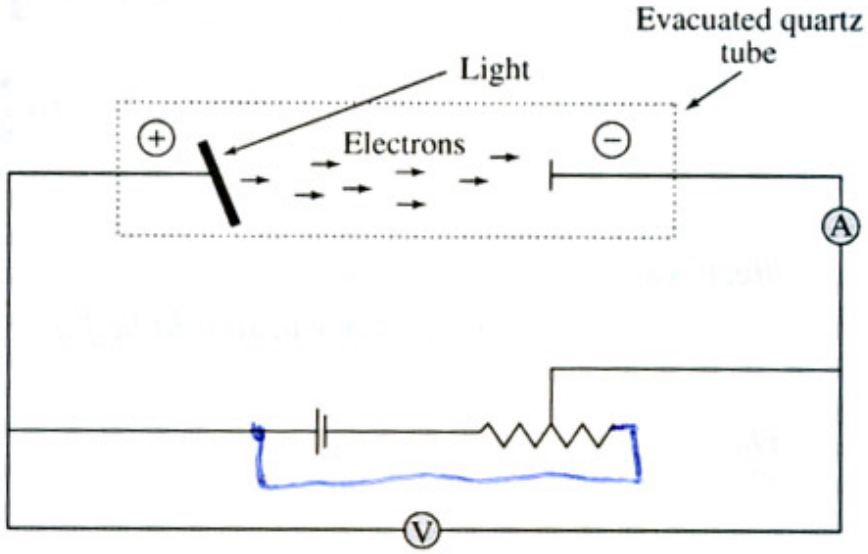
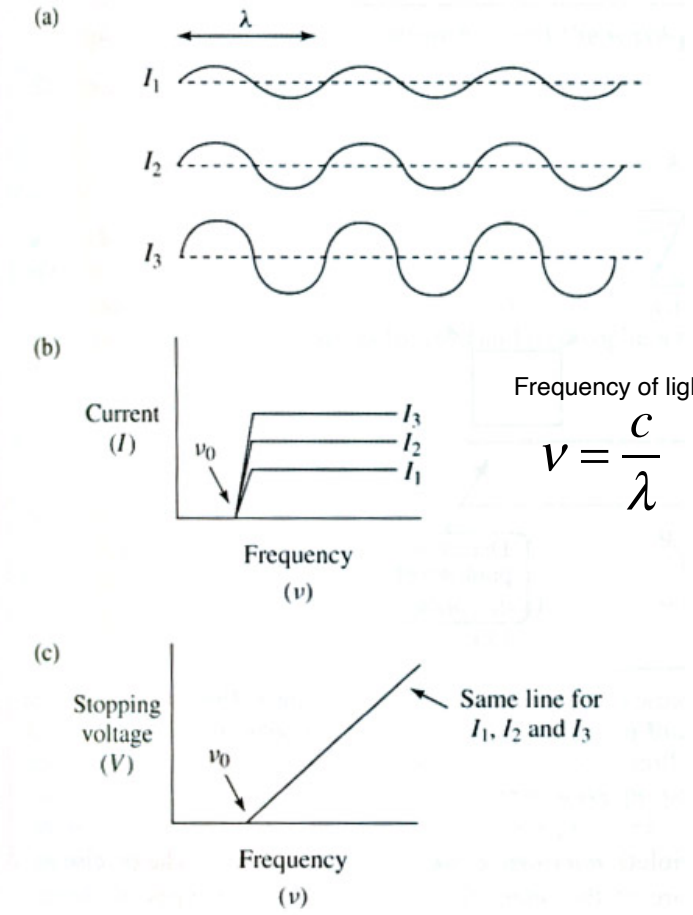


Figure 3.3. Apparatus for the photoelectric effect experiment. When light of a particular intensity (I) and frequency (ν) is shone on a metal plate, electrons are released. These cause a current which may be detected by an ammeter (A). A retarding (or stopping) voltage (V) may be provided which stops the flow of electrons to the cathode. The value of this stopping voltage can be determined with light of different I and ν values (Figure 3.4).



Frequency of light:

$$\nu = \frac{c}{\lambda}$$

Figure 3.4. The photoelectric effect experiment. (a) Light of different intensity (I_1 to I_3) has the same frequency (ν) and wavelength (λ). (b) Current measured in apparatus shown in Figure 3.3 at a variety of ν values. Regardless of the light intensity, current is only detected at values above the threshold frequency ν_0. (c) Stopping voltage increases linearly with ν. Different metals give a line of identical slope but differing ν_0 values. This plot shows that V is proportional to ν at values above ν_0.

The existence of the "photo effect" proves that light must be composed of particles.

What is light?

1. Electromagnetic Waves
==> Interference

2. Particles: Photons
==> Photo Effect

Wavelength

$$\lambda = \frac{c}{\nu}$$

Particle Energy

$$E = h\nu$$

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X-rays, γ	$< 0.0002\ \mu$m = 2 Å	> 5000 eV

Wave – Particle Dualism

Example 3.1 De Broglie's relationship and the wave-particle duality

De Broglie's relationship:

$$\lambda = \frac{h}{p}$$

Where h is Planck's constant (6.626×10^{-27} erg s) implies that *all* matter has *both* a wave and a solid (i.e. particle) nature. We can illustrate this by considering an electron and a golf-ball traveling at the same velocity; 100 m/s. The electron has a mass of 9.11×10^{-28} g while the golf-ball is much larger (10 g). The momentum of each may be calculated by multiplying mass by velocity as follows and de Broglie's relationship can be used to calculate the wavelength associated with the wave nature of each:

Property	Electron	Golf ball
Mass (g)	9.11×10^{-28}	10
Velocity (m/s)	100	100
Momentum (g m/s)	9.11×10^{-26}	1000
Wavelength (cm)	7.27	6.626×10^{-22}

From this table, it is clear that the wave properties of the electron are more significant than its particle properties (e.g. momentum). Conversely, the particle properties of the golf ball are quantitatively more significant than its wave properties. The de Broglie relation means therefore that wave properties are more pronounced at the atomic level while particle properties are more significant at the macroscopic level.

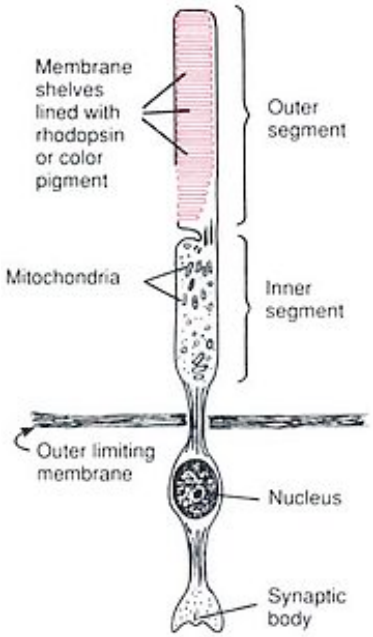
Heisenberg's Uncertainty principle: $\sigma_x \sigma_p \geq \frac{\hbar}{2}$

"If you know, where a particle is (x),
then you cannot know its momentum (p), i.e., mass*speed."

"If you specify exactly the speed and mass of a particle, then you cannot know where it is."

A similar uncertainty relation exists between Energy and Time: If you know the energy of a state, you cannot know how long it will last.

The Human Retina



Rod cell

Rods in the human retina are long and thin, and can be triggered by as little as 6 photons of any color. Rods provide **grey-level** "night vision".

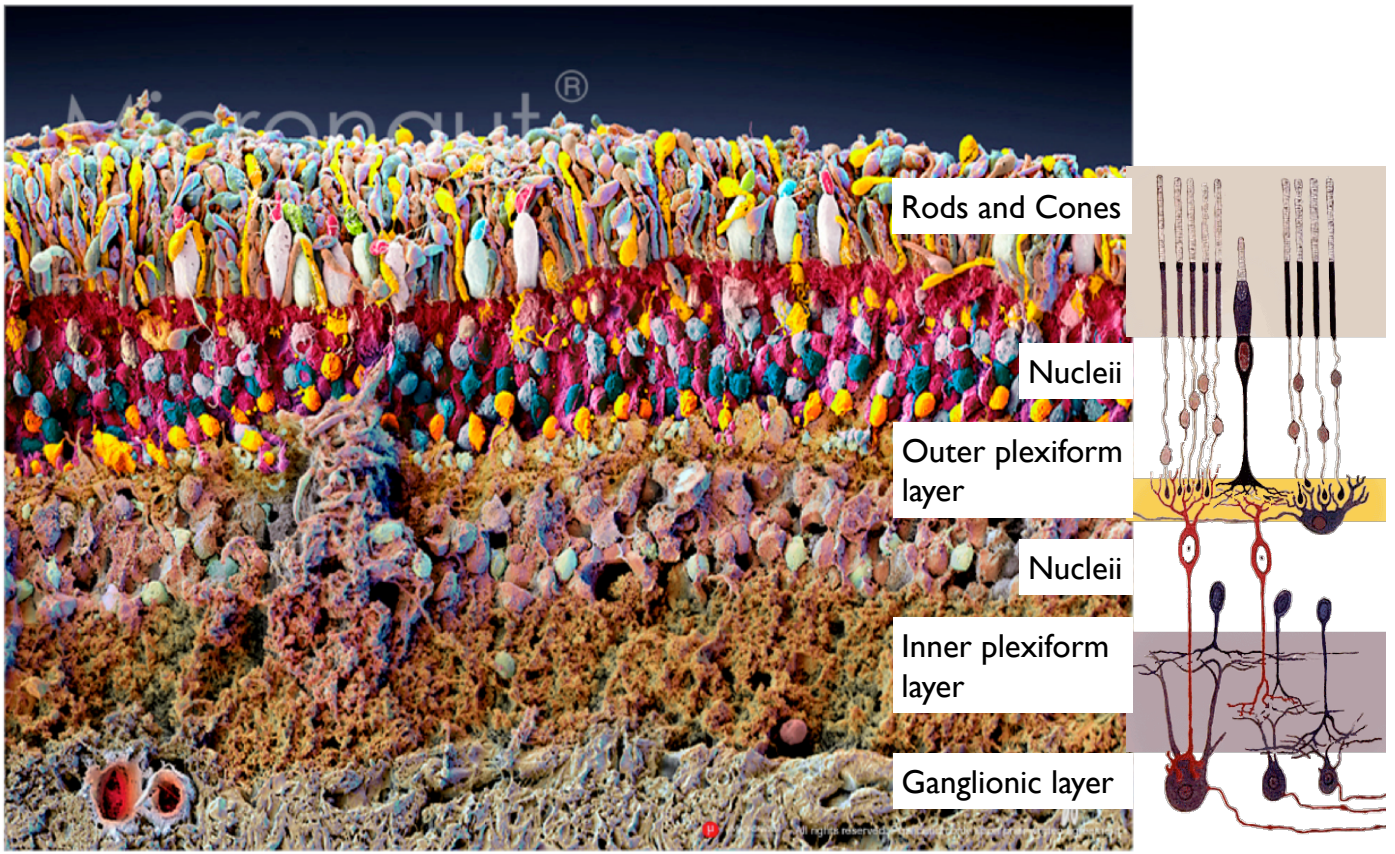
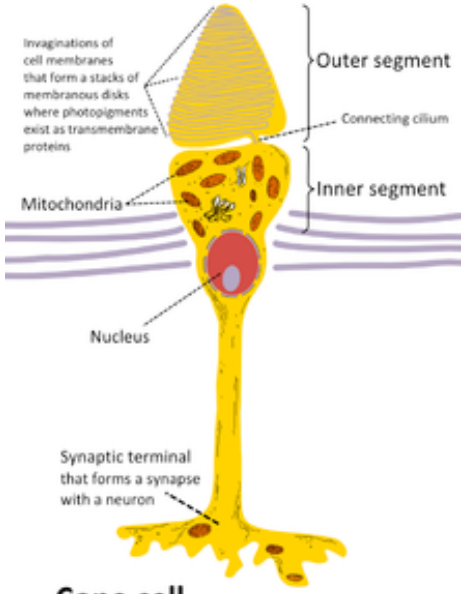
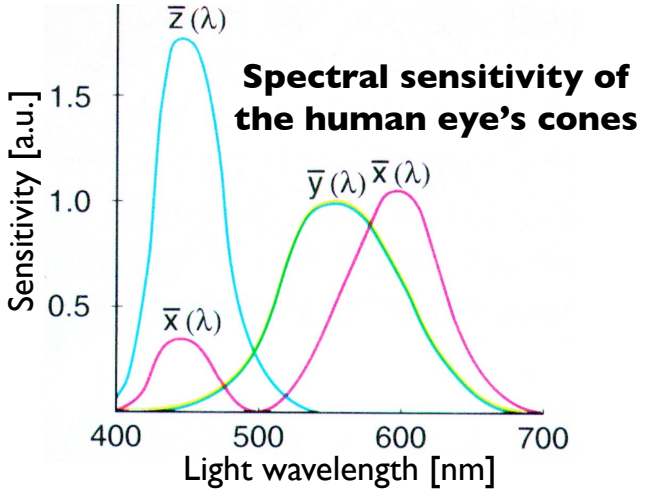


Image from: Micronaut.ch



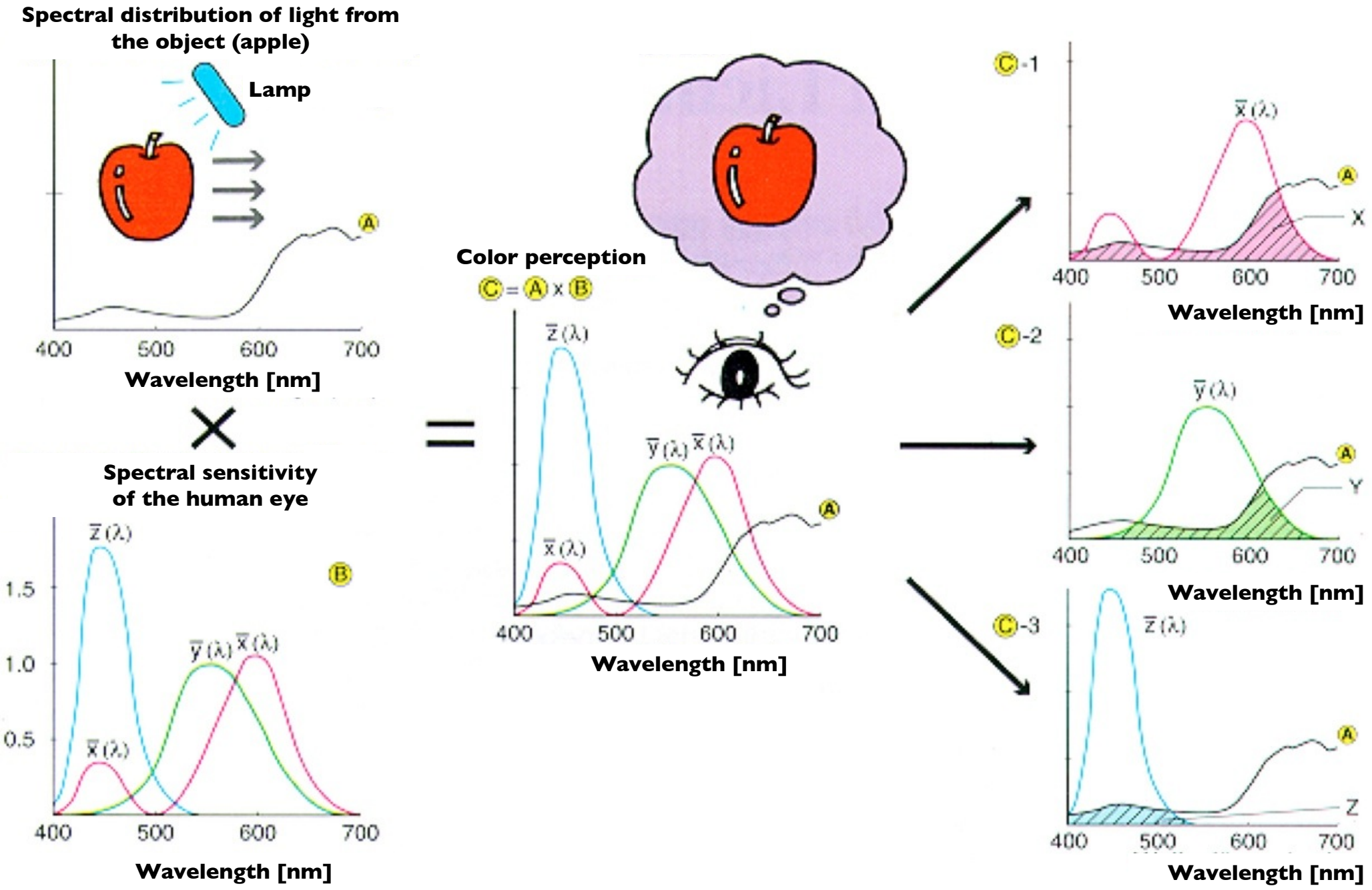
Cone cell

Cones in the human retina are shorter and triangular. Three different types of cones provide **color vision** of three spectral sensitivities, giving Red, Green, and Blue vision. Hence, an RGB signal on a computer monitor provides for humans a perfect "simulation" of the full color spectrum found in nature.



source: wikipedia

The human eye only sees RGB and Grey signals



A basic spectrometer

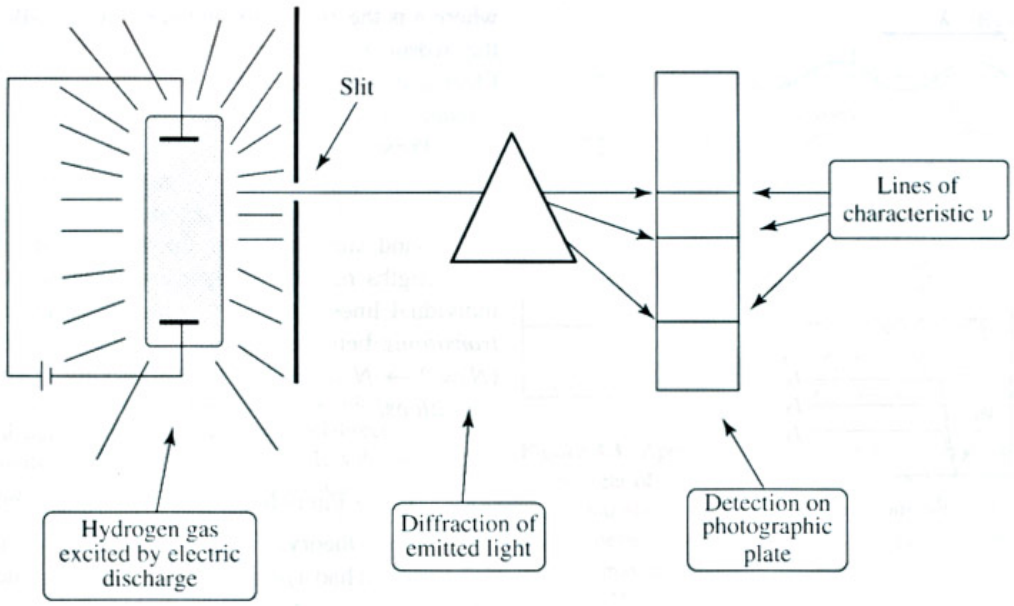


Figure 3.5. The hydrogen emission experiment. When excited by an electric discharge, hydrogen gas emits light. This may be diffracted through a prism and detected on a photographic plate. A series of lines of particular ν and λ are observed rather than a continuum. A completely different spectrum is obtained for helium and other elements.

A spectrometer analysis of the wavelengths within a light beam reveals the nature of the light source.

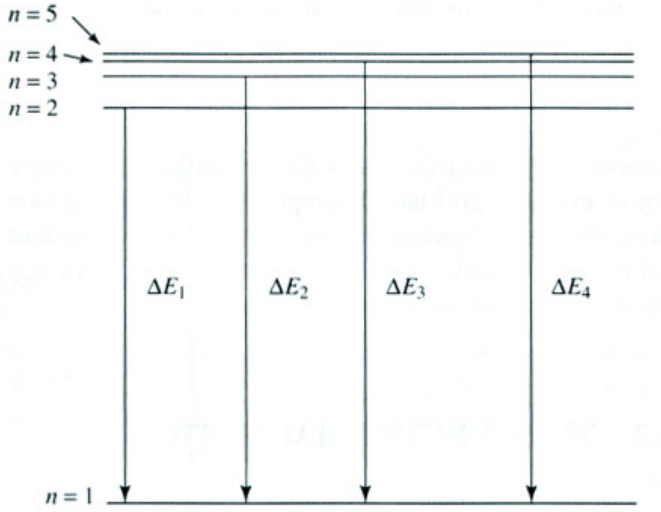


Figure 3.6. Electronic transitions. The hydrogen emission experiment may be explained by postulating that electrons are promoted from their ground state ($n = 1$) to an excited state (e.g. $n = 2$–5). Return of an electron to the ground state involves an electronic transition between quantized energy levels which releases energy (ΔE) corresponding to a light frequency, ν, by the relationship $\Delta E = h\nu$. These beams of light are detected as lines on the photographic plate used in the hydrogen emission experiment.

UV / Vis Absorption Spectroscopy

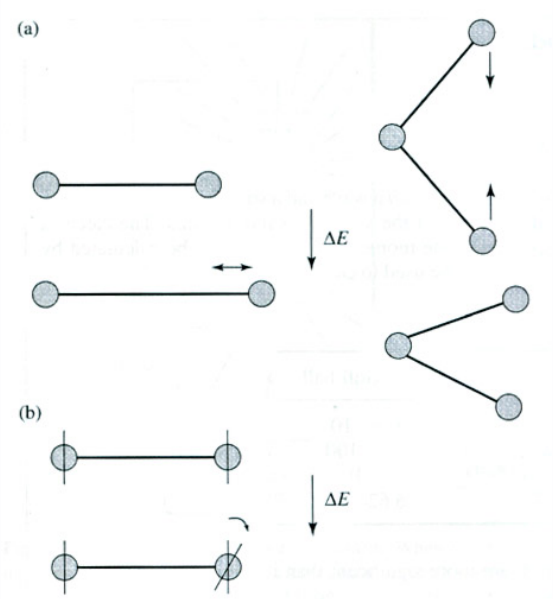


Figure 3.8. Non-electronic transitions. (a) Vibrational transitions involve alternating between a number of allowed bond lengths and bond angles, of differing energy. The energy increment between vibrational energy levels is denoted by ΔE. (b) Rotational transitions. Atoms bonded together can rotate relative to each other. The different rotation states have distinct energies and the energy increment between them is denoted by ΔE.

Table 3.1. Transitions in spectroscopy

Type of transition	Part of spectrum	ΔE (J/mole)	ν (Hz)
Electronic	X-ray	4×10^6	10^{18}
Electronic	Ultraviolet/visible	400×10^3	10^{15}
Vibrational	Infrared	40×10^3	10^{13}
Rotational	Microwave	40	10^{11}
Nuclear spin	Radiowave	4×10^{-4}	10^8

Microwave oven:
2.45 GHz -> 12.2 cm wavelength

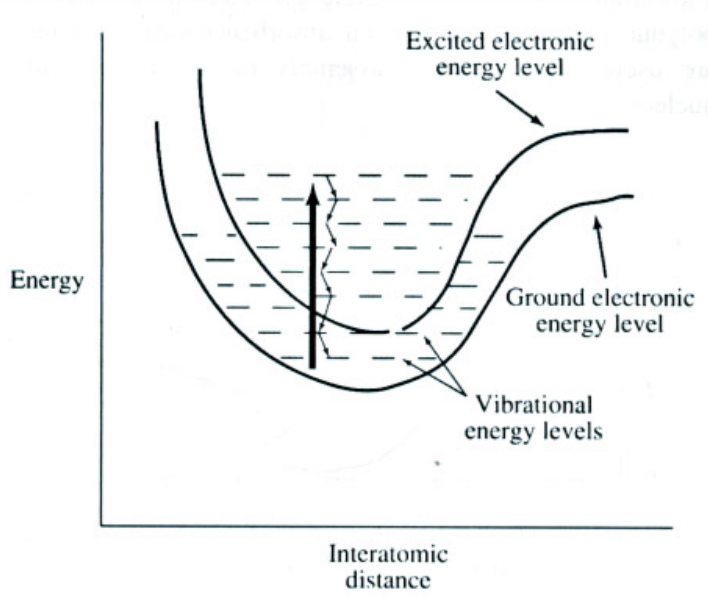


Figure 3.10. Physical basis of absorbance. The electrons of a chromophore may be promoted to a higher energy level as a result of absorption of light energy (large arrow). The electron returns to the ground state by passing through various energy levels (small arrows). Vibrational energy levels of the ground and excited electronic energy levels overlap in flexible molecules. The small increments of energy released as the electron undergoes vibrational transition are lost in collisions with solvent molecules and appear as heat.

UV / Vis Absorption Spectroscopy

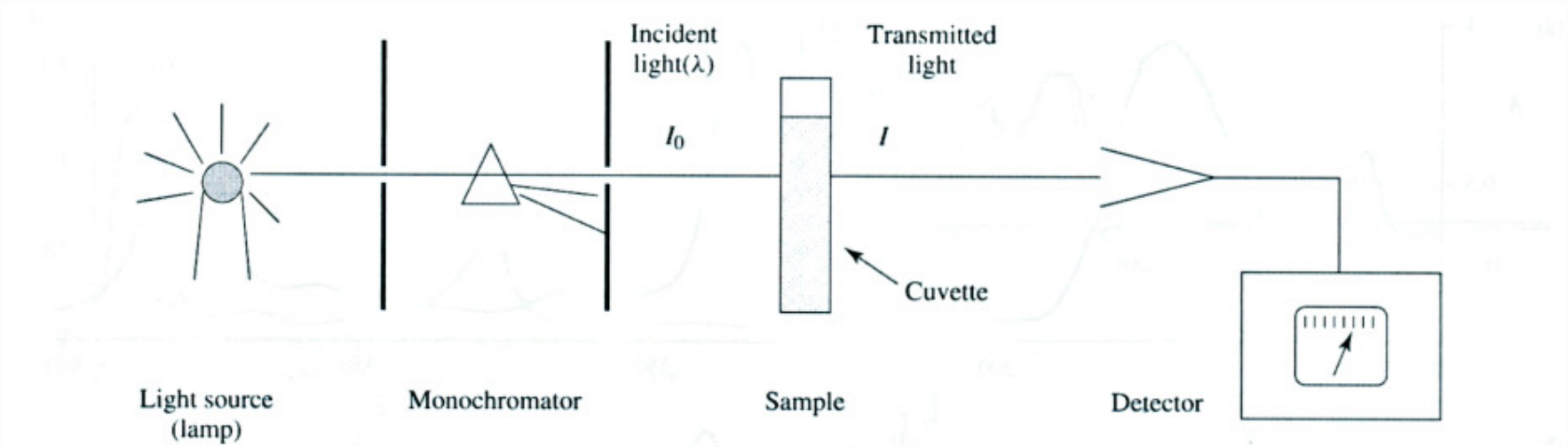


Figure 3.9. Ultraviolet/visible absorption spectroscopy experiment. Light of a single wavelength (λ) and intensity (I_0) is passed through a sample held in a cuvette. Some of this light may be absorbed by the sample. This is detected as a decreased intensity of transmitted light (I) compared to incident light. Log I_0/I is measured as absorbance.

Beer-Lambert law:

$$A = \log_{10}\frac{I_0}{I} = \varepsilon \cdot c \cdot l$$

$$I = I_0 \cdot e^{-A'} = I_0 \cdot e^{-\varepsilon' \cdot c \cdot l}$$

A = *Absorption*; $A' = A\ln(10) = 2.303 * A$

ε = *Characteristic Extinction Coefficient*

$\varepsilon' = \varepsilon\ln(10) = 2.303 * \varepsilon$

c = *Sample Concentration*

l = *Length of Light Path within Sample*

I_0 = *Incident Light*

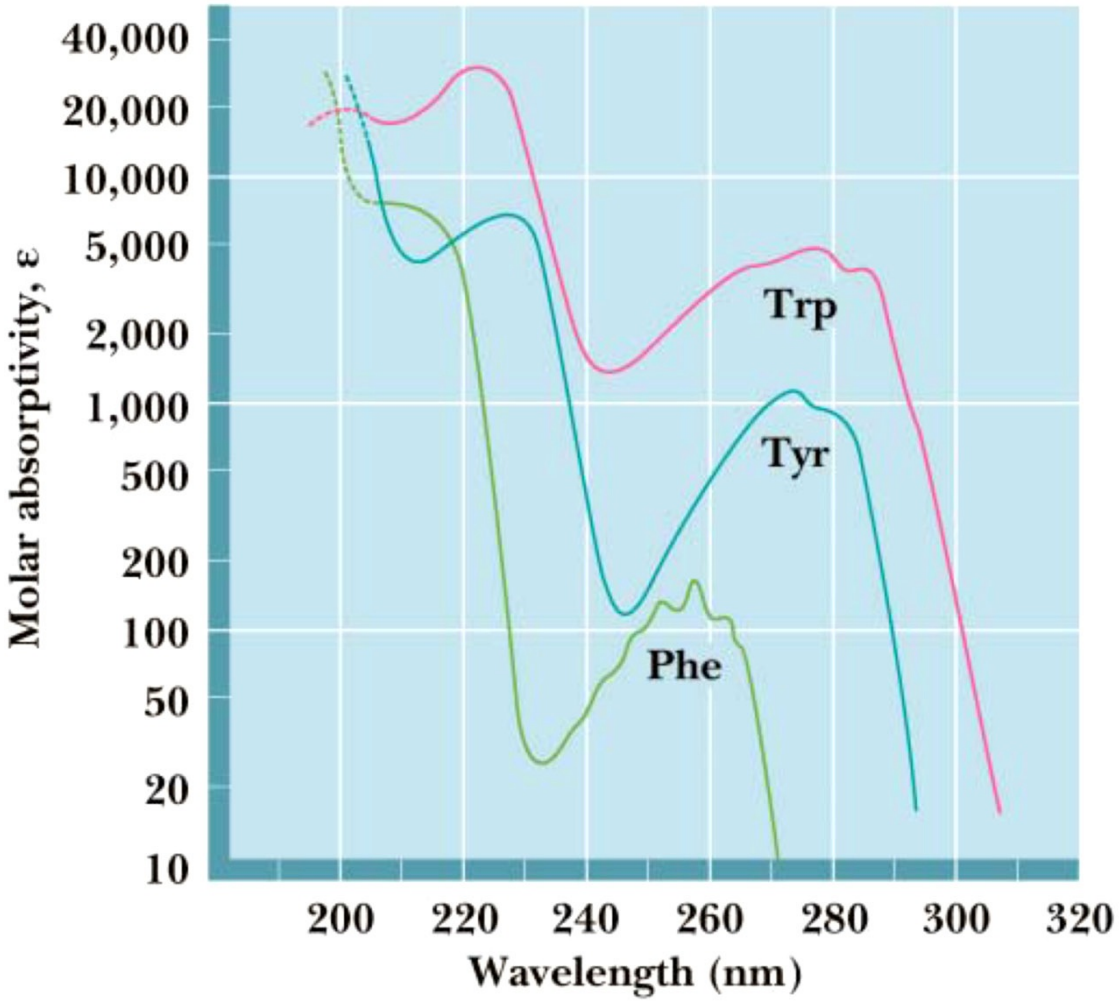
I = *Transmitted Light*

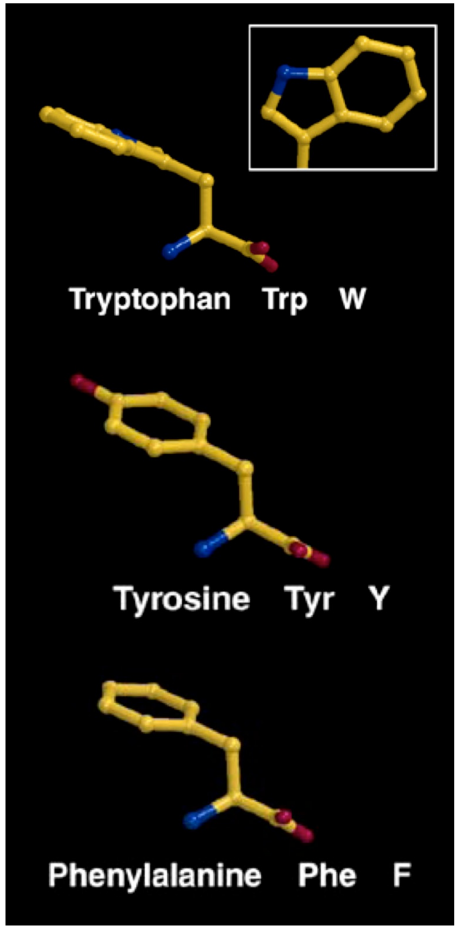
Table 3.2. Some λ_{max} values useful in biochemistry

Chromophore	λ_{max} (nm)	ε ($mM^{-1} \cdot cm^{-1}$)
Tryptophan	280	5.6
	219	47.0
Tyrosine	274	1.4
Phenylalanine	257	0.2
Adenosine	260	14.9
DNA[a]	260	6.6
RNA[a]	260	7.4
Bovine serum albumin	280	40.9

[a] Calculated per mM of 330 Da repeating units.

UV / Vis Absorption Spectroscopy





Only the aromatic amino acids absorb light in the UV region

UV / Vis Absorption Spectroscopy

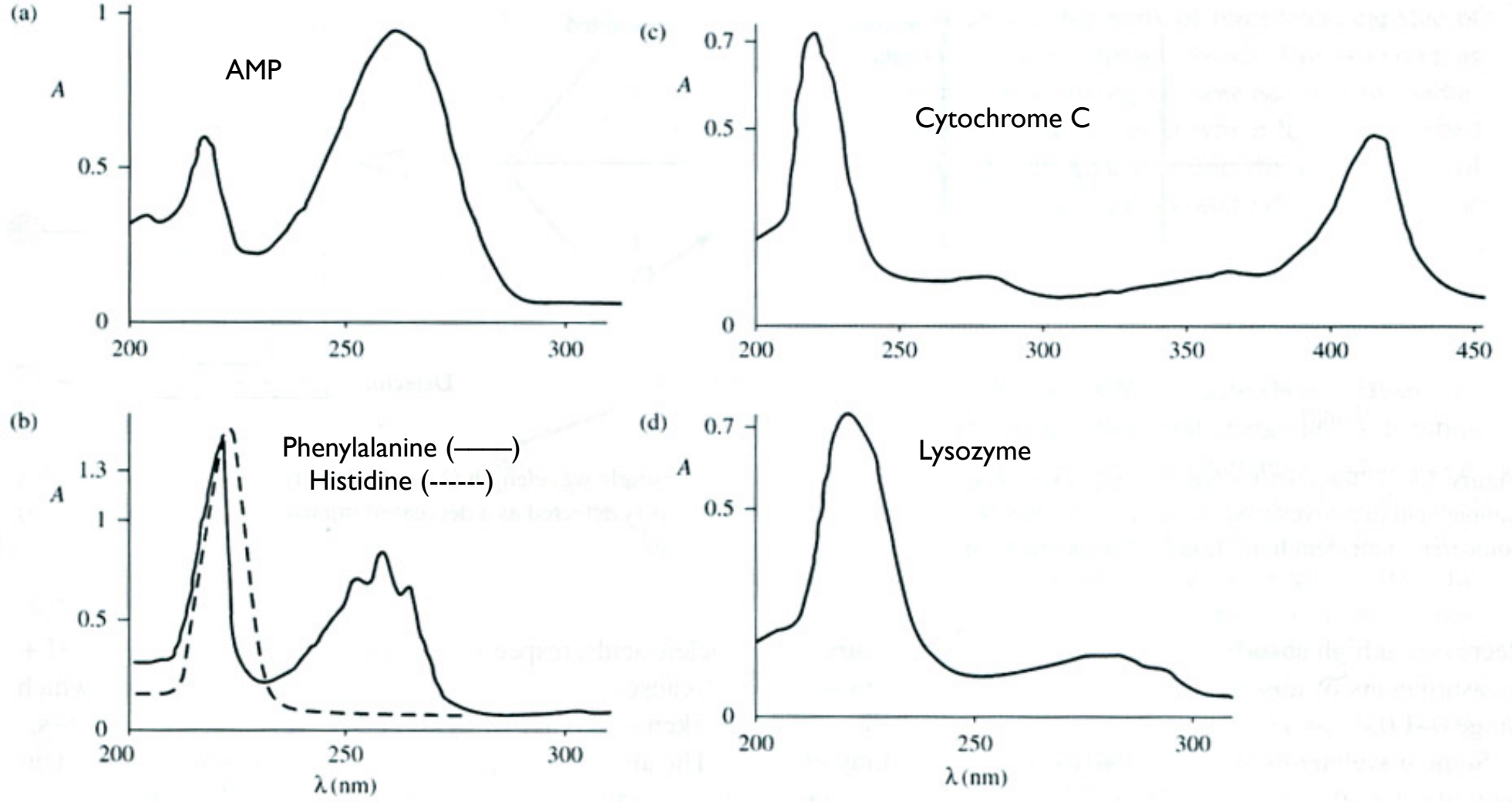


Figure 3.11. Absorption spectra of some biomolecules. Absorbance (A) was measured at pH 5.0 and is plotted against wavelength (λ) for some representative biomolecules. (a) 66 μM adenosine monophosphate (AMP). Note the strong absorbance at 260 nm which is characteristic of nucleotides. (b) 5 mM phenylalanine (solid line) and 0.7 mM histidine (dashed line). Note the λ_{max} at 257 nm which is characteristic for phenylalanine. (c) 70 μg/ml cytochrome C. Note that cytochrome C has a red colour due to the presence of a haem group and has a λ_{max} at 410 nm. (d) 65 μg/ml lysozyme.

UV / Vis Absorption Spectroscopy

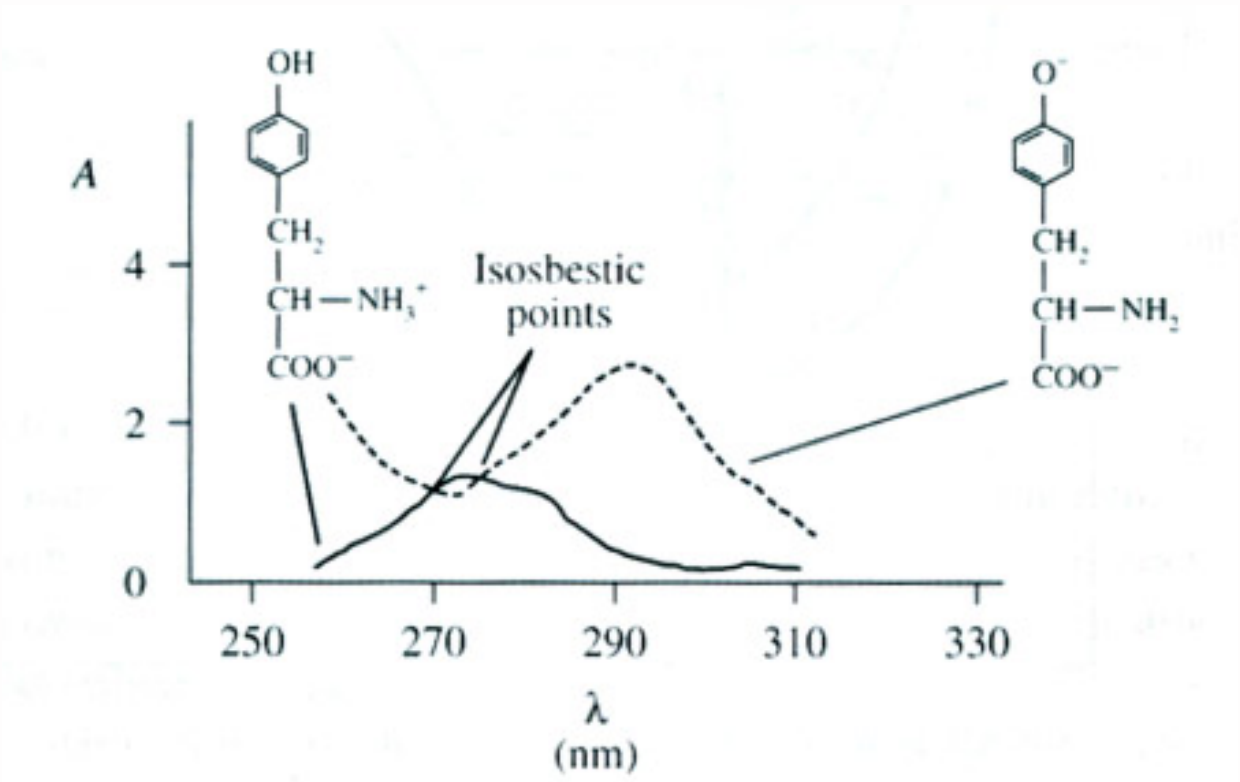


Figure 3.12. Absorbance spectra of tyrosine at pH 6 and 13. Absorbance spectra of 1 mM tyrosine at pH 6 (solid line) and 13 (dashed line) are shown. The structure of the major form of the chromophore at each pH is indicated. Note the λ_{max} at 295 nm for the deprotonated tyrosine anion which is missing from the spectrum of tyrosine. Isosbestic points are wavelengths where the absorbance of both protonated and deprotonated forms of the chromophore are identical.

UV / Vis Absorption Spectroscopy

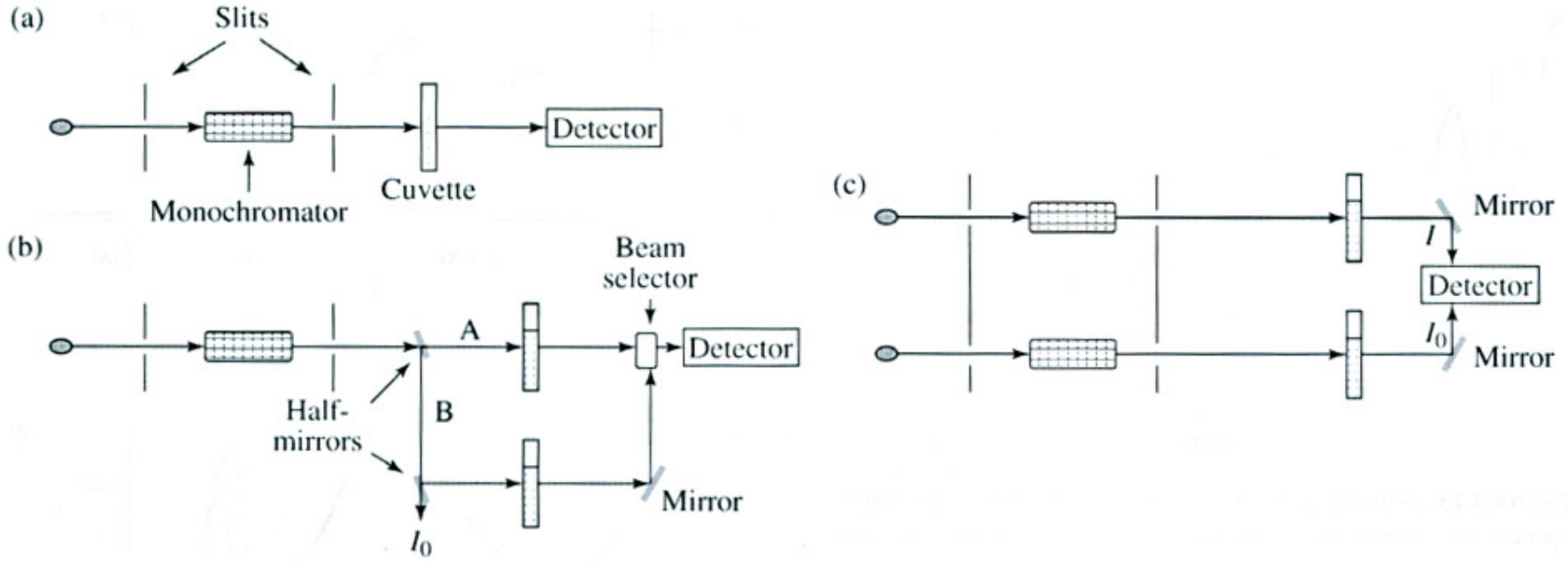


Figure 3.16. Some formats for spectrophotometers. (a) Single-beamed. (b) Split-beamed. Half-mirrors allow some incident light to pass through while reflecting the rest. Two beams of light (A and B) are therefore generated from a single source. One of the cuvettes provides a blank measurement which is automatically subtracted from that of the cuvette containing sample. Note that, at any given moment in time, the detector only measures light from beam A or B as determined by the beam selector. (c) Dual-beamed. Two sources generate individual beams.

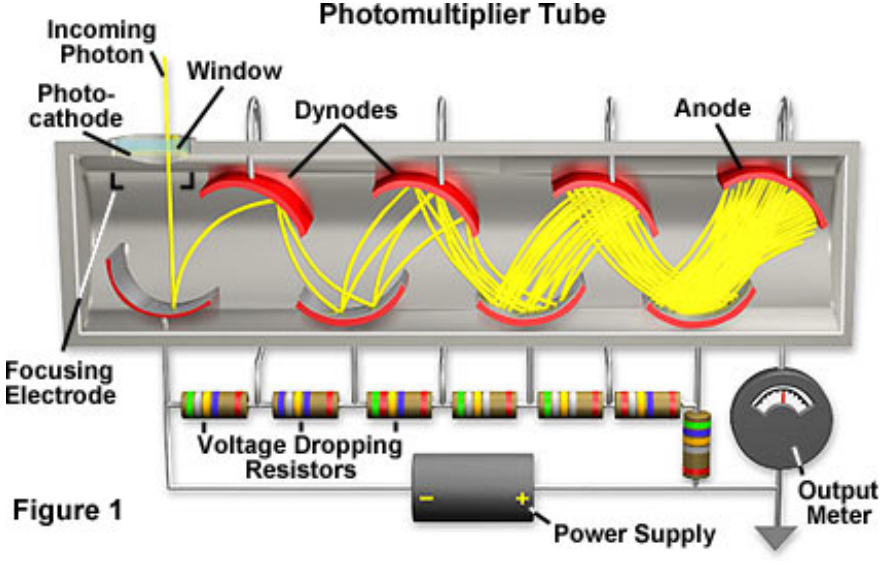
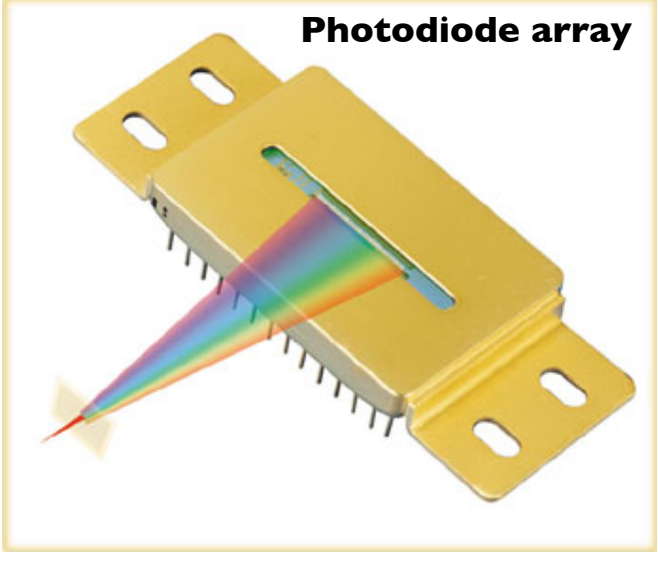


Figure 1



UV / Vis Absorption Spectroscopy

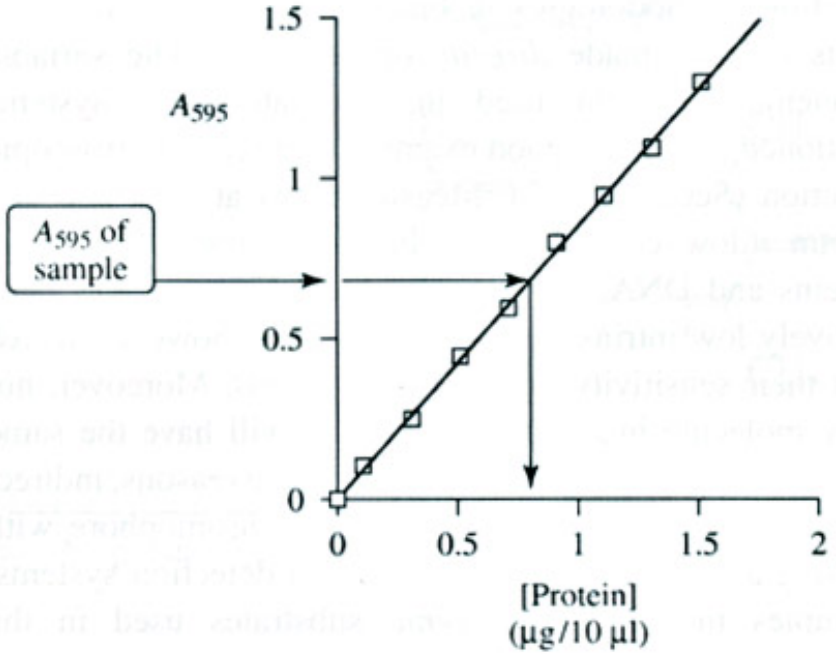
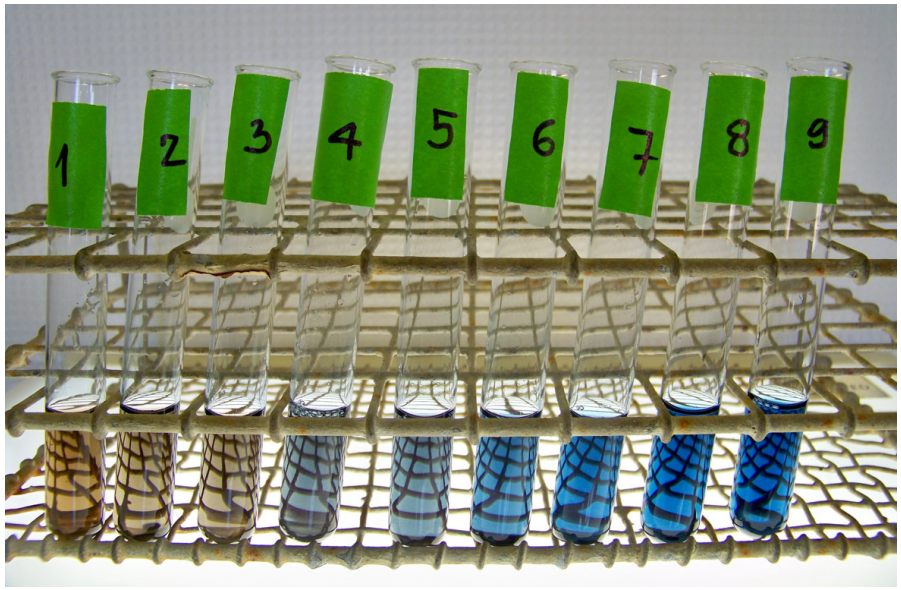
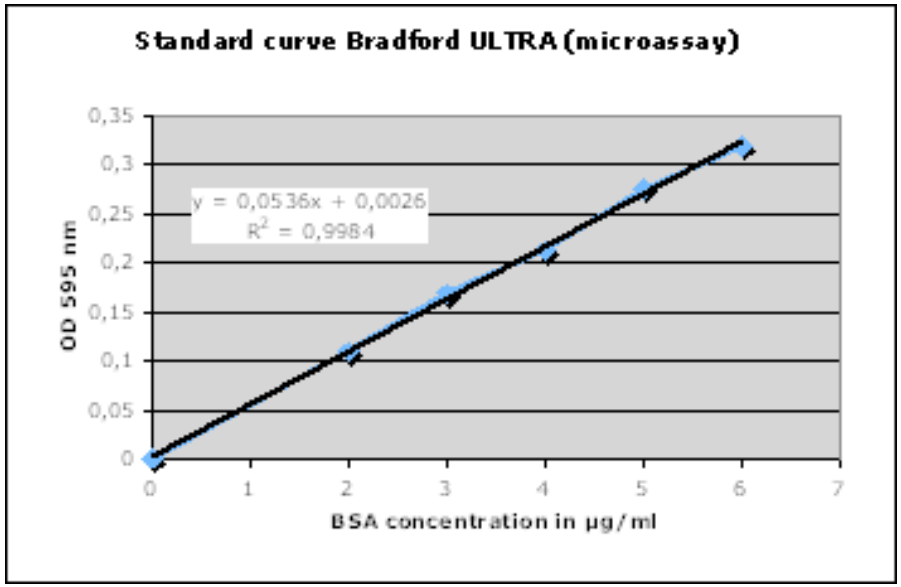


Figure 3.18. Determination of protein concentration using a standard curve – the Bradford assay. Coomassie brilliant blue has a λ_{max} at 596 nm when it complexes with protein. A standard curve is constructed with various known concentrations of bovine serum albumin. The concentration of an unknown sample may be estimated by comparison with this curve (arrows). The use of a standard curve for the relationship between A_λ and concentration depends on the Beer–Lambert law. It is commonly used for estimations in biochemistry.

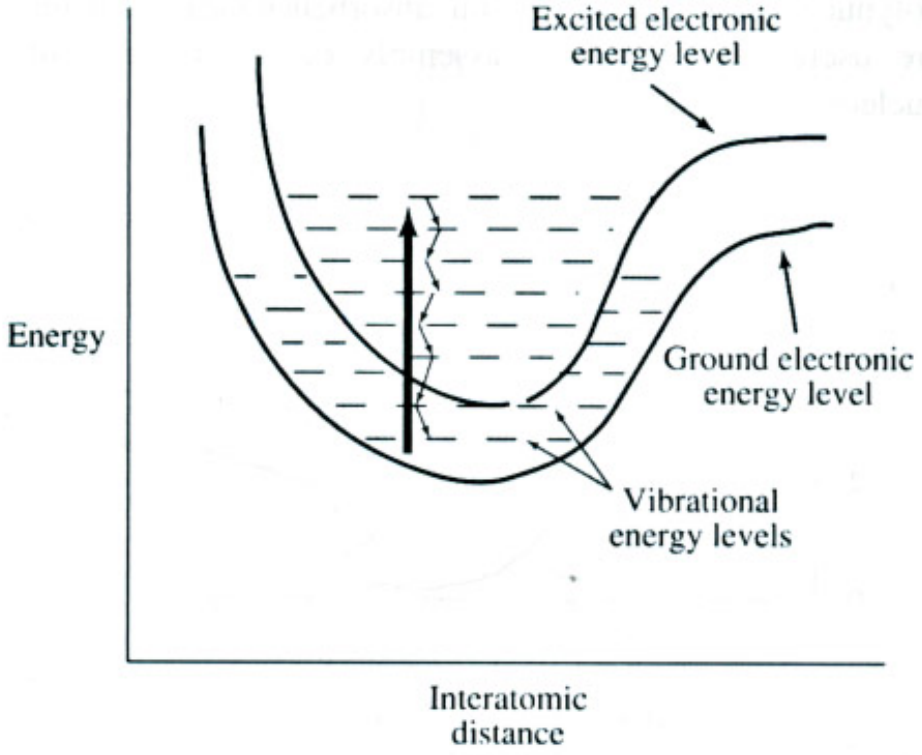




Bradford Assay

Absorbance vs. Fluorescence Spectroscopy

Absorbance



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Fluorescence

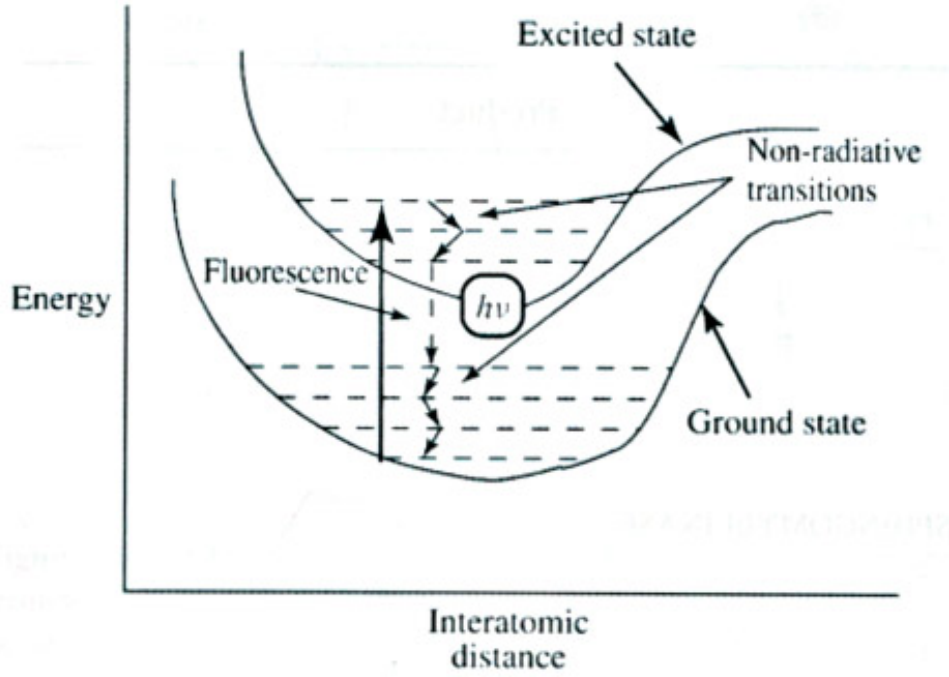


Figure 3.20. Physical basis of fluorescence. If a chromophore has a comparatively rigid structure with few vibrational energy levels such that the electronic energy levels of the ground and excited states do not overlap (*cf.* Figure 3.10), a radiative transition may occur (dashed arrow) which is called fluorescence. This results in emission of radiation of frequency ν, and wavelength λ. Such molecules are called fluors. Phenomena related to fluorescence include phosphorescence and chemiluminescence (*see* text).

Fluorescence Spectroscopy

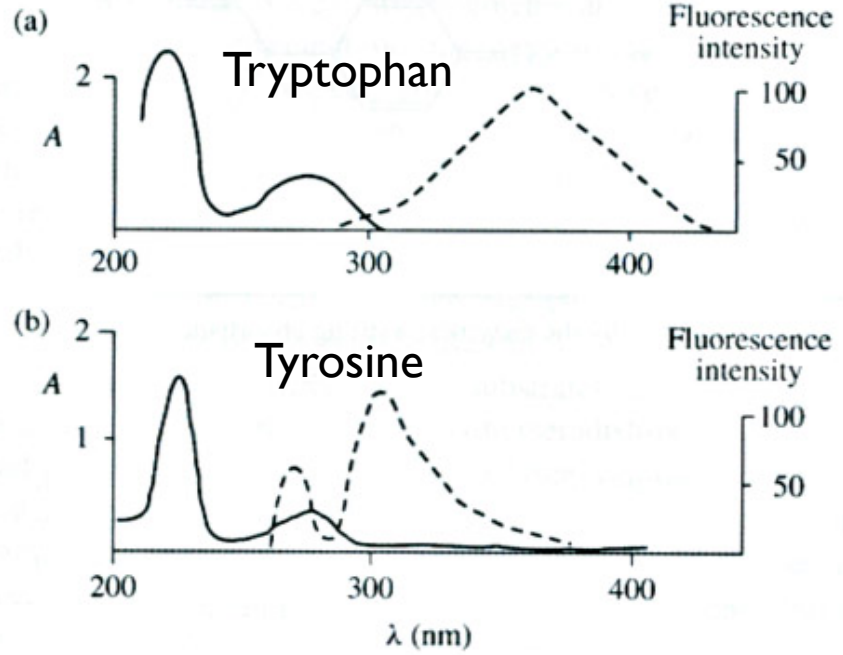


Figure 3.21. Absorbance and fluorescence spectra. Absorbance (solid line) and fluorescence (dashed line) spectra of (a) tryptophan and (b) tyrosine.

Chemiluminescence

$$\text{Luciferin} + \text{ATP} + O_2$$

$$\downarrow \quad \text{(Firefly Luciferase)}$$

$$\text{Oxyluciferin} + \text{AMP} + \text{pyrophosphate} + CO_2 + \text{light}$$

Fluorescence

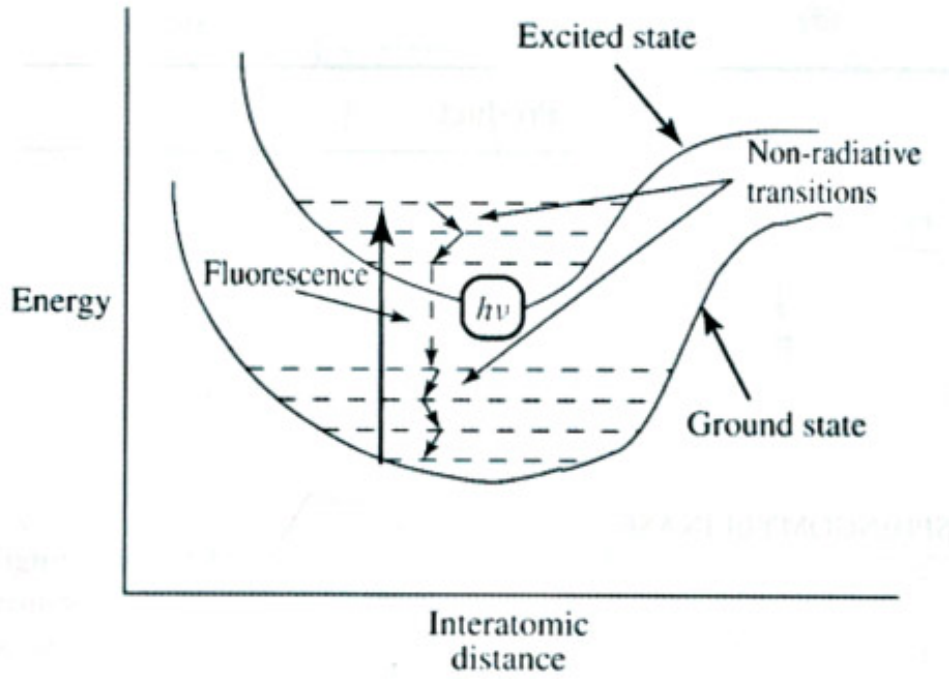
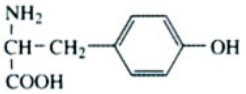
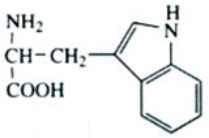
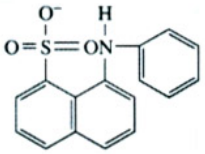
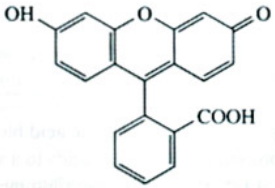
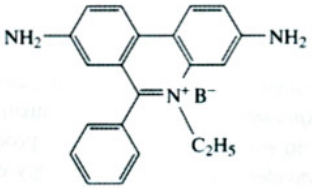
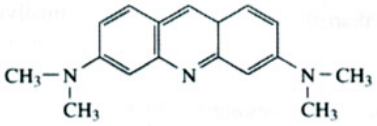
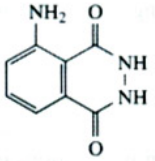
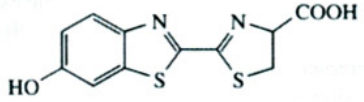


Figure 3.20. Physical basis of fluorescence. If a chromophore has a comparatively rigid structure with few vibrational energy levels such that the electronic energy levels of the ground and excited states do not overlap (*cf.* Figure 3.10), a radiative transition may occur (dashed arrow) which is called fluorescence. This results in emission of radiation of frequency ν, and wavelength λ. Such molecules are called fluors. Phenomena related to fluorescence include phosphorescence and chemiluminescence (*see* text).

Fluorescence Spectroscopy

Table 3.4. Chemical structures of some common fluors and chemiluminescent compounds

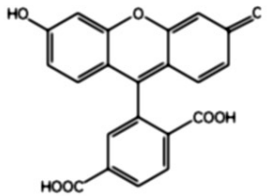
Compound	Structure	Use
Tyrosine		Intrinsic fluor (proteins)
Tryptophan		Intrinsic fluor (proteins)
1-Anilino-8-naphthalene sulphonate (ANS)		Extrinsic fluor (proteins)
Fluorescein		Extrinsic fluor (proteins)
Ethidium bromide		Extrinsic fluor (DNA)
Acridine orange		Extrinsic fluor (DNA)
Luminol (3-Aminophthalhydrazine)		Chemiluminescent substrate (peroxidase)
Luciferin		Chemiluminescent substrate (firefly luciferase)

Intrinsic fluorescence: From the sample itself.
Extrinsic fluorescence: From an additive to the sample

Tyr, Trp, Phe

6-Carboxy Fluorescein:
95% self-quenched >100mM

Fluorescein



Ethidium bromide

Fluorescence Spectroscopy

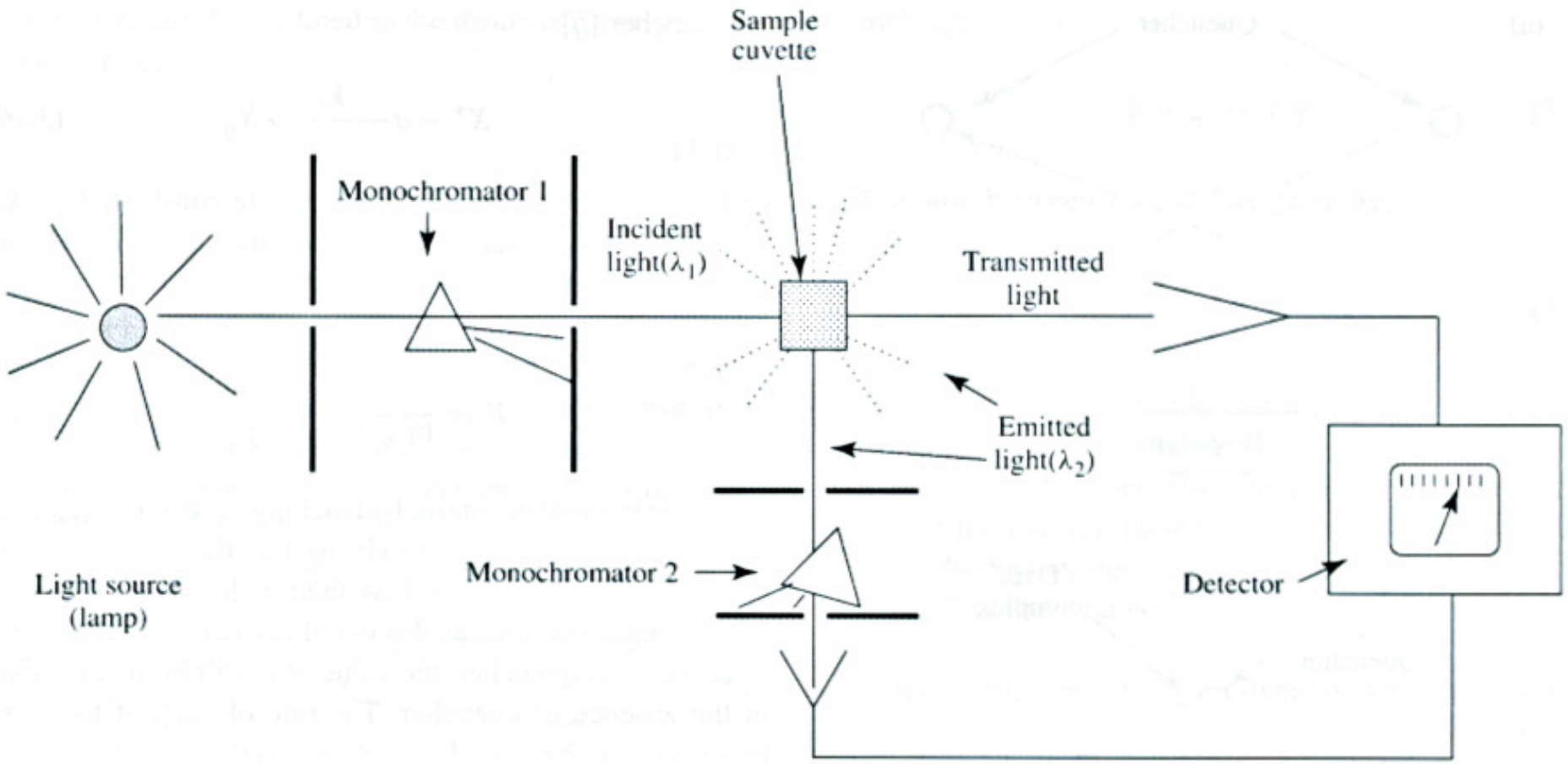


Figure 3.23. Ultraviolet/visible spectrofluorimeter. Light of a single wavelength (λ_1) is passed through and absorbed by a sample held in a cuvette as described in Figure 3.9. At certain values of λ, light is absorbed and the molecule fluoresces. Fluorescent light is emitted in all directions (dashed lines) and is measured at 90° to the incident beam. Emitted light, which is of longer wavelength (λ_2) than absorbed light, is passed through a second monochromator and detected.

Plane-polarized and Circularly Polarized Light

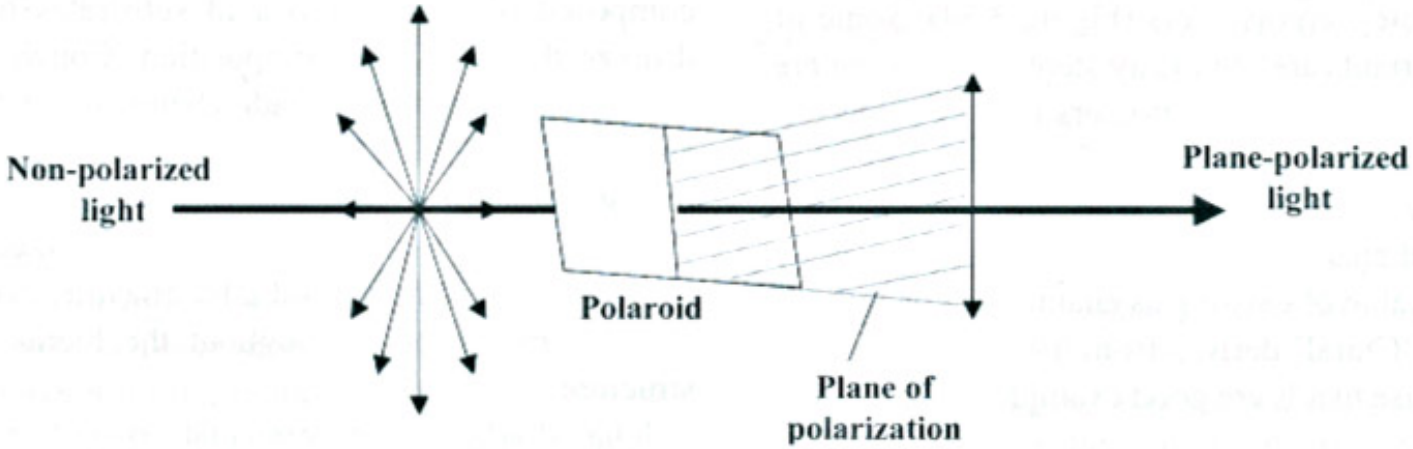
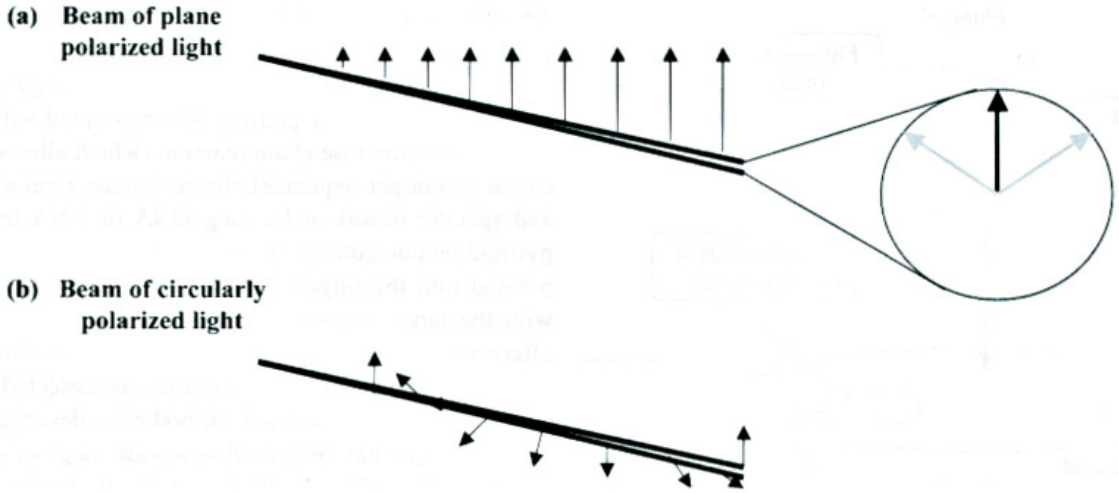
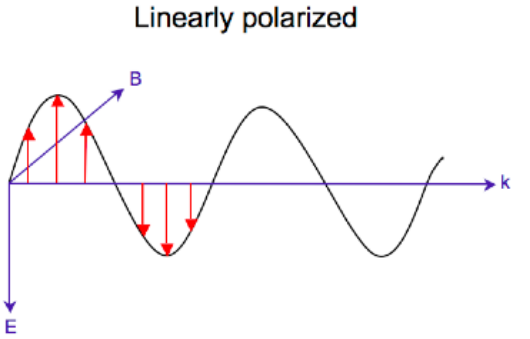


Figure 3.32. Plane polarized light. The electric vectors of a beam of light are shown as arrows. In nonpolarized light, these vectors are orientated randomly through 360°. A polaroid filter selects for a single orientation of electric vectors resulting in a plane of polarization. This is plane-polarized light which is also known as linearly polarized light.





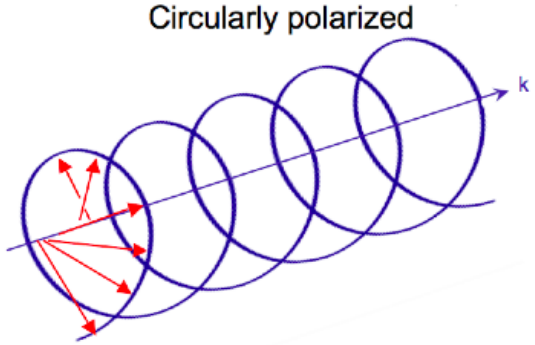


Figure 3.33. Circularly polarized light. (a) The electric vector of plane polarized light (solid arrows) is composed of two equal and opposite circularly polarized components (dashed arrows). (b) The electric vector of circularly polarized light describes an elliptical path along the direction of propogation.

Chirality of Amino Acids

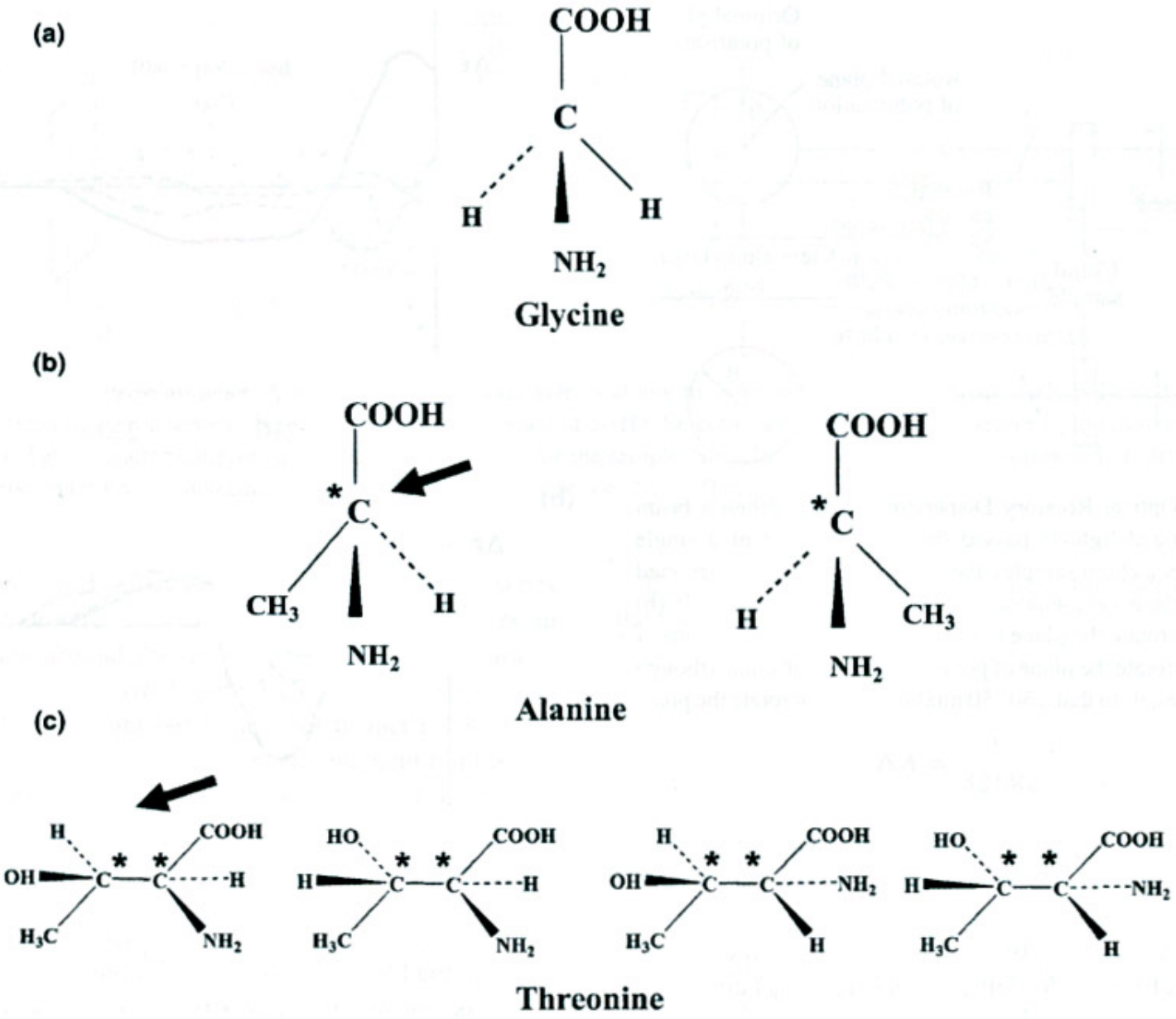
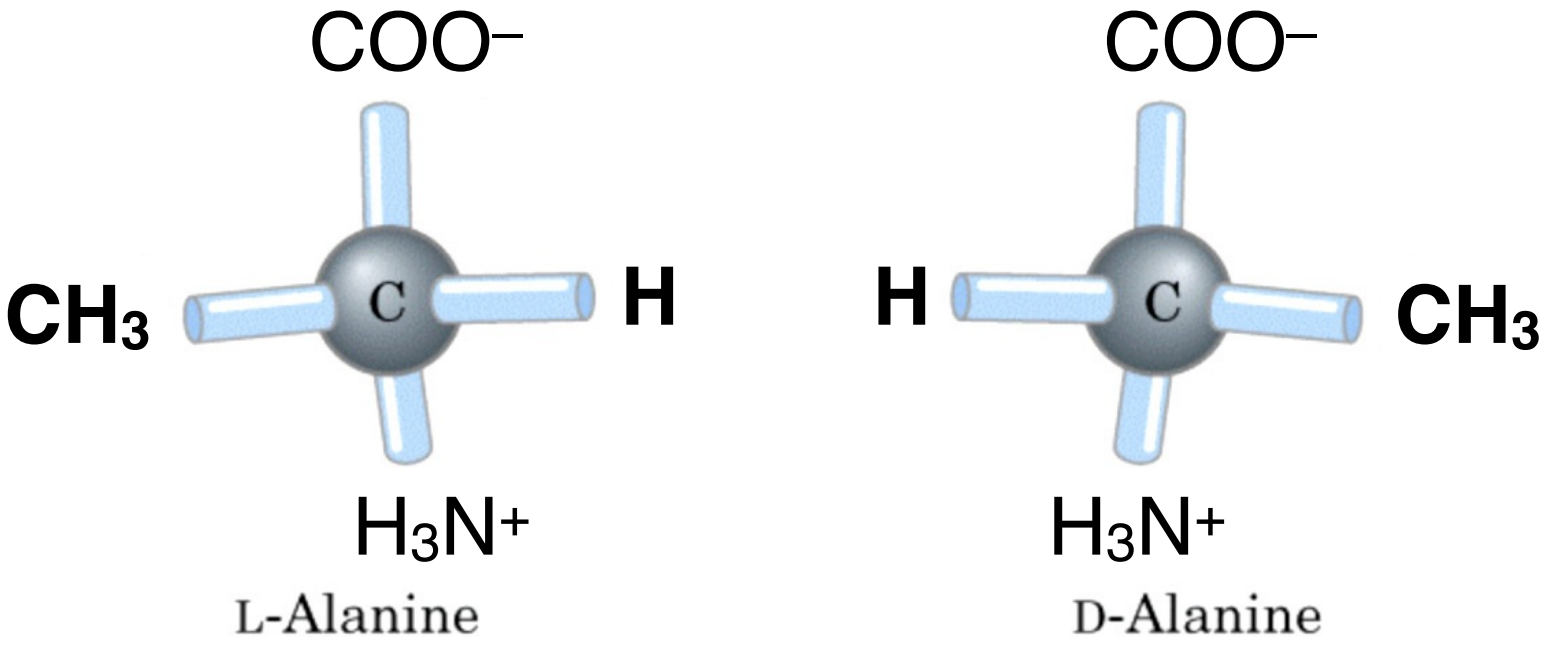


Figure 3.34. Chirality in amino acids. (a) Glycine is an achiral molecule because the four substituents to the α-C are not all chemically different. All the 19 other amino acids commonly found in proteins are chiral. (b) Alanine is a chiral molecule because it has a centre of asymmetry (*). In nature, only the L-enantiomer is commonly found. (c) Threonine has two centres of asymmetry and accordingly has four possible enantiomers, only one of which (arrow) is commonly found in nature.

Optical Activity



- All amino acids except for Glycine rotate polarized light.
- All amino acids except for Proline have an L stereochemical configuration.
- Optically active molecules have an asymmetry such that mirror images are not superimposable.
- Enantiomers (L- / D- forms) are physically and chemically indistinguishable by most techniques (except optics).

Chirality of life

- Ordinary synthesis of chiral molecules produces racemic mixtures, i.e., equal amounts of L- and D- stereoisomers. Ordinary methods do not show stereochemical preference.

- A fact of life: biosynthesis of substances having asymmetric centers produces almost pure stereoisomers, i.e. only one kind.

- Examination of amino acids in meteorites always shows racemic mixtures. Thus, these are not based on life.

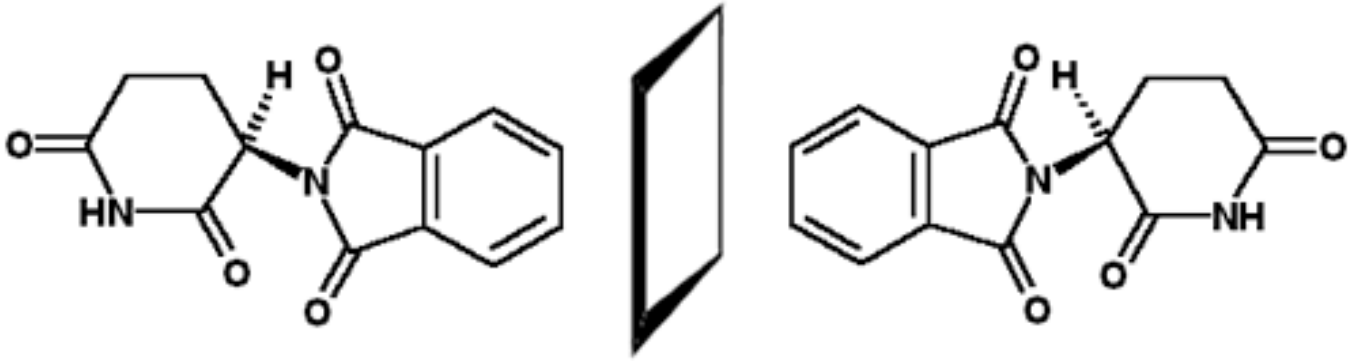
- It is not known why life has shown preference for L-steroisomers.

Thalidomide

- German drugmaker Chemie Grünenthal introduced thalidomide, under the name **Contergan**, to the German market on Oct. 1, 1957
- It was a sedative to treat insomnia as well as to reduce nausea associated with pregnancy.
- In clinical trials, the R-enantiomer had been produced and used. When successful, upscaling the production process produced racemic mixtures, containing the R- and L-enantionmers.
- By 1960, the drug was in use in more than 20 European and African countries.
- When several malformations in newborns were finally connected with Contergan, the drug was taken off the market in 1962.
- More than 10'000 children were born with severe malformations.

Image: WDR.de

R-enantiomer



R-Thalidomide
(sleep-inducing)

L-enantiomer

S-Thalidomide
(teratogenic)

- The L-enantiomer was teratogenic, the R-enantiomer was previously believed to be solely sleep-inducing. However, also the R-enantiomer is now known to cause some malformations.

Optical Rotatory Dispersion (ORD)

ORD measures, how much the sample rotates plane-polarized light.
(Note: ORD does not measure any light absorption.)

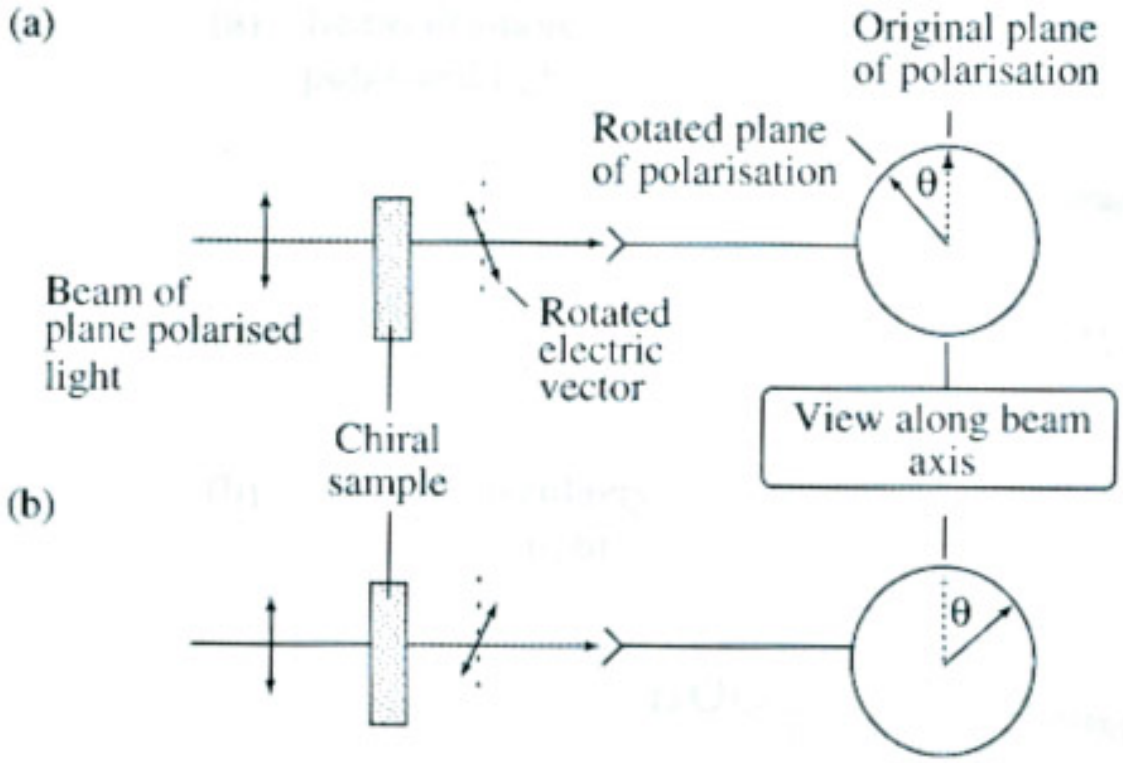


Figure 3.35. Optical Rotatory Dispersion (ORD). When a beam of plane polarized light is passed through a sample of a single enantiomer (i.e. a chiral sample), the plane of polarization is rotated through an angle, θ. (a) L-enantiomers rotate the plane to the left. (b) D-enantiomers rotate the plane to the right. Equal concentrations of an enantiomer rotate the plane of polarization through equal (though opposite) angles, θ, so that a 50 : 50 mixture does not rotate the plane at all.

Circular Dichroism (CD)

Ellipticity (Circular Dichroism)

(CD measures, how much the sample differently absorbs left and right circularly polarized light.)

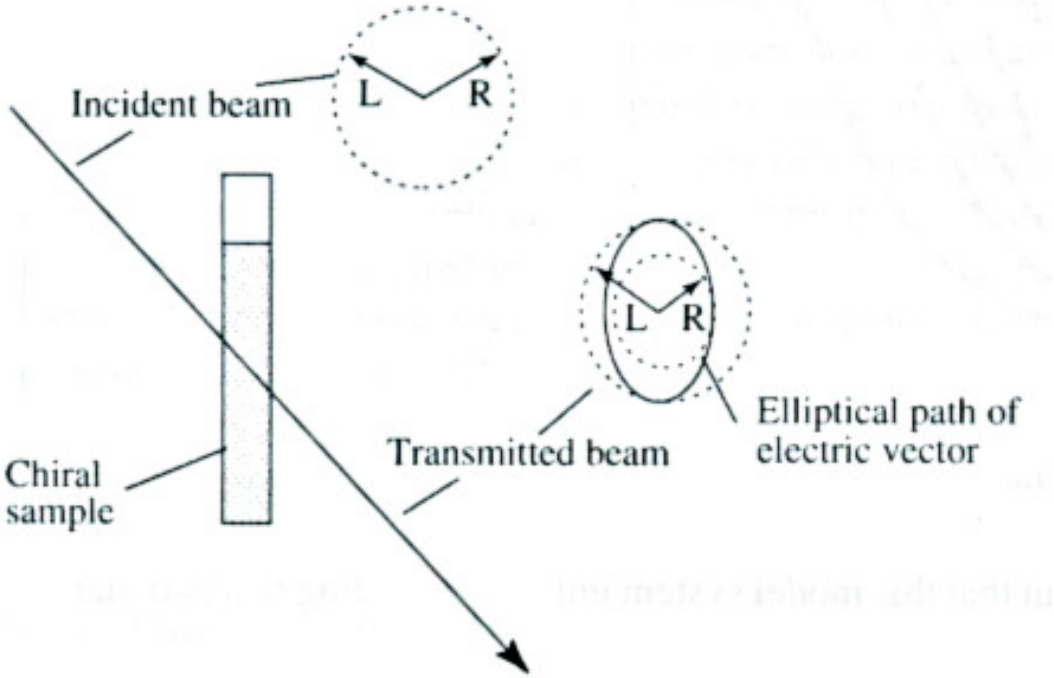


Figure 3.38. Ellipticity. A beam of plane polarized light consists of electric vectors of equal amplitude. When recombined, the resultant vector from the left (L) or right (R) circularly polarized components of plane polarized light would describe a circle (incident beam). If this light is passed through a chiral sample, one circularly polarized component is selectively absorbed. This lowers the amplitude associated with that component (R in the sample above). When recombined, the resultant electric vector now traces an elliptical path. The degree of ellipticity is a measure of circular dichroism.

Beer-Lambert law for Absorption:

$$A = \log \frac{I_0}{I} = \varepsilon \cdot c \cdot l$$

If molar extinction coefficients are different:

$$\varepsilon_L \neq \varepsilon_R$$

we have a difference in the absorbance of left and right circularly polarized light:

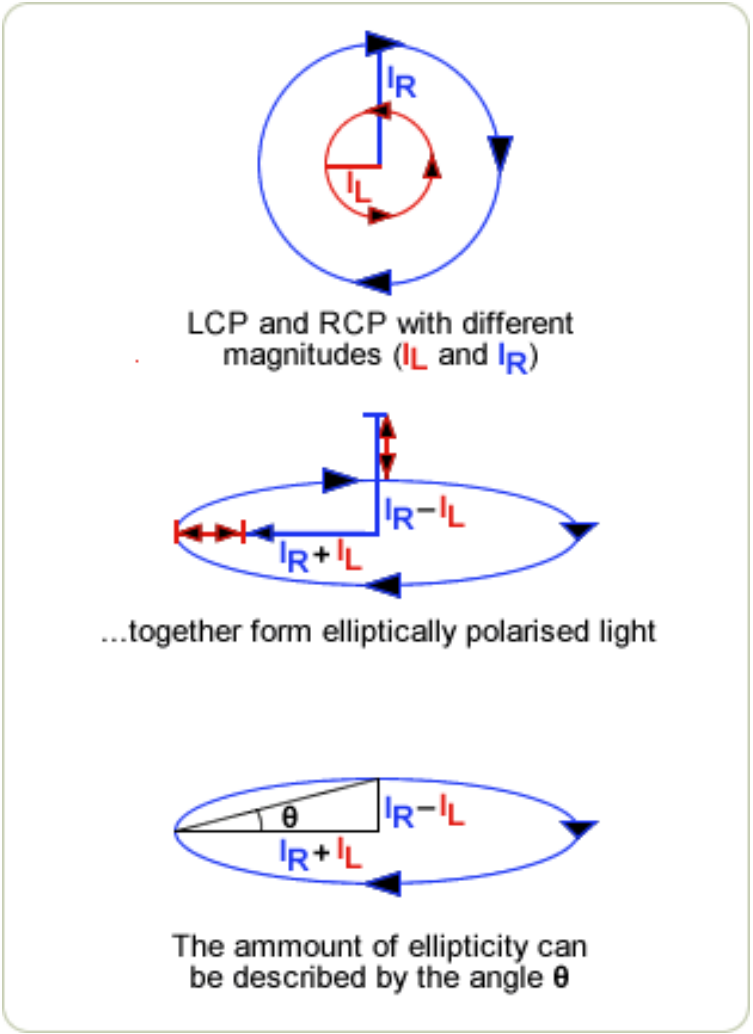
$$\Delta A = \Delta\varepsilon \cdot c \cdot l$$

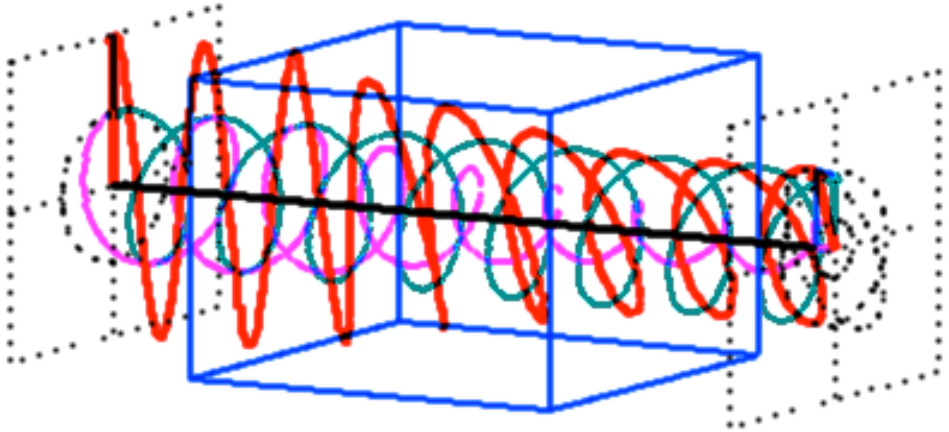
A = Absorption
ε = Characteristic Extinction Coefficient
c = Sample Concentration
l = Length of Light Path within Sample
I_0 = Incident Light
I = Transmitted Light

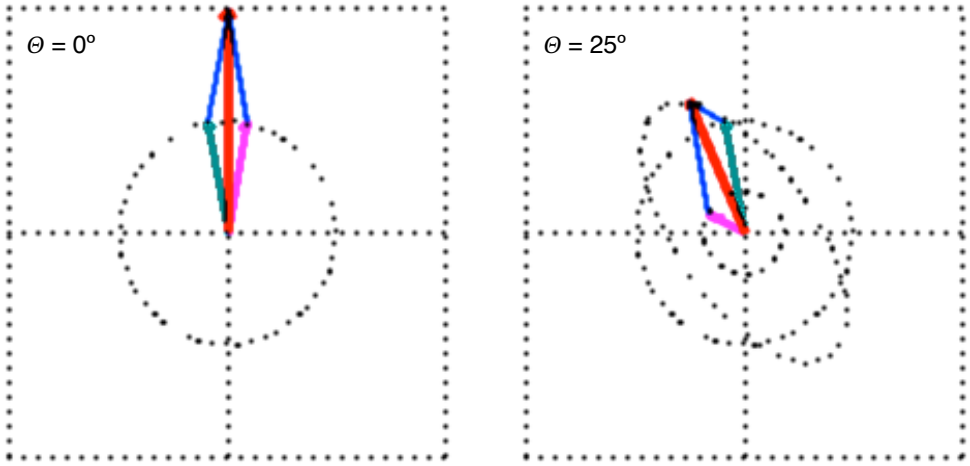
Circular Dichroism (CD)

Ellipticity (Circular Dichroism)

(The sample differently absorbs
left and right circularly polarized light.)







$$\Delta A = \frac{\theta}{32.982}$$

θ = *Amount of ellipticity* [deg]

$$\Delta A = \Delta\varepsilon \cdot c \cdot l$$

$$\Delta\varepsilon = \frac{\Delta A}{c \cdot l}$$

Circular Dichroism (CD)

$\Delta\varepsilon$ is wave-length dependent.

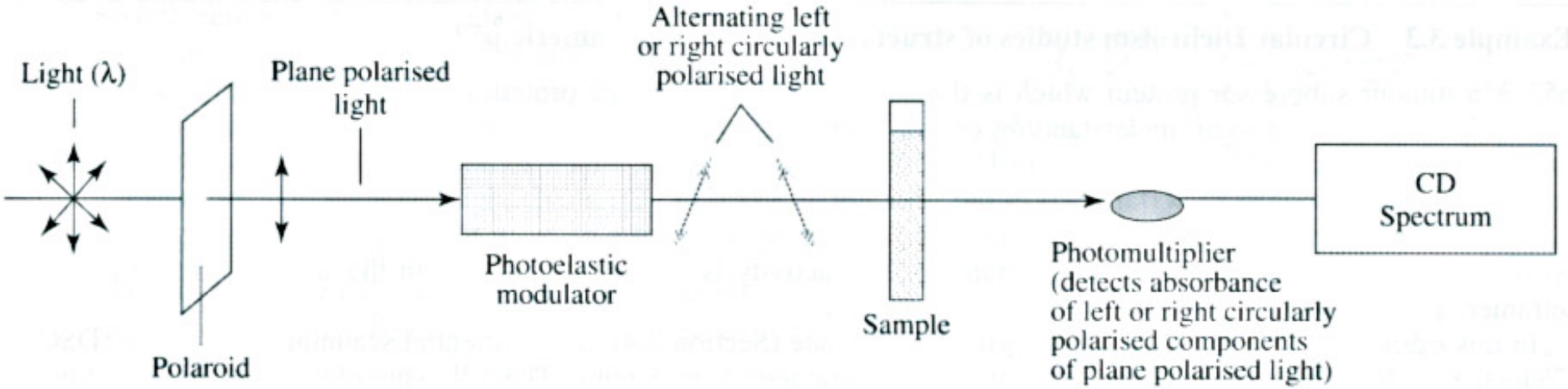


Figure 3.37. CD spectropolarimeter. A photoelastic modulator selects at any time for either left or right circularly polarized components of plane polarized light. It alternates between these at a frequency of 50 Hz. Selective absorption of either left or right circularly polarized light is detected at the photomultiplier and gives a CD spectrum for the sample. Samples which are achiral or composed of 50 : 50 mixtures of enantiomers would give no detectable spectrum in this instrument (i.e. $\Delta A = 0$ at all λ).

Circular Dichroism (CD)

θ or $\Delta\varepsilon$ are wave-length dependent.

CD for protein structures:

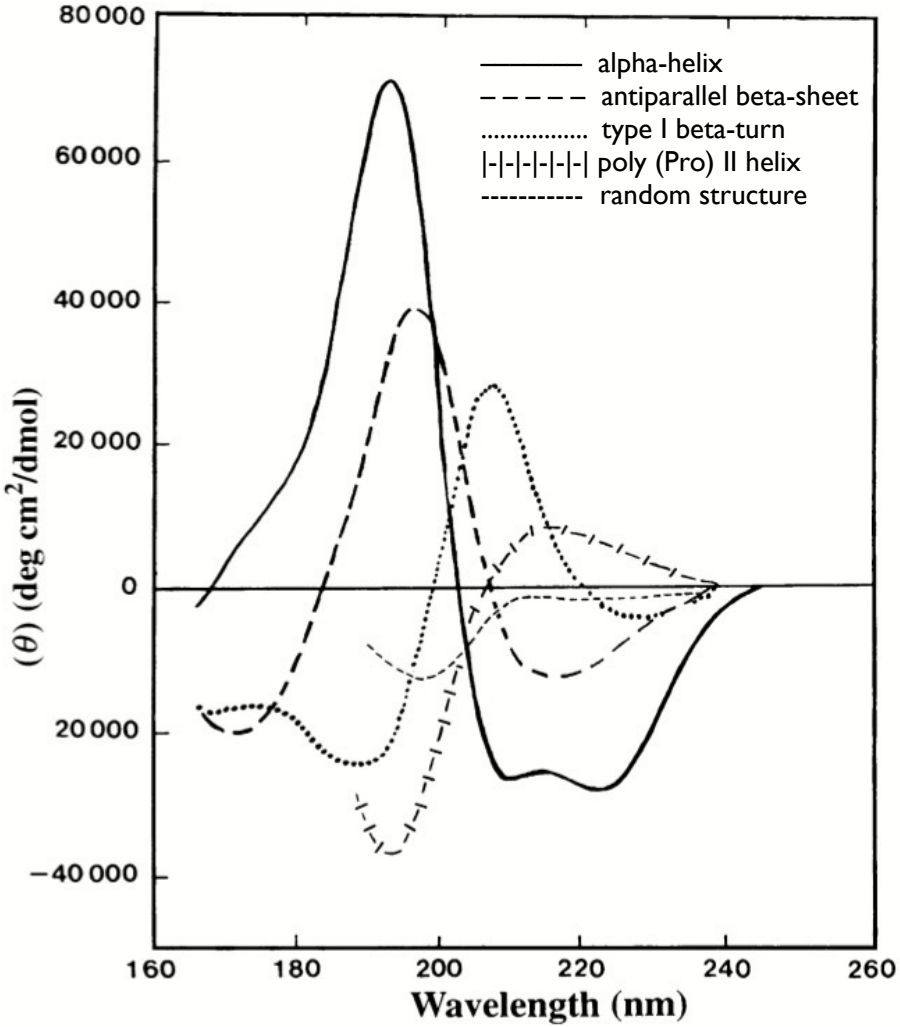
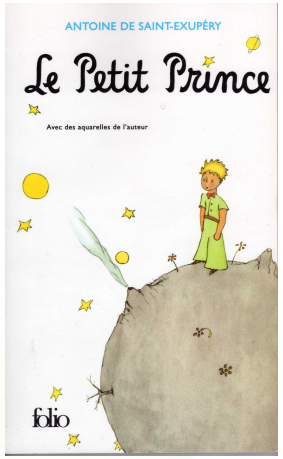


Figure 3: Illustration of graphs showing far-UV CD spectra associated with various types of secondary structure. Solid line, α-helix; long dashed line, antiparallel β-sheet; dotted line, type I β-turn; cross-dashed line, extended 3_1-helix or poly (Pro) II helix; short-dashed line, irregular structure. The data are adapted from Kelly *et al.* (12).



Alpha = Elephant

Circular Dichroism (CD)

θ or $\Delta\varepsilon$ are wave-length dependent.

CD for protein structures:

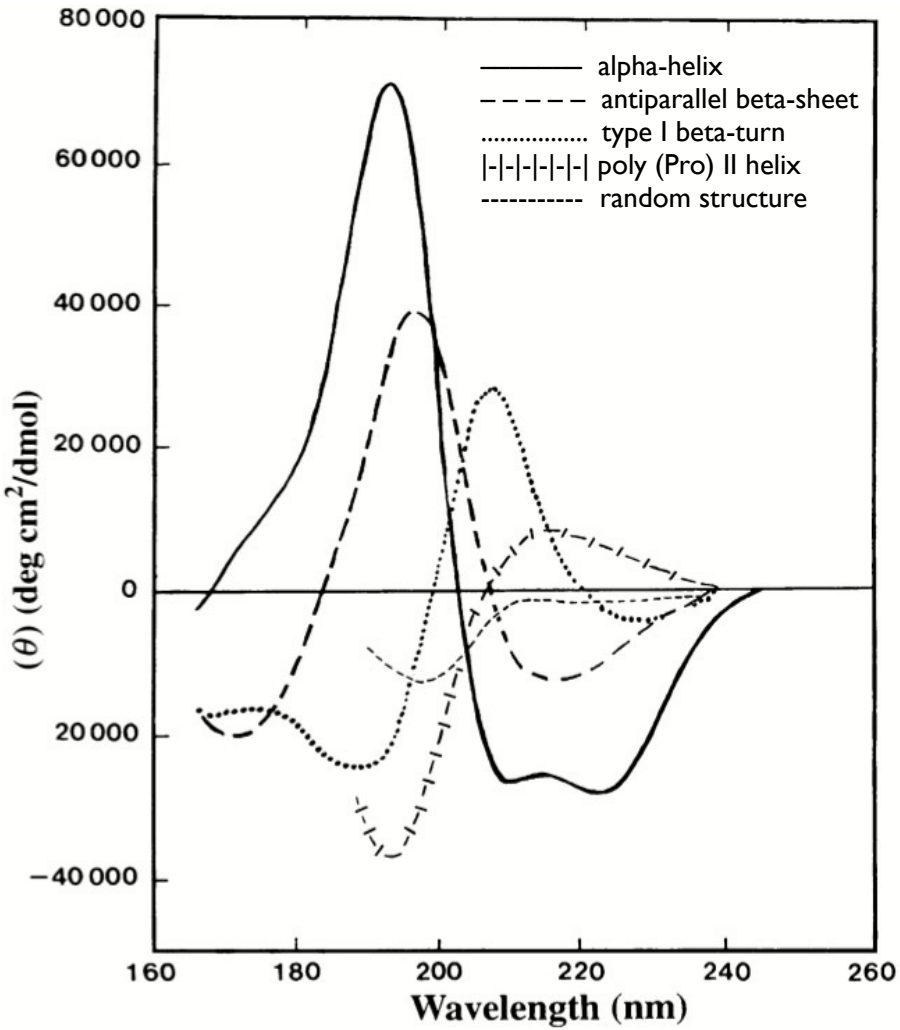


Figure 3: Illustration of graphs showing far-UV CD spectra associated with various types of secondary structure. Solid line, α-helix; long dashed line, antiparallel β-sheet; dotted line, type I β-turn; cross-dashed line, extended 3_1-helix or poly (Pro) II helix; short-dashed line, irregular structure. The data are adapted from Kelly *et al.* (12).

CD for DNA and RNA:

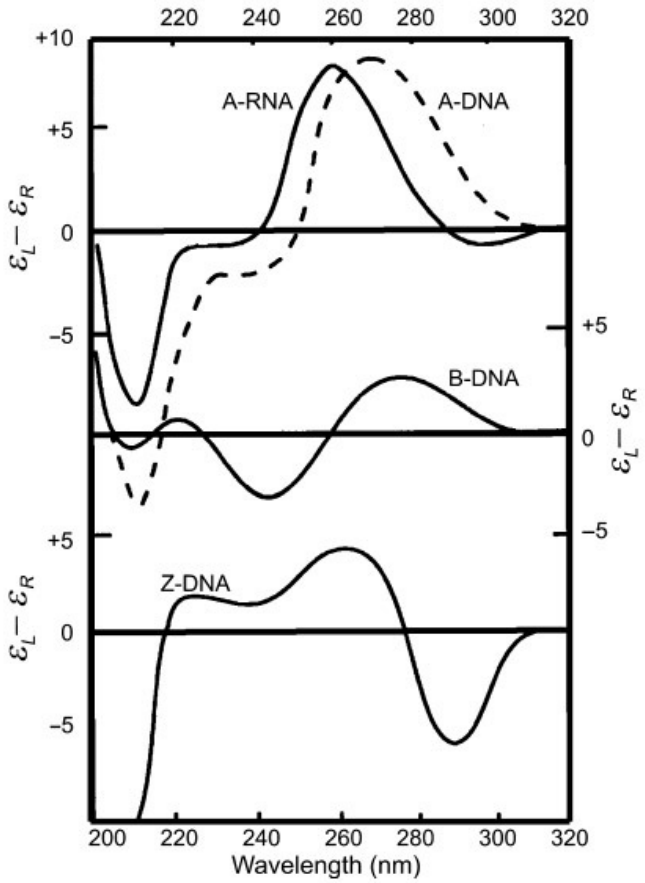
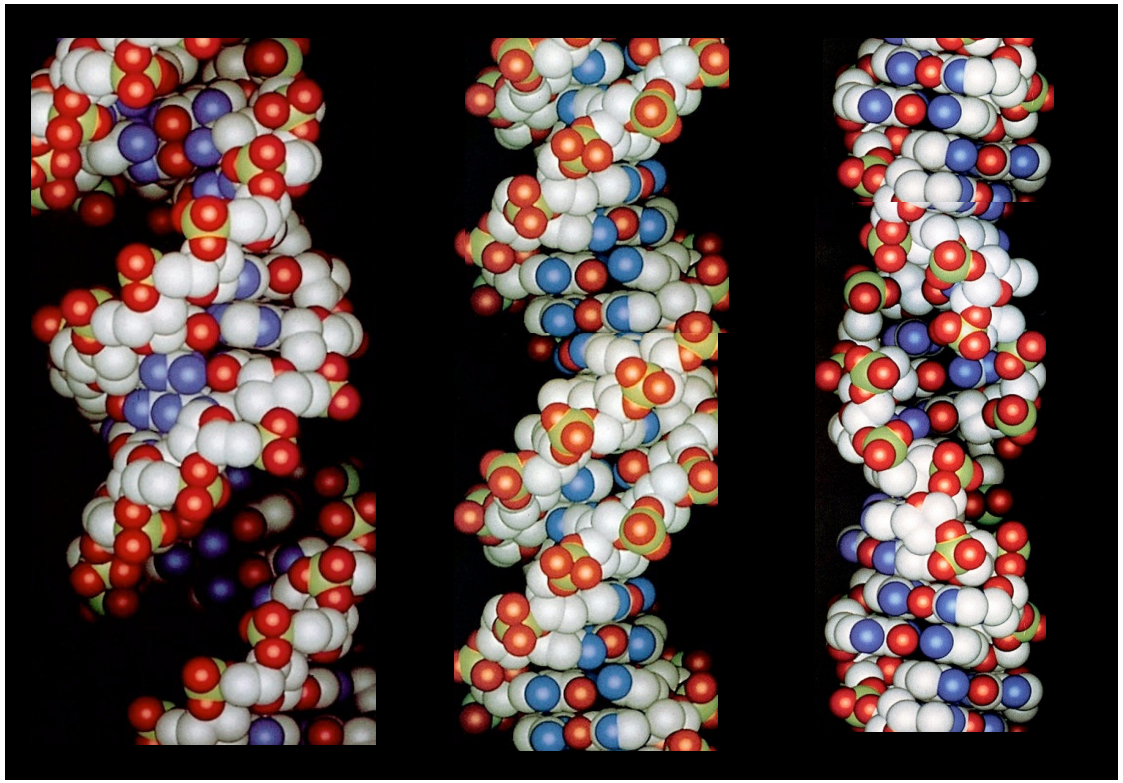


Figure 13: Circular dichroism spectra above 200 nm for right-handed A-RNA and A-DNA, right-handed B-DNA, and left-handed Z-DNA (units are per M/cm/mol of nucleotide) [The data are adapted from Bloomfield *et al.* (59)]. The A-RNA is *Penicillium chrysogenum* fungal virus double-stranded RNA with a G + C content of 54%; it is in 0.01 M Na^+, pH 7 [The data are adapted from Gray *et al.* (61)]. The A-DNA is from *E. coli* with G + C content of 50%; it is in 80% trifluoroethanol, 0.667 M phosphate, pH 7 [The data are adapted from Sprecher *et al.* (62)]. The B-DNA is from *E. coli* DNA in 0.02 M Na^+, pH 7 [The data are adapted from Gray *et al.* (63)]. The Z-DNA is poly [d(CG) d(CG)] in 2 M NaClO, pH 7 [The data are adapted from Riazance *et al.* (64)].

DNA Forms



	A-Form	B-Form	Z-Form
Helical Sense	Right handed	Right handed	Left Handed
Diameter	~26 Å	~20 Å	~18 Å
Base pairs per helical turn	11	10.5	12
Helix rise per base pair	2.6 Å	3.4 Å	3.7 Å
Base tilt normal to helix axis	20°	6°	7°
Prevalence	Only at high Cations or dehydration	Normal form	Forms in high salts (> 2.5 M NaCl)

Circular Dichroism (CD)

Thermal Scans of
the same protein in two different buffers

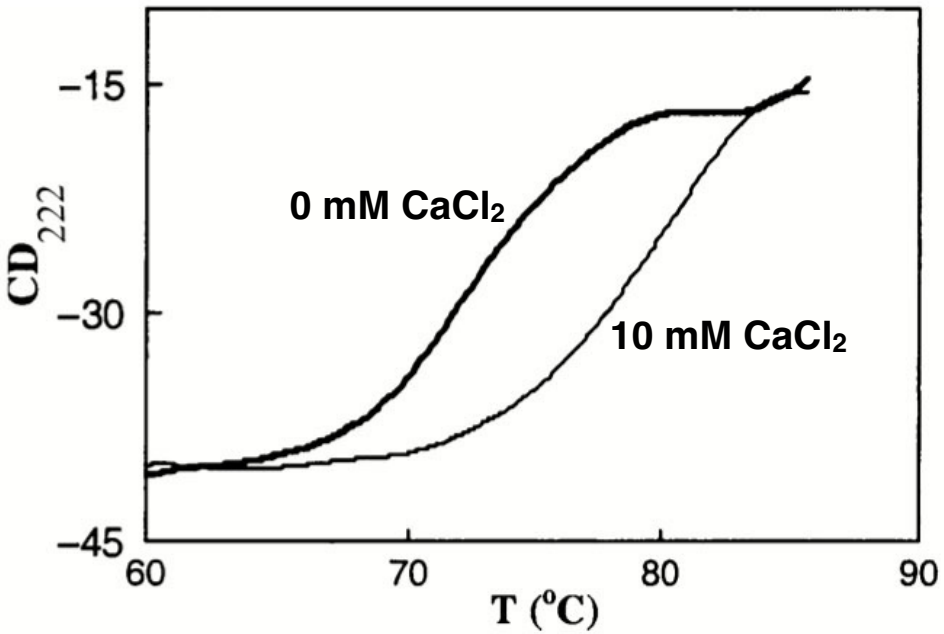


Figure 6: Schematic illustration of thermal scans done for the same protein (BAA) in two different buffers. CD at 222 nm exhibited by BAA at various temperatures in Tris buffer in (thick line) absence and (thin line) presence of 10 mM $CaCl_2$. In the absence and presence of Ca^{2+}, the Tm values of BAA were 71.7 and 80 °C, respectively. Data are adapted from Hassan Sajedi *et al.* (19).

Thermal Scans of
two different mutant forms of a protein

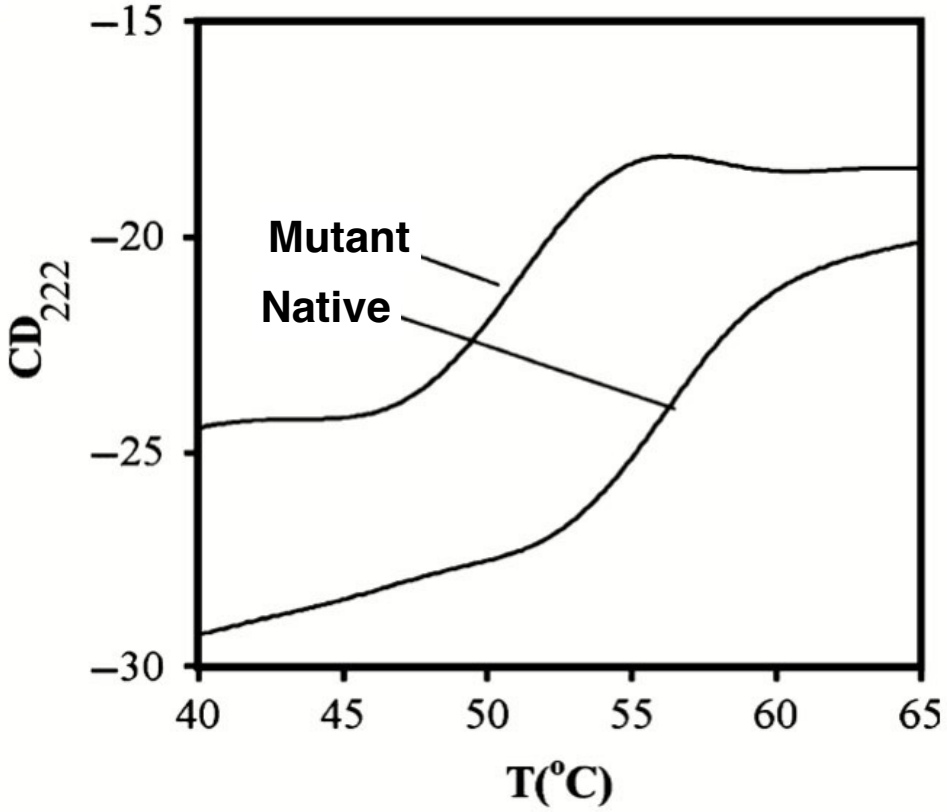
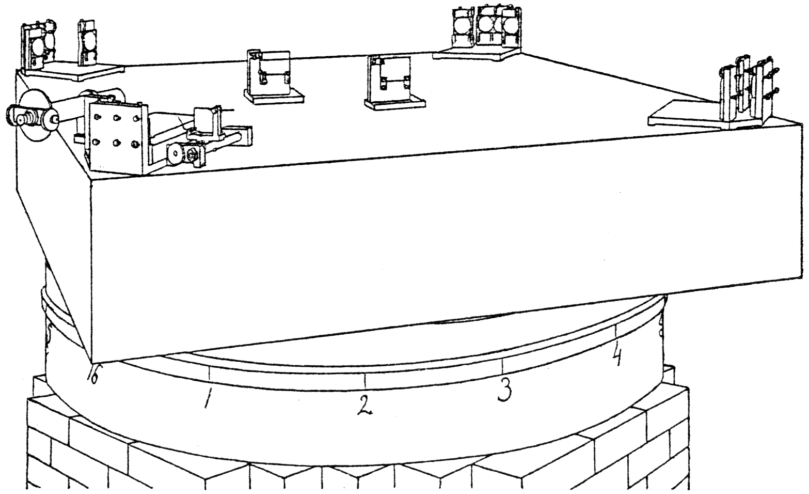


Figure 7: Melting profiles of native (N) and mutant (M) luciferase. Spectra were taken at 25–70 °C by far-UV CD in phosphate buffer (0.95 M, pH 7.0). Data are adapted from Riahi Madvar *et al.* (21).

Michelson Moorley Experiment

Albert Michelson and Edward Moorley
(1887)



Michelson and Morley's interferometric setup,
mounted on a stone slab and floating in a pool of mercury

Earth rotation around
sun at 30 km/s

This is one of the most famous
"failed experiments".
It proved the non-existence
of an aether.

FTIR

Fourier Transform Infrared Spectrometry

Michelson Interferometer

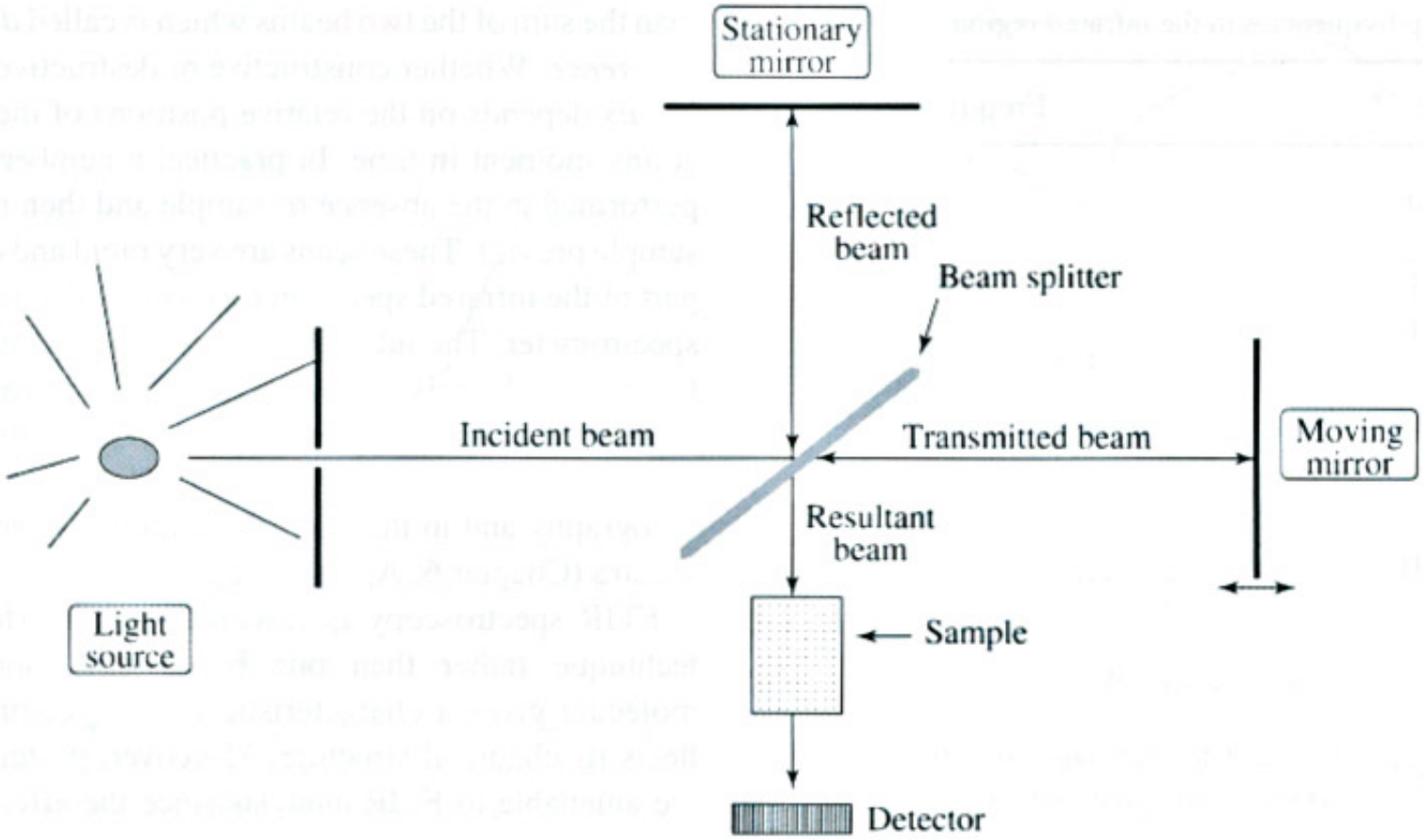


Figure 3.43. The Michelson interferometer as used in FTIR spectroscopy. The beam splitter divides an incident beam into a transmitted and reflected beam, respectively. These are reflected by a stationary mirror and a moving mirror, respectively. The reflected beams are recombined at the beam splitter and the resultant beam passes through the sample. The reflected and transmitted beams can interfere with each other (Figure 3.44) depending on the relative positions of the stationary and moving mirrors. The resultant beams detected are analysed by the Fourier Transform (Appendix 2) to generate an FTIR spectrum which is characteristic of the sample.

FTIR

Fourier Transform Infrared Spectrometry

Phase-shifted beams can extinguish each other through destructive interference.

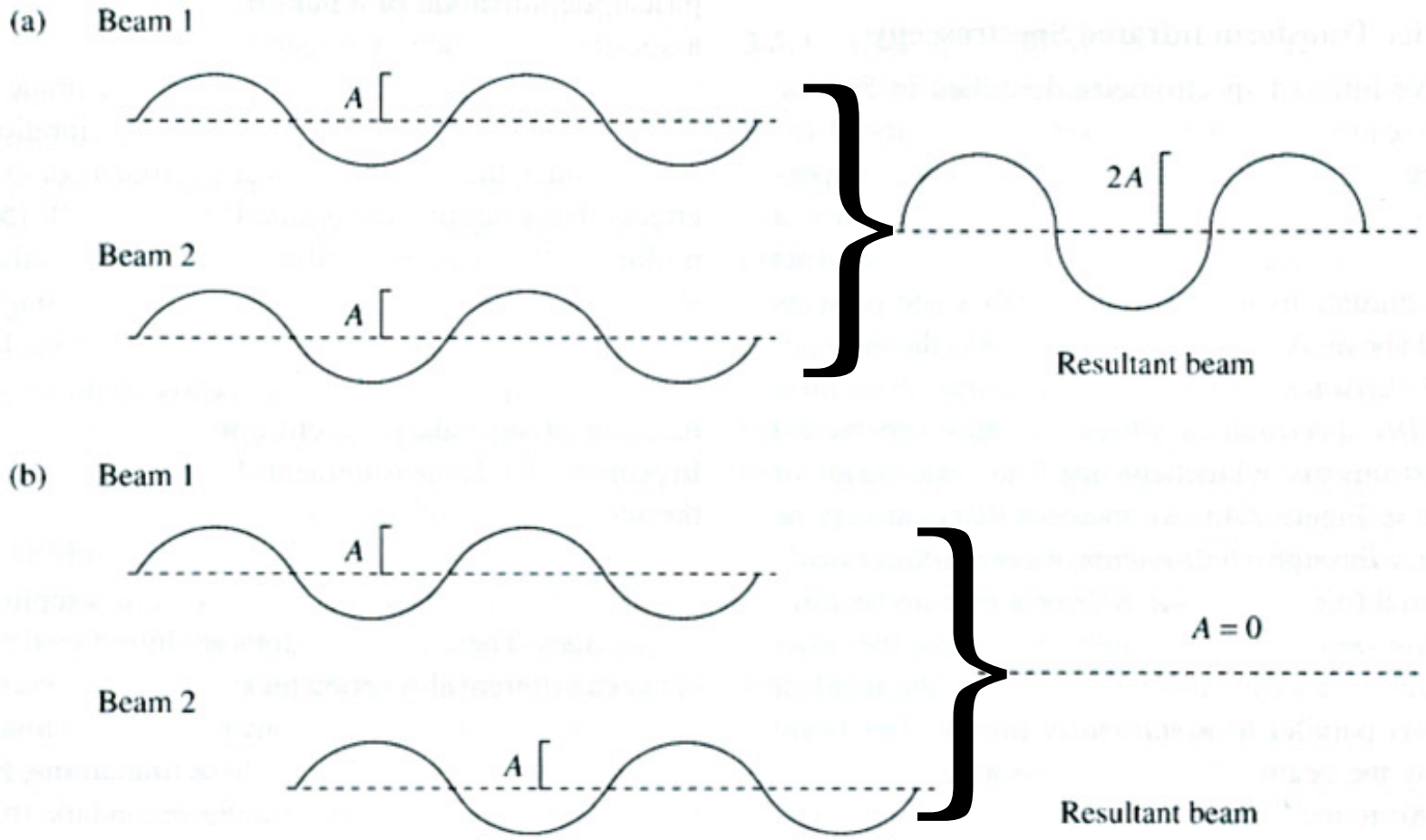
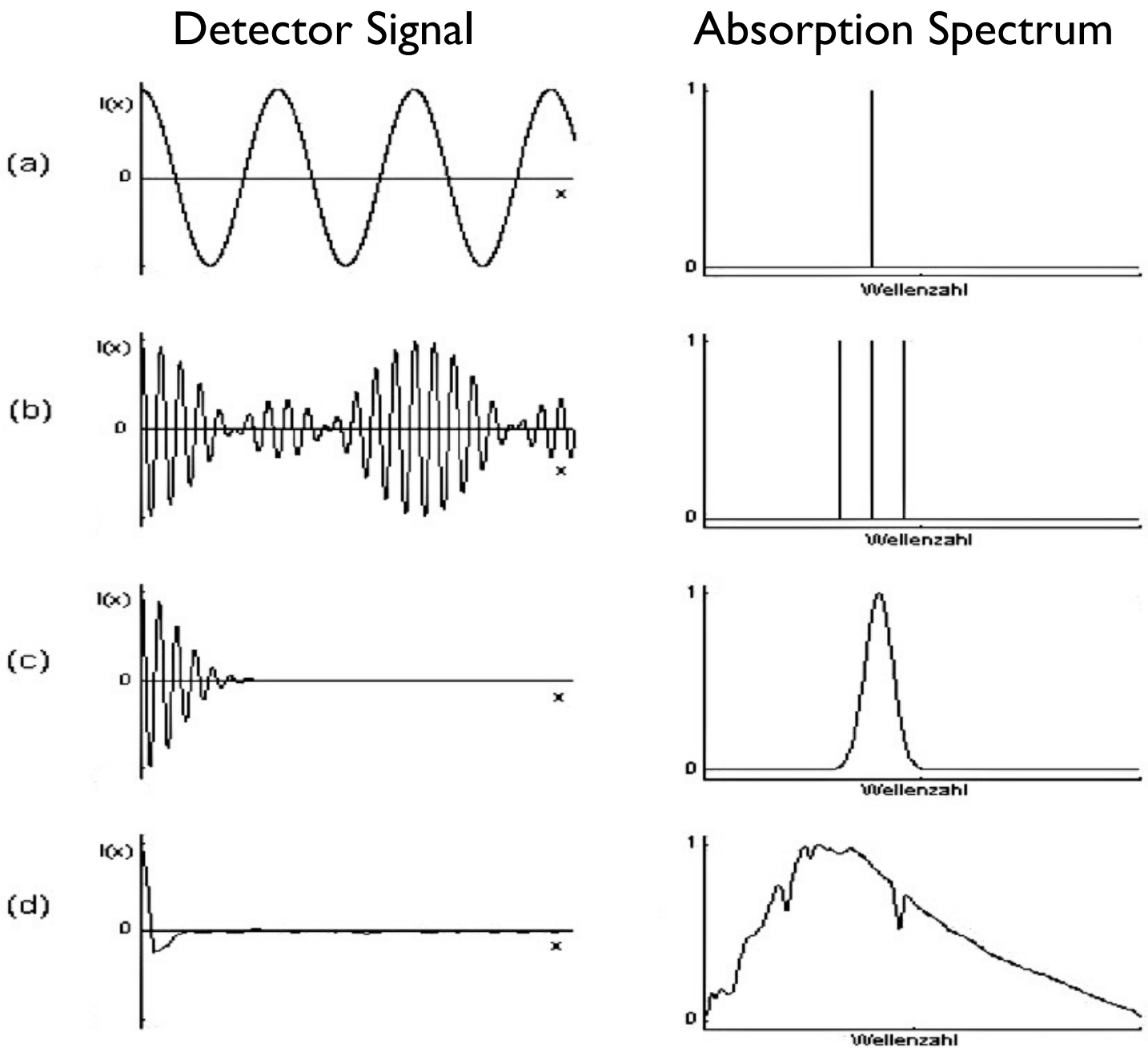


Figure 3.44. Interference between waves. (a) Constructive interference. If superimposed wave beams (of equal amplitude A) are in phase, they reinforce each other such that the resultant beam has amplitude equal to the sum of that of the superimposed waves (i.e. 2A). (b) Destructive interference. If superimposed beams are 180° out of phase, they weaken each other such that the resultant beam has amplitude of 0. Where the beams are less than 180° out of phase, the resultant beam would have amplitude between 0 and 2A.

FTIR

The recorded Interferogram is translated into the Spectrum via the Fourier Transformation.



FTIR

- White light is passed through the Michelson Interferometer.
- The mirror in the interferometer moves from one end to the other.
- This causes the different wavelengths of the white light to flicker with a different frequency: Blue light is blinking faster than red light.
- The white light (composed of different wavelengths that now blink each with a different frequency) passes through the sample, and the total light signal is recorded over time.
- The sample absorbs different wavelengths (i.e., differently blinking frequencies) differently.
- The recorded signal (light intensity over time) is Fourier transformed, which results in the IR absorption spectrum (light intensity over light frequency (wave numbers)). All wavelengths are measured at the same time.
- Since all light frequencies pass through the sample at the same time, recording the FTIR spectrum is very fast.

FTIR

Application: Characterization of samples by comparing FTIR spectra with known spectra.
Here: Samples taken from a Roman sarcophagus.

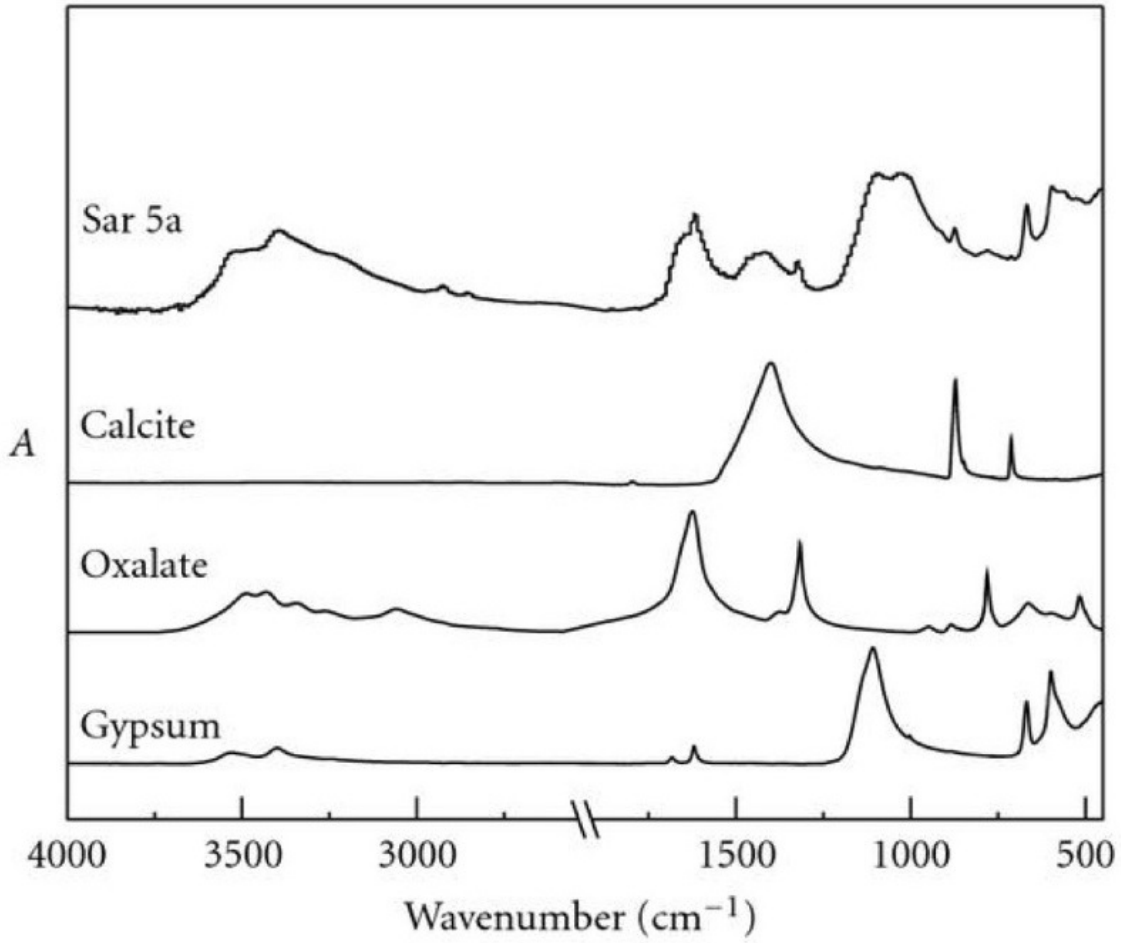


Figure 2: FTIR spectra of samples SG5, Sar 5a reference spectra (taken from ATR-FTIR library) of wax, calcite, oxalate, and gypsum are also reported.

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The Use of FTIR and Micro-FTIR Spectroscopy: An Example of Application to Cultural Heritage

Raman Spectroscopy

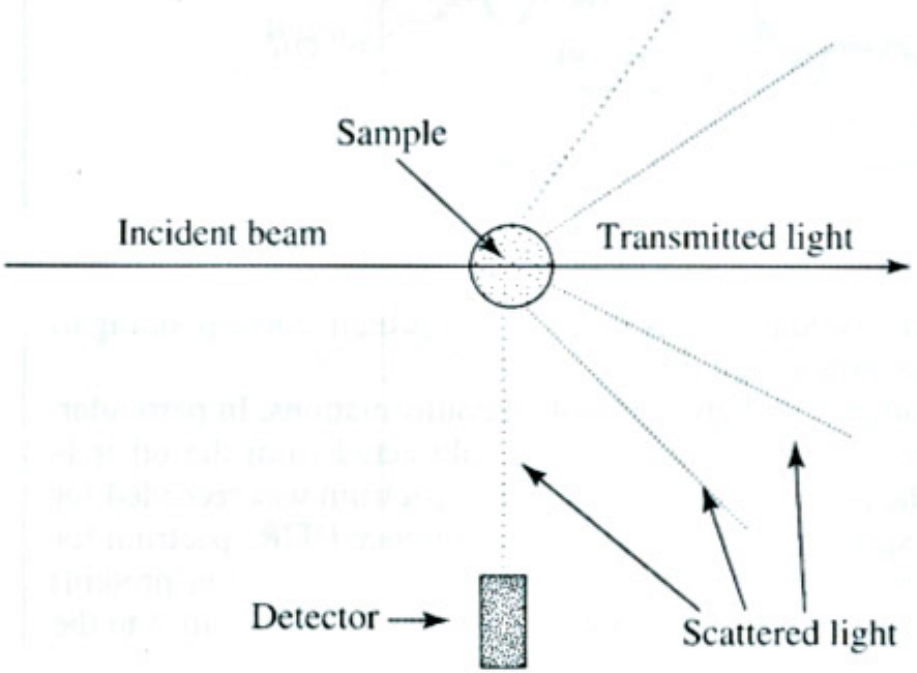


Figure 3.45. Light scattering. Incident light passes through sample solution (solid arrow). A proportion of this light is also scattered in all directions (dashed lines). This scattered light is mostly of the same frequency as the incident beam (Rayleigh scattering). However, occasionally the scattered light has a different frequency to the incident beam (Raman scattering). Measurement of these latter scattered beams is the basis of Raman spectroscopy.

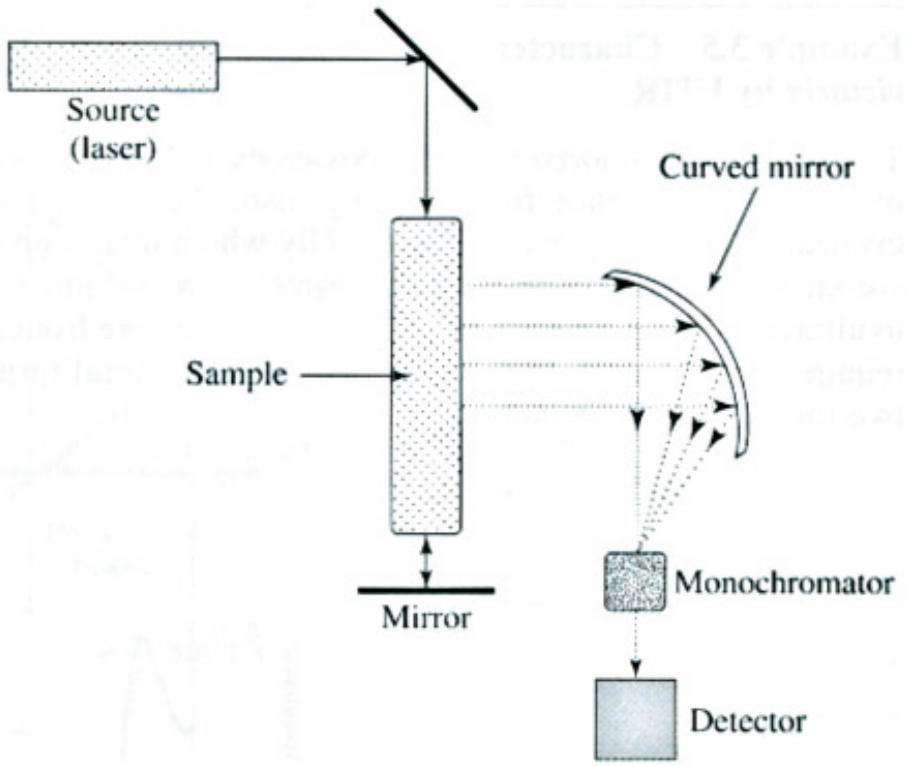
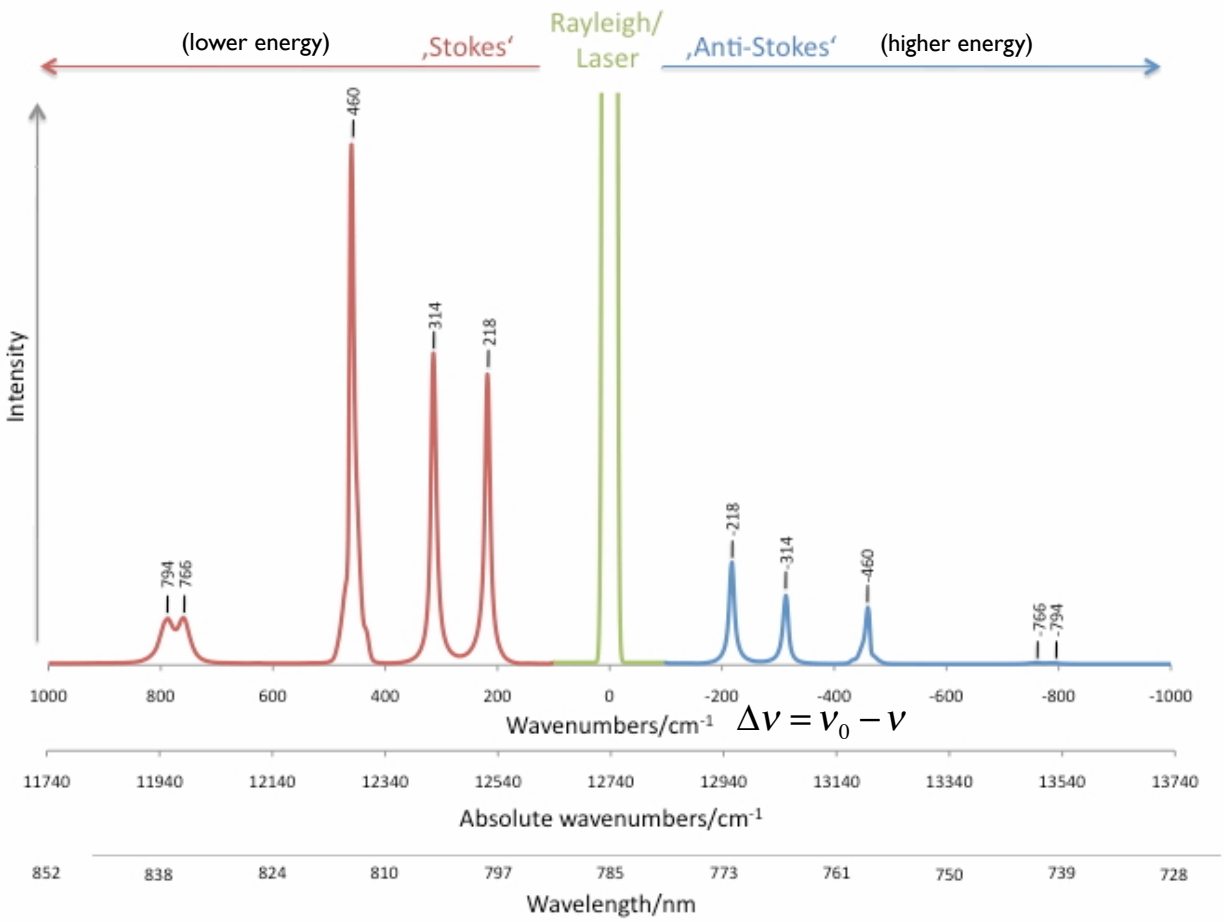
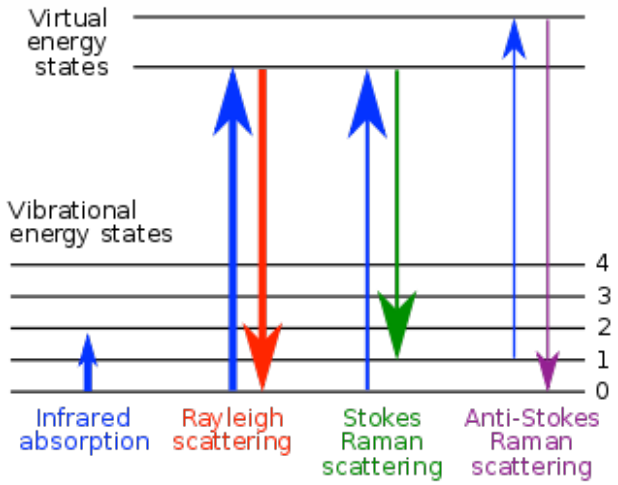


Figure 3.46. Raman spectrometer. Monochromatic light is passed through a concentrated sample. Scattered light (dashed lines) is collected with the aid of a curved mirror. This is then diffracted through a monochromator which allows the identification of frequencies due to both Rayleigh and Raman scattering. Note that the lower mirror reflects transmitted light back through the sample to double the intensity of scattered light.

Raman Spectroscopy





- Sometimes, light is *scattered* at different energies than the incident light beam.
- This effect is so rare that a strong laser light source and a collecting optic is needed to capture the few cases.
- Needs highly concentrated samples (20 mg/ml) (hopefully, the sample doesn't aggregate...)
- Rayleigh line: $\Delta\nu = 0$
- Stokes lines: Lower energy than incident light.
- Anti-Stokes lines: Higher Energy than incident light.
- Anti-Stokes is even less frequent than Stokes.
- Stokes and Anti-Stokes lines show the characteristic vibrational spectrum of the sample.
- Raman spectroscopy can be done in solution (water), on fibers (dry), or on 3D crystals (semi-dry).
- Infrared spectroscopy also shows the vibrational spectrum of a sample, but infrared spectroscopy cannot be done in water, since water absorbs IR light.