Instrumental analysis
Spectrochemistry I.
molecular spectroscopy

Spectroscopy - principles

- Analytical methods deal with the effect of electromagnetic radiation (or the energy of the EM radiation !) on the materials
- As the material absorbs energy: absorbance
- As the material emittes (release) energy: emission
- Widely used methods, while they are selective and sensitive.

The light

- Electromagnetic radiation in the visible wavelenght range called *light*.

- Electronic and magnetic vibrations can be explained by vectors perpendicular to each other and to the propagation.

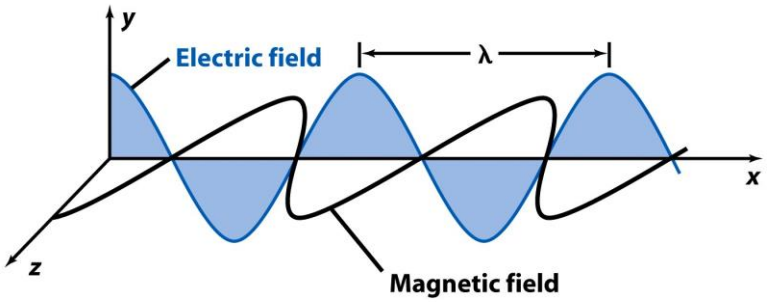


Figure 18-1
Quantitative Chemical Analysis, Seventh Edition

- Have features as wave and as particles also:
  - as particles we are talking about a *photon*
  - as wave it is a *wave* with periodoc changes in time and space.

The light

- A Planck-relation – photon's energy and wavelenght

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

  - E – photon's energy
  - h – Planck-constant (6.62 ×10^{-34} Js)
  - ν (nu) – photon's frequency
  - c – photon's velocity (in vacuum ~ 300.000 km/s)
  - λ (lambda) – photon's wavelengt
  - σ (sigma) – photon's wavenumber ($\sigma = 1/\lambda$)

- Light's intentsity and power:

$$I = \frac{P}{A} = \frac{\Phi E}{A}$$

  - P – radiation's power
  - E – radiation's energy
  - Φ (fi) – number of photons reacing an 'A' surface during a time unit
  - I – radiation's intensity (energy of photons reaching a surface during a time unit)

Light-sample interference

- Irradiated light intensity (I_0) can be divided into three component:

$$I_0 = I_A + I_T + I_R$$

  - I_A – absorbed light intensity
  - I_R – reflected light intensity
  - I_T – transmitted light intensity

The absorbed (or emitted) light's **wavelengt** is **qualitative** information, its **intensity** is **quantitative** information about the sample!

Spectroscopy – qualitative analysis

Spectrometry – quantitative analysis

Light-sample interference
Quantitaitve relations

$I_0 = I_A + I_T + I_R$

transmittance $T = \frac{I_T}{I_0}$

absorption $A_T = \frac{I_A}{I_0} = \frac{I_0 - I_T}{I_0} = 1 - T$

absorbance $A = \lg\frac{I_0}{I_T} = \lg\frac{1}{T}$

reflectance $R = \frac{I_R}{I_0}$

The electromagnetic spectrum

The spectrum

- Its a function, in which the **light intensity** is shows as the function of the **light energy**.

X axis: E, λ, ν, σ

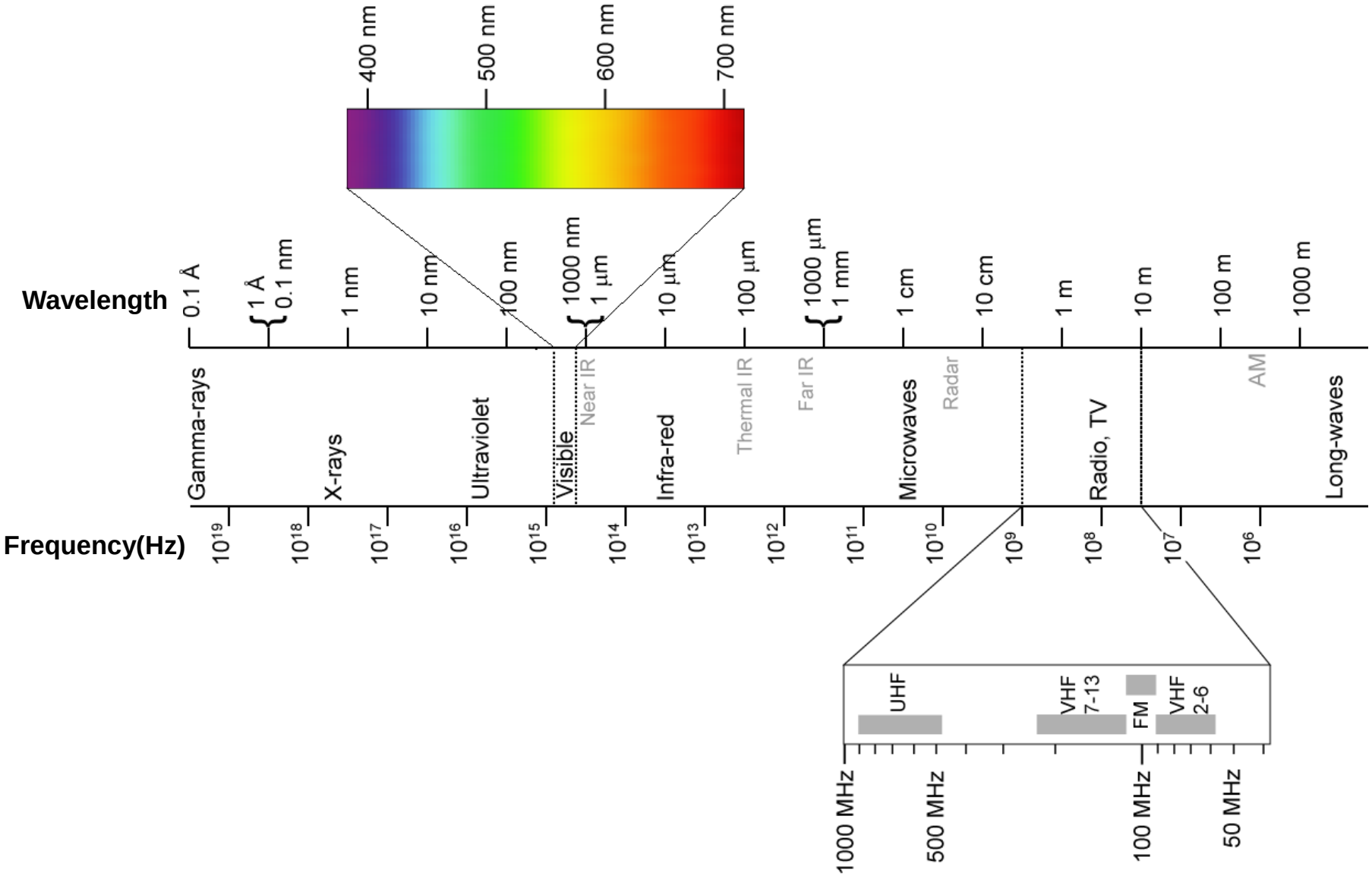
Y axis: I_A, I_E, T (transmittance), A (absorbance)

Molecular spectroscopyc methods

Molecular spectroscopy deals with the effect of known energy of electromagnetic radiation on **molecules**. In the practice the well defined transitions between energetic levels can be analyze through the **emission** of energy or **absorption** a portion of irradiated energy.

- electronexcitation spectrophotometry (UV-Vis range)
- fluorescence and phosphorescence (molecular photoluminescence)
- infrared (IR-) spectrometry
- Raman spectrometry

The electromagnetic spectrum



The effect of the electromagnetic radiation

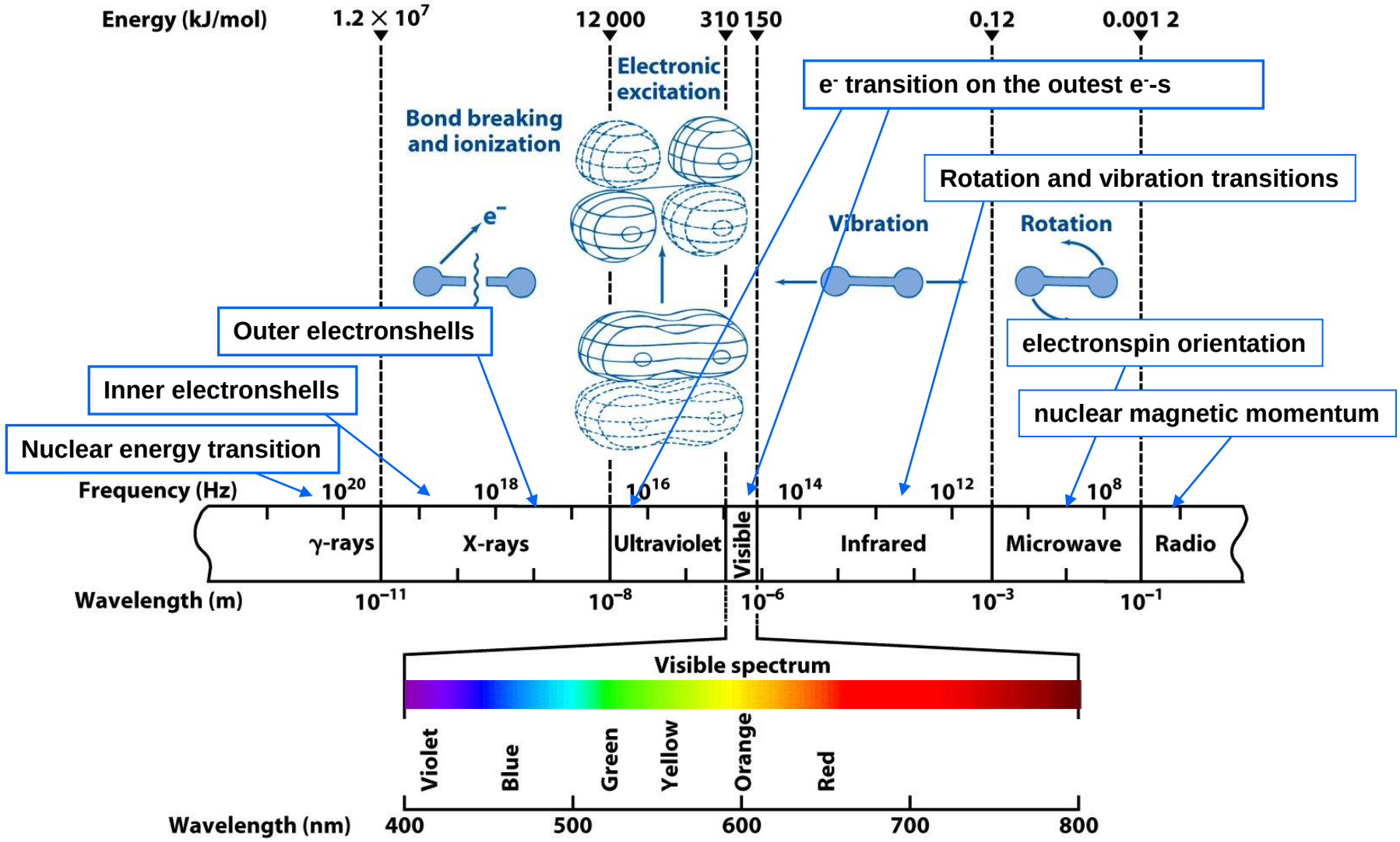


Figure 18-2
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The energy of a molecule

$$E_{total} = E_{electron} + E_{vibration} + E_{rotation}$$

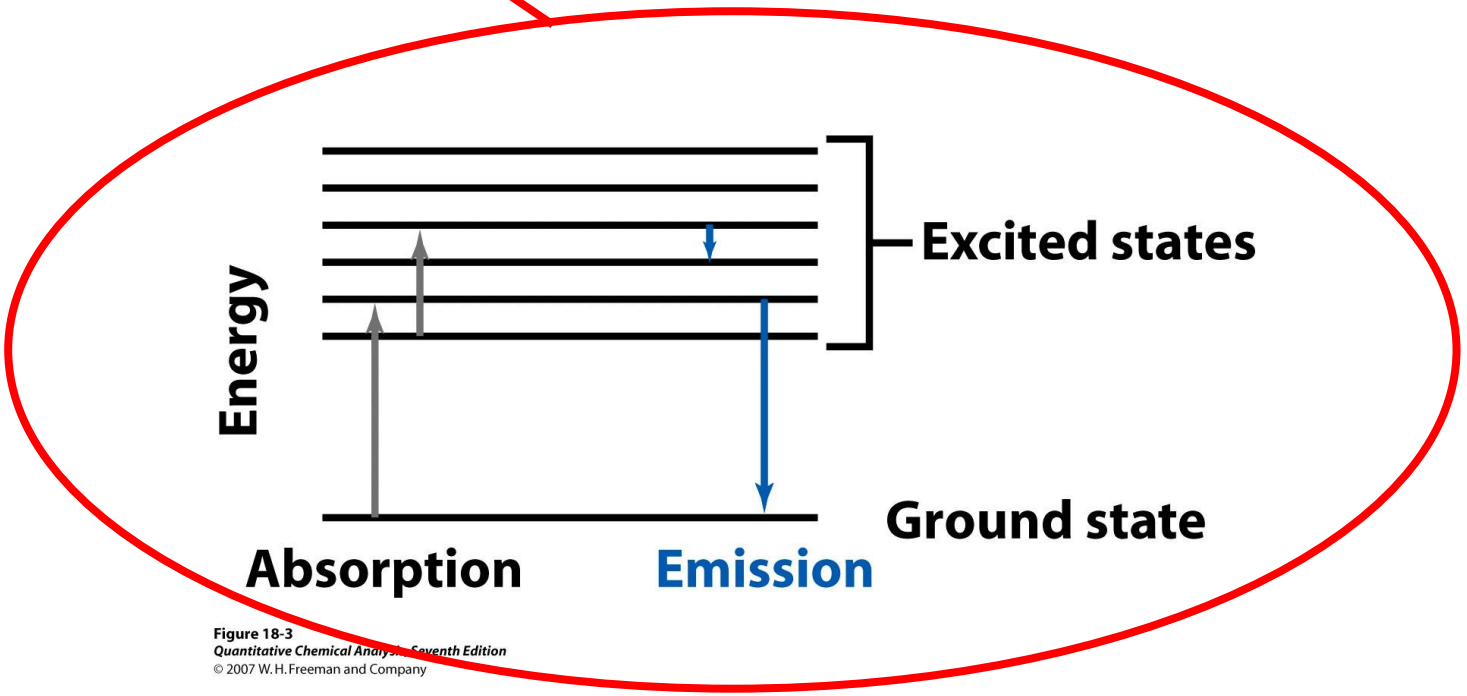

Figure 18-3
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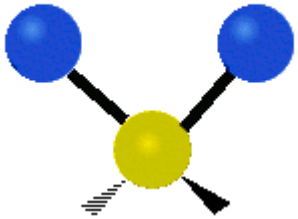
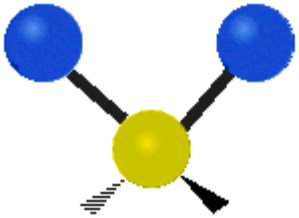
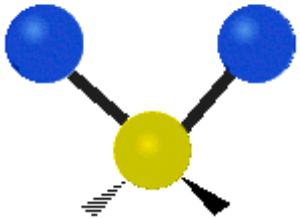
Vibrations (Infrared spectroscopy)



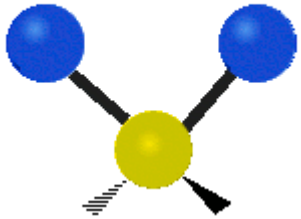
Symmetric stretching



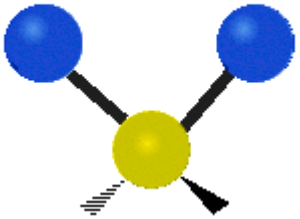
Antisymmetric stretching



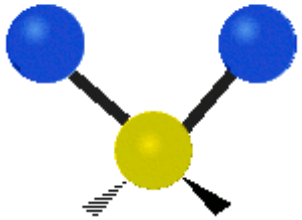
Scissoring



Rocking



Wagging



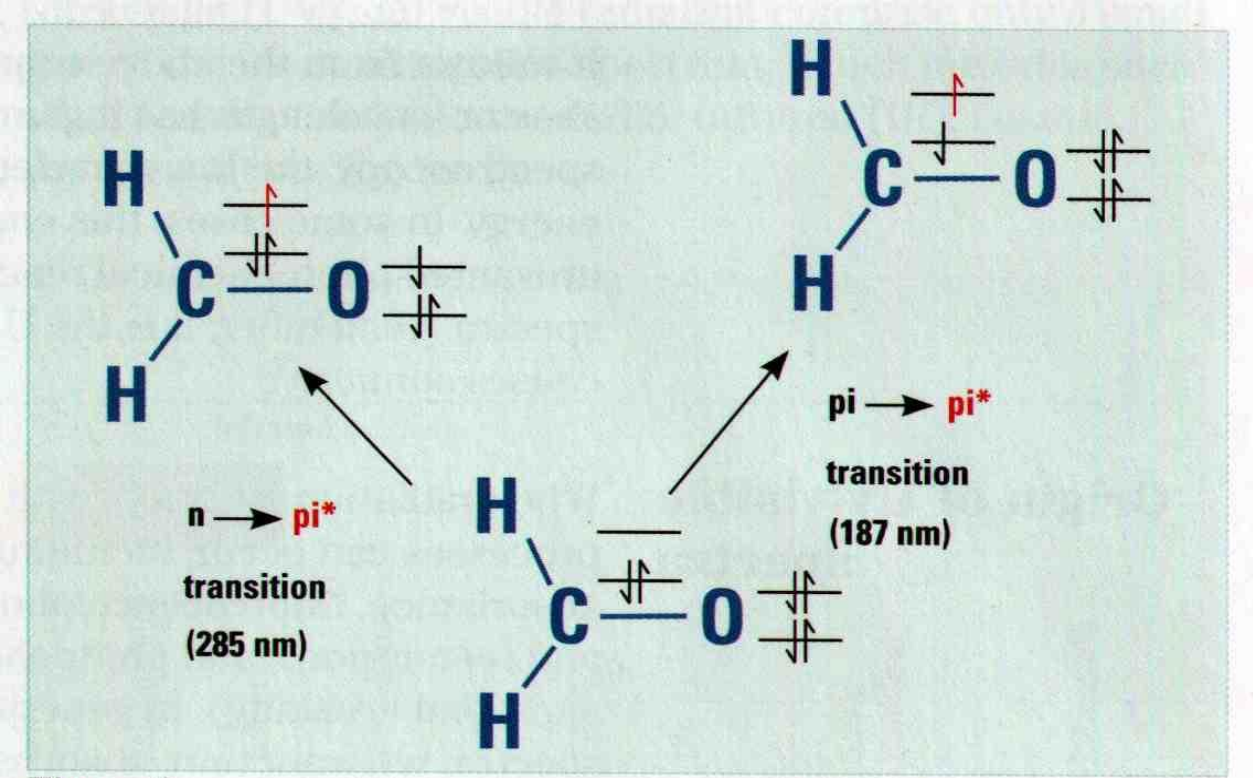
Twisting

Ultraviolet-visible (UV-Vis) spectrophotometry Principles

- Radiation in the 200-800 nm range can be partly absorbed by the sample.
- The energy of the absorbed light is exciting the binding electrons, reaching the excited state.
- As the binding is weaker, lower energy (higher wavelength) can also excite it.
- Materials absorbing light in the 380-780 nm range can see colorful for humans.

- As the material absorbs energy: absorbance
- As the material emittes (release) energy: emission

The molecular spectrum
Electrontransition in a molecule (formaldehyde)



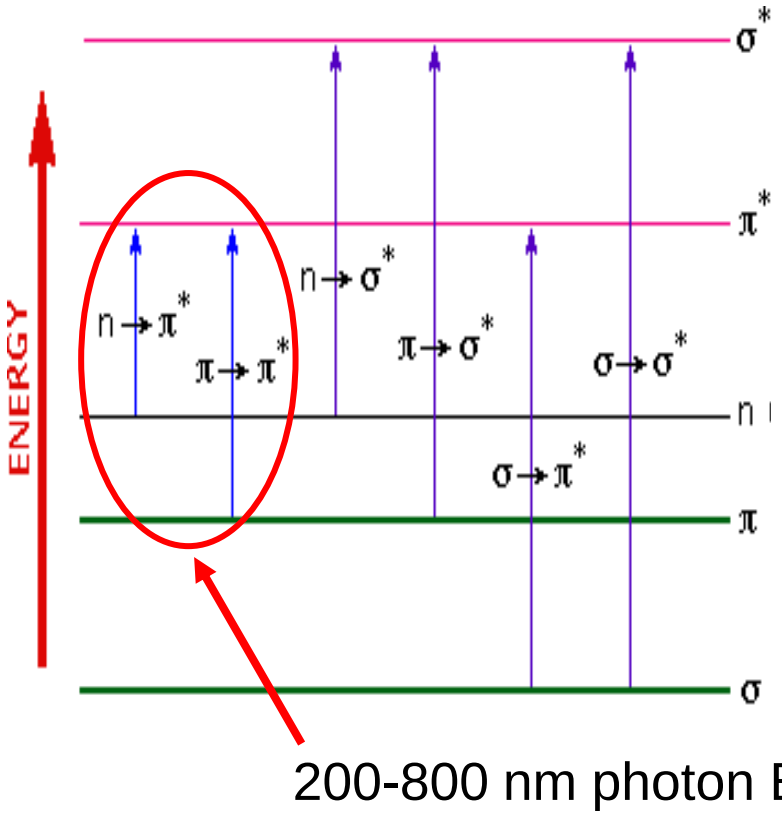
- More, **quantized transition**, e.g.: between bonding and antibonding orbitals
- **By exciting** the electron jumps from an **occupied orbit** to an **unoccupied** one.

Electronexcitation spectrophotometry (UV-Vis)

- Generation of molecules: atomic orbitals → molecular orbitals
- Types of molecular orbitals:
  - **binding** ($E_{binding} < E_{atom}$)
  - **antibinding** ($E_{antibinding} > E_{atom}$)
  - **nonbinding** (n)
- Binding and antibinding orbitals can be also σ and π **orbitals**
  - **Binding orbitals**: σ and π
  - **Antibinding orbitals**: σ* and π*
- Allowed and forbidden transitions
  - Allowed transitions:

    σ → σ*, π → π*, n → σ*, n → π*

Relative energy levels of molecular orbitals

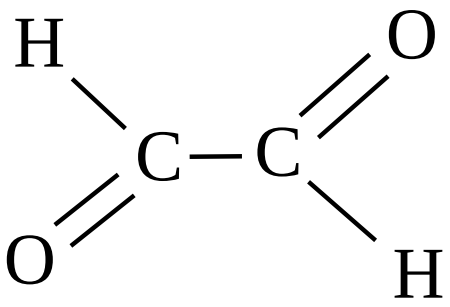


- n → π*: compounds with heteroatoms, aromatic or unsaturated
- π→ π*: unsaturated compounds, e.g. conjugated system (carotene)
  (UV-Vis light)
- n → σ*: saturated compounds with heteroatoms
- σ→ σ*: saturated compounds ,
  greatest ΔE (far UV: <150 nm)

- Other principles for metal ions and complexes!

Excitation of electrons

- Delocalised electron system can be easily excite (π→ π*) → colorful materials
  - **glyoxal** – yellow fluid
  - „simplest colorful organic compound„
  - Electrons are delocalising on four atoms (...O=C-C=O...)
  - These electrons can be easily (=with low energy=Vis range light) excite
- Role of **chromophore groups**
  - In UV-Vis range such groups, which has:
    - π electrons
    - heteroatoms with nonbinding electrons



Absoprtion max. of chromophore groups

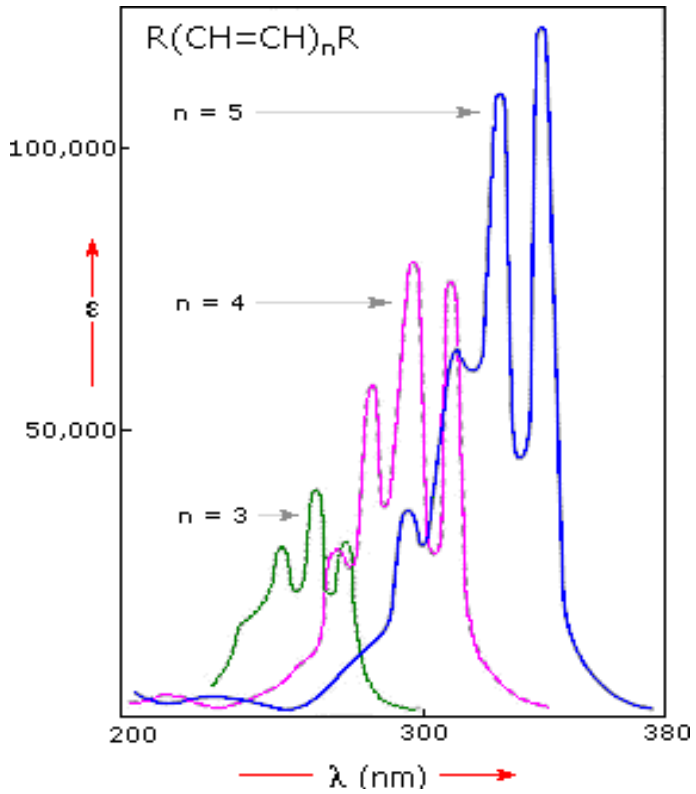
chromophore	example	excitation	λ_{max}, nm	ε	solvent
C=C	ethylene	$\pi \rightarrow \pi^*$	171	15,000	hexane
C≡C	1-hexine	$\pi \rightarrow \pi^*$	180	10,000	hexane
C=O	Acet-aldehyde	$n \rightarrow \pi^*$ $\pi \rightarrow \pi^*$	290 180	15 10,000	hexane hexane
N=O	nitro-methane	$n \rightarrow \pi^*$ $\pi \rightarrow \pi^*$	275 200	17 5,000	ethanol ethanol
C-X X=Br X=I	methyl-bromide, methyil-iodide	$n \rightarrow \sigma^*$ $n \rightarrow \sigma^*$	205 255	200 360	hexane hexane

Absorption max. of chromophore groups

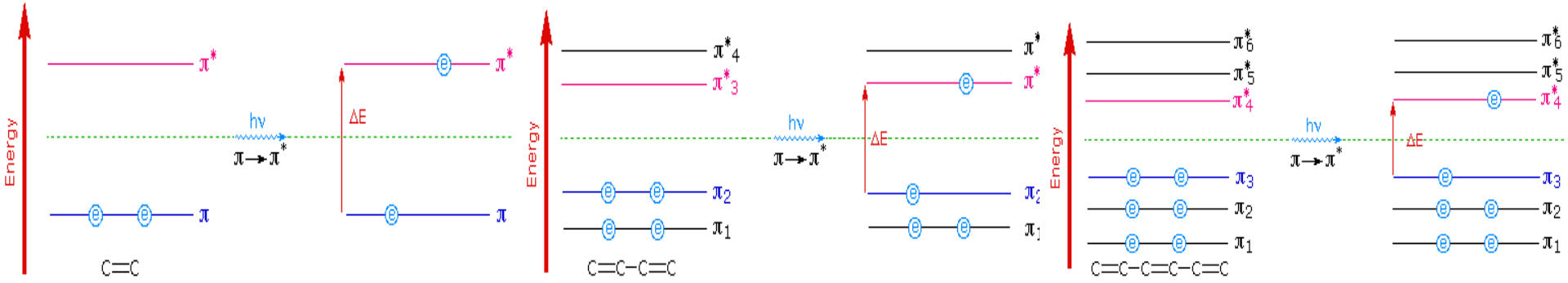
Chromophore	Formula	Example	λ_{max} (nm)
Carbonyl (ketone)	RR'C=O	Acetone	271
Carbonyl (aldehyde)	RHC=O	Acetaldehyde	293
Carboxyl	RCOOH	Acetic acid	204
Amide	$RCONH_2$	Acetamide	208
Ethylene	RCH=CHR	Ethylene	193
Acetylene	RC=CR	Acetylene	173
Nitrile	RC=N	Acetonitrile	< 160
Nitro	RNO_2	Nitromethane	271

- **Chromophore group**: weakly binded π electrons, mostly also conjugated binding (longer chain, every second is double binding).
- **Auxochrome group**: group, which modifying the λ_{max} of the chromophore
  - **hypsochrome** (towards violet), **bathochrome** (towards red), **hyperchrome, hipochrome**

Effect of the conjugated system



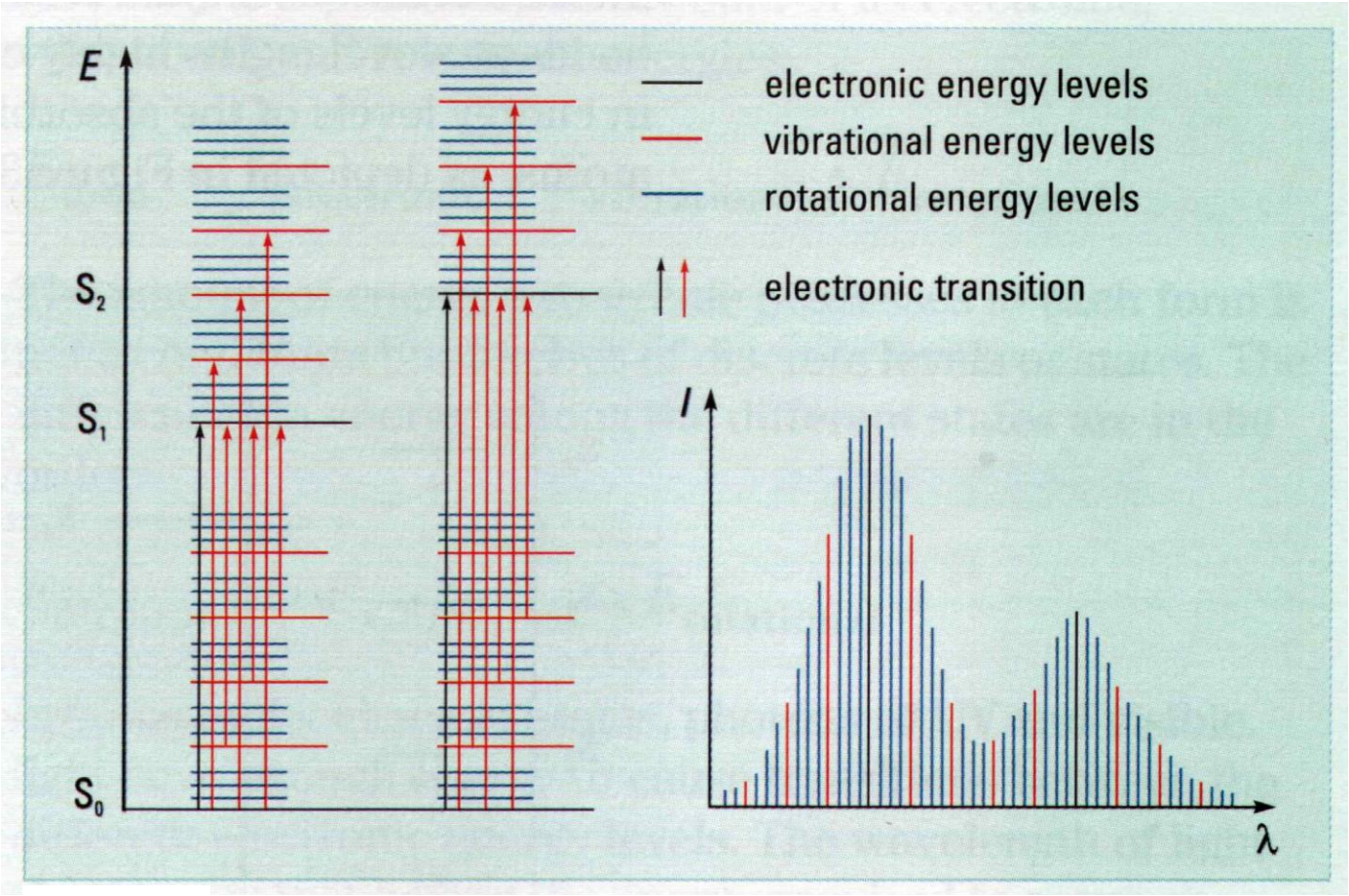
- More electrons in conjugation – stronger batochrome and hiperchrome effect
- More π⟶π* transitions, lower ΔE, lower E photon can also exciting



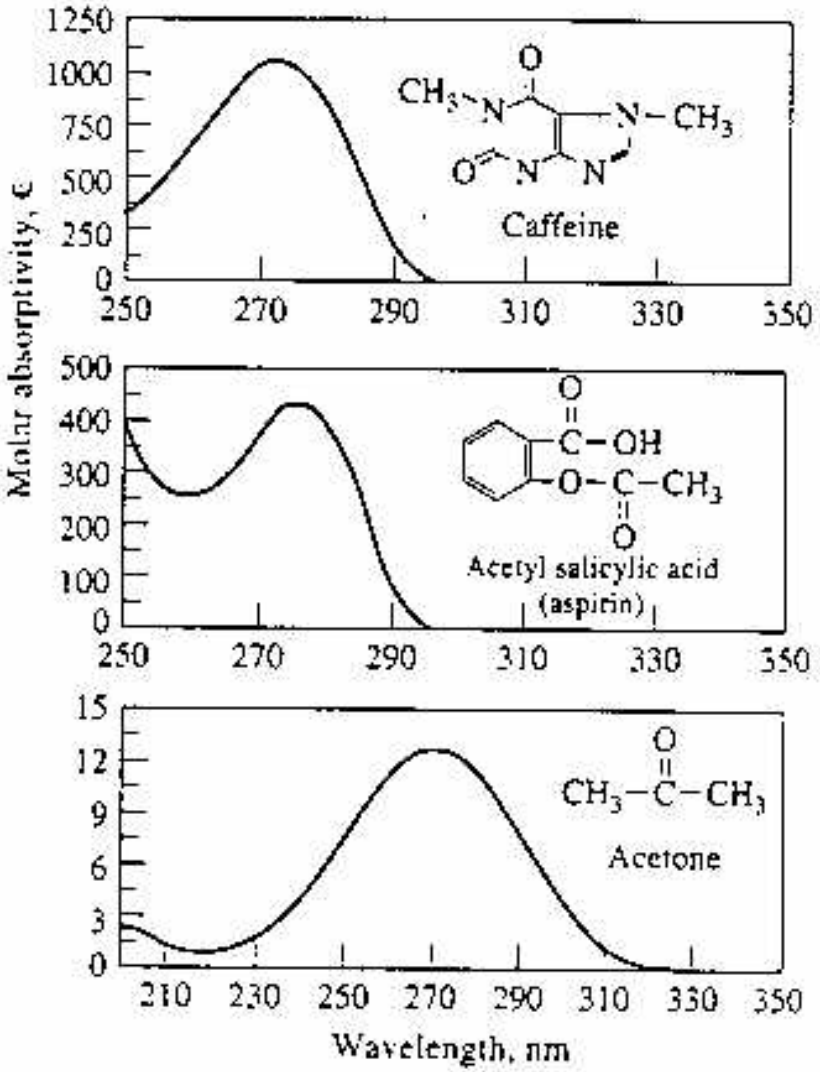
The molecular spectrum

- The molecular spectrum is the summary of the spectrum of the atoms forming the molecules
- Molecules have quantized **rotational** and **vibrational** transitions (atoms do not have)
- These are superimposed to the electrons transitions
- Single lines can not be identified
- Only the envelope curve can be measure
- The molecular spectra are **band spectrum**
- **Solvation** shell pushes the absorption band e.g. iodine is violet in organic solvent (no solvation), yellow-brown in water, ether, alcohol (solvation)
- Widely used in **quantitative analysis**

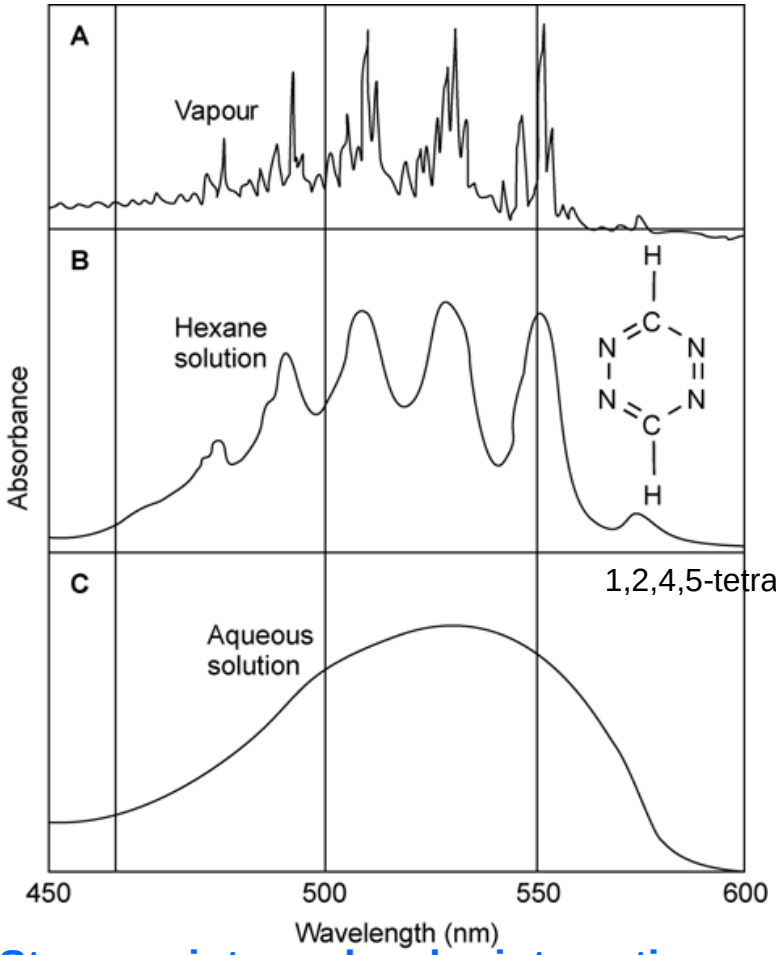
Molecular spectrum



Spectrum in UV and Vis range



- Change of state and solvent effect



1,2,4,5-tetrazine

Stronger intermolecular interactions

(Bouguer-)Lambert-Beer law
(dilute solutions)

- Dissolved ions/molecules are interacting with the photons of the light, absorbing E → light intensity decreases

$$I_T = I_0 10^{-\varepsilon \cdot l \cdot c} \Rightarrow A = \lg \frac{I_0}{I_T} = \varepsilon \cdot l \cdot c$$

A – absorbance

I_0 – incident beam's intensity

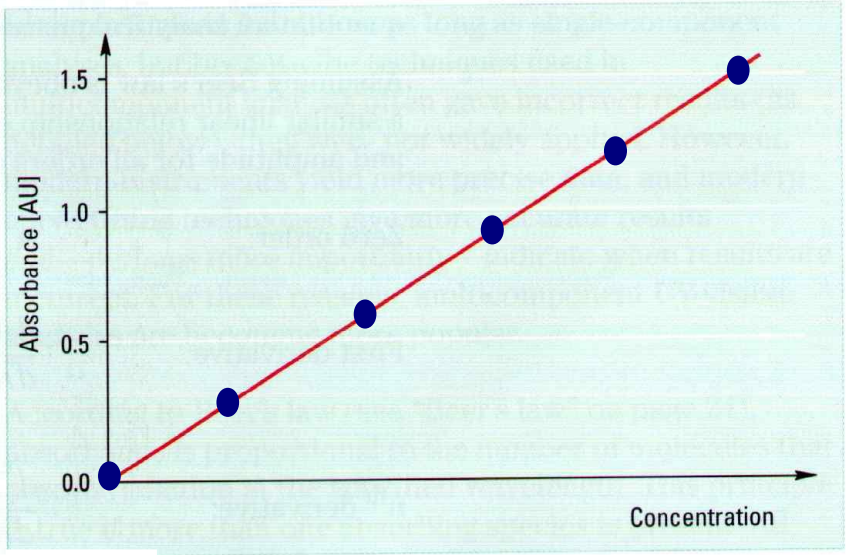
I_T – transmitted beam's intensity

ε – molar absorbance (at given λ)

l – optical path (cm)

c – molar concentration (mol/dm^3)

During measurement $I<I_0$; $A \sim c$



The Beer–Bouguer-Lambert law

The molar absorbance (ε)

- Characteristic to the material property, usually defined as a function of λ
- According to the Lambert-Beer-law it is the absorbance of the 1 M concentration solution at a given wavelength in 1 cm path-length cuvette
- The ε value depends on the **excitation probability** of the electrons
- Value is 10^3 - 10^5 dm^3/mol cm (intensive colorful organic compounds) → 10 dm^3/mol cm (metal ions solution)

Limitations of the Lambert-Beer-law

1. Only in **diluted solutions** (lower than 10^{-3} mol/dm^3) otherwise the **refraction index** (*n*) too high and influencing the results. ***ε*** need to changed to $\varepsilon' = \varepsilon n/(n^2+2)^2$

2. **Chemical reaction** (molecules dissociation, association, protonation, complex formation) of the chromophore group

3. **Changing the solvent** – solvatochrome effect see I_2 color in water (yellow), petrol (viola), acetone (brown)

4. Only for **monochromatic radiation**

5. Only in **molecular solutions**, colloids are causing light scattering.

6. Dependent on the **temperature** – higher temperature, higher energy of the electrons, easier to excite them.

Consequences of the Lambert-Beer Law

$$A = \lg \frac{I_0}{I_T} = \varepsilon \cdot l \cdot c$$

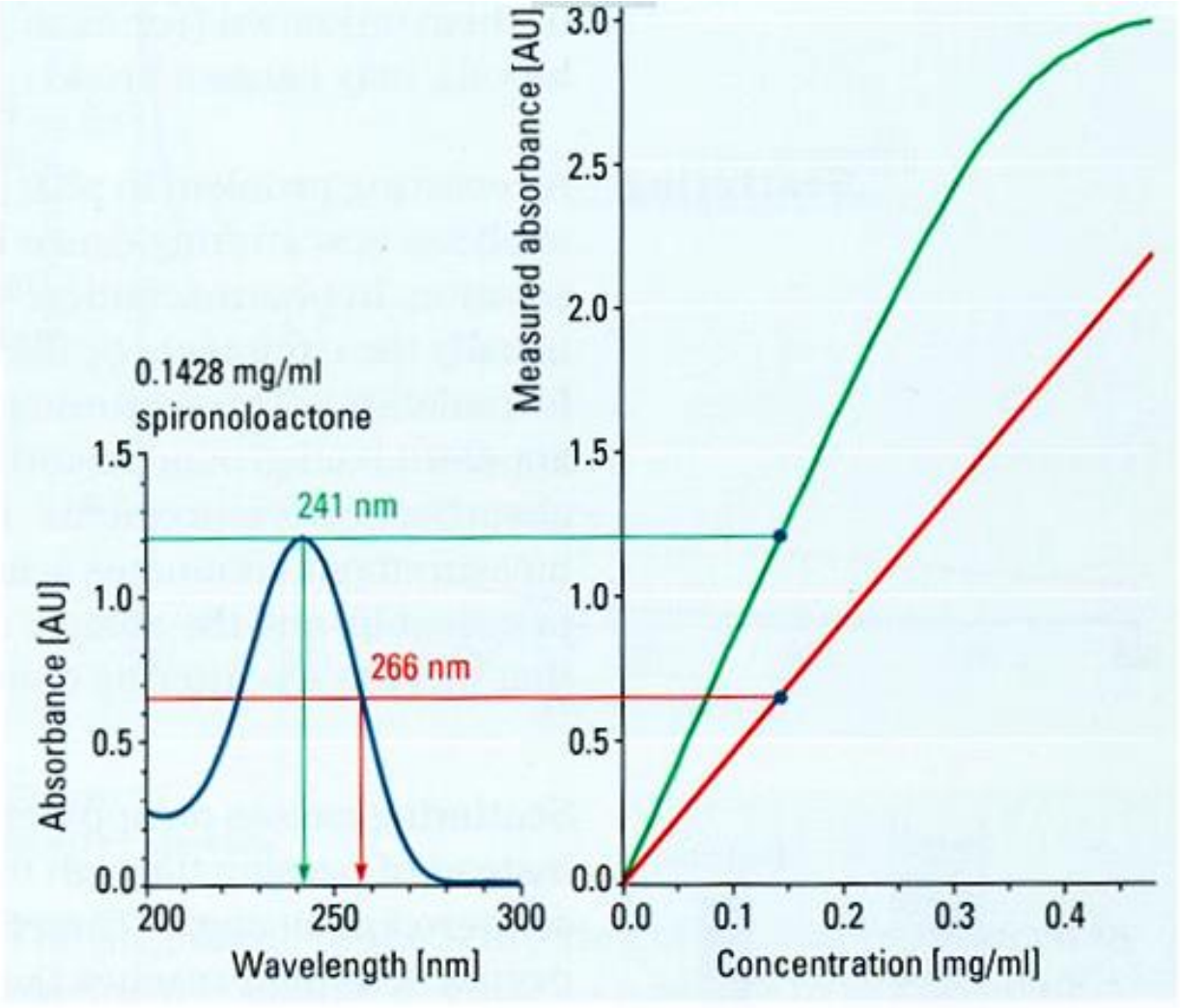
- If **A is low (<0,1),** change in I_T compared to I_0 is small → incorrect
- if **A is high (>1),** few from I_0 reach the detector → incorrect

0.02>A>1.5 ⇒ 2 orders of magnitude in conc. evaluation – not enough!

How to increase?

- Dilution of solution
- Longer cuvette
- Using reagent e.g. 0.1 M Fe^{3+} almost no color (light yellow), but reaction with SCN^- gives (red) 10^{-5} M detection limit → a lot **selective** methods are available

Spectral sensitivity



Simultaneous measurement of multicomponent system

- The absorbance is additive, the absorbance spectrum of a mixture is the sum of the spectrum of its component

  $A_{sum.} = A_1 + A_2 + ... + A_n$

- *n* components can be measured as measuring at least at *n* different wavelength, on each λ:

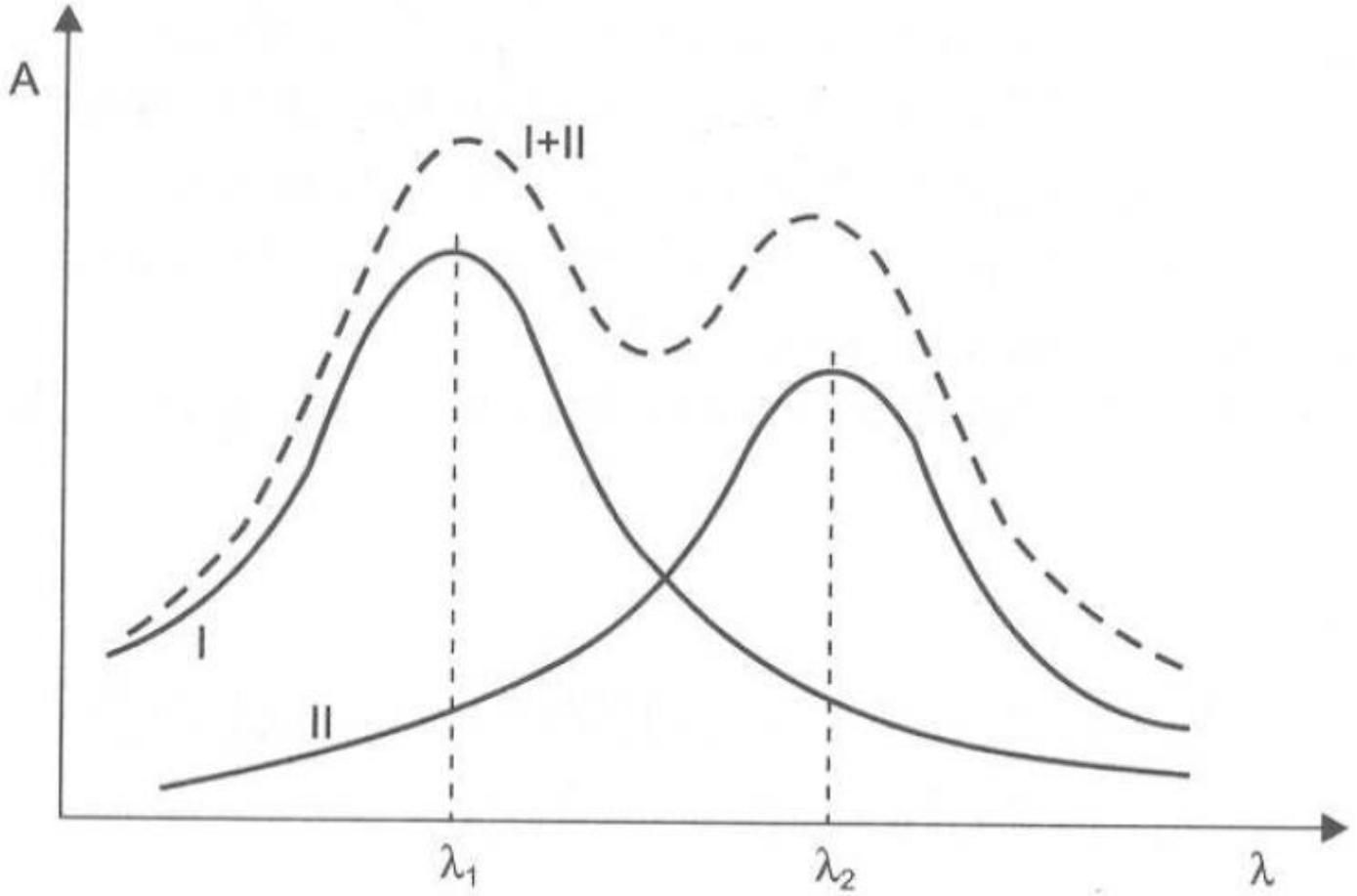
  $A_{sum.} = \varepsilon_1 c_1 l + \varepsilon_2 c_2 l + ...+ \varepsilon_n c_n l$

- From the spectra of known concentration of pure component ε_1, ε_2 can be obtained at any wavelength
- Thus the adsorption of the mixture can be calculated by using equation systems. For two components (A, B materials and λ_1, λ_2 wavelengths):

  $A_{sum\ (\lambda 1)} = \varepsilon_{A(\lambda 1)} c_A l + \varepsilon_{B(\lambda 1)} c_B l$

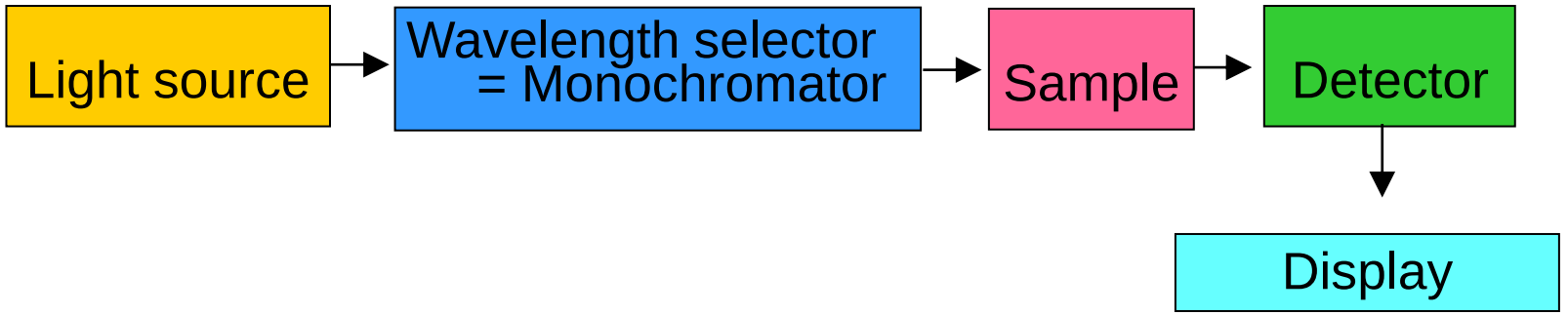
  $A_{sum\ (\lambda 2)} = \varepsilon_{A(\lambda 2)} c_A l + \varepsilon_{B(\lambda 2)} c_B l$

Simultaneous measurement of multicomponent system

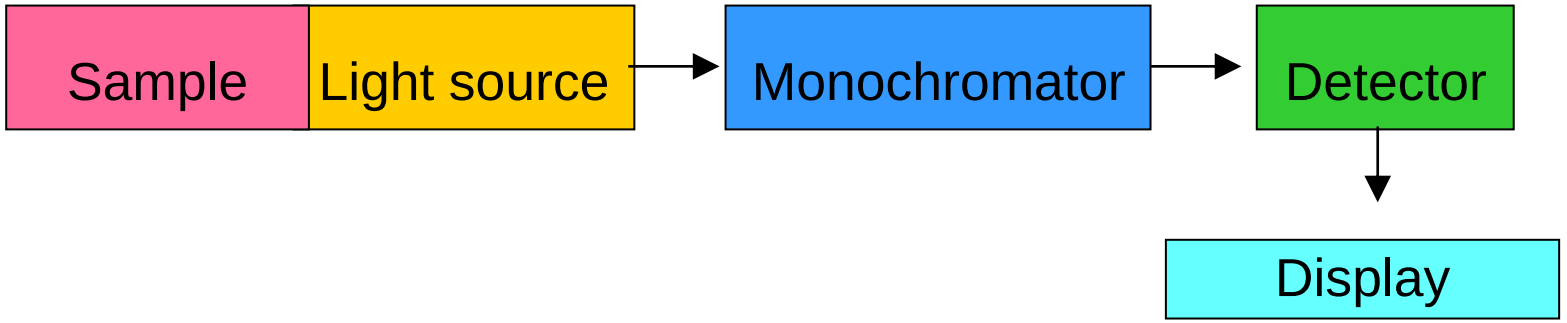


Set-up of a spectrometer

- **Blockdiagram for measuring absorption**



- **Blockdiagram for measuring emission**



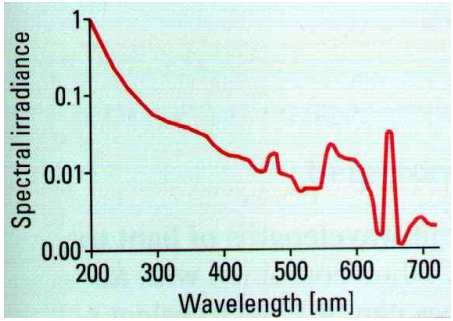
Light sources

- Emission spectroscopy
  - Sample itself is the source of the light

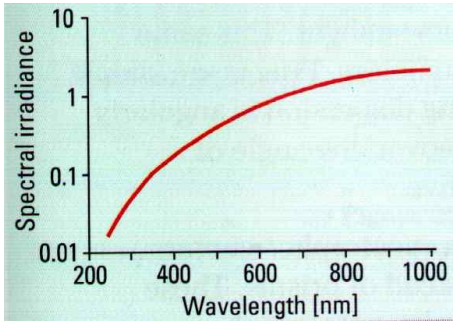
- Absorption spectroscopy
  - High intensity emission in the whole range
  - No change in spectral properties during measurement

  - Different lamp for a given range
    - UV: deuterium lamp
    - visible: tungsten lamp
    - UV+visible: xenon
    - infra: Globar (SiC) lamp
    - Hollow cathode lamp: monochromatic light
    - laser: monochromatic light

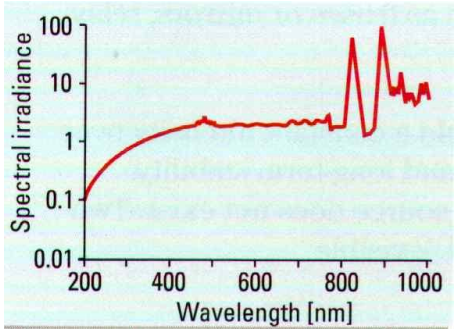
Light sources



Deuterium lamp



Tungsten lamp



Xenon lamp

Emission spectra of deuterium and tungsten lamps

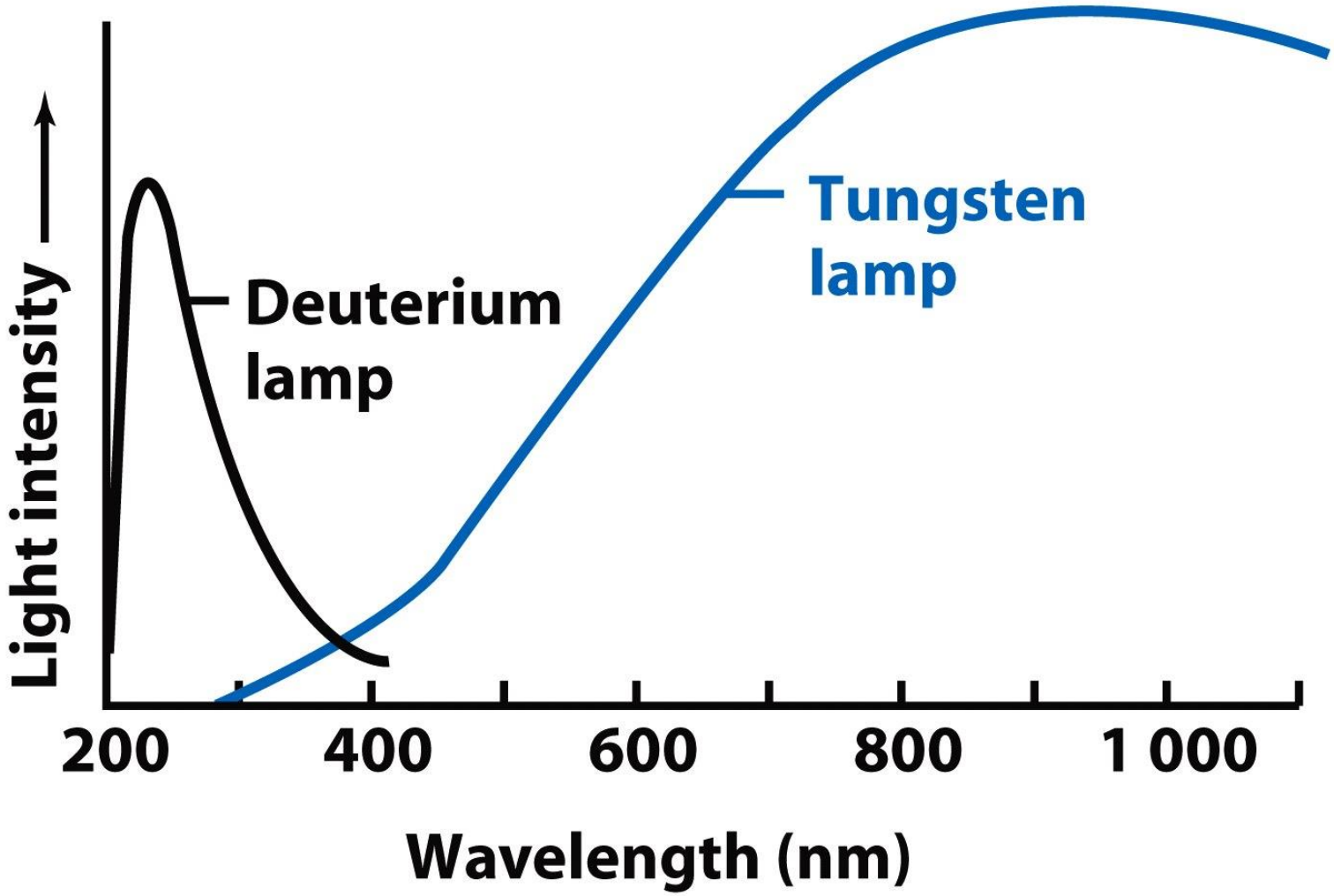


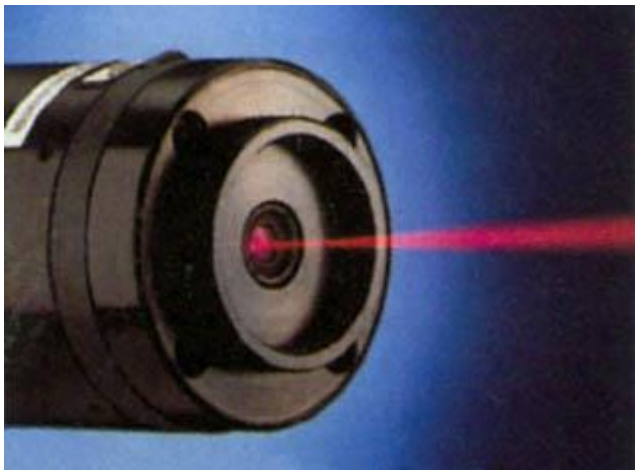
Figure 20-3
Quantitative Chemical Analysis, Seventh Edition

Laser

Light Amplification by Stimulated Emission of Radiation

Laser light's properties:

- monochromatic: single wavelength
- Very bright: high energy at a given wavelength (a general laser is 10^{13}x brighter, then the Sun in the yellow range)
- collimated: parallel rays (<0.05^{o})
- polarized: electric filed is oscillating in one plane
- coherent: all waves have the same phase



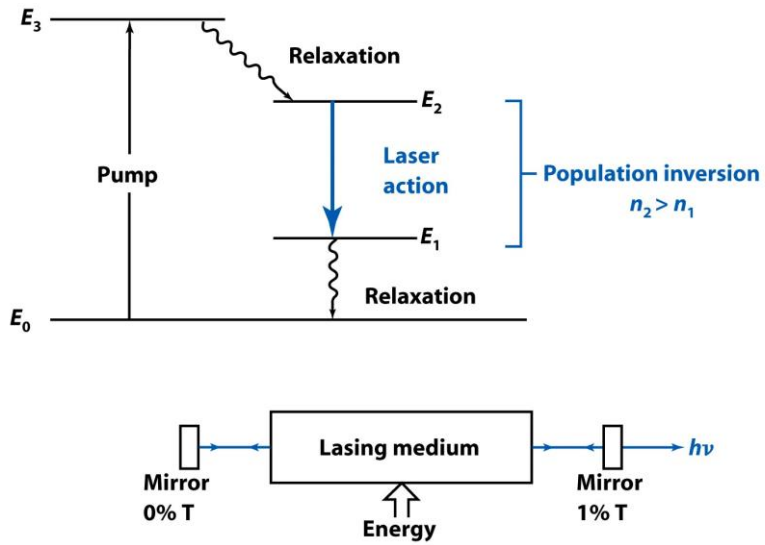


Figure 20-4
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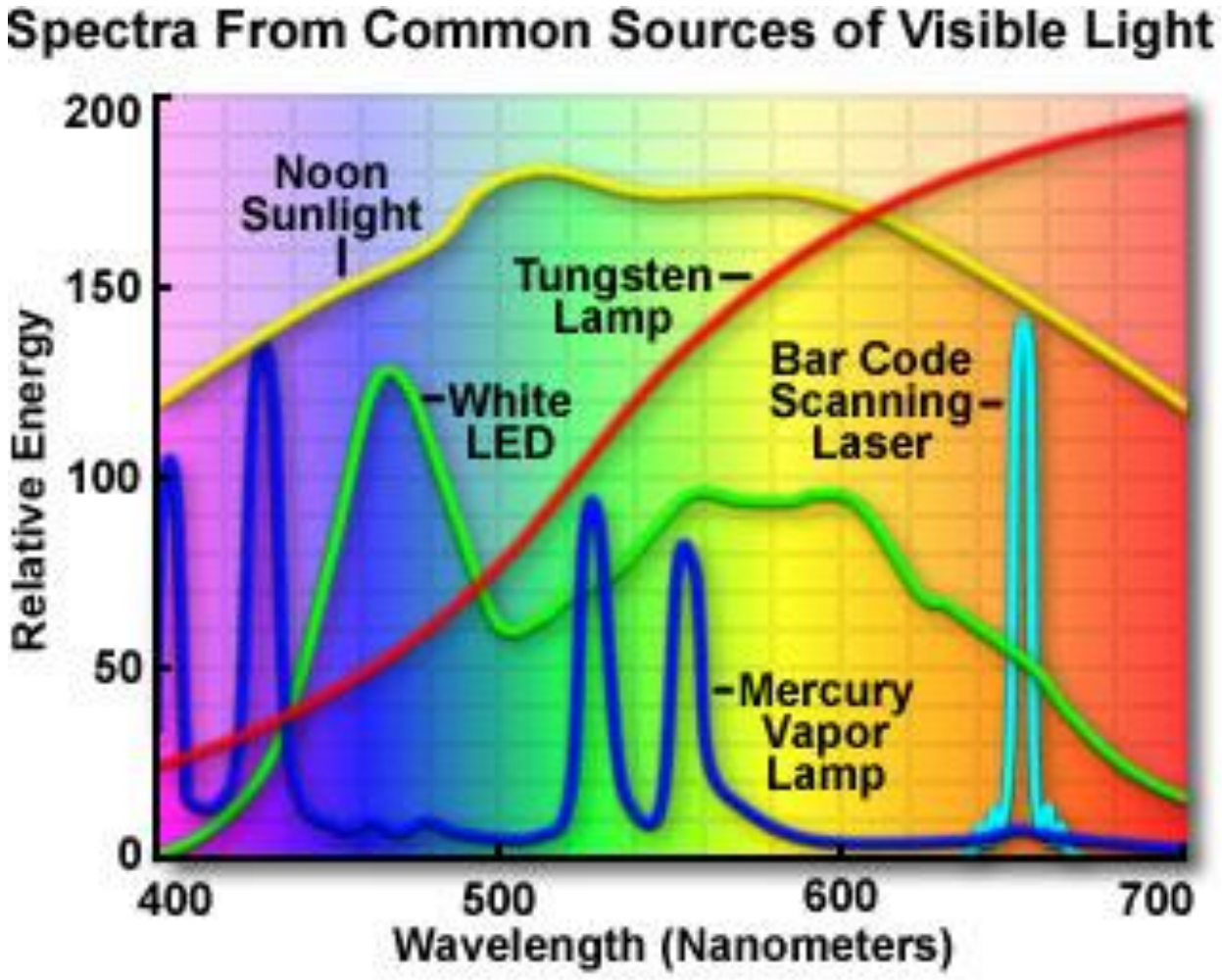
Spectra for light sources



(Poly-) Monochromators

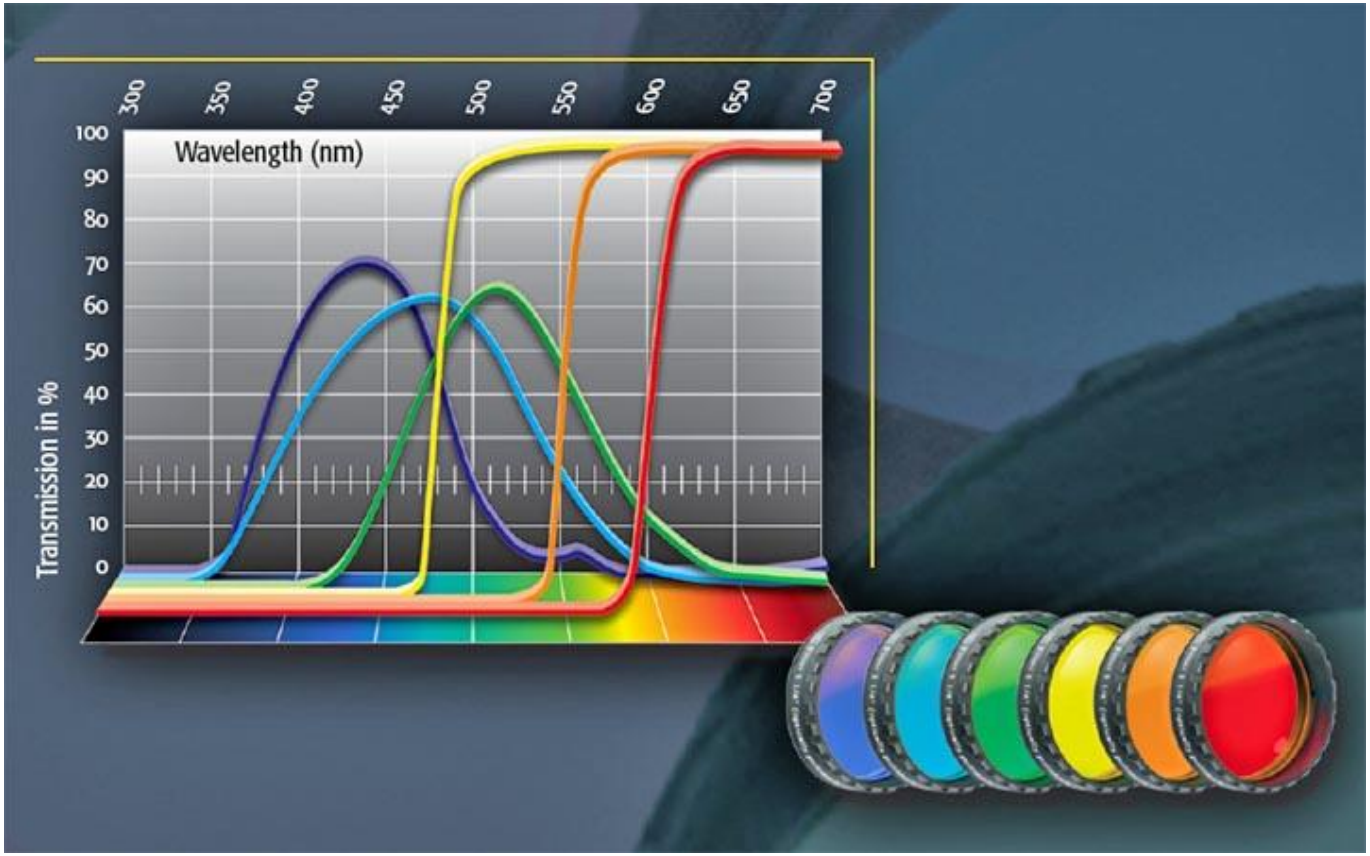
$$A = \lg \frac{I_0}{I} = \varepsilon \cdot l \cdot c$$

„ε" is „constant only at a give wavelenght

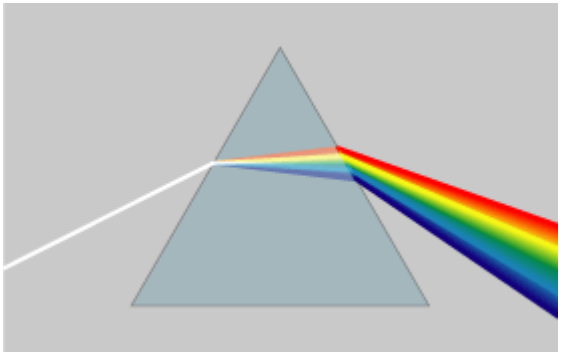
- Generating monochromatic light with the required wavelength from the incident polychromatic light ray
- Characteristic value: full-width at half maximum (FWHM): $\lambda \pm \Delta\lambda \rightarrow$ **$2\Delta\lambda$**
  - filter (**$2\Delta\lambda$** =50-100 nm)
  - prism (**$2\Delta\lambda$=1-2 nm)**
  - optical grating (**$2\Delta\lambda$=<0.2 nm)**

Monochromators with filter

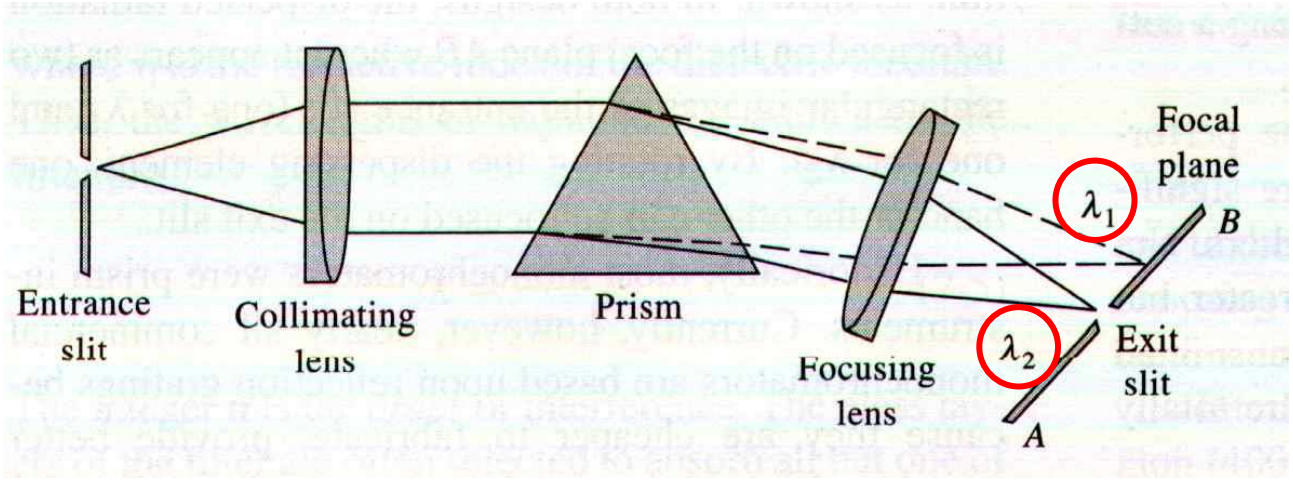
- Usually light is transmitted in a given range, or light is absorbed in a given range



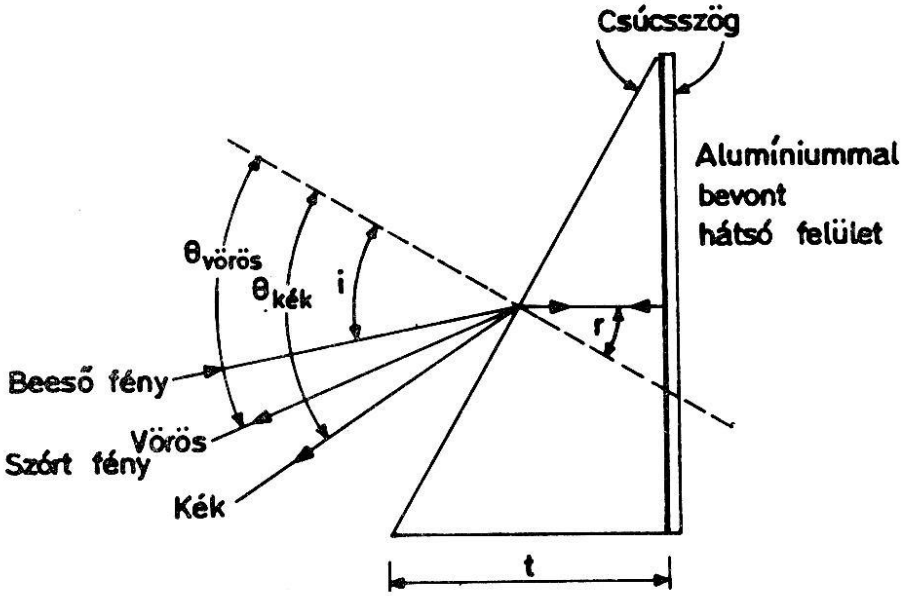
Monochromator with prism



- Refraction of light
- Polychromator !
  (many wavelength)

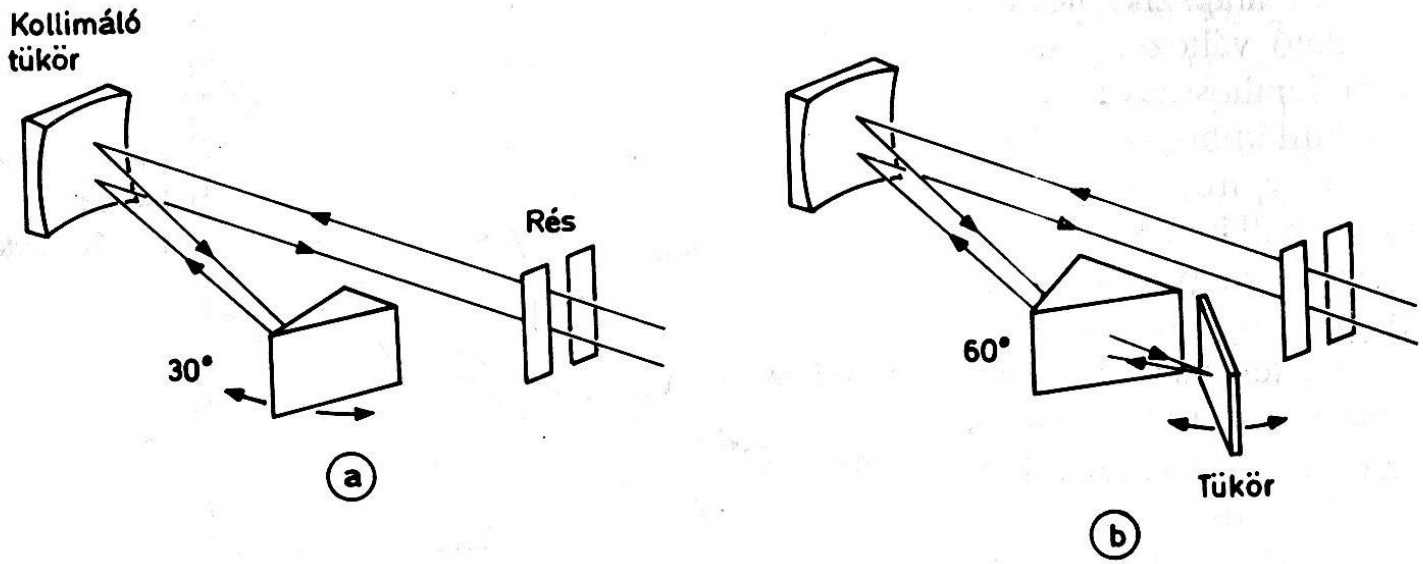


A Littrow-prism



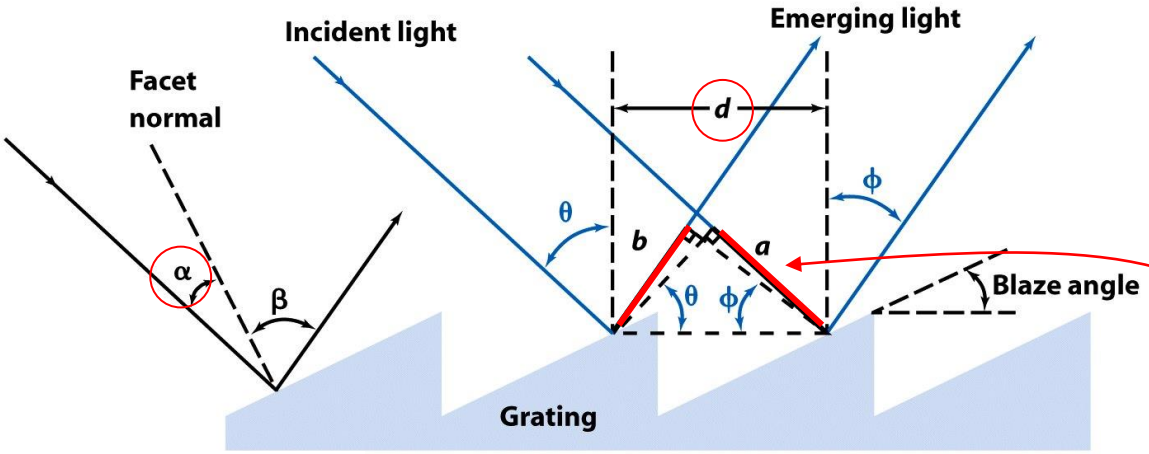
5.11. ábra. A Littrow-prizma fényfelbontása:
i — beesési szög; r — törésszög; t — a prizma alapja (bázis);
θ — az elhajlás szöge; szaggatott vonal a beesési merőleges.

Prism-monochromators with Littrow arrangement



a — 30°-os elfordítható prizma; b — 60°-os prizma, a síktükör fordul el;

Optical grating



- Optical element with closely spaced lines (grooves)
- Diffraction
- Polychromator

Surface is reflective (coated with Al). Each groove is a source of radiation. If the repeat distance (**d**) is in the order of the light wavelength , then the grating is capable to dissolve the incident light – **Diffraction**

Light behave as wave, it has phases, can cause **interference.**

When the adjacent light rays are in phase, they reinforce one another: **constructive i.**

When not in phase, they partially or completely cancel on another: **destructive interf.**

Phase difference is caused by the **different distance** and the wave property.

Constructive interference: $d \sin \alpha_n = 2\, n\, \lambda/2$

Destructive interference: $d \sin \alpha_n = 2\,(n+1)\,\lambda/2$

Interferencing

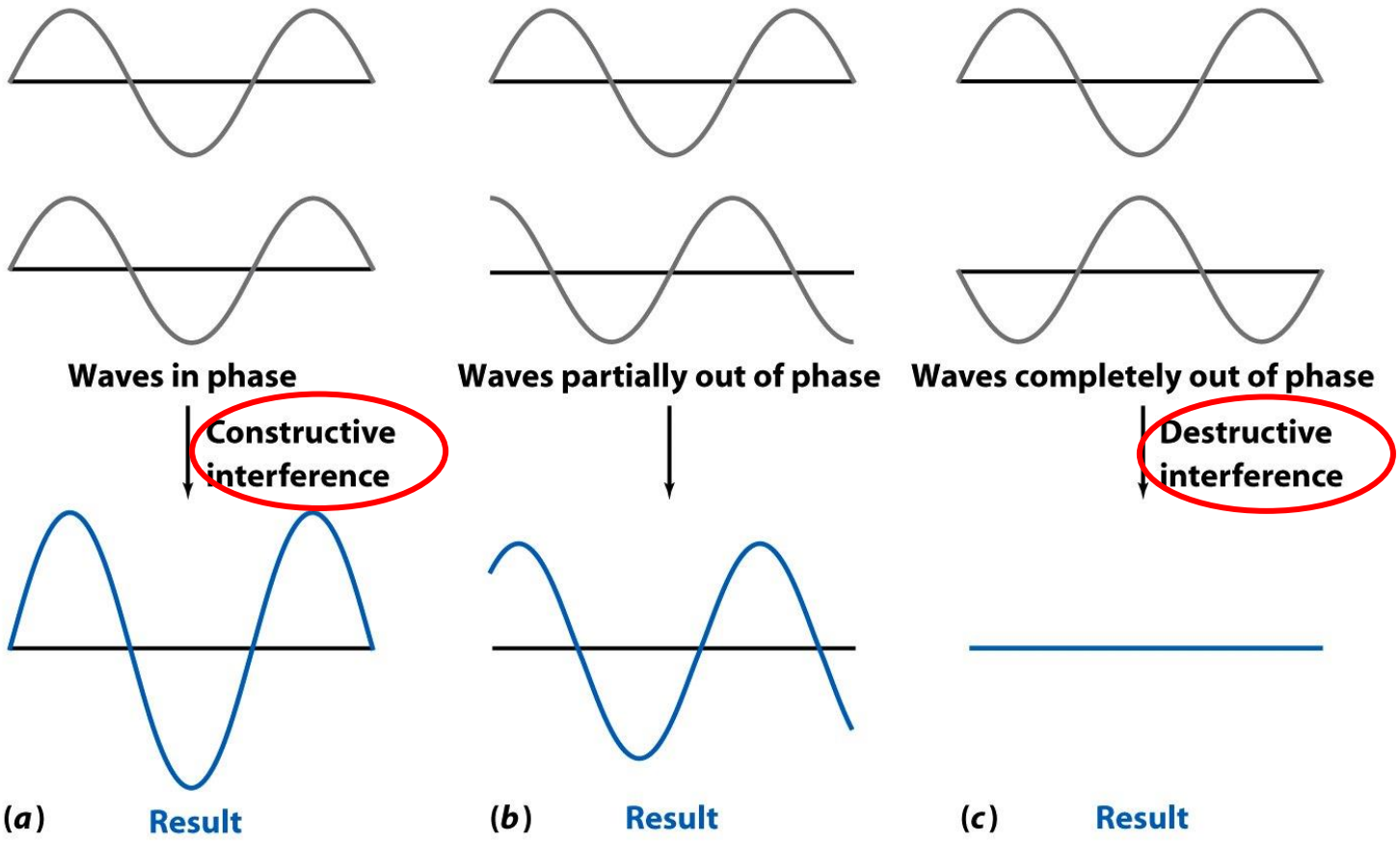


Figure 20-7
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Monochromator with grating

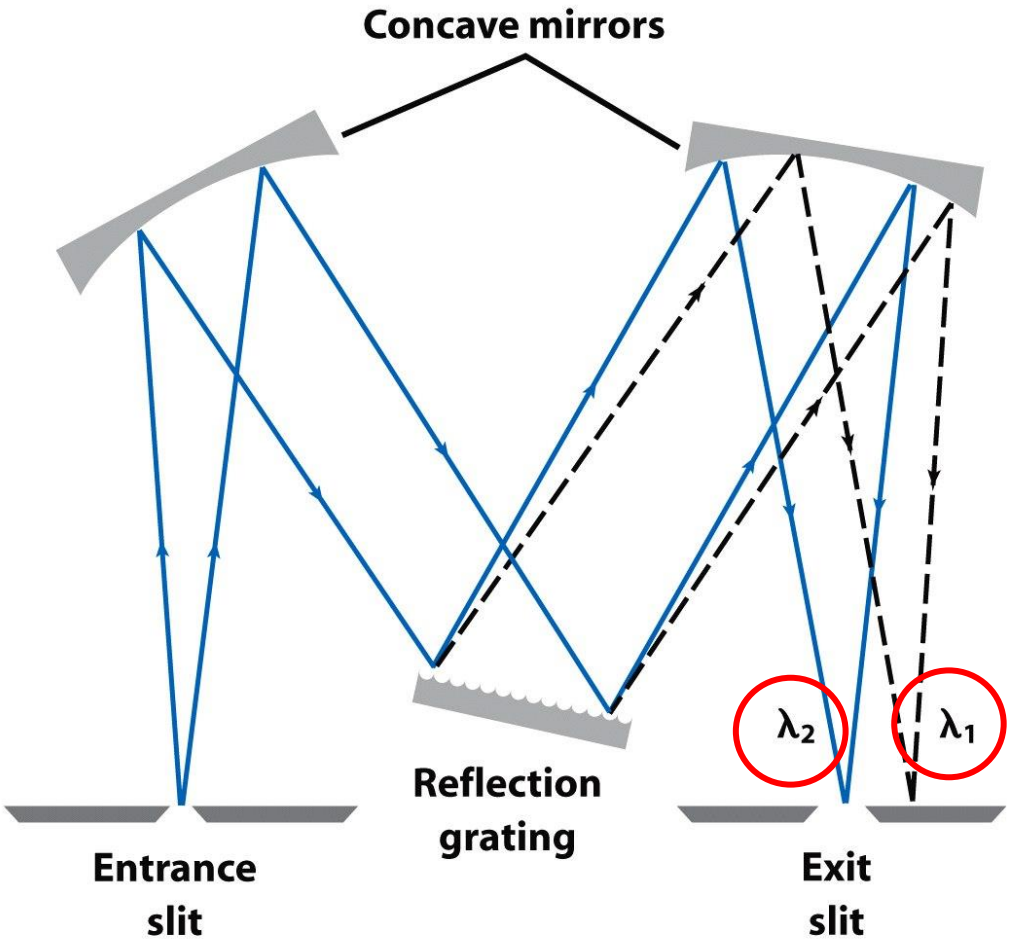
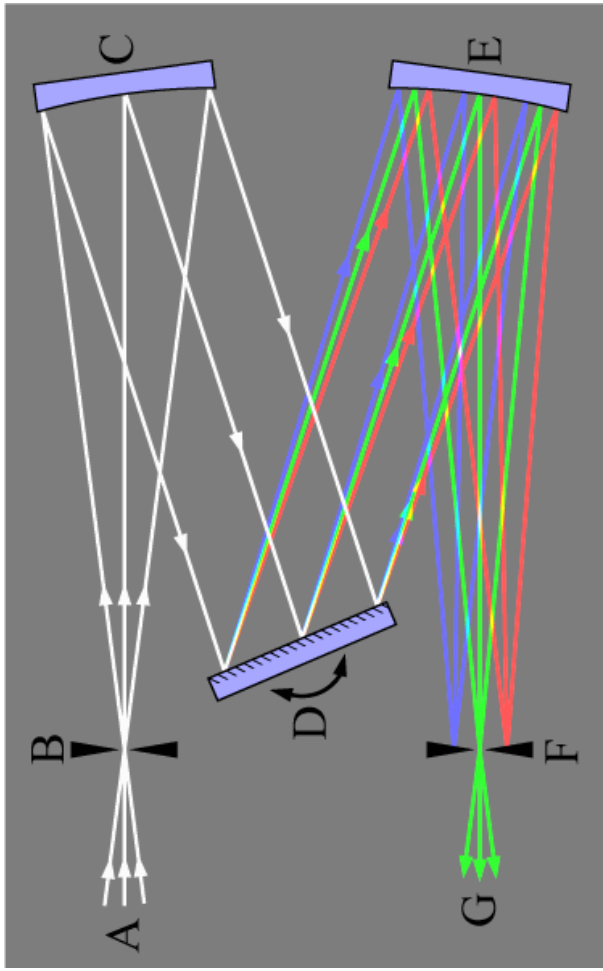
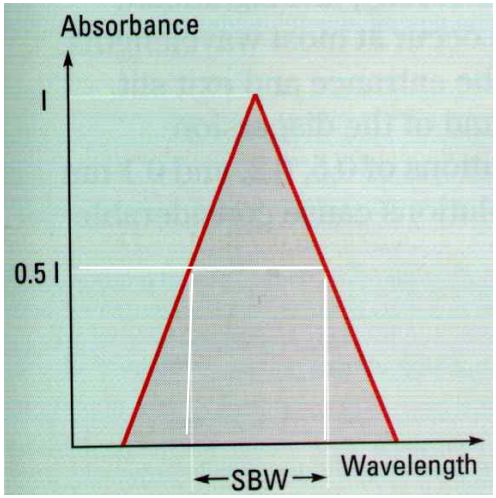


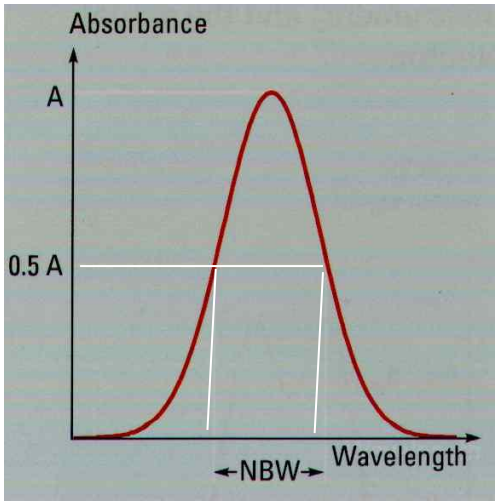
Figure 20-5
Quantitative Chemical Analysis, Seventh Edition



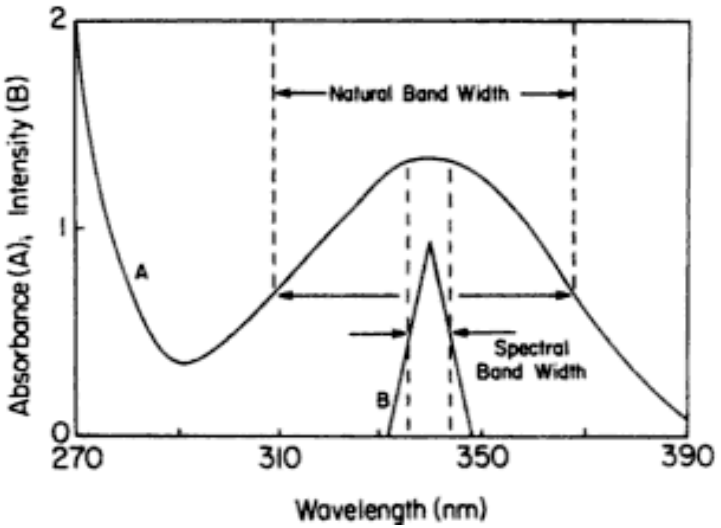
Spectral and natural bandwidth

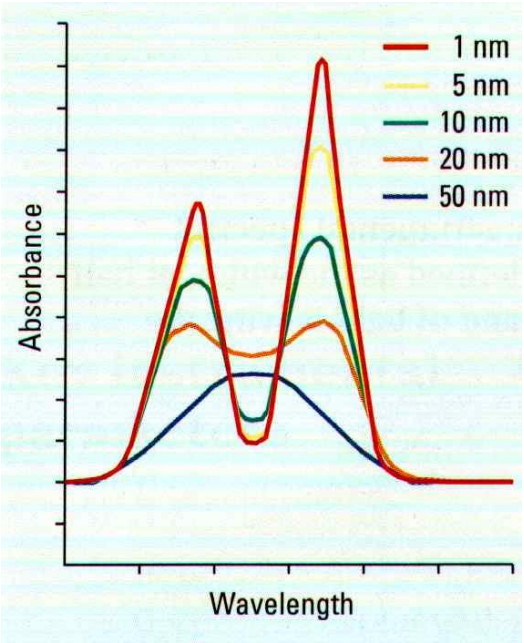


dλ / dx (x-slit size) eg.
On a 0.5 mm wide slit 2 nm is the output ray's width.



One parameter of the sample is the bandwidth at the half of the absorbance. (natural bandwidth)





Spectral bandwidth must be significantly smaller than natural in order to excite or measure correctly.

Effect of the monochromator's bandwidth (slit size) to the spectrum

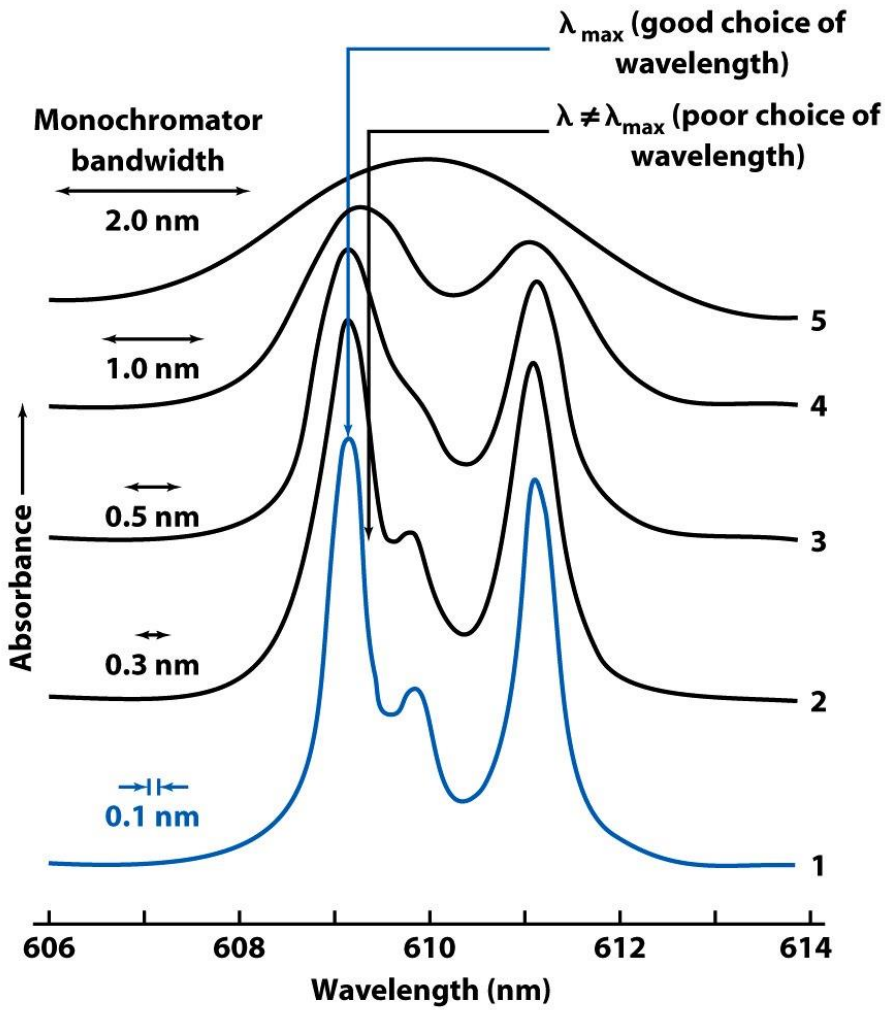


Figure 20-8
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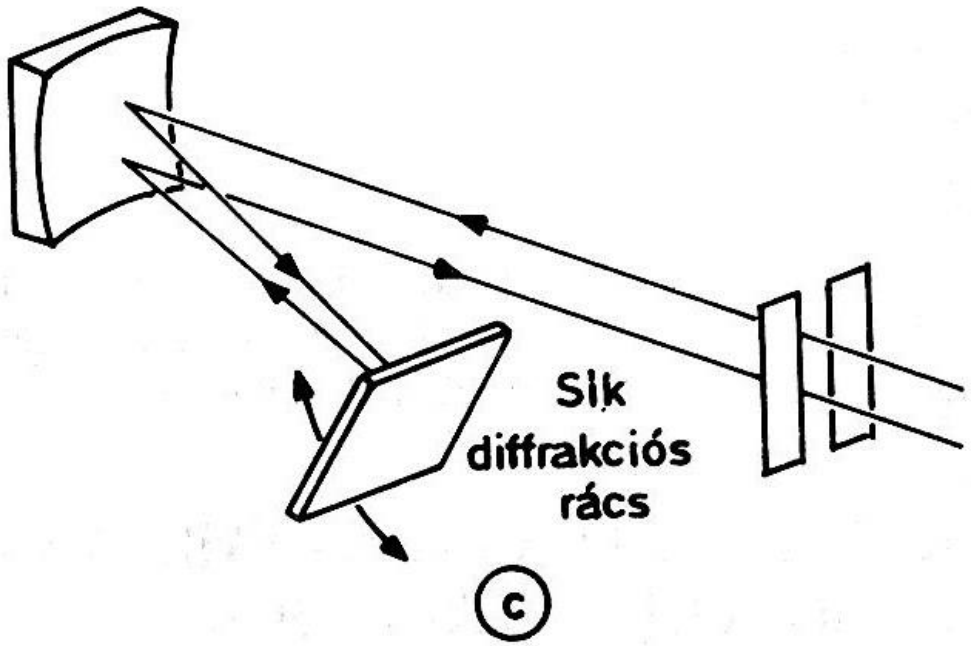
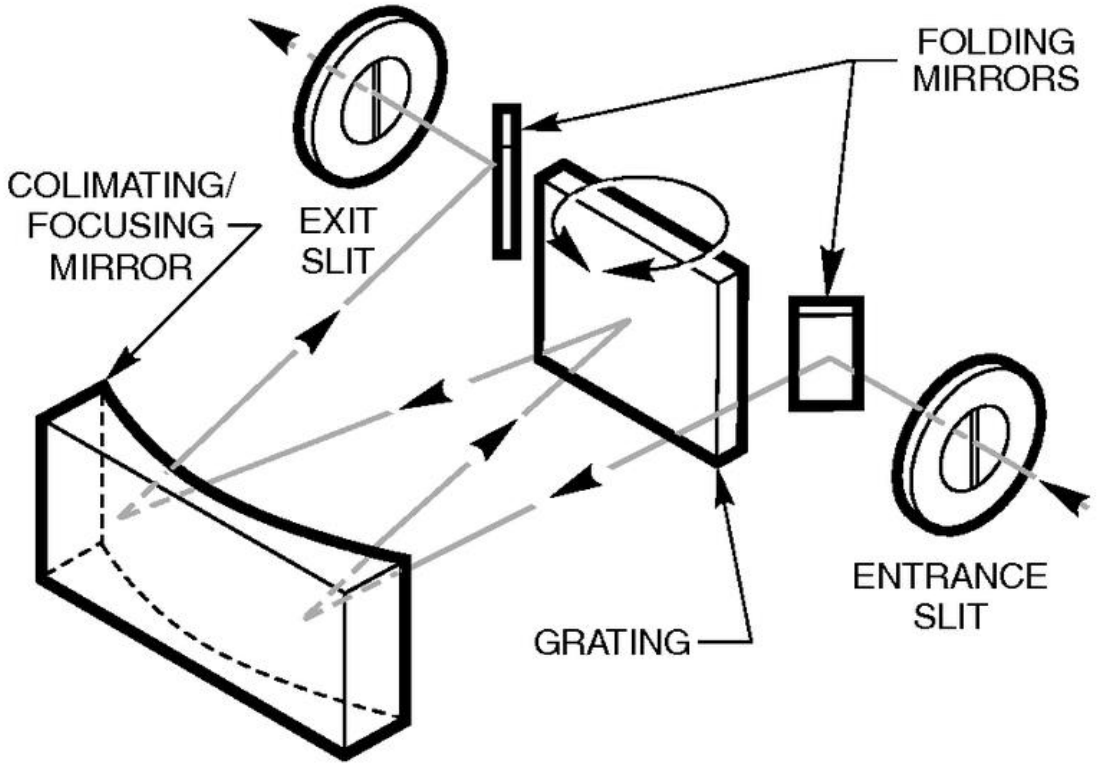
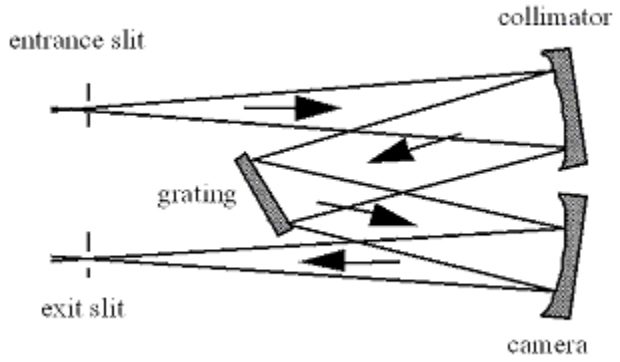
Grid monochromator with Littrow arrangement



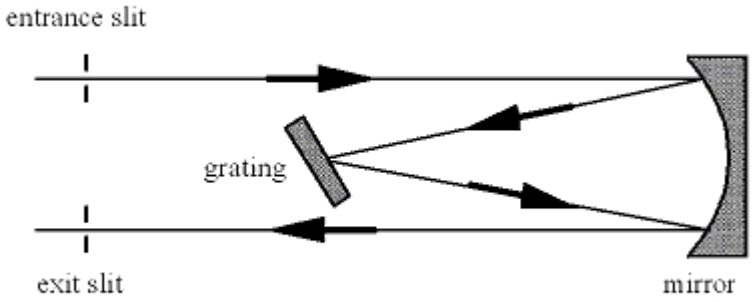
Grid monochromator with Ebert arrangement



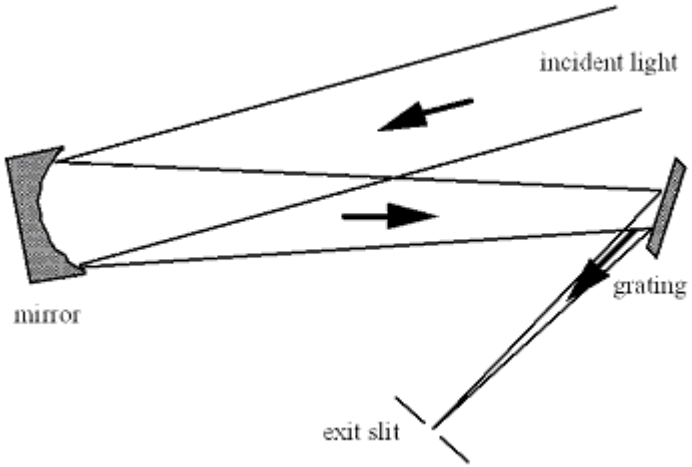
Comparison of monochromator arrangements



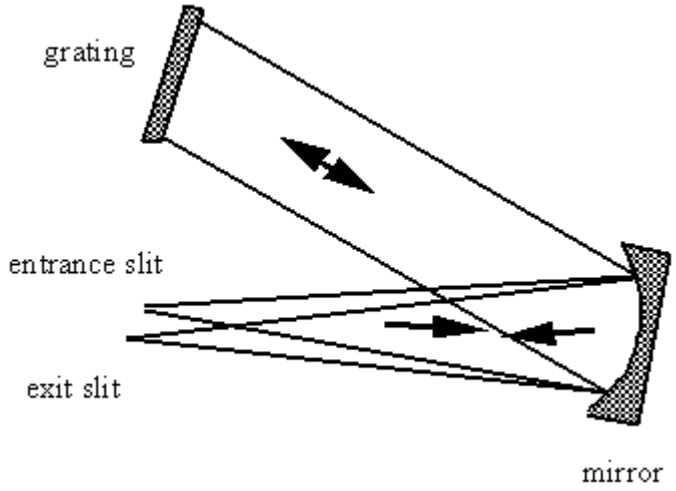
Czerny-Turner



Ebert-Fastie

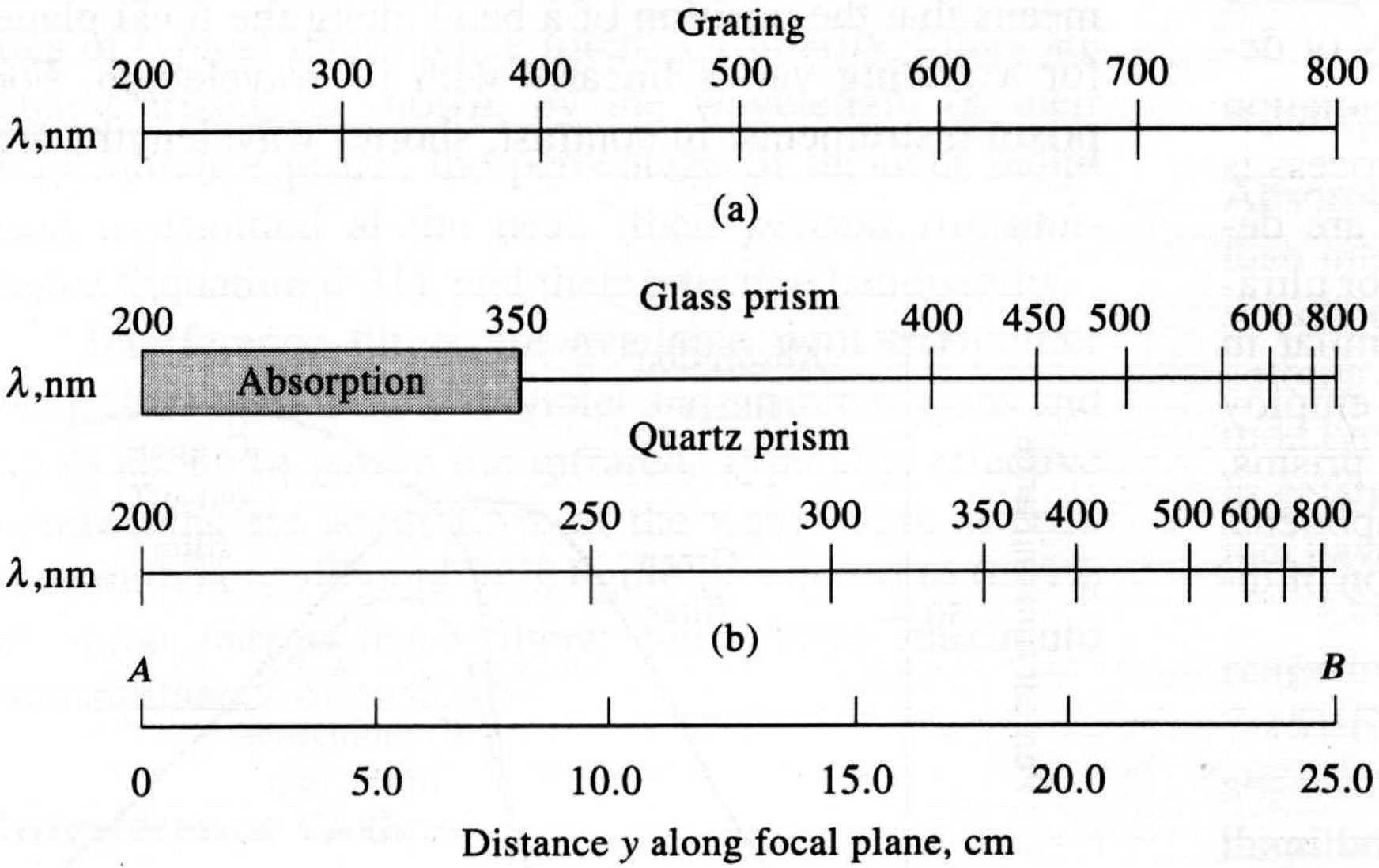


Monk-Gillieson



Littrow

Wavelenght distribution of monochromators



Cuvettes

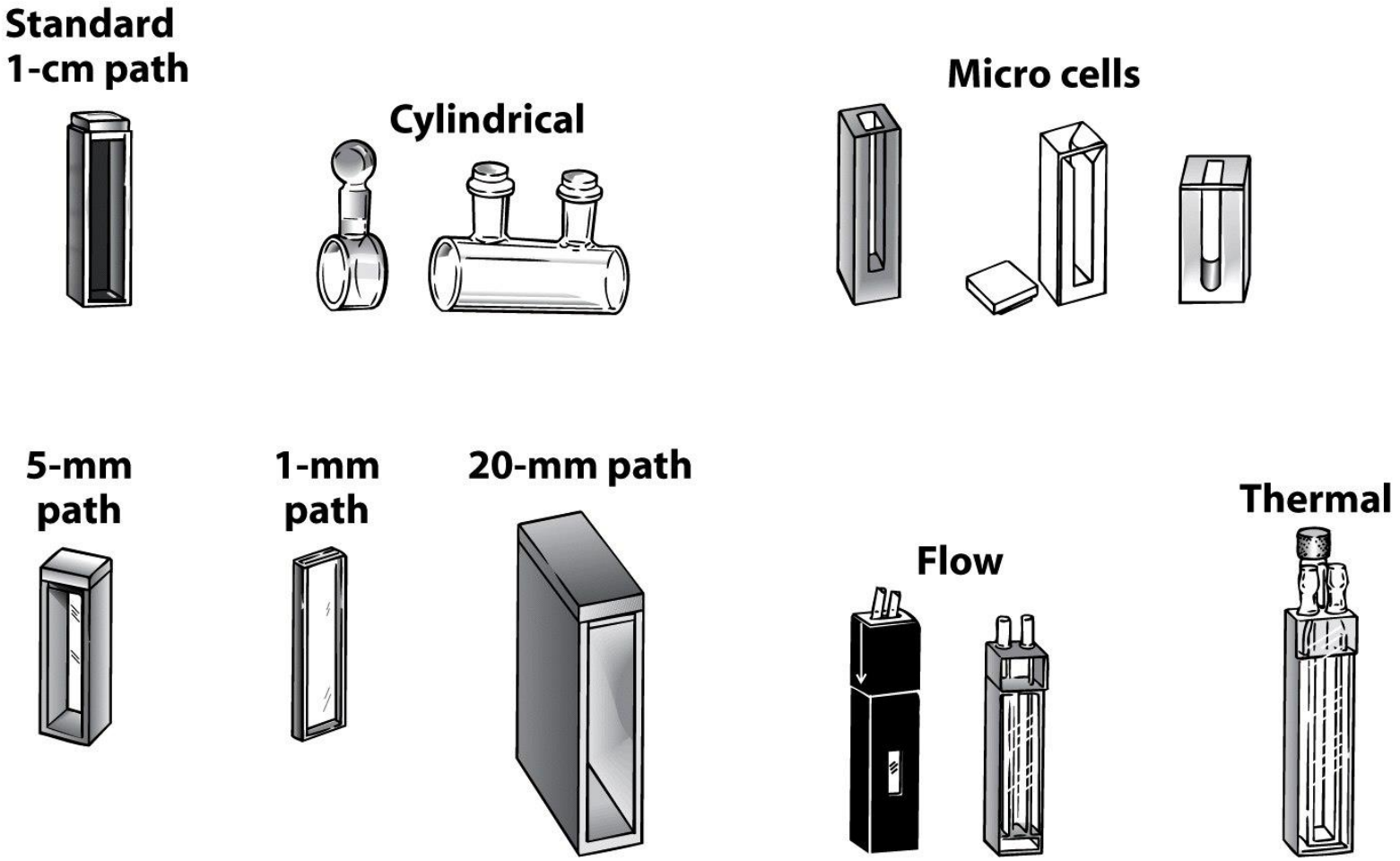


Figure 18-5
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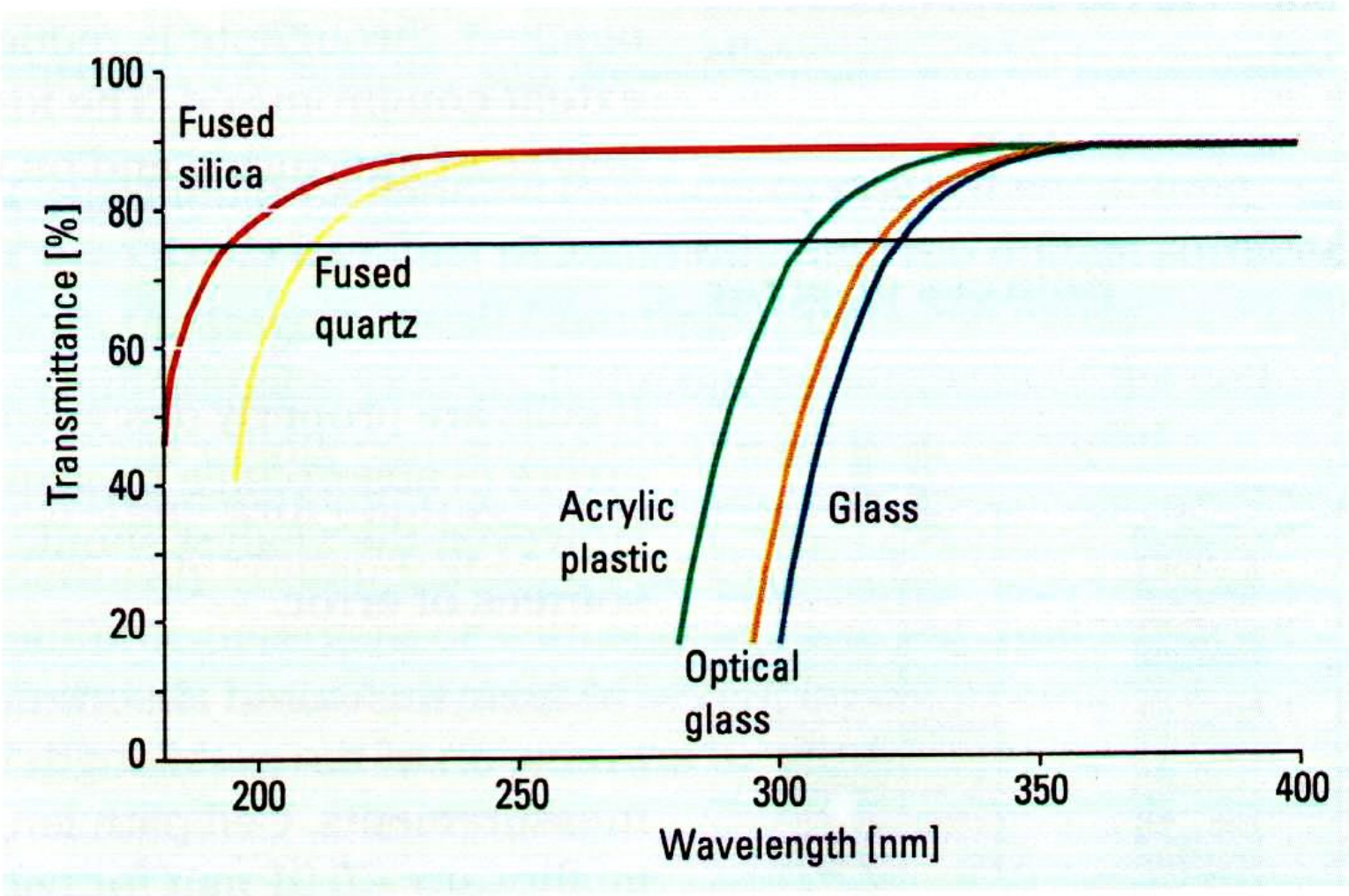
Transmission of cuvettes



Transmission of solvents

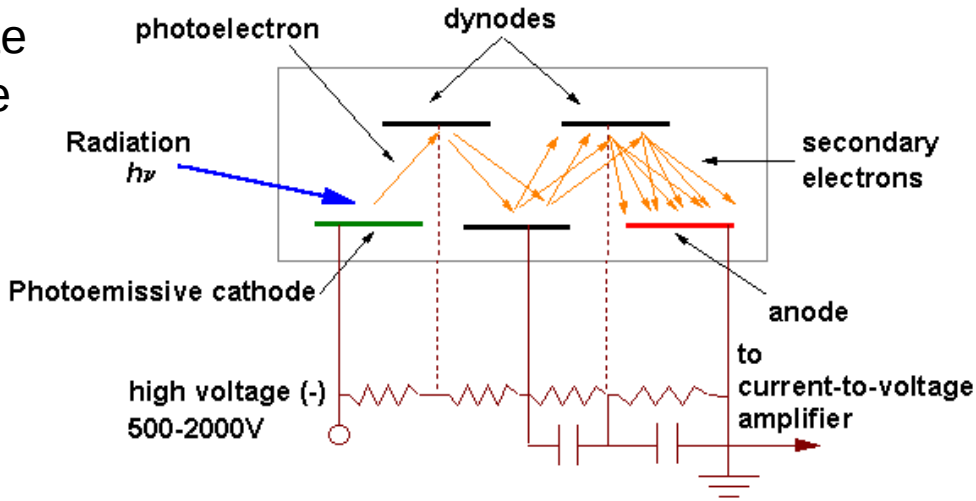
Properties of some common solvents

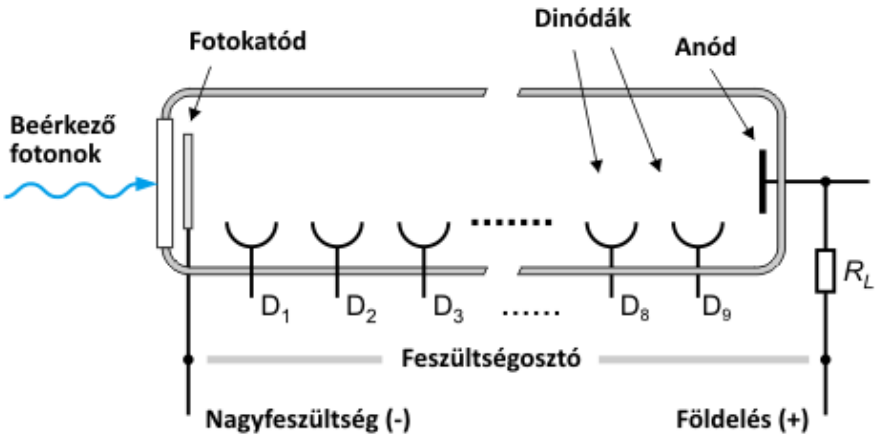
Solvent	Polarity*	Cut-off wavelength (nm)**	Hazard***
Distilled water	78.5	< 195	none
Hexane	1.9	199	F
Ethanol (absolute)	24.3	207	F
Methanol	32.6	210	F
Cyclohexane	2.0	211	F
Chloroform	4.8	246	F/T
Dimethylsulfoxide	none	270	H
Acetone	20.7	331	F

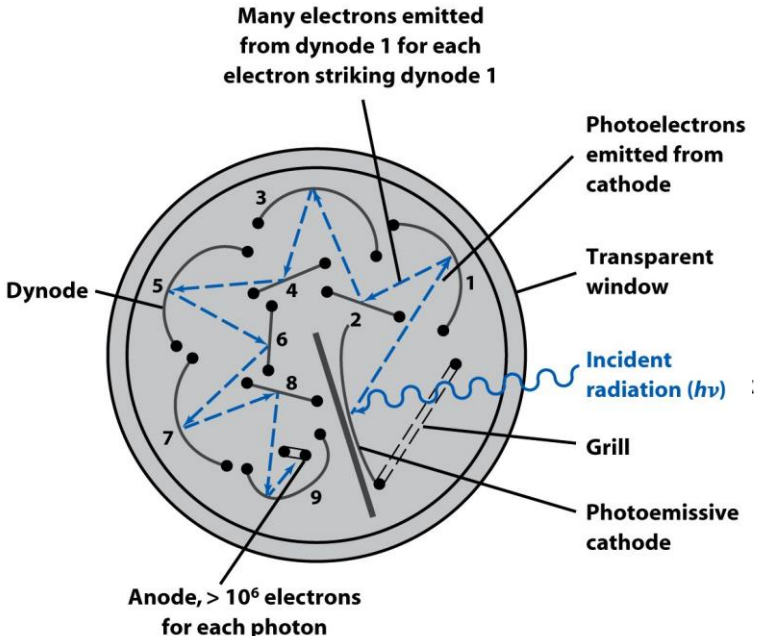
* Dielectric constant at ambient temperature
** Wavelength at which transmittance of 10-mm path length is < 25 %
*** F = flammable; T = toxic; H = health hazard

Detectors - Photomultiplier tube (PMT)

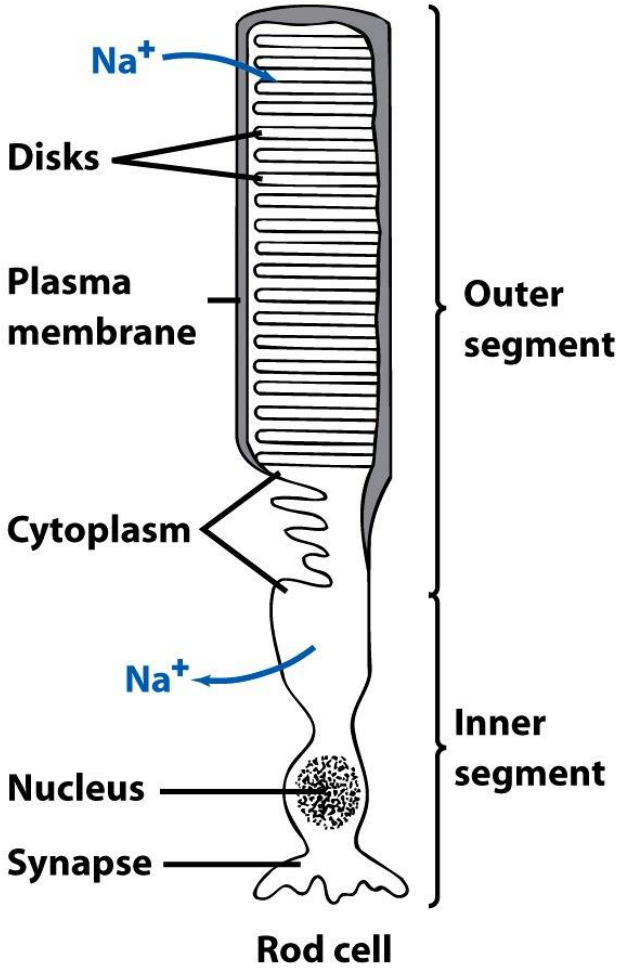
- For measuring light intensity – generate an electrical signal proporional with the incident photon's number – concentration can be calculated



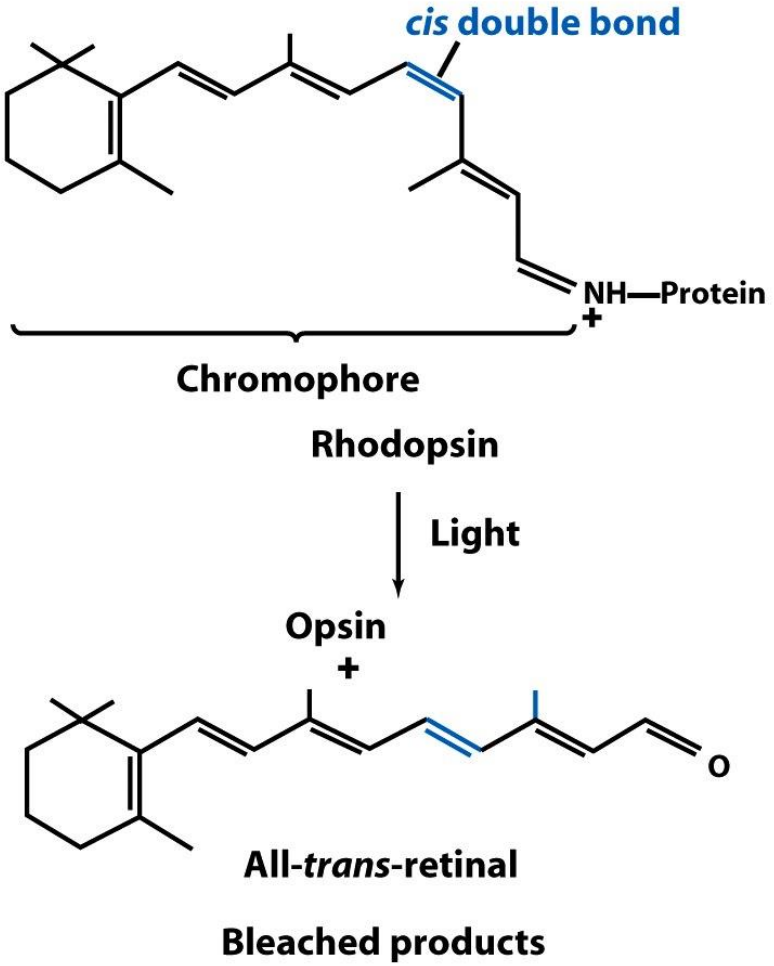




Biological photodetector

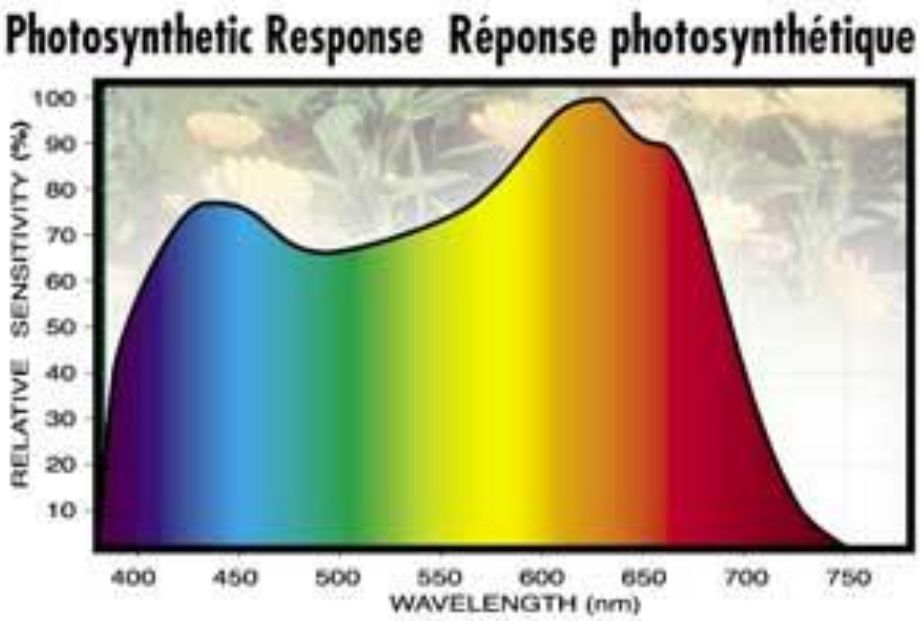


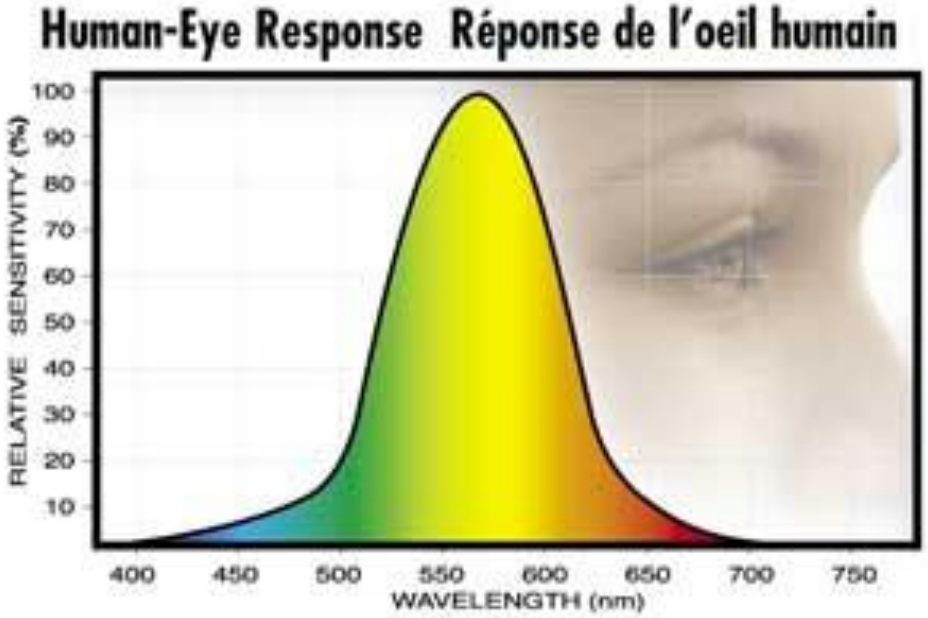
Unnumbered figure pg 435
Quantitative Chemical Analysis, Seventh Edition



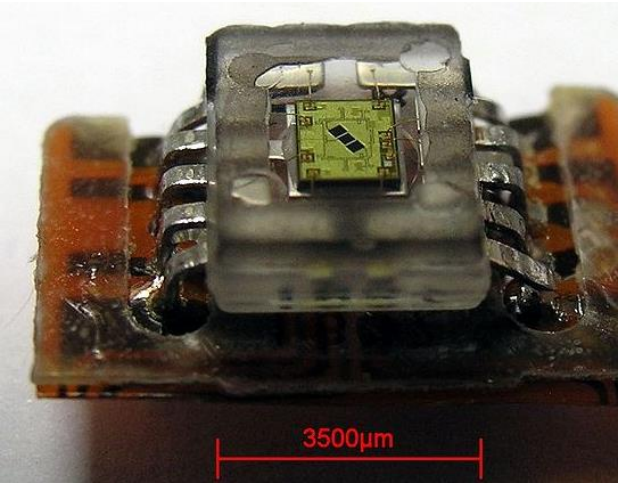
Higher amplification as PMT!

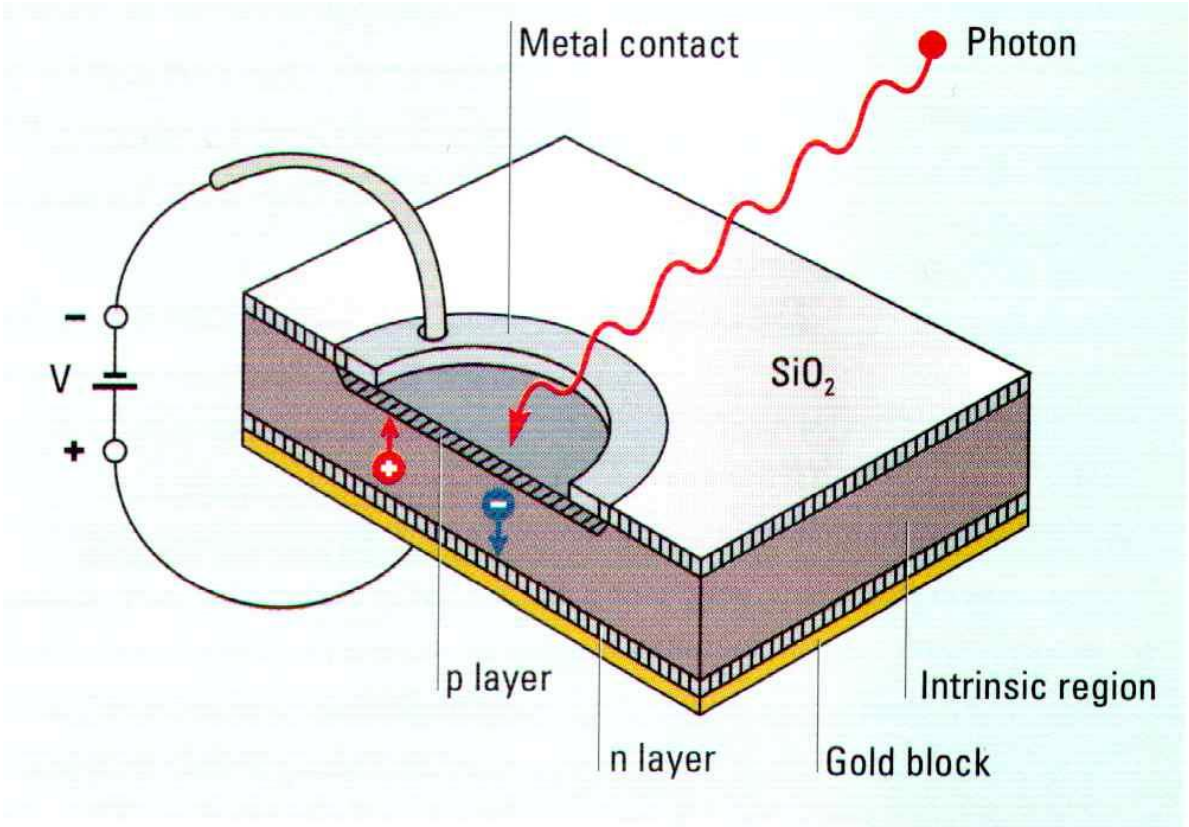
Biological photodetectors





Photodiode

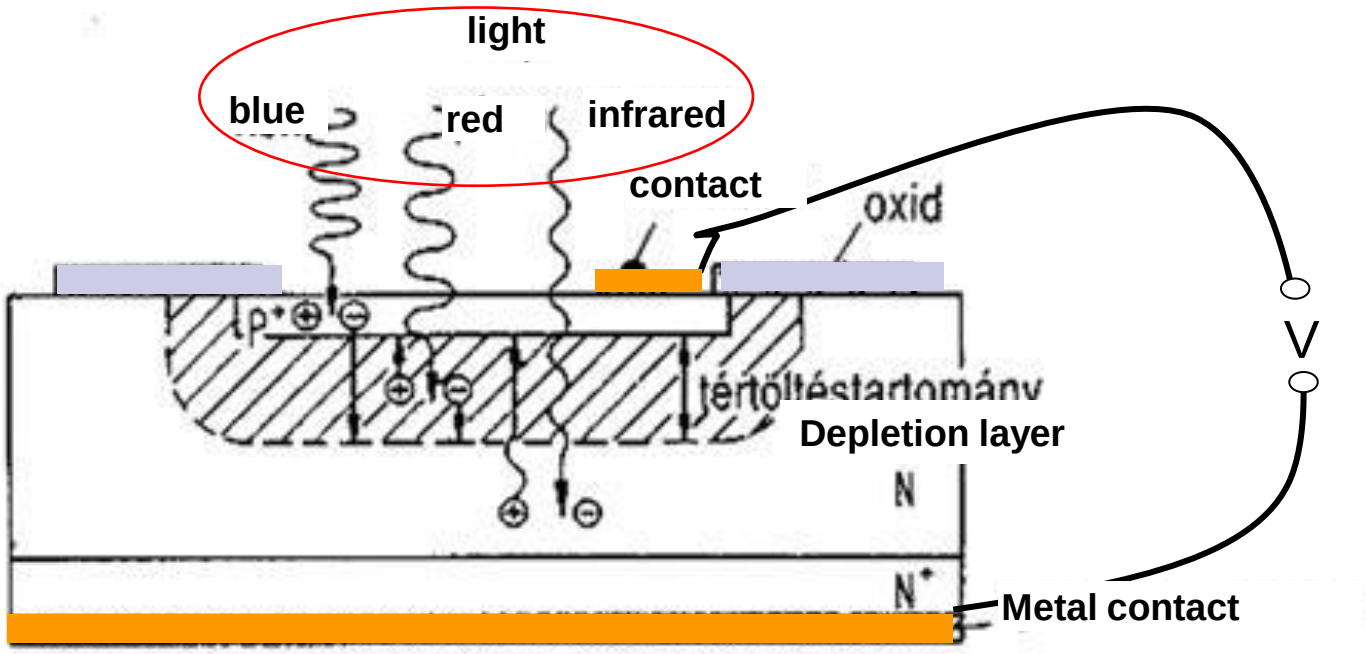




As photons are adsorbed by the diode, electrons are generated and resulted in a lower inner resistance. In this case a higher current is going to flow, which will be proportional with the intensity of the radiation. (response time 10 ms – 1 ns)

Semiconductors – How a photodiode works?

- Voltage is applied on the outer surface. Resulting a thick depletion layer, only low current pass through – dark current.
- Incoming photons are creating charge holders (electrons and holes), these are moving in the electric field - **electrons** to the **n** type, **holes** to the **p** type region. This is called as **photocurrent**.



Photodiode array

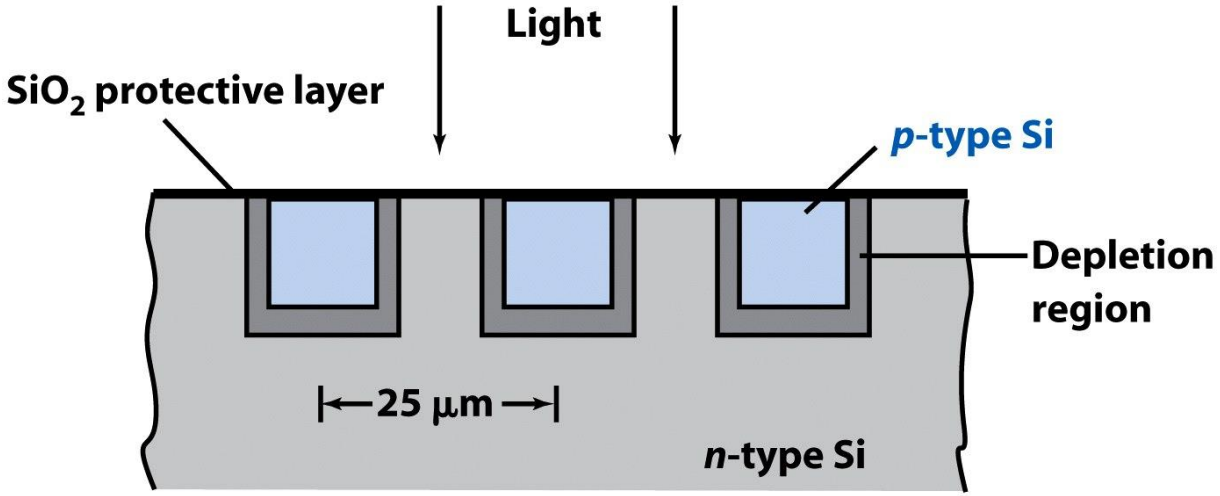


Figure 20-13a
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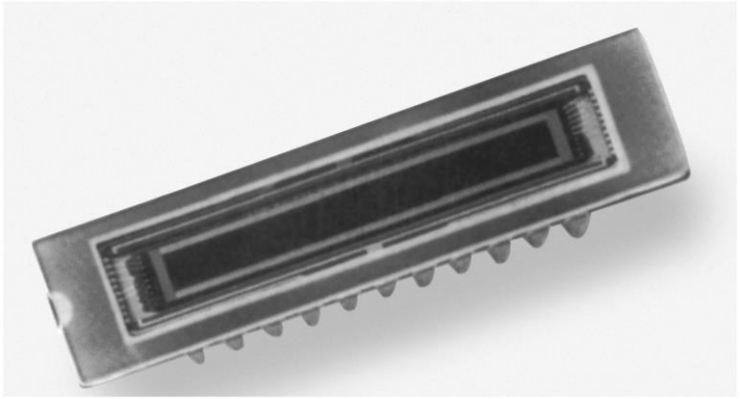

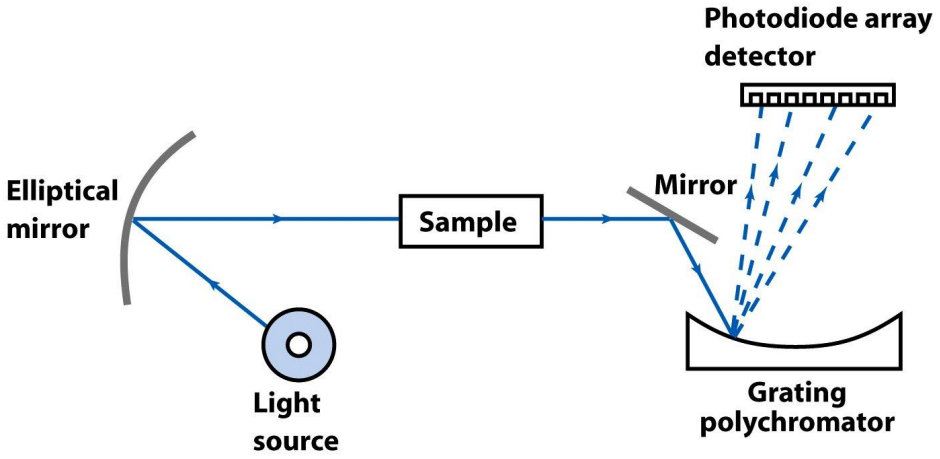

Figure 20-14
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Instrument with photodiode array

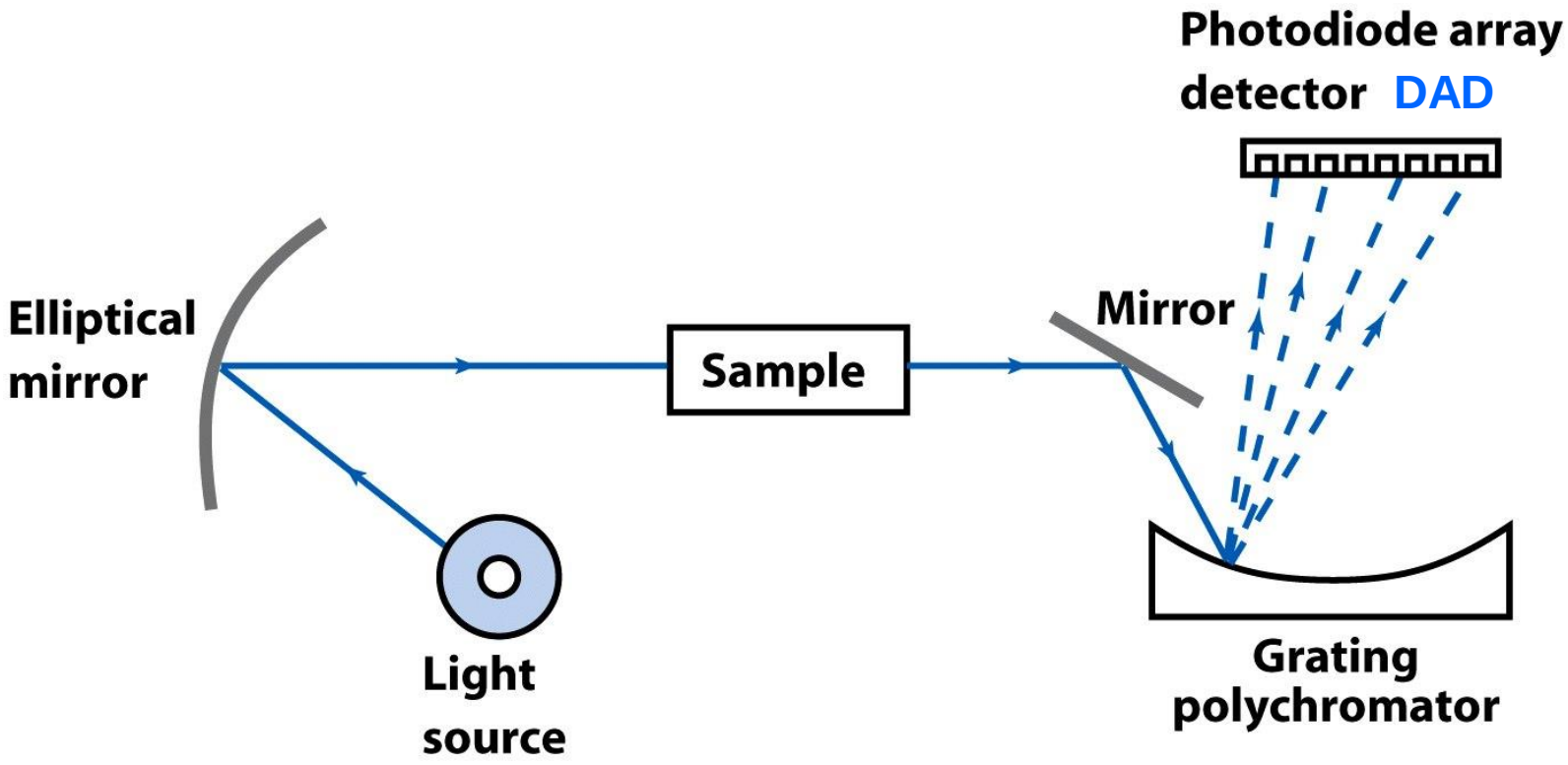

Figure 20-14
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Charge Coupled Device (CCD)

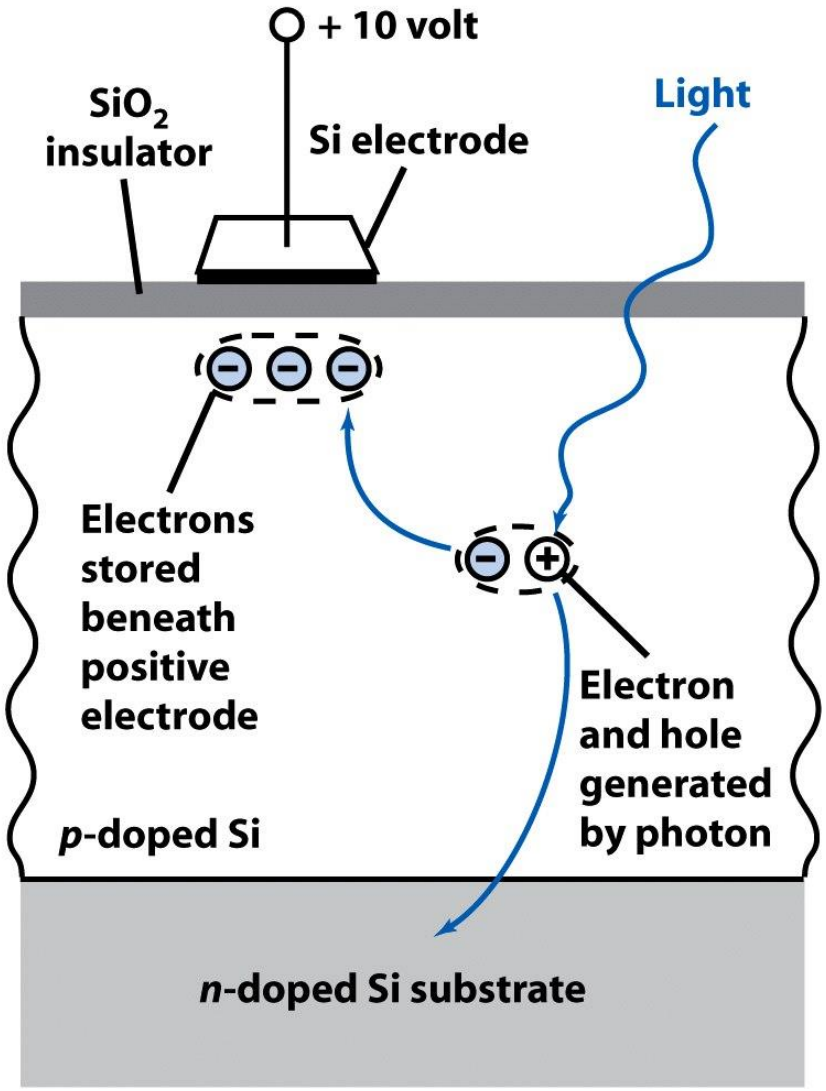

Figure 20-15a
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- Most sensitive detector in the UV/Vis range

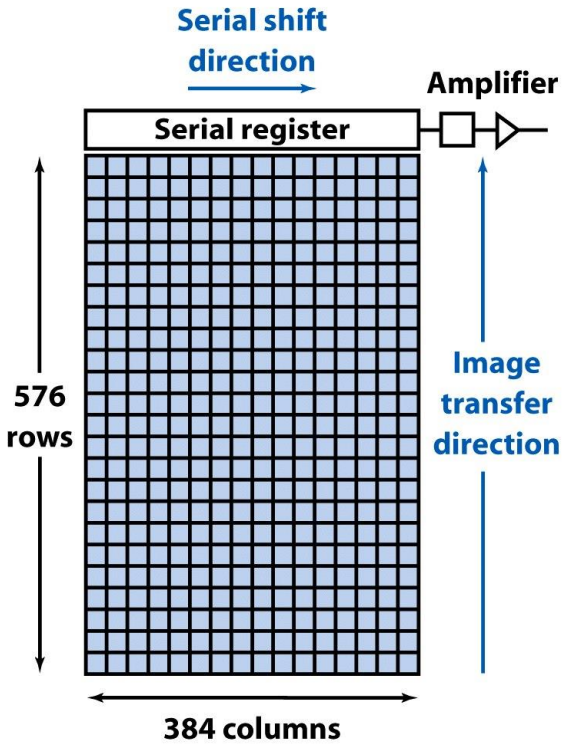
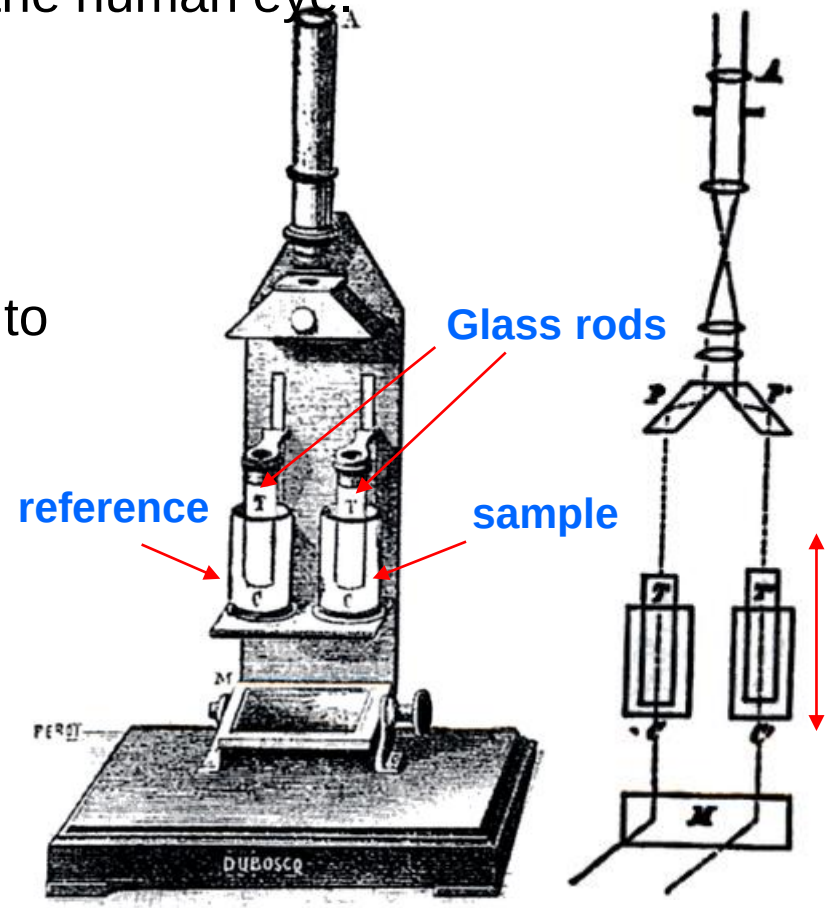


Figure 20-15b
Quantitative Chemical Analysis, Seventh Edition

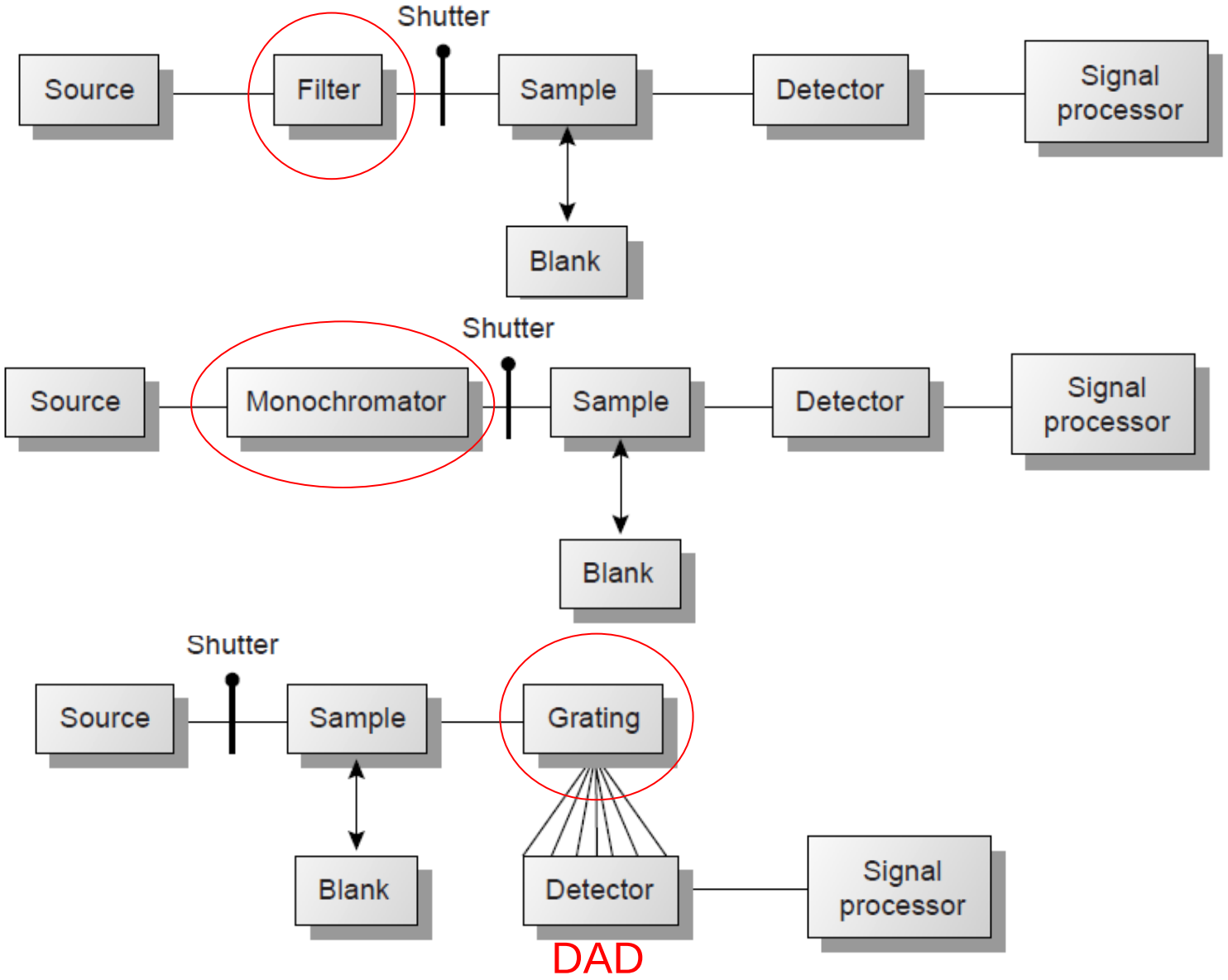
Colorimetry

- One of the oldest absorption method
- No light-dispersion needs, reference and the sample is illuminated with polychromatic light, detector is the human eye.
- Known layer of colorful solution with unknown concentration is compared with the same material's known concentration solution. In the later case the layer thickness is changed to see the same absorption on the two layers.
- Based on the Lambert-Beer-law the layer thicknesses are indirectly proportional to the concentration.

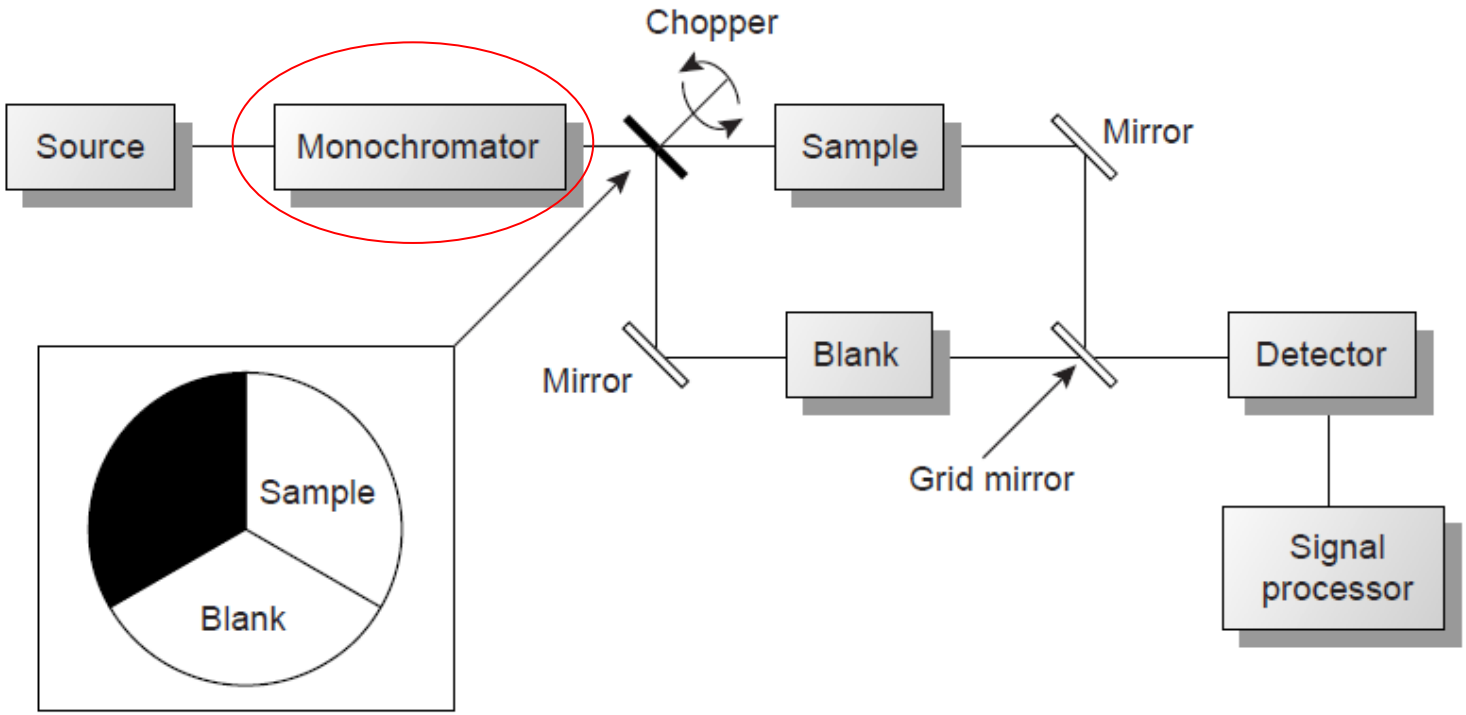
$$A = \varepsilon \, l \, c$$



Photometer's setup – single beam



Photometer's setup – double beam



Photometer's setup – double beam

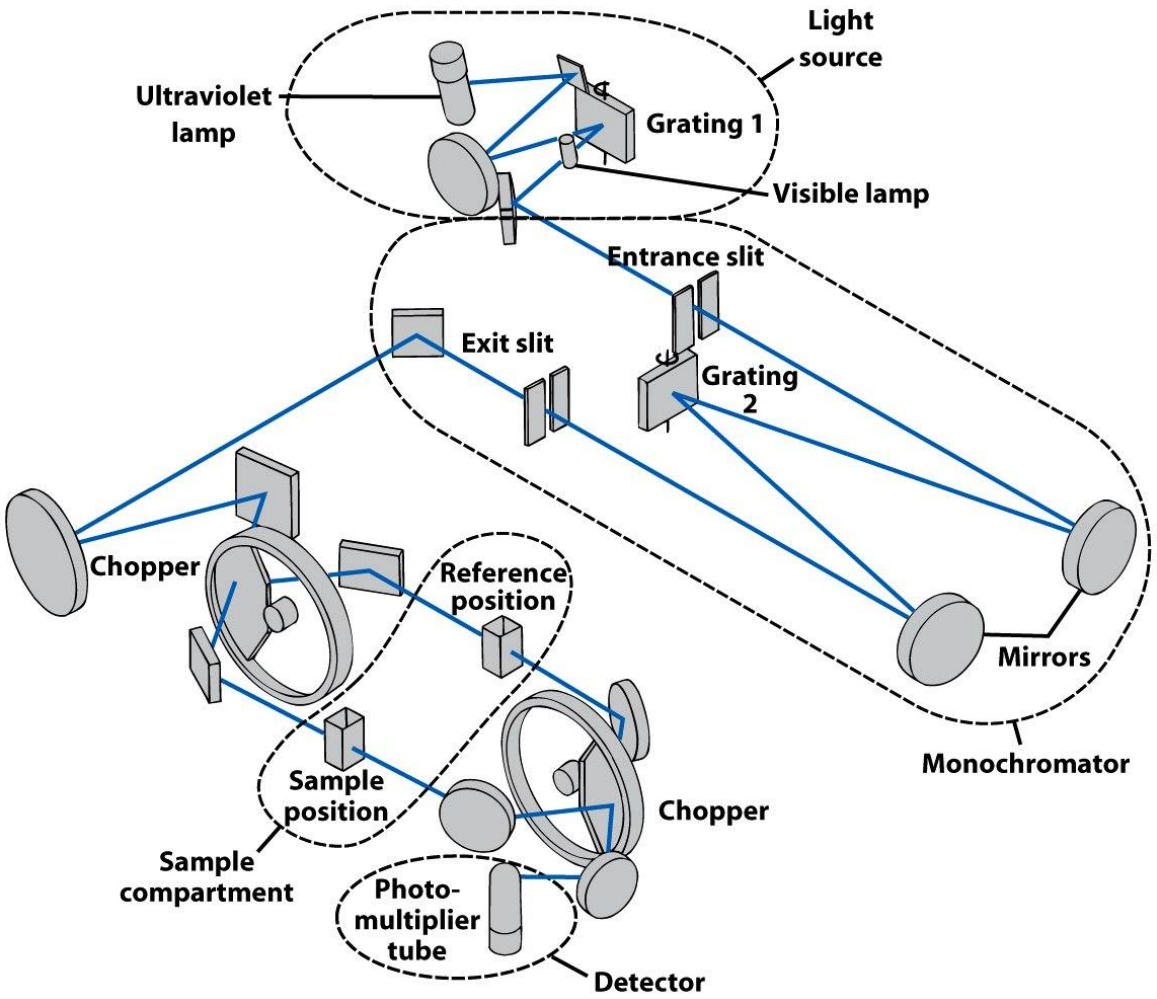


Figure 20-2b
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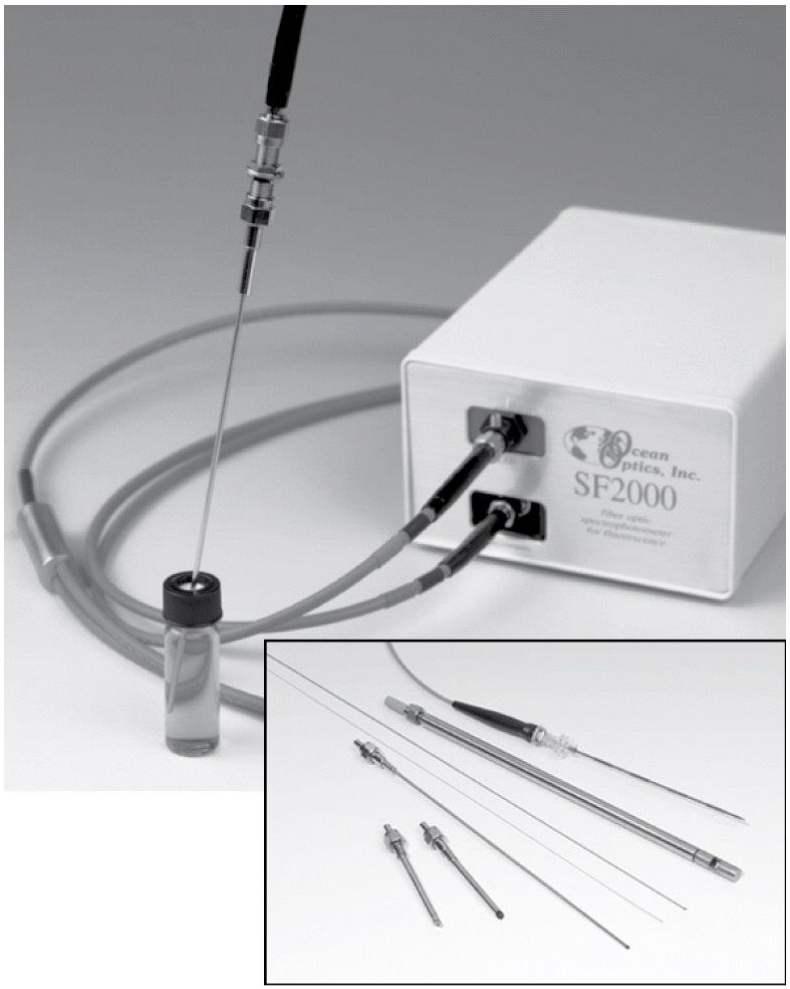
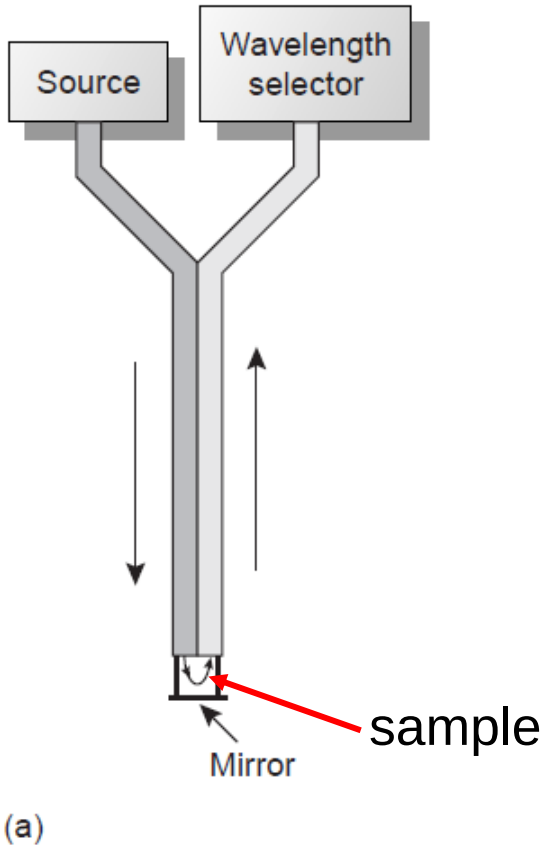
Fiber optic photometer





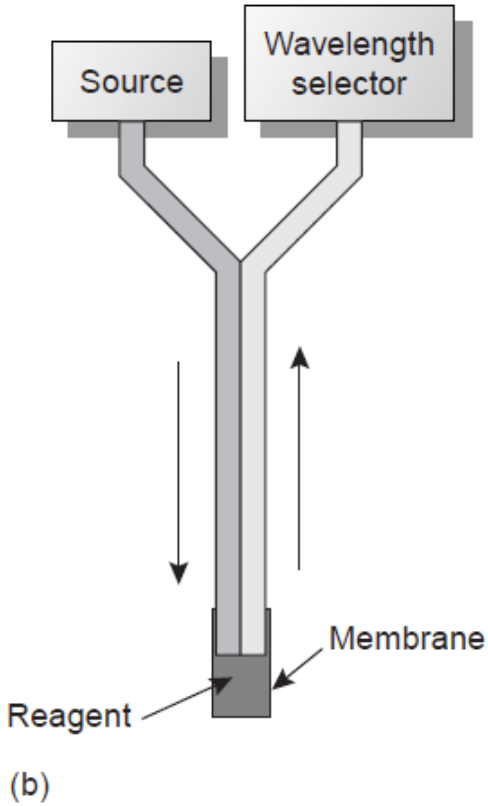


Figure 19-19
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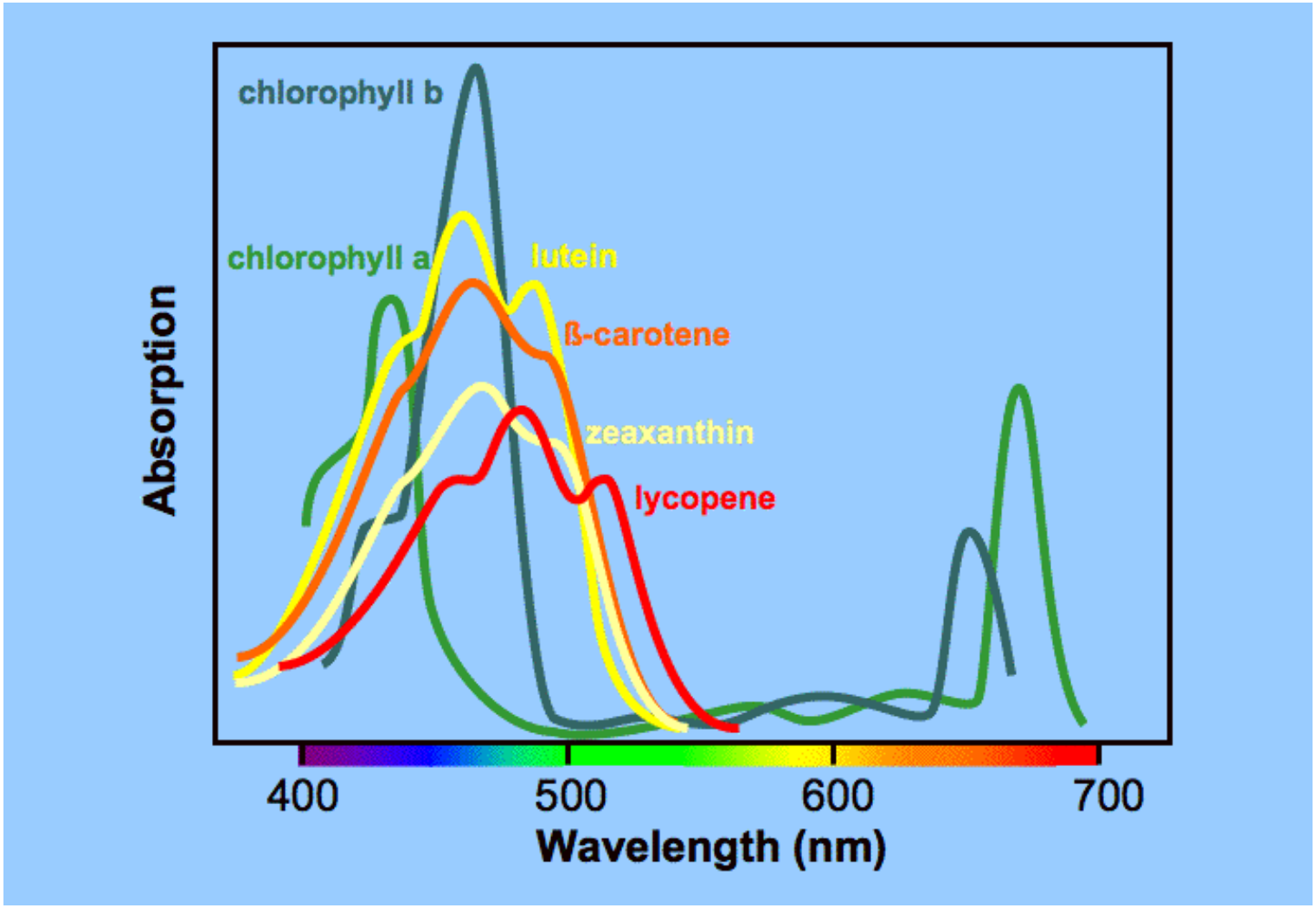
The absorption spectrum

- Information about the material's properties, complex structures
- The aims to create an absorption spectrum are:
- Qualitative analysis
  - **identification**: two materials is identical, if they absoption spectra are completely same.
  - **Contamination:** the adsorption of the contaminant can be also find in the spectrum, however, the detection is not the same for all pollutants.

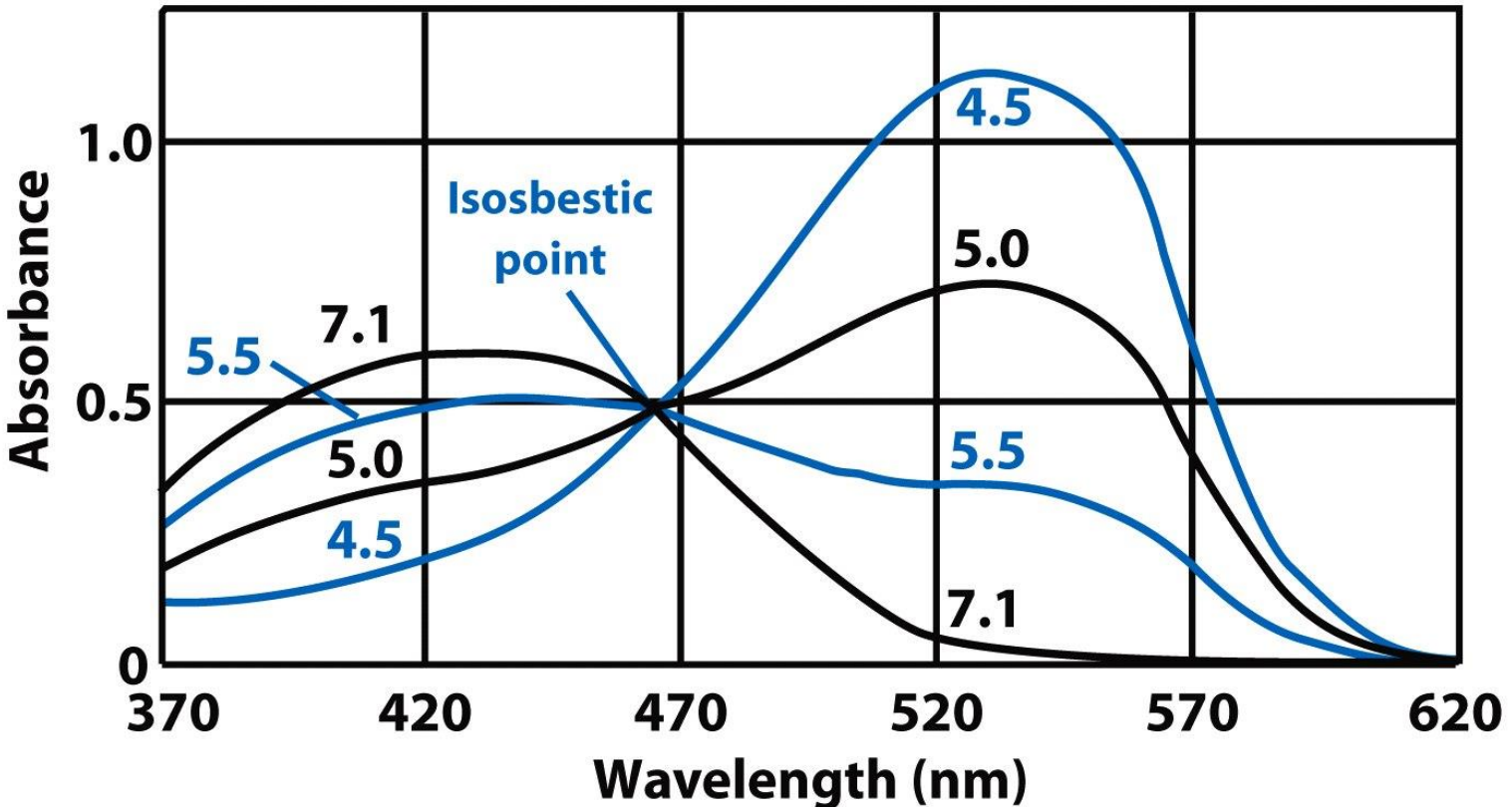
Molecular spectra – plant's pigments



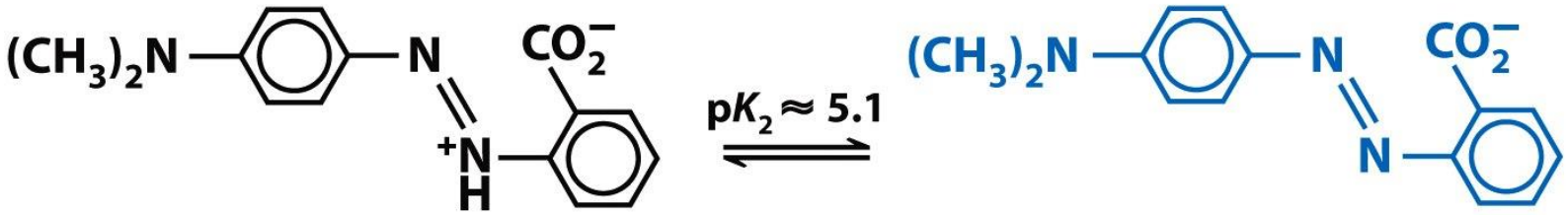
The absorption spectrum

- Quantitative analysis
  - Important to set the **correct wavelength** for a measurement. One needs to measure at the absorption maximum.
  - Analysing a **multicomponent** sample only one component can be measured; to eliminate disturbing effects, the absorption spectra of the **disturbing components** need to know.
  - Quantitative analysis of multicomponent system can be done by the intensity measurement of they absorption spectrum.
  - Classical spectrometrical methods have 1 – 5% relative error, thus mainly used for determination of trace impurities in low concentrations

Spectrum of methyl red – isosbestic points



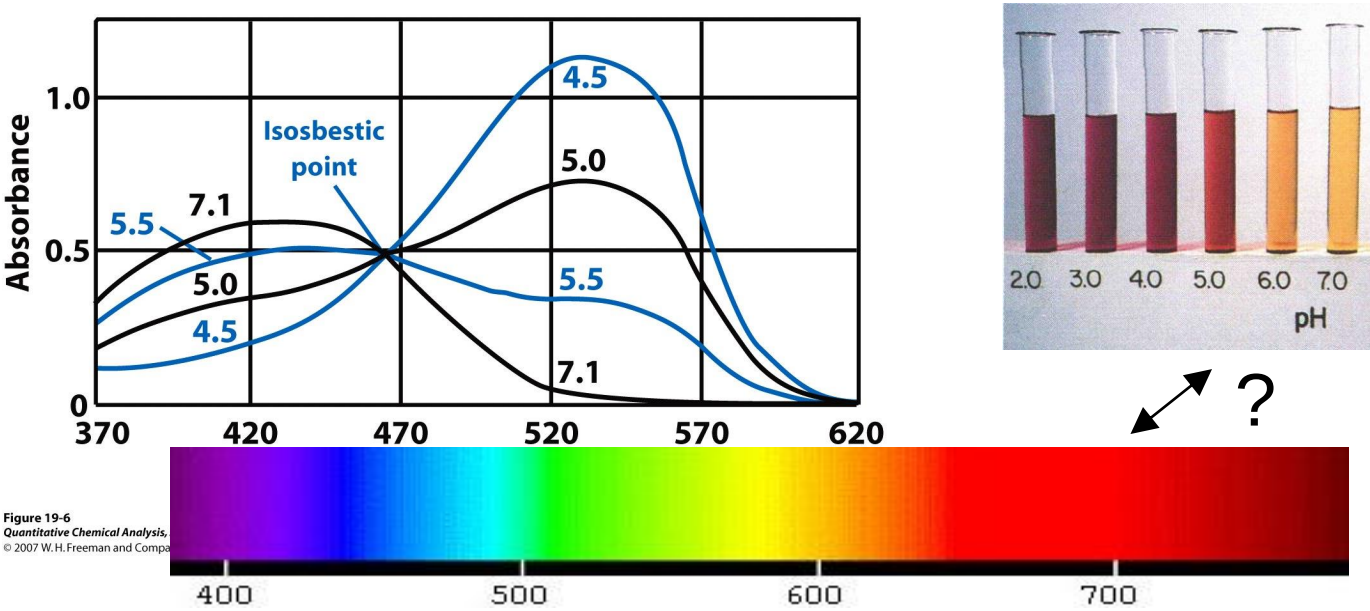
Why does it have a color?



HIn (red)

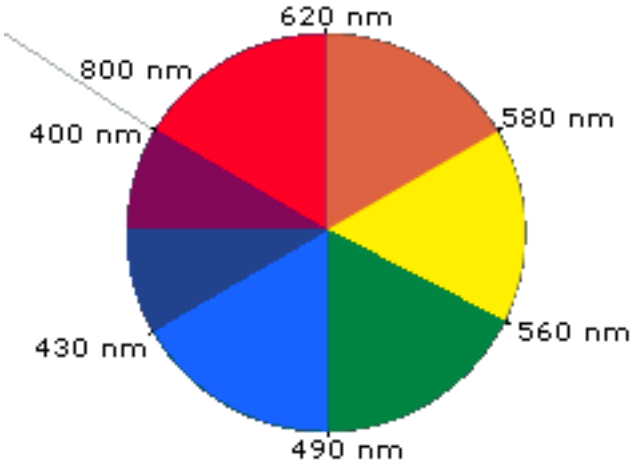
In^- (yellow)

Spectrum of methyl red

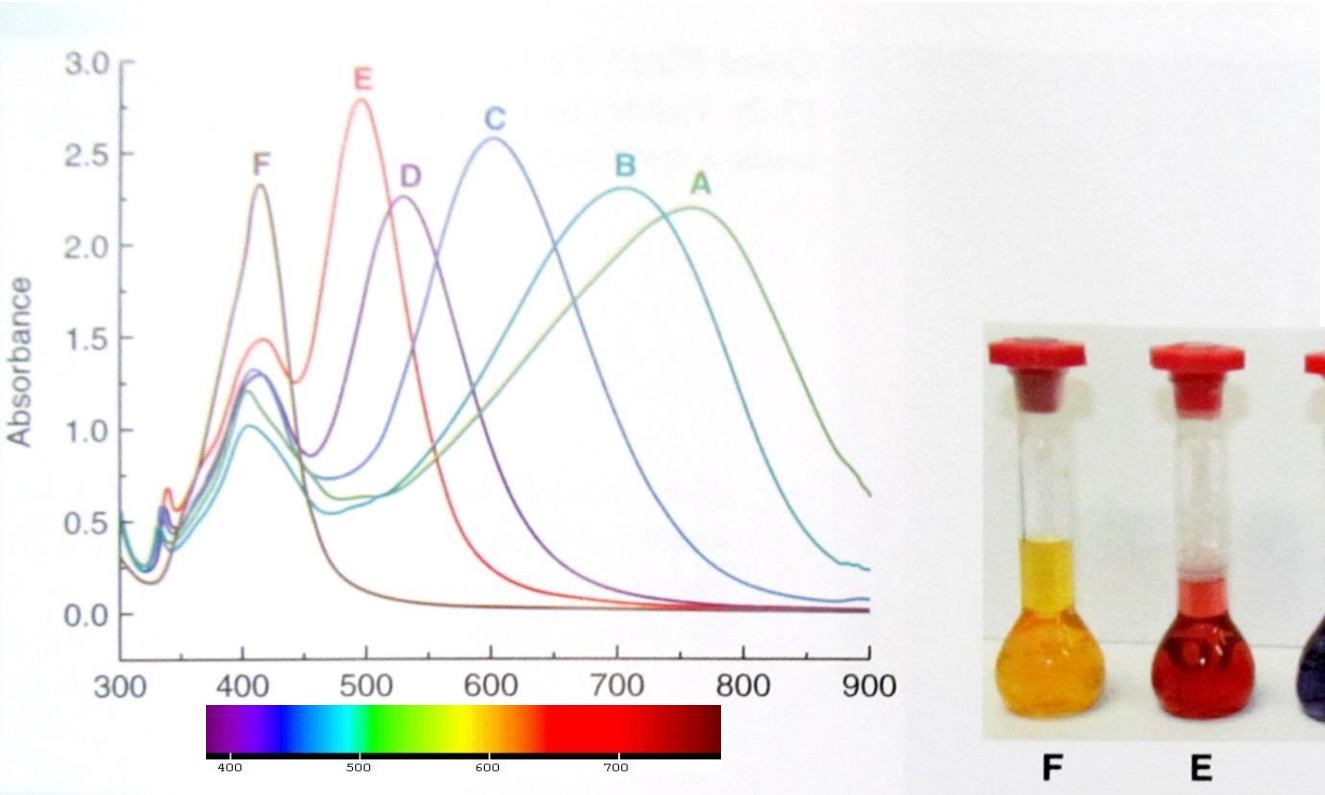


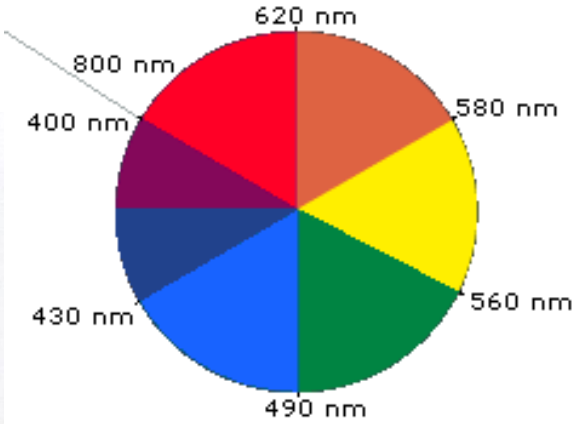
?

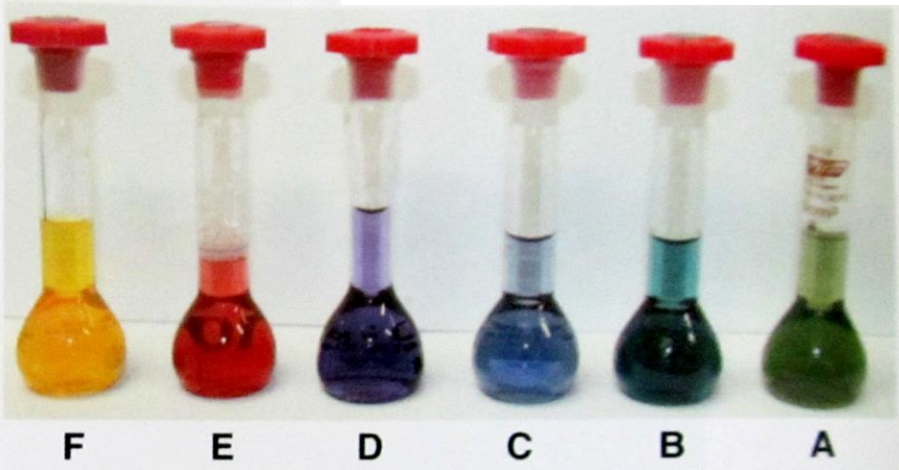
- The absorbed colors can not see! One can see only that colors, which was not absorbed by the sample – **complementary** colors.



Absorption spectra of silver nanoparticles







Color Plate 15 **Absorption Spectra and Color (Section 17-2 and Problem 17-9)** Flasks contain suspensions of silver nanoparticles whose color depends on the size and shape of the particles, which are approximately triangular plates with edge lengths of ~50–100 nm. The visible absorption spectrum of each suspension is shown in the graph. Stable suspensions of nanoparticles are called *colloids* (Demonstration 26-1). [From D. M. Ledwith, A. M. Whelan, and J. M. Kelly, *J. Mater. Chem.* **2007**, *17*, 2459. Courtesy J. M. Kelly and D. Ledwith, Trinity College, University of Dublin.]

Signal-to-noise ratio, spectrum integration

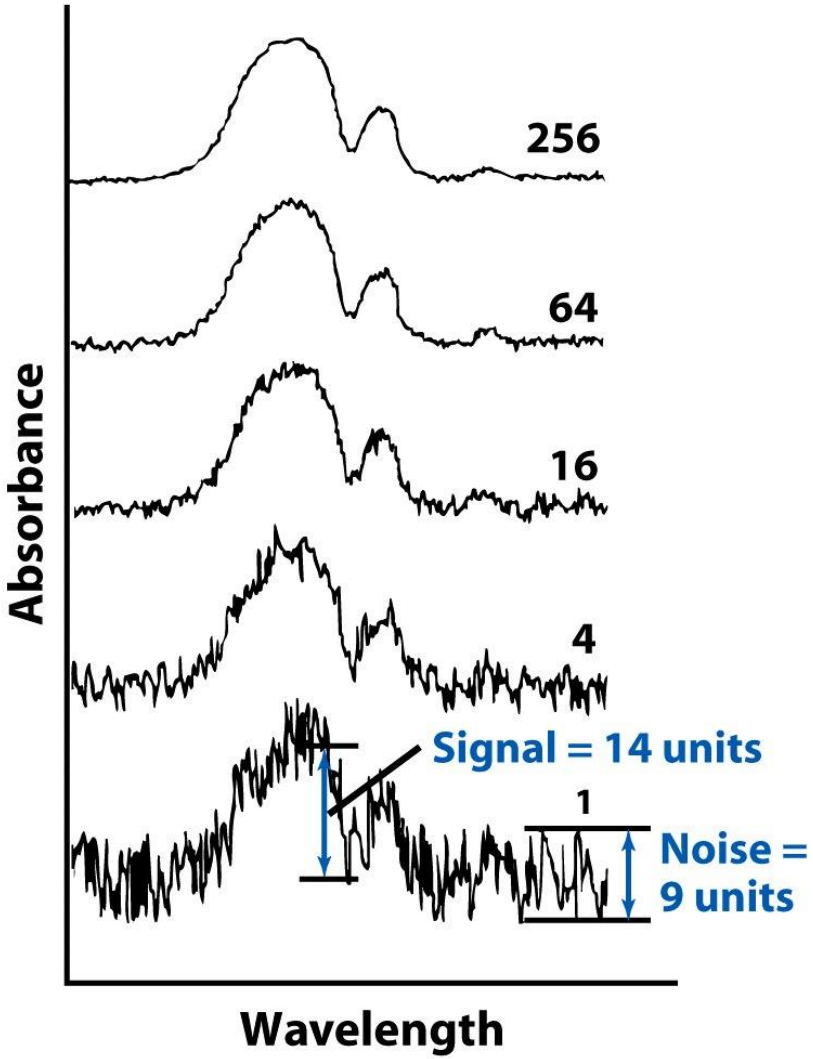
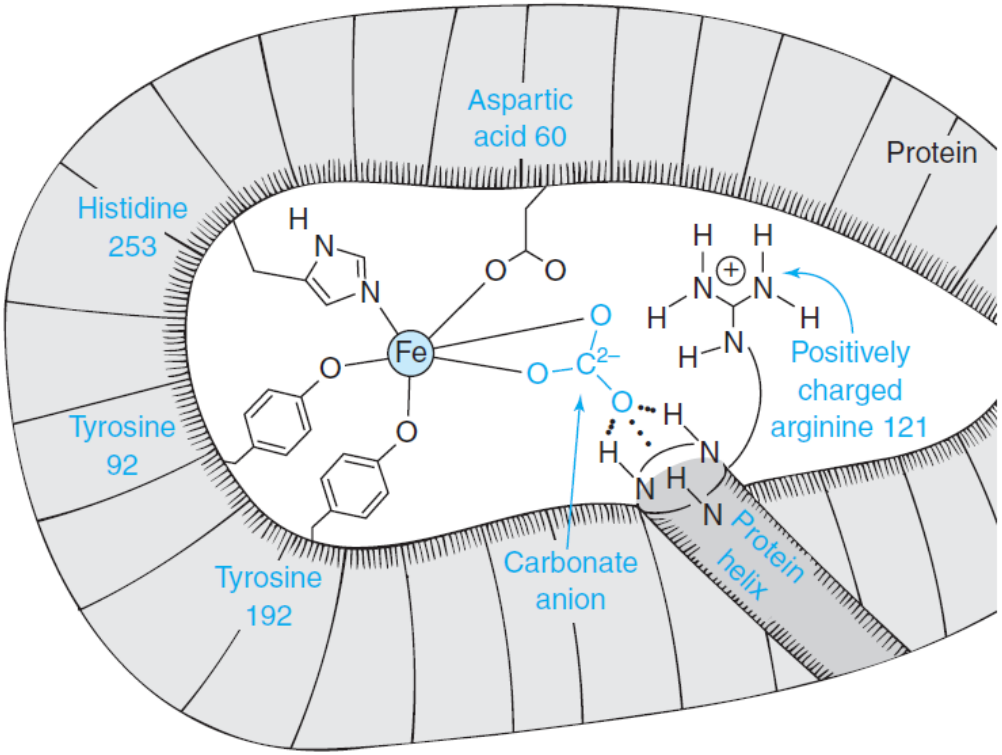


Figure 20-30
Quantitative Chemical Analysis, Seventh Edition

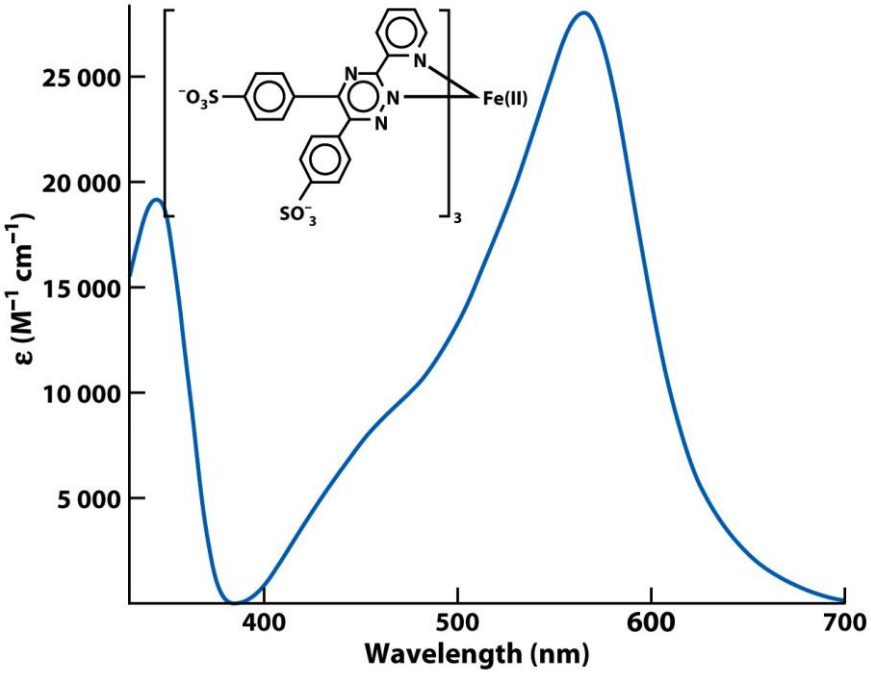
Measuring iron in blood

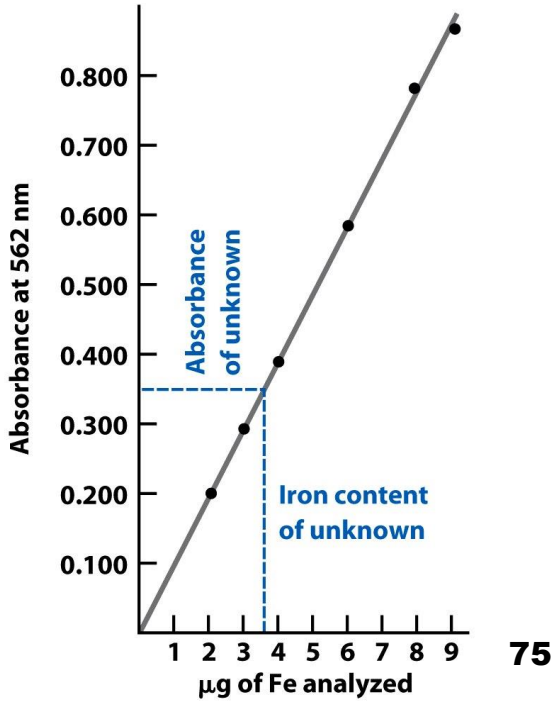
- Fe^{3+} is binded to transferrin (protein), aprox. 1 µg/ml in serum
- Cells in blood and proteins are disturbing the absorption measurement,
- Fe concentration is low, but forming a complex will have more intensive color – can be easily measure.



Measuring iron in blood

1. $Fe^{3+} \rightarrow Fe^{2+}$ reduction e.g. hydroxyamine hydrochloride ($NH_3OH^+Cl^-$), transferrin releases it.
2. Protein precipitation by trichloraacetic acid (Cl_3C-COOH), centrifuging
3. Adding excess ferrozin to the known volume of supernatant, purple complex is formed, measuring at 562 nm.
4. Measuring blank, calibration, calculating the unknown's iron content



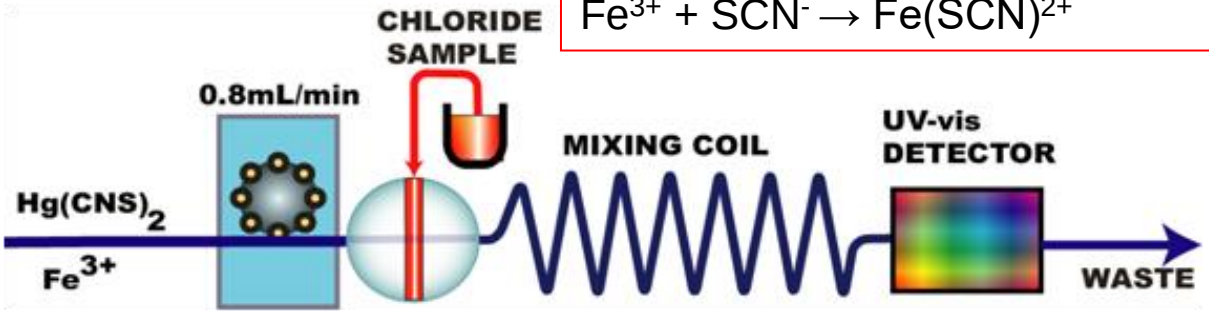


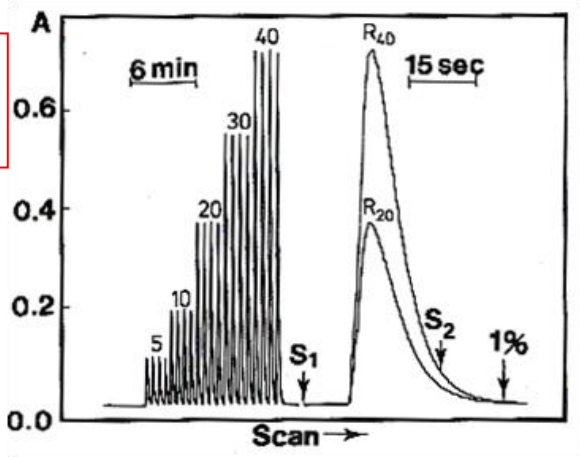
Special applications

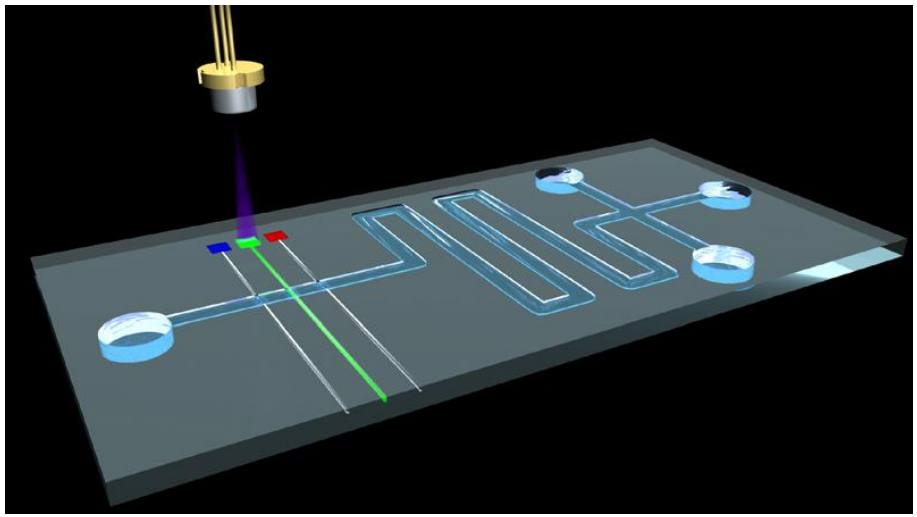
Flow Injection Analysis – determination of chlorid-ion

$Hg(SCN)_2 + 2\ Cl^- \rightarrow HgCl_2 + 2\ SCN^-$

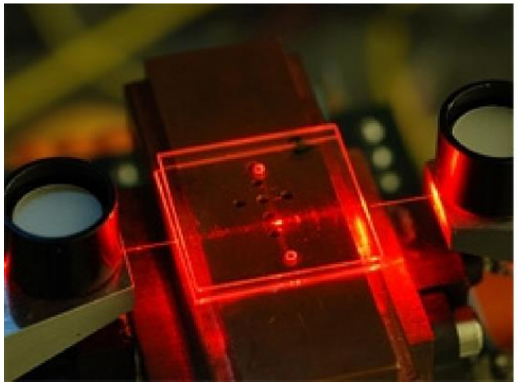
$Fe^{3+} + SCN^- \rightarrow Fe(SCN)^{2+}$

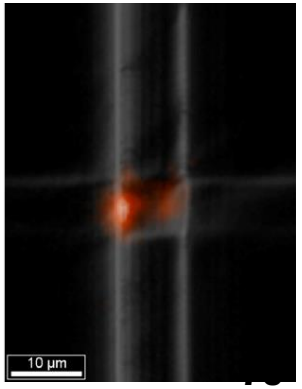






Microfluidics – Lab-on-a-chip





Evaluation of the UV-Vis spectrophotometry

- Advantages
  - High sensitivity
  - High precision
  - Simple
  - Fast
  - Low sample need
  - Can be used as the sample does not have enough absorption, because by oxidation, reduction or complex formation the absorption can be increase.
- Disadvantages
  - Need to have some information about the component in the sample
  - A spectrophotometric method need to check with other analytical methods.
  - Sample preparation can be lengthy
- Advantageous to use in case or **series measurement**.

Fields of application

Molecular spectroscopy (in UV-Vis range)

- „everywhere”
- individually as UV-Vis spectroscopic methods
- present in several instrument as a detector
  - LC, HPLC, capillary electrophoresis – DAD (diode array detector)
- „biological photometers”
  - DNS, RNS, protein measurements