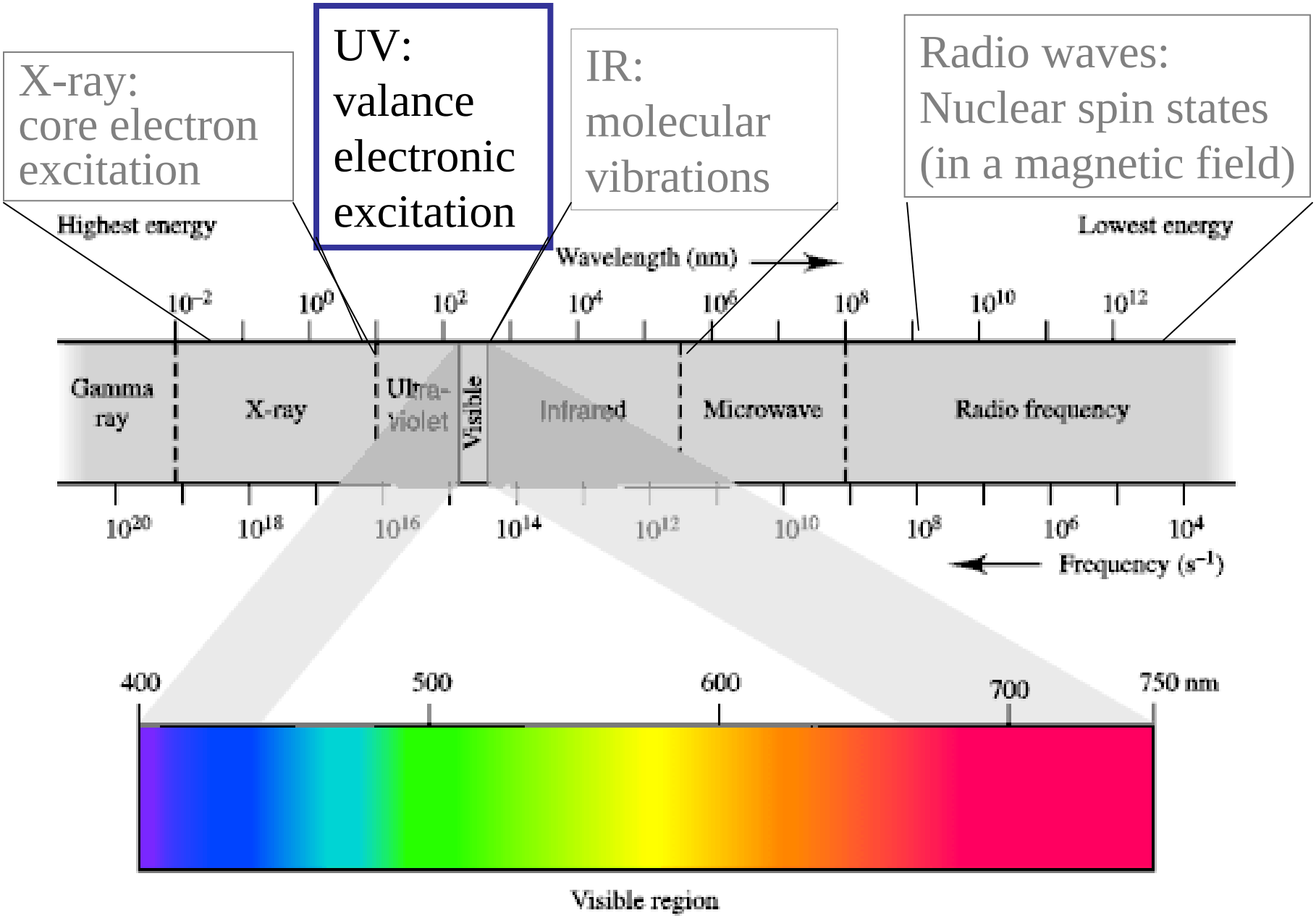
SPECTROSCOPY

Light interacting with matter as an analytical tool

Electronic Excitation by UV/Vis Spectroscopy :



Spectroscopic Techniques and Chemistry they Probe		
UV-vis	UV-vis region	bonding electrons
Atomic Absorption	UV-vis region	atomic transitions (val. e-)
FT-IR	IR/Microwave	vibrations, rotations
Raman	IR/UV	vibrations
FT-NMR	Radio waves	nuclear spin states
X-Ray Spectroscopy	X-rays	inner electrons, elemental
X-ray Crystallography	X-rays	3-D structure

Spectroscopic Techniques and Common Uses		
UV-vis	UV-vis region	Quantitative analysis/Beer's Law
Atomic Absorption	UV-vis region	Quantitative analysis Beer's Law
FT-IR	IR/Microwave	Functional Group Analysis
Raman	IR/UV	Functional Group Analysis/quant
FT-NMR	Radio waves	Structure determination
X-Ray Spectroscopy	X-rays	Elemental Analysis
X-ray Crystallography	X-rays	3-D structure Anaylysis

Different Spectroscopies

- UV-vis – electronic states of valence e/d-orbital transitions for solvated transition metals
- Fluorescence – emission of UV/vis by certain molecules
- FT-IR – vibrational transitions of molecules
- FT-NMR – nuclear spin transitions
- X-Ray Spectroscopy – electronic transitions of core electrons

Spectroscopies in the UV-Vis region

- UV-vis (molecular) absorption spectroscopy
- Solvated metal complexes
- Molecular fluorescence
- Atomic absorption
- Atomic Emission

UV-vis (molecular) absorption spectroscopy

- Quantitative/Beer's Law
- Valence electronic transitions
- Molecular orbital theory
- Effect of conjugation
- Broadness of the spectra (solvent effect and superimposition of vibrational/rotational levels)
- Instrumentation
- Molecular Probes/biokits based on visible absorption spectroscopy
- Examples of some molecular probes

Quantitative Spectroscopy

- Beer's Law

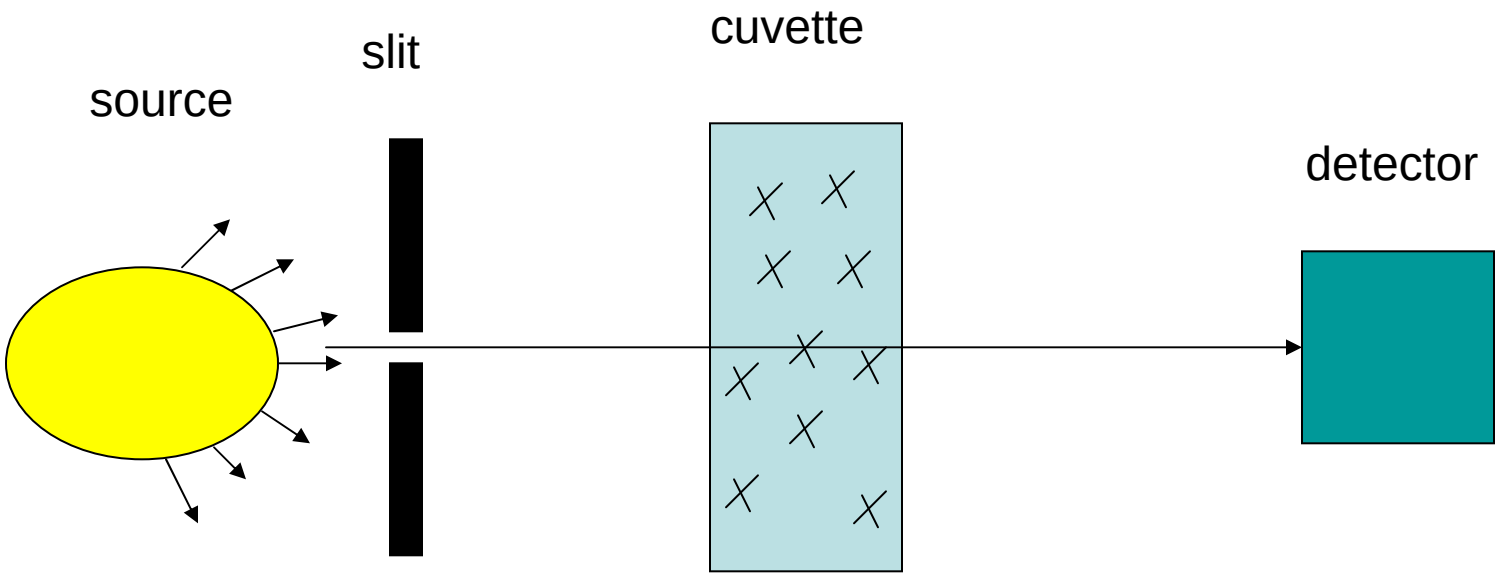
  $A_{\lambda 1} = e_{\lambda 1} bc$

  e is molar absorptivity (unique for a given compound at λ_1)

  b is path length

  c concentration

Beer's Law



- $A = -\log T = \log(P_0/P) = ebc$
- $T = P_{solution}/P_{solvent} = P/P_0$

- $A = -\log T = \log(P_0/P) = ebc$
  - e is molar absorptivity (L/mol·cm)
  - B is the path length (cm)
  - T is the transmittance
  - $T = P_{solution}/P_{solvent} = P/P_0$
- Works for monochromatic light
- Compound x has a unique e at different wavelengths

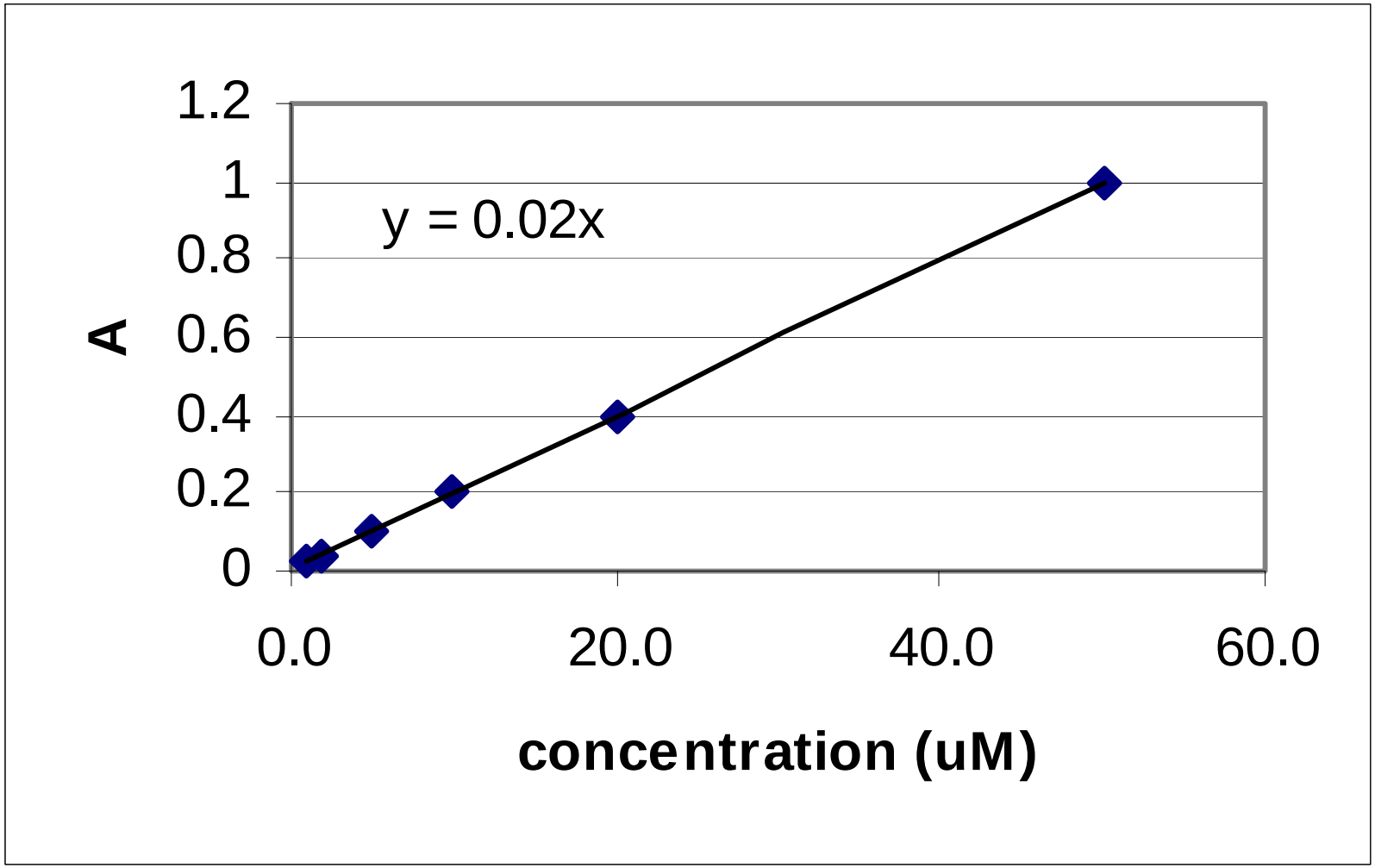
Characteristics of Beer's Law Plots

- One wavelength
- Good plots have a range of absorbances from 0.010 to 1.000
- Absorbances over 1.000 are not that valid and should be avoided
- 2 orders of magnitude

Standard Practice

- Prepare standards of known concentration
- Measure absorbance at λmax
- Plot A vs. concentration
- Obtain slope
- Use slope (and intercept) to determine the concentration of the analyte in the unknown

Typical Beer's Law Plot



UV-Vis Spectroscopy

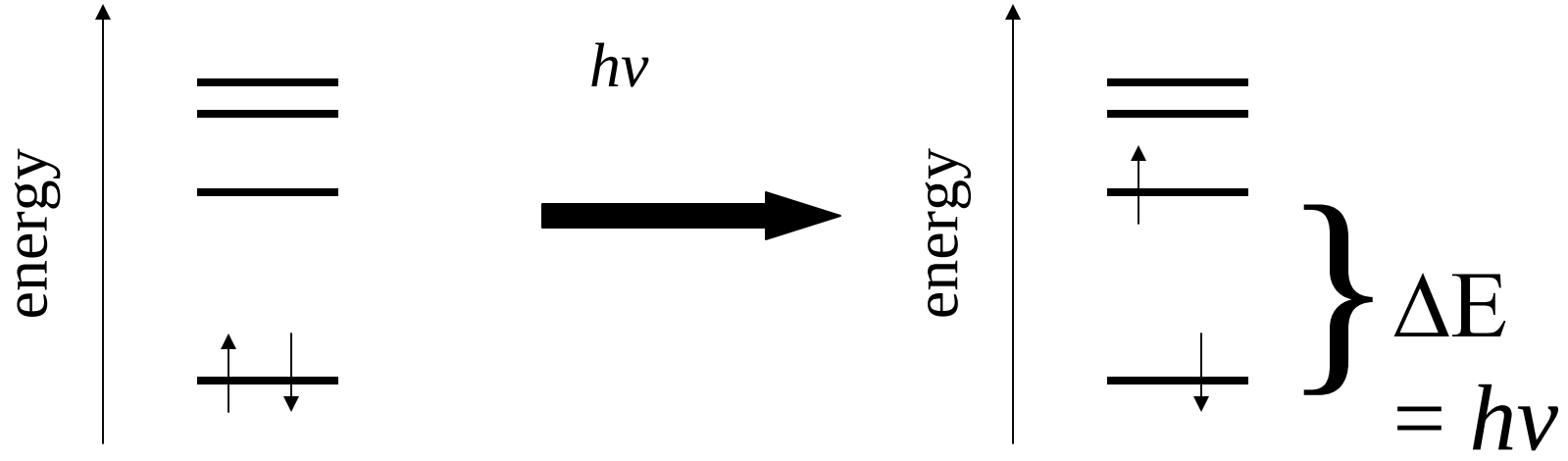
- UV- organic molecules
  - Outer electron bonding transitions
  - conjugation
- Visible – metal/ligands in solution
  - d-orbital transitions
  - Dyes – very high level of conjugation
- Instrumentation

Characteristics of UV-Vis spectra of Organic Molecules

- Absorb mostly in UV unless highly conjugated
- Spectra are broad, usually to broad for qualitative identification purposes
- Excellent for quantitative Beer's Law-type analyses
- The most common detector for an HPLC

Molecules have quantized energy levels:

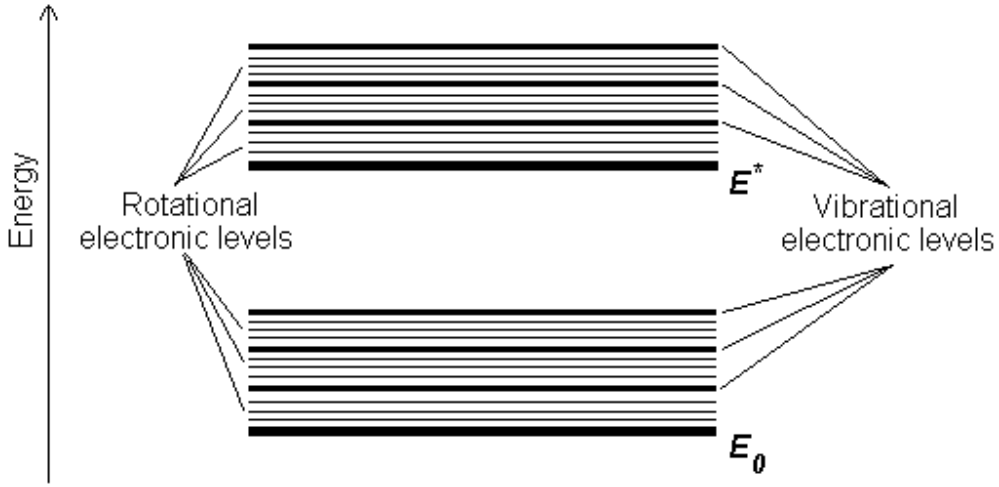
ex. electronic energy levels.



Q: Where do these quantized energy levels come from?

A: The electronic configurations associated with bonding.

Each electronic energy level (configuration) has associated with it the many vibrational energy levels we examined with IR.



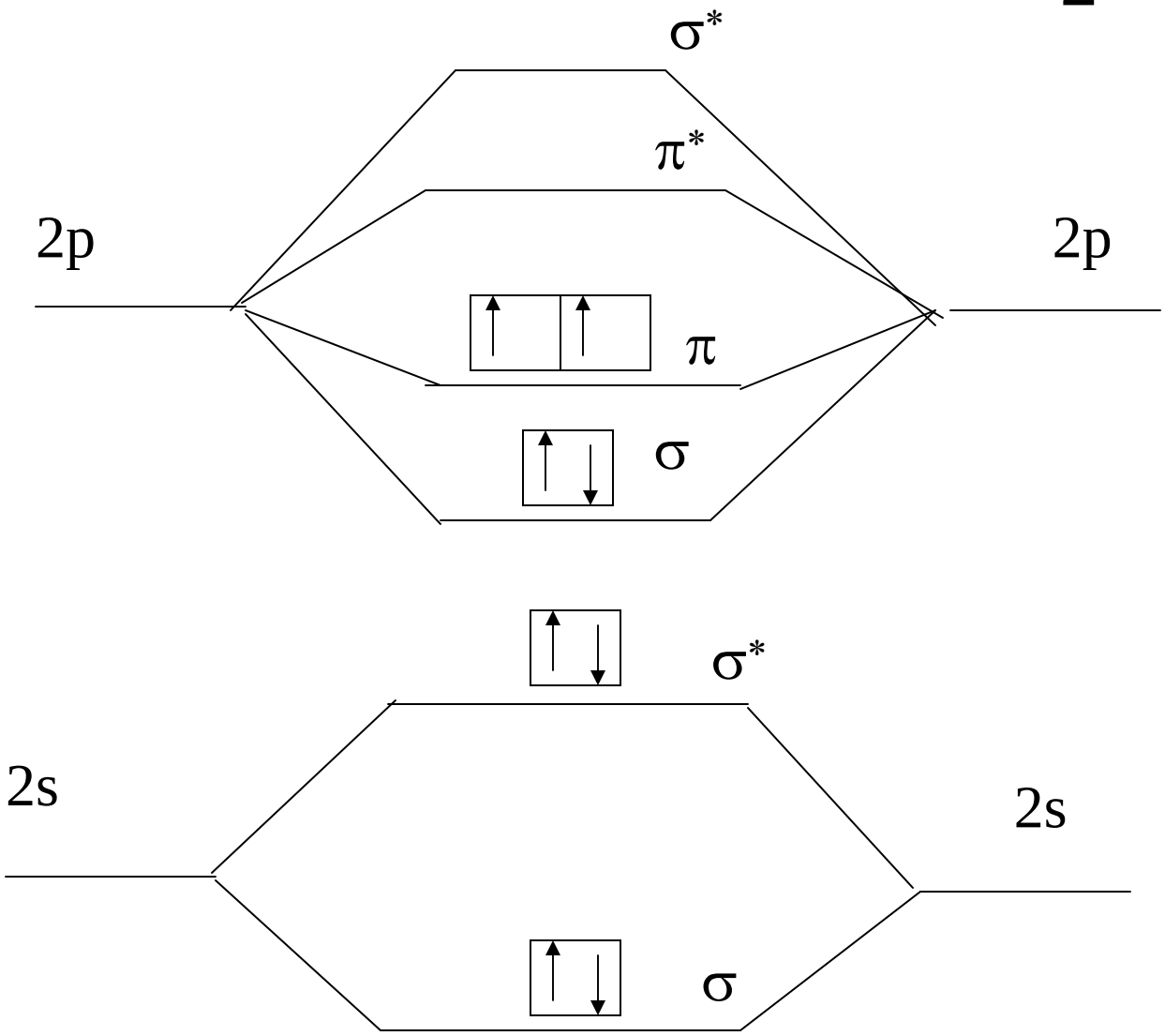
Broad spectra

- Overlapping vibrational and rotational peaks
- Solvent effects

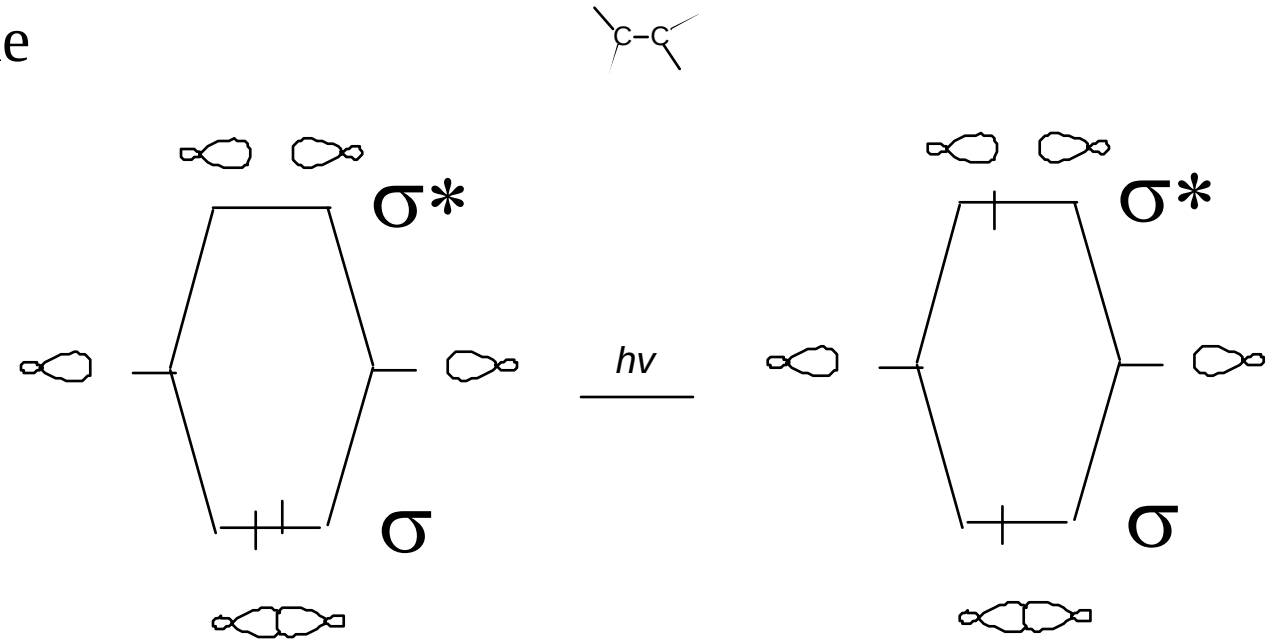
Molecular Orbital Theory

- Fig 18-10

Molecular Orbital for O_2



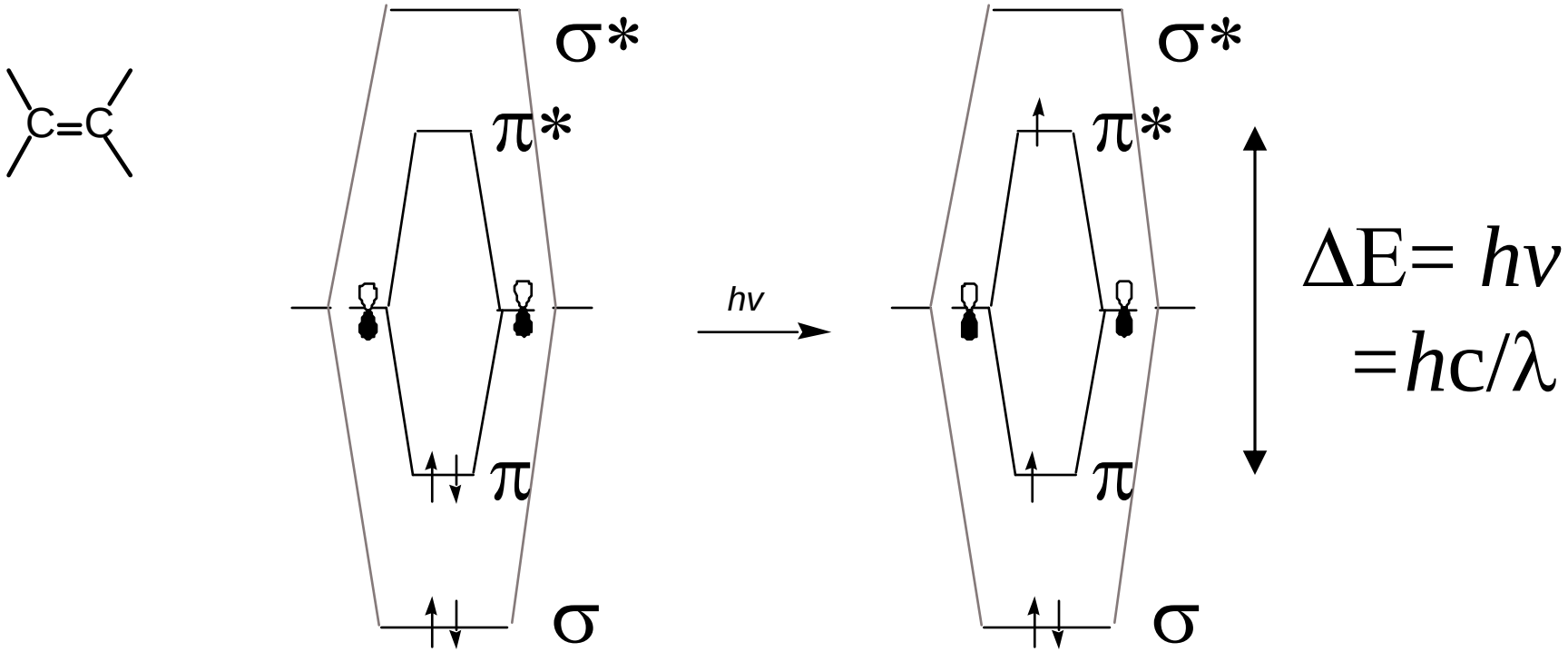
Ethane



$$\sigma \longrightarrow \sigma^*$$

λ_{max} = 135 nm (a high energy transition)

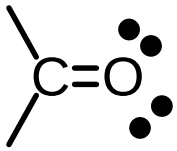
Absorptions having λ_{max} < 200 nm are difficult to observe because everything (including quartz glass and air) absorbs in this spectral region.

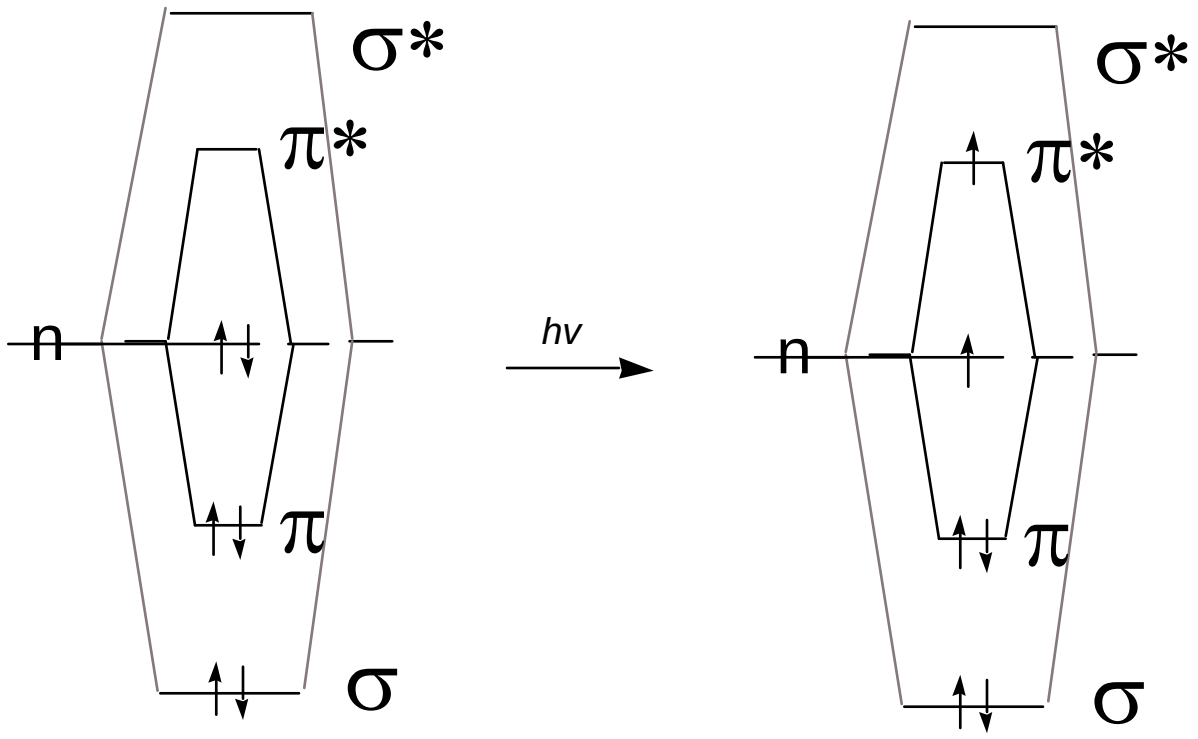


$\pi \longrightarrow \pi^*$

Example: ethylene absorbs at longer wavelengths:

λ_{max} = 165 nm ε= 10,000



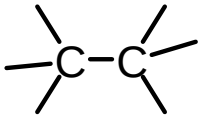
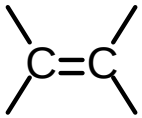
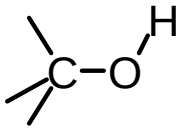
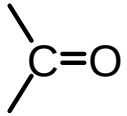


n ⟶ π*

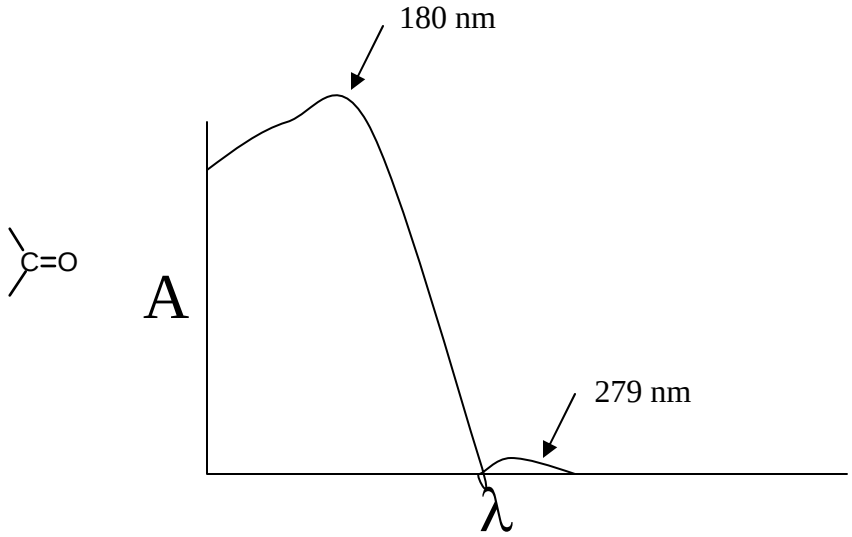
The n to pi* transition is at even lower wavelengths but is not as strong as pi to pi* transitions. It is said to be “forbidden.” Example:

Acetone: n–σ* λ_{max} = 188 nm ; ε= 1860

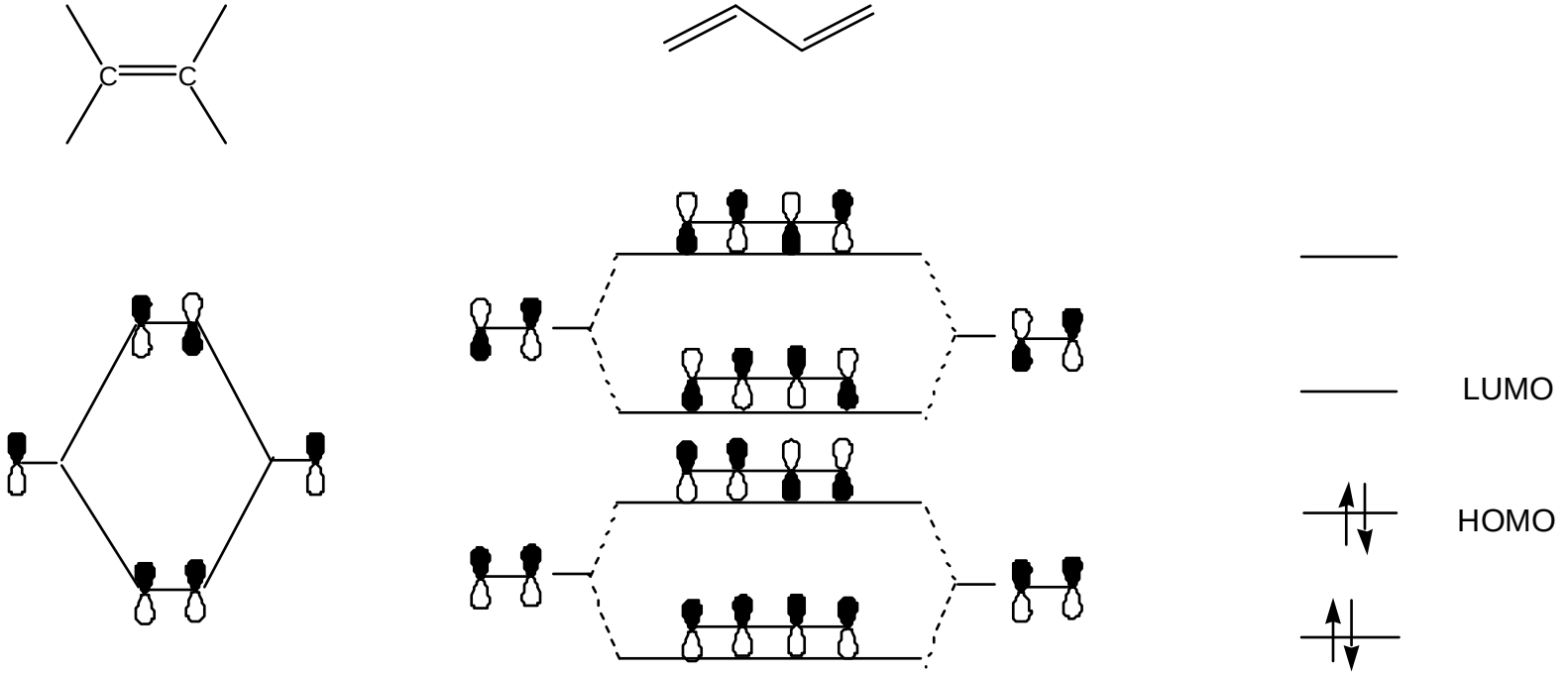
n–π* λ_{max} = 279 nm ; ε= 15

	σ –> σ*	135 nm	
	π –> π*	165 nm	
	n –> σ*	183 nm	weak
	π –> π* n –> σ* n –> π*	150 nm 188 nm 279 nm	 weak

---



Conjugated systems:



Preferred transition is between Highest Occupied Molecular Orbital (HOMO) and Lowest Unoccupied Molecular Orbital (LUMO).

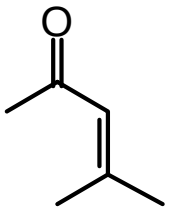
Note: Additional conjugation (double bonds) lowers the HOMO-LUMO energy gap:

Example:

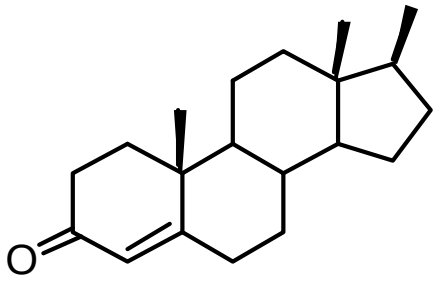
1,3 butadiene: λ_{max} = 217 nm ; ε= 21,000

1,3,5-hexatriene λ_{max} = 258 nm ; ε= 35,000

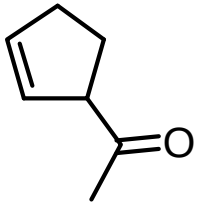
Similar structures have similar UV spectra:



λ_{max} = 238, 305 nm

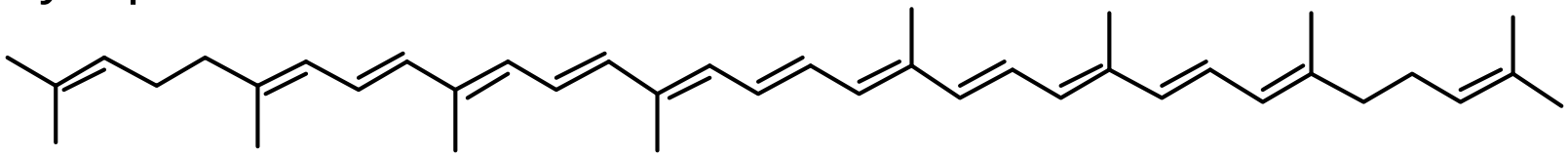


λ_{max} = 240, 311 nm



λ_{max} = 173, 192 nm

Lycopene:

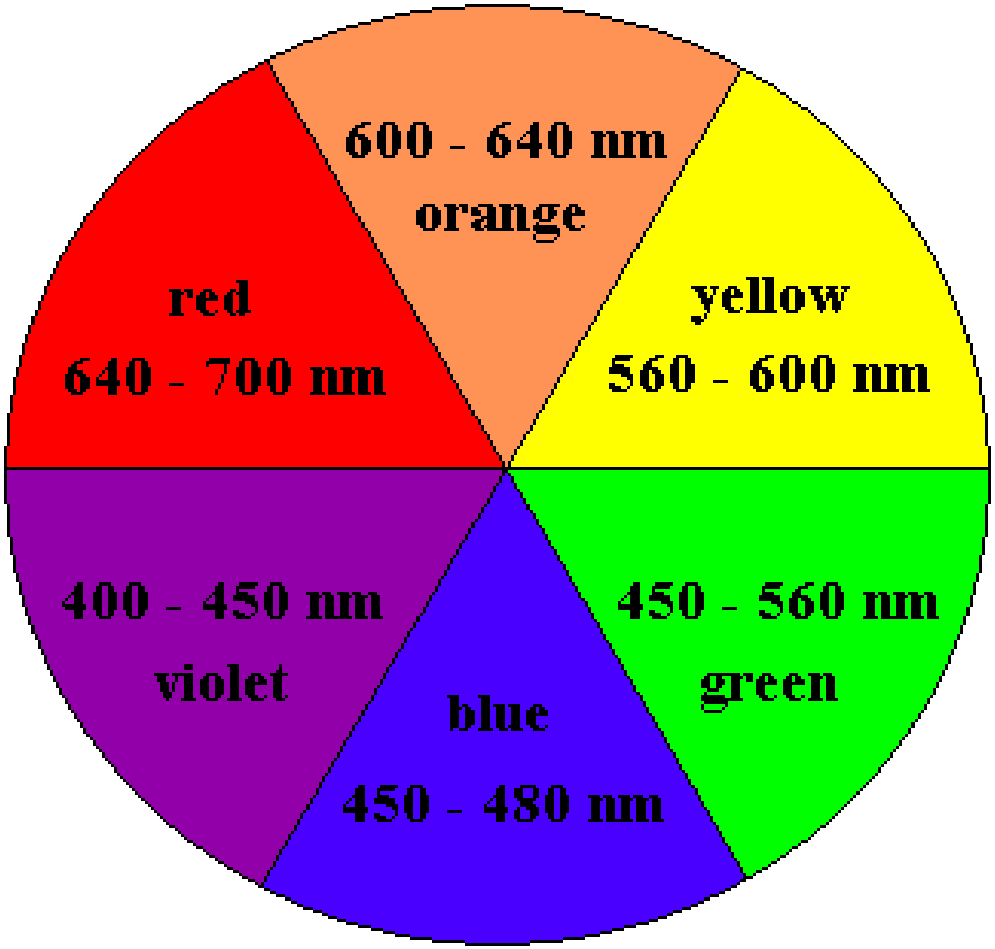


λ_{max} = 114 + 5(8) + 11*(48.0-1.7*11) = 476 nm

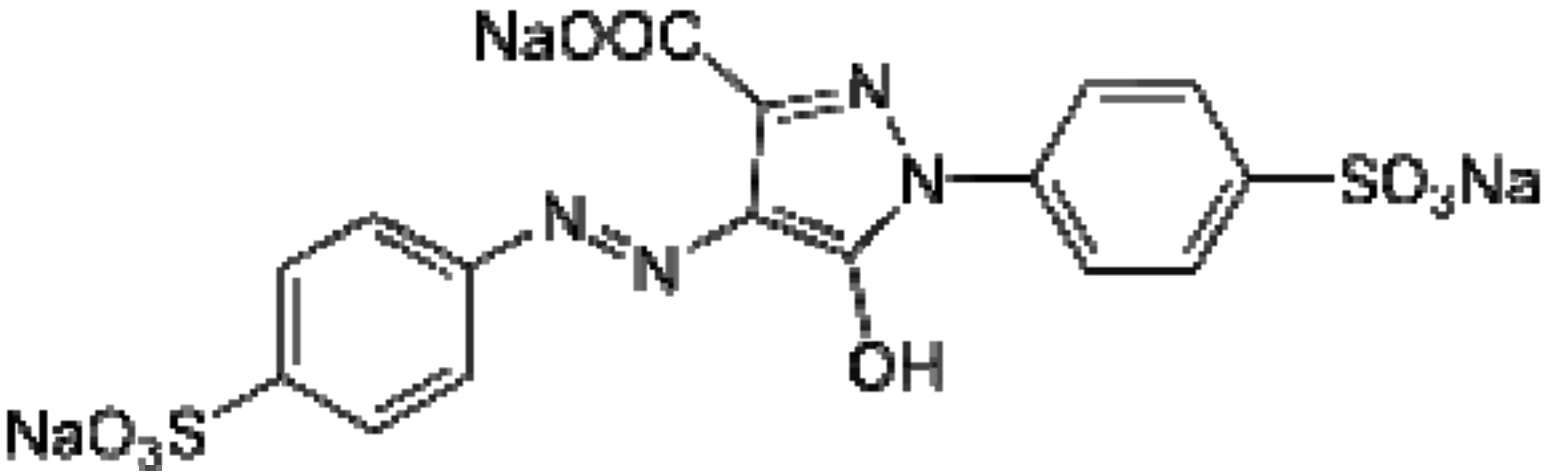
λ_{max}(Actual) = 474.

A solution of lycopene appears orange because it absorbs blue

Color wheel

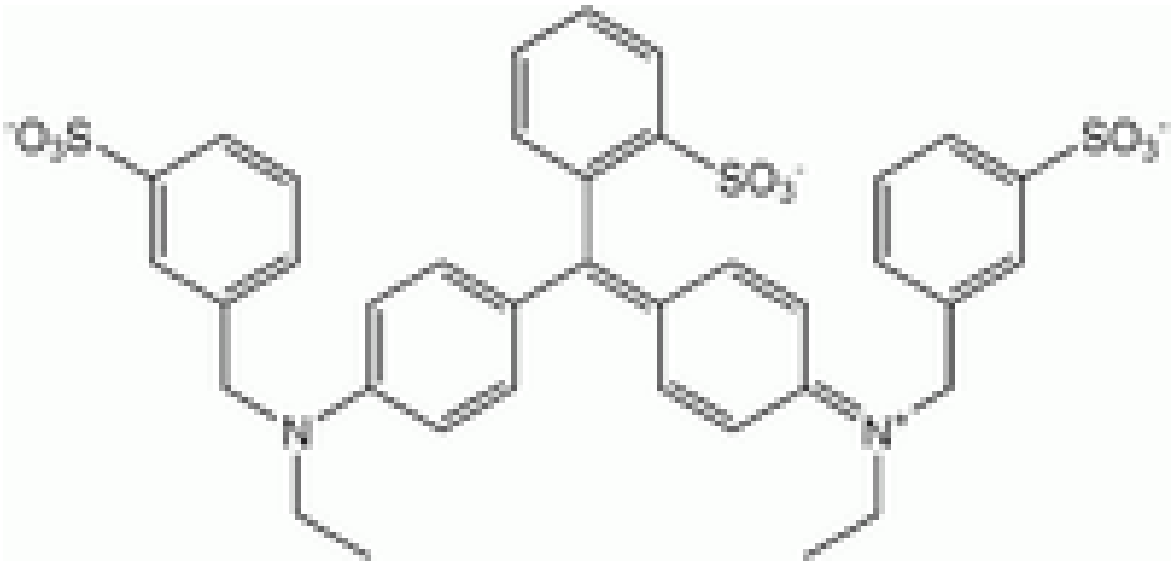

600 - 640 nm
orange
red
640 - 700 nm
yellow
560 - 600 nm
400 - 450 nm
violet
blue
450 - 480 nm
450 - 560 nm
green

Yellow food coloring
Tartrazine



$\lambda_{max} = 427$ nm

Blue food coloring
Brilliant Blue

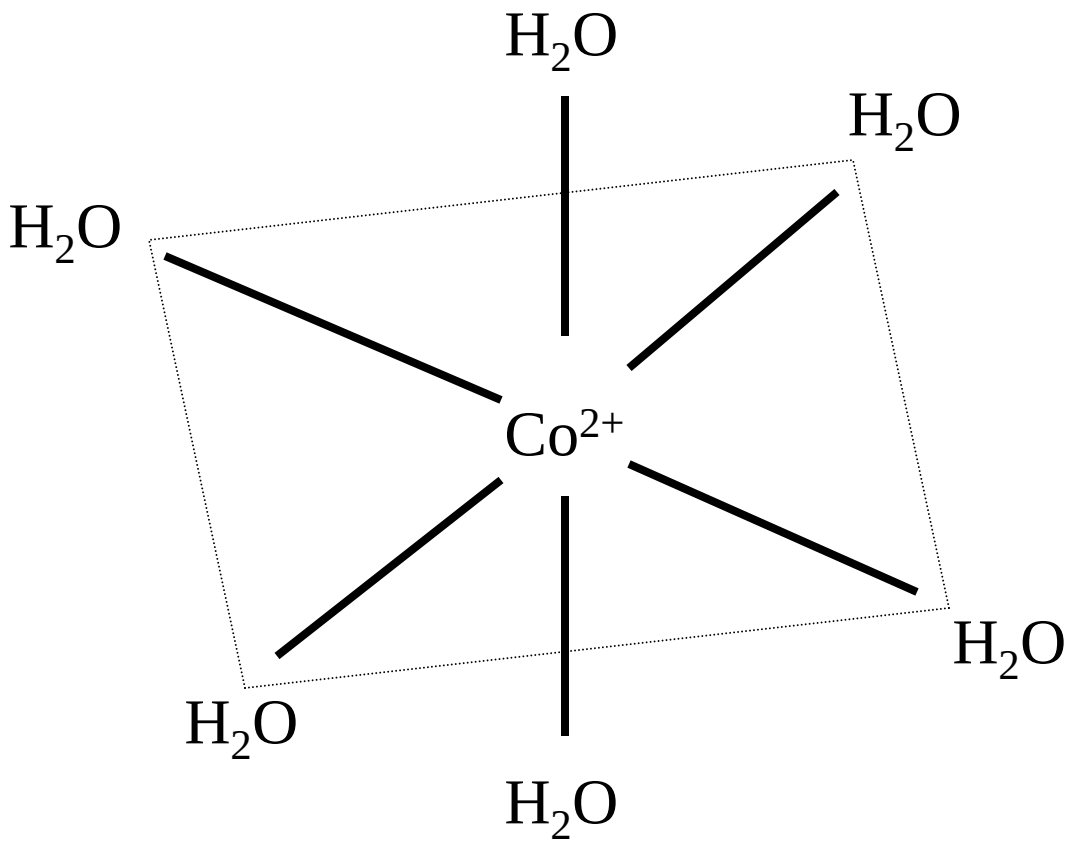


$\lambda_{max} = 628$ nm

Solvated Metal Ions

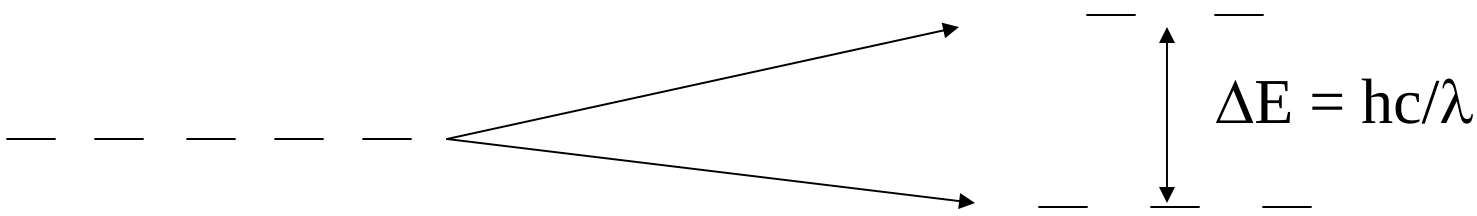
- The Spatial arrangements of ligands around a solvated metal ion causes distortion of the d-orbital
- Consequence: d-orbital splitting
- The ΔE is often in the visible range of the spectrum

Octahedral Geometry



d-orbital splitting

Max. absorbs around 490 nm, solution appears pale pink



Degenerate
D-orbitals
of naked Co

d-orbitals
of hydrated Co^{2+}

Octahedral Configuration

Molecular Probes or TAGs

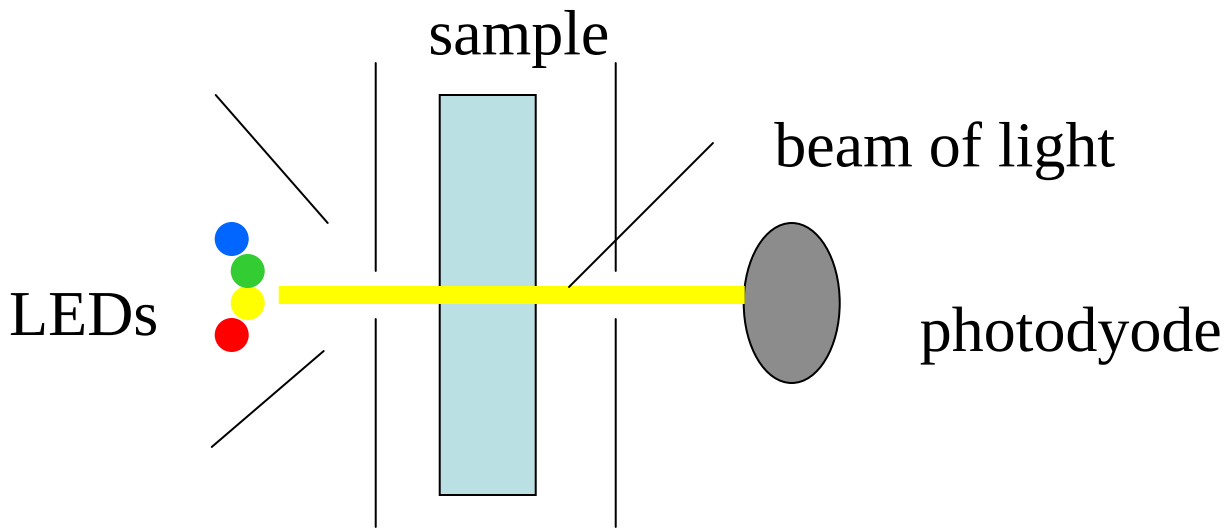
- Chemical reagents that have a detectable handle that can selectively bind to analyte
- Example/ BCA Total protein assay
  - combines reduction of Cu^{2+} to Cu^{+} by protein in an alkaline medium with the highly sensitive and selective colorimetric detection of the cuprous cation (Cu^{+}) by bicinchoninic acid
  - Biuret rxn; chelation complex between amino groups and Cu^{+} (light blue)
  - Cu^{+}/BCA forms a purple complex, intense absorption at 562 nm
  - Microplate reader

Instrumentation

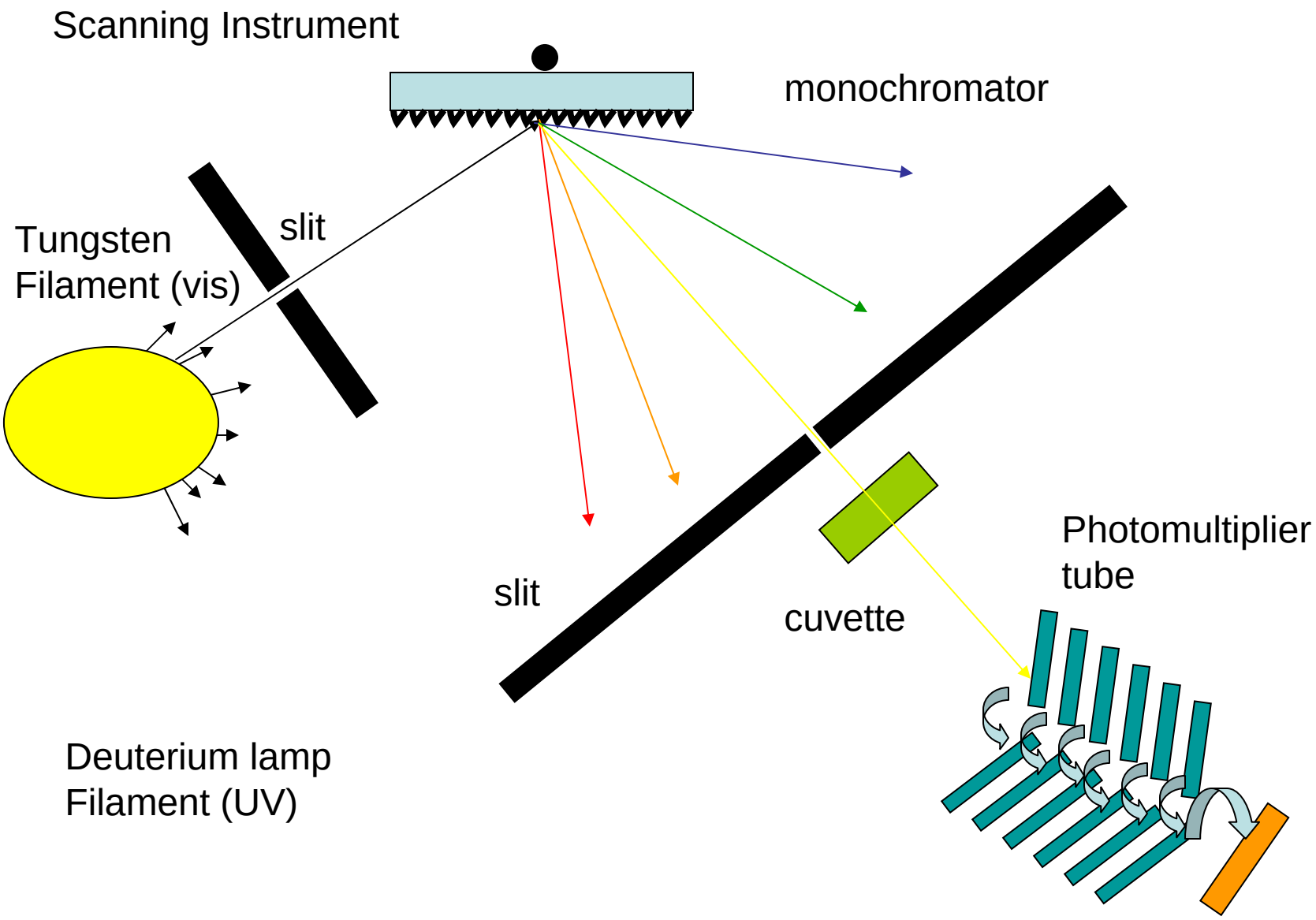
- Fixed wavelength instruments
- Scanning instruments
- Diode Array Instruments

Fixed Wavelength Instrument

- LED serve as source
- Pseudo-monochromatic light source
- No monochrometer necessary/ wavelength selection occurs by turning on the appropriate LED
- 4 LEDs to choose from



Scanning Instrument



sources

- Tungten lamp (350-2500 nm)
- Deuterium (200-400 nm)
- Xenon Arc lamps (200-1000 nm)

Monochromator

- Braggs law, $n\lambda = d(\sin i + \sin r)$
- Angular dispersion, $dr/d\lambda = n / d(\cos r)$
- Resolution, $R = \lambda/\Delta\lambda = nN$, resolution is extended by concave mirrors to refocus the divergent beam at the exit slit

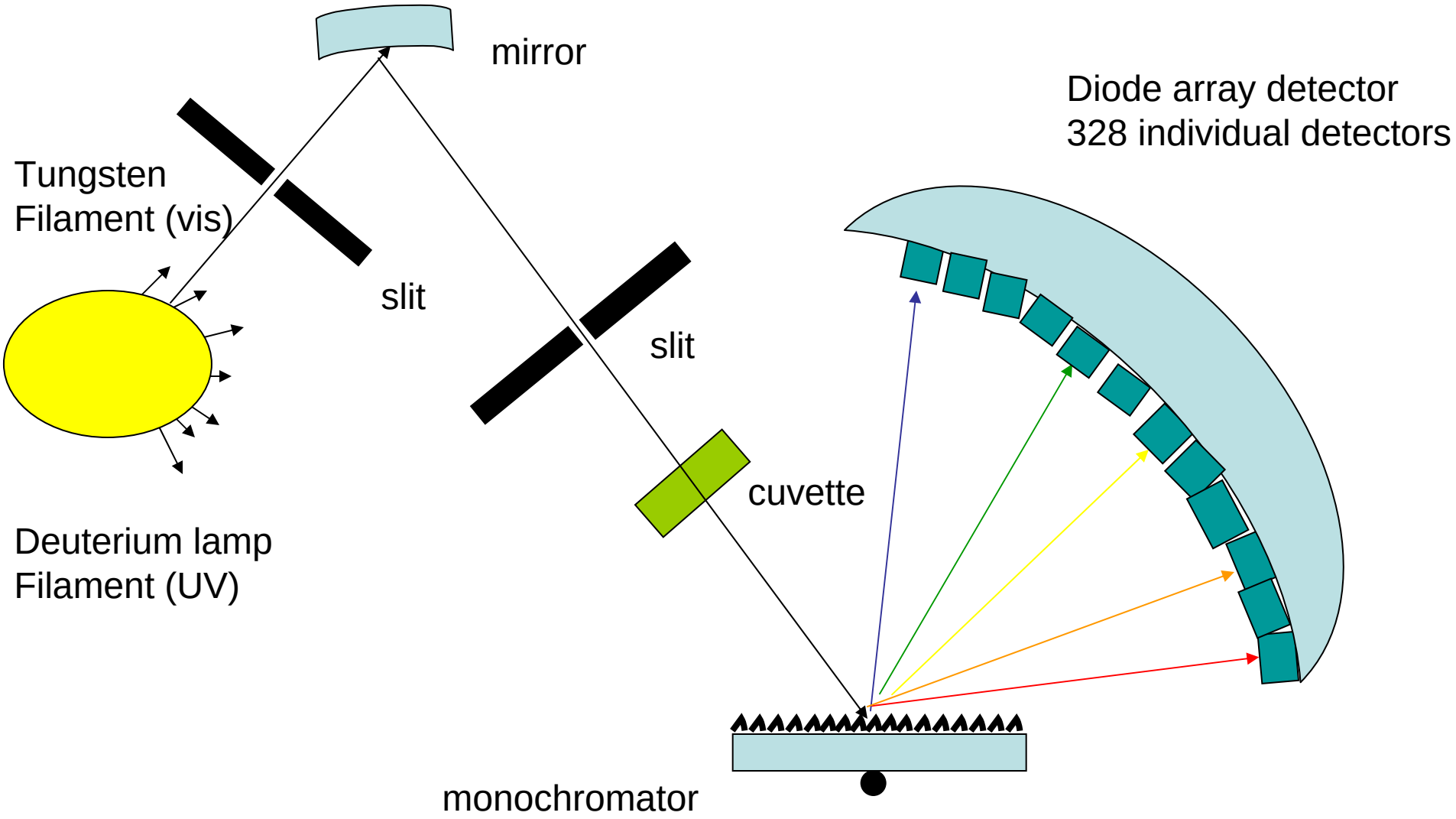
Sample holder

- Visible; can be plastic or glass
- UV; you must use quartz

Single beam vs. double beam

- Source flicker

Diode array Instrument



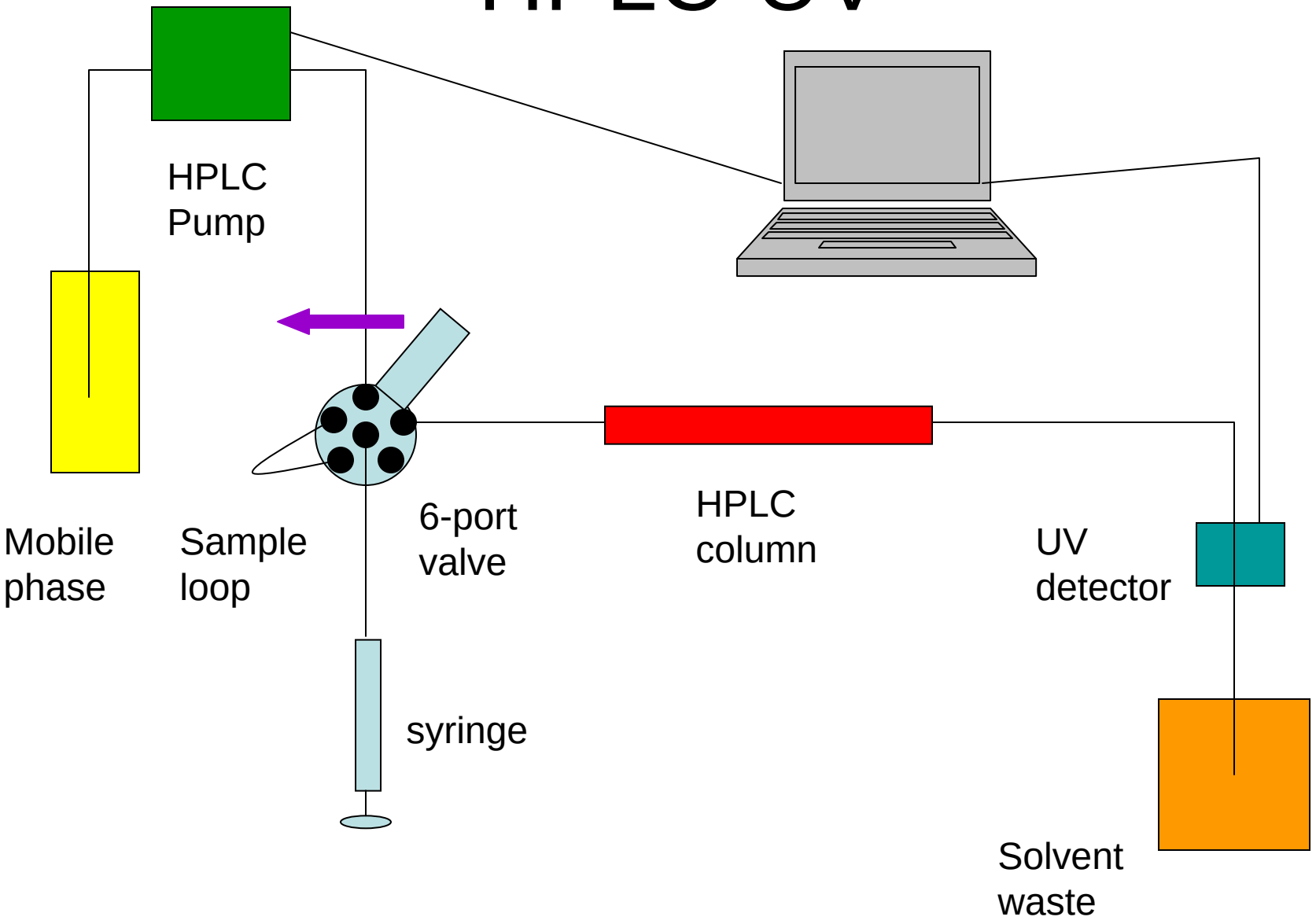
Advantages/disadvantages

- Scanning instrument
  - High spectral resolution (63000), $\lambda/\Delta\lambda$
  - Long data acquisition time (several minutes)
  - Low throughput
- Diode array
  - Fast acquisition time (a couple of seconds), compatible with on-line separations
  - High throughput (no slits)
  - Low resolution (2 nm)

High Performance Liquid Chromatography (HPLC)

- Separation of mixtures
- Because UV-VIS spectra are so broad, components of a mixture need to be separated prior to detection
- Marriage between HPLC and UV/vis detector
  - HPLC separates components
  - Each component can be detected and quantified by UV-VIS detector

HPLC-UV


HPLC
Pump
Mobile
phase
Sample
loop
6-port
valve
HPLC
column
UV
detector
syringe
Solvent
waste