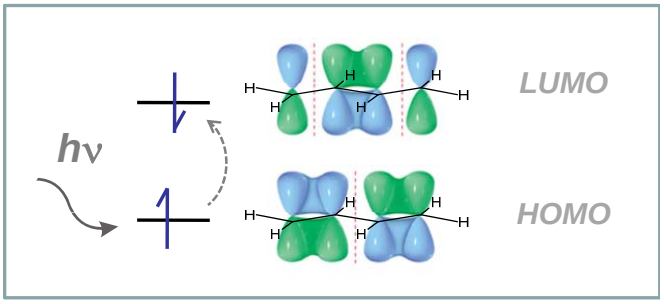
UV-Visible Spectroscopy

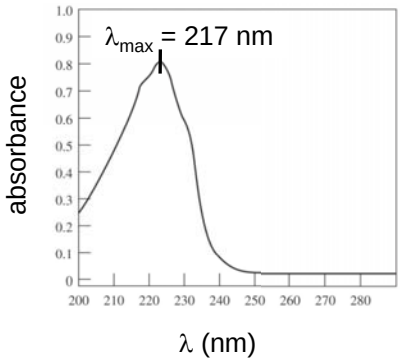
Electronic excitation spectroscopy:

Photon absorption promotes an electron from its ground state to an excited state.



Goal:

Spectrum relating absorbance to photon energy/wavelength.



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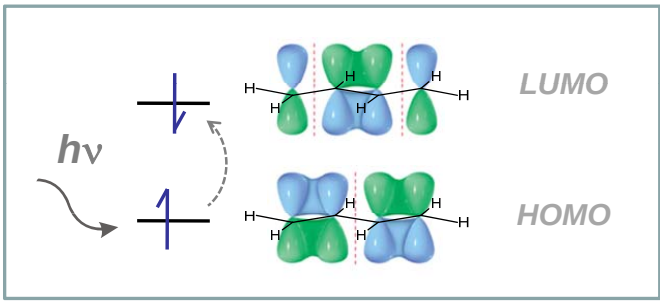


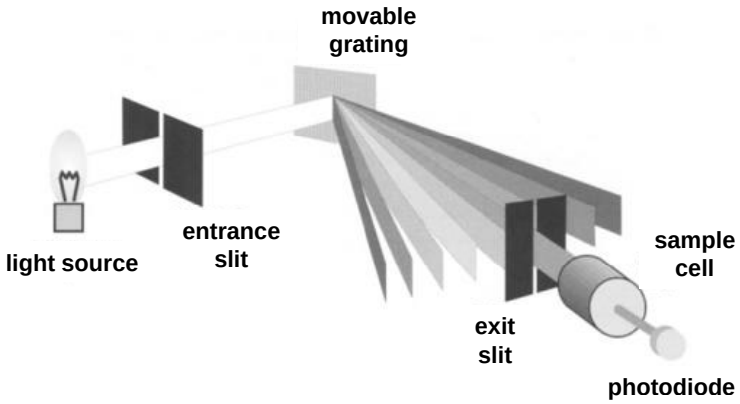
Applications of UV-vis absorption to organic chemists:

- Characterizing chromophores (absorbing functional groups)
- Tuning absorbance detectors in chromatography
- Observing molecules on fluorescent TLC plates

UV/Vis Spectrophotometers

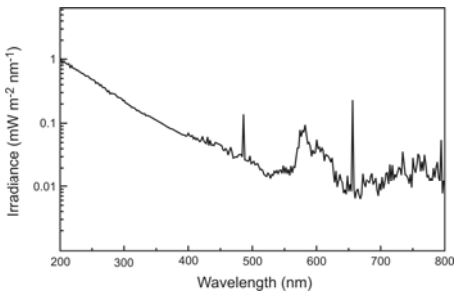
Old style:

Movable grating.

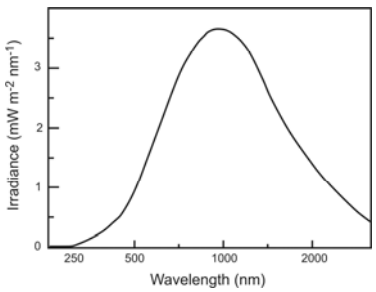


Light Sources:

deuterium lamp



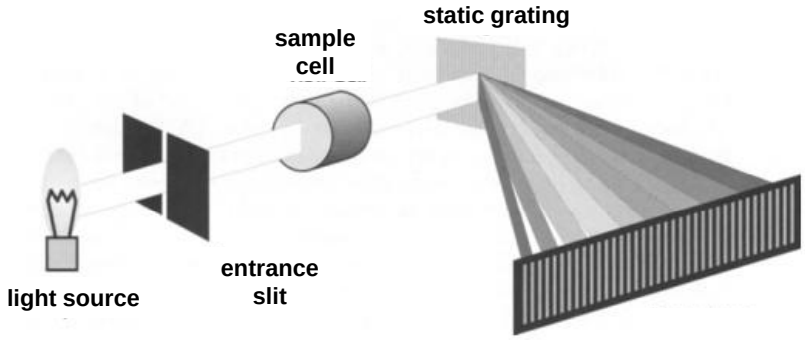
tungsten lamp



UV/Vis Spectrophotometers

New style:

Diode array.



photodiode array
(containing 100s-1000s of array elements)

- No moving parts!
- Each element of the array collects at a different wavelength.
- On-chip integrators sum intensity over a range of positions.
- Extremely fast, so easy to use in chromatography detection.

UV/Vis Spectrophotometry Instruments

Benchtop vs. configurable

Can adapt fiber optic devices to lots of applications.

Beer's Law and Concentration

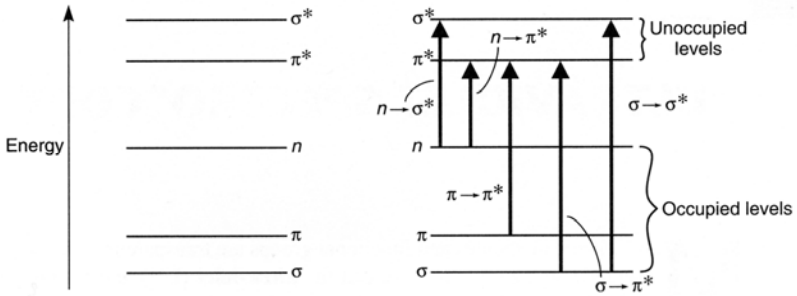
$$A_\lambda = \varepsilon_\lambda \bullet c \bullet \ell$$

- ℓ: *path length* (*cm*)
- A_λ: *measured absorbance;* $A = \log(I_0/I)$ (*unitless*)
- ε_λ: *molar absorptivity (extinction coefficient)* ($M^{-1}\ cm^{-1}$)
- c: *concentration* (*M*)

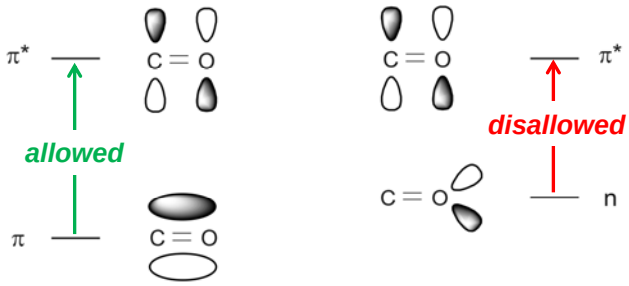
"ε" is often reported at λ_{max}, wavelength of maximum absorbance.

UV-vis Transitions

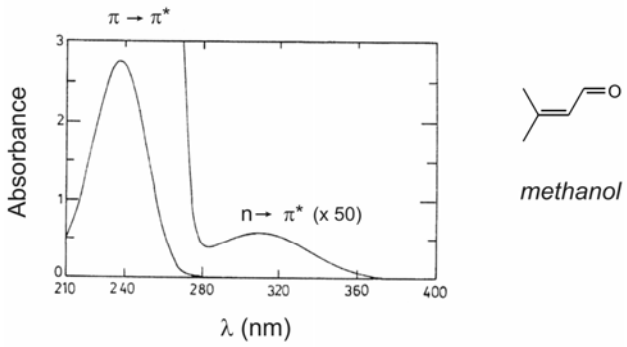
All possible excitations may be observed…

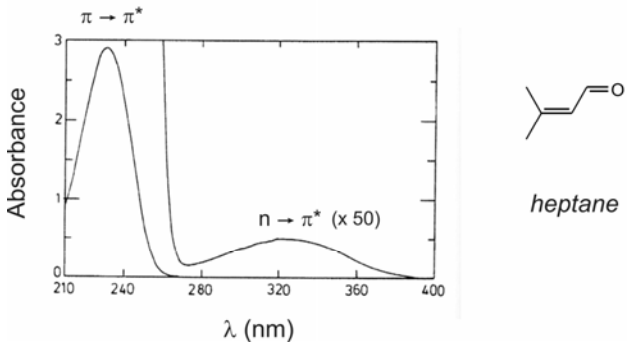


…but intensity (allowed/ disallowedness) dictated by symmetry, overlap.

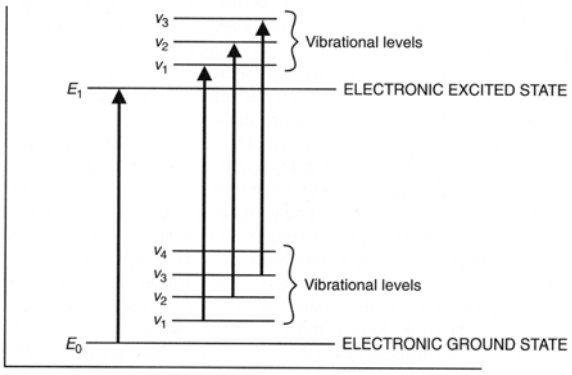


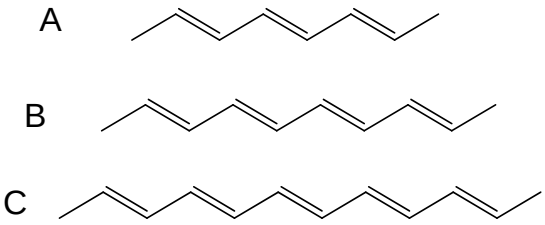
UV-vis Transitions

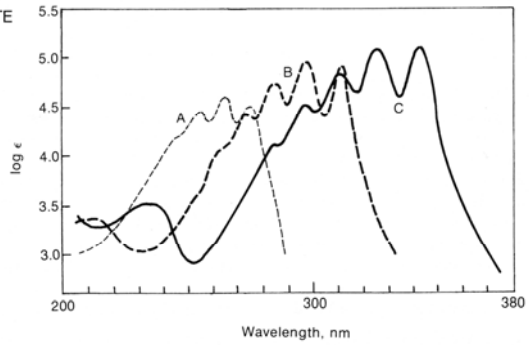




Vibrational Structure in UV-vis







Chromophore	*Compound*	*Transition*	λ_{max} (nm)	ε
C–H	CH_4	σ→σ*	122	
C–C	C_2H_6	σ→σ*	135	
C=C	C_2H_4	π→π*	103	15000
			174	5500
C=C=C	C_3H_4	π→π*	170	4000
			227	630
C≡C	R–C≡C–R´	π→π*	178	10000
			196	2000
			223	160
C–O	R–O–R	*n*→σ*	180	500
C–O	R–O–R´	*n*→σ*	180	3000
C–N	Amino	*n*→σ*	190-200	2500-4000
C–S	R–S–H	*n*→σ*	195	1800
C–S	R–S–R	*n*→σ*	235	180
C=O	Aldehyde/Ketone	*n*→σ*	166	16000
		π→π*	189	900
		n→π*	270	10-20
C=O	Carboxylic acid	*n*→π*	200	50
C=O	Carboxylate	*n*→π*	210	150
C=O	Ester	*n*→π*	210	50
C=O	Amide	*n*→π*	205	200
C=N	$(NH_2)_2C{=}NH$	*n*→π*	265	15
C≡N	$CH_3C{\equiv}N$	π→π*	<170	
N=N	Me–N=N–Me	*n*→π*	350-370	15
N=O	Me_3NO	*n*→π*	300	100
			665	120
N=O	Me_3NO_2	*n*→π*	276	27
C=C=O	$Et_2C{=}C{=}O$	π→π*	227	360
		n→π*	375	20
C–Cl		*n*→σ*	173	200
C–Br		*n*→σ*	208	300
C–I		*n*→σ*	259	400

Bond excitations evaluated on tables.

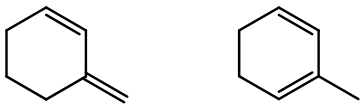
Typical Absorption Maxima

λ_{max} is increased by:

extent of conjugation

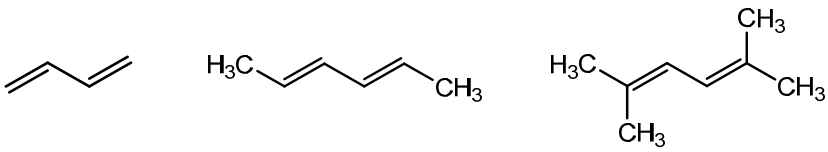
λ_{max} = 171 nm 217 nm 258 nm

forced *s-cis*-arrangement of dienes



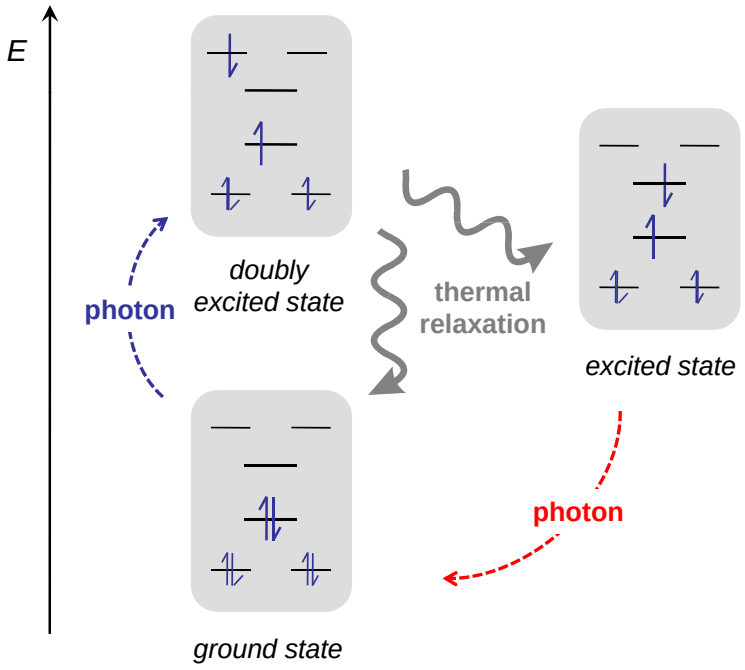
λ_{max} = 232 nm 261 nm

alkyl substitution



λ_{max} = 217 nm 227 nm 241 nm

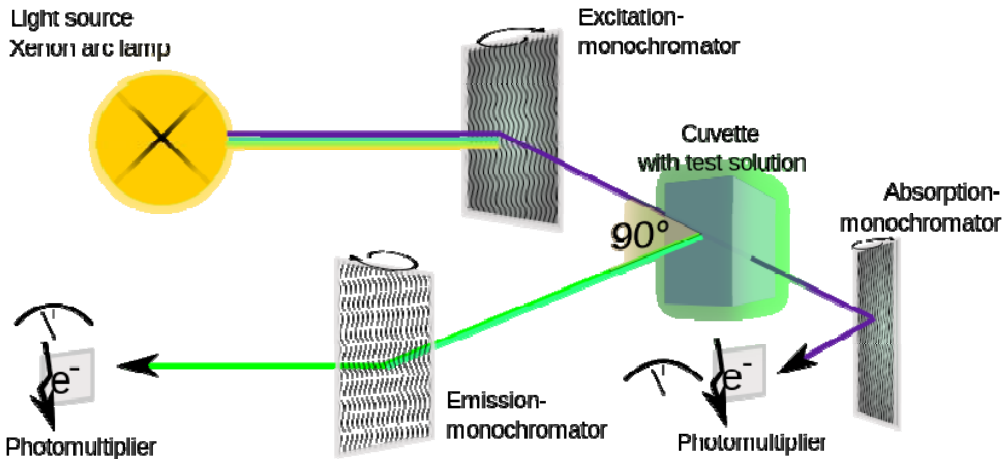
Fluorescence from Excited States



Sometimes, molecule can absorb photon that excites electron above the HOMO.

Thermal relaxation may not dispose of all excess energy; some energy may be re-emitted as (fluorescent) light.

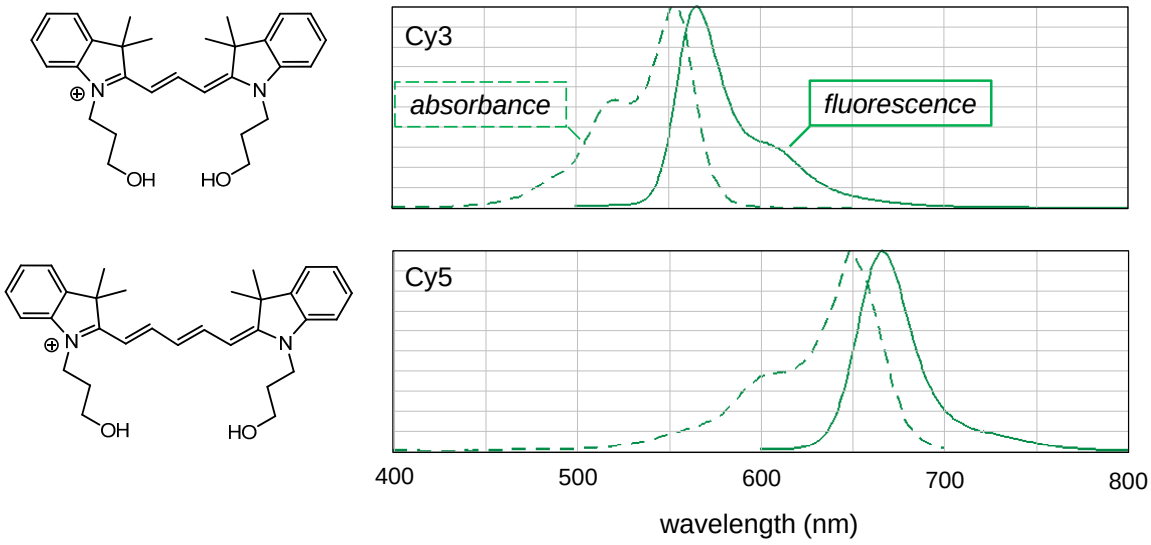
Fluorimeter Configuration



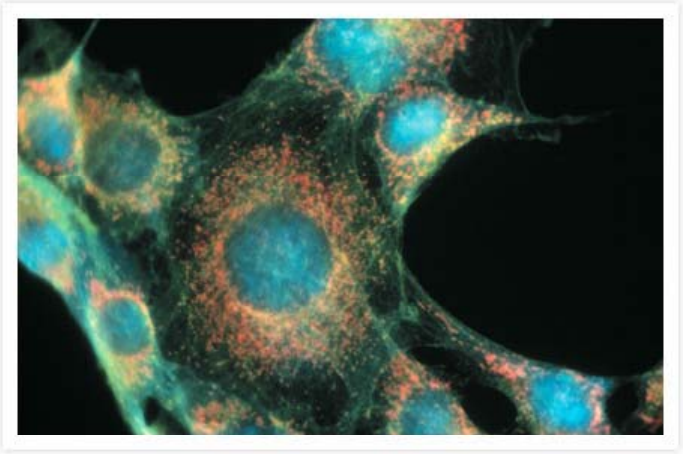
90° measurement angle ensures that excitation light doesn't reach (and overwhelm) photomultiplier.

Fluorescence Spectra

Fluorescent light must be lower in energy (and thus longer in wavelength) than absorbed light.



Fluorescent Molecules Permit Multicolor Imaging & Detection in Biology



NIH 3T3 cells;
mitochondria stained to fluoresce **red**,
cytoskeleton fluoresces **green**,
nuclei fluoresce **blue**.

(Image: Invitrogen Probes catalog)

Fluorescent molecules image sensitively because the incident light wavelength can be blocked with an optical filter.