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# Diffraktion Grating Spectrometer

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Design and Collected  
Spectra

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Theremino System

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## Design and Components

- Webcam : NEW TRUST MEGAPIXEL WEBCAM PRO 1.3MP 1024x1280
- Diffraction gratings : holographic 1000 linee/mm – 600 linee/mm
- Collimating lens
- slit 100micron / slits 0.1–0.5-1mm built with razor blades / micrometric adjustable slit



## Theory of Diffraction Grating

A monochromatic light beam that is incident on a grating gives rise to a transmitted beam and various diffracted beams , at angles that depend on the ratio between the distance between the lines of the grating and the wavelength of the light.

So, if the light beam is composed of multiple wavelengths, the decomposition of the beam into its components is obtained.

The light with a longer wavelength is deflected to a larger angle with respect to the incident direction (angle of diffraction ) . For each wavelength more rows can be observed. The number of rows that are counted from the middle line, which is not skewed with respect to the incident beam and is taken as a reference , it is said "order" and is often denoted by the letter m.

The diffraction gratings can act both for transmission and for reflection of the incident light depending on the light scattering takes place on the same side or the opposite side of the light source . The transmission gratings are composed of a transparent plate on which are created many small strips that do not allow the passage of the radiation. In this way you get many small slits whose figure generated on a screen is solved by a method analogous to that used for the interference .

The reflection gratings are constituted by a reflective layer (mirror) on which are created many small strips or grooves that do not allow the reflection of the radiation. They are used in monochromators and spectrometers .

The distance between the slits, known as "grating pitch" , in the gratings used in spectroscopy is of the same order of magnitude as the wavelength of the light to be analyzed . In practice, the patterns are usually characterized by the number of engravings per unit length , often expressed in lines per millimeter (l/mm).

The fundamental property of the gratings is that the angle of deviation of all the refracted beams depends on the wavelength of the incident light. Thus, a grating separates a beam of polychromatic light in its wavelengths components, so the grating is a dispersive tool.

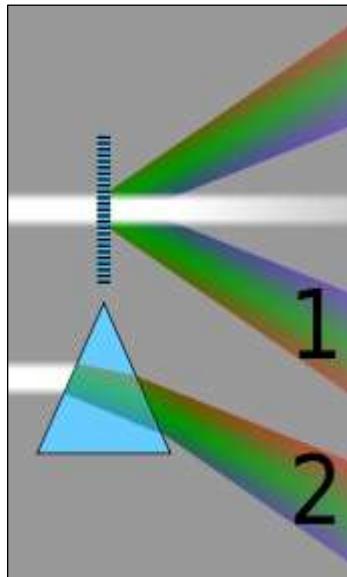
When a light beam is incident on a grating it is diffracted in different beams. The beam corresponding to the direct transmission is called zero-order diffraction . The convention in use denote the not deflected beam with  $m = 0$  . Respect to the direction identified by the reference beam is possible to measure the diffraction angle that characterizes each deflected beam .  $m$  can assume positive or negative values depending on that the deflected beam is to the right or left of the zero order beam (this depends on the convention used for the sign of the angles).

Denoting by  $d$  the grating pitch and  $\lambda$  the wavelength of the incident radiation can be written :

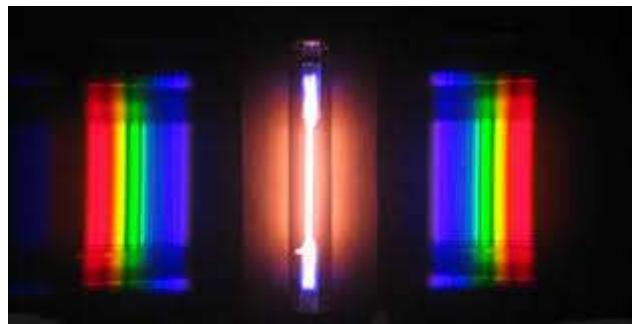
$$d(\sin \theta_m(\lambda) + \sin \theta_i) = m\lambda$$

When the beam hits at angle  $\theta_i$  the grating. The sign in the formula depends on the choice of the Convention on the sign of the angles.

From the previous relation it can be seen that a beam of polychromatic light is divided into its components from violet ( which is the color characterized by shorter wavelength ) till to red; instead in a glass prism the angle of deviation is greater for violet, so the sequence of colors is reversed.



Comparison between the spectra obtained from a diffraction grating (1), and from a refraction prism (2). Longer wavelengths (red) are deflected more, while the shorter ones (violet) are deflected less.



The light from a lamp seen through a transmission grating which shows three diffraction orders. The order  $m = 0$  corresponds to the direct transmission through the grating.

In the first positive order ( $m = +1$ ), colors with longer wavelength (from violet to red) are deflected at higher angles.

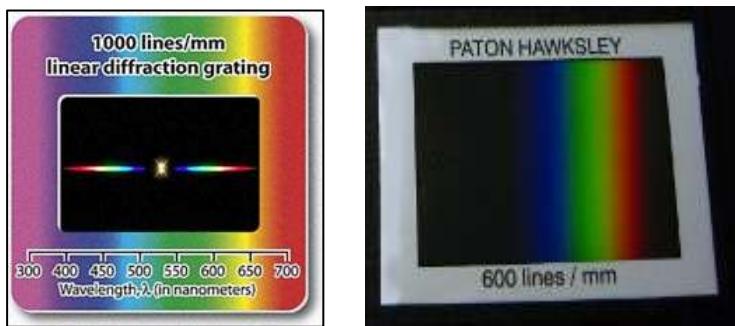
The diffracted beams of different colors and corresponding to consecutive orders can overlap, this phenomenon becomes more likely to grow in the order of diffraction. Moreover, in an experiment the observed diffraction lines are never infinitely narrow ( as expected from the theory ), this phenomenon is a consequence of the not ideal experimental conditions and because of the Doppler effect.

The equation of the grating shows that the diffraction angle depends only on the pitch of the grating and not by the shape of the slits. The efficiency of the grating can also depend on the polarization of the incident light .

## Diffraction Grating Specifications

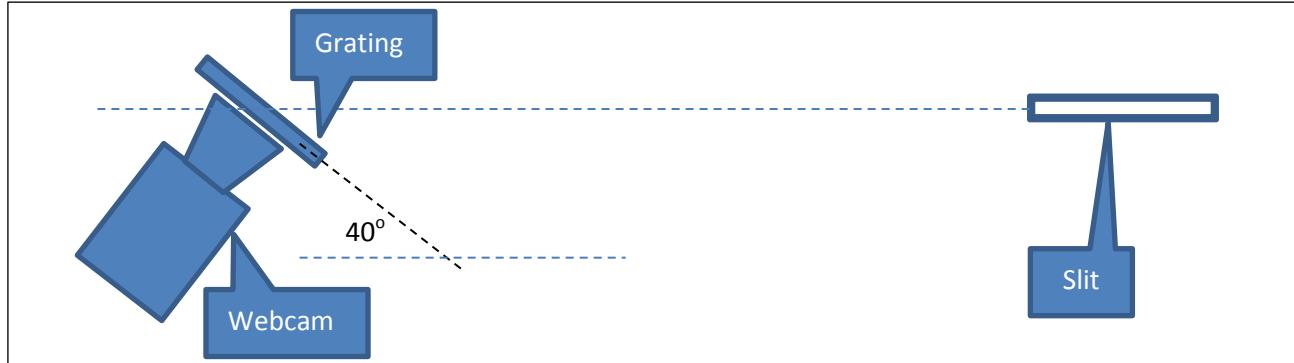
Type of Grating	Lines/mm	Space between lines (nm)	Diffraction for different wavelengths					
			400nm	500nm	600nm	700nm	800nm	900nm
Paton grating	600	1600	14	18	22	26	30	34
Holographic grating	1000	1000	24	30	37	44	53	64

## Gratings in use

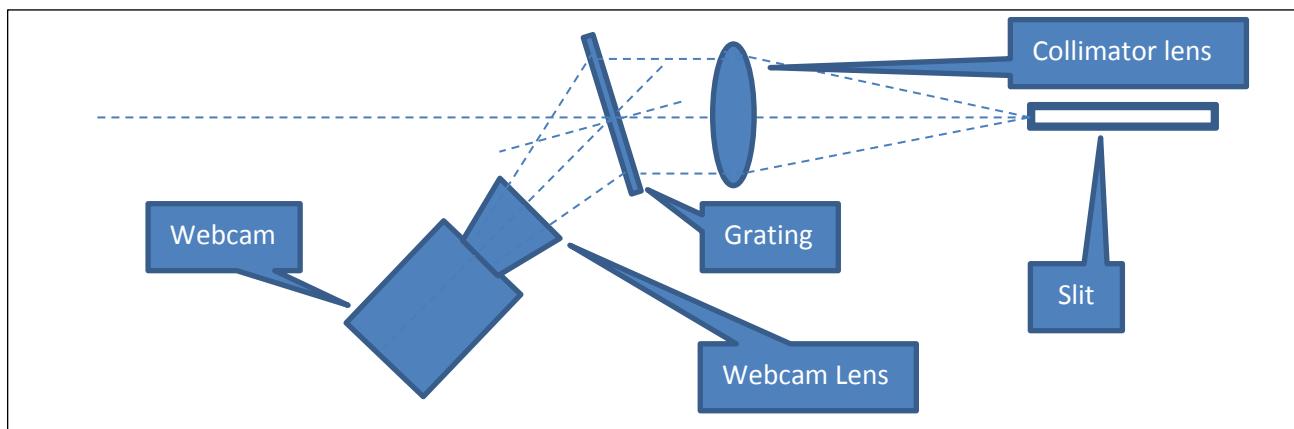


Holographic grating with 1000 lines/mm and holographic Paton grating with 600 lines/mm

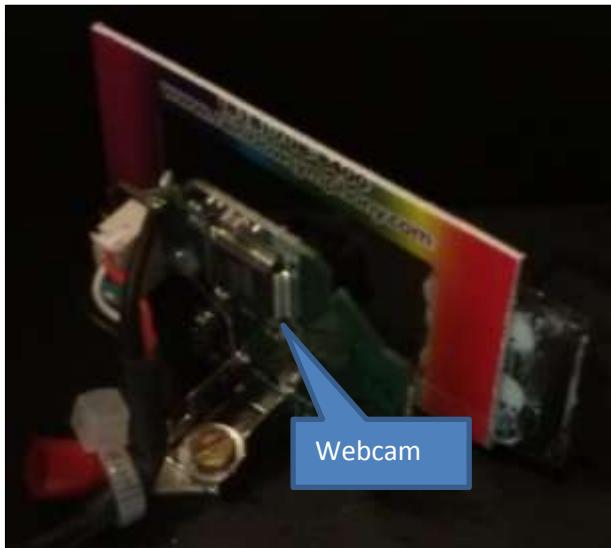
## Portable Spectrometer Design :



## Benchtop Spectrometer Design :



## Portable Spectrometer Construction



**Details of webcam and diffraction grating**

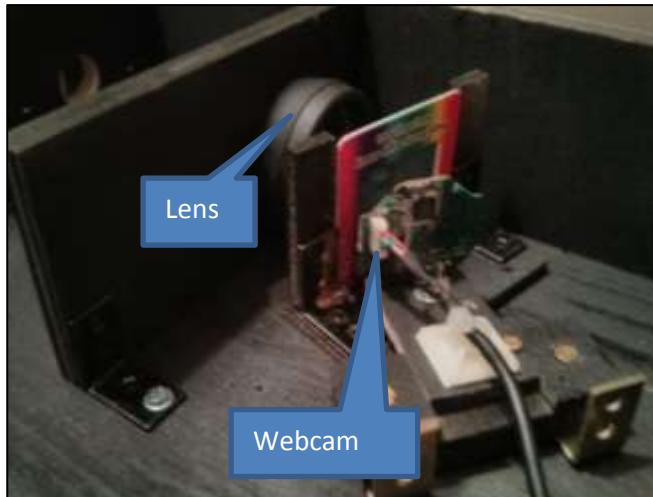


**0.1mm slit, 0.5mm slit and 1mm slit**

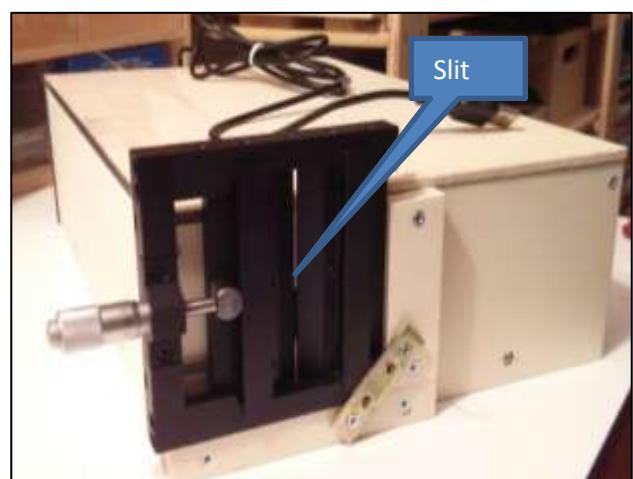
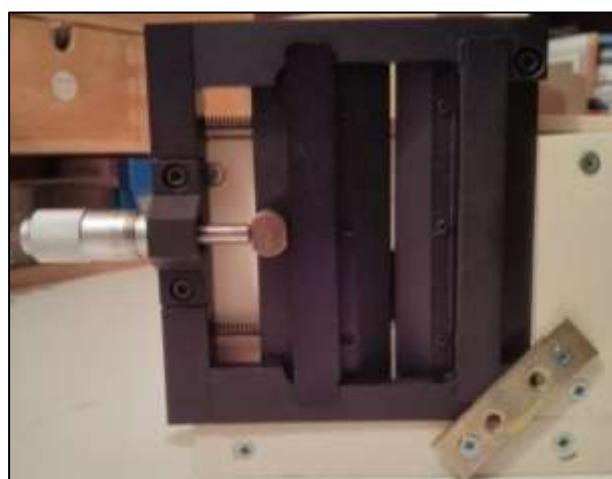


**Scattering screen**

## Benchtop Spectrometer Construction



Inside view with collimating lens, grating and webcam

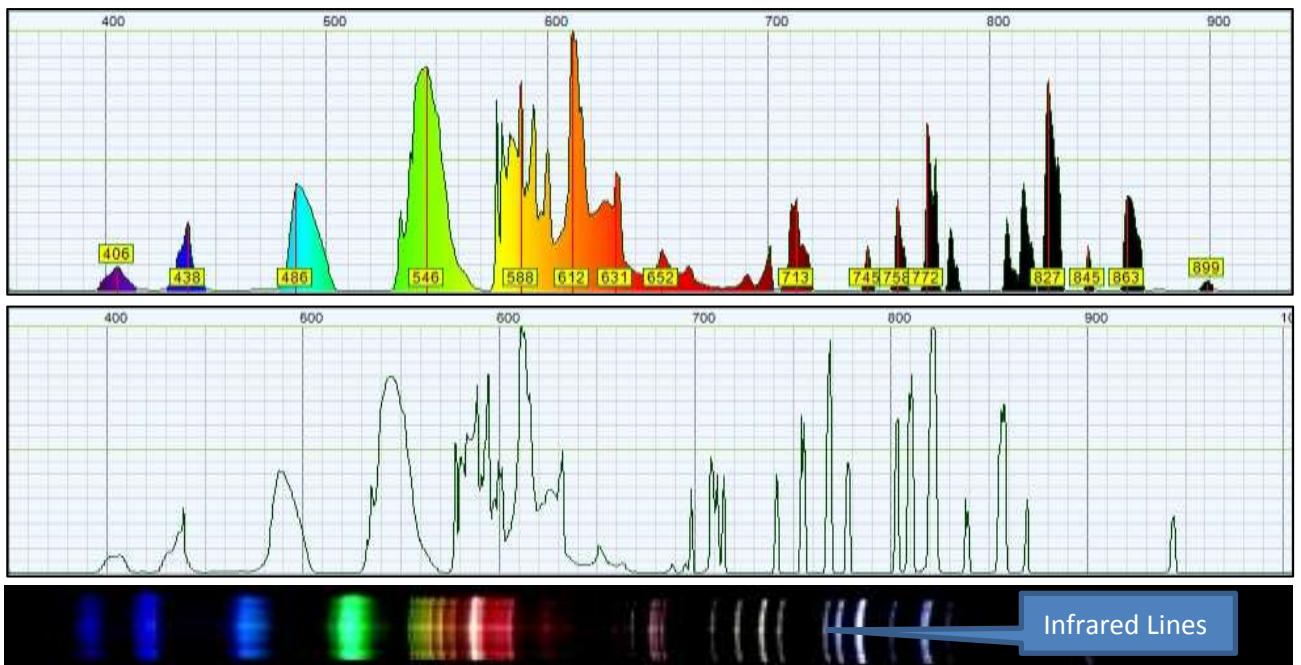


Detail of the micrometric slit and the spectrometer assembled

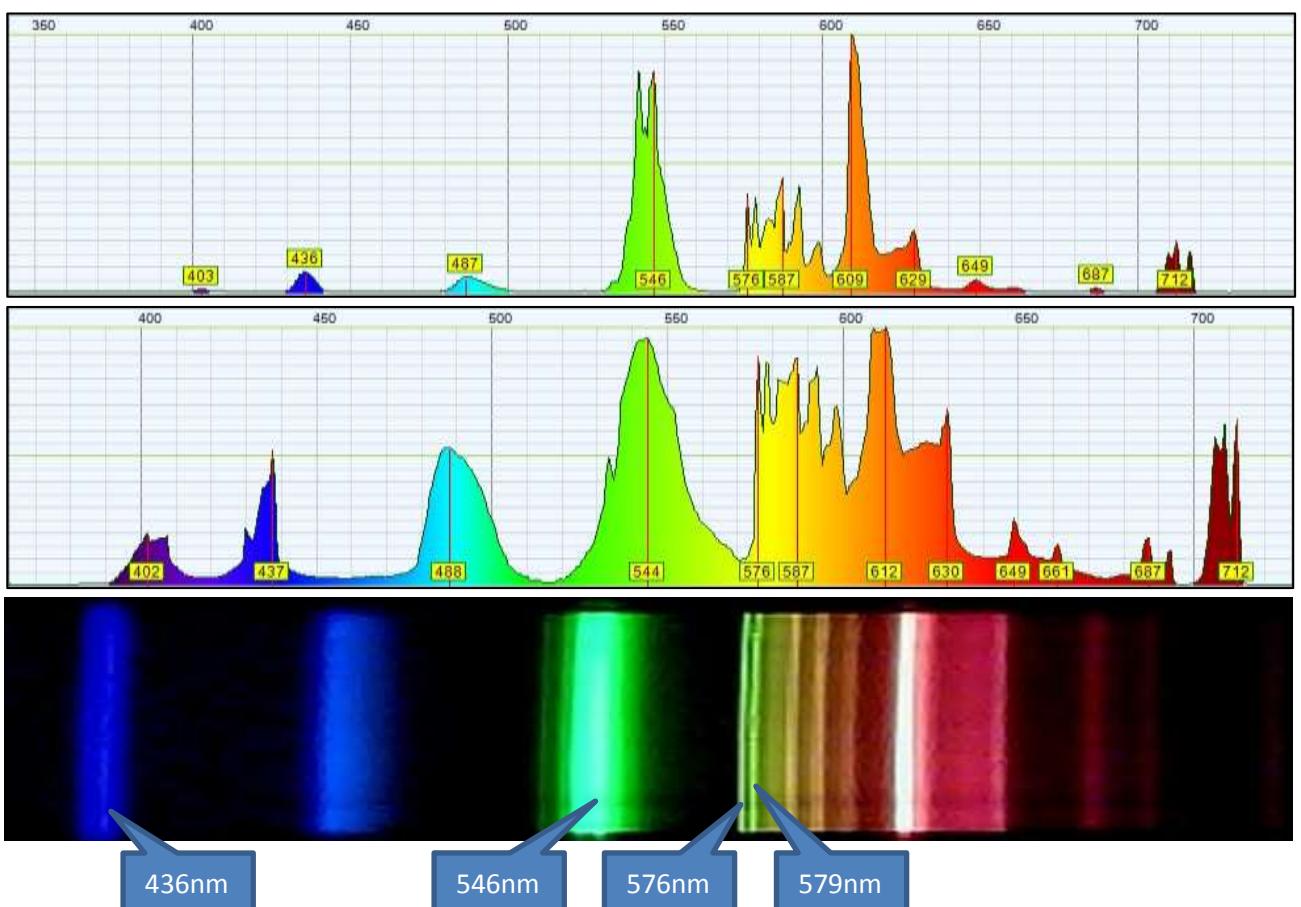


Calibration CFL lamp

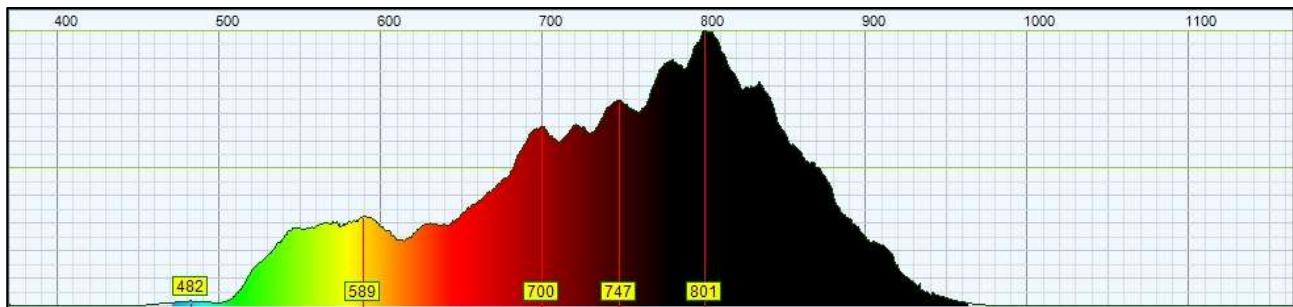
## Spectra of Lamps



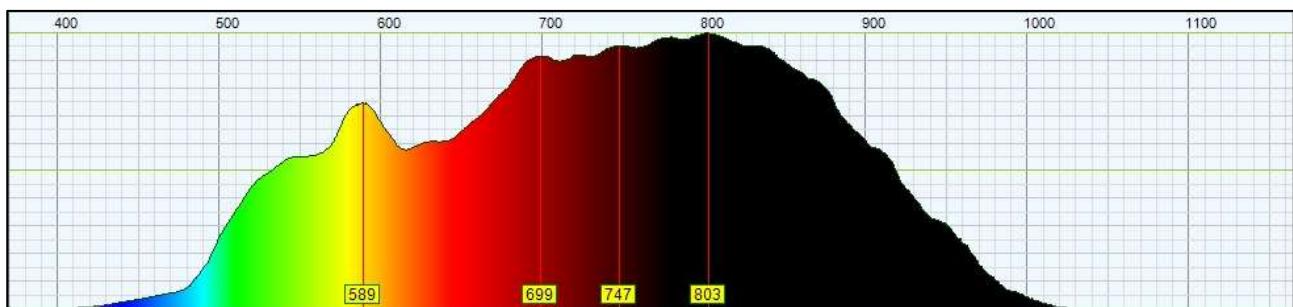
CFL lamp just lit – presence of lines broadened by phosphorus – infrared emission ( $\lambda > 750\text{nm}$ )



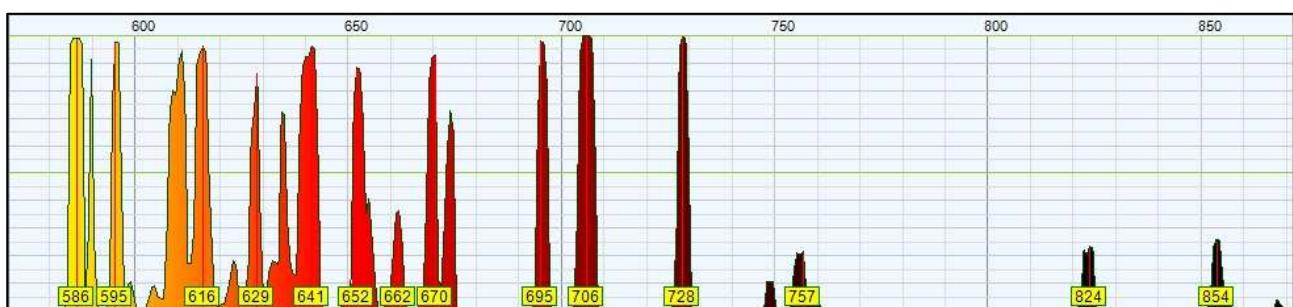
CFL lamp after warm-up (detail 400nm – 800nm) – no infrared emission



Incandescent lamp 25W – continuous spectrum with maximum at ~ 800nm



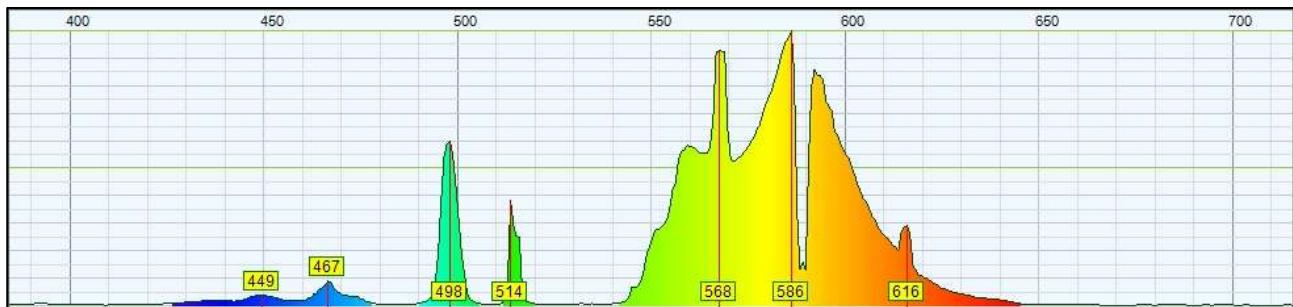
Halogen lamp 70W – continuous spectrum – more emission at shorter  $\lambda$  (temperature is increased)



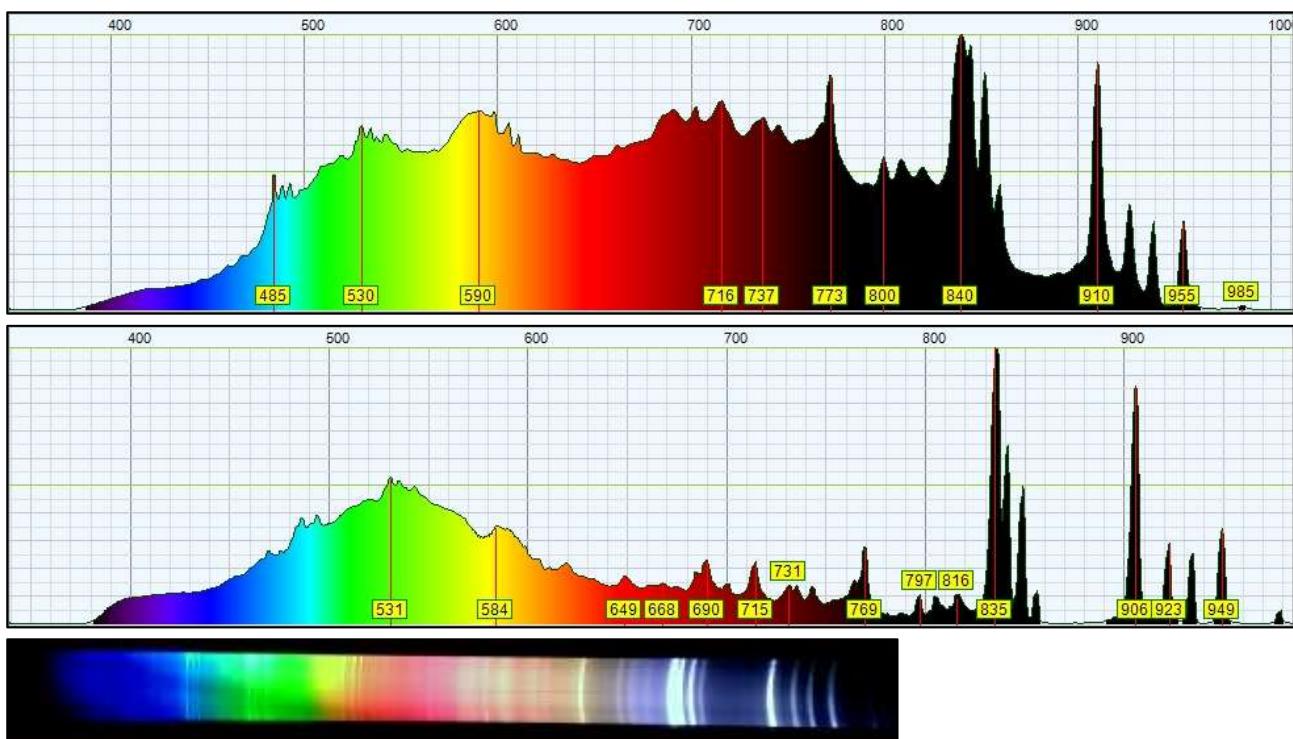
Low pressure neon lamp – discrete spectrum with many narrow lines



Wood lamp – UV emission at 370nm with tiny visible “tail” beyond 380nm



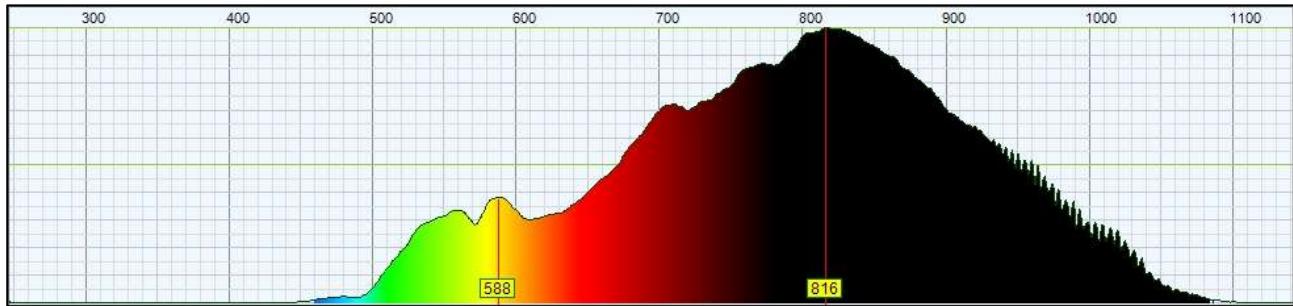
**Sodium-vapor lamp (street lamp) – evidence of the sodium double absorption line at a 589nm – further lines at 568nm, 616nm, 514nm, 498nm all corresponding to emission lines of the sodium spectrum**



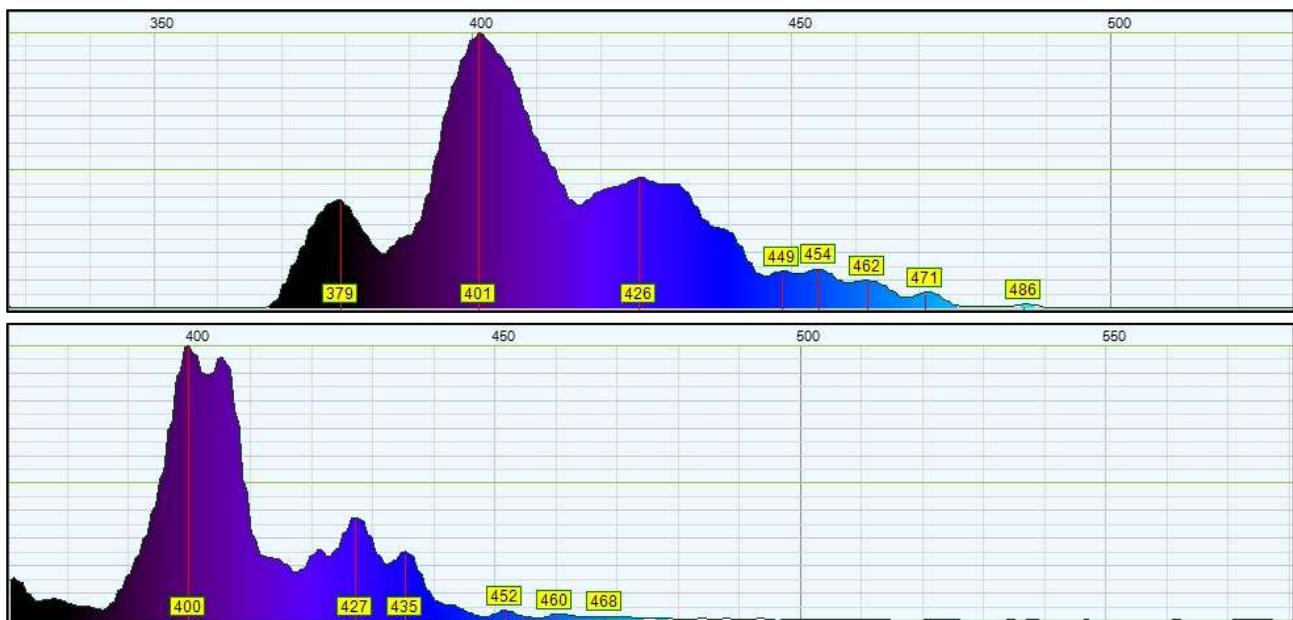
### Xenon lamp

**Due to the broad extension of the emission, from 350nm to 950nm, this lamp is used in absorption and fluorescence spectroscopy**

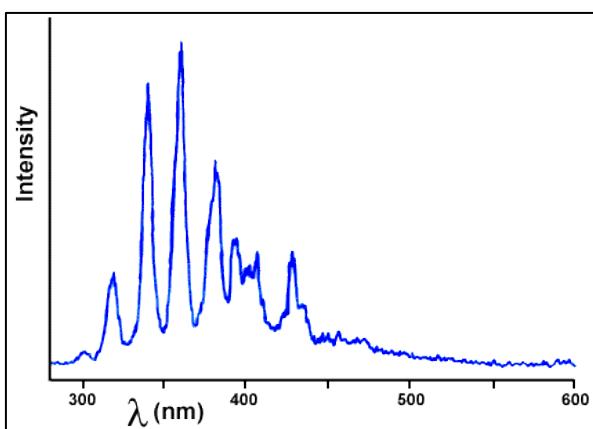
## Spectra of Flames and Plasma



Candle – emission mainly at longer wavelengths

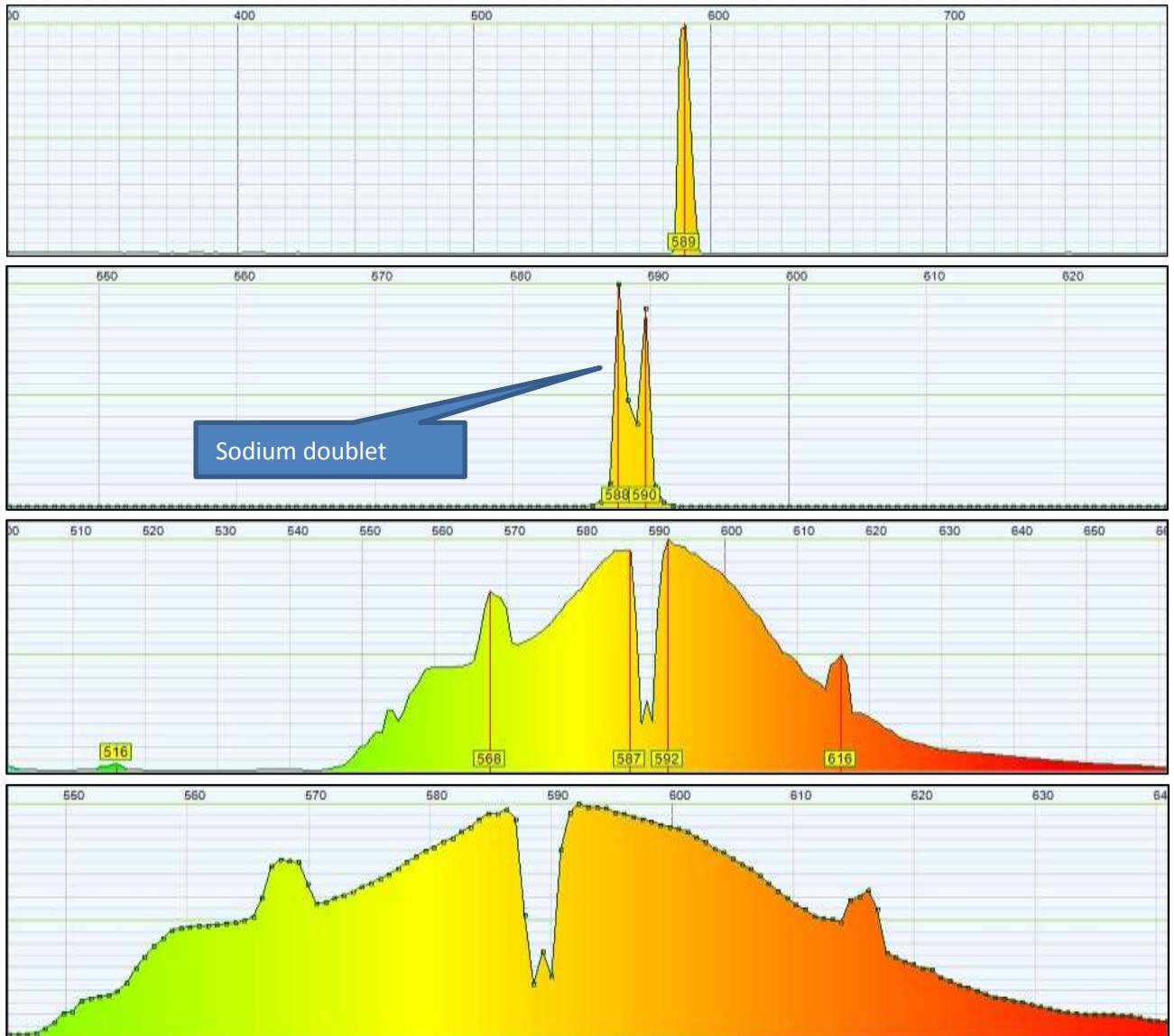


Plasma from high voltage arc – great emission of UV radiation

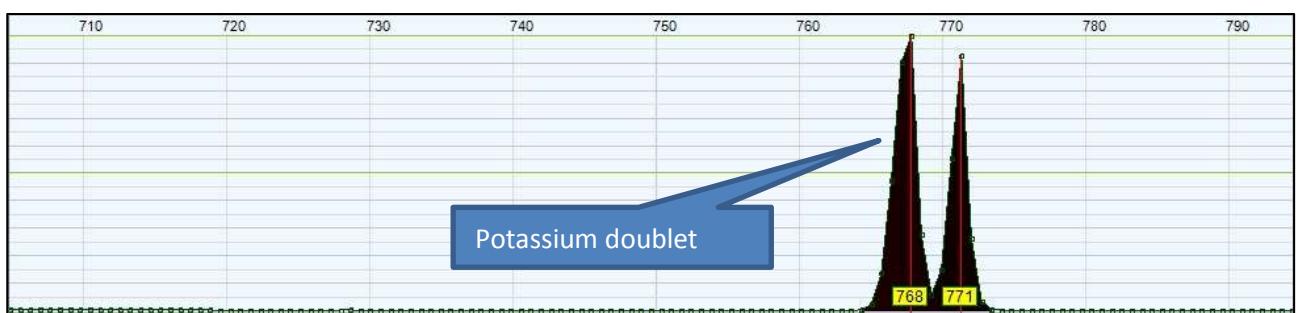


Atmospheric nitrogen emission spectrum (from reference spectra). Correspondence of the emission peaks at 400nm and 430nm. Thus we can infer that the plasma emission is mainly due to the nitrogen emission.

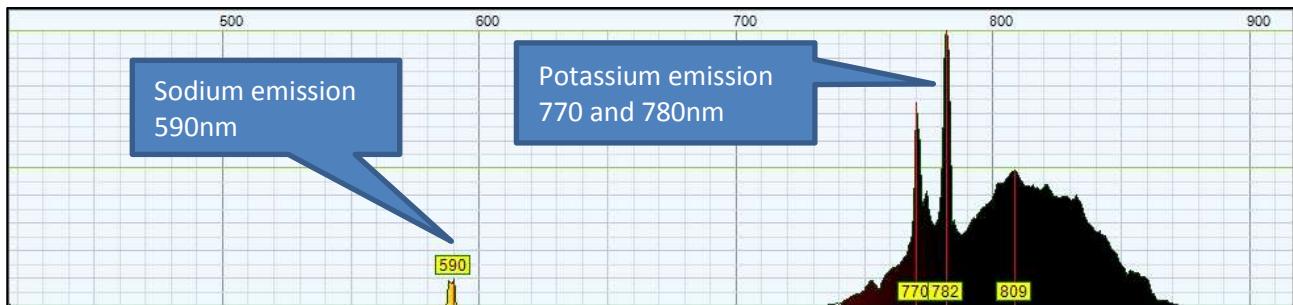
## Emission and Atomic Absorption spectra



In the first spectrum of HV plasma with sodium chloride is evident the sodium line at 589nm. In the second spectrum the detail shows the sodium doublet at 589,0nm and 589,6nm. The third and fourth spectra show the detail of the absorption line in a sodium-vapor lamp

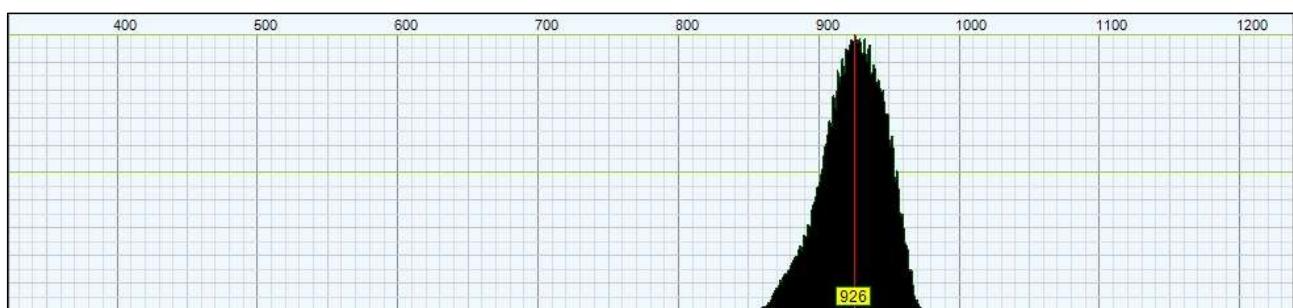


Spectrum of potassium chlorate match – evidence of potassium doublet at 770nm

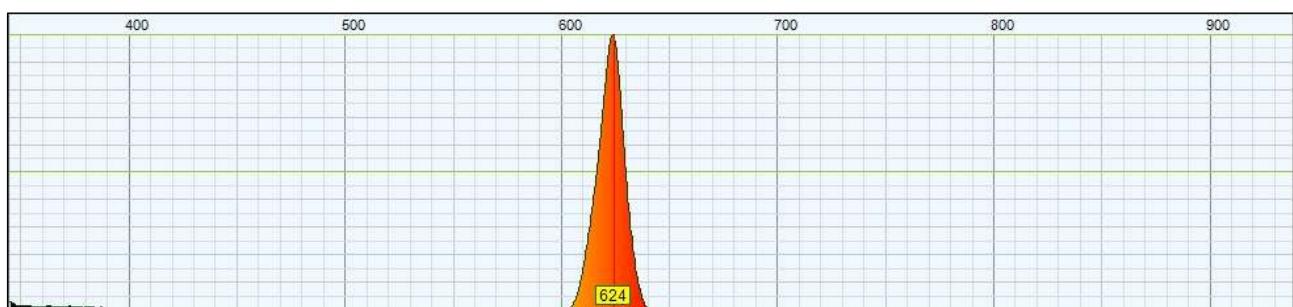


HV plasma spectrum with sodium and potassium emission lines overlapping thermal background

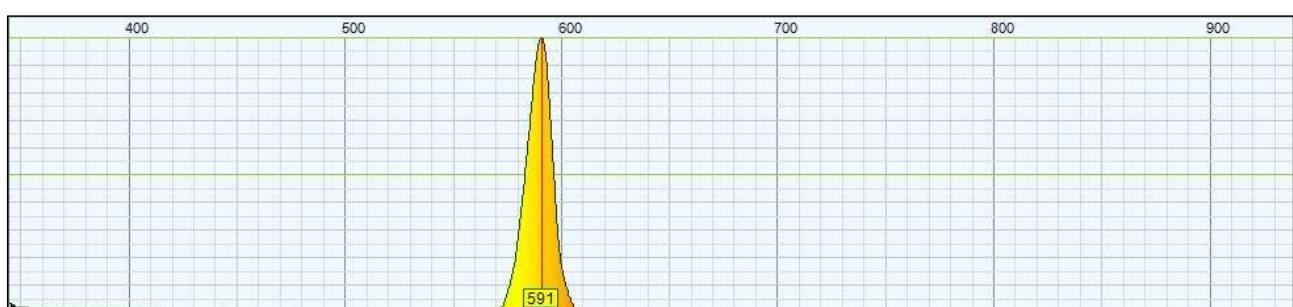
## LED Spectra



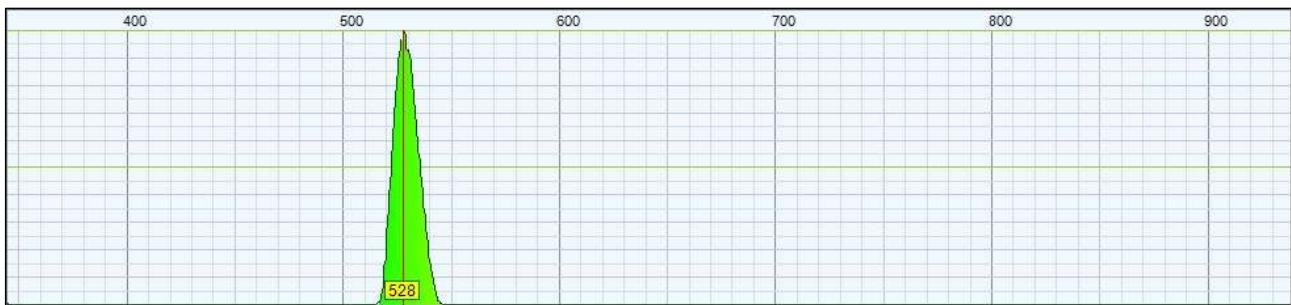
Infrared LED



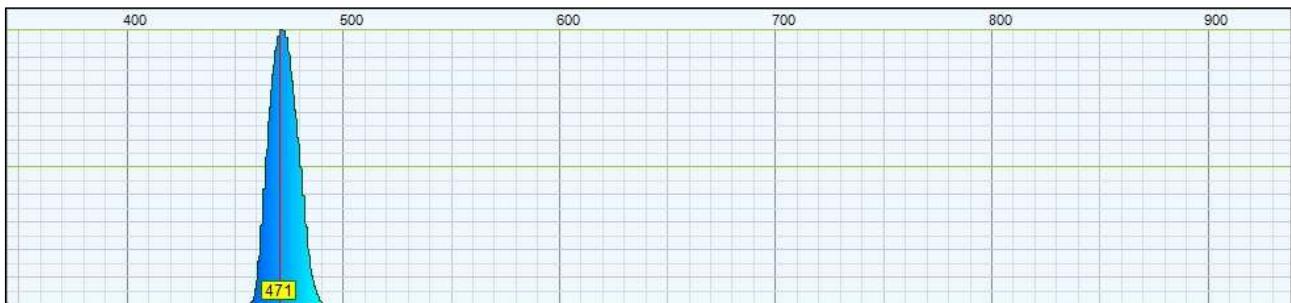
Red LED



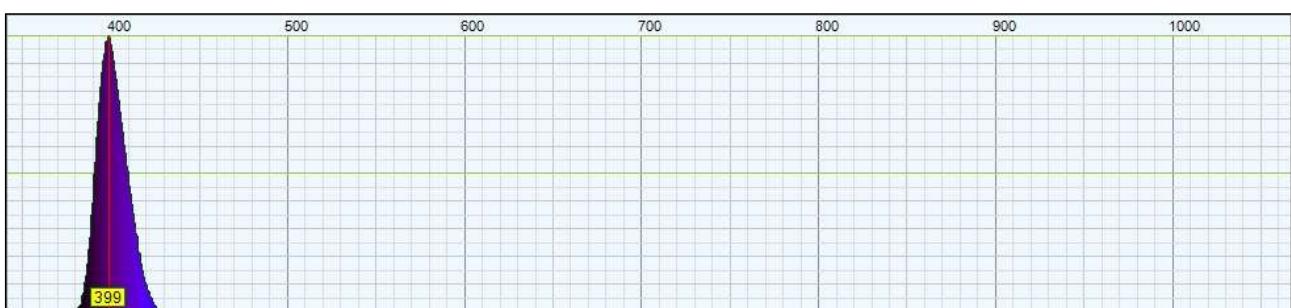
Yellow RED



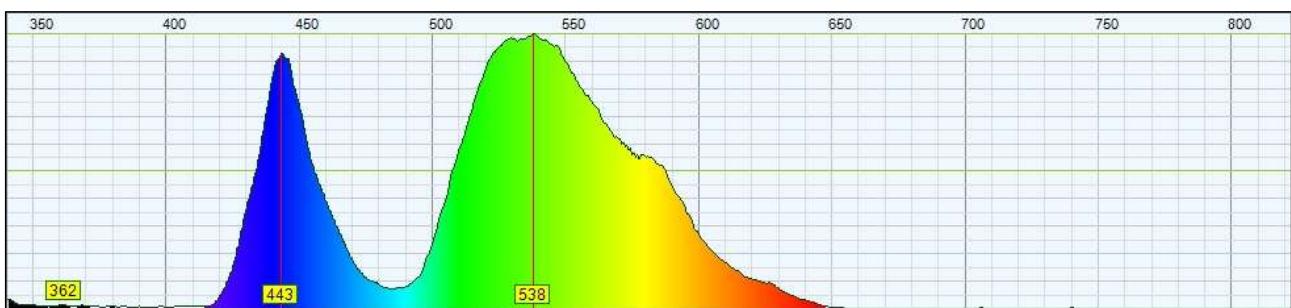
**Green LED**



**Blue LED**



**Violet LED**

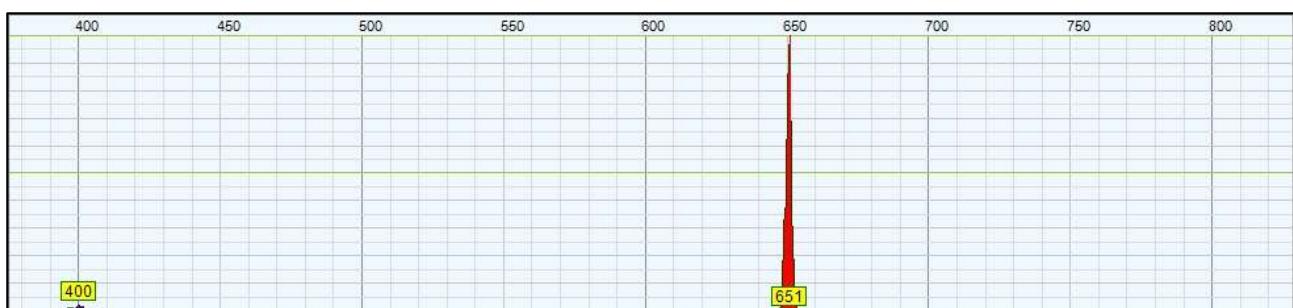


**White LED "cold light"**

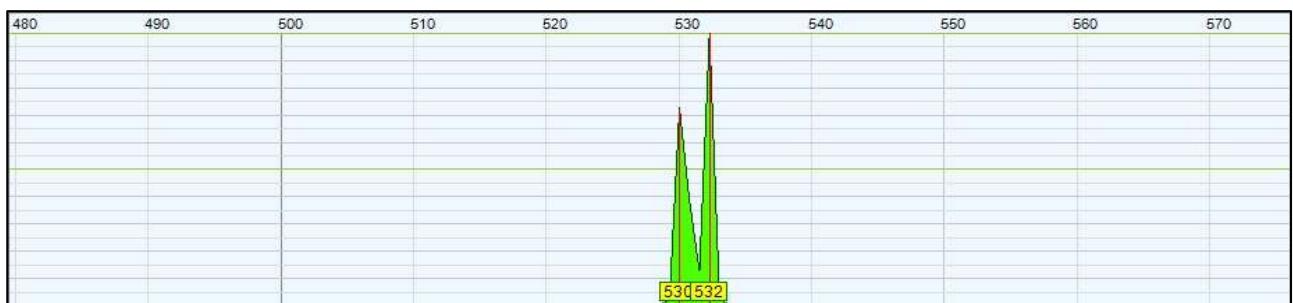
## Laser Spectra



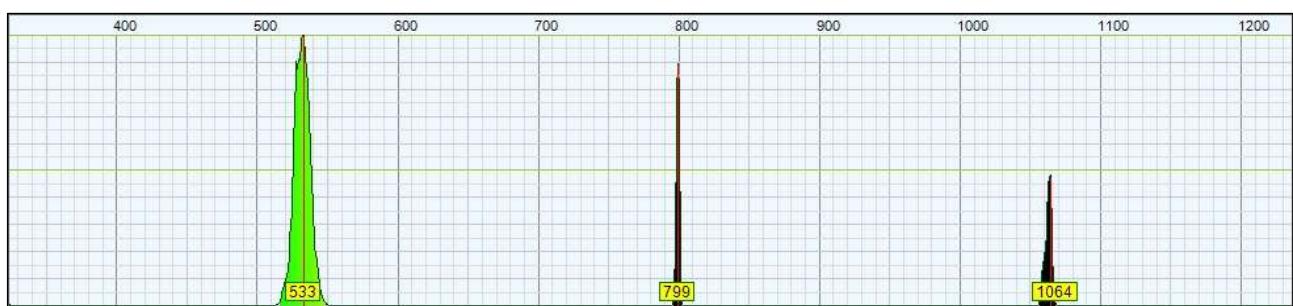
Violet laser diode ~ 410nm



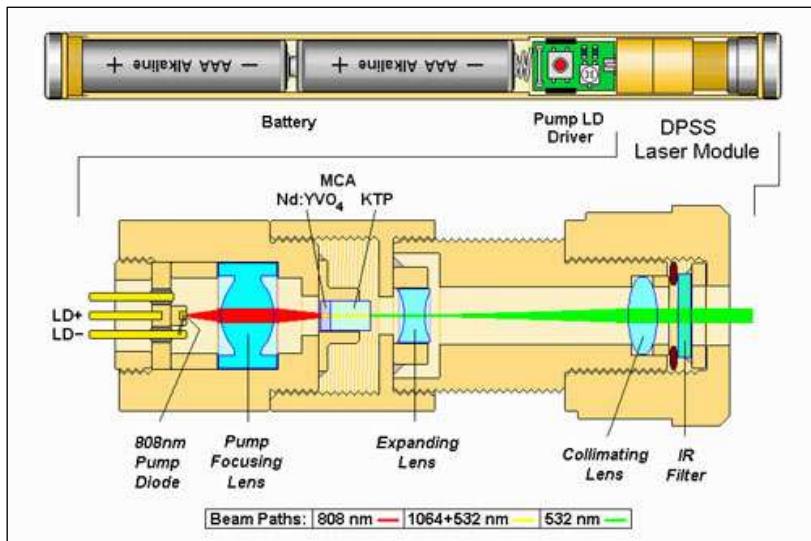
Red laser diode – emission 650nm



Green laser diode – detail of the emission line with two peaks at 532 and 530nm

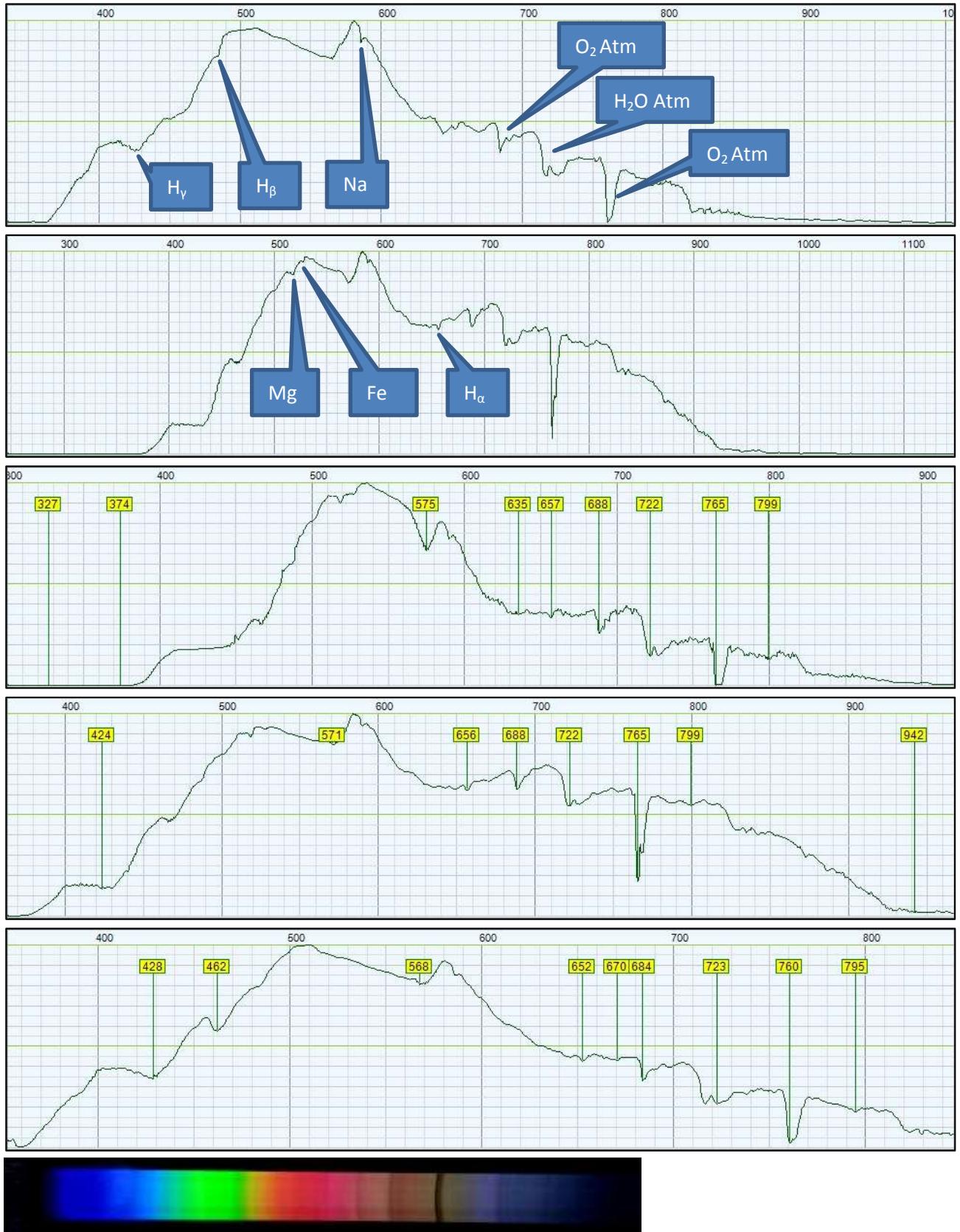


Green laser diode – the pumping emission lines are shown



**532nm green laser diode functioning scheme.** There is IR pumping emission at 800nm. Nd:YVO<sub>4</sub> crystal converts this wavelength into 1064nm emission, the latter is frequency-doubled, at 532nm, from a KTP crystal

# Sun Spectrum



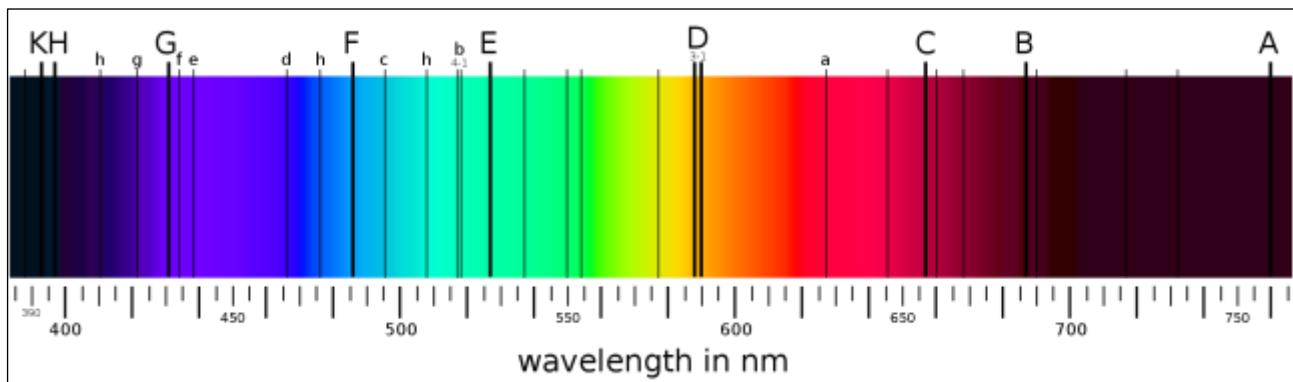
Sun spectra at different times

Maximum at ~ 530nm => T = 5500K<sup>0</sup> (from black body radiation / Wien law)

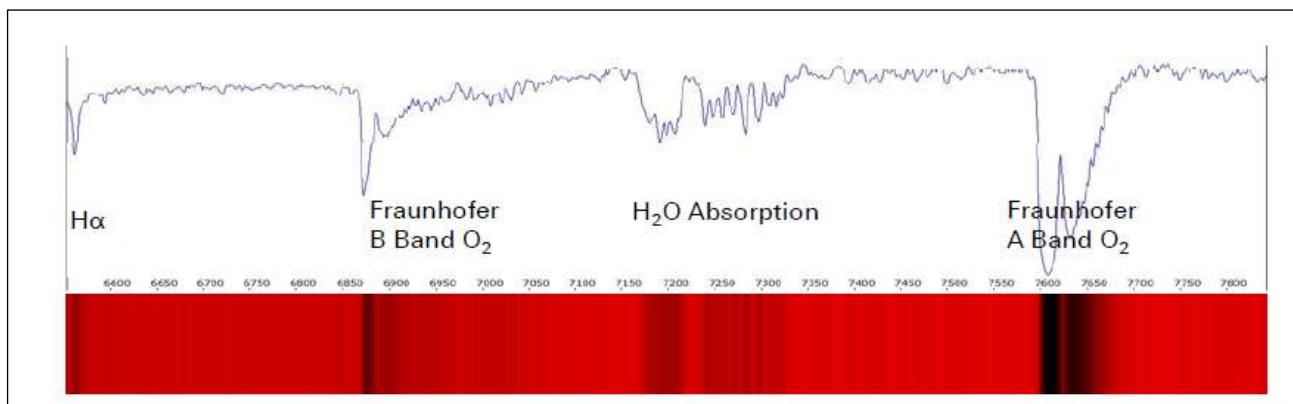
Evidence of UV (<400nm) and IR (>750nm)

Evidence of the following absorption bands / lines :

- Atmospheric oxygen absorption band O<sub>2</sub> 760nm – Fraunhofer A
- Atmospheric water vapor absorption band 720nm
- Atmospheric oxygen absorption band O<sub>2</sub> 684nm – Fraunhofer B
- Absorption H<sub>α</sub> 657nm (Balmer series) Fraunhofer C
- Absorption H<sub>β</sub> 480nm (Balmer series) Fraunhofer F
- Absorption H<sub>γ</sub> 430nm (Balmer series) Fraunhofer G
- Sodium absorption line at 589nm Fraunhofer D
- Iron absorption line at 530nm Fraunhofer E
- Magnesium absorption line at 520nm Fraunhofer b



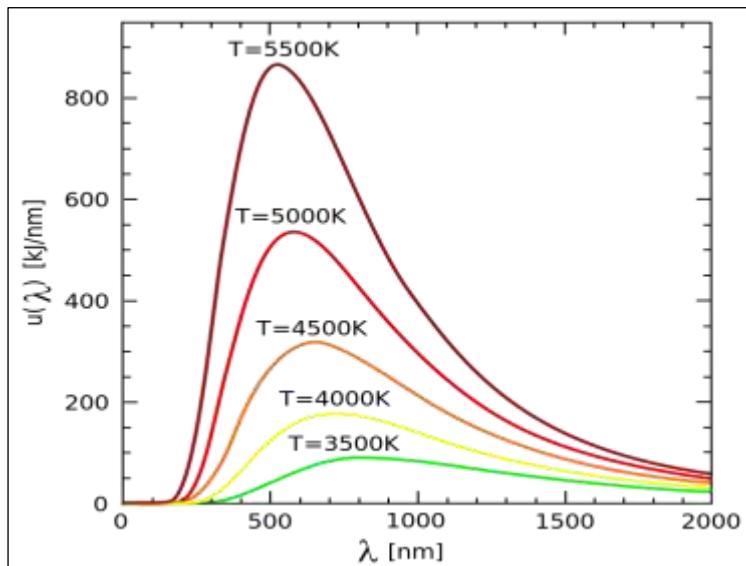
Fraunhofer main lines



Sun spectrum 650nm - 800nm with absorption bands of atmospheric gases

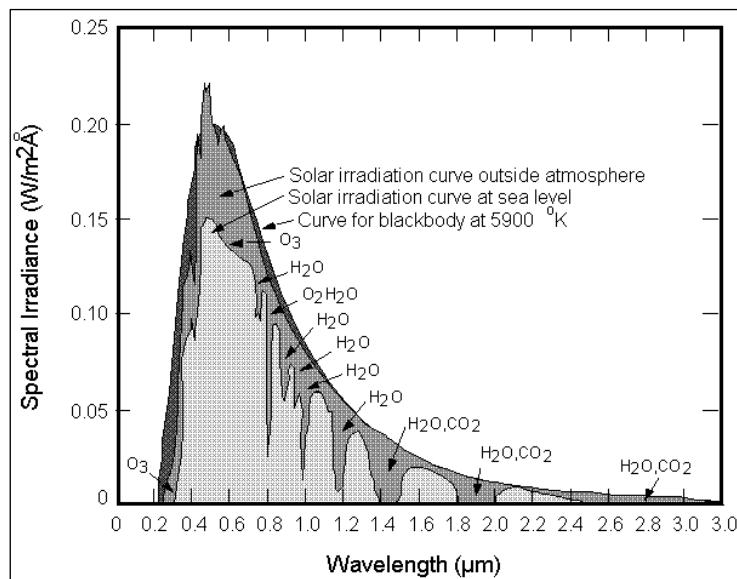
## Wien Law / Black body radiation

Wien's displacement law states that the black body radiation curve for different temperatures peaks at a wavelength inversely proportional to the temperature. The shift of that peak is a direct consequence of the Planck radiation law which describes the spectral brightness of black body radiation as a function of wavelength at any given temperature. However it had been discovered by Wilhelm Wien several years before Max Planck developed that more general equation, and describes the entire shift of the spectrum of black body radiation toward shorter wavelengths as temperature increases.



$$T \cdot \lambda_{max} = b$$

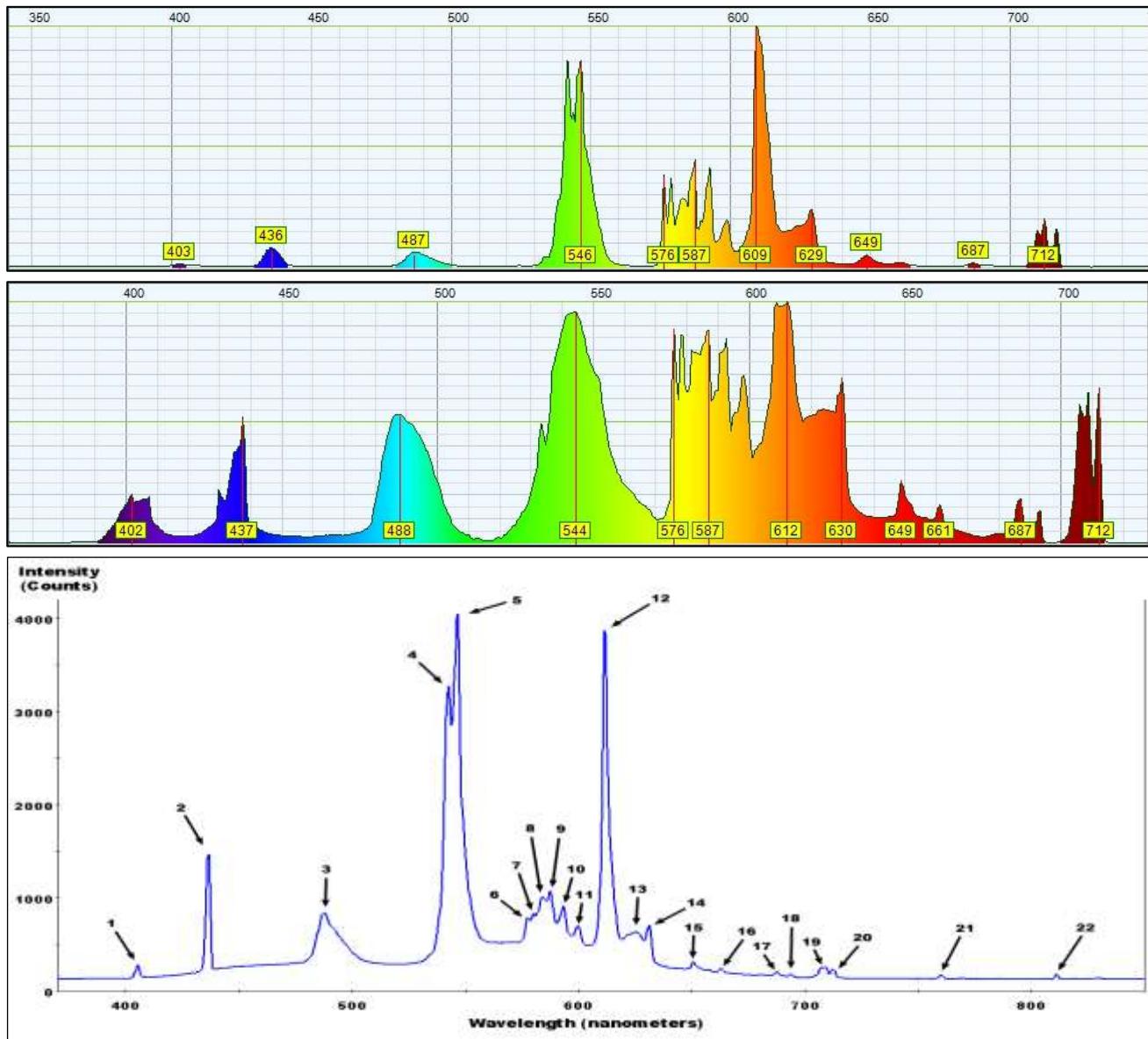
$$b = 2.8977685(51) \times 10^{-3} \text{ m K}$$



**Sun spectrum with absorption bands of atmospheric gases**

## Comparison with Reference Spectra

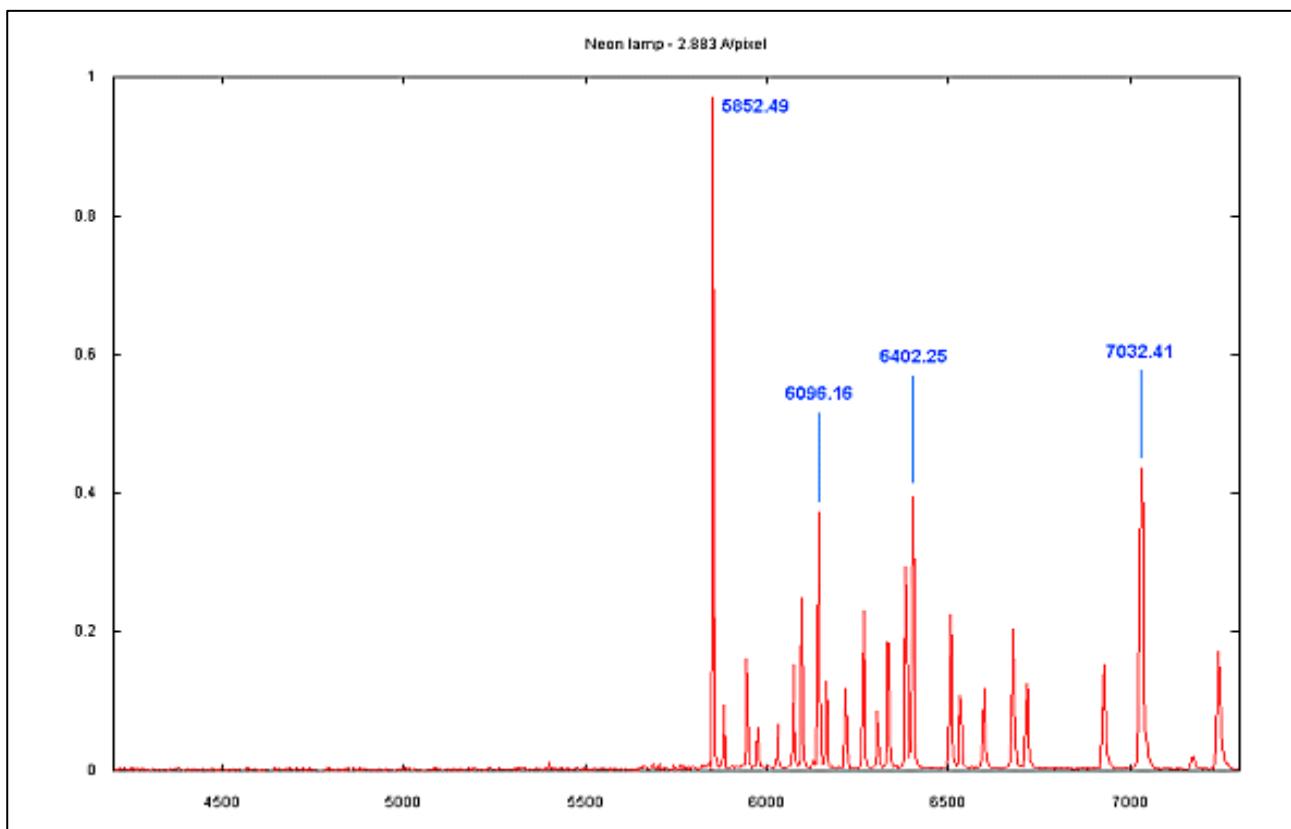
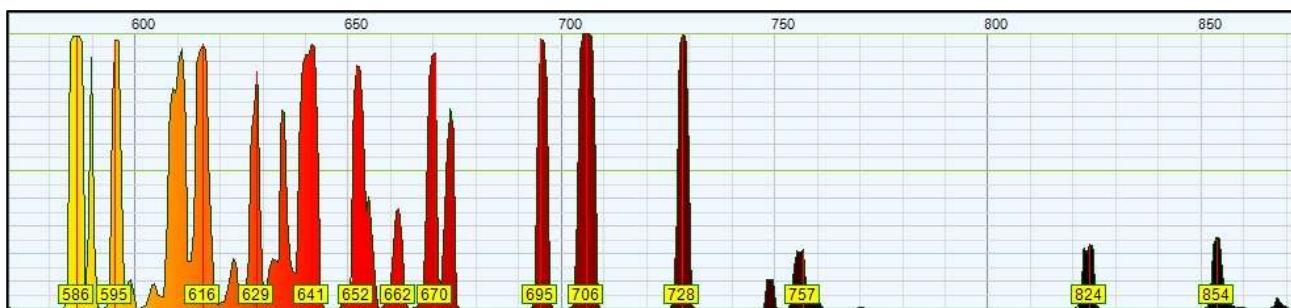
### CFL Lamp



Peak number	Wavelength of peak (nm)	Species producing peak	Actual line location (nm)
1	405.4	mercury	402 - 404
2	436.6	Mercury	437
3	487.7	terbium from Tb <sup>3+</sup>	488
4	542.4	terbium from Tb <sup>3+</sup>	542
5	546.5	Mercury	546
6	577.7	likely terbium from Tb <sup>3+</sup> or mercury	576
7	580.2	mercury or terbium from Tb <sup>3+</sup>	579
8	584.0	possibly terbium from Tb <sup>3+</sup> or europium in Eu <sup>3+</sup> :Y <sub>2</sub> O <sub>3</sub>	584
9	587.6	likely europium in Eu <sup>3+</sup> :Y <sub>2</sub> O <sub>3</sub>	587

10	593.4	likely europium in Eu+3:Y2O3	592
11	599.7	likely europium in Eu+3:Y2O3	600
12	611.6	euroium in Eu+3:Y2O3	612
13	625.7	likely terbium from Tb3+	625
14	631.1	likely europium in Eu+3:Y2O3	630
15	650.8	likely europium in Eu+3:Y2O3	649
16	662.6	likely europium in Eu+3:Y2O3	661
17	687.7	likely europium in Eu+3:Y2O3	689
18	693.7	likely europium in Eu+3:Y2O3	693
19	707 and 709	likely europium in Eu+3:Y2O3	707 – 709
20	712.3	likely europium in Eu+3:Y2O3	712
21	760.0	likely argon	
22	811.0	likely argon	

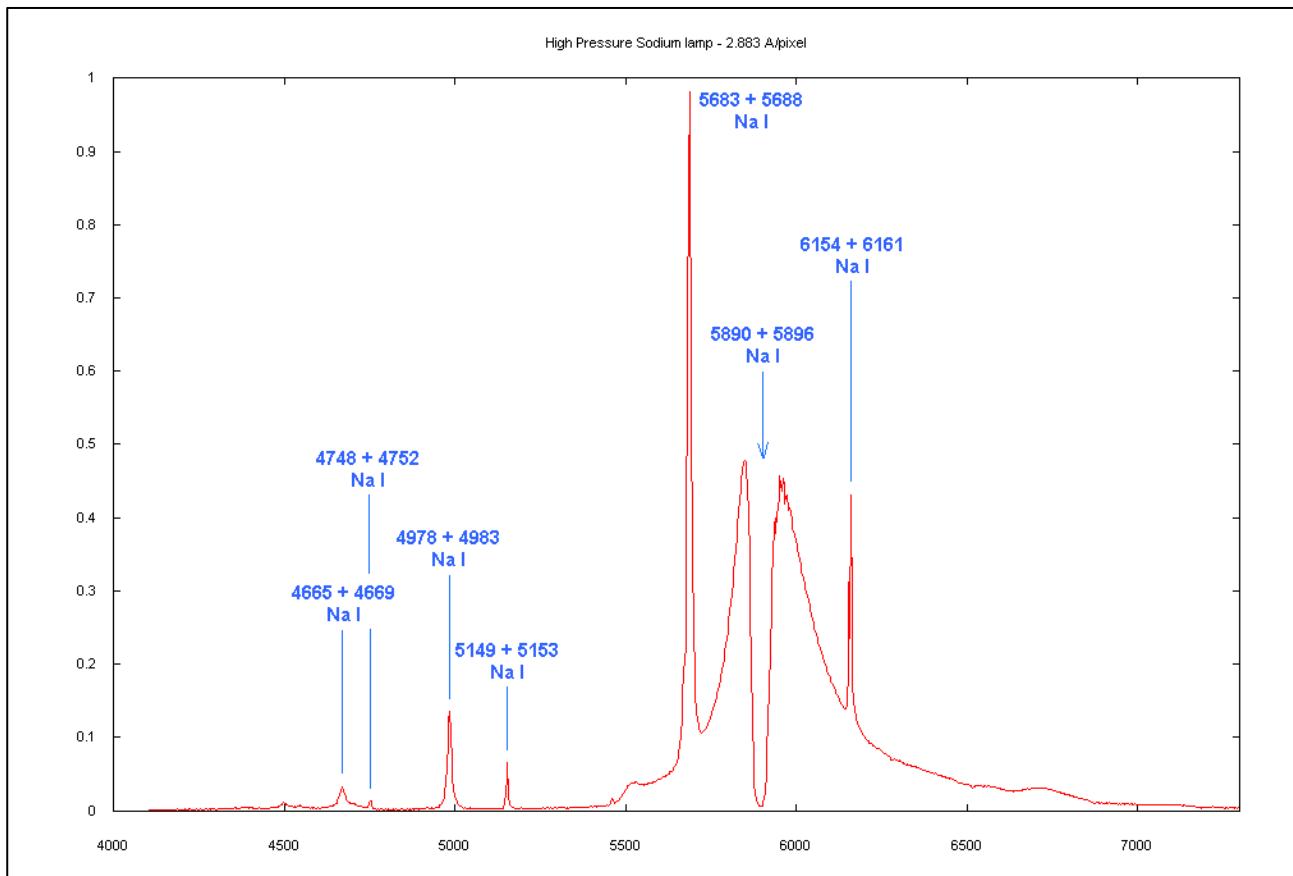
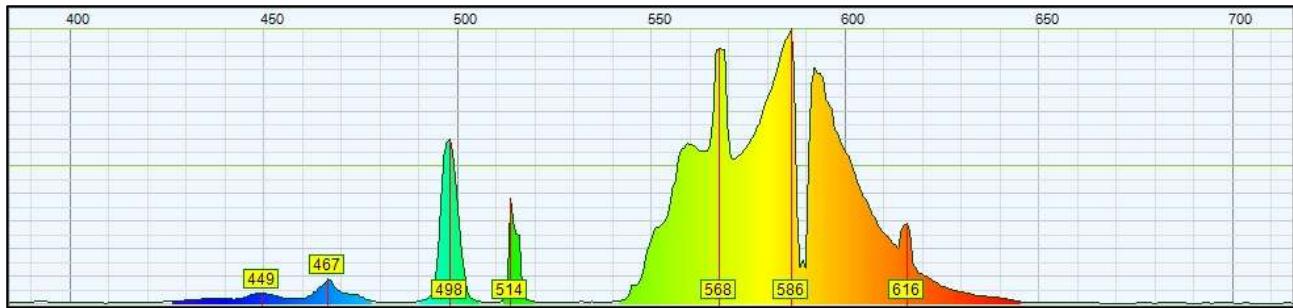
## Neon Lamp



Neon lamp reference spectrum

Correspondence of all emission lines. More than 25 different lines are shown

## Sodium-vapor Lamp

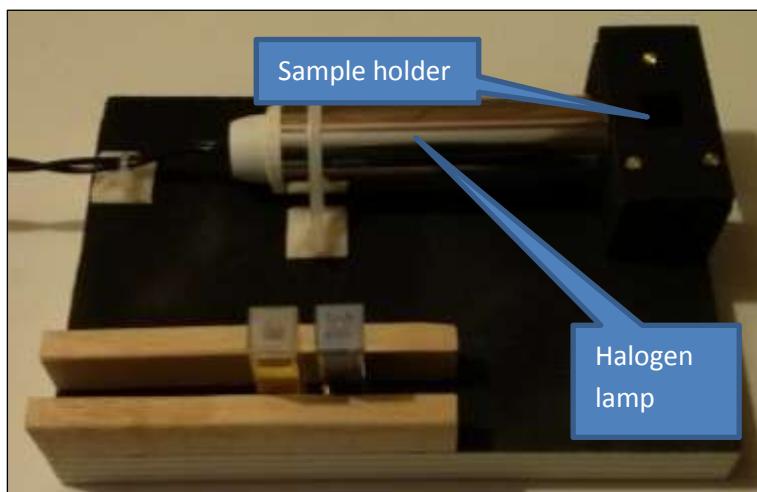
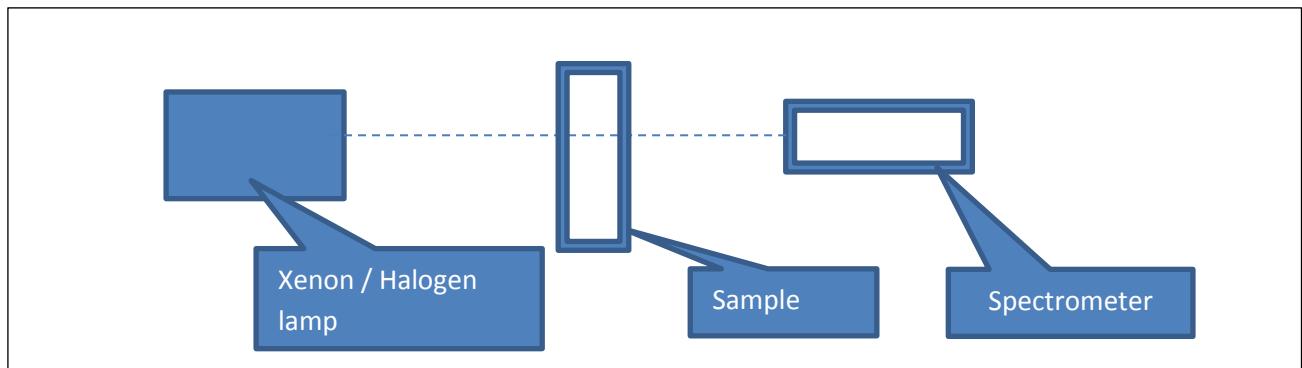


Sodium-vapor lamp – evidence of the double sodium absorption line at 589nm – lines at 568, 616, 514, 498 e 467 nm all correspondent to sodium emission lines

# Absorption Spectroscopy

The absorption spectra are obtained by means of an halogen lamp and a xenon lamp with constant emission over a wide wavelength range. The variations in the emission intensity are compensated with a software algorithm. The useful bandwidth starts at 370nm till to 1000nm.

## Absorption Spectrometer Design

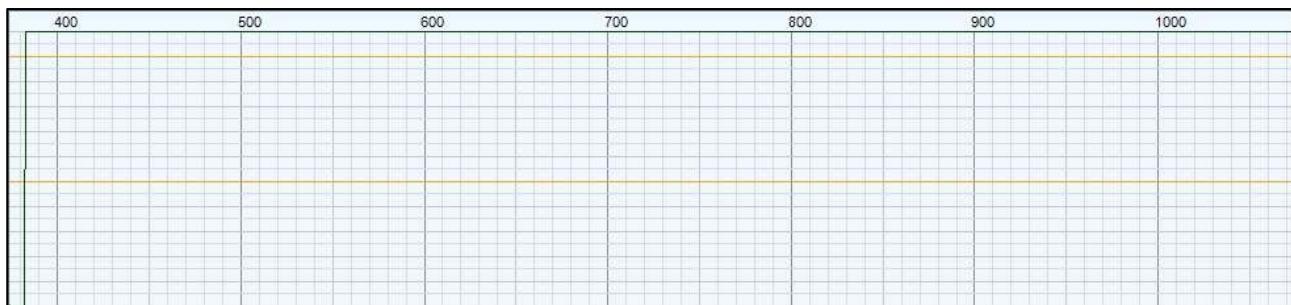


Halogen lamp and sample cuvette holder



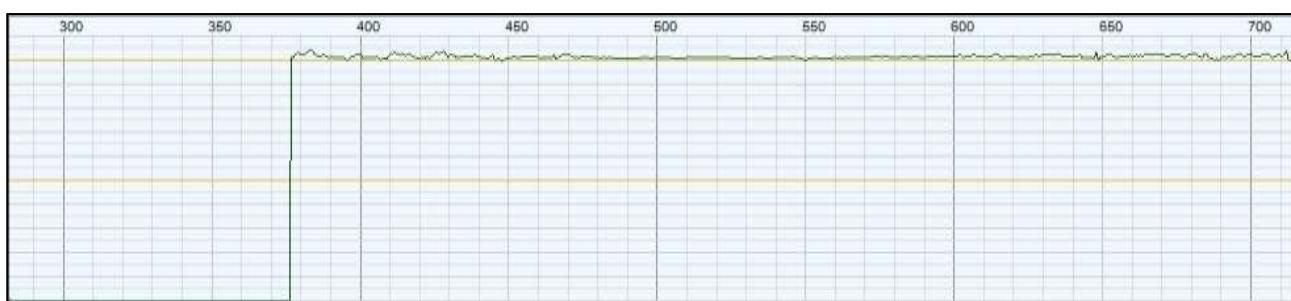
Xenon lamp

## Halogen Lamp Linear Reference



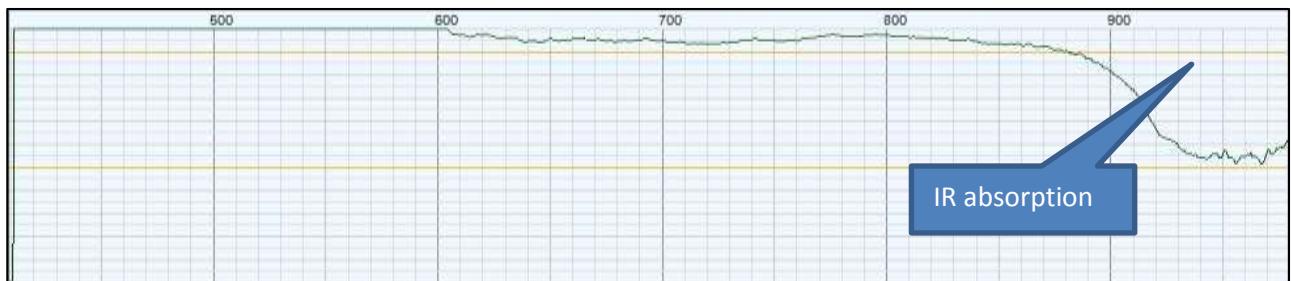
Linear reference from 400nm to 1000 nm

## Xenon Lamp Linear Reference



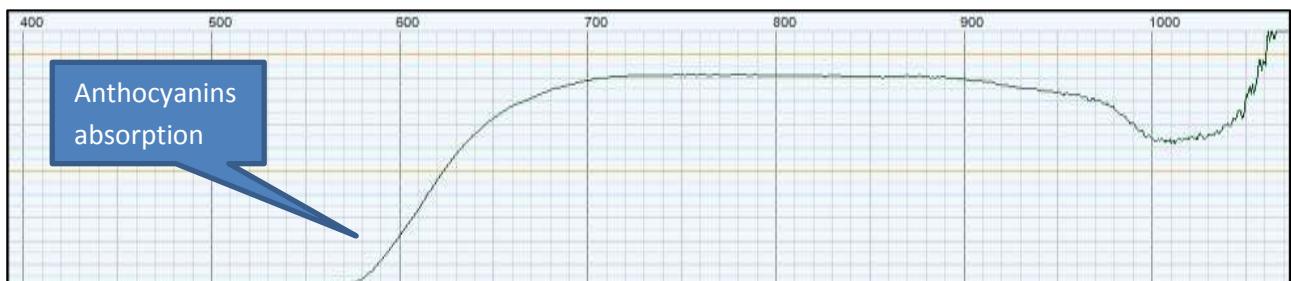
Linear reference from 370nm to 700 nm

## Water



Water absorption (transmission) spectrum. Water absorbs from near IR

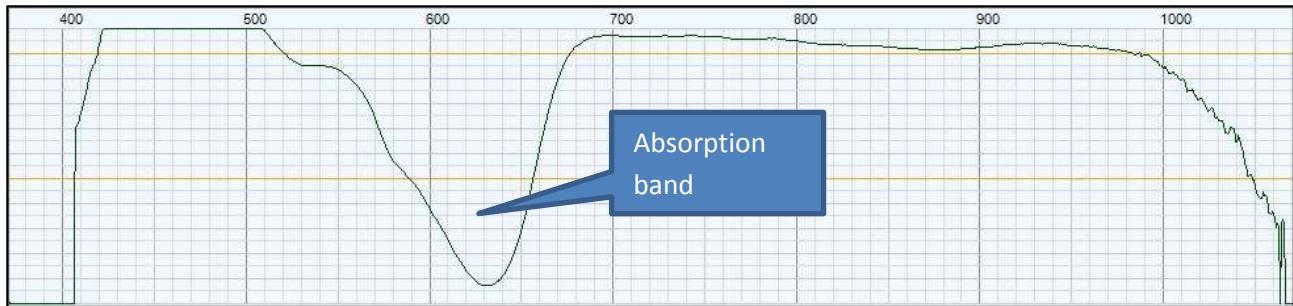
## Red Wine



Red wine absorption (transmission) spectrum

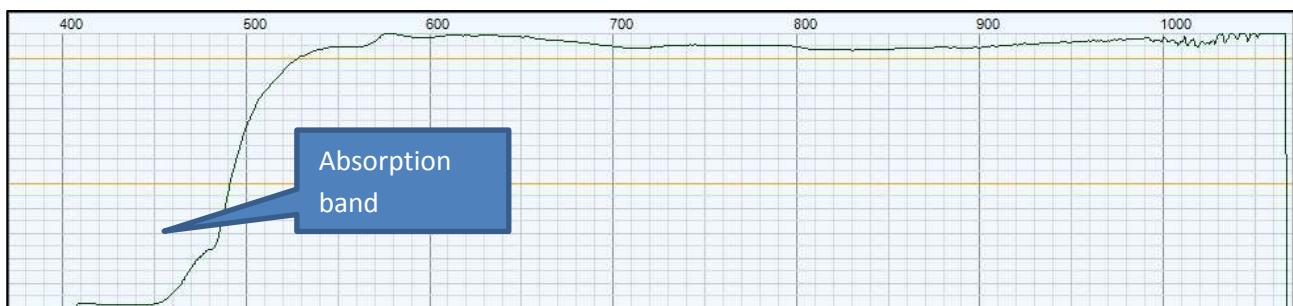
Main absorption band with  $\lambda$  shorter than 550nm due to the content of anthocyanins

## Blue Food Dye



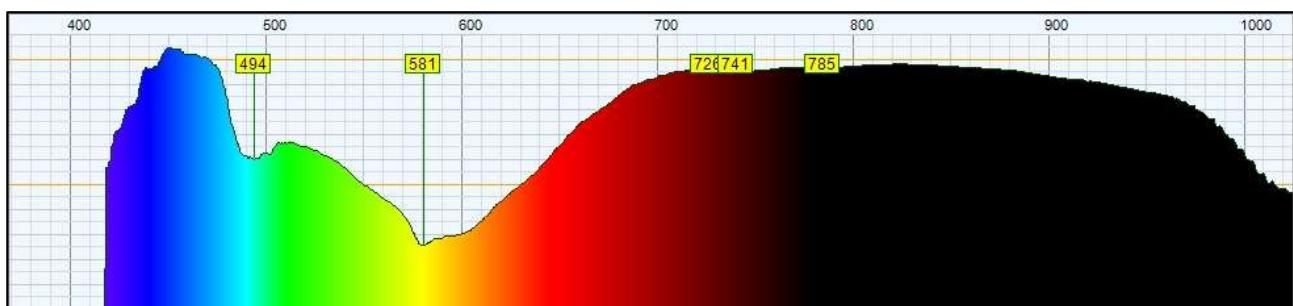
Blue food dye E131 (blue patent V) E514 (sodium sulfate) absorption (transmission) spectrum  
 $\lambda$  between 500nm and 700nm are absorbed

## Yellow Food Dye



Yellow food dye E102 (tartrazine) E110 (orange yellow S) E514 (sodium sulfate) absorption (transmission) spectrum  
 $\lambda$  shorter than 550nm are absorbed

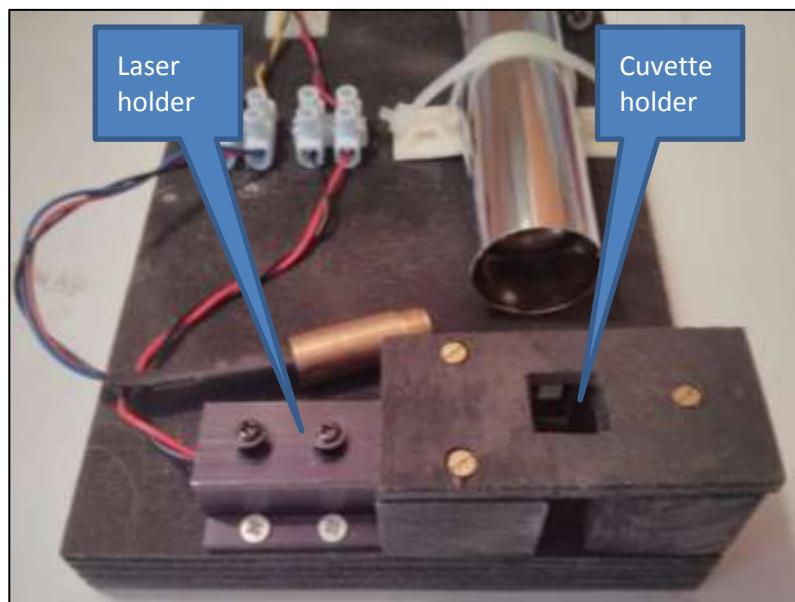
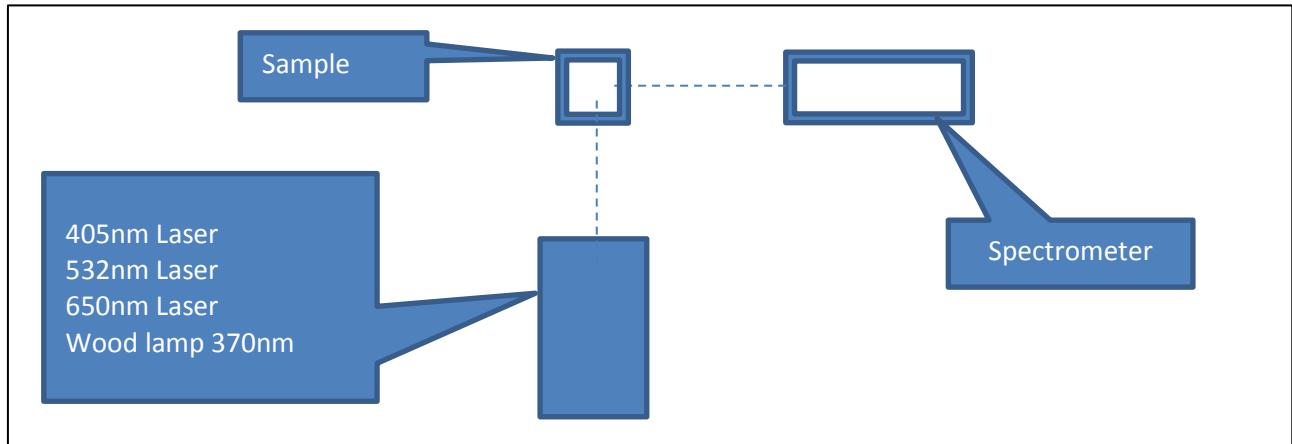
## Blue Ink



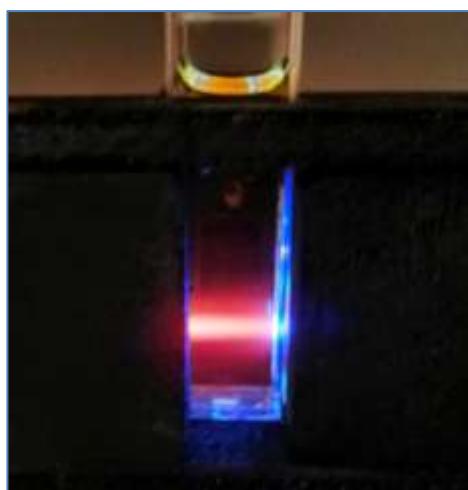
Blue ink absorption (transmission) spectrum  
 $\lambda$  between 470nm and 700nm are absorbed

# Fluorescence Spectroscopy

## Fluorescence Spectrometer Design



Construction scheme with excitation lasers and cuvette holder



Example of fluorescence of a sample (olive oil) excited with a violet laser at 405nm inside a cuvette

## Fluorescence Excitation Sources



### Violet Laser

Power: 20mW  
Wavelength: 405nm  
Current: <280mA @ 2.9 ~ 3V input  
Color: blue violet



### Green Laser

Power: 30mW  
Wavelength: 532nm  
Voltage: 2.9 ~ 3V input  
Color: green



### Red Laser

Power: 5mW  
Wavelength: 650nm  
Voltage: 2.9 ~ 3V input  
Color: red

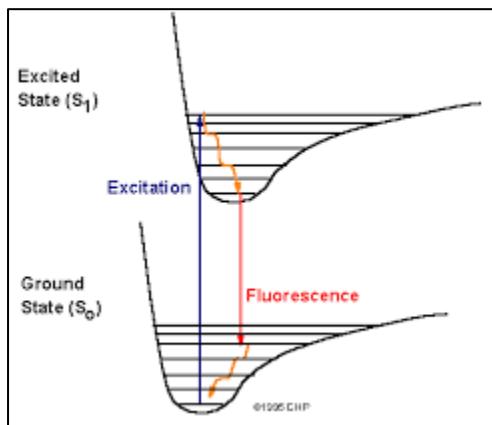


### Wood Lamp

Wavelength: 370nm

## Fluorescence Theory

At room temperature most molecules occupy the lowest vibrational level of the ground electronic state, and on absorption of light they are elevated to produce excited states. The simplified diagram below shows absorption by molecules to produce the first S<sub>1</sub> excited state.



Excitation can result in the molecule reaching any of the vibrational sub-levels associated with each electronic state. Since the energy is absorbed as discrete quanta, this should result in a series of distinct absorption bands. However, the simple diagram above neglects the rotational levels associated with each vibrational level and which normally increase the number of possible absorption bands to such an extent that it becomes impossible to resolve individual transitions. Therefore, most compounds have broad absorption spectra.

Having absorbed energy and reached one of the higher vibrational levels of an excited state, the molecule rapidly loses its excess of vibrational energy by collision and falls to the lowest vibrational level of the excited state. In addition, almost all molecules occupying an electronic state higher than the second undergo internal conversion and pass from the lowest vibrational level of the upper state to a higher vibrational level of a lower excited state which has the same energy. From there the molecules again lose energy until the lowest vibrational level of the first excited state is reached.

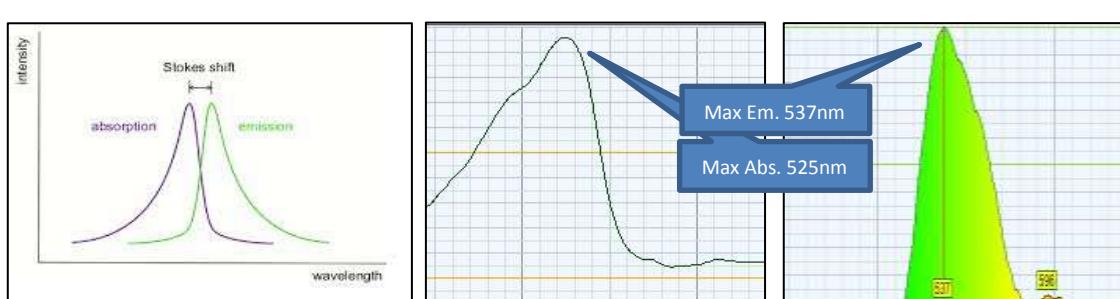
From this level, the molecule can return to any of the vibrational levels of the ground state, emitting its energy in the form of fluorescence. If this process takes place for all the molecules that absorbed light, then the quantum efficiency of the solution will be a maximum, unity. If, however, any other route is followed, the quantum efficiency will be less than one and may even be almost zero.

One transition, that from the lowest vibrational level in the ground electronic state to the lowest vibrational level in the first excited state, the 0 - 0 transition, is common to both the absorption and emission phenomena, whereas all other absorption transitions require more energy than any transition in the fluorescence emission. We can therefore expect the emission spectrum to overlap the absorption spectrum at the wavelength corresponding to the 0 - 0 transition and the rest of the emission spectrum to be of lower energy, or longer wavelength.

In practice, the 0-0 transitions in the absorption and emission spectra rarely coincide exactly, the difference representing a small loss of energy by interaction of the absorbing molecule with surrounding solvent molecules. This difference in wavelength is called **stokes shift**.

$\lambda_{em} > \lambda_a$

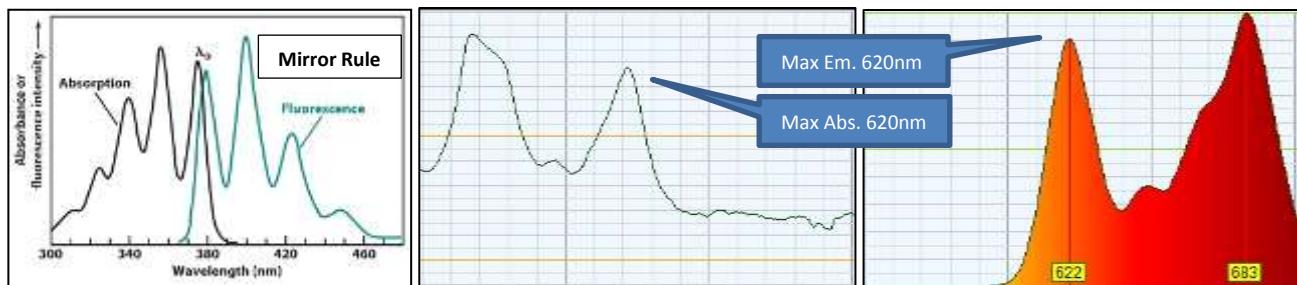
$\lambda_a$  and  $\lambda_{em}$  are absorption and emission spectra peaks



### Example of Stokes Shift

Absorption and emission spectra of **acridine orange**. From the pictures we can see that the difference between the maxima is rather small : stokes shift = 537 – 525 = **12nm**

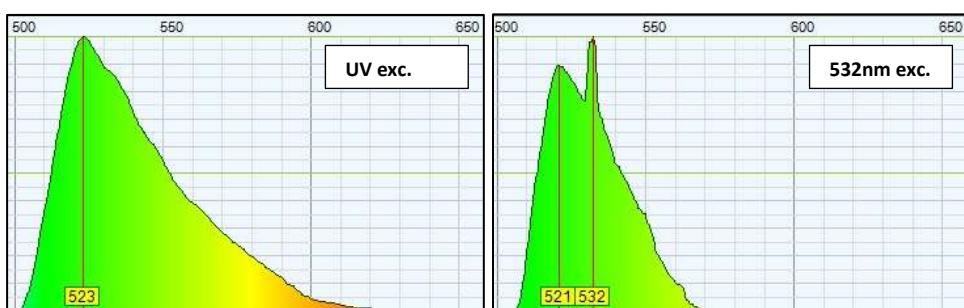
The absorption of energy to produce the first excited state does not perturb the shape of the molecule greatly and this means that the distribution of vibrational levels is very similar in both the ground and first excited states. The energy differences between the bands in the emission spectrum will be similar to those in the absorption spectrum and frequently the emission spectrum will be approximate to a mirror image of the absorption spectrum.



### Example of Mirror Spectra

Absorption and emission spectra of **hematoporphyrin**. There is coincidence of the first peaks of emission and absorption spectra. The shape of the two spectra are in a good agreement to the **mirror-image rule**

Since the emission of fluorescence always takes place from the lowest vibrational level of the first excited state, the shape of the emission spectrum is always the same, despite changing the wavelength of exciting light. This is also known as the Kasha rule

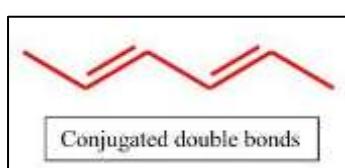


### Example of Emission Spectra Excited by different wavelength

Emission spectra of **fluorescein**. There is coincidence of the maximum and shape of emission spectrum despite different exciting wavelength. In the second spectrum are shown both the anti-stokes (negative shift) emission and the stokes emission (positive shift)

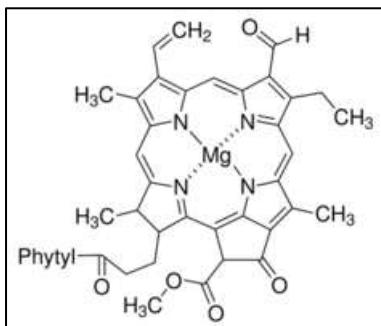
The Kasha rule does not always apply and is violated in many simple molecules, an example is the compost **azulene** which emits fluorescence from transition S<sub>2</sub> - S<sub>0</sub> instead of the usual transition S<sub>1</sub> - S<sub>0</sub>. For details see the paragraph on the chamomile essential oil.

Fluorescence is also influenced by the structure of the molecule. For example the rigid molecules that present systems of **conjugated double bonds**, are well suited to the fluorescence : in particular molecules where there are aromatic structures, in which the resonance phenomenon of the double bonds are **scattered throughout the structure**, if excited give rise to  $\pi \rightarrow \pi^*$  transitions, and thus facilitate the fluorescence.



# Spectroscopy of Organic Pigments

## Chlorophyll

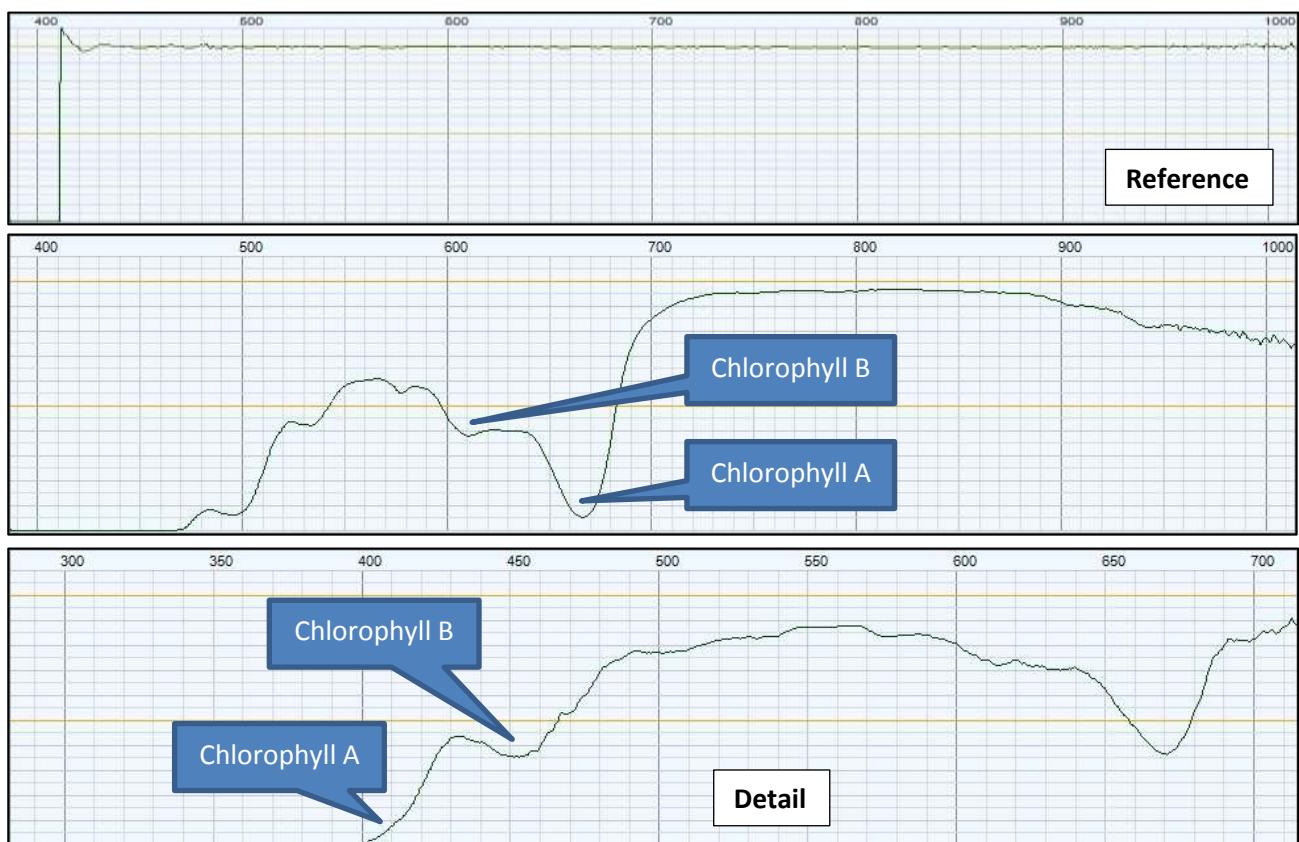


**Chlorophyll** is a green pigment found in chloroplasts of algae and plants. Chlorophyll is an extremely important biomolecule, critical in photosynthesis, which allows plants to absorb energy from light. Chlorophyll absorbs light most strongly in the blue portion of the electromagnetic spectrum, followed by the red portion. Conversely, it is a poor absorber of green and near-green portions of the spectrum, hence the green color of chlorophyll-containing tissues.

Measurement of the absorption of light is complicated by the solvent used to extract it from plant material, which affects the values obtained. In diethyl ether, chlorophyll A has approximate absorbance maximum of 430 nm and 662 nm, while chlorophyll B has approximate maximum of 453 nm and 642 nm.

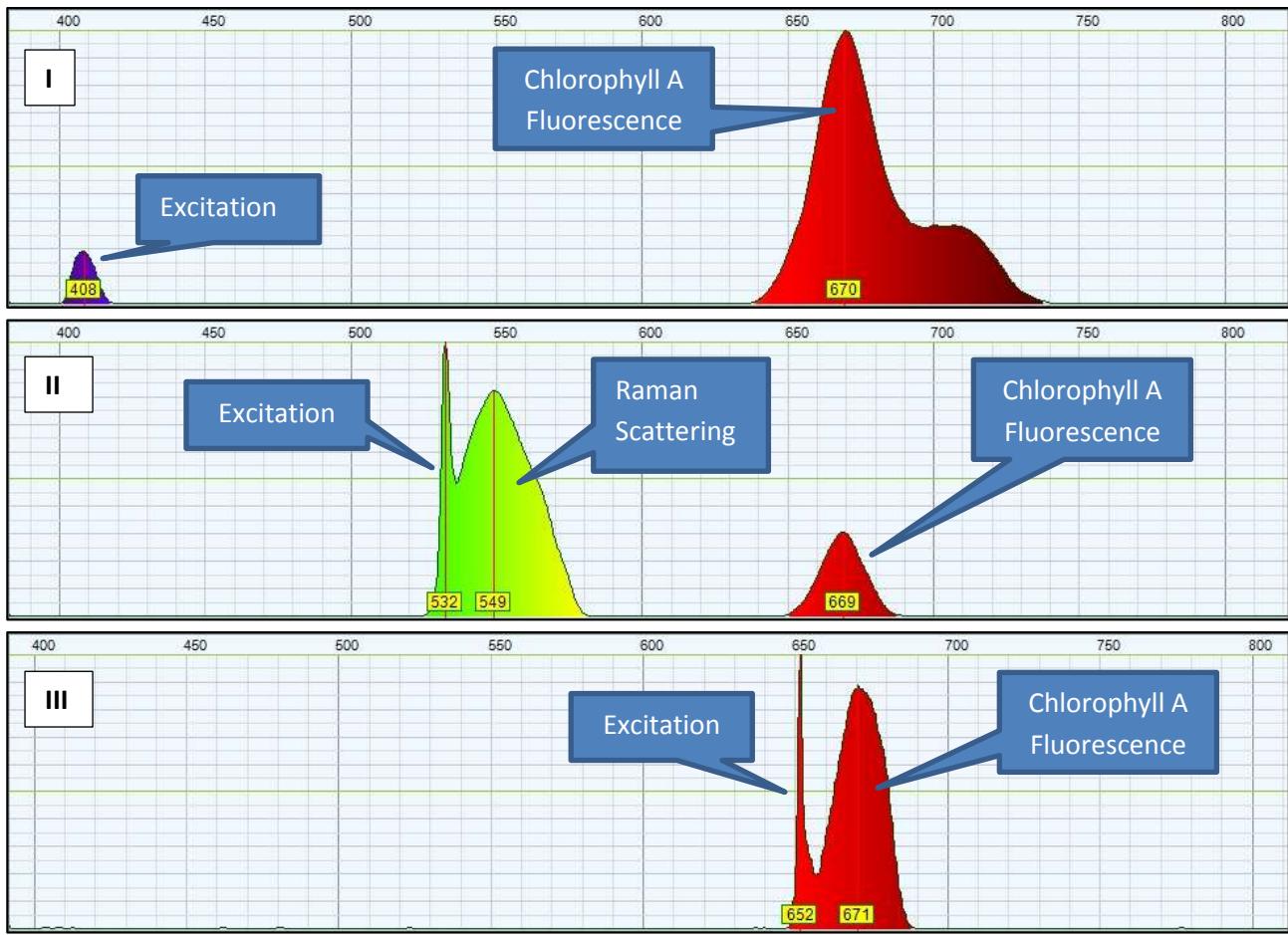
Chlorophyll A fluoresces at 673 nm (maximum) and 726 nm. The different absorption spectra of Chlorophyll A and B achieve a better absorption of the sun radiation in order to enhance the efficiency of the photosynthesis.

The alcoholic chlorophyll solution has been obtained with spinach leaves grinded and macerated in ethanol 95%



Absorption (transmission) spectrum of chlorophyll solution in 95% ethanol

The green band from 500nm till to 600nm passes without absorption. There are two main absorption bands, the first one on the red the second one on the blue. Two maxima between 600nm and 700nm correspond to absorption peaks of chlorophyll A and chlorophyll B. The absorption on the blue band can be explained with the sum of the absorptions of the two chlorophyll types



**I : Chlorophyll fluorescence spectrum excited with laser emission at 405nm**

Emission peak at 670nm due to Chlorophyll A with a second peak around 710nm and tail till to 740nm

**II : Fluorescence spectrum excited with laser emission at 532nm**

Emission peak at 670nm due to Chlorophyll A

**III : Fluorescence spectrum excited with laser emission at 650nm**

Emission peak at 670nm due to Chlorophyll A

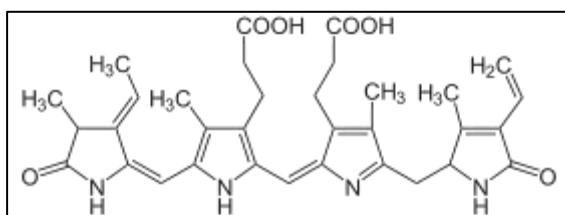
## Phycoerythrin

**Phycoerythrin (PE)** is a red protein-pigment complex from the light-harvesting phycobiliprotein family, present in red algae rhodophytes, accessory to the main chlorophyll pigments responsible for photosynthesis.

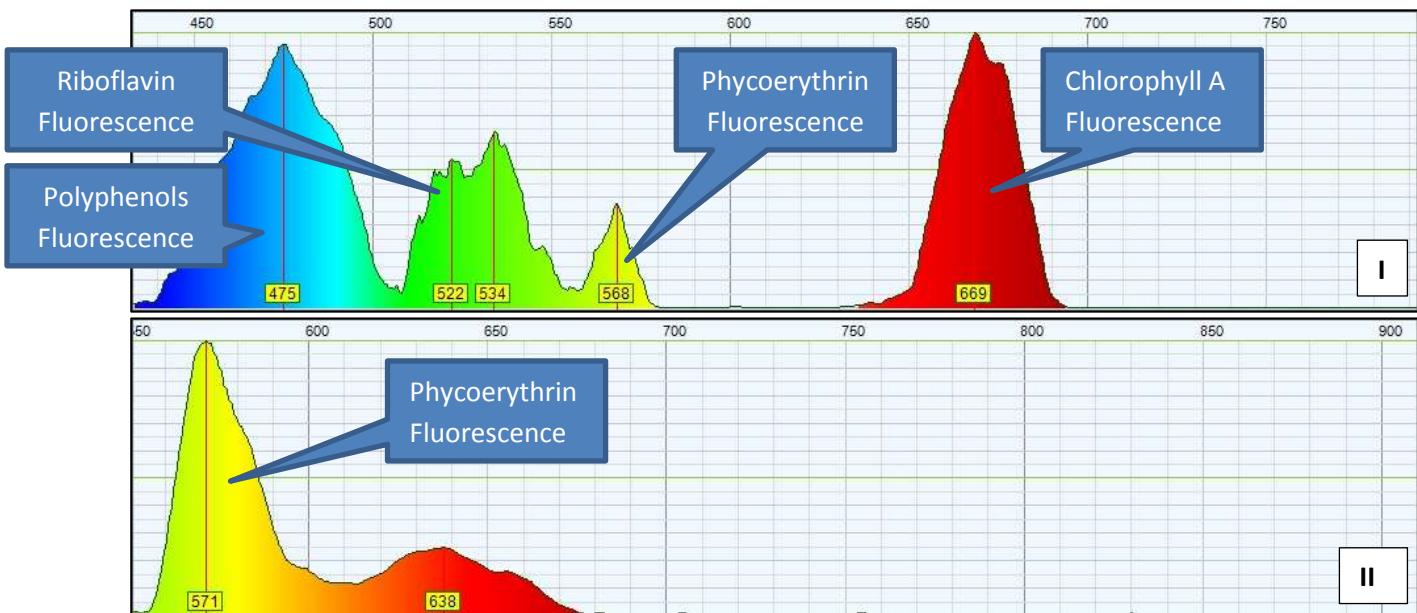
Red algae, like **palmaria palmata**, are red because of the presence of the pigment phycoerythrin; this pigment reflects red light and absorbs blue light. Because blue light penetrates water to a greater depth than light of longer wavelengths, these pigments allow red algae to photosynthesize and live at somewhat greater depths than most other "algae". Some rhodophytes have very little phycoerythrin, and may appear green or bluish from the chlorophyll and other pigments present in them.

Absorption peaks in the visible light spectrum are measured at 495 and 545/566 nm, depending on the chromophores bound and the considered organism. A strong emission peak exists at **575 ± 10 nm**. (i.e., phycoerythrin absorbs slightly blue-green/yellowish light and emits slightly orange-yellow light.)

The aqueous phycoerythrin solution has been obtained with **palmaria palmata** leaves grinded and macerated in water



**Phycoerythrobilin** is the typical chromophore in phycoerythrin. It is similar to porphyrin of chlorophyll for example, but tetrapyrrole is linear, not closed into ring with metal ion in the middle.



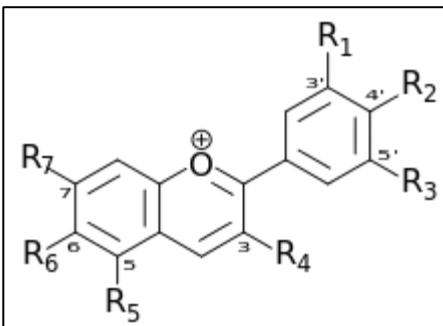
I : Fluorescence spectrum excited with laser emission at 405nm

Different fluorescence bands are shown : around 670nm, due to chlorophyll, around 570nm due to phycoerythrin. The two bands at 475nm and 535nm are likely due to polyphenols and riboflavin

II : Fluorescence spectrum excited with laser emission at 532nm

The 532nm excitation shows the maximum at 570nm due to the phycoerythrin chromophore

## Anthocyanins as pH Indicator



**Anthocyanins** are water-soluble vacuolar pigments that may appear red, purple, or blue depending on the pH. They belong to a parent class of molecules called flavonoids synthesized via the phenylpropanoid pathway; they are odorless and nearly flavorless, contributing to taste as a moderately astringent sensation. Anthocyanins occur in all tissues of higher plants, including leaves, stems, roots, flowers, and fruits. Anthoxanthins are clear, white to yellow counterparts of anthocyanins occurring in plants.

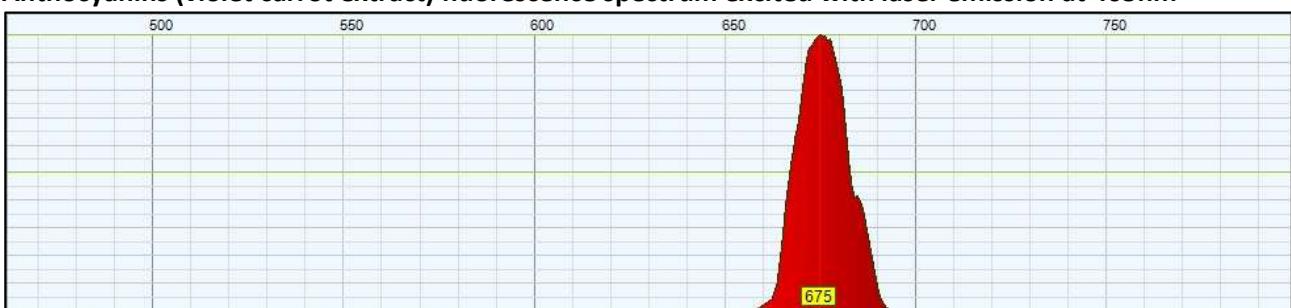
Anthocyanins are derived from anthocyanidins by adding pendant sugars. Anthocyanins are glucosides of anthocyanidins, the basic chemical structure of which is shown in the picture.

The absorbance pattern responsible for the red color of anthocyanins may be complementary to that of green chlorophyll in photosynthetically active tissues. Together with carotenoids the red color become evident in autumn when the chlorophyll degrades and the remaining anthocyanins and carotenoids contribute to the color of the leaves.

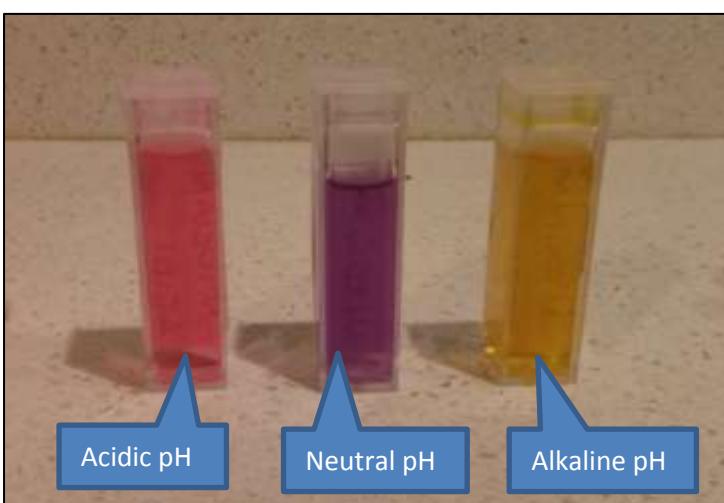
In addition to their role as light-attenuators, anthocyanins also act as powerful antioxidants.

The alcoholic anthocyanins solution has been obtained with red cabbage leaves grinded and macerated in ethanol 95% and with violet carrots also grinded and macerated in ethanol

**Anthocyanins (violet carrot extract) fluorescence spectrum excited with laser emission at 405nm**

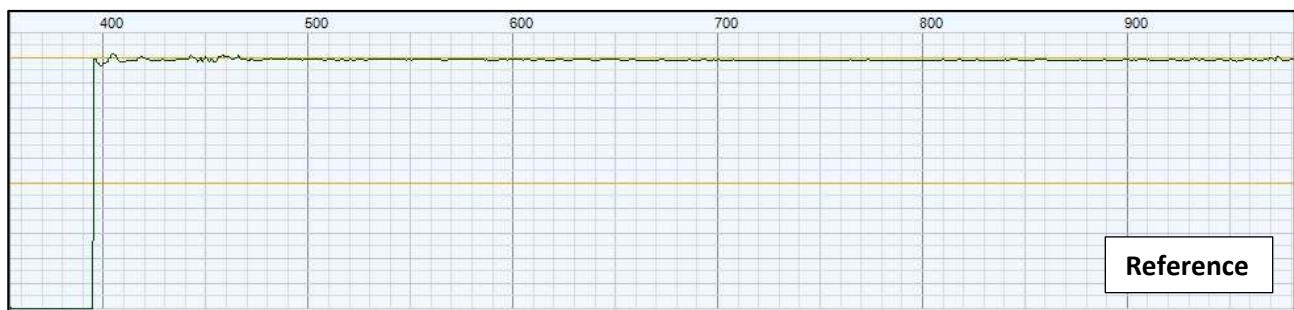


It is evident a red fluorescence that can be attributed to anthocyanins because the carrot extract does not contain chlorophyll

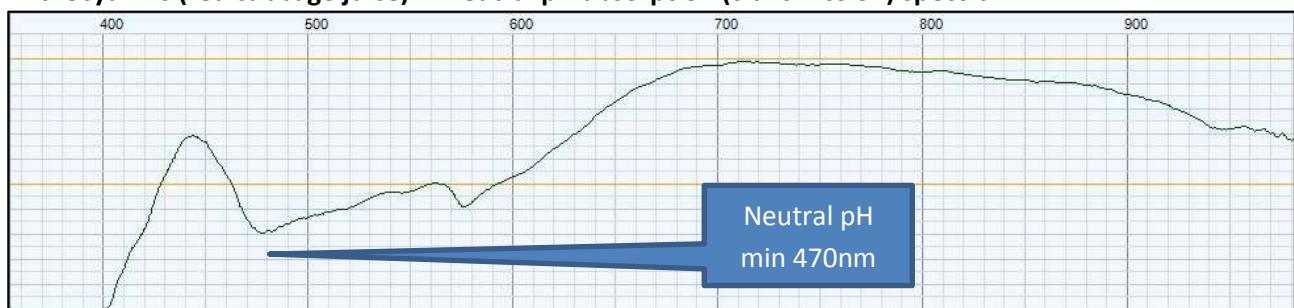


**Ethanol 95% anthocyanins solution in neutral solution, centre of the picture, in acidic solution on the left and in alkaline solution, on the right**

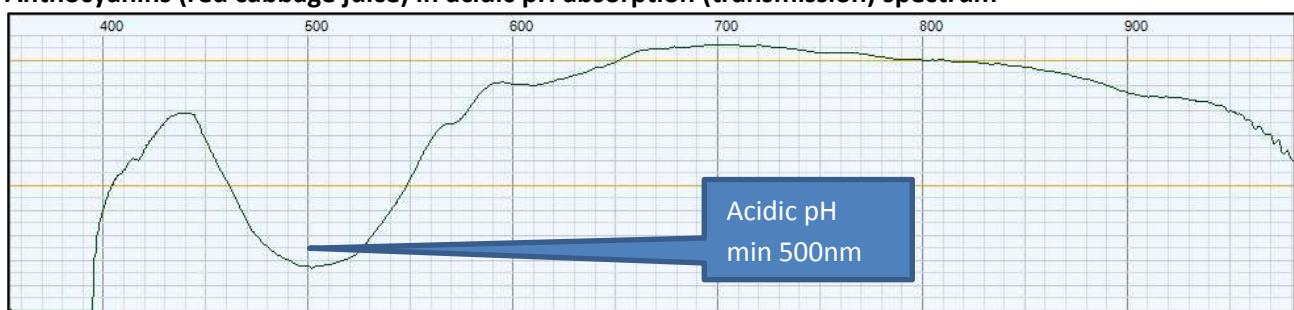
### Reference Spectrum



### Anthocyanins (red cabbage juice) in neutral pH absorption (transmission) spectrum



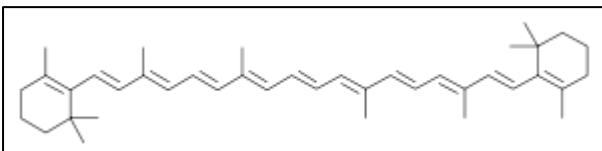
### Anthocyanins (red cabbage juice) in acidic pH absorption (transmission) spectrum



### Anthocyanins (red cabbage juice) in alkaline pH absorption (transmission) spectrum



## Carotenoids



**Carotenoids** are organic pigments that are found in the chloroplasts and chromoplasts of plants and some other photosynthetic organisms, including some bacteria and some fungi.

There are over 600 known carotenoids; they are split into two classes, xanthophylls (which contain oxygen) and carotenes (which are purely hydrocarbons, and contain no oxygen).

In general, carotenoids absorb wavelengths ranging from 400-550 nanometers (violet to green light). They serve two key roles in plants and algae: they absorb light energy for use in photosynthesis, and they protect chlorophyll from photodamage. In humans, three carotenoids (beta-carotene, alpha-carotene, and beta-cryptoxanthin) have vitamin A activity (meaning that they can be converted to retinal), and these and other carotenoids can also act as antioxidants.

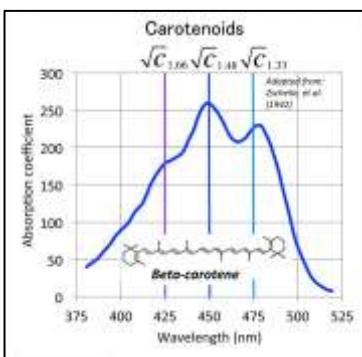
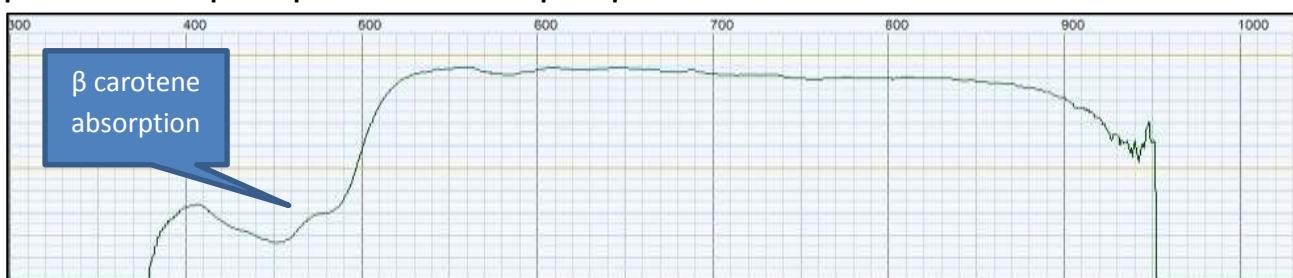
Carotenoids belong to the category of tetraterpenoids (i.e., they contain 40 carbon atoms, being built from four terpene units each containing 10 carbon atoms). Structurally, carotenoids take the form of a polyene hydrocarbon chain which is sometimes terminated by rings, and may or may not have additional oxygen atoms attached.

- Carotenoids with molecules containing oxygen, such as lutein and zeaxanthin, are known as **xanthophylls**.
- The unoxygenated (oxygen free) carotenoids such as  $\alpha$ -carotene,  $\beta$ -carotene, and lycopene, are known as **carotenes**.

Their colour, ranging from pale yellow through bright orange to deep red, is directly linked to their structure. Xanthophylls are often yellow, hence their class name. The double carbon-carbon bonds interact with each other in a process called conjugation, which allows electrons in the molecule to move freely across these areas of the molecule. As the number of conjugated double bonds increases, electrons associated with conjugated systems have more room to move, and require less energy to change states. This causes the range of energies of light absorbed by the molecule to decrease. As more frequencies of light are absorbed from the short end of the visible spectrum, the compounds acquire an increasingly red appearance.

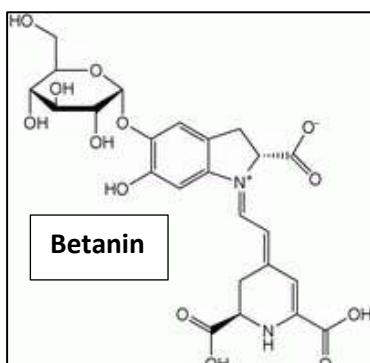
The  $\beta$  carotene solution has been obtained by grinded carrots heated in oil.

**$\beta$  carotene absorption spectrum : two absorption peaks at 480nm and 450nm**



**Reference  $\beta$  carotene absorption spectrum**

## Betalaine



**Betalains** are a class of red and yellow indole-derived pigments found in plants of the Caryophyllales, where they replace anthocyanin pigments. Betalains also occur in some higher order fungi. They are most often noticeable in the petals of flowers, but may color the fruits, leaves, stems, and roots of plants that contain them. They include pigments such as those found in beets.

The name "betalain" comes from the Latin name of the common beet (*Beta vulgaris*), from which betalains were first extracted. The deep red color of beets, bougainvillea, amaranth, and many cactuses results from the presence of betalain pigments. The particular shades of red to purple are distinctive and unlike that of anthocyanin pigments found in most plants.

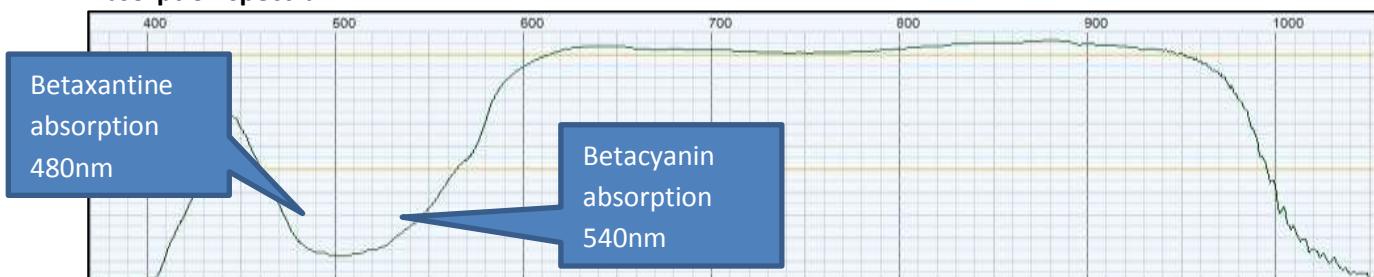
There are two categories of betalains:

- **Betacyanins** include the reddish to violet betalain pigments. Among the betacyanins present in plants include betanin, isobetanin, probetanin, and neobetanin.
- **Betaxanthins** are those betalain pigments which appear yellow to orange. Among the betaxanthins present in plants include vulgaxanthin, miraxanthin, portulaxanthin, and indicaxanthin.

Plant physiologists are uncertain of the function that betalains serve in those plants which possess them, but there is some preliminary evidence that they may have fungicidal properties. Furthermore, betalains have been found in fluorescent flowers.

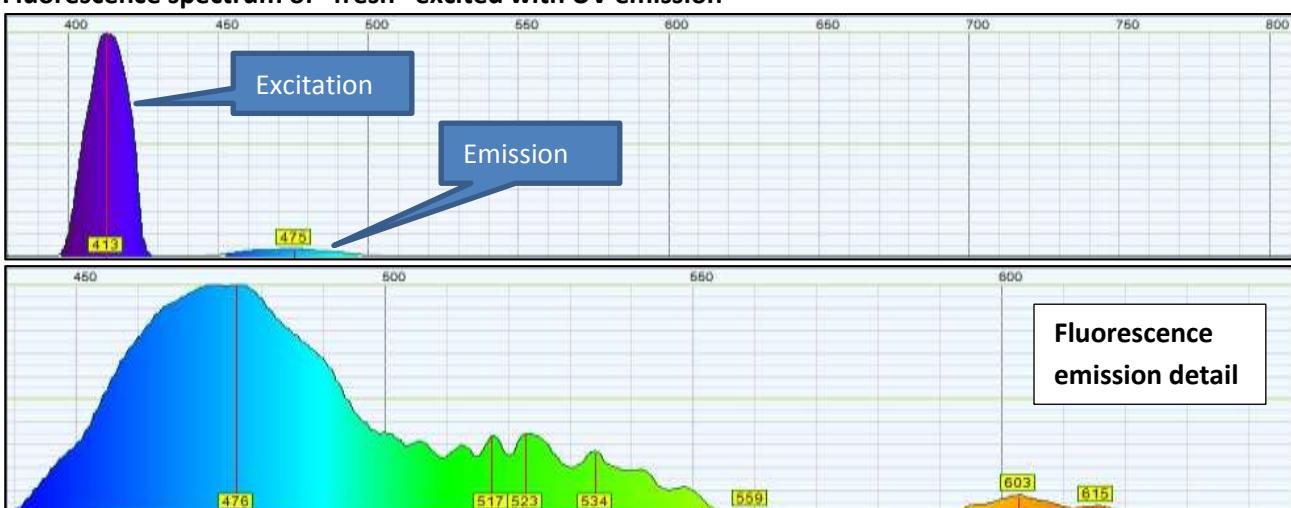
The aqueous betalain solution has been obtained with red beetroot grinded and macerated in water

### Absorption spectrum



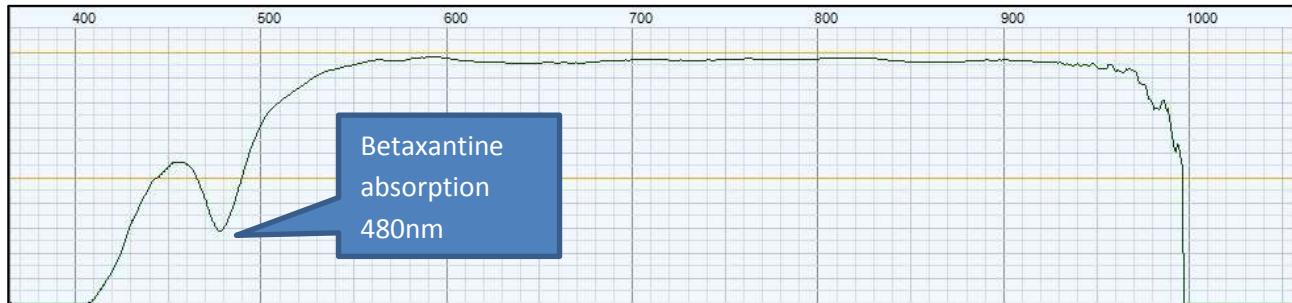
Absorption into visible band centered at 500nm and for wavelengths shorter than 400nm

### Fluorescence spectrum of "fresh" excited with UV emission



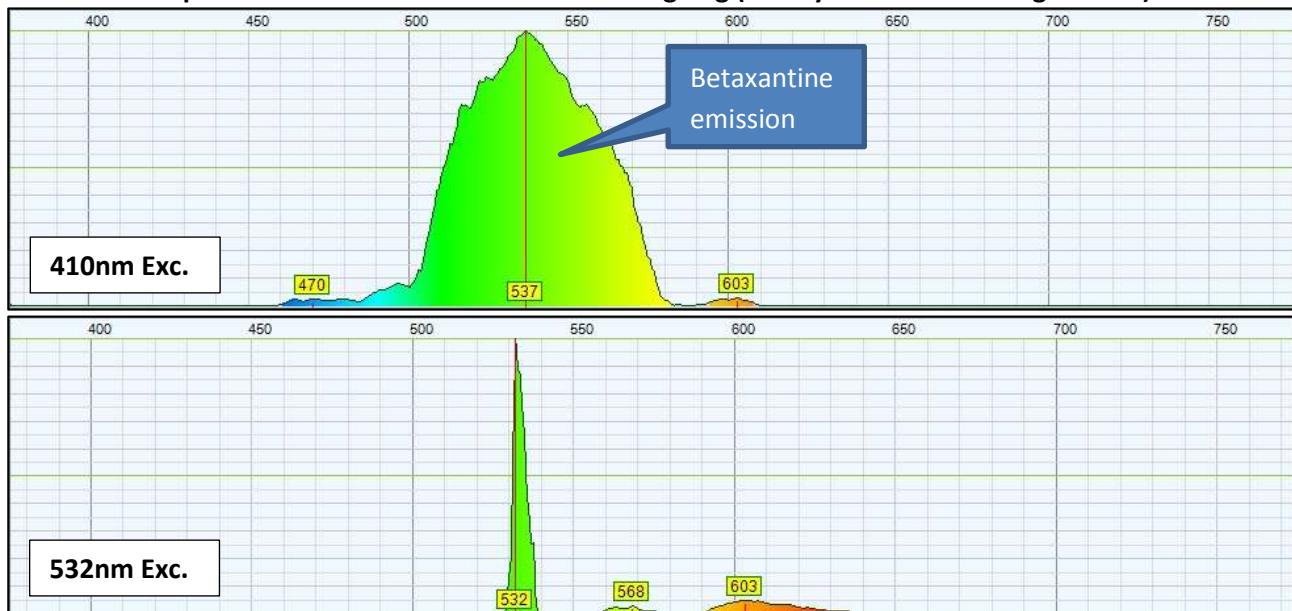
Fluorescent bands at 476nm – 523nm – 603nm. The green-yellow fluorescence, caused by **betaxantine** is partially absorbed by **betacyanin**, which absorbs a band around 540nm

### Absorption spectrum of the betalain solution after ageing (betacyanin has been degraded)



Betaxanthine absorption band with peak at 480nm

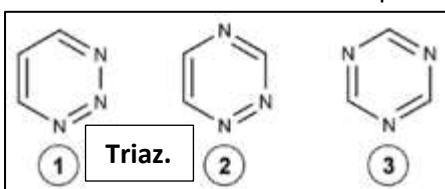
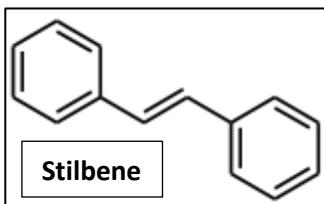
### Fluorescence spectrum of the betalain solution after ageing (betacyanin has been degraded)



The fluorescence maximum at 540 nm is due to the betaxanthine. This fluorescence band is visible in the aged solution because the concentration of betacyanine has been reduced. In the "fresh" solution this peak is not visible because the emission is absorbed by betacyanine, whose absorption peak corresponds to 540 nm. Absence of betacyanine is also evident in the absorption spectrum which shows the betaxanthine absorption peak only. The 532 nm excitation produces only a weak fluorescence at longer wavelength.

# Spectroscopy of Fluorescent Dyes

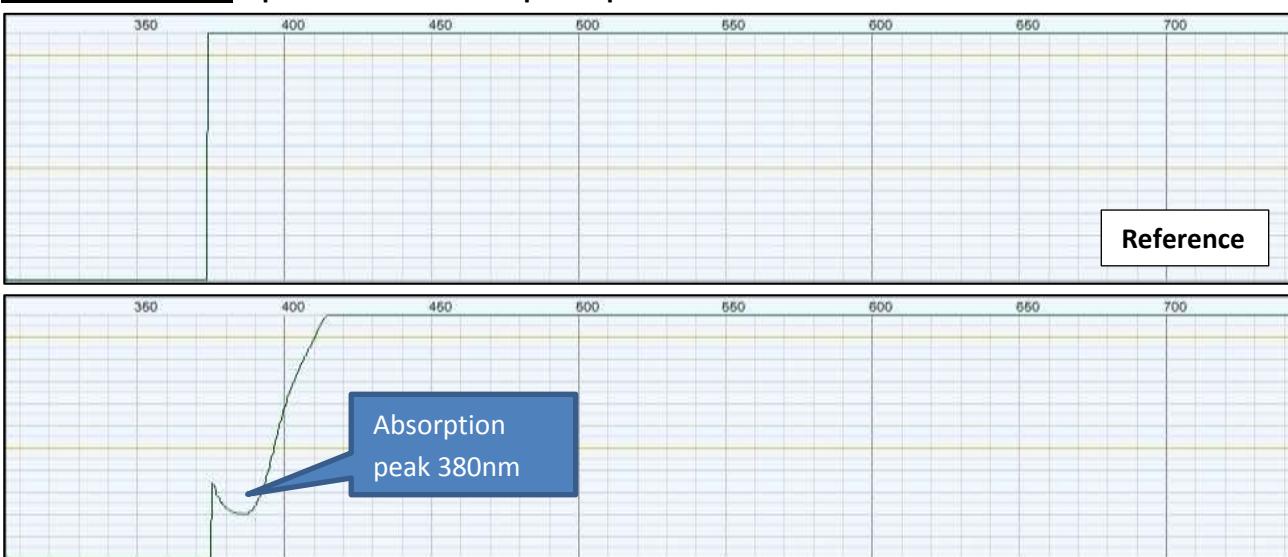
## Stilbenes – Triazine (Optical Brightener)



**Optical brighteners**, optical brightening agents (OBAs), fluorescent brightening agents (FBAs) or fluorescent whitening agents (FWAs) are chemical compounds that absorb light in the ultraviolet and violet region (usually 340-370 nm) of the electromagnetic spectrum, and re-emit light in the blue region (typically 420-470 nm). Fluorescent activity is a short term or rapid emission response, unlike phosphorescence, which is a delayed emission. These additives are often used to enhance the appearance of color of fabric and paper, causing a "whitening" effect, making materials look less yellow by increasing the overall amount of blue light reflected.

The most common classes of chemicals with this property are the **stilbenes-triazine** and older, fluorescent chemical such as umbelliferone, which absorb energy in the UV portion of the spectrum and re-emit it in the blue portion of the visible spectrum. A white surface treated with an optical brightener can emit more visible light than that which shines on it, making it appear brighter. The blue light emitted by the brightener compensates for the diminishing blue of the treated material and changes the hue away from yellow or brown and toward white.

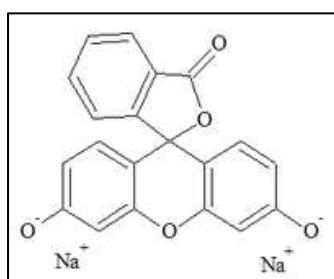
### Stilbenes – triazine aqueous solution absorption spectrum



### Stilbenes – triazine aqueous solution fluorescence spectrum excited with UV emission



## Fluorescein Sodium (Uranine)



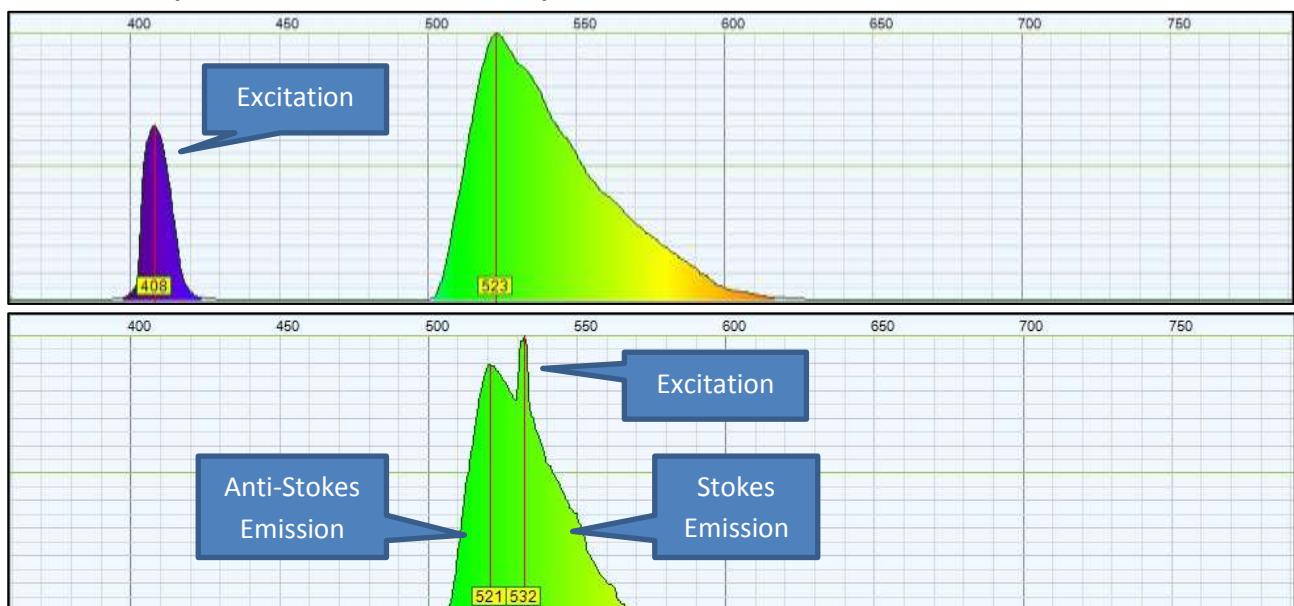
**Fluorescein** is a synthetic organic compound available as a dark orange/red powder slightly soluble in water and alcohol. It is widely used as a fluorescent tracer for many applications.

Fluorescein is a fluorophore commonly used in microscopy, in a type of dye laser as the gain medium, in forensics and serology to detect latent blood stains, and in dye tracing. Fluorescein has an absorption maximum at **494nm** and emission maximum of **521nm** (in water). The major derivatives are fluorescein isothiocyanate (FITC). The disodium salt form of fluorescein is known as uranine or D&C Yellow no. 8. The fluorescence of this molecule is very intense; peak excitation occurs at 494nm and peak emission at 521nm.

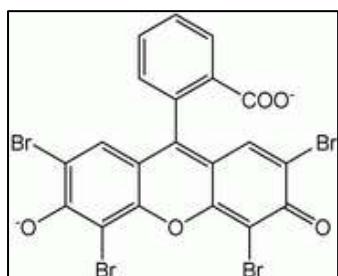
Fluorescein aqueous solution absorption spectrum. Second spectrum with detail in the UV band



Fluorescein aqueous solution fluorescence spectrum, excited from UV radiation and from 532nm laser

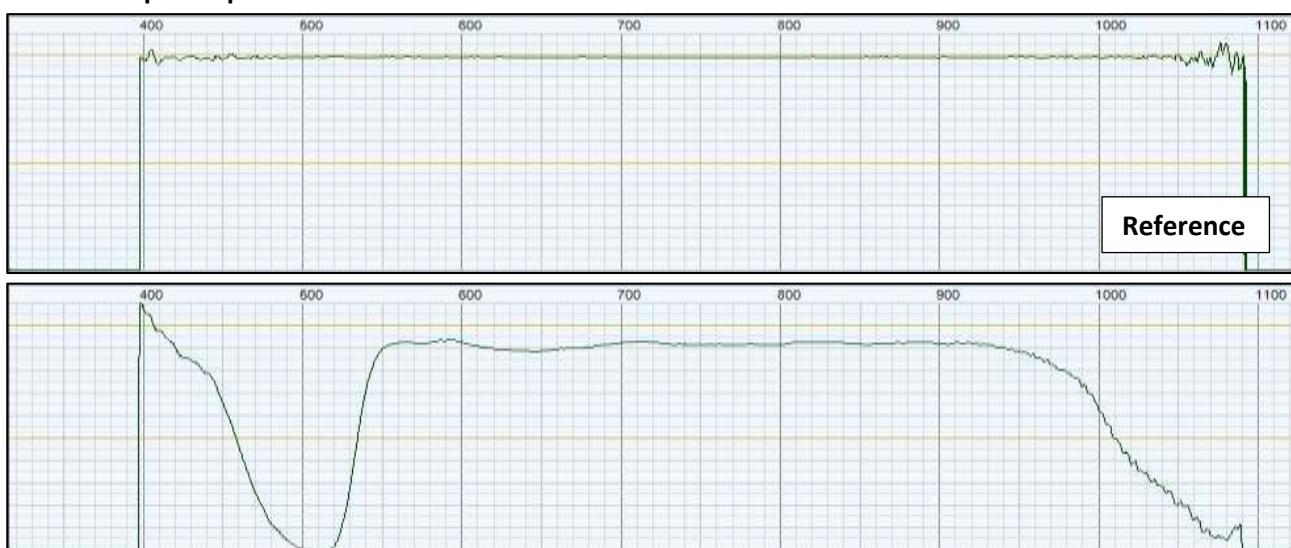


## Eosin

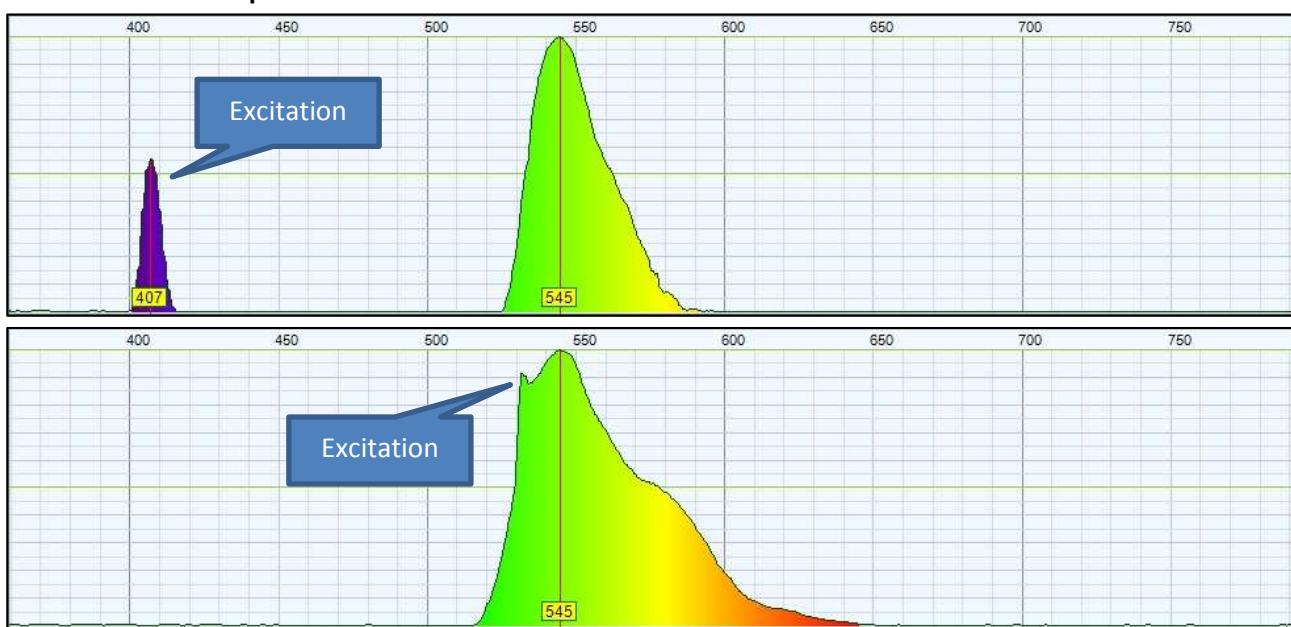


**Eosin** is a fluorescent acidic / negative compound that binds to and forms salts with basic, or eosinophilic, compounds containing positive charges (such as proteins that are basic / positive due to the presence of amino acid residues such as Arginine and Lysine) and stains them dark red or pink as a result of the actions of bromine on fluorescein. In addition to staining proteins in the cytoplasm, it can be used to stain collagen and muscle fibers for examination under the microscope. Structures that stain readily with eosin are termed eosinophilic. There are actually two very closely related compounds commonly referred to as eosin. Most often used is **Eosin Y**. The other eosin compound is **Eosin B**; it has a very faint bluish cast. The two dyes are interchangeable, and the use of one or the other is a matter of preference and tradition. Eosin Y is a tetrabromo derivative of fluorescein. Eosin B is a dibromo dinitro derivative of fluorescein.

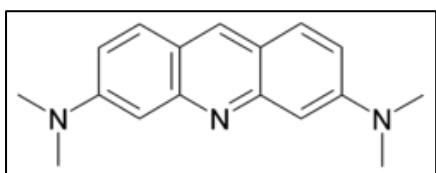
### Eosin absorption spectrum



### Eosin fluorescence spectrum excited from UV radiation and 532nm laser



## Acridine Orange

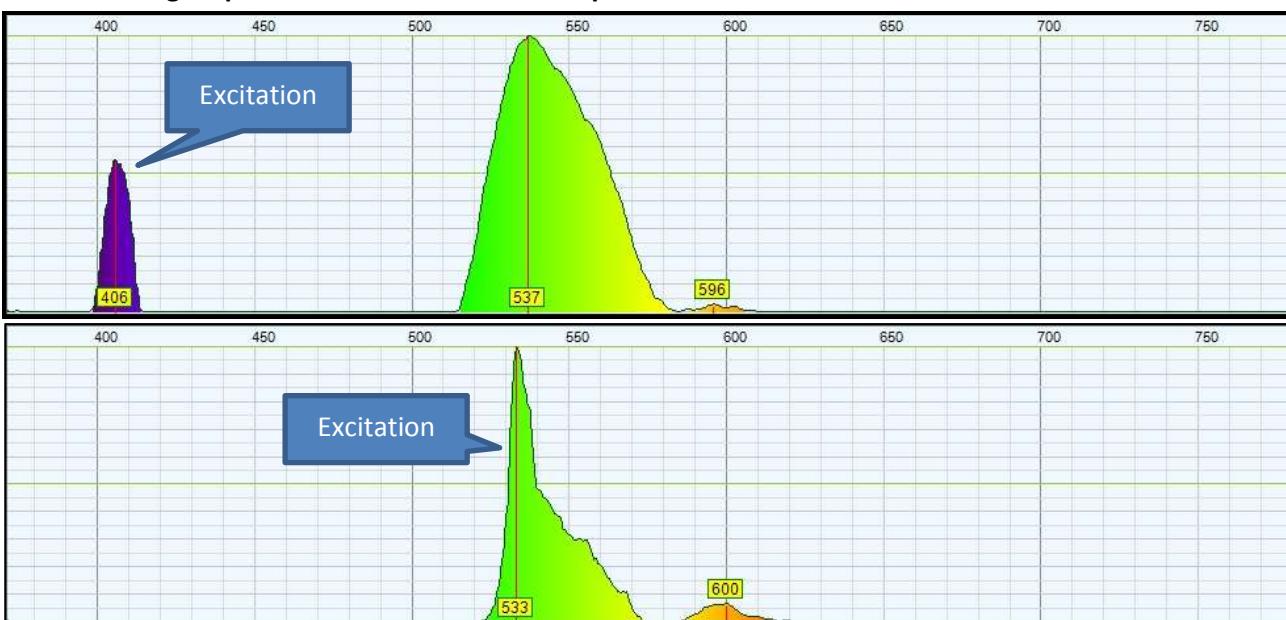


**Acridine orange** is an organic compound. It is used as a nucleic acid-selective fluorescent cationic dye useful for cell cycle determination. It interacts with DNA and RNA by intercalation or electrostatic attractions respectively. When bound to DNA, it is very similar spectrally to fluorescein, with an excitation maximum at 502 nm and an emission maximum at 525 nm (green). When it associates with RNA, the excitation maximum shifts to 460 nm (blue) and the emission maximum shifts to 650 nm (red). Acridine orange has been widely accepted and used in many different areas, such as epifluorescence microscopy.

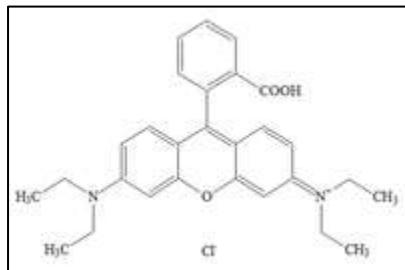
Acridine orange aqueous solution absorption spectrum



Acridine orange aqueous solution fluorescence spectrum excited from UV radiation and 532nm laser



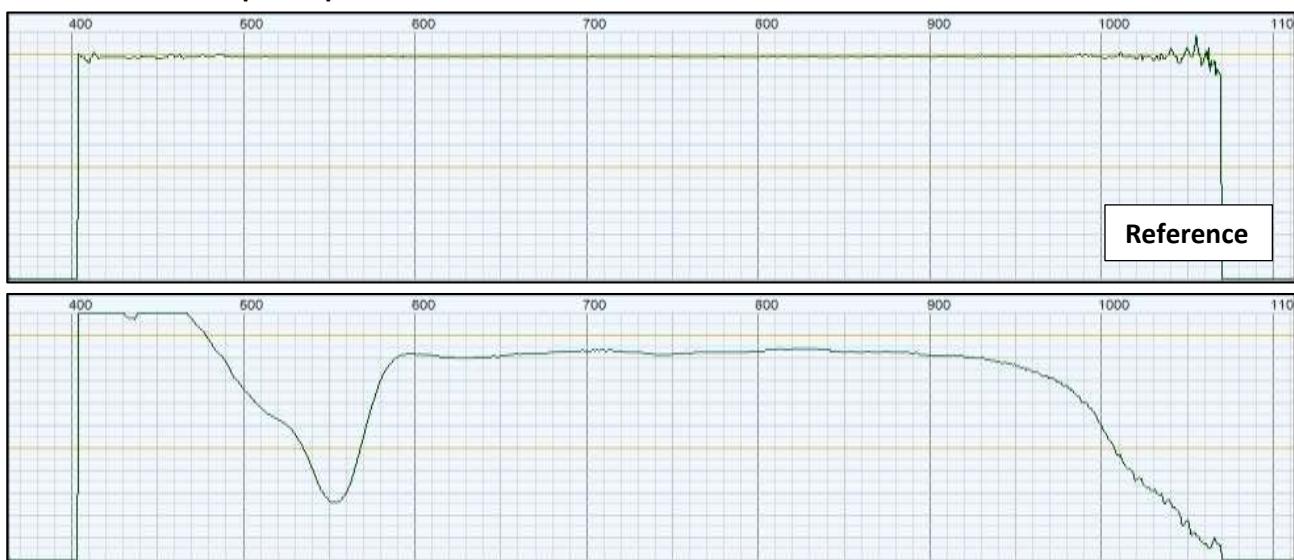
## Rhodamine B



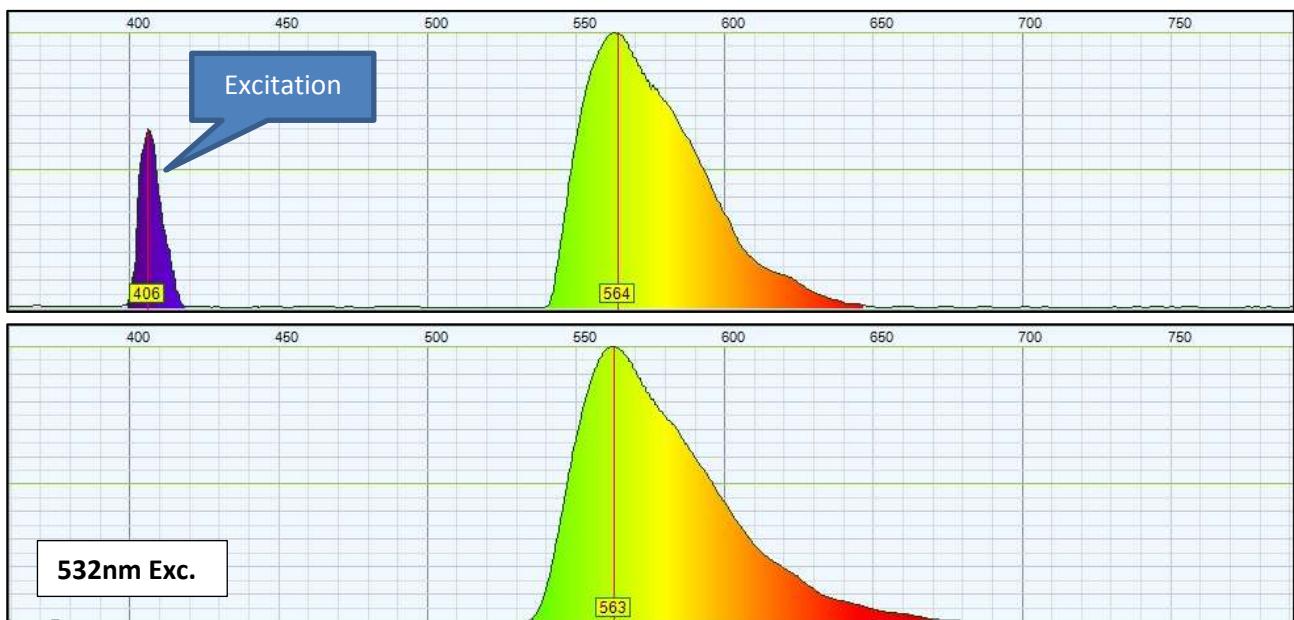
**Rhodamine B** is a chemical compound and a dye. It is often used as a tracer dye within water to determine the rate and direction of flow and transport. Rhodamine dyes fluoresce and can thus be detected easily and inexpensively with instruments called fluorometers. Rhodamine dyes are used extensively in biotechnology applications such as fluorescence microscopy. Rhodamine B is used in biology as a staining fluorescent dye.

Rhodamine B is tunable around 610 nm when used as a laser dye. Its luminescence quantum yield is 0.65 in basic ethanol, 0.49 in ethanol, 1.0, and 0.68 in 94% ethanol. The fluorescence yield is temperature dependent.

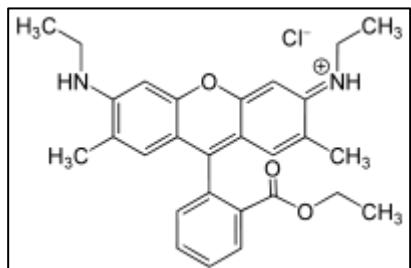
### Rhodamine B absorption spectrum



### Rhodamine B fluorescence spectrum excited by UV emission and by 532nm laser



## Rhodamine 6G



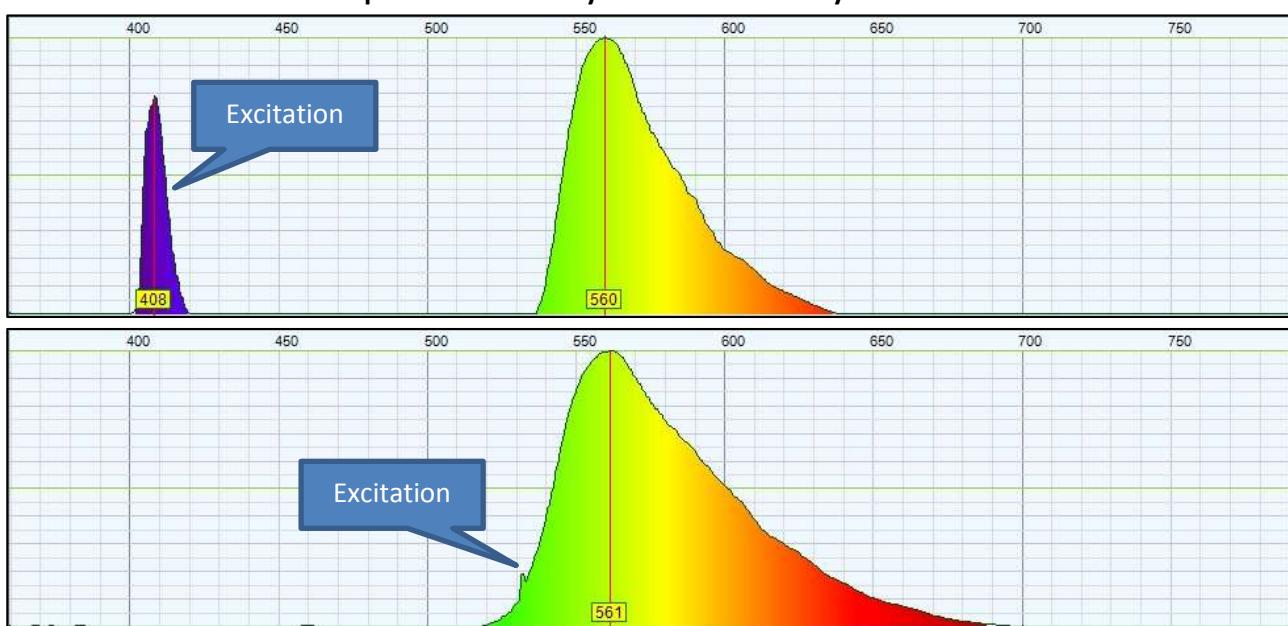
**Rhodamine 6G** is a highly fluorescent rhodamine family dye. It is often used as a tracer dye within water to determine the rate and direction of flow and transport. Rhodamine dyes fluoresce and can thus be detected easily and inexpensively with instruments called fluorometers. Rhodamine dyes are used extensively in biotechnology applications such as fluorescence microscopy, flow cytometry, fluorescence correlation spectroscopy and ELISA.

Rhodamine 6G is also used as a laser dye, or gain medium, in dye lasers, and is pumped by the 2nd (532 nm) harmonic from an Nd:YAG laser or nitrogen laser. The dye has a remarkably high photostability, high fluorescence quantum yield (0.95), low cost, and its lasing range has close proximity to its absorption maximum (approximately 530 nm). The lasing range of the dye is 555 to 585 nm with a maximum at 566 nm.

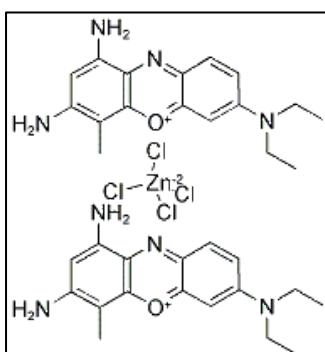
### Rhodamine 6G absorption spectrum



### Rhodamine 6G fluorescence spectrum excited by UV emission and by 532nm laser



## Green Fluorescent Dye



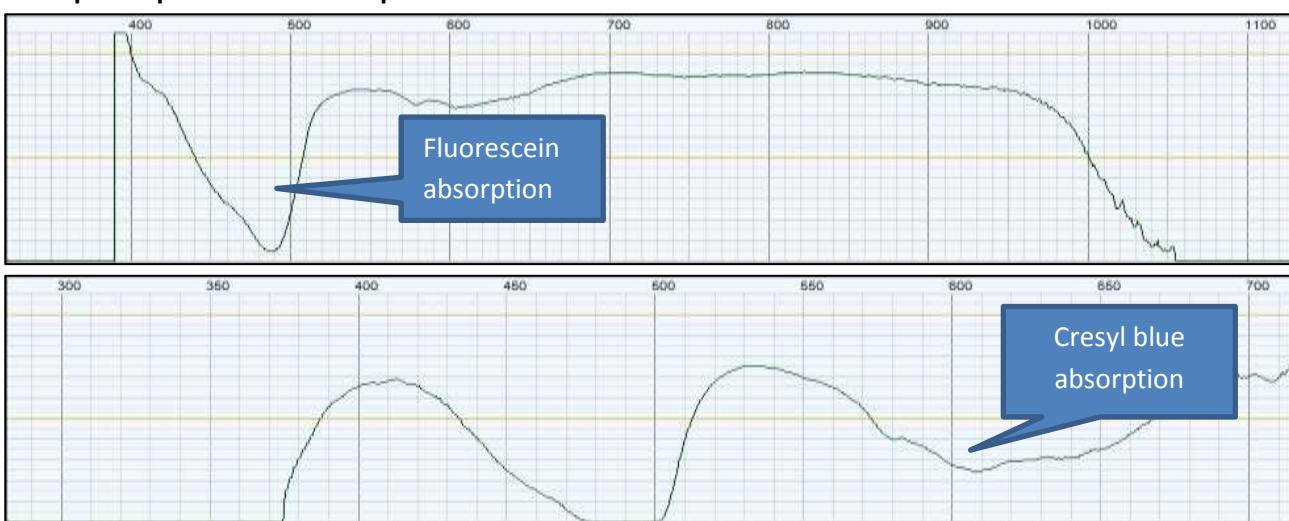
Green fluorescent dye is a blend of fluorescein and cresyl brilliant blue.

**Fluorescein** is a synthetic organic compound.

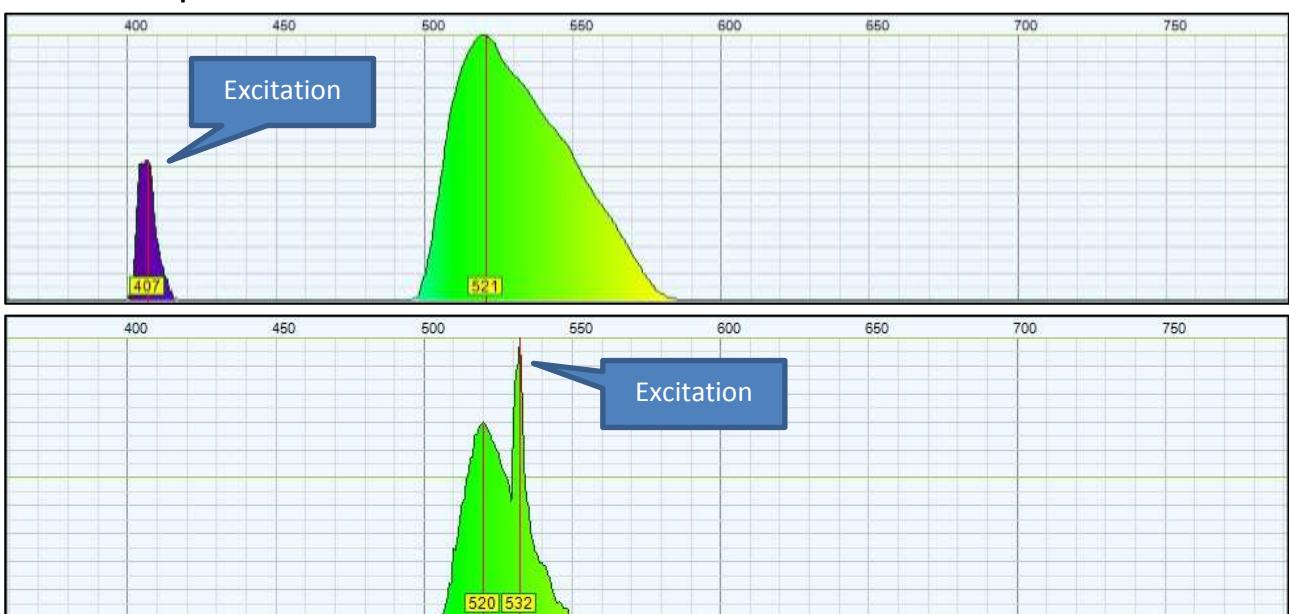
Fluorescein has an absorption maximum at **494nm** and emission maximum of **521nm** (in water). See the paragraph on the fluorescein for the details.

**Brilliant cresyl blue** (shown in the picture) is a dye used in biology for counting reticulocyte

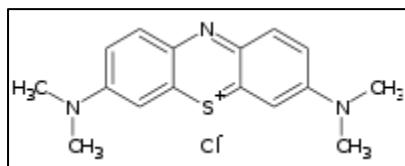
Absorption spectrum. Second spectrum with UV detail



Fluorescence spectrum excited from UV emission and 532nm laser



## Methylene Blue



**Methylene blue** (CI 52015) is a heterocyclic aromatic chemical compound with the molecular formula C<sub>16</sub>H<sub>18</sub>N<sub>3</sub>SCl. It has many uses in a range of different fields, such as biology and chemistry. At room temperature it appears as a solid, odorless, dark green powder, that yields a blue solution when dissolved in water. The hydrated form has 3 molecules of water per molecule of methylene blue. Methylene blue should not be confused with methyl blue, another histology stain, new methylene blue, nor with the methyl violets often used as pH indicators. Methylene blue has a wide range of biological applications.

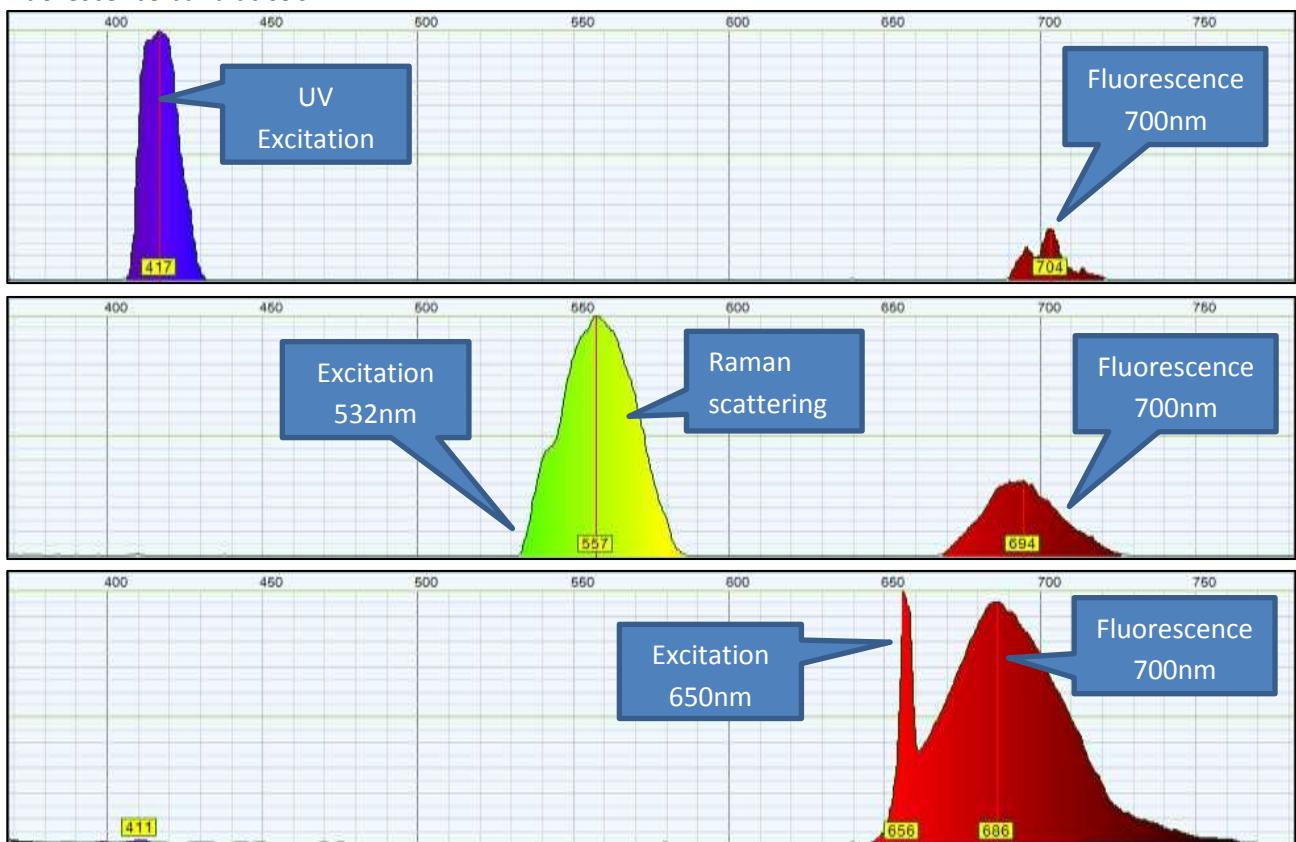
### Methylene blue absorption spectrum (transmission)

Absorption band between 550nm and 700nm with peaks at 665nm and 610nm

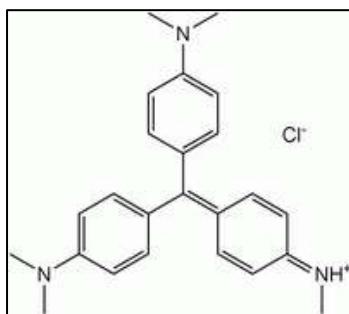


### Methylene blue fluorescence spectrum excited from UV emission, 532nm laser and 650nm laser.

Fluorescence band at 690nm



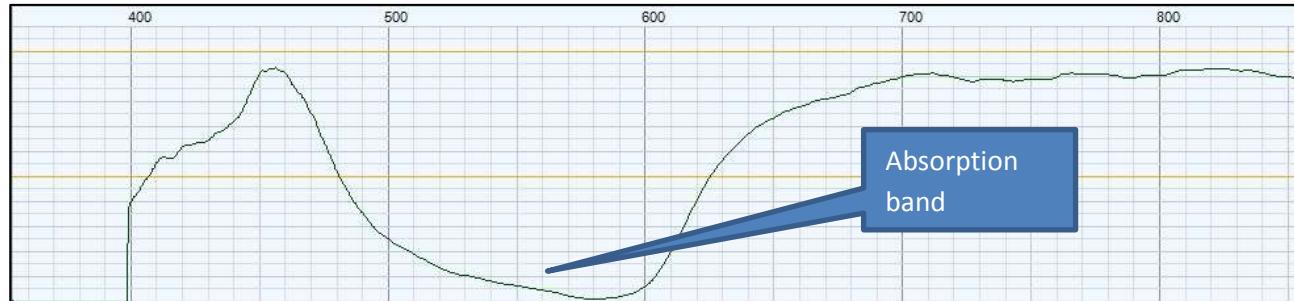
## Crystal Violet



**Crystal violet** or gentian violet (also known as methyl violet 10B, hexamethyl pararosaniline chloride) is a triarylmethane dye. The dye is used as a histological stain and in Gram's method of classifying bacteria. Crystal violet has antibacterial, antifungal, and anthelmintic properties and was formerly important as a topical antiseptic. The medical use of the dye has been largely superseded by more modern drugs, although it is still listed by the World Health Organization. The name "gentian violet" was originally used for a mixture of methyl pararosaniline dyes (methyl violet) but is now often considered a synonym for crystal violet. The name refers to its colour, being like that of the petals of a gentian flower; it is not made from gentians or from violets.

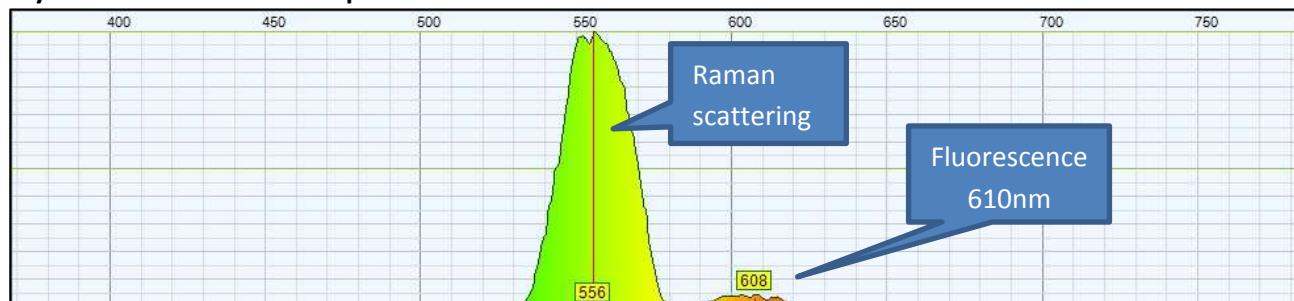
When dissolved in water the dye has a blue-violet colour with an absorbance maximum at 590nm. The colour of the dye depends on the acidity of the solution. At a pH of 1.0 the dye is green with absorption maxima at 420 nm and 620 nm while in a strongly acidic solution (pH of -1), the dye is yellow with an absorption maximum at 420 nm.

Crystal violet absorption spectrum (transmission)



Absorption band between 450nm and 650nm

Crystal violet fluorescence spectrum excited from 532nm laser



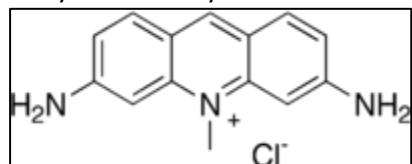
Weak fluorescence at 610nm

## Methylene Blue – Malachite Green – Crystal Violet – Acriflavine (Preparation for Fungal infections)

**Methylene blue** (CI 52015) is a heterocyclic aromatic chemical compound.

**Malachite green** is an organic compound that is used as a dyestuff and has emerged as a controversial agent in aquaculture. Malachite green is traditionally used as a dye for materials such as silk, leather, and paper.

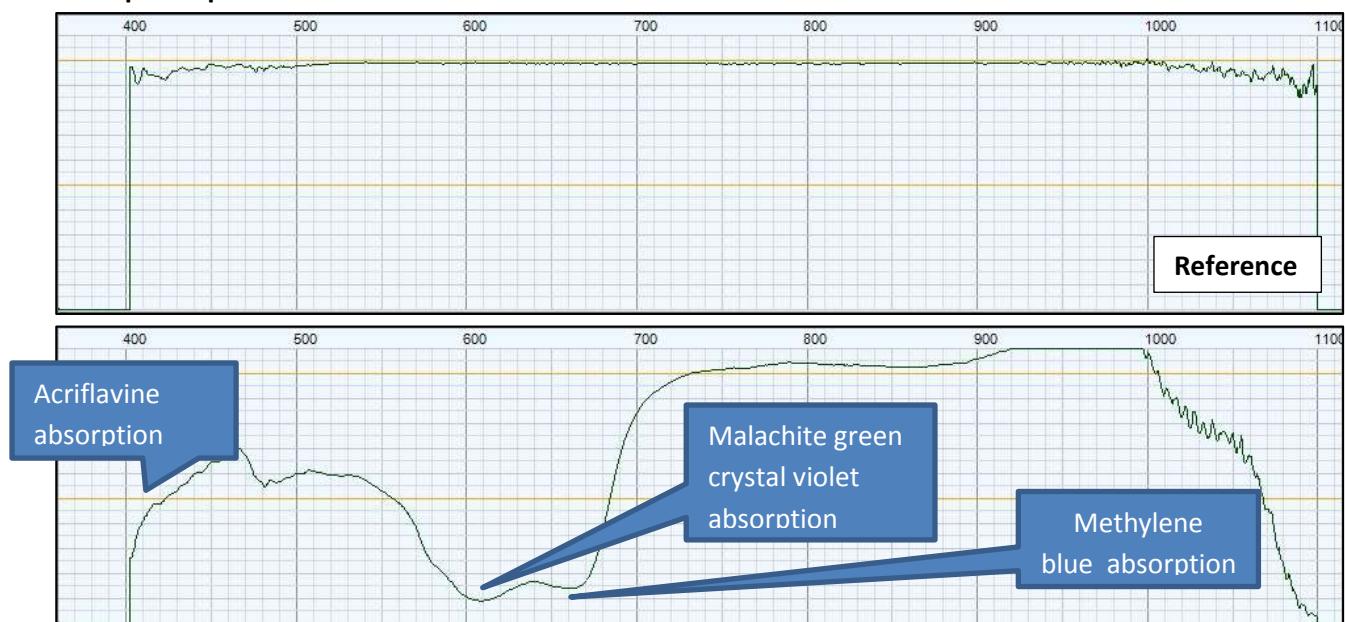
**Crystal violet** or gentian violet (also known as methyl violet 10B, hexamethyl pararosaniline chloride) is a triarylmethane dye.



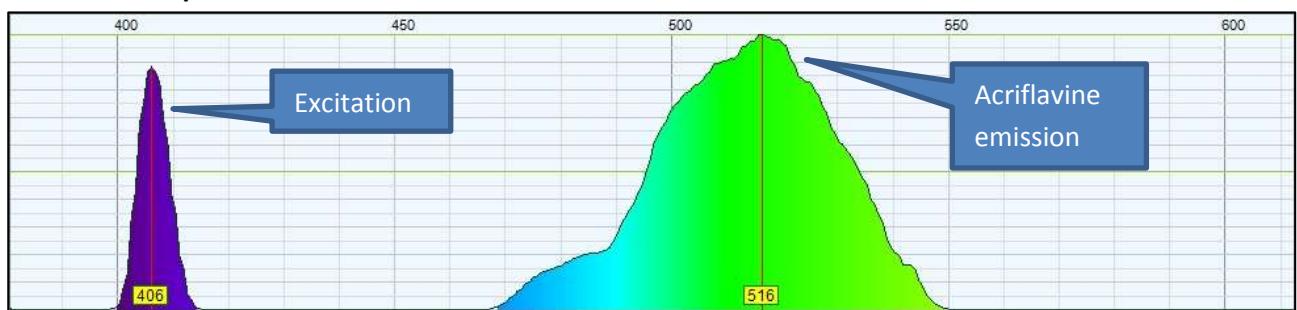
**Acriflavine** (shown in the picture) is a topical antiseptic. It has the form of an orange or brown powder with **green fluorescence**. It may be harmful in the eyes or if inhaled. It is a dye and it stains the skin and may irritate. Commercial preparations are often mixtures with proflavine. It is known by a variety of commercial names.

Acriflavine is also used as treatment for external fungal infections of aquarium fish.

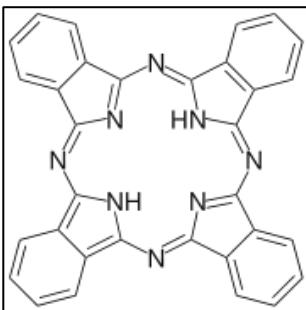
**Absorption spectrum of the mixture**



**Fluorescence spectrum of the mixture**



## Phthalocyanine

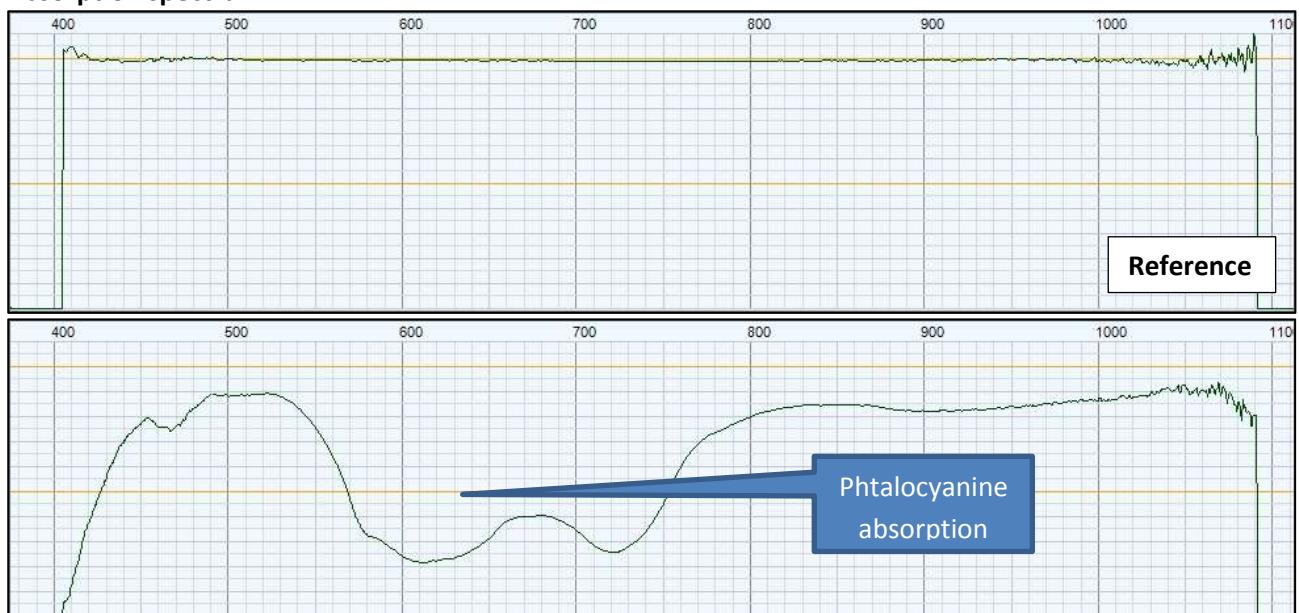


Phthalocyanine is an intensely blue-green-coloured aromatic macrocyclic compound that is widely used in dyeing. Phthalocyanines form coordination complexes with most elements of the periodic table. These complexes are also intensely colored and also are used as dyes or pigments.

Phthalocyanines are organic compounds closely related to other tetrapyrrole macrocycles including porphyrins and porphrazines.

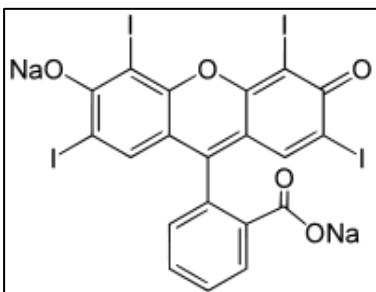
Phthalocyanines strongly absorb light between 600 and 700 nm, thus these materials are blue or green. Approximately 25% of all artificial organic pigments are phthalocyanine derivatives.

### Absorption spectrum



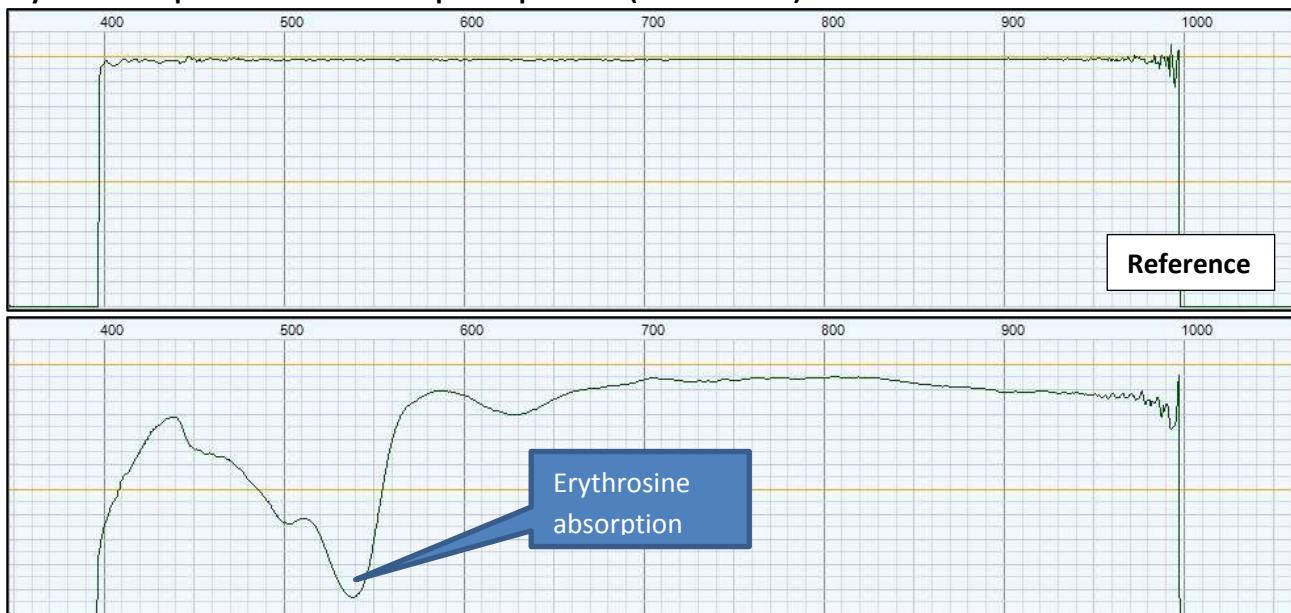
Three absorption bands are shown :  $\lambda < 450\text{nm}$ ,  $620\text{nm}$ ,  $720\text{nm}$

## Erythrosine



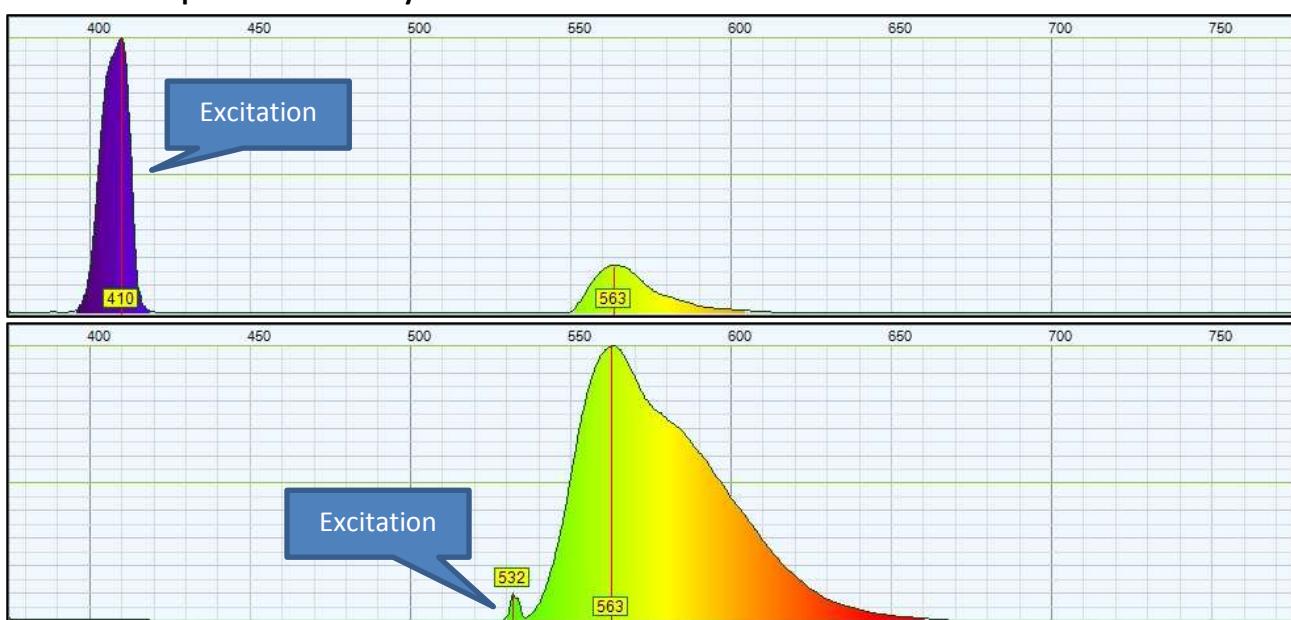
**Erythrosine**, also known as Red No. 3, is an organoiodine compound, specifically a derivative of fluorone. It is cherry-pink synthetic, primarily used for food coloring. Its maximum absorbance is at 530 nm in an aqueous solution, and it is subject to photodegradation. It is used as a food coloring, printing ink, biological stain and dental plaque disclosing agent, just to mention a few.

Erythrosine aqueous solution absorption spectrum (transmission)

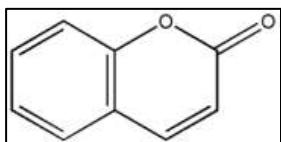


Absorption band with a peak at 530-540nm and a shoulder at 500nm

Fluorescence spectrum excited by UV emission and 532nm laser



## Coumarin



**coumarin** is an aromatic compound. At room temperature is in the form of colorless crystals, with characteristic odor.

Isolated for the first time from *Dipteryx odorata*, whose name was indeed coumarin, coumarin is present in more than 27 families of plants, and is responsible for sweet smell of freshly cut grass.

It is the first of a class of compounds - called coumarins - that share the coumarin chemical structure.

Even **idrossicumarine** are present in many plants : **umbelliferone** , esculetin and scopoletin are the most common in nature. More complex coumarins as **furanocoumarins** are limited to a few families (Rutaceae and Apiaceae); typical example are the phototoxic psoralens which are present in the essential oil of Bergamot (**bergaptene**).

Coumarin is also used as a gain medium in some dye laser and as a sensitizer in photovoltaic technologies. Coumarin absorbs wavelengths less than 400nm and gives strong fluorescence at about 460nm.

**Fluorescence spectrum of coumarin alcoholic excited from UV emission at 405nm**



# Spectroscopy of Edible Oils

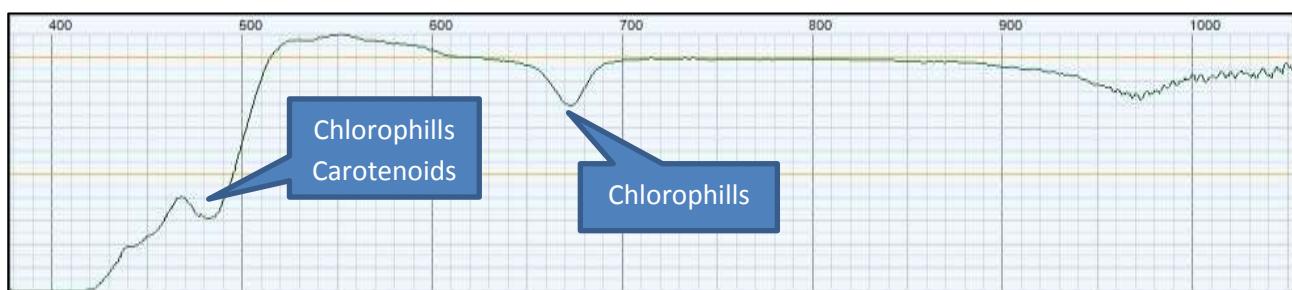
Food oils (olive oil, seed oil) have interesting optical properties (absorption, fluorescence) due to the content of optically active compounds, such as chlorophyll, beta-carotene and others. These properties can be used to recognize and characterize the various types of oil.

In the spectrum of "Extra Vergine" olive oil, obtained by cold pressing you notice the absence of the products of peroxidation of fatty acids, which give fluorescence at about 470nm. This happens both because the oil is cold worked and because the high content of natural anti-oxidants (carotenes and polyphenols ) prevents oil from oxidative degradation.

Seeds oils show all instead, to varying degrees, a clear fluorescence at about 470nm, a sign of the content in peroxides resulting from oxidative degradation of fatty acids, both because they are supposedly hot-worked and because the lower content of molecules anti-oxidants.

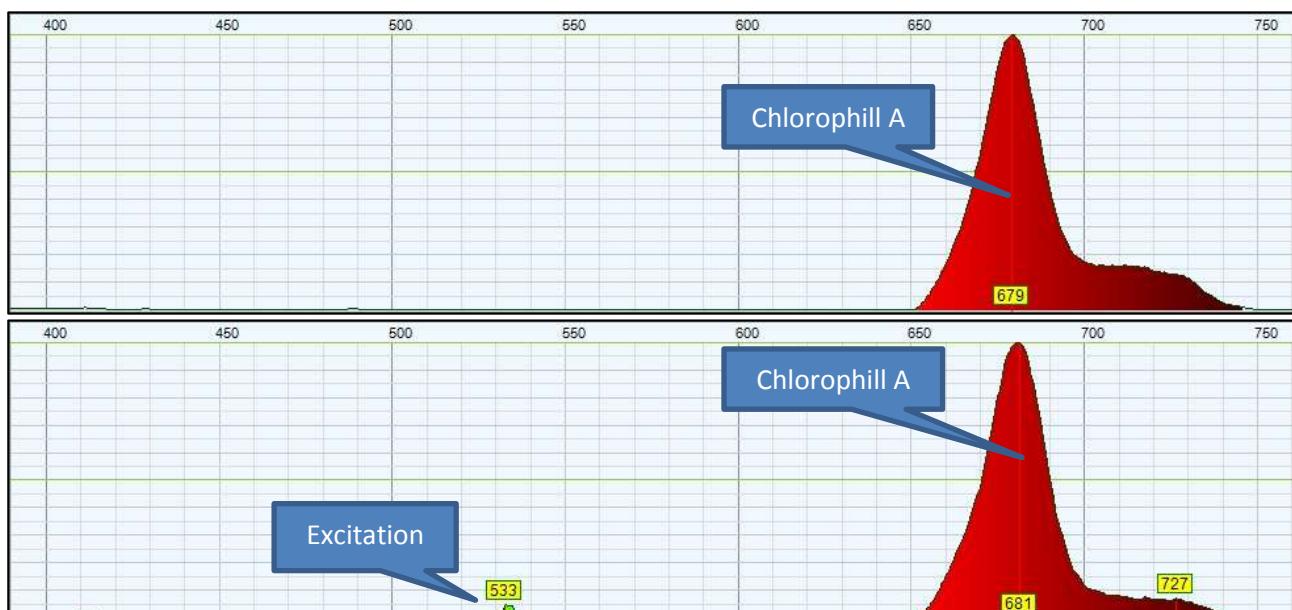
These features make the seeds oils less suitable for use at high temperature.

## "Extra Vergine" Olive Oil



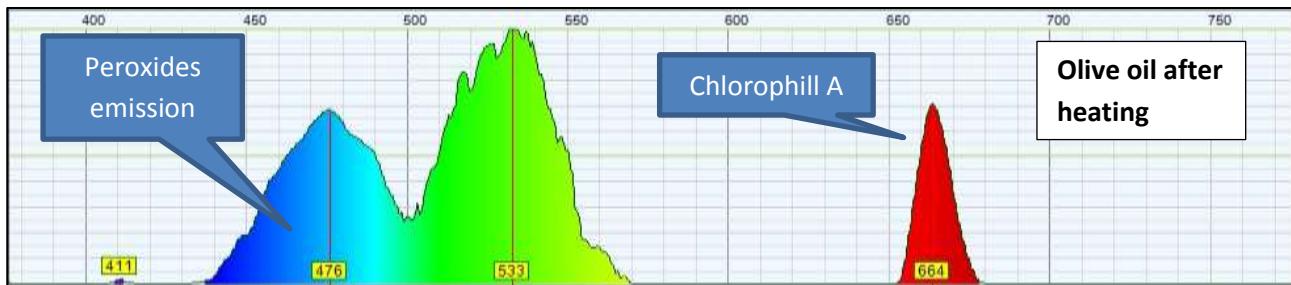
### Olive oil absorption (transmission) spectrum

Absorption maxima in the red band at around 660-670nm and in the blue band, less than 500nm. Olive oil absorption is due to carotenoids and chlorophylls



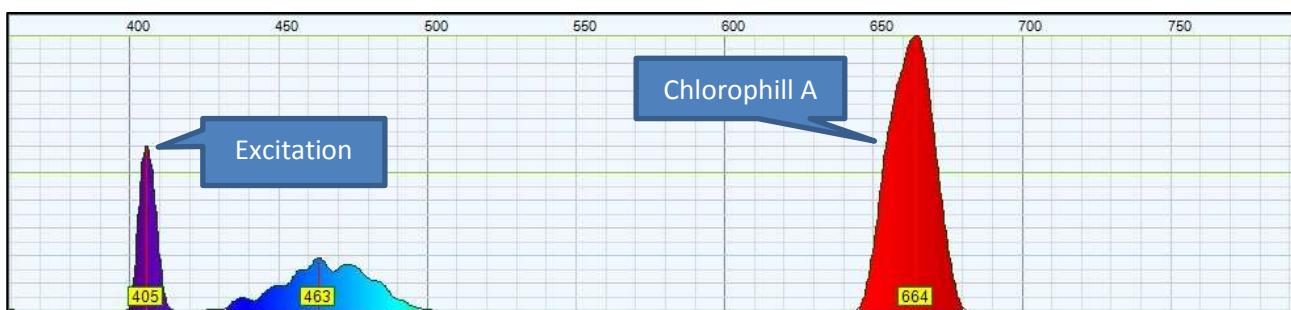
### Olive oil fluorescence spectrum excited by UV emission at 405nm (first spectrum) and by 532nm laser (second spectrum)

The maximum at 680nm is due to chlorophill A



**Fluorescence spectrum of olive oil subjected to heating (thermal degradation) excited by UV emission**  
The chlorophyll fluorescence has been reduced whilst it appeared the signal of the presence of peroxides due to thermal degradation of fatty acids

## Peanut Oil



**Peanut oil fluorescence spectrum excited by UV emission at 405nm**

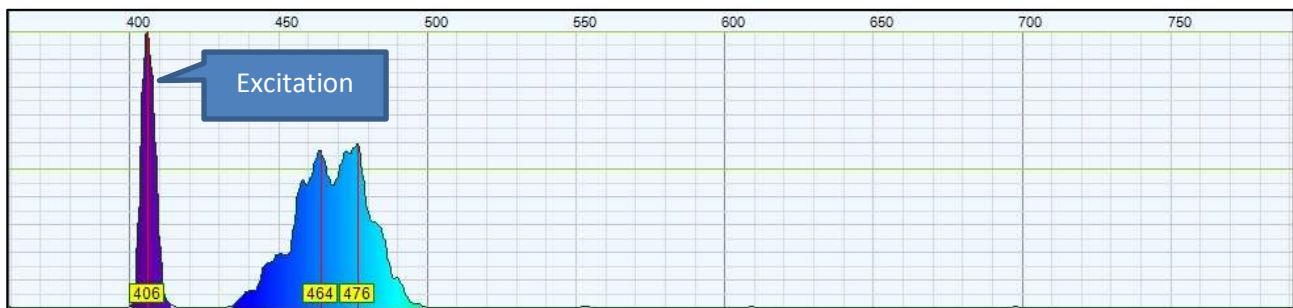
The fluorescence band at 664nm is due to the content in chlorophyll. The fluorescence band with peaks at 463nm and 475nm presumably is due to the products of peroxidation (degradation) of polyunsaturated fatty acids in the oil (oleic and linoleic)



**Fluorescence spectrum of peanut oil subjected to heating (thermal degradation) excited by UV emission**

We note the increase in the intensity of fluorescence at 470nm - 530nm due to thermal degradation of fatty acids and the consequent production of peroxides. The chlorophyll fluorescence has virtually disappeared because the chlorophyll itself has been degraded by heating

## Sunflower Oil



**Sunflower oil fluorescence spectrum excited by UV emission at 405nm**

The fluorescence band with peaks at 464nm and 476nm ca presumably is due to the products of peroxidation (degradation ) of polyunsaturated fatty acids in the oil (oleic and linoleic)

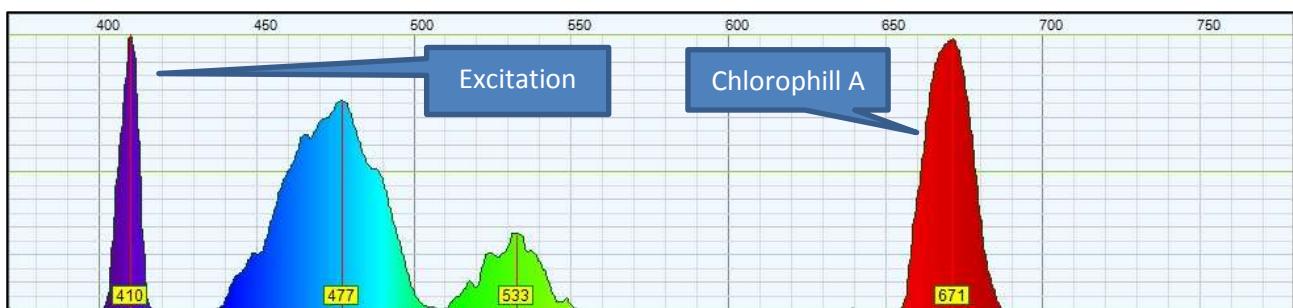
## Corn Oil



**Corn oil fluorescence spectrum excited by UV emission at 405nm**

The fluorescence band with the peak at 464nm is presumably due to the products of peroxidation (degradation) of polyunsaturated fatty acids present in the oil (oleic and linoleic acid). The attribution of the fluorescence band with the peak at 520nm is uncertain and may be also linked to the process of thermal degradation of fatty acids. Alternatively (or in addition) may be due to the compounds of vitamin B2 (flavin)

## Soybean Oil



**Soybean oil fluorescence spectrum excited by UV emission at 405nm**

The fluorescence band with the peak at 477nm is presumably due to the products of peroxidation (degradation ) of polyunsaturated fatty acids present in the oil (oleic and linoleic) . The same applies to the band with a peak at 533nm, to assess the contribution of isoflavones on the emissions of the green fluorescence. See section on soybeans

## Almond Oil



**Almond oil fluorescence spectrum excited by UV emission at 405nm**

The fluorescence band with the peak at 477nm is presumably due to the products of peroxidation (degradation) of polyunsaturated fatty acids present in the oil (oleic and linoleic acid). The fluorescence band with peak at 533nm and 596nm is presumably due to the compounds of vitamin B2 (flavins) - See also section on the fluorescence of riboflavin

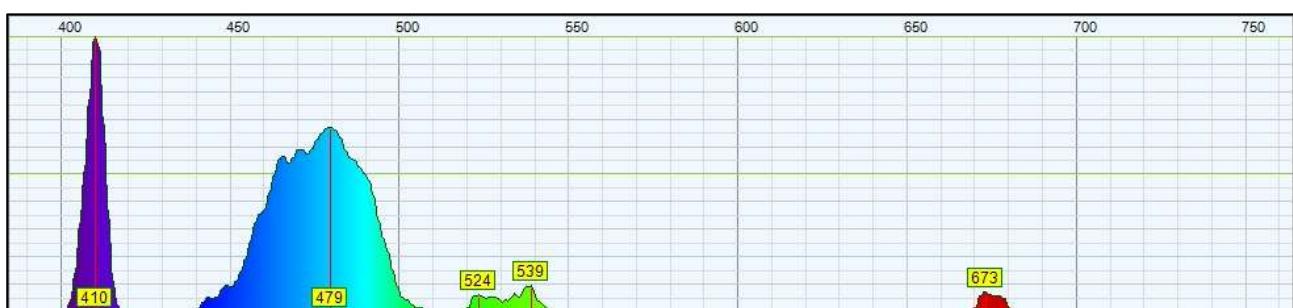
## Sesame Oil



**Sesame oil fluorescence spectrum excited by UV emission at 405nm**

The fluorescence band with the peak at 475nm is presumably due to the products of peroxidation. The attribution of the fluorescence band with the peak at 533nm is uncertain and may be also linked to the process of thermal degradation of fatty acids, or due to the compounds of vitamin B2 (flavin)

## Castor Oil

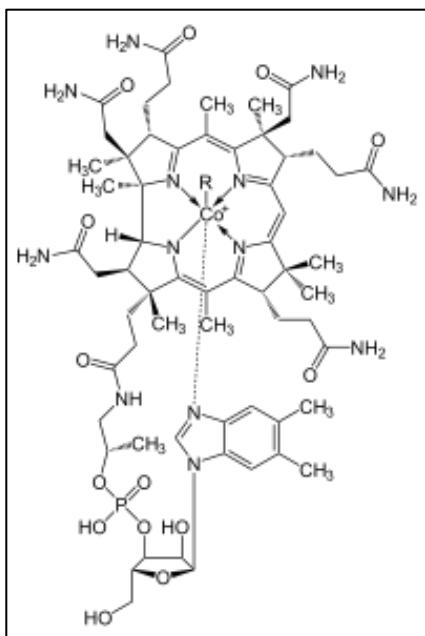


**Castor oil fluorescence spectrum excited by UV emission at 405nm**

The fluorescence band with the peak at 479nm is presumably due to the products of peroxidation

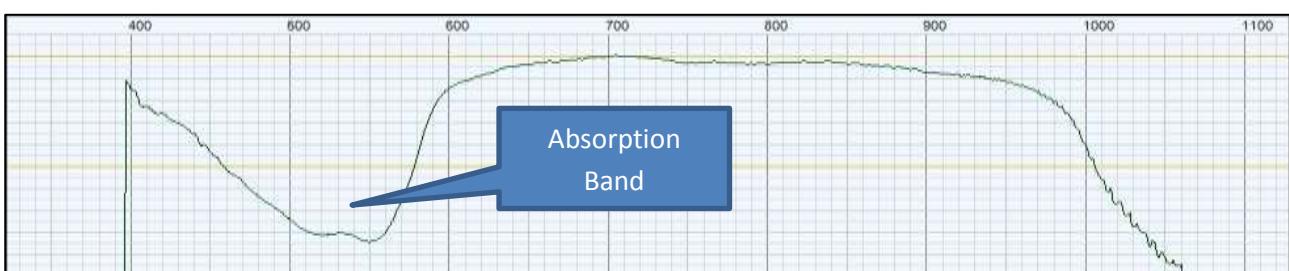
# Spectroscopy of Vitamins

## Cyanocobalamin (Vitamin B12)



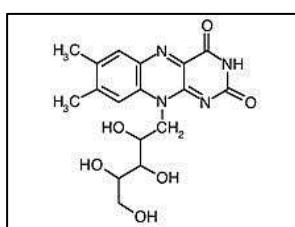
**Vitamin B12**, vitamin B12 or vitamin B-12, also called cobalamin, is a water-soluble vitamin with a key role in the normal functioning of the brain and nervous system, and for the formation of blood. It is one of the eight B vitamins. It is normally involved in the metabolism of every cell of the human body, especially affecting DNA synthesis and regulation, but also fatty acid metabolism and amino acid metabolism.[1] Neither fungi, plants, nor animals are capable of producing vitamin B12. Only bacteria and archaea have the enzymes required for its synthesis, although many foods are a natural source of B12 because of bacterial symbiosis. The vitamin is the largest and most structurally complicated vitamin and can be produced industrially only through bacterial fermentation-synthesis.

**Cyanocobalamin** is the most widely manufactured vitamin in the vitamin B12 family (the family of chemicals that function as B12 when put into the body), because cyanocobalamin is the most air-stable of the B12 forms. It is the easiest to crystallize and, therefore, easiest to purify after it is produced by bacterial fermentation, or synthesized in vitro. It can be obtained as dark red crystals or as an amorphous red powder. Cyanocobalamin is very hygroscopic in the anhydrous form, and sparingly soluble in water (1:80). It is stable to autoclaving for short periods at 121 °C.



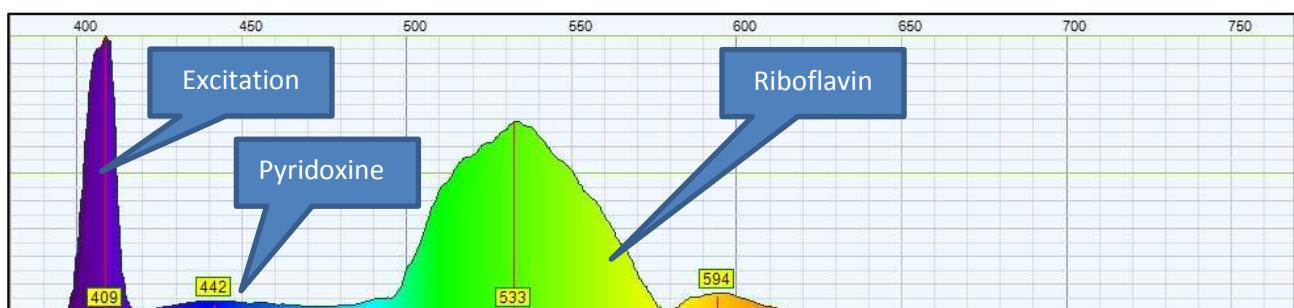
Cyanocobalamin absorption (transmission) spectrum.

## Riboflavin (Vitamin B2) and Pyridoxine (Vitamin B6)



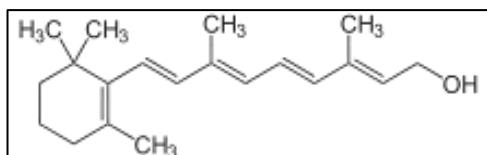
**Riboflavin** (vitamin B2) is part of the vitamin B group. It is the central component of the cofactors FAD and FMN and as such required for a variety of flavoprotein enzyme reactions including activation of other vitamins. Riboflavin is a yellow-orange solid substance with poor solubility in water. It is best known visually as it imparts the color to vitamin supplements and the yellow color to the urine of persons taking it. It shows a strong green fluorescence. The name "riboflavin" comes from "ribose" (the sugar whose reduced form, ribitol, forms part of its structure) and "flavin", the ring-moiety which imparts the yellow color to the oxidized molecule (from Latin *flavus*, "yellow").

**Pyridoxine** is one form of vitamin B6. Its hydrochloride salt pyridoxine hydrochloride is used as vitamin B6 dietary supplement.

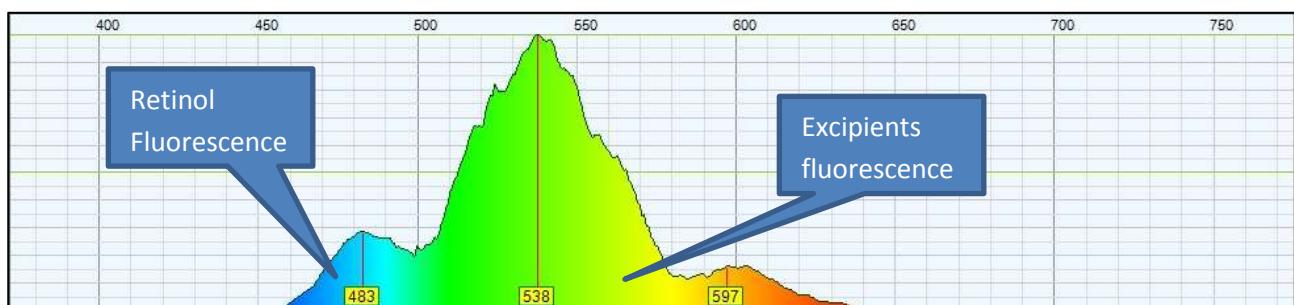


Fluorescence spectrum of a multivitamin compound with group B vitamins excited by UV emission

## Retinol (Vitamin A)



**Vitamin A** is a group of unsaturated nutritional organic compounds, that includes retinol, retinal, retinoic acid, and several provitamin A carotenoids, among which beta-carotene is the most important. Vitamin A has multiple functions: it is important for growth and development, for the maintenance of the immune system and good vision. Vitamin A is needed by the retina of the eye in the form of retinal, which combines with protein opsin to form rhodopsin, the light-absorbing molecule necessary for both low-light (scotopic vision) and color vision.

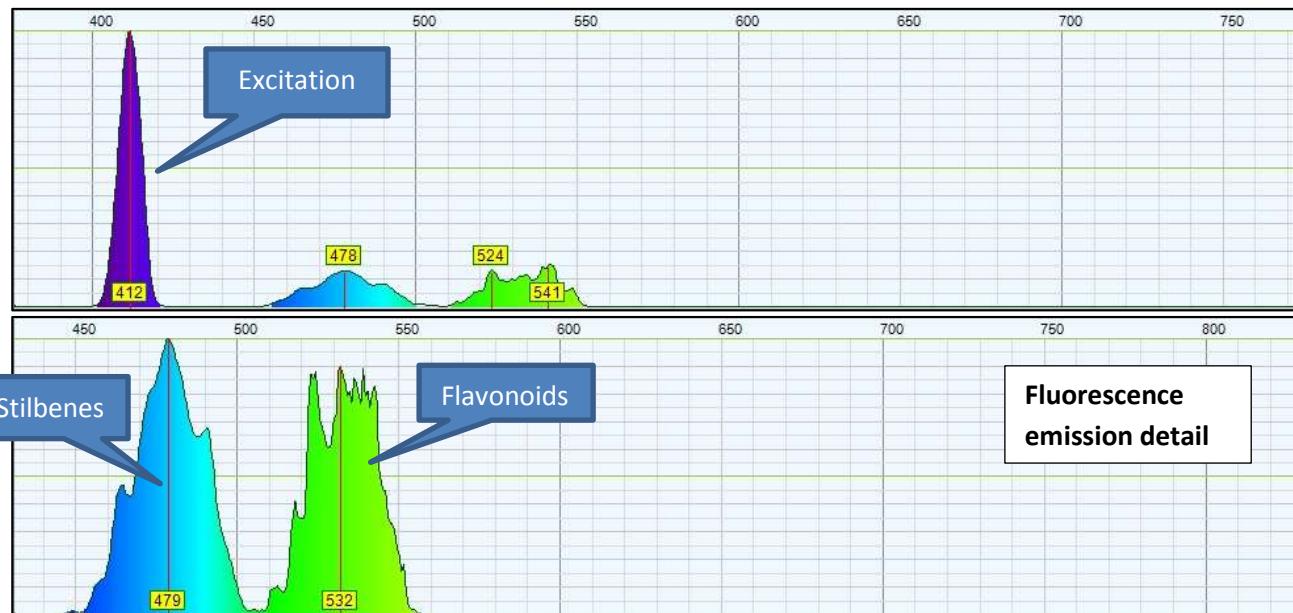


Retinol fluorescence spectrum (with excipients) excited by UV emission with 405nm laser

# Spectroscopy of Food Substances

## White Wine

White wine fluorescence spectrum excited by UV emission



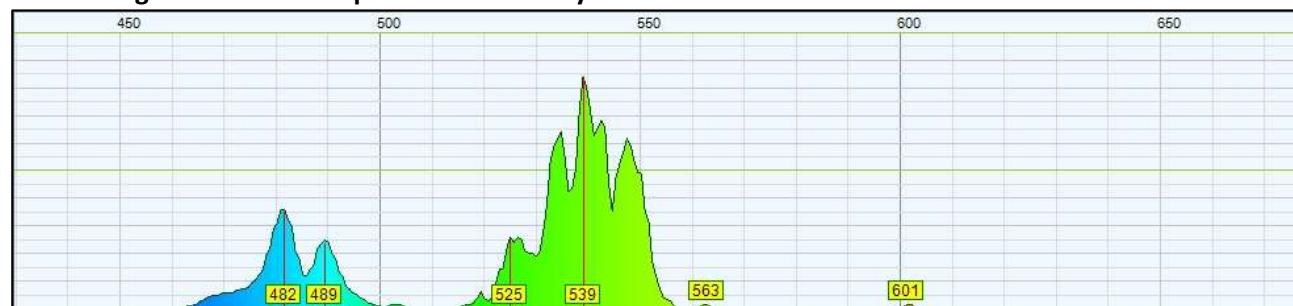
Fluorescence band around 478nm

Fluorescence band around 520-540nm

The fluorescence bands are difficult assignment. The set of organic compounds contained in white wine, for example, stilbenes, flavonoids and polyphenols all contribute to determine the final output. The complexity of the spectrum can also be deduced from jagged edges of the bands, indicating that there are more compounds that emit fluorescence at different wavelengths slightly different from each other.

## Wine Vinegar

Wine Vinegar fluorescence spectrum excited by UV emission

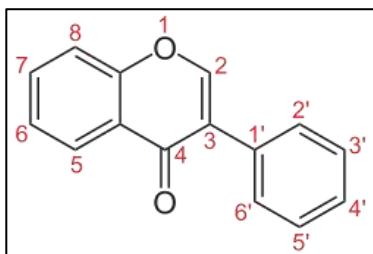


Fluorescence band around 480nm

Fluorescence band around 520-540nm

The Wine Vinegar fluorescence bands are very similar to the wine bands

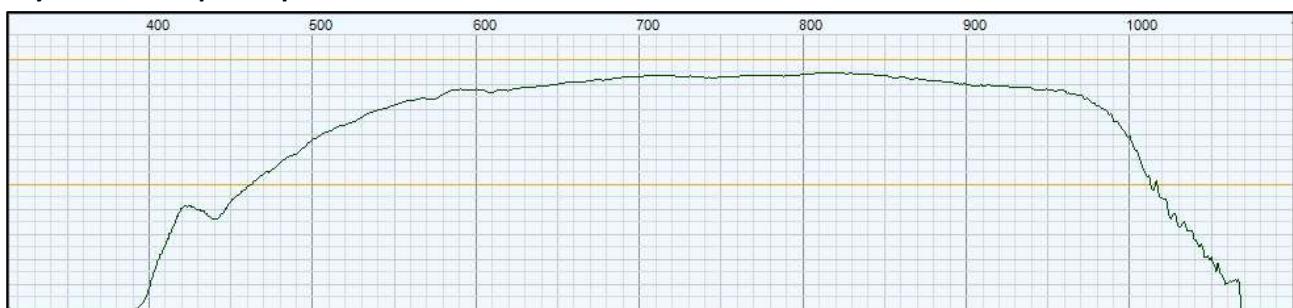
## Isoflavones (Soy Sauce)



**Isoflavones** comprise a class of organic compounds, often naturally occurring, related to the isoflavonoids class compounds. Many act as phytoestrogens in mammals. Some are termed antioxidants because of their ability to trap singlet oxygen. Some isoflavones, in particular soy isoflavones, when studied in populations eating soy protein, have indicated that there is a lower incidence of breast cancer and other common cancers because of its role in influencing sex hormone metabolism and biological activity through intracellular enzymes, protein

synthesis, growth factor actions, malignant cell proliferations, differentiation and angiogenesis. However, the risk reduction in breast cancer from soy isoflavones was only shown in Asian populations (Shanghai Breast Cancer Survival Study). Isoflavones are produced almost exclusively by the members of the Fabaceae (i.e., Leguminosae, or bean) family.

Soy sauce absorption spectrum

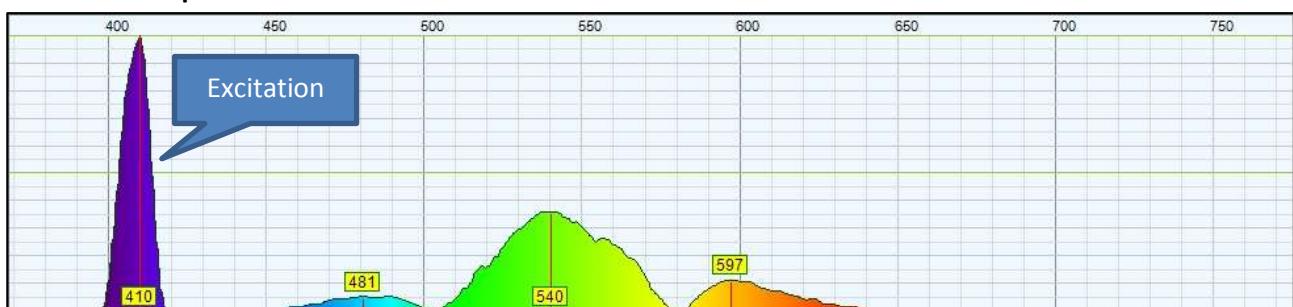


Soy sauce fluorescence spectrum excited by UV emission



## Green Walnut Extract (Liqueur Nocino)

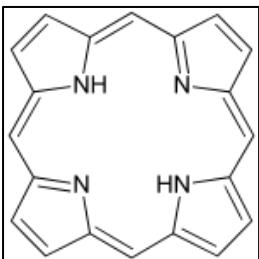
Fluorescence spectrum excited from UV emission



Fluorescence bands at 480nm, at 540nm and at 600nm

The fluorescence bands are difficult assignment. The set of organic compounds contained in the extract, for example flavonoids and polyphenols all contribute to determine the final output

## ProtoPorphyrin (Egg Shell)



**Protoporphyrins** are tetrapyrroles containing the following side chains:

- methyl (4)
- propionic acid (2)
- vinyl (2)

Protoporphyrin IX is a biochemically widely used carrier molecule for divalent cations. Together with iron ( $\text{Fe } 2+$ ) the body of the heme-group of hemoglobin, myoglobin and many other heme-containing enzymes like cytochrome c and catalase are formed. Complexed with magnesium-ions ( $\text{Mg } 2+$ )

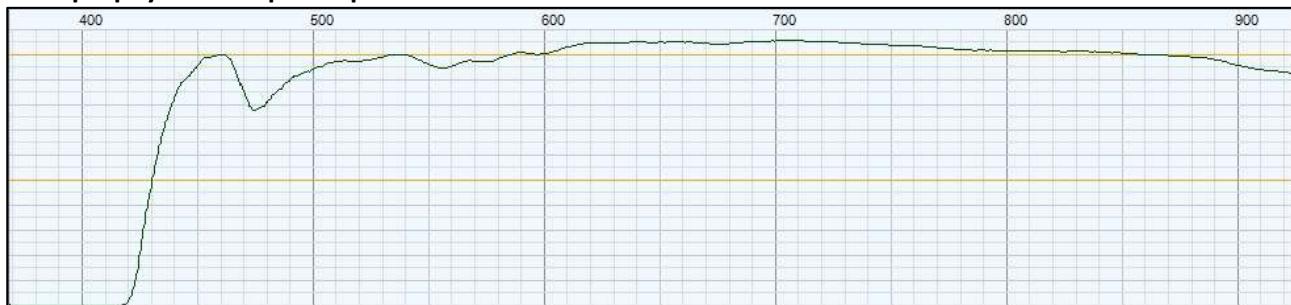
the main part of the Chlorophylls are formed. Complexed with zinc-ions ( $\text{Zn } 2+$ ) it forms Zinc protoporphyrin. Protoporphyrin IX as a direct precursor of heme is accumulated by patients of erythropoietic protoporphyria, which is one of the genetic disorders of the biosynthesis of the heme-pathway. It causes a severe photosensitivity against visible light.

The sensitivity of protoporphyrin IX against light is also used as a therapy against different forms of cancer (photodynamic therapy, PDT).

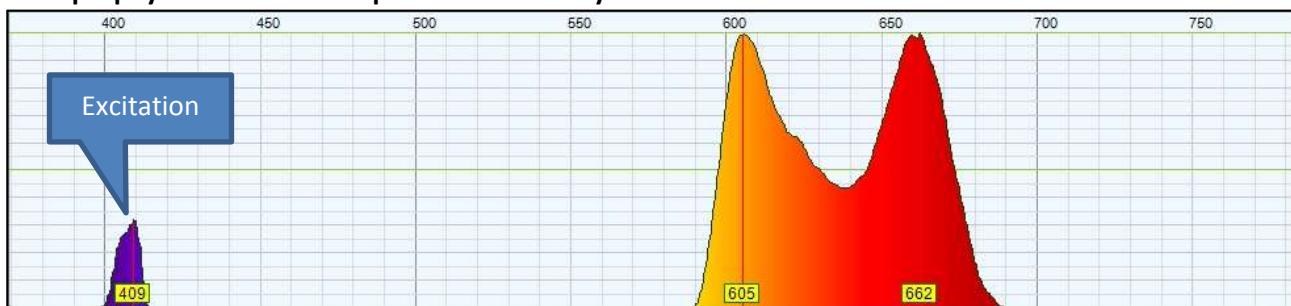
Protoporphyrins are deposited in the shells of the eggs of some birds as a brown or red pigment, either as a ground colour or as spotting. Protoporphyrins strengthen the egg shell, and are deposited where the shell is too thin as a result of calcium shortage. Spotting therefore tend to be heavier where the local soil is calcium-deficient, and in the eggs laid last in a clutch.

The solution of protoporphyrin analyzed was derived from the dissolution of egg shell fragments in hydrochloric acid.

**Protoporphyrin absorption spectrum**



**Protoporphyrin fluorescence spectrum excited by UV emission**

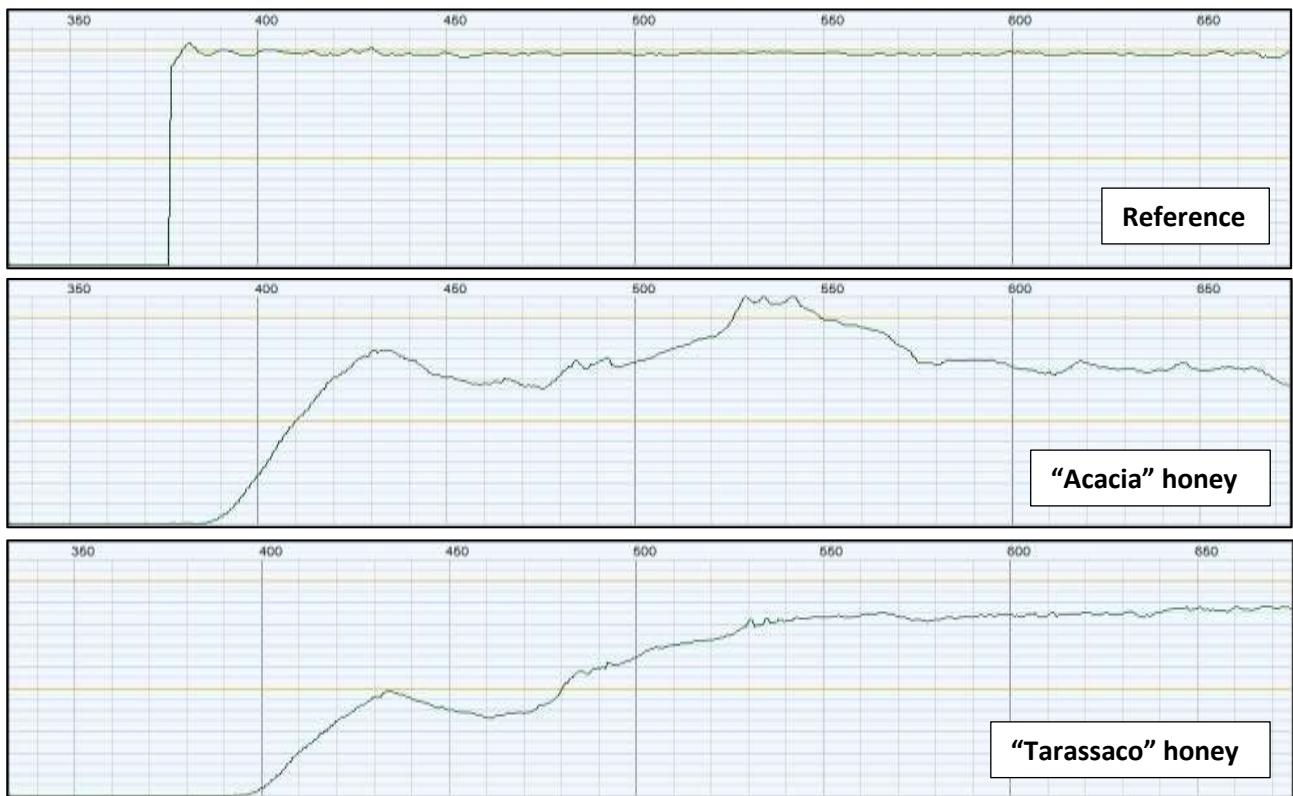


Fluorescence band at 605nm

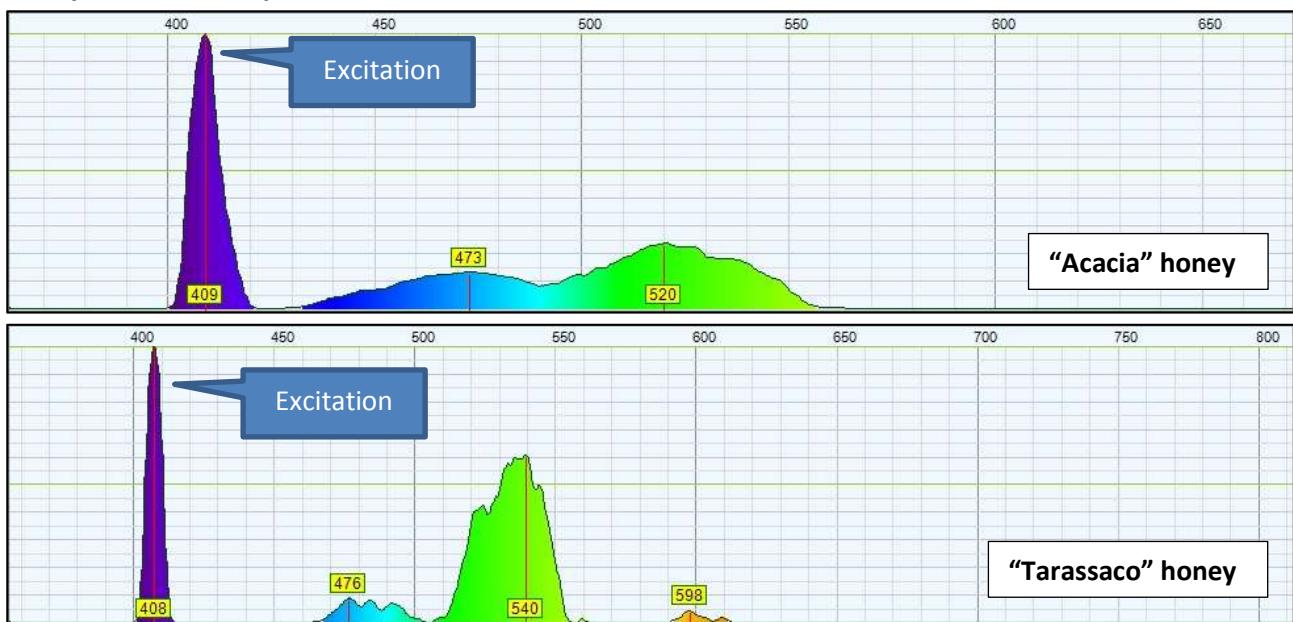
Fluorescence band at 662nm

## Honey

Honey absorption spectrum: absorption bands from 430nm till to 490nm and beyond 550nm



Honey fluorescence spectrum



We can identify two emission peaks : around 475nm and 520-540nm

From the literature the band of green fluorescence at around 520nm could be attributed to the content of flavins (see paragraph on the fluorescence of vitamin B2) , while the band around 470nm may be attributed to the enzyme substance NADH

## Rose Siroop



**Fluorescence spectrum excited by UV emission**

We can identify two emission peaks : around 480nm and 540nm

The band of green fluorescence from 520nm to 540nm could be attributed to the content of flavins , while the band at around 480nm could be attributed to the substance NADH

### NADH

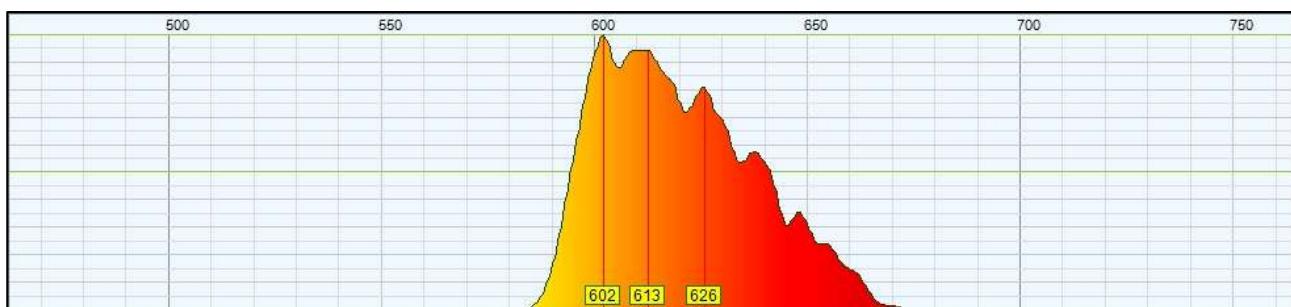
Nicotinamide adenine dinucleotide (NAD) is a coenzyme found in all living cells. The compound is a dinucleotide, because it consists of two nucleotides joined through their phosphate groups. One nucleotide contains an adenine base and the other nicotinamide. Nicotinamide adenine dinucleotide exists in two forms, an oxidized and reduced form abbreviated as NAD<sup>+</sup> and NADH respectively.

NADH, also known as coenzyme 1, is a naturally occurring biological substance. The "H" after NAD stands for hydride, or "high-energy hydrogen". Both NAD<sup>+</sup> and NADH strongly absorb ultraviolet light because of the adenine, NAD<sup>+</sup> and NADH differ in their fluorescence. NADH in solution has an emission peak at 460 nm and a fluorescence lifetime of 0.4 nanoseconds, while the oxidized form of the coenzyme does not fluoresce.

### Flavine

Flavin (from Latin *flavus*, "yellow") is the common name for a group of organic compounds based on pteridine, formed by the tricyclic heteronuclear organic ring isoalloxazine. The biochemical source is the vitamin riboflavin. The flavin moiety is often attached with adenosine diphosphate to form flavin adenine dinucleotide (FAD), and, in other circumstances, is found as flavin mononucleotide(or FMN), a phosphorylated form of riboflavin. It is in one or the other of these forms that flavin is present as a prosthetic group in flavoproteins. In aqueous solution, flavins are yellow-coloured when oxidized, taking a red colour in the semi-reduced anionic state or blue in the neutral (semiquinone) state, and colourless when totally reduced.

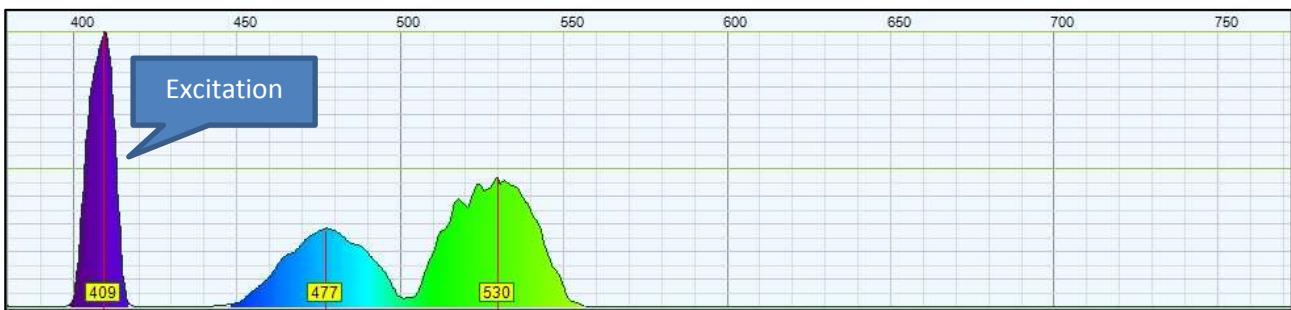
## Rhubarb Extract



**Fluorescence spectrum excited by UV emission at 405nm**

Emission peak at around 600nm due to the compound **anthraquinone**

## Ale Beer



Fluorescence spectrum excited from UV emission

We can identify two emission peaks at about 480nm and 530nm

The fluorescence bands are difficult assignment. The set of organic compounds contained in beer, such as polyphenols and flavonoids all contribute to determine the final output. However, we can speculate that the blue band is due to polyphenols while the band on the green is due to riboflavin.

## Dark Beer



Fluorescence spectrum excited from UV emission

We can identify three emission peaks at about 480nm, 530nm and 600nm

## Stout Beer



Fluorescence spectrum excited from UV emission

We can identify three emission peaks at about 480nm, 530nm and 600nm

## Pistachio Extract



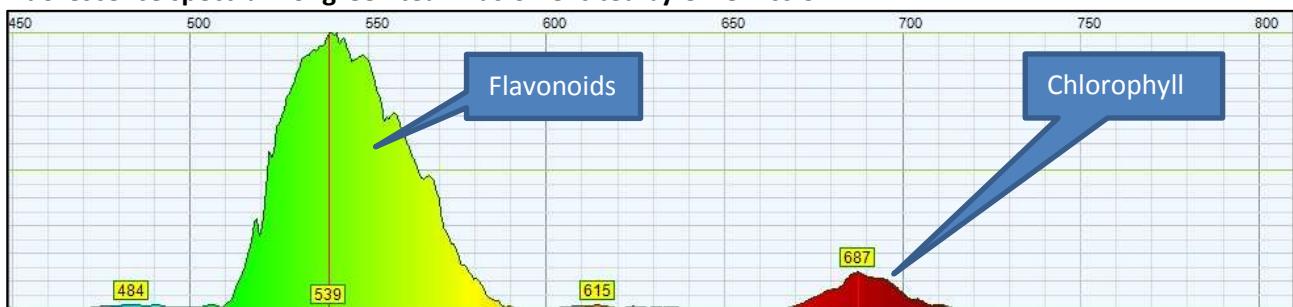
Fluorescence Spectrum excited from UV emission at 405nm

Emission peak at about 670nm

The red fluorescence band at about 670nm is due to chlorophyll which give pistachio the light green color

## Green Tea

Fluorescence spectrum of green tea infusion excited by UV emission



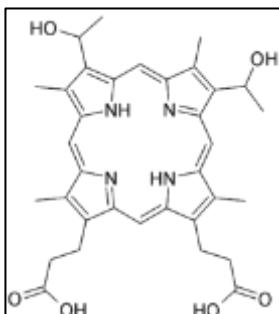
Fluorescence band around 540nm

Fluorescence band around 680-690nm

The band at 680nm is attributed to the content of chlorophyll and the band on the blue-green at 540nm is presumably due to the complex organic substances contained in the leaf of the tea plant, among which polyphenols and flavonoids.

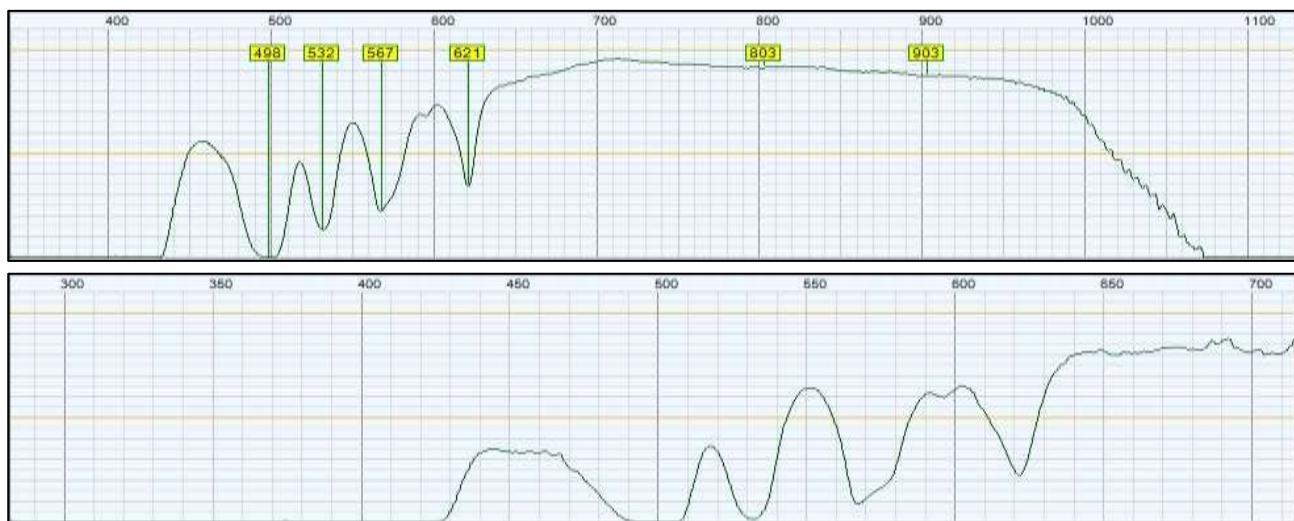
# Spectroscopy of Drugs

## Hematoporphyrin

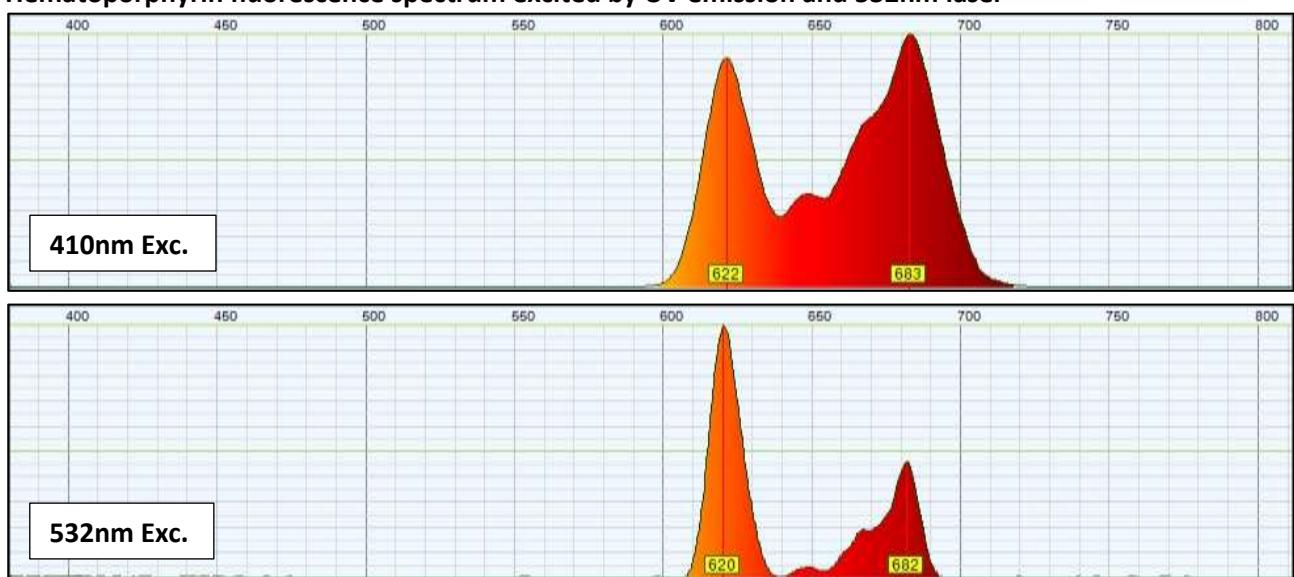


**Hematoporphyrin** is a porphyrin compound. Differs from protoporphyrin hemoglobin for the presence of two hydroxyethyl groups instead of two vinyl groups . Its importance is especially historic because it was the first prepared for porphyrin synthesis (starting at emine) . Its biological role seems minor and his presence in the wild is not currently certain. The hematoporphyrin is used as photosensitizer in photodynamic therapy (PDT ).

Hematoporphyrin absorption spectrum and UV band detail in the second picture

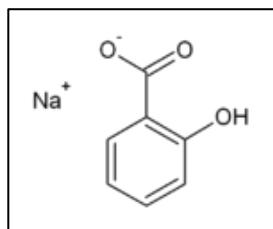


Hematoporphyrin fluorescence spectrum excited by UV emission and 532nm laser



Fluorescence bands at 620nm and 682nm

## Sodium Salicylate

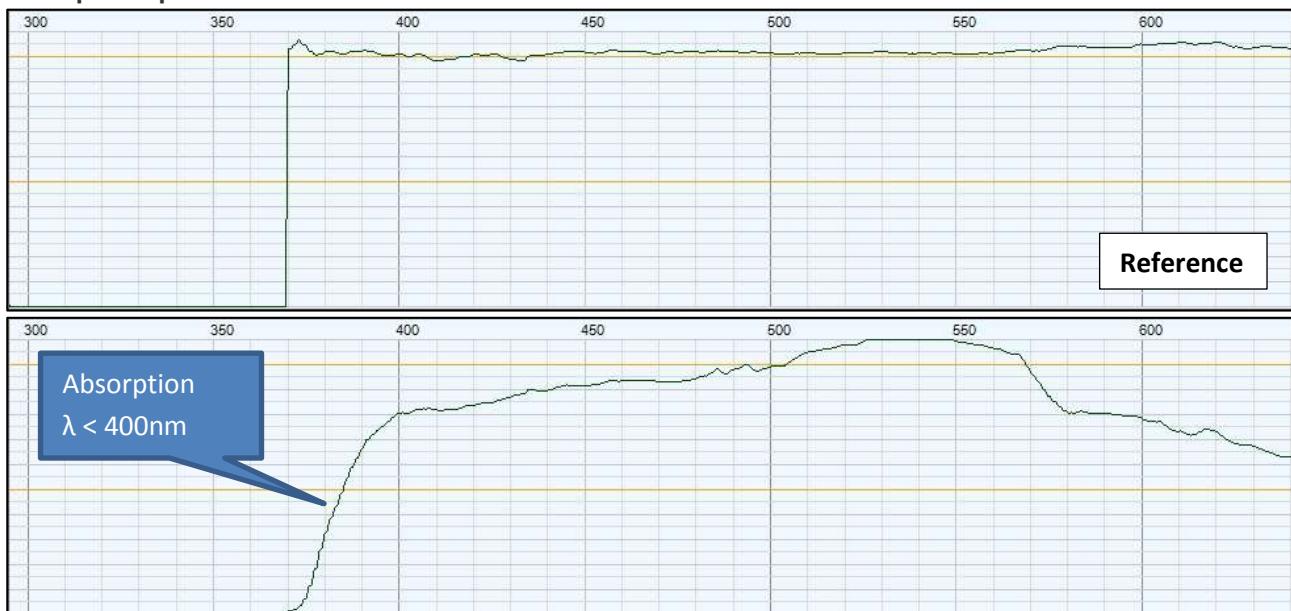


**Sodium salicylate** is the sodium salt of salicylic acid .

At room temperature is presented as a white crystalline solid odorless .

Is obtained by reacting acetylsalicylic acid (the common aspirin) in an aqueous suspension with a basic solution of sodium hydroxide, at the end of the reaction yields a solution of sodium salicylate of pale blue color. It shows blue fluorescence when exposed to UV radiation .

Absorption spectrum

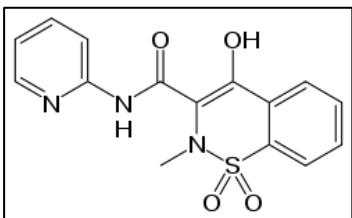


Fluorescence spectrum excited by UV emission



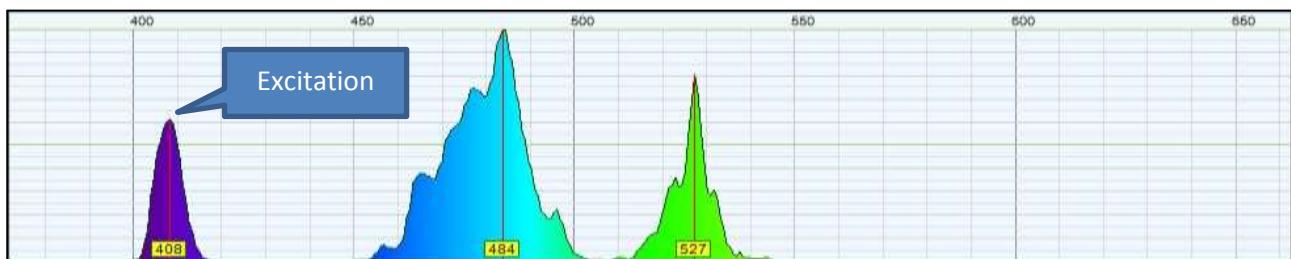
Fluorescence band at around 470nm

## Piroxicam



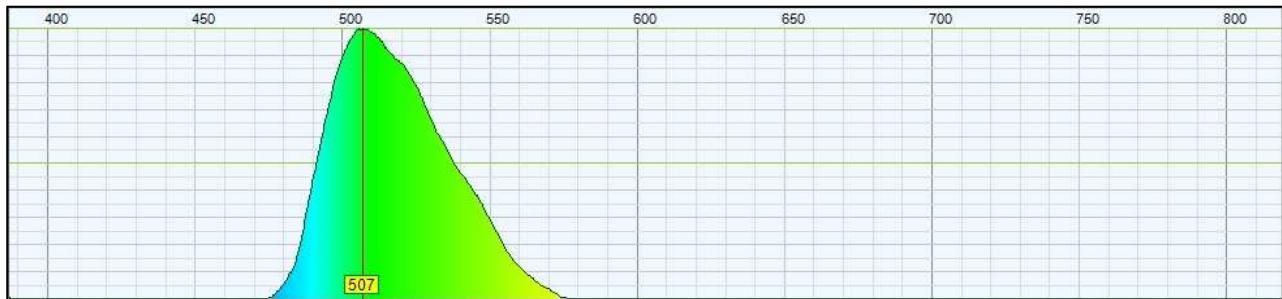
**Piroxicam** is a non-steroidal anti-inflammatory drug , which belongs to the series of oxicams , which has anti-inflammatory , analgesic and antipyretic . It is an amphoteric compound with properties of both the weak acid is of weak base.

### Fluorescence spectrum

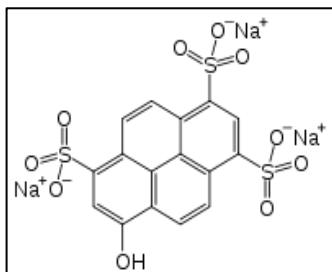


# Spectroscopy of Various Compounds

## Pyranine

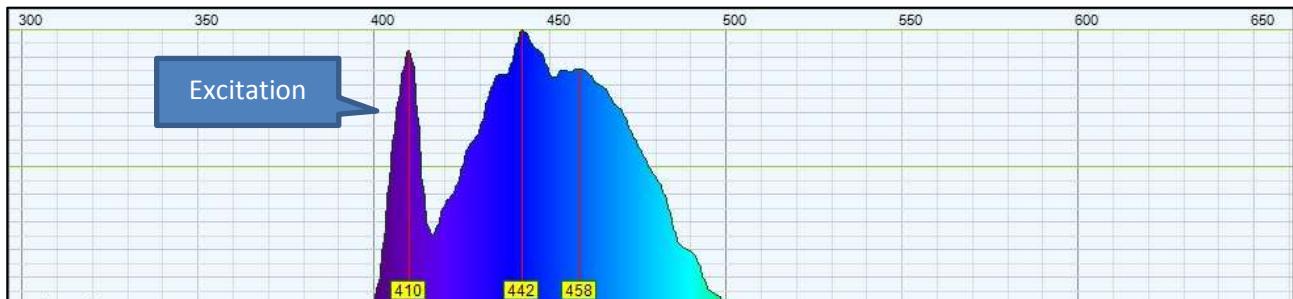


Pyranine fluorescence spectrum excite from UV emission



**Pyranine** is a hydrophilic, pH-sensitive fluorescent dye from the group of chemicals known as arylsulfonates. Pyranine is soluble in water and has applications as a coloring agent, biological stain, optical detecting reagent, and a pH indicator. One example would be the measurement of intracellular pH. Pyranine is also found in yellow highlighters, giving them their characteristic fluorescence and bright yellow-green colour. It is also found in some types of soap.

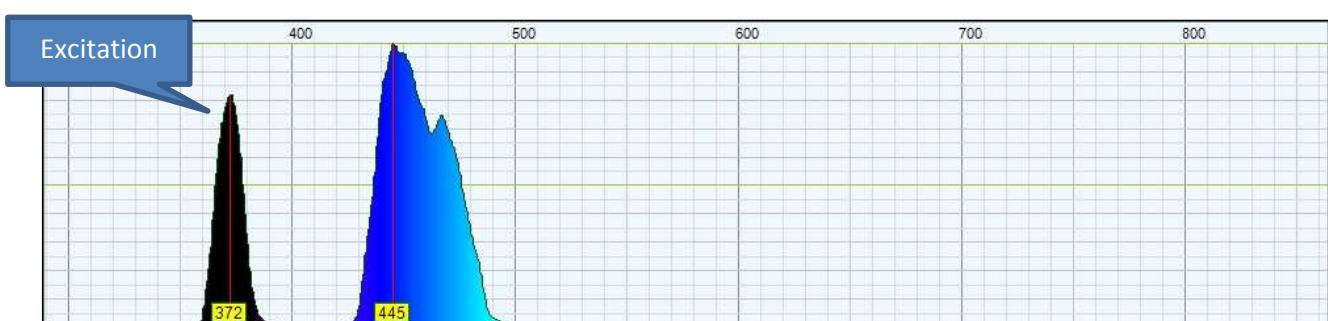
## Perfume



Fluorescence spectrum of a perfume excited from UV emission

The fluorescence is due to **coumarin**

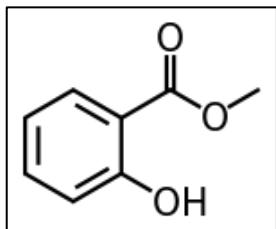
## Bleaching Detergent



Fluorescence spectrum of detergent excited by UV emission at 370nm.

The blue fluorescence is due to the content of stilebene

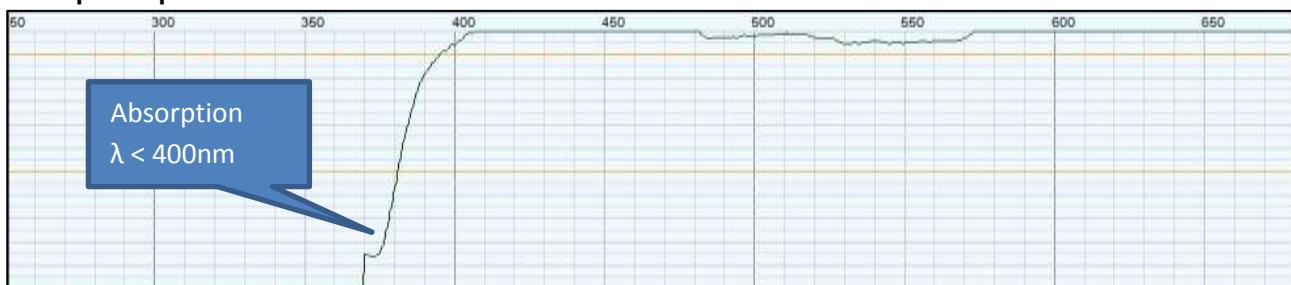
## Methyl Salicylate



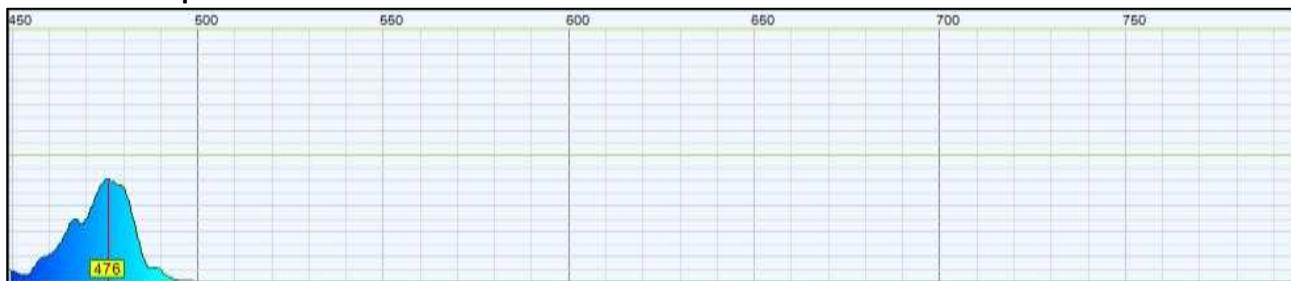
**Methyl salicylate** (oil of wintergreen or wintergreen oil) is an organic ester naturally produced by many species of plants, particularly wintergreens. It is also synthetically produced, used as a fragrance, in foods and beverage.

Numerous plants produce methyl salicylate in very small amounts. Some plants such as gaultheria, produce more. It is used in low concentrations (0.04% and under) as a flavoring agent in chewing gum and mints. When mixed with sugar and dried it is a potentially entertaining source of triboluminescence, gaining the tendency to build up electrical charge when crushed or rubbed. This effect can be observed by crushing wintergreen Life Savers in a dark room. Compare with the section on sodium salicylate.

### Absorption spectrum

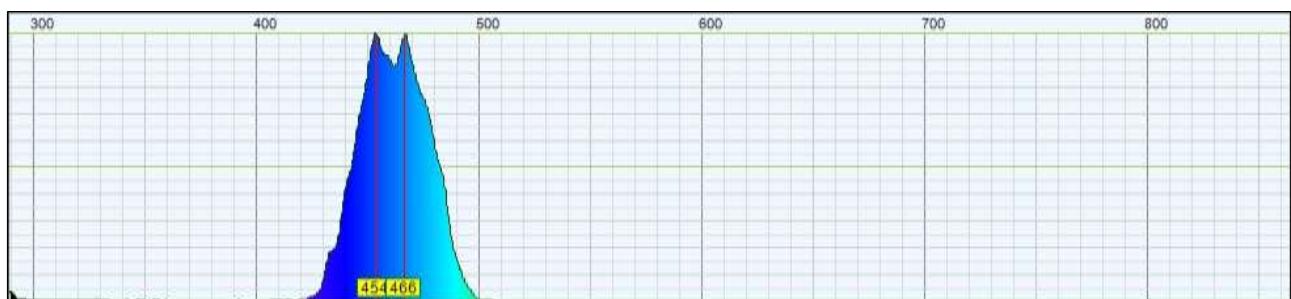


### Fluorescence spectrum excited from UV emission

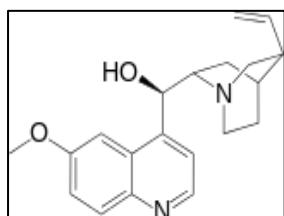


Faint fluorescence band around 476nm

## Quinine



Quinine fluorescence spectrum (tonic water) excited from UV emission at 370nm



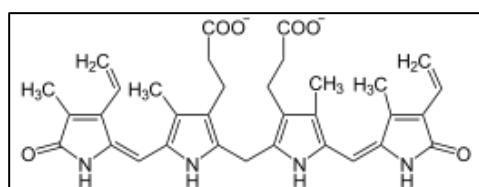
**Quinine** is a natural white crystalline alkaloid having antipyretic (fever-reducing), antimalarial, analgesic (painkilling), and anti-inflammatory properties and a bitter taste. It is a stereoisomer of quinidine, which, unlike quinine, is an antiarrhythmic. Quinine contains two major fused-ring systems: the aromatic quinoline and the bicyclic quinuclidine. Quinine is highly fluorescent (quantum yield ~0.58) in 0.1 M sulfuric acid solution and it is widely used as a standard for fluorescence quantum yield measurement.

## Urine

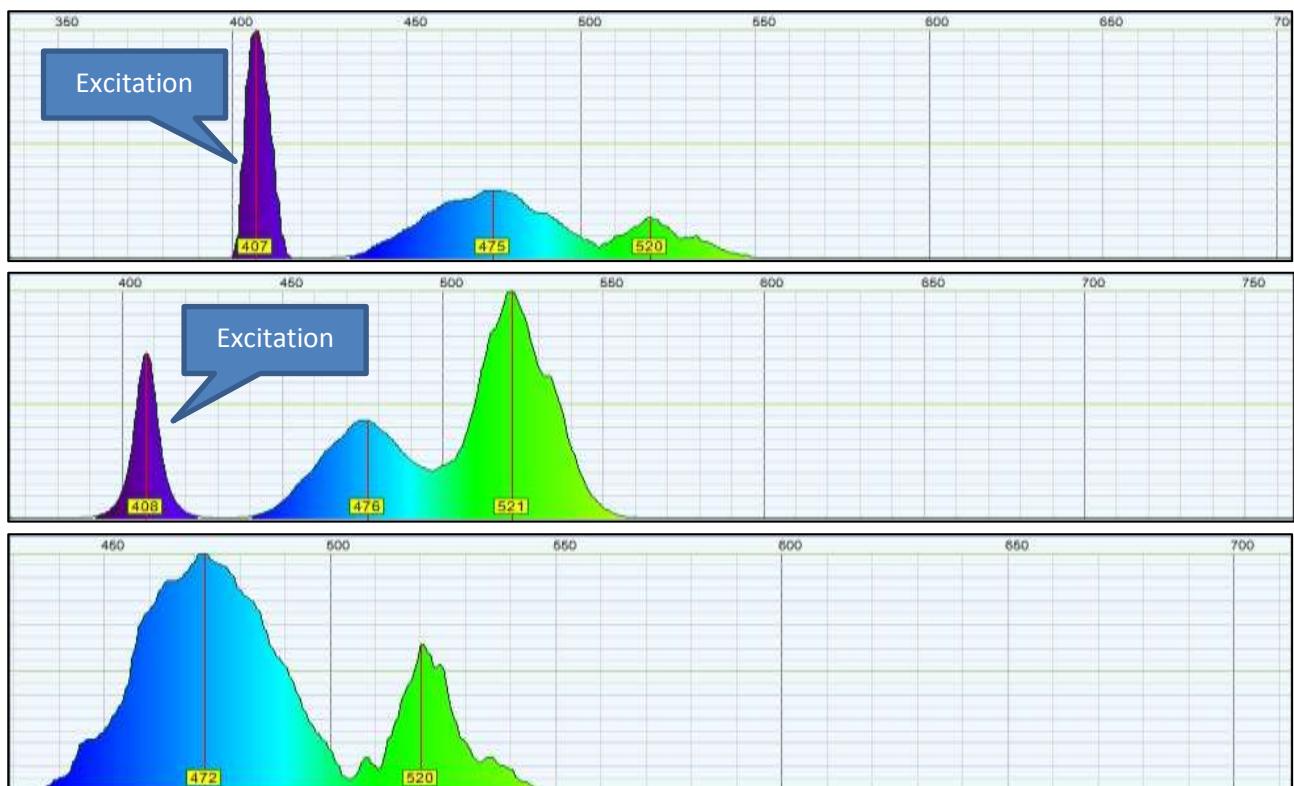


### Urine absorption (transmission) spectrum

Evident the absorption peak at around 460nm due to bilirubin



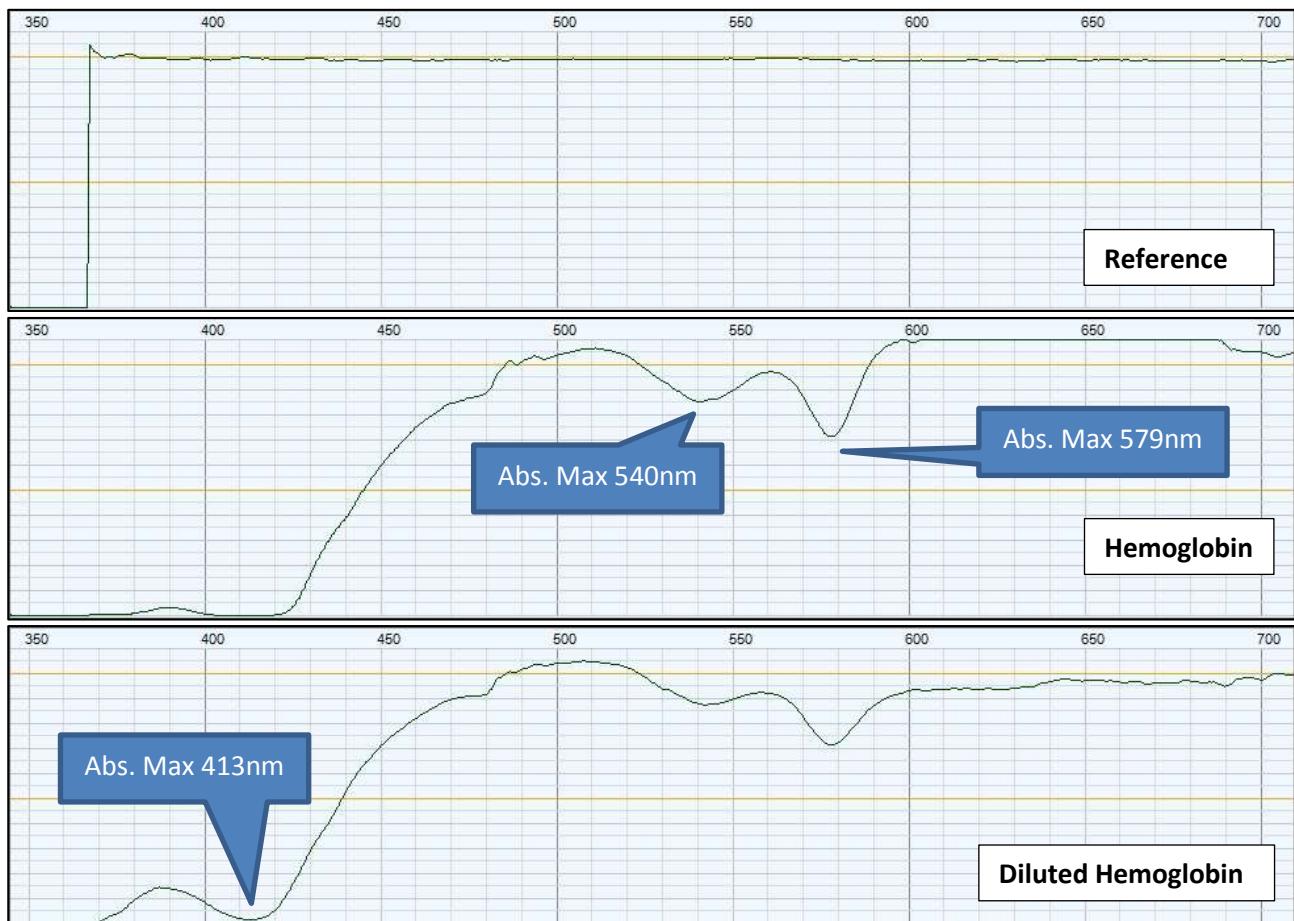
**Bilirubin** is a pigment of yellow-reddish color , contained in bile and is a product of the catabolism of hemoglobin.  
The word comes from the Latin bilis, bile, and ruber, red.



### Urine fluorescence spectrum

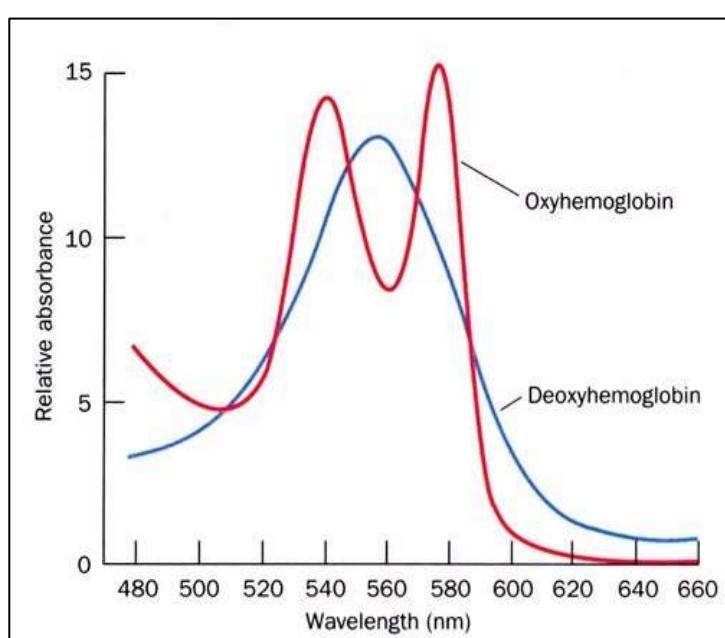
Band at around 480nm, due to NAD(P)H. Band at around 520nm, due to flavine

## Hemoglobin



**Hemoglobin absorption (transmission) spectrum**

Evident the absorption peaks at 579nm, at 540nm and at 413nm and for  $\lambda$  shorter than 390nm



**Hemoglobin Absorption**

**Hemoglobin** is a globular protein whose quaternary structure consists of four subunits. It is soluble, red (is a chromoprotein), and is present in the red blood cells of vertebrates, excluding some Antarctic fish. It is responsible for the transport of molecular oxygen from one compartment with high concentration of O<sub>2</sub> to the tissues that need it. Each of its four protein cells, called globin, contains inside a molecule of **protoporphyrin** coordinating an iron ion Fe (II), located slightly outside the plane of the molecule, collectively called **Eme Group**. When it binds to oxygen hemoglobin is called **oxyhemoglobin**, and it is called **deoxyhemoglobin** in the unbound form .

## Gasoline



Gasoline fluorescence spectrum excited by UV emission

Emission peak at 445nm

Emission peak at 474nm

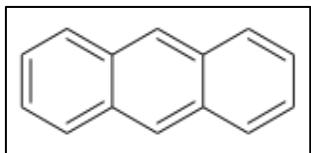
Emission peak at 498nm

The fluorescence of gasoline is due to its content of polycyclic aromatic hydrocarbons. The spectrum that is obtained is a mixture of distinct peaks of varying intensity :

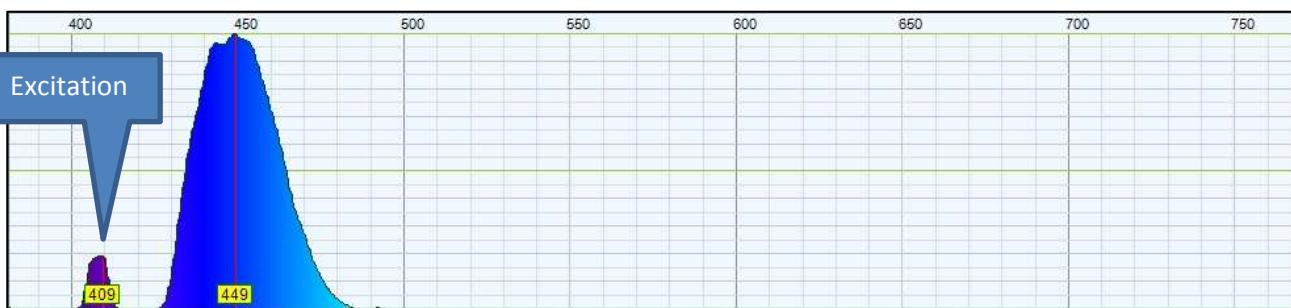
- Monocyclic compounds ( benzene, toluene , ethylbenzene , xylene ) : 250-290nm (known as BTEX)
- Polycyclic compounds (known as IPA)
  - o Two ring compounds (naftalene) : 310-330nm
  - o Phenanthrene (three rings) : 345-355nm
  - o **Anthracene : 447nm**
  - o **Tetracene (Naftacene) : 473nm, 498nm**

The spectrum is obtained by excitation at 405nm and then are shown only the emissions at longer wavelengths, among which recognize the peaks of anthracene and tetracene

## Anthracene

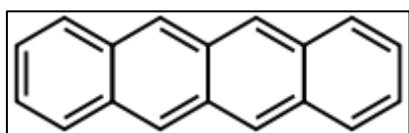


**Anthracene** is a polycyclic aromatic hydrocarbon solid compound (IPA) consisting of three benzene rings condensed and it has a linear structure . The double bonds between the carbon atoms give rise to fluorescence. The anthracene is used in the dye industry for the synthesis of the dye of Alizarin Red. It was also used as a preservative in the timber, thanks to its insecticidal properties. Anthracene is colorless , but has **blue fluorescence** if hit by a source of ultraviolet light. Anthracene has found lately space as the organic semiconductor , it is also used as a scintillator for the detection of electrons and alpha particles.



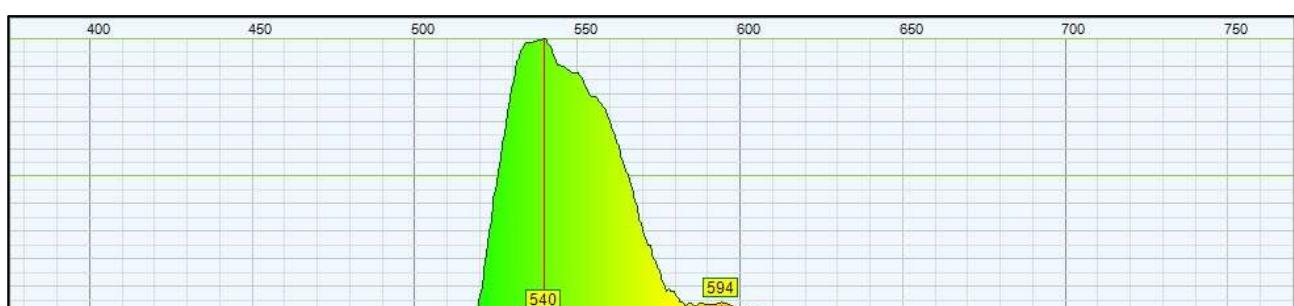
Fluorescence spectrum of diphenylanthracene (from glowstick) excited by UV emission at 405nm

## Tetracene (Naphthacene)



**Tetracene**, also called **naphthacene**, is a polycyclic aromatic hydrocarbon. It has the appearance of a pale orange powder. Tetracene is the four-ringed member of the series of acenes, the previous one being anthracene (tricene) and the next one being pentacene.

Tetracene is a molecular organic semiconductor, used in organic field-effect transistors (OFETs) and organic light-emitting diodes (OLEDs). In May 2007, researchers from two Japanese universities, Tohoku University in Sendai and Osaka University, reported an ambipolar light-emitting transistor made of a single tetracene crystal. Ambipolar means that the electric charge is transported by both positively charged holes and negatively charged electrons. Tetracene can be also used as a gain medium in dye lasers as a sensitiser in chemiluminescence.

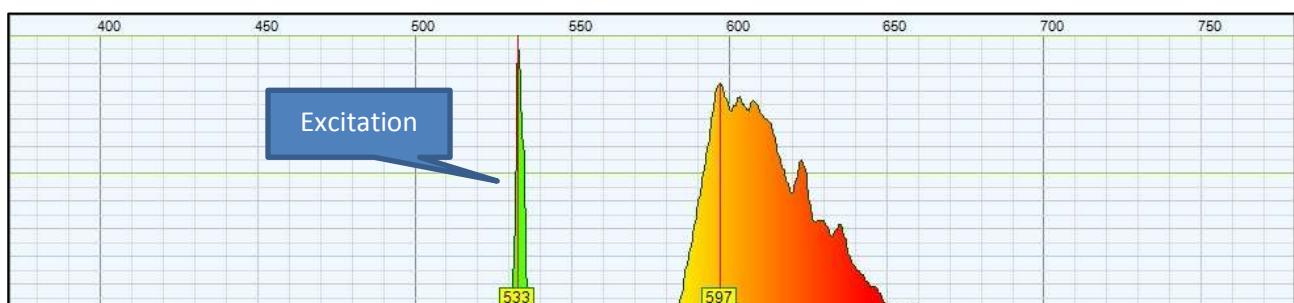


Fluorescence spectrum of tetraphenyl naphthacene (from glowstick) excited by UV emission at 405nm

## Motor Oil



Fluorescence spectrum excited by UV emission



Fluorescence spectrum excited by 532nm laser

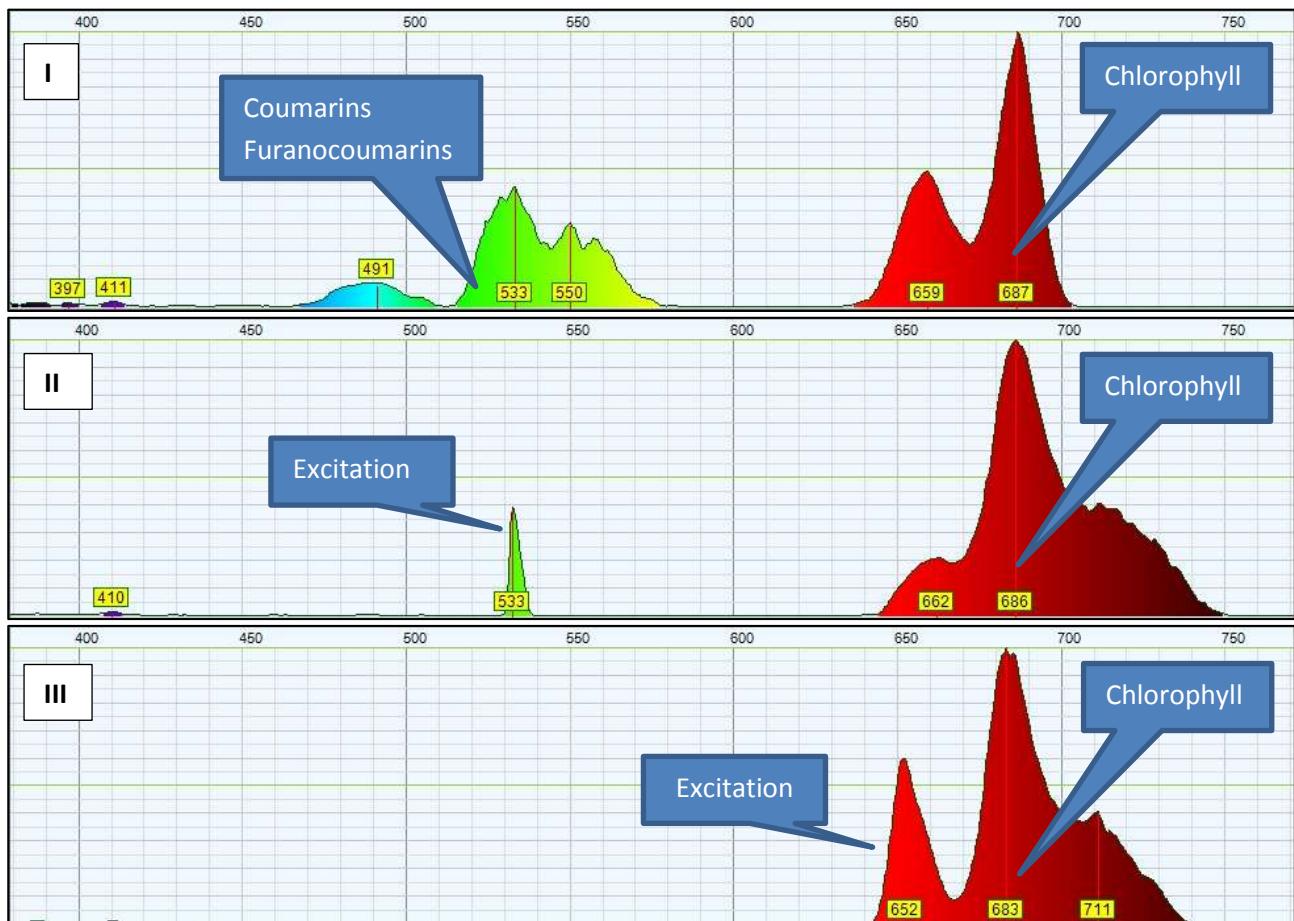
Lubricating oils for engines are added with fluorescent dyes in order to facilitate the search for leaks. From the spectra can be seen the band fluorescence due to these additives . The band on the blue-green color is probably due to a mixture of stilbene / triazine while the orange band could be due to a dye similar to acridine orange.

## Bergamot Essential Oil

The essence is a clear liquid (sometimes there is a deposit formed by waxes) of color ranging from green to greenish yellow. From a chemical point of view it consists for the most part (average 95 %) of a volatile fraction and a fraction (or residual) non-volatile (the remaining 5%). It is a highly complex mixture of many classes of organic substances, particularly terpenes, esters, alcohols and aldehydes, for the volatile fraction.

**Oxygenated heterocyclic compounds, coumarins and furanocoumarins**, for the non-volatile fraction.

The main components of the non-volatile fraction consist of **coumarins** (citropten, 5-Geranyloxy-7-methoxycoumarin) and furanocoumarins (bergaptene, Bergamottin). Furanocoumarins (also known as psoralen) are phototoxic.



I : Fluorescence spectrum excited from laser emission at 405nm.

Emission peak at about 680nm due to chlorophylls.

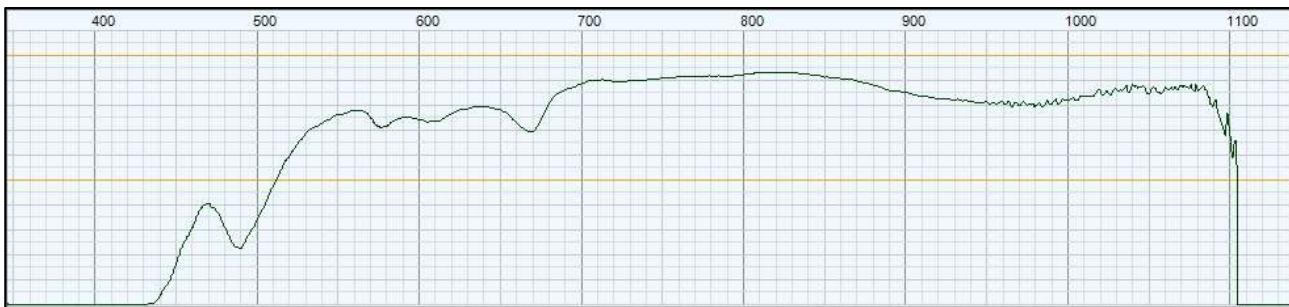
Blue-green-yellow fluorescence bands likely due to **coumarins** and **furanocoumarins**.

II : Fluorescence spectrum excited from laser emission at 532nm.

Emission peak at about 680nm due to chlorophylls.

III : Fluorescence spectrum excited from laser emission at 650nm.

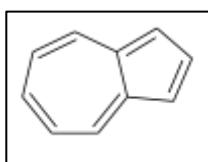
Emission peak at about 680nm due to chlorophylls.



**Absorption spectrum of bergamot essential oil**

Absorption maxima at about 480 and 660nm due to chlorophyll

## Chamomile Essential Oil



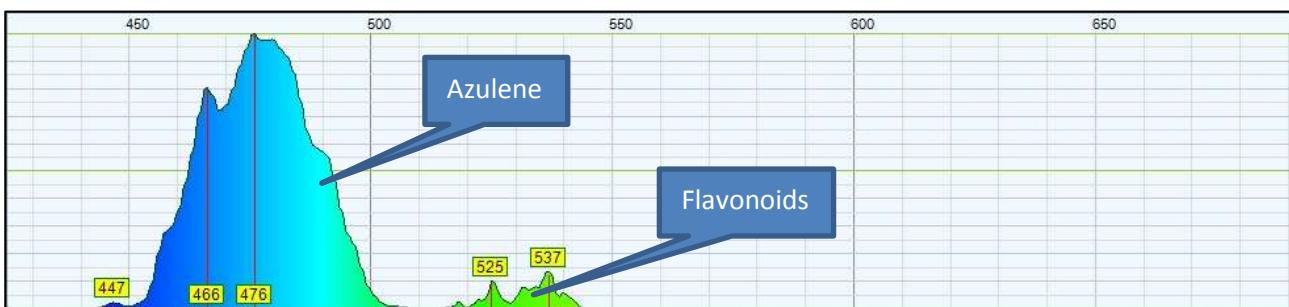
Chamomile essential oil contains interesting substances among which we mention azulene. **Azulene** is an aromatic hydrocarbon, naphthalene isomer. It is also a monoterpenoid. At room temperature is a crystalline solid of intermediate color between blue-violet and black, insoluble in water.

It is mainly used for coloring and in the cosmetics industry.

Its molecule is planar and is composed of two condensed rings, one of 5 carbon atoms, the other of 7. A peculiar feature of azulene is its anomalous fluorescence. Indeed the molecule emits fluorescence from transition S2 - S0 instead of the usual transition S1 - S0.



**Chamomile essential oil absorption spectrum**



**Chamomile essential oil fluorescence spectrum excited from UV emission**

## Orange Essential Oil

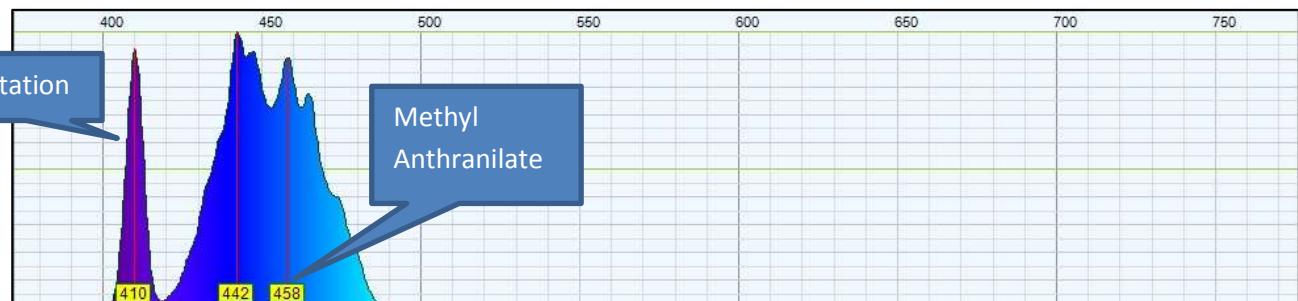
The essential oil of orange (*Citrus aurantium*) is extracted from the rind of the fruit namesake tree , through cold pressing or distillation. Using fruits naturally ripened. Depending on the used fruit type, we have sweet orange essential oil or essential oil of bitter orange.



Fluorescence Spectrum excited from UV emission

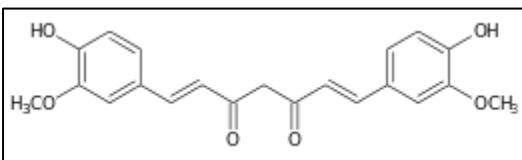
## Orange Blossoms Water

From the distillation of orange blossoms it is obtained a liquid which is composed of two phases : an aqueous phase, which stratifies in the bottom as it is heavier, and an oily phase, which brings high because lighter. Separated from each other, the aqueous phase is precisely the **water of orange blossoms**, while the oily, most valuable, is the essential oil of orange blossom. The essential oil is mainly obtained from the flowers of the bitter orange (**neroli oil**). The violet - blue fluorescence is due to the content of **methyl anthranilate**. Methyl anthranilate is the methyl ester of anthranilic acid. It is used as a repellent for birds. It is considered one of the first artificial flavors, but is present in nature in many essential oils.



Fluorescence Spectrum excited from UV emission

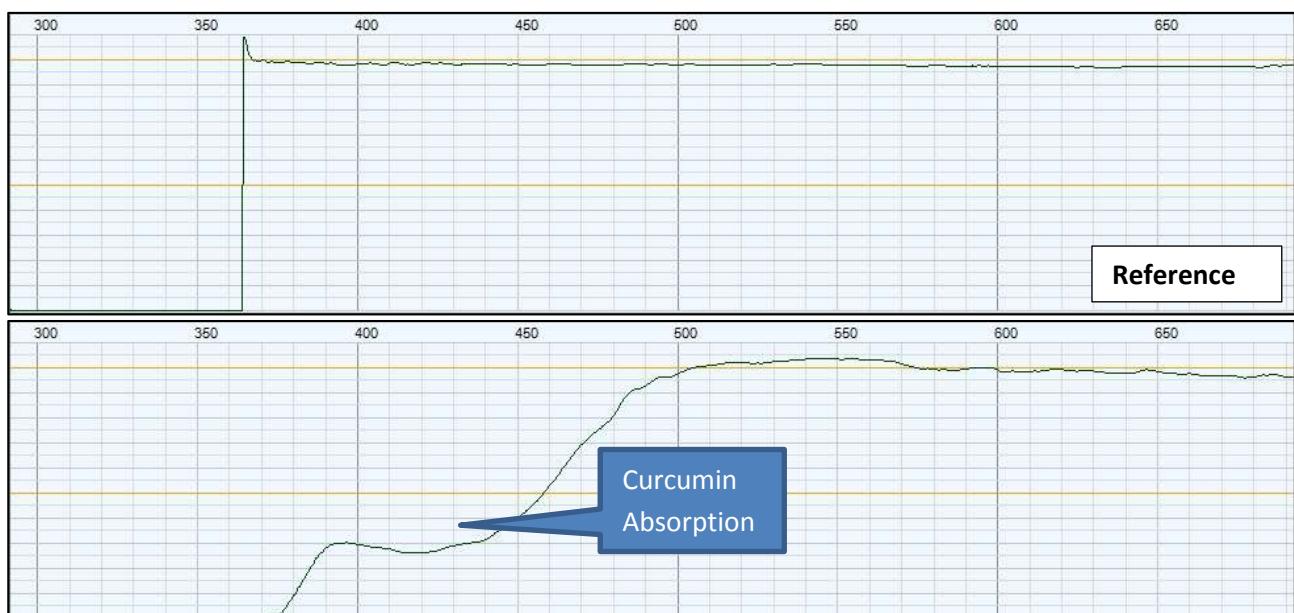
## Curcumin Essential Oil



**Curcumin** is the principal curcuminoid of turmeric, which is a member of the ginger family (Zingiberaceae). Turmeric's other two curcuminoids are desmethoxycurcumin and bis-desmethoxycurcumin. The curcuminoids are natural phenols that are responsible for the yellow color of turmeric.

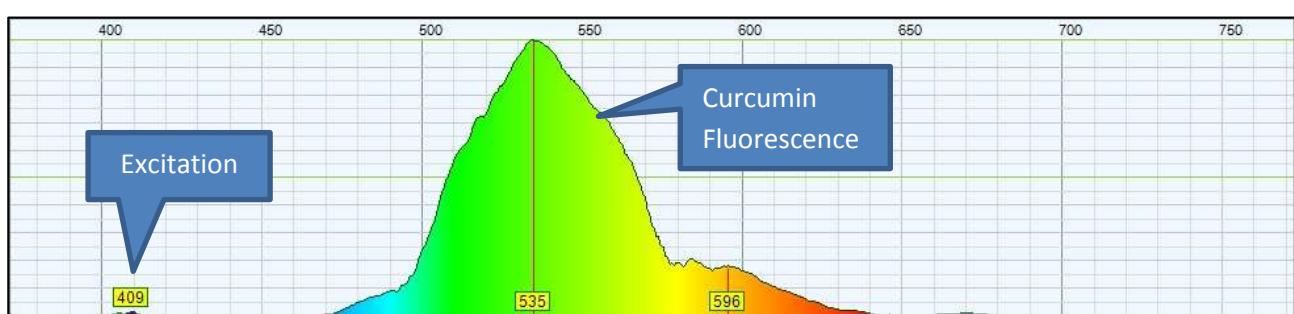
Curcumin can exist in several tautomeric forms, including a 1,3-diketo form and two equivalent enol forms. The enol form is more energetically stable in the solid phase and in solution.

Curcumin can be used for boron quantification in the curcumin method. It reacts with boric acid to form a red-color compound, rosocyanine. Curcumin is a bright-yellow color and may be used as a food coloring. As a food additive, its E number is E100.



Absorption spectrum of curcumin ethanol solution

Absorption maxima at about 420nm and at UV wavelength



Fluorescence spectrum of curcumin ethanol solution excited from UV emission

Maxima at about 535nm

# Spectroscopy of Inorganic Compounds

## Strontium Aluminate

**Strontium aluminate** (SRA, SrAl, SrAl<sub>2</sub>O<sub>4</sub>) is a solid odorless, nonflammable, pale yellow powder, heavier than water. It is chemically and biologically inert. When activated with a suitable dopant (e.g. europium, then it is labeled SrAl<sub>2</sub>O<sub>4</sub>:Eu), it acts as a photoluminescent phosphor with long persistence of phosphorescence.

Strontium aluminate is a vastly superior phosphor to its predecessor, copper-activated zinc sulfide; it is about 10 times brighter and 10 times longer glowing, however about 10 times more expensive than ZnS:Cu. It is frequently used in glow in the dark toys, where it displaces the cheaper but less efficient ZnS:Cu. However, the material has high hardness, causing abrasion to the machinery handling it; coating the particles with a suitable lubricant is usually used when strontium aluminate is added to plastics.

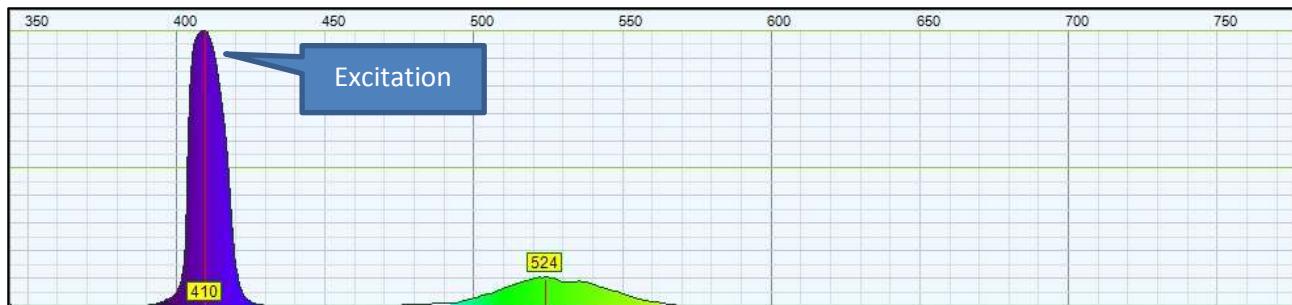
Strontium aluminate phosphors produce green and aqua hues, where green gives the highest brightness and aqua the longest glow time. The excitation wavelengths for strontium aluminate range from 200 to 450nm. The wavelength for its **green formulation is 520nm**, its blue-green version emits at 500nm, and the blue one emits at 490nm. Colors with longer wavelengths can be obtained from the strontium aluminate as well, though for the price of some loss of brightness.

The wavelengths produced depend on the internal crystal structure of the material. Slight modifications in the manufacturing process (the type of reducing atmosphere, small variations of stoichiometry of the reagents, addition of carbon or rare-earth halides) can significantly influence the emission wavelengths.

Strontium aluminate phosphor is fired at about 1250 °C. Subjecting it to temperatures above 1090 °C is likely to cause loss of its phosphorescent properties.

The glow intensity depends on the particle size; generally, the bigger the particles, the better the glow.

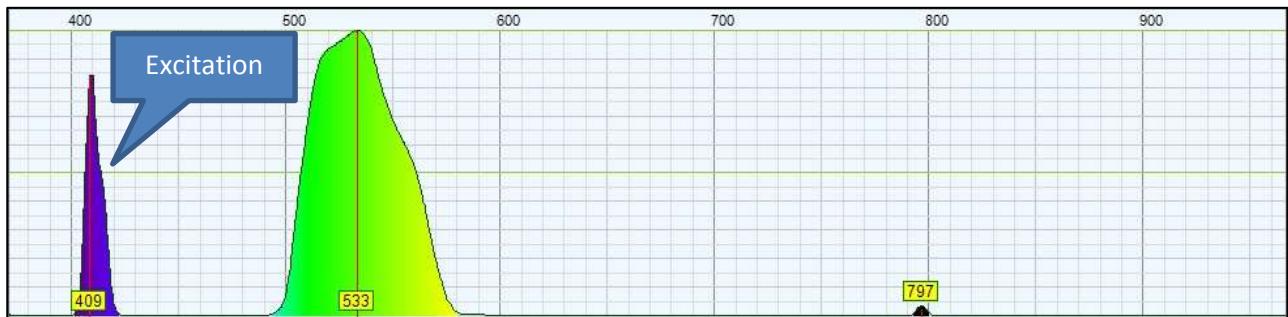
Fluorescence spectrum excited by UV emission



Emission peak detail at around 520nm



## Uranium glass



Fluorescence spectrum of uranium glass excited by UV emission

## Halogen Inner Bulb



Fluorescence spectrum of halogen inner bulb excited by UV emission

The fluorescence is supposedly due to the quartz of the inner ampoule which encloses the tungsten filament

# Raman Spectroscopy

**Raman spectroscopy** is a spectroscopic technique used to observe vibrational, rotational, and other low-frequency modes in a system. It relies on inelastic scattering, or **Raman scattering**, of monochromatic light, usually from a laser in the visible, near infrared, or near ultraviolet range. The laser light interacts with molecular vibrations, phonons or other excitations in the system, resulting in the energy of the laser photons being shifted up or down. The shift in energy gives information about the vibrational modes in the system. Infrared spectroscopy yields similar, but complementary, information. Raman spectroscopy is commonly used in chemistry to provide a fingerprint by which molecules can be identified.

Typically, a sample is illuminated with a laser beam. Electromagnetic radiation from the illuminated spot is collected with a lens and sent through a monochromator. Elastic scattered radiation at the wavelength corresponding to the laser line (**Rayleigh scattering**) is filtered out, while the rest of the collected light is dispersed onto a detector by either a notch filter or a band pass filter.

Spontaneous Raman scattering is typically very weak, and as a result the main difficulty of Raman spectroscopy is separating the weak inelastically scattered light from the intense Rayleigh scattered laser light.

The Raman effect occurs when electromagnetic radiation impinges on a molecule and interacts with the polarizable electron density and the bonds of the molecule in the phase (solid, liquid or gaseous) and environment in which the molecule finds itself. For the spontaneous Raman effect, which is a form of inelastic light scattering, a photon(electromagnetic radiation of a specific wavelength) excites (interacts with) the molecule in either the ground rovibronic state or an excited rovibronic state.

When photons are scattered from an atom or molecule, most photons are elastically scattered (**Rayleigh scattering**), such that the scattered photons have the same energy (frequency and wavelength) as the incident photons. A small fraction of the scattered photons (approximately 1 in 10 million) are scattered by an excitation, with the scattered photons having a frequency different from, and usually lower than, that of the incident photons.

Raman scattering spectrum of ethanol 95% - Excitation 532nm laser



Raman scattering spectrum of chlorophyll alcoholic solution - Excitation 532nm laser



# Chemiluminescence Spectroscopy

**Chemiluminescence** (sometimes "chemoluminescence") is the emission of light (luminescence), as the result of a chemical reaction. There may also be limited emission of heat. Given reactants A and B, with an excited intermediate P



In practice, the reaction leads to the product P in an excited state and the decay to the ground state does not lead to the formation of heat but of a photon ( $h\nu$ ) . It is therefore necessary that the radiative decay mechanisms are competitive compared to non-radiative.

An example of a reaction that leads to chemiluminescence is that of luminol with hydrogen peroxide and a metal catalyst.

When this phenomenon occurs in biological systems, for example in fireflies, it is called bioluminescence. In these cases the reactions are catalyzed by enzymes .

**Chemiluminescence is the base of the so-called "Glowstick".**

The lightstick, also known as starlight, cyalume, glowstick or more simply chemical light, is a cylinder of silicone, or other soft plastic material, self-luminous variable in size from a few cm to over 30 cm.

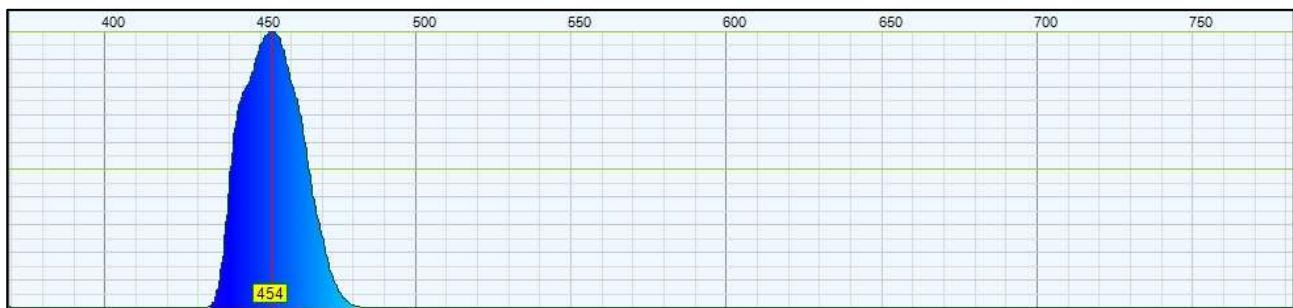
The main chemical components of a glow sticks are hydrogen peroxide , diphenyl oxalate (an ester of oxalic acid) and a fluorescent pigment which determine the color : yellow, blue , green , purple, red , orange or white.

To turn the light is sufficient bending the wand thus breaking the vial contained within it . This makes mixing the chemicals and initiates the luminescent reaction. The mechanism consists in the reaction between the hydrogen peroxide and the ester which provide the energy required to excite electrons of the fluorescent dye . The electrons flow from the fundamental energy level in the excited and returning to the fundamental level emit light.



"Glowsticks"

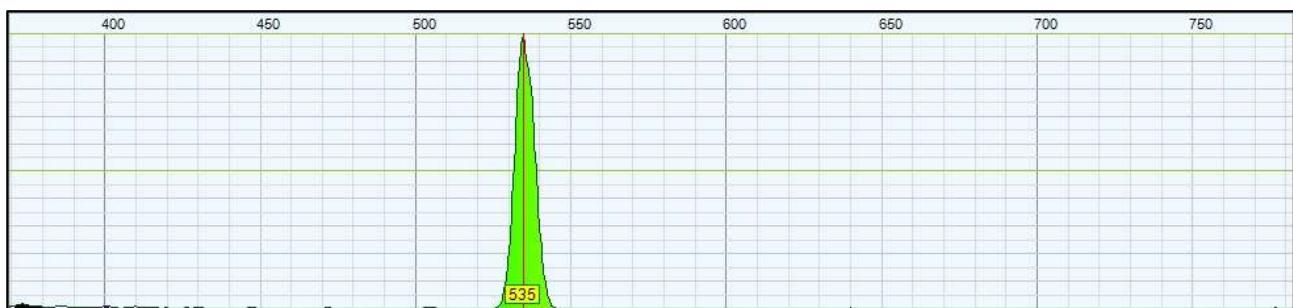
## Blue LightStick



**Blue chemiluminescence spectrum**

The blue chemiluminescence is obtained from the substance (fluorescent dye) **diphenyl anthracene**

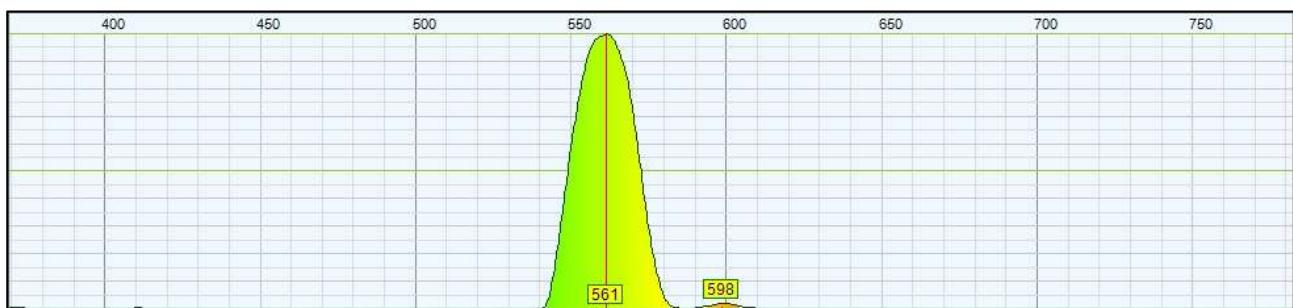
## Green LightStick



**Green chemiluminescence spectrum**

The green chemiluminescence is obtained from the substance (fluorescent dye) **bis phenylethyanyl anthracene**. This aromatic compound has a strong fluorescence and is also used in OLED components

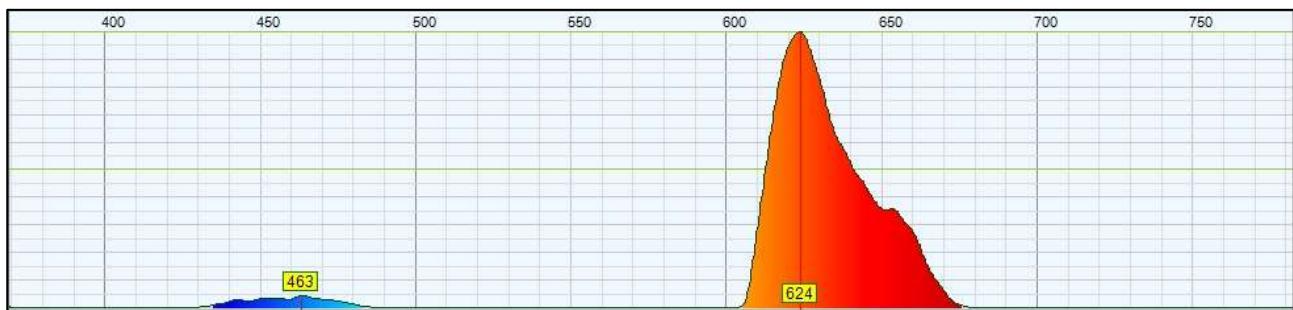
## Yellow LightStick



**Yellow chemiluminescence spectrum**

The yellow chemiluminescence is obtained from the substances (fluorescent dyes) **chloro bis(phenylethyanyl) anthracene** and **tetraphenyl naphthacene**.

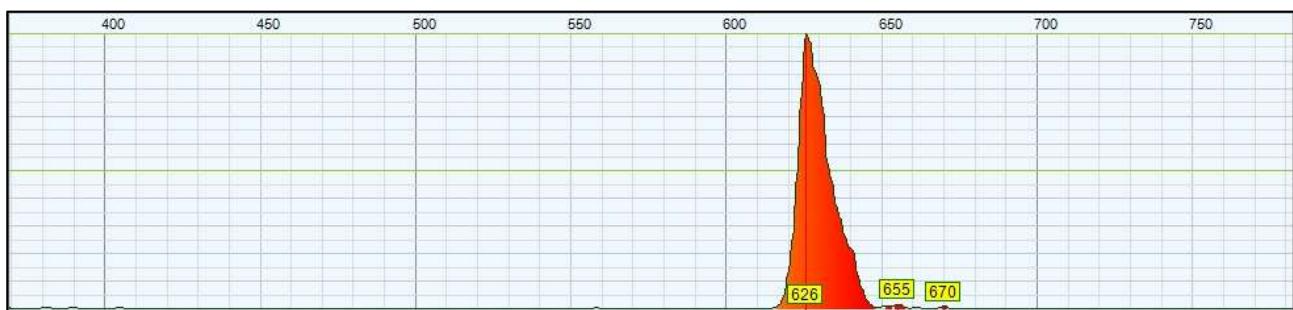
## Purple LightStick



**Purple chemiluminescence spectrum**

The purple chemiluminescence is presumably obtained by mixing the fluorescent dyes **diphenyl anthracene** (blue) and **violanthrone** (orange)

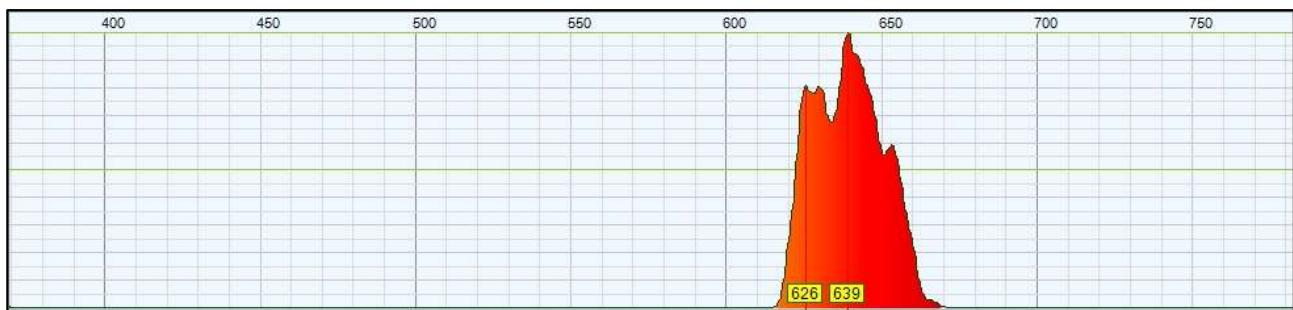
## Orange LightStick



**Orange chemiluminescence spectrum**

The orange chemiluminescence is presumably obtained from the fluorescent dye **violanthrone** (orange)

## Red LightStick



**Red chemiluminescence spectrum**

The red chemiluminescence is probably obtained from a derivative of the substance fluorescent dye **violanthrone**