SAPIENZA Università di Roma
Laurea magistrale in Ingegneria delle Nanotecnologie
A.A. 2019-2020

Biophotonics Laboratory Course

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Applications of optics and photonics

Microscopic Techniques
- Conventional Wide-Field Fluorescence
- TIRF
- FLIM
- FRET, FRAP
- Confocal
- Two-Photon
- Second Harmonic
- Super-resolution (SNOM, STED, PALM, STORM)

Non-Microscopic Label-free
- Surface plasmon Polaritons (SPP)
- Photonic crystals (PC)
- Raman , CARS
- Quantum dots

Non-Microscopic Techniques
- Citofluorimetry
- ELISA
- DNA-Chip
- Cycle-sequencing
- SOLID

Other non Microscopic Techniques
- Southern
- Western
- Northern

All of them make use of the emission of luminescent markers (labels)

LABORATORY WORK 2

Department BBAI

Prof. Rita Petrucci, Dr. Agostino Occhicone

Absorption Spectroscopy

Thursday nov 12 2020
h8.30-h11.00

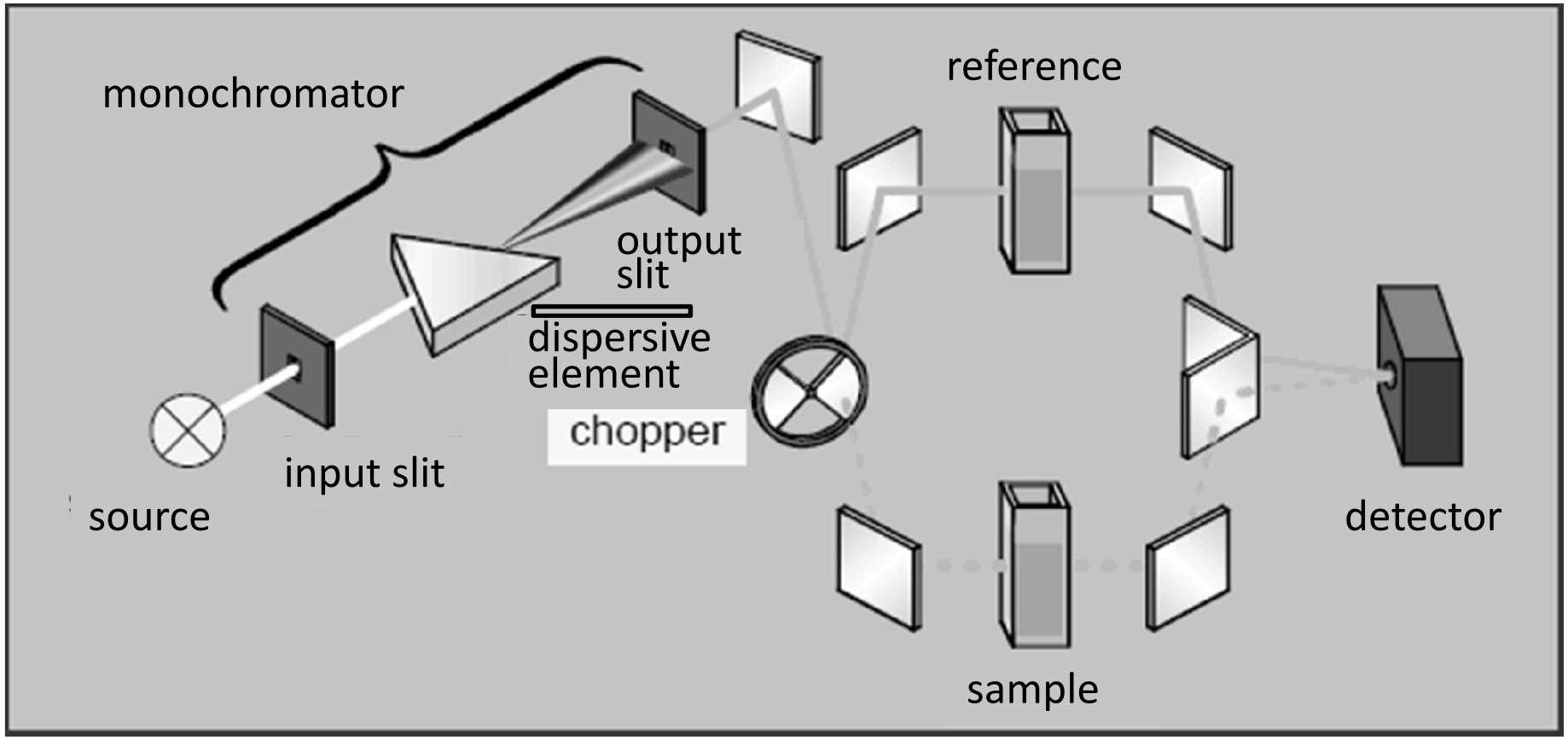
Absorption Spectroscopy

Schedule of the laboratory work

- Description of a UV/VIS spectro-photometer
    - Measurement of the absorption spectrum of Rhodamine 6G in ethanol at different concentrations
    - Measurement of the absorption spectrum of Rhodamine 6G in water at one concentration
    - Measurement of the absorption spectrum of solutions of Rhodamine 6G in water and milk at one concentration

UV/VIS Spectro-photometer

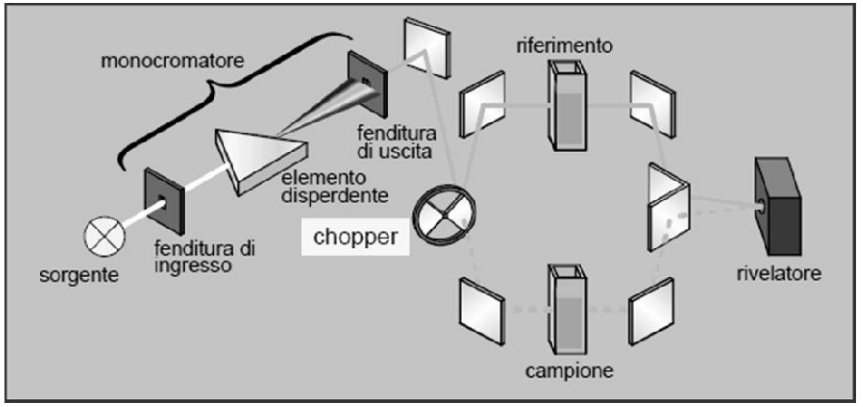
Scheme of a double path spectro-photometer



The radiation emitted by a lamp (deuterium or tungsten) is passed through a mono-chromator and divided in two beams that go through the reference and the sample. The beam splitter is a rotating mirror (chopper), which sends the radiation alternatively in one of the two arms of the instrument. The power of the two beams is measured by means of the same detector.

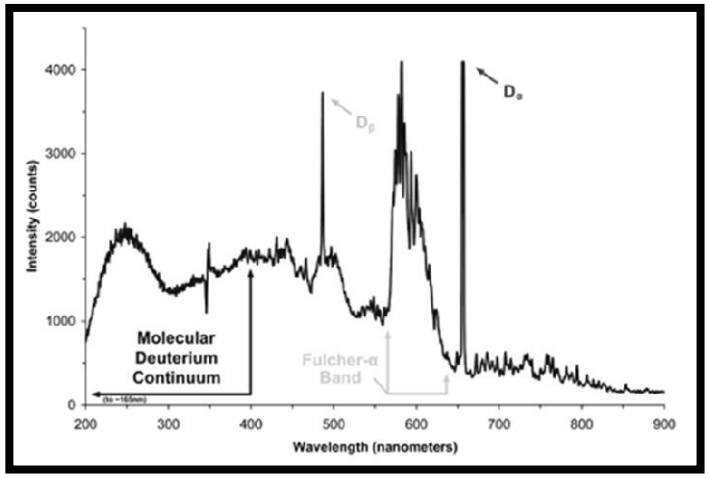
UV/VIS Spectro-photometer

Measure 1 - Calibration

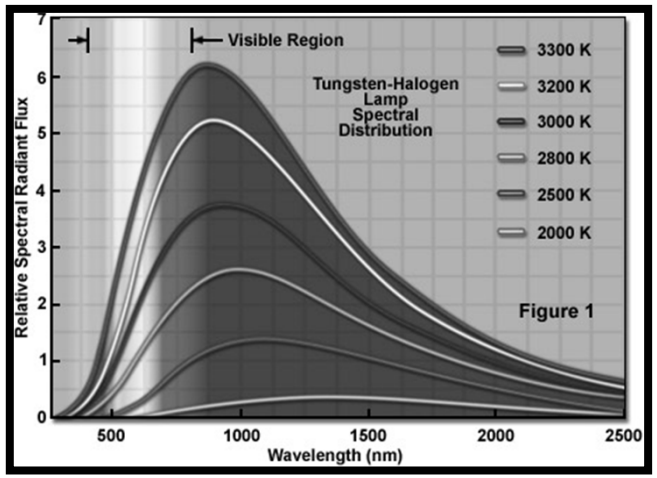


Measure of the power $P_{0,RIF}(\lambda)$ and $P_{0,CAM}(\lambda)$ transmitted through the two arms trasmesse without sample and reference for every wavelenght λ.

$$F_{INSTR}(\lambda) = \frac{P_{0,SAM}(\lambda)}{P_{0,REF}(\lambda)}$$



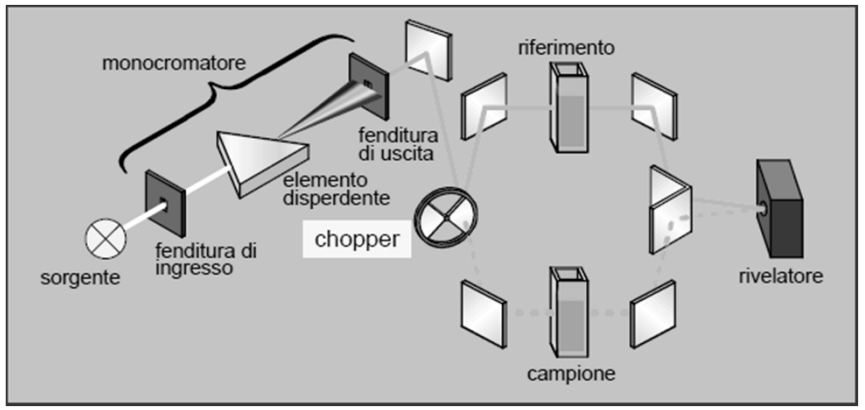
Deuterium Lamp - UV



Halogen Lamp with Tungsten wire - VIS/NIR

UV/VIS Spectro-photometer

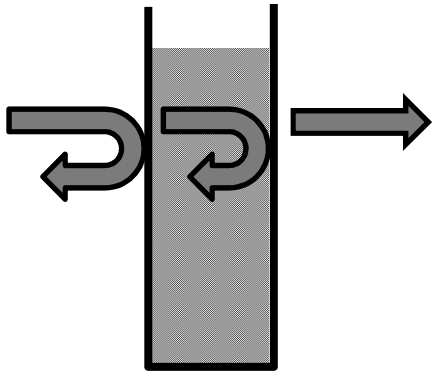
Measure 2 - Transmittance



Measure of the power $P_{RIF}(\lambda)$ and $P_{CAM}(\lambda)$ transmitted by the two arms with sample and reference for every λ.

$$P_{REF}(\lambda) = P_{0,REF}(\lambda)(1 - R_{CUV})e^{-\alpha_{SOLV}(\lambda)h}$$

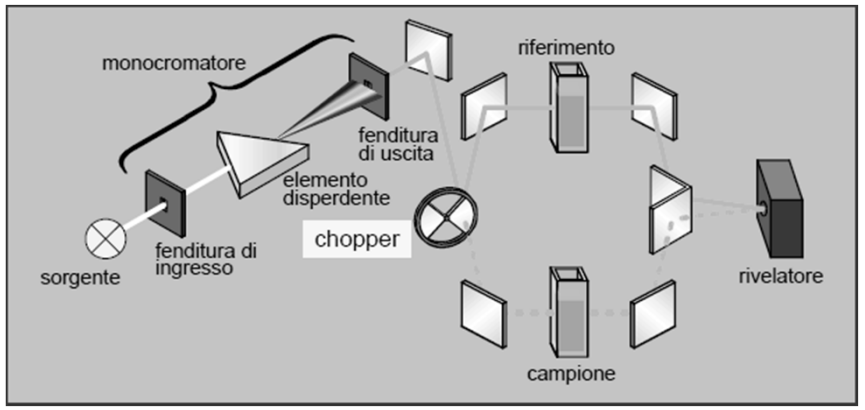
Transmittance of the interfaces of the cuvette



Transmittance of the solvent

UV/VIS Spectro-photometer

Measure 2 - Transmittance



Measure of the power $P_{RIF}(\lambda)$ and $P_{CAM}(\lambda)$ transmitted by the two arms with sample and reference for every λ.

$$P_{REF}(\lambda) = P_{0,REF}(\lambda)(1 - R_{CUV})e^{-\alpha_{SOLV}(\lambda)h}$$

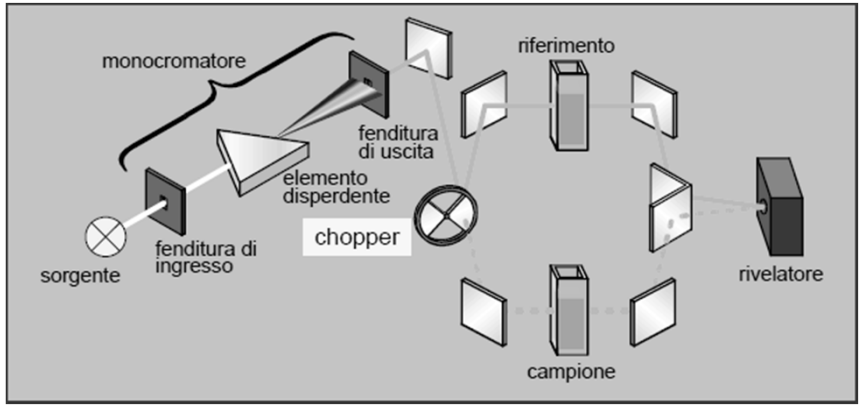
$$P_{SAM}(\lambda) = P_{0,SAM}(\lambda)(1 - R_{CUV})e^{-\alpha_{SOLV}(\lambda)h} \cdot e^{-\alpha_{FLUOROPHORE}(\lambda)h}$$

N.B.

$$\frac{P_{SAM}(\lambda)}{P_{0,SAM}(\lambda)} \neq T_{FLUOROPHORE}(\lambda) = e^{-\alpha_{FLUOROPHORE}(\lambda)h}$$

UV/VIS Spectro-photometer

Measure 2 - Transmittance



Measure of the power $P_{RIF}(\lambda)$ and $P_{CAM}(\lambda)$ transmitted by the two arms with sample and reference for every λ.

$$P_{REF}(\lambda) = P_{0,REF}(\lambda)(1 - R_{CUV})e^{-\alpha_{SOLV}(\lambda)h}$$

$$P_{SAM}(\lambda) = P_{0,SAM}(\lambda)(1 - R_{CUV})e^{-\alpha_{SOLV}(\lambda)h} \cdot e^{-\alpha_{FLUOROPHORE}(\lambda)h}$$

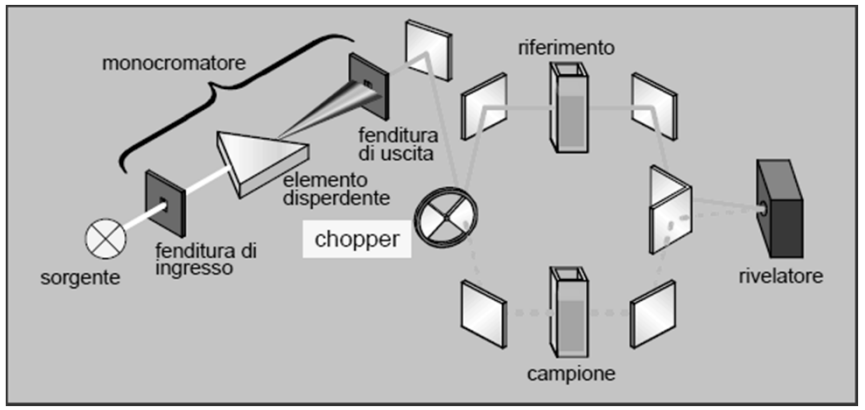
$$\frac{P_{SAM}(\lambda)}{P_{REF}(\lambda)} = \frac{P_{0,SAM}(\lambda)}{P_{0,REF}(\lambda)} e^{-\alpha_{FLUOROPHORE}(\lambda)h} = F_{INSTR}(\lambda) e^{-\alpha_{FLUOROPHORE}(\lambda)h}$$

$$T(\lambda) = e^{-\alpha_{FLUOROPHORE}(\lambda)h} = \frac{P_{SAM}(\lambda)}{P_{REF}(\lambda)} \cdot \frac{1}{F_{INSTR}(\lambda)}$$

Real Transmittance

UV/VIS Spectro-photometer

Parametrs of a Perkin Elmer UV/Vis/NIR LAMBDA 19 spectro-photometer



Spectral Range: 190-3200 nm

Resolution: 0.05 to 5.0 nm with steps of 0.01 nm (UV/VIS)
0.2 to 20 nm in NIR

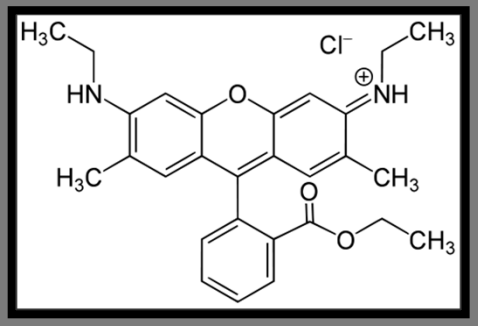
Accuracy on λ: +/- 0.15 nm (UV/VIS)
+/- 0.6 nm (NIR)

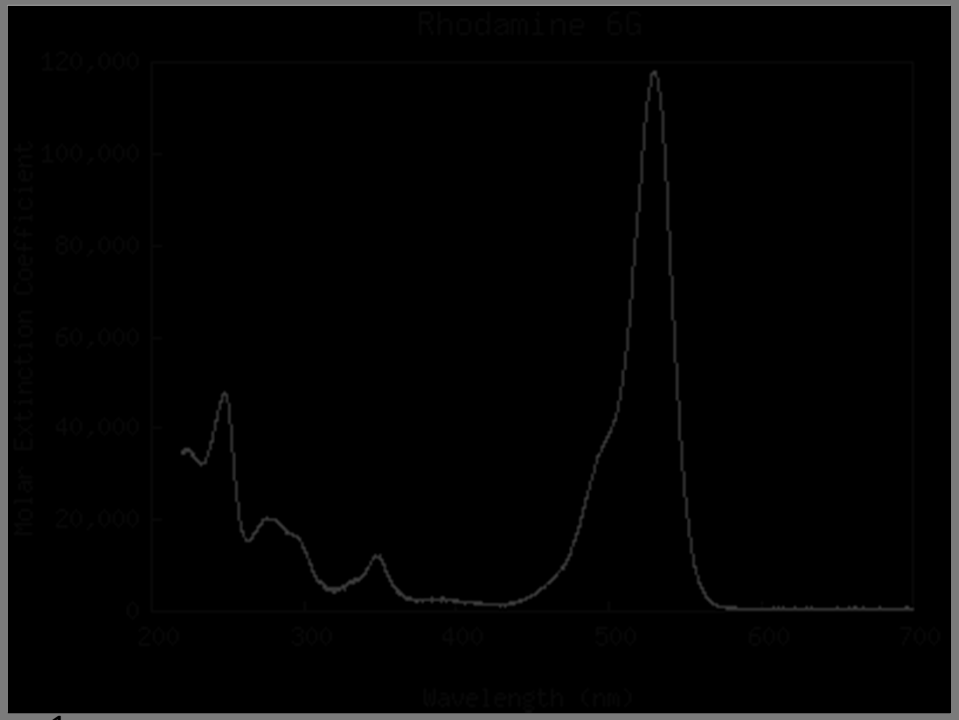
Repeatability λ: better than 0.02 nm (UV/VIS)
better than 0.08 nm (NIR)

Spettrofotometro UV/VIS

EXPERIMENT

Measure of the molar extinction coefficient of Rhodamine 6G





Measured in ethanol

Molecular Weight: 479.02 g/mol

Molar extinction coefficient ε at λ=529.75 nm : 116000 L $mol^{-1}cm^{-1}$

Concentration [C] in ethanol: $2 \cdot 10^{-6}$ mol/L
0.96μg/mL

Absorption Coefficient at λ=529.75 nm: $2.3 \cdot \varepsilon \cdot [C] = 0.54 cm^{-1}$