

TN\_P10; Anna Gakamsky

## Introduction

The absorbance  $A$  or optical density (OD) of a substance is inherent in all aspects of spectroscopy and follows the Beer-Lambert law<sup>1</sup>,  $A(\lambda) = \log[T_{ref}(\lambda)/T_s(\lambda)]$ , where  $T_{ref}(\lambda)$  is the transmission of the reference sample and  $T_s(\lambda)$  the transmission of the sample at the measured wavelength.

## Instrument and procedure

The FS5 Fluorescence Spectrometer is equipped with a silicon detector that allows for transmission and absorbance measurements. Liquid samples can be measured in the standard cuvette holder (SC-05), cuvette holder for coolant circulation (SC-20) and thermoelectrically cooled/heated cuvette holder (SC-25) which can be interchangeably installed in the FS5. Fluorescein solutions were prepared in phosphate buffered saline (PBS) in 1 cm plastic cuvettes. After the transmission scans of the sample and reference are complete, they can be joined to calculate the absorbance through the absorption wizard in the operating software, Fluoracle.

## Results

Figure 1 shows the transmission spectra of fluorescein samples at different concentrations measured in an FS5 fluorescence spectrometer.

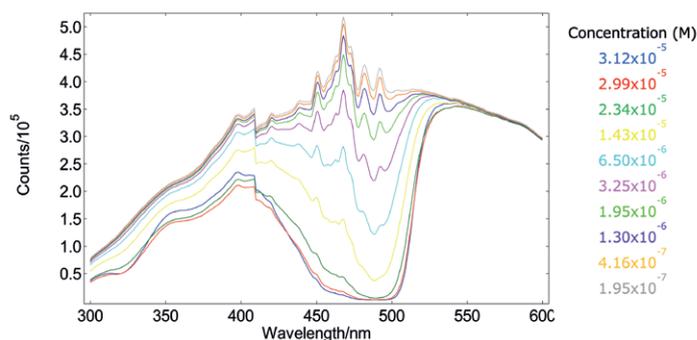


Figure 1: Transmission spectra for different concentrations of fluorescein in PBS.

The transmission spectra were recorded with a band-pass of 3 nm, a step of 1 nm and integration time of 0.2 s. The repeatability of the measurements was confirmed by reference transmission scans to less than 1% relative deviation.

From the transmission spectra, the absorbance spectra of the fluorescein samples in PBS shown in Figure 2 were calculated in the Fluoracle software. The spectra reveal the dianion form<sup>2</sup> of the solution with the absorption peak at 490 nm. The concentration  $c$  was calculated according to the Beer-Lambert law from the peak absorbance as  $c = A / \epsilon l$ , with the molar attenuation coefficient  $\epsilon$  of fluorescein<sup>2</sup>.

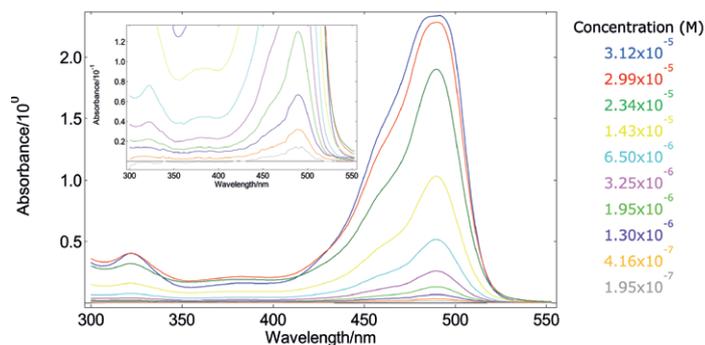


Figure 2: Absorbance spectra for different concentrations of fluorescein in PBS.

## Conclusion

The FS5 Fluorescence Spectrometer enables transmission and absorbance measurements of liquid samples in the wavelength range 230 nm-1000 nm and optical densities of OD 0.01 to OD 2.

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## References

1. Ball, D. W. *The Basics of Spectroscopy*. (SPIE Press, 2001).
2. Sjöback, R., Nygren, J. & Kubista, M. Absorption and fluorescence properties of fluorescein. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* **51**, L7–L21 (1995).

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