Using Laser-Induced Fluorescence Technique for Interdisciplinary Natural Sciences School Experiment

PHOTONICA '23

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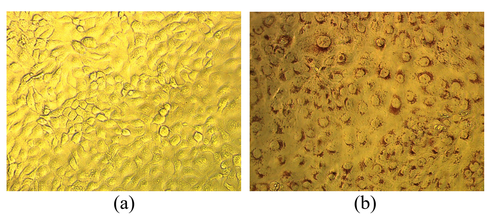
INTRODUCTION

The requirements set down in modern education strengthen the role of experimental methods, research tasks, integrative processes, digital competence and students' skills for independent acquisition of knowledge. In recent years, there is a trend of more extensive focus on the study of chemistry and biology over physics and mathematics. Therefore, the aim of our work is to offer a school practicum in spectroscopy, to implement an integrated education in physics, biology and information technology through students' learning and application of the research method. It is known that research-grade spectrometers are expensive and this makes them out of reach for the vast majority of schools. Therefore, we compare an inexpensive educational spectrometer (Thunder Optics) with a professional research spectrometer (Ocean Optics), from the point of view of the requirements of a school experiment.

LABORATORY TASKS

The first laboratory task involves fluorescence spectrum analysis of various organic materials for the characterization / identification of organic materials. The goal is for students to see the possible applications of this method.



(a) (b)

Figure 1: Microscopic images of (a) control cell lines, (b) treated cell lines with Neutral Red.

The second laboratory task is to visualize the standard cytotoxicity test tool (ELISA reader) using a setup to measure the uptake of a standard Neutral Red dye. This dye is added to the nutrient medium of the cells, it stays for several hours and the living cells take it into their lysosomes. The optical density of the solution is measured, with a higher absorbance corresponding to a higher number of live cells and a higher transparency to a lower number of live cells.

METHODS & RESULTS

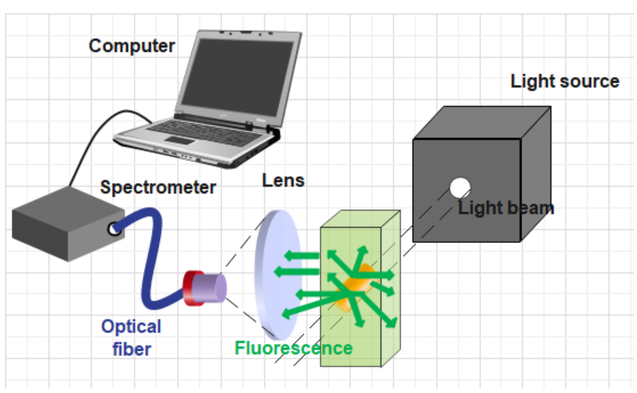


Figure 2: Experimental setup.

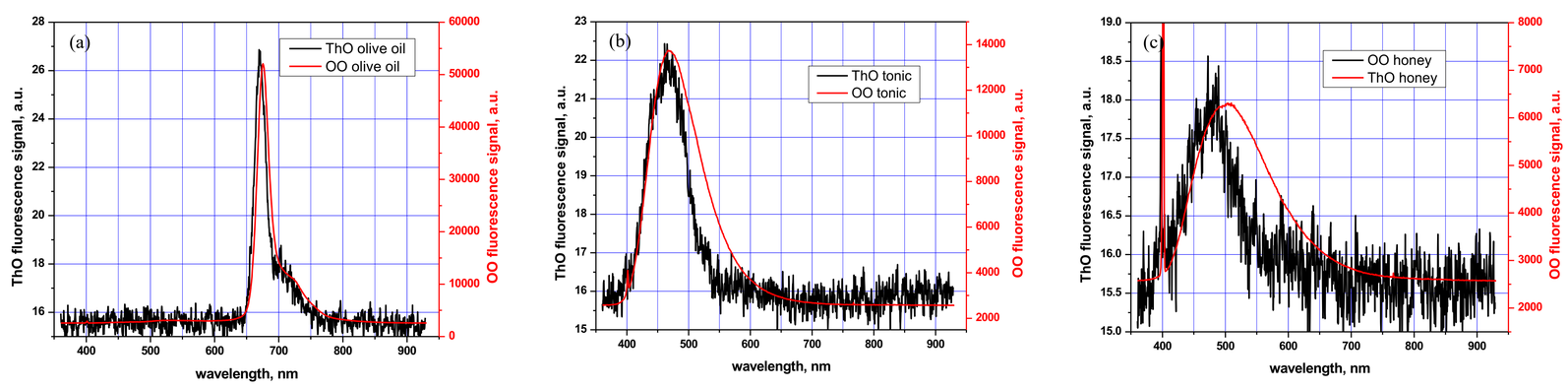


Figure 3: Comparison between the fluorescence signal from Thunder Opics (ThO) and Ocean Optics (OO) of (a) olive oil, (b) tonic and (c) honey.

The setup of the first task is as follows: The light from a laser with a wavelength of 405 nm is fed to a cuvette holder, in which cuvettes with various organic food samples (olive oil, tonic and honey) are placed. Transverse fluorescence is collected using a collimator and fed via fiber to the spectrometer connected to a computer for visualization and post-processing of the data. The results are shown in Figure 3.

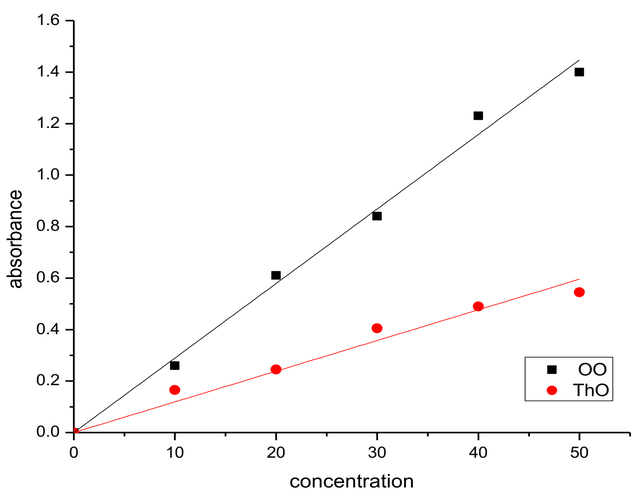


Figure 4: Absorbance of 5 different concentrations of Neutral Red in a lysis buffer.

The light source used for the second task is a halogen lamp. The collimator collects the transmitted light and feeds it to the spectrometer. The samples are cuvettes with a different concentration of Neutral Red in a lysis buffer solution, to simulate the standard situation in Neutral Red Uptake (Figure 5) assay to perform a simplified simulation measurement in our laboratory practicum compared to ELISA reader measurements. From the results (Figure 4) we can say that a single measurement should not be used for concentration estimation, but determination of concentration using a calibration graph is an excellent exercise for students.

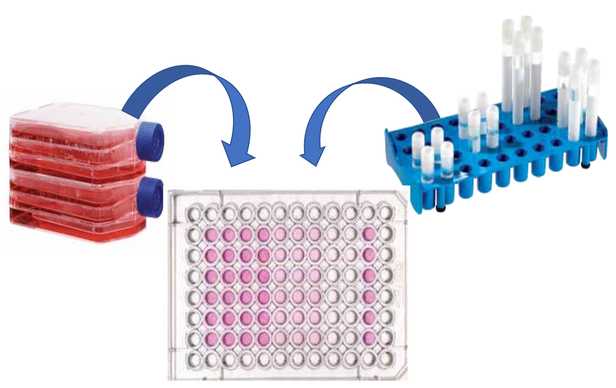


Figure 5: Neutral Red Uptake.

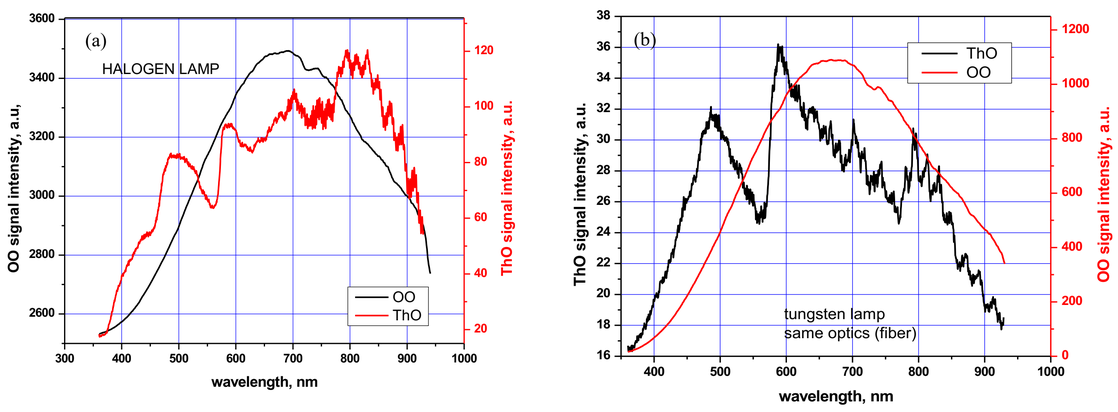


Figure 6: Comparison between ThO and OO, regarding absorbtion signal from (a) halogen lamp and (b) tungsten lamp.

CONCLUSION

We have shown that it is possible to use an educational-grade spectrometer for a number of spectroscopic practical laboratory exercises in schools. Of course, the limitations of the spectrometer should be kept in mind:

- White light demonstration can be illustrated only qualitatively.
- Regarding fluorescence, the most appropriate samples are those with fluorescence in the red region.
- Regarding the absorbance measurements, it is seen that the ThO instrument can provide very good calibration graphs, but the absolute values of the absorbance differ from the values obtained by the OO spectrometer.

ACKNOWLEDGEMENTS

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