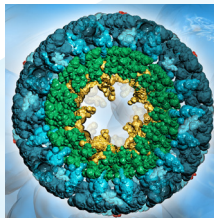


3-in-1 Fluorescence and Absorbance Spectrometer: How does it work?



Application
Note
Life Sciences

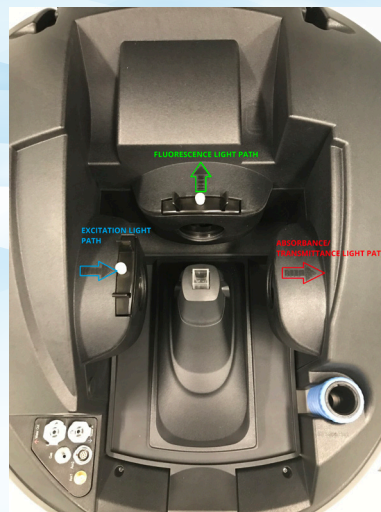


Figure 1: Duetta (left) and the inside of the Duetta sample compartment (right) with direction of the light path

Introduction

Duetta™ is a 3-in-1 fluorescence and absorbance spectrometer from HORIBA. There are several benefits of having two different spectroscopies in one instrument. For high concentration solutions of interest, both primary and secondary inner-filter effects will affect the fluorescence spectrum measured on a standard fluorometer. Having absorbance and fluorescence spectroscopy on the same instrument enables Duetta and EzSpec™ software to apply corrections for inner-filter effect and provide more accurate data, for a wider range of sample concentrations. Another benefit of the two in one instrument is that absorbance results and fluorescence results contain less error since the sample does not move from one instrument to another to get both measurements. Standard fluorescence methods such as emission spectrum and excitation spectrum are available on Duetta, but EzSpec software gives a user the ability to measure the absorbance spectrum as a stand-alone method or simultaneously as any fluorescence method, making Duetta a unique 3-in-1 spectroscopy tool.

Definitions

Fluorescence Emission Spectrum: Exciting a molecule (or population of molecules) with a single wavelength of

excitation energy and the spectrum at longer wavelengths is acquired to measure the distribution of intensity and energy (wavelength) of the photons emitted.

Fluorescence Excitation Spectrum

Acquiring the intensity of photons emitted at a single emission wavelength and scanning the excitation monochromator to excite the population of molecules in the sample with different wavelengths. A fluorescence excitation spectrum is analogous to an absorbance spectrum, but is specific to a single emitting species/wavelength as opposed to collecting all absorbing species in a sample or solution.

% Transmittance Spectrum

This is a ratio, in terms of percentage, of the intensity of transmitted light through an absorbing sample (I) compared to the transmitted light through a blank solvent (I₀).

$$T = (I/I_0) \text{ and } \%T = (I/I_0) \times 100\%$$

Absorbance Spectrum

The spectrum of absorbance is collected using transmission spectrum.

$$A = -\log T$$

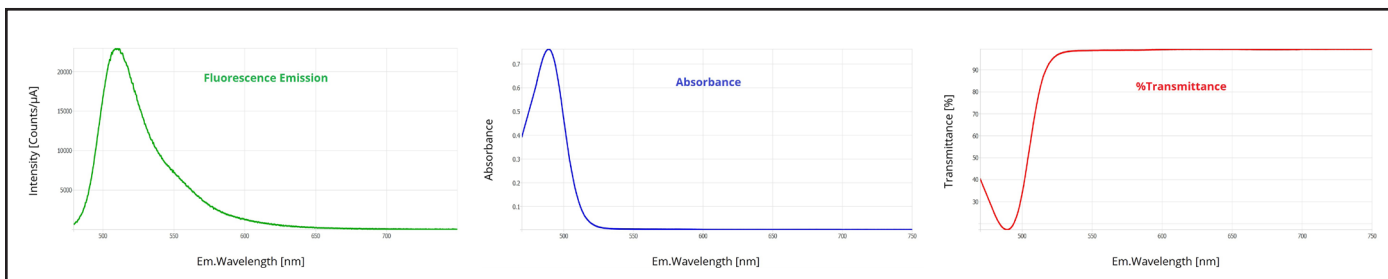


Figure 4: Fluorescence (green), Absorbance (blue), and % Transmittance (red) spectra of fluorescein measured in the same acquisition using Duetta.

Experiment and Results

The fluorescence emission spectrum, absorbance spectrum and % transmittance spectrum were all measured in a single acquisition using Duetta. The intensity of the xenon lamp is measured through a sample and then measured through the blank to get changed intensity (I) and initial intensity (I_0). A solution of 80 μM fluorescein in 0.1 M sodium hydroxide (aq.) solution in a quartz cuvette was the sample and ultrapure filtered water was the blank. The absorbance detector at 180 degrees from the excitation light path was scanned from 750 nm to 470 nm with 1 nm increments for both sample and blank. The fluorescence emission was collected using 470 nm excitation, 3 nm band pass for excitation and emission, and an integration time of 0.05 sec to collect the emission from 480 to 750 nm with CCD detector. The fluorescence spectrum was corrected for detector dark noise, spectral efficiency of the optics (spectral correction) and inner filter effect using the absorbance spectrum collected simultaneously on the same sample.

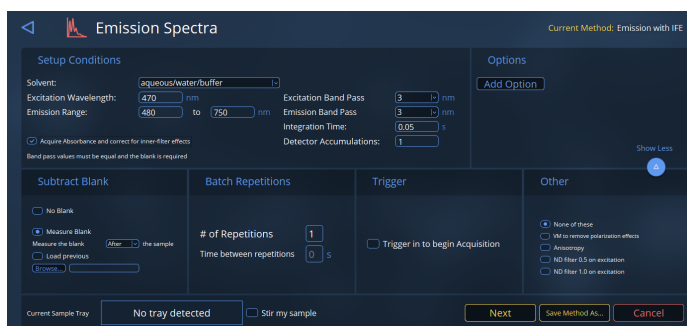


Figure 2: Method for simultaneous acquisition of fluorescence emission, absorbance, and % transmittance spectra with use of inner filter effect correction in EzSpec software

Fluorescence methods on Duetta can add the absorbance and %T spectral acquisition by selecting the “Acquire Absorbance and correct for inner-filter effect” check box in the method setup window as shown in Figure 2. By enabling IFE correction, all three modes (FL/Abs/%T) are acquired and displayed in the resulting graph and table. EzSpec software contains a graph view that can switch between different spectra types with quick button access.

“Figure 3 shows the tool button in EzSpec software where switching between spectral types makes viewing each spectrum easy.”

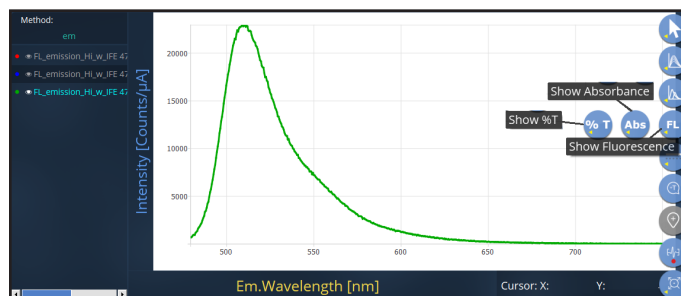


Figure 3: Graph tool buttons allow user to switch easily between different individual spectral types in the same graph

Fluorescein fluorescence emission, absorbance, and % transmittance spectra are shown in Figure 4. By using the modal button in EzSpec software, it is easy to switch between all three data sets or view them together. Figure 5 shows the three spectra normalized to one so that they can be viewed in the same axes.

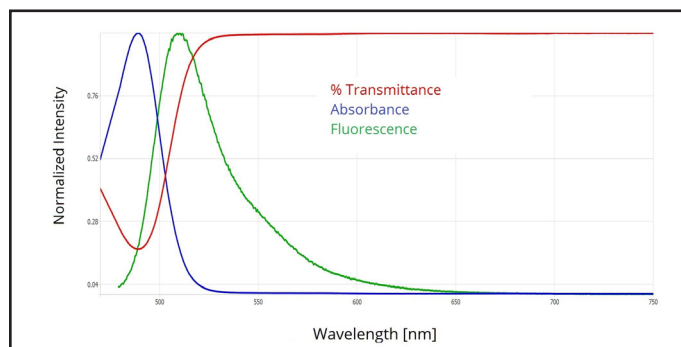


Figure 5: Normalized fluorescence emission, absorbance, and %transmittance spectra of fluorescein measured in the same acquisition using Duetta

Summary

Due to the unique optical design of Duetta, the absorbance, transmittance, and fluorescence spectrum of a sample of interest is acquired simultaneously on the same instrument. In the data shown above, fluorescein is an example showing how the absorbance spectrum can be used to measure both fluorescence and absorbance modes simultaneously, as well as correct the measured fluorescence spectrum for high concentration and high absorbance of the fluorophore. The optical design and flexible software allows users to operate the Duetta in fluorescence mode, UV-Vis absorbance mode, or both, as the application requires.

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