Fluorescence measurements

MF/2022

1. Introduction

The fluorescence has been studied over 150 years. It is defined as emission of light by a substance that has absorbed electromagnetic radiation, and this emitted light typically has a longer wavelength than the radiation that was absorbed. The resulting effect is most noticeable when visible light is emitted after absorption of UV light or how blue light turns to white light in the case of LED lamps. The fluorescence is also used to make objects, like office paper, seem whiter by removing “greyness” or “yellowness”.

In this work you will learn basics of how to use fluorescence spectrometer to measure *Donaldson matrices* (excitation-emission matrix), learn to analyze those matrices and finally use them to recognize various fluorescent substances.

2. Fluorescence Spectrometer

The device used in this work is PerkinElmer FL 8500. It is a very modern fluorescence spectrometer with several functions. Learn more about how to use it and its control software in PerkinElmer FL 8500 manual (below).

3. Theory

Light is a form of electromagnetic radiation characterized by wavelength λ and frequency ν:

$$\nu = \frac{c}{\lambda} \ ,$$

where c is the speed of light.

When light comes to contact with matter, it will either pass through or be absorbed, either entirely or in part. In the latter case energy of the light is transferred to the molecule in the absorption process. In most cases, light of longer wavelengths (above 700 nm) does not possess enough energy to cause electronic promotion to a higher energy level, only stimulating molecular vibrations.

The absorption of light occurs in discreate amounts (quanta) and corresponds to excitation of the molecule from the ground state to an excited state. Similarly, emission of a photon through fluorescence is also measured in terms of quanta. The energy in a quantum is expressed by the equation:

$$E = \ h\nu = \frac{hc}{\lambda} \ ,$$

where E is energy and h is Planck’s constant.

Planck’s formula states that the radiation energy of an absorbed photon is directly proportional to frequency and inversely proportional to the wavelength. The absorption of photon by a molecule is result of an interaction between the oscillating electric field of the light and electrons in the molecule. It can only occur with incident light of specific wavelengths known as absorption bands. If the absorbed photon contains more energy than is necessary for a simple electronic transition, the excess energy is usually converted into vibrational and rotational energy. However, if the photon does not have sufficient energy to promote a

transition, there is no absorption at all. In general, fluorescence measurements are usually performed using excitation wavelengths between 200 to 700 nm.

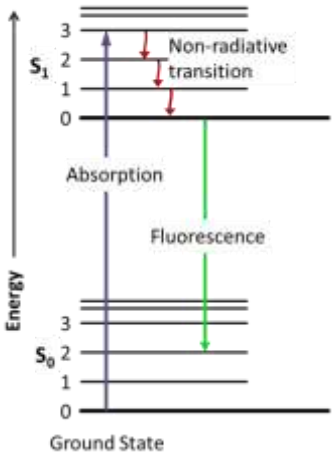


Figure 1. Jablonski diagram of photon absorption and the resulting fluorescence (Source: https://en.wikipedia.org/wiki/Fluorescence).

Ultraviolet or visible light excites molecules to higher vibrational levels of the first S_1 or second S_2 singlet energy state (blue line in Fig. 1). Following the absorption of a photon, several processes will occur. The most likely will be relaxation to the lowest vibrational energy level of the first excited state. This process is known as *internal conversion* or *vibrational relaxation* (loss of energy without emission of light, red lines in Fig. 1). Molecules virtually always undergo complete vibrational relaxation during their excited lifetimes and the excess vibrational energy is converted into heat, which is then absorbed by neighboring molecules.

In the lowest excited singlet state the excited molecule finally relaxes to the ground state (green line in Fig. 1). If this relaxation results to an emission of a photon, the process is known as *fluorescence*. As there are many closely spaced vibrational energy levels in the ground state, there also is a wide range of energies in the emitted photons. Because of this, fluorescence typically produces an emission intensity over a band of wavelengths instead of single sharp line.

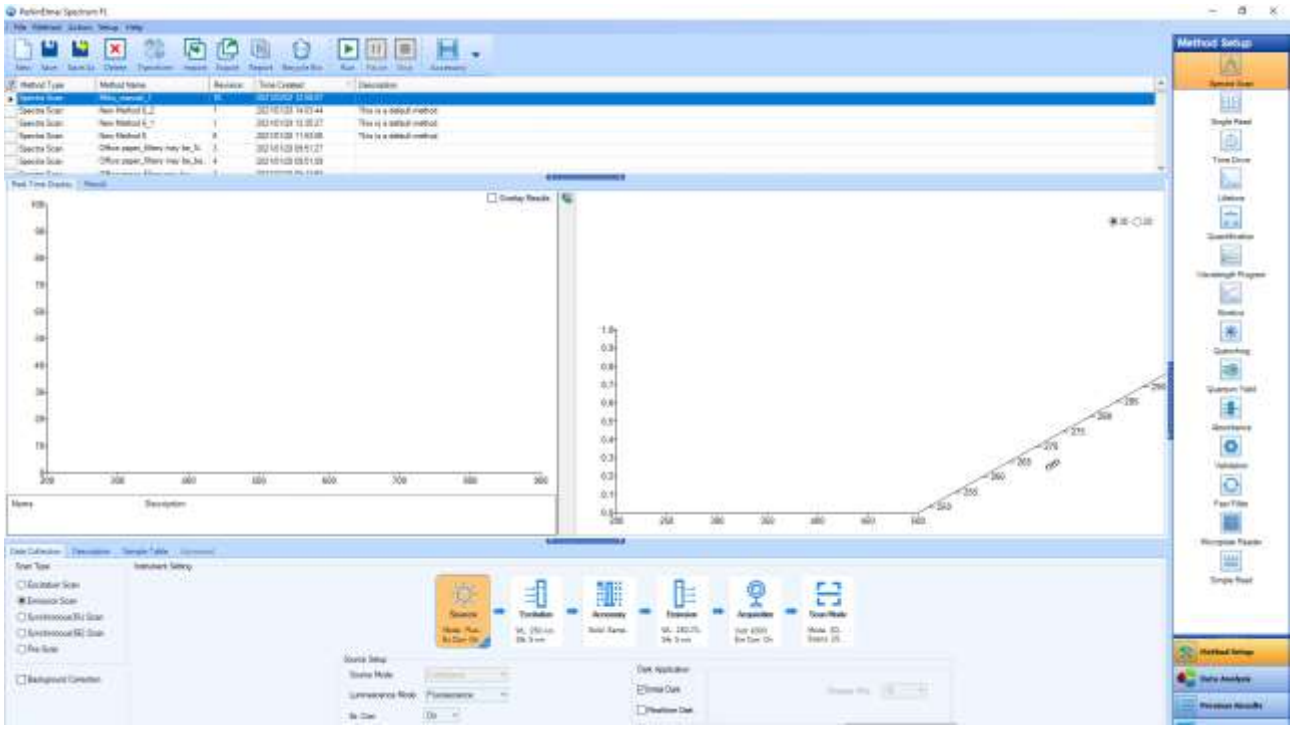
4. Measurements and tasks

In this work you must measure Donaldson matrices for five samples with different fluorescent coatings. Three samples are coated with one paint and two with two paints. There are three different paints in total. Your first task is to analyze the measurement results to find out which samples were coated with two paints and what those paint pairs are. The second task is to calculate what kind of emission spectrum is produced when one of the samples is illuminated by the CIE standard illuminant 1) A and 2) D65. The instructor tells you which of the samples you must use in the second task.

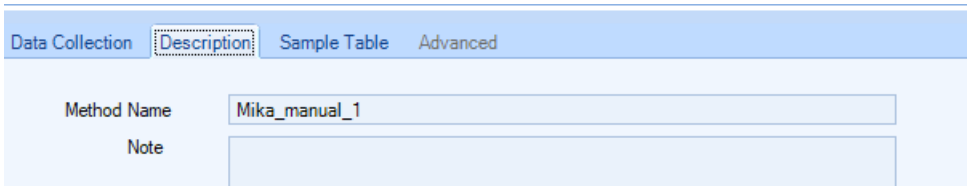
When you do measurements, you need suitable wavelength range and number of scans to get all necessary data, but you must also remember that the larger the range and the number of scans, more time your measurements will take. Follow instructions in the PerkinElmer FL 8500 manual during the measurements and finally export your data and take it with you in the USB flash drive given to you. You can then analyze your results with a suitable program (e.g., Matlab, Octave). Properly analyze the fluorescence in each sample and do the necessary calculations to get results you need for your tasks.

PerkinElmer FL 8500 manual

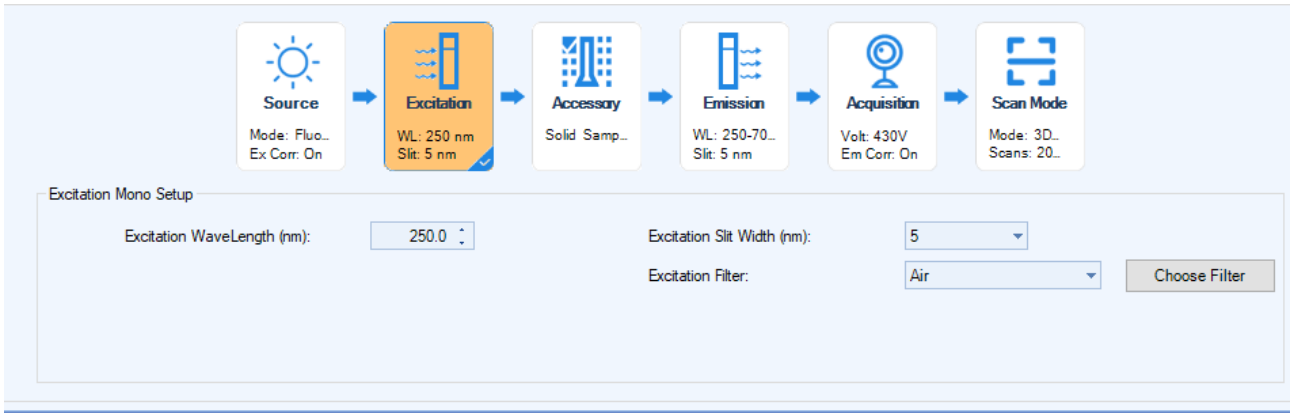
The control software shortcut is shown above. Possible notifications it gives as it starts can be ignored.



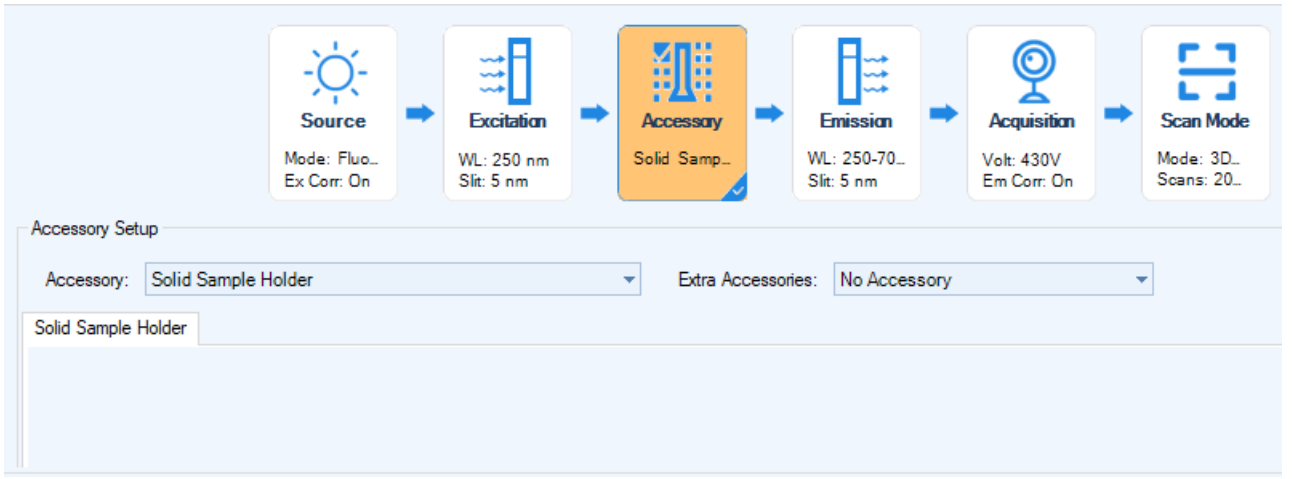
At the first screen, make sure that **Spectral Scan** is selected in the **Method Setup** in the right. In the left, it is possible to select the scan type and whether to use background correction. It is usually not necessary to change the **Source** settings unless they have been changed from above.



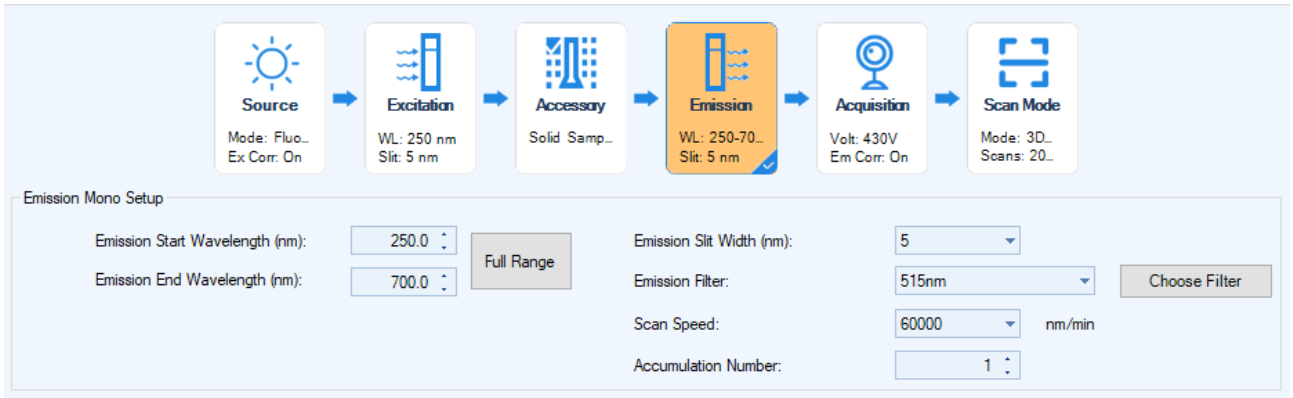
The measurement settings can be named at the **Description** tab. Do this before starting the measurements. Return then to the **Data Collection** tab.



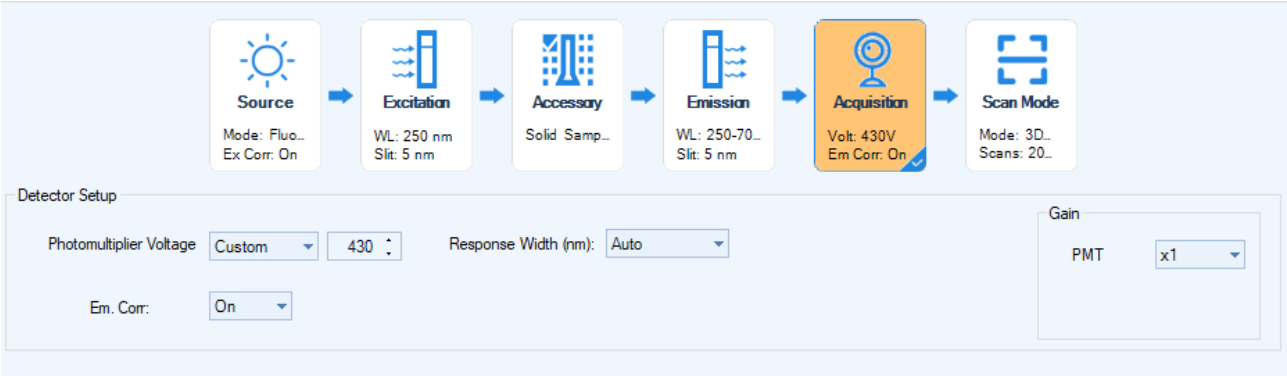
At the **Excitation** settings the excitation wavelength and the used filters can be selected.



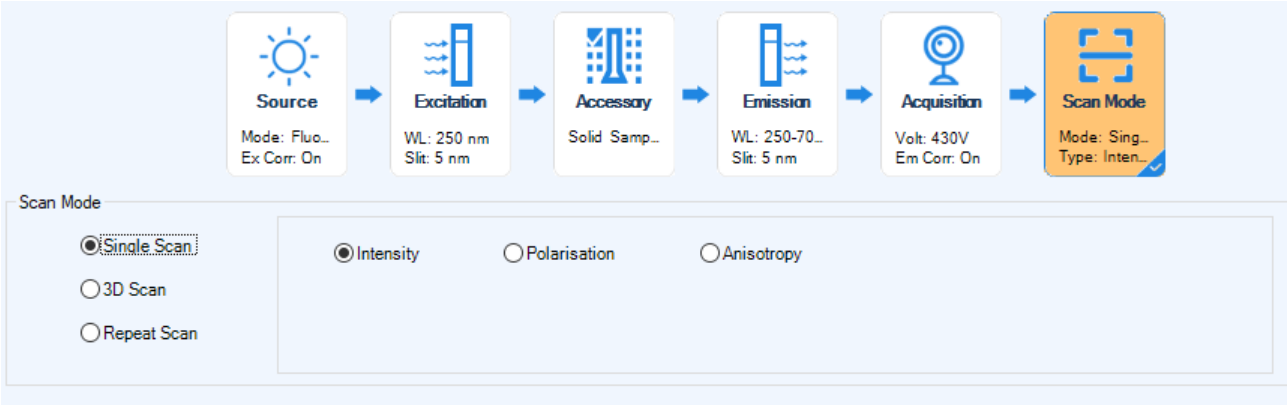
The **Accessory** settings should usually be left as they are above. These settings are changed only when an accessory other the solid sample holder is used.

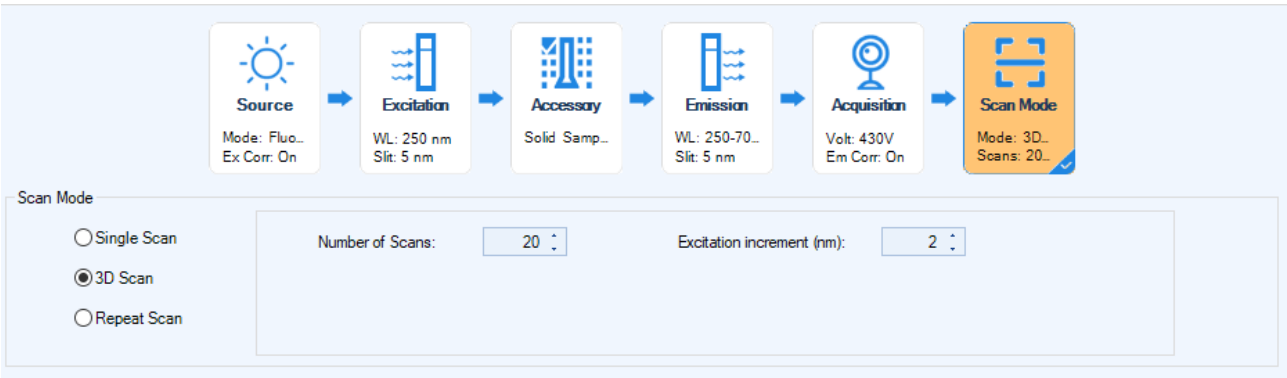


At the **Emission** settings there are several settings, of which the wavelength range for the measurements and the scan speed are the most important. Higher the scan speed, less time measurements take, but results also become less accurate. It is better to keep the **Emission Slit Width** same as the **Excitation Slit Width** at the **Excitation** settings.



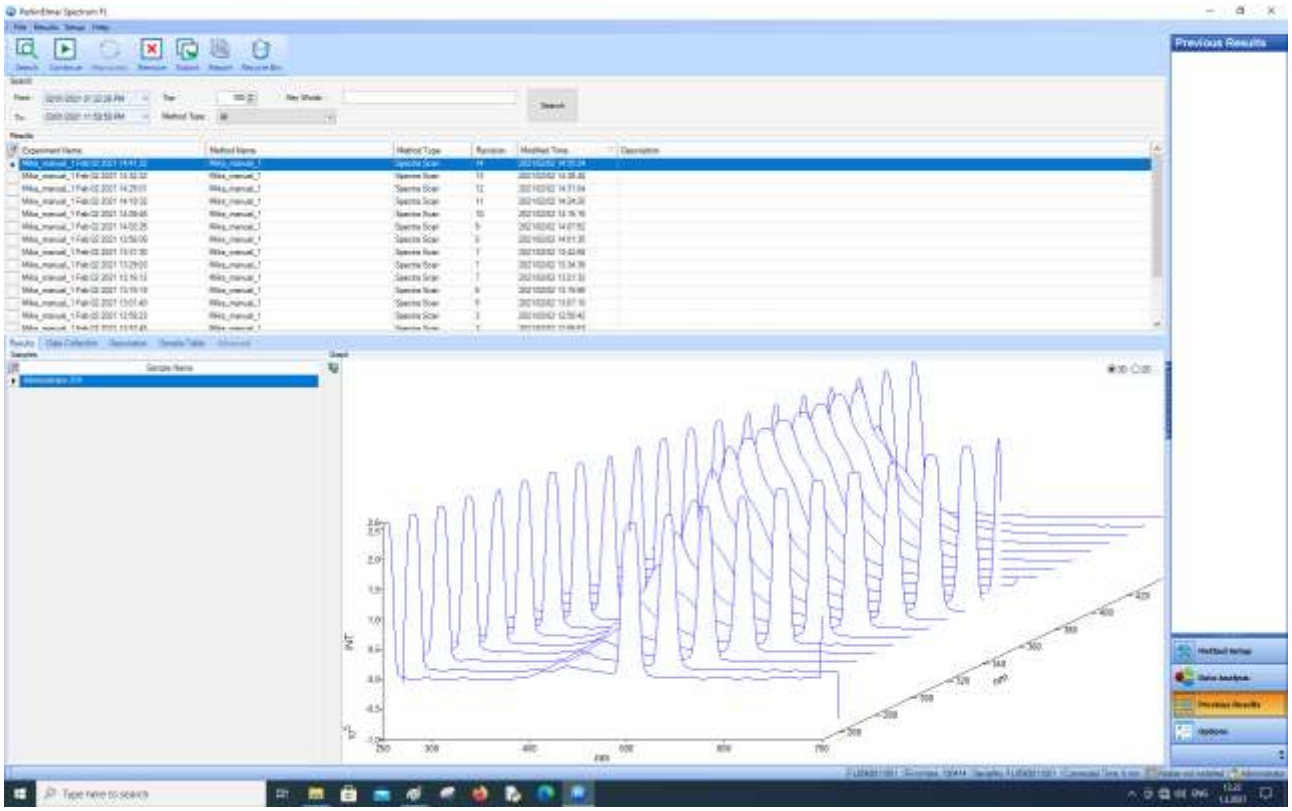
At the **Acquisition** settings it is possible to change photomultiplier voltage and gain PMT.



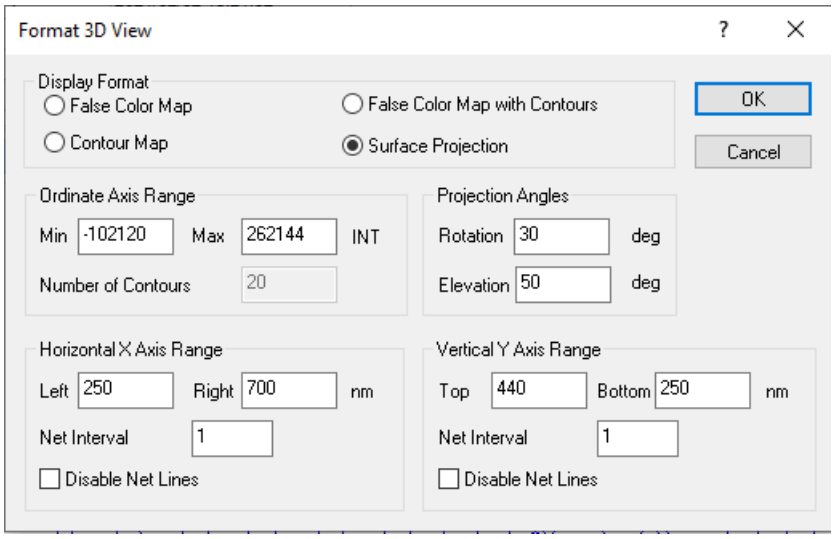


At the **Scan Mode** settings, a single, a 3D (Donaldson matrix), or a repeated scan can be selected. The single scan is much faster and can also be used to test the settings before 3D measurements as those will take a while. For the 3D scan, the number of scans and how much the value of the starting wavelength is increased per scan must be set. Before starting the measurements, save your settings from the **"Save"** button in the top bar. The measurement process starts from the **"Run"** button. If scan speed of 24000 nm/min is used to do about 50 scans, the whole measurement process may take about 15-20 minutes.

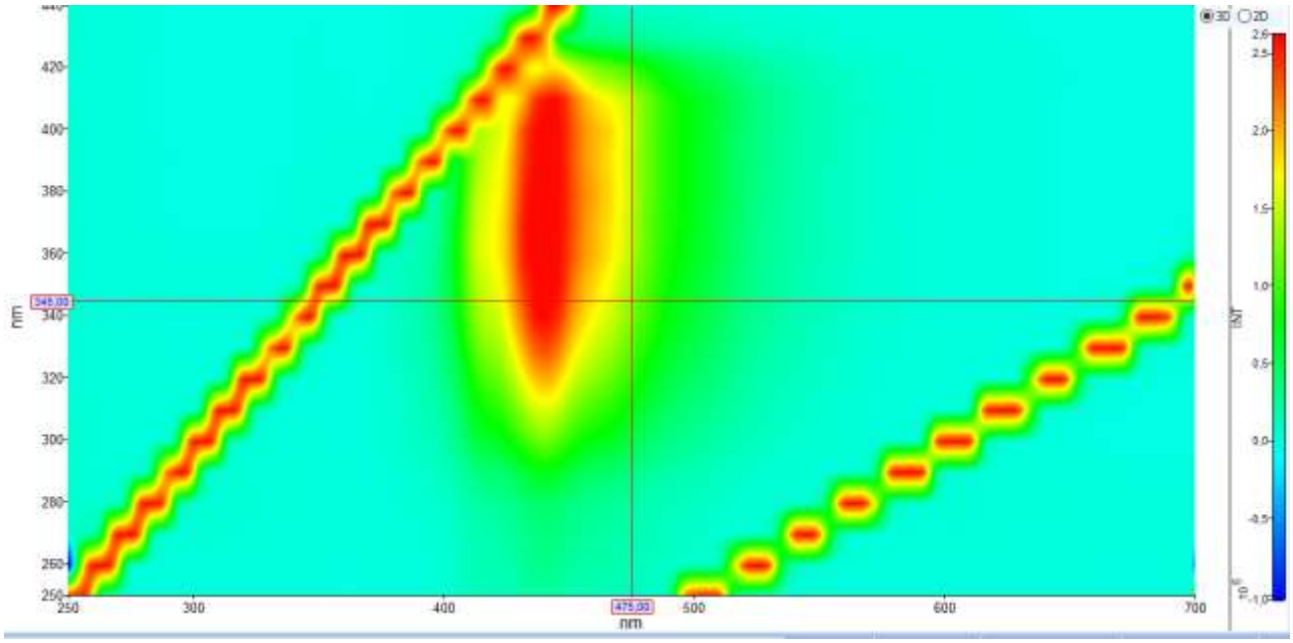
If background correction is selected, device needs to do some calibration without sample. When asked to put the sample in, open the green lid and set the sample as in the images above and tighten it in place with the screws. Try not to touch anything else inside the device and be careful, so you do not make the sample dirty when set it up. Close the lid and make sure also the hatch in it is also closed.

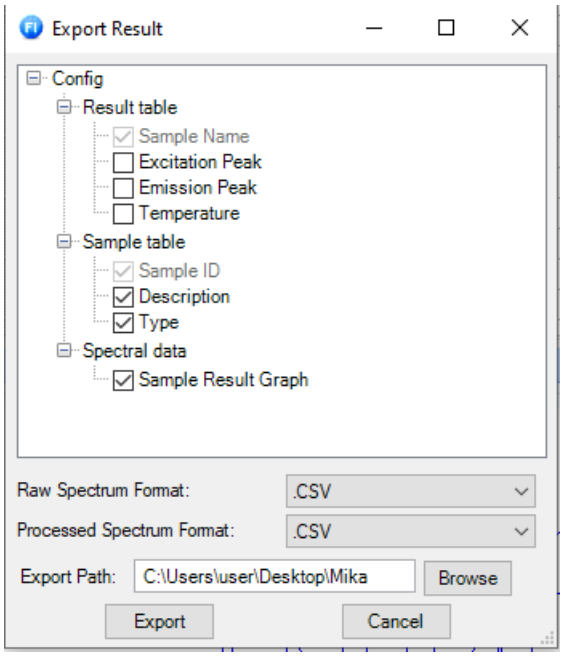


After measurements, the results can be seen in the **Previous Results** page, which can be accessed from the lower right corner. Select the measurement to check from the list and its results can be seen at the bottom image. Measurements can be continued in the **Method Setup** page.



By right clicking the 3D image and choosing **Properties**, it is possible to change the format between the **Surface Projection** (the spectra above) and **False Color Map** (image below).





The results can be exported by clicking **"Export"** button at the top bar. At the export window, ".CSV" must be chosen as the export format (it is not the default setting). Remember to choose a correct folder for your results and create new one if necessary.

After finishing your measurements, you can close the software, but do no turn off the device without permission as it needs some time to warm up before measurements.