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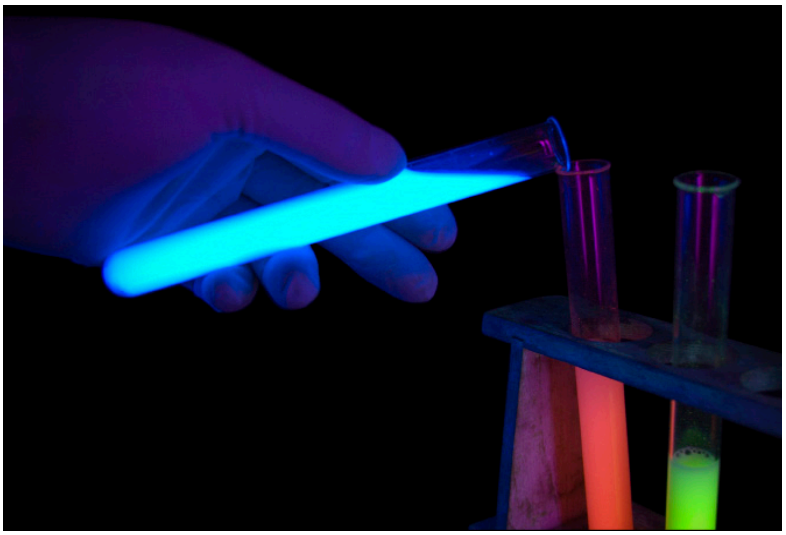
APPLICATION NOTE:
FLUORESCENCE SPECTROSCOPY

Techniques

- Fluorescence Spectroscopy

Keywords

- Fluorescence
- Florimeter
- High Resolution
- Environmental
- Water Monitoring



Introduction

Fluorimeters are widely utilized for the quantitation of biological compounds in solution, with applications ranging from environmental/agricultural water monitoring to DNA sequencing. These devices measure fluorescence by exciting a sample with an integrated light source, and collecting the emitted light using either filtered detectors or embedded spectrometers. Historically laboratory fluorimeters utilized spectrometers due to their high resolution and sensitivity. In contrast, portable and handheld fluorimeters were filter-based to reduce the unit's overall size and power consumption. Recent developments in spectrometer miniaturization, such as the AvaSpec-Mini from Avantes shown in figure 1, have significantly reduced the barriers to full-spectrum analysis, allowing for laboratory-grade measurements in the field. In this application note, we will explore the benefits of miniature spectrometers for portable fluorimeters by briefly reviewing the fundamentals of fluorescence spectroscopy with a particular emphasis on the impacts on fluorimeter design.

Fundamentals of Fluorescence Spectroscopy

Figure 1. Avaspec-Mini fiber-coupled miniature spectrometer, which only consumes 2.5W of power and weighs less than 175g

Since we have already released the application note "Exploration of Fluorescence Theory and System Design," we will not be discussing the fundamentals of fluorescence physics in detail. However, it is still helpful for us to briefly review the subject to understand the inner workings of modern fluorimeters. The most critical concept to understand about fluorescence is that it is a three-step process. First, the analyte must be excited with light at just the right wavelength to be absorbed by the molecule and excite it to a higher electronic energy level. The molecule must then non-radiatively decay within the excited state (from a higher vibrational energy level to a lower one) before decaying back down to the ground energy level. The energy lost due to the intraband decay causes the emitted light to have less energy and a shorter wavelength than the absorbed light. This process, known as a Stokes shift, is illustrated in figure 2, along with the accompanying Jablonski diagram.

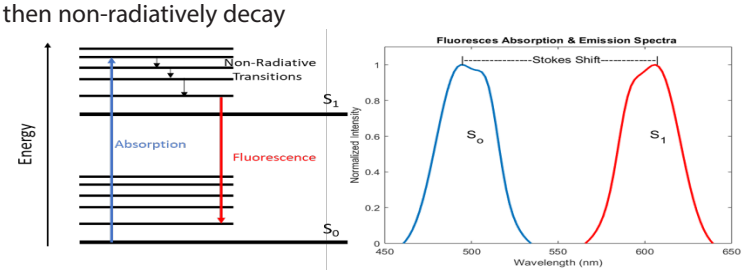


Figure 2. Example Jablonski diagram (left) and the fluoresces absorption/emission spectra (right) displaying the Stokes shift between the absorbed and emitted photons.

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Figure 3: Fluorescence spectra of Coumarin excited at 475nm, Fluorescein excited at 488nm, and Quinine Sulfate excited at 350nm using the AvaFlourimeter, with the spectral bandwidth of a typical fluorescence overlaid1.

Critical Fluorimeter Design

Since fluorescence excitation is wavelength dependent , it is critical to have a wide variety of excitation wavelengths matched to the excitation band of the various molecules of interest via an LED bank or a filtered broadband light source. For laboratory fluorimeters where size and power consumption are not issues, broadband light sources (such as deuterium or xenon lamps) with scanning monochromators or interchangeable filters are widely used. Whereas, in portable systems, individually addressable arrays of ultraviolet and visible LED are typically preferred. It is also critical that the detector (spectrometer) in the fluorimeter be able to differentiate between the various emission bands of the analytes of interest. As discussed above, in portable fluorimeters, this has historically been achieved through the use of filtered single element detectors, resulting in extremely coarse spectral resolution. This limits the system's ability to detect a wide range of molecular species and leads to quantization errors due to overlaps in the fluorescence spectrum from different molecules.

Figure 3 shows example spectra of three common fluorophores measured using an AvaFluorimeter accessory coupled to a AvaSpec-Mini2048CL spectrometer from Avantes. The AvaFluorimeter, shown in figure 4, contains the Avalight-XE-HP-Mini (miniature pulsed Xenon source) and a manually operated monochromator. Each of the spectra are overlaid with the spectral bandwidth of a corresponding filter1 used in a typical portable fluorimeter. As you can see, while these spectra are easily differentiable with the full spectrum, there is significant overlap in each of the filters. Therefore, making it extremely challenging to accurately quantitate the concentration of an analyte in a complex solution using a single filter . While it is possible to use ratioing and other more complex mathematics to improve quantization with multiple filter channels, it increases the system's size, complexity, and cost. Since a typical commercially available fluorescence filter costs approximately $300 per filter, multichannel filter fluorimeters rapidly approach and exceed a miniature spectrometer's cost. In addition, Avantes offers irradiance calibration for all of their spectrometers, which increase a fluorimeter's quantitative capabilities by eliminating unit to unit sensitivity variations and providing a direct spectral energy density readout.

Figure 4: AvaFluorimeter accessory with built-in Xenon

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Additional Spectrometer Consideration

Fluorescence emission is a relatively weak process when compared to other forms of atomic and molecular emissions. As a result, many laboratory fluorimeters utilize TE cooled spectrometers, such as the AvaSpec-Hero (AvaSpec-HSC1024X58-EVO) and the AvaSpec-ULS2048X64-HSC-EVO, to reduce the dark noise and allow for longer integration times. Unfortunately, the power consumption associated with TE cooling often makes this impractical for portable and handheld applications. Luckily, the broad spectral features, which make filters so problematic, are actually an advantage in spectrometer design. As a result, the spectral resolution is not as critical in fluorescence. This allows for the use of a large entrance slit, typically 200 microns, which increases the light collection and decreases integration time. Additionally, to further increase signal collection, a cylindrical detector lens is always recommended to take full advantage of the slit height, further increasing the signal strength.

In addition to the optical sensitivity, for any fluorimeter design, it is essential that the spectrometer allow for easy integration into the system as a whole. At Avantes, all of our spectrometers are available as either standalone units, OEM modules, or integrated multichannel rack mount systems, making them ideal for integration into benchtop, portable and inline fluorescence measurement applications. These units can communicate via USB, Ethernet, and the native digital & analog input/output capabilities the Avantes electronics board provides for a superior interface with other devices. Additionally, the Avantes AvaSpec DLL package, with sample programs in Delphi, Visual Basic, C#, C++, LabView, MatLab, Linux and MacOS APIs, enable users to develop their own code for fluorescence applications.

Contact Us for More Information

For more information about the full range of spectrometer options available from Avantes for fluorescence spectroscopy, please feel free to visit the website at www.avantesusa.com or give us a call at +1 (303)-410-8668 where our knowledgeable applications specialists are standing by to help.

References

- [1] All filter specifications and pricing are based on Semrock's filter selection guide found at https://www.semrock.com/filtersRefined.aspx.