HORIBA Scientific

A Double Monochromator on a Spectrofluorometer

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ELEMENTAL ANALYSIS
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OPTICAL COMPONENTS
CUSTOM SOLUTIONS
PARTICLE CHARACTERIZATION
RAMAN / AFM-RAMAN / TERS
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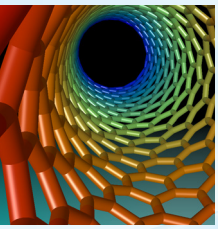
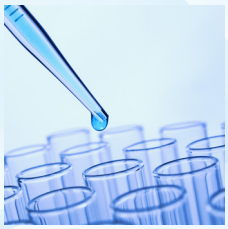
SPR IMAGING

The Importance of an Intermediate Slit on Stray Light

This HORIBA technical note demonstrates the importance of having an intermediate slit and the very important role it plays in the stray light rejection of a true double monochromator.

Introduction

The most compelling reason for using a double monochromator on a spectrofluorometer is to reduce the stray light level. Stray light usually refers to any radiation at wavelengths other than the selected wavelength, which may exit the monochromator. The higher the quality of the monochromator, the lower the amount of the stray light. A classic spectrofluorometer includes two monochromators, one on the illumination side to select the excitation wavelength, and a second one on the detection side to analyze the fluorescence emission.

In a fluorometer the presence of stray light may overlap with the fluorescence signal and, in the case of a very weak emission, may render the signal of interest totally obscured and undetectable. This effect can be particularly destructive for weakly emitting solids and powders, where the scatter is higher, as well as with highly scattering liquid samples, such as micellar or cell suspensions. The stray light exiting the excitation monochromator will be scattered and reflected by the sample towards the emission channel and indiscriminately detected together with the true fluorescence.

Stray light, in a spectrofluorometer, comes from the fact that no filtering element is perfect at its job. For example, every optical bandpass filter passes unwanted photons at all wavelengths, even outside of the center wavelength of the intended filter wavelength. This stray light is usually specified in a rejection of unwanted light expressed as 10^{-3} for example. A 340 nm bandpass filter with 10^{-3} stray light rejection passes mostly light at 340 nm, but it also passes light at all other wavelengths, at an intensity level that is 1,000 times less. In this case if you passed a white light source through the filter with an intensity of 10,000,000 counts per second at 340 nm, then this same filter is by definition also passing 10,000 counts per second at all other wavelengths (assuming an equal intensity output at all other wavelengths). A filter with better stray light rejection will reduce transmission of these unwanted wavelengths, but again no filter is perfect at its job.

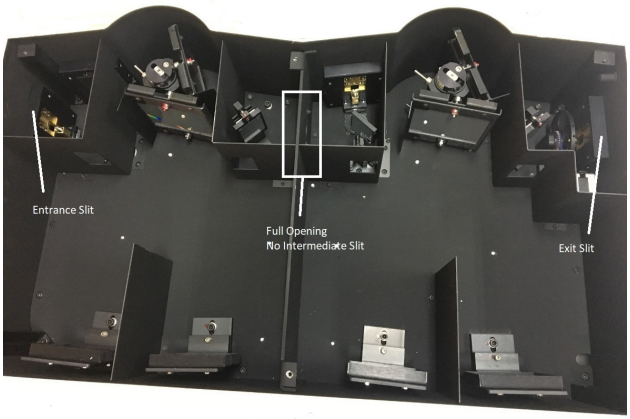
Modular research fluorometers use a scanning monochromator to provide excitation and emission spectra. These monochromators are also not perfect at rejecting stray light. If we look first at the excitation monochromator, most spectrofluorometers use a xenon arc lamp as the light source and this source is projected through an excitation monochromator to act as a tunable illuminator for light which is then directed to a sample. So if we have a monochromator that has 10^{-5} stray light rejection we could imagine that if we tuned the excitation monochromator to 340 nm, then we might have 10,000,000 photons per second at 340 nm that is directed to the sample, but we would also have light at all other wavelengths also directed to the sample on the order of about 100 photons per second (10,000,000 X 10^{-5}). All samples have varying degrees of scattering, with solids, powders and highly scattering solutions having the highest levels. Therefore, light at all wavelengths delivered to a scattering sample is scattering in all directions. If we then have the emission monochromator of the spectrofluorometer tuned to 500 nm, where the expected emission of the fluorescing sample is centered, we know that the excitation light source is delivering light at 500 nm to the sample and this light is happily passing through the emission monochromator and being detected as a “signal”. However, this signal is, in fact, not the fluorescence signal from the sample, but stray,

or unwanted, light that has found its way to the detector and interferes with our ability to measure the true intended signal. This is how stray light negatively impacts an experiment. And this phenomenon also works the other way.

The emission monochromator sitting at 500 nm is mostly passing 500 nm of light, but it is also passing all other wavelengths of light, including the intense excitation signal at 340 nm. So this process is also contributing to stray light.

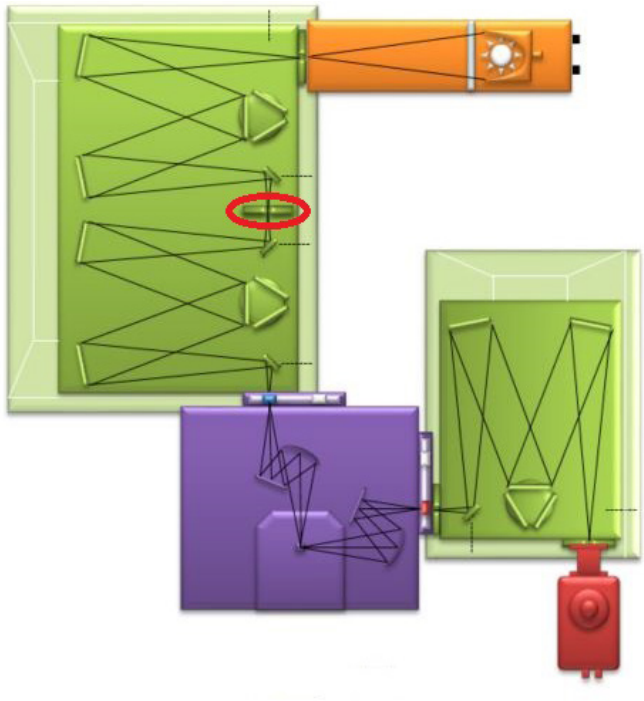
The overall limitation is that as concentrations of fluorescing compounds get lower, and also as some samples are highly scattering, these limitations of optics and physics are met, and the signal becomes lost. The best way to combat this phenomenon is to develop monochromators with better and better stray light rejection, and one of the best ways to achieve this is with a double monochromator. A double monochromator has two dispersive grating elements, one after the other, and it has the theoretical benefit of multiplying the stray light rejection specifications of a single monochromator. So two monochromators with 10^{-5} stray light rejection, designed and implemented in an additive dispersive mode to make a double monochromator, could have, in theory, a stray light rejection of 10^{-10}. A significant improvement in stray light rejection.

Below is a picture of a double monochromator that does not use an intermediate slit in between the two gratings of the double monochromator.



This is a dubious design, and usually is chosen because the two different stages of the monochromator do not track perfectly when the two gratings scan so that there is a characteristic saw toothed up and down artifact to spectral scans produced when a narrow intermediate slit is applied to such a monochromator.

Following is a schematic drawing of the Fluorolog-QM-75-21 model equipped with a double excitation monochromator and a single emission monochromator. The excitation monochromator shows the intermediate slit highlighted in a red ellipse.



The stray light rejection for a typical commercial single monochromator ranges from about 10^{-3} to 10^{-5} depending on the quality of gratings and the opto-mechanical design. The second monochromator in a double monochromator accepts the light exiting from the first monochromator and further reduces the stray light by the same factor of 10^{-3} to 10^{-5}. However, the amount of stray light leaking from the first monochromator will depend on the center slit setting. The broader the opening of the intermediate slit, the more stray light is admitted to the second monochromator. On the other hand, making the center slit narrower than the input slit will reduce the monochromator throughput. The optimum performance is achieved when all three slits are set to the same bandpass. In this scenario the stray light rejection of a well-designed double monochromator is expected to be a product of the stray light rejection of the individual monochromators. The lack of an intermediate (center) slit, or having the intermediate slit bandpass wider than that of the input and output slits will prevent the system from reaching its optimum stray light rejection. As this document will demonstrate, there is no way to achieve the theoretically expected stray light rejection of 10^{-10} without an intermediate slit.

In this publication, we demonstrate how the Rayleigh peak affects the fluorescence spectra at different intermediate slit settings as well as in the absence of an intermediate slit. As a test system we used the Fluorolog-QM-75-22 with double excitation and double emission high performance monochromators with effective focal length of 700 mm each.

Results

In order to illustrate the effect of the intermediate slit on the quality of an emission spectrum, we used phenylalanine, tyrosine (both emitting in UV) and fluorescein (VIS emission) solutions with a small amount of Ludox added in order to simulate a strongly scattering environment.

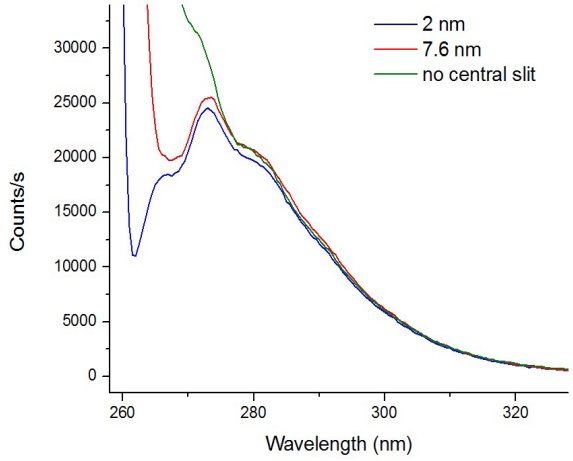


Figure 1: Effect of the intermediate slit setting in double excitation and emission monochromators on fluorescence of 7 x 10^{-4} M phenylalanine (PhA) in deionized H2O with 20 ul of Ludox added with 255 nm excitation wavelength. Entrance and exit slits set at 2 nm.

Scattered light is especially detrimental in the UV region. Figures 1 and 2 show the effect of opening the central slit in the excitation and emission monochromators on the PhA and Tyr fluorescence spectra. While with all three slits set to 2 nm, both spectra are well-resolved, opening the central slits to 7.6 nm leads to a significant spectral distortion and in the case of PhA the short wavelength peak is obliterated. With the central slits removed, practically all spectral information is lost for both samples.

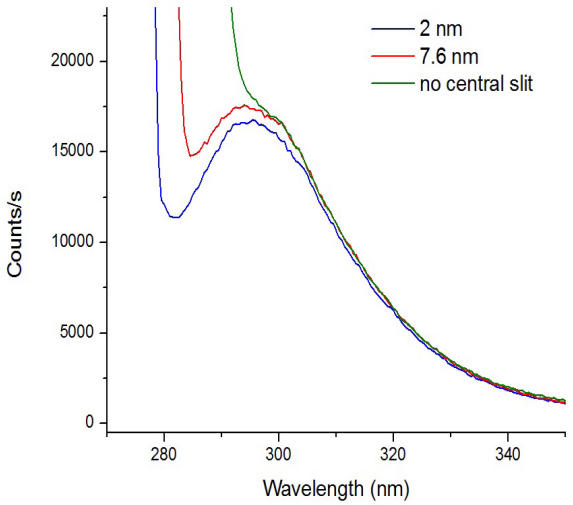


Figure 2: Effect of the central slit setting in double excitation and emission monochromators on fluorescence of 5 x 10^{-6} M tyrosine (Tyr) in deionized H2O with 20 ul of Ludox added using 272 nm excitation wavelength. Entrance and exit slits set at 2 nm.

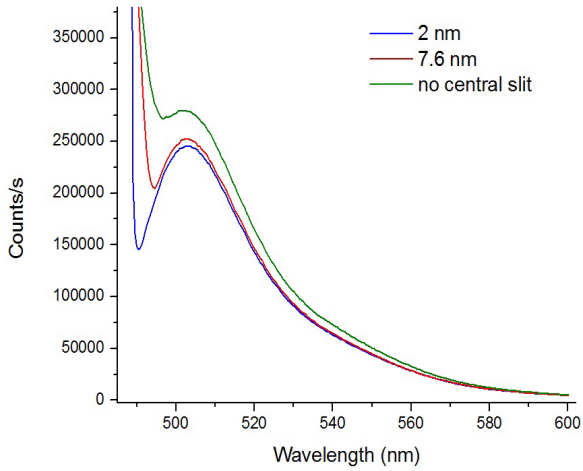


Figure 3: Effect of the central slit setting in double excitation and emission monochromators on fluorescence of 20 nM fluorescein (Fl) in 0.1N NaOH with 20 ul of Ludox added using 485 nm excitation wavelength. Entrance and exit slits set at 2 nm.

In the visible range, the light scattering is usually more forgiving, but for fluorophores with a small Stokes' shift the effect can be as bad as in the UV. Figure 3 illustrates the effect of the central slit settings on the fluorescence emission spectrum. The fluorescence spectrum is gradually degraded as the central slit is set wider and then totally removed.

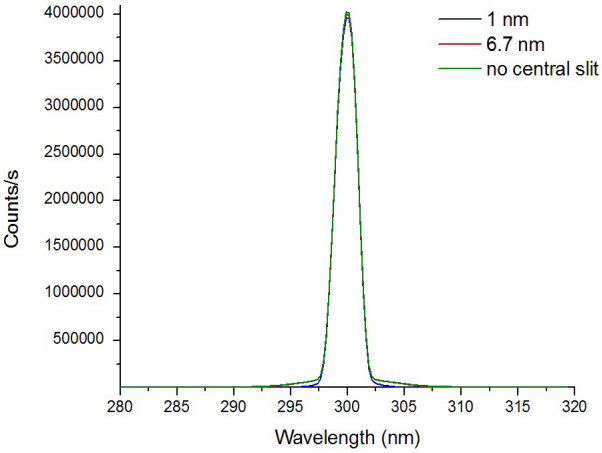


Figure 4: Effect of the central slit setting in double excitation and emission monochromators on the Rayleigh peak with 300 nm excitation and the entrance and exit slits set to 1 nm.

Looking at the Rayleigh peak shape dependence on the central slit, it is easy to understand the reason for the detrimental effect of either too broad or nonexistent center slit on fluorescence spectra. Figure 4 shows that the Rayleigh peak intensity and the FWHM do not change when the central slits are opened or removed. This is a clear indicator of the superior optical design of the Fluorolog-QM double monochromator. However, there is a significant increase in the tail intensity on both sides of the peak (Fig. 5). It illustrates that the lack of the central slit may lower the stray light performance of a double monochromator by orders of magnitude when compared

with the same optical design, which includes the central slit. In other words, a double monochromator without the central slit cannot reach its theoretical stray light rejection potential, which is the square of the single monochromator stray light rejection.

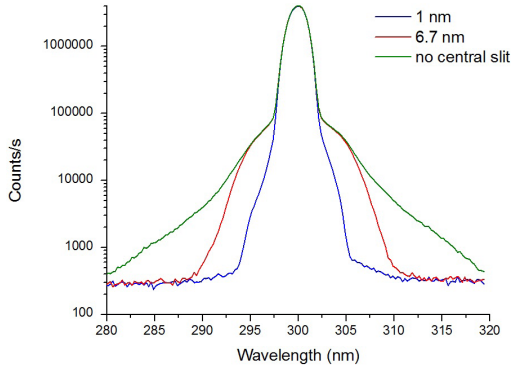


Figure 5: Expanded Rayleigh spectra from Fig. 4 illustrating the increasing short- and long wavelength Rayleigh tails as the central slit is widened and removed. Entrance and exit slits set at 1 nm.

Conclusion

The results presented here clearly indicate the importance of the center (intermediate) slit in a double monochromator. Research grade fluorescence instruments often include double monochromators in order to address difficult and challenging experiments, especially involving low intensity and strongly scattering samples. However, we have shown that a commercial double monochromator that does not utilize an intermediate (center) slit, cannot possibly achieve the stray light rejection theoretically predicted by the square of the stray light rejection of just a single monochromator.

HORIBA has been the leader in optical design and spectroscopy for decades and our research grade spectrofluorometers have always been offered with true double monochromators featuring center slits and providing verifiable optimum performance and maximum stray light rejection. Spectrofluorometer users are advised to always make sure that a center slit is present in a fluorometer featuring double monochromators, and that it is set to the same bandpass as the entrance and exit slits of the double monochromator. Otherwise the optical performance will be degraded, and this degradation can be significant as has been shown. The lack of the central slit can make it impossible to perform quantitative measurements on weak, scattering samples. It can also make fluorescence polarization measurements very difficult, as even small amount of scattered light will make polarization/anisotropy values unreliable.