OMEGA OPTICAL

Custom Filters from Prototype to Production

TECHNICAL NOTE

Optical Density + Flow Cytometry

OD Requirements for Flow Cytometry

What is this OD specification anyway? Optical Density is a measure of how much the light is blocked at a given wavelength. It is the log of the transmittance, or

$$OD = 2 - \log(\%T)$$

OD values are ADDITIVE (Figure 1). Using a dichroic with OD 2.5 blocking at 405 combined with an emission filter with OD 5-6 blocking at 405 will add to give OD 8 blocking at 405 nm as shown in Figure 1 below. Note that OD values above 6 or so are very difficult to measure on a standard spectrometer. Actual blocking may be much higher than the measured values as shown in the right panel below.

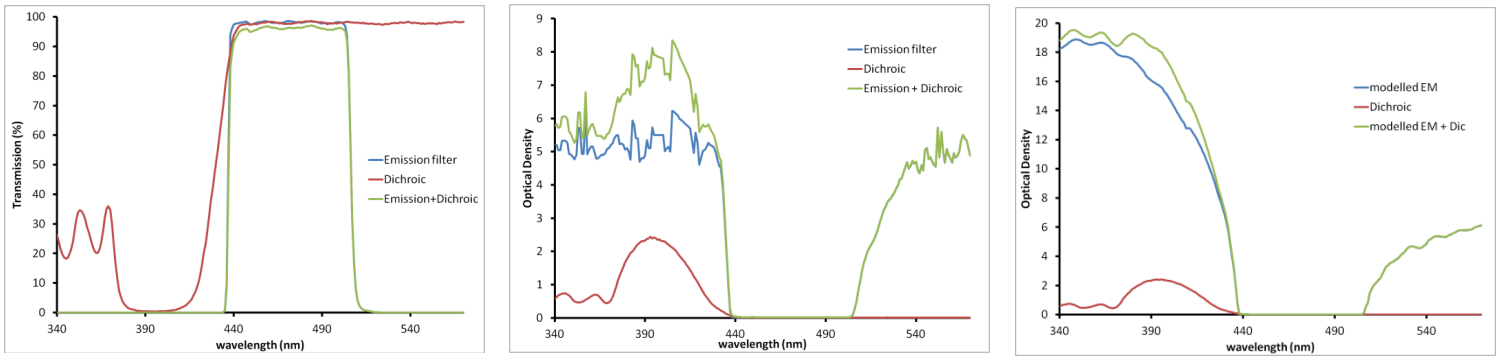

Figure1. Emission and Dichroic filters displayed in Transmission (left) and OD (right two panels). These measurements are related by a log transformation. As %T values get very low, OD values get high. OD is an additive property (green curve on the right). The right panel shows the modeled performance of the emission filter, illustrating the much higher OD that is likely present, especially at lower wavelengths.

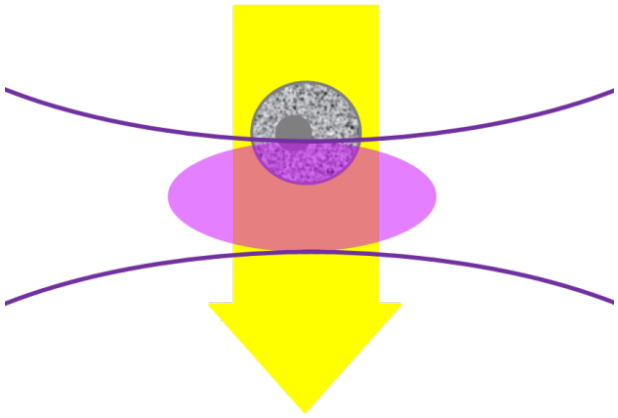

Figure 2. Illustration of the focused laser beam (lavender oval) interacting with the sample in the flow cytometer. The grey cell is moving down through the laser focus.

In this technical note, we will go through an example of how to determine the blocking level needed in a typical flow-cytometer system. Flow cytometers are designed to detect cells and particles in the 1-15 mm range, typically using laser light scatter and fluorescence. The samples pass single file through the detection system as illustrated in Figure 2.

A common flow cytometry laser is the 405 nm laser. At a linear flow rate of 1 m/s (often found in cell counting systems), any point in the cell or particle will interact with the illuminated volume (lavender area above) for approximately 10 ms.

Using 100mW of power, one can easily calculate the number of photons interacting with a fluorophore in the she sample using the following equation:

$$\#photons = \frac{\lambda P t}{hc}$$

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Where power (P) is in Watts, time (t) is in seconds, and wavelength (λ) is in meters. Planck's constant (h) is 6.626x10-34 Js and the speed of light (c) is 3x108 m/s . For the sake of simplicity, we will assume that all of these photons are hitting the fluorophore the whole time.*

There are ~2*1011 photons in the beam focus during the 10 µs transit time. Some fraction of the light will pass through the sample, some will be elastically scattered (Mie and Raleigh scattering off of organelles), some will be inelastically scattered (Raman scattering), some will be absorbed. The absorbed light can be lost to photochemistry or heat, or can be re-emitted in the form of fluorescence which is detected, usually at a right angle to both the laser beam and the direction of travel of the sample particles. In this case, in or out of the plane of the paper.

The worst case scenario would be trying to detect a single photon of fluorescence while trying to simultaneously block the laser beam. In this case, you would need to block 2*1011 photons at 405 nm while simultaneously detecting your fluorescence signal. So, does this mean you need OD 11 blocking in your emission filter?

Not necessarily. As described above, the OD values are additive, so as long as the combined blocking of the dichroic and emission filter add up to OD 11, there will be enough blocking. But that's not the whole story. The amount of scattered light is usually much less than that of a raw laser beam, so less blocking can be used in practice. For instance, if 1% of the light is scattered, the blocking requirement for detecting a single fluorescence photon in the presence of this scattered signal is OD9. But, is it practical to discuss detection of a single photon?

Probably not. Using a typical fluorescence lifetime of 10 ns for a fluorescent probe, a single fluorophore can absorb light (or be excited) 1000 times in the 10 µs transit time. The quantum yield (Φ)of the fluorophore describes how many of the absorbed photons are re-emitted as fluorescence photons that can be detected by the system. It ranges from > 0.9 for FITC (one of the highest) to 0.2 for tryptophan. Using a middle value of 0.5, means 500 photons will be emitted from a single fluorophore. This brings the blocking requirement to OD7. Chances are, each cell, protein or particle will have a number of fluorphores attached, so the number of emitted photons will be even higher, reducing the blocking requirement even more.

One may need to increase the blocking OD if other light in the system is not well-controlled. For instance, the enclosure is not light-tight, there are reflections in the optical system that are not controlled (anti-reflection coatings can help with this, as well as baffles and absorbing materials). Oftentimes though, OD is over-specified, leading to increased coating complexity and cost. If you take the time to account for all the photons in your system, you can maximize signal and minimize filter cost.

*A more thorough approach would include the photon density within the focal volume of the sample

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