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White Paper

Lasers versus LEDs for Bioinstrumentation

Laser Advantage Note No.2 - Spectral Brightness

A laser is a monochromatic source that can yield a clean fluorescence signal with very high signal-to-noise ratio. In contrast, the LED is a broadband source that requires the cost and complexity of extra filters, and presents significant cross-talk challenges when detecting multiple fluorochromes.

Light Sources for Bioinstrumentation

In any instrument based on the detection of fluorescence and/or light scatter, the key to successful operation is the ability to maximize signal-to-noise ratio. This, in turn, is accomplished by delivering the necessary amount of useable excitation light (that is, light that actually excites the fluorescent probe), while minimizing the amount of wasted light that is of the wrong wavelength or in the wrong spatial location. There are several inherent characteristics of the laser that make it a much more efficient source for accomplishing this task than the LED, resulting in *lower instrument costs and superior performance (speed and sensitivity).* Here we examine the role of spectral brightness and its impact on signal to noise (i.e., instrument sensitivity and speed) and optical complexity (i.e., system cost).

Exciting and Detecting Fluorochromes

Fluorescence detection is widely used in bioinstrumentation because it can be quantitative and very sensitive, with high signal-to-noise ratio (SNR). It is also incredibly versatile because of the wide and ever growing range of specialty fluorescent dyes and markers, referred to as fluorophores or fluorochromes. These include stains that preferentially bind to different tissues and sub-cellular structures, indicators whose emission varies with chemical conditions (e.g., Ca^{2+} concentration, pH, membrane potential), fluorescently labeled antibodies for highly specific labeling of cell surface proteins, and genetically modified proteins (e.g., GFP, the m-fruit series), etc.

Whether it's a simple dye or a genetically modified protein, a fluorochrome used in life sciences has a broad absorption (i.e., excitation) spectrum and a broad emission spectrum. When irradiated with light anywhere in the absorption spectrum, part of the light will be absorbed and a very small part of this absorbed light is re-emitted at a longer (Stokes shifted) wavelength. Although usually only a small fraction of the source light eventually ends up as fluorescence, every instrument builder and user knows that *the quality of the data is in the signal-to-noise ratio (S/N)*, not the absolute signal intensity. And this Stokes shift is the key factor that enables the high signal-to-noise capability of fluorescent detection, using simple wavelength discrimination.

On the following page, Figure 1 explains how this works. The absorption and emission spectra of a typical fluorochrome are shown schematically but not to scale; as just noted, the emission spectrum is much weaker. The optics of the instrument are therefore arranged so that none of the excitation light falls on the photodetector, usually by placing the emission collection optics at right angles to the direction of the excitation light. However, there will still be some inevitable light scatter in the instrument that will find its way to the photodetector, and definitely some light scatter by the sample (which is usually quite inhomogeneous). In fact, this sample scatter is used in some cytometry instruments to sort cells by size and/or shape. But, in all other cases, any scatter is unwanted background signal. And, like any signal, it has random (shot) noise associated with it. In a worst case scenario, this background noise can be comparable to the fluorescence signal, potentially compromising its measurement.

Lasers deliver a focused intensity over 100X brighter than a LED – read Laser Advantage No.1 to learn more.

Lasers like BioRay are designed for bioinstrumentation, LEDs are designed for lighting and display – read the final of our three Tech Notes - Laser Advantage No.3 (coming at the end of October) - to learn more.

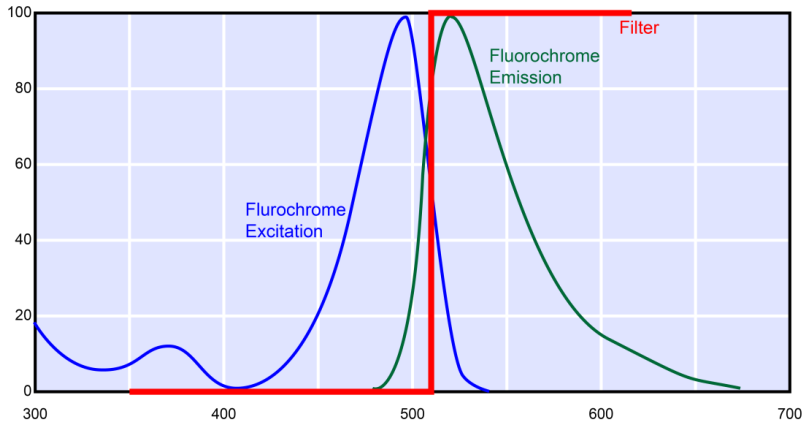

Figure 1. *A typical fluorochrome has a broad absorption spectrum and a broad emission spectrum that is at a longer wavelength, i.e., Stokes shifted. A longpass cut-off filter makes sure that the photodetector efficiently detects fluorescence but is blind to any scattered light at shorter wavelengths an LED has a broad*

Fortunately, because the fluorescence is Stokes shifted, a simple longpass cut-off filter can boost the signal to noise ratio by several orders of magnitude. Specifically, it is chosen to block nearly all of the excitation light and transmit the majority of the fluorescence emission. Even off-the-shelf, low-cost filters made of colored glass are capable of extinction ratios as high as 10^X.

However, the need to enhance signal to noise via this spectral discrimination has a significant impact on the choice of light source – laser or LED.

Laser – a Monochromatic Source

With the exception of specialty examples such as ultrafast lasers, most laser types are extremely monochromatic; simply stated they emit light at a single wavelength. This high spectral brightness is orders of magnitude higher than any LED, and indeed is unmatched by virtually any non-laser source.

This monochromaticity greatly simplifies their use in any fluorescence-based detection scheme, such as in cytometry or genetic sequencing. To detect a specific fluorochrome, all that is required is that the laser wavelength is chosen to be somewhere near the peak of the excitation spectrum. Simple filtering of the Stokes shifted fluorescence, as just described, results in very high S/N data. Moreover, smart lasers specifically optimized for life science applications, such as the Coherent OBIS™ and BioRay™ series, provide off-the-shelf availability of an ever growing list of wavelengths: currently these lasers are offered at over 20 different wavelengths between 355 nm and 785 nm (see table 1).

Model Name	Wavelength(nm)
OBIS 355 LG	355
OBIS 375 LX	375
OBIS 405 LX	405
OBIS 422LX	422
OBIS 445 LX	445
OBIS 458 LX	458
OBIS 473LX	473
OBIS 488 LS	488
OBIS 488 LX	488
OBIS 505 LX	505
OBIS 514 LS	514
OBIS 514 LX	514
OBIS 520 LX	520
OBIS 532 LS	532
OBIS 552 LS	552
OBIS 561 LS	561
OBIS 637 LX	637
OBIS 640 LX	640
OBIS 647 LX	647
OBIS 660 LX	660
OBIS 685 LX	685
OBIS 730 LX	730
OBIS 785 LX	785

This range of closely spaced standard wavelengths provides two advantages in life science applications. First, there is virtually always a laser that matches the absorption peak of even the latest fluorochromes. And, matching the laser to the peak of the absorption spectrum maximizes the fluorescence intensity, and, hence, the S/N of the data. Second, this dense comb of available wavelengths provides even more of an advantage in applications that simultaneously excite and measure several different fluorochromes, as is necessary in some cytometry and DNA sequencing instruments, for example. Here, lasers are chosen to be close to the excitation peaks of each of the target fluorochromes. The signal from the different fluorochromes can then be readily distinguished by ratio-ing the signal from multiple detectors, each with a different longpass or bandpass filter, and, if necessary, by pulsing the lasers and correlating the signal timing.

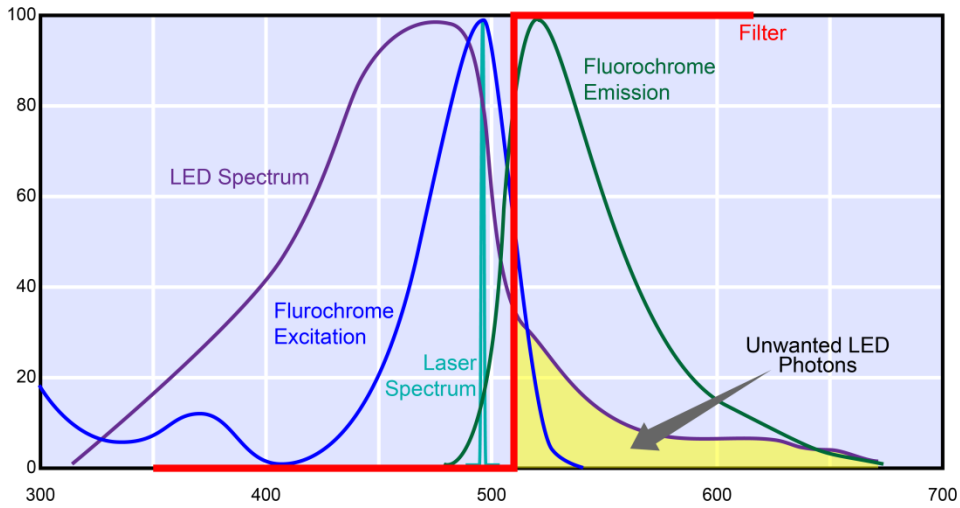

Figure 2. *A laser is a monochromatic source whose wavelength is chosen to be near the absorption maximum of the target fluorochrome. In contrast, an LED has a broad emission spectrum that includes a long tail that usually overlaps with the emission spectrum. These "unwanted" photons complicate the separation of signal and scatter from the source that is key to high SNR in fluorescence detection schemes.*

LED – A Broadband Source

The LED has a broad output spectrum and the shape and center of this spectrum can shift with changes in operating conditions, e.g., operating temperature, operating current, pulsed operation versus continuous wave (CW) operation. This causes several problems. First, the long wavelength part of the LED emission overlaps with the fluorescence emission spectrum potentially compromising the ability to separate fluorescence from source scatter, using the Stokes shift and filtering (see figure 2). To minimize this problem, a shortpass filter must also be included between the LED and the interaction zone to block the long wavelength tail of the LED output – its "bad photons." The end result is an increase in system cost, due to the extra optic, and a reduction in excitation efficiency because some of the LED's output is blocked by this optic.

The situation is even worse when multiple fluorochromes are being interrogated, as in many flow cytometers. This configuration can easily be handled

using multiple lasers as previously described, but, with LEDs, the situation is more complex and messy. Extra front-end filters have to be carefully matched to both the excitation/emission spectra of the fluorochromes and the output spectrum of each of the LED(s), lowering optical efficiency still further.

In addition to this trade-off between signal and cross-talk, any variations in the LED emission spectrum generally requires changes in filter specifications. These variations can be significant between different batches of LEDs, *even of the same nominal product*, and are beyond the control of the instrument manufacturer and even their LED middleman vendor. The only way to ensure the ability to maintain and service an instrument product line to deliver consistent performance over its life, is to purchase a complete reel

of LEDs to cover the anticipated product lifetime, which represents an unwanted investment in inventory. The alternative is to risk having to tweak the filter specifications every time replacement LEDs are required.

Summary

Laser. With a laser, all the light is at a single wavelength. And, with many laser wavelengths to choose from (a) the laser can be matched to the peak absorption of the fluorochrome, maximizing signal, and (b) a low cost longpass filter will completely eliminate any laser scatter, enabling very high signal to noise.

LED. With a LED, the output light is spread over a very broad spectrum with a long wavelength "tail." This means reduces system efficiency, compared to a laser, and means that additional filters must be used so that scattered unwanted photons are separated from the signal and don't reach the photodetector. This becomes even more complex where multiple fluorochromes are targeted.

Result. Even with its higher component cost, a laser enables higher signal, higher S/N and reduced optical complexity. Together with other laser advantages, the end result is that the laser enables a superior instrument with lower true overall costs per measurement.

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