

Bioinstrumentation Light Sources – Lasers or LEDs?

A comprehensive analysis of all the factors involved in designing and building life sciences instrumentation reveals that lasers provide superior performance compared to LEDs, and an overall cost advantage.

Summary: Higher Performance and Lower Cost

Biotech instrumentation manufacturers are currently developing products that feature increased miniaturization and lower costs per use to support worldwide trends in healthcare. For instruments based on fluorescence, this has opened a debate as to whether the laser or LED represents the best choice in light source. While the superior performance of lasers is well-known, it may not be obvious that lasers nearly always also represent a lower cost option.

True Cost

Many instruments that analyze biochemical samples and reactions utilize fluorescent probes. Examples include cytometry, genetic sequencing, hematology, polymerase chain reaction (PCR), high-throughput drug screening, and microarray scanners.

The key to successful operation in all these instruments is the ability to maximize signal-to-noise ratio. This is accomplished by delivering the necessary amount of useable excitation light (light that actually excites the fluorescent probe) into the required illumination sample region, while minimizing the amount of light that ends up focused or scattered outside of this region. For current commercial instruments, these focused spot sizes can range from tens of microns in diameter, to several millimeters in size.

Application	Focused Spot Size
Gene Sequencing/ Medical Diagnostics	1 to 10 x 1 to 10mm
Medical Diagnostics	100's x 100's μ m
Cytometry	30 x 30 μ m

In general, the optical characteristics of the laser make it a much more efficient source for accomplishing this

task than the LED, especially at smaller focused spot sizes. Although the LED source itself might be much less costly than a laser, all the ancillary measures that must be employed to correct for its inherent output deficiencies can end up making the total system cost greater than for a laser-based instrument. The following sections detail why this is the case.

Output Power vs. Rated Power

One important difference between lasers and LEDs is not in how they operate, but in how their output is traditionally specified. Lasers, originally developed by the photonics community, have always been specified by their output power. Simply stated, a 1 Watt laser delivers 1 Watt of light *output*.

In contrast, LEDs are specified by their power *consumption*. Since even the best LEDs rarely surpass 10% efficiency, a 1 Watt LED usually outputs 100 milliwatts or less of light. Moreover, the maximum current and voltage specifications are often specified for pulsed operation. Thus, a 1 Watt LED might only be continuously operable at 750 milliwatts or less, which translates into an optical output of only 75 milliwatts!

Light Throughput: Advantages of a True Point Source

The most significant inherent difference between lasers and LEDs is in the area of light collection and focusing efficiency. The output of a laser is a well-behaved, narrow, low-divergence beam. Such a beam behaves like an ideal, true point source, meaning that virtually all the light can be collected and focused into the precise spot size needed by the application. Similarly, a laser beam can be very efficiently coupled into a single-mode fiber (whose output can be focused to a small, well behaved spot).

On the other hand, a LED is a conventional, extended light source, like a light bulb. The ability to focus an extended source is defined by the size of the emitting surface area and the solid angle into which it radiates in a property called etendue. The important thing about etendue is that, in the best case scenario, it is

preserved across an optical system (and in a real world optical system, it always gets a bit worse). For a perfect, Lambertian source:

$$Etendue = \pi \times \text{emitting area} \times (NA)^2$$

Where the numerical aperture (NA) is the smaller of either the source's emission angle (i.e., source NA) or acceptance angle of the collecting optics (i.e., lens NA). Since real LEDs are never perfect Lambertian sources, this equation represents a theoretical best case.

To see the impact of etendue, consider the case of a 100 milliwatt LED that has an active area of 1 mm² and which emits into a hemisphere (a solid angle of 2π steradians corresponding to a NA=1). If we wish to focus this output into a spot diameter of 100 μm. The etendue equation tells us:

$$\text{Collecting etendue} = \text{Focusing etendue}$$

$$\pi \times 1 \text{ mm}^2 \times \text{Collecting NA}^2 = \pi \times (.1)^2 \text{ mm}^2 \times \text{Focusing NA}^2$$

$$\text{Collecting NA} = .1 \times \text{Focusing NA}$$

The highest theoretically possible NA on the focusing side is a value of 1. Therefore, the *theoretical* maximum for this scenario is a collecting NA of 0.1. This means that about 6% (6 milliwatts) of the LED

output is focused into the desired 100 μm spot (using $NA \cong n \times \sin \theta$).

Still, the NA=1 value for the focusing optics is not practically realizable. Even a value of NA=0.3 would represent a fairly sophisticated and costly optical system. In this case, less than about 2% (2 milliwatts) of the LED output is contained in the 100 μm focused spot.

Due to this phenomenon, it's usually not practical to concentrate even 1% of the output of a typical LED into the focused spot in an instrument. The etendue tradeoff for extended sources, such as LEDs, means that collecting more light means a bigger focused spot, or substantially more complex and costly focusing optics, or both.

This problem can't be avoided by combining multiple LEDs; that increases source size even further, making the problem even worse. What would help is brighter LEDs which produce greater light output without an increase in emitter size. At present, typical LED brightness is increasing by only 10% per year in response to the marketplace. In addition, since most LEDs are used in illumination or display applications that are orders of magnitude greater than the bio-instrumentation market, biotech has no leverage whatsoever to change this situation.

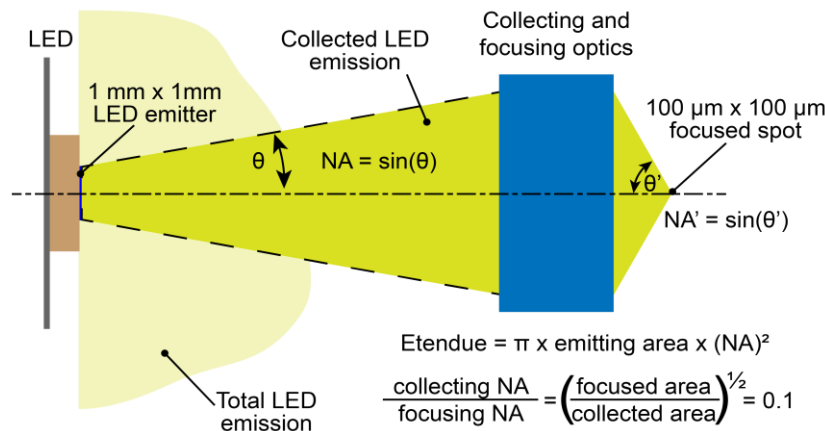


Figure 1

Etendue dictates that if a source is imaged at a magnification of 0.1X, then the collection NA must be 0.1 times the focusing NA. Since the highest possible focusing NA is 1.0, this severely limits the ability to collect all the output from the source LED.

Monochromatic Laser vs. Broadband LED

Another major difference between lasers and LEDs is that the laser is a monochromatic source (it only emits at one wavelength), whereas a LED emits over a much wider spectral range. Why does this matter? Whether it's a simple dye or a genetically modified protein, a fluorophore used in life sciences has a broad absorption (i.e., excitation) spectrum. When irradiated with light anywhere in this spectrum, part of the light will be absorbed and a very small part of this absorbed light is re-emitted as fluorescence at a longer wavelength. With a laser, all that is required is that the laser wavelength is chosen to be somewhere near the excitation maximum. As indicated in the figure, a longpass filter between the interaction zone and the photodetector effectively blocks all scattered laser light and ensures that the photodetector only "sees" the fluorescence, despite the fact that it is far dimmer than the laser excitation. Thus, with a laser, it is very easy to get a high signal-to-noise ratio in the detection system.

With a LED, the output has a broad 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. And, the intensity of this scattered light can

exceed the intensity of fluorescence, which can easily be as weak as 1/1000 of the absorbed light or less. 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 signal-to-noise ratio because some of the LED's output is blocked by this optic.

The situation is even worse when multiple fluorophores are being interrogated, as in most flow cytometers. With lasers, the instrument only needs to incorporate laser wavelengths that are close to the excitation peaks of the fluorophores. The signals from the different fluorophores can be readily distinguished by ratioing the signal from multiple detectors, each with a different longpass filter and, if necessary, by pulsing of the lasers and correlation of the signal timing. 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 fluorophores and the output spectrum 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 are beyond the control of the instrument manufacturer and even their LED vendor.

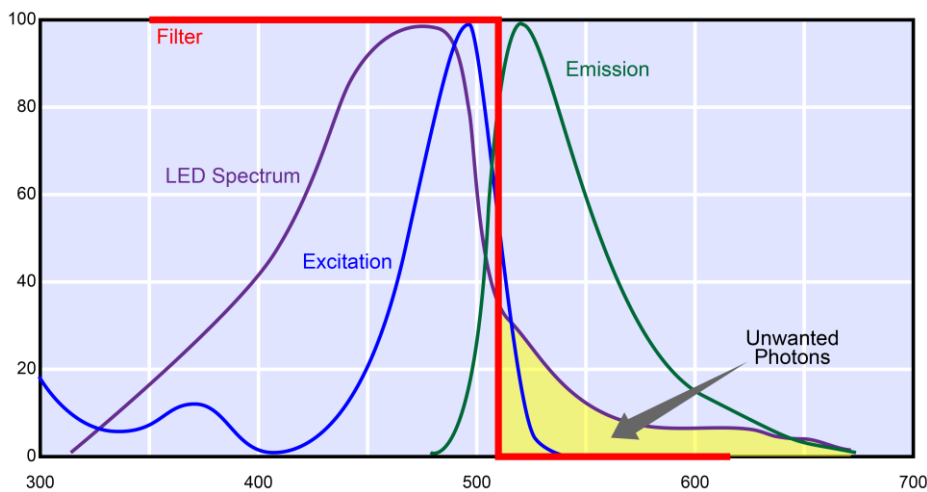


Figure 2
Every fluorophore is characterized by an excitation spectrum and a long wavelength emission spectrum. With a single wavelength laser, it is easy to separate scattered excitation light from the emission spectrum using a long-wave cutoff filter. But with a typical LED, the long wavelength tail of its output overlaps the fluorescence emission and must be somehow eliminated. Plus the size and shape of this tail can vary significantly between different batches of LEDs.

Impact on Detector Costs

Using a LED can also increase the cost and complexity of the photodetection system used to sense the fluorescence and/or scatter, particularly in applications with a small illuminated interaction zone. Specifically, the low brightness of LEDs means that the signal will be correspondingly lower, compared to the use of a laser. Achieving target speed and/or signal-to-noise performance may therefore dictate using a photodetector with higher gain and/or lower noise. For example, a particular instrument may only require a zero-gain photodiode or photodiode array when based on laser excitation, but may need a photomultiplier tube or a CCD array when used with a LED source. For instances where a laser-based instrument uses a room temperature CCD array, a LED version of the same instrument might need an actively cooled CCD array.

Unit-to-Unit Variability: Supply Chain Realities

Today's lasers are characterized by incredible unit-to-unit consistency. All solid-state laser construction and the implementation of automated, robotic assembly and testing methods have largely eliminated individual variations. And, just as important, laser manufacturers like Coherent recognize bio-instrumentation as an important market for our products. For example, lasers are optimized for specific biotech applications and market segments (e.g., research, clinical, point of care). Additionally, specific laser models have a long (multi-year) life cycle with a minimum seven-year life cycle on spare parts and replacement units. This continuity is critical for OEM instrument manufacturers; it allows them to confidently design new instruments and upgrades with no fear of component obsolescence and enables them to service instruments in the field for many years after initial purchase.

In contrast, LEDs are produced by the billions to support high volume application such as displays and lighting fixtures. They are designed specifically to service these high volume applications, which typically have very different performance requirements than a precision biotech instrument. Indeed, the entire bioinstrumentation market is an irrelevantly small size in the eyes of these LED manufacturers. They do not even sell their products at these small (thousands) volumes. Instead, instrument builders have to purchase any LEDs through third party re-sellers, who have no control over product development and product

continuity. Their only "control" over product quality is by occasionally offering testing and pre-selection at a price premium, of course. Thus, while two LEDs might have the same nominal center wavelength, this can vary by several nanometers, and the shape of the entire output spectrum can be very different. Switching between two LEDs that differ in this way often necessitates installing one or more new filters and complete re-calibration.

LEDs are manufactured in large batches called bins and are assigned a bin number. The only way to know that two LEDs are going to be the same is to buy them from the same bin. If a LED-based instrument design is chosen and ready to go into production, the instrument maker needs to advance purchase a lifetime supply of thousands of identical LEDs, i.e., an entire reel of LEDs from the same bin.

Lasers: Scalable Performance

In several applications (e.g., cytometry), instrument makers need to supply instruments to quite a number of different market sectors, such as advanced research, pre-clinical uses, clinical labs, and even point of care tools. These different sectors can have diverse needs from their new instrument, covering factors such as sensitivity, throughput and cost per measurement. This puts different demands on the characteristics and acceptable cost of the light source. With over 50 years supporting life sciences, laser manufacturers like Coherent understand this challenge and provide a very wide range of laser technologies and products to optimally match the needs of all the different instrument types, from the highest performance research instrument to the lowest cost point of care analyzer.

Contrarily, with LEDs there is virtually no range of optical performance to choose from, other than size (power) and center wavelength.

BioRay

In many bioinstrumentation applications, there is a shift from the research laboratory to the clinical laboratory, and, subsequently, to the point of care. This trend manifests itself in greater streamlining, miniaturization and automation. To support this trend, Coherent has recently introduced the BioRay series of lasers that emphasize economy, compact packaging, and simple integration over bleeding-edge performance. These lasers deliver up to 50 milliwatts, and are available at several visible wavelengths (e.g., 405 nm, 450 nm, 488 nm, 520 nm, and 640 nm) that match the optimum excitation of common fluorescent probes and genetically encoded markers. These products are based on laser diode technology, as that is the simplest and lowest cost method of generating CW laser output at these wavelengths and in this power range. Laser diodes also offer the highest efficiency, lowering the required power budget for the final instrument. Since edge emitting laser diodes emit a highly divergent and asymmetric (elliptical) beam, optics are used in the laser head to produce a collimated, elliptical beam. To reduce the complexity of downstream beam delivery optics, each head has an adjustable output lens in order to enable smooth adjustment of the beam waist location.



Figure 3

A new generation of economical CW visible lasers called the BioRay series has been developed with output parameters tailored specifically to meet current and future trends in cost-sensitive biotech instrumentation.

Conclusion

There's certainly no question that it's cheaper to buy a LED than a laser. However, when the total cost of design, the complete bill of materials (BOM), the service strategy, and the true cost of ownership are considered, lasers are often a more cost effective option than LEDs. This is even true for next generation, point of care instrumentation, where component costs are paramount.