# Light Source Characterisation in Life Sciences

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Photonics has become an important tool in many areas of life science, from biological research to biomedical instrumentation and agriculture. Whether the goal is effective UV disinfection, optimal aquarium illumination or accurate blood oximetry, the success of these applications often depends heavily on understanding the performance of the light sources employed. Laser-based techniques used in ophthalmology, optical coherence tomography, multiphoton fluorescence excitation, DNA sequencing and cytometry also begin with a well-characterised light source.

Diode-array spectrometers provide a convenient and compact solution for light source characterisation in research and industry. They can be configured to the specific wavelength range and resolution needs of each application, measuring intensity at all wavelengths simultaneously — even for a single shot of a pulsed light source. By gaining an understanding of best practices for light source characterisation, as well as the factors to consider in configuring a measurement system, the benefits of diode-array spectrometers can easily be applied in life science applications.

## Common Measurement Setups

The best light source measurement setup is modelled after the intended application, i.e., measure your light source in the same way in which it will be used. To illustrate this general rule, consider microscopy, where some light sources are delivered to the instrument with a fibre. For a one-time check of the light source, one can simply connect this fibre to the entrance port of the spectrometer. Measuring through the fibre being used in the system is important to account for transmission of the fibre itself. This is particularly true for ultraviolet light sources transmitted via fibre, as solarisation of the fibre can reduce the amount of light delivered at wavelengths below 400 nm.

Blood oximetry measurements are another good example. As oximeters use the entire output of an LED to determine the blood oxygen level, all angles of LED emission should be captured during characterisation; this is best accomplished using an integrating sphere. Finally, to quantify the illumination at a specific point or distance from a source, as when measuring UV index to assess risk of sunburn, a cosine corrector should be used to collect light for delivery to the spectrometer via a fibre. Eliminating the cosine corrector and using just the bare end of the fibre alone can still provide relative information in this application, such as the wavelength of a peak, but the quantitative intensity information is lost.

## Narrowband Laser Sources

The important performance parameters for a narrowband light source depend upon the application for which it is used. Wavelength and linewidth are the most frequently measured parameters for lasers. In Raman excitation, any shift in the laser wavelength leads to a corresponding shift in the resulting Raman spectral peaks, and thus unreliable or potentially 'blurred' spectra. In flow cytometry, multiple single-wavelength lasers may be used to excite various fluorophores. The detection system then uses complex trains of optical filters to route specific wavelength bands to PMT detectors highly sensitive to residual laser light. In both cases high resolution and wavelength accuracy are of paramount importance for the spectrometer characterising the laser. This can be achieved through the selection of a narrow slit and long optical bench, and by using a high groove density grating (i.e., high lines/mm).

*Figure 1* shows the spectrum of a narrow band laser recorded with high resolution diode-array spectrometer, equipped with a 25 µm slit and an 1800 lines/mm grating to measure the wavelength range 430-560 nm. This configuration results in a spectrometer resolution or FHWM (full width at half maximum) of 0.16 nm. As very little light is needed for this measurement, it might be tempting to simply steer a laser reflection onto the spectrometer entrance slit; however, this will lead to incorrect results. Laser beams are almost perfectly collimated, while spectrometers are designed to image a divergent point source from the entrance slit, via the grating, onto the detector. To avoid this problem, the laser emission shown here was captured with an integrating sphere and delivered to the spectrometer via a 50 µm fibre (to minimise stray light in the spectrometer).

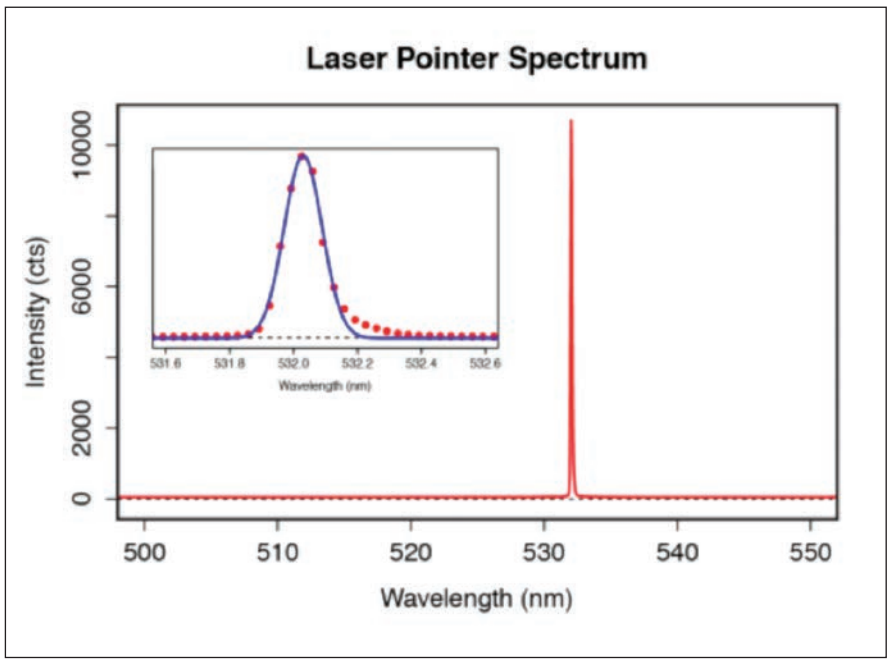


*Figure 1: Spectrum of a diode-pumped solid state laser recorded with an integrating sphere, a 50 µm core fibre and a high-resolution diode-array spectrometer equipped with a 25 µm slit and an 1800 lines/mm holographic grating.*

## Short Pulse Lasers and LEDs

The enormous peak powers delivered by short pulse or 'ultrafast' lasers have enabled new and exciting applications in biomedical microscopy, not the least of which is multiphoton excited fluorescence, a technique used in neuroscience, immunology and cancer research. This technique provides intrinsic three-dimensional spatial resolution (through the position of the laser focus required to generate nonlinear excitation) with deep penetration (thanks to the near-infrared wavelengths). While a spectrometer cannot measure the pulse length of a Ti:Sapphire or short pulse fibre laser directly, both the coherent bandwidth (used as an indication of the minimum pulse length) and the centre wavelength of the tunable laser are of importance for successful research or diagnostics.

Similarly, emission bandwidth and centre wavelength are of most interest when measuring single-wavelength LEDs, typically in the visible or UV range. An LED's centre wavelength and wavelength shape can change with drive current or temperature. *Figure 2* shows

a fibre-coupled 455 nm LED at various drive currents, recorded with a high resolution spectrometer. The relatively broad spectrum of LEDs reduces the resolution requirement for measurement, allowing use of even ultra-compact, moderate resolution spectrometers.

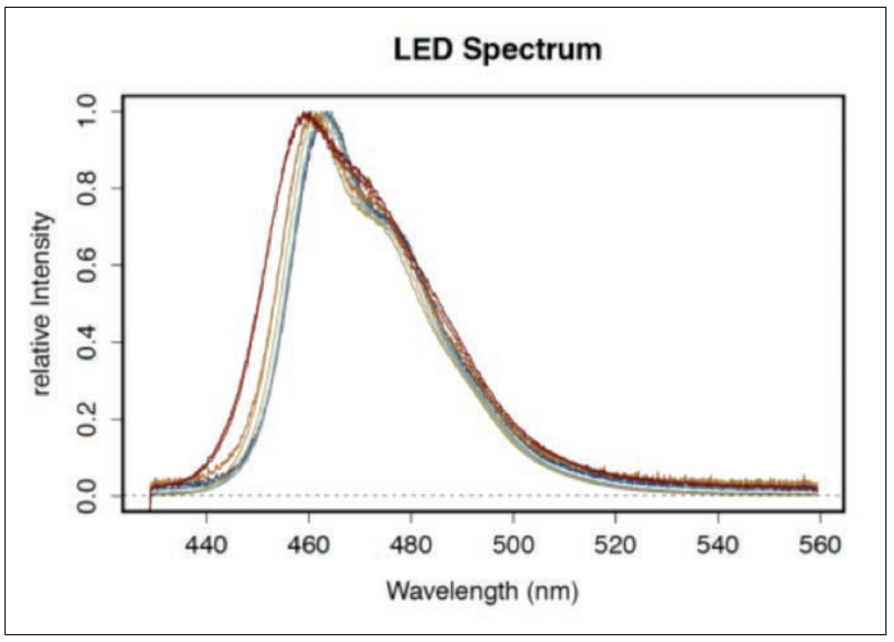


*Figure 2: The output of a single-wavelength LED at different drive currents, showing a shift of the emission wavelength due to temperature changes in the LED. Spectra were recorded with an integrating sphere, a 50 µm fibre and a high resolution diode-array spectrometer equipped with a 25 µm slit and an 1800 lines/mm holographic grating.*

## Tunable Near-Infrared Lasers

The output of tunable short-pulse lasers such as Ti:sapphire lasers often spans the short-wave near infrared (SW-NIR) region up to ~1100 nm, and thus can be measured using a properly configured standard or high resolution diode array spectrometer. In a standard diode-array spectrometer bench, a 600 lines/mm grating, blazed at 1000 nm for highest SW-NIR efficiency, can cover the range 550-1100 nm with a resolution of ~1.3 nm (FWHM). Wavelengths above 1.1 µm — for example, from one of the newer Yb:fibre-pumped OPOs — demand a switch from silicon to InGaAs detectors. NIR diode-array spectrometers typically use a longer optical bench to compensate for the reduced number of pixels. These systems can be configured to cover the missing tuning range from 1100-1500 nm, with a comparable resolution of ~1.5 nm (FWHM) using a 300 lines/mm grating.

## Filtered Broadband Lamps

Broadband emitters such as tungsten halogen or xenon lamps are still by far the most common light sources used in life sciences, with new white light LED sources advancing quickly. Optical filters are often used to select a specific wavelength range for illumination or excitation, and thus careful measurements are required to ensure that sufficient power is emitted at the desired wavelengths while eliminating radiation at any harmful or undesirable wavelengths. The application defines which spectral regions constitute useful or undesirable. For example, UV disinfection requires sufficient UV output in the germicide region (UVC, below 280 nm) for effective sterilisation, while in contrast ophthalmic surgery applications require any UV light below 350 nm to be blocked for to avoid eye damage.

Moving up the rainbow, blue light exposure in the evening from smartphone screens or LED lighting has been associated with sleep disturbances due to its effect on the circadian rhythm. Blue light is essential other cases, as when used as a treatment for neonatal jaundice. On the other end of the spectrum, the majority of the output from tungsten halogen bulbs is composed of invisible near-infrared light. High intensity NIR light can cause thermal damage in the retina and must be minimised in eye surgery tools.

One last example of filtering lamps comes from indoor agriculture. Plants require mainly blue and red light for optimal growth, while any light in the green region is simply reflected (hence their dominant colour). Light sources in this industry are therefore rated by their effectiveness for plant growth, often given as a measure of the photosynthetically active radiation (PAR). PAR quantifies the emission in the range from 400-700 nm, while photosynthetic photon flux density (PPFD) weights the potential photosynthetic action by wavelength.

## Determining Light Source Intensity

Characterisation of a light source for many of these applications also requires absolute irradiance measurements in a certain wavelength region. Diode-array spectrometer systems are ideally suited for this task, as they can be configured specifically for the application. Radiometric calibration over the entire wavelength range can be performed at the factory or by the user using a calibrated lamp. The emission from an LED is best measured with an integrating sphere, while the illumination at a certain distance from the light source is ideally measured with a cosine corrector at the point of use, as discussed previously. It is crucial that any radiometric calibration be made with the exact setup and accessories to be used in the light source measurement, with the calibrated lamp replacing the source to be characterised. Even seemingly insignificant changes, such as disconnecting and re-connecting the fibre will invalidate an absolute irradiance calibration.

Once calibrated, the spectrometer measures the light in absolute units: the emitted energy per time, area, and wavelength slice. A simple integration over the wavelength region of interest yields the desired quantity, i.e., the amount of useful or harmful radiation released from the source. Due to this integration, signal to noise and resolution are of minor importance for the selection of the spectrometer; rather, the analysis requires good linearity, a reproducible 'dark' background and reliable wavelength calibration. While even ultra-compact spectrometers can fulfil these requirements and are good choices for measuring the absolute irradiance in a certain wavelength range, more specialised spectrometers with highly linear detectors can yield slightly more accurate results.

## Measuring Residual Light

If the absence of radiation in a certain wavelength range needs to be tested for safety or performance reasons, stray light will become the most crucial spectrometer specification, which takes more effort to control. As a simple precaution, input fibres with large core diameters could overfill the first mirror in the spectrometer and should be avoided. Similarly, intense light that is not needed for the measurement is best blocked before entering the spectrometer. However, the selection of spectrometer bench and grating probably has the largest impact on stray light performance. In general, holographic gratings tend to cause less stray light than ruled gratings. In addition, some diode-array spectrometer benches are optimised for low stray light, and would be a better choice in these cases.

*Figure 3* depicts an example for the blocking of UV light from a high power xenon source, here performed by inserting safety glasses. The instrument of choice was a low stray light diode-array spectrometer, designed with baffling to minimise stray light in the UV. It was configured with an 1800 lines/mm grating and a 5 µm slit for measurement from 160-310 nm. The spectrometer was radiometrically calibrated and the total intensity from 250-310 nm integrated to quantify the remaining emission in the UV range. Also shown is a less careful measurement without blocking any of the visible light (> 400 nm) using a standard design diode-array spectrometer, leading to higher stray light and an erroneous result.

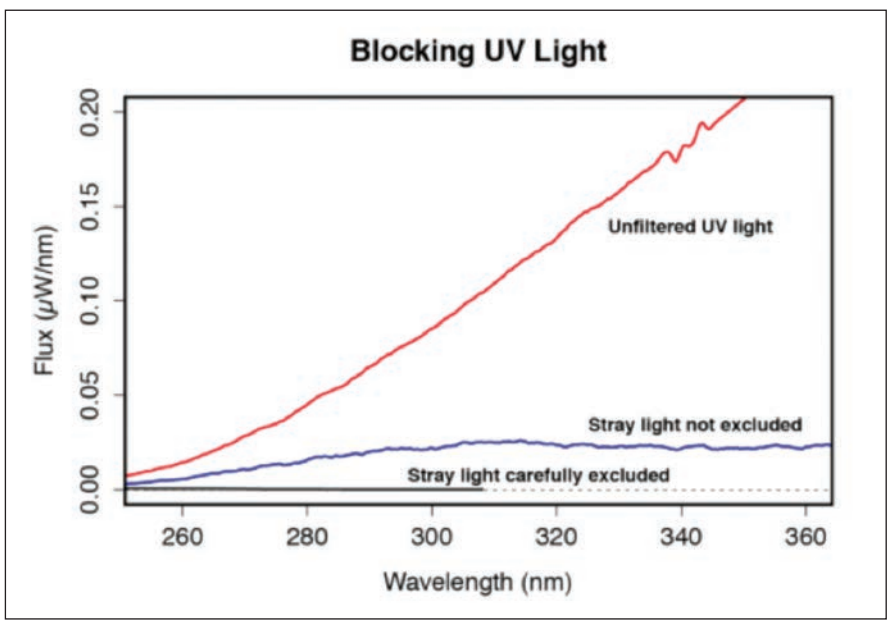


*Figure 3: Emission from a high-power xenon light source (red) with the UV portion blocked for eye safety (black). A measurement preformed without first removing visible light (> 400 nm) leads to significant stray light contributions and an incorrect result (blue).*

## Conclusion

Spectrometers are an excellent tool for rapid light source characterisation in the life sciences. Due to their flexibility in configuring wavelength resolution and range, modular diode-array spectrometers are especially suited to determine the centre wavelength of lasers or LEDs, to measure the bandwidth of the emission, and to quantify broadband lamp irradiance in specific wavelength regions for performance or safety.


### About the Author

A spectroscopist for 20 years, Dieter's experience spans ultraviolet through infrared systems and techniques, including ultrafast and high-resolution spectroscopy, as well as fluorescence microscopy. A specialist in the properties of materials and their interaction with light, he has led research programs that combine simulations and statistical analysis with advanced optical techniques. As a Senior Applications Scientist at Ocean Optics, Dieter develops lab protocols, spectroscopic methods, and data analysis models to support research and OEM programs.