Tech Tip: Stray Light and Absorbance Measurements

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An Introduction to the Impact of Stray Light

Absorbance spectroscopy is a tried and true analytical method used in countless applications around the world. From analytical laboratories to cutting-edge research, from the industrial floor to airborne sensing, absorbance remains the most widely used spectroscopic technique due to its simplicity, accuracy and speed. Long gone are the days of the Spec 20 spectrophotometer measuring one wavelength at a time, carefully and tediously selected by turning a knob by hand. In many of today’s applications, diode array spectrometers measure the entire spectrum simultaneously in a fraction of a second.

The convenience of instantaneous spectral measurement comes at a slight cost. With all wavelengths of light entering the spectrometer at one time, special care must be taken to prevent light of the wrong wavelength from hitting the diode array detector at the wrong location. This unwanted (or “stray”) light falsely increases the signal measured at some wavelengths, which can distort the measurement. While stray light reduction often factors significantly into spectrometer bench design, the user’s actions can also aid in minimizing the impact of stray light on a spectrometer’s performance.

How Does Stray Light Impact Absorbance Measurements?

In Czerny-Turner spectrometer designs, like the Ocean Optics benches, the light enters the completely dark spectrometer at the entrance slit, is collimated by the first spherical mirror, reflected onto the grating, dispersed by wavelength, and focused by the second mirror onto the diode array detector (Figure 1). Every surface that the light bounces off generates a little bit of scatter that results in stray light. In addition to dispersing light into its component wavelengths, the grating produces an undesired mirror-like reflection that also contributes to stray light. High-quality mirrors can reduce the former, while good spectrometer design captures most of the latter through careful placement of baffles (“fingers” that extend into the spectrometer bench to confine unwanted light). Despite the most careful design, some light still escapes these countermeasures and finds its way onto the detector. In this technical note we will discuss the effects of stray light and explore some ways in which it can be reduced through smart choices in the experimental setup.
Stray light constitutes a constant, false addition to the signal level at a given wavelength. At the wavelengths where the analyte absorbs most (often the same wavelength used to determine concentration), the addition of stray light thus causes the recorded intensity to be higher, with the result that the absorbance (the loss of intensity) appears less than expected. The fewer photons transmitted through the analyte (i.e., the higher the absorbance), the larger the relative error of this constant stray light contribution. For high absorbance values, stray light can set an upper limit of the optical density that can be measured with the setup. As the remaining transmitted light intensity drops to levels comparable to the stray light, the recorded absorbance levels off, as can be seen in Figure 2.

Figure 1. A diode array spectrometer with Czerny Turner optical bench design.

Figure 2. The impact of stray light on measured absorbance results in a deviation from linearity at higher absorbance values, plotted here theoretically for stray light values from 0.0001 to 0.1.

How is Stray Light Measured?

Stray light is typically measured by blocking a portion of the incoming spectrum and measuring the residual light at those wavelengths. One method is to use a longpass filter to absorb shorter wavelengths, assigning the recorded intensity in this blocked wavelength region to the stray light generated by the remaining long-wavelength light, and then specifying the ratio of the two. Pharmacopeia, a reference for the pharmaceutical industry, specifies use of a similar procedure in which stray light at 200 nm is measured by absorbing the light below 220 nm using a 1.2% solution of potassium chloride in 1 cm pathlength cuvette.

At Ocean Optics, stray light test measurements are often performed at wavelengths between 400 nm and 600 nm. Typical values range from <0.10% @ 435 nm and <0.05% @ 600 nm for a Flame-series spectrometer to <0.015% @ 400 nm for an ultra-low stray light design like the Maya LSL.

Correcting for Stray Light at a Specific Wavelength

The effects of stray light can be – to some degree – numerically corrected after the measurement. The discussion of a full stray light correction on a given spectrometer is beyond the scope of this introductory technical note, as the amount of stray light in a setup depends on the bench used, the grating in the spectrometer, the starting wavelength, and last, but not least, the spectrum of the light entering the spectrometer (influenced largely by the choice of light source). But even though it is not possible to universally specify the stray light level for a spectrometer, one can determine its value for any given absorbance wavelength of interest with just one additional measurement.

To measure the stray light to be corrected, record the lamp intensity with the blank (reference) solution in the cuvette holder, block the light source and subtract this background level from the reference intensity. With the corrected lamp intensity on display, insert a high-concentration solution of the analyte with an optical density of 5 or more (at the absorbance wavelength of interest) in the cuvette holder. As the solution absorbs all light at the absorbance wavelength of interest, any light intensi-
Minimizing Stray Light Effects Through Experimental Design

Before resorting to numerical corrections, it is best to avoid stray light in the first place through careful design of the experimental setup. While stray light interferes with measurements at high optical densities, low optical densities should also be avoided for different reasons, as even very small relative errors in the provided light intensity translate into large relative errors in the absorbance. A sweet spot for absorbance measurements is therefore an optical density between 0.3 and around 1.0. If the experiment can be modified by adjusting concentrations or the cuvette pathlength to reach this approximate absorbance range, such a simple change will dramatically reduce the stray light sensitivity of the measurement.

Additionally, several modifications of the setup can reduce stray light even further. For one, use of an input fiber of a large core diameter can overfill the first mirror in the spectrometer, generating additional scatter and hence stray light within the bench. Good fiber core diameters are 100 µm (or under) for the USB and Flame benches, 400 µm for the STS spectrometer, and 200 µm for the HR bench.

One of the simplest approaches to stray light reduction is also the least obvious: limit the amount of unneeded light entering the spectrometer in the first place. If a measurement in the UV region is required, turn off the halogen lamp in your light source, increase the UV light level by choosing highly transparent quartz material for cuvettes and fibers, and if possible select a spectrometer optimized for UV measurements. If on the other hand a measurement around 500 nm is needed, use a short-pass filter at 600 nm to eliminate any light above this wavelength. Or better yet, narrow the spectrum with an adjustable combination of short-pass and long-pass filters to limit light input from both sides.

To demonstrate the effect of these methods we recorded a calibration curve (the absorbance at a given wavelength for a series of concentrations) for acetone in water at 280 nm. We used a DH-2000 light source with a deuterium and halogen bulb, a cuvette holder with 1 cm pathlength quartz cuvettes, and a Maya2000 Pro spectrometer with a spectral range of 190-400 nm. Three results are shown: one calibration curve recorded with both UV and halogen bulbs turned on, one with just the deuterium lamp to eliminate visible light in the spectrometer, and one after numerically subtracting the stray light intensity in the absorbance band as recorded with a 0.5 molar concentration of acetone in the same setup. The differences are dramatic, with limits of linearity (as measured in optical density, or AU) of approximately 1, 2, and 3, for the three different approaches.

Figure 3. Elimination of unnecessary illumination wavelengths and subtraction of stray light can greatly improve the linearity of an absorbance calibration curve.

Parting Thoughts

Though stray light is unavoidable when using diode-array spectrometers, a variety of tools are at the disposal of the user for mitigation. From smart experimental choices to numerical stray light correction, the effects of stray light are easily outweighed by the convenience, speed and portability of diode array spectrometers. By following a few simple recommendations, you can optimize measurements with minimal trade-offs.