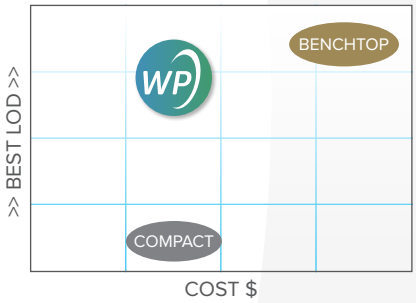
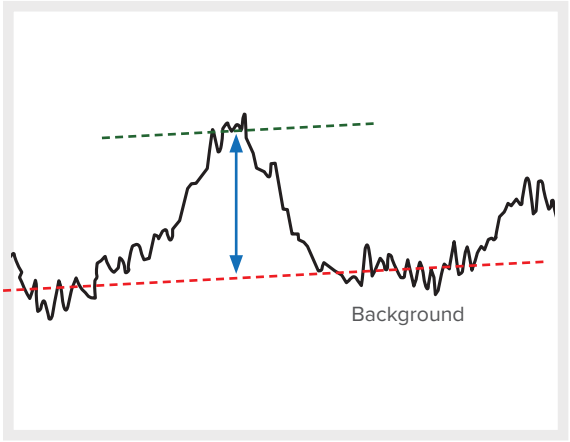
LOD in Fluorescence

One of the first questions users of traditional benchtop fluorimeters ask is, "Does a compact diode array spectrometer have enough sensitivity for my measurement?" Using fluorescein as a benchmark, we show how quickly our WP VIS and quick-fit cuvette holder can collect superior spectra with picomolar sensitivity.

Many compact spectrometer manufacturers claim to have sensitive high quantum efficiency detectors, or low noise due to thermo-electric cooling (TEC) and/or vertical binning of the pixels in a 2D array. While all of these factors can improve the signal to noise ratio (SNR), the essential questions remain unanswered: What is the system limit of detection? What is the lowest concentration it can quantify? How quickly can it be measured?

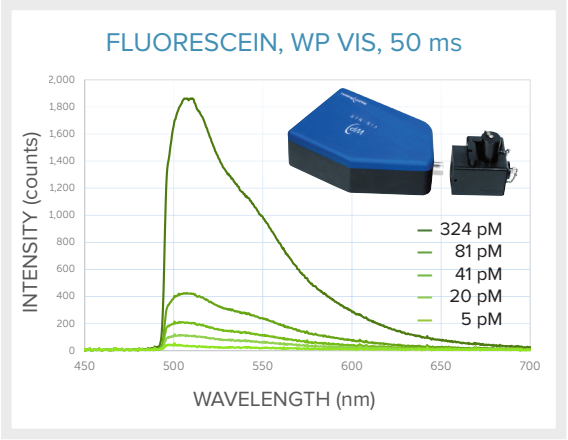
Here, we'll explain how limit of detection (LOD) & limit of quantitation (LOQ) are defined and measured, using fluorescein spectra collected in our WP VIS fluorescence system. By using our own highly efficient volume phase holographic (VPH) gratings in a diffraction-limited transmissive design, our spectrometers maximize sensitivity and reduce stray light significantly as compared to other compact spectrometers of similar cost. The result? Our LOD is within a factor of four of much more expensive benchtop systems!





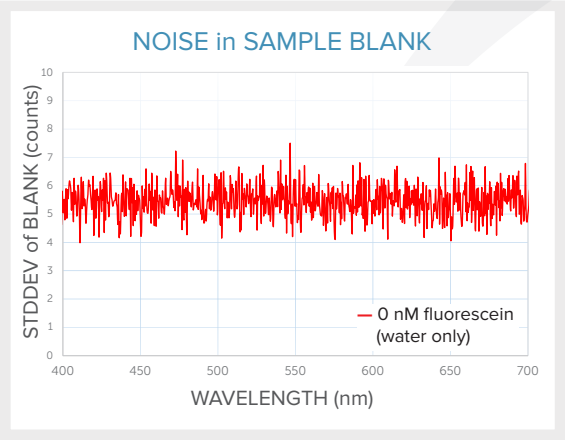
DEFINING LIMIT OF DETECTION (LOD)

Limit of detection (LOD) is defined as the lowest concentration at which the presence or absence of an analyte can be determined with certainty using a given analytical method. The signal arising from the sample (green line) must overcome the baseline noise (red line) by a comfortable margin (blue line) in order to be confident that an analyte is truly being detected. The limit of detection (LOD) is typically defined as the sample concentration at which the signal is equal to 3x the noise level, and is limited by system noise.



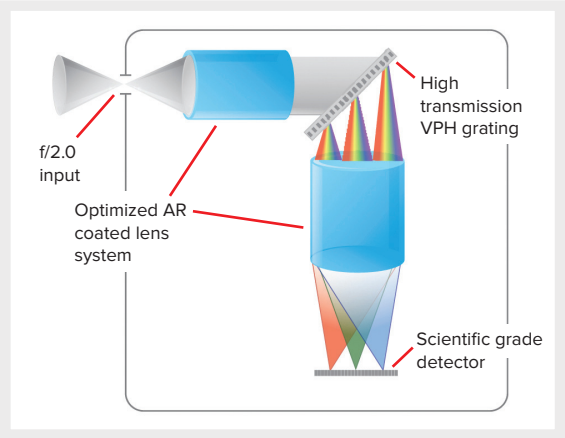
MEASURING FLUORESCEIN

A series of fluorescein dilutions from 5 nM to 5 pM were prepared using 0.1 NaOH solution and measured in a 10 mm pathlength quartz cuvette. Fluorescence measurements were taken with a WP VIS spectrometer, 50 µm slit, using a 450 nm LED for excitation. Our own quick-fit cuvette holder coupled free space to the spectrometer accessed the spectrometer's full f/2.0 field of view for maximum light collection. Fluorescein emission was clearly observed for samples as low as 5 pM, at an integration time of just 50 ms and averaging 50x.



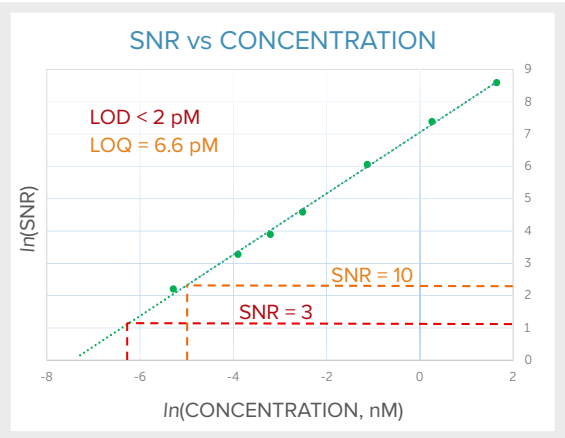
THE SIGNIFICANCE OF NOISE

Noise in a spectrometer measurement may include thermal noise (dark noise, when the detector is not illuminated), shot noise (counting noise, arising from the probability of a photon generating current), readout noise (due to detector circuitry), and fixed pattern noise (diode array dependent). Stray light can also contribute to noise, and should be minimized. The standard deviation (stddev) of our 50 blank measurements of the 0.1 M NaOH reference was calculated as a function of wavelength, and found to be very low: 4 to 7 counts.



MINIMIZING STRAY LIGHT BY DESIGN

Stray light is a key factor that can degrade LOD. That's why we place one of our own perfectly matched, patented volume phase holographic (VPH) gratings at the heart of each spectrometer, using a transmissive design and aberration-corrected optics to keep every possible photon in the optical path. Our VPH gratings offer up to 40% higher efficiency, more uniform response with wavelength and polarization, and ultra-low scatter compared to reflective gratings, enhancing the performance of our robust, thermally stable design.



LIMITS OF DETECTION & QUANTITATION

Signal to noise ratio (SNR) as a function of concentration was plotted for the fluorescein peak, taking the ratio of the signal ("sample" – "blank") to the standard deviation of the blank. Plotting this on a ln-ln scale reveals a highly linear fit, indicating high quality data. Extrapolating to SNR=3, the limit of detection (LOD) of our system was found to be just 2 pM! Limit of quantitation (LOQ) defines the point at which two samples can be said to differ in concentration, and requires SNR=10. The LOQ for our system was found to be 6.6 pM.

CONCLUSION

LOD and LOQ are fairly simple measurements to take, and allow the performance of compact spectrometers to be directly compared to more sensitive benchtop systems. Achieving ~2 pM LOD, the WP VIS fluorescence system proves itself sensitive enough to rival the 0.5 pM LOD typical of an expensive benchtop fluorimeter. Sensitive, compact, and fast, it is an ideal tool for integration into biomedical instrumentation or the field.

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