



Receiver operating characteristic-curve limits of detection[☆]

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ABSTRACT

Using a simple UV LED-excited ruby fluorescence measurement system, we demonstrate that it is easily possible to obtain unbiased detection limits, despite the system deliberately having non-linear response function and non-Gaussian noise. Even when the noise precision model is heteroscedastic, but otherwise only roughly linear, the receiver operating characteristic (ROC) method readily yields results that are in accordance with *a priori* canonical specifications of false positives and false negatives at the detection limit. The present work demonstrates that obtaining unbiased detection limits is not abstruse and need not be mathematically complicated. Rather, detection limits continue to serve a useful purpose as part of the characterization of chemical measurement systems.

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1. Introduction

Detection limits (DL) evoke a variety of responses from experienced analysts. For example, Coleman and Vanatta state “We believe that detection limits should go away. The concept of DLs is an artificial one that has roughly as many definitions and formulations as there are people who talk about and calculate such numbers. Inevitably, discussions devolve into a quest for *the* detection limit; such a value does not exist!” [1]. In a similar vein, Thompson has argued [2] that detection limits are hard to interpret and lead to misconceptions about the measurement process, and therefore should be deemphasized in favor of, e.g., “characteristic functions”.

Frankly, we cannot agree with the above, for one compelling reason: the majority of perceived detection limit issues stem from the published literature on the topic being substantially greater than is merited by its knowledge content. This has led to misconceptions, contradictions, and even sanctioned protocols that are demonstrably biased.

In contrast to the above, Voigtman has demonstrated [3–9] that when a real chemical measurement system (CMS) is well modeled as being univariate, with linear response function, and having additive, Gaussian, white noise (AGWN) as its dominant measurement noise, then Currie's detection limit program [10] may be instantiated quite easily. Several types of heteroscedastic noises were examined in detail, including the “hockey stick” noise precision model (NPM) favored by

Thompson and Ellison [11], along with homoscedastic AGWN. The keys to correctly instantiating Currie's program were the elimination of several plausible long-standing obstructions, plus extensive use of Monte Carlo computer simulations that served to test derived theory and facilitate understanding results from real laser-excited molecular fluorescence experiments that were performed.

Some analysts have questioned the applicability of detection limit theory that assumes, perhaps too uncritically, that a given real CMS necessarily and accurately satisfies *all* of the requisite conditions upon which the theory is predicated [12]. What would happen if, e.g., the response function was non-linear or the noise was non-Gaussian?

Beyond question, this is a valid concern in some cases. However, it has always been incumbent upon experimentalists to exercise their seasoned judgment in deciding whether or not a given relevant theoretical assumption has been accurately satisfied in a real experiment. In the final analysis, some judgment on the part of the experimentalist will always be required. Even so, this raises an important question: “Assuming a univariate CMS, what are the minimum conditions that must be satisfied in order to obtain unbiased detection limits, as per Currie's program [10]?”

One answer is to use a well-studied non-parametric methodology, such as that of the receiver operating characteristic (ROC). Originally developed for radar applications during World War II, ROCs are robust, relatively easy to use, and provide a wealth of useful ancillary information, e.g., “area under curve”. Fraga et al. [13] appear to have been the first to employ ROCs in determining realistic LODs in real world chemical sensing systems. Their paper provides a short, but comprehensive, survey of ROC background information. More recently, Fonollosa et al. [14] employed both ROCs and fundamental information theory principles in their detection limit studies. However, they coupled these with what we consider to be an inapplicable Bayesian analysis: Currie's

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program is fundamentally a dichotomous Neyman–Pearson problem [10,15], with no priors or costs.

Quibbles aside, these papers demonstrate that ROCs provide a powerful means to study limits of detection in CMSs. The purpose of the present work is to demonstrate the main advantage of ROCs, using a real CMS that is almost as simple as possible, yet is deliberately designed to violate the customary simplifying assumptions of linear response function and AGWN. Toward this end, a UV LED-excited ruby laser rod fluorimeter was constructed and used to provide the necessary data for two separate experiments: one with homoscedastic noise and the other with approximately linearly heteroscedastic noise. For brevity, these are referred below as HOM and HET, respectively.

2. Theoretical

2.1. General background

Limits of detection are fundamentally based on probabilities of both false positives (Type 1 errors) and false negatives (Type 2 errors) [10]. Everything else associated with the accurate determination of LODs for a CMS is strictly subordinate to, and in the service of, achieving *a priori* specified probabilities of these two types of mis-categorization errors. As noted above, there is no particular difficulty in instantiating Currie's program when a real CMS is well modeled as being univariate, with linear response function, and having AGWN as its dominant measurement noise. In practice, the common simplifying assumptions are often reasonably well approximated, due to both deliberate experimental design considerations and the “hidden hand” of the central limit theorem. Even when they are not satisfied, the theory still serves as an ideal limiting case for relevant CMSs. However, when the assumptions are clearly not satisfied, the theory is inapplicable and this is where a non-parametric methodology excels: elegant models and some statistical efficiency are sacrificed in favor of robust performance.

2.2. Fluorescence

When a fluorescent substance or medium is appropriately illuminated, it absorbs some of the incident light energy. It subsequently releases, as emitted light, a portion of the absorbed light energy. The basic equations, as approximations for the simplest possible case, are

$$Response(\lambda) \cong K_1 I_{emission}(\lambda) \cong K_2 c_{fluorophor} I_{excitation}(\lambda) \quad (1)$$

where $Response(\lambda)$ is the measured analog or digital response, $I_{emission}(\lambda)$ is the emitted light intensity, $c_{fluorophor}$ is the content (concentration or number density) of the fluorescing moiety, $I_{excitation}(\lambda)$ is the excitation light intensity, λ is the “center” wavelength of the excitation light's spectral profile and both K_1 and K_2 are typically complicated composite instrumental factors [16]. In modern commercial fluorimeter systems, great care is taken to make K_1 and K_2 as constant and large as is feasible or economic.

One of the most frequently encountered chemical fluorescence measurement scenarios involves illumination of a fluorophor-containing specimen with constant intensity light of appropriate constant λ and then measuring the response as a function of fluorophor content. This yields a calibration plot, for wavelength λ , which is simply an estimate of the true underlying response function. It is well known that Eq. (1) is only a satisfactory approximation if, among other things, neither the fluorophor content nor the excitation light intensity are excessively high. As a practical matter, these two restrictions usually pose no significant difficulty and other factors, e.g., light scattering, interferences, and pre-filtering and post-filtering effects are more troublesome.

From Eq. (1), the response is functionally similar for $c_{fluorophor}$ and $I_{excitation}(\lambda)$, i.e., if one of them is held constant, then the other may serve as the independent variable (i.e., measurand) in a calibration plot. Accordingly, if $c_{fluorophor}$ were to be held at a suitably low constant

value, and if λ was constant as well, then any non-excessive increase in excitation light intensity would result in an increased response. In other words, the response would be a strictly monotonic function of $I_{excitation}(\lambda)$ and there would be no fluorescence saturation or “roll-over”. In equation form, this would be expressed as

$$Response = f_{monotonic}(I_{excitation}). \quad (2)$$

If the excitation light source is a light emitting diode (LED), then λ is automatically constant, to an excellent approximation, and $I_{excitation}$ is:

$$I_{excitation} = T_{filter(s)} I_{LED \text{ emission intensity}} \quad (3)$$

where $T_{filter(s)}$ is the constant overall transmittance, at λ , of any optional optical filters that might be utilized to “clean up” the LED's spectral profile. The LED light emission intensity is directly proportional to LED current, i.e.:

$$I_{LED \text{ emission intensity}} = K_3 I_{LED \text{ current}} \quad (4)$$

where K_3 is a proportionality constant. Combining Eqs. (2)–(4), the response is a monotonic function of $I_{LED \text{ current}}$:

$$Response = f_{monotonic}(I_{LED \text{ current}}). \quad (5)$$

When $I_{LED} \equiv 0$, the response is that of the “analytical blank.” Note that since I_{LED} is a current, it is technically a “measurand” rather than as an “analyte”, but this will be treated as a trivial distinction without a difference and the terms will be used interchangeably hereafter.

3. Experimental

3.1. Experimental apparatus

A block diagram of the experimental system is shown in Fig. 1, with component specifications given in Table 1.

Almost all of the components are exactly the same as those used in previously published work [8], with the notable exceptions of the UV LED excitation light source and ruby laser rod fluorophor. The UV LED had peak emission at 397 nm, almost perfectly matched to the strong ruby Cr^{3+} absorption band at about 400 nm. A pair of non-fluorescent blue glass filters removed a low intensity parasitic red emission from the UV LED and also removed a low intensity green fluorescence from the LED's plastic encapsulation. A non-fluorescent red glass filter eliminated any transmitted 397 nm light that may have exited the ruby rod along with the strong 694.3 nm fluorescence emission.

The geometry of the fluorimeter was linear, a configuration that is never used in any standard fluorimeter for various reasons. It was feasible in the present case because of the extraordinarily wide separation between the excitation and emission wavelengths. As a bonus, it had the advantage that the ruby rod acted as its own light pipe, making for an extremely simple optical path and instrument. There was no need for lenses, and, in fact, an aperture had to be added between the red filter and the photodiode in order to reduce the fluorescence intensity.

3.2. Analog circuitry

A key feature of the experiment is the ability to specify and control the behavior of the dominant noise. Toward this end, a simple analog noise generator, shown in Fig. 2 (upper half), was designed to provide non-Gaussian noise with white power spectral density (PSD). The lower half of Fig. 2 shows the transimpedance preamplifier and non-inverting summing amplifier. This circuit is a slightly modified version of that used previously [8], which, in turn, was slightly modified from an even earlier version [7]. Relative to the original version [7], two changes were made: 1) a precision 10 turn potentiometer, buffered fore and aft with TL071 op amp voltage followers, and 2) a 33 Ω resistor

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