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Accurate step wedge calibration for densitometry of electrophoresis gels

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Abstract

Accurate step wedge calibration is important for reliable quantitative densitometry of electrophoresis gels. In this work, we outline the theory and verified by experiment, the effect that the nonlinear response of detectors at low irradiance levels has on accurate optical density (OD) calibration of step wedges. It was found that OD value departures from stated do exist and thus supports the case of calibration. Halogen broadband light was shown to be generally unsuited as light source for step wedge calibration even though applied at highest illumination before saturation, unless correction approaches which add complexity to the process are applied. Monochromatic light was determined best suited for use, provided that the irradiance level was high enough for the calibration to be conducted fully in the detector's linear range.

Keywords: Optical density calibration; Gel densitometry; Step wedge; Monochromatic light

1. Introduction

Laser densitometers [1,2] and charged coupled device (CCD) cameras coupled with stabilized light sources [3,4] are common tools used for the densitometry of electrophoresis gels. The former are essentially specialized equipment with precision optics incorporated for the purpose; and are typically expensive as a consequence. The latter are laboratory type setups that are generally space consuming, expensive (due to the assembly of various specialized components), and with accuracy dependent on the manner with which the optical elements are arranged. Flatbed scanners have been investigated for use in the densitometry of electrophoresis gels for some time due to the obvious advantages of cost and compactness [5–7]. Nevertheless, inconclusive inferences on accuracy from these studies have not encouraged their adoption despite the advantages. A recent work, however, demonstrated that flatbed scanners could achieve measurement accuracies comparable to commercial densitometers through careful consideration of the illumination used and modification of the scanner [8]. This approach also required the scanning in of an optical step wedge to construct a calibration curve that could in turn be used to correctly depict the optical density (OD) of each stained protein [6,8]. This meant that the accuracy of the optical step wedge is crucial in the first instance; although we have found from experience that departures of measured optical density values from stated do exist.

In this work, we investigate the contribution of nonlinear response characteristics of photodetector as well as illumination wavelength in the calibration of optical step wedges. From this, we formulate some caveats needed to accurately calibrate the OD of the step wedges.

2. Photodetector nonlinearity effects on optical density measurements

We assume that photo detection is based on charge coupled devices (CCD). The nonlinear response of CCDs under low illumination has been well investigated [9,10]. The photodetection process, can be described by [9]

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$$N - N_0 = R(\lambda)E(\lambda)t_{\mathrm{exp}} \tag{1}$$

where N is the current of the photodetector, N_0 the detector's dark current, $E(\lambda)$ represents the irradiance, t_{exp} the exposure time and $R(\lambda)$ the responsivity at given wavelength λ. $R(\lambda)$ is dependent on two factors namely, $R^0(\lambda)$, which depends on λ, and $r^{LN}(N)$, which is independent of λ, but depends N. It should be noted that $R(\lambda) = R^0(\lambda)r^{LN}(N)$. Substituting into and rearranging Eq. (1) gives

$$N = R^0(\lambda)r^{LN}(N)E(\lambda)t_{\mathrm{exp}} + N_0 \tag{2}$$

It is often taken that N follows a linear relationship with $E(\lambda)$ in any measurement made using a constant exposure time. Nevertheless, we see from Eq. (2) that this is predicated on $r^{LN}(N)$ being constant regardless of N. If we take $E_{\mathrm{i}}(\lambda)$ as the incident irradiance and $E_{\mathrm{t}}(\lambda)$ as the transmitted irradiance at a certain step of the wedge, the corresponding responses on the photodetector are N_{i} and N_{t}, respectively. Suppose that measurements are made with background subtraction to eliminate dark current. Since measurements are based on the photodetector response rather than irradiance, the optical density $\mathrm{OD}(\lambda)$ is based on the detection of currents N_{i} and N_{t}. OD is expressed as [8]

$$\mathrm{OD}(\lambda) = \log_{10}\left(\frac{N_{\mathrm{i}}}{N_{\mathrm{t}}}\right) \tag{3}$$

The behavior of $r^{LN}(N)$ with respect to N is roughly approximated as shown in Fig. 1a [9,10]. Using Eq. (2), this translates to a typical characteristic of N against $E(\lambda)$ depicted in Fig. 1b. If the calibration procedure is conducted with a hypothetical photodetector that operates linearly throughout the range, the transmitted irradiance E_{t} through a step on the wedge will correspond to the response N_{t2}. If the nonlinear behavior of the detector is taken into account, one would obtain a higher response at N_{t1} instead. Thus, the measured OD can be underestimated at low transmitted irradiance based on Eq. (3). Hence, the measurements must be made with E_{t} falling within the linear range of the photodetector at all times for accurate calibration. This should be achievable with monochromatic illumination, but conceivably more difficult with broadband light due to the wide spread of intensities at the constituent wavelengths.

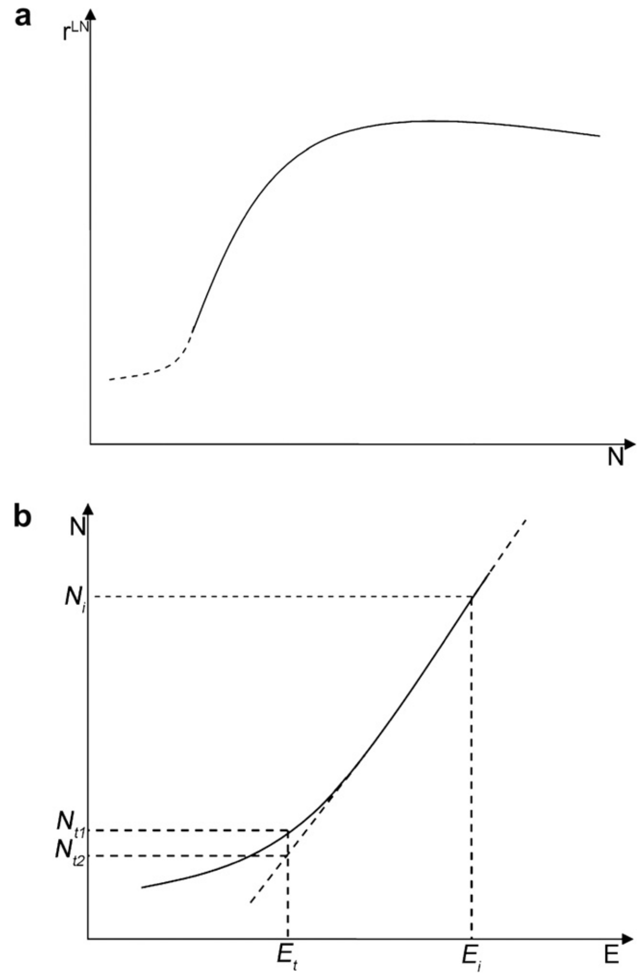

Fig. 1. (a) Typical curve of $r^{LN}(N)$ against N in a CCD photodetector; and (b) corresponding plot of N against $E(\lambda)$ based on (a); wherein the dashed straight line indicates a hypothetical photodetector that operates linearly throughout the range.

3. Experimental procedure

A complete description of the experimental setup is given in Fig. 2. A spectrometer (Ocean Optics model USB4000) was used to register the irradiance throughout. The first experiment was designed to ascertain any departure between measured optical density values from stated in the step wedge. The step wedge (Stouffer Industries Inc., Part No.: T4110C) used had 41 levels of different ODs. A stable 10 mW He–Ne laser was used to illuminate the steps of the wedge and a 1 mm diameter multimode optic fiber used to transmit the light into the spectrometer for recording. The same process was subsequently conducted with light from a stable 150 W halogen broadband light source tuned to 633 nm band pass via a monochromator (Optometrics model DMC1-02 mini) replacing the He–Ne laser. This wavelength was used in order to correspond with the He–Ne laser light.

The second experiment was intended to verify the effects on OD calibration due to detector nonlinearity. The experiment was conducted using a stable 150 W halogen broadband light source at three different levels of illumination, in all cases below the detector saturation. The 1 mm diameter multimode optic fiber remained used to transmit the light into the spectrometer. The OD value at each step of the wedge was calculated under the three illumination levels. The experiment was repeated with the stable 150 W halogen broadband light source kept at the highest illumination before detector saturation without the step wedge introduced. However, a monochromator was now used to band select specific wavelengths to illuminate the wedge steps.

Finally, a third experiment was carried out to investigate measures that could circumvent errors in OD calibration due to photodetector nonlinearity. The stable 150 W halo-