Geometrical-optics approach to increase the accuracy in LED-based photometers for point-of-care testing

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Abstract: A geometrical-optics approach is proposed to increase the accuracy in photometric measurements, using a point-of-care testing (POCT) LED-based sensor. Due to stray-light effects, the measurement accuracy depends on the dimension of the CMOS area, where the radiation is detected. We propose two image processing approaches and evaluate the influence of the sensor area. In addition, we demonstrate that with the same measurement, both absorption coefficient and refractive index can be determined, measuring the beam attenuation and the spot-size enlargement due to ray refraction.

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1. Introduction

Healthcare is becoming more consumer-focused, and requires screening diagnostic tests, that are portable and easy to operate, to provide results in real time and at the site of patient care, far from traditional laboratories [1]. The point-of-care testing (POCT) market is estimated to have grown 9.3% between 2013 and 2018, and focuses on early detection, prevention and managing of chronic illnesses, as well as on environmental monitoring. A rapid screening of infectious diseases is critical in remote or resource-limited areas, where there is not an easy access to a clinical infrastructure, and a prompt treatment may prevent infections from spreading. Malaria and dengue fever diagnosis in remote locations are now possible with new POCTs [2,3], as well as blood typing [4], and water quality monitoring [5,6]. Many POCT devices are based on colorimetric or spectrophotometric analysis of a sample liquid, such as blood or saliva, using reagents to form a colored compound: the biomarker concentration is proportional to the color intensity [7]. Mobile phones are used for colorimetric tests, by taking a picture of the sample and estimating the substance concentration from the RGB pixel values in the image [8].

First photometers for clinical chemistry date back to 1930, and nowadays, they play a fundamental role in many biochemical, pharmaceutical and microbiological experiments that involve DNA, RNA, protein isolation, and enzyme kinetics [9–11]. Spectrophotometry is routinely used to quantify the hemoglobin concentration in blood [12], and in Enzyme-Linked Immunosorbent Assay (ELISA) tests, to measure proteins and antibodies for many diseases ranging from HIV to cancer [13].

Photometric and spectrophotometric sensors measure the number of photons absorbed after that a light beam passes through the sample [14], giving information on the absorption parameter \( \alpha \) at different wavelengths. The transmittance \( T \) is related to the absorbance \( A \), also known as optical density (OD), according to the Beer-Lambert law

\[ A = \log \frac{I_0}{I} = \alpha d \]
where $\Phi_r$ and $\Phi_l$ are the intensities transmitted through the sample and the reference liquid (blank), respectively. The optical depth $\tau = ah$ depends on the effective lightpath $h$ travelled inside the liquid, so that it is strictly required that the monochromatic light beam is a pencil of rays with a reduced waist. Therefore, the analysis is performed over only a small amount of the liquid, and this could be a shortcoming in the case that the solution is not homogeneous.

Most bench-top spectrophotometers use lasers or white sources and monochromators to select the testing wavelength, and some optical architectures are bulky and expensive. Simplifying the optical design and reducing the overall cost is strategic for POCT devices, and different architectures have been proposed for spectrophotometric analysis of a liquid inside a cuvette, using incoherent light sources and CMOS camera [15–17]. However, the accuracy of the absorbance measurements as function of the sensor area has not been fully investigated yet.

We propose a geometrical-optics approach to increase the measurement accuracy in a low-cost optical photometer, composed of a LED, a lens and a CMOS camera. In general, the use of incoherent light hampers the precision of absorbance measurements, due to light diffraction and stray light effects. We demonstrate that a low-complexity image processing can enhance the accuracy of concentration measurements, evaluating not only the beam attenuation, but also the spot-size changes. We consider different dimensions of the sensor area where the beam intensity is detected and analyze how this parameter affects the measurement accuracy. In addition, the spot-size enlargement, due to light refraction and optical ray displacement in the liquid, allows us also to determine the refractive index.

Refractometry has a large number of biomedical and chemical applications, and it is often used to identify a substance, confirm its purity, or measure its concentration. Generally, the change of refractive index is used to quantify the concentration of a solute, such as sugar, in an aqueous solution (Brix degree). It can also be used in the determination of drug concentration in pharmaceutical industry [18].

The POCT photometer is compact and portable, and the image processing is implemented real-time, using a commercial and low-cost data processor, such as a Raspberry Pi.

In the present paper, we consider only homogeneous liquids, and we demonstrate that the beam spot-size does not change with the solute concentration. However, the same device can
be also used to measure the OD of turbid liquids, such as bacterial suspensions [19]. In nephelometric and turbidimetric analysis, the light beam attenuation is due to scattering from particles dispersed in the liquid, the spot-size enlarges, and a different geometrical-optics approach is required to enhance the photometer accuracy [20].

The proposed photometric approach refers to a single wavelength, but it can be used to increase the accuracy of any colorimetric and spectrophotometric POCT devices, using an incoherent source and image sensors.

2. Methods

We use a compact and low-cost spectrophotometric device (WeLab, DNAPhone), with a commercial RGB LED source (Flora RGB Smart NeoPixel version 2, Adafruit) and a 5-Megapixel sensor (OmniVision OV5647, OmniBSI) (Raspberry Pi Camera). The device has a do-it-yourself (DIY) architecture, to enable any user to build a customized low-cost sensor, for different applications [21]. The optical schematics is shown in Fig. 1, and the LED wavelengths and intensities are reported in Table 1. The camera mounts a 2592 × 1944 CMOS image sensor (pixel size 1.4 × 1.4 μm) and 1/4” optics. The lens has focal length \( f = 3.6 \) mm and \( f\text{-number} f/2.9 \). Commercial, low-cost disposable polystyrene cuvette (2xOptical Sarstedt) with \( h = 10 \) mm width have been used. The sensor architecture has very basic optical and mechanical alignment, to reduce the fabrication costs [21]; however, the measurement accuracy can be then improved by additional image processing. Furthermore, the CMOS sensor presents different in-system programming features to adjust the image brightness to a desired range by setting exposure time and gain. Therefore, it is possible to perform an image sensor calibration both in manual and automatic modes, and this is highly recommended when the liquids to be examined may present very different colours and densities.

For each measurement, an 8-bit raw image is acquired at the central 540 × 540 pixels, using a single wavelength illumination, and only the corresponding color in the RGB matrix is considered. In the experiments, we use only red-light source to obtain data compatible with conventional OD at 600nm (OD600) measurements; however, the other spectrophotometric acquisitions, using blue and green wavelengths, can be used for additional colorimetric measurements [16–18].

In front of the LED, there is a diffuser and a pinhole with diameter \( D_1 = 4 \) mm, and we assume that the source can be approximated as a generalized incoherent disk, at a distance \( d \) from the lens, with angular aperture \( \theta_1 = \arctan(D_1/2d) = 4.52 \) deg. The LED luminous flux ranges within the solid angle \( 2\theta_1 \) are reported in Table 1.

In the numerical simulations, we assume that the intensity distribution on the CMOS image sensor has a Gaussian profile (Fig. 2 (a) and (b))
\[ \Phi(X, Y) = \frac{4\Phi}{\pi w_i^2} e^{-\frac{X^2 + Y^2}{w_i^2}}, \tag{2} \]

where \( \Phi_i \) is the average intensity and
\[ w_i = \frac{D_1}{d} d' = 2d'\tan\theta_i = 0.4 \text{mm} \tag{3} \]
is the full beam spot-size, that corresponds to the geometrical image of the \( D_1 = 4 \text{ mm} \) pinhole (magnification factor \( M = d'/d = 0.1 \)). We observe that the Gaussian profile model fits quite well with the measurements, but a Lambertian intensity distribution may also be used [22]. The key parameter of the proposed approach is the beam spot-size, that is evaluated using Eq. (3) in a ray-optics model. If \( X^2 + Y^2 = w_i^2/4 \), the radiant intensity of Eq. (2) is \( \exp(-1) = 0.37 \) of the maximum. Therefore, we measure the beam spot-size as the sensor area (approximated to a circle) covered by the 8-bit pixels (value range 0 - 255) with values larger than \( R = 0.37*255 \sim 94 \).

3. Results

3.1 Absorbance measurements

Figure 2 (c) shows the false-color contour plot of the red-light beam focused on the image sensor (without any cuvette in the lightpath), that is compared with the numerical model of Fig. 2 (b). The raw images of the beam transmitted in vacuum (without cuvette) (Fig. 3 (a)), through a cuvette filled by pure water (Fig. 3 (b)), and cuvettes filled with a food-dye blue-colored water are reported in Fig. 3 (c)-(f), for serial dilution ratios (c) 1:32000, (d) 1:16000, (e) 1:8000, (f) 1:4000, respectively. The axial beam profiles and their normalized values are shown in Fig. 4 (a) and (b), respectively. We observe that the presence of water in the cuvette reduces the maximum intensity and enlarges the beam spot size, due to the ray displacement; this effect will be exploited in the following section to measure the liquid refractive index.

![Fig. 3. Raw images for dye concentration measurements. (a) without cuvette; (b) pure water (blank); dye-colored water with dilution ratios (c) 1:32000; (d) 1:16000; (e) 1:8000; (f) 1:4000.](image-url)
Fig. 4. (a) Axial beam profiles. (b) Axial beam profiles normalized to their own maxima. Data have been smoothed with a moving-average filter only for plot rendering.

We also remark that the light beam is attenuated in colored water, but the beam profile does not change, and the average spot size of the corresponding normalized intensity remains practically the same (see Fig. 4(b)). In fact, for small incident angles $\theta \leq \theta_t$, the absorption parameter only slightly affects the ray displacement related to the Snell’s law [23]. Therefore, we can evaluate the intensities transmitted through the sample $\Phi_t$ and through pure water $\Phi_b$ (blank) on the same sensor area, that should be suitably selected. The smallest area that yields accurate OD measurements corresponds to the beam spot-size $w_r = 2d' \tan \theta_t = 0.4$ mm, that is the image of the pinhole of diameter $D_h$. Since some beam misalignments are possible, the circular area under observation has a diameter of 350 pixels (i.e., sensor area corresponding to the beam spot-size, with 0.49 mm diameter). We gradually increase the area dimension to reach an extended circular region with 540-pixel diameter (full area with 0.76 mm diameter).

We have verified that larger sensor areas include too much noise due to stray-light effect and therefore they are discarded. Therefore, we restrict our analysis to a circular region on the CMOS region, with a diameter that ranges from $N = 350$ to $N = 540$ pixels. The intensity of the pixels outside the selected circular area are all set equal to zero.

According to the Beer-Lambert law of Eq. (1), the absorbance parameter $A$ is evaluated as the ratio between the light intensities transmitted through the sample $\Phi_t$ and through the reference liquid $\Phi_b$.

We consider two different methods to evaluate the absorbance parameter $A$, measuring the pixel intensities $\Phi_i(n,m)$ and $\Phi_t(n,m)$ on the CMOS image sensor:

I) $A$ is calculated as the ratio between the averaged intensities evaluated over the circular sensor area

$$A = \log_{10} \frac{\Phi_t}{\Phi_b} = \log_{10} \frac{\sum_{n,m=1}^{N} \Phi_t(n,m) - \sum_{n,m=1}^{N} \Phi_{dark}(n,m)}{\sum_{n,m=1}^{N} \Phi_t(n,m) - \sum_{n,m=1}^{N} \Phi_{dark}(n,m)}, \quad (4)$$

and

II) using a pixel-by-pixel approach, $A'$ is computed by summing the ratios between the incident $\Phi_i(n,m)$ and transmitted $\Phi_t(n,m)$ intensities for each pixel

$$A' = \log_{10} \frac{\Phi_t}{\Phi_b} = \log_{10} \left( \frac{1}{N^2} \sum_{n,m=1}^{N} \frac{\Phi_t(n,m) - \Phi_{dark}(n,m)}{\Phi_i(n,m) - \Phi_{dark}(n,m)} \right). \quad (5)$$
In both cases, we found that the measurement accuracy is increased if we subtract the intensity $\Phi_{\text{dark}}(n,m)$ measured when the LED is off, that is related to the background dark noise.

The absorbance measurements are compared with those obtained with the commercial spectrophotometer BioPhotometer basic (Eppendorf), that is taken as a reference.

Figure 5 shows the OD measurements obtained with the two approaches of Eq. (4) and (5), considering two sensor areas of diameter $N = 540$ and $N = 350$ pixels. We observe that both approaches give satisfactory results in the linear range ($A, A' = 0.1 \div 1$), and that in both cases the accuracy increases considering a smaller CMOS area. In addition, the average error is less than 2% using Eq. (5) in the linear absorbance range.

Therefore, we can conclude that the optimal sensor area equates the beam spot-size (diameter $N = 350$ pixels), i.e. only pixels with normalized values larger than 0.37 should be included in the evaluation of the absorbance parameter.

In addition, the image processing based on the pixel-by-pixel approach of Eq. (5) yields an accuracy of 2%, that is quite remarkable for a low-cost LED-based device.

We have performed a large set of measurements, also using green and blue LEDs, and other coloring dyes, obtaining similar results.

![Absorbance measurements and error](image)

**Fig. 5.** Absorbance measurements (blue line) and corresponding error (red line) as a function of the CMOS area, where the intensities are measured. Absorbance $A$ is evaluated using Eq. (4) and $A'$ using Eq. (5). The reference absorbance is plotted with black line.

### 3.2 Refractive index measurements

Using a single measurement, we are able to determine both the absorbance and the liquid refractive index. The refractive index $n$ can be evaluated by measuring the enlargement of the beam spot size, due to the light refraction in the liquid. As it is shown in Fig. 1, the presence of liquid inside the cuvette creates a ray displacement $\delta$, that is related to the liquid refractive index $n$ by the Snell’s law [24]

$$n = \frac{\tan \theta_1}{\tan \delta} \sqrt{1 + \tan^2 \delta}$$

(6)

For sake of simplicity, we do not consider the refraction effects on the plastic cuvette, that are quite negligible, and we put the air refractive index equal to 1. The ray displacement is evaluated as a function of the enlarged spot-size $w_2 = 2d' \tan \theta_2$. 
\[
\tan \delta = \frac{D_1 - 2(d - h) \tan \theta_1}{2h}. \tag{7}
\]

Figure 6(a) shows the measured spot-sizes \(w_2\) for four different liquids, evaluated as the averaged area where the pixel values (ranging between 1 and 255) are larger than \(R = 94\).

Table 2. Spot size vs refractive index

| Liquid         | Spot-size increase | Reference refractive index | Measured refractive index (± 0.03 accuracy) |
|----------------|--------------------|----------------------------|--------------------------------------------|
| Pure water     | 15.87%             | 1.33                       | 1.32                                       |
| Ethylene glycol (MEG) | 20.05%             | 1.44                       | 1.44                                       |
| Dimethyl sulfoxide (DMSO) | 21.47%             | 1.48                       | 1.48                                       |
| Benzene        | 22.29%             | 1.50                       | 1.51                                       |

From these measurements, the corresponding refractive index is evaluated using Eqs. (6) and (7), and the results are reported in Fig. 6(b) and in Table 2.

Since \(\theta_1 = 4.52\) deg, we can use the small-angle approximation and evaluate the refractive index as \(n \approx \tan \delta / \tan \theta_1\); in this case, the spot-size variation becomes

\[
\Delta w = \frac{w_2 - w_1}{w_1} = \frac{h}{d - h} \left( n - \frac{n - n_0}{n_0^2} \right) \left( \frac{n_0 - 1}{n_0} + \frac{n - n_0}{n_0} \right) \tag{8}
\]

that can be evaluated as a linear approximation about the point \(n_0\). For the central value \(n_0 = 1.4\), the spot-size variation corresponds to \(-28.08 + 33.43/\text{R.I.U.}\) (refractive index unit). Using the proposed optical architecture, we are able to analyze a broad range that goes from \(n = 1.2\) to 1.6, but with a limited accuracy that depends on the slope of Eq. (8). If the refractive index range is reduced, the linear approximation furnishes more accurate values.

The refractive index interval and measurement accuracy can be varied by changing the distance \(d\) of the LED source from the lens, i.e., the magnification parameter \(M\) (the cuvette width \(h = 10\) mm is a standard parameter). A spot-size increase of at least 2 pixels can be detected, that corresponds to about \(\Delta w = 1\%\) variation, with an average accuracy \(\Delta n = n_0^2 \Delta w (d-h)/h = 0.03\) R.I.U.
4. Summary

Commercial spectrophotometers used in laboratories employ expensive lasers or monochromators and photodetectors. To reduce the costs, portable POCT for colorimetric analysis mount LED sources and CMOS image sensors; however, their accuracy is limited by diffraction and stray-light effects. We propose a geometrical optics approach to evaluate their accuracy and investigate the influence of the dimension of the image sensor area, where the transmitted intensity is detected. We refer to two different real-time image processing strategies, measuring the average intensity on the sensor area, or evaluating the absorbance by a pixel-by-pixel approach. Furthermore, with the same intensity measurements, we can also evaluate the liquid refractive index, measuring the beam spot-size enlargement and identify the liquid.

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Disclosures

The authors declare that there are no conflicts of interest related to this article.

References

1. P. Luppa and R. Junker, *Point-of-care Testing: Principles and Clinical Applications* (Springer, 2018).
2. S. E. McBurney, D. Chen, A. Scholtz, H. Ameri, and A. M. Armani, “Rapid diagnostic for point-of-care malaria screening,” ACS Sens. 3(7), 1264–1270 (2018).
3. A. Thiha and F. Ibrahim, “A colorimetric enzyme-linked immunosorbent assay (ELISA) detection platform for a point-of-care dengue detection system on a lab-on-compact-disc,” Sensors (Basel) 15(5), 11431–11441 (2015).
4. A. Thiha, J. Fernandes, S. Pimenta, F. Soares, and G. Minas, “A complete blood typing device for automatic agglutination detection based on absorption spectrophotometry,” IEEE Trans. Instrum. Meas. 64(1), 112–119 (2015).
5. S. Sumriddetchkajorn, K. Chaitavon, and Y. Intaravanne, “Mobile-platform based colorimeter for monitoring chlorine concentration in water,” Sens. Actuators B Chem. 191, 561–566 (2014).
6. C. Zhao, G. Zhong, D. E. Kim, J. Liu, and X. Liu, “A portable lab-on-a-chip system for gold-nanoparticle-based colorimetric detection of metal ions in water,” Biomicrofluidics 8(5), 052107 (2014).
7. R. S. Khan, Z. Khursid, and F. Y. I. Asiri, “Advancing point-of-care (PoC) testing using human saliva as liquid biopsy,” in *Proceedings Diagnostics* (2017).
8. L. Shen, J. A. Hagen, and I. Papautsky, “Point-of-care colorimetric detection with a smartphone,” Lab Chip 12(21), 4240–4243 (2012).
9. E. R. Holiday, “Spectrophotometry of proteins,” J. Biochem. 30(10), 1795–1803 (1936).
10. W. S. Hoffman, “Recent advances in photometric clinical chemistry,” Amer. J. clin. Path. 12(9), 449–457 (1942).
11. A. T. Trumbo, E. Schultz, M. G. Borland, and M. E. Pugh, “Applied spectrophotometry: analysis of a biochemical mixture,” Biochem. Mol. Biol. Educ. 41(4), 242–250 (2013).
12. E. J. van Kampen and W. G. Zijlstra, “Determination of hemoglobin and its derivatives,” Adv. Clin. Chem. 8, 141–187 (1966).
13. R. M. Lequin, “Enzyme immunoassay (EIA)/enzyme-linked immunosorbent assay (ELISA),” Clin. Chem. 51(12), 2415–2418 (2005).
14. J. A. Räty, K. -E. Peiponen, and T. Asakura, *UV-visible Reflection Spectroscopy of Liquids* (Springer-Verlag Berlin Heidelberg 2004).
15. J. J. Kim, A. H. Kim, H. B. Oh, B. J. Goh, E. S. Lee, J. S. Kim, G. I. Jung, J. Y. Baek, and J. H. Jun, “Simple LED spectrophotometer for analysis of color information,” Biomed. Mater. Eng. 26(1), S1773–S1780 (2015).
16. K. Long, H. Yu, and B. Cunningham, “Smartphone instrument for portable enzyme-linked immunosorbent assays,” Biomed. Opt. Express 5(11), 3792–3806 (2014).
17. C. Zhang, J. P. Kim, M. Creer, J. Yang, and Z. Liu, “A smartphone-based chloridometer for point-of-care diagnostics of cystic fibrosis,” Biosens. Bioelectron. 97, 164–168 (2017).
18. M. D. Green, H. Nettey, O. Villalva Rojas, C. Pamanivong, L. Khounsaknalath, M. Grande Ortiz, P. N. Newton, F. M. Fernández, L. Vongsack, and O. Manolin, “Use of refractometry and colorimetry as field methods to rapidly assess antimalarial drug quality,” J. Pharm. Biomed. Anal. 43(1), 105–110 (2007).

19. E. Berrocal, D. L. Sedarsky, M. E. Paciaroni, I. V. MeGlinski, and M. A. Linne, “Laser light scattering in turbid media Part I: Experimental and simulated results for the spatial intensity distribution,” Opt. Express 15(17), 10649–10665 (2007).

20. M. Lucidi, M. Marsan, F. Pudda, M. Pirolo, E. Frangipani, P. Visca, and G. Cincotti, “A geometrical-optics approach to measure bacteria concentrations using a in LED-based photometer,” to be submitted to Biomed. Opt. Express.

21. https://www.we-lab.it/en/welabmaker

22. I. Moreno and C.-C. Sun, “Modeling the radiation pattern of LEDs,” Opt. Express 16(3), 1808–1819 (2008).

23. S. A. Kovalenko, “Descartes-Snell law of refraction with absorption,” Semicond. Phys. Quant. Electron. Optoelectron. 4(3) 214–218 (2001).

24. S. Nemoto, “Measurement of the refractive index of liquid using laser beam displacement,” Appl. Opt. 31(31), 6690–6694 (1992).