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Portable infrared lightbox for improving the detection limits of paper-based phosphate devices

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ABSTRACT

Herein, we report the development of a portable, iPhone controlled, and inexpensive infrared lightbox for improving the detection limits of paper-based phosphate devices. Commercial devices utilize the molybdenum blue protocol for phosphate detection. Although these devices are user-friendly and have a long shelf life, their standard mode of operation suffers from low sensitivity since it gives semi-quantitative results by comparing to a color chart. To improve the results, we constructed a compact infrared lightbox that communicates wirelessly with a smartphone equipped with a developed colorimetric analyzer. This system measures the absorbance of radiation for the molybdenum blue reaction in the infrared region of the spectrum. By using the infrared lightbox, the detection limits for two popular paper-based commercial devices were improved by a factor of 4 for the Quantofix devices and a factor of 6 for the Indigo units with repeatability of less than or equal to 1.2% RSD.

1. Introduction

Phosphorus pollution is a main environmental concern since phosphorus is a critical nutrient in the eutrophication of aquatic ecosystems. Fertilizers and detergents are the foremost artificial sources of phosphorus contamination which impacts many streams, lakes, bays, and coastal waters. Phosphorus, however, is not present in its elemental form because of its high reactivity [1] but rather it is found in the form of phosphates i.e. phosphate rocks [2]. Therefore, it is significant to monitor the phosphate content in water and soil in order to maintain the quality of water in these environments within an acceptable range [3]. Since the 1960s, researchers have been working on finding accurate and easy to use methods for detecting phosphate in aquatic media.

The colorimetric technique is a method in which a color is generated based on the interaction between a specific analyte of interest and a chromogenic reagent. This method is generally utilized to determine the concentration of a chemical compound or element in a solution. The most widespread colorimetric approach currently used for the determination of soluble phosphate in water is the molybdenum blue method introduced by Murphy and Riley [4]. They suggested a spectrophotometric laboratory method in which phosphate ions first react with an acidic reagent consisting of a mixture of ammonium molybdate and potassium antimony (III) tartrate to generate phosphomolybdate complex. This complex is then consequently reduced by ascorbic acid to form the phospho-antimonyl molybdenum blue (PAMB) compound [5]. The intensity of the blue product formed is directly proportional to the concentration of phosphate present in the solution with higher concentrations of phosphate producing a darker color. Considerable research has been conducted to improve reagent sensitivity, optimize color intensity and formation time, and decrease interferences from other elements for this phosphate detection method [6,7,8,9,10].

In the past two decades, paper-based devices have become very attractive for making inexpensive, disposable and convenient analytical devices for the determination of reactive phosphate in the field. Jayawardane et al. [11] proposed a 3-D microfluidic paper-based analytical device (μPAD) in which two reagents were dried onto filter paper and separated by an interleaving Teflon sheet. A flatbed scanner and a desktop-based image processing software, ImageJ, were used to measure the color intensity of the blue complex produced after removing the Teflon sheet and introducing sample into the device. Although their μPADs are a portable platform for quantitative determination of phosphate in water and wastewater samples, a digital camera or scanner as well as a PC are required to capture and process images. Thus, these kinds of paper-based devices are not fully instrument-free [12]. Furthermore, the storage conditions for reagents and shelf life are challenging issues for these lab-made devices [13,14]. On the other

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hand, several highly inexpensive and with over two-year shelf life commercial paper-based test strips are available in the market for detecting phosphate in water. Such devices, however, are only appropriate to be used as initial monitoring purposes [15]. This is the case since only an approximate concentration of phosphate can be measured by commercial test kits due to subjective color comparison by the operator and the discrete color concentrations in the included color chart. Therefore, a digital camera along with a portable colorimetric analyzer is needed to provide more accurate and precise quantitative results.

Recently, use of smartphones for rapid point-of-care testing has been gaining a lot of interest. Additionally, because of the specific features of smartphones such as portability, availability, and ease of use, various mobile-based analytical software for specific purposes have been developed on different operating systems (i.e. Android and iOS) [16,17,18,19,20]. While some researchers have relied on smartphones for colorimetric analysis and as the illumination source, several reports have proposed a lightbox with light emitting diodes (LED) to provide consistent visible light intensity conditions in order to achieve more reproducible results [21,22,23,24]. Moreover, as an alternative to the conventional methods, combination of smartphone technology and colorimetric paper-based devices is recently receiving more attention to analyze chemical compounds including heavy metals [25,26,27], anions [28], organics [29], biochemical compounds [30,31], and medical applications [32,33]. A summary and comparison between paper-based analytical devices combined with smartphone technology in different applicable area is found in Table 1. There is no report, however, on the use of infrared LEDs in a lightbox to improve the sensitivity of paper-based phosphate analytical devices. Thus, the design and fabrication of an infrared lightbox that enables the capturing of images in the infrared zone while being wirelessly controlled by a smartphone is an attractive system that warrants further development. A combination of lightbox and a smartphone-based colorimetric analyzing application brings about a portable, field-deployable, and low-cost system that enhances the sensitivity of paper-based phosphate devices.

In this work a sophisticated iPhone-based colorimetric analyzer was developed to conveniently and reliably quantify the color measurements of digital photo images by measuring their RGB pixel values. Moreover, for the first time, a standalone infrared lightbox unit that has an onboard power module, mini wireless router, and a camera without an infrared filter (NoIR), wirelessly controlled by a smartphone was fabricated. The objectives of this research were: (i) to produce an inexpensive, portable infrared lightbox system to create uniform and repeatable lighting environments with an infrared camera to take advantage of the peak absorbance of the molybdenum blue reaction. (ii) to develop an iOS application to measure and analyze the RGB pixel values of pictures. (iii) to present a highly accessible worldwide system for tracking and analyzing field measurements, including location and ambient conditions such as temperature, humidity, wind speed, date, time and time zone, operator ID and other parameters, by developing a data architecture that connects to an online data center. (iv) to combine the aforementioned techniques into a portable reader unit for significant improvements in the measuring capabilities of commercial paper-based phosphate devices, which furthermore acts as a substitute for expensive, lab-equipment spectrometers.

Table 1
Summary of paper-based analytical devices (μPADs) coupled with smartphone technology.

Target analyte	Illumination source	Image capturing device	Colorimetric software	Software/Method Feature(s)	Ref.
Six metal ions	Flat LED lamp	iPhone smartphone	ImageJ	Using a LED lamp as an upward lighting source in detecting simultaneously six metal ions	[25]
Lead ions	Ultraviolet lamp	Smartphone	Smartphone application provided by third-party service provider	Fluorescent paper strips with dual-emission carbon dots (CDs)	[26]
Chromium	Black cloth covered box with a LED	Smartphone connected to a microfocus lens	ImageJ	By combing a miniature DC–DC booster converter unit with a portable battery power bank, a portable power supply was assembled in order to produce a stacking mode	[27]
Nitrite and sulfide	N/A	Desktop scanner and Xiaomi smartphone	ImageJ	Paper-based gas-sensing approach	[28]
Glucose and human IgA	Smartphone build-in LED in an imaging box	Smartphone	ImageJ	Utilizing a V-shape optical pipe to guide the light from the LED of a smartphone to the sides of the box to eliminate the direct reflection of light from the surface of the target.	[29]
Glucose in saliva	Smartphone build-in LED	Smartphone	iOS smartphone app*	Homogeneity of color distribution provided by graphene oxide in a paper-based device	[30]
Yeast	Continuously smartphone build-in LED in video mode	iPhone, Samsung and Huawei smartphones	ImageJ	Accessory-free and independent of phone brand/model due to using a rescaling method and color quantification for single-color shifts.	[31]
Ketamine in saliva	Ambient lighting without flash	Smartphone	Android smartphone app *	A white balance correction algorithm was conducted to eliminate color casts due to varying ambient light on the captured images.	[32]
Carcinoembryonic antigen	Smartphone build-in LED as a flashlight	Smartphone	Android smartphone app *	Using a multi-image processing algorithm to reduce the variations caused by ambient light conditions	[33]
Dye concentration	N/A	iPhone smartphone, digital camera, and scanner	ImageJ	Removing gamma correction of imaging devices, correct color cast and variable background color in order to allow for best quantification of colorimetric data	[34]
Dye concentration and potassium ion sensing	Black box equipped with LED light source on the inner side of its roof	Flatbed scanner and iPhone smartphone	ImageJ	Eliminating noise from underlying paper structure to extract accurate absorbance data	[35]
Phosphate	Portable infrared lightbox	No infrared filter camera	iOS smartphone app*	Using infrared LEDs and no infrared camera to take advantage of maximum absorption of molybdenum blue complex, wirelessly control lightbox, and creating online data center	This work

N/A: not available.
* With application software development.

2. Experimental

2.1. Commercial test kits

Paper-based phosphate measuring strips are commercially available. While they are convenient and have an acceptable shelf life, they lack sensitivity and only measure phosphate in the parts per million (ppm) range. In this work, two popular commercial devices for measuring phosphate in water, the Indigo phosphate test strips (Indigo Instruments, Ontario, Canada) and the Quantofix phosphate test kit (Macherey-Nagel, Düren, Germany), were tested in the visible and infrared light spectra.

2.1.1. Indigo instruments phosphate test strip

A simple dip strip provided by this supplier produces a blue colorimetric response upon contact with phosphate ions. The strip is easy to use, and the operating procedure requires the user to only dip the strip into a water sample for one second and then allow color to form in a few minutes. The resulting color on the detection zone can then be compared to that on a provided color chart with 0, 30, 75, 150, and 300 ppm markings.

2.1.2. Quantofix phosphate test kit

The Quantofix phosphate dip strip provides an improved detection limit but requires more user involvement and manipulation of acidic reagents and chemicals. The user is required to perform a sequence of steps involving the test strip, reusable vials, provided chemicals, and the water sample before color is produced in the detection zone. The user can then compare the color formed with the provided color patches with markings of 0, 3, 10, 25, 50, and 100 ppm.

2.2. Solution preparation

A stock solution of phosphate (100 ppm) was created on the day of testing by dissolving 0.0126 g sodium dihydrogen phosphate (Sigma-Aldrich, MO, USA) in 100 mL of deionized (DI) water. This stock solution was then diluted with DI water to phosphate solutions of concentration 0, 0.1, 0.25, 0.50, 0.75, 1, 2.5, 5, 7.5, 10, 25, 50, and 75 ppm.

2.3. Apparatus

2.3.1. Infrared lightbox unit

The spectrophotometric method is perhaps the standard technique for determining low concentrations of orthophosphate in aquatic solutions based on the molybdenum blue method. He and Honeycutt [36] improved on the work of Dick and Tabatabai [37] and they observed that there are two peaks in the absorption spectra of the phosphomolybdate blue complexes. The maximum absorbance peak occurs in the wavelength of around 850 nm which is in the near-infrared range and is 45% higher in value than the other peak occurring at 700 nm in the visible light zone. Recently, some research groups have successfully used the Near-infrared spectroscopy (NIRS) technique to detect phosphorus at lower concentrations in soil or surface water samples [38,39]. However, their methods are not suitable for on-site measurements or those taken on board ships [40]. Thus, using portable instrumentation to make use of maximum absorption of molybdenum blue in the infrared zone is attractive for improving the analysis capabilities of paper-based devices.

The majority of cameras in the market as well as cellphone cameras are equipped with infrared filters in order to reduce the ambient noise and improve the quality of their pictures. For our lightbox device, a Raspberry Pi 3 Model B+ along with a Pi NoIR Camera v2 and four infrared LEDs were used. The NoIR camera is a camera with no infrared filter especially made for Raspberry Pi. LEDs utilized in the lightbox are EVERLIGHT's Infrared Emitting Diode (HIR7393C) with peak wavelength of $\lambda_p = 850$ nm, spectral bandwidth of $\Delta\lambda = 45$ nm, and radiant intensity of $I_e = 7.8$ mW per steradian (mW/sr). A small inexpensive computer board powered by a 2.5 A power supply, the Raspberry Pi can become an operational computer once connected to a monitor, keyboard, and mouse. However, to have a fully portable unit suitable for field measurements, we were able to control the raspberry Pi remotely by cellphone and without use of any peripherals. This was accomplished via the secure shell method (SSH) which provides access to the Raspberry Pi's command-line interface, and virtual network computing method (VNC) which replicates the graphical desktop, so the cellphone would be used instead of peripheral instruments (i.e. monitor, keyboard, and mouse) for the Raspberry Pi. The cellphone and raspberry Pi can be both connected to the mini wireless router while having the same IP address. The cellphone can then issue commands to the Raspberry Pi to turn on the IR LEDs in the lightbox, activate the camera and to capture one or more images at certain settings. Another command from the cellphone directs the raspberry Pi to transfer the images it stored on its SD card to the cellphone's gallery through the file transfer protocol (FTP). Finally, our colorimetric analyzing app is utilized for instant chemical quantification. Fig. 1 shows a schematic of the lightbox consisting of a raspberry Pi 3, a Pi No IR filter Camera v2, four infrared LEDs, a mini router, and a mini external battery as a chargeable resource for supplying power to the raspberry and the router.

2.3.2. Colorimetric analyzer

The colorimetric analyzer was built for iOS using Apple's XCode IDE. An iPhone-based application was chosen since iOS phones are built by one company (Apple) which provides a homogeneity in devices that greatly aids development and testing. This is simply not possible with Android as there are several different vendors with significant contrast in hardware. This is not to say that an Android version will not or cannot be built, but rather that iOS provides several advantages in the research phase prior to a full-scale production release. The application was designed as a simple image processing app capable of analyzing RGB pixel values from photos. The user first picks an image using the app and is then able to analyze the region of interest using an adjustable selection zone. The app then measures the RGB values of all the pixels encompassed by the selection. The average red, green, and blue pixel count along with grayscale are computed for the selection as shown in Fig. 2 (a). These values can then be used to compute phosphate concentrations based on a pre-programmed calibration curve. In addition, pixel histograms are created for even further analysis if necessary. This application was designed with generalization in mind. In other words, the image processing capabilities along with customizable calibration curves can extend the analyzer's use to other colorimetric detection methods and chemicals.

2.3.3. Online data center

An online data center was constructed in order to interface with measurements taken by the app in the field. Whenever a sample is analyzed in the app, data is stored in the backend with GPS coordinates, humidity percentage, temperature, and phosphate levels. This data can then be uploaded to an online data center that aggregates all the measurements found in a network of devices. This system was built in order to quickly provide insights to phosphate levels of various locations in the field. A heatmap of phosphate levels in a body of water for example, can easily be created using such a system (Fig. 2(b)). In addition, the storage of scientific data in a centralized database can be used for advanced analytics by both scientists and the public. This procedure encourages collaboration among fellow researchers and accelerates both the collection and interpretation of field data. One can refer to [13] for further details about the app and online data center.

2.4. Analytical procedure of the colorimetric analyzer

A completely randomized test with three replicates was run for each test kit. All glassware and reusable vials were first washed with a phosphate free detergent. They were then washed with 1 M hydrochloric

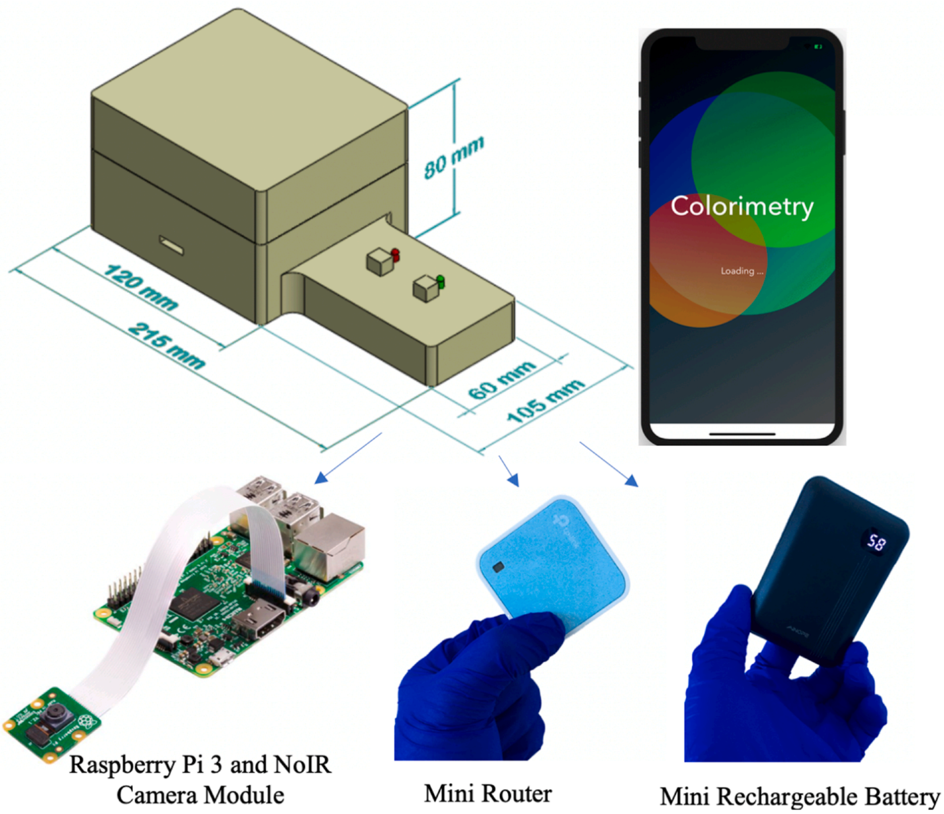


Fig. 1. A schematic of lightbox and its components wirelessly controlled by cellphone.

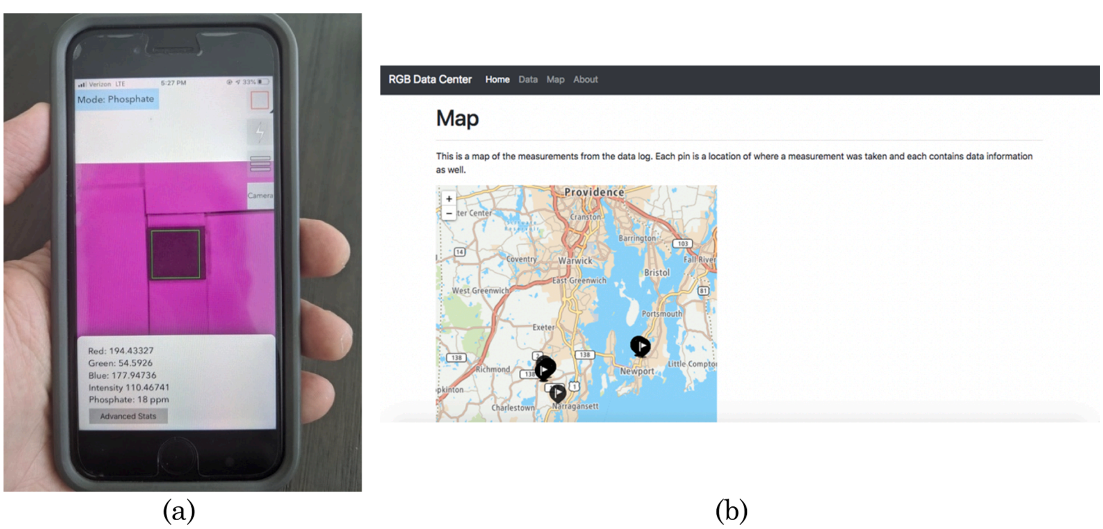


Fig. 2. Main view of the colorimetric analyzer app (a) and map page of the online data center (b).

acid and rinsed with DI water three times. The reusable vials of the Quantofix kit were washed with 1 M HCl three times, rinsing with DI water in between each wash before every test. The tests were run following the manufacturers' instructions. However, in order to achieve the highest sensitivity of the devices, the effect of reaction time was studied. The images were captured from the detection zone of the devices in the visible light spectrum using a desktop scanner (Canon TS6020) at a resolution of 600 DPI and in the infrared zone by using the lightbox unit. The intensity of the red color for the visible light and the grayscale for the infrared light were then measured to compare the results.

3. Results and discussion

3.1. Effect of the reaction time

The suppliers of Indigo and Quantofix test kits have specified the reaction time for their devices. Color produced in the detection zone of Indigo and Quantofix devices should be compared with the provided color scales after 3 min and 1 min, respectively. The reaction time was studied for phosphate concentrations of 50 and 100 ppm for the Quantofix and Indigo test kits, respectively, in which image capturing was performed each minute by the scanner and the lightbox. Fig. 3(a) shows

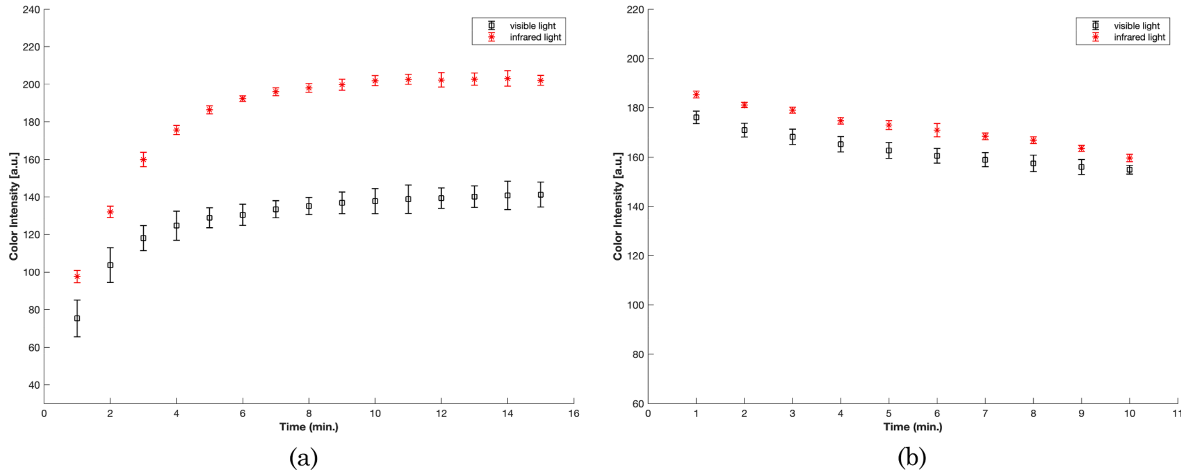


Fig. 3. Effect of reaction time on color intensity in visible and infrared light for Indigo (a) and Quantofix (b) test kits.

that the color intensity for the Indigo test kits kept increasing up to 8 min and no major changes were observed after that. Thus, reaction time of 8 min for this device was selected for further experiments. On the other hand, as shown in Fig. 3(b), the reaction between the reagent and water rapidly occurred in the Quantofix devices and a gradual decrease of color intensity was observed. Therefore, the images were captured within 1 min.

3.2. Effect of distance between the infrared LEDs and strips

Different distances between the infrared LEDs and strips were studied by analyzing 100 ppm phosphate and blank solution with the Indigo test strips. The strips were placed in the center of the infrared lightbox while the distances between the plane that the LEDs are located in and the strips varies in the range of 20–35 mm. Since the position of the camera is fixed in the inside roof of the lightbox, for each set of tests, the focus of the NoIR camera was adjusted by rotating its lens manually. The grayscale intensities at different distance between the LEDs and the strips for 100 and 0 ppm phosphate solutions were measured. The obtained results in Fig. 4 indicated that while the color intensities for 100 ppm phosphate and blank solution at 20 and 35 mm distances are higher than that of at 25 and 30 mm distances, the difference bars between color intensity of these two concentrations are smaller. This is the case because when the position of the strips is too close or too far from the LEDs, they will be located at the beyond of view angle of the LEDs. Therefore, the camera will capture an image at a darker spot from the strips and color intensity seems to be more intense. The different value between the color intensity of 100 ppm and blank solution for the distance 25 and 30 mm were subjected to a one-way analysis of variance. Considering the calculated (2.896) and critical (7.709 at $\alpha = 0.05$) F-values, no significant difference (p-value $= 0.164$) was statistically observed. As a result, 25 mm distance between the infrared LEDs and strips was selected for further experiments.

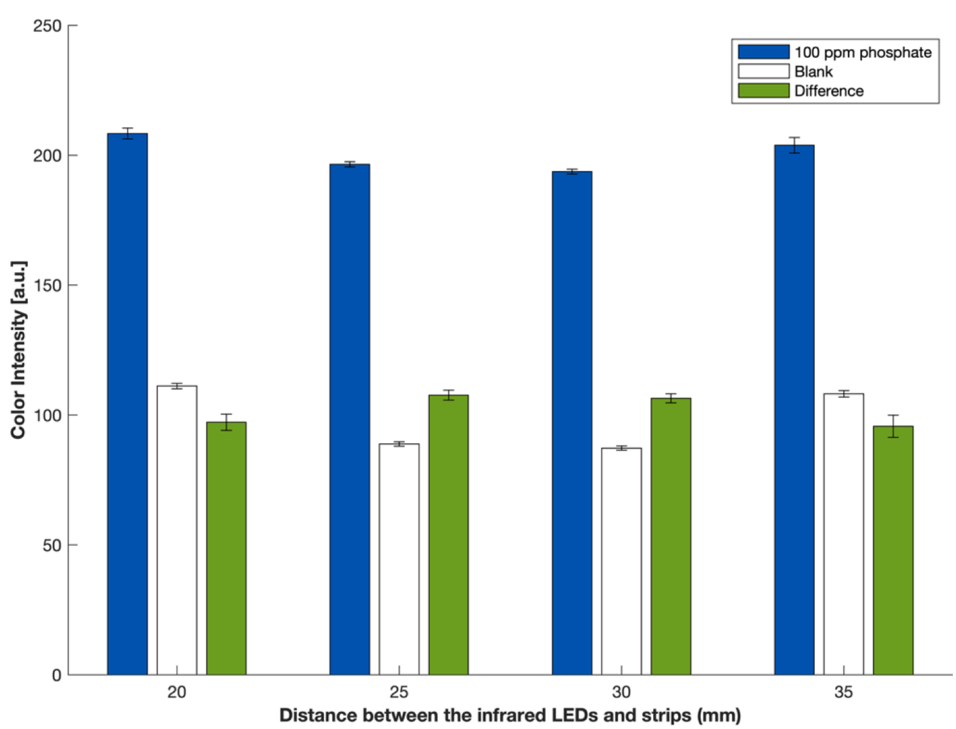


Fig. 4. Effect of distance between the infrared LEDs and strips.

3.3. Effect of light distribution on color intensity

Four levels of constant infrared light intensity inside the lightbox were considered by turning on different numbers of infrared LED (1, 2, 3, and 4 LEDs) in order to study the effect of light distribution on color intensity. The grayscale color observed from the Indigo test strips in 100 ppm phosphate and blank solution was measured under these four light distributions. Fig. 5 shows that different value between obtained results for 100 ppm and 0 ppm phosphate keeps enhancing by increasing the numbers of infrared LEDs which means improving the light distribution in the lightbox. Hence, four infrared LEDs was chosen for further measurements.

3.4. Improvement of phosphate test strips

The advantage of imaging in a remotely controlled infrared lightbox on the analytical performance of two commercial phosphate test kits based on the molybdenum blue method was evaluated. Following suppliers' instructions and the reaction times discussed in 3.1, a series of standard phosphate (1–100 ppm for Indigo test and 0.1–50 ppm for Quantofix) were measured with three replicates under visible and infrared light conditions. The red color intensity was used to create a calibration curve for the images captured by the scanner since the red intensity shows the largest change over the range of concentrations used. In contrast, due to the lack of visible light in the infrared lightbox, all color channels detect the IR radiation. Thus, the intensity of grayscale was selected to depict the calibration graph for the images captured by the NoIR camera in the lightbox. All values were subtracted by a corresponding blank value and represented in Fig. 6 with a logarithmic x scale along with a linear inset plot for lower phosphate concentrations. As shown in this figure, standard deviations for the infrared data are significantly smaller than those in the visible light. For precision evaluation, 100 ppm phosphate for the Indigo and 50 ppm phosphate for the Quantofix test kit were studied with five replicates. The percent of relative standard deviation (%RSD) values for the visible and infrared light were decreased from 3.3% to 1.2% for the Indigo and from 2.1% to 0.51% for the Quantofix test strips, respectively. This proves the reproducibility of light conditions in the box and its superior accuracy for capturing images in the infrared spectrum. In fact, since the lightbox is a close system in which no other light source is available aside from the infrared LEDs then this created a very reproducible lighting conditions and this can be seen in the decrease of %RSD. Moreover, inset plots in Fig. 6 demonstrate that while the color intensities for visible light in the lower concentrations of phosphate are indistinguishable due to either overlapped intensity values or small slope of increasing values, such differences are quite distinct for those in the infrared light.

MATLAB curve fitting toolbox was used to fit an exponential curve of the form $y = a - b \times \exp(-x/c)$ to the entire range of the data. Table 2 shows the values of coefficients for the fitted calibration curves' equations and R^2 values for two test kits in different light conditions. Some researchers [11,22] split their results into two or three concentration ranges in each of which the intensity of analytes vs. concentrations is linear. Then, they utilize linear equations to find the optimum calibration line for each range and the lower range is used to calculate analytical performance of devices (e.g. limit of detection and quantification). Nevertheless, others may fit a model to the entire range of their analytical data using a nonlinear [41] regression especially an exponential curve [42,43]. As indicated in Table 2, the exponential curves were fitted well to the entire range of data ($R^2 > 0.99$). The obtained calibration equations are being used as a single curve for each condition of the different devices in the developed app as a convenient and reliable analytical reader for the determination of phosphate in the field.

Limit of detection (LOD) and quantification (LOQ) were calculated using the following formulas $y_{LOD} = y_{blank} + 3\sigma_{blank}$ and $y_{LOQ} = y_{blank} + 10\sigma_{blank}$. Where y_{blank} and σ_{blank} are the mean and standard deviation of the blank sample i.e. 0 ppm [44]. The symbolic math toolbox on MATLAB was used to find the concentrations that gave the y_{LOD} and y_{LOQ} values calculated by the fitting functions used. Table 3 summarizes the LOD and LOQ calculated for two commercial test strips in the visible and infrared light. An LOD and LOQ of 9.2 and 39.2 ppm phosphate respectively, were obtained using the colorimetric method with visible light for the Indigo test strips. The LOD and LOQ were reduced quite significantly to 1.4 and 7.9 ppm phosphate respectively, in the infrared light, improving the sensitivity of the device by approximately a factor of 5. Similarly, Quantofix devices had an LOD and LOQ of 1.3 and 4.5 ppm phosphate in the visible light. However, these values were

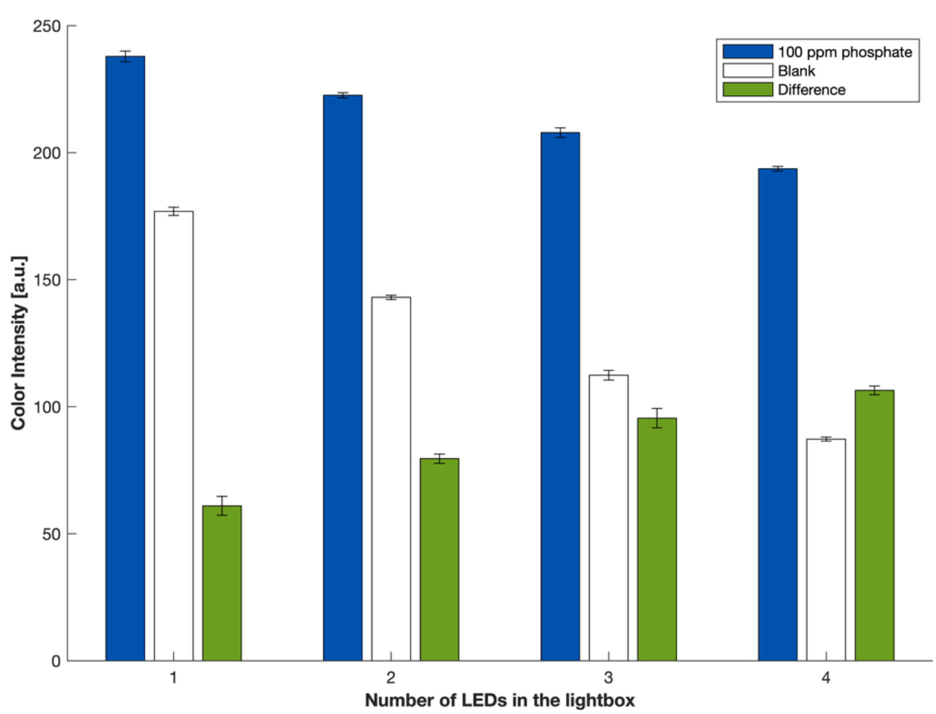
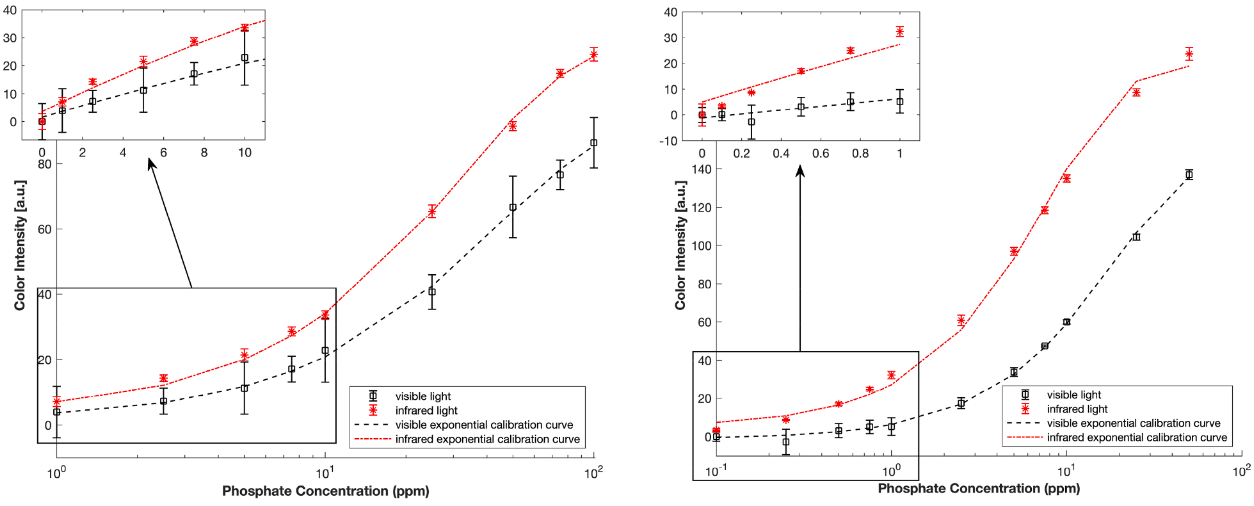


Fig. 5. Effect of the illuminated light intensity on color intensity.



(a) (b)

Fig. 6. Calibration curves for phosphate response in the visible and infrared light conditions for the Indigo (a) and Quantofix (b) test strips.

Table 2
Comparison of the coefficients (with 95% confidence bounds) and the determination coefficients (R^2) for the exponential curve (y = a-b*exp(-x/c)) fit to the visible and infrared data.

Test kit	Visible light				Infrared light			
	a	b	c	R^2	a	b	c	R^2
Indigo	94.5 (±6.7)	93 (±6.2)	42.94 (±7.60)	0.9981	118 (±5.8)	114.4 (±5.4)	32.22 (±4.78)	0.9983
Quantofix	146.7 (±6.1)	147.9 (±5.8)	19.12 (±1.82)	0.9989	194.1 (±9.2)	188.9 (±9.9)	7.98 (±1.11)	0.9968

Table 3
Comparison of Limits of Detection (LOD) and Limits of Quantification (LOQ) in the visible and infrared light conditions.

Test kit	LOD (ppm)			LOQ (ppm)			Testing range (ppm)
	Visible light	Infrared light	Improvement factor	Visible light	Infrared light	Improvement factor	
Indigo	9.200	1.429	6.4	39.154	7.917	4.9	1–100
Quantofix	1.352	0.337	4	4.479	1.182	3.8	0.1–50

improved by a factor of 4 in the infrared light and phosphate concentrations were detected and quantified at 0.3 and 1.2 ppm respectively. This improvement is not limited to the commercial devices and may be applied to other paper-based devices that use the molybdenum method to detect phosphate in water or soil.

Comparing with relevant literature, Jayawardane et al. [11] reported a paper-based device with a working range of 0.6–30 ppm phosphate and can detect phosphate in the parts per billion range. However, their device is not a fully instrument-free portable device and shelf life is a main concern since it ranged from a couple of days in room temperature to around 112 days at temperatures below −20 °C. These issues are not of concern for commercial devices since their shelf life is at least two years. Using the affordable Quantofix test kits and the portable infrared lightbox, one can detect phosphate in parts per billion range in the field. For further assessment, a short comparison between two previously reported paper-based devices and Quantofix device with the infrared lightbox for determination of phosphate in water is provided in Table 4.

The lightbox system can be used to detect other target objects if the peak of the absorbance for their reactions also occur in the infrared or near infrared zones especially between 800 till 900 nm. However, this idea can be used for other target objects by only changing the infrared LEDs with new LEDs which have wavelength compatible with peak absorbance of target reactions.

Table 4
Comparison of using Quantofix strip by the presented method with two previously reported devices.

Device	Working range (ppm)	LOD (ppm)	Repeatability	Reaction time (min)	Shelf life	Ref.
3D paper-based folding device	0.6–30	0.153	Less than 2% RSD	40	122 days stored in freezer at <−20 °C	[11]
2D paper-based device	0.1–10	0.160	N/A	4	35 weeks (245 days) in refrigerator at <4 °C	[45]
Quantofix paper-based dip strip with the infrared lightbox	0.1–50	0.337	Less than 0.51% RSD	1	2 years (730 days) under ambient conditions	This work

N/A: not available (not reported).

4. Conclusions

The portable and low-cost infrared lightbox developed in this paper along with the iPhone-based analyzer app are of great significance since they demonstrate that complex sensors and expensive spectrometers are not required to take advantage of the infrared spectra. The absorption peak of the molybdenum blue reaction in the infrared region was utilized by a remotely controlled lightbox. This leads to a significant improvement in the limit of detection and quantification of two common commercial devices by a factor of up to 6. LOD decreased from 9.2 to 1.4 ppm for the Indigo strips and from 1.35 to 0.34 ppm for the Quantofix phosphate test strips. Moreover, maintaining constant illumination on samples while capturing images by the lightbox provided accurate and repeatable results with RSD values less than 1.2%. This approach is not exclusive to the detection of phosphate since the lightbox and colorimetric analyzer can be further developed in order to account for other colorimetric reactions such as Nitrate and Nitrite. Additionally, the infrared lightbox can be used to improve sensitivity of other commercial as well as lab-made paper-based devices.

CRediT authorship contribution statement

Hojat Heidari-Bafroui: Conceptualization, Methodology, Software, Formal analysis, Investigation, Writing - original draft, Visualization. **Brenno Ribeiro:** Conceptualization, Software, Writing - review & editing. **Amer Charbaji:** Conceptualization, Validation, Formal analysis, Writing - review & editing. **Constantine Anagnostopoulos:** Conceptualization, Supervision, Writing - review & editing, Project administration, Funding acquisition. **Mohammad Faghri:** Conceptualization, Supervision, Writing - review & editing, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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