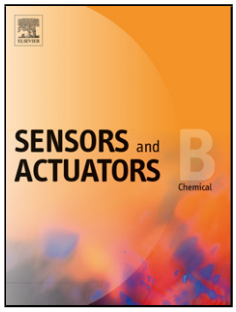

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An Ultra Low-Cost Smartphone Device for In-situ Monitoring of Acute Organophosphorus Poisoning for Agricultural Workers

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Highlights

- A smartphone based optical spectrum detection device with novel lens-less design.
- High accuracy and high sensitivity for IL-6 biomarker detection and spiked organophosphorus poisoning sample diagnosis.
- Assessment of the device for organophosphorus poisoning with blood samples from agricultural workers. The results showed good agreement with that of conventional laboratory equipment.

Abstract

In this work, we present an ultra-low-cost smartphone device for in situ quantification of OP poisoning severity. The performance of the lens-less smartphone spectrum apparatus (LeSSA) is evaluated using standard human Interleukin-6 (IL-6) immunoassay kits. Upon dose-response curve fitting, LeSSA demonstrates an accuracy of 99.5%. The limit of detection (LOD) of LeSSA was evaluated through comparison of 6.4 pg/ml with standard laboratory grade UV-vis spectrophotometer at 5.5 pg/ml. Evaluating the capacity of LeSSA in spike solution by combining plasma cholinesterase (PChE) and human plasma shows consistency at agreement of 97.6% between LeSSA and the laboratory instrument. For application demonstration, the activity of PChE for 24 agricultural workers' plasma samples was measured with LeSSA, showing exceptional agreement (r2=0.92) with the laboratory instrument reference. In addition to near laboratory grade accuracy, the total manufacturing cost of LeSSA is only $20 USD highlighting it's affordability. With LeSSA, clinicians can evaluate OP poisoning severity without the need to transport patient samples to facilities at far distances. Utilizing LeSSA, immediate results can be used for administration of appropriate treatment.

Keywords: Organophosphorus Poisoning; Point-of-care diagnostics; mobile device;

1. Introduction

Acute organophosphorus (OP) poisoning occurs most commonly via deliberate ingestion in attempted suicide, and less commonly by accidental exposure [1]. The World Health Organization (WHO) estimates that there are more than 3 million cases of OP poisoning annually, of which over 250,000 result in death [2,3]. After exposure to OP agents via dermal, respiratory, or oral routes, OP agents can rapidly phosphorylate the serine hydroxyl group of cholinesterase enzymes (ChE), and irreversibly inhibit the activity of the enzyme [4,5]. The inactivation of acetylcholinesterase (AChE) obstructs the degradation of transmitter acetylcholine (ATCl), seriously interfering with central nervous transmission,[6,7] and ultimately leading to acute renal injury, [8,9] organ failure, [10] and even death [11,12]. Onset of poisoning symptoms such as diarrhea, vomiting,

and sweating often appears within minutes to hours. The most common rapid diagnostic test for OP poisoning is to measure plasma ChE (PChE) activity in whole blood or AChE activity in red blood cells [13]. Exposure to OP agents inhibits PChE and AChE, and their activities decrease. The severity of clinical manifestations of OP poisoning is strongly associated with the decrements in PChE or AChE activity [14] The normal ranges of PChE activity for males and females are 10.1-22.1 U/mL (units per milliliter) and 8.3-20 U/mL, respectively [15]. PChE activity in the range of 8.0-10.1 U/mL is considered a borderline low and values less than 8.0 U/mL are considered definitely abnormal. A decrease of 50% or more in PChE activity is considered as severe poisoning, a decrease of 10-50% is graded as moderate poisoning, and less than 10% decreasing PChE activity is classified as mild poisoning [16].

Unfortunately, the hospitals in rural/remote or developing areas where OP poisoning occurs most frequently usually lack central laboratory equipment to perform the rapid testing of PChE and AChE activities. Most poisoned patients or collected samples are transferred or shipped to city hospitals. The turnaround time takes hours or even days and delays treatment of the patient. Inter-hospital transfer leads to longer length of hospitalization stays, worse clinical outcome, and higher mortality rate [17]. The fatality achieves 15% to 30%, dependent upon the delay in initiation of treatment, the type of OP and the amount of exposure [1]. However, this situation can be improved by providing point-of-use diagnostic testing at hospitals or emergency clinics. Here, we propose an ultra-low-cost pocket-size lensless smartphone spectrometer apparatus (LeSSA) to allow clinicians in developing or rural areas to undertake on-site assessment of the severity of OP poisoning.

Recently, mobile-health (mHealth) technology has been considered as the most promising solution to enhance clinical decisions at the point of care and to improve healthcare delivery. mHealth is defined by the WHO Global Observatory for e-health as “medical and public health practice supported by mobile devices, such as mobile phones, patient monitoring devices, personal digital assistants and other wireless devices” [18]. The development of mHealth diagnostics technology allows clinicians to diagnose diseases on site, to immediately transmit data online, and to implement timely treatments [19,20]. The advancement of smartphone technologies and the widespread smartphone

popularity make mHealth diagnostic devices as low-cost, portable on-site diagnostic tools, especially in less-resource remote/rural areas where highly trained personnel and sophisticated equipment aren't available [21,22].

In a conventional diagnostic laboratory, optical spectrometers are one of the most indispensible instruments. Standard laboratory optical spectrometers, which are equipped with highly sensitive detectors (e.g. photomultiplier tubes), dedicated light splitting grating, and wavelength selection mechanisms, are very expensive and bulky. In addition to conventional diagnostic equipment, there is a cholinesterase test kit and portable analyzer available that has been applied to clinical samples [23,24]. To fulfil the on-site diagnosis/detection and transmittable diagnostic results to doctors, much effort has been devoted to developing smartphone optical spectrometers [25-27]. Currently, the most common detection modality for smartphone spectrometers is absorption for colorimetric assays [28-31]. Typical smartphone optical spectrometers use lenses to create focused and collimated light beam [32-34]. The optical lenses effectively enhance the light collection and reduce scattering light. However, glass lenses increased total weight, size, and cost of the device.

In this study, the LeSSA can achieve a clear optical spectrum with no lens needed by using paper apertures to filter out scattering light and to collimate the illumination light. The two aligned paper apertures placed in front and behind the cuvette minimize the scattered light signal. The lensless design ensures the LeSSA has a compact size and ultra-low cost, while maintaining ease-of-operation and sensitivity comparable with standard laboratory spectrometers. For precisely controlling the optical parameters under different circumstances, a smartphone application (App) is developed.

In order to validate the proposed LeSSA technology, we first assayed standards of human Interleukin 6 (IL-6) protein in human serum by enzyme-linked immunosorbent assays (ELISA). IL-6 protein has been proven to be an important biomarker associated with inflammatory diseases, [35] diabetes, [36] and cancers [37,38]. Next, we assessed the LeSSA by measuring PChE enzyme activity, as a demonstration of the application of the LeSSA as a point-of-use diagnostic device for acute OP poisoning. In clinics, the standard diagnostic tests for OP poisoning measure PChE activity from drawn plasma or AChE activity from blood [39]. For the first trial, paraoxon-ethyl was chosen as the OP

model and spiked into PChE standard solutions and purchased human plasma, respectively. Then, we tested PChE activity in plasma samples obtained from 24 OP-exposed agricultural workers. All reading results by the LeSSA were compared with a standard laboratorial microplate reader. The reliability and accuracy of the LeSSA were presented in this work.

2. Material and methods

2.1. Design and fabrication of the Lensless smartphone spectrometer apparatus LeSSA

The LeSSA was designed based on an Apple iPhone 5s (Apple Inc., CA, USA). The iPhone 5s is equipped with a rear iSight 8-megapixel sensor, 1.5 µm pixels and an f/2.2 aperture. The LeSSA is comprised of three main parts: (1) an iPhone 5S holder with a digital versatile disk (DVD) grating embedded, (2) a cuvette holder, and (3) two adapters with apertures embedded. The total length of the LeSSA is 137 mm and the weight is 231 grams, including an iPhone 5S and a xenon light source. The actual LeSSA and the schematic structure are shown in Fig. 1(a) and 1(b), respectively. A commercial 3D printer (Einstart S, Shining 3D, Hangzhous, Zhejiang Province, China) was used for rapid prototyping the smartphone holder and the cuvette holder. The 3D printer filament was biodegradable black opaque 1.75 mm polylactic acid (PLA) (Shining 3D, Hangzhou, Zhejiang Province, China) which can minimize light leakage through the holders. The fused filament fabrication (FFF) method was applied to deposit melted thermoplastic into layers to form a 3D object. A small piece of round shape grating (diameter around 10mm) was cut down at the very edge section of a 16X DVD-R (4.7GB, Kodak, NY, USA) by scissors, the details of operation and characteristics of the grating can be found in [29]. The DVD grating with sub-micron periodic groove pattern generates diffraction when light travel through it. To collimate illumination light and to eliminate unfocused, scattered light in light path, two laser-cut (Trotec Speedy 300, Trotec., MI, USA) black paper apertures (1 mm diameter) were inserted on the both sides of the cuvette holder. A mini xenon bulb flashlight (Solitaire, Mag Instrument Inc., CA, USA) was used to provide a continuous spectrum illumination. The cost, including materials, manufacturing, and components (not including the iphone), of the device is about $20.

Insert Fig. 1(a) and 1(b) here

2.2. Human IL-6 Immunoassays

A commercial ELISA kit for measurement of IL-6 in human serum was obtained from Invitrogen (Carlsbad, CA, USA). The standard protocol from the manufacturer was followed. A calibration series from 3.5 to 125 pg/mL was created via serial dilution of IL-6 standard. This concentration range covers the typical range of IL-6 concentrations in human serum. All samples were assayed in duplicate, and then the absorbance measured by our LeSSA and a standard laboratory microplate reader (Tecan Safire2, Männedorf, Switzerland).

2.3. PChE Enzymatic Activity Assays.

All required materials and reagents, including PChE standard solutions, Acetylthiocholine iodide (ATCl), 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB), paraoxon-ethyl, and phosphate buffer saline (PBS) were purchased from Sigma-Aldrich (St. Louis,MO, USA). PChE standard solution (2 μg/mL), ATCl solution (2 wt‰), DTNB solution (0.2 wt‰), and serial-diluted paraoxon-ethyl standard solutions were all prepared with PBS (pH 7.4). We used paraoxon-ethyl as the OP model and spiked paraoxon-ethyl into PChE standard solution and purchased human plasma, respectively. Paraoxon-ethyl inhibited PChE enzyme and led to PChE activity decreasing. The assay procedure was as follows: 50 μL PChE standard solution/purchased human plasma were added into centrifuge tubes, and then 50 μL paraoxon-ethyl serial-diluted solutions (0-100 nM) were respectively added into each centrifuge tubes to mix and to incubate. After 15 min incubation at 37 ℃, 100 μL ATCl solutions were added into each tube for 15 min incubation (37 ℃). In this step, uninhibited PChE enzyme hydrolyzed ATCl and thiocholine was produced. Then, 200 μL DTNB solutions were added to each tube to react with thiocholine producing the yellow 5-thio-2-nitrobenzoate anion (TNB), which was detected using the microplate reader and the LeSSA at 410 nm wavelength [40-42] .

2.4. Assaying PChE Activity in Plasma from Agricultural Workers.

The plasma samples were collected from agricultural workers in Pakistan who were occupationally exposed to OPs. The assay procedures were as follows: 100 µL 120-fold diluted patients' plasma samples were incubated with 100 µL ATCl for 15 min at 37 ℃, following by adding 200 µL DTNB solution. The color intensity was quantified by both the LeSSA and the microplate reader.

2.5. Device Calibration and Image Analysis.

The wavelength axis for the LeSSA was calibrated by using three laser pointers with wavelengths of 405, 532, and 650 nm. With the DVD diffraction grating, the lasers respectively projected the first-order diffractions on different locations of the image. To map wavelengths and pixels, we measured the distance between the laser projections in pixels shown in Fig. 2(a). There were 978 pixels between red and purple laser projections which mapped to 245 nm wavelength difference. The wavelength was divided by the pixel counts between the red and purple laser projections and the resolution of 0.2505 nm/pixel was obtained. Ambient conditions fluctuate constantly and default smartphone camera software auto adjusts accordingly to take pictures for general consumer. However in scientific measurements such adjustments could create inconsistency for images obtained between samples. To assure the optical settings are the same under different environmental conditions, a photo-taking application (App) was developed to manually adjust optical settings such as controlled burst mode (a number of pictures continuously taken in respect to the exposure time setup), focus, and sensitivity through the ISO number, exposure time (ET), and Red/Green/Blue (RGB) balance shown in Fig. 2(b). A total of five consecutive images were captured for each sample for averaging results. We first captured the images of flashlight spectrum, followed by the assayed samples in the cuvette. A MATLAB (MathWorks, MA USA) program based on a Graphical User Interface (GUI) was coded for analyzing the transmittance spectra. Each pixel of the image is comprised of RGB up to 255 unit per color. The MATLAB program is used to read the intensity of each pixel along the spectrum and details of the algorithm can be

found in the previous work [29]. The read intensity (transmittance T) is then converted to absorbance (A) by Equation (1).

$$A = -\log_{10} T \qquad (1)$$

Insert Fig. 2(a) and 2(b) here

Results & Discussion

2.6. Validation of the LeSSA by Immunoassaying Standard Human IL-6.

The standard IL-6 ELISA kit was used to validate the linearity of the LeSSA. The serial diluted human IL-6 with concentrations from 3.5 to 125 pg/mL were read by the LeSSA and the standard laboratory microplate reader. We examined the relationship between absorbance at wavelength 450 nm and IL-6 concentration obtained from the LeSSA and the microplate reader in Fig 3(a) and 3(b), respectively. The readouts were fitted by a four-parameter logistic (4PL) equation, shown in Equation (2) [43].

$$f(x) = A + \frac{B-A}{1+(klog_{10}c)^s} \qquad (2)$$

where *f(x)* is absorbance related to the IL-6 concentration. A and B represents the maximum and the minimum asymptote, respectively. *k* is the inflection point, *c* is concentration of IL-6, and s is the hill slope to describe the steepness of the dose-response curve. The goodness-of-fit achieved 99.54% by the LeSSA and 99.51% by the laboratory microplate reader. The comparative goodness-of-fit from both instruments strongly suggested that the readout of IL-6 concentration by the LeSSA is as accurate as that from the laboratory instrument. We also investigated the limit of detection ($LOD_{3\sigma}$) of both instruments [44]. We first prepared three blank samples using the same assay kit. The blank samples were used all the reagents and prepared followed all assay steps only without samples. Then the three blank samples were measured absorbance, *f(x)* with standard deviations (σ). The instrument detection limit (IDL=$f(x)_{LOD}$) is determined by $f(x)\pm 3\sigma$. Any signals lower than the IDL is referred to as the noise level. For analytical

purposes, we next convert the IDL from absorbance to the lowest detectable concentration of the analyte ($c_{LOD3\sigma}$) calculated by Equation (3).

$$c_{\mathrm{LOD}_{3\sigma}} = 10^{\frac{1}{k}\left(\frac{B-A}{f(x)-A}-1\right)^{1/s}} \qquad (3)$$

The lowest detectable concentration of human IL-6 ($c_{LOD3\sigma}$) was 5.5 pg/mL by the microplate reader and 6.4 pg/mL by the LeSSA, respectively shown by dashed lines in Fig. 3(a) and 3(b). Thus the LeSSA provides a comparable LOD to the laboratory instrument. Furthermore, to assess the reliability of the LeSSA, the correlation coefficient between the microplate reader and LeSSA is shown in Fig. 3(c) ($r^2 = 0.9711$).

Insert Fig. 3(a), 3(b) and 3(c) here

2.7. Validation of the LeSSA by Measuring PChE enzyme activity

PChE activity was determined by measuring absorbance at 415 nm, as described in the experimental procedure, after OP (paraoxon-ethyl) using both the microplate reader and the LeSSA. Absorbance was converted to the PChE concentration using Beer's Law as shown in Equation (4).

$$\frac{\mathrm{U}}{\mathrm{mL\ blood}} = \frac{(\mathrm{Absorbance})}{\varepsilon\, l} \times \mathrm{dilution\ factor} \qquad (4)$$

where ε is the molar absorption coefficient ($mM^{-1}\ cm^{-1}$) and l is the light path (cm). In this study, ε of Ellman's reagent (DTNB) in dilute salt solutions (0.1 M NaOH or 0.1 M phosphate buffer with 1 mM EDTA, pH 7.27) at 412 nm is 14.15 $mM^{-1}\ cm^{-1}$ [45]. In the first trial, serial-diluted paraoxon-ethyl solutions were spiked into the PChE standard solutions. In Fig. 4(a), PChE activity decreased with increasing OP concentration. The linear correlation (r^2=0.98) achieved by the LeSSA to quantify the OP concentration was comparable to the linearity of the standard microplate reader. (r^2=0.96). In Fig. 4(b), we assess the agreement between the two instruments, the linear correlation between the

LeSSA and the microplate reader showed the strongly agreement with each other (R^2=0.99).

Insert Fig. 4(a) and 4(b) here

In the second trial, we spiked different concentrations of paraoxon-ethyl into the purchased human plasma to simulate real samples. In Fig. 5(a), as expected, the PChE activity decreased linearly with the increase in OP concentration. The linear response measured with the LeSSA (r^2 = 0.99) is comparable to the performance of the lab instrument (r^2=0.98). The agreement between the two instruments is excellent (r^2 =0.98), shown in Fig. 5(b). These two sets of spiked experiments validated the accuracy and reliability of the LeSSA compared with the results of the laboratory instrument.

Insert Fig. 5(a) and 5(b) here

2.8. Assessment of the LeSSA by Testing Agricultural Workers' Plasma Samples

Finally, we used the designed LeSSA to measure PChE activity in plasma samples obtained from a total of 24 agricultural workers who were occupationally exposed to OPs. After running the described assay procedures, the obtained absorbance was converted to PChE activity calculated by Equation (4). Fig. 6 shows the linear correlation between the results of the PChE enzymatic activity measured by the LeSSA and the microplate reader (r^2 = 0.92). It is worth noted that the lowest data point shown in Fig. 6 represents that the instrument limit detection (ILD) of LeSSA is not as low as ILD of the laboratorial microplate reader. While we take advantage of the portable point-of-care benefit of LeSSA, we should aware the results of low PChE activity samples need to further confirm regarding to LeSSA's ILD. This only illustrates that the reportable range of LeSSA is smaller than the laboratorial equipment, not related to the accuracy of LeSSA. We have proved the comparative accuracy between LeSSA and the laboratorial equipment.

Insert Fig. 6 here

3. Conclusions

In this work, we developed an ultra-low-cost lens-less smartphone spectrum apparatus (LeSSA) for assessing the severity of OP poisoning in less-resource areas. The total cost of the LeSSA, is about $20, which is affordable for rural/remote clinics. The analytical results exhibited good linearity and high correlation between the results measured by the LeSSA and the laboratory microplate reader (r^2 = 0.97 in spiked samples, r^2 = 0.92 in clinical specimens). Therefore, the performance of the LeSSA is acceptable for point-of-care and field use, and can provide the clinician with reliable measurements of PChE activity, which in turn allow the clinicians to immediately determine an appropriate course of treatment. Without the delay in inter-hospital transfer or in initiation of treatment, the 15% to 30% increasing fatality rate can be greatly reduced by the LeSSA for the on-site measuring PChE activity. In the future, we aim to modify the LeSSA to achieve better accuracy and reliability, for instance, a constant voltage/current control is desired for consistent light intensity, and a temperature sensor is also desired to compensate for the effects on PChE activity caused variation in ambient temperature.

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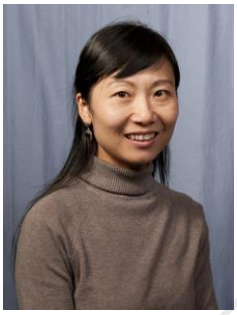
Yu-Chung Chang received B.S. in Materials Science and Engineering in 2014 and M.S. in Mechanical Engineering in 2017 from Washington State University. He continued his Ph.D. study in Materials Science and Engineering at Washington State University. His research interests focus on the design of additive manufacturing polymer composite materials and applications in smartphone-based biosensors for rapid diagnostic.

Assistant Research Professor, **Dr. Li-Ju Wang** joined the School of Mechanical and Materials Engineering at the Washington State University in 2014. She received her Ph.D. in Chemical Engineering at National Taiwan University in 2011. Her current research interests are combining experiments, numerical, and theory in nanomaterials science, colloidal and interfacial science, and nanotechnology to develop diagnostic biosensors and point-of-care rapid detection.



Dr. Annie (Dan) Du, received her PhD degree in Analytical Chemistry in 2005 from Nanjing University, China. She received her training in postdoctoral fellos/scientist in Pacific Northwest National Laboratory, USA. She joined Central China Normal University in 2005 and was promoted to Full Professor in 2011. Currenty, she is a Research Professor at Washington State Universtiy. Her resesarch interests in clude functional nanomaterials for fuel cells and elevtrochemical biosensing.

Dr. Lei Li received a Ph.D. degree in integrated system engineering from the Ohio State University in 2009. He is currently an assistant professor at School Mechanical and Materials Engineering at Washington State University. His research areas include mobile point-of-care technology (MPOCT), additive manufacturing, optical manufacturing, and micro/nano manufacturing.

Dr. Yuehe Lin is a professor at Washington State University and a Laboratory Fellow at Pacific Northwest National Laboratory. He has been actively

working in the nanotechnology area, particularly in the synthesis of functional nanomaterials for energy and environmental applications. His other research activities include development of new biosensors and bioelectronic devices and nanomaterials for biomedical diagnosis and drug delivery. Dr. Lin has published 430 papers, with 42300 total citations and an h-index of 106, according to Google Scholar. He was listed as a highly cited researcher in chemistry on Thompson Reuters and Clarivate Analytics' lists in 2014, 2015, 2016, and 2017.

Figure Captions

Fig. 1. (a) The assembled LeSSA, and (b) schematic structure of the LeSSA.

Fig. 2. (a) The mapping relationship in pixels and wavelength. (b) Self-developed photo-taking App to control the optical parameters.

Fig. 3. Absorbance vs. IL-6 Concentration at 450 nm read by (a) the microplate reader, and (b) the LeSSA. (c) Linear correlation of the microplate reader and the LeSSA.

Fig. 4. (a) Linear regression for PChE activity in PChE standards related to Paraoxon-ethyl concentration, and (b) Linear correlation between PChE activity measured by the LeSSA and the microplate reader.

Fig. 5. (a) Linear regression for PChE activity in purchased human plasma related to Paraoxon-ethyl concentrations, and (b) linear correlation between PChE activity measured by the LeSSA and the microplate reader.

Fig. 6. The linear correlation between PChE activity measured in OP exposed plasma samples from agricultural workers, by the microplate reader and the LeSSA.

Fig. 1.

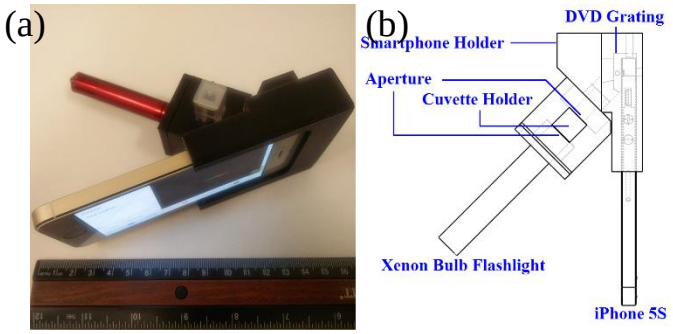


Fig. 2.

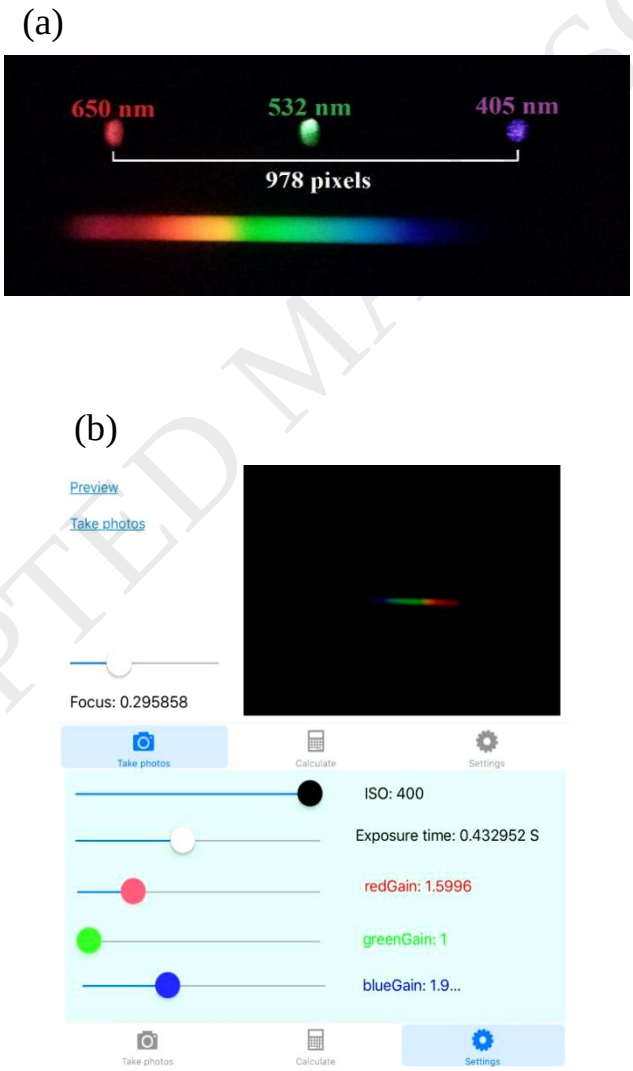


Fig. 3.

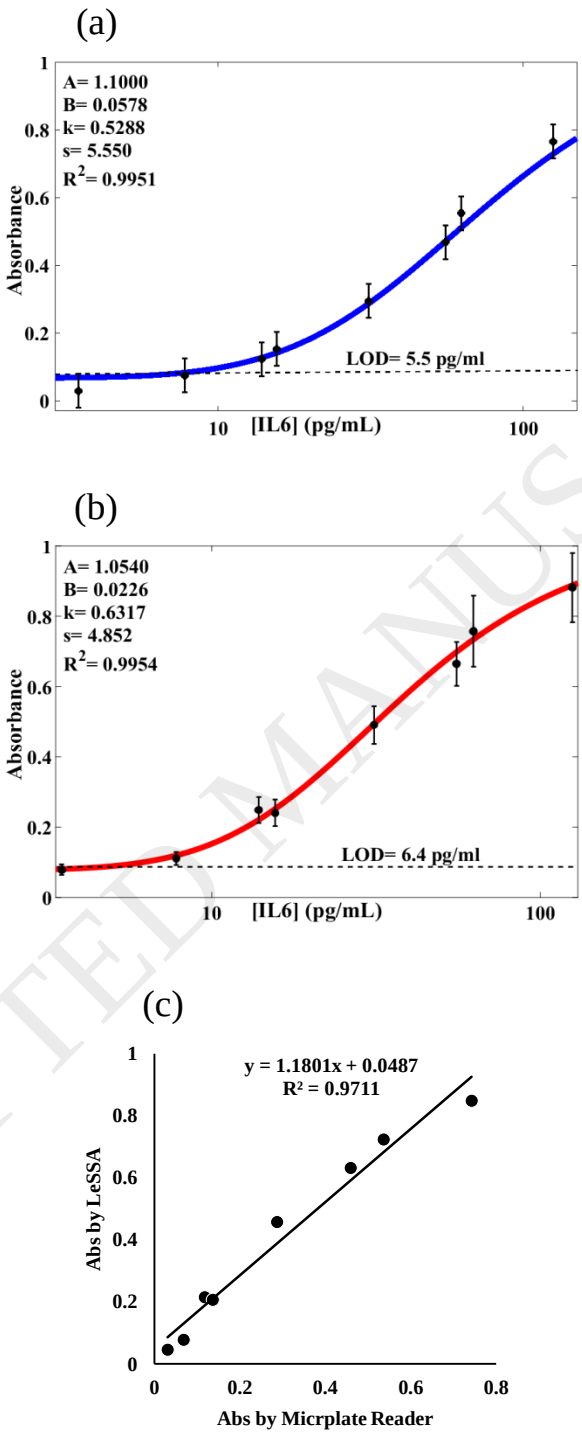
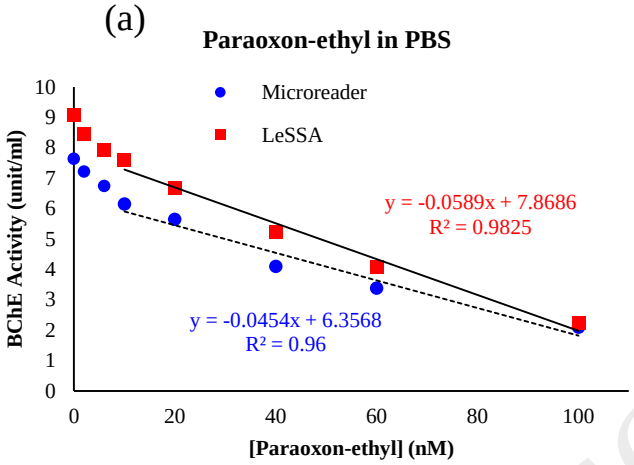


Fig. 4.

(a)



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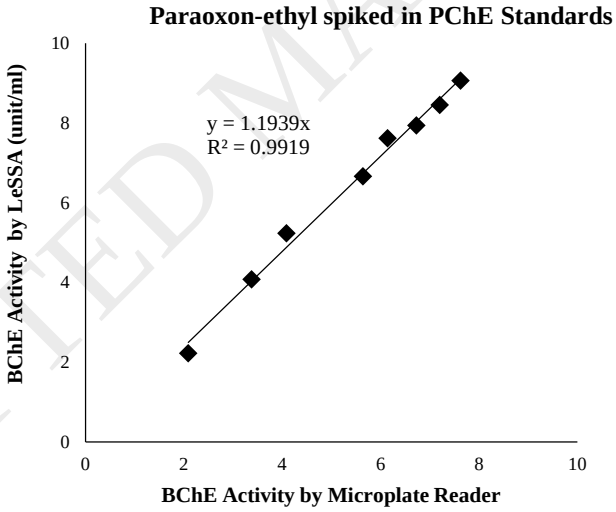
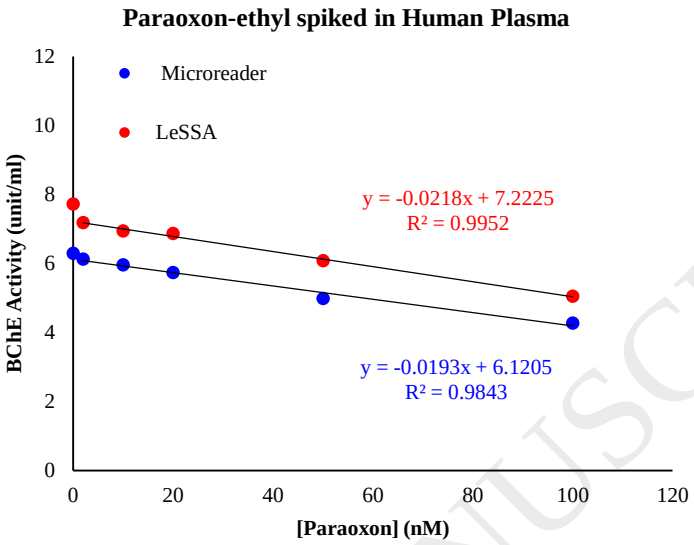


Fig. 5.

(a)



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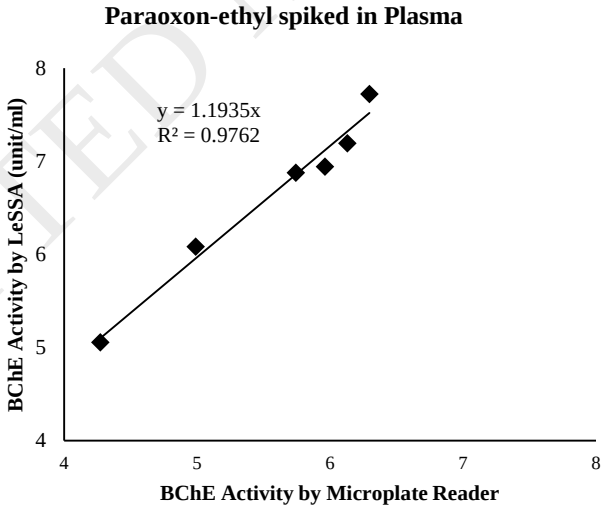
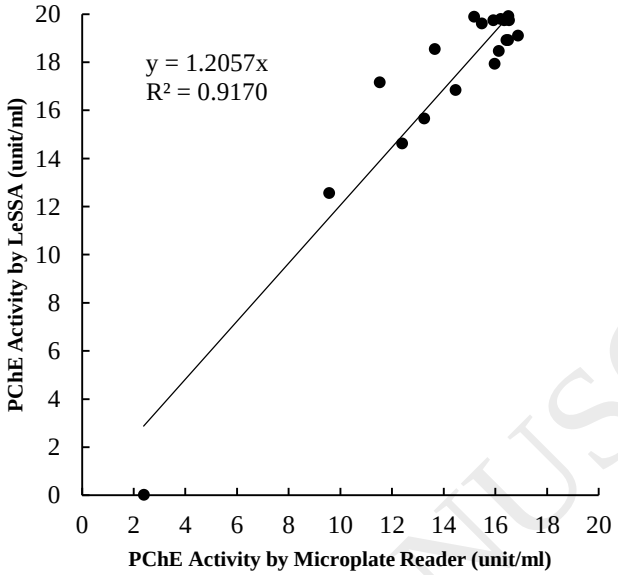


Fig. 6.



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