Original Article

 Fluorescence Nano Particle Detection in a Liquid Sample Using the Smartphone for Biomedical Application

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Abstract
In this paper, we present a Smartphone-based Fluorescence Nanoparticle Detector (SPF-NPD) that can be used for identifying biological agents in biomedical applications. The experimental setup consists of an LED light source and an Eppendorf tube holder placed inside a dark chamber with an optimally located slit for aligning the camera of a smartphone. The camera acquires the fluorescence intensity variations in the target liquid sample placed in the Eppendorf tube and passes it to a dedicated android application running in the smartphone. Using the principle of fluorescence-based pathogen detection, the android application detects the pathogens and displays the results within a few seconds. Since, all smartphones are equipped with high-resolution cameras, the proposed SPF-NPD provides a simple and elegant solution for instantaneous detection of fluorescence nano particles and has a great potential for healthcare applications for live detection of pathogens. The intensity measurement in SPF-NPD algorithm uses 5-pixel method, that is, the center pixel followed by four immediate neighbor pixels, because of which, minimal sample quantity is sufficient for precise measurements. We establish the robustness of SPF-NPD through exhaustive experiments with various smartphone cameras having different resolutions ranging from 8 to 20 Megapixels. The results of the proposed SPF-NPD method are validated against those obtained from standard devices such as Perkin-Elmer Picoflor and Perkin-Elmer Enspire. The advantages of the proposed method are highlighted.

Keywords  Android · Fluorescence · Nanoparticle · Pathogens · Smartphone

Introduction
Human beings are surrounded by numerous pathogens in the form of fungi, bacteria, viruses and many other microorganisms [1]. Many of these microbes survive without causing any damage to the humans, while some conquer our body producing disease [2, 3]. The microorganism growth may take place at a faster rate and hence the need for instantaneous detection of pathogenic agents has increased tremendously [4, 5].

In the medical field, the detection of pathogenic bacteria in food, water, and air in the shortest time has been an important aspect for researchers. Traditional strategies of microorganism detection need analytical laboratories, typically in centralized facilities, which need substantial capital and extremely skilled manpower [6].

Although these achieve sensitive and selective bacterial detection [7], they require a longer time to yield a result [8]. Fluorescence method [2] is well-suited for the detection of pathogenic contamination with excellent sensitivity and minimal analysis time along with an added advantage that there is no need for contact with the sample during the detection [9]. Hence, we have chosen the fluorescence nanoparticle detection method [10] to identify the pathogenic bacteria in liquid samples to determine the presence of impurities and disease conditions [3, 11] with a high level of specificity [12].

Among the software-driven fluorescence nano particle detection methods, the Android-based mobile application detection system has now commanded its application to infectious disease diagnostics [13, 14]. The reason is that Android is a very widely used mobile operating system in the world with more users worldwide than any other mobile operating system [15]. Android based applications have proven to be very successful in the field of biomedical

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instrumentation [2, 16]. Hence, we propose an android mobile application for quantitative analysis of fluorescence intensity in a target liquid sample. We use the pixel intensity method [17, 18] by considering 5 pixels, namely, the center pixel along with four immediate neighbor pixels thereby provide the analysis with minimal sample quantity.

Fluorescent Detection Technique

Fluorescence is the absorption of light energy by a molecule at one wavelength and re-emission at another wavelength [9]. Some amount of energy is dissipated as heat when electrons return to their ground state [7, 19]. Hence, the light produced always has an elongated wavelength than the engrossed light due to limited energy loss by the molecule before radiation [20]. Fluorescence is a three-stage procedure that usually arises in particular molecules called fluorophores or fluorescent dyes [21, 22]. A fluorescent nanoparticle is a fluorophore designed to focus within a specific region of a biological agent and to retort to a specific stimulus [6, 16]. The process responsible for the fluorescent nanoparticles is explained [23] by the simple electronic state diagram shown in Fig. 1.

When photon energy is stimulated by an external light source such as a Light Emitting Diode (LED), fluorophore absorbs its energy creating an excited electronic singlet state \( S_1' \). The energy of \( S_1' \) is partially dissipated as heat, yielding a relaxed singlet excited state \( S_1 \) [24]. After some time, fluorophore returns to the ground state \( S_0 \) emitting a photon having a longer wavelength and lower energy than the excitation energy due to thermal and vibrational losses during the transient exciting lifetime.

The quantity of wavelength of the emitted energy depends on each fluorophore and the chemical setting of the fluorophore [25, 26]. The deterioration of fluorescence intensity as a function of time in a uniform population of molecules excited with a brief pulse of light is described by an exponential function as given in Eq. (1).

\[
I(t) = I(o)e^{-t/T}
\]

where \( I(t) \) is the fluorescence intensity measured at time \( t \), \( I(o) \) is the initial intensity observed immediately after excitation and \( T \) is the fluorescence lifetime.

Stokes’s Shift

Stokes shift is the difference in energy or wavelength between states of the band maxima of the absorption and emission spectra of the same electronic transition [14] as shown in Fig. 2. Usually, fluorophore having a higher stokes shift is preferred.

Materials and Materials

A fluorescence-based system consists of three main components namely: 1) A sample holder to contain the fluorophore for investigation, 2) An excitation source to stimulate the fluorophore, and 3) A smartphone-based mobile application to analyze the target sample. When the sample is excited with the light source, the sample undergoes Stokes shift phenomenon. The basic functional block diagram of the android-based mobile application for fluorescence-based nanoparticle detection system is shown in the Fig. 3.

RGB is an additive colour model in which red, green, and blue are added together to form a broad range of colors. Using the Hue Saturation Value (HSV), also known as Hue Saturation Brightness (HSB), an object colour can be identified and it helps to prevent the influence of light intensity from other sources. The LED excitation source is used to stimulate the fluorophore (which is the fluorescent...
bio-sample in our case) as shown in Fig. 3. The light source is absorbed by fluorophores and light energy of another wavelength is emitted with lower energy compared to excitation energy. This property is suitable for detecting when the absorption or emission process is influenced by analytes of interest [27]. The emitted light energy is captured by the smartphone camera and quantitatively analyzed by the android application.

**Excitation Source**

In order to identify fluorescence with high accuracy, the excitation light should be distinct from the emission light. The source can be any device that emits radiation in the blue wavelength region (450 to 495 nm). We have used white LED with radiation wavelength of 494 nm. We have ensured uniform illumination across the area. The operating characteristics of LED are presented in Table 1.

**Sample Preparation**

Fluorescent dye, Dioctadecyloxacarbocyanine Perchlorate, also called DiOC18, is used in our proposed SPF-NPD method as well as in other well-known methods, to test and compare the performance of our prototype with other methods. This is because an enzyme produced by microscopic organisms helps to break the bonds in these dyes, thus exposing the fluorescing part of the compound [28]. Hence, initially, as a proof of concept, DiOC18 without any biological organisms is detected and analyzed using our prototype. In order to make samples with a wide range of known concentrations of DiOC18, we meticulously prepare the dilutions from a stock solution. The molecular weight of DiOC18 is 376.3 g. Molarity is calculated by using the formula given in Eq. (2)

\[
Molarity = \frac{Grams}{(Molecular\ Weight \times Volume)}
\]

To prepare Molar (1 M) solution, 3.763 g of DioC18 is required. Based on Eq. 2, we prepare the stock solution, which is 1 ml quantity of 10 mM concentration solution, 3.763 mg of DioC18 added with distilled water. To prepare the working solution, serial dilution is done from the stock solution. To prepare 1 ml of 1 mM solution, 100 μl of the stock is dissolved in 900 μl of distilled water. Further serial dilutions can be done for various sample concentrations as per the flowchart given in Fig. 4.

**Smartphone Based Android Application**

In the proposed SPF-NPD method, the smartphone is used as a detector to determine the fluorescent intensity level [29]. The minimum required configuration for a smartphone as a detector is, Android Jellybean mobile operating system

| Table 1 | Operating characteristics for LED |
|---------|----------------------------------|
| Parameter                  | Value                      |
| Product ID                 | L1-0-b5th15-1              |
| Angle                      | 15                         |
| Package                    | 5 mm                       |
| Peak wavelength            | 470 nm                     |
| Luminous intensity         | 3460 mcd typ. @ 20 mA      |
| Max Forward current        | 30 mA                      |
| Forward voltage            | 3.6 V typ. 4.0 V max. @ 20 mA |
| Max reverse current        | 50 μA @ 5 V                |
| Max reverse voltage        | 5 V                        |
| Power dissipation          | 120mW                      |
| Operating temperature      | -30 to +85 °C              |
with camera resolution of 8 megapixels or above. In our experiments, we tested the android application on various android smartphones verified the efficiency and robustness of our method.

**Experimental Setup**

Figure 5 shows the experimental setup of the SPF-NPD method. The dark enclosure shown as ‘A’ in the figure contains the excitation light source and sample holder. A slit is provided in this dark enclosure (marked as ‘B’ in the figure) to enable the smartphone camera to capture the fluorescent intensity level of the sample kept in the

Graphical user interface (GUI) of the android application running in the smartphone is shown in Fig. 6. The intensity level is analyzed and the values of RGB and HSV are displayed on the smartphone screen, marked as ‘C’ and ‘D’ respectively in Fig. 6.

**Software Development**

The application was created in Android studio platform version 1.3.1 using the java programming language which can run on the mobile operating system Android Jellybean API 17 or above. The software detects the color which was emitted by the fluorescent dye or fluorescein. The fluorescent intensity is measured based on the primary colour values of the fluorescence.

The experimental setup along with the sample is aligned with the mobile phone camera. The radiated light from the source passes through the sample where the fluorescein will absorb the light and emit at a different wavelength. The camera captures the emitted intensity and the android application analyzes the fluorescent intensity and displays the RGB and HSV values of the sample. The colour of the sample is detected by the width and height of the pointer radius. Here, we fixed the pointer radius as 5 in pixels. The RGB values can be found out using the YUV420 conversion process as given in Eq. 3.

\[
\text{size.total} = \text{size.width} \times \text{size.height} \tag{3}
\]
This format is the standard image format on the Android camera preview. The Y’UV model defines a colour space in terms of one luma component (Y’) and two chrominance components, called U and V respectively. Luminance is denoted by Y. The prime symbols (’) denote gamma correction, with "luminance" meaning physical linear-space brightness, while "luma" is (nonlinear) perceptual brightness. The developed algorithm gives the RGB value of the image and displays the green component of that image. From the RGB value, we can get the HSV value. The mathematical relationship for RGB to HSV conversion is given in Eqs. 8, 9 and 10.

Hue calculation:

\[
H = \begin{cases} 
60^\circ \times \left( \frac{C' - B'}{\Delta} \mod 6 \right), & C_{\text{max}} = R' \\
60^\circ \times \left( \frac{B' - R'}{\Delta} \mod 6 \right), & C_{\text{max}} = G' \\
60^\circ \times \left( \frac{R' - G'}{\Delta} \mod 6 \right), & C_{\text{max}} = B' 
\end{cases}
\]

Saturation calculation:

\[
S = \begin{cases} 
0, & C_{\text{max}} = 0 \\
\Delta, & C_{\text{max}} \neq 0 \\
C_{\text{max}}, & C_{\text{max}} = 0 
\end{cases}
\]

Value calculation:

\[
V = C_{\text{max}}
\]

Here, R' represents R/255. Similarly, G' represents G/255, and B' represents B/255. The maximum and minimum values are given as follows: \(C_{\text{max}} = \max (R', G', B')\), \(C_{\text{min}} = \min (R', G', B')\), \(\Delta = C_{\text{max}} - C_{\text{min}}\).

**Result and Discussion**

The fluorescent intensity level of various samples with known fluorescein concentrations was measured using SPF-NPD method and the RGB, HSV values, measured in number of pixels, were recorded for all the samples. A calibration chart was prepared from these values in order to measure the fluorescent nanoparticles present in the liquid, by considering the intensity of ‘G’ value alone from the readings.

From the calibration chart shown in Fig. 7, it can be observed that the RGB value increases as the concentration of the sample increases. At a concentration as high as 1000 nM, the RGB values attain their saturation. The calibration of RGB data with the sample concentration is performed and the active range is identified.

The ‘results view’ page of the android application is shown in Fig. 8. It may be observed that the green value...
is equal to the intensity level of fluorescence present in the sample. For various levels of concentrations, equivalent amounts of green values are produced. It is also observed that, higher the concentration level, higher the intensity level i.e., higher the green value.

The sensitivity of SPF-NPD is compared with that of conventional devices for performance analysis. namely: Perkin Elmer enspire and Picofluor. From Fig. 9, it is observed that the sensitivities of Enspire and SPF-NPD are the same. From Fig. 10, it is observed that the sensitivities of Picofluor and SPF-NPD are almost the same.

The Robustness of the SPF-NPD method is verified by testing it with smartphones with different mobile operating systems and different camera resolutions. As shown in Fig. 11, same results are obtained from all the experiments irrespective of the operating system version and camera resolution. The reason is that we have used the minimum
Fig. 9  Comparison of Perkin-Elmer Enspire and SPF-NPD

![Comparison of Perkin-Elmer Enspire and SPF-NPD](image)

\[ y = 14734x + 1915.1 \]
\[ y = 27.182x - 1 \]

Fig. 10  Comparison of Perkin-Elmer Picoflor and SPF-NPD

![Comparison of Perkin-Elmer Picoflor and SPF-NPD](image)

\[ y = 103.34x - 16.987 \]
\[ y = 26.909x + 0.4 \]

Fig. 11  Robustness of SPF-NPD algorithm with various smartphones (different camera MP)

![Robustness testing using different smartphones](image)
configuration for our calibration and all other smartphones have better configurations thereby giving similar results. Thus, the developed android application can be installed with any kind of smartphone which is equivalent to or better than the basic configuration as specified earlier. This proves the robustness and effectiveness of the developed algorithm. Also, comparatively, the proposed SPF-NPD method is much cost-effective, portable, and easy to use when compared to the other methods.

Conclusion

A novel SPF-NPD method for fluorescence detection has been proposed for the detection of fluorescence nanoparticles using an android application developed and installed in a smartphone. Concentrations as low as 100 nM can be detected using the proposed SPF-NPD method. The intrinsic fluorescence detection method is deployed for the detection of fluorescent nanoparticles without the necessity for sample contact. For testing our method, we have used fluorescent dye as nanoparticle. The fluorescent nanoparticle absorbs the light (485 nm wavelength) from the excitation source and emits at a particular wavelength (515 nm). This emitted light is captured using a smartphone to determine the intensity level and hence the nanoparticle count. From the comparison of sensitivity analysis, it is observed that the sensitivities of the prototype developed, and the conventional methods are almost equal. The performance of the prototype was also analyzed with various fluorescence concentration samples (without pathogen). The developed method saturates at 1000 nM concentration. Also, the robustness of the developed application was tested with various camera resolutions ranging from 8 to 20MP.

Authors’ Contributions Anand. G, Thyagarajan. T, Sabitha Ramakrishnan have equally contributed.

Data Availability The datasets generated during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics Approval Not Applicable.

Consent to Participate Not Applicable.

Consent for Publication Not Applicable.

Conflicts of Interest/Competing Interests Not Applicable.

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