Development of a low-cost and portable smart fluorometer for detecting breast cancer cells

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Abstract: Instruments that allow the detection of fluorescence signal are invaluable tools for biomedical and clinical researchers. The technique is widely used in cell biology to microscopically detect target proteins of interest in mammalian cells. Importantly, fluorescence microscopy finds major applications in cancer biology where cancer cells are chemically labelled for detection. However, conventional fluorescence detection instruments such as fluorescence imaging microscopes are expensive, not portable and entail potentially high maintenance costs. Here we describe the design, development and applicability of a low-cost and portable fluorometer for the detection of fluorescence signal emitted from a model breast cancer cell line, engineered to stably express the green fluorescent protein (GFP). This device utilizes a flashlight which works in the visible range as an excitation source and a photodiode as the detector. It also utilizes an emission filter to mainly allow the fluorescence signal to reach the detector while eliminating the use of an excitation filter and dichroic mirror, hence, making the device compact, low-cost, portable and lightweight. The custom-built sample chamber is fabricated with a 3D printer to house the detector circuitry. We demonstrate that the developed fluorometer is able to distinguish between the cancer cell expressing GFP and the control cell. The fluorometer we developed exhibits immense potential for future applicability in the selective detection of fluorescently-labelled breast cancer cells.

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1. Introduction

Fluorescence microscopy which is based on the principle of fluorescence imaging is a powerful tool used by many biologists as well as chemists to monitor cell dynamics and molecules in the field of biology and chemistry. These commercial fluorescent microscopes offer various advantages: live cell imaging, wide field-of-view, sensitive sophisticated cameras for high resolution images, etc. The fluorescently labelled proteins of interest help researchers to investigate any cellular process and aid in extracting meaningful information. However, these microscopes [1,2] are expensive as they utilize sophisticated optics making it affordable only by hospitals/research lab with healthy financial resources. Moreover, operating these microscopes requires trained operator [3] who has an in-depth understanding of the microscope and knows how to optimize the components of microscope to control the wavelength, exposure and hence, retrieve data. A few low-cost fluorescent microscopes [4–6] were also developed to make it accessible to a greater audience from developing countries but low resolution hinders their application.

Fluorimeters, on the other hand, are commonly used in laboratory/controlled environment as well as in nature to observe/monitor the fluorescent signals emitted from the subject of interest [7–11]. Monitoring these signals usually helps to identify the presence of specific molecules in the object of interest. However, these fluorimeters are usually costly and bulky,
and require multiple sophisticated equipment and lab environment. Furthermore, the measurements from these devices may also be susceptible to unreliable variation caused due to the changing environment/atmosphere, sensors, instrument design as well as the calibration of the equipment/parts. Hence, it is still a challenge to build a device from off-the-shelf components which do not require sophisticated equipment or environment.

Breast cancer is the most common form of cancer affecting women with 1 in 8 women expected to be diagnosed with the disease during her lifetime [12]. Canadian cancer statistics project breast cancer to be the second-leading cause of cancer-related deaths among women [13]. In 2017, 26,300 new cases of female breast cancer cases leading to 5000 estimated deaths, were reported in Canada [14]. Though breast cancer mortality rates have declined over the years primarily due to improvements in therapy, early and effective screening is essential to lower the overall incidence and associated mortalities. The most common clinical procedures employed for diagnosing breast cancer include X-ray mammography [15] and Magnetic Resonance Imaging (MRI) [16]. These equipment are large in size, sophisticated, expensive and are accompanied by high costs of operation and maintenance. The application of these diagnostic equipment in a clinical setting is therefore substantially dependent on financial factors and may not necessarily be feasible for use in low-income or developing countries. Therefore, it is imperative that alternate tools and equipment be developed to implement more economical procedures for breast cancer detection.

The use of fluorescence imaging techniques as a clinically viable alternative to detect cancer cells in vivo has gained significant momentum over the years. Several studies have described the detection of cancer cells in vivo by labelling the cells either with a selective cancer cell-permeable fluorescent chemical/dye or a fluorophore targeting a cancer-specific protein of interest [17–19]. However, the ultrasensitive imaging instruments used for the detection of the fluorescence signal are expensive and the entire imaging process is extensively dependent on sophisticated hardware and dedicated software. This therefore warrants the need for the development of a cost-effective instrument as a means to detect fluorescence signals in the cellular and physiological range.
In this work, we designed and fabricated a low-cost, compact, sensitive and portable fluorometer for the detection of fluorescently-labelled breast cancer cells. We present a fluorescence detection system in which the excitation source and the detector (photodiode) are placed on the opposite sides of the sample in a straight line as shown in Fig. 1 to detect breast cancer cells.

2. Materials and methods

The developed device consists of a flashlight (Ultrafire C8) which works in visible range, a photodiode (Mouser, 720-SFH203) to detect fluorescent signal, a microcontroller (Arduino Uno) to read and transmit the data and a LCD screen to display message as major components. The photodiode was chosen based on its spectral characteristics. It responds in the range of 400nm-1,100nm and has a short switching time of 5ns. This makes it an ideal candidate for use in this device. Figure 1 shows the operating principle and the overall block diagram of the system where mainly the excited fluorescence signal is allowed to reach the photodiode with the help of an emission filter, thereby, improving the reliability of the system. The flashlight uses Cree XR-E Q5 type emitter which has 3 switching mode (high, low and strobe). The flashlight was operated in high mode for this experiment. The dimension of flashlight is 14.5cm x 4.5cm x4.5cm and have a luminous flux of 200 lm [20,21]. Figure 2 shows a list of off-the-shelf components that are required to assemble this device successfully. The detector circuitry is placed inside the sample chamber which is printed with 3D printer (CR-10). The complete dimension of the sample chamber is shown in Fig. 3. The sample chamber is black in color (Fig. 2(a)) in order to reduce interference.

![Off-the-Shelf components needed to build the prototype](image)

![Detail dimension of the sample chamber which houses the detector circuitry](image)

Immortalized human breast cancer cells were used to test the fluorometer prototype. The human breast cancer cell line, MDA-MB-231 was procured from ATCC (American Type
Culture Collection, USA). These cells are cultured as a monolayer in vitro. The cells were cultured at 37°C in Dulbecco’s Modified Eagles Medium (DMEM) high glucose media (SH30243.01, Hyclone) supplemented with 10% Fetal Bovine Serum (FBS). The gene encoding the Green Fluorescent Protein (GFP) was stably introduced in these cells via retroviral transduction. Briefly, the gene encoding GFP was first cloned into the retroviral plasmid, pLPC. The plasmid was then transfected in the HEK293 Amphotropic retrovirus packaging cell line, using a 1% Polyethyleneimine (PEI) solution as the transfection reagent. 24-48 hours post-transfection, the culture media containing the retrovirus was harvested and used to transduce the MDA-MB-231 cells. The transduced cells were selected with puromycin and GFP expression in the live cells was confirmed via fluorescence microscopy using a commercial Olympus inverted IX51 fluorescence microscope (Olympus, USA). It should be noted that like all immortalized mammalian (human) cell lines used in biomedical research, the MDA MB-231 cell line is cultured and grows as a monolayer in vitro, using petridishes and coverslips. Furthermore, the MDA MB-231 cells are morphologically homogenous and therefore have a uniform cell size. Changes in cellular shape are observed in instances where the cells undergo proliferation and migration [22]. This however accounts for little variability in the overall cellular population due to homogeneity in growth characteristics. For imaging the cells, the cells were seeded on coverslips (Cat. # 12-540C, FisherScientific, USA) in 6-well culture dishes and cultured overnight as described above. The cells were then fixed using 1% paraformaldehyde (PFA) and the coverslips were mounted on microscope glass slides (Cat. # 12-552-3, FisherScientific, USA). The commercial glass slides and coverslips used have standard dimensions of 75 mm x 25 mm (length x width) and 25 mm x 25 mm (length x width) respectively. The thickness of the glass slides and coverslips were 1 mm and 0.25 mm respectively. Additionally, the thickness of the commercially procured glass slides and cover slips used in this study is highly uniform (identical). These materials are used extensively for imaging purposes and are manufactured with precision to ensure that the products are identical in dimensions. Such precision ensures rigorous reproducibility in the context of imaging quality. The cells were imaged using the Olympus fluorescence microscope. The same samples were used to measure the intensity of the fluorescence signal using the developed fluorometer. The experimental setup is shown in Fig. 4.
3. Results and discussion

Figures 5(a) and 5(b) shows the cultured MDA-MB 231 expressing GFP when viewed under a commercial imaging system (Olympus IX51). The same cell line is then used with our prototype to detect the emitted fluorescent signal and hence confirm whether the cultured cells are fluorescent or not. The excitation and emission maximum of GFP fluorophore is 495nm and 519nm respectively as shown in Fig. 5(c). The filter, light source as well as photodiode were chosen carefully to cover the excitation and emission region of this fluorophore.

![Fig. 5. (a) Shown here is a representative image of the MDA-MB 231 breast cancer cell line visualized under a commercial fluorescence microscope (Olympus IX51 inverted microscope) (b) The same field of view of the breast cancer cells was visualized in bright field (light microscopy) using the same microscope (c) Absorption and emission spectra of Green Fluorescent Protein: This figure shows the absorption and emission spectra of the Green Fluorescent Protein where the peak absorption and peak emission of the cultured sample is 495nm and 519nm respectively (Adapted from [23]).](image)

The confluency of cultured cells on coverslip can also affect the readings of the proposed device. In order to evaluate the minimum level of confluency that is required for the device to successfully determine if the cultured cells were control cells or the cells with GFP, the cells of varying confluency were cultured on different coverslips and were finally seeded on the glass slide. The cultured cells were distributed uniformly throughout the coverslip. The cell confluency were varied as 30%, 40%, 50%, 60%, 70%, 80%, 90% and 95%. 8 samples of each concentration were made and then tested with our device. It was found that a minimum of 60% confluency is required to be able to differentiate with this device successfully. Although the device was able to detect the cells that were less than 60% confluent, the obtained readings from fluorometer were variable and the difference between the cancer cell with GFP and without GFP was less than 700mV. Since, the readings were not consistent, the result were not determined as conclusive. It is important to note that the distance between the excitation source and the sample was kept at 2cm while taking the measurements. After taking these measurements, it is confirmed that this device will be able to differentiate between the cancer cells with GFP and control cells that are minimum of 60% confluent. Thus, all the samples that are used in the measurement, hereafter, are at least 60% confluent.

The developed prototype is a compact and portable fluorometer which provides an alternative to conventional fluorescent microscope. The visible light from flashlight excites the sample with GFP and a fluorescent signal is generated. The emitted fluorescent signal is detected with photodiode which is placed just below the emission filter inside the sample chamber. An emission filter (520nm) is placed in between photodiode and the sample, so that only the emitted fluorescent light from the sample reaches the photodiode, eliminating false detection. The emission filter was selected so that it matches the emission wavelength of GFP, hence ensuring that mainly the emitted fluorescent wavelength is allowed to pass through it. This is to ensure that the light that is received at the photodiode is from the cell line and not from the flashlight. This is confirmed when the GFP expressing cell line is
replaced with the control cell and no signal is received at the receiving end. Although, popular approach is to use excitation light source along with dichroic mirror or excitation filter [4,5,22], this work shows that these filters can be omitted without affecting the performance of this device as it was also shown in [25], if the set-up is done carefully.

A microcontroller (Arduino Uno) is used to control and power the complete system. It controls the detected signal and communicates the measured signal to the display. The analog signal generated by the photodiode is converted by a high-precision 10-bit analog to digital converter which is embedded in the microcontroller. Figure 6 shows the flowchart of the working of the proposed device. The microcontroller is programmed in C language using the Arduino IDE compiler. At first, the device is initialized. After the initial boot is successful and the device is on, a sample of control cell is placed in the holder port of sample chamber. Then the device starts reading. This reading is performed 10 times in order to minimize the errors that can be caused due to movement or interference. An average of measurement is performed by the microcontroller and the value obtained is saved and displayed on the LCD module which will be used later in the calculation. It is to be noted that it is important to hold the flashlight/excitation source on top of the sample. Moving the flashlight away from the sample or not focusing on the desired area will affect the result. The user might need some practice in order to get it right. The next step is to remove the control cell and replace it with cancer cell expressing GFP and the reading is performed again. As in the earlier case, 10
measurements will be taken and averaged. The averaged value is then subtracted from the value obtained from the previously stored value. If the difference is greater than 700mV, “Cancercell found” is displayed. Otherwise, “No Cancer cell” is displayed. The user is then prompted to repeat the process again.

Fig. 7. Results (a) Breast cancer cell without GFP (Control cell) cultured on a glass slide (b) Breast cancer cell conjugated with GFP fluorophore cultured on a glass slide (c) Control cell visualized under commercial microscope (d) Breast cancer cell conjugated with GFP fluorophore visualized under commercial microscope (Olympus IX51) (e) No cancer cell detected with Control cell with the proposed fluorometer (f) Cancer cell detected with Conjugated breast cancer cell with the proposed fluorometer (g) Reading on a waveform oscilloscope.

Figures 7(a) and 7(b) shows the control breast cancer cells and the GFP expressing breast cancer cells. It can be observed that it is not possible to distinguish between these cells with naked eye. However, when the same cell lines are used with the proposed device, we are able
to distinguish between these cells as can be seen in Figs. 7(e) and 7(f). The data from same samples were also read with the help of Waveform Oscilloscope on a computer as shown in Fig. 7(g). The oscilloscope helps in visualization of the difference in the voltage obtained when monitoring cancer cell that are conjugated with GFP and with control cell. The control cells and the breast cancer cells conjugated with fluorophore are visualized in Figs. 7(c) and 7(d) respectively with the help of a commercial fluorescent microscope [1].

To evaluate the performance/effectiveness of the developed device, we employed four measurements that are commonly used in classification. These includes sensitivity, specificity, accuracy and precision. They are calculated as follows:

\[
Sensitivity = \frac{TP}{TP + FN} \tag{1}
\]

\[
Specificity = \frac{TN}{TN + FP} \tag{2}
\]

\[
Accuracy = \frac{TP + TN}{TP + TN + FP + FN} \tag{3}
\]

\[
Precision = \frac{TP}{TP + FP} \tag{4}
\]

where, TP is the number of true positives (cancer cells recognized correctly as cancer cells) and TN is the number of true negatives (i.e., control cells recognized correctly as control cells). FP is the number of false positives (i.e., Cancer cells classified incorrectly as control cells) and FN is the number of false negatives (i.e., control cells classified incorrectly as cancer cells).

Twenty samples (ten of cancer cell expressing GFP and ten of control cell) with confluency greater than 60% were cultured on the glass slide and they were tested first with conventional fluorescent microscope [1] and then with our prototype. Figure 8 shows the confusion matrix drawn from the measurements obtained using the developed prototype. As it can be seen in Fig. 8, the proposed device was able to detect all of the control cell from the samples accurately. On the other hand, the fluorometer detected 9 out of 10 cancer cells with GFP correctly. The sensitivity, specificity, accuracy, and precision of measurement with our device were found out to be 1, 0.91, 0.95, 1 and 1 respectively. False positive reading in this case can be related to inhomogeneous confluency in the field of coverslip. Therefore, uniform confluency of cultured cells is important in successful working of this device.
The list of major components as well as the cost that is needed to assemble this device is shown in Table 1. The total cost of this device can further be reduced by using low cost filter which can provide similar accuracy. Also, the cost can be significantly reduced if the components are bought in bulk. Table 2 shows the comparison of proposed device with other devices that are under research or are already available commercially in relation with weight, size, target of interest, cost, unit (Standalone/requires a personal computer) and power source.

### Table 1. List of major components needed to assemble this device

| Components used     | Model/specifications | Estimated Cost (USD) |
|---------------------|-----------------------|----------------------|
| Emission filter     | 520nm                 | 75                   |
| 3D printed sample   | Printed with CR-10    | 30                   |
| chamber             |                       |                      |
| LCD                 | HC1624, 5V, 16x2      | 3.90                 |
| Breadboard with wires | Microtivity         | 6                    |
| Microcontroller     | Arduino Uno R3       | 16.90                |
| Flashlight          | UltraFire C8          | 6.58                 |
| **Total Cost**      |                       | **138.38**           |

### Table 2. Comparison with other devices that are available in the market or are in research

| Work                        | Weight (kg) | Dimension (cm) | Cost (USD) | Target of interest | Standalone/Requires pc for post-processing | Power Source       |
|-----------------------------|-------------|----------------|------------|--------------------|--------------------------------------------|--------------------|
| Miller et al. [5,24]        | 1           | 7.5x13x18      | $480       | Tuberculosis       | Standalone                                 | Battery Powered   |
| Nunez et al. [26]           | Not known   | 17x18.7x31     | $250       | Imaging Assays     | Needs PC for analysis                      | AC powered        |
| Hasan et al. [4]            | 0.13        | 11x6.5x15      | $358       | Breast Cancer      | Needs PC for visualization                 | Battery Powered   |
| Babbit et al. [27]          | Not known   | Not known      | $772.93$   | Living cells       | Standalone                                 | Battery Powered   |
| Commercial microscope [1]   | 20.5        | 56.5x29x57.8   | $23,300    | Multiple           | Standalone/needs pc for post-processing    | AC powered        |
| Lamb et al. [28]            | Not known   | 4x4           | $3300      | Chlorophyll        | Needs auxiliary component                  | AC powered        |
| Hoadley et al. [29]         | Not known   | Not known      | $712.44    | Chlorophyll        | Standalone/needs pc for post-processing    | AC powered        |
| Blockstein et al. [30]      | Not known   | 7.6x7.6x12.7   | < $500     | Chlorophyll        | Needs a pc with software                   | AC powered        |
| Commercial fluorometer [31] | 55          | 104x59x32      | Not known  | Multiple           | Needs pc with software                      | AC powered        |
| Martin et al. [32]          | Not known   | 22x100x5.3     | > $170b    | Escherichia        | Standalone                                 | AC/Battery powered|
| Proposed Device             | 0.16        | 8x4x3.2c       | $138.38    | Breast Cancer      | Standalone                                 | AC/Battery powered|

*Height is not reported

*bNot reported in detail; so we calculated total cost based on the list of equipment provided in the corresponding paper

|a|not including flashlight
requirement. The table is divided into two categories based on the sensing mechanism used: image based and signal based. The devices mentioned in image based category detects tuberculosis [5,24], imaging assays [26], breast cancer [4] and living cells [27] while the devices that are presented in the signal based category detects chlorophyll [28–30] and Escherichia [32]. In addition to these devices, we have also included commercial microscope/fluorometer for comparison. These commercial devices [1,31] are equipped with sensitive parts and are loaded with many features. However, these devices are expensive and not portable. Hasan et al. [4] detected the same breast cancer cell line that was used with the proposed device by using excitation, emission as well as dichroic mirror. Although this device has imaging capability, the use of sensitive optical filters made this microscope costly when compared with the proposed device. The developed device is lightweight (0.16kg), small in size (8cm x 4cm x 3.2cm) and costs less than other similar devices as can be seen in Table 2. By replacing the light source, optical filters and detector, this device may also be used to detect other types of cancer. These components will be selected based on the fluorophore that is conjugated with the target cancer cell. To the best of authors’ knowledge, this is the first fluorometer of its kind that has been developed to detect breast cancer cells.

This device also removes the need of expensive cameras. The sample chamber occupies only a space of 4x8 cm making it a perfect candidate for bench-top experiments. This device is very easy to use with the instruction displayed on the LCD screen for each step after it is powered on with a 5V supply or USB from a computer through Arduino Uno microcontroller. Alternatively, this device can also be connected to the serial port of the computer via USB port to enable control by the computer and thus, control input and output operations via computer through serial communication. The user needs to manually adjust the flashlight on the sample. As the focusing of the excitation light on the sample is critical in successful working of this device, the user needs some practice to efficiently use this device, hence ensuring that the readings are without error. In future, this limitation can be removed by using a mechanical system which will hold the flashlight on top of the sample at the desired position. This will ensure that the flashlight is held at correct distance and position, further ensuring the consistency of reading across all samples.

We tested functionality of our fluorometer by using this device to detect fluorescence emitted from human breast cancer cells genetically engineered to express the green fluorescence protein. These cells served as a biologically appropriate and technically convenient clinical proxy of patient tissue for the fluorescence-based selective-detection of breast cancer cells. This study involves quantifying the fluorescence signal from cancer cells cultured in vitro. This significantly differs from the morphological and physiological characteristics of cancers found to grow as “tumours” (3D culture of cells) in the human body. Our fluorometer prototype was designed to detect fluorescence emitted from cancer cells cultured in vitro. Since the morphology of the cancer cells as well as the dimensions of glass slides and coverslips used in this study were uniform, these parameters did not have an impact on the fluorometer output. However, in future, further testing may be performed to see the effect on the fluorometer output by varying the thickness of sample. As proof of principle, we first imaged the breast cancer cells using a conventional high-performance inverted fluorescence microscope (at a cost of USD 23,300) procured commercially from Olympus. As shown in Figs. 5(a) and 5(b), the microscope enabled the acquisition of high-resolution images clearly allowing visualization of green fluorescence emitted by the green fluorescent protein. Hence, this device is further simplified when compared with a regular fluorescence microscope [4,5,22] or a fluorometer as it eliminates the need of additional excitation filter as well as dichroic mirror. Furthermore, it is also small in size, costs less and lightweight when compared to those microscopes. Removing the cameras and the filter further eliminates the need of having to adjust light path settings, external arc lamp, and also removing the need of a filter cube to house the different set of filters. These characteristics makes this device attractive.
4. Conclusion

A low-cost, yet reliable, fluorometer is designed and developed in this work which measures the fluorescence signal emitted by cultured breast cancer cells expressing GFP. The goal of this work is to promote the access of fluorometers to medical and research laboratories where resources are limited. In this regard, we demonstrate the applicability of our fluorometer which is compact, lightweight and portable and it can be easily built with low-cost off-the-shelf components. Hence, this device can be relatively easily manufactured by a wide range of laboratories across the globe. This device can also be used to test other types of cancer by simply replacing the filter and photodiode to appropriately accommodate the desired conjugated fluorophore. Overall, our designed fluorometer is not only lightweight, easy-to-use, portable and compact but also entails low cost of operation and fabrication. This fluorometer has broad applications in the fluorescence-based detection of multiple cancer types and will foster research by the clinical and biomedical research community.

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Disclosures

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

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