Development of Smartphone dual-laser waveguide based fluorescent microscopy system using 3D printing

By

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Abstract

Nowadays cellphones are present everywhere, and along with the worldwide network of devices, the concept of mobile health monitoring is changing to reshape the biosensor market. The smartphone's camera is a proven reliable candidate as a detector for the studies performed by various research groups. This study is a proof of concept of the Smartphone detection of two fluorescent dyes which can be used as biomarkers for point-of-care diagnostics through image processing techniques. A smartphone Xiaomi Redmi Note 4 along with two fluorescent dyes DyLight™ 405 NHS Ester and DyLight™ 633 NHS Ester are used in conjunction with two lasers Thorlabs 405 nm and 638nm. The captured pictures were analyzed using Image J. The limit of detection and dynamic range values were calculated for both dyes, 28.39 nM and 20-800 nM for DyLight™ 405 NHS Ester dye and 15.85 nM and 10-600 nM for DyLight™ 633 NHS Ester dye. Then this concept is realized by developing a cheap 3D printed POC device which uses the optical microscopy technology along with a PDMS chip. Hence, this integrated novel innovation which prioritizes accuracy and the ease of usage, can be a game changer for patients who live in countries of limited resources and can moreover aid to the impoverished people who are in dire need of medical help.

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Chapter I: Introduction

The advent of smartphones in the consumer market in late 90s turned the traditional feature-phones into portable computers. These smartphones quickly gained a predominant popularity following the pioneering introduction of Apple's iPhone in 2007. The number smartphone users is estimated to go up from 2.5 billion in 2016 to almost 3.5 billion in 2020 due to the steady rate of increase in usage of smartphones [1]. The figure 3.5 billion is very significant as it is almost equal to half of the total population of the world. Usually, a smartphone is well-equipped with various kinds of sensors like accelerometer, gyrometer, GPS to acquire data for various applications. The foremost broadly utilized sensor by users is likely the optical camera sensor. A smartphone typically also contains fast multicore processor, adequate battery, large memory, audio and USB ports, and touch screen, to supply powerful estimation capability, huge data memory storage, Li-ion battery supply, and interactive user interface to communicate with the users. The obtained smartphone user data can also be communicated to medical professionals or to cloud remote servers through the wireless data transfer systems such as 4G internet service, Bluetooth, Wi-Fi, and near-field communication (NFC), in which medical data can be transferred to local hospitals or medical facilities from remote regions of any country. Therefore, Smartphones have now become more like handheld computers with user friendly operating systems, faster processors with big internal memory, and high-fidelity optical camera systems [2]. The smartphones, however, have more potency of being more attainable and affordable than bulky and expensive analytical laboratory devices. [3]. These versatile, low-cost devices may be utilized to run schedule tests, which are right now carried out by trained professionals utilizing laboratory grade instrumentation such as microscopes and cellular assays. In this manner, they can be utilized to detect and treat the illnesses happening especially in nations with limited resources [4], [6]. Due to these reasons, there is an ever-

increasing curiosity in using smartphones to detect pathological analytes [7]. This includes the biomarker detection to identify cancer, tuberculosis and malaria viruses [8]. The latest advancement of point-of-care (POC) instruments has permitted numerous diagnostic tests to be executed at the point of need, independent of the lab facilities. Complementary metal-oxide semiconductor (CMOS)-based photocameras, has emphasized the use of smartphones as convenient biosensors and analytical appliances [9]. The smartphones are nowadays fitted by other accessories and so in most cases they are not solely functioning analytical instruments. Such accessorized devices have a great prospective as point-of-care platforms in clinical care [10], [11], food toxin detection [12], [13], environmental oversight [14], [15] and bio safety [16], particularly in inaccessible and rural areas. Each year smartphones are growing powerful than before and are also becoming more and more affordable. Thus, general public has very easy accessibility to it and so this serves as another important reason to consider. Within the US, the number of individuals with a mobile phone has reached to 95 % with 77 % particularly smartphone users [1]. This development can be observed in other locales of the world such as Africa, Asia Pacific, and the Middle East that comprises of 80 % of all new mobile phone connections [17]. This massive amount of data clearly illustrates the increased utilization of mobile phones. Smartphones have ended up being an ever-present part of lifestyle around the world. It is basically an impactful, convenient, and network connected device accessible to most of the world. It is evident that there is an immense opportunity to establish this technology to devise POC biosensors that guarantees portability, user friendliness and clinical detection convenience [18], [20]. Therefore, in Point-of-care testing (POCT) and diagnosis, a quick developing area is coming forward as an advanced testing procedure for symptomatic examination and other clinical testing. [21] In comparison to laboratory aided diagnoses, POCT empowers rapid however more exact results at increase

inexpensive rates. Hence, POCT may be a strong elective to laboratory-aided testing in regions with limited resources. Organizations, such as the US Global Health Initiative, the UK Department for International Development, etc., are heavily investing in research and development of POCT [22]. The abilities of smartphones are ever growing, and now it is on the researchers to design special augments to attach on smartphones for analytical biosensing, which supports light sources, signal detectors, or certain interface which has a power supply and also can process information [23]. Taking into account, in the era of quick development, mass production, and broad dissemination for smartphones. In recent times they have given the public a convenient, cost-effective, and user-friendly platforms to homogenize with microfluidics and LOC to construct analytical biosensors for POC applications and overall wellbeing. Biosensors using smartphones have been investigated using various points of view by different research groups. The ideal biosensor format fully coalesces the bio recognition process with the detector (smartphone), for creating a standalone biosensor. But the technical complexity has made research less lucrative although it is considered to be an ideal solution. As an elective, smartphones can essentially be used as versatile detector or as interfacing instrument. In this manner, based on the common expository strategies, the foremost critical modern advancements in this area are of four kinds of smartphone-based expository biosensor frameworks at the POC, i.e., microscopic imaging smartphone camera strategy, colorimetric detection, electrochemical detection, and electrochemiluminescence sensing.

More than 40,000 smartphone apps related to healthcare are accessible in 2020 which shows that there has been a notable increase in these kinds of applications [24]. Several groups have examined this development: Particularly, two research groups, Patrick and Wang evaluated this advance of biomedical imaging methods on the smartphone platform in 2010 [25], [26]. The fast development

of smartphones has ensured the presence of many decent features now, and this has also led to introduction of many new healthcare technologies. Xie group also have investigated the smartphone imaging methods in their review paper [27]. An article by Agu et al. concentrated on the application and usefulness of smartphone for diagnosis with the help of the camera in smartphone or microphone [28] in medical condition. A different publication by Ozcan et al. concentrated on microscopy carried out by smartphone which has the capability to detect single virus. This capability of smartphones to perform imaging/microscopy and optoelectronic/electronic sensing stand out to be an advantage [29].

1.1 Current approaches to smartphone-based point of care diagnosis.

1.1.1 Microscopic imaging sensor using smartphone.

The microscale research of biological samples is performed through microscopy. This method is abundantly used in biochemical experiments for the identification of objects (e.g., cells, bacterium, and parasites) that cannot be detected by unaided eyes [30], [31]. Lab microscopy is considered to be a traditional method and it is comparatively bulky and requires well trained operators due to its approachable application for patient's diagnosis and so it is costly as well. The researchers using smartphones have designed a precise, cheaper and user-friendly tool to deal with microscopy which is appropriate for POC. So, before getting into any definite biochemical diagnostic method developed with the help of smartphone, one must review the progress in microscopic imaging techniques developed from smartphones in recent years.

Almost all the microscopes built using smartphone are optical microscopes which have a visible light source. These smartphones use a lens system to magnify pictures of objects which are in the microscopic scale. The two important parameters to judge the performance of an optical

microscope are field-of-view (FOV) and image resolution. The Smith research group devised a smartphone-based microscope which is integrated and changed the internal lens to 350x magnification along with a visible light spectrometer. [32]. The recorded resolution put out by the microscope was 1.5 μm and with an effective FOV of 150 ×150 μm before image processing and around 350×350 μm after image processing. The progress in microscopy using smartphone highly boosts and broaden the competence of POC detection based on smartphones, particularly in analyzing direct specimens. Microscopy imaging unlocks a pathway in POC diagnostics by smartphone by permitting it the utility to directly test cells, bacteria, and viruses. The Breslauer group developed a light microscope with a smartphone-mount system and realized 1.2 μm resolution with an effective FOV of 180 ×180 μm by introducing a ball-lens into the assembly [33]. Different components are incorporated into a compact platform to form the system. A Light emitting Diode (LED) is possessed by the system of Fluorescence imaging as source of excitation. Additionally, it has a standard microscope eyepiece, a condenser lens, a collector lens and an excitation filter. The apparatus becomes a bright-field microscope when the LED and the filters are removed from it. The researchers analyzed the high-resolution images of P. falciparum malaria-infected blood samples using bright-field technique and fluorescent microscopy of Auramine-O-stained M.-tuberculosis-positive sputum smears were performed [33]. This analysis was done to demonstrate the utility of this device for clinical diagnostics for malaria and tuberculosis detection. Compared to the “gold standard” traditional microscopes, the smartphone platform is used as a possible alternative because of the camera's high resolution, which is sufficient for image analysis of blood cell and microorganism pathology. In developing and rural areas, with finite clinical laboratories but better accessibility to smartphones, smartphone platform could get over the issue of limited access to clinical microscopy. A dark-field imaging and a broad-field fluorescent

technique on a smartphone was illustrated by Zhu et al. [34]. In this case a specimen was charged by a battery-based LED. There is a lens present in front of the built-in camera that helps in imaging of florescent emission from the sample. A large FOV of ~81 mm^2 having an unfiltered resolution of ~10 μm is shown by the smartphone. Wei et al. reported a field-portable fluorescence microscopy platform which can be installed on a smartphone with high structural resolution making it capable of imaging both viruses (fluorescently labeled human cytomegaloviruses) and individual nanoparticles (100 nm of fluorescent particles) [35].

Recently, a pioneering type of microscope have been devised which needs no lens and in turn puts an end to the requirement of lens in a microscope [36]. Tseng research group developed it and it's the first lens free holographic microscope which can be attached to a cell phone which also has 1.5~2 μm spatial resolution with a FOV of ~24 mm^2 [37]. The extra hardware attachments are light weight (~38 grams) and the system is also very cost effective with a cheap LED (at 587 nm) installed in it. The system also has an aperture of ~100 μm which is just in front of the light source. The sample area was vertically illuminated by the group with an LED. At the time of interaction with the sample, the LED light is found to scatter and create refraction. The light waves make their way through the objects taken as sample (e.g. cells). The light waves are also found to interfere with the light that has not dispersed. This process leads to creation of a hologram of each object, which is further detected by the smartphone. The authors illustrated the system's conduct by performing the imaging of different sizes of platelets, white and red blood cells, and parasites.

However, the pixel dimension hinders the spatial resolution of the images, making the platform less precise than the conventional microscope. Furthermore, the process to obtain needs a holographic reconstruction algorithm, which cannot practically be installed on the phone because

it might reduce the processing speed of the phone. This insinuates that the holographic reconstruction methodology should be evaluated off site, e.g. in a remote lab.

A tablet with an expansive screen size however can be applicable, especially when target image analysis is appropriate for diagnosis. For normal usage, a traditional smartphone can work perfectly despite of a small screen size. This group also explained different ways to develop fluorescent microscopes based on smartphones [38], [39], using fiber-optic array [40], [41], rapid-diagnostic-test (RDT) based on smartphones reader platform for LFIA [42], and various ways to detect fluorescence or photometric signals using much more affordable and compact attachments.

An easy to carry smartphone device has been proposed by Erickson et al. [43] which has a smartphone accessory, which comprises of a color-based assay strip, and a cartridge. This smartphone device is based on reflectance photometry. The reaction area is illuminated with the smartphone's integrated flash and there is a diffuser that allows the light to blend in. Thankfully there exists a dedicated app, the device analyses concentrations by correlating the variations of color and brightness.

It is to be noted that the smartphone-based angle-resolved surface plasmon resonance (SPR) is of specific interest. This detection technique was first suggested by Preechaburana et al. [44]. The platform was designed to remain attached to the screen surface of the smartphone, where it is illuminated from a red rectangle on the screen and uses the frontal phone camera as the target where the SPR image is projected. There is an optical element consisting of PDMS and epoxy, which is optical in nature. This is enveloped with a gold-coated glass where the experiments can be executed. A dedicated app was used for image collection. The smartphone's spatial resolution is directly proportional to the SPR's function. Although, the researchers claimed that their

indigenous platform's performance is equivalent to traditional laboratory SPR instruments. They experimented with different assays especially with β2 microglobulin (β2M), a cancer biomarker, and also of various inflammatory conditions and kidney disorder, and calculated a LOD of 0.1 μg/mL [44]. The detection limit is reasonable for clinical testing, in the case of urine which does not require invasive drawing process like in the case of blood serum. The platform's main utility feature is using a onetime use disposable component and does not need any extra attachments and can function on various kinds of smartphones. This makes the device valuable for off-site clinical testing. Despite of the pioneering features, the most important shortfall of this system is its sensitivity. It can be surmised that it could be used only for testing where the target exists in medium-to-high concentrations, as documented by the researchers. There the device has limited practicality.

Of late, Gallegos et al. [45] proposed a phone-based biosensor that utilizes the camera as the detector of a small spectrometer for an unlabeled photonic crystal biosensor. The extra accessory is devised to keep a fixed alignment to keep the smartphone in a definite position along with its optical components. The acquired camera pictures are transformed into photonic crystal transmission spectrum through an app. An antibody which is dependent on concentration and an immobilized protein monolayer bond to a functionalized photonic crystal was detected through the application of this device. The platform is cheap to manufacture, condensed and has high sensitivity. The working principle of this smartphone-based device is based on a dry state and this stands out as its main shortcoming. However, the authors have explained this choice as permitting the analytical sensors to have uncomplicated configuration. It is true that executing the job in a moist state would require to include fluidic attachments, such as valves and pumps. But in retrospect, dry state detection allows assay preparation to be carried out independently, in terms of

time and physical location away from the detection stage, which is desired characteristic for off-site clinical testing. The above-mentioned biosensor formats are described by the fact that the smartphones cannot work alone, but needs accessory devices or attachments, such as external light sources and filters, in order to achieve a response which is analytically and clinically relevant. Roda et al. suggests that in this case, the bio/chemiluminescence (BL/CL) detection technique could be used as a substitution [46].

1.1.2. Colorimetric sensor using smartphone.

The process of biosensing based on color and is very cost effective and so has garnered a lot of attention. Furthermore, it is very simple and practical in nature [47]. It involves in production of visual results and is usually stable, cost effective, and simple to execute. The low-cost CMOS arrays responded to red, green, and blue (RGB). These CMOS arrays were possessed by the smartphone cameras. These cameras incorporate a wide range of automated operations like, such as Auto White Balance (AWB), auto focus (AF) and ISO. The RGB signals are detected at different ratios and are altered by the Auto White Balance (AWB) in order to produce good color. For the purpose of image processing, photo editing and RGB color analysis, mobile apps in huge quantity have been developed. All those apps measures color intensities and changes and thereby examine the digital images and the collection of quantitative data. These biosensors are less sensitive in nature. Therefore, they are relevant to detect analytes present in comparatively higher concentrations in food samples, biological fluids and environmental samples. Even though the smartphones have incorporated the function of balancing the colors, it is not capable of capturing photograph in bright environments and controlling ambient light is also difficult during imaging. This is evidently true outside of controlled environments, such as laboratories. Therefore, it is challenging to perform correct and significant measurements. Additionally, the process of

analyzing images is not always effortless, especially in case of small changes in color. HSV or CIE, are sometimes used instead of RGB as the latter is not appropriate to use in all cases. So, in order to incorporate tests based on colors with smartphones, all the mentioned reasons and conditions must be kept in mind. It is to be noted that external housing elements which are specific to phone are often required. These elements eradicate the fluctuation in camera positioning and lighting condition. All the mentioned shortcomings might be overcome with the help of specific software and additional elements like LED arrays (for reflection and transmission), batteries, and lenses. This might help yielding more specific analysis.

The images with colorimetric changes can be straight up captured, using smartphone camera. Thus, use of the inbuilt camera of the smartphone is very effective for developing efficient colorimetric biosensor. It is to be noted that in order to manage immediate light condition and position of camera, certain external attachments are to be applied at times. The attachments may also include battery packs, LED sources or external lenses. The minute color changes, and various color balancing function of the smartphone might not be detected, so it is difficult to obtain highly accurate analysis. The color estimation in recent smartphone POC colorimetric biosensors can be performed by several designated applications in smartphone that help in the color analysis without much of computer aid or similar technical assistance.

Colorimetric and intensity changes might be brought about by the various biomedical evaluation applications. An E. coli detection smartphone colorimetric based immunoassay was suggested by T. S. Park et al. The detection was done in field water with the help of three channel based microfluidic chip [48]. Two detection channels loaded with low and high concentration of bacteria were fabricated using anti-E. coli conjugated microparticles. In order to draw comparison bovine serum albumin (BSA)- coupled beads were put into the channel in control. Introduction of field

sample triggered the immunoagglutinate phenomenon where the E-coli antigens reacted with the antibody-conjugated beads. By measuring the Mie scatter, the immunoagglutinate level can be analyzed from the pictures taken by the smartphone which is fixated on a specialized angular holder at an optimized distance. The entire assay is completed by 90s and showed promising results when compared with the commercial instruments.

The Vashist group have lately investigated a basic cell phone based colorimetric reader which is applicable for recognizing immunoenzymatic assays for human C-receptive protein (CRP), carried out in a traditional microtiter plate format [49]. They found that the device was performing close to traditional sandwich ELISA and clinically analyzed other immunoassays.

In order to measure the concentration of nitrate and pH, an easy colorimetric technique that involves the use of smartphone camera is reported by N. Lopez, et al. The concerned device is cost effective and is a microfluidic device based on paper [50]. There is no necessity to add any attachment externally. The flash of the smartphone is used as the light source. A customized application is used by the smartphone camera to spot the color detecting areas on the microfluidic paper. The application is also used to extract S (saturation) and H (hue) levels in the HSV platform to analyze pH and nitrite concentration. The outcomes demonstrated the pH resolution to be 0.04 units with a precision of 0.09, and for the nitrite, it accomplished 0.51% at 4.0 mg L−1 of resolution and 0.52 mg L−1 LOD.

1.1.3. Electrochemical sensor using smartphone.

The most efficient scientific devices among the ones that are smartphone based, are those that involve electrochemical based spotting. Electrochemical methods are widely used in analytical chemistry. The idea of coupling the electrochemical method with analytical devices that are

smartphone based has given rise to the origin of various compact and user-friendly systems. A compact medium for detection through electrochemical method was illustrated by Lillehoj et al. It demonstrated how the device is linked with a smartphone by means of a mini-USB port [51]. This setup involves a fabricated, integrated circuit for signal processing and information analysis, and a dispensable microfluidic chip for fluidic processing and biosensing. To execute the quantification, step-by-step guidelines are shown on the cell phone screen, with outcomes displayed at the end of every estimate. The researchers exhibited the relevance of this system by identifying the Plasmodium falciparum histidine-rich protein 2 which is a malaria biomarker [51]. A LOD of 16 ng/mL in human serum was obtained, which is practically similar with other available immunosensors utilizing standard instrumentation. The process can be completed in 15 min. The smaller size and the efficiency make this instrument conceivably valuable for point-of-care testing, particularly in remote and undeveloped regions of the world.

A cost-effective biosensor device based on smartphone was reported in recent times by Sun et al. In this device the attachment can be directly plugged through the audio jack into the smartphone of the user [52]. The screen-printed electrodes (SPE) which is expendable in nature, can be put into action when the device interacts with different spotting elements such as nucleic acids or antibodies. The electrochemical experiments are conducted using a potentiostat present in the device. The potentiostat is further used to analyze, spot and measure biomarkers. The preliminary version of the device is suitable to work with the smartphones that are available in the market. The effectiveness of this device is considered equivalent to that of top level potentiostat that are available.

The world's first medical smartphone was created by Guo et al. This ran with a disposable test strip and could analyze the level of uric acid (UA) in whole blood obtained by pricking the finger of the

subject or patient [53]. The electrochemical module and the smartphone were incorporated together. This was further connected to an expandable electrochemical UA analyzing strip. It required only ~3 μL whole blood obtained by pricking finger to initiate UA characterization. The smartphone served as an analyzer representing the current signal to the blood UA level. Further for telemedicine assistance, this data was saved and uploaded to a personal-health management center. This clinical smartphone device is marketed commercially by Laya Technology Co., Ltd, Chengdu, China.

The reported systems require incorporation of the smartphone with other devices like electrical potentiostats, circuits and electrodes and this stands out to be a disadvantage due to high expenses and complications. Since these devices have a high energy consumption rate, it calls for usage of a lower power microcontroller to redress better power use.

1.1.4. Electrochemiluminescence sensor using smartphone.

The instances where electrochemiluminescence (ECL) is combined with smartphone-based detection are very few even though this technique has been promulgated widely for gene and immune automatic stations and traditional biosensors. Initially, Delaney et al., the developer of a microfluidic device based on paper (or μ-PADs), used smartphone camera as an ECL detector [54]. A very cost effective disposable ECL sensors was constructed using fluidic substrates, screen-printed electrodes and inkjet-printed paper. This ECL sensors may be scanned with a smartphone camera.

A bipolar electrode electrochemiluminescence (P-BPE-ECL) of the size of a palm was introduced by Zhang's group [55]. It was paper based bipolar electrode system that obtained signal with the help of a smartphone. As for the power supply, this small system uses lithium battery. Here the

smartphone works as ECL signal analyzer. For the dispensable strip, the screen printed BPE has been manufactured as the driving electrodes for ECL biosensor. For creating the microfluidic channels on electrode patterned paper substrate, the wax screen printing is used. This system has a good sensitivity, reliability and productivity, and can analyze on a wide range of data. In the proposed system, the limits of detection (LOD) for Glucose in PBS was 0.017 mM and for artificial urine (AU) was 0.030 mM. It might be summarized that the concerned strip with the smartphone can yield very efficient results and thus can be considered to have genuine objectives for mobile health.

A cost-effective system based on smartphone was developed by Doeven et al. to spot, produce and manage ECL [56]. The audible tone pulses were played over the device's audio jack and through this process the power from the phone's Universal Serial Bus (USB) 'On-The-Go' (OTG) port was switched. The switching process was established using a simple tone-spotting integrated circuit. The light that is released is further analyzed using the smartphone camera. The execution capability of an electrochemical cell managed by the smartphone is compared with those, which use a conventional potentiostat. They then used this technique to analyze the ECL constituents of an iridium (III) complex which is soluble in water. This concerned complex causes emission in the spectrum's blue region.

Smart phone POC biosensor can also be classified by using the biological fluids used for diagnosis or identification of the target biomarkers. They are broadly divided into four fluids which are commonly used by the traditional testing systems currently on market.

1.1.5 Type of samples used for smartphone-based POC tests.

1.1.5.1 Blood.

Among bodily fluids, blood is the most commonly used for routine medical analysis. The physiological state of the body can easily be determined by analyzing the basic molecular constituents of the blood such as proteins, metabolites and nucleic acids as they are plentiful in blood [57]. Also, processing of blood is simple, and it is easier to access from patients. As a result, using the detection theories and specific target analysis, the targets in the blood can easily be detected by using a smartphone.

Brightfield technique is the least complex optical microscopy strategy, and therefore, without heavy modification, a smartphone attachment can be devised to make a small POCT device. In view of straightforward guideline, it encourages simple imaging of live cells or big biomolecules in a sample of blood. In view of these merits, a run of the mill microfluidic chip-based smartphone attachment for CD4 recognition using brightfield technique was investigated by Kanakasabapathy et al., [58]. The attachment comprised of a microfluidic chip and multiple optical lenses. A specially built smart phone application (App) was developed to carry out image acquisition, analysis, and count the number of cells in the FOV. The whole processing was finished within 10 s. The calculated resolution of 33 cells for each μL was attained by the experiment.

In a different study, N. A. Switz et al. devised a reverse smart phone camera lens to a smart phone to achieve a 10 mm^2 FOV and a imaging resolution of ≤5 mm. [59] Their prototype model accomplished the identification of RBC and WBC in blood smears and also soil-transmitted

helminth eggs in feces samples. Such methodology brings a stability between lens size and image quality.

A basic cell phone based colorimetric test needs just a light source and image processing. Additionally, the sensitivity should also be high. With the incorporation of a CMOS image sensor, an optical grating, and a range computing method, a complicated cell phone based POCT was created, which comprised of small optical lenses, different individual light sources, and a microfluidic chip which is used as a biosensor [60].

As of late, a POCT tool for the fast identification of IgG antibodies against Ebola by blood testing was investigated by Brangel et al., [61]. The total framework incorporates an immunochromatographic strip with a smartphone. At the point when the Ebola virus antibody serum is released on the strip containing the reagent, a red-purple mark shows up on the detection strip. At that point, a special programmed app calculates the relative strength of the detected line and decides the outcome (positive or negative). The whole procedure takes under 15 min; accordingly, this device is cost effective and does rapid diagnosis and this lateral flow-based assay can be especially utilized in places with finite medical infrastructure. Additionally, it encourages the medical authorities to figure out containment approaches for patients in light of the fact that the app gives access to the location details of the patient. Nonetheless, this device has a limited accuracy in bright environments where the results are affected by it.

Fluorescence detection has some attractive attributes of greater sensitivity, uncomplicated operation, and robust specificity. It is ordinarily utilized in biochemistry to examine blood and its constituents like antibodies or nucleic acids. For instance, Zika, chikungunya, and dengue are extremely infectious as well as hard to make a definite confirmation when clinically tested [62].

A new study investigated the measure of prostate-specific antigen (PSA) by sandwich fluorescence immunoassay [63]. The framework includes a mobile phone platform incorporated with a magnifying lens, a basic illumination source, and a small immunoassay stage known as the microcapillary Film (MCF). The incredible simple nature of operation, transparency, and level geometry of the fluoropolymer MCF, permitted quantification of PSA in whole blood tests with the utilization of a fluorescence substrate.

A mobile phone based electrochemical identification device and paper-based microfluidic chip were created and used to quantify the concentration of β-D-glucose in phosphate-buffer saline [64]. The system transfer information through an audio jack. The calculation rule of glucose concentration depends on chronoamperometry technique. This test is focused on recognition of the glucose level in whole blood for diabetes. Improved dependability ought to widely recognize this low-cost detecting system.

1.1.5.2 Urine.

Urine is another important bodily fluid that is usually utilized in a clinical diagnosis, and its components, for example, urinary hemoglobin, WBC, catalysts, electrolytes, and so on are vital for clinical detection, effectiveness of treatment, and disease prediction [65]. Uniquely different than blood tests, urine testing has the upside of being non-intrusive [66].

Ozcan's research team devised a compact mobile phone-based device for fluorescent identification [67]. They created an albumin analyzer to quantify concentration of albumin in urine tests utilizing a sensitive and explicit fluorescent assay system carried out in a dispensable test tube.

Recently, a research group devised a POC brightfield microscope for S. haematobium eggs detection which runs on a label-free platform for rural regions in Africa [68]. The system used two

LEDs and external lenses. The half-pitch spatial resolution was around 0.87 μm and a FOV bigger than 4 mm^2 was accomplished.

As of late, two research groups detailed smart phone-based techniques for identifying glucose level in human urine. One was a wearable smart device [69] where the framework incorporates a diaper with a self-examining device and a smart phone. This device could investigate the gathered urine samples by means of a specially built app. Right now, colorimetric response is steady for 20–480 min, which is vital for differently abled individuals.

Another popular POCT is detecting Human chorionic gonadotropin (HCG) in urine. The HCG test kits available currently on the market can only give a positive or a negative confirmation. In 2017, a research group developed a smartphone attachment using luminescent phosphor and the cell phone's flash to rapidly quantify the HCG levels in urine [70]. LOD of this framework for HCG is almost ten times better than that of conventional quick pregnancy-test kits. In the event that this device or strategy can be brought to market, it will empower pregnant women to carry out pregnancy tests at home whenever without lining up at the medical clinic, which will end up being a significant direction that POCT is heading and a huge market attraction.

Another smart sensor that can identify uric acid, and dopamine, depending on electrochemical procedure was put forward by Ji et al., [71]. The framework comprised of an expendable sensor, a detector, and a cell phone. The sensor was made out of a screen-printed cathode (SPE) adjusted with reduced graphitic oxide and AuNPs for detecting analog signals. The device further transforms the analog into a digital signal. Ultimately, the smartphone has a special custom app to transform the digital signal into a cyclic volt–ampere and a differential pulse volt–ampere curve.

The whole system was miniaturized and precise. Additionally, this system can possibly be additionally upgraded.

1.1.5.3 Sweat.

Sweat is an essential bodily fluid made of metabolic waste, which constitutes lactic acids, uric acid and different electrolytes [72]. The electrolytic part in sweat is mostly made of sodium particles and chloride particles, and also trace amounts of potassium and calcium particles. For a normal healthy human being, the levels of chloride, sodium, and potassium in human perspiration are 4–60 mmol/L, 10–40 mmol/L, and 9 mmol/L, individually. As of late, the perspiration based POCT has been considered an alternative path for human health services as a result of the advancement of adaptable electronic technology and wearable devices.

Sweat's chloride concentration is a significant detection marker for cystic fibrosis (CF), yet the utilization of POC frameworks for detection is slowed down by the excessive price of available chloride sensors. To defeat this predicament, Zhang and collaborators put inexpensive detection solutions, a synthesis platform using citrate detection, for advancement of novel highly selective fluorescence sensors for chloride was developed. Right now, analyte identification and sign transduction happen by means of fluorescence quenching systems. It was noticed that the existence of chloride in the solution of CA-Cysteine prompted a non-radiative relaxation of the energized fluorophore, bringing about obvious attenuation of fluorescence signal. From that point, the following signal processing was accomplished by summation of the all pixel estimations of the taken pictures to evaluate the intensity of every estimation followed by the calibration curve. The device exhibited a broad linear range of 0.8–200 mM. This test is as of now executed with

traditional sweat testing which is tedious. Notwithstanding, the clinically valid results showed that the cell phone based colorimeter is a rapid and dependable device for the identification of cystic fibrosis and information can be transmitted very easily [73].

As of late, researchers devised a stretchable remote framework for sweat pH surveillance [74]. The whole system could be adequately attached to the skin of the user. It could quantify pH in the scope of 5–9. Nonetheless, the separation distance for effective results was just 2–3 cm; that is the reason, the smart phone ought to be set near the sensor, which makes major difficulty for the end user in regard of real-life situations.

Another recent investigation proposed a wearable smart device for cystic fibrosis and glucose testing [75]. A noteworthy improvement right now, as a comparison to a past research, was iontophoresis for improving proficiency of sweat collection. The fundamental concept of this strategy was that the particle flow was coordinated to diffuse in an electric field driven medium. As indicated by this concept, aggravation agonists could be transmitted to the perspiration glands to invigorate the sweat secretion. The whole system was light weight and uncomplicated to use. It didn't make a harm or caused distress to the skin. These two devices could distinguish various targets in sweat. In addition, the capacity of utilizing sweat was significantly improved because of the upgrades in the strategy for gathering sweat.

1.1.5.4 Saliva.

Saliva is mostly excreted by the salivary organs, transparent and without scent, and the pH extend to a span of 6.6–7.1. Salivation is a complicated fluid, including different proteins, nucleic acids, enzymes, electrolytes, and so on. [76]. A research has demonstrated that saliva incorporates 1116 proteins [77], a large portion of which can be found in the tears and blood. Contrasted with blood

extraction, saliva is less dangerous, more expedient, and without the risk of transmitting the blood-borne infections, because of the nature of collection is non-invasive.

In 2018, a research team additionally put forward an idea of using sweat in an optical biosensor for urea detection [78]. The team used urea and urease as a basis to create ammonia and carbon dioxide. Ammonia is water soluble; the pH ascends from ~6.8 to 8.2. Paper color-based identification carried out by reflectance estimation is a well-known technique. Notwithstanding, this strategy has the issue of poor perceptibility and reproducibility because of inhomogeneity of color formation.

Zangheri and colleagues created a straightforward and precise biosensor dependent on a chemiluminescent (CL)- lateral flow immunoassay (LFIA) technique incorporated in a mobile phone to quantify the identification of salivary cortisol. The immunoassay utilizing peroxidase–cortisol conjugate, is identified by including the chemiluminescent substrate luminol/enhancer/hydrogen peroxide while the cell phone camera was utilized for image procurement and data processing. The framework involves a canister, which holds the LFIA strip, and a cell phone adapter is used with a plano-convex lens and a canister-sliding slot. The technique is easy and rapid, with a dynamic detection range of 0.3–60 ng mL−1, and a LOD of 0.3 ng mL−1 which is sufficient for identifying salivary cortisol in the clinically viable range [79].

A fabric-based biosensor for detecting lactate levels was utilized by Yao et al., [80]. The framework was made of a mobile phone, connectors, a holding compartment, a potentiostat, a device for remote transmission of electrochemiluminescence signal, a fabric-based tool, and a laptop. The LOD of the entire framework was recorded as 0.035 mM. The range of identification of lactic acid was 0.05–2.5 mM. Nonetheless, the inadequacies of this sensor were self-evident.

The entire framework was not miniature and in light of the fact that the cell phone just used for its CMOS camera, in this way its computing power was squandered away. Another inadequacy was that the framework was relatively costly as a result of using a potentiostat. In this way, it is important to additionally streamline the whole framework by utilizing the full computing potential of the cell phone and investigating an alternative way avoiding the use of the potentiostat.

1.2. Current approaches to POC microfluidic device used in smartphones

Smartphones are easy to carry devices that has in-built cameras. It also has internal microprocessors for the purpose of image processing. The smartphones provide more rapid and correct results and thus have turned out to be a better alternative to computers. The smartphones possessing the quality of good connectivity allows easy sharing and yielding immediate results. This is highly applicable for monitoring environment, diagnosing various health issues, immunoassays etc. The idea of incorporation of smartphones and microfluidics has a very promising future. It ensures development of cost effective and user-friendly systems for field analysis. Thus, smartphones serve as very efficient detection tool in microfluidics.

A standard laboratory analysis is quite demanding and so calls for skilled persons to deal with samples and run analysis effectively and timely. To ease the situation in the standard laboratories, Lab-on-a-chip (LOC) has been introduced. This system allows the execution of all the traditional steps with the help of the single chip [81-83]. This system is very distinct, affordable, fast, sensitive, and easy to carry and work with. It is also very effective and so for all these reasons microfluidics is being favored and is also widely applied for specific diagnosis for patients in third world countries [84].

The diagnostics and biological research have received a new dimension with the help of microfluidics [81]. This technology is very precise in terms of handling the fluids in channels that have the dimensions of tens of micrometers. Lab-on-a-chip (LOC) with microfluidics frameworks is a powerful prospect for furnishing the fundamental equipments for these chemical and organic biosensors [85]. Microfluidics is a very time efficient technology and thus can bring about revolutionary improvement in the way of executing disease diagnosis. The fast execution of the process ensures less consumption of bio reagents, power etc. It moreover guarantees greater levels of incorporation and mechanization than standard research facility strategies ordinarily utilized in clinical labs. [86]

In microfluidics the optical and electrochemical detection were used more in comparison to unconventional microfluidic detection methods like Raman, NMR and infrared [87-89]. Here the instrumental methods help to execute accurate detection and the final results are further processed and analyzed with the help of external computers. However, it must be noted that standard computers fail to function outside laboratory. Laptops with long battery life are applicable but smartphones serve as the best alternative because it is portable and is capable of all the necessary functions such as image and date processing and sharing results as well. Apart from executing detection the smartphones are also capable of functions in microfluidics area like, fluid control. The smartphones and microfluidic devices are nowadays incorporated and used for the purpose of testing different constituents present in water, solution, saliva, sweat and even some complex matrix

At first glass and silicon were used for the manufacture of microfluidic chips. However, the revolutionary polydimethylsiloxane (PDMS) has helped the microfluidics to emerge further. Here

is various breakdown of different material that has been used to fabricate and develop the chip structure.

1.2.1 Paper based Microfluidics chip.

Whitesides Group (Harvard University), the developer of the "lab-on-a-paper" has been accredited as the first developer of paper-based microfluidic device. In 1949, Müller and Clegg designed a model by using a filter paper with paraffin as barrier. The experiment showed the restricted channel speeding up the process of diffusion of the sample. The use of sample is shown to decrease in here. Therefore, it won't be wrong to consider this research as the foundation of paper microfluidics [90]. This method of analysis has been embraced by various fields. Various developing countries with poor quality of water has used this method of analysis. This method allows the analysis and detection of any organic or inorganic contaminants present in water like pesticides, viruses, bacteria or any other particular harmful metals etc. In most cases various instrumental methods like AAS, immunoassays, GC, spectrometry. However, microfluidics is also applied for this purpose now [91-93].

The usefulness of paper being used as substrate has been considered in terms of its various qualities like being rapid, sturdy, cost effectiveness. It is easy to use and is disposable and very flexible. Thus, it can be easily used as chip fabrication material in case of large-scale manufacturing [94]. The effectiveness of it has been witnessed through the success of LFA. Pregnancy and other strip tests come under this path.

It is to take note of the fact that the utilization of outside power regarding the capillary action of hydrophilic channel and hydrophobic barriers, which is unnecessary for microfluidic paper-based scientific instruments (μPADs) [95]. But the closed-channel instruments are dependent on wicking

stream have some inescapable impediments in that cellulose strands are semi filled channels. To handle this issue, some open-channel paper based microfluidic instruments have used 'ordinary' microfluidic channel which is made with paper by different methods such as engraving, [96] folding, [97] embossing, [98] or embellishing cellophane techniques. [99]

The optimization of the industrial manufacturing process has made paper-based microfluidic chips very cost effective. Paper stands out to be one of the cheapest materials manufactured for new POC detection and analysis. The paper-based microfluidic chips can be fabricated by paper cutting, photolithography, embossing etc. [93, 100].

The structure of the paper can be described as porous with high explicit region, which prevents the movement of protein and other organic operators. A basic scaled down 56-microwell paper/PMMA half breed microfluidic catalyst connected immunosorbent examine (ELISA) microplate considered for fast and high-throughput identification of irresistible illnesses [101]. The reagents are conveyed and transported through the microwell structure of the porous holes of the paper, and by the hybrid microplate. There the Ab/Ag response might happen quickly to productively catch the biomarkers. The hybrid instrument identifies the hepatitis B surface antigens and immunoglobulin G (IgG) by utilizing an office scanner, which show a similar presentation as business ELISA units.

A paper-based 3D instrument was planned by Wang et al. [102] for breaking down and deciding the centralization of different components with shading reagents. It must be noticed that the identification further reaches of Cu(II), Cr(VI), Cd(II) and Ni(II) is around 0.3 ppm in water test. Then, wax-patterned paper was stuck with both sided sticky tape and were stacked upon each other. This structure empowered the instrument to retain liquids and allotted the equivalent to

discovery zones. The whole procedure is executed with no help from any outside siphons. The tape layers were designed by gaps among the layers of tape. Those openings were stacked with reagents and cellulose powder. By this strategy, association was built up among the diverts present in the paper layers. The incorporation of cell phone and this instrument has yielded a locator that is equipped for recognizing overwhelming metals by dissecting nature.

Bacterial estimation is similarly significant in water examination. A single-cell-level detection was effectively executed using paper based microfluidic device by Park et al. [103]. The instrument has three channels previously filled with BSA-conjugated and anti-Escherichia coli-conjugated globules having fluctuating sums. There were sections driven by narrow channels. A cell phone camera was used to take pictures for the last assessment. The first picture was taken before wastewater test stacking, while the second picture was taken 30 s after example stacking. The immunoagglutination of antibody-conjugated dabs, caused by E. coli antigens were measured by assessing Mie dispersing from computerized pictures taken at an advanced point and separation of the phone. A program coded in MATLAB was used to break down the two concerned pictures. Here, the code calculation changed over the shading picture to a picture which is green in color, perceived channel district, and determined normal force. Each example signal was separated by the foundation sign to offset the contrast between the paper chips. A similar assessment guideline was utilized in a multichannel instrument for Salmonella and E. coli estimation in one instrument [104]. This framework had a bit of leeway over the past framework since it didn't call for any container for the mobile phone or the sensor.

Park et al. [105] structured and created nearly a similar instrument as the one depicted toward the start of this section for the assessment of the flavor of ten diverse red wines, with the utilization of a lot of synthetic colors. Three microliters of each colorant were stacked. They were dried as well.

An amount of 30 μL of red wine was loaded into the vent specimen and allowed to split amongst eight channels. The red wine passing through several channels reach the wells by capillary activity. After the vent filled the entire well and responded with the color, a cell phone took an advanced picture of the microfluidic chip. Twenty-four shading information were taken from each wine test (eight colors × three hues—RGB). The photos were brought into a multivariable examination framework and PCA (head segment investigation) was executed. Prior to the PCA, the connection between RGB shading forces of each color and the convergence of the taste synthetic substances were assessed across fixed wavelengths—red (680nm), green (540 nm) and blue (470nm). Information focuses and their relating hues were very extraordinary for each color. There was relative freedom of the coefficient of assurance to the transmittance regarding three particular hues (RGB). The main important part (PC1) represented the highest among all the factors; the subsequent one (PC2) was not related at all with the former and represented the limit of the remaining difference. The PCA result was contrasted and the flavor map, indicating a generally excellent match. The sweetness of the red wine was explained by the PC1 and the boldness (light or substantial) was explained by PC2.

The paper-based sensor (test paper) is formed from filter paper. By application of the same, Mei et al. [106] demonstrated that radiance recognition of pesticides is additionally conceivable on paper-based instruments. The paper was inundated into the fluid arrangement of NaYF4:Yb/Tm@PAA-Cu nanoprobe and it was made water free at 50°C with ultrasonic sound as the source of energy. A while later, it was set in a tailor-made optical embellishment with the window for the cell phone camera. The accomplished limit of detection for the system was around 0.1 μM.

1.2.2 PDMS based microfluidic chips.

Elastomer degenerate in terms of its shape due to powerless pressure. It then rapidly re-establishes to the previous form, which is inferable from the low Young's modulus and high level of versatility. PDMS is the most utilized elastomer in microfluidics [107]. The rise of soft-lithography techniques establishes PDMS as an extraordinary advancement in the microfluidic field [108]. PDMS is exceptionally flexible and simple to change by oxygen plasma, which can glue two PDMS layers into structures, for example, encased channels, incorporated valves and associated supplies. PDMS empowers valves and siphons and numerous components of fluidic control. It is likewise straightforward to basically the whole range of unmistakable light, taking into consideration optical readouts. PDMS is mostly cell friendly, benign and has an affinity towards O2 and CO2 transportation, which is invaluable for cellular research [109].

The framework comprising of a cell smartphone attachment, an application, and a test-strip for color-based identification of 25-hydroxyvitamin D (25(OH)D) was utilized by AuNP immunoassay detection by Lee et al. [110]. The strip was covered by25(OH)D and the example (altered blood serum) was poured onto the identification territory. This gave results, showing the lone counter acting agent which were not united with the 25(OH)D being caught superficially. The colorimetric sign from AuNP–neutralizer conjugates was then enhanced utilizing silver, which was diminished on the outside of AUNP to expand their size and the affectability of the framework. The entire test strip was set in the smartphone embellishment (vitaAID assistant) to limit the impact of inconstancy in outer lighting and implanted in a PDMS diffuser to enlighten the rear of the test strip. When the serious official of AuNP–anti-25(OH)D was played out, the difference based on color was caught utilizing the cell phone's camera. Prior to the detection the test strip was included in the vitaAID embellishment. Later The grouping of 25(OH)D was assessed by looking at the

recognition and area for instance. The methodology utilizing the microfluidic assay based on gold nanoparticles along with the camera of a smartphone as the identifier was authored by Lu et al. [111].

By coating the essential antibody on the sensor surface, the rest of the immune responses are completed inside the vertical channel. This microfluidic immunoassay can rapidly distinguish biomarkers with less reagents and quicker time [112]. Using the vacuum strategies to driven fluids, there are other vacuum forced microfluidic systems which are exploited [113]. The sandwiched two PDMS layered permeable channel films, vacuum force can quicken the response [114]. The vacuum-accelerated microfluidic immunoassay (VAMI) chip can complete the entire process within 15 mins. A comparable chip permits the synchronous recognition of a series of clinically significant biomarkers at the equivalent time [115].

A comparable method to the aforementioned above was utilized by Park group. [116] in a paper-based chip which was utilized in PDMS instrument for the identification of contagious pathogens straight from adulterated (10%) human blood [117]. Histidine-rich protein 2 (HRP-2), Plasmodium falciparum specific antigen particular to malaria disease, was utilized as a model microbe. HRP-2-infused 10% human blood was blended with Anti-HRP-2- conjugated carboxylated polystyrene beads. The diluted blood and the microbeads were put into independently to individual inlets of the y-channel and blended together in the center compartment with the help of a syringe which is placed externally to the device and fixed to the outlet of the channel. Two mobile phones were utilized, one performing as a white LED source and another one assisting to identify Mie scatter because of immunoagglutination of HRP-2 to antibody-conjugated microbeads. The micro channels were fabricated to be at 45 degrees. Taking the scattering and absorption qualities of blood into account, the blue identification range demonstrated the best outcomes and as far as

possible with a LOD of 1 pg/mL in 10% blood, with the entire process to be completed within roughly 10 min.

The He research group [118] likewise utilized magnetic beads to ensnare pristine exosomes from human blood serum for the identification of three ovarian malignancy biomarkers. A microfluidic chip comprising of a serpentine channel with Y-formed inlets were manufactured with PDMS for blending beads and apprehending exosomes. Then the beads were gathered in a micro-chamber utilizing a powerful magnet, conjugated with three ovarian malignancy exosomal biomarker antibodies and were distinguished by color fluorescence imaging. Similar outcomes to a traditional test for cancer disease versus healthy human samples were collected utilizing the microfluidic chip within a period of 40 min test time. Future endeavors to correspond identification of exosomal marker concentrations with future event of cancer shall be required for detection purposes.

A different research team fabricated a microfluidic PDMS instrument that utilized antibody spiked magnetic beads for ensnaring of Aβ peptides particles, which is marker of Alzheimer's [119]. The chip had a nanoporous hydrogel peptide preconcentrationer, and a micro electrophoresis (μCE) channel for isolating Aβ peptides. 25 ng of Aβ peptide in 100 μL of cerebrospinal fluid (CSF) was recognized by utilizing this unibody device.

Lee research team [120] utilized a 3D helical channel printed chip along with antibody-conjugated magnetic nanoparticle beads to identify E. coli bacteria in milk. The convergence of isolated bacteria dictated by UV-Vis technique, and limit of recognition was 100 cfu/mL in milk. This investigation demonstrates the capability of 3D printed fluidic instruments for bio-marker tests; be that as it may, the dimension of the channels is bigger than in conventional microfluidic channels.

The Wang group [121] exhibited nonintrusive identification of HE4 (human epididymis protein 4), a marker for ovarian malignancy, by a methodology that integrates the microfluidic chip sandwich ELISA assay with smart phone based colorimetric estimation. HE4 concentration in serum has been demonstrated to be associated with the clinical state of ovarian malignancy, however Wang and his team utilized urine as a fluid sample. The microfluidic chips were created from PMMA & a double-sided glue film.

Cell phones are not only utilized for identification in microfluidics, although that is the most significant objective of lab-on-a-chip setup about isn't just to scale down traditional organic and biological tests onto a chip design but also to replace slow built-in fluid handling capacities practically identical with ordinary manual micro-pipetting or automated fluid handling. Essentially, the objective is to accomplish real robotized sample-to-answer activity. For instance, Sia group [122] created a portable device fueled by a 9 V battery which can activate on-chip elastomeric microvalves utilizing solenoid containing incitation units. Pollack and his group [123] introduced a microactuator for quick testing of discrete microdroplets by using electrowetting. The Braille display device created by Gu research tea [124] has been utilized to assemble versatile microfluidic cell culture frameworks. The Li team [125], then again, introduced a handheld robotized microfluidic dealing with framework manipulated by a mobile phone fueled by a 1500 mAh, 12.8 V, Li-ion battery, which was empowered by integrating elastomeric chip valves and a closed pneumatic framework. This instrument is commonly relevant to numerous cell-based tests and biochemical requiring complicated fluid control mechanisms and preparing the samples in various steps.

1.2.3 Glass based microfluidic chips.

The early iterations of microfluidic chip utilized glass or silicon wafers as their chip designing materials using micro-electromechanical frameworks advancements in a clean room. In spite of its good preciseness, this strategy generally needs refined devices, which are not reasonable for large scale manufacturing. The exhaustive exploratory manufacture methodology and the significant expense are most important hindrances while advancing it in numerous applications. In spite of the fact that the expense of chip manufacture is high, glass and silicon are truly dependable material when adding up the applications comprising on-chip responses, bead development, capillary electrophoresis, and solute extraction in some definite radical test environments. A significant group of microfluidic chips are made with these kinds of materials is digitized microfluidics (DMF) [126]. Electrodes are designed on silicon wafers or glass materials. Furthermore, the fluids can move by applying suitable order of potentials to the specific cathodes, in this manner the exact activity of different fluids can be acknowledged. As DMF can be exact and can effectively operate fluids, these instruments have been utilized in clinical assessments, cell research, immunoassays and various organic applications [126,127].

The Kim group [128] created a microchip DNA biosensor with versatile interface that utilized the transduction of DNA hybridization into a promptly recognizable electric signal with the help of a DNA stem-loop structure alteration verification. To show the utilization of this stage, Kim's team utilized it for the isolation of 100 nM E. coli series of DNA and the programmed mapping of the recognition results by means of a versatile smartphone application. The biosensor comprised of two working cathodes made of gold, which includes an immobilized DNA particle in the stem-loop arrangement containing the methylene blue which is close to the terminal surface, which empowers the effective electron movement with high decrease peak current and a platinum counter

and reference electrode. When detection of a specific pathogen is performed, the biosensor is washed with Di water which resets the sensor for subsequent later usage.

1.2.4 Thermoplastics based microfluidic chips.

Thermoplastics is not similar to PDMS. It can be manufactured by the advanced industrial methods involved in production in bulk. In order to fabricate POC chips, various thermoplastics materials have come handy. Conventional thermoplastics can also be utilized to make microchips that includes PMMA, PC, ABS, PET and PS. These materials reflect more convenient solvent compatibility in comparison to PDMS. These possess more choices in terms of union or developing chemical bonds. Complex microfluidic chips can fulfill utilizing these materials. With the end goal of chip fabricating, Centrifugal microfluidic instrument is a kind of microfluidic chip that utilizes thermoplastics as the source material [129]. Centrifugal microfluidic chips can combine numerous activities by utilizing the divergent power, Coriolis force, Euler power and other forces as their source of energy [130]. However, this has not yet commercialized, but it is a fact that centrifugal powered microfluidics may inevitably be a working principle for POC immunoassays.

Another kind of thermoplastics based microfluidic chips uses laser irradiated ferrowax microvalves (LIFM) technology. The chambers used for detection can be opened or closed as per need by the application of LIFM. The completely automatic lab-on-a-disc, possessing valves that open as per need can obtain multiplex immunoassay [131]. This microchip has two analyzing segments. Each of the analyzing segments of the chip is capable of spotting the antibody, therefore has detection capability, causes parting of the sample, has substrate and waste chambers, can clean the buffer. There are dissimilar micro PS beads in each of the reaction chambers for each of the three segments. This device is capable of detecting different biomarkers under 200 µm whole blood

testing samples. This detection process can be executed within a span of 20 mins. It is done with the application of similar LOD and dynamic range in comparison to traditional ELISA.

To show effective applications, improvement must be made in terms of parting technique to attain basic resolution. The DNA fragments are obtained by a method which was reported by a group, showing involvement of microchip, which is gel based. This involved in executing separation as well as preconcentration [132]. The 4 PMMA layers were bonded with the help of heat and this helped in designing the device. Further with the application of the agarose gel electrophoresis, the parting of DNA is being executed. The DNA particles were extracted, using a layer of cellulose ester. Sample's concentration was previously increased before isotachophoresis was performed. A channel having a DNA ladder as reference was used to separate two parallel channels. PCR products reflected 50% of efficiency of extraction upon analysis. This device can help in parting nucleic acid markers in forth coming days if more work in biological fields are performed.

A μCE device was designed by Pagaduan et al. [133] for thymidine kinase 1 (TK1) determination. Thymidine kinase 1 (TK1) is a cancer biomarker. The mAb-TK1 protein complex was separated from the unbound mAb protein. Next, a microchip immunoaffinity analysis was done to measure the mAb-TK1 complex protein. Although this showed parting of purified TK1 protein in buffer solution incubated with mAb off-chip. If this analysis is performed with proper detection limits in an applicable matrix like blood, the method might reflect potential for clinical application.

Thus, by reviewing different types of smartphone designs and biomarkers, it is evident that for low cost rapid diagnostics, cheap 3D printed POC device which uses optical microscopy and PDMS microfluidic chip is an ideal candidate. Among the bodily fluids, blood is easier to assess, and smartphone's camera is a proven, reliable candidate as a detector as per the studies performed by

various research groups. Therefore, an integrated novel technology if developed which prioritizes accuracy with an easy-to-use property, can be a game changer for patients who live in regions of limited resources and can also help impoverished individuals who are in dire need of medical help.

Chapter II: Development of smartphone -based fluorescence sensing system

As discussed in the previous chapter and through the literature survey, it was found that for a cheap 3D printed POC device which uses PDMS chip, smartphone-based microscopy is a great choice for inexpensive, rapid diagnosis of any typical disease or medical condition. Thus, this chapter focusses on advancing a smartphone-based fluorescence sensing system. The proof of concept and development are discussed below:

2.1 Fluorescent dyes, Laser diodes and Optical filters.

To exploit the smartphone-based microscopic sensing, the suitable fluorescent dyes with corresponding wavelength lasers and optical filters need to be chosen to get fluorescence signal for analysis. In addition to choosing the correct optical light wavelength, the dyes also need to have an affinity to react and tag the pertinent biomarkers. In this research, two fluorescent dyes were chosen used which are suitable to tag protein biomarkers. DyLight™ 405 NHS Ester and DyLight™ 633 NHS Ester (Thermofisher Scientific, Rockford, IL, USA) which both contain NHS group to react with amine groups presented in most proteins. DyLight dyes always have brighter fluorescence due to higher quantum yield, than other commercial dyes. DyLight dyes also have better specificity and are less susceptible to photobleaching effect. Photobleaching happens when a fluorophore forever loses the capability to emit energy because of photon-induced damage on orbital electron excitation, or covalent bond re-adjustment [134] which is typically caused by cleaving of covalent bonds or non-specific reactions between the fluorophore and encompassing molecules. Each of the dyes have a peak excitation and peak emission wavelengths of lights along with a band region where they absorb or emit light around the peak. So proper lasers are paired with each of them to exact the proper fluorescence. In order to excite these dyes, L405P150 (405

nm, 150 mW, Ø3.8 mm) and L638P150 (638 nm, 150 mW, Ø3.8 mm), (Thorlabs, Newton, NJ, USA) blue and red laser diodes are chosen respectively. Laser diodes are better than their gas-laser counterpart, because they are cheap, compact in size and shape, and a few milligrams in weight, which is very well suited for developing a smartphone-based optical case. Laser diodes are made of semiconductors and are safe to use. But there is also a disadvantage to use laser diode. The semiconductor diode is susceptible to spontaneous emission which involves emission of multiple wavelengths of light instead of a single desired wavelength. Therefore, optical filters are needed to filter out the unwanted wavelength range, thereby, ensuring the entry of appropriate wavelength exclusively in the POC device and consequently received by the smartphone's camera. For each laser, there are two optical filters, one having the same central wavelength as the laser is attached in front of the laser which is called an excitation (source) filter, and another one is attached in front of the camera which is called emission (camera) filter that let in only the fluorescence emission signal of the respective dye around the peak emission wavelength. The optical filters are essentially bandpass filters, which only lets through a specific narrow band of light. The entry of all the other wavelengths is cut off. They have a central fixed wavelength (CWL), and a FWHM (full width half modulation) value which represents how wide the pass band across the central wavelength is. Three optical filters of dimensions 20mm*5mm with CWL 400nm FWHM 15nm, CWL 440 nm FWHM 15 nm and CWL 680nm FWHM 20nm were purchased from Salvo Technologies, Largo, FL, USA and one filter of dimensions 50mm* 12.50mm with CWL 633nm FWHM 10nm was obtained from Omega optical, Brattleboro, VT, USA.

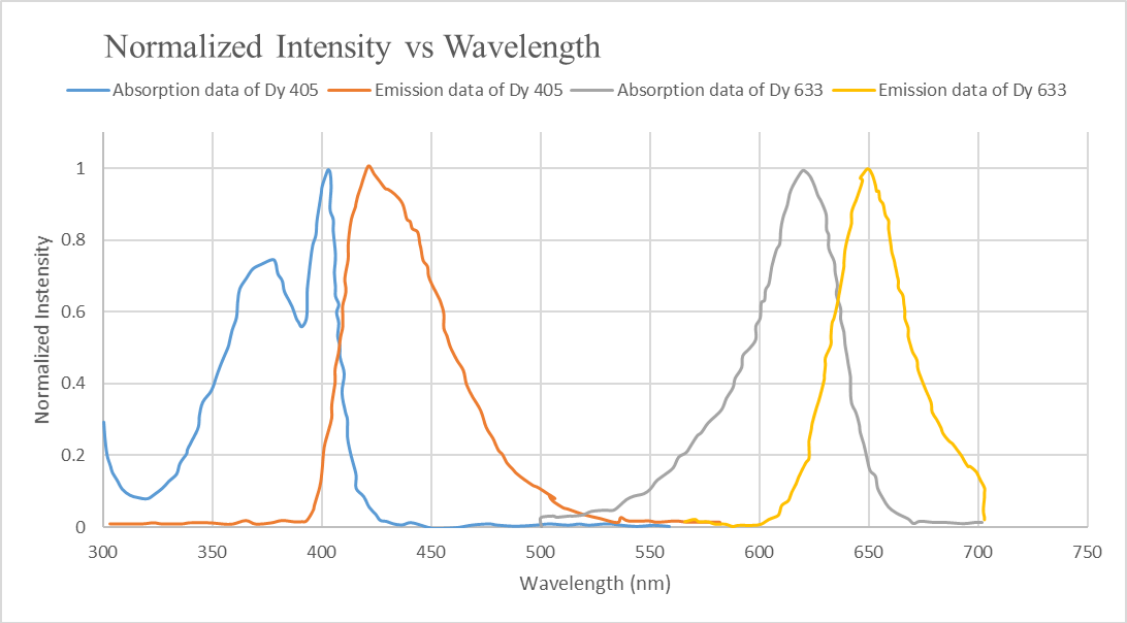


Fig 1: Absorption and Emission wavelength spectral diagram of Dylight 405 and 633 dyes with optical filters as found on Thermofisher's website.

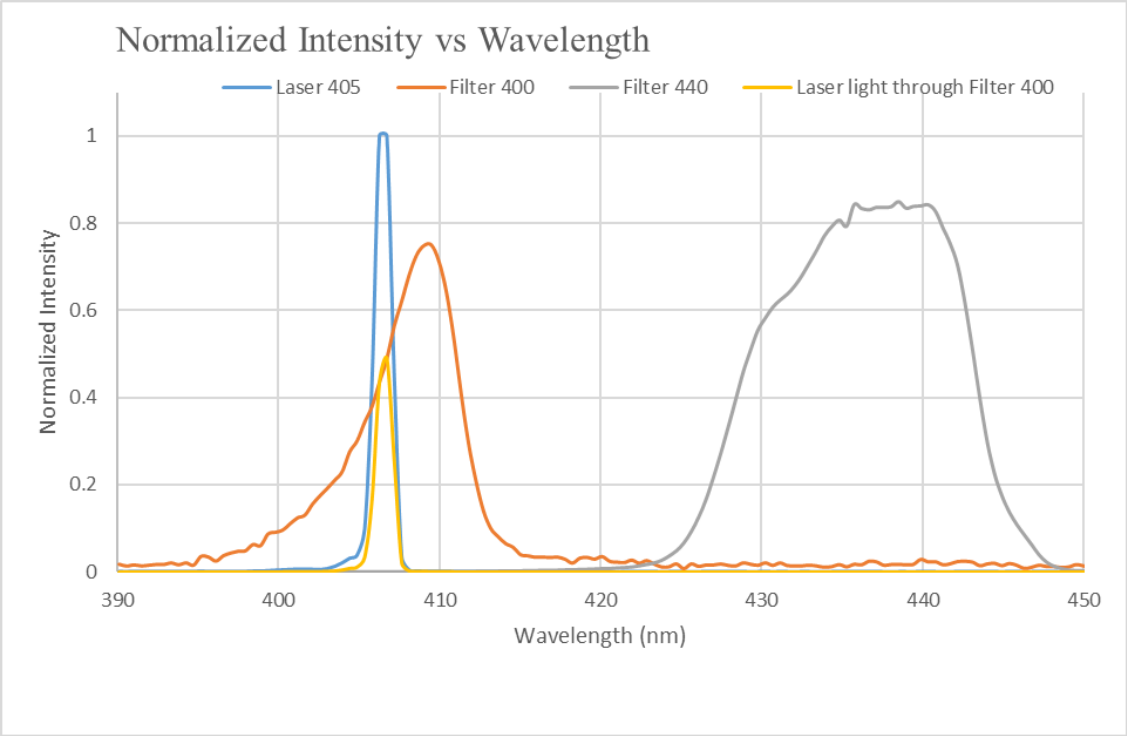


Fig 2: Spectroscopy graph of the blue 405 laser with source 400nm filter and 440 camera filter.

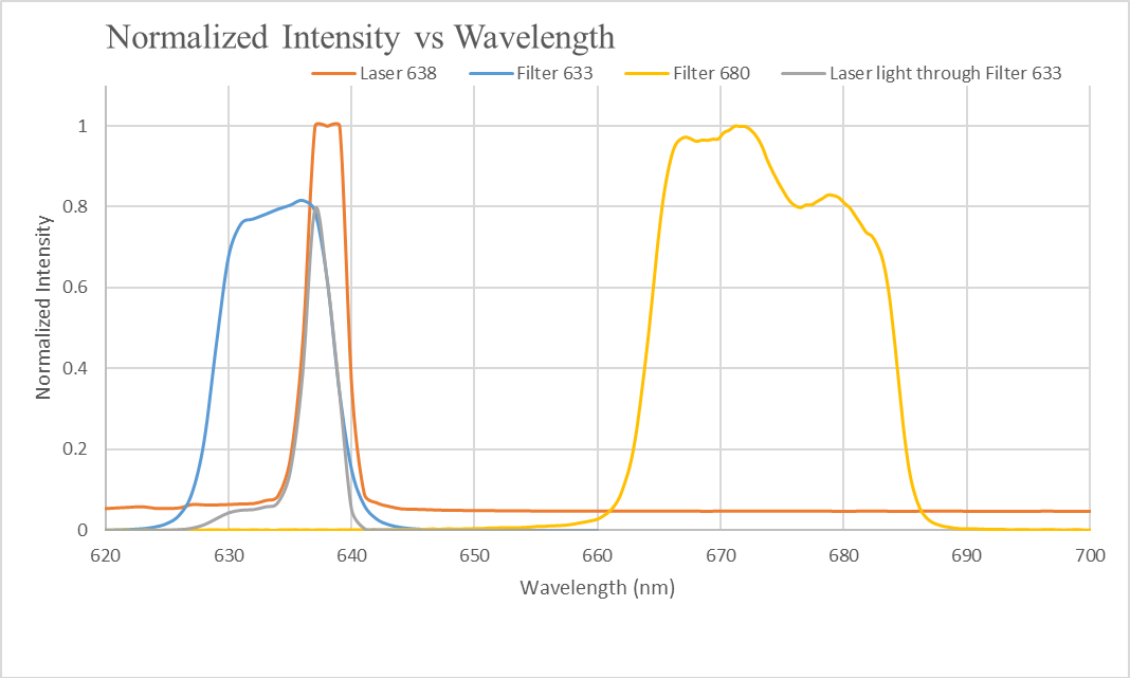


Fig 3: Spectroscopy graph of the red 638 laser with source 633nm filter and 680 camera filter. [135]

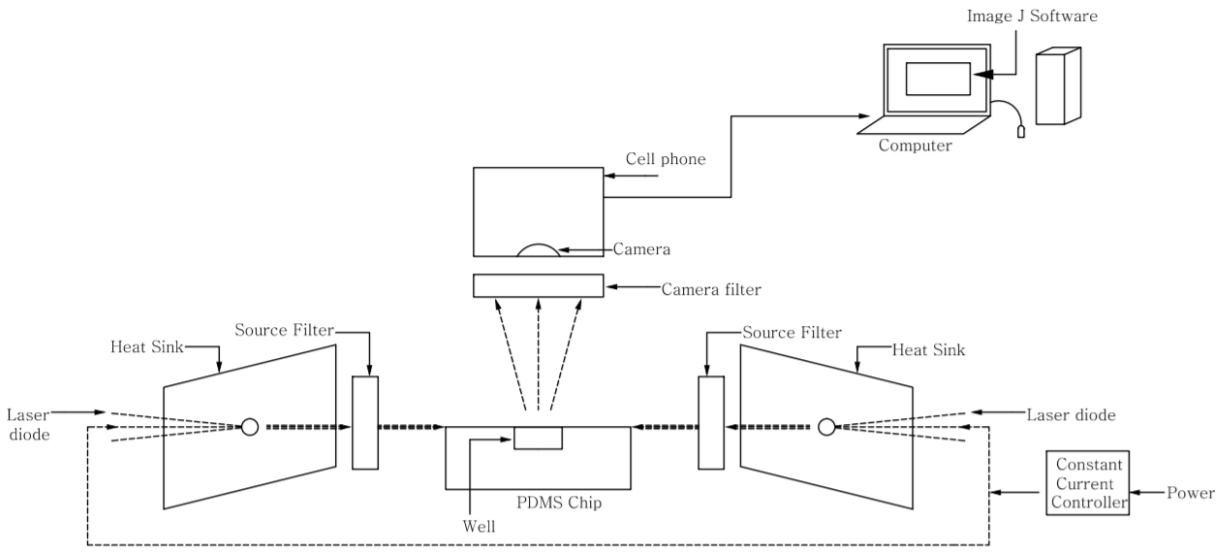


Fig 4: The optical schematic diagram of the proof of concept setup.

The laser diodes although ideally should only let in a definite wavelength of light, but they suffer from spontaneous emission and that's why the source and camera optical filters are used. Figures 2 and 3 display spectroscopy data of the lasers along with the optical filters. As seen in the graphs, using the source filter reduces the intensity of the undesired wavelengths of light thus reduces the noise and improves the sensitivity of the system. The optical schematic diagram of the setup which is used to prove the proof of concept is displayed in Figure 4. Figure 6 displays the picture captured with and without use of source filter for both the lasers along with the camera filters, thus highlighting the importance of using source filters for the setup.

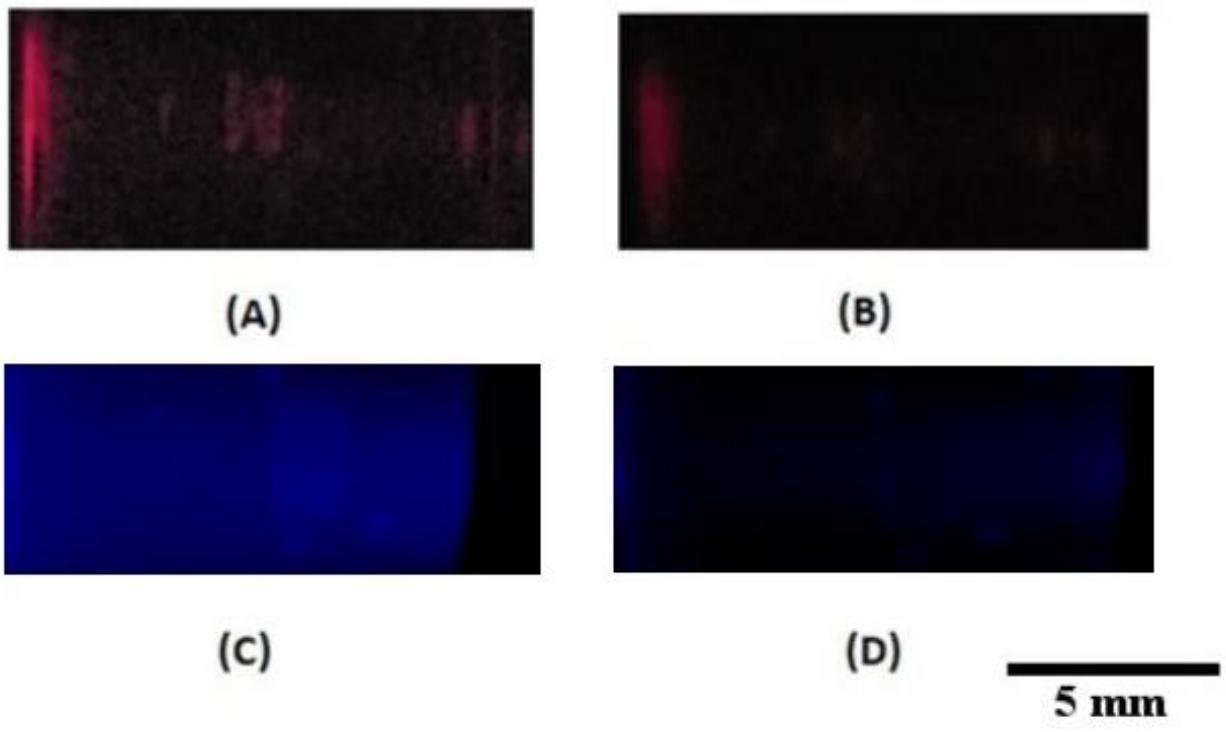
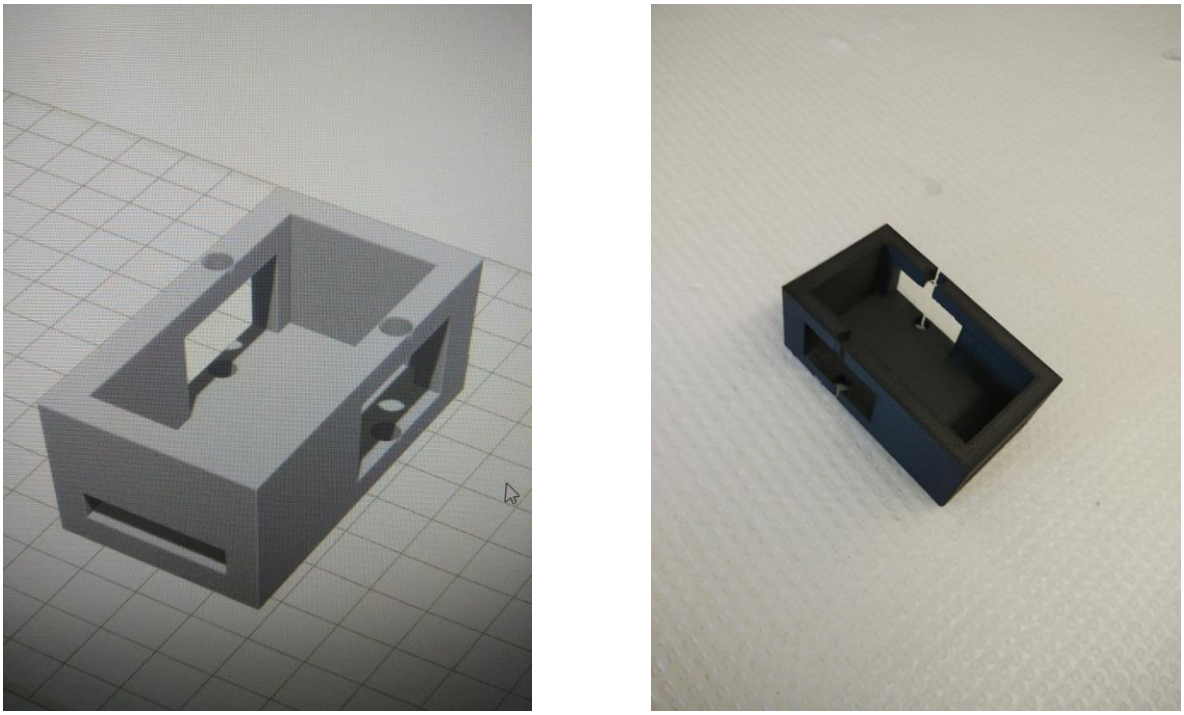


Fig 5: Without and with source optical filter pictures using DI water as blank. (A) and (C) are the without source filters 633 nm and 400 nm pictures and (B) and (D) are with source filters 633 nm and 400 nm pictures.

2.2 Housing Assembly.

Since the aim of this POC biosensor is to be inexpensive alternative to gold standard laboratory tests, it is imperative that all cost cutting measures are taken to produce the housing assembly. 3D printing nowadays has been a reliable source of cheap and effective method to produce prototypes and also can be used to mass produce devices at industry level [136]. SolidWorks™ is a 3D Computer aided design (CAD) program which helps researchers to generate 3D designs which can be then converted into .STL files (3D printer input files). In this case, an Ultimaker 3 printer (Ultimaker B.V., Utrecht, Netherlands) was used to generate the prototype of the housing. Then, the Markforged Oynx One (Markforged, Watertown, MA, USA) printer was used to realize the final design. The proof of concept prototype housing assembly consists of contraptions for the lasers and their heat sinks and a place to hold the PDMS chip. The heat sinks are made of steel and are very important to help the laser diodes to dissipate heat quickly as usually they become really hot if operated continuously for several minutes and save them from significant internal thermal damage. The final desired prototype device is described in figure 7. The Markforged Oynx One printer uses carbon fiber as its filament and utilizes Fused filament fabrication (FFF) printing method. It is tough and is not brittle. It is placed on a table where there is no external vibration and after the lasers are attached to it, the box will contain the PDMS chip and the lasers which are connected to the constant current supply source. The following pictures are a SolidWorks rending with the original box printed off the Markforged Oynx one 3D printer.



(a) (b)

Fig 6: (a) The housing assembly proof of concept final design as created in SolidWorks™ (b) The housing assembly printed using Markforged Oynx One printer.

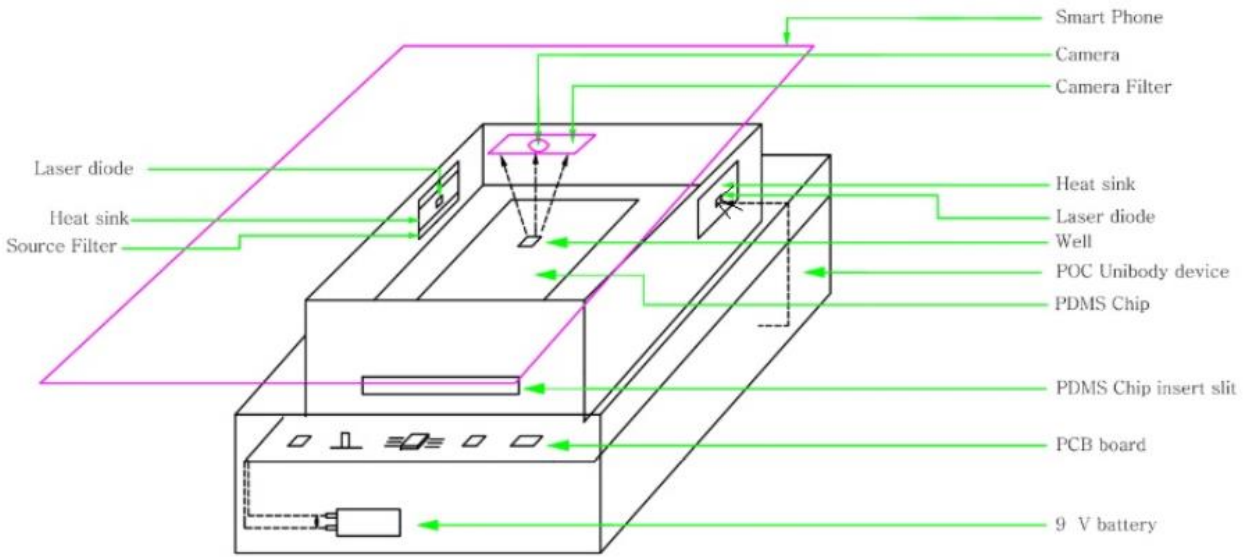
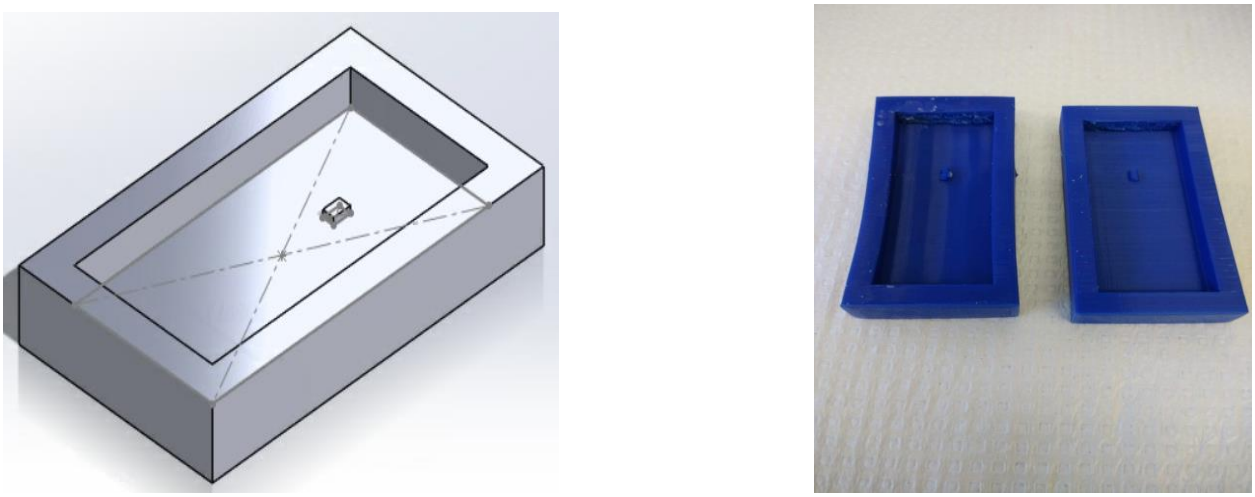


Fig 7: Final sketch design of the POC unibody device.

2.3 PDMS chip and fabrication.

Next for proof of concept, a Polydimethylsiloxane (PDMS) chip is designed to hold the different concentrations of DyLight™ 405 NHS Ester and DyLight™ 633 NHS Ester dyes to procure the calibration curve and to determine the limit of detection of the setup. The idea of the PDMS chip is to have a center well of a desired volume of 12 µl, which will be in parallel to the lasers, so that the light from the lasers will travel in a straight line across the well, which are operated from both sides of the housing box. The design is straightforward as mentioned before and a 3D printed mold design was generated in SolidWorks and an Ultimaker 3 3D printer was used to 3D print the mold using a PLA (polylactic acid) filament using Fused filament fabrication (FFF) printing method. PDMS is a synthetic polymer which is used in many biomedical applications and is a bio inert polymer which can be used as a substrate for many cell culture purposes. Dow SYLGARD™ 184 Silicone Elastomer kit was used to make the final polymer mixture which has two different polymeric parts. At first, 6g of Base Elastomer A of the kit was poured into a plastic cup and measured on a laboratory precise weight measuring scale. Similarly, 0.6g of Elastomer B, a curing agent was added into the cup. Then, they were mixed thoroughly for 10 mins. After a confirmation of a thorough mixture, it was poured into the mold and desiccated for 90 mins. It was then thoroughly checked for bubbles and then put in the oven and baked for 60 mins at 60ºC. Afterwards the mold was taken out of the oven and cooled for 10 mins and the PDMS chip was carefully peeled out. This enabled the required smoothness and transparent nature of the mold through which light can easily pass.



(a) (b)

Fig 8: (a) The PDMS mold final design as created in SolidWorks™ (b) The PDMS mold 3D printed using Ultimaker 3 printer.

(a) (b)

Fig 9: (a) The PDMS chip after peeled out from the mold as seen from the top (b) The PDMS chip as seen from the side.

2.4 Cell phone camera as a detector.

The smartphone which is primarily used in this project is a Xiaomi Redmi Note 4 android phone which has a CMOS 13 MP camera (f/2.0, 1/3.1", 1.12µm) with autofocus. An app was used to take the pictures by the smartphone so that manual control of the camera can be established. The MIUI camera app is a free special app which gives that manual control to the user. The settings which was used in the app for this project are focus 11, ISO 3200 and shutter speed ½ sec. The selected ISO and shutter speed settings are the highest that can be selected in the app so that the maximum amount of fluorescence exhibited by the dye can be aggregated to reduce the noise of the CMOS sensor and to produce a high-quality image. For reproducibility of results across multiple android platforms, another android phone Google Pixel 3 is used with the same apps and settings which has a similar CMOS camera rating (12.2 MP, f/1.8, 1/2.55", 1.4µm).

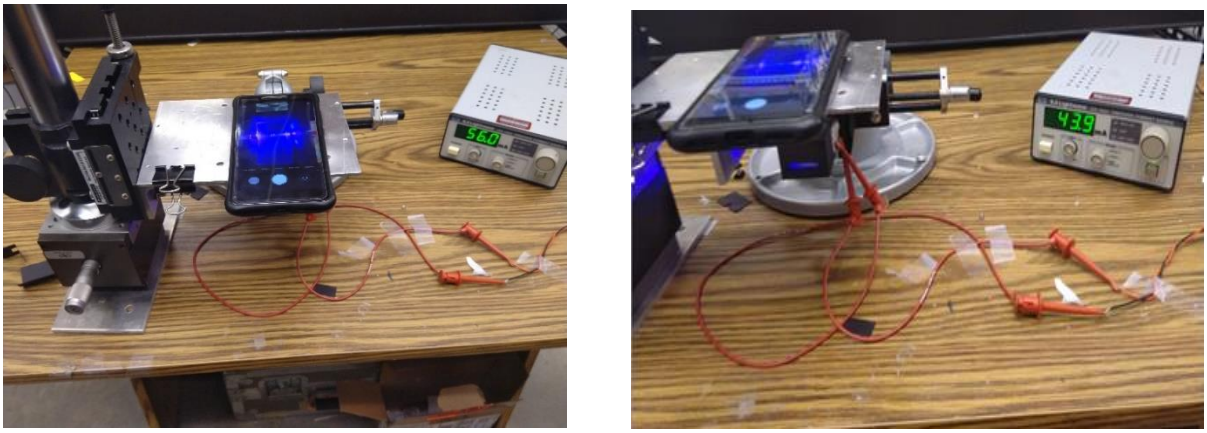
(a) (b)

Fig 10: (a) Xiaomi Redmi Note 4 as advertised on MI global website (b) Google Pixel 3 as advertised on the Google website.

2.5 Setup of the integrated POC system.

For the proof of concept, the whole device is assembled around the housing assembly, with lasers, heat sinks and a constant current source is connected to power up the lasers. The intensity of the emitted light is dependent on the current flowing through the lasers. That's why a constant current source LDX-3412 (Newport, Irvine, CA, USA) is used so that the lasers only produce a fixed intensity of light every time it is turned on. For the blue 405nm laser diode, the constant current is set at 50 mA and for the red 638nm laser diode is at 170 mA. Deionized water (DI water) is used as a solvent and the dyes are mixed into DI water to make the appropriate solutions. DI water is also used as control to measure the baseline. The range of the prepared solutions extend from 10 nM to 1uM concentration of each of the DyLight™ 405 NHS Ester and DyLight™ 633 NHS Ester dyes. 10nM has been used as the lowest concentration for each dye because below that concentration, no significant signal change is detected by the smartphone camera. The process of loading various concentrations onto the PDMS chip is in the ascending order. At first, one of the two dyes are selected. Then, DI water without dye is pipetted into the well of the PDMS chip. Following that, the room is turned dark for accurate measurements, eliminating the possibility of interference from light emitted from other sources than the lasers. In accordance to the dye chosen, the one of the two lasers which is selected in pair with that dye is turned on for approximately 10 secs. The smart phone is mounted on a platform facing the cell phone camera towards the well of the PDMS chip, so that there is no variation of the focal length or the camera angle for each picture which is taken. Three pictures of the fluorescence produced for each concentration are taken per sample which is placed in the PDMS well. Then the laser is turned off and the PDMS chip is cleaned three times using DI water so that no dye residue is leftover which can affect subsequent readings. Thereafter, the process continues till the highest concentration of the dye has its pictures

taken and the same process is repeated with the other pair of dye and laser using the same concentration values in an ascending order. All the pictures are saved into the smartphone and is retrieved using an USB cable onto a computer for further analysis.



(a) (b)

Fig 11: (a) Top and (b) side view of the smart phone based fluorescent microscopy system.

2.6 Analysis of Sensitivity, Limit of detection and Dynamic Range.

After the images are captured for each concentrations for both the dyes, they are transferred to a computer and then analyzed using a software called Image J. Image J is a powerful free image-processing Java based software developed by Wayne Rasband of National Institute of Health which can acquire digital images and can analyze and process them as per user needs. For this project, Image J is used to calculate the intensity of the fluorescence produced after laser light passed through the PDMS well in arbitrary units (A.U.). The captured images are loaded into Image J and intensity measurements are taken of the aforementioned PDMS well. After that the values are exported to a Microsoft Excel sheet where the baseline correction is done by subtracting the intensity of the control from the intensity value of all the other concentrations. A calibration curve is made by plotting all the intensity values in A.U. vs the known concentrations in nanomolar

(nM) units respectively. Below are the calibration graphs of DyLight™ 405 NHS Ester and DyLight™ 633 NHS Ester dyes and their corresponding linear regions.

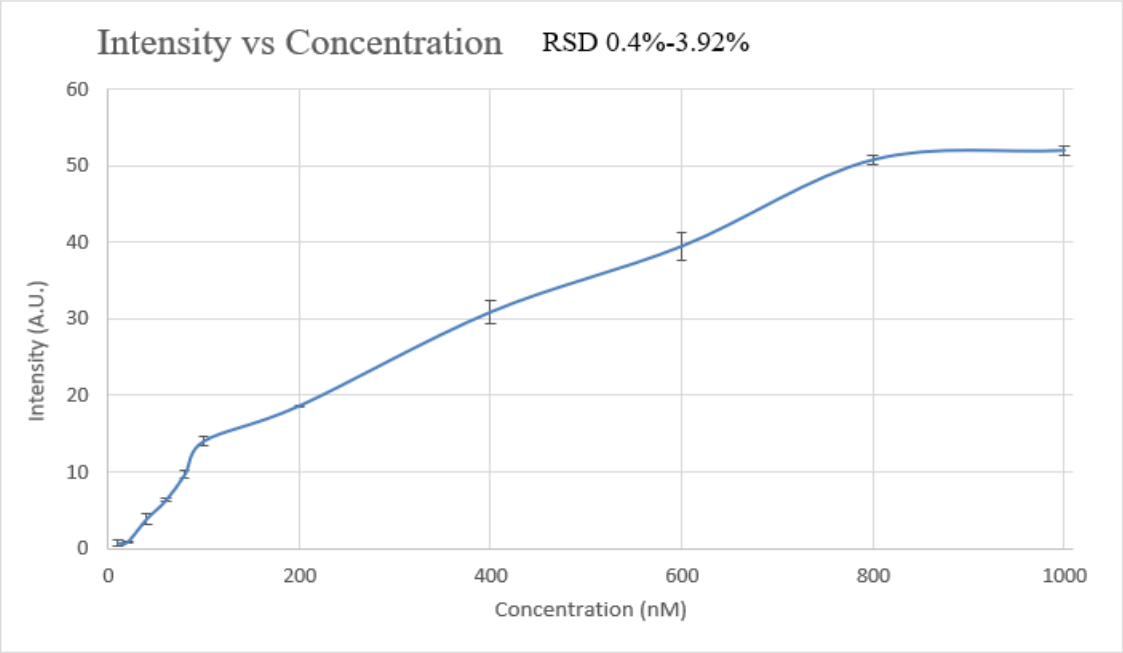


Fig 12: Calibration graph of DyLight™ 405 NHS Ester over the range of concentrations.

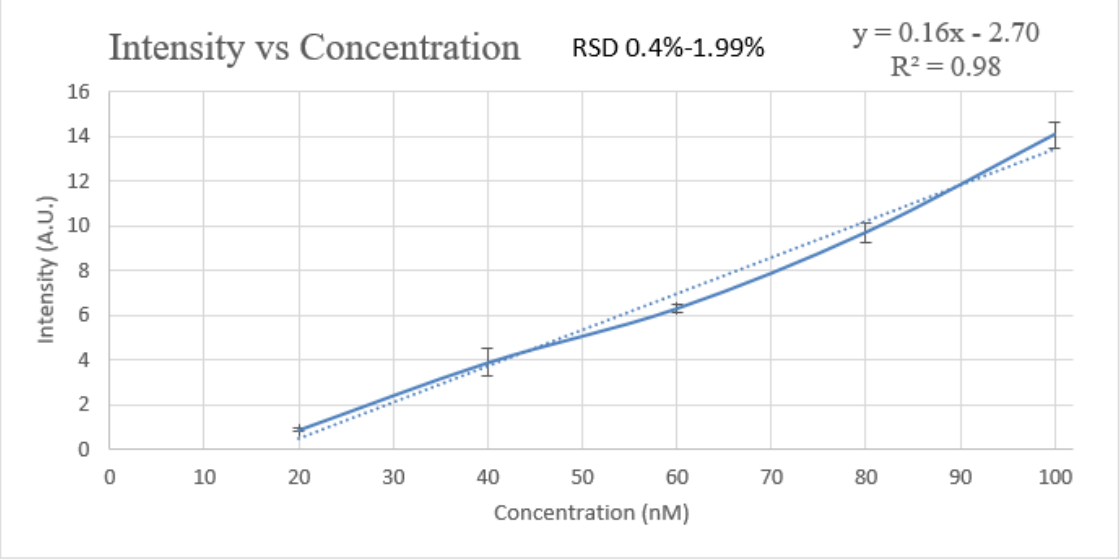


Fig 13: Calibration graph of DyLight™ 405 NHS Ester over the linear region.

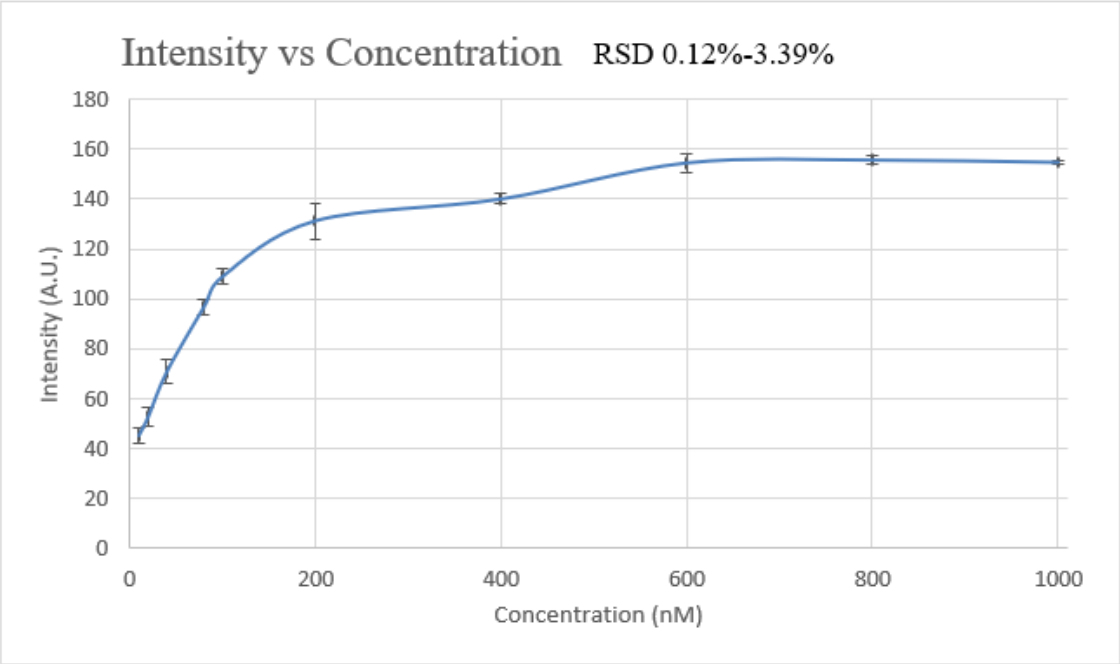


Fig 14: Calibration graph of DyLight™ 633 NHS Ester over the range of concentrations.

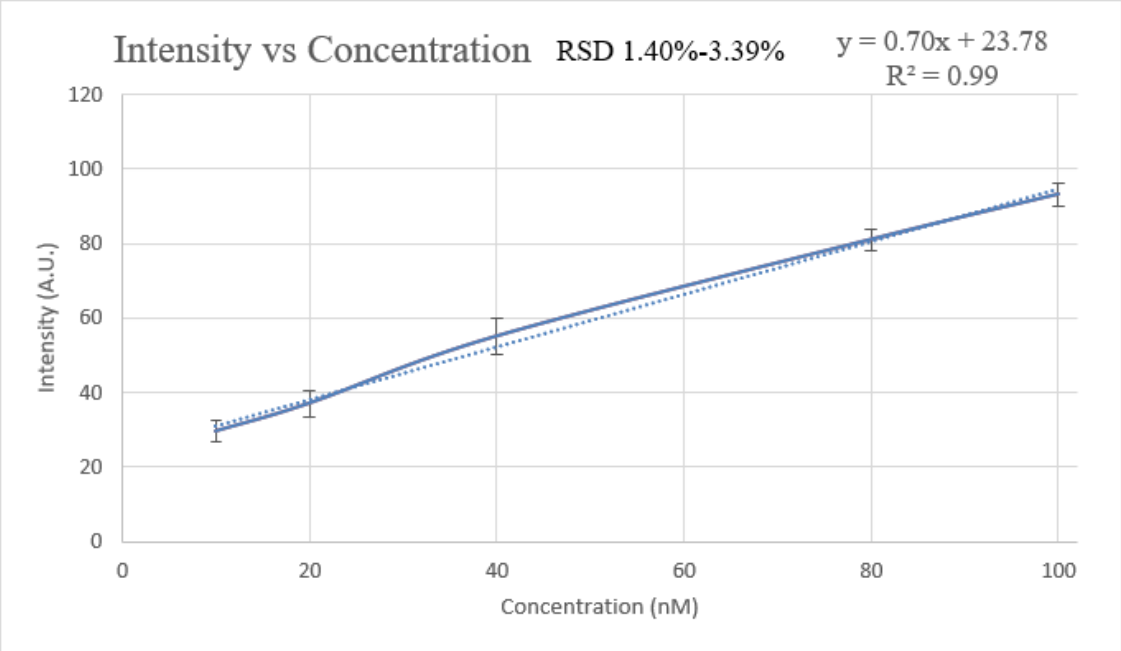


Fig 15: Calibration graph of DyLight™ 633 NHS Ester over the linear region.

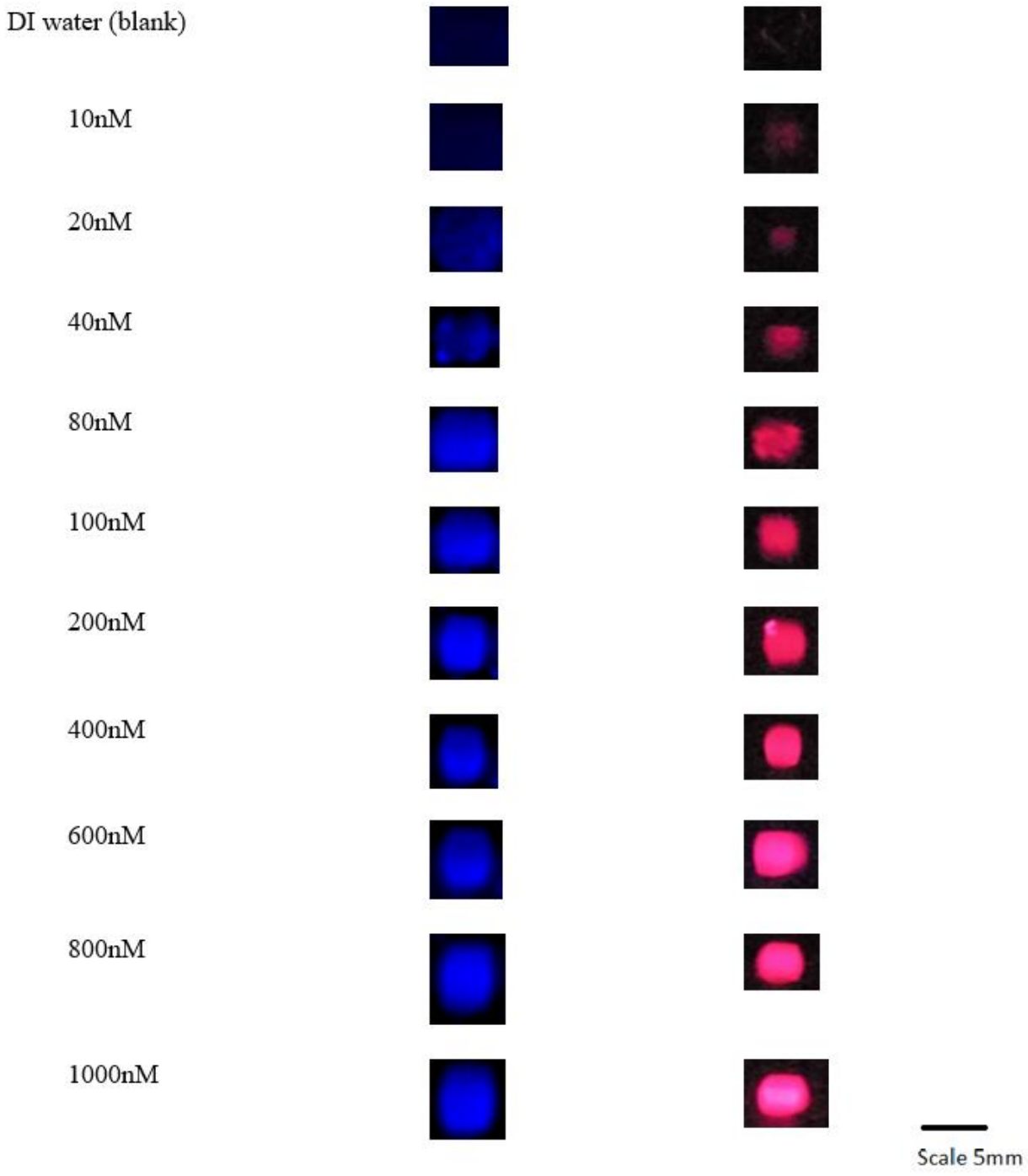


Fig 16: The pictures taken of the well using the proof of concept setup for Dylight 405 and Dylight 633 dyes.

The analytical sensitivity of the sensor is defined as $S = \Delta\lambda/\Delta n$ where $\Delta\lambda$ represents the change of the fluorescence intensity in A.U., and Δn is the change of the known concentration for each dye in nM.

From the linear region of the calibration curve, the best analytical sensitivity found of the DyLight™ 405 NHS Ester dye to be 0.16 A.U/nM and for DyLight™ 633 NHS Ester dye to be 0.70 A.U/nM on average.

The limit of detection (LOD) for each dye is again calculated by regression method of the linear region. The LOD is characterized as the minimum input amount that can be recognized with more than 98% reliability. It is usually three times the standard deviations upon the analytical sensitivity of the system, or $LOD = 3\in/S$, where $\in$ is the standard deviation. The calculated LOD for DyLight™ 405 NHS Ester dye is 28.39 nM and for DyLight™ 633 NHS Ester dye to be 15.85 nM on average.

Dynamic range is another very important metric of a biosensor. The dynamic range of a biosensor portrays the concentration range over which the device can accurately record a variation. The dynamic range for DyLight™ 405 NHS Ester dye is 20-800 nM and for DyLight™ 633 NHS Ester dye is 10-600 nM.

RSD or relative standard deviation is the measure of the standardized dispersion value of a distribution. It is expressed as a percentage of the ratio of standard deviation to the mean or

$$RSD = \frac{Standard\ deviation\ (s.d)}{mean\ (\overline{x})} \times 100\%$$

The calculated RSD values for each graph are mentioned on their caption.

2.7 Analysis of reproducibility of smartphone reading.

To make sure the setup is producing reliable LOD and dynamic range values, a secondary android based smartphone Google Pixel 3 was used to verify the results. The rest of the methodology and setup remained unchanged. The camera rating of Pixel 3 is similar to Redmi Note 4, 12.2 MP (f/1.8, 1/2.55", 1.4µm). The LOD and dynamic range values were calculated to be in the same range, 22.25 nM and 10-800 nM for DyLight™ 405 NHS Ester dye and 28.10 nM and 10-600 nM for DyLight™ 633 NHS Ester dye.

2.8 Comparison of measurement accuracy with laboratory gold standard.

A laboratory fluorometric microplate reader, Synergy H1 Hybrid Multi-Mode Microplate Reader (Biotek, Winooski, VT, USA) is used as a gold standard for the experiment. A fluorometric microplate reader is a benchtop laboratory device which can be utilized to calculate intensity of fluorescence after a fluorescent dye is excited by a specific wavelength of light. The measurements were taken using laboratory protocol and each known solutions were pipetted into the microplate. The same range of concentrations of solutions were used. Then the computer was connected to the reader and the device was turned on. In a few minutes, all the data were visible in the integrated software preloaded on the computer and the data were exported in an Excel sheet. A calibration curve is plotted using the aforementioned principle. Below are the calibration graphs of DyLight™ 405 NHS Ester and DyLight™ 633 NHS Ester dyes and their corresponding linear regions.

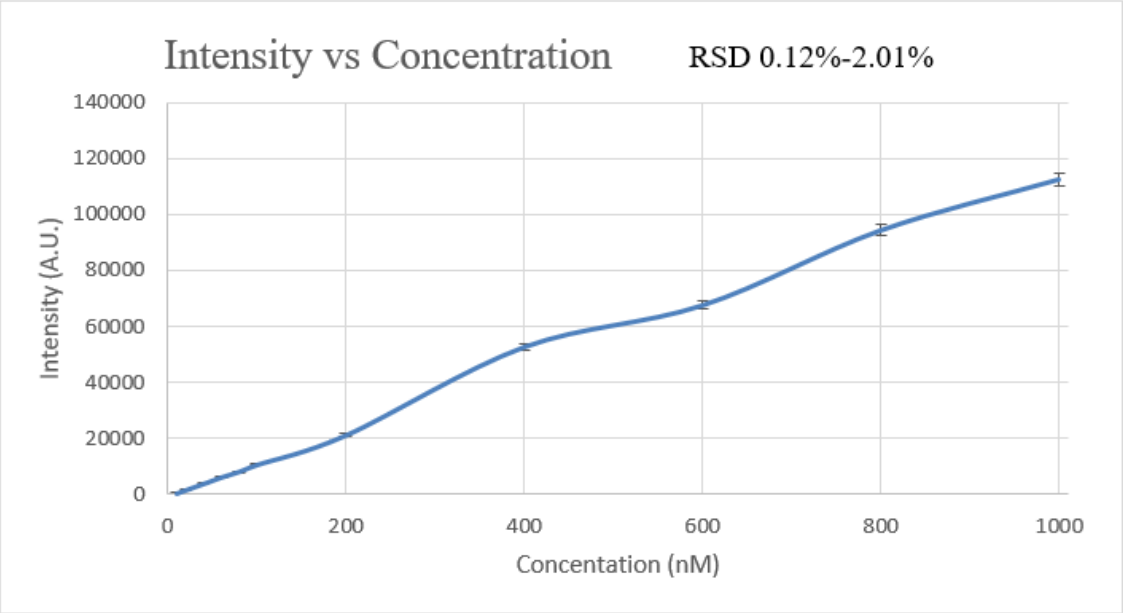


Fig 17: Calibration graph of DyLight™ 405 NHS Ester over the range of concentrations.

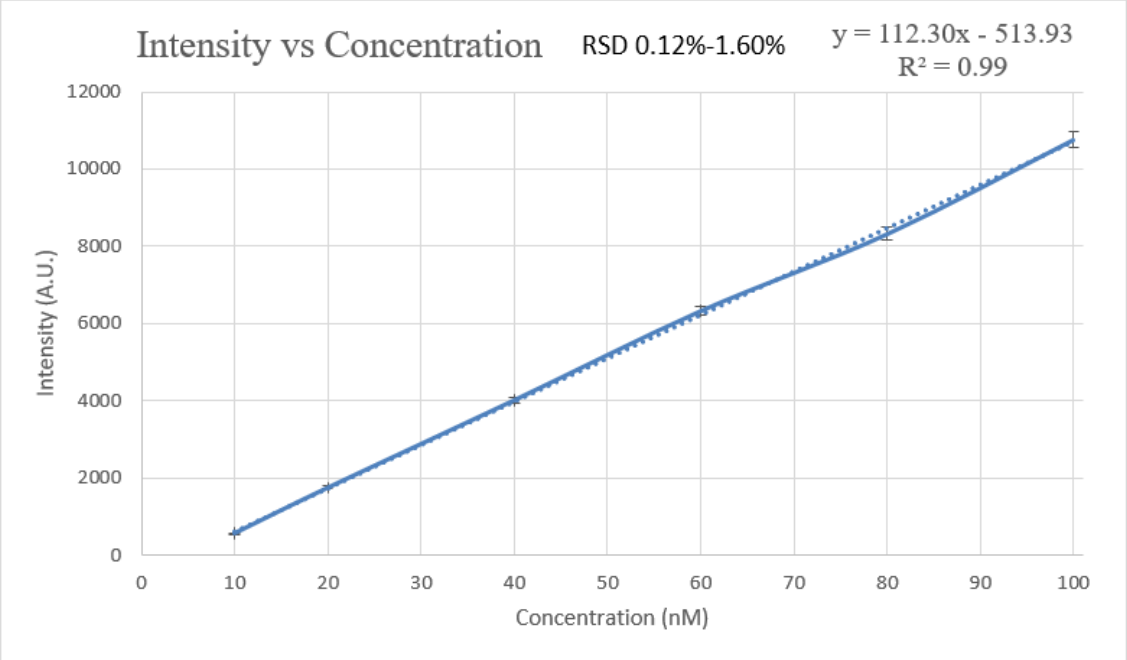


Fig 18: Calibration graph of DyLight™ 405 NHS Ester over the linear region.

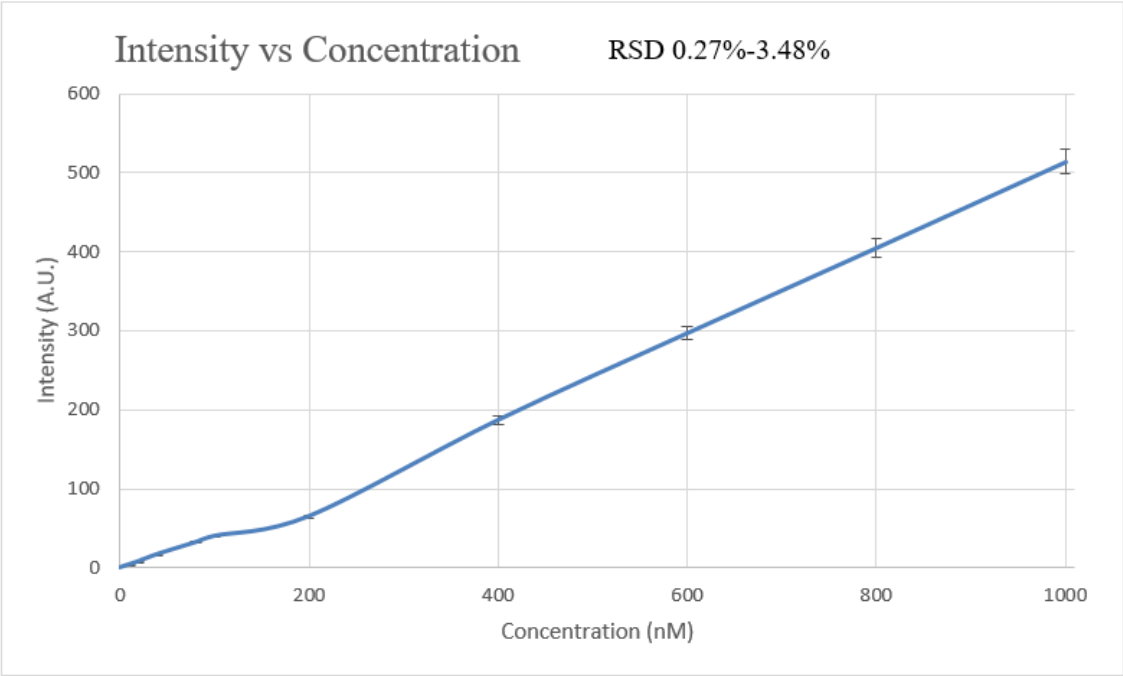


Fig 19: Calibration graph of DyLight™ 633 NHS Ester over the range of concentrations.

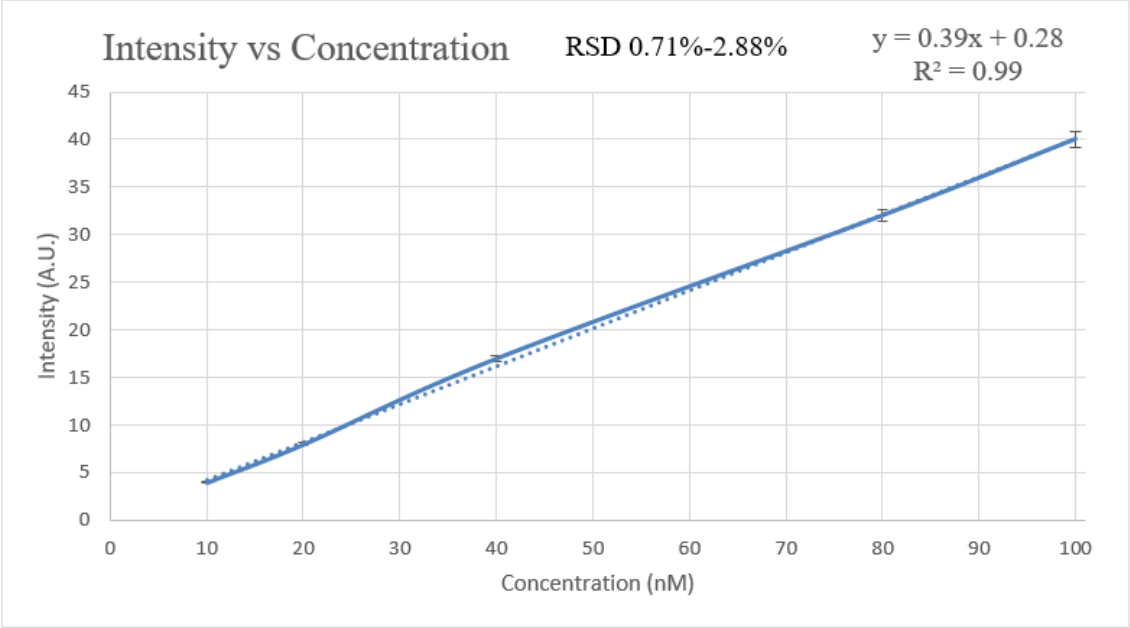
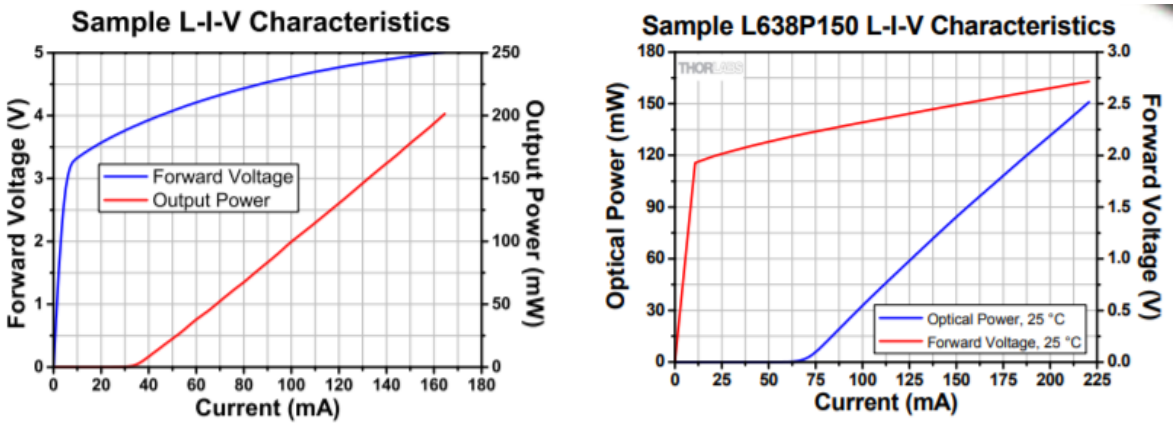


Fig 20: Calibration graph of DyLight™ 633 NHS Ester over the linear region.

The analytical sensitivity, LOD and dynamic range were also calculated using the calibration curve. The analytical sensitivity of the DyLight™ 405 NHS Ester dye is 112.30 A.U/nM and for DyLight™ 633 NHS Ester dye to be 0.39 A.U/nM on average. The calculated LOD for DyLight™ 405 NHS Ester dye is 5.00 nM and for DyLight™ 633 NHS Ester dye to be 6.75 nM on average. The dynamic range for DyLight™ 405 NHS Ester dye and DyLight™ 633 NHS Ester dye is evaluated to be 10-1000 nM.



(a) (b)

Fig 21: The optical power vs current graph for (a) Laser 405 nm and (b) Laser 633 nm from Thorlabs website.

2.9 Theoretical estimation of the absorbed optical power.

Assume the molar attenuation coefficient of Dylight 405 is,

$\varepsilon = 30000 M^{-1} cm^{-1}$ (which means 30000 per [mol] per [cm])

The actual absorption to laser light is,

$\xi = 10^{-\varepsilon c L}$

where c is the molar concentration and L is the thickness of the cell

The laser power absorbed by the sample cell is

$$P_a = S_p(1 - \xi)A$$

where S_p is the optical power density per cm^2 excited on the sample cell, and A is the cross-section of the sample.

Assume 1mW optical power from the laser and the excitation area at the sample cell is 2.5mm x 3mm, the power density of excitation is,

$$S_p = \frac{10^{-3}}{0.25 \times 0.3} = 0.013\ W/cm^2$$

The volume of the sample cell is $12\mu L$, which is $12*10^{-3}$ cm^3

The sample cross section area A = 0.3*0.25= 0.075 cm^2 , length across is L = 0.25cm,

If the molar concentration of sample is c = 10nM= 10^{-8} Mol

$$P_a = 0.013 \times \left(1 - 10^{-30000 \times 10^{-8} \times 0.25}\right) \times 0.075 = 1.68 \times 10^{-7} W = 168\ nW$$

Assume the yield of fluorescence dye is 80%, and the loss of laser light due to surface Fresnel reflection, scattering and PDMS waveguide absorption is 25%, then 60% of laser light will be effective for cell excitation.

$P_a = 168 \times 0.60\ nW = 100.8\ nW$ for every 1mW of laser emission power.

Then there are two optical filters, one at front of the laser and another one in front of the camera, their losses must be accounted as well. The light which passes through the laser filter is around 50% and the light which passes through the camera filter is around 40%, so effective percentage of light passing through is 20%.

Therefore effective $P_a = 100.8 \times 0.2 = 20.16\, nW$.

25 mW laser power is obtained at 50 mA current, so that the fluorescence emission from sample should be on the order of 500 nW.

The number of photons generated for 500nW optical signal at 440nm is approximately:

$N = \frac{P_a}{hv} = \frac{500\times10^{-9}}{6.63\times10^{-34}\times3\times10^{8}/(440\times10^{-9})} = 1.1 \times 10^{12}$ signal electrons per second.

For a cell phone sensor area 16.3 mm^2 ($16.3*10^{-6}$ m^2) with 13 Mega pixels, the pixel size is

1.25 μm^2 ($1.25*10^{-12} m^2$)

The number of photons on each pixel is

$N_{pixel} = \frac{N}{No.of\ pixels} = \frac{1.1\times10^{12}}{13\times10^{6}} = 84615$ electrons per pixel per second

The dark count per pixel is typically on the order of 20 electrons per second which is more than 2 orders of magnitude smaller than the number of signal electrons.

Similarly, the absorbed power can be calculated for Dylight 633 dye, at 90mW optical power which is generated at 170mA current, which is approximately 7812 nW. The number of photons generated for 7812 nW optical signal at 680nm is around 2.67×10^{13} signal electrons per second. The number of photons on each pixel is 2.0×10^{6} electrons per pixel per second.

Chapter III: Design of the circuit and PCB development for smartphone optical readout

In the previous chapter, the concept of smartphone based fluorescent microscopy system has been successfully demonstrated, and reasonably good detection sensitivity is achieved in that process. From a practical application's point of view, a point of care device must be built before this concept can be taken to the end users (patients) so that diagnosis of diseases become more accessible to them.

1. Circuit design

In order to achieve that objective, a driving circuit is designed so that lasers can turn on and the smartphone camera for capturing the picture of the well of the PDMS chip. The circuit is designed using LTSpice (Linear Technology, California, USA) where a 9V battery is used as the power source. The circuit when switched on, will sequentially turn on each laser for 10s, and then turn off both of them. The circuit contains a push button switch to power up the circuit, one LM556CN timer which will turn on each laser diode for 10 secs each sequentially before turning off the entire circuit. The 10s time period is achieved by exploiting the monostable mode of the LM 556 IC. In this mode the LM 556 is utilized as a single shot timer clock to deliver an output voltage of a definite interval. The timespan is set utilizing one capacitor and one resistor. The timespan is set off by applying a low pulse to the trigger pin. The output terminal at that point goes high for rest of the time period. The formula used to calculate the time delay is T = 1.1*R*C where T is the time period in seconds, R is the resistance value in Ohms and C is the capacitance in Farads. R2, R3 and C1, C5 are the resistances and capacitors used for this purpose. Two LM317 voltage regulators are used in the constant current source mode so that each of the two outputs from the

LM556CN timer produces a constant current. LM317 is a multipurpose component, able to supply greater than 1.5A of current even at high temperatures, is profoundly stable, and has a few built in features like current limiting and thermal shutdown features. A resistor R4 is serially attached to the output pin of the first LM317 chip. The adjust pin is then parallelly connected to the resistor. When no load is attached, the chip will preserve the voltage present at the adjust pin at 1.25V, so essentially the voltage across R1 is thus 1.25V. With fluctuation in current, the voltage drop across R1 will usually increase or decrease yet the LM317 quickly modifies the output voltage to recoup according to the change, retaining the voltage drop across R1 at a steady value of 1.25V. By choosing an appropriate value of R1, we can exploit this state to set a 'limit' on the current provided by the voltage regulator chip. This same condition is achieved by the R5 resistor for the second LM317 IC. Using Ohm's law, R = Vref / I, where R is the resistance value in Ohms, I is the desired constant current in Amps and Vref is the reference voltage across the adjust pin which is 1.25V as mentioned previously. A 1N4148 switching diode is used to power off the circuit after 20 seconds. The block diagram used for circuit simulation is given below:

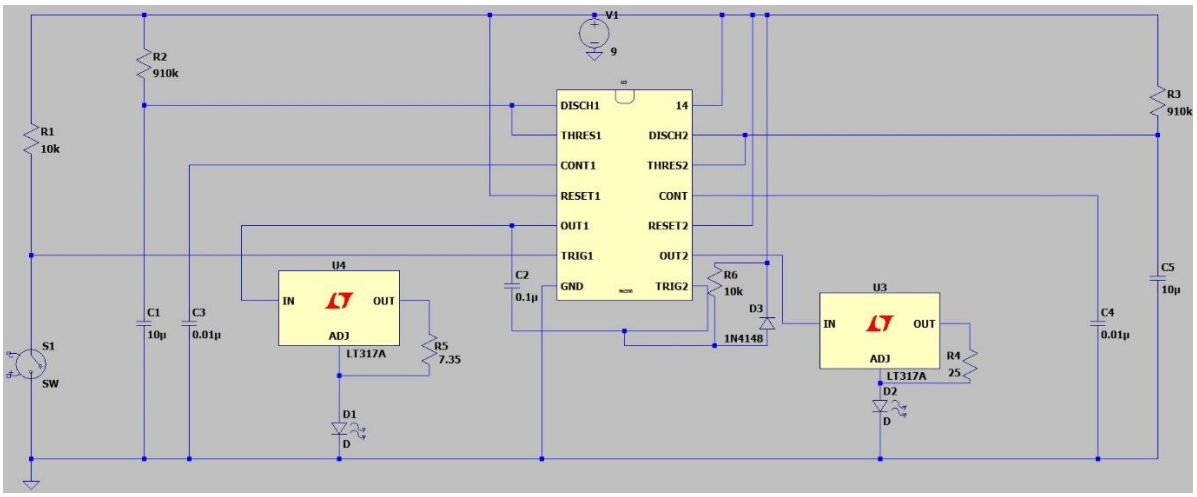


Fig 22: Timing control and laser driving circuit as simulated in the LTSpice software.

2. PCB development and working principle

As previously mentioned, the Printed Circuit Board (PCB) will be powered by a 9V battery. The PCB contains one LM556CN timer (National Semiconductor, California, USA), two LM317 voltage regulators (National Semiconductor, California, USA) are used in the constant current source mode. For the blue 405 nm laser, the desired current is 50mA, therefore the value of R4 is calculated as: $\frac{1.25}{50\times10^{-3}}$ Ohms or 25 Ohm. Using the same formula, the value of R5 is calculated to be 7.35 Ohm for the desired set current for the red 638nm laser which is 170mA. This operation allows the lasers to emit light of constant intensity determined by the constant current over the 10sec time period every time the circuit is turned on. Since both the lasers diode are turned on for 10s each, the values of R2 and R3 are the same and calculated to be 910,000 Ohms and similarly, the capacitance value is same for the capacitors C1 and C5 which is 10 µF. There is an additional 1N4148 switching diode (ON semiconductors, Arizona, USA) to turn off the circuit after 20 seconds. The rest of the components used to design the PCB is a pushbutton switch S1 and two 10,000 Ohms resistors are used as R1 and R6 to prevent shorting of the terminals. With the actual sizes of capacitors, resistors and diode, the final PCB is designed using the EagleCAD (Autodesk, Mill Valley, CA, USA) software and outsourced to 4pcb.com (Advanced Circuits Inc., CO, USA) for fabrication. After receiving the fabricated PCB, all the board components are soldered in and the final product was ready to use for the POC device.

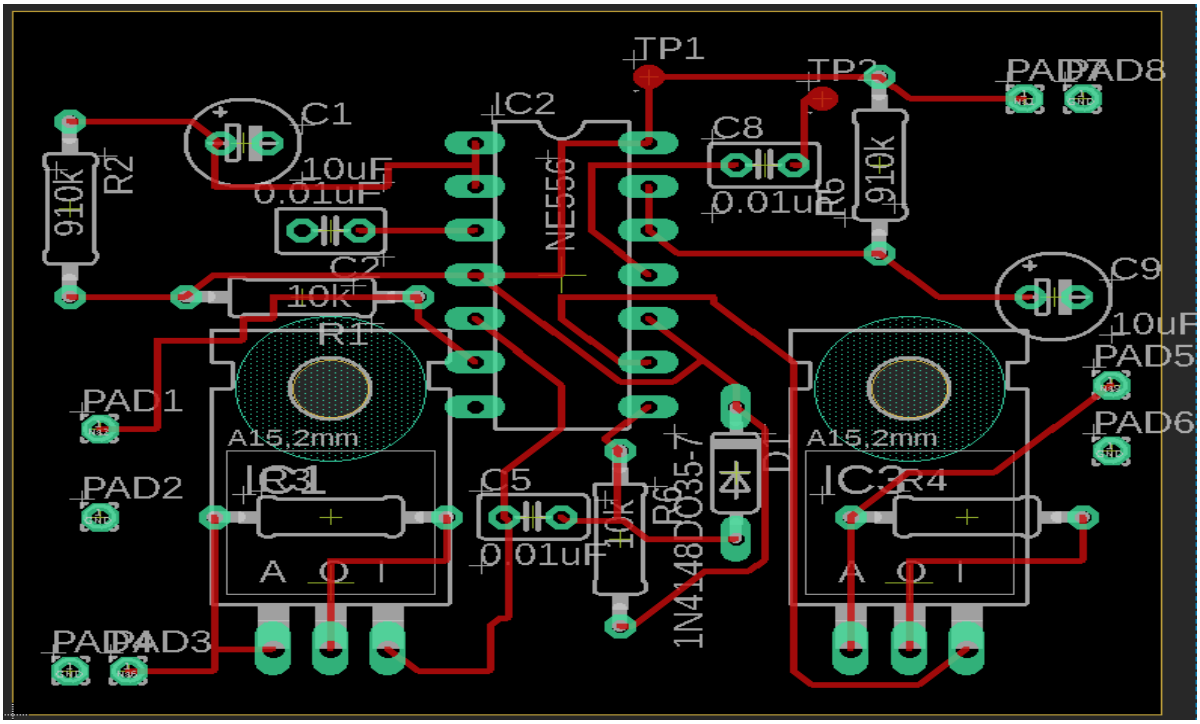


Fig 23: The PCB simulation in Eagle CAD software.

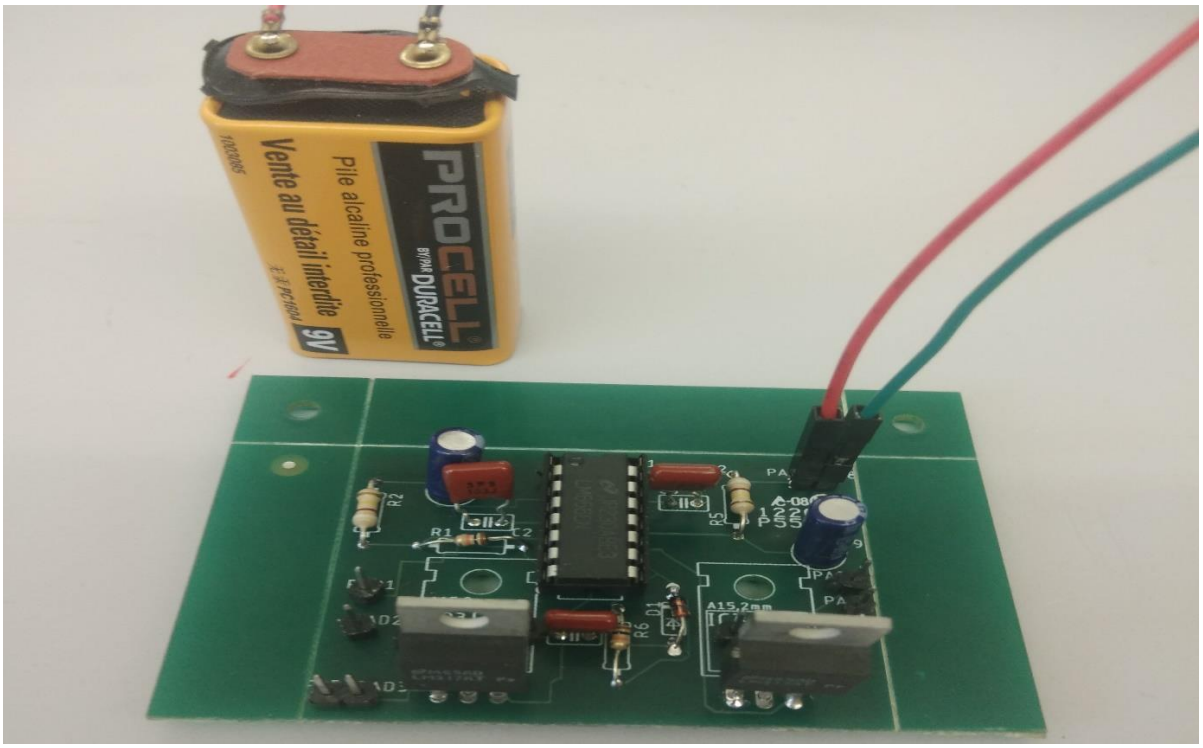


Fig 24: The assembled PCB after receiving from 4pcb.com.

3. **PCB testing**

The PCB is assembled and then tested to find out if it meets the design requirements. The PCB is prepared to power two lasers but for testing purposes, the lasers were replaced by two red LEDs (LED 1 and LED 2) as they are cheaper while having similar current-voltage characteristics as laser diodes. After carrying out the test, a final 3D printed unibody device will be manufactured which will house the lasers with their heatsinks, the PCB module and the 9V battery. The aforesaid design requirements are met when the PCB is tested. The following pictures were obtained during the testing.

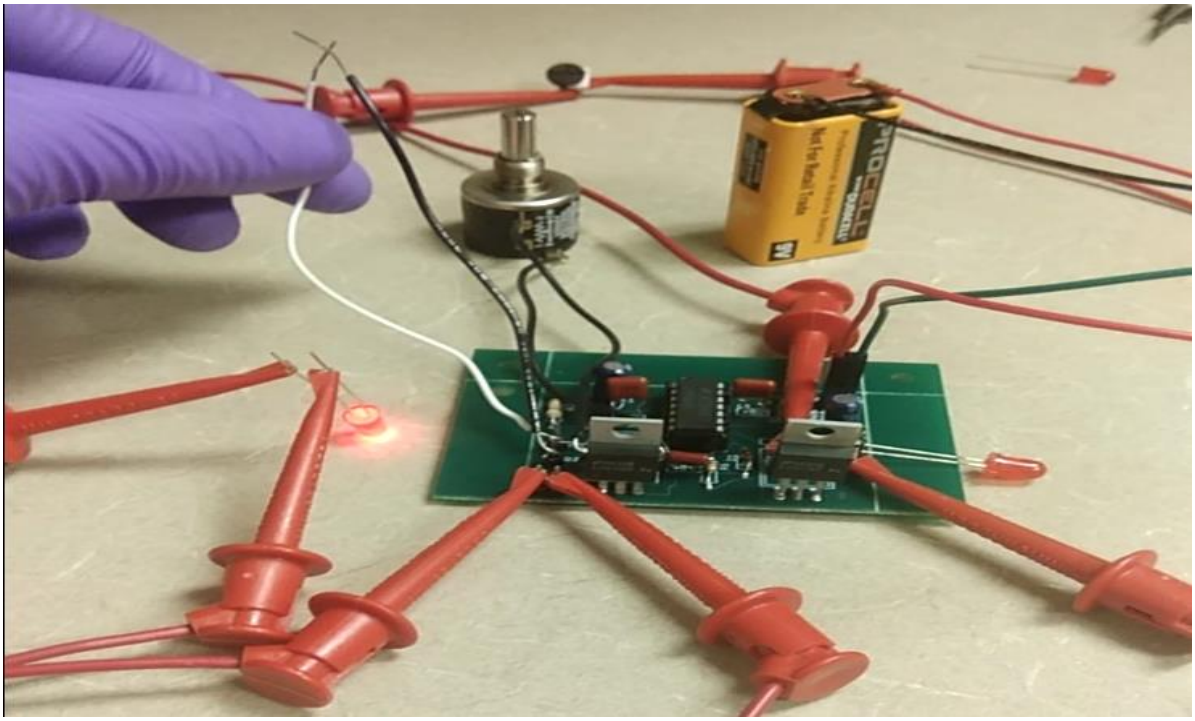


Fig 25: LED 1 (on the left) turns on for 10s when the assembled circuit is shorted (push button action).

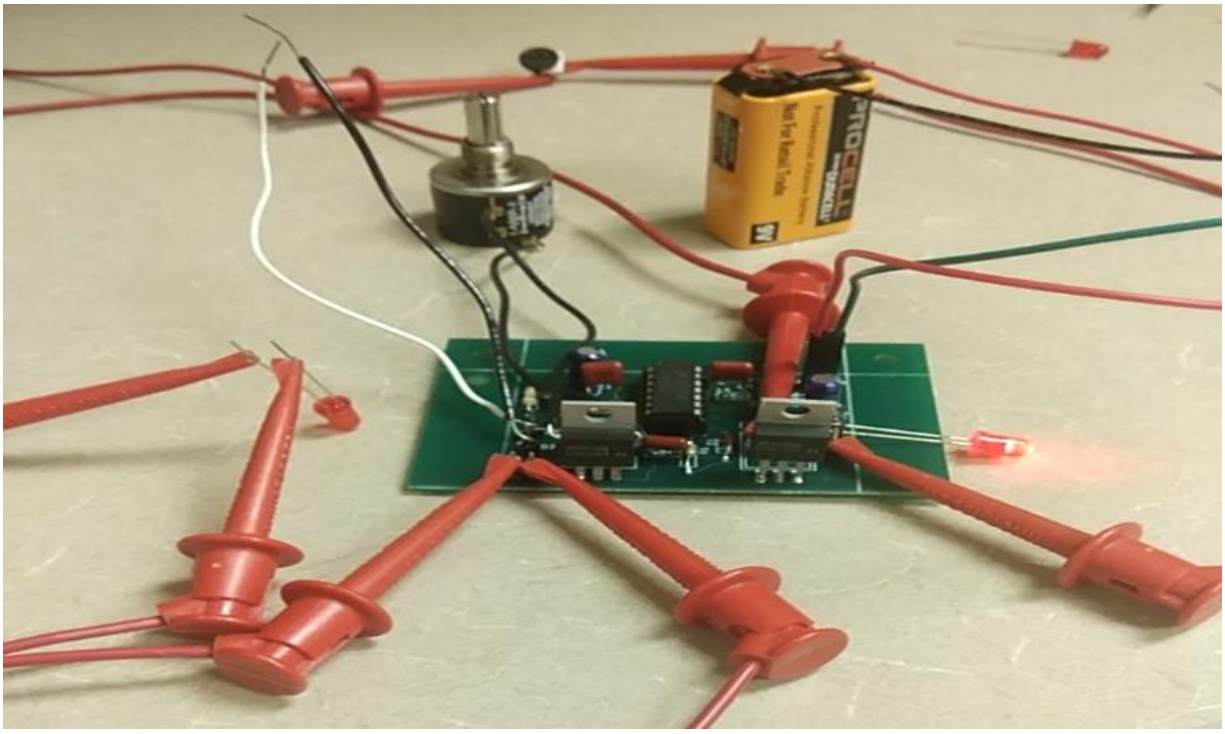


Fig 26: LED 1 (on the left) turns off after 10s and LED 2 (on the right) turns on for 10s before the whole circuit shuts off.

Chapter IV: Discursion and future direction.

Smartphone as described in the first chapter has come a long way since its inception in 2008, so is its camera. The first cell phone camera was a VGA (Visual graphics array) camera, but now smartphones are equipped with powerful camera technology up to 48 Megapixels (MP) in them. The technology used for the camera has also come a long way as charge-coupled device (CCD) detector-based system was popular at first but was expensive, so now almost all the smartphone manufacturers are using complementary metal oxide semiconductor (CMOS) based systems. CCDs produce high quality images but comes at a high cost, whereas CMOS based system is comparatively cheaper. It is to be noted that the sensors of the CMOS based system are not of a higher quality than CCD. CMOS based systems usually needs more light to capture low noise images and its sensors are not that sensitive to incident light. The Carlson group reported that a good CCD camera might be as much as 85 times more sensitive than a cheap CMOS sensor for low light photography [137]. That is why there is a difference between the limit of detection of a laboratory equipment like fluorometer or microplate reader which usually uses a CCD camera and a POC setup using a smartphone. This is also evident from the theoretical calculation that the number of photons generated per pixel using the CMOS sensor is about 10^2 times greater than the number of photons per pixel needed to detect the same 10nM concentration by a CCD camera. It is also believed that the scattering phenomena of the PDMS chip is responsible for higher background noise which saturates the CMOS sensor of the camera, thus increases the PDMS waveguide absorption loss and in turn making it less sensitive. Similar scattering problem is also addressed by the Seiffert group when they exposed a PDMS waveguide to UV emission without using any fluorescent dyes [138]. Therefore, a better system must be designed to address this drawback. For this project an android smartphone is used because they are not only cheaper than

Apple iPhones but also are very popular in most low resource countries. Also, the iOS software platform used by Apple smartphones is less user modifiable unless the user wants to invest in paid apps. So android is the better platform to build this device and eventually can be later expanded into the iOS platform. Even though the cell phone cameras are less sensitive than benchtop expensive laboratory spectrum analyzers, but for rapid, cheap and reliable testing, a product like this can very well work. This study is a preliminary novel work to demonstrate that a 3D printed POC device-based cell phone fluorescent microscopy without using mirrors and focus collectors set at different angles can have low detection limits which is ideal for cellular level testing. The plan is to continue the manufacturing of the POC device by 3D printing a POC unibody device which houses the lasers with the heat sinks, the assembled PCB and the 9V battery. More research is needed to better the design of the setup so that the PDMS scattering can be reduced and in turn the sensitivity of the whole system can be increased. In the future, this study can be expanded into diagnostic domain and can as well be used to detect cellular proteins or pathogens in blood using a microfluidics platform and can serve the humanity by providing low income societies a way to access cheap and reliable testing.

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