MASTER OF BIOMEDICAL ENGINEERING

Design and Optimize a Smartphone-Based Medical Device Using an Aggregation-Induced Emission Bio-probe for CKD Monitoring

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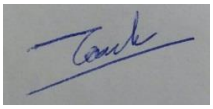
DECLARATION

I certify that this work does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.



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Tran Tam Anh Pham

Abstract

Human Serum Albumin (HSA), a major component of blood plasma, has been used as a potential indicator for the early stages of the Chronic Kidney Disease (CKD) for a considerable period of time. Traditional testing techniques require patients to regularly travel to testing clinics, consuming time and incurring cost. In addition, traditional testing necessitates the availability of trained medical staff and complex diagnostic equipment. However, with the use of an Aggregation-Induced Emission Bio-probe for CKD Monitoring, it may be possible for patients to reliably undertake testing by themselves, at home without the need for intervention from medical professionals. This project therefore proposes the development of a home-based smartphone medical device, which can monitor kidney function, where the patient performs testing by themselves, in an environment which is both convenient using equipment which is accessible and affordable.

A specific Aggregation Induced Emission (AIE) bio-probe, sodium 1,2-bis[4-(3sulfonatoproxyl)phenyl]-1,2-diphenylethene (BSPOTPE), is used to detect serum albumin in urine. BSPOTPE is non-luminescent in urine, but becomes emissive in the presence of HSA. With high resolution in differentiating the concentration levels of HSA in urine (low detection limitation to 1 nM), superior selectivity to Albumin and correlations between light intensity and HSA concentration, the bio probe will be utilised by a smartphone based medical device. The HSA bio-probe will absorb the stimulating light and then emit fluorescence, and the smartphone will be used to record the levels of fluorescent light. Finally, an application installed on a smartphone will be used determine the correlation between light intensity and HSA concentration. The smartphone-based urinalysis device will utilise ASSURED features that are Affordable, Sensitive, Specific, User-friendly, Rapid Robust, Equipment-free and Deliverable to end-user.

In undertaking the project, a number of technical challenges need to be resolved, such as the need for a uniform stimulating light wavelength and high light intensity from the light source, the removal of light contamination from the external environment, as well as the need for an accessible, usable and affordable - physical device to be manufactured. Although there is a need for further development and testing to improve the device's reliability, the project has demonstrated highly encouraging results, in

the correlation between the fluorescent light emitted from the bio-probe and the HSA concentrations that have been detected, in indicating the potential presence of Chronic Kidney Disease.

This device is therefore expected to ultimately help users cost effectively, efficiently and reliably, test and monitor the albumin levels in their urine, and therefore detect the early onset of chronic kidney disease, without unduly impacting their daily lives while improving their kidney health.

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Chapter 1: Introduction

The kidney with its primary function of filtering the blood to remove waste and toxins is one of the most vital organs in the human body. In addition, the kidney is responsible for regulating blood pressure, water balance in the body and vitamin D activation.

Kidney diseases are rarely obvious. Their worsening conditions can silently occur over many years without any apparent medical symptoms. Chronic Kidney Disease is the most deadly of these diseases and has been described as 'the silent killer'. When an individual realizes that they have kidney disease, it is normally so late for effective treatments that dialysis or a kidney transplant is the only remedial options (Kidney Health Australia 2015).

Urinalysis is the most common method employed to detect CKD. If detected in its very early stages, it can be treated with medication or daily intervention activities to support kidney health. To help patients monitor their kidney health routinely, this project therefore aims to design and optimise a smartphone based medical device using an Aggregation Induced Emission (AIE) bio-probe. This device's functions are required to operate in the same way as miniaturized urinalysis, allowing patients to perform the test as and when required and convenient.

This thesis will firstly introduces the background of Chronic Kidney Disease and the technological implications of smartphone based analysis. Next, a new method of kidney failure detection will be explained, followed by the analysis of the opportunity for the design and development of a smartphone based medical device. Following this, the report will briefly review some strengths and weaknesses of previous designs, and so identify the desirable criteria required to be incorporated into a final prototype. After putting the final prototype through a series of evaluation tests for the detection of CKD stages, the report will conclude with an evaluation of the achievements and the drawbacks of this design, as well as a brief overview of the expected future developments for the device.

Chapter 2: Background and Literature Review

2.1. Chronic Kidney Disease (CKD)

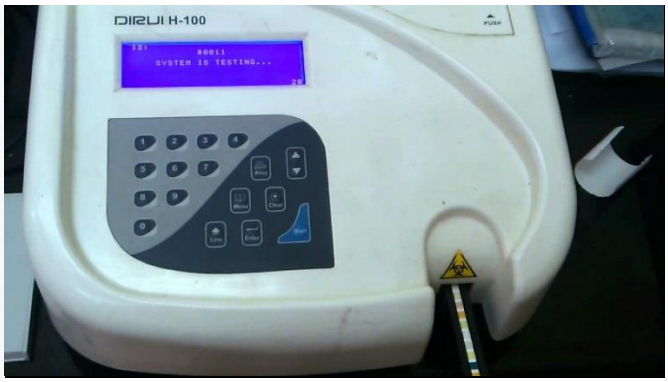
Chronic Kidney Disease has been a growing public health issue. One in three people in Australia has an elevated risk of developing this disease (Kidney Health Australia n.d & World Kidney Day 2017). Although an individual can live well with only one fully functioning kidney, to increase the likelihood of a healthy life, humans are normally born with two. The kidney is one of the vital organs in human body, in terms of filtering the blood to remove waste and excess fluid from the body. However, the symptoms of CKD will not typically present themselves until the patient has lost up to approximately 90% of kidney function, rendering most medical treatments ineffective (Kidney Health Australia 2015). Currently, although there is no cure for this deadly disease, the inevitable deterioration in kidney function could be reduced by as much as 50% and may even be reversible, if patients can detect the CKD symptoms in early stages of the disease (Kidney Health Australia 2017). If the detection of this kind of disease can be made early, treatment with medication, dietary and appropriate changes to their lifestyle can be effective (Kate et al 2012).

2.2. Current Medical Devices for CKD Monitoring and their limitations

Chronic kidney disease monitoring has been undertaken by medical professionals for decades, and most testing methods rely on tests being conducted on urine or blood samples, acquired from high disease-risk patients. A common urine testing method is colorimetric investigation utilising dipstick, a narrow plastic strip equipped with different sensitive-chemical components. This method relies on the colour change of the chemical components, after their interaction with urine. There are many existing types of fully automatic or semi-automatic urine analyzers based on this testing method, such as Dirui, Siemens and Rayto (Medical Expo 2017).

(a) CLINITEK status analyser - Siemens

(b) Rayto RT 150



(c) Dirui H 100 .

Figure 01: The semi-automatic urine analyser

Although this technique is convenient and cost effective, the dipstick approach is limited by the time sensitivity of the chemical components used, and only being able to give qualitative test, not quantitative test, due to its low sensitivity to Albumin at 150 mg/dL (Nabili 2016 & NHS 2014). Due to tests being based on the dipstick technique, the above mentioned equipment is limited in performance. For example, with semi-automatic urine analyzers, measurements must be taken in one minute after the stick has interacted with urine, otherwise the results will be inaccurate. In addition, the equipment's measurement ports are difficult to sterilize after every test, therefore the following sample may be easily contaminated by the previous one, which can lead to inaccurate results. Lastly, in reference to Roberts (2007), the dipstick is reasonably sensitive to environmental air, in that the test is affected when using an out-of-date dipstick, or dipsticks which are stored in previously opened containers. This requires the medical examiner to perform the test repeatedly, if the results are all negative or all positive, or perform an alternative test with microscopy and clinical information because those dipsticks have changed the characteristic due to the environmental effect (Roberts 2007). Although there have been a number of

improvement with semi-automatic urine analysers, for instance the Siemens DCA Vantage Analyser, with launched reagent strip. This test is capable of producing semi-quantitative results, but as they are also designed for doctors to use in clinics, they are costly and not portable enough to be a viable solution for patient self-urinalysis.

With fully-automatic urine analyzers, although they can obtain quantitative results with high rates of reliability, the measurements must be performed in health care facilities, under the strict observation of diagnostic staff. When a patient wants to have the urine test performed, he or she has to make an appointment with a health carer, follow the doctor's instruction in order to correctly prepare the urine sample and then send the sample to a laboratory, and then has to spend time waiting for the results.

For blood tests, the samples are sent to a hospital laboratory and the results may be available within 24 hours (NHS 2014). However, these methods are intended for patients who have already been diagnosed with chronic kidney disease, or to monitor patients who are in the later stages of the disease (NHS 2014).

For current methods of urine testing using urine analyzers, as well as with blood testing for tracking renal failure, they all require patients to visit a medical practicioner's clinic, frequently costing considerable time and money. In addition, high environmental sensitivity can lead to incorrect results, contributing to the patients fear and uncertainty. Hence, there is a need for alternative methods which can compensate for weaknesses in existing systems and equipment.

2.3. New approach for CKD monitoring - Aggregation Induced Emission (AIE) for Human Serum Albumin (HSA) detection

Human Serum Albumin (HSA), a major component of blood plasma and has been used as the potential indicator for the presence of protein in urine for a long time. The Urine Albumin level is a standard test to identify the potential risk of Chronic Kidney Disease, in that, the amount higher than 30 mg/dL will be targeted and called Microalbuminuria (David, et.al 2012; Kidney Health Australia n.d). In normal conditions, kidneys filter-out waste products but retain the beneficial substances required for body health. However, when kidney function decreases, this can allow a certain amount of albumin to leak into urine, leading to Microalbuminuria being present in urine. The higher the microalbuminuria level

identified in urine, the greater the risk of a patient developing CKD. According to the level of albumin concentration leaking into urine, Chronic Kidney Disease has been differentiated into 5 discrete stages. When a trace range with HSA levels lower than 30 mg/dL is detected, this is considered as the safe range for a patient. Meanwhile, the risk level of the disease rises along with the increase of albumin concentrations in urine, corresponding to levels from 1+ to 4+ (Kallen 2015).

Table 1: The semi-quantitative estimation of protein concentration using dipstick (Kallen 2015)

Protein dipstick grading	
Designation	**Concentration**
Trace	5 - 20 mg/dL
1+	30 mg/dL
2+	100 mg/dL
3+	300 mg/dL
4+	More than 1000 mg/dL

Hence, detecting Mircroalbuminuria levels higher than 30 mg/dL in urine, would seem to be an indicator for kidney's function failure (Glassock 2010). A potential indicator for this is the BSPOTPE, which has been reported in some papers for HSA detection and quantitation (Chen et.al 2016; Hong et.al 2010; IEEE Transactions on Industrial Informatics 2015).

In a paper published in 2010, Hong and his group reported on a method using a specific aggregation induced emission (AIE) bio-probe, sodium 1,2-bis[4-(3sulfonatoproxyl)phenyl]-1,2-diphenylethene (BSPOTPE), to detect the serum albumin in urine. It is non-luminescent in urine but becomes emissive in the presence of HSA (Hong et.al 2010). When compared with other methods having low sensitivity such as the colorimetric method, the biosensor tetraphenylethene (TPE), having a broad linear-working range from 0 to 100 nM (0 to 700 mg/dL) and low detection limitation to 1 nM (7×10^{-3} mg/dL), as well

as superior selectivity to albumin, is an effective indicator to detect and quantitate HSA amount in terms of microalbuminuria (less than 30 $\mu g/mL$) (Hong et.al 2010). In this paper, the researchers used a buffer of Phosphate saline (PBS) with pH = 7.0, mixed with HSA to produce a solution of increasing HSA concentrations in μM: 0, 0.001, 0.005, 0.01, 0.05, 0.1, 0.5, 1, 5 and 10, then the solution of 1.0 mM BSPOTPE was added to the mixture of HSA and the resulting solution was incubated for 30 minutes at 25°C. Depending on the target spectral range, a specific excitation wavelength would be used. After being excited by a specific wavelength light of 390nm, the mixture of HSA and BSPOTPE would fluorescent and its intensity was recorded at 475nm. The fluorescence spectra was recorded by a spectrofluorometer. The final results showed that the higher the HSA concentration is, the higher the fluorescence intensity will be. By processing the resulting fluorescence intensity, it is possible to determine the HSA concentration in the buffer solution (Hong et.al 2010). In addition, the experiment has shown that BSPOTPE offers a simple, rapid, effective and economic way of visualizing protein bands. Hence, BSPOTPE is an effective indicator for detecting albumin in urine, even at low concentration.

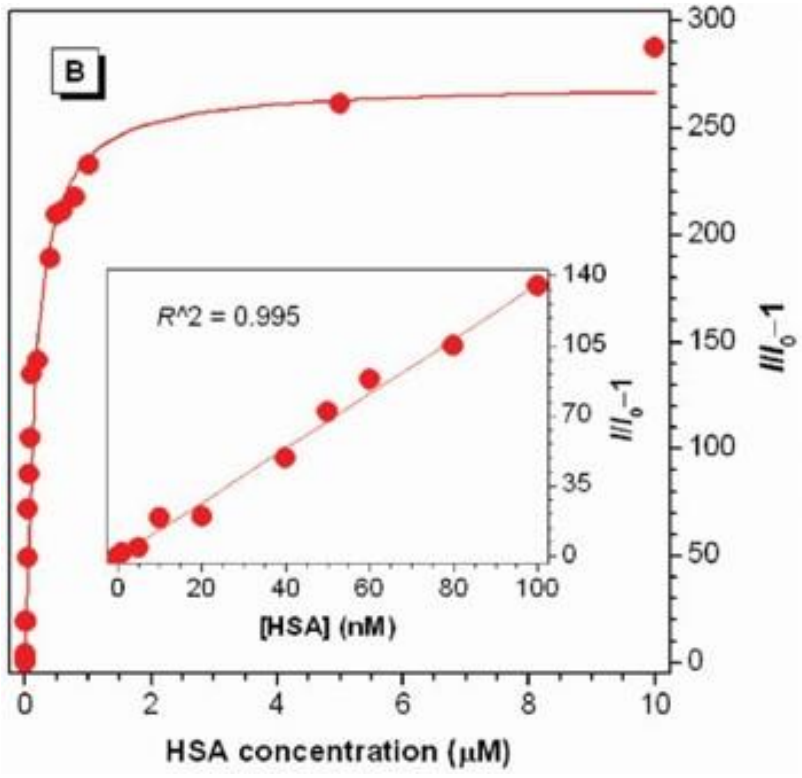


Figure 02: The broad working range of BSPOTPE to different HSA concentrations (Hong et.al 2010).

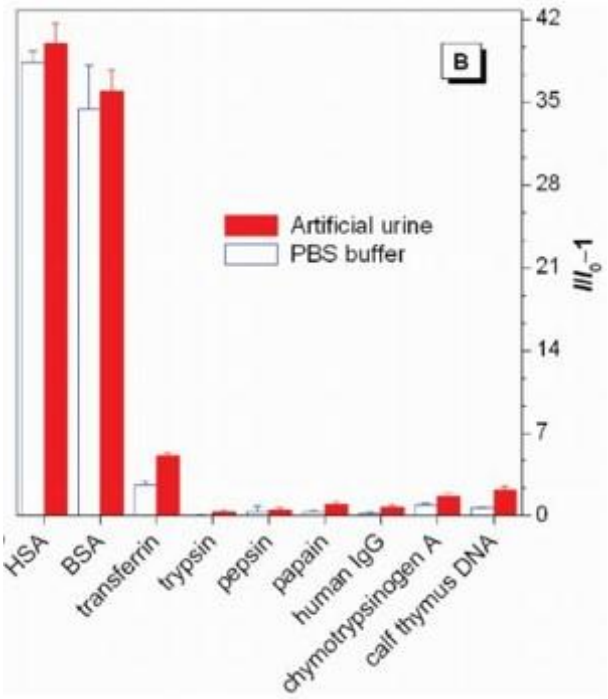


Figure 03: The superior selectivity of BSPOTPE to Albumin when compared with other enzymes (Hong et.al 2010).

Secondly, another report in Faraday Discussion on 5th September 2016, concerning 'Quantitative urinalysis using aggregation induced emission bio-probes for monitoring Chronic Kidney Disease' by Chen and his research group, published in the Royal Society of Chemistry (Chen et.al 2016), also used BSPOTPE as an AIE bio-probe, to quantitate HSA in urine. In this paper, the researcher used two types of TPE salts: iminodiacetic acid functionalised TPE (IDATPE) and BSPOTPE. The indicator solutions were prepared to obtain 25 mM IDATPE solution in acetonitrile and 5 mM BSPOTPE solution in water. The HSA and creatinine solution was prepared by dissolving it in water or artificial urine, to a concentration of 1 M and 30 mM respectively. IDATPE is also an AIE bio-probe, therefore it is non-luminescent in urine at a stable state, but it will become emissive when excited by a specific wavelength. In this case, the wavelength of 262nm was used for exciting the IDATPE and the fluorescent intensity was measured at 375nm. The excitation wavelength for BSPOTPE was 350nm, giving a fluorescent intensity of 475nm. Then performing the experiment as mentioned in the paper above, with the specific wavelengths for excitation. The results indicated that both BSPOTPE and IDATPE are effective

indicators for the detection and quantification of albumin in urine, and therefore exists a promising further evaluation needed to gain a better understanding of the IDATPE, when used to detect urine and urine albumin: creatinine ratio.

Lastly, in the report to IEEE, Transactions on Industrial Informatics in 2015 (IEEE Transactions on Industrial Informatics 2015), researchers stated that BSPOTPE was a powerful indicator for detecting HSA, as it 'allowed direct visualisation of bioanalytes on site and in time and offered useful insights into complex biological structures and processes' (2015). In this research, the researchers used a solution of 30 μM BSPOTPE mixed with HSA solutions having concentrations increasing from 0 to 3000 ng/dL. In addition, the research paper also described the design of a prototype smartphone based device using BSPOTPE, to determine HSA in urine. In this paper, the images from test tubes were observable with different light intensities, due to the various concentrations of HAS as shown in Figure 04:

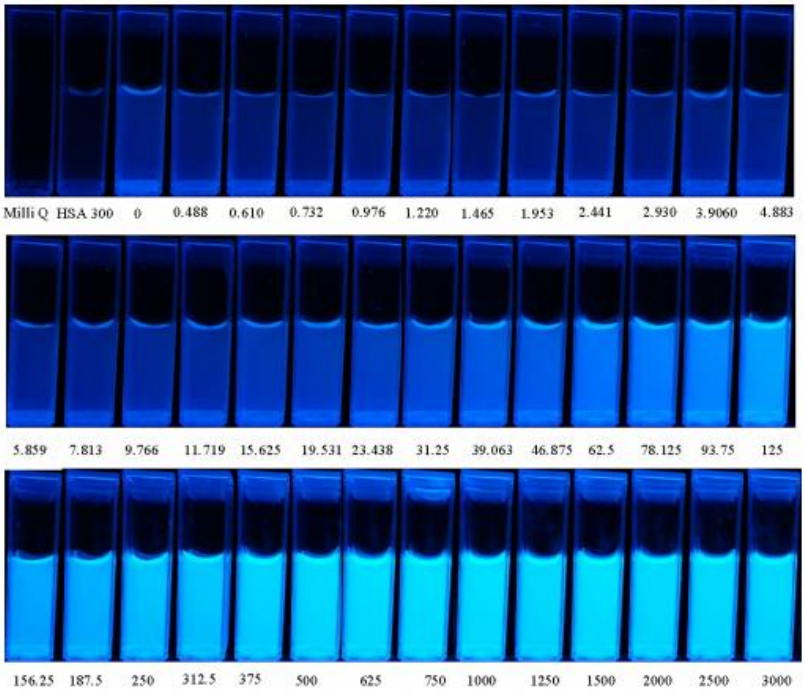


Figure 04: Images of different HSA concentrations (0-3000 g/dL) with 30 μM BSPOTPE taken by smartphone (IEEE Transactions on Industrial Informatics 2015).

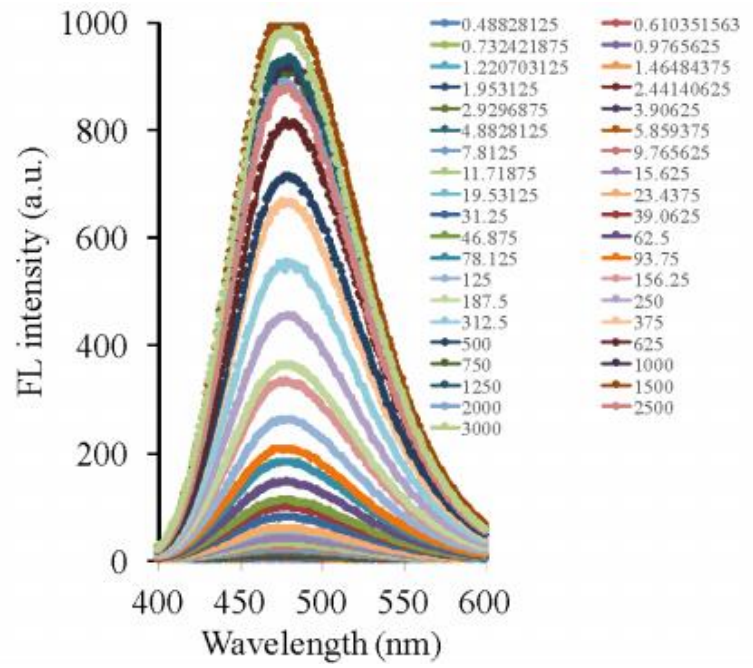


Figure 05: Fluorescent intensity of different HSA concentrations (ng/mL) in artificial urine with 30 μM BSPOTPE (IEEE Transactions on Industrial Informatics 2015).

In Figure 05, the 42 color trends, representing the different concentrations of HSA from 0.488 ng/mL to 3000 ng/mL, have shown the increase in fluorescent intensity within the rise of the HSA concentration in urine.

In regard to this method, the underlying device uses AIE bio-probes to determine HSA, in that there will be a test tube containing a mixed solution of urine, a HSA sample and BSPOTPE, all being kept in a dark container and stimulated by a uniform distributed light. This solution will absorb the incident light, then emit a fluorescent light by itself. By using the camera on a smartphone to capture this fluorescence, combined with a specially developed software application installed on the smart phone to compute the correlation between fluorescent light intensity and HSA concentration, the different levels of albumin are then presented and used for diagnosis.

As a result of the analysis above, there is no doubt that the bio-probes with Aggregation Induced Emission feature, is a powerful method for detecting and quantitating Human Serum Albumin level in urine. BSPOTPE therefore is a promising indicator which can give a fast, simple, rapid, effective and economic way for the visualization of protein bands. In addition, with the extremely high sensitivity

with 1-nM of HSA in urine, as well as the remarkable selectivity to albumin, this method seems to be the most competitive one so far.

2.4. Using smartphone to monitor health conditions

As smartphones have become more common, it has become quite convenient for people to use their 'pocket multifunctional phone' to track their health condition. Being equipped with multi-core CPUs and Graphic Processing Units in factory hardware, the smartphone has the capability to run billions of multifunctional tasks in one second (Agu et.al 2013). In addition, the modern sensor system on smartphones, such as normal or infrared cameras, depth sensing cameras, accelerometers, orientation sensors, light sensors, atmospheric pressure sensors and temperature sensors, which can detect physiological signals from users, could be used to monitor their medical conditions (Agu et.al 2013).

Reviewing reports on the smartphone based accessories and devices for monitoring health status in recent years, has witnessed a significant rise of not only the hardware design, but also app development for smartphones, which relate to measuring physical health conditions such as heart rates, blood pressure, electrocardiography (ECG), blood oxygen, temperature, cough detection and lung function (Health Enews 2014; Harvard Health Publications 2016; Alexander & Joshi 2016; Agu et.al 2013). The two most common smartphone operating systems at this moment are Android and iOS, and this is why most development apps focus on these two major brands (Gadade & Ghodke 2014; Best 2016).

In addition, many developers have been working to transform the smartphone into a 'mobile chemical lab' in that it can measure chemical activities in the body (Burket et.al 2016). In fact, although the concept of transforming a chemistry laboratory to a mobile chemical analysis device was suggested almost 20 years ago, it has just only come into practice, due to the development of modern technology (Erickson et.al 2014). This development has brought disease diagnostic tests which had to be performed in the hospital laboratory, for instance, pH level and glucose concentration in blood, into the mobile pocket-size device - smartphones (Chang 2011; Trojanowicz 2017; Kim, Koo & Yun 2017; Erickson et.al 2014).

Therefore, in regard to the smartphone's advantages such as small pocket size, daily use, easy use, powerful performance, converting a smartphone into a monitor for renal function through urinalysis is a feasible and promising possibility.

2.5. Using smartphone to monitor CKD

The smartphone based urinalyzer is required to be able to track the albumin presence in the early stages of Chronic Kidney Disease, as well as to provide users with a fast, easy and convenient utilisation. Although there is no available commercialized product in the market at the moment, a few research groups and companies are working toward this approach and have published papers on the concept of using a smartphone as a mobile urine analyzers.

The platform 'Albumin Tester', reported by Coskun and his research group from University of California, Los Angeles (2013), have developed a digital sensing platform running on a smartphone that detects albumin level in urine with a high level of sensitivity. Here they use a laser beam to excite a solution sample of urine and the chemical indicator, contained in a test tube laid inside an attachment installed on the camera of the smartphone (Figure 06-d). The test tube will then emit fluorescence which is captured by the camera and the obtained images are processed to determine the different levels of the albumin concentration in the solution. The platform practically shows a very high sensitivity with albumin concentrations of 5-10 $\mu g/mL$, which is one third of the low limitation for the normal range of Albumin level in urine 30 $\mu g/mL$ (David, et.al 2012). This design has the features of fast operation - 5 minutes including sample preparation and testing, easy operation - using a special syringe to take the specific volume of sample for testing, friendly interface - developed on Android operation system, low hazard - button battery to run the laser source, and high sensitivity - 5-10 $\mu g/mL$ (Coskun 2013).

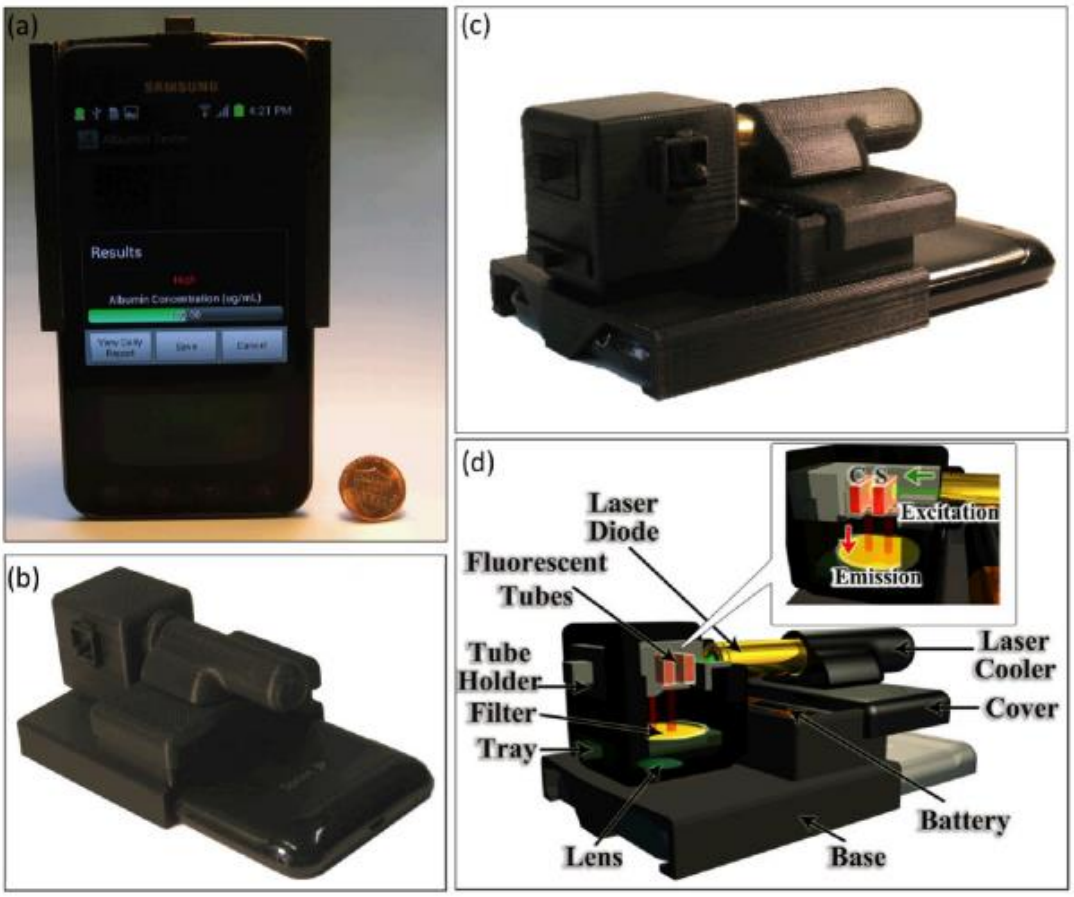


Figure 06: Albumin Tester installed on the Samsung Galaxy S2 (Coskun 2013).

(a) – The interface of the platform; (b, c) The back side of the device attached on smartphone's camera; (d) – The interior sketching of the device's compositions.

Although showing promising and impressive features, and even being reported that the start-up company - Holomic LLC aimed to commercialize computational imaging and sensing technologies licensed from the University of California, Los Angeles, there has been up until now, no further documentation found on this product or similar products in the market. The ongoing research devices are HRDR 300 - Fluorescent reader (Cellmic n.d).

The second highlight application is an algorithm with inter phone repeatability, for both Android and IOS operating systems, which can transform the smartphone into a colorimetric test reader. This application was created by Yetisen and his research group at the University of Cambridge in 2014.

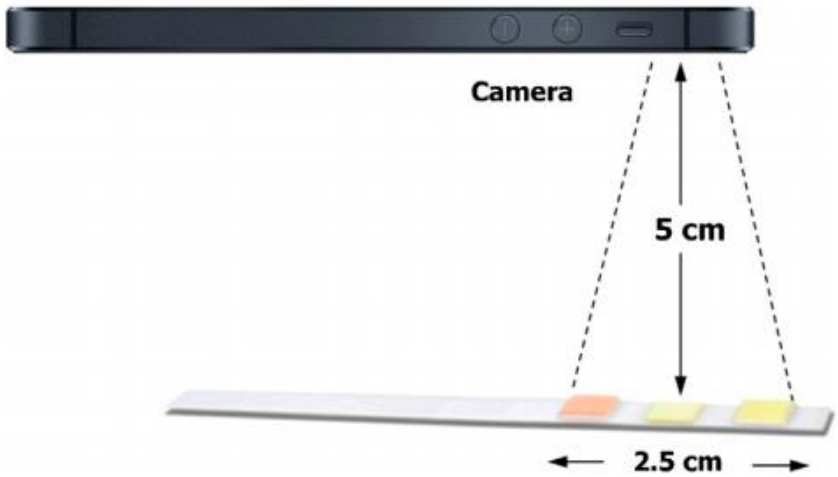


Figure 07: The quantifying colorimetric tests through a smartphone reader (Yetisen 2014).

In this application, Yetisen and his group created an algorithmic application installed on a smartphone, allowing it to run the tests with dipsticks, lateral flow tests, as well as colorimetric tests, that are typically read by spectrophotometers or microplate readers (2014). However, this application has the limitation of the low range for protein detection of 33-41 mg/dL, which is higher than the normal low limit of Chronic Kidney Disease of 30 $\mu g/mL$.

In summary, designing and optimizing the smartphone based urinalyzer is feasible, as there are currently no such devices on the market.

Chapter 3: Project Aims

The main aim of the project is to design, develop and manufacture a smartphone-based urinalysis device with ASSURED features (Affordable, Sensitive, Specific, User-friendly, Rapid and robust, Equipment-free and Deliverable) to detect albumin via the AIE bio-prober. In the mechanism of the project, the solution of urine mixed with Human Serum Albumin (HSA) will generate fluorescent light after being excited by UV light. Next, this fluorescence will be captured by the smartphone through its camera. Finally, an app installed on smartphone will process the obtained image to produce a correlation between the fluorescent intensity and the HSA concentration as shown in Figure 08.

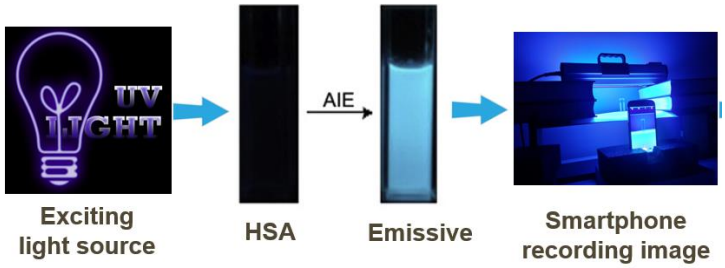


Figure 08: Mechanism for using smartphone-based medical devices, to detect albumin via the AIE bio-prober

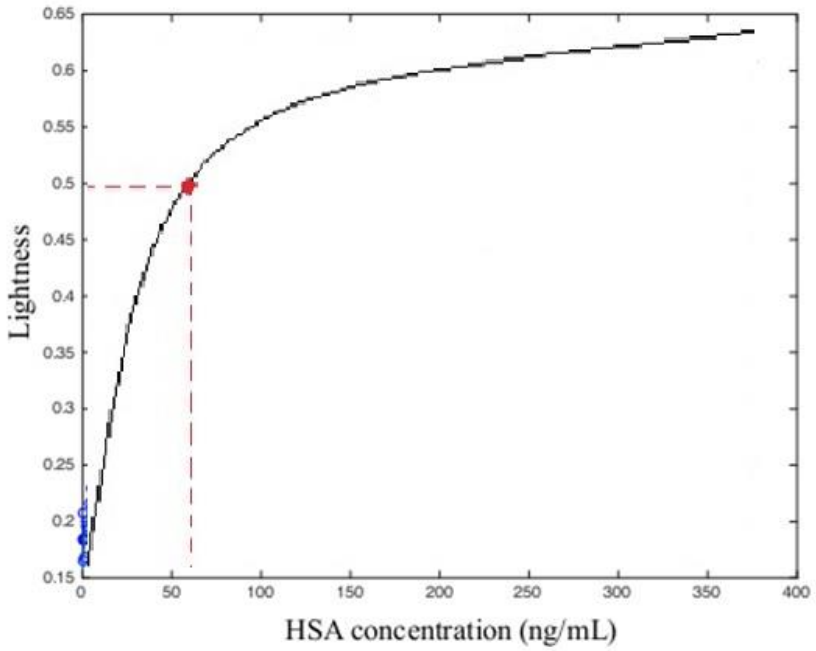


Figure 09: The correlation between the fluorescent intensity and the HSA concentration. For example, the lightness of 0.5 relates to the HSA concentration of around 60 ng/mL.

Therefore, with obtained fluorescent intensity, the app on a smartphone can calculate the HSA concentration in urine, then indicate the stage of CKD corresponding to the 5 ranges of the disease (Figure 09).

The sensitivity feature can be achieved by using BSPOTPE as an AIE bio-probe to detect and measure albumin concentration in urine, in that the sensitivity can be as low as 1nM of albumin concentration (Hong et.al 2010). The duration of the test is less than 5 minutes, due to the rapid reaction of the bio-probe with the excited UV light and the rapid processing of the app installed on the smartphone. For other desirable features, it is important to have a careful design and prototype testing, in order to ensure the ease of accessibility, usability and utilization, ease of manufacture, as well as reducing the price of the product to an affordable price point. Some issues must be addressed are uniform light distribution, the creation of a dark room environment to minimise light ingress from external light sources, the power supply for the excitation light source, ease of use, the accessory to support users, as well as the market researching to ensure that the device is compatible with different brands of smartphone and user types. This device is expected to help users to process a quick and reliable test, to monitor the albumin level in urine to detect the early stages of the chronic kidney disease, then they would be able to make suitable changes in their daily activities, to improve their kidney health.

Chapter 4: Design Conceptualization

4.1. Previous projects of designing a smartphone based device to detect HSA in urine

During previous projects, several prototypes were developed and tested, based on the AIE method where the indicator solution is BSPOTPE.

4.1.1. The first design - Non-adjustable focus length

The first proposal was for an attachment to the imaging-house component of the smartphone. Urine containing HSA and BSPOTPE are mixed in a cuvette, held inside an optomechanical blackbox. The exciting light sources are two LED - The UV LED with wavelength of 365nm and the white light LED.

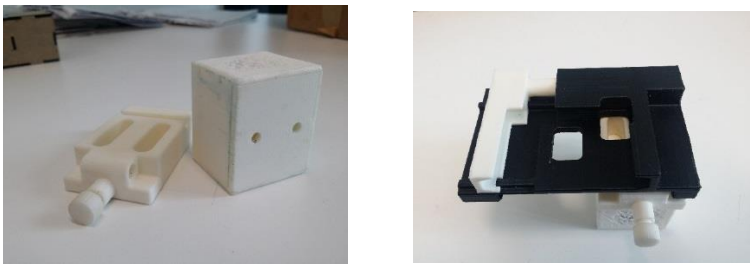


Figure 10: The light source and cuvette component - Equipped with smartphone attachment.

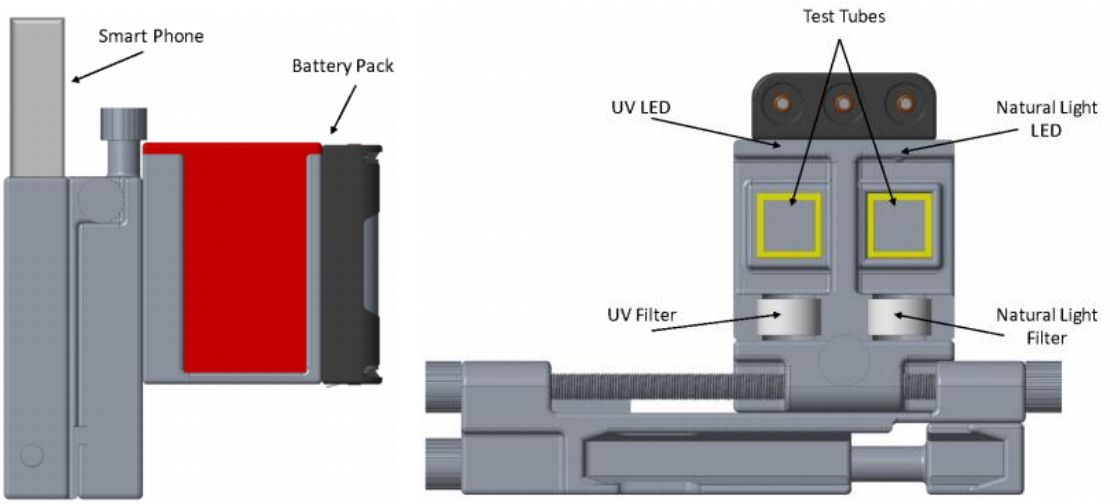


Figure 11: The exterior (left) and interior (right) of the imaging-house of the prototype

(IEEE Transactions on Industrial Informatics 2015)

In this design, the power for LEDs is supplied by 2 AAA batteries with total output of 3V. The light wavelength could be changed by replacing the optical filters to provide a suitable exciting light for the

urine solution. The camera's positions are different among the brands of smartphone so the imagine house is movable to the phone's holder. With this design, the external light is blocked out of the picture taking process to ensure the quality of the result.

Nevertheless, the weakness of this design is that the distance between cuvette and phone camera is non-adjustable, it means the focus length of the camera remains constant regardless of using different makes of smartphone. As mentioned above in mechanism of the project, the quality of picture of the fluorescence is the most significant requirement. The higher the resolution quality is, the more accurate the results would be. For ensuring the quality of the pictures, the distance between camera phone and cuvette - the fluorescence source, must be compatible with the corresponding focus length of different smartphones' cameras. In addition, this project aims to apply to any makes of smartphone, which have been manufactured with different dimensions and camera's positions. Therefore, there exists a challenge for designing in that the phone must be positioned such that the camera would be in focus.

From the research of the focal length of common smartphones in market, most focus lengths vary from 29 to 35 mm and the general rear camera's positions are at the top - middle or left corner of the smartphone (Keen et.al 2016). This research has provided significant information for designing in order to obtain the sufficient quality in result pictures. Moreover, most smartphone cameras nowadays are equipped with auto-focus feature which supports users in taking the high resolution picture as long as the distance between camera and target point is larger than the focal range of that camera. Consequently, the desirable position of the camera is at least 35 mm away from the cuvette containing urine and BSPOTPE, and this would be applied in the later design.

4.1.2. The second design - Fragile assembly and external light contamination

In the second design, the light source are two UV LEDs with wavelength of 365nm, arranged face-to-face in order to increase the exciting light intensity for stimulating the mixture of HSA in urine and BSPOTPE.

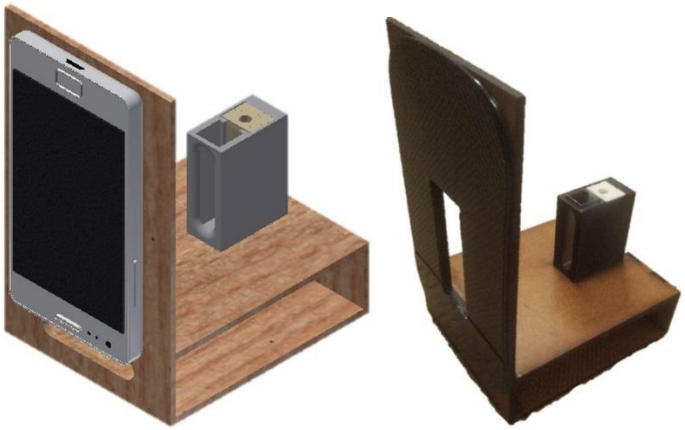


Figure 12: The design using LED to supply the UV light for exciting the mixture of BSPOTPE and HSA in urine (Keen et.al 2016).

One desirable feature is Equipment-free so that the users will have hands-off experience when performing the device. By using polyurethane gel, the anti-slip material, smartphones will be easily and firmly attached to the device, and users can perform the test with both hands. This material is quite common in Australia. It is made from durable polyurethane gel and usually used to prevent objects laid on it from sliding on a moving surface. Besides that, in case of maintenance, this material could be removed from the device and the phone without leaving any dirty marks. However, the first cons in this design is that the smartphone will be held in vertical line so that this material seems to be insufficiently strong for assembling.

The second disadvantage of this design is that the device performance must be taken in the dark room, otherwise the results will be affected by the external light. As mentioned in the project, the major process of the measurement is the works with light in that the stimulating source is the UV light with approximate wavelength of 365nm, and the fluorescent light is emitted from the mixture of HSA and BSPOTPE. These processes are high light-sensitivity and may produce the inaccurate results if there is any contamination of the surrounding light source. In fact, the final product is designed to be used in an open environment so the need exists to make a limited area such that it can block the environmental light away from the measurement. Hence, two shortcomings from this design - the phone securing and the external light prevention, need to be compensated in the later designs.

4.1.3. The third design - Uniform distribution UV source and Interference noise:

The later design is a black-box like prototype which can cover the measurement in a dark room environment. The author also used BSPOTPE as the indicator for HSA presence in urine. With this design, it compensated the issue of dark room environment from the earlier prototype.

Figure 13: The black box design for smartphone based device to detect and quantitate HSA level in urine.

With the horizontal surface for laying smartphones, combined with the anti-slip material from the previous discussion, the smartphone is considered to be firmly assembled with the device in order to provide users the hands-off experience and the stationary phone's stand for taking the fluorescence pictures. In this prototype, the stimulating light source are two UV LEDs, put at the opposite site of the cuvette to the phone's camera. The main body of the device works like a black-box which can shield the external light contamination, allowing the utilization in the open environment. By leaving a small mirror inside the device in angle of 45 degree, the fluorescent light from cuvette will be transmitted from horizontal axis to vertical axis, and coming to the phone's camera laid on the top of the device.

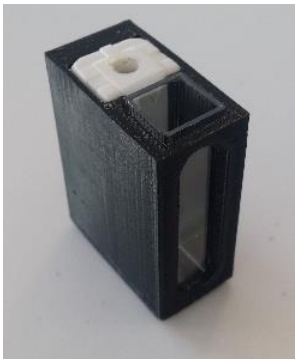


Figure 14: The UV light source with two face-to-face UV LEDs (inside the white component) put on the other side of the cuvette to phone's camera.

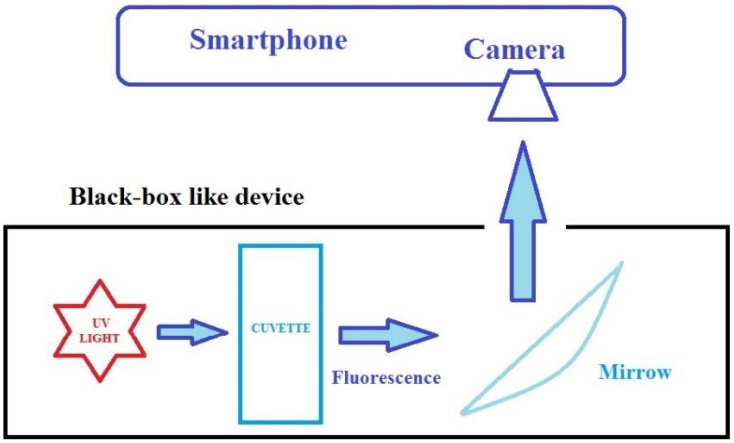


Figure 15: The schematic drawing for mechanism of the third prototype

However, the noise in the result picture is not only caused by the external light, but also the UV exciting light. If the UV light escapes from the stimulating light source and reach the phone camera, even passing through the cuvette, it also adds the interference noise into the light beam coming to camera. So that the result will contain both fluorescent light and the UV light. This interference would undoubtedly affect the brightness of the fluorescence and lead to error in final result of the measurement. In this design, due to arranging the UV light source, the test tube and the camera in one line, UV light also passes through the transparent test tube, then reaches the camera along with the fluorescent light, changing the light intensity in the obtained pictures. It requires an improvement in the later design to get rid of this issue.

The next problem with this design comes from the light source itself. Under stimulation of UV, the test tube filled with HSA and BSPOTPE will become emissive and the phone camera will record the image of that fluorescence. Though, the LED spot light source is not stable and cannot provide a uniform excitation light for the solution, which leads to the uneven fluorescent emission and causes the results to be invalid. The challenge, therefore, is to develop another method to improve the uniform light distribution for the UV source so that the camera could take the picture of test tube with uniform brightness.

4.1.4. The fourth design - Low UV intensity and Limited picture frame size

In this design, the modifications are in the UV light source in that the LEDs position have been moved to form an angle of 90 degrees over the luminous light path and the amount, as well as the arrangement of LEDs have been changed.

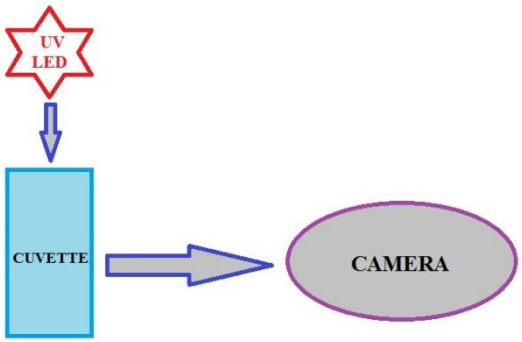


Figure 16: The UV light is perpendicular to luminescent light.

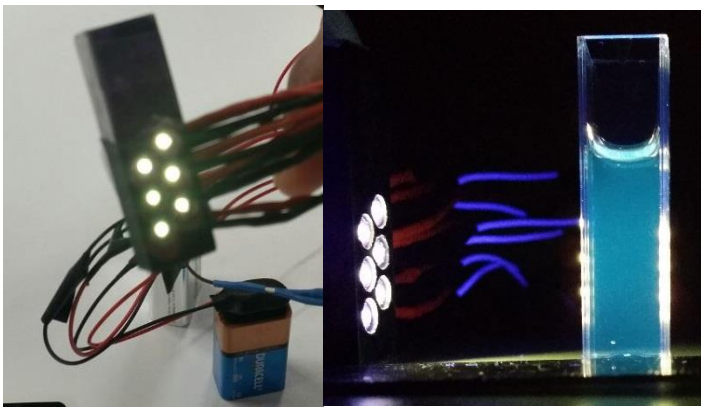


Figure 17: 6 LEDs are arranged in grid system.

By orientating the UV source to be perpendicular to the test tube - phone camera optical path, the resulting image is no longer affected by the UV from LED source. Furthermore, that using large number of LEDs arranged in grid has risen the UV intensity and produced a uniform distribution of the stimulating light, resulting in the final pictures having remarkable and uniform distribution brightness. Nevertheless, there are several negative points in this design in that the UV brightness, firstly, is insufficient to distinguish the HSA levels in low concentration ranges; secondly, the frame size of the picture is limited in only one third of the cuvette's size; and finally, the power for LED system is

currently supplied by 9V batteries, which could not guarantee to generate the stable output and may affect the UV brightness's quality due to the energy running out with use. In the end, these cons require an improvement in the later design in order to provide the better results for the measurement.

4.2. Target user

The project targets patients who are vulnerable to kidney function failure such as people having diabetes, hypertension, family history of kidney failure and cardiovascular disease (Kidney Health Australia 2015). In those groups, elderly people over 60, who have high risk of CKD, as well as the combination of other dangerous diseases, are the major targets, requiring more attention due to their reduced cognitive effectiveness and dexterity in using the device. Therefore, consideration has been given to minimize the operational steps while using the device, such that users can operate the device without or with minimal tutorial. Besides that, in project period, there were some opinions of guiding the users to do the urinalysis with the device by themselves. For instance, there would be a hard-printing of the guide sent along with the device. If users would like to download the device's instruction, they can search for it on the project's website which could be established in the future. Moreover, the user's manual would also be equipped in the project's app, which is expected to run the result processing. In case of users not being familiar with using the app on smartphone, this app's manual could be built in voice-assistance or video, so that the user can listen to the manual, or watch a video of certain instructions and be hand-free to prepare the sample and run the device.

In addition, the device was designed to be used in areas where costly diagnosis machines and manufacture technologies are not readily available. Hence, choosing the less complicated method and economical material to produce the device need to be considered. At Flinders University, there are two common methods of digital fabrication, laser cutting and 3D printing, in that 3D printing has been popular in many countries for a period of time (Johnson 2015).

4.3. Features for designing

From the above discussion, some desirable features should be considered:

Affordable and accessible manufacturing technologies: The design must be usable and marketable to everyone with diverse abilities, and can be manufactured by the potentially technologies such as Laser Cutting and/or 3D Printing.

User-friendly: The design should satisfy most users' preferences, and the user does not need much special experience, training, knowledge, language skill or concentration.

Rapid and robust: Accurate and highly reliable results are available in less than 5 minutes.

Smartphone camera position: Top - Middle and Left Quarter.

Adjustable focus length: Ensure the gap between the test tube and the camera to be over 35mm.

External light contamination prevention.

Uniform and high UV intensity light source.

Consistent light path.

Whole cuvette picture frame.

Stable power supply for light system.

By considering these features, the final prototype has been optimized.

Chapter 5：Development and Testing

The final optimized design that compensates most of those desirable features is shown below.

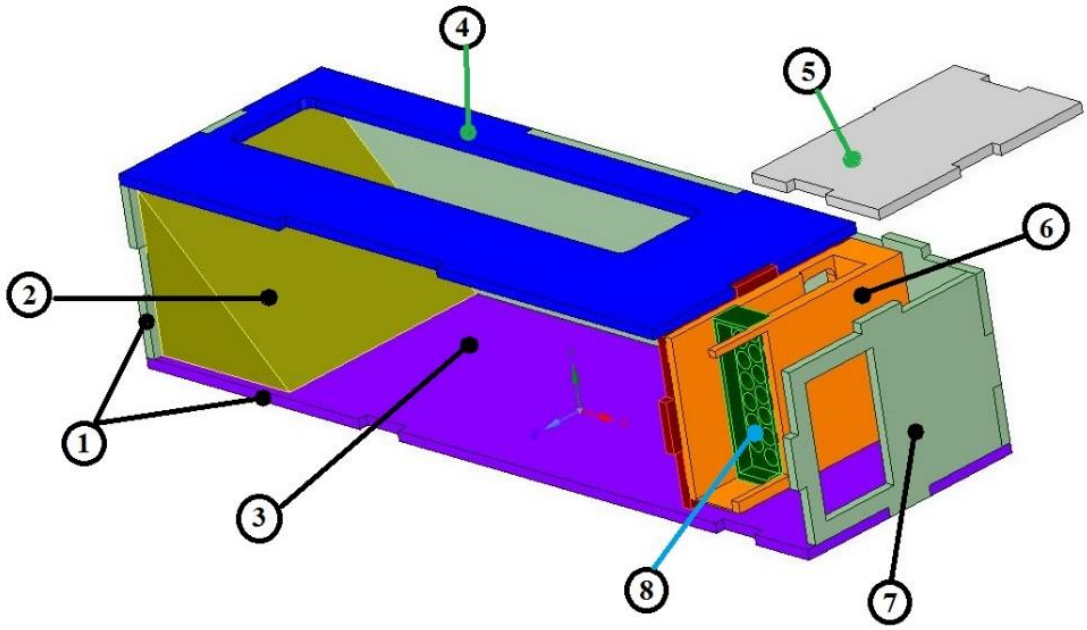


Figure 18: Final optimized design

This design was sketched on Ansys Spaceclaim 2017, and the physical prototype was manufactured by laser cutting and 3D printing, which are equipped in Digital Fabrication Lab G0.7 in Flinders University - Tonsley campus. The main features are:

5.1. The frame (labelled 1)

The device shape is a black rectangular box with dimensions of (250 × 75 × 60) mm. The main frame of the box is made from black acrylic with thickness of 3mm. These acrylic pieces are manufactured by the laser cutter in Digital Fabrication Lab G0.7 in Flinders University - Tonsley Campus, under supervision of Professor Sandy Walker.

The box is divided into two parts: the left part (larger one) works like a dark-room environment while the others (smaller) is the storage for the light system and test tube.

5.2. The small mirror (labelled 2)

The small mirror is laid at an angle of 45 degree to horizon in order to reflect the fluorescent beam from test tube to the smartphone camera placed over the upper surface of the device.

Figure 19: The small mirror sticked on 45 degree wedge.

5.3. The dark room (labelled 3)

This area is built as a dark space, totally isolated from the outer environment such that no any light can enter and affect the fluorescent image recording. It was also experienced that the desirable colour variance should only be the fluorescence emitted from the test tube, and the rest of the image should be a uniform colour. So that, the main material used for this area is chosen to be black, which can absorb all wavelengths in visible range, and non-transparent in order to block all light passing through.

5.4. The smartphone attachment surface (labelled 4)

This is the lid for the dark-room area, was manufactured with a rectangle hole allowing smartphone camera to take the reflecting image of the test tube through the small mirror placed at 45 degree to the horizontal. This lid is under consideration to be equipped with the anti-slip mat to firmly secure the smartphone assembly. Due to the specifications in designing that the distance between the camera and the test tube has to be over 35mm, this long lid aims to suit most of common brands of smartphone in that the test tube would be in focus range for different cameras on smartphone.

5.5. The removable lid (labelled 5)

The smaller lid is used for the storage of light system and cuvette holder. This lid is removable to support the cuvette replacing when the users want to run more than one test. In that case, the users can

remove the small lid to change the cuvette filled with solution of HAS and BSPOTPE, without moving the smartphone from its original position, making sure that the distance from camera to the test tube remains constant between the measurements.

5.6. The test tube and UV LED holder (labelled 6)

This component is the orange part in the drawing, made from black FilaForm Pro ASA material, which helps absorb the reflecting light from the UV source in order to improve the fluorescent photo's quality. This component is designed to place the light source such that the UV light is perpendicular to the fluorescent light. This helps the resulting picture to get rid of the interference noise caused by the UV light passing through the transparent cuvette.

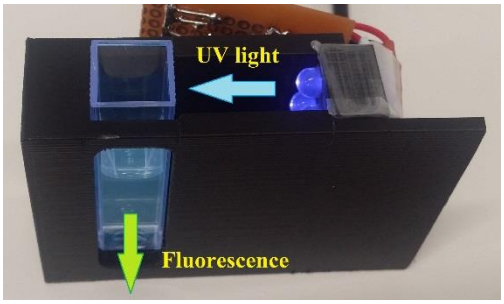


Figure 20: The UV light and the fluorescence are perpendicular.

In the previous prototype, the picture frame just covered 25% of the cuvette's capacity, leading to a lack of the fluorescence data because the tested solution volume is only 3mL, occupying 75% of the cuvette's capacity. For the current design, the area of fluorescence is 75% of the entire cuvette's height so that the researcher can choose the desirable regions to examine in the image processing, widening the opportunities for obtaining the better result in estimation the correlation between the fluorescent brightness and the HAS concentration in urine.

Besides that, this component also specified the distance between the UV light source and the cuvette to be constant at 2 cm, ensuring the UV light intensity to cuvette is stable during the measurement.

5.7. The house for light system and test tube (labelled 7)

This is the smaller container in the device, giving space for the test tube and UV LEDs holder, and the circuit to supply power for the light source.

5.8. The light source (labelled 8)

This is the most important component in the device, generating the UV stimulation for the solution of urine containing HSA and BSPOTPE. It was manufactured by the 3D Printer in Digital Fabrication Lab G0.7.

The desirable material for this component is black FilaForm Pro ASA due to its outstanding characteristics, for example, UV and water resistance, zero warp technology, non-transparent, less reflecting and excellent mechanical properties (Imaginables 2017). With great strength, interlayer adhesion, smooth aesthetics and remarkable commercial price of AUD $50/750g, this ASA material would be the most suitable for the current design (Imaginables 2017).

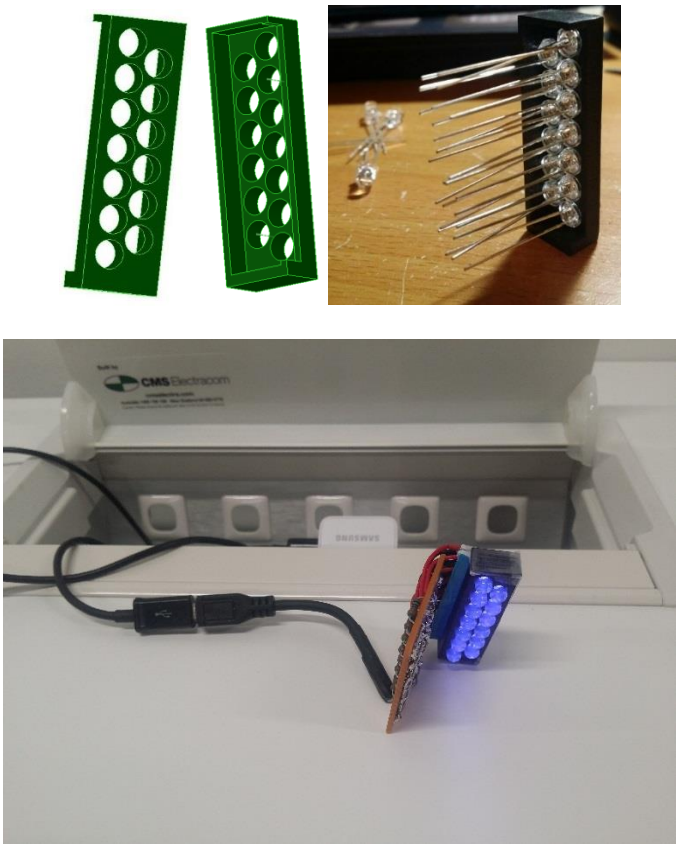


Figure 21: The engineering sketch and the practical grid of LEDs.

For the UV light source, the project uses 13 ultraviolet LEDs with specifications:

Forward voltage: 3.2V.

Forward current: 20mA.

Diameter: 5mm.

Light beam angle: 30 degrees.

This kind of LED has been chosen due to its small size and the reasonable price. That using 13 LEDs with large light beam angle of 30 degree and arranging them in a grid pattern ensure the interference of the ultraviolet rays, helping providing the uniform UV light for the cuvette. When using the spectrometer to measure the wavelength, it shows that these LEDS' wavelength is 371nm, approximately 365nm, this is suitable with the design requirements because it is still in the range of the desirable stimulating light.

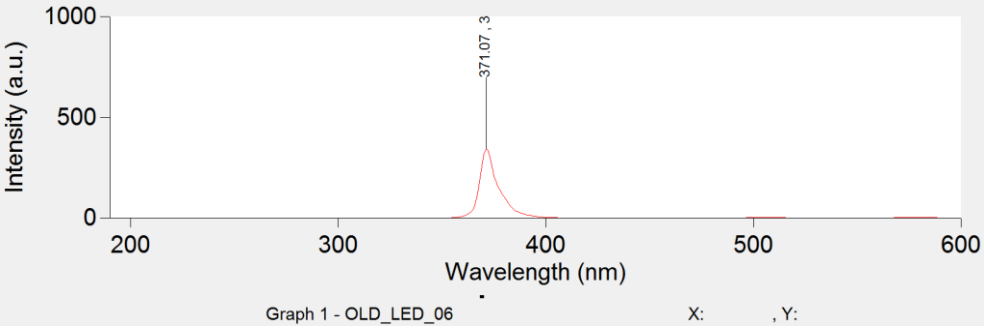


Figure 22 The LEDs wavelength - measured by the spectrometer.

In addition, for ensuring the stable power input for this light source, the project uses the Samsung Mobile-phone Charger as the power supply. This charger provides output of 5.3V and 2.0A through the Micro USB type-B plug. Hence, resistors of 100Ω have been used to establish a circuit for the light source, which is shown in Figure 23.

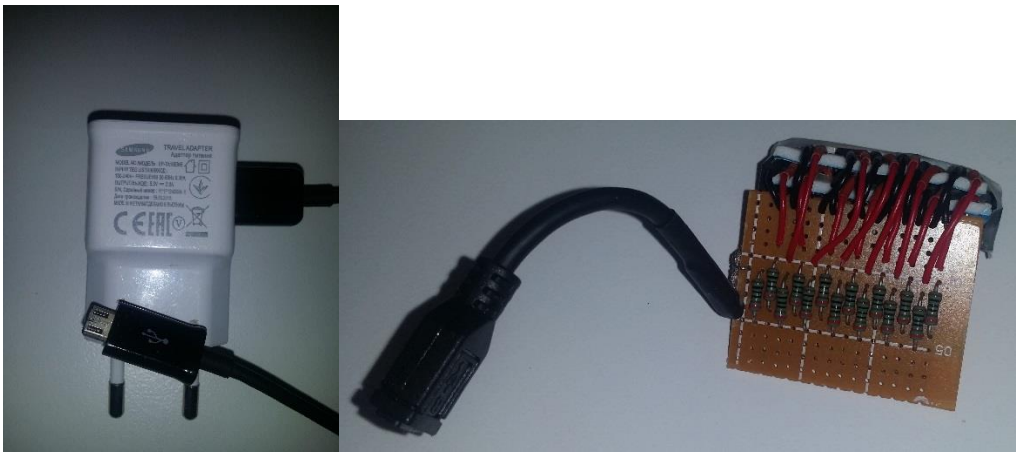
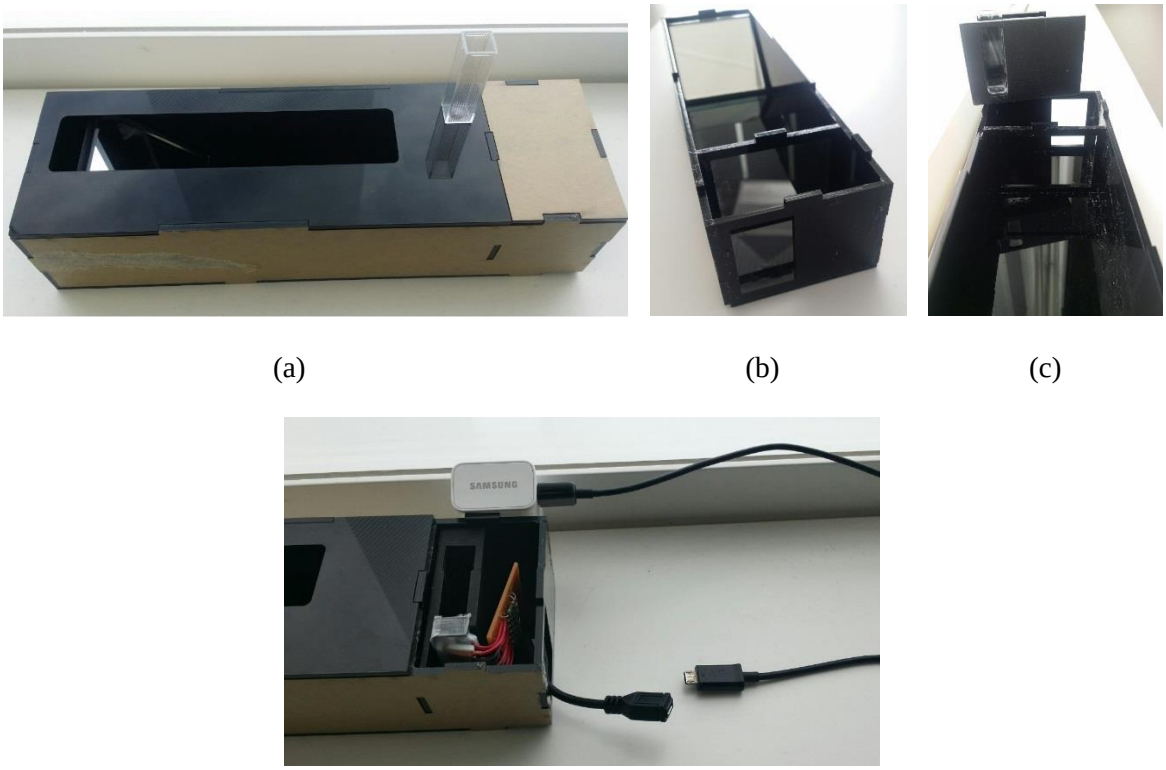


Figure 23: The Samsung Mobile Charger - The Light source with operating circuit.

5.9. The physical device

After designing, the physical device has been manufactured:



(a) (b) (c)

(d)

Figure 24: The practical device (a) - The overview of the device; (b) (c) - The small reflecting mirror and the test tube - light source holder; (d) - The external power supply for UV LEDs system.

The practical process of the device performance

The real operation of the device includes steps as follows:

5.9.1. Sampling

This process is similar with the urine sampling in other urinalysis test. Firstly, users will be provided a sterile container and required not to touch the inside of that container or the lid in order to maintain sterility. For female, she has to wash hands with soap and water, then separate the labial folds. Next is cleansing opening of urethra three times with soap and water. After that, she should allow the initial stream of urine to pass, and finally collect the mid-stream urine into the provided sterile container. For male, he firstly has to retract foreskin and clean glans three times with soap and water in order to get rid of urine contamination. After allowing the initial stream of urine to discharge, he can collect the mid-stream of urine into the provided sterile container. For both cases, users must not collect the last part of the urine flow because it contains a residue which will affect the urine's condition. The urine specimen should be used for testing as soon as possible to prevent the effect from environment, for example, temperature, strong light or bacteria (Specimen Preparation and Collection 2010).

5.9.2. Mixing

The total solution volume is 3000µL, and it requires the ratio of urine and BSPOTPE to be 9:1. So that, the urine specimen is taken out by 2700µL and poured into the provided cuvette, which already contains 300µL BSPOTPE solution. The users then will close the cuvette using the provided lid and shake the cuvette and leave it for incubation at room temperature in 15 minutes in order to ensure the uniform mixing of the chemicals.

5.9.3. Attaching smartphone and device

The device's upper lid is designed with a rectangle hole to fit the smartphone's camera such that the camera len is positioned perfectly to record the whole image of the fluorescent cuvette through the small slanted mirror.

5.9.4. Running the Urinalysis app installed on smartphone to take photo of fluorescence emitted from the test tube

When running the app installed on smartphone, the image recording interface will be on. The users position the camera such that the distance between camera and cuvette is over 35mm, and the image of the cuvette remains in the middle of the screen. By using the auto focus function of the phone camera, the photo could be taken in high quality. The accurate distance between the camera and cuvette will be manufactured in the device.

5.9.5. Obtaining the results

After taking image of the fluorescence from cuvette, the app will calculate to find out the fluorescence intensity, then compare the obtained result to the reference data before indicating the HSA concentration on the phone's screen, as well as the CKD stage the user is having. From this results, the users can consult the healthcare staff to have suitable treatment or proper adjustment in daily activity to improve his kidney's health.

A table of 94 increasing concentrations of Human Serum Albumin (HSA) in urine is established regarding to the table of 5 stages for chronic kidney disease to get ready for testing the device performance (Appendix 2).

In the design, the test-tube is a 4mL cuvette. The total volume of solution used for running one test is 3mL. The fixed concentration for BSPOTPE is $30\mu M$, corresponding to 20.7mg/dL approximately (IEEE Transactions on Industrial Informatics 2015). According to the AIE bio-probe assay (Hong 2010), the ratio between BSPOTPE and urine containing HSA is 1:9 , so that the volume for 30 μM BSPOTPE solution is specified of 0.3mL. The rest of solution is urine mixed with HSA in different concentrations from 5 to 1000 mg/dL, corresponding to 0.0075 μM and 1.5 μM respectively.

Urine having HSA is poured into cuvette, followed by addition of 30 μM BSPOTPE. Then the whole solution is mixed gradually and incubated in room temperature for 15 minutes to guarantee the uniform solution mixing. After that, the testing cuvette with known concentration is laid into the test-tube holder in the small container of the device. When being excited by UV light, this mixture will turn from non-luminescent solution into fluorescent. Next, the fluorescent image is recorded by the smartphone's

camera and then processed by Matlab codes to find out the brightness level. Finally, another table of correlation between the HSA concentration in urine and brightness of the fluorescence is promoted, and used as a reference for the later tests.

In the project, the device performed tests with several makes of smartphone such as Samsung Note 3, Samsung S7, Iphone 6, Samsung Note 4 and Huawei. These are the common makes of smartphone on market, and within the limitation of the project, these are the supported smartphones which will be used. It is no doubt that the later the smartphone's generation is, the higher its technology will be, resulting in the improved quality in obtained picture using auto focus function in the smartphone camera.

Figure 25: The device was working with Samsung S7, Samsung Note 3 and Iphone 6, respectively. (The small lid is left opening to show the light source and the cuvette’s position. In the tests, this lid will be closed completely).

In the above figures, the device has shown performance with two common smartphone brands - Samsung and Iphone with various camera features and positions - the top-middle and top-left quarter. The tests also showed that the smartphone can lay on the device firmly, preventing the scattering noise in photos due to phone moving. In this design, the distance between the camera and the test tube is adjustable and specified around 150mm, showing the effective choice.

Chapter 6: Results and Discussion for Further Development

6.1. The results

In the finalised prototype, the users only spend around 5 minutes on taking the photo of fluorescence and reading the result of the CKD stage on the phone screen.

The obtained images include the frame of fluorescence from the cuvette, as well as the black background. In image processing, only the frame of fluorescence will be used. After removing the black background from the image, only the fluorescence component would be maintained for processing. The Matlab code will then select a homogeneous colour region, and convert the RGB (Red-Green-Blue) values to HSL (Hue, Saturation, Lightness) values, and the mean intensity from HSL values would be measured. These mean intensities are then plotted on the same graph with the HSA concentrations by using a Matlab Algorithm. Different phone models detect different HSA concentration Vs Mean intensity curves, therefore a post-test calibration is performed by using an inv(A) matrix, bringing the data curves closer to the reference phone's curve (SamsungN3).

After observing the results obtained from 3 smartphones: Huawei, Samsung Note 3 (SamN3) and Samsung Note 4 (SamN4), different linear trends in 5 ranges of CKD were identified. For example, in range 3+ (the concentration of HSA in urine ranges from 100 mg/dL to 3000 mg/dL), and the original brightness from three smartphones increases with the increase in HSA concentration.

Table 2: The range 3+ of CKD stages

Range 3+ (100 mg/dL - 300mg/dL)			
Conc of HSA [µM]	**V(HAS) [µL]**	**V(Urine) [µL]**	**V(BSPOTPE) [µL]**
0.150	40.5	2659.5	300
0.181	49	2651	300
0.211	57	2643	300
0.241	65	2635	300
0.270	73	2627	300
0.300	81	2619	300
0.330	89	2611	300
0.359	97	2603	300
0.389	105	2595	300
0.419	113	2587	300
0.450	121.5	2578.5	300

After calibration process using SamsungN3 as the reference phone, it is obvious that this device can work well with different brands of smartphone in that most data from other phones can be calibrated to the reference data. In the experiment, only the smartphones were changed, the other conditions, such as the focus length, the set of samples, the device and the light source, were kept unchanged. The differences in the trends resulted from the various technologies of the smartphones' cameras, such as settings, sensors, processing techniques, which requires further research to find out the most suitable setting in order to obtain the high quality in taking picture.

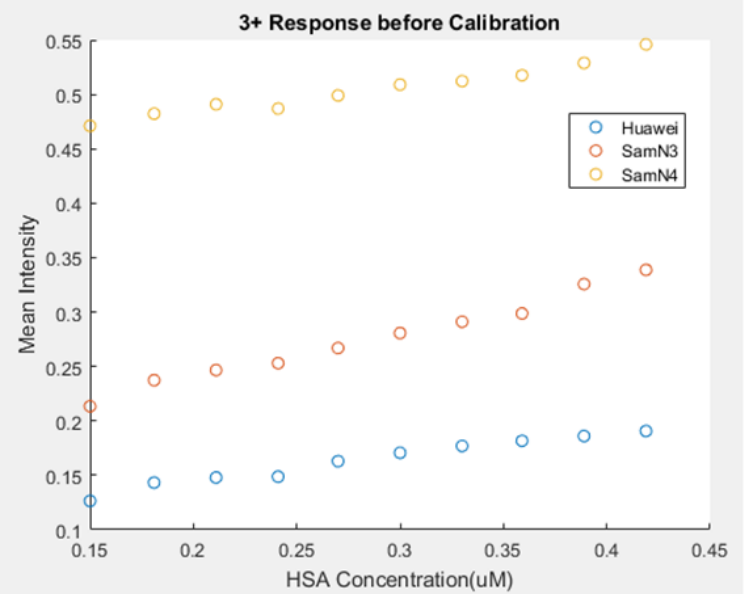


Figure 26: The original data from three smartphone Huawei, SamN3 and SamN4 in range 3+.

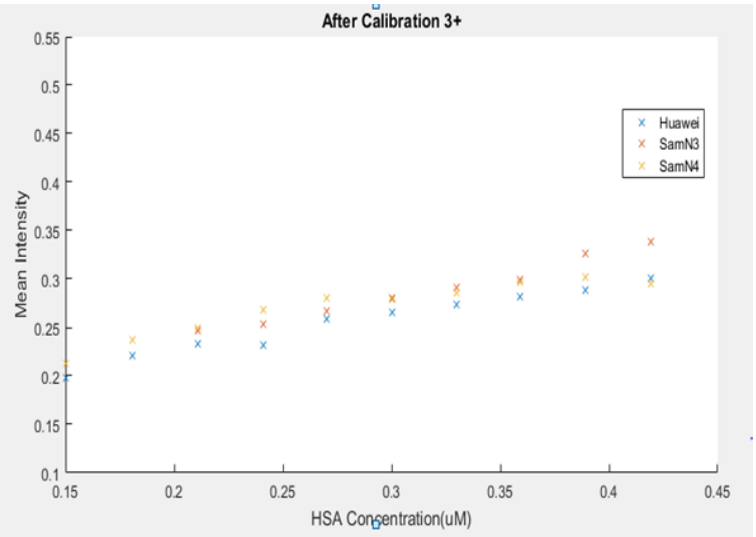


Figure 27: The calibrated data from three phone Huawei, SamN3 and SamN4 in range 3+.

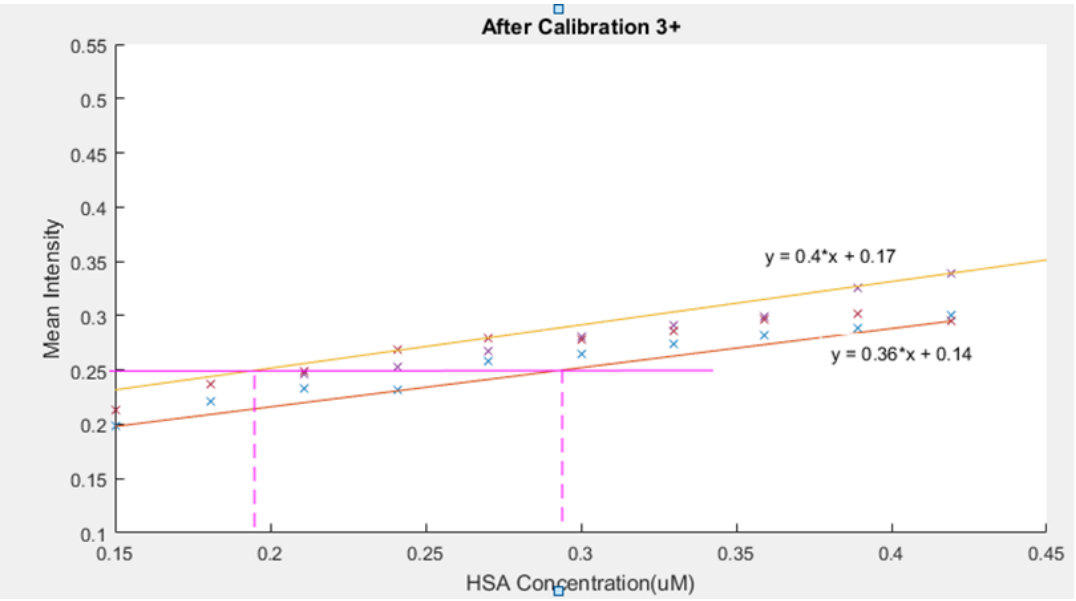


Figure 28: The working range of the data from 3 phones.

From the calibrated trends, it is possible to build up the working range for range 3+ of CKD. For example, when the user uses a smartphone to run the test, and obtain the value of the mean intensity of 0.25, the app will show the HSA concentration is between 0.2µM and 0.5µM, then indicate to the user his kidney disease is in range 3+ and they need to visit a healthcare centre to have treatment or then therapy to support kidney's function.

However, although the sensitivity of the method Aggregation-Induced Emission Bio-probe is remarkably high with resolution down to 1 nM (7×10^{-3} mg/dL), this sensitivity cannot reach that high when working with the device. That is why with value of fluorescent intensity, the app can only notice the range of HSA concentration, this may provide insufficient accuracy for indicating the lower HSA levels.

6.2. Discussion for future development

Although achieving most desirable specifications, the current device also has some imperfections and requires further improvement for the next generation of the device.

6.2.1. Smartphone camera – Auto focus function

Being affected by the camera features, the resulting pictures would absolutely change between brands of smartphones. This exists a requirement for further research about the camera's auto focus function

of the smartphone in order to get the most reliable data from the pictures of fluorescence, and to minimise the error in calculating fluorescent intensity back to HSA concentration.

6.2.2. Optimisation for manufacturing method

The final device is expected to be used in the areas where costly diagnosis machines and manufacture technologies are not readily available, so that choosing the accessible material and manufacturing method could be considered as challenges. The current prototype was produced by using laser cutting and 3D printing technologies which are common at Flinders University. However, the laser cutting machine is not a recommended candidate for commercial feature due to its market price being over $1000 AUD. Meanwhile, the 3D printer machine's prices are from $250AUD to $900AUD popularly. Besides that, according to safety hazard requirement, 3D printer performance does not require as high supervision as laser cutting, resulting in the saving of producing time in that many prototypes can be fabricated in different 3D printers at the same time. In addition, the material used for 3D printer is more diverse and economical than for laser cutting (Takagishi & Umezu 2017; McDermott 2017). Finally, the 3D printing technology has been popular for several years and becoming widespread over the world. 3D printing held 60 percent of market share in North America and Euro, and this percentage is 21 for market share in Japan, China and Korea in 2013 (Grimm 2013). Nowadays, 3D printing technology has been applied in tissue engineering, one of the latest technology of the world, making it to have very few competitors in simple fabrication technologies. According to the wide-spreading and popular condition, 3D printing should be far more accessible than laser cutting machines with suitable materials for manufacturing the prototype in the intended market.

Hence, the next prototype will be designed such that the whole device can be manufactured by 3D printing technology, so that the device will be one component, enhancing the security of the device, contributing to peripheral light penetration prevention.

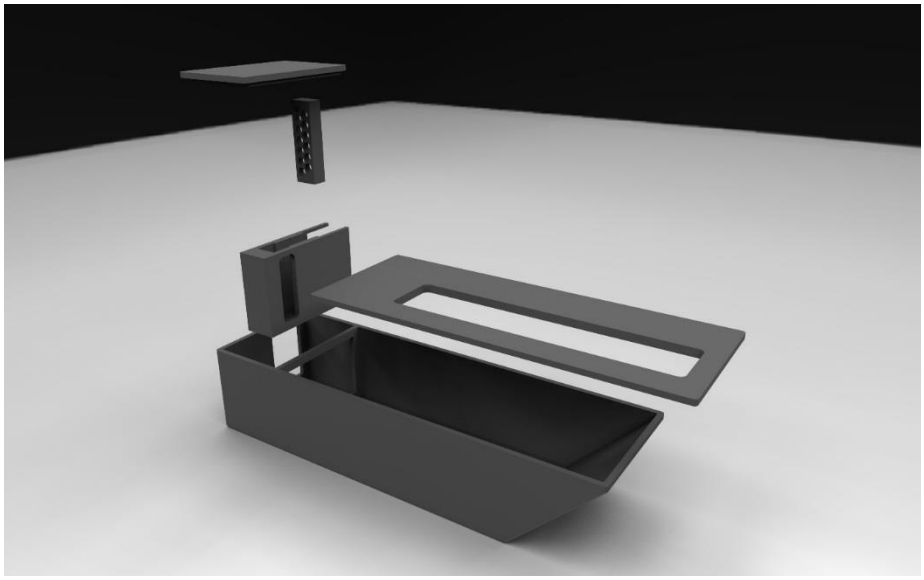


Figure 29: The device by only 3D printing technology, sketched by Spaceclaim – Ansys software.

Dimension: (75×250×60) mm.

6.2.3. Power supply for LEDs

Recently, the device is supported by an external power charger. In next improvement, the project aims to draw current from the smartphone itself. In fact, there are two entrances for power passing through on a smartphone, the charging port and the audio 3.5mm jack.

Firstly, the charging port on the phone allows an input of approximately 5V and over 2A to pass through, which is higher than the required power for LEDs so that it would be convenient to create a voltage divider circuit to draw power from this plug. This plug, however, is just a one-way current input in that it only allows power to enter for battery charging. Hence, this option is incompatible for the design.

The other option is the headphone jack. In the headphone, there is a ring of copper wire attaching to a plastic disc, laying in a circle magnet housing. This setting is called the dynamic driver, the most common transducer type in headphones to convert the electrical signal into an acoustic signal.

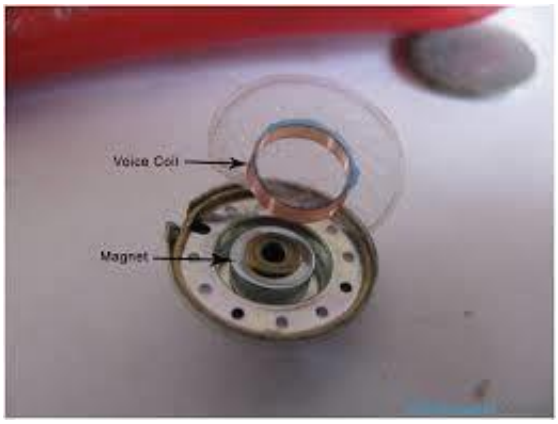


Figure 30: Dynamic transducer in headphone.

When there is an electrical signal sent to headphone, the current will run through the cooper wire, generating an electromagnetic field. The change in current leads to the varying in electromagnetic field and making the coil, as well as the plastic disc to move up and down. This movement causes the disruptions and displacements in air and generates the sound wave. Hence, it is no doubt that when smartphone run an audio file, there exists an alternative current passing through the audio jack. Generating and detecting this current would support the option for drawing power from smartphone to use for LEDs power supply. Though, this current value seems to be insufficient to run the LEDs. In addition, this current is AC while the required supply for LEDs is DC. Therefore, it acquires the amplifier and rectifier circuits to convert this minor AC current into the suitable DC current for LEDs. In fact, different phones will produce different audio output, so there need to be further research to support for this concept of power utilisation.

6.2.4. Solution container

In the estimating tests for the device, chemicals of artificial urine, human serum albumin (HSA) and solution of 30μM BSPOTPE were prepared by laboratory accessories such as pipette, cuvette, stirrer and digital analytical balance. However, most target users are untrained in chemical preparation so it would be a challenge for them to use the lab equipment to take the proper amount of chemicals and mix them together in the cuvette without pouring the solution out. Another concern is about hygiene when manipulate the measurement. In the later design, using a specific syringe with proper volume for taking urine sample, as well as equipping with lab gloves, it helps users not be confusing with sample and reagent volumes. Moreover, the test tube is considered to be equipped with a plastic cap to prevent

solution from pouring out without stopping users from add solutions in. These accessories are disposable so that it requires one set of the accessories for running one test. This set of accessories includes cup for urine sampling, gloves, syringe, cuvette containing specified volume of BSPOTPE. This improvement is predicted to make users feel more comfortable for carrying and performing the solution mixing during the test.

Figure 31: Syringe with specific volume and cuvette with plastic cap.

After testing for precision and the repetition of the results, it is also recommended to provide a control sample vial with the device for instant comparison with the control and for data acquisition.

6.2.5. Light distribution

In the future developments, a Fresnel lens could be inserted between light source and the urine sample, to prevent the uneven dispersion of light from the LED source.

6.2.6. Usability

In designing, the aesthetic is one of the indispensable features, especially the design for a medical device. In the later prototype, although the inner components of the device would still remain black to satisfy the technical requirements such as no light reflecting, UV resistance, interference light absorption, the outside cover is planned to be built in white colour in order to get rid of the negative mental opinion of the concerning hygiene, which may affect the product purchase.

Finally, it is no doubt that the current process of device performance is still complicated to a major group of users. The targets are over-60 elderly so it would be a challenge for them to follow every steps in guiding. Hence, there is necessary requirement to minimize the operation steps, or to introduce a new measurement procedure with a simpler operation in the future design.

Chapter 7: Conclusions

In conclusion, it is no doubt that the project goal has been achieved in that the smartphone based medical device using an aggregation induced emission bio-probe has been designed and optimised for patients to perform the test to monitor the chronic kidney disease (CKD) by themselves when and where they find comfortable, helping indicating the early stage of the disease so that the patients could modify their daily activities to improve their kidney health, or give notice to the doctors to recommend patients what they should follow to prevent kidneys from being more damaged. Although adapting the desirable feature - ASSURE in terms of Affordable material and accessible manufacturing methods, remarkable Sensitivity and Selectivity to human serum albumin, User friendly without or with minimal steps for training and utilisation, Rapid and robust with less than 5 minutes for providing result, Equipment-free and Deliverable to end-user, the current prototype still has some weakness and requires further examination to guarantee the precision of the final results, as well as to find out whether the design would encounter any other failure. Furthermore, this project has shown the promising future with many options for improvement to bring the device into practical utilisation. Lastly, detecting albumin in urine is the first step for further innovation using AIE method to monitor other chemicals in urine or body fluid, providing the basis for the diagnosis of disease in order to have the timely treatment.

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Appendix 1: Smartphone and Camera Information Table

Several common brands of smartphone have been considered to find out their dimensions, cameras positions and cameras' focus length on the rear of smartphone (Keen et.al 2016).

Model	Width (mm)	Thickness (mm)	Focal Length (mm)	Camera Position
HTC				
HTC 10	71.9	9	26	top quarter middle
HTC One X9	75.9	7.99	27.9	top left
HTC One M9	69.7	9.61	27.8	top quarter middle
Microsoft				
Microsoft Lumia 650	70.9	6.9	28	top middle
Microsoft Lumia 950	73.2	8.2	26	top middle
Microsoft Lumia 950 XL	73.2	8.3	26	top middle
Apple				
Iphone SE	58.6	7.6	29	top left
Iphone 3G	62.1	12.3	not provided	top left
Iphone 3GS	62.1	12.3	autofocus	top left
Iphone 4	58.6	9.3	autofocus	top left
Iphone 4 Verizon	58.6	9.3	autofocus	top left
Iphone 4s	58.6	9.3	autofocus	top left
Iphone 5	58.6	7.6	33	top left
Iphone 5s	58.6	7.6	29	top left
Iphone 5c	59.2	8.97	33	top left
Iphone 6	67	6.9	29	top left
Iphone 6 Plus	77.8	7.1	29	top left
Iphone 6s	67.1	7.1	29	top left
Iphone 6s Plus	77.9	7.3	29	top left
Sony				
Xperia E4	74.6	10.5	autofocus	top middle
Xperia E5	69.45	7.99	autofocus	top left
Xperia XA Ultra	79.4	8.4	autofocus	top left
Xperia X Performance	70	8.7	autofocus	top left
Xperia X5	72	7.3	24	top left
Xperia X5 Compact	65	8.9	autofocus	top left
Xperia M5	72	7.6	autofocus	top left
Motorola				
Motorola Moto G4	76.6	9.8	28	top middle
Hauwei				
Hauwei P9	70.9	6.95	27	top left dual lense
LG				
LG G5	73.9	7.7	24	top middle

Model	Width (mm)	Thickness (mm)	Focal Length (mm)	Camera Position
Samsung				
Galaxy A7 (2016)	74.1	7.3	28	top middle
Galaxy A9 (2016)	80.9	7.9	autofocus	
Galaxy J1 (2016)	69.3	8.9	autofocus	top quarter middle
Galaxy J3 (2016)	71.4	7.9	autofocus	top quarter middle
Galaxy J5 (2016)	72.3	8.1	28	top middle
Galaxy J7 (2016)	76	7.8	28	top middle
Galaxy S7 Active	75	9.9	autofocus	top middle
Galaxy Express 3	69.3	8.9	autofocus	
Galaxy Express Prime	71.1	7.9	autofocus	
Galaxy Amp 2	68.6	8.9	autofocus	top quarter middle
Galaxy Amp Prime	71.1	7.9	autofocus	top middle
Galaxy A9 Pro (2016)	80.9	7.9	autofocus	
Galaxy V Plus	62.9	10.7		top quarter middle
Samsung S7	69.6	7.9	26	top quarter middle
Samsung S6	70.5	6.8	28	top quarter middle
Samsung S5	72.5	8.1	31	top middle
Samsung Galaxy Note 55	76.1	7.6	31	top quarter middle

It is no doubt that the common positions of the rear cameras of most smartphones on current market are at one in two area, one is at top-middle of the smartphone, or at top-left corner. Another significant information is that most smartphone cameras' focus length ranges between 23 to 33mm. From these information, the attaching component between smartphone and device could be specially designed so the device could be compatible with most smartphones.

Appendix 2: Five Ranges of Human Serum Albumin for Chronic Kidney Disease:

In the tests of the device's performance, the solutions of different HSA concentrations have been prepared under supervision of Doctor Youhong Tang in the Advanced Materials Laboratory on the 5th floor in Flinders University - Tonsley campus.

Trace (5 - 20 mg/dL)			
Conc of HSA [µM]	**V(HAS) [µL]**	**V(Urine) [µL]**	**V(BSPOTPE) [µL]**
0.0075	67.5	2632.5	300
0.009	81	2619	300
0.01	90	2610	300
0.011	99	2601	300
0.012	108	2592	300
0.013	117	2583	300
0.014	126	2574	300
0.015	135	2565	300
0.016	144	2556	300
0.017	153	2547	300
0.018	162	2538	300
0.019	171	2529	300
0.02	180	2520	300
0.021	189	2511	300
0.022	198	2502	300
0.023	207	2493	300
0.024	216	2484	300
0.025	225	2475	300
0.026	234	2466	300
0.027	243	2457	300
0.028	252	2448	300
0.029	261	2439	300
0.03	270	2430	300

Range 1+ (20 mg/dL - 30mg/dL)			
Conc of HSA [µM]	**V(HAS) [µL]**	**V(Urine) [µL]**	**V(BSPOTPE) [µL]**
0.03	270	2430	300
0.031	279	2421	300
0.032	288	2412	300
0.033	297	2403	300
0.034	306	2394	300

0.035	315	2385	300
0.036	324	2376	300
0.037	333	2367	300
0.038	342	2358	300
0.039	351	2349	300
0.04	360	2340	300
0.041	369	2331	300
0.042	378	2322	300
0.043	387	2313	300
0.044	396	2304	300
0.045	405	2295	300

Range 2+ (30 mg/dL - 100mg/dL)			
Conc of HSA [µM]	**V(HAS) [µL]**	**V(Urine) [µL]**	**V(BSPOTPE) [µL]**
0.045	405	2295	300
0.05	450	2250	300
0.055	495	2205	300
0.06	540	2160	300
0.065	585	2115	300
0.07	630	2070	300
0.075	675	2025	300
0.08	720	1980	300
0.085	765	1935	300
0.09	810	1890	300
0.095	855	1845	300
0.1	900	1800	300
0.105	945	1755	300
0.11	990	1710	300
0.115	1035	1665	300
0.12	1080	1620	300
0.125	1125	1575	300
0.13	1170	1530	300
0.135	1215	1485	300
0.14	1260	1440	300
0.145	1305	1395	300
0.15	1350	1350	300

Range 3+ (100 mg/dL - 300mg/dL)			
Conc of HSA [µM]	**V(HAS) [µL]**	**V(Urine) [µL]**	**V(BSPOTPE) [µL]**
0.150	40.5	2659.5	300
0.181	49	2651	300
0.211	57	2643	300
0.241	65	2635	300

0.270	73	2627	300
0.300	81	2619	300
0.330	89	2611	300
0.359	97	2603	300
0.389	105	2595	300
0.419	113	2587	300
0.450	121.5	2578.5	300

Range 4+ (300 mg/dL - 1000mg/dL)			
Conc of HSA [µM]	**V(HAS) [µL]**	**V(Urine) [µL]**	**V(BSPOTPE) [µL]**
0.45	121.5	2578.5	300
0.5	135	2565	300
0.55	148.5	2551.5	300
0.6	162	2538	300
0.65	175.5	2524.5	300
0.7	189	2511	300
0.75	202.5	2497.5	300
0.8	216	2484	300
0.85	229.5	2470.5	300
0.9	243	2457	300
0.95	256.5	2443.5	300
1	270	2430	300
1.05	283.5	2416.5	300
1.1	297	2403	300
1.15	310.5	2389.5	300
1.2	324	2376	300
1.25	337.5	2362.5	300
1.3	351	2349	300
1.35	364.5	2335.5	300
1.4	378	2322	300
1.45	391.5	2308.5	300
1.5	405	2295	300