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Cell-phone-based measurement of TSH using Mie scatter optimized lateral flow assays

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ABSTRACT

Semi-quantitative thyroid stimulating hormone (TSH) lateral flow immunochromatographic assays (LFA) are used to screen for serum TSH concentration >5 mIU L^{-1} (hypothyroidism). The LFA format, however, is unable to measure TSH in the normal range or detect suppressed levels of TSH (<0.4 mIU L^{-1}; hyperthyroidism). In fact, it does not provide quantitative TSH values at all. Obtaining quantitative TSH results, especially in the low concentration range, has until now required the use of centralized clinical laboratories which require specimen transport, specialized equipment and personnel, and result in increased cost and delays in the timely reporting of important clinical results. We have conducted a series of experiments to develop and validate an optical system and image analysis algorithm based upon a cell phone platform. It is able to provide point-of-care quantitative TSH results with a high level of sensitivity and reproducibility comparable to that of a clinical laboratory-based third-generation TSH immunoassay. Our research approach uses the methodology of the optimized Rayleigh/Mie scatter detection by taking into consideration the optical characteristics of a nitrocellulose membrane and gold nanoparticles on an LFA for quantifying TSH levels. Using a miniature spectrometer, LED light source, and optical fibers on a rotating benchtop apparatus, the light intensity from different angles of incident light and angles of detection to the LFA were measured. The optimum angles were found that the minimized Mie scattering from nitrocellulose membrane, consequently maximizes the Rayleigh scatter detection from the gold nanoparticles in the LFA bands. Using the results from the benchtop apparatus, a cell-phone-based apparatus was designed which utilized the embedded flash in the cell phone camera as the light source, piped the light with an optical fiber from the flash through a collimating lens to illuminate the LFA. Quantification of TSH was performed in an iOS application directly on the phone and verified using the code written in MATLAB. The limit of detection of the system was determined to be 0.31 mIU L^{-1} (never achieved before on an LFA format), below the commonly accepted minimum concentration of 0.4 mIU L^{-1} indicating clinical significance of hyperthyroidism. The system was further evaluated using human serum showing an accurate and reproducible platform for rapid and point-of-care quantification of TSH using a cell phone.

1. Introduction

Iodine deficiency remains a major public health issue, in which 54 countries are still iodine deficient (Benoist, 2004). Iodine deficiency disorders (IDD) affect mental health in children, and may cause stillbirths, spontaneous abortions, and congenital abnormalities (Delange, 1994). While there has been significant progress made in reducing iodine deficiency, primarily through iodized salt, the problem still persists (Delange et al., 1999; Stanbury et al., 1998). Although a 2008 report determined that over 95% of the salt available for human consumption in Uganda contained adequate amounts of iodine to prevent iodine deficiencies, IDDs still remain prevalent in these areas (Benoist et al., 2008). One of the reasons is because expensive equipment, electricity, and trained personnel are required in order to accurately determine the prevalence of goiter in children using ultrasonography. Additionally, measuring iodine secretion levels is conducted in a World Health Organization (WHO) laboratory in South Africa, and samples are difficult to transport and are susceptible to tampering. Thus, a more practical approach is point-of-care diagnostics for neonatal thyroid stimulating hormone (TSH) levels (Ehrenkranz et al., 2011).

Thyroid-stimulating hormone (TSH) is synthesized and secreted by thyrotroph cells in the anterior pituitary gland, and is essential to the biosynthesis and secretion of thyroid hormones, triiodothyronine (T3) and thyroxine (T4). TSH increases the

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activity of sodium-iodine symporters (NIS) on the basolateral membrane of the thyroid follicular cell. This increases iodine trapping and stimulates iodination of thyroglobulin in the follicular lumen. Conjugation of iodinated tyrosines links T4 and T3 to thyroglobulin, which are then secreted into circulation. TSH production is controlled by thyrotropin-releasing hormone (TRH), which is synthesized in the hypothalamus and transported to the anterior pituitary through hypothalamic portal vessels. If an individual is iodine deficient, the body will increase the production of TSH to increase iodine trapping. Thus, increased levels of TSH may indicate hypothyroidism, and decreased levels of TSH may indicate hyperthyroidism.

The thyroid hormones are essential in human development and have a number of biological actions within the body, including, but not limited to growth and maturation, neurological development, control of basal metabolic rate, and musculoskeletal and cardiovascular systems. The most common pathological condition of hypothyroidism (i.e. increased TSH levels) in children is endemic cretinism, in which the child suffers from severe physical and mental growth (Kochupillai and Mehta, 2008). Hypothyroidism (i.e. decreased TSH levels) in adults may result in decreased basal metabolic rate, hypothermia, and cold intolerance. Hyperthyroidism can lead to Graves disease, an autonomic disorder where antibodies mimic TSH.

Lateral flow immunochromatographic assays offer a practical approach to point-of-care measurements of TSH. These are prefabricated strips of a material that contain dry reagents that are then activated by a fluid sample. These assays are not "dip-sticks" but a lateral flow assay (LFA) with high sensitivity and selectivity. The most common application of an LFA is the detection of human chorionic gonadotropin, or commonly referred to as the pregnancy test. Lateral flow immunochromatographic assays have been used in a variety of other applications, including infectious diseases (Shyu et al., 2010; Wilkinson et al., 2003), other hormones, cardiac and tumor markers (Choi et al., 2010), drug screening, and many more. The greatest attribute of LFAs is point-of-care diagnostics: the ability to perform a medical test at the site of the patient. Other benefits of an LFA test are little to no sample pretreatment and no laboratory or trained personnel required to perform the test. However, the tradeoff is that LFAs have reduced sensitivity and a higher percentage of error than their laboratory analogs (Posthuma-Trumpie et al., 2009). This is primarily due to the fact that most LFAs, especially over-the-counter consumer LFAs, are colorimetric and are analyzed by the naked eye. This procedure inherently produces a tremendous amount of variation from user to user.

There have been improvements made to address these issues, primarily in the form of electronic optical detection, to alleviate the issue of human error. In these systems, a simple light emitting diode (LED) and photodetector are used to quantify the intensity of the positive color band on the test strip. While these simple digital LFAs reduce the error rate induced by human variation, the sensitivity is generally on par with the naked eye or less (Cadle et al., 2010). In fact, many critics argue that the use of digital readers actually reduces the detection limit by eliminating subjective user interpretation. More sophisticated systems may use more sensitive reflectance based sensors. Companies such as Detekt Biomedical or Qiagen ESEQuant have developed commercial handheld, PDA-style, quantitative LFA readers/scanners that offer extremely high levels of sensitivity. However, these systems also carry an extremely high price tag, and are counterintuitive for deploying an effective TSH detection system into areas where it is needed, primarily underdeveloped and developing nations. Thus, it is not surprising that they have not been widely adopted.

A more reasonable approach would be to use a readily available digital photodetection system, such as a cell phone with a built in camera. The high-density charge-coupled device (CCD) or complementary metal oxide semiconductor (CMOS) arrays used in cell phone cameras are not optimal for extremely high sensitivity applications; therefore, a technique or methodology needs to be incorporated to significantly improve the sensitivity of LFAs without the addition of expensive components or complicated procedures.

LFAs have been developed for detecting TSH levels with acceptable sensitivity (Kosack et al., 2012); however, because they typically rely on visual inspection or a slightly more sophisticated reflectance sensor for determining the concentration of TSH, the detection of extremely low TSH concentrations (0.4 mIU L^{-1}, hyperthyroidism, Rhee et al., 2012) are often inconclusive (Posthuma-Trumpie et al., 2009). Because of this fact, most TSH LFAs have focused on the detection of hypothyroidism, i.e., high TSH levels.

LFAs rely on the use of nanoparticles (typically colloidal gold), with a mean diameter less than 100 nm. Light scattering microparticle immunoassays have been shown to exhibit an extremely high level of sensitivity and reproducibility using the Mie scatter as the predominant form of light scatter for particles that are larger than the wavelength of incident light (Han et al., 2008). However, because LFAs use gold nanoparticles, in which their mean diameter is significantly smaller than the wavelength of incident light, they exhibit the Rayleigh scatter. The absorption spectrum for gold nanoparticles is predominantly in the green region and slightly in the blue region, and thus they appear pink when irradiated. In previous works, we used simulations employing the analytical solutions to Maxwell's equations (Cai et al., 2008) to determine angles that exhibited maximum intensity change depending on the size of the particles used and the light wavelength (Heinze and Yoon, 2011). In this study, because the particles of interest do not follow the Mie scatter regime due to their small sizes, we cannot use similar simulation methodologies. However, the nitrocellulose membrane follows the Mie scatter regime. Therefore, we can employ the concept of the Mie scatter using an LED and photodiode, or a flash (white LED) and CCD/CMOS image sensor from a cell phone to optimize the detection from a TSH LFA by determining the angles of incident light and detection that minimizes the light scatter from the nitrocellulose membrane (Fig. 1 and 2). This effectively increases the signal of the band regions on the test strip, by improving the signal to noise ratio. In essence, this is a very common practice with home pregnancy LFA tests, where very faint bands may become more pronounced when viewing the test strip at different angles.

In this work, we utilize the Mie scatter to optimize the parameters of our system in order to increase the sensitivity, reproducibility, and usability of the TSH LFA. As a result of determining the optimal parameters, we designed a cost-effective, quantitative detection system for TSH LFA utilizing a cell phone camera as a photodetector and LED flash as a light source with minimal components and cost. Human blood serum with known concentrations of TSH was used to evaluate the accuracy of our cell-phone-based TSH detection system.

2. Materials and methods

2.1. TSH reader

The cell-phone attachment was designed using Solidworks 2011 (Solidworks Corporation, Concord, MA, USA), and then stereolithographically printed using a Stratasys Dimension uPrint 3D printer (Stratasys, Inc., Eden Prairie, MN, USA) in an acrylonitrile–butadiene–styrene (ABS) polymer Fig. 1. An 8.0 mm diameter

positive achromatic doublet lens (Thorlabs, Newton, NJ, USA) was positioned in front of the cell phone camera lens mounted within the reader attachment. A 6.0 mm diameter white core optical light pipe (SparkFun Electronics, Boulder, CO, USA) was used to carry the light from the cell phone camera's flash and irradiate the LFA at a discrete angle to the surface (as determined through the benchtop Mie scatter optimization experiment).

2.2. Benchtop Mie scatter optimization system

Optical positioning stages (Edmund Optics, Inc., Barrington, NJ, USA) were set up to allow rotation of the LFA and an optical detection fiber (Ocean Optics, Inc., Dunedin, FL, USA) about a common axis. A static optical fiber attached to an Ocean Optics LS-450 light source irradiated the LFA. An Ocean Optics USB4000 UV–vis miniature fiber optic spectrometer measured light intensities as the stages were rotated. The LFA stage translated axially to the rotation for measurement of the test band, control band, and non-band regions. SpectraSuite software (Ocean Optics) was used for measuring light intensity from varying regions on the LFA in real-time to reduce temporal variations in band intensities Fig. 3.

2.3. Lateral flow immunochromatographic assay

TSH lateral flow immunochromatographic test strips and control solutions were acquired from ThyroChek (Screening Devices Canada Inc., Hatfield Pt., NB, Canada). Human blood serum was obtained from Interstate Blood Bank, Inc. (Memphis, TN, USA), and used to evaluate the performance of our device. Quantification of TSH from both controls and serum were conducted through The University of Arizona Medical Center,

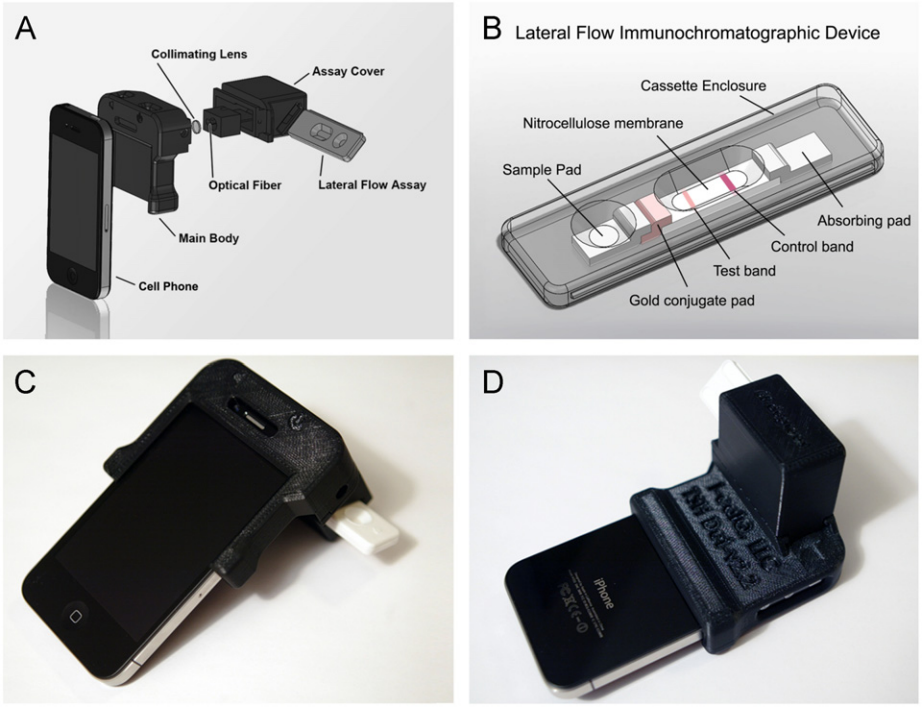


Fig. 1. (A) Exploded view of complete device showing placement of collimating lens and optical fiber (light pipe) set at specific angles in reference to the lateral flow assay cassette. (B) Cheap and disposable lateral flow assay test strip, with gold nanoparticle conjugated antibodies in the gold conjugate pad, anti-TSH immobilized in the test band, and anti-IgG immobilized in the control band. Flow is from left to right. (C,D) Photographs of actual reader attached to cell phone and with a disposable TSH LFA cassette inserted.

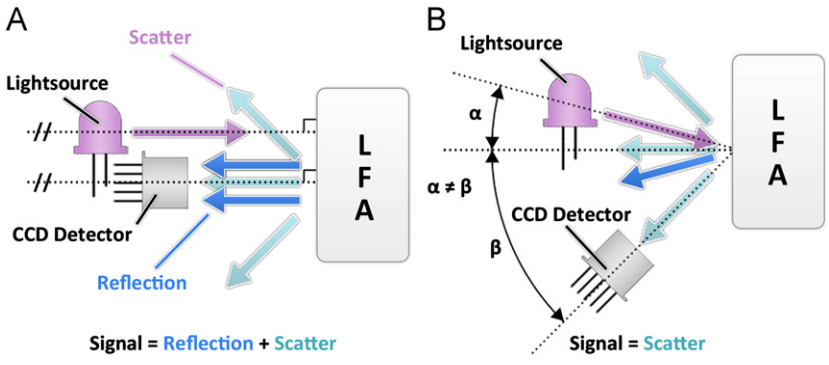


Fig. 2. (A) Reflectometer: standard detection modality for analyzing LFAs that exhibit poor signal to noise ratio due to detector receiving large amount of scattered light from nitrocellulose membrane. (B) Cell-phone-based TSH device: optimized detection modality shows improved signal to noise ratio due to detector being oriented at angle, β, that is optimized for decreased light scatter from nitrocellulose membrane, as well as differs from angle of light source to surface, α.

Department of Pathology using an Architect I2000 (Abbott Laboratories, Abbott Park, IL, USA). Dilutions of the positive control were used to construct a standard curve and to spike the human blood serum. Fig. 4 outlines the standard protocol used to prepare and test a sample using a ThyroChek TSH LFA and our cell-phone-based detection system. After attaching the reader to an iPhone 4 (Apple, Inc., Cupertino, CA, USA), and initializing our iOS application, 50 μL of control or serum was deposited into the test well on the ThyroChek cassette. This cassette was then immediately inserted into the reader, which was automatically detected, starting a 90 s count-down timer. The application yielded an error message if it detected a used cassette was inserted at this stage. After the initial timer, 4 drops of diluent were added to the test well. The reader again automatically detected the presence of a wave-front from the addition of diluent and automatically began a 10 min timer. Upon completion of the timer, the application acquired an image, processed the data, and displayed the results in mIU L^{-1}.

2.4. Image acquisition and data processing

The images acquired using our device were processed using both the MATLAB code and an iOS application that we created using MATLAB R2010a (The Mathworks, Inc., Natick, MA, USA) or iOS Developer Program (Apple, Inc.). The algorithm first creates a red and green band difference of the image. It then locates the control band region and creates a boundary. The dimensions of this boundary are used to map the length of the nitrocellulose region of the image, separating each row perpendicular to the direction of flow, by pixel. The data from the plot created was processed to reduce cassette to cassette variation, including abnormalities such as particle streaking or band offset from loose manufacturing tolerances. Additionally, certain regions of the data plots were isolated to circumvent changes in white levels observed from phone-to-phone CCD/CMOS and other hardware variations.

3. Results and discussion

3.1. Mie scatter optimization

To experimentally determine the optimal irradiation and detection angles, a benchtop system was designed to measure light intensities from the test and control band regions, as well as the nitrocellulose strip. Fig. 3A shows the benchtop device setup. Mechanical limitations of the system, in which the optical fibers and LFA cassette came into contact, prevented all combinations of the two parameters (angle of LFA from irradiation fiber and angle of detection fiber from irradiation fiber) from being tested. However, these limitations are also inclusive in the cell-phone-based TSH device, making the benchtop system an accurate predecessor to the final device design.

Fig. 3B is the results from the benchtop system, in which each data point represents the intensity of the test band from the control band, divided by the background Mie scatter from the nitrocellulose strip. Thus, when the scattered light from the nitrocellulose strip decreases, the signal increases. Certain nodal points were observed, including 45° and 75° (angle of LFA from irradiation fiber and angle of detection fiber from irradiation fiber, respectively), 60° and 95°, and 65° and 110°. Of these, 65° and 110° showed the greatest signal to noise ratio. It appears that if we continued to increase the angle of the detection fiber from irradiation fiber that the signal may increase further; however, beyond 110°, the design of the cassette would impede the transmission of light. These parameters were then used in the design of the device reader by designing the cell phone attachment to orient the LFA cassette at an angle of 45 from the cell phone camera, and piping the light at an angle of 65° above the LFA test strip.

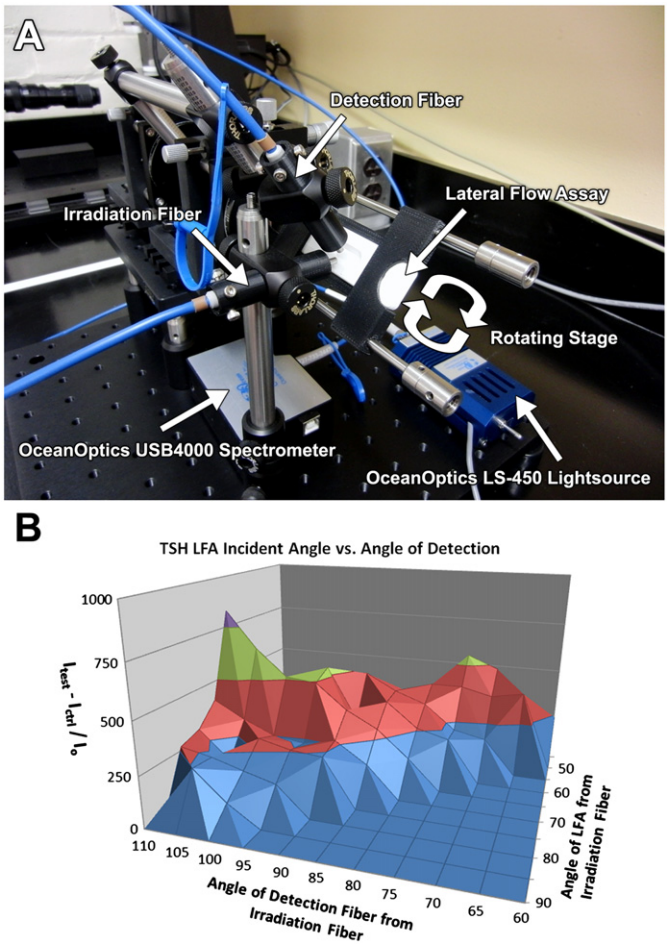


Fig. 3. (A) Benchtop apparatus for experimentally determining optimized irradiation/detection angles of the Mie scatter from LFAs. (B) Results for the nitrocellulose Mie scatter experiments showing multiple nodal points for improved signal to noise ratio. Optimal configuration determined as light source 65° from surface, and detector 110° from surface.

As in similar methodologies employed in the past (You et al., 2011), the Mie scatter simulations using online software (Prahl, 2007) were attempted. However, unlike microparticle immunoagglutination assays with spherical particles, the nitrocellulose fibers are dense, very large and typically cylindrical in shape, making these simulations inappropriate for this application. Yet, the presence of nodal points, and oscillating scatter intensities versus angle, are highly indicative of the Mie scatter regime, and closely follow many trends described in previous work (Heinze et al., 2010; You et al., 2011). Thus, we can conclude that the importance of the optimized Mie scatter to improve signal to noise ratio is extremely important for this application and essential in order to reach clinical laboratory sensitivities with our system.

3.2. TSH detection from control solutions

Using the parameters defined in our benchtop system, we constructed a low-cost reader to quantify cheap and disposable LFA tests. Fig. 5 is the standard curve created using an iPhone 4, our attached reader, and ThyroChek TSH cassettes. The results show a very good linear trend and extremely reproducible results.

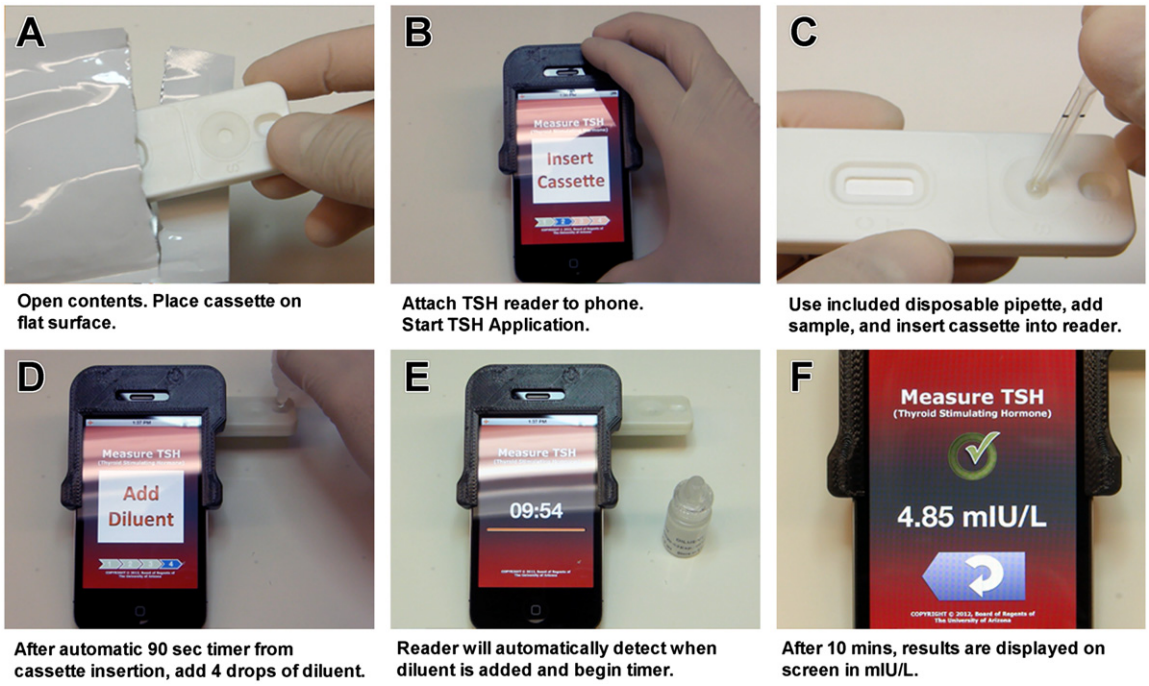


Fig. 4. (A–F) Complete cell-phone-based TSH detection protocol. Total assay time of approximately 12 min.

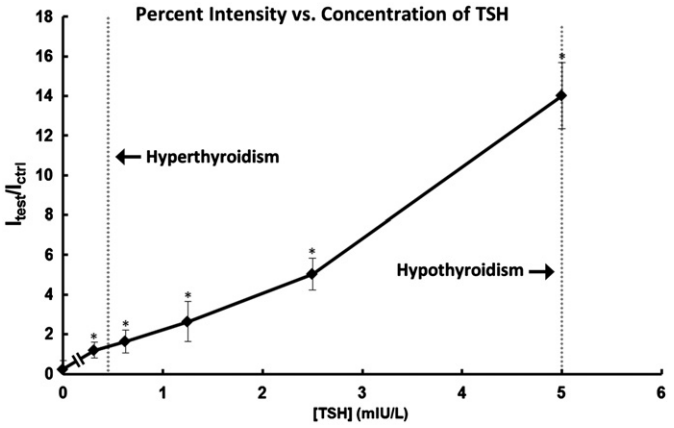
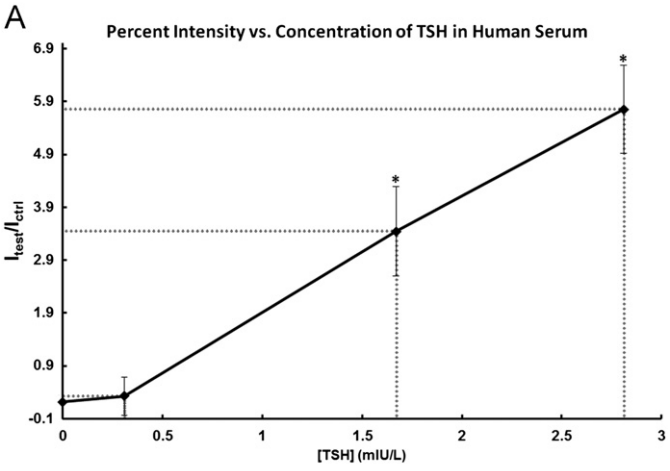


Fig. 5. A standard curve from a cell-phone-based TSH detection system using the standard TSH solutions. A good linear trend with a limit of detection of 0.31 mIU L^{-1} and the ability to detect hyperthyroidism (<0.4 mIU L^{-1}) and hypothyroidism (>5.0 mIU L^{-1}) were demonstrated.



B

Actual TSH Concentration	Calculated TSH Concentration	% Error
0.31	0.29	6.45
1.67	1.64	1.80
2.81	2.89	2.85

Fig. 6. (A) Results from the cell-phone-based TSH detection system using human serum, where the "actual" TSH concentrations were determined by the clinical assay instrument (Architect I2000, Abbott Laboratories). (B) Using the results from Fig. 5 (standard curve), the TSH concentrations of human sera were "calculated" and compared with the "actual" concentrations.

More importantly, the results show good statistical significance ($p<0.05$) of 0.31 mIU L^{-1} over blank, never achieved before on an LFA format, and making a true low-cost point-of-care device able to clinically diagnose hyperthyroidism.

For TSH concentrations greater than 5 mIU L^{-1} the test band appeared within 5 min of adding diluent. The band intensity continued to increase in strength until 10 min. For lower concentrations, the band would not appear until 10 min. Therefore, the time-based kinetics of the reaction may allude to more information than just final band intensity. The iOS application can be programmed to take a series of pictures at certain intervals, and then use this information to further increase the accuracy of the TSH measurement. During calibration, control serums may also be used as a feedback mechanism to ensure that the cassette or phone is functioning properly. Thus, our cell-phone-based system, with the flexibility of iOS programming, can provide many checks and balances to provide a more robust point-of-care platform for diagnostics testing.

3.3. Human serum evaluation

To further evaluate our device under simulated clinical settings, we used human blood serum with the "actual" TSH concentrations determined in a clinical laboratory. Fig. 6A shows the results of three TSH serum concentrations that were tested (0.31, 1.67, and 2.81 mIU L^{-1}). 1.67 and 2.81 mIU L^{-1}

concentrations fall under normal physiological conditions; however, the accuracy in this region remains clinically significant for physicians, and thus the precision of our system in this range is very important. The 0.31 mIU L^{-1} concentration represents an individual with hyperthyroidism.

Using the same platform and algorithm to derive the results in Fig. 5, the three concentrations were analyzed and back-calculated using our standard curve. Fig. 5B shows the actual TSH concentrations of our results, our calculated TSH concentration using our cell-phone-based system and standard curve, and the percent error. In the normal physiological range, the error is very small. Under hyperthyroid conditions, the error is greater, but is well within the range of $\pm 10\%$ that is typically acceptable in medical diagnostics.

Since the standard curves provided reproducible results as indicated in the previous section and the error between the actual and TSH concentrations was less than 10%, there is little need to re-calibrate the system as long as the same types of LFA tests are used. Re-calibration can be easily made, however, if there is such a need, by measuring the signals from a TSH standard and a negative control solutions, i.e. two-point calibration.

4. Conclusions

This study demonstrates the use of cell-phone-based measurements of TSH using the Mie scatter optimized LFAs. More importantly, we successfully demonstrate the ability to use our low-cost, point-of-care device to measure very low concentrations of TSH (< 0.5 mIU L^{-1}). This allows the ability to detect hyperthyroid disorders, which typically require a clinical laboratory. The device exhibited good sensitivity and reproducibility with a detection limit of 0.31 mIU L^{-1} and an assay time of approximately 12 min.

Our research approach uses the methodology of the optimized Mie scatter detection on a nitrocellulose LFA for quantifying TSH levels. Using a miniature spectrometer, LED light source, and optical fibers on a rotating benchtop apparatus, the light intensity from different angles of incident light and angles of detection to the LFA were measured. The optimum angles were found, 65° and 110° (angle of LFA from irradiation fiber and angle of detection fiber from irradiation fiber, respectively) that the minimized Mie scattering from nitrocellulose membrane and the maximized Rayleigh scattering from the gold nanoparticles in the LFA bands. Using the results from the benchtop apparatus, a reader device for cell phone was designed which utilized the embedded flash in the cell phone camera as the light source, piped the light with an optical fiber from the flash through a collimating lens to decrease the focal point and illuminate the LFA. Quantification of TSH was performed in an iOS application directly on the phone and verified in image processing code written in MATLAB. Human blood serum was then used to evaluate the system, in which three concentrations (two physiologically normal, one hyperthyroid) were tested in the cell-phone-based device. The results show high accuracy and excellent reproducibility with an error less than 7% with the hyperthyroid serum, and less than 3% with the physiologically normal serums, making this detection system well within the range of what is clinically acceptable.

Because our system incorporates the use of camera-equipped cell phones, which are readily available throughout the world, there is a potential for broad impact of this device for point-of-care medical diagnostics. In this paper we demonstrate the use of our optimized LFA system for detecting very low concentrations of TSH; however, this methodology can easily incorporate other existing, FDA approved LFA test strips, including infectious diseases, other hormones, cardiac and tumor markers, or drug screening. Furthermore, the algorithm used to process the images acquired by the phone can easily adapt to hardware changes from phone-to-phone, and even phone model-to-phone model. This means that in order to utilize our system on an existing camera phone platform, the only requirement is to create a different low-cost phone attachment, using the same TSH LFA cassettes. Due to the sensitivity, accuracy, cost-effectiveness, and flexibility of this cell-phone-based Mie scatter optimized detection system, the broad impact of this technology is extremely promising, and will assuredly prove to be a capable and powerful tool for point-of-care medical diagnostics.

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