Lab-in-a-Smartphone: Spectroscopic-Based Sensing Utilizing Linear Variable Filters for Point-of-Care Applications

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Abstract

A compact analysis platform for detecting liquid phase absorption and emission spectra using a set of optical linear variable filters atop a CMOS image sensor is presented. This combination has achieved detection of fluorescence down to 0.28 nM for quantum dot emitters and 32 ng/mL for a near-infrared dye. Compared to commercially available spectrometers, the benchtop demonstration shows comparable or even better performance with potentially better portability and reduction in cost for point-of-care applications. Based on the benchtop demonstration, a design suitably sized for use with a smartphone platform is conceptualized and fabricated.

Keywords: Biophotonic sensor; spectroscopy; linear variable filter; fluorescence; absorption; point-of-care applications
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1. Introduction

1.1 Motivation

Diagnostic testing is a vital step to all sources of medicinal care. General practitioners must first make an accurate diagnosis to appropriately treat patients. A variety and growing number of point-of-care (POC) tests that provide rapid and accurate results are used by countries all over the world. A survey was conducted to determine the number of POC tests being used from primary care doctors of Australia, Belgium, Netherlands, United Kingdom (UK), and the United States of America (USA). All of the 2770 primary care doctors who responded to a survey reported the active usage of POC test for diagnosing acute conditions and sometimes chronic conditions. In fact, primary care doctors from the UK and USA reported higher usage and desire for the development of additional POC tests than the rest of the countries [1]. Beyond human health diagnostics, POC technology is also used in food safety, water quality tests, and pathogen detection [2,3].

1.2 Literature Review

POC tests are largely based on spectrally resolved measurements of liquid-phase samples, where fluorescence emission and colorimetric absorption are the most commonly utilized techniques. Systems that are suitable for point-of-care diagnostics in recent literature are expensive, complex to medical practitioners, and inconvenient to carry [4,5,6]. Through the use of optical components and a commercially available image sensor, a low-cost, compact system suitable for POC applications is presented.
2. Linear Variable Filter (LVF) System

2.1 Characterization of LVF atop CMOS Image Sensor

By using linear variable filters (LVF) atop a CMOS image sensor, a spectroscopic system that is compact, low-cost, versatile, and suitable for point-of-care diagnostics can be realized. Distributed Bragg reflector (DBR) stacks of alternating low and high refractive index layers create a highly selective and narrow bandwidth transmission to a certain wavelength depending on the thickness of the stack [7,8]. Figure 1 shows a cross-sectional view of an LVF taken using a scanning electron microscope (SEM). An LVF is created by placing a dielectric wedge in between two DBR stacks [9] as illustrated in Figure 2. As a result, the transmission curve varies spatially across the height gradient of the LVF. As shown in Figure 3, when a monochromatic source is shined perpendicularly onto the LVF, only a select portion of the LVF corresponding to that wavelength will be allowed to transmit through. Thus, the illuminated positions on the CMOS pixel array of the transmitted light passing through the LVF will correspond to the spectrum of the incident light.

Figure 1: LVF cross section taken from a scanning electron microscope (SEM). The alternating layers of the DBR stacks can be easily seen in this image.

Figure 2: Illustrated schematic of an LVF as described in [8].
2.2 Spectroscopic Capabilities of LVF System

The LVF system was characterized for spectroscopic detection. Laser pointers are used for this measurement since they offer single wavelength emission and narrow linewidths. Figure 4 shows the image captures from the LVF system that uses a commercially available CMOS image sensor (PixeLINK, Ottawa, Canada).
Through using mathematical processing software (MATLAB), a spectrum of the incident light can be extracted. The pixel intensity values (0 – 256) are averaged in each vertical column and the resulting horizontal row vector that contains the average pixel intensity represents the spectrum of the incident light. The result of this calculation is plotted and shown in Figure 5.

Figure 5: Spectra generated through the CMOS image captures shown in Figure 4. These are processed through mathematical analysis of the pixel values extracted from the CMOS images. Referencing the positions illuminated on the CMOS pixel array and their peak emission wavelengths, a conversion between pixel position and corresponding wavelength was determined to convert the abscissa.

From left to right, these are the calculated spectra for the violet/blue ($\lambda = 410\text{nm}$), green ($\lambda = 540 \text{ nm}$), and red ($\lambda = 660 \text{ nm}$) laser pointers respectively. Using a commercially available spectrometer, the emission wavelengths of these sources are taken. Thus, through referencing these three sources and their positions illuminated on the CMOS pixel array, a linear fit can be made to convert pixel position to corresponding wavelength. The peak spacing found for the visible (VIS) LVF used for this calculation is 10.07 nm/pixel. Similarly, the peak spacing found for the near-infrared (NIR) LVF is 7.40 nm/pixel. The spectra calculated suggest a monochromatic source with narrow linewidths as expected.
3. Spectroscopic Point-of-Care (POC) Applications

3.1 Fluorescence Emission Measurements

As mentioned, two common detection techniques used for POC applications are fluorescence emission and colorimetric absorption. Two sets of measurements are performed to detect fluorescence emission using the LVF system. The first is through a series of different biological quantum dot emitters (QD520, QD560, QD610, QD740, and QD860). These quantum dot emitters are designed to fluoresce at a single wavelength ranging from 520nm to 860nm. The optical setup of this experiment is shown in Figure 6.

Figure 6: Optical system of fluorescence emission measurement. The fluorescence assay is illuminated by an excitation source. The fluorescence signal is then simultaneously captured by the LVF system and a commercially available handheld spectrometer for comparison. Adapted by permission from [9].
The excitation source is coupled into a fiber that passes through a pair of focusing lenses which illuminates the fluorescence assay. The fluorescence signal is then simultaneously measured by a handheld spectrometer and the LVF system for comparison. The measured signal is collected perpendicularly from the excitation signal to prevent the illumination source from being measured.

In Figure 7, the measured spectra of the handheld spectrometer (left) and LVF system (right) are compared. As shown, the LVF system can resolve the fluorescence signals of various biological quantum dot emitters with comparable performance to the handheld spectrometer. Furthermore, it is also demonstrated that the spectral range of detection can be extended by adding multiple LVFs onto the CMOS image sensor and then concatenating the corresponding spectra.
The second test is a series of diluted fluorescent samples to determine the detection sensitivity of the LVF system. This is done through a series of QD610 and near-infrared dyes for the visible and near-infrared LVF respectively. Samples of QD610 are diluted from concentrations of 760nM to 0.28nM. The spectra of these diluted samples are then measured and plotted to determine the dilution relationship as shown by the LVF system shown in Figure 8.

![Figure 8: Series of diluted fluorescent samples are spectrally measured with the LVF system. The sample that is shown is QD610. The diluted samples range from a concentration of 760 nM to 0.28 nM. A logarithmically scaled plot of the LVF system measurements to show that the dilution relationship can be accurately extracted from these image captures. Adapted by permission from [9].](image)

It is shown that the visible LVF can resolve signals down to concentrations of 1.4 nM before becoming indistinguishable to spectral noise. A logarithmically scaled plot of integrated intensity vs concentration is shown to demonstrate that the dilution relationship can be extracted from the spectra measured with the LVF system.

For the near-infrared LVF, diluted samples of a near-infrared dye ranging from 20 μg/mL down to 32 ng/mL are measured. In Figure 9, the near-infrared LVF can detect solutions down to
32 ng/mL before becoming obscured to noise. Similarly, a logarithmically scaled plot of integrated intensity vs concentration is shown that can be used to calculate the dilution relationship.

![Intensity plot](image1.png)

Figure 9: Series of diluted fluorescent samples are spectrally measured with the LVF system. The sample that is shown is a near-infrared dye. The diluted samples range from a concentration of 20 μg/mL to 32 ng/mL. An intensity plot (left) and a logarithmically scaled plot (right) are shown. Adapted by permission from [9].

### 3.2 Colorimetric Absorption Measurements

![Optical system](image2.png)

Figure 10: Optical system of colorimetric absorption measurement. A white LED is shined incident onto the absorbance assay. The transmitted signal that passes through the absorbance assay is then measured through three different spectroscopic platforms: LVF system, handheld spectrometer, and OSA. Adapted by permission from [9].
In addition to fluorescence, colorimetric absorption measurements are also largely used for POC tests. To determine the LVF system’s capabilities for detecting absorption signals, various food dyes mixed with water are prepared and placed in a similar optical setup as the fluorescence emission measurement. A white LED is used to directly illuminate onto the absorbance assay and collected simultaneously as shown in Figure 10.

A comparison across different spectroscopic platforms (handheld spectrometer, tabletop OSA, and LVF system) is shown in Figure 11. As a consequence of the LVF sitting atop a cover glass, a small cavity lies between the LVF and the CMOS pixel array. This introduces fringes across the intensity plots as seen in Figure 11. However, through calculating the absorbance and converting to transmittance, these fringes are mathematically corrected in the process.

Figure 11: Intensity plots of transmitted signals collected after passing through the absorbance assay. Fringes are introduced due to small cavity between LVF and CMOS pixel array. This is mathematically corrected when calculating transmission. Adapted by permission from [9].
The absorbance is calculated as defined by the equation below:

$$A = \log_{10} \frac{P_0}{P}$$

The incident light power is denoted as $P$ and the transmitted light power is denoted as $P_0$. As such, taking the white light in the intensity plots as the incident light ($P_0$) and the individual food dye solutions as the transmitted light power ($P$), the absorbance of the food dye solutions can be calculated. Then by taking the difference between unity and absorbance, the transmittance can be calculated which is shown in Figure 12.

Figure 12: The absorbance is calculated using the equation mentioned above. The transmittance is then shown across three different spectroscopic platforms. The fringes that appear in the LVF system from Figure 11 are mathematically eliminated through dividing the incident signal by the reference signal. The LVF system provides a better signal-to-noise ratio in comparison to the three spectroscopic platforms.

Comparison of the different spectroscopies reveals tradeoffs for each system. It is shown that the LVF system provides the best signal-to-noise ratio out of the three platforms. While the handheld spectrometer provides the fastest results, it has a decreased sensitivity in the lower wavelength range due to the CCD camera used in its optical system. Lastly, the OSA has the best resolution, but is significantly more expensive and larger than the other two platforms.
3.3 Free-Chlorine Test

As demonstrated, the LVF system can resolve fluorescence and absorption signals with comparable performance. For a comprehensive demonstration of the LVF system, a free-chlorine test is performed with the LVF system to show a frequently used application. Municipal water supplies are mixed with chlorine to inactivate bacteria and certain viruses. As such, a powder is added to a sample of water that creates an onset of a shade of pink dependent on the concentration of the free-chlorine ions. With higher concentrations of free-chlorine ions, the heavier shade of pink appears in the sample. Using the colorimetric absorption setup, the LVF system is compared to the handheld spectrometer.

Figure 13: A free-chlorine ion test is performed on a sample extracted from the Urbana, Illinois municipal water supply. The induced shade of pink is measured through a colorimetric absorption utilizing the optical pathway as illustrated in Figure 10. The results of the LVF system and a handheld spectrometer are compared.
As shown in Figure 13, the results measured from the fluorescence and colorimetric absorption experiments translates well onto real world applications such as free-chlorine tests for the LVF system. Based upon local reports from Urbana, Illinois, the concentration of free chlorine should not exceed a concentration of 3.9 mg/L. A correlation between the absorbance values calculated from the LVF system and the concentration of chlorine in the sample can be extracted from these measurements.
4. Summary and Conclusion

4.1 Current Work

Current work is being done to further miniaturize and make the LVF system increasingly compact without sacrificing significant performance. This development makes the LVF system capable of being integrated onto a smartphone platform. As shown in Figure 14, optical simulations and 3D renderings of such a platform were created through Zemax Optics Studio and Autodesk Inventor respectively.

Figure 14: Simulations and renderings were made to ensure the performance of the fabricated compact LVF system. An optical simulation utilizing Zemax Optics Studio is shown on the left. This shows an optical mount that is capable of the absorption and Surface-enhanced Raman spectroscopy (SERS). On the right is a rendering of the proposed compact LVF system utilizing Autodesk Inventor.

These designs were then fabricated through a 3D printer workshop at the Mechanical Engineering Laboratory at the University of Illinois at Urbana-Champaign. The fabricated design with the LVF integrated onto the CMOS image sensor is shown in Figure 15.
Since the bottom half of the holster houses the electrical board for the CMOS sensor, the area denoted by the dashed lines in Figure 15 shows the compactness of the LVF system for integrating onto a smartphone. There is a metal optical holster that fixes the incident source light and lenses in place. A microfluidic channel is then set above the CMOS image sensor at a designed height such that the fluorescence or absorption signal is illuminated uniformly and fully across the LVF. While this gap restricts the size of the LVF system, the design height as shown is only approximately 9 mm. Thus, even with a height restriction, the LVF system is still compact in size and capable of being integrated onto a smartphone platform.

Figure 15: Fabricated design of the compact LVF system is shown. For smartphone integration, the size of the LVF compact system is outlined by the dashed lines shown on the left picture. On the right, an aerial view of the compact LVF system showing one that is capable of colorimetric absorption measurements is shown.
4.2 Conclusion

A combination of a linear variable filter and a commercially available CMOS sensor is presented. This LVF system is shown to detect a concentration of 0.28 nM for quantum dot emitters and 32 ng/mL for near-infrared dyes. Furthermore, colorimetric absorption measurements were performed that demonstrate the comparable performance of the LVF system against a handheld spectrometer and optical spectrum analyzer. The LVF system showed better signal-to-noise ratio at a lower cost and providing better portability than the other two systems. A free-chlorine test was measured that demonstrated the LVF system’s capabilities for commonly used point-of-care applications. The design of this LVF system was iterated to make the system more compact, capable of being integrated onto a smartphone platform, and fabricated using a 3D printer.
References


