MOBILE APPLICATION
OF COLORIMETRIC SENSING

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Abstract

The current ways of finding a substance’s concentration in a solution are time-consuming, difficult to access, and quite expensive. In this project, we have begun to use ultrasensitive colorimetric plasmonic sensing technologies to develop a mobile-based colorimetric-sensing system that would allow a cost-effective method of concentration detection in any location with a working mobile device.

Ultimately, detections would only require only placing a drop of solution on the plasmonic sensor. By shining a high-intensity light through the semi-transparent sensor, the mobile device can capture the refraction color from the solution. By image analysis and interpolations, the system able to calculate the concentration of the substance within the solution. Above all, this research produces a high-yield, high-portability, and low-cost solution which is a suitable immediate application of ultrasensitive colorimetric plasmonic sensing technique.

Subject Keywords: colorimetric sensing; application; portable device; android
Acknowledgments

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1. Introduction

1.1 Motivation and Purpose

Current medical technologies are at a very advanced stage, many diseases and illnesses can simply be cured if patients can be correctly and promptly diagnosed. However, due to high cost and low portability, many advanced medical examination machines are limited to major hospitals, which limits their accessibility for the general public. Our research group is committed to conducting research and providing solutions to improve the quality of life of all mankind.

In previous research, PhD student Manas Gartia from our group has demonstrated that the ultrasensitive colorimetric plasmonic sensing technology can be used for medical examinations [1]. Currently we can only perform such experiments in a laboratory environment using expensive equipment such as spectrometers, microscopes, and high-resolution microscopic cameras. As a goal of this research, we are developing a portable colorimetric analyzing device based on current mobile technology and ultrasensitive colorimetric plasmonic sensing technology, which can be used in a variety of locations, under diverse conditions to produce highly accurate results. This portable device could be used in many industries, for example, it can be used during medical examination to perform DNA analysis or determine the concentration of glucose in blood samples. For use in environmental protections, this device can be used for tracking the concentration of hazardous substances in chemical wastes from manufacturers.
1.2 Backgrounds

1.2.1 Ultrasensitive colorimetric plasmonic sensing

The Ultrasensitive colorimetric plasmonic sensing technology is inspired by the Lycurgus Cup which was created by the Romans 2000 years ago. Previously, Manas Gartia has demonstrated that ultrasensitive colorimetric plasmonic sensing can produce large transmission and reflection wavelength shifts upon binding of molecules on the nanoscale Lycurgus Cup arrays (nanoLCA) device [1]. This technology enabled human to observe color changes directly by the naked eye and cameras of most mobile devices. Previous experiments have demonstrated that nanoLCA has a high sensitivity and part this project will reaffirm this statement.

1.2.2 Android Platform

According to the market shares research done by the International Data Corporation, The Android platform is currently dominating the market with a 68.8% share of mobile phones and 48.8% share of tablets [2, 3]. Android devices also have a broad price range, which allows users with the all incomes levels to purchase them. In additional, Android supports the manual focusing, constant ISO, and fixed white-balance during image taking starting at application programming interface (API) version 14 which is more convenient to the development and ensure the accuracy of experimental results. According to above reasons, this project was initially built based on the android platform.
1.3 Challenges and Approaches

Since the quality of the colorimetric sensing is highly dependable on the light source, one essential part of this project is finding a high-intensity light source that can evenly illuminate a 3 cm x 3 cm square area and has even intensity of light in all three channels.

The calculation of concentration after calibration requires the mobile device to perform interpolations based on calibration data to find a polynomial equation that can describe the trends of changes on pixel value related to the concentration changes. This requires us to find the right interpolation methods and to write highly efficient code that can allow any mobile device to perform such computation in limited time. The constrained cubic spline interpolation is the mathematical method we are currently using.

1.4 Results

We found a light array panel from Phoenix Image llc that behaves like a perfect, evenly illuminating light source after placing two layers of filters between light source and the sensor. Images captured using HTC ONE X mobile phone can clearly demonstrate the color changes according to the concentration change of solvents in the solution on the sensor. By using the android application, two experiments have been performed using HTC ONE X phone and NaCl solution with silver costing Ultrasensitive Colorimetric Plasmonic sensor. The results show that the accuracy of the sensors is close to ±6.3%. We also conduct experiments using low concentration solution of DNA1: (5'-thiolCAGCAAATGGGCTCGAC-3’) and DNA2: (3’-GTCGGAGCCATTTGCTG-5’-HEX) solutions to evaluate the sensitivity of the colorimetric sensor and HTC ONE X
phone’s camera, results draw from those experiments show the sensor is very sensitive in
detecting changes in concentration to as low as 1 Nano-molar.
2. Literature Review

Many researchers have been working on portable health diagnostic machines or mobile biosensors. This project provides another competitive solution in these research areas. The system that we are building in this project has many advantages compared to other designs, such as the following:

1. Lower cost to manufacture and maintain: the whole hardware design consists of only plastic and one LED light source.

2. Easier to operate: the final design of the phone application will guide the user throughout the whole operating process, which provides a more convenient way for users to perform their measurements.

3. Higher portability: since the hardware component of this design is relatively small, it can be easily carried to any location.

4. More convenience: the mobile application will store all the runtime data and calculate measurement results instantly at the end of the experiment, which can avoid miscalculations and many complex procedures.
3. Descriptions

3.1 Hardware Design

In the past six months, we had designed many setups using AutoCAD and completed the prototypes using a 3D printer. The current setup was built based on single high-intensity LED as the light source. The whole setup is built using black light isolating high-performance composite to reduce the effect of the external environmental conditions on the experimental results. In addition, black composite material can absorb a certain percentage of visible light, which can prevent light from reflecting in random directions inside the system. The setup contains three major pieces including mobile device holder (top tube), sample tube, and bottom stand.

On the top of the setup, there is a mobile device holder which keeps the device in its position when the users are performing measurements. In the middle, there is a 2 mm x 40 mm slot, which is used for inserting the testing sensor. Light source and power supply are placed inside the bottom stand to minimize the space. Since the focus distance of mobile device’s camera can vary, we had designed a retractable top piece.

Fig 1. Current hardware setup for the colorimetric sensing system. This setup uses a single LED light.
As in the image shown in Fig. 2, light source inside the base stand generates light that shines up through the diffuser and illuminates the sensing area of the colorimetric sensor. The mobile device on the holder will be able to capture a clear image of the sensor for future image processing.

Fig 2. Light source placed in the bottom, light shines through a diffuser, and illuminates the sensing area of the sensor.

In this setup, we are able to capture a clear image of the colorimetric sensor as shown in Fig. 3.

Fig 3. Image taken using the setup shown in Fig 2. Substrate we are using in this sample is silver coating nanoLCA. The yellow areas are covered by 5’-thiolCAGCAAAATGGGCTCCGAC-3’
3.2 Light Source

As an essential part of this colorimetric sensing setup, the requirements of light source are strict. The design requires light source to produce high-intensity light and be able to evenly illuminate a 3 cm x 3 cm square area. The light emitted from the source has varied wavelengths from 390 nm to 700 nm.

There are three main factors that will affect our results:

1. **Brightness**: light rays should be able shine through the substrate and semi-transparent solution on top of the substrate. The luminous intensity on the sensing area is within the dynamic range of the mobile devices’ camera.

2. **Evenness**: Light source should be able to evenly illuminate the sensing area of the substrate; the spectrum value detected by the mobile device can only have limited variations in all three channels across the whole sensing area.

3. **Orientation**: The light waves are oscillating in the direction that is perpendicular to the surface of the substrate. Any light wave with different orientation will result in an impact on the final calculation.

In order to get the best results, we have tested many different designs of light sources, such as the ones in Fig. 4. A high-intensity light source (OPT – FLC5050) made by OPT Machine Vision Tech Co., Ltd. is used in the current design as our light source. The illuminant of OPT – FLC5050 are high density LED strips that were placed at inter-edges of its shell [4-5]. Light generated by those LED strips propagates down the light-guide plate by total internal reflection. The light-guide plate has a scattering coating on the bottom of the plate. When light hits the scattering coating, some will emerge out of
the front. Those light will then pass through one additional layer of diffuser and emit a uniform light at the top of the device.

Fig 4. The left column shows the images of the actual devices. The middle column shows the illuminating pattern on a 5 cm x 5 cm square paper. The right column shows average intensity of the illuminating area.

From the graph of average intensity of the illuminating area, we can conclude that the OPT – FLC5050 can illuminate a larger areas with smoother and more uniform pattern.
In addition to the OPT – FLC5050 light source, we added two additional layers of filter to increase the quality of our lighting system. As shown in Fig. 5, on top of the OPT – FLC5050 is a layer of diffuser. The polarizer on top the diffuser will fix the orientation of the light.

![Light source design for current setup](image)

Fig 5. Light source design for our current setup. LED array is using opt-flc5050 from OPT machine vision.

3.3 Application Programming

3.3.1 Development and testing environment

The Android application is developed in Java using Java 1.6 JDE and Android 2.1/4.1 SDK in Eclipse under the Windows 7 environment. The application was tested on the Android Virtual Device, HTC ONE X with Android 4.1 Jelly Bean, and HTC DROID with Android 2.1 Éclair. This application also includes the Efficient Java Matrix Library (EJML), which is a Google open source library. Currently the application only
has the functionality to determine the concentration of a solution if solution type is known. More functionality will be added during future work.

3.3.2 Flow chart of the application

![Software function flow chart]

Fig 6. Software function flow chart
3.3.2 Detailed explanations of individual activities and classes in the Android application package

Main

This activity will display a logo of the Mobofoto for 2 seconds, and during this time the application will ignore all users’ inputs and load all necessary functions in the background. Before this activity is terminated, it will call the Listing activity.

Listing

This activity allows user to select the type of solution they are willing to perform this experiment on. Based on users’ selections this activity will set the solution parameters and call the Calibration activity.

Calibration

After selecting the solution, we will ask the user to perform calibration due to the possible changes of testing environment. Currently Calibration activity will allow user to select image sources by calling android default camera (MediaStore) or gallery CreateChooser() activities. The return URI will be capture by onActivityResult() function and pass to Select activity using Intent class. On the return of the Select activity, this activity will store the average spectrum values and the concentration of the solution into a calibration data array and display it for user references. When the user finished the calibration, the function will call the GetConcentration activity and pass the calibration data array to it.
Select

Select activity will retrieve the URI passed caller activity and load the image based on that URI to an ImageView using onDraw() function in the Drawingtheball class. The application will require the user to select the area of solution on that image and be reported to the average spectrum values back to the Calibration Activity.

Drawingtheball

This class extends the View class in the Android SDK and this class is called when the user wants to examine the average spectrum values of an area on the image. The algorithm used in this class is described in the 3.4 algorithm section below.

GetConcentration

After calibration, the application is ready to help user determine the concentration of the solution. GetConcentration will call the Select activity to sample the image and use functions in Interpolation class to calculate the concentration value.

Interpolation

This class contains interpolation algorithm functions which are described in detail in Section 3.4.
3.4 Algorithm

3.4.1 Image Analysis

Figure 7 is an example image of silver coated sensor with droplets of DNA 1 solution on top, which was captured using HTC ONE X.

![Silver coated sensor with DNA 1 solution](image)

Fig 7. Silver coated sensor with DNA 1 solution

Then the application will ask the user to select the sampling area. There are two ways to select it: circling or auto selecting.

In circling the sample area method, the user will need to place the origin \((x_0, y_0)\) of the circle by tapping the on the ImageView. Then user can use the slide-bar to adjust the radius of the circular selected area. The average RGB function is calculated using following logic.
**Binmap img**: Int pixel; // img is the sampling image

```java
For x = ((y0 - radius) < 0) ? 0: x0 - radius; x < x0 + radius && x > x0 - radius ; y++
For y = ((y0 - radius) < 0) ? 0: y0 - radius; y < y0 + radius && y > y0 - radius ; y++
If (√(x - x0)² + (y - y0)² < radius ) {
    Int pixel = img.getPixel(x,y);
    Separate the pixel value to RBG integers and sum up all RGB value in selected area base on its channel;
}
Divide the sum of spectrum values with number of pixel within the selected area; we will receive the average RGB value.
```

In the auto select method, the user will need to place the origin \((x_0, y_0)\) by tapping the on the ImageView. And the function will retrieve the pixel \((pixel_0)\) value of location \((x_0, y_0)\) on the image. We will also allow the user to select the sensitivity (sensitivity is measured by the percentage of similarity of the two pixel values) in the auto select function. The average RGB function is calculated using following logic.

```java
Binmap img;
Int[][] mask = new int[img.width][ img.height]; // and initialize all value to 0
Int[] RGB = new int[3]; //initialize to 0
Int counter = 0;
Function SUM_RGB (x,y){
    If mask[x][y] !=0
        Return;
    mask[x][y] = 1;
    If int pixel = img.getPixel(x,y) is similar to pixel_0 ( within the sensitivity range) {
        Separate the pixel value to RBG integers and add them to the RGB array base on channels;
        Call average_RGB(x-1,y); average_RGB(x,y+1); average_RGB(x,y-1);
        average_RGB(x+1,y);
        Counter++;
    }
}
```

After calling the SUM_RGB \((x_0, y_0)\) function, the program will get a sum of each of the channel values. The program will retrieve an average by simply dividing RGB by the counter.
3.4.2 Interpolations

Before calculating the concentration of solution, it is required to come up with a mathematic equation that characterizes the relationship between the concentration percentage and the RGB value base on the calibration data. Currently we are only using the red channel value to perform interpolation.

As shown in Table 1, we can observe a clear decrease of red spectrum value as concentration decreases. As shown in Graph 1, the relationship between concentration and red spectrum value is similar to an inverse log function.

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Red</th>
<th>Green</th>
<th>Blue</th>
</tr>
</thead>
<tbody>
<tr>
<td>15%</td>
<td>119</td>
<td>106</td>
<td>111</td>
</tr>
<tr>
<td>10%</td>
<td>115</td>
<td>105</td>
<td>109</td>
</tr>
<tr>
<td>5%</td>
<td>111</td>
<td>108</td>
<td>118</td>
</tr>
<tr>
<td>1%</td>
<td>72</td>
<td>89</td>
<td>126</td>
</tr>
</tbody>
</table>

Table 1. Calibration of the data from NaCl solution

Graph 1. The relationship between concentration and red channel value
Currently the application uses the constrained cubic spline interpolation [6] to characterize the relationship between the concentration and pixel value. The algorithm is shown below:

Given a set of n data points \((x_k, y_k)\) \((1 < k < n)\) and the set is increasing on the order of \(x_i\). We separate the set to n-3 segments; it can be represented by following equation.

\[
p_i(x) = a_i + b_i x + c_i x^2 + d_i x^3 \quad (i \in [1, n-3])
\]

We need to solve for \((a_i, b_i, c_i, d_i)\) using matrix by plugging in \((x_k, y_k)\) \((1 < k < n)\).

\[
\begin{bmatrix}
1 & x_i & x_i^2 & x_i^3 \\
1 & x_{i+1} & x_{i+1}^2 & x_{i+1}^3 \\
1 & x_{i+2} & x_{i+2}^2 & x_{i+2}^3 \\
1 & x_{i+3} & x_{i+3}^2 & x_{i+3}^3
\end{bmatrix}
\begin{bmatrix}
a_i \\
b_i \\
c_i \\
d_i
\end{bmatrix}
= 
\begin{bmatrix}
y_i \\
y_{i+1} \\
y_{i+2} \\
y_{i+3}
\end{bmatrix}
\]

The above matrix can be representing as:

\[
MN = Q
\]

Dividing both sides by M is equivalent to taking the inverse of the left matrix and move it the right.

\[
N = QM^{-1} \quad \text{where} \quad N =
\begin{bmatrix}
a_i \\
b_i \\
c_i \\
d_i
\end{bmatrix}
\]

In our application we treat x as the spectrum value and y as the solution concentration. By plugging in the calibration data to the equation shown above, we can
get $p_i(x)$ $(1 < i < n-3)$. We use the following function logic to decide which $p_i(x)$ function we are going to use in measuring the unknown concentration.

```java
For (int i = 0; i < n-3; i++)
    If $x_i < x_0 < x_{i+3}$ // where $x_0$ is the average spectrum value image of the unknown concentration solution.
    {  
        Concentration = $p_i(x)$;
        Return;
    }
```
4. Research Results

4.4 NaCl Solution Testing

To prove the concept, I performed one set of experiments using NaCl solution. First, I diluted 15% NaCl to form 10%, 8%, 5% and 1% NaCl solutions. I used 15%, 10%, 5% and 1% solutions for the calibration setup and receive the calibration data (Table 1). Then I used 8% solution as unknown concentration solution to verify our results. After calculation, the application estimates the solution concentration to be 8.54%. Compare the result 8.54% to the actual concentration, the error of the result is close to 6.75%. Hence, our results support that our setup can be used to determine the concentration of known solution.

4.5 DNA Solution Testing

The purpose of DNA solution experiment was to test the sensitivity of our colorimetric sensor. I used 3 types of DNA solutions during our experiment. To test the sensitivity of the solution, I diluted them by factor of 10, 100, and 1000. On silver coated sensor, the lowest concentration our phone can detected is 100 pmol. As an example in Table 2, we used DNA 2 (3’-GTCGGAGCCCATTTGCTG-5’-HEX) solutions to demonstrate that the pixel value changes according to concentration down to 100 pmol.

<table>
<thead>
<tr>
<th>Concentration:</th>
<th>Red:</th>
<th>Green:</th>
<th>Blue:</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 nmol</td>
<td>190</td>
<td>180</td>
<td>93</td>
</tr>
<tr>
<td>10 nmol</td>
<td>155</td>
<td>146</td>
<td>80</td>
</tr>
<tr>
<td>1 nmol</td>
<td>136</td>
<td>121</td>
<td>85</td>
</tr>
<tr>
<td>100 pmol</td>
<td>131</td>
<td>131</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 2. Calibration of the data from NaCl solution


Our results as of now are preliminary, we are still working to increase the scale of the testing samples and to provide more solid evidence.
5. Future work

This current project is still ongoing; there are many aspects that we are improving upon, such as more compacted hardware, a better interpolation algorithm, and a user interface.
6. Conclusion

The aforementioned results demonstrate our contribution toward developing a portable colorimetric sensing device and toward supporting the feasibility and sensitivity of the setup. During the process of designing and constructing the hardware setup, we have tested various types of light sources. The one we are currently using, shown in Fig. 4, offers the best resolution. The results we have obtained using the current setup and mobile application is the basis for future commercialized development and application.
References


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