Multispectral 2D Imaging for Oxygen Saturation in Tumor Detection
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Specific Aims

Cancer is a long-lasting problem that the world is facing for decades.[1] Given the fact that tissues near tumor cells generally have greater vascularization and lower blood oxygenation, it is possible to detect tumor/cancer by measuring the change of oxygen saturation (SO_2). using Near-infrared (NIR) spectroscopic imaging technology[2]. SO_2 data can be collected based on the characteristic that oxygenated and deoxygenated hemoglobin exhibit distinctive reflection patterns of light in both near-infrared wavelength and visible red wavelength[3]. Well-established applications of medical devices like pulse oximeters can perform accurate point measurement of oxygen saturation in arterial blood (SaO_2) noninvasively. However, point measurement is limited due to its invisibility of SO_2 distribution over a whole area of biological tissue, which cannot offer an intuitive comparison between cancerous and healthy tissues. Thus, there is a critical need for 2-D measurement that involves imaging of suspicious superficial tumor tissues in the clinical situation.

The goal of this project is to design an optimized method to perform two-dimensional SO_2 mapping over a targeted superficial human tissue by collecting image data under NIR spectroscopic versus images under other wavelengths and being able to distinguish the tumor tissue with normal tissue. Our objective is to acquire an intensity distribution mapping of light reflected from phantoms that are used to mimic healthy/tumor tissues in different wavelengths. Our expected outcome is that the 2D SO_2 imaging technology is capable of producing high-resolution images of targeted tissues based on the difference in vascularization. The hypothesis is based on the existing technology of one-point SO_2 measurement and current papers indicating the potential of creating two-dimensional mapping with the fact that tumor hypoxia indicates its progression[4,6,7,8].Successful design and construction will provide a prototypical instrument for medical biopsy in cancerous disease that is capable of future commercialization. We propose the following aims:

Aim 1: Design and construct a two-dimensional imaging system for SO_2 mapping

Based on the current technology and our hypothesis, a two-dimensional imaging system for SO_2 mapping shall be designed and constructed. The imaging system will consist of a scanner with light emitters and light detectors. Light emitter matrix should be able to emit NIR light favored around 940nm wavelength as well as light emitters of other wavelengths, with embedded system control it should be able to switch quickly between light sources and take multiple pictures at a time. When taking pictures, the light detectors (camera) receive the signals after photons hit the analog-to-digital units, these light signals will be sent as pixel intensity to the back end for further processing.

Aim 2: Analyze collected data and implement algorithms to process 2D imaging and visualize SO_2

With the data collected from the probes, multiple two-dimensional images should be able to be constructed with algorithms. These images indicate the light intensity processed by detectors in different wavelengths. Comparing the images constructed under NIR spectrum with images constructed under non-NIR spectrum, an algorithm should be determined to process these images and be able to map the SO_2 of the targeted tissue. The finalized image should be able to be visualized in the front end with a user-friendly Graphical User Interface (GUI). The GUI should also be capable of receiving commands from users and be able to send the command to the probe.

Aim 3: **Design calibrated phantoms that mimic optical properties of tissue**

Phantoms shall be designed to calibrate the system and to test the veracity of proceeded results. Multiple phantoms should be designed to mimic the optical properties of the tissue. They should include (1) homogeneous phantom mimicking normal tissue with normal SO_2. (2) homogeneous phantom mimicking tumor tissue with minimal saturation. (3) heterogeneous phantom with different extent of 'tumor' material mimicking tumor with different progression.

Aim 4: Validate the device and algorithms on phantoms

The final prototyped device with embedded system and associated algorithm should show good performance when validated with phantoms produced. After our design and construction, we expect that the 2-D imaging system will provide a prototypical instrument for medical biopsy as well as further develop a commercialized product for cancer early detection. It is expected to have a progressive impact in the medical field since existing point measurement is limited due to its invisibility of SO2 distribution over a whole area of biological tissue. We are confident to apply our knowledge and lab experience in biomedical engineering learning to accomplish this task.

Research/Design Strategy

A. Significance

As one of the most common leading causes of death in the world, cancer leads around 10 million deaths worldwide in 2020, with breast cancer as the most common cancer type that has 2.26 million reported cases from WHO.[9,2] Cancer detection is crucial and important. The high growth rate and rapid deterioration of cancer make it essential to be able to be detected and located at an early stage to perform intervention treatments. In general, early diagnosed cancer has a greater opportunity for recovery[10]. Some cancer diagnostic technologies such as endoscopy are invasive[11], and traditional diagnostic methods like whole-body CT and MRI have disadvantages including radiation exposure, high cost, non-routine basis, and potentially low yield in detecting significant abnormalities in these patients[5,13]. As such, any more effective and economic early detection innovation is lifesaving.

Oxygen saturation measurement provides a good way of visualizing suspicious cancerous body tissues[12,14]. Point measurement of SO_2 level like pulse oximetry is well-researched in previous studies, which makes use of photoplethysmographic pulses in the red and infrared regions and utilizes the different spectra of light absorption by oxygenated and deoxygenated hemoglobin[6,7,8,9]. The benefit of this application is that it is noninvasive, without radiation exposure, and more economic, while the disadvantage is apparent, in that it doesn't increase the accuracy of cancer detection, measured SO_2 levels can differentiate by a large degree from the actual value due to light scattering effect[6,16,17]. In recent studies, a 2-D optical device that provides a two-dimensional mapping of HbT and SO_2 in human tissue can reach as much as 92% diagnostic sensitivity and 67% specificity in detecting ductal carcinoma[2]. This supports that 2-D imaging for SO2 in biological Tissues may also improve current detection accuracy in other types of cancerous tissues, for instance, if 2-D imaging for SO2 can be combined with invasive detection methods like endoscopy[14,18]. Furthermore, the 2-D optical device has a potential commercial value since its portability compared to the traditional radiation detection method allows it to reach out to non-governmental organizations and markets with low-resource medical settings[15].

B. Innovation

Current point measurement like pulse oximetry used to measure SO_2 level of a tissue is common in clinical use[6,7,8,9]. However, the point measurement only accounts for an estimation of overall arterial blood SO_2, while few efforts are made in observing the distribution of SO_2 level over an area of biological tissue. Thus, 2-D mapping in our project can challenge current clinical practice paradigms by allowing clinicians to record and visually observe the abnormalities in cancer-risky regions that traditional pulse oximeters cannot. Moreover, this could give a more convenient and prompt way of cancer screening in health examinations even compared to CT scanning and MRI.

<u>Our project is innovative due to the facts that</u>:

(1) We are making use of 2-D mapping technology that is commonly used in biomedical measurement theories but rarely applied in circumstances like tumor tissue detection. This means we put the theory into practice.

(2) We compile algorithms for the image processing, acquiring a 2-D mapping for oxygen saturation distribution instead of a waveform or a prompt of oxygen saturation rate as pulse oximeters do. The algorithms also allow us to modify and update the system of 2-D mapping analysis easily in the future.

(3) The scanning device can take multiple images with different wavelengths in a short scope of time (within ten seconds), so that it enables us to analyze data quickly. This is because we will have multiple light sources on the scanning device that allows us to switch between colors quickly.

The project also combines optical imaging techniques with phantoms techniques. With self-designed phantoms, the system can be furthermore calibrated and will be capable of producing better results[5]. The phantoms can also be used to examine the functionality of the system to ensure the correct output. With such validation mechanisms, the accuracy of the system can be highly manipulated, providing a credible baseline resource for future analysis.

C. Approach
C. 1 Preliminary Data

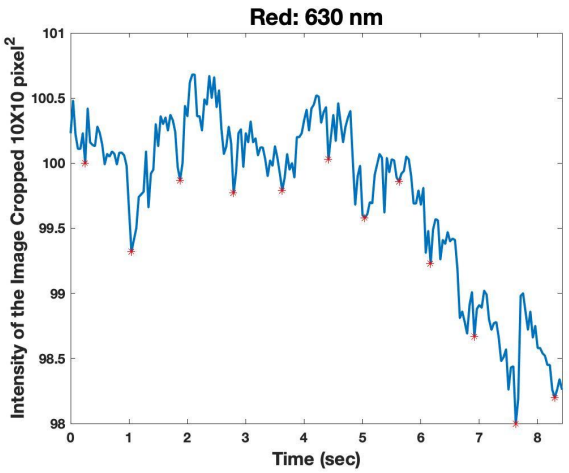


Figure 1-Light intensity plot of the sample with the red light of wavelength 630 nm. using the reflection approach.

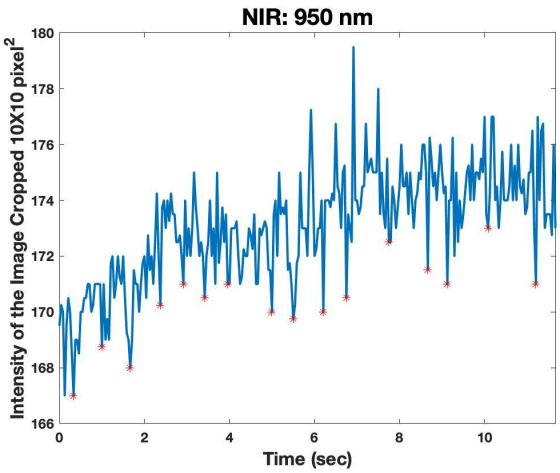


Figure 2-Light intensity plot of the sample with the NIR light of wavelength 950 nm. using the reflection approach.



Figure 3-The sample under the NIR light with the NIR light of wavelength 950 nm. The area of the figure is 5.5 cm ×5.5 cm.

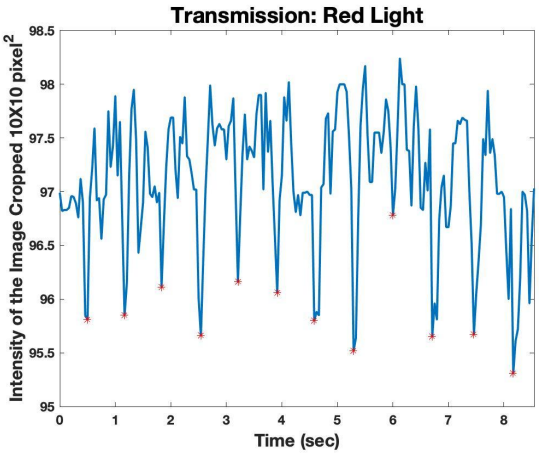


Figure 4-Light intensity plot of the sample with the red light of wavelength 630 nm using the transmission approach.

The first step of our final design was to develop algorithms that analyze the collected data and result in a plot that resembles the heart rate (HR), since the SO2 mapping utilizes the different absorptions of red and NIR lights for each deoxygenated and oxygenated hemoglobin. The video data was taken in a completely dark environment with the camera and the sample, which was the finger, to be stationary during a 10-second period. The primary approach of data collection was the reflection approach, which we shined the light on the finger at the 45° angle and video-recorded the intensity of the reflected light. The wavelengths of lights of our interest were 630 nm, 690 nm, 920 nm, and 950 nm, the first two of which were for the red light and the latter were for the NIR light. In the case of failure of such approach, the alternative was the transmission approach, which the finger was directly in contact with the light and then recorded for the intensity of light that was transmitted through the finger. After collection, it first was taken into a preprocessing code in MATLAB that allowed us to crop the region of interest in an area of 10×10 in pixels. Finally, a processing code processed the selected region and outputted a plot of light intensity based on the light captured in the video; by calculating the difference between two neighboring local minima, the code presented a frequency statistic and eventually the HR of the person whose data was collected.The first set of data was collected via the reflection approach. Our MATLAB codes produced plots as shown in Figure 1 and Figure 2. From both figures, the oscillatory patterns

were observed, which were consistent with the HR oscillation, and we successfully located local minima; the computed HR was 84.32 beats per minute (bpm), which was reasonable and consistent with a normal person's HR. However, the plots had the noise intertwined with the signal, which might be caused by the low-resolution video produced by our PiCamera, as presented in Figure 3. This indicated that we will need to implement a filter to remove the noise. The alternative way that can also improve the performance is to take multiple sets of data for each wavelength of light with the same sample and further take the average of intensity.

Due to the noise presence in Figure 2 and Figure 3, we then attempted the transmission approach and generated the plot in Figure 4. As a result, the plot appeared to have less noise and easier to locate the local minima. The resulting HR analyzed from the plot was 84.60 bpm; the number of pulses existing in the plot is aligned with the expectation.

From the video data collected, both approaches were successfully processed by our MATLAB based on the preliminary plots and the analyzed HR in each case aligned with our expectation. From this stage, we will be able to refine our results via utilizing a filter to remove the noise or taking multiple shots to have an average of light intensity at each frame.

C. 2 Specific Aims

Objective: Design an optimized method to perform two-dimensional SO_2 mapping over a targeted superficial human tissue by collecting image data.

***Specific Aim 1*: Design and construct 2D imaging system for oxygen saturation mapping**

Research/Design Strategy:

The designing and construction of a 2D imaging system requires a series of steps to sequentially build and test the device.

Aim *1.1.* Demonstrate the ability to capture images that can be analyzed. Initial testing is needed to prove the concept that proper images can be captured using given materials and devices. The goal of this stage is to successfully analyze heart rate from the video captured using different methods of fingertips. The experiment test included a camera, a camera holder that fixes the camera, and LEDs in different wavelengths (630 nm, 690nm, 950nm). Video data are gained through different methods as mentioned in the preliminary data part.

Aim *1.2.* Prototyping of Devices that Fix the Camera and LEDs. From the experiment in preliminary data, the vibrations of the camera will produce noise that significantly affects the experiment result. There are two ways to resolve this problem, one is to apply algorithms when analyzing the data to enable movie stabilization through the usage of cross-correlation for image registration; another is through fixing the camera at the initial stage to eradicate the possible jittering produced by muscle trembling if holding by hand. It is essential to prototype structures that act as a stand for camera and LEDs that can hold the elements and are capable of necessary adjusting. Moreover, the fixation provides convenience with later clinical use. The goal at this stage is to engineer a stand that has the flexibility to adjust the camera position and sample position. One possible design is to fix the camera at a certain height and use fiber bundles to send signals to the camera. The fiber bundles' height can be adjusted to leave enough space for sample placing. A sample design is shown in figure 6. Several additional sample drawings will be designed and visualized using CAD software such as SolidWorks. Depending on the shape of the final design and the complexity, it will either be sent to perform a 3D printing or be produced using traditional subtractive machining such as CNC Milling and Lathe. The possible materials for the stand include ABS if choosing 3D printing, metals and acrylics if choosing traditional matching. 303 Stainless Steel and 1018 Steel are all possible choices for metals.



Figure 6- Sample CAD file of proposed stand

Aim *1.3.* Prototyping of Devices that Enable Frequent Switching of LEDs. Another feature that should be included in the design is the ability to switch LEDs in a short time manner. Due to the mechanism of analyzing

oxygen saturation, we need to obtain data under the illumination of LEDs with different wavelengths. The ideal chosen wavelengths should be located when the absorption coefficient of saturated blood and unsaturated blood have a clear difference. According to Figure (7) [26], one clear difference appears at the range of 600-800nm, corresponding to red light. Another significant shift appears between 900-1200nm, located in the near-infrared light (NIR) region. Based on the theory, if applicable data wants to be archived, the wavelength should be identified between those two regions (600-800nm and 900-1200nm). In theory, four wavelengths of LED will be chosen, two in 600-800nm and two in 900-1200nm. To prevent manually loading, those LEDs are expected to perform an automatic quick switch in a 10s-time frame. This is going to be archived by scripting in raspberry pi.

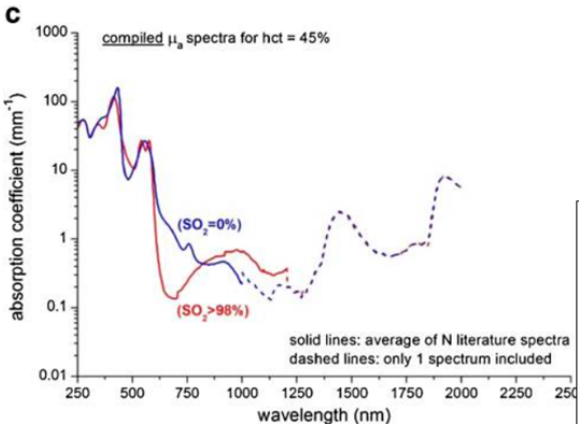


Figure 7 - compiled μ_a spectra for whole blood with a hematocrit of 45 %

Aim *1.4.* Integrating device to ensure its functionality. The previous design stages build a solid baseline for the actual implementation of the device. With the knowledge and experience from the previous stages, an integrated design will be prototyped with careful material selection and well-designed finish. The mechanism of the proposed design is shown as a glass box in figure 8.

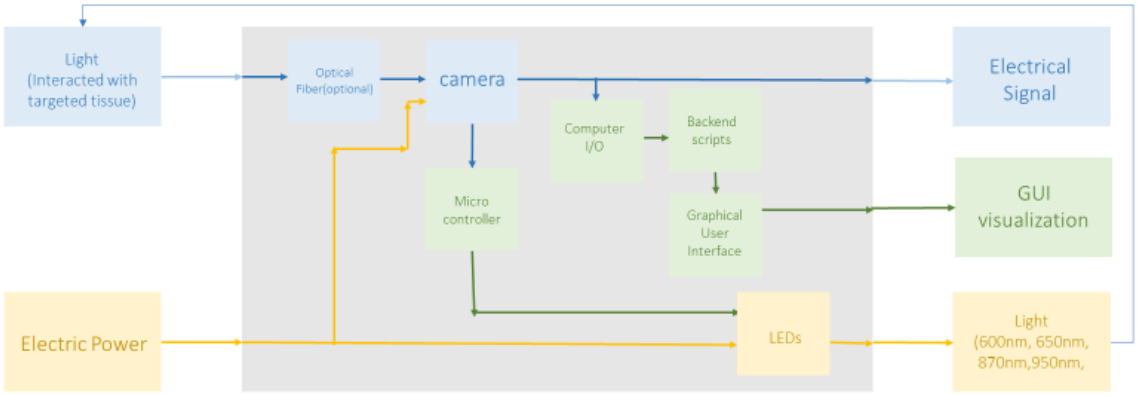


Figure 8-Glass Box Illustration of Proposed Design.Different color indicate different pathways.

Potential Problems and Alternative Approaches:

One possible problem in the designing process is the noise. Several approaches have been implemented to reduce noise. One major source of noise is the environmental light that disturbs measurements. This limitation is resolved in theory test experiments by performing the experiment under an entirely dark environment. However, in clinical cases it is hard and inconvenient to achieve this effect. One way is to indicate the device can only be performed in a darkroom. Another possible solution to this problem is to design light resistant cases covering the device to prevent light interacting with the built-in camera. Another possible problem in this designing stage is that there are other molecules in capillaries and superficial tissue that may also absorb light in similar wavelengths. One example is water. To avoid this problem, we have to carefully search for all the possible factors that may be resulting in data interrupting and collect the absorbance in the selected LED wavelengths. The final selected LED wavelength should not be in an ambiguous range with other materials. What's more, other techniques such as phase shift should also be developed or employed to avoid further interference.

Expected Outcome

The expected outcome of this stage is to design a device that is capable of sending clear signals through the I/O channel to the computer for future processing. What's more, the device should be able to

capture pictures automatically while changing LEDs quickly in a short time. The prototype will then be integrated into a functional and portable device that is user-friendly and clinical practicable.

***Specific Aim 2*: Analyze collected data and implement algorithms to process 2D imaging and visualize SO_2**

Research/Design Strategy
The objective of this aim is to create MATLAB codes to process the collected video data and further produce a light intensity plot and the mapping of oxygen saturation of the region of interest.

Aim *2.1.* Produce light intensity plots consistent with the HR pattern. The collected video data will be processed through two sets of MATLAB codes, one of which is a preprocessing code that allows us to crop the image into an area of 10×10 in pixels and the other obtains the color data from that area. The success will be a light intensity plot that resembles the pattern of HR, which allows further analysis on the frequency of the plot and eventually the HR of the person, and otherwise will be the failure and requires revisions.

Aim *2.2*. Construct Graphical User Interface (GUI) and output a visualization of SO_2. A Graphical User Interface will be constructed so that users can send commands and perform necessary actions. When the user gives a prompt, several series of images will be taken and sent to process. The image records the intensity of the pixels, from the theory Beer–Lambert law's Law[7], Absorbance can be calculated from intensity. With multiple images from different wavelengths, a spectral matrix can be generated. With further calculation, values will be assigned to each pixel. The code will plot the SO_2 mapping in the xy-coordinate system to represent the positions along with a colorbar that shows the SO_2 distribution over the tissue of interest that permits the further tumor detection.

Potential Problems and Alternative Approaches
A potential problem for Aim 2 overall is that MATLAB codes are not able to produce a very clean light intensity plot and clear SO_2 mapping. One possible solution is that we will process the data through ImageJ or Python codes since the camera we will be using is PiCamera.

Expected Outcomes
The expected outcome is that the intensity plot will further be used to count the number of pulses and calculate the HR of the person who contributes the sample that is reasonable for a normal person, and the visualization of SO_2 presents spatial positions on xy-axes and a z-axis shown as color bar to show the saturation level that allows us to detect which region has a relatively low level of oxygen for the application of tumor detection.

***Specific Aim 3*: Design calibrated phantoms that mimic optical properties of tissue**
Research/Design Strategy
The goal in this aim is to produce phantoms that can show similar optical properties as of human body tissue. Materials will be chosen for the best performance of the phantoms. Moreover, we will adopt an existing phantom design idea from an experiment that involves gel-based phantom.

Aim *3.1.* Experiment with optical materials that simulate the healthy/tumor tissue. To construct an optical phantom that functions as real human body tissue, there are several concerns about materials used in the approach. First, the scattering property of tissue is the major contributor to the non-transparency of tissue, which we want to address in the design of the phantom. Polystyrene microspheres may act as good scatterers due to their molecular stability. Their sizes and index of refraction are relatively uniform, making the control and prediction of scattering effect easier. More importantly, another major property we will look at is the absorption, in which hemoglobin has different levels of absorption to various wavelengths of light. A stock of purified human hemoglobin solution will be applied to the phantom to perform this property.

Mixing hemoglobin solution and polystyrene microspheres allow us to combine the scattering and absorption properties together, while the problem is that microspheres have a larger density. This makes a liquid-based phantom approach invalid in our design since all microspheres will eventually sink to the bottom of the solution

and thus fail to uniformly exhibit the scattering property of a tissue. A better approach, adopted from Du Le et al.'s experiment [24], is to make a gel-based phantom utilizing agarose, which has a 100% transmittance and high gel strength. The gel solution will be prepared by mixing Agar in distilled water at 65°C for 2 hours to allow the Agar to be fully dissolved. The concentrated Agar solution was then added to the mixture of hemoglobin and microsphere, which was preheated at the same temperature for 30 seconds. Finally, to vary the SO_2 level in certain areas of the phantom and to generate the effect of oxygen consumption in tumor tissue, a yeast solution will be transported to the prepared gel solution with a pipette to prevent the generation of bubbles on the gel surface. The yeast solution will not be mixed uniformly with other solutions in the gel. A distinction between the section with yeast solution and the part without yeast separately will mimic the optical properties of healthy/tumor tissue. Multiple phantoms with different yeast concentrations will also be produced following the above methods, which helps us for further tests of the effectiveness of our device in identifying tumor regions.

Potential Problems and Alternative Approaches

A possible problem is that the experiment requires a delicate control of the temperature. If the solution gets overheated, the hemoglobin may begin to unfold and thus the concentration of that in phantom may become lower than expected. We can have control of the temperature, but if the proposed solution does not work well, we can use epoxy resin instead. This is because epoxy resin has a more robust mechanical property and good transmittance of light[25].

Expected Outcomes

The experiment is expected to provide us with an optical phantom that has very close absorption and scattering effect as of real human tissue, which will provide us a > 95% oxygen saturation (SO_2) in normal tissue region. For regions that simulate tumor tissue, it is expected to have a significantly lower oxygen saturation level (e.g., around 60%).

***Specific Aim 4*: validate the device and algorithms on phantoms**

Research/Design Strategy

Aim *4.1.* Testify the device and algorithms on the phantoms. Run the algorithms to start the camera, LED light and place a phantom on the testing stand. For each of the 4 LED wavelengths, the incident light will shine on the tested phantom from a 45-degree angle for 10 seconds and quickly switch to the next wavelength. The reflected light will be recorded by the camera from the top of the phantom. At the same time, the algorithms should be able to loop through the image and output the intensity of oxygen saturation for each pixel in terms of gray scale. Repeat the procedure for multiple phantoms with different yeast (tumor) concentrations. Identify the location the grayscale image has the lowest intensity. If the locations of low intensity are coherent with the locations of simulated tumor tissue on the phantoms, then our validation is successful.

Potential Problems

We do not think that there will be significant problems in this aim. If the device and algorithms are properly designed and constructed, it is unlikely that the examination will have unexpected results. It is possible that phantoms need calibration to find the best region of interest, while this does not cause problems to the experiment.

Expected Outcomes

The light reflection can be analyzed by the algorithms. In addition, all measurements will be able to correctly identify the regions of tumor and healthy tissues for various optical phantoms.

Timeline

The entire project takes one year. Detailed timeline during the 8 month period is proposed as below: (1)The first two months involve literacy research to gain background knowledge, during this two months we are also performing initial theoretical tests to justify our theorems.(2) The next two months involve initial prototyping of the device (3) In Month 5-7, algorithm is further developed and phantoms should be engineered to be prepared for the calibration (4)The last month involves the calibration and test of the system using phantoms.

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