Implementing Self-Calibration in an LED-Based Hyperspectral System

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Goal

The aim of this project is to develop a hyperspectral imaging system capable of distinguishing subtle differences between biological tissues. The target application is real time intra-surgical tissue discrimination, for example during prostate cancer surgery. However, precise calibration and semi-automation of the system is necessary for optimal image acquisition before moving to animal models. For this purpose we develop an automated algorithm for calibrating image exposure during the experiment to achieve enhanced information.

Setup

Light interacts with tissues via reflectance, transmittance, and absorption. This hyperspectral system measures the amount of light reflected from the surface (Fig 1c). The light source is a LED panel containing 21 LEDs with varying wavelengths, ranging from 365 to 1050 nm, which includes UV, visible light, and IR (Fig 1b and 2). The LED panel is surrounded by a scattering aluminum foil structure, that serves as wavelength mixer, producing a uniform field of illumination (Fig 3).

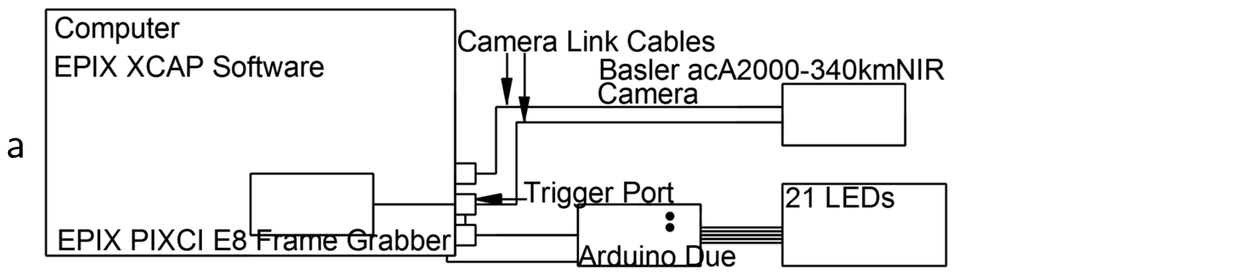

Fig 1: Schematic diagram of the (a) Setup, (b) LED panel, and (c) Light interaction

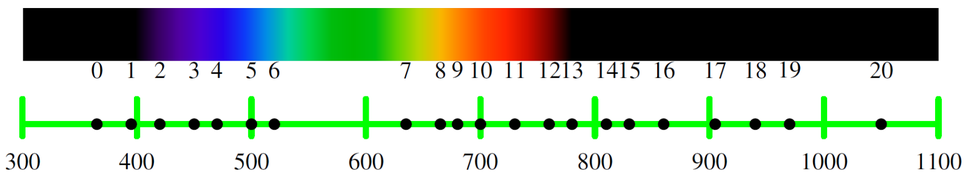

Fig 2: Electromagnetic spectrum of the 21 LEDs

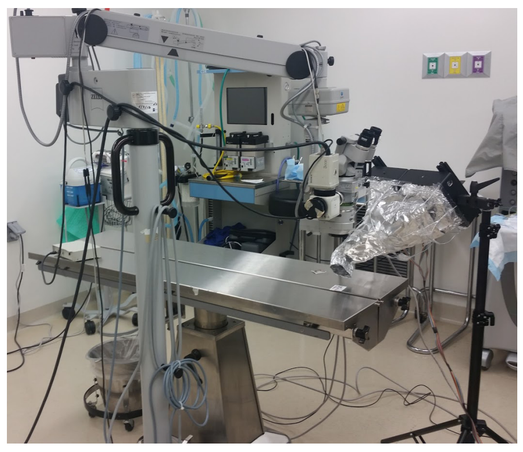

Fig 3: LED structure (camera attached to surgical microscope)

Calibration

The initial exposure times (x_0) for each of the 21 LEDs were found by imaging samples of exposed chicken muscle (Fig 4 and 5).

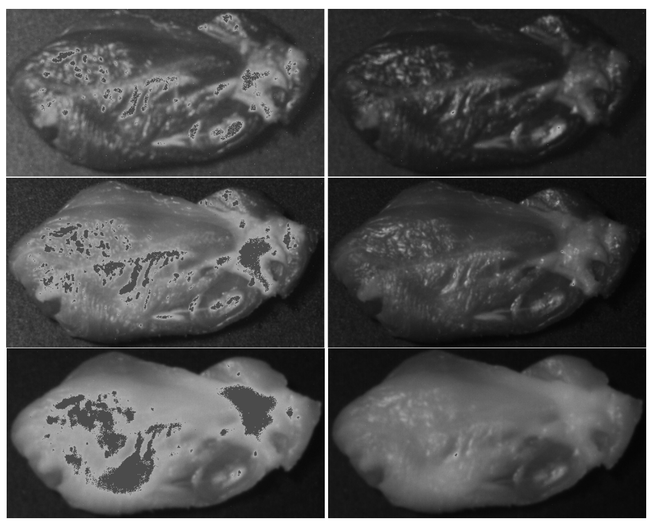

Fig 4: Comparison of saturation by showing y as red for LED #1 (395 nm), LED #4 (470 nm), and LED #17 (860 nm)

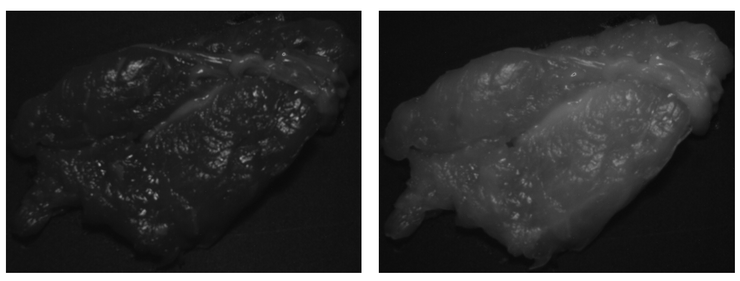

Fig 5: Images using LED #2 (420 nm) and #9 (665 nm)

We characterize each of the 21 LEDs by creating a saturation curve. This curve is obtained by plotting the number of pixel values (y) as a function of different exposure times (x).

The plot was then fitted to a logistic sigmoid curve using the Levenberg-Marquardt algorithm of non-linear least squares curve fitting (Fig 6).

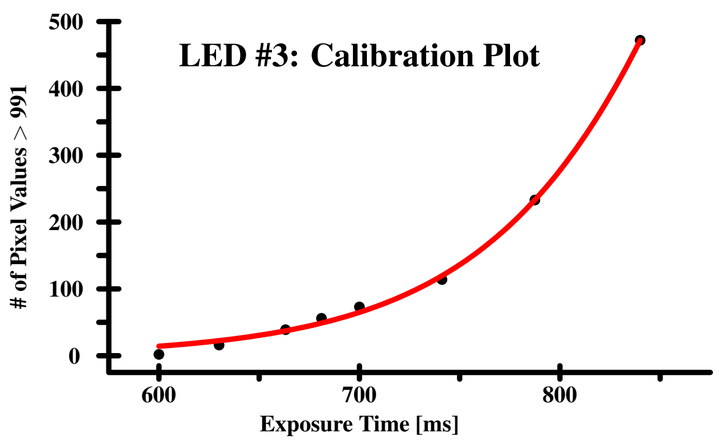

Fig 6: Example of a calibration plot

Exposure Time

The ideal images acquired with a camera should fill the dynamic range of the instrument without under- or over-sampling the amount of light captured. Exposure greatly affects the data acquired, for example, image saturation is a common result of overexposure.

In our software, the image is acquired as 10-bit and saved as 16-bit. Thus, the detected intensity value ranges from 0 to 1023. To optimize the dynamic range, we sequentially adapt the exposure time to allow 0.0098 to 0.019% pixel values greater than 97% (991) of 10-bit.

Experimental Protocol

We use an Arduino Due to trigger each LED light and camera acquisition on/off. The seed exposure time (x_0) for LED #3 (450 nm) is dynamically changed by an algorithm until the ideal imaging parameters are met. The software then displays/saves the images acquired with the optimal exposure time (x_{new}).

In particular:

1) Find the ideal exposure time for LED #3
   a) The down button on the Arduino is pressed and LED #3 (450 nm) is triggered on
   b) Algorithm 1 (free-run) runs and detects x_{new} from looping through these rules:

$$x_{\text{new}} = \begin{cases} 0.5 \cdot x & \text{if } y > 640 \\ 0.99623\, e^{-1.077\times10^{-3}\cdot y} \cdot x & \text{if } 20 \le y \le 640 \\ 1.025641025641 \cdot x & \text{if } 1 < y < 9 \\ 1.1764705882353 \cdot x & \text{if } y = 0 \end{cases}$$

   c) In the Arduino code, x_0 for each LEDs is multiplied by $C = x_{\text{new}}/x_0$ from LED #3
2) Save images of the sample
   a) Algorithm 2 (triggered, trigger width) prepares a rapid sequence of illumination with exposure x_{new} for all LEDs
   b) The up button on the Arduino is pressed, starting the LED sequence

```
int ledOrder[21]={21, 0, 20, 19, 18, 10, 6, 16,
    14, 12, 1, 13, 15, 5, 8, 11, 9, 2, 4, 3, 17};
int exposureTime[21]={2000, 1960, 1960, 1920, 1880,
    1680, 1280, 1240, 1224, 1080, 1040, 1000, 960,
    920, 800, 800, 792, 760, 672, 664, 640};
if(digitalRead(upPin) == HIGH){
 delay(50);

 for (int i=0; i <= 20; i++){
  write_number_matrix(ledOrder[i]);
  digitalWrite(ledPins[ledOrder[i]], HIGH);
  digitalWrite(Camera_Trigger, HIGH);
  delay(exposureTime[i]*C);
  digitalWrite(Camera_Trigger, LOW);
  digitalWrite(ledPins[ledOrder[i]], LOW);
  delay(50);
 }
}
```

Fig 7: Parts of the Arduino code

Results: Oxygen Levels

We tested the system capabilities by measuring tissue oxygenation. We created a control-sample by comparing two fingers: one normal and one wrapped with hemostatic band to cause hypoxia and peripheral cyanosis.

The two fingers were imaged with varying ranges of pixel values using the 21 LEDs (Fig 8). The oxygen deprived finger was found to have a different spectra compared to the normal finger (Fig 9).

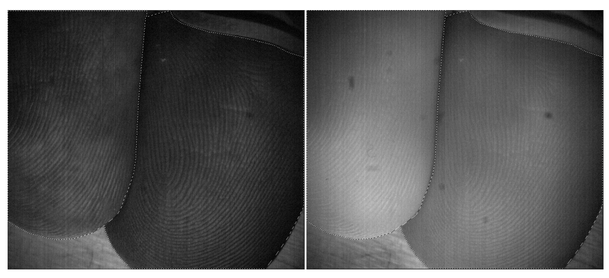

Fig 8: Images using LED #1 (395 nm) and LED #8 (635 nm) with area within each finger specified

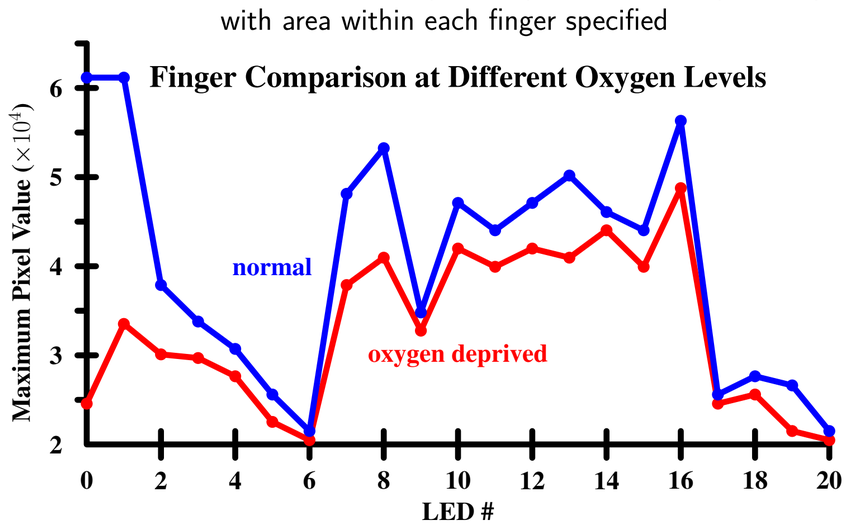

Fig 9: Spectra comparing different oxygen levels

Conclusion

We developed an algorithm and an experimental protocol obtaining the ideal exposure time for images acquired intra-surgically. We tested the system on tissue oxygenation experiments and successfully distinguished hypoxic regions. We plan on testing the performance of the instrument during surgery on rodents to visualize and distinguish sciatic nerve from surrounding muscle tissue.

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