# ESTIMATION OF HAEMOGLOBIN BASED ON OPTICAL SPECTROSCOPY

## AIM

To estimate the concentration of hemoglobin in blood using optical spectroscopy.

## ORJECTIVES

- To estimate the level of oxygenation of blood using optical spectroscopy method
- To analyse the variation in estimation based on the measurement site

## INTRODUCTION

Many investigators have used optical spectroscopy to measure tissue oxygenation and cytochrome redox states. The harmless non-ionizing radiation from light and the relatively inexpensive instrumentation make non-invasive optical measurements attractive for basic studies. in physiology and pathology, and for clinical use. Currently, pulse oximetry, which measures hemoglobin saturation in arterial blood, is commonly used in the hospital setting to evaluate oxygen sufficiency. If oxygenation in the local vascular, intracellular, and mitochondrial spaces could also be known, further refinements to assess efficacies of therapies would be possible in a variety of pathologies. This is especially important in diseases where oxygen supply and/or utilization are compromised, as in shock, peripheral vascular disease, or anemia.

## THEORY

Beer Lambert law describes how a normally incident beam of light is attenuated in a turbid media assuming the beam to be travelling along a single spatial dimension (along z axis) without much scattering along the path.

$$I = I_0 e^{-\mu_{az} z}$$

Where:

$I$ = Transmitted light intensity,

$I_0$ = Incident light intensity

$\mu_a$ = Absorption coefficient and $\mu_a = \varepsilon C$

$\varepsilon$ = Molar extinction coefficient

C = Concentration of the solution

z = path length

This formula is applicable when the transmitted light intensity can be measured with case. As the samples like tissue, we cannot measure I at a depth z to calculate the absorbance. Hence, we define an empirical term called as apparent absorption

$$A_{apparent} = \log_e\left(\frac{1}{Reflectance\ (\lambda)}\right)$$

where Reflectance ($0 \leq$ Reflectance $\leq 1$) from the tissue surface can be measured experimentally. Hence, the application formula to be used in experiment would be

$$A_{apparent} = \mu(\lambda)z$$

## METHODOLOGY

1. Obtain wavelength dependent reflectance from palmar and dorsal surfaces of the palm and interpret the difference in the spectral properties. (Take the spectral data from 400 nm to 880 nm)
2. Identify the spectral peaks corresponding to oxyhemoglobin and deoxyhemoglobin and apply the Beer Lambert's law to find concentration of hemoglobin for an assumed path length of 1 cm. Given: Molar extinction coefficient of oxy and deoxyhemoglobin at corresponding absorption peaks= 52236 $M^{-1}cm^{-1}$ (540 nm HbO2), 50104 $M^{-1}cm^{-1}$ (580 mm HbO2) & 54540 $M^{-1}cm^{-1}$ (556 nm Hb)
3. Find out tissue oxygenation using the formula:

$$SpO_2 \frac{[HbO_2]}{[HbO_2] + [Hb]}$$

### Main Components

- **Spectrometer:** Measures light intensity at different wavelengths for spectral analysis.
- **Light Source:** Provides illumination for the spectrometer to interact with the sample
- **Computer with Ocean View Software:** Controls the spectrometer and analyzes spectral data.

**RESULTS**

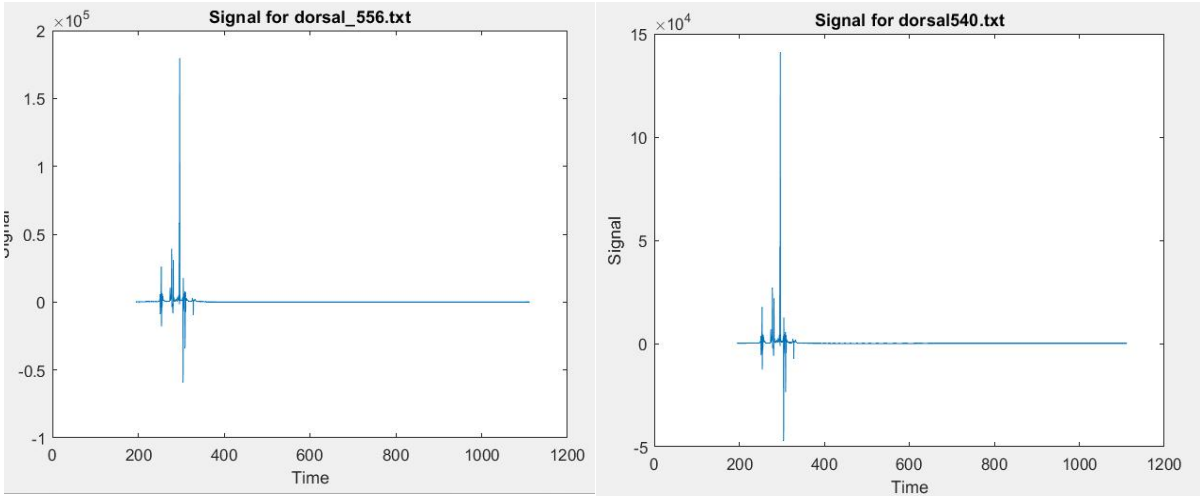
Concentration of Oxygenated hemoglobin in blood

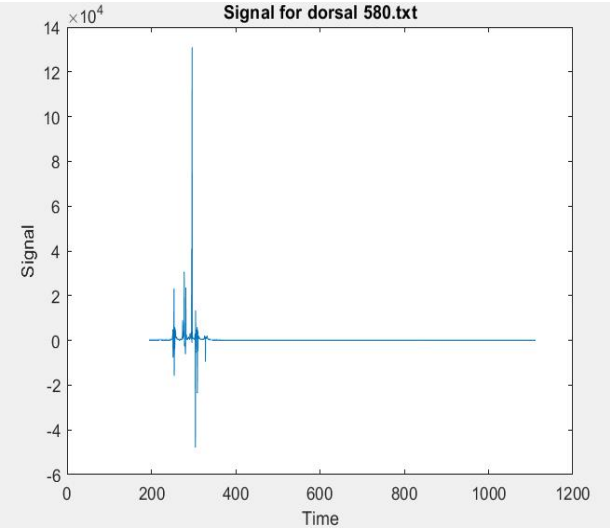
| Surface | % Reflectance | | Absorbance | | % Reflectance | Absorbance | SpO2 |
|---|---|---|---|---|---|---|---|
| | [HbO2] | | | | [Hb] | | |
| | 540 nm | 580 nm | 540 nm | 580 nm | 556 nm | | |
| Palmar | 48.87 | 24.83 | 66.97 | 35.32 | 46.71 | 49.07 | 65.33 |
| Dorsal | 49.67 | 23.22 | 64.84 | 33.65 | 48.97 | 52.13 | 64.36 |

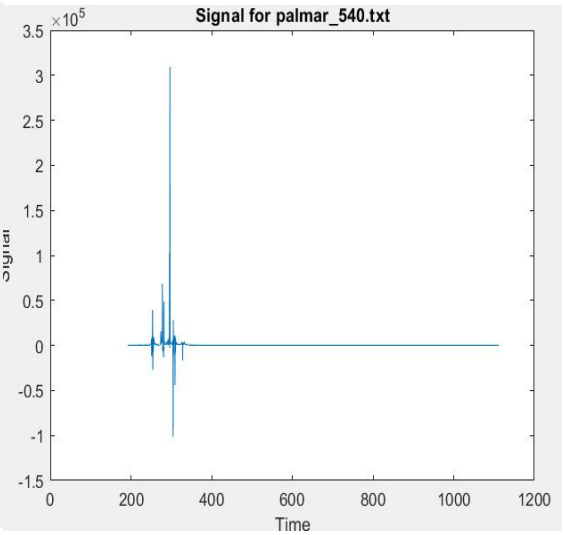
From the above table, we notice that a difference exists between absorbance values of palmar and dorsal sides of the hand. Some of the reasons of that could be:

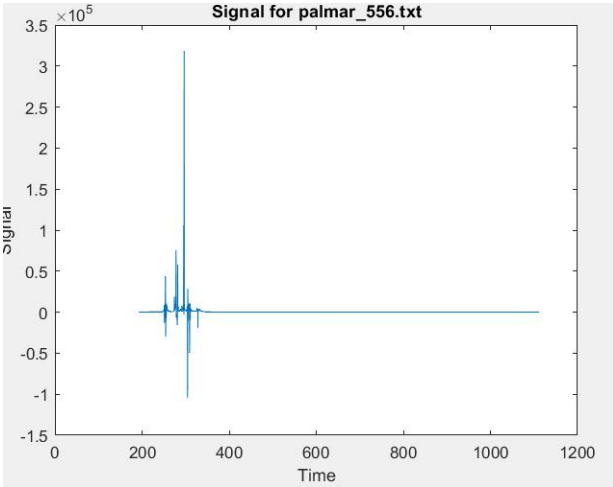
1. Skin Thickness and Composition: The palmar skin is thicker, which can scatter and reflect more light, reducing absorbance. Thinner skin on the dorsal side allows for deeper light penetration and increased interaction with underlying tissues, leading to higher absorbance.

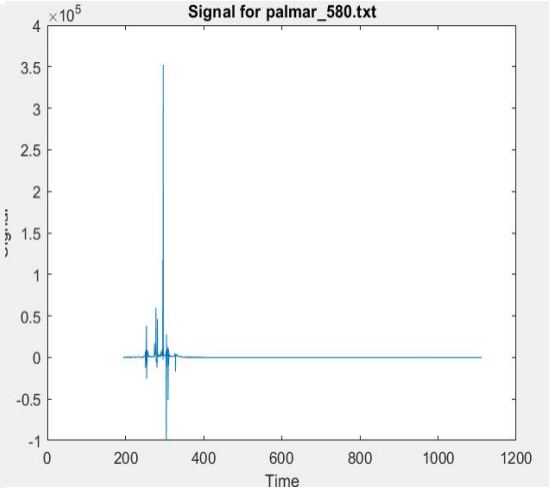
2. Vascularity: The dorsal side of the hand has more superficial blood vessels compared to the palmar side. These blood vessels, particularly the capillaries and veins, contain hemoglobin, which strongly absorbs light.






**MATLAB CODE:**

```
% Define file names
files = {'dorsal540.txt', 'dorsal_556.txt', 'dorsal 580.txt', ...
'palmar_540.txt', 'palmar_556.txt', 'palmar_580.txt'};surfaces = {'Dorsal', 'Dorsal', 'Dorsal',
'Palmar', 'Palmar', 'Palmar'};
wavelengths = [540, 556, 580, 540, 556, 580];
% Initialize variables
reflectance = zeros(1, 6);
absorbance = zeros(1, 6);
path_length = 1; % in cm
% Molar extinction coefficients
epsilon_HbO2_540 = 52236;
epsilon_HbO2_580 = 50104;
epsilon_Hb_556 = 54540;
% Load and process each file
for i = 1:length(files)
try
data = load(files{i});
if size(data, 2) < 2
```

```
error('Data format error: File %s does not have two columns', files{i});
end
time = data(:, 1);
signal = data(:, 2);
catch ME
disp(['Error loading file: ' files{i}]);
disp(ME.message);
continue;
end
% Plot the data
figure;
plot(time, signal);
title(['Signal for ' strrep(files{i}, '_', '\_')]);
xlabel('Time');
ylabel('Signal');
% Calculate reflectance (assuming it's given in the signal)
reflectance(i) = mean(signal);
% Calculate absorbance
absorbance(i) = -log10(reflectance(i));
end
% Apply Beer-Lambert's law to find concentrations
c_HbO2_540 = absorbance(1) / (epsilon_HbO2_540 * path_length);
c_HbO2_580 = absorbance(3) / (epsilon_HbO2_580 * path_length);
c_Hb_556_dorsal = absorbance(2) / (epsilon_Hb_556 * path_length);
c_HbO2_540_palmar = absorbance(4) / (epsilon_HbO2_540 * path_length);
c_HbO2_580_palmar = absorbance(6) / (epsilon_HbO2_580 * path_length);
c_Hb_556_palmar = absorbance(5) / (epsilon_Hb_556 * path_length);
% Calculate tissue oxygenation (SpO2)
SpO2_dorsal = (c_HbO2_540 + c_HbO2_580) / ...
(c_HbO2_540 + c_HbO2_580 + c_Hb_556_dorsal) * 100;
SpO2_palmar = (c_HbO2_540_palmar + c_HbO2_580_palmar) / ...
(c_HbO2_540_palmar + c_HbO2_580_palmar + c_Hb_556_palmar) * 100;
% Create results table
results = table(surfaces', wavelengths', reflectance', absorbance', ...
'VariableNames', {'Surface', 'Wavelength', 'Reflectance', 'Absorbance'});
% Display results
disp(results);
% Add hemoglobin concentrations and SpO2 to the table
HbO2 = [c_HbO2_540; NaN; c_HbO2_580; c_HbO2_540_palmar; NaN;
c_HbO2_580_palmar];
Hb = [NaN; c_Hb_556_dorsal; NaN; NaN; c_Hb_556_palmar; NaN];
SpO2 = [SpO2_dorsal; NaN; SpO2_dorsal; SpO2_palmar; NaN; SpO2_palmar];
results.HbO2 = HbO2;
results.Hb = Hb;
results.SpO2 = SpO2;
```

**CONCLUSION**

The concentration of hemoglobin in blood using optical spectroscopy is estimated.