Department of Physics and Astronomy

BioMedical Physics Program

Course: PHYS 4090/BPHS 5800 3.0 Winter 2025

Laboratory Manual:

In Vivo Near-Infrared Spectroscopy of the Human Brain

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Objective

To measure the in-vivo oxygenation state of haemoglobin, and calculate the change in oxygenation before, during, and after reactive hyperaemia by analyzing the colour content of light diffusely reflected from the skin.

Introduction

Optical methods of skin analysis are ideal because they can be performed non-invasively and in real-time. It is quite intriguing that so much information can be discovered from something as simple as launching some photons at an object and analyzing what comes back. In this lab, you will be exploring the use of light as a non-invasive measurement tool to determine the in-vivo oxygenation status of haemoglobin in your blood. These measurements will be made in a non-invasive sense, so as much as you may enjoy slicing up your lab partner to get at their blood, it will not happen here! In this lab you will get familiar with the concept of light propagation in turbid (scattering) media, as well as gain experience with optical spectroscopy methods. High resolution spectral information can be analyzed to allow semi-quantitative and fully quantitative analysis of biological materials, and is a very powerful technique, useful for a variety of biophysical applications.

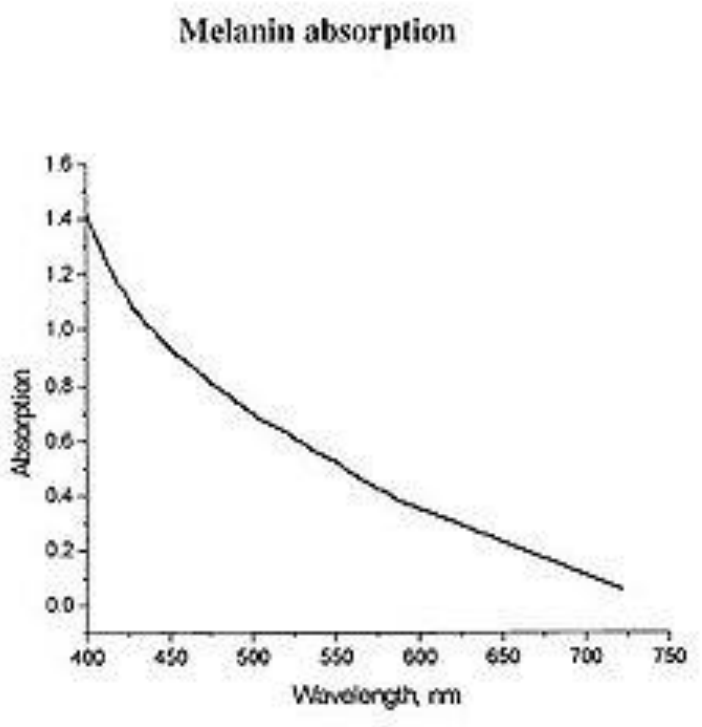

Figure 1. Absorption spectrum typical for melanin.

Determination of physiologically relevant parameters in a quick, reliable and repeatable fashion is of paramount importance in healthcare and biological research. The optical properties of human skin have been the subject of numerous investigations over the years, and two of the most relevant parameters to measure are the haemoglobin (Hb) oxygenation state and melanin content. Hb and melanin are the two major cutaneous chromophores within human skin, which means that their concentrations are essentially responsible for the colour of your skin. Upon exposure to ultraviolet (UV) light, melanocytes increase their production of melanin within the skin. We know this process by its more

common name, a suntan. The absorption spectrum of melanin is shown in Figure 1 and is almost linear over the visible spectrum. It is best measured in the spectral range above 600 nm, as it is the main source of light absorption in the skin at this wavelength and beyond.

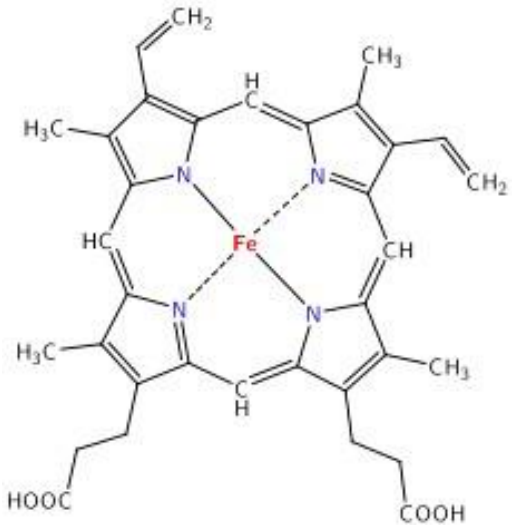

Figure 2. Schematic representation of the heme porphyrin ring in Hb.

The main target we are after in this lab is the oxygenation state of Hb. Hb is the iron-containing protein attached to red blood cells, and aside from giving blood its red colour, it is responsible for transporting oxygen from the lungs to the rest of the body. The mechanism of oxygen binding in Hb is due to a single iron atom, contained within the protein structure of Hb, and just below a porphyrin ring. A 2D representation of the ring and iron is shown in Figure 2. This structure serves to trap an oxygen molecule and hold it for transport around the body. A typical Hb molecule consists of four of these binding sites surrounded by a protein matrix, and the overall structure of the Hb molecule changes when carrying oxygen. This structural change results in a change in the absorption spectrum of haemoglobin in the 700-900 nm spectral range (Figure 3).

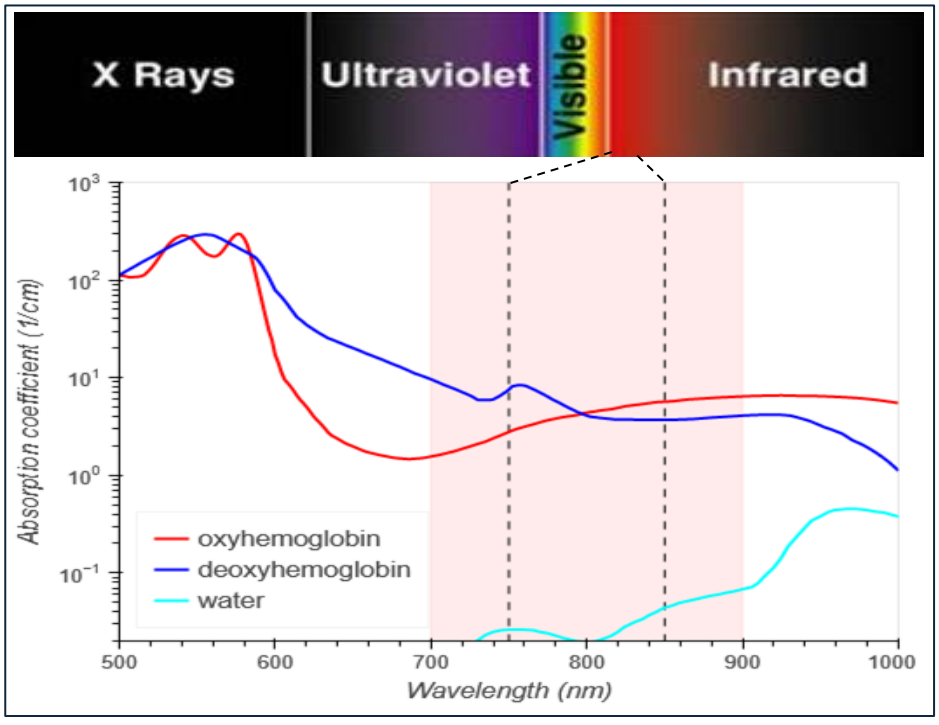

Figure 3. Absorption spectra of oxygenated Hb (red) and de-oxygenated Hb (blue).

There is also a significant shift of the absorption peak in the 400-450 nm spectral range. However, we will focus on measuring the changes in the Near-Infrared (NIR) between 700-900 nm since within this 'optical window' we can penetrate deeper into tissue. The absorption spectrum of oxygenated Hb and de-oxygenated Hb is different within this window. It is the change between these two states that you will quantify using two wavelengths of light (760 and 850 nm), and to do this we will focus on measuring diffusely reflected light from your brain. These diffuse reflectance measurements will allow us to calculate the absorption spectra of Hb, correct for the effects of melanin from different skin types, and monitor the oxygen saturation state of Hb during various tasks. Henceforth we shall call oxygenated Hb, HbO2, and deoxygenated Hb, deoxHb.

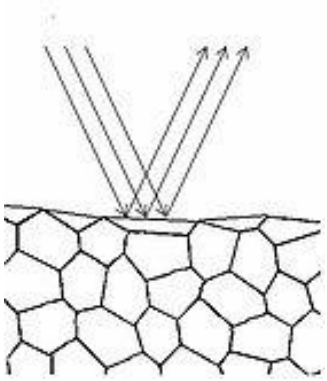

Figure 4. Specular reflection from a surface.

To understand how we can make measurements of absorption by analysing diffusely reflected light, we should first define what is meant by the term reflection. In general, light reflection can be defined in two ways: specular reflection and diffuse reflection. Specular reflection refers to light that has been directly reflected from an interface and is directional. A highly polished metal surface, such as a mirror, is an example of a specular reflector. This type of reflector will follow the law of reflection first described by Descartes, namely that the angle of incidence equals the angle of reflection (Figure 4). Another property of a specular reflector is that it will retain image information, which is why you can see your reflection in a mirror.

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In diffuse reflection, the light can be thought of as penetrating a small distance into the reflector and scattering multiple times before exiting (Figure 5). This type of reflectance is non-directional and does not produce any image since all image information in the wavefront is lost due to multiple scatterings. An example of a diffuse reflector would be a piece of white marble. No matter how much you polish the marble, most of the light striking its surface is diffusely reflected, which is why marble makes for a very poor mirror. A perfect diffuse reflector will reflect light uniformly into the 2π steradian space above it, while a perfect specular reflector will

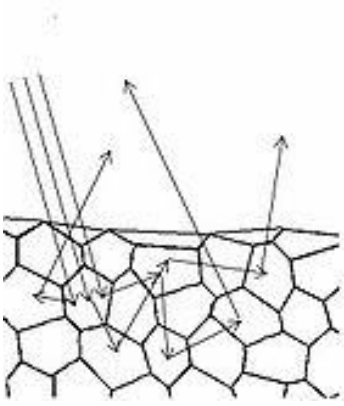


Figure 5. Diffuse reflection from a multi-layer structure.

reflect light at an angle defined by the angle of incidence. In general, most objects will reflect light both specularly and diffusely.

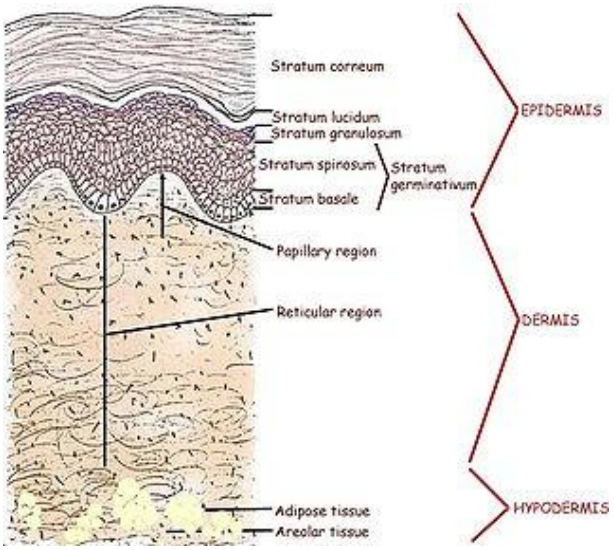


Figure 6. Cross section of human skin showing the major layers and components.

Human skin can also be thought of an object which is diffusely reflective, like the marble, only that it also contains absorbers, namely Hb and melanin. Skin is a heterogeneous, multi-layered structure consisting of three basic layers, each containing numerous sub-layers. The basic layers of skin are the epidermis, which is the outermost layer and provides protection, the dermis, which serves as the location for hair follicles, sweat glands, etc., and the hypodermis, which consists of connective tissue to secure the skin to bones and muscle, as well as blood vessels to deliver oxygen and nutrients to the skin (Figures 6 & 7). Note that it is not important for you to memorize the various layers that make up the skin, but it is important to note where the chromophores we will be measuring reside and originate from.

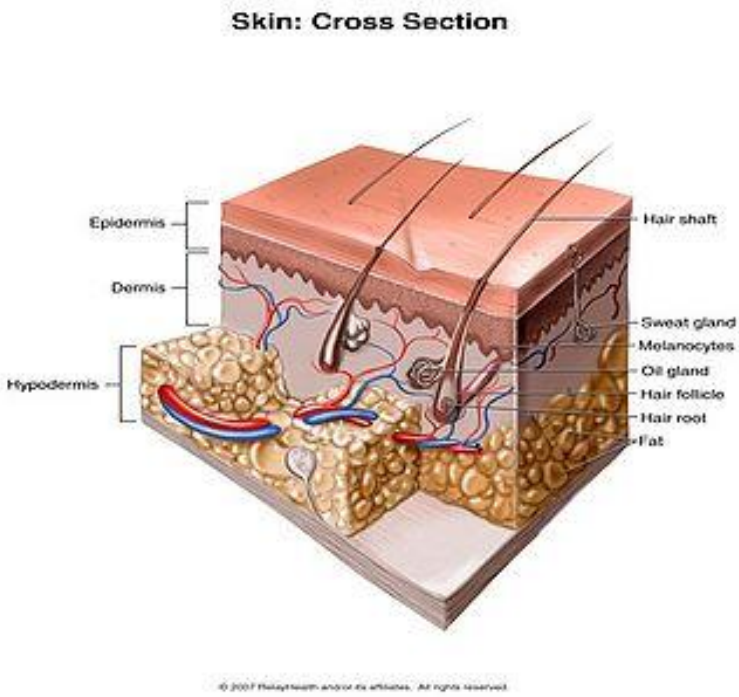


Figure 7. 3D representation of the skin layers and components.

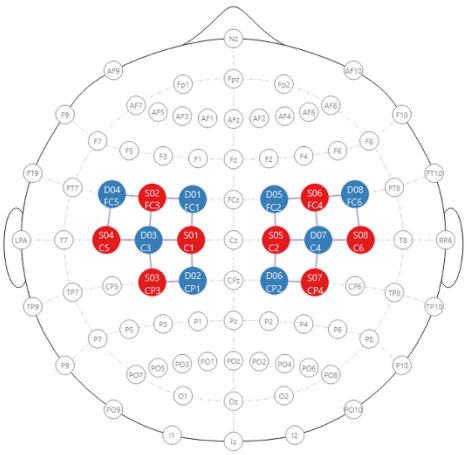
Most of the diffuse reflection you will measure originates from the epidermal layer, which contains no blood vessels or capillaries. The blood diffuses through the dermal layer and into the epidermis, essentially meaning that there is a homogeneous distribution of Hb in the epidermal layer. For the purposes of this lab, you can consider the epidermal layer to be a perfect diffuse reflector, with a uniform distribution of melanin and Hb 'absorbers' present in a given volume. Since the diffusely reflected light is interacting with melanin and Hb as it is scattered within the epidermal layer, there is

information within this light regarding the absorption properties of Hb and melanin.

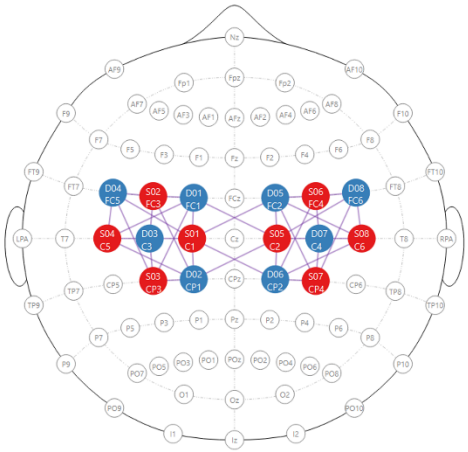
Experiment: In-Vivo Spectroscopy

To measure the diffuse reflectance spectra, we will make use of a functional near-infrared spectroscopy system (fNIRS) known as the NIRSport 2, from NIRx. fNIRS is a non-invasive neuroimaging technique that uses near-infrared light to measure changes in oxygenated-Hb (HbO2) and deoxygenated-Hb (deoxHb) in the brain. By shining light through the scalp and detecting the light that scatters back, fNIRS provides information about cerebral oxygenation, which are indirect indicators of neural activity. Most commercial fNIRS systems utilize continuous-wave technology which does not allow for measuring absolute concentration of oxygenated and deoxygenated hemoglobin.

The NIRSport 2 system consists of eight LEDs (with two wavelength each of 760 and 850 nm) which will be used as an illumination source. The diffuse reflectance will be collected with eight silicon photodiode detectors. The source and detectors will be arranged in the pattern shown in Figure 8a.



(a)



(b)

Source-detector distances [mm]

	D01	D02	D03	D04	D05	D06	D07	D08
S01	39.3	38.9	40.9	85.3	84.8	87.5	112.6	139.7
S02	37.2	85.9	37.6	37.0	101.6	130.9	137.4	144.0
S03	83.6	40.3	36.7	82.1	129.9	111.5	144.2	168.0
S04	82.8	88.4	40.7	34.0	137.2	144.7	158.7	164.8
S05	83.9	87.7	112.7	139.1	39.0	39.2	40.9	85.6
S06	101.4	131.5	138.2	144.0	37.0	86.3	37.1	37.0
S07	129.3	111.6	144.1	167.5	83.2	40.4	37.2	82.4
S08	136.4	145.0	158.8	164.2	81.9	88.8	40.7	33.4

Source-detector distances [mm]

	D01	D02	D03	D04	D05	D06	D07	D08
S01	39.3	38.9	40.9	85.3	84.8	87.5	112.6	139.7
S02	37.2	85.9	37.6	37.0	101.6	130.9	137.4	144.0
S03	83.6	40.3	36.7	82.1	129.9	111.5	144.2	168.0
S04	82.8	88.4	40.7	34.0	137.2	144.7	158.7	164.8
S05	83.9	87.7	112.7	139.1	39.0	39.2	40.9	85.6
S06	101.4	131.5	138.2	144.0	37.0	86.3	37.1	37.0
S07	129.3	111.6	144.1	167.5	83.2	40.4	37.2	82.4
S08	136.4	145.0	158.8	164.2	81.9	88.8	40.7	33.4

Figure 8. Cap montages showing source (red) and detector (blue) arrangements. A source-detector pair forms a channel. (a) Standard channel configuration (corresponding channel distances are shown in green in the table below). (b) Long channel configuration (corresponding channel distances are shown in green in the table below).

Each source and detector pair form a channel, and one source can have multiple channels (for example, source 1 forms 3 channels with detectors 1, 2 and 3). A complete list of channel indices and their source-detector pairs for both montages is provided in a spreadsheet on eClass. In theory, if 8 sources and 8 detectors are available, the maximum number of possible channels is 64. However, in practise, channels longer than 4.5 cm are often masked as the signal-to-noise ratio degrades with the source-detector distance. An example of the same montage with the maximum channel distance set to 10 cm is shown in Figure 8b. This arrangement of sources and detectors is over the motor cortex (region of the brain associated with controlling motor execution) and is often referred to as a motor montage. Source and detectors are secured to a cap via plastic grommets and the caps come in different sizes (based on the head circumference). In this lab, you will have access to two cap sizes (56 and 58 cm). With an 8 source montage, the default sampling rate is 10.2 Hz.

A challenge with fNIRS is that hair between the optodes (an optode is a term used to refer to a source or a detector, i.e., optical electrode) and the scalp can significantly degrade the quality of the data. Hair contains melanin and thick, dark hair can attenuate the signal and lead to poor data quality. In this lab, you will run various experiments to understand the difference between good and bad data quality, as well as to simulate reactive hyperaemia via a Valsalva maneuver and blood pooling effects using a head tilt procedure. A summary of the experiments can be found below. These experiments will be conducted in a block design where there will be periods of rest followed by periods of performing the task. Details on the experimental paradigm can be found below:

Steps to Correctly Setup the Cap on a Participant:

1. Measure your participant head circumference and choose the correct cap size.
2. Shimmy the cap as shown in Figure 9 to comb the sources and detectors through the hair.

Figure 9. Video on setting up the fNIRS cap correctly.

3. Once the participant wears the cap, you can measure from the nasion (between the eyebrows) to the inion (protrusion on the back of the skull) and 50% of this measurement is where Cz (middle of the cap) should be located (see Figure 10).

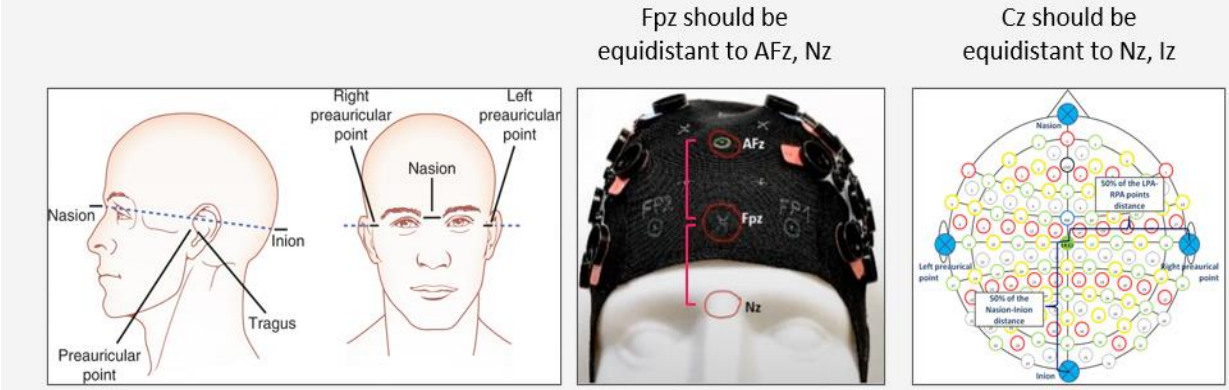


Figure 10. Landmarks on the skull and their position on the cap.

4. In the Aurora software, run an optimization of the sources and detectors by opening the signal level optimization page (dial icon). During the optimization, the participant should remain still and avoid speaking.
5. Assess the quality of the channels by checking the signal and dark noise. For participants with long, thick, or dark hair, the signal for some channels may be critical (red) or acceptable (yellow). Use the hair pin tool to move hair out of the way of optodes and improve the signal. Always reoptimize whenever you make an adjustment.

6. You are now ready to start collecting the data!

Now, you will be conducting 3 different fNIRS experiments as discussed below:

1- **Understanding the effects of source-detector distance separation on data quality.** You will have access to both configurations shown in Figure 8. The setup in Figure 8b includes channels that are much longer than the recommended maximum distance of 4.5 cm on adults. You will investigate the difference in data quality between the standard and long channel configuration.
   a. As a baseline, begin first collecting data in the Standard Channel Configuration.
   b. Navigate to the recording page. Hit record and have the participant sit at rest for 30 s. The HbO2 and deoxHb signals can be clearly observed for each channel in the Lineplot tab.
   c. You will observe natural oscillations in both HbO2 and deoxHb curves. In particular, the HbO2 curve has a more pronounced high frequency oscillation than the deoxHb curve. Why do you think this is?
   d. Next, switch to the Long Channel Configuration. Make sure you rerun the optimization procedure before collecting data!
   e. Hit record and have the participant sit at rest for 30 s. What differences do you observe in signal quality, noise, SNR?

2- **Investigating the effects of blood pooling using a head tilt procedure.** fNIRS is inherently sensitive to changes in blood flow happening in the microvasculature, so tilting your head to one side will result in blood pooling and a large change in the signal (i.e. an increase in oxygenated Hb and a decrease in deoxygenated Hb).
   a. Ensure you are working in the Standard Channel Configuration.
   b. Hit record. Have the participant sit at rest for 15 s. This is an initial rest period before the experiment begins.
   c. Follow the block paradigm: sit upright at rest for 15 s, tilt right for 15 s, sit upright at rest for 15 s, and then tilt left for 15 s.
   d. Repeat this experiment 5x on each side (i.e. 5 tilts to the right and 5 tilts to the left, in total).
   e. Mark each segment in the experiment with a trigger. Triggers are set by pressing the keys F1 (trigger 1), F2 (trigger 2), F3 (trigger 3), and so on. Use the same trigger to mark a particular block of the experiment. For example, in this experiment, use trigger 1 (F1) to mark a rest period, trigger 2 (F2) to mark a right tilt, and trigger 3 (F3) to mark a left tilt. An example of block design and setting triggers is shown for the Valsalva maneuver experiment in Figure 11.

f. Once data collection is complete, you can view an average signal over all channels for a particular trigger by selecting the Block Averages tab. Note, this viewing mode will only be available if triggers are properly set!

3- **Simulating reactive hyperaemia using a Valsalva maneuver.** Valsalva is a vagal maneuver that acts on the vagus nerve to slow your heart rate. To perform it, take a deep breath in and bear down as hard as you can. This maneuver consists of four phases (ref):

- **Phase One:** When you start pushing, pressure rises in your chest and belly. That forces blood out of your heart and down your arms. This causes your blood pressure to go up for a short time.
- **Phase Two:** Your heart pumps less blood with each beat while you're straining. Your blood pressure steadily returns to normal.
- **Phase Three:** When you relax at the end of this maneuver, your heart rate increases.
- **Phase Four:** This is the recovery period. Blood rushes back to your heart. Ideally, your blood pressure rises but then returns to baseline as your heart rate goes back to normal.

The HbO2 concentration will drop and the deoxHb concentration will increase during this maneuver, and immediately following re-perfusion, you will measure a significant jump in Hb oxygenation, then a steady return to normal physiological levels.

a. Ensure you are working in the Standard Channel Configuration.
b. Hit record. Have the participant sit at rest for 20 s. This is an initial rest period before the experiment begins.
c. Follow the block paradigm and set a trigger for each action: sit upright at rest and breathe normally for 20 s, and then perform the Valsava maneuver for 20 s.
d. Repeat 3-5x, depending on the comfort level of the participant.

You may want to practice performing the Valsalva maneuver, ahead of time before the day of the lab, to ensure collecting good data for your final dataset and analysis in your report. If you are uncomfortable performing this maneuver, please let TA/Instructor know.

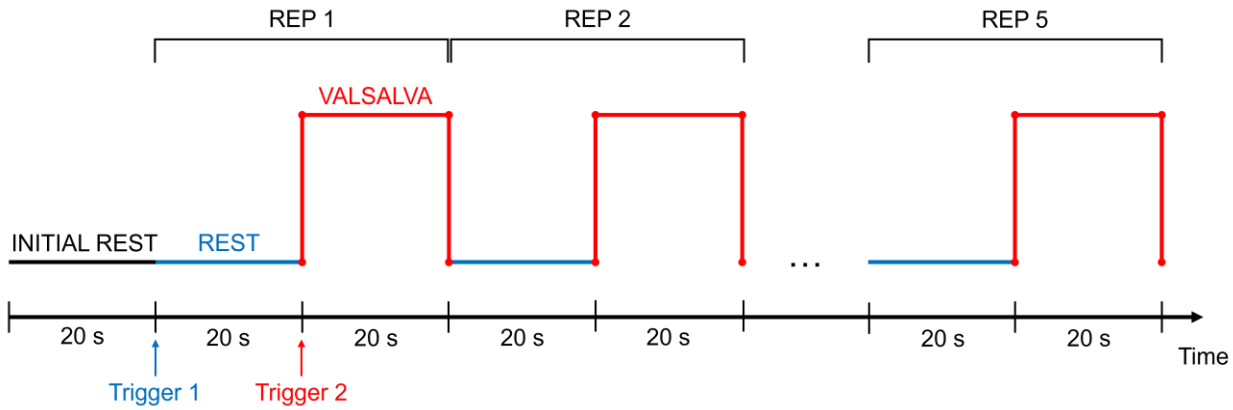


Figure 11. Schematic diagram of the time-sequenced Valsalva maneuver. Suggested that you practice in advance if you are comfortable doing the procedure.

Summary of Data Collection Steps:

1. Open Aurora.
2. Select your device.
3. Select or create a new configuration.
4. Put the cap on your participant using the shimmy method.
5. Run the signal optimization to determine if you need to improve the signal before recording.
6. Run the Refresh setting in signal optimization while parting the hair to improve the signal quality.
7. Begin recording. Include details of the participant and experiment, as necessary.
8. Data is saved automatically in **C:\Users\Username\Documents\NIRx\Data** → data is stored by date of collection.

Data Analysis:

The steps below detail a general routine to analyze your data. You may use any programming method you choose (e.g. MATLAB, Python, etc.). You can also visualize your data in **Aurora** (the acquisition software of the NIRSport 2) "offline".

1- Import your data. The data for each recording session #001, #002, etc. is saved in a folder by date. You can find a description of each recording (i.e. participant and experiment information) in the description file (.json extension), which can be opened in notepad. The raw intensity in Volts for each channel is stored as an array

in the .wl1 and .wl2 files for wavelength 1 (760 nm) and 2 (850 nm), respectively (see Figure 12). Both files can be opened in notepad and saved as text files (append the extensions .wl1.txt and .wl2.txt to the end of the file names), and then imported as data arrays.[1]

$$\begin{array}{c} \\ \text{Time} \downarrow \end{array} \begin{array}{c} \begin{matrix} \text{Channel 1} & \cdots & \text{Channel } n \end{matrix} \\ \begin{bmatrix} I_{1,\lambda_1}(t=0) & \cdots & I_{n,\lambda_1}(t=0) \\ \vdots & \ddots & \vdots \\ I_{1,\lambda_1}(t=T) & \cdots & I_{n,\lambda_1}(t=T) \end{bmatrix} \end{array}$$

Figure 12. Diagram showing how raw intensity is stored in the .wl1 file (identical for .wl2, except $\lambda = \lambda_2$) for a trial of duration $t = T$. Column 1 corresponds to channel 1, column 2 to channel 2, …, and column n *to channel* n *(the source-detector pairs for each channel can be found in the channel indices spreadsheet on eClass).*

2- Define other relevant parameters. The extinction coefficient can be retrieved from this website for both 760 nm and 850 nm. For simplicity, d can be calculated as the average source-detector distance across all channels (different for both montages!). Assume a constant value of 6 for $\mathrm{DPF}(\lambda)$.

3- Calculate the optical density using this equation:

$$\Delta A = -\log_{10}\left(\frac{\text{raw}}{\text{mean(raw)}}\right)$$

4- Use the Modified Beer-Lambert Law (MBLL) to calculate changes in concentration of oxygenated Hb and deoxygenated Hb:

$$\Delta A(\lambda) = \varepsilon(\lambda) \cdot \Delta c \cdot d \cdot \mathrm{DPF}(\lambda)$$

where Δc is the change in hemoglobin concentration, $\varepsilon(\lambda)$ is the extinction coefficient, d is the source-detector distance, and $\mathrm{DPF}(\lambda)$ is the differential path length factor. Then, the concentration changes of the distinct chromophores is:

$$\Delta c = \frac{\Delta A(\lambda)}{\varepsilon(\lambda) \cdot d \cdot \mathrm{DPF}(\lambda)}$$

Here, we are measuring two chromophores (HbO2 and deoxHb) at two different wavelengths; therefore, we have a system of two equations in two unknowns. How

[1] *All* data (raw intensity, wavelengths, source-detector positions, etc.) is stored in a standardized .nirs file. This file can be read into MATLAB as a structure file using the load command: load('filename.nirs', '-mat'). The raw intensity (.wl1 and .wl2), which is all you should need for this lab, can be accessed by dot notation: structName.d

do we write MBLL in this case? Using the files and parameters in the steps above, solve this system of equations to obtain Δc_{HbO2} and $\Delta c_{\mathrm{deoxHb}}$ as a function of time.

As part of your data acquisition and analysis of the 3 experiments, include the following calculations and plots as part of your lab report write up.

1- Understanding the effects of source-detector distance separation on data quality.

➔ Choose 3 source-detector channel pair combinations at :1) short distance, 2) medium distance and 3) long distances of source-detector separation. Obtain a power spectrum for these channels. This involves calculating a Fourier Transform. Note that the sampling frequency for data collection is 10.2 Hz.

2- Investigating the effects of blood pooling using a head tilt procedure.

➔ Plot the Oxy Hb/Deoxy Hb results of the head tilt (blood pooling) from a representative channel on the right side of the head and left side of the head.

3- Simulating reactive hyperaemia using a Valsalva maneuver.

➔ Show plot and calculate saturated oxygenation (SpO2) in brain with Valsalva maneuver choosing one representative data channel to analyze.

Questions/Write Up:

The following questions should be answered in your Results and Discussion section of Lab Report.

1. What is the main function of haemoglobin in the blood? Briefly describe the physical processes involved in haemoglobin's biological functioning as well as what happens to the molecule in its various states of oxygenation.
2. How does the presence of melanin affect the data that you have collected and analyzed? What are the sources of this melanin?
3. Explain what biophotonic measurement you are performing in this lab is? i.e., is this specular or diffuse reflection you are examining and explain why.

4. Discuss why have we chosen to analyze absorption spectra by reflecting light from a surface? What are the advantages of performing reflectance measurements, such as in this lab, versus standard transmission spectroscopy measurements?
5. You will observe natural oscillations in both HbO2 and deoxHb curves. In particular, the HbO2 curve has a more pronounced high frequency oscillation than the deoxHb curve. What are these oscillations, and why is there a difference between the HbO2 and deoxHb curves?
6. Discuss how changing the source-detector pair distance separation affects the SNR and why this is happening? Hint: find a scholarly resource discussing the shape of the diffuse reflectance profile of NIRS light through tissue.
7. In the blood pooling experiments, describe what is happening with the asymmetry observed in the left and right side of the brain. Describe an application of this blood pooling measurement and discuss how such a NIRS instrument could be used both advantageously and disadvantageously, compared to current state-of-the-art technologies.
8. Describe what is happening with the concentration changes of HbO2 and deoxHb during the period of the Valsava maneuver. Are these changes consistent with the physiological effect expected?
9. Can you suggest any improvements to this experimental setup that would make these measurements easier to perform in a clinical setting? What challenges would clinical use of this fNIRS technique and such instrumentation face?

   NB: You MUST cite all sources used.

Useful Reference on fNIRS:

[1] Scholkmann F, Kleiser S, Metz AJ, Zimmermann R, Mata Pavia J, Wolf U, Wolf M. A review on continuous wave functional near-infrared spectroscopy and imaging instrumentation and methodology. Neuroimage. 2014 Jan 15;85 Pt 1:6-27. doi: 10.1016/j.neuroimage.2013.05.004 . Epub 2013 May 16. PMID: 23684868