MEASURING BLOOD PERFUSION IN BONE USING NIRS
BONE OPTICAL SPECTROSCOPY

A Thesis Presented

By

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Contents
List of Tables: ............................................................................................................. v
List of Figures: ........................................................................................................... v
Abstract: ....................................................................................................................... 1
Introduction: ............................................................................................................... 2
Light in bone: ............................................................................................................. 6
  Dominant absorbers in biological tissues: .............................................................. 8
NIRS established applications: ............................................................................... 9
Modified Beer lambert law MBLL: ........................................................................... 10
Differentiated path length: ..................................................................................... 11
DPF: ......................................................................................................................... 13
System: ..................................................................................................................... 13
Design requirements: ............................................................................................. 14
Steady output of light source Intensity: ................................................................. 15
  • Tungsten light source......................................................................................... 15
  • Broadband Laser System.................................................................................... 16
Calibration (White & dark measurements): ........................................................... 17
  • Dark measurements: ......................................................................................... 17
  • White Standards: ............................................................................................... 18
Source detector distances (two layers effect): ....................................................... 20
  • Phantom experiment: ....................................................................................... 21
  • In vivo (ice on skin): .......................................................................................... 23
SMA connectors: .................................................................................................... 25
Probe movement: ................................................................................................... 26
Test system feasibility on paraplegics: ................................................................. 28
System Variability revisited: .................................................................................. 29
  • System error: .................................................................................................... 29
BOSS system: ......................................................................................................... 30
System validation: .................................................................................................. 31
  • Experiment 1. ................................................................................................. 31
  • Experiment 2. ................................................................................................. 32
  • Experiment 3. ................................................................................................. 33
Detecting changes in blood flow: .......................................................................... 34
Typical signal curves as detected by spectrometer: .......................................................... 35
Data representation: ............................................................................................................ 36
Subjects: ............................................................................................................................. 37
  • Steady elevated leg experiment: .................................................................................... 37
  • Running experiment: ...................................................................................................... 39
  • Rowing experiment (Able-bodied): ................................................................................. 42
  • Functional Electrical Stimulation (FES) rowing: ......................................................... 44
Conclusion: ........................................................................................................................ 48
Appendix I: ....................................................................................................................... 51
  BOSS system .................................................................................................................. 51
  Tibia cross sectional area (left leg) .................................................................................. 52
  Steady elevated leg experiment results for detector A: .................................................. 53
  Running Experiment results for detector A: ................................................................. 54
  Rowing Experiment results for detector A: ................................................................. 55
  FES rowing results for detector A: ................................................................................ 56
  Measurements protocol ................................................................................................. 57
    System Initiation: .......................................................................................................... 57
    Steady Elevated Leg experiment: ................................................................................ 57
    Running experiment: .................................................................................................... 58
    Rowing experiment: .................................................................................................... 59
References: ....................................................................................................................... 61
List of Tables:


List of Figures:

1. Schematic representation of vascular organization in the diaphysis of a long bone.
2. Light spectrum.
3. Visible light through air vs. biological tissues
4. Absorption coefficient of HbO2, Hb, water, and lipid.
5. Typical total absorption in human soft tissue.
6. Light penetrating through the non-homogenous biological tissue.
7. Early version of the probe holder.
8. Tungsten light source.
10. White reflectance surface.
11. White standards calibration box.
12. White standards signal reproducibility.
13. Phantom Set up.
15. Ice effects over the anterior surface of tibia at three source-detector distances.
16. Original SMA Connecters design.
17. Probe 2D top surface dimensions
18. Probe holder (sleeve version).
19. Probe.
20. Probe misalignment over SCI subject’s tibia.
21. Probe holder combining the advantages of a sleeve and an arm band.
22. System schematic.
23. Connecting and disconnecting the fiber optics.
24. Experiment 2 set up.
25. System variability due to probe holder movement.
26. Light leakage after including the transparent surface cover.
27. Typical normalized power intensity signal over full range of spectra at different points in time throughout a single experiment.
28. Hypothetical signal to further explain how data is presented for the four experiments conducted.
29. Signal behavior in three distinct wavelengths (720nm, 810nm, & 870nm) over a period of time due to steady elevated leg position at detector B.
30. Signal behavior in three distinct wavelengths (720nm, 810nm, & 870nm) ten minutes after running at detector B.
31. Signal behavior in three distinct wavelengths (720nm, 810nm, & 870nm) before, while and after rowing at detector B (able-bodied).

32. Rowing with Functional Electrical Stimulation.

33. Signal behavior in three distinct wavelengths (720nm, 810nm, & 870nm) before, while and after FES rowing at detector B (Subject one).

34. Signal behavior in three distinct wavelengths (720nm, 810nm, & 870nm) before, while and after FES rowing at detector A & B (Subject two).

35. The BOSS system.

36. Tibia cross sectional area of left leg.

37. Signal behavior in three distinct wavelengths (720nm, 810nm, & 870nm) over a period of time due to steady elevated leg position at detector A.

38. Signal behavior in three distinct wavelengths (720nm, 810nm, & 870nm) ten minutes after running at detector A.

39. Signal behavior in three distinct wavelengths (720nm, 810nm, & 870nm) before, while and after rowing at detector A (able-bodied).

40. Signal behavior in three distinct wavelengths (720nm, 810nm, & 870nm) before, while and after FES rowing at detector A (Subject one).

41. Signal behavior in three distinct wavelengths (720nm, 810nm, & 870nm) before, while and after FES rowing at detector A (Subject two).
Abstract:

The bone optical spectroscopy system (BOSS) uses Near-Infrared (NIR) light to analyze blood flow in the tibia. It aims to detect any changes regarding blood flow in bones due to exercise. Near infrared spectroscopy (NIRS) provides a safe, non-invasive, and inexpensive alternative to analyze hemodynamic changes in the biological tissues using light in the range of 600 to 1000 nm. NIR light can penetrate deeply into the biological tissue. With the use of fiber optic detectors, the scattered diffused light can be collected and relayed to spectrometers where it is decomposed into its spectral components.

In this thesis, I designed the probe holder for the BOSS system, developed the protocol for measurements, analyzed and optimized reproducibility of the system, and determined the system ability to measure changes in blood flow in the tibia, in particular in response to exercise for able-bodied subjects and paraplegic rowing. I conducted four experiments to detect changes due to 1) no movement, 2) high impact exercise, 3) low impact exercise, and 4) bone loading exercise for spinal cord injury patients. When patients are not moving the results illustrate little to no detected changes due to elevated steady leg over time. As for the exercise experiment, the data demonstrated consistent increase in blood volume after engaging the subjects in a high intensity physical exercise. However, the FES rowing experiment is not conclusive enough due to low number of subjects and contradicting results, which will be explained briefly in the upcoming sections.
Introduction:
Bone is a highly vascular organ. As illustrated in fig (1), blood vessels are everywhere within the bone [14]. Bone circulation is very essential to bone physiology and bone formation. Inadequate amount of blood perfusion in bone can be a clear indication of bone pathologies. Traditionally speaking, Osteonecrosis has been the main disease associated with bone circulation. However, recent studies suggest the importance of blood perfusion in long bones with relation to osteoporosis and fracture repair.

![Figure. 1 Schematic representation of vascular organization in the diaphysis of a long bone [14]](image)

Studies have shown high correlation between the decrease in blood perfusion in the formal heads and osteoporosis. Furthermore, osteonecrosis, where old bone cells break down much faster than the formation of new healthy ones, is mainly caused by the insufficient supply of blood perfusion in the joints. Likewise, in Paget’s disease of the
bone where bone cells are rapidly forming in a nanostructured manner, studies have shown an increase of blood flow in the affected extremities [15].

Blood perfusion in bones is a clear indication of the bone’s health, such that the bone acquires all of its essential nutrients necessary for its growth and formation through blood. In this thesis we aim to study and analyze the relative changes in hemoglobin concentration in long bones, human tibia in specific. The tibia was initially chosen due to the minimal influence of skin, lipid, muscle, or any biological tissue for that matter that lies on top of it. We developed a system using the method of Near-infrared Spectroscopy (NIRS) to investigate hemodynamics, changes in concentration of oxygenated hemoglobin HbO2 vs. deoxygenated hemoglobin Hb, and oxygen saturation index.

Many of the pathologies associated with bone can be easily maintained by monitoring the status of blood perfusion in the affected area. However, the commercially available and established methods in the medical field that have the ability to measure blood perfusion in bones are either invasive, expensive, or impractical. Hence our motive is to develop a non-invasive, practical, and affordable system that can monitor blood perfusion in long bones. The following section briefly descripts the methodologies of such techniques and highlights their advantages and disadvantages.
Blood perfusion monitoring techniques:

- **Radioactive or Fluorescent microspheres.**

  Fluorescent particles of 15 micrometer diameters are injected into the circulation and distributed through the arteries. Due to the smaller diameter of the capillaries, the fluorescent particles get trapped there. The number of the microspheres in bone is proportional to its blood flow. Although, this is the golden standard when it comes to monitoring blood perfusion in bone due to its accurate measurements, it is highly invasive as it requires tissue sampling.

- **Magnetic Resonance Imaging (MRI) with contrast media.**

  The embodiment of contrast agent into MRI allows us to evaluate bone circulation. It is non-invasive and non-ionizing. However, this technique requires the injection of contrast agent relatively expensive.

- **Positron Emission Tomography (PET).**

  Specific radiotracers are injected into the circulation to get information about localization and metabolic activities in the tissues. This techniques although non-invasive, it involves a considerable amount of ionizing radiation.

- **Laser Doppler Flowmetry.**

  Such devices are uses the Doppler shift in a laser beam to assess whether a structure such as red blood cells is moving towards or away from the probe. Both direction and speed of the assessed structure can be determined. This technique is neither invasive
nor is it expensive. However, it acquire poor measurement due to its inability to penetrate beyond few millimeters into the tissue.

- **Near Infrared Spectroscopy.**

This method uses the advantage of the respective optical window where light can penetrate into the biological tissue without being fully absorbed. The scattered, partially absorbed light is related to the incident light to calculate the concentration of the absorbers (hemoglobin) in the analyzed region. NIRS is non-invasive, inexpensive, nonionizing, portable, and gives near real time measurements. However, it has yet to be validated to monitor blood perfusion in long bones.

Table (1) below highlights the advantages and disadvantages of blood perfusion monitoring techniques that are commercially available with today’s technology.

<table>
<thead>
<tr>
<th>Method</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radioactive or fluorescent microsphere technique</td>
<td>Gold standard</td>
<td>Invasive – requires tissue sampling</td>
</tr>
<tr>
<td>Magnetic resonance imaging with contrast media</td>
<td>Non-invasive</td>
<td>Contrast media injection</td>
</tr>
<tr>
<td>Positron emission tomography</td>
<td>Non-invasive</td>
<td>Ionizing</td>
</tr>
<tr>
<td>Laser Doppler Flowmetry or Doppler Ultrasonography</td>
<td>Non-invasive and inexpensive</td>
<td>Poor measurement depth</td>
</tr>
<tr>
<td>Near Infrared Spectroscopy</td>
<td>Non-invasive and inexpensive</td>
<td>Not validated for bone yet</td>
</tr>
</tbody>
</table>

Table. 1 Blood perfusion monitoring techniques commercially available [4]
**Light in bone:**

Light ranges over a broad spectrum of wavelengths from vacuum ultraviolet at only a hundred nanometers to Far Infrared at about 14 micrometers fig (2). However, the only optical window where light can transmit through biological tissues without being fully absorbed is in the Near-Infrared region, which starts right after visible light (~700nm) to (~1000nm).

Due to the high scattering coefficient of biological tissues, NIR light takes a banana shape path as it penetrates into muscle, skin, and bone to only be scattered back out of the tissue. To perform a quick examination, one can hold a white LED (visible light) up to his thumb fig (3), and observe the change in color as the light coming out of the other end will surely be red. Such change in color is explained by the absorption of visible light and scattering of NIR light.
Beer lambert law describes how light is attenuated in an absorbing medium as the following:

\[ I(\lambda) = I_0(\lambda) \times \exp(-\mu_a(\lambda) \times D) \]

- \( \lambda \): Wavelength
- \( I \): Transmitted optical power
- \( I_0 \): Incident optical power
- \( D \): Distance traveled through the medium

\[ OD = \mu_a(\lambda) \times D = -\ln\left(\frac{I(\lambda)}{I_0(\lambda)}\right) \]

- OD: optical density of the medium

When photons are being absorbed by the medium, they are typically converted to heat. Furthermore, it can also be released as photons with lower energy. This absorption depends on the molecular structure of the medium. The total absorption of a substance with homogeneously distributed absorbers is given as

\[ \mu_a(\lambda) = w_1 \times \mu_{a,1}(\lambda) + w_2 \times \mu_{a,2}(\lambda) + \cdots \]
- $w_i$: Percentage of the interrogated volume occupied by the absorber

**Dominant absorbers in biological tissues:**

In the case of biological tissues where scattering is orders of magnitude higher than absorption, light is primarily absorbed by blood (Oxy and DeOxy hemoglobin). Approaching higher wavelengths in the NIR optical window, water and lipid play a factor and contribute to the absorption of light. Fig. Presents the absorption coefficients of the four main absorbers in biological tissues. Note: at around 800nm wavelength, the absorption coefficients of both HbO2 and HB intersect making the isosbestic point. The isosbestic point will be later used to illustrate relative changes in volume in hemoglobin.

Figure. 4 Absorption coefficient of HbO2, Hb, water, and lipid.
Fig. Represents the total absorption spectra of the four absorbers indicated above along the region of interest. Notice however, although at 700nm, one would expect a higher total absorption giving the relatively higher absorption coefficient of deoxygenated hemoglobin, total absorption is ~3 times as high around 970nm. This can be explained by the very low concentration of Hb in tissues compared to HbO2 and the water content in the human body.

Given its very unique ability, NIRS is a well-established technique to measure blood perfusion and hemodynamics in biological tissues (i.e. muscles and brain activities). However, its applications in long bones have not been proven yet.

**NIRS established applications:**

- **Soft tissues (Muscle).**

The use of NIRS for the application of monitoring blood perfusion in soft tissues is well established. In order to monitor hemodynamics in muscles, the existing devices use few
distinct wavelengths (two or more) before and after the isosbestic point utilizing the difference in the absorption coefficients for both oxygenated and deoxygenated hemoglobin. Doing such, one can obtain the oxygen saturation index and total hemoglobin concentration. Those devices however, assume a homogeneous medium and could not be used to measure blood perfusion in bone underneath the soft tissues as it does not consider such heterogeneity.

- Functional NIRS (fNIRS).

Further developments were recently conducted into using the NIRS technique to evaluate brain activity with relation to its hemodynamics. In fNIRS, the system accounts for the different layers of biological tissues being tested by incorporating the respective scattering and absorption coefficients. It also uses few distinct wavelength (i.e. 760 and 850nm).

**Modified Beer lambert law MBLL:**

Biological tissue could not be regarded as an absorbing medium due to its optical properties and the very high scattering effect ($\mu_s \gg \mu_a$). That being said, beer lambert law can no longer describe the behavior of light as it travels within the scattered medium. As light scatters to all directions, light will end up taking a longer path as it travels from the light source to the detector. Furthermore, light may even be scattered away from the detector. Beer lambert law is then modified to take into account the scattering effects of the tissues as follows:

$$I(\lambda) = I_0(\lambda) \times \exp(-\mu_a(\lambda) \times L(\lambda) + G(\lambda))$$
• G: describes losses due to scattering

Note. G is hard to obtain, but since we are only interested in relative changes over a specified period of time, G will cancel out eventually, as it will be illustrated later.

• L: differentiated path length

\[ L(\lambda) = D \times DPF(\lambda) \]

• D: physical distance between the source and the detector

• DPF: differentiated path length factor.

Note. DPF varies with wavelength while D is constant.

**Differentiated path length:**

As light travels in the tissue, it takes a banana shape path from the point it penetrates into the skin to the point it exists it. So when shining light into skin at point (A) indicating light source, and holding a detector at point (B) with distance D away from light source, one can vary the depth light is penetrating into the tissue by varying the physical distance between the light source and the detector. Giving the fact that we are interested in measuring blood perfusion in the tibia, we need to factor out the effects of the shallow layer of skin that lies above it. To do so, one can use two detectors at varying distances and subtract away the signal detected at the closer distance as indicated in the fig below.
The measured light collected at both detectors is partially influenced by each layer.

\[ I_A(\lambda) = I_0(\lambda) \times \exp(-\mu_{a,1}(\lambda) \times L_{1,A}(\lambda) - \mu_{a,2}(\lambda) \times L_{2,A}(\lambda) + G_A(\lambda)) \]

\[ I_B(\lambda) = I_0(\lambda) \times \exp(-\mu_{a,1}(\lambda) \times L_{1,B}(\lambda) - \mu_{a,2}(\lambda) \times L_{2,B}(\lambda) + G_B(\lambda)) \]

The measured light collected at both detectors is partially influenced by each layer.

\[ OD_A(\lambda) = -\ln \left( \frac{I_A(\lambda)}{I_0(\lambda)} \right) = \mu_{a,1}(\lambda) \times L_{1,A}(\lambda) + \mu_{a,2}(\lambda) \times L_{2,A}(\lambda) - G_A(\lambda) \]

Assuming that G remains constant over time (i.e. before and after an exercise).

Similarly for: \( OD_B \)

Giving the fact that we are interested in relative changes over time, it is safe to assume that the only chromophore concentration we expect to change is hemoglobin (HbO2 & Hb).
DPF:

Calculation of DPF depends on multiple parameters such as: the homogeneity of the material, the scattering coefficients, and thicknesses of the difference penetrated layers. DPF can be obtained either experimentally using time of flight or frequency domain instruments, or computationally using Diffusion theory or Monte Carlo simulation.

System:

The signal is highly sensitive to the slightest movement of the probe holder. To further summarize, the list below demonstrates the main parameters that plays a factor in the system variability:

- Physiological (what we aim to isolate and quantify)
  2. Oxygen saturation (percentage of oxygen bounded hemoglobin vs. Hb).
- System error.
  1. Angle of fibers with respect to skin.
  2. Power intensity of light source.
    - Laser vs. Tungsten.
  4. Light leakage.
  5. White standards.
Hence, to decouple the system variability and isolate the physiological behavior of the subject being tested, we had to reduce system error to the best of our ability. To be able to trust the data, we aimed to reduce system error to the point where variability associated with physiological behavior is much higher than that associated to system error. Fig (7). Represents the initial design of the probe holder consisting of a white piece of hard plastic with multiple holes to attach the cables for light source and detectors, and two Velcro straps to hold the hard plastic at place. Giving the fact we aim to determine system ability to measure changes in blood flow in response to exercise, maintaining the position of the hard plastic and fiber angels was nearly impossible.

**Design requirements:**

In order to eliminate system error and isolate signal variation associated with physiological behavior, we set out to further develop the probe holder with correspondence to the following design requirements:
• System calibration
  1. Steady output of light source intensity
  2. No light leakage
  3. Fiber holder must be optically absorbing

• Probe holder
  1. Accounts for the two layer effect.
  2. Maintain constant fiber angle with tissue surface
  3. Ensures good skin contact with the source and detector fibers
  4. Position of fibers is exactly the same before and after exercise
  5. Minimize localized pressure by distributing the force
  6. Needs to be “cleanable”
  7. Prober alignment along the axis of the tibia

**Steady output of light source intensity:**

• Tungsten light source.

![Fiber optic bundle](image)

**Figure. 8 Tungsten light source.**

The tungsten light source fig (8), met the requirement of ultimately building an inexpensive system. Likewise the power intensity produced by the light source was stable. Nonetheless, the optical fiber bundle (10mm diameter) that came with it was
relatively heavy introducing the challenge of ever being able to conduct a continuous measurement (while exercise). In addition, depending on the position of the leg relative to the ground, the undesirable weight of the fiber bundle can enforce an unwanted moment that could potentially alter the position of the probe, hence introduce more error to the system. Furthermore, since power intensity is proportional to the surface area of the fiber bundle, the relatively large diameter of the light source can cause irritation and overheating when applied against the skin.

- **Broadband Laser System.**

  Although the broadband laser is orders of magnitude more expensive than the tungsten light source, it had to be switched on for roughly a half an hour to produce light at steady power intensity. On the other hand, light could be transmitted into the skin using a much lighter fiber optic cable (600µm diameter), one that is similar to those used in collecting the light at the detector end.

  In the interest of combining the advantages of the two light sources available, we decided to build an adapter fig (9) to mount a fiber optic cable to the tungsten light source to take advantage of its lighter weight. Arguably enough, the 600µm cables are not capable of transmitting enough optical intensity to the skin. So instead, we purchased a cable with slightly bigger diameter (1000µm) that proved to be sufficient enough.
Calibration (White & dark measurements):

Every time measurements is collected, we would also collect both dark measurements and white standards. The white standards are collected shortly after initializing the system, while the dark measurements are left towards the end after shutting down the light source. The collection of such measurement is intended to further normalize the collected optical intensity when post processing the data as follows:

$$I(\lambda)_{\text{normalized}} = \frac{I(\lambda) - I(\lambda)_{\text{dark}}}{I(\lambda)_{\text{white}} - I(\lambda)_{\text{dark}}}$$

- **Dark measurements:**

The purpose of collecting dark measurements is to account for any light leakage; that is light penetrating into the skin from sources other than the tungsten light source (i.e. sunlight or room lighting). Furthermore, once all measurements needed are collected,
one would turn off the tungsten light source and collect one last measurement for each spectrometer.

- **White Standards:**

  The purpose of collecting white standards is to measure different power intensities produced by the light source and take it into consideration. One might need to vary the intensity to compensate for the different melanin content in skin associated to different skin colors of the tested subjects. Melanin content, being a major contributor to light being absorbed in skin, determines the color of human skin. Darker skin, indicates a higher melanin content, hence higher light absorption.

  ![Figure. 10 white reflectance surface](image)

  To collect white standards, the operator would have to eliminate all sources of light in the room, shine the tungsten light source at a white reflectance surface fig (10) (white standard) and hold the detector where light is reflected. Moreover, to further enhance the system we aimed to standardize the collection the white standards. To do so, we built a box and positioned the white standards at the bottom, drilled two holes
and attached SMA connectors (one for light source and one for the detector). The white standards calibration box fig (11) was painted black such that light shined on the inner walls of the box is fully absorbed and only light reflected from the white standards is collected by the detectors.

![Figure. 11 White standards calibration box.](image)

To further validate the making of the white standards calibration box, few measurement were taken for both spectrometers as we connect the fibers, collect a measurement, and disconnect. This was performed six times for each spectrometer, while maintaining the light intensity throughout. Fig (12) below confirms the ability of the box to produce repeatable measurements, as the percent variation remains under three percent at its worst case.
Source detector distances (two layers effect):

As illustrated in fig (6), we intend to use to spectrometers detecting the transmitted light at two distinct source-detector distances. Ideally speaking, detector A is placed at a distance close enough to light source in such way that absorption of the light detected by its spectrometer is only influenced by the first layer (skin). Detector B, needs to be further away to ensure deeper penetration into the bone.

To justify the two source detector distances chosen for the BOSS system (10mm and 20mm for detectors A and B respectively) we ran the following experiments:
• **Phantom experiment:**

In this experiment, rectangular pieces of bovine cortical bone was cut out and prepared with different thicknesses (2 mm, 4mm, 5mm, and 7mm). A relatively thicker piece was placed towards the bottom. In between, tygon tubing was arranged in such way to replicate blood vessels. Alexa Fluor 750 dye (at 0.8 cm^-1 concentration) was injected into the tygon tubing. The AF750 dye possesses absorption criterion to light at wavelength (750nm). Directly above it, we positioned the already prepared rectangular pieces and took two measurements (one with empty tubes and another after injecting the dye) for both 10mm and 20mm source-detector distance. In order to isolate the effect of varied thickness with respect to different source-detector distances, the experiment was repeated four time using a different thickness (2 mm, 4mm, 5mm, and 7mm) of cortical bone above the tygon tubing. Fig (13) represents the setup of the phantom experiment.
This experiment is aimed to examine how deep can light penetrate into a homogeneous medium (bone) for different source-detector distances. As illustrated in fig. (14) below, it became obvious that at both tested source-detector distances, the effect of the AF750 dye is apparent. Although, it absorbs more light as the source detector distance is increased (20mm).

Figure. 14 Phantom results.
• **In vivo (ice on skin):**

Due to the conduction effect and heat transfer between objects that are in physical contact, skin and muscle is thought to have a higher sensitivity to the application of ice against a particular surface of the body. With that idea in mind, it is hypothesized that if one applied some ice for few minutes (~5 minutes) against the anterior surface of the tibia, blood perfused within the skin layer will have a higher rate of freezing than that in the long bone. And so, we shined light into the skin at various source-detector distances (10mm, 15mm, and 20mm). After collecting measurements, ice was applied to the skin for 5 minutes. Finally one more set of measurements was collected and later compared with the first set of measurements (before applying ice).

![Intensity before and after ice application](image)

*Figure. 15 Ice effects over the anterior surface of tibia at three source-detector distances.*
As presented in the above figure (15), for the first source-detector distance (10mm), the system detected a significant change in the signal before and after the application of ice. On the other hand, for the other source-detector distances (15mm and 20mm), the signal appeared to have no compelling evidence that the application of ice affected the signal at all. The graph above agrees with the aforementioned hypothesis. Note that dotted lines indicate the signal collected by the detector after applying the ice. It appears that when ice is applied, blood perfusion in skin (shallow layer) is weakened, as the vessels were vasoconstricted. Hence, allowing a higher transmission of light.

To conclude, we came to realize that with the smallest source-detector distance (10mm), light absorption is not solely due to blood perfused in the skin and that the signal is partially effected by the absorbance of the second layer (bone). However, due to manufacturing restriction, the first source-detector distance cannot be made smaller than 10mm without disturbing the system stability and adding to its error margin. Furthermore, by shortening the distance; we would also run into the issue of light leakage (light from source to detector without penetrating into the tissue). With that, we agreed to keep the source detector distances at 10mm and 20mm for detectors A and B respectively.
SMA connectors:

It appeared that the signal was extremely sensitive to the angle the fibers are held with respect to the skin. Therefore, in order to enhance the error margin of the system and ensure we can obtain reproducible and repeatable data, it became obvious that we had to find a way to lock the angle between the fiber and the probe. Considering the fiber optics are designed in such way that it had a male SMA connector fig(16) at its tip, it was clear all we had to do is build a new probe and attach female SMA connectors where the fibers are designed to sit. Doing such, we can be certain to maintain the exact same angle at all times.

Fig (17), is a 2D design of the mechanical dimensions of the probe to facilitate the dimensions of the SMA connectors and correspond with the source-detector distances agreed on in the preceding section.
Probe movement:

In order to validate the use of NIRS to measure blood perfusion in bones, we intend to conduct a study on abled body subjects as we collect measurements before and after a particular exercise and check to see whether or not we can detect a change. Depending on the nature of the exercise and its intensity, the probe is bound to move alongside the tibia. To compensate for such movement, we aimed to enhance the probe holder as the original two straps holding the probe at place was not sufficient enough.

Using an adhesive tape to hold the probe at place would be ideal giving the fact it does not alter the physiological behavior of blood. Whereas, the use of a sleeve might occlude the leg as it would apply a considerable amount of pressure against the skin.
However, if one decided to use adhesion, it would have to be strong enough to accommodate for the rapid movement of the leg during the exercise. The stronger the adhesive tape, the tougher it would become to remove it when all measurements are collected, especially if it is applied to hairy legs. Furthermore, since we are only interested to measure blood perfusion in bone, the occlusion effect will primarily affect the first layer (skin). When the data is further post processed, the occlusion effect should eventually subtract out to calculate the relative changes of hemoglobin volume in the tibia. Having come to this conclusion, we decided to use one of the commercially available sleeves that has a stretchable fabric to ensure the force is uniformly distributed around the tibia and enhance stability of the probe throughout the exercise. The sleeve is placed above the probe fig (19) with proper holes to allow for convenient attachment of the fibers to the SMA connectors.

Figure. 18 probe holder (sleeve version).  
Figure. 19 probe.
Test system feasibility on paraplegics:

Spinal cord injury (SCI) patients lose control of their lower extremity immediately after an injury occurs. Due to the lack of loading that undergoes the paralyzed part of the patient’s body, they end up developing osteoporosis at early age compared to abled-body subjects. In a physical exercise (rowing) by using functional electrical stimulation (FES), SCI patients are able to apply load to their legs. Here we measure the effects of this exercise on blood flow in the lower limb.

Having eliminated some of the parameters that contributed greatly to the system error, it was time we took the BOSS system to Spaulding rehabilitation hospital to test its performance on paraplegic (SCI) subjects. Doing such, we encountered the following challenges:

- The hard plastic part was slightly tilted fig (20).
- Sleeve blocked the view.
- Sleeve fixed size.
- No proof the probe remained at one position throughout the exercise.

Figure. 20 Probe misalignment over SCI subject’s tibia.
The main challenge recognized when using our NIRS system on paraplegic subjects is the lack of feedback, as they could not feel the probe pushing against their skin. On the other hand, the sleeve blocks the view and without the feedback of the subject we can never be certain the probe is aligned perfectly along the tibia.

**System Variability revisited:**

- **System error:**
  1. Light leakage.
  2. Lack of feedback (paraplegic).

With the newly introduced challenges, it became clear we needed a semi-sleeve probe holder with a transparent front surface. Additionally, to block all light leakage associated with other sources of light in the room, we designed the new probe with a piece of fabric that naturally falls on top of the transparent surface after having positioned the probe alongside the tibia. Fig (21) resembles our newest design of the probe holder.

![Figure. 21 Probe holder combining the advantages of a sleeve and an arm band.](image)
**BOSS system:**

The BOSS system fig (22) consist of the following components:

- Two Hamamatsu spectrometers.
- Tungsten halogen lamp (similar to most light fixtures).
- Three fiber optic cables.
  - One to transmit light.
  - Two to detect the scattered light.
- Plastic probe to fixate the fibers.
- Probe holder.
- White standards calibration box.
- Computer (to monitor the signal and post-process the data).
- Supporting equipment.

![Figure 22 System schematic.](image)
System validation:

After having improved the system, we ran few experiments to investigate performance of our NIRS system as follows:

- **Experiment 1.**

  Assuming the probe holder remains stationary throughout the experiment, we wanted to investigate the variability introduced into the signal due to the disconnection and reconnection of the fiber cables. Therefore, in this experiment we kept the probe holder at place, asked the subject to remain still to the best of his ability while connecting and disconnecting the fibers 10 times. The graph below, fig (23), shows that the variation of the signal due to such act does not exceed 3.5 percent throughout the NIR optical spectrum (650 – 950nm).

![Figure 23 connecting and disconnecting the fiber optics.](image-url)
• Experiment 2.

Here, we wanted to test the variability caused by moving the probe holder alongside the axis of the tibia. Doing such, we kept the fibers connected at all times and again asked the subject to remain still to the best of his ability. We drew five lines (10mm) apart alongside the axis of the tibia fig (24), and collected five measurement.

![Figure. 24 experiment 2 set up.](image)

The graph below, fig (25), shows that the variation of the signal due to probe movement remains under 10 percent throughout the NIR optical spectrum (650 – 950nm).

![Figure. 25 System variability due to probe holder movement.](image)
• **Experiment 3.**

Lastly, we wanted to investigate the ability of the new probe holder to eliminate any light leakage introduced by all other sources of light (i.e. room lighting). To do so, we collected two dark measurements 1) with room lighting on while all fibers connected and fabric covering transparent surface, 2) by screwing the cap covering the inlet end of the spectrometer. The graph below, fig (26), shows little to no leakage. Note. However the spike in both signals around the 800nm wavelength, is due to manufacturing error of the spectrometer. We contacted Hamamatsu concerning the spike and were assured that since we are normalizing all measurement with respect to both white and dark measurement, any manufacturing error should eventually cancel out.

![Graph of dark measurements spectrometer 1](image)

*Figure. 26 Light leakage after including the transparent surface cover.*
Detecting changes in blood flow:

Having reduced system error and variation, we aim to determine system ability to detect changes in the signal due to physiological behavior. We conducted four experiments:

1. **Steady elevated leg**

To evaluate the physiological behavior of blood perfusion while keeping the leg elevated to chest level for the period of 15 minutes.

2. **High intensity and high impact exercise (running)**

Considering the substantial amount of loading that undergoes the tested tibia, we expect to detect an increase in blood volume after the exercise compared to baseline.

3. **High intensity and low impact exercise (rowing)**

Due to the high impact associated with running, keeping the probe holder at exactly the same place proved to be a challenge. It was rather difficult to compare the signal after running to that collected before the exercise. Hence, we conducted the rowing experiment for its considerable equal intensity but much lower impact on the tibia.

4. **Functional Electric simulator rowing (paraplegic)**

Lastly we took the system to Spaulding rehabilitation hospital to examine the effect of FES rowing on SCI patients.
Typical signal curves as detected by spectrometer:

Figure 27 Typical normalized power intensity signal over full range of spectra at different points in time throughout a single experiment.

Fig (27) above illustrates the normalized light intensity (irradiance) within the NIR optical window at six points in time as captured by detector B. Giving the complexity of investigating the relative changes due to exercise using this many wavelength, we decided to present the physiological behavior of blood perfusion in terms of three distinct wavelengths (720nm, 810nm, and 870nm). The reason behind choosing the previously addressed wavelengths is further explained in the upcoming section.
**Data representation:**

Fig (28) represent the power intensity collected by detector B at three wavelengths (720nm, 810nm, and 870nm) over a period of time (10 minutes) for one subject. The data was normalized to time zero to examine the physiological behavior of blood perfusion for 10 minutes after running. An increase in the signal at 810nm wavelength (isosbestic point) indicates higher transmission of light, hence lower absorption administered by the tissue being tested. Assuming that lipid and water content stays constant within the short period described, such change in absorption is explained by a change in blood volume (being the only other absorber of NIR light within the biological tissue. A decrease in signal indicates the exact opposite. Variation of the signal at the other wavelengths presented is used to calculate oxygen saturation. With reference to the absorption coefficient illustrated in fig (4), deoxygenated hemoglobin
has a higher absorption coefficient before the isosbestic point. Therefore, an increase in the signal at 720nm wavelength indicates lower absorption, thus an increase of oxygen bounded hemoglobin. While detector A corresponds to changes in hemoglobin in the first layer (skin), detector B is used to capture the physiological behavior of blood in the second layer (bone).

**Subjects:**

The able-bodied subjects were all students and faculty members of the northeastern university. For the purpose of investigating the effects of the melanin contents in the skin, individuals of different skin colors were chosen. The hypothesis suggests that the darker the skin color, hence the higher the melanin content, the more light is absorbed as it penetrate the skin. In few cases, the power intensity was increased slightly to ensure enough light is collected by the detectors end.

Due to time restriction, we only received an IRB approval to conduct studies on SCI patients recently. Therefore, we could only run few experiments of two subjects. The results addressed for SCI patients concerning their FES rowing are not conclusive. However, it presents a good pilot study to evaluate the feasibility of such experiment. Future work is intended to take place to schedule more subjects for the study.

- **Steady elevated leg experiment:**

After having established a baseline, the subject is asked to extend his leg and rest it on a table at chest level. Four subjects participated in this experiment with different
skin colors to account for the different melanin contents. The subject is then asked to remain still for the period of 15 minutes to the best of his ability. During which, a measurement is collected every half a second to monitor the physiological behavior of blood perfusion due to no movement.

Figure 29 Signal behavior in three distinct wavelengths (720nm, 810nm, & 870nm) over a period of time due to steady elevated leg position at detector B.  

increase in the signal while one keeps his leg elevated for the considerable period of time. The data shows a decrease in the signal by no more than 5 percent at its most
significant point for all four subjects. The graphs shown above represents the change in light attenuation over the 15 minute time frame captured by detector B. where detector A corresponds to changes in hemoglobin in the first layer (skin), detector B is used to capture the physiological behavior of blood due to the steady elevated light in the second layer (bone).

In the steady elevated leg, variation in the signal over the 15 minutes time period was insignificant. Where blood seems to have increased as the signal declines in the first few minutes, it appears to have resumed its original state as time passes approaching the fifteenth minute. Blood perfusion within the skin shows to be evidently more stable compared to that in the second layer as illustrated by detectors A (appendix I) and B respectively.

• **Running experiment:**

Three able-bodied subjects participated in this experiment. Following a similar method as the steady elevated leg experiment. In this experiment, the subjects were asked to engage their tibia in a physical exercise at both high impact and high intensity. The subjects are asked to walk around the lab for few seconds to establish a baseline before they go for a short run. When running, the subjects must achieve 80 percent of their maximum heart rate and maintain it for three minutes before they can run back to the lab. As soon the subject arrives to the lab, the operator connects the fiber optic cables to the already strapped probe holder. A continuous measurement is then collected for ten minutes to examine the light intensity as the physiological behavior of blood perfused in the tibia trends back to normal. Due to the nature of running and its high impact on the
tibia as the leg hits the ground, we noticed that the position of the probe holder was
deliberately altered for all three subjects when they returned to the lab. Given the fact the
probe holder needed to be readjusted and aligned, the date collected before the exercise is
no longer relevant. We cannot compare to their pre-exercise baseline. So we measure
changes from the time directly after exercise.

The graphs, fig (30), illustrated below present the light captured by detector B to
examine changes taking place in the second layer. An increase in the light intensity
indicates lower absorption as the physiological behavior of blood trends back to normal
(before exercise) than immediately after the exercise.

![Graphs illustrating changes in light intensity](image)

*Figure. 30 Signal behavior in three distinct wavelengths (720nm,
810nm, & 870nm) ten minutes after running at detector B.*
The graphs shown above, illustrate an increase in the power intensity collected at detector B by 26 and 14 percent for subjects one and three respectively. However subject two experiences a decrease in power intensity of light after ten minutes by 1 percent. The results for subjects 1 and 3 agrees with the original hypothesis that by engaging the leg in a physical exercise, more blood is expected to perfuse there to compensate for the rapid increase in the demand of oxygen and nutrients by the lower extremity. Notice however at wavelength 720nm, the power intensity seems to have increased by 24, 12, and 15 percent for subjects one, two and three respectively. Such behavior indicates that the tibia was highly deoxygenated immediately after the exercise.

When engaging the subjects in a physical exercise of both high intensity and high impact on the lower extremity, results demonstrate a greater variation in the physiological behavior of blood perfusion in the tibia compared to that of steady elevated leg. Blood volume indicated by the power intensity at the isosbestic point remained fairly stable within the skin and varied by no more than 8 percent in the bone for the steady elevated experiment. On the other hand, light intensity captured by detector A for the running experiment increased by up to 14 percent as the subject recover back to normal physiological behavior ten minutes after running. Giving the challenge imposed by the high impact on the tibia of the running exercise, the probe holder appeared to have severely moved after running. Due to such challenge, the data collected before the exercise became useless, as we could no longer compare it with the measurements collected after the run. Assuming that the signal would trend back to
normal physiological behavior as time passes by and the subject recover, we can safely use the data presented for time ten minute in place for the before the exercise as we examine the changes in blood volume due to running. Based on such assumption, the result demonstrate an increase in blood volume within the skin by 14, 5, and 8 percent for subjects one, two, and three respectively. The variation in the second layer (bone) appears to be much more significant as blood increased by 26, and 14 percent for subjects one and three respectively after the running exercise. The date however, suggests that the blood volume decreased by roughly one percent in the tibia of subject two due to the exercise. We are not exactly sure as to why this is the case, but since we could not monitor the subjects during the exercise for running experiment, we can only take their words for it that they did run as instructed. The increase in the power intensity at wavelength 720nm ten minutes after the exercise implies highly deoxygenated hemoglobin in the tibia immediately after the exercise. Higher Hb in the tibia immediately after the exercise can be justified by the high demand of oxygen from the muscles during the exercise. Should we ever decide to redo the running experiment, it will have to be in a more controlled environment (i.e. on a treadmill or a physical exercise facility).

- **Rowing experiment (Able-bodied):**

Four subjects participated in the rowing experiment. Here the subjects were engaged in a physical exercise of the same intensity but a much lower impact. In the rowing experiment, the subject would set still for five minutes, rows for five and rests
for ten. Continuous measurements are collected throughout the entire 20 minutes at a half a second time interval. The subjects are asked to maintain 80 percent of their maximum HR using a Polar FT Heart Rate monitor.

The power intensity for the four able-bodied subjects is presented below, fig (31), for detector B. Furthermore, at detector B, the power intensity was decreased by 33, 24, 29, and 19 percent for subjects one, two, three, and four respectively.

Figure. 31 Signal behavior in three distinct wavelengths (720nm, 810nm, & 870nm) before, while and after rowing at detector B (able-bodied).

Having learned from challenge imposed by the high impact due to running, the goal turned into conducting a pilot study on the physiological changes in blood
perfusion within the tibia due an exercise of the same intensity but a much lower impact. Rowing seems to offer the right alternative. In rowing, a continuous measurement is collected for a total of twenty minutes including a before, while and after rowing. The results suggest a consistent increase in blood volume in the tibia for all four subjects. Since we can clearly examine the change in total hemoglobin before and after the exercise. The results implies a much higher change in blood volume in the tibia as it increased by 33, 24, 29, and 19 percent for subjects one, two, three, and four respectively. This evidently suggests that had we instead, collected measurement for a longer time frame after the running experiment, the power intensity would much probably continue to rise before it plateaus corresponding blood perfusion at resting physiological behavior.

- **Functional Electrical Stimulation (FES) rowing:**

  FES rowing fig (32) is designed to maintain cardiovascular health of subjects after a spinal cord injury. SCI patients develop muscle spasm due to the lack of movements of their lower extremities. FES rowing provides an alternative solution for SCI patients to stay active and maintain relatively healthier legs. By engaging the muscles in such physical activity, exhausting both the muscles and the heart, more blood is expected to flow into the lower extremities to feed it with nutrients and oxygen. It is hypothesized that such behavior will maintain cardiovascular health and possibly prolongs bone pathologies associated with SCI (i.e. osteoporosis and osteoarthritis).
FES rowing operates by sending electric signal through electrodes adhered to the surface of thigh muscles (hamstring and quadriceps at the back and front sides of the thigh respectively). The electric signal is set out to contract the muscles in such manner to facilitate both flexion and extension of the knee.

The rowing experiment set up for the SCI patients is similar to that of the able-bodied subjects. However, it was rather challenging getting the subjects achieve 80% of their maximum HR. Due to the limitation addressed above, we disregarded that particular part in the procedure. As getting to row for a considerable amount of time, and collecting measurements before and after the exercise is thought to be sufficient enough to test the feasibility of such study.
For FES rowing, two SCI patients volunteered to participate in the study. For the first subject a continuous measurement of a total of 34 minutes was collected. During that time the subject rowed three times and rested for few minutes in between. It appears that the signal fig (33) of power intensity collected at detector B at the isosbestic point decreased by 6 percent after rowing but eventually resumed its original value at time towards the end (time 34 minutes).

For subject two, we could not collect a continuous measurement due to size limitation of the probe holder. Roughly four minutes of measurements were collected before the FES rowing and five minutes afterward. At detector B, the power intensity fig (34) experienced a decrease by 15 percent immediately after the exercise at wavelength 810nm.

Figure. 33 Signal behavior in three distinct wavelengths (720nm, 810nm, & 870nm) before, while and after FES rowing at detector B (Subject one).
Note. We were only able to examine two SCI patients as they rowed using the FES rowing machine. Due to the time constraint and the low number of subjects participating in the study, the results do not provide conclusive findings, as they did not agree. For the first subject, the FES rowing does not appear to have altered the blood perfusion in the tibia. The power intensity collected by detector A at the isosbestic point resumes its value at time zero shortly after the subject stops rowing or takes a short break. The signal corresponding to the isosbestic point at detector B, suggests an increase in blood volume in the tibia by 6 percent shortly after the subject takes a short break. However the signal captured by detector B appears to have plateaued to its original value at time zero towards the end of the exercise.

The data collected for subject two resembles those obtained for able-bodied subjects. While we could not obtain continuous measurement (before, while, and after)
due to size limitation of the probe holder, we disconnected the fiber optics while the subject rows. After rowing, a continuous measurement of five minutes was collected by both detectors. The data suggests a decrease in the power intensity after rowing. The increase in the blood volume implied by the data collected in the skin (appendix I) is surprisingly higher than that in the bone. Note however, subject two is classified as a chronic SCI patient as he was injured more than ten years ago, thus the leg bone density has greatly declined compared to the acute patient (subject one). One can clearly notice the higher content of skin and lipid above the anterior surface of subject two’s tibia compared to the able-bodied subjects undergoing the rowing experiment.

**Conclusion:**

Due to the high correlation between bone health and its blood perfusion, and the limited resources available in the medical field, we set out to develop a system that can measure blood perfusion in bone. NIRS is an established technique to measure hemodynamics of soft tissue (muscles). Recent developments aimed to illustrate its usefulness in detecting brain activities since due to the fact NIR is extremely safe and non-invasive. In addition, NIRS has proven to be comparatively inexpensive and capable of producing practical and instant measures and results.

In pursuance of keeping the variability at its minimum, we chose the tibia because of the shallow layer of lipid and skin towards its anterior surface. Doing such we can confidently explain the variation in the power intensity of the near infrared region to be due to changes in the total hemoglobin content and its oxygen saturation. After
having eliminated the error imposed by the system, we ran four different experiments. At first, we analyzed the physiological changes in blood perfusion due to rising one leg and keeping it steady for a short period of fifteen minutes. While blood volume seemed to have not changed much when a person raises his or her leg to chest level, the running experiment that follows illustrated an increase in blood volume in the tibia due to running. The high impact associated with the running experiment altered the position the probe holder along the axis of the tibia, thus it was hard to compare the measurement collected before the exercise to that collected afterward. Lastly we aimed to repeat the exercise experiment with rowing instead of running. The probe holder remained at place throughout for all participating subjects. The results suggested a consistent increase in blood volume due to the rowing exercise. The magnitude of the of the blood volume change due to rowing suggests that if we were to collect measurement for a longer period after running, the signal would have continued to raise indicating a higher change in blood volume within the tibia due to running.

The system developed can only measure relative changes in blood perfusion over a short time frame (i.e. due to cooling of the surface or engaging the area into a physical exercise). It also, assumes that the water content and lipid stays constant. Where this may be accepted for relative changes over a short period of time, it might not apply if measurements taken in a longer time frame are to be compared. The BOSS system does not address the index of refraction mismatch between the fibers and skin. Lastly, due to manufacturing constraints, the first source-detector distance (10mm) is
longer than we had wished for it to be. With a 10mm source detector distance, although
the first layer influences majority of the light, some of it is altered by the bone as well.

Future work is necessary to enhance the system and calculate absolute
measurements as oppose to relevant ones. A computational model is being developed
that uses MCX simulation to obtain the differentiated path length factor, and then input
the obtained values in a modified beer lambert law code to calculate both the absolute
total hemoglobin and the oxygen saturation index.

The existing probe holder can only facilitate a small range of cross sectional
areas of legs. At least two more probe holder needs to be built to accommodate big
cross sectional areas and more importantly thinner ones to further facilitate SCI
patients. If the clinical study for SCI patients proceeded, a careful selection of the
subject is necessary ensuring they are all either acute or chronic patients.

In conclusion the BOSS system developed proved it is capable of measuring
changes in blood perfusion in bones. The system has the potential of becoming the gold
standard of monitoring bone health and ultimately bone growth. The BOSS system can
be used to assess bone pathologies associated with blood perfusion (i.e. osteonecrosis,
osteoporosis, osteoarthritis, non-union etc.) in a fast, inexpensive, and non-invasive
manner. Hence, maintain bone health for both able-bodied and SCI patients.
Appendix I:

BOSS system

Figure 3: BOSS system.
Tibia cross sectional area (left leg)

Figure. 36 Tibia cross sectional area of left leg.
Steady elevated leg experiment results for detector A:

The graphs above fig (37) represent the power intensity collected by detector A at three wavelengths (720nm, 810nm, and 870nm) over a period of time (15 minutes) for a total of four subjects.

Figure. 37 Signal behavior in three distinct wavelengths (720nm, 810nm, & 870nm) over a period of time due to steady elevated leg position at detector A.
Running Experiment results for detector A:

In this experiment fig (38), it appears that the power intensity of near infrared light collected by detector A increased by 14, 5, and 8 percent for subjects one, two and three respectively ten minutes after running.

Figure. 38 Signal behavior in three distinct wavelengths (720nm, 810nm, & 870nm) ten minutes after running at detector A.
Rowing Experiment results for detector A:

The intensity at detector A fig (39) decreased by 24, 22, and 9 percent for subjects one, two and four respectively immediately after the exercise compared to time zero. In subject three; the intensity was increased 4 percent after rowing normalized to time zero.

Figure 39: Signal behavior in three distinct wavelengths (720nm, 810nm, & 870nm) before, while and after rowing at detector A (able-bodied).
**FES rowing results for detector A:**

It appears that the signal fig (40) of power intensity collected at detector A increased by 2 percent for after rowing and finally by 4 percent at time 34 minutes compared to time 0.

![Figure 40 Signal behavior in three distinct wavelengths (720nm, 810nm, & 870nm) before, while and after FES rowing at detector A (Subject one).](image)

At detector A fig (40), the power intensity experienced a decrease by 28 percent immediately after the exercise at wavelength 810nm.

![Figure 41 Signal behavior in three distinct wavelengths (720nm, 810nm, & 870nm) before, while and after FES rowing at detector A (Subject two).](image)
Measurements protocol

System Initiation:

1. Turn on the light source on at 60% optical density
2. Wait at least 3 minutes before collecting any measurements.
3. Collect white standards for both spectrometers with 10ms integration time and save as white standards (pa_white & pb_white).
4. Set parameters (integration time for the two spectrometers)
   - Assign spectrometer one (10 mm away from light source) integration time of (10ms)
   - Spectrometer two (20mm) integration time of (100ms)
   - for the purpose of matching the overall period and number of date of the measurements collected by different spectrometers, set parameters for spectrometer (one) in such a way that it averages every 10 measurements into one (10 X 10ms = 100ms).
5. Edit measurement’s spectra (for a 15 minutes (900,000ms) continuous measurements, we would need that divided by 100 (highest int time), thus 9000 measurement).

Steady Elevated Leg experiment:

1. Place the probe holder on subject’s leg.
2. Ensure proper alignment of three holes in the hard plastic along the axis of the tibia.
3. Tighten the Velcro strap around the calf to make sure the probe stays at place throughout the entire experiment.
4. Have the subject walk around the room for few seconds (~15s) to establish a base line.
5. Seat down the subject and cover the transparent area of the probe to eliminate light leakage.
6. Connect the fibers to the SMA connectors (light source towards the distal end of the probe.
7. Subject should have his leg extended and raised to chest level.
8. Allow 20 seconds for signal to stabilize.
9. Assuming the parameters are set up for both spectrometers, click the "measure" button.
10. Ensure little to no movements of subject’s leg.
11. After 15 minutes, screen should make a beeping noise indicating that all measurements have already been collected.
12. Save both sets of data in a different excel file with proper naming ("pa" for 10mm spectrometer, & "pb" for the other one).

13. Turn off the light source and collect a single measurement for both spectrometers and save as dark measurement.

14. Disconnect the fibers and remove the probe holder from subject's leg.

Running experiment:
1. Place transmitter for Heart rate monitor around subject's chest.
2. Ensure HR signal is projected on the watch.
3. Place the probe holder on subject's leg.
4. Ensure proper alignment of three holes in the hard plastic along the axis of the tibia.
5. Tighten the Velcro strap around the calf to make sure the probe stays at place throughout the entire experiment.
6. Have the subject walk around the room for few seconds (~15s) to establish a base line.
7. Seat down the subject and cover the transparent area of the probe to eliminate light leakage.
8. Connect the fibers to the SMA connecters (light source towards the distal end of the probe.
9. Subject should have his leg relaxed and normal to the ground).
10. Allow 20 seconds for signal to stabilize.
11. Assuming the parameters are set up for both spectrometers, click the "measure" button.
   • Note. Collect 10s worth of date before asking the subject to go for a run.
12. Ensure little to no movements of subject's leg.
13. After 10 seconds, screen should make a beeping noise indicating that all measurements have already been collected.
14. Save both sets of data in a different excel file with proper naming ("pa_b" for 10mm spectrometer, & "pb_b" for the other one).
15. Disconnect the fibers, but keep the probe holder attached to subject's leg.
16. Subject should go for a short run (~7-10 minutes).
   • Subjects needs to achieve 80% max heart rate and maintain it for a period of 2 minutes.
17. Utilize the time and edit the measurement spectra.
• 1 minute (60,000ms) continuous measurements, divided by 100 (highest int time), thus 600 measurements.

18. Subject needs run back to the lab to effectively catch the physiological changes associated with the exercise.

19. Have the subject seated on the same chair with leg relaxed and normal to ground.

20. Quickly connect the fibers back to SMA connectors.

21. Click the "measure" button.

22. Ensure little to no movements of subject’s leg.

23. After 1 minute, screen should make a beeping noise indicating that all measurements have already been collected.

24. Save both sets of data in a different excel file with proper naming (“pa_a” for 10mm spectrometer, & “pb_a” for the other one).
   • Note (_b) for before the exercise, and (_a) for after.

25. Turn off the light source and collect a single measurement for both spectrometers and save as dark measurement.

26. Disconnect the fibers and remove the probe holder from subject’s leg.

**Rowing experiment:**

1. Place transmitter for Heart rate monitor around subject’s chest.

2. Ensure HR signal is projected on the watch.

3. Place the probe holder on subject’s leg.

4. Ensure proper alignment of three holes in the hard plastic along the axis of the tibia.

5. Tighten the Velcro strap around the calf to make sure the probe stays at place throughout the entire experiment.

6. Have the subject walk around the room for few seconds (~15s) to establish a base line.

7. Seat down the subject on the rowing machine and cover the transparent area of the probe to eliminate light leakage.

8. Connect the fibers to the SMA connecters (light source towards the distal end of the probe.

9. Allow 20 seconds for signal to stabilize.

10. Assuming the parameters are set up for both spectrometers, click the "measure" button.
11. Note. Collect 180s worth of date before asking the subject to start rowing.

12. Ensure little to no movements of subject’s leg.

13. After 180 seconds, screen should make a beeping noise indicating that all measurements have already been collected.

14. Save both sets of data in a different excel file with proper naming ("pa_b" for 10mm spectrometer, & "pb_b" for the other one).

15. Disconnect the fibers, but keep the probe holder attached to subject’s leg.

16. Subject should start rowing (~5 minutes).
   - Subjects needs to achieve 80% max heart rate and maintain it for a period of 2 minutes.

17. Utilize the time and edit the measurement spectra.
   - 10 minute (600,000ms) continuous measurements, divided by 500 (highest int time), thus 1200 measurements.

18. When subject is done rowing, leave the subject seated on the same chair with leg relaxed.

19. Quickly connect the fibers back to SMA connectors.

20. Click the "measure" button.

21. Ensure little to no movements of subject’s leg.

22. After 10 minute, screen should make a beeping noise indicating that all measurements have already been collected.

23. Save both sets of data in a different excel file with proper naming ("pa_a" for 10mm spectrometer, & "pb_a" for the other one).
   - Note (_b) for before the exercise, and (_a) for after.

24. Turn off the light source and collect a single measurement for both spectrometers and save as dark measurement (pa_dark & pb_dark respectively).

25. Disconnect the fibers and remove the probe holder from subject’s leg.

26. Use disinfecting wipes to clean the probe holder and the heart rate monitor.
References:


