Computational Model of Capillaries: The effect of microvascular geometry on hemoglobin measurements

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**Introduction:**

The microvasculature of the systemic circulatory system is composed of a complex network of capillaries, branching from arterioles and ending in venules. Capillaries have a diameter of 8um and wall thickness of 1um (7). These dimensions of each individual capillary and the large amount of capillaries allow for effective diffusion. The main function of these capillaries is that it acts as a site of molecule exchange, where vital molecules are diffused in and out between the blood and the surrounding tissue. Blood is composed of plasma, leukocytes, platelets and erythrocytes. All erythrocytes contain hemoglobin, a multi-subunit globular protein composed of two alpha and beta subunits, each with a heme group (8). In this heme prosthetic group, oxygen binds to the iron at the center. For hemoglobin there are generally two different states of oxygen saturation. Either oxygenated hemoglobin (HbO₂) and deoxygenated hemoglobin (Hb). The ability of knowing the saturation of hemoglobin in a capillary is important in knowing the distribution of oxygen in a certain block of tissue surrounding the capillary. This information of the saturation is useful in obtaining data of flow rates, hematocrit levels and oxygen diffusion in the tissue. By modeling the saturation of hemoglobin in specific tissues, this can be used to compare different methods of obtaining saturation data and to determine the required sensitivity of these methods. In a model, the exact saturation of a specific volume of tissue can be determined from the known hematocrit and saturation levels in capillaries. Knowing the exact saturation of the specific volume of tissue, different methods can be modeled to compare the accuracy of calculating the oxygen saturation level. The main objective of this project is to create a computational model that accurately estimates the HbO₂ saturation of a single capillary and to investigate the effect of stacked capillary geometry on the sensitivity of estimating HbO₂ saturation. By creating an accurate model, it can be used to model certain disease states and to determine how the heterogeneous geometry of a capillary network in tissue can affect the accuracy of estimating oxygen saturation. By determining this, it can be a start of integrating, further improve and discover various methods of measuring saturation levels with *in vivo* tissue samples.

**Theory:**

The basis of this model in obtaining the mean oxygen saturation of a capillary containing tissue is from the dual wavelength computer-aided videodensitometric method (1). This method involves shining a light on to a thin slice of tissue at two different wavelengths where there are different interactions of the hemoglobin. The absorption of light for any solution, in this case hemoglobin, can be determined from Beer-Lambert’s Law.

\[
\text{OD} = \log_{10}(\frac{I_0}{I}) = \varepsilon \cdot c \cdot d \quad [1]
\]

Where absorbance (which is equivalent to optical density (OD)) is equal to the log of the ratio of the initial incident light and the transmitted light. This is also
equal to the product of $\varepsilon$ the extinction coefficient (mM$^{-1}$cm$^{-1}$), $c$ the concentration of the solution (mM) and $d$ the optical path length (cm). In this equation, there also is a factor added which takes into account of light scattering. Light scattering is mainly due to the non-uniformity of the tissue but also the geometry of erythrocytes, their size and shape. Since we are interested in the calculating the saturation ($S$), it can be quantified by taking the ratio of the optical densities at two specific wavelengths. These wavelengths are specifically chosen so that one at a specific wavelength there is a maximum difference in the extinction coefficients for Hb and HbO$_2$. This wavelength would be oxygen dependent wavelength where the HbO$_2$ will be detected while the other wavelength used would be the isosbestic wavelength. The isosbestic wavelength is the specific wavelength where the absorption for both oxygenated and deoxygenated hemoglobin is the same, and this would be oxygen independent. There is a linear relationship between the hemoglobin oxygen saturation and optical densities. These wavelengths were taken from Ellsworth et al. (2) and were determined to provide the adequate sensitivity and contrast of the erythrocytes the surrounding tissue. The saturation calculation is a simply linear relationship,

$$S = b + a\left(\frac{OD_0}{OD_1}\right)$$  \[2\]

The constants $b$ and $a$ are dependent on the extinction coefficients for Hb and HbO$_2$ at the oxygen dependent and isosbestic wavelengths. This is equation provides the estimated oxygen saturation of hemoglobin in the tissue based on optical density calculations.

**Methods:**
MATLAB was used to create. Very basic code was provided as a template. This code included a single capillary in the center of tissue slab. The model allowed for various inputs of many variables, such as the physical dimensions of the tissue block, the radius, hematocrit and saturation of the capillary. The values of the extinction coefficients, oxygen dependent and isosbestic wavelength and constants in the saturation equation were obtained from Ellsworth et al. Since all the values are experimental, a linear saturation gradient was set in the model to optimize constants for the extinction coefficients. By setting the arteriolar and venule saturation levels, a linear gradient is assumed and the saturation is measured at the middle of the length of the capillary. This is done to check whether the calculation of the mean saturation of the entire tissue is the equal to the actual exact value of the mean saturation of the capillary. The extinction coefficients were optimized until the exact mean saturation of the capillary matched the mean saturation calculation determined from using the optical densities. The resolution of the model was 50 intervals in the x and y axes and 200 along the z axis. For this model, the oxygen dependent wavelength used was 431nm and the isosbestic wavelength was 420nm. (2) For the calculation of the oxygen saturation in this model, the scattering of light was assumed to be zero. The oxygen saturation calculation from this specific information takes ratio of the OD at 431nm and OD at 420nm. For calculating the
individual OD’s, for each point of each interval the OD is calculated in the y-direction, taking all values of x and z in the model, shown in Figure 1.

Figure 1a. 
Basic MATLAB model of single capillary in specific tissue volume specified by xyz axes and showing the direction of incident light and transmitted light used to determine the oxygen saturation of the hemoglobin by using equation [2].

In the model, \( b = 1.78 \) and \( a = -1.56 \) of equation [2] were used. These were determined from the Ellsworth et al. (2) These values were implemented into the code to calculate the mean oxygen saturation of the tissue slab.

The next step was to implement additional capillaries into the model. This was done in a way that the specific locations of each capillary could be easily modified so different spatial geometries of the capillaries can be arranged to see how accurate the estimation of oxygen saturation is to the actual saturation of the specific tissue volume. For each capillary the linear saturation gradient was applied to test the code whether if the mean saturation of the capillary is equal to the saturation calculated at the middle of its length. In the case of two capillaries in the specific tissue volume, a single capillary with fixed parameters (hematocrit, saturation and radius) is used as a reference. As each capillary is added, the mean saturation calculations are based from fixed parameters. Then the second capillary is added so that it is stacked on top of the first capillary, in the y-direction, shown in Figure 1b. With the situation when the capillaries are stacked, the estimated mean saturation measurement based from the optical densities is calculated and compared to the actual mean saturation. Then the parameters of each capillary are changed to model the heterogeneity of the capillary network to see how different values of hematocrit, saturation affect the estimation of the mean saturation of the tissue. This was done exactly in the same way with three capillaries, by having all three stacked on top of each other in the y-direction and changing the saturation and hematocrit of each capillary.
The MATLAB code also included the output of the finite sensitivity based on the optical density calculations of each corresponding wavelength, Figures 2-6. From these graphs, it displays the required sensitivity for accurately estimating the saturation. The sensitivity is represented as the logarithmic ratio of the minimum transmitted light intensity to the incident light. This sensitivity factor is used to determine the certain minimum amount of light that can be detected in order for the estimation of the mean saturation to be accurate. With this output, for each of the different capillaries’ spatial arrangements, the accuracy or required sensitivity to make a proper estimation of the saturation can be determined. This will also show the direct effect of the spatial geometry of capillaries on the accuracy of estimating the mean saturation from optical density measurements. The cut-off of the sensitivity is determined from taking the point where the slope begins to change. This point means that there any point further toward 0 of the x-axis (toward the right) has increasing error in estimating the mean saturation of the tissue.

**Results:**
In the case of two capillaries, comparing the case of no overlap and overlapping (stacked) capillaries, there is a large difference in the cut-off values.
obtained from the two different geometries. The case without overlap had a cut-off value of -9 while for the case with overlap the cut-off was -16. This shows the large change in the required sensitivity and possible error in estimating the exact hemoglobin saturation. This is shown in Figure 2a and 2b, respectively.

In the case of three capillaries, when there was no overlap, the cut-off value was -9. By keeping the hematocrits of the capillaries fixed, changes in the saturation levels did not affect cut-off of the finite sensitivity but had a very small impact on the difference in the calculated estimation of the mean saturation and the actual exact mean saturation of the capillaries in the tissue. This was done by running the code multiple times and reassigning different saturations of each capillary. For all the cases, the cut-off was -25, shown in Figure 3.

In the case where the saturations of each capillary were fixed and the hematocrits varied, the saturation estimation and exact calculation differences did not change, but the finite sensitivity cut-off did change. A trend that was observed was that when the hematocrit was lower in any of the capillaries, the cut-off increased, resulting in a lower required sensitivity, this was done by comparing multiple trials of changing the hematocrit levels, shown in Figures 4a and b. The opposite occurred when the hematocrit levels in any of the capillaries increased, the cut-off decreased, resulting in a higher required sensitivity, shown in Figures 5a and b. This shows in the case of overlapping capillaries, when increases in hematocrit require higher sensitivity and are more prone to error in estimating the mean saturation level of the tissue. Vice-versa when there is a lower hematocrit, the required sensitivity would be lower and less prone to error in estimating the mean saturation level of the tissue.

The integration of both variances in the saturation and hematocrit was also done plus incorporating different linear saturation gradients along the z-axis, in each individual capillary. With the increased heterogeneity of the capillaries’ parameters, there was a decrease in the cut-off and a higher required sensitivity is needed for highly heterogeneous capillary geometry and properties, shown in Figure 6. By combining both variability in saturation and hematocrit levels in the individual capillaries, it greatly increases the required sensitivity for an accurate estimation of the mean saturation by optical density measurements.

An additional note is that when the resolution of the block either increased or decreased, the estimated saturation measurement did not change. Only the finite sensitivity decreased when an extremely coarse resolution was used (2x2x4). In this case, two capillaries would occupy one voxel that resulted in a lower cut-off that would result in a higher required sensitivity to measure the oxygen saturation.

**Discussion:**

The stacked geometry directly causes an increase in error at small cut-offs. The required sensitivity is higher, especially in more heterogenous arrangements.
This shows that there is greater possible error in estimating the mean saturation of the tissue. Knowing this information gives a starting point to understand the importance of having adequate sensitivity in recording devices with a receiver to lower the potential error in calculating the mean saturation. With this information, better models and experimental approaches can be developed to further increase the accuracy of measuring the saturation in tissue samples. By determining the finite sensitivity of different cases, a certain threshold could be made to set a requirement for any measurement being taken that would be used in the future for measuring capillary blood dynamics and oxygen saturation in the surrounding tissue. From the sensitivity cut-offs, it can act as a guide to what appropriate thresholds should be applied when collecting data in an experimental setting and also to provide specific conditions if the microvascular geometry of a sample tissue is known. Possibly combining this information with other imaging techniques, such as spectroscopy, spectrophotometry and photoacoustic microscopy, it can allow for new experimental techniques and methods to be created to measure oxygen saturation of in vivo tissue.

Since microvasculature is one of the most important sites in our body due to its function of molecule exchange, further understanding how the oxygen saturation of a tissue changes in different situations can be extremely useful in predicting certain states the tissue may be in. Once an adequate model is formed, various parameters of the capillaries can be modified to simulate disease states, which can be used to predict and estimate oxygen distribution and saturation within a tissue. The implications of this can benefit various areas of clinical research. This model only uses very simple, parallel arrangements of capillaries but using the data acquired from this simple model, that information could be applied to more complex geometries. Providing a starting point in accurately modeling the coiled capillary networks in liver tissue.

This model has limitations and inaccuracies due to many assumptions that were made, the assumption of ideal flow of erythrocytes through an idealized, cylindrical capillary and uniform distribution of hemoglobin in the capillary. This model only had geometries for identical parallel capillaries, this is applicable to skeletal muscle but for other capillary networks that are specific to tissue, this model does not take into account of more complex geometries. Another large assumption that was made was that the saturation gradient within the tissue decreases linearly across the length of the capillary. This is not the case, knowing Fick’s laws of diffusion; the profile of oxygen levels in surrounding tissue is not linear, which means the profile of hemoglobin oxygenation in the capillary is not linear.

From the results produced by this model, it can be further improved to have the potential to be applicable to clinical settings, discovering new experimental methods and for determining specific thresholds when obtaining this data using optical density measurements. These are all important to help further research and understanding how to measure oxygen saturation in microvasculature. By knowing the methods and requirements needed to accurately model single capillaries and simple geometries, the information obtained can allow for better methods and approaches when measuring oxygen saturation in larger tissue samples that have
more capillaries. In these cases, it will very important to understand how the spatial geometry of capillaries in tissue affects the accuracy in measuring the saturations in the specific tissue since the samples would be larger in size, containing more capillaries.

**Conclusion:**
The main purpose of this project was to create a computational model through MATLAB that accurately estimates the HbO2 saturation for stacked capillaries from optical density calculations at two specific wavelengths and to see the effect of the stacked geometry of capillaries in the required sensitivity to accurately estimate the HbO2 saturation of the tissue. From this project, the model determined that the stacked geometry of capillaries in parallel directly increases the error in estimating the mean oxygen saturation for the tissue. Also the heterogeneity of the capillaries, in terms of differences in hematocrit, individual capillary saturation and the saturation gradient, all cause a large increase in error in estimating the saturation of the tissue. However, changing the resolution of the tissue volume does not change the calculation of the saturation unless an extremely coarse resolution is used.

**References:**


**Appendix:**
Figure 2a.
Oxygen Saturation for two capillaries without overlap, with cut-off value at -9.

Figure 2b.
Oxygen saturation for two capillaries with overlap, with cut-off at -16.
Figure 3.
Oxygen saturation for three capillaries with overlap, same resulting graph for all cases in rearranged individual saturations of each capillary, with cut-off at -25. All capillaries with fixed hematocrits but different saturations in each capillary.

Figure 4a.
Oxygen saturation for three capillaries with overlap, with cut-off at -23
Figure 4b. 
*Oxygen saturation for three capillaries with overlap, with cut-off at -18*

Figure 5a. 
*Oxygen saturation for three capillaries with overlap, with cut-off at -34*
Figure 5b.
Oxygen saturation for three capillaries with overlap, with cut-off at -41

Figure 6.
Oxygen saturation for three capillaries with overlap, all with different hematocrits and saturation gradients, with cut-off at -46
Figure 7. MATLAB output of single capillary representing the optical density for the tissue at the isosbestic wavelength of 420nm. Linear saturation gradient, 0.8 at the arteriolar end (left) to 0.2 at the venule end (right).

Figure 8. MATLAB output of single capillary representing the optical density for the tissue at the oxygen dependent wavelength of 431nm. Linear saturation gradient, 0.8 at the arteriole end (left) and 0.2 at the venule end (right).
Figure 9.
*Transverse cut of tissue slab in the middle showing two capillaries with different saturations (0.3 and 0.6) not in overlap*

Figure 10.
*Transverse cut of tissue slab in the middle showing two capillaries with different saturations (0.3 and 0.6) in overlap*
Figure 11. Transverse cut of tissue slab in the middle showing three capillaries with different saturations (0.3, 0.6 and 0.4) not in overlap.

Figure 12. Transverse cut of tissue slab in the middle showing three capillaries with different saturations (each with different saturation gradient, corresponding to Figure 6), in overlap.