Modeling Oxygen Transport in Three-Dimensional Capillary Networks

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Graduate Program in Medical Biophysics
A thesis submitted in partial fulfillment of the requirements for the degree in Doctor of Philosophy
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MODELING OXYGEN TRANSPORT IN THREE-DIMENSIONAL CAPILLARY NETWORKS

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by

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Graduate Program in Medical Biophysics

A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy

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The thesis by

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Modeling Oxygen Transport in Three-Dimensional Capillary Networks

is accepted in partial fulfillment of the requirements for the degree of
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Chair of the Thesis Examination Board
ABSTRACT

The purpose of this thesis was to examine how the use of real three-dimensional (3D) capillary network geometries affect models of oxygen transport to tissue. In order to generate complex maps of capillary geometry a software package was developed that was capable of reconstructing microvasculature in 3D from intravital video sequences of blood flow. Characterization of the resulting 3D reconstructions demonstrated that capillary density, length and capillary diameter were consistent with previous findings using other measurement methods. Using reconstructed networks as a framework a strategy was devised that utilized red blood cell (RBC) supply rate (SR) as a target metric for blood flow modeling. Applying the RBC SR based flow model on baseline and perturbed flow conditions demonstrated that RBC SR is a major determinant of oxygen delivery that is insensitive to changes in flow redistribution provided total RBC SR is maintained. The resulting flow solutions were used as the basis for comparing oxygen transport in reconstructed 3D networks and synthetic parallel capillary arrays. A variety of physiological conditions were simulated using reconstructions and parallel arrays and it was determined that parallel arrays resulted in oxygen transport solutions with higher mean \( \text{PO}_2 \) due to the homogeneous distribution of vessels longitudinally throughout the network volume. Lastly, to investigate oxygen transport in a complex pathology a model of sepsis was used that tested how incremental perfusion loss, change in consumption and change in RBC SR affect oxygen delivery to diseased tissue. It was shown that incremental perfusion loss did not markedly impair oxygen delivery provided that total RBC SR was maintained. However, a two fold increase in oxygen consumption, similar to
that estimated for sepsis, combined with a loss of perfused capillaries necessitated up to a 4 fold increase in RBC SR to return tissue PO$_2$ to baseline levels. These results have improved our understanding of oxygen transport to tissue in both normal and diseased conditions; it is clear that the use of reconstructed network geometries and direct measurements of blood flow and oxygen saturation in computer models provide different solutions than those arrived at using statistical averages and synthetic networks.

**Keywords:** Blood Flow, Capillary, Computer Modeling, Oxygen Transport, Red Blood Cell, Sepsis
CO-AUTHORSHIP

This thesis contains the following manuscripts that have been published, accepted, or in preparation for submission:


These studies were designed by GM Fraser and CG Ellis. The following co-authors provided experimental and technical support for the studies listed above:

S Milkovich performed a portion of animal surgeries and applied network reconstruction software in Study 1.

D Goldman provided blood flow and oxygen transport modeling software and advised on the application and development of modifications to this software that was used in Studies 2, 3 & 4.

M. Sharpe provided technical expertise, opinion and perspective, based on his role as a clinician treating patients with sepsis for Study 4.

All data analysis, interpretation, and the drafting of manuscripts for the current thesis was completed by GM Fraser. Co-authors also contributed editorial feedback as needed.
ACKNOWLEDGEMENTS

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DEDICATION

This thesis represents the culmination of a journey filled with hard work, persistence and personal sacrifice. There are several people that have helped make this thesis possible and I would like to offer all of them my sincere thanks. To Chris, thank you for openly sharing your knowledge and passion with me, it has been an inspiration that I will strive to pass on. As a mentor you have been a superlative example, giving me the freedom to pursue my own ideas while providing me with direction when I needed it most. To my friends outside of the academic world, you offered support to frustrations you did not necessarily understand and a much needed escape from the empirical and theoretical world of research. To those friends I have made here at UWO, thank you for being there when times were tough, weathering the many challenges we faced together made success possible and more satisfying. To Steph, thank you for your wisdom and friendship, your support helped to shape all of my work. To my family, Sharon, Don, Chris and Leslie, you have always been the foundation of support upon which my entire life has been built; without your love and encouragement this journey would not have been possible. Finally to Jen, you fill my world with joy and laughter, making every day feel rewarding and complete. I look forward to facing the future that lies ahead together, with you.

Graham M Fraser

2012
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LIST OF ABBREVIATIONS SYMBOLS AND NOMENCLATURE

3D – Three-Dimensional
EDL – Extensor Digitorum Longus
GUI – Graphical User Interface
Hb – Hemoglobin
HbO₂ – Oxy-Hemoglobin
Hct - Hematocrit
Hct₀ – Discharge Hematocrit
Hctₗ – Tube Hematocrit
Io – Incident light intensity
IVVM – Intravital Video Microscopy
NRBCSR – Network Red Blood Cell Supply Rate
O₂ – Oxygen
OD – Optical Density
PO₂ – Partial Pressure of Oxygen
RBC – Red Blood Cell
SR – Supply Rate
STI – Space-Time Image
VO₂ – Oxygen Consumption Rate
CHAPTER 1: INTRODUCTION

1.1 Purpose and Hypotheses

Complex organisms deliver oxygen to living tissue through convective transport of oxygen rich blood in the vascular system and diffusive transport of oxygen within the tissue itself. Any impairment or challenge to oxygen delivery can compromise the normal function of tissue and cause hypoxic damage at the cellular, tissue, and organ level. An understanding of convective and diffusive oxygen transport in the microvasculature under normal conditions will elucidate how pathological conditions affecting oxygen transport impact \( O_2 \) delivery to tissue. Furthermore, a thorough understanding of oxygen delivery may aid in determining methods to prevent, reverse or minimize tissue damage caused by impaired oxygen transport.

The geometry and density of microvessels within living tissue is one of the primary determinants of oxygen delivery under normal and increased metabolic demand (38). Specific vascular morphology varies substantially both within areas of the same tissue and between different types of tissue. The general structure of arterioles branching into smaller order vessels and terminating as capillary networks is common across several metabolically active tissues particularly skeletal muscle. Vasculature in skeletal muscle must be capable of meeting a wide range of oxygen demand and as such capillary networks must be configured in such a way to address oxygen delivery both at rest and under working conditions. Early studies on oxygen delivery to skeletal muscle focused
on the density of microvessels and from these measurements proposed the average theoretical area each capillary could provide oxygen to and maximum radius for diffusion under different metabolic condition (38). Absolute capillary density is a valuable measurement that can be used to indicate a tissue’s capacity for oxygen delivery, however, given the marked complexity of networks in vivo and the variable density observed within tissue it would be desirable to directly reproduce microvascular networks for the purposes of mathematical simulations of oxygen delivery given specific network geometry. Specific vascular densities have been represented digitally utilizing various types of parallel vessel arrays (27), though it is unclear how these artificial networks compare with respect to oxygen delivery in actual capillary morphologies as observed in vivo. Precise models of microvascular networks has been achieved through the use of corrosion casting, confocal microscopy and laser scanning techniques (31) though no single methodology is capable of simultaneously acquiring the necessary data to thoroughly study oxygen delivery.

In addition to characterizing the spatial organization of vascular structure in tissue for the purposes of understanding diffusive oxygen transport it is also critical to quantify the volume and potential oxygen delivery to a volume of interest. Oxygen carrying red blood cells (RBC) are fundamental in establishing the concentration gradient that drives diffusive oxygen transport into tissue adjacent to microvessels. Previous efforts have focused on oxygen carrying capacity in terms of blood flow, velocity, and hematocrit to establish a metric for oxygen transport in blood vessels (49). The approaches that have been undertaken to rationalize models of blood flow with experimental measurements
have been fraught with challenges specifically due to the practical difficulty of collecting flow data from numerous blood vessels in a dynamic and unsteady state system. This is particularly a problem in the microvasculature where oscillations, changes in flow distribution, and flow heterogeneity can further confound efforts to quantify blood flow for the purposes of modeling oxygen transport. The transit of RBCs can be more simply represented in terms of RBC supply rate (SR), in cells/s, which can be used to simplify the problem of quantifying convective oxygen transport to tissue particularly in small volumes where diffusion distances between vessels are small. RBC SR combined with measurements of oxygen saturation of hemoglobin within the red cells can be used to quantify how much oxygen is available to diffuse from blood vessels to living tissue by means of maintaining the blood oxygen concentration.

Perturbations to basal oxygen delivery are common physiologically and represent a complicated system, the dynamics of which are still not well understood. Changes in metabolic demand, blood flow, intermittent hypoxia and vascularization changes over time will markedly affect oxygen delivery to skeletal muscle. In addition to normal fluctuations there is a wide range of pathologies and insults that can affect delivery of oxygen to affected tissue. Greater understanding of diseases that can damage living tissue due to insufficient oxygen is of particular interest in medical research.

In the interest of furthering our understanding of oxygen transport, several projects were conceived. The studies outlined below include efforts to develop new tools
and methodology, as well as the application of these strategies to examine oxygen transport using physiologically representative computer models.

**Study 1: Mapping functional capillary geometry in rat skeletal muscle.**

*Purpose:* To create a software package capable of mapping three-dimensional functional microvasculature imaged using intravital video microscopy.

*Goal:* Apply mapping software to quantify microvascular geometry in skeletal muscle such that resulting models can be incorporated into blood flow and oxygen transport simulations.

**Study 2: Microvascular flow modeling using in vivo hemodynamic measurements in reconstructed 3D capillary networks.**

*Purpose:* To develop and test a systematic approach to flow modeling based on in vivo measurements of red blood cell supply rate that could be applied to models of oxygen transport in the microvasculature.

*Hypothesis:* Matching red blood cell supply rate as observed experimentally is a fundamental approach to creating a realistic oxygen transport model that accurately represents oxygen delivery in vivo.

**Study 3: Comparison of generated parallel capillary arrays to 3D reconstructed capillary networks in modeling oxygen transport in discrete microvascular networks.**
Purpose: Quantify the difference in oxygen transport solutions when utilizing reconstructed 3D capillary networks compared to volumetrically equivalent generated parallel arrays of vessels.

Hypothesis: Reconstructed 3D capillary networks, mapped from in vivo data, yield substantially different oxygen transport solutions when compared to synthetic parallel arrays generated using average geometric parameters.


Purpose: Quantify the impact of incremental capillary perfusion loss such as that observed in experimental sepsis and identify whether or not increased red blood cell supply rate is capable of compensating for loss of discrete vessel perfusion.

Hypothesis: Incremental capillary perfusion loss combined with overall decrease of RBC supply rate will result in a decrease in tissue PO$_2$. Increasing RBC supply rate in the presence of capillary perfusion loss can restore tissue PO$_2$ to baseline levels.
1.2 Intravital Video Microscopy

The most fundamental aspect of microvascular research is quantification of the underlying structure and morphology of the arteriolar, capillary and venular networks. Direct observation of the microcirculation is a critical approach to quantifying vascular structure as well as measuring blood flow and oxygen saturation. Recording microscopic fields provides the capacity to make measurements of physiological conditions offline thus maximizing the data that can be collected from a single in vivo experiment. Conducting in vivo experiments for the purpose of obtaining parameters for modeling has significant advantages over the application of histological and statistical data as well as the potential for validating the models with corresponding experimental measurements (24). The intravital microvascular preparation used in the present thesis was first described by Tyml and Budreau (55) and has since been used extensively to study blood flow in skeletal muscle under a range of normal and pathological conditions.

Intravital video microscopy uses a standard light microscope setup capable of directing the resolved image to one or more video cameras. A powerful light source must be fitted for transillumination of the tissue such that structures can be resolved by the camera with sufficient contrast between tissue and red blood cells. While the basic equipment is similar across intravital video microscopy (IVVM) setups there are specific advantages to using a preparation based on that described by Tyml and Budreau, including the isolation of the muscle tissue from the outside environment, quantification
of blood flow in individual vessels and the characterization of blood flow within microvascular networks (55). The following explains IVVM techniques as applied for the purposes of the studies in the current thesis. Young Sprague-Dawley rats were anaesthetized and the extensor digitorum longus (EDL), a skeletal muscle of the lower leg, was gently isolated from surrounding tissue, secured with ligature and reflected intact onto the stage on an inverted microscope (Figure 1.1). The muscle was allowed to equilibrate for 30 minutes and then was transilluminated with a Xenon light source allowing vessels within 100 µm of the muscle surface to be clearly visualized. Light from the objective is channelled through one or more band pass filters chosen to provide high contrast between red blood cells and the surrounding tissue and to facilitate oxygen saturation measurements. A live image of the vasculature was observed on closed circuit monitors of the video camera stream. Regions of interest for data collection were selected on the basis of absence of tissue damage, hyperemia, and foreign objects on the stage surface. Regions with high light levels, relatively discrete network geometries, and with clearly resolvable non-overlapping capillaries (figure 1.2) were preferred over areas with poor light levels, ambiguous vascular connections, indeterminate inlets/outlets, and groups of capillaries with substantial longitudinal overlap. An oscilloscope calibrated to display the magnitude of light intensity across the field in volts was used to ensure sufficient incident light for oxygen saturation calculations. Video was recorded for the desired interval (typically 60 seconds) using digital capture cards connected to a personal computer. Recorded sequences were processed automatically to provide functional images (figure 1.3) that the user could view to ensure both that the capture was successful and that no motion aberrations occurred over the capture period.
**Figure 1.1: Inverted intravital video system.**

Microscope with attached video cameras used for imaging in vivo microvascular blood flow. Inset shows the externalized extensor digitorum muscle reflected across the microscope stage and positioned above the objectives.
Figure 1.2: Intravital video field.
Still image from intravital video sequence of capillary blood flow in extensor digitorum muscle. Red blood cells appear as dark oval object in the field. Square box indicates an in focus capillary and arrows show consistent plasma gaps in a separate vessel. The black region on the left side of the image is a reference area used for quantifying light levels.
Figure 1.3: Variance functional image.

Variance image generated from an intravital video sequence of capillary blood flow. Tissue regions that do not have changing light levels appear black, while vessels with flowing cells appear as lighter areas.
By acquiring video sequences in multiple fields of view it is possible to characterize the flow conditions in dozens of flowing capillaries across a large area of tissue.

Blood flow and functional capillary density recorded in EDL using the described preparation is stable over time and mean RBC velocity in individual capillaries remains consistent over repeated 30 – 60 second observation periods (55). Hematocrit (Hct) and RBC velocity is heterogeneous between vessels across the vascular bed (56) necessitating sampling numerous individual capillaries in order to adequately characterize conditions in the microvasculature. Although flow profiles remain relatively stable over time small temporal variations in flow distribution, hematocrit and velocity are observable particularly over a 60s capture period. Given the three-dimensional (3D) nature of capillary networks in the EDL even small network volumes are spread over several focal planes which, as a result, requires serial acquisition at each plane to record vessel data in the volume of interest. Typically a random selection of capillaries over many fields of view would provide a statistical sampling of conditions within the muscle.

Intravital video microscopy of the EDL muscle in the described preparation has been shown to be a valuable animal model for characterizing blood flow conditions in skeletal muscle in a variety of normal and diseased states (2, 4, 7, 14, 18, 57, 59).
1.3 Measuring Microvascular Blood Flow In Vivo

In order to have a strong basis for modeling blood flow for the purposes of oxygen transport modeling it is critical to obtain accurate measurements of microvascular blood flow in vivo. Calculating blood flow in capillary vessels using intravital video microscopy is possible by measuring tube hematocrit and RBC velocity in the vessels of interest. Individual RBCs in most flowing capillaries tend to be separated by variably sized plasma gaps that have small changes in size over short distances (60) (figure 1.2). Wayland and Johnson first reported an automated method for measuring RBC velocities using photometry from dual slits positioned perpendicular to microvessels in a microscopic projection (60). This approach created an analog tracing of light intensity over the collection period. The tracing was processed using a correlation method to estimate RBC velocity in 15 – 20 µm diameter vessels. Variations of this technique were later adapted to make use of television recording equipment (33), and computer based analysis to make measurements of velocity, hematocrit, lineal density and RBC flux, (17, 32, 58). The main similarity of these early approaches was the analysis of analog or digital waveforms recording the varying light intensity of passing RBCs along limited paths in a microscopic image.

Refinements in the early correlation based velocity measurements and the improvement in computer software methods to analyze digitized video of microvascular blood flow have provided powerful tools to study blood flow in the microcirculation.
The techniques applied in the present thesis use space-time images (figure 1.4) that provide a graphical representation of observed light intensity along the centerline of the vessel segment over time (15). Each streak profile in a space-time images represents individual or groups or RBCs transiting the capillary. RBC velocity can be calculated from the displacement of the light intensity profile along the capillary from one frame to the next, using spatial correlation. The resulting values can be averaged to yield velocity for each cell or group of cells over the collection period (16). Measurement of vessel segment diameter and lineal density is required to determine hematocrit by calculating the luminal volume and determining the volume of RBCs present in a vessel. Capillary diameters can be accurately determined by using variance images which delineate the lumen of a vessel due to changes in optical density over the collection period (34). Lineal density of cells in the capillary is determined in each video frame (vertical line in the space-time image) by dividing the cumulative optical density of each group of one or more cells by a calculated estimate of the optical density for a single RBC in the vessel of interest (16, 35). Mean tube hematocrit can then be calculated from the lineal density, luminal volume and average RBC volume.

RBC velocity, hematocrit and vessel diameters measured in each vessel in vivo can then used as the basis for modeling blood flow in individual vessel and capillary networks.
Figure 1.4: Space time image of capillary blood flow.
Slanted lines in the space time image represent individual or groups of RBCs traveling through a single capillary segment. Each row in the image corresponds to a centreline position along the vessel of interest while the each vertical column shows the light intensity along the centreline for a single video frame.
1.4 Measuring Capillary Oxygen Saturation In Vivo

Quantifying the convective delivery of oxygen in microvessels is a critical aspect of understanding oxygen transport to tissue. In all mammals, oxygen is transported in the blood, bound to hemoglobin within red blood cells, with 2% of available O$_2$ dissolved in plasma (19). Aerobic tissues such as heart, liver, kidney, brain and muscle require consistent supply of oxygen delivered convectively through microvessels to maintain normal organ function. Measuring oxygen levels in capillaries in skeletal muscle provides a greater understanding of necessary physiological oxygen concentrations in oxidative tissues and how delivery of oxygen varies both spatially and temporally.

Oxygen PO$_2$ in tissue can be measured directly through insertion of microelectrodes (61) or through the use of intra vascular phosphorescent probes (43). Both of these methods have great utility for studying the microvascular environment but have specific drawbacks such as the potential for tissue damage when inserting electrodes and the challenge of making blood flow measurements while using phosphorescence to measure plasma or intercellular PO$_2$. Additionally these challenges are compounded if repeated measurements throughout a microvascular network are needed (29). Alternatively it is possible to directly measure the oxygen saturation of hemoglobin within the red cell itself using spectrophotometric techniques based on those first described by Pittman and Duling (42).
The optical absorption spectrum for hemoglobin (Hb) and oxy-hemoglobin (HbO₂) are distinct and vary with wavelength. The Hb and HbO₂ molecules only share the same extinction coefficient at infrequent isosbestic points (Figure 1.5) making it possible to measure oxygen saturation of RBCs in blood (42). Pittman and Duling described measuring saturation in whole blood using three different wavelengths, two of which at isosbestic points. This approach was applied in vivo, in microvessels ranging in size from 15 - 100µm, by comparing optical saturations converted to PO₂ using the HbO₂ dissociation curve and perivascular measurements of PO₂. Since capillaries have a relatively short path length of hemoglobin due to a small diameter, wavelengths in the blue spectrum must be selected to provide contrast for saturation measurements of RBCs (19). Ellsworth et al. describe a method measuring oxygen saturation in microvessels using two wavelengths, one being isosbestic and the other having a maximum difference in extinction coefficient for Hb and HbO₂ (19). The method described by Ellsworth applies the Beer-Lambert Law that describes the absorption of light through a solution to derive an optical density \( OD = \log(Io/I) \) of RBCs at the selected wavelengths. As indicated previously, plasma gaps are ubiquitous in capillary blood flow and therefore allow incident light intensity \( Io \) to be determined when no RBCs intersect with the light path. This allows for oxygen saturation to be calculated using a calibrated ratio of optical density measurements providing a linear relationship for saturation that is path length and concentration independent (19).
Figure 1.5: Extinction Coefficient for hemoglobin and oxy-hemoglobin.
The relative extinction coefficients for hemoglobin and oxy-hemoglobin vary at different wavelengths. An isosbestic point is indicated at 420nm where both hemoglobin and oxy-hemoglobin have the same extinction coefficient. The oxygen sensitive wavelength at 431nm used to determine oxygen saturation is also indicated.
Ellis et al. further improved this technique by designing a system that included a beam splitter for dual video recording on a video cassette recorder synchronized to start on the same video frame (15). In addition to a new acquisition system, Ellis et al. also developed an in vivo calibration method to determine coefficients for the linear saturation relationship with optical density ratio and the selected wavelengths. Finally the technique of measuring oxygen saturation in capillaries was adapted to utilize the space-time images described in the previous section to provide the optical density measurements at each wavelength allowing for both the measurement of hemodynamics and oxygen saturations in a capillary segment over a period of time (16). When this approach is repeated on each capillary in a network it is possible to obtain a detailed description of the convective oxygen transport within vessels across the network of interest.

Measurement of oxygen saturation in individual vessels has been demonstrated to be a reliable technique to characterize the capacity for oxygen delivery of individual vessels within the microvasculature. These methods are limited in that they require serial sampling of vessels that lie in different focal planes. However the valuable data that these techniques provide cannot be understated, particularly the potential to provide detailed spatial and temporal measurements across a network of interest.
1.5 Modeling Microvascular Blood Flow

Understanding how to describe blood flow mathematically is a continuing endeavor that has spanned several centuries. Poiseuille’s work on liquids flowing in glass tubes provided a mathematical description of flow that influenced both the field of fluid dynamics and medicine (41, 51, 52). Poiseuille’s law applies to Newtonian fluids in the absence of turbulent flow (Re < 2000, for $\text{Re} = \frac{\rho v D}{\eta}$) and so is assumed to apply to flow in microvessels given that fluid flow in the microvasculature occurs at Reynolds numbers less than 1 (23). Using Poiseuille’s law, flow in a circular tube can be described by $Q = \frac{\pi Pr^4}{l} \frac{8}{8\eta l}$ where $P$ is the pressure gradient across tube, $\eta$ is the viscosity and $r$ and $l$ are the radius length of the tube. This equation describing flow in circular tubes was the fundamental contribution to modeling blood flow in the microcirculation and provided the basis for calculating blood flow in networks using pressure differentials across the inlets and outlets. Although Poiseuille’s equations appropriately described flow in large arteries and other conduit vessels there existed a discrepancy between blood flow described by Poiseuille’s Law and what was observed experimentally in the microcirculation.

The behaviour of blood in the microcirculation is largely affected by the fact that blood is composed of deformable cells suspended in homogeneous liquid plasma. Early observations noted how blood viscosity varied depending on the tube diameter within
which it flowed. The cause of anomalous apparent viscosity in blood vessels smaller than 300µm was elucidated in part by Fåhraeus when he made the observation RBCs tend towards the center of a flow stream in small diameter vessels, creating a slower marginal layer of plasma near the wall (20). This property, the Fåhraeus effect, is important for the modeling of blood flow and it contributes to the change in viscosity in small vessels (5) which must be considered when calculating pressure changes in small diameter vessels. While the viscosity of blood decreases in vessels smaller than 300 µm, paradoxically it was observed that the pressure drop across capillaries was larger than was predicted by Poiseuille’s law indicating that the reduction in viscosity did not extend to the smallest of microvessels. Landis observed that the viscosity in microcirculation increases in capillaries where RBCs must deform to transit. He theorized that the resistance associated with RBC deformation caused viscosity to increase dramatically in vessels, specifically capillaries, where cells were restricted by narrow lumen (40). These observations by Fåhraeus and Landis were critical in indicating the direction for mathematical descriptions of how the two-phase nature of blood affects flow in vessels.

Further investigation on the Fåhraeus effect, specifically the properties in capillary sized tubes was performed first by Albrecht et al. (1). Albrecht quantified the hematocrit decreases for blood flowing in glass tubes with diameters between 3.3 and 11 µm. Albrecht was careful to define the Fåhraeus effect as deviations in the ratio of tube hematocrit and discharge hematocrit (HT/HD) distinguishing between the effects of plasma skimming and cell screening that may occur at the entrance to capillary tubes. To make the necessary comparisons between tube and discharge hematocrit Albrecht
equated \( \frac{v_b}{v_c} = \frac{H_T}{H_D} \) where \( v_c \) and \( v_b \) are cell velocity and blood velocity respectively. This description of the relationship between velocity and hematocrit was critical for use in blood flow modeling. This identified that diameter was the most important determinant of the Fåhraeus effect and, equally important from a modeling perspective, that the hematocrit reduction reverses in vessels below a critical diameter between 10 and 20 µm (1). The relationship between tube hematocrit and blood viscosity is non trivial and quantification of the Fåhraeus effect is important for the determination of blood viscosity. In order to quantify the ratio between tube and discharge hematocrit Pries et al. derived a parametric fit for the ratio as follows \( \frac{H_T}{H_D} = H_D + (1 - H_D)(1 + 1.7e^{-0.35D} - 0.6e^{-0.01D}) \), where \( D \) is the diameter of the vessel of interest (47). Pries et al. also derived the parametric equation \( \eta_{relative} = 1 + \frac{e^{H_D \alpha}}{e^{0.45 \alpha} - 1} \cdot \left(110e^{-1.24D} + 3 - 3.45e^{-0.035D}\right) \) (where
\[ \alpha = \frac{4}{\left(1 + e^{-0.593(D-6.74)}\right)} \] to describe the dependence of apparent blood viscosity on hematocrit and diameter (Fåhraeus-Lindqvist effect) in microvessels smaller than 100 µm (48). It is relevant to note that calculations of viscosity from in vitro studies did not agree with the fits determined from in vivo data. Pries et al. conjectured that this disconnect was due to irregularities in vessel cross section, the effect of endothelium bound macro molecules, asymmetry of RBC shape in daughter branches and the presence of white blood cells (48). The difference between in vitro and in vivo measurements of viscosity underscores the importance of utilizing in vivo data for modeling efforts. Combined,
these equations enable the calculation of pressure changes across individual microvessels using Poiseuille’s law.

In order to properly describe blood flow within a contiguous microvascular network it is necessary to be able to properly determine how bulk blood flow and RBCs are distributed at discrete bifurcations. While the two phase nature of blood affects hematocrit and viscosity in microvessels it also has an impact on how cells are distributed at branches. Differences in cell trafficking are highly relevant as the spatial distribution of cells will have a direct impact on the delivery of oxygen to tissue. Uneven distribution of cells and plasma at bifurcations was first described as plasma skimming by Krogh (37) and has been since been quantified in several studies in vivo (11, 36, 53). It has been shown that in bifurcations where the diameter of the tube approaches that of the suspended particles the distribution of particles in each branch changes from a linear proportional distribution to a disproportionate sigmoidal shaped distribution. This observation holds true in vivo in narrow capillaries where vessel diameter approaches cell diameter (46) and has been shown to primarily be dictated by the fractional blood flow difference between the two daughter branches (45). In short, the daughter branch with the greater bulk flow tends to receive a disproportionate share of RBCs than the lower flow daughter. Pries et al. provided a parametric equation for flow separation based on a substantial experimental data set which describes the fractional RBC flow ($FQ_E$) at a bifurcation as:

$$FQ_E = \frac{1}{1 + e^{-\left(\frac{Q_a-X_o}{1-X_o}\right)\left(A+B\log(1-X_o)\right)}}$$
where \( Q_b \) is the blood flow of the parent vessel, \( A \) and \( B \) are diameter and hematocrit 
dependant variables that characterize the asymmetry of the distribution, and \( X_o \) defines 
the threshold below which no cells enter the downstream branch (46, 48). This equation 
provides the necessary mathematical description of fractional flow in each daughter 
branch allowing for the appropriate distribution of RBC to be determined within a 
complex branching network.

By combining these descriptions of RBC distribution at bifurcations with the 
aforementioned equations explaining the relationships between pressure, viscosity and 
blood flow, the resulting numerical programs provide the modern basis for modeling 
microvascular flow (28). Many of the base equations utilize aggregate data sets and are 
derived from empirical data resulting in some uncertainty and error when compared to 
direct experimental measurements. Some degree of approximation is necessary due to 
the difficulty of exactly quantifying every variable affecting flow in the microvascular 
environment particularly variations in vessel diameter, the presence of endothelial surface 
proteins and the cumulative affect of downstream resistance variations caused by cell 
trafficking. Despite these limitations, when combined with experimental hemodynamic 
measurements the numerical methods described represent a complete approach for 
characterizing blood flow in the microcirculation. For the purposes of oxygen transport 
modeling, appropriate application of these blood flow models are requisite to adequately 
represent hematocrit, velocities, flow distributions, and RBC flux in capillary networks.
1.6 Modeling Oxygen Transport in Tissue

The use of mathematical methods to predict physiological conditions is an invaluable tool for the understanding of oxygen transport particularly where direct measurements are either impractical or inadequate for investigating the areas of interest. Although experimental methods exist for measuring oxygen tension and RBC saturation within a volume it is currently impossible to obtain a spatially complete data set that provides measurement throughout an appreciably sized volume of interest. To address this substantial problem there have been a multitude of numerical approaches described to provide a mathematical description of oxygen transport from the blood into surrounding tissue. The field of study begins with August Krogh’s foundational work (38) describing oxygen diffusion out of capillaries into cylindrical tissue volumes, and has actively continued across the numerous facets of the broader problem at hand. Due to the extensive body of theoretical descriptions in the literature it is not appropriate to provide an exhaustive historical survey of the field and instead is more practical to refer to the comprehensive reviews by Popel (44) and Goldman (24) which provide an excellent overview of modeling approaches leading up to the present day. The following discussion is restricted to a description of the methods employed in the present thesis and the associated background necessary for appropriate application.
The basic problem of modeling oxygen transport is the description of oxygen efflux from blood into a surrounding tissue volume that is consuming oxygen and thus results in a gradient from the source of oxygen in blood vessels to lower concentrations in the tissue. To address this problem it is necessary to first produce a description of convective transport of oxygen by the blood. As mentioned previously the vast majority of oxygen in blood is bound to hemoglobin within RBCs which can be represented by a percent saturation of the ratio between oxygenated and deoxygenated hemoglobin molecules while partial pressure of oxygen (PO$_2$) is the amount of dissolved O$_2$ within a given medium. The dissociation of oxygen from hemoglobin is a PO$_2$ dependent process and this provides the basis for which to approximate blood PO$_2$ levels based on RBC saturation in blood vessels. The oxygen-hemoglobin dissociation curve (figure 1.6) can be reasonably approximated for hemoglobin using the hill equation which describes the relationship between PO$_2$ and saturation under given physiological conditions. The two-phase nature of blood must be addressed in order to properly represent vascular PO$_2$ for the purposes of diffusional exchange into tissue. A continuous representation of blood is convenient from a modeling perspective and though it does not exactly account for the variation in delivery that would be seen in a particulate based model the oscillations in PO$_2$ resulting from spaced RBCs are small under physiologically relevant hematocrits (30). As such, numerical mass transfer coefficients have been developed to provide realistic approximations of O$_2$ release from non-particulate models of convective blood flow thus accounting for intra capillary resistance (8, 13, 22, 30).
Figure 1.6: Oxy-hemoglobin dissociation curve.
Hemoglobin oxygen saturation is PO2 dependent resulting in a non-linear curve described by the Hill equation \( S(P) = P^n / (P^n + P_{50}^n) \times 100 \) where \( P_{50} \) for rat hemoglobin is 37 mmHg, the Hill exponent \( n \) is 2.7 and \( S \) is the resulting saturation (14). This characteristic of hemoglobin affects the relative affinity hemoglobin has for oxygen at different partial pressures.
The mass transfer coefficient ($\kappa$) used in the present thesis varies with hematocrit and is defined by $\kappa = 1.21 - 4.38H_T + 23.6H_T^2$, which was determined using a quadratic fit to simulations of particulate RBC flow over a range hematocrits by Eggleton et al. (13).

Local flux ($j$) between the capillary and surrounding tissue can then be defined as

$$j = \kappa (P_b - P_w)$$

where $P_b$ is the intracapillary PO$_2$ and $P_w$ is the PO$_2$ at the capillary wall (13). The boundary condition at the capillary wall is defined as $-D\alpha \frac{\partial P_w}{\partial n} = j$ where $D$ and $\alpha$ are the tissue diffusivity and solubility, and $n$ is a vector normal to the capillary surface. Convective oxygen transport can then be described as a mass balance based on the hemoglobin oxygen saturation ($S(\xi,t)$) at a given axial point ($\xi$) along a vessel by

$$\frac{\partial S}{\partial t} = -u \frac{\partial S}{\partial \xi} - \frac{1}{\pi R} \left[ C + \alpha_b \frac{dP_b}{dS} \right]^{-1} \oint j \cdot d\theta.$$  Here $u$ is the mean blood velocity, $R$ is the capillary radius, $\alpha_b$ is the solubility of O$_2$ in plasma, $C$ is the oxygen binding capacity of blood, and $j$ is the oxygen flux out of the capillary which varies with hematocrit as defined by the mass transfer coefficient. (28).

Partial pressure in the tissue is determined using transport equations derived by Popel (44) which determine PO$_2$ in a given tissue volume element ($P(x,y,z,t)$) in a time dependant fashion

$$\frac{\partial P}{\partial t} = \left[ 1 + \frac{C_{Mb} dS_{Mb}}{\alpha dP} \right]^{-1} \left\{ D \nabla^2 P - \frac{1}{\alpha} M(P) + \frac{1}{\alpha} D_{Mb} C_{Mb} \nabla \left( \frac{dS_{Mb}}{dP} \nabla P \right) \right\};$$

tissue oxygenation throughout the volume is therefore restricted by physically defined diffusion according to limitations of oxygen diffusivity ($D$), solubility ($\alpha$) and O$_2$
consumption \((M(P))\) within the simulated volume. This description of oxygen transport also accounts for oxygen bound to myoglobin given the myoglobin concentration \((c_{Mb})\), diffusivity \((D_{Mb})\) and saturation defined by \(S_{Mb}(P) = \frac{P}{P + P_{50,Mb}}\) where \(P_{50,Mb}\) is the \(PO_2\) at which myoglobin is 50% saturated. Non-linearity of oxygen consumption at decreasing \(PO_2\) is addressed through Michaelis-Menten kinetics using

\[ M = M_0 \cdot \frac{P}{P + P_{cr}} \]

where \(P_{cr}\) is the \(PO_2\) at which the rate of consumption, \(M\), is half of the maximum, \(M_0\). A zero flux boundary condition was imposed at the tissue boundary to both simulate the experimental apparatus (muscle surface in contact with a glass stage) and conditions in vivo where the volume would be bounded by similar regions of vascularised tissue. Discritization of the tissue volume into monotonically spaced elements and applying the numerical methods described above, allows for a time dependent solution of \(PO_2\) to be determined within the entire volume (28).

The resulting transport model used in the present thesis can accommodate complex 3D vascular geometries including curvilinear and anastomotic morphologies. Spatial variability in velocities and hematocrits can also be addressed by utilizing the solutions from a steady state flow model to define conditions in individual vessel segments. Using this model of oxygen transport in tissue it is possible to produce a detailed description of tissue oxygenation in specific microvascular environments.
### 1.7 Oxygen Transport Impairment in Sepsis

An important underlying motivation for modeling oxygen transport is the desire to elucidate how pathological states cause deleterious effects to living tissue. One particularly interesting disease with specific and relatively well-defined microvascular sequelae is Sepsis. Sepsis is a disease that has been defined as a systemic inflammatory response resulting from a bacterial infection (10). Sepsis is a very common critical illness with an estimated annual incidence of 750,000 cases and mortality rates ranging between 20 – 50%; a death rate higher than acute myocardial infarction (3). Given the substantial health impact imposed by this disease, any improvement in our understanding of the mechanisms of sepsis serves to enhance clinicians’ ability to effectively treat critically ill patients.

The most pertinent investigation that identifies sepsis as a microvascular disease was conducted by Lam et al. (39). Using a subacute animal model of sepsis Lam found that the proportion of stop flow capillaries increased dramatically in skeletal muscle, reducing perfused vessels by 36% post insult. Increases in mean RBC velocity, an increase in perfused intercapillary distance, and the occurrence of very fast flow vessels (2500 µm/s) in the sepsis group were also noted. Lam also made the important observation that in the sepsis group the distribution of RBC flux was heterogeneous across the vascular bed perhaps leading to areas of hypoxia interspersed amongst relatively healthy tissue. This study clearly identified impairment of microvascular blood
flow and the reduction of perfused capillaries as a defining characteristic of bacterial sepsis. How these changes in microvascular perfusion impact overall oxygen delivery and tissue oxygenation remained unclear.

Several other studies found microvascular dysfunction in animal models of sepsis including increases in stopped flow capillaries (9, 21), decreases in capillary venular end \( \text{SO}_2 \) (14), and increase in capillary oxygen extraction (6, 7, 14). Furthermore several human studies reported a correlation between observed flow dysfunction in sublingual vasculature and patient mortality (50, 54). Also a study employing near infrared spectroscopy to infer the state of oxygen consumption and tissue oxygenation found that oxygen utilization was depressed in severe sepsis as were levels of tissue \( \text{PO}_2 \), particularly in non-survivors (12). This contrasts with findings that indicate in experimental sepsis tissue oxygen consumption is increased (7) which was supported by computer models used to account for the observed capillary oxygen extraction (26). The apparent relationship between changes in microvascular blood flow and mortality in sepsis indicate a promising area of study for oxygen transport modeling due to the possibility of reproducing observed characteristics in silico.

Efforts have been made to model oxygen transport in sepsis using computational models and input parameters that approximate the conditions observed in experimental sepsis. Goldman et al. were the first to apply a mathematical model to simulate loss of capillary perfusion in sepsis (26). Using a synthetic parallel vascular structure generated using statistical averages of capillary density Goldman et al. created non-anastomotic
vascular arrays to represent vascular geometry surrounding fibres in skeletal muscle.

Blood flow characteristics for vessels in the array were defined by assigning normal, stopped, or fast flow velocities to each vessel in the proportions observed experimentally in animal models of sepsis. RBC hematocrit and entrance oxygen saturations were defined uniformly using reported averages from non-septic studies in rat skeletal muscle.

This model predicted large increases in tissue oxygen consumption, 39 – 63% decreases in mean tissue PO$_2$, decreased minimum tissue PO$_2$, and substantial spatial heterogeneity in tissue oxygenation including areas of hypoxia. While these results were consistent with published experimental findings Goldman et al. noted that the applied model could be improved by utilizing a continuous distribution of hematocrits and velocities between individual capillaries and applying measurement based heterogeneity in spacing of capillaries (26). The specific effect these changes would have to the transport solutions was not known though it was suggested that the result would be greater heterogeneity in O$_2$ supply which may produce greater heterogeneity in tissue PO$_2$. As a follow up to this work Goldman et al. utilized similar parallel geometries to examine how spatial heterogeneity in blood flow would affect O$_2$ supply dependency in sepsis (25). Supply dependency occurs when oxygen consumption is limited due to the unavailability of free oxygen. Consistent with this concept, it was found that the resulting supply dependency in models of sepsis was caused by inadequate oxygen delivery to regions within the simulated tissue volume due to increased diffusion distances from the loss of perfused capillaries. This model also predicted that contributing factors to insufficient oxygen delivery included higher consumption, lower overall blood flow and lower inlet oxygen saturation.
The effect of flow dysfunction in sepsis on oxygen delivery has been elucidated largely through experimental studies examining decreases in perfused capillaries, and the resulting changes in capillary oxygen saturations, RBC velocities, hematocrit and tissue consumption. Initial efforts at modeling oxygen transport in simulated sepsis have provided valuable insights into how defects in microvascular blood flow may impact tissue oxygenation and potentially lead to hypoxic regions in tissue. In order to further improve our understanding of oxygen transport in sepsis it is necessary to use more detailed vascular models that can represent the heterogeneity in network geometry and blood flow distribution seen in vivo.
In the current literature, there are no descriptions of oxygen transport modeling in real networks using matched hemodynamic and oxygen saturation measurements made in the modeled network of interest. Intravital video microscopy described in section 1.2 combined with the techniques for measuring blood flow and capillary oxygen saturation described in sections 1.3 and 1.4 provide a unique opportunity to produce data sets detailed enough to fully characterize oxygen delivery to skeletal muscle. By using steady state blood flow modeling (Section 1.5) to approximate the conditions observed in vivo computational models of oxygen transport (Section 1.6) can then be used to predict how oxygen tension would be distributed in living tissue. These approaches serve to answer fundamental questions related to oxygen transport not only in healthy tissue but also in pathological states that affect oxygen utilization and delivery. Among different pathologies sepsis represents one disease that has been characterized to impair oxygen delivery in a non trivial and complex manner. Given the current state of research in these areas the primary question of the current thesis is how does the use of realistic capillary network geometry and RBC supply rate affect mathematical models of oxygen transport to tissue.

The goal of the present thesis is to improve our current understanding of oxygen transport to tissue and to develop new techniques that best represent conditions observed in vivo using computer models. To achieve this goal four studies are presented.
1.9 References


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CHAPTER 2: MAPPING 3D FUNCTIONAL CAPILLARY GEOMETRY IN RAT SKELETAL MUSCLE IN VIVO

(used with permission – see Appendix II)

2.1 Introduction

Since August Krogh first developed a simple model of oxygen diffusion from an idealized capillary into the surrounding tissue (12), researchers have worked to more accurately characterize the microvasculature to develop more realistic models of tissue oxygenation and to provide data for testing these models. In order to provide a clear picture of the microcirculation as a whole a variety of tools have been developed to quantify network morphology and flow hemodynamics and to measure conditions within the blood itself. These tools include stereology, microscopy, histology, spectrophotometry, and a variety of mathematical constructs (7, 8, 13, 17, 19, 24). Drawing on several of these tools, integrated approaches have been made to represent blood flow and oxygen delivery using computer modeling (6, 20, 22). The majority of modeling efforts have been concentrated on the simplification of complex network geometry using predominantly parallel arrays of microvessels constructed using statistical averages from histology and stereology. While these simplified models have provided great insight into convective transport within microvascular networks, they remain somewhat of an abstraction from the conditions found in vivo and likely fail to represent the substantial variability seen in living animals (1, 6).
The specific geometry of a vascular network is integral to its function not only with respect to distribution of blood flow but also for diffusive delivery of vital nutrients and removal of waste products (3). In order to provide a complete picture of convective and diffusive oxygen delivery within a three-dimensional (3D) volume of tissue in vivo, one needs to reconstruct the 3D vascular bed using quantitative data of microvascular network geometry together with measurements of blood flow and oxygen concentrations in these vessels. There are a number of published methods of reconstructing complex 3D vascular networks using fluorescent endothelial labeling and confocal microscopy (2, 4, 10). These approaches are difficult to apply in vivo, and as such the reconstructed networks are derived from ex vivo sections of tissue without direct measurements of blood flow or oxygen levels within individual vessels. Methods using dual photon laser scan are capable of resolving 3D networks and calculating red blood cell (RBC) flux within vessels but still lack the ability to combine these functional measurements with oxygen saturation or PO$_2$ necessary for oxygen transport modeling (10). Furthermore, laser scanning techniques determine hematocrit and RBC flux from a small fraction of labeled RBCs which reduces accuracy of measurements within capillaries with low total RBC flux over the sampling duration.

This paper presents a software package capable of processing functional images from white-light intravital video sequences of microvascular blood flow to reconstruct the functional 3D network geometry within a volume of interest. The reconstructions provide detailed measurements of vessel diameter, vessel length, vascular density as well as an accurate representation of the interconnectivity between individual vascular
segments. These measurements are achieved using an approach that combines automated vessel selection, manual user interaction and quality control algorithms. To illustrate the potential of this approach 7 discrete network reconstructions have been presented that show the variability of geometry in vivo and the scalability of this software. The same video sequences used to provide data for the 3D reconstruction are analyzed for RBC velocity, RBC lineal density, RBC supply rate and RBC oxygen saturation in individual capillaries within the network using existing analysis software (9). Combining geometric data together with parallel analysis of RBC hemodynamics and O$_2$ saturation using a single optical imaging system was an additional objective of this paper. The resulting network data set is a multifaceted characterization of microvascular oxygen transport within a volume of tissue in vivo. Finally we present a simple format for the dataset created by the integrated software. This approach to data management is scalable, adaptable and provides an easy to integrate framework for the analysis of network morphology, for hemodynamic flow modeling and for integrated modeling of oxygen transport in both normal and pathological states.
2.2 Materials and Methods

2.2.1 Experimental Model

Intravital video sequences of capillary networks in the extensor digitorum longus (EDL) muscle of 6 rats were used as the basis for the current work. Animal protocols were approved by the University of Western Ontario’s Animal Care and Use Committee. Male Sprague-Dawley rats (80 - 100g on delivery) were housed in dedicated animal quarters at the University of Western Ontario and were acclimatized for 7 days following delivery. On the day of experiment an animal was randomly selected and weighed to verify a suitable mass between 140 - 180g. Animals were anaesthetized with an initial intra-peritoneal dose of sodium pentobarbital (6.5 mg / 100g body). Tracheotomy was conducted to allow mechanical ventilation at a rate of 73 – 76 breaths per minute with a flow controlled air mixture of 30% O₂, 70% N₂. The left common carotid artery was cannulated with polyethylene tubing and perfused with approximately 1 ml / hr of heparinized saline (1 USP heparin / 1 ml saline) to maintain patency. A pressure transducer connected to the carotid cannula allowed heart rate and mean arterial pressure to be monitored and recorded with an attached printer. Similarly, silastic tubing with a beveled end was used to catheterize the right external jugular vein through which saline was continuously infused for fluid resuscitation (0.5 ml saline / hr / 100g wt). Supplementary doses of anesthetic were delivered via the jugular catheter, as needed, to maintain sufficient anesthesia over the duration of the experiment (3 – 5 hrs). The EDL muscle was blunt dissected and externalized as described by Tyml and Budreau (23).
short, a small section of skin was removed from the lateral side of the right lower hind limb exposing the fascia capsule of the underlying muscles. Superficial dissection of the capsule and blunt separation of the surrounding muscles allowed the EDL to be isolated. Silk ligature was threaded under the intact muscle and secured with a square knot on the distal portion of the EDL tendon. The tendon was then severed between the ligature and muscle insertion leaving the ligature securely attached to the free end of the EDL tendon. Following EDL dissection the animal was transferred to the microscope stage and placed on its right side in a semi-prone position. The ligature secured to the EDL tendon was then taped to the stage such that the lateral side of the muscle was facing the objectives and the muscle maintained a length and angle approximate to the resting position in situ. The muscle was moistened with 37 °C saline and covered on the medial side with a small square of plastic film (~ 2x2 cm, Saran - polyvinylidene chloride) and a glass cover slip in order to isolate the muscle from the external environment.

2.2.2 Dual Spectrophotometric Intravital Video Microscopy

Microvascular blood flow video sequences of in-focus capillaries were simultaneously captured at 2 specific wavelengths (420nm and 431nm) using a system similar to those described elsewhere (4, 9). The 420 nm wavelength is an isosbestic point, and therefore oxygen insensitive, whereas light at 431 nm has differential absorption for rat oxy- and deoxy-hemoglobin. The acquisition system (see Figure 2.1) was composed of a Nikon Diaphot 300 inverted microscope (Nikon objectives 10x/0.25 NA, 20x/0.4 NA) fitted with a 100W Xenon lamp for trans illumination.
Figure 2.1: Digital capture system.

Schematic of microscope and digital capture setup used to record intravital video of muscle blood flow at 2 different wavelengths.
A beam splitter fitted within the light path reproduced the microscope image parfocally to allow for simultaneous video capture with two identical computer acquisition systems. The beam splitter contained a filter cube containing interference filters at each of the aforementioned wavelengths (420 nm and 431 nm). A strip of black electrical tape was affixed vertically to the left side of the light path to provide a region of black reference in the video image. Each system consisted of a charged-coupled device camera (MTI C72). The video signal was captured real-time by an analog to digital capture card (Data Translation 3155) using custom frame capture software (Neovision) and in-house software written in the MATLAB (Mathworks) programming environment. Video images were displayed locally on closed circuit monitors to allow the user to maintain focus before and during the acquisition sequence. Simultaneous frame-by-frame capture was triggered on the capture cards with a digital synchronization pulse from a time code reader attached to an external time code generator (Telecom Research, Burlington, Ontario).

2.2.3 Experimental Protocol for Capture of 3D Network Data

In order to capture the video sequences needed to reconstruct the network in a given volume of interest, the user followed a specific protocol. Using the real time microscopic image of the muscle, an initial pan of the muscle surface was made to identify any damage and to establish that flow within the muscle is normal. A complete arteriolar-to-venular network, a partial network at either end of the capillary bed, or a section in-between was selected that was free of surface artifacts and had a minimum of large underlying vessels that might prevent later analysis of vessel hemodynamics.
Starting at the surface, the user made a recording at each focal plane where one or more microvessels could be clearly resolved. During each video capture, focus was carefully maintained on RBC flow for a period of time, typically 60 seconds. Additional sequences were also made to capture network detail at bifurcations and to track vessels that traverse planes. The result was a serial stack of video sequences within the volume (a single volumetric field of view) composed of unequally spaced focal planes containing vessels that could be used to fully characterize all of the capillaries within that volume of tissue. The typical number of focal planes captured in a volumetric field of view range between 4 – 10. Very short networks or partial networks could be captured using a single volumetric field of view. Larger networks, spanning multiple volumetric fields of view, were acquired starting at the arterial inlets and then repeating the capture process in fields that overlap one or more previously acquired areas (the reconstruction shown in Figure 2.7 required 24 different focal planes). Overlapping fields enabled the user to register each field with respect to one another. A complete series of video sequences spans the entire volume of interest in all three axes.

2.2.4 Video Processing and Functional Images

Videos were processed by the capture software, and offline using separate custom analysis software to provide hemodynamic and oxygen saturation measurements for individual vessels. Video sequences at the isosbestic wavelength (420nm) were processed to produce variance functional images consistent with the method described by Japee et al. (8). Variance images were created by calculating the total variance in each pixel over the video sequence, while mean intensity images show the average pixel value
for each pixel in the frame over capture period. Functional images provide a clear
delineation of the capillary which can be processed to determine the 2 dimensional
coordinates of the lumen and centerline within the field as described below. In focus
capillaries from video sequences were selected and measurements of velocity, hematocrit,
RBC supply rate and oxygen saturation were made using custom software based on the
method described in Japee et al. (9). Briefly, the location of the vessel centerline is used
to extract light intensity values along the vessel from every video frame in the captured
420nm sequence to generate a 420nm space-time image (STI) which describes the
position of RBCs along the vessel centerline over time. The centerline location in the
420nm variance image guides the automatic selection of the centerline location in the
non-isosbestic (431nm) variance image from which a 431nm STI was generated. A
physical black reference region along the left edge of the captured field was used for each
STI to provide the relative zero light intensity value. RBC velocity (mm/sec) and RBC
lineal density (RBC/mm) were calculated from the 420nm STI using a method based on
that described by Ellis et al (5). RBC velocity was calculated by applying a spatial
correlation technique to frame-by-frame light intensity data in the 420nm STI to
determine the displacement of the RBC column from one frame to the next. The RBC
lineal density in each frame was calculated from the STI based on the length and optical
density of single RBCs in each capillary. The product of velocity and lineal density
yields the RBC supply rate (RBC/sec) on a frame-by-frame basis. Hematocrit \((Hct)\) was
also calculated on a frame by frame basis using lineal density to determine the number of
cells in the vessel \(\left(N_{RBC}\right)\), RBC volume \(\left(V_{RBC}\right)\), and the calculated volume of the vessel
segment \(\left(V_{\text{Vessel}}\right)\) using the equation \(Hct = \left(\frac{N_{RBC} \cdot V_{RBC}}{V_{\text{Vessel}}}\right)\).
The temporal and spatial location of plasma gaps in the two STIs were used to extract plasma light intensity data which corresponds to the incident light levels ($I_o$) experienced by the RBCs as they traverse the capillary. Incident light is then used to calculate RBC optical density and hence oxygen saturation (4). A cubic-spline fitting algorithm (MATLAB) was used to interpolate $I_o$ values at locations occupied by RBCs in the STI. Using these interpolated $I_o$ values and the measured intensity values for RBCs ($I_m$) in each STI, the optical density ($OD$) of the RBCs at both wavelengths ($OD_{420}$, $OD_{431}$) were computed using $OD = \log\left(\frac{I_o}{I_m}\right)$. Resulting $OD_{420}$ and $OD_{431}$ STIs were used to generate an $OD$ ratio STI ($OD_{431}/OD_{420}$) from which the hemoglobin oxygen saturation of the RBCs could be calculated using an in vivo calibration acquired in separate experiments.

### 2.2.5 Mapping 3D Network Geometry

#### 2.2.5.1 Calibration and Image Registration

The diagram in Figure 2.2 outlines the process that was followed to reconstruct 3D microvascular network geometries. All aspects of the reconstruction were conducted using a graphical user interface (GUI) for the mapping software package written in the MATLAB programming environment.
Figure 2.2: Network reconstruction process.

Flow chart illustrating the steps for reconstructing a complete microvascular network.

Network geometry is generated in steps 1 – 5, vessel hemodynamics and oxygen conditions are added in steps 6 and 7 while steps 8 – 10 display the completed network reconstruction and format the data set for use in other modeling applications.
Functional images (see Figure 2.3-B and Figure 2.7 middle panel), showing the geometry of individual vessels were generated from the video sequences were used as the basis for mapping the network. Images were registered with respect to each other with the bottom left corner of the first image being used as the origin in 2-dimensions (2D). Subsequent target images were registered by employing a user driven cross-correlation method. Vascular features in functional images are frequently visible across multiple planes of focus which allows this method to be used to register images within the same plane and between overlapping images from different focal planes. The user was prompted to select a single control point from a characteristic vascular feature in the target image and a previously registered image. A small sub-region (30x30 pixels) centered around the control point in each image was then used to perform a normalized 2D cross-correlation of pixel intensity to identify the correct spatial shift to align the two sub-regions (‘normxcorr2’ MATLAB function) using the method described by Lewis (14). The spatial shift and the original position of the sub-region in the target image were combined to register the image in 2D. The user entered the focal depth of each registered image as measured during the experiment using the microscope’s fine focus. This approach provides a complete three-dimensional registration of each source image.

2.2.5.2 Vessel Selection for Diameter and Centerline

With all functional images registered in 3D, step 3 involved defining the location of the lumen of all vessels for the 3D reconstruction as well as for vessel geometry (diameter, length). A vessel lumen (volume available for RBCs flow) is clearly defined in the functional images by white pixels (high variance of light intensity values due to
passage of RBCs) against a dark background of tissue (low variance) and hence an edge
detection algorithm based on the 2D gradient in variance values was used to define the
lumen. By using functional images, the lumen is defined by the passage of the RBC
column and not by the physical location of the endothelial wall (which is rarely visible at
the wavelengths used). The user selects the in-focus sections of individual vessels in
each functional image using a semi-automated user driven technique. A rectangular sub-
region $F(x,y)$ is drawn that bounds the in-focus section of the vessel (Figure 2.3, panel B)
and the software creates a gradient image from the sub-region image using the MATLAB
built in ‘gradient’ function. The function creates a 2-dimensional gradient array in the x
and y directions according to the following equation:

$$\nabla F = \frac{\partial F}{\partial x} i + \frac{\partial F}{\partial y} j$$

where $i$ and $j$ are the pixel indices of the functional image. The x and y gradient arrays
are used to calculate the absolute magnitude (2) and four-quadrant angle (3) of the
gradient vector in 2D using:

$$M_{(j,i)} = \sqrt{\left(\nabla F_{x,(j,i)}\right)^2 + \left(\nabla F_{y,(j,i)}\right)^2}$$

(2)

$$\theta_{(j,i)} = \arctan\left(\frac{\nabla F_{y,(j,i)}}{\nabla F_{x,(j,i)}}\right)$$

(3)

Figure 2.3, panel C shows the gradient image using a threshold such that the low
gradients in the tissue and vessel center are set to zero.
Figure 2.3: Vessel selection process.
The sequence illustrates the semi-automated vessel selection process. Panel A shows the selected region from a single frame of intravital video, B is the variance image for the region generated from the entire video sequence showing the lumen of the vessel, C shows the gradient map while panels D and E show the vessel centerline and edge tracing as determined by the computer overlaid on the variance image panel D and video frame panel E. Average diameter for this vessel was 5.2 ± 0.3 µm. Note, the segment shown is in focus over its length and hence this entire vessel segment lies within the same plane.
The resulting gradient image shows the outline of the vessel and aids the user in determining if the selected vessel was in focus over its entire length (if a portion of the vessel is out of the plane of focus, the gradient falls below the threshold).

The user selects a seed point within the vessel lumen to act as a starting point for the centerline and diameter-tracing algorithm. A single seed point is required for each vessel segment, though segment length is only limited to the size of the field. The algorithm first determines if the vessel was oriented vertically ($45^\circ < \theta < 135^\circ$) or horizontally in the image and then searches for the maximum gradient in the horizontal or vertical direction on either side of the seed point. The edge of the lumen was set as one pixel beyond the location of the maximum gradient. The pixel halfway between the two edges was defined as the centerline for that cross-section. Diameter was calculated by

$$d = C \times XL \times \cos \alpha$$

where $d$ is the calculated diameter, $C$ is the pixel to micron scaling factor, $XL$ is the distance of the vessel cross section (in the vertical or horizontal direction depending on orientation) and $\alpha$ is the average angle of the vessel edges at that point. The algorithm incrementally located vessel edges, centerline and calculated diameter as it moved from the original seed point towards each end of the vessel; this process was repeated until the full lumen of the vessel was traced (Figure 2.3, panels D,E). The location of the centerline for this vessel with corresponding diameters within the 3D volume was stored for reconstruction of the 3D network in step 9. All vessel data were stored in individual files according to a unique vessel identification number (VIN) entered by the user. The automated segmentation process was validated using a digital
video phantom of RBCs flowing through a pre-defined virtual vessel lumen using representative hemodynamic parameters. Cell pixel intensities in the phantom were defined based on typical optical densities for RBCs in video from the described microscopy system and assuming a circular luminal cross section. The background for the phantom was selected from an avascular subregion in a mean image obtained from an intravital video of skeletal muscle. Error rates in edge position detection were calculated for a range of random noise levels applied to each video frame in the phantom video sequence. Percent error was determined by comparing the edge locations identified by the automated algorithm with the known positions as defined by the phantom.

Vessel segments that cannot be selected using the automated process (e.g. vessels that span multiple planes in the vertical direction) were defined manually by the user in a point wise fashion from functional images. Diameters for manually selected vessels were measured directly by the user from the functional images using a built in calibrated point-to-point measurement function.

2.2.5.3 Connecting Vascular Segments

Once individual vessel segments have been acquired it is possible to join vessel segments in a number of ways. Two vessel segments can be joined together to form a continuous vessel where the adjoining section is a straight line between the closest end points of each segment and the diameter at interceding centerline locations is the average of the diameter at the end of each segment. In order to create a bifurcation between three vessels the user identified the vessels that make up the bifurcation and the software
determines the location of the bifurcation by finding the minimum distance (centroid) between the end points of all three vessels using

\[
\text{Centroid}_{x,y,z} = \left[ \frac{(A_x + B_x + C_x)}{3}, \frac{(A_y + B_y + C_y)}{3}, \frac{(A_z + B_z + C_z)}{3} \right]
\]

(4)

where \(A, B\) and \(C\) are the nearest 3D end point coordinates of the three vessels being joined. The vessels making up the bifurcation are extended to the Centroid along the 3D vector between the vessel endpoint and the centroid. Centerline points are placed along the vector in fractional intervals consistent with the source vessel’s centerline point-to-point spacing where the \(n\)th coordinates of the spanning segment are defined as

\[
\text{Span}_{i=1}^{n} = \text{Centroid}_{x,y,z} \left( 1 - \frac{i}{n} \right) - \text{VEnd}_{x,y,z}
\]

(5)

where \(\text{VEnd}_{x,y,z}\) is the 3D coordinate of the original segment end point and \(n\) is the number of centerline points in the span.

Throughout network reconstruction original source videos are reviewed to distinguish between crossing unconnected vessels and bifurcations.

2.2.6 Quality Control

Following selection and joining of all vessels within the volume it is necessary to validate the resulting network for spatial continuity. This is primarily due to vessels being selected from different source images and therefore there can be small spatial errors due to the integer nature of pixel-based registration. Networks can have complex patterns of vessels and a user may miss a segment, fail to connect segments at bifurcations, incorrectly assigned vertical coordinate, or trace the same vessel section
more than once in separate images. To check for these inconsistencies several approaches are used. Initially the distance between all vessel end points is calculated and those that are within 0.5 μm of each other are assumed to be part of the same bifurcation and are fixed to share the same absolute spatial coordinates. The corrected vessel coordinates are then used to validate continuity of vessel end points using a registered array of the end points. Each vessel end point undergoes a logical comparison with all other points in order to determine whether the vessel is an unconnected end, part of a bifurcation or part of an errant connection composed of only two, or greater than three vessel ends. In addition to checks made at end points, all points along each vessel are evaluated to determine if any other vessel within the volume erroneously intersects with the segment wall. The user is provided with a visual map of the reconstructed network with any inconsistencies marked symbolically so the user can rapidly identify problems with geometry (Figure 2.4) or blood flow continuity (Figure 2.5) as well as a textual list of inconsistencies and associated coordinates. Identified inconsistencies must be resolved by the user by making necessary connections, removing errant segments, or correcting blood flow direction before the reconstruction can continue.

2.2.7 Indexing Vascular Functional Data

After the network geometry had been reconstructed completely and all problems with geometric continuity had been resolved it was then possible to index previously measured velocity, hematocrit and saturation data with corresponding vessels within the network.
Figure 2.4: Annotated network geometry features.
Partial section of network G annotated by the mapping software. The top panel indicates features of the geometry which are consistent with expected vessel connections (blue symbols) as well as inconsistent network connections (black symbols). Individual annotations indicate vessel bifurcations (γ), unconnected vessel end points (○), vessels connected with too few or too many connected end points (Λ), and vessel segments that lay too close to one another (≤). The inset shows a rotated and magnified view of vessels where node points in adjacent vessels lay too close to one another. Unconnected end points that do not lie on the boundary are marked in black as inconsistencies since internal vessels are assumed to be continuous throughout the volume.
Figure 2.5: Annotated network flow features.
Partial network segment annotated with arrows to indicate flow direction (arrows), missing measured conditions within vessels (V – Velocity, H – hematocrit, and S – Saturation), and converging or diverging flow discontinuities (⊗).
Each vessel within the reconstructed network was associated with a VIN which was used to index the hemodynamic parameters, blood flow direction, and inflow/outflow saturations to individual vessels. Vessels for which no hemodynamic or saturation data was measured were populated with calculated or mean values from the network or flagged as unknown depending on the intended application of the reconstruction.

Networks of interest were rarely oriented exactly parallel to the y-axis in the field of view and so it was necessary in most cases to rotate and trim the entire geometry, establishing a Cartesian volume that closely bounds the volume of interest in which selected network lies. The network is rotated according to a rotation angle $\phi$ supplied by the user. The $(x,y)$ coordinates of each vessel data file, $V$, are shifted by applying a standard rotation matrix,

$$
\begin{bmatrix}
V_x' \\
V_y'
\end{bmatrix} = \begin{bmatrix}
\cos \phi & -\sin \phi \\
\sin \phi & \cos \phi
\end{bmatrix} \begin{bmatrix}
V_x \\
V_y
\end{bmatrix},
$$

resulting in rotated geometry coordinates that are not otherwise altered. Following rotation the vessels within the network are trimmed such that free ends start and terminate in the plane of the bounding rectangular volume.

### 2.2.8 Visualizing Network Reconstruction

During the reconstruction process, the user could visualize the network in two distinct ways. A 2D composite map of all registered functional images could be dynamically generated with tracings of vessel positions, each labeled with VIN, overlaid on the composite map. Alternatively, the user could generate and interact with the current 3D vascular map using built in zoom, pan and rotate functions in order to inspect
the progress in reconstructing the network geometry. Vessels were rendered for visualization as variable diameter cylinders with surface color indicating relative position within the volume. Cylindrical surfaces of vessels were generated using an open source MATLAB script (21).

2.2.9 Analysis of Reconstructed Network Morphology

In order to calculate the capillary density in a manner consistent with measurements from histological sections (16) an in silico stereological approach was used. Virtual transverse sections were made every 10 µm through the reconstructed 3D geometry (Figure 2.6) and the number of capillaries, vascular area, and Krogh radius were calculated in each slice. Intersecting capillary areas were taken to be elliptical with eccentricity being calculated according to the angle of intersection with the plane. The vascular area was measured for each section and was subsequently used to compute capillary area density. The numbers of capillaries in each slice were used to calculate the number of capillaries per mm² and from those values the Krogh radius for each slice was also determined (12). Capillary volume, surface area, segment lengths and diameter were calculated directly from the geometric data. For the purpose of these calculations capillaries were treated as a series of variable diameter cylindrical sections where any overlaps or gaps at node points was considered to be insignificant. Only vessels with a diameter less than 8 µm were considered for measurements specific to capillaries.
Figure 2.6: Transverse virtual histology slice.
Example of a virtual transverse slice from reconstructed network D (at 800 µm) used to simulate histological capillary area density measurements.
2.2.10 Processing

Network reconstruction and analysis was conducted using an Apple MacBook Pro personal computer with 3 GB of RAM and a minimum of 1 GB of hard drive space. All software used to generate functional images, reconstruct the observed networks, measure hemodynamics and oxygen saturations was written by the authors using current versions of MATLAB (Mathworks). Functional images were generated automatically between successive video captures and were used for offline analysis of hemodynamics and network reconstruction.
2.3 Results

Network reconstruction was carried out on 7 distinct vascular networks from 6 different animals. Total reconstruction time varied between 1 – 10 hours depending on size of the volume of interest and complexity of vascular connections. Completed networks were checked for continuity using aforementioned algorithms. Dimensions of each reconstructed network can be found in Table 2.1. Mean capillary diameter, mean capillary segment lengths, mean capillary area density, capillary volume density, capillary length density and Krogh radius were calculated for each network and are presented in Table 2.2. Error analysis for the vessel segmentation phantom was conducted for noise levels of 4%, 6%, 8%, and 10%, resulting in a percent error of 0.25, 0.25, 1.77, and 5.56 respectively. The small proportion of edge points, as indicated by the percent error, that did not match exactly were within 1 - 2 pixels of the true edge location. Typical noise levels in the described intravital video system range between 5 – 10%.

Registered functional images were combined into composite maps in order to better visualize the spatial position of vessels within the network. The use of functional images allowed for registration of images from within the same focal plane and between images from different planes. The final composite map from network D contains 37 overlapping and registered functional images. A subset of the composite map, 4 functional images from the same plane of focus, is shown in the middle panel of Figure 2.7.
Table 2.1: Summary of network volume dimensions.

Dimensions of the volume of interest for reconstructed networks. Dimensions indicate the absolute boundary of vessels within the volume. All measurements are given in μm (sampling resolution for all networks is ±0.65, 1.3, and 3.0 μm in the X, Y, and Z directions respectively). Networks have been cropped to occupy a single contiguous network and rotated to minimize superfluous volume.

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>327.0</td>
<td>312.9</td>
<td>247.0</td>
<td>255.0</td>
<td>148.9</td>
<td>135.5</td>
<td>117.1</td>
</tr>
<tr>
<td>Y</td>
<td>308.1</td>
<td>283.9</td>
<td>237.6</td>
<td>831.4</td>
<td>505.4</td>
<td>421.5</td>
<td>747.1</td>
</tr>
<tr>
<td>Z</td>
<td>45.0</td>
<td>65.0</td>
<td>40.0</td>
<td>94.0</td>
<td>64.0</td>
<td>65.0</td>
<td>35.0</td>
</tr>
</tbody>
</table>
Table 2.2: Network statistics calculated from reconstructed networks.

<table>
<thead>
<tr>
<th>Network</th>
<th>Capillary Diameter (µm)</th>
<th>Capillary Lengths (µm)</th>
<th>Capillary Area Density (A&lt;sub&gt;ALC/t&lt;/sub&gt; %)</th>
<th>Capillary Volume Density (V&lt;sub&gt;ALC/t&lt;/sub&gt; %)</th>
<th>Capillary Length Density (mm / mm&lt;sup&gt;3&lt;/sup&gt;)</th>
<th>Krogh Radius (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>5.73 ± 0.60 (n = 2144)</td>
<td>196.64 ± 108.31 (n = 20)</td>
<td>2.37 ± 0.40 (n = 31)</td>
<td>2.33</td>
<td>893.27</td>
<td>18.76 ± 1.50 (n = 31)</td>
</tr>
<tr>
<td>B</td>
<td>5.32 ± 0.52 (n = 2440)</td>
<td>146.90 ± 89.80 (n = 26)</td>
<td>1.47 ± 0.18 (n = 29)</td>
<td>1.44</td>
<td>630.67</td>
<td>21.93 ± 0.82 (n = 29)</td>
</tr>
<tr>
<td>C</td>
<td>5.48 ± 0.59 (n = 1389)</td>
<td>165.13 ± 65.73 (n = 11)</td>
<td>1.79 ± 0.28 (n = 24)</td>
<td>1.78</td>
<td>678.97</td>
<td>20.38 ± 1.28 (n = 24)</td>
</tr>
<tr>
<td>D</td>
<td>5.56 ± 0.77 (n = 7266)</td>
<td>137.82 ± 138.61 (n = 96)</td>
<td>1.65 ± 0.39 (n = 84)</td>
<td>1.56</td>
<td>643.93</td>
<td>22.97 ± 1.33 (n = 84)</td>
</tr>
<tr>
<td>E</td>
<td>5.57 ± 0.71 (n = 2433)</td>
<td>85.57 ± 88.31 (n = 46)</td>
<td>2.05 ± 0.73 (n = 51)</td>
<td>1.91</td>
<td>765.63</td>
<td>22.31 ± 3.88 (n = 51)</td>
</tr>
<tr>
<td>F</td>
<td>5.54 ± 0.73 (n = 2143)</td>
<td>113.24 ± 74.11 (n = 31)</td>
<td>1.77 ± 0.61 (n = 43)</td>
<td>1.77</td>
<td>741.71</td>
<td>22.00 ± 1.75 (n = 43)</td>
</tr>
<tr>
<td>G</td>
<td>5.55 ± 0.65 (n = 3598)</td>
<td>137.77 ± 130.23 (n = 38)</td>
<td>2.83 ± 0.85 (n = 75)</td>
<td>2.61</td>
<td>1048.34</td>
<td>17.48 ± 1.31 (n = 75)</td>
</tr>
</tbody>
</table>

Note, diameters and capillary lengths were computed using vessels with a mean diameter < 8.0 µm, area density and Krogh radius was determined using virtual histology at 10 µm spacing, volume density and capillary length density was measured directly from the reconstruction of each network. Krogh radius represents the radius of a theoretical tissue cylinder as determined by the tissue area and number of capillaries in each virtual slice and was determined as follows:

\[
Krogh\,\,radius = \sqrt{\frac{\text{tissue\,area}}{\pi \times \text{number\,of\,capillaries}}}.
\]

Values in parenthesis represent the number of point measurements for diameter, number of vessels for capillary length and the number of virtual slices for area density and Krogh radius.
Figure 2.7: Composite fields and variance images with resulting network reconstruction.

Composite maps of registered frames (left) and variance images (middle) from 4 fields of view in the same focal plane. The corresponding complete reconstruction for network D is shown on the right. Relative scale and image orientation is the same in all three images. Arrows indicate vascular features that are easily distinguishable in each of the three panels. Identified features lay in the focal plane of the registered intravital video fields.
An example of the 3D network reconstruction at bifurcations is shown in Figure 2.8. The panels illustrate a still frame at a bifurcation, the resulting variance image, a view of the reconstruction in the x-y plane and an off axis view that shows relative depth of the three vessel segments. Hemodynamic and saturation data that has been indexed to individual vessels can be visualized in a variety of ways. Figure 2.9 and 2.10 show colorized versions of the 3D network that indicate mean RBC velocity and mean RBC oxygen saturation on the right. Recorded vessel data is stored in text files that follow a simple and uniform structure. The format of the files is shown in Figure 2.11.
Figure 2.8: Vessel reconstruction at a bifurcation.
Image sequence showing separate steps in the reconstruction of an example bifurcation.

Panel A shows the bifurcation in a sub region of a single intravital video frame, individual red cells are visible and the flow direction is from bottom to top of the field.

Panel B shows the variance image of the video sequence illustrating changes in optical density over the recorded 60-second sequence. Panels C and D show the 3D representation of the reconstructed vessels; the view angle in D has been rotated off axis to illustrate how vessels spanning multiple planes are visualized.
Figure 2.9: Velocity network color map.
Mean cell velocity as measured in vivo indexed to individual vessel. Associated color map shows relative velocity in each vessel network geometry (grey indicates no measurement was made).
Figure 2.10: Saturation network color map.
Mean oxygen saturation as measured in vivo indexed to individual vessel. Associated color map shows relative saturation in each vessel network geometry (grey indicates no measurement was made).
Vessel Geometry Data For Segment:
<VID>
Source Image Path:
<Functional Image Path>
XOffset   YOffset (pixels)
0.00       0.00

<table>
<thead>
<tr>
<th>Point#</th>
<th>CentreX</th>
<th>CentreY</th>
<th>CentreZ</th>
<th>Diam</th>
<th>Distance</th>
<th>Vel</th>
<th>HCT</th>
<th>SAT</th>
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<tbody>
<tr>
<td>1</td>
<td>102.6</td>
<td>301.8</td>
<td>5</td>
<td>6</td>
<td>0</td>
<td>97.2</td>
<td>10.3</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td>101.9</td>
<td>300.5</td>
<td>5</td>
<td>6.6</td>
<td>1.5</td>
<td>97.2</td>
<td>10.3</td>
<td>50.3</td>
</tr>
<tr>
<td>3</td>
<td>101.9</td>
<td>299.2</td>
<td>5</td>
<td>6.6</td>
<td>2.8</td>
<td>97.2</td>
<td>10.3</td>
<td>50.6</td>
</tr>
<tr>
<td>...</td>
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<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>n</td>
<td>123.8</td>
<td>105</td>
<td>22</td>
<td>5</td>
<td>215.9</td>
<td>97.2</td>
<td>10.3</td>
<td>51.3</td>
</tr>
</tbody>
</table>

**Figure 2.11: Vessel geometry data file format.**

Example of an individual vessel geometry file illustrating the parameterized format of data for each node point along the vessel length. The vessel files are plain text files that are composed of two parts. A header portion that contains the unique vessel identifier (VID), the directory path of the source image the vessel was selected from, and the x-y shift used to register the source image. The remainder of the file is made up of a collimated structure that contains the node point number, vessel XYZ centerline position, vessel diameter at each node point, cumulative vessel length, mean measured velocity, mean measured hematocrit and RBC oxygen saturation.
2.4 Discussion

2.4.1 Major Findings

This study presents a novel software package capable of reconstructing functional microvascular network geometry in 3D using intravital video images of blood flow within the microcirculation. Reconstructed networks can be utilized for educational and analytical visualization of microvascular morphology and quantitative in vivo measures (velocity, hematocrit and oxygen saturation). The presented 3D networks can be easily quantified to characterize the observed functional microvasculature and thoroughly describe network morphology within discrete volumes. Future use of these reconstructions will add enhanced detail and accuracy to hemodynamic flow modeling, and integrated models of oxygen transport.

The sample reconstructed network in Figure 2.7 demonstrates the great potential of the presented software. The capability to map the geometry of complex microvascular networks spanning over a thousand microns combined with vessel paired functional measurements makes this software a novel tool to investigate oxygen transport. The example network in Figure 2.7 shows the versatility and scalability of generated maps and clearly illustrates how functional images from intravital video can be translated into 3D reconstructions of the network geometry.
Network statistics in Table 2.2 show a large variation between example networks across several presented parameters. Considering the relative volumes of the reconstructions these variations are largely due to the inherent heterogeneity of the microvasculature as a whole. Mean capillary diameters were consistent with previously reported measurements of rat muscle from functional images (9), histological sections (18) and microvascular corrosion casts (19). Similarly, mean capillary length, capillary length density and Krogh radius coincide with the findings of others (7, 15). Comparison between the measured capillary area and volume density illustrates the reliability of the virtual histology (Figure 2.6) in estimating vascular volume and provides an indication of how the volume density measured from the reconstruction would compare to actual histological sectioning.

The reconstructed networks are based on vessels with moving red blood cells. Vessels with stopped RBCs or filled only with plasma do not appear in the functional images and hence do not appear in the final reconstructed network. Thus these are functional networks for delivering O_2 to the tissue but network statistics acquired from these reconstructions may not agree with histology or corrosion casting approaches which presumably measure all vessels. The agreement between the data in Table 2.1 and previous findings supports our observation that in a healthy microvascular bed under normal resting conditions the majority of the capillaries are perfused with RBCs.
2.4.2 Considerations

The current study has several limitations that affect the quality of microvascular network reconstruction. Sampling resolution of the objectives limits the accuracy with which the focal plane can be determined to within approximately 3 µm at 20X magnification. Digital image capture also limits the accuracy of individual point measurements used to calculate vessel diameters and restricts any single measurement accuracy to within 0.65 microns horizontally and 1.3 µm vertically (the width and height between adjacent pixels is different because video fields, not full frames, were captured from analog CCD cameras to eliminate velocity artifacts). Due to the pixel based method used to register overlapping functional images the absolute position of vessels acquired from different images will be subject to an error of the pixel height and width. The effective sampling resolution is 0.65, 1.3 and 3 µm in the X, Y and Z directions respectively. Finer optical slicing and higher digital resolution (objectives with higher numerical aperture and digital video cameras with high pixel density) would ameliorate the accuracy of vessel depth measurement and pixel based measurements. The speed with which a network can be reconstructed has been improved greatly over manual point measurement, however the new software still requires a substantial amount of user input which could be reduced with further automation. Although automatic detection of in-focus vessel segments in each functional image may help speed the process, user input is still needed to guide the reconstruction.

The described approach of sampling only focal planes where vessels lie restricts this method of reconstruction to vascular geometries which have vessel segments
primarily parallel to the X-Y plane. Validation of the vessel selection algorithm using a digital phantom illustrates the robustness of variance image based vessel segmentation. Well ordered vascular geometry, in skeletal muscle and mesentery are ideally suited to this approach. For more complex, randomly oriented vessels other methods using various fluorescence microscopy techniques (2, 11) are available. Our approach that combines functional measurements, made using the same intravital video used in the reconstruction, produces sufficiently detailed vascular maps to achieve our goal of reconstructing 3D networks with matching functional data for use in blood flow and oxygen transport modeling.

2.4.3 Applications

The network reconstructions presented in this paper have the potential to be used for a variety of purposes related to blood flow and oxygen transport. Using real geometry for modeling blood flow in the microvasculature has great appeal particularly when the modeling result can be compared on a vessel-by-vessel basis to in vivo measurements. Furthermore utilizing reconstructed networks for O₂ transport modeling presents an opportunity for improved reproduction of in vivo conditions given that the convective delivery, due to geometry, will more closely match that which was observed in vivo. Since the actual network geometry, capillary hemodynamics and O₂ saturation are acquired from the same microvascular bed in vivo, we have the potential to test the accuracy and relevance of microvascular flow models and O₂ transport simulations.
The present work illustrates a novel method for studying functional network structure based on in vivo data. Other in vivo approaches using fluorescently labeled plasma, quantum dots carried in the plasma or fluorescent tagging of endothelium show all vessels regardless of the amount of RBC flow nor are they useful for measuring hemodynamic or O₂ transport data. These network reconstructions could also be used to follow progressive changes in network perfusion over time in pathological models such as sepsis or shock, which is not possible with histology or in vivo fluorescence imaging. This highlights a major advantage of visualizing the microvasculature and measuring conditions in vivo without the use of intravascular probes or the need for ex vivo sectioning and staining.

2.4.4 Perspectives and Significance

We have achieved our objective of developing a system for digitally characterizing geometry of a microvascular network from in vivo video images of red blood cell flow. The necessary software utilizes a simple graphical user interfaces and can be ran on a basic personal computer capable of running MATLAB. Basic morphological analysis of the reconstructed vasculature agrees with previously reported histological measurements of capillary diameters and capillary densities. The resulting reconstructions are recorded in a versatile and straight forward text format that can be easily translated into other modeling applications. Future work utilizing the reconstructed networks presented here will benefit from highly detailed representations of network geometry and vessel-specific hemodynamic and saturation measurements.
2.5 References


12. Krogh A. The number and distribution of capillaries in muscles with calculations of the oxygen pressure head necessary for supplying the tissue. *J Physiol (Lond)* 52: 409-415, 1919.


23. Tyml K, and Budreau CH. A new preparation of rat extensor digitorum longus muscle for intravital investigation of the microcirculation. *International journal*

CHAPTER 3: MICROVASCULAR FLOW MODELING USING IN VIVO HEMODYNAMIC MEASUREMENTS IN RECONSTRUCTED 3D CAPILLARY NETWORKS

3.1 Introduction

In our previous paper we described an experimental protocol and software package for reconstructing three-dimensional (3D) capillary networks from video recordings of microvascular blood flow in vivo (3). We also described how measurements of red blood cell (RBC) velocity, hematocrit, RBC supply rate (SR), and RBC O$_2$ saturation in these capillaries were acquired from the same videos used to reconstruct the network. By indexing quantitative measurements of hemodynamics (RBC velocity, hematocrit, RBC supply rate) and O$_2$ saturation onto specific vessels within a 3D map of a vascular network, we produce a complete and rich data set on which to base related computational models. However, there are two primary limitations that prevent us from directly using the experimental data in existing O$_2$ transport models (14). First, it is not always possible to acquire a complete hemodynamic dataset for each vessel within a full capillary network due to the topology of the network and hardware limitations under low light levels conditions (14). In capillary networks it is not unusual for one vessel to mask another vessel (independent data cannot be acquired from each vessel) or for there to be very short connecting vessels (there is not enough spatial data for velocity measurements) (14). The wavelengths used for spectrophotometric analysis of O$_2$ saturation result in very low light level conditions in thicker tissues such as the rat.
extensor digitorum longus (EDL) muscle. Some vessels may be too dark to obtain data from, or may require long exposure times and slower framing rates resulting in blurred images of high velocity RBCs. Second, to record a complete vascular map, multiple overlapping fields of view must be acquired sequentially in time to accurately reconstruct the network, and to obtain in-focus video data in as many capillaries as possible. Thus, hemodynamic and O₂ saturation data cannot be acquired simultaneously in all vessels (except for 2D networks entirely visible in one field of view). The inability to simultaneously measure conditions in every vessel, combined with the temporal variability in RBC flow in and among capillaries creates a situation where the collected experimental data may not represent an accurate flow balance throughout a branching network despite best efforts to obtain representative data for each vessel.

Our approach to overcome the above challenges was to apply a steady state flow model that appropriately represents the convective supply of oxygen as observed in vivo across the entire network. The flow model includes rheological dependence of RBC distribution at bifurcations as well as the dependence of viscosity in small vessels on local hematocrit and velocity (4). Since both RBC velocity and capillary hematocrit are highly variable among vessels, we chose to focus on RBC supply rate as the metric to match with our flow model. Our rationale for using RBC supply rate was that supply rate is effectively a direct measure of how much O₂ can be transported in a capillary network. Although velocity measurements have been used by others as the target metric (11, 12), velocity alone cannot be used to calculate O₂ transport in vessels without corresponding hematocrit data to calculate supply rate. The same is true of using hematocrit as the
metric without data on velocity. We also propose that attempting to precisely match supply rate on a vessel-by-vessel basis is unnecessary since diffusional exchange among capillaries will compensate for a mismatch in individual vessel supply rate provided that the total network supply rate is maintained. For this reason we adjusted the driving pressure across the network until the total RBC supply rate computed using the flow model matched the total red blood cell supply rate measured in the network in vivo. In vessels for which we did not have supply rate data, we applied a mass balance with measurements recorded from connected downstream or upstream vessels (14).

The first stage in applying the steady state flow model was to establish this basic mass balance, using hemodynamic measurements in daughter vessels, and test the validity of the resulting calculated blood flow in upstream parent vessels. Second, it was necessary to verify that the rule for distribution of RBC supply rate at bifurcations could be applied to capillary bifurcations in skeletal muscle. Published bifurcation rules acquired for larger vessel bifurcations in different tissues are described by continuous functions that calculate non-discrete cell distributions which cannot directly be rationalized with individual cell transits (8, 9, 11). Select experimental data sets, where data from parent and both daughter vessels could be acquired simultaneously, were used to compare flow distributions measured experimentally with the distributions predicted by empirical rules.

The third stage, was to determine how much effect an error in estimating the total RBC supply rate in the network would have on the computed tissue PO$_2$ distribution by
varying network supply rate by up to ±40%. Finally, since we matched the total RBC SR to the network but not the RBC SR in each individual vessel, we needed to determine how variability in RBC supply rate among capillaries affects the tissue PO\textsubscript{2} distribution. These last two stages were studied using two reconstructed capillary networks and their associated hemodynamic and O\textsubscript{2} saturation data. Results from the O\textsubscript{2} transport model demonstrate that matching RBC supply rate to individual vessels is not necessary, provided that the total RBC supply rate to the network is maintained. Changes to the total network supply rate of ±20% result in errors in tissue PO\textsubscript{2} proportional to approximately 1/2 the change in supply rate. From this we conclude that the approach we outline in this paper is a robust way to apply measured hemodynamic data to reconstructed capillary networks.
3.2 Materials and Methods

3.2.1 Intravital Video Microscopy

Intravital video sequences of capillary networks in the extensor digitorum longus (EDL) from previous work were used in this study (3). The experimental protocol used to visualize blood flow in the microvasculature has been described previously (3). Animal protocols were approved by the University of Western Ontario’s Animal Care and Use Committee. Briefly, male Sprague-Dawley rats were randomly selected on the day of experiment and weighed to verify a suitable mass between 140 – 180g. Animals were anaesthetized with a sodium pentobarbital solution (6.5 mg / 100g body weight) and mechanically ventilated at a mean rate of 74 breaths per minute. A cannula was inserted into the carotid artery to monitor heart rate and blood pressure using an attached pressure transducer. Supplementary saline and anesthetic were delivered as needed via a catheter inserted into the jugular vein. EDL muscle was dissected as described by Tyml and Budreau (13). The isolated muscle was secured with silk ligature and reflected on the microscope stage such that the lateral side of the muscle faced the objectives. The muscle was secured at approximately its in situ resting length and orientation, isolated from the air with polyvinylidene chloride film (Saran Wrap, Dow Chemical) and a glass cover slip, and kept moist with warm saline. At the completion of experimental protocols animals were euthanized using an intravenous injection of Euthanyl Forte (Bimeda MTC).
The video acquisition system utilized a Nikon Diaphot 300 inverted microscope fitted with a 100W white light Xenon lamp for transillumination of the EDL muscle. The intravital image was reproduced via a parfocal beam splitter fitted with 420 nm and 431 nm band pass filters. Simultaneous frame-by-frame video was captured at each wavelength using two identical computer systems, each connected to a charged-coupled device camera through real-time analog to digital capture cards. A live video output was displayed locally on closed circuit monitors to allow the user to select regions of interest and maintain focus during capture.

3.2.2 Mapping Bifurcation and Network Morphology

Vascular morphology for complete networks was determined using custom MATLAB software that has been previously described (3). Briefly, multiple intravital video sequences were recorded in overlapping fields at multiple focal depths in order to capture the complete geometry and connectivity of the network within a region of interest. Variance images were generated offline from each video sequence using the method described by Japee et al. (5). Variance images were used for registration of overlapping fields and fields at different focal planes. Individual vessel segments were selected from functional images and joined using automated and manual methods described elsewhere (3). The resulting reconstructed networks accurately map the functional morphology of contiguous networks observed in vivo.

Isolated divergent bifurcations used to analyze mass balance and cell distribution were captured and mapped (Figure 3.1) using a similar process as that described above.
Figure 3.1: Isolated diverging bifurcation.

Example of a diverging isolated bifurcation mapped using custom 3D microvascular mapping software. Direction of flow is from parent to daughters.
Individual bifurcations were selected on the basis that all three branches lay in approximately the same focal plane.

3.2.3 Capillary Hemodynamic Measurements

Hemodynamic measurements were made offline from video sequences of individual, in-focus capillaries within each field of view. Automated measurements for velocity, lineal density and supply rate were made on a frame-by-frame basis from each 60s sequence using custom analysis software described elsewhere (3, 6). Briefly, capillaries within each field were selected and outlined from variance images generated by processing the intravital video sequences. Variance images provide high contrast delineation between tissue and the luminal space swept out by the passage of RBCs. Space-time images were created for each vessel thereby describing the light intensity along the centerline of a vessel over time. In the resulting space-time images, due to the wavelengths selected (420 and 431 nm), areas containing RBCs have high contrast relative to the surrounding plasma (2, 6). Automated routines were used to segment the RBCs from plasma in order to measure RBC lineal density and to quantify the displacement of the RBC column from frame-to-frame to measure the RBC velocity. Tube hematocrit ($Hct_T$) for each video frame was calculated as the product of lineal density and RBC volume divided by the volume of the vessel segment. Vessel segment volume was calculated using mean vessel diameter and assumed a circular cross section. Frame-by-frame RBC supply rate (RBC/sec) was calculated as the product of the RBC velocity (mm/sec) and RBC lineal density (RBC/mm). Hemodynamic values averaged
over the collection period were indexed to corresponding vessels in reconstructed networks and isolated bifurcations. RBC oxygen saturations were determined using custom software that employs a spectrophotometric technique (calibrated in vivo) which calculates RBC oxygen saturation using RBC optical density as recorded at each wavelength similar to what has been described previously (3, 6).

3.2.4 Mass Balance at Bifurcations

In order to assess the validity of applying a mass balance to supplement direct measurements in a complete network, 8 diverging bifurcations were examined. As previously mentioned, bifurcations were selected on the basis that the parent and both daughter vessels lay within approximately the same plane of focus thus allowing for simultaneous measurement of velocity, hematocrit and RBC supply rate.

The software used to calculate capillary segment hemodynamics determines a mean velocity along the segment of interest. Since the empirical formula used for relative viscosity is based on Poiseuille’s law (10), the effective cylindrical diameter ($D_{cyt}$) for each capillary segment was determined by:

$$D_{cyt} = \left( \frac{n}{\sum_{i=1}^{n} \frac{1}{D_i^4}} \right)^{\frac{1}{4}}$$

(1)

where $n$ represents the number of measured diameters ($D_i$) along the vessel length. Average RBC supply rate was calculated from measured lineal density and velocity in parent and daughter vessels over a 60s sequence, as well as in 1s averages (30 frames)
throughout the entire sequence. The resulting values for average parent RBC supply rate over the entire sequence duration and on a second-by-second basis were then compared with the sums of RBC supply rate in the corresponding daughter vessels over each time period:

\[ SR_p = SR_{d1} + SR_{d2} \]  \hspace{1cm} (2)

Where \( SR_p \) is the calculated RBC supply rate in the parent vessel as determined by the sum of the measured RBC supply rate in the two daughters (\( SR_{d1} \) and \( SR_{d2} \)) over the measurement period. \( SR_p \) was compared against the observed supply rate in the parent vessel using a linear regression (Prism 4.0a, GraphPad Software).

**3.2.5 Cell Distribution at Bifurcations**

The fractional distribution of cells in daughter vessels is primarily determined by the relative downstream flow in each branch. When considering convective transport of oxygen via RBCs in a computational model it is important that an accurate application of this rheological distribution be observed in order for the construct to remain consistent with the observed biophysical properties. This distribution has been previously described in vessels between 7 – 50 \( \mu \)m (7). In capillary bifurcations, RBCs usually arrive at a branch individually or in short trains of a few cells and must traffic down one of the two possible paths. The empirically derived bifurcation rule is continuous in nature and cannot directly account for the discrete distribution of RBCs on a cell-by-cell basis. Due to the necessity of applying such a rule in a mathematical model of blood flow, it was important to determine the relevance of a continuous function compared to discrete
experimental observations in vivo. Using the calculated parent RBC supply rate \( (SR_p) \) defined in equation 2 and the fractional blood flow in each daughter branch described by:

\[
Q_b = \frac{1}{4} \pi \left( D_{cyl} \right)^2 v_b
\]  

(3)

where blood velocity \( (v_b) \) is defined as:

\[
v_b = \left( \frac{Hct_T}{Hct_D} \right) v_c,\]

given measured cell velocity \( (v_c) \), tube hematocrit \( (Hct_T) \) calculated from measured values, and discharge hematocrit \( (Hct_D) \) obtained via Equation 5 below.

The fractional distribution of cells can then be calculated using the aforementioned empirical rule \((7, 8)\):

\[
FQ_E = \frac{1}{1 + e^{-\left( A + B \log \left( \frac{Q_b - X_o}{1 - 2 X_o} \right) \right)}}
\]  

(4)

where the asymmetry of the distribution is represented by:

\[
A = -0.004 - 6.99 \log \left( \left( \frac{D_{cyl_{1}}}{D_{cyl_{2}}} \right) \left/ \right/ D_{cyl_{p}} \right) \quad \text{and} \quad B = 1.01 + 6.716 \left( 1 - Hct_{D} \right) / D_{cyl_{p}}
\]

which characterizes the sigmodal shape of the distribution, and \( X_o = 0.047 - 0.00123 \cdot D_{cyl_{p}} \), which is the fractional blood flow threshold below which no cells enter the downstream branch. This distribution relies on the conversion of the tube hematocrit \( (Hct_T) \) into a discharge hematocrit \( (Hct_D) \) using the relationship described by Pries et al. \((9)\):

\[
\frac{Hct_T}{Hct_D} = Hct_D + \left( 1 - Hct_D \right) \left[ 1 + 1.7 \cdot e^{\left( -0.35D \right)} - 0.6 \cdot e^{\left( -0.01D \right)} \right]
\]  

(5)

as it relates to the apparent viscosity of blood in narrow tubes.
Red blood cell supply rates predicted by the bifurcation rule were compared with measured values in each daughter vessel using linear regression (Prism 4.0a, GraphPad Software).

3.2.6 Modeling Flow in 3D Capillary Networks

Several approaches were employed to produce an accurate representation of the flow conditions observed in vivo. Missing velocity and hematocrit values in parent vessels were determined by using software that applied a mass balance at bifurcations similar to that described by Wetter et al. (14). Parent vessel blood flow \(Q_{bp}\) was taken as the sum of blood flow \(Q_{bd1}\) and \(Q_{bd2}\) from each daughter vessel as calculated by equation 3

\[
Q_{bp} = Q_{bd1} + Q_{bd2} \tag{6}
\]

Values for discharge hematocrit \(Hct_{Dp}\) were calculated by dividing the volume of cell flow in the two daughters \(Q_{cd1}\) and \(Q_{cd2}\) by parent vessel blood flow from equation 6

\[
Hct_{Dp} = \frac{Q_{cd1} + Q_{cd2}}{Q_{bp}} \tag{7}
\]

where the volume of cell flow in each daughter is \(Q_{cd} = V_c \cdot Hct_p \cdot \frac{\pi (D_{ cyl})^2}{4}\).

Similarly, blood velocity in parent vessels \(V_{bp}\) was determined by

\[
V_{bp} = \frac{Q_{bp}}{\pi \left(D_{ cylp}\right)^2} \tag{8}
\]
In cases where data in a daughter vessel was not available, the measured values from the corresponding paired branch were assigned. Where necessary this approach was applied sequentially upstream to provide a calculated hematocrit and velocity in each inlet vessel.

Observed network red blood cell supply rate (SR$_N$) for the volume was calculated from vessels that lay in the network cross-section containing the largest proportion of vessels with direct hemodynamic measurements. Vessels included in the cross section that were not measured experimentally were assigned a mean supply rate calculated manually from the values measured in other vessels within the cross section.

The calculated inlet hematocrits were incorporated into the reconstructed networks and an established flow model created by Goldman and Popel (4), based on equations derived by Pries et al. (11, 12), was applied. Pressure boundary conditions were adjusted iteratively at the network inlets and outlets to approximately match the relative velocity differences in individual vessels observed experimentally. Finally, the boundary conditions were linearly scaled to match the cross-sectional SR$_N$.

Two sets of flow solutions were created. One set closely matched the conditions observed in vivo; approximately matching observed velocity and hematocrit in each vessel, and matching measured total cross sectional SR$_N$ to within < 0.1%. A second set of flow solutions was created that applied an asymmetric increase/decrease in pressure gradient onto the network while maintaining total SR$_N$ in order to test the relative
sensitivity of using cross sectional supply rate as a target for observationally matched solutions.

To test the effect of changes in SR_N on tissue PO_2 an additional series of flow solutions were created that applied changes to SR_N in 10% increments spanning from -40% to +40% of the total cross sectional supply rate matched flow solutions.

3.2.7 Oxygen Transport Model

The O_2 transport model is based on the model described previously by Goldman and Popel (4). Our reconstructed capillary networks have replaced the idealized parallel vessel arrangement, and the flow solutions from the 3D networks have been used as input into the model rather than statistical velocity and hematocrit profile.

The tissue oxygen partial pressure, P(x,y,z,t), is described by:

\[
\frac{\partial P}{\partial t} = \left[1 + \frac{c_{Mb}}{\alpha} \frac{dS_{Mb}}{dP}\right]^{-1}\left\{D \nabla^2 P - \frac{1}{\alpha} M(P) + \frac{1}{\alpha} D_{Mb} c_{Mb} \nabla \cdot \left( \frac{dS_{Mb}}{dP} \nabla P \right) \right\}
\]  

(9)

where D, \alpha, and M(P) are the diffusion coefficient, solubility and consumption rate of O_2 respectively of the tissue. The influence of myoglobin on O_2 transport in the tissue is also included where \( c_{Mb}, D_{Mb}, \) and \( S_{Mb}(P) = P / (P + P_{50,Mb}) \) are the myoglobin concentration, diffusion coefficient and oxygen saturation. The following equation describes the convective O_2 transport in the blood at each constituent axial location (\( \xi \)) using the mass balance equation for blood hemoglobin oxygen saturation \( S(\xi,t) \):
\[ \frac{\partial S}{\partial t} = -u \frac{\partial S}{\partial \xi} - \frac{1}{\pi R} \left[ C + \alpha_b \frac{dP_b}{dS} \right]^{-1} \int j \cdot d\theta \] (10)

where \( u \) is the mean blood velocity, \( R \) is capillary radius, \( j \) is the oxygen flux out of the capillary at the axial location \((\xi, \theta)\), \( C \) is the O\(_2\)-binding capacity of blood, \( P_b \) is the intracapillary PO\(_2\) and \( \alpha_b \) is the solubility of O\(_2\) in plasma.

The exchange of O\(_2\) between capillaries and tissue is given by the following flux equation:

\[ j = \kappa (P_b - P_w) \] (11)

where \( \kappa \) is the mass transfer coefficient and \( P_w \) is the tissue PO\(_2\) at the capillary surface. \( \kappa \) reflects the impact of red blood cell spacing on diffusional exchange between capillary and tissue, and hence is a function of the capillary hematocrit at that location. The boundary condition at the capillary-tissue interface was specified as:

\[ -D \alpha \frac{\partial P_w}{\partial n} = j \] (12)

where \( n \) is the unit vector normal to the capillary surface and \( j \) is defined by Eq. 11. In the current work, the boundary condition at the tissue boundaries was specified as a zero flux boundary condition.

As outlined in Goldman et al. (4) the above O\(_2\) transport equations 9 – 12 were combined with Michaelis-Menten consumption kinetics, \( M = M_0 \frac{P}{(P + P_{cr})} \) and the Hill equation for oxyhemoglobin saturation, \( S(P) = P^n/(P^n + P_{50}^n) \) to define O\(_2\) transport within the 3D volume.
All simulations employed a grid spacing of 2 µm to provide adequate spatial resolution for calculations of tissue PO$_2$ within tissue elements. A summary of network geometry and simulation parameters can be found in Table 3.1. The initial condition for all of the simulations was zero tissue PO$_2$. The simulations were run on an Apple Mac Pro workstation with the approximate runtime to a steady state solution of 18 hours per run depending on the size of the reconstructed network.
Table 3.1: Reconstructed network parameters.
Summary of network geometry and hemodynamic parameters used in oxygen transport simulations.

<table>
<thead>
<tr>
<th>Network</th>
<th>Volume Dimensions (µm x µm x µm)</th>
<th>Volumetric Capillary Density (%)</th>
<th>Calculated RBC SR/Volume (mL RBC x mL tissue(^{-1}) x s(^{-1}))</th>
<th>Entrance Saturation (%)</th>
<th>Consumption Rate (mL O(_2) x mL tissue(^{-1}) x s(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>84 x 168 x 342</td>
<td>1.9</td>
<td>8.3 E-4</td>
<td>63</td>
<td>1.5 E-4</td>
</tr>
<tr>
<td>II</td>
<td>70 x 157 x 268</td>
<td>2.4</td>
<td>8.5 E-4</td>
<td>63</td>
<td>1.5 E-4</td>
</tr>
</tbody>
</table>
3.3 Results

3.3.1 Isolated Bifurcations – Upstream Mass Balance

An example of second-by-second measurements at an isolated bifurcation is shown in Figure 3.2. This example is representative of how the sum of daughter supply rates matches the measured supply rate in the parent vessel over time. Figure 3.3 shows the regression analysis for mean measured red blood cell supply rate in the parent vessel ($SR_p$) versus the mean sum of the corresponding daughter vessels supply rates ($SR_{d1} + SR_{d2}$), applying a mass balance on RBCs at the bifurcation. Regression analysis verified that measurements of $SR_p$ were not significantly different from the sum of supply rates in the daughters (linear regression: $y = 1.184 + 0.91x$, $r^2 = 0.9731; p < 0.0001$) across the bifurcations sampled. The 95% confidence interval for the slope includes 1.0 (0.76 to 1.06) and for the intercept it includes zero (-0.25 to 2.6 RBC/s).

Summary data for each sampled bifurcation can be found in Table 3.2 which indicates effective diameter, hematocrit, velocity, and measured supply rates for each branch of the bifurcation as well as parent supply rate estimated from the mass balance at the bifurcation and the daughter supply rates predicted by the bifurcation rule. Effective vessel diameters of parent vessels ranged from 4.8 – 6.0 µm with mean tube hematocrits from 7.2 ± 3.8 to 20.4 ± 3.8 % and cell velocities ranging from 102.0 ± 48.8 to 351.1 ± 67.0 µm/s.
Figure 3.2: RBC supply rate in an isolated bifurcation.

Measured red blood cell supply rate in each branch of bifurcation H recorded over a 60s period. A mass balance was performed on a second by second basis to produce the $SR_{d1}$ + $SR_{d2}$ curve which shows the sum of the SR in the two daughter vessels over the collection period.
Figure 3.3: Measured vs. predicted RBC supply rates in parent.
Linear regression of the mean measured supply rate for parent vessels in each isolated bifurcation vs. the sum of the mean measured supply rate in the corresponding daughter vessels. N = 8 bifurcations.
### Table 3.2: Summary of isolated bifurcation data.

The table indicates mean values for Effective Diameter (ED), Discharge Hematocrit (Hct), Blood Velocity (Vel), Supply Rate (SR), Predicted Supply Rate by Mass Balance (PSRMB) and Predicted SR by the Bifurcation Rule (PSRBR).

<table>
<thead>
<tr>
<th>Bifurcation ID</th>
<th>$ED_{p,d_1,d_2}$ (µm)</th>
<th>$Hct_{p,d_1,d_2}$ (%)</th>
<th>$Vel_{p,d_1,d_1}$ (µm/s)</th>
<th>$SR_{p,d_1,d_2}$ (cells/s)</th>
<th>$PSRMB_p$ (cells/s)</th>
<th>$PSRBR_{d_1,d_2}$ (cells/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>4.94</td>
<td>17.20 ± 5.36</td>
<td>177.99 ± 80.45</td>
<td>8.67 ± 4.64</td>
<td>9.46 ± 3.17</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>4.86</td>
<td>12.89 ± 3.67</td>
<td>74.64 ± 37.71</td>
<td>3.02 ± 1.80</td>
<td>–</td>
<td>2.69 ± 2.41</td>
</tr>
<tr>
<td></td>
<td>4.79</td>
<td>19.18 ± 4.48</td>
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<td>6.44 ± 2.61</td>
<td>–</td>
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</tr>
<tr>
<td></td>
<td>5.14</td>
<td>17.56 ± 3.14</td>
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<td>3.24 ± 2.54</td>
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</tr>
<tr>
<td></td>
<td>5.66</td>
<td>16.21 ± 3.20</td>
<td>70.68 ± 26.19</td>
<td>4.78 ± 2.1</td>
<td>–</td>
<td>4.49 ± 2.11</td>
</tr>
<tr>
<td>C</td>
<td>6.00</td>
<td>12.76 ± 4.46</td>
<td>156.07 ± 79.59</td>
<td>9.09 ± 4.68</td>
<td>9.23 ± 3.23</td>
<td>–</td>
</tr>
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<td></td>
<td>5.93</td>
<td>11.59 ± 3.81</td>
<td>126.75 ± 35.85</td>
<td>6.61 ± 2.88</td>
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<td>7.98 ± 3.36</td>
</tr>
<tr>
<td></td>
<td>6.06</td>
<td>13.61 ± 3.65</td>
<td>30.02 ± 22.95</td>
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<td>1.19 ± 1.12</td>
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<td>7.47 ± 3.77</td>
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<td>11.45 ± 3.60</td>
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<tr>
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<td>6.19 ± 3.16</td>
<td>229.04 ± 61.06</td>
<td>5.02 ± 2.53</td>
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<td>7.45 ± 3.87</td>
</tr>
<tr>
<td></td>
<td>5.65</td>
<td>9.4 ± 3.13</td>
<td>173.52 ± 31.47</td>
<td>6.45 ± 2.20</td>
<td>–</td>
<td>5.22 ± 2.69</td>
</tr>
<tr>
<td>E</td>
<td>5.50</td>
<td>10.02 ± 6.2</td>
<td>351.08 ± 67.02</td>
<td>13.50 ± 8.53</td>
<td>13.06 ± 4.90</td>
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</tr>
<tr>
<td></td>
<td>5.86</td>
<td>7.26 ± 3.51</td>
<td>176.34 ± 29.27</td>
<td>5.51 ± 2.77</td>
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<td>7.90 ± 4.08</td>
</tr>
<tr>
<td></td>
<td>5.52</td>
<td>8.84 ± 3.44</td>
<td>226.16 ± 42.11</td>
<td>7.58 ± 2.95</td>
<td>–</td>
<td>10.97 ± 5.30</td>
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<td>60.19 ± 32.00</td>
<td>0.99 ± 0.66</td>
<td>–</td>
<td>0.95 ± 1.14</td>
</tr>
<tr>
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<td>5.74</td>
<td>10.30 ± 3.95</td>
<td>79.27 ± 36.34</td>
<td>3.36 ± 2.05</td>
<td>–</td>
<td>2.15 ± 1.72</td>
</tr>
<tr>
<td>G</td>
<td>5.78</td>
<td>20.43 ± 3.81</td>
<td>119.86 ± 31.15</td>
<td>10.16 ± 3.27</td>
<td>10.08 ± 3.42</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>5.49</td>
<td>15.77 ± 2.76</td>
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<td>5.79 ± 2.46</td>
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<td>8.75 ± 2.70</td>
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<td>5.30</td>
<td>19.68 ± 3.70</td>
<td>60.01 ± 16.65</td>
<td>4.34 ± 1.54</td>
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<td>3.89 ± 1.68</td>
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<tr>
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<td>13.4 ± 3.58</td>
<td>206.78 ± 48.68</td>
<td>9.93 ± 3.10</td>
<td>10.43 ± 2.52</td>
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<td>5.72</td>
<td>16.20 ± 3.05</td>
<td>121.95 ± 28.58</td>
<td>8.10 ± 2.10</td>
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<td>7.30 ± 2.45</td>
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<tr>
<td></td>
<td>4.83</td>
<td>8.78 ± 3.19</td>
<td>92.45 ± 31.01</td>
<td>2.36 ± 1.06</td>
<td>–</td>
<td>3.10 ± 1.92</td>
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</tbody>
</table>
3.3.2 Isolated Bifurcations – Downstream RBC Distribution

Supply rate data, represented in Figure 3.2, is plotted in Figure 3.4 in terms of the distribution of RBCs at the bifurcation as a function of the flow distribution for comparison with the predictions of the bifurcation rule for that bifurcation, i.e. the flux or supply rate fraction vs. the flow fraction. Regression analysis was used to compare, for eight bifurcations, the measured RBC supply rate in daughter branches versus the distribution that was predicted by the bifurcation rule (Figure 3.5) given the hemodynamic conditions measured in each parent vessel and the diameter of their daughter vessels. Mean supply rates in the daughter branches were not found to be statistically different than supply rates predicted by the bifurcation rule (linear regression: \( y = -0.86 + 1.27x, r^2 = 0.7517; p = 0.0001 \)). The 95% confidence interval for the slope includes 1.0 (0.85 to 1.69) and for the intercept it includes zero (-3.01 to 1.30 RBC/s). Effective vessel diameters of daughter vessels ranged from 4.8 – 6.1 µm with ratios between daughter vessel diameters between 1.0 and 1.2 (larger relative to smaller). Hemodynamic conditions within daughter branches were variable with mean tube hematocrits ranging from 5.6 ± 2.9 to 19.7 ± 3.7 % and cell velocities ranging from 52.3 ± 37.7 to 229.0 ± 61.1 µm/s.

3.3.3 Reconstructed Networks – Flow Model

Hemodynamic measurements for each vessel were compiled and indexed to the corresponding vessel in the 3D network. Cross sectional network red blood cell supply rate for networks I and II as measured from in vivo data was 47.9 and 38.3 RBCs/s respectively.
Figure 3.4: Measured RBC distribution at a bifurcation.
The fractional RBC distribution vs. fractional blood flow for one daughter branch of bifurcation H with varying hematocrit. The disproportionate distribution of cells follows the prediction of the empirical rule outlined in equation 4. Predicted values are based on the mean hematocrit over the collection period.
Figure 3.5: Measured vs. predicted supply rates in daughters.
Linear regression of measured supply rates in each daughter vessel vs. supply rate predicted by the bifurcation rule given the appropriate flow rate measured in the parent vessel. N = 16 daughter vessels from 8 bifurcations.
Flow model solutions for each network yielded total RBC SR within < 0.1% of the corresponding measured values. Direct comparisons were made between the hemodynamic measurements and flow solutions values on a vessel-by-vessel basis by utilizing indexed data for each 3D network. Figure 3.6 illustrates the difference in supply rate between the measured and modeled result for each vessel in the two test networks; vessels with no in vivo measurement are indicated in grey. The mean RBC supply rate difference per vessel was -1.6 ± 2.7 cells/s in network I and -0.7 ± 2.7 cells/s in network II, and the maximum absolute difference was 9.3 and 5.5 cells/s in network I and II, respectively. Despite these individual differences the total SR difference was less than 0.005 cells/s in both networks.

The flow solutions for the asymmetric case for the two networks were generated by increasing inlet pressure for capillaries on one side of the network while decreasing it for capillaries on the other side (outlet pressure was kept fixed). The goal was to maintain total SR to the network while varying RBC distribution asymmetrically such that SR increased by approximately 20% on one side of the network and decreased by 20% on the opposite side. Figure 3.7 (top panel) shows the difference of SR in the asymmetric cases in each capillary relative to the original flow model solution. Mean absolute difference in SR per capillary between the original and asymmetric perturbations were 27.6 ± 33.3% and 33.2 ± 40.1% in network I and network II, respectively.
Figure 3.6: Difference maps of RBC supply rate.
Colormap of network I (top) and network II (bottom) showing the difference in RBC supply rate in cells/s between in vivo measurements and the flow model result. Grey indicates that there were no in vivo measurements for comparison.
Figure 3.6: (continued)
Figure 3.7: Tissue PO$_2$ differences in test and asymmetric models.

(Previous page) **Top** – RBC supply rate difference (cells/s) between the original solution and the asymmetrically perturbed flow solution for network I (left) and network II (right).

**Middle** – Volumetric tissue PO$_2$ difference map (mmHg) of the oxygen transport model for each network (only regions that have an difference greater than ± 1 mmHg are visible). **Bottom** – Relative distribution of PO$_2$ in the tissue as determined by the oxygen transport model. Black curves represent the PO$_2$ distributions for the original flow solutions while the red curves show the asymmetrically perturbed cases.
3.3.4 Reconstructed Networks – Oxygen Transport Model

Flow solutions for each network were applied to the O₂ transport model. The resulting oxygen profiles in the volumes of interest were treated as controls against the transport models using the asymmetrically perturbed flow solutions. The oxygen transport simulations for the baseline flow solutions of networks I and II resulted in a mean tissue PO₂ of 28.2 ± 4.8 mmHg and 28.1 ± 3.5 mmHg, respectively. For the asymmetrically perturbed cases, mean PO₂ was 27.6 ± 5.17 mmHg and 27.7 ± 3.7 mmHg in networks I and II, respectively. The bottom panels of Figure 3.7 show the distribution of tissue PO₂ for both cases in each network.

Resulting mean PO₂ levels for the incremental changes in SRₙ are shown in Figure 3.8. Increasing supply rate by 10% resulted in a marginal increase in mean tissue PO₂ of 4.0 and 3.4 % for networks I and II, respectively. Similarly, decreasing network supply rate by 10% produced decreases in mean tissue PO₂ of 4.8 and 4.3 % in the two networks. Increasing SR by 40% only caused a mean tissue PO₂ increase of 12.7 and 9.3 % in the networks whereas the SRₙ decrease of 40% lowered mean tissue PO₂ by 29.1 and 29.6 % in networks I and II, respectively.
Figure 3.8: Effect of incremental changes in RBC supply rate.

Mean tissue PO₂ changes predicted by the oxygen transport model for varying changes in SRₙ for networks I and II (error bars are the volume standard deviation).
3.4 Discussion

We have described a systematic approach of using red blood cell supply rate to produce a flow solution that is representative of convective oxygen delivery conditions as observed in vivo. When combined with a 3D model of oxygen transport the use of red blood cell supply rate provides an excellent metric to use when attempting to accurately model oxygen levels within a 3D volume. By perturbing the original flow solutions and comparing results of the oxygen transport model, we were able to show that large supply rate differences in individual vessels did not result in similarly large differences in tissue \( \text{PO}_2 \). This suggests that obtaining accurate inlet oxygen saturation measurements and representative total volume red blood cell supply rate are of paramount importance when modeling oxygen transport in complex networks. The specific distribution of cells within a discrete network does have an impact on oxygen delivery but our results suggest that diffusional exchange between vessels limits the impact of flow redistribution, provided that the overall supply rate remains constant. Small local changes in tissue \( \text{PO}_2 \) are evident given the substantial redistribution of cells within the network (Top panel, figure 3.7), though the resulting change in tissue \( \text{PO}_2 \) is relatively small (< 2% decrease in mean tissue \( \text{PO}_2 \)) compared to baseline solutions.

By applying mass balance on isolated bifurcations we were able to demonstrate that mean downstream measurements in daughter vessels over a 60 second period yield a value that is equal to direct measurements made over the same period in the parent
vessels. While this result was not unexpected it is important to demonstrate that mean measurements of unsteady state flow are relevant to conditions elsewhere in the contiguous network.

Application of an empirical bifurcation rule (8) to predict red cell distribution at capillary branches did not prove to be entirely deterministic. Pries et al. indicated several potential reasons why experimental observations of flow distribution may not agree with the empirical prediction including irregularities in the vessel cross section, retardation of the plasma layer due to endothelial surface proteins and the presence of white blood cells (11). Furthermore, unsteady state flow produces constantly changing downstream conditions (blood flow rate, hematocrit and velocity) and creates inequitable discrepancies that confound efforts to accurately predict flow separation at bifurcations. This highlights the advantage of using measured SR$_N$ as a metric to compare flow solutions, as it less sensitive to local distribution variability.

Recent work by Benedict et al. (1) illustrates the potential utility of applying flow models to reconstructed microvascular networks in order to study the effect of specific pathologies. The present work presents the additional advantage of utilizing fully 3D network geometries and incorporating direct, vessel-by-vessel, hemodynamic measurements into a functional blood flow model.

We were able to demonstrate that substantial changes in total network supply rate do impact total tissue PO$_2$. However, small changes in SR$_N$ only resulted in moderate
alterations to mean tissue $\text{PO}_2$ despite small heterogeneous spatial perturbations to $\text{PO}_2$ distribution. Large decreases to $\text{SR}_N$ did substantially reduce mean tissue $\text{PO}_2$ indicating the continued importance of accurate measurements of RBC supply rate in vivo. Experimental protocols need to focus first on ensuring that the best estimate of $\text{SR}_N$ is obtained before moving on to sample additional vessels.

Accurately quantifying blood flow in discrete vessels throughout a complex 3D capillary network remains a challenge. Currently it is not possible to make simultaneous measurements in all vessels of interest when a given network spans multiple fields of view, with vessels lying in numerous planes of focus. Further, the necessity of observing flow in each vessel for enough time to make accurate measurements of velocity, hematocrit and oxygen saturation, increases the time between individual vessel measurements thus exacerbating the problem of measuring unsteady flow in an inherently dynamic system. We believe that making serial measurements within individual vessels and utilizing total cross sectional red blood cell supply rate as a basis for determining an appropriate flow solution provides a result that represents flow conditions within the network as accurately as current technology will allow. We have shown that $\text{SR}_N$ provides a robust metric for quantifying blood flow in a discrete network, for the purposes of oxygen transport calculations, that is insensitive to errors in flow redistribution and small perturbations in total RBC supply rate.
3.5 References


CHAPTER 4: COMPARISON OF GENERATED PARALLEL CAPILLARY ARRAYS TO 3D RECONSTRUCTED CAPILLARY NETWORKS FOR MODELING OXYGEN TRANSPORT IN DISCRETE MICROVASCULAR NETWORKS

4.1 Introduction

Effectively delivering oxygen in the form of saturated red blood cells is a primary function of the microvasculature. Within skeletal muscle capillaries provide the largest surface area compared to arterioles and venules, for oxygen to diffuse out of the blood and into surrounding tissues. In combination with increased surface area, capillary networks allow for multiple transit paths for erythrocytes to deliver oxygen in a more spatially homogenous fashion. Modeling both normal and impaired oxygen transport in capillary networks can aid in the understanding of the consequences of different disease states as well as the normal functioning of living tissue (4, 8, 9). Precise models utilizing accurate reconstructions of capillary networks and direct measurements made in vivo are critical when examining perturbations to a complex system such as the microvasculature (1, 6, 10, 19). It is unclear whether or not simplified capillary network geometries such as parallel oriented vascular arrays are sufficient to represent the complex morphology observed in vivo.

Several characteristics of blood flow and network morphology have previously been identified as critical to the adequate delivery of oxygen to living tissue. August Krogh’s seminal work on tissue oxygenation identified capillary density and distance between adjacent capillaries as a limiting factor to oxygen delivery in resting and
working muscle (15). Red blood cell supply rate (RBC SR) and oxy-hemoglobin saturation (SO$_2$) directly affect tissue oxygenation and limit the amount of oxygen available for consumption (VO$_2$) by surrounding tissue (3, 5).

Capillary density, VO$_2$, SO$_2$, and RBC SR can be easily manipulated as variables in a computer model and values for these parameters can be readily obtained through mean measurements from histological, in vitro and in vivo means (6, 12, 17, 18). Previous efforts have been made to represent microvascular geometry using parallel networks that approximate what is seen in vivo (6, 10, 11). The resulting computer models of oxygen transport in the microvasculature have provided important insight into both the convective and diffusive delivery of oxygen to tissue.

The goal of this work is to compare the efficacy of using simplified, equivalent parallel capillary arrays compared to three-dimensional network reconstructions mapped using in vivo measurements. Furthermore we apply hemodynamic measurements to produce a flow model for the reconstructed networks that closely represents the flow profile observed experimentally. Using this approach, we hope to illustrate how the result of oxygen transport simulations may differ depending on the characteristics of the capillary geometry.
4.2 Materials and Methods

4.2.1 Intravital Video Microscopy

Capillary networks from rat extensor digitorum longus (EDL) muscle, captured using intravital video microscopy for a previous work were used in this study. The experimental protocol used to observe and record flow conditions in the microvasculature has been described previously (7). Briefly, male Sprague-Dawley rats were randomly selected on the day of experiment and weighed to verify a suitable mass between 140 – 180g. Animals were given an interperitoneal injection of sodium pentobarbital solution for anesthetic. Mechanical ventilation was used to maintain a mean breathing rate of 74 breaths per minute. Cannulas were inserted into the carotid artery, to monitor heart rate and blood pressure, and the jugular vein, to deliver supplementary saline and anesthetic as needed. EDL muscle was dissected as described by Tyml and Budreau (20), the muscle was isolated, secured with silk ligature, and reflected on the microscope stage such that the lateral side of the muscle faced the objectives. The ligature tied to the muscle was secured with surgical tape leaving the muscle in an orientation approximate to rest in situ. The muscle was kept moist with warm saline then covered with plastic film and a glass cover slip to isolate it from the air.

A Nikon Diaphot 300 inverted microscope fitted with a white light, 100W Xenon lamp for transillumination was used to visualize the EDL muscle. The intravital image was reproduced via a parfocal beam splitter fitted with 421 nm and 430 nm band pass filters. Simultaneous frame-by-frame video was captured at each wavelength using two
identical computer systems, each connected to a charged-coupled device camera through real-time analog to digital capture cards. A live video output was displayed locally on closed-circuit monitors to allow the user to select regions of interest and maintain focus during capture.

4.2.2 Mapping Network Morphology

Intravital video sequences were used to create detailed maps of microvascular networks. Discrete networks were mapped from video sequences using a previously described technique (7). Briefly, a series of overlapping video sequences were recorded for a discrete and continuous capillary network. Variance images were generated for each video sequence (13) and characteristic features in functional images were used as fiducial markers allowing for registration between overlapping fields and between different focal planes. Vessel segments within variance images were outlined using a series of automated and manual methods. Connections between vessels were identified by the user allowing for complex interconnected networks to be mapped. During network reconstruction several tools were available to aid in accurate reproduction of the experimental network including dynamic 3D generation of mapped vessels, overlaid transparent composite maps of functional images and review of intravital video to determine appropriate network connections. The resulting reconstructed networks (Figure 4.1) provide a detailed map of the functional morphology of contiguous networks observed in vivo.
Figure 4.1: Vascular maps for reconstructed and parallel networks.
Reconstructed capillary networks I (top left) and II (bottom left) shown beside equivalent parallel capillary arrays I (top right) and II (bottom right) generated to have the same vascular volume as the reconstructions. The foreground of each network image is the arteriolar end.
4.2.3 Capillary Hemodynamic and Oxygen Saturation Measurements

Hemodynamic and saturation measurements were made offline from video sequences using custom software similar to that described previously (2, 3, 7, 14). Briefly, individual in focus capillary segments from each field of view were selected from variance images. Space time images were generated and used to measure velocity, hematocrit and lineal density within individual capillaries (3). Oxygen saturations at inlet capillaries were measured using a spectrophotometric method utilizing a dual camera system recording oxygen sensitive and oxygen insensitive wavelengths (2, 7). Measurements were made on a frame-by-frame basis and averaged over a 60 second collection period. Mean hemodynamic and oxygen saturation measurements were indexed to the corresponding vessels in the network reconstruction.

4.2.4 Equivalent Parallel Arrays

The morphology and hemodynamics of each reconstructed 3D network were quantified using simple software that provided volumetric capillary density, bounding Euclidian X, Y and Z dimensions, mean and standard deviation of capillary diameters, mean red blood cell velocity and mean tube hematocrit. These measured parameters were used as the input for a simple computer program that randomly generating equivalent parallel capillary arrays that matched the source 3D reconstructions in dimensions, capillary density, mean velocity and hematocrit. This method of generating parallel arrays is similar to that described by Goldman and Popel (10) to create straight unbranched vessels populated around hexagonally packed muscle fibres.
4.2.5 Modeling Flow in 3D Capillary Networks

Measurements from the 3D reconstructions made in vivo were used as the basis for modeling flow in the corresponding network geometries. Blood flow and hematocrit values were estimated for vessel segments for which no data was recorded by using mass balance from connected downstream capillaries. Where needed mass balance was applied incrementally from downstream bifurcations until hematocrit and velocity had been estimated for all inlet vessels.

In the previous chapter it was demonstrated that cross sectional RBC SR is a major factor in determining oxygen delivery to a given tissue volume. RBC SR was calculated from in vivo measurements of vessels that lay within a cross section of the network that intersected a majority of vessels for which direct hemodynamic measurements had been made. Vessels in the cross section for which no measurement was recorded experimentally were assigned a mean supply rate calculated from the measurements made in other vessels in the cross section.

Hematocrits and velocity values for inlet vessels were calculated by recursively applying a mass balance to upstream vessels until the inlet values were determined. Resulting inlet hematocrits were set for the reconstructed networks and an existing flow model (11) was applied to calculate hemodynamic conditions throughout the network vessels. Boundary pressures were adjusted iteratively at the inlets and outlets of the network to roughly match the velocities observed in vivo. Cross-sectional RBC SR was matched to the experimental measurements by linearly scaling boundary pressures. Flow
solutions for 3D reconstructions matched in vivo measurements of total cross sectional RBC SR to within < 0.1%. Similarly for the parallel array networks cell velocity was linearly scaled such that the RBC SR was identical to the supply rate determined by the flow solution for each corresponding 3D reconstruction. Unique flow solutions were created for rest, exercise, and ischemia for each network; the hypoxic case utilized the same flow profile as rest. Parameters used in each simulation can be found in Table 4.1.
Table 4.1: Summary of parameters used in oxygen transport models.
Summary of network geometry and hemodynamic parameters used in oxygen transport simulations.

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<th></th>
<th>Volume Dimensions (µm x µm x µm)</th>
<th>Volumetric Capillary Density (%)</th>
<th>Capillary Length Density (mm x mL)</th>
<th>RBC SR/Volume (mL RBC x mL tissue⁻¹ x s⁻¹)</th>
<th>Entrance Saturation (%)</th>
<th>Consumption Rate (mL O₂ x mL tissue⁻¹ x s⁻¹)</th>
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<td>Network I</td>
<td>84 x 168 x 342</td>
<td>1.9</td>
<td>778.2</td>
<td>8.3 E-4</td>
<td>63</td>
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<td>736.5</td>
<td>8.3 E-4</td>
<td>63</td>
<td>1.5 E-4</td>
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<td>(Ischemia: 5.1 E-4)</td>
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4.2.6 Oxygen Transport Model

A finite difference model of oxygen transport developed by Goldman and Popel (11) was used to calculate $PO_2$ in the tissue surrounding the networks. The flow conditions generated for each case were used as the input to the oxygen transport model to yield oxygen transport solutions describing the $PO_2$ within the tissue volume. The volume was discretized into a series of grid points each representing $8 \, \mu m^3$ volume. At each time step $PO_2$ was calculated using equations describing oxygen diffusion between individual volume elements. Oxygen levels in the blood were determined within each vessel using a convective mass balance equation based on provided hemodynamics, vessel geometry, oxy-hemoglobin saturation and flux of oxygen out of the vessel into the tissue. A baseline oxygen consumption rate (Table 4.1) was selected such that the resulting capillary $SO_2$ throughout the network fit with experimental observations. Simulations were run to convergence on an Apple Mac Pro workstation with approximate runtimes of 18 – 36 hours needed to approximate steady state conditions determined by a 0 slope in $PO_2$ values over time within the corners of the simulation volume.
4.3 Results

Figures 4.2 – 4.5 illustrate tissue PO$_2$ distributions in each of the two reconstructed networks and corresponding parallel arrays for the 4 simulation cases (rest, hypoxic challenge, ischemia and exercise). In each set of simulations the tissue PO$_2$ in the 3D capillary network reconstructions were left shifted compared to the solutions for the corresponding equivalent parallel capillary array.

Mean tissue PO$_2$ in the resting simulation for the 3D reconstructions were 28.15±4.78 and 28.07±3.53 mmHg for networks I and II respectively compared to the equivalent parallel arrays means values of 31.24±4.54 and 30.57±3.44 mmHg (Figure 4.2). The PO$_2$ distributions in the ischemia cases spanned a wider range compared to rest with minimum PO$_2$ values 3.1 – 6.51 mmHg lower in the 3D reconstructions (Figure 4.3). The 40% flow reduction resulted in decreased mean tissue PO$_2$ of 19.81±7.63 and 19.90±4.92 in the reconstructions versus 24.64±6.89 and 23.62±5.07 mmHg in the associated parallel arrays.

Tissue PO$_2$ distributions in the hypoxia simulations were comparable to the ischemic case with wide ranges of PO$_2$ values (Figure 4.4). Again, reconstructed networks a had lower mean PO$_2$ values of 14.22±5.84 and 14.06±4.11 mmHg, compared to mean values determined for the parallel capillary arrays of 18.07±4.79 and 17.08±3.78 mmHg.
Figure 4.2: Tissue oxygen distribution for rest simulations.
Tissue PO₂ distributions for reconstructed (solid black line) and parallel array (broken red line) networks I (top) and II (bottom).
Figure 4.3: Tissue oxygen distribution for 40% ischemia simulations. Tissue PO$_2$ distributions for reconstructed (solid black line) and parallel array (broken red line) networks I (top) and II (bottom).
Figure 4.4: Tissue oxygen distribution for 40% hypoxia simulations.
Tissue PO₂ distributions for reconstructed (solid black line) and parallel array (broken red line) networks I (top) and II (bottom). Inlet SO₂ for hypoxia simulations was 37.8%.
Exercise simulations resulted in the widest PO$_2$ distributions (Figure 4.5) with minimum PO$_2$ values approaching 0 in reconstructed networks I and II, and parallel array I. The relatively high minimum PO$_2$ (7.5 mmHg) in parallel array II is caused by a counter current flow vessel which was set to mimic a shorter counter current vessel segment present in reconstructed network II. Under simulated exercise conditions mean tissue PO$_2$ in the reconstructed capillary networks was 10.07±5.37 and 12.61±5.70 mmHg compared to 15.32±7.26 and 18.76±5.25 in the equivalent parallel networks.

Capillary density in reconstructed networks varied longitudinally through the volume. The arteriolar end of the vascular bed had lower capillary density increasing towards the venular end. Figure 4.6 shows the relative vascular area in 10 µm sections taken longitudinally along the network. Mean tissue PO$_2$ in longitudinal X-Z planes was higher in parallel networks due to the uniform capillary density from arteriolar to venular end. Figure 4.7 illustrates the longitudinal difference in mean tissue PO$_2$ for each X-Z slice between reconstructed networks and parallel arrays for the resting case.
Figure 4.5: Tissue oxygen distribution for exercise simulations.
Tissue PO$_2$ distributions for exercise (6X VO$_2$, 5X RBC SR) in reconstructed (solid black line) and parallel array (broken red line) networks I (top) and II (bottom). Note that the parallel array for Network II has a higher minimum tissue PO$_2$ which is due to a counter current vessel that spans the volume.
Figure 4.6: Longitudinal capillary density for networks.
Capillary density for reconstructed networks and associated parallel arrays along the long axis of the network volumes. Vessel to tissue percentage was calculated for slices every 10 µm. The longitudinal position of arteriolar (A) and venular (V) ends of the network have also been indicated.
Figure 4.7: Mean longitudinal tissue PO$_2$.
Mean tissue PO$_2$ in each longitudinal X-Z slice for reconstructed networks (solid black line) and parallel arrays (broken red line) for resting case in networks I (top) and II (bottom). Tissue PO$_2$ in the parallel networks is higher due to greater capillary density at the arteriolar end and uniform spacing throughout the network volume.
4.4 Discussion

We have presented a comparison of tissue oxygen distribution within reconstructed 3D capillary network volumes and equivalent parallel capillary arrays. Utilizing resting conditions based on in vivo measurement our model has shown that parallel capillary arrays result in a higher overall tissue PO$_2$ in the volume of interest when compared to the 3D reconstructions. Capillary density at the upstream end of the tissue volumes was higher in the parallel capillary arrays resulting in higher tissue PO$_2$ in tissue surrounding inlet vessels (Figure 4.8). The higher tissue PO$_2$ is likely due to reduced diffusion distance between vessels creating a more homogeneous PO$_2$ profile in tissue at the inlet end of the volume. The simple geometry of parallel arrays results in a more uniform oxygen gradient longitudinally due to the fixed distance between vessels along their length. Similarly, since the outlet end of parallel array volumes have the same spatial distribution of vessels and uniform diffusion distances, we observed higher tissue PO$_2$ levels at the venous end of parallel networks compared to reconstructions. Mean PO$_2$ levels under resting conditions for parallel arrays (31.2 and 30.6 mmHg) are consistent with modeling results reported by Ellsworth et al. (27 – 30 mmHg) using experimentally measured input parameters applied to parallel vessel models (6). In addition, the mean tissue PO$_2$ in the reconstructed networks under rest conditions (28.2 and 28.1 mmHg) is similar to average PO$_2$ measured in spinotrapezius muscle (27.8 mmHg) using Whalen-type oxygen microelectrodes (16).
Figure 4.8: Tissue PO$_2$ at arteriolar end of network volume.
Network volumes for Network I (top) and the equivalent parallel array (bottom) illustrating volumetric regions with PO$_2$ values between 35 – 40 mmHg for baseline conditions. All other PO$_2$ values are transparent to show the range of interest.
Figure 4.8: (continued)
Under simulated ischemia, hypoxia and exercise conditions we also observed higher mean tissue PO\textsubscript{2} in each of the two parallel array models compared to the corresponding 3D reconstructed networks. Lower flow in the ischemic simulations yielded an increased difference in mean tissue PO\textsubscript{2} between reconstructed and parallel networks compared to rest. Larger differences in mean tissue PO\textsubscript{2} between reconstructions and parallel arrays compared to rest were also seen in simulated hypoxia models.

Of the four input conditions exercise was the only perturbation to create regions of anoxia in a test volume. Mean tissue PO\textsubscript{2} for reconstructed networks (10.1 and 12.6 mmHg) was lower than previous calculated values for mild exercise (17 mmHg) but consistent with heavy exercise conditions (12 mmHg) (6). Similarly Goldman et al. reported mean tissue PO\textsubscript{2} of 23.2 mmHg for simulated working conditions in fibre-structured vessels (analogous to parallel networks in this study) which is somewhat higher than what was found under exercise conditions in the equivalent parallel arrays (15.3 and 18.8 mmHg). There are however some differences in the blood flow and VO\textsubscript{2} values used in this study compared to previous reports. For comparison, the 10 fold increase in VO\textsubscript{2} applied by Goldman and Ellsworth (6, 10) results in an oxygen consumption of 15.7E-4 (mL O\textsubscript{2} x mL tissue\textsuperscript{-1} x s\textsuperscript{-1}); 70% higher than that used in the present study. Furthermore the 5 fold blood flow increase described previously (10) yields a RBC SR of 62E-4 (mL RBC x mL tissue\textsuperscript{-1} x s\textsuperscript{-1}), which is 50% higher than the RBC SR per unit volume used for exercise conditions in this study. With these
differences in mind the relative scaling of blood flow and oxygen consumption is comparable between this and previous studies.

An additional consideration for interpretation of these results is the presence of a single counter current vessel in Network II which was mimicked in the equivalent parallel array. The imposed counter current vessel spans the full length of the parallel volume, compared to a short counter current segment in the corresponding reconstructed network. This likely prevented oxygen levels from falling to 0 in the parallel array II exercise simulation. Counter current exchange between this vessel and other vessels increased the mean tissue $\text{PO}_2$ substantially compared to the 3D reconstruction. It has been noted previously that the presence of counter current flow significantly affects the distribution of oxygen within a volume (10). It is important to note that while the difference in mean tissue $\text{PO}_2$ between parallel and reconstructed networks increased in each of the three perturbed cases compared to rest, the overall magnitude the distributions shifted was relatively similar between parallel and reconstructed networks in each case.

A previous study compared several variations of computer generated capillary networks to study the effect of anastomoses and tortuosity on oxygen delivery (10). Goldman et al. applied oxygen transport simulations using straight unbranched capillary networks, similar to those used in the current study, and showed that networks with anastomotic vessels yielded mean tissue $\text{PO}_2$ values 1 mmHg higher than the comparable simulations with straight unbranched vessels. Similarly networks with tortuous vessels resulted in a mean tissue $\text{PO}_2$ 1 mmHg higher than straight unbranched networks. This
effect did not appear to be additive, in that network cases tortuosity and anastomoses did not further increase mean tissue $\text{PO}_2$ beyond 1 mmHg above the straight and unbranched case. Goldman et al. concluded that the presence of tortuous and anastomotic vessels increased mean tissue $\text{PO}_2$. In the present study we found that parallel arrays (equivalent to straight unbranched networks) delivered more oxygen when compared to the reconstructed networks. From this it is reasonable to conclude that adding tortuosity and anastomoses to our parallel arrays would further increase the difference compared to real networks reconstructed from in vivo data. Ellsworth et al. also noted that the homogeneity of vessel $\text{SO}_2$ increased longitudinally in a parallel vessel model of oxygen transport (6). The cause of this uniformity was attributed to a high degree of diffusional exchange amongst adjacent capillaries causing a convergence of $\text{SO}_2$ levels towards the venular end of the network. This effect is likely to similarly impact tissue $\text{PO}_2$ longitudinally causing more uniform gradients of oxygen perpendicular to the direction of flow resulting in higher tissue $\text{PO}_2$ throughout the tissue volume. Morphological differences in reconstructed networks, specifically varying capillary density longitudinally along the network and a more heterogeneous distribution of vessels throughout the volume, therefore have a marked impact on oxygen delivery in vivo. Parallel networks that utilize a homogeneous and ordered distribution of vessels throughout the network volume yield more uniform oxygen delivery that may not correspond as closely to what can be represented by a reconstructed vascular geometry.

The method used to generate parallel networks was based on the absolute capillary volume in the corresponding reconstructed network. Alternative metrics could
be used to create parallel arrays that could be considered equivalent. We consider producing networks with equal vascular volume to be a realistic approach though we acknowledge it does not directly compare to the approach of using the number of vessels per cross sectional area. Regardless of the method employed to generate a parallel array it is important to realize that the collection and characterization of a given network with respect to specific geometry and hemodynamic parameters was first accomplished experimentally in order to identify representative values to apply to the resulting generated arrays. We would assert that models are made more realistic when they employ a representative range of network morphologies and input parameters; ideally reconstructed 3D geometries and flow conditions calculated using direct in vivo measurements would be used. The limited number of reconstructed networks used in this study certainly does not encompass all of the diversity that may be seen in living muscle. However, we believe the ability to utilize specific cases and accurately represent a degree of variation is a step forward from the application of mean values to represent what is certainly a widely variable system that exhibits a distinct heterogeneity of vascular density and relative flow distribution.

The use of reconstructed 3D capillary networks can provide a more precise spatial picture of oxygen delivery within the microvasculature under a variety of normal and pathological conditions. Heterogeneities in oxygen tension within a given geometry could be used to explore angiogenesis and other physiological responses to hypoxic challenges. The future possibility of combining several distinct network geometries with models of larger vessels could aid in producing more integrated models of blood flow in
whole tissue as well as providing a framework for exploring control mechanisms that involve oxygen sensing in the microvasculature. Existing parallel array models continue to have great utility when simulating large vascular beds although modelers should attempt to incorporate observed diversity in morphology and blood flow. The availability of reconstructed network geometries and associated in vivo data sets, will improve the predictive power of existing models and facilitate the validation of the resulting solutions.

Using a computational oxygen transport model we have demonstrated that the source and structure of the underlying vascular geometry will produce distinctly different results. The application of 3D reconstructed capillary networks and matching blood flow profiles created using in vivo data, consistently yielded lower mean tissue PO$_2$ compared to parallel arrays generated using equivalent volumetric and hemodynamic parameters. Perturbations to oxygen delivery and consumption increased the observed difference in mean tissue PO$_2$ between reconstructed and parallel source geometries. The results of this study illustrate the value of using precise representations of vascular structures when modeling oxygen transport. Continued efforts to directly measure larger and more complex microvascular networks will provide a broader scope of input data allowing existing models to reflect the structural and functional variability seen in vivo.
4.5 References


15. **Krogh A.** The number and distribution of capillaries in muscles with calculations of the oxygen pressure head necessary for supplying the tissue. *J Physiol (Lond)* 52: 409-415, 1919.


CHAPTER 5: IMPACT OF INCREMENTAL PERFUSION LOSS ON OXYGEN TRANSPORT IN 3D CAPILLARY NETWORKS

5.1 Introduction

Mortality from sepsis and septic shock remains high despite improved diagnosis using biomarkers (12) and implementation of early goal directed therapies (13, 16, 17, 19). Sepsis has been defined as a systemic inflammatory response to an invading microorganism (4) and is a common cause of mortality in a variety of patient groups. Dysfunction of the microcirculation has been hypothesized to be the underlying pathophysiology leading to multi-organ dysfunction which is associated with the systemic inflammatory response in sepsis. The most notable aspect of blood flow disruption in sepsis is the loss of capillary perfusion in tissue remote to the site of infection (2, 11). Observable changes to blood flow within the microcirculation has been observed clinically within hours of infection onset (22). The loss of perfused capillaries in the microvasculature impairs oxygen delivery and is followed by deleterious effects caused by hypoxia in living tissue (5). Inadequate oxygen delivery in sepsis has therefore been hypothesized as a factor contributing to patient mortality and as such has received considerable attention from researchers and clinicians.

Accordingly, several animal models of sepsis have been developed in order to quantify the impact sepsis and the resulting flow dysfunction has on tissue oxygenation. Varying degrees of perfusion loss has been reported in skeletal muscle, liver, brain and
intestinal microcirculation (1, 5, 20, 21). However there are significant challenges to making direct measurements of tissue oxygen in vivo which has led to the use of computer models in order to quantify how loss of capillary perfusion impacts oxygen delivery to living tissue. Previous modeling efforts by Goldman et al. utilized generated parallel capillary arrays and experimental measurements to provide a detailed description of oxygen tension within three-dimensional (3D) tissue volumes with loss of functional capillary density (FCD) consistent with that observed in animal models of sepsis (7, 8). Goldman et al. estimated tissue consumption using a computer model to match observed oxygen extraction ratios from skeletal muscle capillaries in a rat cecal ligation and perforation model of sepsis. They concluded that increased oxygen consumption as predicted by the model and decreased oxygen supply causes increased susceptibility to tissue hypoxia (7).

In the previous chapter we demonstrated that computer models using equivalent parallel capillary arrays result in artificially high estimates of tissue PO$_2$ when compared to actual capillary geometries reconstructed from intravital video recordings of blood flow in vivo. The discrepancy between calculated tissue PO$_2$ in parallel arrays and reconstructed network geometries was shown under normal conditions as well as under simulated exercise, ischemia and hypoxia. We concluded that real network geometries reconstructed from experimental observations, produced oxygen transport solutions that more accurately represent the spatial PO$_2$ distribution and mean PO$_2$ values in vivo compared to equivalent parallel arrays generated using mean data from the same experimental data sets. Given this result, computational models should utilize
reconstructed or realistic network geometries, and corresponding experimental measurements of velocity, hematocrit and oxygen (SO$_2$ or PO$_2$) whenever possible.

In the present study we examine the impact of progressive functional capillary perfusion loss on oxygen transport in 3D reconstructed capillary networks. The impact of incremental FCD loss on oxygen delivery in vivo is not known, though computer models utilizing parallel arrays have shown decreased tissue PO$_2$ as a result of FCD loss (7, 8). By utilizing reconstructed networks we are able to simulate septic flow dysfunction in real capillary geometries. Application of a physical flow model was used to determine blood flow redistribution resulting from stoppages in individual capillary segments. We then compared the resulting oxygen transport changes to solutions produced using baseline red blood cell supply rates obtained from direct vessel-by-vessel hemodynamic and oxygen measurements made from the sample networks in vivo.

Recent clinical studies have measured venous O$_2$ saturation in septic patients and used this metric as a target measurement for early goal directed therapies. Central venous SO$_2$ is an indirect measurement of the balance between oxygen delivery and oxygen demand of tissues. Clinically central venous SO$_2$ has been used as a trigger for blood transfusion and has been shown to increase following fluid resuscitation which has been attributed to improved oxygen delivery to tissue (13, 16, 18). In the current study we were able to quantify how increasing red blood cell supply rate affects capillary outflow saturation (cvSO$_2$) in discrete capillary networks. The relationship between cvSO$_2$ and
restoration of basal tissue PO$_2$ levels can be compared to clinical measurements of mixed venous saturation as a target for treatment of septic patients.
5.2 Materials and Methods

5.2.1 Intravital Video Microscopy

Capillary networks from rat extensor digitorum longus (EDL) muscle, captured using intravital video microscopy, reconstructed in 3D, and modeled in previous chapters, were used as the basis for new flow solutions and oxygen transport simulations. The methodology for recording and measuring microvascular blood flow in skeletal muscle has been described previously (6). In short, male Sprague-Dawley rats were allowed to acclimatize for 1 weeks in animal quarters before being randomly selected on the day of experiment. Selected animals weighing between 140-180g were anesthetized using sodium pentobarbital delivered via interperitoneal injection. Following tracheotomy, animals were ventilated at 74 breaths per minute and cannulas were inserted to monitor heart rate and blood pressure. The EDL was blunt dissected and isolated as described previously (6, 23) such that the muscle could be transilluminated and the lateral surface of the muscle observed through microscope objectives. The externalized muscle was bathed in warm saline and covered with an oxygen impermeable film and glass cover slip.

An inverted microscope (Nikon Diaphot 300) with a 100W white light source was used to image blood flow in the EDL. The intravital image was passed through a beam splitter and band pass filters at 420 and 431nm wavelengths. Video sequences were
recorded on identical personal computers via video capture cards connected to charged-coupled device cameras.

5.2.2 3D Network Reconstruction

Capillary networks were reconstructed for use in blood flow and oxygen transport simulations using the methods described previously (6). Briefly, video sequences of microvascular flow were processed to create variance images and overlapping fields of view and focal planes were registered to produce a mosaic map of the volume of interest. Characteristic features in each image were used for registration allowing for vessels to be tracked between images and focal planes. Vessels in each image were segmented using both manual and automated methods. Connections between vessel segments and at bifurcations were created using automated algorithms creating contiguous maps of network geometries. Completed geometries were processed with quality control software to ensure contiguous geometry, and proper flow directions. Resulting vascular maps provide an accurate representation of the structure and conditions observed experimentally.

5.2.3 Capillary Hemodynamic and Oxygen Saturation Measurements

Individual vessels within the network geometry were analyzed to measure hematocrit, cell velocity and oxygen saturation using an existing custom software package similar to that described previously (3, 6, 10). Vessel segments were selected from a variance image of microvascular flow and space-time images were created from 60 second long sequences. Space-time images were used to measure cell velocity and
hematocrit over the capture period. Video captured from the dual-wavelength microscopy system was processed to create space-time images at the isosbestic (420nm) and an oxygen sensitive wavelengths (431nm) allowing oxygen saturations to be calculated from the optical density of cells at each wavelength. Mean values measured for each vessel were indexed to the network geometry such that recorded data for each vessel was paired with the specific capillary within the 3D geometry.

5.2.4 Modeling Flow in 3D Capillary Networks

Network reconstructions were processed to create a node-to-node description of the geometry for use in a steady state flow model. Velocity and hematocrit for inlet vessels was calculated using a recursive mass balance upstream to the inflow nodes. An existing steady state flow model (9) was applied using the flow equations described by Pries et al (14, 15). Inlet and outlet pressures were adjusted to approximate the velocity profile observed in vivo and to create a starting point for further scaling of the flow model. RBC supply rates were recorded from the in vivo data in a cross section of the network and the total network RBC supply rate was determined. The pressure boundary conditions were incrementally adjusted until the RBC supply rate matched the experimentally measured values within < 0.1%.

In order to simulate the loss of functional capillary density (FCD) observed in the experimental models of sepsis, a random sampling of vessels were set to be stopped for a given perfusion loss. Individual stop flow capillaries were selected from the group of vessels within the network that intersected in projection with two sampling lines.
perpendicular to the network volume Y axis (Figure 5.1). Blood flow was arrested in the target vessels by increasing intracapillary resistance such that the resulting flow solution calculated 0 hematocrit and 0 velocity in each of the target stop flow capillaries.
Figure 5.1: Projection of Network I showing sampling lines.

(Previous page) Reconstruction of network I illustrating the simulated 40% FCD loss; stopped flow capillaries are indicated in grey (color gradient of other vessels indicates relative depth in network volume). Dashed sampling lines dividing the network into thirds are used to identify the total FCD from the two cross sections and to identify stopped capillaries for simulated FCD loss based on experimental data.
Percent of FCD loss was calculated by counting the number of stopped flow vessels divided by the total number of vessels that intersected each sample line. Levels of FCD loss were created to approximately correspond to 10%, 20%, 30% and 40% FCD loss. Unique flow solutions were created for each network at four levels of FCD loss, and for the 40% range of FCD loss at 1X, 2X, 3.5X and 4X baseline RBC supply rates. A single counter current inlet vessel in Network II was clamped at the originally measured RBC supply rate and the driving pressure at the inlets at the arterial end were adjusted to increase total RBC supply.

5.2.5 Oxygen Transport Model

Oxygen tension in tissue was calculated using a finite difference model of oxygen transport described previously by Goldman and Popel (9). Flow solutions generated for each case described above were used as the input for the transport models in order to describe tissue PO$_2$ within the sample volumes under each simulated condition. Finite elements were defined for the entire volume resulting in a monotonic 2 µm grid spacing. Simulations were run from a starting state of zero PO$_2$ throughout the volume and diffusion was calculated between capillaries and tissue elements incorporating consumption for each case as described in Table 5.1 and 5.2. Changes in vessel oxygenation were calculated at each time step and convective transport of oxygen was accounted for based on hemodynamics calculated by the flow model and oxyhemoglobin saturation within the vessel segments.
Table 5.1: Variables for Network I simulations.

Variables for Network I (NI) simulations outlining the four different levels of perfusion loss (PL1 – PL4); high consumption (HC) simulations are indicated with associated increasing levels of RBC supply rate ranging from baseline RBC flux (F1) to four times baseline RBC flux (F4).

<table>
<thead>
<tr>
<th>Network I Simulations</th>
<th>FCD Loss (%)</th>
<th>VO$_2$ (mL O$_2$/mL tissue/s)</th>
<th>RBC Supply Rate (mL RBC/mL tissue/s)</th>
<th>Mean Pressure Drop Across Network (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NI-F1</td>
<td>0</td>
<td>1.5E-4</td>
<td>8.25E-04</td>
<td>3.00</td>
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<tr>
<td>NI-PL1</td>
<td>6.7</td>
<td>1.5E-4</td>
<td>7.67E-04</td>
<td>3.00</td>
</tr>
<tr>
<td>NI-PL2</td>
<td>20</td>
<td>1.5E-4</td>
<td>7.43E-04</td>
<td>3.00</td>
</tr>
<tr>
<td>NI-PL3</td>
<td>26.7</td>
<td>1.5E-4</td>
<td>6.83E-04</td>
<td>3.00</td>
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<tr>
<td>NI-PL4</td>
<td>40</td>
<td>1.5E-4</td>
<td>6.07E-04</td>
<td>3.00</td>
</tr>
<tr>
<td>NI-PL4F1</td>
<td>40</td>
<td>1.5E-4</td>
<td>8.25E-04</td>
<td>4.03</td>
</tr>
<tr>
<td>NI-PL4HCF1</td>
<td>40</td>
<td>3.0E-4</td>
<td>8.25E-04</td>
<td>4.03</td>
</tr>
<tr>
<td>NI-PL4HCF2</td>
<td>40</td>
<td>3.0E-4</td>
<td>1.65E-03</td>
<td>7.93</td>
</tr>
<tr>
<td>NI-PL4HCF3</td>
<td>40</td>
<td>3.0E-4</td>
<td>2.89E-03</td>
<td>13.74</td>
</tr>
<tr>
<td>NI-PL4HCF4</td>
<td>40</td>
<td>3.0E-4</td>
<td>3.30E-03</td>
<td>15.69</td>
</tr>
</tbody>
</table>
Table 5.2: Variables for Network II simulations.

Variables for Network II (NII) simulations outlining the four different levels of perfusion loss (PL1 – PL4); high consumption (HC) simulations are indicated with associated increasing levels of RBC supply rate ranging from baseline RBC flux (F1) to four times baseline RBC flux (F4).

<table>
<thead>
<tr>
<th>Network II Simulations</th>
<th>FCD Loss (%)</th>
<th>VO$_2$ (mL O$_2$/mL tissue/s)</th>
<th>RBC Supply Rate (mL RBC/mL tissue/s)</th>
<th>Mean Pressure Drop Across Network (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NII-F1</td>
<td>0</td>
<td>1.5E-4</td>
<td>6.84E-04</td>
<td>1.98</td>
</tr>
<tr>
<td>NII-PL1</td>
<td>7.1</td>
<td>1.5E-4</td>
<td>6.62E-04</td>
<td>1.98</td>
</tr>
<tr>
<td>NII-PL2</td>
<td>21.4</td>
<td>1.5E-4</td>
<td>6.12E-04</td>
<td>1.98</td>
</tr>
<tr>
<td>NII-PL3</td>
<td>28.6</td>
<td>1.5E-4</td>
<td>5.91E-04</td>
<td>1.98</td>
</tr>
<tr>
<td>NII-PL4</td>
<td>42.9</td>
<td>1.5E-4</td>
<td>4.37E-04</td>
<td>1.98</td>
</tr>
<tr>
<td>NII-PL4F1</td>
<td>42.9</td>
<td>1.5E-4</td>
<td>6.84E-04</td>
<td>2.96</td>
</tr>
<tr>
<td>NII-PL4HCF1</td>
<td>42.9</td>
<td>3.0E-4</td>
<td>6.84E-04</td>
<td>2.96</td>
</tr>
<tr>
<td>NII-PL4HCF2</td>
<td>42.9</td>
<td>3.0E-4</td>
<td>1.37E-03</td>
<td>5.70</td>
</tr>
<tr>
<td>NII-PL4HCF3</td>
<td>42.9</td>
<td>3.0E-4</td>
<td>2.39E-03</td>
<td>8.41</td>
</tr>
<tr>
<td>NII-PL4HCF4</td>
<td>42.9</td>
<td>3.0E-4</td>
<td>2.74E-03</td>
<td>11.13</td>
</tr>
</tbody>
</table>
Each simulation was run to a steady state condition established when the slope of tissue PO$_2$ over time in corner elements was equal to zero.
5.3 Results

Steady state oxygen transport simulations were completed, for each reconstructed network geometry, under four incrementally increasing levels of functional capillary density loss with normal oxygen consumption conditions. Tissue PO$_2$ distributions calculated for each case under normal consumption are shown in Figure 5.2. Increasing FCD loss and concomitant RBC supply rate decrease resulted in a progressive reduction in median tissue PO$_2$ levels from baseline values of 28.1 mmHg and 27.5 mmHg for networks I and II respectively. At the highest level of FCD loss (40% for network I and 42.9% for network II) median tissue PO$_2$ was reduced to 22.9 mmHg, in network I, and 20.1 mmHg in network II. In both network geometries the highest level of FCD loss caused the span of the fourth quartile of PO$_2$ values to widen and tissue PO$_2$ values residing in the third quartile to fall below the median value at baseline (Figure 5.2). When boundary pressures were increased to restore RBC supply rate to baseline levels for 40.0% and 42.9% FCD loss, median tissue PO$_2$ increased to 26.7 mmHg in network I and 26.8 mmHg in network II; returning median PO$_2$ to within 5% of baseline levels.

Color PO$_2$ surface maps in Figure 5.3 illustrate PO$_2$ conditions with normal consumption in the network I sample volume under baseline and the resulting drop in PO$_2$ following a 40% FCD loss. A substantial shift in the lower quartile was seen for both sample network volumes dropping from 23.8 and 25.0 mmHg under baseline conditions with normal consumption, to 17.2 and 17.4 mmHg with a 40% FCD loss.
Figure 5.2: Tissue PO$_2$ with baseline consumption at each level of FCD loss.
Tissue PO$_2$ values at each level of FCD loss with baseline consumption for networks I (top) and II (bottom) as predicted by the oxygen transport model. Supply rate weighted mixed capillary outflow saturation (cvSO$_2$) at each level of FCD loss with baseline consumption for networks I and II as predicted by the oxygen transport model are indicated by red markers scaled to the right Y-axis.
Figure 5.2: (continued)
Figure 5.3: Example color maps for network I with baseline consumption.
Surface color maps of tissue PO$_2$ (mmHg) for network volume I with baseline consumption as viewed from the venular end. Shown are 0% FCD loss with baseline consumption and RBC SR (top), 40% FCD loss with associated decrease in RBC SR (middle), 40% FCD loss with baseline consumption and baseline RBC SR (bottom).
Figure 5.3: (continued)
The decrease in the lower quartile tissue PO\textsubscript{2} values for network I are clearly visualized in Figure 5.2 showing the fall of tissue PO\textsubscript{2} levels at the venous end of the network following perfusion loss. Minimum tissue PO\textsubscript{2} fell to 13.5 and 15.4 mmHg, compared to the baseline levels of 19.5 and 22.4 mmHg in networks I and II respectively. The lower panel of Figure 5.2 shows the tissue PO\textsubscript{2} of network I with 40% FCD loss following RBC supply rates being restored to baseline. The upper limit of the lower quartile for networks I and II, with 40.0% and 42.9% FCD loss and baseline RBC supply rate, increased to 22.04 and 24.5 mmHg, while minimum tissue PO\textsubscript{2} similarly increased to 18.9 and 22.5 mmHg respectively.

Mixed capillary outflow saturation (cvSO\textsubscript{2}) decreased progressively with increasing FCD loss (Figure 5.2). By imposing a 40.0% and 42.9% FCD loss the corresponding drop in RBC supply rate caused cvSO\textsubscript{2} to drop to 12.9 and 13.2 percent saturated compared to 23.8 and 26.9 percent saturated under baseline conditions. With baseline RBC supply rate restored in network I at 40.0% FCD loss and network II at 42.9% FCD loss, cvSO2 increased to 23.5 and 27.4 percent saturation in the two networks respectively.

A second set of steady state oxygen transport simulations were completed, for each network volume, with the highest level of FCD loss (40.0% and 42.9%) and double the baseline oxygen consumption, at incrementally increasing RBC supply rates (Figure 5.4). Median tissue PO\textsubscript{2} levels for double consumption and baseline RBC supply rate was 11.5 mmHg for network I and 8.9 mmHg for network II.
Figure 5.4: Tissue PO$_2$ with 2X consumption with varying RBC SR.
Tissue PO$_2$ values at 40% and 42.9% FCD loss with double the baseline consumption and 1X, 2X, 3.5X and 4X RBC supply rate for networks I (top) and II (bottom) respectively. Supply rate weighted mixed capillary outflow saturation (cvSO$_2$) at 40% (network I) and 42.9% (network II) FCD loss with 2X baseline consumption and 1X, 2X, 3.5X and 4X RBC supply rate are indicated by the red markers and scaled to the right Y-axis. Baseline result (0 FCD loss with baseline consumption and RBC supply rate), is shown for comparison.
Figure 5.4: (continued)
High consumption cases with baseline RBC supply rates caused the lower quartile to decrease to 2.7 and 3.3 mmHg, with simulations resulting in minimum PO\textsubscript{2} of 0.0 and 0.4 mmHg for network I and II respectively. A doubling of the baseline RBC supply rate was not sufficient to restore median tissue PO\textsubscript{2} levels in either network with the median rising to 20.6 and 22.6, lower quartiles of 11.7 and 19.7, and minimum tissue PO\textsubscript{2} of 9.8 and 15.0 mmHg, in networks I and II respectively. A 3.5 fold increase in RBC supply rate restored median tissue PO\textsubscript{2} in network II (27.9 mmHg) whereas a 4-fold increase was necessary to restore baseline levels in network I (28.0 mmHg). Increasing RBC supply rate resulted in distinctly narrowed PO\textsubscript{2} distributions in each network volume, particularly in the second and third quartiles. Color PO\textsubscript{2} surface maps in Figure 5.5 show the spatial distribution in tissue PO\textsubscript{2} for 2X consumption cases and FCD loss under baseline RBC SR, 2X baseline RBC SR and 4X RBC SR. conditions.

With doubled consumption rate and baseline RBC supply rate the cvSO\textsubscript{2} fell to 0.0 and 0.4 percent saturation in networks I and II (Figure 5.4). Doubling RBC supply rate dramatically increased cvSO\textsubscript{2} in both networks, restoring outflow saturations to 16.9 and 23.5 percent saturation in networks I and II respectively. Higher RBC supply rates resulted in cvSO\textsubscript{2} well above baseline levels. The 3.5 fold increase in RBC supply rate caused cvSO\textsubscript{2} of 34.5 percent saturation in network I and 38.6 percent saturation in network II, while 4 times baseline supply rate raised outflow saturations to 44.4 and 41.3 percent saturation in the two networks respectively.
Figure 5.5: Example color maps for network I with 2X consumption.
Surface color maps of tissue PO$_2$ (mmHg) for network volume I with 2X consumption as viewed from the venular end. Shown are 40% FCD loss with 2X consumption and baseline RBC SR (top), 40% FCD loss with 2X consumption and 2X baseline RBC SR (middle), and 40% FCD loss with 2X consumption and 4X baseline RBC SR (bottom).
Figure 5.5: (continued)
Pressures needed to create RBC flux conditions in each simulation are shown in Tables 5.1 and 5.2 for networks I and II respectively. Under baseline boundary pressures increasing perfusion loss (PL) resulted in decreased RBC supply rate in each network. This change in supply rate was due to increased resistance from to stopped vessels and causing the decrease in RBC supply rate to be non linear with FCD loss. In order to increase RBC supply rate in the high consumption (HC) simulations inlet boundary pressures were increased. The pressure increases necessary to reach the desired RBC SR resulted in mean pressure drops in the high flow simulations several factors larger than the baseline difference.
5.4 Discussion

Using real microvascular geometry, and associated experimental measurements from rat extensor digitorum longus we have shown how FCD loss with concomitant decrease in RBC supply rate would impact tissue oxygenation. This approach simulates the FCD loss that has been observed in experimental models of sepsis (3, 5, 11). Further we have demonstrated that restoration of baseline RBC supply rate will largely restore original tissue $PO_2$ levels and that in order to compensate for increased metabolic consumption with FCD loss it is necessary for blood flow to increase several fold. With normal consumption, the highest tested FCD loss caused up to a 27% decrease in median tissue $PO_2$ and 31% decrease in minimum $PO_2$ (NII-PL4). By increasing the total RBC supply rate to the simulated tissue volume under normal consumption conditions and the highest level of FCD loss (NI-PL4F1), median tissue $PO_2$ was restored to $< 5\%$ below baseline, and minimum tissue $PO_2$ was returned to within 3\% of baseline in network I whereas restoration of baseline RBC SR caused tissue $PO_2$ to increase by 1\% in network II (NII-PL4F1). This suggests that the microvasculature is capable of maintaining tissue $PO_2$ levels given a substantial loss of perfused capillaries, provided that the necessary increase in RBC SR can be achieved. This suggests that the loss of capillary flow directly impairs oxygen delivery by decreasing the total RBC flux and not necessarily by increasing diffusion distance to flowing capillaries. Furthermore, the presence of anastomoses between adjacent capillaries allow for blood flow to redistribute around stopped flow vessels limiting the impact capillary perfusion loss may have on oxygen
delivery. Utilizing reconstructed capillary networks is advantageous when modeling this type of injury as it allows for realistic flow redistribution to occur both with and without the restoration of RBC supply rates.

In the present study increased oxygen consumption in the presence of high FCD loss was used to simulate a severe septic insult. Experimental models of sepsis have previously shown lower capillary SO\textsubscript{2} and higher capillary oxygen extraction which has been attributed to increased tissue oxygen consumption due to septic injury (3, 5). The oxygen consumption increase in the EDL was previously estimated using data from experimental sepsis (8) which predicted 2 – 4 fold consumption increases under pathological conditions. To simulate the conditions observed in experimental sepsis we applied high FCD loss and a 2 fold increase in O\textsubscript{2} consumption to our model and examined whether or not increases in RBC supply rate could restore tissue PO\textsubscript{2}. With baseline flow conditions, higher oxygen consumption caused a radical drop in tissue PO\textsubscript{2} creating anoxic regions in both sample networks and resulting in substantial hypoxia throughout the tissue volumes while reducing median tissue PO\textsubscript{2} to 41% (NI-PL4HCF1) and 32% (NII-PL4HCF1) of baseline levels. This result is consistent with a previous work that demonstrated oxygen supply dependency in a blood flow impaired model of septic injury (7). By doubling RBC supply rate, tissue PO\textsubscript{2} was partially recovered in simulations for both network volumes, restoring tissue oxygen levels to 73% and 82% of baseline in networks I and II respectively. In order to return median tissue PO\textsubscript{2} back to baseline under high consumption and high FCD loss conditions it was necessary to increase RBC supply rate by 4 fold in network I (NI-PL4HCF4) resulting in a tissue PO\textsubscript{2}
99.6% of baseline while an increase of 3.5 fold was necessary in network II (NII-PL4HCF3) to restore median tissue PO$_2$ to 101% of baseline levels. This clearly illustrates that a substantial increase in RBC flux is necessary to compensate for the combination of observed FCD loss and the estimated high consumption present in microvascular septic injury.

We examined mixed capillary outflow saturations in each of the presented simulation cases. Outflow saturations are in some ways comparable with clinical measurements of mixed venous saturation in that the relative changes in mixed venous saturation is presumed to reflect the quality of oxygenation in tissues throughout the body. Clinical studies examining early goal directed therapy in septic patients have used mixed venous blood saturations as a specific target for patient treatment (17). We found that progressive functional capillary density loss caused tissue PO$_2$ to drop resulting in a corresponding decrease in mixed capillary outflow saturation of up to 50% compared to baseline. Decreases in mixed capillary outflow saturation can be compared with clinical findings that show a drop in mixed venous saturation in patients with severe sepsis. Low mixed venous saturation and rising serum lactate levels (an indicator of anaerobic metabolism) have been shown to be an indicator of higher patient mortality (16). In the simulation cases where high consumption was used to simulate severe sepsis we found that increasing RBC supply rate by a factor of 2 restored median tissue PO$_2$ to within 18% - 27% below baseline and this was reflected in a similar recovery of cvSO$_2$ to 14% - 29% below baseline values. However it is important to note that the blood flow increases needed to restore median tissue PO$_2$ to baseline levels increased cvSO$_2$ to 40% - 87%
higher than baseline levels. Current early goal directed therapy strategies suggest the administration of dobutamine (an inotrope used to increase myocardial contractility and heart rate) and blood transfusions in order to achieve a normal mixed venous saturation of 70% (16). Both of these interventions improve oxygen delivery by increasing RBC supply rate and it is analogous to the high RBC supply rate cases with high consumption we examined in the present study. We believe that high cvSO₂ accompanying the restoration of tissue PO₂ in the high consumption and FCD loss simulation cases is illustrative of the outcomes seen clinically following successful application of early goal directed therapies in septic patients.

Previous work modeling perfusion loss in sepsis utilized parallel capillary arrays generated using specific capillary densities and structured in a relatively uniform hexagonal distribution (8). Goldman et al. examined the effect of perfusion loss in sepsis and found that tissue PO₂ decreased in simulated average sepsis (tissue consumption $M_0 = 3.86 \times 10^{-4}$ mL O₂/mL/s) to a mean value of 35.4 mmHg from 43.0 mmHg in control cases compared to our findings of 10.3 – 11.5 mmHg in high consumption/high perfusion loss sepsis versus 28.1 – 28.2 mmHg at baseline (8). There are two main reasons for the differences in mean tissue PO₂ determined in this study compared to previous findings. First, Goldman et al. utilized an inlet blood saturation of 69%, which is slightly higher than our experimentally measured value of 63% for the specific networks used in our simulations. Higher inlet saturation would result in slightly elevated tissue PO₂ than what our simulations predict. Second, the relative capillary density determined for the real networks used in the current study were 665 cap/mm² and 855 cap/mm² compared to the
density chosen by Goldman et al. of 1000 cap/mm$^2$ and 1500 cap/mm$^2$. The high vascular density, blood velocities and hematocrit used to determine blood flow in the previous study result in much higher RBC supply rates than those used as the input for our simulations. Furthermore, we have previously shown that parallel arrays with mean vessel hematocrits and velocities tend to yield higher estimates of tissue PO$_2$ than reconstructed network geometries using modeled flow parameters based directly on experimental measurements. While our approach provides an accurate representation of conditions in vivo in our networks, the spatial variability in vascular density, oxygen consumption and blood flow observed in living tissue cannot be fully represented by a small number of networks. Therefore examination of our results in conjunction with Goldman’s allows for a greater context of the delivery of oxygen in vivo in regions of varying vascularization and metabolic demand.

The modeling approach that we have outlined in the current work is a strong foundation for future efforts in studying the impact sepsis has on oxygen delivery as a result of microvascular injury and flow dysfunction. We have produced a model using real network geometries and utilized an approach that creates an experiment based blood flow solutions that includes arresting RBC flux in specific blood vessels which simulates the stoppage of flow observed in experimental sepsis. This approach provides a clearer picture of how perfusion loss and changes to metabolism affect tissue oxygenation, and the extent to which hypoxia are present in tissue during a septic insult. Further improvements to this model will similarly require precise maps of vascular geometry combined with direct measurements of individual vessels within the same geometry from
septic animals in vivo. We have previously outlined a strategy for applying blood flow models to sparse hemodynamic data sets which will undoubtedly be necessary due to the practical challenge of collecting simultaneous measurements from multiple vessels within a three-dimensional geometry, however tracking the specific perfusion loss within a given geometry will provide a further context the current study is unable to provide. The current work represents the most realistic simulations that can currently be achieved without tracking loss of perfusion in individual capillaries which ultimately would be ideal to characterize the impact in vivo. It will also be necessary to adjust consumption in the tissue volume to match the observed oxygen extraction in the specific volume of interest as observed in vivo. In future works, by combining a complete map of microvascular geometry, careful measurements within the majority of vascular segments, a flow model guided by experimental measurements, and an adjusted consumption to reflect the observed conditions, it will be possible to create an even more complete picture of tissue oxygenation in this and other pathological states. Ultimately these approaches should be adapted to study how vital organs such as heart, liver, kidney and diaphragm are affected by capillary perfusion loss and changes to tissue oxygen consumption.

We have described a comprehensive and detailed model of oxygen transport that utilizes capillary networks reconstructed from intravital video of rat skeletal muscle combined with a flow model that allows hemodynamic and oxygen saturation measurements to accurately represent conditions measured in the network in vivo. Using this model of oxygen transport we have simulated the blood flow dysfunction observed in
skeletal muscle during experimental sepsis and reproduced varying degree of functional capillary density loss and increase in oxygen consumption estimated previously (8). In cases with normal oxygen consumption and 40% FCD loss our model predicts a decrease in median tissue PO$_2$ of up to 27% primarily due to the decrease in RBC supply rate that results from stopped flow capillaries. In simulations where RBC supply rate was restored by increasing boundary pressures on inlet vessels tissue oxygenation was recovered to within 5% of baseline. When high FCD loss was combined with high consumption, simulations predicted large drops in tissue PO$_2$ decreasing median PO$_2$ by up to 41%, creating large regions of hypoxia and driving tissue elements at the venous end to anoxia. Only by increasing RBC supply rate by 3 – 4 times baseline was it possible to return median tissue PO$_2$ to baseline levels under high consumption and high FCD loss. We have examined mixed capillary outflow saturations predicted by our simulations and shown that cvSO$_2$ returns to baseline prior to complete restoration of median tissue PO$_2$. This finding can be compared with clinical targets in early goal directed therapy in which interventions designed to increase mixed venous PO$_2$ have been shown to be associated with reduced patient mortality. While the results of our simulations cannot be directly translated to patient treatment, our findings provide insight into how tissue oxygenation is affected by flow dysfunction in sepsis and how tissue conditions can be recovered by increasing tissue perfusion.
5.5 References


CHAPTER 6: FINAL SUMMARY

Modeling oxygen transport in the microvasculature is a complicated undertaking that requires detailed in vivo measurements coupled with the careful application of modeling strategies to simulate the convective and diffusive delivery of oxygen to tissue. A fundamental starting point for modeling microvascular flow is the precise reproduction of microvascular networks in order to construct a network that closely replicates what was observed in vivo. By combining reconstructed 3D networks with a blood flow model that represents conditions in real tissue volumes it is possible to utilize oxygen transport models that predict how oxygen is distributed within a sample tissue. Using reconstructed networks and measurement based flow models it is possible to make comparisons against simpler models that utilize statistical data applied to parallel arrays of vessels. With these principles in mind the current thesis has endeavored to describe novel software and approaches to produce oxygen transport models that accurately represent conditions as observed in vivo and apply these approaches to examine the effect that specific perturbations to blood flow, consumption, perfusion and oxygen saturation have on delivery to well defined microvascular networks.
6.1 Summary of Results

This thesis presents a novel and unique approach to oxygen transport modeling that combines a broad range of in vivo measurements into a cohesive and rational modeling framework. The first study describes 3D reconstruction software that was used to produce detailed microvascular maps from rat skeletal muscle and combine the resulting maps with indexed measurements of hematocrit, RBC velocity and oxygen saturation made in the muscle in vivo. A novel approach was developed to map complete networks using only a sparse sampling of planes where vessels lay avoiding the need for finely spaced Z-stacks to reconstruct microvascular geometry. The described software utilized functional images from intravital video to register video field positions in three dimensions allowing individual vessel segments to be selected from each field using a semi-automated segmentation algorithm. Vessel segmentation algorithms were validated using a digital vessel phantom that was used to conduct error analysis comparing the effectiveness of the automated approach and the defined phantom edge location. Low error rates from the phantom vessel edge detection demonstrate reliability of the automated segmentation over the range of noise levels present in the acquisition system. Capillary density and diameter measurements from the reconstructed networks were consistent with previous finding using corrosion casting and histological techniques (9, 10) and clearly show the physiological variability found in capillary networks in vivo. Finally a simple and versatile file format was presented that was adapted for use as an input for blood flow and oxygen transport modeling in Studies 2, 3 and 4. Combining in
vivo measurements with reconstructed network geometries is a powerful tool for examining complex data sets and a necessary preliminary step in creating initial conditions for blood flow and oxygen transport models. Developing this mapping package achieved our goal for Study 1 allowing for the use of the resulting reconstructions and functional measurements as a basis for Study 2. The next step for creating a physical model of capillary oxygen transport would require the reconstructed contiguous microvascular networks, and hemodynamic measurements in order to produce a flow modeling approach that was experiment based and yielded a mass balanced flow solution that was consistent with conditions observed in vivo.

To address modeling in vivo blood flow data, Study 2 examined how calculated steady state blood flow in isolated bifurcations compared with direct measurements of RBC distribution at bifurcations. Flow separation at bifurcations had been previously described and an empirical equation had been derived to calculate distribution of cells at bifurcations (8, 9) in microvessels. The mapping software described in Study 1 was used to reconstruct isolated capillary bifurcations with vessels diameters less than 6 µm. Reconstructed bifurcations made it possible to test both mass balance and the empirical bifurcation rule to understand the efficacy of each in modeling capillary blood flow. The strong agreement found from comparing the additive mass balance of downstream daughters with the measured RBC supply rates in the parent vessel suggest that that the analysis software is highly effective at accounting for RBC flux over a given time period and provides a high degree of confidence in determining upstream conditions using a calculated mass balance. Similarly the flow distribution predicted by the bifurcation rule
correlated well with the values measured experimentally although there was a greater variability which is likely due to the integer nature of cell distribution in capillaries bifurcations when compared to a continuous steady state function. The approach of matching total network RBC flux measured in experimental networks was successful and produced blood flow models with representative RBC supply rates and spatial distribution of cells throughout the sample networks. Examining how asymmetrically perturbed blood flow would alter tissue PO$_2$ showed that moderate changes in RBC distribution in a microvascular network result in small spatial changes to tissue PO$_2$, this suggests that small volumes of tissue are relatively unaffected by flow redistribution provided that all vessels remain perfused and total RBC supply rate is unchanged. Study 2 also predicts that changes to total RBC supply rate in a network will have an affect on tissue PO$_2$, particularly if RBC supply rate is decreased. These observations led to Study 3 of how vascular geometry affects tissue oxygenation when compared to parallel capillary networks with equivalent vascular volumes and equal total RBC supply rates.

Modern models of oxygen transport have largely relied on parallel capillary arrays for examining oxygen transport in silico (2, 6, 7), as a result it is important to understand how the use of synthetic networks may affect the predicted tissue oxygenation given the same absolute oxygen supply and RBC flux to the sample tissue. To study how the results of oxygen transport models may differ when using reconstructed networks versus equivalent parallel capillary arrays 4 different physiological conditions were simulated. It was shown that parallel capillary networks consistently produced higher mean tissue PO$_2$ under all simulated physiological conditions when compared to
reconstructed 3D networks. Higher tissue PO$_2$ in parallel networks were most likely a result of more homogenous capillary geometry longitudinally and a higher density of vessels at the inlet end of the parallel networks. These finding highlight the importance of using real network geometries, ideally with matching functional data as achieved in Study 1.

The final study in this thesis examined the impact of incremental perfusion loss due to stopped flow capillaries on tissue PO$_2$ as is seen in experimental models of sepsis. In addition to that primary goal an additional question was to determine whether restoration of baseline RBC supply rate and increase in RBC supply rate would be capable of returning tissue PO$_2$ to basal levels. Consistent with what was found in Study 2, if RBC supply rate is maintained tissue oxygenation remains relatively unaffected despite loss of perfusion in 40% of vessels, however maintenance of baseline supply rate required increase in driving pressure and proved insufficient when tissue oxygen consumption increased beyond baseline levels. Doubling oxygen consumption combined with vessel stoppage caused drastic decreases in tissue PO$_2$ requiring up to a 4 fold increase of RBC supply rate in order to restore baseline tissue oxygenation. Furthermore, consistent with clinical findings of above baseline mixed venous SO$_2$ in septic patients with positive outcomes (11) it was shown that the mixed capillary outflow PO$_2$ was similarly above baseline levels in simulations where oxygen consumption was doubled and RBC supply rate was increased to restore baseline tissue PO$_2$. These finding illustrate the utility of computer simulations to explore how therapeutic interventions may impact oxygen delivery to tissue under a variety of complex pathological states.
6.2 Considerations

The intention of any modeling research is to create a reasonably accurate representation of a real world system using a well-defined construct that utilizes actual physical properties of the system of interest. Given the complexity of microvascular blood flow, oxygen transport and utilization in living tissue, and the scale of the system under study, there are some practical and methodological limitations that must be taken into consideration when interpreting the results of the present thesis. The current work focuses on small regions of interest within skeletal muscle, specifically areas close to the muscle surface where discrete and contiguous capillary networks can be observed and measured; for the purposes of the present thesis, conditions within these capillary networks are taken to be representative of what can be observed throughout the muscle and capture a reasonable subset of the existing physiological variability. As shown in Study 1 there is an appreciable range of capillary densities and morphological differences within vascular networks, given this fact efforts were made to select capillary geometries with typical structural characteristics for modeling blood flow and oxygen transport for Studies 2 – 4. These efforts were limited in part by the acquisition protocol outlined in Study 1 which was designed to concentrate on capillary networks and specifically avoid areas with overlapping venules or arterioles. This was intended to simplify data collection and modeling of the observed microvasculature. However, the presence of venules or arterioles crossing a microvascular network would have an impact on oxygen delivery through diffusional exchange with capillaries and tissue. Furthermore, it is not
possible to account for oxygen delivery from regions adjacent to the volume of interest, as such zero flux boundary conditions were imposed at the tissue boundary for Studies 2 – 4. The assignment of a zero flux boundary condition represents an assumption which must be acknowledged since diffusion of oxygen between tissue regions outside the volume of interest is certain.

As described in Study 1 measuring conditions within a network in vivo requires multiple acquisitions at different focal planes and in different fields of view. This methodology allows for the capture of video to measure vascular geometry, hemodynamics and oxygen saturation throughout a network, but as indicated in Study 2 presents the problem of dealing with data sets that have elements collected at different time points; as such there is the potential for temporal variability to create discontinuities in an inherently unsteady state system. To resolve this problem the approach described in Study 2 produces steady state blood flow solutions that rationalize physical flow with respect to mass balance and the conditions observed over the acquisition periods. This approach made it possible to model the various physiological conditions described in Study 3 and to explore the complex problem of loss of perfused capillaries described in Study 4. With the limitations of the capture system and procedure outlined in this thesis, it is not possible to reproduce small variations in blood flow in individual vessels, and the use of a steady state approximation was necessary in order to conduct the research presented.
In all the studies presented in this thesis data from naïve animals was used as the basis for 3D network reconstruction and measurement of baseline conditions used in Studies 3 and 4. Perturbing these initial conditions in specific and deliberate ways was done to simulate a variety of physiological states and explore the resulting impacts on oxygen delivery to tissue. Furthermore, blood flow distribution was altered in Study 4 by changing resistance in individual vessel segments in an effort to reproduce the loss of perfusion observed in experimental models of sepsis (5, 8). In each case these perturbations were imposed on data sets from healthy animals and as such may not exactly replicate the changes in blood flow, oxygen saturation and oxygen consumption that may occur in a live animal subjected to the actual interventions being simulated. Further work should be done using measurements collected in vivo to further understand how the simulated perturbations presented in this thesis, particularly those representing disease states, impact oxygen delivery in the presence of a dynamic and reactive vascular system.

The EDL muscle characterized and modeled throughout the present thesis is composed of oxidative and glycolytic fibre types which have varying oxygen consumption rates (1, 3). Experimentally it was not possible to differentiate or spatially quantify muscle fibre types using the described intravital microscopy system. As such for the purposes of the oxygen transport model used in Studies 2 – 4 tissue consumption was assumed to be homogenous throughout the volume. Ideally the distribution of fibre types could be quantified and applied to the oxygen transport model such that the tissue regions have spatially variable oxygen consumption representative of oxidative and
glycolytic muscle fibres. This is particularly relevant for investigations under working conditions where oxidative fibres may cause greater heterogeneity in the oxygen consumption.

The convective oxygen transport equations used in Studies 2 - 4 employ a mass transfer coefficient that approximates the intracapillary resistance to oxygen diffusion into surrounding tissue. This is necessary due to the two-phase nature of blood where plasma gaps carry relatively little oxygen compared to RBCs. It has been previously shown that the mass transfer coefficient changes substantially with hematocrit (4). The hematocrit dependence of the mass transfer coefficient has been accounted for by using a numerical fit over a range of hematocrit values (25 – 55%). However hematocrits in the microvasculature can fall below the ranges to which the mass transfer coefficient has been calculated. This is a particular limitation for modeling oxygen transport in networks with very low hematocrits.

These considerations provide context to the results presented in the current thesis and should be noted when making comparisons with similar studies. Furthermore each limitation represents an aspect that could be altered or improved upon in future works.
6.3 Future Directions

There are several new research directions that could be undertaken to further the findings presented in the current thesis. The variability illustrated in capillary networks in Study 1 suggests that further efforts to characterize 3D capillary network morphology, and the broader scope and breadth of the observed variability, would lead to a better understanding of normal vascular geometry and provide a standard with which to compare microvascular structure and changes in blood flow under various pathological conditions. The presented methodology for network reconstruction is effective and achieves the stated goals, however future development focusing on further automation of 3D vascular reconstruction and blood flow analysis would make compiling expansive libraries of vascular data less time consuming and more easily accessible to other researchers (7).

Although Study 2 demonstrated that in vivo blood flow measurements made over time in multiple fields of view can be rationalized into a RBC balanced steady state blood flow model, development of new acquisition techniques allowing for simultaneous capture of blood flow measurements throughout the volume could lead to the application and validation of unsteady state flow models that more closely replicate conditions observed in vivo. The relative agreement between simultaneous in vivo measurements of RBC distribution at bifurcations with predictions from the empirically derived
bifurcation rule, further illustrates the potential to develop improved algorithms that account for downstream effects as well as the integer nature of cell distribution.

Finally, there is enormous potential to apply the methods and techniques as they have been presented in the current thesis to examine how specific pathologies (sepsis, diabetes, etc.) affect microvascular blood flow and oxygen delivery. Reconstruction of 3D network geometries and measurement of hemodynamics and oxygen saturation in animal disease models can be used to directly quantify how changes in blood flow observed within a given network in vivo alter tissue oxygenation in the volume of interest. Furthermore, the sample networks, baseline flow conditions and oxygen transport simulations described in this thesis can be used to conduct virtual experiments that can aid in generating meaningful hypotheses for experimental work as well as providing a reference for experimental outcomes under a range of physiological conditions.
6.4 Final Conclusions

It is possible to produce a detailed and comprehensive physical based model of oxygen delivery in living tissue using detailed vascular maps, RBC supply rate balanced steady state blood flow and a finite difference model of oxygen transport. The studies outlined in this thesis improve our current understanding of modeling oxygen delivery and the importance of using experimental measurements of network geometry and flow conditions to produce realistic models that accurately reproduce conditions observed in vivo. Modeling blood flow using experimentally measured RBC supply rates produced blood flow conditions that were insensitive to changes in flow distribution within the experimental volume and elucidated how changes in RBC supply rate impact tissue oxygenation. Oxygen transport modeling using reconstructed network geometries produced substantially different tissue PO$_2$ levels compared to models using equivalent parallel vessel arrays, highlighting the advantage of modeling using real network geometry. These methods can be applied to simulate a variety of pathological states, and as demonstrated with the model of perfusion loss in sepsis, show how specific therapeutic interventions targeted at improving tissue oxygenation by blood transfusion or increased blood pressure would ameliorate poor oxygen delivery.
6.5 References


APPENDIX I

ETHICS APPROVALS
Dear Dr. Ellis

Your Animal Use Protocol form entitled:

**Microvascular Oxygen Exchange**

has been approved by the Animal Use Subcommittee.
This approval is valid from **06.01.08** to **05.31.09**
The protocol number for this project remains as **2006-052-05**

1. This number must be indicated when ordering animals for this project.
2. Animals for other projects may not be ordered under this number.
3. If no number appears please contact this office when grant approval is received.
   If the application for funding is not successful and you wish to proceed with the project, request that an internal scientific peer review be performed by the Animal Use Subcommittee office.
4. Purchases of animals other than through this system must be cleared through the ACVS office. Health certificates will be required.

**ANIMALS APPROVED FOR 1 YEAR**

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<th>Housing/Use Locations</th>
<th>Animal # Total for 1 Year</th>
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**REQUIREMENTS/COMMENTS**

Please ensure that individual(s) performing procedures on live animals, as described in this protocol, are familiar with the contents of this document.

cc. Approved Protocol
Approval Letter

*This is the 2nd Renewal of this protocol
*A Full Protocol submission will be required in 2010
Dear Dr. Ellis

Your Animal Use Protocol form entitled:

**Optical Imaging of Microvascular Oxygen Transport in Skeletal Muscle**

has been approved by the Animal Use Subcommittee.

This approval is valid from **06.01.08 to 05.31.09**

The protocol number for this project remains as **2006-055-05**

1. This number must be indicated when ordering animals for this project.
2. Animals for other projects may not be ordered under this number.
3. If no number appears please contact this office when grant approval is received.
   If the application for funding is not successful and you wish to proceed with the project, request that an internal scientific peer review be performed by the Animal Use Subcommittee office.
4. Purchases of animals other than through this system must be cleared through the ACVS office. Health certificates will be required.

**ANIMALS APPROVED FOR 1 YEAR**

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**REQUIREMENTS/COMMENTS**

Please ensure that individual(s) performing procedures on live animals, as described in this protocol, are familiar with the contents of this document.

c.c. Approved Protocol - C Ellis, S Milkovich, W Lagerwerf

Approval Letter - S Milkovich, W Lagerwerf

---

*This is the 2nd Renewal of this protocol
*A Full Protocol submission will be required in 2010

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The University of Western Ontario
Animal Use Subcommittee / University Council on Animal Care
Health Sciences Centre, • London, Ontario • CANADA – N6A 5C1
PH: [Redacted] • FL: [Redacted] • www.uwo.ca / animal
Dear Dr. Ellis

Your Animal Use Protocol form entitled:

Oxygen Supply Dependency: mechanisms and modulation

has had its yearly renewal approved by the Animal Use Subcommittee.

This approval is valid from 09.01.08 to 08.31.09

The protocol number for this project remains as 2007-061

1. This number must be indicated when ordering animals for this project.
2. Animals for other projects may not be ordered under this number.
3. If no number appears please contact this office when grant approval is received.
4. If the application for funding is not successful and you wish to proceed with the project, request that an internal scientific peer review be performed by the Animal Use Subcommittee office.

REQUIREMENTS/COMMENTS

Please ensure that individual(s) performing procedures on live animals, as described in this protocol, are familiar with the contents of this document.

c.c. S Milkovich, W Lagerwerf
APPENDIX II

PERMISSIONS
February 21, 2012

The American Physiological Society grants to Dr. Graham Fraser, and University of Western Ontario permission to include the following published article in a thesis:

> Graham M. Fraser, Stephanie Milkovich, Daniel Goldman, and Christopher G. Ellis
Mapping 3-D functional capillary geometry in rat skeletal muscle in vivo

The American Physiological Society, by policy, allows original authors to republish their article(s) within a thesis document without charge and without requesting permission, provided that full acknowledgement of the source is given in the thesis. Please see our website for the full policy http://www.the-aps.org/mm/Publications/Copyright-and-Permissions.

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HONOURS AND AWARDS:

2008 Microcirculatory Society Zweifach Student Travel Award

2008 Graduate Student Teaching Award
PUBLISHED ARTICLES:


PUBLISHED ABSTRACTS:


3. Fraser GM, Goldman D, and Ellis CG. Examining the effect of varying red blood cell supply rate on tissue PO2 using a computation model of oxygen transport. *World Congress of Microcirculation.* 2010


5. Fraser GM, Steinback CD, Dias GM, Goldman D, and Ellis CG. Computational model of tissue oxygenation in rat skeletal muscle: application of a functional microvascular imaging system. *High Alt Med Biol.* 2009 (Accepted)


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11. **Fraser GM**, Goldman D, and Ellis CG. Progression of functional capillary loss in a five-hour sepsis model. *FASEB J.* 2005

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