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UNIVERSITY OF CALIFORNIA, SAN DIEGO

Long-Term Oxygen Sensor Implantation in the Porcine Subcutaneous Environment

A dissertation submitted in partial satisfaction of the requirements for the degree
Doctor of Philosophy

in

Bioengineering

by

Lucas Stefan Kumosa

Committee in charge:

Professor David A. Gough, Chair
Professor Karen Christman
Professor William Hodgkiss
Professor Andrew Mizisin
Professor Geert Schmit-Schoenbein

2011

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The Dissertation of Lucas Stefan Kumosa is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

Chair

University of California, San Diego

2011

DEDICATION

To my family and friends...

EPIGRAPH

“Quae nocent, saepe docent”

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ABSTRACT OF THE DISSERTATION

Long-Term Oxygen Sensor Implantation in the Porcine Subcutaneous Environment

by

Lucas Stefan Kumosa

Doctor of Philosophy

University of California, San Diego, 2011

Professor David A. Gough, Chair

The effect of long-term implantation on metabolically active devices is of utmost importance for not only the success of implanted glucose sensors used in diabetic therapy, but also for the development of artificial tissues and encapsulated cell devices. Such devices are dependent on the constant, predictable supply of metabolites from the local vasculature. Long-term implantation leads to the formation of a foreign body capsule whose purpose is the protection of the host and isolation of the foreign material

from local tissue resources. While this encapsulation is successful in protecting tissues from invading agents, metabolite flow continues, albeit at greatly reduced levels. The understanding of this encapsulation process is critical for the design and successful implementation of active implants dependent on metabolite supply.

The project goal is to utilize implantable wireless telemeters designed and manufactured by Glysens, Inc. to understand changes in oxygen levels of the surrounding subcutaneous tissues over the course of implantation in pigs. This is performed in three distinct, yet interrelated parts, namely: the analysis of oxygen signals collected from the long-term implantation of telemeters, the histological analysis of serial tissue samples collected from regions adjacent to the telemeter-like shams over the course of implantation, and the investigation of an accurate model for the dynamics of metabolite supply to implanted devices over the long-term.

Oxygen signals collected from sensors implanted into the subcutaneous tissues of pigs were found to contain several salient features. First, a long-term trend exhibiting exponential decay properties was discovered and is attributed to the impedance of mass transfer by the formation of a thick, avascular, fibrous foreign body capsule. The existence of such a capsule is demonstrated via histological examinations, and its impact on mass transfer of oxygen from the underlying vasculature to implant surface is investigated utilizing analytical and numerical methods. Second, a dominant frequency with a period of oscillation of 7-14 days was found for most of the signals examined. A model is proposed detailing the critical components of such an oscillations and how they might correlate with specific stages of the wound healing response.

The successful completion of this project provide a better understanding of the tissue changes that occur during the foreign body reaction, and the findings will be of direct benefit to the implant and device community allowing for better design parameters and device performance.

CHAPTER 1 –

Introduction

1.1 Background

Within the last 5 decades we have come to expect more from our implants. Performing a simple mechanical support function is no longer enough and these devices are carefully designed to integrate and interact with the body. We require cellular ingrowth and anchorage, adaptation to surrounding tissue mechanics, the leaching of factors, promotion of tissue restructuring surrounding the implant, transfer of mechanical information, metabolic transfer to and from the implant, etc. [1-3]. Our implants are now becoming more a part of the intricate functioning of the human body than ever before. However, despite the above mentioned expectations and functions one problem still exists: we do not fully understand the response of the body to the intimate placement of foreign devices into living tissues.

Of the many functions that engineered implants have come to perform, this work investigates the use of implants that require a metabolite to interact on its surface. With the current development of glucose sensors for diabetic monitoring [4-12], oxygen sensors for monitoring of proper oxygenation in hospital intensive care environments [13], lactose sensors for monitoring of fatigue under physical stress [14], stem-cell constructs for replacement/regulation of biological function [15-18], β -cell

islet devices for pancreatic supplementation [19-21], etc., it is clear that this field has a bright future ahead of it. One of the best ways to improve performance and usefulness of such devices is to better understand the complex relationship between an implant and its host tissue, in this case through the transfer of metabolites to and from the metabolically active implant. Specifically, the interaction of an implanted electrochemical oxygen sensor within subcutaneous tissues will be examined.

1.1.1 Electrochemical oxygen sensor

The electrochemical oxygen sensor has been around for many decades [22, 23]. The Clark electrode design discussed here, involves the presence of a cathode platinum electrode surface that is held at a constant negative potential with respect to a silver-chloride (AgCl) counter electrode [24, 25]. As oxygen comes in contact with the surface, it is instantly reduced in the presence of water into hydrogen peroxide, creating a current flow. This current can then be readily measured. The overall chemical reaction is as follows:



In order to prevent the adsorption of proteins and increase selectivity to oxygen, the electrodes are coated in a hydrolyte/PDMS coating that is highly permeable to oxygen

molecules and impermeable to larger molecules. A schematic representation of the electrode setup is shown in **Figure 1**.

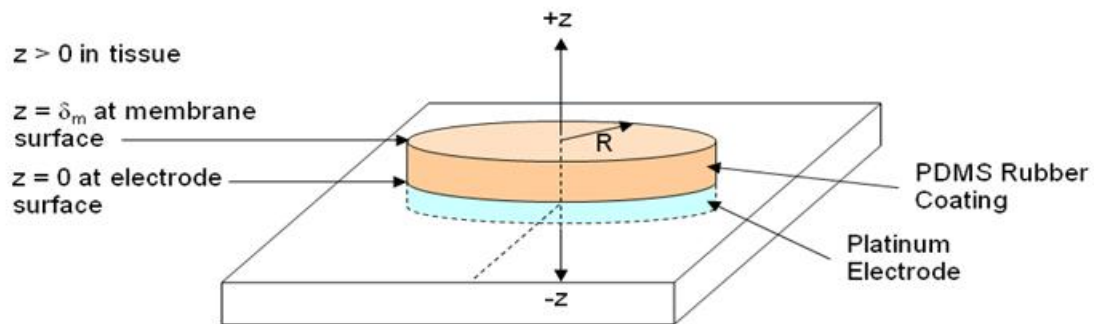


Figure 1. Oxygen electrode schematic illustrating the PDMS rubber coating and showing necessary boundaries and orientations.

The flux of oxygen to the electrode surface, j , can be mathematically described using one-dimensional Fick's law of diffusion:

$$j = -D \frac{\partial c}{\partial z} \quad (\text{Eq. 2})$$

where D is the diffusion coefficient of oxygen and c is the concentration of oxygen.

Using the following initial and boundary conditions:

$z = z_e = 0$ at the electrode surface, $c = 0$,

$0 < z < z_m$, $D = D_m$,

$z = z_m$ at the membrane boundary, $c = c_m$,

$z_m < z < z_t$, $D = D_t$,

$z \rightarrow z_t$ in the bulk tissue, $c = c_t$,

and evaluating this equation at steady state separately for oxygen flux, j , between the electrode and the membrane surface, and oxygen flux between the electrode and the bulk tissues:

$$j = D_m \left. \frac{\partial c}{\partial z} \right|_0^{z_m} = D_m \frac{c_m}{\delta_m} \text{ where } \delta_m = z_m - z_e \quad (\text{Eq. 3})$$

and

$$j = D_t \left. \frac{\partial c}{\partial z} \right|_{z_m}^{z_t} = D_t \frac{c_t - c_m}{\delta_t} \text{ where } \delta_t = z_t - z_m \quad (\text{Eq. 4})$$

we can solve for the total flux at the electrode surface. By inspection [26] of Equations 3 and 4 we obtain:

$$(c_m) + (c_t - c_m) = j \delta_m / D_m + j \delta_t / D_t \quad (\text{Eq. 5a})$$

$$j = \frac{c_t}{\left(\delta_m / D_m + \delta_t / D_t \right)} \quad (\text{Eq. 5b})$$

which represents the total flux through the tissue and membrane layers. Defining the Biot number, Bi , as the ratio of internal to external permeability and recognizing the linear relationship between flux at the electrode surface and generated current [25]:

$$j = i / nFA \quad (\text{Eq. 6})$$

via rearrangement we obtain:

$$i = \frac{nFAc_t D_m / \delta_m}{(1 + Bi^{-1})} \quad (\text{Eq. 7})$$

where n is the electron equivalent per mole of oxygen (in this case 4), F is Faraday's constant and A is the area of the electrode surface.

The implants utilized in the proceeding work are shown in **Figure 2**. The electrodes rely on this basic electrochemical principle, however many individual electrodes are arranged in an array fashion, allowing multiple signals to be collected

simultaneously. This provides redundancy in case of electrode malfunction and accounts for tissue heterogeneity. 18 platinum oxygen electrodes are present on each array, eight of which are dedicated to oxygen) with a 300 μ m diameter. A schematic representation of an electrode array is shown in **Figure 3**, where the 8 working oxygen electrodes are located on the left half (colored red). These electrodes are held at a constant -500 mV potential with respect to the counter electrode and the resulting current required to maintain this potential, equal to the current flow caused by consumption of oxygen at the surface, is recorded. Each array also contains a battery power supply capable of lasting the length of the implantation. Variable measurement and data recording rates can be selected, depending on the data needed, and remaining battery life. Internal telemetry is also present that allows for wireless data transmission to an external receiver. Sensor signal data is recorded and wirelessly transmitted in a continuous fashion to a nearby receiver. Data is output in terms of voltage (mV) which can be readily converted to the current produced at the electrode surface using a linear conversion.

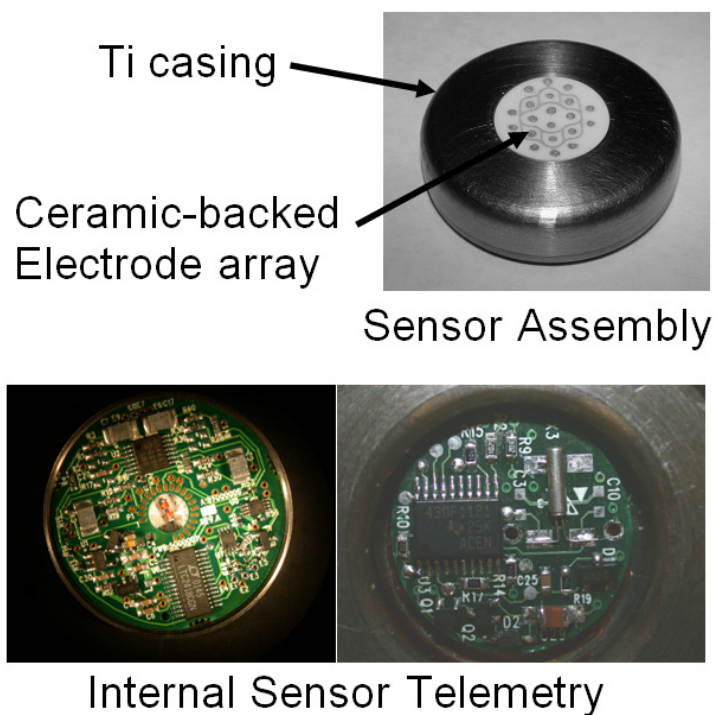


Figure 2. Photographs of the electrochemical oxygen sensor assembly.
(courtesy of Glysens, Inc.)

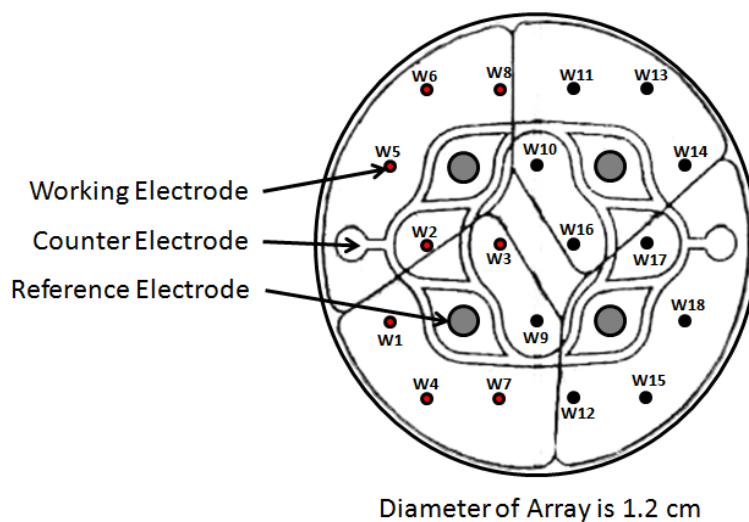


Figure 3. Schematic representation of the electrode array of the implantable telemeters. 18 working electrodes are present on the surface, with the left hemisphere (W1 – W8, colored red) dedicated to oxygen measurement.

1.1.2 Oxygenation of the subcutaneous tissues

Oxygen is a small, non-polar molecule that readily passes through aqueous environments and lipid membranes. After inspiration in the lungs, oxygen enters the bloodstream by passing through permeable alveolar tissues and is pumped through the vasculature into the tissues and organs. There, the oxygen tension between the highly oxygenated blood and the hypoxic tissues drives the outwardly flow of oxygen. This flow is dictated by numerous factors including the permeability of the tissues and vasculature, the consumptive rates of the tissues, the distances that oxygen has to travel, the distribution of sources, blood flow rate and pressure, etc. Obviously, this is an extremely complicated process and as a result has been extensively studied over the years. For this work, we are primarily interested in the tissue morphology and related properties that affect how oxygen travels from a nearby vessel, through the tissue bulk, to an implanted device.

The supply of oxygen from the vasculature is variable in the subcutaneous tissues. Over the years, the work of Dr. Marcos Intaglietta's group has yielded much information regarding the supply and distribution of oxygen in the microcirculation and the surrounding tissues [27-30]. The effects of longitudinal and radial distributions, vessel order, blood oxygenation [28, 29], vascular motion [31], etc. have all been shown to contribute. Most of these effects however are constrained to the short time-scale (<6 hours), and their effects on oxygen signals is currently being investigated by other researchers. Frequencies related to vasomotion on the order of seconds to minutes

have been identified in both hamster and pig subcutaneous tissues for a limited number of signals however further analysis is required.

The distribution of vessels in the tissues, and their order (diameter), can have a pronounced effect on oxygen flux trends lasting from days to months. Data on the spatial distributions of vessels in subcutaneous tissues adjacent to an implant is limited. Ertafai and Gough [32, 33], using serial histology of tissue collected from the rat window chamber model, demonstrated a characteristic distribution of blood vessels as a function of distance from the surface of an implant. This distribution shows that after 10 days of implantation, the majority of blood vessels occur closest to the implant.

Using micro-needle electrodes, Tsai and her colleagues tracked oxygen concentrations from the lumen of various sized vessels, out towards the vessel walls, through the endothelial and smooth-muscle into the extracellular space adjacent to the vessel. A correlation was found between the vessel diameter and the oxygen concentration at its external wall surface, and is shown in **Figure 4** [29]. This relationship between increasing vessel diameter and its corresponding oxygen supply can be used to guide the modeling of oxygen supply from vessels in tissues.

The experimentally determined diffusion coefficients of oxygen through subcutaneous tissues have previously been reported comparable to that of oxygen diffusion through water ($D_{O_2/H_2O} = 2.3 \cdot 10^{-5} \text{ cm}^2/\text{s}$, at physiologic temperature and pressure) [24, 34]. This value is deemed acceptable due to the relatively non-fibrous, highly aqueous nature of bodily tissues.

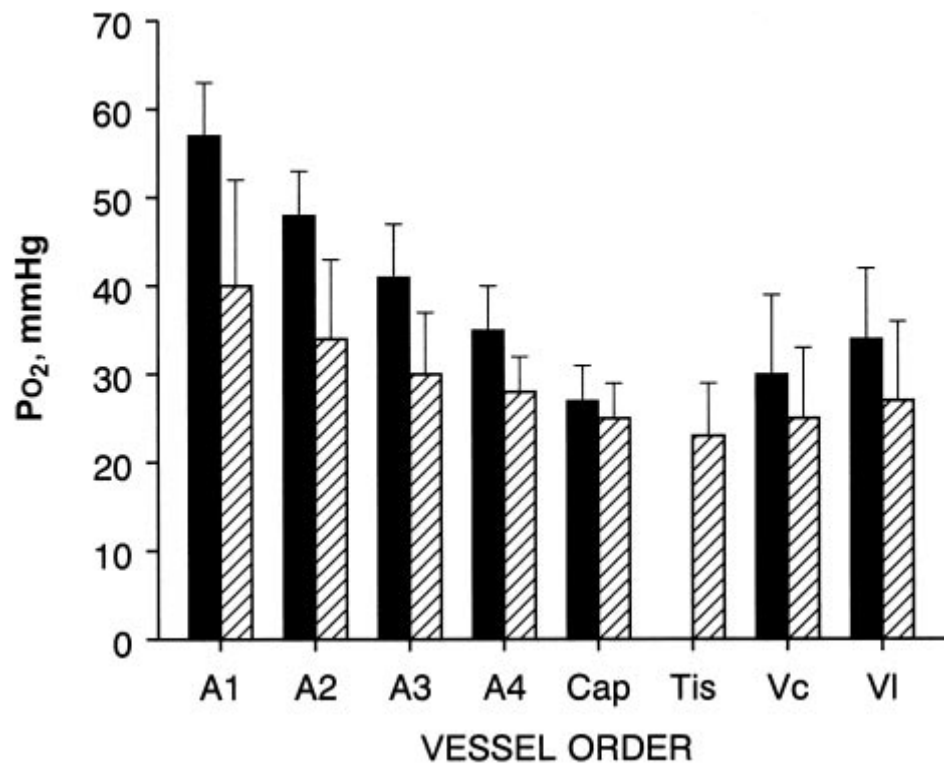


Figure 4. Distribution of oxygen in the microcirculation of hamster skinfold preparation. Intravascular values are shown as solid bars, and extravascular values immediately outside the wall are shown as hatched bars. The vessel diameters for each order of branching are as follows (in μm , means $\pm$ SD): A1, 85.1 ± 23.1 ; A2, 28.4 ± 11.7 ; A3, 9.8 ± 3.2 ; A4, 6.4 ± 2.1 ; Vc, 21.0 ± 5.9 ; VI, 88.7 ± 34.5 . A1 through A4, arteriolar orders; cap, capillary; tis, tissue; Vc, collecting venule; VI, large venule. PO₂ values can be readily converted to units of mol/cm^3 using Henry's Law and range from approx. $4 - 8 \cdot 10^{-7} \text{ mol}/\text{cm}^3$. (Reprinted from Tsai et al. [29])

1.1.3 Injury, wound healing and the foreign body response

The implantation of a device, such as the oxygen telemeter discussed in this work, triggers very specific and effective defense mechanisms by the body [1, 2, 35-48]. The initial injury involved in creating an implantation site starts a cascade of events intended to protect the area from excessive bleeding, and begin cleanup and repair of

the site as soon as possible. Previous literature demonstrates the dynamic nature of the wound site [37, 48]. The time scale for such an event is 10-14 days, during which time the prominence of various cells and structural components in the wound site change dramatically. In addition, the long-term isolation of an implant from bodily resources and prevention of further infection and damage lead to what is known as the foreign body response; which is in essence a modified wound healing event.

Wound healing has been previously identified to occur in several distinct stages, namely: onset, progression and resolution [35, 37, 48]. Onset involves initial bleeding following injury and an immediate clotting and vasoconstriction response. Blood plasma components (fibrin, IgG, platelet, etc.) adhere to the implant surface and signal for inflammatory action. This includes the release of powerful chemokines such as complement proteins (C3a, C3b) which trigger the fibrin cascade and reverse vasoconstriction following clotting, and cellular chemottractants (C5a, histamine, thrombin). Progression brings about the influx of leukocytes (granulocytes, macrophages/monocytes, etc.), endothelial proliferation and remodeling, and the signaling of fibroblasts. The tell-tale signs of inflammation (edema, macrophage proliferation, etc.) occur. Hypoxic conditions also bring about angiogenesis in an attempt to better oxygenate starved tissues and meet metabolic demands. The arrival of fibroblasts instigates fibrosis with the deposition of type I and III collagen at the wound site and remodeling of the ECM.

Oxygen in the wound site drops sharply immediately following the initial injury [45, 46]. However, since elevated O_2 levels are critical for the metabolic needs of

inflammatory, immune and remodeling cells, vasodilation and vessel permeability are quickly restored. Oxygen is a necessary component for generating energy equivalents via the Krebs cycle and is the electron donor in the electron transport chain within tissues. The production of reactive oxygen species (ROS; eg. peroxide anion, hydroxyl ion, etc.), a common class of oxidative molecules used to signal for the degradation of foreign substances, is especially vital for the cleansing of the wound site. The activity of many cell types involved in the wound healing response is strongly correlated with the levels of available oxygen (eg. neutrophils, macrophages, fibroblasts). While temporary local hypoxia may occur in isolated locations in order to instigate angiogenesis, the sufficient oxygenation of the overall wound site is critical for full, healthy repair.

As the wound progresses it can either resolve acutely, leading to a return to normal tissue conditions, or become arrested in a perpetual stage of granulation/inflammation [35-37, 46]. The presence of an implant leads to what has been coined in the literature as a “chronic wound” where the final resolution step is halted. Fibrosis continues and a tough collagenous capsule is created (the extent of which has been shown to vary with material type, shape, size, etc. [41-43]). Additionally, a continued presence of macrophages/giant cells engulfing the implant is also maintained. It is this capsule formation and long-term presence of inflammatory cells that is suspected to cause a measureable impact on oxygen signals reported by implanted telemeters. Not only is the fibrotic tissue relatively avascular, it is also believed to increase the distance between existing vessels and the implant surface, thus increasing the diffusion path of oxygen. Therefore, if the stability of the sensors

themselves is unaltered [34], then any changes in oxygen signals reported must be due to changes in the tissues within which the sensors are placed.

1.2 Need

The effect of long-term implantation on metabolically active devices is of utmost importance not only for the success of implanted enzymatic sensors such as glucose sensors used in diabetic therapy [4], but also for the development of artificial tissues and encapsulated cell devices [15, 19, 49]. Such devices are dependent on the constant, predictable supply of metabolites from the local vasculature [21]. Till now, the common understanding has been that implants placed into subcutaneous tissues become walled off within 1-2 weeks, and that long-term implantation is not viable for sufficient supply and measurement. As a result, for clinical glucose monitoring purposes, cumbersome direct blood-draw and percutaneous measurement methods are used, and full implantations require more invasive locations. Determining the available supply characteristics of the subcutaneous tissues is needed for use of implants in these favorable locations.

A direct need for oxygen sensing also exists in the field of pulmonary diseases. Chronic obstructive pulmonary disease (COPD) is one such disease where the constant presence of foreign particles or gases (such as those encountered during smoking or in polluted environments) causes an abnormal inflammatory response [50]. Fishman states in his recent book, that “COPD is the fourth leading cause of chronic morbidity and mortality in the United States” [51]. By 2020 it is projected that COPD will become

the world's third leading killer. The inability of such patients to adequately oxygenate tissues and organs presents immense stress on the organism leading to damage and possible necrosis, weakness, and a variety of other maladies possibly leading to death. The effective monitoring and measurement of not only the oxygen levels at a given time point, but also to track oxygenation dynamics would be of tremendous benefit both in and out of a hospital setting. Oxygen levels could be tracked at key organs that are particularly susceptible to hypoxic damage, and care could ideally be administered before the onset of significant damage. The use of implanted oxygen sensors for monitoring of patients suffering from COPD or other pulmonary disorders could be ideal for such treatment and therapy.

1.3 Hypothesis and project methodology

It is hypothesized that O_2 flux from the porcine subcutaneous tissue environment to the surface of an implanted oxygen electrode (and the resultant collected signals) correlates with the tissue structure – specifically the vascular density and distribution, cellular content, collagen content, and dimensions – over the long-term. Any fluctuations in the oxygen signals can be tracked via changes in the tissues within which sensors are placed. This will be investigated in three interrelated specific aims.

Aim 1: The characterization of signals from long-term implanted oxygen sensors into the porcine subcutaneous environment

Data from eleven sensor arrays, each comprised of eight oxygen electrodes, has been provided from Glysens, Inc. Removing any inactive or defective oxygen sensor signals, a total of 60 working electrode signals were provided for analysis. The 11 arrays were implanted into three animals at one of four specific locations on the animals back: right cephalic, left cephalic, right caudal, left caudal. Oxygen current signals were then recorded as a function of time for a period of 13 plus weeks. The signals are analyzed for the trend/pattern characteristics in the following time scales: >4 weeks, 1-4 weeks, and <1 week (but >6 hours). Trend analysis includes the use of the following signal processing tools:

- filtering and decimation to achieve desired sampling rates and remove unwanted noise,
- signal auto-correlation analysis to determine signal periodicity and pattern repetition, and cross-correlation analysis is used to identify patterns occurring between different electrodes, arrays and animals,
- power-spectrum analysis to identify most powerful frequencies within the signals,
- and wavelet analysis to determine whether any oscillatory behavior changes in time or remains constant.

Aim 2: Analysis of Encapsulation Dynamics Surrounding Long-Term Implanted Devices via Quantitative Histology

The encapsulation process that occurs in the porcine subcutaneous tissue surrounding the implant is quantified in order to mathematically represent the changes in cellular density, fibrous tissue density and vascular distribution. This is accomplished experimentally by implanting sham sensor constructs into the dorsal subcutaneous tissues of pigs. These shams are then serially removed along with adjacent tissues. The shams are designed to mimic the shape, size and tissue-adjacent structure of functional sensor arrays. The tissues removed along with each sham are then prepared for standard and immunological histology. Specifically, stains are utilized to best identify the distribution and density of the following structures: cell nuclei, collagen, endothelial cells, granulocytes and macrophages.

Aim 3: Computational analysis of oxygen flux dynamics due to long-term subcutaneous porcine environment remodeling

Computational and analytical models are utilized in order to examine various tissue/sensor geometries and conditions in an attempt to better understand the oxygen diffusion process in the tissues and to provide explanations for the dynamic changes and in-sensor variations that have been observed in the long term implanted sensor data. Data and findings from Aims 1 & 2 are used to guide the computational simulations and necessary values for oxygen supply, diffusion in the tissues and consumption are obtained from the literature.

The modeling concentrates on four areas of interest regarding the long-term implantation of oxygen sensors. Specifically, how the oxygen flux to the electrode surface is affected by:

- the mass transfer properties of the bulk as defined by the permeability (dimension to diffusion ratio),
- the physical distribution of the microvascular metabolite supply (due to rearrangement, shunting of blood vessels, etc.),
- the metabolic consumption rates of encapsulation tissues,
- and vessel size and location.

1.4 Goal

The understanding of this encapsulation process and the resulting changes in metabolite supply is critical for the design and successful implementation of active implants dependent on metabolite supply. The goal of this project is to elucidate the complex behavior of the implant/subcutaneous tissue environment. This is accomplished by utilizing actual long-term signals of oxygen current (and as a result flux), examining the dynamics in tissue structures and morphology that occur over the course of implantation, and devise a mathematical model that attempts to predict and explain the effect of encapsulation on the behavior of metabolically active implants. Specifically, we aim to answer the following question: how will the encapsulation process affect the mass transfer properties of oxygen between tissue and implant?

1.5 Scope

This thesis tackles a very broad, involved, and complicated subject. The contents span a vast number of technical and multidisciplinary fields, including: electrical engineering, materials science, signals processing and analysis, standard and immunochemical histology, automated image processing, statistics, computational model analysis (both analytical and numerical), etc. It would be very easy to get lost in the minutia of any one of these aspects of the project. The reasoning behind the Bioengineering approach is to pick and choose selected tools from each of these diverse fields and apply them to the investigation and understanding of a biomedical problem. Attempts have been made to customize the tools to aid in the timely and accurate processing and analysis of the collected data, however the intent of this project is not to improve already well developed fields of study.

In addition, the immense amount of data provided by others (Glysens, Inc.) and collected during the thesis work leads to numerous analytical directions that could be taken. This thesis will deal with the relationship between the signals collected from live oxygen sensors and the changes in the underlying tissues that could result in such signal dynamics. A rigorous materials comparison, the development of new and novel signals processing techniques tailored to biological signals, the development of new and novel histological procedures, modifications to finite element techniques, etc. is avoided. This does however open up the possibility of much work utilizing this data set for the future.

CHAPTER 2 –

Characterization of oxygen signals

2.1 Methodology

An affiliated biotech company specializing in the development of wireless implantable metabolite sensors (Glysens, Inc.) provided sensor recordings from telemeter arrays that were implanted into the dorsal subcutaneous tissues of Yorkshire farm pigs. For more information regarding the experimental procedures, please refer to the Appendix, Section 7. Data from 60 functioning electrodes spanning eleven arrays implanted into one of three pigs were supplied. The signals contain up to 13 weeks of information, depending on battery life and electronic stability. Given that battery life is a concern, the telemeters have variable data transmission rate capability, and for this experiment one data point was recorded every 2 minutes to ensure 3 months of continuous data. The data was transmitted in the form of millivolts linearly proportional to the oxygen current (consistent with Equation 7) with a corresponding time stamp from which elapsed time was calculated. In accordance with the goals of this project, we were primarily interested in the long-term trends in the signals, here defined as anything occurring on or greater than a half-day time-scale. cursory observations were first made by plotting the data and are discussed in Section 2.2 followed by more rigorous quantitative analysis presented in Section 2.3.

For this rigorous analysis, both well established and novel signal processing and time-series analysis techniques are utilized. Cross-correlation and Fourier based power estimation techniques are employed to identify the presence of dominant frequencies in the collected signals. Wavelet analysis, a relatively new approach previously used to investigate heart beat [52], blood flow [53, 54] and glucose dynamics in the blood [55], is employed for simultaneous time/frequency analysis. In addition, similarities between individual electrodes and between arrays are examined to determine larger tissue and animal dependent trends.

2.2 Data

2.2.1 Recorded Signals

Of the eleven sensors that were implanted across four animals, 60 functioning oxygen electrode signals were recorded. The signals are composed of measurements made every two minutes for a period of over 13 weeks. Due to the volume of data, and the fact that our interest extended only to the long-term information present in these signals, the data was averaged to more manageable sets. This averaging is indicated where necessary. Four example signals are shown in **Figure 5**, where it can be readily observed that signal behavior varies from array to array. The four following characteristics are displayed in **Figure 5a-d**, respectively: (a) a large initial drop followed by a low equilibrium point, (b) slow signal decay reaching equilibrium slightly below the starting value, (c) a large initial drop followed by slow decay reaching equilibrium, (d) decay to low equilibrium followed by recovery/deviations. However, it is interesting to

note, that despite some minor differences, the signals from the electrodes on one array demonstrate the same long-term trends. *Moreover, in all the collected signals there is a pronounced decay from the initial value at the start of the experiment, to the equilibrium level at the end of the experiment.*

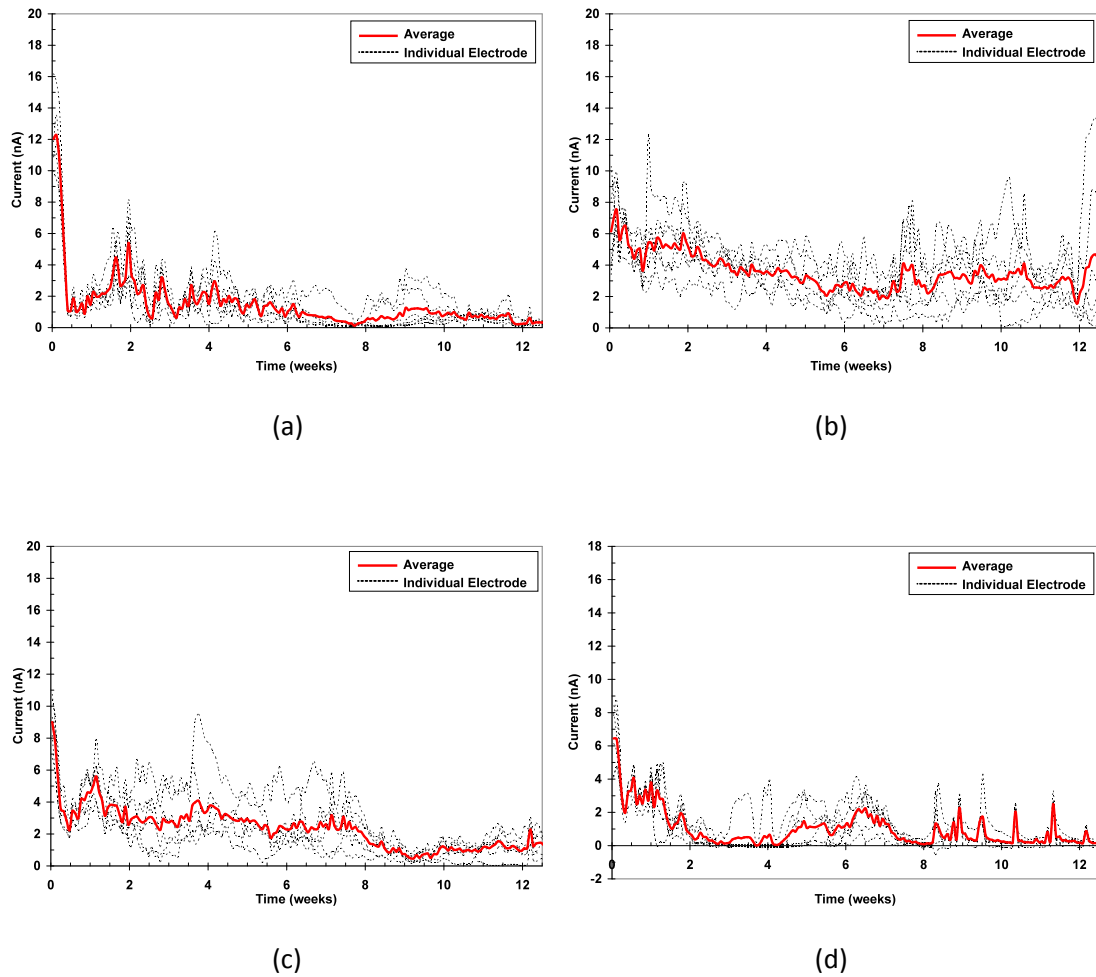


Figure 5. Examples of oxygen electrode signals from four sensor arrays illustrating various signal characteristics.

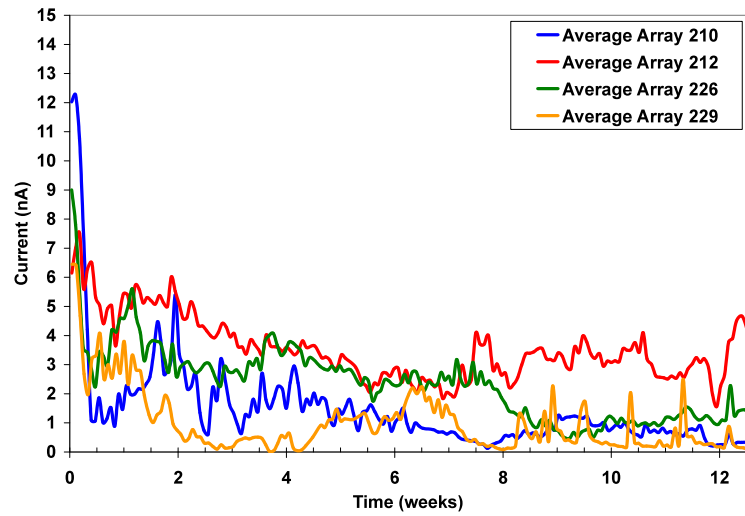
(a) Large initial drop followed by low equilibrium point, (b) slow signal decay reaching equilibrium slightly below starting value, (c) large initial drop followed by slow decay reaching equilibrium, (d) decay to low equilibrium followed by recovery/deviations.

2.2.2 Exponential decay

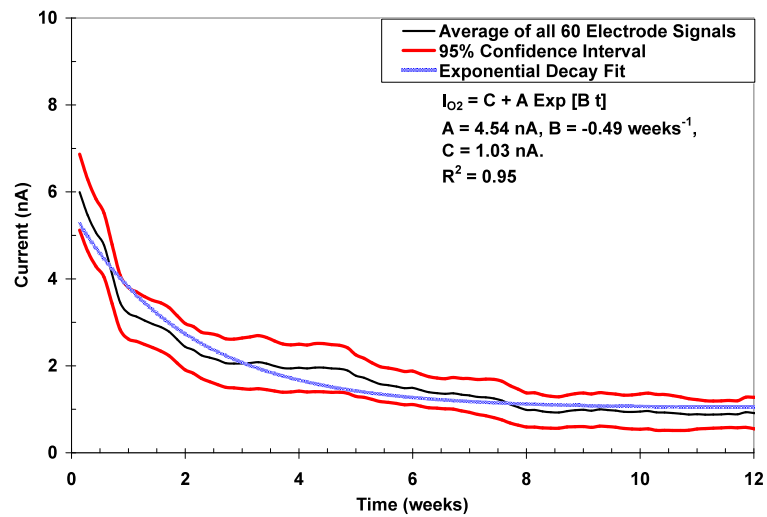
This decay can be readily seen when all the signals are averaged together, as is shown in **Figure 6**. When observing the individual array averages (**Figure 6a**) and the cumulative average of all 60 electrode signals (**Figure 6b**), the decay characteristics become pronounced. *In particular, there is a clear empirically observed exponential decay in the oxygen current, i_{O_2} , that can be readily fitted using a 1st order exponential equation:*

$$i_{O_2} = C + A \cdot e^{[B \cdot t]} \quad (\text{Eq. 8})$$

where A represents the magnitude change from initial position to final equilibrium, C , via time constant, B . These parameters are included in **Figure 6b** along with the R^2 value indicating the goodness of fit. In addition, the 95% confidence interval is provided on the figure, which being narrow indicates the average value is quite highly repeatable from a similar sample set. Fitting was performed using non-linear regression analysis in Mathematica [56].



(a)



(b)

Figure 6. Long-term oxygen decay trends.

(a) Examples of 4 averaged arrays illustrating the various trends that occur in the signals, (b) smoothed average of all 60 signals spanning all eleven arrays including the 95% confidence interval.

2.2.3 Selection of signals for further analysis

As stated earlier, all sensor arrays were calibrated before and after the implantation (see Appendix, Section 7.1.3 for more information). Only those sensors with a magnitude change in calibration less than 30% were chosen for the remainder of the analysis (see **Table 6**). The average of these 12 identified signals is presented in **Figure 7** with a similar exponential decay fit and 95% confidence interval. Here the exponential decay fit remains quite similar to the fit for all 60 electrode signals, but with a slightly higher equilibrium value. The confidence interval however is much broader, partly due to the lower number of signals analyzed, and in part due to the greater standard deviation.

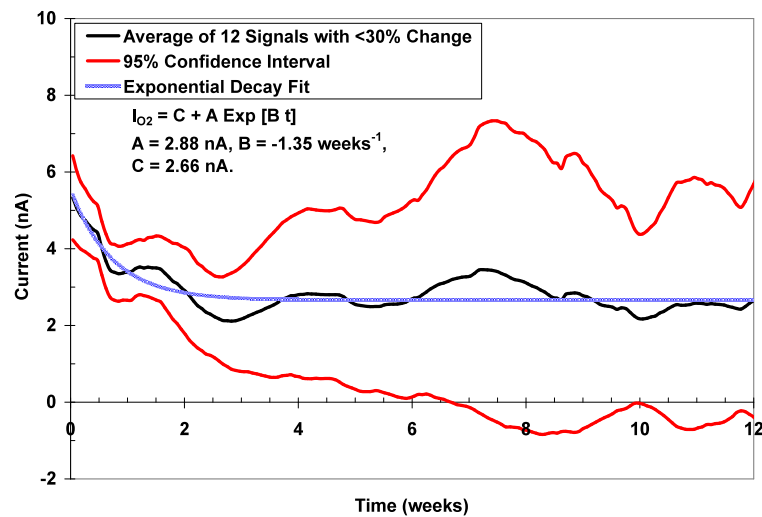


Figure 7. Smoothed average of selected 12 signals with a less than 30% magnitude change in calibration.

The 95% confidence interval bounds are included.

2.3 Analysis

2.3.1 Data processing and preparation

2.3.1.1 Signal filtering and decimation

For all numerical analysis of signal characteristics in this manuscript, the aforementioned 12 selected signals were reduced to one point per hour. This was done so as to minimize calculation time given the fact a complete sensor signal is composed of approximately 65,000 data points and to minimize high frequency noise in the computation of frequency characteristics, yet still retain enough data resolution for the intended signals analysis. Signal reduction was performed using a low-pass equiripple filter created in Matlab [57] with an appropriate 1-hour cutoff frequency followed by decimation in order to reduce the data set to the desired sampling rate. The Nyquist sampling theorem dictates that the highest frequency which can be identified within a discrete signal is half the sampling frequency [58]. Given the 1-hour final sampling rate of the signals analyzed, the Nyquist frequency limit is 0.5 hour^{-1} , well above our imposed long-term time-scale limit of 1 day^{-1} . Validation of the filtering/decimation method is presented later in this manuscript in section 2.3.3.2 entitled “Frequency characterization”.

2.3.1.2 Signal trend removal

The long-term trend (> 4 weeks) needed to be removed to unmask the low frequency characteristics of the signals. These trends, which have been largely identified as exponential in nature, were individually calculated for each signal by

performing averages of the data at 1 week intervals, then using a cubic spline to interpolate the points between in order to match the number of data points to the original signals. At this point these long-term decay trends could be either subtracted from the original signals (implying an additive effect of the trend) or the original signals could be divided by the trends and then de-measured (implying a multiplicative effect).

Multiplicative trends are readily explained using the mass transfer paradigm of the sensor/tissue interface in question. If the hypothesis that the signals recorded by the electrodes are dependent on the mass transfer characteristics of the tissues and subject to any dynamics thereof is accurate, an attenuation of the signal would be expected. The signal decay would also decrease the signal deviation from the trend as the decay process progresses. An additive trend, on the other hand, would imply electronic noise interference of the sensor system which would more likely have no impact on the range of deviation around the trend, regardless of the decay process. Therefore, both the multiplicative and additive trend removal was performed and compared for each signal.

Figure 8 illustrates two sample signals (a and b) that have had their trends removed using both methods. A minimum and maximum value for a moving 1-day window of the 1 sample per hour filtered and decimated signals is plotted versus time. The normalized average of all 12 selected signals is presented in **Figure 8c**. Note that the spread for the signals with the removed additive trend is greater at the start of the implant experiment and decreases over time, whereas the signals with the removed multiplicative trend show no visible change in spread. *This indicates that the trend is*

multiplicative, supporting the hypothesis that the signals are attenuated by physical mass transfer alterations over the course of implantation. Multiplicative trend removal was carried out for the remainder of this work.

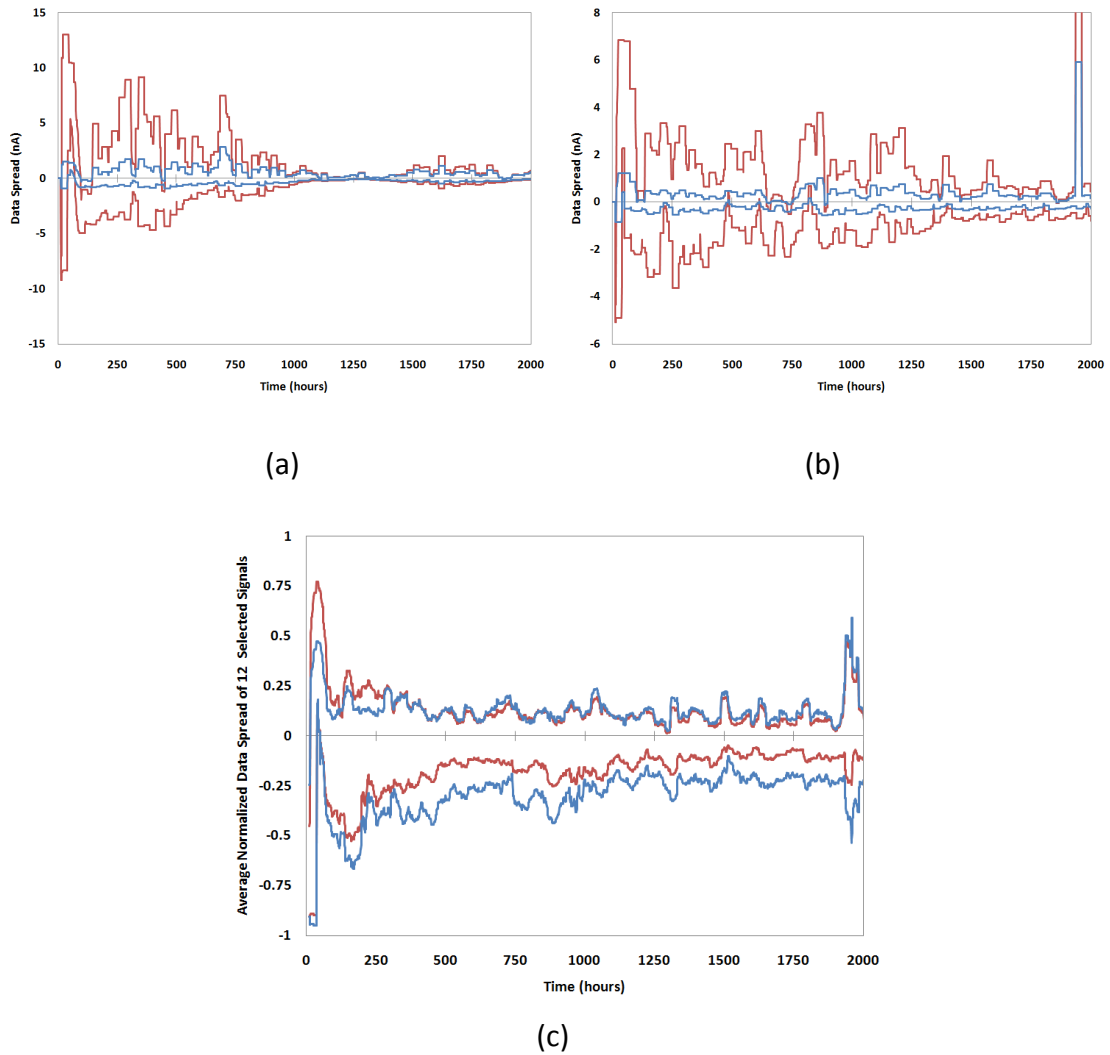


Figure 8. Analysis of data spread in the 12 selected signals. (a and b) Data spread of two sample signals which have been detrended using both the additive (red) and multiplicative (blue) methods, (c) normalized, averaged data spread of all 12 selected signals after additive (red) and multiplicative (blue) detrending.

2.3.2 Frequency analysis methodology

2.3.2.1 Autocorrelation

In order to further identify the information contained within the signals over the course of the 3+ month implantation, a series of signal analysis techniques was applied. The first was autocorrelation of the signals (convolution of a signal with itself) in order to identify any type of repetitive pattern that might exist over the course of the implantation. The autocorrelations of detrended signals were performed using the following method [57]. Signals x and y ($x = y$ for autocorrelation) are of length N shifted with respect to each other by time-lag τ , $R_{xy}(\tau)$ is the correlation coefficient defined by:

$$R_{xy}(\tau) = \begin{cases} \sum_{n=0}^{N-\tau-1} x_{n+\tau} \cdot y_n^* & \tau \geq 0 \\ R_{yx}^*(-\tau) & \tau < 0 \end{cases} \quad (\text{Eq. 9})$$

The correlation is then scaled so that a zero-lag ($\tau=0$) autocorrelation of the reference signal produces a value of 1.

The resulting autocorrelelograms were subsequently analyzed to identify periods of oscillation. The first five (if available) full, identifiable oscillatory periods were identified by hand, and piece-wise fitted to the following sinusoidal function:

$$R_{xx} = c \cdot (\text{Cos}[2\pi \cdot a \cdot (\tau + b)]), \quad (\text{Eq. 10})$$

where R_{xx} is the autocorrelation coefficient, τ is the time-shift (days) and a , b and c are constants representing the period (days), offset (days) and magnitude (scaled correlation coefficient), respectively. The oscillatory period was calculated using a nonlinear regression algorithm [56] for each of these segments and an R^2 value was obtained to judge the quality of the fit.

2.3.2.2 Frequency Characterization

2.3.2.2.1 Welch Method for Power Spectrum Analysis

Using the Fourier-based Welch approximation algorithm, power spectra of the 12 selected signals were computed identifying the frequencies with maximum power. The Welch method was chosen for its noise reducing properties and ease of calculation, the details of which are presented elsewhere [59]. The Welch algorithm effectively transforms discrete time series data into the frequency space using a sinusoidal basis. Frequency power is plotted either linearly or logarithmically as a function of frequency. This method presents no information regarding where in these signals these frequencies temporally occur; for that more advanced techniques are required.

2.3.2.2.2 Wavelet Analysis

Wavelet analysis is a useful technique for not only corroborating the information given by standard Fourier-based approaches, but for additionally identifying the times at which powerful frequencies occur. A detailed explanation of the fundamentals and

intricate details of wavelet analysis can be found elsewhere [52, 60, 61]; only a superficial explanation is given here.

Whereas the basis for Fourier-based approaches is set for the user as sinusoidal, much of the power of wavelet analysis comes from the fact that the basis is user selected. Several features such as whether the wavelet is orthogonal, complex or “wide”, or its shape play a key role in its usefulness in analyzing a specific signal. Non-orthogonal wavelets are particularly adept for signals with many overlaying frequencies as they produce smooth, undisturbed variations in amplitude, whereas orthogonal wavelets will produce discontinuities as scale is changed. Additionally, for non-orthogonal wavelets spectra are conserved if aperiodic shifts in time occur, which is not the case for orthogonal wavelets. Complex wavelets can provide useful amplitude and phase information regarding the oscillations in a signal. The “width” of the wavelet refers to its resolution as judged by the ratio of width in the time domain to its width in the frequency domain. This gives rise to the notion that no two wavelets will have the same temporal and frequency resolution. Finally, a qualitative judgment of the shape of the wavelet function is needed to ensure it matches the shape of features in the signal of interest. The user can decide the basis that best suits the data set and necessary analysis, as long as the function obeys certain rules and conditions [52, 57, 60].

Wavelet analysis of a discrete time series such as an oxygen signal $s(t)$ is performed by the following continuous transform to compute the wavelet coefficient, $W_b(a)$, at scale a and position b :

$$W_b(a) = \sum_{b=0}^{N-1} s(t) \psi * \left[\frac{(t-b)\delta t}{a} \right] \quad , \quad (\text{Eq. 11})$$

where ψ is the wavelet of interest. The squared magnitude of the amplitude gives the associated power of the wavelet spectrum. The information provided by wavelet analysis is in the form of a 3 dimensional graph with time on the x-axis, scale (related to, but not the same as Fourier scale) on the y-axis and power is represented on the z-axis. This data can then be analyzed to show not only which high power frequencies are present, but also when in the signal they occur. The imaginary (phase) components of the complex spectra can also be analyzed if necessary; however this has been omitted in this work.

Unfortunately, with the choice of a basis comes the detriment of wavelet analysis. For general trends and identifying ranges for both frequency and time resolution, the choice of wavelet is not so critical. If on the other hand it is necessary to identify specific and accurate frequencies and times, wavelet selection becomes key. Moreover, in general no wavelet will provide both good frequency and good time resolution simultaneously. Here we chose both the Morlet and Mexican Hat (a 2nd derivative of the Gaussian function) wavelets for further analysis where both wavelets are non-orthogonal, however Morlet is complex whereas the Mexican Hat is not [57, 60] and the two functions have different “widths”. The Mexican Hat wavelet is defined as:

$$\psi_0(\eta) = \left(\frac{2}{\sqrt{3}} \pi^{-1/4} \right) (1 - \eta^2) e^{-\eta^2/2}, \quad (\text{Eq. 12})$$

where η is a non-dimensional time parameter. The Morlet Wavelet is defined as:

$$\psi_0(\eta) = \frac{1}{\sqrt{\pi f_b}} e^{2if_c\eta} e^{-\eta^2/f_b}, \quad (\text{Eq. 13a})$$

where f_b and f_c are the bandwidth parameter and wavelet center frequency, respectively. In the case of the built-in implementation of the Morlet wavelet in Matlab, simplification and isolation of the real component of the equation is performed to obtain one that allows for faster computation [57], namely:

$$\psi_0(\eta) = C \cdot e^{-\eta^2/2}, \quad (\text{Eq. 13b})$$

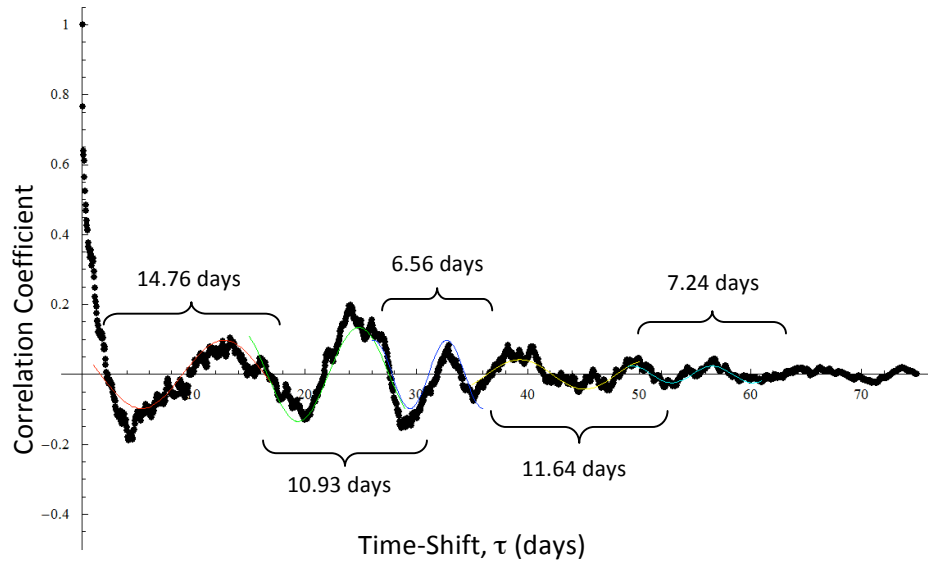
where C is a normalization constant. The resulting wavelet scale, a , is related to Fourier time-scale, λ (1/frequency) and can be computed [60]. In the case of the Morlet wavelet $\lambda = 1.23a$, which is nearly identical to the Fourier time-scale. For the Mexican Hat wavelet $\lambda = 4a$, indicating that the wavelet scale is 4 times smaller than the Fourier time-scale. The derivations and a more detailed description of these calculations can be found elsewhere [57, 60].

2.3.3 Analysis results

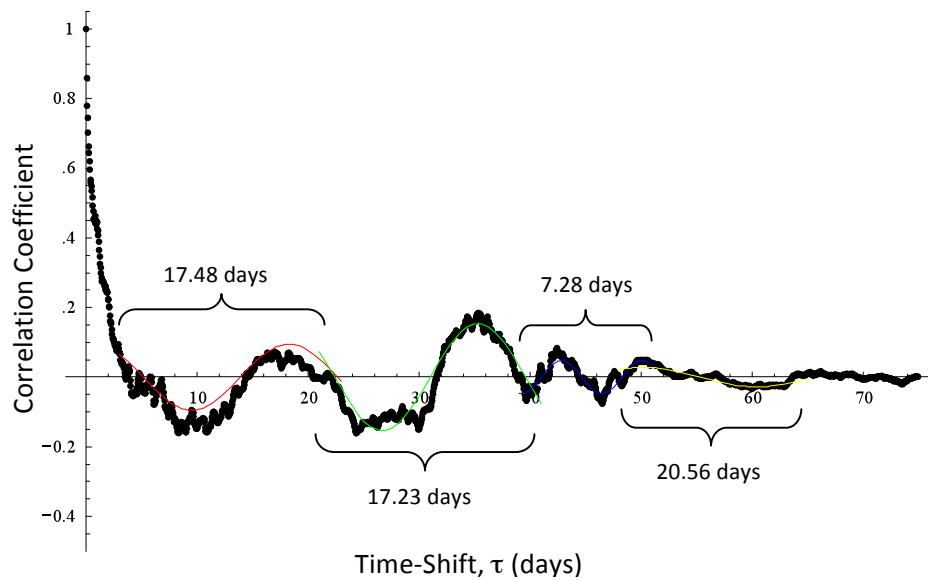
2.3.3.1 Autocorrelation

Two examples of the autocorrelelograms are presented in **Figure 9**. A clear sinusoidal pattern can be observed in both, indicating reasonably strong periodicity in the signals. This observation was found for all but one of the 12 signals chosen for signals analysis. In order to identify the period of oscillation, a sinusoidal function was fitted to the autocorrelation results using non-linear regression. These fits are also presented in **Figure 9** with labels indicating the oscillatory period in days.

The period of oscillation for the twelve selected signals was calculated and ranges from 5-30 days as shown in Table 3. For those signals that demonstrated this periodicity, the amount of said periodicity is highly variable. Some signals demonstrate periodicity for a majority of the temporal offset, while others have 2 or 3 oscillations and then show no further trend. *The average period of oscillation for those regions that could be clearly identified is 12.29 days with a standard deviation of 5.4 days.* The autocorrelation results provide a cursory indication that there is strong periodic nature to the signals; a more detailed analysis can be obtained using various frequency analysis techniques.



(a)



(b)

Figure 9. Examples of two autocorrelelograms for the detrended signals. Sinusoidal segments that were individually fitted to the data averaged to 1 point per hour using non-linear regression and the period (in days) of those fits are also displayed.

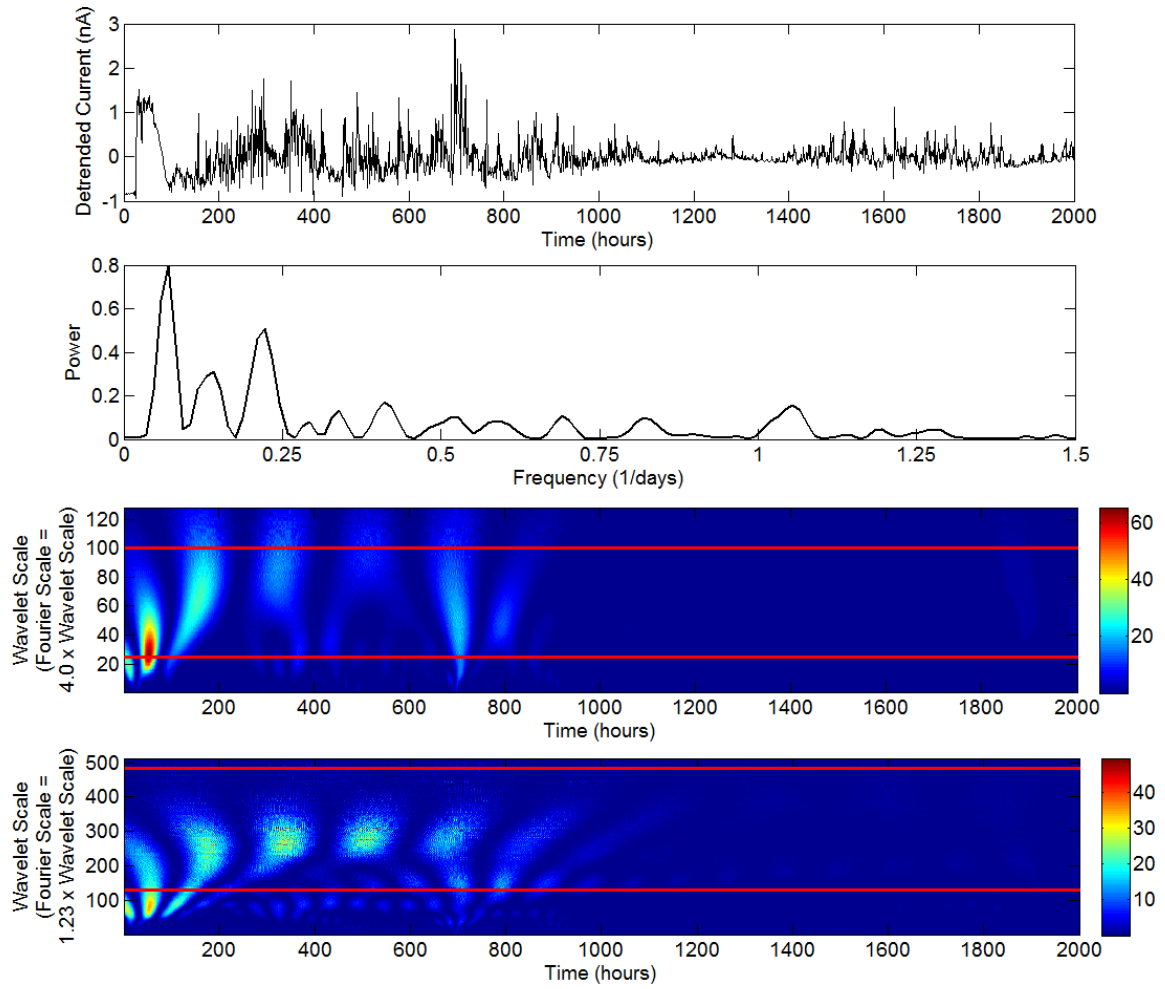
Table 1. Oscillatory periods in oxygen signal autocorreleleograms.
 Accompanying R^2 values and standard deviation values (where applicable) are included.
 $\dagger, \ddagger$ represent signals from the same array (226 and 230, respectively).

Signal	1 st	2 nd	3 rd	4 th	5 th	Average
No.	Oscillation [days] (R^2)	Oscillation [days] (R^2)	Oscillation [days] (R^2)	Oscillation [days] (R^2)	Oscillation [days] (R^2)	$\pm$ S.D. [days]
1	17.48 (0.72)	9.50 (0.77)	-	-	-	13.49 $\pm$ 5.64
2	-	-	-	-	-	-
3	13.37 (0.38)	32.15 (0.39)	17.85 (0.82)	-	-	21.12 $\pm$ 9.81
4	7.76 (0.85)	7.11 (0.66)	8.82 (0.73)	7.51 (0.75)	-	7.8 $\pm$ 0.73
5	6.09 (0.59)	17.14 (0.63)	20.46 (0.68)	12.84 (0.83)	-	14.13 $\pm$ 6.20
6 $\dagger$	17.48 (0.62)	17.23 (0.91)	7.28 (0.78)	20.56 (0.84)	-	15.64 $\pm$ 5.77
7 $\dagger$	10.73 (0.85)	6.78 (0.25)	10.40 (0.92)	10.24 (0.81)	-	9.54 $\pm$ 1.85
8 $\dagger$	14.76 (0.78)	10.93 (0.84)	6.56 (0.61)	11.64 (0.68)	7.24 (0.72)	10.23 $\pm$ 3.37
9	12.61 (0.52)	20.13 (0.66)	-	-	-	16.37 $\pm$ 5.32
10 $\ddagger$	10.03 (0.88)	10.11 (0.93)	8.06 (0.86)	-	-	9.4 $\pm$ 1.16
11 $\ddagger$	9.07 (0.91)	12.32 (0.12)	8.69 (0.73)	-	-	10.03 $\pm$ 2.00
12 $\ddagger$	9.49 (0.76)	13.85 (0.64)	10.46 (0.61)	-	-	11.27 $\pm$ 2.29
Average of all oscillations across the 12 selected signals						12.29 $\pm$ 5.40

2.3.3.2 Frequency characterization

Examples of two signals and their corresponding frequency information are presented in **Figure 10**. For the signals in **Figure 10a** and **e**, the power spectra, are presented in **Figure 10b** and **f**. For both signals presented, as for all 12 selected signals, the maximum power occurs for frequencies well below 0.5 days^{-1} . Several signals also show a pronounced peak at 1 day^{-1} , an observation consistent with biological signals due to the diurnal cycle important in many living organisms.

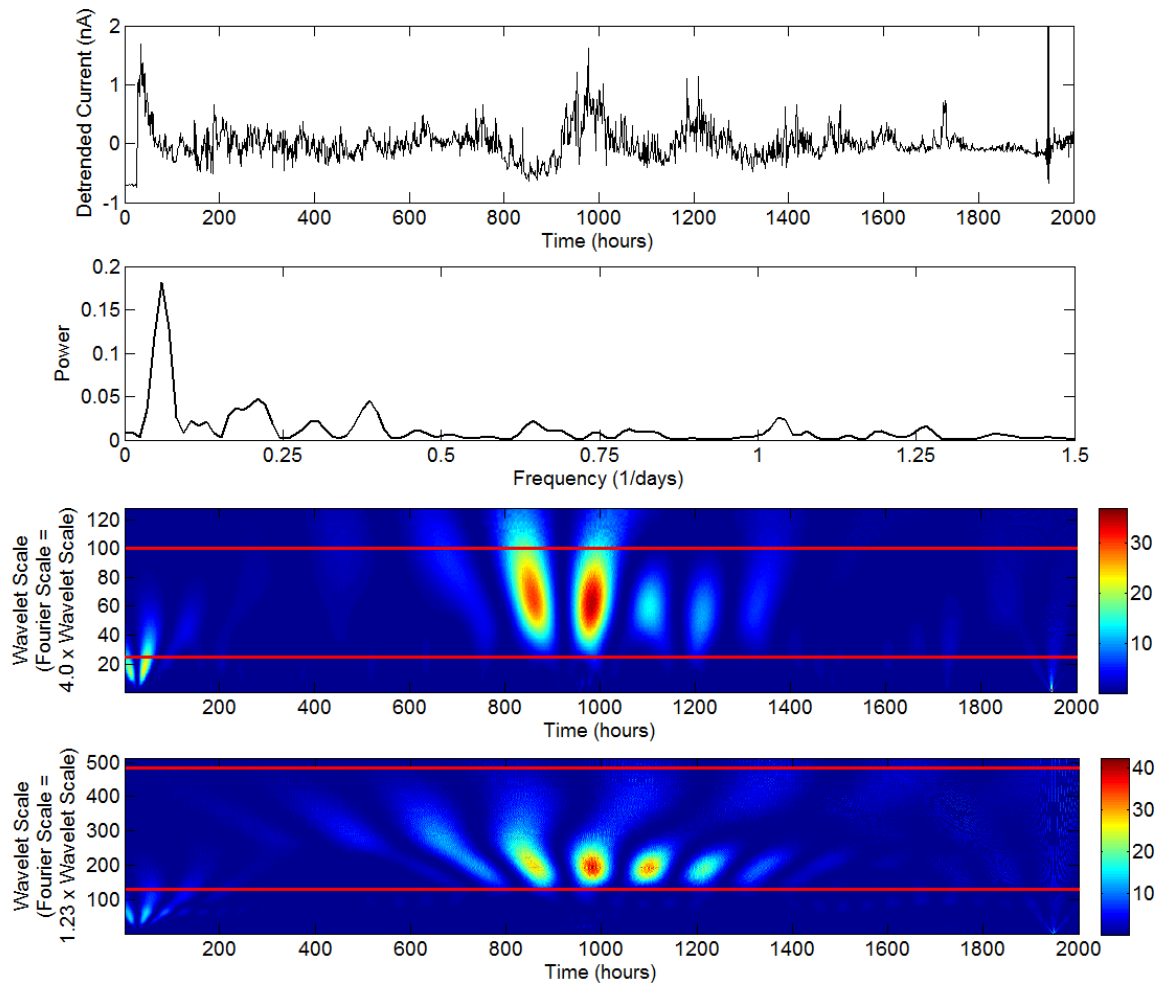
For the two signals previously subjected to Fourier-based analysis, two wavelet power spectra are presented as well. The first is computed using the Morlet wavelet (**Figure 10c** and **g**), the second using the Mexican Hat wavelet (**Figure 10d** and **h**). Comparing the two spectra for each signal, one can readily see that the Mexican Hat-based spectra provide much better time localization without the “echoes” due to separation of the real and imaginary components that are seen in the Morlet-based spectra (for example, see **Figure 10d** at times of 500 and 700 hours). Even though both functions are non-orthogonal and have similar “width”, the spectra clearly have different time and frequency resolutions. The Morlet-based spectra provide much cleaner discretization of frequency information; whereas the Mexican Hat-based spectra tend to “smear” the frequencies (see **Figure 10c** at a scale of 100-200 and time of 950 hours). It should also be noticed that the scale values from the Mexican Hat-based spectra do not coincide with the scale values from the Morlet-based spectra. Therefore, equivalent Fourier scales of 100-400 hours are indicated with the red lines in **Figure 10c**, **d**, **g** and **h**.



(a, b, c and d – top to bottom)

Figure 10. Fourier and wavelet frequency analysis of the 12 selected signals.

(a,e) Two examples of the detrended oxygen current vs. time signals averaged to 1 point per hour, (b,f) the associated Fourier power spectra computed using the Welch algorithm, (c,g) and the associated wavelet analysis using the Mexican Hat wavelet (DOG, $n=2$) and (d,h) the Morlet wavelet. Red colored lines in (c,g) and (d,h) indicate equivalent Fourier time-scales of 100 – 400 hours.



(e, f, g and h – top to bottom)

Figure 10 (continued). Fourier and wavelet frequency analysis of the 12 selected signals. (a,e) Two examples of the detrended oxygen current vs. time signals averaged to 1 point per hour, (b,f) the associated Fourier power spectra computed using the Welch algorithm, (c,g) and the associated wavelet analysis using the Mexican Hat wavelet (DOG, $n=2$) and (d,h) the Morlet wavelet. Red colored lines in (c,g) and (d,h) indicate equivalent Fourier time-scales of 100 – 400 hours.

2.3.3.3 Validation of filtering/decimation regime

In order to validate this filtering procedure, the power spectra of the unfiltered, averaging filtered and low pas filtered signals were plotted and examined in **Figure 11a** for 3 sample signals. The intended cutoff frequency is 1 hour (24 days^{-1}). It can be readily seen that the filtering process using the averaging filter disturbs the higher frequency information starting at 5 days^{-1} for all three signals (blue unfiltered spectrum compared with red averaging filtered spectrum). The low-pass filtering (see green spectra in **Figure 11a**) on the other hand do not disturb the signal before the cutoff frequency. The un-decimated low-pass filtered signal was then subtracted from the original signal leaving the remaining residuals, which were binned and plotted using a histogram. **Figure 11b** shows these binned residuals for the three sample signals. Note that the distribution of residuals is symmetric with no skew in either direction, although a difference is seen in distribution width. Analysis revealed that high frequency information has little bearing on low frequency characteristics of these signals and filtering cleanly reduces the data sets with no distortion of the low frequency characteristics of interest.

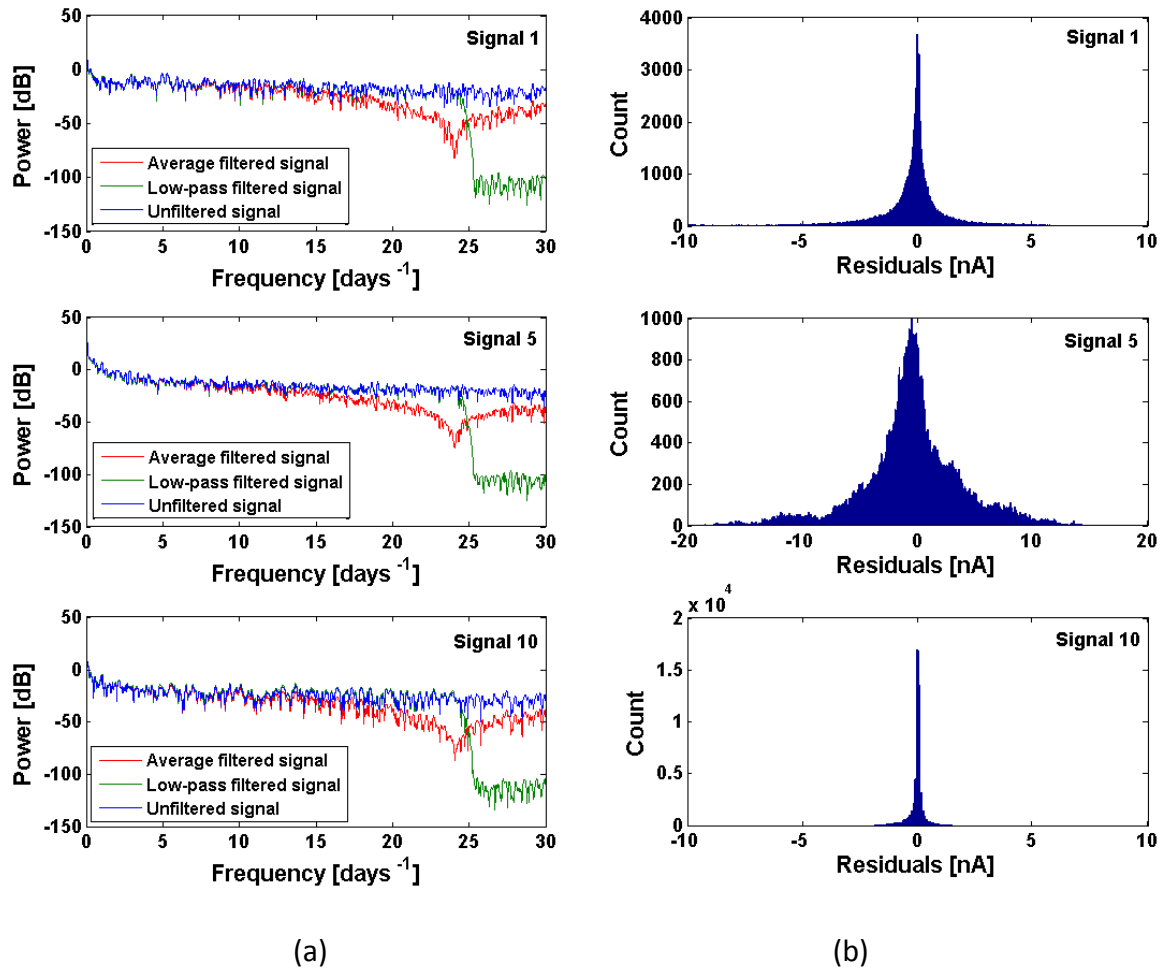


Figure 11. Validation of filtering method using power spectrum and residuals analysis.
 (a) Power spectra for three sample unfiltered (blue), 1-hour averaging filtered (red), 1-hour low-pass filtered (green) signals and (b) histograms of 1-hour low-pass filtered residuals.

2.3.3.4 Comparison of 12 selected signals

Wavelet spectra for all 12 signals were computed just like for the two signals in **Figure 10**. Then the mean power and standard deviation of each spectrum was calculated, and then any values greater than the mean plus one standard deviation were set to 1 and all other values were set to 0. This in effect identified the most contributing areas of the spectra for each signal. These data sets were then added together, indicating the levels of similarity amongst all 12 signals for both Mexican Hat-based spectra and the Morlet-based spectra. The average Fourier-based power spectrum, along with these Wavelet similarity plots are presented in **Figure 12**. From **Figure 12a**, we can readily identify that the *frequencies with the most power occur between 0.05 and 0.15 days⁻¹, which corresponds to a 6-20 day time-scale*. Additionally, a significant peak is present at 1 days⁻¹, an expected feature of long term mammalian biological signals. The information gathered from the wavelet analysis (see **Figure 12b** and c) corroborate this finding. *The most powerful frequencies occur between 100-400 hours on the Fourier time-scale (see horizontal red lines), and these most powerful frequencies tend to be clustered within the first 1000 hours (approx. 40 days, see vertical red lines) of the experiment*. That is not to say that there is no information in the later stages of the experiment, just that the signals show more distinct behavior from 1000 hours onward.

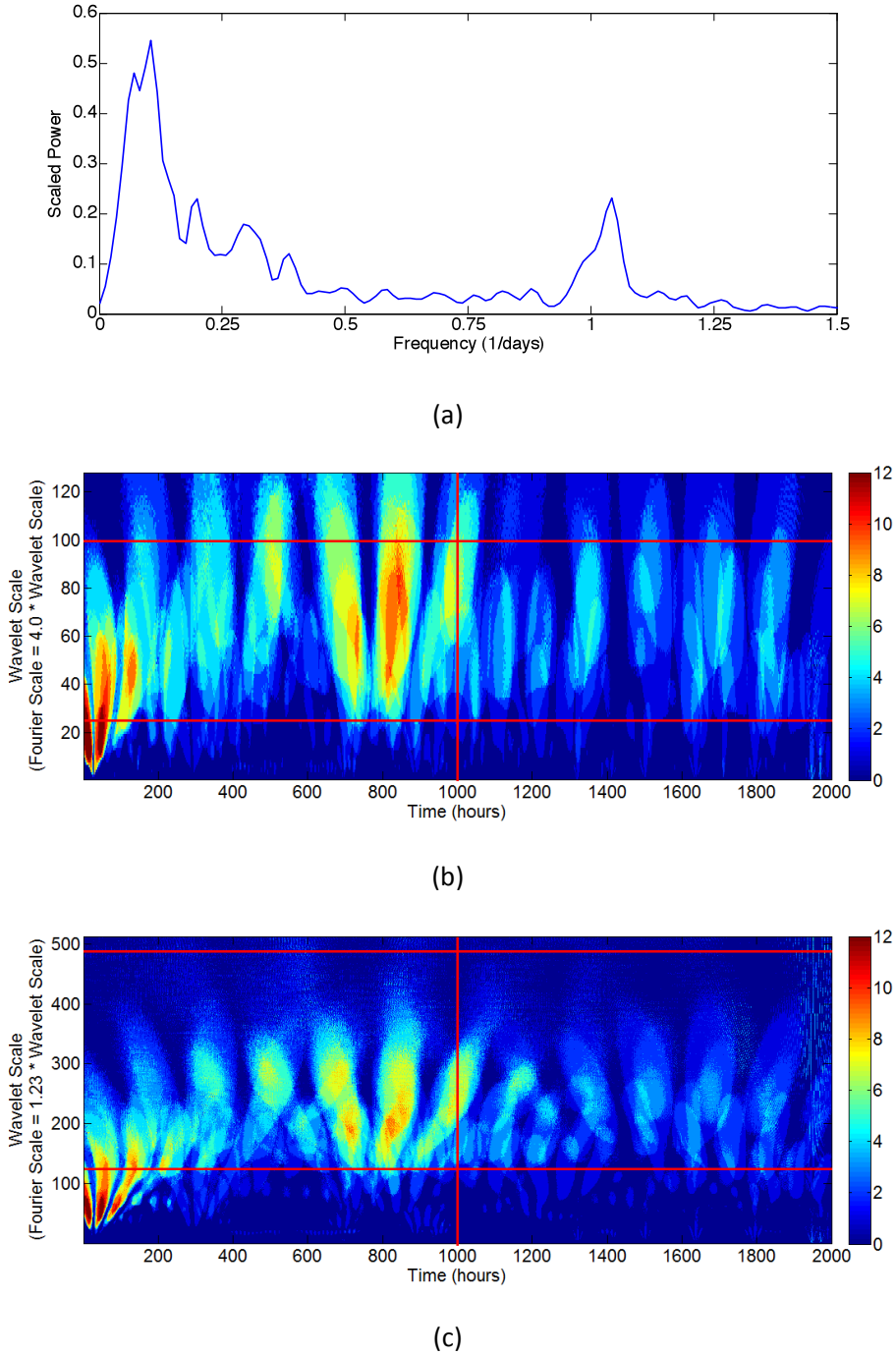


Figure 12. Quantitative frequency comparisons of all 12 selected signals. (a) Averaged and scaled Welch power spectra of 12 selected signals sampled once per hour indicating maximum signal power in the 0.06-0.15 days⁻¹ frequency range and a pronounced peak at 1 days⁻¹, (b) similarity in regions of Mexican Hat wavelet power spectra where the values are greater than the mean plus one standard deviation, (c) same as (b) but using the Morlet wavelet. The horizontal red lines in (b) and (c) indicate equivalent Fourier time-scales of 100 – 400 hours and the vertical lines indicate the 600 hour time point.

2.3.3.5 Comparison of signals originating from the same arrays

To examine the effect of electrode proximity and array variability, signals that originated from the same arrays were compared using both cross correlation and wavelet analysis techniques described earlier. Of the 12 signals used for signals analysis in this study, two sets of three signals from two different arrays were selected. The two arrays (labeled 226 and 230) were located on different animals, in different anatomical locations. It should be noted that electrodes on one array can be no more than 1 cm apart (see **Figure 3**).

Correlation and wavelet analysis consistent with the techniques described previously were applied to these signals separately in order to investigate signal similarity. Correlation results are shown in **Figure 13a** and **b** for Array 226 and 230, respectively. Three sets of curves are shown for each array, one set calculated with respect to each individual electrode. Wavelet analysis is presented in **Figure 14a-d**. *Both arrays show similarity amongst their respective electrode signals, as can be observed in both the wavelet and cross-correlation plots.* However, array 230 shows much stronger similarity both in terms of the cross-correlations, which are almost identical and wavelet power spectra comparisons, which contain considerably more overlap.

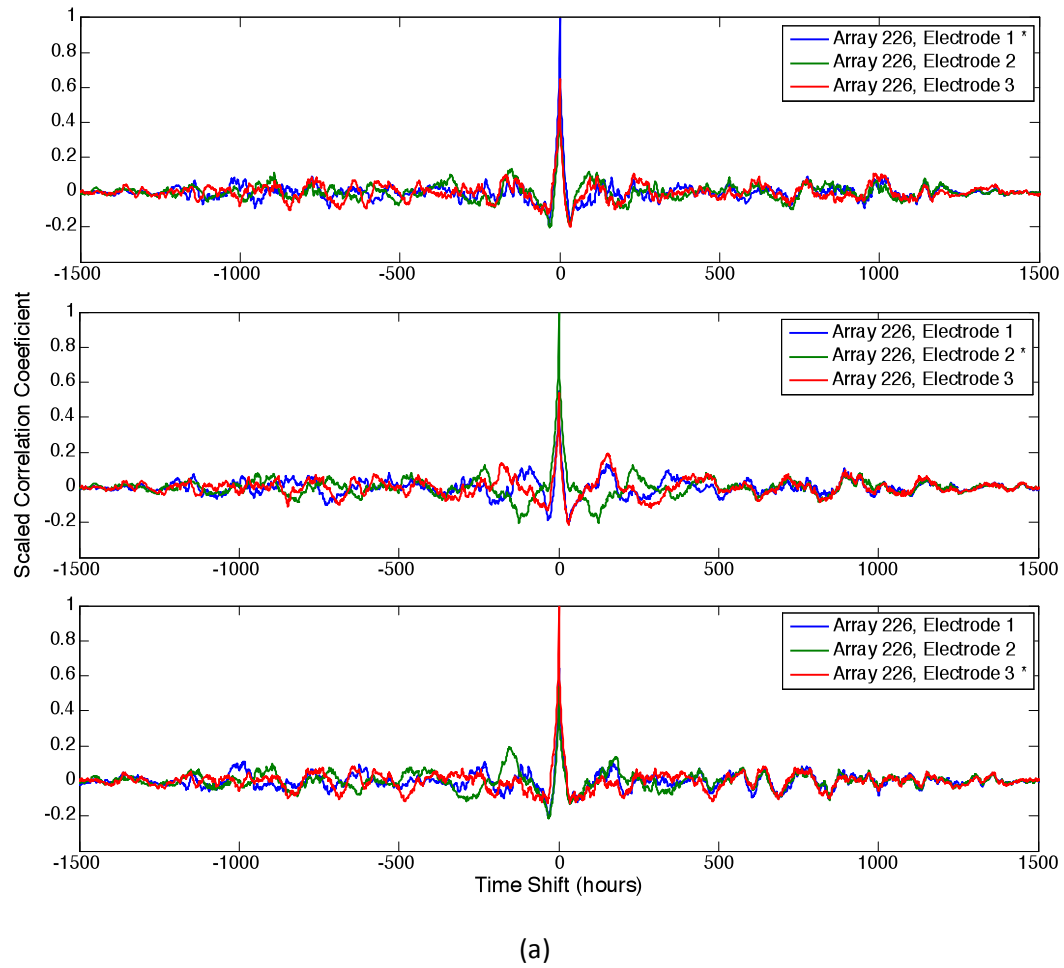


Figure 13. Cross-correlation between 3 signals originating from the same array.
 (a) Array 226 and (b) Array 230. The star (*) denotes the signal against which all three signals are cross-correlated.

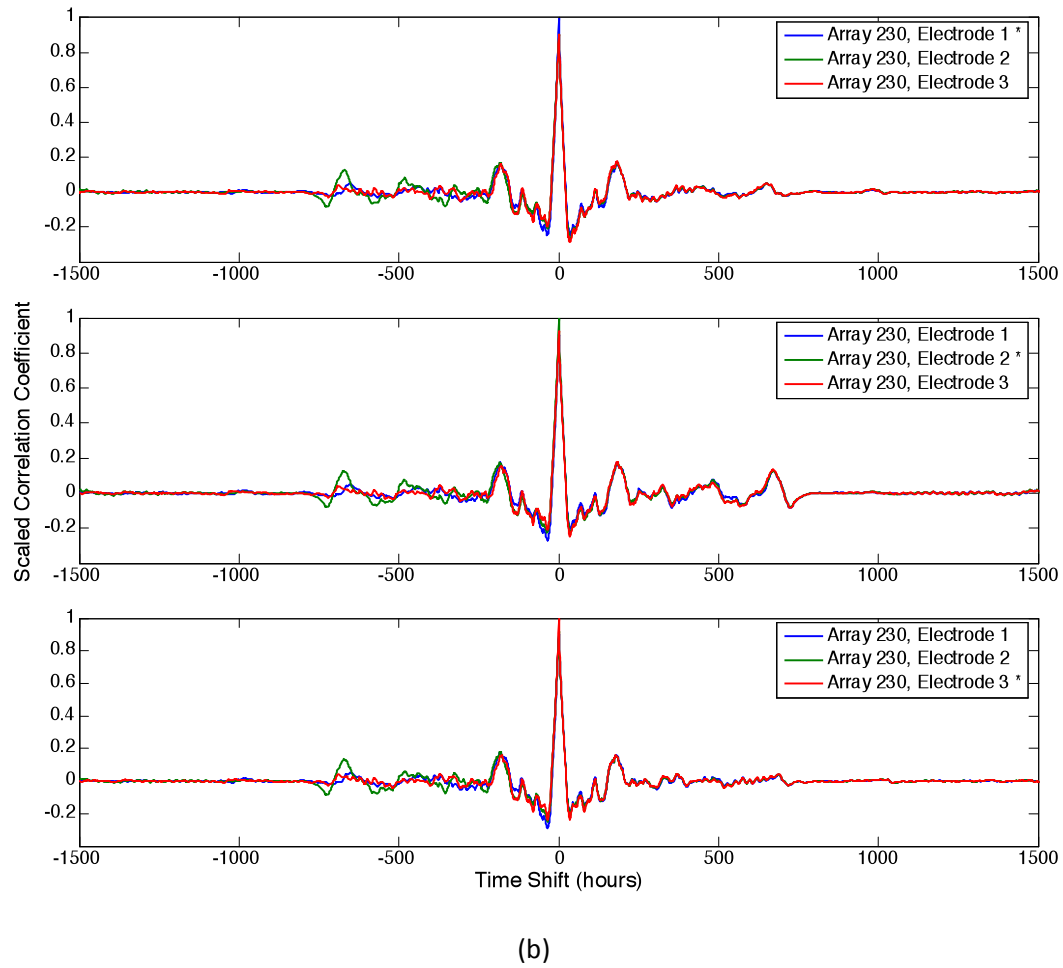
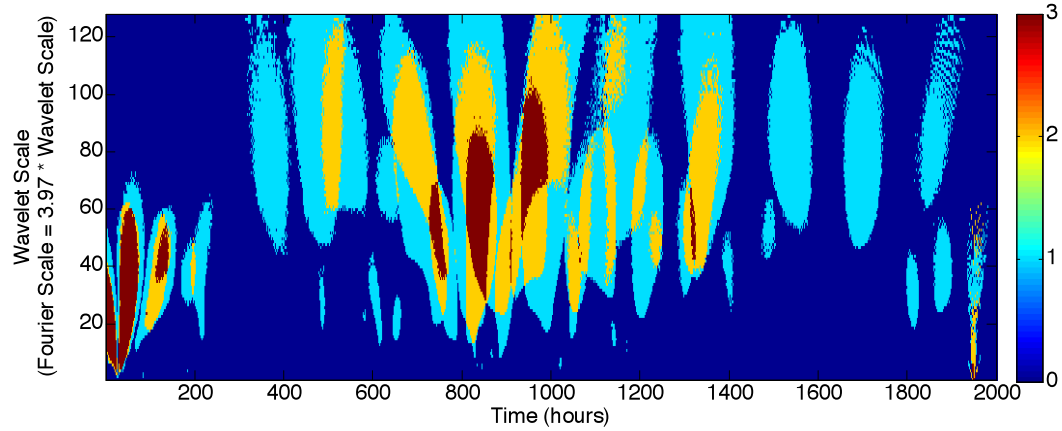
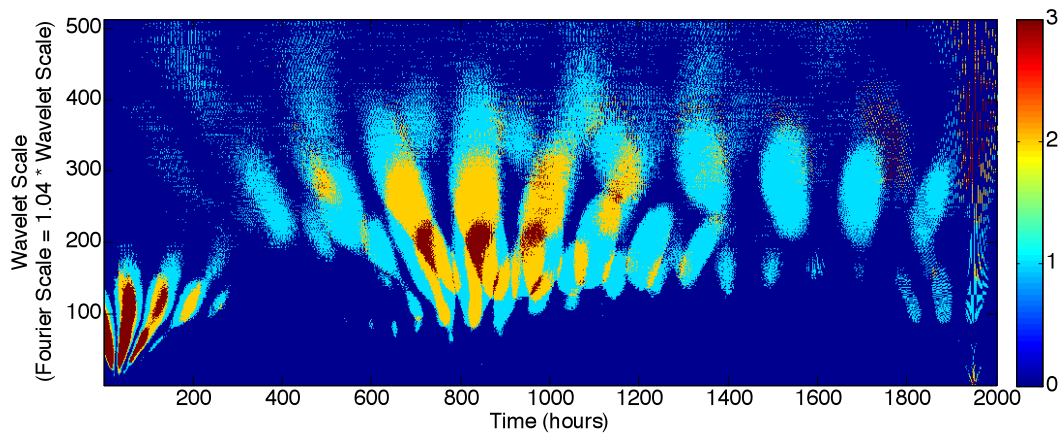


Figure 13 (continued). Cross-correlation between 3 signals originating from the same array. (a) Array 226 and (b) Array 230. The star (*) denotes the signal against which all three signals are cross-correlated.

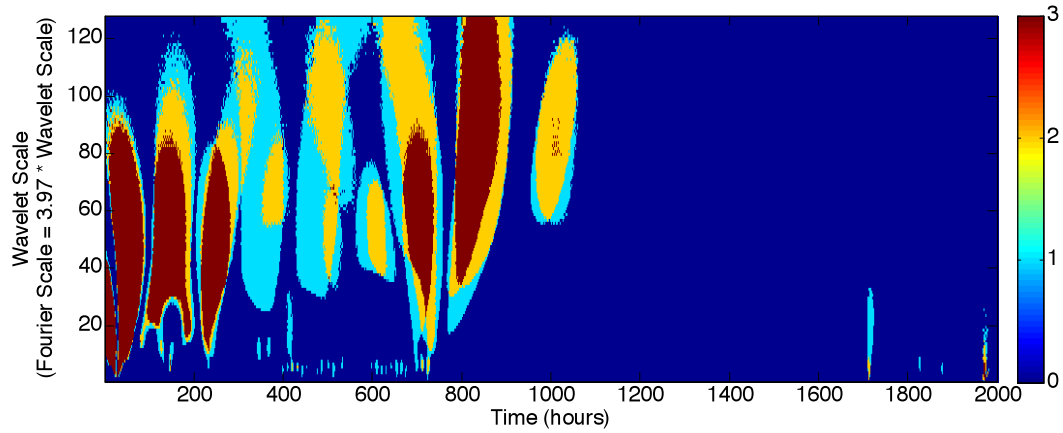


(a)

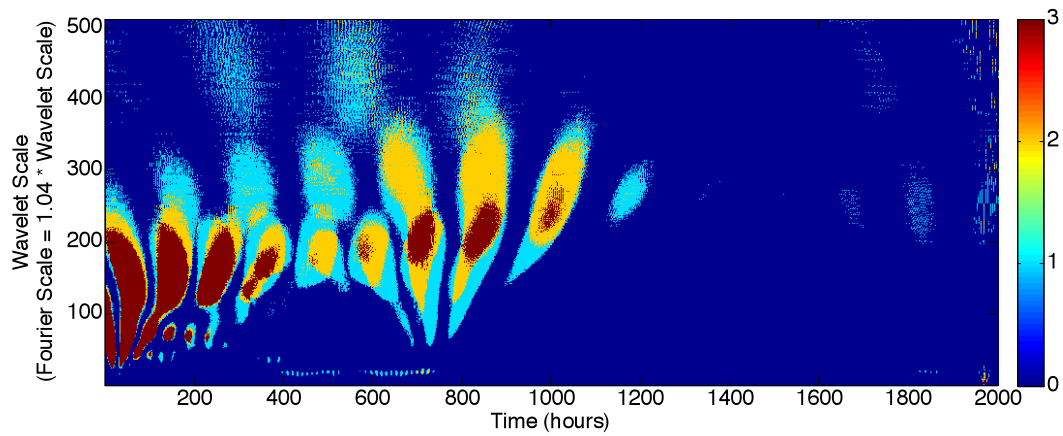


(b)

Figure 14. Wavelet comparison of 3 signals originating from the same array. Mexican Hat (3° D.O.G.) (a and c) and Morlet (b and d) wavelet analyses of 3 signals on same array; (a and b) Array 226 and (c and d) Array 230. The colors depend on how many of the 3 signals exhibit the same power characteristics (light blue = 1, yellow = 2 and red = 3).



(c)



(d)

Figure 14 (continued). Wavelet comparison of 3 signals originating from the same array. Mexican Hat (3° D.O.G.) (a and c) and Morlet (b and d) wavelet analyses of 3 signals on same array; (a and b) Array 226 and (c and d) Array 230. The colors depend on how many of the 3 signals exhibit the same power characteristics (light blue = 1, yellow = 2 and red = 3).

CHAPTER 3 –

Analysis of encapsulation dynamics via quantitative serial histology

3.1 Methodology

The vast majority of histological studies have been performed at a single terminal time point after the removal of an implant, with little focus on the change in tissue composition and morphology over the course of implantation. Due to attenuating effects of prolonged implantation on oxygen signal strength of implanted oxygen telemeter arrays that have been previously reported [4] and presented earlier in this work (see Section 2.2.2), a need for understanding the complex dynamics of encapsulation tissue formation and progression has arisen. It is hypothesized that the mass transfer characteristics of the tissues themselves would be responsible for the changes in reported metabolite levels at the implant surface due to hindered metabolite flow through the tissues. Therefore, it is necessary to examine and quantify the development and progression of capsule formation, and specifically answer the following questions: what are the critical cell types present during the progression of wound healing, encapsulation and immune response?, how do their distributions change?, what are morphological dynamics of the implant site?, and what trends can be identified in the tissues that could account for the decay that is seen in the oxygen signals over the course of long-term implantation?

Specifically, the following features were selected for analysis: overall cellular density, collagen density, vascular (endothelial) density and distribution, macrophage and granulocyte density and distribution, granular layer dimensions. Each of these variables could account for a large change in the mass transfer of oxygen (and other metabolites) to the tissues. A large increase of deposited collagen over time, for example, could inhibit permeability of oxygen and increase diffusion distance, thus lowering the amount of oxygen reaching the sensor surface and lessening the resultant generated signal. Similarly, the development of a strong immune/inflamed state with the presence of many metabolically active white blood cells could potentially decrease the amount of oxygen making its way to the implant surface as it is consumed along the way. The ability to quantify these compositional and morphological dynamics will allow for a deeper understanding on encapsulation and its effect on mass transfer of metabolites.

To accomplish this goal, we have devised a time-course experiment where six shams, in essence structurally and materialistically identical to functional array telemeters sans electronics, are implanted into the subcutaneous tissues on the back of an adult Yucatan mini-pig. At six predetermined time-points, chosen to coincide with notable points of interest on the oxygen signal decay curve (see **Figure 6**) [4], one of the shams is removed along with surrounding tissue samples. These samples are then subjected to rigorous histological analysis aimed at identifying and quantifying each of the features of interest. A combination of standard and immunological histology is

implemented in this study to not only determine general morphology and composition of the tissues, but also to identify specific cell types of interest.

Standard image processing algorithms are employed in Matlab [57] for color channel separation, object identification, analysis (shape, size, solidity, orientation, etc.), sorting and exclusion to quantify our histological findings. Image analysis is commonly employed to quantify and analyze biological findings [62-69]. Our methodology combines many of these techniques for the expressed purpose of not only quantifying the levels that certain histological features are expressed, but to also determine the distributions with respect to implant location. Ideally, changes in not only the amount of a constituent but its distribution throughout the surrounding tissue will provide a better understanding of the impact on mass transfer of metabolites.

3.2 Materials/methods

3.2.1 Shams

Six sham implants, designed to mimic the size, weight and texture of functioning oxygen telemeters and assembled using the same materials and techniques with which real telemeters are built were used in this study. Medical grade titanium is used for the housing which is comprised of two halves that are welded together for a hermetic seal. Next, a spin coating of PDMS is applied to the top surface in order to mimic the PDMS coated sensor array. The titanium backside of the sham is left exposed save for four polyester velour tabs which were adhered to the backside in order for tissue growth into the fabric to hold the sham in place to prevent sliding within the tissue pocket. The

shams were sterilized using an FDA approved chemical sterilant procedure prior to implantation [70].

3.2.2 Implant procedure

At the start of the procedure, six implant locations (see **Figure 15a**) were chosen on the dorsal skin of one anesthetized adult female Yucatan mini-pig, offset on either side of the spine. The animal was 56 weeks old and weighed 62.1 kg at the start of the experiment. The implant locations were kept a minimum of 15 cm away from each other. A 5 cm long, 1-2 cm deep incision was made in each location, splitting open the skin, and exposing the dermal layers, as shown by the schematic in **Figure 15b**. A pocket was created using blunt dissection between the sub dermal fat and underlying muscle while not disturbing the fascia. Each implant location was kept as similar to every other location as possible, however variability due to the manual nature of the implantation procedure was to be expected. The shams were placed into the pockets with the PDMS surface facing in towards the muscle layer. Once the sham was seated in the pocket, the skin was sutured together, cleaned, and bandaged. **Figure 15c** presents a cross-section photograph of a pocket at week 13 that has been totally excised after sacrificing the animal. *No severe abnormalities, defects or injury can be seen indicating healthy tissue and implant pocket.*

In addition to the implants, a modified dual-lumen Hickman catheter (Bard Access Systems) was introduced into the central vena cava for blood sampling and fluid infusion, with the catheter ports exteriorized at the midscapular region. The catheter

was maintained patent between uses with a dilute solution of heparin. Sterile technique was used in the procedures, and all animal activities were performed pursuant to NIH guidelines [71].

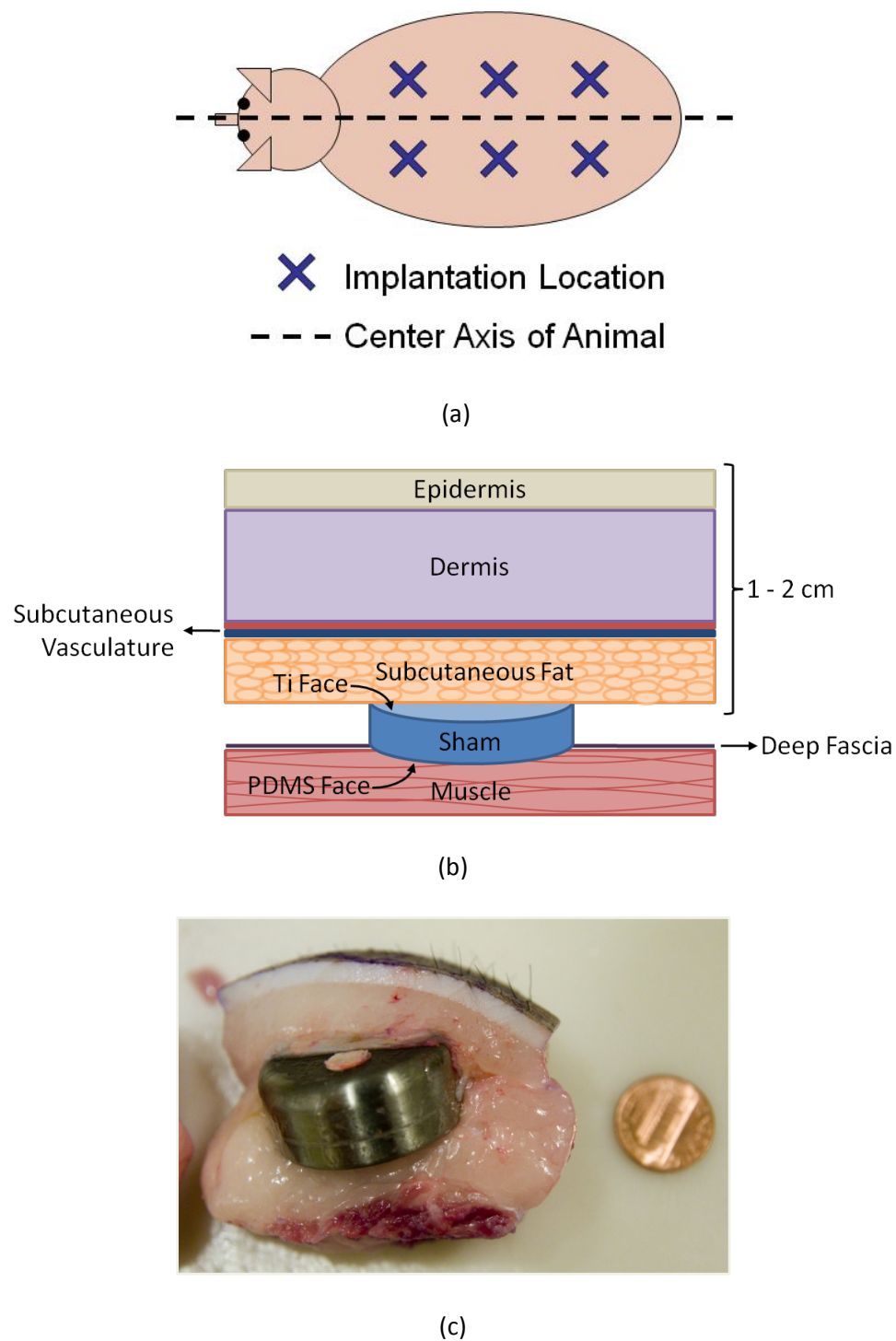


Figure 15. Details of the sham implantation.

The implantation of shams was performed at six sites in the dorsal skin off-center from the spine as shown in (a). Each implant was placed into a pocket created between the subcutaneous fat and underlying fascia/muscle via blunt dissection exposing the layers of the skin as illustrated in (b). A cross-section photograph of the implant pocket with sham at week 13 is presented in (c).

3.2.3 Explantation times

Explantation of the implants was timed with the oxygen signal decay characteristics demonstrated in earlier (see **Figure 6b**) where it has been shown that oxygen signals lose approximately half their initial values within 4 weeks of implantation and that the signals have plateaued to their minimum by 12 weeks. Therefore, the six shams were removed at times corresponding to the decay trend. Specifically removal of the shams was performed at 1, 2, 4, 6, 8 and 13 weeks. Locations were picked at random for sham removal. After 13 weeks of implantation the animal weighed 77.1 kg.

3.2.4 Tissue collections

At each explantation the animal was given a combination of general and local anesthetic at the wound site. An incision in the skin directly over the sham sensor was made, exposing the sham underneath. The sensor was removed and examined for any damage or anomalies, and the PDMS surface was dabbed with glass slides to collect any cellular material present. Once the sham was out of the way, tissue samples were excised from regions of the pocket adjacent to the PDMS and Ti surfaces and immediately placed in the according fixative. The incision was sutured and wound site cleaned with antibiotic fluid. Sterile techniques were used for all implant and explant procedures maintaining absolute cleanliness of the wound sites and materials in direct contact with the animal.

3.2.5 Histology

3.2.5.1 Tissue fixation and preparation

The subcutaneous tissue was fixed in either 10% neutral buffered formalin (Fisher Scientific) for standard histological staining, or in tris-based buffer with zinc ions (BD PharMingen IHC Zinc Fixative, BD Biosciences) for immunohistochemical processing. The tissues were fixed for 24 hours according to manufacturer recommendations for both fixatives. Following fixation, the tissues were slowly dehydrated in graded alcohol solutions and xylene, and then embedded in paraffin blocks. The use of isotonic fixatives and a slow dehydration protocol allowed us to minimize tissue shrinkage and maintain the proportions of salient features during sectioning and staining [72]. The tissue samples were aligned in the blocks to allow cross-sections to be sliced through the subcutaneous layers. For both standard and immunohistochemical samples, 5 μ m thick slices were professionally cut.

3.2.5.2 Staining, microscopy and imaging

Standard hematoxylin and eosin (H&E) staining was performed for analysis of general morphology and cell nuclear density. To investigate collagen content of the tissues, PROTOCOL Gomori Trichrome Stain (Fisher Scientific) was used as instructed by the manufacturer.

Immunohistochemical staining was utilized in order to identify endothelial cells, macrophages and granulocytes in the tissues. Antibodies targeting specific epitopes on

cell surfaces were employed (see **Table 2**). Endothelial cells were labeled using BD PharMingen purified mouse anti-rat CD31 unconjugated antibodies, which cross react with pig CD31 (BD Biosciences). CD31 (also known as PECAM-1) is a cell surface receptor that, in part, is present on the internal walls of blood vessels allowing for the binding of platelets in cases of vessel damage and provides adhesion points for white blood cell “rolling” and extravasation [73, 74]. Macrophages were labeled using purified mouse anti-pig CD4 unconjugated antibodies, (Abcam, Inc.). CD4 is a cell surface receptor present on several white blood cells including monocytes/macrophages [75]. Granulocytes were labeled using anti-pig 6D10 unconjugated antibodies (Abcam, Inc.). 6D10 is a surface marker found of porcine granulocytes in various stages of development [76]. Staining was completed using a HRP/DAB enzymatic detection kit (Abcam, Inc) with goat anti-mouse IgG biotinylated antibodies. Counterstaining of was achieved using Mayer’s hematoxylin solution (Sigma-Aldrich). No antigen retrieval steps were required.

Table 2. Salient details regarding antibodies utilized in immunohistochemistry. Included are the features of interest, targeted epitope, antibody type, vendor information and associated references

Feature of Interest	Epitope	Antibody	Vendor Info.	References
Vascular Endothelial Cells	CD-31 (PECAM-1)	Mouse anti-rat (cross reacts with pig)	BD Biosciences, Inc.	[73, 74]
Macrophages/ T-Cells	CD-4	Mouse anti-pig	Abcam, Inc.	[75]
Granulocytes	6D10	Mouse anti-pig	Abcam, Inc.	[76]

The resulting slides were qualitatively observed using an Olympus VANOX-S slide microscope at 40, 100, 200 and 400x magnifications. Imaging of entire slides was performed using an Olympus E-30 DSLR with microscope lens mount. The images were taken using the 4x objective on the microscope providing a scale factor of 0.34 $\mu\text{m}/\text{pixel}$. Constant exposure and sensor sensitivity settings were maintained during the imaging of a slice to maintain identical light levels as measured by the camera's internal light meter. Depending on the size of the tissue slice, between 10 and 50 full resolution images were captured in a raster pattern in order to cover the entire sensor interface region and were then stitched together via software (Adobe Photoshop CS2, Adobe, Inc.). Regions of interest that were used for further analysis on the CD4 and 6D10 antibody labeled slides were additionally imaged using the 10x objective with a final magnification factor of 0.14 $\mu\text{m}/\text{pixel}$. After digital stitching of the images, brightness and contrast levels were adjusted to maintain consistency between slides of a given

stain, however natural differences in staining intensity were present and some discrepancy still exists.

3.2.5.3 Quantification methods

Once a slide was digitally imaged, five regions of 250 μ m width and 500 μ m depth (away from the sensor surface) were selected for quantitative histology analysis in Matlab. Region selection was kept random, however regions with staining artifacts and tissue damage were avoided, and whenever possible selection was avoided towards the edges of samples to minimize effect of tissue distortion due to manhandling. Of the five selected regions, one region was chosen as a reference (based on quality of staining and representative feature content) to which the other four regions' color histograms were later equalized.

The region to be analyzed was first split into RGB color channels, and each channel was passed through a median filter to reduce noise and preserve edges. Each channel's histogram was equalized to the corresponding channels of the reference region, and the image was then reconstructed. The reconstructed image was horizontally sliced into 20 regions (250x25 μ m each), corresponding to 25 μ m depth increments away from the sensor surface.

Each slice was split into RGB and NTSC channels, and thresholds for the RGB and NTSC channels were chosen and applied to each slice. The applied thresholds were manually chosen for every region and verified by comparing an image of the original

region to an image of the region following threshold filtering. This manual method with user chosen thresholds ensured effective filtering of the areas of interest. Variability in the applied thresholds was necessary due to variations in stain intensity, slice thickness, digital imaging, etc. The area, solidity ratio, eccentricity ratio, and orientation of the remaining regions were calculated, and additional restrictions to these morphology values were applied. The number, area, eccentricity ratio, solidity ratio, and orientation of the remaining parts of each slice were then calculated and output for data analysis. This process is summarized in **Figure 16**.

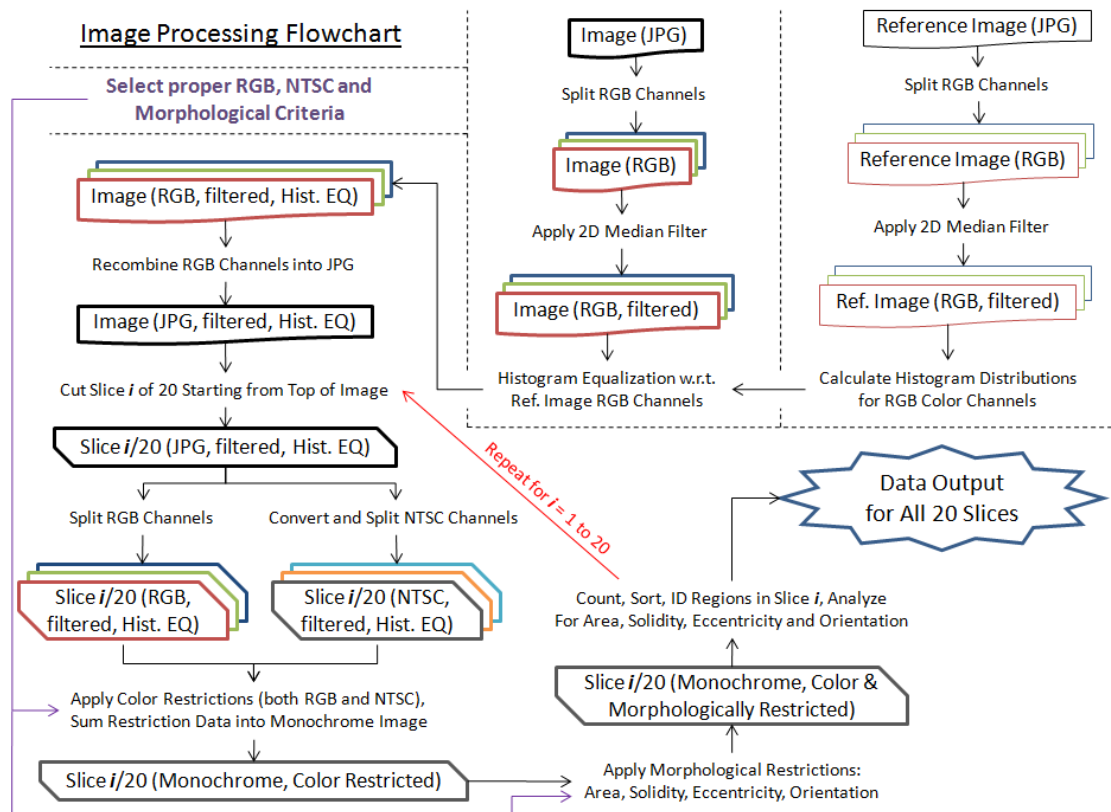
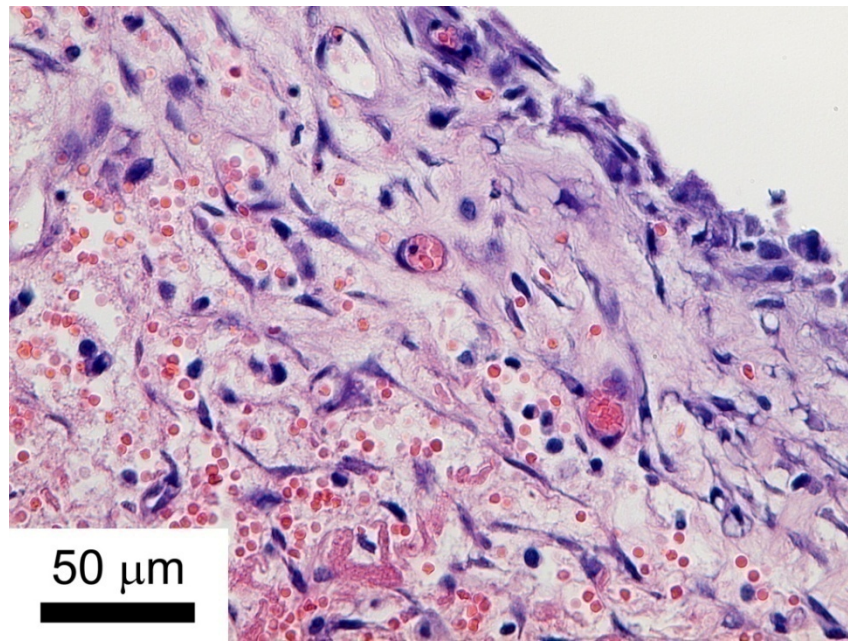


Figure 16. Flow chart diagram of the computational image processing workflow.

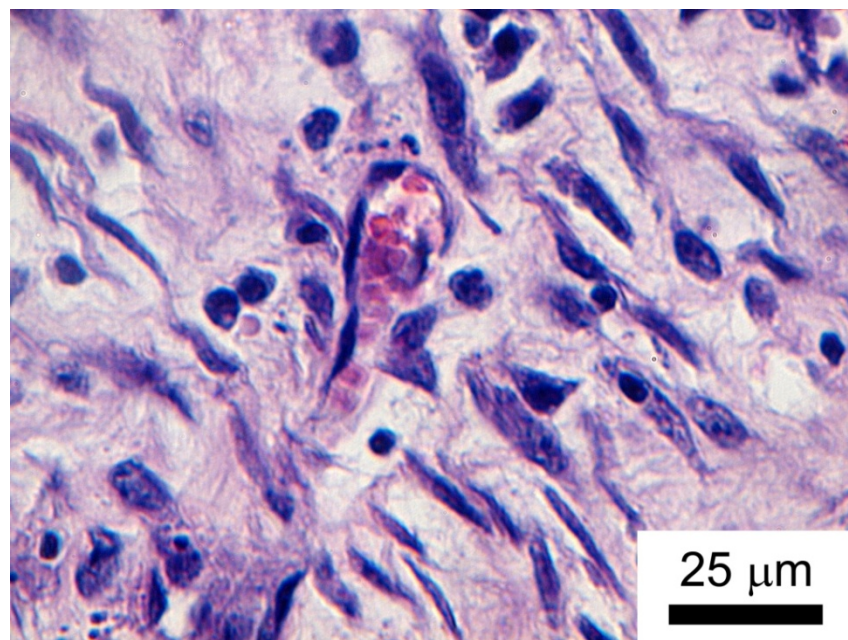
3.3 Results

3.3.1 Histology images

High magnification images (either 40x, 0.03 $\mu\text{m}/\text{px}$ or 20x, 0.07 $\mu\text{m}/\text{px}$) are shown of all five different stains illustrating feature coloration and morphology that is of interest in this study. Note the nuclear location and definition in the H&E stained slides stained dark blue/purple demonstrating the cellular density of the tissues (see **Figure 17**). The Gomori Trichrome (GTC) stained slides shown in **Figure 18** demonstrate excellent collagen identification with a strong bluish/green cast that readily stands out from the reddish/gray background. **Figure 19** illustrates the labeling of various sized microvessels where the strong contrast of the brown HRP/DAP stain against the light blue hematoxylin counterstain is ideal for image analysis. **Figure 20** exceptionally demonstrates the presence of granulocytes in the tissues (labeled with a T), in the lumen of a vessel (labeled with an L) and as they extravasate (labeled with an E). Finally, macrophages in the tissues are shown in singular form in **Figure 21a**, and as multinucleated giant cells (labeled with an M) engulfing polyester velour fibers (labeled with a P) in **Figure 21b**.

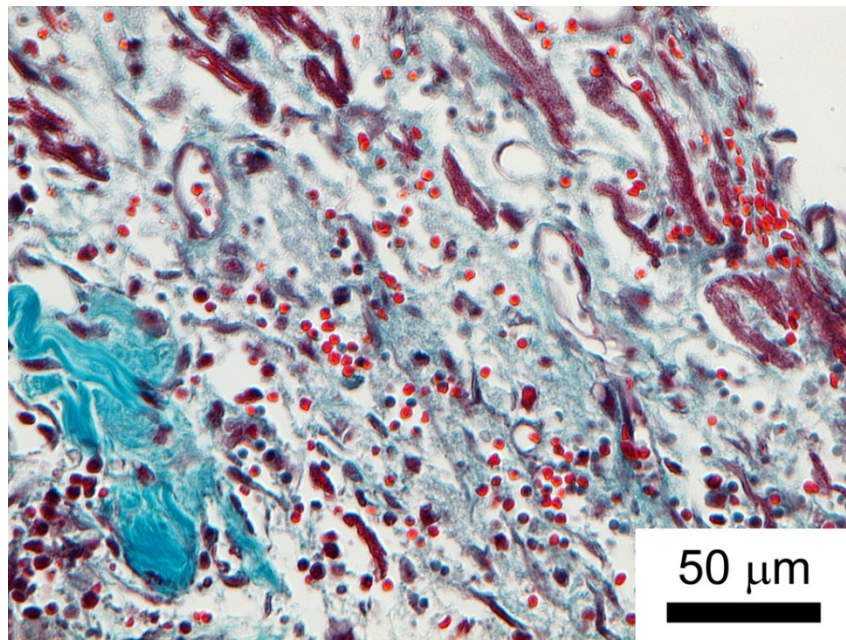


(a)

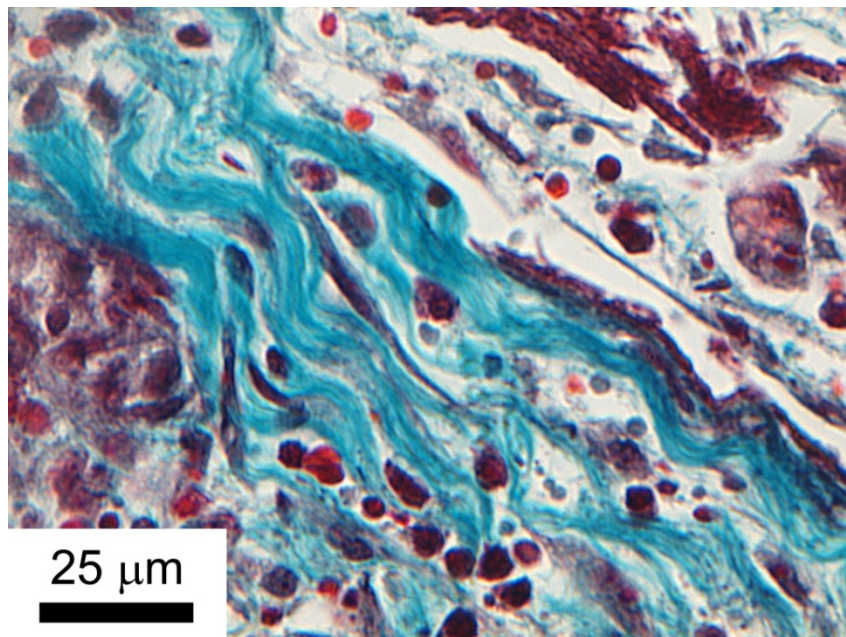


(b)

Figure 17. Subcutaneous tissue stained with standard H&E process illustrating general morphology and composition. Note the dark blue/purple stained nuclei. (a) 0.03 μm/px, (b) 0.07 μm/px resolution.



(a)



(b)

Figure 18. Subcutaneous tissue stained with Gomori's Trichrome process meant to identify extracellular collagen.

Note the blue/green collagen deposit (C). (a) 0.03 μm/px, (b) 0.07 μm/px resolution.

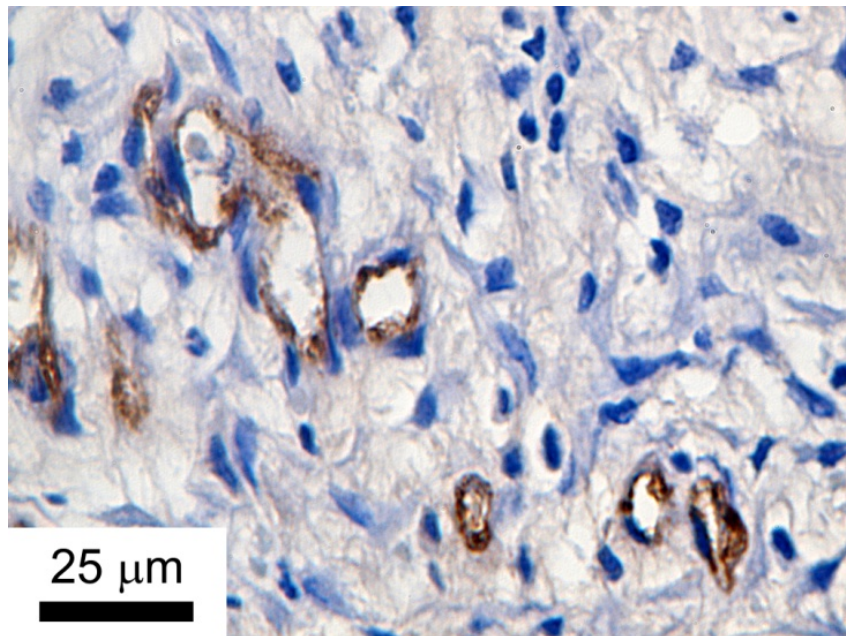


Figure 19. Subcutaneous tissue endothelial cells labeled with CD31 antibodies. Stained with HRP/DAP and hematoxylin counterstain.

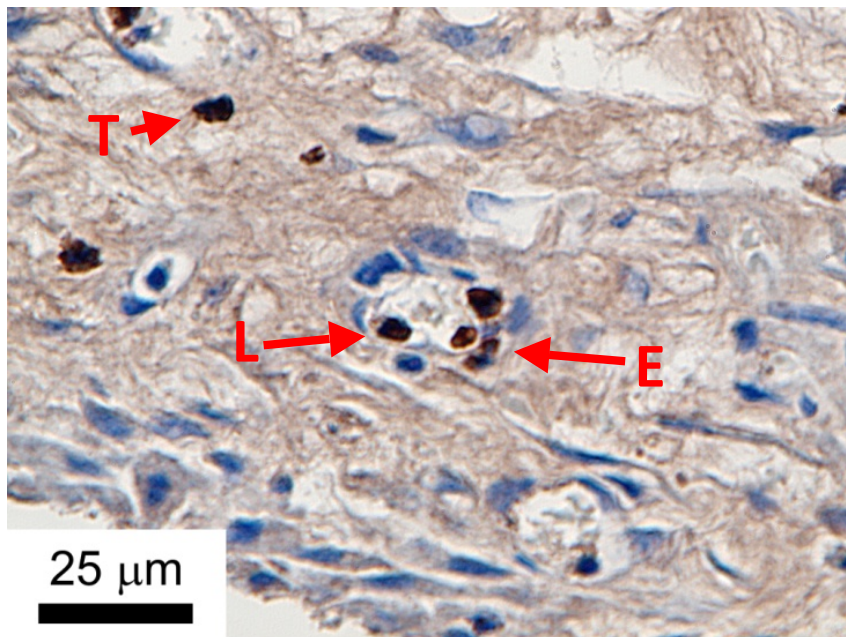
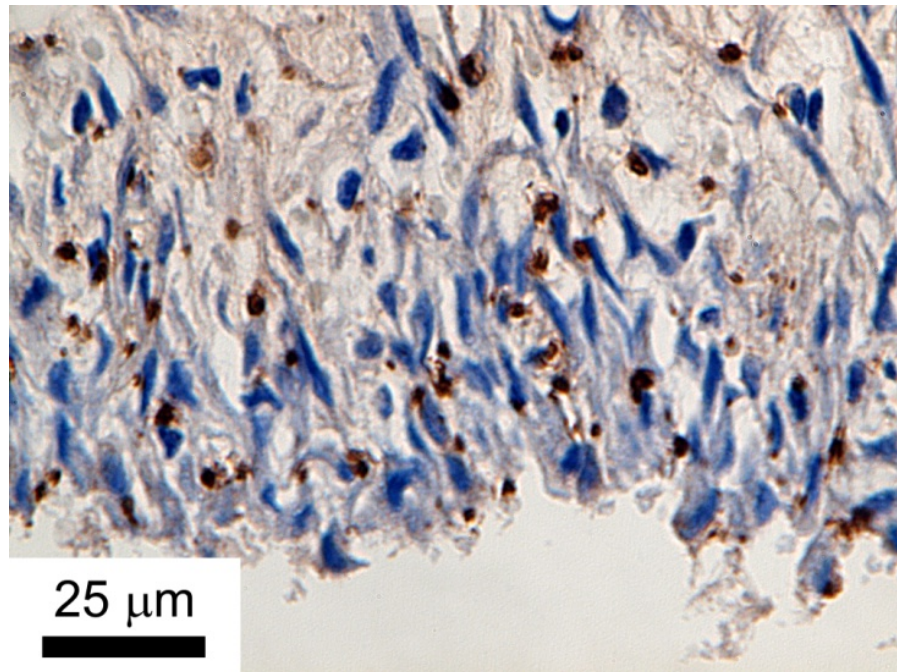
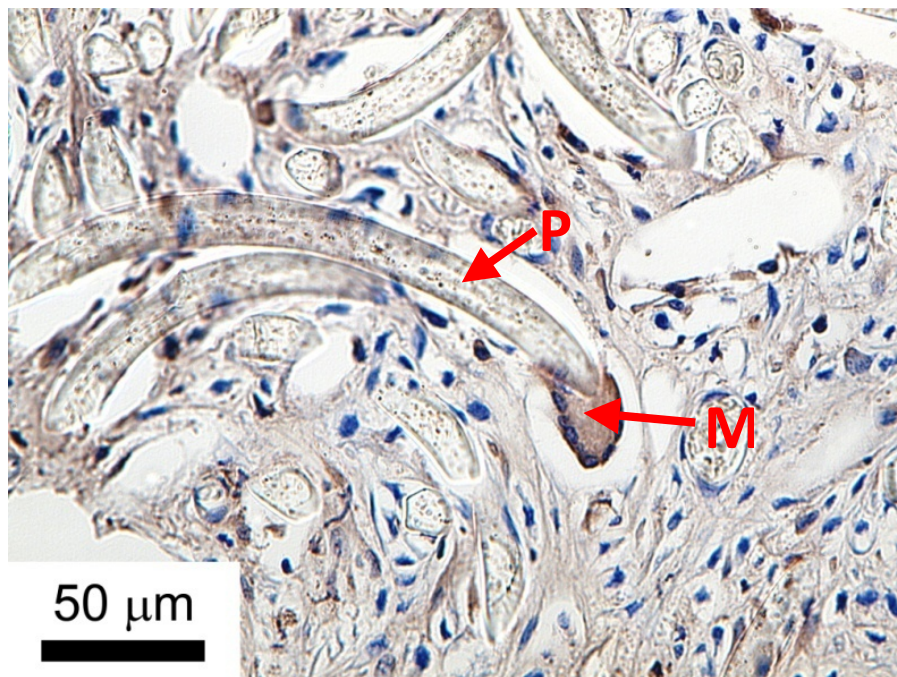


Figure 20. Subcutaneous tissue granulocytes labeled with 6D10 antibodies. Stained with HRP/DAP and hematoxylin counterstain. Granulocytes in the tissues (labeled with a T), in the lumen of a vessel (labeled with an L) and as they extravasate (labeled with an E).



(a)

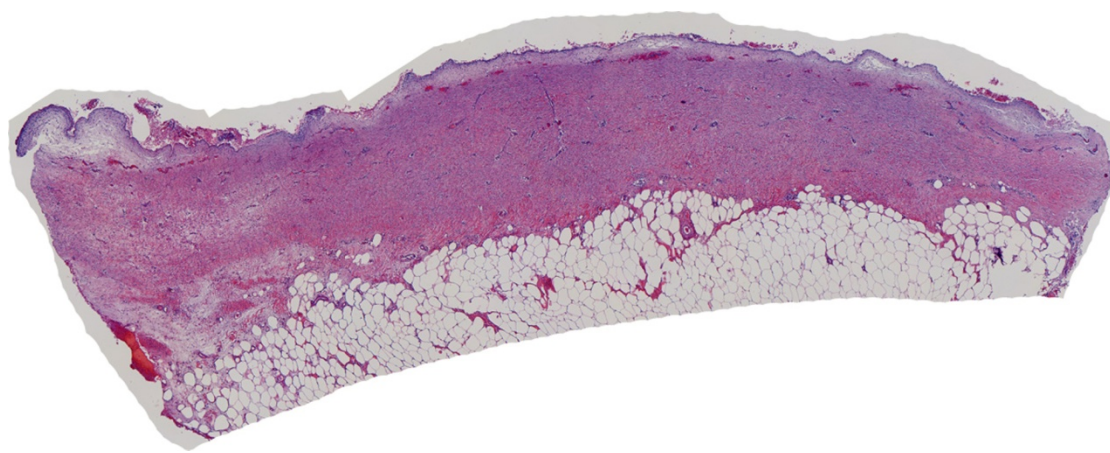


(b)

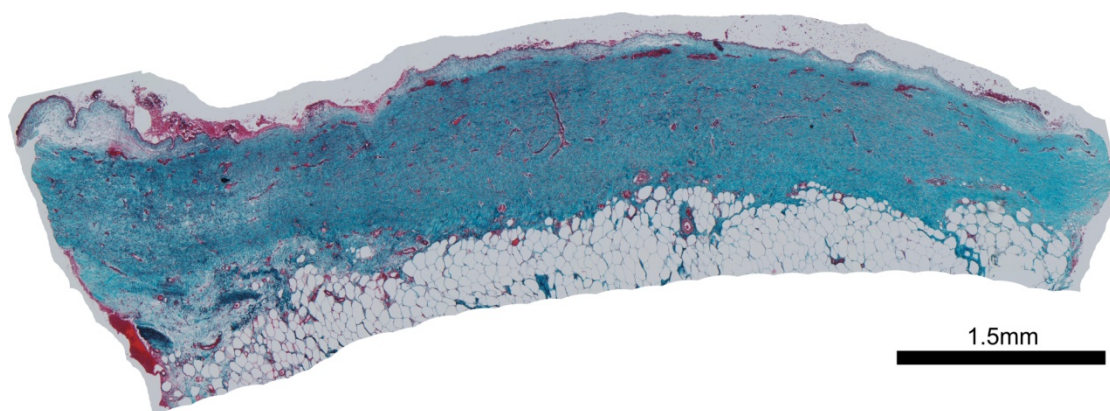
Figure 21. Subcutaneous tissue macrophages labeled with CD4 antibodies. (a) Normal tissue and (b) engulfing embedded Dacron fibers. Stained HRP/DAP and hematoxylin counterstain. Multinucleated giant cells (labeled with an M) engulfing polyester velour fibers (labeled with a P)

Each time point (1, 2, 6 and 13 weeks) and each staining (H&E, GTC, CD31, CD4 and 6D10) resulted in a composite image made up of many individual high resolution micrographs. Two sample mosaics of H&E and GTC stained PDMS adjacent tissues at Week 4, composed of at least 25 individual micrographs each, are presented in **Figure 22**. This figure illustrates the scope of imaging such a slide. In both mosaics, the sham was in contact with the tissue along the top surface. The regions for analysis were kept as consistent as possible between the different stains, but local tissue damage and staining abnormalities did not always allow for this.

A progression of representative 250 x 500 μm H&E, GTC and CD31, 6D10 and CD4 images over the 13 week course of implantation are presented illustrating the morphology of the tissues surrounding the sham implant. Images from tissues adjacent to both the titanium (see **Figure 23**) and PDMS (see **Figure 24**) surfaces are shown where the top surface is adjacent to the implant. These images were subsequently subjected to image analysis for quantification.



(a)



(b)

Figure 22. Full-slide mosaic images of PDMS adjacent tissue collected at week 4 stained with H&E (a) and GTC (b).

From these mosaics, individual regions adjacent to the implant (top edge in both images) were chosen for further analysis. Mosaics were created from 25+ micrographs with $0.34\text{ }\mu\text{m}/\text{pixel}$ resolution.

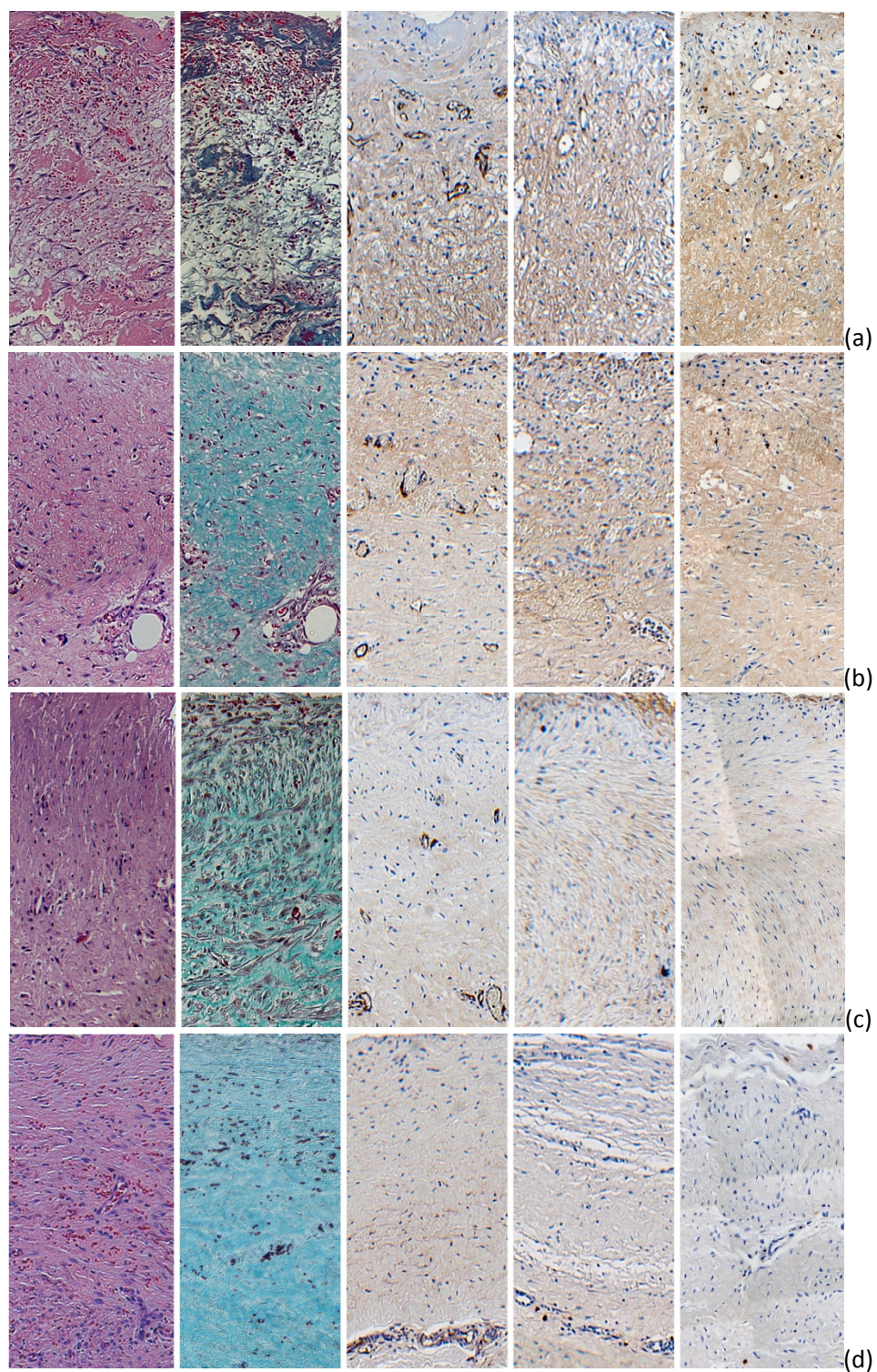


Figure 23. Histology micrographs of tissues adjacent to the titanium surface subjected for quantification via image analysis.

The five images from left to right represent H&E, GTC, CD31, CD4 and 6D10 staining for (a) week 1, (b) week 2, (c) week 6 and (d) week 13. All images are 250 x 500 μm in scale.

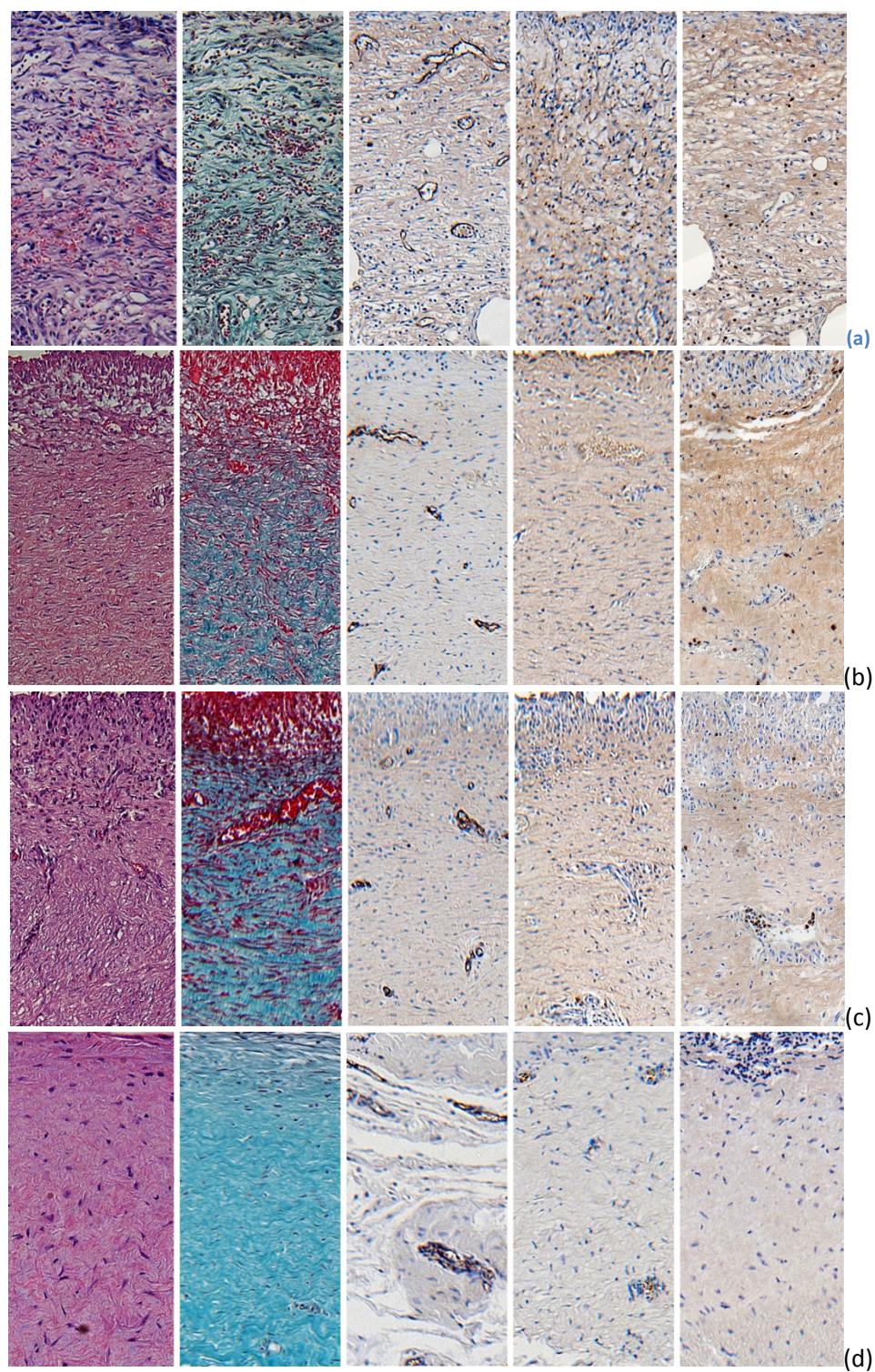


Figure 24. Histology micrographs of tissues adjacent to the PDMS surface subjected for quantification via image analysis. The five images from left to right represent H&E, GTC, CD31, CD4 and 6D10 staining for (a) week 1, (b) week 2, (c) week 6 and (d) week 13. All images are 250 x 500 μm in scale.

3.3.2 Data and analysis

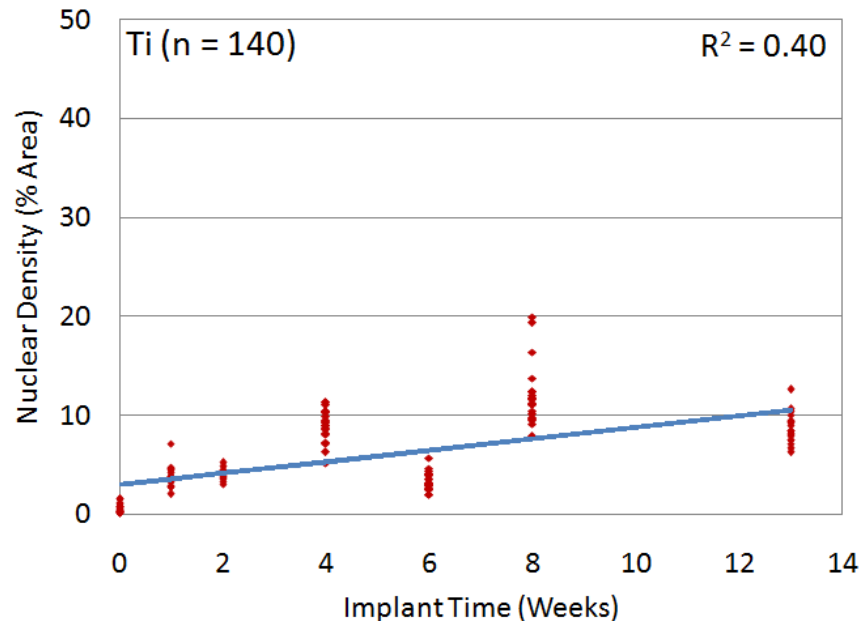
3.3.2.1 Standard histology: H&E/nuclear density and GTC/collagen

Qualitative analysis of the H&E stained slides indicates no significant abnormalities in staining for the titanium and PDMS adjacent slides. The PDMS adjacent tissues present a higher number of interstitial red blood cells (RBCs) than their titanium adjacent counterparts. This can be readily seen when observing the GTC stained slides at weeks 2 and 6 (see **Figure 24b** and c, second images from the left). A very prominent red stain is seen that, when magnified, clearly shows large quantities of red blood cells, both individual and fused (clotted). It is not clear if this presence of RBCs is related to the material in question, but by week 13 (see **Figure 24d**) this “bleeding” is absent, indicating that whatever caused the event either dissipates within the time course of the experiment or was localized to those two implant locations and sham surfaces.

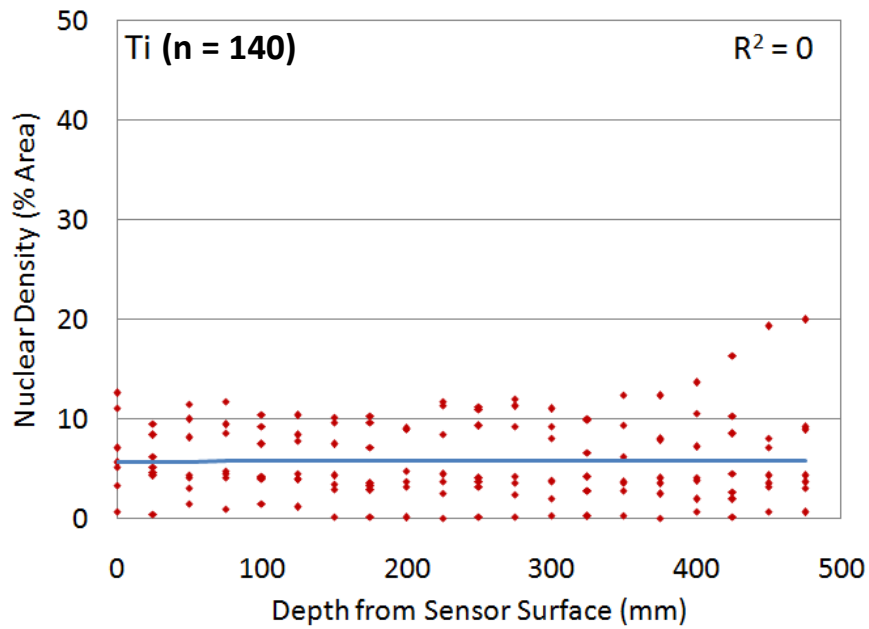
For both H&E and GTC stained slides, the features of interest (cell nuclei and collagen deposits) appear clearly and distortion free. Visual assessment of H&E stained slides provided no clues as to any nuclear density trends in the tissues. GTC stained slides, when visually inspected, readily demonstrated increases in the blue/green collagen deposits indicating the presence of a trend.

Image quantification was performed for nuclear density on the H&E stained slides. This was done by isolating the dark purple/black nuclei from the red/pink/light purple background. By relating the nuclear presence to tissue activity, seeing as the nuclei in H&E staining are not distinguished from each other, we can approximate how tissue activity relates to the presence of the sham over the course of the implantation.

The percent nuclear density is presented as a function of time and as a function of depth for both the titanium (see **Figure 25a** and **b**) and PDMS (see **Figure 25c** and **d**) adjacent tissues. Linear regressions were performed on this data, and R^2 and p values for the student's t-test were calculated for each fit. *For titanium adjacent tissues, a positive trend was found for nuclear density as a function of time ($p < 0.001$), but no trend was found with respect to depth. For PDMS adjacent tissues, a negative trend was found with respect to depth ($p < 0.001$) and no trend was present with respect to time.*

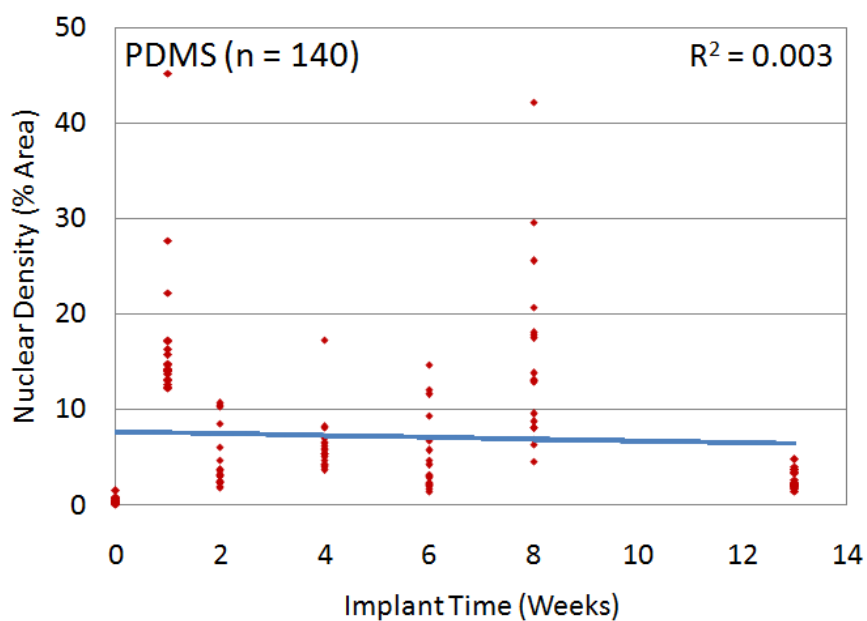


(a)

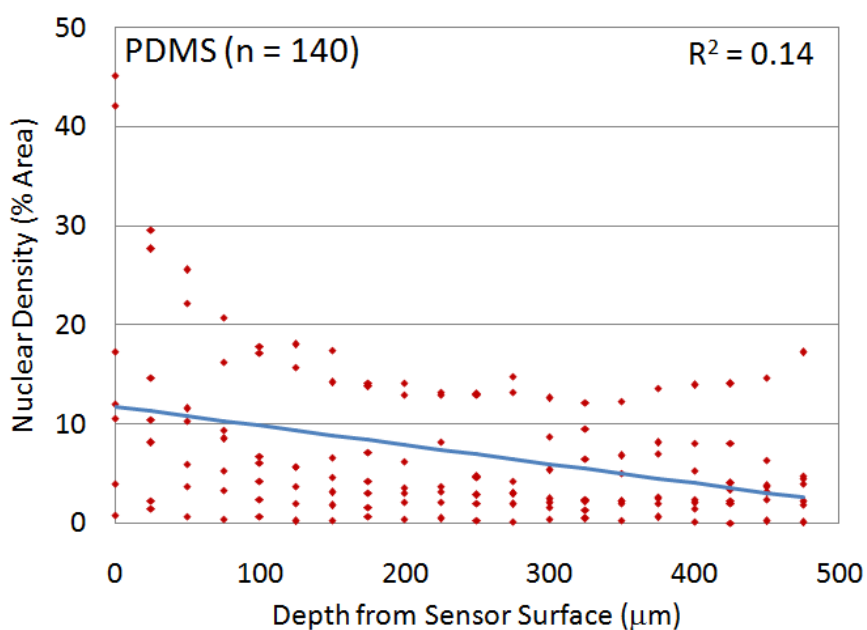


(b)

Figure 25. Quantification results for the nuclear density in H&E stained slides. Presented as nuclear density as a function of (a) implant time for Ti adjacent tissue, (b) depth from sensor surface for Ti adjacent tissue, (c) implant time for PDMS adjacent tissue, and (d) depth from sensor surface for PDMS adjacent tissue. Included are the linear regression fits and corresponding R^2 values.



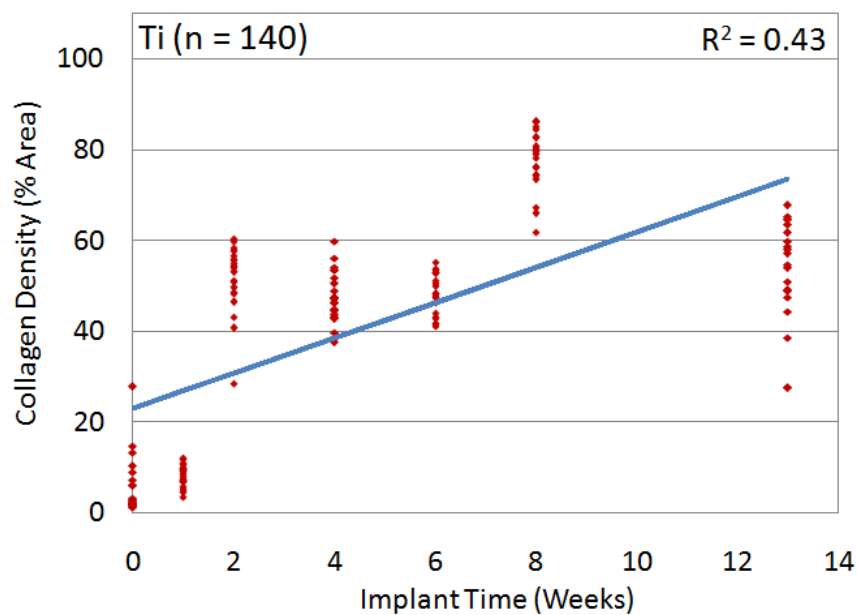
(c)



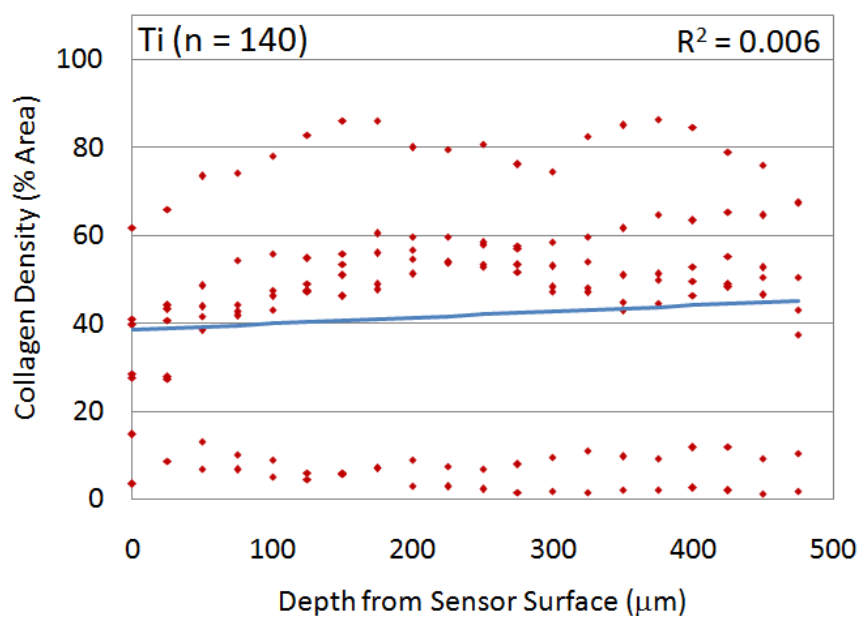
(d)

Figure 25 (continued). Quantification results for the nuclear density in H&E stained slides. Presented as nuclear density as a function of (a) implant time for Ti adjacent tissue, (b) depth from sensor surface for Ti adjacent tissue, (c) implant time for PDMS adjacent tissue, and (d) depth from sensor surface for PDMS adjacent tissue. Included are the linear regression fits and corresponding R^2 values.

Collagen density was calculated from the GTC stained slides. Collagenous material appears blue/green in these images (see **Figure 18**) and can be readily isolated from the remaining pink/red/purple background. Similar to nuclear density, percent collagen density is presented as a function of time and as a function of depth for both the titanium (see **Figure 26a** and **b**) and PDMS (see **Figure 26c** and **d**) adjacent tissues. Again, R^2 and p values are presented for the corresponding linear regression fits and student's t -tests. *For titanium adjacent tissues, only a positive trend was found for collagen density with respect to time ($p < 0.001$), whereas PDMS adjacent tissues showed both a strong trend with respect to time ($p < 0.001$) and a less prominent, yet statistically significant trend with respect to depth ($p < 0.01$).*

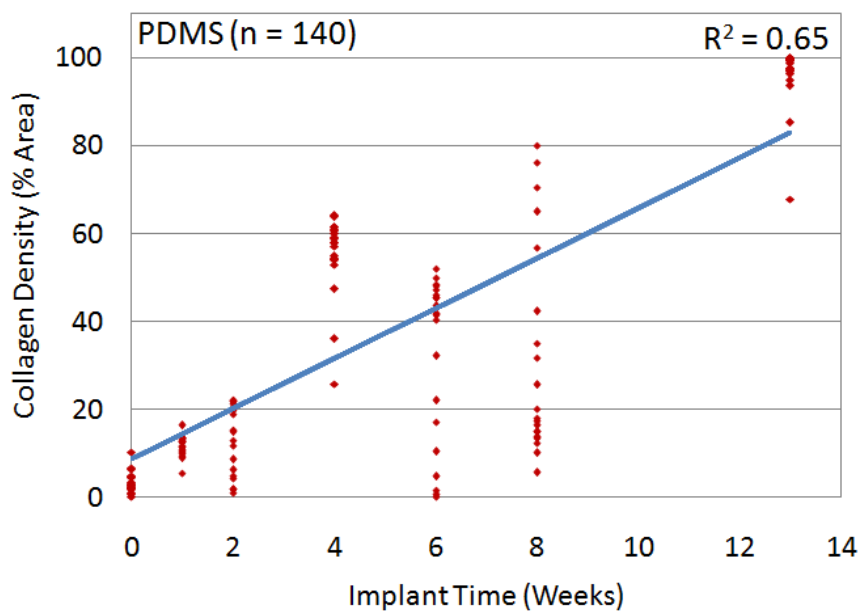


(a)

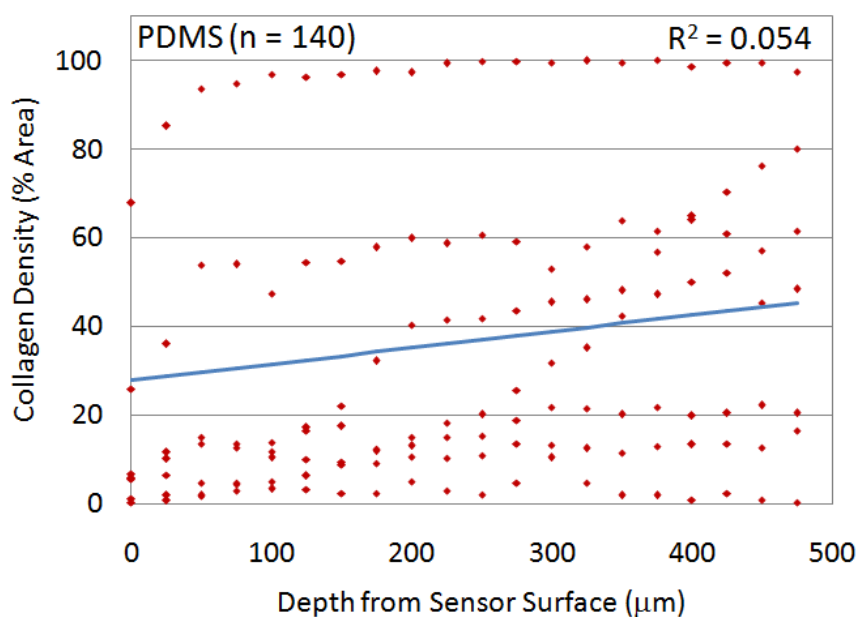


(b)

Figure 26. Quantification results for the collagen density in GTC stained slides. Presented as nuclear density as a function of (a) implant time for Ti adjacent tissue, (b) depth from sensor surface for Ti adjacent tissue, (c) implant time for PDMS adjacent tissue, and (d) depth from sensor surface for PDMS adjacent tissue. Included are the linear regression fits and corresponding R^2 values.



(c)



(d)

Figure 26 (continued). Quantification results for the collagen density in GTC stained slides. Presented as nuclear density as a function of (a) implant time for Ti adjacent tissue, (b) depth from sensor surface for Ti adjacent tissue, (c) implant time for PDMS adjacent tissue, and (d) depth from sensor surface for PDMS adjacent tissue. Included are the linear regression fits and corresponding R^2 values.

3.3.2.2 Immunohistochemical staining: CD31, CD4 and 6D10 antibody stains

3.3.2.2.1 CD31 Ab/endothelial cells

Qualitative observation of the CD31 labeled and immunohistochemically stained slides demonstrated clear staining of the features of interest. Few staining artifacts are present (save for the edges and regions of damage). Vessels of various dimensions and shapes are present and stain intensity is conserved among them. This is especially important for the smaller, single-celled capillaries. Faint stain intensity would most readily impact identification of such small structures, but this was not seen to be the case. Trends were difficult to grade visually as they seem to change somewhat moving from one end of the sample to the other. The selection of random regions for further quantitative analysis was therefore deemed necessary to ensure a representative trend for an entire tissue slice.

CD31 antibody labeled tissues were quantified in the same manner as the H&E and GTC tissues except, the individual distributions as a function of time were examined. The distributions are presented in **Figure 27** for both the titanium (a-d) and PDMS (e-h) adjacent tissues. Percent of CD31 antibody labeled image as a function of depth from sensor surface is reported for weeks 1, 2, 6 and 13. The distributions were smoothed using a 5-point moving average to remove some of the noise due to the small number of samples per point. The error bars on the distributions represent the minimum and maximum values illustrating the range of occurrence. The narrowest depth ranges of the distributions which contain 50% or more of the CD31 labeled tissue were calculated and are highlighted. *It can be readily observed that the distributions*

shift over time with more heavily stained tissues occurring at larger depths over the course of the experiment. Similar distribution shifts occur for both titanium and PDMS adjacent tissues, however for titanium adjacent samples, the depth of labeled tissue at 1 week is 100 μm , whereas the PDMS adjacent tissues contain significant staining near the surface of the implant.

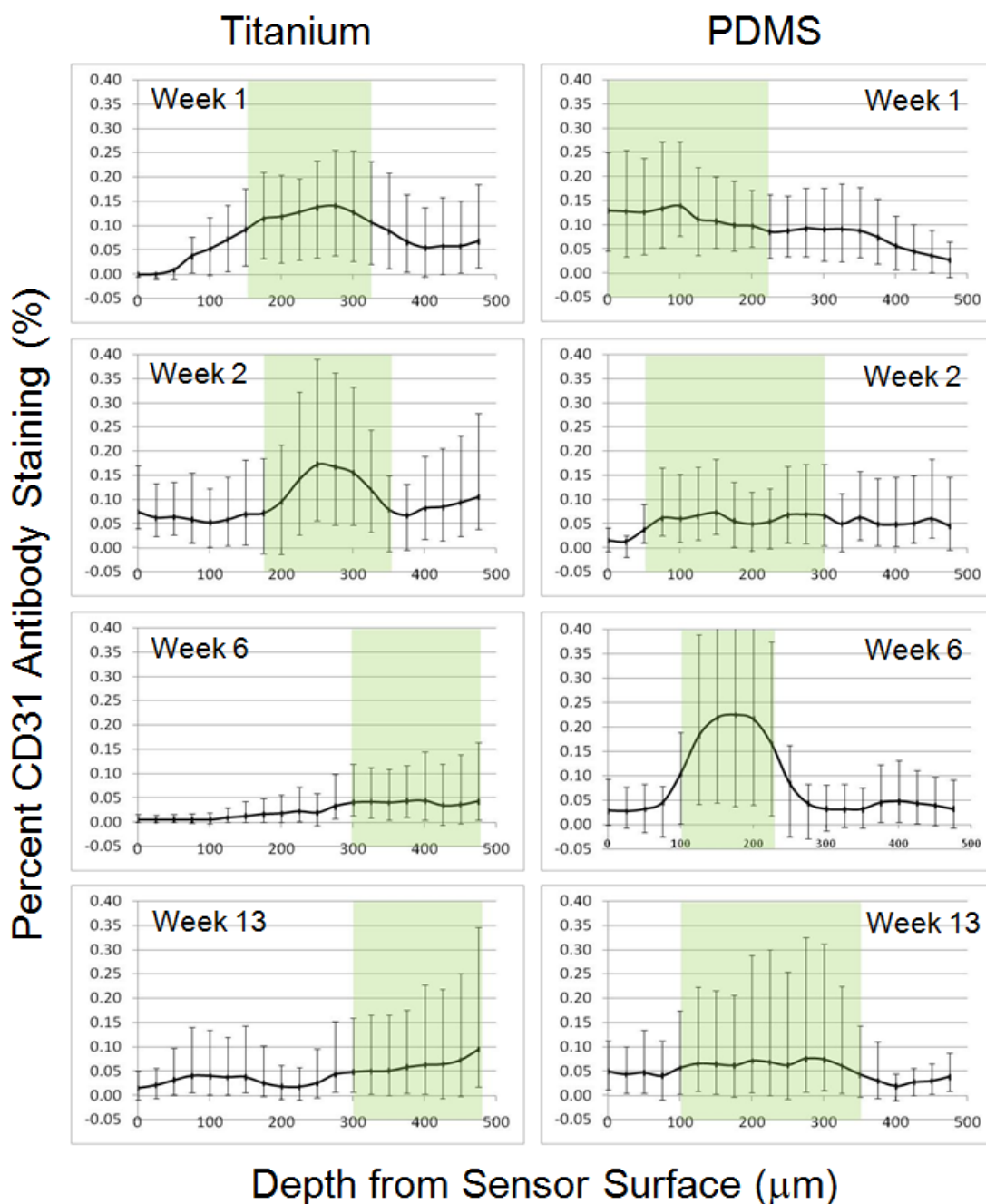


Figure 27. Quantification results for endothelial cell density from CD31 antibody stained slides. Percentage of 25 x 250 μm tissue sections stained with CD31 antibodies as a function of depth from sham implant edge. Shaded regions represent the smallest distance of tissue with at least 50% of available staining. (Error bars represent minimum and maximum values)

3.3.2.2.2 CD4 Ab/macrophages

A preliminary visual inspection of the CD4 antibody labeled and immunohistochemically stained slides indicated minimal cellular presence and no readily discernable trends. For select cases (see **Figure 23b, c** and **Figure 24b**, top surface is the interface with the sham) an increased staining at the tissue/sham interface is present which is consistent with the findings of other researchers describing macrophage engulfment of foreign material. The slides in general present no staining artifacts (again, if the edges and regions of slide damage are avoided) and positively stained cells were readily identified. Of interest is a sample collected on week 2 from the titanium surface that happened to include the velour patch with ingrown tissue. Labeling of this tissue with the CD4 antibodies demonstrated excellent examples of macrophage/multinucleated giant cells attempting to engulf the velour fibers (**Figure 21b**) and serves as positive control for the stain.

*The macrophages were clearly and readily observed and identified computationally. They exist in very few numbers and are sporadically distributed. No obvious trends can be visibly discerned from the distribution data shown in **Figure 29** and statistical analysis confirmed no trends with respect to either depth or time (linear regression with t-test, results not shown). Averages across all depths and times for both the titanium and PDMS adjacent tissues were calculated with titanium adjacent tissues showing 0.0074% with a standard deviation of 0.0051% and PDMS adjacent tissues showing 0.0080% with a standard deviation of 0.0076%. The large spread in these values makes any definitive statements unwarranted at this time.*

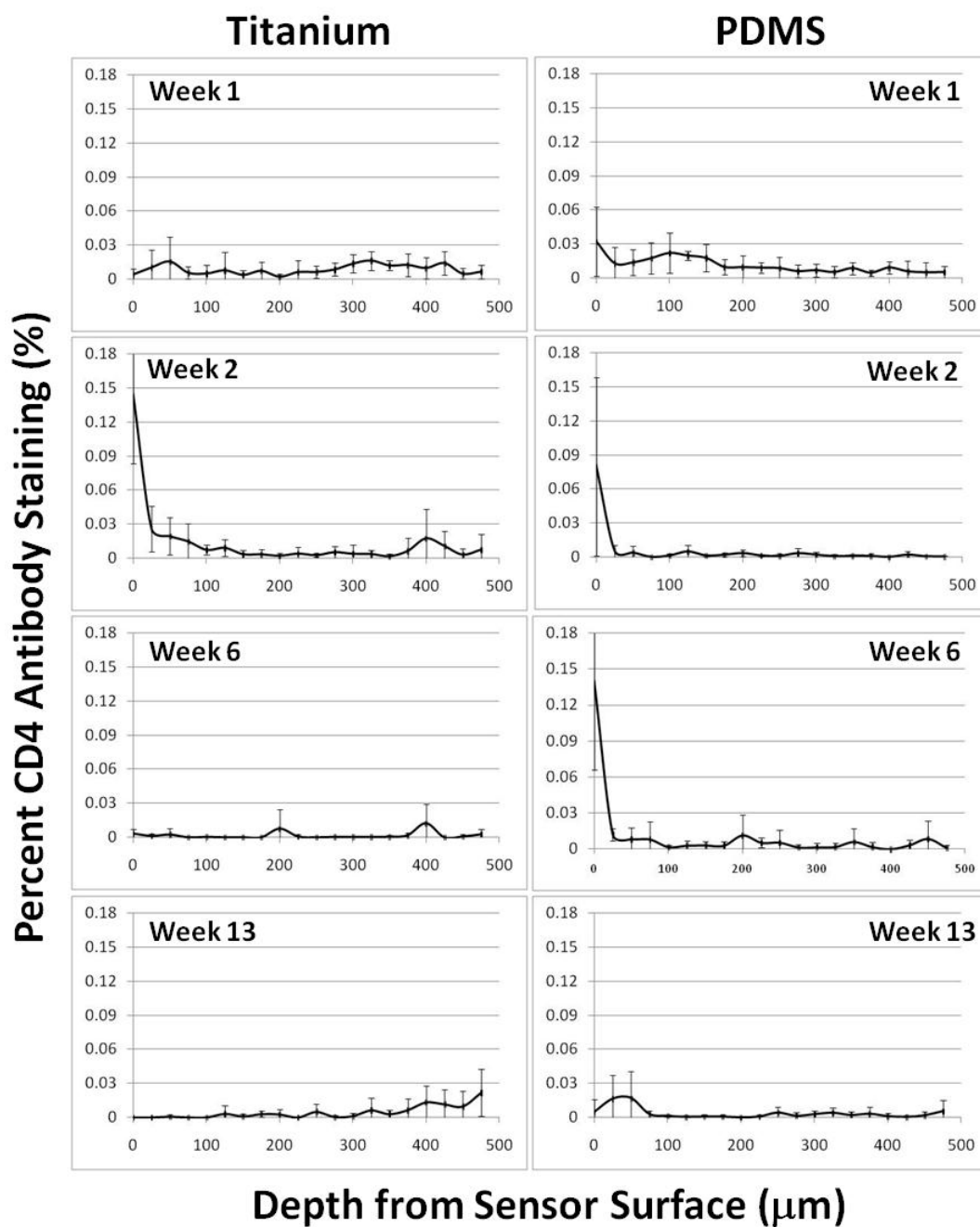


Figure 28. Quantification results for macrophage density from CD4 antibody stained slides. Percentage of 25 x 250 μm tissue sections stained with CD31 antibodies as a function of depth from sham implant edge. (Error bars represent plus/minus one standard deviation)

3.3.2.2.3 6D10 Ab/granulocytes

Similar to the analysis performed for the CD31 and CD4 antibody labeled tissues, 6D10 antibody labeled tissues were also analyzed for their distributions. *While the cells were clearly and readily observed and identified computationally, they exist in very few numbers and are sporadically distributed. No obvious trends can be visibly discerned from the distribution data shown in **Figure 29** and statistical analysis confirmed no trends with respect to either depth or time (linear regression with t-test, results not shown).* Averages across all depths and times for both the titanium and PDMS adjacent tissues were calculated with titanium adjacent tissues showing 0.0040% with a standard deviation of 0.0056% and PDMS adjacent tissues showing 0.0073% with a standard deviation of 0.0090%. Due to the small number of granulocytes present in the tissues, and the limited number of data points, too much spread is present to warrant any definitive statements.

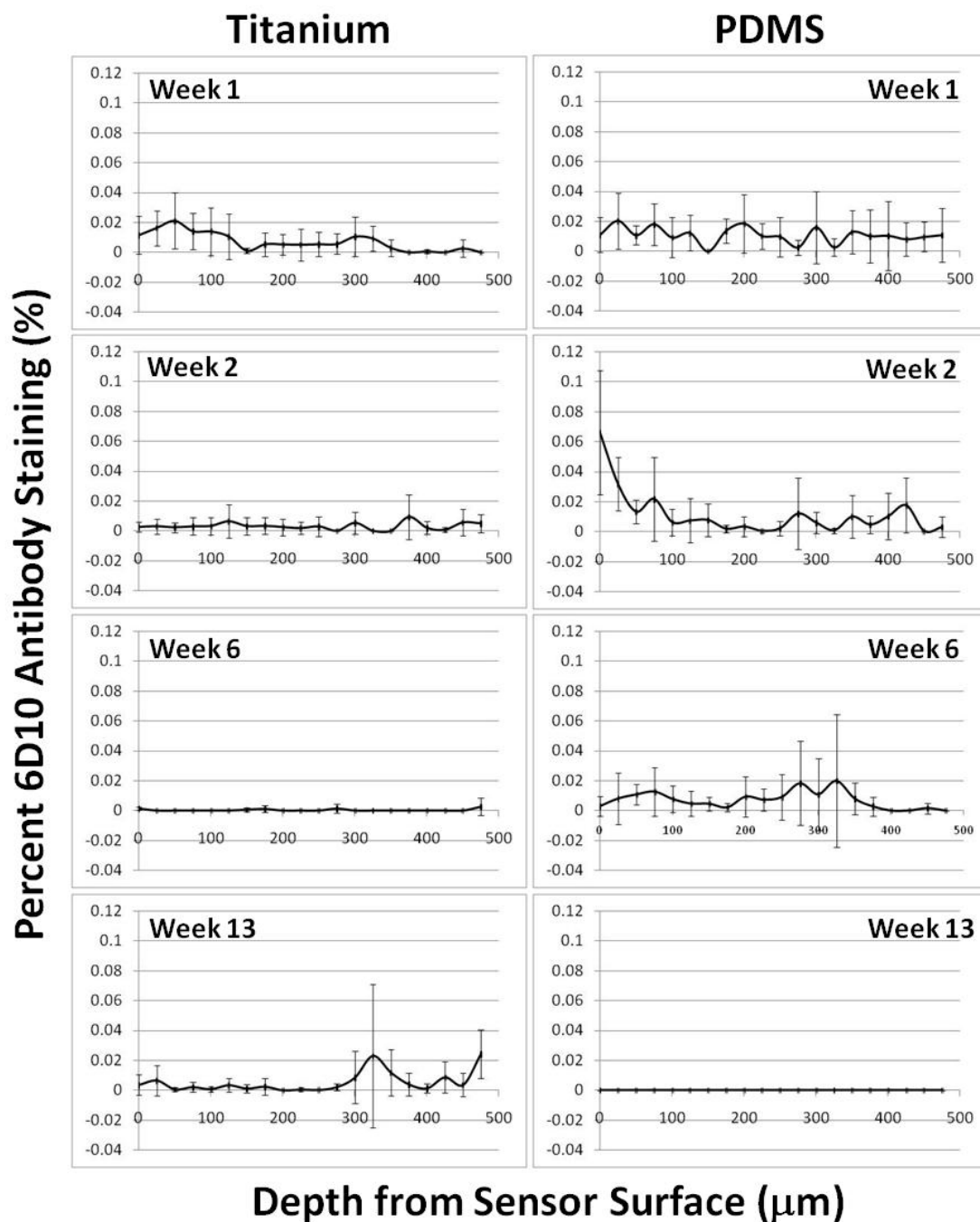


Figure 29. Quantification results for granulocyte density from 6D10 antibody stained slides. Percentage of $25 \times 25 \mu\text{m}$ tissue sections stained with CD31 antibodies as a function of depth from sham implant edge. (Error bars represent plus/minus one standard deviation)

CHAPTER 4 –

Computational analysis of oxygen flux dynamics

4.1 Methodology

Findings from signals analysis (Chapter 2) and quantitative serial histology (Chapter 3) have to this point supported the hypothesis that oxygen signals (and resultant flux, see Equation 7) are affected by the changes in the tissues that arise from the foreign body response. The exponential decay that was described in Section 2.2.2 can therefore be further mathematically modeled in order to explain and predict the long-term decay trends that arise from changes in tissue morphology and composition. Specifically, we will look at the changes in the tissues that might affect the diffusion coefficient of oxygen through the tissues, D_{O_2} (tissue density, collagen content), the diffusion distance, δ_t (vascular distribution, granular layer thickness), oxygen concentration, c_t (vessel size, type) and consumption of oxygen (metabolic rates of tissues) (see **Table 4**). Both analytical and numerical computational techniques are used to quantifiably elucidate the impact that each of these variables might have on long-term oxygen signals reported from an implanted oxygen sensor.

4.2 Histological data used for mathematical analysis

4.2.1 Microvessel distribution and location

The CD31 staining distributions shown in **Figure 27** were fitted to two common distribution types in an attempt to quantifiably relate the prominence of staining with the depth away from the sensor surface, δ_t . The normal (Gaussian) and beta distributions were used to create probability density functions (PDFs) to aid in the mathematical modeling of microvessel distributions within tissues [57]. The finding of Ertefai and Gough [32] discussed previously (see Section 1.1.2) was the impetus for the use of PDF functions to mathematically describe vessel distributions within the tissues. The normal (Guassian) distribution, $G_{PDF}(\delta_t)$ was chosen for its modifiable nature, where the peak position and width can be mathematically related using the mean, μ , and standard deviation, σ , as a function of distance from sensor surface, δ_t :

$$G_{PDF}(\delta_t) = \frac{1}{\sigma\sqrt{2\pi}} \cdot \text{Exp}\left[-\frac{(\delta_t - \mu)^2}{2\sigma^2}\right]. \quad (\text{Eq. 14})$$

The Beta distribution, $B_{PDF}(\delta_t)$ was also chosen for its modifiable nature, where the peak position and width can be readily described mathematically using two parameters, α and β , as a function of distance from sensor surface, δ_t :

$$B_{PDF}(\delta_t; \alpha, \beta) = \frac{1}{B(\alpha, \beta)} \delta_t^{\alpha-1} (1 - \delta_t)^{\beta-1} \quad (\text{Eq. 15})$$

The parameters, α and β , are related to the mean, μ , and standard deviation, σ of the function by the following equations:

$$\mu = \frac{\alpha}{\alpha + \beta} \quad (\text{Eq. 16a})$$

$$\sigma = \sqrt{\frac{\alpha\beta}{(\alpha + \beta)^2(\alpha + \beta + 1)}} \quad (\text{Eq. 16b})$$

The eight distributions were fitted seeing as the data tends to not follow one distribution strictly. R^2 values were calculated for the fits to judge quality. Examples of this fitting presented in **Figure 30** and the accompanying parameters α , β , μ and σ are included along with the corresponding R^2 values in **Table 3**. Note that both the quantity of staining and the depth away from the sensor surface in **Figure 30** have been normalized to unity. Shifts in peak position, seen in changes in μ , from sensor proximal to sensor distal occur over the course of 13 weeks. No trend in the width of the peaks (standard deviation), σ , can be seen as a function of time or material, most likely due to the highly variable nature of the measurement and the limited amount of data available. Of importance is the lack of consensus between distribution type and quality of fit. Therefore, both the normal and beta distributions will be examined in simulation.

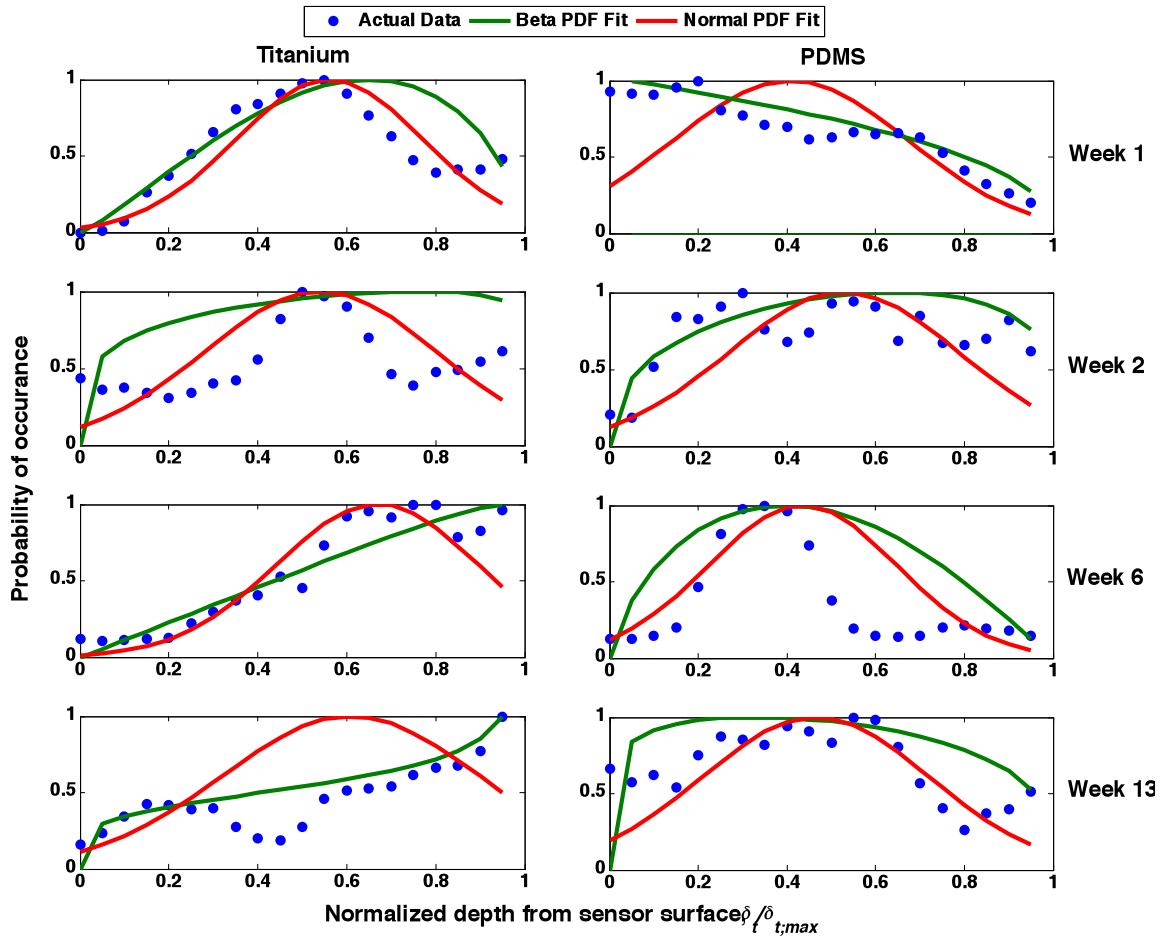


Figure 30. Normal PDF, $G_{PDF}(\delta/\delta_{t,max})$, and Beta PDF, $B_{PDF}(\delta/\delta_{t,max})$, fitted to all eight normalized CD31 stain distributions as a function of normalized distance from sensor surface, $\delta/\delta_{t,max}$. Titanium (left) and PDMS (right) adjacent tissues are presented for weeks 1, 2, 4 and 13 (top to bottom)

Table 3. Normal PDF, $G_{PDF}(\delta_t/\delta_{t,max})$, and Beta PDF, $B_{PDF}(\delta_t/\delta_{t,max})$, best fit parameters. Parameters α , β , μ and σ for both the titanium and PDMS adjacent tissues at weeks 1, 2, 6 and 13 and the corresponding R^2 values for each are presented.

PDF Type	Titanium		PDMS	
	Normal PDF	Beta PDF	Normal PDF	Beta PDF
Week 1	$\mu = 0.56$	$\alpha = 2.28$	$\mu = 0.41$	$\alpha = 0.99$
	$\sigma = 0.21$	$\beta = 1.70$	$\sigma = 0.27$	$\beta = 1.43$
	$R^2 = 0.80$	$R^2 = 0.51$	$R^2 = -0.49$	$R^2 = 0.90$
Week 2	$\mu = 0.54$	$\alpha = 1.23$	$\mu = 0.53$	$\alpha = 1.41$
	$\sigma = 0.26$	$\beta = 1.06$	$\sigma = 0.26$	$\beta = 1.22$
	$R^2 = -0.05$	$R^2 = -2.41$	$R^2 = -0.34$	$R^2 = 0.24$
Week 6	$\mu = 0.67$	$\alpha = 2.05$	$\mu = 0.44$	$\alpha = 1.70$
	$\sigma = 0.23$	$\beta = 1.05$	$\sigma = 0.21$	$\beta = 2.07$
	$R^2 = 0.78$	$R^2 = 0.89$	$R^2 = 0.17$	$R^2 = -0.54$
Week 13	$\mu = 0.61$	$\alpha = 1.20$	$\mu = 0.46$	$\alpha = 1.14$
	$\sigma = 0.29$	$\beta = 0.79$	$\sigma = 0.26$	$\beta = 1.30$
	$R^2 = -2.05$	$R^2 = 0.55$	$R^2 = 0.27$	$R^2 = -0.77$

4.2.2 Vessel diameter and number

As discussed earlier, a relationship was observed between the microvessel diameter (order) and the potential deliverable oxygen supply (oxygen concentration at vessel/tissue interface) (see Section 1.1.2, **Figure 4**). An analysis was performed on the CD31 histology presented earlier by an undergraduate volunteer, investigating the size and shape distributions of vessels in the tissues via hand selection of vessels in the micrographs. For a further description, please see the Appendix, Section 7.2. The number of vessels in a $250 \times 500 \mu\text{m}$ region of tissue adjacent to sham implants was found to be 11.5 ± 4.3 . Additionally, a full range of diameter distributions in the CD31

stained tissues presented in this thesis was demonstrated to be consistent with the findings of Tsai et al. [29].

4.2.3 Collagen and cellular density

The cellular (nuclear) density in the tissues demonstrated little to no change with regards to time or depth over the course of the 13-week implantation for both the titanium and PDMS materials (see Section 3.3.2.1). Collagen density on the other hand showed considerable change. There is a pronounced increase in collagen content for both the materials with respect to time (titanium 40%, PDMS 80%) and depth (titanium 5%, PDMS 15%). However, no direct correlation of this change to tissue oxygen diffusivity is currently available. Dense, collagenous materials, whether natural [77, 78] or synthetic [79] have demonstrated much lower (by as much as 3 fold) oxygen diffusion than that of water. Using these literature obtained values for synthetic and natural collagenous materials as a sample range, the D_{O_2} is varied between $0.5 - 2.3 \cdot 10^{-5} \text{ cm}^2/\text{s}$ (the upper representing the diffusion of oxygen through water, commonly applied to low density tissues).

Table 4. Variables used for mathematical sensitivity analysis of oxygen transfer in tissues.

Variable of Interest	Biological Features Responsible	Range of Variance	References
δ_t	Vascular distribution, and position, number	$0 - 5 \cdot 10^{-3}$ cm (position) (Distribution width) $4 - 32$ (number)	[33]
D_{O_2}	Collagen and cellular density	$0.2 - 2.3 \cdot 10^{-5}$ cm ² /s	[24, 34, 77-79]
c_t	Vessel diameter and perfusion	$4 - 8 \cdot 10^{-8}$ mol/cm ³	[29, 30]
R	Tissue metabolic rate	$4 \cdot 10^{-10} - 1.25 \cdot 10^{-6}$ mol/cm ³ /s	[60, 80]

4.3 1-D Tissue/implant interface model

Using the data from the literature along with the findings from the histological analysis, the flux observed at the electrode surface (and corresponding generated current) was simulated for various conditions using Equation 7. A rigorous sensitivity analysis is presented comprised of an investigation of each of these variables that attempts to identify which, if any, has a dominant effect on the resultant reported oxygen signals.

The following assumptions have been made for the proposed model:

- Temperature and pressure were invariant.
- No convection is present in either the tissue bulk or the vessels. This is appropriate due to the time-scales in question; any convection effects occur on the seconds-days time-scale.

- Electrode surface is diffusion limited and consumes all oxygen that comes in contact.
- Partition coefficients are incorporated into “effective” diffusion coefficients.
- Vessel surface concentrations, c_t , are kept proportional to their diameter, per the findings of Tsai et al [29].

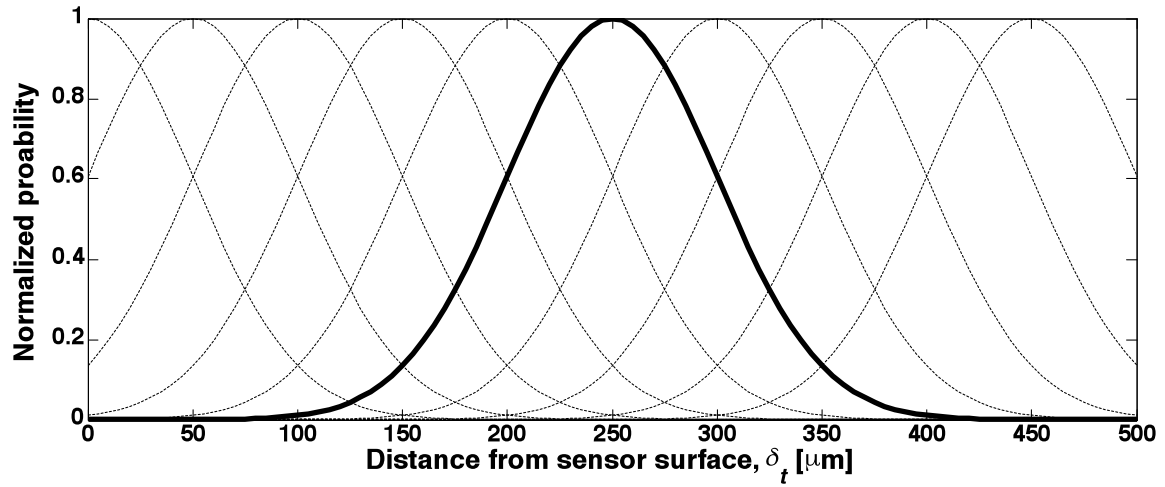
4.3.1 Diffusion path length, δ_t

First, the diffusion path length, δ_t , was varied. The number and distribution of vessels within a region of interest surrounding the electrode is of importance. In a 1-D system, this cannot be investigated using traditional methods, therefore the use of random number selection from a pool in accordance with the aforementioned normal and beta PDFs (see section 4.2.1) was employed. For each simulated “depth”, a distribution is calculated with an associated mean (peak position), μ , and standard deviation (width), σ . From this distribution, n random distances are chosen and for each, a flux is calculated (as discussed in Section 4.2.2). The average of these fluxes is calculated by:

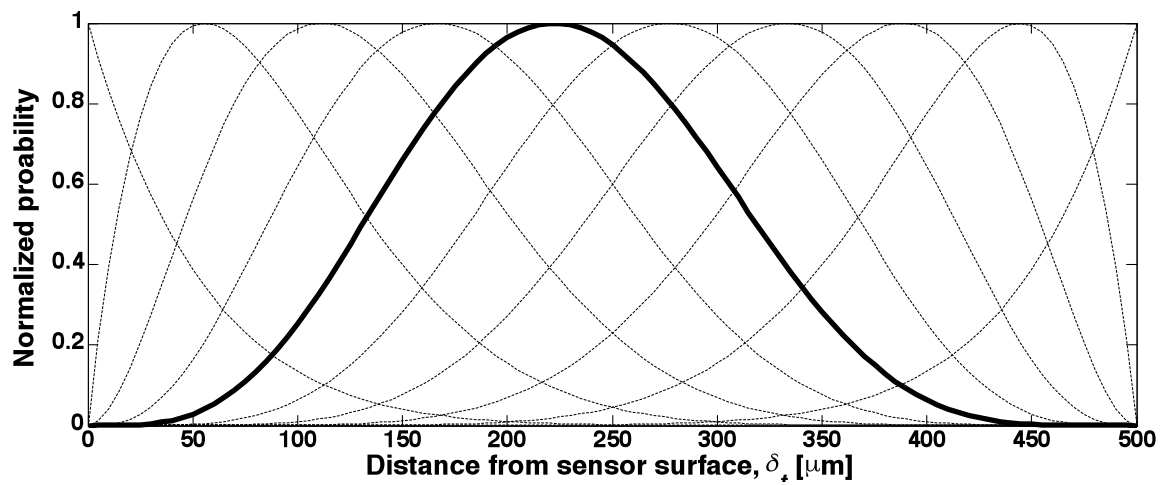
$$j = \frac{\sum_n j_n}{n} \quad (\text{Eq. 17})$$

where j_n is calculated from equation 7 for each vessel as discussed in Section 1.1.1. The mean and standard deviation are calculated in order to effectively gauge the variability. Ten simulated “depths” were calculated for each distribution type. Sample profiles of both the normal and beta distributions are presented in **Figure 31**.

The calculated flux for these distribution “depths” are presented in **Figure 32**. Note the decay process seen in these flux plots, reminiscent of the decay seen in the long-term oxygen current signals presented earlier in **Figure 6**. Flux can be readily converted to current using Equation 6 and was calculated for each “depth” value, δ_t , using the following constants: $D_m = 3.2 \cdot 10^{-5} \text{ cm}^2/\text{s}$, $D_m = 2.3 \cdot 10^{-5} \text{ cm}^2/\text{s}$, $c_t = 4.3 \cdot 10^{-8} \text{ mol/cm}^3$, $\delta_m = 35 \text{ }\mu\text{m}$, no reaction term present ($R = 0$).



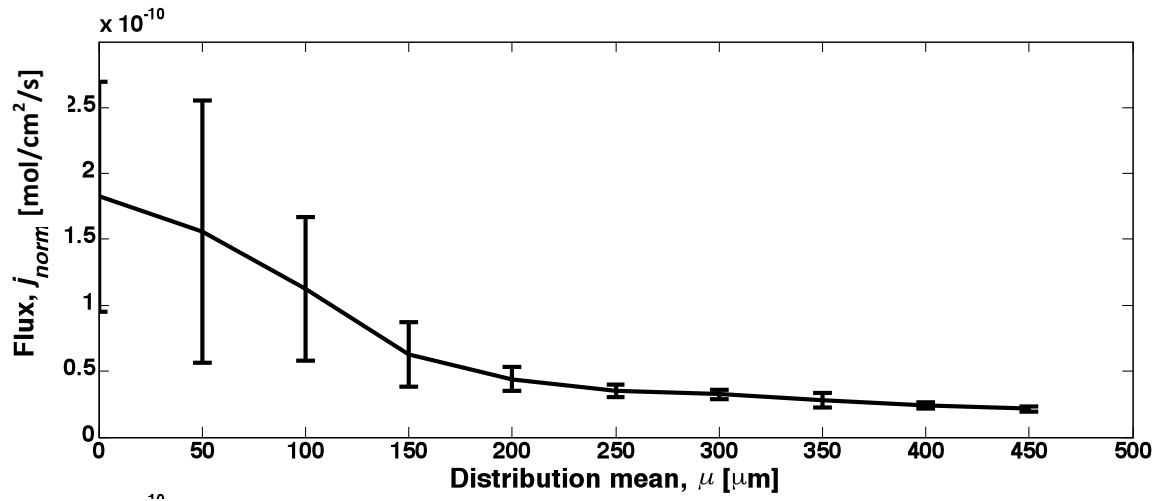
(a)



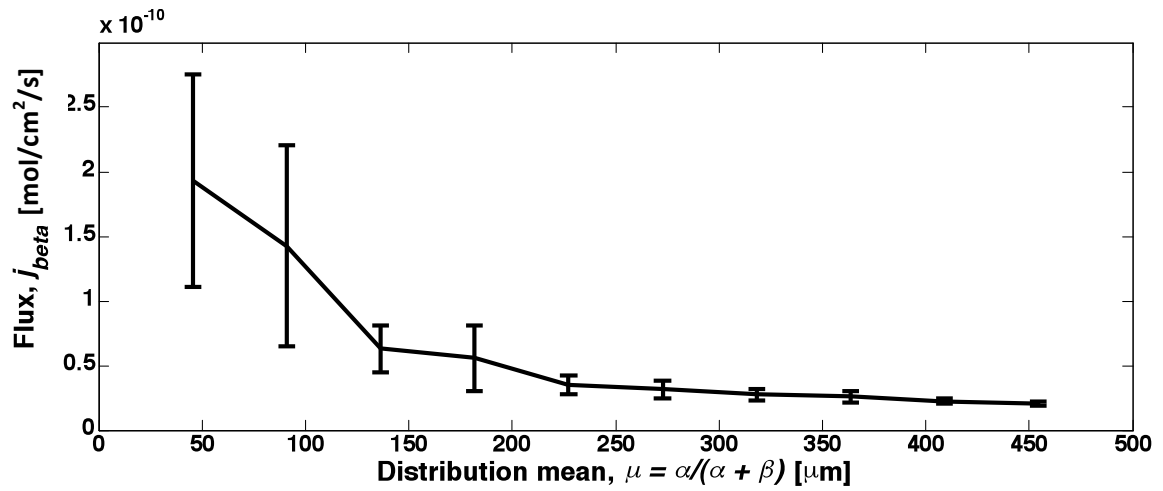
(b)

Figure 31. Sample distributions with varying peak position as a function of distance from sensor surface.

Distributions based on the (a) normal PDF (solid line has $\mu = 250 \mu\text{m}$ and $\sigma = 62.5 \mu\text{m}$) and (b) beta PDF (solid line has $\mu = 227.25 \mu\text{m}$ and $\sigma = 100 \mu\text{m}$).



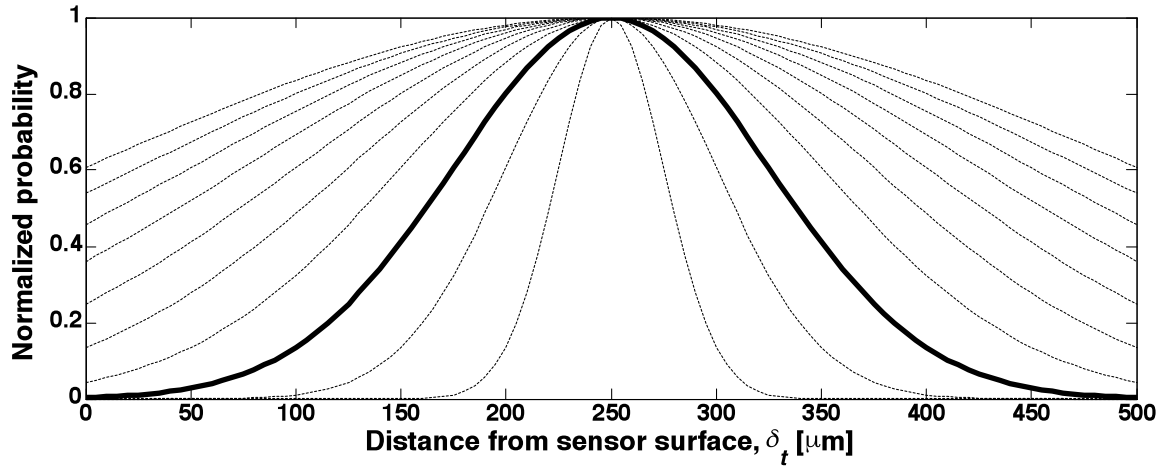
(a)



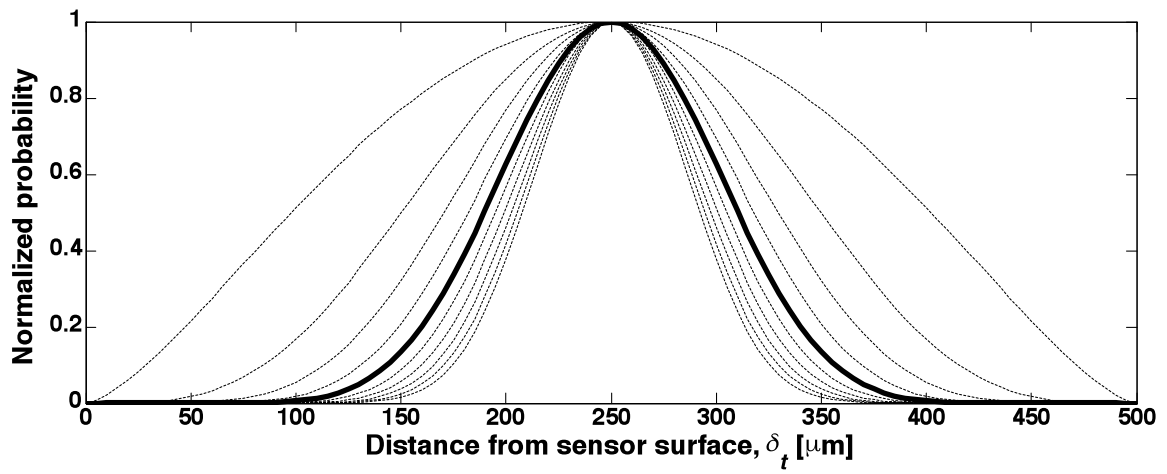
(b)

Figure 32. Flux calculations for the corresponding “depth” distributions shown in Figure 30. Calculations based on the (a) normal PDF and (b) beta PDF are presented as a function of distribution mean, μ . Error bars represent plus/minus one standard deviation of the calculations.

Examining the CD31 distributions in **Figure 27** (CD31 distribution in Chapter 3), we see that there is a noticeable difference in the spread of the distributions. For example, the titanium adjacent tissues at week 1 and the PDMS adjacent tissues at week 6 show significantly different distribution types, where Ti week 1 has a broad distribution to its peak, spanning almost the entire depth range. PDMS week 6 on the other hand is much narrower, spanning 150 μm of the entire depth range. To examine this effect of peak spread on the simulated flux calculations, the following two distribution sets in **Figure 33** were created, for the normal and beta PDF, respectively. The corresponding flux calculations were carried out and plotted as a function of standard deviation (proportional to width); they are shown in **Figure 34**. *The flux stays constant as the distribution standard deviation increases; however, the error bars indicate that the standard deviation of the calculation increases. In essence, as the width of the distribution increases, the repeatability of the flux calculation decreases.*



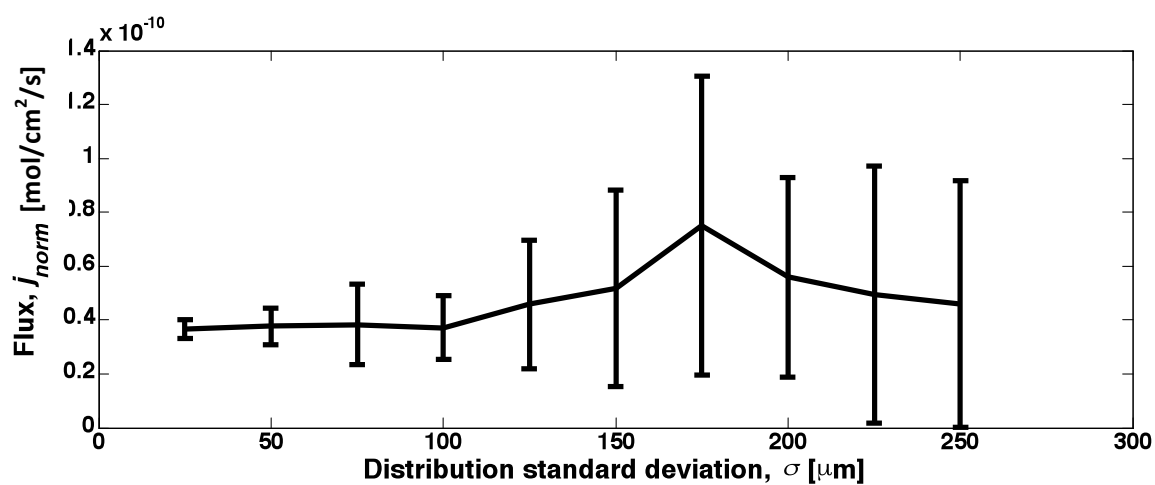
(a)



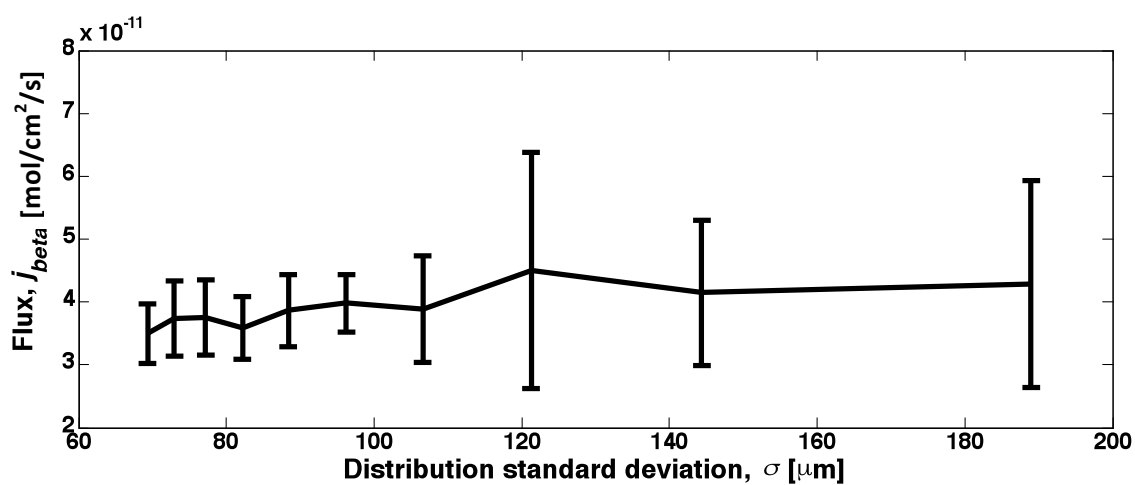
(b)

Figure 33. Sample distributions with varying width as a function of depth from sensor surface as represented by the mean, σ .

Distributions based on the (a) normal PDF (solid line has $\mu = 250$ μm and $\sigma = 75$ μm) and (b) beta PDF (solid line has $\mu = 250$ μm and $\sigma = 96.25$ μm).



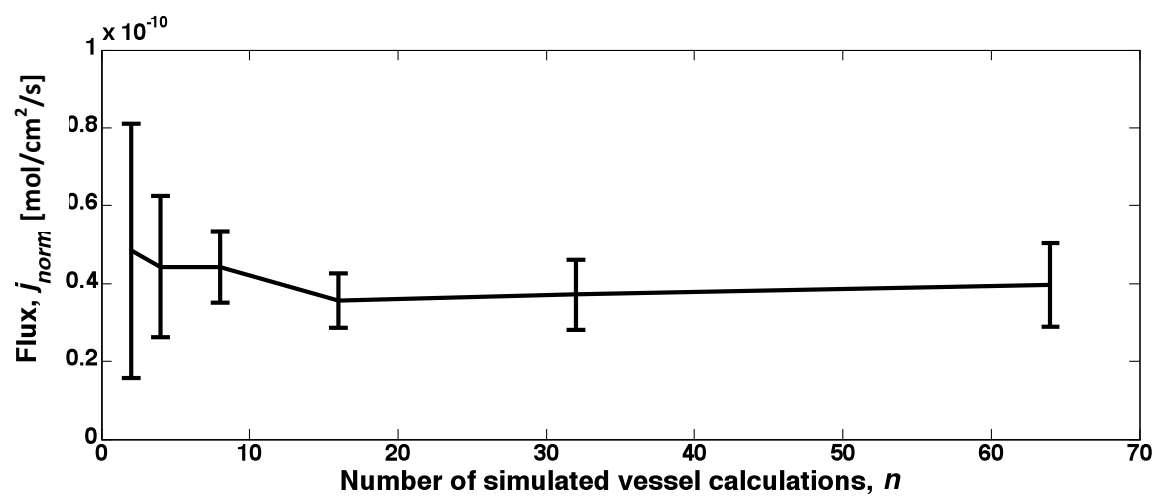
(a)



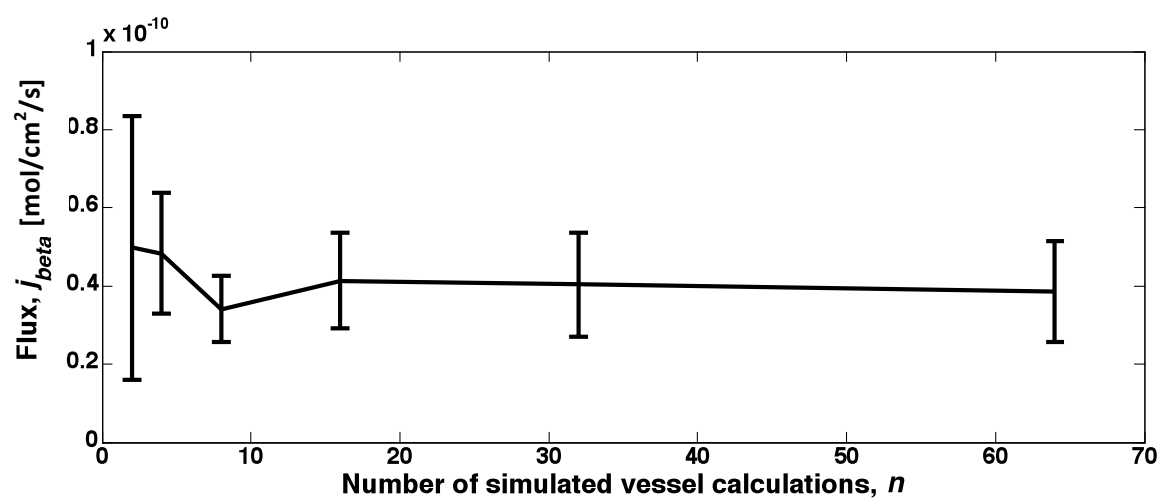
(b)

Figure 34. Flux calculations for the corresponding “depth” distributions shown in Figure 32. Calculations based on the (a) normal PDF and (b) beta PDF are presented as a function of distribution standard deviation, σ . Error bars represent plus/minus one standard deviation of the calculations.

The number of oxygen supplying vessels within the immediate vicinity of the electrode is also of importance. The CD31 staining discussed in Section 3.3.2.2.1 marks the vast majority of endothelial lined structures in the tissues, however the number of actively blood-perfused vessels versus unperfused (shunted, damaged) or non-blood carrying vessels (lymph, etc.) can vary [ref]. It has been found that there is on average 11.5 ± 4.3 vessels in a $250 \times 500 \mu\text{m}$ region of tissue, a region of tissue deemed critical for the generation of an electrode signal. The number of vessels used for the calculations was varied by factors of 2 from 2 to 64, encompassing a wide range of possibilities in order to investigate the relationship between number of vessels and the resultant calculated flux. The simulated flux as a function of number of simulated vessel calculations, n , is presented in **Figure 35**. *Apart from an expected noticeable effect on the variability in calculations with small numbers of vessel calculations, n , the effect of vessel number on resultant flux is negligible for the 1-D analytical model.*



(a)

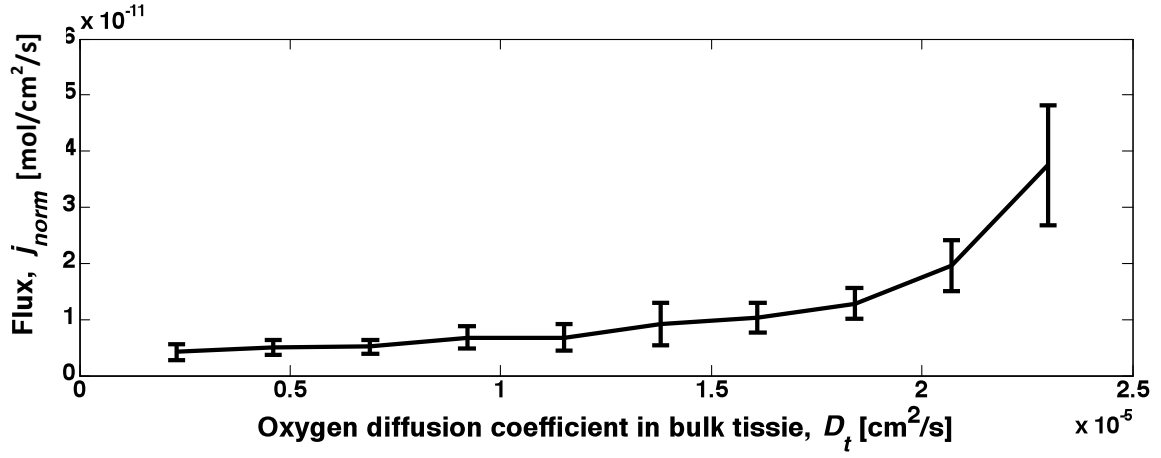


(b)

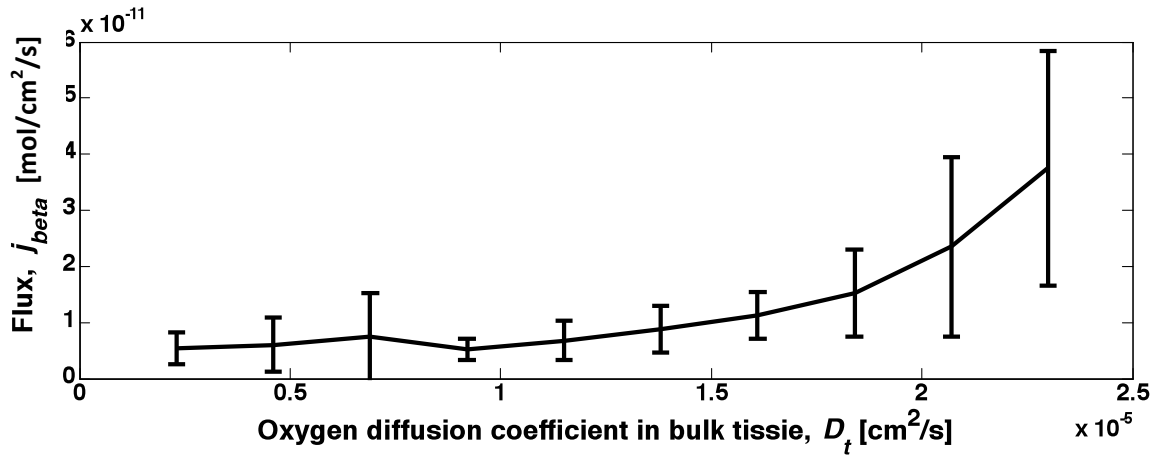
Figure 35. Simulated oxygen flux as a function of the number of simulated vessel calculations, n . Flux calculations based on the (a) normal PDF ($\mu = 250 \mu\text{m}$ and $\sigma = 75 \mu\text{m}$) and (b) beta PDF (α and $\beta = 5$) distributions. Error bars represent plus/minus one standard deviation of the calculations.

4.3.2 Oxygen diffusion coefficient in the bulk tissue, D_t

The oxygen diffusion coefficient in the bulk tissue, D_t , was varied for one normal and one beta distribution, both with $\mu = 250 \text{ } \mu\text{m}$, and similar σ values. A maximum D_t value of $2.3 \cdot 10^{-5} \text{ cm}^2/\text{s}$ equal to the diffusion coefficient of oxygen in water at physiological temperature and pressure was chosen as a starting point, and successively reduced to $1/10^{\text{th}}$ the original value. Oxygen flux was calculated for each value using the following constants: $D_m = 3.2 \cdot 10^{-5} \text{ cm}^2/\text{s}$, $c_t = 4.3 \cdot 10^{-8} \text{ mol}/\text{cm}^3$, $\delta_t = 250 \text{ } \mu\text{m}$ (according to μ and σ), $\delta_m = 35 \text{ } \mu\text{m}$, no reaction term present ($R = 0$). These flux calculations are presented in **Figure 36**, where a strong dependence of D_t on the resultant flux is present. *While it is highly unlikely that a 10 fold decrease in D_t occurs in the tissues, even a 10% decrease results in an approximately 50-75% drop in calculated flux, for both distribution types, which is within the range of physiologically possible effects [78].*



(a)



(b)

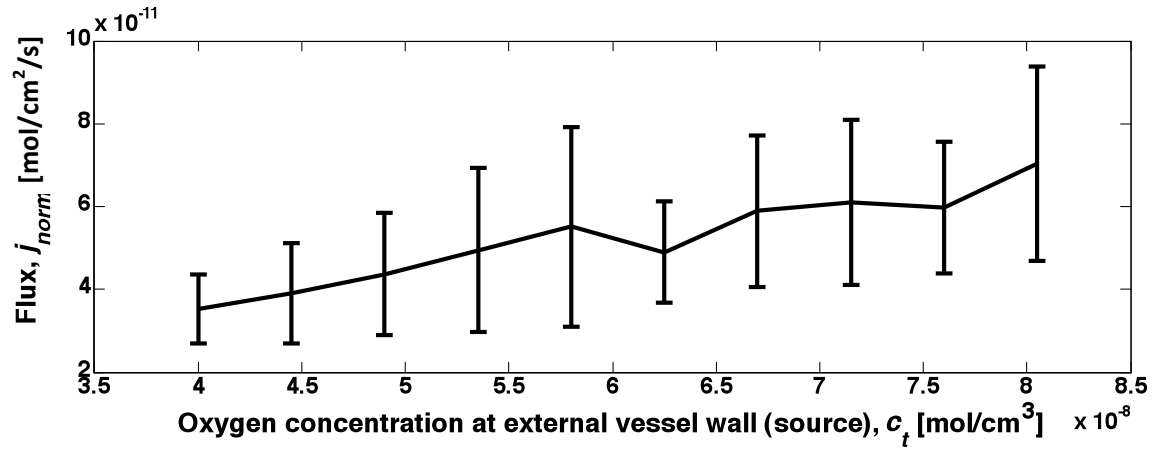
Figure 36. Simulated oxygen flux as a function of the bulk tissue oxygen diffusion coefficient, D_t . Flux calculations based on the (a) normal PDF ($\mu = 250 \mu\text{m}$ and $\sigma = 75 \mu\text{m}$) and (b) beta PDF (α and $\beta = 5$) distributions. Error bars represent plus/minus one standard deviation of the calculations.

4.3.3 Oxygen concentration in tissues at vessel/tissue interface, c_t

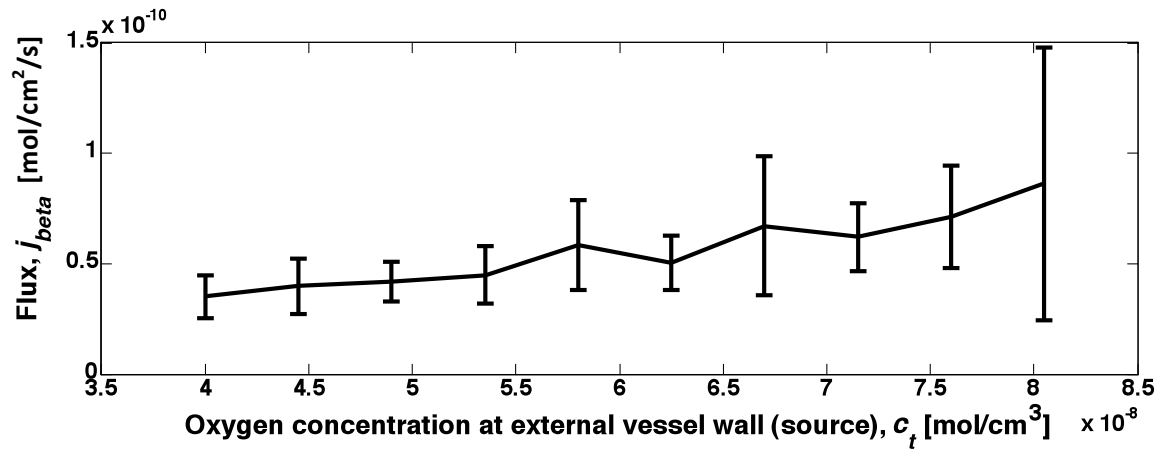
The range of oxygen source concentrations was defined consistent with the findings of Tsai et al. shown in **Figure 4** [29]. Capillaries, defined as vessels with diameters less than 6 μm , were found to have an average oxygen concentration, c_t , at their external wall surface of approximately $4 \cdot 10^{-8} \text{ mol/cm}^3$. Large A1 arterioles, with a diameter greater than 70 μm , were found to have an average oxygen concentration, c_t , at their external wall surface as high as $8 \cdot 10^{-8} \text{ mol/cm}^3$. It should be noted these results were produced from the hamster skin-fold chamber model, and values in the pig model might be significantly different. However, the relationship between the size of the vessel in question and the oxygen tension on its external wall surface is assumed to remain for the pig subcutaneous model.

The oxygen concentration at the external vessel wall (source) in the bulk tissue, c_t , was varied for one normal and one beta distribution, both with $\mu = 250 \text{ }\mu\text{m}$, and similar σ values. A minimum c_t value of $4 \cdot 10^{-8} \text{ mol/cm}^3$ and successively increased to $8 \cdot 10^{-8} \text{ mol/cm}^3$. Oxygen flux was calculated for each value using the following constants: $D_m = 3.2 \cdot 10^{-5} \text{ cm}^2/\text{s}$, $D_t = 2.3 \cdot 10^{-5} \text{ cm}^2/\text{s}$, $\delta_t = 250 \text{ }\mu\text{m}$ (according to μ and σ), $\delta_m = 35 \text{ }\mu\text{m}$, no reaction term present ($R = 0$). These flux calculations are presented in **Figure 37**. *The dependence of the resulting flux calculation appears to be linear with respect to the changing concentration, c_t , as is readily apparent by observing Equation 7. This change is in fact lost within the error bounds (error bars represent plus/minus one standard deviation). Thus the effect of changing the source oxygen concentration is not nearly as*

critical for the resultant flux as is the diffusion distance, d_t , or bulk tissue diffusion coefficient, D_t .



(a)



(b)

Figure 37. Simulated O₂ flux as a function of oxygen concentration at the external vessel wall, c_t . Flux calculations based on the (a) normal PDF ($\mu = 250 \mu\text{m}$ and $\sigma = 75 \mu\text{m}$) and (b) beta PDF (α and $\beta = 5$) are presented as a function of distribution standard deviation, σ . Error bars represent plus/minus standard deviation of the calculations.

4.4 2-D FEM simulations of the tissue/implant interface

Due to the complexity of the source distributions found in the histological analysis presented earlier, a validation of the 1-D approach is necessary. The 1-D analytical model simplifies the source distribution by averaging all the individual flux contributions into one final flux value. In order to ascertain whether this is an appropriate simplification, a more rigorous 2-D approach is employed that allows better simulation of the 2-D histological data found in Chapter 3 and accounts for the complex spatial distributions of vessels. For this the finite element method (FEM) is utilized.

4.4.1 Proposed 2-D tissue/sensor interface model

Mathematical investigation of the mass transfer in the tissue/implant interface is performed using FEM. The system is governed by the following partial differential equation:

$$\frac{\partial c}{\partial t} + \nabla \cdot (-D \nabla c) = R \quad (\text{Eq. 18})$$

where, and R is the reaction term. Because we are interested in the concentration distributions and resultant fluxes at specific time-points, and the time-scale is very long compared to the rate of physical modification in the tissues, this equation can be

simplified to its steady-state form where $\partial c / \partial t = 0$. For a 1-D problem with either no reaction term, or a linear one, this solution does not present much of a problem and can be readily solved analytically. Due to the complex geometries imparted by the random placement of vessels according to a specified distribution, however, the solutions become quite complex. The following 2-D model is proposed: a bulk tissue with a uniform diffusion coefficient for oxygen, D_t , and dispersed vessels throughout with a boundary oxygen supply, c_t , adjacent to a membrane coating with uniform diffusion coefficient for oxygen, D_m , on an underlying electrode with a constant oxygen drain, $c_e = 0$. The geometry of the various boundaries and surfaces is varied accordingly. A schematic is presented in **Figure 38**.

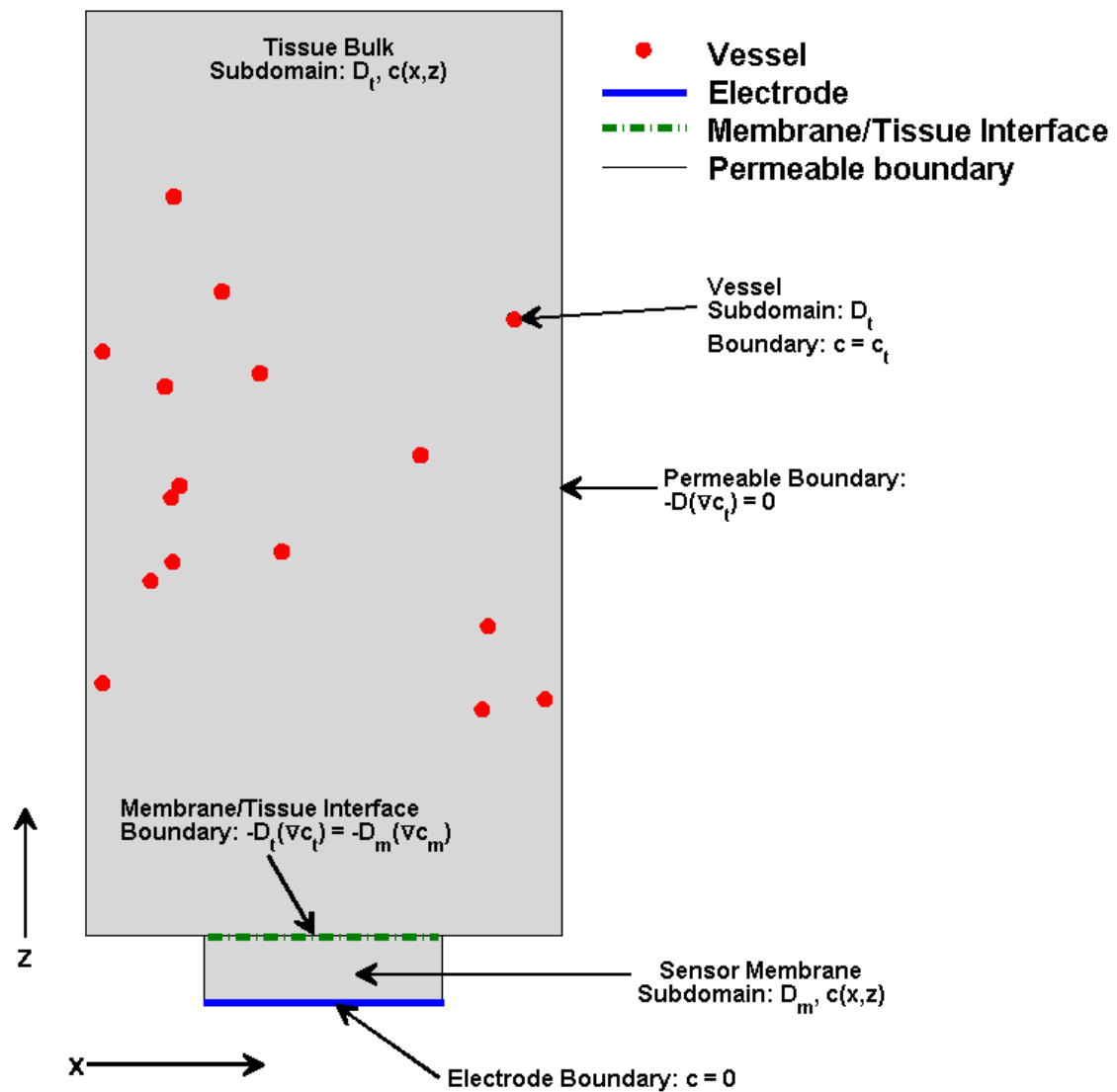


Figure 38. A proposed 2-D model of the tissue/sensor interface. Included are labels of pertinent subdomains and boundaries and the accompanying initial and boundary conditions used for analysis.

4.4.2 Model criteria affecting the finite element analysis

A detailed description of the finite element method can be found elsewhere as it is a technique commonly employed to solve complex non-linear problems and problems involving variable system geometries [81]. In brief, the three steps involve defining the problem (either graphically or in code) and assigning appropriate initial and boundary conditions, breaking down a complex structure into smaller simple geometrical segments (mesh elements), and solving the problem using iterative techniques. Care must be taken at each step to ensure a stable, converging solution. Particularly, a dense enough mesh must be drawn of appropriately selected mesh elements to ensure minimal singularities and boundary discrepancies, proper solver tolerances must be obeyed to balance computation time and solution accuracy, and any solution must be examined to ensure physically accurate results [82]. All FEM computations were performed using a combination of COMSOL Multiphysics 3.2b and Matlab 2007b [57, 82].

The following assumptions have been made for the proposed model:

- Temperature and pressure were invariant.
- No convection is present in either the tissue bulk or the vessels. This is appropriate due to the time-scales in question; any convection effects occur on the seconds-days time-scale.

- Tissue bulk is homogenous (bulk tissue subdomain and boundary settings are kept homogenous throughout each individual simulation, but may be varied between different simulations, e.g. D_t' c_t)
- Electrode surface is diffusion limited and consumes all oxygen that comes in contact.
- Partition coefficients are incorporated into “effective” diffusion coefficients.
- Uniform concentration gradient fields are assumed and allow for the use of less complex, computationally efficient mesh elements (triangular Lagrangian-quadratic elements are used).
- Vessel surface concentrations, c_t , are kept proportional to their diameter, per the findings of Tsai et al [29].

4.4.3 Vessel distribution and number effects

As in the case of the 1-D analytical solution, the vessel position was patterned using PDF functions. For the 2-D FEM models, only the beta distributions were utilized, seeing as the differences in the final calculations between the normal and beta distributions were minimal. Vessels were distributed with equal probability along the x-axis of the model, and with probability consistent with the beta PDF along the z-axis. Again, α and β parameters were varied to control the mean, μ , and the standard deviation, σ . In **Figure 39**, 8 models created using the beta PDF with varying α and β are shown and where σ is kept relatively constant and μ is altered from sensor proximal to

sensor distal. Meshing was performed to illustrate the adaptive nature of the mesh to feature size.

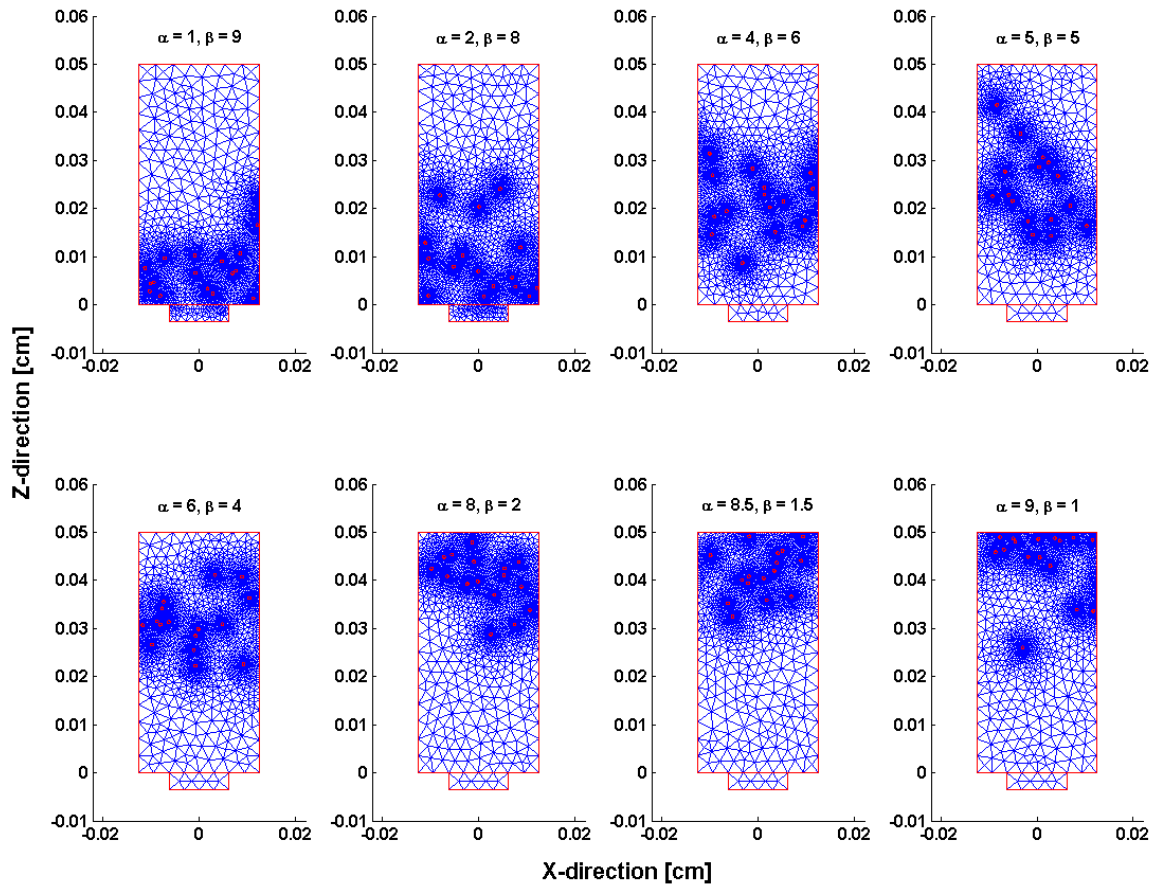


Figure 39. 2-D meshed models of the sensor/tissue interface spanning 8 different beta PDF-based vessel distributions with varying distribution mean, μ .

These distributions range from sensor proximal (top row, left) to sensor distal (bottom row, right).

A solution to one such model is presented in **Figure 40**. Specifically, α and β values of 5 were used and 16 distinct vessels (n) are dispersed within that distribution (see **Figure 40a** and **b**). The following subdomain and boundary conditions were used to obtain the solutions in this section: $D_t = 2.3 \cdot 10^{-5} \text{ cm}^2/\text{s}$, $D_m = 3.2 \cdot 10^{-5} \text{ cm}^2/\text{s}$, $c_t = 4 \cdot 10^{-8} \text{ mol/cm}^3$, no reaction term present ($R = 0$). Upon inspection of the concentration profile (**Figure 40c**) we see that the tissue bulk above the first group of vessels nearest the sensor is homogenously supplied with oxygen. In contrast, between that first group of vessels and the tissue/sensor membrane interface there is a drop-off of concentration as a strong gradient is established between the vasculature and the electrode surface. Looking at the arrow plot of diffusive flux (**Figure 40d**), we see that the flux values confirm the existence of this gradient, and increase strongly close to the electrode surface. Integrating the flux along the entire electrode surface, and normalizing for electrode size, we can calculate a normal diffusive flux, j (units of $\text{mol}/(\text{cm}^2 \cdot \text{s})$). This value represents the total flux at the electrode normalized by the electrode area.

*Plotting normal diffusive flux at the electrode surface as a function of the distribution peak mean relative to the sensor surface (see **Figure 41**), we see the same trend that was observed in the 1-D analytical simulations presented in **Figure 32**. Not only is the trend conserved, but the flux values are well within an order of magnitude, albeit slightly higher. This difference is a result of the previously mentioned undue prominence that distant vessels are given relative to those vessels closer to the sensor*

surface in the 1-D approach. *It is believed that the 2-D FEM model results in more accurate calculations based on the distributed vessels.*

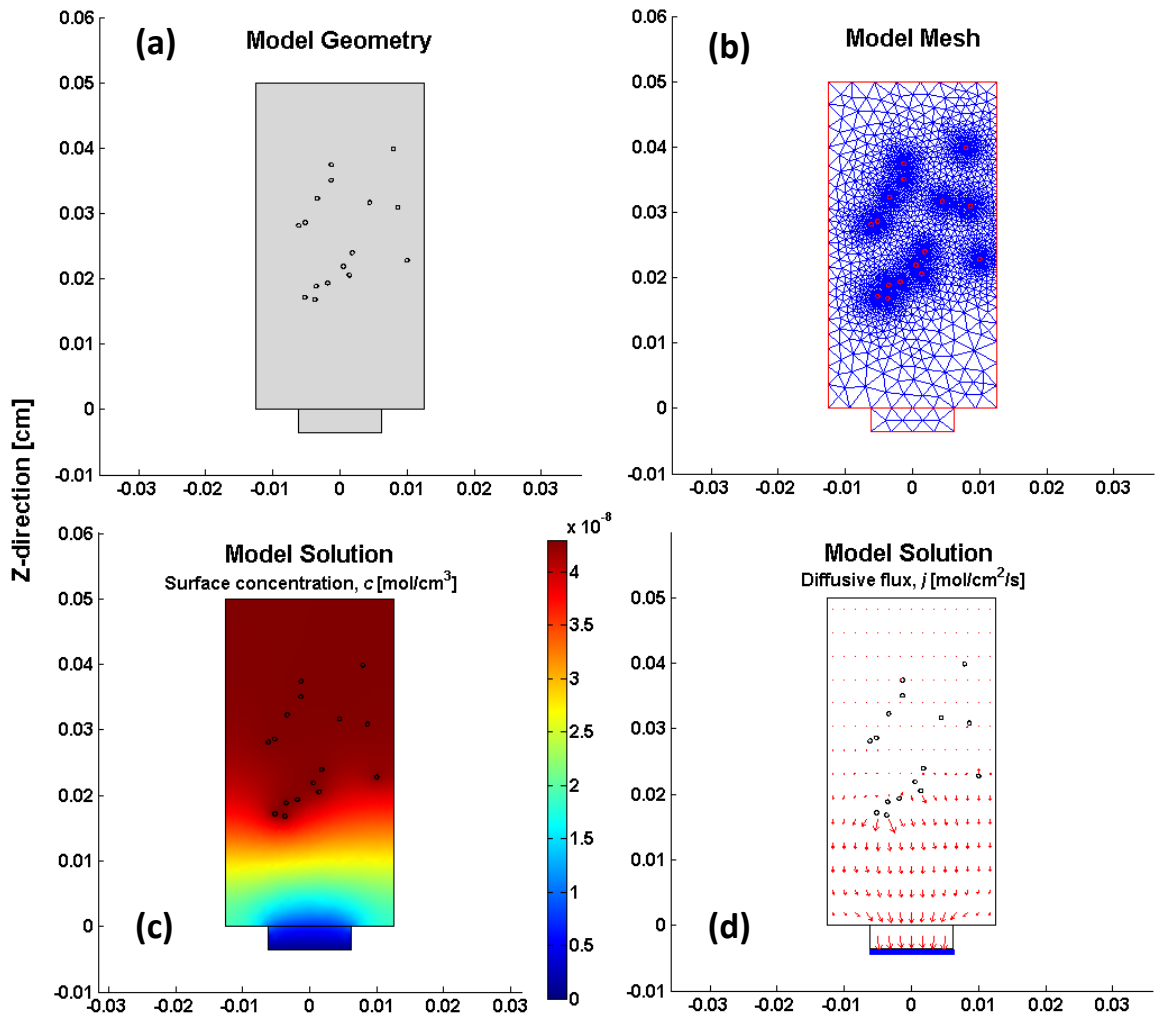


Figure 40. FEM solution to a sample 2-D tissue/sensor interface model.
 (a) Model geometry, (b) model mesh using triangular elements, (c) model solution for surface concentration, and (d) model solution for diffusive flux.

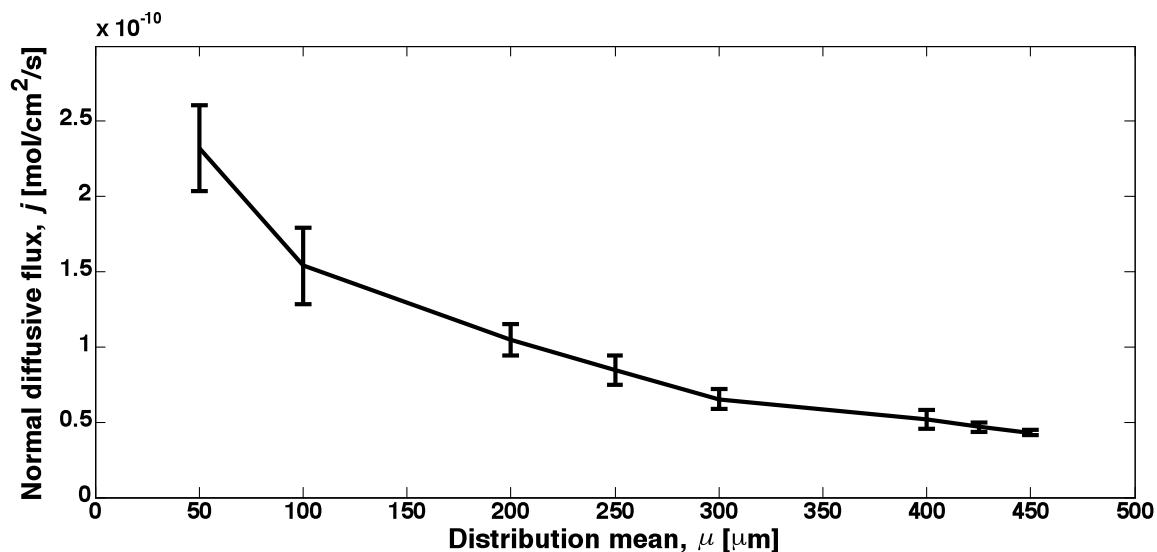


Figure 41. Normal diffusive flux, j , as a function of distribution mean, μ , from sensor surface. Error bars represent plus/minus one standard deviation of 5 repeated calculations.

The effects of distribution spread are investigated by modeling distributions with constant mean, μ , but varying the standard deviation, σ . Eight meshed models based on such distributions are presented in **Figure 42** where $\mu = 250$ μm, but σ ranges from 25 to 133 μm. *In the 1-D analytical model, the effect of distribution spread on flux was minimal, only the range of calculated fluxes was affected. For the 2-D FEM models, there is a clear impact of a wider spread distribution on the calculated flux at the electrode surface, as is demonstrated in **Figure 43**. Again, this is attributed to the heavier prominence that proximal vessels have over distal ones in the 2-D approach.*

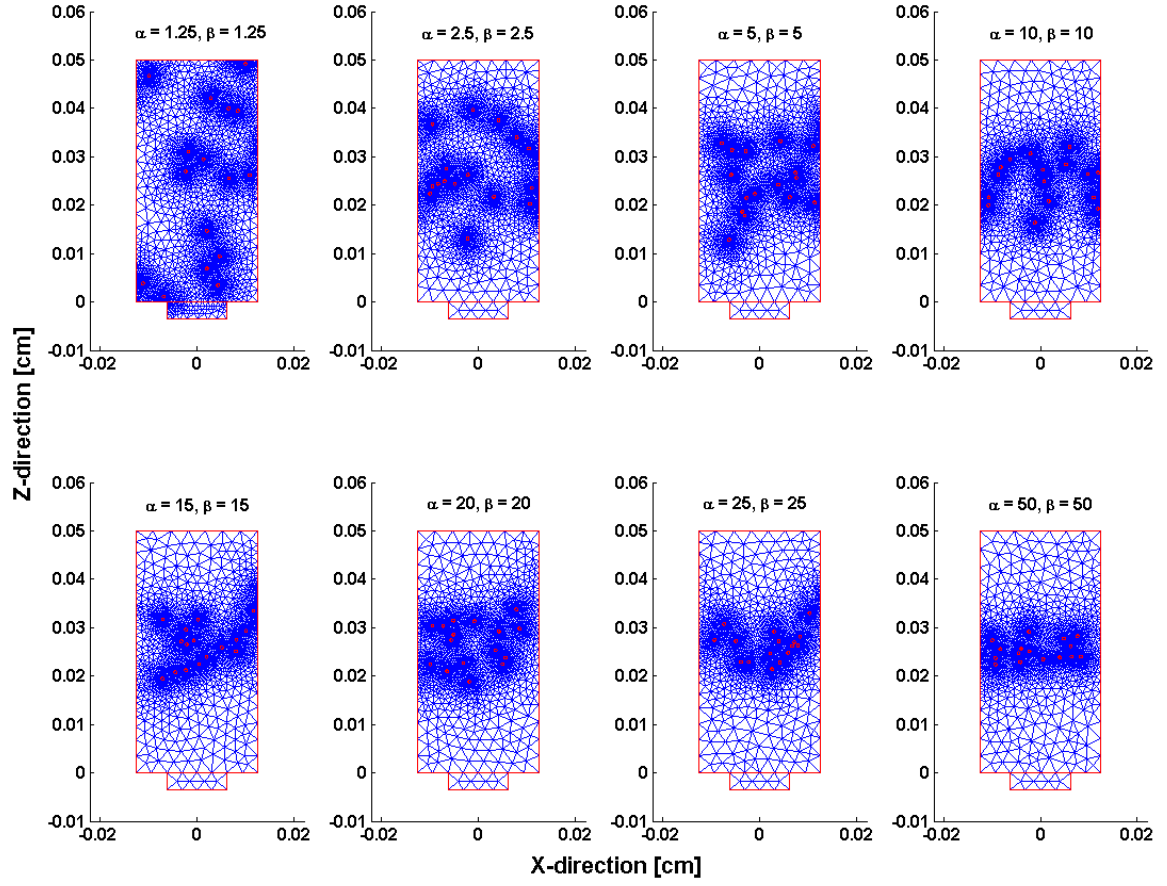


Figure 42. 2-D meshed models of the sensor/tissue interface spanning 8 different beta PDF-based vessel distributions with varying distribution spread, σ . These distributions range from wide spread (top row, left) to narrow spread (bottom row, right).

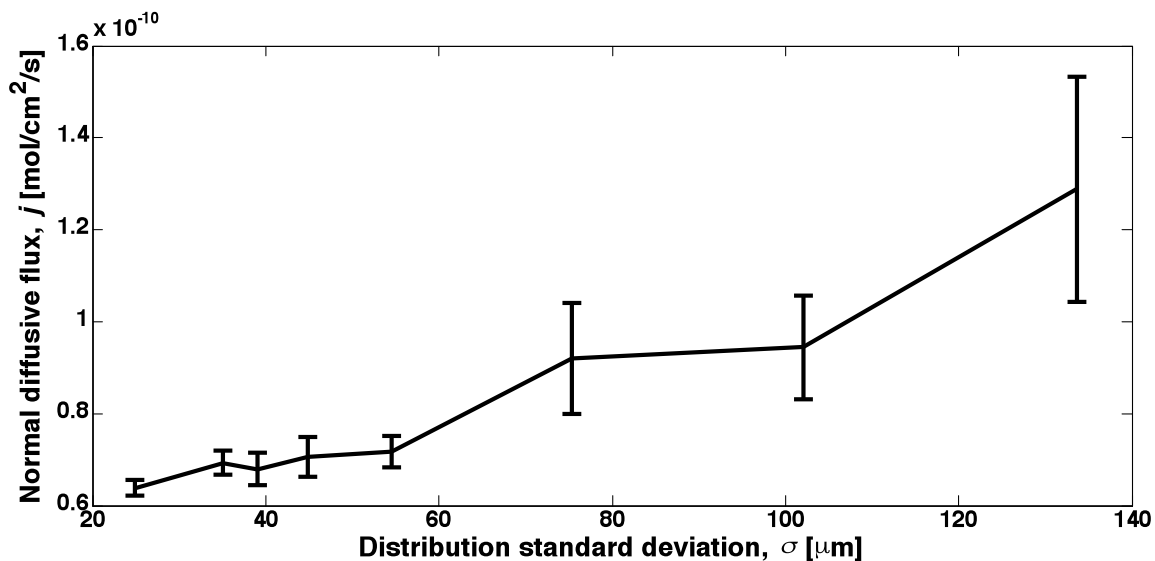


Figure 43. Normal diffusive flux, j , as a function of distribution standard deviation, σ , for constant mean, μ .

Error bars represent plus/minus one standard deviation of 5 repeated calculations.

The effects of varying the number of vessels within a distribution are investigated by modeling distributions with constant mean, μ , and standard deviation, σ , but varying the number of vessels, n . 7 meshed models based on such distributions are presented in **Figure 44** where $\mu = 250 \mu\text{m}$, $\sigma = 55 \mu\text{m}$, and n ranges from 2 to 128. In the 1-D analytical model, the effect of vessel number on flux was minimal, only the range of calculated fluxes was affected. *For the 2-D FEM models, there is a clear impact of having more vessels within the distribution up to a certain point on the calculated flux at the electrode surface. As the number of vessels surpasses 30, the flux stops changing as rapidly, as is demonstrated in Figure 45.* As with the effects of spread, this is

attributed to the heavier prominence that proximal vessels have over distal ones in the 2-D approach.

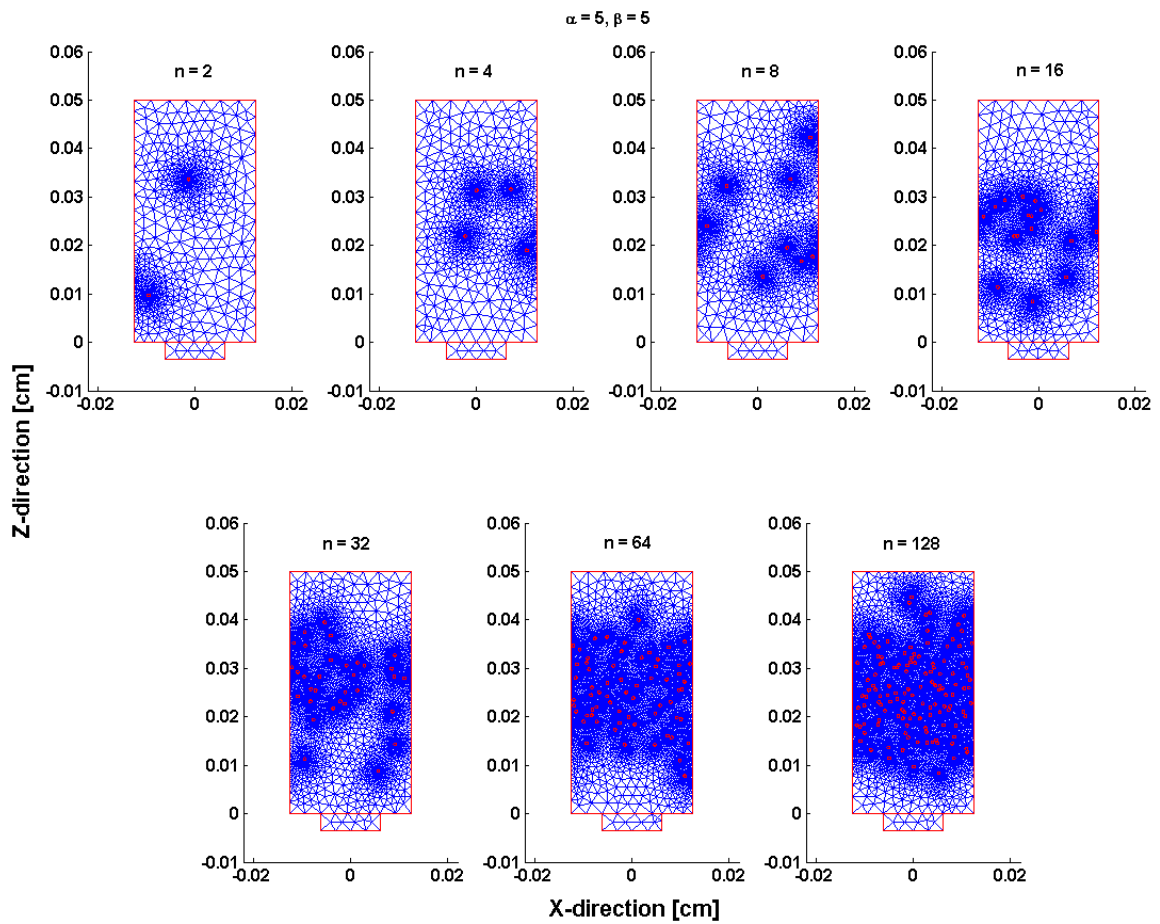


Figure 44. 2-D meshed models of the sensor/tissue interface spanning 7 different beta PDF-based vessel distributions with varying vessel numbers, n . These distributions range from wide spread (top row, left) to narrow spread (bottom row, right).

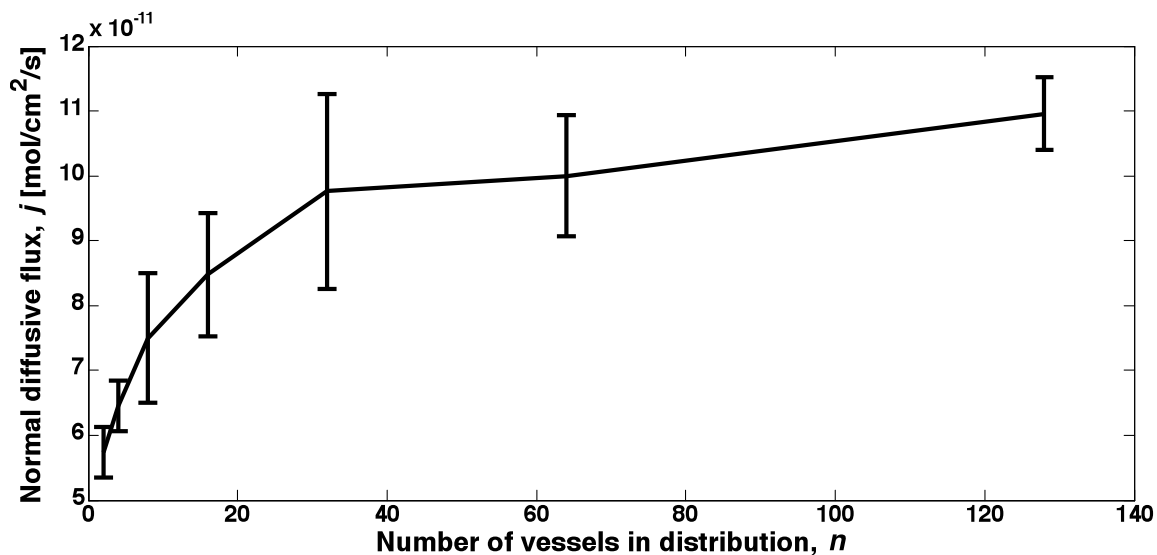


Figure 45. Normal diffusive flux, j , as a function of number of vessels in distribution, n . σ and μ are held constant. Error bars represent plus/minus one standard deviation of 5 repeated calculations.

The effect of vessel distributions was found to be influential for the 1-D analytically simulated flux calculated using Equation 7, where small disturbances in the distance away from the sensor surface resulted in very noticeable changes in flux. The use of a 1-D approximation in the previous section gives us some idea of the range of values that could result, but unfortunately, taking an average of n random vessel calculations gives each vessel inaccurately equal prominence in the resulting value of flux. This is problematic due to the concentration gradients that are expected in such a vessel distribution field. Even if two vessels are located in line normal to the electrode surface, the concentration gradient between the proximal vessel and the electrode surface is very large. The gradient established between the proximal and distal vessels

however is small (or nonexistent) producing little to no oxygen flux. Therefore the assumption in the 1-D component of this work that each vessel has an equal contribution on the resulting flux is insufficient. As a result, a more rigorous 2-D approach as described in this section is required.

4.4.4 Effects of tissue oxygen consumption rates on flux to the electrode

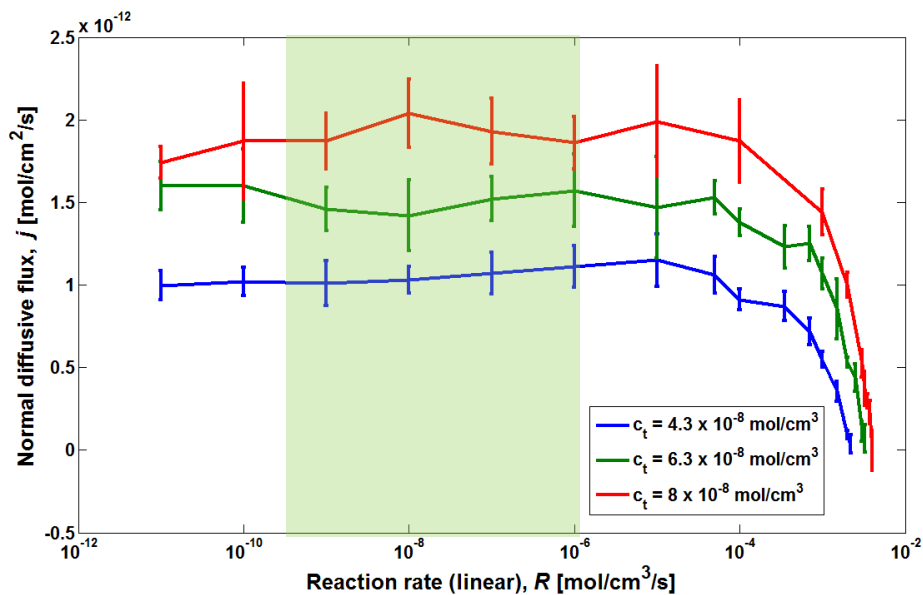
The tissue consumption of oxygen can potentially have a significant effect on the reported flux values of the electrodes. Tissue reaction rates have been identified in the literature for rat mesentery and vessel wall proximal [80], rat loose connective and mesentery [28] tissues. These tissues are believed to represent a good range of activity, with tissues near vessel walls expected to consume more than mesentery and loose connective tissue. Converting the values found in the literature to consistent units, a range of $4 \cdot 10^{-10} - 1.25 \cdot 10^{-6} \text{ mol/cm}^3/\text{s}$ was identified for analysis. Along with the following additional subdomain and boundary conditions: $D_t = 2.3 \cdot 10^{-5} \text{ cm}^2/\text{s}$, $D_m = 3.2 \cdot 10^{-5} \text{ cm}^2/\text{s}$, $c_t = 4 \cdot 10^{-8} \text{ mol/cm}^3$, this oxygen consumption “reaction” rate, R , was instituted in the models as the following linear bulk tissue subdomain condition:

$$R = \begin{cases} R_t & \text{if } c(x, z) > 0 \\ 0 & \text{if } c(x, z) = 0 \end{cases} \quad (\text{Eq. 19})$$

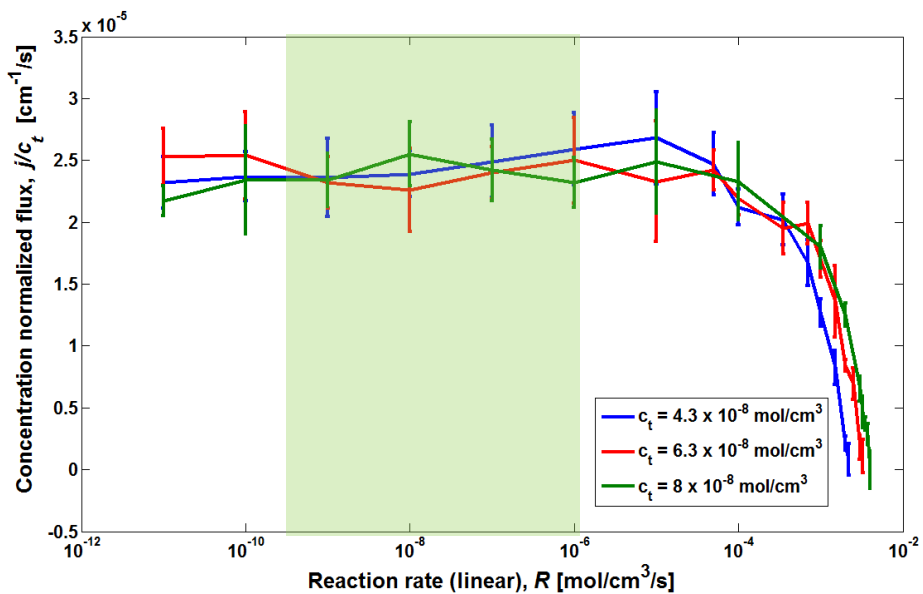
A constant distribution of 16 vessels was maintained with α and β equal to 5 for a μ of $250 \text{ } \mu\text{m}$ and a σ of $38 \text{ } \mu\text{m}$. Three various oxygen concentrations at the external vessel

wall, c_t , and three various bulk tissue diffusion coefficients, D_t , are simulated to illustrate the effect of changing oxygen supply and permeability.

The results from these simulations are presented in **Figure 46** and **Figure 47**. *The flux values remain stable regardless of a reaction term from 10^{-11} to 10^{-4} mol/cm³/s. This includes the region of interest identified from the literature, marked as the shaded region in the figure. Only at values 3 orders of magnitude greater than those found in the literature for similar tissue types do the calculated fluxes begin to rapidly approach zero.* The effect of changing the vessel wall concentration has a predictable upwards shift in flux (see **Figure 46a**), however it also produces a slight right shift in the flux drop-off as the reaction rate is increased which is easily observed when dividing normal diffusive flux by the concentration (see **Figure 46b**). A similar, yet more pronounced shift is seen when different diffusion coefficients are simulated (see **Figure 47a** and **b**).

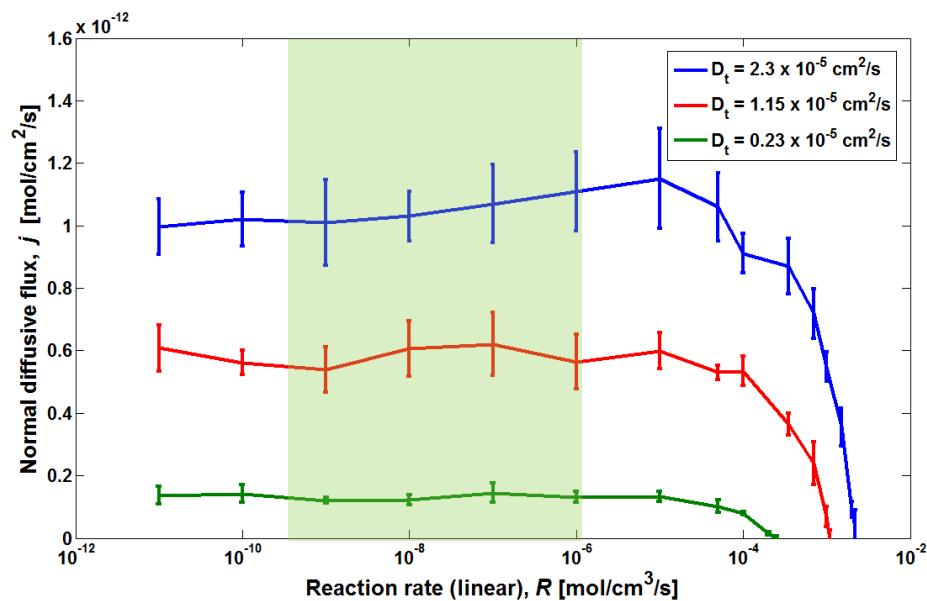


(a)

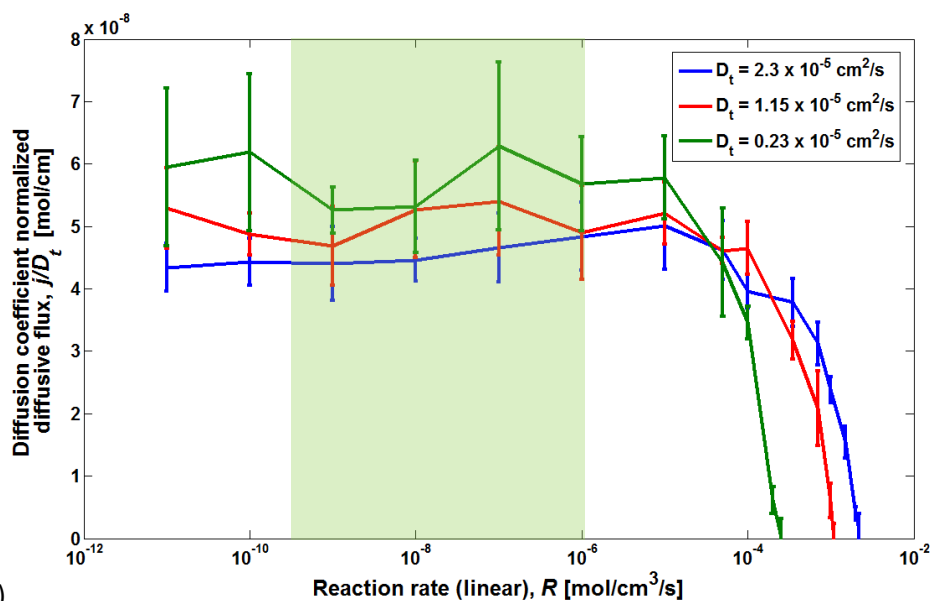


(b)

Figure 46. Normal diffusive flux, j , as a function of a linear reaction rate, R , in the bulk tissue and the oxygen concentration at the external vessel wall, c_t . σ and μ are held constant and three various oxygen concentrations at the external vessel wall, c_t , are simulated. (a) Normal diffusive flux and (b) concentration normalized diffusive flux are shown where the green shaded range of reaction rates represents the literature derived range of interest mentioned in the text. Error bars represent plus/minus one standard deviation of 5 repeated calculations.



(a)



(a)

(b)

Figure 47. Normal diffusive flux, j , as a function of a linear reaction rate, R , in the bulk tissue and diffusion coefficient of oxygen in the bulk tissue, D_t .

σ and μ are held constant and three various bulk tissue diffusion coefficients, D_t , are simulated. (a) Normal diffusive flux and (b) diffusion coefficient normalized diffusive flux are shown where the green shaded range of reaction rates represents the literature derived range of interest mentioned in the text. Error bars represent plus/minus one standard deviation of 5 repeated calculations.

CHAPTER 5 –

Discussion

5.1 Signals content

Findings from the rigorous analysis of the collected oxygen signals demonstrate two important levels of dynamic information contained within. The first is the long-term decay trend with a time-scale ranging from 1 to 2 months. It is hypothesized that this type of signal behavior is due to the response of the surrounding tissues via formation of a foreign body capsule to the placement of the sensor array. The second level of information contained within these signals is a 1-2 week repeatable oscillation with readily identified characteristics that is hypothesized to be due to wound healing resultant from minor injury caused by animal repositioning of the implant, aggravation to the wound site (scratching), and general impact from daily animal activity and manhandling. The following presents a thorough discussion of these signals features, and the corroborating evidence from both quantitative histology and computational simulations of the mass transfer behavior in the tissue/sensor interface.

5.2 Exponential decay in oxygen signals

Observing all the oxygen signals obtained for this work, whether they are individual electrodes, averaged arrays, or an average of all signals collected, there is a

clearly visible decay process that takes place over the course of the implantation.

During those three months the signals drop by approximately 80% of their original levels (see Figure 5b) and reach equilibrium near the 2-month mark. This decay process is hypothesized to occur due to several possible reasons, all resulting from the wound healing and foreign body response triggered by the implantation.

The current (i_{O_2}) measured by the electrodes is related to the oxygen flux as was shown earlier by Equations 6 and 7. The formation of the foreign body capsule around the implanted sensor array results in the deposition of a dense, fibrous, avascular tissue in front of the sensor. This is expected to decrease the flow of oxygen (and other metabolites) to the electrode surfaces caused by (1) decreased diffusivity in tissues (D_t) due to the formation of dense fibrous tissue, (2) increased diffusion distance (δ_t) from the nearby vascular supply by either shunting blood flow away or remodeling the tissues adjacent to the sensor, (3) increasing the presence of highly metabolically active cells (macrophages, neutrophils, fibroblasts, etc.) that consume metabolites (R) before they reach the electrodes, (4) buildup of a fluid exudate between the sensor and tissue thus further increasing diffusion distance (δ_t).

5.2.1 Tissue histology and computer simulation as corroboration

Any change in the composition of the tissues requires some form of cellular activity, whether it is fibroblast deposition of new fibrous material, white blood cell activation to a foreign body, granulocyte breakdown of damaged tissues, macrophage

engulfment of particles, etc. H&E staining was used to investigate this, as it is adept at illustrating nuclear, cytoplasmic and extracellular components. Nuclear density was shown to increase slightly in titanium adjacent tissue, but no change was found for tissues adjacent to PDMS (see **Figure 25a,b**). This is not a surprising result as both materials are deemed biocompatible and have been utilized extensively in the past. With respect to depth, there is a slight decrease in cellular density in the tissue adjacent to PDMS and no change in tissue adjacent to titanium (see **Figure 25c,d**). Again, the changes, if any, are very slight and consistent with the biocompatibility of both materials. Even though no significant changes in the overall nuclear density were found, this does not imply that the ratios of individual cell types did not change. Therefore the changes seen in the signals are due to factors other than the change in the number of cells within adjacent tissues.

The transport of solutes and metabolites to and from the tissues is dependent on the permeability of the tissues themselves. It has been previously shown that mass transfer through various collagenous substances, both natural and manmade, is directly dependent on the collagen content [77-79]. Values for the diffusion of oxygen through tissues have been for the most part assumed to be equal to that of oxygen through water at physiological temperature and pressure ($2.3 \cdot 10^{-5} \text{ cm}^2/\text{s}$). This is believed reasonable due to the prominently cellular and loose extracellular composition where a small, non-polar molecule like O_2 would very easily pass through interstitial fluids, cell membranes, cytosol, etc. However, in tissues and artificial structures with very high collagen content, the diffusion rate drops by as much as 50% (for example, D_{O_2} in aortic

valve cusps is reported as $1.06 \cdot 10^{-5} \text{ cm}^2/\text{s}$ [78]). A dense, solid structure like a collagenous network would impede that flow. Ergo, a thorough understanding of the rate and distribution of collagen deposition near the sensor surface is vital. The results from the image analysis of GTC stained slides presented in **Figure 26** indicate that collagen levels increase with implant time from approximately 10% at 1 week post implantation to >50% adjacent to titanium and >90% adjacent to PDMS after 13 weeks. Additionally, in the case of both materials, collagen increases as a function of depth. Particularly in the PDMS adjacent tissues, the collagenous layer encompassed the entire 500 μm image analysis region. Both findings lead to the conclusion that the permeability of the tissues decreases substantially as encapsulation progresses.

Computer simulations of the tissue/sensor interface attest to this. The effect of the diffusion coefficient of oxygen in the tissues, D_t , on resultant flux calculations is quite pronounced. Even a slight decrease in the diffusivity of oxygen in tissues can result in significant downward changes in flux. The histology results demonstrate strong increases in collagen deposits within the tissues at the sensor interface. While this will not cause a 10-fold drop in diffusion coefficient as was simulated earlier, a 10% decrease of the coefficient is not unreasonable. Such a drop could result in upwards of a 50% decrease in flux (see **Figure 36**).

As metabolites pass from the vessels to the surface of the implant, consumption occurs by metabolically active cells in between. As the deposition of collagen, the formation of new vasculature and infiltration of phagocytic and inflammatory cells to

the area occurs, the metabolic cost increases and a need for nutrients rises. Therefore, a portion of the metabolic supply provided by the vasculature is consumed before ever reaching the implant. The change in vessel distribution and the increase in collagen content are both present as demonstrated by GTC and CD31 antibody staining, however consumption by tissues is a much more difficult factor to investigate. We can infer about consumption rates of various cell types present, but a rate of consumption itself cannot be determined from histological slides. Given previous studies, we have identified the macrophage and the granulocyte as cell types of interest given their primary response to the wound site. In particular, macrophages are of interest due to their continued presence throughout the duration of the implant as they fuse forming multinucleated giant cells and attempt to engulf the entire foreign body.

Slides labeled with CD4 and 6D10 antibodies showed clear and readily identifiable cells present in relatively similar levels throughout the implant duration (see **Figure 20** and **Figure 21**). No significant changes occurred with respect to depth or time for the 6D10 stained slides indicating that the presence of granulocytes remains fairly constant during the course of implantation. Macrophages cluster near the surface of the tissue directly at the implant/tissue interface, consistent with the findings of others in the field; however, this only occurs in certain histology samples. Their distribution deeper in the tissues is consistent with those of 6D10 labeled granulocytes. It is believed that the layer of macrophages/giant cells directly at the sensor/tissue interface is very delicate and easily disturbed and lost in the sham removal, fixation and staining process. These cells occurred in such low numbers that a positive control was beneficial

to ensure the staining was accurate. **Figure 21b** illustrates CD4 antibody staining of tissue ingrown into the polyester velour pad on the surface of one of the explanted sensors. There is clearly positive staining for CD4+ cells that are morphologically consistent with macrophage/giant cell structure.

The computational simulations of tissue oxygen consumption (via a linear reaction rate term, R) presented in **Figure 46** and **Figure 47** indicated that levels of oxygen consumption commonly reported in the literature for loose connective, mesentery and vessel wall adjacent tissues in rats are well below those required to affect the resultant flux. In fact, reaction rates 3 orders of magnitude higher are required in order to impart any change in flux levels at the sensor surface. The points at which the flux values begin to drop off are influenced by the oxygen concentration adjacent to the vessel wall, c_t , and by the bulk tissue oxygen diffusion coefficient, D_t . However even these effects only occur at much greater reaction rates than are likely to occur in subcutaneous tissue.

How far a solute needs to travel from source to sink (electrode surface) is also critical. In subcutaneous tissues the oxygen supply is the microvasculature. This includes capillaries, arterioles of various sizes and larger feeding arteries in the vicinity, all of which can be found in the subcutaneous tissues (see **Figure 19** for an example of several various sized vessels in close proximity). CD31 antibody staining yielded some fascinating results. For tissues adjacent to both titanium and PDMS, there is a pronounced shift in endothelial cell distribution from near the implant surface to implant distal as implantation progresses. Looking at the depth ranges containing 50%

of CD31 staining on **Figure 27**, the shift in distribution is approximately 200 μm in depth. This is a considerable increase in distance that oxygen now must traverse before reaching the implant surface. Taking this in conjunction with the permeability decrease due to collagen deposition over the same time-frame, the decay in oxygen flux to the surface of a metabolically active implant can be assumed to be substantial. This could conceivably account for the decay in oxygen signals measured by long-term implanted electrodes as reported in Chapter 2 and in [4].

Additionally, there is an almost 200 μm difference in initial endothelial cell distribution between the two materials. For titanium adjacent tissues, endothelial cell presence begins almost instantly at the implant interface. However, in the PDMS adjacent tissues a 200 μm region of avascular tissue exists. It is not clear why this difference exists, but it is most likely due to the blunt dissection used to create the pocket for implant placement. The subcutaneous tissue of mammals has a very regular, stratified structure that would lend itself well to separation at repeatable locations along any fibrous boundaries. The surgeon in charge of the implantation has had much experience with placing these implants at repeatable depths in numerous animals, and it is believed that the same strata were exposed during the initial placement of the implants. Since the respective materials of an implant face opposing directions (PDMS faces down into the core of the animal, titanium faces the exterior), different strata are experienced by both materials. Regardless, a similar shift in distribution occurs in both cases, most likely as a result of fibrous tissue deposition in front of the implant.

The investigation of the diffusion path, δ_t , via both the position and spread of vessel distributions indicates that the oxygen flux (and therefore reported current) is highly sensitive to variations in the distance of the vessels away from the electrode surface. Interestingly, in the case of the 1-D simulations the spread of the distribution (see **Figure 31**) has little effect on the magnitude flux change (see **Figure 32**), however, the variability in calculated fluxes becomes considerable with greater spread. In the 2-D simulations this is not the case (see **Figure 43**). Additionally, the number of vessels present within a tissue area of influence near the electrode surface is also of importance. There is a discrepancy when observing the distribution spread results from the 1-D analytical and 2-D FEM simulations. The 1-D solution shows no change in calculated flux as the number of vessels within the distribution increases (see **Figure 35**), whereas the flux calculated from the 2-D FEM approach (shown in **Figure 45**) is very susceptible to low numbers of vessels and stabilizes from about 30 vessels on.

These differences between the 1-D analytical and 2-D numerical approaches are attributed to the manner in which the complex PDF based distributions are simulated in the 1-D approach, where each calculated vessel is given equal prominence on the final mean flux result. When the 2-D FEM model is solved, this is not shown to be the case. **Figure 40c** and **d** show the solution to one sample model. There is a strong concentration gradient established between the sensor and the proximal vessels, causing high oxygen flux; however the distal vessels are in a homogeneously oxygenated region of tissue and little to no resultant flux occurs. Flux calculations plotted versus the

spread of the vessel distributions used to generate the models are presented in **Figure 34** for the 1-D analytical model and in **Figure 43** for the 2-D FEM model. The 1-D solution shows no change in mean flux as the spread increases, whereas the 2-D solution shows considerable effect. This indicates the mean peak position, while important for flux calculation, is heavily modulated by its spread and where the closest vessels occur. Therefore, for geometry unrelated variables such as subdomain and global boundary conditions the 1-D model is sufficient to investigate effects. However, complex vessel distributions such as the ones presented in this work require more complex 2-D approaches such as the FEM.

5.2.1.1 Comparison with histology collected by Glysens, Inc.

Histological findings from tissues surrounding the live sensors from which oxygen signals reported in Chapter 2 were collected (see Appendix, Section 7.1.4) indicate that there is in fact a thickening of the tissue adjacent to the sensor/PDMS regions of the implants as compared to the titanium regions (see **Figure 50**). Observing the GTC stained slides, a considerable increase in collagen deposition is present as well. Comparing the tissue regions adjacent to the titanium case, and those adjacent to the sensor/PDMS, a much greater collagen concentration is visible (blue/green coloration in **Figure 50b** and c). This is consistent with the findings reported from sham adjacent tissues in Chapter 3. However, a considerable increase in cellular density near the proximity of the sensors indicates a highly active tissue zone, which most likely corresponds to a high rate of metabolic activity (dark blue coloration in **Figure 50b-e**).

This was not observed in the histology collected from the implanted shams presented in Chapter 3.

The live sensors differed from the shams in one especially important characteristic. Half of the live sensor electrode array surface was dedicated to oxygen measurement, and smooth PDMS is used to coat that half. The opposing half however (see **Figure 3**, working electrodes 11-16) is dedicated to the measurement of a glucose oxidase modulated oxygen current, where the immobilized enzyme consumes oxygen before it strikes the electrode surface. This immobilization is achieved in glutaraldehyde cross-linked albumin wells, which are believed to leach small, yet irritating, amounts of glutaraldehyde and enzyme into the surrounding tissues initiating a strong cellular response. Since it was not possible at the time of tissue collection to track the precise location from which histology was analyzed (the actual working electrodes underneath), it is possible that the histology presented in **Figure 50** came from glucose sensor adjacent tissue.

5.3 Signal periodicity

5.3.1 Analysis technique independence

The analysis of the oxygen signals yielded the same findings from three independent techniques (see Chapter 2, Section 2.3.3). There is a pronounced oscillatory nature in the signals with a period of 7-15 days, as shown in the autocorrelation and Fourier based approaches (**Table 1** and **Figure 10a**, respectively). In addition, most of the power in these signals occurs early on in the experiment, well

before 1000 hours of implantation as demonstrated by wavelet analysis (see **Figure 10b**, c). This is also where most of the similarity in the signals occurs. After 1000 hours, the signals become much more diverse with some signals showing powerful trends all the way through the end of the implantation, while others die out and equilibrate steadily for the remainder of the experiment. There is a clear indication, that even though the signals lose much of their initial strength within the first 2-4 weeks as shown in **Figure 6**, there are still measurable, resolvable oxygen values throughout the entire 3+ month implantation. This finding in and of itself is quite remarkable. Up till now it has been widely thought that the encapsulation process of tissues around implants is so efficient and pronounced, that there is an almost complete cut-off of nutrients (oxygen, glucose, etc.) to the implant site [2, 35, 36, 47]. However, the results from these long-term oxygen sensor implantation experiments clearly show that albeit decreased from original levels, after 3 months of implantation and encapsulation there is still a strong, resolvable oxygen signal that can be measured.

5.3.2 Physiological periodicity

Animal activity is not to be ignored. The sensors are placed within the dorsal skin off-center of the spine at a relatively shallow depth of 1-2 cm. The implantation locations are protected by wrapping the animals in gauze bandage, foam, and adhesive tape, however this would not prevent compression that would occur from the animal placing its weight on the skin tissue in question for an extended period of time (e.g. overnight), or shifting the array in the pocket from a strong impact. If the damage to

the sensor pocket is severe enough, re-aggravation of the wound site would most surely take place. This would restart the wound healing process, bringing on bleeding, inflammation, white blood cell recruitment, proliferation of fibroblasts, and renewed fibrosis and extra cellular matrix deposition/remodeling as discussed earlier (see Section 1.1.3).

The wound healing response has been shown to have implications on the oxygen levels at the wound site [45, 46]. Wound healing is therefore hypothesized to have a measurable impact on oxygen signals generated from sensors implanted in the subcutaneous tissues. These signals are characterized by 7-14 day oscillations in the signals; whereas previous work on wound healing has identified a 12-day time course for the process of wound onset, progression and resolution [35, 37, 48]. **Figure 48** illustrates a representative oxygen signal with its long-term decay trend removed. At approximately 700 hours, an oscillatory event begins with a pronounced drop in oxygen current from the baseline. This event is magnified in the bottom plot of **Figure 48** and key events are marked.

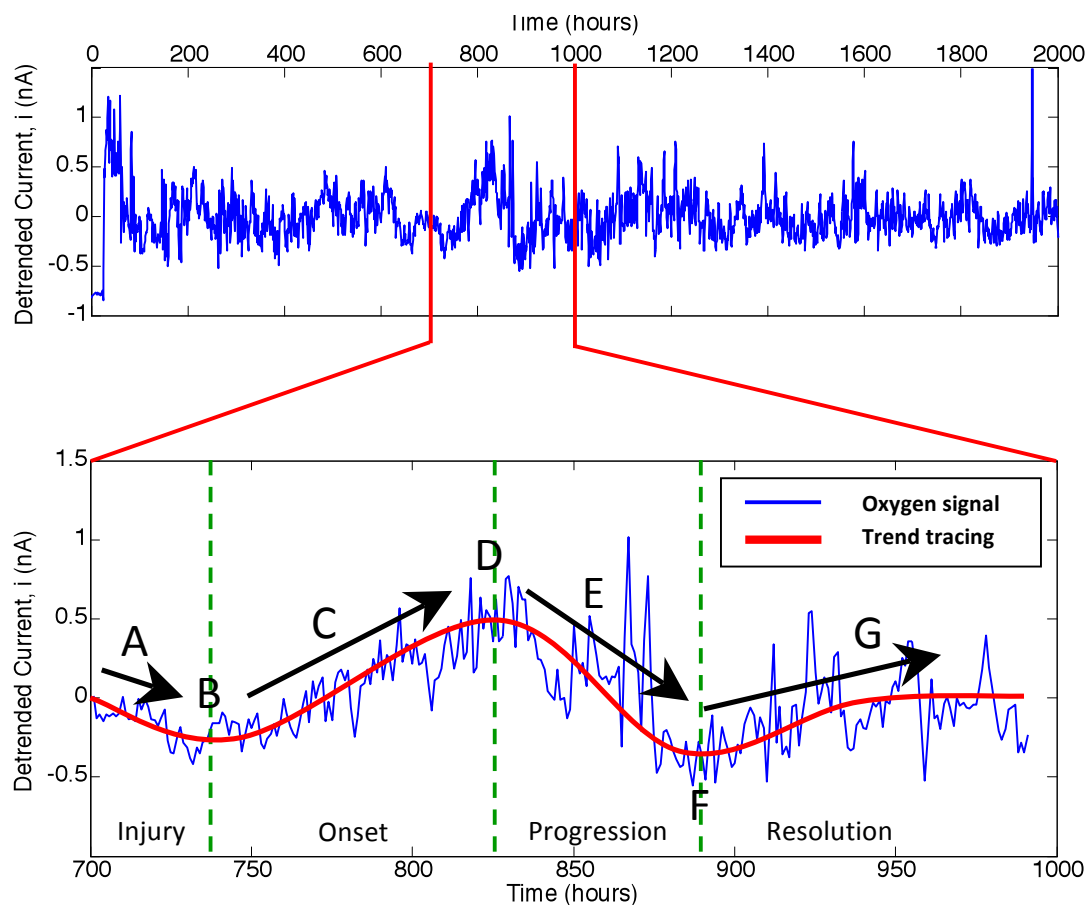


Figure 48. Proposed model of oxygen signal response to an acute wound healing event at the implant location.

A representative signal from one electrode is depicted in the top plot, and the oscillation beginning at 700 hours is magnified and shown in the bottom plot. The trend tracing was performed by hand to illustrate behavior of the trend over the course of the 12 days. A-G represent important points in the 4 proposed stages of signal oscillation due to wound healing, namely: injury, onset, progression and resolution (see text for further description).

The steps of oxygen signal oscillation and corresponding hypothesized wound response stage presented in the preceding figure are as follows.

- Injury (Days 0-2):
 - A) The initial dip in oxygen signal occurs due to the injury of tissues surrounding the sensor. Initial bleeding and sensor/tissue interface disruption via protein adsorption ceases direct flow of O_2 . In addition, vasoconstriction reduces perfusion to the area.
- Onset (Days 2-6):
 - B) Within 1-2 days, the formation of a clot and protein aggregation near the sensor face results in the signal reaching a local minimum.
 - C) Release of chemokines leads to the onset of wound healing. Formation of a fibrin network is initiated causing release of thrombin and other vasodilators. Protein adsorption, including IgG and platelets, cytokine release (C3a and C5 cofactors), etc. cause edema and recruitment of leukocytes (inflammation). Hypoxia dependent vascular remodeling begins with proliferation of endothelial cells. As a result oxygen supply begins to steadily resume.
 - D) As the numbers of phagocytic, immune, and endothelial cells increase, the oxygen requirements increase and levels of available O_2 to the sensor stabilize at a maximum.
- Proliferation (Days 6-9):

- E) Repair and remodeling is fully underway and oxygen is consumed significantly. Oxygen measured at electrode surface drops significantly as new cells infiltrate. Fibroblast presence increases as scar tissue is deposited.
- F) Maximum numbers of macrophages and fibroblasts in the area occur; oxygen values reach another local minimum.

- Resolution (Days 9-12):

- G) Macrophage, fibroblast and endothelial activity is concluded and cells exit repaired wound site. This leads to the restoration of oxygen to levels prior the oscillatory event.

If another injury is sustained from continued animal activity, this process could potentially restart before full resolution, appearing in the signals as a continued oscillation (see **Figure 10a**).

In most of the signals studied, the oscillatory behavior is strongest at the onset of the experiment and weakens over time. The progression of fibrous encapsulation and hypothesized signal attenuation would result in a corresponding decrease in magnitude as the experiment progressed. Additionally, it should also be noted that the animals used to collect these signals increased in mass more than three-fold over the course of the experiment (see **Table 5**), where most of this weight gain has been previously attributed to fat [83, 84]. As fat is deposited in the subcutaneous tissues, the effects of force transfer to the sensor from scratching, rubbing and strong impacts would be greatly lessened leading to much milder wound healing, thus lessening disruptions in oxygen flow. However, this weakening occurs even after the signals were

subjected to multiplicative trend removal shown in **Figure 8**. This form of detrending is expected to normalize the entire signal with respect to the global exponential decay trend identified. The presence of oscillatory attenuation indicates that an additional phenomenon is responsible. Since the animals from which these signals were collected increased in mass several-fold, a natural protective layer around each telemeter was most likely created and lessened the damage sustained from scratching, impacts, etc. A further analysis on oscillatory amplitude as a function of signal time would be necessary for a more adequate explanation.

While the strong 12 day oscillations contained the most power (see **Figure 10**), there is a noticeable peak at the 0.9 to 1.1 day⁻¹ frequency. This is consistent with the findings of other researchers who have investigated the frequency content of long-term biological signals in mammals [85, 86]. The diurnal activity of human and animal alike is strong with a sleep – feeding – activity pattern. The pigs used in this study were no different. The animals would become very excited when fed, which occurred at a regular daily intervals, the animals would sleep at night and be active during the day.

The animals were simultaneously subjected to a myriad of other testing at the same time as the oxygen signals were collected. At regular intervals, the animals were collected from their pens, herded into a sling and subjected to blood collections, intravenous glucose infusions for glucose sensor testing, weighing, etc. Understandably, this can be an exciting process for the animals, raising their heart rates, blood pressure, changing the local blood flow patterns and thus altering the resulting oxygen signals. Depending on the amount of time that the animals required to in effect “calm down”

from this process, the magnitude of the signal oscillations could alter dramatically. This series of tests was performed twice weekly and could account for much of the intermediate frequency information seen in the power spectra (see **Figure 12a**).

While it cannot be presently stated with absolute certainty that these shorter course animal behaviors and occurrences are the definite causes of the intermediate oscillations, it is safe to assume that local changes in blood flow due to animal activity (whether due to changes in blood pressure, heart rate, local shunting etc.) have a strong impact. The work of Persson et al. [87] demonstrated in dogs, that over a period of 30 days daily maximum mean arterial pressures (MAP) can vary by as much as 20 mmHg or approximately 15-20. The effects of this change to the microvasculature could lead to disturbances in flow near individual electrodes affecting the resultant signals. It is not clear whether this type of behavior has periodicity to it, but this definitely seems plausible and warrants further investigation. Utilizing this oxygen sensor setup would be most beneficial for this type of work as blood flow measurements could be corroborated with full-time oxygen signals produced by the telemeters. Further work is required for a full analysis of the oscillatory nature of long-term oxygen signals.

5.3.3 Tissue heterogeneity

The oscillations are also shown to be conserved between signals from the same array. **Figure 13** and **Figure 14** illustrate that as many as three individual electrodes on an array show very similar signal behavior, despite differences in electrode location, leading us to believe that these oscillatory events affect the entire array, and not just

individual electrodes. Moreover, individual electrodes can be as far as 1 cm away from each other; a significant distance with respect to the mass transfer limitations of oxygen in tissues. This is consistent with the proposed model of wound healing modulated oscillations. If the sensor is jarred to such an extent that damage to the underlying tissues occurs, it stands to reason that all electrodes on the array within that pocket will be affected by compositional and morphological changes that result. This does not bar electrode local tissue behavior from introducing differences between signals, as is seen when examining Array 226 in **Figure 13** and **Figure 14** which demonstrates noticeably weaker similarity among its signals than does Array 230.

5.4 Limitations of this work

Numerous limitations imposed on this work are presented throughout where appropriate. These range from limitations of selected techniques, time constraints, financial constraints, etc. However, most of these revolve around one central issue. The bulk of the histological information presented within this thesis was collected from one animal. This was due to the expense of both maintaining an animal of this size, the costs associated with the surgeries, materials, and husbandry. The use of one animal greatly limits statistical impact of the results. While great care was taken to maximize data collection and ensure representative selection for analysis, the results still derive from one animal. The findings presented in this work are deemed representative for mammalian tissue based on similar results reported in the literature (especially for the histological aspects of this work). Absolute numbers generated from the histology are

generally to be taken with speculation; however trends and relationships are believed to hold true if repeated.

In Chapter 3, tissue at each individual time point comes from a different location on the animals back and therefore variability in skin tissue cannot be discounted. While the animal was observed to be healthy with no skin abnormalities, scars, growths, etc. of any kind pre-operatively, it cannot be discounted that the locations differed somewhat in cellular and compositional morphology. The implant locations span the majority of the animal's dorsal length originating near the base of the neck and extending to the lower portion of the rib cage. The removal of the shams was performed randomly with respect to location and no correlation between changes in composition and location along the animals back were found. It is therefore believed that the skin was of uniform constitution at the start of the experiment, and any changes observed are due to the encapsulation process.

CHAPTER 6

Conclusions and future directions

6.1 Conclusions

A rigorous signal processing methodology is presented in Chapter 2 for analyzing complex biological signals with multileveled characteristics. Using cursory visual observations of the signals, a filtering and decimation routine is presented and validated for the handling of large data sets containing more information than necessary. Also, appropriate trend removal is discussed and presented illustrating its impact on signals from complex biological tissues. In order to obtain irrefutable evidence of signal characteristics, the use of several relatively novel and well established techniques (the Welch algorithm, cross-correlation and wavelet analysis) is presented as an example of such workflow for future investigations.

Subsequently, a novel sham implantation experiment is described in Chapter 3 that provides for tissue sample removal at specific time points chosen to coincide with corresponding signal features. The use of standard and immunochemical histology processes is detailed capable of providing a wealth of information regarding the tissues collected. In order to deal with such an abundance of information obtained, several automated and semi-automated image analysis processes are developed for efficient workflow.

Chapter 4 presents the analytical and numerical investigation of oxygen flux through subcutaneous tissues. The novel use of normal and beta probability density functions to model the change in vascular distributions found in histology is detailed in an attempt to quantify the shift in vessel position during implantation. Despite the significant variability in the histological data, expected for a complex biological system, such PDFs lend themselves rather well to such a task. Using these distribution models, an in-depth sensitivity analysis of the various mass transfer properties of tissues is performed and discussed.

Analysis of oxygen signals from sensors implanted over the long term in porcine subcutaneous tissues revealed several salient features that have changed our understanding of metabolic behavior and sensing in tissues. Primarily, contained within signals oxygen electrodes implanted for a period of 13 weeks there is a pronounced decay process that strongly displays an exponential behavior with a greater than 80% reduction in signal strength and a decay constant of approximately 2 weeks. Histological evidence from tissues excised after removal of the sensors indicates that a deposition of collagenous material in front of the PDMS coated electrodes in addition to a pronounced increase in vascular depth is the most likely culprit for this decay. Surprisingly, minimal cellular activity change, including macrophage and granulocyte activity, is seen in the histology. Computer simulations of the mass transfer properties within the subcutaneous tissue/sensor interface in conjunction with histological findings further corroborate the collected signals and strongly support our hypothesis that it is in

fact morphological and compositional changes in the tissues that affect the mass transfer of metabolites.

Moreover, strong evidence of periodicity is present in the signals with most oscillatory power occurring in the 7 - 15 day and 0.9 - 1.1 day periods. It is believed that these oscillations are due to the wound healing and diurnal processes, respectively. A model is proposed that attempts to synchronize the critical events in the wound-healing process with specific milestones in one oscillatory cycle. In particular, the initial injury and subsequent onset, progression and resolution steps of wound healing are shown to correlate with the specific falling and rising oxygen levels near a sensor during one of these events. Comparisons between signals collected from the same telemeters demonstrate that these oscillations affect entire arrays indicating this is a wound site-specific event and not localized to only one electrode.

6.2 Future Directions

The investigation of oxygen transfer through subcutaneous tissues during the presence of a metabolically active implant has provided a wealth of information regarding the complex nature of encapsulation, wound healing and mass transfer dynamics. It stands to reason that a similar investigation be performed for the numerous other metabolites currently under investigation for clinical use in implanted device modalities. Using the methodology presented, similar studies could be performed on the behavior of glucose, lactose, signaling factors, etc. as they are impacted by the placement of a foreign body. Glucose, for which wireless,

subcutaneously implanted sensors are currently being developed, is subject to many of the same effects that we see for oxygen. It stands to reason it's passage from vasculature to implant, through the tissues, will also be affected by the development of a foreign body capsule and modulated by wound healing events.

As mentioned previously, this study is statistically limited by the use of one animal for the sham and histology experiment presented in Chapter 3. It would be beneficial to perform that work with more animals to provide more statistical power to the results. Additionally, the histological samples were collected from subcutaneous tissues adjacent to non-functioning shams. The findings were then compared against signals collected from electronically functional sensors implanted into different animals. The use of live sensors, where the signals collected correlate directly with tissue samples retrieved upon excision would be extremely beneficial for such studies. Until now this has been limited by the prohibitive cost and availability of such sensor telemeters, but as commercialization progresses, this limitation will be removed.

Moreover, the investigation of other tissues besides the subcutaneous would be of benefit to various fields where oxygen flow, thorough oxygenation and hypoxia are of serious concern. Brain and lung tissue come to mind, among a myriad of sensitive organs, for studies investigating pulmonary disorders where patients are unable to inspire requisite amounts of oxygen and hypoxic stress occurs. COPD, as discussed briefly in Chapter 1, is caused by elevated inflammation of the airways and a correlation between inflammation severity graded from histology and oxygen signals collected in an

organ of choice could be informative. This type of study could be of enormous benefit in the future.

APPENDIX

7.1 Supplemental information pertaining to Chapter 2.

The sensor design, implantation and removal, and signal collection presented in Chapter 2 was performed by Glysens, Inc. The following experimental procedure and necessary information is provided for the reader, but was not performed by the dissertation author. Permission has been obtained for reproduction in this document.

7.1.1 Animals

Three female juvenile Yorkshire farm pigs (company providing animals) were used for this study. The animals were approximately 2-3 months old at the start of the experiment and were kept under a weight of 30 kg. The pertinent details concerning the animals are included in **Table 5**.

Table 5. Animal age, weight information (at start and end of experiment) and duration of sensor implantation.

Subject #	Age at Implant	Weight at Implant	Weight at Explant	Implant Duration
1	11 weeks	28 kg	93 kg	151 days
2	7 weeks	17 kg	73 kg	136 days
3	7 weeks	17 kg	68 kg	136 days

7.1.2 Implantation procedure

The sensor arrays (see **Figure 2**) were implanted into one of four pockets created via blunt dissection in the subcutaneous tissue on the dorsal side of juvenile Yorkshire farm pigs. These pockets were created about 1 cm below the surface of the skin, between the underlying fat and muscle layers. Orientation was kept such that the electrode array faced into the core of the animal while the antenna on the back of the case faced out of the body. No mechanical fastening was applied to the sensors in order to anchor them to the tissues; the pocket was sized just large enough to fit the array so fastening was unnecessary. The sensors were then left implanted in the animals for a period of 13+ weeks. The animals were kept in their pens, however they were not restrained. Twice a week the animals were subjected to glucose testing procedures which required their removal from their pens, herding into a lab facility, suspension in a sling, and the injection of glucose and collection of serial blood samples.

7.1.3 Sensor calibration

To verify that any changes in the oxygen signals were due to the surrounding tissues and not changes in the performance of the oxygen sensors themselves, calibrations were performed before implantation and after removal for each array. This process involved placing the active sensor array into a physiologic buffer solution through which various known oxygen concentration gas mixtures were sparged. The resulting currents measured by each electrode were recorded and a calibration slope was measured for current as a function of oxygen concentration. These slopes were

then compared before and after implantation for each functioning electrode. **Table 6** presents the magnitude calibration change from initial calibration performed before implantation to final calibration after removal.

Table 6. Magnitude of calibration change (%) for all 8 electrodes spanning the 11 implanted arrays.

Red boxes indicate those electrodes where calibration could not be calculated both before implantation and/or after removal, gray boxes indicate that magnitude change was too great, yellow boxes indicate appropriate magnitude change however signals were not available, and green boxes indicate signals who have appropriate magnitude change and were available.

Array No.	Electrode 1	Electrode 2	Electrode 3	Electrode 4	Electrode 5	Electrode 6	Electrode 7	Electrode 8
205 *	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
210	N/A	51%	65%	-57%	26%	-49%	N/A	-16%
212	66%	56%	83%	-62%	86%	-39%	8%	-15%
213	N/A	N/A	-1%	-39%	7%	-53%	N/A	N/A
214 *	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
222 *	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
223	N/A	N/A	57%	-45%	15%	-63%	106%	24%
226	-39%	-31%	31%	12%	-36%	-6%	-48%	4%
228 *	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
229	62%	124%	110%	-29%	53%	-46%	-46%	-33%
230	29%	9%	45%	9%	21%	-39%	-43%	37%

* Arrays damaged upon removal, calibration could not be completed.

7.1.4 Histology collection

At the conclusion of the experiments, the animals were euthanized via an overdose of anesthesia. The sensor arrays and the surrounding tissue were then surgically excised, the sensors carefully removed to protect the delicate PDMS coating, and the tissue sectioned as shown in **Figure 49**. The two quadrants of tissue adjacent to the electrode face were fixed in 10% buffered formalin, paraffin embedded, sliced, and stained using either H&E or GTC. The slides were then imaged at high magnification and composite images were created allowing a macroscopic view.

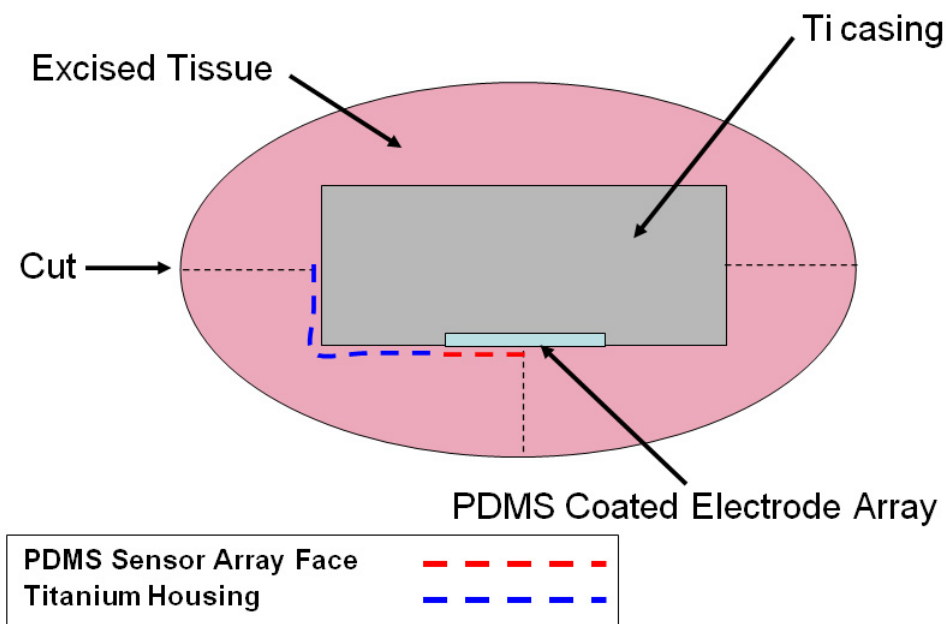


Figure 49. Schematic of sensor array/tissue removal. Procedure for sensor removal, histological cutting planes, and identifying orientation of sensor with respect to collected histology is illustrated.

Figure 50a shows an example of one of these tissue segments. This image has been labeled indicating the regions of tissue adjacent to the titanium casing of the sensor, and those regions adjacent to the PDMS coated electrode array. In addition, two regions are identified where magnified views of the cellular structure are shown in **Figure 50b-e**. It can be clearly seen that there is a distinct difference in the subcutaneous tissue adjacent to the titanium casing from the tissue adjacent to the PDMS coated electrode array. Taking a close look at **Figure 50b** and **d**, H&E and GTC stains of the same region of titanium adjacent tissue respectively, we can see a regular and dispersed nuclear presence (dark blue/black color in **Figure 50c**) and intracellular collagen (light blue/green color seen in **Figure 50e**). Observing the corresponding images from the PDMS/electrode array adjacent tissue (**Figure 50b** and **d**), it is clear that there is a marked increase in cellular density, seen in the heavy concentration of cell nuclei in the H&E stained image, and in the lack of prominent intracellular collagen in the GTC stained image.

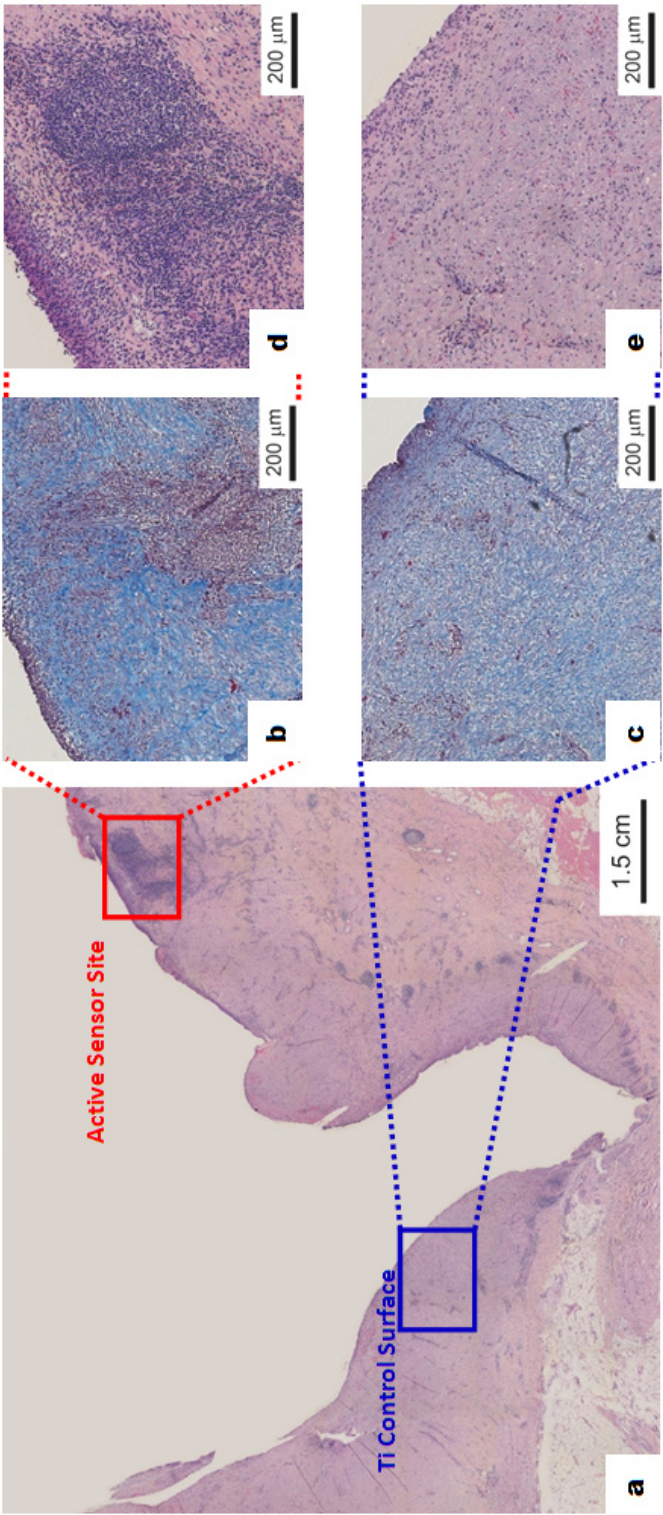


Figure 50. H&E and trichrome histology from tissues collected at live sensor array removal.

(a) Macro view of the implant location indicating the regions of tissue in contact with the titanium casing and those in contact with the PDMS membrane. Higher magnification histology images of tissue adjacent to the PDMS-coated sensor array face (b,d) (blue region in (a)) and adjacent to the titanium casing of the telemeter (c,e) (red region in (a)). The tissues were stained using H&E (b,c) and GTC (d,e).

7.2 Vessel size and number distribution analysis

Akshay Chaudhari, an undergraduate volunteer in the Biosensors Lab at the Dept. of Bioengineering, UCSD performed a rigorous analysis of vessel size and number in the CD31 stained tissues. The following is a more in-depth description of his work, the findings of which are utilized in Chapter 4.

The goal of this part of the study was to study the distribution patterns of capillaries from histology that was obtained from porcine specimens. This was undertaken in order to see if there existed a correlation between vasculature distribution and the signal decay of the oxygen sensor. Several vasculature characteristics were evaluated towards this end. Image manipulation was done using Adobe Photoshop Creative Suite 4. Image analysis was conducted using MATLAB v2008b and the MATLAB Image Processing Toolbox.

CD-31 stained porcine histological images were obtained 1, 2, 6 and 13 weeks after sensor implantation, from either side of the sensor. Ten evenly spaced sections (see **Figure 51a** and **b**) of dimensions $271\mu\text{m} \times 488\mu\text{m}$ were chosen from each of the larger histological images (except for the PDMS week 1 and PDMS week 13 samples, which had 15 and 8 image sections, respectively). For each of the sectioned images, a separate image layer was created overlapping the original image. In this new layer, the outlines of capillaries from the original section were drawn manually. Once all the capillaries were drawn onto the new layer, the layer was saved individually and this was the image that was analyzed in MATLAB (see **Figure 51c-e**). In this way, a semi-automated procedure for the analysis of the vasculature was created.

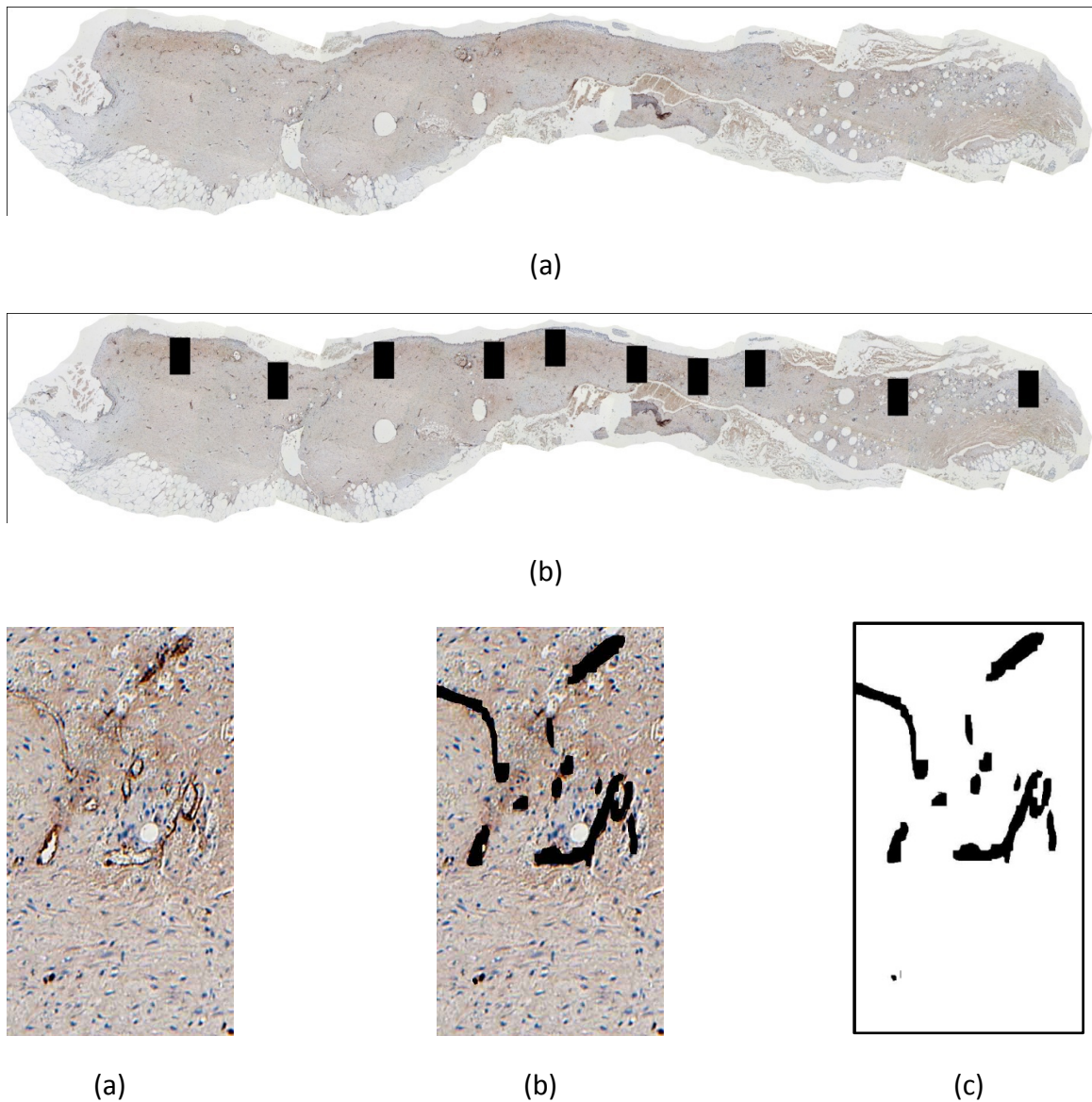


Figure 51. Microvessel image analysis.

a) An original histological sample collected from the Titanium surface at 2 weeks post implantation. b) 10 evenly spaced out sections, each with dimensions of $271\mu\text{m} \times 488\mu\text{m}$, were created. c) Each section (Section 4 in this case), where the capillaries are represented by the dark brown areas, was used for capillary distribution analysis. d) A new image layer was created, in which the capillaries are highlighted, which was overlaid on top of the original image. e) Only the highlighted image layer from Figure 1d. This is the image used for the image analysis.

7.3 Matlab code

The following is a collection of code written by the author with help from numerous collaborators (mentioned individually where applicable). The code is annotated as necessary (“%” in the code indicates a line of annotation and not functioning code) and is presented in a way that can be readily comprehended by the reader with the necessary background in Matlab.

7.3.1 Signals processing code

The following program performed all the necessary filtering, decimation, autocorrelation, and wavelet analysis presented in Chapter 2. Power spectrum estimation was performed using the “Signal Processing Toolbox”. All charts, graphs and data were later extracted and generated by hand from the workspace in Matlab.

Begin Matlab code for SigPower_filt.m

```
clear, clc
tic
A1=xlsread('[path to *.xls file]');
sizeA1 = size(A1);
% -----Check for NaN-----
for n = 1 : sizeA1(1,1)
    for m = 1 : sizeA1(1,2)
        if isnan(A1(n,m)) == 1
            A1(n,m) = 0;
        end, end, end
    % -----
% -----Averaging Filter-----
windowSize = 30; % Sampling Freq. = 0.5 min-1, windowSize = 30 for 1 hour averaging
```

```

AvgFlt = ones(1,windowSize)/windowSize;
B1a(:,1) = A1(:,1);
for n = 2 : sizeA1(1,2)
    B1a(:,n) = filter(AvgFlt,1,A1(:,n));
end
% resampling at 1/30 of original sample rate
B1b = B1a;
B1b = resample(B1b,1,30);
% -----

% -----Lowpass Filter-----
lowpass % calls lowpass.m, a low-pass equiripple filter designed to cut off at 1/30 samples

C1a(:,1) = A1(:,1);
for n = 2 : sizeA1(1,2)
    C1a(:,n) = filter(ans, A1(:,n));
end
% resampling at 1/30 of original sample rate
C1b = C1a;
C1b = resample(C1b,1,30);
% offset application
for i = 2 : sizeA1(1,2)
    C1_min(i) = min(C1b(:,i));
    if C1_min(i) < 0
        C1b(:,i) = C1b(:,i) + (C1_min(i)*(-1));
    end, end
% -----

% -----Residuals-----
% Averaging Filter residuals
D1 = A1 - B1a;
% Lowpass Filter residuals
E1 = A1 - C1a;
% -----

% -----Averaging-----
ASF = (24*7); % averaging rate
sizeB1b = size(B1b);
for n = 1 : (sizeB1b(1,1)/ASF)
    for m = 2 : sizeB1b(1,2)
        F1a(1,m) = mean(B1b(1:ASF/2,m));
        F1a(1,1) = 1;
        F1a(n+1,m) = mean(B1b((n-1)*ASF+1:n*ASF,m));
        F1a(n+1,1) = (n*ASF)-(ASF/2);
        F1a(n+2,m) = mean(B1b((sizeB1b(1,1)-(ASF/2)):sizeB1b(1,1),m));
    end
end

```

```

F1a(n+2,1) = sizeB1b(1,1);
G1a(1,m) = mean(C1b(1:ASF/2,m));
G1a(1,1) = 1;
G1a(n+1,m) = mean(C1b((n-1)*ASF+1:n*ASF,m));
G1a(n+1,1) = (n*ASF)-(ASF/2);
G1a(n+2,m) = mean(C1b((sizeB1b(1,1)-(ASF/2)):sizeB1b(1,1),m));
G1a(n+2,1) = sizeB1b(1,1);
end, end
sizeF1a = size(F1a);

F1b = zeros(sizeB1b(1,1),sizeB1b(1,2));
G1b = zeros(sizeB1b(1,1),sizeB1b(1,2));
for n = 1 : sizeF1a(1,1)
    F1b(F1a(n,1),:) = F1a(n,:);
    G1b(G1a(n,1),:) = G1a(n,:);
end
% -----

% -----Cubic Spline Interpolation-----
sizeF1b = size(F1b);
for n = 1 : sizeF1b(1,1)
    F1b(n,1) = n;
    G1b(n,1) = n;
end
sizeF1a = size(F1a);
sizeF1b = size(F1b);
for n = 2 : sizeF1a(1,2)
    for i = 1:sizeF1a(1,1)
        x(i) = F1a(i,1);
        y(i) = F1a(i,n);
        xg(i) = G1a(i,1);
        yg(i) = G1a(i,n);
        for j = 1 : sizeF1b(1,1)
            xx(j) = F1b(j,1);
            xxg(j) = G1b(j,1);
        end, end

        yy = spline(x,y,xx);
        yyg = spline(xg,yg,xxg);
        F1c1 = ['SplineF', num2str(n-1), ' = yy;'];
        eval(F1c1);
        G1c1 = ['SplineG', num2str(n-1), ' = yyg;'];
        eval(G1c1);
    end
end
for n = 2 : sizeF1b(1,2)

```

```

for m = 1 : sizeF1b(1,1)
    F1c5 = ['F1b(',num2str(m),',',num2str(n),') = SplineF', num2str(n-1),('(',num2str(m),')');];
    eval(F1c5);
    G1c5 = ['G1b(',num2str(m),',',num2str(n),') = SplineG', num2str(n-1),('(',num2str(m),')');];
    eval(G1c5);
end, end
% -----

% -----DETRENDING-----
% -----Subtracting Trend from Original Data-----
% -----(additive trend)-----
for n = 1 : sizeF1b(1,1)
    for m = 2 : sizeF1b(1,2)
        F1d(n,1) = F1b(n,1);
        F1d(n,m) = B1b(n,m) - F1b(n,m);
        G1d(n,1) = G1b(n,1);
        G1d(n,m) = C1b(n,m) - G1b(n,m);
    end, end
% -----Dividing out Trend from Original Data-----
% -----(multiplicative trend)-----
for n = 1 : sizeF1b(1,1)
    for m = 2 : sizeF1b(1,2)
        F1e(n,1) = F1b(n,1);
        F1e(n,m) = B1b(n,m) / F1b(n,m);
        G1e(n,1) = G1b(n,1);
        G1e(n,m) = C1b(n,m) / G1b(n,m);
    end, end

    for m = 2 : sizeF1b(1,2)
        MeanF1e(m) = mean(F1e(:,m));
        F1e(:,m) = F1e(:,m) - MeanF1e(m);
        MeanG1e(m) = mean(G1e(:,m));
        G1e(:,m) = G1e(:,m) - MeanG1e(m);
    end
% -----

% -----Cross-Correlation Detrended-----
sizeF1d = size(F1d);
XCS = -(sizeF1d(1,1)-1):(sizeF1d(1,1)-1);
for j = 2:sizeF1d(1,2)
    for i = 2:sizeF1d(1,2)
        XCTempF = ['XCF',num2str(j-1),('(',',',num2str(i),')=xcorr(F1d(:,j),F1d(:,',num2str(i),'),"coeff");];
        eval(XCTempF);
        XCTempG = ['XCG',num2str(j-1),('(',',',num2str(i),')=xcorr(G1d(:,j),G1d(:,',num2str(i),'),"coeff");];
        eval(XCTempG);
    end
end

```

```

end, end
% -----

% -----1-D Continuous Wavelet -----
sizeG1e = size(G1e);

for i = 2 : sizeF1b(1,2)
    SigTemp = ['Sig', num2str(i-1), ' = G1e(:,i);'];
    eval(SigTemp);
end
for i = 1 : sizeF1b(1,2)-1
    WaveTemp1a = ['Sig', num2str(i), '_mexh256_coeffs = cwt(Sig', num2str(i), ', 1:256, "mexh");'];
    eval(WaveTemp1a);
    WaveTemp1b = ['Sig', num2str(i), '_mexh256_scales = 1:256;'];
    eval(WaveTemp1b);
    WaveTemp1c = ['Sig', num2str(i), '_mexh256_power = Sig', num2str(i), '_mexh256_coeffs.^2;'];
    eval(WaveTemp1c);
    WaveTemp1d = ['Mean1 = mean(mean(Sig', num2str(i), '_mexh256_power));'];
    eval(WaveTemp1d);
    WaveTemp1e = ['STD1 = mean(std(Sig', num2str(i), '_mexh256_power));'];
    eval(WaveTemp1e);
    WaveTemp1f = ['COEFFSP1 = Sig', num2str(i), '_mexh256_power;'];
    eval(WaveTemp1f);
    WaveTemp2a = ['Sig', num2str(i), '_morl512_coeffs = cwt(Sig', num2str(i), ', 1:512, "morl");'];
    eval(WaveTemp2a);
    WaveTemp2b = ['Sig', num2str(i), '_morl512_scales = 1:512;'];
    eval(WaveTemp2b);
    WaveTemp2c = ['Sig', num2str(i), '_morl512_power = Sig', num2str(i), '_morl512_coeffs.^2;'];
    eval(WaveTemp2c);
    WaveTemp2d = ['Mean2 = mean(mean(Sig', num2str(i), '_morl512_power));'];
    eval(WaveTemp2d);
    WaveTemp2e = ['STD2 = mean(std(Sig', num2str(i), '_morl512_power));'];
    eval(WaveTemp2e);
    WaveTemp2f = ['COEFFSP2 = Sig', num2str(i), '_morl512_power;'];
    eval(WaveTemp2f);

% -----Wavelet Comparison of All Signals-----
SizeCOEFFSP1 = size(COEFFSP1);
for n = 1 : SizeCOEFFSP1(1,1)
    for m = 1 : SizeCOEFFSP1(1,2)
        if COEFFSP1(n,m) > Mean1 + (1 * STD1)
            Eval5 = ['Sig', num2str(i), '_mexh256_Mean1STD(n,m) = 1;'];
            eval(Eval5);
        else
            Eval6 = ['Sig', num2str(i), '_mexh256_Mean1STD(n,m) = 0;'];

```

```
eval(Eval6);
end, end, end
```

```
SizeCOEFFSP2 = size(COEFFSP2);
for n = 1 : SizeCOEFFSP2(1,1)
for m = 1 : SizeCOEFFSP2(1,2)
if COEFFSP2(n,m) > Mean2 + (1 * STD2)
Eval5 = ['Sig',num2str(i),'_morl512_Mean1STD(n,m) = 1;'];
eval(Eval5);
else
Eval6 = ['Sig',num2str(i),'_morl512_Mean1STD(n,m) = 0;'];
eval(Eval6);
end, end, end, end
```

```
SumSigWave_mexh256_Mean1STD = Sig1_mexh256_Mean1STD + Sig2_mexh256_Mean1STD + ...
    Sig3_mexh256_Mean1STD + Sig4_mexh256_Mean1STD + Sig5_mexh256_Mean1STD + ...
    Sig6_mexh256_Mean1STD + Sig7_mexh256_Mean1STD + Sig8_mexh256_Mean1STD + ...
    Sig9_mexh256_Mean1STD + Sig10_mexh256_Mean1STD + Sig11_mexh256_Mean1STD + ...
    Sig12_mexh256_Mean1STD;
SumSigWave_morl512_Mean1STD = Sig1_morl512_Mean1STD + Sig2_morl512_Mean1STD + ...
    Sig3_morl512_Mean1STD + Sig4_morl512_Mean1STD + Sig5_morl512_Mean1STD + ...
    Sig6_morl512_Mean1STD + Sig7_morl512_Mean1STD + Sig8_morl512_Mean1STD + ...
    Sig9_morl512_Mean1STD + Sig10_morl512_Mean1STD + Sig11_morl512_Mean1STD + ...
    Sig12_morl512_Mean1STD;
```

```
% -----
```

End Matlab code.

7.3.2 Image processing code

The following image processing program performed all the computations presented in Chapter 3 as outlined in the workflow diagram illustrated in **Figure 16**.

Mark Chapman (NSF REU Student 2009, B.S. Biomedical Engineering, U. of Minnesota) and Robert Turner (B.S. Bioengineering, UC San Diego) were instrumental in the writing, testing and implementation of this code. Dr. Jared Goor's work on computational image processing (HistoQuant 0.96 [69]) was heavily influential in the writing of our code, and a great debt of thanks is extended.

Begin Matlab code for improc.m:

```
tic
clear, clc,
NumReg = 20; % Number of Region Images for analysis
filename_big = 'Image.jpg';
filename_Ref = 'Reference_Image.jpg'; % filename of reference image used in histogram equalization
IM_Big = imread(filename_big);
IM_Ref = imread(filename_Ref);
SizeBig = size(IM_Big);
SizeRef = size(IM_Ref);

% ----- Split RGB Channels of Original Image-----
for i = 1:3
    if i == 1
        IM_Red_Big_Prefilt = IM_Big(:, :, i);
        IM_Ref_Red_Prefilt = IM_Ref(:, :, i);
    elseif i == 2
        IM_Green_Big_Prefilt = IM_Big(:, :, i);
        IM_Ref_Green_Prefilt = IM_Ref(:, :, i);
    elseif i == 3
        IM_Blue_Big_Prefilt = IM_Big(:, :, i);
        IM_Ref_Blue_Prefilt = IM_Ref(:, :, i);
    end, end

IM_Ref_Red = 0;
IM_Ref_Green = 0;
IM_Ref_Blue = 0;
```

```

% -----Median Filter & Histogram Equalization-----
IM_Red_Big_histeq = 0;
IM_Green_Big_histeq = 0;
IM_Blue_Big_histeq = 0;

IM_Red_Big = medfilt2(IM_Red_Big_Prefilt);    % medfilt2 function for 2D Median Filter
IM_Green_Big = medfilt2(IM_Green_Big_Prefilt);
IM_Blue_Big = medfilt2(IM_Blue_Big_Prefilt);

IM_Ref_Red = medfilt2(IM_Ref_Red_Prefilt);    % medfilt2 function for 2D Median Filter
IM_Ref_Green = medfilt2(IM_Ref_Green_Prefilt);
IM_Ref_Blue = medfilt2(IM_Ref_Blue_Prefilt);

IM_Red_Big_histeq = histeq(IM_Red_Big,imhist(IM_Ref_Red));    % Histogram equalization to a
Reference image (IM_Ref)
IM_Green_Big_histeq = histeq(IM_Green_Big,imhist(IM_Ref_Green));
IM_Blue_Big_histeq = histeq(IM_Blue_Big,imhist(IM_Ref_Blue));

IM_Big_EQ(:,:,1) = IM_Red_Big_histeq;    % Reconstructed IM_Big after histogram equalization for
% comparison
IM_Big_EQ(:,:,2) = IM_Green_Big_histeq;
IM_Big_EQ(:,:,3) = IM_Blue_Big_histeq;
IM_Big_EQ = uint8(IM_Big_EQ);

% -----Section Image into NumReg Slices-----
for z = 1 : NumReg
    fs1 = ['Slice',num2str(z),' = imcrop(IM_Big_EQ,[0 SizeBig(1)/NumReg*(z-1) SizeBig(2)...
SizeBig(1)/NumReg]);'];
    eval (fs1);
    fs2 = ['imwrite(Slice',num2str(z),'','Slice',num2str(z),'.jpg','jpg');'];
    eval (fs2);
end

clear a;
try
clf(1), clf(2), clf(3), clf(4), clf(5),...
clf(6), clf(7), clf(8), clf(9), clf(10)
end

for a = 1 : NumReg
    fn1 = ''Slice';
    fn2 = '.jpg';
    fn3 = 'filename = ';
    fn4 = [fn1,num2str(a),fn2,';'];
    fn5 = [fn3,fn4];
    eval (fn5);
    IM_Orig = imread(filename);
    SizeOrig = size(IM_Orig);
    TotArea = SizeOrig(1)*SizeOrig(2);

```

```

% ----- RGB Min and Max Values -----
Red_Min = 0; % Default = 0
Red_Max = 120; % Default = 255
Green_Min = 100; % Default = 0
Green_Max = 255; % Default = 255
Blue_Min = 0; % Default = 0
Blue_Max = 175; % Default = 255

% ----- NTSC Min and Max Values -----
Y_Min = 0; % Default = 0
Y_Max = 1; % Default = 1
I_Min = -0.596; % Default = -0.596
I_Max = 0.05; % Default = 0.596
Q_Min = -0.523; % Default = -0.523
Q_Max = 0.523; % Default = 0.523

% ----- Morphology Min and Max Values -----
Area_Min = 20; % area in pixels
Area_Max = Inf; % area in pixels, 'Inf' is infinite
Solid_Min = 0; % solidity ratio
Solid_Max = 1; % solidity ratio
Eccentr_Min = 0; % Eccentricity ratio
Eccentr_Max = 1; % Eccentricity ratio

% ----- Split RGB Color Channels & Display -----
IM_Red = 0;
IM_Green = 0;
IM_Blue = 0;

for i = 1:3
    if i == 1
        IM_Red = IM_Orig(:,i);
    elseif i == 2
        IM_Green = IM_Orig(:,i);
    elseif i == 3
        IM_Blue = IM_Orig(:,i);
    end
end

% ---- Red Color Restriction Application -----
SIZE_Red = size(IM_Red);
IM_Red_RES = 0;
IM_Red_RES_BIN = 0;

for j = 1 : SIZE_Red(1)
    for k = 1 : SIZE_Red(2)
        if IM_Red(j,k) < Red_Min
            IM_Red_RES(j,k) = 0;
            IM_Red_RES_BIN(j,k) = 0;
        elseif IM_Red(j,k) > Red_Max
            IM_Red_RES(j,k) = 0;
        end
    end
end

```

```

IM_Red_RES_BIN(j,k) = 0;
else
IM_Red_RES(j,k) = IM_Red(j,k);
IM_Red_RES_BIN(j,k) = 1;
end, end, end

% ---- Green Color Restriction Application -----
SIZE_Green = size(IM_Green);
IM_Green_RES = 0;
IM_Green_RES_BIN = 0;

for j = 1 : SIZE_Green(1)
for k = 1 : SIZE_Green(2)
ifIM_Green(j,k) <Green_Min
IM_Green_RES(j,k) = 0;
IM_Green_RES_BIN(j,k) = 0;
elseifIM_Green(j,k) >Green_Max
IM_Green_RES(j,k) = 0;
IM_Green_RES_BIN(j,k) = 0;
else
IM_Green_RES(j,k) = IM_Green(j,k);
IM_Green_RES_BIN(j,k) = 1;
end, end, end

% ---- Blue Color Restriction Application -----
SIZE_Blue = size(IM_Blue);
IM_Blue_RES = 0;
IM_Blue_RES_BIN = 0;

for j = 1 : SIZE_Blue(1)
for k = 1 : SIZE_Blue(2)
ifIM_Blue(j,k) <Blue_Min
IM_Blue_RES(j,k) = 0;
IM_Blue_RES_BIN(j,k) = 0;
elseifIM_Blue(j,k) >Blue_Max
IM_Blue_RES(j,k) = 0;
IM_Blue_RES_BIN(j,k) = 0;
else
IM_Blue_RES(j,k) = IM_Blue(j,k);
IM_Blue_RES_BIN(j,k) = 1;
end, end, end

% ----- Split NTSC Color Channels & Display -----
IM_Orig_NTSC = rgb2ntsc(IM_Orig);

for i = 1:3
if i == 1
IM_Y_Prefilt = IM_Orig_NTSC(:, :, i);
elseif i == 2
IM_I_Prefilt = IM_Orig_NTSC(:, :, i);
elseif i == 3

```

```

IM_Q_Prefilt = IM_Orig_NTSC(:,:,i);
end, end

IM_Y = IM_Y_Prefilt;      % medfilt2 function for 2D Median Filter
IM_I = IM_I_Prefilt;
IM_Q = IM_Q_Prefilt;

% ---- Luminance (Y) Restriction Application -----
SIZE_Y = size(IM_Y);
IM_Y_RES = 0;
IM_Y_RES_BIN = 0;

for j = 1 : SIZE_Y(1)
for k = 1 : SIZE_Y(2)
if IM_Y(j,k) < Y_Min
    IM_Y_RES(j,k) = 0;
    IM_Y_RES_BIN(j,k) = 0;
elseif IM_Y(j,k) > Y_Max
    IM_Y_RES(j,k) = 0;
    IM_Y_RES_BIN(j,k) = 0;
else
    IM_Y_RES(j,k) = IM_Y(j,k);
    IM_Y_RES_BIN(j,k) = 1;
end, end, end

% ---- Chrominance (I) Restriction Application -----
SIZE_I = size(IM_I);
IM_I_RES = 0;
IM_I_RES_BIN = 0;

for j = 1 : SIZE_I(1)
for k = 1 : SIZE_I(2)
if IM_I(j,k) < I_Min
    IM_I_RES(j,k) = 0;
    IM_I_RES_BIN(j,k) = 0;
elseif IM_I(j,k) > I_Max
    IM_I_RES(j,k) = 0;
    IM_I_RES_BIN(j,k) = 0;
else
    IM_I_RES(j,k) = IM_I(j,k);
    IM_I_RES_BIN(j,k) = 1;
end, end, end

% ---- Chrominance (Q) Restriction Application -----
SIZE_Q = size(IM_Q);
IM_Q_RES = 0;
IM_Q_RES_BIN = 0;

for j = 1 : SIZE_Q(1)
for k = 1 : SIZE_Q(2)
if IM_Q(j,k) < Q_Min

```

```

        IM_Q_RES(j,k) = 0;
        IM_Q_RES_BIN(j,k) = 0;
    elseif IM_Q(j,k) > Q_Max
        IM_Q_RES(j,k) = 0;
        IM_Q_RES_BIN(j,k) = 0;
    else
        IM_Q_RES(j,k) = IM_Q(j,k);
        IM_Q_RES_BIN(j,k) = 1;
    end, end, end

    % ----- Restrictions -----
    % Color restrictions only
    IM_RES_Temp = IM_Red_RES_BIN + IM_Green_RES_BIN + IM_Blue_RES_BIN + ...
        IM_Y_RES_BIN + IM_I_RES_BIN + IM_Q_RES_BIN;
    MAX_IM_RES_Temp = max(max(IM_RES_Temp));
    if MAX_IM_RES_Temp < 6    % 'if' statement to prevent MAX_IM_RES_Temp matrix from being
        % comprised of only zeroes.
    IM_RES_Temp(1,1) = 6;
    end

    for l = 1 : SIZE_Green(1)
        for m = 1 : SIZE_Green(2)
            if IM_RES_Temp(l,m) == 6
                IM_RES_BIN(l,m) = 1;
            else
                IM_RES_BIN(l,m) = 0;
            end, end, end

    MAX_IM_RES_BIN = max(max(IM_RES_BIN));

    % ----- Calculating Morphology Properties -----
    if a >= 2
        clear('IM_RES_BWLABEL'), clear('IM_RES_BWLABEL_MAX'),
        clear('IM_RES_BWLABEL_REGIONPROPS_STRUCT'), ...
        clear('IM_RES_BWLABEL_REGIONPROPS_DUB'), clear('IM_RES_BWLABEL_REGIONPROPS'), ...
        clear('IM_RES_BWLABEL_REGIONPROPS_SUMAVG')
    end

    [IM_RES_BWLABEL, IM_RES_BWLABEL_MAX] = bwlabel(IM_RES_BIN);
    IM_RES_BWLABEL_REGIONPROPS_STRUCT = regionprops(IM_RES_BWLABEL, ...
        'Area', 'Solidity', 'Eccentricity', 'Orientation');    % regionprops function

    for n = 1 : IM_RES_BWLABEL_MAX
        IM_RES_BWLABEL_REGIONPROPS_DUB(1,n) = 1;
        IM_RES_BWLABEL_REGIONPROPS_DUB(2,n) = IM_RES_BWLABEL_REGIONPROPS_STRUCT(n).Area;
        IM_RES_BWLABEL_REGIONPROPS_DUB(3,n) = ...
            IM_RES_BWLABEL_REGIONPROPS_STRUCT(n).Eccentricity;
        IM_RES_BWLABEL_REGIONPROPS_DUB(4,n) = IM_RES_BWLABEL_REGIONPROPS_STRUCT(n).Solidity;
        IM_RES_BWLABEL_REGIONPROPS_DUB(5,n) = ...
            IM_RES_BWLABEL_REGIONPROPS_STRUCT(n).Orientation;
        if IM_RES_BWLABEL_REGIONPROPS_DUB(2,n) < Area_Min

```

```

        IM_RES_BWLABEL_REGIONPROPS(1:5,n) = 0;
    elseif IM_RES_BWLABEL_REGIONPROPS_DUB(3,n) < Eccentr_Min
        IM_RES_BWLABEL_REGIONPROPS(1:5,n) = 0;
    elseif IM_RES_BWLABEL_REGIONPROPS_DUB(4,n) < Solid_Min
        IM_RES_BWLABEL_REGIONPROPS(1:5,n) = 0;
    elseif IM_RES_BWLABEL_REGIONPROPS_DUB(2,n) > Area_Max
        IM_RES_BWLABEL_REGIONPROPS(1:5,n) = 0;
    elseif IM_RES_BWLABEL_REGIONPROPS_DUB(3,n) > Eccentr_Max
        IM_RES_BWLABEL_REGIONPROPS(1:5,n) = 0;
    elseif IM_RES_BWLABEL_REGIONPROPS_DUB(4,n) > Solid_Max
        IM_RES_BWLABEL_REGIONPROPS(1:5,n) = 0;
    else
        IM_RES_BWLABEL_REGIONPROPS(1:5,n) = IM_RES_BWLABEL_REGIONPROPS_DUB(1:5,n);
    end, end

for i = 1 : 5
    IM_RES_BWLABEL_REGIONPROPS_SUMAVG(i,1) = ...
        sum(IM_RES_BWLABEL_REGIONPROPS(i,1:IM_RES_BWLABEL_MAX));
    IM_RES_BWLABEL_REGIONPROPS_SUMAVG(i,2) = ...
        sum(IM_RES_BWLABEL_REGIONPROPS(i,1:IM_RES_BWLABEL_MAX))...
./IM_RES_BWLABEL_REGIONPROPS_SUMAVG(1,1);
end

    tmp1 = ['IM_RES_BWLABEL_REGIONPROPS_SUMAVG_Slice',num2str(a),' = ...
        IM_RES_BWLABEL_REGIONPROPS_SUMAVG;'];
eval(tmp1);
    tmp2 = ['IM_RES_BWLABEL_REGIONPROPS_Slice',num2str(a),' = IM_RES_BWLABEL_REGIONPROPS'';'];
eval(tmp2);

% -----Caclulating Morphology Restrictions-----
IM_RES_BWLABEL_RES = IM_RES_BWLABEL;

for n = 1 : IM_RES_BWLABEL_MAX
    if IM_RES_BWLABEL_REGIONPROPS(1,n) == 0
        [r,c,v] = find(IM_RES_BWLABEL == n);
        IM_RES_BWLABEL_RES(r,c) = 0;
    end, end

MAXofMAX(a) = IM_RES_BWLABEL_MAX;

% -----Combining Morphology and Color Restrictions-----
IM_RES_Temp2 = IM_RES_BIN + IM_RES_BWLABEL_RES;

for l = 1 : SizeOrig(1)
    for m = 1 : SizeOrig(2)
        if IM_RES_Temp2(l,m) >= 2
            IM_RES_BIN2(l,m) = 1;
        else
            IM_RES_BIN2(l,m) = 0;
        end, end, end

```

```

% -----Original Image minus Restricted area display-----
for i = 1 : SizeOrig(1)
for j = 1 : SizeOrig(2)
for k = 1 : SizeOrig(3)
if IM_RES_BIN2(i,j) == 0
IM_Orig_MinusRES(i,j,k) = 0;
else
IM_Orig_MinusRES(i,j,k) = IM_Orig(i,j,k);
end, end, end, end

IM_Orig_MinusRES = uint8(IM_Orig_MinusRES);
end

b = max(MAXofMAX);

% -----Data Prep for Excel-----
for a = 1 : NumReg
if a == 1
    AREA_PREXLS = zeros(b,NumReg);
    ECCEN_PREXLS = zeros(b,NumReg);
    SOLID_PREXLS = zeros(b,NumReg);
    NUMBER_PREXLS = zeros(b,NumReg);
    ORIENT_PREXLS = zeros(b,NumReg);
end

    tmp3 = ['NUMBER_PREXLS(1:MAXofMAX(a),a) =
IM_RES_BWLABEL_REGIONPROPS_Slice',num2str(a),'{:,1}'];
eval(tmp3);
    tmp4 = ['AREA_PREXLS(1:MAXofMAX(a),a) =
IM_RES_BWLABEL_REGIONPROPS_Slice',num2str(a),'{:,2}'];
eval(tmp4);
    tmp5 = ['ECCEN_PREXLS(1:MAXofMAX(a),a) =
IM_RES_BWLABEL_REGIONPROPS_Slice',num2str(a),'{:,3}'];
eval(tmp5);
    tmp6 = ['SOLID_PREXLS(1:MAXofMAX(a),a) =
IM_RES_BWLABEL_REGIONPROPS_Slice',num2str(a),'{:,4}'];
eval(tmp6);
    tmp6 = ['ORIENT_PREXLS(1:MAXofMAX(a),a) =
IM_RES_BWLABEL_REGIONPROPS_Slice',num2str(a),'{:,5}'];
eval(tmp6);
end

% -----Excel Spreadsheet Export-----
Header = {'Slice 1','Slice 2','Slice 3','Slice 4','Slice 5','Slice 6','Slice 7','Slice 8','Slice 9',...
'Slice 10','Slice 11','Slice 12','Slice 13','Slice 14','Slice 15','Slice 16','Slice 17','Slice 18',...
'Slice 19','Slice 20'};
warning off MATLAB:xlswrite:AddSheet
xlswrite('Cellspecs.xls', Header, 'Number');
xlswrite('Cellspecs.xls', NUMBER_PREXLS(1:b,1:NumReg), 'Number', 'A2');
xlswrite('Cellspecs.xls', Header, 'Area');
xlswrite('Cellspecs.xls', AREA_PREXLS(1:b,1:NumReg), 'Area', 'A2');

```

```

xlswrite('Cellspecs.xls', Header, 'Eccentricity');
xlswrite('Cellspecs.xls', ECCEN_PREXLS(1:b,1:NumReg), 'Eccentricity', 'A2');
xlswrite('Cellspecs.xls', Header, 'Solidity');
xlswrite('Cellspecs.xls', SOLID_PREXLS(1:b,1:NumReg), 'Solidity', 'A2');
xlswrite('Cellspecs.xls', Header, 'Orientation');
xlswrite('Cellspecs.xls', ORIENT_PREXLS(1:b,1:NumReg), 'Orientation', 'A2');
winopen('Cellspecs.xls');
toc

```

End Matlab code.

7.3.3 Matlab/COMSOL code for 2D tissue models

The following code, a combination of Matlab and COMSOL functions used in unison, performed all FEA computations of the 2D electrode/tissue interface geometry presented in Chapter 4. The code was modularized due to its length and complexity.

RCGM.m is the controlling code and calls the following successive codes:

COMSOL_version.m, COMSOL_modgeom.m, COMSOL_circdraw.m or

COMSOL_linedraw.m, COMSOL_solve.m, and COMSOL_savedata.m. Each of these is presented here. The program is capable of representing capillaries and vessels either in circular or line-segment fashion with variously constrained geometries. Capillaries and vessels are drawn at random utilizing the appropriate probability density functions (PDFs).

Begin Matlab code for RCGM.m

```

% Generating n random, non-overlapping capillaries (line-segments or
% circles) in a model of the sensor surrounded by bulk tissue. The bulk
% has dimensions of BW x BH and there is a sensor of dimensions MW x MH.
% The capillaries have a max length of 2 x CR and there is an edge
% tolerance of ET. The random distribution of capillaries in the y
% direction is based on a beta distribution with parameters A and B.
clear
tic

```

```

% -----MODEL SETTINGS-----
% For Steady State, ST = 1. For Transient, ST = 2.
ST = 1;
% -----
% For circular capillaries, CAP = 1. For Line Segment capillaries, CAP = 2.
CAP = 1;
% -----
% If capillaries are line segments, HZA = 1 for random horizontal
% arrangement, HZA = 2 for only horizontal capillaries, and HZA = 3 for
% capillaries rotated by +/- ROTDEG from the horizontal.
HZA = 1;
ROTDEG = 22.5;
% -----
% Geometry Values (units of cm)
% Note: CR has to be less than sqrt(((MH*0.99)^2)/2) or 0.00245 in
% order to ensure that the proper boundary values are applied
BW = 0.025    % Bulk Width
BH = 0.05;    % Bulk Height
MH = 0.0035;  % Membrane Height
MW = 0.0125;  % Membrane Width
CR = 0.0003;  % Capillary radius/length
ET = 0.0001;  % Edge Tolerance
% -----
% Reaction Term for the bulk
% Example: RTERM = '-(1.5e-4)*(c>0)'
RTERM = 0; % '-(1.5e-2)*(c>0)';
% -----
% Beta Distribution Width and Height Factor
% idxD = 1 for A+B=5
% idxD = 2 for A+B=10
% idxD = 4 for A+B=20
% idxD = 10 for A+B=50
idxD = 2;
% -----

for idxN = 1:7 % Scaling the number of capillaries from 2-128 (1-7)
    if idxN == 1,
        n=2;
    elseif idxN == 2,
        n=4;
    elseif idxN == 3,
        n=8;
    elseif idxN == 4,
        n=16;
    elseif idxN == 5,
        n=32;
    elseif idxN == 6,
        n=64;
    else idxN == 7,
        n=128;
    end

```

```

for idxA = 1:1 % Scaling of bulk height idxA*0.1 cm (1-3)
  for idxB = 1:5 % 5 - number of repetitions (1-5)
    for idxC = 1:8 % Full range of beta distributions (1-8)
      BH=BH*idxA;
      if idxC == 1,
        A = 0.5*idxD;
        B = 4.5*idxD;
      elseif idxC == 2,
        A = 1*idxD;
        B = 4*idxD;
      elseif idxC == 3,
        A = 2*idxD;
        B = 3*idxD;
      elseif idxC == 4,
        A = 2.5*idxD;
        B = 2.5*idxD;
      elseif idxC == 5,
        A = 3*idxD;
        B = 2*idxD;
      elseif idxC == 6,
        A = 4*idxD;
        B = 1*idxD;
      elseif idxC == 7,
        A = 4.25*idxD;
        B = 0.75*idxD;
      else idxC == 8,
        A = 4.5*idxD;
        B = 0.5*idxD;
      end

      idxA
      idxB
      idxC
      BH
      n
      A
      B

      flclear fem

      COMSOL_version
      COMSOL_modgeom

      if CAP == 1
        COMSOL_circdraw
      elseif CAP == 2
        COMSOL_linedraw
      end

      COMSOL_solve

```

```

        end
    end

    COMSOL_savedata

end
end
toc

```

Begin Matlab code for COMSOL_version.m

```

% COMSOL version
clear vrsn
vrsn.name = 'COMSOL 3.2';
vrsn.ext = 'b';
vrsn.major = 0;
vrsn.build = 304;
vrsn.rcs = '$Name: $';
vrsn.date = '$Date: 2006/04/04 14:56:13 $';
fem.version = vrsn;

```

Begin Matlab code for COMSOL_modgeom.m

```

% Model Geometry
g1=rect2(BW,BH,'base','corner','pos',{(-BW/2),0},'rot','0');
g2=rect2(MW,MH,'base','corner','pos',{(-MW/2),(-MH)},'rot','0');

clear c s Gs
s.objs={g1, g2};
s.name={'R1', 'R2'};
s.tags={'g1', 'g2'};

Gs = '';
Bs = '';
GsA = '';
cobjs='c.objs=';
cname='c.name=';
ctags='c.tags=';

```

Begin Matlab code for COMSOL_circdraw.m

```

% Generating n random circles
i=1;
while i <= n+2
    try
        sobjs='s.objs=';
        sname='s.name=';
        stags='s.tags=';
    end
    i=i+1;
end

```

```

if i > 2
    x1=(((BW-ET)/2)-CR)*rand*randsrc+ET/2;
    y1=((BH-(CR)-ET)*betarnd(A,B))+CR+ET/2;
    eval(['g',int2str(i),'=circ2(x1,y1,CR);']);
end

% Generating the commands "s.objs", "s.name", and "s.tags".
if i >= 2
    Gs = [Gs, 'g', num2str(i-1), ', '];
    Bs = [Bs, "'R', num2str(i-2), ''', ' '];
    GsA = [GsA, "'g', num2str(i-1), ''', ' '];
end

sobjs = [sobjs, Gs, 'g', num2str(i), ''];
sname = [sname, Bs, "'R', num2str(i-1), ''', ' '];
stags = [stags, GsA, "'g', num2str(i), ''', ' '];

eval(sobjs)
eval(sname)
eval(stags)

% Verifying the number of boundaries and subdomains. If too
% many are created, indicates overlap and last drawn element is
% deleted and redrawn.

fem.draw=struct('s',s);
fem.geom=geomcsg(fem);
[nmr,nbs] = geominfo(fem.geom, 'Out', {'nmr','nbs'});

if i < n+2
    clear sobjs sname stags
end

if i > 2
    if nmr > i
        clearfxn = ['clear g', num2str(i)];
        eval(clearfxn)
        i = i
    elseif nbs > 9+((i-2)*4)
        clearfxn = ['clear g', num2str(i)];
        eval(clearfxn)
        i = i
    else
        i = i+1;
    end
else
    i=i+1;
end
end
end

```

```
end

eval(sobjs)
eval(sname)
eval(stags)

%fem.draw=struct('c','c','s','s');
fem.draw=struct('s','s');
fem.geom=geomcsg(fem);

% Calculating the number of subdomains (nmr) and boundary segments (nbs)
[nmr,nbs] = geominfo(fem.geom, 'Out', {'nmr','nbs'});

% Initialize mesh
fem.mesh=meshinit(fem);

% Application mode 1
clear appl
appl.mode.class = 'FIDiffusion';
appl.border = 'on';
appl.assignsuffix = '_di';
clear bnd
bnd.type = {'N0','cont','C','C'};
bnd.c0 = {0,0,0,4.3e-8};

IndB = '';
for i=10:nbs
    bndind = 'bnd.ind = [1,1,1,1,3,2,1,1,1,'];
    IndB = [IndB, '4', ', ', ''];
end

bndind = [bndind, IndB, ''];
eval(bndind)

% bnd.ind = [1,1,1,3,1,3,4,2,3,1,3,1,1,5,5,5,5,5,5,5];

appl.bnd = bnd;
clear equ
equ.D = {2.3e-5,3.2e-5,2.3e-5};
equ.init = {0,0,4.3e-8};
equ.R = {RTERM,0,0}; % Reaction terms for subdomains. Example: equ.R = {'-(1.5e-4)*(c>0)',0,0};

IndD = '';
for i = 3:nmr
    equind = 'equ.ind = [1,2,'];
    IndD = [IndD, '3', ', ', ''];
end

equind = [equind, IndD, ''];
```

```

eval(equind)

% equ.ind = [1,2,3,1,4,4];

appl.equ = equ;
fem.appl{1} = appl;
fem.frame = {'ref'};
fem.border = 1;
fem.outform = 'general';
fem.units = 'cgs.m';
EBN = 5;

```

Begin Matlab code for COMSOL_linedraw.m

```

% Generating n random line-segments
i=3;
while i <= n+3
    try
        cobjs='c.objs=';
        cname='c.name=';
        ctags='c.tags=';

        if HZA == 1
            % Random rotational orrientation
            x1=(((BW/2)-CR)*rand*randsrc);
            x2=x1+(rand*CR*randsrc);
            y1=((BH-(2*CR))*betarnd(A,B))+CR;
            y2=y1+(rand*randsrc*CR);
            eval(['g',int2str(i),'=curve2([x1,x2],[y1,y2]);']);
        elseif HZA == 2
            % Purely horizontal rotational orrientation
            x1=(((BW/2)-CR)*rand*randsrc);
            x2=x1+(rand*CR*randsrc);
            y1=((BH-(2*CR))*betarnd(A,B))+CR;
            y2=y1;
            eval(['g',int2str(i),'=curve2([x1,x2],[y1,y2]);']);
        elseif HZA == 3
            % Horizontal orrientation +/- ROTDEG degrees
            x1=(((BW/2)-CR)*rand*randsrc);
            x2=x1+(rand*CR*randsrc);
            y1=((BH-(2*CR))*betarnd(A,B))+CR;
            y2=y1;
            PR=[((x1+x2)/2), ((y1+y2)/2)];
            eval(['g',int2str(i),'=curve2([x1,x2],[y1,y2]);']);
            eval(['g',int2str(i),'=rotate(g',int2str(i),' , randsrc*rand*2*pi*(ROTDEG/360), PR);']);
        end

        % Generating the commands "c.objs", "c.name", and "c.tags".
        if i >= 4
            Gs = [Gs, 'g', num2str(i-1), ', '];

```

```

    Bs = [Bs, "'C', num2str(i-3), '' , ' '];
    GsA = [GsA, "'g', num2str(i-1), '' , ' '];
end

cobjs = [cobjs, Gs, 'g', num2str(i), ''];
cname = [cname, Bs, "'C', num2str(i-2), '' , ' '];
ctags = [ctags, GsA, "'g', num2str(i), '' , ' '];

eval(cobjs)
eval(cname)
eval(ctags)

fem.draw=struct('c','c','s','s');
fem.geom=geomcsg(fem);
[nmr,nbs] = geominfo(fem.geom, 'Out', {'nmr','nbs'});
clear fem.geom

if i < n+3
    clear cobjs cname ctags
end

% Checking for excess domains and boundaries indicating
% overlap. If overlap detected, the last geometry object
% drawn is deleted and re-drawn.
if i > 2
    if nmr > 2
        clearfxn = ['clear g', num2str(i)];
        eval(clearfxn)
        i = i
    elseif nbs > 9+(i-2)
        clearfxn = ['clear g', num2str(i)];
        eval(clearfxn)
        i = i
    else
        i = i+1;
    end
else
    i=i+1;
end
end
end

eval(cobjs)
eval(cname)
eval(ctags)

%fem.draw=struct('c','c','s','s');
fem.draw=struct('c','c','s','s');
fem.geom=geomcsg(fem);

```

```

% Calculating the number of subdomains (nmr) and boundary segments (nbs)
[nmr,nbs] = geominfo(fem.geom, 'Out', {'nmr','nbs'});
% Calculating the vertex numbers (se) and vertex coordinates (mp)
% for each boundary segment
[se,mp] = geominfo(fem.geom, 'Out', {'se','mp'});

% Calculating the length of each boundary segment
for j = 1:(n+10)
    BIG1(j,1) = j;
    BIG1(j,2) = se(1,j);
    BIG1(j,3) = se(2,j);
end
for j=1:((2*n)+10)
    BIG2(j,1) = mp(1,j);
    BIG2(j,2) = mp(2,j);
end
for j=1:(n+10)
    BIG4(j,1)=j;
    for k=1:((2*n)+10)
        if BIG1(j,2) == k;
            BIG4(j,1) = j;
            BIG4(j,2) = BIG2(k,1);
            BIG4(j,3) = BIG2(k,2);
        end
        if BIG1(j,3) == k;
            BIG4(j,4) = BIG2(k,1);
            BIG4(j,5) = BIG2(k,2);
        end
    end
end
for j=1:(n+10)
    BIG4(j,6) = sqrt(((BIG4(j,4) - BIG4(j,2))^2) + ((BIG4(j,5) - BIG4(j,3))^2));
end

% Initialize mesh
fem.mesh=meshinit(fem);

% Application mode 1
clear appl
appl.mode.class = 'FIDiffusion';
appl.border = 'on';
appl.assignsuffix = '_di';
clear bnd
bnd.type = {'N0','cont','C','C'};
bnd.c0 = {0,0,0,4.3e-8};

% Assigning boundary conditions based on the length of the boundary
% segment and identifying the boundary number of the electrode
IndB = ' ';
j=1

```

```

while j <= (n+10)
    BIG4(j, 6);
    j;
    if BIG4(j,6) < MH + (0.01 * MH) & BIG4(j,6) > MH - (0.1 * MH)
        IndB = [IndB, '1,'];
        A = 'case1';
        j=j+1;
    elseif BIG4(j,6) >= (BW-MW)/2
        IndB = [IndB, '1,'];
        A = 'case2';
        j=j+1;
    elseif BIG4(j,6) < MH
        IndB = [IndB, '4,'];
        A = 'case3';
        j=j+1;
    elseif BIG4(j,6) < MW + (0.01 * MW) & BIG4(j,6) > MW - (0.01 * MW) & BIG4(j,3) < 0 + 0.0001...
        & BIG4(j,3) > 0 - 0.0001
        IndB = [IndB, '2,'];
        A = 'case4A';
        j=j+1;
    elseif BIG4(j,6) < MW + (0.01 * MW) & BIG4(j,6) > MW - (0.01 * MW) & BIG4(j,3) > -...
        (MH + (0.01 * MH)) & BIG4(j,3) < -(MH - (0.01 * MH))
        IndB = [IndB, '3,'];
        A = 'case4B';
        EBN = j;
        j=j+1;
    end
end

bndind = 'bnd.ind = [';
bndind = [bndind, IndB, ','];
eval(bndind)

%Assigning domain conditions
appl.bnd = bnd;
clear equ
equ.D = {2.3e-5,3.2e-5};
equ.init = {0,0};
equ.R = {RTERM,0}; % Reaction terms for subdomains. Example: equ.R = {'-(1.5e-4)*(c>0)',0,0};
equ.ind = [1,2]; % equ.ind = [1,2,3,1,4,4];

appl.equ = equ;
fem.appl{1} = appl;
fem.frame = {'ref'};
fem.border = 1;
fem.outform = 'general';
fem.units = 'cgs.m';

```

Begin Matlab code for COMSOL_solve.m

```
% Multiphysics
fem=multiphysics(fem);

% Extend mesh
fem.xmesh=meshextend(fem);

if ST == 1;
    % Solve problem
    fem.sol=femlin(fem, 'solfile','on', 'solcomp',{'c'}, ...
        'outcomp',{'c'});

    % Save current fem structure for restart purposes
    fem0=fem;

    % Integrate
    BIF(idxC,idxB)=postint(fem,'ndflux_c_di', ...
        'dl',[EBN], 'edim',1);
else
    % Solve problem
    fem.sol=femtime(fem, 'solfile','on', 'solcomp',{'c'}, ...
        'outcomp',{'c'}, 'tlist',[0:5:1750], ...
        'atol',{'1e-8'}, 'rtol',1e-7, 'tout','tlist');

    % Save current fem structure for restart purposes
    fem0=fem;

    % Integrate
    BIF(idxC,idxB)=postint(fem,'ndflux_c_di', 'dl',[EBN], ...
        'edim',1, 'solnum','end');
end
```

Begin Matlab code for COMSOL_savedata.m

```
% File saved as BIFfile_nN_BHXXX_BetaY__STZ_CAPW_HZAV_DATE.mat where N is the number of
% capillaries modeled, XXX is the thickness of the bulk tissue, Y
% is the Beta Distribution form (A+B), Z is the model solution type
% (steady state or transient), W is the capillary model form, V
% is capillary horizontal alignment, and DATE is the date the calculation was completed on.
eval(['save BIFfile_n', num2str(n),'_BH',num2str(idxA),'00_', 'Beta',num2str(idxD*5),'_ST',...
    num2str(ST), '_CAP',num2str(CAP),'_HZA', num2str(HZA),'_Date_',date, ' BIF']);
```

End Matlab code.

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