INVESTIGATING THE TEMPORAL EVOLUTION OF THE CEREBRAL HEMODYNAMIC RESPONSE USING DIFFUSE OPTICAL TOMOGRAPHY

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Abstract

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The primary goal of this dissertation is to provide evidence to support the hypothesis that near-infrared optical measurement of brain function relies upon similar neurovascular coupling mechanisms and measures the same hemodynamic changes as does functional magnetic resonance imaging. To test this assertion, instrumentation was constructed and methodologies were developed to replicate a sequence of rodent experiments in which an electrical stimulus to the forepaw elicited a spatially localized hemodynamic response within the somatosensory region of the cerebral cortex. The temporal evolution of this response, previously measured with functional magnetic resonance imaging, was then measured using diffuse optical tomography, and the results of both measurements are compared and discussed.

In order to perform these experiments, a number of technical issues had to be addressed. Instrumentation capable of acquiring noninvasive real-time imagery of vascular events within the cerebral cortex with minimal temporal distortion had to be designed and tested. This investigation led to the evolution of a series of optical tomographic instruments and the development of a new optical source encoding technique, Pulse TDM, which provides for advanced capabilities like individualized gain control, while significantly reducing the degree of temporal skew in the optical data.

Special surgical and anesthetic techniques which maintained normal cerebral hemodynamic activity while providing sufficient analgesia for stereotactic headgear had to be learned and mastered. To meet this need, custom surgical, biomonitoring, anesthesia, and stimulus equipment was developed.

Although the temporal hemodynamic measurements themselves comprise the core of this dissertation, they represent only a small fraction of the work involved in reaching that goal. To emphasize this point, the dissertation includes chapters which discuss topics vital to biophysical experimentation, including vascular physiology, electro-optical instrument design, and anesthesia.

The ethical implications of experimentation with live subjects is a topic which is often eschewed by many researchers in the field of biophysics. Because this is an important topic, the ethical and legal responsibilities involved in animal and human experimentation are discussed in the Appendix.

Since some electronic components are common to many DOT instruments, a number of datasheets are included in the Appendix as a convenience for the more technically-minded readers. Likewise, complete schematics of the bioinstrumentation developed for these measurements is provided, in an effort to assist others in replicating this circuitry for their own research.

Acknowledgements

As an inevitable consequence of my decision to pursue my doctoral degree part-time, the majority of my efforts were spent at home or in the lab. I thus had little time to enjoy the freedoms of being a graduate student, and probably appeared to others as impatient at times (which was true, since I had to accomplish in one day the various and sundry beaurocratic tasks which others had five days to perform). As a result, I needed to rely on the assistance of my colleagues and friends, perhaps in different ways than would others. To all of you whom I may have offended or annoyed in my quest for alacrity – thank you for putting up with me. I hope that the Tootsie Rolls helped . . .

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Table of Contents

List of Tables

List of Figures

List of Abbreviations

ABG	Arterial Blood Gas (measurement)
AC	Alternating Current
ADC, A/D	Analog-to-Digital Converter
ADP	Adenosine Diphosphate
AM	Amplitude Modulation.
ANS	Autonomic Nervous System
APD	Avalanche Photodiode
AR	Antireflection (optical coating)
ART	Algebraic Reconstruction Technique
ATP	Adenosine Triphosphate
B/G	Blood/Gas partition coefficient
BNC	Bayonet Nut Connector (a standard coaxial connector)
BOLD	Blood Oxygen Level Dependent
BP	Blood Pressure
BW	Bandwidth
CBF	Cerebral Blood Flow
CBV	Cerebral Blood Volume
cc, ml	Cubic Centimeter, Milliliter (interchangeable)
CGA	The Compressed Gas Association (standardizes gas valve fittings)
cGMP	Cyclic Guanosine Monophosphate
CMOS	Complementary Metal Oxide Semiconductor
CMRglu	Cerebral Metabolic Rate of glucose consumption
$CMRO_2$	Cerebral Metabolic Rate of Oxygen consumption
CMRR	Common-Mode Rejection Ratio
CNS	Central Nervous System
CO_2	Carbon Dioxide
cP	CentiPoise, 0.01P (a measure of viscosity)
CPAP	Constant Positive Airway Pressure
CPV	Cerebral Plasma Volume
CSF	Cerebrospinal Fluid
C-T	Computed (X-ray) Tomography
CW	Continuous Wave
CW1, CW4	Internal designations for our continuous wave DOT instruments
dB	Decibel, = 0.1 Bel
DC	Direct Current
dl	Deciliter, 100ml

DN Digital Number (a single digital count, 1 LSB)
DNA Deoxyribonucleic Acid
DNR Dynamic Range
DOT Diffuse Optical Tomography
ECG Electrocardiogram
EDRF Endothelium-Derived Relaxing Factor
EEG Electroencephalogram
EMI Electromagnetic Interference
EPI Echo-Planar Imaging (MRI)
etCO_2 End Tidal CO_2 (concentration)
eV Electron-Volt
FBR The Foundation for Biomedical Research
FDA The United States Food and Drug Administration
^{18}FDG Fluoro Deoxy Glucose, made with ^{18}Fluorine, a radioactive isotope
FDM Frequency-Division Multiplexing
FFT Fast Fourier Transform
fiO_2 Fractional inspired oxygen concentration
FM Frequency Modulation
fMRI Functional Magnetic Resonance Imaging
FOV, IFOV Field Of View, Instantaneous Field Of View
FWHM Full Width at Half-Maximum
g The anisotropy factor, for angular scattering (unitless)
GBWP Gain-Bandwidth Product
H_2O Water
H_2S Hydrogen Sulfide
Hb, HbR Deoxyhemoglobin
HbCO, COHb Carboxyhemoglobin
HbO_2 Oxyhemoglobin
Hbt Total Hemoglobin
Hi Methemoglobin, Hemiglobin
HPD Hematoporphyrin Derivative
HR Heart Rate
HR High Reflectivity (optical coating)
HSD Harlan Sprague-Dawley (rat)
Hz Hertz, cycles/sec
IACUC Institutional Animal Care and Use Committee
I/E Inspiration/Expiration (ratio)
I/Q In-phase + Quadrature (demodulation).
ICG Indocyanine Green (dye)

IDC	Insulation Displacement Connector
IEC	The International Electrotechnical Commission (a standards organization)
IF	Intermediate Frequency (demodulation)
IGC	Individualized Gain Control
IM	Intramuscular (injection)
IP	Intraperitoneal (injection)
IRB	Internal Review Board
IV	Intravenous (injection)
kcal	Kilocalorie, = 1 Calorie
kg	Kilogram, = 1000 grams
kHz	Kilohertz, = 1000Hz
ksps	Kilosamples Per Second (ADC sampling rate)
LD	Laser Diode
LDPE	Low Density Polyethylene
LED	Light Emitting Diode
LIDAR	Light Detection And Ranging
LSB	Least Significant Bit
LSF	Least Square Fit (line)
MAC	Minimum Alveolar Concentration (anesthesia)
MAP	Mean Arterial (blood) Pressure
mg	Milligram
mg/dl	Milligrams per Deciliter
MGH	Massachusetts General Hospital
MHz	Megahertz, = 1000kHz
mHz	Millihertz, = 0.001Hz
mm	Millimeter
mmHg	Millimeters of mercury column, same as Torr
MRI	Magnetic Resonance Imaging
MUR	Maximum Unambiguous Range (radar/sonar)
MUX	Multiplexer
mW	Milliwatts
n	The index of refraction, in the near-IR spectral band
N_2O	Nitrous Oxide
NAD	Nicotinamide Adenine Dinucleotide (the reduced form of NADH)
NADH	The protonated form of Nicotinamide Adenine Dinucleotide
ND	Neutral Density (attenuating filter)
NIR	The Near-Infrared spectral band, 600nm to 900nm
NIRS	Near-Infrared Spectroscopy
NIRS3	Our internal designation for a near-infrared spectroscopic instrument

nm	Nanometer
NO	Nitric Oxide
NTC	Negative Temperature Coefficient (thermistor)
O/W	Oil/Water partition coefficient
O_2	Oxygen
OCT	Optical Coherence Tomography
OFM	The Optical Fetal Monitor (equipment)
Paw	Proximal airway pressure
PCB, PWB	Printed Circuit Board, same as Printed Wiring Board
PCMCIA	Personal Computer Memory Card International Association
pCO_2	The partial pressure of carbon dioxide
PDF	Probability Density Function
PE	Polyethylene
PEEP	Positive End Expiratory Pressure
PET	Positron Emission (transaxial) Tomography
PeTA	People for the ethical Treatment of Animals
PETN	Pentaerythritol Tetranitrate
pH	The negative log of the hydrogen ion (proton) concentration
PIN	Positive-Intrinsic-Negative (lateral doping density within a photodiode)
PM	Phase Modulation
PMMA	Poly Methyl Methacrylate (acrylic plastic used for constructing optical fibers)
PMT	Photomultiplier Tube
PNS	Peripheral Nervous System
pO_2	The partial pressure of oxygen
psi	Pounds per Square Inch. 1psi = 60mmHg
PTFE	Polytetrafluoroethane, Teflon
Pulse-TDM	Pulse-Modulated Time-Division Multiplexing
PVC	Polyvinyl Chloride
QRS	The portion of the ECG waveform generated by ventricular depolarization
RBCs	Red Blood Cells, erythrocytes
RC	Resistor-Capacitor (filter)
RF	Radio Frequency
RFI	Radio Frequency Interference
RGD	Rayleigh-Gans-Debye (scattering)
RTV	Room Temperature Vulcanizing (silicone)
saO_2	Arterial oxygen saturation
SC	Subcutaneous (injection)
SCR	Silicon Controlled Rectifier (a silicon reverse-blocking triode thyristor)
SCUBA	Self-Contained Underwater Breathing Apparatus

S-D	Source-Detector (with regard to optode placement)
SHb	Sulfhemoglobin
SIRT	Simultaneous Iterative Reconstruction Technique
SMA	Surface Mount, type A (a connector style used for both RF and fiberoptics)
SNR	Signal to Noise Ratio
spO_2	Pulse-based arterial oxygen saturation
SS-TDM	Switched-Source Time-Division Multiplexing
SVD	Saturated Vapor Dilution (vaporizer), Singular Value Decomposition (math)
T	Tesla
TCG	Truncated Conjugate Gradient
TDM	Time-Division Multiplexing
TiO_2	Amorphous Titanium (IV) dioxide, also referred to as Anatase
TPSF	Temporal Point Spread Function
TSVD	Truncated Singular Value Decomposition
u_a	The absorption coefficient, with units of 1/length
um, μm	Micron, Micrometer (same)
US	Ultrasound
u_s'	The reduced scattering coefficient, with units of 1/length
UV	Ultraviolet
VAC	Volts of Alternating Current
VDC	Volts of Direct Current
VGA	Variable Gain Amplifier
VHF	Very High Frequency (a band extending from 30MHz to 300MHz)
VIC	(Anesthetic) Vaporizer-Inside-Circuit
VOC	(Anesthetic) Vaporizer-Outside-Circuit
V-Q	Ventilation-Perfusion (matching)
W	Watts (for power measurement)
ΔX	The change in "X," with respect to a previous value
[x]	The molar concentration of "x," in units of moles/liter

1: Introduction

Diffuse Optical Tomography

Diffuse optical tomography (DOT) is a noninvasive neuroimaging technique which exploits both the spectrally varying absorption and diffuse scattering nature of near-infrared light. DOT can directly and simultaneously measure concentration changes in deoxy-hemoglobin ([Hb]), oxy-hemoglobin ([HbO_2]), and total hemoglobin ([HbT] = [Hb] + [HbO_2]) in cortical tissue with excellent temporal resolution [1].

Other neuroimaging modalities, including functional Magnetic Resonance Imaging [2, 3], Positron Emission Tomography (PET) [4], magnetoencephalography (MEG) [5-7] and electroencephalography (EEG) [6] are also capable of monitoring neural and/or metabolic activity within the brain, however DOT offers a number of important advantages over many of these well-established techniques. These advantages, along with some disadvantages, will be discussed in further detail below.

fMRI has led to significant advances in neuroimaging during the last decade. It allows near real-time observation of the hemodynamic response to neuronal activation. The blood oxygen level dependent (BOLD) fMRI signal scales approximately with absolute changes in [Hb], however questions still remain about the exact relationship between the BOLD signal and the vascular response. Likewise the coupling between neural activity and the vascular response itself is poorly understood. Since MRI and DOT are based upon different fundamental principles, cross-validation can help us to better understand both the neurophysiology behind cortical activation and the biophysics behind the measurement techniques themselves.

Others have observed qualitative correspondence between the fMRI BOLD signal and diffuse optical measures of [Hb] and [HbO_2] in humans, however to date there has been no quantitative examination of the temporal correlation between fMRI and DOT. Prior measurements have revealed much about the time course and spatial extent of cerebral blood volume and BOLD signals following median nerve stimulation in a rodent model. Since this preparation has been well characterized with fMRI, it was chosen as a convenient model to cross-validate fMRI and DOT. Therefore the primary objective of the research presented in this dissertation will be to quantitatively examine the temporal correlation between fMRI and DOT measurements of the cerebral hemodynamic response. To this end, measurements of the temporal evolution of the hemodynamic response to electrical forepaw stimulation in rat somatosensory cortex with fMRI and DOT will be compared and discussed.

Spatial and temporal resolution of current neuroimaging techniques

The temporal and spatial performance of four neuroimaging techniques is depicted in Figure 1.1 [8]. MEG provides excellent temporal resolution but only modest spatial resolution [7]. fMRI offers better spatial resolution than DOT, however decreasing MRI voxel size significantly reduces the contrast-to-noise ratio of the measurements. This necessitates the averaging of multiple trials to provide useable images. DOT can provide excellent temporal response, however its spatial resolution is limited by the diffuse nature of scattered light, which must travel through the scalp and skull twice before being detected. Since PET measurements entail the injection of a short-lived radionuclide and the monitoring of its subsequent nuclear decay, its temporal response is on the order of many minutes, which is inadequate for capturing cerebral metabolic activity in real-time.

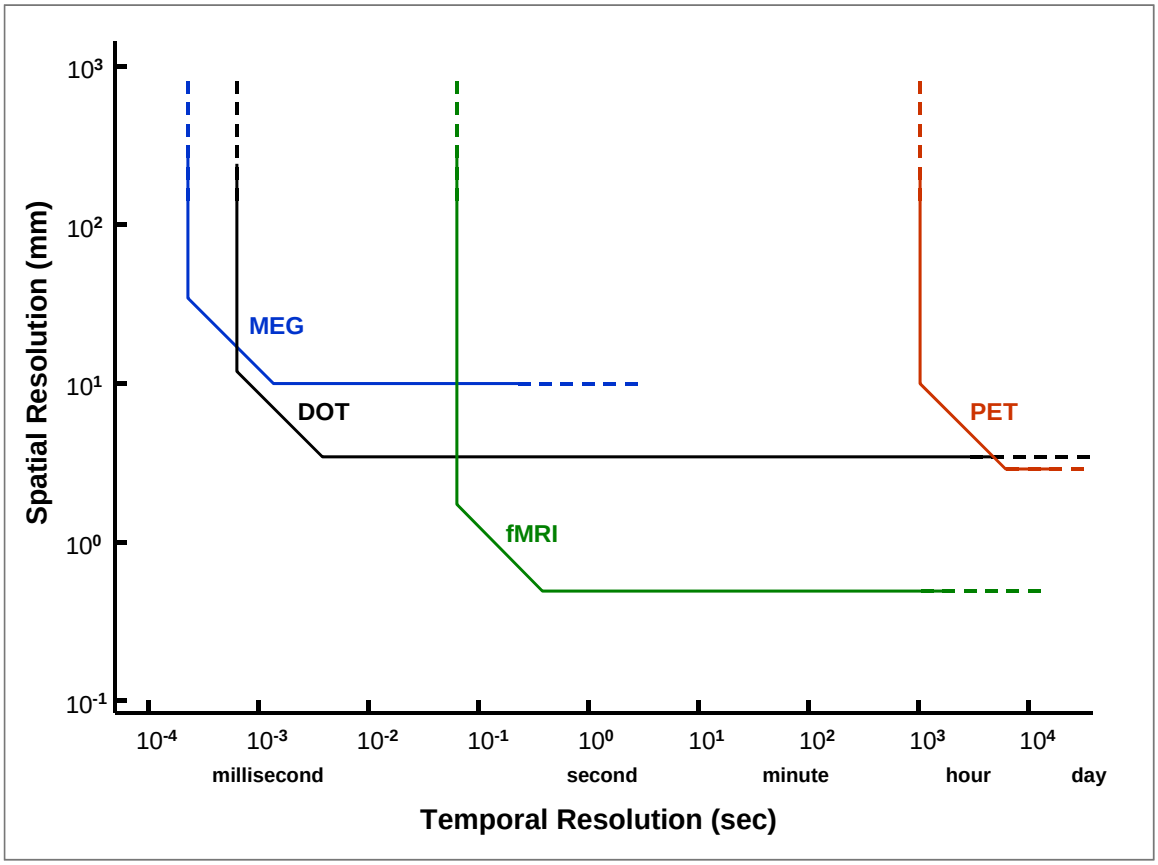

Figure 1.1. A notional diagram comparing the spatial and temporal performance of four minimally invasive neuroimaging techniques. The diagonal "chamfers" represent the improvement in spatial resolution with increased measurement time, a feature of all four modalities. MEG can provide excellent temporal resolution, but only modest spatial resolution. fMRI offers better spatial resolution than DOT, but this comes with a cost of only modest temporal response. DOT can provide excellent temporal response, perhaps rivaling MEG, however its spatial resolution is limited by the diffuse nature of scattered light in tissue [8]. PET can monitor cerebral metabolic activity with spatial resolution comparable to DOT, however each measurement requires about 10 minutes to complete, so real-time monitoring of metabolic activity is not possible [4].

Potential disadvantages of MEG and fMRI as neuroimaging tools

DOT offers a number of important advantages over both MEG and fMRI, both of which involve the use of large, heavy, and expensive equipment. Often, an entire portion of a building must be dedicated to these measurements, since elaborate magnetically shielded enclosures must be constructed to both shield the equipment from ambient magnetic fluctuations and in certain cases (high-field MRI) to protect the outside environment from the emanation of stray magnetic fields.

Both require the use of cryogens or mechanical coolers to maintain temperatures low enough to sustain superconduction. Modern fMRI systems employ superconducting magnets to provide the large (1.5 to 7 Tesla) static magnetic field to induce proton precession and MEG systems use superconducting Josephson junctions to detect very small changes in magnetic field caused by electronic and ionic currents within the brain [7].

Both MEG and fMRI also require that the subject remain motionless to within the order of a voxel over the duration of the experiment, or else spatial resolution could be severely compromised. This makes both MRI and MEG measurements of uncooperative subjects (animals and infants, for example)

nearly impossible without pharmacologic intervention. Although anesthesia will render a subject motionless for many hours, it can also severely affect normal neural function, while making voluntary response measurements nearly impossible.

Potential advantages of DOT over MEG and fMRI for neuroimaging

DOT systems can be constructed to be physically compact, lightweight, and energy-efficient [9, 10]. Portable DOT systems can be constructed to conform to just about any environment which is compatible with human life. Since DOT can employ flexible fiberoptic light guides, a modest amount of motion can be tolerated during DOT measurements [8, 10]. Monitoring the regional cerebral blood volume and oxygenation of infants in the NICU, astronauts in orbit, or technical divers undergoing decompression – feats clearly difficult or even impossible with fMRI or MEG – are possible with DOT.

An important feature of DOT not shared by fMRI or MEG is the capability to rapidly and simultaneously measure changes in both local cerebral blood volume (CBV), deoxyhemoglobin concentration ([Hb]), and oxyhemoglobin concentration ($[HbO_2]$) within the brain [8, 11]. fMRI can measure changes in blood volume, but is only sensitive to absolute changes in deoxyhemoglobin, from which blood oxygenation changes must then be inferred [12]. PET can measure the cerebral metabolic rate of glucose consumption (CMRglu), the cerebral metabolic rate of oxygen consumption ($CMRO_2$), and cerebral blood flow (CBF), however each measurement can take more than 10 minutes to complete [4, 13].

Since DOT can provide capabilities unavailable with any other neuroimaging techniques, there is significant interest in better understanding the capabilities and limitations of DOT as a neuroimaging modality.

Features of DOT

DOT is painless, noninvasive, and simple to use. It can be performed inexpensively and safely on nearly any patient at any time, in contrast to other imaging modalities like C-T and MRI. If the optode assemblies are properly designed, patients are afforded more freedom of movement during DOT imaging than with either C-T or MRI. Since DOT equipment can be portable, the DOT system can be wheeled to the patient, instead of vice-versa, thus reducing the added risk to the patient.

Spectroscopic information about the sample can be obtained in real-time. This can then be processed to yield three-dimensional maps of both blood oxygenation and CBV vs. time. With DOT, exogenous contrast agents are not required, and metabolic information can be monitored continuously, unlike with PET.

Limitations of DOT

DOT can provide only limited spatial resolution, which decreases with increasing penetration depth. This is an unavoidable consequence of the nature of diffusely scattered light: there is an inexorable increase in entropy, and hence a loss in spatial information, as the result of every scattering event which occurs inside the tissue. Although DOT cannot replace either MRI or C-T for obtaining deep tissue metabolic and structural information, it will likely serve as an adjunct to such modalities, providing details on metabolism and perfusion in human subjects not easily obtained otherwise.

Problems with performing accurate DOT include compensating for partial-volume effects and the loss in hemodynamic specificity at the sub-millimeter level, possibly due to significant contributions from surface vessels [14]. (fMRI has shown that CBF changes remain specific, even down to sub-mm functional domains [15]).

Uncertainties with fMRI and DOT as neuroimaging tools

fMRI is currently one of the preferred modalities for neuroimaging because it can provide both structural and metabolic information. Since fMRI and DOT are both believed to monitor brain function indirectly through measurements of blood flow and blood oxygenation changes within the brain, yet require vastly differing financial investments to construct and operate, it is important to explore the relative merits of DOT as a modality for neuroimaging applications. If fMRI and DOT share similar spatial, temporal, chemical, and vascular sensitivities throughout the cerebral cortex, then DOT could serve as a simple, safe, and low-cost substitute for fMRI in many neuroimaging applications.

If, on the other hand, fMRI and DOT differ in one or more of these sensitivities, then the possibility exists that, to the extent of their orthogonality, both techniques may complement each other, thus providing an augmented neuroimaging capability unavailable from each technique used independently.

However, in order to exploit the benefits of either case, the exact nature of how and what each modality measures in-vivo must be determined. Simply combining the data from a new, yet poorly understood, modality with that from another well-characterized modality would result in little, if any, additional knowledge.

fMRI appears to be uniformly sensitive to blood flow and oxygenation changes within the volume of the brain, except in the regions surrounding large blood vessels [12]. Although the mechanisms underlying the use of superparamagnetic contrast agents to monitor changes in Cerebral Blood Volume (CBV), or more correctly, Cerebral Plasma Volume (CPV) are well understood [12, 16, 17], evidence for the origin of the Blood Oxygen Level Dependent (BOLD) signal is still not conclusive [12]. It is believed to result from the metabolic reduction of slightly diamagnetic oxyhemoglobin (HbO_2) to form weakly paramagnetic deoxyhemoglobin (Hb). The Hb then perturbs the local magnetic environment, thus generating the BOLD signal. As a result, the carrier to noise ratio of the CBV measurement is proportional to the fractional change in CBV, while the carrier to noise ratio of the BOLD signal is proportional to the absolute change in [Hb] [12].

DOT, on the other hand, shows greater sensitivity to flow and oxygenation changes in vessels closer to the cortical surface [14, 18], although the sensitivity near large vessels may actually be reduced [18]. This follows from the volume weighting function for DOT, illustrated by Figures 1.1 and 2.2, which show proportionally greater sensitivity to blood volume and oxygenation changes occurring nearer to the optodes and within the "banana." The light transiting larger vessels may be so strongly absorbed that, despite the large modulation depth imposed by the high blood volume fraction, the amount of light actually reaching the sensing optode may fall below the detector noise floor and thus be missed [18].

Research objectives and issues to be addressed within this dissertation

This dissertation will attempt to answer some of these questions by comparing DOT and fMRI measurements of the temporal evolution of the cerebral hemodynamic response.

Many researchers have observed qualitative spatial [19-21] and temporal [22] correspondence between the fMRI BOLD signal and diffuse optical measures of deoxyhemoglobin concentration ([Hb]) and oxyhemoglobin concentration ($[HbO_2]$) in humans. However to date there has been no quantitative examination of the temporal correlation between fMRI and DOT in similar preparations. The observation that the rise in Cerebral Blood Flow (CBF) in upper cortical layers preceded the rise in lower layers [23] suggests that differences in the volume weighting functions between fMRI and DOT may be revealed through comparison of temporal measurements of the cerebral hemodynamic response by both modalities.

Previous measurements of rat somatosensory cortex have revealed much about the time course and spatial extent of CPV and BOLD signals following median nerve stimulation in a rodent model [12, 16, 17, 24-27].

Since the rodent forepaw stimulation preparation has been well characterized with fMRI, and due to its geometry, would afford a similar spatial matching in voxel size between fMRI and DOT, it was chosen as a convenient model to cross-validate fMRI and DOT. Thus the principal objective of this dissertation will be to quantitatively compare the temporal evolution of the cerebral hemodynamic response as measured with fMRI and DOT using the rodent preparation, and to use these measurements to better understand both the matching of sensitivities between fMRI and DOT and to learn more about the origin and nature of the cerebral hemodynamic response.

Researchers using other optical imaging modalities have observed an amplitude dependence of the vascular response on stimulation parameters such as the pulse frequency and stimulus duration [28]. This is likely a fruitful topic that is relatively easy to explore with our preparation, so the effects of stimulus repetition rate, duration, and magnitude will also be investigated, both to determine the robustness of this preparation and to further explore the physiology underlying the neurovascular response.

Preparing for these rodent measurements required a significant hardware development effort. New, faster DOT systems capable of recording at frame rates sufficient to capture the temporal features of the hemodynamic response were required. Anesthesia and biomonitoring instrumentation with performance superior to that available commercially was developed in order to maintain hemodynamic stability during these experiments. Hence the secondary objective of this dissertation will be to discuss the development of the diffuse optical and biomedical instrumentation specifically designed for in-vivo DOT measurements.

Chapter summary

The first five chapters of this dissertation were written to both educate the reader in preparation for the discussion of the experimental results presented in Chapter 6, and to explore the evolution of DOT instrumentation which ultimately enabled spatially resolved measurements at frame rates fast enough to examine the temporal features of the hemodynamic response. Chapter 7 summarizes the many conclusions drawn and lessons learned throughout this dissertation, and the Appendix contains supplementary information relevant to a number of topics which may also interest the reader.
Introduction

Diffuse optical tomography is based upon the diffusive transport of light in tissue, so the principles behind DOT, and the unique nature of diffuse imaging, are briefly covered in the Introduction. In order to compare DOT with other neuroimaging techniques, the principles behind other metabolic and neuroimaging modalities, including functional magnetic resonance imaging (fMRI) in particular, are also presented. The relative merits of fMRI and DOT are mentioned, with emphasis on their similarities as neuroimaging tools. Prior research citations are then used to reveal the current level of uncertainty concerning the temporal, vascular, and spatial sensitivities of both modalities, which leads to the specific questions which motivated the pursuit of this dissertation.

Chapter 2

The concept of diffuse imaging is introduced in Chapter 2 , and the basic principles of CW, RF, and time-domain instrumentation are all discussed. Chapter 2 begins by introducing the reader to the basic optical principles behind optical imaging, and then discusses light propagation and scattering in turbid media. Issues such as the pathlength correction factor and scattering anisotropy are also covered. Distinctions are made between how ballistic, “snake,” and diffusely scattered light are best modeled. The relative merits and disadvantages of both ballistic and diffuse imaging are also explored.

Since the quality of DOT image reconstruction is a direct function of the accuracy with which diffusive light transport through the medium is modeled, Chapter 2 presents a number of optical transport and scattering models for light propagation through scattering media. Multiple scattering models are then discussed, and the concepts behind the basic diffusion theory are introduced. Since the DOT measurements discussed in this dissertation were all performed in living tissue, the many sources of optical absorption and scattering in biological tissues are then presented, and the important distinction between static and dynamic absorbers and scatterers is made.

DOT image reconstruction is then discussed at an overview level. The theoretical and numerical techniques involved in reconstructing images from raw optical measurements are reviewed. Both the forward and inverse problems are explained, and a number of common algorithms are discussed. The complexities introduced by the Ill-posed and underdetermined nature of the matrices involved in DOT image reconstruction are explained, and the concept of regularization is then discussed.

Chapter 3

In order to draw meaningful conclusions from diffuse optical measurements of brain function, the physiology behind neurovascular coupling and the cerebral hemodynamic response must be understood, so Chapter 3 provides a comprehensive introduction to vascular and metabolic physiology, with specific emphasis on circulation and the mechanisms underlying the hemodynamic response. Since metabolic activity generates both desirable and undesirable hemodynamic changes, Chapter 3 also addresses the issue of biogenic interference and presents some of the ways in which its effects can be mitigated.

Chapter 4

Before useful DOT measurements can be made, instrumentation capable of acquiring noninvasive real-time imagery of vascular events within the cerebral cortex with minimal temporal distortion had to be designed and tested. Many of the issues involved in the design and development of DOT instrumentation are covered in Chapter 4. An investigation into better methods of source encoding led to the development of a new optical encoding technique, Pulse-TDM, which allows for advanced capabilities like individualized gain control while providing very high dynamic range and minimal temporal skew in the optical imagery.

Chapter 4 also covers the design and construction of a variety of DOT instruments, ranging in evolution from the first tomographic system we constructed to the flexible high-speed DOT system used to perform the temporal hemodynamic response measurements discussed in Chapter 6 and still in use today. The history behind their development, plus the results of in-vivo performance evaluations and laboratory measurements used to characterize some of these instruments are also included in Chapter 6.

Chapter 5

Since custom surgical, biomonitoring, anesthesia, and stimulus equipment was required in order to perform the rodent DOT measurements, the design and development of the equipment necessary for performing in-vivo measurements of brain function in rodents are all discussed in detail in Chapter 5. Although the DOT measurements themselves are noninvasive, the need for tight metabolic monitoring and control over an uncooperative subject (a rat) necessitated the use of both stereotactic restraint and invasive procedures to obtain vascular access, so both sedation and analgesia were required. Surgical and anesthetic techniques that preserved normal cerebral hemodynamic function while providing analgesia sufficient for the use of stereotactic headgear are described.

Chapter 5 begins by discussing the pharmacology, physics and physiology behind anesthesia, followed by the design, assembly and testing of a custom anesthesia/ventilator system suitable for rodents and other small animals. Since monitoring the physiologic status of the subjects is critical to

both managing anesthesia and maintaining hemodynamic stability, the topic of biomonitoring is discussed, followed by the design and development of a rodent biomonitoring system.

This rodent preparation employed forepaw stimulation to generate a reproducible cerebral hemodynamic response in a localized region of the somatosensory cortex, so the issues involved in delivering stable and repeatable electrical stimuli over long periods of time are addressed, followed by the design and construction of a galvanically isolated forepaw stimulator. Complete schematics of the bioinstrumentation are provided to encourage future investigators to replicate this circuitry for their own research.

Chapter 6

Once all of the DOT, anesthesia, biomonitoring, and stimulus equipment has been constructed and tested, quantitative DOT measurements could then be performed and analyzed. Since much experience was gained from forepaw stimulation measurements performed on earlier DOT systems, Chapter 6 begins by presenting forepaw stimulation data collected using our original DOT instrument: CW1. This work is then followed by a detailed description of the temporal hemodynamic response measurements performed with CW4, a faster and more flexible frequency-encoded DOT system. The imagery and temporal data are presented and the mechanisms believed to be responsible for the temporal features visible in the data are discussed and compared to similar forepaw measurements collected with functional MRI. The effects of stimulus repetition rate, duration, and magnitude were examined in an effort to determine the robustness of the rodent forepaw stimulus preparation and to further understand the physiology underlying the neurovascular response.

Chapter 7

Chapter 7 summarizes the many conclusions drawn and lessons learned throughout the body of this dissertation. The hardware section covers topics related to optical source and detector selection, source encoding, anesthesia, biomonitoring, and stimulation. The experimental section then presents conclusions drawn from the rodent somatosensory cortical measurements, including observations concerning the effects of variations in forepaw stimulus current and stimulation frequency.

Appendix

The Appendix includes information that is important, yet not sufficiently salient to the main topics, to be included in the body of the dissertation. The ethical implications of experimentation with live subjects are a topic that is often eschewed by many researchers in the field of biophysics. Because this is an important topic, the ethical and legal responsibilities involved in animal and human experimentation are discussed here. Datasheets for many of the optical sources, detectors, and sensors used to construct the DOT instrumentation discussed above are also included for convenience, should the reader wish to refer to these for more information.