INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps. Each original is also photographed in one exposure and is included in reduced form at the back of the book.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6” x 9” black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

UMI
A Bell & Howell Information Company
300 North Zeeb Road, Ann Arbor MI 48106-1346 USA
313/761-4700 800/521-0600
RICE UNIVERSITY

Oblique-Incidence Fiber-Optic Reflectometry for Measuring Absorption and Scattering in Turbid Media

by

Shao-Pow Lin

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE

Master of Science

APPROVED, THESIS COMMITTEE:

Frank K. Tittel, Chairman
Professor of Electrical and Computer Engineering

John W. Clark
Professor of Electrical and Computer Engineering

Antonios Mikos
Professor of Chemical Engineering

Houston, Texas
July, 1996
Abstract

Oblique-Incidence Fiber-Optic Reflectometry for Measuring Absorption and Scattering in Turbid Media

by

Shao-Pow Lin

Oblique-incidence, fiber-optic reflectometry is a simple and accurate method for measuring the absorption and reduced scattering coefficients, $\mu_a$ and $\mu_s'$, of semi-infinite turbid media. Obliquely incident light produces a spatial distribution of diffuse reflectance that is not centered about the point of light entry. The shift in the center of diffuse reflectance is related to the medium's diffusion coefficient, $D$. We developed a fiber-optic probe to deliver light obliquely and sample the profile of diffuse reflectance. From a relative profile, we measure $D$, deduce the effective attenuation coefficient, $\mu_{\text{eff}}$, then calculate $\mu_a$ and $\mu_s'$. This method was verified with Monte Carlo simulations and tested on tissue phantoms. Measurements at 632.8 nm were accurate to within 5% for $D$ and $\mu_{\text{eff}}$, resulting in 10% and 5% accuracy for $\mu_a$ and $\mu_s'$. In addition, $\mu_a$ and $\mu_s'$ spectra were deduced from wavelength-resolved measurements of the diffuse reflectance from a white light source.
Acknowledgments

I would like to express my appreciation to Drs. Frank Tittel and Lihong Wang for two years of keeping me on track whenever I seemed to get lost in a maze of unanswered questions. With their level-headed advice and the freedom to explore on my own, I have learned more in the past two years about accomplishing a goal than in four years of college. I would also like to thank Drs. Steven Jacques and Sharon Thomsen for their support as well. It is to their constructive criticism of my papers, posters, and talks that I owe any ability I might have in explaining my scientific work to others.

I am especially grateful to Jon Schwartz, Martin Ostermeyer, and Beop-Min Kim for all I have learned from them about performing an experiment from beginning to end, for the innumerable ideas they provided me, and just for putting up with me in the laboratory. Finally, thanks to my family for believing in me, and especially to Nancy, for putting up with countless hours of my frustration.
Table of Contents

1 FUNDAMENTALS OF TISSUE OPTICS ................................................................. 1

1.1 INTRODUCTION .................................................................................. 1
1.2 MATHEMATICAL DEFINITIONS OF BASIC TERMS IN TISSUE OPTICS ........... 7
  1.2.1 Directional Photon Density and Beer’s Law ........................................ 7
  1.2.2 Radiance ..................................................................................... 10
  1.2.3 Flux .......................................................................................... 11
  1.2.4 Fluence Rate ............................................................................. 12
1.3 THE RADIATION TRANSPORT EQUATION ........................................... 13
  1.3.1 Intuitive Derivation of the Transport Equation ..................................... 13
  1.3.2 The Source Term ....................................................................... 14
  1.3.3 The Phase Function ................................................................... 16
1.4 APPLICATION OF THE DIFFUSION APPROXIMATION TO TRANSPORT THEORY ............................................................................. 18
  1.4.1 The Diffusion Approximation ......................................................... 18
  1.4.2 Fick’s Law, Flux Balance, and Energy Balance ................................. 21
  1.4.3 Solution for an Isotropic Point Source in an Infinite Medium .......... 28

2 CURRENT METHODS FOR MEASURING OPTICAL PROPERTIES ............... 33

2.1 INTRODUCTION .................................................................................. 33
2.2 COLLIMATED TRANSMISSION ............................................................. 35
2.3 INTERSTITIAL FLUENCE MAPPING ..................................................... 39
2.4 ADDED ABSORBER ......................................................................... 42
2.5 INTEGRATING SPHERES ..................................................................... 43
  2.5.1 Direct Measurement .................................................................... 43
  2.5.2 Indirect Measurement .................................................................. 45
2.6 SPATIALLY-RESOLVED DIFFUSE REFLECTANCE ................................ 48
  2.6.1 Theory and Modeling .................................................................. 48
  2.6.2 Normal Incidence Reflectometry .................................................... 54
2.7 SUMMARY .......................................................................................... 57

3 SINGLE WAVELENGTH OBLIQUE-INCIDENCE REFLECTOMETRY ............ 59

3.1 THEORY OF OBLIQUE-INCIDENCE REFLECTOMETRY .......................... 59
  3.1.1 Introduction ................................................................................ 59
  3.1.2 Mathematical Development ............................................................. 60
  3.1.3 Monte Carlo Simulations ................................................................. 67
3.2 EXPERIMENTAL VERIFICATION OF OBLIQUE-INCIDENCE REFLECTOMETRY .................................................................................. 70
  3.2.1 Materials and Methods ................................................................ 70
  3.2.2 Results ....................................................................................... 73
3.3 DISCUSSION ...................................................................................... 75
3.3.1 The Optical Fiber Probe .......................................................... 75
3.3.2 Index Matching ................................................................. 76
3.3.3 Collection Fiber Calibration ............................................... 79
3.3.4 Error Propagation ............................................................. 80

4 MULTIPLE WAVELENGTH OBLIQUE-INCIDENCE REFLECTOMETRY .......... 81
4.1 Introduction ........................................................................ 81
4.2 Multi-wavelength Oblique-incidence Reflectometry ................. 83
  4.2.1 Review of Oblique-incidence Reflectometry ....................... 83
  4.2.2 Materials and Methods .................................................. 86
  4.2.3 Results ....................................................................... 92
4.3 Discussion and Conclusions ............................................... 96
  4.3.1 General Discussion ....................................................... 96
  4.3.2 Discussion of Results for Water-based Phantoms ............. 98
  4.3.3 Discussion of Results for Resin Phantom ....................... 102
  4.3.4 Discussion of Results for Chicken Breast ...................... 103
  4.3.5 Conclusion ................................................................ 105

5 MULTIPLE FIBER IMAGING SPECTROGRAPH SYSTEM ....................... 107
5.1 Motivation and Explanation ............................................... 107
5.2 Complete Parts List ............................................................ 112
5.3 Schematics ...................................................................... 114
5.4 Discussion ...................................................................... 118

6 CONCLUSIONS ..................................................................... 122

APPENDIX A VECTOR CALCULUS REFERENCE ..................................... 126

APPENDIX B OBLIQUE-INCIDENCE REFLECTOMETRY SOFTWARE ........ 128
B.1 Sample Output .................................................................. 128
B.2 Source Code ..................................................................... 131
  B.2.1 Headers.h ................................................................. 132
  B.2.2 Main.c ....................................................................... 135
  B.2.3 Files.c ....................................................................... 137
  B.2.4 GetExpData.c ............................................................ 142
  B.2.5 Fitting.c ..................................................................... 145
  B.2.6 PLPlotter.c ............................................................... 154

REFERENCES ....................................................................... 160
List of Tables

Table 3-1  Fitted results from Monte Carlo Generated Data...........................................69

Table 3-2  Results from phantom experiments. The sample size for each measurement was four. * error in $\mu_{eff}$ drops to $-2.6\%$ and error in $\mu_a$ drops to $-8.2\%$ when fiber nearest source is not included...........................................................................................................74

Table A-1. Table of basis vectors and metric coefficients for the cartesian, cylindrical, and spherical coordinate systems. The vectors and coefficients are substituted in the general forms of the vector operations grad, div, curl, and Laplacian..................................................126
List of Figures

Figure 1-1. The flux into each side of the cube leaves the opposite side attenuated by $\mu_a dx$ (or $dy$, $dz$, depending on which side). Summing the power deposited in the cube from all directions results in Eq. 1-16. .................................................................12

Figure 1-2. a. Given the source and detector positions, the irradiance at $r$ without the intervening tissue is "known" i.e. it can be easily measured. b. With the tissue in place, the irradiance at $r$ is attenuated by the factor $e^{-\mu l}$. Note that the term "irradiance" refers to primary light directly from the source. .................................15

Figure 1-3. When a photon scatters off a spherically symmetric particle, the probability of its final trajectory being at an angle $\theta$ from its original trajectory is the same regardless of the angle $\phi$. ..................................................................................16

Figure 1-4. a. $\hat{u}$ is the unit vector designating the orientation of the perturbation $\varepsilon(r)$. b. Graphical depiction of one of the vectors to be summed by the integral in Eq. 1-29. Its magnitude is $\cos \theta$ and direction is $\hat{s}$. c. Diagram representing all the vectors to be summed by the integral. From (c) it is clear that the result of the vector integration is a vector in the $\hat{u}$ direction. In addition, it is clear that only the components of the vectors in the $\hat{u}$ direction (magnitude = $\cos^3 \theta$) need be accounted for (all other components cancel out). The result of these observations is the final integral expression of Eq. 1-29 where the integral over all solid angles has been converted to a spherical coordinate system (see Appendix A for details on integration over solid angles). .................................................................20

Figure 1-5. Graphical interpretation of the integral in Eq. 1-40. Since $\hat{s}$ is a unit vector, the gradient has no radial component and hence is always in the $\hat{\theta}$ direction, perpendicular to $\hat{s}$. ..................................................................................24

Figure 1-6. Graphical representation of the integral in brackets in Eq. 1-43. a. $\hat{s}'$ is the original photon direction. b. Example of one of the vectors to be summed. The magnitude is $p(\hat{s},\hat{s}')\hat{s}$, and it is pointing in the $\hat{s}$ direction (the final direction, which is the variable of integration). c. Depiction of all the vectors to be summed. The important observation is the symmetry about $\hat{s}'$, as illustrated in Figure 1-3. This particular drawing indicates somewhat forward directed scattering. d. Since all of the vector components perpendicular to $\hat{s}'$ cancel out, what is left to be integrated points in the $\hat{s}'$ direction and has a magnitude = $p(\hat{s},\hat{s}')\hat{s} \cdot \hat{s}'$. .................................................................25
Figure 2-1  Schematic of experimental set up to perform collimated transmission measurements.................................................................36

Figure 2-2  Diagram of fluence mapping experiment. Since the fiber tips are isotropic detectors, their orientation is irrelevant. The only sensitive factor is the separation, \( \Delta z \), which must be along the axis of illumination.................................................................40

Figure 2-3  Single integrating sphere set up for direct measurement of a) \( \mu_a \) and b) \( \mu_t \). The baffle inside the sphere blocks singly-scattered light from being detected...........44

Figure 2-4  (a) Single integrating sphere set up for measuring \( R_d \) of an optically thick sample. (b) Single integrating sphere set up for measuring \( T_d \) of an optically thick sample. (c) Double integrating sphere set up for measuring \( R_d \) and \( T_d \) simultaneously.................................................................45

Figure 2-5  a) The distribution of light in a semi-infinite tissue from an incident pencil beam can be modeled by a single, buried, isotropic source at a depth of \( z_0 \). b) The distribution of light from an isotropic point source in a semi-infinite tissue (\( z>0 \)) can be approximated by the distribution from a dipole source in an infinite tissue. See the text for details about the distances \( z_0 \) and \( z_b \).................................................................48

Figure 2-6  a) Diagram of the vectors involved in the integral of Eq. 2-17. The vector of integration, \( (\cos \theta) \hat{s} \) can be split into its components, one b) parallel to \( \hat{z} \), and one c) perpendicular to \( \hat{z} \). Only the dot product of \( \tilde{\mathbf{A}} \) and the component of \( (\cos \theta) \hat{s} \) parallel to \( \hat{z} \) contributes to the integral. In (c), at any given \( \mathbf{q} \), every dot product cancels out with its negative as we integrate around in the \( \mathbf{f} \) direction.................................................................52

Figure 2-7  If the fluence rate as a function of \( z \) is approximately linear in the range \( z=[0,z_b] \), then we can say that \[ \frac{\partial \phi}{\partial z} = \frac{\phi_1 - \phi_2}{z_1 - z_2} = \frac{2D \partial \phi / \partial z}{-z_b}, \] and \( z_b = -2D \).................................................................53

Figure 2-8  a) Schematic of system for video reflectometry measurement. b) Schematic of system optical fiber reflectometry measurement. The source could be a laser or a white light source such as a halogen or xenon lamp. The detector could be a photomultiplier tube or an optical multi-channel analyzer.................................................................55

Figure 3-1  Diffuse reflectance from a semi-infinite tissue for normally incident light, as modeled by Monte Carlo simulation vs. the two-source diffusion theory model. \( \mu_a = 0.4 \text{ cm}^{-1}, \mu'_s = 8 \text{ cm}^{-1}, \) and \( 1/mfp' = 0.123 \text{ cm} \). (a) Plot of Monte Carlo and diffusion theory curves. (b) Plot of \% difference in two curves, (Monte Carlo - Diffusion theory)/Diffusion Theory. From both (a) and (b), it is clear that the two curves agree well within \( 2/mfp' \) of the center of diffuse reflectance, which coincides with the light entry point for normal incidence.................................................................51
Figure 3-2  (a) Positions of point sources in diffusion theory model for normal incidence.  
(b) Positions of point sources in diffusion theory model for oblique-incidence. The y axis points out of the page..........................................................62

Figure 3-3  Diffuse reflectance from a semi-infinite tissue for obliquely incident light as modeled by Monte Carlo simulation vs. the two-source diffusion theory model. $\mu_a = 0.4 \text{ cm}^{-1}$, $\mu_s' = 8 \text{ cm}^{-1}$, and $1 \text{ mfp}' = 0.123 \text{ cm}$. (a) Plot of Monte Carlo and diffusion theory curves. (b) Plot of % difference in two curves, (Monte Carlo - Diffusion theory)/Diffusion Theory. From both (a) and (b), it is clear that the two curves agree well within 2 mfp' of the center of diffuse reflectance, which does not coincides with the light entry point for oblique-incidence.............................................64

Figure 3-4  Three dimensional perspective view of tissue surface, coordinate axes, and positions of light delivery and collection. The arrows represent the fibers in the probe. The arrows pointing up are the collection fibers and the dashed arrow is the source fiber .............................................................................65

Figure 3-5  (a) Sample of Monte Carlo simulated data after removal of noise far from point of entry as well as data within 1.5 mfp of the center of diffuse reflectance. The curve shown is the fitted result generated by our algorithm. To emphasize how well these curves agree with what we expected, in (b) we have plotted just the expected and fitted curves without the simulation data. This particular simulation used $(\mu_s', \mu_a) = (6 \text{ cm}^{-1}, 0.6 \text{ cm}^{-1})$.................................................................69

Figure 3-6  (a) Sample of data taken with oblique-incidence fiber optic probe. As in Figure 3-5, the curve shown is the fitted result generated by our algorithm. In (b) we have plotted just the expected and fitted curves without the data to reiterate how well they agree. This particular phantom had $(\mu_s', \mu_a) = (6 \text{ cm}^{-1}, 0.6 \text{ cm}^{-1})$. .................72

Figure 3-7  Plot of total diffuse reflection coefficient $R_d$ as a function of $n_1/n_2$. Notice that there is no reflection for a matched boundary. The reflection coefficient approaches 1 asymptotically for $n_1 > n_2$, whereas it approaches 1 very quickly for $n_1/n_2 < 0.5$. The best place to be for index matching is where $n_1/n_2$ is just slightly less than 1........................................79

Figure 4-1  Schematic of experimental apparatus. The probe was placed on the surface of the tissue phantom.........................................................................................87

Figure 4-2  a. Surface plot of spectral data collected for a phantom with $(\mu_s', \mu_a) = (4 \text{ cm}^{-1}, 0.407 \text{ cm}^{-1})$. Each fiber collects a spectrum at a specified position. There are no detection fibers, and hence no data, between -0.92 and +0.19 cm.  b) An example of one spectral slice (at 633 nm) through the data in (a). The data at each individual
wavelength looks and is processed exactly like the single wavelength data from Chapter 3............................88

Figure 4-3 Plots of the wavelength dependence of absorption and reduced scattering for the following water-based phantoms: \((\mu'_a, \mu_a) = a,b\) \((4 \text{ cm}^{-1}, 0.407 \text{ cm}^{-1})\), \(c,d\) \((6 \text{ cm}^{-1}, 0.194 \text{ cm}^{-1})\), \(e,f\) \((6 \text{ cm}^{-1}, 0.614 \text{ cm}^{-1})\), \(g,h\) \((8 \text{ cm}^{-1}, 0.195 \text{ cm}^{-1})\), \(i,j\) \((8 \text{ cm}^{-1}, 0.610 \text{ cm}^{-1})\), and \(k,l\) \((10 \text{ cm}^{-1}, 0.405 \text{ cm}^{-1})\).................................................................92

Figure 4-4 Measured spectra for the four flat surfaces of the two cylindrical solid resin phantoms. a) Surface 1-1. b) Surface 1-2. c) Surface 2-1 d) Surface 2-2 .................94

Figure 4-5 Results from chicken breast experiment. The absorption and reduced scattering spectra were determined with the probe aligned at a) \(0^\circ\), b) \(30^\circ\), c) \(60^\circ\), and d) \(90^\circ\) to the direction of the muscle fibers.................................................................95

Figure 4-6 Plots of the reduced scattering spectra for the following water-based phantoms: \((\mu'_a, \mu_a) = a\) \((4 \text{ cm}^{-1}, 0.407 \text{ cm}^{-1})\), \(b\) \((6 \text{ cm}^{-1}, 0.194 \text{ cm}^{-1})\), \(c\) \((6 \text{ cm}^{-1}, 0.614 \text{ cm}^{-1})\), \(d\) \((8 \text{ cm}^{-1}, 0.195 \text{ cm}^{-1})\), \(e\) \((8 \text{ cm}^{-1}, 0.610 \text{ cm}^{-1})\), and \(f\) \((10 \text{ cm}^{-1}, 0.405 \text{ cm}^{-1})\). In contrast to Figure 4-3, in each of these plots the expected curve has been shifted down 45 nm to illustrate how an incorrect size for the polystyrene spheres could account for discrepancies between the expected and measured scattering spectra.................................................................100

Figure 4-7 Plot of expected reduced scattering spectra calculated from Mie theory. For simplicity, the following were assumed constant (over all wavelengths and for each sphere size): index of refraction of the polystyrene spheres = 1.5746, index of refraction of water = 1.3324, density of polystyrene = 1.05, density of water = 1.0, and concentration of spheres by weight = 0.102. The sphere size was varied from 900 to 1000 to 1150 nm. Notice that a) the period of undulations in the curves decreases with sphere size and b) the relative amount of scattering also decreases with sphere size.................................................................101

Figure 4-8 Spectra for oxy- and deoxyhemoglobin. The peaks in the range from 500 – 600 nm look similar to the absorption spectrum from chicken breast..................104

Figure 5-1 Sample screen capture from multi-fiber imaging spectrograph system. Each linear spectrum is the output from a single detection fiber illuminated by an incandescent tungsten bulb.................................................................108

Figure 5-2 Sample screen captures from multi-fiber imaging spectrograph system with detection fibers illuminated with a) \(594 \text{ nm} \) HeNe and b) \(633 \text{ nm} \) HeNe lasers. Distortion at the top and bottom of the array is due to spherical aberration in the spectrograph. Shown in the insets are plots of the intensity along the line indicated in each figure...............................109
Figure 5-3  Schematic diagram of entire multi-fiber imaging spectrograph system. The following figures give detailed views of the bulkhead connector, probe, entrance, and exit assemblies.

Figure 5-4  Plans for bulkhead connector. All dimensions are given in inches  a) Side view.  b) Front view. The holes are drilled all the way through and filled with SMA bulkhead adapters (Amphenol 905-120-5003).  c) Bottom view. The 1/4-20 tap allows the bulkhead connector to be attached to a standard 1 inch rule 1/4-20 tapped optical table.

Figure 5-5  Plans for constructing oblique-incidence optical fiber probe.  a) Side view of the two parts of the probe. The tip is sharpened to decrease the size of the probe’s footprint on the sample.  b) Inside surface of part 1. There is a groove for the source fiber. The circle is milled to the same depth of 0.040. It allows the source fiber to make a 45 degree bend.  c) Inside surface of part 2. There are straight grooves for all of the detection fibers. To assemble the probe, the fibers are snapped into their respective grooves, and the two parts are glued together with Duro Master Mend Epoxy. After curing, the tips can be polished.

Figure 5-6  The detection fibers from the probe are coupled to another set of fibers through the bulkhead with SMA connectors. The second set of fibers are brought together into a linear array which is embedded in casting resin. The polished tips of the linear array comprise the object to be imaged by the spectrograph.

Figure 5-7  a) Complete entrance assembly. The Nikon zoom lens is attached to a C mount adapter, which is attached to our custom entrance tube which in turn is attached to the outside of the filter mount of the spectrograph. Behind the filter is the entrance slit upon which the lens system images the object.  b) End view of the entrance tube. The holes are for connection to the adapter plate.  c) Adapter plate. It is screwed to the entrance tube as well as the filter holder.

Figure 5-8  Diagram of 1 inch continuous neutral density filter from Reynard Corporation. The filter ranges from 0 O.D. at the bottom to 4 O.D. at the top.

Figure 5-9  a) Complete exit assembly. The cylindrical lens corrects the astigmatism of the spectrograph. It is glued in place with clear silicone sealant (Dow Corning). Exit tube 1 slides into the exit port of the spectrograph and is held in place by a set screw in the slot. Exit tube 2 slides into exit tube 1 and is also held in place be set screws. At the end of exit tube 2 is a focusing lens that actually drops within the C mount of the camera.  b) Detailed view of exit tube 2.

Figure 5-10  Collected spectra from an incandescent tungsten bulb. Light was collected with a single fiber which was then imaged by the spectrograph onto the CCD camera.
Shown are sample screens from NIH Image where we have plotted the amount of light collected vs. wavelength. In a) we used a 300 groove/mm grating, whereas in b) we used a 600 groove/mm grating.

**Figure A-1.** Diagrams of differential volumes for three coordinate systems. a) Cartesian, b) Cylindrical, and c) Spherical.

**Figure B-1** Sample dialog screen from Oblique-Incidence Reflectometry fitting program. We have chosen to perform a fit on data from input file 6/0.6_oirl at 632.4 nm. Figure B-2 is the graphics screen.

**Figure B-2** Graphical output for a single wavelength fit. Plotted are the data points (squares), the center of diffuse reflectance, the expected, and the fitted curves.

**Figure B-3** More of the sample dialog box. We have chosen to repeat the fit, but this time to fit for all the available wavelengths at once. The program outputs the results in text for each wavelength. Only three are shown here. The graphical output screen is in Figure B-4.

**Figure B-4** Graphical output for multi-wavelength fits. The first plot shows the absorption spectrum, and the second shows the reduced scattering spectrum.
Chapter 1  Fundamentals of Tissue Optics

1.1 Introduction

The ability to noninvasively view the inside of the human body is among the most important developments in the history of medical diagnosis. Current imaging techniques include computed x-ray tomography (CT), magnetic resonance imaging (MRI), ultrasonography (US), and positron emission tomography (PET). Each of these methods rely on unique physical principles and produce images based on different mechanisms of contrast.

CT imaging takes advantage of variations in tissue density and hence provides primarily structural information. Abnormal structure can indicate disease processes, but at the cost of exposure to x-rays, a form of ionizing radiation. MRI relies on nuclear magnetic resonance and spin relaxation to distinguish between tissues based on water content. Current developments in MRI also include functional and flow imaging. While no health risks have been associated with MRI, its biggest disadvantages are its exceptionally high cost and the length of time patients must remain isolated inside the coil during the scan. US measures differences in mechanical impedance and hence provides only anatomic information. It offers relatively poor spatial resolution, and imaging of particular organs may be difficult based on a high impedance mismatch between the organ of interest and its surrounding tissue. PET scans detect the accumulation of injected radioactive compounds. The primary application of this technique is to quantitatively
analyze physical processes, metabolism in particular. Since PET requires intravenous exposure to radioactive substances, there is also a safety risk involved. In addition, PET scans do not offer as high a resolution as CT or MRI. In contrast to CT, MRI, and PET, ultrasound is the only imaging study whose equipment is both inexpensive and portable [1]. Despite these drawbacks, imaging studies have risen to a position of tantamount importance for quality medical care, and this fact encourages us to work towards building upon the present imaging arsenal to produce new, reliable, convenient, and inexpensive techniques for noninvasive diagnosis, specifically optical imaging and spectroscopy.

In optical studies, light, primarily in the visible and near infrared region of the electromagnetic spectrum, is used to probe biological tissues. After passing through the body, light can emerge bearing information about the medium it has just traversed, and it is this information that must be extracted and utilized. The primary advantages to the use of light for imaging are that prolonged exposure is virtually harmless, much of the equipment required for optical work can be made portable, and optical imaging and spectroscopy has the potential to provide both anatomic and physiologic information about a tissue [2].

In the field of biomedical optics, the most fundamental issue is the distribution of light energy within an irradiated tissue. This problem can be posed in a deceptively simple form: given the relevant optical properties and boundary conditions of a biological tissue, how many photons per second will reach a particular chromophore and be absorbed? The unique problem in biomedical optics is that tissues are optically opaque, or turbid. To solve this problem, there are four basic processes that must be accounted
for. Within the tissue there is 1) photon scattering and 2) photon absorption. At the
tissue boundaries, there is 3) reflection and 4) transmission. As with most questions in
physics, there are two sides to the same problem. The first task is to find a way to
calculate how much light energy per unit area per unit time reaches a particular target,
given the pertinent optical properties of the tissue. The second task involves actually
measuring the optical properties of tissues *in vivo* [3, 4]. This chapter covers the
fundamentals of light propagation in turbid media such as biological tissues, and Chapter
2 will serve as an introduction to techniques for measuring tissue optical properties. The
remainder of this work focuses on a simple, new technique for quickly and noninvasively
determining the optical properties of turbid media: oblique-incidence, fiber-optic
reflectometry. Our particular goals were to 1) prove that our method is theoretically valid
with Monte Carlo simulations, 2) demonstrate the accuracy, sensitivity, and simplicity of
our method with monochromatic experiments on water-based tissue phantoms, and 3)
show that the technique is very easily extended to multi-wavelength measurements on
solids and soft tissues.

A mathematically rigorous approach to the problem of light propagation within
biological tissue and other turbid media entails treating light as an electromagnetic wave
that is interacting with a spatially and temporally varying dielectric medium. An exact
solution requires a model of the spatial, temporal, and size distribution of all tissue
structures, their absorbing properties, and their scattering properties. [The scattering
properties are actually implicit in the size and refractive indices (or dielectric constants)
of the tissue structures]. Probably the best representation of tissue would be as a
randomly varying continuum because it is a condensed medium with a complex dielectric microstructure that has spatial variations on the order of the thickness of a cellular or organellar membrane [3]. Because the dimensions of fluctuation which are significant at optical wavelengths are microscopic, it is very difficult to actually measure the complete statistical distribution of dielectric variations in a tissue for use in a model. As a result, very little progress has been made with this approach in the optical regime.

A somewhat simpler model considers tissue as an ensemble of randomly distributed, discrete, scattering and absorbing particles suspended in a uniform, non-attenuating medium. Electromagnetic theory can provide exact expressions for the absorbing and scattering parameters of single dielectric particles, and the solution for multiple scattering can, in principle, be obtained by solving the single scatter fields and superposing the fields for all of the particles [5-7]. It is worth noting that the final result will be a statistical average of field quantities because each wave-particle interaction will have a particular probability associated with it. This approach is conceptually satisfying because it preserves the wave characteristics of the optical field, most notably the effects of diffraction and interference. Despite the elegance of this formalism, it has not led to as yet solvable equations for any cases of interest in biomedical optics, nor has there been much progress in developing approximate solutions for these equations for the propagation of light in biological media [4, 8].

Since rigorous solutions with electromagnetic wave theory have proven to be intractable, an alternate, heuristic approach that utilizes the particle aspect of wave-particle duality has been applied to the problem of biomedical optics. This approach,
called radiation transport theory, simplifies the problem of light propagation in dielectric media yet another step — not only is the medium considered to be a random ensemble of discrete absorbing and scattering centers, but light itself is considered as composed of discrete packets of energy with no wave properties. Each light particle, or photon, can be locally scattered (elastically or inelastically) or absorbed during an interaction with a particle in the medium, and these interactions are described in terms of either scattering and absorption cross sections or, more commonly, scattering and absorption interaction coefficients \( \mu_s \) and \( \mu_a \). Along with the effect of scattering comes an added parameter, the angular distribution of scattering, which is often described by the scattering phase factor \( g \) [5, 9, 10]. These terms will be clarified in the following sections. It is important to reiterate that radiation transport theory does not account for the amplitude and phase of a photon, but instead only deals with power and intensity, the net result being that there can be no correlation between radiation fields, and wave effects such as diffraction and interference are subsequently ignored.

Despite not having a direct connection to electromagnetic theory (as of yet), radiation transport theory has proven to be a useful and consistent framework for studies of light propagation in biological tissue. That is to say, if the optical parameters of a tissue are measured in accordance with the transport theory model, then these parameters are subsequently used in the transport model to determine the spatial variation of fluence rate (given adequate information about boundary conditions), the result agrees well with experimental findings [4, 11]. An important caveat is that this form of experimental consistency does not imply general validity. A simple case in point is that radiation
transport theory seems to break down in highly structured tissues where scattering is not random and interference effects are non-negligible [12]. We were actually able to detect the optical anisotropy of chicken breast tissue with our oblique-incidence reflectometer (Chapter 4, section 4.2.2.3).

Assuming that radiation transfer theory is adequate for modeling optical transport in tissue, there are still no exact, general solutions to the transport equation upon which to build. Nonetheless, transport theory maintains its utility in the fact that there are relatively simple analytic approximations and numerical methods which can be applied to particular situations. These techniques have historically been developed for the fields of nuclear reactor theory and atmospheric physics [5, 10], and they translate relatively well into the field of biomedical optics. The question of deciding what kind of approximation is appropriate for any given situation is, of course, not clear cut, but a common convention is to divide the problem into three regimes: tenuous media where the volume fraction of scatterers is less than 0.1%, intermediate media where the volume fraction is between 0.1% and 1%, and dense media where the volume fraction is greater than 1%. As might be expected, tenuous media are the simplest to work with, since the light fluence rate at a particular point in space can be approximated by first-order multiple scattering theory. That is, only the attenuation of the incident irradiation is considered, and light scattered into the region of interest from other parts of the medium are not counted. Unfortunately, biological tissues do not fall into this regime. Intermediate media are the most difficult to work with, since it is harder to apply simplifying approximations. Some
attempts have been made to find solutions to special cases, but they also have found little
generalization to many cases of biomedical interest [4].

From this point on, we will only discuss dense media, the regime into which most
soft tissues fall at optical wavelengths. For dense media in which the albedo is high and
the distance between scattering centers is small relative to the particular source-tissue-
detector geometry, photons can be considered to be highly scattered, and the diffusion
theory approximation can be used to solve the radiation transport equation, either
numerically or analytically (for some special cases). The remainder of this chapter is
dedicated to defining the terms pertinent to radiation transport theory and deriving the
diffusion theory approximation upon which oblique-incidence reflectometry is grounded.

1.2 Mathematical Definitions of Basic Terms in Tissue Optics

1.2.1 Directional Photon Density and Beer's Law

In this section, the relevant parameters used to describe light transport are defined
and their relations to one another clarified in an intuitive manner. This material was
compiled and reorganized from Optical-Thermal Response of Laser Irradiated Tissue and
Volume 1 of Ishimaru's Wave Propagation and Scattering in Random Media. The
Appendix contains a brief review of coordinate systems and vector calculus.
The first and most fundamental transport term is necessary to quantify not only how much light is present at a particular position, but also in what direction that light is propagating:

Eq. 1-1

\[
N(\vec{r}, \hat{s}) = \frac{\text{# of photons}}{\text{cm}^3 \text{sr}} \quad \text{or, more explicitly,}
\]

\[
\frac{\text{# of photons in unit volume moving in } \hat{s} \text{ dir. within a unit of solid angle centered about } \hat{s}}{\text{(size of unit volume)(size of unit solid angle)}}
\]

In other words, \( N \) is the "directional photon density per differential solid angle", or simply the "directional photon density", and

Eq. 1-2

\[
N(\vec{r}, \hat{s})(c \cdot dA)(d\omega)(h\nu) \Rightarrow \left[ \frac{J}{s} = W \right]
\]

where \( c \) is the speed of light, and the resulting power is the amount of photon energy that propagates per second through an area \( dA \) located at position \( \vec{r} \), within the solid angle \( d\omega \), in the \( \hat{s} \) direction. If \( d\vec{A} \) (the normal vector to the differential area) is not parallel to \( \hat{s} \), then the additional term \( \cos \theta \) must be included, where \( \theta \) is the angle between \( d\vec{A} \) and \( \hat{s} \).

Given this fundamental transport quantity, we can explore the effects of absorption and scattering interaction coefficients as a function of position. This exercise is best carried out as a one-dimensional problem where \( N(\vec{r}, \hat{s}) \) reduces to \( N(s) \). If we consider a purely absorbing medium, then the dependence of directional photon density on position is as follows:

\[
\lim_{\Delta r \to 0} [\Delta N = N(s + \Delta s) - N(s)]
\]

Eq. 1-3

\[
ds = -N(s)(\text{probability of absorption in distance } ds) = -N(s)(\mu_a ds)
\]
where $\mu_a \,[\text{cm}^{-1}]$ is the interaction coefficient for absorption. Eq. 1-3 is identical to the well known equation describing radioactive decay, and has the following solution known as Beer's Law:

**Eq. 1-4**

$$N(s) = N_0 e^{-\mu_a s}$$

From Eq. 1-4 it is easy to derive the probability distribution function $F(s)$ and the probability density function $f(s)$, both of which are important for Monte Carlo modeling of light transport [13-15].

$$F(s) = \text{probability of absorption between 0 and } s = \frac{\text{\# of photons absorbed}}{\text{\# of incident photons at } s = 0}$$

**Eq. 1-5**

$$F(s) = \frac{N_0 - N_0 e^{-\mu_a s}}{N_0} = 1 - e^{-\mu_a s}$$

$f(s) = \text{probability of absorption between } s \text{ and } s + ds \text{ divided by } ds \text{ is the slope of } F(s)$

**Eq. 1-6**

$$f(s) = \frac{d}{ds} F(s) = \mu_a e^{-\mu_a s}$$

There is another way to think about the probability of absorption in a differential path length $ds$ from Eq. 1-3:

$$\text{probability of absorption in } ds = \frac{ds}{\text{average distance between absorption events}} = \frac{ds}{mfp}$$

where $mfp$ is the mean free path between absorption events. Thus it follows that

**Eq. 1-7**

$$mfp = \frac{1}{\mu_a} \Rightarrow \left[ \text{cm}^{-1} \right]$$

If the medium both scatters and absorbs, then the situation is substantially more complex because scattered photons do not simply disappear, and hence they must be kept account of until they are either absorbed or escape the bounds of the tissue. This complication is also well illustrated by the one-dimensional model. First of all, if for some reason, we could assume that all scattered photons were annihilated, then scattering
would effectively be no different from absorption, and Beer's law would still apply with one minor modification.

Eq. 1-8

\[ N(s) = N_0 e^{-(\mu_a + \mu_s) s} \]

Eq. 1-8 is the expression used to describe situations that approximate single scattering.

For this situation, the mean free path becomes

Eq. 1-9

\[ mfp = \frac{1}{\mu_a + \mu_s} \Rightarrow \left[ \text{cm}^{-1} \right] \]

and describes the average distance between interactions (either scattering or absorption).

In addition, it is the distance along \( s \) in which the directional photon density decreases by \( 1/e \) (again, only in the single scatter approximation). If multiple scattering occurs, that is, if a photon can scatter from the \( +s \) direction into the \( -s \) direction and back to the \( +s \) direction, then Eq. 1-8 does not apply and we must develop a more complicated formalism (see the next section “Radiation Transport Theory”). At this point, rather than going into a detailed discussion of one-dimensional transport theory [9], we will define the remaining relevant terms, derive the vector transport equation, and develop the diffusion approximation to the transport equation.

1.2.2 Radiance

A term similar to the directional photon density, \( N(\vec{r},\hat{s}) \), is the radiance:

Eq. 1-10

\[ L(\vec{r},\hat{s}) = N(\vec{r},\hat{s})h\nu c \Rightarrow \left[ \frac{W}{cm^2sr} \right] \]

where \( h \) is Planck’s constant and \( \nu \) is the frequency. Radiance is a very important term because it provides a convenient and intuitive way to quantify not only the amount of
light energy (or power) that is intercepted by an object such as a scattering particle, but also the amount of energy that falls within that object's particular cone of acceptance. This is especially useful when the object is a detector because the collected power can be expressed as:

\[ dP(\vec{r},\hat{s}) = L(\vec{r},\hat{s}) \hat{s} \cdot (d\vec{A} \cdot d\omega) \Rightarrow [W] \]

Eq. 1-11

Eq. 1-11 is in fact identical to Eq. 1-2, and setting \( d\omega \) equal to \( 2\pi \) gives the total amount of power that passes through the area \( d\vec{A} \), in the \( \hat{s} \) direction, regardless of the acceptance cone.

1.2.3 Flux

The next term we will define is the net flux vector, which results from the vector sum of the radiance from all directions at the point \( \vec{r} \) in space.

\[ \vec{F}(\vec{r}) = \int_{4\pi} L(\vec{r},\hat{s})\hat{s}d\omega \Rightarrow \left[ \frac{W}{cm^2} \right] \hat{s}' \]

\( L(\vec{r},\hat{s})\hat{s}d\omega \) is the elemental flux vector in the direction \( \hat{s} \). After integration over all directions, it gives the vector net power transfer per unit area. The component of net flux in a given direction \( \hat{n} \) is simply \( \vec{F}(\vec{r}) \cdot \hat{n} \). On the other hand, to calculate the total, hemispherical flux in the direction \( \pm \hat{n} \) (such as forward or backward across a tissue boundary), then the following expressions should be used:

\[ F_{\pm\hat{n}}(\vec{r}) = \int_{2\pi} L(\vec{r},\hat{s})(\hat{s} \cdot \pm\hat{n})d\omega \Rightarrow \left[ \frac{W}{cm^2} \right] \]

Eq. 1-13

where the component of net flux in the \( \hat{n} \) direction is simply:

\[ \vec{F}(\vec{r}) \cdot \hat{n} = F_{+\hat{n}}(\vec{r}) - F_{-\hat{n}}(\vec{r}) \]

Eq. 1-14
1.2.4 Fluence Rate

Returning to the originally posed question of how many photons per second will reach a particular chromophore and be absorbed, it is now clear that we can approach the first part of the problem in terms of the radiance and define yet another term, the fluence rate, as the scalar sum of the incident power at a point $\vec{r}$ in space from all directions.

Eq. 1-15

$$\phi(\vec{r}) = \int_{4\pi} L(\vec{r}, \hat{s}) d\omega \Rightarrow \left[ \frac{W}{cm^2} \right]$$

The fluence rate is given in watts per square centimeter, and multiplication by the cross-sectional area of a spherical target particle will give the total incident power upon that particle (assuming that the particle size is small on the scale of variation in $\phi(\vec{r})$). As for the second part of the question, consider a cube at position $\vec{r}$ (Figure 1-1). The net flux into one face of the cube is $F_{+,z}(\vec{r})(dydz)$ (with similar expressions for the other five faces), and the amount of power absorbed during traversal of the cube is

**Figure 1-1.** The flux into each side of the cube leaves the opposite side attenuated by $\mu_s dx$ (or $dy$, $dz$, depending on which side). Summing the power deposited in the cube from all directions results in Eq. 1-16.
\( F_{\pm z}(\vec{r})(dydz)(\mu_a dx) \). Summing up the total amount of power deposited into the cube from all directions:

\[
power \text{ absorbed} = (\mu_a d\nu)(F_{+x} + F_{-x} + F_{+y} + F_{-y} + F_{+z} + F_{-z})
\]

\text{Eq. 1-16} \quad \frac{power \text{ absorbed}}{d\nu} = \mu_a(\vec{r})\phi(\vec{r})

where the sum of the hemispherical fluxes inward from each face of the cube equals the fluence rate at \( \vec{r} \) in the limit of an infinitesimally small cube.

### 1.3 The Radiation Transport Equation

#### 1.3.1 Intuitive Derivation of the Transport Equation

Now that all the relevant definitions have been made and it is clear what quantities need to be solved for to characterize the distribution of light in an irradiated tissue (fluence rate and flux in particular), we will derive the transport equation and investigate how to deduce the quantities of interest given \( \mu_a, \mu_s, \) and \( g \), the basic optical parameters of the medium. In the last section, Beer's law for a single scattering medium (Eq. 1-8) was derived from the basic differential equation for exponential decay (Eq. 1-3). To include the effect of \textit{multiple} scattering, the equation describing the incremental change in the light field per differential path length in the \( \hat{s} \) direction \((ds)\) simply needs an additional term to account for photons from the rest of the tissue volume that, at position \( \vec{r} \), scatter into the direction of interest, \( \hat{s} \). This equation will be derived in terms of the
radiance $L(\hat{r},\hat{s})$, rather than the directional photon density $N(\hat{r},\hat{s})$ that we used previously (recall that the two are related by the constant $h\nu c$, Eq. 1-10).

$$dL(\hat{r},\hat{s}) = -L(\hat{r},\hat{s})(\mu_a + \mu_r)ds + \text{radiance at } \hat{r} \text{ scattered from } \hat{s}' \text{ dir into } \hat{s} + \text{ any sources}$$

**Eq. 1-17**

$$dL(\hat{r},\hat{s}) = -L(\hat{r},\hat{s})(\mu_a + \mu_r)ds + \int_{4\pi} [p(\hat{s},\hat{s}')(\mu_a, \mu_r) L(\hat{r},\hat{s}') d\omega' + S(\hat{r},\hat{s})] ds$$

The first two terms in Eq. 1-17 are exactly analogous to Eq. 1-8. The third term requires some explanation. Without the terms in the square brackets, the integral is simply the sum of all the light energy incident upon a differential volume at position $\hat{r}$, or the fluence rate at $\hat{r}$ (Eq. 1-15). Looking into the brackets, $\mu_a ds$ is the probability that a photon will scatter within the differential path length $ds$, and $p(\hat{s},\hat{s}')$ is the probability (given that a scattering event has occurred) of scattering from the direction $\hat{s}'$ into the direction of interest, $\hat{s}$. So the third term is actually the sum of the light energy incident upon the differential volume at position $\hat{r}$ from all directions $\hat{s}'$, weighted by the probability of scattering into the direction $\hat{s}$. The last term accounts for any sources in the tissue.

Rearranging Eq. 1-17 results in the more common form of the radiation transport equation:

**Eq. 1-18**

$$\frac{dL(\hat{r},\hat{s})}{ds} = -\mu_a L(\hat{r},\hat{s}) - \mu_r L(\hat{r},\hat{s}) + \mu_r \int_{4\pi} p(\hat{s},\hat{s}') L(\hat{r},\hat{s}') d\omega' + S(\hat{r},\hat{s}) \Rightarrow \left[ \frac{W}{cm^3 sr} \right]$$

### 1.3.2 The Source Term

To find an expression for the source term, consider a source with a known irradiance $E_0(\hat{r},\hat{s}_0)$ [W/cm$^2$] *in the absence of tissue*. This irradiance could be from a collimated laser beam or a diffusing fiber-optic tip – the actual form is not important at the moment, only
Figure 1-2. a. Given the source and detector positions, the irradiance at \( \vec{r} \) without the intervening tissue is "known" i.e., it can be easily measured. b. With the tissue in place, the irradiance at \( \vec{r} \) is attenuated by the factor \( e^{-\mu_l} \). Note that the term "irradiance" refers to primary light directly from the source.

that it can be considered a "known". When used to irradiate a tissue, either from the surface or interstitially, there will be two resulting "types" of radiance within the tissue: radiance from primary, unscattered photons and scattered radiance.

Eq. 1-19  
\[
L(\vec{r}, \hat{s}) = L_p(\vec{r}, \hat{s}) + L_s(\vec{r}, \hat{s}) \quad \text{and} \quad \phi(\vec{r}, \hat{s}) = \phi_p(\vec{r}, \hat{s}) + \phi_s(\vec{r}, \hat{s})
\]

The relationship between the two is that primary radiance becomes scattered radiance after one scattering event. Writing an equation to describe \( L_p(\vec{r}, \hat{s}) \) results in (essentially) the familiar Eq. 1-8—Beer’s law for single scattering. On the other hand, the equation for \( L_s(\vec{r}, \hat{s}) \) must account for multiple scattering and therefore has the form of the transport equation, Eq. 1-18, with \( L_s(\vec{r}, \hat{s}) \) instead of \( L(\vec{r}, \hat{s}) \). The only source of scattered radiance is previously unscattered, primary radiance, so the source term is (see Figure 1-2):
Figure 1-3. When a photon scatters off a spherical particle, the probability of its final trajectory being at an angle $\theta$ from its original trajectory is the same regardless of the angle $\phi$.

$$S(\vec{r},\hat{s}) = \mu_s p(\hat{s},\hat{s}_0) E_0(\vec{r},\hat{s}_0) e^{-\mu_s l} \Rightarrow \left[ \frac{W}{cm^3 sr} \right]$$

$\mu_s p(\hat{s},\hat{s}_0) = \text{(probability of scattering)(probability of scattering from } \hat{s}_0 \text{ into } \hat{s})$

$E_0(\vec{r},\hat{s}_0) = \text{irradiance at } \vec{r} \text{ if the tissue were absent (non-attenuating)}$

$l = \text{path length for unattenuated photons from point of entry to } \vec{r}$

$\mu_s = \mu_a + \mu_s = \text{the "total interaction coefficient"}$

$e^{-\mu_s l} = \text{attenuation by absorption and single scattering events}$

The final result is a transport equation for scattered light with a defined source term. This is the transport equation we will use from now on, and all future references to radiance will be to the scattered radiance unless otherwise explicitly specified [16].

$$\text{Eq. 1-21} \quad \frac{dL_s(\vec{r},\hat{s})}{ds} = -\mu_s L_s(\vec{r},\hat{s}) + \mu_s \int \frac{p(\hat{s},\hat{s}') L_s(\vec{r},\hat{s}')}{4\pi} d\omega' + \mu_s p(\hat{s},\hat{s}_0) E_0(\vec{r},\hat{s}_0) e^{-\mu_s l}$$

1.3.3 The Phase Function

$p(\hat{s},\hat{s}')$ is known as the "phase function" of scattering, it is in units of [1/sr], and it has some important properties and relationships that need to be elucidated. First of all, when a photon scatters from a particle, the probability distribution of the final trajectory must be symmetric about the original trajectory, see Figure 1-3. That implies that the
phase function \( p(\mathbf{s}, \mathbf{s}') \) can be expressed more simply as \( p(\cos \theta) \), where \( \theta \) is the angle between \( \mathbf{s} \) and \( \mathbf{s}' \). From this observation follows Eq. 1-22 (see Appendix A for a review of integration over solid angles \( d\omega \)):

\[
\text{Eq. 1-22} \quad \int_{4\pi} p(\mathbf{s}, \mathbf{s}') d\omega' = \int_0^{2\pi} \int_0^\pi p(\cos \theta) \sin \theta d\theta d\phi
\]

\[
= 2\pi \int_{-1}^1 p(\cos \theta) d(\cos \theta) = 2\pi \int_{-1}^1 p(\mu) d\mu \equiv 1
\]

Up to this point, we have been working toward a way to solve the transport equation given the relevant optical parameters of \( \mu_a, \mu_s, \) and \( g \). Solution of the transport equation would provide an (at least approximate) expression for the radiance which could further be used to find the fluence rate and fluxes in a tissue. Careful inspection of Eq. 1-21 reveals that all the terms are either assumed to be given as "knowns" or are the unknown itself, \( L(\mathbf{r}, \mathbf{s}) \), except for the phase function, from which the anisotropy parameter, \( g \), is defined:

\[
\text{Eq. 1-23} \quad g = \int_{4\pi} p(\mathbf{s}, \mathbf{s}') (\mathbf{s} \cdot \mathbf{s}') d\omega' = 2\pi \int_{-1}^1 p(\cos \theta) (\cos \theta) d(\cos \theta)
\]

Eq. 1-23 is simply a sum over all possible cosines weighted by the probability of scattering at the angle \( \theta \). In other words, \( g \) is the expectation value of the cosine of the angle of scattering. For purely isotropic scattering, the phase function must be constant for Eq. 1-22 to hold:

\[
p(\cos \theta) = \text{constant} = \frac{1}{4\pi}
\]

Substitution into Eq. 1-23 reveals that \( g = 0 \) for isotropic scattering. For purely forward scattering
Eq. 1-24  \[ p(\cos \theta) = 0, \text{ if } \theta \neq 0 \text{ and } p(\cos \theta) = \frac{1}{2\pi}, \text{ if } \theta = 0 \]

The value of \( p \) at \( \theta = 0 \) makes sense if you think of Eq. 1-22 in terms of the "impulse filter function":

\[ \int_a^b K \delta(x - x_0) f(x) \, dx = K f(x), \text{ where } x_0 = [a, b] \]

Substitution into Eq. 1-23 reveals that \( g = 1 \) for pure forward scattering. There are a number of phase functions that have been used to model the angular distribution of single scattering in tissue, most notably the Henyey-Greenstein and delta-Eddington functions [16-19]. All of these phase functions are strongly biased toward forward scattering. This topic will not be developed further because a mathematical expression for the phase function (or anisotropy parameter \( g \)) will not be necessary to understand the remaining chapters.

1.4 Application of the Diffusion Approximation to Transport Theory

1.4.1 The Diffusion Approximation

Now we are prepared to apply the diffusion approximation to the radiation transport equation. The end result will not be an approximate solution to the transport equation per se, but rather will be the steady-state diffusion equation with all the constants defined in terms of the optical transport parameters of the medium \( \mu_a, \mu_s, \) and \( g \). The advantage to this seemingly indirect approach is that there are a number of
numerical methods as well as known analytic solutions to the diffusion equation (for certain geometries and boundary conditions).

For radiation transport to be approximately equivalent to a diffusion process, there are a few conditions that must hold. As stated earlier in Section 1.1 regarding "dense media", the average distance between scattering centers \((1/\mu_s)\) must be very small relative to the dimensions of the problem, namely the distances to the boundaries and between the sources and detectors. Similarly, the amount of scattering should be significantly higher than the amount of absorption (the albedo \(= \mu_s/(\mu_s+\mu_a)\) should be close to 1) such that any given photon is expected to scatter many times before either being absorbed by or escaping from the tissue. Given these conditions, the radiance at a particular point in the tissue should have an approximately isotropic angular dependence because a large number of scattering events serves to randomize the direction of photon propagation. It is important to point out that the angular dependence of the radiance cannot be exactly isotropic, otherwise the net flux at \(\vec{r}\) would be zero, and there would be no net transfer of energy. An approximate expression for the slightly anisotropic radiance at \(\vec{r}\) has the form:

\[ L(\vec{r}, \hat{s}) = L_0(\vec{r})(1 + \epsilon(\vec{r})\hat{u} \cdot \hat{s}) \]

where \(L_0(\vec{r})\) is a term for isotropic radiance and \(\epsilon(\vec{r})\hat{u}\) is added as a slight perturbation in the \(\hat{u}\) direction. Substitution of Eq. 1-25 into Eq. 1-12, the definition of net flux, yields a simple expression relating total radiance, the isotropic component of radiance, and the net flux vector.

\[ \hat{F}(\vec{r}) = \int_{4\pi} L(\vec{r}, \hat{s})\hat{s}d\omega = \int_{4\pi} (L_0(\vec{r}) + L_0(\vec{r})\epsilon(\vec{r})\hat{u} \cdot \hat{s})\hat{s}d\omega \neq 0 \]
Figure 1-4. a. \( \hat{u} \) is the unit vector designating the orientation of the perturbation \( \epsilon(\vec{r}) \). b. Graphical depiction of one of the vectors to be summed by the integral in Eq. 1-29. Its magnitude is \( \cos \theta \) and direction is \( \hat{s} \). c. Diagram representing all the vectors to be summed by the integral. From (c) it is clear that the result of the vector integration is a vector in the \( \hat{u} \) direction. In addition, it is clear that only the components of the vectors in the \( \hat{u} \) direction (magnitude = \( \cos^2 \theta \)) need be accounted for (all other components cancel out). The result of these observations is the final integral expression of Eq. 1-29 where the integral over all solid angles has been converted to a spherical coordinate system (see Appendix A for details on integration over solid angles).

Splitting up the integral:

Eq. 1-27  \[ \int_{4\pi} L_0(\vec{r}) \hat{s} d\omega = 0 \]

Eq. 1-28  \[ \int_{4\pi} (L_0(\vec{r}) \epsilon(\vec{r}) \hat{u} \cdot \hat{s}) \hat{s} d\omega = L_0(\vec{r}) \epsilon(\vec{r}) \int_{4\pi} (\hat{u} \cdot \hat{s}) \hat{s} d\omega \]

Eq. 1-29  \[ \int_{4\pi} (\hat{u} \cdot \hat{s}) \hat{s} d\omega = \hat{u} \int_{0}^{\frac{3\pi}{2}} d\phi 2 \int_{0}^{\pi/2} (\cos^2 \theta)(\sin \theta d\theta) = \frac{4\pi}{3} \hat{u} \]

In Eq. 1-29 the integral over \( \theta \) is changed from an integral over \([0,\pi]\) to an integral over \([0,\pi/2]\) multiplied by 2 to properly account for the negative dot product \( \hat{u} \cdot \hat{s} \) when \( \theta > \pi/2 \) (See Figure 1-4 and Appendix A for an explanation of Eq. 1-29). So:

Eq. 1-30  \[ \vec{F}(\vec{r}) = L_0(\vec{r}) \epsilon(\vec{r}) \frac{4\pi}{3} \hat{u} \]

Eq. 1-31  \[ L(\vec{r}, \hat{s}) = L_0(\vec{r}) + \frac{3}{4\pi} \vec{F}(\vec{r}) \cdot \hat{s} \]

after combining Eq. 1-25 and Eq. 1-30.
1.4.2 Fick’s Law, Flux Balance, and Energy Balance

1.4.2.1 Fick’s Law

With Eq. 1-31 in hand, the derivation of the steady state diffusion equation in terms of the optical parameters follows after two more steps. Both steps begin with the radiation transport equation: the first step involves derivation of a flux balance equation, and the second involves derivation of an energy balance equation. There is a heuristic approach to understanding how to get from the transport equation to equations involving flux and energy. Since the transport equation, Eq. 1-21, is written in terms of radiance, we can invoke the definitions of net flux and fluence rate (Eq. 1-12 and Eq. 1-15), both of which are also in terms of radiance, as an indication of how to proceed. The flux balance equation is derived by multiplying the transport equation by $\hat{s}$ and integrating over $4\pi$ steradians, and the energy balance equation is derived by simply integrating the transport equation over $4\pi$ steradians. Note that the fluence rate, which is the scalar integral of radiance, is essentially a measure of energy density.

Before actually deriving the flux and energy balance equations in terms of the optical parameters, it is worthwhile to first get an idea of what their final form will be. Fick’s law of diffusion states simply that:

$$\tilde{F}(\vec{r}) = -D(\text{grad } X) + \text{ a source term}$$

Eq. 1-32

$$\tilde{F}(\vec{r}) = -D\nabla \phi(\vec{r}) + \tilde{S}_i(\vec{r})$$

$D$ is the diffusion constant and is a property of the particular diffusing medium. There are a number of quantities that can be used as “$X$” in our formalism of Fick’s law, such as the radiance or directional photon density, but we choose to use the fluence rate because
we are after flux and energy balance equations. Eq. 1-32 is the general form for flux balance. The steady-state energy balance equation is derived as follows:

\[ \text{power that diffuses away from } \vec{r} + \text{power absorbed at } \vec{r} = \text{power from source} \]

Eq. 1-33

\[ \nabla \cdot \vec{F}(\vec{r}) + \mu_s \phi(\vec{r}) = S_s(\vec{r}) \]

Eq. 1-32 and Eq. 1-33 can be combined to give the energy balance equation in terms of \( D \) and \( \phi \) alone.

Eq. 1-34

\[ D \nabla^2 \phi(\vec{r}) - \mu_s \phi(\vec{r}) + [S_s(\vec{r}) - \nabla \vec{S}_s(\vec{r})] = 0 \]

After derivation of the flux and energy balance equations from the radiation transport equation (utilizing the diffusion approximation), we will return to the general forms given in Eq. 1-32 through Eq. 1-34 to assign definitions to \( D, \vec{S}_s(\vec{r}), \) and \( S_s(\vec{r}) \) in terms of the optical parameters \( \mu_o, \mu_w, \) and \( g. \)

1.4.2.2 Flux Balance Equation

We begin by multiplying Eq. 1-21 by \( \hat{s} \) and integrating over \( 4\pi \) steradians.

Eq. 1-35

\[ \int_{4\pi} \frac{dL(\vec{r},\hat{s})}{ds} \hat{s} d\omega = -\int_{4\pi} \mu_L(\vec{r},\hat{s}) \hat{s} d\omega + \mu_s \int_{4\pi} \int_{4\pi} p(\hat{s},\hat{s}') L(\vec{r},\hat{s}') \hat{s}' d\omega' d\omega \]

\[ + \mu_o p(\hat{s},\hat{s}_0) E_0(\vec{r},\hat{s}_0) e^{-\mu_o \lambda} \hat{s} d\omega \]

Starting with the first term, the integral can be rewritten:

Eq. 1-36

\[ \int_{4\pi} \frac{dL(\vec{r},\hat{s})}{ds} \hat{s} d\omega = \int_{4\pi} (\hat{s} : \nabla L(\vec{r},\hat{s})) \hat{s} d\omega \]

This transformation follows directly from the definition of gradient (see Appendix). The radiance in Eq. 1-36 can be replaced with the expression from Eq. 1-31, which was derived from our diffusion approximation, to yield:
Eq. 1-37
\[ \int_{4\pi} \left[ \hat{s} \cdot \nabla (L_0(\vec{r})) + \frac{3}{4\pi} \vec{F}(\vec{r}) \cdot \hat{s} \right] \hat{s} d\omega = \int_{4\pi} \left[ \hat{s} \cdot \nabla L_0(\vec{r}) \right] \hat{s} d\omega \]
\[ + \frac{3}{4\pi} \int_{4\pi} \left[ \hat{s} \cdot (\nabla \vec{F}(\vec{r}) \cdot \hat{s}) \right] \hat{s} d\omega \]

The first integral is identical in form to Eq. 1-29 with the vector \( \nabla L_0(\vec{r}) \) in place of \( \hat{u} \), so:

Eq. 1-38
\[ \int_{4\pi} \left[ \hat{s} \cdot \nabla L_0(\vec{r}) \right] \hat{s} d\omega = \frac{4\pi}{3} \nabla L_0(\vec{r}) = \frac{\nabla \phi(\vec{r})}{3} \]

Where we replaced the isotropic radiance with fluence rate because:

Eq. 1-39
\[ \phi(\vec{r}) = \int_{4\pi} L(\vec{r}, \hat{s}) d\omega = \int_{4\pi} L_0(\vec{r}) d\omega + \int_{4\pi} (L_0(\vec{r}) \epsilon(\vec{r}) \hat{s} \cdot \hat{u}) d\omega \]
\[ = 4\pi L_0(\vec{r}) + L_0(\vec{r}) \epsilon(\vec{r}) \int_{0}^{\pi} (\cos \theta \sin \theta) d\theta = 4\pi L_0(\vec{r}) \]

The second integral has the general form:

Eq. 1-40
\[ \int_{4\pi} \left[ \hat{s} \cdot \nabla (\vec{A} \cdot \hat{s}) \right] \hat{s} d\omega \]

which turns out to be the sum of vectors pointing in the \( \hat{s} \) direction with magnitude
\[ \hat{s} \cdot \nabla (\vec{A} \cdot \hat{s}) = \hat{s} \cdot \nabla |\vec{A}| \cos \theta \] (remember that \( \hat{s} \) is a unit vector! Eq. 1-41 does not hold for an arbitrary vector \( \vec{s} \).) Expanding Eq. 1-40 into spherical coordinates, we get:

Eq. 1-41
\[ \int_{0}^{2\pi} d\phi \int_{0}^{\pi} (\hat{s} \cdot \nabla \vec{A} \cos \theta) \hat{s}(\sin \theta) d\theta = 2\pi \int_{0}^{\pi} (\hat{s} \cdot \hat{\theta} - \sin \theta \frac{\sin \theta}{R}) \hat{s}(\sin \theta) d\theta = 0 \]

because 1) \( \hat{s} \) is a unit vector so \( R=1 \) and 2) \( \hat{s} \) is always radial so \( \hat{s} \cdot \hat{\theta} = 0 \). Figure 1-5 provides a graphical depiction of the integral in Eq. 1-41.

The second term of Eq. 1-35 is significantly easier to evaluate, since it is nothing more than the definition of net flux.

Eq. 1-42
\[ \int_{4\pi} \mu L(\vec{r}, \hat{s}) \hat{s} d\omega = -\mu \vec{F}(\vec{r}) \]
Recall that we are actually only working with the scattered radiance, so here \( \vec{F}(\vec{r}) \) is really the net \textit{scattered} flux vector.

The third term is not so obvious:

\[ \text{Eq. 1-43} \quad \int_4\int_4 \mu_s \int_4 p(\hat{s}, \hat{s}') L(\hat{r}, \hat{s}') \hat{s} d\omega' d\omega = \mu_s \int_4 L(\hat{r}, \hat{s}') \left[ \int_4 p(\hat{s}, \hat{s}') \hat{s} d\omega \right] d\omega' \]

The tricky part of this term is the integral between the square brackets. It is the vector sum of vectors in all directions \( \hat{s} \), weighted by the probability of scattering from \( \hat{s}' \) into \( \hat{s} \). From Figure 1-6 it is clear that the resulting sum will be in the \( \hat{s}' \) direction, and only those vector components parallel to \( \hat{s}' \) need be taken account of because all perpendicular components cancel out. These observations are expressed in Eq. 1-44 by 1) changing the integration into a scalar sum of the components parallel to \( \hat{s}' \) (via the dot product) and 2) defining the final result to be in the \( \hat{s}' \) direction.

\[ \text{Eq. 1-44} \quad \hat{s}' \int_4\int_4 (p(\hat{s}, \hat{s}') \hat{s}) \cdot \hat{s}' d\omega = \hat{s}' \int_4 p(\hat{s}, \hat{s}') (\hat{s} \cdot \hat{s}') d\omega = g \hat{s}' \]

by the definition of \( g \), Eq. 1-23. Substitution back into Eq. 1-43 yields:
Figure 1-6. Graphical representation of the integral in brackets in Eq. 1-43. a. $\hat{s}'$ is the original photon direction. b. Example of one of the vectors to be summed. The magnitude is $p(\hat{s}, \hat{s}')\hat{s}$, and it is pointing in the $\hat{s}$ direction (the final direction, which is the variable of integration). c. Depiction of all the vectors to be summed. The important observation is the symmetry about $\hat{s}'$, as illustrated in Figure 1-3. This particular drawing indicates somewhat forward directed scattering. d. Since all of the vector components perpendicular to $\hat{s}'$ cancel out, what is left to be integrated points in the $\hat{s}'$ direction and has a magnitude $= p(\hat{s}, \hat{s}')\hat{s} \cdot \hat{s}'$.

Eq. 1-45

$$\mu_s \int L(\vec{r}, \hat{s}') g\hat{s}' d\omega' = \mu_s g\vec{F}(\vec{r})$$

The final term to evaluate from Eq. 1-35 is the source term. Since the integral is over $d\omega$ only, there are constant terms that can be removed from the integral to leave:

Eq. 1-46

$$\int \mu_s p(\hat{s}, \hat{s}_0) E_0(\vec{r}, \hat{s}_0) e^{-\mu_s \hat{s} d\omega} = \mu_s E_0(\vec{r}, \hat{s}_0) e^{-\mu_s \hat{s} d\omega} \int p(\hat{s}, \hat{s}_0) \hat{s} d\omega$$

This integral is identical in form to the bracketed integral in Eq. 1-43, with $\hat{s}_0$, the constant direction from which the tissue is being irradiated by the source, instead of the variable $\hat{s}'$. So:

Eq. 1-47

$$\mu_s E_0(\vec{r}, \hat{s}_0) e^{-\mu_s \hat{s}_0 d\omega} = \mu_s g E_0(\vec{r}, \hat{s}_0) e^{-\mu_s \hat{s}_0}$$

Collecting all the results to substitute back into Eq. 1-35 yields the following equation:

Eq. 1-48

$$\frac{\nabla \phi(\vec{r})}{3} = -\mu_s \vec{F}(\vec{r}) + \mu_s g\vec{F}(\vec{r}) + \mu_s g E_0(\vec{r}, \hat{s}_0) e^{-\mu_s \hat{s}_0}$$
which can be rearranged:

$$\tilde{\Phi}(\vec{r})(\mu_0 + (1 - g)\mu_s) = -\frac{\nabla \phi(\vec{r})}{\mu_s} + \mu_s g E_0(\vec{r}, \hat{s}_0) e^{-\mu_s l \hat{s}_0}$$

We then define:

$$\mu_s' = (1 - g)\mu_s = \text{the "reduced scattering coefficient"}$$

$$\mu_l' = \mu_s + \mu_l' = \text{the "reduced total interaction coefficient"}$$

so the final result is:

$$\tilde{\Phi}(\vec{r}) = -\frac{\nabla \phi(\vec{r})}{3\mu_l'} + \frac{\mu_s g}{\mu_l'} E_0(\vec{r}, \hat{s}_0) e^{-\mu_s l \hat{s}_0}$$

This final result has the same form as the general equation of flux balance shown in Eq. 1-32 with:

$$D = \frac{1}{3\mu_l'}$$

$$\tilde{S}_l(\vec{r}) = \frac{\mu_s g}{\mu_l'} E_0(\vec{r}, \hat{s}_0) e^{-\mu_s l \hat{s}_0}$$

Eq. 1-53 is approximate because the fluence rate term in Eq. 1-52 comes from the first term in Eq. 1-35, which was evaluated via the diffusion approximation, expressed in Eq. 1-31. The fact that the expression for the diffusion constant is approximate is an important one which will resurface later in the discussion of oblique-incidence reflectometry.

### 1.4.2.3 Energy Balance Equation

To derive the energy balance equation from the transport equation, we need only integrate the transport equation over $4\pi$ steradians.
\textbf{Eq. 1-55} \quad \int_{4\pi} \frac{dL(\tilde{r}, \tilde{s})}{ds} \, d\omega = -\int_{4\pi} \mu_s L(\tilde{r}, \tilde{s}) \, d\omega + \int_{4\pi} \mu_s \int_{4\pi} p(\tilde{s}, \tilde{s}', L(\tilde{r}, \tilde{s}') \, d\omega' \, d\omega \\
+ \int_{4\pi} \mu_s p(\tilde{s}, \tilde{s}_o) E_0(\tilde{r}, \tilde{s}_o) e^{-\mu_s l} \, d\omega

The first integral can be evaluated after a simple transformation using the definition of divergence (see Appendix A).

\textbf{Eq. 1-56} \quad \int_{4\pi} \frac{dL(\tilde{r}, \tilde{s})}{ds} \, d\omega = \int_{4\pi} \nabla \cdot L(\tilde{r}, \tilde{s}) \, d\omega = \nabla \cdot \int_{4\pi} L(\tilde{r}, \tilde{s}) \, d\omega = \nabla \cdot \tilde{F}(\tilde{r})

The second integral is the definition of fluence rate, Eq. 1-15, with an added constant.

\textbf{Eq. 1-57} \quad -\int_{4\pi} \mu_s L(\tilde{r}, \tilde{s}) \, d\omega = -\mu_s \phi(\tilde{r})

The third term also turns out to be essentially equivalent to Eq. 1-15.

\textbf{Eq. 1-58} \quad \int_{4\pi} \mu_s \int_{4\pi} p(\tilde{s}, \tilde{s}') L(\tilde{r}, \tilde{s}') \, d\omega' \, d\omega = \mu_s \int_{4\pi} L(\tilde{r}, \tilde{s}') \left[ \int_{4\pi} p(\tilde{s}, \tilde{s}) \, d\omega \right] \, d\omega' \\
= \mu_s \int_{4\pi} L(\tilde{r}, \tilde{s}) \, d\omega' = \mu_s \phi(\tilde{r})

The bracketed integral in Eq. 1-58 is equal to 1 by definition (Eq. 1-22).

The final source term is evaluated similarly:

\textbf{Eq. 1-59} \quad \int_{4\pi} \mu_s p(\tilde{s}, \tilde{s}_o) E_0(\tilde{r}, \tilde{s}_o) e^{-\mu_s l} \, d\omega = \mu_s E_0(\tilde{r}, \tilde{s}_o) e^{-\mu_s l} \int_{4\pi} p(\tilde{s}, \tilde{s}_o) \, d\omega = \mu_s E_0(\tilde{r}, \tilde{s}_o) e^{-\mu_s l}

Collecting all the terms together to put back into Eq. 1-55, we get:

\nabla \cdot \tilde{F}(\tilde{r}) = -\mu_s \phi(\tilde{r}) + \mu_s \phi(\tilde{r}) + \mu_s E_0(\tilde{r}, \tilde{s}_o) e^{-\mu_s l}

which simplifies to:

\textbf{Eq. 1-60} \quad \nabla \cdot \tilde{F}(\tilde{r}) + \mu_s \phi(\tilde{r}) = \mu_s E_0(\tilde{r}, \tilde{s}_o) e^{-\mu_s l}

This final result has the same form as the general equation for energy balance shown in

\textbf{Eq. 1-33} with:

\textbf{Eq. 1-61} \quad S_2(\tilde{r}) = \mu_s E_0(\tilde{r}, \tilde{s}_o) e^{-\mu_s l}
Now we finally have expressions for all the terms in the steady-state diffusion equation, Eq. 1-34, and can rewrite it in terms of the optical parameters of the tissue and the irradiance.

\[
\text{Eq. 1-62} \quad \frac{\nabla^2 \phi(\vec{r})}{3\mu_t} - \mu_a \phi(\vec{r}) + \left[ \mu_s E_0(\vec{r}, \hat{S}_0) e^{-\mu_l} \frac{\mu_g}{\mu_t} E_0(\vec{r}, \hat{S}_0) e^{-\mu_l} \hat{S}_0 \right] = 0
\]

### 1.4.3 Solution for an Isotropic Point Source in an Infinite Medium

Eq. 1-62 is the steady-state diffusion equation which we have been working towards throughout this chapter. By applying the diffusion approximation of Eq. 1-31 to the transport equation, Eq. 1-21, we were able to deduce expressions for the diffusion constant and source terms defined in terms of the optical parameters of the tissue and the irradiance. In this last section of Chapter 1, we will solve the simplest situation modeled by the diffusion equation: a single, isotropic point source buried in an infinite medium of known optical properties. This model will prove essential to understanding methods for measuring tissue optical properties via reflectance measurements.

We assume that the constant $D$ is known, and for a buried, isotropic point source, the sources $\nabla \cdot \hat{S}_1(\vec{r})$ and $S_2(\vec{r})$ must add up to a delta function at the origin, weighted by the source power $S_0$. Thus, Eq. 1-34 is rewritten:

\[
\text{Eq. 1-63} \quad D \nabla^2 \phi(\vec{r}) - \mu_a \phi(\vec{r}) = -S_0(\vec{r}) = -S_0 \delta(\vec{r})
\]

Despite the symmetry of the problem, which allows all the terms to be functions of $r$ alone, any integration must still be done in spherical coordinates. The effective attenuation coefficient is defined:
\[ \mu_{\text{eff}}^2 = \frac{\mu_s}{D} = 3\mu_s \mu_t, \]

and Eq. 1-63 can be rewritten again, with \( k^2 = -\mu_{\text{eff}}^2 \), to give the inhomogeneous Helmholtz equation:

\[ \nabla^2 \phi(\vec{r}) + k^2 \phi(\vec{r}) = -\frac{S_0}{D} \delta^3(\vec{r}) \]

with \( \phi(\vec{r}) \) being the Green's function, that is, the solution to the inhomogeneous partial differential equation when the source is an impulse function, denoted by \( \delta^3 \) because we are considering a three-dimensional problem. The term \( S_0/D \) is simply a scaling factor which must be taken into account for this particular situation. The significance of the Green's function is that it is the impulse response of the system (an infinite medium), and hence completely characterizes it. In fact, a solution of Eq. 1-34 for an arbitrary source can be assembled by linear superposition of the impulse response. We can thus take advantage of a well-known method for finding the impulse response of an electrical system – it requires rewriting Eq. 1-65 in terms of an integral transform (we'll use the Fourier transform here), finding the transform of the solution, then inverse-transforming back to the solution [20]. The first step is to write the "input" (source) and "output" (fluence rate) functions in terms of a Fourier transform.

\[ \phi(\vec{r}) = \frac{1}{(2\pi)^3} \int \Phi(\vec{u}) e^{i\vec{u} \cdot \vec{r}} d^3 \vec{u} \]

\[ \delta(\vec{r}) = \frac{1}{(2\pi)^3} \int \Delta(\vec{u}) e^{i\vec{u} \cdot \vec{r}} d^3 \vec{u} = \frac{1}{(2\pi)^3} \int e^{i\vec{u} \cdot \vec{r}} d^3 \vec{u} \]

In Eq. 1-67 we have taken advantage of the fact that the Fourier transform of a delta function is 1. Substituting these transforms into Eq. 1-65 gives:
Eq. 1-68 \[ \frac{1}{(2\pi)^3} \int \bar{\Phi}(\vec{u})e^{i\vec{u}\cdot\vec{r}}d^3\vec{u} = -\frac{S_0}{D} \frac{1}{(2\pi)^3} \int e^{i\vec{u}\cdot\vec{r}}d^3\vec{u} \]

The Laplacian on the left hand side should be evaluated in spherical coordinates. The result is:

Eq. 1-69 \[ \frac{1}{(2\pi)^3} \int \bar{\Phi}(\vec{u})e^{i\vec{u}\cdot\vec{r}}d^3\vec{u} = -\frac{S_0}{D} \frac{1}{(2\pi)^3} \int e^{i\vec{u}\cdot\vec{r}}d^3\vec{u} \]

From this equation we can algebraically deduce the transform \( \Phi(\vec{u}) \), which now be inverse-transformed to yield the Green’s function, \( \phi(\vec{r}) \).

Eq. 1-70 \[ \Phi(\vec{u}) = \frac{S_0}{D} \frac{1}{u^2 - k^2} \]

Eq. 1-71 \[ \phi(\vec{r}) = \frac{S_0}{D} \frac{1}{(2\pi)^3} \int \frac{1}{u^2 - k^2} e^{i\vec{u}\cdot\vec{r}}d^3\vec{u} \]

The inverse transform should be evaluated in spherical coordinates:

\[ \phi(r,\theta,\phi) = \frac{S_0}{D} \frac{1}{(2\pi)^3} \int_0^{2\pi} d\phi \int_0^\pi d\theta \int_0^\infty u^2 du \left[ \int_0^\pi e^{iur \cos \theta} \sin \theta d\theta \right] \]

\[ = \frac{S_0}{D} \frac{1}{(2\pi)^3} \int_0^\infty u^2 du \left[ \frac{1}{iu} \sin \theta \right] \left[ \frac{1}{iu} - \frac{1}{iu} \right] \]

Eq. 1-72 \[ = \frac{S_0}{D} \frac{1}{(2\pi)^3} \int_0^\infty u^2 du \left[ \frac{1}{iu} \sin \theta \right] \left[ \frac{1}{iu} - \frac{1}{iu} \right] \]

\[ = \frac{S_0}{D} \frac{1}{(2\pi)^3} \int_0^\infty u^2 du \left[ \frac{1}{iu} - \frac{1}{iu} \right] \]

\[ = \frac{S_0}{D} \frac{1}{8\pi^2i} \int_0^\infty \left( \frac{ue^{iur}}{u^2 - k^2} - \frac{ue^{-iur}}{u^2 - k^2} \right) du \]

In the last step, the limits of integration were changed from \([0,\infty]\) to \([-\infty,\infty]\) with an added factor of 1/2. This is possible because the integrand is an even function. To complete the evaluation of Eq. 1-72, there are two integrals

Eq. 1-73 \[ \int \frac{ue^{iur}}{(u+k)(u-k)} du \quad \text{and} \quad \int \frac{ue^{-iur}}{(u+k)(u-k)} du \]
that must be evaluated using the Residue Theorem from complex analysis (it is sometimes called Cauchy’s Integral Formula) [21, 22].

\textbf{Eq. 1-74} \quad \oint_C f(u) du = 2\pi i (\text{sum of the residues enclosed by } C)

Where a residue is the coefficient of each term \((u-k)^{-1}\) in the expansion of \(f(u)\) in the neighborhood of each isolated singularity, or pole, caused by that term. The poles in Eq. 1-73 occur at \(u = \pm k\), and evaluation of the two integrals using the Residue Theorem yields:

\textbf{Eq. 1-75} \quad 2\pi i \sum_{n=1}^{\infty} \text{Res} \left[ \frac{ue^{ikr}}{(u+k)(u-k)} \right] = 2\pi i \left[ \frac{ke^{ikr}}{2k} - \frac{-ke^{-ikr}}{-2k} \right] \Rightarrow i\pi e^{ikr}

and

\textbf{Eq. 1-76} \quad 2\pi i \sum_{n=1}^{\infty} \text{Res} \left[ \frac{ue^{-ikr}}{(u+k)(u-k)} \right] = 2\pi i \left[ \frac{ke^{-ikr}}{2k} - \frac{-ke^{ikr}}{-2k} \right] \Rightarrow -i\pi e^{ikr}

Since it does not make physical sense for there to be a term \(e^{-ikr} = e^{\mu_r} e^{-\sigma r}\) (the wave must propagate away from the point source, and the fluence rate must decrease), those terms are left out in the final summation of residues. Additional minus signs are added when evaluating the residues at \(u = -k\) because the theorem specifies poles for terms of the form \((u-k)^{-1}\) with positive \(u\), not \((u+k)^{-1}\) with negative \(u\). This is equivalent to reversing the direction of integration. So finally,

\textbf{Eq. 1-77} \quad \phi(r, \theta, \phi) = \frac{S_0}{D} \frac{1}{8\pi^2 \rho^4} \left[ (i\pi e^{-ikr} - (-i\pi e^{ikr}) \right] = \frac{S_0}{D} \frac{e^{ikr}}{4\pi r}

or, in terms of the effective attenuation coefficient, recall that \(k^2 = -\mu_{\text{eff}}^2\), so:

\textbf{Eq. 1-78} \quad \phi(r) = \frac{S_0}{D} \frac{e^{-\mu_{\text{eff}} r}}{4\pi r}

which is Green’s function for an isotropic point in a diffusing medium of infinite extent.
At this point, we are prepared to move on to Chapter 2, which utilizes the theoretical background material covered in this chapter to review several of the methods currently in use for measuring the optical properties of turbid media such as biological tissue.
Chapter 2  Current Methods for Measuring Optical Properties

2.1 Introduction

The measurement of the optical properties of biological tissues remains a central problem in the field of tissue optics. As covered in Chapter 1, before the distribution of light in a tissue can be accurately described by a model of light transport and sufficient boundary conditions (most importantly, the incident irradiance), the intrinsic optical properties of the tissue itself must be known. Having derived an adequate light distribution model, measurement of these properties is what was described as “the second task of tissue optics”. In the radiation transport model, the fundamental properties include the absorption coefficient ($\mu_a$), the scattering coefficient ($\mu_s$), the index of refraction ($n$), and the single-scattering phase factor ($g$). From these basic constants are derived some often more commonly used terms: the total interaction coefficient ($\mu_t$), the mean free path ($mfp$), the reduced scattering coefficient ($\mu_s'$), the transport mean free path ($mfp'$), the effective attenuation coefficient ($\mu_{\text{eff}}$) and the diffusion coefficient ($D$) (refer to Chapter 1 for details).

Armed with the knowledge of what the optical properties of a tissue are, we can then use them in the model of light transport to calculate fluence rate and flux in a tissue given the source power and tissue geometry. Therapeutic procedures such as photocoagulation, photoablation, and photodynamic therapy call for real-time monitoring of tissue optical properties (at all the wavelengths in use) for proper dosimetry of light because the absorption and scattering parameters, which are necessary to calculate the transport of
power, are subject to change during the course of a procedure due to the action of the procedure itself. Diagnostic procedures, including forms of optical imaging and spectroscopy, also often require knowledge of tissue optical properties for accurate image reconstruction or other forms of information processing. In addition, the optical properties themselves can potentially provide sufficient information to distinguish between diseased and normal tissues (and hence be the basis of image contrast). In particular, there is evidence to suggest that certain forms of cancer can be diagnosed via optical properties determined from spectrally resolved reflectance measurements [23, 24]. It is known that visible and near infrared light interact with both the molecular and subcellular components of tissue, and therefore it is not unreasonable to expect that characteristic changes in tissue optical properties at particular wavelengths are indicative of disease. Thus, for both therapeutic and diagnostic applications, it is important to have an efficient way to monitor tissue optical properties at many wavelengths simultaneously.

There are currently a number of standard methods that have been developed for the purpose of measuring optical properties. This chapter briefly reviews the theory underlying a few of the techniques that are relevant for comparison to the method that we have developed, oblique-incidence optical fiber reflectometry. That is to say, of the many photometric, photoacoustic, and photothermal means of determining one or more of the aforementioned properties of turbid media, reviewed here is only a sampling of the photometric methods. In discussing these methods, there are five main issues that should be kept in mind for each: 1) which optical properties can be measured, 2) are multiwavelength measurements possible, and if so, how convenient, 3) what equipment is
necessary to perform the measurement, 4) can the measurement be performed in vivo, and 5) are the optical properties measured directly or indirectly? A direct measurement allows one to obtain the property of interest in a manner that is independent of any model of light transport, whereas in an indirect measurement the optical properties must be reconstructed by solving an “inverse problem” – some parameter, usually reflection or transmission, is measured, and a particular model of light transport is used to predict what the values of the optical properties must have been to yield the measured results. Indirect measurements are thus only as accurate as their associated models and curve-fitting procedures. These five points are emphasized in the discussion of each of the following techniques: collimated transmission, interstitial fluence mapping, added absorber, integrating spheres and spatially-resolved diffuse reflectance. The majority of this chapter was compiled from *Optical-Thermal Response of Laser-Irradiated Tissue* and *Medical Optical Tomography: Functional Imaging and Monitoring.*

### 2.2 Collimated Transmission

Measurement of optical properties by collimated transmission is a simple but not highly reliable technique for real tissue samples. A collimated beam of light such as that from a laser is first aligned with a collimated photodetector, for example, a photomultiplier tube with either a pinhole or an entrance tube. The sample is then placed in the path of the beam. With smaller solid angles of collection, higher degrees of accuracy are achieved, so the detector aperture size should be minimized while the sample to detector distance should be maximized. By using an optically thin sample, i.e. thin
enough so that we can assume no photons scatter more than once during traversal of the sample, we can determine the total interaction coefficient, $\mu_t$. For a sample to be optically thin, the thickness $d$ must be significantly smaller than the average distance between scattering events: $d << 1/\mu_s$. Given the ideal situation of a single-scattering sample, a perfectly collimated source beam, and a perfectly collimated (and aligned) detector, we assume that only those photons that are neither absorbed nor scattered by the sample will be detected (Figure 2-1). In this case, the amount of detected light can be predicted by Beer's law, Eq. 1-8:

$$\text{Collected power} = \text{Incident power} \ e^{-(\mu_s + \mu_t) d}$$

It follows that:

$$\% \text{Transmitted} = T_c = e^{-\mu_t d},$$

so

$$\mu_t = \frac{-\ln(T_c)}{d}$$

Eq. 2-1

There are two fundamental problems with this technique. First of all, the optical scattering coefficient for soft tissues typically ranges anywhere from 100 to 1000 cm$^{-1}$. To be certain that a sample is actually "optically thin", it would have to be on the order of 0.001 cm (10 µm) thick. There are only two ways to reliably obtain tissue samples of
this thickness: 1) microtome sectioning of frozen tissue and 2) homogenizing the tissue to a semiliquid state and placing it in a microcuvette [25]. Both of these methods have potential to alter the optical properties of the excised tissue, which in fact may not even be the same as the \textit{in vivo} optical properties to begin with. In addition, the thickness of a frozen section may change significantly upon thawing.

The second fundamental problem with this technique is the assumption that once a photon has undergone a scattering event, it is no longer part of the collimated beam. The mean scattering cosine ($g$), for tissues is typically 0.85 or higher, so there is a very high probability that a photon will be "forward scattered" and, in fact, not scatter out of the main beam, thus yielding high values of $T_c$ and correspondingly low values of $\mu_c$. Despite these issues, it is possible to quantify the error associated with problems such as these as well as the non-ideal situation of imperfectly collimated sources and detectors [26]. By doing so, a collimated transmission measurement can theoretically be made within predefined, arbitrary limits on the error.

While this technique for measuring the total interaction coefficient of real biological tissues tends to be somewhat unreliable, mainly as a result of problems with tissue preparation, it is ideally suited for measurements on tissue phantoms, liquids in particular. As long as solid (or gel) phantoms can be reliably cut sufficiently thin, this method will work quite well for them too. This technique is ideal for liquids because 1) they can be placed in precision cuvettes and 2) they can be diluted. As a result, the interaction coefficient can be determined from many measurements at sequential dilutions, as opposed to a single measurement. Beer's law can be modified to take into account the dilution of a phantom:
Eq. 2-2

\[ T_e = e^{-C_r \mu_t d} \]

where \( C_r \) is the serial dilution factor (which equals 1 for the undiluted phantom). Of course, \( C_r \) must be sufficiently small to approximate a single-scatter situation. Rearranging Eq. 2-2, we get:

Eq. 2-3

\[ C_r \mu_t = -\frac{\ln(T_e)}{d} \]

Since the thickness \( d \) is constant, we see that \( \mu_t \) is essentially the slope of a plot of \( \ln(T_e) \) vs. \( C_r \). As long as the points lie in a straight line, we can be confident that the single-scattering condition was satisfied.

Finally, let us discuss the five main issues mentioned in the introduction. As already pointed out, this method measures only the total interaction coefficient of a sample, and hence cannot provide values for absorption or scattering alone unless the sample is a pure absorber or scatterer. (For pure liquid absorbers, this technique is very accurate and is employed by commercial spectrophotometers.) Since the only mathematical expression employed by this technique is Beer's law, we can say that this method measures \( \mu_t \) directly, but not \textit{in vivo} because thin slices of tissue are required. Next, it would be relatively easy to obtain total interaction spectra with this technique. If the measurement were performed with a collimated white light source and an automated optical multi-channel analyzer (OMA), then acquisition of a spectrum requires the same amount of work as a single wavelength measurement. A time-consuming alternative would be to use a white light source and manually scan a monochromator, either on the source or detector. In summary, the equipment required to perform monochromatic measurements is minimal: laser(s) at the desired wavelength(s), a collimated photodetector, and either a
precision cuvette (for liquids) or a microtome. To perform multi-spectral measurements, a wavelength resolving instrument is necessary, be it a simple grating or prism, a spectrograph/monochromator, or an OMA.

2.3 Interstitial Fluence Mapping

Interstitial fluence mapping is primarily used as a simple technique for measuring the effective attenuation coefficient, $\mu_{\text{eff}}$, of tissues in vivo. In Chapter 1, we derived the Green’s function for a diffusing medium of infinite extent, Eq. 1-78:

$$\phi(r) = \frac{S_0 e^{-\mu_{\text{eff}}}}{D} \frac{e^{-\mu_{\text{eff}} D}}{4\pi r}$$

For a one dimensional problem, which can be approximated in an experiment by broad beam illumination.

Eq. 2-4

$$\phi(z) = K \frac{e^{-\mu_{\text{eff}} z}}{z}$$

By placing two isotropic detection fibers in a tissue with a known separation in the $z$ direction (along the axis of illumination), $\Delta z$ (see Figure 2-2), $\mu_{\text{eff}}$ can be determined as follows:

$$\frac{\text{Detected Power (Fiber 1)}}{\text{Detected Power (Fiber 2)}} = \frac{e^{-\mu_{\text{eff}} z_0}}{e^{-\mu_{\text{eff}} (z_0 + \Delta z)}}$$

Eq. 2-5

$$\ln \left( \frac{P(1)}{P(2)} \right) = \Delta z \mu_{\text{eff}}$$

Since this technique requires the use of the Green’s function for an infinite diffusing medium, it is not model independent, and hence is considered an indirect method. There are two important points to keep in mind when using this method to measure $\mu_{\text{eff}}$. First
of all, recall that the model of tissue as a homogeneous, isotropically diffusing medium (the diffusion approximation to radiation transport theory) is only accurate when the source – detector separation is much larger than the average distance between scattering centers, $1/\mu_t$. In addition, the boundaries must be sufficiently far away such that the solution for an infinite medium is approximately correct. So whereas for the collimated transmission measurement one of the main limiting factors is the ability to get a thin enough sample slice, for the fluence mapping measurement the sample must be large enough to approximate an infinite medium. The second point is that although insertion of the fiber tips into a tissue can be done in vivo, it is a rather invasive procedure. Not only could the fibers themselves perturb the light field, but the trauma following penetration could also cause the optical properties of the tissue to change from their normal values.

Similarly to collimated transmission, this technique requires minimal equipment for monochromatic measurements – just an expanded monochromatic beam, isotropic detection fibers, and a photodetector. The detection fibers can be made very easily from normal fused silica optical fiber dipped into a mix of epoxy and titanium dioxide (there are
of course other methods for making isotropic tips as well as methods for testing them [27]). This method is simple to extend to perform multi-spectral measurements, given the requisite equipment: a broad beam white light source and an OMA. Of course, if an OMA is not available, then the wavelengths of interest must somehow be scanned manually.

Finally, it is worth pointing out that this technique can also potentially be extended to measure additional optical properties. To determine a second property, another measurement must be built into the previously described procedure. One possibility is to use a narrow illumination beam instead of a broad beam. The fluence distribution in the tissue would then become a two-dimensional, cylindrically symmetric problem. The additional information provided by the lateral spread of light could be used to determine a second optical property by fitting the sampled two-dimensional fluence distribution against expected distributions based on either diffusion theory calculations or Monte Carlo simulations. A serious drawback to this approach is that mapping of the two-dimensional fluence distribution is very invasive. An alternative is to use broad beam illumination and the one-dimensional problem while including an absolute measurement of fluence at a known depth in the sample. The absolute measurement adds a scaling factor to the original measurement, so not only can the slope of the decrease in light be measured ($\mu_{\text{eff}}$), but the correct amplitude is also now known. These two pieces of information can be translated into the absorption and reduced scattering coefficients, $\mu_a$ and $\mu_s'$ [25]. The following section covers yet another extension of the fluence mapping technique to determine both the absorption and reduced scattering of a tissue.
2.4 Added Absorber

As the name of this technique suggests, it requires the sequential addition of a dye with a known absorption coefficient to either a tissue homogenate or a sample fluid (such as a water-based phantom). This method will not work with solids or semi-solids because the added dye must be distributed uniformly in the medium. In addition, the total volume must be known to calculate the absorption coefficient of the diluted dye. From Eq. 1-64:

$$\mu_{\text{eff}}^2 = 3\mu_a (\mu_s + \mu_s')$$

If the albedo of a sample is high, then:

Eq. 2-6

$$\mu_{\text{eff}}^2 = 3\mu_a \mu_s' = 3\mu_{at}\mu_{st}'+3\mu_{ad,\mu_{st}}'$$

where the subscript "t" refers to the properties of the tissue itself, and the subscript "d" refers to the properties of the diluted dye. It is assumed that the dye is purely absorbing. Since $\mu_{at}$ and $\mu_{st}'$ are constants, a plot of $\mu_{\text{eff}}^2/3$ vs. $\mu_{ad}$ for sequentially increasing amounts of dye should yield a line a with slope of $\mu_{st}'$ and a y-intercept at $(\mu_{at}, \mu_{st}')$. Both the absorption and reduced scattering coefficients can thus be deduced with this indirect measurement, which can be performed with the fluence mapping technique to determine $\mu_{\text{eff}}$. 
2.5 Integrating Spheres

2.5.1 Direct Measurement

An integrating sphere is a hollow ball whose inside surface is coated with a highly reflective and diffusing coating. When assembled, there are a number of ports in the wall of the sphere through which to introduce or detect light. If a single sphere were set up as depicted in Figure 2-3 with no sample inside, all the incident collimated light would strike the back wall of the sphere and reflect in random directions within the sphere. The detector would record an output power just slightly less than the total input power because of light scattered out through the input port or absorbed in the sphere.

\[ \eta = \frac{P}{P_o} = \text{fraction of incident power that is detected} \]

Eq. 2-7

With an optically thin sample placed in the middle of the sphere, the power detected at the output port corresponds to a known percentage (\( \eta \)) of the photons that are either scattered or transmitted (i.e. not absorbed) by the sample. Going back to Beer's law, it follows that:

\[ \text{detected power (corrected for sphere losses)} = \frac{P}{\eta} = P_0 e^{-\mu_a d} \]

Eq. 2-8

\[ \mu_a = -\frac{1}{d} \ln \left( \frac{P}{\eta P_0} \right) \]

The scattering coefficient can be measured in a similar manner. If a third port is opened directly behind the sample such that all transmitted photons escape the sphere, then a known percentage of the scattered (but not absorbed or transmitted) photons will...
be detected. There is an expression similar to Eq. 2-8 for $\mu_s$. Whereas in the expression for absorption, we have:

$$\mu_a = -\frac{1}{d} \ln(\% \text{ of light NOT absorbed})$$

For scattering the expression becomes:

$$\mu_s = -\frac{1}{d} \ln(\% \text{ of light NOT scattered})$$

Eq. 2-9

$$\mu_s = -\frac{1}{d} \ln \left(1 - \frac{P}{\eta P_0}\right)$$

Disregarding the issue of obtaining and mounting an optically thin tissue sample inside the integrating sphere, the main problem with this approach is that it assumes each photon will traverse the sample only once. This, of course, is not the case, so absorption is overestimated. In addition, for the scattering measurement, it is incorrect to assume that only non-scattered light escapes through the rear port. Not only is scattering highly forward directed, as we pointed out in section 2.2, but photons that have been multiply scattered in the sphere could also escape.
As with the previously described methods, the instrumentation required is minimal for monochromatic measurements: a collimated light source, an integrating sphere, and detectors. Clearly, this technique can be extended to perform multi-wavelength measurements in the same manner as the previously described methods.

2.5.2 Indirect Measurement

An integrating sphere can also be employed to make diffuse reflectance and transmittance measurements on optically thick tissue slabs \((d \gg \text{optical penetration depth} = 1/\mu_{eff})\) as shown in Figure 2-4a and b. With these two measurements, the absorption and reduced scattering coefficients of the tissue can be obtained in a number of
ways, all of which involve the use of some particular physical model, and hence all of which are indirect.

It is quite easy to measure the absolute diffuse reflectance and transmittance with an integrating sphere. For $R_d$, first a diffuse reflectance standard is used as the sample. After this calibration, the absolute diffuse reflectance of the tissue slab is simply:

\[ \text{Eq. 2-10} \quad R_d = (\text{reflectance of standard}) \frac{(\text{signal from sample})}{(\text{signal from standard})} \]

For the transmission measurement, the standard for 100% transmission is no sample at all:

\[ \text{Eq. 2-11} \quad T_d = (1) \frac{(\text{signal with sample})}{(\text{signal without sample})} \]

The factor $\eta$, which was necessary for the direct measurement, cancels out for these measurements.

Instead of using a single integrating sphere, as shown in Figure 2-4a and b, many groups have been using a double integrating sphere system, as shown in Figure 2-4c [28-30]. With this set-up, $R_d$ and $T_d$ can be measured simultaneously, but cross-talk between the two spheres increases the signal in both spheres over the corresponding single sphere measurement. This problem must be accounted for either in the calculations or through careful calibration with standards.

Once $R_d$ and $T_d$ have been obtained, their connection to the optical properties of the tissue slab can be modeled analytically using the Kubelka-Munk multiple flux models [5, 31, 32], diffusion theory [5, 16, 33], or the adding-doubling method [34]. An alternative is to use statistical Monte Carlo modeling. While the analytic methods are easy to work with because the diffuse reflectance and transmittance are expressed in closed form as
functions of $\mu_a$ and $\mu_s'$, there are no existing analytic inverse solutions. So regardless of
the model chosen, the only way to reconstruct the optical properties from $R_d$ and $T_d$ is
through either an iterative fitting procedure or interpolation in precomputed maps of the
three dimensional spaces defined by $\mu_a$, $\mu_s'$, and either $R_d$ or $T_d$ [25]. For quick,
approximate solutions, a multiple-flux model can be used in an iterative procedure [28],
but for maximum accuracy, the errors associated with the particular model and the
physical set-up should calculated, or preferably, the exact source – sample – detector
geometries should be modeled with Monte Carlo simulations [29].

The instrumentation required to do integrating spheres measurements on thick tissue
slabs is identical to that required for the direct, thin sample, measurement for both
monochromatic and multiple wavelength measurements. For the most part, these
measurements are performed in vitro, but reflectance measurements alone have been
performed in vivo to characterize the difference in reflectivity between neoplastic and
non-neoplastic skin lesions [23, 24]. Finally, the double integrating sphere can be
modified slightly by the addition of an exit port for collimated, transmitted light (similar
to the port introduced for the measurement of scattering in the previous section) to allow
the simultaneous deduction of $\mu_a$, $\mu_s$, and $g$ [28].
2.6 Spatially-resolved diffuse reflectance

2.6.1 Theory and Modeling

We begin by taking Eq. 1-34, the diffusion theory energy balance equation, as our mathematical model for photon migration.

\[ D \nabla^2 \phi(\vec{r}) - \mu_e \phi(\vec{r}) + \left[ S_1(\vec{r}) - \nabla \cdot \vec{S}_1(\vec{r}) \right] = 0 \]

Solution of this equation involves proper characterization of the source terms and satisfaction of the boundary conditions. For a pencil beam incident upon a semi-infinite medium, the source terms for scattered fluence will comprise a distribution of point sources along the z axis (the axis of the beam). See Figure 2-5. Since these point sources
represent the first scattering interactions of the incident photons (recall from Chapter 1 that we are working with scattered fluence and ignoring primary fluence in these equations), the most physically correct source distribution would be a line of point sources with exponentially decreasing strength as depth into the tissue increases (because of Beer’s law for single scattering). To find an expression for the spatially-resolved diffuse reflectance, the next step is to find the Green’s function for a point source in a semi-infinite medium. With the Green’s function in hand, any source distribution can be “built-up” by superposition.

For simplicity, we can choose to lump our source distribution into a single isotropic point source located at a depth of $z_0$ below the tissue surface, as depicted in the figure. For a beam at normal incidence, $z_0$ is one transport mean free path ($mfp'$):

\[ mfp' = \frac{1}{\mu_s + \mu_t'} \]

Eq. 2-13

Just as the reduced scattering coefficient is the probability of isotropic scattering per differential path length, the transport mean free path is the average path length a photon must travel before its direction is effectively randomized. From Eq. 1-53:

\[ D = \frac{1}{3\mu_t'} = \frac{1}{3(\mu_s + \mu_t')} \]

so the depth of the isotropic source can also be expressed as $3D$. This is an important point that will be discussed further in Chapter 3. Since the Green’s function for an isotropic point source in an infinite medium is already known (Chapter 1, section 1.4.3), rather than solve for the Green’s function of an isotropic point source in a semi-infinite medium, we would like to find a way to approximate the fluence rate in the semi-infinite medium with a clever distribution of isotropic point sources in an infinite medium. It has
been shown that this can be done by the introduction of a negative image source on the axis of the incident beam at some height (in the infinite medium) above where the actual tissue boundary for the semi-infinite medium resides (Figure 2-5b) [35].

To find the Green's function for this dipole source in an infinite medium, it is necessary to know the separation between the two sources. This problem reduces to finding the position $z_b$ of the midpoint of the dipole. Anywhere on the plane $z=z_b$, the fluence rate must be zero, so this plane is called the "extrapolated zero-fluence boundary". We proceed by elucidating the relationships between the fluence rate, fluxes, and diffuse reflectance for the semi-infinite medium. Fick's law, Eq. 1-32, without the extra source term, is:

$$\bar{F}(\bar{r}) = -D \nabla \phi(\bar{r})$$

The reflectance from a tissue is the outward flux across its surface, which is simply the perpendicular component of flux at the surface

$$R(r) = \bar{F}(r) \cdot (-\hat{z}) = D \frac{\partial \phi(r,z)}{\partial z} \text{ at the tissue surface. } z=0 \tag{Eq. 2-14}$$

Note that the positive $z$ direction is into the tissue, and that the position vector $\bar{r}$ has been replaced by $r$, the radial distance from the beam, and the height $z$. The fluence rate itself (rather than its derivative) can also be related to the hemispherical flux (and hence reflectance) across the tissue boundary [16]. Eq. 1-13 is the definition of hemispherical flux:

$$F_{\perp}(\bar{r}) = \int_{2\pi} L(\bar{r},\hat{\omega})(\hat{\omega} \cdot \hat{z}) d\omega$$

Substituting in Eq. 1-31 from the diffusion approximation:

$$L(\bar{r},\hat{\omega}) = L_0(\bar{r}) + \frac{3}{4\pi} \bar{F}(\bar{r}) \cdot \hat{\omega}$$

yields
\[ F_{z+}(\vec{r}) = \int_{\vec{z} \geq 0} L_0(\vec{r})(\hat{z} \cdot \hat{z}) d\omega + \int_{\vec{z} \geq 0} \frac{3}{4\pi} \vec{F}(\vec{r}) \cdot \hat{z}(\hat{z} \cdot \hat{z}) d\omega \]

The first integral is quite simple:

\[ \int_{\vec{z} \geq 0} L_0(\vec{r})(\hat{z} \cdot \hat{z}) d\omega = L_0(\vec{r}) \int_0^{2\pi} \int_0^{\pi/2} (\cos \theta) \sin \theta d\theta d\phi = \pi L_0(\vec{r}) \]

The second integral is tricky. It has the general form:

\[ \int_{\vec{z} \geq 0} \vec{A} \cdot \hat{z}(\hat{z} \cdot \hat{z}) d\omega = \int_0^{2\pi/\gamma} \int_0^{\pi/2} [\vec{A} \cdot (\cos \theta) \hat{z}] \sin \theta d\theta d\phi \]

To solve this integral, break the variable vector \((\cos \theta)\hat{z}\) into components parallel and perpendicular to \(\hat{z}\). It turns out that only the component parallel to \(\hat{z}\) contributes to the integral. Integration of the dot product of the perpendicular component with \(\vec{A}\) is exactly zero (Figure 2-6). So we are left with:

\[ \text{Component of } \hat{z} \parallel \hat{z} = (\cos^2 \theta) \hat{z} \]

\[ \int_0^{2\pi/\gamma} \int_0^{\pi/2} (\vec{A} \cdot (\cos^2 \theta) \hat{z}) \sin \theta d\theta d\phi = 2\pi (\vec{A} \cdot \hat{z}) \int_0^{\pi/2} \cos^2 \theta \sin \theta d\theta \]

\[ = \frac{2\pi}{3} (\vec{A} \cdot \hat{z}) \]

The second integral of Eq. 2-15 is thus:

\[ \int_{\vec{z} \geq 0} \frac{3}{4\pi} \vec{F}(\vec{r}) \cdot \hat{z}(\hat{z} \cdot \hat{z}) d\omega = \frac{\vec{F}(\vec{r}) \cdot \hat{z}}{2} \]

And the expressions for the hemispherical fluxes are (the negative hemispherical flux is solved in exactly the same way as the positive):

\[ F_{z+}(\vec{r}) = \pi L_0(\vec{r}) + \frac{\vec{F}(\vec{r}) \cdot \hat{z}}{2} = \frac{\phi(\vec{r})}{4} + \frac{\vec{F}(\vec{r}) \cdot \hat{z}}{2} \]

\[ F_{z-}(\vec{r}) = \pi L_0(\vec{r}) - \frac{\vec{F}(\vec{r}) \cdot \hat{z}}{2} = \frac{\phi(\vec{r})}{4} - \frac{\vec{F}(\vec{r}) \cdot \hat{z}}{2} \]

We have taken advantage of Eq. 1-39 which relates fluence rate with the term for isotropic radiance:
\[ \phi(\vec{r}) = 4\pi L_0(\vec{r}) \]

Finally, addition of Eq. 2-20 with Eq. 2-21 yields the following expression:

**Eq. 2-22**

\[ \phi(\vec{r}) = 2\left(F_{+,z}(\vec{r}) + F_{-,z}(\vec{r})\right) \]

If the semi-infinite tissue is index-matched to the (presumably non-scattering) ambient medium, then at the tissue surface, \( F_{+,z} \) (the diffuse flux directed into the tissue) equals zero because none of the photons incident upon the boundary from below will reflect. \( F_{-,z}(r) \) is the diffuse reflectance at the surface, where again the position vector has been replaced with \( r \), the radial distance from the incident beam, and \( z \). So by combining Eq. 2-14 with Eq. 2-22:

**Eq. 2-23**

\[ \phi(r,z) = 2R(r) = 2D \frac{\partial \phi(r)}{dz} \bigg|_{z=0} \]

If we assume that the fluence rate is approximately linear in the region between \( z = z_b \) (the extrapolated zero-fluence boundary) and \( z = 0 \), (this is the same as saying the gradient constant), then \( \phi \) goes to zero at \( z_b = -2D \). See Figure 2-7.
at $z = 0$, $\phi = 2D \frac{\partial \phi}{\partial z}$

Figure 2-7: If the fluence rate as a function of $z$ is approximately linear in the range $z=[0,z_b]$, then we can say that $\frac{\partial \phi}{\partial z} = \frac{\phi_i - \phi_2}{z_1 - z_2} = \frac{2D \partial \phi / \partial z}{-z_b}$, and $z_b = -2D$.

If there is an index mismatch between the tissue and the ambient, then a correction in the height of the zero-fluence boundary must be made to account for the increased amount of light in the region $z>0$ due to internal reflection. This amounts to an increased separation between the positive and negative sources due to an increased $z_b$ [36, 37]:

Eq. 2-24

$$z_b = 2AD$$

Eq. 2-25

$$A = \frac{1 + R_d}{1 - R_d}$$

Eq. 2-26

$$R_d = \frac{-1.44}{n_{rel}^2} + \frac{0.71}{n_{rel}} + 0.668 + 0.0636n_{rel}$$

Eq. 2-27

$$n_{rel} = \frac{n_{tissue}}{n_{ambient}}$$

$R_d$ is the diffuse (power) reflection coefficient for unpolarized light. When the boundary is matched, $A$ goes to one, as expected.

Once the positions of the two sources are known, their individual Green's functions can be added together to yield the Green's function for a dipole.

Eq. 2-28

$$\phi(r, z) = \frac{S}{4\pi D} \left[ \frac{e^{-\mu_d \rho_1}}{\rho_1} - \frac{e^{-\mu_d \rho_2}}{\rho_2} \right]$$
\( S \) is a scaling factor for the strength of the sources (which are equal). \( \rho_1 \) and \( \rho_2 \) are the distances from the positive and negative sources to the point of interest. In terms of \( z \) and \( r \):

\[
\begin{align*}
\rho_1 &= \sqrt{(z - z_0)^2 + r^2} \\
\rho_2 &= \sqrt{(z + (z_0 + 2z_0))^2 + r^2}
\end{align*}
\]

**Eq. 2-29**

To get the reflectance, combine Eq. 2-14 with Eq. 2-28:

\[
R(r) = D \frac{\partial}{\partial z} \left( \frac{S}{4\pi D} \left( \frac{e^{-\mu_d \rho_1}}{\rho_1} - \frac{e^{-\mu_d \rho_2}}{\rho_2} \right) \right)_{z=0}
\]

**Eq. 2-30**

After differentiation with respect to \( z \) at \( z=0 \), the final result is a closed form, analytic expression for the diffuse reflectance from a semi-infinite tissue irradiated with a narrow beam of light [35]:

\[
R(r) = \frac{S}{4\pi} \left[ z_0 \left( \mu_{\text{eff}} + \frac{1}{\rho_1} \right) \frac{e^{-\mu_d \rho_1}}{\rho_1} + (z_0 + 2z_0) \left( \mu_{\text{eff}} + \frac{1}{\rho_2} \right) \frac{e^{-\mu_d \rho_2}}{\rho_2} \right]
\]

**Eq. 2-31**

### 2.6.2 Normal Incidence Reflectometry

Eq. 2-31 is the basic equation behind spatially-resolved diffuse reflectometry for measuring the absorption and reduced scattering of tissues. There are two methods to obtain a measurement of \( R(r) \): video reflectometry [38] and optical fiber reflectometry [39, 40]. To perform a video measurement, the output of a laser is directed normal to the surface of a test sample. A CCD camera, placed slightly off-axis with the laser to reject specular reflectance, captures an image of the diffuse reflectance, \( R(r) \), in arbitrary units. See Figure 2-8a. Due to the short penetration depth of visible light into tissues, the CCD camera typically requires 16 bits of dynamic range (4 orders of magnitude) to accurately
Figure 2-8  a) Schematic of system for video reflectometry measurement.  b) Schematic of system optical fiber reflectometry measurement. The source could be a laser or a white light source such as a halogen or xenon lamp. The detector could be a photomultiplier tube or an optical multi-channel analyzer.

record (without saturating or hitting the noise floor) the diffuse reflectance within a radius of a few centimeters of the beam. If the camera has insufficient dynamic range, it is possible to individually capture small slices of the diffuse reflectance profile by sequentially changing the source power with neutral density filters. The major drawback of this method is that there is no convenient or elegant way to make multi-spectral measurements.

The alternative to using video reflectometry is optical fiber reflectometry. Light can be delivered and collected through a fiber optic probe. One fiber serves as the source, and five to ten detection fibers sample the diffuse reflectance at known distances from the point of light incidence. All the fibers need only be cleaved and polished, and the probe must be aligned normally to the tissue surface (Figure 2-8b). Each detection fiber is then
coupled to a photodetector. As we pointed out for a number of the previously discussed techniques, optical fiber reflectometry can also be extended to perform multi-wavelength measurements with a white light source and some form of wavelength-resolving detector. A potential problem with using an optical fiber probe is the lack of collimation of the source beam. Since a typical mean free path for tissues falls in the range of 10 – 100 μm and a typical numerical aperture for fused silica fiber is 0.37, the beam actually diverges very little before it begins to be diffused.

Once \( R(r) \) is obtained, another measurement must be performed to allow us to deduce both the absorption and reduced scattering coefficients. One option is to measure the total diffuse reflectance from the sample with a photodiode placed significantly farther from the sample than the penetration depth of light into the sample (at least a foot away). Comparison of the sample reflectance with a reflectance standard yields the total diffuse reflectance of the sample. From a plot of the total diffuse reflectance vs. \( \mu_s' / \mu_a \) generated by the adding-doubling method [34], \( \mu_s' / \mu_a \) can be determined. Eq. 2-31 can be rewritten in terms of this known ratio, which we label as \( N' \), leaving two unknown parameters, \( \mu_a \) and a scaling factor, which can then be determined through a two parameter curve fit.

Eq. 2-32

\[
R(r) = \left( \mu_a \sqrt{3(N'+1)} + \frac{1}{\rho_1} \right) e^{-\rho_2 \sqrt{3(N'+1)}} + \left( 1 + \frac{4}{3} A \right) \left( \mu_a \sqrt{3(N'+1)} + \frac{1}{\rho_2} \right) e^{-\rho_2 \sqrt{3(N'+1)}}
\]

where

\[
\rho_1 = \sqrt{\frac{1}{\mu_a^2 (N'+1)^2 + r^2}}
\]

Eq. 2-33
\[ \rho_2 = \frac{(1 + 4A/3)^2}{\mu_0^2(N' + 1)^2 + r^2} \]

A second option, which is most convenient with the fiber optic approach, is to calibrate the \( R(r) \) curve in absolute units. Either a neural network can be trained to reconstruct the optical properties from the absolute curve of diffuse reflectance \([39, 41]\), or they can be reconstructed through a two parameter curve fitting procedure \([40]\).

For either the video or fiber optic approach, the same pair of optical properties, \( \mu_a \) and \( \mu_a' \), can be determined, but for multi-wavelength measurements, the optical fiber approach is significantly more efficient. The equipment required for a multi-wavelength optical fiber system is comparable to that required for the single wavelength video system: in particular, a CCD camera with a large dynamic range. In addition, the fiber optic probe is much more suitable for in vivo applications than video reflectance measurements. With all these factors taken into account, the optical fiber approach is far superior, and it is this approach that we have adopted in our oblique-incidence reflectometry experiments which are described in the remaining chapters.

2.7 Summary

There have been many methods devised for measuring the optical properties of tissues. This chapter contains a sampling of the most notable techniques to date, of which the integrating spheres measurement is probably the most common. This is most likely because it has only moderate demands on instrumentation and the set-up allows the simultaneous measurement \( \mu_a \), \( \mu_a' \), and \( g \). It is also relatively easy to perform these
measurements multi-spectrally. A unifying thread in all of these measurements has been the need for an independent measurement (or calibration) for each optical property desired. In the next 3 chapters, we will describe oblique-incidence optical fiber reflectometry, a new technique which is based on the measurement of spatially resolved diffuse reflectance. It does not require an additional measurement of total diffuse reflectance or a calibration to absolute units to deduce both the absorption and reduced scattering coefficients with a high degree of accuracy.
Chapter 3  Single Wavelength Oblique-incidence Reflectometry

3.1  Theory of Oblique-incidence Reflectometry

3.1.1  Introduction

As discussed in Chapter 2, the ability to accurately measure the optical properties, absorption and reduced scattering, of tissues in vivo is of tantamount importance to both therapeutic and diagnostic applications of light in medicine. To date, this task has remained a challenge, and current methods for performing this measurement have not overcome drawbacks such as requiring tissue excision, sensitive instrument calibrations, and expensive equipment. This chapter introduces the concept of oblique-incidence reflectometry as originally conceived by Wang [42] and presents its continued development [43]. Whereas the original paper considered measurement of only the reduced scattering coefficient of turbid media at a single wavelength via video reflectometry, our current work provides a method for determining both absorption and reduced scattering coefficients at multiple wavelengths simultaneously with a prototype fiber optic probe. In contrast to normal incidence reflectometry, obliquely incident light produces a spatial distribution of diffuse reflectance that is not centered about the point of light entry, and the amount of shift in the center of diffuse reflectance is directly related to the medium’s diffusion length, D. We developed a fiber optic probe to deliver light obliquely and sample the relative profile of spatially distributed diffuse reflectance. Since
we already have a measure of \( D \), calibration absolute units is not necessary for deducing both optical properties (also in contrast to normal incidence reflectometry). From the profile of diffuse reflectance, it was possible to measure \( D \), perform a curve fit for the effective attenuation coefficient, \( \mu_{\text{eff}} \), then calculate \( \mu_a \) and \( \mu_s' \). Our measurements of \( D \) and \( \mu_{\text{eff}} \) had an accuracy of approximately 5%, thus giving us 10% and 5% accuracy for \( \mu_a \) and \( \mu_s' \), respectively. This chapter focuses on the theory of oblique-incidence reflectometry and its verification with Monte Carlo simulations and single wavelength measurements on tissue phantoms. A discussion can be found in section 3.3. Chapter 4 presents experimental results from multiple wavelength measurements, and Chapter 5 describes a simple and relatively inexpensive multi-fiber imaging spectrograph system for performing virtually instantaneous multi-wavelength measurements.

3.1.2 Mathematical Development

When light enters a semi-infinite tissue, it will generally scatter a number of times before either being absorbed or escaping the tissue surface at a point other than its point of entry. The multiply scattered light that escapes is called diffuse reflectance. Monte Carlo simulations of light transport accurately reproduce spatially resolved diffuse reflectance data, but iterative algorithms taking advantage of this accuracy to determine optical properties are impractical because the simulations are computationally intensive and relatively slow [13-15, 44]. In this work we utilize the simple dipole-source diffusion theory model of spatially resolved, steady-state diffuse reflectance [35]. This diffusion theory model does not accurately model near diffuse reflectance, that is, reflectance that falls in the range of 1-2 transport mean free paths (\( mfp' \)) of the source.
Figure 3-1 Diffuse reflectance from a semi-infinite tissue for normally incident light, as modeled by Monte Carlo simulation vs. the two-source diffusion theory model. $\mu_s = 0.4 \text{ cm}^{-1}$, $\mu'_s = 8 \text{ cm}^{-1}$, and $1 \text{ mfp}' = 0.123 \text{ cm}$. (a) Plot of Monte Carlo and diffusion theory curves. (b) Plot of % difference in two curves, $(\text{Monte Carlo - Diffusion theory})/\text{Diffusion Theory}$. From both (a) and (b), it is clear that the two curves agree well within $2 \text{ mfp}'$ of the center of diffuse reflectance, which coincides with the light entry point for normal incidence.

However, the model's simplicity makes up for this drawback by providing an efficient and conceptually clear means of reconstructing optical properties. Moreover, this disadvantage can be avoided as long as no attempt is made to fit data too close to the point of light entry to the diffusion theory model. See Figure 3-1. From this point on, any reference to "diffuse reflectance" specifically excludes near diffuse reflectance.

Since it is easier to model isotropic scattering than anisotropic scattering, we work with the "reduced" or "transport" scattering coefficient, $\mu'_s$. Recall from Chapter 1 that $\mu'_s$ is the equivalent isotropic scattering coefficient of an otherwise anisotropically scattering medium. $\mu'_s = \mu_s(1-g)$, where $\mu_s$ is the scattering coefficient and $g$ is the average scattering cosine. As covered in Chapter 2 for normal incidence reflectometry, the spatial distribution of diffuse reflectance of normally incident light can be modeled by two isotropic point sources, one positive source located $1 \text{ mfp}'$ below the tissue surface and one negative image source above the tissue surface [35]. The buried, positive source represents a source of isotropically scattered radiance, hence its placement $1 \text{ transport}$
Figure 3-2 (a) Positions of point sources in diffusion theory model for normal incidence. (b) Positions of point sources in diffusion theory model for oblique-incidence. The y axis points out of the page.

mean free path below the tissue surface. The negative image source is included to match the boundary condition, that is, to force the fluence rate to zero at a plane parallel to the tissue surface. The height in \( z \) of the image source and the zero fluence plane depends on the boundary condition, and the fluence rate for the single source in a \textit{semi}-infinite medium is given as the sum of the fluence rates for the positive and image sources calculated in an infinite medium (see Chapter 2 for a complete development of this formalism). This “method of images” is very similar to that used to solve boundary value problems in electromagnetics.

For obliquely incident light, the positive, buried source should be located at the same depth, with this depth now measured along the new optical path as determined by Snell's law. The net result is a change in the positions of the isotropic point sources of the
photon dipole pair, particularly a shift in the x direction (see Figure 3-2). Wang's original presentation of oblique-incidence reflectometry, using Monte Carlo simulation and least squares fitting to this model, showed that the buried source is actually located at a depth (measured along the optical path) which we define as

\[
3D = \frac{1}{0.35 \mu_a + \mu'_s} \neq 1 \text{ mfp}'
\]

Eq. 3-1

The factor of 0.35 has a significant effect for media with moderately high absorption relative to scattering. Looking back at Eq. 1-53, take note of the fact that our original expression for \( D \)

\[
D = \frac{\text{mfp}'}{3}
\]

was based on the diffusion approximation, so it is not unreasonable to suggest that there is in fact an empirically more accurate expression. We believe that for both normal and oblique-incidence, the more correct expression for the depth of the positive point source is \( 3D \) (as given in Eq. 3-1) rather than \( 1 \text{ mfp}' \). Both cases are diagrammed in Figure 3-2

The diffuse reflectance profile for oblique-incidence is centered about the position of the virtual point sources, so the shift, \( \Delta x \), can be measured by finding the center of diffuse reflectance relative to the light entry point. As is the case for normal incidence, the diffusion theory model, when shifted by \( \Delta x \), also agrees with Monte Carlo results outside of \( 1-2 \text{ mfp}' \) from the center of diffuse reflectance, which it is important to reiterate is no longer at the point of entry (Figure 3-3).

Referring back to Eq. 2-31, we have the general form for the steady-state, spatially resolved diffuse reflectance:
Figure 3-3 Diffuse reflectance from a semi-infinite tissue for obliquely incident light as modeled by Monte Carlo simulation vs. the two-source diffusion theory model. $\mu_a = 0.4$ cm$^{-1}$, $\mu'_s = 8$ cm$^{-1}$, and $1 \text{ mfp}' = 0.123$ cm. (a) Plot of Monte Carlo and diffusion theory curves. (b) Plot of % difference in two curves. (Monte Carlo - Diffusion theory)/Diffusion Theory. From both (a) and (b), it is clear that the two curves agree well within 2 mfp' of the center of diffuse reflectance, which does not coincide with the light entry point for oblique-incidence.

$$R(r) = z_0 \left( \mu'_{eff} + \frac{1}{\rho_1} \right) \frac{e^{-\rho_1 \mu'_{df}}}{\rho_1^2} + \left(z_0 + 2z_b\right) \left( \mu'_{eff} + \frac{1}{\rho_2} \right) \frac{e^{-\rho_2 \mu'_{df}}}{\rho_2^2}$$

which can be scaled arbitrarily to fit a relative reflectance profile that is not in absolute units. $\rho_1$ and $\rho_2$ are the distances from the two sources ("1" corresponds to the positive source and "2" corresponds to the negative) to the point of interest (the point of light collection), and the boundary condition is included in the term $A$ [36, 37, 45]. Using a depth of $3D$ for the position of the positive source, $z_0$, gives the following expression:

Eq. 3-2

$$R(r) = 3D \left( \mu'_{eff} + \frac{1}{\rho_1} \right) \frac{e^{-\rho_1 \mu'_{df}}}{\rho_1^2} + \left(3D + 4AD\right) \left( \mu'_{eff} + \frac{1}{\rho_2} \right) \frac{e^{-\rho_2 \mu'_{df}}}{\rho_2^2}$$

When light is incident at an oblique angle, a minor modification must be made. Most importantly, the depth of the positive source must account for the angle of incidence.

The height of the extrapolated zero-fluence boundary remains the same because $A$ and $D$ are intrinsic properties of the media, as is the effective attenuation coefficient, $\mu_{eff}$. The distances $\rho_1$ and $\rho_2$ are still the respective distances from the positive and negative sources to the point of interest. The modified expression is thus:
Figure 3-4 Three dimensional perspective view of tissue surface, coordinate axes, and positions of light delivery and collection. The arrows represent the fibers in the probe. The arrows pointing up are the collection fibers and the dashed arrow is the source fiber.

Eq. 3-3 \[ R(r) = 3D \cos \theta_{tissue} \left( \mu_{eff} + \frac{1}{\rho_1} \right) e^{-\frac{\rho_1}{\mu_{eff}}} + \left( 3D \cos \theta_{tissue} + 4AD \right) \left( \mu_{eff} + \frac{1}{\rho_2} \right) e^{-\frac{\rho_2}{\mu_{eff}}} \]

As can be seen in Figure 3-2, the diffusion coefficient can be calculated from \( \Delta x \), the shift between the actual center of diffuse reflectance and the point of light entry. We can measure \( \Delta x \) directly, and \( \theta_{tissue} \) is known as long as we know all the relevant parameters for Snell's law.

Eq. 3-4 \[ D = \frac{\Delta x}{3 \sin \theta_{tissue}} \]

Substituting back into Eq. 3-3 we get

Eq. 3-5 \[ R(r) = \frac{\Delta x}{\tan \theta_{tissue}} \left( \mu_{eff} + \frac{1}{\rho_1} \right) e^{-\frac{\rho_1}{\mu_{eff}}} + \left( \frac{\Delta x}{\tan \theta_{tissue}} + \frac{4}{3} A \frac{\Delta x}{\mu_{eff} \sin \theta_{tissue}} + \frac{1}{\rho_2} \right) e^{-\frac{\rho_2}{\mu_{eff}}} \]

where
\[ \rho_1 = \sqrt{(x - \Delta x)^2 + \Delta y^2 + z_0^2} \]
\[ \rho_2 = \sqrt{(x - \Delta x)^2 + \Delta y^2 + (z_0 + 2z_h)^2} \]
\[ = \sqrt{(x - \Delta x)^2 + \Delta y^2 + \left( \frac{\Delta x}{\tan \theta_{\text{issue}}} + \frac{4}{3} A \frac{\Delta x}{\sin \theta_{\text{issue}}} \right)^2} \]

Eq. 3-6

In Eq. 3-6, \( x \) is the current position and the dependent variable, \( \Delta x \) is the shift in the center of diffuse reflectance, \( \Delta y \) is any offset between the plane of incidence and the plane of collection, as depicted in Figure 3-4, and the \( z \) term is the distance from each point source to the tissue surface (not the extrapolated boundary).

Assuming that the angle of refraction, \( \Delta y \), and \( A \) are known, our overall method is outlined as follows:

1. sample the diffuse reflectance profile at known positions from the light entry point
2. search for the center of diffuse reflectance
3. measure \( \Delta x \)
4. calculate the positions of the buried source and the image source (these distances are the coefficients of the two terms in Eq. 3-3)
5. perform a non-linear least-squares fit with the Levenberg-Marquardt method on Eq. 3-5 to determine \( \mu_{\text{eff}} \) [46]
6. solve for \( \mu_a \) and \( \mu_s' \) from the expressions

\[ \mu_a = D \mu_{\text{eff}}^2 \]
\[ \mu_s' = \frac{1}{3D - 0.35 \mu_a} \]

Eq. 3-7
Eq. 3-8

where \( D \) is calculated from Eq. 3-4, and Eq. 3-1 is used to relate \( \mu_a \) and \( \mu_s' \).
3.1.3 Monte Carlo Simulations

As a first test of the validity of our reconstruction algorithm, Monte Carlo simulations of light transport were run on a Sun Sparcstation 10. Monte Carlo simulations are considered accurate provided a sufficient number of photons are launched to be statistically significant. In addition, mathematically the simulations bear no resemblance to our diffusion-approximation/photon-dipole model of diffuse reflectance. We performed simulations in “tissues” with defined optical properties, inputted the Monte Carlo generated reflectance “data” into the fitting routine, and received the original set of optical properties back as output. This consistency between our fitting algorithm and Monte Carlo simulations proved that, at least theoretically, it is possible to use the analytically simple diffusion theory model to reconstruct optical properties from oblique-incidence reflectance data.

Over six simulations the optical properties were varied from 0.2 cm\(^{-1}\) to 0.6 cm\(^{-1}\) for \(\mu_a\) and from 4 cm\(^{-1}\) to 10 cm\(^{-1}\) for \(\mu'_s\). The number of photon packets launched varied from \(6 \times 10^6\) to \(10 \times 10^6\) depending on the optical properties; higher scattering media required more total photons to keep the signal to noise ratio roughly equal for all the simulations over a predefined maximum source-detector separation of 2 cm. Light was launched from a refractive index matched ambient (to give a matched boundary condition, \(n_{rel} = A = 1\)) into the tissue at an angle of 51°. This angle was chosen to match the refracted angle of transmission from our fiber optic probe (see Section 3.2.1) and thus gave us the same optical path inside the turbid media for both the simulations and experiments.
The reflectance data were collected in a plane parallel to the plane of incidence, but offset by a small distance, \( \Delta y \), see Figure 3-4. This offset was also chosen to match the physical dimensions of the probe. Diffusely reflected photons were counted in bins extending 0.1 \( mfp' \) long in \( x \) and 0.03735 cm wide in \( y \). We chose to allow the bin size to vary in \( x \) to reflect the scale of each individual simulation – the shift in the center of diffuse reflectance for any measurement will always be on the order of a transport mean free path, so a bin size of 0.1 \( mfp' \) assured us that we would attain adequate resolution in \( x \) for each simulation. The bin size was held constant in \( y \) so we could collect data in the same predefined collection plane for each simulation.

Before testing our reconstruction algorithm, the data had to undergo a little preprocessing. Generally speaking, the noise level in the data tended to increase significantly around 0.9 cm or farther from the point of incidence, so all data beyond 0.9 cm for all the simulations were simply removed. In addition, any data within 1.5 \( mfp' \) of the center of diffuse reflectance (not the light entry point) were removed to avoid the “near diffuse reflectance” region where the diffusion approximation model is least accurate. Finally, the simulated data were inputted to our fitting algorithm which plots both the expected and fitted curves based on the diffusion theory model. The expected curve was based on the optical properties defined for the simulated tissue. The program, which is implemented in C (and has been ported to both UNIX and Macintosh operating systems), also outputs relative errors for all of the fitted parameters. A printout of the code and sample output is provided in Appendix B.
Figure 3-5 (a) Sample of Monte Carlo simulated data after removal of noise far from point of entry as well as data within 1.5 mfp' of the center of diffuse reflectance. The curve shown is the fitted result generated by our algorithm. To emphasize how well these curves agree with what we expected, in (b) we have plotted just the expected and fitted curves without the simulation data. This particular simulation used \((\mu_r', \mu_a) = (6 \text{ cm}^{-1}, 0.6 \text{ cm}^{-1})\).

Figure 3-5 is a sample of the output curves for a typical simulation. Clearly, the fitting routine works well. Table 3-1 contains the compiled results from six Monte Carlo simulations. Included are all fitted values, expected values, and their respective errors for both the directly fitted parameters \(\Delta x\) and \(\mu_{\text{eff}}\) and the final calculated values of \(\mu_a\) and \(\mu_r'\).

The majority of errors fell in the range of \(-2\%\) to \(+2\%\), with the largest errors of 3.3% and

<table>
<thead>
<tr>
<th>Fitted Parameter</th>
<th>Values</th>
<th>Expected Values of ((\mu_r', \mu_a)) (cm(^{-1}),cm(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\Delta x) (cm(^{-1}))</td>
<td>Expected</td>
<td>(4, 0.4)</td>
</tr>
<tr>
<td></td>
<td>Fitted</td>
<td>0.188</td>
</tr>
<tr>
<td></td>
<td>% Error</td>
<td>0.4</td>
</tr>
<tr>
<td>(\mu_{\text{eff}}) (cm(^{-1}))</td>
<td>Expected</td>
<td>2.229</td>
</tr>
<tr>
<td></td>
<td>Fitted</td>
<td>2.262</td>
</tr>
<tr>
<td></td>
<td>% Error</td>
<td>1.5</td>
</tr>
<tr>
<td>(\mu_a) (cm(^{-1}))</td>
<td>Expected</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>Fitted</td>
<td>0.414</td>
</tr>
<tr>
<td></td>
<td>% Error</td>
<td>3.4</td>
</tr>
<tr>
<td>(\mu_r') (cm(^{-1}))</td>
<td>Expected</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td>Fitted</td>
<td>3.980</td>
</tr>
<tr>
<td></td>
<td>% Error</td>
<td>-0.5</td>
</tr>
</tbody>
</table>
3.4% in the determination of $\mu_a$. A discussion of error propagation is in section 3.3.4. These results are fairly convincing evidence that the diffusion theory/photon dipole based reconstruction algorithm is valid. We now move on to the experimental verification.

3.2 Experimental Verification of Oblique-incidence Reflectometry

3.2.1 Materials and Methods

The second step in validating our method for tissue optical property reconstruction is through experiment. Since our model is based on a homogeneous, semi-infinite medium, we prepared homogeneous water-based suspensions as tissue phantoms and made sure that the extent of the phantoms significantly exceeded the expected radial spread of light. The radius of each phantom was a minimum of eight 1/e penetration depths. Even though it is unrealistic to expect real biological tissue to be either homogeneous or semi-infinite, this experiment serves as actual proof that, at least under carefully controlled conditions, the optical properties of a medium can accurately be measured with fiber optic, oblique-incidence reflectometry.

Tissue phantoms with optical properties similar to those of biological tissue were prepared from water, Trypan Blue dye (an essentially pure absorber) and 900 nm diameter Polystyrene spheres (an essentially pure scatterer from Bristol Colloid Centre). We measured the absorption coefficient of the Trypan Blue stock solution with a spectrophotometer (HP8452A Diode Array Spectrophotometer driven by a Macintosh
II), calculated the scattering anisotropy of the spheres with Mie theory [47], and measured the scattering coefficient of the sphere stock solution by collimated transmission. The dilutions necessary to mix phantoms with the desired optical properties were calculated from the absorption and reduced scattering coefficients of the stock solutions. We made ten phantoms with $\mu_a$ varying from 0.2 cm$^{-1}$ to 0.6 cm$^{-1}$ and $\mu_s'$ from 4 cm$^{-1}$ to 10 cm$^{-1}$. Six of the ten sets of optical properties were the ones used in the simulations. To decrease the error in the expected values for each phantom, the absorber was diluted first and an independent measurement of absorption was taken with the spectrophotometer before addition of the scatterer. The expected phantom optical properties were thus derived from (1) an accurate, independent measurement of absorption, (2) the assumption that the scatterer was accurately diluted to the desired values, and (3) the assumption that there is no interaction between the absorber and scatterer to change the final optical properties of the phantoms, once all the components have been mixed.

We constructed a prototype oblique-incidence optical fiber probe out of black delrin and 600 μm diameter, low-loss optical fiber (Spectran HCP-M0600T-08). There was one source fiber which was bent to an angle of 45°, and there were nine collection fibers, all aligned normal to the probe face and offset from the plane of incidence by a small distance $\Delta y$. The tilt in the source fiber resulted in a transmitted angle after refraction of 51°, the same angle we used in the simulations. The collection fiber positions were measured relative to the source fiber with a resolution of twenty microns from a digitized video image of the probe face. A 632.8 nm HeNe laser was the light source and an SMA coupled photodetector (3M Photodyne 88XLC Radiometer/Photometer) measured the
Figure 3.6 (a) Sample of data taken with oblique-incidence fiber optic probe. As in Figure 3.5, the curve shown is the fitted result generated by our algorithm. In (b) we have plotted just the expected and fitted curves without the data to reiterate how well they agree. This particular phantom had \((\mu_r', \mu_a) = (6 \text{ cm}^{-1}, 0.6 \text{ cm}^{-1})\).

amount of light collected in each detection fiber. The exposed fiber tips on the probe face were placed on the surface of the tissue phantoms, and to approximate a matched boundary condition (no light reflected back from the tissue surface) we floated a piece of black, exposed x-ray film on top of each tissue phantom. The film had a small window through which to place the probe in contact with the phantoms. See section 3.3.1 for a discussion of the probe characteristics and section 3.3.2 for a discussion of this method of boundary matching.

The diffuse reflectance profile for each phantom was collected four times. Each measurement was independent from the last, meaning the entire procedure of stirring the phantom, putting the matching film in place, and aligning the probe was repeated for each measurement. The four phantoms with \((\mu_r', \mu_a) = (6 \text{ cm}^{-1}, 0.374 \text{ cm}^{-1}), (8 \text{ cm}^{-1}, 0.386 \text{ cm}^{-1}), (7 \text{ cm}^{-1}, 0.178 \text{ cm}^{-1})\) and \((7 \text{ cm}^{-1}, 0.589 \text{ cm}^{-1})\) were used as calibration standards and the remaining six as test standards. The calibration standards were used to calculate correction factors for the collection fibers, and the test standards were used as knowns against which to compare our measured optical properties. Correction factors were
necessary because each fiber had slightly different collection efficiency due to imperfections in polishing and differences in coupling efficiency to the detector. The calibration factors, or $k_i$'s (where $i$ is the fiber number) were obtained by calculating the scaling terms required to make each point on the measured reflectance curve exactly match the expected curve for each calibration phantom (with an arbitrary scaling factor). We then calculated the average $k_i$'s over the four calibration phantoms and used these average values as the calibration factors for the remainder of the experiment. The raw data from each of the remaining six test standards were first corrected with the calibration factors, then inputted to the same fitting algorithm used on the simulated data from the previous section. A discussion of the calibration is in section 3.3.3.

### 3.2.2 Results

In Figure 3-6 there is a sample of the data, expected, and fitted curves for a phantom measurement. Table 3-2 contains the compiled results from the experiments with the fiber optic probe. The four phantoms with optical properties ($\mu_s', \mu_a$) = (6 cm$^{-1}$, 0.374 cm$^{-1}$), (8 cm$^{-1}$, 0.386 cm$^{-1}$), (7 cm$^{-1}$, 0.178 cm$^{-1}$) and (7 cm$^{-1}$, 0.589 cm$^{-1}$) were used for calibration as described in Section 3.2.1. The table gives the expected values, average fitted values, their associated errors, and the standard deviation for $\Delta\tau$, $\mu_{\text{eff}}$, $\mu_a$ and $\mu_s'$. The majority of errors easily fell in the range from $-5\%$ to $+5\%$, with the largest error of $-11.8\%$ in the determination of $\mu_a$ for the phantom with a scattering of 4 cm$^{-1}$ and absorption of 0.381 cm$^{-1}$. The mfp' for this phantom is 0.228 cm, and after taking into account the expected shift in the center of diffuse reflectance of 0.188 cm, it becomes clear that to eliminate data within 1.5 mfp' of the center of diffuse reflectance, the source fiber
Table 3-2 Results from phantom experiments. The sample size for each measurement was four. * error in $\mu_{eff}$ drops to −2.6% and error in $\mu_a$ drops to −8.2% when fiber nearest source is not included.

<table>
<thead>
<tr>
<th>Fitted Parameter</th>
<th>Average Values</th>
<th>Expected Values (4, 0.4)</th>
<th>(6, 0.2)</th>
<th>(6, 0.6)</th>
<th>(8, 0.2)</th>
<th>(8, 0.6)</th>
<th>(10, 0.4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\Delta x$ (cm$^{-1}$)</td>
<td>Expected 0.188</td>
<td>0.128</td>
<td>0.125</td>
<td>0.096</td>
<td>0.095</td>
<td>0.077</td>
<td>0.182</td>
</tr>
<tr>
<td>Std. Deviation</td>
<td>0.001</td>
<td>0.002</td>
<td>0.001</td>
<td>0.003</td>
<td>0.001</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td>% Error</td>
<td>−3.2</td>
<td>1.2</td>
<td>−1.5</td>
<td>2.3</td>
<td>0.6</td>
<td>5.0</td>
<td></td>
</tr>
<tr>
<td>$\mu_{eff}$ (cm$^{-1}$)</td>
<td>Expected 2.174</td>
<td>1.824</td>
<td>3.277</td>
<td>2.149</td>
<td>3.847</td>
<td>3.414</td>
<td>2.091</td>
</tr>
<tr>
<td>Fitted</td>
<td>0.021</td>
<td>0.099</td>
<td>0.033</td>
<td>0.032</td>
<td>0.023</td>
<td>0.021</td>
<td></td>
</tr>
<tr>
<td>Std. Deviation</td>
<td>−4.5*</td>
<td>−0.4</td>
<td>−2.4</td>
<td>−0.02</td>
<td>0.7</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>% Error</td>
<td>−11.8*</td>
<td>0.8</td>
<td>−6.1</td>
<td>2.3</td>
<td>2.0</td>
<td>5.1</td>
<td></td>
</tr>
<tr>
<td>$\mu_a$ (cm$^{-1}$)</td>
<td>Expected 0.381</td>
<td>0.183</td>
<td>0.577</td>
<td>0.191</td>
<td>0.601</td>
<td>0.383</td>
<td>0.341</td>
</tr>
<tr>
<td>Fitted</td>
<td>0.008</td>
<td>0.023</td>
<td>0.016</td>
<td>0.010</td>
<td>0.012</td>
<td>0.012</td>
<td></td>
</tr>
<tr>
<td>Std. Deviation</td>
<td>−11.8*</td>
<td>0.8</td>
<td>−6.1</td>
<td>2.3</td>
<td>2.0</td>
<td>5.1</td>
<td></td>
</tr>
<tr>
<td>% Error</td>
<td>−11.8*</td>
<td>0.8</td>
<td>−6.1</td>
<td>2.3</td>
<td>2.0</td>
<td>5.1</td>
<td></td>
</tr>
<tr>
<td>$\mu_s'$ (cm$^{-1}$)</td>
<td>Expected 4.0</td>
<td>6.0</td>
<td>6.0</td>
<td>8.0</td>
<td>8.0</td>
<td>10.0</td>
<td>4.155</td>
</tr>
<tr>
<td>Fitted</td>
<td>0.022</td>
<td>0.102</td>
<td>0.070</td>
<td>0.230</td>
<td>0.095</td>
<td>0.234</td>
<td></td>
</tr>
<tr>
<td>Std. Deviation</td>
<td>3.9</td>
<td>−1.2</td>
<td>1.8</td>
<td>−2.2</td>
<td>−0.6</td>
<td>−4.8</td>
<td></td>
</tr>
<tr>
<td>% Error</td>
<td>3.9</td>
<td>−1.2</td>
<td>1.8</td>
<td>−2.2</td>
<td>−0.6</td>
<td>−4.8</td>
<td></td>
</tr>
</tbody>
</table>

can be no closer than 0.155 cm to the nearest collection fiber (the first one to the right of the source in Figure 3-4). The distance from the source fiber to the nearest collection fiber is only 0.186 cm ± 0.002 cm, and the collection fiber size is 600 μm. Thus the fiber tip actually extends from a position of 0.156 to 0.216 cm (± 0.002 cm) and hence samples the reflectance right at the edge of the region 1.5 mfp’ from the center of diffuse reflectance. Removing this data point for this particular phantom decreased the average error in $\mu_{eff}$ from −4.5% to −2.6% and the average error in $\mu_a$ from −11.8% to −8.2%. The errors for $\Delta x$ and $\mu_s'$ remained unchanged.
3.3 Discussion

3.3.1 The Optical Fiber Probe

There are a number of points that should be clarified involving the collection and manipulation of data for both the Monte Carlo simulations and experiments. The first issue is that of the $y$ offset between the plane of incidence and the plane of collection. Addition of this offset has one major effect: it increases the effective distances, $\rho_1$ and $\rho_2$ in Eq. 3-5, between the virtual sources and the point of interest without increasing the size of the probe in the $x$ dimension. The small increase in the thickness of the probe in the $y$ dimension is negligible compared to the width. This is an advantage because it allows us to make more compact probes without compromising the requirement that data too close to the source be ignored. This probe shows better performance than our previous "all-oblique" design [48] for two reasons: a) the earlier design did not incorporate the additional $y$ offset for the collection fibers and b) the earlier design required each collection fiber to have a diffusing tip. Not only is it inconvenient to fabricate very small, truly diffusing tips, but the diffuser also dramatically decreases light throughput. The next generation probe should incorporate another improvement – replacement of the bent source fiber with a straight, side firing fiber. We have experimented with beveled-edge fiber tips for this purpose and found the source beam to be unacceptable. Perhaps the best solution would be to place a GRIN lens and an angled mirror or prism on the tip of the source fiber. This is beyond our current micro-machining capabilities, but is certainly possible in the commercial realm.
Since any given probe has predefined collection fiber positions, it is also important to realize that there is a physical limitation to the range of optical properties that can be accurately measured. One limiting factor is the distance from the source fiber to the nearest collection fiber. If the diffusion theory model is accurate only in the region farther than 1-2 mfp' from the center of diffuse reflectance, then there is a maximum value for the mfp' of the tissue under investigation which can be measured correctly. The maximum value of mfp' for our probe is approximately 0.23 cm, as described in section 3.2.2, and removal of data too close to the source improves the results significantly. In the simulations, all data within 1.5 mfp' of the expected center of diffuse reflectance was removed, and the fitted results were very good. This shows that, within these parameters, our fitting algorithm for reconstruction of the optical properties is valid. In an actual measurement, if the fitted results were to yield a mfp' on the order of the maximum measurable value of 0.23 cm, then the results would be prone to relatively high error and a larger probe should be used. At the other extreme, as the mfp' decreases, the only physical limitation is the sensitivity of the photodetector. If the collection fibers farthest from the source do not collect a detectable amount of signal, then the probe is too large. So again, in an actual measurement if the lowest signal were below some threshold of unacceptability, then the results would be prone to high error and a smaller probe should be used.

3.3.2 Index Matching

In both the simulations and experiments, a matched boundary condition with $A = 1$ was used to avoid the issue of what $A$ should be for a tissue-air interface. There have
been both analytical and empirical approaches to quantifying $A$ as a function of index mismatch at a surface, but as yet no definitive method [36, 37, 45, 49, 50]. In our experiments, the matched boundary condition was approximated by floating a black, exposed piece of x-ray film on the phantom surfaces. The refractive index of the film was 1.5, and it is fairly easy to show that this situation approximates a matched boundary quite well.

For the sake of simplicity, consider a surface that is uniformly irradiated by diffuse light. Right away, two things are clear: 1) the radiance, $L$, is a constant (not a function of direction or position), and 2) the total incident power per unit area, which is simply the hemispherical flux in the $\hat{z}$ direction, can be expressed as a function of radiance.

**Eq. 3-9**

$$F_z = \int_0^{2\pi} L(\hat{s} \cdot \hat{z}) d\omega = L \int_0^{\pi/2} d\phi \int_0^\pi \cos \theta \sin \theta d\theta = \pi L$$

The total reflected power is simply the integral of the incident power weighted by the Fresnel coefficient of specular reflection for unpolarized light (the power reflection coefficient), $R_s(\theta)$.

$$\text{reflected power} = \int_0^{2\pi} \int_0^{\pi/2} L(\hat{s} \cdot \hat{z}) d\omega \int_0^{\pi/2} R_s(\theta) \cos \theta \sin \theta d\theta$$

**Eq. 3-10**

$$= 2\pi L \int_0^{\pi/2} R_s(\theta) \cos \theta \sin \theta d\theta$$

where

**Eq. 3-11**

$$R_s(\theta) = \frac{1}{2} \left[ \frac{\tan^2(\theta - \sin^{-1}((n_1/n_2)\sin(\theta)))}{\tan^2(\theta + \sin^{-1}((n_1/n_2)\sin(\theta)))} + \frac{\sin^2(\theta - \sin^{-1}((n_1/n_2)\sin(\theta)))}{\sin^2(\theta + \sin^{-1}((n_1/n_2)\sin(\theta)))} \right]$$

$$= \frac{(n_1 - n_2)^2}{(n_1 + n_2)^2} \quad \text{for} \ \theta = 0$$

By combining Eq. 3-9 and Eq. 3-10 we get the total diffuse reflection coefficient:
$$R_d = \frac{\text{reflected power/unit area}}{\text{incident power/unit area}} = 2 \int_0^{\pi/2} R_\gamma(\theta) \cos \theta \sin \theta d\theta$$

Eq. 3-12
$$= \int_0^{\pi/2} R_\gamma(\theta) \sin(2\theta) d\theta$$

If the incident medium happens to have a higher index of refraction than the second medium such that $n_1/n_2 > 1$, then the integral in Eq. 3-12 must be split at the critical angle, which yields the following expression for the total diffuse reflection coefficient:

$$R_d = \int_0^{\theta_c} R_\gamma(\theta) \sin(2\theta) d\theta + \int_{\theta_c}^{\pi/2} (1) \sin(2\theta) d\theta$$

Eq. 3-13
$$= \int_0^{\theta_c} R_\gamma(\theta) \sin(2\theta) d\theta + \frac{1}{2} (\cos(2\theta_c) + 1)$$

With Eq. 3-12 and Eq. 3-13, we can calculate the total diffuse reflection coefficient for any tissue/ambient index of refraction pair. Figure 3-7 is a numerically generated plot of $R_d$ over the range $n_1/n_2 = [0,2]$. For our tissue phantoms, $n = 1.33$, so in air $n_1/n_2 = 1.33/1.0$, where we are calculating the internal total diffuse reflectance coefficient $R_{id} = 0.47$. With the matching film, $n_1/n_2 = 1.33/1.5 = 0.8867$ and $R_{id} = 0.03$. So only around 3% of the light incident upon the matching film from the phantom is reflected back into the phantom, whereas almost 50% is internally reflected from the phantom-air interface. The primary reason for the great disparity in the two total internal diffuse reflection coefficients is that there is a critical angle for the phantom to air interface, but not for the phantom to film interface.

Thus, as long as the film is thick enough such that photons from inside the phantom do not effectively "see" the film-air interface (i.e. there are no reflections from the film-air interface back into the phantom), then the matching film should serve its purpose well. The optical density of the film was over 4 O.D. (the limit of our spectrophotometer), so
Figure 3-7 Plot of total diffuse reflection coefficient $R_d$ as a function of $n_1/n_2$. Notice that there is no reflection for a matched boundary. The reflection coefficient approaches 1 asymptotically for $n_1 > n_2$, whereas it approaches 1 very quickly for $n_1/n_2 < 0.5$. The best place to be for index matching is where $n_1/n_2$ is just slightly less than 1.

light reflecting off the film-air interface back into the phantom would be attenuated by at least eight orders of magnitude, and is hence negligible.

3.3.3 Collection Fiber Calibration

After the collection of raw data, it is important to calibrate the probe for maximum accuracy. The primary reason is to correct for variability in fiber collection efficiency. Since the fibers collect light in a fixed orientation relative to the inhomogeneous diffuse illumination from the source, the simplest way to calibrate is to mimic the situation of an actual measurement. The calibration factors thus obtained theoretically should hold true for any optical properties being measured. In fact, since the fitting routine only requires a relative diffuse reflectance profile, all the wavelength variability in our signal also becomes irrelevant. This is a very important property that we will take advantage of in Chapter 4. In a multiwavelength measurement, the spectrum of the broadband source and the
responsivity of the detector would be the only wavelength dependent elements in our system (other than the optical properties themselves, of course), so any wavelength at which the optical properties are known could be used to find a set of \textit{wavelength independent} calibration factors. Finally, to decrease any small variations in the calibration factors that may be present, it is desirable to calibrate against several phantoms (or wavelengths) whose properties span the region of interest.

### 3.3.4 Error Propagation

The final issue of discussion is that of error propagation. From Table 3-1 and Table 3-2 it should be clear that the range of error in calculating $\mu_a$ is 2-3 times higher than that obtained for $\mu_a'$ and the directly measured parameters of $\Delta x$ and $\mu_{\text{eff}}$. The reason is evident from Eq. 3-7. $\mu_a = D\mu_{\text{eff}}^2$, so the error in $\mu_a$ is on the order of the error in $D$ (or $\Delta x$, which $D$ is proportional to) plus twice the error in $\mu_{\text{eff}}$. This is derived as follows:

$$\mu_a = D\mu_{\text{eff}}^2$$  \hspace{1cm} \text{Eq. 3-14}

$$\ln(\mu_a) = \ln(D) + 2\ln(\mu_{\text{eff}})$$

$$\frac{\Delta \mu_a}{\mu_a} = \left( \frac{\Delta D}{D} \right) + 2\left( \frac{\Delta \mu_{\text{eff}}}{\mu_{\text{eff}}} \right)$$  \hspace{1cm} \text{Eq. 3-15}

In contrast, the error in $\mu_a'$ is on the order of the error in $D$ (or $\Delta x$), particularly for tissues with high albedo. This follows similarly from Eq. 3-8.
Chapter 4  Multiple Wavelength Oblique-incidence Reflectometry

4.1 Introduction

In this chapter we continue the development of oblique-incidence reflectometry for measuring the optical properties of turbid media. Chapter 3 showed that the original presentation of oblique-incidence reflectometry [51] as a means of measuring the reduced scattering coefficient of biological tissues at a single wavelength could be extended and improved upon to measure simultaneously both the absorption and reduced scattering coefficients of a tissue-like turbid medium with accuracies of 10% and 5%, respectively. Recall that after delivering obliquely incident laser light to a tissue phantom and sampling its spatial distribution of diffuse reflectance with optical fibers, we were able to 1) measure the shift in the center of diffuse reflectance, which is proportional to the diffusion coefficient of the medium \(D\) and 2) fit the distribution to a diffusion theory model to determine the effective attenuation coefficient of the medium \(\mu_{\text{eff}}\). From \(D\) and \(\mu_{\text{eff}}\), we calculated \(\mu_a\) and \(\mu'_s\). This chapter investigates the use of oblique-incidence reflectometry to deduce absorption and reduced scattering spectra from multi-spectral measurements of the relative diffuse reflectance profile of white light obliquely incident upon tissue phantoms [52]. By using white light and taking multi-spectral measurements, the wavelength variations in absorption and reduced scattering can be obtained with the same amount of work that it takes to find the optical properties at a single wavelength.
See section 4.2 for a detailed description of the experiment and results. Chapter 5 describes a multiple fiber imaging spectrograph system that we have designed and built that allows us to measure the spectral response of all of the collection fibers at the same time, and hence has potential to improve the efficiency of our measurements even further.

The motivation for developing oblique-incidence optical fiber reflectometry is a need for a convenient, inexpensive, reliable, and accurate method for measuring the tissue optical properties of absorption and reduced scattering in vivo. Several techniques already exist for multi-wavelength measurement of tissue optical properties [25], some of which we have discussed in detail in Chapter 2. The integrating sphere measurement provides for multi-spectral determination of $\mu_a$ and $\mu_s'$, but this technique requires a tissue slice and hence is not suited for non-invasive diagnostics [28, 30]. Non-invasive integrating sphere reflectance measurements can be taken alone (without transmission measurements) on in vivo tissue samples, but they fail to allow separation of the absorption characteristics from the scattering characteristics, and are also somewhat inconvenient to perform. Normal incidence video reflectometry, a method which is based on the dipole-source diffusion theory model [35], provides a non-invasive way to determine both optical properties, but it requires a measurement of both the spatial distribution of diffuse reflectance and the total diffuse reflectance at each desired wavelength, which makes this procedure quite inconvenient and time-consuming for multi-spectral analysis [38]. Another technique circumvents this problem through the use of white light and a normal incidence fiber-optic probe coupled to a spectrograph. If the diffuse reflectance is sampled in absolute units, then both optical properties could be
deduced through curve fitting, where the absolute units provide the necessary scaling factor [40]. An alternative to the curve fitting approach is to train a neural network to deduce the optical properties [41, 53]. With either of these methods, the calibration in absolute units is a sensitive procedure that may not be well suited for a clinical setting. Similarly, time-resolved and frequency domain techniques require instrumentation that may not be cost effective for non-research applications. In contrast, fiber-optic oblique-incidence reflectometry allows for fast and non-invasive multi-spectral measurements of optical properties without the need for a sensitive calibration procedure or expensive instrumentation.

4.2 Multi-wavelength Oblique-incidence Reflectometry

4.2.1 Review of Oblique-incidence Reflectometry

This section contains a brief summary of the underlying theory of oblique-incidence reflectometry which is described in detail in Chapter 3. All of the current methods that use reflectometry to deduce the optical properties of turbid media require a means of measuring the spatial distribution of diffuse reflectance of an incident light beam from tissue, either with a CCD camera or with optical fibers coupled to a power meter. All of these methods also require an additional measurement to deduce both the absorption and reduced scattering coefficients; otherwise, only a single parameter such as the transport mean free path or the effective attenuation coefficient, which are functions of both
absorption and scattering, can be determined. This second measurement consists of either a calibration to absolute units, a measurement of total diffuse reflectance, or, in our case, a measurement of the shift in the center of diffuse reflectance from the point of light incidence.

As we already know, light interacting with molecules or cellular organelles in a tissue are either absorbed or scattered. Photons that escape the tissue after multiple scattering events comprise what is called diffuse reflectance. We have utilized a diffusion theory based photon dipole expression for the steady-state spatial distribution of diffuse reflectance to model the diffuse reflectance of light delivered through an optical fiber to a semi-infinite tissue medium [35]. Recall from Chapter 3 that, for normally incident light, the profile of diffuse reflectance from a semi-infinite medium can be modeled by two isotropic point sources in an infinite medium. A positive source is buried in the tissue and a negative image source is above the position of the tissue surface. However, if light is delivered obliquely, the positive source is at the same depth into the tissue, but this depth is measured along the optical path determined by Snell’s law rather than normal to the tissue surface. As a result, the fundamental difference between normal and oblique-incidence is a shift in the positions of the point sources (and the center of diffuse reflectance) in the $x$ direction, as depicted in Figure 3-2. For both normal and oblique-incidence, this model was shown to be accurate for source-detector separations greater than 1.5 transport mean free paths. See Figures 3-3 and 3-5.

Given a light delivery and collection probe with a known configuration such as that shown in Figure 3-4, the process of deducing the absorption and reduced scattering
coefficients from the profile of diffuse reflectance was a relatively simple one. With $\theta$ (the angle of refraction from the source fiber tip into the tissue), $\Delta y$ (the offset between the plane of incidence and the plane of collection), and $A$ (the boundary condition) in hand, we were able to reconstruct the optical properties of tissue phantoms as follows:

1. the diffuse reflectance profile was sampled at known positions from the point of light incidence (the source fiber position) by the collection fibers

2. the raw data from each collection fiber was scaled by a calibration factor, $k_i$ (which was previously determined as described in section 3.2.1)

3. the center of diffuse reflectance was found, and its shift from the point of light incidence, $\Delta x$, was measured

4. the diffusion coefficient, $D$, was calculated using Eq. 3-4:

$$D = \frac{\Delta x}{3 \sin \theta_{\text{tissue}}}$$

5. the distances in $z$ of the positive, buried source and the negative, image source from the actual tissue surface were calculated with the following expressions from Eq. 3-5:

$$z_0 = \text{depth of positive source} = \frac{\Delta x}{\tan \theta_{\text{tissue}}}$$

$$z_0 + 2z_b = \text{height of negative source} = \frac{\Delta x}{\tan \theta_{\text{tissue}}} + \frac{4}{3} A \frac{\Delta x}{\sin \theta_{\text{tissue}}}$$

Eq. 4-1

6. a non-linear least-squares fit using the Levenberg-Marquardt method was performed on the following equation (Eq. 2-31) to determine $\mu_{\text{eff}}$:

$$R(r) = z_0 \left( \mu_{\text{eff}} + \frac{1}{\rho_1} \right) e^{-\rho_1 \mu_{\text{eff}}} + \left( z_0 + 2z_b \right) \left( \mu_{\text{eff}} + \frac{1}{\rho_2} \right) e^{-\rho_2 \mu_{\text{eff}}}$$

7. $\mu_a$ and $\mu_s'$ were calculated with Eqs. 3-7 and 3-8:

$$\mu_a = D \mu_{\text{eff}}^2$$
4.2.2 Materials and Methods

4.2.2.1 Measurements on Water-Based Tissue Phantoms

Experiments were performed on phantom solutions with optical properties designed to be similar to those found in actual biological tissues. The tissue phantoms we used for these multi-spectral measurements were prepared in exactly the same manner as the phantoms we used for the single wavelength measurements. That is to say, 1) they consisted of Trypan Blue dye and 900 nm diameter Polystyrene spheres (Bristol Colloid Centre) diluted in water, 2) the values of absorption and reduced scattering for the stock solutions were measured at 632.8 nm with a spectrophotometer (HP8452A) and by collimated transmission, respectively, and 3) Mie theory was used to calculate g. To minimize the uncertainty in the optical properties of the phantoms, the absorption of each was measured with the spectrophotometer after dilution of the dye but prior to the addition of the scatterer. Thus, assuming that there is no significant interaction between the components of the phantoms, we had a set of standards with the reduced scattering coefficient known to the accuracy of the dilution process and an independently measured absorption coefficient, both at 632.8 nm. Ten phantoms were mixed with the following sets of optical properties at 632.8 nm: \((\mu_s', \mu_a) = (4 \text{ cm}^{-1}, 0.407 \text{ cm}^{-1}), (6 \text{ cm}^{-1}, 0.194 \text{ cm}^{-1}), (6 \text{ cm}^{-1}, 0.413 \text{ cm}^{-1}), (6 \text{ cm}^{-1}, 0.614 \text{ cm}^{-1}), (7 \text{ cm}^{-1}, 0.195 \text{ cm}^{-1}), (7 \text{ cm}^{-1}, 0.616 \text{ cm}^{-1}), (8 \text{ cm}^{-1}, 0.195 \text{ cm}^{-1}), (8 \text{ cm}^{-1}, 0.415 \text{ cm}^{-1}), (8 \text{ cm}^{-1}, 0.610 \text{ cm}^{-1})\), and \((10 \text{ cm}^{-1}, \)
Figure 4-1  Schematic of experimental apparatus. The probe was placed on the surface of the tissue phantom.

0.405 cm\(^{-1}\)). These in fact were designed to be the same properties as those used in the single wavelength experiment – the only reason we made fresh phantoms is because the optical properties are prone to vary slightly over time, primarily due to loss of water.

To perform the multi-spectral measurement, we used a broadband (white) light source instead of a laser (Dolan Jenner Quartz-Halogen Fiber Lite 170-D), and the light collected by each detection fiber was directed to an optical multichannel analyzer (OMA) for spectral resolution. Our experimental system is shown schematically in Figure 4-1. The exposed x-ray film was again floated on the surface of each tissue phantom to approximate a matched boundary (see section 3.3.2). The white light was both delivered and collected with the same black delrin and 600 \(\mu\)m diameter fiber-optic, oblique-incidence probe we used in the previous experiment. The OMA was composed of a spectrograph (Instruments SA HR320 100 mm spectrograph with variable entrance slit and f-matcher), an intensified CCD camera (Princeton Instruments IRY-7006/PAR/R Linear Array Intensified CCD), and a computer controller (Princeton Instruments ST115
Figure 4-2  a. Surface plot of spectral data collected for a phantom with \((\mu'_s, \mu_a) = (4 \text{ cm}^{-1}, 0.407 \text{ cm}^{-1})\). Each fiber collects a spectrum at a specified position. There are no detection fibers, and hence no data, between -0.92 and +0.19 cm.  b) An example of one spectral slice (at 633 nm) through the data in (a). The data at each individual wavelength looks and is processed exactly like the single wavelength data from Chapter 3.

OSMA detector controller). The controller, which was interfaced with a Macintosh IIx, automatically scanned the wavelength range from 400 nm – 800 nm to yield a spectrum of the collected light. The wavelength resolution was 6.2 nm. For calibration purposes we
also made certain that one of the wavelength bins was approximately centered about 632.8 nm (it was actually centered at 632.4 nm). Each collection fiber from the probe was sequentially coupled with an SMA connector to the OMA, which can accommodate only one input at a time. The slit opening of the spectrograph was fixed at a position that ensured that the fiber with the highest throughput (the one nearest the source fiber) would not saturate the detector, even for the highest albedo phantoms \((\mu'_a, \mu_a) = (10, 0.405)\) and \((8, 0.610)\). The measurement from each fiber was performed with 40 accumulations, thus maximizing the signal to noise ratio. In addition, each phantom was measured independently four times. By determining the spectrum collected by each individual detection fiber, we actually acquired the spatial distribution of diffuse reflectance at many wavelengths with exactly the same amount of effort it took to do the same at a single wavelength, as presented in the previous chapter.

To correct for slight variations in collection efficiency from one detection fiber to the next, a wavelength independent correction factor, \(k_i\), was calculated for each fiber. To calculate the \(k_i\)'s, the diffuse reflectance curves measured at 632.4 nm for four of the phantoms were scaled to match their expected curves at 632.8 nm (the wavelength at which the optical properties of the phantoms were targeted). The four calibration standards were \((\mu'_a, \mu_a) = (6 \text{ cm}^{-1}, 0.413 \text{ cm}^{-1}), (7 \text{ cm}^{-1}, 0.195 \text{ cm}^{-1}), (7 \text{ cm}^{-1}, 0.616 \text{ cm}^{-1}),\) and \((8 \text{ cm}^{-1}, 0.415 \text{ cm}^{-1})\). The \(k_i\)'s derived for each of the calibration standards were averaged, and the raw data for the remaining six test standards (at all wavelengths) was corrected before curve fitting. From the corrected data, the relative diffuse reflectance profile at each desired wavelength was extracted and analyzed independently as outlined
in section 4.2.1 (and Chapter 3). Each “spectral slice” through the data allowed us to deduce the optical properties of the medium at that particular wavelength (Figure 4-2). This procedure was repeated automatically for each desired wavelength by the fitting program. The absorption and reduced scattering spectra are presented in the results section 4.2.3.1.

4.2.2.2 Measurements on a Resin-Based Solid Phantom

In addition to the measurements on the water-based phantoms, measurements were also performed on a solid phantom made from casting resin to demonstrate that this method will work on solids (such as tissues) as well as fluids. The resin phantoms were prepared from clear liquid plastic casting resin (Castin' Craft), titanium dioxide (DuPont TIPure), and black ink (which happens to be a near-infrared dye with a relatively flat visible absorption spectrum) [54]. It was difficult to come up with "stock" values of scattering and absorption for these components because they were water-insoluble powders. Thus, we did not have very reliable "expected values". Nonetheless, our target value was \((\mu'_s, \mu_a) = (10 \text{ cm}^{-1}, 0.06 \text{ cm}^{-1})\). The phantom was cast in a PVC drainage pipe with an inner diameter of 7.5 cm. A rubber cap was placed on the bottom to prevent leakage during curing. 5 mg of the dye was dissolved in a small amount of ethanol and sonicated. 800 mg of TiO\(_2\) was also dissolved in ethanol and sonicated. These two components were mixed in the pipe with 800 ml of resin and allowed to sit for several hours before the addition of 45 drops of the resin-curing catalyst. At room temperature, the phantom cured within 24 hours. After hardening, the phantom was removed from the
PVC pipe, cut into two 7.5 cm tall cylinders, and polished. Measurements were taken on all four flat surfaces by coupling the plastic to both the probe and the index-matching film with microscope immersion oil (Stephens Scientific). The index of refraction of the matching oil was 1.51, and the index of the resin was 1.58 (measured with a gem-identification refractometer). Results are in section 4.2.3.2.

**4.2.2.3 Measurements on Chicken Breast**

For our final experiment, we took measurements from fresh, raw, lean chicken breast meat. The skin and fat was removed from four freshly frozen chicken breasts, and the breasts were stacked in a small black bucket such that the muscle fibers were aligned. The bucket was then filled with warm water to fully submerge the chicken, and it was left for 30 minutes to thaw. Measurements were taken with our oblique-incidence fiber-optic probe, and the chicken was left underwater to 1) ensure good coupling between the separate pieces of chicken and 2) to index match to the top surface. On top of the water we floated the exposed x-ray film to approximate a matched boundary condition. Since water is essentially optically clear in the visible wavelength range, its only effect was to act as index matching fluid between the chicken and the x-ray film. Four measurements were performed with the probe oriented at different angles relative to the muscle fibers: 0° (parallel), 30°, 60°, and 90° (perpendicular). Each measurement was repeated once, and the average values are shown in Figure 4-5 (section 4.2.3.3).
4.2.3 Results

4.2.3.1 Water-Based Phantoms

![Graphs showing absorption properties of water-based phantoms across different wavelengths.](image-url)
Figure 4-3 Plots of the wavelength dependence of absorption and reduced scattering for the following water-based phantoms: \((\mu'_a, \mu_a) = a,b\) (4 cm\(^{-1}\), 0.407 cm\(^{-1}\)), \(c,d\) (6 cm\(^{-1}\), 0.194 cm\(^{-1}\)), \(e,f\) (6 cm\(^{-1}\), 0.614 cm\(^{-1}\)), \(g,h\) (8 cm\(^{-1}\), 0.195 cm\(^{-1}\)), \(i,j\) (8 cm\(^{-1}\), 0.610 cm\(^{-1}\)), and \(k,l\) (10 cm\(^{-1}\), 0.405 cm\(^{-1}\)).

Figure 4-3 contains the absorption and reduced scattering spectra obtained for each of the six test standards. The expected absorption and reduced scattering curves were derived from a) a scaled version of the absorption spectrum of Trypan Blue dye as measured by spectrophotometer, and b) a scaled version of the reduced scattering spectrum of 900 nm spheres as calculated by Mie theory. Both expected spectra were scaled to match the expected \(\mu_a\) and \(\mu'_a\) at 632.8 nm. The measured values shown are the average values calculated at each wavelength over four measurements ± one standard deviation. In addition to the expected curve, also shown is the expected curve ± 10%.

Looking at the absorption spectra, we first notice that the measured peak coincides very well with the expected peak. There is no clear trend relating the expected absorption or scattering to whether or not the measured absorption curve is slightly lower or higher.
than expected, but it is worth noting that for both phantoms with $\mu_a = 0.2 \, \text{cm}^{-1}$, the measured result was not only high, but fell slightly outside of the $\pm 10\%$ envelope for a significant wavelength range (roughly 400-620 nm). On the other hand, the result for the remaining phantoms tended to remain within the $\pm 10\%$ envelope except for slight excursions below the expected in the range from 630-720 nm.

The results for reduced scattering were also very good. For phantoms $(\mu_s', \mu_a) = (4 \, \text{cm}^{-1}, 0.407 \, \text{cm}^{-1}), (6 \, \text{cm}^{-1}, 0.614 \, \text{cm}^{-1})$, and $(8 \, \text{cm}^{-1}, 0.610 \, \text{cm}^{-1})$, the measured reduced scattering fell within the $\pm 10\%$ envelope for all wavelengths. For the remaining phantoms with $(\mu_s', \mu_a) = (6 \, \text{cm}^{-1}, 0.194 \, \text{cm}^{-1}), (8 \, \text{cm}^{-1}, 0.195 \, \text{cm}^{-1})$, and $(10 \, \text{cm}^{-1}, 0.405 \, \text{cm}^{-1})$, the measured results were low for all wavelengths, and actually dropped out of the $\pm 10\%$ envelope for wavelengths below 470 nm. See section 4.3.2 for a further discussion.
Figure 4-5 Results from chicken breast experiment. The absorption and reduced scattering spectra were determined with the probe aligned at a) 0°, b) 30°, c) 60°, and d) 90° to the direction of the muscle fibers.

4.2.3.2 Resin Phantoms

Figure 4-4 shows the measured absorption and reduced scattering spectra for all four of the flat surfaces from the solid resin phantom, which was cut into two cylinders. Expected values are not shown. See section 4.3.3 for a discussion.

4.2.3.3 Chicken Breast

In Figure 4-5 are plots of the absorption and reduced scattering spectra measured from fresh chicken breast at different angles relative to the alignment of the muscle fibers. There are no expected values. When the probe was oriented parallel to the muscle fibers (0°), the absorption was found to be approximately 0.4 cm⁻¹ in the wavelength range from 450 to 550 nm. Beyond 600 nm the absorption essentially disappeared. The reduced
scattering ranged from 12 down to 8 cm\(^{-1}\) when the wavelength was between 450 and 575 nm. Above 600 nm the reduced scattering decreased gradually from 5 to 3 cm\(^{-1}\) at 800 nm. When the probe was rotated to 30°, the absorption in the range from 450 to 550 nm increased to over 0.6 cm\(^{-1}\), and the reduced scattering in the same range decreased to 5 – 7 cm\(^{-1}\). At 60°, the results were only slightly different from 30° – the absorption decreased by a few percent, and the reduced scattering increased a by a few percent. Finally, when the probe was oriented perpendicular to the muscle fibers, the absorption peak from 450 to 550 nm increased to over 0.7 cm\(^{-1}\), while the reduced scattering remained quite close to the values obtained at 30 and 60°.

4.3 Discussion and Conclusions

4.3.1 General Discussion

This section contains an in depth discussion of several issues regarding data collection, data manipulation, and the resulting errors in our measurements. Beginning with the light source, it is important to determine whether or not the output spectrum of the source itself must be taken into account to obtain accurate results. It turns out that this is not the case. Regardless of how “white” the source beam is, since the optical properties at any given wavelength are reconstructed from the relative diffuse reflectance profile at that wavelength, the fitted optical properties will be the same regardless of the amount of source power at that wavelength. Thus, to measure the optical properties
monochromatically at 600 nm and 500 nm independently (i.e. using a monochromatic source for each wavelength), there would be no need to either a) ensure that the source powers at the two wavelengths were equal or b) calibrate in absolute power units. In Figure 4-2a there are 3 distinct regions where the diffuse reflectance drops significantly: at the ends of the visible spectrum and in the middle, around 600 nm. On the other hand, in Figure 4-3, none of the spectra show any significant increase in absorption or reduced scattering to account for the dips in the data, except at 600 nm where the Trypan Blue absorption peak occurs. It turns out that the spectrum of the quartz-halogen lamp begins to cut off at roughly 450 nm and 750 nm, and this is the reason the unreconstructed data in Figure 4-2a rolls off at the ends of the spectrum. Nonetheless, we made no correction for the lamp spectrum, and it had absolutely no effect on the reconstructed optical properties. A similar argument explains why the spectral responsivity of the detector and the fibers themselves is also irrelevant to our measurement.

This leads to the next issue: is calibration at a single wavelength (632.8 nm) adequate? We believe that it is because the algorithm we used to reconstruct the optical properties requires only a relative profile of diffuse reflectance, and hence any wavelength to wavelength variability in total light throughput cancels out in the end. On the other hand, the fact that the relative errors for \( \mu_a \) were low for all of the phantoms in the wavelength region around 633 nm but not elsewhere suggests that perhaps a single correction factor \( k_i \) is insufficient, but rather each fiber requires a calibration spectrum. A possible explanation for this is the wavelength variability in the indexes of refraction of the fused silica fibers and the phantom itself. For fused silica, the index varies from
approximately 1.47 at 400 nm to 1.45 at 800 nm. For water, the index varies from 1.3421 at 400 nm to 1.3289 at 800 nm. Taking these variations into account, the refracted angle of light in the sample would vary less than ±1%. Also, if each fiber did require a calibration spectrum, then we would expect to see a systematic error at wavelengths other than that at which the calibration was performed. Not only are the errors at other wavelengths not systematic, but they are also small enough relative to the amount of error we can realistically expect to achieve in the expected properties of the phantoms to preclude any judgment.

4.3.2 Discussion of Results for Water-based Phantoms

Recall from the discussion in Chapter 3 that each probe has an upper and lower bound to the optical properties that it can measure accurately. Since we used the same probe in these multi-spectral experiments as we did for the single wavelength experiments, and since the optical properties of the phantoms are also the same, there should have been no problem with the probe size. While the absorption does drop to virtually nothing at wavelengths above and below the absorption peak at 580 nm, the transport mean free path (which is the factor we must take into account when assessing probe size) is dominated by the reduced scattering, which is at a minimum of 3.4 cm\(^{-1}\) at 800 nm for the phantom \((\mu_r^s, \mu_a) = (4 \text{ cm}^{-1}, 0.407 \text{ cm}^{-1})\). Thus, the effect of incorrect probe size could only account for a small amount of error at long wavelengths for that particular phantom.
For the most part, the error in absorption was quite low. In addition, the standard deviation for the measured values was also low, so the measurements were also repeatable. The standard deviation did increase around the absorption peak, especially for phantoms with short transport mean free paths, due to decreased signal. Also notice that in all of the phantoms, the measured absorption shows a small peak centered about 770 nm and an even smaller peak centered about 420 nm, neither of which appear in the expected spectrum. This peak may be due to absorption by the Polystyrene spheres, but it is difficult to test because such small amounts of absorption relative to the total amount of scattering would not show up on a spectrum of total attenuation, $\mu_a + \mu_s$. On the other hand, it is possible that the error at 420 and 770 nm is simply due to the fact that a) the lamp spectrum is near cut-off and hence the signal is very low and b) the expected absorption coefficients at these wavelengths are very small, so any small deviations due to noise would give a large error. Since the errors at these two wavelengths is consistently positive for all of the phantoms, the first explanation is more likely.

Careful comparison of all the measured reduced scattering curves reveals that they all have the same overall shape, as expected, so the errors are most likely simply errors in dilution (and hence the expected values). In addition, comparison of the shape of the measured curves with the shape of the expected curves yields an interesting observation: the shapes are very similar, but they appear to be shifted in wavelength. In Figure 4-6 are plotted the same curves that appear in Figure 4-3, but with the expected reduced scattering spectra shifted down by 45 nm. Due to the slope of the curve, one effect of this is simply to decrease the expected value of scattering at each wavelength. A second
effect is to move the small peaks in the curve. With this adjustment, it is quite evident that the measured curves match the expected curves even better. In fact, the results for all of the phantoms now fall within the ±10% envelope for the majority of the wavelength range. Since the wavelengths of the expected and measured absorption peaks matched so well, nonlinearity or miscalibration in the wavelength drive of the OMA cannot explain this phenomena. The only explanation is that the expected curves are incorrect.
Figure 4-7 Plot of expected reduced scattering spectra calculated from Mie theory. For simplicity, the following were assumed constant (over all wavelengths and for each sphere size): index of refraction of the polystyrene spheres = 1.5746, index of refraction of water = 1.3324, density of polystyrene = 1.05, density of water = 1.0, and concentration of spheres by weight = 0.102. The sphere size was varied from 900 to 1000 to 1150 nm. Notice that a) the period of undulations in the curves decreases with sphere size and b) the relative amount of scattering also decreases with sphere size.

With all other factors constant, a change in the size of the spheres tends to both shift the expected Mie scattering spectrum, change the overall amount of scattering, and cause minor alterations in the shape of the expected curve, namely a change in the period of the undulations (Figure 4-7). In Figure 4-6, there is an obvious mismatch that appears in the range of 560-600 nm for all of the phantoms. If the shape of the expected curve were correct and only the decrease in the expected reduced scattering due to the shift were important, then a) this mismatch would not exist and b) the most likely explanation for our error is simply in the expected concentration of the stock polystyrene spheres. Assuming that this is the case, then a possible explanation for the increased measured scattering from 560-600 nm is scattering by the Trypan blue dye. Not only is the mismatch located at the absorption peak, but the mismatch is slightly larger for the
phantoms with higher absorption. Since the dye molecules are so much smaller than the wavelength of visible light though, this explanation is not likely.

An alternate explanation is that the sphere size is actually slightly larger than 900 nm. Larger spheres give rise to a smaller period (in nm) of variation in scattering as well as less scattering overall. Thus, this situation would explain both the extra peak in the measured reduced scattering spectrum as well as the fact that the measured value for reduced scattering was slightly smaller than what we originally expected for most of the phantoms. Further experimentation with these phantoms should be preceded by verification of the size of the polystyrene spheres by scanning electron microscopy.

4.3.3 Discussion of Results for Resin Phantom

Since the two cylindrical resin phantoms were originally one, by measuring the optical properties we can identify which two of the four surfaces were originally in contact as well as which of the remaining two surfaces was on the bottom during curing. From Figure 4-4, since the absorption is the virtually the same for all four surfaces, we know that the absorber was mixed very well throughout the resin, and that it did not settle much. Since the absorption for surface 1-1 is lower than the others in the range of 750 – 800 nm, this suggests that 1-1 was the top of the original cylinder. Looking at the scattering, right away we can see that surface 2-2 has the highest scattering, and hence must have been the bottom of the original, uncut cylinder. The titanium dioxide must have settled quite a bit. The measured reduced scattering from the other three surfaces is almost the same. With this information, it is fairly simple to deduce that surface 1-2 was
originally in contact with surface 2-1. Note that this would have been a difficult assessment to make with information on the scattering or absorption alone because a) the absorber was so well mixed into the resin that only one surface gave some slightly lower values than the others and b) there also appears not to have been much of a gradient in TiO₂ concentration throughout the phantom, only a build-up on the bottom.

It is also worth noting that the measured results are consistent with the values we originally estimated for the absorption and reduced scattering of the resin phantom. While we estimated an absorption of 0.06 cm⁻¹ and reduced scattering of 10 cm⁻¹, we measured the absorption to range from 0.2 to 0.5 cm⁻¹ and the reduced scattering to range from 6.5 to 10 cm⁻¹. Thus, with this simple experiment we have shown qualitatively that our method for measuring the wavelength variation of the optical properties works equally well for solid as well as liquid media, and that the technique is sensitive enough to detect small gradients in the phantoms due to settling during the curing process.

4.3.4 Discussion of Results for Chicken Breast

The most striking result of this experiment is that the optical properties measured with the probe oriented at different angles relative to the muscle fibers varied significantly, especially in the range from 450 – 550 nm. This result is not surprising, though, because the reconstruction algorithm relies on an approximate diffusion process in the tissue. Due to the highly ordered nature of muscle tissue, it would be unrealistic to expect the photons to spread isotropically. Despite this drawback, our measurements still gave us reasonable approximate values for the bulk tissue which are consistent with
Figure 4-8  Spectra for oxy- and deoxyhemoglobin. The peaks in the range from 500 – 600 nm look similar to the absorption spectrum from chicken breast.

previously published results [55]. Another very interesting point is that the absorption peak(s) in the range from 500 – 600 nm are almost certainly due to hemoglobin. Oxyhemoglobin has peaks at 542 and 577 nm with a shallow “valley” in between. Deoxyhemoglobin has a peak at 550 nm (Figure 4-8). Figure 4-5 clearly demonstrates features reminiscent of an oxy-/ deoxyhemoglobin mix, and it is quite reasonable to expect that our technique could also be developed into an oximetry system.

Regarding the measurement itself, there are a few issues to be discussed. First of all, since the measured reduced scattering of the tissue dropped below 4 cm\(^{-1}\) at long wavelengths, we can expect that the amount of error there is slightly higher than at shorter wavelengths. On the other hand, there was strong absorption in the blue to blue–green, and the decreased signal caused a rather large increase in the amount of noise. This could only be corrected by a) repeating the experiment with more accumulations and b) averaging the results over many independent measurements.
There were also some important issues regarding the tissue under test. Since the chicken was submerged in deionized water, which is not isosmotic with tissue, the chicken swelled during the course of the experiment. In retrospect, it would have been better to submerge the chicken in 0.9% saline or ringer's solution. In addition, due to the relatively small size of the chicken breasts, the tissue could not truly be considered semi-infinite. In fact, it was clear that light was escaping the sides of the tissue. This problem may become very important in true in vivo applications in which the size of a contiguous piece of the same type of tissue could be quite small relative to the optical penetration depth. There has been some work done to tackle this problem by modeling layered tissues [13, 15, 45, 56], and tissues with arbitrary geometries can be modeled through Monte Carlo simulations. At this point in time, though, it is unclear how this issue will develop. It is plausible that while very accurate measurements of absorption and reduced scattering may prove to be impossible on tissues in vivo, the averaged bulk measurements may prove to be adequate for most applications.

4.3.5 Conclusion

In conclusion, we have demonstrated that the absorption and reduced scattering spectra of a turbid medium can be measured simultaneously with oblique-incidence optical fiber reflectometry. The multi-spectral measurement is accomplished simply by extending our method for monochromatic measurements to work with a white light source. We have also shown that this method can be easily applied to solid phantoms and real tissues as well as to water-based phantoms. In the following chapter we will
describe a multi-fiber OMA system which we have built to perform these same multispectral measurements virtually instantaneously.
Chapter 5  Multiple Fiber Imaging Spectrograph System

5.1 Motivation and Explanation

To further increase the efficiency of the oblique incidence optical fiber method that we have developed, we have also built a multiple fiber imaging spectrograph system for simultaneous, wavelength-resolved measurements from each collection fiber. In Chapters 3 and 4, the photodetectors were capable of handling only one input at a time. That is to say, there was no way to simultaneously take measurements (with or without spectral resolution) from more than one detection fiber at a time, so the fibers had to be manually coupled to the detector to measure their output sequentially. One way to solve this problem for monochromatic measurements is to use a linear photodiode array with a one-to-one correlation between the photodiodes and the detection fibers. In other words, the light collected by the detection fibers could be measured simultaneously by parallel processing with multiple detectors. Similarly, multi-spectral measurements could also be performed in parallel, but that would require multiple OMA’s and hence be very impractical. Thus we have endeavored to construct an OMA-type system that can measure simultaneously the spectra of light collected by a number of fibers [39, 40].

The first step for the multiple fiber imaging spectrograph system is to arrange the output of the detection fibers into a linear array. This array of points can be imaged by an imaging spectrograph system (as opposed to a standard monochromator/spectrometer) onto a two-dimensional CCD array (as opposed to a linear array). In the final image, each input point, which corresponds to a single detection fiber, is spread into a linear
Figure 5-1 Sample screen capture from multi-fiber imaging spectrograph system. Each linear spectrum is the output from a single detection fiber illuminated by an incandescent tungsten bulb.

spectrum. The result is a two-dimensional image with spectral information along one axis and spatial information (corresponding to the position of each detection fiber) along the other (Figure 5-1).

In designing our multi-fiber system, we were not aware of the important differences between imaging spectrographs and the more common monochromator/spectrometers. Imaging spectrographs are equipped with toroidal mirror systems for removal of spherical aberration, coma, and astigmatism that otherwise severely degrade an image as it is processed by the system. They are usually designed such that any object (with an extent on the order of 1-2 cm) placed at the entrance slit of the spectrograph is imaged with a 1:1 magnification at the exit. The 1:1 magnification is only along the axis perpendicular to the direction of spectral resolution, of course. In the other dimension, each object point
becomes a linear spectrum. There is also usually space left at the spectrograph input for various filters. Since we had already acquired a non-imaging spectrograph, we decided to correct for the problems inherent in our system and make the additions necessary for it to function relatively well as an imaging spectrograph.

To explain each component of the system, we will work backwards from the detector to the source. Refer to section 5.3 for schematics. The final images were captured by a Macintosh IIci desktop computer equipped with a frame grabber card from Data Translation, Inc. (model 06973) and NIH Image version 1.57. The controller unit of an 8 bit CCD camera from Dage-MTI (model CCD-72) negotiated the connection between the camera and the computer. This unit had independent electronic control of gain and black level. The camera was connected via a standard C mount to a custom exit assembly on a Czerny-Turner spectrograph from Thermo-Jarrell Ash Corporation (model Monospec 18). The spectrograph had a focal length of 156 mm and an aperture of F/3.8.
It was equipped with a 300 groove/mm and 500 nm blaze grating giving it a full spectral range of 250 to 950 nm and a linear dispersion of 18 nm/mm. The exit assembly was composed of a cylindrical lens (f = 75 mm) to correct for astigmatism and a spherical focusing lens (f = 1.25 in) to demagnify the final image onto the CCD chip. The distances between the lenses and the chip were made variable. With a 25 μm entrance slit to the spectrograph, we could capture a 280 nm wavelength range in a single image with a point spread function of 10 pixels/nm (assuming the laser bandwidth to be 1 nm). See Figure 5-2.

To accurately measure the spatially-resolved diffuse reflectance up to a distance of several transport mean free paths from the light input point, a detector with a dynamic range of 4 orders of magnitude (16 bits) is required. To work with an 8 bit CCD, we equipped the entrance of the spectrograph with a custom-made continuous gradient neutral density filter ranging from 0 to 4 O.D. from Reynard Corporation. This 1 inch square filter was held in place by a custom holder from Thermo-Jarrell Ash. By filtering the output of the detection fibers nearest the source fiber through the darker portions of the gradient ND filter, we approximately equalized the amount of light that reached the camera from each detection fiber. In this way, readings could be taken from all the fibers without pushing the detector either up to saturation or down to the noise floor. The problem with this trick is that the filter must be mounted outside of the spectrograph, in front of the entrance slit, which is also the object plane. Because of this, a real image of the object had to be relayed onto the entrance slit by a lens system. Our solution was to design a custom mount for a Nikon Micro-Nikkor 55 mm zoom lens with a bayonet
flange. The flange connected to a C Mount adapter which in turn was connected to our custom entrance adapter assembly that was mounted in front of the ND filter.

The object which we imaged was a 1 cm tall linear array of the tips of nine 600 \( \mu \)m diameter optical fibers. The fibers were embedded in a cylinder of casting resin (Castin’ Craft clear liquid plastic casting resin) and polished. The opposite ends of these fibers were terminated with Surgimedics brass crimp sleeves (model 921425-000) glued inside Amphenol 630 \( \mu \)m SMA connectors (model 905-150-5005) with LocTite Speed Bonder 325 structural adhesive. The SMA connectors were screwed into a custom bulkhead connector designed to be mounted on a standard 1 inch ruled 1/4-20 tapped optical table. The detection fibers from the optical fiber probe, which were also SMA terminated, were connected to the other side of the bulkhead connector. The bulkhead connector provides a convenient way to switch between different fiber optic probes without the need for realignment of any part of the detection system.

The next part of the system is the source. Any broadband white light source would do, preferably one with as flat a response in the visible as possible, although this is not required. We chose to use a 150 W Xenon arc lamp from Oriel Instruments (model 77822) which we coupled into the source fiber of the probe with a focusing tube and SMA adapter (Oriel model #s 77800 and 77670). For calibration purposes, we used two HeNe lasers from Uniphase, one at 632.8 nm and one at 594 nm (model #s 1135 (red) and 1677 (yellow)).

Finally, the probe itself was custom designed and built out of black delrin and 600 \( \mu \)m diameter low-loss optical fiber from Spectran (model HCP-M0600T-08). The ends
of the fibers in the probe were cleaved and polished, and the opposite ends were
terminated with SMA connectors.

5.2 Complete Parts List

Sources

150W Xe arc lamp  
Focusing tube  
SMA adapter  
632.8 nm HeNe laser  
594 nm HeNe laser  

Oriel Instruments  
Oriel Instruments  
Oriel Instruments  
Uniphase  
Uniphase  

77822  
77800  
77670  
1135  
1677

Probe

Black delrin  
Fused silica fiber  
630 µm SMA connectors  
Brass crimp sleeves  

not otherwise specified  
Spectran  
Amphenol  
Surgimedics  

HCP-M0600T-08  
905-150-5005  
921245-000

Entrance Assembly

Bulkhead connector  
SMA bulkhead adapters  
Linear fiber array  

custom built, aluminum  
Amphenol  
fused silica fiber, SMA terminated at one end and
embedded in resin at the other  
905-120-5003
Casting resin
55 mm zoom lens
Bayonet to C adapter
C mount adapter to spectrograph
0 – 4 O.D. gradient ND filter
1 inch square filter mount

**Spectrograph**

Monospec 18
Grating mount
300 grv/mm, 500 nm blaze grating
25 μm entrance slit

**Exit Assembly**

f = 75 mm cylindrical lens
Alignment/focusing tubes
(includes f = 1.25 in. focusing lens)

**Camera System**

8 bit CCD camera/controller
Macintosh Computer
Frame grabber card
NIH Image 1.57

Castin' Craft
Nikon
Nikon
custom built, aluminum
Reynard Corporation
custom
Scientific Measurement Sys.
custom
Scientific Measurement Sys. 82-479
Scientific Measurement Sys. 00-6845
Scientific Measurement Sys. 985413922
Scientific Measurement Sys. 12-509
Oriel Instruments
Scientific Measurement Sys. custom
43930

Dage-MTI
Apple Computer
Data Translation
ftp://zippy.nimh.nih.gov/pub/nih-image
CCD-72
IIci
06973
5.3 Schematics

![Diagram of imaging spectrograph system](image)

**Figure 5-3** Schematic diagram of entire multi-fiber imaging spectrograph system. The following figures give detailed views of the bulkhead connector, probe, entrance, and exit assemblies.

---

![Diagram of bulkhead connector](image)

**Figure 5-4** Plans for bulkhead connector. All dimensions are given in inches. a) Side view. b) Front view. The holes are drilled all the way through and filled with SMA bulkhead adapters (Amphenol 905-120-5003). c) Bottom view. The 1/4-20 tap allows the bulkhead connector to be attached to a standard 1 inch rule 1/4-20 tapped optical table.
Figure 5-5 Plans for constructing oblique-incidence optical fiber probe. a) Side view of the two parts of the probe. The tip is sharpened to decrease the size of the probe's footprint on the sample. b) Inside surface of part 1. There is a groove for the source fiber. The circle is milled to the same depth of 0.040. It allows the source fiber to make a 45 degree bend. c) Inside surface of part 2. There are straight grooves for all of the detection fibers. To assemble the probe, the fibers are snapped into their respective grooves, and the two parts are glued together with Duro Master Mend Epoxy. After curing, the tips can be polished.

Figure 5-6 The detection fibers from the probe are coupled to another set of fibers through the bulkhead with SMA connectors. The second set of fibers are brought together into a linear array which is embedded in casting resin. The polished tips of the linear array comprise the object to be imaged by the spectrograph.
Figure 5-7  a) Complete entrance assembly. The Nikon zoom lens is attached to a C mount adapter, which is attached to our custom entrance tube which in turn is attached to the outside of the filter mount of the spectrograph. Behind the filter is the entrance slit upon which the lens system images the object. b) End view of the entrance tube. The holes are for connection to the adapter plate. c) Adapter plate. It is screwed to the entrance tube as well as the filter holder.

Figure 5-8  Diagram of 1 inch continuous neutral density filter from Reynard Corporation. The filter ranges from 0 O.D. at the bottom to 4 O.D. at the top.
a) Exit assembly

![Diagram of exit assembly with dimensions and labels]

- 2 in. Circular cylindrical lens
- \( f = 75 \text{ mm} \)
- Oriel #43930
- Exit tube 1
- Exit tube 2
- 1-32 C Mount

b) Detail view, Exit tube 2

![Diagram of detail view with dimensions and labels]

**Figure 5-9**  
a) Complete exit assembly. The cylindrical lens corrects the astigmatism of the spectrograph. It is glued in place with clear silicone sealant (Dow Corning). Exit tube 1 slides into the exit port of the spectrograph and is held in place by a set screw in the slot. Exit tube 2 slides into exit tube 1 and is also held in place by set screws. At the end of exit tube 2 is a focusing lens that actually drops within the C mount of the camera.  
b) Detailed view of exit tube 2.
5.4 Discussion

There were two fundamental problems that we came up against in implementing this system. The first one involves what appears to be an inherent flaw in the design of the Monospec 18 spectrograph. It was discovered that some form of second order effect from the gratings was causing an unexpected artifact in the spectra we collected. What we found was a very regular, periodic amplitude modulation in the spectral dimension. This modulation was present even when we measured the spectrum of an incandescent Tungsten light bulb, which is essentially a black body. The reason we suspect that the problem is caused by the gratings is that we actually replaced our 300 groove/mm grating with a 600 groove/mm grating and found that the period of oscillation doubled as the linear dispersion decreased from 18 nm/mm to 9 nm/mm. See Figure 5-10. At this point in time, neither we nor Scientific Measurement Systems has found the solution to this problem. Despite this irregularity in the response of our detection system, there will be
no effect on the final reconstructed absorption and reduced scattering spectra because, as we mentioned in previous chapters, the spectral responsivity of the detector cancels out and hence is not critical.

The second problem we came up against was the issue of the effective dynamic range of our detection system. Our first solution to this problem utilized a gradient neutral density filter to normalize the throughput from each of the detection fibers. The major drawback to this method was the need to consequently relay the image of the object (the linear array of detection fiber tips) through the filter onto the entrance slit of the spectrograph. Two possible alternatives to this approach are 1) to use a gradient slit which is narrower at one end and wider at the other and 2) to change the size of the detection fibers as a function of distance from the source fiber, with small fibers near the source. With either of these choices, the need for the zoom lens and entrance assembly is removed, and the detection fiber tips could be placed right at the entrance slit. Despite this advantage, these approaches are not necessarily superior. First of all, the spectral resolution is a function of slit width, so a gradient slit would yield a different spectral resolution for each detection fiber. Secondly, using fibers of various diameters (assuming all the desired sizes were even available) would prove exceptionally inconvenient to implement because each connector would also be of a different size. Finally, there is a certain degree of flexibility that the zoom lens provides for the number, size, and separation of the detection fibers that would otherwise be lost.

All three of the aforementioned methods can be used to deal with the differences in the amount of light collected by each detection fiber due to proximity to the source fiber, but there is another issue to consider as well: the wavelength to wavelength variation in
throughput for any individual fiber. None of these methods would work well if the samples under test contained, for example, a very strong absorber with a relatively narrow absorption bandwidth. Between the wavelengths where absorption occurs and the wavelengths where there is no absorption, the amount of light collected at any given position could vary over many orders of magnitude, even for typical values of $\mu_a$. There is nothing that can be done to normalize throughput as a function of wavelength at the spectrograph entrance, before the light has been spectrally resolved. It is for this particular reason that we currently have no measurements on our tissue phantoms.

The simplest solution is to replace the gradient ND filter and 8 bit camera with a 16 or 18 bit camera. These instruments are very expensive, but fortunately there are still some alternatives for use with an 8 bit camera. One alternative is to scan small wavelength regions at a time, adjust the gain and black level for each region as necessary, and to re-scale and piece together the spectra on the computer. This method can even be automated. A second alternative is to modify the exit assembly. In the exit tube, rather than focusing the spectra directly onto the CCD array, their images could be relayed through a two-dimensional mask which has a higher optical density everywhere that the light throughput is high, i.e. corresponding to the fiber positions closest to the source and the wavelengths where there is the lowest absorption. The mask would have to be placed in the image plane previously occupied by the CCD array, and the modified, real image relayed again to the camera. A separate mask would have to be made for each "class" of samples measured, depending on where the absorption peaks occur. This method is rather simple in concept, but would be difficult to actually implement, primarily because there is no obvious way to make the masks. A possibility is to place unexposed film in
the position to be occupied by the mask and expose it with light collected from a typical sample. The exposed film negative could then be used as the mask.

In conclusion, we have designed and built a multiple fiber imaging spectrograph system to expedite the multi-wavelength determination of tissue optical properties. The total cost of our system was under $4000 (it is difficult to give an exact figure due to the many custom parts and the relatively old Macintosh) and hence well within the realm of instrumentation that would be practical to add to doctor’s offices and operating rooms as well as laboratories working in the field of biomedical optics. At this point in time, we have solved a large number of the problems that we have encountered in assembling this system, and have also proposed solutions to solve the remaining problems in the future.
Chapter 6  Conclusions

In conclusion, we have developed an inexpensive method for quickly and non-invasively measuring the absorption and reduced scattering coefficients of turbid media such as biological tissues with an oblique-incidence optical fiber probe. Previously devised techniques for measuring tissue optical properties, both directly and indirectly, require independent measurements for each property deduced. For example, the widely used integrating sphere technique with optically thick samples requires a measurement of both diffuse reflectance and transmission to deduce two optical properties: absorption and scattering. For the determination of $g$, yet another measurement must be performed. These three measurements can be performed together fairly conveniently, which is why this method has remained popular. The major drawback of this technique is that it is indirect, and there is not a good analytic model upon which to base the optical property reconstruction. For direct measurements, all of the calculations are based on Beer's law, and the only way deduce any individual optical property is to set up an experiment that isolates that one property, either absorption or scattering.

An alternative to using an independent measurement to deduce a second optical property is to calibrate the first measurement in absolute units. This method is utilized primarily in measurements of spatially-resolved diffuse reflectance with an optical fiber probe. The absolute units remove the need for an arbitrary scaling factor in the curve fit to the expression for diffuse reflectance, $R(r)$, so what remains is a two-parameter fit for two optical properties, ostensibly absorption and reduced scattering.
The spatially-resolved oblique-incidence technique has a distinct advantage over these other methods: the second measurement that is required to deduce the second optical property really is not a second measurement at all — it is "built-in" to the measurement of the diffuse reflectance profile in arbitrary units. The fundamental principle is that the shift in the center of diffuse reflectance from the light entry point gives us a measure of the diffusion length, while a curve fit to the dipole-source diffusion theory model gives us a measure of the effective attenuation coefficient, $\mu_{\text{eff}}$, which primarily determines the slope of the fall-off of the spatially-resolved diffuse reflectance profile. Thus, with only a relative measurement of the diffuse reflectance profile, we can measure two parameters and hence deduce two optical properties, $\mu_a$ and $\mu_s'$. 

In addition to the added efficiency created by the removal of one measurement, the fact that an absolute calibration is not required also makes this technique more reliable for use in a clinical setting. Rather than having a system that must be professionally calibrated to absolute units periodically to provide accurate measurements, the oblique-incidence optical fiber system can be calibrated (to remove fiber to fiber variations in throughput, but not in absolute units) very easily by the operator before each measurement. This can be accomplished simply by providing solid resin phantoms with known optical properties to be used as calibration standards.

The fiber-optic implementation of oblique-incidence reflectometry also provides all the same advantages that were discussed for normal incidence spatially-resolved reflectometry. With a white light source and a detector capable of spectral resolution such as the OMA we used in Chapter 4, we could measure the optical properties as a function of wavelength. This process could be further expedited by a multi-fiber OMA
system, such as that described in Chapter 5. With an imaging spectrograph and a CCD camera, a spectrally resolved image of each detection fiber could be simultaneously recorded and automatically processed to yield the optical properties as a function of wavelength. Yet another advantage is that the range of optical properties that can be measured is essentially limitless as long as the probe size and configuration is consistent with the criteria for the diffusion approximation – that is, the nearest sample point is a minimum of one transport mean free path from the position of the virtual sources.

At this point in the development of our oblique-incidence optical fiber reflectometry system, we have proven with Monte Carlo simulations and single wavelength measurements on water-based tissue phantoms that our concept is sound. We have also shown that the extension to multi-wavelength measurements follows naturally. With these multi-wavelength measurements, we have demonstrated that not only can the method be used to measure the optical properties of liquids, but it works equally well for solids as well as actual biological tissue. In fact, those measurements were shown to be sensitive enough to detect 1) the gradient of absorber and scatterer in our resin phantom caused by settling during the curing process, 2) the directional variation of the optical properties of chicken muscle, a highly organized tissue, and 3) the presence of oxy- and deoxyhemoglobin in chicken muscle. Future work will involve optimization of the multi-fiber OMA followed by its application to a number of different areas of research. In particular, the 8 bit CCD and gradient neutral density filter should be replaced with an 18 bit CCD, and the customized spectrograph should be replaced with a true imaging spectrograph. After completion of this tool, the research can continue in a number of directions including, but not limited to: 1) in vivo comparisons of the optical properties of
normal and cancerous tissues, 2) the development of endoscopic oblique-incidence probes, 3) the \textit{in vivo} measurement of physiological parameters such as oxygenation status and glucose concentration, and 4) the development of a system for studying the effect of polarization on photon migration. In short, now that the fundamental issues around oblique-incidence reflectometry have been elucidated, and the technique has been proven to be both accurate and efficient, this method has considerable potential for medical diagnostic applications.
Appendix A  Vector Calculus Reference

Table A-1. Table of basis vectors and metric coefficients for the Cartesian, cylindrical, and spherical coordinate systems. The vectors and coefficients are substituted in the general forms of the vector operations grad, div, curl, and Laplacian.

<table>
<thead>
<tr>
<th>Coordinate System</th>
<th>Cartesian</th>
<th>Cylindrical</th>
<th>Spherical</th>
</tr>
</thead>
<tbody>
<tr>
<td>Base Vectors</td>
<td>$\hat{u}_1$</td>
<td>$\hat{x}$</td>
<td>$\hat{r}$</td>
</tr>
<tr>
<td></td>
<td>$\hat{u}_2$</td>
<td>$\hat{y}$</td>
<td>$\hat{\phi}$</td>
</tr>
<tr>
<td></td>
<td>$\hat{u}_3$</td>
<td>$\hat{z}$</td>
<td>$\hat{z}$</td>
</tr>
<tr>
<td>Metric</td>
<td>$h_1$</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Coefficients</td>
<td>$h_2$</td>
<td>1</td>
<td>$r$</td>
</tr>
<tr>
<td></td>
<td>$h_3$</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Differential Volume</td>
<td>$d\nu$</td>
<td>$dxdydz$</td>
<td>$rdrd\phi dz$</td>
</tr>
</tbody>
</table>

Gradient

Eq. A-1

$$\nabla V = \hat{u}_1 \frac{\partial V}{\partial u_1} + \hat{u}_2 \frac{\partial V}{\partial u_2} + \hat{u}_3 \frac{\partial V}{\partial u_3}$$

Divergence

Eq. A-2

$$\nabla \cdot \vec{A} = \frac{1}{h_1 h_2 h_3} \left[ \frac{\partial}{\partial u_1} (h_2 h_3 A_1) + \frac{\partial}{\partial u_2} (h_1 h_3 A_2) + \frac{\partial}{\partial u_3} (h_1 h_2 A_3) \right]$$

Curl

Eq. A-3

$$\nabla \times \vec{A} = \frac{1}{h_1 h_2 h_3} \begin{vmatrix} \hat{u}_1 h_1 & \hat{u}_2 h_2 & \hat{u}_3 h_3 \\ \frac{\partial}{\partial u_1} & \frac{\partial}{\partial u_2} & \frac{\partial}{\partial u_3} \\ h_1 A_1 & h_2 A_2 & h_3 A_3 \end{vmatrix}$$
Figure A-1. Diagrams of differential volumes for three coordinate systems. a) Cartesian, b) Cylindrical, and c) Spherical

Scalar Laplacian

Eq. A-4 \[ \nabla^2 V = \nabla \cdot \nabla V = \frac{1}{h_1 h_2 h_3} \left[ \frac{\partial^2}{\partial u_1^2} \left( h_2 h_3 \frac{\partial V}{h_2 \partial u_1} \right) + \frac{\partial^2}{\partial u_2^2} \left( h_1 h_3 \frac{\partial V}{h_1 \partial u_2} \right) + \frac{\partial^2}{\partial u_3^2} \left( h_1 h_2 \frac{\partial V}{h_3 \partial u_3} \right) \right] \]

Solid Angles

\[ \Omega = \frac{\text{area on surface of sphere}}{r^2} \Rightarrow [\text{steradians (sr)}] \]

Eq. A-5 \[ d\Omega \text{ or } d\omega = \frac{(r \partial \theta)(r \sin \theta d\phi)}{r^2} = \sin \theta d\theta d\phi \]
Appendix B  Oblique-incidence Reflectometry Software

B.1 Sample Output

![Image of MacDRI. fat.rei.out]

Oblique Incidence Reflectometry on the Macintosh!
Generates curve fits for data from oblique-incidence optical fiber probes
To deduce the $\mu_a$ and $\mu_s$

by: Lihong Wang and Shao-Pow Lin
Last Modified: 6/96

1: Generate R(r) data from Farrell diffusion equation
2: Fit wavelength dependent data
   • Single wavelength data gives expected & fitted R(r) curves
   • Multiple wavelength data gives $\mu_a$ and $\mu_s'(\lambda)$ curves

--> See calibration.template and data.template files for help

Your choice: 2
Input datafile: 6/0.2 airy
Output datafile: output

Number of wavelengths entered = 80

Wavelength (enter 0 if doing _FIT_ for multiple wavelengths): 532.4
$\mu_a$ [1/cm], at 632.40 nm: 0.194
$\mu_s'$ [1/cm], at 632.40 nm: 0

1. Load calibration factors from a file
2. Calculate calibration factors from data
Your choice: 1
Input calibration datafile: CalibrationFile
Macintosh Window

Done!

1: Start over
2: Quit

Your choice: 1

Figure B-1 Sample dialog screen from Oblique-Incidence Reflectometry fitting program. We have chosen to perform a fit on data from input file 6/0.6 airy at 632.4 nm. Figure B-2 is the graphics screen.
Figure B-2 Graphical output for a single wavelength fit. Plotted are the data points (squares), the center of diffuse reflectance, the expected, and the fitted curves.
Figure B-3 More of the sample dialog box. We have chosen to repeat the fit, but this time to fit for all the available wavelengths at once. The program outputs the results in text for each wavelength. Only three are shown here. The graphical output screen is in Figure B-4.
Figure B-4 Graphical output for multi-wavelength fits. The first plot shows the absorption spectrum, and the second shows the reduced scattering spectrum.

B.2 Source Code

The code included here is our original code. We have also made use of the following routines from Numerical Recipes in C [46]: nrutil.h, nurutil.c, mrqmin.c, mrqcof.c, gaussj.c, and covsrt.c. The plotting routines make calls to the public domain PLPlot
library, which we have compiled for the Macintosh [57]. The software is available by anonymous ftp from ftp://dino.ph.utexas.edu/.

B.2.1 Headers.h

#include <stdio.h>
#include <stdlib.h>
#include <math.h>
#include <string.h>
#include "ntrutil.h"

#define PI 3.1415926
#define REFL_MIN 1E-5
#define SQ(x) ((x)*(x))
#define MIN(x, y) (((x) < (y)) ? (x):(y))

/ * Here I'm defining macros to solve for:
  * rho: the distance from either the positive or image source to the point of interest
  * J1: the reflectance due to either the positive or image source
  * R: the total reflectance
  * dJ1: pretty self explanatory
  *
  * To get the EXPECTED reflectance at x, for example, use the call:
  * R(x, Optics_xs_exp, Optics_ys, Optics_zsp_exp, Optics_zsi_exp, Optics.mueff_exp)
  *
  * To get the FITTED reflectance at x, use the call:
  * R(x, Optics_xs_fit, Optics_ys, Optics.zsp_fit, Optics.zsi_fit, Optics.mueff_fit)
  */
#define rho(zs, x, xs, ys) sqrt(SQ(zs) + SQ((x) - (xs)) + SQ(ys))
#define J1(zs, mue, Rho) (zs*(1 + mue*Rho)*exp(-mue*Rho)/(4*PI*pow(Rho, 3)))
#define R((x,ys, zsp, zsi, mue, D) J1(zsp, mue, rho((zsp),(x),(xs),(ys)))/
     + J1((zsi),(mue),rho((zsi),(x),(xs),(ys)))
#define dJ1dmue(zs, mue, Rho) (-zs*mue*exp(-mue*Rho)/(4*PI*Rho))

/ * Truly global variables (accessible by _all_ source code are defined this way:
  * In main() I #define DEFINEGLOBAL, in this header file, which is included in
  * ALL source codes, the following structure is declared as a global for the piece
  * of code containing main(), and as extern for all other source code.
  */
ifdef DEFINEGLOBALS
#define extern
#endif

/ *
  * n: refractive index
  * mua: absorption coefficient [1/cm]
  * musp: reduced scattering coefficient [1/cm]
  * mueff: effective attenuation coefficient, sqrt(mua/D)
  * D: diffusion constant, 1/(3*(0.35mua + musprm))
  * x: or delta_x, horizontal shift of the lumped point source [cm]
* zsp: depth of lumped source [cm]
* zsi: height of imaged lumped source [cm]
* zb: distance between virtual and real boundaries [cm]
* ys: offset of the source fiber from the collection plane.
*/
extern struct {
  double A;
  double lambda;  /* current wavelength*/
  double n_ambient, n_tissue;
  double nrel;    /* indices. nrel = nt/na. */
  double angle_tissue;  /* refractive angle [rad]. */
  double mua_exp, musprm_exp;  /* expected values. */
  double amplitude_exp;
  double mueff_exp;
  double xs_exp, zsp_exp, zsi_exp, zb_exp;
  double mua_fit, musprm_fit;  /* fitted values. */
  double amplitude_fit;
  double mueff_fit;
  double xs_fit, ys, zsp_fit, zsi_fit, zb_fit;
  double stderr_x, error_mueff;
} Optics;

/* The following functions are found in GetExpectedData.c */
void GenerateData();
void GetExpOptics(short fit);

/* The following functions are found in Fitting.c */
void FitData(FILE *Filepin, FILE *Filepout);
void GetKFactors(short *Index_xplus_p);
void LocateCenterLine(short Index_xplus, short *I_min_p, float *Refl, float *Xctr, double Lambda);
short Intersect(float x1, float x2, float y1, float y2, float *Xpos_p);
void ComputeFittedOptics(float *Xctr, short I_min, short Index_xplus);
void FitForOtherOptics(float *Refl, short Index_xmin, short Index_xplus);
void funcR(float X, float a[], float *Rp, float dRda[], int na);

/* The following functions are found in files.c */
FILE *
GetAndOpenFile(char *filename, char mode);
void WriteExpData (FILE *Filepout);
short CountLambda (FILE *Filep);
void GetConstFromFile (FILE *Filepin, short *Index_xplus_p, short firstCall);
short
SkipComments(FILE *Filepin, char *Buf);
short
GetDataFromFile(FILE *Filepin, double *Lambda_p, float *Refl, short StillCalib);

/* These functions are for screen graphics. They are located in plplot.c */
void
PointPlotter(float *Refl, float *Xctr, int Index_xmin, int Index_xplus);
void
CurvePlotter(int Npts, float *X, float *Y1, float *Y2);

/* The following function is in nrqmin.c - it needs to be prototyped for
 * some reason, but I have not fiddled with the original Numerical Recipes
 * code at all, I just copied the function declaration to here.
 */
void nrqmin(float x[], float y[], float sig[], int ndata, float a[], int ia[],
            int ma, float **covar, float **alpha, float *chisq,
            void (*funcs)(float, float [], float *, float [], int), float *alamda);
B.2.2 Main.c

/*
 * Macintosh Version (FAT) for Oblique Incidence Reflectometry
 *
 * This version should allow you to:
 * 1. Generate dummy data with the Farrell diffusion expression
 * 2. Fit data for either a single wavelength or
 * 3. Multiple wavelength measurement.
 * Both 2 and 3 allow a variable number of calibration phantoms.
 * Template files are located in the MacIR folder as well.
 */

#define DEFINEGLOBAL
#include "headers.h"
#undef DEFINEGLOBAL

void
main(int argc, char *argv[])
{
    short menu_id;
    short again;
    char mode;
    char infname[128], outname[128];
    FILE *filein, *fileout;

    puts("Oblique Incidence Reflectometry on the Macintosh!\n"
        "Generates curve fits for data from oblique-incidence optical fiber probes\n"
        "To deduce the \mu_a and \mu_s\"\n"
        "by: Lihong Wang and Shao-Pow Lin\"
        "Last Modified: 6/96\"");

    /* Loop program as many times as user wants */
    do
    {
        again = 0;
        puts("1: Generate R(r) data from Farrell diffusion equation \n"
            "2: Fit wavelength dependent data\n"
            "*Single wavelength data gives expected & fitted R(r) curves\n"
            "*Multiple wavelength data gives mua and mus'(lambda) curves\n"
            "*See calibration.template and data.template files for help\"");

        /* Loop input line until valid entry */
        do
        {
            printf("Your choice: ");
            scanf("%hi", &menu_id);
        } while ((menu_id!=1)&&(menu_id!=2));

    printf("\n");
    if (menu_id==2)
    {
        /* Get Name of Input file and open it in read mode (only for choice 2) */
        printf("Input datafile: ");
    

mode = 'r';
if ( !(filepin = GetAndOpenFile(infname, mode)) )
exit(1);
}

/* Get Name of Output file and open it in write mode (for BOTH choices)*/
printf("Output datafile: ");
mode = 'w';
if ( !(fileout = GetAndOpenFile(outfname, mode)) )
exit(1);

switch (menu_id)
{
case 1:
    GenerateData(fileout); /* in GetExpData.c */
    break;

case 2:
    FitData(filepin, fileout); /* in Fitting.c */
    break;
}

/* Only close files that you have actually opened */
fclose(fileout);
if (menu_id==2)
fclose(filepin);

/* Loop input line until valid entry */
do
{
    puts("1: Start over"
         "2: Quit\n");
    printf("Your choice: ");
    scanf("%hi", &again);
} while ((again!=1) &&(again!=2));

} while (again==1);

ExitToShell();
}
B.2.3 Files.c

#include "headers.h"

/*
 * This routine is passed a variable for a filename (usually 128
 * long char string) and a char for "mode" (r, w, a). It returns
 * a pointer to the new file, which is already opened.
 */

FILE *
GetAndOpenFile (char *filename, char mode)
{
    FILE    *filep = NULL;

    do {
        scanf("%s", filename);
        if (strlen(filename) == 1 && filename[0] == '.')
            break;
        if (mode == 'w')
            filep = fopen(filename, "w");
        else if (mode == 'r')
            filep = fopen(filename, "r");
    } while (filep == NULL);

    return (filep);
}

/* This function reports expected reference values to output file. It
 * is called whenever an output file is written.
 */
void
WriteExpData (FILE *Filepout)
{
    fprintf(Filepout, "#Refractive index of top ambient = %5.2lf\n", Optics.n_ambient);
    fprintf(Filepout, "#Refractive index of tissue = %5.2lf\n", Optics.n_tissue);

    if (Optics.lambda>0)
    {
        fprintf(Filepout, "#Expected absorption coefficient at %5.2lf nm: mua = %5.2lf/cm\n", 
                Optics.lambda, Optics.mua_exp);
        fprintf(Filepout, "#Expected reduced scattering coefficient at %5.2lf nm: mus = 
                %5.2lf/cm\n", Optics.lambda, Optics.musprm_exp);
    }
    else
        fprintf(Filepout, "#Multiple wavelength fit - no single expected values for mua, mus\n");

    fprintf(Filepout, "#Angle of refraction = %5.2lf deg\n", Optics.angle_tissue * 180 / PI);
    fprintf(Filepout, "#Shift between source and detectors, ys = %5.2lf cm\n", Optics.ys);
}

/* This routine counts up the number of wavelengths that are included
 * in the input file, then rewinds the file */
short
CountLambda (FILE * Filep)
{

}
char      buf[128];
short     numLambda = 0;

/* find out number of wavelengths. */
while ((fgets(buf, 127, Filep) != NULL)
{
    if (((buf[0] != '#') && (buf[0] != '\n'))
        numLambda++;
}

rewind(Filep);
return (numLambda - 5);        /* 5 lines at beginning for info (not comments)
                          * other than reflectance data*/

/* This routine goes into the input data file and extracts Refractive indexes,
* the angle of Refraction, Optics.ys, x[] (fiber positions), and the number of
* collection fibers. It is called by k factor routine as well as fitting routine
* itself, and makes sure that constants used to calculate k factors are consistent
* with those used to do fit.
*/

void
GetConstFromFile (FILE *Filepin, short *Index_xplus_p, short firstCall)
{
extern short     numFibers;
extern float     *x;

    char      buf[128];
    float     *xTemp;          /* temporary holding pen for x positions
                        (b/4 I know numFibers. */

struct {
    double    n_ambient;
    double    n_tissue;
    double    angle_tissue;
    double    ys;
    short     numFibers;
} Temp;

    /* temporary holding pen for all other values */

char      *begin, *end;        /* temporary pointers to char to point to
                        beginning and end of each fiber position
                        (used to convert chars into double */

int       i;
short     notEof = 0;          /* flag for hitting end of file */

/* get Optics.n_ambient, n_tissue, angle_tissue, ys */

/* first skip past all the comment lines, and watch for end of file
* SkipComments returns 1 on success, 0 on failure (hits EOF) */
if (!SkipComments(Filepin, buf))
{
    printf("Hit end of file while searching for n_ambient!n");
    exit(1);
}
/* then read in value */
sscanf(buf, "%f", &Temp.n_ambient);
/* repeat for other parameters */
if (!SkipComments(Filepin, buf))
{
    printf("Hit end of file while searching for n_tissue\n");
    exit(1);
}
sscanf(buf, "%lf", &Temp.n_tissue);

if (!SkipComments(Filepin, buf))
{
    printf("Hit end of file while searching for angle_tissue\n");
    exit(1);
}
sscanf(buf, "%lf", &Temp.angle_tissue);
Temp.angle_tissue *= PI / 180;

if (!SkipComments(Filepin, buf))
{
    printf("Hit end of file while searching for ys\n");
    exit(1);
}
sscanf(buf, "%lf", &Temp.ys);

/* count the number of collection fibers and get their positions */
if (!SkipComments(Filepin, buf))
{
    printf("Hit end of file while searching for fiber positions\n");
    exit(1);
}
Temp.numFibers = 0;
xTemp = vector(1, 128); /* make it large so it won't overflow
this big vector will be copied into x then disposed of with this function */
begin = buf;
while (*begin != '\n')
{
    Temp.numFibers++;
    xTemp[Temp.numFibers] = strtod(begin, &end);
    /* check for first +x position, if so, assign to index_xplus */
    if (((xTemp[Temp.numFibers]>0) && (*index_xplus_p==0))
        *index_xplus_p = Temp.numFibers;
    begin = end++;
    /* point begin at tab/b/tw positions */
}

/* make sure values are consistent for calibration and data files */
if (!firstCall)
{
    if (((Temp.n_ambient != Optics.n_ambient) ||
         (Temp.n_tissue != Optics.n_tissue) ||
         (Temp.angle_tissue != Optics.angle_tissue) ||
         (Temp.ys != Optics.ys) ||
         (Temp.numFibers != numFibers))
    {
        printf("Inconsistency in constants!\n");
        exit(1);
    }
}
for (i=1; i<=Temp.numFibers; i++)
    if (xTemp[i]!=x[i])
    {
        printf("Inconsistency in fiber positions!");
        exit(1);
    }
else
{
    /* assign values to permanent variables if it's the first set read
     * in, if subsequent passes pass the consistency test, there's no
     * need to reassign the variables, b/c they are already right! */
    Optics.n_ambient = Temp.n_ambient;
    Optics.n_tissue = Temp.n_tissue;
    Optics.nrel = Optics.n_tissue/Optics.n_ambient;
    Optics.angle_tissue = Temp.angle_tissue;
    Optics.ys = Temp.ys;
    numFibers = Temp.numFibers;
    x = vector (1,numFibers);
    for (i=1; i<=numFibers; i++)
        x[i] = xTemp[i];

    /* the next call to fgets will be from the first line following the
     * line containing fiber positions */
}

/* This function does one thing: reads in one line of file and checks
 * to see if it's a comment or blank. Returns the first non-comment
 * line and 1 on success, or 0 for failure (hit EOF) */

short
SkipComments(FILE *Filepin, char *Buf)
{
    while (fgets(Buf, 127, Filepin) != NULL)
    {
        if ((Buf[0] != '#')&&(Buf[0] != 'u'))
            return(1);              /* didn't hit EOF, so we're OK */
    }
    return(0);
}

/* This function moves the file pointer from its last position
 * (which should be pointing to line after fiber positions) to the correct
 * wavelength for a single wavelength fit.
 * "StillCalib" is 1 if called by calibration routine (GetKFactors),
 * otherwise it is 0.
 *
 short
GetDataFromFile(FILE *Filepin, double *Lambda_p, float *Refl, short StillCalib)
{
    extern float *k;

    char buf[128];            /* lambda is originally set to an impossible value */
    short notEof = 0;          /* flag for hitting end of file */
    char *begin, *end;        /* pointers to char, which hold line positions */
short i = 0;

/* Make sure you don't hit the EOF */
while (fgets(buf, 127, Filep) != NULL) {
    if ((buf[0] != '#') && (buf[0] != '
')) /* skip comment lines */
    {
        begin = buf;
        while (*begin != '
')
        {
            if (i == 0)
                *lambda_p = strtod (begin, &end);
            /* the first number is wavelength */
            else if (StillCalib)
                /* the rest are reflectance[i] */
                refl[i] = strtod (begin, &end);
            /* don't mult by k[i] if a calibrating */
            else
                refl[i] = strtod (begin, &end) * k[i];
                i++;
        begin = end++; /* point begin at tab b/tw positions */
        } not_eof = 1; /* flag successful retrieval (no EOF) */
    break;
    }
}
return(not_eof);
B.2.4 GetExpData.c

#include "headers.h"

/* This function generates data from the Farrell equation and inputted
* optical parameters. All parameters are put in the Optics._-exp
* globals out of convenience (and overlapping "meaning").
*/

void
GenerateData(FILE *Filepout)
{
    double xstart, xstop, X, dx; /* start value, stop value, current position, spacing */
    int xnumber; /* number of points */

    /* Get relevant data on tissue, probe, etc. */
    GetExpOptics(0); /* the zero flags that we are generating data, and
    * hence need to ask for ALL PARAMETERS */

    /* Get range information to calculate expected reflectance */
    printf("Input range of X from low value to high value.\n");
    printf("X position to start from [cm]: ");
    scanf("%lf", &xstart);
    printf("X position to stop at [cm]: ");
    scanf("%lf", &xstop);
    printf("Number of points: ");
    scanf("%i", &xnumber);

    /* Output assorted info to file for future reference */
    WriteExpData(Filepout); /* located in files.c */
    /* Write more data that is specific for this function */
    fprintf(Filepout, "#Start position: %5.2lf\n", xstart);
    fprintf(Filepout, "#Stop position: %5.2lf\n", xstop);
    fprintf(Filepout, "#Number of points: %i\n\n", xnumber);
    fprintf(Filepout, "#x[cm]\n\n[x]\n\n");

    /* Calculate spacing between points to generate data */
    dx = (xstop-xstart) / (xnumber-1);

    /* Loop through from xstart to approximately xstop */
    X = xstart;
    while (X <= xstop)
    {
        fprintf(Filepout, "%lf\n", X, R(X,Optics.xs_exp,Optics.ys,Optics.zsp_exp,
                                        Optics.zsi_exp,Optics.mueff_exp));
        X+=dx;
    }

    printf("\nDone!\n");
}

/* This function asks for input to all constants in Optics structure
* and calculates dependent expected values as well. It is called
* by all three options - Generate Data, Fit Single Wavelength, and
* Fit Multiple Wavelength.
*/
void GetExpOptics(short fit) /* if fit=1, that means this is called by a fitting routine
* and hence we can skip some inputs which
* are available from input file */
{
  double ri; /* ri is internal reflection parameter */

  /* Get relevant constant parameters of tissue, probe
  See headers.h for definitions of Globals */
  if (!fit)
  {
    printf("Refractive index of top ambient: ");
    scanf("%lf", &Optics.n_ambient);
    printf("Refractive index of tissue : ");
    scanf("%lf", &Optics.n_tissue);
    Optics.nrel = Optics.n_tissue / Optics.n_ambient;

    /* This angle could actually be variable for multiple wavelength
    measurements b/c of variations in index of refraction of both
    fused silica and tissue! For NOW, ignore this effect. */
    printf("Angle of refraction [deg, - if light points toward -x]: ");
    scanf("%lf", &Optics.angle_tissue);
    Optics.angle_tissue *= PI / 180;

    printf("Shift of source from the detection plane (ys) [cm]: ");
    scanf("%lf", &Optics.ys);
  }

  do
  {
    printf("Wavelength (enter 0 if doing _FIT_ for multiple wavelengths): ");
    scanf("%lf", &Optics.lambda);
  } while (Optics.lambda<0); /* repeat until you get a valid response */

  /* Only get expected values if single wavelength measurement */
  if (Optics.lambda>0)
  {
    printf("mua [1/cm], at %5.2lf nm: ", Optics.lambda);
    scanf("%lf", &Optics.mua_exp);
    printf("mus* [1/cm], at %5.2lf nm: ",Optics.lambda);
    scanf("%lf", &Optics.musprm_exp);

    /* Calculate dependent terms */
    Optics.mueff_exp = sqrt((3 * Optics.mua_exp * (0.35 * Optics.mua_exp +
                              Optics.musprm_exp)));

    /* Note that negative angles give negative shift xs --
    * Note that in the remaining definitions of zb, zs's, the
    * negative xs in combo with either sine or tangent gives
    * a positive result, so there's no need for fabs() anywhere! */
    Optics.xs_exp = sin(Optics.angle_tissue) / (0.35 * Optics.mua_exp +
                                        Optics.musprm_exp);
    Optics.zsp_exp = Optics.xs_exp / tan(Optics.angle_tissue);
  }
}
/* Set A exactly to 1 for matched boundary, otherwise use Groenius approx */
/* need to do this for ANY situation: generating data, fitting 1 or fitting many */
if (Optics.nrel == 1)
    Optics.A = 1;
else
{
    ri = -1.440 / SQ(Optics.nrel) + 0.710 / Optics.nrel + 0.668 + 0.0636 * Optics.nrel;
    Optics.A = (1 + ri) / (1 - ri);
}

/* more dependents to calculate only if single wavelength fit */
if (Optics.lambda>0)
{
    Optics.zb_exp = 2 * Optics.A * Optics.xs_exp / (3 * sin(Optics.angle_tissue));
    Optics.zs1_exp = (Optics.zsp_exp + 2 * Optics.zb_exp);
    Optics.amplitude_exp = 1;
}

B.2.5 Fitting.c

/* This set of functions does all the fitting work and calls
 * all of the other --.c parts of the program as necessary
 */

#include "headers.h"

/* I declared these three variables as GLOBALS because their
 * values don't change after their first definition, but they are
 * used repeatedly. They are "externed" in files.c/GetConstFromFile,
 * files.c/GetDataFromFile, and plplot.c/
 */
short numFibers=0;                           /* number of collection fibers */
float *k, *x;                                 /* calibration factors and positions */

void FitData(FILE *Filepin, FILE *Filepout)
{

double lambda;                                 /* current wavelength */
float *refl;                                   /* data from the file. */
float *xctr;                                   /* center line. */
short index_xplus = 0;                         /* index to the 1st +x point. */
short index_xmin;                              /* the index to the min refl. in -x (usually 1) */
short i;
  /* vectors to store multiwavelength results */
short numLambda;
  /* used to define the vectors to store multiwavelength results */

numLambda = CountLambda(Filepin);
printf("%snNumber of wavelengths entered = %sh\n", numLambda);
Lambda_store = vector(0, numLambda-1);
  /* used 0 to numLambda-1 b/c that's what CurvePlotter() requires */
Mua_store = vector(0, numLambda-1);
Musprm_store = vector(0, numLambda-1);

GetConstFromFile(Filepin,&index_xplus, 1);
/* located in files.c. the "1" flags the function that
 * this is its first call */
GetExpOptics();                                 /* the "1" flags this routine to skip some
 * queries for parameters that are provided in
 * the input file */
GetKFactors(&index_xplus);

xctr = vector(1, index_xplus-1);
refl = vector(1, numFibers);
  /* I didn't make refl a Global b/c I want
   to be more careful about it's use - it
   is variable between calls if you are doing
   a multi lambda fit. (x, k, numFibers are not) */

WriteExpData(Filepout);
/* write more data on fiber positions, k factors */
fprintf(Filepout,"%Fiber\nPosition\tK factor\n");
for (i=1; i<=numFibers; i++)
  fprintf(Filepout,"%hn\t%f\t%f\n", i, x[i], k[i]);
fprintf(Filepout,"\n#Lambda\dx\terror_dx\tmueff\terror_mueff\tmua\tmus\n\n");  

i=0;  
while (GetDataFromFile(Filepin, &lambda, refl, 0))  
{  
    if (((Optics.lambda==0)||(Optics.lambda>0)&&(lambda==Optics.lambda)))  
    {  
        LocateCenterLine (index_xplus, &index_xmin, refl, xctr, lambda);  
        /* gets xs */  
        ComputeFittedOptics (xctr, index_xmin, index_xplus);  
        /* computes zsi,zsp, zb from xs */  
        FitForOtherOptics (refl, index_xmin, index_xplus);  
        /* fits for mueff and computes mua, mus' */  
        fprintf(Filepout, "%5.3f\n%5.3f\n%5.3f\n%5.3f\n%5.3f\n", lambda, Optics.xs_fit, Optics.stderr_x,  
                Optics.mueff_fit, Optics.error_mueff, Optics.mua_fit,  
                Optics.musprm_fit);  
        if ((Optics.lambda>0)&&(lambda==Optics.lambda))  
        {  
            fprintf(Filepout,"Percent Error in mua = %5.3f\n",  
                    100*(Optics.mua_fit-Optics.mua_exp)/Optics.mua_exp);  
            fprintf(Filepout,"Percent Error in mus' = %5.3f\n",  
                    100*(Optics.musprm_fit-Optics.musprm_exp)/  
                    Optics.musprm_exp);  
        }  
        /* store results regardless of single or multiple fit */  
        Lambda_store[i] = lambda;  
        Mua_store[i] = Optics.mua_fit;  
        Musprm_store[i] = Optics.musprm_fit;  
        i++;  
    }  
    /* uses first +x fiber (presumably the peak) to calculate amplitude factor to scale plot  
    * of expected curve */  
    Optics.amplitude_exp = refl[index_xplus]/R(x[index_xplus],  
                Optics.xs_exp,Optics.ys,Optics.zsp_exp,  
                Optics.zsi_exp,Optics.mueff_exp);  
    if (Optics.lambda>0)  
    {  
        /* since we know we just processed the only */  
        PointPlotter(refl, xctr, index_xmin, index_xplus);  
        /* data that will be processed, do the plot now */  
        /* before while loop reads in any more */  
    }  
    if (Optics.lambda == 0)  
    {  
        /* if it was multiwavelength fit, now plot mua, mus' vs lambda */  
        CurvePlotter(numLambda, Lambda_store, Mua_store, Musprm_store);  
    }  
    fprintf("\nDone!\n");  
}  

/* This function goes through any number of calibration files of identical form  
* to data files and 1. checks to make sure that all relevant constants are  
* consistent, 2. picks out the line of data where mua/mus' are known (which  
* wavelength) and calculates k[fiber index] by averaging the k values found  
* for each fiber for each calibration measurement.  
*  
* This function needs simple modification to make it accept multiple calibration
* measurements from the same file, but I haven't done it yet.
*/

void GetKFactors (short *Index_xplus_p)
{
    short numFiles;  /* numFiles is number of calibration files, each
                      * with 1 relevant line of data */
    FILE *filepin, *filepout, *callFilepin;
    char inname[128], outname[128];  /* buffer for file names */
    char buf[128];  /* buffer for loaded_k factors */
    char *begin, *end;  /* pointers for reading through a line of calibration file */
    double temp_lambdas;
    double scale;  /* used to scale k factors to around 1 */
    short i, j, notToff = 0;
    char mode;  /* mode to open file (read/write) */
    short choice = 0, dummyVar;

    struct {
        double lambda;  /* current wavelength */
        double mua_exp, musprm_exp;  /* expected values */
        double mueff_exp;
        double xs_exp, zsp_exp, zsi_exp, zb_exp;
        float refl;
    } cal;

    /* skip this procedure if a) this isn't the first time and b) you want to reuse old k factors
     * I'm using k[0]=-1 as the marker for whether or not this is the first time we've tried to
     * get calibration factors */
    if (k[0]==-1)
    {
        do
        {
            printf("n1. Reuse same calibration factors ");
            printf("n2. New set of calibration factors ");
            printf("nYour choice: ");
            scanf("%d",&choice);
            if (choice == 1)
                return;
        } while (choice != 2);
    }
    do
    {
        printf("n1. Load calibration factors from a file");
        printf("n2. Calculate calibration factors from data");
        printf("nYour choice: ");
        scanf("%d",&choice);
        if (choice == 1)
        {
            k = vector(0,numFibers);
            k[0] = -1;

            /* Get Name of Input file and open it in read mode */
            printf("Input calibration datafile: ");
            mode = 'r';
            if (!((callFilepin = GetAndOpenFile(outname, mode))
                exit(1);
for (i=1 ; i<=numFibers ; i++)
{
    /* first skip past all the comment lines, and watch for end of file
    * SkipComments returns 1 on success, 0 on failure (hits EOF) */
    if (!SkipComments(calFilepin, buf))
    {
        printf("Hit end of file while searching for
               K factors!\n");
        exit(1);
    }
    /* then read in value */
    begin = buf;
    while (*begin != 'n')
    {
        k[i] = strtod (begin, &end);
        /* keep reading in values - last on line will be k */
        begin = end++;
        /* point begin at tab b/tw positions */
    }
    return;
}
} while ((choice != 2) && (choice != 1));

/* everything past this point is only executed if we are not reusing an old set of
 * k's, either from the previous fit or loaded from a file */

k = vector(0,numFibers);
k[0] = -1; /* remember that k is "local" global */
for (i=1 ; i<=numFibers ; i++)
k[i]=0;

cal.refl = vector(1,numFibers);

printf("Enter the number of calibration files: ");
scanf("%d", &numFiles);

for (i=1 ; i<=numFiles ; i++)
{
    /* get name of calibration file and open it for reading */
    printf("Enter name of calibration file number %d: ", i);
    mode = 'r';
    if (!((filepin = GetAndOpenFile(infname, mode)))
        exit(1);

    GetConstFromFiles(filepin, Index_xplus_p, 0);

    printf("Wavelength to use for calibration: ");
    scanf("%f", &cal.lambda);
    printf("mua [1/cm], at %f nm: ", cal.lambda);
    scanf("%f", &cal.mua_exp);
    printf("mum [1/cm], at %f nm: ", cal.lambda);
    scanf("%f", &cal.musprm_exp);
    cal.mueff_exp = sqrt(3 * cal.mua_exp * (0.35 * cal.mua_exp +
                      cal.musprm_exp));
    cal.xs_exp = sin(Optics.angle_tissue) / (0.35 * cal.mua_exp + cal.musprm_exp);
cal.zsp_exp = cal.xs_exp / tan(Optics.angle_tissue);
cal.zb_exp = 2 * Optics.A * cal.xs_exp / (3 * sin(Optics.angle_tissue));
cal.zsi_exp = (cal.zsp_exp + 2 * cal.zb_exp);

while (GetDataFromFile(filepin, &temp_lambda, cal.refl, 1))
{
    if (temp_lambda == cal.lambda)
    {
        for (j=1 ; j<=numFibers ; j++)
            k[j] += R(x[j],cal.xs_exp,Optics.yS,cal.zsp_exp,
                    cal.zsi_exp,cal.mueff_exp)/cal.refl[j];
        notEof = 1;
    }
}
if (!notEof)
{
    printf("Hit end of file without finding calibration wavelength!
            ");
    exit(1);
}
}
for (i=1 ; i<=numFibers ; i++)
    k[i] /= numFiles;

/* automatically scale k factors so they are around 1 */
/* use fiber #index_xplus, this is arbitrary */
scale = k[*Index_xplus_p];
for (i=1 ; i<=numFibers ; i++)
    k[i] /= scale;

/* option to save a file with just the calibration factors for future use
   * this is most useful when doing MANY runs, with multiple calibration files.
   * Only does this if this is a new set of k's. Otherwise, the program
   * never gets here. */
do
{
    printf("n1. Save calibration factors to a file");
    printf("n2. Don't save calibration factors");
    printf("nYour choice: ");
    scanf("%d", &choice);
    if (choice == 1)
    {
        /* Get Name of Output file and open it in write mode */
        printf("nOutput calibration datafile: ");
        mode = 'w';
        if (!((filepout = GetAndOpenFile(outfilename, mode)))
            exit(1);

        fprintf(filepout,"#This file contains calibration factors\n");
        fprintf(filepout,"#Fiber Number\nK factor\n");
        for (i=1 ; i<=numFibers ; i++)
            fprintf(filepout,"%dC%lf\n", i, k[i]);
        fclose(filepout);
    }
} while ((choice != 2) && (choice != 1));
/*  
* This function goes through all the fibers in negative x, draws a line  
* of constant Refl for each and finds the +x position at which this line  
* intersects the reflectance curve. The midpoint of these lines are stored  
* in Xctr[i]. Result is that Xctr[i] contains points that line up at SHIFTED  
* center of diffuse reflectance.  
* The index of the Xctr[i] is: i = 1->_max-1. The index of the X[i] is:  
* i = 1->numFibers. The index of the Refl[i] is: i = 1->numFibers.  
*/

void LocateCenterLine(short Index_xplus, short *Index_xmin_p, float *Refl, float *Xctr, double Lambda)
{
    short ineg, ipos;
    float xpos;           /* +x position where Refl [ineg]  
                           * hits reflectance curve */
    short intersected;

    for (ineg = Index_xplus - 1; ineg >= 1; ineg--)
        /* step backwards through fibers in -x */
        {
            /* what interpolated +x position corresponds to the Refl[ineg]? */
            for (ipos = Index_xplus; ipos <= numFibers-1; ipos++)
                if ((intersected = Intersect(x[ipos], x[ipos + 1],
                   log10(Refl[ipos]), log10(Refl[ipos + 1]),
                   log10(Refl[ineg]), &xpos))
                    break;

            if (intersected)
                {
                    Xctr[ineg] = 0.5 * (x[ineg] + xpos);
                    *Index_xmin_p = ineg;
                    /* i_min ends up being the index for lowest reflectance in -x THAT INTERSECTS*/
                }
            else
                {
                    /* get an error message, but fit still works ok - just doesn't use points that don't intersect */
                    printf("Trouble locating shifted center of diffuse reflectance at \%5.2lf\n", Lambda);
                    printf("There's no intersection for Fiber \%");
                    ineg);
                    break;
                }
        }

    /* This function gets x,y coordinates for 2 fibers on +x side, the reflectance 
* on the -x side that we're trying to match, and a pointer to a variable xpos 
* which will be filled with the interpolated position of the intersection, if 
* there is one. 
*/

short Intersect(float x1, float x2, float y1, float y2, float y, float *Xpos_p)
{
    if ( ((y1 <= y) && (y <= y2)) || ((y2 <= y) && (y <= y1)) )
        {
            if (y1 == y2)
                *Xpos_p = 0.5 * (x1 + x2);
            else
                *Xpos_p = x1 + (x2 - x1) * (y - y1) / (y2 - y1);
/* linear interpolation here,
   * but remember that we passed
   * in the log(Refl) */

    return (1);
}
else
    return (0);
}

/* This function is pretty obvious - given the Xctr[i] vector, it averages the
 * values that physically make sense to give Optics.xs_fit. It then computes
 * the standard error from the data point it used ONLY. With Optics.xs_fit,
 * Optics.zb_fit, Optics.zsi_fit and Optics.zsp_fit can be calculated.
 */

void
ComputeFittedOptics(float *Xctr, short Index_xmin, short Index_xplus)
{
    short  i;

    /* basically average Xctr[i], but leave off
    * any fibers where the reflectance goes back up
    * when it shouldn't (more negative x) I.E. when there's no intersection */
    Optics.xs_fit = 0;
    for (i = Index_xmin; i <= Index_xplus - 1; i++)
        Optics.xs_fit += Xctr[i];
    Optics.xs_fit /= (Index_xplus - Index_xmin);

    Optics.stderr_x = 0;
    for (i = Index_xmin; i <= Index_xplus - 1; i++)
        Optics.stderr_x += SQ(Xctr[i] - Optics.xs_fit);
    Optics.stderr_x = 3 * sqrt(Optics.stderr_x) / (Index_xplus - Index_xmin);

    Optics.zsp_fit = Optics.xs_fit / tan(Optics.angle_tissue);
    Optics.zb_fit = 2 * Optics.A * Optics.xs_fit / (3 * sin(Optics.angle_tissue));
    Optics.zsi_fit = (Optics.zsp_fit + 2 * Optics.zb_fit);
}

/*
 * mrqmin() is called three times: 1. to initialize by setting alamda to
 * -1.2. to enter a loop. 3. to obtain covariance matrix by setting
 * alamda to 0. GLOBALS such as x and numFibers are passed as parameters
 * anyway just because the Numerical Recipe calls for it that way.
 */

void
FitForOtherOptics(float *Refl, short Index_xmin, short Index_xplus)
{
#define MAXGOODFITS 10

    float  *a, *sigma;
    int    i, *ia, num_good_fits = 0;
    float  **covar, **alpha, chiq, chiq_old, a2_old;
    float  alamda = -1;

    a = vector(1, 2); /* what we are fitting for: a[2] = mueff, a[1] = amplitude */

    /* Make initial guesses as to what a[1,2] are */
/* Use last two fibers in +x to make initial guess at mueff */
Optics.amplitude_fit = 1;
/* Use second to last fiber in +x to make initial guess at amplitude */
a[1] = Refl[numFibers - 1] / R(x[numFibers-1],Optics.xs_fit,Optics.ys,Optics.zsp_fit, Optics.zsi_fit,Optics.mueff_fit);

/* What about Optics.mueff_fit at this point? Unassigned...
* It's zero, so a[1] uses R = zsp/4pi(rho^3) +zsi/4pi(rho^3)
*/

ia = ivector(1, 2);
ia[1] = ia[2] = 1;  /* fit both amp. & mueff. */
covar = matrix(1, 2, 1, 2);
alpha = matrix(1, 2, 1, 2);
sigma = vector(1, numFibers);
for (i = 1; i <= numFibers; i++)
  sigma[i] = Refl[i];
mrqmin(x, Refl, sigma, numFibers, a, ia, 2, covar, alpha, &chisq, funcR, &alamda);

while (num_good_fits <= MAXGOODFITS)
{
  chisq_old = chisq;
  a2_old = a[2];
  mrqmin(x, Refl, sigma, numFibers, a, ia, 2, covar, alpha, &chisq, funcR, &alamda);
  if(chisq > chisq_old)  /* failed, restart counting. */
    num_good_fits = 0;
  else if(fabs(chisq_old - chisq) < 0.1)
    num_good_fits++;
}
alamda = 0;
mrqmin(x, Refl, sigma, numFibers, a, ia, 2, covar, alpha, &chisq, funcR, &alamda);
Optics.amplitude_fit = a[1];
Optics.mueff_fit = a[2];
Optics.error_mueff = sqrt(covar[2][2]);

/* in calculating mua, mus', note that if Optics.angle_tissue is negative, so is
* Optics.xs, and both results come out positive.
*/
Optics.mua_fit = SQ(Optics.mueff_fit) * Optics.xs_fit / (3 *sin(Optics.angle_tissue));
Optics.musprm_fit = sin(Optics.angle_tissue) / Optics.xs_fit - 0.35 *Optics.mua_fit;

/*
* To be called by the fitting routine mrqmin(). rhop: Distance between
* (x, 0, 0) & point source (xs_fit, ys, zsp_fit). rohi: Distance between
* (x, 0, 0) & imaginary source (xs_fit, ys, zsi_fit).
* passed x[i] into here (one at a time as X) b/c it had to be passed into mrqmin,
* this is how mrqmin calls this function.
*/
void funcR(
  float  X,
float *Rp, /* R(x). */
float dRda[], /* derivative wrt amplitude & mueff. */
int na /* # of fitting param = 2. */

} /* Derivative wrt the amplitude. */
dRda[1] = R(X, Optics.xs_fit, Optics.ys, Optics.zsp_fit, Optics.zsi_fit,a[2]);
*Rp = a[1] * dRda[1];
/* Derivative wrt mueff. */
dRda[2] = a[1] * (dJ1dmue(Optics.zsp_fit, a[2], rho(Optics.zsp_fit, X, Optics.xs_fit, Optics.y)),
            dJ1dmue(Optics.zsi_fit, a[2], rho(Optics.zsi_fit, X, Optics.xs_fit, Optics.y))) ;
B.2.6 Plplotter.c

#include "headers.h"
#include <plplot.h>
#define npts 100

/* number of points plotted in generated expected and fitted curves for
   single wavelength plot */
#define SYMBOL 4

/* code for what symbol to use to designate points in plot */

/* define codes for available colors */
#define black 0
#define red 1
#define yellow 2
#define green 3
#define aquamarine 4
#define pink 5
#define wheat 6
#define grey 7
#define brown 8
#define blue 9
#define blueviolet 10
#define cyan 11
#define turquoise 12
#define magenta 13
#define salmon 14
#define white 15

/* define scale numbers from default size for ticks, characters, and symbols */
#define MAJTICK_SCALE 0.5
#define MINTICK_SCALE 0.5
#define CHAR_SCALE 1.2
#define SYMBOL_SCALE 1.6

extern short numFibers;
extern float *x;

/* This function plots data points, expected and fitted reflectance curves
   * for a single wavelength fit. It is EXTENSIVELY COMMENTED. Some of which
   * are taken directly from the plplot distribution example "tutor.c" */
void
PointPlotter(float *Refl, float *Xctr, int Index_xmin, int Index_xplus)
{
    float xmin, xmax, ymin, ymax, xdiff, ydiff; /* used to define window */
    int i; /* counters */
    /* temporary holding for values */
    /* to pass into plopt() */
    /* holding vectors for generated */
    /* expected and fitted curves, which */
    /* will be passed to CurvePlotter() */
    float dx;

    /* x, Refl vectors are from 1 to numFibers. plpoin needs to start at zero */
    TempX = vector(0, numFibers-1);
    TempY = vector(0, numFibers-1);
    TempXctr = vector(0, (Index_xplus - Index_xmin - 1));
TempYctr = vector(0, (Index_xplus - Index_xmin - 1));
for (i=1; i<numfibers; i++)
{
    TempY[i-1] = log10(Refl[i]); /* convert to log base 10 */
    TempX[i-1] = x[i];
}
for (i = Index_xmin; i <= Index_xplus - 1; i++)
{
    TempXctr[i-Index_xmin] = Xctr[i];
    TempYctr[i-Index_xmin] = log10(Refl[i]);
}

/* ============= Graph the data. ============= */

/*
* Set graph to landscape orientation. (Default actually is landscape.)
* (Portrait is usually desired for inclusion in TeX documents.)
* 0 = landscape, 1 = portrait
*/
plson(0);

/* Initialize plplot */

pldev("mac"); /* select output device */
plfontid(0); /* I = load Extended character set, 0 = standard set */
plsub(1,1); /* ensures that page is made up of only 1 subpage */
plinit(); /* initialize plplot */
plfont(1); /* Pick from Extended character set - Roman Font */
    * I = standard, 3 = italic, 4 = script */

/*
* We must call pladv() to advance to the first (and only) subpage.
* You might want to use plenv() instead of the pladv(), plvpor(),
* plwind() sequence. But this actually looks better, I think.
*/
pladv(0);

/*
* Set up the viewport. This is the window into which the data is
* plotted. The size of the window can be set with a call to
* plvpor(), which sets the size in terms of NORMALIZED subpage
* coordinates. I want to plot the lines on the upper half of the
* page and I want to leave room to the right of the figure for
* labelling the lines. We must also leave room for the title and
* labels with plvpor(). Normally a call to plvsta() can be used
* instead.
*/
plvpor(0.2, 0.9, 0.15, 0.85);

/*
* We now need to define the size of the window in user coordinates.
* To do this, we first need to determine the range of the data
* values.
*/

xmin = xmax = TempX[0];
ymin = ymax = TempY[0];
for (i = 1; i < numFibers; i++)
{
    if (TempX[i] < xmin)
        xmin = TempX[i];
    if (TempX[i] > xmax)
        xmax = TempX[i];
    if (TempY[i] < ymin)
        ymin = TempY[i];
    if (TempY[i] > ymax)
        ymax = TempY[i];
}

/*
 * Now set the size of the window. Leave a small border around the
 * data.
 */
xdiff = (xmax - xmin) / 20.;
ydiff = (ymax - ymin) / 15.;
plwind(xmin - xdiff, xmax + xdiff, ymin - ydiff, ymax + ydiff);

/*
 * Call plbox() to draw the axes (see the PLPLOT manual for
 * information about the option strings.)
 */
plcol(white);   /* Background is black, set axis color */
plwid(2);       /* Sets pen width (1 is smallest) */
plsmaj(0.0, MAJ_TICK_SCALE);  /* Sets length of major ticks in mm - (default, scale) */
plsmin(0.0, MIN_TICK_SCALE);  /* Sets length of minor ticks in mm - (default, scale) */
plchr(0.0, CHAR_SCALE);      /* Sets character size - (default, scale) - 0.0 means use auto default */
plbox("bcnst", 0.0, 0, "abcest", 0.0, 0);
/* defines plot area - see the plplot manual */

/*
 * Label the axes and title the graph. The string "#gm" plots the
 * Greek letter mu, all the Greek letters are available, see the
 * Plplot manual.
 */
plwid(1);
plcol(yellow);
pllab("X Position [cm]", "log Reflectance [AU]", "Spatial Distribution of Diffuse Reflectance");

/*
 * Plot the data. plpoin() draws a symbol at each point. plline()
 * connects all the points.
 */
plwid(2);
plcol(green);    /* set color of points */
plssym(0.0, SYMBOL_SCALE);  /* make point markers a little bigger */
plpoin(numFibers, TempX, TempY, SYMBOL);
plcol(blueviolet);
pline(Index_xplus_index_xmin, TempXctr, TempYctr);

/* Generate expected and fitted curves with npts (defined at top) points */
CurveX = vector(0, npts-1);
ExpCurveY = vector(0, npts-1);
FitCurveY = vector(0, npts-1);

/* Calculate spacing between points to generate data */
dx = (xmax - xmin) / (npts-1);

/* Loop through from xmin to approximately xmax */
for (i = 0; i < npts; i++)
{
    CurveX[i] = xmin + (i*dx);
    ExpCurveY[i] = log10(Optics.amplitude_exp * R(CurveX[i], Optics.xs_exp, Optics.ys,
                                  Optics.zsp_exp, Optics.zsi_exp,
                                  Optics.mueff_exp));
    FitCurveY[i] = log10(Optics.amplitude_fit * R(CurveX[i], Optics.xs_fit, Optics.ys,
                                  Optics.zsp_fit, Optics.zsi_fit, Optics.mueff_fit));
}

/* plot expected and fitted curves on same graph */
plwid (1);
plcol (magenta);
pline (npts, CurveX, ExpCurveY);
plcol (cyan);
pline (npts, CurveX, FitCurveY);
plend();

/* This function plots mua and musprn curves vs. lambda for multiwavelength fit */
void CurvePlotter(int Npts, float *X, float *Y1, float *Y2)
{
    float xmin, xmax, y1min, y1max, y2min, y2max, xdiff, ydiff;
    short i;

    printf("n");
    for (i=0; i < Npts; i++)
        printf ("lambda = %lf, mua = %lf, mus' = %lf\n", X[i], Y1[i], Y2[i]);

    /* see function PointPlotter for comments on the following commands */
    plsort(0);
    plsdev("mac");
    plfontd(0);
    plssub(1,2); /* splits page into 2 stacked subpages */
    plinit();
    plfont(1);

    /* calculate mins and maxes for ALL curves */
    xmin = xmax = X[0];
    y1min = y1max = Y1[0];
    y2min = y2max = Y2[0];
    for (i = 1; i < Npts; i++)
{
    if (X[i] < xmin)
        xmin = X[i];
    if (X[i] > xmax)
        xmax = X[i];
    if (Y1[i] < y1min)
        y1min = Y1[i];
    if (Y1[i] > y1max)
        y1max = Y1[i];
    if (Y2[i] < y2min)
        y2min = Y2[i];
    if (Y2[i] > y2max)
        y2max = Y2[i];
}

/* advances to FIRST subpage */
pladv(0);
plvpor(0.2, 0.9, 0.15, 0.85);
xdiff = (xmax - xmin) / 20.;
ydiff = (y1max - y1min) / 10.;
plwind(xmin - xdiff, xmax + xdiff, y1min - ydiff, y1max + ydiff);

plcol(white);
plwid(1);
plsmaj(0.0, MAJICK_SCALE);
plmin(0.0, MINTICK_SCALE);
plchr(0.0, CHAR_SCALE);
plbox("bcnst", 0.0, 0, "bcnstv", 0.0, 0);
plwid(1);
plcol(yellow);
pllab("Wavelength [nm]", "#gm#da#u [cm#u-1#d]", "Absorption vs. Wavelength");

plwid (1);
plcol (magenta);
plssym (0.0, SYMBOL_SCALE);
plpoin (Npts, X, Y1, SYMBOL);
plline (Npts, X, Y1);

/* advances to SECOND subpage */
pladv(0);
plvpor(0.2, 0.9, 0.15, 0.85);
xdiff = (xmax - xmin) / 20.;
ydiff = (y2max - y2min) / 10.;
plwind(xmin - xdiff, xmax + xdiff, y2min - ydiff, y2max + ydiff);

plcol(white);
plwid(1);
plsmaj(0.0, MAJICK_SCALE);
plmin(0.0, MINTICK_SCALE);
plchr(0.0, CHAR_SCALE);
plbox("bcnst", 0.0, 0, "bcnstv", 0.0, 0);
plwid(1);
plcol(yellow);
pllab("Wavelength [nm]", "#gm#ds#u [cm#u-1#d]", "Reduced Scattering vs. Wavelength");

plwid(1);
plcol (cyan);
plssym (0.0, SYMBOL_SCALE);
plpoin (Npts, X, Y2, SYMBOL);
plline (Npts, X, Y2);
plend();
References


[29] P. van der Zee, “Methods for measuring the optical properties of tissue samples in the visible and near infrared wavelength range,” in *Medical optical tomography: Functional imaging and monitoring*, vol. IS 11, SPIE Institutes for advanced optical technologies, G. Muller, B. Chance, R. Alfano, S. Arridge, J. Beuthan, E. Gratton, M.


