Custom Silicon Annular Photodiode Arrays for Spatially Resolved Diffuse Reflectance Spectroscopy

by

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Dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Electrical and Computer Engineering Duke University in the Graduate School of Duke University 2016
ABSTRACT

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Abstract

Diffuse reflectance spectroscopy (DRS) is a simple, yet powerful technique that has the potential to offer practical, non-invasive, and cost effective information for optical diagnostics and therapeutics guidance. Any progress towards moving DRS systems from their current laboratory settings to clinical settings, field settings and ambitiously to home settings, is a significant contribution to society in terms of reducing ever growing healthcare expenditures of an aging society. Additionally, improving on the existing mathematical models used to analyze DRS signals; in terms of speed, robustness, accuracy, and capability in accounting for larger feature space dimensionality (i.e. extraction of more tissue-relevant information) is equally important for real-time diagnosis in the desired settings and to enable use of DRS in as many biomedical applications (e.g. skin cancer diagnosis, diabetics care, tissue oxygenation monitoring) as possible. Improving the reflectance signal complexity and density through novel DRS instrumentation, would facilitate development of the desired models or put the existing ones built on simulations in practical use; which otherwise could not go beyond being a theoretical demonstration.

DRS studies tissue morphology and composition through quantification of one or more (ideally all of them) of the tissue-and wavelength-specific optical properties: absorption coefficient ($\mu_a$), reduced scattering coefficient ($\mu'_s$), scattering anisotropy ($g$), tissue thickness, and scattering phase function details (e.g. higher order moments of the scattering phase function). DRS involves sampling of diffusely reflected
photons which experience multiple scattering and absorption as they travel within the tissue, at the tissue surface. Spatially resolved diffuse reflectance spectroscopy (SRDRS) is a subset of general DRS technique, which involves sampling of diffuse reflectance signals at multiple distances to an illumination source. SRDRS provides additional spatial information about the photon path; yielding depth-resolved tissue information critical to layered tissue analysis and early cancer diagnostics. Existing SRDRS systems use fiber optic probes, which are limited in accommodation of large number and high-density collection fibers (i.e. yielding more and dense spatially resolved diffuse reflectance (SRDR) measurement data) due to difficulty of fiber multiplexing. The circular shape of the fibers restricts the implementable probe geometries and reduces the fill factor for a given source to detector (i.e. collection fiber) separation (SDS); resulting in reduced light collection efficiency. The finite fiber numerical aperture (NA) reduces the light collection efficiency well as; and prevents selective interrogation of superficial tissues where most cancers emerge. Additionally, SRDR systems using fiber optic probes for photon collection, require one or more photodetectors (i.e. a cooled CCD); which are often expensive components of the systems.

This thesis deals with development of an innovative silicon SRDRS probe, which partially addresses the challenge of realizing high measurement density, miniaturized, and inexpensive SRDRS systems. The probe is fabricated by conventional, flexible and inexpensive silicon fabrication technology, which demonstrates the feasibility of developing SRDRS probes in any desired geometry and complexity. Although this approach is simple and straightforward, it has been overlooked by the DRS community due to availability of the conventional fiber optic probe technology. This new probe accommodates large number and high density of detectors; and it is in the form of a concentric semi-annular photodiode (PD) array (CMPA) with a central illumination aperture. The CMPA probe has 24 semi-annular PDs; which function
both for photon collection and detection, eliminating the need for another detection component in the system offering a reduction in system cost and size. The 24 PDs are grouped into three sets based on the PD annulus width (w): 1) eight inner photodiodes of (w) = 50 µm; 2) eight middle photodiodes of w = 100 µm; and 3) eight outer photodiodes of w = 250 µm. The CMPA probes with illumination aperture diameters of 100 µm, 400 µm, and 750 µm were fabricated with 100% fabrication yield and with dark currents ranging from 1-20 pA and responsivities between 0.26-0.37 A/W for the wavelength (λ) range of 450-600 nm, with comparable performance to commercial photodiodes. This is the first multiple source-detector spacing Si SRDRS probe reported to date, and the most densely packed SRDRS probe reported to date for all types of SRDRS systems. The closely spaced and densely packed detectors enable higher density SRDR measurements compared to fiber-based SRDR probes, and the higher PD NA compared to that of fibers results in a higher SNR increasing light collection efficiency. The higher NA of the PDs and the presence of PDs positioned at very short distances from the illumination aperture center enable superficial tissue analysis as well as depth analysis.

First, the back-illumination induced photocurrents at the side walls of the CMPA illumination aperture were characterized, and the probe was tested on 99% diffuse reflectance standard (puck) using an illumination source of a Xenon lamp coupled to a monochromator. The through-aperture optical power with this source was measured as <=2.5 µW (lower than those of typical DRS systems) for wavelength range of 370-990 nm; yet the puck measurements yielded signal to noise (SNR) ratios larger than 40 dB in the entire λ range for all but the two inner PDs. An extra effort to optimize the light source would further decrease the measurement uncertainty. The CMPA probe was validated on benign and malignant breast tissue mimicking liquid phantoms with a mean error between the measurements and the forward Monte Carlo (MC) simulations less than 9%.
Next, extensive spatially resolved diffuse reflectance spectroscopy (SRDRS) phantom measurements using this most densely packed and only custom silicon photodiode array reported to date are performed. These SRDRS measurements, using a broadband Xenon lamp, performed at 41 wavelengths using custom, closely spaced photodetectors, produced over 800 data points for each of 30 phantoms, with good agreement between these measurements and forward Monte Carlo modeling. The measured phantoms cover a wide range of optical properties with a $\mu'_{s}$ range of 0.6-2.8 mm$^{-1}$ and a $\mu_{a}$ range of 0-7.58 mm$^{-1}$, which is the most extensive $\mu_{a}$ range reported to date for SRDRS. The dense SRDRS measurements provide unique reflectance data for more than 96% of the measured $\mu'_{s}$ and $\mu_{a}$ pairs, which is promising for rapid empirical model development, facilitating real-time feature extraction for clinical use.

Finally, spatially resolved diffuse reflectance measurements were performed on bulk and two-layered skin tissue-mimicking phantoms using the densely packed CMPA probe with 400 $\mu$m aperture diameter. The two-layer phantoms were assembled by stacking thin phantom layers with thicknesses ranging from 0.150 mm to 1.828 mm on the bulk phantom. The average error between the measured reflectance data and the forward MC simulation results were less than 6.2% for all the two-layer phantoms and the bulk phantom. The maximum measured signal contrasts between the bulk phantom and the two-layer phantoms were >80% for thick top layers. For a very thin top layer of 0.15 mm thickness, the signal contrast is higher than 20%; and for this top layer thickness, 0.05 mm increase in top layer thickness induces 10% change in the signal contrast enabling detection of small changes in thickness. The described probe is promising for low cost, portable SRDRS systems and has the potential to enable both superficial and layered tissue analysis.
To my husband Seckin, who sees deep into my eyes and whose company makes the best of me.

And, to those who strive to navigate through their life rightfully
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List of Abbreviations and Symbols

Symbols

\( \omega \) Photodiode annulus width.
\( \lambda \) Optical wavelength.
\( \mu_a \) Absorption coefficient.
\( \mu_s \) Scattering coefficient.
\( \mu'_s \) Reduced scattering coefficient.
\( \sigma_{sc} \) Scattering cross section.
\( \rho \) Particle density.
\( g \) Anisotropy factor.

Abbreviations

AR Antireflection
CCD Charge coupled device
CMPA Concentric semi-annular photodiode array
DRS Diffuse reflectance spectroscopy
MC Monte Carlo
NA Numerical aperture
NIR Near infrared
OCT Optical coherence tomography
PD Photodiode
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<tr>
<td>PMT</td>
<td>Photomultiplier tube</td>
</tr>
<tr>
<td>SDS</td>
<td>Source to detector separation</td>
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<tr>
<td>Si</td>
<td>Silicon</td>
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<td>SNR</td>
<td>Signal to noise ratio</td>
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<td>SpRDRS</td>
<td>Spectrally resolved diffuse reflectance spectroscopy</td>
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<td>SRDR</td>
<td>Spatially resolved diffuse reflectance</td>
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<td>SRNRS</td>
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1 Introduction

This chapter provides a motivation for the development of concentric multi-pixel photodiode array (CMPA) spatially resolved diffuse reflectance probes and gives an outline for the thesis. Additionally, it gives an introduction to diffuse reflectance spectroscopy (DRS) and DRS instrumentation.

1.1 Motivation

Healthcare is one of the major areas in which many countries of the world are spending a significant portion of their GDPs: for example, in 2013, USA expenditures on healthcare were 17.1% of GDP, increasing from 9% in 1980 [1]. The world population with persons aged 65 and older has been increasing dramatically: by 2040, this group is expected to constitute 14% of the total population, where they constituted only 7% of the world population in 2008 [2], increasing the pressure on healthcare budgets. New approaches that improve overall cost efficiency will help to constrain healthcare expenditures as our populations age.

One approach to future diagnostics will focus on improving care by creating novel physiological sensors and measurement methods, and, based upon this data, creat-
ing model-based decisions to support and optimize diagnosis and therapy selection. Healthcare providers are interested in new technology if it improves at least one of these metrics: clinical outcomes, processes, and financial returns.

Currently, a multitude of medical diagnostics and therapeutics tools are based on optical techniques and their number continues to grow rapidly. A few examples of optical methods currently employed in clinical settings are optical coherence tomography (OCT), photoacoustic microscopy, photodynamic therapy, and laser surgery. Research towards establishing new optical techniques and improving existing technologies for personalized diagnosis and therapeutics, user friendliness, and cost-effectiveness is underway. Biomedical optical technologies will continue to play a key role in future healthcare systems.

Diffuse reflectance spectroscopy (DRS) is a non-invasive biomedical tissue diagnostic technology that has the potential to offer practical and cost-effective diagnostic information. DRS is a well-established technique that studies tissue morphology and biochemical composition. It quantifies the tissue reduced scattering coefficient ($\mu'_s$) and the absorption coefficient ($\mu_a$) of tissue, which provide quantitative information about cellular and sub-cellular tissue structures and tissue chromophore types, as well as their concentrations. DRS is promising for many clinical applications, including diagnosis of various cancer types, e.g. skin cancer [3–5], breast cancer [6–8], and cervical cancer [9, 10], early cancer diagnosis [11], monitoring tissue oxygenation [12], characterizing tumor response to therapy [13], and intra-operative breast cancer margin assessment [14, 15].

DRS utilizes optical photons traversing tissue for diagnostic purposes. Photons launched into tissue undergo absorption and multiple scattering events as they traverse the tissue. A fraction of these diffusely reflected photons exit the tissue surface and are collected by one or more detectors or fibers. Figure 1.1 illustrates DRS schematically, showing photon launch, scattering, absorption, and collection using a
source fiber and a collection fiber (the simplest DRS probe implementation). Extraction of tissue optical properties is achieved by fitting the detected reflectance to a mathematical model or by comparison to a database [16]. These models include diffusion approximation analytical models [17, 18], numerical models based on Monte-Carlo simulations [19–21], and empirical models [22].
Figure 1.1: A schematic representation of diffuse reflectance spectroscopy (DRS). Photon launch, scattering, absorption (black x) and collection, and representative optical property extraction data and methods are illustrated.
The majority of reported DRS instruments, in their current forms, are not being employed in clinical settings because of their large size and high cost. Typical DRS systems include a broad-band light source, an imaging spectrograph, a CCD detector array, and a fiber optic probe with both illumination and collection fibers. The most common DRS fiber optic probe design has a central illumination fiber circularly surrounded by multiple collection fibers [23–25] for photon collection. However, the circular fiber core, the presence of the fiber cladding, and the limited fiber numerical aperture (NA) results in a probe that has a fill factor that is less than one for a given detection region radius, and has a low photon collection efficiency.

For example, for improved photon collection efficiency, Yu et al. replaced the collection fibers with a commercial rectangular Si photodiode (PD) that was positioned next to an illumination fiber, which was an innovative approach to DRS instrumentation [26]. The higher NA of the Si PD compared to a typical fiber (0.96 versus 0.22) and the PD in contact with the tissue significantly improved the light collection efficiency and eliminated the need for a cooled CCD detector and a spectrograph, resulting in a significant reduction in cost and size. In their following work [27] aiming for a practical probe for intra-operative breast cancer margin assessment, a spectral DRS imaging system was built using a 3x3 array of commercial Si PDs with optical fibers inserted to drilled apertures through the PDs for illumination. In this system, in addition to the elimination of the collection fibers, CCD, and spectrograph, the monochromator was replaced by bandpass filters resulting in further reduction in cost and size. Figure 1.2 compares the fiber-based and this reported commercial Si PD-based proof-of-concept DRS imaging systems.
The commercial Si PD based system facilitated cost and size reduction; yet it came with its own disadvantages: The predefined geometries of the commercial PDs resulted in higher pixel-to-pixel optical crosstalk and mechanical drilling of the PDs resulted in non-identical pixel geometries and degradation in PD performance. For taking advantage of Si PDs for size and cost reduction, yet avoiding its limitations, our group (Dhar et al.) developed a custom diffuse reflectance spectral imaging probe
comprised of a 4x4 array of annular Si PDs with central illumination apertures for intraoperative breast cancer margin assessment [29]. The illumination aperture and the PD diameter were customized to optimize the system spatial resolution for a tolerable optical crosstalk. Unlike the use of fiber multiplexing for illumination, which was proved to be disadvantageous for increased scalability (i.e. each pixel on the array was addressed by an individual fiber becoming tedious as the number of pixels increase); in this custom system, a fiber bundle-coupled free space absorptive tube (which is also optimized to reduce optical crosstalk by filtering the photons with higher divergence) was used for light delivery. The source illuminated the tissue through the backside of the PD array (through the PD central apertures), and the front sides of the PDs face the tissue. Additionally, the PD spectral response was customized to compensate for the variation in the optical output of the illumination source across the measurement wavelength spectrum, yielding increased signal to noise ratios at all wavelengths.

To further increase the clinical applicability of the system, our group developed thin film PDs on transparent Pyrex (which did not interfere with the back illumination design) aiming to address some of the shortcomings of the previous system: During measurement of the tissue specimen, leaking of blood through the PD aperture caused variation in illumination conditions and problems with measurement repeatability. In this system, the Pyrex substrate served as a barrier avoiding blood getting through the aperture. Moreover, decreased silicon thickness improved the optical power through the aperture. Another advantage of thin film array is the applicability of transparent surface protection coatings on them; which improves the array lifetime under stringent clinical cleaning protocols of clinical applications. Additionally, our group (Dhar et al.) reported thin film annular Si PDs on flexible substrates, which opens the door for conformal DRS imaging [30].

Although, the previous works of our group dealt with one specific biomedical
application (intra-operative breast cancer margin assessment using a DRS spectral imaging probe); from these works, it is obvious that utilization of custom photodiodes would provide unique advantages for various other DRS instruments targeting different biomedical applications.

Spatially-resolved diffuse reflectance spectroscopy (SRDRS) is a subset of general DRS technique, which provides additional spatial information about the photon path. Typical SRDRS probes are fiber-based, and incorporate multiple source-detector pairs (typically one source fiber and three to eight collection fibers) [21, 31, 32]. Each detector selectively collects scattered light, which can be viewed as interrogating tissue from different depths. If the tissue is layered, then SRDR can interrogate various layers of the tissue under study, as shown in Figure 1.3, and can yield information associated with the interrogated layers. Thus, SRDR measurements enable depth-resolved tissue analysis [33–35], which is important for detailed study of layered tissues such as the skin and cervix. Early cancer diagnosis is another domain that could significantly benefit from layered tissue analysis, and several groups have already reported on use of DRS for this purpose [36]. Intraepithelial neoplasia is a precursor of cancer, and several publications report that it causes tissue optical property changes in the epithelium and in the underlying stroma [37]. Understanding these changes and enabling superficial and layered tissue optical parameter extraction is of critical importance for early cancer diagnosis. Additionally, simultaneous use of spectral and spatial information in DRS was shown to increase the accuracy of optical property extraction [31].
Currently available SRDRS systems utilize fiber-based probes, which have difficulty in accommodating high density and closely spaced source-detector pairs. In addition, fiber SRDRS probes have low light collection efficiency because of their low NA compared to Si PDs. Similar to fiber-based systems, free space SRDRS systems also suffer from low light collection efficiency because of the limited NA of the lenses. Additionally, free space SRDRS systems are bulky and expensive for clinical use.

Use of photodiode-based probes in SRDRS systems would address some of the mentioned problems, in a similar way the annular custom PDs contributed to the breast cancer margin assessment probe. Each independent pixel on the reported custom 4x4 DRS Si probe [29] is in the form of a single source (central aperture)/detector (annular Si PD) pair, as in Figure 1.4a. Each single Si PD has photons passing through the central hole, and these photons interrogate a bulk portion of the sample under study. The diffusely reflected photons are detected by the single PD; this PD measurement does not provide information regarding the photon path, except that
the photon path resulted in photon detection defined by the PD detecting area.

This thesis reports on the design, fabrication, characterization, and validation of an innovative Si PD probe consisting of a concentric multi-pixel array (CMPA) of PDs with a central illumination aperture that enables SRDRS measurements (i.e. pixilated form of the individual pixel of the 4x4 array). Figure 1.4b shows a simple schematic representation of a concentric multi-pixel array (for simplicity, showing fewer pixels than the actual probe has). This is the first multiple source-detector separation Si SRDRS system reported to date, and the most densely packed SRDRS probe reported to date for all types of SRDRS systems. This results in much more SRDRS data from this probe than other SRDRS probes.

Pixels on the probe are positioned in radially increasing distances, and each pixel interrogates the tissue to a certain depth. Pixels are positioned at very short distances from the illumination aperture center, enabling superficial tissue analysis as well as depth analysis. Additionally, the closely spaced and densely packed detectors enable higher density SRDR measurements compared to fiber-based SRDR probes, and the higher PD effective NA compared to fibers results in a higher SNR than fiber probes, enabling a higher range of measurable scattering and absorption. The next section gives a brief outline of the thesis.
Figure 1.4: Microscopic image of one of the PDs of a 4x4 thick Si PD array currently employed in the clinic for intra-operative tumor margin assessment [29]; b) Illustration of a representative concentric detector with three sub-pixels. It is composed of a centered illumination aperture and three concentric circular PDs.

1.2 Outline of the Thesis

The objective of this thesis research was to design, fabricate and test innovative custom spatially resolved diffuse reflectance (SRDR) probes based on silicon pn junction concentric multi-pixel photodiode arrays (CMPA) for dense and depth-resolved reflectance measurements on human tissue phantoms. Testing the probe involves diffuse reflectance measurements on homogenous liquid phantoms with a wide optical property range, and layered solid phantoms for probe validation purposes. The organization of this thesis is as follows:

Chapter 1 provides a motivation for the research work presented in this thesis. Additionally, it provides a brief introduction to diffuse reflectance spectroscopy and its current instrumentation.

Chapter 2 presents the background and status of the various technologies relevant to this thesis. Section 2.1 describes the principles of tissue optics and diffuse reflectance spectroscopy. Section 2.2 reviews the semiconductor photodetector tech-
nologies utilized in DRS systems, and presents a brief literature review for miniaturized and cost effective spectroscopy systems.

Chapter 3 outlines the details of the design, fabrication, and test of a CMPA spatially resolved diffuse reflectance probes. The spectral response, dark current, and back illumination-induced photocurrents of the semi-annular concentric Si PDs are presented. The CMPA probe was tested on diffuse reflectance standards and two breast tissue-mimicking phantoms. The effects of specular reflectance on wavelength calibration were assessed.

Chapter 4 presents a comprehensive multi-spectral phantom study using the CMPA SRDRS probe. The constructed phantoms cover a broad range of tissue optical properties with $\mu_s$ range of 0.6-2.8 mm$^{-1}$ and $\mu_a$ range of 0-7.58 mm$^{-1}$, which is the largest range of SRD measurements reported to date. Experimental measurement results are compared to forward Monte Carlo modeling results.

Chapter 5 deals with a layered phantom study using the CMPA SRDRS probe. The constructed solid phantoms represent the human skin with two different layers representing the epidermis and the dermis. Experimental measurement results are compared to forward Monte Carlo modeling results.

Chapter 6 concludes the thesis and provides a perspective for future research opportunities towards the development of customized SRDRS probes based on Si PD arrays and potential biomedical applications.
This chapter provides a background and the status of the technologies related to this thesis. Subsection 2.1 deals with the details of photon transport in biological tissues and the principles and the instrumentation of DRS, with a focus on active research topics in the DRS field. Subsection 2.2 presents the physics and current status of semiconductor photodetector technologies as possible detection components of biomedical probes. Several detection technologies are compared to understand their applicability to the specific goal of this thesis, namely, of developing custom, cost-effective SRDRS probes incorporating both photon collection and detection.

2.1 Tissue Optics and Diffuse Reflectance Spectroscopy

The three main steps in optical diagnostics are photon penetration into the tissue, interrogation of the tissue as the photons travel within it, and photon emission from the tissue. In contrast, the main steps in therapeutics are photon penetration and depositing energy to the relevant portion of the tissue. Identifying the tissue optical properties is crucial to the proper design of optical diagnostic and therapeutic tools, the interpretation of diagnostic measurements, and therapeutic protocols. With
tissue optical property specification, a mathematical light transport model can be used to predict the light distribution and energy deposition in the tissue.

The following subsections provide the details of photon transport in biological tissues and diffuse reflectance spectroscopy, which is a well-established method to measure tissue optical properties.

2.1.1 Fundamentals of Tissue Optics

Tissue optical properties are described by the refractive index ($n$), the scattering coefficient ($\mu_s$), the scattering phase function ($p(\theta,\phi)$), and the absorption coefficient ($\mu_a$). Photon transport in tissue can be modeled using these three optical parameters.

Scattering

Scattering occurs due to the presence of local refractive index mismatch and results in a direction change of the photon incident upon the mismatched interface. Refractive index mismatch in tissue occurs, for example, between extracellular fluid and cell membranes, and between collagen fibers and microstructures. Features and particles causing scattering vary in size and concentration, and are specific to a given type of tissue.

The total scattering cross section ($\sigma_{sc}$) quantifies the amount of scattering, and it is described as the ratio of total scattered power in all directions to the intensity of incident light. In principle, $\sigma_{sc}$ can be calculated using classical electromagnetic theory if the refractive indices of the host medium and the scatterer, and the shape of the scatterer are known values. For example, Mie theory solves Maxwell equations for electromagnetic (EM) radiation scattering from a spherical particle, and Rayleigh scattering is the small-size limiting case of Mie scattering.

Typically, practical applications deal with a volume of particulate media rather than a single particle, where defining a volume total scattering cross-section (the
sum of the cross sections of all the particles in that volume) is more meaningful. The scattering coefficient ($\sigma_{sc}$) is defined as the total scattering cross section per unit volume of the particulate medium. Assuming a homogenous medium (i.e. a medium where the particle density is constant throughout the medium), $\sigma_{sc}$ (in units of mm$^{-1}$) is the product of the total cross section and the particle density ($\rho$) of the medium [38]:

$$\mu_s = \rho \sigma_{sc}$$

(2.1)

And, the inverse of the scattering coefficient, $l_s = \frac{1}{\mu_s}$ is the average distance a photon traverses before undergoing scattering (i.e. the mean free path).

In cases where the amount of the scattering into a certain direction is of interest, the scattering phase function, $p(\theta, \phi)$ (sr$^{-1}$) is utilized. Typically, it is given in spherical coordinates where $\theta$ is the polar angle and $\phi$ is the azimuthal angle. It describes the amount of scattered intensity into a given direction, and is normalized such that its integral over a unit sphere is unity [38]:

$$\frac{1}{4\pi} \int_\theta p(\theta, \phi) dS = 1$$

(2.2)

The phase function information is crucial when describing events involving single or few scattering events, i.e. confocal reflectance microscopy. Multiple scattering averages out the phase information, and the scattering directionality information can be described by a constant rather than a complex function. In randomly oriented media, $\phi$ is completely averaged out, removing the $\phi$ dependence of the phase function (i.e. it is sufficient to describe phase function as $p(\theta)$), and in the diffuse regime, multiple scattering averages $\theta$ such that the anisotropy constant of the scattering medium ($g = \langle \cos \theta \rangle$) suffices to characterize the scattering directionality. The anisotropy factor (or the first order Legendre moment of the phase function ($p(\theta)$))
is given by the following equation [39]:

\[
g = \int_{\theta=0}^{\pi} p(\theta)\sin\theta\cos\theta d\theta
\] (2.3)

For calculation of \( g \), \( p(\theta) \) must be normalized such that \( \int_{\theta=0}^{\pi} p(\theta)\sin\theta d\theta = 1 \). Typically, in biological tissues \( g \) varies between 0.75 and 0.99, implying highly forward scattering (i.e. \( g = 0 \) means the medium is isotropic, \( g = 1 \) means it is forward scattering, and \( g = -1 \) means it is backward scattering).

**Absorption**

The absorption process involves the extraction of energy from light by the molecular species (chromophores). The three basic processes that are responsible for absorption are electronic, vibrational, and rotational transitions. The spectral regions where these transitions occur are known as absorption bands, and are specific to particular atoms or molecules. Thus, the types and concentrations of the chromophores in a given tissue specifies its absorption characteristics and serve as a fingerprint that can guide the choice of photon wavelengths for diagnostic and therapeutic applications.

The Beer-Lambert Law correlates the transmission \( (T) \) through a medium containing an absorber species to the concentration of that species and the thickness of the medium \( (l) \) as given by the equation below [39]:

\[
T = \frac{I}{I_0} = e^{C\varepsilon l}
\] (2.4)

where \( \varepsilon \) is the molar extinction coefficient \([\text{cm}^2\cdot\text{mol}^{-1}]\) of the absorber, and \( C \) is the molar concentration of the absorber \([\text{mol}\cdot\text{cm}^{-3}]\), and \( I \) and \( I_0 \) are the transmitted and the incident light, respectively. The Beer-Lambert Law is applied to spectrophotometer measurements to analyze the concentration of a species with given absorption spectrum, or the absorption spectrum of an absorber with known concentration.
This law starts to fail for higher absorber concentrations: for higher concentrations, the molecules aggregate and start to interact with each other, resulting in a change of the absorption characteristics [40].

Typically, biological tissues contain more than one chromophore (e.g., hemoglobin, melanin, and water), which are collectively responsible for the absorption within the tissue. The optical absorption coefficient \( \mu_a \) [mm\(^{-1}\)] describes the tissue absorption, and using the Beer-Lambert Law (in its limits), it can be calculated by the equation [39]:

\[
\mu_a(\lambda) = \sum_n C_n \varepsilon_n(\lambda)
\]

(2.5)

where \( n \) represents each different absorber species. The reciprocal of \( \mu_a \) is absorption mean free path \( (l_a) \), and describes the average distance that a photon travels before being absorbed.

2.1.2 Light Transport in Tissue

The radiative transport equation (RTE, also known as the Boltzman equation) treats light propagation as the transport of photons (as particles) and it describes the energy flow associated with the photon travel in biological tissues based on conservation of energy in an analytical form. The RTE is given as [41]:

\[
\frac{\partial L(\vec{r}, \vec{s}, t)}{\partial t} = -\vec{s} \cdot \nabla L(\vec{r}, \vec{s}, t) - \mu_t L(\vec{r}, \vec{s}, t) + \\
\mu_s \int_{4\pi} L(\vec{r}, \vec{s}', t) P(\vec{s}', \vec{s}) d\Omega' + S(\vec{r}, \vec{s}, t)
\]

(2.6)

where \( L \) is the radiance at a position \( \vec{r} \) propagating in direction \( \vec{s} \), \( \mu_t = \mu_a + \mu_s \) is the extinction coefficient, and \( S \) describes the light source. \( P(\vec{s}', \vec{s}) \) is the phase function describing the probability of light propagating in the \( \vec{s} \) direction being
scattered into solid angle $d\Omega$ around the $\vec{s}$ direction. At steady state, the time $(t)$ dependence is eliminated, and the term on the left side of the equation becomes equal to zero. Additionally, for a solution in a source-free region, the last term is also zero. The remaining terms describe the spatial change in radiance at the position $\vec{r}$.

The first term represents the outflow of energy from the position $\vec{r}$ in the direction of $\vec{s}$. The second term on the right side of the equation describes the extinction of energy at a position $\vec{r}$ traveling in a direction $\vec{s}$ (per unit solid angle) per unit area normal to $\vec{s}$, which is reduced by absorption and scattering at that position. The third term indicates that radiance at a position $\vec{r}$ traveling in a direction $\vec{s}$, is increased by light scattered from $\vec{s}$ directions into direction $\vec{s}$.

The diffuse reflectance from a scattering medium with known optical properties was initially described by diffusion theory, based on the diffusion approximation in the RTE. The diffusion approximation assumes that the dominant photon transport mechanism is scattering, and that the absorption is sufficiently small to let photons scatter many times so that they are considered to be diffused into the scattering medium. The regime where this assumption is valid is known as diffusive regime. In the diffusion approximation, the phase information and $\mu_s$ are combined into a single variable (known as reduced scattering coefficient) described by the following equation [38]:

$$\mu_s' = \mu_s(1 - g) \quad (2.7)$$

This equation is known as similarity principle which states that observed reflectance measurements for any combination of $\mu_s$ and $g$ pairs, resulting in the same $\mu_s'$, are equivalent.

In general, diffusion theory successfully predicts diffuse reflectance from tissues in the NIR spectral range, but it starts to fail in the UV-VIS spectral regions where
hemoglobin is strongly absorbing. It also fails to describe diffuse reflectance for small source detector separations, where photons that scatter only once or few times are collected (i.e. not diffused into the medium). Thus, numerical methods have been developed to model photon transport for an extended range of tissue optical properties and complex probe geometries. Compared to diffusion theory, numerical methods are powerful, yet computationally expensive.

Monte Carlo (MC) modeling is a numerical simulation method which is currently accepted as the gold standard for modeling photon transport in biological tissues. [42]. The Monte Carlo method can successfully model photon transport for a wide range of scattering and absorption coefficients, it can be utilized for the entire UV-VIS-NIR spectral regions, and it also work successfully for complex geometries. Monte Carlo methods involve repeated random sampling and photon transport in a medium is described as a random walk. The statistical nature of the simulations requires a large number of photons (typically $> 10^5$) for accurate results; thus unlike diffusion theory, MC simulations are computationally intensive. In MC simulations of the photon transport, the model parameters are the absorption coefficient, the scattering coefficient, and the phase function. The Henyey-Greenstein phase function $p_{HG}(\cos\theta)$ is the most widely used phase function in MC modeling of biological tissues due to its simple representation, which is described by the equation below [43]:

$$p_{HG}(\cos\theta) = \frac{1}{4\pi} \frac{1 - g^2}{(1 + g^2 - 2g\cos\theta)^2}$$

Using this form of the phase function, the selection of the $g$ value is sufficient to describe the probability distribution of the scattering angles.

Ideally, individual MC simulations are required for each set of model parameters ($\mu_a$, $\mu'_s$), reducing the computational practicality of the method. One approach to
increasing the computational efficiency is to use a scaling procedure, which uses the results of a single MC simulation (i.e. for a single set of parameters) to predict the outcome for mediums with different set of optical parameters [44]. To further increase the computational efficiency of the MC simulations, similarity principles involving first or higher order Legendre moments of the phase function have been utilized by several groups [45–47]. However, the effect of the phase function details on the reflectance measurements have been questioned and the limits of various similarity principles were investigated [43]. Some of these results, in particular, in relation to probe geometries, will be discussed in more detail in the following section dealing with the principles and the instrumentation of DRS.

2.1.3 Diffuse Reflectance Spectroscopy

Diffuse reflectance spectroscopy (DRS) has been studied over two decades, and is a well established tissue characterization technique. Simply put, in DRS, a source illuminates the studied sample, and a portion of the photons re-emitted at the sample surface is detected, yielding information about the physical structure and the chemical content of the sample. DRS relies on quantitative methods to extract information from the measured reflectance data, which is inherently an ill-posed inverse problem. In order to condition the problem, various DRS measurement methods are employed: time-resolved DRS, frequency-domain DRS, and steady-state DRS. In steady-state methods, the problem is either spatially-constrained (spatially-resolved DRS (SR-DRS)), or spectrally-constrained, or constrained in both domains. Time-resolved [48] and frequency-domain [49] DRS instruments are complex, bulky and expensive for clinical use; and they are not the topic of this thesis. However, one motivation for the thesis work herein is the demonstration of an efficient, small, simple, and low cost system compared to time-resolved and frequency-resolved DRS measurement systems. Herein, only steady-state methods are explored.
A typical DRS instrument incorporates a broadband light source, which is connected to a dispersive element (e.g. monochromator), a fiber optic probe for light delivery and collection, a spectrograph, and a detector. Figure 2.1 shows a schematic illustration of a typical DRS instrument. Some SRDRS systems also employ single or multiple laser diodes separately or together with a broadband source for illumination. In DRS systems, the most commonly utilized detection elements are fibers connected to charge-coupled devices (CCD), providing spatial information or connected to photo-multiplier tubes (PMT) with superior sensitivities, preferred especially in laboratory settings. Both CCDs and PMTs are advantageous in terms of performance, yet their cost and size make them undesired for DRS applications. The challenge addressed in this thesis is to demonstrate a micro-scale resolution system that has high efficiency and low cost through leveraging typical Si manufacturing technology.
Figure 2.1: Schematic illustration of a typical DRS system incorporating a light source, a dispersive element, a probe with source and detector fibers, an imaging spectrograph and a detector.

The desired tissue volume and depth to be interrogated vary vastly among DRS applications, i.e. early cancer diagnosis in epithelial tissues requires selective collection of the photons from thin epithelial layers (100-500 µm), whereas breast cancer margin assessment requires interrogation of the tissue at least up to 2 mm depth. The light delivery and collection characteristics of the probe play a key role in addressing the requirements of a target application. The source and detector size (e.g. collection fiber diameter for fibers), source-to-detector separation (SDS), number of sources and detectors within the probe, and their numerical apertures (NAs) are the parameters to be optimized to address the given requirements. Figure 2.2 shows an illustration of simple probe geometry with the given parameters labeled.
A typical fiber-optic based DRS probe incorporates a central source fiber (typically with diameter of 200 µm, and NA=0.22), and multiple collection fibers forming an annulus around the source fiber to increase the light-collection efficiency for a given SDS (i.e. source center to the annulus midpoint). In SRDRS fiber probes, typically one source fiber and multiple collection fibers (between three and eight fibers) are arranged linearly, with a different SDS between the source and each of the collection fibers. Figure 2.3 shows various DRS fiber optic probes used in previously published DRS work [50–53].
Figure 2.3: Pictures of some of the previous DRS fiber-optics designs [50–53].

The DRS signal decreases exponentially as a function of increasing SDS, resulting in a significant magnitude difference between the measured signals of the fibers with the shortest and longest SDSs, which requires a high dynamic range for detections, especially for the SRDRS probes with very large SDSs (> 1 cm). In some systems, this is achieved by using neutral density filters of varying optical density (OD) to avoid saturation [54], or by omitting the readings of any detectors that are either below the lower detection limit, or above the detection linear region [31].

In addition to the probe parameters given above, researchers have reported on novel probe designs aiming for control of the illumination and collection direction for depth-selective interrogation, which is of crucial importance for studying layered tissues. Example to these probes are a ball-lens coupled fiber optic probe designed
for depth-resolved spectroscopy of epithelial tissues [55], and an obliquely oriented fiber probe for improved depth resolution DRS [56].

The probe geometry also influences the selection of the modeling technique, and the model parameters for feature extraction. The accuracy of the extraction strongly depends on how accurately the forward model predicts the reflectance for a given source/detector pair and tissue optical properties. For example, the diffusion approximation fails to predict reflectance accurately for short SDSs and low albedo (\( \frac{\mu_s}{\mu_s + \mu_a} \)) samples [43]. For similar conditions (i.e. source-detector separation < 1 mm in tissue), many groups have recently reported on the failure of the similarity principle (as given by Eq. 2.7) and MC models, which both approximate the scattering phase function to the first order Legendre moment or anisotropy factor (\( g \)) [43, 45]. Thus, to describe the reflectance predicted by MC simulations more accurately for smaller source detector separations, higher Legendre moments were taken into account resulting in a new similarity principle involving an additional parameter given as [57]:

\[
\gamma = \frac{1 - g_2}{1 - g_1} \tag{2.9}
\]

where \( g_1 \) is the first order moment or anisotropy factor (\( g \)), and \( g_2 \) is the second order Legendre moment of the phase function, given as [58]:

\[
g_2 = \int_{0}^{\pi} p(\theta) \sin(\theta) \left( \frac{1}{2} (3 \cos^2 \theta) - 1 \right) \tag{2.10}
\]

The use of this similarity principle simplifies the calculations such that the solution for any combination of \( g_1, g_2, \) and \( \mu_s \) resulting in the same \( \mu'_s \) and \( \gamma \) are deemed equivalent. Recent research showed that \( \gamma \) can provide a larger source of contrast between different tissues compared to \( g \) [43]. For example, for a given \( \mu'_s \), the reflectance at
SDS=250 μm significantly changes with varying γ and constant g (i.e. reflectance or γ=2.3 is 6 times larger than that of γ=1.2), yet the variation in reflectance is very small when g is varied and γ is held constant [43]. In addition to computational work exploring γ, recent experimental research has demonstrated the significance of γ as a sensitive metric yielding information about the tissue ultrastructure [59].

The widely used Henyey-Greenstein (HG) phase function is described by a given anisotropy factor (g), and the selection of g automatically determines the rest of the higher-order Legendre moments ($g_{n,HG} = g^n_1$; where $g^n_1$ is the $n^{th}$ order moment of the HG phase function and $n > 2$), and thus fixes the value of γ. The possible γ values for the HG phase function are limited to a range between 1 and 2. Additionally, the HG phase function has been found to underestimate large-angle backward scattering [60, 61]. To address this shortcoming, a modified version of the HG phase function (MHG), the $p_{MHG}(\cos\theta)$, has been developed [45]:

$$p_{MHG}(\cos\theta) = \beta p_{HG}(\cos\theta) + (1 - \beta)\frac{3}{4\pi}\cos^2\theta$$  

where $\beta$ describes the fractional contribution of the HG phase function, and $1 - \beta$ describes the contribution of Rayleigh scattering, which accounts for the backward scattering. The MHG provides the flexibility of selecting $g_1$ and γ independently, yet similar to HG phase function, the γ values are limited to the same range between 1 and 2.

Because the MHG phase function γ is restricted to less than 2, Mie phase function which is more representative of tissue (i.e. it can be constructed for a wider range of γ and can represent tissue scattering more realistically) was also investigated, yet at the expense of increased computational cost [43].

In addition to MC models accounting for the scattering directionality by employing different phase functions, developing empirical models is another research topic
actively studied by DRS researchers. Although empirical models do not directly provide information of physical origin, their prediction accuracies are comparable to MC models with the advantage of reduced computational cost, enabling real-time optical property extraction. Empirical models are trained on experimentally measured data or, most commonly, on MC simulations applying regression techniques, such as partial least squares (PLS), support vector machines (SVM), and artificial neural networks (ANN).

In addition to modeling light propagation in bulk media, of which optical properties are a point of interest, a multitude of recent publications deal with light propagation in layered turbid media and layer-specific optical property extraction. As the number of layers increases, the dimensionality of the inverse problem increases as well: the layer thicknesses and the corresponding $\mu_s$ and $\mu_a$ pairs for all of the layers need to be calculated. Approaches to optical property extraction from multilayer media include: (1) sequential optical property extraction, which, for example, in a two layer sample, extracts the top layer optical properties using the measurement of one probe channel, then uses this information to calculate the bottom layer properties with the measurement of another channel, [56] and (2) the use of look-up tables (generated based on MC simulations) with increased dimensionality (i.e. top layer thickness, top layer $\mu_a$) [62].

2.2 Semiconductor Photodetector Technologies

This thesis deals with development of an SRDRS probe, which combines the photon collection and photon detection into a single element. The more general goal is to realize a higher level of cost reduction and user friendliness for DRS systems intended for specific biomedical applications, as well as facilitating the realization of complex and more accurate probes desired for increased functionality.

One approach to an SRDRS probe with both photon collection and detection
is to use photodetectors as the probe. The first part of this section describes the fundamentals of photodetection, and the remainder deals with the advantages and disadvantages of several photodetector technologies with regards to developing custom probes for DRS applications.

2.2.1 Fundamentals of Semiconductor Photodetectors

Photodetectors (PDs) are important components of biomedical spectroscopy systems. The choice of a detector depends upon the application, which dictates wavelength range, responsivity, dark current, size, acquisition speed, and acceptable noise. Semiconductor photodetectors operate through three main processes: 1) photon induced carrier generation, 2) carrier separation and transport, and 3) carrier extraction as an output signal.

Following electron-hole generation, the electrons and holes are separated by an
electric field to avoid recombination. Using either an external electric field or an internally built-in field, the carriers drift across the semiconductor material to the electrodes, where they are collected, resulting in a photocurrent with intensity proportional to that of incoming light.

The probability of generation of an electron-hole pair that contributes to photocurrent per incident photon (having sufficient energy) is defined by the external quantum efficiency ($\eta$), which is given by the following equation [63]:

$$\eta = \frac{I_{ph}/q}{P_{opt}/h\nu}, 0 \leq \eta \leq 1$$  \hspace{1cm} (2.12)

where $P_{opt}$ is the incident optical power and $I_{ph}$ is the induced photocurrent. For an ideal photodetector, the external quantum efficiency would be 1. However, a fraction of the incident photons reflect back from the device surface, don’t get absorbed, and do not contribute to photocurrent. To decrease back reflection and increase external quantum efficiencies, antireflection coatings are deposited onto the photodetector input surface. Non-radiative recombination of photogenerated electron-hole pairs (for example, recombination at trap centers) also prevents carriers from contributing to the photocurrent. Trap centers that are present at the semiconductor material surfaces can be minimized by careful surface cleaning, controlling contaminants during device fabrication, and passivation.

The responsivity ($R(\lambda)$) of a PD at a particular wavelength is defined as the photocurrent induced per unit optical power. Responsivity is linearly proportional to the quantum efficiency and the free-space wavelength. The spectral response is the responsivity as a function of wavelength.

In addition to quantum efficiency and responsivity, the noise characteristics of a detector determines its overall performance and appropriateness for a desired application. Random photocurrent fluctuations are regarded as noise, and quantified by
the standard deviation in photocurrent ($\sigma_i = \langle (i - \bar{i})^2 \rangle$). The main sources of noise in photodetectors include shot noise, thermal noise, and low-frequency noise.

Shot noise (Poisson noise) arises because of the discrete nature of the light and electrical current. The incoming photons arrive randomly with a mean photon flux ($\phi = \frac{P_{\text{in}}}{h\nu}$ (photons/s)); and the fluctuations in photon arrival are dictated by the light source. The number of photons within an integration period of $t_{\text{int}}$ (n), incoming from an ideal laser or a thermal light source with a spectral bandwidth much greater than $t_{\text{int}}$ obeys the Poisson distribution with a mean of $n = \phi t_{\text{int}}$ and variance of $\sigma_n^2 = n$. The randomness in photon arrival results in a similar randomness in carrier generation with mean generated carrier number (in interval $t_{\text{int}}$) of $\bar{m} = \eta n$ and variance of $\sigma_m^2 = \eta \bar{m}$.

Thermal noise (Johnson-Nyquist noise) arises from the random motions of carriers due to thermal fluctuations of the medium and results in randomness in the electrical current. The variance in electrical current in a medium with electrical resistance of $R_{\text{el}}$ at temperature $T$ is described by $\sigma_i^2 \approx 4kTB/R_{\text{el}}$, where $B$ is the bandwidth, and $k$ is the Boltzmann constant.

Low-frequency noise (flicker noise) or $1/f$ noise has spectral density with $1/f$ dependence. The surface properties of the semiconductor material primarily dictate the $1/f$ noise. Amplification of the high frequency signal components is used to reduce $1/f$ noise, whereas in continuous-signal systems with a large $1/f$ component, chopper stabilization techniques can be used.

Photodetectors generate electrical current in the absence of optical illumination as well, and this current is known as the dark current and contributes to photodetector noise. Dark current noise results from random nature of electron-hole pair generation due to thermal or tunneling processes.

Extraneous light sources (i.e. sunlight, roomlight) can also contribute to noise. This noise is known as background noise, and it can become significant at near- and
far-infrared wavelengths due to the significant thermal radiation of objects.

2.2.2 Semiconductor Photodetectors

PN/PIN Junction Photodiodes and Miniaturized Detection Technologies for Biomedical Applications

Photodiodes are attractive for biomedical applications since they are easy to fabricate, inexpensive, easily scalable, can be fabricated in arrays for spatial resolution, can have high responsivity and low dark current, and leverage low cost semiconductor manufacturing technology.

A p-n junction photodiode is a semiconductor device with a structure composed of adjacent n-doped and p-doped regions, and a p-i-n junction photodiode is a diode with an undoped region between the n-doped and p-doped regions. The diffusion of carriers (electrons from the n-doped region and holes from the p-doped region) to the oppositely doped adjacent region forms a space charge region (also called depletion region) free of carriers. This diffusion current is counter-balanced by a drift current induced by the built-in potential formed in the depletion region at equilibrium. Figure 2.4 illustrates the principle of operation of a pn junction photodiode. P-i-n junction photodiodes typically have wider depletion regions defined by the undoped regions.
The built-in junction potential ($V_0$), is governed by the equation [63]:

$$V_0 = \frac{kT}{q} \ln \frac{N_A N_D}{n_i^2} \quad (2.13)$$

where $N_A$ and $N_D$ are the acceptor and the donor concentrations of the p and n regions, respectively, $n_i$ is the intrinsic carrier concentration, $k$ is the Boltzmann constant, $T$ is the temperature, and $q$ is the electronic charge. The width of the depletion region ($W$) is:

$$W = \sqrt{\frac{2\varepsilon V_0}{q} \left( \frac{1}{N_A} + \frac{1}{N_D} \right)} \quad (2.14)$$

where $\varepsilon$ is the permittivity of the medium.

When photons with energies greater than the semiconductor material bandgap are incident on the photodiode, they are absorbed and generate electron-hole pairs. If this electron-hole pair generation occurs in the depletion region or within one diffusion length of the depletion region, these carriers drift because of the built-in electric field in the depletion region and can be collected by the electrodes, producing...
a photocurrent. Reverse bias on the diode increases the depletion width, resulting in reduced junction capacitance and response time. However, reverse biasing results in an increase of the dark current. The current-voltage relationship of a p-n junction photodiode is governed by the following equation [63]:

\[
I = I_0(e^{\frac{qV}{kT}} - 1) - I_{ph}
\] (2.15)

where, \(I_0\) is the diode saturation current and \(I_{ph}\) is the photocurrent, which is governed by the following equation [63]:

\[
I_{ph} = \eta_e \frac{eP_{opt}}{h\nu}
\] (2.16)

where the external quantum efficiency \(\eta_e\) is expresses as [63]:

\[
\eta_e = \eta_{coll}(1 - R)T_h(1 - e^{-\alpha W})
\] (2.17)

where, \(\eta_{coll}\) is the photogenerated carrier collection efficiency, \(R\) is the surface reflectivity, and \(T_h\) is the optical transmittance to the photodiode. Silicon photodiodes with appropriate anti-reflection (AR) coatings can approach 100% external quantum efficiency in the near infrared region.

Si photodiodes can be inexpensively miniaturized and integrated with biological and biomedical detection, spectroscopy, and imaging systems. Publications reporting integrated photodiodes for various biological and biomedical applications are increasing steadily with time. Some examples include a solution processed thin film photodiode as a compact and integrated detector for antioxidant screening [64], and a semiconductor p-i-n junction photodetector integrated with a vertical-cavity-surface-emitting laser for biomedical fluorescence sensing [65].

Biomedical imaging and spectroscopy researchers focus on cost-effective miniaturized systems, which can be employed at the bedside or at the point-of-care, both
of which are ideal uses for non-invasive optical monitoring. Examples of such systems include a fluorescence and diffuse reflectance spectroscopy system integrating thin-film optical filters and silicon photodiodes for early cancer detection [66], a flexible probe holding light-emitting-diodes (LEDs) and photodiodes for bedside monitoring of the newborn brain [67], and a hand held probe of eight dual-wavelength lasers and eight silicon photodiodes for near infrared tissue imaging [68]. Other reported systems include a ring-shaped photodiode with illumination LEDs used for reflectance pulse oximetry [69] and an implantable flexible probe with two LEDs and two photodiodes for multimodal measurements combining near-infrared spectroscopy and electrocorticography in a single apparatus [70].
A concentric multi-pixel photodiode array (CMPA) semiconductor probe was designed, fabricated, and tested for spatially resolved diffuse reflectance (SRDR) measurements. The concentric multi-pixel photodiode array (CMPA) probe has 24 pixels, and it is the most densely packed SRDRS probe reported to this date. We validated the probe on two tissue mimicking liquid phantoms. The maximum signal contrasts between the two phantoms were higher than 100%, and the average error between the Monte Carlo simulations and the experiments was less than 9%. The described probe is promising for low cost, portable SRDRS systems and has the potential to enable both superficial and layered tissue analysis. This chapter is modified from “Concentric Multi-Pixel Silicon Photodiode Array Probes for Spatially Resolved Diffuse Reflectance Spectroscopy” published in The Journal of Selected Topics in Quantum Electronics, 2016 [71].
3.1 DRS and SRDRS Systems

The majority of reported DRS instruments include a broadband light source, an imaging spectrograph, a CCD detector array, and a fiber optic probe with both illumination and collection fibers. The most common DRS fiber optic probe design has a central illumination fiber circularly surrounded by multiple collection fibers [23, 24, 27] for efficient photon collection and high signal-to-noise ratio (SNR). However, the circular fiber core, the presence of the fiber cladding, and a limited numerical aperture (NA) results in a probe that has a fill factor that is less than one for a given detection region radius, and has a low photon collection efficiency. For improved photon collection efficiency, Yu et. al. replaced the collection fibers with a commercial rectangular Si photodiode (PD) that was positioned next to an illumination fiber [26]. The higher NA of the Si PD compared to a typical fiber (0.96 vs. 0.22) and the PD direct contact with the tissue significantly improved the system performance. In addition to the elimination of the collection fibers, the expensive imaging spectrograph and the CCD could also be removed from the instrument, since the Si PD both collected and detected the reflected phonons, resulting in a significant reduction in cost and size. For further performance improvement and increased scalability, our group (Dhar et. al.) developed a custom diffuse reflectance spectral imaging probe comprised of a 4x4 array of annular Si photodiodes with central illumination apertures for intra-operative breast cancer margin assessment [29]. In this system, the source illuminated the tissue through the backside of the PD array (through the PD central apertures), and the front sides of the PDs face the tissue. The customized annular detector shape and the optimized PD spectral response further improved the system performance compared to that of commercial PDs and fiber optic probes. Additionally, our group (Dhar et. al.) reported thin film annular Si PDs on flexible substrates, which opens the door for conformal DRS imaging [30].
Each independent pixel on the reported custom 4x4 DRS Si probe [29] is in the form of a single source (central aperture)/detector (annular Si photodiode) pair. Each single large area photodiode has photons passing through the central hole, and these photons interrogate a bulk portion of the sample under study (the photons interrogate layers at multiple depths). The diffusely reflected photons are detected by the single PD; this PD measurement does not provide spatial information regarding the photon path.

In contrast, a typical fiber SRDRS probe incorporates multiple source-detector pairs (typically one source fiber and three to eight collection fibers) [21, 31, 52], and provides spatial information on the photon path. Each detector selectively collects scattered light from various layers of the tissue under study, as shown in Figure 3.1, and yields information specific to the interrogated layers. Thus, SRDRS measurements enable depth-resolved tissue analysis [31, 33, 34], which is important for detailed study of layered tissues such as the skin and cervix. Currently available SRDRS systems utilize fiber-based probes, which have difficulty in accommodating high-density and closely spaced source-detector pairs. In addition, fiber SRDRS probes have low light collection efficiency because of their finite numerical aperture (NA).
In this thesis chapter, we report on the design, fabrication, characterization, and validation of a new silicon (Si) concentric multi-pixel photodiode array (CMPA) probe with a central illumination aperture that enables SRDRS measurements. Pixels on the probe are positioned in radially increasing distances, and each pixel interrogates the tissue to a certain depth. The probes also have pixels positioned at very short distances from the illumination aperture center, enabling superficial tissue analysis as well as depth analysis. Additionally, closely spaced and densely packed detectors enable higher density SRDRS measurements compared to fiber-based SRDRS probes, and the higher PD effective NA compared to fibers results in a higher SNR than fiber probes under similar illumination conditions.
3.2 CMPA Probe Design

The design of the CMPA probe reported in this thesis was based on Monte Carlo simulations, which use the known (measured and reported in the literature) optical constants of benign and malignant breast tissue at wavelengths of 450 nm and 600 nm [28], the lower and the upper ends of the spectrum that is used in the clinical miniaturized spectral imaging system for breast cancer margin assessment. At these wavelengths, the major breast tissue chromophores oxy-hemoglobin (HbO₂) and deoxy-hemoglobin (Hb) exhibit high (λ=450 nm) and low absorption (λ=600 nm) relative to each other, which results in low and high diffuse reflectance signals correspondingly, and informed the detector dynamic range required for breast cancer margin assessment as one example practical application. The tissue optical coefficients used in the simulations are tabulated in Table 3.1.
Table 3.1: Typical optical properties of adipose and malignant breast tissues used in Monte Carlo simulations.

<table>
<thead>
<tr>
<th>Wavelength</th>
<th>Adipose</th>
<th>Malignant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\mu_a$ (cm$^{-1}$)</td>
<td>$\mu'_a$ (cm$^{-1}$)</td>
</tr>
<tr>
<td>450nm</td>
<td>11.29</td>
<td>7.44</td>
</tr>
<tr>
<td>600nm</td>
<td>0.55</td>
<td>6.45</td>
</tr>
</tbody>
</table>

The simulations showed logarithmically decreasing diffuse reflectance signals as a function of increasing radial distance ($r$) from the source. Figure 3.2 shows the simulated diffuse reflectance intensity versus radial distance for the illumination spot diameters of 100 $\mu$m, 400 $\mu$m and 750 $\mu$m. These simulations were performed by Dr. Justin Lo, in collaboration with Professor Nimmi Ramanujam at Duke University. For the most highly absorbing case, which is malignant breast tissue at $\lambda=450$ nm, the simulated diffuse reflectance per unit area at $r=0.5$ mm was 104 times that at $r=3$ mm, necessitating larger detecting areas for PDs positioned at farther distances from the source to achieve a target minimum detectable signal level resulting in an SNR of 20 dB (using area-scaled dark current).
Figure 3.2: Monte Carlo simulated reflectance normalized to input intensity vs. radial distance for adipose and malignant breast tissue optical properties at 450 nm and 600 nm. Different curves represent various illumination aperture sizes for the mentioned wavelengths and tissues. Simulations were conducted by Dr. Justin Lo.

The SRDRS probe designed for this thesis had an illumination aperture at the center, and 24 concentric semi-annular photodiodes grouped into three sets: 1) eight inner photodiodes with each photodiode of width (w)=50 µm; 2) eight middle photodiodes of w=100 µm; and 3) eight outer photodiodes of w=250 µm. The schematic illustration of the probe geometry is shown in Figure 3.3, and the width (w) was defined as the distance between the outer and the inner radius of each PD annulus.
Figure 3.3: Illustration of the CMPA probe. The probe contains three sets of photodiodes with annulus widths of 50, 100, and 250 µm. Each set is represented by different colors. The white circle in the middle represents the illumination aperture.

The theoretical current levels for each pixel of the CMPA probe design were calculated using the Monte Carlo reflectance data and the responsivities of p-n junction Si photodiodes reported previously by our group, which are 0.25 A/W for λ=450 nm, and 0.35 A/W for λ=600 nm [28]. Figure 3.4 shows the calculated photocurrent at each pixel of the design for illumination spot diameters (detector vias) of 100 µm, 400 µm and 750 µm. The simulated illumination optical power is assumed to be 100 µW for the 100 µm aperture diameter and scales up as the illumination aperture area scales up, in order to maintain constant optical power per unit area. The available
optical power (Xenon lamp, lasers) and the maximum permissible exposure for tissue as a function of wavelength dictate the maximum illumination light intensity per unit area. The presence of three discontinues regions in each of the CMPA simulations are due to the varying detector width in the three regions of the array. As Figure 3.4d shows, the calculated photocurrents are above 100 pA for all but the last three pixels of the array with a 100 µm aperture diameter in the worst case scenario, where $\mu_s'$ is low and $\mu_a$ is high. The calculated current levels are well above the expected dark currents, which are 1-20 pA.
Figure 3.4: Calculated photocurrents based on previously published Si photodiode responsivities [29] for three different illumination aperture diameters: a) 100 µm b) 400 µm c) 750 µm and d) comparison of the photocurrents for three apertures for the lowest signal case, which is malignant tissue interrogated by 450 nm light.

3.3 CMPA Probe Fabrication

Three different CMPA arrays with different aperture diameters (100 µm, 400 µm, and 750 µm) were fabricated, although results from only the 750 µm diameter CMPA are reported in this chapter. Figure 3.5 shows the schematic illustration of the fabrication process flow. The detector array fabrication started with RCA cleaning of an n-type epitaxial silicon wafer (5-7 µm thick epilayer, n-type 5-9 ohm-cm on a 600-610 µm thick substrate, n-type 0.025-0.045 ohm-cm). A 150 nm thick SiO₂ layer grown by thermal oxidation on both sides of the sample served as a diffusion mask.
The SiO$_2$ layer on the backside of the wafer was removed by buffered oxide etchant (BOE). In order to form an n+ back contact, the wafer backside was coated with phosphorous doped spin-on-glass (SOG) with $n=5\times10^{20}$ cm$^{-3}$ (Emulsitone). The SOG was annealed at 1050 $^\circ$C for 20 min under a N$_2$ ambient for dopant drive-in. The SOG on the back substrate face was then removed using buffered oxide etch (BOE). For defining the PD regions, a 130-150 nm thick diffusion mask SiO$_2$ layer on the front surface was then patterned by photolithography and partially etched using reactive ion etch (RIE), and the etch was completed using BOE to minimize surface damage. Next, a boron doped SOG with $p=5\times10^{19}$ cm$^{-3}$ (Emulsitone) was spin-coated on the front side of the wafer and annealed at 950 $^\circ$C for 15 minutes under a N$_2$ ambient to dope the p region, thus forming the pn junction. Next, the 150 nm layer of p-SOG was thinned to 50 nm using RIE. The PD absorbing area was then exposed using photoresist patterned using photolithography (using the same mask pattern) and the remaining SOG on top of the absorbing area was removed by BOE. The photoresist was removed, and the sample was then cleaned in a piranha solution, which helps to minimize dark currents. Next, semi-circular top contacts were patterned by photolithography on the front surface, and 250 nm of aluminum was deposited by e-beam evaporation followed by lift-off. The Al contacts were annealed at 530 $^\circ$C for 4 minutes under a N$_2$ ambient. Then, contact leads and wirebonding pads were patterned by photolithography and a Ti/Ni/Au (40nm/50nm/250nm) metal stack was deposited by e-beam evaporation followed by lift-off in acetone. A Ti/Au (80 nm/250 nm) blanket metal stack was deposited as a back contact. The through-wafer illumination aperture located in the center of the concentric PD array was patterned using 10 $\mu$m thick positive AZ9260 resist and photolithography, and a hole through the Si substrate was etched using a deep reactive ion etching Bosch process (DRIE, SPTS Pegasus). Finally, a 52 nm thick SiN$_x$ layer was deposited by plasma enhanced chemical vapor deposition (PECVD), to function as both an anti-
reflection (AR) coating and a surface passivation layer. The wirebonding pads were masked to avoid nitride deposition onto the wire bonding pads.
**Figure 3.5**: A schematic illustration of the CMPA fabrication process flow. Each material used is color-coded as illustrated at the side of the figure.
Figure 3.6a shows a photograph of a completed array, and Figure 3.6b shows a close-up photomicrograph of the same array, showing the illumination aperture. The blanket backside contact was silver epoxied and bonded to the printed circuit board, and the top contacts were wire-bonded to pads on a gold-coated FR-4 board for the electrical readout. Wire-bonds were potted with UV-curable epoxy for protection. Figure 3.6c is a photograph of the packaged CMPA probe. Additionally, Figure 3.7 shows close-up micrographs of the 100 µm and 750 µm aperture diameter CMPAs with all the 24 pixels. Multiple individual photomicrographs were stitched together to generate the pictures.
Figure 3.6: (a) A photograph showing the entire CMPA array with PDs and wire-bonding pads. The illumination aperture diameter of the array is 750 μm; (b) A close-up microphotograph showing the illumination aperture and the first few pixels of the array; (c) A photograph of the packaged array with connectors.
3.4 CMPA Probe Characterization

Both the PD dark currents and photocurrents were measured using the Keithley 4200 source measure units. Figure 3.8 shows the dark current vs. voltage measurements for all 24 pixels, where the plots of inner pixels are in green, those for middle pixels are in red, and those for the outer pixels are in blue. The average measured dark currents at 0 V bias of all the 24 photodiodes were between 1-20 pA (20 measurements per pixel were averaged).
Figure 3.8: Dark current vs. voltage measurements for all the 24 pixels, where the plots of inner pixels are in green, those for middle pixels are in red, and those for the outer pixels are in blue.
For surface normal responsivity characterization, a 450 W Xenon lamp (MAX-302, Asahi Spectra) with eight discrete bandpass filters (< 10 nm bandwidth) was used as the light source. A fiber bundle connected to the lamp, illuminated the each pixel separately through a 50 X microscope objective, and the induced photocurrents (at 0 V bias), were measured at eight different wavelengths of 470 nm, 480 nm, 490 nm, 510 nm, 560 nm, 580 nm, 590 nm, and 600 nm. The surface normal responsivities were calculated by dividing the average of the 20 photocurrent measurements by measured optical illumination power using a calibrated photodetector. The average responsivity of the 24 pixels are presented in Figure 3.9. The calculated responsivities were between 0.25 A/W to 0.35 A/W for the given wavelength range, and are comparable to commercial Si photodiode responsivities. The responsivity variations across the 24 pixels were less than 3%.
3.5 Experimental Phantom Testing and Probe Validation

For characterizing the probe performance, we conducted measurements on a 99% Spectralon reflectance standard (SRS-99-010, Labsphere, Inc., North Sutton, New Hampshire) and two liquid phantoms mimicking adipose and malignant breast tissues. All of the measurements reported in this chapter were performed using the CMPA probe with a 750 µm diameter aperture. A 450 W Xenon lamp connected to a monochromator (Gemini 180-Jobin Yvon Horiba) was used as the illumination source in all of the experiments. The light delivery used an optical light guide with a 1 mm diameter and a numerical aperture of 0.39. One end of the light guide was connected to the monochromator exit aperture and the other end was fixed to the backside of the FR-4 board above the CMPA illumination aperture using an aluminum collar. The typical optical power through the aperture was measured as
2.5 µW at a wavelength of 550 nm with ∆λ < 10nm. Eight Keithley 4200 source measure units were used for current measurements, and eight PDs were measured at once. For measuring the entire 24 PDs, the three groups of channels were switched manually. Back illumination caused photon absorption at the aperture sidewalls, resulting in undesired background signals that were detected primarily by the PDs closest to the illumination aperture. These currents (I_{BI}) were measured at 33 wavelengths, ranging from 350 nm to 990 nm with 20 nm steps, and each measurement was repeated 10 times. Figure 3.10 shows the I_{BI} as a function of pixel radial distance for wavelengths of 370 nm, 530 nm, 730 nm, 950 nm, and 970 nm. At shorter wavelengths, the measured back illumination induced currents were negligible for all PDs due to shorter photon penetration depths. As the wavelength increased, the photon penetration depth increased, resulting in increased I_{BI} at the inner pixels. At wavelength of 970 nm, the measured I_{BI} values for the first five pixels ranged from 1 nA to 16 nA.
Figure 3.10: Back illumination generated photocurrent (nA) as a function of PD radial distance (µm). Different colors represent the data for different wavelengths.
For wavelength calibration, reflectance measurements were performed on a 99% reflectance standard, which was placed 4.5 mm away from the detection plane using spacers. The same 33 wavelengths were used. The calculated SNRs [SNR=$20\log(\text{mean signal/standard deviation})$] based on the 99% reflectance standard measurements were above 40 dB for all of the 24 pixels at measurement wavelengths shorter than 650 nm, as shown in Figure 3.11. At the longer wavelengths, the increase in $I_B$ degraded the SNRs of the three innermost pixels, and the SNRs of these pixels ranged from 25 dB to 40 dB at these wavelengths.

Figure 3.11: The signal-to-noise-ratio (SNR) measured on 99% reflectance standard at $\lambda = 370, 530, 730, 950,$ and $970$ nm
To assess the effect of the puck to CMPA distance on the specular reflectance, we measured the photodetector signals (for all PDs) at each wavelength for different 99% puck to CMPA distances: 0.51, 1.02, 1.59, 4.5, and 5.82 mm. For all puck distances, the sums of the measured photocurrents of the 2nd-8th pixels at all wavelengths \( (i_{puck(2-8)}(\lambda)) \) were normalized to the maximum sum of the photocurrents measured at \( (\lambda) \). The Figure 3.12a below shows the normalized reflectance standard measurements \( \left( \frac{i_{puck(2-8)}(\lambda)}{i_{puck(2-8)}(\lambda_{max})} \right) \) for the given puck distances. The resulting spectra for all the distances agree very well (the largest difference at \( \lambda=690 \text{nm} \) is < 3.5%), and it indicates that the specular reflection is negligible. The first detector was not included due to back illumination noise. When pixels 2-24 were averaged, the largest difference in normalized reflectance standard measurements was as high as 15% (again, at \( \lambda = 690 \text{ nm} \)). Figure 3.12b shows the normalized reflectance standard measurements for pixels 2-24.
Figure 3.12: Normalized 99% puck measurements vs. wavelength (nm) for five varying puck distances of 0.5, 1.02, 1.59, 4.5, and 5.82 mm. a) summed over pixels 2-8, b) summed over pixels 2-24. The data points were connected to aid in the data viewability.
Since the responsivity of the pixels (separately measured) was uniform across all pixels, we can use a subset of the pixel puck reflectance that is less affected by specular reflectance to calibrate the measurements. So, to correct the wavelength dependence for the data presented for the phantoms, we used the readings from the $2^{nd}$-$8^{th}$ pixels (from the 99% reflectance standard (puck)). Next, two liquid phantoms which are representative of benign and malignant breast tissue were measured; targeting previously reported tissue optical properties [28]. Both the scattering and absorption coefficients of the malignant phantom were higher than those of the benign. Two 1 $\mu$m diameter polystyrene sphere scatterers (07310-15, Polysciences, Inc.) with different weight to weight concentrations (malignant:0.1124 and benign:0.0754), and two human Hb absorbers (H0267, Sigma Co.) with different weight to weight concentrations (malignant:0.0064 and benign:0.0034) were mixed in deionized water to create the phantoms. The scattering coefficients were calculated using Mie theory, and the Hb absorption spectrum was obtained by spectrophotometric measurements of a diluted Hb solution. The scattering coefficients of the phantoms ranged from 45 cm$^{-1}$ to 160 cm$^{-1}$, and the absorption coefficients ranged from 0.001 cm$^{-1}$ to 45 cm$^{-1}$ for the wavelength range of 350 nm to 850 nm. Figure 3.13 shows the $\mu_s'$ and $\mu_a$ spectra for both of the phantoms.
Figure 3.13: Scattering coefficient ($\mu_s$) and absorption coefficient ($\mu_a$) spectra of the malignant and the benign tissue mimicking phantoms

The liquid phantoms were contained in 14 ml black vials, which were capped with 40-60 $\mu$m thick transparent Teflon film (23-FEP-2-36, CS Hyde Company). The vials were inverted onto the CMPSA PD array, and the diffuse reflectance induced photocurrents were measured at the same 33 wavelengths, ten times per wavelength. The measured photocurrents ranged from 2 pA to 35 nA, as shown in Figure 3.14.
Figure 3.14: Photocurrent measured at $\lambda=370, 450, 570, 590, 630, \text{ and } 750 \text{ nm}$ for the two liquid phantoms vs. PD radial distance. The circles represent the data for malignant tissue mimicking phantom, and the squares represent the data for benign tissue mimicking phantom. Different colors represent different wavelengths.

For wavelength calibration, measured photocurrents (A) on the phantoms were normalized with respect to the photocurrent (A) measured on the 99% reflectance standard, yielding the scaled reflectance. The following equations describe the normalization with respect to puck measurements. The photocurrent registered by the pixel located at radial distance $\rho$ due to diffusely reflected flux from the phantom/sample at wavelength $\lambda$ can be described by the equation given below:

$$i_{\text{phantom, } \rho}(\lambda) = R_{\text{phantom}}(\lambda, \rho) I_o(\lambda) r(\lambda)$$  \hspace{1cm} (3.1)

where $I_o(\lambda)$ is the input flux (W) through the illumination aperture at wavelength $\lambda$, $r(\lambda)$ is the responsivity (A/W) of the photodiodes at wavelength $\lambda$, and $R_{\text{phantom}}(\lambda, \rho) = \frac{I_r(\lambda)}{I_o(\lambda)}$ is the dimensionless absolute diffuse reflectance which is the ratio between the
flux diffusely reflected back from the phantom and collected by the pixel located at
distance $\rho$ and the input flux at $\lambda$.

To calculate the wavelength normalization factor, the puck measurements from
pixels 2-8 were used, which were minimally affected by specular reflection as ex-
plained previously. The total collected puck photocurrent from pixels 2-8 due to the
diffusely reflected flux from the puck (calibration standard) located at 4.5 mm away
from the detection plane is:

$$i_{\text{puck-col}}(\lambda) = I_0(\lambda) r(\lambda) \sum_\rho R_{\text{puck-col}}(\lambda, \rho)$$  \hspace{1cm} (3.2)

where $R_{\text{puck-col}}(\lambda, \rho)$ is the dimensionless diffuse reflectance, which is the ratio be-
tween the flux diffusely reflected back from the puck and collected by the pixel located
at distance $\rho$ and the input flux at $\lambda$.

$R_{\text{puck-col}}(\lambda) = \sum_\rho R_{\text{puck-col}}(\lambda, \rho) = R_{\text{puck-col}}$ is the total reflectance registered by
pixels 2-8 and it is constant over the measurement wavelengths as given by the puck
vendors specifications. In the experiments, a 99 \% reflectance standard was used, and
$R_{\text{puck-col}}$ ideally would be 0.99. However due to the finite spatial detection range, a
smaller portion of the 99\% back reflected flux could be collected, and $R_{\text{puck-col}} < 0.99$.

Therefore, we can obtain the scaled or relative diffuse reflectance, $S_c R_{\text{phantom},\rho}(\lambda)$,
from the phantom for each pixel and $\lambda$ as follows (actual corresponding diffuse re-
reflectance values are scaled by $R_{\text{puck-col}}$ for all conditions):

$$S_c R_{\text{phantom},\rho}(\lambda) = \frac{i_{\text{phantom},\rho}(\lambda)}{i_{\text{puck-col}}(\lambda)} = \frac{R_{\text{phantom}}(\lambda, \rho)}{R_{\text{puck-col}}(\lambda, \rho)} = R_{\text{puck-col}}^{-1} R_{\text{phantom}}; \quad \hspace{1cm} (3.3)$$

Finally, to take into account varying PD areas, the scaled reflectance was normal-
ized with respect to photodiode area (mm$^2$), yielding the scaled reflectance per unit
area. Figure 3.15 shows the scaled reflectance (mm$^{-2}$) as a function of PD radial distance at six wavelengths: $\lambda = 370$ nm, 450 nm, 570 nm, 590 nm, 630 nm, and 750 nm for both phantoms (M=malignant, B=benign). The data shown in Figure 3.15 have a faster decay at wavelengths where the measured absorptions are higher (highest at 370 nm among the 6 wavelengths) as predicted by Beers law. The variance in the data is less than 4%, thus the error bars are not visible in the plot. Figure 3.16a and 3.16b show the full 33 wavelength data as functions of both the PD radial distance and the wavelength for the malignant and benign tissue-mimicking phantoms, respectively.
Figure 3.15: Scaled reflectance ($\text{mm}^{-2}$) (measured photocurrent normalized to 99% puck photocurrent measurements and photodetector area) versus detector mid-position at $\lambda = 370, 450, 570, 590, 630$, and $750$ nm. The data both for the malignant and the benign tissue mimicking liquid phantoms are shown.
Figure 3.16: Scaled reflectance (mm$^{-2}$) versus detector mid-position and wavelength (in linear scale). The shown data obtained from a) the malignant tissue-mimicking phantom; b) the benign tissue-mimicking phantom.
The signal contrast between different tissues is an important figure of merit for differentiation between benign and malignant tissues. The contrast between these signals was calculated using the malignant and benign breast tissue mimicking liquid phantom measurements for all 24 pixels and 33 wavelengths. The equation below describes the signal contrast:

\[ S_{i,\lambda} = 100 \frac{M_{i,\lambda} - B_{i,\lambda}}{B_{i,\lambda}} \] (3.4)

where \( M_{i,\lambda} \) is the signal measured by PD i at wavelength \( \lambda \) on the malignant tissue mimicking phantom, and \( B_{i,\lambda} \) is the signal measured on the benign tissue mimicking phantom. Figure 3.17 shows the signal contrast as functions of PD radial distance and the wavelength for all 33 measured wavelengths. For \( \lambda > 600 \) nm, the Hb absorption is negligible, and the major source of contrast is the scattering. Since the scattering of the malignant phantom is higher compared to that of the benign phantom, the measured signals on the malignant phantom are higher in this wavelength range, resulting in a positive signal contrast. In this region, the middle group of PDs provides the maximum contrast, which is as high as 60%. For \( \lambda < 450 \) nm, the Hb has significant absorption, and it is the major contrast source, resulting in negative signal contrast. In this wavelength region, the outermost PDs provide the maximum signal contrast, which is higher than 100% (this is negative in Figure 3.6). In the region between \( \lambda = 450 \) nm and 600 nm, the Hb absorption increase (\( \Delta \mu a \)) in the malignant phantom is slightly larger than that of the benign phantom. Photodetectors positioned at shorter radial distances collect photons with shorter path lengths that undergo less absorption. Thus, for the innermost detectors, the signal increase due to increased scattering is higher than the signal decrease due to absorption, yielding a positive signal contrast. As the PD radial distance increases, the photon path lengths increase, resulting in higher photon absorption, yielding a
higher signal decrease. Hence, the signal contrast becomes zero (white regions of
the graph, where signal increase due to increase in scattering is balanced by signal
decrease due to absorption) as the photon path length increases (for intermediate
PD distances). Then, the contrast becomes negative for further increases in distance
(i.e. for the outermost PDs).
3.6 Comparison of Theory to Experiment

To compare the measured results to theory quantitatively, Monte Carlo simulations were conducted using the commercially available ray tracing software, ZEMAX. The built-in bulk (volumetric) scattering model and Henyey-Greenstein phase function were used to simulate the tissue. The input parameters of the model are the anisotropy factor \( g \), mean free path \( \mu \), and the transmission \( T \), known also as albedo in tissue optics. The variable \( g \) is the average scattering angle \( \langle \cos \theta \rangle \), which is calculated based on the phantom constituents using Mie theory. The mean free path and the transmission are calculated using the following equations:

\[
\mu = \frac{1}{n} \int n \cos \theta \, d\nu \\
T = \frac{1}{\rho} \int \rho \cos \theta \, d\nu
\]
\[ M = \frac{1}{\mu_s + \mu_a} \quad (3.5) \]

\[ T = \frac{\mu_s}{\mu_s + \mu_a} \quad (3.6) \]

The simulation geometry was a 40 mm x 40 mm x 40 mm cube with absorptive bottom and side faces and a transparent front face, and 1x10⁶ photons were launched. The photon fluxes collected by each of the modeled 24 annular detectors (with 2 \( \mu m \) radial resolution and 1 angular resolution) were normalized to the input flux, resulting in the calculated absolute diffuse reflectance values for each pixel for the corresponding wavelengths. In order to mimic the experimental normalization, we also modeled the 99% reflectance standard measurements using a 99% reflective lambertian surface; which is used to normalize the calculated absolute reflectance values. Figure 3.18 shows both the Monte Carlo simulation results and the measured data for scaled reflectance (mm⁻²) as a function of PD radial distance at \( \lambda = 450 \) nm, 570 nm, and 590 nm. We calculated the mean error over these three wavelengths and 24 detectors to be less than 9%. The scattering from Teflon film surface is the main source of this error.
Figure 3.18: Scaled reflectance (mm$^{-2}$) (measured photocurrent normalized to puck measurements and photodetector area) (circles) and Zemax Monte Carlo simulations (dashed lines) versus detector mid-position at $\lambda$ = 450 nm, 570 nm, and 590 nm for the malignant tissue mimicking liquid phantoms.
High Density Spatially Resolved Diffuse Reflectance Measurements Using a Custom Silicon Probe

This chapter reports on extensive spatially resolved diffuse reflectance spectroscopy (SRDRS) phantom measurements using the most densely packed, and only custom silicon photodiode array (CMPA) reported to date. The measurements agree well with the forward Monte Carlo simulations. The measured phantoms cover a wide range of optical properties with a $\mu'_s$ range of 0.6-2.8 mm$^{-1}$ and a $\mu_a$ range of 0-7.58 mm$^{-1}$, which is the most extensive $\mu_a$ range reported to date for SRDRS. The dense SRDRS measurements provide unique reflectance data for more than 96% of the measured $\mu'_s$ and $\mu_a$ pairs, which is promising for rapid empirical model development, facilitating real-time feature extraction for clinical use.

4.1 Introduction

Diffuse reflectance spectroscopy (DRS) enables minimally-/non-invasive medical tissue diagnostics, and has been extensively investigated for practical clinical implementation. DRS typically quantifies the reduced tissue scattering coefficient ($\mu'_s$)
and the absorption coefficient ($\mu_a$) in the visible and near-infrared regimes, which are indicative of tissue morphology (i.e. cellular and sub-cellular tissue structures) and tissue composition (i.e. chromophores and their concentrations). DRS research includes theoretical/computational modeling of photon propagation in tissue [72–76], developing fast and robust algorithms for real-time optical property extraction to facilitate the clinical use of the technique [24, 77], and developing improved functionality and clinically implementable time-resolved [78], frequency domain [79], and steady-state [80, 81] DRS instruments. Applications include developing custom DRS systems for specific clinical targets such as intra-operative breast cancer margin assessment [14, 82], diagnosis of various types of cancer (e.g. skin cancer [4, 83], and cervical cancer [84]), monitoring tissue oxygenation [12], and characterizing tumor response to cancer therapy [13]. Recent publications have reported on the use of DRS for the investigation of epithelial tissue, where a majority of cancers emerge [85], aiming for early cancer diagnosis, and on quantifying higher order moments of the tissue scattering phase function [59], which provides additional contrast for tissue discrimination.

In all DRS methods (time-resolved, frequency-domain, steady-state), a source illuminates the tissue under study, and the launched photons are scattered or absorbed as they traverse the tissue. A fraction of these diffusely reflected photons exit the tissue surface and are collected by one or more fibers or detectors. In general, time-resolved and frequency-domain DRS instruments are more complex, bulky and costly in comparison to those of steady-state DRS methods, making them less attractive for clinical settings, and these approaches require larger sampling volumes to average out tissue inhomogeneities [48], limiting the source-detector spacing to greater than 1 cm, and limiting their use to non-endoscopic applications. Thus, research has been mostly focused on steady-state DRS methods: Spatially-resolved DRS (SRDRS), spectrally-resolved DRS (SpRDRS), and the two methods combined.
that is spatially- and spectrally-resolved DRS (SSRDRS). For certain $\mu_s$ and $\mu_a$ values, simultaneous use of spectral and spatial information in SSRDRS was shown to increase the accuracy of optical property extraction [31], which is typically achieved by fitting the resulting reflectance data to a forward mathematical model or by comparison to a pre-calculated database [81], where the types and the absorption spectra of the chromophores are a-priori information. These forward models include analytical models using the diffusion approximation [41] and numerical models based on Monte-Carlo (MC) simulations (currently considered state-of-the-art [42]).

In addition to these well-established physical models, to enhance clinical applicability through real-time optical property quantification, researchers have developed more rapid and robust empirical models with property extraction accuracies comparable to those of inverse physical models [86, 87]. However, these models consider only low tissue absorption ($< 1 \text{ mm}^{-1}$) levels. Empirical models are typically trained on experimental or, most commonly, on MC simulated reflectance datasets (i.e. simulations mimicking the experimental data for a given probe geometry) applying regression techniques including partial least squares (PLS) [88], support vector machines (SVM) [88], and artificial neural networks (ANN) [87, 89–92]. ANN is a commonly used technique, and typically uses spatially-resolved diffused reflectance (SRDR) measurements (i.e. reflectance measurements at multiple source-detector spacings (SDSs)), and does not require a-priori information about the sample under test, such as chromophore types and absorption spectra. In the majority of ANN publications, the models were trained, validated, and tested on MC simulated reflectance with a known probe geometry.

The number and range of the SDSs used in these reported models varied extensively, and there are not any systematic studies showing how the density and the spatial range of reflectance measurements affect the extraction accuracy for an extensive optical property range. In a recent publication, Chen et al. reported a significant
improvement in the accuracy of the ANN model when the simulated reflectance at SDSs of 1 mm and 2 mm were used in the model instead of those at 2 mm and 3 mm [86]. In this work, 0.5% noise was assumed (i.e. added to the simulated reflectance to mimic experimental measurements), and results only for a very limited optical property range ($\mu_a < 0.5 \text{ mm}^{-1}$) were reported. Typically, the noise is larger for higher absorption samples, and it is not clear whether the measurement spatial density would be sufficiently predictive beyond the limited absorption range reported to produce accurate results. In general, for model training, MC simulations were preferred over measured data because of the lack of extensive experimental data, however this introduces the problem of overlooking possible experimental biases. For example, the inverse ANN model trained on a simulated dataset in [54] resulted in a higher extraction error (27%) when implemented on experimentally measured reflectance data, whereas the error was lower (19%) for simulated test data.

Recent collaborations between research groups with expertise in modeling and those producing extensive experimental data facilitated development and comparison of empirical models based on experimental data [88], yet this work is still limited to simple probe geometries. The availability of experimental data obtained on tissue-mimicking phantoms with a wide range of optical properties and using probes with dense geometries would facilitate development of improved models and validation of models on experimental data. For example, Jager et al. [89] developed an ANN model trained on simulated data that used derivatives of diffuse reflectance measurements at two SDSs whose values are not constant, but rather, are determined based on the optical properties to be extracted, thus requiring reflectance measurements at many SDSs when a wide range of optical properties are considered. Thus, a densely spaced SRDRS probe used to measure a wide range of phantom properties could be a significant step forward for SRDRS model validation. This chapter reports on the most dense SRDRS probe reported to date, with extensive phantom testing,
including the largest absorption coefficient range ever reported for SRDRS.

Previously reported SRDRS/SSRDRS instruments are based on non-contact free-space optical (hyperspectral for SSRDRS) imaging systems [35], and on fiber-based probes incorporating a source fiber and multiple collection fibers [21, 31, 32]. Generally, non-contact free space systems require additional system characterization (i.e. characterization of the point spread function (PSF) of the camera system) [93], and they are less desirable in clinical settings due to their large size, complexity and cost. Fiber-based systems are convenient for clinical settings, however they have lower light collection efficiency due to low fill-factor (i.e. the circular shape of the fibers and the fiber cladding reduces the percentage of the collection area compared to the total area) and due to the low fiber numerical aperture, which is small compared to photodiode probe systems.

The use of photodetectors (PDs) for simultaneous photon collection and detection is advantageous due to the high numerical aperture of PDs compared to fibers, and the PDs can be implemented with high fill factors and customized sizes and geometries. The use of PDs also enables the elimination of the expensive imaging spectrograph and CCD instruments present in fiber-based systems, resulting in significant reductions in system size and cost [29]. Photodiode-based systems are inexpensive and compact, and are appropriate for clinical, field, and home settings, and can be implemented in endoscopic implementations. Annular Si PDs, including thin film devices heterogeneously integrated on rigid and flexible substrates have been reported by our group [30], enabling flexible conformal probes and endoscopic probes.

This chapter reports on a comprehensive multi-spectral phantom study using the new concentric multi-pixel Si photodiode array (CMPA) SRDRS probe. This probe is the most densely packed SRDRS probe reported to date, and the only custom photodiode-based probe reported to date. This custom probe does not use commer-
cial PDs, but rather a PD array specifically designed and fabricated for SRDRS. In the SRDRS research reported in Chapter 3, the initial tests of this probe were reported for a limited set of absorption and scattering coefficients, (i.e. the measurements were conducted for $\mu_s'$ range of 0.45-1.6 mm$^{-1}$ and $\mu_a$ range of 0-4.5 mm$^{-1}$; the validation was performed at three discrete $\mu_s'/\mu_a$ pairs: 16.0/0.63 mm$^{-1}$, 13.48/0.46 mm$^{-1}$ and 19.96/0.13 mm$^{-1}$) [71]. Herein, the phantoms measured and validated cover a broad range of tissue optical properties, with a $\mu_s'$ range of 0.6-2.8 mm$^{-1}$ and a $\mu_a$ range of 0-7.58 mm$^{-1}$, which is a wider range of $\mu_a$ than has been reported by any other SSRDRS system to date. Previously reported SSRDRS systems used a limited range of absorption coefficients (less than 1 mm$^{-1}$), which excludes some tissues, e.g. skin (0.001 < $\mu_a$ < 10 mm$^{-1}$) [39, 94].

The photodetectors within CMPA probe face the target tissue/phantom, and light illuminates the target through the aperture in the Si. The diffusely scattered photons are collected by the concentric photodetectors, which are in contact with the target. The experimentally measured data and the corresponding forward MC simulations are compared for a total of 1230 $\mu_s'$ and $\mu_a$ pairs in the given range to assess the probe limits and experimental accuracy. This data could potentially be used for side-by-side comparison of empirical optical property extraction models trained on simulated data and measured data. Additionally, to provide guidance for the use of this large experimental data set for model development, we report on the availability of unique reflectance data for the measured 1230 $\mu_s'$ and $\mu_a$ pairs.

4.2 Materials and Methods

4.2.1 Experimental Setup

The custom Si CMPA probe is composed of twenty-four concentric, semi-annular photodiodes (PDs) positioned around a 750 $\mu$m diameter illumination aperture etched through a 600 micron thick Si wafer; fabrication details are available in Chapter 3.
The PDs are arranged in three sets with radially increasing annulus widths to compensate for the exponentially decaying signal as a function of radial distance. The first set of eight photodiodes (numbers 1-8) has annulus width (w) of 50 µm for each PD, the middle set of eight photodiodes (numbers 9-16) has w of 100 µm, and the outermost set of eight photodiodes (numbers 17-24) has w of 250 µm; concentric PD 1-12 are shown in the photomicrograph in Figure 4.1.

The experimental setup used a 450 W Xenon lamp connected to a monochromator (Gemini 180-Jobin Yvon Horiba) with a light guide for the illumination source, the CMPA probe for reflected light collection and detection, and Keithley 4200 source measure units (SMUs) for measurement of photodiode currents, as shown in Figure 4.1. The optical light guide had a 1 mm diameter and N.A. of 0.39. The distal end of the light guide was connected to the monochromator exit aperture and the other end was fixed at the backside of the probe above the illumination aperture using an aluminum mount. The typical optical power through the aperture was measured as 2.5 µW at wavelength of 550 nm with bandpass < 10 nm.
Figure 4.1: The experimental setup used to perform liquid phantom measurements: a Xenon lamp with a monochromator for the source, light guide delivery to the CMPA, the CMPA, Keithley Source-Measure Units for current measurement, and a computer for instrument control and data collection. The inset is a photomicrograph of the CMPA showing the first 15 concentric pixels and the aperture and the contacts to the photodetectors (gold on the blue background).

4.2.2 Phantom Construction

The homogenous liquid phantoms, which are representative of human tissue optical properties in the UV-VIS range [39], were prepared using 1 µm diameter polystyrene spheres (07310-15, Polysciences, Inc., Warrington, Pennsylvania) as the scatterers, and either human Hemoglobin (Hb) (H0267, Sigma Co., St. Louis, Missouri) or water-soluble Nigrosin powder (N4754, Sigma Co., St. Louis, Missouri) as the absorber. In total, six phantom groups were prepared. Internal to each group, the scattering properties are similar, and in each group, there were five phantoms with varying absorber levels. In each group, the first phantom (base phantom) was prepared by suspending a target amount of polystyrene spheres in deionized (DI) water that did not contain any absorber. Next the remaining four phantoms in each group were prepared by adding four titrations of a constant amount of absorber solution. Note that each titration slightly decreased the scatterer concentration within each set. Table 4.1 summarizes the $\mu'$ and $\mu_a$ values of the prepared phantoms. The $\mu'$,
values were calculated by Mie theory and the $\mu_a$ values were calculated based on spectrophotometer measurements of the Hb and Nigrosin absorber stock solutions over the wavelength range of 400-800 nm. The liquid phantoms were contained in glass vials with a diameter of 1.3 cm and height of 3 cm, and capped with 40-60 $\mu m$ thick transparent Teflon film (23-FEP-2-36, CS Hyde Company) to avoid liquid spilling during the measurements.
Table 4.1: Average $\mu_s'$ and $\mu_s$ range for the wavelength range of 400-800 nm for the six phantom groups composed of five phantoms each.

<table>
<thead>
<tr>
<th>Phantom Group</th>
<th>Average $\mu_s'$ (mm$^{-1}$)</th>
<th>$\mu_s$ range (mm$^{-1}$)</th>
<th>Absorber</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.119</td>
<td>0.921-1.412</td>
<td>Hb</td>
</tr>
<tr>
<td>2</td>
<td>0.752</td>
<td>0.619-0.949</td>
<td>Nigrosin</td>
</tr>
<tr>
<td>3</td>
<td>1.133</td>
<td>0.933-1.430</td>
<td>Nigrosin</td>
</tr>
<tr>
<td>4</td>
<td>1.492</td>
<td>1.228-1.883</td>
<td>Hb</td>
</tr>
<tr>
<td>5</td>
<td>1.810</td>
<td>1.489-2.284</td>
<td>Nigrosin</td>
</tr>
<tr>
<td>6</td>
<td>2.230</td>
<td>1.835-2.814</td>
<td>Nigrosin</td>
</tr>
</tbody>
</table>
Table 4.2: Average $\mu_a$ and $\mu_a$ range for the wavelength range of 400-800 nm for five different absorption levels of the phantom groups. Phantom groups 1 and 4 use hemoglobin absorbers, while phantom groups 2, 3, 5, 6 use nigrosin absorbers.

<table>
<thead>
<tr>
<th>Phantom Group</th>
<th>$\mu_a$ level</th>
<th>Average $\mu_a$ (mm$^{-1}$)</th>
<th>$\mu_a$ range (mm$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.088</td>
<td>0.003-0.869</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.130</td>
<td>0.004-1.279</td>
<td></td>
</tr>
<tr>
<td>(Hb absorber)</td>
<td>4</td>
<td>0.166</td>
<td>0.005-1.628</td>
</tr>
<tr>
<td>5</td>
<td>0.208</td>
<td>0.006-2.048</td>
<td></td>
</tr>
<tr>
<td>2,3,5,6</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.484</td>
<td>0.242-0.689</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.926</td>
<td>0.463-1.1319</td>
<td></td>
</tr>
<tr>
<td>(Nigrosin absorber)</td>
<td>4</td>
<td>1.327</td>
<td>0.664-1.891</td>
</tr>
<tr>
<td>5</td>
<td>1.701</td>
<td>0.851-2.424</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.128</td>
<td>0.004-1.254</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.197</td>
<td>0.006-1.935</td>
<td></td>
</tr>
<tr>
<td>(Hb absorber)</td>
<td>4</td>
<td>0.385</td>
<td>0.012-3.778</td>
</tr>
<tr>
<td>5</td>
<td>0.772</td>
<td>0.023-7.581</td>
<td></td>
</tr>
</tbody>
</table>
4.2.3 Forward Monte Carlo Simulations

Our forward model uses the known phantom optical properties, $\mu_a$ and $\mu_s$, to model the diffuse reflectance signal measured at the surface of the target samples. In this study, forward Monte Carlo (MC) simulations were performed using the Virtual Tissue Simulator (VTS) Matlab Package [95]. The Henyey-Greenstein phase function (with anisotropy ($g$) values calculated by Mie theory for each wavelength) was used to model the probability distribution of single scattering angles. In the simulations, the refractive indices of the phantom and the Teflon cladding were 1.335 and 1.365, respectively. Forward MC simulations were performed for each detector for a total of 1230 $\mu_s$ and $\mu_a$ pairs (30 phantoms x 41 wavelengths) using a point source, and the photon flux at the surface of the modeled target was recorded for source to detector distances which are within 3.7 mm, divided into 1 $\mu$m bins. All of the photons exiting the surface were collected by the probe for computational efficiency, and the effect of detector NAs were taken into account during probe calibration. For each simulation, $5 \times 10^5$ photons were launched into the two-dimensional simulation geometry (radial symmetry was assumed) with a radius of 3.7 mm and depth of 3 cm. Next, the simulated impulse responses were convoluted with the measured beam profile, and the respective currents for each of the PDs in the CMPA probe were calculated by summing the readings of corresponding bins, which fall spatially into a particular detector area. Finally, the calculated sum was normalized to the detector annulus area, resulting in reflectance per unit area. The final results were compared to the experimentally measured data to validate the model.

4.3 Results and Discussions

The experiments were conducted in the following order: First, PD currents were measured without a target while the probe was illuminated from the back side to
assess back illumination induced photocurrent (due to photons absorbed at the 600 
µm thick aperture side walls) and the PD dark currents. Next, for calibration pur-
poses, photocurrent measurements were performed on a 99% Spectralon reflectance 
standard (SRS-99-010, Labsphere, Inc., North Sutton, New Hampshire) positioned 
4.5 mm away from the detection plane. Finally, the teflon-clad phantom container 
was inverted onto the upwards facing front (PD) side of the CMPA probe, and re-
flectance measurements were performed. Each measurement was repeated ten times. 
Measurements on each phantom (41 wavelengths) took 10 minutes. For assessing 
whether bead sedimentation occurred during the measurement period, we performed 
a second measurement on the phantom using the first measured probe channel, which 
incorporates the closest set of PDs to the illumination aperture. This first channel 
is the most sensitive one to the sedimentation due to the proximity of the sampled 
volume to the teflon cladding, since the container was inverted and the beads would 
accumulate on the teflon cladding, facing the PD, if sedimentation occurred. The 
difference between the two measurements was less than 4%, indicating that there is 
no significant sedimentation.

The measured photocurrent was normalized by the puck measurements and the 
PD area, resulting in a scaled diffuse reflectance per unit area ($R_s$) given by the 
equation below:

$$R_s^i(\lambda) = \frac{I_{ph}^i(\lambda) - I_{BI}^i(\lambda)}{A^i \sum_{k=2}^{8} I_{puck}^k(\lambda)}$$

(4.1)

where $I_{ph}^i(\lambda)$ is the photocurrent measured by the $i^{th}$ detector at wavelength $\lambda$, $I_{BI}^i,\lambda$ is 
the back illumination induced photocurrent (and includes the dark current), $A^i$ is the 
area of the $i^{th}$ PD, $I_{puck}^k,\lambda$ and is the photocurrent measured on the puck by the $k^{th}$ PD 
at wavelength $\lambda$. We used the readings of the 2$^{nd}$ – 8$^{th}$ pixels for puck calibration, 
since these pixels exhibit minimal specular reflection: when measured at different
puck to detection plane distances (0.5-5.8 mm), the difference was less than 3.5%.

This comprehensive experiment yielded a large quantity of data, of which we will publish herein a subset. The measured data produces diffuse reflectance scaled to the puck measurements, whereas the forward MC simulations yield absolute reflectance values, necessitating a calibration between the two to perform a comparison. We calibrated the forward Monte Carlo model by taking the ratio of the diffuse reflectance spectra measured on a reference phantom and the corresponding MC simulation spectra for the same set of optical properties for each photodiode. Next, to make the simulated spectra equivalent to the spectra measured on target phantoms, the simulation results were multiplied by this ratio. To ensure that the optical properties used in the calibration set did not bias the results, twelve out of the 30 measured phantoms (phantoms from the first two absorption levels of the six phantom sets) were used as reference phantoms. The remaining 18 (phantoms from the third, the fourth and the fifth absorption levels of the six phantom sets) were not included in the reference set due to their significantly high absorption coefficients, which result in very low signals on the outer PDs (i.e. below the detection target for the outermost ones), which could bias the calibration. In each calibration cycle (twelve cycles in total), one of these twelve phantoms was used as the reference phantom, and the mean percent error (MPE) between the simulation results and the experimental data were calculated for the remaining 29 phantoms.

The measured scaled diffuse reflectance spectra are in good agreement with the corresponding simulation results. Figures 2a and 2b show the pairs of $\mu'_s$ and $\mu_a$ for measured phantom groups 4 and 5 as an example. The values plotted in blue are the $\mu'_s$ values, and in red are the $\mu_a$ values. The phantoms within each group were positioned such that the leftmost set of plots belong to the $\mu_a$ level 1 (from Table 4.2), and the rightmost positioned ones belong to the $\mu_a$ level 5 (from Table 4.2). Figures 2c and 2e are the measured data (points) and forward MC simulations (dashed lines)
for the $\mu'_s$ and $\mu_a$ values shown in Figure 4.2a, for phantom group 4 (Hemoglobin absorber) for PDs with source detector separations (SDSs) of 0.6 mm and 1.25 mm, respectively. Figures 4.2d and 4.2f show the measured (data points) and forward MC simulation (dashed lines) for phantom set 5 (Nigrosin absorber) for the $\mu'_s$ and $\mu_a$ values in Figure 4.2b, again at SDSs of 0.6 mm and 1.25 mm, respectively. The error bars in the measured data are less than 2%, and are not visible in the figure. The simulation results shown in Figure 4.2c-f were calibrated using the mean of all of the ratios calculated by twelve calibration cycles. In Figures 4.2c-4.2e, the blue (uppermost) data corresponds to the first $\mu_a$ level (no absorption), and the brown (bottommost) data corresponds to the fifth $\mu_a$ level (highest absorption).

The data shown in Figure 4.2 indicates that PDs 5 and 13 (with SDS=0.6 mm and 1.25 mm) exhibited sufficient dynamic range to measure diffuse reflectance from phantoms with a wide range of optical properties (i.e. high-scattering/low-absorption to low-scattering/high-absorption). These are two examples of PDs from the array that exhibit sufficient dynamic range; some PDs did not exhibit sufficient dynamic range due to low signals at a few wavelengths with high absorption (discussed below). The simulation results and the measured data are in good agreement for both PDs and for both phantom sets with two different absorber species. The mean percent errors (MPE) between the simulations and the measured data including all five $\mu_a$ levels are 9.7% and 7.8% for the PD with SDS of 0.6 mm for the phantom sets 4 and 5, respectively, and the errors for the PD with SDS of 1.25 mm are 9.4% and 11%.

A minimum signal to noise ratio (SNR) criterion was used to qualify a PD for this MPE analysis, since some of the outermost PDs in the CMPA did not detect sufficient photons for an accurate result. In the MPE calculations, the PD measurements with SNR $< 25$ dB were excluded from analysis since an SNR of 25 dB is reported in the literature as necessary for extraction of tissue absorption and scattering parameters.
where SNR is defined as:

$$SNR = 20\log\frac{\text{mean}(I_{ph} - I_{BI})}{\text{std}(I_{ph} - I_{BI})}$$

(4.2)

where \(\text{mean}(I_{ph} - I_{BI})\) is the mean value of ten repeated measurements of the photocurrent due to diffuse reflectance at a given phantom and wavelength, and \(\text{std}(I_{ph} - I_{BI})\) is the standard deviation in these ten measurements. As the minimum SNR increases, the tissue extraction errors decrease, however, fewer measurements are allowed. Thus, for every system there is an optimization between the data density and the extraction error. In this chapter, tissue parameters are not extracted, and thus, an analysis of minimum SNR was not completed, and the published minimum SNR of 25 dB was used as a starting point. In the calculation of MPEs reported above, measurements of the PD with SDS=1.25 mm at \(\lambda=400, 410\) and 420 nm (note that there is very high Hb absorption at these wavelengths) on phantom group 4 were excluded.

The CMPA probe as reported herein measured SRDR over a more dense spatial range and a larger absorption range than ever previously reported for SRDRS. The full set of measured and simulated data was evaluated for use in MC modeling using mean percent error (MPE) calculations. These calculations were performed for the full set of measured and simulated data across all values in Table 4.1 and Table 4.2 using MC forward modeling with the HG phase function. Measurements with SNR < 25 dB were excluded, and six PDs in the array of 24 were not functional. The PDs with SDS < 1.05 mm (first twelve PDs) had sufficient dynamic range to measure the diffuse reflectance induced photocurrents for all the \(\mu_s'\) and \(\mu_a\) pairs (i.e. 30 phantoms and 41 wavelengths) and met the 25 dB SNR criterion; thus none of the measurements on these PDs were excluded in the comparison of modeling and experiments. In total, 2215 data points out of 22140 (10% of all measurements) were
excluded, for high absorption phantoms, due to SNR < 25 dB (3/1230 readings of PD 13, with SDS=1.25 mm, 14/1230 readings of PD 14 with SDS=1.35 mm, 18/1230 readings PD 15 with SDS=1.45 mm, 71/1230 readings of PD 18 with SDS=1.98 mm, 148/1230 readings of PD 19 with SDS=2.23 mm, 228/1230 readings of PD 20 with SDS=2.48 mm, 405/1230 readings of PD 2 with SDS=2.74 mm, 370/1230 readings of PD 22 with SDS=2.99 mm, and 958/1230 readings of PD 23 with SDS=3.25 mm were excluded). As expected, for higher absorption levels, the light level at the outer PDs (e.g. PD with SDS=3.25 mm) was very small, and the SNR was less than 25 dB for many of the measurements.
**Figure 4.2**: a-b) The $\mu_s$ and $\mu_a$ spectra for all five $\mu_a$ levels of phantom groups 4 and 5, respectively. c-d) The measured (triangles) and simulated (dashed lines) diffuse reflectance obtained by the PD with SDS=0.6 mm for all the five $\mu_a$ levels of phantom groups 4 and 5, respectively. e-f) The measured and simulated diffuse reflectance from the PD with SDS=1.25 mm for all the five a levels of phantom groups 4 and 5, respectively.
When the phantoms with $\mu_a$ levels 1-3 (in Table4.2) of all six phantoms were considered, all of the PDs within SDS $< 2.5 \text{ mm}$ exhibit MPE $< 10\%$. When the fourth $\mu_a$ level was included, which included high $\mu_a$ values, the maximum value of the SDS with mean MPE $< 10\%$ decreases to $1.5 \text{ mm}$, which means that the errors between theory and experiment were larger for higher absorption coefficients farther from the illumination area. When all five $\mu_a$ levels were used, including the highest absorption values (the fifth level), all of the PDs with SDS $< 1.5 \text{ mm}$ showed mean MPEs $< 15\%$.

The sensitivity of each PD to the choice of a reference phantom was evaluated by calculating the variation in MPEs estimated for varying reference phantoms. Overall, the inner 8 PDs and the middle 8 PDs in the probe (PDs each with annulus widths of 50$\mu\text{m}$ and 100$\mu\text{m}$) were the least sensitive, with a standard deviation in MPE $< 2.5\%$ over all reference phantoms. The outermost PDs had higher deviations, varying between $4\% - 8.5\%$, increasing with the increase in SDS.

Critical to optical property extraction models is a unique reflectance value that correlates to a given $\mu'_s$ and $\mu_a$ pair (i.e. we do not want multiple $\mu'_s$ and $\mu_a$ pairs to result in the same (indistinguishable) reflectance data). The advantage of the CMPA probe reported herein is that the high number of densely packed PDs increases the number of $\mu'_s$ and $\mu_a$ pairs that result in unique reflectance values. For example, Figure 4.3 shows the measured scaled diffuse reflectance (colored heat map values) as a function of phantom $\mu'_s$ and $\mu_a$ pairs for PD 5 (SDS=0.6 mm, $w=50 \mu\text{m}$) (4.3a), PD 13 (SDS=1.25 mm, $w=100 \mu\text{m}$) (4.3b), and PD 23 (SDS=3.25 mm, $w=250 \mu\text{m}$) (4.3c). The sensitivity of each of these different PDs to changes in $\mu'_s$ and $\mu_a$ is different, as evidenced by the difference in color changes for each graph in Figure 4.3, in particular, how the same reflectance (i.e. the same color) on each graph is different. The reflectance for $\mu_a$ values greater than 0.6 $\text{mm}^{-1}$ were at the lower detection limit of PD 23 (SDS=3.25 mm), so conclusions cannot be drawn for this
PD at higher absorption values. Figure 4.3d shows a detailed plot for PD 23, where \( \mu_a < 0.8 \text{ mm}^{-1} \).

![Figure 4.3](image1.png)

**Figure 4.3:** The measured scaled diffuse reflectance (in logarithmic color scale) as a function of the \( \mu'_s \) and \( \mu_a \) values of the phantoms for PDs with a) SDS=0.6 mm and PD annulus width=50 \( \mu \text{m} \), b) SDS=1.25 mm and PD annulus width=100\( \mu\text{m} \), c) SDS=3.25 mm and PD annulus width=250\( \mu\text{m} \). d) a detailed plot showing scaled reflectance in (c) for a smaller range of \( \mu_a \).

If two reflectance measurements were within one standard deviation of another, they were deemed non-unique. The large number and high density of PDs in the CMPA probe resulted in less non-unique reflectance data with increasing number and density of PDs, which improves the models. To demonstrate this, the uniqueness of the reflectance was examined as a function of the number and density of the PDs in the CMPA array by examining the reflectance for five different collections of PDs, shown in Table 4.3. Collections 1 and 2 cover the spatial ranges SDSs < 1.25 mm and SDSs > 1.25, respectively, and collection 3 covers the possible entire SDS range. Collection 4 includes one PD from all the three sets of eight PDs, and it also covers
the entire range of SDSs of the probe. In collection 5, the number of PDs from each set of eight doubled and the measurement density increased in comparison to collection 4, while the SDS range is the same. Finally, collection 6 includes all of the functional PDs (PD1 and PD2 with higher back illumination currents were excluded for not biasing the calculations) for the highest CMPA data density.

Table 4.3: Six PD collections chosen for demonstration of improvement in uniqueness condition with increase in detector density. Quantity of PDs in each collection, SDS of each PD within a collection, and PD number as numbered starting with the closest one to the illumination aperture are shown.

<table>
<thead>
<tr>
<th>Collection</th>
<th>Number of PDs in the Collection</th>
<th>PD SDS values (mm)</th>
<th>PD numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>0.6, 1.25</td>
<td>5,13</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>1.25, 3.25</td>
<td>13,23</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>0.6, 3.25</td>
<td>5,23</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>0.6, 1.25, 3.25</td>
<td>5,13,23</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>0.6, 0.71, 1.25, 1.45, 2.48, 3.25</td>
<td>5,7,13,15,20,23</td>
</tr>
<tr>
<td>6</td>
<td>12</td>
<td>0.6-3.25</td>
<td>5,6,7,8,9,10,11,13,14,15,19,20,21,22,23</td>
</tr>
</tbody>
</table>
Highly different $\mu_s'$ and $\mu_a$ pairs that result in the same, non-unique reflectance can increase modeling errors; thus, minimization of the percent difference between different $\mu_s'$ and $\mu_a$ values with the same reflectance (indistinguishable pairs) is important. To illustrate the advantages of more PDs and more dense PDs, the percent difference between indistinguishable $\mu_s'$ and $\mu_a$ pairs is shown in Figure 4.4 as a function of $\mu_s'$ and $\mu_a$ for different PD collections (i.e., number and density of PDs). The color bars in Figures 4.4 a, c, e are the maximum percent difference for $\mu_s'$, and in Figures 4.4 b, d, f, are the maximum percent difference for $\mu_a$. The percentages of the indistinguishable pairs for collections 1 through 3 are 24.80%, 21.30%, and 15.45%. Among these three collections composed of two PDs, collection 3, which covers the entire CMPA SDS range, has the smallest percentage of indistinguishable pairs, which indicates that more diversity in SDS yields a lower number of indistinguishable pairs. Table 4.4 summarizes the percentage of indistinguishable pairs, maximum and average percent differences of $\mu_s'(\mu_a)$ pairs for all six collections of PDs. As the number and density of PDs increases, the number of indistinguishable pairs decreases, and the maximum percent differences in $\mu_s'$ and $\mu_a$ also decrease. This is highlighted in Figure 4.4, which shows that the number of indistinguishable pair data points decrease with increasing collection number (1,5,6), to a minimum of 3.82% for collection 6.
Figure 4.4: Max percent difference between $\mu'_s$ (or $\mu'_s$) of $\mu'_s$ and a pairs for which a unique reflectance is not available for a (or b) collection 1, c(or d) collection 5, and e(or f) collection 6.
Table 4.4: Summary of indistinguishable $\mu_s$ and $\mu_a$ pairs for PD collections: 1, 2, 3, 4, 5 and 6

<table>
<thead>
<tr>
<th>Collection</th>
<th>% of non-unique pairs</th>
<th>Max % difference in $\mu_a$ value</th>
<th>Average difference in $\mu_a$ value</th>
<th>Max difference in $\mu_s$ value</th>
<th>Average difference in $\mu_s$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>24.80</td>
<td>2143</td>
<td>33.51</td>
<td>30.70</td>
<td>3.60</td>
</tr>
<tr>
<td>2</td>
<td>21.30</td>
<td>240.46</td>
<td>14.66</td>
<td>14.66</td>
<td>2.70</td>
</tr>
<tr>
<td>3</td>
<td>15.45</td>
<td>164.93</td>
<td>15.4</td>
<td>31.11</td>
<td>3.26</td>
</tr>
<tr>
<td>4</td>
<td>9.02</td>
<td>47.34</td>
<td>5.84</td>
<td>10.87</td>
<td>1.49</td>
</tr>
<tr>
<td>5</td>
<td>7.56</td>
<td>47.34</td>
<td>4.18</td>
<td>9.57</td>
<td>0.91</td>
</tr>
<tr>
<td>6</td>
<td>3.82</td>
<td>9.62</td>
<td>1.35</td>
<td>1.39</td>
<td>0.40</td>
</tr>
</tbody>
</table>
4.4 Conclusion

In this chapter, the most densely packed SRDRS probe and also the first custom semiconductor photodiode probe reported to date is used to conduct a comprehensive multi-spectral phantom study covering a broad range of tissue optical properties. This is the largest range of $\mu_a$ reported to date with SRDRS, with a $\mu_a$ range of 0-7.58 mm$^{-1}$, and a $\mu_r$ range of 0.6-2.8 mm$^{-1}$. For the $\mu_r$ range of 0.6-2.0 mm$^{-1}$ and $\mu_a$ range of 0-2.5 mm$^{-1}$, all of the PDs with SDS < 1.5 mm had mean percent errors < 10%. For the $\mu_r$ range of 0.6-2.5 mm$^{-1}$ and $\mu_a$ range of 0-1.59 mm$^{-1}$, all of the PDs with SDS < 2.5 mm had mean percent errors < 10%. These dense SRDR measurements result in unique reflectance data for more than 96% of the measured $\mu_r$ and $\mu_a$ pairs, which is promising for rapid empirical model development, facilitating real-time feature extraction for clinical use.
This chapter presents experimental SRDR measurements on two-layer PDMS skin tissue-mimicking phantoms of varying top layer thicknesses (200 µm-1000 µm) and optical properties using concentric multi-pixel photodiode array (CMPA) probes. Experimental measurements are compared to corresponding forward MC simulations, and the contrasts between the signals measured on varying top layer thicknesses are estimated.

5.1 Introduction

Many biological tissues (e.g. skin and cervix) have layered structures; and each layer (e.g. epidermis, dermis, and subcutaneous layers of the skin) has different microstructures and chromophore constituents, resulting in layer specific optical properties. Ad-
ditionally, epithelial tissue (where the majority of the cancers emerge) covers many of
human organs, and has different optical properties than those of the remaining bulk
tissue portion. Cancer induces distinctive changes in the optical properties of each
layer at the various cancer stages [37]. Assessment of these changes independently
in each layer, and of the tumor thickness, may enable early cancer diagnosis and
provide tumor removal guidance, which motivates depth-resolved tissue analysis.

DRS analysis of layered tissues is a significant challenge due to the increased
parameter space (e.g. each layer add three more parameters: \( \mu'_s, \mu_a \) and layer thick-
ness) compared to that of homogenous bulk tissues (typically \( \mu'_s \) and \( \mu_a \) are the only
two parameters). Analysis of two-layer tissues (i.e. the simplest case) is an active re-
search topic in the field. The typical approaches to tackle this problem are two-fold:
1) splitting the overall problem into parts of reduced dimensionality, and solving
each one with an existing mathematical method, and 2) developing new mathemati-
cal techniques that handle the increased dimensionality. Typically, the first approach
involves developing DRS systems and probes that can selectively interrogate various
tissue layers yielding layer-specific information. These probes enable the use of the
existing mathematical models repeatedly (i.e. MC modeling) on the multiple re-
fectance spectra selectively measured from target layers. The main interest is in
selective interrogation of superficial layers (e.g. the epidermis), where the majority
of diseases emerge, inducing changes in its optical properties and its thickness [85].
This requires sampling of photons traveling within the superficial layer, necessitating
measurements at shorter SDSs. However, even with short SDSs (e.g. 300 \( \mu \)m), fiber
probes are still limited in their capability of interrogating superficial tissue due to
their finite NA (e.g. 0.22; photons which traverse the tissue shallowly are not guided
through the fiber). Examples to fiber optic DRS probes designed to overcome this
limitation include a ball-lens coupled fiber optic probe [55] and an obliquely oriented
fiber probe [56]. Figure 5.1 presents a schematic illustration of the limitation of the
conventional probes and the selective interrogation principle of the given examples. For example, the second system (using an obliquely oriented fiber probe) performs two consecutive measurements and mathematical inversion procedures: first, the reflectance spectrum measured by the first probe with angled illumination and collection fibers (with short SDS<0.3 mm to selectively interrogate top layer) is fed into an inverse MC model and the top layer $\mu_s'$ and $\mu_a$ are extracted. Next, the reflectance spectrum measured by a second probe with regular flat top illumination and collection fibers (with a longer SDS, greater than 1mm, to preferentially interrogate the bulk bottom layer) is fed in to a second model that uses the estimated information from the previous step. This approach enabled property extraction from superficial layers by use of existing models, yet the extraction errors were high. For the best case of top layer thicknesses ranging from 0.2 mm-0.5 mm, the extraction errors in optical properties including thickness were around 20%, in contrast to the errors of homogenous bulk tissue layers, which are typically <10%. Additionally, the sequential procedure and the angled probe fabrication are cumbersome.
The second approach involves developing novel mathematical models for layered tissue analysis. For example, in a recent study, Sharma et al. reported on an inverse mathematical model which accounts for extraction of all of the optical properties in a single calculation step [62]. They created a four-dimensional look up table (LUT) based on MC simulated reflectance data assuming an existing SRDRS probe geometry with two collection fibers (flat top, and SDS=0.37 mm and 0.74 mm) for inversion. The LUT free parameters included top layer thickness, $\mu_a$s of the top and bottom layers, and a common $\mu'_a$. Although $\mu'_a$ differs considerably between the layers, it was assumed to be the same so as to reduce the dimensionality of the problem. This study reported on a significant improvement on top layer thickness prediction accuracy in the case of using the spectra from both of the collection fibers simultaneously compared to those using the individual spectra, and significant dependence of the extraction accuracy on the top layer thickness. However, even with the assumption of the common $\mu'_a$ for both layers (the phantoms were prepared are in compliance with this assumption) and within the appropriate thickness range (best case scenario), the reported errors were in the range of 12%-25%, which motivated the authors to point out the need for including different SDS ranges and more detectors for improved results.
The CMPA SRDR probe addresses this need by incorporating a large number of detectors with SDSs ranging from 225 \( \mu \text{m} \) (the innermost detector in the CMPA) to 3.5 mm (the outermost detector in the CMPA). The CMPA was used to perform diffuse reflectance measurements on skin tissue mimicking two-layer PDMS phantoms provided in collaboration by Gage Greening and Dr. Tim Muldoon. The high density and the large SDS ranges of the PDs within the probes yielded more data to provide a more accurate solutions to the higher dimensional problems of layered tissue optical property estimation. Additionally, the significantly higher N.A. of the photodiodes compared to that of the typical fibers (0.96 vs. 0.22), increases the sensitivity to the shallower portion of the tissue and eliminates the need for complex probes.

5.2 Materials and Methods

This section describes the experimental measurement setup and the details regarding the optical properties and the assembly of the two-layer PDMS phantoms.

5.2.1 Experimental Setup

The experimental setup is composed of the CMPA probe with illumination aperture diameter of 400 \( \mu \text{m} \), an inexpensive laser diode (LD) with wavelength (\( \lambda \)) of 660 nm (HL6544FM, Thorlabs, Inc.), since, at this wavelength, phantom optical properties are well characterized, a DC current source for driving the LD (Keithley 6220), and the Keithley 4200 source-measure units (SMUs) for the photocurrent readout. The entire 24 PDs were measured by switching three groups of eight PDs manually. The LD was pigtailed to the illumination aperture directly from the backside of the FR-4 board using an aluminum fixture. The optical power through the illumination aperture was measured as 250 \( \mu \text{W} \).
5.2.2 Two Layer PDMS Phantoms

Two different PDMS phantom mixtures were prepared using nigrosin powder (the absorption agent) and titanium dioxide (the scattering agent) by our collaborators, Gage Greening and Dr. Tim Muldoon. Table 5.1 lists the absorption and the scattering agent concentrations, the corresponding absorption coefficients ($\mu_a$) and reduced scattering coefficient values ($\mu'_s$) at $\lambda=659$ nm. The listed optical properties represent the optical properties of the dermis (mixture-1) and the epidermis (mixture-2) of the human skin at $\lambda \sim 650$ nm. The details of the phantom preparation and characterization were published in [97]. One 15 mm thick bulk phantom (bulk1) was prepared using mixture-1; and several thin layer phantoms (thickness ranging from 0.15 mm to 0.935 mm) were prepared by spin-coating mixture-2. Another bulk phantom (bulk2) was assembled by stacking four 0.935 mm thick phantoms prepared from mixture-2. Finally, ten two-layer phantoms (indexed as phantom1-phantom10) were assembled by stacking thin layer phantoms of mixture-2 on the bulk1 phantom. The top layer thicknesses of the phantoms ranged from phantom1 to phantom10 are 0.150 mm, 0.205 mm, 0.271 mm, 0.364 mm, 0.476 mm, 0.662 mm, 0.809 mm, 0.914 mm, 1.576 mm, and 1.828 mm.
Table 5.1: The concentrations of nigrosin powder and titanium dioxide used to make the phantom; and the respective phantom optical properties

<table>
<thead>
<tr>
<th></th>
<th>Concentration of Nigrosin Powder per PDMS Base [mg/g]</th>
<th>Concentration of Titanium Dioxide per PDMS Base [mg/g]</th>
<th>$\mu_a$ 659nm mm$^{-1}$</th>
<th>$\mu_s'$ 659nm mm$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mixture-1</td>
<td>0.026</td>
<td>9.6756</td>
<td>0.05</td>
<td>2.0</td>
</tr>
<tr>
<td>Mixture-2</td>
<td>0.084</td>
<td>14.677</td>
<td>0.2</td>
<td>3.0</td>
</tr>
</tbody>
</table>
5.2.3 Forward Monte Carlo Simulations

To compare the experimentally obtained results with the theory, forward Monte Carlo simulations were performed using the publicly available Virtual Tissue Simulator (VTS) Matlab package [95]. The simulations were performed on a two-dimensional geometry with radial symmetry. The probability distribution of the scattering angle for individual scattering events was modeled using the Henyey-Greenstein phase function with anisotropy ($g$) of 0.8. For a given $\mu_s$ value, the variance in the simulated MC reflectance with respect to tissue relevant $g$ (i.e. 0.7-0.95) was shown to be negligible [43]; thus wavelength dependence of $g$ was ignored, and a constant $g$ value was input to the simulations. A separate simulation was performed for each phantom; and in each simulation, 1 million photons were launched into the simulation geometry by a point source. The photons exiting the modeled phantom surface were detected in the radial range of 0-3.5 mm, which was divided into 3500 equal bins, and the resulting impulse response was convolved by the measured beam profile. Lastly, the bins that fall into a particular CMPA pixel were summed and normalized to the pixel annulus width, resulting in diffuse reflectance per unit area for the corresponding pixel.

The computed results are absolute diffuse reflectance values for each pixel (i.e. normalized to the input power), whereas the measured results are scaled diffuse reflectance values (i.e. normalized to the portion of optical power from the reflectance standard), necessitating a calibration between the two. The mean of the ratios between the $R_{sc}^{i}$ values measured from a selected reference phantom and the calculated diffuse reflectance values using the corresponding phantom optical properties was used as a scaling coefficient ($SC$) to calibrate the rest of the computational results. The $SC$ is given in the equation below:
\[
SC = \frac{\sum_{i=1}^{24} R_{i,sc,ref}}{\sum_{i=1}^{24} R_{i,MC,ref}}/24
\]  
(5.1)

where \( R_{i,sc,ref} \) is the measured scaled diffuse reflectance [mm\(^{-2}\)] from the reference phantom by the \( i^{th} \) pixel, and \( R_{i,MC,ref} \) is the absolute diffuse reflectance [mm\(^{-2}\)] calculated by using the known optical properties of the reference phantom for the \( i^{th} \) pixel.

5.3 Experimental Results and Discussions

During the measurements, the CMPA probe was oriented such that the PD detection surfaces were facing upwards, and it was back-illuminated by the LD. This orientation, relying on the weight of the phantom positioned on the probe, facilitated making gap-free contacts between the surfaces of the PDs and the sample under study. Additionally, it hindered the undesired photocurrents induced by back reflections during the back-illumination induced photocurrent characterization, since the photons absorbed at the illumination aperture sidewalls induce photocurrent on the closest pixels to the aperture. First, photocurrent measurements were performed in the absence of the phantom for background measurement, since the measured photocurrent is a sum of PD dark current and back-illumination induced photocurrent. Next, for calibration and normalization purposes, measurements were performed on a 99\% reflectance standard (SRS-99-010, Labsphere, Inc.), which was placed 1 mm away from the detection plane using spacers. Finally, we placed the phantom of interest on the CMPA probe, and measured the photocurrent resulting due to diffusely reflected light from the phantom. To obtain reflectance values, the background induced photocurrent was subtracted from the photocurrent resulting from the phantom measurements, and the resulting value was normalized to the puck measurements.
and PD area (similar to the normalization procedure described in Chapter 4) as given by the equation:

\[
R_{sc}^i = \frac{I_{ph}^i - I_b^i}{A^i \sum_{k=2}^{8} I_{puck}^k}
\]

where, \(I_{ph}^i\) is the photocurrent measured from the phantom by the \(i^{th}\) pixel, \(I_b^i\) is the background induced photocurrent on \(i^{th}\) pixel, \(A^i\) is the surface area of the \(i^{th}\) pixel, and \(\sum_{k=2}^{8} I_{puck}^k\) is the sum of photocurrents measured by \(2^{nd}-8^{th}\) (set of inner pixels) from the reflectance standard. \(R_{sc}^i\) is the resulting scaled diffuse reflectance (in units of \(\text{mm}^{-2}\)) registered by the \(i^{th}\) pixel. The inner set of pixels was found to be immune to surface reflections from the reflectance standard, thus their readings were used for normalization. The first pixel reading was excluded due to its higher back-illumination induced photocurrent.

Measurements on twelve phantoms were performed, including two bulk phantoms and ten two-layer phantoms, and each measurement was repeated ten times. The signal-to-noise ratio (\(SNR = 20 \log \frac{I_{meas,\text{mean}} - I_{b,\text{mean}}}{I_{ph,\text{std}}}\)) measured on all twelve phantoms were higher than 60 dB, and those measured on the 99% reflectance standard were greater than 68 dB for all of the 23 functioning pixels. Pixel 18 became irresponsible during measurements, and its readings are not reported. \(I_{meas,\text{mean}}\) is the mean of the ten photocurrent measurements on the phantom or puck, and \(I_{meas,\text{std}}\) is the variance in the measured photocurrent.

Figure 5.2 shows the measured and simulated scaled diffuse reflectance as a function of source-detector separation (SDS) for all twelve phantoms. The circles and solid lines represent the experimental data and simulation results for the bulk phantoms, respectively, and the stars and dashed lines represent the same for the two-layered phantoms. The error bars representing one standard deviation of the experimental data is very small (<1%), and they are not visible in the graph. The
simulation data of phantom bulk2 and the ten two-layer phantoms as plotted in Figure 5.2 were obtained by calibration using the phantom bulk1 as the reference phantom. For calibration of the simulation results of bulk phantom1, bulk phantom2 was used as the reference phantom. The data for the two-layer phantoms with thin top layers is similar to that of phantom bulk1, and it begins to look similar to that of phantom bulk2 as the top layer thickness increases. The experimental results and simulations are in very good agreement with overall mean percent error. The errors for all the 23 pixels and twelve phantoms were averaged, and were less than 6.2%. Figure 5.3a shows the mean percent errors, averaged over twelve phantom measurements as a function of source-detector spacing SDS. Figure 5.3b shows the mean percent errors, averaged over 23 pixels, for the individual phantoms. For all 23 pixels and all of the twelve phantoms, the mean errors were smaller than 10%.
FIGURE 5.2: The measured scaled diffuse reflectance data and the forward MC simulation results as a function of PD radial distance on the twelve phantoms. The markers show the measured data (circles: bulk phantoms, triangles: two-layer phantoms), and the lines show the simulation results.
Figure 5.3: The mean percent errors between the measured reflectance data and the forward MC simulation results. a) The mean percent error (averaged over twelve phantom measurements) as a function of PD radial distance. b) The mean percent error (averaged over 23 PD readings) as a function of phantom index.
The probe sensitivity to varying tissue optical properties and varying thicknesses is an important figure of merit for differentiation. Figure 5.4 shows the experimental and simulated signal contrasts (\(\text{SignalContrast} = 100 \times \frac{R_{\text{bulk}} - R_{\text{ph}}}{R_{\text{bulk}}}\)) between the phantom bulk1 and the ten two-layer phantoms (phantom1-phantom10). The farthest positioned PDs exhibited greater contrast due to longer paths of the collected photons. Signal contrasts higher than 80% were observed for two-layer phantoms with top layers thicker than 0.9 mm. The signal contrast for a top layer thickness of 0.15 mm is >20%, and a 0.05 mm thickness increase can be sensed for a top layer thickness <0.6mm. The signal contrasts are negative for the closer PDs and increases as the PD distance increases. The closer PDs register a signal increase (negative signal contrast) due to higher scattering of the top layer, whereas farther PDs register a signal decrease due to higher absorption of the top layer.
Figure 5.4: The measured and simulated signal contrasts between the phantom bulk1 and ten two-layered phantoms with top layer thicknesses ranging from 0.15(red color)-1.828(cyan color) mm. The stars show the measured data, and the dashed lines show the simulation results.
5.4 Conclusion

Spatially resolved diffuse reflectance measurements were performed on two bulk and ten two-layered skin tissue-mimicking phantoms using the densely packed CMPA probe. The average error between the measured reflectance data and the forward MC simulation results are less than 6.2%. The maximum measured signal contrasts between the bulk1 phantom and the two-layer phantoms are >80% for thick top layers. For a very thin top layer of 0.15 mm thickness, the signal contrast is higher than 20%; and for this top layer thickness, a 0.05 mm increase in top layer thickness induces a 10% change in the signal contrast, enabling detection of small changes in thickness.
Conclusions and Future Work

This chapter concludes the thesis and presents an outline for future research, which propels the work described in this thesis.

6.1 Conclusion

DRS is a well-established technique that studies tissue morphology and biochemical composition, and it has the potential to offer practical, non-invasive and cost-effective diagnostic information. SRDRS, a subset of the general DRS technique, provides additional spatial information about the photon path, yielding depth-resolved tissue information critical to the analysis of layered tissues (e.g. skin and cervix) for early cancer diagnosis. SRDRS involves sampling of diffuse reflectance signals at multiple distances to an illumination source.

Currently reported SRDRS technologies use fiber optic probes typically with 3-8 collection fibers. The complexity of optical fiber multiplexing impedes a larger number and high-density reflectance measurements. The circular fiber geometry complicates development of custom probes and reduces the fill factor, resulting in a reduction in detection area for a given source-detector spacing and poor light col-
lection efficiency compared to PDs. Finite fiber NA also reduces light collection efficiency and prevents interrogation of superficial tissue layers, resulting in a further increase in probe fabrication complexity and cost if superficial thin layer characterization is desired. Additionally, optical fibers function only as photon collectors and an additional photodetector is needed in the system, which adds to cost and size of the system.

SRDR measurements can be performed at a single wavelength, expanding the group of optical sources (e.g. discrete laser diodes) alternative to broadband sources, which may provide a better fit to a diagnostic settings. This also contributes to a reduction in system size and cost. Multi-wavelength SRDR measurements can extend the range of optical properties with acceptable extraction accuracies compared to that of single SDS measurements performed at the same wavelengths. Mathematical models, which strive to address the problem of layer specific property extraction and real time tissue analysis, were developed based on both multiple and single wavelength SRDR simulations. There is also demand for densely measured DRS data for evaluation of empirical models built on simulation data.

Existing SRDRS technologies based upon optical fibers are limited due to difficulties in accommodating a large number and high density of collection fibers, as desired for accurate depth-resolved and real-time optical property extraction from biological tissues. The reviewed advantages of SRDRS could be fully exploited through realization of SRDRS systems enabling high density measurements, which is difficult to implement with optical fibers. Additionally, technologies that can miniaturize and simplify the desired high density measurement SRDR systems would result in significant steps toward utilization of these systems in clinical, field, and perhaps even in home settings.

This thesis reported on the development of an innovative SRDRS probe to begin to address the challenges of realizing high measurement density in a miniaturized,
inexpensive SRDRS system. This new probe accommodates a large number and high density of detectors, and it is fabricated by conventional and inexpensive silicon manufacturing technologies, exemplifying the feasibility of developing low-cost SR-DRS probes in any desired geometry and complexity. The probe is in the form of a concentric semi-annular photodiode array (CMPA) with a central illumination aperture. CMPA probes with illumination aperture diameters of 100 µm, 400 µm, and 750 µm were fabricated with dark currents ranging from 1-20 pA and responsivities between 0.27-0.35 A/W for the wavelength range of 450-600 nm.

This is the first multiple source-detector spacing Si SRDRS system reported to date, and the most densely packed SRDRS probe reported to date for all types of SRDRS systems. The closely spaced and densely packed detectors enable higher density SRDR measurements compared to fiber-based SRDR probes, and the higher PD effective NA compared to fibers results in a higher SNR than fiber probes, enabling a higher dynamic range of scattering and absorption. The higher NA of the PDs and the presence of PDs positioned at very short distances from the illumination aperture center enabled superficial tissue analysis as well as depth analysis.

The CMPA was fully characterized and tested on both liquid and solid layered phantoms. First, back-illumination induced photocurrents at the sidewalls of the CMPA illumination aperture were characterized, and the probe was tested on a 99% diffuse reflectance standard (puck) using an Xenon lamp illumination source coupled to a monochromator. The optical power through the aperture with this source was measured as <=2.5 µW (lower than those of typical DRS systems) for the wavelength range of 370-990 nm, yet the puck measurements yielded signal to noise (SNR) ratios larger than 40 dB in the entire λ range for all but the two inner PDs. Additional effort to optimize the light source for increased optical throughput would further decrease the measurement uncertainty. The CMPA probe was validated on benign and malignant breast tissue mimicking liquid phantoms with a mean error between
the measurements and the forward Monte Carlo (MC) simulations of less than 9%.

Next, the densely packed CMPA probe with illumination aperture diameter of 750 µm and a Xenon lamp coupled to a monochromator was used to conduct a comprehensive multi-spectral phantom study covering a broad range of tissue optical properties. The phantom optical properties covered a $\mu'$ range of 0.6-2.8 mm$^{-1}$ and a $\mu_a$ range of 0-7.58 mm$^{-1}$, which is the largest range of $\mu_a$ reported to date with SRDRS. For the $\mu'$ range of 0.6-2.0 mm$^{-1}$ and $\mu_a$ range of 0-2.5 mm$^{-1}$, all of the PDs with SDS<1.5 mm had mean percent errors <1% (between the experiment anf forward MC modeling). And, for the $\mu'$ range of 0.6-2.5 mm$^{-1}$ and $\mu_a$ range of 0-1.59 mm$^{-1}$, all of the PDs with SDS<2.5 mm had mean percent errors <10%. For the $\mu'$ range of 0.6-2.5 mm$^{-1}$ and $\mu_a$ range of 0-1.59 mm$^{-1}$, all of the PDs with SDS<2.5 mm had mean percent errors <10%. These dense SRDR measurements result in unique reflectance data for more than 96% of the measured $\mu'$ and $\mu_a$ pairs, which is promising for rapid empirical model development, facilitating real-time feature extraction for clinical use.

Finally, spatially resolved diffuse reflectance measurements were performed on bulk and two-layered skin tissue-mimicking phantoms using the densely packed CMPA probe with a 400 µm aperture diameter and a red laser diode as the illumination source. The two-layer phantoms were assembled by stacking thin phantom layers with thicknesses ranging from 0.150 mm to 1.828 mm on the bulk phantom. The use of a larger optical power laser diode (250 µW) enabled a decrease in measurement uncertainty with SNRs greater than 68 dB on 99% puck measurements, and larger than 60 dB on all of the measured phantoms for all of the pixels. The average error between the measured reflectance data and the forward MC simulation results were less than 6.2% for all of the two-layer phantoms and the bulk phantom. The maximum measured signal contrasts between the bulk phantom and the two-layer phantoms were > 80% for thick top layers. For a very thin top layer of 0.15
mm thickness, the signal contrast was higher than 20%; and for this top layer thickness, a 0.05 mm increase in top layer thickness induced a 10% change in the signal contrast, enabling detection of small changes in thickness. The described probe is promising for low cost, portable SRDRS systems and has the potential to enable both superficial and layered tissue analysis.

6.2 Future Work

There are several aspects of this thesis that would benefit from further research and development.

This thesis reported on dense SRDRS measurements on tissue mimicking phantoms covering an extensive optical property range and on corresponding forward MC simulations, with a good agreement between the two. Additionally, the analysis of the experimental data revealed that more than 96% of the $\mu_s'$ and $\mu_a$ pairs result in unique reflectance data, which is promising for fast and robust mathematical models critical to real time applications. However, for practical applications, tissue optical property information must be extracted through inverse mathematical modeling of the measured reflectance data. Thus, inverse modeling of the experimental data would complement the work performed in this thesis for the practical use of the CMPA probe. Additionally, the dense SRDRS measurements can be used to compare a variety of mathematical models for optimized model selection.

As an initial step towards optical property extraction, two dimensional (2D) reflectance lookup tables (LUT) with free parameters of $\mu_s'$ and $\mu_a$ were created for each pixel on the CMPA, based on forward MC simulations, and one out of the 30 measured phantoms was used as a reference phantom for calibration of LUTs. In our approach, a simple matrix subtraction operation between the two 3D matrices of simulated and experimental reflectance data (i.e. first matrix: 2D LUTs of all pixels were concatenated, resulting in a 3D matrix of simulated reflectance and second
matrix: 1D array of reflectance measurements from each pixel was repeated along the dimensions of the LUT) yielded the optical properties of interest, which are the $\mu_s'$ and $\mu_a$ pair, minimizing the result of subtraction summed over all pixels. The mean extraction error in $\mu_s'$ was 8.1% in $\mu_s'$ range of 0.6-2.8 mm$^{-1}$, and the mean extraction error in $\mu_a$ was 5.2% for a $\mu_a$ range of 0-2.5 mm$^{-1}$. In this inversion procedure no a priori information (e.g. absorber type and spectral shape) or spectral information was used and this is the first and the only inversion procedure that does not use optimization algorithms to the best of our knowledge. This procedure used the reflectance data from the entire set of PDs within CMPA for inversion. Further research investigating the extraction accuracies with subsets of the CMPA pixels would provide guidance to probe design for target biomedical applications.

Similarly, the reflectance data measured on two-layer tissue mimicking phantoms can be used to validate a higher dimensional LUT model (layer thickness, $\mu_s'$ and $\mu_a$ the additional layer can be incorporated in the model resulting in a five dimensional LUT) for extracting the optical properties from layered tissues. Regression techniques (e.g. artificial neural networks), as alternative approaches, can be used as for feature extraction from layered tissues, and comparisons between several techniques can be performed.

Finally, the CMPA probe can be used with a custom designed transimpedance amplifier and a switch for photocurrent read out and a laser diode as the illumination source, to realize a practical, cost effective and small footprint SRDRS system.
Bibliography


Biography

Ozlem Senlik was born in Zile, Tokat, Turkey, on July 4, 1984. She received her Bachelor of Science Degree in Electrical and Electronics Engineering, in 2006, and Master of Science Degree in Materials Science and Nanotechnology in 2008, both from Bilkent University, Turkey.

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