Contact and non-contact methods for optical spectroscopy and imaging in epithelial cancer diagnosis and tissue viability assessment

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**Contact and non-contact methods for optical
spectroscopy and imaging in epithelial cancer
diagnosis and tissue viability assessment**

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**SCHOOL OF CHEMICAL & BIOMEDICAL
ENGINEERING**

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**Contact and non-contact methods for optical
spectroscopy and imaging in epithelial cancer
diagnosis and tissue viability assessment**

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Dedication

To my parents and my wife, Hanni

Contact and non-contact methods for optical spectroscopy and imaging in epithelial cancer diagnosis and tissue viability assessment

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Under the supervision of Assistant Professor Liu Quan

At Nanyang Technological University, Singapore

This dissertation presents a series of studies in the development of contact and non-contact optical measurement techniques, and Monte Carlo (MC) based data analysis methods in visible diffuse reflectance and auto-fluorescence spectroscopy/imaging for tissue characterization. Their applications in early epithelial cancer diagnosis and tissue viability prediction are demonstrated.

Firstly, the background in epithelial cancer and flap tissue viability as well as the principles/state of art of optical spectroscopy and imaging in tissue optics was presented in Chapter 1. Then a general survey on the capability of MC modeling of light transport in tissues was provided in Chapter 2 due to the importance of MC modeling in the field of tissue optics. The recent progress in the development of methods for speeding up MC simulations and the potential directions of future development were discussed.

Based on the literature review on MC methods given in the previous Chapter, we developed a MC method to simulate diffuse reflectance and fluorescence from a layered tissue with embedded objects to mimic an early epithelial cancer model in Chapter 3. With the help of this MC method, a series of numerical studies were performed to provide the guidelines for the selection of a proper epithelial cancer model in MC simulations.

However, it is very time consuming to use the standard MC method for the simulation of light transport in a layered tissue with embedded object. To overcome this problem, a hybrid method, in which the scaling method and the perturbation MC method were integrated coherently, was proposed and validated to accelerate the simulations of diffuse reflectance from a layered tissue with embedded objects in Chapter 4. This method is suitable for simulating diffuse reflectance spectra or creating a MC database to extract optical properties of an early epithelial cancer model.

All the above studies were designed for contact optical measurements using fiber-optics. However, inconsistent probe-sample contact could induce significant errors in diagnosis of early epithelial cancer. To address this problem, lens based setup was investigated for non-contact optical measurements in Chapter 5 and Chapter 6. We firstly developed a MC method to simulate diffuse reflectance and fluorescence measurements by convex lenses based non-contact setup. Then a series of numerical studies were performed to achieve depth sensitive diffuse reflectance and fluorescence measurements on the early epithelial cancer model. After that experimental studies were performed by a lens based optical system to validate the MC results and confirm the findings obtained in simulation study.

We further extended the lens based non-contact spectroscopy system to an imaging setup with a larger field of view to perform depth sensitive color imaging on an early epithelial cancer phantom in Chapter 7. In the proposed setup, a micro-lens array was used to induce multi-focal illumination and a tunable lens was utilized to map multiple light foci into the tissue phantom at a range of depths. Another imaging lens was used to image light into a 3-CCD camera. The study performed on the

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In addition to the numerical and phantom studies in epithelial cancer diagnosis, an animal study was also performed in Chapter 8 to predict tissue viability in flap surgery using a dual-modal system capable of performing both visible diffuse reflectance and auto-fluorescence spectroscopy. The results showed that either visible diffuse reflectance spectroscopy or auto-fluorescence spectroscopy alone can predict the skin viability accurately; however, auto-fluorescence spectroscopy was more sensitive to tissue changes in the first two hours after the induction of ischemia. It was feasible to predict flap failures in the first two hours when using auto-fluorescence spectroscopy alone. Moreover, it is possible to predict flap failures even in the first 15 minutes with high accuracy when using diffuse reflectance and auto-fluorescence spectroscopy simultaneously.

In Chapter 9, we summarized that UV-visible diffuse reflectance and auto-fluorescence spectroscopy or imaging are promising tools for early epithelial cancer diagnosis and tissue viability prediction. Further refinement of these techniques would help advance the use of optical spectroscopy and imaging in clinical settings for tissue characterization in an even larger range of clinical applications.

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Abstract

This dissertation presents a series of studies in the development of contact and non-contact optical measurement techniques, and MC based data analysis methods in visible diffuse reflectance and auto-fluorescence spectroscopy/imaging for tissue characterization. Their applications in early epithelial cancer diagnosis and tissue viability prediction are demonstrated.

Firstly, the background in epithelial cancer and flap tissue viability as well as the principles/state of art of optical spectroscopy and imaging in tissue optics was presented in Chapter 1. Then a general survey on the capability of MC modeling of light transport in tissues was provided in Chapter 2 due to the importance of MC modeling in the field of tissue optics. The recent progress in the development of methods for speeding up MC simulations and the potential directions of future development were discussed.

Based on the literature review on MC methods given in the previous Chapter, we developed a MC method to simulate diffuse reflectance and fluorescence from a layered tissue with embedded objects to mimic an early epithelial cancer model in Chapter 3. With the help of this MC method, a series of numerical studies were performed to provide the guidelines for the selection of a proper epithelial cancer model in MC simulations.

However, it is very time consuming to use the standard MC method for the simulation of light transport in a layered tissue with embedded object. To overcome this problem, a hybrid method, in which the scaling method and the perturbation MC method were integrated coherently, was proposed and validated to accelerate the simulations of diffuse reflectance from a layered tissue with embedded objects in

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In addition to the numerical and phantom studies in epithelial cancer diagnosis, an animal study was also performed in Chapter 8 to predict tissue viability in flap surgery using a dual-modal system capable of performing both visible diffuse reflectance and auto-fluorescence spectroscopy. The results showed that either visible

diffuse reflectance spectroscopy or auto-fluorescence spectroscopy alone can predict the skin viability accurately; however, auto-fluorescence spectroscopy was more sensitive to tissue changes in the first two hours after the induction of ischemia. It was feasible to predict flap failures in the first two hours when using auto-fluorescence spectroscopy alone. Moreover, it possible to predict flap failures even in the first 15 minutes with high accuracy when using diffuse reflectance and auto-fluorescence spectroscopy simultaneously.

In summary given in Chapter 9, UV-visible diffuse reflectance and auto-fluorescence spectroscopy or imaging are promising tools for early epithelial cancer diagnosis and tissue viability prediction. Further refinement of these techniques would help advance the use of optical spectroscopy and imaging in clinical settings for tissue characterization in an even larger range of clinical applications.

Chapter 1: Introduction

1.1 Background in epithelial cancer and flap tissue viability

1.1.1 Epithelial cancer and the current clinical diagnostic methods

As one of the major tissue types, epithelial tissue covers the entire surface of the human body. Epithelial tissue is made up of a set of cellular and extracellular components specialized in the exchange of materials between the tissue and its environment [1]. Epithelial tissue covers many important human organs including the cervix, colon, skin and oral cavity. It is reported that over half of human cancers arise in the epithelium and more than 2 million patients with non-melanoma cancers in epithelial tissues are identified each year in the US alone [2]. Squamous cell carcinoma (SCC) and basal cell carcinoma (BCC) are two commonly seen epithelial cancers. SCC usually arises from the basement membrane of the epithelium. The SCC tumor first proliferates upward; after the entire epithelium is occupied, the tumor will invade into the basement membrane towards the stroma [3]. Thus in the early stage, the tumor usually is located in the epithelial layer. The BCC tumor originates from the basal layer of the epidermis and frequently grows downward deeply into the dermis [4]. Skin BCC tumor is the most common skin cancer due to the fact that it constitutes almost 80% of all non-melanoma skin cancers [2]. Since the detection of epithelial cancer at an early stage could significantly reduce its morbidity and mortality, the early detection of epithelial cancer has been a hot research area in the past decades.

Currently, the standard procedure for diagnosis of epithelial cancer is visual inspection followed by tissue biopsy. An endoscope might be needed if the cancer is inside the body. Several disadvantages are associated with the current procedure. Firstly, visual inspection can only observe the tissue surface; Secondly, the visual

inspection is subject to the expertise and experience of the clinical staff. Moreover, the tissue biopsy removal is invasive and has a risk of infection.

1.1.2 Flap tissue viability and the current clinical assessment methods

Tissue viability is the term used to describe the preservation and the restoration of tissue integrity [5]. Tissue viability is determined by the ability of its microcirculatory which is affected heavily by blood-oxygen supply. The tissue could become dead if it suffers from an inadequate blood-oxygen supply. The diagnosis and monitoring of tissue viability is vitally important in reconstructive plastic surgery. Skin flaps are frequently used to provide well vascularized tissues for wound coverage in plastic surgery. However, it is reported that 6% to 25% of flaps require surgical re-exploration for vascular compromise and approximately 10% of flaps are not salvageable [6, 7]. Most flap losses occur within 72 hours after the surgery [8], and these flaps could be saved and the cost can be reduced significantly if the accurate tissue viability diagnosis can be made early.

The periodical clinical examination of capillary refill, flap color, temperature and bleeding patterns is the current gold standard method for the assessment of flap viability [9]; however the current gold standard method relies heavily on the skill and the availability of hospital staff.

1.2 Principles of optical spectroscopy/imaging in tissue optics

Optical spectroscopy/imaging techniques are very attractive for clinical tissue characterization due to several unique advantages. Firstly, optical measurements are non-invasive. Secondly, optical spectroscopy/imaging are sensitive to the physiological changes associated with many diseases. Thirdly, it is practical to

achieve real time measurement due to the technical advances in light sources and detectors.

The basis of optical spectroscopy and imaging technique for tissue characterization is built on light-tissue interactions, which mainly include absorption, scattering and fluorescence. In an absorption process, the excitation light photons are absorbed by a molecule while no new photons will be emitted. The major light absorbers in tissue are oxygenated hemoglobin and de-oxygenated in the UV-Visible optical spectrum [10]. In a scattering process, incident photons are deflected after interacting with a particle in the medium. Several parameters, i.e. the absorption coefficient and scattering coefficient, are frequently used to characterize absorption and scattering properties. The tissue optical properties can be described by the absorption coefficient, scattering coefficient and anisotropy value. The absorption coefficient (μ_a) is the probability of absorption event occurring per unit length and the unit of the μ_a is cm^{-1} . The scattering coefficient (μ_s) is the probability of scattering occurring per unit length, the unit is also cm^{-1} . The anisotropy factor (g) is the measure of angular dependence of scattering, whose value varies from 0 to 1. The g value of human tissues is typically around 0.9 in the UV-VIS spectrum, which suggests that the scattering events in human tissues are highly forwarding. The reduced scattering coefficient defined by $\mu_s*(1-g)$ can be viewed as a measure of effective scattering. Fluorescence usually involves multiple wavelengths, it refers to the emission of light that result from the return of a molecule from a singlet excited state to the ground state. The excitation wavelength is always shorter than emission wavelength. Tissue fluorescence is much more difficult to interpret because the fluorescence signal is usually distorted by absorption and scattering.

A tissue can be treated as the mixture of absorbers and scatterers. The diffuse reflectance from tissue refers to the fraction of photons escaped from the same surface as the incident side after travelling through the tissue due to the scattering nature. During the traveling process, photons can encounter absorbers and scatterers. The reflectance measured at multiple wavelengths comprises a spectrum. The diffuse reflectance spectrum contains information related to tissue optical properties, which can be used to characterize the tissue condition. Fluorescence is quite different. When excitation photons are launched into the tissue and undergo multiple absorption and scattering events, a fraction of these photons can be absorbed by a fluorophore and emitted a fluorescent photon. The fluorescent photons can also undergo multiple absorption and scattering events and part of them could be remitted from the tissue surface and collected by detectors. The measured fluorescence signals not only reflect the intrinsic fluorescence but also the indirect effects of absorption and scattering.

Diffuse reflectance spectroscopy is sensitive to the absorption and scattering properties of tissue. It has been reported that there is an increase in the scattering coefficient in the epithelium of pre-cancer tissues relative to that in normal cervical tissues [11] which is attributed to the increase of the nuclear size and chromatin texture. Moreover, there is an increase in blood vessel growth in the stroma in cervical cancer. In addition, the thickness of the epithelium in tissues decreases with patient age and menopausal status and increases with neoplasia [12], thus the depth distribution of endogenous absorption and scattering contrast will be changed. The knowledge of physiological changes with disease and its relation to the tissue optical properties can be helpful to build a physiological basis in using diffuse reflectance spectroscopy for epithelial cancer detection because diffuse reflectance spectroscopy provides a measure of tissue absorption and scattering.

1.3 State of art in optical spectroscopy/imaging for tissue characterization

1.3.1 Optical spectroscopy/imaging for epithelial cancer diagnosis

Optical spectroscopy techniques have been explored extensively for the diagnosis of human cancers in the past decades. The following review will focus on diffuse reflectance and fluorescence spectroscopy techniques for the diagnosis of epithelial cancers. These techniques can be generally divided into two groups according to the methods of data analysis. In the first group, diffuse reflectance and /or fluorescence spectra measured from tissue samples are analyzed by statistical classification algorithms to discriminate the cancerous tissues from normal tissues. For example, Drakaki et al. [13] used laser-induced fluorescence and reflectance spectroscopy to discriminate the basal cell carcinoma from surrounding normal skin tissue. Their study shows that neoplastic tissues can be discriminated from normal skin tissue if an appropriate classification analysis method is used. Skala et al. [14] performed an *in vivo* study for the diagnosis of epithelial neoplasia using diffuse reflectance spectroscopy alone. The principal component analysis method was used for the analysis of diffuse reflectance spectra and a high accuracy of 95% was obtained.

In the second group, light transport models are applied to measured diffuse reflectance or fluorescence to derive biochemical parameters such as total hemoglobin concentration and hemoglobin oxygen saturation. Zonios et al. [15] modified the diffusion theory to model diffuse reflectance with the concentrations of oxygenated and deoxygenated hemoglobin and scattering properties as variables from a semi-infinite tissue model. They applied the analytical model to diffuse reflectance spectra measured from adenomatous colon polyps and normal colonic mucosa of patients undergoing colonoscopy to estimate total hemoglobin concentration, oxygen

saturation, effective scatters density and effective average scatter size. They found that hemoglobin concentration and effective scatter size from the normal and adenomatous tissue sites exhibited differently. Wang et al. [16] combined the diffusion approximation and a hybrid P3 diffuse reflectance model developed by Hull and Foster [17], then they applied the model to estimate the total hemoglobin concentration and oxygen saturation *in vivo* in 27 patients with colorectal cancer. They found that the hemoglobin concentration increased and the oxygen saturation decreased from normal sites to premalignant tissue and then to malignant tissues. Chang et al. [18] developed a mathematical model to calculate the relative concentration of light scatterers, light absorbers, and fluorophores in the epithelium and stroma. In their model, the total detected fluorescence is expressed as the sum of fluorescence contribution from the epithelial and stromal layers. The fluorescence contribution from each layer is function of each layer's optical properties and the concentration of biochemical components. They applied this model to estimate the contribution of several optical biomarkers by analyzing fluorescence spectra from human cervical tissue. Their study demonstrated that epithelial flavin adenine dinucleotide fluorescence, epithelial light scattering, stromal hemoglobin light absorption has increased; while epithelial keratin fluorescence, stromal collagen fluorescence has decreased in cancerous tissue. Chang et al. [19] applied numerical model on diffuse reflectance spectra to identify contrasts in optical biomarkers at different grades of cervical intraepithelial neoplasia (CIN). Their study showed that total hemoglobin was statistically higher in CIN 2(+) compared to normal and CIN1 sites. Scattering was significantly reduced in CIN1 and CIN 2(+) compared with normal sites.

Due to the advantages in the field of view and spatial resolution, spectral imaging techniques have been explored for epithelial cancer diagnosis. Costas Balas [20] presented a novel optical imaging method for the *in vivo* early detection, quantitative staging, and mapping of cervical cancer and pre-cancer. Their clinical trials showed that optical contrast enhancement results in a notable improvement of the sensitivity in detecting incipient lesions. It was also observed that the measured temporal characteristics of the phenomenon contain specific information, which enables the differentiation between neoplastic and non-neoplastic lesions, as well as between neoplasias of different grade. Yaroslavsky et al. [21] presented a spectral imaging method for the detection of non-melanoma cancers. They combined the multispectral polarized light imaging and confocal microscopy to localize the non-melanoma skin cancer. Salomatina et al. [22] investigated the feasibility of combining multimodal reflectance and fluorescence polarization imaging with spectroscopic analysis of reflectance images for facilitating intraoperative delineation of BCCs. According to their study, they presented that reflectance polarization images provided more detailed information on skin morphology, with the appearance of skin structures resembling that of histopathology. Fluorescence polarization images exhibited higher contrast of cancerous tissue as compared to reflectance images.

1.3.2 Optical spectroscopy/imaging for prediction of flap tissue viability

Optical spectroscopy and imaging could provide information about tissue status complementary to clinical examination and have shown excellent potential for assessing tissue viability. The most common commercial tool used for clinical flap monitoring now is laser Doppler flowmetry and imaging, which has been considered as non-invasive techniques [6]. Laser Doppler flowmetry assesses flap viability [6, 23-25] by measuring the Doppler frequency shift of a laser beam reflected from a

tissue. However, the laser Doppler technique has inherent limitation in assessing flap viability since it can measure only one physiological parameter, i.e. blood flow, which is just one of several physiologically relevant parameters affecting tissue viability. Moreover, this technique requires careful interpretation for recording of laser Doppler flowmetry [26] and it cannot distinguish between the venous and arterial occlusion [6]. To overcome the limitations associated with laser Doppler, multiple other optical techniques have been explored to provide additional parameters for monitoring flap viability. Near infrared (NIR) diffuse reflectance spectroscopy has been explored extensively for assessing tissue viability by measuring hemoglobin oxygen saturation [27-29], hemoglobin concentration [28], and tissue hydration [29]. Visible light spectroscopy has been used to measure tissue oxygenation and total hemoglobin concentration for tissue viability characterization [9, 30]. Compared to NIR diffuse reflectance spectroscopy, visible diffuse reflectance spectroscopy offers higher sensitivity to the change in hemoglobin parameters at the cost of smaller penetration depth. Fluorescence spectroscopy utilizing exogenous fluorophore [31] and endogenous fluorophore [32, 33] have also been reported as tools to characterize flap viability by measuring the metabolic rate of tissues during the past years.

1.3.3 Methods for analysis of tissue spectra and images

Several important biochemical and biophysical parameters in tissue such as hemoglobin concentration, oxygenation, and average scatter size, can be quantitatively extracted from diffuse reflectance spectra under certain assumptions. Another set of fluorescence based parameters such as fluorophore concentrations and redox ratio can be estimated from fluorescence spectra. These parameters could be correlated with the physiology of tissues thus it could provide insight into tissue status including cancer development. In addition, these parameters could also be used for

cancer diagnosis as an alternative to pure statistical classification. The methods used to estimate the tissue optical properties from optical measurements can be, in general, divided into two groups, i.e. analytical model based methods and MC based method.

For the processing of diffuse reflectance spectra, analytical model based methods apply the diffusion theory, modified diffusion theory or some other approximation theories to compute optical properties from diffuse reflectance spectra. The diffusion theory model was presented by Farrell et al. [34] more than twenty years ago and it has been widely used for the inverse calculation of optical properties of tissues from optical measurements. The diffusion theory model was derived from Boltzmann radiative transport equation by making a few simplifying assumptions. They calculated the diffuse reflectance from a narrow beam of light incident on the surface of a semi-infinite turbid medium under the diffusion approximation, and the following expression for the diffuse reflectance radial density $R(\lambda, r)$ at a distance r from the point of incidence had been obtained:

$$R(\lambda, r) = \frac{z_0}{4\pi} \frac{u_s'}{u_s' + u_a} \left[\left(u + \frac{1}{r_1}\right) \frac{\exp(-ur_1)}{r_1^2} + \left(1 + \frac{4}{3}A\right) \left(u + \frac{1}{r_2}\right) \frac{\exp(-ur_2)}{r_2^2} \right] \quad (1.1)$$

with

$$u = [3u_a(u_a + u_s')]^{\frac{1}{2}}, z_0 = \frac{1}{u_s' + u_a}, r_1 = (z_0^2 + r^2)^{\frac{1}{2}}, r_2 = [z_0^2(1 + \frac{4}{3}A) + r^2]^{\frac{1}{2}} \quad (1.2)$$

The parameter A depends on the refractive index of the medium but $R(\lambda, r)$ does not depend strongly on the parameter. The parameter μ_s' in the equation refers to the reduced scattering coefficient which is defined by $\mu_s(1-g)$. The examples of using the diffusion theory to extract optical properties of tissue from diffuse reflectance spectra can be found in [35-37]. Zonios et al. [15] modified the diffusion theory to model

diffuse reflectance with the concentrations of oxygenated and deoxygenated hemoglobin and scattering properties as variables. They estimated the optical properties of tissue by performing a method of nonlinear regression such as the Gauss-Newton algorithm. Finlay and Foster et al. [38, 39] used the P3 approximation of the transport equation to model light transport and the inversion was also done by nonlinear regression. Zonios et al. [40] presented a simple and practical model to model the light transport in a semi-infinite turbid. The model, shown in the following equation, is a simpler one compared to other models available for describing diffuse reflectance measured with an optical fiber probe.

$$R = \frac{1}{k_1 \frac{1}{u_s} + k_2 \frac{u_a}{u_s}} \quad (1.3)$$

where the parameters k_1 and k_2 are determined with the optical fiber probe configuration. The model performed well in the tissue phantom study and *in vivo* human tissue study. Later, they further developed this model to describe light transport in two-layered biological tissues [41, 42].

These analytical model based methods are fast thus it is possible to perform diagnosis using these methods in real time. However, there are several limitations associated with the analytical model based methods. The first limitation is that the diffusion theory based methods are not valid when the source-detector separation is small or when the absorption and scattering coefficients are comparable, such as in the case of diffuse reflectance spectroscopy in the UV-VIS spectral region. The second limitation is that the tissue has to be assumed to be a semi-infinite homogenous layer. Therefore, it is not appropriate to apply the analytical model when

the tissue structure becomes complex, such as a multi-layered tissue model with tumor-like heterogeneities described earlier.

The process of fluorescence spectra by analytical model based methods will be more complicated than that for diffuse reflectance. The reason is that the fluorescence is influenced by not only the fluorophores but also the background optical properties. To remove the effect from the background absorption and scattering properties, usually additional diffuse reflectance measurements are involved. Weersink et al. [43] proposed a simple fluorescence/reflectance ratio technique to estimate the concentration of fluorescent compounds in turbid media. In their method, both of diffuse reflectance and fluorescence would be measured at different source-detector separations, and then the ratios of fluorescence and diffuse reflectance at optimal source-detector distance were used to characterize the fluorophore concentrations. Jun and Muller et al. [44, 45] reported a photon migration theory based method to extra intrinsic fluorescence from turbid media. They developed an analytical model based on the MC simulations to describe the relationship between the bulk fluorescence spectrum and the diffuse reflectance spectrum for arbitrary geometries and boundary conditions. De Veld et al. [46] tried to obtain the first order approximation of intrinsic auto-fluorescence spectra by dividing the auto-fluorescence spectra by diffuse reflectance spectra recorded at the same anatomical location to a power of specific values. Valdes et al. [47] reported a dual-band normalization technique for *in vivo* estimation of protoporphyrin IX (PpIX) concentrations during brain tumor resection procedures. Their method can be described by following equation,

$$\Phi(\lambda) = \frac{\Phi^{Fluo}(\lambda)}{\Phi_x^{Re} * (\Phi_m^{Re})^a} \quad (1.4)$$

where $\Phi(\lambda)$ is the intrinsic fluorescence, $\Phi^{Fluo}(\lambda)$ is the measured fluorescence at each wavelength, Φ_x^{Re} and Φ_m^{Re} represent the spectrally integrated reflectance signals over the fluorophore excitation band and emission band, respectively. The assumption of this method is that most of the change in fluorescence magnitude is due to tissue absorption at the excitation wavelength and that the impact of light scattering can be corrected by further dividing or multiplying the raw fluorescence spectra by a power function of Φ_m^{Re} . Liu et al. [48, 49] proposed a ratio metric method to estimate hemoglobin concentration and redox ratio from fluorescence measurements only.

The MC method has been widely used to analyze the data obtained in either the diffuse reflectance measurements or fluorescence measurements. A more detailed review can be found in Chapter 2. If the MC based methods are used for the inversion calculation, a diffuse reflectance or fluorescence database is usually built first by running forward MC simulations. For given diffuse reflectance measurements, the corresponding optical properties of tissue could be found by looking up the database. Among the MC methods presented by different groups, the most widely used MC code was developed by Wang and Jacques, their open-source code, i.e. MC code for Multi-Layered media (MCML) [50], can simulate diffuse reflectance from multi-layered media. Examples for the use of MC based methods for the processing of spectral data can be found in [51-53].

1.4 Organization of the dissertation

The dissertation is organized as follows. Chapter 2 gave a general survey of MC modeling of light transport in tissues. The recent progress in the development of methods for speeding up MC simulations and the future development directions were discussed too in this Chapter. Based on the literature review in Chapter 2, Chapter 3 introduced a MC method to simulate both diffuse reflectance and fluorescence from a

layered tissue model with embedded objects, which was intended to mimic early epithelial cancer model. With the help of this MC method, a series of simulation studies were performed to provide guidelines for the selection of a proper epithelial cancer model. Chapter 4 explored a hybrid method to accelerate the MC simulation of diffuse reflectance measurements from the layered tissue model with embedded objects as introduced in Chapter 3. In Chapter 5, we investigated the capability of a lens based non-contact setup for depth sensitive diffuse reflectance measurements. First, a new MC method was developed to simulate the diffuse reflectance measurements by lens based non-contact setup. Then a series of numerical studies were performed to achieve depth sensitive diffuse reflectance measurements in an early epithelial cancer model. Chapter 6 extended the setup introduced in Chapter 5 from diffuse reflectance into fluorescence measurements; moreover, an experimental phantom study was performed to validate the MC method and confirm the findings obtained in the simulation study. Chapter 7 further extended the lens based non-contact spectroscopy system to a imaging setup with a larger field of view to perform depth sensitive color imaging on an early epithelial cancer phantom. In the proposed setup, a micro-lens array was used to induce multi-focal illumination and a tunable lens was utilized to map multiple foci into the tissue phantom at a range of depths. The validation on an epithelial cancer phantom demonstrated that our method could be potentially used as a clinical tool for the diagnosis of early epithelial cancer. Then an animal study was performed in Chapter 8 to predict tissue viability in flap surgery using a dual-modal system capable of performing both UV-visible diffuse reflectance and auto-fluorescence spectroscopy. It was found possible to predict flap failures in the first 15 minutes with high accuracy when using diffuse reflectance and auto-fluorescence spectroscopy simultaneously. In the last Chapter, i.e. Chapter 9, we

summarized our achievements and further discussed the future work for the proposed techniques.

Chapter 2: Review of MC modeling of light transport in tissue

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2.1 Introduction

MC modeling was an important data analysis method which has been explored extensively for the data processing in the field of tissue optics, in this Chapter we would like to give our attention on the survey of the principles of MC modelling, the developments of MC modelling in the past decades to get a comprehensive understanding of MC modeling.

MC methods are a category of computational methods that involve the random sampling of a physical quantity [54, 55]. The term “the Monte Carlo method” can be traced back to 1940s [54], in which it was proposed to investigate the neutron transport through various materials. Such a problem cannot be solved by conventional and deterministic mathematical methods. Due to its versatility, this method has found applications in many different fields [56] including tissue optics. It has become a popular tool for simulating light transport in tissues for more than two decades [57] because it provides a flexible and rigorous solution to the problem of light transport in turbid media with complex structure. The MC method is able to solve radiative transport equation (RTE) with any desired accuracy [58] assuming that the required computational load is affordable. For this reason, this method is viewed as the gold standard method to model light transport in tissues, results from which are frequently used as reference to validate other less rigorous method such as diffuse approximation to the RTE [34, 59]. Due to its flexibility and recent advances in speed, the MC method has been explored in tissue optics to solve both the forward and inverse

problems. In the forward problem, light distribution is simulated for given optical properties; while in the inverse problem; optical properties are estimated by fitting the light distribution simulated by the MC method to experimental measured values. A general illustration for MC modeling of light transport in tissue was shown in Fig. 2-1.

Figure 2- 1 The movement of one photon through a homogenous medium

In this literature review, the principles of MC modeling for the simulation of light transport in tissues, including the general procedure of tracking an individual photon packet, common light-tissue interactions that can be simulated such as light absorption and scattering, frequently used tissue models, common contact and non-contact illumination and detection setups, and the treatment of time resolved and frequency domain optical measurements, are described in details to help interested readers achieve a quick start. Following that, a variety of methods for speeding up MC simulations, including scaling methods, perturbation methods, hybrid methods, variation reduction techniques, parallel computation, and special methods for fluorescence simulations, and their respective advantages and disadvantages are discussed. Then the biomedical applications of MC methods, including the simulation of optical spectra, estimation of optical properties, simulation of optical measurements in laser Doppler flowmetry, simulation of light dosage in photodynamic therapy

(PDT), simulation of signal source in optical coherence tomography (OCT) and diffuse optical tomography (DOT), are surveyed. Finally, the potential directions for the future development of MC methods are discussed, which are based on the current status in the literature survey and the authors' anticipation. It should be pointed out that this review is intended to give a general survey on the capability of MC modeling in tissue optics while paying a special attention on methods for speeding up MC simulations since the time consuming nature of common MC simulations could limit its applications. The more detailed and basic description of MC method in tissue optics has been explained very well by Lihong Wang et al [50], the readers are encouraged to refer to the reference for more basic and detailed information about MC method if necessary.

2.2 Principles of MC modeling of light transport in tissues

2.2.1 General procedure of steady state MC modeling of light transport in tissues

In the general procedure of MC modeling, light transport in tissues is simulated by tracing the random walk steps that each photon packet takes when it travels inside a tissue model. For each launched photon packet, an initial weight is assigned as it enters the tissue model as illustrated in Figure.2-2. The step size will be sampled randomly based on the optical properties of the tissue model. If it is about to hit a boundary, either of the following two methods could be used to handle this situation. In the first method, the photon packet will either transmit through or be reflected from the boundary. While in the second method, a fraction of the photon packet weight will always be reflected and the remaining fraction of the photon packet weight will transmit through. The probability of transmission or reflection in the first method, and the fraction of the photon packet weight transmitting through or being reflected in the

second method is governed by Snell's law and Fresnel's equations. At the end of each step, the photon packet weight is reduced according to the absorption probability; meanwhile the new step size and scattering angle for the next step will be sampled randomly based on their respective probability distributions. The photon packet propagates in the tissue model step by step until it exits the tissue model or is completely absorbed. Once a sufficient number of photon packets are launched, the cumulative distribution of all photon paths would provide an accurate approximation to the true solution of the light transport problem and the contribution averaged from all photons can be used to estimate the physical quantities of interest.

Figure 2- 2 Flow chart for MC modeling of the propagation of a single photon packet, in which no wavelength change is involved

2.2.2 Common light-tissue interactions in MC modeling

Several types of common light-tissue interactions, including light absorption, elastic scattering, fluorescence and Raman scattering, have been simulated by the MC methods previously. The absorption coefficient μ_a (unit: cm^{-1}) and the scattering coefficient μ_s (unit: cm^{-1}) are used to describe the probability of absorption and

scattering, respectively, occurring in a unit path length. The anisotropy factor g , which is defined as the average cosine of scattering angles, determines the probability distribution of scattering angles to the first-order approximation. In addition, the refractive index mismatch between any two regions in the tissue model or at the air-tissue interface will determine the angle of refraction. The fraction of photon packet weight that escapes from the same side of the tissue model as the incident light after traveling in the medium is scored as diffuse reflectance. In contrast, the fraction of photon packet weight that travels through the medium and escapes from the other side of the tissue model is scored as transmittance [50, 58, 60].

To simulate fluorescence emission, one additional parameter, which is fluorescence quantum yield [61, 62], needs to be incorporated to describe the probability that the absorbed photon packet weight can be converted to a fluorescence photon at a different wavelength. If time resolved fluorescence is simulated, the life time of fluorescence needs to be defined. The initial direction of the fluorescence photon is isotropic due to the nature of fluorescence emission. As illustrated in Figure. 2-3, the MC modeling of fluorescence propagation in tissues involves three steps [62-64]. The first step involves a general MC simulation to simulate light propagation with optical properties at the excitation wavelength. In the second step, a fluorescence photon may then be generated upon the absorption of an excitation photon with a probability defined by the quantum yield and time delay defined by the life time of fluorescence. The third step involves again a general MC simulation to simulate fluorescence light propagation with optical properties at the emission wavelength. It is clear that simulated fluorescence from a tissue model will be related to the absorption and scattering properties of the tissue model in addition to the fluorescence quantum yield and life time. Fluorescence simulation is typically much more time consuming

than the simulation of diffuse reflectance due to extra fluorescence photon propagation.

To simulate Raman emission, a parameter similar to fluorescence quantum yield, named as Raman cross section [65-68], is needed to describe the probability that a Raman photon will be generated at each step. A phase function for Raman photons needs to be determined. The MC simulation procedure for Raman light propagation will be similar to that for fluorescence.

Bioluminescence refers to the phenomenon of that living creatures producing light which results from the conversion of chemical energy to bioluminescence photons [69], which has also been investigated using MC modeling. Because bioluminescence does not need an external light source for excitation, the first step in MC simulation of bioluminescence is to generate bioluminescence photons package according to the distribution of bioluminescence sources [70, 71]. After that, the simulation of bioluminescence photon propagation in a tissue model is exactly the same as the simulation of diffuse reflectance.

If the polarization property of light is considered in MC modeling, the polarization of a photon can be represented by Stokes vectors and the polarimetry properties of the tissue model can be described by Jones matrix or Mueller matrices [72, 73], which will not be expanded in this review.

Figure 2- 3 Flow chart for MC modeling of the propagation of a single photon packet, in which one set of wavelength change is involved. λ_{exc} indicates the excitation wavelength and λ_{em} indicates the emission wavelength. The new photon packet with a different wavelength corresponds to fluorescence or Raman light at a single emission wavelength.

2.2.3 Common tissue models in MC modeling

Common tissue models used in MC simulations include the homogeneous and non-homogeneous tissue models. The optical properties in a homogeneous tissue model are equal everywhere [57, 74]. In contrast, the optical properties in a non-homogeneous tissue model vary with the tissue region. The following survey is focused on non-homogeneous tissue models because of its high preclinical and clinical relevance.

The most commonly used non-homogeneous tissue model is perhaps the multi-layered tissue model [50, 60, 63, 64, 75-81], which is frequently employed to mimic epithelial tissues. In a multi-layered tissue model, each tissue layer is assumed to be flat with uniform optical properties and it is infinitely large on the lateral dimension. This assumption works fine when the source-detector separation is small so that the

spatial variation in the optical properties within the separation is negligible. However, it could cause significant errors if the optical properties change significantly in a small area, such as in dysplasia or early cancer [82] and port wine stain model [83]. To overcome this limitation, tissue models including heterogeneities with well defined shapes have been used to mimic complex tissue structures from different organs. For example, Smithies et al. [83] and Lucassen et al. [84] independently proposed MC models in which simple geometric shapes were incorporated into layered structures to model light transport in port wine stain (PWS) model. In their PWS models, infinitely long cylinders were buried in the bottom dermal layer to mimic blood vessels. Wang et al. [85] reported a MC model in which a sphere was buried inside a slab to model light transport in human tumors. Zhu et al. [82, 86] proposed a MC model in which cuboid tumors were incorporated into layered tissues to model light transport in early epithelial cancer models including both squamous cell carcinoma and basal cell carcinoma.

Voxelated tissue models have been also explored to simulate irregular structures. Pfefer et al. [87] reported a three dimensional (3-D) MC model based on modular adaptable grids to model light propagation in geometrically complex biological tissues and validated the code in a port wine stain model. Boas et al. [88] proposed a voxel-based 3-D MC model to model arbitrary complex tissue structures and tested the code in an adult head model. Patwardhan et al. [89] also proposed a voxel-based 3-D MC code for simulating light transport in non-homogeneous tissue structures and tested the code in a skin lesion model. The three voxel based MC codes above showed great flexibility in a range of applications. However, to model tissue media with curved boundaries in a voxel-based MC model, the grid density will have to be increased, which requires more memory and computation. A few other approaches have been

explored to accommodate this situation. Li et al. [70] reported a public MC domain, named MOSE, to model bioluminescent light transport in a living mouse model. The mouse model consists of several segmented regions that are extended from several building blocks such as ellipses, cylinders, and polyhedrons. This platform is particularly suitable for small animal imaging. Margallo-Balbas et al. [90] and Ren et al. [91] have developed triangular surface based MC methods to model light transport in complex tissue structures. The triangular surface based approach allows an improved approximation to the interfaces between domains but it is not able to model complex media with continuously varying optical properties. Moreover, it could be time consuming to determine ray-surface intersection because a range of triangles will have to be scanned. To overcome the limitations associated with triangular surface based MC method, most recently, Shen et al. [92] as well as Fang [93] have presented mesh-based MC methods, by which one can model much more complex structures and situations.

2.2.4 Common illumination and detection setups in MC modeling

One important advantage of MC modeling, as compared to other non-numerical methods such as diffuse approximation, is its capability to faithfully simulate a variety of contact and non-contact illumination and detection setups for optical measurements. Note that the contact setup requires the direct contact between the tip of an optical probe and tissue samples. In contrast, the non-contact setup enables optical measurements from a tissue sample without directly contacting it.

2.2.4.1 Contact setup for illumination and detection

Fiber-optic probes are commonly used in contact illumination and detection configurations as demonstrated in many previous reports [94]. In general these fiber-

optic probes could be divided into two groups. In the first group, the same fiber or fiber bundle is used for both illumination and detection [81, 95, 96], While in the second group, separate fibers are used for illumination and detection [51, 63, 97, 98]. There is no difference in the treatment of these two groups of fiber-optic probes from the point of view of modeling because the first group of probes can be viewed as two separate and identical fibers or fiber bundles for illumination and detection that happen to locate at the same spatial position.

The key parameters in simulated fiber-optic probes include the radii, numerical apertures (NA), tilt angles of illumination and detection fibers and the center-to-center distance between the two sets of fibers (which is called the source-detector separation), as well as the refractive indices of these fibers relative to that of the tissue model. The radius and NA of the illumination fiber in combination with the radial and angular distributions of photons coming out of the fiber define the locations and the incident angles of incident photons. For a commonly used multi-mode fiber, the spatial locations and incident angles of launched photons are typically assumed to follow uniform distribution and Gaussian distribution. Both spatial locations and incident angles need to undergo spatial coordinate transformation when the tilt angle of the illumination fiber is larger than zero. Here the tilt angle of a fiber refers to the angle of the fiber axis relative to the normal axis of the tissue model. The incident beam could be also assumed to be collimated or focused.

Light detection by a fiber usually contains two steps. The first step is to determine whether an exiting photon could enter the area defined by the radius of the detection fiber. If it is true, the second step is to determine whether the exiting direction of the photon falls within the acceptable angle of the detection fiber calculated from the NA and refractive index of the fiber. If the tilt angle of the

detection fiber is larger than zero, the exiting location and angle are subject to spatial coordinate transformation.

2.2.4.2 Non-contact setup for illumination and detection

Non-contact setups usually employ various lenses for illumination and detection. In these setups, an adjunct lens or a combination of lenses is usually placed between a fiber-optic probe and the tissue sample to achieve non-contact measurements while maintaining the well defined illumination and detection geometry. Jaillon et al. [99] proposed a method to simulate a beveled fiber-optic probe coupled with a ball lens to achieve depth sensitive fluorescence measurements from layered tissue models. Later the same group incorporated a half-ball lens into the beveled fiber-optic probe to achieve the same purpose with a higher sensitivity [100]. Zhu et al. [101] proposed a method to simulate a fiber-optic probe coupled with convex lenses to achieve non-contact depth sensitive diffuse reflectance measurements from early tumors in an epithelial tissue model. By manipulating the lens combination, an ordinary cone configuration and a special cone shell configuration were investigated. It was found that the cone shell configuration provides higher depth sensitivity to the tumor than the cone configuration.

2.2.5 Time-resolved and frequency-domain MC modeling

Time-resolved optical measurements such as fluorescence life time imaging (FLIM) [102] and the complementary frequency domain measurements such as frequency domain photon migration (FDPM) have received increasing attention recently, which have been also investigated in MC modeling. A time domain technique usually measures the temporal point spread function (PSF) or the spreading of a propagating pulse in time [103, 104]. A frequency domain technique measures

the temporal modulation transfer function or the attenuation and phase delay of a periodically varying photon density wave [105, 106]. The two techniques are related by Fourier transform. Several groups have developed time domain MC models [88, 107-110] and frequency domain MC models [111-115] to simulate light transport in tissue. In the MC simulation of time resolved measurements, all the steps are the same as in steady-state measurements except that one additional parameter, i.e. time, is used to keep track of the time at which each event occurs [88, 107-110]. The refractive index in each tissue region will influence the time that photons take to travel through. In the simulation of FLIM, it needs to be pointed out that the time delay from photon absorption to fluorescence generation should follow the probability density distribution defined by the fluorescence life time [116, 117]. In the frequency domain measurements, the modulation and/or phase delay of detected waves were analyzed. The modulation and phase delay can be simulated in either a direct approach [114] or an indirect approach, i.e. using Fourier transformation from a time domain MC simulation [115].

2.3 Methods for the acceleration of MC simulation

While the MC method is the gold standard method to model light transport in turbid media, the major drawback of the MC method is the requirement of intensive computation to achieve results with desirable accuracy due to the stochastic nature of MC simulations, which makes it extremely time consuming compared to other analytical or empirical methods. Significant efforts have been made to speed up the MC simulation of light transport in tissues during the past decades. These acceleration methods can be roughly divided into several categories as follows.

2.3.1 Scaling methods

A typical scaling method requires a single or a few baseline MC simulations, in which the histories of survival photons such as trajectories or step sizes are recorded. Then diffuse reflectance or transmittance for a tissue model with different optical properties can be estimated by applying scaling relations on the recorded photon histories. These methods take advantage of the fact that the scattering properties determine photon paths and the absorption property only influence the weights of survival photons. Graaff et al. [118] proposed a limited scalable MC method for fast calculation of total reflectance and transmittance from slab geometries with different optical properties. It was demonstrated that the trajectory information obtained in a reference MC simulation with a known albedo, i.e. $\mu_s / (\mu_a + \mu_s)$, can be used to find the total reflectance and total transmittance from slabs with other albedos. Kienle et al. [119] extended Graaff's theory to simulate space- and time- resolved diffuse reflectance from a semi-infinite homogeneous tissue model with arbitrary optical properties. Their approach was based on scaling (for different scattering coefficients) and re-weighting (for different absorption coefficients) a discrete representation of the diffuse reflectance from one baseline MC simulation in a non absorbing semi-infinite medium. It is powerful but both the discrete representation and interpolation could introduce errors that are often amplified in scaling. Pifferi et al. [120] proposed a similar approach to estimate space- and time-resolved diffuse reflectance and transmittance from a semi-infinite homogeneous tissue model with arbitrary optical properties. Different from Kienle's method, the evaluation of reflectance and transmittance in Pifferi's approach is based on interpolation of results from MC simulations for a range of different scattering coefficients, and scaling is performed for absorption coefficients. This approach increases the accuracy of results for

different scattering coefficients at the cost of a significantly increased number of baseline MC simulations.

The methods reviewed above are fast but the binning and interpolation involved introduce errors. In order to improve the accuracy of these methods, Alerstam et al. [110] improved Kienle's method by applying scaling to individual photons. In this method, the radial position of the exiting location and the total path length of each detected photon are recorded and the trajectory information of each photon will be individually processed to find the survival photon weight for tissue media with other sets of optical properties. Martinelli et al. [121] derived a few scaling relationships from the radiative transport equation and their derivation showed that a rigorous application of the scaling method requires rescaling to be performed for each photon's biography individually. Two basic relations for scaling a survival photon's exit radial position r and exit weight w are listed in Eq. (2.1) and (2.2) below [51],

$$r' = r \bullet \frac{\mu_t}{\mu'_t} \quad (2.1)$$

$$w' = w \cdot \left(\frac{\alpha'}{\alpha} \right)^N \quad (2.2)$$

in which r , w , μ_t and α are the exit radial position, exit weight and transport coefficients and albedo in the baseline simulation, while r' , w' , μ'_t and α' are those in the new simulations. N is the number of collisions recorded in the baseline simulation before the photon exits. Two relations essentially assume that the same set of random numbers sampled in the baseline simulation are also used in the new simulation and everything remains unchanged in two simulations except the absorption and scattering coefficients.

Illumination and detection geometries have been also incorporated into the scaling procedure. Palmer et al. [51] extended Graaff's scaling method from illumination by a pencil beam to that by an optical fiber and they also extended the original scaling method from the total reflectance to the reflectance detected by an optical fiber by combining scaling and convolution. Wang et al. [122] proposed two convolution formulas for the scaling MC method to calculate diffuse reflectance from a semi-infinite medium for a single illumination–detection fiber. Nearly all the previous papers about scaling only dealt with a homogeneous tissue model. Liu et al. [123] developed a method that applies the scaling method to multi-layered tissue models. In this method, the homogeneous tissue model in a single baseline MC simulation is divided into multiple thin pseudo layers. The horizontal offset and the number of collisions that each survival photon experienced in each pseudo layer are recorded and used later to scale for the exit distance and exit weight of the photon in a multi-layered tissue model with different set of optical properties. The method has been validated on both two-layered and three-layered epithelial tissue models.

2.3.2 Perturbation MC methods

Similar to the scaling method, the perturbation MC (pMC) method requires one baseline simulation, in which the optical properties are supposed to be close to the optical properties in the new tissue model so that the approximation made by perturbation is valid [124]. The trajectory information including the exit weight, path length and number of collisions of each detected photon spent in the region of interest will be recorded in the baseline simulation. Then the relation between the survival weight in the baseline simulation and that in the new tissue model based on the perturbation theory [125, 126], i.e.

$$w_{new} = w \cdot \left(\frac{\mu'_s}{\mu_s} \right)^j \cdot \exp[-(\mu'_t - \mu_t)S] \quad (2.3)$$

is used to estimate diffuse reflectance from the tissue model in which the optical properties of the interesting region are perturbed. In Eq. (2.3), w , μ_s and μ_t are the exit weight, scattering coefficient and transport coefficient in the baseline simulation, while w_{new} , μ'_s and μ'_t are those in the new simulation. S and j are the photon path length and the number of collisions that a detected photon experienced in the perturbed region, respectively, recorded in the baseline simulation. It should be pointed out that the pMC is an approximation in nature so its accuracy depends on the magnitude of difference in the optical properties between the perturbed optical properties in the new tissue model and the original optical properties in the baseline simulation. In contrast, the scaling method is precise in nature regardless of the differences in optical properties because no approximation is made in scaling. One important advantage of the pMC is its simplicity and fast speed when the perturbed region is small therefore it has been explored in the inverse problem of light transport to estimate optical properties in the perturbed region as surveyed below.

Sassaroli et al. [125] proposed two perturbation relations to estimate the temporal response in diffuse reflectance from a medium, in which scattering or absorbing inhomogeneities are introduced, from the trajectory information obtained from the baseline simulation of a homogeneous medium. Hayakawa et al. [126] demonstrated that the perturbation relation can be directly incorporated into a two-parameter Levenberg-Marquardt algorithm to solve the inverse photon migration problems in a two-layered tissue model rapidly. Recently, the same group [127] demonstrated the use of this method for extraction of optical properties in a layered phantom mimicking an epithelial tissue model for given experimental measurements

of spatially resolved diffuse reflectance. This method was found effective over a broad range of absorption (50% to 400% relative to the baseline value) and scattering (70% to 130% relative to the baseline value) perturbations. However, this method requires both the thickness of the epithelial layer and the optical properties of one of the two layers.

Many other groups also proposed pMC based methods for the recovery of the optical properties in various tissue models. Kumar et al. [128] have presented a pMC based method for reconstructing the optical properties of a heterogonous tissue model with low scattering coefficients and the method was validated experimentally [80]. Their results show that a priori knowledge of the location of inhomogeneities is important to know in the reconstruction of optical properties of a heterogeneous tissue. More recently, Sassaroli et al. [129] proposed a fast pMC method for photon migration in a tissue model with an arbitrary distribution of optical properties. This method imposes a minimal requirement on hard disk space thus it is particularly suitable to solve inverse problems in imaging, such as DOT. Zhu et al. [86] proposed a hybrid approach combining the scaling method and the pMC method to accelerate the MC simulation of diffuse reflectance from a multi-layered tissue model with finite-size tumor targets. Besides the advantage in speed, a larger range of probe configurations and tumor models can be simulated by this approach compared to the scaling method or the pMC method alone.

2.3.3 Hybrid MC methods

Hybrid MC methods incorporate fast analytical calculations such as diffuse approximation into a standard MC simulation. Flock et al. [130] proposed a hybrid method to model light distribution in tissues. In this model, a series of MC simulations for multiple set of optical properties and geometrical parameters were

performed to create a coupling function. Then this coupling function was used to correct the results computed by diffusion theory. Wang et al. [131] proposed a conceptually different hybrid method to simulate diffuse reflectance from semi-infinite homogeneous media. Wang's method combined the strength of MC modeling in accuracy at locations near the light source and the strength of diffusion theory in speed at locations distant from the source. Wang et al. [132] later extended this method from semi-infinite media to turbid slabs with finite thickness, which is more useful than the previous method in practice. Alexandrakis et al. [112] proposed a fast diffusion-MC method for simulating spatially resolved reflectance and phase delay in a two-layered human skin model, which facilitates the study of frequency-domain optical measurements. This method has been proven to be several hundred times faster than a standard MC simulation. Hayashi et al. [133] presented a hybrid method to model light propagation in a head model that contains both high-scattering regions and low scattering regions. Light propagation in high-scattering regions was calculated by diffusion approximation and that in the low-scattering region, i.e. cerebrospinal fluid layer, was simulated by the MC method. Since the time-consuming MC simulation is employed only in part of the head model, the computation time is significantly shorter than that of the standard MC method. Donner et al.[134] presented a diffusion-MC method for fast calculation of steady-state diffuse reflectance and transmittance from layered tissue models. In their method, the steady-state diffuse reflectance and transmittance profiles of each individual layer were calculated and then convolved to generate the overall diffuse reflectance and transmittance to eliminate the need of considering boundary conditions. Luo et al. [135] introduced an improved diffusion model derived empirically. Then the modified diffusion model was combined with the MC method to estimate diffuse reflectance

from turbid media with a high ratio of the absorption coefficient to the reduced scattering coefficient, which can be as large as 0.07. Di Rocco et al. [136] proposed a hybrid method to speed up MC simulations in slab geometries including deep inhomogeneities. In this approach, the tissue model was treated as two sections, i.e. the top layer with a thickness of d in which there is no inhomogeneity and the bottom layer with the inhomogeneity. Propagation up to the given depth d , i.e. the top layer, is replaced by analytical calculations using diffusion approximation. Then photon propagation is continued inside the bottom layer using MC rules until the photon is terminated or detected. Tinet et al. [108] adapted the statistical estimator technique used previously in the nuclear engineering field to a fast semi-analytical MC model for simulating time-resolved light scattering problems. There were two steps in this approach. The first step was information generation in which, the contribution to the overall reflectance and transmittance was evaluated for each scattering event. The second step was information processing in which the results of first step were used to calculate desired results analytically. Chatigny et al. [137] proposed a hybrid method to efficiently model the time- and space-resolved transmittance through a breast tissue model which was divided into multiple isotropic regions and anisotropic regions. In this hybrid method, the standard MC method incorporated with the isotropic diffusion similarity rule was applied to the area that contains both isotropic and anisotropic regions; while the analytical MC which is similar to Tinet's method was used for the area that contains isotropic regions only.

2.3.4 Variance reduction techniques

In addition to hybrid methods reviewed above, multiple variance reduction techniques, which were initially applied in modeling neutron transport [138], have been also investigated in the MC modeling of light transport in tissues. For example,

the weighted photon model and Russian roulette scheme have been employed in the public-domain MC code, MCML [50]. Liu et al. [139] have used one of the oldest and the most widely used variance reduction techniques in MC modeling, i.e. geometry splitting, to speed up the creation of a MC database to estimate the optical properties of a two-layered epithelial tissue model from simulated diffuse reflectance. In this strategy, the tissue model is separated into several volumes and the technique can reduce variances in certain important volumes by increasing the chance of sampling in important volumes and decreasing the chance of sampling in other volumes. Chen et al. [140] proposed a controlled MC method in which an attractive point with an adjustable attractive factor was introduced to increase the efficiency of trajectory generation by forcing photons to propagate along directions more likely to intersect with the detector, which is similar to geometry splitting in principle. They first demonstrated this approach in the transmission geometry [140] and then in the reflection geometry [141]. Behin-Ain et al. [142] extended Chen's method for the efficient construction of the early temporal point spread function created by the visible or near infrared photons transmitting through an optically thick scattering medium. More recently, Lima et al. [143, 144] incorporated an improved importance sampling method into a standard MC for fast MC simulation of time-domain optical coherence tomography (OCT) by which several hundred times of acceleration has been achieved.

2.3.5 Parallel computation based MC methods

Parallel computation has received increasing attention recently in the study of speeding up MC simulations due to advances in computer technology. The acceleration due to parallel computation is independent of all previous techniques thus could be used in combination with them to gain extra benefit. Kirkby et al. [145]

reported a approach by which one can run a MC simulation on multiple computers simultaneously aiming to utilize the unoccupied time slots of networked computers to speed up MC simulations. This method has reduced simulation time appreciably. However, it can be time consuming to wait for all computers to update the result files in order to get the final result. Moreover, the requirement of saving disk space imposes the use of binary files and this raised compatibility issues across in various types of computers. Colasanti et al. [146] explored a different approach to address the limitations associated with Kirkby's method. They developed an MC multiple processor code that can be run on a computer with multiple processors instead of running on many single-processor computers. The results showed that the parallelization reduced computation time significantly.

Considerable efforts have also been made to implement MC codes in graphics processing unit (GPU) environment to speed up MC simulations. Erik et al. [147] proposed a method which was executed on a low-cost GPU to speed up the MC simulation of time resolved photon propagation in a semi-infinite medium. The results showed that GPU based MC simulations were 1000 times faster than those performed on a single standard central processing unit (CPU). The same group [148] further proposed an optimization scheme to overcome the performance bottleneck caused by atomic access to harness the full potential of GPU. Martinsen et al. [149] implemented the MC algorithm on an NVIDIA graphics card to model photon transport in turbid media. The GPU based MC method was found to be 70 times faster than a CPU based MC method on a 2.67 GHz desktop computer. Fang et al. [150] reported a parallel MC algorithm accelerated by GPU for the simulation of time resolved photon propagation in an arbitrary 3-D turbid media. It has been demonstrated that GPU based approach was 300 times faster than the conventional

CPU approach when 1792 parallel threads were used. Ren et al. [91] presented a MC algorithm that was implemented into GPU environment to model light transport in a complex heterogeneous tissue model in which the tissue surface was constructed by a number of triangle meshes. The MC algorithm has been tested and validated in a heterogeneous mouse model. Leung et al. [151] proposed a GPU based MC model to simulate ultrasound modulated light in turbid media. It was found that a GPU based simulation was 70 times faster compared to CPU based approach on the same tissue model. Most recently, Cai et al. [152] implemented a fast perturbation MC method proposed by Angelo [129] on GPU. It has been demonstrated that the GPU based approach was 1000 times faster compared to the conventional CPU based approach.

Besides using GPU to speed up the MC simulations, some researchers have explored using field-programmable gate arrays (FPGA) to accelerate MC simulations. For example, Lo et al. [153] implemented a MC simulation on a developmental platform with multiple FPGAs. The FPGA based MC simulation was found to be 80 times faster and 45 times more energy efficient on average than the MC simulation executed on a 3-GHz Intel Xeon processor.

Recently, internet based parallel computation has gained increasing attention for fast MC modeling of light transport in tissues. Pratz et al. [154] reported a method for performing MC simulation in a massively-parallel cloud computing environment based on MapReduce developed by Google. For a cluster size of 240 nodes, an improvement of 1258 times in speed was achieved as compared to the single threaded MC program. Doronin et al. [155] developed a peer-to-peer (P2P) MC code to provide multi-user access for the fast online MC simulation of photon migration in complex turbid media. Their results showed that this P2P based MC simulation was three times faster than the GPU based MC simulations.

2.3.6 Acceleration of MC simulation of fluorescence

The methods reviewed above are all about the acceleration of MC simulation of diffuse reflectance or transmittance. Compared to diffuse reflectance, fluorescence simulation is more complex and much more time consuming due to the generation of fluorescence photons upon each absorption event of an excitation photon. A number of groups [62-64, 81, 107, 156-158] have employed MC modeling to simulate fluorescence in tissues due to the growing interest in fluorescence spectroscopy or imaging for medical applications [159-162]. As a consequence, multiple groups have investigated various methods to speed up the MC simulation of fluorescence in biological tissues. Swartling et al. [64] proposed a convolution based MC method to accelerate the simulation of fluorescence spectra from layered tissues. Their method exploited the symmetry property of the problem, which requires the multi-layered tissue model to be infinite in the radial dimension. Different from the conventional fluorescence MC code, this method computed the excitation and emission light profiles separately, from which the spatial distribution of absorption and emission probabilities were obtained. Then a convolution scheme will be applied on the absorption probability and emission probability data to get the final fluorescence signals. Swartling's method has been used by Palmer et al. [53, 163] to create a MC database for fluorescence spectroscopy to estimate the fluorescence property of a breast tissue model from fluorescence measurement using a fiber-optics probe. Liebert et al. [107] developed a MC code for fast simulation of time resolved fluorescence in layered tissues. In this method, both the spatial distribution of fluorescence generation and the distribution of times arrival (DTA) of fluorescence photons at the detectors were calculated along the excitation photons' trajectories. Then the distribution of fluorescence generation inside the medium and DTA as well

as the fluorescence conversion probability were used to calculate the final fluorescence signal. It should be noted that the reduced scattering coefficients at the excitation and emission wavelengths have to be approximately equal in this method.

2.3.7 Comparison of methods for MC acceleration

Most methods surveyed in the previous sections have been compared and summarized in table 2-1 with respect to their acceleration performance, relative error in simulated optical measurements, respective advantages and limitation. It should be noted that those parallel computation based methods were not listed in this table because its performance highly depends on the computing architecture and all the methods summarized in this table can be further accelerated by applying parallel computation

Table 2- 1.Comparison of various methods in MC acceleration

Methods	Acceleration relative to standard MC	Relative error in simulated optical measurements	Advantages	Limitations
Scaling MC	~200 [123]	Less than 4% [123]	No approximation is made, and it is accurate and fast.	Applicable to layered tissue models only so far.
Perturbation MC (pMC)	~1300 [129]	Can be less than 4% depending on the magnitude of perturbation [129]	It is applicable to tissue with complex structures.	Sensitive to perturbation in scattering properties.
Hybrid MC	~300 [132]	Around 5% [132]	It has a larger applicable range than pMC.	Relatively complicate computation; The particular region has to be homogeneous.
Variance reduction	~300 [143, 144]	Around 5% [143, 144]	There are a variety of choices available.	Limitation varies with the specific technique.

Note: The improvement relative to standard MC was defined as the fold of improvement in computation speed compared to a standard MC simulation in order to obtain results with comparable

variance. GPU based methods were not listed in this table because all the methods summarized in this table can be further accelerated by GPU.

2.4 Application of MC methods in tissue optics

The most common application of MC method in the tissue optics is the simulation of optical measurements such as diffuse reflectance, transmittance and fluorescence for a given tissue model and illumination/detection geometry, which is considered as a forward problem. In this situation, MC simulations could provide guidelines for the selection of optimal illumination/detection geometry for selective optical measurements [97, 99, 164-167]. In contrast, MC simulations can also provide data to estimate the optical properties of a tissue model from optical measurements, which is considered as an inverse problem. Solving an inverse problem typically involves the use of a non-linear least square error algorithm [51, 139] or a similar algorithm to find a set of optical properties that would yield optical measurements in MC simulations best matching the actual measurements. Due to the slow speed of traditional MC simulations, a database is frequently created *a priori* in such an inverse problem to speed up the inversion process [168]. Most of the acceleration methods discussed above can be employed in the creation of such a MC database.

The MC method has been frequently used to find the optimal optical configuration in laser Doppler flowmetry (LDF), one of the oldest techniques in biomedical optics during the past decade. Jentink et al. [169, 170] used MC simulations to investigate the relationship between the output of laser Doppler perfusion meters and the optical probe configuration as well as the tissue scattering properties. Stern et al. [171] used MC modeling to simulate the spatial Doppler sensitivity field of a two-fiber velocimeter by which an optimal fiber configuration was identified. Similar applications can be also found in other references [172-174].

Recently, MC method has been incorporated into LDF to estimate blood flow [175, 176] or the phase function of light scattering [177].

The MC method plays an important role in the selection of optimal configuration for photodynamic therapy (PDT) because it can generate light distribution in a complex tissue model for PDT dosage determination. Barajas et al. [178] simulated the angular radiance in tissue phantoms and human prostate model to characterize light dosimetry using the MC method. Liu et al. [179] used the MC method to simulate the temporal and spatial distributions of ground-state oxygen, photosensitizer and singlet oxygen in a skin model for the treatment of human skin cancer. Valentine et al. [180] simulated *in vivo* protoporphyrin IX (PpIX) fluorescence and singlet oxygen production during PDT for patients with superficial basal cell carcinoma. Later, the same group [181] used the MC method to identify optimal light delivery configuration in PDT on non-melanoma skin cancer.

The MC method has also been investigated to simulate the optical coherence tomography (OCT) signals [182, 183] and images [184-186] during past years due to its flexibility and high accuracy. Moreover, with the development of efficient MC methods, researchers have started to explore the MC method for image reconstruction in diffuse optical tomography (DOT) [187, 188].

2.5 Potential future directions and discussions

Due to advances in computing technology, it is expected that the applications of the MC method will be expanded in the near future. A few potential directions in the development of the MC method are discussed as follows.

2.5.1 Phase function of Raman scattering

Raman spectroscopy has been explored extensively for tissue characterization [66, 68, 189, 190] including cancer diagnosis [4, 65, 191-197]. Depending on whether the excitation light is coherent or incoherent, Raman scattering can be broken down into two categories, i.e. spontaneous Raman scattering or coherent Raman scattering. The signal generated out of spontaneous Raman is typically very weak, in which the probability of generating a Raman photon for every excitation photon is lower than 10^{-7} [198, 199]. Different from that, coherent Raman techniques utilize laser beams at two different frequencies to produce a coherent output, which result in much stronger coherent Raman signals compared to spontaneous Raman scattering. Because of the high chemical specificity of Raman spectroscopy, it is anticipated that there will be more studies using the MC method for Raman spectroscopy to optimize experimental setup. One important issue in these studies is that, the phase function of Raman scattering from biological components in tissues have not been systematically studied. Recent MC studies on Raman scattering [65, 66] assumed isotropic Raman emission. This assumption should work fine for spontaneous Raman scattering according to a numerical study [200]. However this assumption is not valid for coherent Raman scattering since the angular distribution of Raman emission is affected by both the wavelength of the pump light source and the propagating beam geometry [200-202]. A systematic study on the phase function of Raman scattering on the molecule level for Raman active biological molecules such as protein and DNA and on the subcellular level for organelles such as mitochondria will be very helpful, in which one or a couple of key parameters similar to the anisotropy factor in elastic scattering could accurately describe the angular distribution of Raman scattering in most common cases. The use of such validated phase functions in MC simulations will yield more useful information than the simplistic treatment in the current literature.

2.5.2. Incorporation of more realistic elastic light scattering model into the MC method

Despite the exploration of various inhomogeneous tissue models discussed above, including the multi-layered tissue model, voxel based and mesh based tissue models, these tissue models are all based on a few simple optical coefficients including the scattering coefficients and anisotropy factor to characterize optical scatterers. A complete phase function could be used to provide the comprehensive information related to the morphology of optical scatterers but it is inconvenient for use and its physical meaning is not straightforward. From these scattering properties, the scatterer size and density can be derived [51, 203] if they are assumed to be uniformly distributed spheres with homogeneous density. In many scenarios, these assumptions are not valid. For example, it is commonly known that the size and shapes of cells vary significantly with the depth from the tissue surface and they also change with carcinogenesis. From this point of view, the superposition of multiple phase functions [204] or the fractal distribution of the scatterer size [205] have been proposed to accommodate special situations. An equiphase-sphere approximation for light scattering has been also proposed by Li et al. [206] to model inhomogeneous microparticles with complex interior structures. Later the same group reported two stochastic models [207] , i.e. the Gaussian random sphere model and the Gaussian random field model, to simulate irregular shapes and internal structures in tissues. The incorporation of these more realistic elastic light scattering models into the MC method will expand its capability and offer more accurate information about light scatterers in tissues.

2.5.3. Exploration of the MC method in imaging reconstruction

In most current applications of the MC method, the tissue model is assumed to be a simple layered model or determined *a priori*, which does not fully exploit the potential of the MC method in preclinical or clinical imaging/spectroscopy. When the MC method becomes adequately fast in the near future, which might be mostly attributed to the combination of the accelerated MC methods and parallel computing discussed above, the MC method could be used to reconstruct the optical properties of a complex tissue in optical tomography, in which the morphological structure could be obtained in real time by fast imaging techniques such as Optical coherence Tomography (OCT) for superficial regions of interest or magnetic resonance imaging (MRI) for deep regions of interest in a large tissue volume. Those voxelated tissue models [70, 87-93], which are more realistic than simplified homogeneous or layered tissue models, can be readily used in such reconstruction. The speed of reconstruction might be comparable to that using diffusion approximation reported in the current literature but the accuracy would be considerably higher in a tissue model on the millimeter scale.

In summary, the principles of MC modeling for the simulation of light transport in tissues were described at the beginning. Then a variety of methods for speeding up MC simulations were discussed to overcome the time consuming weakness of MC modeling. Then the applications of MC methods in biomedical optics were briefly surveyed. Finally, the potential directions for the future development of the MC method in tissue optics were discussed.

Chapter 3: Finite-size tumor model in diagnosis of early epithelial cancer

[Copyright permission from: C. Zhu and Q. Liu, “Validity of the semi-infinite tumor model in diffuse reflectance spectroscopy for epithelial cancer diagnosis: a Monte Carlo study,” Opt Express 19, 17799-17812 (2011).]

3.1 Introduction

As we reviewed in Chapter 2, the MC method has been considered as a gold standard tool to study light transport in tissues since 1980s [208] for providing guidance on the design of optical setups for tissue measurements [123]. Compared to analytical models such as diffusion theory, the MC method can be used in a much broader range of optical properties and measurement geometry. Hence it is frequently used to validate the results from analytical models [58]. Moreover, one unique advantage of the MC method is its ability to simulate light fluence rate distribution inside a complex tissue model. Because MC simulations are generally time consuming, a variety of methods have been developed to accelerate the simulations so that this method could be used to solve inverse problems, for example, to determine the optical properties of a tissue sample for optical diagnosis [51, 126, 139].

The most common tissue model used in three-dimensional MC simulations assumes a layered structure, in which the tissue consists of one or more layers with homogeneous optical properties within each layer [50, 209]. Every tissue layer is assumed to be semi-infinite, which infers infinite width and length, but finite thickness. While this semi-infinite model works fine for large tumors, it may result in significant deviation from actual measurements when applied on small tumors at early stage. This would consequently cause inaccurate diagnosis.

The focus of this study is to investigate the validity of the semi-infinite tumor model in two commonly seen epithelial cancers, i.e. squamous cell carcinoma (SCC) and basal cell carcinoma (BCC). Two fiber-optic probe configurations, including one with fibers perpendicular to the tissue surface and the other with tilted fibers, were examined. The dependence of diffuse reflectance on the variation of the tumor width, ranging from zero (corresponding to the case of no tumor) to infinity (corresponding to a semi-infinite tumor), was evaluated in both SCC and BCC models. Moreover, the effects of the tumor thickness, the source-detector separation, and the tilt angles of source and detector fibers on the validity of the semi-infinite tumor model were studied. Two look-up tables, which relate the validity of the semi-infinite tumor model to the tumor width in terms of the source-detector separation, were given to guide the tumor model selection in diffuse reflectance spectroscopy. Finally, the effects of the top layer's thickness and the emission wavelength on the simulation results were discussed.

3.2 Material and methods

3.2.1 MC method for finite-size tumor model

To perform the study proposed above, a 3D MC code based on MCML was developed to simulate light transport in layered tissue with embedded objects which is used to mimic finite-size tumor model. The finite-size tumor model is made from several layers while containing a finite tumor-like heterogeneity. The location and size of the cuboid tumor like target can be specified. The flowchart of the new MC code was illustrated in Figure.3-1 as follows. Generally, the flow chart of the MC code can be divided into five sections: (1) initial photon launch; (2) photon migration at the air-tissue boundary; (3) photon movement within the tissue and photon-tissue

interaction; (4) photon migration at the interfaces between layers as well as between the target and surrounding tissue layers; (5) photon termination. In this flowchart, the boundary refers to the interface between two tissue layers; while the inner interface refers to the interface between the buried target and its surrounding tissue.

Figure 3- 1 Flow chart of the MC code for layered tissue with embedded object

To verify our code, we compared our results with the published data [87] under the same tissue model. This is a skin model that consists two layers with different optical properties: an epidermis of 60 μm and a very thick (2 mm) dermis. A blood vessel with a diameter of 120 μm is buried 260 μm under the epidermis. In our simulations, the blood vessel was specified to be a cuboid with a surface area of 120 $\mu\text{m} \times 120 \mu\text{m}$ which is very close to a cylindered blood vessel used in the reference we picked for the verification. The cuboid was located 260 μm under the epidermis. The

optical properties for the skin model were exactly same as that used in the reference. The results shown in Figure 3-2 agree very well both quantitatively and qualitatively with the published data.

Figure 3- 2 Comparison of simulation result with published data[87]

3.2.2 Fiber probe configurations

Two commonly used probe configurations were examined, as shown in Figure.3-3. In Figure.3-3(a), both fibers were perpendicular to the tissue surface thus the tilt angles of both fibers relative to the normal axis of the tissue surface were 0 degree. The center-to-center distance between the source and detector fibers (S-D), was varied from 200 μm to 800 μm with an increment of 200 μm . In Figure.3-3(b), the tilt angles of both fibers relative to the normal axis of the tissue surface were 45 degrees and the S-D was varied from 400 μm to 600 μm , and then to 800 μm . The diameters of all fibers were 200 μm and the numerical aperture (NA) was 0.22. The refractive indices of all the fibers were set to 1.47. Ten million photons were launched in all simulations.

Figure 3- 3 Probe configurations with tilt angles of both fibers at (a) 0 degree and (b) 45 degrees, relative to the normal axis of the tissue surface. The two cylinders in both sets represent the source and

detector fibers and the arrows indicate the direction of light propagation. The acronym S-D represents the center-to-center distance between source and detector fibers.

3.2.3 Tissue models

3.2.3.1 Squamous cell carcinoma (SCC) tissue model

An epithelial tissue is typically composed of two layers, the epithelium on the top and the stroma at the bottom. The basement membrane separates the two layers. In the development of an SCC, the tumor usually originates from the basement membrane of the epithelium [3]. The tumor first proliferates upward; after the entire epithelium is occupied, the tumor will invade into the basement membrane towards the stroma. Therefore an epithelial tissue model with SCC used in the literature [165] consists of three layers, i.e. the epithelium on the top, the tumor in the middle and the stroma at the bottom. Each of these three layers was assumed to be semi-infinite in which the thickness is finite while the width and length are infinite.

We studied two SCC models, as shown in Figure.3-4, with (a) a semi-infinite and (b) a finite-width tumor in our simulation. The epithelial thickness was set to be 300 μm and the thickness of the stroma was set to be 2050 μm to mimic a thick tissue. The optical properties of each layer were obtained from the literature [210] and listed in Table.3-1. A refractive index of 1.4 was used in all tissue layers [211]. The anisotropy factors for the epithelium and the tumor were set to 0.97 and an anisotropy factor of 0.8 was used for the stroma [211]. In the semi-infinite tumor model [Figure.3-4 (a)], the epithelium, tumor and stroma were assumed to have infinite width and length. For the finite-width tumor model [Figure.3-4 (b)], an infinite-length tumor with finite thickness and width, was introduced into the epithelium. In all

simulations, the central axis of the tissue model bisects the source and detector fibers in Figure.3-3 and the tumors in Figure.3-4 on the cross section view.

Totally two sets of simulations were performed on every SCC tissue models, one for each probe configuration. In each set, the thickness of the tumor was varied from 100 μm to 200 μm to investigate the effect of the tumor thickness. For each thickness, the width of the tumor as in Figure.3-4 (b) was varied from zero to a large value with uneven increments to find the minimum width of the tumor required for the validity of the semi-infinite tumor model as in Figure.3-4(a). A threshold value of the tumor width was determined for all tumor thicknesses to guide the selection of the semi-infinite tumor or finite-width tumor in the SCC tissue model, based on the tumor size.

Figure 3- 4 Cross section schematics of the squamous cell carcinoma (SCC) tissue models (a) with a semi-infinite tumor and (b) with a finite-width tumor. The central dashed lines in both (a) and (b) give the central axes of the tissue model used in the simulations, which bisects the source and detector fibers in Figure.3-3 and the tumor in Figure.3-4 on the cross section view. In the finite-width model, the tumor has a specified finite thickness (h) and width (w).

Table 3- 1 Optical properties of the SCC tissue model at 500nm [210]

Tissue	Optical properties		
	$\mu_a(cm^{-1})$	$\mu_s(cm^{-1})$	g
Epithelium	2.0	35.6	0.97
Tumor	2.0	106.8	0.97
Stroma	9.1	223.7	0.8

Note: μ_a : absorption coefficient; μ_s : scattering coefficient; g : anisotropy

3.2.3.2 Basal cell carcinoma (BCC) tissue model

Basal cell carcinoma is a common form in non-melanoma cancer of skin, which consists of a BCC tumor sandwiched in between the superficial epidermis and the underlying dermis. The tumor in basal cell carcinoma originates from the basal layer of the epidermis, frequently grows downward deeply into the dermis [4, 212].

Two BCC models were examined in our simulations, as shown in Figure.3-5, with (a) semi-infinite tumor and (b) a finite-width tumor. The width and length of the epidermis were assumed to be infinitely large, while the epidermal thickness was set to be 80 μm and the thickness of the dermis was set to be 2000 μm to mimic a thick skin tissue. These values are the typical skin thickness for BCC frequently occurs on the neck and back [213], although the epidermal thickness could vary with organ sites. In addition, the absorption coefficient, scattering coefficient and anisotropy used in our simulation were taken from the literature and listed in Table.3-2 [214]. A refractive index of 1.4 and an anisotropy factor of 0.8 were used in all the three layers [214]. In the BCC model with a semi-infinite tumor [Figure.3-5(a)], the tumor has an infinite width. In the BCC tissue model with a finite-width tumor, an infinite-length tumor with finite thickness and width was introduced into the dermis [Figure.3-5 (b)]. Similar to the SCC models, the central axis of the tissue model bisects the source and detector fibers as in Figure.3-3 and the tumors in Figure.3-5 on the cross section view.

The BCC tissue model simulations were performed on fiber probe configurations with tilt angles of 0 degree and 45 degrees. The thickness of the tumor was varied from 200 μm to 400 μm for each fiber probe configuration. The tumor width was increased from zero [finite-width tumor in Figure.3-5(b)] to infinity [semi-infinite tumor in Figure.3-5(a)] with uneven increments for thicknesses of 200 μm and 400 μm ,

respectively, to determine the minimum threshold of the tumor width for the validity of the semi-infinite tumor model.

Figure 3- 5 Cross section of the basal cell carcinoma (BCC) tissue model (a) with a semi-infinite tumor and (b) with a finite-width tumor. The central dashed lines in both (a) and (b) represent the central axes of the coordinate systems used in the simulations, bisects the source and detector fibers as in Figure. 1 and the tumors in Figure. 3 on the cross section view. In finite-width tumor model, the tumor has a specified finite thickness (h) and width (w).

Table 3- 2 Optical properties of BCC tissue model at 500nm [214]

Tissue	Optical properties		
	$\mu_a(cm^{-1})$	$\mu_s(cm^{-1})$	g
Epidermis	7.0	350	0.8
Tumor	3.1	160	0.8
Dermis	3.5	250	0.8

Note: μ_a : absorption coefficient; μ_s : scattering coefficient; g : anisotropy

3.3 Results

In the following results for both SCC and BCC models, the diffuse reflectance values simulated for each fiber configuration and tumor model were plotted as a function of the tumor width in terms of the source-detector separation (S-D). The diffuse reflectance corresponding to the finite tumor widths were compared to the last data point in each curve that corresponds to the semi-infinite tumor model by performing the unpaired two-sample t test. For those data points corresponding to small tumor widths that are likely to be different from the last data point, five repeated

simulations have been performed to estimate the means and standard deviations for the construction of error bars. Those circled data points in Figure.3-6 through Figure.3-9 indicate statistically significant differences from the last data point with a p-value smaller than 0.05. The threshold value is defined as the smallest tumor width at which the finite tumor model is statistically equivalent to the semi-infinite tumor model in terms of simulated diffuse reflectance.

3.3.1 Results for the SCC tissue model

Simulated diffuse reflectance values as a function of the tumor width for the SCC tissue model are shown in Figures.3-6 and 3-7, which correspond to tilt angles of 0 degree and 45 degrees, respectively.

Figure.3-6 shows that there are no significant differences in simulated diffuse reflectance values between the SCC tissue models with a finite-width tumor at different widths and that with a semi-infinite tumor for the probe configuration with zero-degree tilt angle. The diffuse reflectance value decreases with the increment of S-D. Interestingly, the diffuse reflectance changes only minimally when the tumor thickness increases from 100 μm to 200 μm . This should be mainly due to the fact that the absorption coefficients of the epithelium and the tumor were equal. Moreover, the small thickness and the high anisotropy factor of the epithelium minimized the effect of different scattering coefficients on detected diffuse reflectance.

In contrast, Figure.3-7 reveals that the probe configuration at 45-degree tilt angle in the SCC tissue model shows significant differences in diffuse reflectance between the models with a semi-infinite tumor and those with a finite-width tumor for tumor widths smaller than a threshold value as highlighted by circled data points. The threshold values of the tumor width for S-D at 400 μm , 600 μm and 800 μm were

around 0.75, 1 and 1 time of S-D when the tumor thickness was 100 μm . These threshold values did not change when the tumor thickness was increased to 200 μm .

Figure 3- 6 Simulated diffuse reflectance as a function of the tumor width in terms of the source-detector separation (S-D) when the tumor thickness was fixed at (a) 100 μm and (b) 200 μm in a squamous cell carcinoma (SCC) tissue model. The tilt angles of all fibers were fixed at 0 degree and the S-D was varied from 200 μm to 800 μm with an increment of 200 μm . Each curve is divided into four segments according to the increment of the tumor width on the horizontal axis. The error bars at

initial data points and the last data point represent the standard deviation of the data. The label “Inf” on the horizontal axis stands for “Infinity”.

Figure 3- 7 Simulated diffuse reflectance as a function of the tumor width in terms of the source-detector separation (S-D) when the tumor thickness was fixed at (a) 100 μm and (b) 200 μm in a squamous cell carcinoma (SCC) tissue model. The tilt angles of all fibers were fixed at 45 degrees and the S-D was varied from 400 μm to 800 μm with an increment of 200 μm. Each curve is divided into four segments according to the increment of the tumor width on the horizontal axis. The error bars at initial data points and the last data point represent the standard deviation of the data. Among these data points, the circled ones are statistically different from that for the SCC tissue model with a semi-infinite tumor, which corresponds to the last data point in each subplot. The label “Inf” on the horizontal axis stands for “Infinity”.

3.3.2 Results for the BCC tissue model

Simulated diffuse reflectance as a function of the tumor width for the BCC tissue model are shown in Figures.3-8 and 3-9, which correspond to tilt angles of 0 degree and 45 degrees, respectively.

Different from the results for the SCC tissue model, the simulated diffuse reflectance changes significantly with the tumor width in the BCC tissue model as shown in Figure.3-8 even when the tilt angles of source and detector fibers are zero degree. Consequently, a significant difference can be observed between the BCC tissue models with finite-width tumors and that with a semi-infinite tumor when the tumor width was smaller than a threshold value as highlighted by circled data points in Figure.3-8. The threshold values of the tumor width for the S-D of 200 μm , 400 μm , and 600 μm and 800 μm were around 3, 2, 1.25 and 1.25 times of S-D when the thickness of tumor was 200 μm . The threshold values changed to around 2, 1.75, 1.5 and 1.25 times of S-D when the tumor thickness was 400 μm .

When the tilt angles of source and detector fibers were 45 degrees, both the detected diffuse reflectance and the threshold values changed as shown in Figure.3-9 compared to the case of zero-degree tilt angles in Figure.3-8. The threshold values of the tumor width for the S-D of 400 μm , 600 μm and 800 μm were around 1, 1.75 and 1 times of S-D when the tumor thickness was 200 μm . The threshold values changed to around 1.25, 1 and 1 times of S-D when the tumor thickness was 400 μm . Similar to Figure.3-8, it appears that the threshold values changed only moderately with the tumor thickness.

Figure 3- 8 Simulated diffuse reflectance as a function of the tumor width in terms of the source-detector separation (S-D) when the thickness of tumor was fixed at (a) 200 μm and (b) 400 μm in a basal cell carcinoma (BCC) tissue model. The tilt angles of all fibers were fixed at 0 degree and the S-D was varied from 200 μm to 800 μm with an increment of 200 μm . Each curve is divided into four segments according to the increment of the tumor width on the horizontal axis. The error bars at initial data points and the last data point represent the standard deviation of the data. Among these data points, the circled ones are statistically different from that for the BCC tissue model with a semi-infinite tumor, which corresponds to the last data point in each subplot. The label “Inf” on the horizontal axis stands for “Infinity”.

Figure 3- 9 Simulated diffuse reflectance as a function of the tumor width in terms of the source-detector separation (S-D) when the tumor thickness was fixed at (a) 200 μm and (b) 400 μm in a basal cell carcinoma (BCC) tissue model. The tilt angles of all fibers were fixed at 45 degrees and the S-D was varied from 400 μm to 800 μm with an increment of 200 μm. Each curve is divided into four segments according to the increment of the tumor width on the horizontal axis. The error bars at initial data points and the last data point represent the standard deviation of the data. Among these data points, the circled ones are statistically different from that for the BCC tissue model with a semi-infinite tumor, which corresponds to the last data point in each subplot. The label “Inf” on the horizontal axis stands for “Infinity”.

Tables.3-3 and 3-4 summarize the threshold values for the various tumor thicknesses and probe configurations in the SCC tissue model and the BCC tissue model, respectively. The zero threshold value implies that the SCC tissue models with finite-width tumors are similar (statistical difference with a p-value greater than 0.05)

to that with a semi-infinite tumor in diffuse reflectance for all tumor widths under evaluation. In summary, the variations in the S-D, tumor thickness and tilt angle of source and detector fibers, have an effect on the threshold value of tumor width/S-D for the validity of the semi-infinite tumor model.

Table 3- 3 Threshold value of tumor width for the valid semi-infinite SCC tumor model

Tumor thickness	100 μm		200 μm	
	Tilt angle (degrees)			
S-D(μm)	<i>0</i>	<i>45</i>	<i>0</i>	<i>45</i>
200	0	NA	0	NA
400	0	0.75	0	0.75
600	0	1	0	1
800	0	1	0	1

Note: "NA" stands for "not available"

Table 3- 4 Threshold value of tumor width for the valid semi-infinite BCC tumor model

Tumor thickness	200 μm		400 μm	
	Tilt angle (degrees)			
S-D (μm)	<i>0</i>	<i>45</i>	<i>0</i>	<i>45</i>
200	3	NA	2	NA
400	2	1	1.75	1.25
600	1.25	1.75	1.5	1
800	1.25	1	1.25	1

Note: "NA" stands for "not available"

3.4 Discussions

The validity of the semi-infinite tumor model and diffuse reflectance values in the SCC and BCC tissue model are affected by the variations in S-D, tumor thickness and the tilt angle of source and detector fibers. The trends in the threshold value of tumor width threshold for semi-infinite tumor validity and the diffuse reflectance in

the SCC and BCC tissue models can be explained by considering light transport in the tissue models as following.

In the SCC tissue models, the simulated diffuse reflectance values are similar for probe configuration with zero-degree tilt angles, between the semi-infinite SCC tissue model and the finite-width SCC tissue models when S-D varies from zero to infinity. This observation can be explained by the fact that photons detected in this probe configuration travel only short paths in the tumor arising from the high anisotropy value of 0.97 of the epithelium and tumor. In contrast, significant differences (p-value smaller than 0.05) are observed for the probe configuration with 45-degree tilt angle as highlighted by circled data points in Figure.3-7, in the diffuse reflectance values between the finite-width and semi-infinite SCC tissue models. Tilted fibers allowed more photons primarily traveled in the top layer to be detected compared to the probe configuration with zero-degree tilt angle. Therefore, it can be inferred that the probe configuration with 45-degree tilt angle is much more sensitive to the superficial tumor than that with zero-degree title angle, which agrees with previous publications [139, 166, 215, 216].

In Figure.3-7 where the tilt angles of fibers were 45 degrees, it is observed that the threshold value is lower for smaller SD separations in general, which is due to the fact that the fiber-optic probe with a smaller S-D separation probes the tissue volume in a smaller horizontal range.

The diffuse reflectance simulated for finite-width BCC tissue models in the case of the probe configuration with zero tilt angles are different from that for the semi-infinite BCC tissue model as highlighted by circled data points in Figure.3-8. This trend is different from that in the SCC tissue model as in Figure.3-6. The reason is that the epidermis has an anisotropy value of 0.8 (in comparison to an anisotropy

value of 0.97 in the epithelium of the SCC model) thus photons were likely to propagate towards and travel through the BCC tumor. So the probe configuration with a zero-degree tilt angle was more sensitive to the BCC tumor than to the SCC tumor. In addition, the optical properties of the BCC tumor are different from those of the surrounding dermis.

The threshold values in the BCC tissue model for the probe configuration with 45-degree tilt angle are in general smaller than those for the probe configuration with zero-degree tilt angle as shown in Table.3-4. This is likely due to the fact that the fiber-optic probe with 45-degree tilt angle examines the tissue volume in a smaller horizontal range than that with zero-degree tilt angle.

In the SCC tumor model, we used a commonly cited value, i.e. 300 μm , for the epithelial thickness. However, the epithelial thickness varies significantly, with a typical range from 200 μm to 500 μm [217]. It should be noted that the change of the epithelial thickness may affect the results in this study and this effect is discussed as following. Assuming that the tumor size is fixed, the tumor depth will increase when the total epithelial thickness is increased because the SCC tumor is located at the bottom of the epithelium. Due to the high anisotropy value in the top layer, the effective mean free path in the top layer for the optical properties in table 3-1, defined as $1/[\mu_s(1-g)]$, is about 0.94 cm, which is much larger than 500 μm . Therefore most photons would travel directly through the top layer and spend much longer path in the bottom layer just like when the top layer was 300 μm thick in the SCC model. For this reason, we expect that the trend in simulated diffuse reflectance as a function of the tumor width would be similar to Figure.3-6 and 3-7 when the epithelial thickness changes. However, the exact threshold values will be different.

The threshold value depends on the contribution of the tumor to total diffuse reflectance. Only if the contribution of the tumor to simulated diffuse reflectance is significant, there will be a difference in simulated diffuse reflectance between the finite-width tumor model and the semi-infinite tumor model. Due to the numerical aperture of the fibers, a light delivery cone and a light acceptance cone will be formed at the end of the source and detector fibers, respectively. The overlapping region of the light cones between the source and detector fibers determines the origination of simulated diffuse reflectance, which will be called the detection region in the following discussion. The tumor volume covered by the detection region relative to the total detection region indicates the contribution of the tumor to simulated diffuse reflectance.

For the probe configuration with a zero degree tilt angle, the contribution of the tumor to simulated reflectance is small since the majority of diffuse reflectance is contributed by the deeper stroma as indicated by the tumor volume covered by the detection region in Figure.3-10 (a). The contribution of the tumor will increase when the total thickness is increased from 300 μm to 500 μm because a larger tumor volume will be covered by the detection region. Thus the probe configuration will become more sensitive to the tumor and the threshold value will be likely to increase to a non-zero value. In contrast, the contribution of the tumor will decrease when the total thickness is decreased from 300 μm to 200 μm , thus the probe configuration will be still insensitive to the tumor and the threshold value will be still zero.

For the probe configuration with a 45-degree tilt angle and a small S-D, a large portion of the tumor volume is covered by the detection region, as shown in Figure.3-10(b). As the epithelial thickness is increased, the tumor will gradually move out of the detection region. When the tumor is entirely out of the detection region,

simulation diffuse reflectance will be insensitive to the tumor width. Before the tumor is out of the detection region, the threshold value of the tumor width depends on the tumor volume covered by the detection region, whose trend is difficult to predict because both the complex shape of the detection region and the tumor height may influence that. It is noted that the detection region shown in Figure.3-10(b) will change with the S-D, which will further complicate the prediction of the trend. Similarly, the threshold values will change when the epithelial thickness is decreased from 300 μm to 200 μm but the exact values are difficult to predict.

Figure 3- 10 Schematic of the SCC tumor (light gray color) relative to the detection region (black color) with an increasing epithelial thickness for (a) the probe configuration with a zero-degree tilt angle and (b) the probe configuration with a 45-degree tilt angle. It is assumed that the tumor thickness was fixed to be 100 μm when the epithelial thickness was increased from 300 μm to 500 μm in both (a) and (b).

In the BCC model, the epidermal thickness may affect the results obtained in this study just as in the SCC model. The effective mean free path in the epidermis for the optical properties in Table.3-2 is around 142 μm . When the epidermal thickness is close to this value or smaller, an incident photon will not change its direction significantly when approaching the tumor thus it is expected that the trends in Figure.3-8 and 3-9 will not change much. However when the epidermal thickness is much larger than 142 μm , the photon will change the direction significantly before it reaches the tumor. In this case, the trends in Figure.3-8 and 3-9 may change dramatically.

Another parameter that usually affects simulation results is the emission wavelength. But we expect that the trends shown in Figure.3-6 through Figure.3-9 will not change significantly with the emission wavelength. The reason is that the scattering coefficient and the anisotropy value of the tissue models, which are the major factors affecting the amount of photons reaching the tumor change slowly in the visible spectrum in both the SCC tissue model [218] [219] and the BCC tissue model [214].

In the current clinical practice, the size of a skin tumor a clinician can see is usually larger than 1 mm. However, diffuse reflectance spectroscopy as a potential tool for early epithelial cancer diagnosis, could detect skin cancer smaller than 1 mm, which is beyond the capability of the current clinical practice. Moreover, in the epithelial tissues covering many organs such as the cervix or oral cavity, the dysplasia could be smaller than 1 mm at early stages when it is bounded in the epithelium. In these cases, the results from this study will be directly applicable because the S-D values in this study are smaller than 1 mm and the threshold values in Tables 3-3 and 3-4 are comparable to the S-D. When the S-D value is much larger than 1 mm, it will be difficult to tell whether the guideline will work without further investigation because the simple interpretation in terms of geometrical optics as shown in Figure. 3-10 may not work anymore. When the S-D value is very large such as 5 mm, the MC code used in this study may not be the best tool to investigate this problem because very few photons would be detected in MC simulations at such a large S-D. This would result in unacceptable uncertainty in simulated diffuse reflectance. Other methods such as phantom experiments may be preferred.

Based on the results obtained in this study, it is important to know the tumor width and epithelial thickness (or epidermal thickness) to select an appropriate tumor

model. The epithelial thickness and tumor width could be obtained by other imaging modalities, such as optical coherence tomography [220] and magnetic resonance imaging [221], or estimated from clinical examination. Then the following guideline for the validity of the semi-infinite tumor model could be applied in diffuse reflectance spectroscopy, which is based on the threshold values shown in the table 3-3 and table 3-4. For the SCC tissue model with a 300- μm thick epithelium, the semi-infinite SCC model generates statistically equal diffuse reflectance as finite-width SCC models for the probe configuration with a zero-degree tilt angle. This observation implies that this probe configuration is not sensitive to the superficial SCC tumor. For the probe configuration with a 45-degree tilt angle, the minimum threshold values for the semi-infinite tumor model to be valid are 0.75, 1 and 1 time of S-D, respectively. A finite-width tumor model will be more appropriate if the tumor width is smaller than the threshold value. For the BCC tissue model with an 80- μm thick epidermis, both the probe configuration with a zero-degree tilt angle and that with a 45-degree tilt angle are sensitive to the BCC tumor. Table 3-4 provides the minimum threshold values of the tumor width for the semi-infinite BCC tumor model to be valid. It should be aware that varying the epithelial or epidermal thickness might change the threshold value. When the epithelial or epidermal thickness is different from what have been studied here, the discussion earlier about its effect on the threshold values will help estimate the new threshold value.

In this study, all the finite-width tumors were assumed to be infinite in the length dimension, which does not affect the validity of the guideline. This can be explained by the fact that the dimension of the probed volume in the length dimension, i.e. the direction perpendicular to the plane containing source and detector fibers, is typically comparable to the fiber diameter. Hence, an infinite-length tumor is

equivalent to a finite tumor with equal width and thickness and a length comparable to the fiber diameter in terms of light propagation. Since most threshold values are comparable to or larger than the fiber diameter (Tables 3-3 and 3-4), the validity of the guideline is still justified for the infinite-length tumor assumption. Nevertheless, future studies are warranted to validate the guideline in tumors with finite size in all dimensions.

In conclusion, we have investigated the validity of the semi-infinite tumor model in diffuse reflectance spectroscopy for epithelial cancer diagnosis. Two common epithelial tissue models, including a squamous cell carcinoma tissue model and a basal cell carcinoma tissue model, were examined. It was demonstrate that the validity of the semi-infinite tumor model depends on both fiber-optic probe configuration and tumor dimensions. Two look-up tables were derived to guide the selection of appropriate tumor models and fiber-optic probe configurations in the optical diagnosis of early epithelial cancers. It should be aware that the threshold values in the look-up tables could change when any key parameter in the tissue model, such as the organ site and tumor stage, or probe specifications, such as the fiber size and numerical aperture, is varied.

Chapter 4: Acceleration of MC simulation of diffuse reflectance from Finite-size tumor model in diagnosis of early epithelial cancer

[Copyright permission from: C. Zhu and Q. Liu, “Hybrid method for fast MC simulation of diffuse reflectance from a multilayered tissue model with tumor-like heterogeneities,” J Biomed Opt 17, 010501 (2012).]

4.1 Introduction

As we reported in Chapter 3, proper selection of the finite-size tumor model for modeling the early epithelial cancer could provide more accurate results for the diagnosis of early epithelial cancer. For analysis of spectra obtained from finite-size tumor model, the best choice would be MC method due to the fact that no analytical models would be applicable to this finite-size tumor model up to now. However, the main drawback of the MC method is the requirement of intensive computation to achieve results with desirable accuracy which makes it extremely time consuming.

Several methods have been proposed to speed up the MC method for modeling light transport in complex tissue models. Liu et al.[123] presented a scaling method for fast MC simulation of diffuse reflectance spectra from multi-layered turbid media. Hayakawa et al. [126] proposed a perturbation MC (pMC) method to solve inverse photon migration problems in a two-layered tissue model based on spatially resolved diffuse reflectance and validated this method experimentally[127]. Sassaroli et al.[129] proposed a fast pMC method for photon migration in a tissue model with an arbitrary distribution of optical properties. To our best knowledge, there has been no effort in the literature to speed up the MC method in multi-layered tissue model with finite-size tumor-like heterogeneities. Theoretically, the pMC method may be used in this

case, but the applicable range of optical properties in the tissue model and the heterogeneity will be limited. Moreover, the probe configuration is always fixed in the previous pMC methods, which would cause significant inconvenience for a fiber-optics probe involving multiple configurations such as multiple source-detector separations. In this Chapter, we present a hybrid method that combines the multi-layered scaling method [123] and the pMC method [126] mentioned above for fast MC simulation of diffuse reflectance from a multi-layered tissue model with tumor-like heterogeneities.

4.2 Principle of the acceleration method

Our method consists of two steps as shown in Figure. 4-1. The first step applies the multi-layered scaling method on a set of photon trajectory information including the exit weight, the x and y offsets in each random walk step of all survival photons escaping from the top surface of the tissue model, generated from a single baseline simulation to scale the exit weight and exit distance of photons for the multi-layered tissue model without heterogeneities. In the second step, a convolution scheme is used first to determine the probability of a survival photon collected by the fiber-optic probe geometry of interest [51] . Then the second set of photon trajectory information including the locations of all collision events for each collected photon, which is generated from the same baseline MC simulation, will be processed to determine the path length and the number of collisions of photon spent in the tumor. Finally, the scaling result, i.e. the exit weight of collected photons, as well as the path length and the number of collisions spent in the tumor will be utilized by pMC method to compute the diffuse reflectance for the given probe configuration.

Figure 4- 1 Schematic representation of the hybrid method. The single baseline simulation is run on a homogeneous tissue model as shown in the left block.

4.3 Validation and performance of the method

4.3.1 Probe configuration and tissue model

A previous MC code [123] was modified to create a photon trajectory database for scaling and perturbation. A single simulation was run for a homogeneous baseline tissue model, in which $\mu_a = 0 \text{ cm}^{-1}$, $\mu_s = 100 \text{ cm}^{-1}$, and the anisotropy factor $g = 0.8$. The refractive indices of the medium above the tissue model, the tissue model and the medium below the tissue model were set to be 1.47, 1.4 and 1.4, respectively. These two values represent the refractive indices of the fiber material, i.e. glass in this case and the tissue at 500 nm. The thickness of the tissue model was set at 4 cm to mimic a semi-infinite medium. A total of 10^7 photons were launched at the origin of a Cartesian coordinate system to obtain the impulse response of the tissue model in diffuse reflectance. When a photon exits from the top surface of the tissue model, its exit angle relative to the z-axis will be calculated. If the exit angle is smaller than the cut-off angle defined by an NA of 0.22, the relevant trajectory information of this photon will be stored in a numerical array. Approximately 2.4×10^5 photons were detected in this manner and a total memory of 10 gigabytes (GB) was needed for the storage of the trajectory data. Then based on the stored trajectory data, the multi-layered scaling method [123] and the pMC method [126] will be sequentially carried out as described previously to estimate diffuse reflectance from the multi-layered

tissue model with finite-size heterogeneities. Both the scaling and pMC methods were coded and run in Matlab 10 (Mathworks, Natick, Massachusetts, US).

A basal cell carcinoma (BCC) skin tissue model was used to evaluate the effectiveness of the hybrid method. The BCC usually originates from the basal layer of the epidermis and frequently grows downward deeply into the dermis [4, 212], thus it is induced into the dermis in our theoretical tissue model as shown by the cross-sectional view in Figure. 4-2. The thickness of the epidermis was set to be 80 μm and the thickness of the dermis was set to be 4 cm to mimic a thick skin tissue. The epidermal thickness is representative of that on the neck and back [213] . The length, width and thickness of BCC tumor were all set to be 400 μm . The optical properties of the epidermis and dermis were selected from the literature [214] and listed in Table 4-1. A refractive index of 1.4 and an anisotropy factor of 0.8 were used in the entire tissue model including the BCC tumor. The absorption and scattering coefficients of the tumor were varied sequentially to investigate the valid range of the hybrid method. The fiber configuration was shown in Figure.4-2, the source and detector fibers were placed side by side, both of which were perpendicular to the tissue surface. The bisecting line between the two fibers overlaps with the middle line of the tumor in the width dimension, i.e. the x dimension in Figure.4-2. The two fibers both had a core diameter of 200 μm and a NA value of 0.22. The refractive indices of the fibers were set to be 1.47. Totally two sets of tests were performed. In the first set, the absorption coefficient, i.e. μ_a , of the tumor was varied from 1% to 400% of that of the dermis, while the scattering coefficient, i.e. μ_s , of the tumor was kept identical to that of the dermis. In the second set, the scattering coefficient, i.e. μ_s , of the tumor was varied from 25% to 190% of that of the dermis, while the absorption coefficient, i.e. μ_a , of the tumor was kept identical to that of the dermis. Diffuse reflectance values, which

refer to the ratio between detected and incident powers in this paper, calculated by the hybrid method were compared to those from independent MC simulations (no scaling or perturbation methods were used) which were run by using a previously validated MC code[222], to evaluate the effectiveness of the hybrid method. Each independent simulation was run five times and 10 million photons were used.

Figure 4- 2 Cross-sectional view of the theoretical BCC model and fiber configuration

Table 4- 1 Optical properties of BCC tissue model at 500 nm [214]

Tissue	μ_a (cm ⁻¹)	μ_s (cm ⁻¹)	g
Epidermis	7.0	350	0.8
Dermis	3.5	250	0.8

Note: μ_a : absorption coefficient; μ_s : scattering coefficient; g: anisotropy

4.3.2 Performance of the hybrid method

The percent deviation in diffuse reflectance between results calculated by the hybrid method and those simulated independently was calculated by Eq. (4.1) to quantify the accuracy of the calculated results.

$$\text{Percent Deviation} = \frac{\text{Hybrid-Simulated}}{\text{Simulated}} * 100 \quad (4.1)$$

where “Hybrid” refers to the diffuse reflectance value calculated by the hybrid method and “Simulated” refers to the mean of simulated diffuse reflectance values from five runs of the independent simulation on the same tissue model. The percent deviations of five individually simulated diffuse reflectance values relative to their mean were also calculated in the same manner. The 95% confidence interval (CI) of

the percent deviation of simulated diffuse reflectance values relative to their mean was then estimated by Eq. (4.2).

$$95\% \text{ CI} = [\text{mean} - 1.96 \times \frac{\text{std}}{\sqrt{m}}, \text{mean} + 1.96 \times \frac{\text{std}}{\sqrt{m}}] \quad (4.2)$$

where m is the number of runs ($m=5$), and “mean” and “std” refer to the mean and standard deviation of the percent deviations for simulated diffuse reflectance values, respectively. The comparisons between the hybrid method and independent MC method are shown in Figure.4-3 and Figure.4-4.

Figure 4- 3 Comparison in (a) diffuse reflectance and (b) percent deviation, between the hybrid method, i.e. “Hybrid”, and independent MC simulations, i.e. “Simulated”, with varying μ_a in the tumor. “PD” refers to percent deviation. The error bars in (b) indicates the 95% CI of the percent deviations for diffuse reflectance values from independent MC simulations as calculated by Eq. (2)

Figure 4- 4 Comparison in (a) diffuse reflectance and (b) percent deviation, between the hybrid method, i.e. “Hybrid”, and independent MC simulations, i.e. “simulated”, with varying μ_s in the tumor. “PD” refers to percent deviation. The error bars in (b) indicates the 95% CI of the percent deviations for diffuse reflectance values from independent MC simulations as calculated by Eq. (2).

Figure 4-3 (a) shows the comparison in diffuse reflectance values between the hybrid method and independent MC simulations for μ_a varying from 1% to 400% of the dermal value. The two sets of symbols completely overlap at nearly every point, which indicates the high accuracy of the hybrid method. Figure 4-3(b) shows the percent deviations of diffuse reflectance calculated by the hybrid method according to Eq. (1) and the 95% CIs of the percent deviations of simulated diffuse reflectance calculated by Eq. (2) as indicated by the error bars. The percent deviations for the hybrid method are always smaller than 5% when μ_a is varied from 1% to 390% of the dermal value. Moreover, they are all close to or within the 95% CIs of the percent deviations of simulated diffuse reflectance values.

Figure 4-4(a) compares diffuse reflectance values between the hybrid method and independent MC simulations for μ_s varying from 25% to 190% of the dermal value. Figure 4-4(b) compares the percent deviations of diffuse reflectance values obtained by the hybrid method with the 95% CIs of the percent deviations for independently simulated diffuse reflectance values as indicated by the error bars. The percent deviations for the hybrid method are all less than 5% when μ_s is varied from 30% to 180% of the dermal value. Moreover, they all fall within or close to the 95% CIs of the percent deviations of simulated reflectance values. The following interesting trends have been observed in Figs. 3-3 and 3-4. The hybrid method overestimates the reflectance value when the absorption coefficient of the tumor is larger than that of the dermis and underestimates the reflectance value when the absorption coefficient of the tumor is smaller than that of the dermis. The trend for the scattering coefficient is opposite. Similar trends have been observed for the pMC method [125, 126]. Thus the trends are most likely caused by the perturbation part of the hybrid method.

All tests were performed on a laptop computer with an Intel Core i5 CPU and 4GB memory. Ten hours were needed to run the baseline simulation and generate the photon trajectory information. It should be noted that the major portion of the time in the baseline simulation was spent in saving data but not the simulation. It took about 1.5 minutes for the scaling step, 15 seconds for the convolution scheme to calculate the probability of each survival photon being collected and about 6 minutes to determine the path length and the number of collisions spent in the tumor. The pMC step took about 90 milliseconds for each set of optical properties to yield the final diffuse reflectance. In contrast, it took about 30 minutes to run one independent MC simulation to get the same value. The hybrid method will significantly shorten simulation time if a database is needed for such a tumor model in which lots of simulations are needed. For example, if a total of 500 sets of optical properties for the tumor need to be simulated, it will only take around 10 hours (baseline simulation) plus 10 minutes (hybrid calculation) to finish all the simulations by using the hybrid method. In contrast, it will take around 250 hours to finish all the simulation by using the standard MC method. The more set of optical properties one needs, the more time the hybrid method will save.

4.4 Discussions

The hybrid method takes advantage of the proven high accuracy of the multi-layered scaling method in the simulation of a multi-layered tissue model so that the perturbation method is applied to only the heterogeneities instead of the entire tissue model. This feature significantly expands the range of applicable tissue models compared to pMC methods alone. Moreover, the hybrid method only requires a single baseline simulation to generate the photon trajectory information required and applies

to a multi-layered tissue model embedded with tumor like heterogeneities, in which all tissue layers and tumors could have arbitrary absorption and scattering coefficients and dimensions. This advantage will speed up the computation by several orders of magnitude. Therefore the method is suitable for simulating diffuse reflectance spectra or creating a MC database to extract optical properties of a multi-layered tissue model with tumor-like heterogeneities from diffuse reflectance measurement.

Chapter 5: Non-contact depth sensitive diffuse reflectance spectroscopy for early epithelial cancer diagnosis

[Copyright permission from: C. Zhu and Q. Liu, “Numerical investigation of lens based setup for depth sensitive diffuse reflectance measurements in an epithelial cancer model,” Opt Express 20, 29807-29822 (2012).]

5.1 Introduction

As discussed in previous Chapters, diffuse reflectance spectroscopy has been investigated for the early diagnosis of epithelial cancer in the past two decades [15, 16, 223]. Due to its non-invasiveness and capability to provide quantitative information about the physiological and pathological status of tissues in real time, this technique has great potential to be widely used in clinical settings. In a typical diffuse reflectance setup, a fiber-optic probe serves as the conduit for the delivery of illuminating light and collection of emitted light [94]. The fiber-optic probe is a metal cylindrical tube enclosing one or multiple optical fibers [224], in which some fibers are used for delivering light onto a tissue surface and the same fibers or other fibers depending on the probe design are used for collecting light emanating from the tissue surface.

Although fiber-optic probes are widely used in optical spectroscopy due to their flexibility and high efficiency [94], the uncertainty in measurements due to inconsistent probe-sample pressure is difficult to remove. It has been reported that the inconsistent pressure could induce significant distortions in measured spectra, which consequently would cause large errors in diagnosis [225-228]. To reduce such an

artifact, several groups have developed lens based setups for non-contact diffuse reflectance measurements. Andree et al. [229] reported a lens based setup involving a spherical and a flat folding mirror for illumination while two achromatic lenses for detection. An illumination fiber was placed at the focal point of the spherical mirror to deliver the white light to tissue surface. A detection fiber was placed at the focal point of the top achromatic lens to transmit diffusely scattered light to a spectrometer. The distance between the illumination and the detection area can be varied continuously; moreover, both source and detection fibers with different diameters can be used. Therefore this non-contact lens based setup is able to perform spatially resolved diffuse reflectance measurements without physically contacting a tissue sample. Bish et al. [230] recently proposed a different setup for non-contact diffuse reflectance measurements. Two collimating lenses were used to image the illumination and collection fibers onto the tissue surface and serve as a non-contact probe to eliminate the influence of inconsistent probe-tissue pressure that would be present in a contact probe. To overcome the limit of the lens in focal depth, a customized autofocus mechanism was incorporated in the setup. Mazurenka et al. [231] introduced a non-contact lens based setup for time-resolved diffuse reflectance measurements, in which laser scanning was used to achieve imaging. Besides the reports reviewed above, some other proposed techniques such as low coherence enhanced backscattering [232], diffuse backscattering [233], and confocal technique [234] have also been explored for non-contact diffuse reflectance measurements. Turzhitsky et al. [234] examined the dependence of the penetration depth of low coherence enhanced backscattering signals on optical properties using MC modeling, but they did not look into the details on the simulation of lens based illumination and detection.

The literature review above demonstrates that the lens based setup is a promising tool for non-contact diffuse reflectance measurements without distortion due to inconsistent probe-sample contact. However, there have been no reports describing the details of MC modeling of lens based non-contact setup for depth sensitive diffuse reflectance measurements to the best of our knowledge. In this study, we first presented a MC method to model non-contact diffuse reflectance measurements in a lens based setup with a cone or cone shell configuration. Then this method was used to simulate diffuse reflectance measurements from a squamous cell carcinoma (SCC) tissue model in the cone shell, cone and hybrid configurations, in which the cone shell configuration has not been previously proposed in optical spectroscopy. The performance of three configurations in terms of the sensitivity to the tumor and the stroma was compared to each other.

5.2 Material and methods

5.2.1 MC method for modeling lens based non-contact setup

5.2.1.1 Lens based non-contact diffuse reflectance measurement setup

The schematic of the proposed lens based set up is shown in Figure.5-1(a). White light from a point source, i.e. S , was first collimated by Lens 1 into a polarizing cube beam splitter, and then focused into a tissue sample via Lens 2. Note that Lens 2 is also called the imaging lens in this paper according to its role in measurements. Diffusely reflected light from the tissue sample was collected by Lens 2 and collimated into the polarizing cube beam splitter, which was then focused into a detection fiber by Lens 3. The detection fiber would deliver the light into a spectrometer. The polarizing cube beam splitter reflected one component of light with a particular polarization, for example the S component, while allowing the other

component of light, for example the P component, to transmit through. The role of the beam splitter in this setup was to minimize the contribution of specular reflectance. Lenses 2 and 3 were assumed to possess equal focal distances. The shape of the intersection between light and the sample volume in an optically transparent sample is typically a cone, in which case light intersecting with the plane of Lens 2 forms a circle as shown in the top graph of Figure.5-1 (b). In this study, we would like to propose a configuration, in which the shape of intersection between light and sample volume is a cone shell and consequently light intersecting with the plane of Lens 2 forms a ring as shown in the bottom graph of Figure.5-1(b).

In practice, a mask can be placed between the beam splitter and Lens 2 to change the dimensions of the cone or cone shell configuration. For example, a diaphragm serving as the mask can be used to control the radius of the circle in the cone configuration, i.e. r in Figure.5-1(b); while a ring slider with multiple rings each has different dimensions as the mask can be used to control the ring dimensions including the ring radius R and the thickness t in the cone shell configuration. Note that both illumination and detection are affected by the mask in Figure.5-1(a) because it is located right next to the imaging lens, i.e. Lens 2. If a ring slider is placed between Lens 1 and the beam splitter and a diaphragm is placed between Lens 3 and the beam splitter at the same time, this will create a hybrid configuration with the cone shell configuration for illumination and the cone configuration for detection.

The sample stage can be moved up and down to vary the imaging depth. Alternatively, Lens 2 can be adjusted to change the depth of focal point in the tissue sample and achieve the same purpose. In principle, most non-contact setups for diffuse reflectance spectroscopy mentioned earlier are similar to this setup in terms of the illumination and detection configuration. In this sense, the methodology and most

results in this study are generally applicable to many other non-contact lens based systems for diffuse reflectance measurements.

Figure 5- 1 (a) Schematic of the lens based set up for non-contact diffuse reflectance measurements; (b) The circular (top) and ring (bottom) shapes of the mask between Lens 2 and the beam splitter in the cone and cone shell configurations. In (b), light can pass through the white area but is blocked in the gray area. The symbols, “ r ”, “ R ” and “ t ” represent the radius of the circle in the cone configuration, and the ring radius and the ring thickness in the cone shell configuration. The cone configuration can be seen as a special case of cone shell configuration in which the ring radius is zero.

5.2.1.2 Cone shell illumination in MC simulation

MC method for simulating focused light beam, i.e. the cone configuration for illumination, has been described by Wang et al. [164], in which the detection configuration was not considered. In this study, Wang’s method is extended from the cone illumination to the cone shell illumination; then a numerical method is developed to simulate the cone shell detection.

Only the cone shell configuration is described here because the cone configuration can be treated as a special case of the cone shell configuration in which the ring radius is zero. The ring radius, ring thickness, the imaging lens’s focal length and the depth of focal point in the tissue can uniquely define a cone shell configuration. The four parameters were denoted by R , t , f and Z_f respectively. It should be noted that the maximum value of R cannot be larger than the radius of lens. The lens used here was treated ideal thus the lens thickness was not taken into

account. A Cartesian coordinate system was set up in the simulation to facilitate tracking the positions of photons. The origin was the center of the incident light beam on the surface of the tissue model. The Z axis was the normal of the surface pointing toward the inside of the turbid. The x-y plane was located on the surface of the tissue model. The focused light beam formed a ring with a radius of ρ_{ring} and a thickness of t_{new} on the tissue surface as shown in Figure.5.2. The radius and thickness of the illumination ring on the tissue surface can be calculated from R and t as follows.

$$\frac{\rho_{ring}}{R} = \frac{Z_f}{f} \quad (5.1)$$

$$\frac{t_{new}}{t} = \frac{Z_f}{f} \quad (5.2)$$

The irradiance was assumed to be uniform on the surface of the turbid medium and the radial position of a photon packet was sampled by

$$\rho = \sqrt{[(\rho_{ring} + t_{new})^2 - \rho_{ring}^2] \epsilon_\rho + \rho_{ring}^2} \quad (5.3)$$

where ϵ_ρ was a random number uniformly distributed between 0 and 1. The azimuthal angle of the photon packet was sampled by

$$\theta = 2\pi\epsilon_\theta \quad (5.4)$$

where ϵ_θ was a random number uniformly distributed between 0 and 1. The Cartesian coordinates of the incident point were then

$$x = \rho \cos(\theta) \quad (5.5)$$

$$y = \rho \sin(\theta) \quad (5.6)$$

The directional cosines were set to

$$u_x = -x / \sqrt{\rho^2 + Z_f^2} \quad (5.7)$$

$$u_y = -y / \sqrt{\rho^2 + Z_f^2} \quad (5.8)$$

$$u_z = Z_f / \sqrt{\rho^2 + Z_f^2} \quad (5.9)$$

If the ambient medium and the tissue had the same refractive index, the directional cosines did not need to change when the photon entered the tissue. Otherwise, the directional cosines were changed based on the Snell's law and the specular reflection was taken into account based on the Fresnel law. Once the photon was launched into the tissue model, the treatment of photon tracing was exactly same as that done by Wang et al. [50] thus was not repeated here.

Figure 5- 2 Cone shell illumination schematic

5.2.1.3 Cone shell detection in MC simulation

Besides the parameters associated with the lens mentioned above, i.e. R , t , f and Z_f (Figure.5-2), two additional parameters associated with the detection fiber (Figure.5-1), including the fiber diameter and the numerical aperture (NA), were also used to define the cone shell detection. The detection part of the setup can be simplified as shown in Figure.5-3(a).

Figure 5- 3 (a) Schematic of the cone shell detection; (b) Detailed view of the cone shell detection at the interface of Lens 2 and the tissue model

To simplify the problem, the thickness of the mask was assumed to be zero. Since both the imaging lens and the mask were treated as infinitely thin, the two components can be merged into Lens 2 as shown in Figure.5-3(a). The detection fiber D is placed at the focal plane of Lens 3. An image of the fiber D can be formed in the tissue model on the focal plane of Lens 2, which is denoted as D' in Figure.5-3(a). Because Lenses 2 and 3 possess equal focal distances, D and D' have equal size. Due to the reciprocity of ray tracing, finding whether a photon is detected by fiber D is equivalent to identifying whether the photon can be traced back to its image D' and the latter problem is easier to solve. Figure.5-3(b) illustrates the detail about how an exiting photon can be traced back to D'. Once a photon exits the tissue surface in a direction of $\vec{r}$ from position P, two steps will be performed to determine whether this photon could be detected by the detector. The first step is to determine whether this photon can pass through the ring. This can be done by moving the photon from P to the plane of Lens 2 along $\vec{r}$ and find the intersection with the plane of Lens 2, which is denoted by P₁. If P₁ is located within the ring, it suggests that the photon can pass through the ring then the photon will continue to go through the second step, otherwise the detection procedure for this photon will be terminated. The second step is to perform ray retracing to determine whether this photon could be detected by the detection fiber. This can be done by moving the photon from P along vector $-\vec{r}$ towards the plane of $Z=Z_f$ and checking whether the intersection with the plane, which is denoted by P₂, is located within the fiber tip area. If it is, the exiting angle of this photon will be compared to the acceptance angle of the fiber, which is calculated from the NA value. If the exiting angle is also smaller than the fiber acceptance angle,

the photon will be counted as being detected by the fiber; otherwise the photon will be rejected. If a photon is detected by the fiber, all related trajectory information will be recorded. It should be noted that the mismatch of refractive indices of the tissue model and the ambient medium above the tissue does not affect the ray retracing step proposed above. This is due to the fact that D' was an image of D . In the retracing procedure, the virtual medium around D' , which is the “image” of the medium around D , should have the same refractive index as that of the medium around D , which happens to be equal to the refractive index of the ambient medium above the tissue.

5.2.1.4 Validation of the MC method

To validate the MC method introduced above, several simulations were performed. The illumination scheme described above was validated against results published by Wang et al. [164], in which the agreement between our results and published ones was excellent (results not shown to save space).

To validate the detection scheme, we performed another set of simulations in a semi-infinite homogeneous tissue model. The optical properties of the tissue model were $n_t=1.4$, $\mu_a=1.0 \text{ cm}^{-1}$, $\mu_s=100 \text{ cm}^{-1}$, and $g=0.9$. The refractive index of the ambient medium was also set to 1.4 such that there was no refractive index mismatch on the top surface of the tissue model. The diameter of the detection fiber was 2 mm and the NA was set to 0.6. The radii of lenses 2 and 3 were 10 mm while the focal lengths were set to 20 mm. Both the ring radius and the ring thickness were set to 5 mm. The depth of focal point of the imaging lens, i.e. Lens 2, in the tissue model was varied from 0.5 mm to 1.0 mm at an increment of 0.5 mm. In each simulation, 200 million photons were launched and the grid sizes were 0.04 mm in x, y, and z dimensions. The absorption distribution contributed only by detected photons was shown in Figure.5-4. Figure.5-4(a) shows the result for the first simulation in which the depth

of focal point in tissue was 0.5 mm. Based on the setting described above, the light beam focused onto the turbid medium surface should form a ring with a radius of 0.125 mm and a ring thickness of 0.125 mm. Figure.5-4(a) shows that, the shape and size of light beam focused onto the medium surface approximately agree with our predictions, in which the blurred boundary of the cone shell was due to the turbidity of the tissue model. It is also clear that the brightest spot in the tissue model is located at a depth of around 0.5 mm, which agrees with the depth of the focal point in the tissue model set in the simulation parameters. The result for the other situation, i.e. when the depth of the focal point in the tissue model was 1.0 mm, was shown in Figure.5-4 (b). Based on the setting for this situation, the light beam focused onto the turbid medium surface should form a ring with a radius of 0.25 mm and a ring thickness of 0.25 mm. Figure.5-4(b) shows that, the shape and size of light beam focused onto the medium surface approximately agree with our predictions. It can be observed that there was no single obvious focus in the absorption distribution and the distribution shifted towards the superficial area. Moreover the bottom edge of the absorption distribution was located around 0.8 mm, which was likely due to the joint effects of tissue turbidity and the cone shell detection scheme. Based on the results shown in Figure.5-4, it can be seen that the MC method for the cone shell configuration of illumination and detection has been validated.

Figure 5- 4 Absorption distribution of detected photons for (a) the depth of focal point in the tissue model was 0.5 mm; and (b) the depth of focal point in the tissue model was 1.0 mm

5.2.2 Tissue model and simulations

5.2.2.1 Early Squamous Cell Carcinoma model

An epithelial tissue typically consists of two layers, the top epithelium and the bottom stroma. The basement membrane separates the two layers. The squamous cell carcinoma (SCC) usually originates from the basement membrane of the epithelium [3]. Previously our group has utilized a simplified early squamous cell carcinoma model in which the SCC tumor is assumed to be a cuboid target whose dimensions and position can be specified [82]. The actual dimensions and position of an SCC tumor in an epithelial tissue varied considerably among stages during tumor development. The SCC model used in this study is shown in Figure.5-5. The epithelial thickness was set to 0.5 mm [217]. The stromal thickness was set to 2.5 mm to represent a semi-infinite layer. The tumor width and length were both set to 0.5 mm and the tumor thickness was set to 0.3 mm. This SCC model represents a tumor model in an early stage. The optical properties of each region as listed in table 5-1 were obtained from the literature [210] at a wavelength of 420 nm. This wavelength was chosen because it is close to the absorption peak of hemoglobin, which is the major absorber in human tissues. A refractive index of 1.4 was used in all tissue regions [211]. The anisotropy factors of the epithelium and the tumor were set to 0.97 and an anisotropy factor of 0.8 was used for the stroma [211].

Figure 5- 5 Cross section view of the squamous cell carcinoma (SCC) tissue model. The tumor width and length were set to 0.5 mm while the tumor thickness was set to 0.3 mm. This model represents a tumor in an early stage.

Table 5- 1 Optical properties of the SCC tissue model at 420nm [210]

Tissue	Optical properties		
	$\mu_a(cm^{-1})$	$\mu_s(cm^{-1})$	g
Epithelium	3.0	42.4	0.97
Tumor	3.0	127.2	0.97
Stroma	9.09	266.3	0.8

Note: μ_a : absorption coefficient; μ_s : scattering coefficient; g : anisotropy

5.2.2.2 Criteria for the evaluation of depth sensitivity in a non-contact setup

To characterize the depth sensitivity of the non-contact set up, two criteria that have been reported previously [167] were used. The first criterion was the weighted fraction of photon-scatterer collisions for detected photons, which is mainly determined by the scattering properties of the tissue model, spent in each region. The numbers of photon-scatterer collisions in the epithelium, the SCC and the stroma were recorded separately for each detected photon, and then the weighted average number of collisions in the each region can be calculated as follows:

$$\overline{NC} = \frac{\sum_{i=1}^N W_i * NC_i}{\sum_{i=1}^N W_i} \quad (5.10)$$

where W_i is the exit weight of each detected photon, NC_i is the number of collision spent in the region and N is the total number of detected photons. The fraction of collisions spent in the tumor (FCT) was obtained according to the following equation:

$$FCT = \frac{\overline{NC}_{tumor}}{\overline{NC}_{epithelium} + \overline{NC}_{tumor} + \overline{NC}_{Stroma}} \quad (5.11)$$

The fraction of collisions spent in the epithelium (FCE) and the stroma (FCS) can be obtained similarly. The second criterion was the weighted fraction of path length spent in each region for detected photons. The path length of each detected photon

spent in the epithelium, the SCC and the stroma were recorded, and then the weighted average photon path length in each region for all detected photons can be calculated as follows:

$$\overline{PL} = \frac{\sum_{i=1}^N W_i * PL_i}{\sum_{i=1}^N W_i} \quad (5.12)$$

where W_i is the exit weight of each detected photon, PL_i is the path length spent in the region and N is the total number of detected photons. The fraction of path length spent in the tumor (FPLT) was obtained by:

$$FPLT = \frac{\overline{PL}_{tumor}}{\overline{PL}_{epithelium} + \overline{PL}_{tumor} + \overline{PL}_{stroma}} \quad (5.13)$$

The fraction of path length spent in epithelial (FPLE) and in the stroma (FPLS) can be obtained similarly.

5.2.2.3 Simulation parameters for the cone shell, the cone, and the hybrid configurations

To investigate the depth sensitivity of each configuration in diffuse reflectance measurements, a series of simulations were performed on the SCC model. In all simulations, the refractive index of the ambient medium was set to 1.0 that represented the refractive index of air, both the radius and the focal length of the imaging lens were set to 10 mm thus the half angle of light cone formed by the lens was 45 degrees for incident light, the NA value of the detection fiber was set to 1.0 to increase the efficiency of photon detection without losing generality. The central line of the Lens 2 and Lens 3 (Figure.5-1) always overlap with the middle line of the tumor (dashed line shown in Figure.5-5). The parameters investigated in the simulations for the cone shell configuration, the cone configuration and the hybrid

configuration involving the cone shell illumination and cone detection, were listed in Tables 5-2, 5-3 and 5-4 respectively. It should be noted that the cone radius for detection in the hybrid configuration was always equal to the radius of the lens, i.e. 10 mm. In each independent simulation, 10 million photons were used, which was repeated five times to estimate the means and standard deviations for the construction of error bars shown in the results section.

Table 5- 2 Simulations for the cone shell configuration

Parameters	Values under investigation
Ring radius (mm)	0, 2, 4, 6
Ring thickness (mm)	1, 2, 3
Depth of focal point in tissue (mm)	0.1, 0.3, 0.5, 1.0
Detection fiber diameter (mm)	0.1, 0.2, 0.4

Table 5- 3 Simulations for the cone configuration

Parameters	Values under investigation
Cone radius (mm)	2, 4, 6, 8
Depth of focal point in tissue (mm)	0.1, 0.3, 0.5, 1.0
Detection fiber diameter (mm)	0.1, 0.2, 0.4

Table 5- 4 Simulations for the hybrid configuration*

Parameters	Values under investigation
Ring radius (mm)	0, 2, 4, 6
Ring thickness (mm)	1, 2, 3
Depth of focal point in tissue (mm)	0.1, 0.3, 0.5, 1.0
Detection fiber diameter (mm)	0.1, 0.2, 0.4

*Note: *The cone radius for detection in the hybrid configuration was always equal to the radius of the lens, i.e.10 mm*

5.3 Results

5.3.1 Diffuse reflectance intensity and depth sensitivity in the cone shell configuration

Simulated diffuse reflectance intensity from the SCC tissue model as a function of the ring radius for the cone shell configuration is shown in Figure.5-6. The error bars associated with most data points in Figure.5-6 are too small to see. An overall decreasing trend in diffuse reflectance with increasing ring radii can be observed. This is expected because an increasing ring radius would result in a longer photon path thus detected photons would experience larger attenuation. A depth of 0.3 mm for the focal point in the tissue model produces the highest diffuse reflectance intensity in all cases. In contrast, a depth of 1 mm produces the lowest diffuse reflectance intensity.

Figure 5- 6 Diffuse reflectance as a function of the ring radius for a range of depths of the focal point in the tissue model in a cone shell configuration. Each line represents the results for a different depth value as indicated in the legends. The ring thickness and the diameter of detection fiber were fixed at 2 mm and 0.2 mm, respectively.

Figure.5-7 shows the fraction of collisions spent in the tumor, the epithelium and the stroma for the cone shell configuration. Figure.5-7(a) suggests that the fraction of collisions in the tumor does not change considerably when the depth of focal point in the tissue model is varied from 0.1 mm to 0.5 mm; however it drops significantly when the depth value is increased to 1.0 mm. A depth value of 0.3 mm with a large

ring radius, i.e. 6 mm in Figure.5-7(a), yields the most significant contribution from the tumor. Figure.5-7(c) shows that the fraction of collisions in the stroma increases significantly when the ring radius is increased for the depth of 1.0 mm. The largest ring radius at 6 mm produces the highest collision fraction, i.e. around 73%, from the stroma. The fraction of collisions in the epithelium is significantly affected by the finite width of the tumor. When a small ring radius close to zero is used, the SCC model can be treated as three layers including the thin epithelium, for which a smaller focus depth produce higher collision fraction in the epithelium. When a larger ring radius is used, the trajectory of most detected photons may get around the tumor. In this case, the SCC model could be treated as two layers including the thick epithelium and the stroma only thus the trend becomes quite different.

Figure 5- 7 Fraction of collisions in (a) the tumor, (b) the epithelium and (c) the stroma layer in the cone shell configuration. Each line represents the results for a different depth value as indicated in the legends. The ring thickness and the diameter of detection fiber were fixed at 2 mm and 0.2 mm, respectively.

Figure.5-8 shows the fractions of path length spent in the tumor, epithelium and stroma in the cone shell configuration. It is easy to see that the trends in Figure.5-8 agree with those shown in Figure.5-7. Therefore only the fraction of collisions spent in each region will be shown in the subsequent sections to represent the depth sensitivity of the non-contact setup.

Figure 5- 8 Fraction of path length in (a) the tumor, (b) the epithelium and (c) the stroma layer in the cone shell configuration. Each line represents the results for a different depth value as indicated in the legends. The ring thickness and the diameter of detection fiber were fixed at 2 mm, and 0.2 mm respectively.

5.3.1.1 Effect of the ring thickness on the depth sensitivity of the cone shell configuration

Because it has been found that the depths of focal point of 0.3 mm and 1.0 mm yield the best sensitivity for the tumor and stroma respectively, only the FCT for a depth of 0.3 mm and the FCS for a depth of 1.0 mm are shown below. The effects of ring thickness on the depth sensitivity for cone shell configuration were shown in Figure.5-9. It can be found that the FCT and FCS did not change too much when the ring thickness varied.

Figure 5- 9 Effect of the ring thickness on the fraction of collisions in (a) the tumor and (b) the Stroma for the cone shell configurations. In (a), the depth of focal point in the tissue model, i.e. Z_f , is 0.3 mm; while in (b), the depth of focal point in the tissue model, i.e. Z_f , is 1.0 mm. Each line represents the results for a different ring thickness as indicated in the legends. The diameter of detection fiber was fixed at 0.2 mm.

5.3.1.2 Effect of the detection fiber size on the depth sensitivity of the cone shell configuration

The effect of the detection fiber size on the depth sensitivity of the cone shell configuration was shown in Figure.5-10. It can be seen from Figure.5-10 (a) that, a smaller detection fiber size always provides a higher fraction of collision in the tumor. In Figure.5-10(b), an opposite trend is observed but the changes in the fractions in the stroma are insignificant.

Figure 5- 10 Effect of the detection fiber size on the fraction of collisions in (a) the tumor and (b) the Stroma for the cone shell configurations. In (a), the depth of focal point in the tissue model, i.e. Z_f , is 0.3 mm; while in (b), the depth of focal point in the tissue model, i.e. Z_f , is 1.0 mm. Each line represents the results for a different detection fiber size as indicated in the legends. The ring thickness was fixed at 2 mm.

5.3.2 Depth sensitivity in the cone configuration

The depth sensitivity for the cone configuration in both illumination and detection is shown in Figure.5-11. Figure.5-11(a) shows that a smaller detection fiber size always provides a higher fraction of collisions in the tumor region. Figure.5-11(b) shows that the changes in the fraction of collisions in the stroma are insignificant.

Figure 5- 11 Effect of the detection fiber size on the fraction of collisions in (a) the tumor and (b) the Stroma for the cone configurations. In (a), the depth of focal point in the tissue model, i.e. Z_f , is 0.3 mm; while in (b), the depth of focal point in the tissue model, i.e. Z_f , is 1.0 mm. Each line represents the results for a different detection fiber size as indicated in the legends.

5.3.3 Depth sensitivity in a hybrid configuration with the cone shell illumination and cone detection

Figure.5-12 shows the effect of the ring thickness on the depth sensitivity of the hybrid configuration. Figure.5-12 shows that a larger ring thickness provides a slightly higher fraction of collisions in the tumor region and the stroma, but the changes in the fraction of collisions are not significant.

Figure 5- 12 Effect of the ring thickness on the fraction of collisions in (a) the tumor and (b) the stroma for the hybrid configuration. In (a), the depth of the focal point in the tissue model, i.e. Z_f , is 0.3 mm; while in (b), the depth of the focal point is 1.0 mm. Each line represents the results for a different ring thickness as indicated by the legends. The diameter of the detection fiber was fixed at 0.2 mm. The radius of the cone for detection is equal to the radius of the imaging lens, i.e. 10 mm.

Figure.5-13 demonstrates the effect of the size of the detection fiber on the depth sensitivity of the hybrid configuration. Figure.5-13(a) shows that a smaller detection fiber size always provides a higher collision fraction in the tumor region. Figure.5-13(b) illustrates a similar trend in the fraction of collisions in the stroma but the changes are insignificant.

Figure 5- 13 Effect of the detection fiber size on the fraction of collisions in (a) the tumor and (b) the stroma for the hybrid configuration. In (a), the depth of the focal point in the tissue model, i.e. Z_f , is 0.3 mm; while in (b), the depth of the focal point is 1.0 mm. Each line represents a different size of the detection fiber as indicated by the legends. The ring thickness was fixed at 3 mm. The radius of the cone for detection is equal to the radius of the imaging lens, i.e. 10 mm.

5.3.4 Comparison of depth sensitivity between the cone shell, cone and hybrid configuration

Tables.5-5 and 5-6 show the comparison of the best sensitivity between all above configurations for the tumor and the stroma, respectively. The data in the table are extracted from relevant figures shown earlier. Besides FCT and FCS, a new criterion, i.e. tumor contrast (TC), is included to evaluate the sensitivity to tumor because of the general interest in tumor detection. The tumor contrast (TC) is defined as the percent deviation for diffuse reflectance which was calculated based on equation (5.14),

$$TC = \frac{|R_{tumor} - R_{control}|}{R_{control}} \times 100\% \quad (5.14)$$

where R_{tumor} is the diffuse reflectance simulated from the tumor model and $R_{control}$ is the diffuse reflectance simulated from the control tissue model. The two models are exactly identical except that there is no tumor buried in the epithelial layer in the control tissue model. Table.5-5 shows that there was no significant difference between any two of the three configurations in terms of FCT. However, the hybrid configuration and cone shell configuration performed noticeably better than the commonly used cone configuration in terms of TC. Table.5-6 shows that the hybrid configuration and cone shell configuration yielded similar FCS for the stroma, and they both performed better than the commonly used cone configuration

Table 5- 5 The best FCT between the cone, the hybrid and the cone shell set up for a depth of focus of 0.3 mm

Configuration	Cone	Hybrid	Cone shell
Specifications			
Radius	8 mm	6 mm	6 mm
Detector size	0.1 mm	0.1 mm	0.1 mm
Ring thickness	NA	3 mm	2 mm
FCT	49% $\pm$ 0.1%	49% $\pm$ 0.1%	51% $\pm$ 0.1%
TC	24.9% $\pm$ 0.4%	29.9% $\pm$ 0.4%	31.0% $\pm$ 0.6%

Note: FCT: The fraction of collisions in the tumor; NA: Not applicable; TC: tumor contrast. The row header "radius" refers to the cone radius in the cone configuration and the ring radius in both the cone shell configuration and hybrid configuration. In the rows of "FCT" and "TC", the first percentage is the mean while the second percentage is the standard deviation of the corresponding quantity.

Table 5- 6 The best FCS between the cone, the hybrid and the cone shell set up for a depth of focus of 1.0 mm

Configuration	Cone	Hybrid	Cone shell
Specifications			
Radius	8 mm	6 mm	6 mm
Detector size	0.1 mm	0.4 mm	0.4 mm
Ring thickness	NA	3 mm	3 mm
FCS	68% $\pm$ 0.2%	76% $\pm$ 0.2%	74% $\pm$ 0.1%

Note: FCS: The fraction of collisions in the stroma; NA: Not applicable. The row header "radius" refers to the cone radius in the cone configuration and the ring radius in both the cone shell

configuration and hybrid configuration. In the rows of “FCS”, the first percentage is the mean while the second percentage is the standard deviation of the corresponding quantity.

5.4 Discussions

The findings shown in the results section can be explained by considering the light transport in the tissue model as follows.

Figure.5-6 shows simulated diffuse reflectance as a function of the ring radius for a range of depths of focal point in the tissue model for the cone shell configuration. A depth of 0.3 mm produces the highest diffuse reflectance intensity in all cases, while a depth of 1 mm yields the lowest diffuse reflectance due to the larger attenuation associated with the longer photon path length in this case. Because a depth of 1 mm happens to yield a high sensitivity to the stroma according to Figure.5-7 and 5-8, a tradeoff will have to be made between the diffuse reflectance intensity and the sensitivity to the stroma for the choice of the depth value. A similar tradeoff needs to be made between the diffuse reflectance intensity and the sensitivity to the tumor for the choice of the ring radius in the cone shell configuration. In contrast, no tradeoff needs to be made for the choice of the depth value for the tumor because a depth of 0.3 mm yields both high sensitivity to the tumor and high diffuse reflectance intensity.

Figure.5-7 shows that depth sensitive diffuse reflectance measurements could be achieved by adjusting: (1) the depth of the focal point in the tissue model; and (2) the cone radius in the cone configuration or ring radius in the cone shell configuration. When the focal point of the imaging lens is located inside the tumor, e.g. $Z_f = 0.3$ mm, most launched photons can reach and travel in the tumor region and the fraction of collisions spent in tumor is large. When the focal point is located in the stroma, e.g. $Z_f = 1.0$ mm, most launched photons travel around the tumor and reach the stroma due to the high anisotropy factor of the tumor and the epithelium, i.e. 0.97. Consequently the

fraction of collisions spent in the tumor is very small and the fraction of collisions spent in the stroma is large. It is observed in Figure.5-7(a) that a larger ring radius yields a higher tumor collision fraction for a depth of the focal point of 0.3 mm. The opposite trend is observed for a depth of 1.0 mm. This phenomenon can be explained by Figure.5-14, in which the intersection between the cone shell region and tumor drawn in black determines the contribution of the tumor to measured diffuse reflectance. As shown in Figure.5-14(a), a focal point with a depth of 0.3 mm is located inside the tumor region. When the ring radius is increased, the intersection of the cone shell region and the tumor, i.e. the black area in Figure.5-14(a), increases thus the fraction of collisions of detected photons spent in the tumor would increase accordingly. For a focal point with a depth of 1.0 mm, the beam can get around the central portion of the tumor and reach the tumor as shown in Figure.5-14(b). The intersection between the cone shell region and the tumor is small thus few detected photons travel through the tumor. That explains the small fraction of collisions in the tumor when the depth of the focal point is 1.0 mm. The results for the fraction of collisions in the stroma as in Figure.5-7(c) can be explained in a similar way.

Figure 5- 14 Intersection of the cone shell region and the tumor when the depth of the focal point in the tumor model is (a) 0.3 mm and (b) 1.0 mm.

Figure.5-9 and 5-12 both show that a cone shell configuration or a hybrid configuration with a thicker ring performs slightly better in depth sensitivity regardless of the depth of the focal point. This may be explained as follows. For a small depth such as 0.3 mm, the angle between the light beam and the normal axis would be larger when the ring is thicker. The intersection between the cone shell region and the tumor is thus larger as shown in Figure.5-15, so the depth sensitivity for tumor measurement will be better. Similarly, for a large depth of the focus such as 1.0 mm, the intersection between the cone shell region and the tissue model is mainly located within the stroma. A thicker ring would mean more contribution from the stroma. It should be pointed out that the interpretation by Figure.5-14 and 5-15 relies on the assumption that photons roughly maintain the original direction before reaching the target region. This is true if the desired depth is comparable to or smaller than the reciprocal of the reduced transport coefficient.

Figure 5- 15 Intersection of the cone shell region and the tumor when the ring thickness is different. The symbols “ α ” and “ β ” refer to the angles between the light beam and the normal axis for a thin and a thick ring respectively. The two shaded areas refer to the light beam formed by two sets of cone-shell illumination models with different ring thickness.

Figure.5-10(a), Figure.5-11(a) and Figure.5-13(a) show that a smaller detection fiber performed better for a depth of the focal point at 0.3 mm, which could be explained as follows. The detection fiber can be imaged into the tumor region

according to the ray retracing method as shown in Figure.5-3. The image of a smaller fiber in the tumor region is more likely to yield photons traveling in the tumor region thus a higher fraction of collisions in the tumor. In contrast, the image of a larger detection fiber is more likely to yield photons traveling in other regions thus a smaller fraction of collisions in the tumor.

Tables.5-5 and 5-6 show that the cone shell and hybrid configurations perform better than the cone configuration in terms of sensitivity. For sensitive measurements from tumor, a cone shell configuration and hybrid configuration with a large ring radius yields much better performance than the cone configuration. This could be attributed to the fact that the cone shell configuration and hybrid configuration provides a relatively larger intersection between the cone shell region and the tumor compared to the cone configuration. It should be pointed out that the comparison between the cone shell configuration and the cone configuration is analogous to that between a fiber-optic probe configuration with two obliquely placed fibers and that with two straight fibers [139, 166, 215] to certain extent. For sensitive measurements from the stroma, a cone shell configuration or hybrid configuration with a large ring radius yields much better performance than the cone configuration. This is due to the fact that the light beam in the cone shell configuration or hybrid configuration is able to propagate around the finite-width tumor and reach the stroma thus maximizing the contribution of the stroma to measured diffuse reflectance.

Another parameter that may affect simulation results is the emission wavelength. The simulations presented in this report were carried out for a wavelength of 420 nm which is close to the absorption peak of hemoglobin. The scattering coefficient of the tumor and the absorption coefficient of the stroma at 420 nm are particularly high [218, 219], thus an enhanced sensitivity to the embedded

tumor and the stroma can be achieved if the optimal configuration was used. At longer wavelengths, the scattering coefficient of the tumor and the absorption coefficient of the stroma would decrease [218, 219]. It is quite likely that it will be more challenging to achieve the enhanced sensitivity to the tumor or the stroma. In that case, a new series of simulations may be needed to find the exact sensitivities.

Although the tumor size was fixed at a small value to represent an early tumor in this study, the rules of thumb for achieving enhanced sensitivity to different regions (but not necessarily every figure) should remain unchanged even if the tumor size changes because they agree with the analysis of illumination and detection geometry based on light propagation as shown in Figure.5-14 and 5-15. They are supported by the previous publications [97, 139, 215, 235] in which fiber-optic probes with related geometries were simulated. The exact sensitivity to each region may change with the tumor size, which can only be obtained by a new batch of simulations.

In this study, the central line of the Lens 2 and Lens 3 (Figure.5-1) always overlap with the middle line of the tumor. It can be predicted that the sensitivity to the lesion would decrease when the focal point is located on the margin of a lesion. In a real application, the focal point can be scanned across a tumor in the lateral dimension with a small step size. During scanning, some measurements will be taken from the middle of the lesion and others will be taken from the margin of the lesion or outside the lesion. The difference between these measurements as quantified by “Tumor contrast” defined in Eq. 5-14 could be used to find the tumor margin. The optimal configuration identified in this study will maximize such a difference to increase the contrast of the tumor region relative to the normal region.

The method for simulating lens based illumination and detection is generally applicable to any similar lens based setups for optical measurements. In addition, the

following rules of thumb can be of general interest to any application in which the high sensitivity to a target tissue region is desirable. Based on the results shown in this report, it can be seen that the two parameters, i.e. the depth of focal point in the tissue model and the cone (in the cone configuration) or ring (in the cone shell configuration) radius, are important to the achievement of sensitive measurements from a given region. To achieve enhanced sensitivity to a target region, the focal point needs to be located in the region. Moreover, a large cone or ring radius, which corresponds to a small f-number for a fixed focal length, would help achieve high sensitivity. These rules of thumb are applicable to a layer tissue model with different optical properties if the desired depth is comparable to or smaller than the reciprocal of the reduced transport coefficient. This condition will ensure that photons roughly maintain the original direction before reaching the target region.

In summary, we have developed a MC based method to investigate depth sensitive diffuse reflectance measurements using a non-contact lens based system in an SCC model. Three lens based configurations, i.e. the traditional cone configuration, a novel cone shell configuration and a hybrid configuration involving the cone shell illumination and the cone detection, were studied using the method. It is shown that depth sensitive measurements were achieved by adjusting the following two parameters: (1) the depth of focal point of the imaging lens in the SCC model; and (2) the cone radius in the cone configuration or the ring radius in the cone shell configuration. It was demonstrated that the cone shell and the hybrid configurations in general have better depth sensitivity than the more commonly used cone configuration for diffuse reflectance measurements in the SCC model. The MC method and the findings for different configurations can be useful in guiding the development of a non-contact lens based system for the optical diagnosis of early epithelial cancer.

Chapter 6: Non-contact depth sensitive fluorescence spectroscopy for early epithelial cancer diagnosis

6.1 Introduction

In Chapter 5, we have demonstrated that lens based non-contact setup was able to perform depth sensitive diffuse reflectance measurement for early epithelial cancer diagnosis. In this Chapter, firstly we extended our MC method to simulate depth sensitive fluorescence measurements from an early epithelial cancer model and then we performed phantom study by a customer designed lens based non-contact setup to validate the findings obtained in MC simulations.

Fluorescence spectroscopy has been widely explored for the detection of precancers and cancers in human epithelial tissues. Being an optical spectroscopic technique, fluorescence spectroscopy is capable of rapidly and non-invasively quantifying biochemical and morphological changes in human tissues due to the rich endogenous fluorophore contents in epithelial tissues that contain vital diagnostic information for the identification of tissue malignancy. Generally, an epithelial tissue consists of an outer epithelial layer that rests on the basement membrane and an underlying stromal layer. The distribution of these fluorophores in an epithelial tissue is depth dependent and can be affected by several factors, such as the age, menopausal status [236, 237] and disease progression [12, 238]. The optical scattering property of epithelial tissues further complicates the *in vivo* localization of these fluorophores. Thus, an optical setup of illumination and detection with excellent depth sensitivity is

highly preferable to improve the diagnostic accuracy of this technique in epithelial precancer and cancer.

Depth sensitive fluorescence measurements have previously been demonstrated using contact fiber-optic based probe geometries by varying the source-detector separation [239], aperture diameter [165], and tilt angle of illumination and collection fibers [215]. However, a fiber-optic based probe requires contact with the sample surface and a past study has shown that the inconsistent pressure exerted on biological tissues can cause spectral distortion that severely affects the diagnostic accuracy of this technique [240]. Besides that, the contact between the probe and the patient's skin especially on infectious sites may cause probe contamination and elevate the risk of disease transmission across patients. To overcome these complications, alternative non-contact measurement methods have been investigated by using lens-based setups. Bish et al. [230] performed non-contact diffuse reflectance measurements on tissue phantoms and human skin by using a lens based probe in an effort to eliminate diagnostic inconsistency due to the exertion of probe pressure. Mazurenka et al. [231] demonstrated non-contact time-resolved diffuse reflectance measurements with a lens based setup, and laser scanning was used to achieve imaging. Generally, these lens-based setups use the combination of lenses to achieve the excitation and collection volumes that would form cones in an optically transparent medium.

To effectively reproduce the experimental configuration of these optical measurements without incurring significant cost, the MC method has been developed and employed in the past few decades, as a versatile computational tool to model light propagation in turbid tissue-like media. Optical configurations and light-tissue interactions (scattering, absorption and fluorescence) can be modeled to simulate light distribution in the numerical model of human tissues, which has been extremely

useful in helping the design and optimization of various experiments and optical setups. A comprehensive review of MC modeling has been given in Chapter 2. In achieving depth sensitive fluorescence measurement, Zhu et al. [98] used MC simulations to model different contact fiber-optic based probe geometries in fluorescence measurements from different depths in epithelial tissues. Recently, Zhu et al. [241] has simulated the cone and cone shell configuration implemented by a convex lens in a non-contact imaging geometry using the MC method and the results showed that the cone shell configuration yields a larger sensitivity to diffuse reflectance from deep layers. Although the MC method has been widely employed to study light propagation in turbid media, limited works on the experimental validation of MC modeling have been reported. Liu et al. [215] used MC modeling to simulate fluorescence measured from turbid tissue phantoms by an angled fiber-optic probe and demonstrated that depth selectivity can be achieved by varying the illumination angle. The simulation results have been verified with experiments by using two-layered epithelial tissue phantoms. Liu et al. [63] have also used the MC method to simulate fluorescence and diffuse reflectance values measured by several different fiber-optic probe geometries that were designed to sample small tissue volumes. They quantitatively compared the numerically simulated and experimentally measured results to validate the MC model.

Even though there has been an increasing interest in using the MC method to simulate fluorescence light transport in turbid media, most previous reports focused on using fiber-optic based probe geometries for illumination and detection. Investigation on non-contact lens-based probe geometries using the MC method has been so far very limited. The goal of this study is to experimentally verify MC modeling of fluorescence measurements involving non-contact lens-based probe

geometries from turbid media with a layered structure. We have developed a MC model to simulate different combinations of illumination and detection configurations, involving both the cone and cone shell geometry, on a tissue model. In particular, simulations and experiments were carried out to assess the depth sensitivity performance of different combination of illumination and detection configuration in a non-contact lens based probe geometry, implemented by convex lenses, in a two-layered turbid medium mimicking the optical properties of human epithelial tissue. In this work presented, we further expand our previous investigation both numerically and experimentally. The simulation results are validated with the experimental results in terms of fluorescence intensities at the emission peaks and the depth sensitivity to a given layer. The experimental results provide useful insights to the change in depth sensitivity achieved using different types of lenses and illumination-detection geometry. The development and validation of this MC code provides a fast, inexpensive, reliable and robust computational platform that can assist the planning and optimization of optical designs involving the cone or cone shell illumination and detection geometries prior to the physical development of an optical system for real experiments.

6.2 Materials and Methods

6.2.1 Optical setup and measurements

In this study, we investigated two different combinations of illumination and detection configurations involving the cone and coneshell geometries implemented by a convex lens. The two configuration pairs are shown in Figure. 6-1.

Figure 6-1 Schematic diagram of two illumination–detection configurations implemented using convex lens: (a) Coneshell – Cone, (b) Cone – Cone. The solid arrow represents the excitation laser; the dashed arrow represents the collected fluorescence.

The non-contact probe for configurations (a) and (b) was coupled to a diode laser (iFlex-2000, Point Source Ltd., Hamble, UK) with a maximum output power of 50 mW at 405 nm, as shown in Fig. 6-2. The output laser light with a beam diameter of around 1 mm was expanded using a 30x beam expander before passing through a 405 nm bandpass filter and then deflected by a dichroic mirror towards a convex lens ($f = 35$ mm) with a diameter of 25.4 mm, in which the lens was slightly overfilled. A piece of aluminium foil with a diameter of 23.4 mm was placed along the excitation light path, between the beam expander and bandpass filter, to create a cone shell illumination configuration as shown in Fig. 6-1 (a). The fluorescence signal was then collected through the same convex lens, which then passed through the dichroic mirror and a long pass filter before being focused onto the core of a collection fiber, with a diameter of 400 μm and NA of 0.22, by a convex lens ($f = 35$ mm).

Figure 6- 2 Schematic diagram of the probe configuration based on the combination of convex lenses

During measurements, the phantom was placed on a translational stage underneath the probe. The probe-sample distance was varied to measure from different depths in the phantom by raising the stage towards the probe. The first measurement started at 0 mm where the focal spot of the excitation light was located on the surface of the phantom. Then, for every subsequent measurement, the stage was raised by 0.5 mm until it reached a depth of 4 mm beneath the surface. The integration time for each spectrum was one second and the laser shutter was closed for ten seconds between each measurement to allow fluorescence recovery in case of the possible photo-bleaching of fluorophores.

6.2.2 Tissue phantoms

A two layered agar phantom was prepared according to the recipe and procedure published in earlier report [242]. The optical properties of the phantom was made to mimic human squamous cervical tissue [243]. The phantom was made in a cylindrical plastic petri dish with a diameter of 30 mm and the top layer (epithelium) was made to be 500 μm while the bottom layer (stroma) was made to be 10 mm to represent a semi-infinite medium. Two different fluorophores, flavin adenine dinucleotide (FAD) with peak emission at 530 nm and protoporphyrin IX (PpIX) with peak emission at

630 nm, were added into the top and bottom layers, respectively, for the ease of fluorescence signal discrimination from each layer. The concentration of FAD was 33.2 μM and the concentration of PpIX was 32.3 μM . Polysphere and Nigrosin was used to mimic the light scattering and absorption properties of the epithelium and stroma of squamous cervical tissue at 530 nm [243]. The concentration of polysphere needed to mimic the scattering coefficient of tissue at 530 nm was estimated using Mie theory and the concentration of nigrosin used to mimic the absorption coefficient of the tissue was measured experimentally. The absorption and scattering spectra of epithelial tissue vary differently with those of the elastic scatterers (polyspheres) and light absorbers (nigrosin) across the excitation wavelength (405 nm) to the emission peak wavelengths (530 and 630 nm). Thus, optical properties of the tissue phantom was mimicked at the central wavelength, around 530 nm, in order to simulate and investigate the depth sensitive fluorescence measurement as close to the real human tissue as possible. A piece of plastic wrap was placed between the top and bottom layer to prevent the diffusion of phantom contents across the two layers. The thickness of the plastic wrap was measured to be 10 μm and no fluorescence signal from the plastic wrap was observed in the experiment. The optical properties of top and bottom layers of the tissue phantom at excitation and emission wavelengths were listed in Table 6-1.

Table 6- 1 Optical properties of the tissue phantom [244]

	405 nm (Excitation)	530 nm (FAD)	670 nm (PpIX)
	μ_a , μ_s	μ_a , μ_s	μ_a , μ_s
Top layer	1.2, 39.7	1.9, 34.2	1.8, 23.1
Bottom layer	1.4, 250.2	2.2, 215.1	2.1, 174.0

μ_a : absorption coefficient in cm^{-1} ; μ_s : scattering coefficient in cm^{-1} .

6.2.3 MC simulations

The details of MC method for simulation of light illumination and detection by a convex lens have been described earlier in Chapter 5 thus it will not be repeated here. The simulation of fluorescence is based on a method proposed by Liu et al. earlier [63]. All of the simulated parameters such as radius of cone, cone shell, focal length of lenses, and distance between the focal point and tissue surface are exactly same with those we used in our experimental study. The optical properties used in the simulation are same with that shown in Table 6-1. A total of 0.5 million photons are used for each simulation and each simulation was repeated three times to get a standard deviation.

In MC simulations, the quantum yield of the fluorophores was always set to 1 and a single absorption coefficient was used to account for the absorption coefficients of the absorber and fluorophores. To compare the simulation results to the experimental results, the quantum yield of FAD and PpIX at both emission wavelengths has to be rescaled to the measured value and the absorption coefficients contributed separately by Nigrosin and fluorophores (FAD and PpIX) in the tissue phantom have to be converted to a combined absorption coefficient for use in the MC simulations. The independent absorption coefficients indicate the probability of an excitation photon being absorbed by Nigrosin or the FAD and PpIX fluorescent molecules, while the quantum yield of a fluorophore indicate the probability of an absorbed photon being converted into fluorescence light. The conversion of absorption coefficient and rescaling of quantum yield have been discussed in detail in previous publication [63]. The independent absorption coefficients of Nigrosin, FAD and PpIX at the excitation wavelength were measured using a UV-VIS spectrophotometer (UV-2450, Shimadzu Corp., Kyoto, Japan) at the concentrations

used in tissue phantom. The probability of excitation light being absorbed by FAD molecules in the top layer was calculated by taking the ratio of absorption coefficient of FAD to the sum of absorption coefficients of Nigrosin and FAD. Similarly, the probability of excitation light being absorbed by PpIX in the bottom layer was calculated by taking the ratio of absorption coefficient of PpIX to the sum of absorption coefficients of Nigrosin and PpIX.

6.2.4 Data analysis

Background subtraction was performed on all measured spectra. The background spectrum was acquired for every set of experiments with the excitation laser source switched off. The peak intensities at 530 nm and 630 nm were used for the plotting of FAD and PpIX fluorescence signal changes over a range of targeted depths in each configuration. The FAD peak intensity measured at every depth was divided by the maximum among these intensities to achieve normalization. Similarly, the PpIX peak intensity measured at every depth was divided by the maximum among these intensities to obtain the normalized PpIX intensity. This method of normalization facilitated the comparison of experimental results across different experimental setups by cancelling out the effect of different excitation laser powers on the sample due to the variation in optical setup. To compare the depth sensitivity achieved by each configuration, the sensitivities of measured fluorescence to the top and bottom layer as a function of the targeted depth were computed. The sensitivity to the top or bottom layer at a particular depth was computed by dividing the normalized FAD or PpIX intensity at this depth to the sum of the normalized FAD and PpIX intensities at the same depth.

To determine the quantum yield of FAD and PpIX in the tissue phantom for the purpose of simulation, the fluorescence spectra of FAD and PpIX were measured at

very low concentrations first to minimise the secondary absorption effect of the fluorophore itself on emitted fluorescence. Background subtraction was performed on the spectra then their intensities were scaled up to the fluorophores concentrations used in the tissue phantom given that the fluorescence intensity is linearly proportional to the concentration of the fluorescence molecule in the concentration range in this study. The quantum yield of FAD at 530 nm was determined by taking the ratio of FAD fluorescence intensity to the sum of FAD and PpIX fluorescence intensities at 530 nm. The quantum yield of PpIX at 530 nm was computed by taking the ratio of PpIX fluorescence intensity to the sum of FAD and PpIX fluorescence intensities at 530 nm. The quantum yields of FAD and PpIX at 630 nm were determined similarly.

The simulated results, after the correction for both the absorption coefficient and quantum yield, were normalized in the same manner as in the processing of experimental data, as described earlier to facilitate the comparison of FAD and PpIX fluorescence trends between computational and experimental results. Then, the sensitivities to the top and bottom layer of each simulated configuration were computed using the same method as described above to further validate the MC modeling that we have developed.

6.3 Results

Figure 6-3 shows the trends of measured FAD and PpIX fluorescence intensity and the sensitivity to the top and bottom layer in a cone–cone configuration. The experimental results are represented in solid line and the simulation results are represented in dotted line. The experiments were repeated three times at different

locations on the same phantom to generate the standard errors in the experimental results; however the error bars are too small to see.

In Figure 6-3 (a), it can be seen that the measured FAD fluorescence rises slightly from phantom surface and to reach its maximum at around 0.7 mm before decreases gradually to around 0.22 at a focal depth of 5.5 mm. In Figure 6-3 (b), the measured PpIX fluorescence is highest between 0 to 0.7 mm and then decreases slowly to 0.38 at a focal depth of 5.5 mm. The simulations results agreed well with the experimental results.

Figure 6- 3 (a) Normalised FAD fluorescence and (b) normalised PpIX measured from tissue phantom using Cone–Cone configuration, and corresponding MC simulation results. (c) Sensitivity to top layer and (d) sensitivity to bottom layer computed from the experimental results and simulation results. Focal depth refers to targeted depth of focus beneath the phantom surface, which has been corrected for refractive mismatch between the sample and air.

Figure 6-3 (c) and (d) show experimental and computational sensitivity to the top and bottom layer in cone–cone configuration. The experimental sensitivity to top layer is 0.49 at the surface and reaches its peak at 0.5 at focal depth of 0.7 before

decreases to 0.38 at focal depth of 5.5 mm, while the experimental sensitivity to the bottom layer is the lowest, 0.5 at focal depth of 0.7 mm and increases to 0.62 at focal depth of 5.5 mm. The overall trends of the sensitivity to the top and bottom layer in experimental results and MC simulation agree with each other except for the first measurement point at the surface. This difference is probably due to the inaccuracy in the experiment to focus accurately at the surface of the phantom. As the 0 mm position in the experiment was determined visually at the height where laser spot was smallest on the phantom, it might be slightly different from the actual 0 mm position which can be set accurately in MC simulation.

Figure 6-4 shows the trends of measured FAD and PpIX fluorescence and the sensitivity to the top and bottom layer in a coneshell–cone configuration implemented by convex lens. The experimental results are represented in solid line and the simulation results are represented in dotted line.

Figure 6- 4 (a) Normalised FAD fluorescence and (b) normalised PpIX measured from tissue phantom using Coneshell–Cone configuration, and corresponding MC simulation results. (c) Sensitivity to top

layer and (d) sensitivity to bottom layer computed from the experimental results and simulation results. Focal depth refers to targeted depth of focus beneath the phantom surface, which has been corrected for refractive mismatch between the sample and air.

In Figure 6-4 (a), it can be seen that the measured FAD fluorescence rises from 0.88 at the phantom surface and reaches its maximum at around 0.7 mm before decreases gradually to around 0.11 at a focal depth of 5.5 mm. It should be noted that the FAD fluorescence in this configuration decreases faster after the maximum at 0.7 mm than those in the cone–cone configuration as shown in Figure 6-3 (a). This shows that a coneshell geometry in the illumination geometry can be used to reduce the contribution from the shallower layers when performing deep measurement in a turbid medium, which agrees well with our previous report [245]. In Figure 6-4 (b), the measured PpIX fluorescence rises from 0.94 at 0 mm to its maximum at 0.7 mm and then decreases slowly to 0.25 at a focal depth of 5.5 mm. The simulated FAD and PpIX fluorescence trends are in close similarity to the measured results with a rise from the surface to the maxima at 0.7 mm and decreases after that.

The experimental and computational sensitivity to the top and bottom layer of this coneshell–cone configuration are shown in Figure 6-4(c) and (d). Both experiment and simulation shows a sensitivity to the top layer of 0.48 at the sample surface and 0.5 at a focal depth of 0.7 mm. Then the experimental sensitivity to the top layer falls to 0.3 at focal depth of 5.5 mm while the computational sensitivity drops to 0.28 at the same focal depth. The sensitivity to the bottom layer for both experiment and simulation is 0.52 at 0 mm and drops to 0.5 at focal depth of 0.7 mm before increases to 0.70 and 0.72 at focal depth of 5.5 mm, respectively. The trends in which the sensitivity to the top and bottom layers shift upon increase in focal depth for the experimental results and simulation results agree with each other and the relative higher range of sensitivity achieved as shown in Figure 6-3 (c) and (d)

suggested that a coneshell illumination configuration is able to reduce fluorescence from overlaying layers in a turbid medium during deep measurement hence improving the contrast of targeted subsurface fluorescence.

6.4 Discussion and conclusion

In this study, we have investigated the depth sensitivity of two illumination and detection configurations involving the conventional cone and novel coneshell geometry implemented by a convex lens, in a turbid medium. The results show that a coneshell illumination configuration was able to reduce the fluorescence contribution from the overlaying layers when performing deep measurements as compared to a cone illumination configuration. A coneshell detection configuration can reject undesired fluorescence from off-focus regions, hence, giving the best depth sensitivity in conjunction with a coneshell illumination configuration. The use of convex lens in implementing the illumination-detection configurations has no profound effect on the depth sensitivity. To facilitate the optimization of optical system, we have developed a MC simulation to model the convex lens in implementing the cone and coneshell illumination and detection configurations. The simulation results were validated against the experimental results in term of fluorescence intensity and depth sensitivity. This MC code will be a useful tool that helps in planning and optimization of optical designs involving cone or cone shell configurations and convex lenses prior to the real experimental study.

Chapter 7: Non-contact depth sensitive fluorescence imaging for early epithelial cancer diagnosis

[Copyright permission from: C. Zhu, Yi Hong Ong and Q. Liu, “Multifocal noncontact color imaging for depth-sensitive fluorescence measurements of epithelial cancer,” Opt Letters 20, Vol. 39 (11) ,p.3250-3253, (2014).]

7.1 Introduction

In previous Chapters, we have demonstrated that lens based non-contact setup will be able to perform both of depth sensitive diffuse reflectance and sensitive fluorescence spectroscopy in a point measurement system for early epithelial cancer diagnosis. However the point measurement system is slow when optical imaging in a large field of view is desired. This is especially true in the diagnosis of early epithelial cancer where spatial context in optical images may provide important information for clinical diagnosis. In this Chapter, we extended the setup from point measurement system into an imaging system to achieve depth sensitive fluorescence imaging on a larger field of view. In our proposed multi-focal non-contact setup, the combination of a microlens array and a tunable lens enables the depth of the multi-focal plane to be conveniently adjusted without any mechanical movement of the imaging lens or the sample. This advantage is particularly desirable in the clinical setting. Results from the phantom study demonstrated that the setup can achieve depth sensitive color imaging for fluorescence measurements, which was further confirmed by spectral measurements. Color values could be also used to reconstruct full spectra using the previously developed algorithm for rapid spectral imaging.

Depth sensitive optical spectroscopy has attracted increasing interests for the diagnosis of epithelial cancers in the past years [165, 246, 247]. Because the distribution of molecules such as endogenous fluorophores in epithelial tissues is depth dependent and it varies significantly with disease stage [215], depth sensitive optical measurements may yield higher sensitivity to malignant growth in epithelial tissues than common optical measurements in which optical signals are averaged throughout the volume being interrogated. A common setup for optical spectroscopy uses a fiber-optic probe for the delivery of illuminating light and collection of emitted light [94, 162]. In a fiber-optic spectroscopy setup, it is possible to achieve depth sensitive measurements by varying the source-detector separation [246], the effective aperture diameter of fibers [165] and the illumination and collection angles [215]. However, the uncertainty in measurements due to inconsistent probe-sample pressure could induce significant distortion in measured spectra, which consequently would cause large errors in diagnosis [225]. Lens based setups have been investigated to perform non-contact optical measurements to overcome this problem. Andree et al.. [229] performed spatially resolved diffuse reflectance measurements without physically contacting a tissue sample by using a non-contact setup, which involves a spherical and a flat folding mirror for illumination while two achromatic lenses for detection. Bish et al.. [230] achieved non-contact diffuse reflectance measurements on tissue phantoms and human skin by a lens based non-contact probe. Mazurenka et al.. [231] pursued time-resolved diffuse reflectance measurements by a non-contact lens based setup, in which laser scanning was used to achieve imaging. Although these reports addressed the problems of inconsistent probe-sample contact, none of them are suitable for depth sensitive measurements because of the lack of change in the depth of light focus. Our previous numerical study [101] demonstrated that it is possible to

use a lens based non-contact setup to obtain depth sensitive diffuse reflectance measurements on an epithelial cancel model by adjusting the depth of light focus under the tissue surface. We also developed a special lens based non-contact fiber-probe [247] to achieve depth sensitive fluorescence spectroscopy on human skin model without physically moving the imaging lens. Unfortunately, most above setups were designed for point measurements, which is slow when optical imaging in a large field of view is desired. In this report we demonstrated a multi-focal non-contact setup to perform depth sensitive fluorescence imaging on tissue phantoms in a large field of view for the diagnosis of early epithelial cancer. Moreover, our setup does not require the mechanical movement of any optical components or sample to achieve depth sensitive optical measurements thus would be convenient in the clinical setting.

7.2 Materials and Methods

7.2.1 Imaging setup

The schematic of our proposed setup is shown in Figure.7-1. In the illumination module, a 405-nm laser (iFlex-2000, Point Source Ltd., Hamble, UK) with a maximum output power of 20 mW was used as the excitation source. The laser light was coupled onto a beam expander to achieve a beam diameter of 4 mm before passing through the dichroic mirror. Next to the dichroic mirror, a microlens array was used to get a multifocal illumination plane, which was imaged onto a tissue sample by a tunable lens (HR EL-10-30, Optotune, Dietikon, Switzerland). The microlens array (Customized model, Wuxi Opton Tech Ltd, Jiang Su, China) contained around 20×20 microlenses and each single microlens had a diameter of 250 μm and a focal length of 1000 μm , the filling factor of the microlens array was around 75%. The focal length of the tunable lens can be varied precisely by changing the current applied on it. When the focal length of the tunable lens was changed, the

depth of the focal plane of the microlens array under the sample surface, which will be named as the focal depth in the rest of the paper, would vary accordingly. With the help of the tunable lens, the focal depth inside the sample can be varied easily and precisely without any mechanical movement of the imaging lens or the sample. In the detection module, fluorescence from the sample was first imaged onto the microlens array's focal plane by the tunable lens, then defocused by the microlens array and finally imaged by a convex lens onto a color camera (AT-200 GE, JAI, USA) equipped with 3-CCD, which captured images in Red, Green and Blue channels, after passing through a long pass filter to remove the excitation light. The distance between the focal plane of the microlens array and the tunable lens, labeled as u in Figure.7.1, was fixed at 5.5 cm, while the distance between the sample surface and the tunable lens, labeled as d in Figure.1, was set to 4.5 cm. The focal length of the tunable lens was varied from 2.45 cm to 2.60 cm with an increment around 0.25 mm. Consequently, it can be calculated that the focal depth varied from -0.8 mm to 5.2 mm approximately with an increment of 0.8 mm assuming that the sample surface corresponded to a focal depth of zero. It should be noted that the filling factor of the microlens array we used is only 75%, which means that a large portion of excitation light passes through the array would stay parallel. It was necessary to reduce the amount of the parallel excitation light hitting the tunable lens; otherwise this portion of light would form a strong focal spot after passing through the tunable lens and serve as the background in the subsequent depth sensitive measurements. In order to solve this problem, a blocker made of aluminium foil with a diameter of around 4.0 mm was placed immediately above the tunable lens to block the parallel light. The distance between the microlens array and tunable lens in our setup was large enough so that the portion of light focused by the microlenses formed a light beam with a size

of 10 mm in diameter on the top surface of the tunable lens, while the portion of parallel light remained 4 mm in diameter on the same surface. This ensured that all parallel light has been effectively blocked. It should be noted that a small amount of focused light from the microlenses was blocked, but the major portion of focused light passing through was sufficient for fluorescence imaging.

Figure 7- 1 Schematic of the multifocal noncontact imaging setup. Solid lines with arrows represent excitation light flow while green dotted lines represent emission light flow. Light propagation is illustrated for one microlens only in the region below the microlens array for clarity.

7.2.1 Tissue phantoms and measurements

The setup was evaluated on a two-layered agar tissue phantom. The phantom was prepared following the procedure published in an earlier publication [242] in which the optical properties were representative of human epithelial tissues. The thickness of the top layer was 500 μm . The thickness of the bottom layer was 1 cm and the lateral dimensions of both layers were larger than 3 cm, which were large enough to represent a semi-infinite medium. Protoporphyrin IX (PpIX) was added into the top layer at a concentration of 71.1 μM while flavin adenine dinucleotide (FAD) was added into the bottom layer at a concentration of 25.5 μM . PpIX and FAD, which can be found naturally in the skin, were

chosen in this study as that their non-overlapping emission peaks were located at 670 nm and 530 nm, respectively. Thus it was easy to facilitate the discrimination of fluorescence signals originated from each layer in this study. The concentrations of both fluorophores were chosen so that the magnitudes of both emission peaks fell within the same order. Polystyrene spheres (07310, Polysciences, Warrington, PA, USA) and Nigrosin (N4754, Sigma-Aldrich, St. Louis, MO, USA) were added into each layer at different concentration to mimic the light scattering and absorption properties of the epithelium and stroma of cervical tissues at 530 nm [244]. A plastic wrap about 10- μ m thick was used to separate the top and bottom layer to prevent the diffusion of fluorophores and nigrosin molecules across the interface between the two layers. The optical properties of the top and bottom layers in the tissue phantom at the excitation wavelength and the peak emission wavelengths of FAD and PpIX were listed in Table 7-1.

Table 7- 1 Optical properties of the tissue phantom [244]

	405 nm (Excitation)		530 nm (FAD)		670 nm (PpIX)	
	μ_a	μ_s	μ_a	μ_s	μ_a	μ_s
Top layer	1.2,	39.7	1.9,	34.2	1.8,	23.1
Bottom layer	1.4,	250.2	2.2,	215.1	2.1,	174.0

μ_a : absorption coefficient in cm^{-1} ; μ_s : scattering coefficient in cm^{-1} .

All experiments were performed in a dark room. The focal length of the tunable lens was controlled precisely by changing the current applied on it via the software provided by the manufacturer. Within the range of the tunable lens's focal length, a total of eight color images were acquired, one image for each focal depth taking one second. There was a 10-sec time interval between two consecutive measurements to minimize photo bleaching. The acquired color images were processed using ImageJ software first to pick up the regions of interest and then further processed by a custom designed image processing code written in Matlab (R2010, MathWorks, U.S).

7.3 Results

A sequence of color images with different focal depths were shown in Figure.7-2 (a). In each subfigure of Figure.7-2 (a), every circular bright spot refers to one focal spot imaged from the surface or the inside of the tissue phantom. When the focal depth was varied from 0 mm (i.e. tissue surface) to 4.3 mm, slight color changes can be observed. As the light focus moved deeper, the bright spots gradually varied from slightly red to slightly green. This trend was more obvious in the color values as shown in Figure.7-2(b), in which the color values in nine different focal regions were averaged calculate the mean and standard deviation. It can be seen clearly that when the focal depth was increased from -0.8 mm to 5.2 mm, both R and G values increased to their peak first and then decreased. However R value (mainly from PpIX in the top layer) reached the peak earlier than G value (mainly from FAD in the bottom layer); moreover R value decreased faster than G value. This observation directly demonstrated that our setup was capable of discriminating fluorescence from various depths with different sensitivities, i.e. depth sensitive fluorescence imaging. B value was relative low and changed only slightly with the focal depth. This was due to the fact that the two selected fluorophores in this report contributed little to the blue channel and most blue light was blocked by the long pass filter. It is interesting to see that the signal from top layer, i.e. R value shown in Fig. 2(b) does not reach the maximum until the focal depth is increased to 0.8 mm that is slightly larger than the thickness of top layer (note that the data points between 0 mm and 0.8 mm are not shown for clear visualization). This is likely because the top layer had a considerably large scattering coefficient that could have affected the distribution of excitation light.

Figure 7- 2 (a) Color images acquired at different focal depths; (b) Raw RGB values averaged for bright spots at a range of focal depths. Scale bar is $400\ \mu\text{m}$.

To characterize the depth sensitivity of the new setup, the percentage of each of R, G and B values relative to the summation of all was calculated as shown in Figure.7-3. Since B value changed very slightly, only the percentages of R and G were shown. Figure.7-3 shows that the percentage of R value increased a little when the focal plane was moved from the air to the tissue surface, then decreased all the way when the focal plane went deeper from the tissue surface to the bottom layer. In contrast, the percentage of G has the opposite trend. The trends in these ratios explained why the color of images in Figure.7-2(a) changed from slightly red to slightly green when the focal depth increased. The percentage change as a function of focal depth was mainly affected by the numerical aperture of the microlens array, which was 0.14 in our setup. Each single microlens can be treated as an objective lens. It is well known that an objective lens with larger numerical aperture possesses a stronger focusing power thus such a lens would yield a better spatial resolution. Similarly a microlens array with a larger numerical aperture should yield better depth sensitivity because of the stronger focusing power. As a matter of fact, we also tried another microlens array with a smaller numerical aperture (MLA150-5C, Throlabs, Sterling, Virginia, USA) and found that this microlens array has much smaller depth sensitivity (results not shown

in the manuscript) which demonstrated that the percentage change could be much greater if the microlens array with a considerably larger numerical aperture was used.

Figure 7- 3 Percentage of R and G values as a function of focal depth

To clarify the source of color changes shown in the Figure.7-2, fluorescence spectra were measured from the same phantom using a setup similar to that in Figure.7-1 except two differences. The first difference was that the 3-CCD was replaced by a spectrometer (QE 65 Pro, Ocean Optics,Dunedin, Florida, US). The second difference was that a 100- μ m pinhole was placed above the microlens array to select only one microlens for illumination and detection. The exposure time used for each measurement was two seconds, and a total of eight spectra were acquired. Detected fluorescence spectra for the same range of focal depths as in Figure.7-3 were shown in Figure.7-4. It is interesting to see that PpIX emission peak around 670 nm and FAD emission peak around 530 nm both increased to their maximum first and then decreased when the focal depth increased. PpIX emission peak reached its maximum when the focal depth was 0.80 mm while FAD emission peak reached its maximum later when the focal depth was 1.68 mm. The change in the intensity of PpIX peak was more dramatic than that of FAD peak, which was likely due to the fact that the PpIX was in the top layer. It is straightforward to see that the trends in the

changes of fluorescence peaks in Figure.7-4 agree very well with those of color values as shown in Figure.7-2 (b).

Figure 7- 4 Fluorescence spectra for a range of focal depths, the legends show the values of focal depths.

7.4 Discussion and conclusion

Results shown in Figure.7-2 (a) demonstrated that our setup is able to acquire multi-focal color images rapidly. Moreover, Figure.7-2 (b) and Figure.7-3 showed that depth sensitive optical imaging could be achieved in our setup by varying the focal distance of the tunable lens and in turn the depth of the multiple focal planes in the sample. Color values of tissues could be used directly for cancer diagnosis [248, 249]. Furthermore, color values could be used to reconstruct the full spectrum using a published algorithm [250]. Therefore our setup could potentially achieve depth sensitive spectral imaging rapidly which would be faster and cheaper than the traditional spectral imaging setup. Currently the field of view of our setup was around 4 mm in diameter, which was limited by the size of the microlens array and tunable lens we used. In the 4-mm field of view, totally around 10×10 microlenses were covered, which corresponded to a spatial resolution of around 0.4 mm. The spatial

resolution could be easily improved by changing the distances u and d in Figure.7-1 or using a microlens array with smaller individual microlenses. Currently, most spectral imaging setups utilized uniform illumination [251], which offers no changes in depth sensitivity thus no advantages in epithelial cancer diagnosis. Another advantage of the proposed setup is the lower requirement on the excitation laser power because the excitation power is focused only on a finite number of focal spots rather than the entire tissue area.

Another potential issue in this setup is the variation in the focal length of the tunable lens with wavelength. By using the law for approximation of focal length of lenses [252], it was found that the focal length of the tunable lens changed around 4% when the wavelength was varied from 405 nm to 670 nm. In our fluorescence imaging setup, the effect could be eligible since such variation might affect the detection efficiency only because the illumination light was a laser beam with single wavelength. However, this effect could not be ignored in a diffuse reflectance imaging setup since the illumination light is wavelength dependent.

In summary, we proposed a multi-focal non-contact setup capable of performing depth sensitive fluorescence imaging on a two-layered epithelial tissue model. The combination of a microlens array and a tunable lens enables the depth of the multi-focal plane to be conveniently adjusted without any mechanical movement of the imaging lens or the sample. This advantage is particularly desirable in the clinical setting. Results from the phantom study demonstrated that the setup can achieve depth sensitive color imaging, which was further confirmed by spectral measurements. Color values could be also used to reconstruct full spectra using the previous algorithm for rapid spectral imaging.

Chapter 8: Dual-modal spectroscopy for early prediction of skin tissue viability

[Copyright permission from: Caigang Zhu, Shuo Chen, Christopher Hoe-Kong Chui, Bien-Keem Tan and Quan. Liu, "Early prediction of skin viability using visible diffuse reflectance spectroscopy and auto-fluorescence spectroscopy", *Plastics and reconstructive surgery*, 134: 240e, Aug, 2014]

8.1 Introduction

In previous Chapters, we have explored several optical measurement techniques and MC based data analysis methods in UV-visible diffuse reflectance and auto-fluorescence spectroscopy/imaging for early epithelial cancer diagnosis. In this Chapter, we further validated our optical measurement techniques on another important clinical application, i.e. tissue viability diagnosis.

Skin flaps are frequently used to provide well vascularised tissues for wound coverage in plastic surgery. One problem with skin flap surgery is that 6% to 25% of flaps require surgical re-exploration for vascular compromise and approximately 10% of flaps are not salvageable [6, 7]. Most flap losses occur within 72 hours after the surgery [8], thus it is critical to determine as early as possible whether or not a skin flap will survive to maximize the chance of flap salvage. The periodical clinical examination of capillary refill, flap color, temperature and bleeding patterns is the current gold standard method for the assessment of flap viability; however the current gold standard method relies heavily on the skill and the availability of hospital staff. To overcome this limitation, several adjunct instrumental methods have been introduced for the monitoring of skin flaps.

Laser Doppler flowmetry and imaging have been widely used as an adjunct tool for noninvasive assessment of flap viability [6, 23-25] by measuring the Doppler

frequency shift of a laser beam reflected from a tissue. However, the Laser Doppler technique has inherent limitation in assessing flap viability since it can measure only one physiological parameter, i.e. blood flow, which is just one of several physiologically relevant parameters affecting tissue viability, thus it is likely to further improve the accuracy of skin flap assessment by measuring other important parameters as well. Multiple other techniques have been reported to provide additional parameters for monitoring flap viability. Near infrared (NIR) diffuse reflectance spectroscopy has been explored extensively for assessing tissue viability by measuring hemoglobin oxygen saturation [27-29], hemoglobin concentration [28], and tissue hydration [29]. Visible light spectroscopy has been used to measure tissue oxygenation and total hemoglobin concentration for tissue viability characterization [9, 30]. Compared to NIR diffuse reflectance spectroscopy, visible diffuse reflectance spectroscopy offers higher sensitivity to the change in hemoglobin parameters at the cost of smaller penetration depth. Fluorescence spectroscopy utilizing exogenous fluorophore [31] and endogenous fluorophore [32, 33] have also been reported as tools to characterize flap viability by measuring metabolic rate of tissues during the past years. Both diffuse reflectance and autofluorescence spectroscopy provide complementary information about tissue status and have shown excellent potential for assessing tissue viability separately. These two techniques could share the fiber-optic probe and spectrometer due to the overlapping spectral regions involved. However their simultaneous use to characterize the skin flap metabolism and their respective accuracies in flap viability assessment has not been studied to our best knowledge. Since these two sets of techniques involve the use of two different light sources and optics, understanding of the individual roles of visible diffuse reflectance spectroscopy and autofluorescence spectroscopy would enable the tailoring of such

equipment for a particular skin flap problem to reduce the cost of equipment and operation complexity.

This is the first pilot study to evaluate the simultaneous use of both visible diffuse reflectance and autofluorescence spectroscopy on a reverse MacFarlane rat dorsal skin flap model in the early prediction of skin viability, to our best knowledge. A total of 62 flap measurement sites from eleven Sprague Dawley rats were monitored for 72 hours. Both statistical analysis using measured spectra and quantification of physiologically relevant tissue parameters using empirical methods were performed. The statistical analysis results suggest that either visible diffuse reflectance spectroscopy or autofluorescence spectroscopy alone can predict the skin viability accurately; however, autofluorescence spectroscopy is more sensitive to tissue changes in the first two hours after induction of ischemia. The pilot study shows that it is feasible to predict flap failures in the first two hours when using autofluorescence spectroscopy alone; and it is possible to predict flap failures even in the first 15 minutes with high accuracy when using diffuse reflectance and autofluorescence spectroscopy simultaneously. Meanwhile, several physiologically relevant parameters including hemoglobin oxygenation, total hemoglobin concentration and redox ratio indicators estimated from diffuse reflectance and autofluorescence spectra show distinctively different trends over time for non-viable and viable skin. These findings will be helpful to clinicians for making precise judgment on flap viability. Furthermore, our results highlight the advantage of using autofluorescence spectroscopy in the early prediction of skin flap viability relative to diffuse reflectance spectroscopy.

8.2 Materials and Methods

8.2.1 Animal model and surgical procedure

All animal experiments were conducted in compliance with the SingHealth Institutional Animal Care and Use Committee (IACUC) animal welfare committee's requirement for the care and use of laboratory animals in research.

In this study, eleven Sprague Dawley rats at an age of 16 weeks were acclimated three days prior to the flap surgery. All the procedures were done under 1.2-2% isoflurane inhalational anesthesia. Thirty minutes prior to the surgery, the dorsa of the rats were shaved and the sites at which optical measurements would be made were marked on the exposed skin. Meanwhile the baseline optical measurements were performed and these data were used as control values in the subsequent analysis. A cutaneous flap that was 2-cm wide and 8-cm long was raised based on the caudal skin attachment. The flap was then repositioned over the closed wound and held in place with sutures. During the 72-hour postoperative study period, the animals were housed separately. Final tissue viability was determined at the end of the study period prior to euthanasia of the animals.

8.2.2 Non-invasive optical measurements

Diffuse reflectance and fluorescence spectra were measured by a custom designed optical measurement system on the marked positions one by one shortly before the surgery, in the first 15 minutes, then approximately once every one hour in the first four hours and finally once every four hours over the next 68 hours after the creation of the flap. The scheme of the entire optical measurement system and custom bifurcated fiber probe was shown in Figure.8-1.

Figure 8- 1 (a) scheme of optical spectroscopy measurement system; (b) custom bifurcated Fiber probe

The spectra were collected with a spectrometer (USB4000, Ocean Optics Inc., Dunedin, Florida, US) using a custom bifurcated fiber optic probe. The fiber probe consisted of one central illumination fiber and four surrounding detection fibers. The core diameter of the illumination and detection fiber was 200 μm . The source-detection separation was around 490 μm . The diameter of stainless steel ferrule at the common end was about 2.5 mm. A DAQ card (USB-6501, National Instruments Corporation, Austin, Texas) provides transistor-transistor logic (TTL) control signals to synchronize illumination and detection.

In fluorescence measurements, a diode laser that generates excitation light at 405nm was used for illumination. The excitation light was delivered onto the skin through the excitation channel of the fiber-optic probe. The backscattered fluorescence light was collected and sent to the spectrometer by the emission channel of the probe. An inline long pass filter, placed between the emission channel and the spectrometer, was used to block the excitation light. In diffuse reflectance measurements, a Tungsten Halogen white light source (HL-2000-FHSA, Ocean Optics Inc., Dunedin, Florida, US) was used for illumination. The same probe was

used for both illumination and detection. Different from fluorescence measurements, no filter was used between the emission channel and the spectrometer. A 99% reflectance standard (SRS-99-010, Labsphere Inc., New Hampshire, US) was used as a reference to generate reflectance spectra for the purpose of calibration.

A custom programmed graphical user interface (GUI) software written in Labview (Labview 10.0, National Instruments Corporation, Austin, Texas) was used to control the entire system. The GUI software generated TTL signals to modulated the light sources and synchronize the light sources and the spectrometer. The output from a light source would reach its maximum if the TTL signal sent to the light source controller was high. In contrast, the output would be nearly zero when the TTL signal was low. For every pulse sent to the light source, the GUI software sent two pulses to the spectrometer to collect two optical spectra, one for the situation where the output of the light source reached the maximum and the other for the situation when the output from light source was nearly zero. The former spectrum serves as the original sample spectrum. The latter spectrum serves as the background. The true sample spectrum, which was obtained by subtracting the background spectrum from the original sample spectrum, was least subject to the effect of ambient light. Six sample spectra were taken in each measurement and then averaged to improve the signal to noise (SNR) ratio. The integration time for each sample spectrum was 200 ms.

A total of around 20 sets of spectra at each measurement site were acquired in 72 hours. Along with optical measurements, a corresponding color image of the rat's dorsum was taken at each time point, which was used as a reference for clinical examination. The total time to carry out a full set of diffuse reflectance and fluorescence measurements at all marked locations in one animal was around 15 minutes. Totally 62 flap measurement sites were monitored. Among all the monitored

flap sites, 31 flap sites were completely non-viable and the other 31 sites were still viable after 72 hours based on the examination by the clinician.

8.2.3 Data analysis

The qualitative analysis on diffuse reflectance spectra was restricted to a wavelength range of 450-700 nm which covers most of the visible light range, while the analysis on autofluorescence spectra was restricted to an emission wavelength range of 420-700 nm which covers the emission peaks for most of tissue fluorophores. Each spectrum was smoothed using a digital median filter first. Then each smoothed diffuse reflectance spectrum was calibrated by dividing the spectrum by the diffuse reflectance spectrum measured from a reference standard (SRS-99-010, Labsphere Inc., New Hampshire, US) first, then the calibrated spectrum was normalized by dividing the entire spectrum point by point by the peak intensity value. In contrast, the smoothed fluorescence spectrum was calibrated by multiply it by a the ratio of the given spectrum of a standard lamp current source (RS-4, Gamma Scientific, San Diego, US) and the spectrum measured using the emission channel of the probe and the spectrometer at each wavelength of fluorescence measurements, then the calibrated spectrum was normalized by dividing the entire spectrum by the peak intensity value wavelength by wavelength. Next, the partial least square (PLS) analysis [253] was conducted on the normalized diffuse reflectance or autofluorescence spectra to find principal components (PCs) to represent the measured spectra. The first 15 PCs of each spectrum were retained, which accounted for over 99% variance in the original spectra data. A Wilcoxon rank-sum test [254] was applied to identify a subset of the PCs scores that show statistically significant differences ($p < 0.05$) between the non-viable and viable groups, which would be

diagnostically important. This subset of PCs scores up to a range of early time points were then fed into a linear discriminant analysis [255] (LDA) classifier to predict skin viability at the end of 72 hours. A leave-one-out cross validation method [256] was used in the analysis to obtain an unbiased estimate of the prediction accuracy. The reason why we use the PCs scores instead of the raw spectra for prediction purpose is that not every spectral information contains useful diagnostic information, the PLS and Wilcoxon rank-sum test would help us to find out the data contain the most meaningful diagnostic information which could provide higher accuracy. The non-viable group was treated as positive case while the viable group was treated as negative case in the calculation of sensitivity and specificity. The overall accuracy, sensitivity and specificity of prediction using diffuse reflectance spectra alone, autofluorescence spectra alone and their combination were compared.

Measured diffuse reflectance spectra were used to estimate the indicators of total hemoglobin concentration (THB) and hemoglobin oxygen saturation (StO₂) as previously described [257]. THB was derived from autofluorescence spectra using a ratio metric method [48]. In addition, the indicator of redox ratio was also estimated from autofluorescence spectra. The reduction-oxidation (redox) ratio in tissues is an important metabolic biomarker which has been widely used for tissue characterization. It is indicative of the redox state and reflects cellular metabolism and oxygen consumption of the tissue. The redox ratio measurement typically involves the autofluorescence intensities of two major fluorophores in tissues, i.e. reduced nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FAD). It is commonly defined by the following equation [49],

$$redox \text{ ratio} = \frac{[FAD]}{[FAD] + [NADH]}$$

where the [FAD] and [NADH] represent the concentrations of FAD and NADH respectively. In this report, the measured autofluorescence intensity around 530 nm and 470 nm were used to represent the FAD and NAD concentrations since these two wavelengths corresponded to the emission peaks of FAD and NADH respectively.

The trends of these four derived parameters over time were shown in table 8.2 to table 8.5 and Figure. 8.3 to Figure.8-6 respectively. Every data point shown in the tables or figures has been divided by the baseline value in the same set that was measured shortly prior to the surgery. Although the four parameters were reported as relative to the baseline value instead of absolute concentrations, it is sufficient to demonstrate the trend over time. The p-values shown in the tables were obtained by conducting a t-test on the data for the non-viable and viable groups.

8.3 Results

8.3.1 Flap viability

Using this model, an area approximately half the length of the flap (furthest from the pedicle) always became necrotic by 72 hours. Figure.8-2 shows a typical skin flap created in this study at different time points. The area of necrosis is identified by the black eschar. A total of 62 flap measurement points from eleven Sprague-Dawley rats were assessed for 72 hours. Among these points, 31 were deemed non-viable and 31 deemed viable after 72 hours.

Figure 8- 2 A typical flap shown at 15 minutes, 4 hours, 12 hours and 72 hours post flap elevation. Spectroscopic measurement sites are indicated by blue circles on the flap. The non-viable skin at the cranial half of the flap becomes black and dark after 72 hours.

8.3.2 Classification accuracy obtained by PLS regression

Table 8-1 shows the accuracies calculated from LDA classification between non-viable and viable groups using those PCs scores obtained by PLS regression and Wilcoxon rank-sum test that demonstrated statistically significant differences with a p-value of 0.05. The overall classification accuracy when using diffuse reflectance spectra alone was increased with time and always higher than 90%. The accuracy when using autofluorescence spectra alone was always higher than 92 %. It was found that the accuracy when using autofluorescence spectra alone reached a maximum of 97.6% in just 2 hours after flap elevation. In terms of sensitivity, autofluorescence spectroscopy always provided slightly better or equal accuracy in the first 12 hours. In terms of specificity, autofluorescence spectroscopy provided significantly better accuracy in first 3 hours and equal accuracy from 4 hours to 12 hours.

Table 8- 1 The overall classification accuracy, sensitivity and specificity

Time (h)	0.25	1	2	3	4	8	12
Overall accuracy (%)							
R	90.3	90.2	90.2	92.7	93.5	95.2	98.4
F	95.2	92.7	97.6	97.6	95.2	95.2	96.8
R+F	98.4	92.7	97.6	95.1	95.2	95.2	90.2
Sensitivity (%)							
R	93.5	95.7	91.3	95.7	93.5	96.8	96.8
F	100	95.7	95.7	95.7	96.8	96.8	96.8
R+F	100	95.7	95.7	91.3	96.8	93.5	90.3
Specificity (%)							
R	87.1	83.3	88.9	88.9	93.5	93.5	100
F	90.3	88.9	100	100	93.5	93.5	96.8
R+F	96.8	88.9	100	100	93.5	96.8	90.3

Legend: “Time” = number of hours after flap elevation; “R” = diffuse reflectance data alone; “F” = autofluorescence data alone; “R+F” = both diffuse reflectance and autofluorescence data.

8.3.3 Oxygen saturation indicator

The mean oxygen saturation indicator as derived from reflectance spectra for the non-viable group decreased to less than 32.3% of the baseline value within the first hour of ischemia and remained low. In contrast, the mean oxygen saturation for the viable points decreased to no less than 61.2% of the baseline value within the first hour and then stabilized as shown in Table 8-2 and Figure.8-3.

Table 8- 2 Normalized oxygen saturation indicators derived from reflectance spectra

Time (h)	0	0.25	1	2	3	4	8	12
Mean $\pm$ Standard Deviation of normalized oxygen saturation indicator								
Non-viable	1.0 $\pm$ 0.0	0.45 $\pm$ 0.1	0.32 $\pm$ 0.1	0.29 $\pm$ 0.1	0.23 $\pm$ 0.1	0.29 $\pm$ 0.1	0.22 $\pm$ 0.1	0.24 $\pm$ 0.2
Viable	1.0 $\pm$ 0.0	0.75 $\pm$ 0.2	0.75 $\pm$ 0.3	0.69 $\pm$ 0.3	0.61 $\pm$ 0.3	0.61 $\pm$ 0.3	0.67 $\pm$ 0.3	0.69 $\pm$ 0.2
p-value	NA	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Legend: “NA” = “not available”

Figure 8- 3 StO₂ indicator derived from diffuse reflectance spectra over time from flap elevation.

8.3.4 Total hemoglobin concentration indicator

Mean THB from reflectance spectra for the non-viable group increased gradually after flap elevation. In contrast, mean THB for the viable skin group plateaued at around 1.5 as shown in Table 8-3 and Figure.8-4.

Table 8- 3 Normalized THB indicators derived from reflectance spectra

Time (h)	0	0.25	1	2	3	4	8	12
Mean $\pm$ Standard Deviation of normalized THB indicator								
Non-viable	1.0 $\pm$ 0.0	1.89 $\pm$ 0.6	1.93 $\pm$ 0.8	2.23 $\pm$ 1.0	2.39 $\pm$ 1.1	2.82 $\pm$ 1.4	4.46 $\pm$ 2.9	5.61 $\pm$ 3.8
Viable	1.0 $\pm$ 0.0	1.45 $\pm$ 0.5	1.62 $\pm$ 0.8	1.45 $\pm$ 0.5	1.50 $\pm$ 0.5	1.44 $\pm$ 0.6	1.47 $\pm$ 0.8	1.46 $\pm$ 0.7
p-value	NA	<0.01	>0.05	<0.01	<0.01	<0.01	<0.01	<0.01

Legend: "NA" = "not available"

Figure 8- 4 THB indicator derived from diffuse reflectance spectra over time from flap elevation.

The results obtained from autofluorescence spectra were consistent with those obtained from reflectance spectra; see Table 8-4 and Figure.8-5.

Table 8- 4 Normalized THB indicators derived from autofluorescence spectra

Time (h)	0	0.25	1	2	3	4	8	12
Mean $\pm$ Standard Deviation of normalized THB indicator								
Non-viable	1.0 $\pm$ 0.0	1.34 $\pm$ 0.1	1.43 $\pm$ 0.2	1.46 $\pm$ 0.1	1.48 $\pm$ 0.2	1.42 $\pm$ 0.3	1.60 $\pm$ 0.4	1.64 $\pm$ 0.5
Viable	1.0 $\pm$ 0.0	1.23 $\pm$ 0.2	1.24 $\pm$ 0.2	1.23 $\pm$ 0.2	1.21 $\pm$ 0.1	1.19 $\pm$ 0.1	1.16 $\pm$ 0.2	1.16 $\pm$ 0.2
p-value	NA	<0.01	<0.01	<0.01	<0.05	<0.01	<0.01	<0.01

Legend: "NA" = "not available"

Figure 8- 5 THB indicator derived from autofluorescence spectra over time from flap elevation.

8.3.5 Redox ratio indicator

The mean redox ratio value of the non-viable group decreased to 76.7% in the first hour and kept decreasing. In contrast, the mean redox ratio value for the viable group decreased to 88.0% in the first hour but then normalized to the baseline level by 12 hours. See Table 8-5 and Figure.8-6.

Table 8- 5 Normalized redox ratio indicators derived from autofluorescence spectra

Time (h)	0	0.25	1	2	3	4	8	12
Mean $\pm$ Standard Deviation of normalized redox ratio indicator								
Non-viable	1.0 $\pm$ 0.0	0.81 $\pm$ 0.1	0.77 $\pm$ 0.1	0.75 $\pm$ 0.1	0.75 $\pm$ 0.2	0.72 $\pm$ 0.1	0.70 $\pm$ 0.2	0.64 $\pm$ 0.2
Viable	1.0 $\pm$ 0.0	0.92 $\pm$ 0.2	0.88 $\pm$ 0.1	0.90 $\pm$ 0.1	0.90 $\pm$ 0.1	0.91 $\pm$ 0.1	0.97 $\pm$ 0.2	1.00 $\pm$ 0.2
p-value	NA	<0.01	<0.05	<0.01	<0.01	<0.01	<0.01	<0.01

Legend: "NA" = "not available"

Figure 8- 6 Redox ratio indicator derived from autofluorescence spectra over time from flap elevation.

8.4 Discussions

Successful reconstructive surgery requires vigilant postoperative monitoring that accurately detects early disturbances in tissue perfusion. An ideal monitor would allow continuous monitoring of tissue metabolism, allow rapid and accurate detection of irregularities in perfusion, and be minimally invasive.

In this pilot study we have found it is feasible to predict flap failures in the first two hours when using autofluorescence spectroscopy alone; moreover, it is possible to predict flap failures even in the first 15 minutes with high accuracy when using diffuse reflectance and autofluorescence spectroscopy simultaneously. This technology has great potential for clinical use in flap monitoring or intra-operatively to determine tissue viability. Although flap failures usually occur within the first 72 hours after surgery, if and when it occurs is anyone's guess. In order to salvage a failing flap, it needs to be identified in a timely manner so that the disturbance in perfusion can be corrected with as little ischaemic injury to the flap as possible. This technology has the potential to provide almost continuous monitoring of skin cellular metabolism and alert the surgeon within 15 mins of a threat to flap viability.

From Figure.8-3 to Figure.8-6, clear trends in the indicators of StO₂, THB and redox ratio with time were observed after flap elevation. The trend of StO₂ was consistent with previous findings [9, 27, 29]. Increases in THB are most likely due to venous congestion, and when the venous outflow is inadequate it can lead to eventual skin necrosis [9]. The measuring of redox ratio using autofluorescence spectroscopy to predict skin viability has not been reported previously, but it would seemingly hold great potential. In addition to its accuracy, it can non-invasively provide an indication of *in vivo* cellular metabolism without requiring administration of exogenous fluorophores and measurements can be repeated frequently with no morbidity to the patient. Inadequate oxygen supply, which is a common consequence in ischemia, would cause a slower oxidation and in turn a decrease in redox ratio in autofluorescence spectroscopy [258, 259]. Our findings in the trends of redox ratio for flaps shown in Figure. 6 are consistent with several published reports [32, 260].

Diffuse reflectance spectroscopy typically is a lower cost system compared to autofluorescence spectroscopy, however, autofluorescence spectroscopy offers the advantage of earlier detection of tissue ischemia, it would be preferred for early diagnosis in a non-contact setup [101]. The preference of one or the other technique depends on the tradeoff between the cost and performance. Diffuse reflectance spectroscopy alone at a low cost provides a decent overall accuracy for early assessment of skin viability in the first 2 to 3 hours and an excellent overall accuracy within 12 hours. Autofluorescence spectroscopy alone, at a relatively high cost, offers an excellent overall accuracy even in the first 2 hours. The combination of both techniques yields an excellent overall accuracy even in the first 15 minutes.

At this time, there is no single commercially available technology that has widespread use in flap monitoring and clinical monitoring is still considered the gold

standard. The laser Doppler flowmetry is one non-invasive technique that has shown promise[6]. In an animal study involving islanded flaps in pigs, Jia et al. [261]found that laser Doppler scanning could predict skin viability with an accuracy of 91.3%. However, improved accuracy has been achieved when combined with tissue spectroscopy to measure oxygen saturation [23]. Laser Doppler flowmetry requires careful interpretation [26]even though it can only measure the blood flow, which is not inadequate for flap assessment. Moreover, it cannot distinguish the venous and arterial occlusion [6]which is very important in flap monitoring. In contrast, our proposed technique would overcome most of the limitations associated with laser Doppler and give considerable higher accuracy. For the point measurement system used in this report, the area we can measure each time is around 1 mm as shown in Figure.8-1. It should be noted that the point measurement system may not be a perfect choice for clinical use due to the low resolution and slow speed in case of a large tissue region to be examined. We are currently developing the technique in an imaging setup that would have a much larger field of view (around multiple cm² or even larger) and much higher spatial resolution, which is more suitable for clinical use.

In summary, we performed both visible diffuse reflectance and autofluorescence measurements on a reverse MacFarlane rat dorsal skin flap model to identify their respective values in the early assessment of skin flap viability. Our results show that fluorescence spectroscopy is more sensitive to flap changes in the first a couple of hours when a fiber-optic probe with small source-detector separation was used. These findings demonstrated the great potential of fluorescence spectroscopy in the early assessment of skin flap viability in plastic surgery when high-resolution imaging is desired. In contrast, diffuse reflectance spectroscopy is less sensitive to flap changes in the first a couple of hours compared to fluorescence spectroscopy when a fiber-

optic probe with small source-detector separation was used. In short, the advantages of diffuse reflectance spectroscopy lie in its low cost and decent performance in early assessment; in contrast, the advantages of fluorescence spectroscopy include its excellent performance and readiness for imaging with high spatial resolution. A tradeoff has to be made between the cost and performance when only one of these two techniques can be used in early assessment. In this study, the combination of two do not yield considerably better performance than fluorescence spectroscopy alone except in the first 15 minutes, which needs to be further confirmed in a systematic study.

Chapter 9: Conclusions and Future directions

9.1 Conclusions

This dissertation presents a series of studies in the development of contact and non-contact optical measurement techniques, and MC based data analysis methods in visible diffuse reflectance and auto-fluorescence spectroscopy and imaging for tissue characterization. Their potential applications in early epithelial cancer diagnosis and tissue viability prediction have been demonstrated in both phantom and animal studies. The main conclusions are highlighted as follows.

First, we provided a comprehensive background in epithelial cancer and flap tissue viability as well as the principles/state of art of optical spectroscopy and imaging in tissue optics. And then we gave a survey on the MC modeling of light transport in tissues, especially in the methods for speeding up MC simulations in Chapter 2. Chapter 3 introduced a series of numerical studies to provide the guidelines for the selection of a proper epithelial cancer model. Based on previous Chapters, we proposed a hybrid method to accelerate the simulation of diffuse reflectance measurements from a layered tissue model with embedded objects. The validation study show that the hybrid method is suitable for simulating diffuse reflectance spectra or creating a MC database to extract optical properties of an early epithelial cancer model. After Chapter 4, we paid more attention on methods for non-contact optical measurements. Chapter 5 introduced a novel MC method for simulation of lens based non-contact optical setups. Depth sensitive diffuse reflectance and fluorescence measurements have been demonstrated on the early epithelial cancer model numerically and experimentally in Chapter 5 and 6. In Chapter 7, the lens

based non-contact point measurement system was expanded into an imaging setup with a larger field of view to perform depth sensitive spectral imaging on an epithelial cancer phantom. Meanwhile, a dual-modal optical system capable of performing both UV-visible diffuse reflectance and auto-fluorescence spectroscopy was developed in Chapter 8 for tissue viability prediction in an animal model. The results showed that it was feasible to predict flap failures in the first two hours when using auto-fluorescence spectroscopy alone; while it was possible to predict flap failures even in the first 15 minutes with high accuracy when using diffuse reflectance and auto-fluorescence spectroscopy simultaneously.

Since the entire research theme shown in this thesis is application-oriented, the feasibility of each category of techniques for clinical applications is discussed as following.

A contact setup as shown in the Chapter 8 in this thesis usually includes a light source, a spectrometer, a fiber-optic probe, and a laptop. The entire setup could be very compact thus it has high portability and is convenient for clinical uses. Meanwhile, the cost for such a point system is relative low thus it has great possibility for commercialization. However, one limitation of such a contact setup is the effect of pressure caused by the inconsistent probe-tissue contact, thus the precise control on the pressure is a necessary concern for the setup design. Another limitation for the point system is the slow scanning speed for a large tissue sample. One potential solution is the use of multi-channel fiber-optic probes [262]; however the use of such a multi-channel probe and the associated system will significantly increase the cost of the system.

Non-contact imaging setups could solve the two limitations associated with the contact setup as discussed above. However, the cost for a non-contact spectral

imaging setup is usually higher than a contact setup because of the requirement of a light source with a high power, a tunable filter or equivalent, and a 2D detector array such as a CCD camera to capture images from the sample. The entire system could be bulky thus it has a relative lower portability. A new approach involving wide-band imaging and spectral reconstruction [263] may help solve this problem to improve the portability and reduce the cost, which may pave a way to implement spectral imaging in clinical settings due to its advantages as discussed in the thesis.

9.2 Future directions

In this series of study, we mainly focused on the applications of contact and non-contact optical techniques in early epithelial cancer diagnosis and skin tissue viability prediction. The future directions described next are also about these two applications.

MC modelling has been proven to be a great tool for simulating light transport in turbid media. In our studies, we mainly focused on the forward problem, which is the simulation of diffuse reflectance or fluorescence measurements from epithelial tumor models with given optical properties. However, MC method could also be useful in the inverse problem, which is the extraction of optical properties from diffuse reflectance or fluorescence measurement from a tissue sample. The extraction of epithelial tissue optical properties could provide more quantitative parameters about the tissue samples than spectra alone. The major drawback of MC method is its slow speed. We have developed a hybrid method to speed up the simulations of diffuse reflectance from early epithelial cancer model; however the method for fast simulation of fluorescence is still needed. It is worth exploring new MC method to speed up fluorescence simulation from an early epithelial cancer model and these new

MC methods can be further incorporated within the inverse algorithm for the extraction of optical properties of early epithelial cancer tissues.

We developed a depth sensitive color imaging system for the diagnosis of early epithelial cancer. The setup was evaluated on a two layered tissue phantom, while more work need to be done to push this technique into clinical use. Firstly, the system specifications including the field of view and the imaging speed need to be optimized according to the need of clinical application. A fast data processing method will also need to be developed prior to any clinical study.

In our study, we have tested the diffuse reflectance and auto-fluorescence spectroscopy for early prediction of skin flap viability. The study showed that it was feasible to predict flap failures in the first two hours when using auto-fluorescence spectroscopy alone; while it was possible to predict flap failures even in the first 15 minutes with high accuracy when using diffuse reflectance and auto-fluorescence spectroscopy simultaneously. However, the optical measurement in this study can only be performed point by point, which could be very time consuming if the map information of large field of view is needed. This point measurement system needs to be further developed to an imaging system to fill the gap. In the future work, we aim to develop a spectral imaging setup for the assessment of tissue viability in flap surgery.

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