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# Effects of Spatial and Spectral Frequencies on Wide-field Functional Imaging (WiFI) Characterization of Preclinical Breast Cancer Models

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## ABSTRACT

A common strategy to study breast cancer is the use of the preclinical model. These models provide a physiologically relevant and controlled environment in which to study both response to novel treatments and the biology of the cancer. Preclinical models, including the spontaneous tumor model and mammary window chamber model, are very amenable to optical imaging and to this end, we have developed a wide-field functional imaging (WiFI) instrument that is perfectly suited to studying tumor metabolism in preclinical models. WiFI combines two optical imaging modalities, spatial frequency domain imaging (SFDI) and laser speckle imaging (LSI). Our current WiFI imaging protocol consists of multispectral imaging in the near infrared (650-980 nm) spectrum, over a wide (7 cm x 5 cm) field of view. Using SFDI, the spatially-resolved reflectance of sinusoidal patterns projected onto the tissue is assessed, and optical properties of the tissue are determined, which are then used to extract tissue chromophore concentrations in the form of oxy-, deoxy-, and total hemoglobin concentrations, and percentage of lipid and water. In the current study, we employ Monte Carlo simulations of SFDI light propagation in order to characterize the penetration depth of light in both the spontaneous tumor model and mammary window chamber model. Preliminary results suggest that different spatial frequency and wavelength combinations have different penetration depths, suggesting the potential depth sectioning capability of the SFDI component of WiFI.

**Keywords:** Tissue optics, speckle, cancer, preclinical, Monte Carlo, computational model, laser Doppler perfusion imaging

## 1. INTRODUCTION

Breast cancer is the second most common cancer worldwide and the most common cancer specific to women. Worldwide, there were an estimated 1.29 million cases of breast cancer in 2008<sup>1</sup>. In 2009, an estimated 192,370 new cases of invasive breast cancer will be diagnosed in the United States and an estimated 40,610 American women will die from the disease<sup>1</sup>. Over the course of their lifetime, American women have a roughly 1 in 8 chance of being diagnosed with breast cancer. It is clear that breast cancer is a major health issue both in the United States and worldwide and as a result, much effort has been put into studying breast cancer.

The use of the preclinical model is an invaluable tool in the study of breast cancer. These models are significant since they provide a physiologically relevant model with which to test and study the components of breast cancer ranging from the molecular to the tissue level. Preclinical models also provide a well controlled system that can be customized to the study. Additionally, preclinical models are an avenue in which to study the effects of novel therapies and combination therapies that are have not yet reached trials in the clinic. Preclinical models could potentially be very useful in determining customized treatment for an individual. The mouse is the preclinical model of choice due to its well known and studied genome, ease of breeding and maintenance, and physiological parallels to humans.

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Two notable preclinical models are the transgenic spontaneous tumor model<sup>4</sup> and the mammary window chamber model<sup>5</sup>. The transgenic spontaneous tumor model typically has one or multiple knockout genes that, due to the loss of function mutation, will lead to the development of a tumor. The tumors will then develop subcutaneously and in the desired tissue. By contrast, the mammary window chamber model is a variant of other window models, most notably the dorsal and cranial window models. A typical window model involves removal of the skin and/or other tissues in order to directly expose the tissue to be interrogated and often times a metallic or plastic frame to keep the incision open. The incision is then covered with a glass or plastic window that is sutured to the skin. This model allows for direct visualization of the underlying tissue and these models have been frequently used to study the microvasculature.

We postulate that both preclinical models are amenable to study with our wide-field functional imaging (WiFI) system. The WiFI system consists of two components: spatial frequency domain imaging (SFDI) and laser speckle imaging (LSI). SFDI provides information about the biochemical composition of the tissue in question while LSI provides information about blood perfusion to the tissue. Previously, we used WiFI to monitor changes in tumor hemodynamics during application of a hyperoxic respiratory challenge on a spontaneous tumor model<sup>4</sup>. While we were able to successfully monitor tumor hemodynamics, we were unsure of what tissue depth the optical information we acquired was originating from.

To this end, the current study reports on the use of Monte Carlo simulations of light propagation to get a first-order approximation of the depth of tissue being probed in both the spontaneous and mammary window chamber tumor models.

## **2. MATERIALS AND METHODS**

### **2.1 Instrumentation**

#### **2.1.1 Spatial frequency domain imaging**

SFDI makes use of spatially varying patterns (i.e. sinusoidal) of broadband light that are projected onto the tissue. These patterns are projected at different intervals, or spatial frequencies<sup>2,3</sup>. Each of these spatial frequencies is then shifted by a certain spatial offset, or phase. Depending on the spatial frequency, different depths of tissue can be interrogated; low frequency patterns probe deeper into the tissue (maximum of 5 mm) while high frequency patterns probe shallower depths of tissue. Near IR reflectance images of the tissue are then acquired from the light projected at each of the different frequency and phase combinations onto the tissue using a camera equipped with a tunable liquid crystal filter set for the NIR regime. Optical properties (absorption and reduced scattering) of the tissue can be determined by demodulating the various frequency/phase images. From these optical properties, tissue chromophore concentrations can be calculated using a diffusion-based model of light propagation in tissue<sup>2,3</sup>. The tissue chromophores include oxyhemoglobin (HbO<sub>2</sub>), deoxyhemoglobin (Hb), total hemoglobin (THb), lipid, and water. Based on HbO<sub>2</sub> and Hb values, we compute oxygen saturation (S<sub>t</sub>O<sub>2</sub>).

### **2.2 Animal models**

#### **2.2.1 Spontaneous animal model**

The basis of the spontaneous animal model geometry used in this study is the p53/Brcal knockout model<sup>4</sup> developed in Dr. Eva Lee's lab. Breast tumors develop spontaneously at subcutaneous depths ranging between 1 and 10 mm.

#### **2.2.2 Mammary window chamber model**

The basis of the mammary window model geometry used in the Monte Carlo simulations is the mammary window chamber model<sup>5</sup> preparation. This model facilitates an appropriate tumor microenvironment for breast tumor development and ease with which to perform optical imaging. Briefly, this model first involves removal of the skin overlying the mammary tissue and subsequent implantation of tumor cells into the underlying mammary tissue. A circular acrylic window is then sutured over the incision, sealing the environment. Advantages of the mammary window chamber model include a well-defined day 0 of tumor development and precise control of the tumor location.

Table 1: Summary of animal models

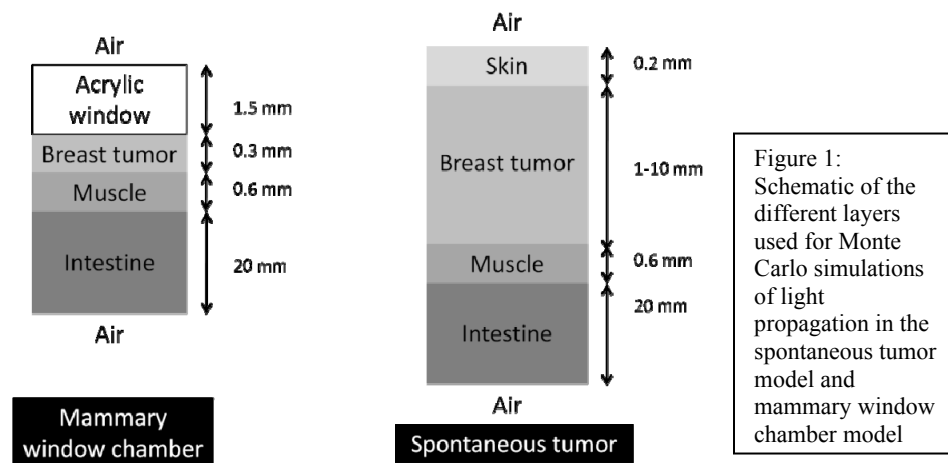
	<b>Spontaneous Model</b>	<b>Mammary Window Chamber</b>
<b>Tumor</b>	Spontaneous	Orthotopic
<b>Overlying skin?</b>	Yes	No
<b>Physiological microenvironment?</b>	Sometimes	Yes
<b>Biologically relevant model?</b>	Yes (p53/BRCA1)	Yes
<b>Direct visualization?</b>	No	Yes
<b>Conduct early stage tumor studies?</b>	No	Yes
<b>Controllable model?</b>	No	Yes

### 2.3 Computational model

A multi-layer, Monte Carlo simulation code developed by the Virtual Photonics Core at the Beckman Laser Institute was used for this study. This code uses a forward model approach to solve the radiative transport equation using Monte Carlo methods. Parameters of the simulations are given in Table 2. A schematic of the model geometries used in the Monte Carlo simulations for both the spontaneous tumor model and mammary window chamber models are shown in Figure 1.

Table 2: Summary of Monte Carlo model parameters

<b>Layers</b>	4
<b>Source</b>	Pencil beam with axisymmetric geometry
<b>Wavelengths</b>	758 nm, 800 nm, 978 nm
<b>Number of photons</b>	$10^6$
<b>Optical properties</b>	Published literature, unpublished diffuse optical spectroscopy measurements



### 3. DATA

Plots of the fluence of different projected spatial frequencies in each of the model geometries at different wavelengths were generated. During modeling of the spontaneous tumor, the size of the tumor was varied from 1-10 mm. Additionally, fluence plots at each spatial frequency were generated at different wavelengths. Each line in the plot corresponds to a specific spatial frequency and plots were shaded to highlight the fluence values in a specific region of the model corresponding to a specific tissue type. Below is a representative plot of the fluence versus tissue depth of 785 nm illumination of four different spatial frequencies in a 1 mm spontaneous tumor.

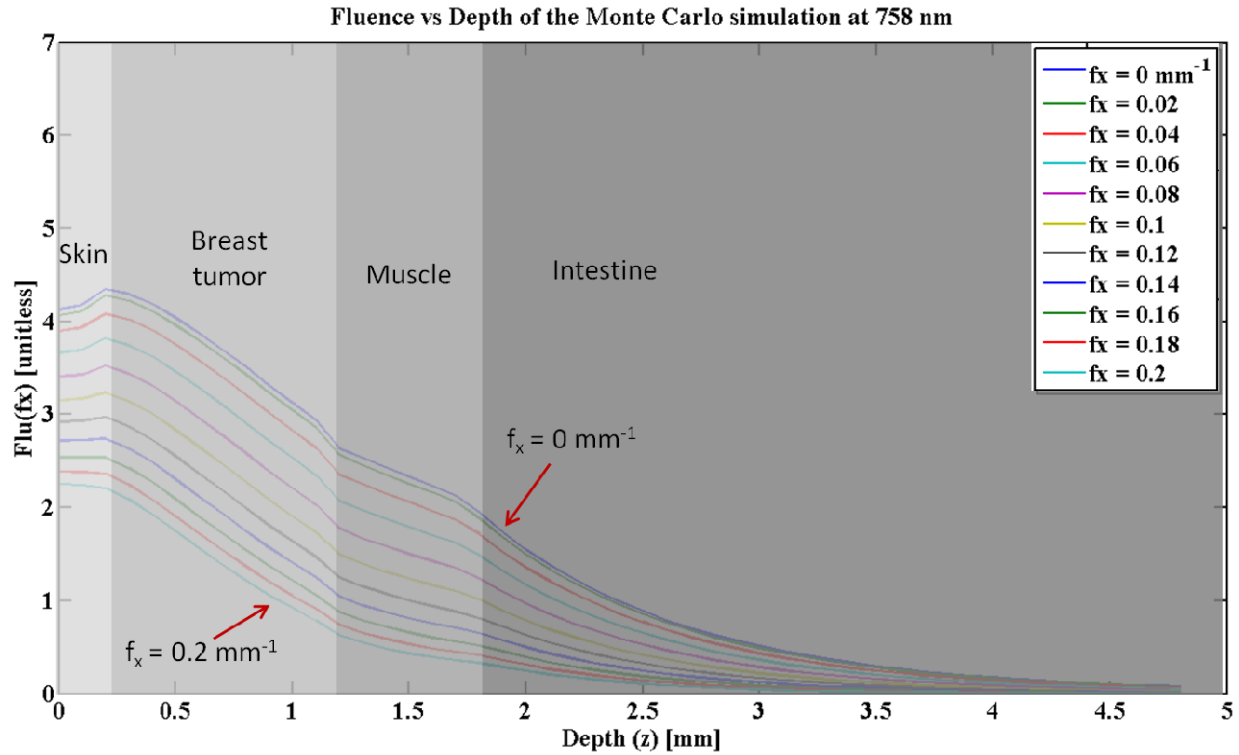


Figure 2: Representative plot of fluence, as a function of spatial frequency, versus penetration depth (mm)

Using these plots, the penetration depth, defined as the depth where the fluence was equal to 37% of the maximum fluence, was determined. The following table summarizes the estimated penetration depth of selected wavelengths of light into the mammary window chamber model, a 1 mm spontaneous tumor model, and a 10 mm spontaneous tumor model.

Table 3: Summary of spatial frequency penetration depth of 758 nm, 800 nm, and 978 nm wavelengths

Wavelength (nm)	Spatial frequency (mm <sup>-1</sup> )	Mammary Window Chamber (tumor)	Spontaneous Tumor (1 mm)	Spontaneous Tumor (10 mm)
<b>758</b>	0	1.6 mm	2 mm	> 5 mm
	0.06	1.6 mm	1.8 mm	2.6 mm
	0.14	1.4 mm	1.2 mm	1.3 mm
	0.2	1.3 mm	1 - 1.1 mm	0.9 - 1 mm
<b>800</b>	0	1.6 mm	1.7 mm	> 5 mm
	0.06	1.6 mm	1.6 mm	2.6 mm
	0.14	1.4 mm	1 mm	1.3 mm
	0.2	1.3 mm	0.9 mm	1 mm
<b>978</b>	0	1.5 mm	1.7 mm	3.8 mm
	0.06	1.5 mm	1.6 mm	2.4 mm
	0.14	1.4 mm	1.1 mm	1.4 mm
	0.2	1.3 mm	0.9 - 1 mm	1.1 mm

#### 4. DISCUSSION

In general, the simulation results agree with expected results and previously acquired empirical data. Mammary window chamber geometry results are almost identical for the different spatial frequencies at the three wavelengths. Of note is that the data presented here are absolute depths, which means that in the case of the mammary window chamber model, there will be a contribution from the tissue layers underlying the breast tissue (i.e. muscle and intestine) to the detected tissue signal.

In the case of the spontaneous tumor geometry, the simulations suggest wavelength dependence, but only for wavelengths relatively far from each other, and tumor size dependence. The results from the 758 nm and 800 nm simulations do not differ significantly, but the data taken at 978 nm is different at the 0 spatial frequency. Additionally, at high spatial frequencies, while the data suggests a superficial depth penetration in both the 1 mm and 10 mm spontaneous models, at lower spatial frequencies there is a clear difference in the depth of tissue being interrogated. This result suggests that we can use different spatial frequency and wavelength combinations to probe different depths of the tissue, which further suggests that WiFi has potential in vivo depth sectioning capability. More simulation studies are required to accurately characterize the depth sectioning potential of WiFi and to further elucidate the light-tissue interactions of WiFi.

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