Development of near infrared diffuse optics-based flowmetry for non-invasive deep tissue perfusion assessment

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**DEVELOPMENT OF NEAR INFRARED DIFFUSE
OPTICS-BASED FLOWMETRY FOR
NON-INVASIVE DEEP TISSUE PERFUSION
ASSESSMENT**

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SUMMARY

The continuous running of blood in the cardiovascular system is very important since it carries oxygen as well as other nutrition to support the metabolic activity of the other organs of the body. Consequently, the monitoring of blood flow becomes critical in clinical cases. For the past decades, plenty of well-known and notable methodology had been developed for non-invasive measurement of blood flow. For example, laser Doppler flowmetry and laser speckle flowmetry are developed for shallow blood flow measurement, and diffuse correlation spectroscopy (DCS) has been recognized as a standard technique for deep tissue perfusion monitoring. In DCS, the autocorrelation of speckle fluctuations of the scattered light from a coherent source is measured to obtain the information of the motion of red blood cells. So far, instrument for measuring relative blood flow in deep tissue has been already built, involving usage of hardware autocorrelator and other optical components. However, on one hand, there was no real-time device available for non-invasive deep tissue perfusion assessment; on the other hand, the expense was always the issue along with the increasing number of channel especially when applying the technology to clinical settings. Therefore, this project is focused towards development of near infrared diffuse optics-based flowmetry for non-invasively assessing deep tissue blood perfusion. The capability of non-invasively assessing blood flow information in deep tissue in real-time will benefit the diagnosis as well as medical treatment directly.

The series of studies in this thesis has unfolded as follows. It initially began with a motivation of building a flexible software autocorrelator-based single channel real-time diffuse correlation spectroscopy system. The system was then utilized in monitoring the tumor response during photodynamic therapy treatment in animal model. As real-time DCS system was able to record the raw photon count signal, complexity measures such as sample entropy analysis was subsequently used on this raw signal as a possible alternative of autocorrelation-based DCS. Furthermore, with the help of laser speckle contrast imaging (LSCI) we explored the diffuse speckle contrast analysis for deep tissue blood flow measurement which has great potential for multi-channel system. More detailed description of each study is following.

Firstly, in order to balance the cost and performance, the software autocorrelator-based diffuse correlation spectroscopy system was developed using Fast Fourier Transform (FFT), followed by applying the system to monitor the tumor response in photodynamic therapy (PDT) that was conducted on mice model. Instrumentation-wise, besides the basic optical components, only a reasonably fast counter is sufficient so that the cost can be reduced. The whole system, being controlled by LabVIEW, can be operated in real-time to obtain the relative blood flow (*rBF*). In addition, as the raw photon count signal is recorded, it provides more room to explore other methods to obtain blood perfusion information in deep tissue.

Sample entropy (SampEn), which gains increasing popularity in physiological time series, is a novel approach we applied to the photon count signals that are obtained from software autocorrelator based DCS system. It has been well known that the complexity of the system can be characterized by entropy. Since the detected photons which undergo scattering events in the sample carry the information of moving scatterers, this project applied sample entropy analysis to diffuse reflectance signals. To our knowledge, this is the first time to correlate the sample entropy to tissue blood perfusion information. Compared to DCS, SampEn is simple in concept because it is not based on model fitting which is commonly utilized in DCS. Moreover, SampEn holds the merits of being computationally light weight, thus it is also suitable for real-time analysis. As an alternative approach, this model-free method for quantifying the relative flow will be useful in clinical setting where real-time flow assessment is necessary.

In an effort to exploit deep tissue flowmetry with multiple channels based on the concept of LSCI, a CCD-based diffuse speckle contrast analysis (DSCA) method was ingeniously developed. This method not only alleviates the requirement of instrumentation, but also paves the way for depth-sensitive blood flow measurement in the future work.

GLOSSARY

α	fraction of dynamic photon scattering events in medium
g	scattering anisotropy factor
μ_a	absorption coefficient
μ_s	scattering coefficient
μ_s'	reduced scattering coefficient
v	speed of light in tissue
G_0	homogeneous Green's function
l_{tr}	transport mean-free path
$D(r)$	photon diffusion coefficient
D_b	Brownian diffusion coefficient
ε	extinction coefficient
λ	wavelength of light (nm)
ω	laser/source modulation frequency
$L(r, \hat{\Omega}, t)$	light intensity traveling in the $\hat{\Omega}$ direction at position r and time t
$Q(r, \hat{\Omega}, t)$	power per volume emitted by sources in the $\hat{\Omega}$ direction at position r and time t
$f(\hat{\Omega}, \hat{\Omega}')$	normalized differential cross-section for single light scattering events in the medium
$\phi(r, t)$	photon fluence rate at position r and time t
$J(r, t)$	photon flux
$E(r, t)$	total light electric field at position r and time t
$I(r, t)$	light field intensity at position r and time t
$G_1(r, t)$	electric field temporal auto-correlation function
$G_2(r, t)$	intensity auto-correlation function
$g_1(r, t)$	normalized electric field temporal auto-correlation function

$g_2(r, t)$	normalized intensity auto-correlation function
$\langle \Delta r^2(\tau) \rangle$	mean-square displacement in time τ of the scattering particles
ASL-MRI	arterial spin labeling MRI
ApEn	approximate entropy
APD	avalanche photodiode
BFI	blood flow index
Ce6	chlorin e6
CW	continuous wave
DCE-MRI	dynamic contrast enhanced MRI
DCS	diffuse correlation spectroscopy
DOS	diffuse optical spectroscopy
DOT	diffuse optical tomography
DRS	diffuse reflectance spectroscopy
DPF	differential pathlength factor
DPDW	diffuse photon density wave
DSCA	diffuse speckle contrast analysis
FD	frequency domain
HbO ₂	oxy-hemoglobin concentration
Hb	deoxy-hemoglobin concentration
LSCI	laser speckle contrast imaging
MRI	magnetic resonance imaging
OD	optical density
PDT	photo dynamic therapy
PET	positron emission tomography
PMT	photomultiplier tube
RBCs	red blood cells
RF	radio frequency
RTE	radiation transport equation
rBF	relative blood flow
StO ₂	tissue blood oxygen saturation

SPECT	single photon emission computed tomography
SampEn	sample entropy
THC	total hemoglobin concentration
TMRO ₂	tumor metabolic rate of oxygen
TD	time domain
TPSF	temporal point spread function
TTL	transistor-transistor logic
XeCT	xenon-enhanced computed tomography

Chapter 1 Introduction

Near-infrared diffuse optics is an emerging functional technology concerned with non-invasively monitoring the changes in the state of biological tissues using light in the range of 600 nm to 900 nm. According to the object it detected, diffuse optics can be categorized into “static” and “dynamic” regimes. For the “static” case, diffuse optics was primarily focused on the measurement of slow variations of absorption and scattering of a sample, and techniques such as diffuse optical spectroscopy (DOS) and diffuse optical tomography (DOT) which provide information of oxy- and deoxy-hemoglobin concentrations in 2D or 3D respectively were based on the measurements of absorption and scattering coefficient. On the other hand, diffuse optics was also utilized to probe the motions of scatterers and this is the so-called “dynamic” regime. Particularly in the case of tissue, red blood cells (RBCs) are the critical moving scatterers. To date, the main technique has been developed to detect the motion of RBCs in deep tissue is diffuse correlation spectroscopy (DCS), which provides relative blood flow (rBF) via measuring the correlation of speckle fluctuations of the scattered light from coherent sources.

During the last several decades, near-infrared diffuse optics has been rapidly developed and widely applied, ranging from neuroscience [1-9], therapy or wound monitoring [10-17], cancer detection [18-25] to muscle study [26-28]. There have been several instruments implemented for the “static” oxy- and deoxy-hemoglobin concentration detection, such as the hand-held remission

systems [19, 29-32] and full 3D tomographic systems [21, 33-36]. However, instrument for “dynamic” blood flow measurement in deep tissue is still in early research. Comparing to the other non-optics based modalities for tissue perfusion assessment such as positron emission tomography (PET), Xenon-enhanced computed tomography (XeCT) and Arterial-spin labeled MRI (ASL-MRI), DCS has non-ionizing radiation, needs no contrast agents, additionally it has no interference with commonly used medical devices such as pacemaker and metal implants. These advantages make DCS suitable for cancer therapy monitoring, surgical monitoring. The DCS instrument which has been built so far involved usage of some optical components such as optical fibers and lens as well as a hardware autocorrelator [2, 3, 12, 14, 15, 28, 37-43]. The simplicity in instrumentation makes DCS portable and brings big potential to be widely used for bedside monitoring. However, before translating the instrument to clinical settings, there are some gaps existing: firstly, the current DCS is not able to obtain rBF in real-time; secondly, the extraction of rBF using DCS is based on model-fitting, but it is still debatable that which model could describe the motions of RBCs better; thirdly, the cost of the system can be burdensome with increasing number of channels. In order to tackle the above mentioned problems, this project is geared towards development of near infrared diffuse optics-based flowmetry for assessing deep tissue blood perfusion in a non-ionizing, inexpensive, portable and non-invasive manner.

The theory of diffuse optics is firstly reviewed in Chapter 2 to establish a basic background framework, including the physical model which describes the photon migration inside the tissue, the Green's function solution in semi-infinite geometry and the way of determination of chromophore concentrations and motion of scatterers. In addition, the experimental modes of diffuse optical methods as well as various kinds of instrumentation and applications are also introduced in this chapter.

From Chapter 3 to Chapter 6, the different phases of project are elaborated. The Fast Fourier Transform (FFT) software autocorrelator based diffuse correlation spectroscopy (DCS) system was first developed and demonstrated in Chapter 3. The system can be operated in real-time to obtain rBF ; moreover, it is capable of recording raw photon count signal. Through the simulation, a flow phantom and *in-vivo* experiments, the system was validated while balancing both the cost and performance. The DCS system was then applied together with DOS to monitor the tumor response of mice which underwent the photodynamic therapy (PDT) treatment in real-time as shown in Chapter 4.

The raw photon count signal log obtained by the above mentioned DCS system provides more room to explore other methods to obtain blood perfusion information in deep tissue. Thus in Chapter 5, for the first time, the Sample entropy (SampEn) analysis is applied to the photon count signals. Sample entropy is a relatively new approach and gains more popularity in physiological time series data analysis. The detected diffuse reflectance signal undergoes

scattering events in the sample and carries the information of moving scatterers. It has been well known that the complexity of the system can be characterized by entropy; as a result, the motion of scatterers is correlated to SampEn. The SampEn analysis was conducted on flow phantom as well as *in-vivo* study. Via the comparison to DCS, it has been proven that SampEn resembles the flow behavior which is obtained from DCS. In addition, this approach is not based on model fitting process which is commonly utilized in DCS. The fact that it is computationally light weight also permits its application where real-time flow assessment is necessary.

For majority of clinical cases, the measurement of rBF from a single channel is not sufficient. Apparently, the cost of instrument will increase as the number of channel increases because either more sources or detectors are necessary to enable the simultaneous measurement at different channels. Besides the increasing cost, it tends to make the system bulky and less portable. Therefore, a novel approach named as diffuse speckle contrast analysis (DSCA) is introduced in Chapter 6. The concept of DSCA originated from that of laser speckle contrast imaging (LSCI), namely the statistics of laser speckles is correlated with the flow beneath the surface. Briefly, the same light source with long coherence length as DCS was used; however, instead of avalanche photodiode (APD) or photomultiplier tube (PMT), a CCD camera was ingeniously applied. Instrumentation-wise, the utilization of DSCA reduces the

hardware requirement considerably. Furthermore, this method paves the way for depth-sensitive blood flow measurement in the future.

Lastly, in Chapter 7, the overall concluding remarks are made from the above studies, as well as future potential development directions.

Chapter 2 Literature Review

In 19th century, Bright [44] firstly exploited the ability of light to penetrate tissue, by noting that light could be transmitted through the head of a child with hydrocephalus. After the first employment of light on tissue, utilizing transillumination technique to diagnose hydrocephalus and intraventricular haemorrhage became more and more acceptable. Later in 1977, Jobsis pointed out that the oxygenation status of chromophores can be measured by using the “near-infrared window” between approximately 600-900 nm [45]. And this was regarded as the most significant breakthrough in optical imaging. In this chapter, the theoretical background of diffuse optics will be reviewed first followed by various experimental techniques and current applications of this technology.

2.1 Basic Theory

This section provides the theoretical framework of diffuse optics, which is based on the photon propagation model in tissues. Equations and calculations required to obtain optical parameters are presented for the various diffuse optical techniques.

2.1.1 Model of light transport in tissue

Light propagation through human tissue has been described successfully via the photon diffusion equation. It can be derived from the radiation transport

equation (RTE), which is a conservation equation for the radiance in each infinitesimal volume element within the sample [46], i.e.

$$\begin{aligned} \frac{1}{v} \frac{\partial L(r, \hat{\Omega}, t)}{\partial t} + \hat{\Omega} \cdot \nabla L(r, \hat{\Omega}, t) = & -(\mu_a + \mu_s) L(r, \hat{\Omega}, t) + Q(r, \hat{\Omega}, t) \\ & + \mu_s \int_{4\pi} L(r, \hat{\Omega}', t) f(\hat{\Omega}, \hat{\Omega}') d\Omega' \end{aligned} \quad (1)$$

where v represents the speed of light in the turbid medium, $L(r, \hat{\Omega}, t)$ is the light intensity traveling in the $\hat{\Omega}$ direction at position r and time t , $Q(r, \hat{\Omega}, t)$ is the power per volume emitted by sources in the $\hat{\Omega}$ direction, $f(\hat{\Omega}, \hat{\Omega}')$ is the normalized differential cross-section for single light scattering events in the medium. Both the absorption coefficient μ_a and the scattering coefficient μ_s determine the change of light intensity at each point.

When the source-detector separation is much greater than the transport mean-free path l_{tr} (i.e. the average length it takes for a photon's direction to become random), equation (1) can be reduced to diffusion approximation to the RTE, i.e.

$$\frac{1}{v} \frac{\partial \phi(r, t)}{\partial t} + \nabla \cdot J(r, t) + \mu_a(r) \phi(r, t) = S(r, t) \quad (2)$$

where $\phi(r, t) = \int_{4\pi} L(r, \hat{\Omega}, t) d\Omega'$ is defined as the photon fluence rate, which refers the total power per area moving radially outward from the infinitesimal volume element at position r and time t . $J(r, t) = \int_{4\pi} L(r, \hat{\Omega}, t) \hat{\Omega} d\Omega'$ is the

photon flux, which is a vector sum of the radiance emerging from the infinitesimal volume. $S(r, t)$ is an isotropic source term.

Equation (2) can be further converted to the photon diffusion equation via Fick's law of diffuse $J(r, t) = -D\nabla\phi(r, t)$, which is as follows [47, 48],

$$\nabla \cdot (D(r)\nabla\phi(r, t)) - \nu\mu_a(r)\phi(r, t) - \frac{\partial\phi(r, t)}{\partial t} = -\nu S(r, t) \quad (3)$$

In the above equation, D is the diffusion coefficient defined as $D(r) = \nu / 3(\mu_a(r) + \mu'_s(r))$. Here, reduced scattering coefficient $\mu'_s(r)$ is the reciprocal of the “photon random walk step length” and defined as $\mu'_s(r) = (1 - \langle \cos\theta \rangle)\mu_s$ where θ is the angle between scattered and incident directions and $\langle \cos\theta \rangle$ represents the anisotropy factor. In tissues, anisotropy factor lies between 0.8 and 0.98 typically [49].

The photon diffusion model is valid only when the source is isotropic and scattering is much higher than absorption [50]. This assumption is not valid at locations near the surface or near the source.

2.1.2 Diffuse photon density waves

Diffuse photon density wave (DPDW), which is produced by an amplitude-modulated light source, can propagate spherically outwards from the source through the highly scattering medium. The photons then combine

macroscopically to produce a scalar wave of light energy density with well-defined amplitude and phase although they constantly experience scattering in the medium. Measuring distortions in the DPDW wavefront which is caused by heterogeneities allows the detection and characterization of them [51-53].

An intensity modulated point source can be expressed in the following form [52]:

$$S(r, t) = S_{dc}(r) + S_{ac}(r)e^{-i\omega t} \quad (4)$$

Likewise, the solution $\phi(r)$ also has dc and ac components, namely,

$$\phi(r, t) = \phi_{dc}(r) + \phi_{ac}(r)e^{-i\omega t} \quad (5)$$

where ω is the modulation frequency.

As a consequence, the photon diffusion equation becomes:

$$(-D(r)\nabla^2 - i\omega + v\mu_a(r))\phi_{ac}(r) = vS_{ac}(r) \quad (6)$$

$$(\nabla^2 + k^2)\phi_{ac}(r) = -vS_{ac}(r) / D \quad (7)$$

where $D(r)$ is the diffusion coefficient, μ_a is the absorption coefficient, v is the average speed of light in medium, and $k^2 = (-v\mu_a + i\omega) / D$ is the wavenumber.

Assuming that D is homogeneous, the solution to this Helmholtz Equation in an infinite medium is given by [52]:

$$\phi_{inf}(r) = \frac{-vS_{ac}e^{ikr}}{4\pi rD} \quad (8)$$

Here, k is the complex wavevector with real and imaginary components, i.e.

$$k = \Re(k) + i\Im(k) \quad , \quad \text{thus} \quad k^2 = \Re(k)^2 - \Im(k)^2 + 2\Re(k)\Im(k)i = (-v\mu_a + i\omega) / D \quad .$$

Solving $\Re(k)^2 - \Im(k)^2 = -v\mu_a / D$ and $2\Re(k)\Im(k) = \omega / D$,

$$\text{Real part: } \Re(k) = \left(\frac{v^2\mu_a^2 + \omega^2}{D} \right)^{1/4} \sin \left(\frac{1}{2} \tan^{-1} \left(\frac{\omega}{v\mu_a} \right) \right) \quad (9)$$

$$\text{Imaginary part: } \Im(k) = \left(\frac{v^2\mu_a^2 + \omega^2}{D} \right)^{1/4} \cos \left(\frac{1}{2} \tan^{-1} \left(\frac{\omega}{v\mu_a} \right) \right) \quad (10)$$

Ignoring higher-order terms, both the phase ϕ , and the log of the product of the source-detector distance and amplitude $\ln(r|\phi(r)|)$, vary linearly as a function of the source-detector distance. The slopes of these lines provide the real and imaginary parts of k respectively:

$$\text{phase}(\phi(r)) = \Re(k)r \quad (11)$$

$$\ln(r|\phi(r)|) = -\Im(k)r + \ln(v) - \ln(4\pi D) \quad (12)$$

Once k is known, the absorption and reduced scattering coefficients of the medium can be calculated from the equations below [51]:

$$\mu_a = \frac{\omega}{v} \tan \left(2 \tan^{-1} \left(\frac{\Re(k)}{\Im(k)} \right) \right)^{-1} \quad (13)$$

$$\mu'_s = \frac{\Re(k)^2 + \Im(k)^2}{3(\mu_a^2 + \frac{\omega^2}{v^2})^{1/2}} \quad (14)$$

In theory, the phase, dc and ac components of the signal need to be measured only at two distances to determine the slopes. However, use of more source-detector separations offers better fitting of the lines. In addition, more distances can improve the signal-to-noise ratio (SNR) of the measurement and its accuracy as well as the reliable performance.

It is important to outline the conditions required for the theory and the above equations to be valid. It is assumed that:

- i. The medium is highly scattering,
- ii. The source-detector distance is larger than the photon transport length (the travel distance before the transport direction gets randomly, roughly in the order of 0.1 cm of tissue).

2.1.3 Green's function solutions for semi-infinite geometry

A common technique employed to solve the frequency-domain photon diffusion equation (equation (3)) is to first find their respective Green's function for the chosen geometry, and then make use of the Green's function to construct more general solutions [54, 55]. In homogeneous media with a

specific geometry, the frequency-domain Green's function $G_0(r, r_s)$ satisfies the equation:

$$(\nabla^2 + k^2)G_0(r, r_s) = -\delta(r - r_s) \quad (15)$$

as well as the extrapolated zero boundary conditions [56-58]. The final solution for $\phi(r)$ is equivalent to the convolution of this Green's function with the source term vS_{ac}/D .

In a homogeneous semi-infinite medium, the solution of the photon diffusion equation can be obtained by using image sources, while simultaneously satisfying the extrapolated zero boundary condition.

Figure 2.1 The extrapolated zero boundary condition

As shown in Figure 2.1, source light is injected into the tissue via a fiber at $z=0$, which is well approximated by an isotropic point source at $z=z_0$. A negative image of the real point source is introduced at $z=2z_b+z_0$ via reflection of the real source about the extrapolated zero boundary in order to satisfy the extrapolated zero boundary condition. The superposition of the homogeneous infinite solutions (equation 8) for both the real and image sources results in the homogeneous semi-infinite Green's function for a detector at a distance of ρ from source [58],

$$\phi_{semi}(r) = \frac{vS_0 e^{ikr}}{4\pi D r^2} [-2ik(z_0 z_b + z_b^2)] \quad (16)$$

where $k^2 = 3\mu'_s(-i\omega / v + \mu_a)$.

In the special case when $\omega = 0$ which means the phase is always zero and there is only amplitude information, it corresponds to a continuous wave (CW) system (will be introduced in next section). Under CW condition the DPDW solutions produced in a homogeneous infinite and semi-infinite medium can be written as follows [51, 52]:

$$\phi_{inf}^{CW}(r) = \frac{vS_0 e^{-kr}}{4\pi D r} \quad (17)$$

$$\phi_{semi}^{CW}(r) = \frac{vS_0}{4\pi D} \left(\frac{e^{-kr_1}}{r_1} - \frac{e^{kr_2}}{r_2} \right) \quad (18)$$

where, $r_1 = \sqrt{\rho^2 + z_0^2}$, $r_2 = \sqrt{\rho^2 + (z_0 + 2z_b)^2}$, and $z_b = \frac{2}{3\mu'_s} * \frac{1 - R_{eff}}{1 + R_{eff}}$. R_{eff} refers

to the effective reflection coefficient, which accounts for the index mismatch between tissue and air. In this expression,

$R_{eff} \approx -1.440n^{-2} + 0.710n^{-1} + 0.668 + 0.00636n$, and $n = n_{in} / n_{out}$ is equivalent

to the ratio of the refractive index inside to that outside the turbid medium.

However, $k^2 = 3\mu_a\mu'_s$ is inadequate for a unique determination of both unknown absorption (μ_a) and reduced scattering (μ'_s) coefficients.

2.1.4 Determination of tissue chromophore concentrations

The tissue absorption linearly depends on the concentrations of chromophores. Particularly, the absorption coefficient which is wavelength-dependent is given by the Beer-Lambert Law:

$$\mu_a(\lambda) = \sum_i \varepsilon_i(\lambda)c_i \quad (19)$$

where $\varepsilon_i(\lambda)$ is the wavelength-dependent extinction coefficient and c_i is the concentration of i^{th} chromophore. By measuring μ_a at different wavelengths, the unknown chromophore concentrations can be obtained by solving the Beer-Lambert equation. Generally, in order to estimate the number of N chromophores, at least N different wavelengths are necessary for the determination of wavelength-dependent μ_a . Therefore, in the case that main absorbers considered are oxy- and deoxy- hemoglobin in the range of NIR, two

wavelengths are sufficient for hemoglobin concentration determination. However, more wavelengths permit the inclusion of water and lipid and other chromophores. Also, the use of more wavelengths improves the accuracy of the hemoglobin measurements since systematic errors can be reduced by redundant measurements. The most commonly used geometry is the reflection geometry, where $\mu_a(\lambda)$ is derived by employing the homogeneous semi-infinite media analysis.

In many cases, people are more interested in the temporal variations of physiological quantities, and thus we need to look into the changes in optical properties, namely $\Delta\mu_a$ and $\Delta\mu'_s$ respectively [46]. The differential pathlength approach (DPF) can be applied directly to obtain these relative changes. The differential pathlength is defined as:

$$d_a = \frac{\partial OD(\mu_a, \mu'_s)}{\partial \mu_a} \quad (20)$$

The Tayler series expansion of the optical density with known source-detector separation ρ to first order in μ_a and μ'_s is:

$$\begin{aligned} & OD(\mu_a^{(0)} + \Delta\mu_a, \mu_s'^{(0)} + \Delta\mu'_s, \rho) \\ & \approx OD(\mu_a^{(0)}, \mu_s'^{(0)}, \rho) + \frac{\partial OD(\mu_a^{(0)}, \mu_s'^{(0)}, \rho)}{\partial \mu_a} \Delta\mu_a + \frac{\partial OD(\mu_a^{(0)}, \mu_s'^{(0)}, \rho)}{\partial \mu'_s} \Delta\mu'_s \end{aligned} \quad (21)$$

And thus,

$$\Delta OD(\lambda, t, \rho) = \frac{\partial OD(\mu_a^{(0)}, \mu_s^{(0)}, \rho)}{\partial \mu_a} \Delta \mu_a + \frac{\partial OD(\mu_a^{(0)}, \mu_s^{(0)}, \rho)}{\partial \mu_s'} \Delta \mu_s' \quad (22)$$

where $\Delta OD(\lambda, t, \rho) = -\ln(I(r_d, t) / I(r_d, t = 0))$.

According to modified Beer-Lambert law, i.e.

$$\begin{aligned} \Delta OD(\lambda, t) &= \sum_i (\varepsilon_i(\lambda) \Delta c_i(t)) d_a(\rho, \lambda) + \Delta \mu_s'(\lambda, t) d_s(\rho, \lambda) \\ &\approx \sum_i (\varepsilon_i(\lambda) \Delta c_i(t)) DPF(\lambda) \rho \end{aligned} \quad (23)$$

As a result, by measuring ΔOD at multiple wavelengths, we can determine the changes in chromophore concentrations via inverting modified Beer-Lambert law. DPF and ε_i , which are inherent properties of a material, can be obtained from literature [59, 60].

2.1.5 Diffuse correlation spectroscopy

Near-infrared diffuse correlation spectroscopy has been studied extensively as a potential non-invasive probe of blood flow in deep tissue. As an emerging technique, DCS offers high temporal (~ 100 ms) and moderate spatial ($\sim$ mm) resolution in tissues. Basically, when a laser beam with constant intensity shines on the surface of a turbid solid sample, photons migrate over different path lengths through the medium before emerging from the surface. As a result,

the emerging photons at the detector will interfere constructively or destructively and form speckles as shown in Figure 2.2. Furthermore, the movements of the scattering particles result in the fluctuation of these speckles. Hence, the motion of blood cells can be extracted [12, 51].

Figure 2.2 Speckle pattern illustration. The bright spots correspond to the strong light intensity, while the dark spots correspond to the regions of little intensity [61].

In detail, when diffusing photons scatter from moving scatterers, they experience phase shifts and cause the speckle fluctuation at the detector side. The motion information below the tissue surface is carried in electric field of diffuse light $E(r, \tau)$ and can be extracted from the electric field autocorrelation

function, which is defined as $G_1(r, \tau) = \langle E(r, t) E^*(r, t + \tau) \rangle$. It has been shown that $G_1(r, \tau)$ satisfies a correlation diffusion equation, i.e. [39, 62-64]

$$\left(-\frac{1}{3\mu'_s} \nabla^2 + \mu_a + \frac{1}{3} \alpha \mu'_s k_0^2 \langle \Delta r^2(\tau) \rangle \right) G_1(r, \tau) = S(r) \quad (24)$$

Here k_0 is the wavenumber of the light in medium, α is the fraction of photon scattering events from moving scatterers out of total scatterers. $\langle \Delta r^2(\tau) \rangle$ represents the mean square displacement of the moving scatterers, and is commonly described using two different models, namely the Brownian motion and random flow model in biological tissues. Brownian motion model considers the motion of scatterers as diffusive motion and $\langle \Delta r^2(\tau) \rangle = 6D_B \tau$ [65, 66], where D_B is the effective diffusion coefficient of scatterers. Meanwhile, random flow model assumes the random ballistic motion of scatterers and $\langle \Delta r^2(\tau) \rangle = \langle V^2 \rangle \tau^2$ [67, 68] where $\langle V^2 \rangle$ represents the mean square velocity of the scatterers. Experimentally, the diffuse light electric field autocorrelation function is usually derived from measured normalized intensity autocorrelation $g_2(r, \tau) = \langle I(r, t) I(r, t + \tau) \rangle / \langle I \rangle^2$ by using the Siegert relation [2, 39, 69]:

$$g_2(r, \tau) = 1 + \beta \frac{|G_1(r, \tau)|^2}{\langle I \rangle^2} \quad (25)$$

where $I(r, t)$ is the detected diffusing light intensity at position r and time t , the angle bracket $\langle \dots \rangle$ denotes an ensemble average. β here is a numerical factor related to detector geometry, number of detected speckles and other experimental parameters. The assumption for applying the Siegert relation to obtain the electric field autocorrelation function is that the system is ergodic, which means the time average is equal to the ensemble average. The analytic solution of the correlation diffusion equation for a point light source upon a semi-infinite medium is [39, 70]

$$G_1(r, \tau) = \frac{3\mu'_s}{4\pi} \left(\frac{e^{-k_D r_1}}{r_1} - \frac{e^{-k_D r_2}}{r_2} \right) \quad (26)$$

where $k_D = \sqrt{3\mu'_s\mu_a + 6\mu_s'^2 k_0^2 \alpha D_B \tau}$, $r_1 = \sqrt{\rho^2 + z_0^2}$ ($r_2 = \sqrt{\rho^2 + (z_0 + 2z_b)^2}$) are the distance between source (image source) and detector, respectively, in which ρ

is the source detector separation, $z_0 = \frac{1}{\mu'_s}$, $z_b = \frac{2(1 - R_{eff})}{3\mu'_s(1 + R_{eff})}$ and R_{eff} still

represents the effective reflectance. Although physically the motion of blood cells may be considered as random flow, but practically the Brownian motion model showed better fitting in majority of cases, ranging from brain [2, 6, 9, 16, 38, 39, 71-73] to muscle [27, 74] and tumor model [12, 14, 43, 75]. Therefore the measurements of $G_1(r, \tau)$ can be fitted to yield a blood flow index ($BFI = \alpha D_B$) to parameterize the relative blood flow.

The BFI and β were obtained by minimizing the difference between the analytical model of autocorrelation in the reflectance geometry $g_{1,m}(r, \tau)$ and the measured autocorrelation function $g_{1,\text{exp}}(r, \tau)$, namely

$$\chi^2 = \sum [g_{1,m}(r, \tau) - g_{1,\text{exp}}(r, \tau)]^2.$$

2.2 Experimental modes of diffuse optical methods

Based on the instruments employed, the experimental techniques are broadly categorized into three groups: continuous-wave (CW), time-domain (TD) and frequency-domain (FD), as shown in Figure 2.3.

Figure 2.3 Transmission and reflection geometries, and various measurement domain [46].

2.2.1 Continuous-wave (CW) measurement

Continuous-wave systems require a source with constant intensity or modulation at a low frequency of a few kHz, in order to provide high sensitivity inherent in phase-locked detection methods. The intensity of transmitted light is measured, which only contains information about the amplitude attenuation of the incident light. It also offers the advantages of fast data acquisition and simple instrumentation. Nevertheless, it is unable to resolve both absorption and scattering contrasts simultaneously, due to only a single amplitude measurement for each source-detector pair. Besides, the measured light intensity can be greatly affected by skin hair and other surface coupling parameters.

2.2.2 Frequency –domain (FD) measurement

Frequency domain (FD) systems require a continuous light source as well, but the light intensity is amplitude-modulated at frequencies in the order of tens to hundreds of megahertz. The corresponding changes in amplitude decay and phase shift of transmitted light are detected several centimeters away from the source. Information about the absorption and scattering properties of tissue are obtained from these changes. It has been verified that for typical tissues and at frequency below 200MHz, the phase shift is linear to the mean total path length [76]. Theoretically, frequency-domain measurements obtained at multiple frequencies and time-domain measurements sampled at multiple times provide

the same information, as they are related by the Fourier Transform [77]. However, the complexity of RF instrumentation makes it difficult to produce RF systems with multiple frequencies. Thus, most frequency-domain systems are single-frequency and therefore generally have less information content relative to time-domain systems.

2.2.3 Time-domain (TD) measurement

In time domain or time-resolved systems, very short (picoseconds-order) laser pulses are introduced into tissue. Going through various tissue layers, the narrow pulses are broadened and attenuated and the intensity of emerging light is detected as a function of time, namely the temporal point spread function (TPSF) or the temporal distribution of photons with picoseconds resolution [78]. The tissue absorption and scattering information can be extracted from the shape of this distribution. The TDS can provide better spatial resolution [79] and more accurate separation of absorption and scattering contrast, but the system costs much more than CW. So far, majority of time-resolved instruments have only been developed in the laboratory. However, some advances in technology have been made commercial products possible such as a single-channel TDS instrument which is used in clinical applications [80, 81].

2.3 Applications of diffuse optical measurements

2.3.1 Application of diffuse optical spectroscopy

One of the first clinical applications of diffuse optical techniques for functional brain monitoring was the investigation of fetal, neonatal and infant cerebral oxygenation and functional activation [1]. It holds the obvious advantage because ultrasound can only provide anatomical information and MRI needs to move a sick infant out of the intensive care unit. On the other hand, employing diffuse optics in monitoring adults with ischemic and hemorrhagic stroke is underway [82, 83]. Hence, diffuse optical approach offers an alternative as a bedside system to isolate infants at risk, diagnose brain injury and monitor therapeutic treatment [84]. In addition, diffuse optics has been used on other basic research domains to help unearth the developmental alterations in the cerebral hemodynamic response to auditory, visual and other stimulations [85].

Besides, because malignant tumors are always associated with increased blood supply [86], 3D diffuse optical tomography (DOT) provides the ability in cancer detection, imaging and monitoring through the measurement of blood volume and blood oxygen saturation. The instrumentation for 3D optical imaging of the human breast with malignant cancer has been developed by means of the innovative reconstruction algorithm [46]. However, it was demonstrated that a reliable screening tool must be able to identify tumors smaller than 1 cm as mortality escalates rapidly for tumors beyond this size

[87], while optical mammography can only offer moderate spatial resolution now. Therefore, improving spatial resolution is one of the major concerns in optical mammography.

Moreover, combining different imaging modalities is underway to overcome structural resolution limitations of DOT. The multi-modal approach can also provide further physiological information on tissues; DOT has been combined with MRI [88, 89], 3D X-ray mammography [90, 91] and ultrasound [92, 93], which leads to a better understanding of the relationship between the functional contrast obtained by optical imaging and the structural contrast provided by other forms of imaging.

2.3.2 Application of diffuse correlation spectroscopy

The typical DCS system includes the continuous-wave laser with a long coherence length as the source, as well as the avalanche photodiode (APD), the photomultiplier tube (PMT), or the charge coupled device (CCD) as the detector. The detected signals need to be connected to a hardware autocorrelator or software autocorrelator [94] to compute the auto-correlation function. The APD and PMT are extremely sensitive detectors with relatively high acquisition speed, when compared to the CCD. Therefore, in APD or PMT-based DCS systems, single mode fibers are always employed and a time-averaged light intensity auto-correlation function is also calculated for increased accuracy. On the other hand, the multispeckle nature of the CCD detection enables parallel

detection at multiple spatial positions so that a position-averaged light intensity can be performed in this case [95].

To date, DCS has been studied extensively as a potential non-invasive probe of blood flow in deep tissue due to its portability and cost-effectiveness compared to existing techniques. The first direct validation of relative blood flow (rBF) measurement by DCS in human has been done in recent years [28]. Moreover, it has been successfully used over a wide range of tissue types such as the brain [39], human skeletal muscle [27], skin [62], as well as the breast [40]. In addition, blood flow is a key physiological parameter which can provide information about tumor metabolism and the micro-environment. Therefore, DCS can also serve as an alternative method for bedside monitoring of tumor therapies such as chemotherapy in human breast cancer and photodynamic therapy in patients with head and neck tumors.

Chapter 3 FFT Software Autocorrelator Based Diffuse Correlation Spectroscopy

As an emerging non-invasive deep tissue blood flow probing technique, DCS is based on the time-averaged intensity autocorrelation function of the fluctuating diffuse reflectance signal. This chapter describes a Fast Fourier Transform (FFT)-based software autocorrelator DCS system in which the graphical programming language LabVIEW (National Instruments) is utilized to complete data acquisition, recording and processing tasks. The validation and evaluation experiments were also conducted under the acquisition rate of $\sim 400\text{kHz}$. The merits from both instrumentation and data processing perspective will be discussed. The results indicate that an FFT-based software autocorrelator can be an alternative solution to the conventional hardware ones in DCS system with considerable benefits.

3.1 Introduction

Near-infrared diffuse correlation spectroscopy (DCS), also known as diffusing-wave spectroscopy (DWS), is established on the well-known spectral window in the near-infrared (NIR, 600-900nm) range, wherein the relatively low biological tissue absorption enables deep penetration of light. It is an emerging technique for continuous noninvasive measurement of blood flow in biological tissues. In the last decade or so, DCS technology has been developed [62, 63, 96], extensively validated and vigorously employed to noninvasively probe the

blood flow information in deep tissue vasculature such as brain [2, 6, 9, 39, 71-73, 97-105], muscle [74, 106-110], and breast [111, 112]. Compared to other blood flow measurement techniques, such as positron emission tomography (PET), single photon emission computed tomography (SPECT), and xenon-enhanced computed tomography (XeCT), DCS uses non-ionizing radiation and needs no contrast agents. In contrast to dynamic susceptibility contrast magnetic resonance imaging (DSC-MRI) and arterial spin labeling MRI (ASL-MRI), it has no interference with commonly used medical devices such as pacemaker and metal implants [113]. Therefore, it has also been applied on cancer therapy monitoring [43, 75, 114, 115] and bedside monitoring in clinical settings [116].

The typical setup of DCS technique consists of a laser with a long coherence length as light source, photon-counting avalanche photodiode (APD) or photomultiplier tube (PMT) as detector, and a hardware autocorrelator. As one of the critical components, autocorrelator computes the autocorrelation function of the temporal fluctuation of the light intensity which was subject to multiple scattering events before emerging from the investigated position on the sample surface. There are many commercial correlators available such as the ones manufactured by ALV (Langen, F. R. Germany) and by Correlators.com (Bridge-water, New Jersey). To date, most published works have used these hardware autocorrelators for autocorrelation calculation [2, 6, 39, 42, 75, 97, 106, 110, 112], although a few of them had designed a multi-tau software

correlator for dynamic light scattering and validated it by particle size measurement [117, 118]. The hardware correlators utilized multi-tau scheme, so they could be operated at a fast sampling speed thus offered a real time computing across a wide range of lag times from several nanoseconds to hours. However, they are relatively costly and not flexible since the fixed number of bits per channel results in fixed lag time scale. Meanwhile, the software autocorrelator mentioned earlier was capable of measuring correlation functions over time scale of ~ 5 microseconds in real time [117, 118], but it was not capable of recording the raw photon-count signal and therefore had limitations in terms of pre-processing of the raw photon count and post-measurement sanity checking.

In this chapter, we demonstrate an alternative approach of DCS analysis using a software correlator based on Fast Fourier Transform (FFT). It is cost-effective, flexible, and can be easily implemented with other technologies. Furthermore, the ability of recording raw photon-count signal holds the potential of signal pre-processing before autocorrelation calculation, such as filtering out the noise caused by fluctuation of laser source. It also provides room for investigating the methods other than autocorrelation to extract flow information from raw signals. To achieve data acquisition, recording and autocorrelation calculation, we used the graphical programming language LabVIEW (National Instruments), which is one of the most popular and powerful tools for signal acquisition and processing. The FFT, which is one of the most efficient algorithms known in

the history of signal processing, was applied for autocorrelation calculation. The sampling rate of the system can reach up to $\sim 400\text{kHz}$ and thus enables the minimum lag time of $\sim 2.5\ \mu\text{s}$, which is significantly shorter than the decay constant (between tens to hundreds of microseconds) in DCS applications. Through comparison with a simulated hardware autocorrelator that works the same way as hardware correlator (correlator.com) does, we evaluated the performance of FFT-based software autocorrelator. For the purpose of validation, in-house flow phantom experiment, human arm cuff occlusion experiment as well as photodynamic therapy (PDT) response monitoring experiment were conducted.

3.2 Development of real-time diffuse correlation spectroscopy system

3.2.1 FFT-based software autocorrelator

The unnormalized intensity autocorrelation, represented as $G_2(\tau) = \langle I(t)I(t+\tau) \rangle$, resembles convolution operation, except that in convolution calculation, the second term being multiplied has to be the time-reversal of the original function $I(t)$, namely $I(-t)$. Therefore, the same autocorrelation can be obtained by convolution between two functions $I(t)$ and $I(-t)$. Then, by convolution theorem, one can obtain the autocorrelation function based on Fourier Transform as follows:

$$\begin{aligned}
G_2(\tau) &= (I(t) * I(-t))(\tau) \\
&= F^{-1}[F\{I(t)\}F\{I(-t)\}] \\
&= F^{-1}\{\tilde{I}(\nu)\tilde{I}^*(\nu)\} \\
&= F^{-1}\left\{\left|\tilde{I}(\nu)\right|^2\right\}
\end{aligned} \tag{27}$$

where F and F^{-1} refer to forward and inverse Fourier transform, respectively, and $\tilde{I}(\nu)$ is the Fourier transform of $I(t)$. Note that the last equality of equation (27) is also called Wiener-Khinchin theorem and real valued $I(t)$ always results in real values of G_2 .

Since the Fourier transform can be very efficiently implemented by Fast Fourier Transform (FFT), one can obtain the linear autocorrelation function in a very fast and stable manner using above equation.

3.2.2 Hardware autocorrelator simulation

In order to examine the behavior of FFT-based software autocorrelator with a known input signal, generated either by simulation or experiment, the hardware autocorrelator simulation was done according to its working principle. In this section, the working principle of the hardware correlator board will be briefly described, based on the instruction manual of a most commonly used commercial product (Flex99R-12) from correlator.com. The algorithm behind the hardware correlator will be simulated by software approach, and its performance will be compared against our new FFT-based approach.

The typical hardware correlators such as the ones manufactured by ALV and Correlator.com are based on multi-tau scheme, and have multiple tier structure which contains a certain amount of registers as shown in Figure 3.1.

Figure 3.1 The diagram of hardware autocorrelator inner structure, with several tiers of registers where the delay times are the (n-1)-th power of two times of initial delay time T_0 where n is the index of tiers.

If we take the one from Correlator.com for instance, the first tier has 32 registers, and higher tiers have 16 registers each. The incoming photon count starts filling up the registers in the first tier from the left with a given delay time T_0 (typically 160ns). When it reaches the right end, the second tier fills up from the left at a twice as slow rate, since the sum of two register values in the right end of the first tier constructs the first register value of the second tier. Likewise,

the first register value of n^{th} tier is constructed by sum of the last two register values in $(n-1)^{\text{th}}$ tier, which means the registers of n^{th} tier is updated every $2^{n-1}T_0$. The hardware correlator can have up to 31 tiers, which means there are 512 ($=1 \times 32 + 30 \times 16$) registers with 512 different delay times. However, registers at the 31st tier updates every $2^{30}T_0$, which is too long with a practical T_0 values of $\sim 100\text{ns}$, so later tiers are hardly used in practice.

For each shift that occurs in the register, the unnormalized intensity autocorrelation coefficient for the i^{th} register, $G_2(t_i)$, is calculated as average value of $n_i \cdot n_0$, where n_i indicates the photon count in the i^{th} register, n_0 is the photon count at zero delay time with the same bin width as the i^{th} register, and t_i is the delay time between n_i and n_0 [113]. The delay time t_i is calculated as the summation of all the bin widths on the left of the i^{th} register. With a few hundred register channels (512 channels for 31 tier system, for example), a delay time that ranges from hundred of nanoseconds to minutes can be achieved, greatly reducing the computational load compared to linear autocorrelator.

The above mentioned hardware correlator algorithm is implemented in Matlab (Mathwork, Inc.). The number of tiers, N , was given as a variable (normally set around 10), and a cell array variable with a size of $16(N+1)$ (32 for 1st tier, and $16(N-1)$ afterwards) was allocated for register channels. The Matlab script initiates a loop, where the register channels in the first tier shifts to the right by one register per iteration and a new data is read in the left-most register. When the iteration number reaches 2^{n+1} , registers in n^{th} tier become shifted to the right

by one register, after which a new value (sum of two register values at the end of $(n-1)^{\text{th}}$ tier) is assigned to the left-most register in n^{th} tier. Figure 3.2 shows a snapshot of the register profile in different tiers, where the register values are represented in logarithmic scale to better visualize the wide-range of the values.

Figure 3.2 A snapshot of the registers of simulated hardware correlator. Color represents the logarithm of the photon count in each register. Ignore the blue portion on the left where there are no registers.

When the register fully fills up, the autocorrelation coefficient between i^{th} register and the 0^{th} register (with the same bin width, as described previously in hardware correlator section) is calculated. The autocorrelation curve for whole register is calculated for each shift event in the last tier, and they are averaged over hundreds of trials before being plotted.

Using multi-tau approach simulating hardware correlator board and the FFT-based linear approach, the comparison between the two normalized intensity autocorrelation $g_2(\tau)$ calculations is shown in Figure 3.3. The data used in Figure 3.3(a) was from a flow phantom experiment, acquired by NI counter board (PCI-6602) at the sampling rate of 400kHz. For FFT-based approach, the data size used was about 4×10^5 , covering about 1 second. For hardware simulating approach, the delay time was only calculated up to about 30 ms due to the small number of tiers (10 tiers were used), but it was averaged over 500 autocorrelation calculations, and total length of data used is about the same. The FFT-based calculation always ends up showing a spike near the highest delay time, but that is due to the cyclic nature of the FFT-based result, and our visualization in Figure 3.3 only shows the first half thereby removing the unwanted peak. Both of them show nice convergence to the value 1 for long delay times, and they nicely overlap with each other. For experimental data in general, we were able to observe a trend that hardware (multi-tau) algorithm tends to show higher fluctuation for small delay times, whereas the FFT-based algorithm always show smoothest behavior for small delay times due to its single-tau calculation. A small shoulder that is observed between 100 μ s and 1 ms is attributable to the presence of another dynamic layer in the sample, which is the stationary part of the flow phantom in this case. We observe it more frequently with a small source-detector distance where the contribution from the stationary part becomes more important.

The comparison was also performed using simulated data based on Markov chain with a Gaussian noise, and the result was always overlapping unless the average count value of the data was too low. When average was low, we were able to regain the equivalence between the two methods by binning the data thereby increasing the average photon count value. Typical autocorrelation curve for simulated data are shown in Figure 3.3(b).

Figure 3.3 Comparison between hardware and software autocorrelation calculation. (a) Autocorrelation calculated from a typical flow phantom data. (b) Autocorrelation calculated from a simulated data. Hardware-simulating algorithm (red) was implemented under the assumption of 10 tier register, averaged over 500 different autocorrelations. Sampling rate was 400kHz.

3.2.3 Real-time diffuse correlation spectroscopy system

The DCS system consisted of a continuous-wave laser with a long coherence length ($>10\text{m}$) operating at 785 nm (DL785-100-S, $\sim 100\text{mW}$, CrystaLaser, Reno, Nevada, USA) as source and a photon-counting APD (SPCM-AQRH-13-

FC, Perkin-Elmer, Vudreuil-Dorion, Quebec, Canada), whose output was a stream of transistor-transistor logic (TTL) pulses, as detector. The light from the source was coupled to a multi-mode optical fiber (400 μ m diameter) and contacted with sample surface. The detecting fiber was single-mode operated at 785 nm (9 μ m diameter) which was located several centimeters away from source fiber and fed to APD. The TTL output generated by APD was connected to the 32-bit, eight input channels counter/timer board with the maximum input rate of 80MHz (PCI-6602, National Instruments). The counter/timer board was connected to PC (CPU: Intel Core 2 Duo, RAM: 3-Gbyte) through a shielded I/O connector block for DAQ devices (SCB-68, National Instruments). The schematic of DCS setup is shown in Figure 3.4.

Figure 3.4 Schematic of DCS experimental setup.

The instrument was controlled by LabVIEW (National Instruments) which is one of the most popular and powerful program tools and suitable for interactive data acquisition and signal processing. The TTL output of the detector was counted over sampling time τ by the counter/timer board. The PCI-6602 has

timebase of 80 MHz which means the fastest input pulses that can be counted are 12.5ns ($1/80,000,000$ s) apart. The PCI-6602 is the 32-bit counter and can count up to 4,294,967,295 ($2^{32}-1$) before it rolls over. The counter can count the events continuously and store the data in the buffer. Due to the limitation of buffer size, time resolution of anything less than 2 μ s would fill up the buffer in less than 1 s. For the purpose of stable data acquisition we conducted experiments using 2.5 μ s. In data acquisition program, we applied “queue” structure to link “producer” loop and “consumer” loop, which were in charge of reading photon counts and writing results to a file, respectively. By using this kind of structure, the two loops were able to work in parallel so that faster speed can be achieved. Simultaneously, another LabVIEW program detected the latest written photon-counting file and calculated autocorrelation function by FFT algorithm. The result was then displayed and saved to a specified folder. Running on our current system, one core of CPU was fully occupied to acquire the data, while the other core was used for autocorrelation calculation. The screen shot of FFT software autocorrelator is shown in Figure 3.5.

Figure 3.5 Screen shot of the LabVIEW interface of FFT-based autocorrelation calculation.

3.3 Experiment

3.3.1 Phantom design

Phantom, which is the tissue-simulating object to mimic the properties of human or animal tissues, has played an important role in the evolvement of diagnostic systems and most physical therapeutic interventions. It can be used for the purposes of initial testing of system design, optimizing signal to noise ratio (SNR) in existing system and so on [119]. In order to validate and assess the ability of our system, a flow phantom was built as shown in Figure 3.6. The optical properties of solid flow phantom were $\mu'_s = 8cm^{-1}$ and $\mu_a = 0.03cm^{-1}$ (measured by OxiplexTS, ISS Inc, at 830 nm), which satisfied $\mu'_s \gg \mu_a$ criterion, and the photon diffusion equation remains valid.

Figure 3.6 The in-house silicon flow phantom, in which an acrylic cylindrical tube was embedded to mimic the blood vessel. The tube was filled up with glass beads with diameter of 3mm (Sigma Aldrich) in order to make random flow direction.

Based on literature [120], the phantom making procedure was as followed,

1. Prepare a mold to contain the phantom as illustrated by Figure 3.7. Insert the acrylic cylinder tube onto the two side holes of the mold which is fixed 1 cm from the base of the mold. Fill up acrylic cylinder with transparent glass-beads.

Figure 3.7 Mold Design for Random Flow Phantom

2. To make 1,100 mL silicon phantom, 1000 mL RTV 12A and 100 mL RTV 12C (Momentive Performance Materials) are required (the ratio of RTV12A and RTV 12C is 10:1).
3. Weigh 4.675 mg carbon black to reach $\mu_a = 0.05 \text{ cm}^{-1}$.
4. Weigh 2.56 gram TiO_2 to obtain the desired $\mu_s' = 8 \text{ cm}^{-1}$.
5. Dissolve 4.675 mg carbon black to 40 mL RTV 12C.
6. Dissolve 2.56 gram TiO_2 in 40 mL RTV 12C.
7. Stir the two solutions for 30 seconds using glass stirrer. Do not use magnetic stirrer as the plastic cover of the stirrer may react with the RTV 12C.

8. Cover these two solutions with plastic cover or parafilm to prevent it from air exposure. RTV 12C may cause white precipitate in the phantom if it is left open to air for too long.
9. Sonicate both solutions in ice water 3 times for 10 minutes each. Add more ice block for every 10 minutes as the ice will have melted after 10 minutes sonification.
10. While waiting for the sonification process, pour 1000 mL RTV 12A into mixing pail inside the fume hood. Stir gently to remove air bubbles.
11. Add both sonicated solutions to RTV 12A.
12. Add 20 mL RTV 12C to the solution.
13. Stir the solution for 30 minutes to let it mixed well.
14. Pour the solution into mold and stir it for another 30 minutes to remove air bubbles.
15. Leave the phantom in fume hood for 1 day.
16. Take out the phantom from the mold and connect the acrylic cylinder to pump.

3.3.2 In-house flow phantom experiment

Figure 3.8 Schematic of in-house flow phantom experiment with software autocorrelator.

The schematic of DCS experimental setup is shown in Figure 3.8. The Lipofundin N 20% (B.BraunMelsungen AG, Germany) with concentration of 0.6% was pumped through the cylindrical tube, with flow speeds of 0ml/s, 0.05ml/s, 0.125ml/s, 0.2ml/s and 0.325ml/s. The source-detector pair was centered over the solid phantom surface at a distance of 1cm from the flow tube surface. A reflection geometry was adopted in which incident light was injected into the phantom by the source fiber and detected 3.0 cm away along with the cylindrical tube direction.

3.3.3 *In-vivo* cuff occlusion experiment on human arm

After flow phantom experiment, we conducted the validation study on human subject. The *in-vivo* cuff occlusion experiment was done on the arm of a 28 year-old healthy male subject. In order to vary the blood flow speed, a cuff on

subject's upper arm was constricted for 15 seconds for temporary arterial occlusion. The source-detector separation was 1.5cm and the probes were located on forearm. The cuff inflation pressure was 200mmHg.

3.4 Results and discussion

3.4.1 In-house flow phantom experiment

Figure 3.9 shows the normalized intensity autocorrelation functions in semi-logarithm plot with varying flow speed and source-detector separation of 3 cm. It can be observed that the normalized intensity autocorrelation function $g_2(\tau)$ showed an initial constant plateau but decayed exponentially with increasing delay time. As speed increased, the decay rate of autocorrelation curve increased. If we recall the definition of autocorrelation, finite data length will always cause the copy of data shift out of data window thus eventually the autocorrelation function will go down to zero naturally. However, by employing FFT, one can actually get the cyclic autocorrelation, thus it avoids edge effect caused by data finiteness and offers faster calculating speed. Ideally, the zero flow (0 ml/s) was not supposed to show any decay, but we observed a decay that is mainly attributed to the intrinsic fluctuation of laser source and the room temperature Brownian motion of the Lipofundin remaining in tubing. The autocorrelation function calculating speed was less than 20 milliseconds for 800,000 data points with the type of double-precision integer, which is faster than data writing speed (new file generating speed) and enables the display of

the autocorrelation curve in almost real-time. We note from the autocorrelation curves, that increasing flow causes the plateau region of the curves to be shorter and makes it difficult to determine the β value. Therefore, it might cause inaccuracy in measuring high flow, but one can obtain stable plateaus for most of the relevant flow range *in-vivo*.

Figure 3.9 Autocorrelation curves of the phantom with varied speed of flow (0ml/s, 0.05ml/s, 0.125ml/s, 0.2ml/s and 0.325ml/s) as a function of delay time, τ

Since FFT-based autocorrelation calculation provided smoother starting and ending plateaus, it obviously helped with curve fitting. For better comparison between the Brownian motion and random flow model, we performed curve

fitting by using both models. The Levenberg-Marquardt algorithm was applied in Matlab (Mathwork, Inc., *nlinfit* function). As indicated by Figure 3.10, which was the autocorrelation function of the flow with speed of 0.325ml/s, the Brownian motion model fitted the experimental data better than the random flow model did. This result also suggested our self-designed phantom is able to mimic the capillary flow of human body. Therefore we conducted curve fitting by using Brownian motion model, and blood flow index ($BFI=\alpha D_B$) was obtained. The fitting result in general depends highly on the methods of data sampling. In order to obtain better fitting with a uniform weightage in logarithmic time scale, we sampled entire autocorrelation function equally on 2-base logarithm scale as shown in Figure 3.10. This sub-sampling helps with faster fitting speed without compromising its accuracy.

Figure 3.10 Comparison between Brownian motion and random flow model. The autocorrelation function was calculated from the flow phantom data with speed of 0.325ml/s. The entire autocorrelation data was sampled and shown as dots.

In order to compare the goodness of fitting of FFT-based software autocorrelator with that of simulated hardware autocorrelator, we calculated 95% confidence interval of fitted parameters by using *nlparci* function in Matlab (Mathwork, Inc.). As shown in Figure 3.11, by using speed of 0ml/s as base, relative αD_B and $\sqrt{\alpha \langle v^2 \rangle}$ with their standard deviations at different flow speeds were calculated in both software and hardware cases. It can be found that in either Brownian motion or random flow model, the FFT-based software autocorrelator and simulated hardware autocorrelator showed the similar results, but the standard deviations of fitted parameter in former correlator were always

smaller than in the latter one. This is mainly due to the smoothness of autocorrelation curve. As aforesaid, the bigger fluctuation shown at the beginning in autocorrelation curve calculated by hardware autocorrelator resulted in bigger standard deviation of the fitting results. Concurrently, in the same autocorrelator, the parameter fitted for Brownian motion had smaller standard deviation than that for random flow model and it also can be observed from individual fitting as shown in Figure 3.11. Moreover, we performed linear regression of the relative αD_B and $\sqrt{\alpha \langle V^2 \rangle}$. It showed higher linearity to flow speed in software autocorrelator case than in the hardware one. In addition, as mentioned previously, the autocorrelation calculating speed is faster than data writing speed, our instrument holds the potential to make the full use of this time to accomplish data fitting and provide the rBF values in real-time.

Figure 3.11 Linearity test of relative BFI (either αD_B or $\sqrt{\alpha \langle V^2 \rangle}$) vs. flow speed.
(a) and (c) are obtained using software autocorrelator (shown in blue), while (b) and (d) are obtained using hardware autocorrelator (shown in red). All $rBFI$ s are normalized to the BFI value at zero flow.

3.4.2 *In-vivo* cuff occlusion experiment on human arm

Figure 3.12(a) shows the normalized intensity autocorrelation function $g_2(\tau)$ of three stages of in-vivo cuff occlusion experiment, namely baseline, cuff inflation and cuff deflation. The baseline curve was averaged over first 15

seconds, inflation curve was averaged over 15th to 35th seconds, while deflation curve was averaged of only 35th to 45th seconds. The decay rate of $g_2(\tau)$ shows significant change. Figure 3.12(b) reveals rBF which was obtained by fitting $g_2(\tau)$ to the solution of photon diffusion correlation equation. The first 15 seconds, the blood flow was constant as no constriction was applied. Once the constriction was applied to the cuff, the blood flow dropped down sharply and when constriction was released, the blood flow immediately showed an overshoot followed by a slow decrease to the baseline due to homeostasis.

Figure 3.12 (a) Autocorrelation functions of cuff occlusion stages, namely baseline (blue solid line), cuff inflation (red dash-dot line) and cuff deflation (green dashed line). (b) Representative time response of relative blood flow (rBF) during cuff occlusion experiment, including baseline recording, cuff inflation (cuff occlusion period on the figure) and cuff deflation stages.

3.5 Summary

In this chapter, we have demonstrated an implementation of DCS system with an FFT-based software autocorrelator. The operation of the system was controlled by LabVIEW. The software autocorrelator has undergone the validation and assessment by an in-house flow phantom experiment and *in-vivo* cuff occlusion experiment on human arm. It is easy to operate and relatively

cost-effective. Besides, although the data sampling speed is moderate compared to conventional hardware ones ($\sim 100\text{ns}$), actually the minimum lag time is much shorter than the decay constant thus the extraction of desired parameter is still valid. We also expect the counting speed to improve by using a buffer with the bigger size. Furthermore, smoother starting and ending plateaus improve the accuracy in data fitting, and hence in blood flow index. In addition, the system still holds the potential of being a real-time flow indicator and can be used for future DCS system in research and clinical settings.

Chapter 4 Non-invasive Hemodynamic Response Monitoring of Chlorin e6-mediated Photodynamic Therapy using diffuse optical and correlation spectroscopy

Photodynamic therapy (PDT) is an emerging technique which is used in malignant tumor treatment. It involves the photochemical interaction among light, photosensitizer and tissue oxygen to destroy tumor tissues through direct cytotoxicity, vascular shutdown and stimulation of the body's immune response. Tumor response is dependent on treatment conditions; hence response monitoring is important for optimizing PDT efficacy. In this chapter, we apply the diffuse correlation spectroscopy (DCS) system which introduced in previous chapter, incorporating diffuse optical spectroscopy (DOS) to monitor the tumor response during PDT treatment. The hybrid system enables concurrent monitoring of relative blood flow (rBF), tissue oxygenation (StO_2) and total hemoglobin concentration (THC). Mouse models bearing xenograft tumors were subjected to chlorin e6 (Ce6)-mediated PDT. The study has two phases, CW-DOS and DCS were applied in phase I where FD-DOS and DCS were used in phase II. In phase I study, the StO_2 and rBF were presented pre- and post-PDT. Although there was no conclusive result due to small sample size, it was a good attempt to apply diffuse optics to PDT monitoring and validate instrumentation. However, in phase II study, by using StO_2 , THC and rBF , the tumor metabolic rate of oxygen ($TMRO_2$) can be extracted. The results showed: 1) At 3 hours post-PDT, relative StO_2 ($rStO_2$), rBF and relative $TMRO_2$

($rTMRO_2$) exhibited sharp Ce6-PDT induced decreases in responders (tumors with more than 40% reduction in volume). However, there were relatively small decreases in non-responders (tumors with re-growth) and minor fluctuations from baseline in controls (drug-only). Statistical result showed significantly different $rStO_2$ and rBF between responders and non-responders as well as responders and controls, while there was no significant change shown in $rTMRO_2$. 2) Comparing with the gradual recovery of $rStO_2$, rBF and $rTMRO_2$ from ~24 hours post-PDT in non-responders, those values in responder group maintained low value up till 48 hours post-PDT. Long-term study showed tumor $rStO_2$, rBF and $rTMRO_2$ initiative recovery at 1 week post-PDT and full recovery to baseline values at 2 weeks post-PDT. While the definite conclusion seems early to be drawn from small sample size, we correlate decreases up to ~48 hours post-PDT in these parameters to the long-term tumor control. The results suggest the hybrid diffuse optical system is not only capable of the real-time treatment monitoring, but also able to extract tumor metabolic rate of oxygen to provide more insights about therapy mechanisms. Translation of this technique to the clinic could make real-time prediction of treatment outcome feasible and help in clinical decisions on treatment optimization.

4.1 Introduction

Photodynamic therapy (PDT) is an emerging treatment modality for cancer. The U.S. Food and Drug Administration has approved PDT for the treatment of microinvasive lung cancer [121-123], obstructing esophageal cancer [124, 125],

and other cancers [126]. It destroys tumor tissues through direct cytotoxicity, vascular shutdown and immune response [127]. Recently, PDT gained popularity in malignant tumor treatment because of its advantages such as fewer side effects compared with radiation therapy and other conventional systemic therapies, and the treatment can be repeatedly employed in case a single treatment fails [128]. The PDT-induced damage in individual patients with a specific type of photosensitizer and treatment protocol is a result of the photochemical interaction of treatment light, photosensitizer dose and tissue oxygenation level. Therefore, monitoring the response of hemodynamic parameters such as blood flow and blood oxygenation is important for assessing efficacy of PDT [43, 129].

DOS, also known as diffuse reflectance spectroscopy (DRS), has recently emerged as a contender for tumor diagnosis and tumor therapy monitoring due to its several merits such as portability, cost-effectiveness, simplicity and capability of bedside monitoring. There are several research groups employing DOS in monitoring of PDT response [37, 130-132]. So far, most published works have applied continuous-wave DOS (CW-DOS), by fitting the detected light spectrum to the Green's function using the Mie Scattering assumption, or by applying the differential pathlength factor (DPF) method with the assumption of a constant scattering coefficient [133-135]. Unlike CW-DOS, frequency domain DOS (FD-DOS) and time-domain DOS (TD-DOS) are able to obtain both absorption and scattering information simultaneously.

Particularly, in FD-DOS, the light source intensity is modulated, and amplitude attenuation together with phase shift are detected. In TD-DOS, a light pulse with very narrow band (picoseconds) shines on sample surface, and the expansion of the light pulse is detected. Both techniques are slightly costly, but still contain the advantages of CW-DOS.

On the other hand, DCS is a promising technique for noninvasive measurement of deep tissue blood flow and has been successfully applied in hemodynamic studies of the brain [2, 38, 39, 136], human skeletal muscle [27, 28], and measurement of tissue burn depth [62]. The DCS technique relies on using the time-averaged intensity autocorrelation function of the fluctuating diffuse reflectance signal to extract tissue flow information with high temporal (~100ms) and moderate spatial (~mm) resolution. DCS has several intrinsic merits over other dynamic sensitive modalities such as Doppler Ultrasound, dynamic contrast enhanced magnetic resonance imaging (DCE-MRI) and positron emission tomography (PET) due to its non-ionizing radiation, no requirement of contrast agent, and moreover, its portability and cost-effectiveness. Therefore, this makes DCS an alternative method for bedside monitoring of tumor therapies, and in particular, in PDT response monitoring [15, 43, 133].

The hemodynamic changes in the tumor caused by PDT can modify treatment outcome, it is thus anticipated that methods sensitive to tumor hemodynamic post cancer therapy are of interest for early prediction of treatment outcome,

enabling oncologists to optimize and customize treatment regimens. In order to non-invasively and repeatedly measure PDT response, we utilized DOS and DCS in this study. Mouse models bearing xenograft tumors were subjected to chlorin e6 (Ce6)-mediated PDT treatment. In phase I study, CW-DOS and DCS were combined. The hemodynamic responses were monitored on PDT-treated tumors and tissue oxygen saturation (StO_2) and relative blood flow (rBF) during until 2 hours after PDT will be reported. While in phase II study FD-DOS and DCS were applied. The short term (up to 48 hours after PDT) and long term (up to 2 weeks after PDT) hemodynamic responses were monitored on treated and drug-only treated tumors. The hemodynamic changes, namely relative tissue oxygen saturation ($rStO_2$), relative blood flow (rBF), relative total hemoglobin concentration ($rTHC$) are reported together with the tumor size measurement. Moreover, with the functional parameters we obtained, the tumor metabolic rate of oxygen is able to be calculated in phase II study. Therefore, this study paves the way to aid clinicians to optimize and individualize treatment by adjusting treatment parameters with information of real-time hemodynamic responses.

4.2 Photodynamic therapy and mechanism of photodynamic action

The treatment process of photodynamic therapy (PDT) begins by the injection of the photosensitizer (also called PDT drug) into the bloodstream. With sufficient incubation time (also called drug light interval), high concentration of the drug will be accumulated at the target tumour site through the blood

circulation. Under this condition, it is optimal for laser treatment. The laser light with specific wavelength shines on the lesion tissue and is absorbed by the photosensitizer which induces cytotoxic species and kills the cells selectively. The whole procedure of PDT is reflected in Figure 4.1.

Figure 4.1 Procedures of PDT [137]

The effect of photosensitizer is caused by photodynamic action with cancerous cell. To date, there has been two proposed processes that explain the mechanism of cytotoxicity generation using photosensitizer [138, 139]. As shown in Figure 4.2, Upon absorption of the light, the PDT drug is excited and

turns singlet state photosensitizer; through intersystem crossing it populates a long lived triplet state. The type I process postulates the triplet state photosensitizer in the presence of water generates free radicals and peroxides, while type II process states that the triplet state photosensitizer reacts with oxygen in the tissue to convert normal triplet state oxygen to highly reactive singlet state oxygen. The product will result in the cytotoxicity via oxidation of cellular components. Note that so far type II process has been deemed to play a major part in destroying malignant tissues [139].

Figure 4.2 Two processes involved in generation of cytotoxicity for PDT [139]

There are three ways in which PDT is used to treat cancer. 1) Killing the cells directly through cytotoxicity of reactive oxygen species; 2) Destruction of tissue vasculature of the cancer tissue and 3) Activation of body immune system against cancerous tissue. In this study, we mainly focus on the hemodynamic response of the treated tumor.

4.3 Phase I: Tumor response monitoring by means of CW-DOS and DCS

4.3.1 Instrumentation

In CW-DOS system, a continuous wave tungsten halogen lamp with an SMA connector (HL-2000, Ocean Optics, USA) was applied and coupled into a multimode fiber (400 μm) as source. Another multimode fiber (400 μm) detected reflected signal at 0.8 *cm* away from source fiber and connected to a 16-bit A/D resolution spectrometer (USB 4000, Ocean Optics, USA). The single channel data collection was over a large range of wavelength, from 345 nm to 1041 nm; and the integration time used for the experiment was set to 3 seconds.

Figure 4.3 Data Acquisition Flow of CW-DOS

The system was controlled by LabVIEW, dealing with data acquisition and preprocessing. The working flow is shown in Figure 4.3. The electrical dark and dark signals were firstly required. “Electrical dark” (edark) file contains the background electrical noise without the light source turned on, namely the power of light is on but the shutter that controls light is off. The “dark” file contains noise from back reflected light within the lenses projection extension; it requires the light source is turned on. The rationale of saving each different data type is to rule out the noise. Subsequently, the following process was firstly made to eliminate the noise:

$$\begin{aligned}
 dark &= dark_m - edark \\
 R &= data_m - edark - dark = data_m - dark_m
 \end{aligned}
 \tag{28}$$

where $dark_m$ and $data_m$ were the measured spectrum of dark and sample, respectively, thus R was the reflected amplitude. After tabulating reflectance signal, the “ R ” file was subjected to moving window average with window size of 5, followed by trimming process where data points corresponding to the wavelength between 650 and 900 nm were remained. To extract hemoglobin concentrations, $\chi^2 = \sum_{\lambda} \left| R(\lambda, \mu_a, \mu'_s) - R_c(\lambda, \mu_a, \mu'_s) \right|^2$ was minimized using Nelder-Mead Simplex algorithm in Matlab (Mathwork, Inc.). R_c is the calculated diffuse reflectance in the semi-infinite geometry which has been reviewed in section 2.1.3. It was also assumed that scattering follows a Mie-type behavior and $\mu'_s = A\lambda^{-B}$ where $B=0.8$ was used. After calculating the concentration of chromophores (in our case are oxy- and deoxy-hemoglobin concentration namely $[HbO_2]$ and $[Hb]$, respectively), the physiological parameter tissue oxygen saturation StO_2 can be subsequently obtained,

$$StO_2(\%) = \frac{[HbO_2]}{[HbO_2] + [Hb]} \times 100\% \quad (29)$$

The DCS setup was the same as we mentioned in previous chapter.

The source-detector separation of both CW-DOS and DCS probes were 0.8 cm, so that the same sensitive volume was studied. The fiber connectors were held by a piece of rubber so that the distance between source and detector was fixed. During measurement, the hand-held rubber was slightly bended and gently placed on the tumor surface.

4.3.2 Measurement protocol

The experimental PDT treatment was approved by the SingHealth Institutional Animal Care and Use Committee. It was done on a male Balb/c mouse bearing xenograft tumors on the flanks. The mouse received Chlorine e6 (Ce6) at a dose of 5 mg/kg as photosensitizer. Approximately 4 hours later, the light treatment started. The whole PDT treatment was lasting for 65 minutes long, consisting of alternating 5 minutes exposure time and 10 minutes dark time. The treatment light used for PDT is a 665 nm medical laser (Biolitec, Germany). The light dose used was $100\text{J}/\text{cm}^2$, delivered at a rate of $80\text{mW}/\text{cm}^2$ via an optical fiber. The treatment light was expanded to 2.5 cm diameter on the tumor surface. Noninvasive diffuse optical measurements were carried out before and immediately after each exposure, as well as at 30 and 60 minutes after the last treatment. CW-DOS and DCS measurements were conducted alternately, as shown in Figure 4.4.

Figure 4.4 Measurement protocol of PDT treatment by using CW-DOS and DCS system.

Each measurement was last for 1 minute, with updating rate of 1 second. The mean $\pm$ standard deviation within 1-min duration will be reported.

4.3.3 Results and discussion

Figure 4.5 (a) Blood oxygen saturation (StO_2) and (b) relative blood flow (rBF) measured by CW-DOS and DCS pre- PDT, during PDT and post-PDT.

Figure 4.5 demonstrates the tissue oxygen saturation (StO_2) and relative blood flow (rBF) response during Ce6-mediated PDT treatment, shadow columns in the figure represent PDT light interval. The StO_2 decreased by $\sim 30\%$ with respect to the pre-PDT treatment, and $\sim 20\%$ increase of StO_2 can be observed 60 minutes after PDT concluded. The decrease of StO_2 reflects higher oxygen consumption, it may caused by vessels or photosensitizer, but it suggests Ce6 induced vascular changes in tumor. Nevertheless, compared to immediate previous dark period, a raising trend in StO_2 was observed right after each PDT light exposure. It provides another evidence of the vessel recovery. On the other hand, the mean tumor rBF decreased by $\sim 50\%$ immediately after the whole PDT, and descent to $\sim 40\%$ of baseline 60 minutes afterward. During each PDT dark interval, blood flow changes show minor fluctuation. They indicate impermanent vessel occlusion during PDT light intervals. These temporary vessel occlusions can be reversible, and it allows flow recovery during dark intervals. A similar trend was observed using laser Doppler measurements although different animal models were used in each case [140]. However, the results after 60 minutes suggest that permanent vessel occlusion had occurred due to PDT.

In the process of curve fitting to extract chromophore concentrations, the result was highly dependent on the choice of number of unknown parameters, initial

guess and other variables. Sometimes the negative value was obtained which did not make sense physically as the concentration was not supposed to be less than zero. Therefore, a more precise technique was necessary. In next session, the frequency domain DOS (FD-DOS) will be introduced, in which the scattering information was able to be separated from absorption information and the spectrum fitting process was not needed.

4.4 Phase II: Tumor response monitoring by means of FD-DOS and DCS

4.4.1 Instrumentation

In phase II study, a commercialized frequency domain tissue oximeter (OxiplexTS, ISS Inc., USA) with customized optical probe was used for DOS. The operating wavelengths of the system were 690 nm and 830 nm. Water concentration was assumed to be 75%. The contact optical probe was a fiber bundle consisting of 8 illuminating fibers and 1 detector fiber. The tips of the illuminating and detector fiber bundles in the probe are in alignment, with the source fibers at varying distances of 0.5mm to 4mm from the detector fiber. The schematic is shown in Figure 4.6.

Figure 4.6 Schematic of FD-DOS probe tip and illuminating area.

In this DOS system, the intensity modulated light at a frequency of 110MHz and a multi-distance method is used to acquire measurements of the phase shift and attenuation of the detected light to describe tissue optical properties. The method utilizes a physical model and does not depend on prior estimation of the scattering contribution or pathlength. By placing multiple source fibers at dissimilar distances from the single detector fiber, the amplitude of the light intensity oscillations AC, average light intensity DC, and phase shift ϕ of the detected light-intensity signal, can be measured as function of distance d . These measured parameters are related to the tissue optical properties by the diffusion approximation for semi-infinite homogeneous media as reviewed in section 2.1.2.

According to the Beer-Lambert law, once μ_a is determined, the concentrations of oxy-hemoglobin (HbO_2) and deoxy-hemoglobin (Hb) can be calculated:

$$[HbO_2] = \frac{\mu_a^{\lambda_1} \epsilon_{Hb}^{\lambda_2} - \mu_a^{\lambda_2} \epsilon_{Hb}^{\lambda_1}}{\epsilon_{HbO_2}^{\lambda_1} \epsilon_{Hb}^{\lambda_2} - \epsilon_{HbO_2}^{\lambda_2} \epsilon_{Hb}^{\lambda_1}} \quad (30)$$

$$[Hb] = \frac{\mu_a^{\lambda_2} \varepsilon_{Hb}^{\lambda_1} - \mu_a^{\lambda_1} \varepsilon_{Hb}^{\lambda_2}}{\varepsilon_{HbO_2}^{\lambda_1} \varepsilon_{Hb}^{\lambda_2} - \varepsilon_{HbO_2}^{\lambda_2} \varepsilon_{Hb}^{\lambda_1}} \quad (31)$$

where ε_{HbO_2} and ε_{Hb} are the extinction coefficients of oxy- and deoxy-hemoglobin respectively.

The DCS setup was the same as mentioned in previous chapter.

As measurements with hemodynamic and metabolic parameters can enhance sensitivity and specificity in therapeutic monitoring, we introduced a useful index that is tumor metabolic rate of oxygen consumption ($TMRO_2$) [14]. $TMRO_2$ provides valuable insight about tumor physiological responses to therapy, and hence explores dynamic signatures of tumors. It is calculated from the product of the fractional conversion of oxygen from arterioles to venules and tumor blood flow:

$$TMRO_2 = ([O_2]_A - [O_2]_V) \cdot BF \quad (32)$$

Here $[O_2]_A$ and $[O_2]_V$ are the tumor arteriolar and venous concentrations of oxygen respectively, and BF is the tumor blood flow. In steady-state, equilibrium between oxygen concentration and oxy-hemoglobin saturation in the arteriolar and venous compartments is assumed:

$$TMRO_2 = (S_A O_2 - S_V O_2) \cdot BF \quad (33)$$

Assuming that the changes of BFI index are proportional to the tissue blood flow changes, the tumor metabolic rate of oxygen becomes:

$$TMRO_2 = (S_A O_2 - S_V O_2) \cdot BFI \quad (34)$$

With the assumption that $S_A O_2 = 1$, the tumor metabolic rate of oxygen can be expressed as:

$$\begin{aligned} TMRO_2 &= (1 - S_V O_2) \cdot BFI \\ &= \frac{[Hb]}{THC} \cdot BFI \end{aligned} \quad (35)$$

4.4.2 Measurement protocol

The *in vivo* experiment was performed on male Balb/c mice (27g to 35g). EMT-6, a tumorigenic mouse mammary tumor cell line, was implanted subcutaneously in the rear flank of these mice 12 days before PDT. Tumors were treated when their diameters reached approximately 7 to 8 mm. Fur at the tumor region was depilated using a hair removal cream (Nair) before tumor cell inoculation and PDT treatment. This study was conducted according to the guidelines provided by the *SingHealth Institutional Animal Care and Use Committee (IACUC)*.

Mice were anesthetized with an intraperitoneal injection of ketamine/valium cocktail before each monitoring time point to ensure smooth measurements throughout both PDT and optical monitoring measurements. Before light

delivery, the photosensitizer Chlorin-e6 (Ce6) (ApoCare GmbH, Germany) was administered intravenously at a dose of 1 mg/kg or 5 mg/kg in mice. The drug-light interval of ~3 hours allowed selective accumulation in tumor tissue. The treatment light was delivered by a 665 nm medical-grade laser (Biolitec, Germany). The light dose used was (85-130) J/cm², delivered at a rate of (105-200) mW/cm² through an optical fiber. The treatment light illuminated a diameter of 2.5 cm on the treatment field for a continual duration of 25 minutes.

DOS and DCS were used to monitor the hemodynamic responses pre- and post-PDT. They were conducted before Ce6 injection, 2.5 hours after Ce6 injection, and 1-hour, 3-hour, 24-hour and 48-hour after finishing of PDT. Follow-up measurements were made until two weeks post-PDT and thereafter if further treatments were required. The tumor sizes, measured every 2 days beginning from 2 days prior to PDT, were calculated as $\frac{\pi}{6} [(d_1 \times d_2 \times d_3)]$ where d_1 , d_2 and d_3 refer to the length, width, and height of the tumor, respectively. The mouse models were categorized into responders (those with more than 40% tumor shrinkage at 48 h after PDT) and non-responders (those with tumor re-growth). For responders, 7 identical measurements were made to obtain the standard error of the mean ($\pm$ SE) distribution whereas for non-responders, 6 repeated measurements were acquired.

4.4.3 Results and discussion

Short-term study

The tumor volumes of PDT-treated mice at different time points are shown in Table 4.1.

Mouse No.	Pre-PDT baseline (mm³)	48 h after PDT (mm³)	% of baseline volume	Responder
1	239.1	97.9	40.9	Yes
2	164.6	46.0	27.9	Yes
3	158.8	40.2	25.3	Yes
4	142.7	81.4	57.0	Yes
5	156.2	81.5	52.2	Yes
6	123.5	70.3	56.9	Yes
7	184.9	109.3	59.1	Yes
8	287.0	435.4	151.7	No
9	155.1	183.8	118.5	No
10	108.4	158.2	145.9	No
11	113.8	117.7	103.4	No
12	252.8	362.6	143.4	No
13	118.5	182.3	153.8	No

Table 4.1 Tumor volumes before and after PDT

Figure 4.7 Averaged traces of (a) $rStO_2$, (b) rBF , (c) $rTHC$ and (d) $rTMRO_2$ normalized to baseline using 5mg/kg Ce6 in responders (n=7) and non-responders (n=6) pre- and post- PDT. Ce6-PDT was performed on tumors with total fluence of (105-200) J/cm² and a fluence rate of (85-130) mW/cm².

The mice were categorized into responder and non-responder groups according to predefined criteria. The averaged $rStO_2$, rBF , $rTHC$ and $rTMRO_2$ before and after PDT in tumors that were treated using a drug dose of 5mg/kg are shown in

Figure 4.7. PDT-induced changes were followed for up to 48 hours after the conclusion of treatment.

The $rStO_2$ levels in the responder group at 1, 3, 24 and 48 hours post-PDT were $81.4 \pm 4.2\%$, $65.9 \pm 5.3\%$, $78.3 \pm 2.4\%$ and $71.4 \pm 9.8\%$, whereas $rStO_2$ levels in non-responder group were $93.4 \pm 2.4\%$, $87.9 \pm 9.1\%$, $89.0 \pm 5.1\%$ and $87.2 \pm 8.9\%$ at the same time points. The rBF levels at 1, 3, 24 and 48 hours post-PDT were $101.5 \pm 6.7\%$, $55.1 \pm 5.7\%$, $42.3 \pm 3.6\%$ and $33.8 \pm 3.9\%$ in the responder group and $99.5 \pm 3.0\%$, $70.4 \pm 2.8\%$, $74.4 \pm 2.4\%$ and $84.5 \pm 2.7\%$ in the non-responder group. For $rTHC$, it was $113.0 \pm 9.3\%$, $116.9 \pm 4.6\%$, $167.4 \pm 11.9\%$ and $153.6 \pm 12.2\%$ in the responder group whereas $88.0 \pm 6.6\%$, $74.7 \pm 9.5\%$, $108.0 \pm 8.0\%$ and $120.3 \pm 4.0\%$ in the non-responder group at 1, 3, 24 and 48 hours post-PDT. Besides, we observe that the $rTMRO_2$ in the responder group at post-PDT time points initially increased to $108.2 \pm 5.2\%$ followed by substantial decreases to $69.0 \pm 3.4\%$, $50.1 \pm 2.4\%$ and $56.4 \pm 3.6\%$ at 3, 24 and 48 hours post-PDT, respectively. In non-responder group, the $rTMRO_2$ also showed an initial increase to $108.4 \pm 4.1\%$ but less decrease to $78.3 \pm 3.7\%$, $75.3 \pm 4.9\%$ and $88.8 \pm 6.8\%$ afterwards. The general behavior of $rTMRO_2$ was pretty similar to rBF in both groups.

It has been studied that the rapid response to the injury is to send mediators of host defense, leukocytes and plasma proteins, to the site of injury. This demand of delivery triggers changes in the vascular caliber, leading to an initial increase of blood flow to activate and spread the immune response deep into the inside

of tumor volume [141], as it portrayed in both rBF and $rTMRO_2$ at 1h post-PDT. In addition, we observed that at 3h post-PDT, both $rStO_2$ and rBF decreased below baseline in all treated tumors. In the responder group, an averaged dip of 34.1% from baseline in $rStO_2$ at 3h post-PDT implied more massive PDT-induced vessel destruction than the $rStO_2$ in the non-responder group which only decreased by 13.1%. Similar to the greatly reduced $rStO_2$ at 3h post-PDT, rBF in the responder group was measured to be a low $55.1 \pm 5.7\%$ whereas in non-responder group it was $70.4 \pm 2.8\%$ of baseline values. The considerably larger decrease in both $rStO_2$ and rBF levels in responders than that of non-responders at 3h post-PDT implies a greater degree of PDT-induced vessel damage in responders. The results demonstrated that animals with larger declines in $rStO_2$ and rBF at 3h post-PDT exhibited a more favorable treatment response. Therefore, we hypothesis that the amount of declines of $rStO_2$ and rBF at 3h post-PDT can be used to analyze how post-PDT tumor oxygenation and tumor blood flow correlate with treatment response; particularly the evident decrease indicates a favorable tumor response.

Responder vs Non-responder	$rStO_2$	rBF	$rTMRO_2$
1h post-PDT	0.0874	0.3540	0.3773
3h post-PDT	0.0047*	0.0336*	0.0664

Table 4.2 Comparison of $rStO_2$, rBF and $rTMRO_2$ at 1h and 3h post-PDT between responder and non-responder group, reported in p-value.

The t-test was conducted on $rStO_2$, rBF and $rTMRO_2$ at both 1h and 3h post-PDT. As shown in Table 4.2, the significant differences of $rStO_2$ and rBF between responder and non-responder group can be found at 3h post-PDT ($p<0.05$) but not at 1h post-PDT. The $rTMRO_2$ didn't show statistically significant difference at both 1h and 3h post-PDT.

In literature, in-depth investigations on monitoring of tumor oxygenation and blood flow in mouse models have been conducted using CW-DOS and DCS systems respectively [142, 143]. In these studies, apparent reductions in $rStO_2$ and rBF at 1h, 3h and 6.5h post-PDT were correlated with delayed tumor regrowth. In our case, although there weren't significant changes in hemodynamic parameters especially in rBF and $rTMRO_2$ indices at 1h post-PDT due to the overlapped distributions, the Receiver Operating Characteristic (ROC) curve was used compute area under the curve (AUC) so that performance of these indices in characterizing tumor response could be determined. At 1h post-PDT, it was 0.82 and 0.71 for rBF and $rTMRO_2$ in responders while 0.84 and 0.74 respectively in non-responders. In addition, we found the rBF and $rTMRO_2$ maintains low value in responder group, whereas those of non-responders started to recover from 24h post-PDT and back closely to baseline reading at 48h post-PDT. We assumed this behavior was correlated to the long-term tumor response, which will be elaborated in long-term study section.

In another short-term study involving mice with two tumors each, only one tumor was irradiated. We present the average values of $rStO_2$, rBF , $rTHC$ and $rTMRO_2$ (Figure 4.8) in the treated and drug-only tumors in the mice from the responder group.

Figure 4.8 Averaged traces of (a) $rStO_2$, (b) rBF , (c) $rTHC$ and (d) $rTMRO_2$ normalized to baseline using 5mg/kg (n=7) Ce6 in treated and drug-only tumor in responder group pre- and post- PDT. Ce6-PDT illuminated mice tumors with (105-200) J/cm² at (85-130) mW/cm²; drug-only controls received Ce6, but were not irradiated. Data are presented as means of $\pm$ SE.

While average tumor $rStO_2$, rBF and $rTMRO_2$ progressively decreased with time after PDT in treated tumors, minor fluctuations about the baseline were found in the drug-only controls. The average $rStO_2$ and $rTMRO_2$ deviated within 13.7% and 28.7% across all time points while rBF varied within 9.4% from baseline. Table 4.3 shows the p -value calculated by applying paired t-test on $rStO_2$, rBF and $rTMRO_2$ at both 1h and 3h post-PDT. Similar to the result of responder vs non-responder group, at 3h post-PDT, both $rStO_2$ and rBF showed significant difference between treated-tumor and drug-only group but not for $rTMRO_2$. However, at 1h post-PDT, there are no significant differences of $rStO_2$, rBF and $rTMRO_2$ between two groups.

Treated-tumor vs drug-only	$rStO_2$	rBF	$rTMRO_2$
1h post-PDT	0.2422	0.6341	0.3678
3h post-PDT	0.0021*	0.0334*	0.0750

Table 4.3 Comparison of $rStO_2$, rBF and $rTMRO_2$ at 1h and 3h post-PDT between treated-tumor and drug-only group among responders, reported in p-value.

The antitumor effects of PDT result from the combination of direct killing of malignant cells, destruction of tumor vasculature, and immunologic effects. PDT can have an influence on tumor vasculature, as reactive oxygen species (ROS) generated may cause vascular shutdown, thus depriving the tumor of

nutrients. Comparing to the small variation around baseline in control mouse, monitoring hemodynamic response in real-time facilitates in-depth understanding of tumor physiological responses to therapy. The similar declines in prostate StO_2 and rBF were observed after illumination in the study done on prostate patients [144], although the PDT illumination was given at several intervals for the whole gland to be treated. These anti-vascular effects can be correlated with tumor regression, on human as well as mouse model.

Variation with photosensitizer dose

To compare effects of varying the photosensitizer dose, we administered a dosage of 5mg/kg and 1mg/kg in 2 groups of mice. Figure 4.9 represents the values of $rStO_2$, rBF , $rTHC$ and $rTMRO_2$ using these two doses.

Compared to the tumor treated with 5mg/kg Ce6, both $rStO_2$ and rBF levels of the tumor treated with 1mg/kg Ce6 dose showed small decreases at 3h post-PDT. At 24h post-PDT, steep decreases to levels nearly as low as the readings in the 5mg/kg group were seen. This sharp decrease of $rStO_2$ and rBF from 3h to 24h post-PDT might be due to hypoxia rendered by PDT. Hypoxia could occur when hypoxic cells are not adequately exposed to anticancer drugs. It has been demonstrated that the surviving hypoxic subpopulation promotes tumor propagation as it increases mutation rates and expression of genes associated with angiogenesis, and promotes migration of tumor cells to more distant sites, thereby permitting the cells to escape their hostile environment. By enhancing

metastasis, treatment outcome is compromised [145]. Hence, the development of vasculature repair or angiogenesis could explain the increase of $rStO_2$ and rBF toward baseline at 48h post-PDT in low dosage group.

Figure 4.9 Average (a) $rStO_2$, (b) rBF , (c) $rTHC$ and (d) $rTMRO_2$ levels of treated tumors using drug doses of 5mg/kg (n=7) and 1mg/kg (n=2) at pre- and post-PDT. Mice were subjected to a (105-200) J/cm² light dose administered at a fluence rate of (85-130) mW/cm².

The $rTMRO_2$ in both treated groups show distinct differences at 3h and 24h post-PDT, with the high dose group displaying steep decreases and the low dose group stabilizing at baseline levels. However, the stability in the low dose group was not maintained at 48h post-PDT as the $rTMRO_2$ drops by 27.6%. This decline could be due to reoxygenation induced by tumor angiogenesis.

Long-term study

Recall the results of the short term study, we had demonstrated that animals with larger declines in the $rStO_2$, rBF and $rTMRO_2$ at 3h, 24h and 48h post-PDT exhibited a good treatment response. We hypothesized that concurrent decreases in $rStO_2$ and rBF levels from baseline up till 48h post-PDT would correlate to long-term tumor control. To verify this, the correlation between vascular response during PDT and the long-term response to treatment was explored.

Generally, tumor response in responders for long-term study behaved in a similar manner as of that in the responders for the short-term measurement. In Figure 4.10(a), $rStO_2$ decreased to $83.9 \pm 10.4\%$ at 1h post-PDT; the 3h post-PDT depression was most representative of tumor hypoxia and vascular shutdown induced by PDT. At 24h post-PDT and 48h post-PDT, $rStO_2$ showed minor increases to $68.5 \pm 5.1\%$ and $64.4 \pm 6.5\%$ respectively, but remained lower than the pretreatment levels. Similarly, rBF in Figure 4.10(b) started with $100.7 \pm 10.8\%$ at 1h post-PDT and made a substantial decrease to $41.1 \pm 2.2\%$ at

3h post-PDT that suggests tumor vascular shutdown. At 24h post-PDT and 48h post-PDT, rBF remained extensively lower than baseline. Full recovery to baseline was observed at approximately 14 days after PDT. In Figure 4.10(d), $rTMRO_2$ followed the behavior of rBF . It was $110.1 \pm 7.0\%$ at 1h post-PDT, but remained low value of $61.7 \pm 4.4\%$, $68.7 \pm 10.9\%$ and $51.4 \pm 12.8\%$ at 3h, 24h and 48h post-PDT, respectively. All the functional parameters began their recovery towards baseline at 7 days post-PDT, and returned toward baseline at approximately 14 days after PDT.

Figure 4.10 Long-term variations of (a) $rStO_2$, (b) rBF , (c) $rTHC$ and (d) $rTMRO_2$ levels of the treated tumor at pre- and post-PDT. A total fluence of (105-200) J/cm^2 was delivered to the animals at a fluence rate of (85-130) mW/cm^2 . Post-PDT response monitoring was made up till two weeks post-PDT (n=3). Error bars represent the standard error of the mean.

The tumor response to PDT treatment corresponded with a black eschar at the treatment site 48 h after treatment (Figure 4.11(a)) followed by eradication of tumor and healing of lesion at 2 weeks post-PDT (Figure 4.11(d)). A complete response to PDT was observed, with no sign of tumor recurrence up to 3 months post-PDT.

Figure 4.11 Corresponding tumor images of the treated tumor in Fig. 7 at (a) 2 days, (b) 4 days, (c) 1 week, and (d) 2 weeks after PDT.

Comprehensive understanding of the relationship between the degree of PDT-induced vascular damage and tumor response has been reported in many studies. In these studies, treatments that are unsuccessful in inducing complete vascular shutdown will not bring about long-term tumor control [146-149]. As such, the importance of vascular-mediated damage in tumor eradication after PDT is demonstrated and therefore monitoring tumor blood flow post therapy is essential in prediction of long term cure. In our long term study, our data shows that better tumor responses to PDT correlate with larger decreases in $rStO_2$, rBF and $rTMRO_2$ at 3h, 24h and 48h post-PDT. The initiation of recovery at 1 week post-PDT and their full recovery to baseline values at 2 weeks post-PDT are consistent with our hypothesis that the decreases in these parameters up till 48h post-PDT correlated to long-term tumor control. Similarly, in a study with head and neck tumors [12], decreases of StO_2 and rBF readings in the complete responders starting from 2 weeks after PDT were correlated with complete shutdown of tumor vessels and consequently effective tumoricidal effect. On

the other hand, the partial responders that had delayed tumor re-growth instead of tumor eradication, showed a gradual increase in the StO_2 and rBF values throughout the weeks post therapy. Therefore, we could find the potential of diffuse optical instrumentation in distinguishing responders and non-responders, even partial responders from complete responders. The combination of tumor $rStO_2$, rBF and $rTMRO_2$ can be indicative of PDT efficiency in producing long-term regression of treated tumors.

Limitation

Comparing to the CW-DOS measured system which was introduced in section 4.2 and more commonly used in most published works, FD-DOS has its intrinsic advantages since it is able to separate absorption information from scattering, namely the absorption coefficient μ_a and reduced scattering coefficient μ'_s can be obtained simultaneously. The exact value of μ_a and μ'_s will benefit the DCS fitting directly. However, since our FD-DOS is the system with only two wavelengths, it is only capable of obtaining maximum two chromophore concentrations. In PDT treatment, the appearance of photosensitizer Ce6 might affect the calculated results of oxygenated and deoxygenated hemoglobin concentrations because Ce6 is an absorber agent also. Considering the extinction coefficient of Ce6 was about 5240 and 39.6 at 690 nm and 830 nm, in order to assess how much effect is caused by Ce6, we conducted the simulation by varying the concentration of Ce6 from 0 to 10^{-5} molar. The result of simulation is shown in Figure 4.12. As Ce6 concentration

changes from 0 (0%) to 10^{-5} molar (100%), the corresponding changes of HbO_2 , Hb and StO_2 are 2.33%, -4.56% and 2.47%, respectively. They are neglectable comparing to the significant hemodynamic changes we have reported in previous sections. Therefore, although it would be better if three wavelengths system could be applied here, our results still remain valid using current dual wavelengths (690 nm and 830 nm) FD-DOS system.

Figure 4.12 Simulation result of the corresponding changes of HbO_2 , Hb and StO_2 when the concentration of photosensitizer Ce6 varied from 0 (0%) to 10^{-5} molar (100%).

4.5 Summary

In this study, we have utilized a combined optical system incorporating both DOS and DCS which can noninvasively measure in vivo hemodynamic changes

in real-time. By applying multi-modal diffuse optical instrument, noninvasive monitoring of the *in vivo* hemodynamic parameters during PDT can be achieved. In phase I study, CW-DOS and DCS were used. The results reflected the hemodynamic parameters changes mediated by Ce6 for mouse, by showing the decrease of both StO_2 and rBF after PDT treatment. However, as complicated fitting process was necessary and could affect the result significantly, the phase II study was conducted, where FD-DOS and DCS were applied and more precise measurement protocol was made. The combined diffuse optical system facilitated not only rapid real-time determination of tissue oxygen saturation, total hemoglobin concentration and blood flow measurement in tumors, but also enable the extraction of tumor metabolic rate of oxygen. The measurements show that tumor response can be predicted as early as 3h post PDT by assessing variations in $rStO_2$, rBF and $rTMRO_2$ levels. Significant changes in these levels from 3h to 48h post-PDT can substantiate tumor response predicted by the hemodynamic responses at 3h post-PDT. In long-term study, although the definite conclusion seems early to be drawn in this case due to small sample size, we observed the initiative recovery of in $rStO_2$, rBF and $rTMRO_2$ at 1 week post-PDT and full recovery to baseline values at 2 weeks post-PDT in responders, which were consistent with the hypothesis that the decreases in these parameters up till 48h post-PDT correlated to long-term tumor control.

Further experimental studies using increased sample size are ongoing. Current data exhibits a vascular component to PDT response, suggesting a potential role

for tumor oxygenation and blood flow monitoring as a mean for real-time PDT treatment planning or rapid efficacy assessment. By presenting promising therapeutic usefulness of noninvasive tumor oxygenation and blood flow measurements, we expect the monitoring of the in vivo hemodynamic changes within tumors before and after PDT to provide information on the extent of cellular and vascular damage and lead to improved strategies. Furthermore, translation of this technique to the clinic could make feasible real-time prediction of treatment outcome using hemodynamic measurements and manage clinical decisions on treatment optimization.

Chapter 5 Sample Entropy Analysis of Diffuse Reflectance Signal

Sample Entropy (SampEn) is a measurement of complexity, gaining increasing popularity in biomedical time series data analysis. In this chapter, we apply SampEn to the diffuse-reflected photon count signal which was obtained by our DCS system and study whether this quantity can be used for deep tissue blood flow assessment. SampEn holds the merits such as computationally light weight, simple in concept (model-free), and suitable for real-time analysis. It has been shown in this study that the SampEn can be a good indicator for relative blood flow, supported by flow phantom experimental data and *in-vivo* cuff occlusion data on human forearm and toe.

5.1 Introduction

The utilization of entropy in the assessment of physiological time course data has been increasing over the past decades. As one of the non-linear complexity measures, approximate entropy (ApEn) was introduced by Pincus in 1991 [150]. It determines the conditional probability of similarity between a chosen data segment and the rest with the same data length and provides solutions to the problems with short and noisy data which often appears in physiological processes. The sample entropy (SampEn), a modified version of ApEn, is a less biased statistic because it avoids counting self-matches of segments, and has other advantages such as data length-independence [151] and robustness under

noise [152]. It has been applied on several physiological time series analysis such as heart rate variability (HRV) in diseased and aging condition [153], cardiac arrhythmias such as atrial fibrillation (AF) [154, 155], the electroencephalographic background activity in Alzheimer's patients [156], and most recently skin micro-vascular function detected by laser Doppler flowmetry (LDF) [157].

On the other hand, in an effort to extract tissue blood flow information out of diffuse reflectance signal, researchers have been developing and utilizing diffuse correlation spectroscopy (DCS) technology that use time varying optical transmission signal [158]. It is established based on photon diffusion theory in highly scattering medium. When a beam of coherent light is incident on the surface of a turbid medium, photons migrate over different path lengths before emerging and form speckles at the detector positions. The movements of the scattering particles result in fast fluctuation of these speckles in time. A faster movement of the scattering particles causes more rapid speckle fluctuation, and it renders the time-series data to lose its self-similarity sooner. Hence, blood flow information in tissue can be extracted by measuring the decay time of the autocorrelation function of photon count signal. To date, DCS has been a promising blood flow probing modality on variety of tissues such as breast [14], brain [38, 159] and muscle [160].

In typical DCS system, in order to obtain autocorrelation function with small delay times (hundreds of nanoseconds to several microseconds), the high speed

photon count detecting system is required. For calculating autocorrelation of detected photons, besides most of the work in literature use hardware correlator [42], we demonstrated a software correlator approach with a promising performance in previous chapter and it has been reported [94]. However, in order to obtain a stable tail in the autocorrelation function, long data acquisition time is necessary. The blood flow information can be subsequently extracted by fitting the autocorrelation function with either Brownian motion or random flow model. There are some challenges in this step: firstly, these models assumed that the system is ergodic so that the Siegert relation could be applied to make the relationship between autocorrelation function of electric field and that of light intensity, but it could not be tested rigorously. Secondly, the autocorrelation curve fitting deviates much from either Brownian motion or random flow model as shown in previous chapter. So far, based on the goodness of fit, majority of researchers have used Brownian motion model to obtain the flow information, though the random flow model makes more sense for capillary flow. Lastly, model fitting requests the optical properties of sample, which is normally assigned with empirical values or obtained by combining other diffuse optical modality [42]. Therefore, a complementary instrumentation is necessary to make DCS result more accurate. All these challenges in DCS instrumentation and data analysis lead us to explore an alternative approach to obtain relative blood flow information, more efficient in speed, and free in model as well as sample optical properties.

In this chapter, instead of calculating autocorrelation function and subsequently fitting the model to assess the flow, we demonstrate the use of the Sample Entropy (SampEn). From instrumentation perspective, it does not require high speed photon counting detector since the sampling time requirement is relatively low and the SampEn can be calculated with a relatively small number of data. From signal processing perspective, it is not based on fitting the model and it does not require the input of sample optical properties. Therefore, these advantages taken together, it is possible to make the DCS instrumentation less costly and faster, by using a reasonably fast photodiode and by using less number of data points, respectively; and enable the independent application of DCS.

5.2 Theoretical background of Sample Entropy

5.2.1 Sample Entropy

Sample entropy (SampEn) is a measure of unpredictability of a given time series data. In other words, it is a measure of complexity [151]. It is the negative logarithm of the probability that, given the fact that two patterns of length m are close to each other within the distance of r , the distance between two patterns of length $m+1$ will be still less than r . Here the pattern length m is called embedded dimension, and r is called tolerance. More precise definition is given below.

Let us assume a time series data of length N , $\{x_1, x_2, \dots, x_N\}$ with a time interval of τ . A template vector of length m within the time series is defined as $x_m(i) = \{x_i, x_{i+1}, \dots, x_{i+m-1}\}$, and we count the number of template vector pairs that are within the distance r from one another. For the distance function $d[x_m(i), x_m(j)] (i \neq j)$, the Chebychev distance (maximum coordinate difference) is normally used. One counts the number of template vector pairs that satisfies $d[x_m(i), x_m(j)] < r$, and SampEn is defined as follows:

B = number of length m template vector pairs that satisfies $d[x_m(i), x_m(j)] < r$.

A = number of length $m+1$ template vector pairs that satisfies $d[x_{m+1}(i), x_{m+1}(j)] < r$.

$$SampEn(m, r) = -\ln \frac{A}{B} \quad (36)$$

Note that SampEn is always positive value, since A is always smaller than B .

5.2.2 Multiscale Sample Entropy

On top of the normal definition of SampEn described above, one slight modification can be made such that template vectors are defined with an interval between each element, in which a tunable parameter δ is introduced [161, 162]. In other words, we can sample the data with a skipping parameter δ , as $x_{m,\delta}(i) = \{x_i, x_{i+\delta}, x_{i+2\delta}, \dots, x_{i+(m-1)*\delta}\}$. Then the sample entropy can be defined with another argument such that

$$SampEn(m, r, \delta) = -\ln \frac{A_\delta}{B_\delta} \quad (37)$$

where A_δ and B_δ are defined the same way as above, with modified template vector $x_{m,\delta}(i)$. Typical parameter values being used are $m=2$ and $r=0.2 \times std$, where std is the standard deviation of the total time series data. The reason behind this multiscale definition of sample entropy is because the normal SampEn is highly affected by the degree of autocorrelation, as will be explained in later sections. A simple implementation of multiscale SampEn in MatLab can be found on the web [163].

5.3 Experiment

In order to obtain the transmitted photon count signal, the same datasets from in-house flow phantom as mentioned in Chapter 3 section 3.3.2 and from *in-vivo* cuff occlusion experiment on human arm as mentioned in Chapter 3 section 3.3.3 were analyzed. Besides, a control cuff occlusion experiment was also conducted on the toe of a healthy human subject, by applying the cuff pressure around calf. The source-detector distance was 1.5 cm and the maximum inflating cuff pressure was 220 mmHg as well.

5.4 Results

5.4.1 Flow phantom results

SampEn was calculated from the time series photon count data. This data was obtained through the flow phantom experiment with varying amount of steady flow. The effective sampling rate was 100 kHz (400 kHz sampling with software binning of 4), making the sampling time to be $10\ \mu\text{s}$. The parameters for SampEn calculation were $m=2$, $r=0.2 \times std$, and δ was varied among 1, 2, 4 and 8, which translate into delay times of 10, 20, 40, and $80\ \mu\text{s}$, respectively. A data length of $N = 4096$ was used for one SampEn calculation, and ten data points were used to calculate the average and standard deviation.

The result is shown in Figure 5.1. A nonlinear behavior is observed between the SampEn and flow, and higher δ value results in higher SampEn, which is consistent with the decaying behavior of the autocorrelation function. Since their relationship is monotonous, the graphs in Figure 5.1 can be used as a calibration curve between SampEn and flow, which enables conversion of measured SampEn value into the flow value. Note, however, that this calibration curve cannot be used for obtaining the absolute flow value, because the data in Figure 5.1 is from a specific geometry of the phantom we used. The volume fraction α of moving particles within the whole sensitivity volume of the source-detector pair will highly affect the transmission measurement as well.

Despite the limitation, we attempted to extract an empirical model of the SampEn-flow relationship so that *in-vivo* SampEn results could be translated into flow values. We used logarithmic function to fit the data in Figure 5.1, and used the fitted function as a calibration curve. The *in-vivo* result from the cuff experiment will be shown in the next section, where this calibration curve will be used.

Figure 5.1 SampEn calculated from flow phantom data using different delay times. The photon count data were binned by 4 registers, rendering the effective sampling time to be $\tau_0 = 10\mu\text{s}$. Therefore, delay times for $\delta = 1, 2, 4$, and 8 are $10, 20, 40$, and $80\mu\text{s}$, respectively. SampEn increases as delay time increases, as it loses self-similarity. SampEn shows nonlinear but monotonously increasing behavior as a function of flow.

The outcome of SampEn calculation depends on such arbitrary parameters as pattern length m and tolerance r , but so far there has been no consensus in literature as what values to use. In practice, m is chosen to maximize B (number of length m template vector pairs that satisfies $d[x_m(i), x_m(j)] < r$) in order to have a better estimate of the conditional probability and r must be larger than most of the noise. Generally, one could intuitively consider the number of matches increases if relatively small m and large r are chosen. Since SampEn is an assessment as to how tougher it becomes to find matching patterns when the pattern length increases by one, small tolerance r tends to generate higher SampEn (i.e., harder to find pattern match) while large r tends to render SampEn to approach 0 (i.e., most of the existing match still remains matching after pattern length increases by one). In order to investigate the effect of m and r in our case, we varied them individually using fixed delay time ($\delta = 4$) at flow speed of 0.125 ml/s. As shown in Figure 5.2(b), SampEn decreased fast as r increased, while it showed mild decrease as m increased. Note the errorbar size is actually similar between Figure 5.2(a) and Figure 5.2(b).

Figure 5.2 Dependence of SampEn of (a)length m (with fixed $r = 0.2 \times \text{std}$) and (b)tolerance r (with fixed $m = 2$), calculated from flow phantom data using fix delay time $\delta = 4$ (40 μs) at speed of 0.125 ml/s. SampEn decrease as pattern length and tolerance increases.

5.4.2 Cuff occlusion result

The Figure 5.3(a) shows SampEn values during baseline, cuff occlusion and cuff release on human arm with varying delay factor δ . The parameters for SampEn calculation were the same as flow phantom experiment, namely $m=2$, $r=0.2 \times \text{std}$, and $N = 4096$. Errorbars represent the standard deviation over ten SampEns. Figure 5.3(b) shows the value of $g_2(\tau)-1$ during the cuff occlusion experiment, where $g_2(\tau)$ is the normalized intensity autocorrelation function of

the optical transmission data. They were averaged over the first 5 seconds for baseline, over 6 to 13 seconds for inflation, and over 14 to 18 seconds for deflation.

Figure 5.3 SampEn and autocorrelation function during arm cuff experiment. (a) Three representative datasets are shown for baseline, occlusion, and release period. Different colors correspond to different time delay used for SampEn calculation, as shown in legend. (b) Autocorrelation functions for the same three representative datasets. The corresponding delay times are indicated on the autocorrelation curve for comparison. The time delays of $4\tau_0$ and $8\tau_0$ show the best contrast in SampEn, which can be understood from the contrasts in the autocorrelation curve.

One can observe that with the same delay factor δ , SampEn showed good contrast between different stages of the cuff occlusion protocol. Especially, the biggest contrast in SampEn appeared at $4\tau_0$ and $8\tau_0$ delay times, which can be understood from the contrasts in the autocorrelation curve in Figure 5.3(b). We also note that for any given stage, higher δ value results in higher SampEn value, just as results in flow phantom. This is also due to the loss of self-similarity as the delay factor increases.

To better visualize the time behavior of SampEn during cuff occlusion experiment, the SampEns were calculated with parameters of $m=2$, $r=0.2 \times std$, $N = 4096$, and $\delta=4$, and plotted with time resolution of 1 second in Figure 5.4(a). Since the sampling time is $4\tau_0 = 40 \mu s$, one SampEn calculation covers about 160 ms, generating five SampEn data per second, out of which the average and standard deviation can be calculated. For the first 5 seconds, the SampEn was quite stationary as no cuff pressure was applied. Once the pressure was applied to the cuff, the SampEn dropped down to a significantly lower value, and when it was released, it immediately showed an overshoot (reactive hyperemia), followed by a slow decrease to the baseline value due to homeostasis. As mentioned in the previous chapter, we attempted to translate the SampEn into the relative flow value by using the phantom result (Figure 5.1) as the calibration curve. The solid line in Figure 5.4(b) reveals the converted relative flow during cuff protocol. The overall pattern of the whole behavior was preserved by this conversion, but significant change in standard

deviation is observed due to the nonlinearity of the calibration curve. The variations of relative flow during cuff occlusion stage were smaller while in release stage it became larger than that of SampEn. Comparing to the result obtained by fitting the autocorrelation function to Brownian model (dashed line in Figure 5.4(b)), the estimated blood flow index (BFI) exhibited the consistency with the relative blood flow (rBF) obtained by DCS.

Figure 5.4 (a) SampEn calculated from cuff occlusion data. (b) Solid line: same data converted into relative flow value by the relationship obtained from phantom experiment. Dashed line: the rBF obtained by fitting the autocorrelation function to Brownian motion model, and the data value refers to the right y-axis. Yellow lines indicate starting and ending of the cuff occlusion.

Figure 5.5 shows the time behavior of SampEn during cuff occlusion experiment on toe. For this time, the cuff inflation time was 1 minute and we monitored the deflation up to 1 minute also, mainly due to the assumption that response on the toe is slower because it locates at far extremities distal to the cuff position. The SampEns were calculated with parameters of $m=2$, $r=0.2 \times std$, $N = 4096$ and $\delta=4$, also. The similar behavior was observed as the cuff experiment on the arm, except the response of occlusion and release of cuff came later than it was on the arm. (Note the different time scale between Figure 5.4 and Figure 5.5) It suggests that the blood perfusion on extremity has significantly more time delay.

Figure 5.5 SampEn calculated from toe measurement during cuff occlusion on calf. Yellow lines indicate starting and ending of the cuff occlusion.

5.5 Discussion

First, a few remarks must be made regarding the procedure of normal DCS data analysis. The correlation diffusion equation was derived by Boas et al. from correlation transportation theory [51] and formed the basis of the current DCS technology. Briefly, the blood flow index ($=\alpha D_B$, in Brownian motion case) is obtained by fitting the electric field temporal autocorrelation function (calculated from measured temporal intensity autocorrelation function via Siegert relation) to the Green's function of the correlation diffusion equation that satisfies the boundary conditions in semi-infinite homogeneous media. It involves the fitting process of the two parameters (β and αD_B) that are related to detector size and flow respectively, as well as two optical parameters (absorption and reduced scattering coefficient) of the tissue. Reconstruction of all the four parameters at the same time is considered impractical because of the ill-posed nature of the problem, and normally the two optical parameters are fixed to the values that are measured by other methods such as frequency-domain diffuse optical spectroscopy. So far, the DCS technology based on this two-parameter fitting method has been known to be quite stable and it was applied in many clinical areas including brain, breast, and muscle. But the model-fitting procedure inherently increases computational load, and puts limitations in real-time operation and quantitative measurement.

In search of better ways to extract flow information from the same time series data, we borrowed the concept of entropy from information theory and applied

it to DCS analysis. To our knowledge, it is the first time to apply the SampEn to diffuse reflectance signal to quantify the flow information. The main advantage of our approach lies in the simple algorithm of the analysis and light-weight computation. In our analysis, we have used the data length of $N = 4096$ regardless of the delay time, a significantly lower number of data compared to the one we used for autocorrelation calculation ($N \sim 100,000$). The algorithm of SampEn calculation requires $O(N^2)$ number of pair comparison for given data length, but it takes only $O(N)$ comparison for updating the SampEn for each added data point afterwards. Therefore, SampEn analysis is suitable for real-time analysis of DCS signal. Also, our results in Figure 5.4 show that delay time of 40 to 80 μs was optimal to view the blood flow contrast in *in-vivo* cuff protocol, suggesting that a regular avalanche photodiode with a data acquisition (DAQ) card of reasonable speed will work just as well for practical purpose of monitoring physiological blood flow change. This means a significant cost reduction is possible compared to the current DCS paradigm which uses photon-counting avalanche photodiode and a hardware correlator (or a fast counter with software correlator).

The fact that SampEn analysis does not require an underlying model leads us to very simply necessities for analysis: the data itself, and no curve fitting or input of sample optical properties. As the converted flow value is based on the calibration curve, more accurate curve is critical to improve the accuracy of SampEn approach. From this preliminary study, we only claim that SampEn

analysis is useful in semi-infinite geometry with fixed optical properties and further study needs to be done for other geometries as well as varied optical properties. In transmission geometry, for example, we expect the SampEn to behave similarly as semi-infinite geometry because the decay rate of the autocorrelation function would still increase as flow increase, but the calibration curve between SampEn and real flow might look quite different. However, as most of the clinical optical probing in practice is done in reflection mode, our semi-infinite geometry result will be the most useful and suitable for real time blood perfusion assessment during a procedure or treatment.

We used the stable result of flow phantom experiment as a calibration curve to calculate the relative flow value of *in-vivo* cuff occlusion experiment. Ideally, the boundary condition and the probe configuration such as source-detector separation should be considered before converting SampEn value into flow value, but we can still use SampEn as a surrogate indicator of deep tissue blood flow in relative sense. Without performing complicated model fitting, the flow information was shown to be obtained easily with a reasonable accuracy; additionally the hardware constraint and the computational load could be effectively decreased. Further studies to investigate the use of SampEn in general case are underway, in order to establish it as a more robust indicator of flow.

5.6 Summary

In this chapter, we have applied sample entropy (SampEn), a measure of complexity, to the analysis of diffuse reflected photon count signal for the first time. Using the same experimental setup as diffuse correlation spectroscopy (DCS), we have demonstrated that SampEn is a good indicator for relative flow: signals from higher flow resulted in higher complexity. Both the flow phantom and *in-vivo* cuff occlusion experiments support it. We examined the dependence of SampEn on the delay parameter δ , and verified that higher δ results in higher SampEn value which makes a good sense from the autocorrelation argument. Besides, through the analysis of dependence of SampEn on the pattern length m and tolerance r , we found the SampEn tends to decrease as m or r increase. Using the result of phantom as calibration function, we were able to estimate the relative flow values from the SampEn, which are highly consistent with the results we obtained from conventional DCS fitting method. The authors believe this model-free method for quantifying the relative flow has tremendous merit in terms of reducing the computational load and hardware requirements, and it will be useful in clinical setting where real-time flow assessment is necessary.

Chapter 6 Deep Tissue Flowmetry Based On Diffuse Speckle Contrast Analysis

Either the FFT software autocorrelator based DCS or sample entropy analysis on diffuse reflectance signal, they are both operated in single channel. Albeit they are more cost-effective comparing to other DCS systems, as long as simultaneous working of multiple channels is needed, more sources and detectors are unavoidable. In an effort to exploit deep tissue flowmetry with multiple channels in an ingeniously way, rely on the concept of laser speckle contrast imaging (LSCI), a method named diffuse speckle contrast analysis (DSCA) will be introduced in this chapter. By means of CCD camera, it shows that the same level of deep tissue flow information can be obtained from much simpler analysis on the spatial distribution of the speckles by presenting both the flow phantom experiment and in-vivo cuff occlusion data. The establishment of DSCA not only is the requirement of instrumentation reduced considerably, but also paves the way for depth-sensitive blood flow measurement in the future work.

6.1 Introduction

Diffuse correlation spectroscopy (DCS) is a non-invasive technique to obtain the relative blood flow (rBF) in deep tissue, which has already been widely adopted in clinical studies. Taking advantage of low power near infrared (NIR) laser and flexible geometry of source and detector, DCS is particularly suitable for in-vivo bedside monitoring study on human subjects, including infants.

In typical DCS system, rBF is extracted from temporal autocorrelation function of the fluctuating transmission signal, which is normally obtained by a hardware correlator [164] or a counter board together with FFT-based software correlator [94] as shown in literature review section. Both methods need a very sensitive detector, such as photon-counting avalanche photodiode (APD) or photomultiplier tube (PMT), and multiple detectors are needed for concurrent multi-channel operation. Thus a typical DCS setup is relatively expensive, especially for multiple-channel detectors which is necessary to achieve depth-selective flow measurement.

To provide an alternative approach to assess deep tissue perfusion, a diffuse speckle contrast analysis (DSCA) method, which estimates the flow value based on the analysis of the speckle pattern in spatial domain, is proposed. In this system, the same light source with a long-coherence-length as typical DCS system was applied. But different from typical DCS system, a high sensitivity CCD camera was used instead of the photon-counting detector. In order to acquire the diffuse laser speckle pattern, the CCD takes images of a small area

on the surface of the subject to. As a result, rBF of certain depth can be extracted from diffuse laser speckle pattern.

6.2 Theoretical Background

In traditional DCS, the fluctuation of one single speckle is detected. By applying Siegert relation, the electric field autocorrelation function $G_1(\tau)$ can be obtained from intensity autocorrelation function $G_2(\tau)$. Furthermore, since $G_1(\tau)$ satisfies the correlation diffusion equation as reviewed in section 2.1.5, the blood flow index can be derived.

Meanwhile, flow-induced speckle fluctuations will also result in the reduction of laser speckle contrast in space for a given exposure time, where the speckle contrast is defined as the ratio of the standard deviation to the mean intensity across many detectors or pixels, namely

$$K_s = \frac{\sigma_s}{\langle I \rangle} \quad (38)$$

Here, the subscript s refers to the spatial, as opposed to temporal, variations [165, 166]. To establish the relationship between K_s and normalized temporal electric field autocorrelation function $g_1(\tau)$, starting from the variance of intensity across the pixels is a simple way [167, 168]. For a given exposure, the signal of each pixel from an image array is proportional to the total number of photons it receives. As a result, from the beginning of exposure ($t=0$) with the

total duration of the exposure T , the signal at pixel i is proportional to the time-average of the intensity $I_i(t)$:

$$S_{i,T} = \frac{1}{T} \int_0^T I_i(\tau_1) d\tau_1 \quad (39)$$

An image array with total number N of pixels, contains N sets of $\{S_{i,T}\}$ where the index i ranges from 1 to N . Thus, the n th-moment of the distribution of pixel signals is

$$\langle I^n \rangle_T = \frac{1}{N} \sum_{i=1}^N (S_{i,T})^n \quad (40)$$

We should note that here these moments represent an ensemble average over pixels for fixed exposure duration. Especially, the first-moment is the average of intensity, namely

$$\langle I \rangle = \frac{1}{N} \sum_{i=1}^N S_{i,T} \quad (41)$$

The second-moment is the average over pixels of $(S_{i,T})^2$, where

$$(S_{i,T})^2 = \frac{1}{T^2} \int_0^T \int_0^T I_i(\tau_1) I_i(\tau_2) d\tau_1 d\tau_2 \quad (42)$$

As this is the ensemble average, the Siegert relation (equation 25) is valid.

Using the definition of normalized autocorrelation function of intensity $g_2(\tau)$

and of temporal electric field $g_1(\tau)$, Siegert relation can be rewritten as following:

$$\langle I_i(\tau_1)I_i(\tau_2) \rangle = \langle I \rangle^2 \left\{ 1 + \beta [g_1(\tau_1 - \tau_2)]^2 \right\} \quad (43)$$

As a result, the given intermediate result for the second-moment becomes

$$\langle I^2 \rangle_T = \frac{\langle I \rangle^2}{T^2} \int_0^T \int_0^T \left\{ 1 + \beta [g_1(\tau_1 - \tau_2)]^2 \right\} d\tau_1 d\tau_2 \quad (44)$$

Note that $g_1(\tau)$ is an even function, by doing the integration the normalized variance can be defined as:

$$\begin{aligned} V(T) &= [K_s(T)]^2 \equiv \frac{1}{\beta} \left[\langle I^2 \rangle_T / \langle I \rangle^2 - 1 \right] \\ &= \frac{1}{T^2} \int_0^T \int_0^T [g_1(\tau_1 - \tau_2)]^2 d\tau_1 d\tau_2 \\ &= \frac{2}{T} \int_0^T (1 - \tau / T) [g_1(\tau)]^2 d\tau \end{aligned} \quad (45)$$

The above equation shows that the variance of pixel intensities measured with exposure time T can be regarded as a weighted average of $|g_1(\tau)|^2$, with higher weightage towards $\tau = 0$. In LSCI a wide field illumination is used rather than point illumination, and $g_1(\tau)$ is commonly assumed as a simple exponential form. Thus, normal LSCI is suitable for flow measurement of superficial layer

inside relatively transparent medium. Note that equation 45 holds in general cases where $g_1(\tau)$ might deviate from simple exponential decay, although it has been most useful in single scattering situation so far.

Moreover, according to equation 45, K_S of the speckle pattern on the sample's surface is determined by $g_1(\tau)$ of the same position. If we recall the theory of DCS in section 2.1.5, Green's function solution in semi-infinite geometry of the correlation diffuse equation is given by [46]

$$G_1(r, \tau) = \frac{3\mu'_s}{4\pi} \left[\frac{\exp(-k_D(\tau)r_1)}{r_1} - \frac{\exp(-k_D(\tau)r_2)}{r_2} \right] \quad (46)$$

Under Brownian motion assumption, $k_D = \sqrt{3\mu'_s\mu_a + 6\mu_s'^2 k_0^2 \alpha D_B \tau}$.

The normalized $G_1(\tau)$ which is given by

$$g_1(r, \tau) = G_1(r, \tau) / G_1(r, 0) \quad (47)$$

will be used in equation 45. Therefore, within the same medium, K_S is determined by flow rate, source-detector separation and exposure time.

6.3 Experiment

6.3.1 Comparison of diffuse correlation spectroscopy and diffuse speckle contrast analysis

Figure 6.1 (a) Schematic of a traditional DCS setup, where D is a photon counting detector and a hardware correlator. (b) Schematic of DSCA setup, where the CCD camera takes images of the speckle pattern on the sample surface. Shaded region in the sample represents the sensitivity volume of the source-detector pair.

Comparison between proposed DSCA setup and traditional DCS setup is shown in Figure 6.1. DSCA shares the same light source with DCS system, which is fiber-coupled point illumination of long coherence length laser. In contrast to the traditional DCS which uses single mode fiber as detector, however, DSCA uses lens coupled CCD chip to take images of the speckle pattern on the surface of the sample. The banana-shaped sensitivity volume for a source-detector pair is drawn in Figure 6.1 as shaded region. Although the sensitivity volume in Figure 6.1(b) has more spread shape near the detector, we can divide the CCD image into small segments and treat each segment as a separate detector, which

has a sensitivity volume similar to the one shown in Figure 6.1(a). Since the CCD image covers relatively large area, we can acquire multi-channel detection from single exposure by this segmentation.

6.3.2 Experimental setup of diffuse speckle contrast analysis

To validate the DSCA method, a control experiment was conducted on a flow-phantom which was designed and made in our lab. Details about the in-house phantom building were described in section 3.3.1. [94]. The optical properties of the solid body were $\mu'_s = 8\text{cm}^{-1}$ and $\mu_a = 0.03\text{cm}^{-1}$. The hollow tube was filled up with small beads to randomize the flow direction.

The experimental setup is shown in Figure 6.2. The light source was a continuous-wave laser with a long coherence length ($>10\text{m}$) operating at 785 nm (DL785-100-S, $\sim 100\text{mW}$, CrystaLaser, Reno, Nevada, USA). An EMCCD (Cascade 512F, Photometrics) was used as detector. A $f=35\text{ mm}$ biconvex lens was used as imaging lens, with a magnification of 1, and a lens tube (not shown in figure) was used along the light path between the sample and CCD to prevent exposure to stray lights. The EMCCD chip size, which was the same as the imaging area, was about $8 \times 8\text{ mm}^2$, where the individual pixel size was $16 \times 16\text{ }\mu\text{m}^2$. The imaging region covered a source-detector separation range from 1.6 ~ 2.4 cm, within which the sensitivity volume of larger separation overlapped more with the flow region in phantom (see Figure 6.2). In addition, the experiment was also conducted on other surface (the lower surface in Figure

6.2) of the phantom, where the minimum distance from the surface to the flow cylinder is 3 cm, with the same source-detector geometry.

Figure 6.2 Schematic of the flow-controlled phantom experiment. The raw speckle image is shown beside the setup in pseudocolor. The processed K_s distribution, where each point is calculated from 100×50 pixels, is given at the top right corner.

During experiment, Lipofundin N 20% (B.BraunMelsungen AG, Germany) with concentration of 0.6% was pumped through the cylindrical tube, with different rates ranging from 0 to 0.10 ml/s in steps of 0.02 ml/s. The light source and detector were placed on the upper surface of the phantom, and 10 images were taken for every single flow rate, with exposure time of 0.1 ms. For comparison, the same protocol was applied on the lower side instead of the upper side of the phantom, so that sensitivity volume does not overlap with the actual flow. We kept all the conditions the same as the actual flow experiment,

including the flow rate, in order to examine the effect of sensitivity volume only.

For better illustration of the data acquisition and processing in DSCA system, Figure 6.2 also shows the raw speckle image from the imaging area and the processed K_S distribution. The raw speckle images are normalized by the smooth intensity background which is averaged over 100 speckle images. K_S is calculated from every segment of $100(\text{vertical}) \times 50(\text{horizontal})$ pixels, and the processed speckle contrast distribution is shown at the top right part in Figure 6.2. Thus we have 10 different source-detector separations from each row.

6.3.3 *In-vivo* cuff occlusion experiment on human arm

To validate the biomedical application of DSCA system, as shown in Figure 6.3, in-vivo experiment on human arm was performed together with a normal time-domain DCS system [94] simultaneously, sharing the same light source and source-detector separation of 1.8 cm. The imaging rate of DSCA system could reach up to 30 Hz, while the update rate of the DCS system was 1 Hz. The protocol which included three stages were as follow: baseline lasted for 90 seconds, cuff occlusion lasted for 90 seconds, and cuff release lasted for 120 seconds. Arm cuff pressure we applied on the subject's upper arm during the occlusion period was 200 mmHg.

Figure 6.3 *In-vivo* cuff occlusion experiment on human arm with simultaneous normal time domain DCS and proposed DSCA measurements. They share the same light source which was guided through multimode fiber (source fiber in the figure). For traditional DCS, the detector fiber was a singlemode fiber which was connected to a photo-count module; for proposed DSCA the detector was a CCD camera.

6.4 Results and discussion

6.4.1 Phantom study

The main results of phantom study are summarized in Figure 6.4. Figure 6.4(a) shows experimental $1/K_S^2$ values as a function of flow rate for two different

situations. When the flow region is within the sensitivity volume, $1/K_s^2$ increases as flow rate increases (blue line). However, with the same source-detector separation, when the flow depth is out of the sensitivity volume, $1/K_s^2$ is not affected by the flow rate (black line). Therefore, it can be confirmed that the DSCA system is only sensitive to the flow within the sensitivity volume.

The result of phantom study with varied flow speed within sensitive volume is plotted in Figure 6.4(b). Particularly, the dependence of $1/K_s^2$ on the flow rate is shown for different source-detector separations. It can be observed that the $1/K_s^2$ behaves linearly along with changing the flow rate over a broad range of the imaging area. On the other hand, the sensitivity which can be regarded as the slope of each line increases as source-detector separation increases.

Figure 6.4 Summary of experimental data. (a) $1/K_s^2$ plotted against flow rate, for comparison between the flow depth inside and outside the sensitivity volume, at the same source-detector separation of 2.32 cm. (b) $1/K_s^2$ as a function of the flow rate for 3 source-detector separations. The slope can be regarded as the sensitivity to the flow rate.

In addition, according to numerical simulation of equation 41, the simulation was conducted where $g_1(\tau)$ was invoked by equation 42 with varied αD_b (Brownian motion model was assumed here) over a large range. As shown in Figure 6.5, $1/K_s^2$ tends to exert linearly as the change of αD_b . Comparing with the result of flow phantom experiment, it behaved consistently with simulation result.

Figure 6.5 Numerical simulation of $1/K_s^2$ as a function of αD_b , with exposure time T of 0.1 ms.

Moreover, in accordance with our definition of sensitivity, where the slope of the $1/K_s^2$ -flow rate curve was used, the dependence of sensitivity on source-detector separation was also studied. The flow sensitivities calculated from all

ten available source-detector separations ranging from 1.6-2.4 cm in a single CCD image are plotted in Figure 6.6, where it is clearly shown that larger overlap with the flow region results in higher sensitivity to the flow. This demonstrates that multi-depth measurements can be conveniently achieved by single exposure in DSCA system, which may enable a depth-specific measurement of deep tissue blood flow.

Figure 6.6 Flow sensitivities measured at 10 different source-detector separations.

6.4.2 *In-vivo* cuff occlusion validation on human arm

Figure 6.7 Simultaneous measurement of rBF by DCS and DSCA systems during arm cuff occlusion protocol.

$1/K_s^2$, which is used as the indicator for blood flow dynamics in DSCA system, is obtained from an area of 50×50 pixels. Good correlation between the rBF measured by normal DCS system and DSCA system is demonstrated in Figure 6.7. Similar to LSCI, exposure time plays an important role in DSCA system. From reported in-vivo DCS results on human limbs, $g_1(\tau)$ is most sensitive to rBF in $\tau = 0.02 \sim 1$ ms range [46, 94]. In this experiment, 0.1 ms was chosen as the exposure time, considering the balance between SNR and speckle contrast. Comparing with LSCI, relatively bigger averaging pixel number is necessary to guarantee the accuracy of K_s , possibly compromising the imaging resolution. Note, however, the purpose of DSCA is to obtain the average deep-tissue flow

rate midway between the source and detector, and resolution is not immediate concern. If needed, higher imaging magnification can be used to overcome the resolution issue, although it will shorten the span of source-detector separation.

6.5 Summary

In conclusion, by combining with diffuse correlation theory we applied laser speckle contrast analysis (LSCA) in rBF measurement inside deep tissue for the first time. Comparing with DCS system, the new proposed DSCA system significantly reduced the cost, increased the sampling rate, and simplified the analysis. The validation study of the DCSA system was performed using an in-house flow phantom and human arm cuff occlusion protocol. The inherent multi-channel measurement capability may find applications in real-time depth-sensitive flow measurement.

Chapter 7 Conclusion and Future Perspectives

To sum up, under this project, near infrared diffuse optics-based flowmetry for non-invasive deep tissue perfusion assessment has been developed and presented. In brief, starting from building FFT software autocorrelator based single channel real-time diffuse correlation spectroscopy (DCS) system which followed by utilization on tumor response monitoring during photodynamic therapy (PDT) on mice, sample entropy (SampEn) analysis was subsequently used on raw photon count signal which was obtained from above mentioned software autocorrelator based DCS system. Moreover, based on the concept of laser speckle contrast image (LSCI), a new method named as diffuse speckle contrast analysis (DSCA) was explored, and a multi-channel system based on this new approach for deep tissue blood flow measurement was initiated. The performance of diffuse optics-based flowmetry is promising, while utilizing non-ionizing, inexpensive, rapid, portable and non-invasive instrumentation.

The FFT software autocorrelator based DCS system was operated by LabVIEW, and the validation study was conducted by an in-house flow phantom experiment and *in-vivo* cuff occlusion experiment on human arm. It is easy to operate and relatively cost-effective. Moreover, smoother starting and ending plateaus of autocorrelation curve obtained from software autocorrelator improve the accuracy in data fitting, and hence in rBF .

In terms of application of our DCS system, the tumor response monitoring during PDT treatment was conducted on mice model. In Phase I study, the DCS system was combined with a continuous wave diffuse optical spectroscopy (CW-DOS) system, whereas in Phase II study the combination of a frequency domain (FD)-DOS was utilized. The results showed the capability of non-invasively assessing blood flow information during PDT treatment in real-time, and it is believed to be beneficial to the diagnosis as well as medical treatment.

Afterwards, for the first time in author's knowledge, the SampEn analysis was applied to diffuse reflected photon count signal which was recorded by our DCS system. With the same experimental setup as DCS, we have demonstrated via both flow phantom and *in-vivo* cuff occlusion experiments that SampEn is a good indicator for relative flow: signals from higher flow resulted in higher complexity. This model-free method for quantifying the relative flow has tremendous merit in terms of reducing the computational load and hardware requirements, and it will be useful in clinical setting where real-time flow assessment is necessary.

Although both DCS and laser speckle contrast image (LSCI) technologies have been around for decades, to our knowledge there has been little study to apply laser speckle contrast analysis in deep tissue flow measurement. The new proposed DSCA relies on the concept of LSCI, by means of combining with diffuse correlation theory, an inherent multi-channel flowmetry was implemented by using point NIR laser source illumination and CCD detection

to enable measurement of rBF in deep tissue. The new system was validated by flow phantom as well as *in-vivo* experiments together with our aforesaid DCS system. The DSCA system significantly reduced the cost, increased the sampling rate, and simplified the analysis. Not only can it be regarded as an alternative approach of conventional DCS, but also it is possible to be applied in real-time depth-sensitive flow measurement.

For future work, we can take the cue from SampEn to study further on extracting blood flow information without model fitting, either from raw photon count signal itself or from autocorrelation function of the raw signal. On one hand, it will simplify the data processing procedure; on the other hand, without the extra information such as optical properties of the sample we may obtain motion information of scatterers directly.

In terms of the development of multi-channel diffuse optics-based flowmetry, DSCA would be a good approach. The CCD detection enables the inherent multi-channel property of the system. However, more optimization studies are necessary before applying the instrument to the clinical settings. For example, the study of optimal exposure time would be important since it relates to the temporal resolution directly. In addition, by making use of flow phantom with several flow layers embedded, the depth-sensitive flow measurement can be realized in a more precise way.

Moreover, as the near infrared diffuse optics-based flowmetry has been built, we can broaden its application to several clinical cases where real-time flow measurement is essential. Besides the tumor response monitoring during PDT treatment we have demonstrated in Chapter 4, for instance, it can also be utilized to angioplasty surgery which is one of a treatment options for peripheral artery disease (PAD). Especially for lower limb PAD patient, the tissue blood perfusion on lower extremity is very important for assessment of treatment effect. Thus, a real-time instrument to provide instant deep tissue blood perfusion information in a non-invasive manner is quite crucial. Besides, we can also apply the technology to brain study by easily combining with other instrument. We believe the big potential in clinical practice will point the future direction of instrumentation.

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R. Bi, **J. Dong**, K. Lee. “*Deep Tissue flowmetry based on diffuse speckle contrast analysis*”, Optics Letters, Vol. 38, Issue 9, pp. 1401-1403 (2013)

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- **Book Chapter**

J. H. Ho, **J. Dong**, K. Lee. “Diagnostic and Therapeutic Breast Cancer Imaging, Vol. 4 - Diffuse Optical Imaging in Breast: Recent Progress,” Multimodality Breast Imaging- Diagnosis and Treatment, *SPIE Press*.

- **Conference Proceedings and Presentations**

J. Dong, R. Bi, K. Lee. “Sample Entropy of Light Transmission as a Blood Flow Indicator”. *Proceedings of SPIE Vol. 8580, Photonics West 2013*

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