Application of gain-modulated image intensifier in biomedical imaging

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**APPLICATION OF GAIN-MODULATED IMAGE
INTENSIFIER IN BIOMEDICAL IMAGING**

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SUMMARY

In recent years, the incorporation of image intensifiers into charge-coupled-device (CCD) devices has resulted in intensified CCD (ICCD) systems, which possess high sensitivity in low light conditions, as well as high temporal resolution. Such ICCD systems are typically used in fluorescence applications such as fluorescence lifetime imaging (FLI), but seldom used in the context of diffuse optical tomography (DOT). Thus, this project is focused towards developing novel experimental methods and instrumentation for both DOT and FLI systems, especially with the incorporation of image intensifiers.

Diffuse optical tomography (DOT) is a promising, non-invasive biomedical imaging technique that is especially suitable for imaging of the breast, brain, muscle and joints. DOT makes use of transmission measurements for various source-detector pairs on the sample surface, which can be used to reconstruct a 3D spatial distribution of optical properties within the sample, namely absorption and scattering coefficients. Since accurate determination of both absorption and scattering properties in homogeneous diffuse medium is critical in DOT applications, we came up with a new experimental technique to measure both absorption and reduced scattering coefficients (μ_a and μ_s') in transmission geometry, based on a homodyne approach using a RF-modulated laser source and a gain-modulated image intensifier. In addition, we also explored similar setups for

DOT image reconstruction, in various domains and geometries, and presented the corresponding experimental procedures and image reconstruction results.

On the other hand, fluorescence lifetime imaging (FLI) is a powerful imaging technique which can provide valuable information about the molecular microenvironment of a fluorophore within a medium. More specifically, it is sensitive to local pH changes and the presence of quenchers such as oxygen and ions, which can be potentially useful for diagnosis and monitoring of diseases. Frequency-domain systems have significant advantages over time-domain systems in terms of cost savings and simpler instrumentation. Thus, in this report, we presented our own frequency-domain experimental setup and procedures, and conducted a preliminary study on fluorescence dyes for system validation. This provides an initial insight into the design of FLI experiments using the image intensifier, as well as paves the way for future work involving frequency-domain FLI systems.

1. Introduction

The first attempts to use gain modulation in the image intensifier for frequency-domain massive data acquisition can be found in Sevick-Muraca's group [1-3], although they still used detector fibers on different locations of the sample surface which delivered fluorescence signal directly onto the CCD chip. More recently, there have been reports of totally non-contact schemes utilizing either heterodyne [4] or homodyne [5] measurements. For instance, homodyne work from Hielscher's group [5] utilized modulation frequencies up to 1 GHz, and presented good results especially with small size samples. In general, although heterodyne measurements provide higher SNR than their homodyne counterparts, they are constrained in terms of the range of CCD exposure times that can be used, since the resulting image is changing in intensity at the cross-correlation frequency.

Thus, this project is geared towards building homodyne frequency-domain DOT and FLI systems, with the incorporation of image intensifiers, in order to allow simultaneous massive parallel data acquisition and phase-sensitive detection. Although such modulated ICCD systems are typically used in fluorescence lifetime imaging (FLI) [6, 7], they are seldom used in DOT. The newly developed DOT system may significantly improve the accuracy of DOT in the quantification of tissue optical properties, via using multiple harmonics to improve the level of resolution between absorption and scattering. On the other hand, the FLI system is

used in preliminary studies which provide an initial insight into the design of FLI experiments using the image intensifier, as well as pave the way for future work involving frequency-domain FLI systems.

In order to establish a basic background framework, Chapter 2 explains in detail the internal workings of the image intensifier, its major components, as well as fundamental operational principles. In addition, Chapter 3 provides an extensive review of related literature under DOT, which includes the background theoretical model and related derivations, the various kinds of instrumentation and image reconstruction algorithms, as well as related physiological parameters.

Accurate determination of both absorption and scattering properties in homogeneous diffuse medium is critical in DOT applications. Thus in Chapter 4, we report a new method to measure both absorption and reduced scattering coefficients (μ_a and μ_s') in transmission geometry, based on a homodyne approach using a RF-modulated laser source and a gain-modulated image intensifier. We demonstrate an efficient way to analyze the phase-sensitive images using an approximate formula from the well-known analytical Green's function solution in slab geometry. Furthermore, we discuss possible improvements in measurement accuracy using the higher harmonics of the modulation frequency, hence leading to multi-frequency diffuse optical imaging.

Equipped with a method that can be used to determine optical properties of a turbid medium accurately, as discussed in Chapter 4, we can then proceed to explore similar setups for DOT image reconstruction, in various domains and geometries. Thus in Chapter 5, we present in depth the corresponding experimental procedures and image reconstruction results and also compare the image quality of different reconstructed images obtained from changing reconstruction parameters.

In Chapter 6, we provide a brief introduction to fluorescence lifetime imaging (FLI), the underlying theoretical framework as well as the kinds of instrumentation. We also present our frequency-domain FLI experimental setup and procedures, based on a homodyne approach using a RF-modulated laser source and a gain-modulated image intensifier. We have also conducted a preliminary study which investigates the possibility of fluorescence emission from one dye being absorbed by adjacent dyes, which in turn may affect the final fluorescence lifetime results. This is achieved via comparing the results obtained from simultaneous excitation of all dyes, with results from separate excitation of individual dyes. The comparison shows that there is little or no influence.

Lastly, in Chapter 7, we present our overall concluding remarks from the above studies in both DOT and FLI, as well as future potential development directions.

2. The Image Intensifier

2.1 Introduction

An image intensifier is essentially a vacuum tube, typically with a bore of diameter in the range of 16-25 mm [8]. It encompasses a coating of semiconductor or multi-alkali layers on the interior of the input window, also called the photocathode input, as well as a fluorescing phosphor screen on the interior of the output window, as shown in Figure 1 [9]. In addition, simple grid-shaped electrodes in the early days or a more complex electron-multiplying microchannel plate (MCP) (Figure 2) [10] developed more recently have been incorporated, in order to accelerate electrons through the tube.

Figure 1. Image Intensifier's Interior [9].

Figure 2. Microchannel Plate [10].

In general, the operation of the image intensifier is as follows [8]. A fraction of the incident photons striking the photocathode causes the release of electrons, due to the photoelectric effect. Such electrons are then accelerated via electrodes, and also multiplied in more recent MCP-based intensifiers, thus moving towards the phosphor screen, where they strike the coating and cause the release of light. This released light can provide many times more photons for every single incident photon striking the photocathode.

Over the years, the demand for night vision within the military has brought about the development of image intensifiers [11]. Military imaging devices have been

specifically customized for use in the near-infrared (NIR) range (650-900 nm), which corresponds to the main illumination conditions in night battles. This popularity of image intensifiers in military contexts has resulted in the embrace of their military naming conventions in non-military applications as well. In the order of technology development, the various kinds are divided into categories known as generations, which currently comprise of Gen I, Gen II, Super-Gen II (or Gen II+), and Gen III [11].

It started with electrostatically focused Gen I tubes featuring high image resolution, a wide dynamic range and low noise [11]. Gen I passive devices, introduced during the Vietnam War, relied on ambient light instead of an infrared light source. Using an S20 photocathode made of potassium-caesium-antimony and sodium-potassium-antimony, their image intensifiers produced a light amplification of around 1,000 times. In addition, the S20 photocathode had a sensitivity of around 150 to 200 $\mu\text{A}/\text{lm}$. The additional sensitivity made these tubes usable with limited light such as moonlight, while still being suitable for use with low-level infrared illumination.

Gen II image intensifiers used the same multi-alkali photocathode as that of Gen I tubes [11]. However, by using thicker layers of the same material, the S25 photocathode was developed, which provided extended red response and reduced

blue response, making it more suitable for military applications. In addition, the micro-channel plate (MCP) was introduced for much higher gain in Gen II image intensifiers. By using the MCP with a proximity focused tube, amplifications of up to 30,000 times with a single MCP layer were possible. By increasing the number of MCP layers, additional amplification to well over 1,000,000 times could be achieved.

Although not formally recognized under the U.S. generation categories, Super-Gen II was developed in 1989 by Jacques Dupuy and Gerald Wolzak [11]. This technology improved the tri-alkali photocathodes to more than double their sensitivity and reduced the noise level. This allowed such tubes, which were more economical to manufacture, to achieve comparable results to Gen III tubes.

Gen III image intensifiers were fundamentally similar to Gen II tubes, except for two major differences [11]. Firstly, they used a gallium arsenide photocathode which is more sensitive in the 800-900 nm range than Gen II photocathodes. Secondly, the photocathode exhibits negative electron affinity (NEA) which provides photoelectrons excited to the conduction band with a free ride to the vacuum band, as the caesium oxide layer at the edge of the photocathode causes sufficient band-bending. This makes the photocathode very efficient at creating photoelectrons from photons. However, the drawback was that they are seriously

degraded by positive ion poisoning. Due to the high electrostatic field stresses in the tube and the operation of the MCP, this led to failure of the photocathode within a short period - as little as 100 hours before the photocathode sensitivity dropped to below Gen II levels. To protect the photocathode from positive ions and gases produced by the MCP, they introduced a thin film of sintered aluminium oxide attached to the MCP. The high sensitivity of this photocathode, which is greater than 900 $\mu\text{A}/\text{lm}$, allows more effective low light response, though this was offset by the thin film, which typically blocked up to 50% of electrons.

In the context of non-military applications, the coupling of Gen III image intensifiers to highly sensitive charge-coupled-device (CCD) cameras has led to intensified CCD (ICCD) systems in spectroscopic and imaging applications, which can provide high temporal resolution of less than 2 ns, as well as high sensitivity in low light conditions. Such ICCD systems are currently used in fluorescence applications such as fluorescence lifetime imaging microscopy (FLIM) [6, 7, 12], laser-induced fluorescence (LIF) [13-15], and single-molecule fluorescence imaging [16, 17].

2.2 Components

2.2.1 Photocathode

The first key element of an image intensifier is the photocathode [8, 18]. The coating on the photocathode converts a fraction of the incident light photons impinging on the photocathode into electrons. The remaining incident photons not captured by the photocathode are lost from the final intensifier output signal. Thus, an important parameter for intensifiers is known as quantum efficiency (QE), which is termed as the proportion of photons impinging on the photocathode which are converted into electrons. Early-stage intensifiers used multi-alkali coatings with satisfactory photo-conversion performance at visible and ultraviolet wavelengths, but relatively unsatisfactory performance in the NIR region [19]. For instance, S20 (Gen I) and Super S25 (Super-Gen II) have a QE of less than 20% [19] in the NIR region, as shown in Figure 3. In later generations, the Gen III GaAs series of photocathodes have higher QE (25-50%) [19] in both visible and NIR regions, when compared to earlier generations. In practice, this means either an improvement in the lifetime accuracy or a shorter exposure time for Gen III, under similar settings for both Gen III and Gen II.

Figure 3. Sensitivity versus wavelength (nm) for various photocathodes [8, 19].

2.2.2 Microchannel plate (MCP)

The MCP is a thin layer made of glass, with a diameter and thickness of roughly 2 cm and 0.5 mm respectively, and it is mildly conductive [8, 18]. It comprises of millions of parallel traversing micro-channels coated with a secondary electron emitter such as caesium iodide and copper iodide on their wall interiors. Electrons from the photocathode are accelerated through the channels via a constant electric field applied to the MCP with a voltage of 600-900 V [18]. A fraction of the electrons going through the channels then collide with the walls, resulting in the formation of more electrons. Multiple collisions lead to many thousands of electrons that eventually exit the MCP, for every electron which undergo collision with the walls, as shown in Figure 4. In addition, the MCP input surface, which encompasses up to 50% of the MCP, is normally coated with nichrome with a low secondary electron emission coefficient, in order to provide electrical contact among the micro-channels. This means that electrons missing channel openings and striking the input surface are still able to create secondary electrons, which can be pulled into neighbouring channels via electrostatic forces. This provides substantial recovery of electronic charge that will otherwise be lost due to electrons missing channel openings. In the case of a photomultiplier tube, it consists of a number of electrodes called dynodes. Each dynode is held at a more positive voltage than the previous one. The electrons leave the photocathode, having the energy of the incoming photon (minus the work function of the photocathode). As

the electrons move toward the first dynode, they are accelerated by the electric field and arrive with much greater energy. Upon striking the first dynode, more low energy electrons are emitted, and these electrons in turn are accelerated toward the second dynode. The geometry of the dynode chain is such that a cascade occurs with an ever-increasing number of electrons being produced at each stage. Finally, the electrons reach the anode, where the accumulation of charge results in a sharp current pulse indicating the arrival of a photon at the photocathode. Thus in principle, each MCP channel operates analogously to a PMT (Figure 4).

Figure 4. MCP channel versus Photomultiplier tube (PMT) [8, 18].

2.2.3 Phosphor screen

The phosphor screen is the third key element of an image intensifier [8, 18]. Electrons exiting the MCP are driven forward by a constant voltage of 5-8 kV before impinging on the phosphor screen, where they are converted back into light photons for CCD detection. Phosphor screens are normally manufactured from rare earth oxides or halides (gadolinium, lanthanum, yttrium), with decay times in the order of a few hundred nanoseconds (ns) to a few milliseconds (ms). A material can emit light either through incandescence, where all atoms radiate, or by luminescence, where only a small fraction of atoms, called emission centers or luminescence centers, emit light [20]. In inorganic phosphors, these inhomogeneities in the crystal structure are created usually by addition of a trace amount of dopants, impurities called activators. (In rare cases dislocations or other crystal defects can play the role of the impurity.) The wavelength emitted by the emission center is dependent on the atom itself, and on the surrounding crystal structure. The above general discussion gives a brief overview of the compatibility of materials towards certain wavelengths, while a detailed explanation of how individual phosphor coatings are manufactured to cater towards specific wavelengths is beyond the scope of this thesis. The fluorescence emission spectra for several phosphor screen coatings are displayed in Figure 5, with their corresponding fluorescence decay times shown in Table 1. From Figure 5, it can be seen that P45 and P47 have higher spectral emission and thus, more suitable for use

in the visible region, whereas P43 and P20 are catered towards the NIR region for the same reason. When applications require high frame rates (>300 Hz) or frames to be captured with short inter-frame times, a phosphor with a fast decay time in the order of microseconds such as P46 or P47 is required [8, 18]. Otherwise, for most applications, P43 is a suitable phosphor as it provides the highest emission and closely matches the peak sensitivity of the CCD.

Figure 5. Phosphor screen coatings' emission spectra. P20 = $\text{Y}_2\text{O}_2\text{S}:\text{Eu}$, P46 = $\text{Y}_3\text{Al}_5\text{O}_{12}:\text{Ce}$, P43 = $\text{Gd}_2\text{O}_2\text{S}:\text{Tb}$, P47 = $\text{Y}_2\text{SiO}_5:\text{Ce}$, P45 = $\text{Y}_2\text{O}_2\text{S}:\text{Tb}$ [8, 18]

Phosphor Decay	Time Down to 1%
P20	60 msec
P43	3 msec
P46	2 μsec
P47	0.4 μsec

Table 1. Phosphor material's decay times [8, 18].

2.3 Operations

2.3.1 Coupling of image intensifiers to CCD cameras

In a ICCD system, the image intensifier can be coupled to the CCD camera either via a fiberoptic bundle or a lens (Figure 6) [18]. Fiberoptic-coupled ICCD systems offer very high light sensitivities up to single-photoelectron detection, with a much higher signal-to-noise ratio (SNR) than that of lens-coupled systems, since most of the light generated from the phosphor screen is captured within the optical fiber's acceptance angle. However, the fiberoptic coupling is permanent and the system must be operated in dry, non-inert and non-vacuum settings. On the other hand, lens-coupled systems provide a higher level of flexibility in terms of configuration. In other words, the image intensifier can be removed so that the CCD camera can be used as a standard imaging device, or the image intensifier can be added to an existing CCD camera in a cost-effective manner. However, lens-coupled systems provide lower light throughput (<10%) since some light signal is lost in transmission, and produce increased stray light within the system (Figure 6).

Figure 6. Fiberoptic-coupled (top) versus lens-coupled (bottom) ICCD cameras [8, 18].

2.3.2 Time-gated image intensifier systems

Gating is the term used to refer to the capability of switching the image intensifier on and off very rapidly [10]. When the photocathode is positively biased compared to the MCP, electrons cannot enter the MCP and this means that the intensifier gate is closed. When the photocathode is negatively biased on the other hand, electrons will be accelerated into the MCP and the intensifier gate is now open. The fast shuttering or gating capability of intensifiers allows capture of transient phenomena such as fluorescence or luminescence immediately following the excitation of a sample. Gating is also used to effectively eliminate ambient light. Typical fast-gated intensifiers have minimum FWHM (full width at half maximum) gate widths of roughly 2 ns, which is fast enough for planar laser-induced fluorescence of OH radicals [18] for instance, whereas the FWHM of slow-gated devices is about 50 ns. In comparison, our frequency-domain image intensifier is modulated at 70 MHz, which is equivalent to a slower gating speed of $1/(70 \times 10^6) \approx 1.43 \times 10^{-8}$ s. The shortest gate times are limited by the speed of switching the gate potential. Intensifier manufacturers have addressed this issue by lowering the impedance of the photocathode using a conductive nickel undercoat on one of the photocathode surfaces [21]. However, this approach involves a trade-off because nickel absorbs light, which lowers the effective QE. Slow-gated intensifiers have neither a Ni layer nor its effects on QE.

2.3.3 Frequency-domain image intensifier systems

The frequency domain system requires a modulated light source and a modulated image intensifier as detector [18]. Both the light source and the image intensifier gain are modulated in intensity at high frequencies in the order of a few hundred MHz, and the resulting output signal is detected at several phase steps over an entire cycle during a small integration period of the CCD camera. The output signal will show a phase-shift (delay in time) and a decrease in modulation-depth with respect to the excitation light, which will be measured. In the phase step that the light source is in phase with the image intensifier gain, a high output signal level is detected. Conversely, if the light source is out of phase with the image intensifier gain, a low output signal level will be detected.

2.3.4 Focusing of lens-coupled image intensifier

After attaching the image intensifier to a CCD camera in a lens-coupled system, the image intensifier needs to be carefully focused using a grid image (Figure 7) before actual data acquisition. The grid image is pasted on a box around 50 cm away from the CCD and is used as a visual guide to achieve the best focusing.

Figure 7. Actual grid image and focused CCD image of grid.

By providing uniform input illumination via a torch and adjusting the CCD (Exposure time = 100 ms, Gain = 1x), and image intensifier (Cathode DC voltage = - 0.7 V, MCP gain = 500 V) parameters, a visible CCD image of the grid with a suitable intensity can be obtained through rough focusing. After that, a sharper image (Figure 7) can be obtained via the following fine focusing steps [22]. Firstly, the aperture and focal length of the attached lens are set at f/2.8 and 50 mm respectively. On the optical input side, the C-mount (Figure 8, Part 5) is pre-set for

a regular objective lens to be mounted. On the other side of the image intensifier, the camera is screwed almost completely onto the C-mount thread (Figure 8, Part 1) as a starting point. Following that, the optimal focusing position is obtained by previewing the live CCD image while adjusting the distance between the camera and the image intensifier via the screw threads. After the optimal focusing position is found, the camera can be secured by screwing the camera locking ring (Figure 8, Part 2) against the camera body. The position of the camera locking ring can be fixed with 3 set screws (Figure 8, Part 3).

Figure 8. Image intensifier unit. 1. Relay lens with C-mount thread to be attached to camera. 2. Camera locking ring to secure attached camera after focusing. 3 and 4. Set screws for focusing. 5. C-mount ring for mounting objective lens on optical input side. 6. Cable inputs to supply high voltage and RF signals [22].

3. Diffuse Optical Tomography

3.1 Introduction

This chapter provides an extensive review of related DOT literature, which includes the background theoretical framework, instrumentation and image reconstruction algorithms, as well as related physiological parameters.

3.2 Modelling light transport in tissues

Light propagation through human tissues has been described successfully via the photon diffusion equation. It can be derived from the radiation transport equation (RTE), which is as follows [23]:

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

where v represents the speed of light in the turbid medium, $L(r, \hat{\Omega}, t)$ is the radiance traveling in the $\hat{\Omega}$ direction, $f(\hat{\Omega}, \hat{\Omega}')$ is the normalized differential cross-section for individual light-scattering events in the medium, and $Q(r, \hat{\Omega}, t)$ is the power per volume emitted by sources in the $\hat{\Omega}$ direction. The change in light intensity at each point depends on the scattering coefficient μ_s (reciprocal of the scattering length) and the absorption coefficient, μ_a (reciprocal of the absorption length) at the same 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, the light propagation through tissues can be modeled by the diffusion approximation to the radiation transport equation, 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 $\phi(r, t) = \int_{4\pi} L(r, \hat{\Omega}, t) d\Omega$ refers to the photon fluence rate, $J(r, t)$ is the photon flux, and $S(r, t)$ represents an isotropic source term at position r and time t .

Via Fick's law of diffusion $J(r, t) = -D \nabla \phi(r, t)$, Equation 2 can be converted to the following photon diffusion equation [24, 25]:

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

In the above equation, $D(r)$ refers to the diffusion coefficient defined as $D(r) = v / 3(\mu_a(r) + \mu_s'(r))$, where $\mu_s'(r) = (1 - g) \mu_s$ where μ_s is the light scattering coefficient, which is the reciprocal of the scattering length and $g = \langle \cos \theta \rangle$ (θ is the angle between scattered and incident directions) represents the anisotropy factor. The closer g is to unity, the higher possibility for a photon to be scattered in the forward direction. In tissues, g lies between 0.8 and 0.98 typically [26]. The photon diffusion model is valid only when the source is

isotropic. This happens when scattering is much higher than absorption ($\mu_s' \gg \mu_a$) and when the distances of travel for photons within the medium are large enough when compared to l_{tr} . As a rule of thumb, $\mu_s' / \mu_a > 10$ is required to apply the photon diffusion model accurately [27].

3.3 Continuous wave, time domain and frequency domain

In order to properly apply the photon diffusion model, light is detected at known distances away from point sources. The extreme left of Figure 19 shows source–detector pairs in two different geometries – transmission versus reflection [28]. In transmission mode, light is typically injected into the medium via a laser and detected on the other side via a CCD camera system, whereas in reflection mode, light is introduced into the medium via a fiber-coupled laser and detected a distance ρ away with another fiber-coupled photomultiplier tube (PMT) or avalanche photodiode (APD). Although the directional light from a fiber seems to defy the isotropic source assumption for the diffusion model, it can still be reasonably approximated by an equivalent isotropic light source at a depth equal to the transport mean-free path l_{tr} into the medium [25]. Practically, the source–detector separation should be at least three times l_{tr} in order to accurately apply the diffusion model [29].

Figure 9. Transmission and reflection geometries, and various measurement domains [28].

Diffuse optical systems can be broadly divided into 3 main categories: continuous-wave, time frequency-domain and time-domain systems. Each technique has its own advantages and disadvantages, which are explained in further detail below.

Continuous-wave (CW) 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 [30-33]. It also offers the advantages of fast data acquisition and simple instrumentation. However, it is unable to resolve both absorption and scattering contrasts simultaneously, due to only a single amplitude measurement for each source-detector pair [34, 35]. This is due to the fact that only the effective attenuation factor $k = (3\mu_a\mu_s')^{1/2}$, can be determined

from the amplitude information, which is insufficient for a unique determination of both unknown absorption (μ_a) and reduced scattering (μ_s') coefficients.

On the other hand, frequency-domain (FD) systems involve modulating the source amplitude at high frequencies in the order of a few hundred MHz, and measuring the corresponding change in amplitude and phase shift of the detected output [36, 37]. At a specific source-detector separation, both the amplitude and phase can be determined, which can be used to obtain both the imaginary and real parts of the wavenumber k (covered in Section 3.3), which in turn enables simultaneous determination of absorption and scattering properties [34, 35].

In time-domain (TD) systems, the temporal point spread function (TPSF) is measured, which is defined as the photon distribution generated as a function of time when an ultra-short light pulse of a few picoseconds is shone through a highly scattering medium [38, 39]. The pulse temporally broadens as it propagates through the medium. At the detector, the pulse shape contains the necessary information to determine both μ_a and μ_s' from a single source-detector pair. It contains the most information content among the three domains (Figure 9), since it is equivalent to intensity-modulated sources scanned over the entire gamut of modulation frequencies present in the pulse, via the Fourier transform. However, there is a trade-off between instrument complexity and information content, as TD

systems also requires complicated instrumentation and high implementation costs. Thus we have decided to build our system in frequency-domain using the image intensifier, and the theoretical framework for the diffuse photon density waves (frequency-domain) will be covered in the next section.

3.4 Diffuse photon density waves

An amplitude-modulated light source produces a diffuse photon density wave (DPDW) which propagates spherically outwards from the source through the highly scattering medium. Although the photons experience scattering in the medium, they combine macroscopically to produce a scalar wave of light energy density with a well-defined amplitude and phase. The measurement of distortions in the DPDW wavefront caused by heterogeneities allows them to be detected and characterized [23, 40, 41].

An amplitude-modulated point source has both dc (S_{dc}) and ac (S_{ac}) components and thus can be expressed in the following form as a function of distance r and time t [40]:

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

where ω is the modulation frequency.

Likewise, the solution $\phi(r)$ will also have dc (ϕ_{dc}) and ac (ϕ_{ac}) components:

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

Considering only ac components and substituting eq. (5) into eq. (3), the photon diffusion equation becomes:

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

where $D(r)$ is the diffusion coefficient, v is the wave velocity in the medium, and $\mu_a(r)$ is the absorption coefficient.

For infinite homogeneous media, it results in:

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

where $k^2 = (-v\mu_a + i\omega)/D$ refers to the wavenumber.

The particular solution to eq. (7) in a homogeneous infinite medium with a single intensity-modulated point source at the origin ($S_{ac}(r) = S_{ac}\delta(r)$), has the form of a damped, scalar, spherical wave [40]:

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

where $k = k_r + ik_i$ is the complex wavevector, with real (k_r) and imaginary (k_i) components. Thus, $k^2 = (k_r^2 - k_i^2) + 2k_r k_i i = (-v\mu_a + i\omega)/D$.

Solving $k_r^2 - k_i^2 = -v\mu_a/D$ and $2k_r k_i = \omega/D$,

$$k_r = \left(\frac{v\mu_a}{2D}\right)^{1/2} \left[\left(1 + \left[\frac{\omega}{v\mu_a}\right]^2\right)^{1/2} + 1 \right]^{1/2}$$

$$k_i = \left(\frac{v\mu_a}{2D} \right)^{1/2} \left[\left(1 + \left[\frac{\omega}{v\mu_a} \right]^2 \right)^{1/2} - 1 \right]^{1/2} \quad (9)$$

An analogous expression for k is as follows [23]:

$$\begin{aligned} k &= \sqrt{\frac{-v\mu_a + i\omega}{D}} \\ &= (-1)^{1/2} \left(\frac{v^2\mu_a^2 + \omega^2}{D^2} \right)^{1/4} \exp \left(-\frac{i}{2} \tan^{-1} \frac{\omega}{v\mu_a} \right) \\ &= \left(\frac{v^2\mu_a^2 + \omega^2}{D^2} \right)^{1/4} \left[\sin \left(\frac{1}{2} \tan^{-1} \frac{\omega}{v\mu_a} \right) + i \cos \left(\frac{1}{2} \tan^{-1} \frac{\omega}{v\mu_a} \right) \right] \end{aligned} \quad (10)$$

where

$$\begin{aligned} k_r &= \left(\frac{v^2\mu_a^2 + \omega^2}{D^2} \right)^{1/4} \sin \left(\frac{1}{2} \tan^{-1} \frac{\omega}{v\mu_a} \right), \\ k_i &= \left(\frac{v^2\mu_a^2 + \omega^2}{D^2} \right)^{1/4} \cos \left(\frac{1}{2} \tan^{-1} \frac{\omega}{v\mu_a} \right) \end{aligned}$$

A practical application of the above theoretical framework is that the optical properties of a homogeneous turbid medium can be obtained by measuring the amplitude and phase of DPDWs at multiple source-detector separations. Writing the left-hand side of eq. (8) in the form $\phi_{ac}(r) = Ae^{i\theta}$, we can see that the logarithm of the product of the detected light amplitude $A(r)$ and source-detector separation r , as well the detected phase $\theta(r)$ vary linearly as a function of r [40], i.e.

$$\ln[rA(r)] \propto -k_i r, \quad \theta(r) \propto k_r r \quad (11)$$

since $A = \frac{vS_{ac}e^{-k_i r}}{4\pi Dr}$ and $\theta = k_r r$.

Since both the amplitude and phase can be experimentally determined, plotting $\ln[rA(r)]$ and $\theta(r)$ as a function of r produces straight lines, in which the slopes can be used to obtain the imaginary (k_r) and real (k_i) parts of k respectively. The absorption (μ_a) and reduced scattering coefficients (μ_s') can then be calculated from the following relations [23]:

$$\mu_a = \frac{\omega}{v} \left[\tan \left(2 \tan^{-1} \frac{k_r}{k_i} \right) \right]^{-1}, \quad \mu_s' = \frac{k_r^2 + k_i^2}{3 \sqrt{\mu_a^2 + \left(\frac{\omega^2}{v^2} \right)}} \quad (12)$$

3.5 Boundary Conditions

In non-invasive DPDW clinical applications, the source and detector must be placed on the tissue surface to be examined. The physics tells us that for diffusive light at the interface between scattering and non-scattering media, any light passing from the scattering medium through the interface into the non-scattering medium will not return into the scattering medium. Therefore if the boundary is perfectly transmitting with no Fresnel reflection, the radiance at the boundary must fall to zero when the source direction points into the scattering medium from the non-scattering medium outside. Generally there are three boundary conditions often used for this photon diffusion problem, namely the zero, partial-flux and extrapolated zero boundary conditions [42-44]. The zero boundary condition, in which the photon fluence vanishes at the interface between the scattering and non-scattering media, is relative simple but not physically correct. The partial-flux boundary condition (Robin boundary condition) where the radiance vanishes at the interface, is a mixed Dirichlet-Neumann boundary condition which relates the photon intensity to its gradient at the boundary [42, 43]:

$$\phi = z_b \hat{n} \cdot \nabla \phi, \quad (13)$$

where $z_b = \frac{2}{3} \frac{1+R_{eff}}{1-R_{eff}} l_{tr}$, and 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. Although the partial-flux boundary condition accurately depicts practical scenarios, it makes the derivation of analytical solutions to the diffusion equation complicated. Thus, an alternative simpler boundary condition has been proposed instead, which is called the extrapolated zero boundary condition [42, 45]:

$$\phi(z = -z_b) = 0 \quad (14)$$

This means that the photon intensity can be considered negligible at an extrapolation distance z_b , outside the tissue, as shown in Figure 10 below. Haskell et. al. [43] has shown that the partial-current and the extrapolated boundary conditions give solutions that are almost equal to each other with an error of within 3%, when the source-detector distance is larger than 5 mm for tissue optical properties. By making use of this new interface, it is feasible to use the method of images to obtain analytic solutions in various geometries, as shown in the next section.

3.6 Green's function solutions for various geometries

A common technique employed to solve the frequency-domain photon diffusion equation (Equation (6)) is to first obtain the respective Green's function for the chosen geometry, and then make use of the Green's function to derive a more general solution [46, 47]. 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 [42, 43, 48]. The final solution for $\phi(r)$ is then equivalent to the convolution of this Green's function with the source term, $vS_{ac}(r)/D$.

In diffuse optical imaging, a homogeneous semi-infinite or infinite slab geometry may be used to model certain tissues. In both geometries, the method of images can be utilized to obtain the respective Green's functions, subject to the extrapolated zero boundary condition (Equation (14)) [48].

For a semi-infinite medium, the solution of the photon diffusion equation can be obtained by using image sources, while satisfying the extrapolated zero boundary condition at the same time. Light is injected into the tissue via a fiber at $r = (\rho = 0, z = 0)$, which is well approximated by an isotropic point source $S = S_0$

at $r_s = (\rho_s = 0, z_s = l_{tr})$, as shown in section 3.2. Then, in order to satisfy the extrapolated zero boundary condition, a negative image of the real point source is introduced at $z_s = -(2z_b + l_{tr})$ via reflection of the real source about the extrapolated zero boundary, as shown in Figure 10. 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 $r_d = (\rho_d, z_d)$:

$$\phi_{semi}(r_d) = \frac{\nu S_0}{4\pi D} \left[\frac{e^{ikr_{1+}}}{r_{1+}} - \frac{e^{ikr_{1-}}}{r_{1-}} \right], \quad (16)$$

where $r_{1+} = \sqrt{(z_d - l_{tr})^2 + \rho_d^2}$ and $r_{1-} = \sqrt{(z_d + l_{tr} + 2z_b)^2 + \rho_d^2}$.

Figure 10. The extrapolated zero boundary condition [28].

For a semi-infinite geometry, Arridge et al [48] has demonstrated that when the source-detector separation $r \gg 1/\mu_s'$,

$$\phi_{semi}(r) = \frac{vS_0 e^{ikr}}{4\pi D r^2} \left[-2ik(z_0 z_b + z_b^2) \right], \quad (17)$$

from which $\ln[r^2 A_{semi}(r)] \propto -k_i r$ and $\theta_{semi}(r) \propto k_r r$ are obtained. In a similar fashion to that discussed in section 3.3, the slopes of the straight lines $\ln[r^2 A_{semi}(r)]$ and $\theta_{semi}(r)$ versus r can be used to obtain the imaginary (k_i) and real (k_r) parts of k respectively, which in turn give the absorption (μ_a) and reduced scattering coefficients (μ_s') of the homogeneous medium.

In the special case when $\omega = 0$, which corresponds to a CW system, there is only amplitude information, which means that the phase is always zero. For clarity purposes, the DPDW solutions produced by a CW source in a homogeneous infinite and semi-infinite medium can be rewritten as follows [23, 40]:

$$\phi_{inf}^{CW}(r) = \frac{vS_0 e^{-kr}}{4\pi D r} \quad (18)$$

$$\phi_{semi}^{CW}(r_d) = \frac{vS_0}{4\pi D} \left[\frac{e^{-kr_{1+}}}{r_{1+}} - \frac{e^{-kr_{1-}}}{r_{1-}} \right] \quad (19)$$

In this case, the effective attenuation factor k can be determined from the above slope method. However, this attenuation factor, $k = (3\mu_a \mu_s')^{1/2}$, is insufficient for a

unique determination of both unknown absorption (μ_a) and reduced scattering (μ_s') coefficients.

On top of the above mentioned two geometries, the method of images can also be extended to obtain the frequency-domain Green's functions in other geometries, such as the infinite slab and cylindrical geometries [49]. For instance, the DPDW solution for an infinite slab will be [23, 40]:

$$\phi_{slab}(r_d) = \frac{\nu S_0}{4\pi D} \sum_{m=-\infty}^{\infty} \left[\frac{e^{ikr_{m+}}}{r_{m+}} - \frac{e^{ikr_{m-}}}{r_{m-}} \right] \quad (20)$$

In later chapters, these geometries will be discussed in greater detail, and methods for simplifying the above expressions, in order to solve the photon diffusion equation analytically, will be proposed and evaluated.

3.7 Image reconstruction algorithms

The goal of diffuse optical tomography (DOT) is to reconstruct the 3D spatial distribution of optical properties within the tissue from measurements of detected light intensity on the tissue surface. This image reconstruction procedure is typically known as solving the inverse problem.

In contrast, the forward problem refers to the calculation of the photon flux on the tissue surface, given a forward model (radiative transport/photon diffusion equation) and prior knowledge of internal optical properties and sources. The forward problem can be solved using numerical finite-difference methods (FDM) [50], finite-element methods (FEM) [51], boundary element methods (BEM) [52] or Monte Carlo simulations [53]. On the other hand, inversion approaches are classified as linear or nonlinear; as well as analytical or numerical. In terms of quantification, nonlinear methods are preferred, since the DOT inverse problem is inherently nonlinear. However, they are computationally intensive. In the limit that the optical properties of the volume elements are close to those of the background, the inverse problem is approximately linear and thus, can be solved using linearized methods. Over the years, many linearized methods have been employed for inverting the ill-posed Jacobian matrix, which include singular value decomposition (SVD), algebraic reconstruction technique (ART) as well as simultaneous iterative reconstruction technique (SIRT) [54]. These methods have

been used in optical mammography applications, in which optical properties can change in response to external factors.

In addition, symmetry in some systems has been exploited by some researchers, in an attempt to boost the speed and efficiency in solving the inverse problem. These include translational symmetry in a slab and rotational symmetry in a cylinder or sphere. Such initiatives have been developed for Green's functions, which are analytical inversion kernels for solving the inverse problem [55-59]. Markel and Schotland [57, 59] have demonstrated that the symmetry of the slab geometry can be used to transform the original 3-D integral equation into a series of 1-D integral equations in Fourier space. These linear equations are then merged to form a final solution, which can be transformed into image space via inverse Fourier Transform. Similar schemes can be employed for cylindrical and spherical geometries as well, in which Fourier and spherical harmonic waves form the integral components respectively.

On the other hand, when the optical properties of the volume elements differ a lot from those of the background, the linearized approach becomes inappropriate as an inversion model. In order to circumvent this problem, nonlinear iterative methods, such as Jacobian-based and gradient-based schemes are required. In essence, nonlinear Jacobian-based schemes involve comparing calculated photon flux with

corresponding measured values and updating the calculated optical properties iteratively till convergence [50], whereas gradient-based schemes encompass finding the optical properties that minimize an objective function along a search direction and updating them repeatedly as well till convergence [34, 60, 61]. Both the gradient-based and Jacobian-based schemes are utilized in popular DOT reconstruction packages mostly written in MATLAB, such as TOAST [62] and NIRFAST [63]. In addition, the exact analytic solution for the inversion problem can also be written as a nonlinear infinite series [64]. Although incorporating higher-order terms in the series can lead to higher accuracy, this has the limitation of being more computationally intensive. However, for certain geometries, the inherent symmetry can be exploited in a manner similar to that of linearized analytic inversion techniques, which boosts image reconstruction with higher order terms [65]. On top of the various image reconstruction techniques mentioned above, there are other critical aspects which can also affect the quality of reconstructed images. In general, inherent noise within data incurred during experiments may lead to image artifacts when solving the inverse problem, which can be reduced via regularization methods [66, 67]. In addition, various research groups are also combining DOT with other imaging methods such as MRI [68, 69] and ultrasound [70, 71], in order to incorporate prior data into the inversion process. This improves the accuracy and resolution of the results.

3.8 Physiological parameters

From DOT, tissue absorption information obtained can be translated into information about the concentrations of key tissue chromophores, i.e. oxy- and deoxy- haemoglobin, total haemoglobin concentration, water, and lipid, whereas tissue scattering properties can offer insights into important morphological data, such as collagen density and orientation.

In particular, the wavelength-dependent absorption coefficient μ_a is given by the Beer-Lambert Law:

$$\mu_a(\lambda, r) = \sum_{i=1}^N \varepsilon_i(\lambda) c_i(r), \quad (21)$$

where $\varepsilon_i(\lambda)$ is the extinction coefficient, $c_i(r)$ is the concentration of the i^{th} chromophore and N is the total number of chromophores. The known extinction coefficients from literature [72] are wavelength-dependent and have units in $[cm^{-1}\mu M^{-1}]$, while the concentrations have units of $[\mu M]$.

On the other hand, the reduced scattering coefficient μ_s' follows the Mie-scattering theory as follows:

$$\mu_s'(\lambda, r) = A(r) \lambda^{-b(r)}, \quad (22)$$

where $A(r)$ is defined as the scattering prefactor and $b(r)$ is the scattering power. The scattering power is dependent on the particle size and density, as well as refractive index of scatterers and the medium.

In traditional single-spectral schemes as highlighted above, absorption and scattering coefficients at a specific wavelength are first determined from image reconstruction in DOT, before using the obtained absorption coefficients to extract chromophore concentrations. This can be extended to a multi-spectral approach, in which the chromophore concentrations and scattering properties are reconstructed directly in a single step using acquired experimental data at multiple wavelengths. This multi-spectral technique provides benefits of reducing inter-parameter crosstalk and providing unique solutions, even for the CW case. Once chromophore concentrations are obtained, more useful physiological parameters can be derived, such as the tissue blood oxygen saturation $StO_2 = c_{HbO_2} / THC$, as well as the total haemoglobin concentration $THC = c_{Hb} + c_{HbO_2}$.

4. Determination of Homogeneous Optical Properties in Turbid Medium

4.1 Introduction

In this chapter, we report a new method to measure both absorption and reduced scattering coefficients (μ_a and μ_s') in transmission geometry, based on a homodyne approach using a RF-modulated laser source and a gain-modulated image intensifier. We demonstrate an efficient way to analyze the phase-sensitive images using a truncated Green's function solution in slab geometry and investigate the use of inherent higher harmonics in the laser source and image intensifier gain waveform.

With the emergence of diffuse optical tomography (DOT) as a promising non-invasive medical imaging modality, the accurate measurement of background optical properties has also become crucial, both in tabletop and clinical experiments [28, 73]. For example, in clinical settings, there have been many studies on background optical properties of the human breast, in order to observe its correlation with demographic parameters and to find a good reference for tumor contrast [36, 73-76].

The attempt to determine accurate homogeneous optical properties, namely the absorption (μ_a) and reduced scattering coefficient (μ_s'), has a long history of over 20 years [45, 48, 77]. To list some of the milestone developments in history,

Fantini's group has published a series of papers focusing on frequency-domain measurements of optical properties of highly scattering medium containing methylene blue [78] and methemoglobin [79]. In addition, they derived and verified experimentally the theoretical expressions for the optical properties in a semi-infinite medium based on the diffusion model [80]. Influence of layered structure in semi-infinite medium were also studied theoretically [81-83] and were applied in-vivo [84, 85] for more accurate tissue optical property assessment. In terms of instrument development, Tromberg's group [86] utilized an avalanche photodiode (APD) for detection in a portable frequency-domain photon migration (FDPM) instrument, in order to perform quantitative measurements of breast tissue optical properties, while Pogue's group [87] designed a photomultiplier-tube (PMT)-based breast cancer screening device and validated its performance on test phantoms. In addition, some have made use of frequency-domain multiplexing (FDM) systems [88, 89] to determine the optical properties of tissue more efficiently, while others have used time-resolved spectroscopy using pulsed light source [90, 91]. In the above mentioned works and many other excellent works, it should be noted that most of works are performed in half-space geometry using reflection-based probes [77, 92-95]. A limitation of this method is that the probed volume is highly biased towards the surface, and thus, the data measurements might give values which deviate significantly from the whole volume average value.

On the other hand, transmission-based measurement provides a more uniform spatial distribution of the sensitivity volume, which makes it more suitable for the determination of homogeneous optical properties. Transmission geometry is also more amenable to the use of CCD cameras for massive parallel detection, although some use of CCD camera in reflection geometry is possible where there is no harsh dynamic range issue as in recently developed spatial modulation imaging [96-98]. Although transmission geometry is not possible for inaccessible or non-squeezable organs, it is suitable for investigating accessible tissues such as in breasts and finger joints. One limitation with CCD-based transmission geometry has been that it is difficult to perform phase-sensitive measurements (frequency-domain) to obtain μ_a and μ_s' separately. There has been a hybrid approach by Yodh's group [33, 93] employing both frequency-domain (in half-space) and continuous-wave (in transmission) measurements in a 3D breast imaging system, but genuine frequency domain transmission geometry measurement can only be readily achieved via the use of the image intensifier.

The first attempts to use gain modulation in the image intensifier for frequency-domain massive data acquisition can be found in Sevick-Muraca's group [1-3], although they still used detector fibers on different locations of the sample surface which delivered fluorescence signal directly onto the CCD chip. More recently, there have been reports of totally non-contact schemes utilizing either heterodyne

[4] or homodyne [5] measurements. For instance, homodyne work from Hielscher's group [5] utilized modulation frequencies up to 1 GHz, and presents good results especially with small size samples. In general, although heterodyne measurements provide higher SNR than their homodyne counterparts, they are constrained in terms of the range of CCD exposure times that can be used, since the resulting image is changing in intensity at the cross-correlation frequency.

In this paper, we present a homodyne DOT system incorporating a gain-modulated image intensifier, which is more suitable for breast imaging and works well at modulation frequencies below 120 MHz. We built a solid phantom with homogeneous optical property and show our phase-sensitive images can be used to accurately retrieve the optical properties. We developed an approximate analytic form of Green's function in transmission geometry, and demonstrated how it simplifies our homogeneous slab analysis. Furthermore, we observed that both the modulated laser source and image intensifier gain generally show high level of distortion, indicating high harmonic content. This enables us to obtain multi-frequency information from a single experimental dataset, instead of conducting experiments repeatedly at different modulation frequencies. We present a comprehensive multi-harmonic data analysis, which provides more accurate measurements of homogeneous optical properties, and can also be extended to 3D diffuse optical image reconstruction.

4.2 Theoretical background

The turbid medium is assumed to have boundaries at $z = 0$ and $z = d$ as shown in Figure 11, and the extrapolated zero boundary condition is used as a suitable approximation of partial current boundary conditions. In Figure 11, the various parameters are defined as below:

$$\begin{aligned}
 d' &= d + 2z_b \\
 r_u &= r_0 - \Delta r \approx r_0 - z_b \sin \theta \\
 r_d &= r_0 + \Delta r \approx r_0 + z_b \sin \theta \\
 r_{1+} &\approx r_u - (z_b + l_{tr}) \sin \theta = r_0 - (2z_b + l_{tr}) \sin \theta \\
 r_{1-} &\approx r_u + (z_b + l_{tr}) \sin \theta = r_0 + l_{tr} \sin \theta \\
 r_{2+} &\approx r_d + (z_b + l_{tr}) \sin \theta = r_0 + (2z_b + l_{tr}) \sin \theta \\
 r_{2-} &\approx r_d - (z_b + l_{tr}) \sin \theta = r_0 - l_{tr} \sin \theta, \tag{23}
 \end{aligned}$$

where $z_b, l_{tr} \ll d'$ is assumed, r_0 , r_u and r_d are shown in Figure 11, θ is an angle between the r_0 vector and the $z = -z_b$ line, and $r_{n,\pm}$ ($n=1,2$) refers to the distance to the detector from each image source.

Figure 11. Image sources used to calculate the Green's function in infinite slab geometry.

The red and blue dots represent the positions of isotropic sources, with the three dots lying outside of the medium as image sources. The solid green square denotes the detector position, whereas the empty green square represents the position where effects from two dipoles (denoted as 1 and 2) exactly cancel out (drawn not to scale).

The analytic solution for the Green's function in homogeneous infinite slab geometry has already been well established using an infinite series of image sources [43, 99]:

$$G_{slab}(r_0) = \frac{1}{4\pi D} \sum_{m=-\infty}^{\infty} \left[\frac{e^{ikr_{m+}}}{r_{m+}} - \frac{e^{ikr_{m-}}}{r_{m-}} \right] \quad (24)$$

Here we present an approximate form of it using a truncated series. For a detector positioned at $z = 0$, the Green's function can be represented as a sum of first four dominant terms as shown below, where $G_0(r) = \frac{1}{4\pi D} \frac{e^{ikr}}{r}$:

$$\begin{aligned} G_{slab}(r_0) &\approx G_o(r_{1+}) - G_o(r_{1-}) + G_o(r_{2+}) - G_o(r_{2-}) \\ &\approx G'_o(r_0 - (z_b + l_{tr}) \sin \theta) \cdot (-2z_b \sin \theta) \\ &\quad + G'_o(r_0 + (z_b + l_{tr}) \sin \theta) \cdot (2z_b \sin \theta) \\ &\approx 2z_b \sin \theta \cdot G''_o(r_0) \cdot 2(z_b + l_{tr}) \sin \theta \\ &\quad , \quad (25) \\ &= \frac{d'^2 k^2 z_b (z_b + l_{tr})}{\pi D r_0^3} \left(-1 - \frac{2i}{kr_0} + \frac{2}{k^2 r_0^2} \right) e^{ikr_0} \\ &\approx - \frac{d'^2 k^2 z_b (z_b + l_{tr})}{\pi D r_0^3} e^{ikr_0} \end{aligned}$$

where $\sin \theta = d'/r_0$, and $|kr_0| \gg 1$ was used in the last step.

In order to estimate the accuracy of this approximate truncated form, assuming some initial values for the input parameters ($z_b = 0.175 \text{ cm}$, $l_{tr} = 0.1 \text{ cm}$, $r_0 = 7 \text{ cm}$), the value of this truncated series is computed and found to be within 94.2% accuracy, when compared to the actual value of the entire infinite series. Thus this truncated form is verified to be reasonably accurate, which can be used in further analysis. In addition, note that this approximate slab Green's function is proportional to e^{ikr_0} / r_0^3 . It can be compared to the fact that the Green's functions for both homogeneous infinite and semi-finite geometries are proportional to e^{ikr_0} / r_0 and e^{ikr_0} / r_0^2 respectively, as shown in Section 3.4 and 3.6. One can think of free-space as a monopole field and half-space as a dipole field, where the expression of the field magnitude in this case is analogous to the Yukawa potential field in particle physics [100]. Extending this analogy, the slab geometry can be thought of as two-dipole field where the $z = -z_b$ plane corresponds to the exact middle plane between the two vertical dipoles where the potential vanishes. Therefore, in this regime of high $|kr_0|$, $\ln|r_0^3 G_{slab}(r_0)|$ becomes a linear function of r_0 , which facilitates the analysis tremendously, as we will see in the results and discussion section. In addition, with reference from Chapter 3, we see that the imaginary (k_i) and real (k_r) parts of the diffuse wavenumber $k = k_r + ik_i$ can be directly obtained from the linear slopes of the plots of $\ln|r_0^3 G_{slab}(r_0)|$ and phase (ϕ) versus r_0 , respectively, for a given frequency, as shown below:

$$\ln \left| r_0^3 G_{slab}(r_0) \right| = -k_i r_0 + c_0 \quad (26)$$

$$\phi(r_0) = k_r r_0 + \phi_0 \quad (27)$$

Following this, μ_a and μ_s' can be calculated from k_r and k_i , as previously described in Chapter 3.

4.3 Phase detection with image intensifier

The amplitude and phase of incoming light modulated at a specific modulation frequency can be measured via a gain-modulated image intensifier. Since the output signal is proportional to the product of incoming light intensity and the gain of the image intensifier, the image intensifier acts as a mixer, producing a down-converted signal (Figure 12).

Figure 12. Frequency down-conversion with image intensifier (LO: local oscillator, ω_c & ω_g : source & gain modulation frequencies).

Supposing that the light detected by the image intensifier is modulated at an angular frequency of ω_c while the gain of image intensifier is modulated at an angular frequency of ω_g , the light intensity and gain can be expressed in terms of:

$$I(t) = I_0 + I_1 \sin(\omega_c t + \phi_c)$$

$$g(t) = g_0 + g_1 \sin(\omega_g t + \phi_g) \quad (28)$$

Subsequently, the output signal from the image intensifier can be expressed as a product of incoming light intensity and the gain of the image intensifier:

$$\begin{aligned} S(t) &= I(t) \times g(t) \\ &= I_0 g_0 + I_1 g_0 \sin(\omega_c t + \phi_c) + I_0 g_1 \sin(\omega_g t + \phi_g) \\ &\quad + \frac{1}{2} I_1 g_1 (\cos((\omega_c - \omega_g)t + \phi_c - \phi_g) - \cos((\omega_c + \omega_g)t + \phi_c + \phi_g)) \end{aligned} \quad , \quad (29)$$

Since the response time of the image intensifier phosphor screen is approximately 1 ms, it acts as a low-pass filter and thus, Equation 29 effectively gets time-averaged to give:

$$S(t) = I_0 g_0 + \frac{1}{2} I_1 g_1 (\cos \Delta\omega t + \Delta\phi), \quad (30)$$

where $\Delta\omega = \omega_c - \omega_g$ and $\Delta\phi = \phi_c - \phi_g$.

Under homodyne detection ($\Delta\omega = 0$), the signal will remain constant over time and vary as a function of $\Delta\phi$ only. Thus, the data signal can be collected for different phase delays and fitted to a sinusoidal curve, in order to obtain the amplitude and phase. The total number of phase steps should cover the whole 360° cycle with equal step size and is preferred to be in the order of powers of 2 to ensure efficiency of the Fast Fourier Transform (FFT) algorithm.

4.4 Signal-to-noise ratio

The signal-to-noise ratio (SNR) of photomultiplier tubes (PMTs) has been researched thoroughly [101] and similar concepts have been extended to derive the SNR for ICCD detectors [102]. This analogy is appropriate because the photocathode and MCP of the ICCD have similar physical mechanisms as the photocathode and dynodes of the PMT. The incident radiation follows Poisson statistics, an inherent source of shot noise due to the quantum nature of light. The intensifier noise is the dominant noise source of the detection system when using an intensifier. CCD-related noise (e.g. read-out noise, dark current and digitization error) is often assumed to be negligible in comparison to the photon shot noise and intensifier noise.

Employing interdependent noise propagation for a cascade of sequential events, the SNR of our image intensifier-based DOT system as a whole is expressed as [101]:

$$SNR \approx \sqrt{\frac{\eta \cdot \alpha \cdot N_0}{1 + \left(\frac{\sigma_g}{g}\right)^2}} \quad (31)$$

where N_0 is the mean rate of photons incident upon the photocathode of the intensifier, g includes both the gain of the MCP and the quantum efficiency of the phosphor with variance σ_g^2 , η is the quantum efficiency of the photocathode, and α is the fraction of photoelectrons that undergo electron multiplication inside the MCP (i.e. α is modulated by the voltage applied to the photocathode of the intensifier and this controls the gain modulation). The effective image intensifier gain in Equation 28 is then [101]:

$$g(t) = \alpha(t) \cdot \eta \cdot g \quad (32)$$

which is equivalent to the ratio of the number of photons emitted from the phosphor per incident photon on the photocathode).

Notably, the low quantum efficiency of the photocathode, η , for converting fluorescence photons into photoelectrons degrades the SNR, as shown in Equation

31 [101]. Effectively, the MCP sees a reduction in particle counts due to the low quantum efficiencies of the photocathode (≈ 0.4). Thus, Equation 31 is consistent with intuition that the SNR should decrease following a reduction in the particle counts (in accordance with Poisson statistics). As expected, the signal amplification further degrades the SNR, as illustrated in the $(\sigma_g/g)^2$ term in the denominator of Equation 31.

4.5 Experimental setup

In the experimental setup (Figure 13), we used a 660 nm laser source (56ICS254 Melles Griot) intensity-modulated at 70 MHz, along with a gain-modulated image intensifier (III18MDS Lambert Instruments) that is lens-coupled to an electron-multiplying CCD camera (Cascade 512, Photometrics). In order to achieve maximum modulation depth, the intensifier gain was modulated by applying a sinusoidal signal to the cathode, via a modulation technique commonly used in fluorescence lifetime imaging microscopy (FLIM) [103]. The cathode DC bias voltage was fixed near the cutoff voltage of the image intensifier. The cutoff voltage is the voltage at which electrons generated at the photocathode are just beginning to be accelerated towards the MCP, or more quantitatively, the voltage generating 1% of the image intensity that results from normal operating conditions (cathode DC voltage = -10 V). Figure 14 shows the estimated waveform of the

modulated gain when a perfect sinusoidal RF wave is applied to the cathode. Since the resulting gain waveform (top right graph in Figure 14) deviates from the shape of a sinusoidal wave, it contains both the fundamental frequency and higher harmonic components. This presence of higher harmonics is further verified by observing the laser source waveform and its corresponding Fourier transform (Figure 15).

Figure 13. Experimental setup for determination of optical properties in turbid medium.

Figure 14. Measured DC gain profile of image intensifier (upper left) and estimated input-output curve. Internal structure of image intensifier (lower right).

Figure 15. Modulated laser waveform and corresponding Fourier transform.

The exact experimental parameters used for the image intensifier are: cathode DC voltage = -0.6 V, MCP voltage = 500 V, modulation frequency = 70 MHz, phase interval = 10°, and cathode AC voltage = high; while the lens-coupled CCD parameters are: aperture = f/4.0, exposure time = 200 ms, and focal length = 50 mm.

The solid silicone phantom with a thickness of 3.2 cm was made from RTV 12A (base compound) and RTV 12C (curing agent) (Momentive Performance Materials) in the ratio of 20:1. The weight ratio of 20:1 of RTV 12A : RTV 12C is recommended by the manufacturer. RTV 12A is 80 % polydimethylsiloxane, 10 % MQ resin, benzene and toluene. RTV 12C is 5 % dibutyl tin oxide solution, 20 % ethyl silicate 40, 20 % aminopropyltriethoxysilane, 5 % 1,2,4-trimethylbenzene and naphtha (mineral spirits 60 %). Carbon black and titanium dioxide were added to provide the desired μ_a and μ_s' respectively. The amount of carbon black and titanium dioxide to be added can be calculated by [104]:

$$carbonblack(mg) = \frac{\mu_a + 0.0068}{0.0203} \times \frac{phantomvolume(ml)}{1650(ml)} \quad (33)$$

$$TiO_2(g) = 3.6(g) \times \frac{\mu_s'}{7.5} \times \frac{phantomvolume(ml)}{1650(ml)} \quad (34)$$

where the phantom volume is 480 ml, and the desired μ_a and μ_s' are 0.03 cm⁻¹ and 4 cm⁻¹ respectively. Both the required amount of carbon black and titanium dioxide

are added to RTV 12C and the resulting mixture is vortexed, sonicated for 1 hour and finally combined with RTV 12A. The final mixture is poured into a mould and left to cure for 1 to 2 days.

We acquired phase-sensitive transmission images (Figure 16) with a fixed source position on the solid phantom, with changing phase delays in steps of 10° over the entire 360° cycle. The resulting images were sampled and the Fourier components (fundamental and higher harmonic frequencies) were calculated for each pixel, in order to extract the corresponding amplitudes and phases. The following formulas are employed for amplitude and phase calculations, in a similar manner to FLIM data pre-processing procedures [6, 7].

In addition, the experiment has been conducted for a few times on different positions on the phantom using the same parameters for both image intensifier and CCD camera, in order to test for system consistency and repeatability of results. Multiple experiments demonstrated that the system is reasonably stable and reliable, producing consistent results.

Figure 16. Raw CCD images (top) taken with varying RF phase delays between the two arms, and corresponding plot of image intensity at center pixel versus phase (bottom).

In general, if the total number of phase steps is K :

$$F_{\sin,n} = \sum_k I_k \sin(2\pi nk / K)$$

$$F_{\cos,n} = \sum_k I_k \cos(2\pi nk / K), \quad (35)$$

where I_k is an image in the k -th phase step.

From the equations above, the phase and amplitude can be obtained:

$$\phi_n = \tan^{-1}(F_{\sin,n} / F_{\cos,n})$$

$$A_n = \sqrt{F_{\sin,n}^2 + F_{\cos,n}^2}, \quad (36)$$

where ϕ_n and A_n represent the phase and amplitude of the n -th order harmonic, respectively.

4.6 Results and discussion

Amplitude and phase images for different harmonics were obtained using Equations 36, as shown in Figure 17. The radial distribution and the level of noise can be directly observed from this figure. Note that the DC and AC amplitude images are visualized in logarithmic scale, whereas the phase images are in linear scale, for better comparison among images. We sampled 30 pixels at fixed intervals radially away from the centre position and generated plots of $\ln|r_0^3 A_n|$ and phase (ϕ_n) versus source-detector separation r_0 , for the first three harmonic frequencies (Figure 18). The intensity graph for DC component is also drawn in the amplitude plot. Both graphs show linear behavior as expected from theoretical treatment. Note that the $\ln|r_0^3 A_n|$ versus r_0 graph shows higher values for $n = 3$ than for $n = 2$, due to the gain waveform in Figure 14 resembling a square wave and hence having higher odd-harmonic components. The laser source waveform was also observed to have significant harmonic content, but its harmonic content was monotonously decreasing with harmonic number regardless of its even or odd nature. Hence the effect of $A_3 > A_2$ is solely attributable to the image intensifier gain waveform.

Figure 17. Logarithmic amplitude (left) and phase (right) images (30 x 30 pixels) for the DC component ($n=0$) and three different harmonics ($n = 1, 2$ and 3).

In Figure 17, $n = 0$ corresponds to the DC component, while $n = 1$ corresponds to the fundamental frequency of 70 MHz. The same color scale applies to DC and AC amplitude image. Note that starting from the centre position towards the sides, the amplitude decreases and phase increases as expected. Also note that the amplitude for $n = 3$ is greater than that for $n = 2$ because of higher odd harmonic content.

Figure 18. (upper) Plots of (a) $\ln|r_0^3 A_n|$ and (b) phase (ϕ_n) versus source-detector distance, r_0 , and (lower) resulting values of μ_a and μ_s' with error bars based on 95% confidence intervals, computed at different Fourier components.

In Figure 18(a), the $\ln|r_0^3 A_n|$ for $n = 3$ is greater than that for $n = 2$ because of higher odd harmonic content. In (b), the second and third harmonics have higher variance, when compared to the fundamental component. In (c), both μ_a and μ_s' were calculated from the slopes and plotted for each harmonic. The DC component provides a constraint for the product between μ_a and μ_s' via $3\mu_a\mu_s' = k_i^2$, which is plotted as a black curve with its tolerance represented as gray shade. The DC, fundamental and third harmonic components overlap at around $\mu_a = 0.041 \text{ cm}^{-1}$ and $\mu_s' = 4.0 \text{ cm}^{-1}$. On the other hand, the second harmonic component generates an outlying data point with a large uncertainty. Thus, multiple harmonics can yield independent multi-frequency datasets from a single-frequency experiment, which can be useful in measuring absorption and scattering properties more accurately and even potentially reducing the level of cross-talk between the two. More specifically, data at each frequency provides an independent constraint on the wavevector k in the complex plane, which helps to reduce the level of cross-talk.

The resulting μ_a and μ_s' values were compared to a reflection-based measurement taken by a commercial tissue oximeter (OxiplexTS, ISS), which is about 0.03 cm^{-1} and 4.1 cm^{-1} , respectively at 690 nm. These results match quite well, especially in μ_s' . Note, however, the significant differences in probing depths and sensitivity volume shapes between the two measuring schemes will cause deviations in

measurement in general, since the custom-made solid phantom can have a spatial distribution of optical properties that deviates from perfect homogeneity. Measurements using the commercial tissue oximeter were done in reflection geometry and thus intuitively, it may be possible to change the experiment setup from transmission to reflection geometry to have similar probing depths, since probing depth is dependent on system geometry. However in reflection geometry, the large dynamic range of the signal which can easily lead to signal saturation makes the experiment impractical to implement.

4.7 Discussion

In conclusion, we have shown that simultaneous measurement of μ_a and μ_s' can be achieved in transmission geometry by using a gain-modulated image intensifier and a simple linear regression analysis from the phase-sensitive data. In terms of experiment, this method does not require long data acquisition time because many data with different source-detector separation are simultaneously acquired by multi-pixel detection. Instead, the data acquisition time is determined by the size of the phase step one decides to use. If higher harmonic measurements are intended, one should ensure that the measurements are performed with enough number of phase steps in order to extract high frequency components without aliasing errors. In terms of analysis, although analytic formulation of the photon diffusion model is becoming less important due to the high computational power that is accessible today, finding an approximate linear relationship that is present in the complicated model gives us more insights on photon diffusion in transmission geometry. The validity of this approximate linear model increases with modulation frequency. Although the RF frequency in our system is bound by 120 MHz, significant higher harmonic components can be acquired from the distorted gain oscillation curve, and can be utilized for increasing the measurement accuracy.

Higher harmonic analysis shown in this paper can yield abundant independent datasets (multi-frequency data) from a single experimental run, which can be useful in measuring absorption and scattering properties more accurately. In addition, data at each frequency provides an independent constraint on the wavevector k in the complex plane, which can help to reduce the level of cross-talk. For this purpose, it would be necessary to further optimize the image intensifier parameters such as the DC bias and MCP voltage, so that one can obtain a higher signal-to-noise ratio for higher harmonics. 3D image reconstruction work is underway using the same experimental setup with a diffuse medium containing an inhomogeneity, and the background measurement scheme described in this paper will play a vital role in achieving better quality image reconstruction.

5. Image Reconstruction of Heterogeneities in Turbid Medium

5.1 Introduction

In this chapter, we present in depth DOT instrumentation for different domains and geometries, corresponding experimental procedures such as data calibration and acquisition, as well as successfully reconstructed results of absorption and scattering contrast. In addition, we also provide a discussion on the dependence of image quality on reconstruction parameters, such as the pixel basis and SVD truncation parameter (together with accompanying figures). In the last section, we also incorporate the higher harmonic content (inherent in the laser source and image intensifier gain waveforms) into the analytic code, in order to perform multi-frequency DOT reconstruction and present our reconstruction results.

5.1.1 The finite element method and NIRFAST

Near Infrared Fluorescence and Spectral Tomography (NIRFAST) is a simulation and image reconstruction software toolbox, which has been commonly utilized to model the propagation of light in biological tissues, based on the finite element method (FEM) [63]. In general, the fundamental principle of the finite element method is based on the discretization of the target volume, via dividing the volume into many elements with their vertices as nodes. The goal of the DOT inverse problem is then to recover the absorption (μ_a) and reduced scattering (μ_s') coefficients at each node within the volume using light intensity measurements on

the tissue surface. Within NIRFAST, this inversion is realized via least-square minimization with a modified-Tikhonov regularization scheme. If ϕ^M is the measured light intensity at the tissue surface and ϕ^C is the forward data computed from the forward solver, then the standard Tikhonov minimization function is given by [63]:

$$\chi^2 = \min_{\mu} \left\{ \sum_{i=1}^{NM} (\phi_i^M - \phi_i^C)^2 + \lambda \sum_{j=1}^{NN} (\mu_j - \mu_0)^2 \right\}, \quad (37)$$

where NM and NN are the total number of measurements and FEM nodes respectively. $\lambda = \sigma_{\phi^M}^2 / \sigma_{\mu}^2$, which is defined as the ratio of the variances of the measurement data and optical properties, is called the Tikhonov regularization parameter [105]. μ_0 can be an initial estimate of the optical properties, typically known from data calibration [106], or an a priori optical property distribution, if available. If the initial estimate μ_0 , lies close to the actual parameter distribution, it can be neglected [69, 107].

By setting the first-order derivative equal to zero ($\frac{\partial \chi^2}{\partial \mu} = 0$) and ignoring higher order terms, χ^2 is minimized with respect to μ . The first-order condition is then as follows [63]:

$$\left(\frac{\partial \phi^C}{\partial \mu} \right)^T (\phi^M - \phi^C) - \lambda (\mu - \mu_0) = 0 \quad (38)$$

The derivative matrix $\frac{\partial \phi^c}{\partial \mu}$, is typically called the weight or sensitivity matrix, or the Jacobian matrix, J . Applying linear approximation and solving the problem in an iterative fashion leads to [63]:

$$(J^T J + \lambda I) \delta \mu = J^T \delta \phi - \lambda(\mu_i - \mu_0), \mu_{i+1} = \mu_i + \delta \mu \quad (39)$$

where $\delta \mu$ is an incremental update for the optical properties and $\delta \phi$ is the difference between model and experimental values at the current iteration.

The iterative procedure is repeated until experimental values match model values within a tolerance level set as 2% in NIRFAST. Figure 19 below [63] shows a flowchart outlining the NIRFAST commands used in the process of image reconstruction in general.

Figure 19. Flowchart outlining the NIRFAST commands used in the process of image reconstruction in general [63].

5.1.2 Analytic symmetry inversion

Symmetry in certain geometries can be exploited to improve the speed and efficiency in solving the DOT inverse problem. Specifically, it enables the reconstruction of massive data sets with a very high number of source-detector pairs, which cannot be achieved via iterative methods due to their high computational complexity. Studies have shown that the transverse resolution is heavily dependent on the step size of the grid lattice for the placement of sources and detectors. In other words, a large number of source-detector pairs is needed to attain the best spatial resolution possible.

Thus, various initiatives have been developed for Green's functions in different geometries, which are analytical inversion kernels for solving the inverse problem [56, 57, 59]. These include translational symmetry in a slab and rotational symmetry in a cylinder or sphere. In particular, the only critical property used in the derivation of the Green's function G_0 is translational or rotational invariance. For instance, in the infinite slab geometry, G_0 is invariant in terms of translations parallel to the transverse plane, whereas in the infinite cylindrical geometry, G_0 is invariant in terms of translations parallel to and rotations about the cylinder axis. Further details on how to make use of these derivations in solving the DOT inverse problem are presented and discussed in the later sections of this chapter.

5.2 Continuous-wave in infinite slab geometry

5.2.1 Objectives and theoretical background

The objective of this DOT experiment is to reconstruct the absorption coefficient $\mu_a(\vec{r})$ from a set of measurement data $\phi(\vec{r})$ on the detector plane of the slab, according to the framework described below.

As mentioned in an earlier chapter, the diffusion approximation to the radiative transport equation is used to model light propagation in a highly scattering medium. The diffusion equation in CW domain is as follows:

$$[-\nabla \cdot D(\vec{r})\nabla + v\mu_a(\vec{r})]\phi(\vec{r}) = S(\vec{r}), \quad (40)$$

where $D(\vec{r})$ = diffusion coefficient, v = average speed of light in medium and $S(\vec{r})$ = source intensity.

In addition, the Robin boundary condition, $\phi = z_b \hat{n} \cdot \nabla \phi$, must be satisfied, where z_b = extrapolation distance and $\hat{n}$ = vector normal to plane at boundary. Since the medium is highly scattering, $\mu_a \ll \mu_s'$, where μ_s' is the reduced scattering

coefficient, and $D = v / 3(\mu_a + \mu_s') \approx v / 3\mu_s'$. In addition, if μ_s' is the same for both target and medium, $D \approx D^{(0)}$ is constant.

In the experiment, light was shone into the tank at the position defined by $\vec{r}_s = (\vec{\rho}_s, z_s)$, while the CCD camera measured the light intensity coming out from the opposite surface at the position $\vec{r}_d = (\vec{\rho}_d, z_d)$. In this coordinate system, $\vec{\rho}_s$ and $\vec{\rho}_d$ are the 2D radius-vectors defining the transverse coordinates of the source and detector respectively, while the z-axis defines the direction perpendicular to the slab.

Theoretically, the measured intensity $I(\vec{r}_d, \vec{r}_s)$, is related to the Green's function, $G(\vec{r}_d, \vec{r}_s)$, by $I(\vec{r}_d, \vec{r}_s) = C_d C_s G(\vec{r}_d, \vec{r}_s)$, where C_d and C_s are unknown coupling coefficients. For two arbitrary positions on the surface or inside the medium $\vec{r}$ and $\vec{r}'$, $G(\vec{r}, \vec{r}')$ is defined by:

$$[-D^{(0)}\nabla^2 + v\mu_a(\vec{r})]G(\vec{r}, \vec{r}') = \delta(\vec{r} - \vec{r}'), \quad (41)$$

where $\delta(\vec{r} - \vec{r}')$ represents a delta point source.

In addition, the absorption coefficient $\mu_a(\vec{r})$ can be broken down into two components, as $\mu_a(\vec{r}) = \mu_a^{(0)} + \delta\mu_a(\vec{r})$, where $\mu_a^{(0)}$ denotes the background absorption coefficient in the medium while $\delta\mu_a(\vec{r})$ represents a spatial variation caused by the target. As a result, the Green's function satisfies the Dyson equation:

$$G(\vec{r}_d, \vec{r}_s) = G_0(\vec{r}_d, \vec{r}_s) - \int_V G_0(\vec{r}_d, \vec{r}) v \delta\mu_a(\vec{r}) G(\vec{r}, \vec{r}_s) d^3r, \quad (42)$$

where G_0 is the Green's function when there is no target in the medium, meaning that $\delta\mu_a(\vec{r}) = 0$ and $\mu_a(\vec{r}) = \mu_a^{(0)}$.

Although the left-hand side of the above equation can be measured using the CCD camera, $G(\vec{r}, \vec{r}_s)$ on the right-hand side cannot be found, as the position defined by $\vec{r}$ lies inside the medium. Since $G(\vec{r}, \vec{r}_s)$ is heavily dependent on $\delta\mu_a(\vec{r})$, the right-hand side becomes a non-linear function of $\delta\mu_a(\vec{r})$, making the forward problem difficult to solve. In order to simplify the solving process, the first Rytov approximation is used, resulting in the following expression:

$$G(\vec{r}_d, \vec{r}_s) = G_0(\vec{r}_d, \vec{r}_s) \exp \left[\frac{-\int_V G_0(\vec{r}_d, \vec{r}) v \delta\mu_a(\vec{r}) G_0(\vec{r}, \vec{r}_s) d^3r}{G_0(\vec{r}_d, \vec{r}_s)} \right] \quad (43)$$

This expression can be manipulated to form the data function $\varphi(\vec{r}_d, \vec{r}_s)$:

$$\begin{aligned}\varphi(\vec{r}_d, \vec{r}_s) &= -G_0(\vec{r}_d, \vec{r}_s) \ln \frac{I(\vec{r}_d, \vec{r}_s)}{I_0(\vec{r}_d, \vec{r}_s)} = -G_0(\vec{r}_d, \vec{r}_s) \ln \frac{G(\vec{r}_d, \vec{r}_s)}{G_0(\vec{r}_d, \vec{r}_s)} \\ &= \int_V G_0(\vec{r}_d, \vec{r}) v \delta\mu_a(\vec{r}) G_0(\vec{r}, \vec{r}_s) d^3r\end{aligned}\quad (44)$$

where $I(\vec{r}_d, \vec{r}_s)/I_0(\vec{r}_d, \vec{r}_s)$ is the ratio of detected intensity with target to that without target. Note that over here, relative image reconstruction (not absolute) is used to eliminate source (C_s) and detector (C_d) coupling coefficients, which is reflected in the fact that $I(\vec{r}_d, \vec{r}_s)/I_0(\vec{r}_d, \vec{r}_s) = C_s C_d G(\vec{r}_d, \vec{r}_s)/C_s C_d G_0(\vec{r}_d, \vec{r}_s)$.

5.2.2 Experimental setup

A non-contact, single-wavelength (660 nm), continuous-wave DOT system was built in parallel plate geometry using a galvanometer-steered laser and a CCD camera (Figure 20). 2 targets (1 cm x 1 cm x 1 cm) 2.5 cm apart, with higher absorption coefficients than the surrounding medium were positioned in the middle of a slab container (30 cm x 25 cm x 6 cm) filled with highly scattering Intralipid solution. The targets are secured on a nylon thread going through their centres. Their absorption coefficients are approximately five times the background absorption coefficient. A CCD image was captured for each source position on a regular 15 x 15 grid, when there was no target in the medium, and the whole image

capturing process was repeated with the targets submerged in the medium. The collected data is then preprocessed and used to reconstruct a 3D image of the absorption coefficient (μ_a).

In addition, the absorption and reduced scattering coefficients of the background Intralipid medium had been obtained using a commercial tissue oximeter (OxiplexTS, ISS), which is about 0.03 cm^{-1} and 4.1 cm^{-1} respectively. On the other hand, the target absorption coefficients had been obtained using a commercial spectrometer, which is about 0.16 cm^{-1} (~5x absorption contrast from background), while the target Intralipid concentration is set at the same value as that of the background, which gives an equivalent reduced scattering coefficient of 4.1 cm^{-1} . The spatial sampling needs to be carefully chosen, as there is a tradeoff between computational complexity and reconstructed image quality. The total imaging area ($8.4 \text{ cm} \times 8.4 \text{ cm}$) is configured to have complete coverage over the targets and the spatial sampling interval (0.6 cm) is chosen to be smaller than the target physical size ($1 \text{ cm} \times 1 \text{ cm} \times 1 \text{ cm}$) for better resolution. A finer sampling interval can certainly improve the final image quality, but our chosen sampling interval is reasonable enough to resolve the targets, considering computational limitations. The acquisition time for one CCD image is 1 second, which includes the time taken for the rotation of galvanometer mirrors, the CCD exposure time, as well as a short time interval delay before the next image. Since there are 15×15 source positions

and the total acquisition time for each source position is 1 second, the total data acquisition time amounts to 225 (15 x 15) seconds.

For data calibration, relative image reconstruction was performed to remove the effects of unequal coupling coefficients, as explained in the previous section. In addition, a uniform CCD pixel sensitivity is assumed, which is valid as previous studies on vignetting effects (decrease in CCD sensitivity towards the sides) have been conducted and proven such effects to be minimal.

Figure 20. Experimental setup of continuous-wave DOT in slab geometry (left). 15 x 15 source grid relative to target positions (right).

Experimental Protocol:

- No. of sources - 15 x 15 grid, with interval of 0.6 cm
- No. of detectors - 15 x 15 grid, with interval of 0.6 cm (sampled CCD pixels)
- Imaging area dimensions – 8.4 cm x 8.4 cm (8.4 cm = 14 x 0.6 cm)
- Exposure time = 200 ms
- Total data acquisition time = 15 x 15 x 1 s = 225 s (~ 4 min)
- Two absorbing blobs (1 cm x 1 cm x 1 cm) with ~ 5x absorption contrast ($\mu_a = 0.16 \text{ cm}^{-1}$, $\mu_s' = 4.1 \text{ cm}^{-1}$)
- Background optical properties ($\mu_a = 0.03 \text{ cm}^{-1}$, $\mu_s' = 4.1 \text{ cm}^{-1}$)

5.2.3 Analytical image reconstruction

As mentioned earlier in Section 5.2.1, in CW analytical image reconstruction, our experimental data function is defined as:

$$\phi(\vec{r}_d, \vec{r}_s) = -G_0(\vec{r}_d, \vec{r}_s) \ln[T(\vec{r}_d, \vec{r}_s)], \quad (45)$$

where $T(\vec{r}_d, \vec{r}_s) = I(\vec{r}_d, \vec{r}_s) / I_0(\vec{r}_d, \vec{r}_s)$ is the ratio of detected intensity with target to that without target, which is also known as the transmission function. $G_0(\vec{r}_d, \vec{r}_s)$ is the Green's function, which is a solution to the photon diffusion equation when a homogeneous medium (no targets) is illuminated with a delta function point source.

After simplification, the following linear integral equation is obtained [55-57, 59]:

$$\phi(\vec{r}_d, \vec{r}_s) = \int_V G_0(\vec{r}_d, \vec{r}) \nu \delta\mu_a(\vec{r}) G_0(\vec{r}, \vec{r}_s) d^3r \quad (46)$$

In this equation, $\phi(\vec{r}_d, \vec{r}_s)$ can be measured experimentally, while the kernel $\Gamma(\vec{r}_d; \vec{r}_s; \vec{r}) = G_0(\vec{r}_d, \vec{r}) G_0(\vec{r}, \vec{r}_s)$ is known analytically.

Based on the translational symmetry of the slab, G_0 can be rewritten as [55-57, 59]:

$$G_0(\vec{\rho}, z; \vec{\rho}', z') = \int g(\vec{q}; z, z') \exp[i\vec{q} \cdot (\vec{\rho}' - \vec{\rho})] \frac{d^2 q}{(2\pi)^2}, \quad (47)$$

$$\text{where } g(\vec{q}; z, z') = \frac{\sinh[Q(L - |z - z'|)] + Q\ell \cosh[Q(L - |z - z'|)]}{\sinh(QL) + 2Q\ell \cosh(QL) + (Q\ell)^2 \sinh(QL)} \quad [57],$$

$$Q = \sqrt{q^2 + k^2} \text{ and } L = \text{slab thickness.}$$

After performing a 4-D Fourier transform with respect to $\vec{\rho}_s$ and $\vec{\rho}_d$, the following equation is obtained [55-57, 59]:

$$\tilde{\phi}(\vec{q}_d, \vec{q}_s) = \nu \int_{z_d}^{z_s} g(\vec{q}_s; z_s, z) g(\vec{q}_d; z, z_d) \delta\tilde{\mu}_a(\vec{q}_s + \vec{q}_d, z) dz, \quad (48)$$

where the Fourier transforms are defined as:

$$\tilde{\phi}(\vec{q}_d, \vec{q}_s) = \sum_{\vec{\rho}_d, \vec{\rho}_s} \phi(\vec{\rho}_d, z_d; \vec{\rho}_s, z_s) \exp[i(\vec{\rho}_d \cdot \vec{q}_d + \vec{\rho}_s \cdot \vec{q}_s)], \quad (49)$$

$$\delta\tilde{\mu}_a(\vec{q}_s + \vec{q}_d, z) = \int \delta\mu_a(\vec{\rho}, z) \exp(i\vec{\rho} \cdot (\vec{q}_s + \vec{q}_d)) d^2 \rho$$

With the change of variables $\vec{q}_s = \vec{q} + \vec{p}$, $\vec{q}_d = -\vec{p}$, the data function becomes:

$$\psi(\vec{q}, \vec{p}) = \int_{z_d}^{z_s} \kappa(\vec{q}, \vec{p}; z) \delta\tilde{\mu}_a(\vec{q}, z) dz, \quad (50)$$

where $\psi(\vec{q}, \vec{p}) = \phi(\vec{q} + \vec{p}, -\vec{p})$ and $\kappa(\vec{q}, \vec{p}; z) = \nu g(\vec{q} + \vec{p}; z_s, z) g(-\vec{p}; z, z_d)$.

In this whole process, the problem has been reduced to inverting a set of linear 1-D integral equations with known kernels $\kappa(\vec{q}, \vec{p}; z)$. The pseudo-inverse of $\kappa(\vec{q}, \vec{p})$ is constructed via singular value decomposition (SVD). Subsequently, $\delta\tilde{\mu}_a(\vec{q}, z)$ undergoes inverse Fourier transform, resulting in a spatial map of the absorption coefficient $\delta\mu_a(\vec{\rho}, z)$.

In the actual image reconstruction process, a 4-D data function based on 50,625 (= 15^2 sources x 15^2 detectors) data images was defined, since the source and detector grids are both 15 x 15 in terms of dimensions, and the inverse problem was solved via singular value decomposition in the Fourier domain, in order to obtain a final map of the absorption coefficient (μ_a). The entire reconstruction process took less than 4 seconds with MatLab coding executed on a 1.99 GHz AMD processor, and the 2 target blobs can still be seen, as shown in Figure 21 below. This result has proven that this CW analytical image reconstruction technique is reasonably fast

and reliable and thus, it can be further developed and modified to make it more versatile, efficient and accurate.

Reconstruction Protocol:

- Minimum singular value as criteria for SVD truncation, $\lambda_{min} = 2 \times 10^{-7}$
- No. of $\vec{q}$ sampled = 9
- No. of $\vec{p}$ sampled = 5

Figure 21. Reconstructed images of absorption coefficient (μ_a) at different depths, with 15 x 15 source positions. The 2 blobs can be clearly seen at $z = 3$ cm.

From Figure 21, it can be seen that the reconstructed target and background absorption coefficients are approximately 0.15 cm^{-1} and 0.02 cm^{-1} respectively, which is quite close to the corresponding values of 0.16 cm^{-1} and 0.03 cm^{-1} , pre-determined using commercial instruments. Thus, the reconstructed absorption contrast compared to the background is around 7-9 times, which is also close to the initial pre-determined contrast of roughly 5-6 times. However, there are some regions with negative absorption coefficients (-0.2 cm^{-1}), which corresponds to reconstruction artifacts. These can be potentially minimized via post-processing filtering, as well as tweaking pre-reconstruction parameters such as the minimum singular value as the criteria for SVD truncation for instance. More details on how this reconstruction parameter can affect the final reconstruction images will be shown in Section 5.4.3.

5.3 Continuous-wave in infinite cylindrical geometry

After carrying out continuous-wave DOT studies in slab geometry, we extended our analysis to the cylindrical geometry, since the cylindrical geometry is very suitable for containing small mice in animal studies. On top of this, the inherent rotational symmetry in the cylindrical geometry boosts the speed and efficiency in solving the inverse problem, and also contributes to image stability by acquiring data from various angles of projections.

5.3.1 Objectives

The objective is similar to that as mentioned above for the slab geometry, i.e. to reconstruct $\mu_a(\vec{r})$ from a set of measurement data $\phi(\vec{r})$ on the curved cylindrical surface. The same diffusion equation in CW domain is used as the model for photon propagation within the highly scattering medium, subject to the same Robin boundary condition. In the experiment, light is shone into the cylinder at the position defined by $\vec{r}_s = (\varphi_s, z_s)$, while the CCD camera measures the light intensity coming out from the opposite surface at the position $\vec{r}_d = (\varphi_d, z_d)$.

5.3.2 Experimental setup

A non-contact, single-wavelength (660 nm), continuous-wave DOT system was built using a galvanometer-steered laser and a CCD camera (Figure 22). 2 targets (1 cm x 1 cm x 1 cm) 2.5 cm apart, with higher absorption coefficients than the surrounding are positioned in the middle of a cylindrical container ($r = 3.5$ cm, $h = 30$ cm) filled with highly scattering Intralipid solution. The targets are secured on a nylon thread going through their centres. Mirrors are used to obtain additional projection data at different angles which are not visible from the camera direction, which has previously been exploited in Hillman's work [108, 109] with regards to small-animal optical imaging as well. On top of obtaining additional detector positions, we can also effectively expand the dynamic range by selectively attenuating the mirror view using optical filters. Normally, the mirror view shows saturation in the image since it is closer to the light source. In addition, virtual detectors positioned in the overlapping region between the direct view and mirror-reflected view can be used for data calibration, which will be elaborated in a later paragraph. Ray-tracing due to the curvature of the cylinder is displayed using arrows in Figure 22. The CCD exposure time (100 ms) and gain (2x) are carefully chosen, so as to utilize the entire dynamic range (16 bits) of the CCD, while preventing CCD saturation at the same time. The laser source moves to a new position within a fixed time interval, from top to bottom, while the CCD camera captures a corresponding image for each position. Then, the container rotates by a

fixed angular displacement of 20° , via the rotational stage and the image capturing process is repeated again. This continues till the container has completed a full turn of 360° . This whole sequence of image capture is performed 2 times, once without targets and once with targets in the medium. In this manner, surface intensity measurement data will be collected from various projection angles, which is then preprocessed and used to reconstruct a 3D image of the absorption coefficient (μ_a).

In addition, the absorption and reduced scattering coefficients of the background Intralipid medium had been obtained using a commercial tissue oximeter (OxiplexTS, ISS), which is about 0.03 cm^{-1} and 4.1 cm^{-1} respectively. On the other hand, the target absorption coefficients had been obtained using a commercial spectrometer, which is about 0.16 cm^{-1} ($\sim 5\times$ absorption contrast from background), while the target Intralipid concentration is set at the same value as that of the background, which gives an equivalent reduced scattering coefficient of 4.1 cm^{-1} .

An important experimental consideration is that there are intensity differences in CCD measurements between the direct (I_{direct}) versus indirect ($I_{indirect}$) projection cases due to differences in path lengths between the two cases, as well as the reflection coefficients of the mirrors. This discrepancy was resolved by measuring

the intensities of multiple corresponding points between the two cases, obtaining the corresponding intensity ratio $I_{direct} / I_{indirect}$ for each point and then deriving a calibration model for the system. This calibration models the cylinder surface as a Lambertian ideal diffusely reflecting surface, where the radiant intensity observed from the surface is directly proportional to the cosine of the angle between the observer's line of sight and the surface normal and inversely related to the pathlength. The data is carefully preprocessed using this calibration model, before being fed into the image reconstruction procedure.

Figure 22. Experimental setup of continuous-wave DOT in cylindrical geometry. Ray-tracing due to the curvature of the cylinder is displayed using

arrows. For image registration, the yellow and red boxes show corresponding points for direct projection onto CCD and indirect projection via mirror reflection.

Figure 23. Sample CCD image with 2 additional mirror projections on the sides, with 9 (vertical) x 18 (horizontal) grid showing source and detector positions. For image registration, the yellow and red boxes show corresponding points for direct projection onto CCD and indirect projection via mirror reflection. Please refer to Figure 22 for schematic illustration of this idea via ray-tracing.

Experimental Protocol:

- No. of sources - 9 (v) x 18 (h), with vertical interval of 0.5 cm and angular interval of 20° (v – vertical; h - horizontal)
- No. of detectors - 9 (v) x 11 (h), with vertical interval of 0.5 cm and angular interval of 20°
- CCD exposure time = 100 ms
- CCD gain – 2x
- Total data acquisition time = 9 x 18 x 1 s = 162 s (~ 3 min)
- Two absorbing blobs (1 cm x 1 cm x 1 cm) with ~ 5x absorption contrast ($\mu_a = 0.16 \text{ cm}^{-1}$, $\mu_s' = 4.1 \text{ cm}^{-1}$)
- Background optical properties ($\mu_a = 0.03 \text{ cm}^{-1}$, $\mu_s' = 4.1 \text{ cm}^{-1}$)

5.3.3 Finite-element-based image reconstruction

In order to incorporate experimental data into the NIRFAST image reconstruction process, the objective function to be minimized has to be defined in a modified manner, as follows:

$$\begin{aligned}
\chi^2 &= \min_{\mu} \left\{ \sum_{i=1}^{NM} \left\{ \ln \left[(\varphi_i^M / \varphi_i^{M_0}) (\varphi_i^{C_0} / \varphi_i^C) \right] \right\}^2 + \lambda \sum_{j=1}^{NN} (\mu_j - \mu_0)^2 \right\} \\
&= \min_{\mu} \left\{ \sum_{i=1}^{NM} \left\{ (\ln \varphi_i^M - \ln \varphi_i^{M_0} + \ln \varphi_i^{C_0}) - \ln \varphi_i^C \right\}^2 + \lambda \sum_{j=1}^{NN} (\mu_j - \mu_0)^2 \right\}, \quad (51) \\
&= \min_{\mu} \left\{ \sum_{i=1}^{NM} (\phi_i^M - \phi_i^C)^2 + \lambda \sum_{j=1}^{NN} (\mu_j - \mu_0)^2 \right\}
\end{aligned}$$

where $\phi_i^M = \ln \varphi_i^M - \ln \varphi_i^{M_0} + \ln \varphi_i^{C_0}$ is the preprocessed data to be fed into NIRFAST. Here φ_i^M and $\varphi_i^{M_0}$ refer to the surface intensity data obtained from the experiment with and without targets respectively, whereas $\varphi_i^{C_0}$ refers to the forward data computed from the NIRFAST forward solver for the same geometry with no targets.

In order to generate the forward data $\varphi_i^{C_0}$, a mesh with 6012 nodes and 27459 elements was first created within NIRFAST. This mesh is defined to mimic the actual turbid medium, by having the same dimensions as the cylinder, as well as the same background optical parameters ($\mu_a = 0.03 \text{ cm}^{-1}$ and $\mu_s' = 4.1 \text{ cm}^{-1}$) as the Intralipid medium, which is pre-determined using commercial instruments, as

mentioned in the previous section. In addition, the actual source and detector positions are also input into NIRFAST, enabling the forward data $\varphi_i^{C_0}$ to be calculated.

In the image reconstruction process, the modulation frequency is set at 0 MHz, since it is CW case, and the initial regularization parameter is set at 0.1. In addition, the reconstruction basis used is a pixel basis with dimensions [18 18 18]. The pixel basis instead of the mesh basis is chosen, so as to reduce the number of variables to be solved. The mesh basis is not used, as the number of nodes is too large and thus, not computationally feasible. In addition, the size of the pixel basis is maximized as far as possible in order to improve the resolution, while at the same time maintaining the image reconstruction procedure well within computational limitations. Interpolation between pixels is utilized for visualization of results and the 2 blobs can be clearly seen in Figure 24 below.

Figure 24. Reconstructed absorption coefficient (μ_a) in infinite cylindrical geometry using NIRFAST (a) Top view at different planes along the z-axis. (b) Top view of μ_a contour (c) Front view of μ_a contour.

As shown in the z-slices in Figure 24 above, the two blobs are clearly resolved at the correct location, 2.5 cm apart from each other near the $z = 0$ plane. In addition,

the reconstructed absorption coefficient ($\mu_a \approx 0.1 \text{ cm}^{-1}$) is approximately 3 to 4 times the reconstructed background absorption coefficient ($\mu_a \approx 0.03 \text{ cm}^{-1}$), which is a good match to the pre-determined corresponding value of 5 times (target $\mu_a = 0.16 \text{ cm}^{-1}$, background $\mu_a = 0.03 \text{ cm}^{-1}$).

5.3.4 Analytical image reconstruction

The same data function $\phi(\vec{r}_d, \vec{r}_s) = \nu \int_V G_0(\vec{r}_d, \vec{r}) \delta\mu_a(\vec{r}) G_0(\vec{r}, \vec{r}_s) d^3r$ [37, 38 and 40] for

CW analytical image reconstruction for infinite slab geometry, is also employed here. For the cylindrical geometry with coordinate system (z, ρ, φ) and radius R , where sources and detectors are located on the measurement surface $\rho = R$, via exploiting rotational symmetry, the Green's function can be expressed as [55-57, 59]:

$$G_0(\vec{r}, \vec{r}') = \sum_{m=-\infty}^{\infty} \int g(m, q; \rho) \exp[im(\varphi - \varphi')] \exp[iq(z - z')] \frac{dq}{(2\pi)^2}, \quad (52)$$

$$\text{where } g(m, q; \rho) = \frac{1}{R} \left[\frac{I_m(Q\rho)}{I_m(QR) + Q\ell I'_m(QR)} \right] \quad [57],$$

$I_m(Q\rho)$ = modified Bessel function of the 1st kind, z = cylinder height and ρ = cylinder radius.

After performing a 4-D Fourier transform with respect to $\varphi_s, \varphi_d, z_s, z_d$, the following equation is obtained [55-57, 59]:

$$\tilde{\phi}(m_s, q_s; m_d, q_d) = \nu \int_0^R \rho g(m_s, q_s; \rho) g(m_d, q_d; \rho) \delta\tilde{\mu}_a(m, q) d\rho, \quad (53)$$

where the Fourier transforms are defined as:

$$\delta\tilde{\mu}_a(m, q) = \int \delta\mu_a(\varphi, z) \exp[i(m_s\varphi + m_d\varphi + q_s z + q_d z)] d\varphi dz, \quad (54)$$

$$\tilde{\phi}(m_s, q_s; m_d, q_d) = \int_{-\infty}^{\infty} dz_s dz_d \int_0^{2\pi} d\varphi_s d\varphi_d \phi(\varphi_s, z_s, \varphi_d, z_d) \exp[i(q_s z_s + q_d z_d + m_s \varphi_s + m_d \varphi_d)]$$

With the change of variables $q_s = q + p$, $q_d = -p$, $m_s = m + n$ and $m_d = -n$, the data function becomes:

$$\psi(m + n, q + p; -n, -p) = \int_0^R \kappa(m + n, q + p; -n, -p; \rho) \delta\tilde{\mu}_a(m, q) d\rho, \quad (55)$$

where $\psi(m + n, q + p; -n, -p) = \tilde{\phi}(m_s, q_s; m_d, q_d)$,

$$\kappa(m + n, q + p; -n, -p; \rho) = v\rho g(m_s, q_s; \rho) g(m_d, q_d; \rho).$$

In this whole process, the problem has been reduced to inverting a set of linear 1-D integral equations. The pseudo-inverse is constructed via singular value decomposition (SVD). Subsequently, $\delta\tilde{\mu}_a(m, q)$ undergoes inverse Fourier transform, resulting in a spatial map of the absorption coefficient $\delta\mu_a(\varphi, z)$.

Before performing image reconstruction on actual experimental data, we validated our theoretical framework using simulated data, based on one absorbing target in a highly scattering medium in cylindrical geometry. The model is a single spherical absorbing blob 0.75 cm in diameter, located at a radial distance of 0.5 cm from the cylinder axis, with an absorption coefficient of 0.16 cm^{-1} (~5x absorption contrast from background absorption coefficient of 0.03 cm^{-1}). The aim of this sub-study is to verify the practical feasibility of the analytical reconstruction algorithm in cylindrical geometry, before moving on to reconstruction based on real experimental data. The reconstruction results are shown in Figure 25 (below).

Reconstruction Protocol:

- Minimum singular value as criteria for SVD truncation, $\lambda_{min} = 5 \times 10^{-7}$
- No. of q sampled = 5
- No. of p sampled = 5
- No. of m sampled = 4
- No. of n sampled = 4

Figure 25. Results of feasibility study - reconstructed images of absorption coefficient (μ_a) from simulated data at different radii. The single absorbing blob can be clearly seen at roughly $r = 0.5 \text{ cm}$.

In Figure 25 above, the reconstruction images in the form of cylindrical strips at different radii starting from $r = 0.5 \text{ cm}$ to $r = 3 \text{ cm}$ are displayed accordingly from

top to bottom. In the left column, the cylindrical strips are unwrapped into rectangular strips for better visualization. The single blob is most clearly resolved at the correct location in the $r = 0.5$ cm strip, with a reconstructed absorption coefficient of approximately 0.15 cm^{-1} , whereas the reconstructed background absorption coefficient is about 0.02 cm^{-1} respectively. These values are quite close to the corresponding pre-defined target and background absorption coefficients of 0.16 cm^{-1} and 0.03 cm^{-1} respectively. However, there are some regions with negative absorption coefficients (-0.2 cm^{-1}) especially near the cylinder axis in the $r = 0.5$ cm strip and at the boundaries in the $r = 3$ cm strip, which corresponds to reconstruction artifacts. Thus, these results demonstrate the practical feasibility of the cylindrical analytical reconstruction algorithm using simulated data, which allows us to move on to image reconstruction using experimental data involving the two absorbing blobs ($\sim 5\times$ absorption contrast) next.

In the actual image reconstruction process using experimental data, a 4-D data function based on $9 \times 9 \times 9 \times 11$ ($\approx 8,000$) data images was defined, and the inverse problem was solved via singular value decomposition in the Fourier domain, in order to obtain a final map of the absorption coefficient (μ_a). The entire reconstruction process took less than 15 seconds with MatLab coding executed on

a 1.99 GHz AMD processor, and the reconstruction results are shown in Figure 26 below.

Figure 26. Reconstructed images of absorption coefficient (μ_a) from experimental data at different radii. The 2 blobs can be clearly seen at roughly $r = 1\text{ cm}$.

In Figure 26 above, the reconstruction images in the form of cylindrical strips at different radii starting from $r = 0.5$ cm to $r = 3$ cm are displayed accordingly from top to bottom. In the left column, the cylindrical strips are unwrapped into rectangular strips for better visualization. The two absorbing blobs (2.5 cm apart) are clearly resolved in the $r = 1.25$ cm strip, with a reconstructed absorption coefficient of approximately 0.15 cm^{-1} , whereas the reconstructed background absorption coefficient is about 0.03 cm^{-1} respectively. These values are quite close to the corresponding target and background absorption coefficients of 0.16 cm^{-1} and 0.03 cm^{-1} respectively, pre-determined using commercial instruments. Thus, these results show that the two blobs are reconstructed successfully at the right location with relatively accurate values of absorption coefficients.

As we compare the results from NIRFAST in Figure 24 and analytical image reconstruction in Figure 26, we find that the two blobs are well resolved at the correct location (2.5 cm apart from each other near the $z = 0$ plane) in both cases. In terms of contrast, the reconstructed absorption coefficient ($\mu_a \approx 0.1 \text{ cm}^{-1}$) is approximately 3 to 4 times the reconstructed background absorption coefficient ($\mu_a \approx 0.03 \text{ cm}^{-1}$) in the case of NIRFAST, whereas the reconstructed absorption coefficient ($\mu_a \approx 0.15 \text{ cm}^{-1}$) is approximately 5 times the reconstructed background absorption coefficient ($\mu_a \approx 0.03 \text{ cm}^{-1}$) in the analytical image reconstruction case.

Thus the analytical image reconstruction algorithm is a slightly better match to the pre-determined corresponding absorption contrast of 5 times (target $\mu_a = 0.16 \text{ cm}^{-1}$, background $\mu_a = 0.03 \text{ cm}^{-1}$). In addition, the analytical image reconstruction is also much faster in terms of speed (less than 15 seconds), although it has lower image quality with more artifacts, when compared to NIRFAST.

5.4 Frequency-domain in infinite slab geometry

After obtaining successful results from analytic image reconstruction in continuous-wave DOT in both slab and cylindrical geometry, we also extended the study to frequency-domain DOT, which can be used to resolve absorption and scattering contrast simultaneously and quantitatively.

5.4.1 Objectives

The objective of this DOT experiment is to reconstruct both the absorption coefficient $\mu_a(\vec{r})$ and reduced scattering coefficient $\mu_s'(\vec{r})$ from a set of measurement data $\phi(\vec{r})$ on the detector plane of the slab. The diffusion equation in FD domain is used as the model for photon propagation within the highly scattering medium:

$$[-\nabla \cdot D(\vec{r})\nabla - i\omega + v\mu_a(\vec{r})]\phi(\vec{r}) = S(\vec{r}) \quad (56)$$

Other parameters and details remain unchanged.

5.4.2 Experimental setup

A non-contact, single-wavelength (660 nm), homodyne frequency-domain DOT system was built in parallel plate geometry using a galvanometer-steered laser and a CCD camera (Figure 27). The source and the image intensifier gain are modulated at the same modulation frequency of 70 MHz, which is commercially compatible with current electronic systems. 2 cylindrical targets (diameter – 1 cm, length – 4 cm) 2.5 cm apart, with different absorption and reduced scattering coefficients than the surrounding medium are positioned in the middle of a slab container (30 cm x 25 cm x 6 cm) filled with highly scattering Intralipid solution. The targets are secured on a nylon thread going through their centres. The CCD exposure time (200 ms) and gain (2x) are carefully chosen, so as to utilize the entire dynamic range (16 bits) of the CCD, while preventing CCD saturation at the same time. CCD intensity data are acquired for 9 x 9 source grid positions at different phase delays over one cycle, once without targets and once with targets in the medium. The amplitude and phase measurements for each CCD pixel are obtained by fitting the detected intensity values corresponding to different phase delays for that pixel, to a sine wave. 3D images of the absorption (μ_a) and reduced scattering coefficient (μ_s') are then reconstructed from the amplitude and phase measurements.

In addition, the absorption and reduced scattering coefficients of the background Intralipid medium had been obtained using a commercial tissue oximeter (OxiplexTS, ISS), which is about 0.03 cm^{-1} and 4.1 cm^{-1} respectively. On the other hand, the target absorption coefficients had been obtained using a commercial spectrometer, which is about 0.1 cm^{-1} ($\sim 3\times$ absorption contrast from background), while the target Intralipid concentration is set at 3 times the background concentration, which gives a reduced scattering coefficient of 12 cm^{-1} roughly.

Figure 27. Homodyne frequency-domain DOT setup in slab geometry (left). (A) CCD camera (B) Image intensifier (C) Targets in Lipofundin medium; (D) Galvanometer (E) 660 nm laser. 9 x 9 Source grid relative to target positions (right).

Experimental Protocol:

- No. of sources - 9 x 9 grid, with interval of 1.2 cm
- No. of detectors - 19 x 19 grid, with interval of 0.6 cm
- No. of phases – 18 with interval of 20°
- Modulation frequency = 70 MHz
- CCD exposure time = 200 ms
- CCD gain – 2x
- Total data acquisition time = 9 x 9 x 18 x 1 s = 1458 s (~ 24 min)
- Two cylindrical objects with ~3x absorption and scattering contrast ($\mu_a = 0.1 \text{ cm}^{-1}$, $\mu_s' = 12 \text{ cm}^{-1}$)
- Background optical properties ($\mu_a = 0.03 \text{ cm}^{-1}$, $\mu_s' = 4.1 \text{ cm}^{-1}$)

5.4.3 Analytical image reconstruction

In FD analytical image reconstruction, our experimental data function is defined as:

$$\phi(\vec{r}_d, \vec{r}_s) = -G_0(\vec{r}_d, \vec{r}_s) \ln \left[\frac{I(\vec{r}_d, \vec{r}_s)}{I_0(\vec{r}_d, \vec{r}_s)} + i(\theta(\vec{r}_d, \vec{r}_s) - \theta_0(\vec{r}_d, \vec{r}_s)) \right], \quad (57)$$

where $I(\vec{r}_d, \vec{r}_s) / I_0(\vec{r}_d, \vec{r}_s)$ is the ratio of the detected intensity with targets to the detected intensity without targets (reference) and $\theta(\vec{r}_d, \vec{r}_s) - \theta_0(\vec{r}_d, \vec{r}_s)$ is the phase

difference between the target and reference data. Note that relative image reconstruction (not absolute) is used to eliminate coupling coefficients, which is reflected in the fact that $I(\vec{r}_d, \vec{r}_s) / I_0(\vec{r}_d, \vec{r}_s) = G(\vec{r}_d, \vec{r}_s) / G_0(\vec{r}_d, \vec{r}_s)$.

After simplification, the following linear integral equation is obtained [55-57, 59]:

$$\phi(\vec{r}_d, \vec{r}_s) = v \int_V G_0(\vec{r}_d, \vec{r}) [\delta\mu_a(\vec{r}) - \nabla \cdot \delta D(\vec{r}) \nabla] G_0(\vec{r}, \vec{r}_s) d^3r \quad (58)$$

In this equation, $\phi(\vec{r}_d, \vec{r}_s)$ can be measured experimentally, while the kernel $\Gamma(\vec{r}_d; \vec{r}_s; \vec{r}) = G_0(\vec{r}_d, \vec{r}) G_0(\vec{r}, \vec{r}_s)$ is known analytically. In the process of simplification, the unknown coupling coefficients C_d and C_s are eliminated, since $I(\vec{r}_d, \vec{r}_s) / I_0(\vec{r}_d, \vec{r}_s) = G(\vec{r}_d, \vec{r}_s) / G_0(\vec{r}_d, \vec{r}_s)$.

After performing a 4-D Fourier transform with respect to $\vec{\rho}_s$ and $\vec{\rho}_d$, the following equation is obtained [55-57, 59]:

$$\begin{aligned} \tilde{\phi}(\vec{q}_d, \vec{q}_s) = & v \int_{z_d}^{z_s} g(\vec{q}_s; z_s, z) g(\vec{q}_d; z, z_d) \delta\tilde{\mu}_a(\vec{q}_s + \vec{q}_d, z) dz \\ & + v \int_{z_d}^{z_s} \frac{\partial g(\vec{q}_s; z_s, z)}{\partial z} \frac{\partial g(\vec{q}_d; z, z_d)}{\partial z} - \vec{q}_s \cdot \vec{q}_d g(\vec{q}_s; z_s, z) g(\vec{q}_d; z, z_d) \delta\tilde{D}(\vec{q}_s + \vec{q}_d, z) dz \end{aligned} \quad (59)$$

where the Fourier transforms are defined as:

$$\tilde{\phi}(\vec{q}_d, \vec{q}_s) = \sum_{\vec{\rho}_d, \vec{\rho}_s} \phi(\vec{\rho}_d, z_d; \vec{\rho}_s, z_s) \exp[i(\vec{\rho}_d \cdot \vec{q}_d + \vec{\rho}_s \cdot \vec{q}_s)], \quad (60)$$

$$\delta\tilde{\mu}_a(\vec{q}_s + \vec{q}_d, z) = \int \delta\mu_a(\vec{\rho}, z) \exp(i\vec{\rho} \cdot (\vec{q}_s + \vec{q}_d)) d^2\rho$$

$$\delta\tilde{D}(\vec{q}_s + \vec{q}_d, z) = \int \delta D(\vec{\rho}, z) \exp(i\vec{\rho} \cdot (\vec{q}_s + \vec{q}_d)) d^2\rho$$

With the change of variables $\vec{q}_s = \vec{q} + \vec{p}$, $\vec{q}_d = -\vec{p}$, the data function becomes:

$$\psi(\vec{q}, \vec{p}) = \int_{z_d}^{z_s} \alpha(\vec{q}, \vec{p}; z) \delta\tilde{\mu}_a(\vec{q}, z) + \beta(\vec{q}, \vec{p}; z) \delta\tilde{D}(\vec{q}, z) dz, \quad (61)$$

where $\psi(\vec{q}, \vec{p}) = \phi(\vec{q} + \vec{p}, -\vec{p})$, $\alpha(\vec{q}, \vec{p}; z) = v g(\vec{q} + \vec{p}; z_s, z) g(-\vec{p}; z, z_d)$ and

$$\beta(\vec{q}, \vec{p}; z) = v \left[\frac{\partial g(\vec{q} + \vec{p}; z_s, z)}{\partial z} \frac{\partial g(-\vec{p}; z, z_d)}{\partial z} - (\vec{q} + \vec{p}) \cdot (-\vec{p}) g(\vec{q} + \vec{p}; z_s, z) g(-\vec{p}; z, z_d) \right].$$

In this whole process, the problem has been reduced to inverting a set of linear 1-D integral equations with known kernels $\alpha(\vec{q}, \vec{p}; z)$ and $\beta(\vec{q}, \vec{p}; z)$. The pseudo-inverse is constructed via singular value decomposition (SVD). Subsequently, $\delta\tilde{\mu}_a(\vec{q}, z)$ and $\delta\tilde{D}(\vec{q}, z)$ undergoes inverse Fourier transform, resulting in a spatial

map of the absorption coefficient $\delta\mu_a(\vec{\rho}, z)$ and the diffusion coefficient $\delta D(\vec{\rho}, z)$ respectively. The reduced scattering coefficient can also be obtained via the relation $\delta D(\vec{\rho}, z) = v/3[\delta\mu_a(\vec{\rho}, z) + \delta\mu_s'(\vec{\rho}, z)]$.

In the actual image reconstruction process, a 4-D data function based on $9 \times 9 \times 19 \times 19$ ($\approx 29,000$) data images was defined, and the inverse problem was solved via singular value decomposition in the Fourier domain, in order to obtain a final map of the absorption coefficient (μ_a) and reduced scattering coefficient (μ_s'). The entire reconstruction process took less than 1 minute with MatLab coding executed on a 1.99 GHz AMD processor, and both absorbing and scattering contrast are successfully resolved, although there is significant level of crosstalk, as shown in Figure 28 below.

Reconstruction Protocol:

- Minimum singular value as criteria for SVD truncation, $\lambda_{min} = 2 \times 10^{-7}$
- No. of $\vec{q}$ sampled = 9
- No. of $\vec{p}$ sampled = 5

Figure 28. Reconstructed images of (a) absorption contrast ($\mu_a/\mu_a^{(0)}$) and (b) reduced scattering coefficient contrast ($\mu_s'/\mu_s'^{(0)}$) at different depths from left (close to source plane, $z = 1\text{ cm}$) to right (close to detector plane, $z = 5\text{ cm}$).

From Figure 28, it can be seen that the reconstructed absorption and reduced scattering contrast are approximately 3 and 2.5 times the background respectively, which is a good match to the pre-determined corresponding values (3 times). In order to investigate the dependence of image quality on reconstruction parameters, image reconstruction is repeated several times using different values for SVD truncation.

The SVD truncation values are carefully chosen based on the desired balance between image resolution and level of noise artifacts. The minimum singular value (λ_{min}) in the SVD diagonal matrix to be retained is used as a criterion for SVD truncation. Singular values larger than λ_{min} are retained for matrix inversion while those less than λ_{min} are removed. A higher value of λ_{min} results in a smaller number of singular values retained, which in turn leads to a smoother image with less noise, but with lower image resolution. On the other hand, a smaller λ_{min} value causes a larger number of singular values to be utilized, which in turn gives rise to higher image resolution, but with more high-frequency noise artifacts. This concept can be seen as an analogy to regularization within least-squares minimization. A comparison of reconstructed absorption and reduced scattering contrast images based on different SVD truncation values is shown in Figure 29 and 30.

Figure 29. Comparison of reconstructed absorption contrast ($\mu_a/\mu_a^{(0)}$) at different depths from left (close to source plane, $z = 1\text{ cm}$) to right (close to detector plane, $z = 5\text{ cm}$), based on different minimum singular values for SVD truncation, $\lambda_{min} =$ (a) 3×10^{-5} (high value), (b) 6×10^{-7} (medium value), (c) 2×10^{-7} (low value).

From Figure 29, it can be seen that the absorption contrast is better resolved at lower λ_{min} values (b and c), as compared to higher λ_{min} values (a). The recovered

absorption contrast is around 2 to 4 times the background, which is a good fit to the pre-determined absorption contrast of 3 times via a commercial spectrometer.

Figure 30. Comparison of reconstructed reduced scattering contrast ($\mu_s'/\mu_s^{(0)}$) at different depths from left (close to source plane, $z = 1\text{ cm}$) to right (close to detector plane, $z = 5\text{ cm}$), based on different minimum singular values for SVD truncation, $\lambda_{min} =$ (a) 8×10^{-5} (high value), (b) 2×10^{-5} (medium value), (c) 8×10^{-6} (low value).

From Figure 30, it can be seen that there are more high-frequency ripple artifacts at lower λ_{min} values (b and c), as compared to higher λ_{min} values (a). The recovered reduced scattering contrast is around 1.6 to 2.5 times the background, which is a good match to the pre-determined reduced scattering contrast of 3 times.

5.4.4 Multi-frequency DOT using higher harmonics

In addition, we also plotted the intensity values for the centre pixel with different phase delays over one cycle, which may look like a sine wave but actually contains higher harmonic components, as shown in Figure 31 below.

Figure 31. Image intensity versus phase for centre CCD pixel over one cycle.

Thus, we can make use of the higher harmonic components by incorporating them into the analytic code, in order to perform multi-frequency DOT reconstruction. However, we have to take special care in the sub-sampling of useful data points, as well as in phase-unwrapping, which becomes more difficult with increasing modulation frequencies. Nevertheless, we manage to reconstruct the desired

absorption contrast (roughly 3 times the background) from higher harmonic data obtained from experimental data acquired at a single modulation frequency. However, there is significant level of crosstalk between absorption and scatter, as shown in Figure 32 below.

Figure 32. Reconstructed images of absorption contrast ($\mu_a/\mu_a^{(0)}$) and reduced scattering coefficient contrast ($\mu_s'/\mu_s'^{(0)}$), using higher harmonics data. Left - 70 MHz (1ω), middle - CW (DC) + 70 MHz (1ω), right - CW (DC) + 70 MHz (1ω) + 140 MHz (2ω).

From Figure 32, it can be seen that the absorption contrast is reconstructed successfully with relatively accurate values (3 times the background), using only

the first harmonic (left column of Figure 32), as well as the DC component and the first harmonic combined (centre column of Figure 32). However, there is a small amount of cross-talk in the reduced scattering contrast. As for the combined three-component ($DC + 1\omega + 2\omega$) case (right column of Figure 32), the images suffer from poor resolution with high level of noise artifacts. Since there is high odd inherent harmonic content as shown in the laser and gain waveforms in the previous chapter, the incorporation of the second harmonic which has low inherent information, into the image reconstruction process will not contribute much to image resolution and may even degrade image quality, as shown in Figure 32.

6. Frequency-Domain Fluorescence Lifetime Imaging

6.1 Introduction

The phenomenon of fluorescence occurs when a fluorophore absorbs energy of a particular wavelength (excitation), and subsequently emits energy at a different wavelength (emission). The energy level and wavelength of the emission depend on both the fluorophore itself, as well as the microenvironment of the fluorophore, which is the reason why this technique is popular in biochemistry and protein studies. The time interval between excitation and emission is extremely short, typically in the order of nanoseconds (ns). In addition, the emitted radiation is normally of a longer wavelength, as compared to that of the absorbed radiation [110]. From the Planck–Einstein equation, $E = hc / \lambda$, where E represents the energy of the electromagnetic radiation, h is a proportionality constant called the Planck's constant, c is the light speed and λ refers to the wavelength, we can see that E is inversely related to λ . This means that the emitted radiation is of a lower energy level, when compared to that of the absorbed radiation. Thus, there is energy loss during fluorescence.

Figure 33. Fluorescence phenomenon.

After a fluorophore has been excited by a photon to a higher energy state, it will eventually return to its ground state through one of several decay pathways, which can be radiative, non-radiative or combination of both. In fluorescence emission, the return pathway must be accompanied by the spontaneous emission of a photon [111] (Figure 33). The average time that the molecule stays in its excited state before going back to its ground state and emitting a photon is known as the fluorescence lifetime.

The fluorescence emission signal has a first-order exponential decay with respect to time as shown below.

$$F(t) = F_0 \exp(-t / \tau), \quad (62)$$

where $F(t)$ represents the fluorescence intensity at time t , F_0 refers to the initial fluorescence intensity, and τ is the fluorescence lifetime.

In terms of FLI implementation, there are typically two main categories – time-domain and frequency-domain. In time-domain measurements [12, 112-115], the changes of the fluorescence signal over time can be observed. The sample is excited sequentially by short excitation pulses with a pulse width much shorter than the fluorescence lifetime. The fluorescence emission signal is subsequently measured in several time windows by fast detection circuitry, which can be used to obtain decay characteristics. This technique requires costly instrumentation and the number of channels or time windows required is very large, especially for multi-exponential lifetime measurements [116].

A simpler and cheaper alternative would be frequency-domain measurements [110, 117], in which the fluorophore is excited by an intensity-modulated excitation source. The source is modulated at very high frequency in the order of MHz, in a sinusoidal manner (Figure 34). Since the excited state of the fluorophore has its own lifetime, a phase shift and a decrease in modulation depth (demodulation) are observed for the fluorescence emission with respect to the excitation (Figure 35).

Figure 34. Fluorescence emission and lifetime exponential decays (red) as a result of sinusoidal-modulated excitation (blue).

Figure 35. Phase shift ϕ ($\rightarrow$) & demodulation M ($\downarrow$) observed between fluorescence emission (red) and excitation (blue).

The phase shift and modulation depth depends on the modulation frequency and fluorescence lifetime, according to the equations below [118]:

$$\phi = \tan^{-1} \omega \tau \quad (63)$$

$$M = \frac{1}{\sqrt{1 + \omega^2 \tau^2}},$$

where ω represents the modulation frequency, ϕ refers to the phase shift for emission, $M = \frac{M_{em}}{M_{ex}}$ refers to the demodulation - ratio of modulation depth for excitation to that for emission, and τ is the fluorescence lifetime. The equation parameters can be represented in an AB-plot where $A = m \sin \phi$ and $B = m \cos \phi$, as shown in Figure 36. M represents the distance of the point on the semicircle from the origin while ϕ represents the angle in a counterclockwise direction from the positive x-axis.

Figure 36. AB-plot.

The semicircle above is known as the universal curve for individual lifetime components. The location with coordinates $(1,0)$ correspond to a component with zero lifetime, while that with coordinates $(0,0)$ correspond to a component with infinite lifetime. Thus, lifetime values increase in a counterclockwise direction along the semicircle from the right to the left.

For multi-component lifetimes, the final lifetime value is an intensity-weighted average of the combinations of vectors representing individual components. Thus, the resulting coordinates corresponding to the final lifetime will lie inside instead of on the semicircle. Points lying outside the semicircle are practically impossible, corresponding to invalid lifetime combinations. In the dual-component case, the coordinates representing all the various combinations of the ratios of the two lifetime components will lie on the line between them, as shown in Figure 37 below.

Figure 37. AB-plot for dual-component lifetimes [119].

From the above equations for modulation depth and phase shift, as well as AB-plots, we can see that a shorter fluorescence lifetime will result in smaller phase shift, but larger demodulation and vice-versa. As for modulation frequency, there exists an optimum value $\omega = \frac{1}{\tau}$, which depends on the fluorescence lifetime as well [120]. Thus, the range of modulation frequencies which is employed is typically in the radio-frequency (RF) range from one to several hundred MHz, depending on the values of the fluorescence lifetime usually in the order of nanoseconds or picoseconds.

Using Equations 63, from the phase shift and modulation depth, fluorescence lifetimes are calculated pixel-by-pixel, and subsequently displayed in the form of a colormap (Figure 38).

Figure 38. Fluorescence lifetime values ($\tau_1 - \tau_4$) calculated at individual CCD pixels.

6.2 Theoretical background

In frequency-domain FLI, both the image intensifier and the excitation source are modulated at the same frequency. A CCD image sequence is then recorded at equal phase intervals over one complete cycle, and the phase and modulation depth can be obtained from these resulting images via Fourier analysis. The Fourier coefficients are computed at every single pixel, according to the following equations [6]:

$$F_{DC} = \sum_k I_k \quad (64)$$

$$F_{\sin,n} = \sum_k I_k \sin(2\pi nk / K)$$

$$F_{\cos,n} = \sum_k I_k \cos(2\pi nk / K),$$

where I_k is an image in the k -th phase step, n is the harmonic of interest, and K is the total number of phase steps.

Fluorescence lifetime values are usually calculated based on the fundamental frequency only, when $n=1$. However, information on additional lifetime components can also be acquired from higher harmonics inherent in the signal,

especially in multi-exponential decays [121]. Subsequently, the phase shift and modulation depth can be obtained via the following relations:

$$\phi_n = \tan^{-1}(F_{\sin,n} / F_{\cos,n}) \quad (65)$$

$$M_n = \frac{2\sqrt{F_{\sin,n}^2 + F_{\cos,n}^2}}{F_{DC}},$$

where ϕ_n and M_n represents the phase and modulation depth of the n -th order harmonic, respectively.

In order to calculate the fluorescence lifetime for one particular fluorophore (target), another fluorophore (reference) whose lifetime is already known is used as reference. The lifetimes of the target fluorophore from phase (τ_ϕ) and modulation depth (τ_M) information are computed using the equations below:

$$\tau_\phi = \frac{1}{\omega} \tan \left[(\phi - \phi_{ref}) + \tan^{-1}(\omega \tau_{ref}) \right] \quad (66)$$

$$\tau_M = \frac{1}{\omega} \left[\frac{M_{ref}^2}{M^2} (1 + \omega^2 \tau_{ref}^2) - 1 \right]^{1/2},$$

where τ_{ref} is the known lifetime of the reference from literature, ω represents the modulation frequency, ϕ and ϕ_{ref} refer to the phase shift for the target and

reference respectively, and M and M_{ref} refer to the modulation depth for the target and reference respectively.

When a reflecting or scattering object is used as a zero-lifetime reference instead of a fluorophore reference, $\tau_{ref} = 0$ and the fluorescence lifetime equations become:

$$\tau_{\phi} = \frac{1}{\omega} \tan(\phi - \phi_{ref}) \quad (67)$$

$$\tau_M = \frac{1}{\omega} \left[\frac{M_{ref}^2}{M^2} - 1 \right]^{1/2},$$

It is noteworthy that in the case of monoexponential decay, whereby only a single lifetime component is present, $\tau_{\phi} = \tau_M$ [6, 118]. On the other hand, in the case of multi-exponential decay, whereby more than one lifetime component may be present, $\tau_{\phi} < \tau_M$. In this case, both τ_{ϕ} and τ_M correspond to the weighted averages over all the lifetime components in the sample.

6.3 Experimental procedures

The objective of the experiment is to investigate the possibility of fluorescence emission from one dye being absorbed by adjacent dyes, which may affect the final fluorescence lifetime results. This is achieved via comparing the results obtained from simultaneous excitation of all dyes, with results from separate excitation of individual dyes. In addition, we also want to find out more about the influence of the choice of reference dye on the accuracy of final lifetime values. Lastly, we also want to compare the accuracy of results obtained from modulation depth with those from phase.

The experiment was performed via obtaining relative lifetime measurements based on simultaneous excitation of all samples, as well as separate excitation of individual dyes. The experimental setup for fluorescence lifetime imaging is in Figure 39 below.

Figure 39. Free-space experimental setup for fluorescence lifetime imaging. (1) 660 nm laser (2) beam expander and collimator (3) iris (4) emission filter (5) image intensifier (6) CCD camera (obscured under metal plate) (7) sample dyes (7a) single dye for individual excitation (7b) Alexa-Fluor 680 dye (7c) ATTO 680 dye (7d) DyLight 680 dye.

The 3 fluorescent dyes – ATTO 680, Alexa Fluor 680 and DyLight 680, are chosen as samples, since they can be simultaneously excited by the 660 nm laser and have similar absorbance and fluorescence spectra suitable for comparison. The corresponding spectra for ATTO 680 are shown in Figure 40 as reference [122].

Figure 40. Absorbance and fluorescence spectra of ATTO 680.

At the beginning of the optical path, the laser beam was expanded and collimated, in order to provide an area of illumination large enough to excite all the dyes at once. A long-pass emission filter with a cut-off wavelength at 700 nm was placed in front of the image intensifier to filter out the backscattered 660 nm excitation light, as well as allow the 710 nm fluorescence emission signal to pass through to be detected by the CCD camera.

Before commencing the actual experiment, both the image intensifier and the CCD camera were first focused carefully to provide a suitable imaging view, with the samples omitted in the setup initially. In addition, the aperture size, shutter speed and the gain of the CCD camera were adjusted appropriately, in order to avoid saturation in images during acquisition over the entire phase cycle. This was done

via first determining the phase step which corresponds to the maximum fluorescence emission signal, and subsequently adjusting the parameters such that the detected intensity lies well within the CCD dynamic range. After mounting the 3 samples, the iris was kept closed till the start of the experiment, in order to prevent photo-bleaching and degradation of the samples.

During the process of data acquisition, both the laser and the image intensifier gain were modulated at the same frequency of 70 MHz via a homodyne scheme, and a background intensity CCD image was first obtained, without the laser illuminating the sample. Following this, the iris was opened to allow the laser to excite the dyes, and the fluorescence emission signals from the dyes were captured via CCD images taken at each phase interval with an equal phase step of 30° over a complete cycle. Then the collected data was preprocessed by performing background intensity subtraction using the background image acquired earlier. The Fourier coefficients are then computed, according to the equations mentioned earlier in the previous section:

$$F_{DC} = \sum_k I_k, \quad F_{\sin,n} = \sum_k I_k \sin(2\pi nk / K), \quad F_{\cos,n} = \sum_k I_k \cos(2\pi nk / K), \quad (68)$$

where I_k is an image in the k -th phase step, n is the harmonic of interest, and K is the total number of phase steps.

Subsequently, the phase shift and modulation depth are also calculated:

$$\phi_n = \tan^{-1}(F_{\sin,n} / F_{\cos,n}), \quad M_n = \frac{2\sqrt{F_{\sin,n}^2 + F_{\cos,n}^2}}{F_{DC}}, \quad (69)$$

where ϕ_n and M_n represents the phase and modulation depth of the n -th order harmonic, respectively.

For relative lifetime measurements, one dye is chosen to be the reference dye, while another dye whose lifetime is to be found is the target dye. The lifetimes of the target dyes from phase (τ_ϕ) and modulation depth (τ_M) information are then computed using the equations below:

$$\tau_\phi = \frac{1}{\omega} \tan\left[(\phi - \phi_{ref}) + \tan^{-1}(\omega\tau_{ref})\right], \quad \tau_M = \frac{1}{\omega} \left[\frac{M_{ref}^2}{M^2} (1 + \omega^2\tau_{ref}^2) - 1 \right]^{1/2}, \quad (70)$$

where τ_{ref} is the known lifetime of the reference dye from literature, ω represents the modulation frequency, ϕ and ϕ_{ref} refer to the phase shift for the target and reference dye respectively, and M and M_{ref} refer to the modulation depth for the target and reference dye respectively.

6.4 Image analysis and discussion

Figure 41. Simultaneous excitation on Alexa Fluor (reference) & ATTO (sample). Top row from left to right: modulation depth, M (reference), phase, ϕ (reference, radians), lifetime from modulation depth, τ_M (seconds). Bottom row from left to right: modulation depth, M (target), phase, ϕ (target, radians), lifetime from phase, τ_ϕ (seconds).

Figure 42. Separate excitation on Alexa Fluor (reference) & ATTO (sample). Top row from left to right: modulation depth, M (reference), phase, ϕ (reference, radians), lifetime from modulation depth, τ_M (seconds). Bottom row from left to right: modulation depth, M (target), phase, ϕ (target, radians), lifetime from phase, τ_ϕ (seconds).

From the figures above, we observed that the approximate mean lifetimes from phase shift ($\tau_\phi = 1.91ns, 2.05ns$) yielded more accurate results compared to those from modulation depth ($\tau_M = 4.56ns, 3.02ns$), since the expected lifetime value ($\tau = 1.8ns$) from literature is much closer to τ_ϕ than τ_M . In addition, the lifetimes obtained from phase shift also showed lower uncertainty with lower standard

deviations (in the order of 10^{-11}), compared to those from modulation depth with higher standard deviations (in the order of 10^{-10}). Thus, lifetimes values from phase shift are more reliable than those from modulation depth.

In addition, we also want to find out more about the influence of the choice of reference dye on the accuracy of final lifetime values. We observed that the lifetime values obtained from phase shift are more accurate when the reference lifetime is less than the sample lifetime ($\tau_{ref} < \tau_{\phi}$). For instance, when Alexa-Fluor ($\tau_{\phi} = 1.2ns$) is used as reference while ATTO ($\tau_{\phi} = 1.8ns$) is the target dye, $\tau_{\phi,experiment} = 1.91ns, 2.05ns \approx \tau_{\phi,actual} = 1.8ns$. However, the two dyes are swopped in terms of their roles and that is, Alexa-Fluor as target and ATTO as reference (figures not shown), $\tau_{\phi,experiment} = 2.52ns, 2.67ns$, which are roughly twice the expected value of 1.2 ns.

Moreover, as mentioned earlier, we want to investigate the possibility of fluorescence emission from one excited dye interfering with the excitation of other samples in the situation of simultaneous excitation. This was done via comparing the results from simultaneous versus separate excitation. Considering the mean lifetime values from phase shift, both experiments under simultaneous and separate excitation yield quite similar results ($\tau_{\phi,sim} = 1.91ns \approx \tau_{\phi,sep} = 2.05ns$ and

$\tau_{\phi, sim} = 2.52 ns \approx \tau_{\phi, sep} = 2.67 ns$). Thus, the fluorescence emission signals have minimal effects, in terms of excitation of the surrounding dyes.

For clarity and completeness, the means and standard deviations for all the phases, modulation depths and corresponding lifetimes are computed and displayed in Table 2 and Table 3.

<i>Reference</i>	Alexa Fluor	Atto	<i>Reference</i>	Alexa Fluor	Atto
<i>Fluorescent sample</i>	Atto	Alexa Fluor	<i>Fluorescent sample</i>	Atto	Alexa Fluor
meanMR	0.6269	0.5745	meanLM	4.5593×10^{-9}	0
stdMR	0.0030	0.0015			
meanMF	0.5745	0.6269	stdLM	1.1886×10^{-10}	0
stdMF	0.0015	0.0030			
meanPR	-1.0689	-1.1378	meanLP	1.9056×10^{-9}	2.5212×10^{-9}
stdPR	0.0038	0.0022			
meanPF	-1.1378	-1.0689	stdLP	3.9680×10^{-11}	4.0713×10^{-11}
stdPF	0.0022	0.0038			

Table 2. Means and standard deviations of phases, modulation depths and corresponding lifetimes under simultaneous excitation.

<i>Reference</i>	Alexa Fluor	Atto	<i>Reference</i>	Alexa Fluor	Atto
<i>Fluorescent sample</i>	Atto	Alexa Fluor	<i>Fluorescent sample</i>	Atto	Alexa Fluor
meanMR	0.5847	0.5635	meanLM	3.0170×10^{-9}	0*
stdMR	0.0051	0.0034			
meanMF	0.5635	0.0051	stdLM	3.2249×10^{-10}	0*
stdMF	0.0034	0.0030			
meanPR	-0.9276	-1.0101	meanLP	2.0473×10^{-9}	2.6667×10^{-9}
stdPR	0.0038	0.0063			
meanPF	-1.0101	-0.9276	stdLP	6.9299×10^{-11}	7.1241×10^{-11}
stdPF	0.0063	0.0038			

Table 3. Means and standard deviations of phases, modulation depths and corresponding lifetimes under separate excitation.

The zeros with asterisks (*) as superscripts imply the presence of complex numbers involved in computation, which is irrelevant to our analysis.

7. Conclusion and Future Perspectives

In summary, under this project, we have presented homodyne frequency-domain DOT and FLI systems incorporating a gain-modulated image intensifier. Using the DOT system, we have shown that simultaneous measurement of both μ_a and μ_s' in homogeneous turbid medium can be achieved in transmission geometry by using a gain-modulated image intensifier. This method does not require long data acquisition time because many data with different source-detector separation are simultaneously acquired. In addition, we have developed a novel theoretical framework for frequency-domain DOT in transmission geometry, which utilizes an approximate analytic form of Green's function in transmission geometry and simplifies our homogeneous slab analysis. Moreover, we also presented a comprehensive multi-harmonic data analysis, which provides more accurate measurements of homogeneous optical properties. The main advantage of this kind of analysis is simplicity and robustness, because it uses stable linear regression rather than complex model fitting.

In terms of image reconstruction, we have conducted careful calibration of our frequency-domain DOT system, especially for the cylinder geometry with mirrors, before moving on to analytical reconstruction using real experimental data, which has not been performed previously in cylindrical DOT. We have demonstrated the

feasibility of our DOT systems by presenting reconstructed 3-D maps of absorption and scatter, for both infinite slab and cylinder geometries. The reconstructed absorption and scattering contrast values and spatial locations are found to be close to pre-determined values of optical properties and known locations of the targets respectively. In addition, reconstructed images obtained via tweaking certain reconstruction parameters have been presented, in order to demonstrate the influence of reconstruction parameters on the reconstructed results. The incorporation of higher harmonics into the image reconstruction process has also been explored and yielded better image quality if only odd harmonics are used, which is inherent in the image intensifier gain waveform.

On the other hand, under FLI, we have built a non-contact homodyne frequency-domain FLI system, by incorporating the gain-modulated image intensifier. We have conducted a preliminary study which investigates the possibility of fluorescence emission from one dye being absorbed by adjacent dyes, which in turn may affect the final fluorescence lifetime results. This is achieved via comparing the results obtained from simultaneous excitation of all dyes, with results from separate excitation of individual dyes. The comparison showed that there is little or no influence. We have also shown that fluorescence lifetime information obtained from phase data are more reliable, when compared to that

from modulation depth data, since it yields results closer to the actual sample lifetime with smaller percentage errors.

For future work, we can make use of the higher harmonics inherent in the image intensifier output signal for more accurate measurements of optical properties, as well as for multi-frequency DOT reconstruction. For this purpose, odd harmonics are preferred over even harmonics, as the image intensifier gain waveform has high inherent odd harmonic content and the first and third harmonics have been verified to provide more accurate measurements of optical properties, when compared to the second harmonic. This has been demonstrated in the results under Chapter 4. In addition, it would be necessary to further optimize the image intensifier parameters such as the cathode DC bias and MCP voltage, so that we can obtain higher signal-to-noise ratios for higher harmonics.

In addition, current DOT experimental techniques face the limitation of slow data acquisition, since a large number of source-detector pairs is required to achieve reasonable image quality. Thus, the use of spatially-modulated light can be explored, in order to reduce the acquisition time and computational complexity of the inverse problem. Additionally, the measured datasets can be compressed to further boost the image reconstruction speed.

Furthermore, the above mentioned image intensifier techniques and use of structured illumination can also be extended to fluorescence DOT (fDOT) with the use of indocyanine green (ICG) as an exogenous contrast dye. In these situations, the image intensifier can be utilized in frequency-domain fDOT systems in a similar homodyne technique, whereas the structured illumination approach can be used to speed up data acquisition.

As for FLI, more careful studies have to be conducted, in order to further verify the the feasibility and limitation of the instrumentation system, as well as the reliability of the obtained lifetime values. Since the in-vivo lifetimes are very sensitive to many factors, the effects of background autofluorescence and photobleaching are potential future directions for investigation as well.

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9. Appendices

9.1 Matlab implementation for modified Bessel function I_m in cylindrical reconstruction

As mentioned earlier, the Green's function for infinite cylindrical geometry is:

$$G_0(\vec{r}, \vec{r}') = \sum_{m=-\infty}^{\infty} \int g(m, q; \rho) \exp[im(\varphi - \varphi')] \exp[iq(z - z')] \frac{dq}{(2\pi)^2},$$

where $g(m, q; \rho) = \frac{1}{R} \left[\frac{I_m(Q\rho)}{I_m(QR) + QI'_m(QR)} \right]$ [57],

$I_m(Q\rho)$ = modified Bessel function of the 1st kind, z = cylinder height and ρ = cylinder radius.

In general, $I_m(x) = i^{-n} J_m(ix)$, where $J_m(ix)$ is the Bessel function of the 1st kind.

Under Matlab implementation, the series form of $I_m(x) = \sum_{s=0}^{\infty} \frac{1}{s!(s+m)!} \left(\frac{x}{2}\right)^{2s+m}$ is

used, while its first derivative is derived based on the recurrence relation,

$I'_m(QR) = [I_{m-1}(QR) + I_{m+1}(QR)]/2$. The figure below shows plots of $I_m(x)$ against

x for various values of m .

Figure 43. Plots of $I_m(x)$ against x .

9.2 Matlab codes for FLI

```
clear all

%Input parameters
np = 9;           %no of phases
w = 70*10^6;      %modulation frequency

%Read background intensity
% B = imread('bg.tif');

%Read intensity values from images & remove background
intensity
S = cell(1,np);
S_g = cell(1,np);
S_s = cell(1,np);

for i = 1:np
    S{i} = imread(['s' num2str(i,'%04.0f'), '.tif']);
    I{i} = I{i} - B;
    S_g{i} = double(S{i}(:, :, 2));
    S_s{i} = S_g{i}(1376:1425, 326:375);
end

%Sine-fitting
xdata = zeros(50,50,np);
ydata = zeros(50,50,np);
Sy = zeros(50,50,3);

for i = 1:np
    xdata(:, :, i) = 360/np*(i-1);
    ydata(:, :, i) = S_s{i}(:, :);
end

for j = 1:50
    for k = 1:50
        Sy(j,k,:) = lsqcurvefit(@(Sy,xdata)
            Sy(1)*sin(xdata*pi/180 + unwrap(Sy(2))) +
            Sy(3), [50,0,0], xdata(j,k,:), ydata(j,k,:));
    end
end
Smod1 = Sy(:, :, 1) ./ Sy(:, :, 3);
Sphil = Sy(:, :, 2);

%Normal fourier analysis
```

```

Fdc = zeros(50,50);
Fsin2 = zeros(50,50);
Fcos2 = zeros(50,50);

for i = 1:np
    Fdc = Fdc + S_s{i};
    Fsin2 = Fsin2 + S_s{i}.*sin(2*pi*i/np);
    Fcos2 = Fcos2 + S_s{i}.*cos(2*pi*i/np);
end

Sphi2 = atan(Fsin2./Fcos2);
Smod2 = 2*sqrt(Fsin2.^2 + Fcos2.^2)./Fdc;

%Read intensity values from images & remove background
intensity
F = cell(1,np);
F_g = cell(1,np);
F_s = cell(1,np);

for i = 1:np
    F{i} = imread(['f' num2str(i,'%04.0f'), '.tif']);
    %    I{i} = I{i} - B;
    F_g{i} = double(F{i}(:,:,2));
    F_s{i} = F_g{i}(1376:1425,326:375);
    %    I_s{i} = G{i}(1001:1050,596:645);
end

%Normal fourier analysis
Fdc = zeros(50,50);
Fsin2 = zeros(50,50);
Fcos2 = zeros(50,50);

for i = 1:np
    Fdc = Fdc + F_s{i};
    Fsin2 = Fsin2 + F_s{i}.*sin(2*pi*i/np);
    Fcos2 = Fcos2 + F_s{i}.*cos(2*pi*i/np);
end

Fphi2 = atan(Fsin2./Fcos2);
Fmod2 = 2*sqrt(Fsin2.^2 + Fcos2.^2)./Fdc;

%Calculate lifetimes from modulation depths and phases
Smod1_mean = mean(mean(Smod1));
Smod2_mean = mean(mean(Smod2));
Sphi1_mean = mean(mean(Sphi1));
Sphi2_mean = mean(mean(Sphi2));

```

```
Tau1_phi = tan(abs(Fphi1 - Sphi1_mean))./w;  
Tau2_phi = tan(abs(Fphi2 - Sphi2_mean))./w;  
Tau1_mod = sqrt(1./((Smod1_mean./Fmod1).^2-1))./w;  
Tau2_mod = sqrt(1./((Smod2_mean./Fmod2).^2-1))./w;  
Tau1_avg = (Tau1_phi + Tau1_mod)/2;  
Tau2_avg = (Tau2_phi + Tau2_mod)/2;
```

10. List of Publications

1. **Jun Hui Ho**, Hooi Ling Chin, Jing Dong, and Kijoon Lee, “Multi-harmonic homodyne approach for optical property measurement of turbid medium in transmission geometry,” *Optics Comm.* 285, 2007-2011 (2012).
2. **Jun Hui Ho**, Jing Dong and Kijoon Lee, “Diagnostic and Therapeutic Breast Cancer Imaging, Vol. 4 - Diffuse Optical Imaging in Breast: Recent Progress,” *SPIE Book Chapter*, In Press.
3. Jing Dong, **Jun Hui Ho**, Renzhe Bi, Kijoon Lee, “Diffuse Correlation Spectroscopy with an FFT-based software autocorrelator,” *JBO*, In Press.
4. Jing Dong, **Jun Hui Ho**, Siong Luong Ting, Patricia S.P. Thong and Kijoon Lee, “Noninvasive Diffuse Optical Measurement of Hemodynamic Parameters during Photodynamic Therapy,” *Proc. Of the First International Symposium on Bioengineering (ISOB 2011)*.

Conference Presentations and Posters

1. **Jun Hui Ho**, Jing Dong and Kijoon Lee, “A Quantitative Analysis on Image Quality in an Analytical Diffuse Optical Tomography System,” *BIOS-SPIE Photonics West 2011*.
2. **Jun Hui Ho**, Jing Dong and Kijoon Lee, “Analytical Image Reconstruction in Diffuse Optical Tomography,” *IEEE Photonics Global Conference 2010*.
3. **Jun Hui Ho**, Jing Dong and Kijoon Lee,” Analytical Continuous-Wave Diffuse Optical Tomography in Cylindrical Geometry, *BIOS-SPIE Photonics West 2010*.
4. **Jun Hui Ho** and Kijoon Lee, “Fast Analytic Image Reconstruction of Absorbers in Diffuse Medium,” *IEEE Photonics Global Conference 2008*.